Edited by

MONIKA SUJKA
WALDEMAR GUSTAW
URSZULA PANKIEWICZ
KAROLINA WÓJCIAK

FOOD IN A SUSTAINABLE WORLD

KRAKÓW 2025

International Conference
“Food in a Sustainable World”

accompanying

the 20th anniversary celebrations of
the Faculty of Science
and Biotechnology,
University of Life Sciences in Lublin

Monika Sujka, Waldemar Gustaw,

Urszula Pankiewicz, Karolina Wójciak
(editors)

September 23-24, 2025, Lublin

Scientific reviewers:

prof. dr hab. Urszula Gawlik
prof. dr hab. Paweł Glibowski
prof. dr hab. Małgorzata Karwowska
prof. dr hab. Małgorzata Materska
prof. dr hab. Michał Świeca
prof. dr hab. Karolina Wójciak
dr hab. Ewa Baranowska-Wójcik, prof. uczelni
dr hab. Aldona Sobota, prof. uczelni
dr hab. Dariusz Stasiak, prof. uczelni
dr hab. Monika Sujka, prof. uczelni

Editorial preparation, typesetting and proofreading:

dr inż. Katarzyna Lisiecka
dr inż. Małgorzata Sierocka

Cover design:

Mariusz Witczak

Publishing house:

Polskie Towarzystwo Technologów Żywności
Wydawnictwo Naukowe PTTŻ
31-149 Kraków, ul. Balicka 122
http://wydawnictwo.pttz.org

ISBN 978-83-953897-7-1

Content

Changes in Physicochemical Parameters During Roasting of Coffee of Caturra Variety

Krzysztof Barabosz, Dominika B. Matuszek, Łukasz Biłos 6

Spent coffee grounds as a cosmetic raw material

Łukasz Biłos, Renata Kulesza, Barbara Krzysiak-Warzała, Dominika Matuszek, Julia Owsiak, Zuzanna Sykulska , Krzysztof Barabosz, Krzysztof Szkutnik 18

The knowledge, lifestyle and nutritional behavior of pregnant women

Monika Bojanowska, Małgorzata Kostecka, Marzena Pabich, Monika Sachadyn-Król 31

Bioactive and sensory peptides from microalgae proteins: an *in silico* profile and hydrolysis perspective

Łukasz Byczyński..50

Nutritional management of Crohn's disease

Anna Ciołek, Alicja Skrzypek, Dawid Leszkowicz 77

Students' knowledge and attitudes regarding energy drinks in the context of public health prevention

Renata Czeczko, Karolina Worku 91

Green solvent systems for extraction of bioactives from common thyme (*Thymus vulgaris* L.) in sustainable food production

Radosław Kowalski, Grażyna Kowalska, Marzena Włodarczyk-Stasiak, Artur Mazurek..105

Comparison of knowledge and dietary habits in a group of healthy and cancer patients

Małgorzata Kostecka, Monika Bojanowska, Monika Sachadyn-Król, Marzena Pabich 125

Probiotic-mediated biosynthesis of metal nanoparticles

Anna Krzepiłko, Agata Święciło 140

Cross-sectional study of body composition using bioelectrical impedance among high school students in Lublin (2023–2025)

Justyna Libera, Karolina Nowosad, Katarzyna Iłowiecka, Dorota Teterycz, Paulina Łysakowska 153

Role and mechanisms of inositol supplementation in women with PCOS

Karolina Nowosad, Justyna Libera, Agnieszka Malik 164

Knowledge of patients with SIBO or IMO about nutrition and supplementation

Marzena Pabich, Małgorzata Kostecka, Monika Bojanowska, Monika Sachadyn-Król 172

Dietary Fiber Content and Its Role in the Nutritional Therapy of Gastrointestinal Diseases – A Review of Recent Studies

Monika Sachadyn-Król, Małgorzata Kostecka, Monika Bojanowska, Marzena Pabich 184

Dietary interventions in Alzheimer's disease: a review of preventive and therapeutic potential

Alicja Skrzypek, Joanna Matysiak 203

The Use of Kefir Grains for the Production of Non-milk Fermented Beverages

Bożena Sosnowska, Katarzyna Czerwińska 216

The influence of spice plants on the prevention of non-communicable diseases development

Jessica Świerc 227

Nutritional Deficiencies in Celiac Disease: Insights from a Decade of Research (2015–2025)

Dorota Teterycz 238

Authors'Index……………………….……………………………………...…252

Changes in Physicochemical Parameters During Roasting of Coffee of Caturra Variety

Krzysztof Barabosz[1,3*], Dominika B. Matuszek[2], Łukasz Biłos[2,3]

[1]Faculty of Mechanical Engineering,
Opole University of Technology, Opole, 45-271 Poland.
[2]Faculty of Production Engineering and Logistics,
Opole University of Technology, Opole, 45-271 Poland.
[3]Hard Beans Sp. z o.o., Opole, 45-071 Poland.
[*]corresponding author: k.barabosz@hardbeans.com

Abstract

The aim of this study was to compare the roasting dynamics of two Guatemalan specialty Coffees (Caturra varietal) processed using the natural and washed methods. All samples were roasted under identical conditions using a Roest L100 Plus sample roaster with three profiles: Regular (73% power), Fast (88% power) and Slow (66% power). Key physicochemical parameters were monitored: bean temperature, time to yellow phase (175°C), rate of temperature increase (RoR), number of cracks, mass loss, moisture, water activity (a_w) and final roast color lightness. Washed beans (higher density, lower initial moisture and water activity) reached yellow phase faster and showed higher initial RoR. Naturally processed beans absorbed heat slower but underwent more intensive Maillard reactions, resulting in greater mass loss and darker color. Fast profile intensified cracking in natural coffees, while in washed ones it gave lighter roast. The Slow profile promoted sustainable development, especially for washed beans. The post-harvest processing method significantly affects the roasting dynamics, so adapting the roasting profiles to the processing method can optimize the final quality of the coffee.

Key words: coffee roasting, specialty coffee, roast profile, physicochemical parameters.

Introduction

Coffee roasting is a complex thermal transformation process involving simultaneous heat and mass transfer that fundamentally alter the physicochemical properties of green coffee beans [Bustos-Vanegas et al. 2025]. It also determines the sensory and commercial value of the final product [Mehaya and Mohammad 2020]. During this key processing step, numerous reactions occur, including moisture evaporation, Maillard browning, caramelization, and pyrolytic decomposition, each of which is governed by specific kinetic parameters and activation energies that respond differently to temperature-time profiles [Várady et al. 2022]. Scientific understanding of these transformation is becoming very important as the global Specialty coffee market continues to grow [Duarte et al. 2024]. For this study, we selected coffees sourced directly from producers in Guatemala. The Huehuetenango region exemplifies the combination of exceptional terroir and precise coffee cherry processing techniques, achieving high quality on the global coffee market. Located at an altitude of 1350 to 2000 meters above sea level, the region benefits from volcanic soils and distinctive microclimates that contribute to the creation of complex flavor profiles [Rusinek et al. 2022]. The roasting process begins with the evaporation of water content, followed by subsequent phases, including Maillard reactions, caramelization, and pyrolysis, with each step contributing to the development of characteristic color, aroma, and flavor compounds [Bustos-Vanegas et al. 2025; Tarigan et al. 2022]. Significant changes in physiochemical properties occur after the first crack, which is defined as the popping sound of the beans during roasting [Herawati et al. 2019]. It occurs when the accumulated steam pressure causes the beans to crack [Münchow et al. 2020]. Importantly, the post-harvest processing method significantly affects the composition of coffee metabolites, including chlorogenic acids and trigonelline contents, which directly influence roasting behavior and the final sensory quality [Worku Wondimkun et al. 2022]. The roasting profile, defined as the relationship between temperature and time, strongly influences the dynamics of chemical changes [Várady et al. 2022; Obando et al. 2024]. Understanding these mechanistic relationships is crucial to optimizing roasting protocols [Mutovkina and Bredikhin 2023]. The aim of this study is to further explain these dynamics by comparing two specialty, caturra variety coffees from the Huehuetenango region in Guatemala.

2. Materials and methods

2.1. Coffee samples

Two specialty Arabica Caturra coffees from Huehuetenango, Guatemala, representing natural and washed post-harvest processing methods were used. Bulk density of green beans was measured using a coffee moisture analyzer BeanPro 6070 (Sinar Technology, UK), obtaining 715 g/l for natural and 725 g/l for washed samples. Initial moisture content was assessed using the same instrument, recording 10.3% for natural and 9.3% for washed beans. Water activity was assessed at 19°C using a calibrated Rotronic HP23-AW-A (Process Sensing Technologies PST SAS, France) water activity meter, obtaining values of 0.497 for natural and 0.423 for washed samples (table 1). The setup of conducted research is presented in figure 1.

Table 1. Characteristic of green coffee beans used in research.

Parameter	Green coffee	
	Washed	Natural
Bulk density [g/l]	725±2.867	715±2.055
Moisture [%]	9.3±0.037	10.3±0.025
Water activity	0.423±0.003	0.497±0.007

2.2. Roasting procedure

Roasting was performed in a Roest L100 Plus (Roest Coffee, Norway) drum sample roaster in batches of 100 g. The starting temperature was 180°C (measured with a PT-100 resistive temperature detector), air flow 75% (PWM controlled fan) and drum stirrer speed 63 rpm. An example of a roasting curve and settings is presented in the figure 2. Three power profiles (fast: 88%, regular: 73%, slow: 66%) and three development times after the first crack (1 s, 75 s, 180 s) were applied. The first crack was detected by the built-in microphone VM3000 (Vesper Technologies Inc., USA) with a frequency range 20 Hz – 8 kHz, and its onset was normalized to five distinct cracks; a 5% power drop was applied immediately after the first crack.

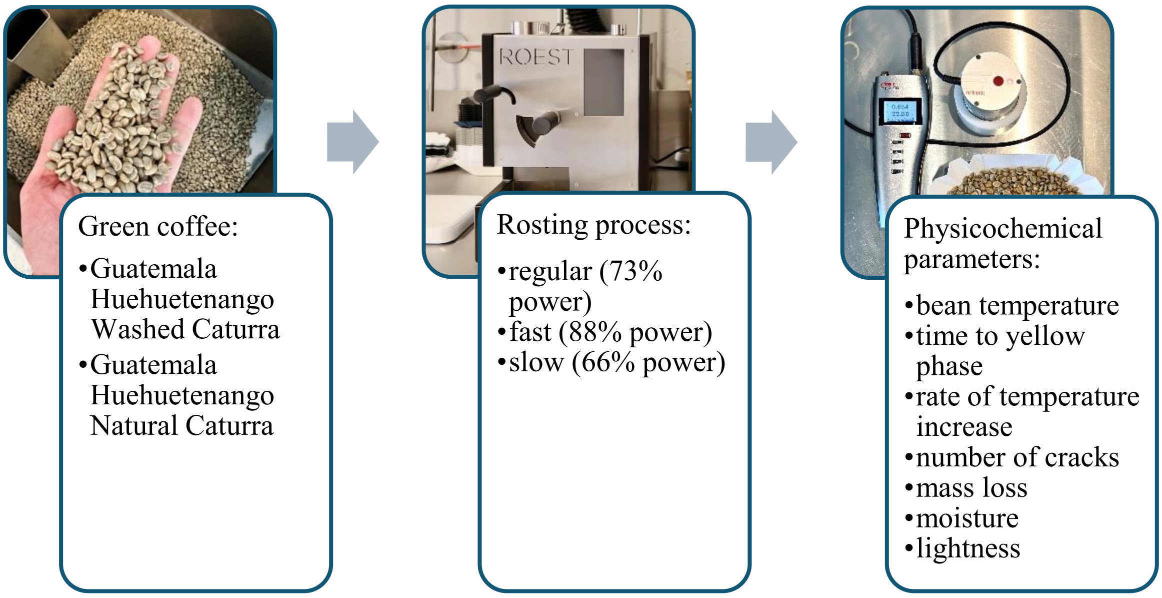

Figure 1. Methodology of conducted research – setup.

2.3. Physicochemical analyses of roasted coffee

The temperature of the beans was recorded every second using a K-type thermocouple embedded in the mass of beans, based on which the rate of temperature increase (RoR), time to yellowing (175°C) and to first crack were automatically calculated by the built-in sample roaster software. Weight loss was determined gravimetrically using a certified precision scale WTC 2000 (Radwag, Poland) with an accuracy of 0.01 g. Moisture content after roasting was re-evaluated using a coffee moisture analyzer BeanPro 6070 (Sinar Technology, UK). Water activity of roasted samples was measured using a Rotronic HP23-AW-A (Process Sensing Technologies PST SAS, France) water activity meter. The lightness of roasted coffee was assessed on ground samples using a colorimeter 3NH NH310 (Shenzhen Threenh Technology Co., Ltd.). All measurements were performed three times, and the results were averaged.

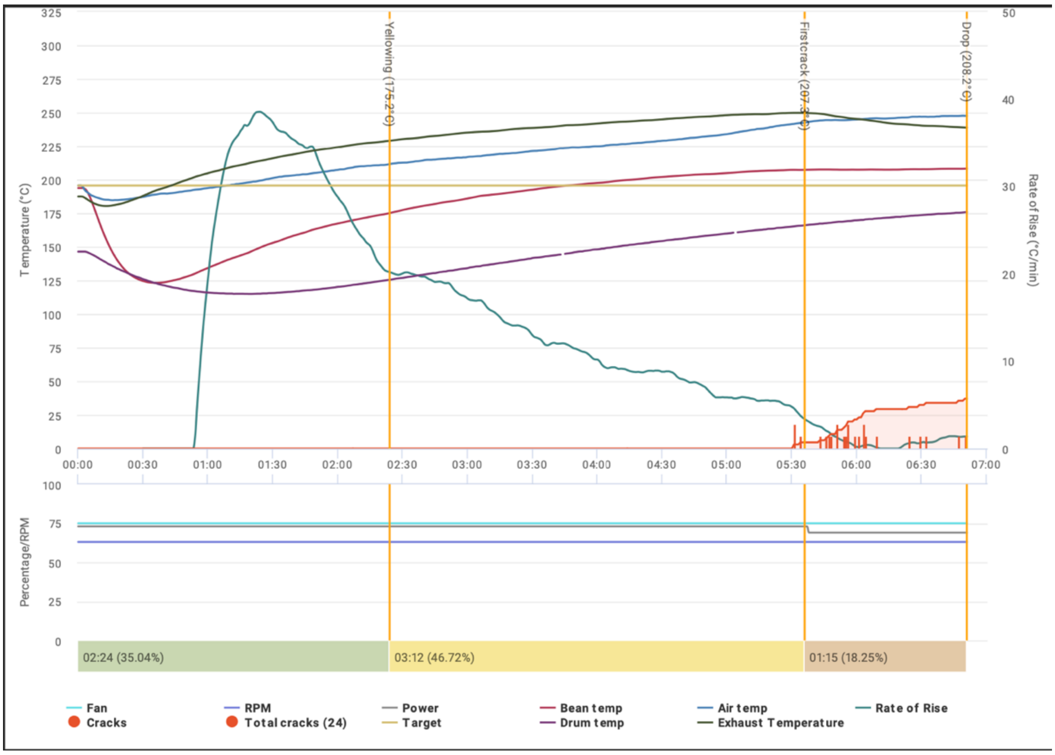

Figure 2. Example of a roasting profile.

3. Results

3.1. Initial physicochemical properties of green coffee beans

Natural beans had lower density, higher initial moisture content and higher water activity (table 1). These differences may directly affect thermophysical properties during roasting. Higher initial moisture requires more energy input to the endothermic drying phase and modulates the kinetics of the Maillard reaction [Geiger et al. 2005]. The lower density in natural beans may also change the conductive heat flux [Mudjijana et al. 2025].

3.2. Effect of development time using regular roasting profile (73% power)

Using regular power setting, the development time (DT) after the first crack was varied (1 s, 75 s, 180 s) to evaluate its effect on thermophysical parameters and structural transformations. Results are presented in table 2.

Table 2. Parameters of coffee grains after roasting process (regular profile, 73% power).

Parameter	Development time (DT), s					
	1		75		180	
	Washed coffee	Natural coffee	Washed coffee	Natural coffee	Washed coffee	Natural coffee
Bean temperature [°C]	207.00±0.00	204.00±0.71	208.75±0.78	211.30±1.27	220.8±1.27	226.60±0.49
Weight loss [%]	8.60±0.28	10.30±0.28	12.00±0.14	13.65±0.21	14.80±0.14	17.40±0.14
Moisture [%]	2.40±0.00	2.35±0.0007	1.95±0.0007	1.95±0.0007	1.75±0.0007	1.75±0.0007
Water activity	0.136±0.001	0.119±0.005	0.132±0.005	0.115±0.001	0.118±0.002	0.073±0.005
Lightness L*	40.45±0.015	38.28±0.011	35.21±0.010	34.43±0.015	33.46±0.005	31.44±0.015
Number of cracks	3.50±2.12	5.00±0.00	21.00±4.24	30.00±5.66	30.5±9.19	72.00±7.07

Roasting time and final grain temperature: Higher DT increased the total roasting time and final grain temperature, respectively. For naturally treated grains, the roasting time increased from 341 s (final temperature 205.5°C) at 1 s DT to 500 s (227.0°C) at 180 s DT. Washed coffee beans showed a similar trend (339 s at 207.0°C to 518 s at 219.9°C), reflecting the continuous energy input causing further changes.

Weight loss (WL): WL strongly correlates with DT, indicating increased water evaporation and loss of volatiles. WL of naturally processed beans increased from 10.3% (1 s DT) to 17.4% (180 s DT); WL of washed beans increased from 8.6% to 14.8%. Natural coffee consistently showed higher WL (e.g. ~13.65% vs. 12.0% at 75 s DT), which can be attributed to higher initial moisture content and greater release of volatiles during the roasting process.

Final moisture content (MC) and water activity (a_w): Final MC and aw decreased with extended DT, reflecting continued diffusion-driven drying. The MC content of naturally processed coffee decreased from 2.35% (a_w 0.119) at 1 s DT to 1.75% (a_w 0.073) at 180 s DT. The MC content of washed beans decreased from 2.4% (a_w 0.136) to 1.75% (a_w 0.118). The more significant reduction in MC content in natural beans suggests effective internal water migration, probably through the developing porous structure.

Color development (Lightness value): Bean lightness decreased with increasing DT, indicating an increase in Maillard and caramelization reactions. Naturally processed beans consistently achieved lower lightness values (darker) than washed beans at equivalent DT (e.g. L* 34.43 vs. 35.21 at 75 s DT), suggesting more intense reactions driven by their initial composition.

Structural changes (number of cracks): Audible crack emissions, demonstrating thermal stress-induced cracking when internal pressure exceeds structural integrity, increased with DT for natural coffee (5 at 1 s DT to 72 at 180 s DT). Crack counts for washed coffee increased from 3,55 to 30,5 and then plateaued. The higher number of cracks in natural beans suggests greater internal gas generation and potentially increased brittleness due to polysaccharide degradation.

3.3. Influence of Roast Profile (Heat Application Rate) at Fixed Development Time (75 s)

With DT fixed at 75 s, varying heat input rates: Fast (88% power), Regular (73% power), and Slow (66% power), were studied. Results are presented in table 3.

Roast time and final bean temperature: The fast profile resulted in the shortest roast times and highest final temperatures (natural: 307 s, 216.8°C; washed: 274 s, 212.0°C). In contrast, the slow profile resulted in the longest durations and lowest final temperatures (natural: 583 s, 203.3°C, washed: 670 s, 201.7°C), which directly reflects how the rate of energy input dictates the thermal trajectory.

Table 3. Parameters of coffee grains after roasting process 75 s (fast profile 88% power, slow profile 66% power).

Parameter	Roast profile			
	Fast		Slow	
	Washed coffee	Natural coffee	Washed coffee	Natural coffee
Bean temperature [°C]	211.08±0.20	217.45±0.65	201.45±0.25	202.00±1.30
Weight loss [%]	11.20±0.00	14.05±0.25	12.35±0.00	13.60±0.10
Moisture [%]	2.10±0.00	1.90±0.00	1.90±0.00	1.95±0.00
Water activity	0.134±0.005	0.080±0.001	0.102±0.005	0.075±0.005
Lightness L*	36.85±0.015	33.05±0.005	34.12±0.025	33.48±0.005
Number of cracks	30.50±2.50	66.00±3.00	3.50±0.50	10.00±1.00

Weight loss (WL): For naturally processed coffee, WL was relatively constant across all profiles (~13.5–13.8%). The WL of washed beans increased with the slower profiles (11.2% Fast to 12.4% Slow), suggesting that the extended exposure of slow roasts facilitated greater moisture/volatile diffusion, potentially limited by shorter exposure or a tight surface in the case of fast roasts.

Final Moisture Content (MC) and Water Activity (a_w): Washed beans had the highest final MC (2.1%) and a_w (0.134) after fast roasting, likely due to steeper thermal gradients resulting in less even moisture removal. Naturally treated beans consistently achieved lower a_w than washed beans (0.08 Fast, 0.075 Slow).

Color Development (Lightness value): The effect of profile on color varied significantly. For natural beans, the fast profile produced the darkest roast (L* 33.05). For washed beans, the slow profile was the darkest (L* 34.12), while the fast profile produced the lightest (L* 36.85). This highlights the different reaction kinetics; rapid heating of washed beans may not provide enough time for the full Maillard reaction and caramelization process [Yulianti et al. 2023].

Structural changes (number of cracks): Crack frequency scaled by thermal intensity. The fast profile, inducing steeper thermal gradients and rapid internal evaporation, generated the most cracks (natural: 63; washed: 29). The slow profiles produced the fewest. Naturally treated beans consistently cracked more intensely, indicating a greater susceptibility to mechanical stress.

Based on the results of the determined physicochemical parameters, statistical comparative analysis (Fisher's test) was performed at the level of significance (table 4).

Table 4. Results of statistical comparative analysis.

Parameters	Washed coffee		Natural coffee	
	F	p	F	p
Bean temperature [°C]	209.52	0.00060	62.11	0.00362
Weight loss [%]	83.40	0.00235	1.92	0.29033
Moisture [%]	36.31	0.00790	0.50	0.64952
Water activity	13.00	0.03327	58.06	0.00399
Lightness L*	1241.33	0.00004	5439.64	0.00000
Number of cracks	5920.45	0.00000	92.92	0.00200

The results of the comparative analysis show statistically significant differences in all quality parameters of washed coffee beans roasted with varying heat input rates. In the case of grains after natural processing, statistically significant differences were obtained for the parameters: bean temperature, water activity, lightness and number of cracks (table 4).

3.4. Summary of Thermomechanical Behavior

Naturally processed coffee beans with higher initial moisture content and lower density exhibited greater thermal reactivity and mechanical instability, as evidenced by greater mass loss, darker color, and more cracks. This is consistent with their properties requiring more energy to dry and drive more extensive exothermic reactions. The denser, initially drier, wet-processed grains exhibited different heat transfer characteristics, which may facilitate faster internal temperature increases but require specific time and temperature combinations for full chemical development.

These findings emphasize that the initial thermophysical properties of grains significantly determine roasting reactions. Matching roasting to these internal properties is essential to control heat/mass transfer, structural changes, and final product quality, consistent with the principles of thermal processing in porous media, where moisture, structure, and heat flux govern the kinetics of transformation.

4. Discussion

The different roasting dynamics of naturally and washed beans underscores the important influence of initial physicochemical properties on thermomechanical behavior. Higher initial moisture and water activity in naturally treated beans required more energy to reduce water content during early drying, which affected the rate of rise (RoR) before exothermic reactions became dominant. When water activity dropped below critical thresholds, naturally treated beans, with potentially higher residual sugars, showed intense chemical activity, as evidenced by rapid mass loss and development of darker color [Tarigan et al. 2022].

The consistently higher mass loss in natural processed beans suggests more extensive water evaporation and release of volatiles during the pyrolysis process [Geiger et al. 2005]. This, combined with a more significant chemical transformation, likely contributed to their darker color. The Fast profile (88% power) increased browning in naturally treated beans (L* 33.05) but resulted in lighter washed beans (L* 36.86). This discrepancy highlights how the reaction kinetics are related to heat transfer and bean composition; rapid heating of washed beans may not provide sufficient time for the full Maillard/caramelization reaction due to kinetic or precursor constraints [Hofmann 1998]. The differences in roasting profile responses illustrate the interplay between heat input and bean structure. Rapid roasting, with rapid heat input, rapidly generated high internal pressures, leading to more severe structural damage (cracks). Natural beans cracked more extensively (63 cracks) than washed beans (29 cracks). Slow roasting, with gentler thermal gradients, allowed for a more gradual pressure release, minimizing cracks in washed beans (2 cracks). This indicates that the mechanical integrity of the beans and their response to internal vapor pressure are significantly modulated by the heating rate and intrinsic properties. These results are consistent with roasting science, where aw is key in modulating reaction rates [Tarigan et al. 2022]. From a process engineering perspective, this requires tailored roasting strategies. Understanding these thermomechanical reactions is key to optimizing roasting profiles for specific bean types, ensuring consistent quality by managing heat and mass transfer, structural changes and reaction kinetics.

5. Conclusions

This study shows that the initial physicochemical properties of coffee beans, including moisture content, water activity, and bulk density, significantly affect their thermomechanical behavior during the roasting process. Naturally processed beans, which in this study had higher moisture content and lower density, required greater thermal input to initiate and maintain the roasting process, resulting in slower initial heating rates but more pronounced mass loss, darker final color, and ultimately a higher incidence of structural cracks. Washed beans, on the other hand, showed faster early heating and reached the yellow phase more quickly, but required longer exposure or milder profiles to achieve uniform levels of.

A comparison of three roasting profiles (fast, regular development, and slow) under controlled conditions showed that roasting dynamics are not linearly transferred between bean types. Fast roasting increased thermal gradients and internal pressure, leading to increased crack formation and uneven color development—particularly in natural beans. Slow profiles allowed for more even moisture diffusion and reduced mechanical stresses, which was particularly beneficial for denser washed beans, facilitating more controlled browning.

These findings confirm the need for differentiated roasting strategies tailored to the beans' internal thermophysical parameters. The selection of appropriate energy, airflow, and development time should be based on the bean origin and post-harvest treatment method to optimize heat and mass transfer, control structural transformation, and ultimately ensure consistent quality in specialty coffee production.

6. References

[1] Bustos-Vanegas J.D., Martins M.A., Corrêa P.C., Baptestini F.M., de Oliveira G.H.H.: Modeling and simulation of coffee bean heating during roasting: effect of heat generation. Frontiers in Food Science and Technology, 2025, 5, 1-7. https://doi.org/10.3389/frfst.2025.1603783

[2] Duarte R.N., Guimarães E.R., do Valle M.R., Galina S.V.R.: Unveiling the coopetition pathway: the stakeholder influence in the specialty coffee market. Benchmarking: An International Journal, 2025, 32(3), 1045-1068. https://doi.org/10.1108/BIJ-03-2023-0138

[3] Geiger R., Perren R., Kuenzli R., Escher F.: Carbon Dioxide Evolution and Moisture Evaporation During Roasting of Coffee Beans. Journal of Food Science, 2025, 70(2), E124–E130. https://doi.org/10.1111/j.1365-2621.2005.tb07084.x

[4] Herawati D., Giriwono P.E., Dewi F.N.A., Kashiwagi T., Andarwulan N.: Critical roasting level determines bioactive content and antioxidant activity of Robusta coffee beans. Food Science and Biotechnology, 2019, 28(1), 7–14. https://doi.org/10.1007/s10068-018-0442-x

[5] Hofmann T.: Studies on the Influence of the Solvent on the Contribution of Single Maillard Reaction Products to the Total Color of Browned Pentose/Alanine SolutionsA Quantitative Correlation Using the Color Activity Concept. Journal of Agricultural and Food Chemistry, 1998, 46(10), 3912–3917. https://doi.org/10.1021/jf980478a

[6] Mehaya F.M., Mohammad A.A.: Thermostability of bioactive compounds during roasting process of coffee beans. Heliyon, 2020, 6(11), e05508. https://doi.org/10.1016/j.heliyon.2020.e05508

[7] Mudjijana M., Kusni M., Wirasena M.A., Nugraha A.D., Darmanto S., Das S., Nugroho A.D., Alandro D., Ramadhan M.A., Rashyid M.I., Himarosa R. A., Muflikhun M.A.: The Characteristics of Moisture and Hardness of the Coffee Bean with Different Treatment Related to Roasting and Storage Time: Study Case from Coffee Arabica and Coffee Canephora. Engineering Headway, 2025, 21, 57–67. https://doi.org/10.4028/p-ynBc18

[8] Münchow M., Alstrup J., Steen I., Giacalone D.: Roasting Conditions and Coffee Flavor: A Multi-Study Empirical Investigation. Beverages, 2020, 6(2), 29. https://doi.org/10.3390/beverages6020029

[9] Mutovkina E.A., Bredikhin S.A.: Analysis of coffee thermophysical changes during roasting using differential scanning colorimetry. Food Science and Technology, 2023, 43. https://doi.org/10.1590/fst.119722

[10] Várady M., Tauchen J., Frankova A., Kloucek P., Popelka P.: Effect of method of processing specialty coffee beans (natural, washed, honey, fermentation, maceration) on bioactive and volatile compounds. LWT – Food Science and Technology, 2022, 172. https://doi.org/10.1016/j.lwt.2022.114245

[11] Rusinek R., Dobrzański B., Oniszczuk A., Gawrysiak-Witulska M., Siger A., Karami H., Ptaszyńska A.A., Żytek A., Kapela K., Gancarz M.: How to Identify Roast Defects in Coffee Beans Based on the Volatile Compound Profile. Molecules, 2022, 27(23), 8530. https://doi.org/10.3390/molecules27238530

[12] Tarigan E.B., Wardiana E., Hilmi Y.S., Komarudin N.A.: The changes in chemical properties of coffee during roasting: A review. IOP Conference Series: Earth and Environmental Science, Volume 974, 2022, The 2nd International Conference on Sustainable Plantation, Bogor, Indonesia, September 2-3, 2021, 012115. https://doi.org/10.1088/1755-1315/974/1/012115

[13] Obando A.M., Figueroa J.G.: Effect of Roasting Level on the Development of Key Aroma-Active Compounds in Coffee. *Molecules,* 2024, *29(19)*, 4723. https://doi.org/10.3390/molecules29194723

[14] Wondimkun Y.W, Emire S.A, Teferra T.F, Stoecker B., Esho, T.B.: Influence of genotype and processing on bioactive compounds of Ethiopian specialty Arabica coffee. International Journal of Food Properties, 2022, 25(1), 2574–2588. https://doi.org/10.1080/10942912.2022.2147540

[15] Yulianti Y., Adawiyah D.R., Herawati D., Indrasti D., Andarwulan N.: Detection of Markers in Green Beans and Roasted Beans of Kalosi-Enrekang Arabica Coffee with Different Postharvest Processing Using LC-MS/MS. International Journal of Food Science, 2023, 1–12. https://doi.org/10.1155/2023/6696808

Spent coffee grounds as a cosmetic raw material

Łukasz Biłos[1*], Renata Kulesza[2], Barbara Krzysiak-Warzała[2], Dominika Matuszek[1], Julia Owsiak[4], Zuzanna Sykulska[4] , Krzysztof Barabosz[1], Krzysztof Szkutnik[3]

[1]*Faculty of Production Engineering and Logistics, Opole University of Technology, Prószkowska 76, 45-758 Opole, Poland.*
[2]*Łukasiewicz Research Network- Institute of Heavy Organic Synthesis "Blachownia", Energetyków 9, 47-225 Kędzierzyn-Koźle, Poland.*
[3]*Hard Beans Sp.z o.o. 35 Armii Krajowej Street, 45-071 Opole, Poland*
[4]*BJB Cosmetics 3 Kopalina Street, 46-053 Chrząstowice, Poland*
[*] *corresponding author: l.bilos@po.edu.pl*

Abstract

The aim of this study is to investigate the potential of spent coffee grounds as a raw material for various industrial applications. This study explores the potential use of spent coffee grounds in the cosmetic industry. Spent coffee grounds still contain a rich matrix of ingredients with properties desirable for the proper functioning of the skin. Food grounds can be used in cosmetic products not only as carriers of active ingredients, but also as abrasives in various scrubs. In the study, an extraction process was used to extract active substances from spent coffee grounds. The drying was optimized and carried out at 70 °C, for 10-12 hours, under atmospheric pressure, until a moisture content of about 5% was reached. Coffee oil was extracted using methylene chloride as a solvent for 30min, at room temperature using ultrasound. A study of the composition of the spent coffee grounds compared to ground coffee shows that the amount of coffee oil obtained by extraction in the Soxhleth apparatus using methylene chloride is comparable in both cases. This therefore indicates that the spent coffee grounds have a composition comparable to ground coffee. The solvent mixture that allows the biggest amount of caffeine and chlorogenic acid to leach out is a mixture of 50% ethanol: 50% water. The appropriate extraction method, the time and temperature of this process, as well as the type of solvent, significantly affect the matrix of components of the extracted coffee oil, its quality and the efficiency of the process. Caffeine is the compound that leaches out of the grounds to the greatest extent. In addition, chlorogenic acid and trigonelline can be isolated, as well as phenolic compounds and flavonoids

Keywords: Spent coffee grounds, upcycling, extraction, coffee, cosmetic raw material

1. Introduction

Coffea arabica is a perennial shrub characterized by opposite leaves and small, fragrant white flowers. This species thrives in the tropical regions known as the "coffee belt," situated between the Tropic of Cancer and the Tropic of Capricorn. Typically, *C. arabica* begins to bear fruit between three and five years after planting. The mature coffee cherry contains green seeds, which, after harvesting and processing, are used to produce coffee extract [Hoffmann, J. 2018]. Recent literature indicates that brewing roasted coffee, whether through cold brew (CB) methods at room temperature or batch brew (BB) techniques at temperatures ranging from 80 to 96 °C, leaves significant amounts of bioactive compounds in the spent grounds [Anh-Dao et al. 2024]. A study of the composition of grounds needs to be carried out, however, caffeine, trigonelline and chlorogenic acid are the main active ingredients, which also have a beneficial effect on improving skin condition [Pujol, D., et al. 2013, Carvalho Neto et al. 2021]. Trigonelline has a protective effect against oxidative DNA damage and shields cells from photodamage caused by UVB radiation. It may act as a potentially powerful cosmetic agent in this regard [Malik et al. 2023]. Caffeine can increase apoptosis (programmed death) of damaged skin cells, which may prevent cancer [Lu, Y. et al. 2002]. Chlorogenic acid reduces the production of reactive oxygen species (ROS) and levels of pro-inflammatory cytokines, which protects skin fibroblasts from UV damage and slows down the skin aging process [Girsang et al. 2021]. Selecting the appropriate oil extraction method from the grounds and the physicochemical parameters of the process significantly influences the quantitative chemical composition of the obtained raw material. Proper optimization of these parameters enables the production of a cosmetic raw material with the desired composition [Krinski et al. 2025]. Once extracted and dried, the grounds - due to their moisture-retaining capacity - can be used as an abrasive agent in various types of cosmetic scrubs. The development of a cosmetic product formulation is a complex process that involves selecting appropriate raw material quantities, defining technological parameters, and determining the optimal time and temperature for each processing stage [Nowak et al. 2022]. This formulation process will be discussed in a separate work. Defining composition parameters is crucial for optimizing the production process of the raw material, which will subsequently be used in the formulation of the cosmetic product as an active ingredient. The brewing process determines the moisture content of the waste coffee grounds, which can range from 50-85% [Al -Hamamre, Z., et al. (2012), Gómez-de la Cruz et al. 2015]. It is possible to purchase raw material that is consistent in terms of particle size, moisture content and bulk density - dried coffee grounds are available on the market [Ecobean (2025)]. When comparing moisture levels in grounds, retail coffee shop grounds have lower levels than waste grounds from instant coffee production [Al -Hamamre, Z., et al. (2012), Gómez-de la Cruz et al. 2015]. Most of the methods for extracting substances from grounds described in the literature cite a dry raw material as a necessity, so the aforementioned moisture content is a hindrance to the use of grounds both as a raw material but also for extracting coffee oil (the lipid fraction) [Del Catillo M. D et al. 2013; Zarebska et al. 2022]. Different drying methods are used in laboratories, which show their advantages as well as disadvantages of use: convection, microwave, infrared or contact drying.

2. Materials and Methods

The study used methods commonly recognised and practised in analytical laboratories.

2.1. Raw materials and solvents

Raw material: The coffee used in the study was Coffeea Arabica segment Specialty coffee sourced from the coffee roaster Hard Beans from Opole, which carries out coffee research [Stanek et al. 2021]. The coffee, although of the same botanical variety came from different sources and the samples were labelled Ethiopia Kello, Brazil Guariroba, Costa Rica El Higueron.

Extraction: Ethanol, 96%, pure for analysis, P.P.H. „Stanlab", CAS: 64-17-5

Liquid chromatography: In the study the following materials were used: Chlorogenic acid, 3-CQA, Sigma Aldrich, 99%, CAS: 327-97-9, Neochlorogenic acid, 5-CQA, Sigma Aldrich, 99.5%, CAS: 906-33-2, Caffeine, Fluka, 99%, CAS: 58-08-2, Trigonelline, 99.8%, ChromaDex, CAS: 535-83-1 Water, Ultrapure Direct-Q, CAS: 7732-18-5, Methanol, POCH, HPLC purity, CAS: 67-56-1, Formic acid, Fisher Scientific, LC/MS purity, CAS: 64-18-6.

2.2. Preliminary studies

The composition of the grounds was compared to ground coffee using infrared spectroscopy and liquid chromatography as preliminary tests. The grounds were left overnight in a fume cupboard, after being drained on laboratory blotting paper and spread on porcelain cuvettes. The resulting free-flowing powder was placed in an oven at 70°C for 10-12h so that the moisture content was approximately 5%, which was checked on a moisture analyzer. The grounds prepared in this way were subjected to solvent extraction. The extraction of dried grounds and ground coffee in a Soxhlet apparatus using methylene chloride as extractant was also compared. The extraction was carried out until the colour of the solvent disappeared. Subsequently, evaporation of the solvent from the extracts was carried out on a vacuum evaporator. The quantities of "coffee oil" obtained from the dry grounds were about 12% and from the ground coffee about 12%. The spectra of the oils obtained are shown in Figure 1. In addition, the dry grounds were also extracted with water in a Soxhlet apparatus and 2.8 % "coffee oil" was thus obtained.

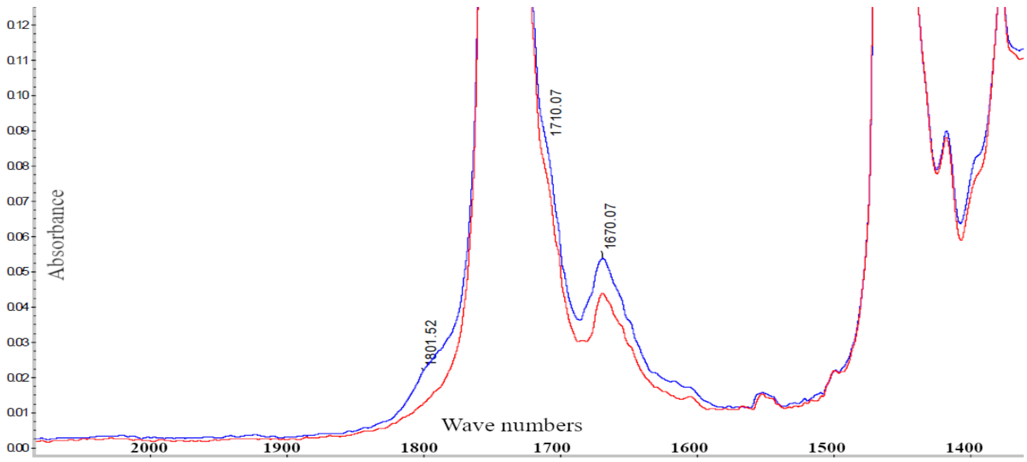

Figure 1. Comparison of FTIR spectra from extracts of dried grounds and ground coffee. Source: own elaboration.

For comparison, hot water extraction of ground coffee and dried grounds was carried out. The results obtained are shown in Table 1. The conclusion of the process is that grounds are a rich source of caffeine and, relative to brewed coffee, this accounts for about 11%. The other determined compounds represent ca. 3-4%.

Table 1. Comparison of chlorogenic acid, caffeine and trigonelline content of coffee and grounds obtained from this coffee.

	Content [mg / 100g]				
	5-CQA	3-CQA	4-CQA	caffeine	trigonelline
Ground coffee	596	1824	726	1313	1445
Dried grounds	18	53	27	138	44
%	3%	3%	4%	11%	3%

The dried grounds were mixed with the eluent in a proportion determined by the system being implemented and shaken or assisted by ultrasound. Such a system was then filtered and the eluent evaporated on a rotary evaporator. Time variants of 10, 30 and 60 minutes and a 50/50 ethanol/water system were adopted as the extraction systems. Water and ethanol in 100% systems with shaking applied were taken as reference. Samples of extracts were analyzed by HPLC in triplicate, in reversed-phase, using a DAD detector (HPLC-DAD). Qualitative as well as quantitative analysis of chlorogenic acid in the form of three isomers: 3-CQA, 4-CQA and 5-CQA were performed based on a modified method proposed by Meinhart A.D., et al. [14 Identification of individual compounds was carried out based on retention times. Analyte contents were determined by the external standard method on a Kinetex XB C-18, 100x4.6 mm, 3.5-µm column at 30^{o}C. 0.1% formic acid, pH 2.4, and methanol were used as eluents. The flow rate was 1.0 ml/min.A DAD detector was used, which at 325 nm allowed the determination of 3-CQA, 4-CQA and 5-CQA, while at 272 nm trigonelline and caffeine were determined.

3. Results

Table 2 shows the results of the determination of chlorogenic acid isomers, caffeine and trigonelline by HPLC in the tested systems leaching active substances from coffee grounds.

Figure 2 illustrates the solution separation of an example extract of a sample of grounds obtained on a chromatography column of the C-18 type. Part A is the chromatogram obtained at 325 nm, which shows the peaks of chlorogenic acids in the order: peak 1 - 5-CQA, peak 2 - 3- CQA and peak 3 - 4-CQA. A chromatogram collected at 272 nm is described as B, which shows peaks from trigonelline - peak 4 and from caffeine - peak 5. Caffeine was the compound that eluted from the grounds in the highest amount 23.88 - 201.6 mg/100g. Next were 3-CQA acid and trigonelline, of which the amounts were close to each other. The lowest concentrations were recorded for 5-CQA and 4-CQA.
The best extraction efficiency was observed using the ethanol-water solvent system. In Figure 3, the amounts determined in the ethanol-water extracts of leached compounds are trending: caffeine > 3-CQA > trigonelline and 4-CQA > 5-CQA. Comparing the times of running the process, it can be concluded that the differences in the amounts of leached compounds between extraction carried out for 30min and 60min are so small that from an economic point of view it is better to carry out a shorter extraction with similar efficiency.

Table 2. Results of HPLC determinations.

Solvent system	Time [MIN]	Content [mg/100g]									
		5-CQA	±SD	3-CQA	±SD	4-CQA	±SD	caffeine	±SD	Trigo-nelline	±SD
W100%	10	17,7	0,48	51,63	0,75	22,34	0,64	148,65	0,97	46,71	0,13
W50% E50%	10	12,0	3,41	61,06	0,8	45,66	0,21	187,26	2,84	58,03	0,14
E 100%	10	0	0	2,92	0,29	1,58	0,01	23,88	0,23	0,16	0,33
W100%	30	18,6	0,56	54,28	2,07	23,49	1,22	0	0,23	47,22	0,19
W50% E50%	30	9,3	0,05	71,83	0,73	49,66	1,47	196,2	1,21	51,38	0,79
E 100%	30	0,3	0,03	3,12	0,11	2,53	0,04	25,88	7,05	3,48	0,46
W100%	60	19,3	1,28	55,49	1,5	24,24	1,36	164,61	1,23	46,12	3,02
W50% E50%	60	15,0	0,78	68,54	0,92	50,12	0,31	201,6	8,71	60,52	0,57
E 100%	60	0,19	0	3,33	0,27	5,05	1,5	28,79	5,5	2,66	1,6

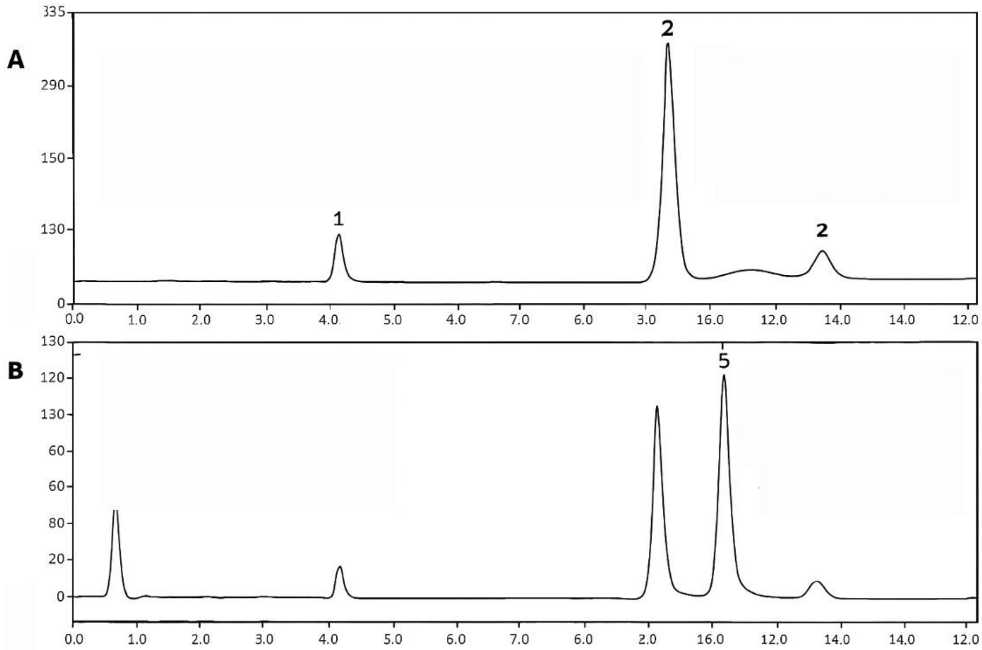

Figure 2. Chromatographic separation of a solution of an example extract of a sample of grounds obtained on a C-18 type chromatographic column. Source: own elaboration.

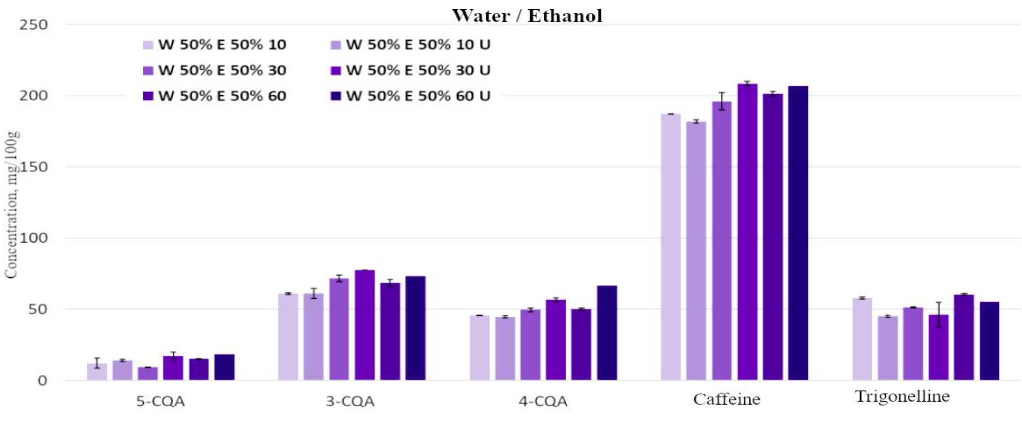

Figure 3. Extraction results in the analyzed systems. Source: own elaboration.

3.1. Qualitative analysis of the obtained oil

Laboratory-scale coffee oil was created using ultrasound-assisted extraction of coffee grounds at room temperature for 30min using methylene chloride. The extract was then drained and washed with solvent. Finally, the solvent was evaporated on a vacuum evaporator at a solvent boiling point of 35°C, at a pressure of about 240 mbar. The resulting oil was left in an open vessel under a fume cupboard at room temperature for a period of several hours. The Figure 4 shows the FTIR spectrum of the obtained coffee oil.

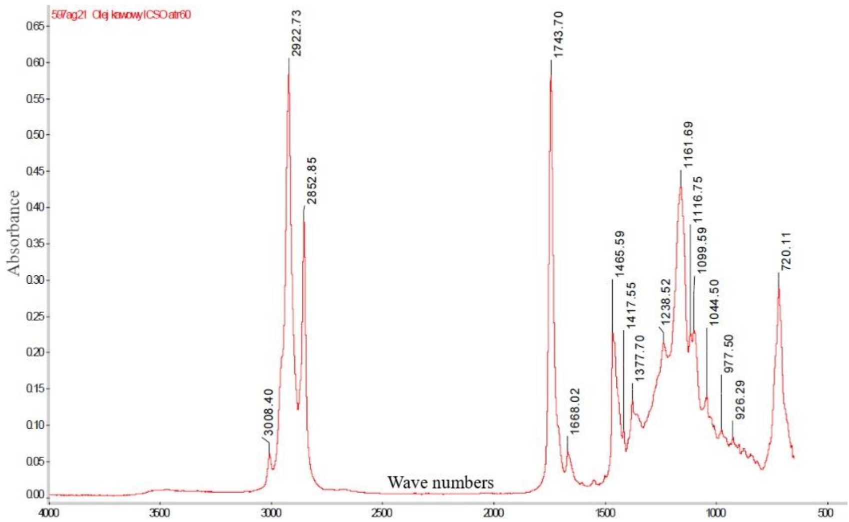

Figure 4. FTIR spectrum of the obtained coffee oil. Source: own elaboration.

Main bands in coffee oils: around 3500 cm^{-1} – overtone in glycerides C=O, 3009 cm^{-1} – C-H of HC=CH groups in cis-olefins, 2955 cm^{-1} – C-H of CH_3 groups in aliphatic chains, 2923, 2853 cm^{-1} – C-H of CH_2 groups in aliphatic chains, 1744 cm^{-1} – C=O in O-C=O of triglycerides, 1654 cm^{-1} – C=C in cis RHC=CHR, cis-olefins, 1465 and 1377 cm^{-1} – C-H of -CH_3 and -CH_2- groups in alkanes, 1237 and 1161 cm^{-1} – C-O in ester groups, 1118 and 1099 cm^{-1} – C-O in ester groups, 720 cm^{-1} – out-of-plane vibrations in cis-olefins and C-H of $(CH_2)n \geq 4$ groups.

3.2. Physicochemical analyses of raw materials

To obtain and use the grounds and extract as a cosmetic raw material, the resulting extract was concentrated on a rotary evaporator adjusting the temperature to completely remove the solvent from the extract. Determination of the physicochemical parameters of the post-extraction grounds made it possible to make a cosmetic raw material for skin exfoliating products. Tables 3 and 4 show the characteristics of the obtained fractions - spent coffee grounds and coffee oil.

Table 3. Characteristics of the cosmetic raw material - spent coffee grounds.

Specification	Result
State of matter	Solid, powder
Color	Brown
Odor	Characteristic
Moisture content	≤ 5%
pH (aqueous extract, concentration 1 g/100 ml, 20°C)	5,4
Solubility	Partially soluble in water

Table 4. Characteristics of the cosmetic raw material - coffee oil.

Specification	Result
State of matter	Liquid, possible sediment
Color:	Brown to dark brown
Odor:	Characteristic of roasted coffee
Density (20°C):	0,95 g/cm^3
pH (direct measurement at 20°C):	4,3
Kinematic viscosity (20°C):	83 mm^2/s
Refractive index:	1,4777
Solubility:	Non-soluble in water
Flash-point	77 (±5) °C (closed crucible)
Acid number	3,55 mg KOH/g
Peroxide value	< 1 meq O_2/kg

4. Discussion

After the study, the process parameters for drying coffee grounds and extracting coffee oil from coffee grounds were optimized. Drying was carried out at 70°C, for 10-12h, at atmospheric pressure until a moisture content of about 5% was reached. Coffee oil was extracted using methylene chloride as a solvent for 30min, at room temperature using ultrasound. A study of the composition of the spent coffee grounds compared to ground coffee shows that the amount of coffee oil obtained by extraction in the Soxleth apparatus using methylene chloride is comparable in both cases. This therefore indicates that the spent coffee grounds have a composition comparable to ground coffee. The solvent mixture that allows the biggest amount of caffeine and chlorogenic acid to leach out is a mixture of 50% ethanol: 50% water, while for trigonelline it is a combination of 25% glycerin: 75% water. An increase in temperature and the length of the extraction run resulted in an increase in the amount of compounds leached, but there are no such observations with ultrasound. Similarly, as for phenolic compounds and flavonoids, the solvent system 50% ethanol: 50% water allowed the greatest amount of these substances to leach out, and the obtained also had the highest antioxidant activity values. In the study carried out by Al-Hamamre et al., the best coffee oil extraction yield from coffee grounds was obtained using a non-polar solvent, hexane (15.28%), with an extraction time of 30 minutes[Al -Hamamre, Z., et al. 2012]. However, it is desirable to move away from toxic solvents, replacing them with more environmentally friendly solvents that can be recovered. Modification of the temperature, solvent and process time can affect the extraction efficiency, as shown by the results obtained by Krinski et al., who, when carrying out the extraction process using ethanol as a solvent, obtained an efficiency of 14.57% at 60ºC [Krinski et al. 2025]. The efficiency results obtained indicate a slight difference between processes with different parameters and solvents used. However, ethanol is definitely less toxic than hexane. Confirmation that ethanol is a safe, yet inexpensive and repeatable solvent extraction method is provided by studies conducted by Meinhart et al. [Meinhart, A.D., et al. 2017]. The research carried out by Krinski confirms that ethanol is a safe, inexpensive and repeatable solvent extraction method.

Based on Krinski's research, the efficiency of the process can also be increased by reducing the size of the coffee grounds particles [Krinski et al. 2025]. During our research, we also noticed that grinding the grounds did not affect the composition of the oils extracted from them. This research allowed us to develop two cosmetic raw materials from coffee waste: coffee grounds as an abrasive material for cosmetic scrubs, and recycled coffee oil. Below, in Figure 5, there are cosmetic products made from raw materials obtained from coffee grounds.

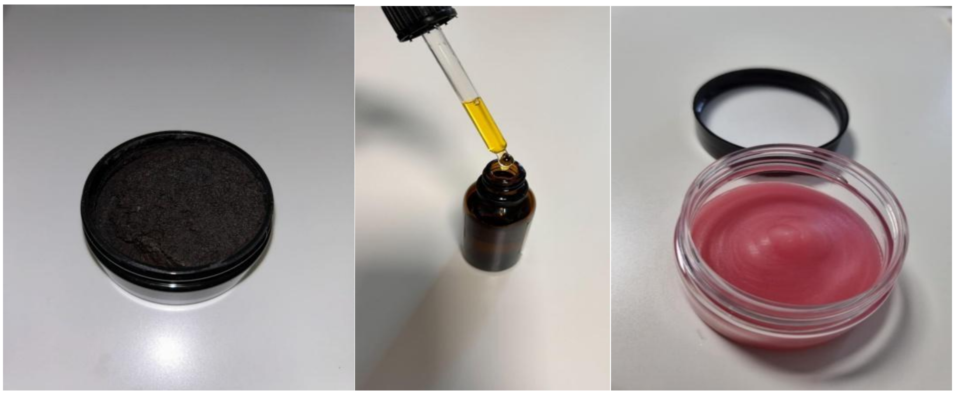

Figure 5. Coffee grounds scrub, Coffee grounds oil, Eye butter with coffee oil - Cosmetic products made in up-cycle trend.

5. Conclusions

This study shows that choosing the right parameters such as time, temperature and pressure for drying grounds significantly affects their final moisture content and the speed of the process. The drying process itself affects the content of active substances in spent coffee grounds. The appropriate extraction method, the time and temperature of this process, as well as the type of solvent, significantly affect the matrix of components of the extracted coffee oil, its quality and the efficiency of the process. Caffeine is the compound that leaches out of the grounds to the greatest extent. In addition, chlorogenic acid and trigonelline can be isolated, as well as phenolic compounds and flavonoids. The active ingredients present in the extracted cosmetic raw materials have properties that significantly improve the condition of the skin.

6. References

[1] Anh-Dao, L.T., Chi-Thien, T., Nhut-Truong, N., Tu-Chi, T., Minh-Huy, D., Thanh-Nho, N., Cong-Hau, N. (2024). Changes in the total phenolic contents, chlorogenic acid, and caffeine of coffee cups regarding different brewing methods. Food Research, 8, 71–79.

[2] Al -Hamamre, Z., et al. (2012). Oil extracted from spent coffee grounds as a renewable source for fatty acid methyl ester manufacturing. Fuel, 96, 70–76.

[3] Ballesteros, L.F., Teixeira, J.A., Mussatto, S.I. (2014). Chemical, functional, and structural properties of spent coffee grounds and coffee silverskin. Food and Bioprocess Technology, 7, 3493–3503.

[4] Bio-bean.com. https://bio-bean.com/renrwals/inficaf

[5] Campos -Vega, R., Loarca-Piña, G., Vergara-Castañeda, H.A., Oomah, B.D. (2015). Spent Coffee Grounds: A Review on Current Research and Future Prospects. Trends in Food Science & Technology, 45, 24–36.

[6] Carvalho Neto, D.P., Gonot-Schoupinsky, X.P., Gonot-Schoupinsky, F.N. (2021). Coffee as a Naturally Beneficial and Sustainable Ingredient in Personal Care Products: A Systematic Scoping Review of the Evidence. Frontiers in Sustainability, 2.

[7] Del Catillo M. D., Ibañez M. E., Amigo-Benavent M., Herrero M., Plaza M., Ullate M., (2013). Application of products of coffee silverskin in anti-ageing cosmetics and functional food. WO2013004873 A1.

[8] Girsang, E., Ginting, C.N., Lister, I.N.E., Gunawan, K.Y., Widowati, W. (2021). Anti-inflammatory and antiaging properties of chlorogenic acid on UV-induced fibroblast cell. PeerJ, Jul 7;9:e11419.

[9] Gómez-de la Cruz, F.J., Cruz-Peragón, F., Casanova-Peláez, P.J., Palomar-Carnicero, J.M. (2015). A vital stage in the large-scale production of biofuels from spent coffee grounds: the drying kinetics. Fuel Processing Technology, 130, 188–196.

[10] Hoffmann, J. (2018). The World Atlas of Coffee: From Beans to Brewing - Coffees Explored, Explained and Enjoyed (2nd ed.). Mitchell Beazley: London, UK. https://imp.dayawisesa.com/wp-content/uploads/2023/07/The_world_atlas_of_coffee_from_beans_to_brewing_coffees_explored-1.pdf (accessed on 9 July 2025).

[11] Krinski, I.M., Leite, V.R., Moura, L.M., Mariani, V.C. (2025). Critical Extraction Parameters for Maximizing Oil Yield from Spent Coffee Grounds. Energies, 18, 1346.

[12] Lu, Y., Lou, Y., Xie, J., Peng, Q., Liao, J., Yang, C.S., Huang, M., Conney, A.H. (2002). Topical applications of caffeine or EGCG inhibit carcinogenesis and selectively increase apoptosis in UVB-induced skin tumors in mice. PNAS, 99, 12455–12460.

[13] Malik, A.T., Rashid, H., Nazir, L.A., Archoo, S., Shahid, N.H., Gupta, R., Umar, S.A., Tasduq, S.A. (2023). Trigonelline, a plant-derived alkaloid prevents ultraviolet-B-induced oxidative DNA damage in primary human dermal fibroblasts and BALB/c mice via modulation of phosphoinositide 3-kinase-Akt-Nrf2 signalling axis. Experimental Gerontology, 171, 112028.

[14] Meinhart, A.D., et al. (2017). Multivariate optimization of chlorogenic acid extraction from Brazilian coffee. Food Analytical Methods, 10(9), 2943–2951.

[15] Nowak, I., Latanowicz, K., Bętlewski, A., Feliczak-Guzik, A., Wawrzyńczak, A. (2022). Produkt kosmetyczny – od koncepcji w laboratorium do produkcji przemysłowej. Wiadomości Chemiczne, 76, 7–8.

[16] Pujol, D., et al. (2013). The chemical composition of exhausted coffee waste. Industrial Crops and Products, 50, 423–429.

[17] Rodrigues, R., Oliveira, M.B.P.P., Alves, R.C. (2023). Chlorogenic Acids and Caffeine from Coffee By-Products: A Review on Skincare Applications. Cosmetics, 10, 12. https://doi.org/10.3390/cosmetics10010012

[18] Shahnamdeh, M. (2022). Coffee Market: Worldwide Production 2003/04-2020/21. https://www.statista.com/statistics/263311/worldwide-production-of-coffee/ (accessed on 26 November 2022).

[19] Stanek, N., Zarębska, M., Biłos, Ł., et al. (2021). Influence of coffee brewing methods on the chromatographic and spectroscopic profiles, antioxidant and sensory properties. Scientific Reports, 11, 21377. https://doi.org/10.1038/s41598-021-01001-2.

[20] Zarebska, M., Stanek, N., Barabosz, K., et al. (2022). Comparison of chemical compounds and their influence on the taste of coffee depending on green beans storage conditions. Scientific Reports, 12, 2674. https://doi.org/10.1038/s41598-022-06676-9.

The knowledge, lifestyle and nutritional behavior of pregnant women

Monika Bojanowska*, Małgorzata Kostecka, Marzena Pabich, Monika Sachadyn-Król

Faculty of Food Science and Biotechnology
University of Life Sciences in Lublin,
**corresponding author: monika.bojanowska@up.lublin.pl*

Abstract

Pregnancy is a period of particular sensitivity, in which a woman's eating habits, lifestyle and level of health knowledge have a significant impact on the mother's health and proper development of the fetus. This work discusses key nutrients that have a significant impact on fetal development, as well as the issue of nutritional programming. The proper course of pregnancy and the health of the child after birth and in the future are also influenced by the woman's lifestyle, including the use of substances with toxic effects (such as alcohol consumption or smoking). The aim of this article was to analyze the relationship between the level of nutritional knowledge, lifestyle and dietary practices of pregnant women. The study included a group of women of different ages, in different trimesters of pregnancy, who completed a questionnaire regarding eating habits and supplementation used, their lifestyle and their knowledge about nutrition during pregnancy. Most women declare that they are aware of the importance of proper nutrition during pregnancy, but there are clear discrepancies between the declared knowledge and actual behaviors. Women most often drew their knowledge from the Internet, less often from doctors or dietitians. The conclusions emphasize the need to intensify educational activities, both at the individual and systemic level. Regular consultations with medical personnel and easy access to reliable sources of knowledge can significantly improve the lifestyle and health of pregnant women and their children.

Keywords: knowledge, pregnant women, diet, healthy eating, nutrition awareness

1. Introduction

Pregnancy is considered a special period in a woman's life. The physiological changes that occur in a woman's body at that time are associated with a change in the way she eats during this period. Normal body weight values during the procreative period support the proper functioning of the reproductive system and maintain metabolic and hormonal balance. Normalizing body weight before a planned pregnancy is important because both underweight and overweight, including obesity, can negatively affect fertility, the course of pregnancy and the child's health. Therefore, women planning a pregnancy should take care of proper nutrition and lifestyle changes (including giving up stimulants or introducing regular physical activity). The mother's diet determines the health of the developing child, affects the general course of pregnancy, and after delivery, the course of lactation [Jensen 2022; Marsshall et al. 2022]. During pregnancy, the demand for energy, basic nutrients, vitamins and minerals increases. And the reason for this condition is the development of the fetus, placenta, as well as the mother's tissues. A pregnant woman should eat meals regularly 4-5 times a day and compose them using all product groups, especially vegetables and fruits rich in vitamin C (including kiwi, berries, citrus fruits), products containing full-value protein (fish, veal, lean poultry), cereal products, dairy products, eggs, vegetable fat, nuts and legumes [Marshall et al. 2022]. It is important to balance the diet, determine the energy demand appropriate for a given period of pregnancy and the amount of macronutrients (proteins, fats, carbohydrates) and ingredients of particular importance such as: iodine, omega-3 acids, iron, vitamin D and folic acid, the supplementation of which is recommended for every woman of reproductive age. Nutritional deficiencies can lead to premature birth, placental insufficiency, and developmental defects in the fetus. Underweight during pregnancy increases the risk of anemia. Osteoporosis, hypertension, and postpartum depression are more common in underweight women, as well as problems with lactation [Weker 2021]. Insufficient weight gain during pregnancy is associated with an increased risk of fetal growth restriction and hypotrophy, and with a higher rate of perinatal morbidity and mortality [Sossa et al. 2023]. Nutritional programming emphasizes the importance of adequate nutrition in the prenatal period and early childhood for health in later life. Changes in metabolism, programmed in response to nutritional deficiencies, can lead to the development of metabolic diseases and other health problems in adulthood. Children with low birth weight, often the result of maternal malnutrition during pregnancy, are more likely to develop metabolic diseases in adulthood (cardiovascular diseases, type 2 diabetes, osteoporosis, and even cancer) [Apostolopoulou et al. 2024].

Obesity and inappropriate weight gain during pregnancy can cause various problems for both the mother and the child. Obese women are at increased risk of developing gestational diabetes, hypertension, and miscarriage. These women are also more susceptible to venous diseases such as thrombosis. In turn, the fetus may experience growth problems and even intrauterine death [Cochrane et al. 2024]. Excess body weight may be associated with macrosomia (birth weight ≥ 4000 g), which affects about 12% of newborns of healthy mothers, and in women with gestational diabetes, this percentage is up to 45% of newborns. This increased risk of macrosomia in children is mainly due to increased insulin resistance in the mother, due to the greater amount of glucose in the blood, which passes through the placenta into the fetal circulation and is stored as adipose tissue [Kc et al. 2015; Wanaditya et al. 2023].

During pregnancy, it is important to eliminate ingredients that pose a risk to the health and life of the child, and these are: unpasteurized dairy products, blue cheeses that pose a risk of infection with the Listeria monocytogenes bacteria, raw meat, fish, seafood - parasites, Toxoplasma gondii, Salmonella, Capylobacter) and eggs - Salmonella), liver, due to the high content of vitamin A, which in excess can be teratogenic [Mandryk and Węgrzyn 2023; Weker 2021]. A healthy lifestyle for a pregnant woman also involves undertaking moderate physical activity, if there are no contraindications [Sass and Mączka, 2017]. Women in this special period should stop drinking alcohol and coffee [Wojtyła and Wojtyła 2017; Surma et al. 2022].

Alcohol consumption by pregnant women contributes to the development of fetal alcohol syndrome (FAS) in children, which is characterized by improper functioning of the nervous, cardiovascular and genitourinary systems, as well as skeletal defects [Hur et al. 2022]. Excessive caffeine consumption during pregnancy or while breastfeeding may be associated with negative effects on the health of the fetus or infant. Pregnant women metabolize caffeine much slower [Qian et al. 2020]. According to scientists, caffeine increases the concentration of catecholamines in the blood, which in turn disrupts blood flow through the placenta and causes the fetus to receive fewer nutrients [Surma et al. 2022]. Thus, excessive coffee consumption may cause low birth weight, miscarriage or premature birth [Qian et al. 2020]. According to the EFSA position (and many other expert groups), it is worth limiting caffeine consumption to 200 mg per day during pregnancy [EFSA 2015]. However, a 2020 literature review shows that a safe dose of caffeine during pregnancy cannot be determined [James 2020].

Trans fats in a pregnant woman's diet are harmful and should be avoided. Their consumption during pregnancy can lead to health problems in the mother, such as pregnancy-induced hypertension, preeclampsia, gestational diabetes, and can also negatively affect the child's development, including its birth weight and nervous system. They can also interfere with lactation in breastfeeding women. During pregnancy, highly processed products containing trans fats should be avoided, e.g. crackers, chips, wafers, deep-fried products, or fast food [Cohen et al. 2011; Marshall et al. 2022].

The aim of the study was to assess the level of knowledge among pregnant women regarding the role of selected nutrients that are crucial for the health of both mother and child, as well as the influence of lifestyle and dietary behaviors on pregnancy course.

2. Materials and Methods

The research method was a survey, and the research instrument was a survey questionnaire that was made available on social media, mainly in groups for pregnant women. The study lasted 3 months, from November 2024 to January 2025, and completion of the survey was voluntary and anonymous. The sample was selected using the purposive sampling method due to the specificity of the group, the main qualifying criterion was female gender and pregnancy or postpartum status (immediately after the birth of a child).

The author's survey questionnaire consisted of a personal data sheet containing basic information: age, place of residence, education, height and weight, as well as sections on eating habits and supplementation used, lifestyle, and women's knowledge about nutrition during pregnancy. The questionnaire contained single- and multiple-choice closed-ended questions.

204 correctly completed surveys were obtained. The survey results were processed using Microsoft Excel Office 365 and StatSoft Statstica 13. The Pearson chi-square test was used to test the statistical significance between the analyzed variables.

3. Results and discussion

The age of the surveyed women varied, from 19 to 41 years, with the majority of participants being in the 24-27 age group (33%). The surveyed women were primarily residents of cities with more than 50,000 inhabitants, who assessed their financial situation as good. More than half of the women declared higher education (Table 1).

Table 1. Characteristics of the study group.

Age (years		
	Number of women	Participation %
19-23 years	34	16,7%
24-27 years	73	35,8%
28-30 years	50	24,5%
>30 years	47	23%
Place of living		
Rural area	101	25%
City up to 50 thousand inhabitants	100	24%
City with more than 50 thousand inhabitants	206	51%
Education		
higher	131	64%
secondary	56	27%
vocational	14	7%
primary	3	22%
Financial situation		
Very good	29	14,3%
Good	122	59,1%
Average	51	25,6%
Bad	2	1%

The vast majority of the surveyed women (98%) were pregnant at the time of the survey, the remaining women had recently given birth. For almost 62% of the surveyed women, the current pregnancy was their first in their lives, and for 93 pregnant women, it was their second pregnancy. 30 respondents declared having 2 or more children.

In pregnant women, weight gain is a very important aspect to ensure proper development of the fetus, it also determines the woman's health (Table 2). Based on the body weight of the respondents before pregnancy and their height, the BMI was calculated. More than half of the respondents had normal BMI values, overweight was found in 34, obesity in 24 women, and underweight in 5% (Fig. 1). Also in the study conducted by Kobus-Cisowska et al. [2016] irregularities in body weight were found. Out of 233 patients examined by Lautenbach et al. [2017b], 74 women had abnormal body weight before pregnancy, overweight was found in 34 of them and obesity in 21. In pregnant women with excess body weight, a higher percentage of maternal complications was found, in the form of gestational diabetes and pregnancy-induced hypertension, than in women with normal body weight. In contrast, patients with a lower BMI were at greater risk of preterm birth. In the study by Machado et al. [2019] assessing the effect of pre-pregnancy BMI on the course of pregnancy in women with gestational diabetes (GDM) in Portugal, out of 3103 women, 29.6% were overweight and 27.3% were obese. Compared to normal weight, overweight and obese mothers had a higher rate of gestational hypertension, cesarean section, macrosomia and large for gestational age (LGA) newborns. This shows that in pregnant women with GDM, pregnancy complications increase with pre-pregnancy BMI.

Table 2. BMI characteristics and recommended weight gain during pregnancy [IOM 2009].

Category	BMI [kg/m^2]	Total weight gain during pregnancy [kg]	Weight gain in the second and third trimester [kg/tydzień]
Underweight	< 18,5	12,5 - 18	0,5 - 0,6
Normal body weight	18,5 – 24,9	11,5 - 16	0,4 – 0,5
Overweight	25,0 – 29,9	7,0 - 11,5	0,2 - 0,3
Obesity	> 30	5,0 – 9,0	0,2 - 0,3

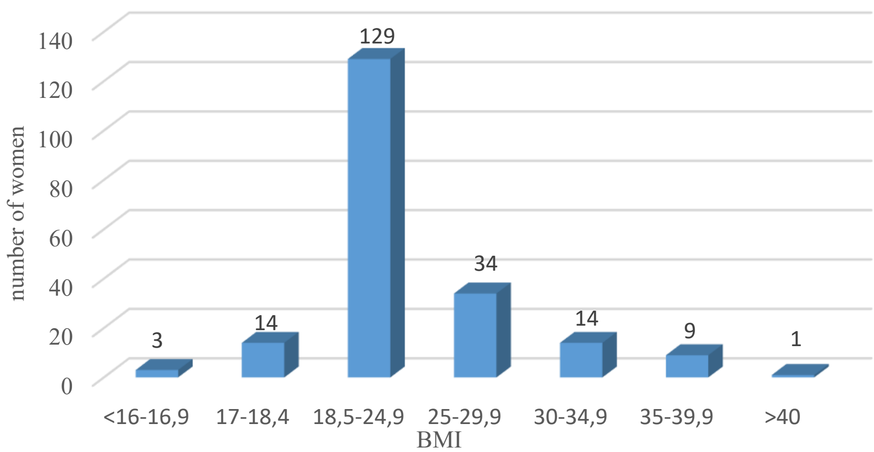

Figure 1. Pre-pregnancy BMI.

The weight gains in the individual groups of women are presented in Table 3 and ranged on average from 6.9 to 9.7. The respondents did not indicate the exact week of pregnancy, so it is impossible to assess the correctness of these gains, although they may be too small - in the case of underweight women or too large in pregnant obese women. The results were compared with the work of Berner-Trąbska [2009], where the average body weight before pregnancy of patients from the group with a normal BMI was 61.6 kg. At the end of the last trimester, the women weighed 15.5 kg more, which is within the norm. The average body weights of overweight and obese pregnant women were 76.5 kg and 88.3 kg, respectively, and the achieved gains were too large - 14.4 kg and 10.2 kg. In the survey conducted by Lautenbach et al. [2017a] among patients of the maternity ward of the Medical University of Gdańsk, only 47% of respondents achieved weight gain in line with the currently applicable recommendations. An alarmingly high percentage of women were those whose weight gain was too high (34%), because regardless of the initial BMI, it is a factor increasing the risk of complications. Respondents with a pre-pregnancy BMI above the norm most often manifested excessive weight gain during pregnancy, which may indicate a lack of improvement in the way of nutrition during pregnancy. Proper nutrition of a woman in the period preceding pregnancy and during it is of great importance for the optimal development of the child, the course of pregnancy and for maintaining the health of the mother.

Table 3. Average body mass values in individual BMI categories before pregnancy and in the third trimester.

Kategoria BMI [kg/m^2]	Pre-pregnancy body weight [kg]	Pre-pregnancy BMI [kg/m^2]	Body weight in the third trimester [kg]	Weight gain [kg]
17 - 18,4	49,4	17,8	58,7	9,3
18,5 - 24,9	61,7	21,7	71,4	9,7
25 - 29,9	73,3	27,1	80,6	7,3
30 - 34,9	90,9	32,1	97,8	6,9
35 - 39,9	104,7	37,6	112,0	7,3

For most of the respondents, the primary source of knowledge in this area is the media, such as the Internet or television, and nearly half of the women also used information obtained from a doctor supervising the pregnancy or a midwife. Next, women mentioned members of their family and friends, and finally dietitians. It is disturbing that in the group of women surveyed there were some who were not interested in the topic of nutrition during pregnancy at all, which may affect their dietary choices and, consequently, the course of pregnancy and the child's health (Fig. 2). Similar observations were noted in the work of Lemska et al. [2019]), where the Internet was used as a source of knowledge about pregnancy, childbirth, postpartum period and child care by almost 90% of the respondents. In the study by Kobus-Cisowska et al. [2016], on the other hand, magazines came first (58%), and 45% of women indicated the Internet. More pregnant women chose a doctor and/or dietician and specialist literature as a source of knowledge. Comparing the cited study results, it can be stated that women often used the Internet, but medical consultations and specialist literature were equally popular. According to the authors of the cited works, women do not have sufficient knowledge about the proper course and nutrition during pregnancy. Borkowska and Ostrowska [2019] also showed that the knowledge of the surveyed young women about proper eating habits during pregnancy is insufficient. Large discrepancies were found in the answers given regarding the possibility of consuming selected food products during pregnancy.

A pregnant woman should take care of a healthy, varied diet, providing the right amount of calories and nutrients. It is important to eat meals regularly, which helps maintain energy and supports the development of the child. Such a diet should be rich in vegetables, fruits, whole grains, protein and healthy fats [Weker 2021; Marshall et al. 2021]. 36% of surveyed women declared that they eat 5 or more meals, and 43% - 4 meals, 7 pregnant women only had 2 meals a day. Many studies on the nutritional behavior of pregnant Polish women show similar results [Kobus-Cisowska et al. 2016, Dereń et al., 2017, Misan et al. 2019]. But Sossa et al. [2023] also showed in their study that the majority of pregnant women (88%) were not aware of their nutritional needs during pregnancy. Only 49.6% of them ate at least three meals and two snacks a day. The National Institute of Public Health recommends eating smaller portions, but more often throughout the day. Eating just one or two meals can lead to consuming a large amount of calories in a short period of time, because it is easier to reach for a quick satisfaction of hunger.

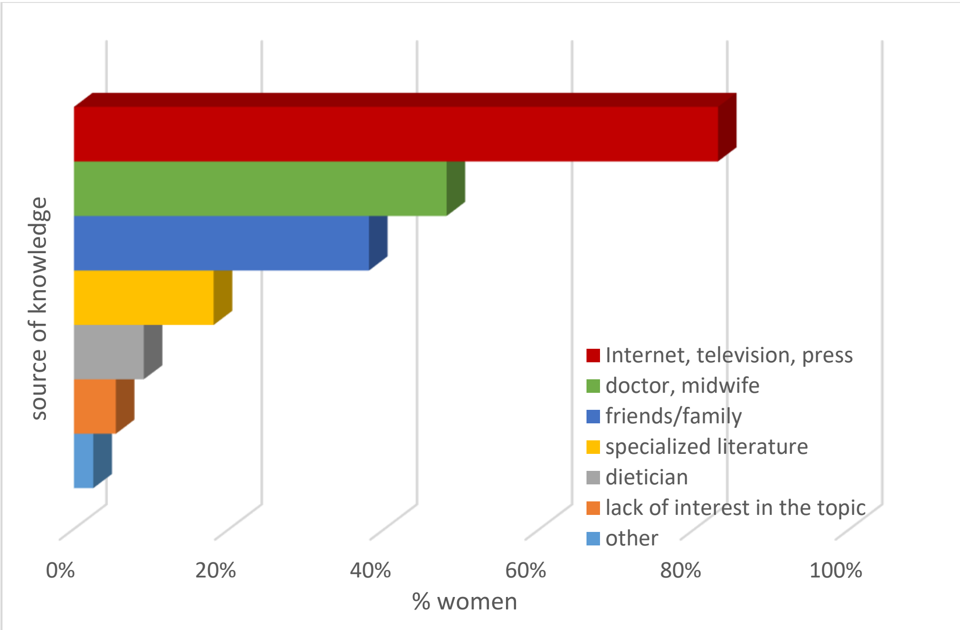

Figure 2. Sources of knowledge in the field of nutrition.

In addition, such a way of eating can increase the appetite for sweet snacks. Therefore, it is worth trying to eat regularly, in smaller portions, to better control appetite and take care of health.

The majority of respondents admitted to snacking between meals, including sweets. The respondents reached for these products too often, as many as 139 of them declared eating them once a day or 2-4 times a week. Sweets not only provide simple sugars, which quickly raise blood glucose levels, and their excess can lead to obesity, insulin resistance and type 1 diabetes, but also contain saturated fatty acids and trans fats. The latter are often found in processed sweets and can increase the risk of heart disease, raise the level of bad cholesterol and have a negative impact on the circulatory system.

Saturated fatty acids can also contribute to increased cholesterol and increase the risk of cardiovascular diseases. In expectant mothers, excessive consumption of these fats can have a negative impact on their health, as well as on the course of pregnancy, increasing the risk of complications and affecting the child's development [Pieczyńska et al. 2017].

During pregnancy, the demand for many important vitamins and minerals increases significantly, and diet is not enough to cover these needs. Therefore, the use of vitamin and mineral supplements is very important - it helps maintain a healthy pregnancy and can prevent many complications [Makowska-Donajska and Hirnle 2017; Wiesner and Paśko 2021]. As many as 62% of respondents received recommendations for supplementation from their doctor, but 16 women did not take any supplements. The research shows that pregnant women are quite aware of the importance of supplementation during pregnancy. In the works of Grzelak et al. [2016], Charkiewicz er al. [2016], a similar number of pregnant women supplemented their diet with vitamins, minerals, or other essential food ingredients. Women know how important it is to take the right vitamins and minerals to ensure healthy development for themselves and their child. This awareness translates into their decisions regarding the use of supplements, which is a very positive signal.

The most popular were multi-component dietary supplements for pregnant women (77%), which is also confirmed by other authors [Charkiewicz et al. 2016; Makowska –Donajska et al. 2017]. About 40% of respondents also used preparations with folic acid and vitamin D (Fig. 3). The choice of folic acid as the most important supplement was noted in the study by Grzelak et al. [2016]. Folic acid plays a key role in pregnancy. It is essential for the proper development of the child's nervous system, especially in the first weeks, when the brain and spinal cord are formed. Taking appropriate doses of folic acid helps prevent neural tube defects, such as spina bifida or anencephaly. Therefore, it is recommended that women planning a pregnancy and during it regularly supplement folic acid, which significantly increases the chances of healthy development of the child and minimizes the risk of complications [Wiesner and Paśko 2021]. Folic acid before pregnancy was supplemented by 42% of the respondents. In pregnancy, the number almost doubled, but most women did not start the supplementation until 5-6 weeks of gestation. Before pregnancy, almost all subjects used single folic acid preparations, whereas during pregnancy they used vitaminmineral preparations for expectant women [Wierzejska and Wojda 2020].

The need for vitamins and minerals is an individual feature of each pregnant woman, depending on her diet, health condition and lifestyle, which is why starting supplementation should be consulted with a specialist. It is worth remembering that supplementation is only a supplement to the diet. In the results of the research conducted by Makowska-Donajska and Hirnle [2017], multi-ingredient preparations were the most popular among pregnant women, but almost 22% of women took several different multi-ingredient supplements at the same time, without consulting a specialist. The effects of such behavior have not been studied, however, the lack of control over the dietary supplements consumed during pregnancy may affect the effects of other medications and supplements taken.

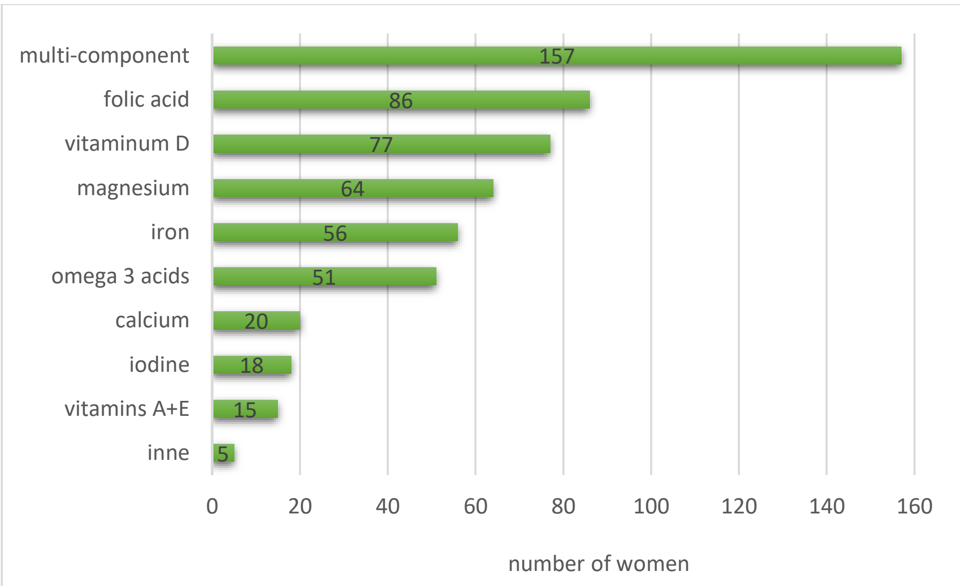

Figure 3. Types of supplements chosen by pregnant women.

For many years, it was believed that there was a "safe" dose of caffeine during pregnancy, but recently several studies have been published that suggest that there is no such thing [James 2020; Kukkonen et al. 2024]. Excessive caffeine consumption by the mother can have a negative impact on the health of the child, increasing the risk of various complications. Therefore, it is currently recommended that pregnant women limit or completely stop drinking caffeine to provide the child with the best possible conditions for development. Alcohol has a teratogenic effect on the child, which means that it can cause serious damage and malformations during pregnancy. When a woman consumes alcohol during pregnancy, these substances cross the placenta and end up in the body of the developing child. Alcohol can interfere with the normal development of the brain and other organs, which has long-term effects on the health and functioning of the child. Therefore, the safest and most recommended option is to completely avoid alcohol during pregnancy to ensure a healthy start in life for the child [Sbrana et al. 2016].

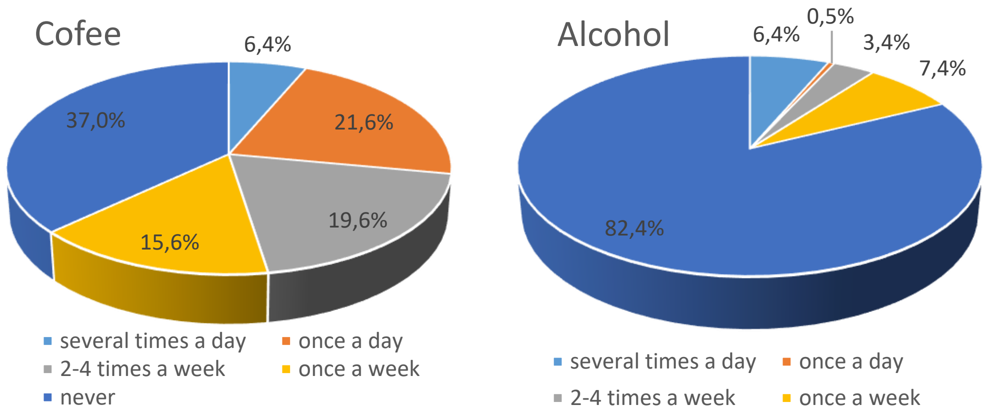

Figure 4. Frequency of coffee and alcohol consumption.

The results obtained show that the surveyed women do not give up consuming products that are considered harmful during pregnancy (Fig. 3). Only 37% of the surveyed women avoid consuming coffee. 6% of the surveyed women drink more than 1 coffee a day, the rest of the respondents drink it less often. In total, as many as 130 women declared coffee consumption during this special period. Other studies analyzing the dietary habits of pregnant women also show that women do not give up consuming this beverage during pregnancy, although sometimes they reduce the frequency of its supply [Wyka et al. 2015, Charkiewicz et al. 2016]. In the study by Vitti et al. [2018], 4908 (64.5%) mothers consumed caffeine, of which 143 (2.9%) reported high consumption, which in turn was significantly associated with lower education, as well as alcohol consumption and cigarette smoking. Kukkonen et al. [2024] showed that in the first trimester of pregnancy as many as 67.9% of women drank coffee, while in the third trimester this rate was almost unchanged (67.4%). In addition, the authors found that caffeine intake was exceeded in one third of all women participating in the study and almost half of coffee drinkers in early pregnancy, which was associated with a higher risk of giving birth to a SGA baby (too small for gestational age).

In the case of alcohol, the results are different, with 82% of respondents declaring they had stopped drinking alcohol, while the data on its consumption several times a day, which was recorded in 13 pregnant women, are worrying (Fig. 3). The results of studies by other authors show that pregnancy is not a time of complete abstinence for many women, although the frequency and type of alcohol consumption change significantly. Of the women surveyed by Wojtyła and Wojtyła [2017], 54.7% declared that they had consumed alcohol during pregnancy, while 45.3% declared abstinence. Over 35% of women consuming alcohol during pregnancy consumed it once a month or less often, and almost 6% at least twice a month during pregnancy. In the study conducted by McDonald and Watson [2020], 19% of mothers were abstinent, over half stopped when they found out they were pregnant, and nearly 30% continued to consume alcohol. Daily consumption was associated with increased obesity in mothers. Sbrana et al. [2016] showed that 23% of women consumed alcohol during pregnancy. Consumption occurred mainly in the first trimester (14.8%) and decreased as pregnancy progressed.

The knowledge of the surveyed women was also checked in questions about the dietary sources of nutrients important during pregnancy. The results presented in Table 4 show that education had an impact on greater awareness of the sources of vitamin D, iron, calcium, folic acid, omega-3, iodine, $p<0.05$), such a relationship was not obtained in the case of magnesium ($p=029451$). People with higher education more often correctly indicated the sources of vitamin D in food (oily fish) - 49%. Only 19% of people with secondary education indicated the correct source of this vitamin. In general, 149 women indicated the correct answer. It was similar in the case of iron, calcium, folic acid, omega-3 acids, iodine and magnesium. The best knowledge was demonstrated by women with higher education and they were the ones who most often indicated the correct sources of individual nutrients. In the case of calcium, the correct answer was indicated by 170 of all women (58% with higher education). About half of all respondents were able to indicate the best sources of omega-3 acids, iron and magnesium, while in the case of iodine and folic acid, this was a group of almost 70% of women. The obtained results confirm the work of other authors, in which education was a key factor determining the knowledge of pregnant women [Kobus-Cisowska et al. 2016]. Women's knowledge and awareness of proper nutrition during pregnancy are still too low [Sossa et al. 2023] and are not always determined by education [Książek et al. 2014]. This phenomenon is particularly noticeable among women living in rural areas [Momora and Krupa 2019].

In the survey, women were asked whether they believed that the mother's diet had an impact on the course of pregnancy and the health of the unborn child. The majority, about 83%, answered that it was very important, which shows that many women are aware of the importance of proper nutrition during this special period. However, other pregnant women do not see such a relationship. This may be due to a lack of knowledge, insufficient education about the impact of diet on health during pregnancy, or the belief that genetic factors or other aspects are more important. It is important to educate expectant mothers, because conscious dietary choices can significantly affect the health of their child and the course of pregnancy. Insufficient knowledge was also reflected in the answers to the question about the increase in energy demand in the second and third trimesters of pregnancy. The latest nutritional standards from December 2024 include changes in the recommended calorie requirements for pregnant and breastfeeding women. In the first trimester, the value dropped from 85 kcal to 70 kcal, in the second trimester from 285 kcal to 260 kcal, and in the third trimester it increased from 475 kcal to 500 kcal. For breastfeeding women, the change was from 505 kcal to 500 kcal [Rychlik et al. 2024]. It is worth noting that the survey was prepared a little earlier, and at the time it was made available, new standards for calorie requirements in pregnancy appeared. Therefore, the question included older values that did not reflect the latest guidelines. Despite this, only 59 women gave the correct answer regarding the increase in calorie requirements in the second and third trimesters of pregnancy.

Table 4. Education and knowledge about the nutritional sources of selected food ingredients.

Test performed	Chi-square	p
Education and knowledge about sources of vitamin D		
Pearson Chi^2	70,49216	0,00000
Chi^2 NW	19,16941	0,00389
Spearman's R rank	0,1617238	0,02084
Education and knowledge about iron sources		
Pearson Chi^2	34,75239	0,00000
Chi^2 NW	29,46309	0,00005
Spearman's R rank	0,1193931	0,08896
Education and knowledge about calcium sources		
Pearson Chi^2	33,54451	0,00001
Chi^2 NW	27,41157	0,00012
Spearman's R rank	0,3106794	0,00001
Education and knowledge about sources of folic acid		
Pearson Chi^2	19,35210	0,00361
Chi^2 NW	15,69022	0,01552
Spearman's R rank	-0,063920	0,36373
Education and knowledge about omega-3 sources		
Pearson Chi^2	32,72217	0,00001
Chi^2 NW	17,53497	0,00751
Spearman's R rank	-0,013477	0,84827
Education and knowledge about iodine sources		
Pearson Chi^2	19,66992	0,00317
Chi^2 NW	18,22567	0,00569
Spearman's R rank	0,1791607	0,01035
Education and knowledge about magnesium sources		
Pearson Chi^2	7,294024	0,29451
Chi^2 NW	6,917870	0,032851
Spearman's R rank	0,1791607	0,01035

Also in the study by Borkowska and Ostrowska [2019], a significant proportion of the young women surveyed declared that they had no knowledge about changes in energy requirements during pregnancy or claimed that it increased at the beginning of pregnancy and remained at a constant level until delivery. This means that many women are fully aware of the increased energy needs during these stages of pregnancy. Failure to follow these recommendations can lead to nutrient deficiencies, which in turn can have serious consequences for the health of the mother and the developing child. Calorie deficiencies can result in lower birth weight, a weakened immune system, and delayed fetal development.

The last question in the survey concerned the subjective assessment of one's own diet during pregnancy, assessed on a five-point numerical scale. The majority of respondents (over half) assessed their diet at level 4, i.e. as good. Only 19 women considered their eating habits as very good. For 36% of pregnant women, the diet was correct, the remaining women assessed their diet during pregnancy as bad or very bad. The respondents' financial status had a significant impact on the assessment of their diet. The chi-square statistical test was used to check whether the assessment of the diet of pregnant women was influenced by their financial status (chi-square test $\chi^2=31.38$, $p=0.001$). Women with good and very good financial conditions more often declared that their diet during pregnancy was good. Additionally, it was checked whether the subjective assessment of the women's diet translated into the frequency of eating sweets ($\chi^2=34.964$, $p=0.004$). Respondents who consumed sweets every day or several times a week most often assessed their diet as good or correct.

5. Conclusions

1. The main source of knowledge of pregnant women about nutrition during pregnancy was the Internet.
2. Higher education of women was a factor determining the correct choice of products that were the best source of ingredients important in the course of pregnancy.

3. Women's awareness of nutritional behavior during pregnancy is increasing, but it is still insufficient - many women consume alcohol and coffee during pregnancy.
4. Every fourth woman had an increased BMI before getting pregnant, indicating overweight, which could have influenced the nutritional choices made during pregnancy.
5. Most women used supplements during pregnancy, most often choosing multi-ingredient preparations.
6. Despite assessing their own way of eating during pregnancy as good, sweets were relatively often present in the diet of the respondents.
7. Women declaring good and very good financial status more often assessed their diet during pregnancy as good.
8. There is a need to intensify educational activities, both at the individual and systemic level. Regular consultations with medical personnel and easy access to reliable sources of knowledge can significantly improve the lifestyle and health of pregnant women and their children.

6. References

[1] Apostolopoulou A., Tranidou A., Tsakiridis I., Magriplis E., Dagklis T., Chourdakis M.: Effects of nutrition on maternal health, fetal development, and perinatal outcomes. Nutrients, 2024, 16, 375.
[2] Berner-Trąbska M., Kowalska-Koprek U., Karowicz-Bilińska A., Brzozowska M., Estemberg D., Orłowska K., Kuś E.: Wpływ nadwagi lub otyłości u ciężarnych na przebieg ciąży i okresu okołoporodowego z uwzględnieniem stanu urodzeniowego noworodka w oparciu o materiał kliniczny. Ginekol. Pol., 2009, 80, 845–850.
[3] Borkowska U., Ostrowska L.: Ocena wiedzy młodych kobiet na temat wybranych aspektów prawidłowego żywienia w okresie ciąży. Med. Ogólna Nauki o Zdrowiu, 2019, 25(4), 245–250.
[4] Charkiewicz A.E., Omeljaniuk W.J., Piotrowska I.K.: Determinanty zdrowia kobiet w ciąży w Białymstoku. Bromatol. Chem. Toksykol., 2016, 49(1), 42–51.
[5] Cochrane A.L.K., Murphy M.P., Ozanne S.E., Giussani D.A.: Pregnancy in obese women and mechanisms of increased cardiovascular risk in offspring. Eur. Heart J., 2024, 45(48), 5127–5145.
[6] Cohen J.F., Rifas-Shiman S.L., Rimm E.B., Oken E., Gillman M.W.: Maternal trans fatty acid intake and fetal growth. Am. J. Clin. Nutr., 2011, 94(5), 1241–1247.
[7] Dereń K., Gaweł M., Łuszczki E., Jarmakiewicz S., Sokal A., Polak E., Wyszyńska I.J.: Nutritional behavior of pregnant women from the Podkarpacie province. Eur. J. Clin. Exp. Med., 2017, 15(4), 322–329.
[8] EFSA: Scientific opinion on the safety of caffeine. EFSA J., 2015, 13(5), 4102.
[9] Grzelak T., Suliga K., Sperling M., Pelczyńska M., Czyżewska K.: Ocena stosowania suplementów diety wśród kobiet ciężarnych lub planujących ciążę. Via Medica, 2016, 7(1), 8–15.

[10] Hur Y.M., Choi J., Park S., Oh S.S., Kim Y.J.: Prenatal maternal alcohol exposure: diagnosis and prevention of fetal alcohol syndrome. Obstet. Gynecol. Sci., 2022, 65(5), 385–394.
[11] Institute of Medicine (IOM) Food and Nutrition Board. Weight gain during pregnancy: reexamining the guidelines. Washington: DC National Academy Press; 2009.
[12] James J.: Maternal caffeine consumption and pregnancy outcomes: a narrative review with implications for advice to mothers and mothers-to-be. BMJ Evid. Based Med., 2020.
[13] Jensen M.E.: The guidelines for balanced diet and healthy lifestyles during pregnancy: the management of health and morbidity in pregnancy. 2022. ISBN 978-3-0365-5502-7 (PDF).
[14] Kobus-Cisowska J., Kmiecik D., Przeor M., Jędrusek-Golińska A., Waszkowiak K., Żołna H.: Ocena poziomu wiedzy żywieniowej i sposobu żywienia kobiet w okresie ciąży. Bromatol. Chem. Toksykol., 2016, 49(3), 531–535.
[15] Kc K, Shakya S, Zhang H. Gestational diabetes mellitus and macrosomia: a literature review. Ann Nutr Metab. 2015, 66 Suppl 2, 14-20.
[16] Książek P., Kozłowiec J., Kozłowiec M.: The nutritional knowledge of pregnant women. Pol. J. Public Health, 2014, 124(4), 191–194.
[17] Kukkonen A., Hantunen S., Voutilainen A., Ruusunen A., Backman K., Kirjavainen P.V., Ylilauri M., Voutilainen R., Pasanen M., Keski-Nisula L.: Maternal caffeine intake during pregnancy and the risk of delivering a small for gestational age baby: Kuopio Birth Cohort. Arch. Gynecol. Obstet., 2024, 310(1), 359–368.
[18] Lautenbach D., Kardasz K., Rolnik A., Kaźmierczak A., Malczewska B., Preis K.: Ocena wpływu nieprawidłowej masy ciała przed zajściem w ciążę na masę urodzeniową noworodków oraz występowanie powikłań matczynych. In: Zagrożenie życia i zdrowia człowieka. Eds. Tatarczuk J., Zboina B., Dąbrowski P. Wydawnictwo Naukowe NeuroCentrum, Lublin, 2017, 203–216.
[19] Lautenbach D., Rolnik A., Kaźmierczak A., Kardasz K., Krzysztof Preis K.: Zależność między przyrostem masy ciała w ciąży a występowaniem nieprawidłowej masy urodzeniowej noworodków oraz powikłań matczynych. In: Zagrożenie życia i zdrowia człowieka. Eds. Tatarczuk J., Zboina B., Dąbrowski P. Wydawnictwo Naukowe NeuroCentrum, Lublin, 2017, 185–202.
[20] Lemska M., Kiełbratowska B.G, Sioma-Markowska U., Nowak A.: Sposoby pozyskiwania informacji przez kobiety na temat ciąży i macierzyństwa. Gin. Pol Med. Project, 2019, 3 (53), 053-057.
[21] Machado C., Monteiro S., Oliveira M.J.: Impact of overweight and obesity on pregnancy outcomes in women with gestational diabetes – results from a retrospective multicenter study. Arch. Endocrinol. Metab., 2020, 64(1), 45–51.
[22] Makowska-Donajska M., Hirnle L.: Suplementacja witamin i składników mineralnych podczas ciąży. Ginekol. Perinatol. Prakt., 2017, 2(4), 166–172.
[23] Mandryk I., Węgrzyn I.: Awareness of pregnant women about proper nutrition habits and rational diet. J. Educ. Health Sport, 2023, 16(1), 49–61.
[24] Marshall N.E., Abrams B., Barbour L.A., Catalano P., Christian P., Friedman J.E., Hay W.W. Jr, Hernandez T.L., Krebs N.F., Oken E., Purnell J.Q., Roberts J.M., Soltani H., Wallace J., Thornburg K.L.: The importance of nutrition in pregnancy and lactation: lifelong consequences. Am. J. Obstet. Gynecol., 2022, 226(5), 607–632.
[25] McDonald B.W., Watson P.E.: Maternal alcohol intakes before and during pregnancy: Impact on the mother and infant outcome to 18 months. Nord. Stud. Alcohol Drugs, 2020, 37(2), 153–171.
[26] Misan N., Paczkowska K., Szmyt M., Kapska K., Tomczak L., Breborowicz G., Ropacka-Lesiak M.: Nutritional behavior in pregnancy. Ginekol. Pol., 2019, 90(9), 527–533.
[27] Momora A., Krupa J.: Zwyczaje żywieniowe kobiet ciężarnych zamieszkałych na obszarach wiejskich. Pediatr. Med. Rodz., 2019, 15(2), 164–170.
[28] Pieczyńska J., Sozański R., Kłósek A., Grajeta H.: Wpływ zaawansowania ciąży na strukturę spożycia kwasów tłuszczowych z dietą przez kobiety ciężarne. Probl. Hig. Epidemiol., 2017, 98(1): 73-80.
[29] Qian J., Chen Q., Ward S.M., Duan E., Zhang Y.: Impacts of caffeine during pregnancy. Trends Endocrinol. Metab., 2020, 31(3), 218–227.
[30] Rychlik E., Stoś K., Woźniak A., Mojska H.: Normy żywienia dla populacji Polski. Narodowy Instytut Zdrowia Publicznego PZH – Państwowy Instytut Badawczy, 2024.

[31] Sass A., Mączka M.: Physical activity of pregnant women in the light of scientific research – a review of the literature. J. Educ. Health Sport, 2017, 7(8), 550–565.

[32] Sbrana M., Grandi C., Brazan M., Junquera N., Nascimento M.S., Barbieri M.A., Bettiol H., Cardoso V.C.: Alcohol consumption during pregnancy and perinatal results: a cohort study. Sao Paulo Med. J., 2016, 134(2), 146–152. DOI: 10.1590/1516-3180.2015.02040211.

[33] Sossa C.J., Salami L., Metonnou C.G., Tozoukan E., Azandjeme C., Mongbo V., Paraïso M.N.: Knowledge, attitudes, and practices relating to food and nutrition among pregnant women attending antenatal clinics at Menontin Hospital (Benin). Health, 2023, 15, 1202–1217.

[34] Surma S., Witek A.: Coffee consumption during pregnancy – what the gynecologist should know? Review of the literature and clinical studies. Ginekol. Pol., 2022, 93(7), 591–600.

[35] Vitti F.P., Grandi C., Cavalli R.C., Simões V.M.F., Batista R.F.L., Cardoso V.C.: Association between caffeine consumption in pregnancy and low birth weight and preterm birth in the birth cohort of Ribeirão Preto. Rev. Bras. Ginecol. Obstet., 2018, 40(12), 749–756.

[36] Wanaditya G.S., Putra W.A., Dwi Aryana M.B., Mulyana R.S.: Obesity in pregnant women and its impact on maternal and neonatal morbidity. EJMED, 2023, 5(3), 17–21.

[37] Weker M.: Żywienie kobiet w okresie ciąży – teoria i praktyka. Wydawnictwo Lekarskie PZWL, Warszawa, 2021.

[38] Wierzejska R., Wojda B.: Folic acid supplementation in pregnancy and prevention of fetal neural tube defects. Przegl. Epidemiol., 2020, 74(2), 362–369.

[39] Wiesner A., Paśko P.: Stosowanie suplementów u kobiet ciężarnych w świetle najnowszych rekomendacji Polskiego Towarzystwa Ginekologów i Położników. Farm. Pol., 2021, 77(1), 40–47.

[40] Wojtyła C., Wojtyła A.: Alcohol consumption by pregnant women in Poland. J. Health Inequal., 2017, 3(1), 51–57.

[41] Wyka J., Misiarz M., Malczyk E., Zołoteńka-Synowiec M., Całyniuk B., Smółka B., Mazurek D.: Ocena spożycia alkoholu, kawy i palenia papierosów wśród kobiet w ciąży. Bromatol. Chem. Toksykol., 2015, 48(3), 578–582.

Bioactive and sensory peptides from microalgae proteins: an *in silico* profile and hydrolysis perspective

Łukasz Byczyński*

Department of Biotechnology and General Food Technology, Faculty of Food Technology, University of Agriculture in Krakow, Al. Mickiewicza 21, 31-120 Kraków
corresponding author: lukasz.byczynski@urk.edu.pl

Abstract

This study focuses on the *in silico* analysis of the potential of selected proteins derived from *Spirulina* (*Arthrospira platensis*) and *Chlorella* (*Chlorella pyrenoidosa*) microalgae to generate bioactive and flavour peptides through simulated enzymatic hydrolysis. We utilized bioinformatic methods to predict the fragmentation of microalgal proteins (including dominant proteins such as phycocyanin from *Spirulina* and photosynthetic and structural proteins from *Chlorella*) under the action of proteolytic enzymes typical of digestive and industrial processes. Based on protein sequences obtained from the UniProt database (www.uniprot.org), profiles of potential biological activity were generated using the Analysis BIOPEP-UWM module. Subsequently, simulated hydrolyses were performed using the Enzyme(s) action module (BIOPEP-UWM), and the resulting peptide sequences were analysed for their known bioactive properties (e.g., antioxidant, hypotensive – ACE-inhibitory, immunomodulatory, antidiabetic activity) and sensory profile (especially umami and bitter taste). The obtained results confirm that both *Arthrospira* and *Chlorella* constitute a valuable source of potential bioactive peptides. We also identified peptides that may contribute to improving the flavour profile of products, particularly in terms of umami taste, making protein hydrolysates from these microalgae attractive natural flavour enhancers. On the other hand, the presence of bitter-tasting peptides may pose a challenge, indicating the need for process optimization during hydrolysis. This *in silico* analysis provides a foundation for further experimental studies *(in vitro* and *in vivo*) to verify the predicted peptide properties.

Keywords: bioactive peptides,sensory peptides, microalgae, *in silico*

1. Introduction

Consumer demand for microalgae-derived food products is growing, primarily due to their perceived health benefits. While there are over 30,000 species of microalgae, only a few are approved for sale in the European Union with authorization from the European Food Safety Authority (EFSA). Among the microalgae classified as 'Not Novel Food' are *Arthrospira platensis* (*Spirulina*) and several *Chlorella* species (*vulgaris,*

pyrenoidosa, luteoviridis). These selected microalgae are characterized by a very high protein content (65-77% of dry mass), which significantly surpasses the protein content found in soy flour (37%), skimmed milk (36%), or fish (24%) [Bleakley 2021].
Until now, microalgae have primarily been used as dietary supplements or as additives aimed at improving the functional properties of finished products. Utilizing microalgae directly as a food additive has its advantages, such as a lower material processing cost, and such an additive is comprehensive in nature – containing not only protein but also minerals (iron, iodine), vitamins (B, E, C), and dietary fiber (peptidoglycan, cellulose)[Andrade et. al. 2018]. However, various studies have shown that such an addition can lead to disadvantages in the form of changes in product color, odor, and texture, which may be unacceptable to consumers [Byczyński et. al. 2025]. Currently, there is a trend focusing on the selective isolation of specific components from microalgae and seeking their applications in food, cosmetology, and pharmacology. Besides the isolation of fiber and lipid fractions (which contain pigments), a promising direction is the isolation and processing of protein derived from microalgae. Preliminary studies indicate that even direct protein isolates can exhibit bioactive properties, such as inhibiting angiotensin-converting enzyme-I (ACE-I) activity or possessing antioxidant properties [Ryan et. al. 2011]. Isolated microalgal proteins can be subjected to hydrolysis using digestive enzymes or industrial proteases (e.g., papain, bromelain, subtilisin A) to obtain peptides with diverse activities and sensory characteristics. Bioactive peptides can also be released from proteins during technological processes (acidification, heating) and during fermentation. The released peptides may exhibit antimicrobial, antiamnesic, antioxidant, opioid activities, as well as impart taste (salty, sweet, bitter, or umami) [Iwaniak et. al. 2016].
The biological function of peptides obtained through protein hydrolysis can also include inhibiting enzymatic activity, e.g., amylase (EC 3.2.1.1), lipase (EC 3.1.1.3), or angiotensin-converting enzyme I (ACE; EC 3.4.15.1). Hence, it is believed that these peptides can act as regulators of the digestive, circulatory, or nervous systems [Awosika and Aluko, 2019]. Lipase and glucanase inhibitors are becoming helpful in the fight

against obesity, while ACE inhibitors can contribute to lowering blood pressure in humans and animals [Iwaniak et. al. 2014; Iwaniak et. al. 2018].

The aim of this study is to analyze the sequences of selected, most frequently occurring proteins from Arthrospira platensis (Spirulina) and Chlorella microalgae to determine their potential biological activity profiles and sensory peptide profiles. An additional objective was to assess the possibility of releasing bioactive and sensory peptides from the analyzed microalgal proteins through the hydrolysis of these proteins using various proteases and a set of digestive enzymes, whose action was simulated using bioinformatics tools from the BIOPEP database.

Due to their broad biological activity, particularly useful in the prevention of diet-related diseases, active biopeptides obtained through hydrolysis may find future application as ingredients in functional foods, supplements, and pharmaceuticals [Grija 2018; Iwaniak et. al. 2018].

2. Materials and Methods

Protein sequences were collected from the UniProt database [UniProt Consortium 2025] (https://www.uniprot.org/; access 2025.06.01) [Bateman et. al. 2022] and they were processed in a manner similar to that described by Minkiewicz et al. [2023]. Protein UniProt accession numbers, name, length, mass and sequnence are provided in the table 1 and 2. Their sequences were submitted to analysis via the BIOPEP-UWM database [Iwaniak et. al. 2024] of bioactive and sensory peptides (provider: University of Warmia and Mazury in Olsztyn, Poland; accessed 2025.06.14). The database generate list of specific activity and location of bioactive fragments, frequency of bioactive fragments, and activities of fragments obtained by proteolysis.

Table 1. List of selected *Spirulina* protein used for bioactive peptide analysis

Protein name	Access. no in UniProt	Length (aa)	Mass (Da)
Allophycocyanin alpha chain	P72504 (PHAA_ARTPT)	161	17392
Sequence:_MSIVTKSIVNADAEARYLSPGELDRIKSFVTSGERRVRIAETMTGARERIIKEAGNQLFQKRPDVVSPGGNAYGEEMTATCLRDLDYYLRLITYGIVAGDVTPIEEIGVVGVREMYKSLGTPIEAVAEGVRAMKSVATSLLSGEDAAEAGAYFDYLIGAMS			
C-phycocyanin beta subunit	P72508 (PHCB_ARTPT)	172	18094
Sequence:_MFDAFTKVVSQADTRGEMLSTAQIDALSQMVAESNKRLDAVNRITSNASTIVSNAARSLFAEQPQLIAPGGNAYTSRRMAACLRDMEIILRYVTYAVFAGDASVLEDRCLNGLRETYLALGTPGSSVAVGVGKMKEAALAIVNDPAGITPGDCSALASEIASYFDRACAAVS			
Ferredoxin	P00246 (FER_ARTPT)	99	10634
Sequence:_MATYKVTLINEAEGINETIDCDDDTYILDAAEEAGLDLPYSCRAGACSTCAGTITSGTIDQSDQSFLDDDQIEAGYVLTCVAYPTSDCTIKTHQEEGLY			

Table 2. List of selected *Chlorella* protein used for bioactive peptide analysis

Protein name	Access. no in UniProt	Length (aa)	Mass (Da)
Photosystem II protein D1	P56318 (PSBA_CHLVU)	353	38986
Sequence:_MTAILERRESASLWARFCEWVTSTENRLYIGWFGVLMIPTLLTATSVFIIAFIAAPPVDIDGIREPVSGSLLYGNNIISGAIIPTSNAIGLHFYPIWEAASLDEWLYNGGPYQLIVCHFFLGICSYMGREWELSFRLGMRPWIAVAYSAPVAAATAVFIIYPIGQGSFSDGMPLGISGTFNFMIVFQAEHNILMHPFHMLGVAGVFGGSLFSAMHGSLVTSSLIRETTENESANEGYKFGQEEETYNIVAAHGYFGRLIFQYASFNNSRSLHFFLAAWPVVGIWFTALGISTMAFNLNGFNFNQSVVDSQGRVINTWADIINRANLGMEVMHERNAHNFPLDLAVVEAPAVNG			
Photosystem II CP43 reaction center protein	P56308 (PSBC_CHLVU)	473	52048
Sequence:_MKNLYSLRRFYHVETLFNGSLVVGGRDQESTGFAWWAGNARLINLSGKLLGAHVAHAGLIVFWAGAMNLFEVAHFVPEKPMYEQGLILLPHLATLGYGVGPGGEVIDTYPYFVSGVLHLISSAVLGFGGVYHSLVGPETLEESFPFFGYVWKDKNKMTTILGIHLIVLGFGAWLLVWKAMYFGGIYDTWAPGGGDVRIISNPTVSPGVIFSYILKSPFGGDGWIVSVDNMEDVIGGHIWIGTLCIFGGIWHILTKPWAWARRAFVWSGEAYLSYSLGAIALMGFTACCMSWFNTTAYPSEFYGPTGPEASQSQTFTFLVRDQRLGANVASAQGPTGLGKYLMRSPTGEIIFGGETMRFWDFRGPWLEPLRGPNGLDLNKLKNDIQPWQERRAAEYMTHAPLGSLNSVGGVATEINAVNYVSPRSWLATSHFCLGFFFFVGHLWHAGRARAAAAGFEKGIDRDNEPVLSMRPLD			
Tubulin alpha chain	Q9ZRJ4 (TBA_CHLVU)	451	49712
Sequence:_MREVISIHIGQAGIQVGNACWELYCLEHGIQPDGQMPSDKTIGGGDDAFNTFFSETGAGKHVPRCVFLDLEPTVIDEVRTGTYRQLFHPEQLISGKEDAANNFARGHYTIGKEIVDLCLDRIRKLADNCTGLQGFLVFNAVGGGTGSGLGSLLLERLSVDYGKKSKLGFTVYPSPQVSTAVVEPYNSVLSTHSLLEHTDVSVMLDNEAVYDICRRSLDIERPTYTNLNRLIAQVISSLTASLRFDGALNVDVTEFQTNLVPYPRIHFMLSSYSPVISAEKAYHEQLSVAEITNSAFEPASMMAKCDPRHGKYMACCLMYRGDVVPKDVNAAVATIKTKRTIQFVDWCPTGFKCGINYQPPTVVPGGDLAKVQRAVCMISNSTAIAEVFSRLDHKFDLMYAKRAFVHWYVGEGMEEGEFSEAREDLAALEKDYEEVGAESAEADGEDEGEEY			

All sequences of proteins were analyzed using the following procedure: Proteins tab → Analysis → Calculations → For your sequence → paste the protein sequence → Report. This procedure enabled the calculation of the parameter A, which indicates the potential of the protein as a source of bioactive peptides. A full detailed list of the protein sequences and frequency of all bioactive and sensory fragments, in the analyzed sequences is available in the BIOEP-UWM database of proteins (https://biochemia.uwm.edu.pl/en/biopep-uwm-2/; access 2025.06.01). The results generated by the analysis module BIOPEP-UWM included the location of bioactive fragments in protein chains and their predicted activity [Minkiewicz et. al., 2019]. The frequencies of bioactive fragments in the protein sequence (A) were calculated according to equation (1):

$$A = a/N \quad (1)$$

where: "a" is the number of fragments with a given activity in the analyzed protein sequence, and "N" is the number of amino acid residues in the chain of this protein [Dziuba et. al. 2003]. The predicted frequency of release of bioactive fragments using proteolytic enzymes (A_E) was calculated according to equation (2):

$$A_E = d/N \quad (2)$$

where "d" is the number of peptides with a given activity (e.g. antioxidants) released as a result of the action of a specific enzyme or enzymes, and "N" is the number of amino acid residues in the tested protein [Minkiewicz et. al. 2011]. The relative frequency of release of fragments with given activity by selected enzymes was calculated according to equation (3):

$$W = A_E/A \quad (3)$$

where: A_E is the frequency of release of fragments with given activity by selected enzymes and "A" is previously counted A the frequency of bioactive fragments occurrence in protein sequence. Theoretical degree of hydrolysis (DHt) was calculated according to equation (4):

$$DHt = d/D \cdot 100\% \quad (4)$$

where: “d” is a number of hydrolyzed peptide bonds and “D” is the total number of peptide bonds in a protein chain.

Proteolysis simulations of the studied proteins and calculations of quantitative parameters were performed using calculation modules included in the BIOPEP-UWM database. Protein proteolysis was simulated by the action of coupled action of digestive enzymes: pepsin at pH > 2 (EC 3.4.23.1, BIOPEP-UWM ID 39), trypsin (EC 3.4.21.4, BIOPEP-UWM ID 12) and chymotrypsin A (EC 3.4.21.1, BIOPEP-UWM ID 11), according to the INFOGEST experimental protocol [Brodkorb 2019]. Among the predicted proteolysis products, bioactive fragments and sensory consistent with the data contained in the BIOPEP-UWM database were searched for [Minkiewicz et. al. 2019].

3. Results

Potential of biological activity

Sequence analysis of the tested proteins for potential biological activity indicates that all tested proteins, both from *Spirulina* (P72504 PHAA, P72508 PHCB, P00246 FER) and *Chlorella* (P56318 PSBA, P56308 PSBC, Q9ZRJ4 TBA), exhibit various potential biological activities (Table 3 and 4). The total sum of potential biological activities (Σ) is highest for *Chlorella* proteins, especially for P56308 PSBC (1122) and Q9ZRJ4 TBA (811), compared to *Spirulina* proteins, where the highest sum is for P72504 PHAA (348). Detailed data on the activity attributed to a given peptide are available in the BIOPEP database of bioactive and sensory sequences [Minkiewicz et. al. 2019].

The highest frequency of occurrence (parameter A) in the tested proteins was characterized by the activity of dipeptidyl-peptidase IV inhibitor. This activity was very often observed in all analyzed proteins, with a particularly high frequency (A) in *Chlorella* proteins: P56308 PSBC (0.7209), P56318 PSBA (0.6799), Q9ZRJ4 TBA (0.6231). In *Spirulina* proteins, this is also the dominant activity, with the highest frequency in the P72508 PHCB protein (0.6453), slightly lower in the P72504 PHAA protein (0.6087), and the lowest, but still high, frequency in the P00246 FER protein

(0.5859). Another activity that frequently appears in the analyzed microalgae protein sequences is ACE inhibitor activity. It is particularly visible in the *Chlorella* proteins: P56308 PSBC (0.6025) and P56318 PSBA (0.5467), and the *Spirulina* protein P72504 PHAA (0.5404).

Among other high-frequency activities in individual proteins, antioxidant activities, dipeptidyl-peptidase III inhibitor, and neprilysin inhibitor are also notable. For example, the P56308 PSBC protein from *Chlorella* shows a high frequency (0.1607) for antioxidant activity, and among *Spirulina* proteins, the P72504 PHAA protein stands out, for which the A parameter for this activity is 0.1056. In turn, the P56308 PSBC (*Chlorella*) and P72504 PHAA (*Spirulina*) proteins show a significant frequency (0.1142 and 0.1118, respectively) for dipeptidyl-peptidase III inhibitor activity. In contrast, the P72504 PHAA (0.1304) protein from Spirulina and P56308 PSBC (0.0951) from *Chlorella* exhibit neprilysin inhibitor activity with high frequency.

Some activities, such as ACE 2 activator, AChE inhibitor, alpha-amylase inhibitor, or immunomodulatory activities, are rare or absent in some of the tested proteins. For example, ACE 2 activator was detected only in the P56318 PSBA sequence from *Chlorella* (frequency 0.0028).

It is worth noting that activities with potentially adverse effects, e.g., embryotoxic or toxic, are very rare or occur with very low frequency. *Chlorella* proteins (P56318 PSBA, P56308 PSBC, Q9ZRJ4 TBA) seem to have a wider range and higher frequency of some biological activities, especially those related to dipeptidyl peptidase IV and ACE inhibition. In turn, the P72504 PHAA protein from *Spirulina* is distinguished by a relatively high frequency of neprilysin inhibitor (0.1304) and dipeptidyl peptidase III inhibitor (0.1118) activity.

Table. 3. Profile of potential biological activity of selected Spirulina proteins.

Type of potential biological activity	Type of protein					
	P72504 · PHAA (Spirulina)		P72508 · PHCB (Spirulina)		P00246 · FER (Spirulina)	
	a	A	a	A	a	A
ACE inhibitor	87	0.5404	81	0.4709	36	0.3636
activating ubiquitin-mediated proteolysis	1	0.0062	4	0.0233	1	0.0101
alanine carboxypeptidase inhibitor	3	0.0186	3	0.0174	1	0.0101
alpha-glucosidase inhibitor	7	0.0435	5	0.0291	4	0.0404
anti-inflammatory	0	0.0000	0	0.0000	1	0.0101
antiamnestic	2	0.0124	3	0.0174	0	0.0000
antibacterial	2	0.0124	5	0.0291	2	0.0202
anticancer	1	0.0062	0	0.0000	1	0.0101
antioxidative	17	0.1056	9	0.0523	9	0.0909
antithrombotic	2	0.0124	3	0.0174	0	0.0000
antiviral	1	0.0062	1	0.0058	0	0.0000
binding	2	0.0124	0	0.0000	2	0.0202
calpain 1 inhibitor	0	0.0000	1	0.0058	0	0.0000
CaMPDE inhibitor	1	0.0062	0	0.0000	1	0.0101
citrate lyase deacetylase inhibitor	2	0.0124	0	0.0000	2	0.0202
D-Ala-D-Ala dipeptidase inhibitor	1	0.0062	4	0.0233	1	0.0101
dipeptidyl peptidase III inhibitor	18	0.1118	14	0.0814	4	0.0404
dipeptidyl peptidase IV inhibitor	98	0.6087	111	0.6453	58	0.5859
glutamate carboxypeptidase II inhibitor	12	0.0745	7	0.0407	9	0.0909
glutamate carboxypeptidase inhibitor	8	0.0497	1	0.0058	3	0.0303
haemolytic	1	0.0062	1	0.0058	0	0.0000
hypotensive	5	0.0311	4	0.0233	1	0.0101
hypouricemic	4	0.0248	5	0.0291	3	0.0303
immunomodulating	1	0.0062	0	0.0000	0	0.0000
immunostimulating	2	0.0124	0	0.0000	1	0.0101
inhibitor of cytosol alanyl aminopeptidase	2	0.0124	4	0.0233	1	0.0101
inhibitor of tripeptidyl peptidase II	7	0.0435	10	0.0581	3	0.0303
lactocepin inhibitor	1	0.0062	0	0.0000	1	0.0101
Leucyltransferase inhibitor	1	0.0062	2	0.0116	0	0.0000
neprilysin 2 inhibitor	0	0.0000	1	0.0058	0	0.0000

neprilysin inhibitor	21	0.1304	10	0.0581	5	0.0505
neurolysin inhibitor	1	0.0062	0	0.0000	0	0.0000
neuropeptide	6	0.0373	3	0.0174	4	0.0404
neuroprotective	2	0.0124	3	0.0174	0	0.0000
PAM inhibitor	2	0.0124	3	0.0174	0	0.0000
pancreatic lipase inhibitor	1	0.0062	0	0.0000	1	0.0101
peptidylprolyl isomerase isnhibitor	0	0.0000	1	0.0058	0	0.0000
phospholipase A2 inhibitor	0	0.0000	0	0.0000	1	0.0101
regulating	6	0.0373	4	0.0233	1	0.0101
renin inhibitor	3	0.0186	6	0.0349	2	0.0202
stimulating	9	0.0559	7	0.0407	5	0.0505
thymidylate synthase inhibitor	2	0.0124	0	0.0000	2	0.0202
toxic	1	0.0062	0	0.0000	1	0.0101
tubulin-tyrosine ligase inhibitor	5	0.0311	2	0.0116	1	0.0101
Σ	348		318		168	
N	161		172		99	

Σ – sum of potential biological activities for a given protein (some peptides show several activities);
N - number of amino acid in the chain of protein
A - frequency of occurrence of a given type of biological activity in a protein sequence
a - number of fragments with a given activity in the analyzed protein sequence

Table. 4. Profile of potential biological activity of selected Chlorella proteins.

Type of potential biological activity	Type of protein					
	P56318 · PSBA (Chlorella)		P56308 · PSBC (Chlorella)		Q9ZRJ4 · TBA (Chlorella)	
	a	A	a	A	a	A
ACE 2 activator	1	0.0028	0	0.0000	0	0.0000
ACE inhibitor	193	0.5467	285	0.6025	218	0.4834
ACE2 inhibitor	1	0.0028	2	0.0042	0	0.0000
AChE inhibitor	0	0.0000	1	0.0021	0	0.0000
activating ubiquitin-mediated proteolysis	5	0.0142	11	0.0233	5	0.0111
acylaminoacyl peptidase inhibitor	1	0.0028	6	0.0127	3	0.0067
alanine carboxypeptidase inhibitor	2	0.0057	9	0.0190	5	0.0111
alkaline phosphatase inhibitor	1	0.0028	5	0.0106	0	0.0000
alpha-amylase inhibitor	1	0.0028	2	0.0042	0	0.0000
alpha-glucosidase inhibitor	8	0.0227	19	0.0402	12	0.0266

anti-inflammatory	1	0.0028	1	0.0021	3	0.0067
antiamnestic	1	0.0028	11	0.0233	1	0.0022
antibacterial	7	0.0198	7	0.0148	4	0.0089
anticancer	1	0.0028	0	0.0000	1	0.0022
antidiabetic	2	0.0057	0	0.0000	0	0.0000
antioxidative	37	0.1048	76	0.1607	30	0.0665
antithrombotic	1	0.0028	11	0.0233	2	0.0044
antiviral	1	0.0028	4	0.0085	1	0.0022
bacterial permease ligand	0	0.0000	0	0.0000	1	0.0022
BChE inhibitor	0	0.0000	1	0.0021	0	0.0000
binding	2	0.0057	1	0.0021	3	0.0067
calpain 1 inhibitor	4	0.0113	1	0.0021	3	0.0067
CaMPDE inhibitor	3	0.0085	2	0.0042	4	0.0089
chymotrypsin inhibitor	0	0.0000	1	0.0021	0	0.0000
citrate lyase deacetylase inhibitor	2	0.0057	1	0.0021	3	0.0067
D-Ala-D-Ala dipeptidase inhibitor	6	0.0170	4	0.0085	3	0.0067
dipeptidyl peptidase III inhibitor	21	0.0595	54	0.1142	39	0.0865
dipeptidyl peptidase IV inhibitor	240	0.6799	341	0.7209	281	0.6231
embryotoxic	0	0.0000	0	0.0000	1	0.0022
glutamate carboxypeptidase II inhibitor	5	0.0142	10	0.0211	18	0.0399
glutamate carboxypeptidase inhibitor	3	0.0085	8	0.0169	11	0.0244
haemolytic	1	0.0028	3	0.0063	1	0.0022
HMG-CoA reductase inhibitor	1	0.0028	2	0.0042	1	0.0022
hypolipidemic	0	0.0000	1	0.0021	2	0.0044
hypotensive	12	0.0340	20	0.0423	7	0.0155
hypouricemic	8	0.0227	24	0.0507	9	0.0200
immunostimulating	2	0.0057	3	0.0063	2	0.0044
inhibitor of cytosol alanyl aminopeptidase	9	0.0255	10	0.0211	7	0.0155
inhibitor of tripeptidyl peptidase II	30	0.0850	27	0.0571	23	0.0510
lactocepin inhibitor	4	0.0113	7	0.0148	3	0.0067
Leucyltransferase inhibitor	2	0.0057	7	0.0148	4	0.0089
neprilysin 2 inhibitor	4	0.0113	1	0.0021	3	0.0067
neprilysin inhibitor	18	0.0510	45	0.0951	35	0.0776
neurolysin inhibitor	1	0.0028	1	0.0021	1	0.0022
neuropeptide	10	0.0283	11	0.0233	11	0.0244

neuroprotective	0	0.0000	2	0.0042	1	0.0022
opioid	1	0.0028	1	0.0021	0	0.0000
PAM inhibitor	0	0.0000	3	0.0063	1	0.0022
pancreatic lipase inhibitor	5	0.0142	4	0.0085	1	0.0022
peptidylprolyl isomerase isnhibitor	3	0.0085	2	0.0042	0	0.0000
phospholipase A2 inhibitor	1	0.0028	1	0.0021	2	0.0044
pseudolysin inhibitor	2	0.0057	4	0.0085	1	0.0022
regulating	8	0.0227	19	0.0402	9	0.0200
renin inhibitor	15	0.0425	13	0.0275	12	0.0266
stimulating	20	0.0567	27	0.0571	15	0.0333
thymidylate synthase inhibitor	2	0.0057	1	0.0021	3	0.0067
toxic	1	0.0028	0	0.0000	0	0.0000
tubulin-tyrosine ligase inhibitor	3	0.0085	6	0.0127	5	0.0111
xaa-pro inhibitor	2	0.0057	3	0.0063	0	0.0000
Σ	715		1122		811	
N	353		473		451	

Σ – sum of potential biological activities for a given protein (some peptides show several activities);
N - number of amino acid in the chain of protein
A - frequency of occurrence of a given type of biological activity in a protein sequence
a - number of fragments with a given activity in the analyzed protein sequence

Potential of sensory activity

The analysis of the potential for releasing sensory peptides from the tested microalgal proteins is presented in Tables 5 and 6. All analyzed proteins, from both *Spirulina* and *Chlorella,* demonstrate the potential to release peptides with diverse sensory activities. The total sum of potential sensory activities (Σ) is significantly higher for *Chlorella* proteins than for Spirulina. For *Chlorella*, Σ ranges from 499 (P56318 · PSBA) to 723 (P56308 · PSBC), whereas for *Spirulina*, it ranges from 167 (P00246 · FER) to 309 (P72504 · PHAA). The N parameter (number of amino acids in the protein chain) is generally higher for *Chlorella* proteins, which may explain the greater sum of sensory activities. Bitter-tasting peptides are the dominant sensory activity in all tested proteins, both in terms of the number of fragments (a) and their frequency of occurrence (A). In *Spirulina* proteins, the frequency A for bitter taste ranges from 0.4747 (P00246 · FER) to 0.7267 (P72504 · PHAA). In *Chlorella* proteins, the frequency A for bitter taste is even higher, reaching from 0.6807 (Q9ZRJ4 · TBA) to 0.8372 (P56308 · PSBC).

The second most frequently occurring activity is sweet taste. In *Spirulina*, the frequency A for sweet taste ranges from 0.2626 (P00246 · FER) to 0.3895 (P72508 · PHCB). In *Chlorella*, the frequency A for sweet taste is also high, ranging from 0.3229 (P56318 · PSBA) to 0.3784 (P56308 · PSBC). Third in line is umami taste. In *Spirulina*, the frequency A for umami ranges from 0.1860 (P72508 · PHCB) to 0.3737 (P00246 · FER). In *Chlorella*, the frequency A for umami ranges from 0.1522 (P56308 · PSBC) to 0.2594 (Q9ZRJ4 · TBA).

Table 5. Profile of potential sensory activity of selected *Spirulina* proteins.

Type of sensory activity	P72504 · PHAA		P72508 · PHCB		P00246 · FER	
	a	A	a	A	a	A
astringent	6	0,0373	4	0,0233	2	0,0202
bitter	117	0,7267	98	0,5698	47	0,4747
bitterness suppressing	21	0,1304	17	0,0988	5	0,0505
salt enhancer	8	0,0497	5	0,0291	1	0,0101
salty	11	0,0683	11	0,0640	20	0,2020
sour	35	0,2174	25	0,1453	27	0,2727
sweet	62	0,3851	67	0,3895	26	0,2626
sweetness suppressing	3	0,0186	1	0,0058	2	0,0202
umami	46	0,2857	32	0,1860	37	0,3737
umami enhancing	0	0	0	0	0	0
Σ	309		260		167	
N	161		172		99	

Σ – sum of potential biological activities for a given protein (some peptides show several activities);
N - number of amino acid in the chain of protein
A - frequency of occurrence of a given type of biological activity in a protein sequence
a - number of fragments with a given activity in the analyzed protein sequence

Chlorella proteins, especially P56308 · PSBC and Q9ZRJ4 · TBA, exhibit a higher total sum of peptides with sensory activity (Σ) and frequency (A) across most taste categories compared to *Spirulina* proteins. P00246 · FER from *Spirulina* has a relatively high frequency of salty taste occurrence (A=0.2020) compared to other *Spirulina* proteins and most *Chlorella* proteins. In the Chlorella protein Q9ZRJ4 · TBA, the highest frequency for this taste is A=0.0843. Furthermore, the protein P00246 · FER from *Spirulina* showed the greatest potential for releasing sour taste (A=0.2727), while for *Chlorella*, it was Q9ZRJ4 · TBA (A=0.2395) and P56308 · PSBC (A=0.1311). The most bitterness-suppressing peptides can potentially be obtained from *Chlorella* proteins Q9ZRJ4 · TBA (A=0.1042) and P56308 · PSBC (A=0.0782) compared to the analyzed *Spirulina* proteins. Umami-enhancing activity is rare and does not occur in any of the *Spirulina* proteins, whereas in *Chlorella* proteins, it appears in P56308 · PSBC (a=3, A=0.0063) and Q9ZRJ4 · TBA (a=1, A=0.0022). Astringent peptides, on the other hand, are present in *Spirulina* proteins, but in smaller quantities and with a low frequency of occurrence (A) compared to *Chlorella* proteins (with the exception of P56318 · PSBA = 1 peptide).

Table 6. Profile of potential sensory activity of selected *Chlorella* proteins.

Type of sensory activity	P56318 · PSBA		P56308 · PSBC		Q9ZRJ4 · TBA	
	a	A	a	A	a	A
astringent	1	0,0028	13	0,0275	19	0,0421
bitter	255	0,7224	396	0,8372	307	0,6807
bitterness suppressing	19	0,0538	37	0,0782	47	0,1042
salt enhancer	5	0,0142	14	0,0296	9	0,0200
salty	11	0,0312	17	0,0359	38	0,0843
sour	36	0,1020	62	0,1311	108	0,2395
sweet	114	0,3229	179	0,3784	156	0,3459
sweetness suppressing	2	0,0057	2	0,0042	6	0,0133
umami	56	0,1586	72	0,1522	117	0,2594
umami enhancing	0	0	3	0,0063	1	0,0022
Σ	499		723		691	
N	353		473		451	

Σ – sum of potential biological activities for a given protein (some peptides show several activities);
N - number of amino acid in the chain of protein
A - frequency of occurrence of a given type of biological activity in a protein sequence
a - number of fragments with a given activity in the analyzed protein sequence

Chlorella proteins, especially P56308 · PSBC and Q9ZRJ4 · TBA, exhibit a higher total sum of peptides with sensory activity (Σ) and frequency (A) across most taste categories compared to *Spirulina* proteins. P00246 · FER from *Spirulina* has a relatively high frequency of salty taste occurrence (A=0.2020) compared to other *Spirulina* proteins and most *Chlorella* proteins. In the Chlorella protein Q9ZRJ4 · TBA, the highest frequency for this taste is A=0.0843. Furthermore, the protein P00246 · FER from *Spirulina* showed the greatest potential for releasing sour taste (A=0.2727), while for *Chlorella*, it was Q9ZRJ4 · TBA (A=0.2395) and P56308 · PSBC (A=0.1311). The most bitterness-suppressing peptides can potentially be obtained from *Chlorella* proteins Q9ZRJ4 · TBA (A=0.1042) and P56308 · PSBC (A=0.0782) compared to the analyzed *Spirulina* proteins. Umami-enhancing activity is rare and does not occur in any of the *Spirulina* proteins, whereas in *Chlorella* proteins, it appears in P56308 · PSBC (a=3, A=0.0063) and Q9ZRJ4 · TBA (a=1, A=0.0022). Astringent peptides, on the other hand, are present in *Spirulina* proteins, but in smaller quantities and with a low frequency of occurrence (A) compared to *Chlorella* proteins (with the exception of P56318 · PSBA = 1 peptide).

In silico **hydrolysis – bioactive peptides**

The proteins selected for analysis, both from *Spirulina* (allophycocyanin alpha chain, C-phycocyanin beta subunit, ferredoxin) and *Chlorella* (Photosystem II protein D1, Photosystem II CP43 reaction center protein, tubulin alpha chain), when subjected to in silico digestion, released diverse sets of bioactive peptides (Tables 7 and 8). The degree of hydrolysis (DHt values) for each protein was high, ranging from 75.5% for *Spirulina* ferredoxin to 80.6% for Spirulina allophycocyanin alpha chain. For *Chlorella*, the DHt values ranged from 76.4% (Tubulin alpha chain) to 77.6% (Photosystem II protein D1) and 78.2% (Photosystem II CP43 reaction center protein).

Table 7. The result of in silico proteolysis of selected *Spirulina* proteins for the release of bioactive peptides.

Spirulina protein name	DHt [%]	Type of peptide bioactivity	Amount of peptides	Ae	W
Allophycocyanin alpha chain (P72504)	80,6	dipeptidyl peptidase IV inhibitor	15	0,0932	0,1531
		inhibitor of tripeptidyl peptidase II	3	0,0186	0,4276
		ACE inhibitor	12	0,0745	0,1379
		renin inhibitor	1	0,0062	0,3333
		regulating	2	0,0124	0,3324
		glutamate carboxypeptidase inhibitor	2	0,0124	0,2495
C-phycocyanin beta subunit (P72508)	76,6	regulating	4	0,0233	1
		dipeptidyl peptidase IV inhibitor	20	0,1163	0,1802
		inhibitor of tripeptidyl peptidase II	3	0,0174	0,2995
		calpain 1 inhibitor	1	0,0058	1
		antithrombotic	3	0,0174	1
		ACE inhibitor	14	0,0814	0,1729
		antiamnestic	3	0,0174	1
		stimulating	3	0,0174	0,4275
		neprilysin 2 inhibitor	1	0,0058	1
		neuropeptide	1	0,0058	0,3333
		PAM inhibitor	3	0,0174	1
Ferredoxin (P00246)	75,5	dipeptidyl peptidase IV inhibitor	9	0,0909	0,1551
		inhibitor of tripeptidyl peptidase II	1	0,0101	0,3333
		ACE inhibitor	5	0,0505	0,1389
		stimulating	2	0,0202	0,4
		phospholipase A2 inhibitor	1	0,0101	1
		renin inhibitor	1	0,0101	0,5
		neuropeptide	2	0,0202	0,5
		anti inflammatory	1	0,0101	1
		glutamate carboxypeptidase inhibitor	1	0,0101	0,3333
		hypouricemic	1	0,0101	0,3333
		antioxidative	1	0,0101	0,1111

Table 8. The result of in silico proteolysis of selected *Chlorella* proteins for the release of bioactive peptides

Chlorella protein name	DHt [%]	Type of peptide bioactivity	Amount of peptides	Ae	W
Photosystem II protein D1 (P56318)	77,6	dipeptidyl peptidase IV inhibitor	44	0,1246	0,181
		inhibitor of tripeptidyl peptidase II	9	0,0255	0,3
		calpain 1 inhibitor	4	0,0113	1
		ACE inhibitor	29	0,0822	0,1473
		antioxidative	6	0,017	0,1622
		renin inhibitor	5	0,0142	0,3341
		CaMPDE inhibitor	2	0,0057	0,6706
		stimulating	3	0,0085	0,1499
		phospholipase A2 inhibitor	1	0,0028	1
		dipeptidyl peptidase III inhibitor	1	0,0028	0,0449
		neuropeptide	3	0,0085	0,2724
		alpha-glucosidase inhibitor	2	0,0057	0,2511
		anti inflammatory	1	0,0028	1
		regulating	6	0,017	0,7489
		neprilysin 2 inhibitor	4	0,0113	1
		ACE2 inhibitor	1	0,0028	1
		xaa-pro inhibitor	2	0,0057	1
		lactocepin inhibitor	2	0,0057	0,5044
		neprilysin inhibitor	1	0,0028	0,0549
Photosystem II CP43 reaction center protein (P56308)	78,2	regulating	7	0,0148	0,3682
		dipeptidyl peptidase IV inhibitor	62	0,1311	0,1819
		inhibitor of tripeptidyl peptidase II	6	0,0127	0,2224
		calpain 1 inhibitor	1	0,0021	1
		antithrombotic	2	0,0042	0,1803
		ACE inhibitor	42	0,0888	0,1474

		antiamnestic	2	0,0042	0,1803
		antioxidative	11	0,0233	0,145
		stimulating	8	0,0169	0,296
		phospholipase A2 inhibitor	1	0,0021	1
		alpha-glucosidase inhibitor	6	0,0127	0,3159
		renin inhibitor	1	0,0021	0,0764
		dipeptidyl peptidase III inhibitor	6	0,0127	0,1112
		neuropeptide	5	0,0106	0,4549
		anti inflammatory	1	0,0021	1
		neprilysin 2 inhibitor	1	0,0021	1
		pancreatic lipase inhibitor	2	0,0042	0,4941
		ACE2 inhibitor	1	0,0021	0,5
		PAM inhibitor	2	0,0042	0,6667
		xaa-pro inhibitor	3	0,0063	1
		lactocepin inhibitor	3	0,0063	0,4257
		hypouricemic	6	0,0127	0,2505
		neprilysin inhibitor	1	0,0021	0,0221
Tubulin alpha chain (Q9ZRJ4)	76,4	dipeptidyl peptidase IV inhibitor	43	0,0953	0,1529
		inhibitor of tripeptidyl peptidase II	8	0,0177	0,3471
		calpain 1 inhibitor	3	0,0067	1
		ACE inhibitor	28	0,0621	0,1285
		antioxidative	6	0,0133	0,2
		renin inhibitor	1	0,0022	0,0827
		CaMPDE inhibitor	1	0,0022	0,2472
		stimulating	3	0,0067	0,2012
		phospholipase A2 inhibitor	1	0,0022	0,5
		dipeptidyl peptidase III inhibitor	8	0,0177	0,2046
		neuropeptide	1	0,0022	0,0902
		alpha-glucosidase inhibitor	2	0,0044	0,1654

		anti inflammatory	1	0,0022	0,3284
		regulating	4	0,0089	0,445
		neprilysin 2 inhibitor	3	0,0067	1
		anticancer	1	0,0022	1
		glutamate carboxypeptidase inhibitor	1	0,0022	0,0902
		hypouricemic	2	0,0044	0,22
		neprilysin inhibitor	1	0,0022	0,0284

The dominant activities in all analyzed proteins were dipeptidyl peptidase IV (DPP-IV) inhibitors and ACE inhibitors, both in terms of the number of released peptides and their effective activity (Ae). For *Spirulina* proteins, the DPP-IV inhibitor amounts were: 15 peptides (Ae: 0.0932) for allophycocyanin, 20 peptides (Ae: 0.1163) for C-phycocyanin beta, and 9 peptides (Ae: 0.0909) for ferredoxin. For *Chlorella* proteins, these values were even higher: 44 peptides (Ae: 0.1246) for Photosystem II protein D1, 62 peptides (Ae: 0.1311) for Photosystem II CP43 reaction center protein, and 43 peptides (Ae: 0.0953) for tubulin alpha chain. In contrast, for *Spirulina*, the ACE inhibitor amounts were: 12 peptides (Ae: 0.0745) for allophycocyanin, 14 peptides (Ae: 0.0814) for C-phycocyanin beta, and 5 peptides (Ae: 0.0505) for ferredoxin. For *Chlorella* proteins, the values were significantly higher: 29 peptides (Ae: 0.0822) for Photosystem II protein D1, 42 peptides (Ae: 0.0888) for Photosystem II CP43 reaction center protein, and 28 peptides (Ae: 0.0621) for tubulin alpha chain. Among the other activities, tripeptidyl peptidase II inhibitor can also be distinguished, which is frequently released from all tested proteins.

Chlorella proteins, particularly Photosystem II protein D1 and Photosystem II CP43 reaction center protein, appear to release a greater quantity and broader spectrum of bioactive peptides compared to *Spirulina* proteins. For instance, activities such as alpha-glucosidase inhibitor, neprilysin 2 inhibitor, and xaa-pro inhibitor were detected, which are absent or rarer in *Spirulina* protein hydrolysates. Peptides with antioxidant activity are primarily released from *Chlorella* proteins (e.g., 6 peptides from D1, 11 peptides from CP43, 6 peptides from alpha tubulin) and from Spirulina ferredoxin – 1 peptide. Regulating activity is present in all proteins, but its quantity and effectiveness (Ae) vary. In *Chlorella*, Photosystem II protein D1 releases 6 regulating peptides, while Photosystem II CP43 reaction center protein releases 7 peptides. Peptides with calpain inhibitor activity are released from *Chlorella* proteins (4 from D1, 1 from CP43, 3 from alpha tubulin) and from *Spirulina* C-phycocyanin beta subunit (1 peptide). Peptides with anti-inflammatory effects are released from all *Chlorella* proteins and from *Spirulina* ferredoxin. However, anticancer activity was detected only in *Chlorella's* tubulin alpha chain (1 peptide).

The W index indicates what proportion of the potential activity was released as a result of enzyme action. A value of 1 signifies 100% effectiveness. Many activities, especially those released in smaller quantities, exhibit high W values (e.g., calpain 1 inhibitor, antithrombotic, antiamnestic, neprilysin 2 inhibitor, PAM inhibitor, phospholipase A2 inhibitor, anti-inflammatory, renin inhibitor, anticancer, xaa-pro inhibitor, hypouricemic). This suggests that, despite their smaller quantity, these peptides can influence the potential bioactive effect.

In silico **hydrolysis – sensory peptides**

Tables 9 and 10 present the results of *in silico* digestion of the analyzed *Spirulina* and *Chlorella* proteins regarding the release of sensory peptides. All tested proteins released peptides with diverse sensory activity as a result of in silico hydrolysis. The most dominant sensory activity, in terms of both the number of released peptides (Amount of peptides) and effective activity (Ae), across all analyzed proteins was bitter taste. Notably, *Chlorella* proteins showed a higher quantity and frequency of release (Ae) for these peptides. The W values (relative frequency of release) for bitter taste are moderate (approx. 0.3-0.4).

Regarding sweet taste, higher release frequencies were observed for *Spirulina* proteins: Allophycocyanin alpha chain (31 peptides, Ae=0.1925), C-phycocyanin beta subunit (33 peptides, Ae=0.1919), and *Chlorella's* Photosystem II CP43 reaction center protein

(97 peptides, Ae=0.2051). *Chlorella's* Ferredoxin, Photosystem II protein D1, and Tubulin alpha chain had lower Ae parameters (0.1515, 0.1643, and 0.1707, respectively). The W values for sweet taste are also moderate (approx. 0.49-0.57), but higher than those for bitter taste.

Table 9. The result of in silico proteolysis of selected *Spirulina* proteins for the release of sensory peptides.

Spirulina protein name	DHt [%]	Type of sensory peptide	Amount of peptides	Ae	W
Allophycocyanin alpha chain (P72504 · PHAA_ARTPT)	80,6	bitter	38	0,2360	0,3248
		sour	23	0,1491	0,6858
		umami	21	0,1304	0,4564
		salty	7	0,0435	0,6369
		sweet	31	0,1925	0,4999
		astringent	4	0,0248	0,6649
		bitterness suppressing	13	0,0807	0,6189
		salt enhancer	3	0,0186	0,3742
C-phycocyanin beta subunit (P72508 · PHCB_ARTPT)	76,6	bitter	43	0,25	0,4388
		sour	20	0,1163	0,8004
		umami	18	0,1047	0,5629
		salty	9	0,0523	0,8172
		sweet	33	0,1919	0,4927
		astringent	4	0,0233	1
		bitterness suppressing	14	0,0814	0,8239
Ferredoxin (P00246 · FER_ARTPT)	75,5	bitter	15	0,1515	0,3191
		sour	15	0,1515	0,5556
		umami	14	0,1414	0,3784
		salty	7	0,0707	0,35
		sweet	15	0,1515	0,5769
		astringent	1	0,0101	0,5
		bitterness suppressing	1	0,0101	0,2

Table 10. The result of in silico proteolysis of selected *Chlorella* proteins for the release of sensory peptides.

Chlorella protein name	DHt [%]	Type of sensory peptide	Amount of peptides	Ae	W
Photosystem II protein D1 (P56318 · PSBA_CHLVU)	77,6	bitter	102	0,2890	0,4001
		sour	25	0,0708	0,6941
		umami	26	0,0737	0,4647
		salty	3	0,0085	0,2724
		sweet	58	0,1643	0,5088
		astringent	1	0,0028	1
		bitterness suppressing	12	0,034	0,632
Photosystem II CP43 reaction center protein (P56308 · PSBC_CHLVU)	78,2	bitter	144	0,3044	0,3636
		umami	37	0,0782	0,5138
		sour	43	0,0909	0,6934
		salty	12	0,0254	0,7075
		sweet	97	0,2051	0,542
		astringent	13	0,0275	1
		bitterness suppressing	30	0,0634	0,8107
		umami enhancing	2	0,0042	0,6667
		salt enhancer	2	0,0042	0,1419
Tubulin alpha chain (Q9ZRJ4 · TBA_CHLVU)	76,4	bitter	118	0,2616	0,3843
		sour	71	0,1574	0,6572
		umami	60	0,133	0,5127
		salty	20	0,0443	0,5255
		sweet	77	0,1707	0,4935
		astringent	16	0,0355	0,8432
		bitterness suppressing	30	0,0665	0,6382
		umami enhancing	1	0,0022	1
		salt enhancer	1	0,0022	0,11

Umami peptides generally exhibited higher Ae parameters for *Spirulina* (ranging from 0.1047 to 0.1414), with the exception of the tubulin alpha chain protein (0.1330). W values for umami are generally higher than for bitter taste (0.37-0.56). Similarly, for sour taste, W values are often very high (above 0.5, and even close to 0.8 in some cases), meaning that a large proportion of potentially sour fragments are efficiently released.

Salty peptides occur in sequences in smaller quantities but are often released with high relative frequency (W). For example, *Spirulina's* C-phycocyanin beta subunit (W=0.8172) and *Chlorella's* Photosystem II CP43 reaction center protein (W=0.7075) show particularly high efficiency in releasing salty peptides. Similarly, for bitterness-suppressing peptides, which are quantitatively fewer but are released by proteins from both microalgae with high efficiency (high W values, often above 0.6, and even 0.8 in some cases), which is beneficial given the dominance of bitter taste.
Both *Spirulina* and *Chlorella* proteins contain fragments with potential astringent activity that can be released during enzymatic hydrolysis. The analyzed *Chlorella* proteins exhibit a larger quantity of this type of peptide, and the relative release frequency is 100% or close to it (Tubulin alpha chain W = 0.8432). Only *Chlorella* proteins (Photosystem II CP43 reaction center protein, Tubulin alpha chain) release umami-enhancing and salt-enhancing peptides, which is not observed for *Spirulina.* Overall, *Chlorella* proteins (e.g., Photosystem II CP43 reaction center protein) generally release a higher number of peptides with sensory activity (e.g., 144 bitter peptides, 97 sweet peptides) compared to *Spirulina* proteins (e.g., C-phycocyanin beta subunit: 43 bitter peptides, 33 sweet peptides).

4. Discussion

Currently, due to their chemical composition, microalgae play a key role in aquaculture, as a component of feed. Fish fed with microalgae show faster growth and are characterized by an increased content of valuable nutrients in their meat [Dohaish et. al. 2018]. These beneficial features of microalgae have not yet found their full application, and they can be relatively easily used as a valuable food additive as a source of colorant, vitamins, minerals and protein [Gouveia et. al. 2010; Pehlivanov et. al. 2024]. As food additives, *Chlorella* and *Spirulina* can impart an intense odor and alter the natural color and texture of the product. [Byczyński et. al. 2025; Çelekli et. al. 2024]. These changes are usually undesirable. Therefore, applications for microalgae-derived isolates (proteins, oils, cell wall polysaccharides) are currently being explored. Specially microalge are promising as source of protein, aminoacids and active peptide because of high (60-75%) protein content [Andrade et. al. 2018]. The introduction to the use of algal proteins may be in silico analyses, which may provide an answer to the bioactivity potential of peptides generated from microalgal proteins.

It should be noted at the outset that *in silico* analysis provides a cost-effective and rapid tool for preliminary screening to identify the potential of specific proteins from a given matrix. Such methods are used to predict peptide profiles from specific protein sequences and under specific hydrolysis conditions. However, it should be noted that these models generate predictions and do not confirm actual biological activity, bioavailability, stability in complex food matrices, or *in vivo* efficacy. The hydrolysis process and product activity are influenced by additional factors, such as absorption, metabolism, and potential interactions with other food components—these aspects are typically not considered in simple *in silico* models. Nevertheless, such methods can be valuable in the search for new sources of bioactive and sensory peptides.

The proteins used in the analysis were characterized by a high potential content of bioactive fragments (over 300 for two *Spirulina* proteins and over 700 for *Chlorella* proteins) and frequency of occurrence in the sequence (maximum 0.64 for *Spirulina* and 0.72 for *Chlorella*). For comparison, in a study on rainbow trout proteins [Borawska et al. 2015], only collagen achieved high parameters (number of sequences 1999, A=0.73), while proteins such as myosin, actin, and creatine kinase had parameters similar to or lower than those of algal proteins (number of sequences: 475, 227, 218; parameter A: 0.34, 0.41, 0.37, respectively).

The observed higher amount of potential bioactivities and sensory peptides in *Chlorella* proteins than in *Spirulina* proteins is mainly related to the number of amino acids in the protein chain (N). Chlorella proteins usually have longer chains than those from Spirulina, which may lead to a greater number of potential activities. The described result is consistent with the findings of Dziuba and Darewicz (2007), who observed that the longer the protein sequence, the higher the probability of detecting a bioactive fragment. All proteins have high DHt parameters, such a high degree of hydrolysis indicates the efficient release of peptides from the proteins discussed.

The most promising activities, in terms of both the number of released peptides (Amount of peptides) and effective activity (Ae), are dipeptidyl peptidase IV (DPP-IV) inhibitors and ACE inhibitors. These activities are dominant in all analyzed proteins from both *Spirulina* and *Chlorella.* Furthermore, *Chlorella* proteins exhibited even higher values for DPP-IV and ACE inhibitors compared to *Spirulina* proteins, indicating their greater potential. The high presence and effectiveness of DPP-IV and ACE inhibitors in the hydrolysates suggest their potential in treating type 2 diabetes (DPP-IV) and hypertension (ACE).

Both *Spirulina* and *Chlorella*, after hydrolysis, can provide peptides with taste-modifying effects. The dominant bitter profile in both types of microalgae suggests that protein hydrolysates may require additional processing to reduce undesirable bitterness, unless it is a desired product characteristic. The moderate W parameter values for bitter-tasting peptides indicate that although they are abundantly released, not all potentially bitter fragments in the original protein are efficiently liberated. The high relative efficiency of releasing bitterness-suppressing peptides is also interesting, which is beneficial given the dominance of bitter taste.

The coincidence of a high frequency of dipeptidyl peptidase IV (DPP-IV) inhibitors and ACE inhibitors with a high content of bitter-tasting peptides seems interesting. It turns out that these are often the same peptides [Iwaniak et al. 2014]. This situation raises dilemmas such as: "should food taste good or have health-promoting properties?

The presence of bitterness-suppressing and umami-enhancing peptides is promising, as they can contribute to improving the overall flavor profile of products. Higher W values for sweet, umami, and sour tastes compared to bitter suggest that significant proportions of potential fragments with these tastes are released relative to their full potential.

The release of a greater number of peptides with sensory activity from *Chlorella* proteins (e.g., 144 bitter peptides, 97 sweet peptides from Photosystem II CP43) compared to *Spirulina* proteins (e.g., C-phycocyanin beta subunit: 43 bitter peptides, 33 sweet peptides) is again linked to the larger number of amino acids in *Chlorella's* protein chains. Furthermore, the fact that umami and salt enhancers are released only from *Chlorella* proteins could be a key feature for food applications.

Despite the relatively low frequency of occurrence of astringent fragments in the original protein sequence (parameter A), the *in silico* digestion process appears to be very effective in releasing them. High W values (often equal to 1 or close to 1) for astringent peptides indicate that a significant, and in many cases complete, portion of potential astringent fragments is released. *Chlorella* proteins, particularly Photosystem II CP43 reaction center protein and Tubulin alpha chain, appear to release a larger number of astringent peptides (13 and 16 respectively) compared to *Spirulina* proteins (maximum 4 peptides). This means that *Chlorella* hydrolysates may have stronger astringent properties. The presence of astringent peptides can affect mouthfeel, causing a dry or puckering sensation. This is an important factor to consider in the context of food applications for these hydrolysates.

5. Conclusions

The *in silico* analysis results confirm that both *Spirulina* and *Chlorella* are rich sources of proteins that, after enzymatic digestion, can release peptides with a broad spectrum of potential biological activities. The most promising activities, due to the number and effectiveness of released peptides, are DPP-IV and ACE inhibitors. This suggests the potential of these hydrolysates in treating type 2 diabetes (DPP-IV) and hypertension (ACE). *Chlorella* proteins appear to be characterized by a greater diversity and number of released peptides, indicating their broader therapeutic potential compared to the analyzed Spirulina proteins.

Chlorella proteins seem to offer a greater overall number and diversity of sensory peptides, additionally including unique activities such as umami and salt enhancers, and a larger number of astringent peptides, which makes *Chlorella* potentially a more versatile source for applications in functional food.

Bitter taste is dominant among the released peptides, regardless of the microalga type, which may necessitate further processing steps to minimize undesirable bitterness.

The presence of sweet, umami, salty, sour peptides, and importantly, bitterness-suppressing and umami-enhancing peptides, creates potential for utilizing microalgal hydrolysates as functional ingredients influencing the flavor profile of food.

The analyses presented in this work provide a solid basis for further *in vitro* and *in vivo* experimental studies to confirm the biological and sensory activity of these peptides and to assess their potential application in the food, pharmaceutical, or nutraceutical industries.

6. References

[1] Andrade, L.M.; Andrade, C.J.; Dias, M.; Nascimento, C.A.; Mendes, M.A. Chlorella and Spirulina Microalgae as Sources of Functional Foods, Nutraceuticals, and Food Supplements; an Overview. MOJ Food Process. Technol. 2018, 6, 45–58.

[2] Awosika, T.O.; Aluko, R.E. Inhibition of the in vitro activities of -amylase, -glucosidase and pancreatic lipase by yellow field pea (Pisum sativum L.) protein hydrolysates. Int. J. Food Sci. Technol. 2019, 54, 2021–2034.

[3] Bateman, A.; Martin, M. J.; Orchard, S.; et al.et al. UniProt: the Universal Protein Knowledgebase in 2023. Nucleic Acids Res. 2022, 51(D1), D523−D531

[4] Bleakley, S.; Hayes, M. Functional and Bioactive Properties of Protein Extracts Generated from Spirulina platensis and Isochrysis galbana T-Iso . Appl. Sci. 2021 , 11 , 3964.

[5] Borawska J.; Pliszka M.; Świtaj M.; Darewicz M. Rainbow trout proteins as potential source of biologically active peptides. Med Og Nauk Zdr. 2015;21(3), 322-327

[6] Brodkorb, A.; Egger, L.; Alminger, M.; Alvito, P.; Assunção, R.; Ballance, S.; Bohn, T.; Bourlieu-Lacanal, C.; Boutrou, R.; Carrière, F., Clemente A., Corredig M., Dupont D., Dufour C., Edwards C., Golding M., Karakaya S., Kirkhus B., Le Feunteun S., Lesmes U., Macierzanka A., Mackie A.R., Martins C., Marze S., McClements D.J., Ménard O., Minekus M., Portmann R., Santos C.N., Souchon I., Singh R.P., Vegarud G.E., Wickham M.S.J., Weitschies W., Recio I. INFOGEST static in vitro simulation of gastrointestinal food digestion. Nature Protoc. 2019, 14, 991–1014.

[7] Byczyński Ł., Duliński R., Smoleń S. In Vitro Analysis and Assessment of the Bioavailability of Selected Minerals and B Vitamins in Kefir Enriched with Microalgae, Applied Sciences-Basel, 2025, 15(12), 1-17.

[8] Çelekli, A.; Özbal, B.; Bozkurt, H. Challenges in Functional Food Products with the Incorporation of Some Microalgae. Foods 2024, 13, 725.

[9] Dohaish, E.; Dhahri, M.A.; Omar, H. Potential application of the blue-green alga (Spirulina platensis) as a supplement in the dietof nile tilapia (Oreochromis niloticus). Appl. Ecol. Environ. Res. 2018, 16, 7883–7902

[10] Dziuba M., Darewicz M., 2007 Food proteins as precursors of booactive peptides – classification into families. Food Sci. Technol. Int., 13 (6), 393-404.

[11] Dziuba, J.; Iwaniak, A.; Minkiewicz, P. Computer-aided characteristics of proteins as potential precursors of bioactive peptides. Polimery 2003, 48, 50−53.

[12] Girija, A.R. Peptide nutraceuticals. In Peptide Applications in Biomedicine, Biotechnology and Bioengineering; Koutsopoulos, S., Ed.;Woodhead Publishing: Cambridge, UK, 2018; pp. 157–181.

[13] Gouveia, L.; Marques, A.E.; Sousa, J.M.; Moura, P.; Bandarra, N.M. Microalgae—Source of Natural Bioactive Molecules as Functional Ingredients. Food Sci. Technol. Bull. Funct. Foods 2010, 7, 21–37.
[14] Iwaniak, A., Darewicz, M. and Minkiewicz, P. Peptides Derived from Foods as Supportive Diet Components in the Prevention of Metabolic Syndrome. Comprehensive Reviews in Food Science and Food Safety, 2018, 17: 63-81.
[15] Iwaniak, A.; Minkiewicz, P.; Darewicz M. Food-originating ACE inhibitors, including antihypertensive peptides, as preventive food components in blood pressure reduction. Compr. Rev. Food Sci. Food Safety. 2014, 13(2), 114-134.
[16] Iwaniak, A.; Minkiewicz, P.; Darewicz, M. BIOPEP-UWM database present and future. Curr. Opin. Food Sci. 2024, 55, No. 101108
[17] Iwaniak, A.; Minkiewicz, P.; Darewicz, M.; Hrynkiewicz, M. Food protein-originating peptides as tastants–Physiological, technological, sensory, and bioinformatic approaches. Food Res. Int. 2016, 89, 27–38.
[18] Iwaniak A. , Mogut D., Darewicz M., Hrynkiewicz M, Wykorzystanie biologicznie aktywnych peptydów z białek żywności w profilaktyce zespołu metabolicznego. Zeszt. Probl. Post. Nauk Roln., 2016, 585, 75-85.
[19] Minkiewicz, P.; Darewicz, M.; Iwaniak, A. In Silico Analysis of Individual Fractions of Bovine Casein as Precursors of Bioactive Peptides—Influence of Post-Translational Modifications. Appl. Sci. 2023, 13, 8091.
[20] Minkiewicz, P.; Dziuba, J.; Michalska, J. Bovine meat proteins as potential precursors of biologically active peptides–a computational study based on the BIOPEP database. Food Sci. Technol. Int. 2011, 17 (1), 39−45.]
[21] Minkiewicz, P.; Iwaniak, A.; Darewicz, M. BIOPEP-UWM Database of Bioactive Peptides: Current Opportunities. Int. J. Mol. Sci. 2019, 20, 5978.
[22] Pehlivanov, I.; Gentscheva, G.; Nikolova, K.; Andonova, V. Some Applications of Arthrospira platensis and Algae in Pharmaceutical and Food Technologies. Biointerface Res. Appl. Chem. 2024, 14, 32.
[23] Ryan, J.T.; Ross, R.P.; Bolton, D.; Fitzgerald, G.F.; Stanton, C. Bioactive peptides from muscle sources: Meat and fish. Nutrients 2011, 3, 765.

Nutritional management of Crohn's disease

Anna Ciołek*, Alicja Skrzypek, Dawid Leszkowicz

Faculty of Food Science and Biotechnology,
University of Life Sciences in Lublin,
Akademicka 15, 20-950 Lublin, Poland.
*corresponding author: anna.ciolek@up.lublin.pl

Abstract

Crohn's disease (CD) is a type of inflammatory bowel disease (IBD). The inflammatory lesions, typically granulomatous, can affect any segment of the gastrointestinal tract. It is a chronic disease with exacerbations and remissions. The development of the disease is most likely due to an excessive pathological immune response to intestinal bacteria in individuals with a genetic predisposition. Symptoms depend on which section of the gastrointestinal tract is affected, and may include weakness, abdominal pain, diarrhoea, fever, anaemia and weight loss. Treatment includes lifestyle modification, diet, pharmacological and surgical treatment. CD may lead to a range of complications, such as fistulas, interloop abscesses, intestinal strictures and, rarely, colorectal cancer. There is no specific diet designated for Crohn's disease. The diet differs between flare-ups and remission phases. During exacerbations, a low-residue, easily digestible diet is required, involving multiple food eliminations and limiting cooking techniques to boiling and steaming. During remission, the diet should be more varied, although individual triggers should be avoided. The nutritional goal is to correct numerous deficiencies (iron, folic acid, vitamin B12) and improve the patient's overall nutritional status. Appropriate dietary prophylaxis based on the principles of the Mediterranean diet is important.

Keywords: Crohn's disease, Mediterranean diet, enteral nutrition, Crohn's disease exclusion diet.

1. Introduction

Elements of the Western lifestyle, such as a diet high in saturated fats, simple sugars, highly processed foods and red meat, while low in fibre, vegetables and fruit, promote increased inflammatory processes and increase the risk of autoimmune diseases. The intestinal microbiota, which is a complex and dense population of microorganisms, plays a key role in food digestion, nutrient production and immune substances such as sodium butyrate, which promotes regeneration of the intestinal mucosa. An imbalance between beneficial and pathogenic bacteria, known as intestinal dysbiosis, can lead to a dysregulation of the immune system and contribute to the development of various conditions, including inflammatory bowel disease (IBD) [Corsello et al. 2020]. IBD patients, including Crohn's disease (CD), face a number of problems in their professional and personal lives due to pain and other symptoms of the disease. In addition, decreased appetite, insufficient intake in relation to increased needs, impaired absorption (i.e. the effects of active inflammatory disease) can lead to malnutrition. these dimensions.

Crohne disease can develop at any age, with a peak incidence between 20 and 35 years of age. However, the disease is affecting increasingly younger children and carries a number of risks. Children are at risk of stunted growth, impaired bone metabolism, delayed puberty, malnutrition and micronutrient and vitamin deficiencies are often associated with pediatric inflammatory bowel disease.

The aim of this paper was to describe selected nutritional strategies for people suffering from Crohn's disease, taking into account the diverse needs during periods of exacerbation and remission of the disease.

2. Characteristics of inflammatory bowel diseases

Inflammatory bowel disease (IBD) is a complex chronic condition that includes: ulcerative colitis (CU) and Crohn Disease (CD). They are diseases with an incompletely known aetiology, associated with multiple gastrointestinal and systemic complications. Both are chronic and progressive diseases, meaning that they can last a lifetime, often with non-specific symptoms making diagnosis difficult. The course of IBD is characterised by cyclic recurrent gastrointestinal inflammation with alternating phases of exacerbation and remission.

In ulcerative colitis, inflammation is most often localised to the rectum and sigmoid colon, although it may involve the entire colon. The onset is limited to the mucosa and superficial layers of the submucosa. Some patients develop ulcerations. The typical symptom of this disease is diarrhoea with an admixture of blood and mucus, which may be accompanied by abdominal pain and fever [Wils et al. 2022].

In Crohn's disease, inflammatory lesions are segmental and can affect the entire gastrointestinal tract, from the oral cavity to the perianal area. In about 30 % of patients, the lesions are granulomatous and transmural. In about half of the patients, within 20 years of diagnosis, alternating inflammation and scarring leads to complications such as fistulas, strictures and abscesses. In the classic disease phenotype, with involvement of the terminal ileum, intestinal symptoms include acute abdominal pain, diarrhoea occasionally with an admixture of blood and mucus and a palpable lump in the right iliac fossa. Symptoms of CD also include fever, weakness and weight loss. CD sometimes presents with a range of extra-intestinal symptoms that can be diagnosed even before the diagnosis of IBD. These can include ophthalmic complications (scleritis, choroiditis, uveitis), recurrent aphthae, stomatitis, skin lesions (especially erythema nodosum), gangrenous dermatitis and complications such as anaemia, sarcopenia, osteoporosis and malnutrition. People with CD also have about twice the incidence of the life-threatening complication venous thromboembolism compared to the general population [Ranasinghe et al. 2024, Malik and Aurelio 2024].

The mechanism of the disease is not fully understood. According to the current aetiopathogenetic concept, the development of the disease occurs as a result of an excessive pathological immune response to intestinal bacteria - which constitute the intestinal flora - in individuals with a genetic predisposition. The response directed against bacteria, which in this case are commensals, is determined, among other things, by mutations in the NOD2, ATG16L1 and IRGM genes responsible for innate immunity and autophagy. The dysfunction of these genes hinders the elimination of bacteria with the subsequent development of chronic inflammation in the intestinal wall. In addition, environmental factors, poor diet and lack of hygiene in life play an important role. All this leads to chronic inflammation, which impairs intestinal function and nutrient absorption. This results in malnutrition and deterioration of the patient's health. Inflammatory bowel disease is also associated with an increased risk of developing colorectal cancer, with the most important predisposing factor being the duration of the disease [McDowell et al. 2023].

Initially occurring as sporadic cases in the UK and northern Europe, inflammatory bowel disease has gradually increased in number and geographical spread. So has the adaptation of the modern, so-called western, model of life by industrialisation, globalisation and technological advances linked to changes in agriculture, production and dietary patterns [Kirsner 2001, Kaplan 2015].

Research confirms a systematic and marked increase in disease rates during the 20th century. Shivashankar et al. [2017] conducted an analysis of the number of Olmsted County residents with Crohn's disease or ulcerative colitis between 1970 and 2010, confirming this trend. Currently, IBD affects nearly 2.5 million people in the United States. Of course, the higher number of diagnosed cases may be due not only to potential changes in environmental factors such as diet, lifestyle and the gut microbiome, but also to better diagnosis and increased awareness of the disease among physicians and patients [Ananthakrishnan et al. 2024].

In developed regions, inflammatory bowel diseases have reached a phase of stability in the number of infections, passing through all stages of epidemiological evolution. In contrast, developing countries have seen a continuous increase in the burden of these diseases, which may reflect changes in lifestyle and processes associated with increasing industrialisation. The increase in cases among ethnic groups and populations where these diseases were previously rare, in regions such as South America, Eastern Europe, Asia and Africa, provides new data on the pathogenesis and impact of environmental factors in different parts of the world. The observed epidemiological changes, especially noticeable in newly industrialised countries and among Asian immigrants in Western countries, are reminiscent of trends observed in Western countries more than half a century ago, when they were accompanied by rapid socio-economic development [Kaplan 2015, Siew et al. 2017].

According to the analysis by Zagórowicz et al. [2022], in Poland, the problem affects approximately 100 000 people, which is 0.25% of the total population and means that 1 in 400 people suffer from IBD. The authors confirmed a count in 2020 of 23,574 patients with Crohn's disease and 73,235 with ulcerative colitis. Over the period 2012-2018, the largest increase in the number of patients with CD was recorded among the 20-29 and 30-39 age groups, while with UC among patients in the 60-69 and 70+ age groups.

3. Dietary therapy in inflammatory bowel disease

Lack of appetite occurring during disease exacerbations can exacerbate nausea, vomiting, abdominal pain and diarrhoea, leading to reduced food intake, fluid and electrolyte loss. The consequence is often patient malnutrition. Macro- and micronutrient deficiencies that occur can also be a side effect of the drug treatment used. A common problem for patients with IBD is a fear of eating due to the fear of abdominal pain and increased diarrhoea. In addition, the patient's personal dietary beliefs and restrictions on food choices may influence nutritional status [Razack and Seinder 2007]. In people with Crohn's disease, malabsorption syndrome is very common. If the disease involves the small intestine, it interferes with the absorption of nutrients. Changes in the intestinal mucosa, such as loss of epithelial integrity and disruption of its transport function, can lead to problems with fluid and electrolyte movement. In addition, the chronic inflammation caused by the disease contributes to constant blood loss, which exacerbates the symptoms of malnutrition. Patients with inflammatory bowel disease often experience difficulties due to a lack of knowledge about appropriate nutrition to support treatment. As a result, they make dietary decisions on a trial-and-error basis, tailoring their diet to their current mood, often without consulting their doctor or dietician. This approach encourages the formation of erroneous eating habits [Balestrieri et al. 2020]. People who significantly restrict or completely exclude certain foods without consulting their doctor and nutritionist are at particular risk of developing nutrient deficiencies [Kaliora 2023].

The mechanisms leading to inflammatory reactions in the gut are not limited to the effects of the food itself, but also include additives, such as emulsifiers, which can induce inflammation. Although research on the effects of emulsifiers on the microbiome is in its early stages, there is evidence to suggest their potential effects on the mucus layer in the gut [Besedin et al. 2024].

Deficiencies due to malabsorption syndrome in CD include iron deficiencies. Diagnosis should include assessment of iron, transferrin and ferritin levels to distinguish iron deficiency from anaemia associated with chronic disease. Sources of this element are found in many foods, such as beef, liver, fish, poultry, eggs and legumes. Many experts prefer intravenous iron administration, fearing an exacerbation of IBD after oral iron administration. Recent studies confirm the efficacy and safety of modern oral preparations. Intravenous iron administration should therefore be used mainly in patients who cannot tolerate or do not respond to oral supplementation [Bertani et al. 2021]. Malabsorption and chronic diarrhoea in CD, as well as an insufficient supply of selected foods, can result in deficiencies of vitamin B12, folic acid, calcium, magnesium, zinc, sodium and potassium [Rudzki et al. 2020].

4. Nutritional management in disease exacerbation

The assessment of nutritional status in patients with inflammatory bowel disease should be comprehensive and multifaceted, involving several key elements. A detailed dietary analysis that takes into account the patient's daily calorie intake and level of energy expenditure is an essential step. Body composition and overall nutritional status should be accurately assessed. A bioelectrical impedance analysis (BIA) or a densitometric examination should be used for this purpose. An additional element should be the assessment of biochemical parameters, which makes it possible to accurately determine the need for individual nutrients and to better tailor dietary interventions to the patient's individual needs.

A theoretical monograph should be a synthetic, original, and creative overview of knowledge on a given topic.

It is important to stress that in the case of CD, there is no one-size-fits-all diet that can be universally recommended to induce remission in the active phase of the disease. An individualised approach that takes into account the specific needs of the patient, his or her medical condition and possible nutritional deficiencies is crucial. The diet should be tailored to prevent nutritional deficiencies, support the patient's overall health and improve their quality of life. This holistic approach enables optimisation of dietary therapy and effective support in the management of inflammatory bowel disease [Bischoff et al. 2023].

5. Nutritional management in disease exacerbation

The main aim of diet therapy in the treatment of CD, is to reduce the intestinal load by using a balanced semi-liquid or liquid diet. In the severely exacerbated moment of the disease, the first choice is to include exclusive enteral nutrition, (EEN). This involves the use of specialised nutritional formulas with a specific quantitative and qualitative composition and good bioavailability of ingredients. Enteral nutrition is the only source of nutrition, to the total exclusion of the normal diet, for a period of six to eight weeks, depending on the patient's condition. It provides 100% of caloric requirements in liquid form. During disease severity, resting energy expenditure is approximately 25-30 kcal/kg body weight. The preparations used contain protein hydrolysates and fats containing medium-chain fatty acids (MCTs), and do not contain fibre. In the treatment of Crohn's disease in children, EEN has fully proven dietary efficacy comparable to corticosteroid therapy. Clinical remission and endoscopically confirmed healing occurred in 50-70% of cases. The use of this dietary regimen modifies the microbiome and has an impact on intestinal permeability and the immune system [Miele et al. 2018]. At the start of management, the patient's growth age, physical activity and protein-energy requirements related to the existing inflammation should be assessed. Enteral nutrition should be introduced within a few days, gradually increasing the supply of industrial diets until the optimal volume is reached, covering the energy requirements. In addition, polymeric diets are recommended to keep the gastrointestinal tract continuously working (monomeric diets should be used for strict indications, such as severe food allergy) [Rudzki et al. 2020].

The restrictiveness, limited acceptability and duration of use of EEN have contributed to the search for other dietary solutions effective in affecting remission in CD. The Crohn's disease exclusion diet (CDED), used in the active phase of CD in combination with 50% partial bowel feeding, has the best documented efficacy. CDED is a long-term strategy and consists of several consecutive 6-week phases. The strategy involves excluding certain ingredients from the diet that may negatively affect intestinal homeostasis, while specific whole foods are included [Levine et al. 2019]. CDED involves the introduction of high amounts of high-quality protein, a reduction in dietary fat and the inclusion of foods rich in complex carbohydrates supplemented with varying amounts of polymeric formula, in proportions dependent on the CDED phase. The diet ensures that the energy requirements and additional supply of protein, calcium and vitamin D are achieved, in addition to beneficial fibre and the substrates necessary for SCFA synthesis. An inventory of obligatory, allowed and contraindicated foods is developed, appropriate for each stage of CDED. Foods such as chicken, eggs, potatoes, rice, fruit and vegetables are included in the diet. The composition of the diet, as well as the quantity in variable amounts depending on the specific phase of treatment [Herrador-Lopez et al. 2020].

Researchers also studied the CD treatment-with-eating diet (CD-TREAT). An oral diet was developed that mapped the composition of the most popular European industrial mix used in EEN (Module IBD, Nestle Vevey, Switzerland). This was achieved by excluding certain ingredients from the diet (e.g. gluten, lactose, alcohol) and matching others from ordinary foods as closely as possible. Maltodextrin, which is absent in natural foods, was replaced by starch-rich and fibre-poor foods, with a lower proportion of carbohydrates and a higher proportion of protein in the daily supply, which did not differ from the composition of other mixtures used in EEN. Multivitamin supplementation was introduced ensuring an adequate supply of micronutrients. Positive results on the feasibility of CD-TREAT were obtained by studying healthy subjects, analysing an animal model and results in several children with active CD [Svolos et al. 2019].

Fluid supplementation to compensate for water and electrolyte losses due to diarrhoea is important. If oral rehydration proves insufficient, intravenous fluids may be necessary. It is advisable to avoid substances that can aggravate intestinal spasms, such as caffeine, and products containing fermentable sugars, such as fruit juices.

Due to possible damage to the intestinal mucosa during an exacerbation, lactose intolerance is common. Therefore, dairy products should be kept to a minimum, as lactose fermented by intestinal bacteria is converted into lactic acid, which can further stimulate intestinal peristalsis and exacerbate symptoms [Baczewska-Mazurkiewicz and Rydzewska 2011]. To meet calcium requirements, the dairy industry has introduced lactose-free products, which are made by adding exogenous lactase (β-galactosidase) that pre-degrades the lactose contained in milk. In addition, products containing only trace amounts of lactose, such as hard cheeses, yoghurts, as well as plant-based products such as rice, soya, oats, almonds, cashew nuts or other nuts, can be reached [Facioni et al. 2020].

Adequate water supply plays a key role, especially due to the risk of dehydration resulting from chronic diarrhoea and electrolyte loss. During periods of disease exacerbation, it is important to adapt fluid intake to the individual patient's needs, taking into account age, physical activity, disease severity and the presence of complications such as fistulas or bowel resections. Regular drinking of water, mild vegetable juices, broths and rehydration solutions is recommended. Alcohol, sweetened fizzy drinks and caffeinated products should be eliminated, as they can exacerbate the symptoms of diarrhoea or have a dehydrating effect. For severe diarrhoea, oral rehydration solutions or even intravenous fluid supplementation is necessary. It is important to drink fluids slowly and avoid using straws, which can introduce air into the digestive tract, causing bloating and discomfort. Excessive intake of hypotonic fluids, such as water alone, can lead to hyponatraemia (sodium deficiency), so it is advisable to choose electrolyte-enriched drinks [Müller-Nothmann 2007].

6. Nutritional management in disease remission

During remission, the aim of diet is to achieve and maintain adequate nutrition. A well-balanced diet plays a key role in therapy, influencing the length of remission and the rate of recovery from exacerbations. During this period, nutrition should be healthy and balanced, providing all necessary nutrients. It is important to provide adequate amounts of protein, vitamins and micronutrients, as well as omega-3 fatty acids. There is no universal diet, and recommendations must be individual, with elimination only of those products that the patient tolerates poorly. Nevertheless, there are attempts to develop dietary indications to maintain the remission period as long as possible and to improve the nutritional status and comfort of the patient. Numerous dietary nutrients such as vitamins, amino acids, polyphenols, fibre and fatty acids have been shown to play an important role in regulating the immune response in the gastrointestinal mucosa. Depending on the stage and phase of the disease or induction, the requirements for the nutrients in question change [Lee et al. 2015].

Among the most favourable dietary options often recommended during disease remission is the Mediterranean diet (MD). This predominantly plant-based diet is based on whole grains, fruit and vegetables including weekly portions of legumes and nuts; protein sources in the form of fish, poultry, eggs or plant-based alternatives with a limitation of red, processed meat; fat consumption in the form of olive oil and a limitation of sweetened drinks and alcohol. As research shows, not only is a health-promoting diet associated with a lower risk of CD, but it also has positive effects after the onset of the disease. The Mediterranean eating style is distinguished by its abundance of vitamins, minerals and antioxidants. Studies suggest that serum levels of these nutrients are significantly reduced in patients with active forms of IBD. Vitamins A, D, E, K and C are crucial for supporting immune responses in the context of IBD. Adherence to a Mediterranean diet has a beneficial effect on the profile of the gut microbiota, promoting the production of short-chain fatty acids, which play a key role in preventing the development of inflammatory bowel disease [Çelik et al. 2023].

Dietary fibre, present in fruit and vegetables, plays a key role in the normal functioning of the intestines. This is because it is an important substrate for the production of butyrate and other short-chain fatty acids, which exhibit strong anti-inflammatory properties by inhibiting cytokine transcription. Dietary fibre deficiency can lead to degradation of the intestinal mucosal layer, resulting in increased permeability of the mucosal barrier. As a result, there is an increased risk of gut bacteria coming into contact with the epithelium, which can induce inflammation and tissue damage [Parada et al. 2019]. Organisations such as the Academy of Nutrition and Dietetics, the American Crohn's Disease and Inflammatory Bowel Disease Foundation and the World Organisation of Gastroenterology all agree that patients with inflammatory bowel disease in remission should not restrict fibre intake, provided there is no intestinal stenosis. However, fibre restriction is recommended for exacerbations [Lewis and Abreu 2016].

Strong anti-inflammatory effects are demonstrated by omega-3 fatty acids, including plant-derived α-linolenic acid and eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids from fish. Mechanisms of action of n-3 PUFAs include improved intestinal barrier function, reduced expression of pro-inflammatory cytokines and modulation of the intestinal microbiome towards beneficial bacteria. Monounsaturated fatty acids, contained for example in olive oil, have also been shown to have a protective effect in CD diet therapy [Lewis and Abreu 2016]. A substance that is recognised as a potential dietary ingredient for the treatment of inflammatory bowel disease is curcumin, present in turmeric. Due to its anti-inflammatory and immunomodulatory properties, it may inhibit key inflammatory processes [Holt 2016].

A study by Çelik et al. further confirms that the use of MD by patients can improve the balance of the gut microbiome and also significantly increase their quality of life [Çelik et al. 2023]. A health-promoting lifestyle based on the Mediterranean diet implies practising well-chosen physical activity. A study was conducted on patients with inflammatory bowel disease in remission or with mildly active disease. The essence of the study was a change in dietary habits based on a patient-personalised Mediterranean diet and moderate physical activity. Differences in participants' lifestyles and fitness levels were taken into account. Dieticians and physiotherapists set the goal of therapeutic management, provided feedback and knowledge to achieve a sustainable change in habits. The physical activity introduced did not worsen the wellbeing of the subjects; on the contrary, the majority of participants evaluated the intervention very positively. Additionally, the improvement in diet quality had a noticeable impact on

daily functioning, reducing the negative impact of the disease and fatigue levels, highlighting the effectiveness of the holistic lifestyle approach [Lamers et al. 2022].
Attempts have been made to use the Specific Carbohydrates Diet (SCD) in patients with mild to moderate CD. SCD was originally developed for patients with coeliac disease. It is an elimination diet and involves the exclusion of poly- and disaccharides from the diet, which can lead to intestinal bacterial overgrowth. Elimination includes all cereals and starchy vegetables (potatoes, yams), dairy, sucrose and processed foods. Foods containing easily absorbed: simple sugars are allowed: fruit, some vegetables and honey. In a randomised trial comparing the effects of the above two diets in patients with CD, no better results were obtained for SCD compared to MD. The Mediterranean diet was superior to SCD in terms of ease of use and numerous additional health benefits and should be recommended [Lewis et al. 2021].

7. Summary

In the development of Crohn's disease, it is important to link the onset of the disease to specific dietary patterns. Certain dietary components may influence inflammatory bowel activity, hence the importance of promoting a healthy diet such as the Mediterranean diet.
Crohn's disease, through the discomfort experienced and possible complications, greatly affects the working activity of sufferers. Social activity is also often problematic due to the numerous dietary restrictions. There is a high risk of deficiencies in patients, both for organic reasons and the use of elimination diets without consultation with a doctor and dietician can be fraught with a high risk of deficiencies, consequently exacerbating the inflammation and worsening the condition. Dietary intervention is therefore important, both in terms of improving clinical outcomes and life comfort.

8. References

[1] Ananthakrishnan A.N., Gerasimidis K., Ho S.M., Mayer E., Pollock J., Soni S., Wu G.D., Benyacoub J., Ali B., Favreau A., Smith D.E., Oh J.E., Heller C., Hurtado-Lorenzo A., Moss A., Croitoru K.: Challenges in IBD research 2024: Environmental triggers. Inflamm. Bowel Dis., 2024, 30 (Suppl 2), 19-29.

[2] Baczewska-Mazurkiewicz D., Rydzewska G.: Nutritional problems in inflammatory bowel disease patients. Gastroenterol. Rev., 2011, 6 (2), 69-77.

[3] Balestrieri P., Ribolsi M., Guarino M.P.L., Emerenziani S., Altomare A., Cicala M.: Nutritional aspects in inflammatory bowel diseases. Nutrients, 2020, 12 (2), 372-387.

[4] Bertani L., Ribaldone D.G., Bellini M., Mumolo M.G., Costa F.: Inflammatory bowel diseases: Is there a role for nutritional suggestions? Nutrients, 2021, 13 (4), 1387.

[5] Besedin D., Shah R., Brennan C., Panzeri E., Hao T.T., Eri R.: Food additives and their implication in inflammatory bowel disease and metabolic syndrome. Clin. Nutr. ESPEN, 2024, 64, 483–495.

[6] Bischoff S.C., Bager P., Escher J., Forbes A., Hébuterne X., Hvas C.L., Joly F., Klek S., Krznaric Z., Ockenga J., Schneider S., Shamir R., Stardelova K., Bender D.V., Wierdsma N., Weimann A.: ESPEN guideline on Clinical Nutrition in inflammatory bowel disease. Clin. Nutr., 2023, 42 (3), 352–379.

[7] Çelik K., Güveli H., Erzin Y., Kenge E.B., Özlü T.: The Effect of Adherence to Mediterranean Diet on Disease Activity in Patients with Inflammatory Bowel Disease. Turk. J. Gastroenterol., 2023, 34 (7), 714-719.

[8] Corsello A., Pugliese D., Gasbarrini A., Armuzzi A.: Diet and nutrients in gastrointestinal chronic diseases. Nutrients, 2020, 12 (9), 2693-2711.

[9] Facioni M.S., Raspini B., Pivari F., Dogliotti E., Cena H.: Nutritional management of lactose intolerance: the importance of diet and food labelling. J. Transl. Med., 2020, 18 (1), 260-283.

[10] Głażewski T, Dyrla P., Gil J.: Podstawowe zasady żywienia pozajelitowego. Pediatr. Med. Rodz. 2017, 13 (1), 29–39.

[11] Herrador-López M., Martín-Masot R., Navas-López V.M.: EEN Yesterday and Today … CDED Today and Tomorrow. Nutrients, 2020,12 (12), 3793.

[12] Holt PR.: Curcumin for inflammatory bowel disease: A caution. Clin. Gastroenterol. Hepatol., 2016, 14 (1), 168.

[13] Kaliora A.C.: Nutrition in inflammatory bowel diseases; Is there a role?. Best Prac. Res. Clin. Gastroenterol., 2023, 62-63, 101827.

[14] Kaplan G.G.: The global burden of IBD: from 2015 to 2025. Nat. Rev. Gastroenterol. Hepatol., 2015, 12 (12), 720-727.

[15] Kirsner J.B.: Historical origins of current IBD concepts. World J. Gastroenterol., 2001. (2), 175-184.

[16] Lamers C.R., De Roos N.M., Heerink H.H., Van De Worp-Kalter L.A.,. Witteman B.J.M.: Lower Impact of Disease on Daily Life and Less Fatigue in Patients With Inflammatory Bowel Disease Following a Lifestyle Intervention. Inflamm. Bowel Dis. 2022, 28 (12), 1791–1799.

[17] Lee D., Albenberg L., Compher C., Baldassano R., Piccoli D., Lewis J.D., Wu G.D.: Diet in the pathogenesis and treatment of inflammatory bowel diseases. Gastroenterology, 2015, 148 (6), 1087–1106.

[18] Levine A, Wine E, Assa A, Levine A., Wine E., Abitbol G., Dunn K.A., BielawskiJ.P., Van Limbergen J.: Crohn's disease exclusion diet plus partial enteral nutrition induces sustained remission in a randomized controlled trial. Gastroenterology, 2019, 157, 440–450.

[19] Lewis J.D., Abreu M.T.: Diet as a trigger or therapy for inflammatory bowel diseases. Gastroenterology, 2017, 152 (2), 398-414.

[20] Lewis J.D., Sandler R.S., Brotherton C., Brensinger C. Braly K, Nessel L., McCauley M., McKeever L., Herfarth H.: A Randomized Trial Comparing the Specific Carbohydrate Diet to a Mediterranean Diet in Adults With Crohn's Disease. Gastroenterology, 2021, 161 (3), 837 – 852.

[21] Malik T.F., Aurelio D.M.: Extraintestinal manifestations of inflammatory bowel disease. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, https://www.ncbi.nlm.nih.gov/books/NBK568797/

[22] Miele E., Shamir R., Aloi M.: Nutrition in pediatric inflammatory bowel disease: a position paper on behalf of the Porto Inflammatory Bowel Disease Group of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition. J. Pediatr. Gastroenterol. Nutr., 2018; 66, 687–708.

[23] McDowell C., Farooq U., Haseeb M.,:. Inflammatory Bowel Disease. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Accessed: 31.05 2023. https://www.ncbi.nlm.nih.gov/books/NBK470312/

[24] Parada Venegas D., De la Fuente M.K., Landskron G., González M.J., Quera R., Dijkstra G., Harmsen H.J.M., Faber K.N., Hermoso M.A.: Short chain fatty acids (SCFAs)-mediated gut Eeithelial and immune regulation and its relevance for inflammatory bowel diseases. Front. Immunol., 2019, 10:277.

[25] Ranasinghe I.R., Tian C., Hsu R.: Crohn Disease. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, Accessed: 31.05 2023, https://www.ncbi.nlm.nih.gov/books/NBK436021/

[26] Razack R., Seidner D.L.: Nutrition in inflammatory bowel disease. Curr. Opin. Gastroenterol.,2007, 23 (4), 400-405.

[27] Rudzki B., Żwirełło A., Budrn G., Kazimierczak-Siedlecka K., Lubiniecka-Archutowska E., Lebiedzińska A.: The use of enteral nutrition in pediatric patients with Crohn's disease. Farm. Pol., 2020, 76 (3), 170-174.

[28] Shivashankar R., Tremaine W.J., Harmsen W.S., Loftus E.V. Jr.: Incidence and Prevalence of Crohn's Disease and Ulcerative Colitis in Olmsted County, Minnesota From 1970 Through 2010. Clin. Gastroenterol. Hepatol., 2017, 15 (6), 857–863.

[29] Siew N.G., Shi H.Y., Hamidi N., Underwood F.E., Tang W., Benchimol E.I., Panaccione R., Ghosh S., Wu J.C.Y, Chan F.K.L., Sung J.J.Y., Kaplan G.G.: Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet, 2017, 390 (10114), 2769–2778.

[30] Svolos V., Hansen R., Nichols B., Gaya D.R., Milling S.,Russell R.K., Gerasimidis K.: Treatment of active Crohn’s disease with an ordinary food-based diet that replicates exclusive enteral nutrition. Gastroenterology, 2019, 156, 1354–1367.

[31] Wils P., B. Caron, F. D'Amico, S. Danese, L. Peyrin-Biroulet, : Abdominal Pain in Inflammatory Bowel Diseases: A Clinical Challenge, J. Clin. Med., 2022, 11(15), 4269-4275.

[32] Zagórowicz E., Walkiewicz D., Kucha P., Perwieniec J., Maluchnik M., Wieszczy P., Reguła J.: Nationwide data on epidemiology of inflammatory bowel disease in Poland between 2009 and 2020. Pol. Arch. Intern. Med., 2022, 132 (5), 16194.

Students' knowledge and attitudes regarding energy drinks in the context of public health prevention

Renata Czeczko[1*], Karolina Worku[2]

[1]*University of Life Sciences, Faculty of Food Science and Biotechnology, Akademicka 13, 20-950 Lublin, Poland*
[2]*Cardinal Stefan Wyszynski University, Faculty of Theology, Dewajtis 5, 01-815 Warsaw, Poland*
[*]*corresponding author: renata.czeczko@up.lublin.pl*

Abstract

Contemporary youth are growing up in an environment characterized by intense stimulation, a fast-paced lifestyle, and widespread access to products that affect the nervous system, including energy drinks. The popularity of these beverages has been steadily increasing, particularly among young adults and adolescents, who often consume them to enhance alertness, improve concentration, or combat fatigue.The aim of the study was to assess health awareness and consumption behaviors related to energy drink intake among university students. The research was conducted using a diagnostic survey method with a proprietary questionnaire, which included questions on the frequency of consumption, motives for using energy drinks, awareness of their ingredients, and adverse effects associated with their intake. The analysis encompassed responses from 262 university students. The results showed that 28% of respondents consume energy drinks regularly, at least once a week. The primary reasons for consumption were the need to increase energy levels (74.7%) and to improve concentration (38%). A significant difference was observed between genders: women demonstrated a higher level of awareness regarding the health effects of energy drinks (82.8% of women vs. 19.7% of men). Moreover, only 9% of participants were aware of the maximum recommended caffeine dose, and approximately 50% reported experiencing alarming symptoms following energy drink consumption. The findings indicate a high prevalence of energy drink consumption among students and insufficient knowledge about their composition and potential adverse effects. These results underscore the need for educational initiatives aimed at raising health awareness within this age group.

Keywords: energy drinks, caffeine, hazards health toxicity, side effects.

1. Introduction

Energy drinks (EDs) are non-alcoholic beverages characterized by a high content of substances with stimulating effects on the nervous and cardiovascular systems. The most common ingredients found in these beverages include caffeine, taurine, glucuronolactone, B vitamins, guarana, and significant amounts of sugar [Cadoni and Peana

2023]. The declared purpose of ED consumption is to increase energy, enhance concentration, reduce fatigue, and improve psychophysical performance. However, the consumption of EDs, particularly among young individuals, has raised growing concerns and health-related controversies. The effects of energy drinks are primarily attributed to their high caffeine content—often exceeding 150 mg per serving—which stimulates the central nervous system and increases adrenaline secretion [Hałasiński et al. 2022]. Caffeine (1,3,7-trimethylxanthine) is generally well tolerated by healthy adults when consumed in moderation. Nevertheless, excessive caffeine intake may elevate the risk of adverse effects, including increased blood pressure, tachycardia, insomnia, irritability, anxiety, gastrointestinal disturbances, and, in some cases, arrhythmias and psychotic symptoms [Sankararaman et al. 2018, Cadoni and Peana 2023]. In individuals with pre-existing cardiovascular diseases, excessive caffeine consumption can be particularly harmful due to its potential to elevate blood pressure and precipitate other cardiac events [Grandner et al. 2014]. Moreover, scientific reports have suggested a possible association between energy drink consumption and the development of aneurysms, arterial dilatation, dissection, and even rupture of large arteries [Alsunni 2015]. Taurine, the second most common ingredient in energy drinks (EDs), influences neurotransmission and may act synergistically with caffeine, thereby enhancing its stimulating effects. At the same time, EDs are a significant source of sugar, which, when consumed regularly, contributes to obesity, dental caries, and the development of insulin resistance [Puupponen et al. 2023]. The consumption of EDs has also been associated with an increase in risky behaviors among adolescents. As demonstrated by Puupponen et al. [2023], even occasional ED consumption by teenagers significantly raises the likelihood of psychoactive substance use (including tobacco, marijuana, and alcohol), sleep disturbances, breakfast skipping, and problematic use of digital media. The risk escalates proportionally with the frequency of ED consumption. The authors describe EDs as part of a so-called “package of risky health behaviors,” which also includes unhealthy diets, lack of physical activity, and disruptions in circadian rhythms. In addition to physical consequences, psychosocial effects are equally significant. Regular ED consumers are more likely to experience symptoms of anxiety, depression, irritability, and report difficulties with concentration and learning [Cadoni and Peana 2023, Puupponen et al. 2021]. Studies from the United States indicate that adolescents who drink EDs tend to have lower academic performance, increased absenteeism, and reduced motivation to study [Seifert et al. 2011]. This association is even stronger when EDs are combined with alcohol or consumed during periods of sleep deprivation, which is commonly observed among older adolescents and university students. Research also suggests that ED consumption does not mitigate the effects of sleep loss; on the contrary, it may lead to increased daytime sleepiness and reduced alertness [Mansour et al. 2019]. Apart from overdose symptoms, some individuals may develop dependence and withdrawal symptoms. Dependence on EDs correlates with the age of caffeine initiation; the earlier caffeine is introduced to the body, the higher the risk of developing dependence [Reissig et al. 2009]. The reasons why young people consume energy drinks are complex. On one hand, adolescents often report a desire to boost energy, improve concentration, and combat fatigue—particularly in the context of academic work or physical activity. On the other hand, social factors play a substantial role, including peer pressure, advertising messages, and prevailing trends [Puupponen et al. 2023, Cadoni and Peana 2023]. For many young people, EDs are not perceived as potentially harmful products but rather as a "superior version of a soft drink." The authors of the cited studies consistently emphasize the low level of awareness among adolescents regarding the composition, effects, and potential side effects of EDs. Young consumers often cannot distinguish between energy drinks, isotonic beverages, and sports supplements. They also lack knowledge about safe daily caffeine limits, possible interactions with medications, and the risks associated with combining EDs with alcohol. Therefore, it is recommended to introduce mandatory warning labels, restrict advertising targeted at adolescents, and prohibit the sale of EDs to minors—measures that have already been implemented in several European countries [Cadoni and Peana 2023]. In Poland, such a ban was enacted on January 1, 2024 (Dz.U.2024.1670) [Holt 2023].

The aim of the study was to assess the behaviors and knowledge levels of students at Lublin universities regarding energy drinks. In particular, the study focused on the frequency and reasons for consumption, awareness of side effects, knowledge of ingredients (especially caffeine), and possible interactions with alcohol and medications. It also aimed to explore what alternatives to EDs students consider and what factors influence their choice of specific brands.

2. Materials and Methods

The study employed a method utilizing a proprietary research tool in the form of an original questionnaire, which consisted of twenty questions—eighteen closed-ended and two open-ended. The closed-ended questions included both single-choice and multiple-choice formats. This approach aimed to obtain the most accurate representation of respondents' knowledge without implying that only one response could be correct. The survey was conducted among 430 students aged 18–20 years, enrolled at universities in Lublin. Participation in the survey was voluntary and anonymous. The first section of the questionnaire contained demographic questions regarding age, gender, and place of study. Subsequent questions addressed the respondents' knowledge about energy drinks and issues related to their consumption of these beverages. The questionnaire was distributed via student groups affiliated with Maria Curie-Skłodowska University, the University of Life Sciences in Lublin, the Medical University of Lublin, Lublin University of Technology, and John Paul II Catholic University of Lublin. This distribution method proved to be the most effective and convenient for participants to complete the form. The collected data were processed using Microsoft Excel 2013 and subjected to statistical analysis with StatSoft Statistica 13 software. To assess the statistical significance between variables, Pearson's chi-square test was applied, adopting a significance level of $p<0.05$.

3. Results

A total of 430 questionnaires were collected and analyzed. The analysis revealed that approximately 39% of respondents reported not consuming energy drinks, while one-third of the students reported occasional consumption (a few times per month). Regular consumers, defined as those drinking energy drinks at least once per week, accounted for a total of 28% of the surveyed population (figure 1).

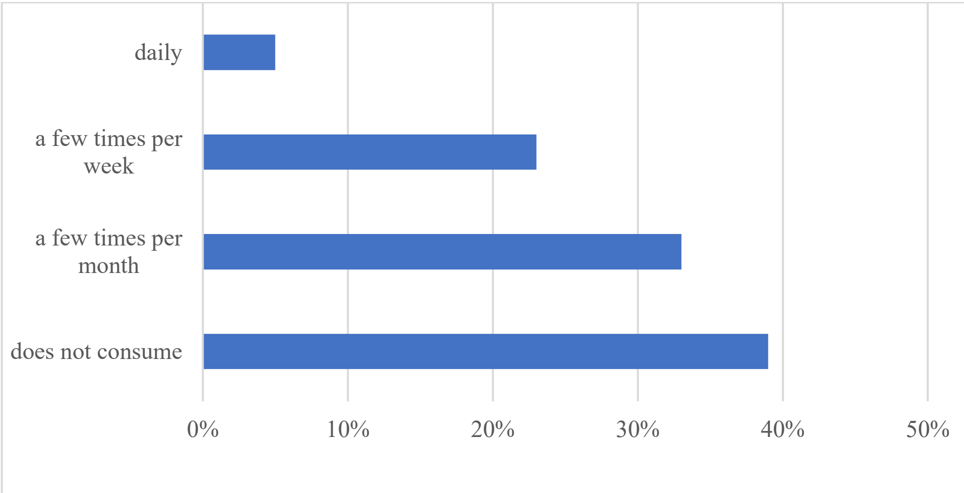

Figure 1. Frequency of energy drink consumption (% of responses).

For further analysis, only the questionnaires from respondents who declared consuming energy drinks were included, yielding a sample of 262 participants. Of these, 145 were women (55%) and 117 were men (45%). It was observed that 98% of respondents were aged 18–20 years, with 19-year-old students constituting the largest age group (73%). Figure 2 illustrates the relationship between gender and the perceived impact of energy drinks on human health.

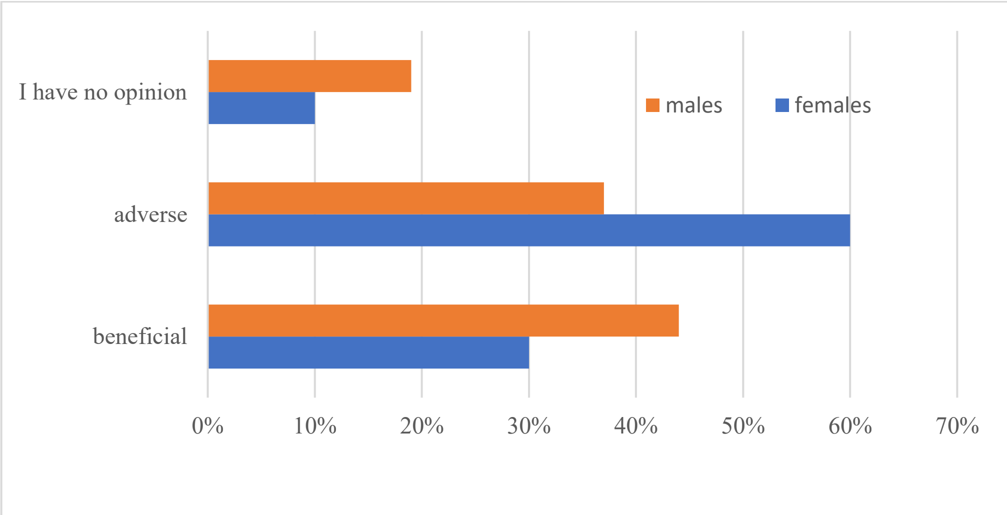

Figure 2. Effects on human health of consuming energy drinks (% of responses).

The results of the statistical analysis ($p = 0.0008$, $p < 0.05$) indicate a significant association between these variables. This suggests that gender influences perceptions of the health effects of energy drink consumption. Women were more likely to perceive the impact of these beverages as negative (60%), whereas men more often viewed them as beneficial (44%) or admitted lacking knowledge on the subject (19%).

Table 1. Analysis of student consumption habits related to energy drinks.

		total		female		male		
		n=262	%*	n=145	%*	n=117	%*	ch2 test, p value
Purpose of use	reduced drowsiness	234	89,31	131	90,3	103	88	0,6886
	improved physical performance	183	69,85	85	58,6	98	83,8	0
	improved mental performance	114	43,51	64	44,1	50	42,7	0,9185
	improved well-being	93	35,5	54	37,2	39	33,3	0,5979
	appealing taste	74	28,24	48	33,1	26	22,2	0,0708
Motivations to purchase	flavor	207	79,01	115	79,3	92	78,6	1.0000

	price	184	70,23	102	70,3	82	70,1	1.0000
	ingredients (no sugar)	143	54,58	120	82,8	23	19,7	0
	advertising	120	45,8	74	51	46	39,3	0.0771
	Popularity among peers	60	22,9	20	13,8	40	34,2	0,0002
	Black	194	74,05	84	57,9	110	94	0
	Tiger	153	58,4	60	41,4	93	79,5	0
	Monster	98	37,4	46	31,7	52	44,4	0,0469
	Dzik	83	31,68	21	14,5	62	53	0
	RedBull	78	29,77	50	34,5	28	23,9	0,0853
	BePower	63	24,05	32	22,1	31	26,5	0.4914
	Burn	27	10,31	12	8,3	15	12,8	0.3180
Most frequently selected brands	Frugo	23	8,78	15	10,3	8	6,8	0.4367

p<0,05 statistically significant, *values cannot be summed up to 100% due the ability to indicate more than one answer

As shown in table 1, men were more likely than women to report consuming energy drinks to stimulate physical activity (83.8% vs. 58.6%). In other consumption contexts, the purposes of use showed no statistically significant differences between women and men. Regarding purchase motives, notable differences emerged in the importance placed on popularity among peers (13.8% of women vs. 34.2% of men). Women were significantly more likely to pay attention to product composition and to favor beverages labeled as sugar-free (82.8% of women vs. 19.7% of men). Brand preferences analysis revealed that men more frequently chose brands such as Black (94.0% vs. 57.9%), Tiger (79.5% vs. 41.4%), and Dzik (53.0% vs. 14.5%). Women's preferences were more diverse; however, no significant differences were found for brands such as Red Bull, Burn, or Frugo, suggesting similar perceptions of these brands across both groups.
The question regarding perceived side effects after consuming energy drinks revealed that 45.4% of respondents reported experiencing no adverse effects. Meanwhile, 27% of participants reported palpitations, 15.5% declared sleep disturbances, and 7.6% reported headaches. Occasionally, respondents experienced nervousness or diarrhea. Less

commonly reported side effects included vomiting, heightened stress levels, skin reactions, or abdominal pain. A few individuals also mentioned constipation and hand tremors.

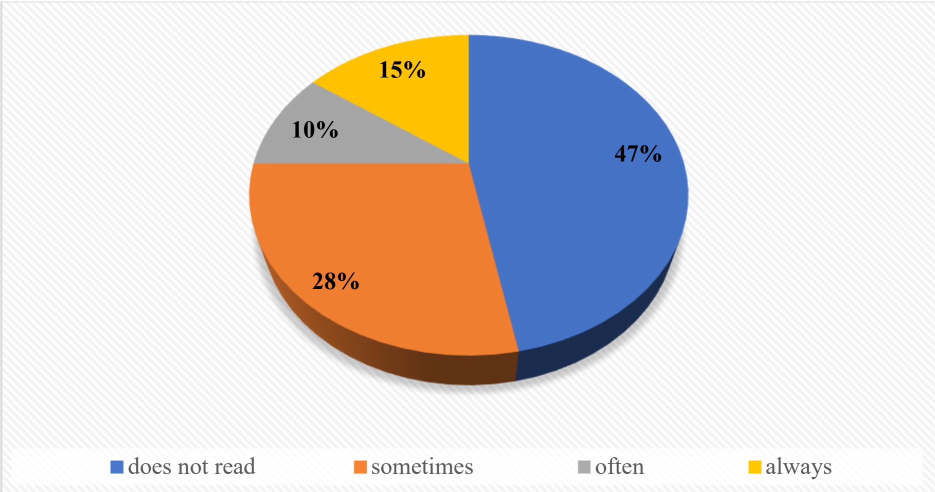

Figure 3. Frequency of label reading among consumers.

Students were also asked whether and how often they read the labels on energy drink packaging. Nearly half of the respondents (47%) admitted that they never read labels, while 25% reported reading them always or often, and 28% stated they do so occasionally (figure 3).

The majority of respondents (71%) claimed to know the active ingredients contained in energy drinks. Among those who reported such knowledge, caffeine and taurine were the most widely recognized active ingredients, identified by 98.9% and 86.5% of these respondents, respectively. More than half (57.3%) were also aware of the frequent presence of guarana. By contrast, only a small proportion of this group knew about the addition of L-carnitine and inositol in the energy drinks they consumed. Among all respondents, 29% admitted having no knowledge of any active ingredients in energy drinks. When asked about combining energy drinks with alcohol, most respondents (81%) denied such behavior; however, 19% admitted to having mixed energy drinks with alcohol at least once. Similarly, 16% of respondents reported having combined energy drinks with medications, while 84% stated they had never engaged in this practice. In response to the question about the safe daily dose of caffeine for a healthy adult, answers varied. Approximately 42% of respondents indicated they did not know the recommended daily limit. Of the remaining respondents, 49% believed the safe dose to be between 0 and 200 mg, while only 9% selected the option of 300–400 mg. Participants were also asked what factors influence the quantity of energy drinks they consume.

Table 2. Consumer preferences regarding alternatives to Energy drinks.

Responses	Frequency of responses
Coffee	90
Tea black\green	30\2
Yerba mate	22
Physical activity, sport	14
Balanced diet	10
I don’t know	12
Getting enough sleep	11
Water	10
Juices/fruits	9
Cola	5
Nutritional supplements: with caffeine,taurin,guarana\others	5\2
Isotonic drinks	4
Chocolate	4
Sugar in other forms	4
Nuts	3
Herbs	3

Analysis of responses showed that exam sessions were the most frequently cited factor (46%). Other commonly mentioned factors included various types of stress (16%), fatigue, sports activity, and sleep deprivation. A smaller group of respondents (5%) declared that their consumption was driven by curiosity or habitual use. The final question in the survey was open-ended, allowing respondents to express their opinions regarding possible alternatives to energy drink consumption (table 2). The most frequently suggested substitute was coffee. A total of 41 respondents recommended tea, with 75% specifying black tea and the remaining respondents preferring green tea. Yerba mate was also mentioned immediately after tea as a potential alternative. Some participants suggested adopting a healthier lifestyle characterized by increased physical activity, a balanced diet, and adequate sleep.

Other alternatives proposed included water, fruit juices, or consuming fresh fruits. Cola, chocolate or other sweets, isotonic beverages, and dietary supplements were also mentioned as replacements for energy drinks. The least frequently cited alternatives were nuts and herbal remedies.

4. Discussion

The results of the present study confirm that the prevalence of energy drink (ED) consumption among students is very high, with 61% of respondents reporting intake. Similar findings were reported by Chang et al. [2017], Douglas et al [2018] and Pavlović [2023] who observed consumption rates exceeding 50% in their respective studies. The observation that 28% of respondents declared regular consumption (at least once per week) aligns with results from other academic populations. For instance, Buxton and Hagan [2012] found that 26% of surveyed students consumed energy drinks at least once weekly. Such widespread use of EDs provides alarming data, particularly given that young people are in a highly sensitive stage of physical and psychological development. This could lead to numerous harmful consequences in the future. The frequency and motivations for ED consumption showed significant gender differences. The analysis of the relationship between gender and perceptions of the health impact of energy drinks ($p = 0.0008$) suggests greater health awareness among women compared to men. These findings are consistent with observations by Visram et al. [2016], who, in a qualitative study, found that women were more likely to perceive energy drinks as harmful and reported greater caution in their consumption. Similarly, Malinauskas et al. [2017] reported that women paid significantly more attention than men to product composition.

This trend may also explain findings in the present study, where 82.8% of women reported reading labels to verify ingredients, compared to only 19.7% of men. These results are in agreement with multiple analyses from various countries. For example, Borlu et al. [2019] and Martins et al. [2018] also found that energy drink consumption was higher among men than women. Particularly noteworthy is the finding that 47% of surveyed students never read energy drink labels, supporting conclusions from Pawlas et al. [2017], who indicated that a lack of interest in product composition is typical among young adults, especially males. Chang et al. [2017] similarly reported low levels of interest in the composition of energy drinks among Taiwanese university students, linking poor awareness of ingredients to an increased risk of excessive caffeine and sugar intake. Although 71% of respondents in the present study claimed to be aware of the active ingredients in energy drinks, only 9% correctly identified the recommended daily caffeine dose (300–400 mg). This highlights functional knowledge gaps, similar to those reported in other student populations [Costa-Valle et al. 2022, Kraak et al. 2020]. Regarding the declared side effects—such as palpitations reported by 27% of respondents and sleep disturbances by 15.5%—the results are consistent with observations by Seifert et al. [2011], who documented a high incidence of adverse effects following energy drink consumption, particularly among young individuals. Similar side effects were reported by Higgins et al. [2011], and Hammond et al. [2018] emphasizing that high doses of caffeine, especially in combination with other ingredients like taurine or guarana, may provoke negative cardiovascular responses. An interesting aspect of the study is the analysis of consumption motives, where the dominant reasons included stimulation for physical activity and reduction of sleepiness. This phenomenon has been extensively described in the literature. Slijvo et al. [2020], Degirmenci et al. [2018], and Mahoney et al. [2019] noted that young adults often perceive energy drinks as a convenient and accessible source of energy during periods of increased intellectual or physical effort. Equally alarming is the finding that 19% of respondents admitted to mixing energy drinks with alcohol—a practice recognized as particularly dangerous due to the risk of alcohol poisoning and the masking of its effects [Scalese et al. 2021]. The results concerning alternatives to energy drinks highlight the significant role of traditional caffeine-containing beverages, such as coffee and tea, as well as an emerging awareness of the importance of a healthy lifestyle. This includes reducing the consumption of highly caffeinated beverages in favor of natural energy sources and healthier habits. The findings of the study confirm gender-based differences in energy drink consumption patterns and underscore critical gaps in students' health literacy. It appears necessary to undertake educational initiatives targeted at young adults, with particular emphasis on the risks associated with combining energy drinks with alcohol and the importance of reading product labels as part of informed consumer decision-making.

5. Conclusions

The conducted study enabled a comprehensive assessment of university students' consumption habits related to energy drink intake, their health awareness, and their knowledge of the composition of these products. The results indicate that although a substantial proportion of respondents (approximately 40%) declared no consumption of energy drinks, as many as 28% of students reported regular use (at least once per week), while one-third consumed them occasionally. Such a high level of consumption in this age group is a cause for concern from a public health perspective. Significant gender-related differences were observed. Female students were more likely than male students to perceive the health impact of energy drinks as negative, showed greater interest in the product composition (82.8% of women vs. 19.7% of men), and less frequently combined energy drinks with alcohol or medications. In contrast, male students more often emphasized their usefulness in the context of physical activity (83.8% vs. 58.6%). These findings underscore the need for differentiated educational strategies tailored to the specific characteristics of individual groups. Another area of concern is the limited knowledge of respondents regarding the ingredients of energy drinks and the safe levels of caffeine consumption. More than 49% of participants incorrectly estimated the maximum permissible dose of caffeine, while 29% reported having no knowledge at all about the active ingredients of these products. Furthermore, 45.4% of students reported experiencing no side effects after consuming energy drinks, which may contribute to underestimating potential health risks. The findings of this study confirm the necessity of implementing educational initiatives among students to raise awareness of the potential hazards associated with excessive consumption of energy drinks, their combination with alcohol or pharmaceuticals, and to promote healthier alternatives such as coffee, tea, yerba mate, physical activity, or adequate sleep. Moreover, these results may serve as a foundation for further in-depth analyses in larger study samples, including other age and occupational groups, to identify broader determinants of energy drink consumption.

6. References

[1] Alsunni A.A.: Energy drink consumption: beneficial and adverse health effects. Int J Health Sci., 2015, 9(4), 468–474, available: https://pubmed.ncbi.nlm.nih.gov/26715927

[2] Borlu A., Oral B., Gunay O.: Consumption of energy drinks among turkish university students and its health hazards. Pak J Med Sci., 2019,35(2), 537–42, available: doi:10.12669/pjms.35.2.638

[3] Buxton C. and Hagan J. E.: A survey of energy drinks consumption practices among student-athletes in Ghana: lessons for developing health education intervention programmes. *Journal of the* Int. Soc. Sports Nutr., 2012, 9(1), 9, available: https://doi.org/10.1186/1550-2783-9-9

[4] Cadoni C. and Peana A.T.: Enargy drinks at adolescence avareness or unavarenes. Front Behav Neurosci., 2023, 17, 1080963, available: 10.3389/fnbeh.2023.1080963

[5] Chang Y. J., Peng C. Y., Lan Y. C.: Consumption of energy drinks among undergraduate students in taiwan: related factors and associations with substance use. Int. J Environ Res Public Health., 2017, 14(9), avalable: https://doi.org/10.3390/ijerph14090954

[6] Costa-Valle T., Gomes J.F., Rodrigues De Oliveira K., et al.: Energy drinks and alcohol in a binge drinking protocol in Wistar rats: Male and female behavioral and reproductive effects. Pharmacol Biochem Behav., 2022, 221, 173487, available: DOI:10.1016/j.pbb.2022.173487

[7] Degirmenci N, Fossum I.N, Strand T.A, et al.: Consumption of energy drinks among adolescents in Norway: a cross-sectional study. BMC Public Health., 2018,18:1391, available: https://doi.org/10.1186/s12889-018-6236-5

[8] Douglas K.E., Nkporbu A.K.: Energy drink consumption among medical and dental students at the University of Port Harcourt, Nigeria. Int. J. Med. Sci. Health Res., 2018, 2, 149–168, available: https://ijmshr.com/uploads/pdf/archivepdf/2020/IJMSHR_02_74.pdf

[9] Grandner M.A., KnutsonK.L., Troxel W., Hale L., Miller E.K.: Implications of sleep and energy drink use for health disparities. Nutr Rev*, 2014, 72(1), 14-22*, available: https://doi.org/10.1111/nure.12137

[10] Hałasiński P., Musielak M., Piotrowski I.: Energy drinks among young people: trends and pharmacological mechanisms of adverse effects on the cardiovascular system. Review of the literaturę, Folia Cardiol, 2022, 17(3), 163-169, available: DOI: 10.5603/FC.a2022.0013

[11] Hammond D., Reid J.L., Zukowski S.: Adverse effects of caffeinated energy drinks among youth and young adults in Canada: a Web-based survey. Can. Med. Assoc. J. Open, 2018, 6, 19–25, available: DOI:https://doi.org/10.9778/cmajo.20160154

[12] Higgins J. P., Tuttle T. D., Higgins C. L.: Energy beverages: content and safety. *Mayo Clinic Proceedings, 2011, 85*(11), 1033-1041, available: DOI:10.4065/mcp.2010.0381

[13] Holt E.: Poland bans energy drinks for under 18s. Lancet., 2023, 401(10376), 540, available: doi:10.1016/S0140-6736(23)00322-7

[14] Kraak V.I, Davy B.M., Rockwell M.S., Kostelnik S., Hedrick V.E.: Policy recommendations to address energy drink marketing and consumption by vulnerable populations in the United States. J Acad Nutr Diet., 2020, 120(5),767–77, available: doi:10.1016/j.jand.2020.01.013

[15] Mahoney C.R.; Giles G.E.; Marriott B.P.; Judelson D.A.; Glickman E.L.; Geiselman P.J.; Lieberman H.R.: Intake of caffeine from all sources and reasons for use by college students. Clin. Nutr., 2019, 38, 668–675, available: https://doi.org/10.1016/j.clnu.2018.04.004

[16] Malinauskas B. M., Aeby V. G., Overton R. F., Carpenter-Aeby T., Barber-Heidal K.: A survey of energy drink consumption patterns among college students. *Nutr J, 2017, 6*, 35: *available:* 10.1186/1475-2891-6-35

[17] Mansour B.; Amarah W.; Nasralla E.; Elias N.: Energy drinks in children and adolescents: demographic data and immediate effects. Eur. J. Pediatr., 2019, 178, 649–656, available: 10.1007/s00431-019-03342-7

[18] Martins A., Ferreira C., Sousa D., Costa S.: Consumption patterns of energy drinks in portuguese adolescents from a city in Northern Portugal. Acta Med Port, 2018, 31(4), 207-212, available: https://doi.org/10.20344/amp.9403

[19] Pavlović N., Miskulin I., Jokić S., Kovacevic J., Miskulin M.: Consumption of energy drinks among university students in Eastern Croatia. *Appl. Sci.* 2023, *13*(2), 1124, available: https://doi.org/10.3390/app13021124

[20] Pawlas K., Hołojda P., Brust K.: Ocena spożycia napojów energetycznych oraz ich wpływu na zdrowie człowieka na podstawie badania wśród studentów wrocławskich uczelni. Med Srod., 2017, 20(2), 39–45, available: DOi: 10.19243/2017205

[21] Puupponen M., Tynjälä J., Tolvanen A., Välimaa R., Paakkari L.: Energy drink consumption among finnish adolescents: prevalence, associated background factors, individual resources, and family factors. Int J Public Health., 2021, 66, 620268, available:https://doi.org/10.3389/ijph.2021.620268

[22] Puupponen M., Tynjälä J., Tolvanen A., Välimaa R., Paakkari L.: Associations between adolescents' energy drink consumption frequency and several negative health indicators. BMC Public Health., 2023, 23, 258, availaible: 10.1186/s12889-023-15055-6

[23] Reissig C.J., Strain E.C., Griffith R.R.: Caffeinated energy drinks--a growing problem. Drug Alcohol Depend, 2009, 99(1-3), 1-10, available: doi:10.1016/j.drugalcdep.2008.08.001

[24] Sankararaman S., Syed W., Medici V., Sferra T.J.: Impact of energy drinks on health and well-being. Curr. Nutr. Rep. 2018, 7, 121–130, available: 10.1007/s13668-018-0231-4

[25] Scalese M., Cerrai S., Biagioni S., Benedetti E., Bastiani L., Potente R., Cutilli A., Molinaro S.: Trends in energy drink and combined alcohol and energy drinks consumption among Italian High School Students, 2008–2019. Drug Alcohol Depend. 2021, 228, 10906, available: https://doi.org/10.1016/j.drugalcdep.2021.109061

[26] Seifert S. M., Schaechter J. L., Hershorin E. R., Lipshultz S. E.: Health effects of energy drinks on children, adolescents, and young adults. *Pediatrics, 2011, 127*(3), 511–528, available: doi: 10.1542/peds.2009-3592

[27] Šljivo A., Kulo A., Mrdović L., Muhić A., Dujić T., Kusturica, J.: Patterns of energy drinks consumption in leisure, sports and academic activities among a group of students attending University of Sarajevo, Bosnia and Herzegovina. Ann Ig, 2020, 32(2), 141-156, available: https://doi.org/10.7416/ai.2020.2338

[28] Visram S., CheethamM., Riby D. M., Crossley S. J., Lake A.: Consumption of energy drinks by children and young people: a rapid review examining evidence of physical effects and consumer attitudes. *BMJ Open, 2016, 6*(10), e010380, available: 10.1136/bmjopen-2015-010380

Green solvent systems for extraction of bioactives from common thyme (*Thymus vulgaris* L.) in sustainable food production

Radosław Kowalski[1*], Grażyna Kowalska[2],
Marzena Włodarczyk-Stasiak[1], Artur Mazurek[1]

[1]*Department of Analysis and Evaluation of Food Quality,
University of Life Sciences in Lublin,
8 Skromna Street, 20-704 Lublin, Poland.*
[2]*Department of Tourism and Recreation,
University of Life Sciences in Lublin,
15 Akademicka Street, 20-950 Lublin, Poland*
[*]*corresponding author: radoslaw.kowalski@up.lublin.pl*

Abstract

The aim of the study was the qualitative evaluation of low-processed (LP) extracts of *T. vulgaris* obtained with "green" solvents as alternative products to commercial dense extracts (DER 4:1) from the perspective of reducing production costs and environmental footprint. Seven batches of dried thyme (T1–T7) were verified for total polyphenol content (TPC), total flavonoid content (TFC) and essential-oil content (EOC) in order to select the sample with optimal chemical composition. The selected herb (T1, TPC = 124 mg · g⁻¹, TFC = 100 mg · g⁻¹, 1.88 % v/w) was then extracted (2 g · 100 mL⁻¹, 10 min) with water, nanowater, 65 % glycerol–water and 50 % ethanol–water over a temperature range adapted to the properties of the solvent systems (25 °C, 50 °C, 80 °C, 100 °C). Yields were referenced to an exhaustive 70 % methanol extract and compared with two dense market extracts. The highest recoveries for LP extracts were obtained for water at 100 °C – 38.6 % (TPC) and 33.2 % (TFC). In contrast, dense extracts reached maximal TPC and TFC recoveries of 12.8 % and 11.6 %, respectively, despite approximately 66-fold higher TPC and about 70-fold higher TFC concentrations compared with classical thyme infusions, indicating considerable losses of the plant's phytochemical potential during concentration. The results show that the traditional hot-water infusion enables recovery of up to one-third of the bioactive compounds (TPC and TFC) present in thyme herb without the costly evaporation and complex purification steps employed in the production of dense extracts. LP extracts therefore represent a cost-effective, more environmentally friendly ingredient for functional foods and can replace or complement dense extracts, especially in products oriented towards sustainable development.

Keywords: thyme herb, polyphenols, flavonoids, low-processed plant extracts

1. Introduction

Common thyme (*Thymus vulgaris* L.) of the Lamiaceae family has enjoyed an enduring reputation for centuries, both as a culinary spice and as a medicinal raw material [Najar et al. 2021]. The plant's popularity stems primarily from its exceptionally rich phytochemical profile. The herb contains more than 2 % essential oil dominated by thymol, carvacrol, p-cymene, γ-terpinene, linalool, β-myrcene and terpinen-4-ol [Najar et al. 2021; Niksic et al. 2021], along with numerous phenolic metabolites—rosmarinic, chlorogenic and caffeic acids, a wide range of flavonoids (luteolin and apigenin glycosides) and triterpenes such as ursolic and oleanolic acids [Roby et al. 2013; Shimada et al. 2022]. Such chemical diversity translates into a broad spectrum of biological activity, ranging from antibacterial and antifungal properties through antioxidant and anti-inflammatory effects to expectorant and spasmolytic actions. This is reflected in the growing interest in using thyme and its extracts as natural preservatives and antioxidants in food technology [Waheed et al. 2024]. Rising consumer expectations for "clean-label" products and worldwide efforts to lower chemical inputs are prompting the food industry to seek gentle, environmentally friendly methods for obtaining plant ingredients [Khalid et al. 2024]. Traditionally used organic solvents can be effective, but they are associated with high purification costs and the risk of solvent residues. Hence, increasing attention is being directed toward low-processed extracts obtained by minimal-processing methods. Such extracts—produced via infusions, hydro-alcoholic macerates or extraction with water whose physicochemical properties have been modified—preserve the natural proportions of metabolites and do not require energy-intensive purification steps. The literature highlights the particular potential of "green" solvents [Kowalska et al. 2021b; Kowalska et al. 2021c], including water, nanowater, glycerol–water mixtures and ethanol–water mixtures at concentrations permitted by food legislation. Thyme phenolics and volatiles dissolve readily in these media, which are themselves biodegradable, low-toxic and suitable for direct use in food products.

To date, most research on *T. vulgaris* has focused on intensive extraction techniques—ultrasound-assisted, microwave-assisted or supercritical CO_2 extraction [Gavrila et al. 2023]. Although these methods deliver high concentrations of active compounds, they are not always compatible with industrial settings oriented toward low energy consumption and operational simplicity. Studies comparing simple, energy-saving infusion procedures with commercially available highly processed extracts—especially in terms of metabolite-recovery efficiency using food-grade solvents—are lacking. The present study offers a comprehensive evaluation of the chemical composition of low-processed extracts from thyme herb obtained using water, nanowater, a 65 % glycerol–water solution and 50 % v/v ethanol. Preliminary screening of seven raw-material batches determined total polyphenol (TPC) and total flavonoid (TFC) contents as well as the main essential-oil constituents, thymol and p-cymene; the batch with the most advantageous bioactive profile was selected. This material was extracted at three temperatures (50, 80 and 100 °C), enabling assessment of how thermal parameters influence extraction yield. The resulting extracts were compared with two commercially available concentrated extracts with a DER of 4 : 1, regarded as representatives of the highly processed category.

This experimental design allows verification of the extent to which classical water infusion and its modifications can compete with intensive technologies while adhering to the paradigm of sustainable functional-food production. The findings provide arguments for wider application of thyme as a natural additive possessing antioxidant and preservative properties and demonstrate the potential of "green" solvents for simple, economical extraction of bioactive compounds. This study evaluates the efficiency of green solvent systems (water, nanowater, glycerol–water, ethanol–water) in recovering key bioactives from T. *vulgaris*, compared to dense DER 4:1 extracts.

2. Materials and Methods

2.1. Plant Material And Reference Extracts

The study was carried out on seven batches of commercially available common thyme (*Thymus vulgaris* L.) herb in the form of cut dried drug, labelled T1–T7 (Table 1). Two viscous commercial extracts with a declared drug-extract ratio (DER) of 4 : 1, coded ET1 and ET2 and standardised to 6 % total polyphenols (TPC), served as reference samples. All materials were stored in a dry, shaded environment (22 ± 2 °C, relative humidity < 50 %). The plant material was further standardised by pooling and milling to obtain a homogeneous fraction, after which the moisture content of each product was determined for conversion to dry matter using a WPS 50 SX moisture analyser (RADWAG, Radom, Poland).

Table 1. Characteristics of plant materials and extracts.

Research material / description	Supplier (year)	Sample code
Thyme herb		
Common thyme herb, cut dried	Dary Natury (2020)	T1
Common thyme herb, cut dried	Krautex (2020)	T2
Common thyme herb, cut dried	Krautex (2020)	T3
Common thyme herb, cut dried	Greenvit Sp. z o.o. (2020)	T4
Common thyme herb, cut dried	Greenvit Sp. z o.o. (2020)	T5
Common thyme herb, cut dried	Thympol (2020)	T6
Common thyme herb, cut dried	Greenvit Sp. z o.o. (2020)	T7
Thick thyme extracts		
*Thick thyme extract, DER 4 : 1 (standardised to 6 % total polyphenols)	Greenvit Sp. z o.o. (2020)	ET1
*Thick thyme extract, DER 4 : 1 (standardised to 6 % total polyphenols)	Greenvit Sp. z o.o. (2020)	ET2

*The thick thyme extract was produced using a confidential in-house manufacturing protocol. The extraction was carried out at 40–70 °C over 4–8 hours.

2.2. Experimental design

The study was carried out in two stages. Preliminary screening. Total polyphenol content (TPC), total flavonoid content (TFC), essential-oil yield and the thymol/*p*-cymene ratio were determined in the seven thyme batches T1–T7. On the basis of these results, the sample displaying the most favourable bioactive profile (T1) was selected for further work.Main experiment. Sample T1 was extracted using four green solvent systems (Table 2): distilled water, nanowater, a 65 % (v/v) glycerol–water mixture and a 50 % (v/v) ethanol–water mixture. Each system was tested at three temperatures (50, 80 and 100 °C), except for the ethanol–water mixture, for which—owing to the boiling point of the solvent—initial temperatures of 25 °C and 50 °C were applied. The low-processed extracts obtained were compared with the reference extracts ET1 and ET2 with respect to total polyphenol and total flavonoid contents.

Table 2. Extraction systems employed in the experiment.

Extraction system / temperature	Composition of the extraction medium (%)		
	water	glycerol	ethanol
Distilled water 50°C, 80°C, 100°C	100	0	0
Nanowater* 50°C, 80°C, 100°C	100	0	0
Glycerol**–water mixture 65 % v/v 50°C, 80°C, 100°C	35	65	0
Ethanol–water mixture 50 % v/v 25°C, 50°C	50	0	50

*Plasma-activated water with an elevated singlet-oxygen content (Nantes Medical, Bolesławiec).

**Plant-derived glycerol Ph. Eur. 99.5 % (TechlandLab, Tarnobrzeg)

2.3. Extraction

Thyme-herb extraction was carried out according to the procedure described previously [Kowalska et al. 2021b]. Precisely weighed portions (2.0 g) of thyme herb were placed in 250 mL Erlenmeyer flasks, after which 100 mL of the appropriate extraction solvent—prepared as specified in Table 2—were added. The extraction was performed in a thermostatically controlled shaker for 10 min, maintaining the temperature at the preset level. Aliquots of 50 mL were withdrawn, filtered through medium-porosity filter paper, and the filtrate was used for further analyses. Each extract was prepared in triplicate.

In parallel, an exhaustive extract was prepared: 2 g of plant material were refluxed with 100 mL of 70 % (v/v) methanol–water for 4 h; the extract was decanted and the procedure was repeated twice on the residue. The combined methanolic fractions were brought to 100 mL. This extract was regarded as the reference extract (100 % recovery of constituents).

The extraction yield of the test systems (W_1) was calculated with Eq. (1), whereas the theoretical yield of the commercial DER 4 : 1 extracts (W_2) was calculated with Eq. (2). The extraction yield (recovery W_1) for polyphenols and flavonoids was expressed as the percentage share of bioactive compounds in the test extract relative to their content in the methanol–water reference extract. The 70 % methanol–water mixture was treated as the reference solvent, assumed to afford optimal extraction of active substances; therefore the concentration obtained in the reference extract was taken as 100 % yield, and the yield achieved with each solvent system applied in this study was calculated from Eq. (1):

$$W_1[\%] = \frac{C_{Sol} * 100\%}{2 * C_{Met}} \qquad (1)$$

where:

C_{Met} – content of the constituent in thyme herb [mg · g⁻¹] obtained with exhaustive extraction using 70 % methanol (reference solvent)

C_{Sol} – concentration of the constituent [mg GA · L⁻¹] in the extract obtained with the selected extraction system

In addition, the theoretical extraction yield (recovery W_2) was calculated for the viscous commercial extracts (DER 4 : 1). A DER of 4 : 1 denotes that 1 kg of extract is produced from 4 kg of thyme herb. It was assumed that the total polyphenol and total flavonoid contents in the raw material used for extract manufacture were identical to those in the experimental herb (T1). It was an important assumption to relate the experimental extract results to those of the commercial extract. The phytochemical parameters of the selected raw material did not deviate from the ranges reported in the literature

The theoretical yield for the commercial extracts was calculated according to Eq. (2):

$$W_2[\%] = \frac{C_{DER} * 100\%}{400 * C_{Met}} \quad (2)$$

where:

C_{Met} – content of the constituent in thyme herb [mg · g⁻¹] obtained with exhaustive extraction using 70 % methanol (reference solvent)

C_{DER} – concentration of the constituent [mg · L⁻¹] in the viscous commercial extract (DER 4:1)

2.4. Evaluation of Total Polyphenols, Flavonoids and Essential Oil Content

Total polyphenols (TPC) in thyme herb and in the extracts were determined spectrophotometrically with the Folin–Ciocalteu reagent (λ = 725 nm) according to the method described previously [Singleton and Rossi 1965; Kowalska et al. 2021b]; the results are expressed as mg of gallic acid (GA). Total flavonoids (TFC) were assayed spectrophotometrically by the aluminium-chloride complexation method (λ = 425 nm) following the same authors' protocol [Polish Pharmacopoeia VIII; Kowalska et al. 2021b]; the results were recalculated as mg of quercetin (QE). Additionally, TPC and TFC values obtained for the extracts were recalculated per gram of dry plant material.

The essential-oil content (EO) was determined by steam distillation for 3 h and expressed as % v/w per 100 g of raw material, in accordance with the procedure reported earlier [Kowalska et al. 2021b]. All analyses were performed in triplicate.

The volatile profile—expressed as the percentage shares of thymol and *p*-cymene—was analysed by GC–MS (ITMS Varian 4000 GC-MS/MS, Varian, USA) and GC-FID (Varian 3800 GC, Varian, USA) under the chromatographic conditions described previously [Kowalska et al. 2021a].

GC–MS: CP-8410 auto-injector; VF-5 ms column, 30 m × 0.25 mm i.d., 0.25 µm film (Varian, USA); carrier gas He at 0.5 mL min^{-1}; injector, ion-source and interface at 250 °C, 200 °C and 250 °C, respectively; split 1 : 50; injection 1 µL; temperature programme 50 °C (1 min) → +4 °C min^{-1} → 250 °C, hold 10 min; ionisation 70 eV; mass range 40–870 Da; scan time 0.80 s.

GC-FID: CP-8410 auto-injector; DB-5 column, 30 m × 0.25 mm, 0.25 µm film (J&W

Scientific, USA); carrier gas He at 0.5 mL min^{-1}; injector and detector 260 °C; split 1 : 100; injection 5 μL; temperature programme 50 °C (1 min) → +4 °C min^{-1} → 250 °C, hold 10 min.

Qualitative identification was based on MS spectra [Kowalska et al. 2021a] matched against the NIST/EPA/NIH library and literature data; compound identities were confirmed by retention indices taken from the literature and by comparison with our own standards. Quantitative analysis was carried out by the internal-standard method with *n*-alkanes C12 and C19 following the procedures of Kowalska et al. [2021a].

2.5. Statistical analysis

Data were analyzed using SAS version 9.4 (SAS Institute, Cary, NC, USA). One-way analysis of variance (ANOVA) was performed to test for differences between means, and Duncan's multiple-range test was applied at $p \leq 0.05$ to separate homogeneous groups. In addition, Pearson's correlation coefficients (r) were calculated on the mean values of each dataset using the PROC CORR procedure to evaluate the strength and direction of associations among all measured variables. Significance was accepted at $p \leq 0.05$, with $|r| > 0.8$ interpreted as strong, 0.5–0.8 as moderate, and < 0.5 as weak correlation.

3. Results and Discussion

Within the seven thyme batches (Fig. 1) a marked quantitative variability in secondary metabolites was observed. The mean total polyphenol content (TPC) was 126 mg GA · g^{-1}; this value falls within the 42–241 mg GA · g^{-1} range reported in the literature [Koşar et al. 2005; Roby et al. 2013; Spiridon et al. 2011; Ulewicz-Magulska and Wesołowski 2019]. The T6 sample, richest in TPC, contained 164 mg GA · g^{-1}, whereas T4 contained only 112 mg GA · g^{-1}. Flavonoid profiles proved even more diversified, from 57 mg QE · g^{-1} (T3) to 100 mg QE · g^{-1} (T1), with a mean of 73 mg QE · g^{-1}. All values exceeded the 4–37 mg g^{-1} range reported for Central-European materials [Kameníková et al. 2015; Tohidi et al. 2017; Vábková and Neugebauerová 2013], probably owing to the phenological stage of the herb and favourable growing conditions.

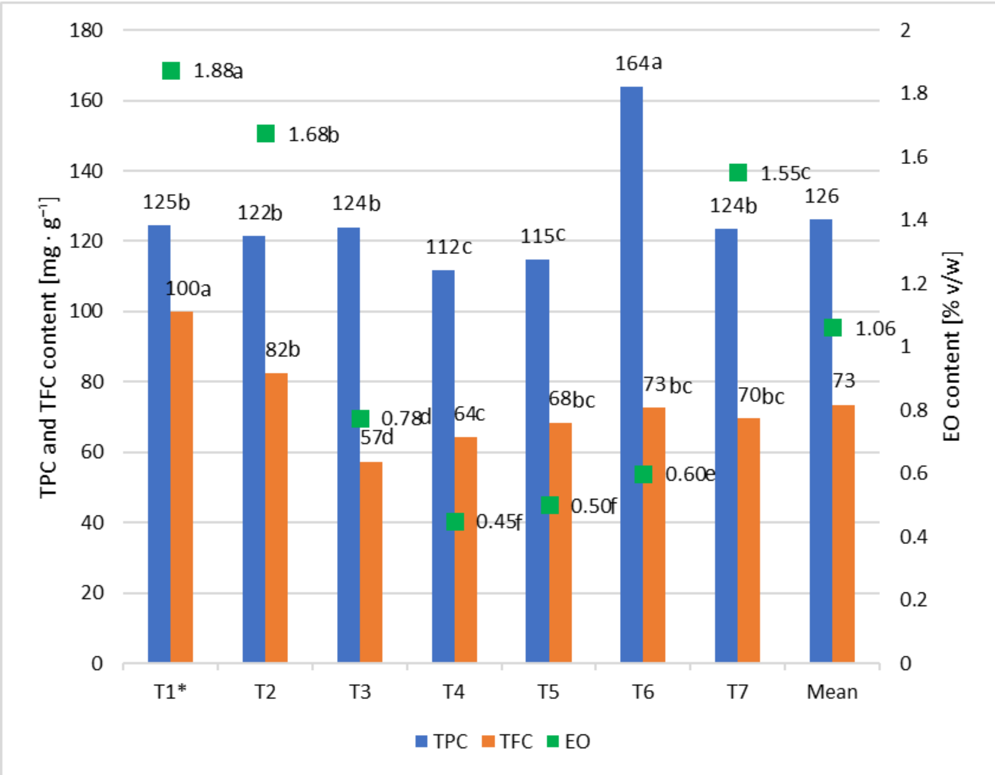

Figure 1. Total polyphenol content, total flavonoid content and essential-oil yield in thyme herb.
a, b, c… - values marked with the same letter are not significantly different ($p > 0.05$).

Essential-oil content varied from 0.45 to 1.88 % v/w (mean 1.06 % v/w), i.e. within the classical range [Najar et al. 2021; Niksic et al. 2021]. GC–MS profiling (Fig. 2) confirmed a thymol chemotype: thymol exceeded 50 % in every sample, but its share decreased as p-cymene (the biosynthetic precursor of thymol) increased, corroborating earlier data [Sárosi et al. 2013]. The share of p-cymene correlated negatively with thymol ($r = -0.88$). The highest thymol proportion (84.8 %) was noted in T4, whereas the highest p-cymene level (21.5 %) occurred in T6. Such variation suggests that different factors may shift the monoterpene pathway equilibrium towards intermediate or final metabolites. Ahmad et al. [2014] likewise found thymol to be the major component of thyme oil (60.18 %), followed by p-cymene (15.44 %), values close to those obtained here. Król and Kiełtyka-Dadasiewicz [2015] studied the effect of drying on oil composition, reporting 59.5 % thymol and 11.24 % p-cymene in fresh herb, versus 50.8 % and 21.45 %, respectively, in dried material. Statistical analysis showed that Pearson's correlation coefficients (r) ranged from –0.726 ($p = 0.065$) for p-cymene vs. thymol—indicating a strong inverse trend—to 0.753 ($p = 0.051$) for total flavonoids (TFC) vs. oil yield (EO). This confirms that as p-cymene increases, thymol proportion falls (reflecting distinct chemotypes), and that higher flavonoid levels are associated with greater oil yield, albeit at the borderline of statistical significance.

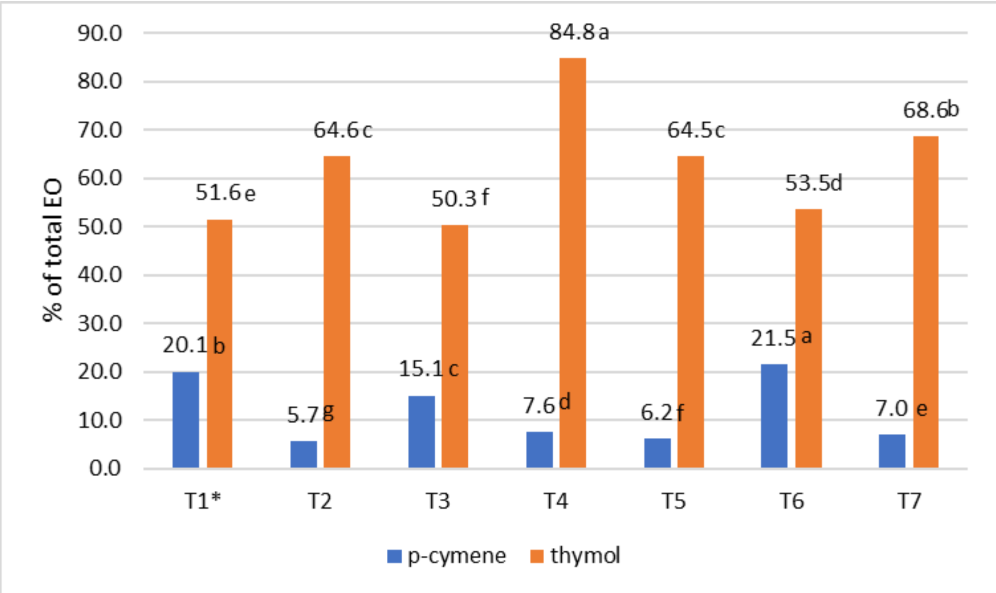

Figure 2. Percentage shares of p-cymene and thymol in essential oil (EO) isolated from the thyme-herb samples.

a, b, c… - values marked with the same letter are not significantly different ($p > 0.05$)

Comparison of parameters identified T1 as the reference raw material: it contained the highest levels of flavonoids and essential oil, while its polyphenol level was close to the median, thus ensuring a balance between phenolic and volatile fractions important for functional formulations.

Sample T1 was extracted with four green solvent systems (distilled water, nanowater, 65 % glycerol–water, 50 % ethanol–water) at three temperatures (50, 80, 100 °C; for ethanol 25 and 50 °C).

The TPC values in the resulting extracts (Table 5) increased significantly with temperature in the aqueous and glycerol systems, reaching a maximum of 962 mg GA · L^{-1} in the 100 °C water infusion. This value is below the 2000 mg GA · L^{-1} recorded by Kulišić et al. [2006], who extracted 15 g plant material with 150 mL water for 30 min followed by concentration. Nevertheless, the TPC values obtained here exceeded by several-fold those reported by Sonmezdag et al. [2018], confirming the strong influence of contact time and plant-to-solvent ratio. In the study of Sonmezdag et al. [2018] thyme infusions prepared with 3 g herb in 200 mL water for 1–5 min contained 9.50–141.36 mg GA · L^{-1}. These figures are far lower than those achieved in the present work for water extraction (394 mg GA · L^{-1} at 50 °C and 962 mg GA · L^{-1} at 100 °C) and nanowater extraction (346 mg GA · L^{-1} at 50 °C and 475 mg GA · L^{-1} at 100 °C). Nanowater yielded results 10–20 % lower than distilled water.

Statistical analysis showed that Pearson's correlation coefficients (r) for temperature–TPC relationships were exceptionally high: $r = 1.000$ ($p < 0.01$) in nanowater and $r = 0.996$ ($p \approx 0.06$) in water, while TFC exhibited similarly high but non-significant correlations with temperature in water ($r = 0.993$; $p \approx 0.07$). All other pairwise correlations were non-significant ($p > 0.05$). This confirms that increasing the extraction temperature is the primary driver of polyphenol and flavonoid release in aqueous systems, with nanowater showing a perfectly linear response and water a nearly linear one.

Table 5. Concentrations of total polyphenols (TPC) and total flavonoids (TFC) in thyme extracts obtained with different green solvent systems at various temperatures.

Extraction temperature	Concentration				
	TPC (mg GA · L^{-1})				
	Water	Glycerol–water	Nanowater	Ethanol–water	Exhaustive extraction
20 °C				175i	2490
50 °C	394e	32k	346f	241h	
80 °C	782b	69j	425d		
100 °C	962a	298g	475c		
	TFC (mg QE · L^{-1})				
	Water	Glycerol–water	Nanowater	Ethanol–water	Exhaustive extraction
20 °C				431cd	1999
50 °C	407de	360e	360e	461c	
80 °C	536b	387e	506b		
100 °C	665a	386e	485c		

a, b, c… - values marked with the same letter are not significantly different ($p > 0.05$)

It should be emphasised that the “commercial” nanowater purchased for the study has a mineralisation level comparable with that of tap water, and it can therefore be assumed that the presence of cations contributed to the changes observed in the series arranged in descending concentrations of the target bio-active compounds. Samsonowicz and Regulska [2016] demonstrated that the presence of, inter alia, calcium and magnesium ions modifies the spectrophotometrically determined concentration of polyphenolic compounds. Metal cations can form complexes with phenolics or activate specific functional groups, which in turn may affect both the measured concentrations of these constituents and the antioxidant activity of the extracts [Samsonowicz and Regulska 2016]. Similar relationships were described by Wyrostek and Kowalski [2021].

Nanowater is produced in a low-temperature plasma reactor in which water cluster structures are disrupted; the resulting structural modification imparts specific physicochemical properties, including the ability to enhance plant uptake of soil nutrients [Murawski et al. 2015]. The available literature contains very little information on the use of nanowater for extracting bio-active compounds from herbs. The evidence published to date suggests that nanowater could markedly influence the isolation of such constituents; however, the results obtained in the present work indicate that, as an extraction medium, nanowater does not outperform ordinary water and in fact shows a lower extraction efficiency in this case.

The glycerol–water mixture at 50 °C produced the lowest TPC (32 mg GA · L^{-1}), but increasing the extraction temperature to 100 °C raised the concentration almost ten-fold, a trend consistent with earlier data for glycerol–water extracts from peppermint and nettle leaves [Kowalska et al. 2021b]. This behaviour is linked to the high viscosity of glycerol, which at lower temperatures limits phenolic diffusion; an analogous phenomenon was reported by Manousaki et al. [2016] for waste plant materials. Although only a few publications deal with the use of glycerol for extracting biologically active substances, interest in this topic is growing, and the first studies on extraction kinetics with glycerol–water systems—especially from the perspective of green chemistry and the valorisation of plant processing waste—are now appearing [Manousaki et al. 2016].

Extraction with 50 % ethanol yielded 241 mg GA · L^{-1} at 50 °C, confirming the universal ability of this solvent system to dissolve both medium-polar phenolics and the lipophilic terpenoid fraction. Depending on the chemical nature of the target compounds, the extractant is selected to ensure optimum yield while also meeting other criteria, e.g. economic (cost) or legal (safety, environmental protection). Alcoholic solvents are widely used to extract phenolics from natural sources, providing high total-extract yields even though they are not highly selective for phenols. In particular, alcohol–water mixtures have proved more efficient for phenolic extraction than the corresponding single-component solvents [Pinelo et al. 2005 a; Pinelo et al. 2005 b; Yilmaz and Toledo 2006]. Spigno et al. [2007] showed that adding water to ethanol improved extraction yield; however, an excessively high water proportion increased the co-extraction of other compounds and subsequently reduced polyphenol concentrations in the extracts. Using an alcohol–water mixture offers the advantage of modulating solvent polarity, and the solubility of polyphenols depends chiefly on the presence of hydroxyl groups, molecular size and the length of the hydrocarbon chain.

The same solvent–temperature relationships observed for TPC were also found for TFC. The highest total flavonoid concentrations were detected in aqueous extracts (water and nanowater), ranging from 360 mg QE · L^{-1} (nanowater, 50°C) to 665 mg QE · L^{-1} (water, 100°C). Glycerol–water and ethanol–water extracts contained lower flavonoid levels, which depended on the extraction temperature; increasing solvent temperature led to higher flavonoid concentrations in the extracts.

On the basis of the TPC values in thyme herb T1, the theoretical concentration of total polyphenols in the extracts, assuming 100 % extraction efficiency, was calculated at 2490 mg GA · L^{-1}. The corresponding theoretical concentration for total flavonoids was 1999 mg QE · L^{-1}. The extraction yield for polyphenols (Table 6) in the 100 °C water infusion was 38.6 %, while that for flavonoids reached 33.2 % (Table 6). Although these values do not exceed 50 %, the process is attractive to the clean-label sector owing to its simplicity and zero use of organic solvents. Economic aspects—such as the low cost of brewing—and the minimal environmental impact further enhance its practical value. Nanowater, despite its modified cluster structure, did not improve yield; the negative effect can be explained by the mineralisation of the plasma medium [Wyrostek and Kowalski 2021]. In the glycerol system, the increased yield at 100 °C confirms that viscosity is the limiting factor; once viscosity is reduced, glycerol matches water in performance and, thanks to its multiple hydroxyl groups, further stabilises phenolic molecules [Huamán-Castilla et al. 2020]. Experience indicates a correlation between polyphenol and flavonoid concentrations and increasing extraction temperature. Authors relate the positive temperature effect to enhanced solubility of phenolic compounds in the solvent system and to intensified diffusion of phenolics from the plant cells into the solvent phase [Antony and Farid 2022; Zheng et al. 2024].

Table 6. Extraction yield (%) of polyphenols (TPC) and flavonoids (TFC) obtained with green solvent systems at various temperatures.

Extraction yield (%)				
Extraction temperature	TPC			
	Water	Glycerol–water	Nanowater	Ethanol–water
20 °C				7.0i
50 °C	15.8e	1.3k	13.9f	9.7h
80 °C	31.4b	2.8j	17.1d	
100 °C	38.6a	12.0g	19.1c	
	TFC			
	Water	Glycerol–water	Nanowater	Ethanol–water
20 °C				21.5cd
50 °C	20.3de	18.0e	18.0e	23.0c
80 °C	26.8b	19.4e	25.3b	
100 °C	33.2a	19.3e	24.2c	

a, b, c… - values marked with the same letter are not significantly different ($p > 0.05$)

The study showed that the total polyphenol concentration in the viscous thyme extracts (DER 4 : 1) amounted to 63 774 mg GA · L^{-1} and 63 941 mg GA · L^{-1}, respectively, with a calculated extraction yield of about 12.8 % (Table 7). In turn, the total flavonoid concentration in these viscous extracts reached 46 388 mg QE · L^{-1} and 23 639 mg QE · L^{-1}, corresponding to extraction yields of approximately 11.6 % and 5.9 %. Consequently, compared with the infusions, the commercial viscous extracts contain roughly 66-fold higher total-polyphenol concentrations and about 70-fold higher total-flavonoid concentrations. Achieving such high levels of active compounds in the commercial extracts results from the fact that producing 1 kg of a DER 4 : 1 extract consumes 4 kg of plant material, followed by costly volume reduction by evaporation. From a life-cycle-assessment (LCA) perspective, water infusions—which require only a 10-min heating step and generate no solvent effluents—exhibit a far smaller carbon footprint. Although concentrated herbal extracts are valuable semi-products for multiple industrial sectors, it should be emphasised that their extraction yields are markedly lower than those obtained for low-processed extracts, which has implications for overall economic balance, natural-resource use and environmental impact.

Heating 1 L of water for an infusion needs only ≈ 0.12 kWh (0.43 MJ) of electricity when an 80 %-efficient induction kettle is used (Vik 2022). At the current average EU grid intensity (≈ 255 g CO_2 kWh^{-1}), that equates to ≈ 30 g CO_2 e L^{-1} (average EU mix 2024) [Ember 2024], perfectly in line with the 21 g CO_2 e reported for a black-tea cup brewed with just the required water (Berners-Lee and Clark 2010). By contrast, concentrating commercial thyme extracts to DER 4 : 1 requires evaporating roughly 3 kg of water per kg of extract. Even an energy-optimised two-effect falling-film evaporator still consumes ≈ 0.32 kg steam kg^{-1} water, i.e. ≈ 0.72 MJ (0.20 kWh); the overall energy demand therefore reaches ≈ 2.4 MJ kg^{-1} extract, corresponding to ≈ 0.17 kg CO_2 e kg^{-1} (Tetra Pak 2024).

Laboratory LCAs confirm the steep rise in impacts when more intensive techniques are employed. For spruce-bark polyphenols, global-warming potential per gram of extract jumps from 8 kg CO_2 e (ultrasound assisted) to ≈ 21 kg (Soxhlet) and 44 kg (NaOH solid–liquid extraction) (Barjoveanu et al. 2020). Essential-oil studies tell a similar story: producing 1 g rosemary oil via hydro-distillation requires 166 MJ and emits 8 kg CO_2 e, whereas supercritical-CO_2 extraction demands 290 MJ and 15 kg CO_2 e for the same functional unit (Moura et al. 2022). Against these benchmarks, the 10-min thyme infusion developed in this work—yielding 38.6 % of total polyphenols and 33.2 % of flavonoids—offers a carbon footprint two orders of magnitude lower while fully eliminating volatile-organic-solvent residues.

These quantitative comparisons substantiate the claim that low-processed water (or water–glycerol) extracts satisfy "clean-label" expectations with minimal energy input and the smallest life-cycle impacts currently documented for thyme-derived ingredients.

Table 7. Concentration of total polyphenols (TPC) and total flavonoids (TFC) in commercial thick thyme extracts (DER 4 : 1) and extraction yield.

Thick thyme extract	TPC		TFC	
	Concentration (mg GA · L^{-1})	Extraction yield (%)	Concentration (mg QE · L^{-1})	Extraction yield (%)
ET1	63774a	12.8a	46388a	11.6a
ET2	63941a	12.8a	23639b	5.9b

a, b, - values marked with the same letter are not significantly different ($p > 0.05$)

Summarising the results obtained, it can be stated that simple, classical, low-processed thyme extracts—infusions—are products rich in polyphenols and flavonoids. The manufacture of such simple extracts is characterised by moderate extraction yields (30–39 %) at incomparably lower energy costs and with no use of solvents classified as volatile organic compounds. Nanowater did not surpass distilled water in extraction performance, which suggests that further modification (e.g. de-mineralisation) is required before its practical application. The glycerol–water mixture demands higher temperatures but may be a valuable alternative in products for which ethanol is undesirable and liquid viscosity is not a technological limitation (e.g. sauces, herbal syrups). *Thymus vulgaris* therefore remains a valuable source of natural antioxidants, and straightforward, low-processed water extractions (or water with added glycerol) can effectively compete with DER extracts in ecological and economic terms while providing a product consistent with the "clean-label" market demand and the strategy of sustainable foods.

4. Conclusions

Green-solvent infusions (water, nanowater, glycerol–water) recovered up to 39 % of TPC and 33 % of TFC in a 10-min protocol, eliminating the need for organic solvents. These low-processed extracts offer a viable clean-label ingredient for sustainable food formulations.

5. References

[1] Ahmad A., Van Vuuren S., Viljoen A.: Unravelling the complex antimicrobial interactions of essential oils — the case of Thymus vulgaris (thyme). Molecules, 2014, 3 (19), 2896–2910.

[2] Antony A., Farid M.: Effect of temperatures on polyphenols during extraction. Appl. Sci., 2022, 4 (12), 2107.

[3] Barjoveanu G., Pătrăuțanu O.-A., Teodosiu C., Volf I.: Life cycle assessment of polyphenols extraction processes from waste biomass. Sci. Rep., 2020, (10), 13632.

[4] Berners-Lee M., Clark D.: What's the carbon footprint of a cup of tea or coffee? Green Living Blog, The Guardian. [online]. Published: 17 June 2010. Accessed: 3.07.2025. https://www.theguardian.com/environment/green-living-blog/2010/jun/17/carbon-footprint-tea-coffee

[5] Ember: European Electricity Review 2024. Ember–Climate & Energy Think Tank, London, UK, 2024, Accessed: 3.07.2025. https://ember-climate.org/insights/research/european-electricity-review-2024.

[6] Gavrila A.I., Chisega-Negrilă C.G., Maholea L., Gavrila M.L., Pârvulescu O.C., Popa I.: Enhancing the extraction process efficiency of thyme essential oil by combined ultrasound and microwave techniques. Agronomy, 2023, 9 (13), 2331.

[7] Huamán-Castilla N.L., Mariotti-Celis M.S., Martínez-Cifuentes M., Pérez-Correa J.R.: Glycerol as alternative co-solvent for water extraction of polyphenols from Carménère pomace: hot pressurized liquid extraction and computational chemistry calculations. Biomolecules, 2020, 3 (10), 474.

[8] Kameníková M., Fialová S., Ťažký A., Čičová I.: Polyphenolic compounds and essential oil analysis of selected species of the genus Thymus. Acta Fac. Pharm. Univ. Comen., 2015, s9 (62), 12–17.

[9] Khalid M.Z., Saima H., Nadeem M.T., Saeed K., Arshad M.S., Khalid W., Rafique H., Fatima M., Alsulami T., Alzahrani A.: Clean-label extraction of bioactive compounds from Chenopodium album and their role in the characterization and stability of ostrich meat. Front. Sustain. Food Syst., 2024, 8, 1472110

[10] Koșar M., Dorman H.J.D., Hiltunen R.: Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected Lamiaceae species. Food Chem., 2005, 3 (91), 525–533.

[11] Kowalska G., Baj T., Kowalski R.: Comparison of chemical composition of essential oil acquired from single-component pharmaceutical products, food products, and from the cultivation of sage Salvia officinalis L. from Poland. J. Essent. Oil Bear. Plants, 2021a, 6 (24), 1235–1247.

[12] Kowalska G., Baj T., Kowalski R., Szymańska J.: Optimization of glycerol–water extraction of selected bioactive compounds from peppermint and common nettle. Antioxidants, 2021b, 5 (10), 817.

[13] Kowalska G., Wyrostek J., Kowalski R., Pankiewicz U.: Evaluation of glycerol usage for the extraction of anthocyanins from black chokeberry and elderberry fruits. J. Appl. Res. Med. Aromat. Plants, 2021c, 100296.

[14] Król B., Kiełtyka-Dadasiewicz A.: Effect of drying method on sensory characteristics and essential oil composition of thyme (Thymus vulgaris L.). Żywność. Nauka. Technologia. Jakość / Food. Science. Technology. Quality, 2015, 1 (101), 64.

[15] Kulišić T., Dragović-Uzelac V., Miloš M.: Antioxidant activity of aqueous tea infusions prepared from oregano, thyme and wild thyme. Food Technol. Biotechnol., 2006, 4 (44), 485–492.

[16] Manousaki A., Jancheva M., Grigorakis S., Makris D.: Extraction of antioxidant phenolics from agri-food waste biomass using a newly designed glycerol-based natural low-transition-temperature mixture: a comparison with conventional eco-friendly solvents. Recycling, 2016, 1 (1), 194–204.

[17] Moura B., Monteiro H., Mata T. M., Martins A. A.: Life cycle energy and carbon emissions of essential oil extraction from rosemary. Energy Rep., 2022, (8), 291–297.

[18] Murawski M., Schwarz T., Grygier J., Patkowski K., Oszczęda Z., Jelkin I., Kosiek A., Gruszecki T.M., Szymanowska A., Skrzypek T., Zieba D.A., Bartlewski P.M.: The utility of nanowater for ram semen cryopreservation. Exp. Biol. Med., 2015, 5 (240), 611–617.

[19] Najar B., Pistelli L., Ferri B., Angelini L.G., Tavarini S.: Crop yield and essential oil composition of two Thymus vulgaris chemotypes along three years of organic cultivation in a hilly area of central Italy. Molecules, 2021, 16 (26), 5109.
[20] Niksic H., Becic F., Koric E., Gusic I., Omeragic E., Muratovic S., Miladinovic B., Duric K.: Cytotoxicity screening of Thymus vulgaris essential oil in brine shrimp nauplii and cancer cell lines. Sci. Rep., 2021, 1 (11), 13178.
[21] Polish Pharmacopoeia VIII; 2008. Polish Pharmaceutical Society, Warsaw, Poland.
[22] Pinelo M., Fabbro P., Manzocco L., Núñez M., Nicoli M.: Optimization of continuous phenol extraction from by-products. Food Chem., 2005a, 1 (92), 109–117.
[23] Pinelo M., Rubilar M., Jerez M., Sineiro J., Núñez M.J.: Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. J. Agric. Food Chem., 2005b, 6 (53), 2111–2117.
[24] Roby M.H.H., Sarhan M.A., Selim K.A., Khalel K.I.: Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (Thymus vulgaris L.), sage (Salvia officinalis L.) and marjoram (Origanum majorana L.) extracts. Ind. Crops Prod., 2013, (43), 827–831.
[25] Samsonowicz M., Regulska E.: Evaluation of influence of selected metal cations on antioxidant activity of extracts from savory (Satureja hortensis). Chem. Pap., 2016, 6 (70), 10.1515/chempap-2016-0001.
[26] Sárosi Sz., Sipos L., Kókai Z., Pluhár Zs., Szilvássy B., Novák I.: Effect of different drying techniques on the aroma profile of Thymus vulgaris analyzed by GC-MS and sensory profile methods. Ind. Crops Prod., 2013, (46), 210–216.
[27] Shimada A., Ueno H., Inagaki M.: Glutaminase inhibitory activities of pentacyclic triterpenes isolated from Thymus vulgaris L. Nat. Prod. Res., 2022, 11 (36), 2864–2868.
[28] Singleton V.L., Rossi, J.A.Jr.: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic., 1965, 16(3), 144–158.
[29] Sonmezdag A.S., Kelebek H., Selli S.: Characterization of bioactive and volatile profiles of thyme (Thymus vulgaris L.) teas as affected by infusion times. J. Food Meas. Charact., 2018, 4 (12), 2570–2580.
[30] Spigno G., Tramelli L., De Faveri D.M.: Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. J. Food Eng., 2007, 1 (81), 200–208.
[31] Spiridon I., Bodîrlău R., Teacă C.A.: Total phenolic content and antioxidant activity of plants used in traditional Romanian herbal medicine. Open Life Sci., 2011, 3 (6), 388–396.
[32] Tetra Pak: Evaporators. In: Dairy Processing Handbook. Tetra Pak Processing Systems AB, Lund, Sweden, 2024, Chapter 7.6.
[33] Tohidi B., Rahimmalek M., Arzani A.: Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of Thymus species collected from different regions of Iran. Food Chem., 2017, (220), 153–161.
[34] Ulewicz-Magulska B., Wesołowski M.: Total phenolic contents and antioxidant potential of herbs used for medical and culinary purposes. Plant Foods Hum. Nutr., 2019, 1 (74), 61–67.
[35] Vábková J., Neugebauerová J.: Determination of total phenolic content, total flavonoid content and FRAP in culinary herbs in relation to harvest time. Acta Univ. Agric. Silvic. Mendel. Brun., 2013, 1 (60), 167–172.
[36] Vik T.: How much energy do you need to boil a litre of water? [online]. blog.viktomas.com. Published: 2022. Accessed: 3.07.2025. https://blog.viktomas.com/graph/boiling-litre-of-water/
[37] Waheed M., Hussain M.B., Saeed F., Afzaal M., Ahmed A., Irfan R., Akram N., Ahmed F., Hailu G.G.: Phytochemical profiling and therapeutic potential of thyme (Thymus spp.): a medicinal herb. Food Sci. Nutr., 2024, 12 (12), 9893–9912.
[38] Wyrostek J., Kowalski R.: The effect of water mineralization on the extraction of active compounds from selected herbs and on the antioxidant properties of the obtained brews. Foods, 2021, 6 (10), 1227.
[39] Yilmaz Y., Toledo R.T.: Oxygen radical absorbance capacities of grape/wine industry by-prod ucts and effect of solvent type on extraction of grape seed polyphenols. J. Food Compos. Anal., 2006, 1 (19), 41–48.

[40] Zheng L., Jacquier J.-C., Harbourne N.: Preparation of polyphenol-rich herbal beverages from white willow (Salix alba) bark with potential Alzheimer's disease inhibitory activity in silico. Beverages, 2024, 3 (10), 75.

Comparison of knowledge and dietary habits in a group of healthy and cancer patients

Małgorzata Kostecka*, Monika Bojanowska, Monika Sachadyn-Król, Marzena Pabich

Faculty of Food Science and Biotechnology,
University of Life Sciences in Lublin, Poland
**corresponding author: malgorzata.kostecka@up.lublin.pl*

Abstract

Cancer and its treatment place a strain on the patient's body. One of the first effects of the disease is weight loss, which is primarily associated with impaired nutrient supply and reduced food intake, which can result in nutritional deficiencies, leading to a poorer response to treatment and a deterioration in the patient's quality of life. For this reason, nutritional intervention is a very important measure, carried out in parallel with conventional treatment. The aim of this study is to compare nutritional knowledge among people diagnosed with cancer and the healthy population. The research tool was a questionnaire based on the KomPAN questionnaire, completed in paper form. The study was conducted between October 2023 and December 2024 on a group of 389 people. Nearly 90% of respondents believed that diet is an etiological factor in cancer, and 84% believed that diet can be a chemopreventive factor (more often among patients than healthy individuals, $p<0.05$). Almost all respondents (95.5%) believed that cancer patients should not consume highly processed foods containing additives (e.g. nitrates, nitrites, monosodium glutamate, hydrogenated fats, glucose-fructose syrup). Among the products not recommended for cancer patients, respondents indicated heavily salted products and dishes (87%), heavily baked products (82.5%), smoked products (80.5%), cured (78.5%), marinated (75%), repeatedly reheated (72%) and traditionally grilled in smoke (61.5%). On the other hand, among the dietary components beneficial for cancer patients, respondents indicated antioxidants (79.5%) and omega-3 fatty acids (71%), vitamins C and E (59%), bioactive substances, including coenzyme Q10, polyphenols, phytosterols (58%).

Keywords: cancer, diet, nutrition knowledge, malnutrition

1. Introduction

Cancer is a group of diseases characterised by the uncontrolled growth of genetically altered cells in the body. These changes include both morphological and functional features resulting from disorders at the genetic code level [Jeziorski, 2015; Przystaś et al., 2019]. Depending on the nature of the changes, cancers are divided into benign, locally malignant and malignant, as well as epithelial and non-epithelial (www1). An increase in cancer incidence is observed particularly in older age groups. The main factors influencing the development of the disease include external (exogenous) factors, such as UV radiation, environmental pollution, unhealthy diet, smoking, alcohol consumption, and viral and bacterial infections, as well as internal (endogenous) factors such as age, genetic predisposition, hormone levels, immune system function, and prolonged stress [Kulik and Latalski, 2009; Przystaś et al., 2019]. Reducing exposure to carcinogens can extend life by up to 10–20 years [Przystaś et al., 2019].

According to the classification of the International Agency for Research on Cancer (IARC), a carcinogen is a substance or mixture of substances that can cause cancer or increase its incidence. The IARC divides them into five risk groups: from confirmed carcinogens (group 1) to those that are unlikely to be carcinogenic (group 4). Although the classification has no legal force, it is an important reference point in risk assessment [Olędzki, 2017; Kowalska et al., 2019]. Statistics from the National Cancer Registry indicate a systematic increase in the incidence of cancer in Poland, which is due, among other things, to the increasing average life expectancy, the ageing of the population and civilisational changes – a sedentary lifestyle, poor diet, environmental pollution and chronic stress [Surwiłło and Wawrzyniak, 2014; Didkowska et al., 2021]. In Poland, there are over 171,000 cases and 100,000 deaths per year. In the Polish male population in the second half of the 20th century, the risk of lung cancer grew the fastest. The last three decades have seen a halt to this unfavourable trend and, after 1997, a reversal to a downward trend. Prostate cancer is characterised by the highest growth rate in incidence, particularly in the last decade, and since 2016 it has remained the most common cancer in men. At the same time, mortality has stabilised and survival rates have improved significantly in the last decade. Colon cancer is a disease with a significant increase in incidence and the second most common cause of death. Stomach cancer, the fourth most common cancer, has seen a decline in incidence and mortality over the last half-century [National Cancer Registry].

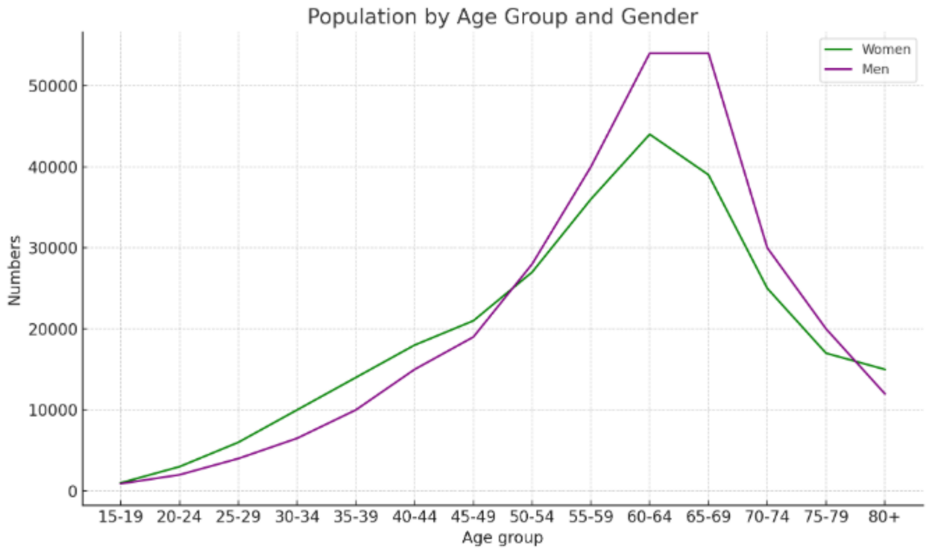

Figure 1. Number of malignant cancer cases in Poland by age group in 2020–2021 [National Cancer Registry 2025].

Preventive measures taken in Poland include health programmes (e.g. National Health Programme 2021–2025, National Cancer Control Programme 2016–2024), social campaigns and the activities of non-governmental organisations such as the Rak'n'Roll Foundation [Przystaś et al., 2019]. The aim of these initiatives is to raise awareness, improve screening uptake and promote a healthy lifestyle. Early detection of cancer is crucial, as it significantly increases the chances of a complete cure. Regular preventive examinations, such as mammography, cytology, colonoscopy and tumour marker tests, are the basis for effective prevention and early diagnosis [Przystaś et al., 2019].

An unhealthy diet and low physical activity are among the key etiological factors of cancer, accounting for about one-third of cases, including colorectal cancer [Choi and Hua, 2021; Meder, 2018; Przystaś et al., 2019]. A diet rich in meat, fats, sugars and preservatives promotes the development of the disease and also reduces the effectiveness of treatment [Surwiłło and Wawrzyniak, 2014]. A strong correlation between healthy or unhealthy eating patterns and lifestyle and many socio-demographic factors was observed in young men (aged 19-40, the study group consisted of 235 men) in a study conducted by Lennie et al. [2018]. This population group is particularly vulnerable to an increased risk of unhealthy behaviours. The authors showed that the most health-promoting dietary and lifestyle pattern depended on family status – it concerned men in stable marriages. Nutrition education is key to cancer prevention.

Table 1. Food products or nutritional components whose regular consumption increases the risk of malignant tumours [Szczygieł, 2013].

Type of cancer	Food products or food ingredients
Colorectal cancer (colon and rectum)	Red meat (beef, pork, mutton) and processed meat (smoked, canned, salted or containing added preservatives)
Stomach cancer	Excessive salt in the diet
Prostate cancer	Excessive dietary calcium intake (unbalanced diet of intensive sports people)
Liver cancer	Foods containing aflatoxins (mouldy cereal grains, nuts and pulses)
Cancer of the mouth, throat, larynx, oesophagus, liver, colon, breast	Alcohol
Oesophageal cancer (especially in smokers)	Yerba mate tea with temp >85°
Lung cancer (in smokers)	Β-carotene in food supplements

An excessive amount of fat in the diet (usually expressed as the total energy intake in the diet) correlates with a higher risk of developing breast, prostate and colon cancer, among others. This relationship is primarily determined by their composition and fatty acid content. For this reason, it is important to be aware of the impact of specific fats on human health, as some have a positive effect (they are recommended in the diet, mainly unsaturated fatty acids from the omega-3 and omega-6 families, commonly found in meat, fish, eggs and vegetable oils, including sunflower oil), while others have a negative effect (they should be limited, e.g. fats rich in saturated acids, present in red meat or dairy products). In addition, the fat content in the diet determines the level of hormones in the body. This includes, among other things, an increase in oestrogen concentration, which contributes to an increased risk of breast cancer [Malczyk and Majkrzak, 2015]. There is sufficient scientific evidence to indicate that eating processed meat can cause colon cancer – a daily intake of 50 g increases the risk by 18%, which is why it is recommended to avoid this group of products in the diet. In addition, a strong correlation has been shown between the consumption of processed meat and red meat and many cancers, including colorectal cancer, pancreatic cancer, stomach cancer and prostate cancer. The consumption of red meat during the week should not exceed 500 g after cooking [Jarosz, 2018]. The risk of cancer is associated with a high glycaemic index diet, which increases blood insulin levels, leads to insulin resistance and increases the secretion of insulin-like growth factor (IGF-1), which is also detrimental to the carcinogenesis process [Malczyk and Majkrzak, 2015].

The impact on cancer pathogenesis has been proven, among others, in relation to zearalenone (ZEA). This compound, synthesised by certain species of Fusarium, is a secondary metabolite of mould and a strong pathogen of cereal crops. It enters the human and animal body through the consumption of contaminated plant-based food. In humans, the risk also applies to the consumption of contaminated animal tissues. Zearalenone is classified as a non-steroidal oestrogen or micro-oestrogen, and its oestrogenic activity is several times stronger than that of naturally occurring oestrogens, which can cause damage to the reproductive organs and reproductive disorders.

Table 2. Food products or nutritional components whose regular consumption reduces the risk of malignant tumours [Szczygieł, 2013].

Type of cancer	Food or food ingredient
Cancer of the mouth, pharynx, larynx, oesophagus, stomach	Low starch vegetables (broccoli, lettuce, cucumber, tomatoes, cabbage, cauliflower, courgettes, aubergines, peppers, turnips, asparagus, onions, garlic, parsley, radishes, leek, radish and alfalfa sprouts
Cancer of the mouth, pharynx, larynx, oesophagus, stomach, lung	Fruit
Cancer of the stomach	Onion vegetables (onions - all types, garlic, leek, chives)
Cancer of the large intestine (colon, rectum)	Garlic
Cancer of the oesophagus	Foods containing vitamin C (fruits and vegetables)
Cancer of the pancreas	Foods containing folate (dark green vegetables, pulses, nuts, yeast, avocados, bananas, raspberries, oranges)
Cancer of the mouth, pharynx, larynx, lung	Foods containing carotenoids (carrots, yams, pumpkin, peppers, parsley, kale, spinach, tomatoes, apricots, broccoli)

This compound has been classified by the International Agency for Research on Cancer (IARC) as Group 3 – a potential carcinogenic hazard to humans. Exposure to ZEA may increase the incidence of hormone-dependent cancers, such as breast cancer in women and prostate cancer in men [Mruczyk et al., 2018].

Diet is an important factor in the development of breast cancer, among other things. According to data from the International Agency for Research on Cancer (IARC), poor diet was a factor in 25% of breast cancer cases. Modifying one's diet in the case of breast cancer can reduce the risk of developing the disease by up to 50% [Malczyk and Majkrzak, 2015].

In a study conducted by Jeżewska-Zychowicz et al. on a representative group of 1,017 people, it was found that almost 40% of respondents were concerned about the onset of cancer. This mainly concerned women (42% compared to 34% of men), overweight and obese people, people with high health and nutritional risks, and people with extensive nutritional knowledge [Jeżewska-Zychowicz et al., 2018]. The perception of health considerations and food prices (mainly when choosing meat, meat products, dairy products, vegetables and fruit) was more important the greater the respondent's fear of developing cancer. At the same time, those who showed the greatest concerns paid attention primarily to health considerations, declared more correct eating habits, and price was an important factor when choosing food, but did not differentiate their eating habits [Jeżewska-Zychowicz et al., 2018].

A properly balanced diet should be introduced in oncology patients as early as possible – preferably immediately after diagnosis. The aim is to maintain a healthy body weight and prevent malnutrition, which affects up to 80% of patients [Kłęk and Kapała, 2019]. Nutritional deficiencies worsen the prognosis, increase the risk of infections, complicate treatment and prolong hospitalisation. Nutritional interventions are key – an oral diet is preferred, and if necessary, enteral or parenteral nutrition [Dutkiewicz and Grzelak, 2021]. The care of a clinical dietitian is also essential.

The diet in cancer should be easily digestible, based on the principles of healthy eating, properly balanced and, if necessary, supplementing any nutritional deficiencies [Key et al., 2020]. In certain cases, it is necessary to modify the nutritional intervention, e.g. by increasing the proportion of liquid, semi-liquid or mushy meals, especially in patients with chewing and swallowing difficulties [Dutkiewicz and Grzelak, 2021]. The caloric intake of cancer patients should be 25-30 kcal/kg of body weight/day. Some patients require special diets, e.g. lactose-free diets, low-fat diets, modified BRAT diets (especially in patients with chronic diarrhoea; unripe bananas, white rice, baked apples, wheat toast), low FODMAP diets. Sometimes certain restrictions are required, such as limiting the intake of insoluble dietary fibre or simple sugars. In other cases, supplementation with bioactive compounds is essential, depending on medical indications. The diet should be consulted and introduced by a qualified dietitian, especially in the case of restrictive diets. It is necessary to establish the right balance of protein and energy. The use of unbalanced alternative diets (e.g. the Gerson diet, the Budwig diet, ketogenic diet, fasting diets) or alternative supplements (e.g. high doses of vitamin C) may be associated with high risk and may result in deterioration of nutritional status, the development of many complications, and postponement or interruption of therapy due to health conditions [Kłęk and Kapała, 2019]. The diet of cancer patients should be balanced, supportive of treatment and aimed at maintaining a healthy body weight It is recommended to avoid sweetened beverages and high-energy foods, especially highly processed foods that are rich in sugars and fats and low in fibre. BMI should be maintained between 18.5 and 24.9 kg/m². Your daily menu should be based on plant-based products – vegetables, fruit, whole grain cereal products and legumes. It is recommended to consume at least 5 portions of different coloured vegetables and fruit per day (400–600 g) and a minimum of 25 g of fibre. It is worth regularly consuming dairy products (milk, kefir, yoghurt, low-fat cheeses), which provide calcium and reduce the risk of colon cancer. It is also recommended to eat sea fish at least twice a week as a source of vitamin D. In the UK, vitamin D supplementation (800–2000 IU/day) is necessary from October to March or throughout the year if skin synthesis is insufficient. Red meat consumption should be limited to 500 g per week (after preparation), and processed meat products (cold cuts, bacon, salami) should be avoided altogether. Meat dishes should not be fried or grilled for long periods of time; the recommended cooking technique is boiling or steaming. Salt should also be limited – no more than 5 g per day;

herbs are recommended instead of salt. Mouldy grains, nuts and seeds should be avoided due to the presence of mycotoxins. Drinking very hot beverages (>65°C) is not recommended as they can damage the mucous membrane of the digestive tract. Dietary supplements should only be used in justified cases, under the supervision of a doctor or dietitian, as all scientific studies indicate that supplementation without indications does not protect against cancer. Adequate hydration is recommended through the consumption of water, light teas or compotes. Coffee (2–4 cups a day), without sugar or additives, can have a beneficial effect on health due to its polyphenol content [Jarosz, 2018].

Table 3. Dietary recommendations for the most common ailments associated with cancer treatment.

Problem	Dietary advice
Nausea and vomiting	-Start proper hydration 2-3 days before chemotherapy, liquids with ginger, ice cubes, coca-cola, avoid mint infusions - small and frequent meals, cold - Delicate foods: milk and fruit cocktails, soups, mousses, jellies, pancakes, addition of FSMPs limination of foods that are hard to digest, fried, high in fat and fibre
Diarrhoea	- modification of the BRAT diet (bananas, wheat toast, apples, rice) -low FODMAP diet -sometimes lactose elimination (chemotherapy regimens containing 5-FU, irinotecan, capecitabine) -probiotics, oral electrolyte solutions -blackberry vapour, natural cocoa on water
Constipation	-after constipation has been regulated (pharmacological, mechanical), incorporation of a diet with increased fibre content (soluble and insoluble) and adequate hydration -natural sorbitol from prunes, pears, birch sugar -natural silages

Cancer chemoprevention is a growing field of cancer research that involves prevention through the use of synthetic compounds or substances that occur naturally in the diet. These compounds have anti-carcinogenic properties [Malczyk and Majkrzak, 2015]. Cruciferous vegetables from the Cruciferae family are a particularly valuable source of anti-cancer substances. These vegetables, such as white cabbage, red cabbage, Chinese cabbage, savoy cabbage, broccoli, cauliflower, Brussels sprouts, radishes and turnips, play an important role in the diet of Central Europeans and are available all year round [Malczyk and Majkrzak, 2015]. Unprocessed foods contain bioactive ingredients (nutraceuticals), both nutritious and anti-nutritious, which have a beneficial effect on the body. These include polyphenols (e.g. curcumin, lignans, resveratrol, quercetin), flavonoids, glucosinolates, sesquiterpenes and steroid metabolites. They have health-promoting effects in heart disease, hypertension, obesity, type 2 diabetes, osteoporosis and cancer [Drywień and Górnicka, 2018; Wróblewska, 2018]. During digestion, enzymes and intestinal bacteria release these compounds, which then affect inflammatory mechanisms and regulate gene expression. Their action depends on the physiological state of the body. Products rich in bioactive ingredients include milk and dairy products, meat, eggs, fish (tuna, sardines, herring, salmon), cereals (wheat, corn, rice), soybeans, pumpkin, amaranth, sorghum and fermented products [Drywień and Górnicka, 2018; Wróblewska, 2018]. More and more studies also emphasise the importance of fructans in the prevention of colon cancer. Fructans (inulin, fructooligosaccharides, oligofructose) are a soluble fraction of fibre based on fructose molecules. They have a preventive and supportive effect in treatment – they alleviate the side effects of cancer therapy. They support the development of beneficial intestinal microflora (especially Bifidobacterium) and increase the concentration of short-chain fatty acids, e.g. butyric acid, in the colon. In addition, they improve the frequency and volume of bowel movements [Glibowski, 2018].

One of the main dietary contraindications in cancer is alcohol consumption. Complete abstinence is recommended, as there is no safe dose – even small amounts increase the risk of developing certain cancers, such as breast cancer [Jarosz, 2018]. Caution should also be exercised when using vitamin C in so-called mega-doses. Despite decades of research, its effectiveness in treating cancer has not been conclusively proven. However, intravenous administration of vitamin C may improve the quality of life of cancer patients and reduce the side effects of therapy, such as fatigue, loss of appetite, nausea and pain. On the other hand, in some cases, its use may accelerate the progression of the disease, e.g. in people with acute myeloid leukaemia, colorectal cancer or melanoma

[Kalemba-Drożdż, 2018]. Special dietary recommendations apply to patients after surgical removal of a tumour, especially one located in the digestive tract. For about a month after surgery, heavy, bloating and fatty foods should be avoided. Contraindicated foods include wholemeal bread, raw vegetables and fruit, cruciferous vegetables (cabbage, cauliflower, Brussels sprouts, broccoli), onions, garlic, chives, legume seeds (peas, beans, lentils, soybeans), fatty meats (pork, mutton), cold cuts (sausages, pâté, bacon), fatty cheeses (yellow, blue, processed), cream, mayonnaise, lard, fried foods (chips, breaded cutlets), cakes, sweets, chocolate, salty snacks, hot spices, vinegar, alcohol, coffee, cocoa, strong tea, carbonated drinks [Szczygieł, 2013]. Adherence to dietary recommendations plays an important role in the treatment and recovery of cancer patients.

The aim of this study is to compare nutritional knowledge among people diagnosed with cancer and the healthy population.

2. Materials and methods

The research tool was a questionnaire based on the KomPAN questionnaire, completed in paper form. The study was conducted between October 2023 and December 2024 on a group of 540 people. A total of 389 correctly completed questionnaires were received, from 178 people diagnosed with cancer and 211 healthy people. The survey results were analysed using Microsoft Excel Office 365 and StatSoft Statstica 13. Pearson's chi-square test was used to examine the statistical significance between the analysed variables.

3. Results and discussion

The majority of respondents were women, who accounted for 63.1% of the patient group and 75.1% of the healthy group. In terms of age, respondents aged 46–55 predominated (25.5%, n=100), followed by those aged 36–45 (23%, n=89) and 56–65 (22%, n=86). The smallest group consisted of people aged 18–25 (7%, n=27). In terms of education, most respondents had completed a master's degree (41%, n=158) or a bachelor's degree

(27.5%, n=107). The majority of respondents came from rural areas (40%, n=156).

In the group of cancer patients, more than half had breast cancer, 17.1% had lung cancer, 15.6% had colon cancer, and 13.3% had prostate cancer. The respondents almost unanimously (99%) agreed that diet has an impact on overall health and that an unhealthy diet can contribute to the development of diseases. Almost 95% of respondents (n=370) indicated diet as an etiological factor in cancer. Statistically significant differences ($p<0.05$) were observed between the groups of patients and healthy individuals in this regard – patients were more likely to recognise the relationship between diet and cancer. According to 81% of respondents diet can act as a chemopreventive factor, with cancer patients significantly more likely to believe this, and over 10% of respondents (n=39) admitted that they had no knowledge on this subject. In terms of the impact of harmful substances in food, over 90% of respondents believed that mould and mycotoxins can contribute to the development of cancer, with healthy people more likely to declare a lack of knowledge ($p=0.023$). The majority of respondents (94%) also believed that cancer patients should avoid highly processed foods with chemical additives (nitrates, nitrites, flavourings, monosodium glutamate, hydrogenated fats, etc.), but no statistically significant differences were observed between the healthy and sick groups ($p>0.05$).

Professional dietary counselling based on scientific evidence should be recommended for every patient diagnosed with malignant cancer, regardless of the stage of the disease. Dietary care should be part of the overall care of cancer patients [Poulsen et al., 2014]. Among cancer patients, as many as 91% (n=162) reported changing their diet after diagnosis, and over 50% reported following a specialised diet that eliminated one of the main macronutrients, with high-protein ($p=0.011$) and low-fat ($p=0.028$) diets being significantly more common. According to research, individualised dietary counselling has a positive effect on nutritional status and quality of life, compared to no counselling or standard, one-time dietary advice provided by a nurse [Langius et al., 2013].

Respondents most often indicated that people with cancer should avoid: heavily salted products (87%), heavily charred products (82.5%), smoked products (80.5%), cured products (78.5%), marinated products (75%), repeatedly reheated (72%) and smoke-grilled (61.5%) foods. Only in the case of cured and grilled products was there a significant difference in knowledge, with cancer patients demonstrating greater awareness. Among healthy individuals, there were also responses regarding the avoidance of raw vegetables and products cooked in salted water.

The most beneficial dietary components in cancer were considered to be: antioxidants (79.5%), omega-3 fatty acids (71%), vitamins C and E (59%), bioactive substances: coenzyme Q10, polyphenols, phytosterols (58%), and vitamin D (53%). Significantly more correct answers were given by healthy people ($p=0.019$). In the context of products with anti-cancer properties, most respondents (92%) indicated 'all of the above' from the list: green tea (89.5%), blueberries and raspberries (87.5%), green vegetables (87.5%), ginger (82.5%) and turmeric (79.5%).

Nearly three-quarters of respondents believed that sick people should eat 4–5 small meals every 3–4 hours; illness was not a significant differentiating factor among respondents. Similarly, three-quarters of respondents believed that cereal products should be included in most meals throughout the day and that the diet should be rich in protein products. Sick people more often indicated lean meats and dairy products, while healthy people more often indicated eggs and red meats. The correct amount of vegetables and fruit (400–600 g per day) was indicated by two-thirds of respondents, with sick people giving correct answers significantly more often ($p>0.05$). In terms of meal temperature, as many as 80% of respondents indicated the need to consume cooled dishes (neither hot nor cold). Recommendations for cancer patients indicate the need to increase the calorie content of the diet and meet protein requirements, which in patients at risk of malnutrition or malnourished with a lack of appetite, with an unchanged or reduced diet volume, may be difficult to apply in practice [Arends et al., 2017]. More than two-thirds of respondents believe that sick people should not give up meat and fish because they are sources of protein. The methods of increasing the amount of protein in the diet are primarily all the indicated options: the addition of cream, powdered milk, eggs, and chopped meat. The correct answer was given significantly more often by patients ($p<0.05$). The most frequently recommended sources of fat were olive oil (76%), pumpkin seed oil (74.5%), linseed oil (70.5%) and avocado (67%). Margarine (3.5%), mayonnaise (4%) and refined rapeseed oil (9.5%) were considered the least beneficial. In the study, for more than two-thirds of people with cancer, the main sources of knowledge were the internet and friends, while only 19% cited their doctor and one in ten cited a dietitian. Research conducted by Cong et al. showed that in the analysed population, the main sources of knowledge about diet in cancer patients were doctors (26% of respondents), the internet (18.5%) and television (16.1%) [Cong et al., 2018].

4. Conclusions

The study confirms the high level of public awareness of the importance of nutrition in the context of cancer prevention and treatment.

Both sick and healthy people demonstrate a great deal of knowledge about the components of the diet, contraindicated and beneficial products, as well as the impact of processed food on health. People with cancer with higher education are significantly more likely to follow a specialist diet.

After being diagnosed with cancer, the model of nutrition has changed in most patients.

People with cancer have greater knowledge than healthy people about the model of nutrition in cancer diseases.

Healthy people assess their nutritional knowledge worse compared to sick people.

5. References

[1] Arends J., Baracos V., Bertz H., Bozzetti F., Calder P. C., Deutz N. E. P., Erickson N., Laviano A., Lisanti M. P., Lobo D. N., McMillan D. C., Muscaritoli M., Ockenga J., Pirlich M., Strasser F., de van der Schueren M., Van Gossum A.,Vaupel P., Weimann A.: ESPEN expert group recommendations for action against cancer related malnutrition. Clinic Nutr., 2017, 36, 1187-1196.
[2] Choi J.W., Hua T.N.M.: Impact of life style behaviors on cancer risk and prevention. J. Lifestyle Med., 2021, 11 (1), 1-7.
[3] Cong M., Wang J., Fang Y., Liu Y., Sun M., Wu Q., Wang K., Huang Y., Ling Y., Liu Y., Li Q., Liu Y., Zhu J., Zhu L., Zheng Z., Li L., Liu D., Liu Z., Shi H., Yuan P.: A multi-center survey on dietary knowledge and behavior among inpatients in oncology department, Supportive Care in Cancer, 2018, 5.

[4] Didkowska J., Wojciechowska U., Olasek P., Santos F.C., Michałek I.: Malignant tumours in Poland in 2019. National Cancer Registry. Maria Skłodowska-Curie National Research Institute of Oncology. Warszawa. Accessed: 11.06.2025. http://onkologia.org.pl/wp-content/uploads/Nowotwory_2019.pdf
[5] Drywień M.E., Górnicka M.: Biologically active compounds in juices and vegetables. In: Hamułka J., Przybyłowicz K., Wądołowska L. 2018. Nutrition and cancer. Dilemmas in human nutrition science – today and tomorrow. Eds. Polish Society of Nutrition Sciences, Warszawa, 2018.
[6] Dutkiewicz A., Grzelak T.: Dietary preferences of patients with anorexia nervosa and malignant tumours, and recommendations for the dietary treatment of malnourished individuals. Medycyna Ogólna i Nauki o Zdrowiu, 2021, 27(2), 175-181.
[7] Gardocka-Jałowiec A., Stańczyk P., Szalonka K.: The impact of nutrition and food on health in the light of research. In: Szalonka K., Nowak W. 2020. Health and lifestyles. Determinants of life expectancy. E-Monograph, Eds. University of Wrocław, 2020.
[8] Glibowski P.: The role of fructans in the prevention of colorectal cancer. In: Hamułka J., Przybyłowicz K., Wądołowska L. 2018. Nutrition and cancer. Dilemmas in human nutrition science – today and tomorrow. Eds. Polish Society of Nutrition Sciences, Warszawa, 2018.
[9] Jarosz M:. Nutritional recommendations for cancer prevention. In: Hamułka J., Przybyłowicz K., Wądołowska L. 2018. Nutrition and cancer. Dilemmas in human nutrition science – today and tomorrow. Eds. Polish Society of Nutrition Sciences, Warszawa, 2018.
[10] Jeziorski A.: Oncology. A handbook for nurses.,Eds. PZWL, Warszawa, 2015.
[11] Jeżewska-Zychowicz M., Drywień M., Hamułka J.: Perception of cancer risk and the importance of price and health considerations in food choices among adults. In: Hamułka J., Przybyłowicz K., Wądołowska L. 2018. Nutrition and cancer. Dilemmas in human nutrition science – today and tomorrow. Eds. Polish Society of Nutrition Sciences, Warszawa, 2018.
[12] Kalemba-Drożdż M.: Vitamin C in cancer treatment and prevention of non-communicable diseases. Państwo i Społeczeństwo, 2018, 3, 91-104.
[13] Key T.J., Bradbury K.E., Perez-Cornago A., Sinha R., Tsilidis K.K., Tsugane S.: Diet, nutrition, and cancer risk: what do we know and what is the way forward? BMJ, 2020, 368, m511.
[14] Kłęk S., Kapała A.: Nutrition treatment. Varia Medica, 2019, 3(1), 77-87.
[15] Kowalska J., Jeżewska A., Woźnica A.: Occupational exposure to carcinogenic and mutagenic substances. Methods for determining selected chemical substances. Guide. Central Institute for Labour Protection – National Research Institute, Warszawa, 2019.
[16] Kulik T., Latalski M.: Public health. A handbook for students and graduates of nursing and health sciences departments at medical academies.. Eds. Czelej, Lublin, 2019.
[17] Langius J. A., Zandbergen M. C., Eerenstein S. E., van Tulder M. W., Leemans C. R, Kramer M. H., Weijs P. J.: Effect of nutritional interventions on nutritional status, quality of life and mortality in patients with head and neck cancer receiving (chemo)radiotherapy: a systematic review. Clinical Nutrition, 2013, 32(5), 671-678.
[18] Lennie M., Wądołowska L., Kowalkowska J., Bandurska-Stankiewicz E.: The interdependence between sociodemographic factors and dietary and lifestyle patterns in young men: the MeDiSH study. In: Hamułka J., Przybyłowicz K., Wądołowska L. 2018. Nutrition and cancer. Dilemmas in human nutrition science – today and tomorrow. Eds. Polish Society of Nutrition Sciences, Warszawa, 2018.
[19] Malczyk E., Majkrzak Ż.: Nutritional risk factors for breast cancer. Probl. Hig. Epidemiol., 2015, 96(1), 67-76.
[20] Meder J.: Education, prevention and cancer screening in relation to food and nutrition issues. In: Hamułka J., Przybyłowicz K., Wądołowska L. 2018. Nutrition and cancer. Dilemmas in human nutrition science – today and tomorrow. Eds. Polish Society of Nutrition Sciences, Warszawa, 2018.
[21] Mruczyk K., Mizgier M., Cisek-Woźniak A., Wójciak R.: The presence of zearalenone in food and its impact on the pathogenesis of malignant tumours. In: Hamułka J., Przybyłowicz K., Wądołowska L. 2018. Nutrition and cancer. Dilemmas in human nutrition science – today and tomorrow. Eds. Polish Society of Nutrition Sciences, Warszawa, 2018.
[22] Olędzki R.: Hazardous substances in food that are carcinogenic and mutagenic. In: Maciąg M., Maciąg K. Threats and problems of civilisation in the 21st century – overview and research. Eds. TYGIEL Scientific Publishing, Lublin, 2017.

[23] Poulsen G.M., Pedersen L.L., Østerlind K., Bæksgaard L., Andersen J.R.: Randomized trial of the effects of individual nutritional counseling in cancer patients, Clin. Nutrition, 2014, 33(5), 749-753.
[24] Przystaś B., Sander-Grabowska M., Chwiałkowska M.: Aspects of health promotion and preventive examinations in the context of cancer incidence and mortality in Poland. Innovations in Nursing and Health Sciences, 2019, 4(2), 116-130.
[25] Surwiłło A., Wawrzyniak A.: Assessment of nutritional awareness among people with cancer. Probl. Hig. Epidemiol., 2014, 95(1), 75-80.
[26] Szczygieł A.: Nutrition and cancer. Guide for patients and their families. Wyd. PRIMOPRO, Warszawa, 2013.
[27] Wróblewska B.: Bioactive food components and cancer. In: Hamułka J., Przybyłowicz K., Wądołowska L. 2018. Nutrition and cancer. Dilemmas in human nutrition science – today and tomorrow. Eds. Polish Society of Nutrition Sciences, Warszawa, 2018.
[28] www1: http://onkologia.org.pl/nowotwory-wprowadzenie/ (dostęp 5.05.2025).

Probiotic-mediated biosynthesis of metal nanoparticles

Anna Krzepiłko[1*], Agata Święciło[2]

[1]*Faculty of Food Sciences and Biotechnology*
University of Life Sciences in Lublin
[2]*Department of Environmental Microbiology*
University of Life Sciences in Lublin
**corresponding author: anna.krzepilko@up.lublin*

Abstract

Numerous probiotic bacterial species are known for their ability to produce lactic acid and exhibit beneficial probiotic properties. These bacteria are Gram-positive, non-spore-forming, and highly adaptable to low pH environments, with the capacity to grow under both aerobic and anaerobic conditions. In recent years, biological methods for nanoparticle synthesis have gained increasing interest due to their environmentally friendly nature and low production costs. Probiotic bacteria have emerged as promising sources of natural reducing and stabilizing agents in the biosynthesis of metal nanoparticles. This study investigates the ability of various probiotic strains to synthesize both metal and metal oxide nanoparticles. The synthesis process is strongly influenced by culture conditions, which affect the size and morphology of the nanoparticles - key factors determining their physicochemical and biological properties. The bacteria utilize natural enzymes and metabolites to reduce metal ions to their elemental (zero-valent) forms. The resulting nanoparticles are often coated with biomolecules, such as proteins and polysaccharides, which enhance their stability. The article presents the mechanisms and conditions involved in both intracellular and extracellular nanoparticle synthesis, characterizes the physical and chemical properties of the synthesized nanoparticles, and outlines examples of their potential applications.

Keywords: probiotic bacteria, green synthesis, metal nanoparticles, metal oxide nanoparticles

1. Probiotic Bacteria

Probiotic bacteria most commonly occur naturally in the gastrointestinal tract of humans and animals, as well as in fermented products such as yogurt, kefir, sauerkraut, and in specialized pharmaceutical preparations and dietary supplements (Vera-Santander et al., 2023). They play a crucial role in maintaining human health by supporting the balance of the intestinal microflora, protecting against colonization by pathogenic microorganisms, enhancing immune function, and influencing numerous metabolic processes (Inchingolo et al., 2023).

Among probiotic bacteria, species within the genus *Lactobacillus* are particularly significant. In recent years, the taxonomic classification of probiotic bacteria previously grouped under *Lactobacillus* has been revised. Based on genetic analyses, the genus has been divided into several new genera, including *Lacticaseibacillus, Lactiplantibacillus, Limosilactobacillus, Levilactobacillus*, and others (Zheng et al., 2020). In the remainder of this publication, species names are presented in accordance with the original terminology used by the cited authors.

Probiotic bacteria of the genus *Bifidobacterium* are especially important for infants and young children, as they naturally dominate the gut microbiota of breastfed newborns (Vera-Santander et al., 2023). Other relevant species include *Streptococcus thermophilus*, commonly used in yogurt production, and certain strains of *Enterococcus faecium*, which are employed in selected probiotic formulations, particularly in veterinary medicine (Das et al., 2022).

The multifaceted impact of probiotic bacteria on the host includes supporting digestion and nutrient absorption, facilitating lactose metabolism, positively influencing intestinal motility, exerting anti-inflammatory effects, maintaining the integrity of the gut barrier, and promoting the natural microbiota (Vera-Santander et al., 2023; Inchingolo et al., 2023). In cases of microbiota disruption caused by antibiotic therapy, gastrointestinal infections, or irritable bowel syndrome, probiotics may aid in restoring microbial balance. Numerous studies also highlight their potential influence on cognitive function, mood regulation, and even the alleviation of anxiety and depressive symptoms (Inchingolo et al., 2023).

2. Benefits of probiotic bacteria-mediated biological synthesis of nanoparticles

Nanotechnology represents one of the most innovative and groundbreaking fields in modern scientific development. Conventional methods of nanoparticle synthesis, which rely on chemical and physical approaches, often require expensive equipment, toxic reagents, and extreme conditions such as high temperatures and pressures. In contrast, biological synthesis methods- central to the so-called green synthesis paradigm - are gaining increasing popularity. These approaches utilize living organisms or their derivatives to produce nanoparticles under mild, environmentally friendly conditions (Purohit et al., 2019).

This process is inherently eco-friendly, as synthesis occurs under gentle conditions, typically at bacterial culture temperatures and standard atmospheric pressure. Chemical stabilizers are generally unnecessary, and the risk of releasing toxic substances into the environment is significantly lower compared to chemical methods (Bahrulolum et al., 2021). Moreover, nanoparticle synthesis via biological routes does not require specialized equipment and can be easily implemented in both laboratory and industrial settings. Production costs are relatively low, while the resulting nanoparticles demonstrate good stability and high biocompatibility with other cells.

Probiotic bacteria belonging to the LAB (Lactic Acid Bacteria) group are among the most commonly used microorganisms in the biosynthesis of metallic nanoparticles. Because they naturally occur within the human body, products derived from their activity can be safely applied in direct contact with living tissues, for example, as components of pharmaceuticals, implants, or wound dressings with antimicrobial properties. The biological synthesis of nanoparticles relies on the metabolic activity of bacteria, which naturally reduce metal ions to their zero-valent forms. These reduced ions nucleate and subsequently lead to nanoparticle formation (Iravani et al., 2020). Notably, certain bacterial species can synthesize nanoparticles even in highly toxic environments with elevated concentrations of heavy metal ions, converting them into less toxic nanoparticle forms (Kapoor et al., 2021). In such cases, nanoparticle biosynthesis likely serves as a cellular defence mechanism against the harmful effects of these xenobiotics. In biosynthesis processes, various bacterial components can be employed, including whole cells, cell-free supernatants obtained after centrifugation, or extracts from lysed cells (Tab. 1-3). Probiotic bacteria, including LAB strains, are easy to culture and do not require advanced laboratory infrastructure. They grow efficiently on simple nutrient media, which significantly reduces production costs. The use of whey as an alternative substrate to conventional microbiological media has also yielded promising results (El Fadly et al., 2024).

Table 1. Biological synthesis of metal nanoparticles using whole cultures of probiotic bacteria or their post-culture supernatants – examples.

Bacterial species	Synthesized nanoparticles	Process conditions	Product characteristics	Literature
Bifidobacterium bifidum	TiO_2NPS	the supernatant was added to the TiO_2 solution and stirred for 30 minutes, then incubated at 37 °C for 48 hours.	average size 81nm, oval shape, antimicrobial activity	Ibrahem et al., 2020
Lactobacillus delbruecki	AgNPs	extracellular synthesis was carried out using bacterial culture and silver nitrate solution at 37°C. The nanoparticles were separated by centrifugation.	sizes of 54–113nm, spherical shape, antimicrobial activity	Saravanan et al., 2011
Lactobacillus casei	SeNPs	sodium selenite (Na_2SeO_3), 200 mg/l was added to the bacterial culture in MRS broth medium for 48 h at 37 °C	spherical SeNPs with maximum size distribution at 100 nm, synergic effect of SeNPs and LAB against Cd toxicity.	Laslo et al., 2022
Lactobacillus paracasei	SeNPs	Na_2SeO_3solution was mixed with bacterial pellet and incubated at 35 °C for 40 h	average size 56.91nm hexagonal, antifungal	El-Saadony et al., 2021
Lactobacillus bulgaricus	AgNPs	silver nitrate was mixed with cell-free culture supernatant. The solution was incubated overnight at room temperature.	sizes of 54–113nm, spherical shape, antimicrobial activity	Naseer et al., 2021
Lactobacillus plantarum	ZnONPs	solution of zinc sulfate was added to the *L. plantarum* culture. The mixture was heated in a water bath to approximately 80 °C for 10 minutes. The suspension was then left to precipitate at 37 °C for 12 hours.	sizes of 7-19nm, hexagonal or spherical shape, stable nanoparticles with a protein-based protective coating	Selvarajan, Mohanasrinivasan, 2013

Nanoparticle biosynthesis using probiotics takes place in aqueous environments, eliminating the need for organic solvents or harsh chemical reagents. The synthesis can occur both intracellularly and extracellularly.

In intracellular synthesis, a solution containing metal ions is introduced to bacterial cells, which take up the ions. Inside the cytosol and along the cytoplasmic membrane, enzymatic systems reduce the metal ions to their elemental form (Korbekandi et al., 2012). The formed metal atoms associate, resulting in the formation of stable nanoparticle structures within the cytoplasm. In extracellular synthesis, the nanoparticles produced intracellularly are secreted outside the cell.

The primary enzymes involved in the reduction process are NADPH-dependent reductases, such as nitrate reductases (Ali et al., 2019). Additionally, the reduction of metal ions can occur through intracellular reducing agents, including sulfur-containing amino acids (cysteine, methionine), thiol-containing proteins and peptides, as well as cell wall polysaccharides. These metabolites may also contribute to stabilizing the forming nanoparticles, influencing their size, shape, and physicochemical properties (Prakash et al., 2010). Electron microscopy analyses have demonstrated that silver nanoparticles are synthesized on the inner side of the cell membrane, within the cytoplasm, and outside the cells (Behzadi et al., 2017). The extracellular synthesis process is more advantageous regarding final product recovery, as it does not require cell disruption (Table 1). For biological nanoparticle synthesis, the post-culture supernatant obtained by removing cells from the culture medium via centrifugation - can also be employed (Saifuddin et al., 2009; Prakash et al., 2010). Bioactive molecules present in the supernatant participate in the reduction of metal ions and the subsequent formation of nanoparticles. Nanoparticles can also be synthesized using cell extracts obtained after cell wall disruption, such as mechanical lysis of microbial cells (Table 2). These extracts contain enzymes and metabolites that act as reducing and stabilizing agents during nanoparticle biosynthesis (Escárcega-González et al., 2018). Utilizing post-culture fluids or cell extracts allows for more precise control over the synthesis process and facilitates easier purification of the resulting nanoparticles.

Various species of probiotic bacteria are employed in the synthesis of nanoparticles such as silver, zinc oxide, copper oxide, and selenium (Tables 1-3). These processes occur under mild conditions - using gentle reagents, at room temperature, and at neutral pH and can take place extracellularly, intracellularly, or through the use of cell-free fluids or extracts. The nanoparticles produced through these methods can be functionalized with bacterial-derived components, such as fragments of cell membranes, which significantly enhance their stability in biological systems and reduce immunological responses (Shanmugam et al., 2023; Markus et al., 2023).

Table 2. Examples of nanoparticle synthesis using whole probiotic bacteria cells or cell extracts.

Bacterial species	Synthesized nanoparticles	Process conditions	Product characteristics	Literature
Lactobacillus kimchicus	AuNPs	the bacterial cells were centrifuged, and the biomass was mixed with gold salts. The mixture was incubated at 30 °C with shaking. The cells were then ultrasonicated and centrifuged. AuNPs were collected by centrifugation and purified with 80% methanol.	sizes of 5-30nm, spherical shape, non-toxic to murine macrophage, free radical scavengers against 2,2-diphenyl-1-picrylhydrazyl (DPPH)	Markus et al., 2023
Lactobacillus fermentum	ZnONPS	bacterial cells with the medium were mixed with precursors: zinc acetate and sodium hydroxide. The mixture was incubated for 10 minutes at room temperature, after which the pellet was washed, and the cells were disrupted by ultrasound. Nanoparticles were then separated by centrifugation.	size 90 -100nm, antimicrobial activity, highest level of antimicrobial activity was noted against a *Vibrio harveyi* strain	Shanmugam et al., 2023
Lactobacillus casei	ZnO NPs	0.1 M $ZnSO_4 \cdot H_2O$ solution and the post-culture supernatant were added to the flask, followed by heating in a water bath at 80°C for 5–10 minutes and incubation at 37°C for 12 hours	moderately stable, hexagonal phase, roughly spherical with maximum particles in size range within 7–19 nm in diameter.	Salman,Taj Al-Deen 2019
Lactobacillus plantarum	CS-LP-AgNPs encapsulated with chitosan	cell extracts were incubated with AgNO3 solution for 48 h at room temperature in the dark. AgNPs were encapsulated with chitosan	spherical shape, size from 15.3 nm to 55.0nm, crystalline structure, strong anti-cancer properties	Ravi, et al., 2024
Lactobacillus fermentum	Fe_2O_3NPs	the cytoplasmic extract of *Lactobacillus fermentum*, obtained via the freeze-thaw method, was combined with a 10^{-3} M iron(III) sulfate solution at a 10% (v/v) ratio and incubated for three weeks at 37 °C under 5% CO_2.	sizes from 10-15nm, spherical shape, environmentally friendly,	Fani et al., 2019
Lactobacillus plantarum	TeNPs	the bacterial cells were grown in MRS medium supplemented with K_2TeO_3	the average size of 45.7nm , spherical antihyperlipidemic activity	Mirjani et al., 2015
Lactobaciluus acidophilus	AgNPs	the bacterial cells were suspended in water. The cell extract was obtained by filtration. Silver nitrate was added to the extract and stirred at 30 °C. Nanoparticles were then centrifuged and dried.	sizes of 45- 60nm, spherical shape, non-toxic to genomic DNA	Namasivayam et al, 2010
Lactobacillus casei subsp. *casei*	CuONPs	the bacterial cells and 1 mM $CuSO_4$ were mixed and incubated at 37 °C for 48 hours.	spherical morphology, a size range of 40-110 nm, antibacterial activity, anticancer	Kouhkan et al., 2019

Moreover, these bacterial components can confer additional functionalities to the nanoparticles, including selectivity toward cancer cells, the ability to cross the blood-brain barrier, enhanced antioxidant activity, and antimicrobial or antifungal properties (Namasivayam et al. 2010; Saravanan et al., 2011; Markus et al., 2023; Anjana et al.2025). An additional advantage of biological synthesis is the ability to control the size and shape of the resulting nanoparticles by manipulating culture parameters such as pH, incubation time, temperature, and medium composition. This enables the production of highly specialized nanomaterials tailored for specific applications. Due to their biological origin, nanoparticles synthesized by probiotic bacteria are largely bioavailable and more readily transformed within environmental or physiological systems. Microorganisms can modify nanoparticles through processes such as dissolving metal oxides and reconstituting metallic nanoparticles into ionic forms. Furthermore, probiotic bacteria secrete stabilizing substances - including lipid membranes, lipopolysaccharides, and surface proteins - that contribute to nanoparticle stability. Proteins present in the culture environment or on cell surfaces can also affect the properties of metallic nanoparticles by forming a so-called "protein corona" on their surfaces (Duran et al., 2015). Protein molecules adsorb onto the surface of metal nanoparticles; if the affinity is high, a "hard corona" forms, while lower-affinity interactions lead to dynamic binding and unbinding, resulting in a loosely associated "soft corona." Bacterial metabolites additionally prevent particle aggregation and endow nanoparticles with bioactive properties. Consequently, these nanoparticles exhibit not only desirable chemical and physical characteristics but also increased biocompatibility, making them well-suited for integration into biological systems (Escárcega-González et al., 2018; Ali et al., 2019).

Particularly significant in the production of protein-coated nanoparticles are bacteriocins, low-molecular-weight proteins with antimicrobial properties (Table 3). A wide variety of bacteria produce numerous bacteriocins, which are classified into groups based on their molecular weights, sizes, modes of action, producing organisms, and spectrum of activity. Both Gram-positive and Gram-negative bacteria can produce bacteriocins; however, lactic acid bacteria are the primary producers (Mossallam et al., 2014). The most promising bacteriocins are those produced by LAB. Bacteriocins from Gram-positive bacteria - which are more numerous and diverse than those from Gram-negative bacteria are comparable to the antimicrobial peptides produced by eukaryotes. They typically range in size from 2 to 6 kDa and are cationic, amphiphilic, and membrane-permeable peptides (Ahmed et al., 2023). Nanoparticles conjugated with bacteriocins usually exhibit stronger antibacterial properties than either nanoparticles or bacteriocins alone, offering a promising approach for innovative food preservation techniques and the treatment of life-threatening infectious diseases (Table 3).

Table 3. Conjugates of Metal Nanoparticles with Bacteriocins Produced by Probiotic Bacteria.

Bacterial species producent bacteriocin	Conjugates	Process conditions	Product characteristics	Literature
L. sakei	bacteriocins - AgNPs	the bacteriocin of *L. sake*i, in the presence of silver nitrate and $NaBH_4$ under visible light for 30 to 48 hours,	forms spherical or polyhedral silver nanoparticles conjugated with the bacteriocin, ranging in size from 20.20–60.61 nm to 28.14–51.41 nm. These conjugates exhibit stronger bacteriostatic and bactericidal activity than either the bacteriocin or silver nanoparticles (AgNPs) alone.	Youssef et al., 2022
Lactobacillus acidophilus	Bac10307-AgNP	$AgNO_3$ was mixed with bacteriocin 10307 at room temperature for 1 hour and then exposed to UV light. The mixture was centrifuged to remove silver ions and unbound bacteriocin..	spherical nanoparticles with irregular shapes and varying sizes, ranging in diameter from 9 to 20 nm, exhibited stronger bactericidal, antioxidant, and anticancer properties compared to nanoparticles or bacteriocin alone.	Siddiqui et al., 2023
Lactobacillus plantarum isolated from raw milk	Bac23-capped AgNPS	silver nitrate ($AgNO_3$) was mixed with purified Bac23 and incubated for 1 hour, followed by UV irradiation. The solution was centrifuged to remove unbound silver ions and bacteriocin.	irregular, spherical in shape, and varied in size, stronger antibacterial activity compared to the individual components	Sidhu, Nehra, 2021
L. pentosus S6,	bacteriocins - AgNPs	the purified bacteriocin was mixed with a 0.1 mM	the synthesized silver nanoparticles (AgNPs) were	Sharma et al., 2023

Lactococcus crustorum F11, Lactococcus spicheri G2		silver nitrate solution and incubated at 30°C for 24 hours.	round or spherical, appearing either as individual particles or in clusters. The mean particle size was approximately 20 nm for G2 AgNPs, 10 nm for S6 AgNPs, and 5 nm for F11 AgNPs, respectively. Bacteriocin-AgNP conjugates demonstrated greater antibacterial efficacy compared to either component used alone.	
Lactobacillus acidophilus CH1	Bacteriocin-AuNPs	a mixture of AuNPs was combined with a bacteriocin solution at a 1:18 ratio (v/v) at pH 7.5 and incubated for 10–15 minutes. Unbound bacteriocin was removed by centrifugation.	spherical smooth surfaced nanoparticles with average size 18.3–22 nm (mean particle size of 20.15 nm), stronger anti-microsporidial effects bacteriocin/Au-NPs than that of pure bacteriocin	Mossallam et al., 2014
Lactobacillus plantarum	plantaricin-incorporated silver nanoparticles PL-AgNPs	PL-AgNPs were prepared at room temperature by successive addition of freshly prepared 3 mM silver nitrate (15 µL) and 0.6 mM NaBH4 (100 µL) to 1 mL extracted plantaricin solution in water, natural visible light, 30 min,	PL-AgNPs particles have approximately spherical shapes with approximately mean diameter of 78.7 nm, enhanced antibacterial activity against tested foodborne pathogenic bacteria,	Amer 2021
Lactococcus lactis	AgNP-nisin conjugates	a solution of commercial nisin was mixed with commercial AgNPs at a 1:0.25 ratio and incubated overnight with shaking. Unbound nisin and nanoparticles were separated by centrifugation.	enhanced antibacterial properties against both Gram-positive and Gram-negative bacteria, with increased antimicrobial activity against nisin-resistant strains.	Arakha et al.,2016;

Therefore, combinations of bacteriocins and nanosized metals are expected to exert a synergistic effect on antibacterial activity (Thirumurugan et al., 2013; Siddiqui et al., 2023). Nanotechnology enhances antimicrobial activity, improves the physicochemical properties of bacteriocins, increases their applicability, broadens their antimicrobial spectrum, and promotes stability (Youssef et al., 2022, Zelin et al. 2024). Bacteriocins in nanoformulations have been experimentally used in wound treatment; for example, silver nanoparticles conjugated with nisin have been applied to treat S. epidermidis skin infections in mice. Nanofibers composed of ethylene oxide and polylactic acid, along with nisin or plantaricin, have shown potential in treating deep, infected wounds caused by *S. aureus*. Several bacteriocins are also used as probiotics in place of antibiotics, as they play a key role in strengthening the immune system (Amer, 2021; Arakha et al., 2016; Sharma et al., 2023).

3. Applications and potential risks associated with the presence of metal nanoparticles synthesized using probiotic bacteria

Nanoparticles synthesized with the involvement of probiotic bacteria have potential applications across various fields, with their antibacterial and antifungal properties being among the most extensively studied (Saravanan et al., 2011; Kouhkan et al., 2019; Ibrahem et al., 2020; Naseer et al., 2021; Shanmugam et al., 2023). For example, biogenic copper oxide nanoparticles (CuONPs) have been shown to induce DNA damage and alter membrane permeability in pathogenic bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Kouhkan et al., 2019). Similarly, TiO_2 nanoparticles produced using *Bifidobacterium bifidum* extract demonstrated bactericidal activity against *P. aeruginosa, Acinetobacter baumannii,* and *Klebsiella pneumoniae* (Ibrahem et al., 2020). Biogenic silver nanoparticles (AgNPs) have also been effective in inhibiting the growth of *Salmonella typhi*, *S. aureus, S. epidermidis* (Naseer et al., 2021). Selenium nanoparticles (SeNPs) synthesized by *Lactobacillus paracasei* significantly suppressed the growth of fungal species such as *Candida albicans, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis, Fusarium oxysporum,* and *F. solani* (El-Saadony et al., 2021).Thanks to their antimicrobial properties, these nanoparticles have found applications in medicine, particularly in wound dressings and implant surface coatings (Das et al., 2022; Vera-Santander et al., 2023). They also serve as carriers for anticancer drugs, antibiotics, and anti-inflammatory compounds (Vera-Santander et al., 2023). Silver and zinc oxide nanoparticles are especially effective in disrupting bacterial biofilms, making them useful for treating chronic infections and non-healing wounds. In the food industry, nanotechnology is more frequently applied in packaging rather than processing. Probiotic-based nanoparticles can be used to develop active packaging that extends shelf life or functions as natural preservatives with antimicrobial and antifungal effects (Das et al., 2022). In environmental applications, biogenic nanoparticles may be employed to remove pollutants such as heavy metals, industrial dyes, and pesticides. Their surfaces can be functionalized to bind specific chemical compounds, making them useful for purifying groundwater and wastewater. Looking ahead, the biosynthesis of nanoparticles using probiotic bacteria could see broader application, particularly in targeted therapy, personalized nanomedicine, tissue engineering, and the development of artificial organs (Inchingolo et al., 2023). Despite their many advantages, biologically synthesized nanoparticles—like those produced industrially - may also pose potential risks to human and animal health. Their long-term environmental impact remains insufficiently understood. Therefore, further research is necessary to assess the risks associated with their use and to ensure safety for both humans and the environment.

4. References:

[1] Ahmed, M. E., Al-Awadi, A. Q., Abbas, A. F. (2023). Focus on synergistic bacteriocin-nanoparticles enhancing antimicrobial activity assay. Mikrobiol. J. 85(6), 95-104.
[2] Ali, J., Ali, N., Wang, L., Waseem, H., Pan, G. (2019). Revisiting the mechanistic pathways for bacterial mediated synthesis of noble metal nanoparticles. J. Microbiol. Meth. 159, 18-25.
[3] Amer, S. A., Abushady, H. M., Refay, R. M., and Mailam, M. A. (2021). Enhancement of the antibacterial potential of plantaricin by incorporation into silver nanoparticles. J. Genet. Eng. Biotechnol. 19, 13.
[4] Anjana, M Abiyoga, M., Rajalakshmy, K., Kumari, P. Saravana. (2025). Lactobacillus sp., mediated synthesis of zinc oxide nanoparticles and their anticancer activity on a549 lung cancer cell lines. Asian J. Chem. 37. 661–672. 10.14233/ajchem.2025.33297.
[5] Arakha, M., Borah, M.S., Saleem, M., Jha N.A., Jha S. (2016). Interfacial assembly at silver nanoparticle enhances the antibacterial efficacy of nisin. Free Radic Biol Med. 101:434–445.
[6] Bahrulolum, H., Nooraei, S., Javanshir, N., Tarrahimofrad, H., Mirbagheri, V. S., Easton, A. J., Ahmadian, G. (2021). Green synthesis of metal nanoparticles using microorganisms and their application in the agrifood sector. J. Nanobiotechnol. 19, 1-26.
[7] Behzadi, S., Serpooshan, V., Tao, W., Hamaly, M. A., Alkawareek, M. Y., Dreaden, E. C., Mahmoudi, M. (2017). Cellular uptake of nanoparticles: journey inside the cell. Chem. Soc. Rev., 46(14), 4218-4244.
[8] Das, T. K., Pradhan, S., Chakrabarti, S., Mondal, K. C., Ghosh, K. (2022). Current status of probiotic and related health benefits. Appl. Food Res. 2(2), 100185.
[9] Durán, N., Silveira, C. P., Durán, M., & Martinez, D. S. T. (2015). Silver nanoparticle protein corona and toxicity: a mini-review. J. Nanobiotech. 13(1), 55.
[10] El-Fadly, E.B., Salah, A.S., Abdella, B. (2024). Mapping a sustainable approach: biosynthesis of lactobacilli-silver nanocomposites using whey-based medium for antimicrobial and bioactivity applications. Microb. Cell Fact **23**, 195.
[11] El-Saadony, M.T., Saad, A.M., Taha, T.F., Najjar, A.A., Zabermawi, N.M., Nader, M.M., AbuQamar, S.F., El-Tarabily, K.A., Salama, A., (2021). Selenium nanoparticles from Lactobacillus paracasei

HM1 capable of antagonizing animal pathogenic fungi as a new source from human breast milk. Saudi J.Biol.Sci. 28.

[12] Escárcega-González, C. E., Garza-Cervantes, J. A., Vázquez-Rodríguez, A., Morones-Ramírez, J. R. (2018). Bacterial exopolysaccharides as reducing and/or stabilizing agents during synthesis of metal nanoparticles with biomedical applications. Int. J. Pol. Sci. 2018(1), 7045852.

[13] Fani, M., Ghandehari, F., Rezaee, M. J. J. M. C. S. (2018). Biosynthesis of iron oxide nanoparticles by cytoplasmic extract of bacteria lactobacillus fermentum. J. Med. Chem. Sci. 1(2), 28-30.

[14] Ibrahem, K. H., Ali, F. A., Sorchee, S. M. A. (2020). Biosynthesis and characterization with antimicrobial activity of TiO2 nanoparticles using probiotic Bifidobacterium bifidum. Cell. Mol. Biol. 66(7), 111-117.

[15] Inchingolo, F., Inchingolo, A. M., Malcangi, G., De Leonardis, N., Sardano, R., Pezzolla, C., Dipalma, G. (2023). The benefits of probiotics on oral health: Systematic review of the literature. Pharm. 16(9), 1313.

[16] Iravani, S., Varma, R. S. (2020). Bacteria in heavy metal remediation and nanoparticle biosynthesis. ACS Sustain. Chem. Eng. 8(14), 5395-5409.

[17] Kapoor, R. T., Salvadori, M. R., Rafatullah, M., Siddiqui, M. R., Khan, M. A., Alshareef, S. A. (2021). Exploration of microbial factories for synthesis of nanoparticles–a sustainable approach for bioremediation of environmental contaminants. Front. Microb. 12, 658294.

[18] Korbekandi H, Iravani S, Abbasi S., (2012). Optimization of biological synthesis of silver nanoparticles using lactobacillus casei subsp. casei. J. Chem. Technol. Biotechno. 2012;87:932–7.

[19] Kouhkan, M., Ahangar, P., Babaganjeh, L.A., Allahyari-Devin, M., (2019). Biosynthesis of copper oxide nanoparticles using Lactobacillus casei subsp. casei and its anticancer and antibacterial activities. Curr. Nanosci.16 (1), 101-111.

[20] Markus, J., Mathiyalagan, R., Kim, Y. J., Abbai, R., Singh, P., Ahn, S., ... Yang, D. C. (2016). Intracellular synthesis of gold nanoparticles with antioxidant activity by probiotic Lactobacillus kimchicus DCY51T isolated from Korean kimchi. Enz. Microb. Tech. 95, 85-93.

[21] Mirjani, R., Faramarzi, M.A., Sharifzadeh, M., Setayesh, N., Khoshayand, M.R., Shahverdi, A.R., (2015). Biosynthesis of tellurium nanoparticles by Lactobacillus plantarum and the effect of nanoparticle-enriched probiotics on the lipid profiles of mice. IET Nanobiotech. 9 (5), 300-305.

[22] Mossallam, S. F., Amer, E. I., Diab, R. G. (2014). Potentiated anti-microsporidial activity of Lactobacillus acidophilus CH1 bacteriocin using gold nanoparticles. Exp. Parasitol. 144, 14-21.

[23] Namasivayam, S. K. R., Gnanendra, E. K., Reepika, R. (2010). Synthesis of silver nanoparticles by Lactobaciluus acidophilus 01 strain and evaluation of its in vitro genomic DNA toxicity. Nano-Micro. Letters, 2, 160-163.

[24] Naseer, Q. A., Xue, X., Wang, X., Dang, S., Din, S. U., Kalsoom, Jamil, J. (2021). Synthesis of silver nanoparticles using Lactobacillus bulgaricus and assessment of their antibacterial potential. Braz. J. Biol. 82, e232434.

[25] Prakash, A., Sharma, S., Ahmad, N., Ghosh, A., Sinha, P. (2010). Bacteria mediated extracellular synthesis of metallic nanoparticles. Int. Res. J. Biotechnol. 1(5), 071-079.

[26] Purohit, J., Chattopadhyay, A., Singh, N. K. (2019). Green synthesis of microbial nanoparticle: approaches to application. Microbial Nanobionics: Volume 2, BRA 35-60.

[27] Ravi, A. K., Muthukrishnan, S., Gunasangkaran, G., Arumugam, V. A., Shanmugam, V., Sakthivel, K. M., Kaliyaperumal, A. (2024). Biosynthesis of chitosan encapsulated silver-nanoparticles using probiotic-Lactobacillus plantarum strain and it's in vitro anticancer assessment on HeLa cells. Med. Microecol. 22, 100117.

[28] Saifuddin, N., Wong, C. W., Yasumira, A. N. (2009). Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. J. Chem. 6(1), 61-70.

[29] Salman, Z., Taj Al-Deen, W. R. (2019). Biosynthesis of zinc oxide nanoparticles by Lactobacillus casei. Biochem. Cell. Arch. 19, 2259-2265.

[30] Saravanan, M., Nanda, A., Kingsley, S. J. (2011). Lactobacillus delbrueckii mediated synthesis of silver nanoparticles and their evaluation of antibacterial efficacy against MDR clinical pathogens. In International Conference on Nanoscience, Engineering and Technology (ICONSET 2011) (pp. 386-390). IEEE.

[31] Selvarajan, E., Mohanasrinivasan, V. J. M. L. (2013). Biosynthesis and characterization of ZnO nanoparticles using Lactobacillus plantarum VITES07. Mater. Lett. 112, 180-182.

[32] Shanmugam, R., Munusamy, T., Jayakodi, S., Al-Ghanim, K. A., Nicoletti, M., Sachivkina, N., Govindarajan, M. (2023). Probiotic-bacteria (Lactobacillus fermentum)-wrapped zinc oxide nanoparticles: biosynthesis, characterization, and antibacterial activity. Fermentation. 9(5), 413.

[33] Sharma, S., Sharma, N., & Kaushal, N. (2023). Utilization of novel bacteriocin synthesized silver nanoparticles (AgNPs) for their application in antimicrobial packaging for preservation of tomato fruit. Front. Sustain. Food Syst. 7, 1072738.

[34] Siddiqui, A.J., Patel, M., Adnan, M., Jahan, S., Saxena, J., Alshahrani, M.M., Abdelgadir, A., Bardakci, F., Sachidanandan, M., Badraoui, R. (2023) Bacteriocin-nanoconjugates (Bac10307-AgNPs) biosynthesized from Lactobacillus acidophilus-derived bacteriocins exhibit enhanced and promising biological activities. Pharmaceutics.15, 403.

[35] Sidhu, P. K., Nehra, K. (2021). Purification and characterization of bacteriocin Bac23 extracted from Lactobacillus plantarum PKLP5 and its interaction with silver nanoparticles for enhanced antimicrobial spectrum against food-borne pathogens. LWT. 139, 110546.

[36] Thirumurugan, A., Ramachandran, S., Gowri, A. S. (2013). Combined effect of bacteriocin with gold nanoparticles against food spoiling bacteria-an approach for food packaging material preparation. Int. Food Res. J. 20, 1909–1912.

[37] Vera-Santander, V. E., Hernández-Figueroa, R. H., Jiménez-Munguía, M. T., Mani-López, E., López-Malo, A. (2023). Health benefits of consuming foods with bacterial probiotics, postbiotics, and their metabolites: a review. Molecules, 28(3), 1230.

[38] Youssef, G. A., Farag, A. M., Saad, A. H., El-Shenawy, M. A., El-Aassar, S. (2022). Production, characterization and biosynthesis of silver nanoparticles bacteriocin of Lactobacillus sakei isolated from fish samples. Egypt. J. Aquat. Biol. Fish. 26(4), 135.

[39] Zelin, G., Yu W., Pinglan Li, Nan Shang. (2024). Lactobacillus plantarum derived nanoparticles deliver class IIa bacteriocin as a potential natural food preservatives. LWT, Volume 207, 116675.

[40] Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M., Harris, H. M., Mattarelli, P., Lebeer, S. (2020). A taxonomic note on the genus Lactobacillus: description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. Int. J. Syst. Evol. Microbiol. 70(4), 2782-2858.

Cross-sectional study of body composition using bioelectrical impedance among high school students in Lublin (2023–2025)

Justyna Libera[1*], Karolina Nowosad[2], Katarzyna Iłowiecka[3], Dorota Teterycz[1], Paulina Łysakowska[1]

[1]*Department of Engineering and Cereals Technology*
Faculty of Food Science and Biotechnology
University of Life Sciences in Lublin, Skromna 8, 20-704 Lublin, Poland
[2]*Department of Biotechnology, Microbiology and Human Nutrition*
Faculty of Food Science and Biotechnology
University of Life Sciences in Lublin, Skromna 8, 20-704 Lublin, Poland
[3]*Department of Food and Nutrition*
Faculty of Medical Sciences
Medical University of Lublin, Chodzki 4a, 20-093 Lublin, Poland
**corresponding author: justyna.libera@up.lublin.pl*

Abstract

The aim of the study was to assess body composition using bioelectrical impedance analysis (BIA) among high school students (n = 135) in Lublin. The research was conducted at the university's dietetic counselling center between October 2023 and February 2025. Participants' age breakdown: 27% (ages 15–16), 63% (17–18), and 10% (19); by gender: 39% male and 61% female. Body composition was assessed using a foot-to-hand BIA analyzer (SECA mBCA515, GmbH & Co. KG, Hamburg, Germany). The parameters evaluated included relative and absolute fat mass, fat-free mass and skeletal muscle mass. In addition, body mass index (BMI), fat mass index (FMI) and fat-free mass index (FFMI) were calculated. Results showed that 41% of participants had a normal BMI (18.5–24.9 kg/m²), 33% were overweight, 9% were obese, and 18% were underweight. Although underweight was more prevalent among girls than boys, more than 23% of males had an FFMI below 15 kg/m², indicating too low lean mass. The average absolute fat mass was higher in females (21 kg) than in males (18 kg), while the mean fat-free mass, expressed as FFMI, was similar in all sexes and averaged approximately 16 kg/m². Elevated fat mass, defined as an FMI above 8.7 kg/m², was observed in 44% of female participants. Skeletal mass was proportional to total body mass, and was highest among participants with a BMI above the normal range, averaging 21 kg, compared to 19 kg in those with normal BMI and 15 kg in underweight individuals.
In conclusion, BIA is a rapid, non-invasive, and effective method for assessing body composition and represents a promising screening tool for identifying body weight abnormalities in adolescents. These findings may be highly valuable in practice for health promotion and for monitoring the nutritional status of individuals.

Keywords: Body composition, bioimpedance, BMI, fat mass index, free-fat mass index, adolescents

1. Introduction

The most commonly used indicator for classifying body weight is the Body Mass Index (BMI). It is calculated by dividing body weight in kilograms by height in meters squared (kg/m^2). According to the World Health Organization (WHO), normal weight is defined as a BMI between 18,5 and 24,9 kg/m^2. BMI is easy to calculate, as it requires only basic anthropometric measurements and no specialized equipment. However, a significant limitation of this method is its inability to distinguish between fat mass and lean body mass. It can also be unreliable in individuals undergoing dialysis, due to fluctuations in body water content. Although BMI is correlated with various anthropometric indicators, body fat percentage may provide a more accurate assessment of abnormal body weight than the WHO classifications based on BMI [WHO 2005].

Body composition describes the relative contribution of fat, muscle, bone and water to an individual's body volume. Several body composition techniques are available, based on assumptions of weight (hydrostatic weighing), water content (isotope dilution), volume (air displacement plethysmography), energy attenuation (Dual-Energy X-Ray Absorptiometry; DXA), and imaging techniques like computer tomography (CT) and magnetic resonance imaging (MRI) [Kasper et al. 2021, Liu et al. 2024, Moonen and Van Zanten 2021].

Gold standard techniques to determine body composition, such as hydrostatic weighing and deuterium dilution, are costly and time-consuming [Lee et al. 2008]. Therefore, alternative non-invasive methods of body composition assessment that are easier and safer to administer have been developed. DXA provides a reliable estimate of total body composition. This technique is quick, accurate, and carries a low risk of exposure to radiation [Bredella et al. 2013, Namwongprom et al. 2013, Norcross and Van Loan 2004]. Hydro-densitometry is oldest indirect method of underwater weighing, using assumed densities. This technique is based upon the understanding that when an object is submerged underwater, the measurement of the 'buoyant force' is deemed to be equal to the weight of the water that it displaces [Kasper et al. 2021]. Air displacement

plethysmography (ADP) is an alternative to hydrostatic weighing and has more practicality in applied sport, where instead of water, air is utilized to measure body density. Some consider ADP to overcome several of the aforementioned limitations of hydro-densitometry [Gibby et al. 2017].
Bioelectric impedance analysis (BIA) is quick, non-invasive and relatively inexpensive, which is why it currently seems the most feasible body composition measurement technique [Day et al. 2018]. BIA relies on the differences in the impedance of an electric current between adipose tissue and fat-free body mass. Both adipose tissue and extracellular water lack capacitive reactance (Xc) but exhibit active electrical resistance (R) [Shafer et al. 2005]. Reactance is generated by the cell membranes of tissues with high water content and primarily influences the phase shift of the applied alternating current, whereas resistance causes a voltage drop [Lee et al. 2008]. BIA represents the total resistance of the body to an alternating current [Moonen and Van Zanten 2021]. Resistance depends on the electrical conductivity of the current through body water and the electrolytes dissolved therein. Reactance, in turn, is produced by tissues that function as capacitors. The third component of impedance is the phase angle (φ), defined as the phase shift between the maximum current and maximum voltage values due to the capacitive properties of body tissues [Barbosa-Silva et al. 2005, Rosa et al. 2025].
The aim was a cross-sectional study of body composition using bioelectrical impedance with the SECA mBCA 515 analyzer among the high school-aged population of Lublin.

2. Materials and Methods

The participants of the study were students at nine high schools located within the Lublin area, aged between 15 and 19 years, interested in the free body composition assessment (BIA). They were recruited by dietetics students from the University of Life Sciences in Lublin. The BIA tests were conducted by dietetics at the university's counselling center between October 2023 and February 2025. Prior to participation, all individuals were informed about the study procedures and provided with detailed explanations regarding possible health implications related to the examination. Written informed consent was obtained from all participants; for minors, written consent from a parent or legal guardian was mandatory. This study was reviewed and approved by the Ethics Committee of Scientific Research Involving Humans at University of Life Sciences in Lublin (Resolution No. KE/10/2023) and conducted in accordance with the ethical principles of the Declaration of Helsinki. The study included a total of 135 participants, of whom 61% were female. The age distribution was as follows: 15–16 years (27%), 17–18 years (63%), and 19 years (10%).

Body composition was assessed using a foot-to-hand BIA analyzer (SECA mBCA515, GmbH & Co. KG, Hamburg, Germany). The parameters evaluated included relative and absolute fat mass, fat-free mass and skeletal muscle mass. Prior to each measurement, the device was disinfected to ensure hygiene standards. The examination lasted approximately 16 seconds and involved the participant standing barefoot on the analyzer while holding the electrodes with their hands to initiate the measurement. Subsequently, each participant's height was measured using a SECA stadiometer, and waist circumference was recorded using a SECA measuring tape. All measurements at the dietetic at the university's counselling center were performed by qualified personnel—registered dietitians. Participants received a detailed report of their test results along with an interpretation of their body composition data. If the results significantly deviated from established normative values, participants were offered a free consultation with a dietitian. Those with results within the normal range were invited to undergo a follow-up assessment one year later.

Based on the BIA reports, body mass index, fat mass index (FMI), and fat-free mass index (FFMI) were calculated [Bosy-Westphal et al. 2017]. The collected data were analyzed using ANOVA, , taking into account participant age and BMI. Statistical calculations were conducted using Microsoft Office Excel 2013 (Microsoft Corporation, Redmond, WA, USA) and Statistica 10 (StatSoft Polska Sp. z o.o., Kraków, Poland). Post hoc analysis was performed using Tukey's test at $p \leq 0.05$.

3. Results and discussion

The results of body composition analysis performed using bioelectrical impedance analysis (BIA) among participants, divided into three age groups: 15–16, 17–18, and 19 years were presented in Table 1. Among adolescents aged 15–16 years (n = 36), male individuals were taller than females (172 cm vs. 164 cm). Although fat-free mass (FFM) was higher in males (49 kg) compared to females (45 kg), the absolute fat mass values were similar between genders. In the 17–18-year-old group (n = 85), sex differences in body composition were more pronounced. This is due to the dynamic phase of boys' development at this time. They were significantly taller (178 cm) than females (165 cm) Males also had substantially higher FFM and skeletal muscle mass compared to females. Despite higher FFM, males had lower absolute fat mass (19 kg) than females (23 kg). Waist circumference was in norm range (<80 cm for women, <94 cm for men). The 19-year-old subgroup (n = 14) followed similar trends. Males exhibited significantly greater height (181 ± 5 cm vs. 163 ± 6 cm), weight (71 kg vs. 56 kg), and FFM (51 kg vs. 40 kg) compared to females. Muscle mass was similar in males (21 kg) and females (19 kg).

The obtained results indicate that with the physiological development of adolescents, the disproportion in body height and weight between girls and boys increases, which is characteristic of the puberty period. Across all age groups, males demonstrated higher fat-free mass, skeletal muscle mass, than females, which is consistent with sex-based physiological differences in muscle development and body composition during adolescence. Notably, despite higher body weight in males, absolute fat mass was similar. These findings highlight the importance of using detailed body composition analysis rather than relying solely on weight or BMI, especially in adolescent populations where growth and development can significantly influence these metrics. The use of BIA offers valuable insight into both fat and lean mass, enabling more precise assessment of nutritional status and potential risk factors for obesity [Chula de Castro et al. 2018].

Table 1. BIA body composition analysis results based on participants' age. Data are presented as means ± standard deviations (X ± SD).

	Females	Males	All
Aged 15-16, years	*(n = 23)*	*(n = 13)*	*(n = 36)*
Weight, kg	62.64^a ± 15.11	67.31^a ± 9.28	64.33 ± 13.35
Height, cm	164^a ± 6	172^a ± 8	167 ± 7
Fat-free mass value, kg	44.83^a ± 5.03	48.66^a ± 5.92	46.22 ± 5.60
Fat mass value, kg	17.81^a ± 11.98	18.65^a ± 5.40	18.11 ± 10.02
Skeletal muscle mass, kg	18.13^a ± 4.03	18.87^a ± 3.76	18.43 ± 3.90
Waist circumference value, cm	80^a ± 9	82^a ± 14	80 ± 11
Aged 17-18, years	*(n = 53)*	*(n = 32)*	*(n = 85)*
Weight, kg	67.40^a ± 13.53	71.31^a ± 12.64	68.87 ± 13.26
Height, cm	165^a ± 5	178^a ± 7	170 ± 9
Fat-free mass value, kg	44.04^a ± 5.34	52.81^a ± 7.00	47.34 ± 7.35
Fat mass value, kg	23.36^a ± 9.93	18.50^a ± 8.41	21.53 ± 9.63
Muscle mass, kg	19.83^a ± 2.91	21.90^a ± 5.91	20.61 ± 4.37
Waist circumference value, cm	80^a ± 11	88^a ± 15	83 ± 13
Aged 19, years	*(n = 6)*	*(n = 8)*	*(n = 14)*
Weight, kg	55.73^a ± 14.4	70.08^a ± 9.11	63.93 ± 13.37
Height, cm	163^a ± 6	181^b ± 5	173 ± 10
Fat-free mass value, kg	40.14^a ± 6.40	51.13^a ± 4.51	46.42 ± 7.65
Fat mass value, kg	15.59^a ± 8.14	18.95^a ± 5.83	17.51 ± 6.84
Muscle mass, kg	18.88^a ± 4.00	21.49^a ± 4.34	20.37 ± 4.25
Waist circumference value, cm	71^a ± 11	81^a ± 11	77. 12

Explanatory notes: the same letter in the row means do not differ statistically among themselves by the Tukey test ($p \leq 0.05$)

A similar study was conducted among adolescents in the United States by Unick et al. [2006], in which the body composition of 13- to 18-year-olds (n = 77) was assessed using the Tanita foot-to-foot analyzer. The results showed that the percentage of body fat was significantly lower in males (14%) compared to females (26%). In our study, body fat mass was higher, at 26% in males and 31% in females. These differences may be attributed to the use of different body composition analyzers and variations in study conditions. In contrast, research conducted on healthy Taiwanese children by Lee et al. (2017) showed that the percentage of fat-mass measured using BIA ranged on average from 19% to 24% in boys and from 20% to 25% in girls, depending on the body composition analyzer used.

Table 2. presents the results of body composition analysis (BIA) in the study participants (female n = 82, male n = 53), divided into four groups defined on the basis of BMI: underweight (< 18.49 kg/m^2), normal weight (18.50 – 24.99 kg/m^2), overweight (25.00 – 29.99 kg/m^2) and obese (> 30.0 kg/m^2). Results showed that 41% of

participants had a normal BMI, 18% were underweight, 33% were overweight and 9% were obese. In the female group, 72% of participants (n = 82) were categorized as either normal weight or overweight, indicating a predominance of these two BMI classifications. Among male participants (n = 53), the largest proportion—49%—were within the normal weight range. The BIA findings underscore the limitations of BMI as a sole indicator of nutritional and health status in adolescents [Yang et al. 2025]. BIA is an extremely useful tool for screening body composition in adolescents [Nie et al. 2025]. It can be used not only to diagnose malnutrition or obesity but also to assess the risk of metabolic syndrome, eating disorders and dermatology problems in young adults [Namwongprom et al. 2014, Štefanová et al. 2020, Pugliese et al. 2023].

Table 2. BIA body composition analysis results based on participants' BMI values. Data are presented as means ± standard deviations (X ± SD).

	Underweight	Normal	Overweight	Obese
Females	*(n = 15)*	*(n = 29)*	*(n = 30)*	*(n = 8)*
Weight, kg	$46.2^{a} \pm 4.8$	$60.0^{b} \pm 6.8$	$73.3^{c} \pm 5.0$	$90.1^{d} \pm 11.9$
BMI, kg/m^2	$17.2^{a} \pm 1.5$	$21.9^{b} \pm 1.7$	$26.9^{c} \pm 1.4$	$34.0^{d} \pm 4.2$
Fat-free mass value, kg	$38.3^{a} \pm 4.8$	$43.1^{ab} \pm 4.5$	$46.1^{bc} \pm 4.0$	$50.0^{c} \pm 3.3$
FFMI, kg/m^2 *(range: >15 kg/m^2)**	14.3 ↓	15.8	16.9	18.9
Fat mass value, kg	$7.8^{a} \pm 4.8$	$16.9^{b} \pm 4.9$	$27.2^{c} \pm 3.7$	$40.0^{d} \pm 10.6$
Fat mass value, %	16.6	27.8	37.1	43.9
FMI, kg/m^2 *(range: 3.8 – 8.7 kg/m^2)**	2.9 ↓	6.1	10.0 ↑	15.1↑↑
Skeletal muscle mass, kg	$15.0^{a} \pm 1.5$	$18.4^{b} \pm 2.2$	$21.2^{bc} \pm 2.7$	$23.1^{c} \pm 1.8$
Males	*(n = 9)*	*(n = 26)*	*(n = 14)*	*(n = 4)*
Weight, kg	$59.9^{a} \pm 4.9$	$67.0^{a} \pm 7.6$	$77.1^{b} \pm 6.2$	$94.5^{c} \pm 9.8$
BMI, kg/m^2	$17.9^{a} \pm 0.4$	$21.3^{b} \pm 1.5$	$25.6^{c} \pm 0.7$	$30.4^{d} \pm 0.3$
Fat-free mass value, kg	$48.1^{a} \pm 4.6$	$49.9^{a} \pm 5.7$	$56.2^{b} \pm 6.0$	$57.0^{b} \pm 8.2$
FFMI, kg/m^2 *(range: >17 kg/m^2)**	14.4 ↓	15.9 ↓	18.6	18.3
Fat mass value, kg	$11.7^{a} \pm 4.9$	$17.0^{b} \pm 3.7$	$20.9^{b} \pm 3.4$	$37.5^{c} \pm 4.4$
Fat mass value, %	19.4	25.3	27.2	39.8
FMI, kg/m^2 *(range: 1.2 – 5.6 kg/m^2)**	3.5	5.4	6.9 ↑	12.1 ↑↑
Skeletal muscle mass, kg	$15.8^{a} \pm 1.3$	$20.4^{b} \pm 3.5$	$22.7^{b} \pm 4.6$	$32.1^{c} \pm 6.2$

Explanatory notes: * the standard range is consistent with the recommendations of the manufacturer of the SECA mBCA 515 analyzer; ↓ - means low value, ↑ - means increased value, ↑↑ - means high value; the same letter in the row means do not differ statistically among themselves by the Tukey test ($p \leq 0.05$)

As expected, both body weight and BMI increased progressively across the BMI categories, from 46.2 kg (BMI: 17.2 kg/m²) in the underweight group to 94.5 kg (BMI: 30.4 kg/m²) in the obese group. These values confirm appropriate classification based on WHO BMI standards.

In the group of girls, both absolute and relative fat mass showed a significant increase in relation to BMI. Females classified as underweight had 7.8 ± 4.8 kg of fat mass (16.6% of body weight), whereas obese girls had 40.0 ± 10.6 kg, which constituted 43.9% of body weight. Fat-free mass also correlated with BMI, ranging from 38.3 ± 4.8 kg in underweight girls to 50.0 ± 3.3 kg in the obese group. The FFMI value is a very useful indicator for assessing body composition, correcting FFM for height. Importantly, the FFMI in the underweight group was 14.3 kg/m², below the acceptable threshold of >15 kg/m², indicating a low proportion of lean body mass, particularly skeletal muscle.

Participants in underweight males group showed significant deficits in fat-free mass, with FFMI well below the recommended minimum of 17 kg/m², indicating underdevelopment of lean tissue, especially muscle. Despite being underweight, fat mass values were not critically low, suggesting a possible imbalance between lean and fat mass typical of sarcopenic profiles or delayed muscular development. In second group (normal BMI) the average FFMI was still below the optimal cut-off for males, suggesting that some participants might have insufficient muscle mass relative to their height. Interestingly, the FMI was approaching the upper limit, indicating relatively high fat mass even in the absence of excess weight. This group may include 'normal-weight obesity' profiles—individuals with normal BMI but increased fat mass and reduced muscle tissue. This condition is associated with higher cardiometabolic risk despite a normal BMI.

Overweight participants exhibited adequate development of lean body mass (FFMI >17); however, their FMI values exceeded reference norms, indicating excessive fat accumulation. Potential contributing factors may include a positive energy balance resulting from excessive caloric intake, insufficient levels of physical activity, sedentary behaviors, and frequent consumption of energy-dense foods. Obese boys demonstrated markedly elevated fat mass, both in absolute and relative terms. Although their FFMI remained within the normal range, the disproportionately high FMI and body fat percentage reflect pronounced adiposity and a potentially increased risk of metabolic complications. This group also presented with the highest skeletal muscle mass (32.1 ± 6.2 kg), likely as a consequence of the greater mechanical load imposed by excess body weight. Nevertheless, this increased muscle mass should not be interpreted as improved physical fitness or functional capacity but rather as a passive adaptation to higher body mass [Mahmoud et al. 2021]. Changes in fat mass may be related to adipocyte hypertrophy due to intrinsic changes within these cells during maturation [Dipali et al. 2019]. Even among individuals classified as normal weight, imbalances between fat and lean mass were observed, which may predispose to long-term health risks [Shafer et al. 2008]. These results highlight the importance of incorporating comprehensive body composition indices, such as FFMI and FMI, into routine adolescent health assessments to enable the early detection of sarcopenia, excess adiposity, or concealed metabolic risk [Namwongprom et al. 2013]. Potential causes of low fat-free mass and muscle mass could be insufficient protein and energy intake, low engagement in resistance-based physical activity and rapid growth without proportional muscle gain. Because BMI does not distinguish between adipose tissue and muscle, BIA—along with derived indices such as FMI and FFMI—provides a more accurate assessment of body composition in adolescents. Participants classified as underweight exhibited deficiencies in both fat and lean tissue, pointing to a possible risk of undernutrition. Conversely, those with overweight and obesity had excessive fat mass, with FMI values significantly above reference norms. BIA can predict alterations in body composition effectively, even without strict protocols. This makes it a useful tool for large-scale population studies where detailed protocols may not be feasible [Gonçalves et al. 2013]. Additionally, it has been found to be a reliable method for assessing body composition in various settings, including field and clinical environments, provided that population-specific equations are used [Rosa et al. 2025].

4. Conclusions

The findings revealed notable sex and age-related differences, with males consistently presenting higher fat-free and muscle mass across all age groups. Despite a majority of participants falling within the normal BMI range, deviations in body composition were observed, particularly among male adolescents. Some males with normal BMI demonstrated low fat-free mass index (FFMI) values and elevated fat mass index (FMI), suggesting suboptimal muscle development and hidden adiposity. These findings point to the presence of normal-weight obesity—a phenotype linked to increased metabolic risk despite a healthy BMI. Underweight individuals also showed reduced lean tissue, indicating potential undernutrition or inadequate physical activity. Conversely, overweight and obese adolescents exhibited excessive fat accumulation, with FMI values exceeding recommended thresholds. The use of BIA proved valuable in revealing such discrepancies, offering a more nuanced understanding of adolescent nutritional status. Overall, the results emphasize the need for comprehensive body composition monitoring in youth, beyond standard BMI assessment.

5. References

[1] Barbosa-Silva M.C., Barros A.J., Wang J., Heymsfield S.B., Pierson R.N. Jr. Bioelectrical impedance analysis: population reference values for phase angle by age and sex. Am. J. Clin. Nutr., 2005, 82 (1), 49-52.

[2] Bosy-Westphal A., Jensen B., Braun W. Pourhassan M., Gallagher D., Müller M.J. Quantification of whole-body and segmental skeletal muscle mass using phase-sensitive 8-electrode medical bioelectrical impedance devices. Eur. J. Clin. Nutr., 2017, 71, 1061–1067.

[3] Bredella M.A., Gill C.M., Keating L.K., Torriani M., Anderson E.J., Punyanitya M., Wilson K.E., Kelly T.L., Miller K.K. Assessment of abdominal fat compartments using DXA in premenopausal women from anorexia nervosa to morbid obesity. Obesity, 2013, 21 (12), 2458-2464.

[4] Chula de Castro J.A., de Lima T.R., Silva D.A.S. Body composition estimation in children and adolescents by bioelectrical impedance analysis: A systematic review, J. Bodyw. Mov. Ther., 2018, 22 (1), 134 – 146.

[5] Day K., Kwok A., Evans A., Mata F., Verdejo-Garcia A., Hart K., Ward L.C., Truby H. Comparison of a bioelectrical impedance device against the reference method dual energy X-ray absorptiometry and anthropometry for the evaluation of body composition in adults. Nutrients, 2018, 10 (10), 1469.

[6] Dipali S.S., Ferreira C.R., Zhou L.T., Pritchard M.T., Duncan F.E. Histologic analysis and lipid profiling reveal reproductive age-associated changes in peri-ovarian adipose tissue. Reprod. Biol.

Endocrinol., 2019, 17, 46.

[7] Gibby J.T.. Njeru D.K., Cvetko S.T., Heiny E.L., Creer A.R., Gibby W.A. Whole-body computed tomography–based body mass and body fat quantification: A comparison to hydrostatic weighing and air displacement plethysmography. J. Comput. Assist. Tomogr., 2017, 41, 302–308.

[8] Gonçalves V.S., Faria E.R., Franceschini S., Priore S.E. Predictive capacity of different bioelectrical impedance analysis devices, with and without protocol, in the evaluation of adolescents. J. Pediatr. (Rio J), 2013, 89 (6), 567-574.

[9] Kasper A.M., Langan-Evans C., Hudson J.F., Brownlee T.E., Harper L.D., Naughton R.J., Morton J.P., Close G.L. Come back skinfolds, all is forgiven: A narrative review of the efficacy of common body composition methods in applied sports practice. *Nutrients,* 2021, 13 (4), 1075.

[10] Lee K., Lee S., Kim Y.J., Kim Y.J. Waist circumference, dual-energy X-ray absortiometrically measured abdominal adiposity, and computed tomographically derived intra-abdominal fat area on detecting metabolic risk factors in obese women. Nutrition, 2008, 24 (7-8), 625-631.

[11] Lee L.W., Liao Y.S., Lu H.K., Hsiao P.L, Chen Y.Y., Chi C.C. Validation of two portable bioelectrical impedance analyses for the assessment of body composition in school age children. PLoS ONE, 2017, 12 (2), e0171568.

[12] Liu X., He M., Li Y. Adult obesity diagnostic tool: A narrative review. Medicine (Baltimore), 2024, 26, 103(17):e37946.

[13] Mahmoud I., Al-Wandi A., Gharaibeh S., Mohamed S. Concordances and correlations between anthropometric indices of obesity: a systematic review. Public Health., 2021, 198, 301–306.

[14] Moonen H.P.F.X., Van Zanten A.R.H. Bioelectric impedance analysis for body composition measurement and other potential clinical applications in critical illness. Curr. Opin. Crit. Care, 2021, 27(4), 344-353.

[15] Namwongprom S., Rerkasem K., Wongthanee A., Pruenglampoo S., Mangklabruks A. Relationship between total body adiposity assessed by dual-energy X-ray absorptiometry, birth weight and metabolic syndrome in young Thai adults. J. Clin. Res. Pediatr. Endocrinol., 2013, 5(4), 252-257.

[16] Namwongprom S., Rerkasem K., Wongthanee A., Pruenglampoo S., Mangklabruks A. Relationship between body composition parameters and metabolic syndrome in young Thai adults. J. Clin. Res. Pediatr. Endocrinol., 2014, 6 (4), 227-232.

[17] Nie M.J., Sun R.Z., Fan C.Q., Wang J.J., Fei X., Hu Z.K., Li H.J. Diagnostic performance of anthropometric measurements for identifying obesity in high-altitude pediatric populations: evidence from Tibet via bioelectrical impedance analysis. Lipids Health Dis., 2025, 24(1), 216.

[18] Norcross J., Van Loan M.D. Validation of fan beam dual energy X-ray absorptiometry for body composition assessment in adults aged 18-45 years. Br. J. Sports Med., 2004, 38, 472–476.

[19] Pugliese C., Delgado A.F., Kozu K.T., Campos L.M.A., Aikawa N.E., Silva C.A., Maluf Elias A. Body composition and phase angle: How to improve nutritional evaluation in juvenile dermatomyositis patients. Nutrients., 2023, 15(13), 3057.

[20] Rosa G.B., Lukaski H.C., Sardinha L.B. The science of bioelectrical impedance-derived phase angle: insights from body composition in youth. Rev. Endocr. Metab. Disord., 2025, 10.

[21] Shafer K.J., Siders W.A., Johnson L.K., Lukaski H.C. Validity of segmental multiple-frequency bioelectrical impedance analysis to estimate body composition of adults across a range of body mass indexes. Nutrition, 2009, 25 (1), 25-32.

[22] Štefanová E., Bakalár P., Baška T. Eating-disordered behavior in adolescents: Associations with body image, body composition and physical activity. Int. J. Environ. Res. Public Health., 2020, 17 (18), 6665.

[23] Unick J. L., Utter A. C., Schumm S., McInnis T. Evaluation of leg-to-leg BIA in assessing body composition in high-school-aged males and females. Res. Sports Med., 2006, 14 (4), 301–313.

[24] Yang R., Yu J., Luo C. Oj W., Yang D., Xue H., Ni X., Yu S., Du W., Zhang F. Correlations and consistency of body composition measurement indicators and BMI: a systematic review. Int. J. Obes., 2025, 49, 4–12.

[25] World Health Organization (WHO): Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. WHO Technical Report Series, 1995; 854, Geneva, Switzerland.

Role and mechanisms of inositol supplementation in women with PCOS

Karolina Nowosad*, Justyna Libera, Agnieszka Malik

Faculty of Food Science and Biotechnology,
Uniwersity of Life Sciences in Lublin
corresponding author:karolina.nowosad@up.lublin.pl

Abstract

Polycystic ovary syndrome (PCOS) is a multifactorial endocrine disorder affecting reproductive, metabolic, and psychological health in women of reproductive age. Its complex pathogenesis involves insulin resistance, hyperandrogenism, and chronic low-grade inflammation. Diet and lifestyle interventions play a central role in PCOS management, particularly in improving insulin sensitivity, regulating hormonal function, and reducing cardiometabolic risk.
Among the non-pharmacological approaches, inositol supplementation—particularly with myo-inositol (MI) and D-chiro-inositol (DCI)—has gained considerable interest. These stereoisomers are involved in insulin signaling, glucose metabolism, and ovarian function. The present work summarizes current dietary recommendations for women with PCOS and reviews available clinical evidence on inositol's therapeutic potential. While several studies report positive effects on ovulation, metabolic parameters, and hormonal balance, recent meta-analyses reveal inconsistent findings. Most trials confirm the safety and tolerability of inositol, and some suggest comparable outcomes to metformin in selected populations.
Although inositol cannot yet be considered a definitive therapy, it appears to be a promising, well-tolerated supplement that may complement dietary and lifestyle strategies. Based on current evidence, its clinical use should be personalized and accompanied by further research. This paper highlights the importance of an integrated, evidence-based approach to PCOS treatment, emphasizing the role of nutrition and the potential value of inositol in improving patient outcomes.

Keywords: polycystic ovary syndrome, inositol, myo-inositol, insulin resistance, nutrition therapy, metabolic health

1. Introduction

Diagnostic criteria for PCOS

The diagnosis of polycystic ovary syndrome (PCOS) in women is based on diagnostic criteria that were first developed in 1990 and have since been repeatedly modified by scientific societies. Currently, the most commonly used are the Rotterdam criteria, according to which a diagnosis requires the presence of at least two out of the following three features:

- clinical and/or biochemical signs of hyperandrogenism,

- oligoovulation or anovulation (infrequent or absent ovulation),
- polycystic ovarian morphology on ultrasound (i.e., ≥12 follicles or an ovarian volume greater than 10 ml) [The Rotterdam ESHRE/ASRM 2003].

Polycystic ovary syndrome is associated with the occurrence of numerous symptoms, including **metabolic disturbances**. In many cases, **tissue insulin resistance** is observed, which consequently leads to **elevated insulin levels** in the blood. High levels of **androgens** - whose excess causes symptoms such as **hirsutism** (excessive hair growth), **acne, seborrhea, male-pattern baldness,** and **excessive hair loss** - are also common. **Overweight and obesity**, influenced by **hyperinsulinemia** and **insulin resistance**, are frequently observed. Other common features include **dyslipidemia, irregular menstrual cycles, anovulatory cycles**, and **infertility** [Nowotnik 2012].

PCOS is currently recognized as a multifactorial endocrine disorder with clinical and metabolic characteristics overlapping with those of type 2 diabetes and obesity. Its pathogenesis is influenced by both genetic susceptibility and environmental factors. From a pathophysiological perspective, PCOS is regarded as a heterogeneous condition of androgen excess, accompanied by varying degrees of gonadotropin and metabolic dysregulation [Dasgupta et al. 2008].

Environmental contributors to the development and progression of PCOS include a diet rich in saturated fats and simple carbohydrates, insufficient physical activity, perinatal stress, and exposure to environmental endocrine disruptors such as xenoestrogens [Dasgupta et al. 2008].

Several non-exclusive pathophysiological models have been proposed to explain the mechanisms underlying PCOS. The gonadotropin model suggests abnormalities in luteinizing hormone (LH) secretion and the biological activity of follicle-stimulating hormone (FSH). The ovarian model focuses on impaired androgen synthesis and metabolism within the ovaries. The insulin-dependent model emphasizes dysregulation in insulin secretion and action as a central factor in the syndrome's development [Nestler 2005].

The role of diet in the management of PCOS

According to research, between 35% and 65% of women with polycystic ovary syndrome (PCOS) are affected by overweight or obesity [Azziz et al. 2004]. As such, dietary intervention plays a central role in PCOS management. Studies have shown that weight reduction to a BMI within the normal range (18.5–24.9 kg/m²) significantly improves insulin sensitivity and ovarian function in women with excess body weight [Beydoun et al. 2010].

Elevated insulin levels stimulate ovarian androgen production, contributing to hyperandrogenic symptoms such as menstrual irregularities, hirsutism, and acne. Diets with a low glycemic index can help regulate insulin levels, exert antiandrogenic effects, and thereby alleviate many of these clinical manifestations [Dutkowska et al. 2019].

PCOS is also associated with elevated markers of systemic inflammation, including C-reactive protein (CRP), proinflammatory cytokines, chemokines, and increased white blood cell count. Anti-inflammatory dietary patterns have been shown to reduce insulin resistance, oxidative stress, and weight gain, while enhancing the body's anti-inflammatory response [Salama et al. 2015]

Metabolic disturbances, such as increased LDL cholesterol, triglycerides, and total cholesterol levels, along with decreased HDL cholesterol, are frequently observed in PCOS and may predispose individuals to type 2 diabetes and cardiovascular disease [Greff et al. 2023]. Dietary strategies aimed at reducing the intake of trans fats, saturated animal fats, and ultra-processed foods have demonstrated therapeutic potential [Chavarro 2007]. Moreover, omega-3 polyunsaturated fatty acids have been shown to support hormonal regulation and offer cardiovascular protection [Dutkowska et al. 2019].

Inositol as a potential supplement in the treatment of PCOS

Inositol, formerly referred to as vitamin B8, is no longer classified as a B vitamin due to its endogenous synthesis in the human body. It belongs to the group of sugar alcohols and is naturally produced in the brain, kidneys, and testes. It is also found in various dietary sources, including legumes, whole grains, wheat bran, nuts, and citrus fruits. Although nine isomeric forms of inositol exist, two are of primary physiological importance: myo-inositol, predominantly located in glucose-demanding tissues (e.g., brain, heart, ovaries), and D-chiro-inositol, found mainly in glucose-storing tissues such as the liver and muscles. Inositol plays essential roles in cell membrane structure, intracellular signal transduction, and neuronal function. It also contributes to glucose homeostasis by enhancing insulin sensitivity and supports lipid metabolism by reducing triglyceride levels [Januszewski et al. 2019].

Both myo- and D-chiro-inositol are involved in ovarian signaling pathways, including oocyte maturation, fertilization, and implantation, as well as hormonal synthesis. Given these properties, inositol supplementation has emerged as a promising therapeutic strategy in PCOS management [Zdravko et al. 2020].

PCOS, as an endocrine disorder, affects not only physical health but also psychological well-being. Combining a low glycemic index, anti-inflammatory diet with inositol supplementation improves insulin resistance, which positively influences energy levels and mood stability. Weight reduction achieved through diet may also help regulate menstrual cycles, reduce hyperandrogenism, and improve body image and self-esteem in women with overweight or obesity [DiNicolantonio 2022].

For women struggling with infertility, the combination of dietary intervention and inositol supplementation offers a non-pharmacological avenue to enhance reproductive outcomes. Clinical studies report that myo-inositol supplementation induces ovulation in up to 69.5% of cases. Therefore, a holistic approach that integrates nutrition and targeted supplementation is considered more effective than either intervention alone [Nowotnik 2012].

Biological activity and therapeutic use of inositol in women with PCOS

Inositol exerts its biological effects through several insulin-related pathways, which are particularly relevant in the context of PCOS. One of the key mechanisms involves the epimerization of myo-inositol to D-chiro-inositol, a process that takes place primarily in insulin-sensitive tissues such as the liver, skeletal muscles, and bloodstream. This transformation supports tissue-specific roles in glucose uptake and metabolism [Watkins et al. 2020].

The binding of insulin to its receptor initiates phosphorylation events and the activation of insulin receptor substrates, leading to the translocation of GLUT4 glucose transporters to the cell membrane. Inositol is essential in this signaling cascade. A deficiency in inositol has been associated with impaired glucose uptake and reduced insulin sensitivity. Additionally, inositol derivatives are involved in glycogen synthesis and act as secondary messengers in insulin signaling pathways. Given its biochemical roles, inositol - particularly myo-inositol and D-chiro-inositol - has gained attention as a dietary supplement in the treatment of PCOS. The recommended clinical dose is typically 4 grams per day, with a 40:1 ratio of myo- to D-chiro-inositol providing optimal metabolic and reproductive outcomes [Gunalan et al. 2018]. In many studies, supplementation is also combined with 400 μg of folic acid, which offers additional antioxidant benefits [Farshchi et al. 2007].

Although inositol is generally well tolerated, evidence regarding long-term safety remains limited. The highest tested doses - up to 12 grams per day of myo-inositol - have been associated with only mild gastrointestinal disturbances such as nausea, vomiting, and diarrhea. These effects did not appear to intensify with increasing dosage. As such, inositol is considered safe at standard therapeutic levels. Nonetheless, clinical decisions regarding inositol supplementation should be tailored to individual needs, and further research is warranted to optimize dosing strategies and evaluate long-term outcomes [Carlomagno 2011].

Clinical evidence on inositol supplementation in PCOS

There is a growing body of clinical research exploring the role of inositol supplementation in women with PCOS. Numerous randomized controlled trials and systematic reviews have assessed its effects on metabolic, hormonal, and reproductive outcomes, aiming to establish evidence-based guidance for its use in clinical practice.

A meta-analysis by Fitz et al (2024) critically assessed the efficacy of inositol - either alone or in combination with other interventions - for the treatment of PCOS. The analysis included 30 randomized controlled trials (n=2230 participants), of which 19 studies were combined in quantitative meta-analyses. Key results showed that both myoinositol and D-chiroinositol produced statistically significant improvements in metabolic parameters. D-chiroinositol showed potential benefits for ovulation. Interestingly, compared with metformin, inositol was associated with fewer gastrointestinal adverse events, although metformin still demonstrated superiority in reducing waist-to-hip ratio and hirsutism. Importantly, the evidence did not confirm a clear effect on BMI. Overall, the evidence supporting routine inositol supplementation in PCOS was rated as limited and inconclusive. The authors emphasized the importance of shared decision-making between physicians and patients, taking into account individual values, preferences, and current uncertainty in the evidence base.

The meta-analysis by Jethaliya et al. (2022) also aimed to assess the efficacy of myoinositol supplementation on anthropometric, metabolic, and endocrine outcomes in women diagnosed with polycystic ovary syndrome (PCOS). The study included 17 randomized controlled trials with a total of 1083 participants. Myoinositol was compared with folic acid, metformin, and oral contraceptives in different studies. The outcomes analyzed included a wide range of clinical markers, including body mass index (BMI), waist-to-hip ratio, fasting glucose and insulin, HOMA-IR, as well as reproductive hormones such as LH, FSH, estradiol, SHBG, DHEA-S, and total testosterone. The results did not show statistically significant or clinically relevant improvements in most of the parameters assessed. In some studies, only small reductions in serum androstenedione and prolactin were observed. Importantly, there were no consistent effects on key markers of insulin resistance or hormonal imbalances that are commonly targeted in PCOS treatment.

A meta-analysis by Fatima et al. (2023) evaluated eight randomized controlled trials involving a total of 1,088 women with PCOS. Of the participants, 460 received metformin, 436 received myoinositol, and 192 received combination therapy. The authors analyzed a range of metabolic and endocrine outcomes, including body mass index (BMI), fasting glucose, and luteinizing hormone/follicle-stimulating hormone (LH/FSH) ratio. The results did not show a statistically significant difference between the two agents in terms of efficacy. Both myoinositol and metformin were associated with improved metabolic control and hormonal balance, with neither treatment showing clear superiority. In addition, both supplements were generally well tolerated, and the incidence of adverse events - mostly gastrointestinal - was mild.

2. Conclusions

Inositol, especially in its myo- and D-chiro- forms, shows potential as a supportive therapy in PCOS due to its role in improving insulin sensitivity and hormonal balance. Combined with dietary interventions, it may help alleviate both metabolic and reproductive symptoms.

Although some clinical studies and meta-analyses report beneficial effects, the overall evidence remains inconclusive. Inositol appears safe and may be a suitable alternative to metformin in selected patients, but further high-quality research is needed to confirm its efficacy.

3. References

[1] Azziz R., Carmina E., Dewailly D., Diamanti-Kandarakis E., Escobar- Morreale H.F., Futterweit W., Witchel, S.F.: The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil. Steril., 2009, 91 (2), 456-488.

[2] Beydoun H.A., Beydoun M.A.: Gender differences in the association of PCOS and obesity with insulin resistance among university students. Obes. Res. Clin. Pract., 2010, 4 (4), e267-e278.

[3] Chavarro J.E, Richh-Edwards J.W., Rosner B.A., Willett W.C.: A prospective study of dairy foods intake and anovulatory infertility. Hum. Reprod., 2007, 22 (5), 1340-1347.

[4] Dasgupta S., Reddy B.M.: The role of epigenetics in the etiology of polycystic ovarian syndrome. J. Genet. Couns., 2008, 17 (3), 295-304.

[5] DiNicolantonio J.J., O'Keefe, J.H.: Myo-inositol for insulin resistance, metabolic syndrome, polycystic ovary syndrome and gestational diabetes. Open. Heart., 2022, 9 (1), e001989.

[6] Dutkowska A., Konieczna A., Breska-Kruszewska J., Sendrakowska M., Kowalska I., Rachoń D.: Recommendations on non-pharmacological interventions in women with PCOS to reduce body weight and improve metabolic disorders. Endokrynol. Pol., 2019, 70 (2), 198-212.

[7] Fatima K., Jamil Z., Faheem S., Adnan A., Javaid S.S., Naeem H., Ochani S.: Effects of myo-inositol vs. metformin on hormonal and metabolic parameters in women with PCOS: a meta-analysis. Ir. J. Med. Sci., 2023, 192 (6), 2801-2808.
[8] Farshchi H.R., Taylor M.A., McDevitt R.M.: The role of inositol and folic acid in health and disease. Nutr. Rev., 2007, 65 (12), 516-527.
[9] Fitz V., Graca S., Mahalingaiah S., Liu J., Lai L., Butt A., Ee, C.: Inositol for Polycystic Ovary Syndrome: a systematic review and meta-analysis to inform the 2023 update of the International Evidence-Based PCOS Guidelines. J. Clin. Endocrinol. Metab., 2024, 109 (6), 1630-1655.
[10] Gunalan K., Sivaswamy L., Ravi R. Inositol: Clinical relevance and its effect on metabolic disorders. Journal of Clinical Endocrinology & Metabolism, 2018, 103(12), 4625-4632.
[11] Greff D., Juhász A.E., Váncsa S., Váradi A., Sipos Z., Szinte J., Horváth, E.M.: Inositol is an effective and safe treatment in polycystic ovary syndrome: a systematic review and meta-analysis of randomized controlled trials. Reprod. Biol. Endocrinol., 2023, 21 (1), 10.
[12] Januszewski M., Issat T., Jakimiuk A.A., Santor-Zaczynska M., Jakimiuk A.J. Metabolic and hormonal effects of a combined Myo-inositol and d-chiro-inositol therapy on patients with polycystic ovary syndrome (PCOS). Ginekol. Pol., 2019, 90 (1), 7-10.
[13] Jethaliya H., Gajjar N., Patel V., Deshpande S., Patel R.: Efficacy of myo-inositol on anthropometric, metabolic, and endocrine outcomes in PCOS patients: a meta-analysis of randomized controlled trial. Reprod. Sci., 29 (8), 2022, 2282-2298.
[14] Nestler J.E.: Metformin for the treatment of the polycystic ovary syndrome. N. Engl. J. Med., 2005, 353 (5), 555-556.
[15] Nowotnik A. Wielowymiarowość doświadczenia zespołu policystycznych jajników u kobiet w wieku rozrodczym: przegląd badań. NL., 2012, 81 (3) 268–272.
[16] Salama H.H., Makled A.K., Zaki A.A.: Markers of inflammation and insulin resistance in women with polycystic ovary syndrome. J. Obstet. Gynaecol. Res., 2015, 41 (6), 919-922.
[17] Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group.: Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Fertil. Steril., 2004, 81 (1), 19-25.
[18] Watkins C.O., Yong H.E.J., Sharma N., Shiao-Yng C.: A review of the role of inositols in conditions of insulin dysregulation and in uncomplicated and pathological pregnancy. Crit. Rev. Food Sci. Nutr., 2020, 62 (6), 1626-1673.
[19] Zdravko K., Smith J., Williams, P.: Inositol and its impact on ovarian function and insulin signaling. Reprod. Biol. Endocrinol., 2020, 18 (1), 27.

Knowledge of patients with SIBO or IMO about nutrition and supplementation

Marzena Pabich*, Małgorzata Kostecka, Monika Bojanowska, Monika Sachadyn-Król

Faculty of Food Science and Biotechnology,
University of Life Sciences in Lublin
**corresponding author: marzena.pabich@up.lublin.pl*

Abstract

Small intestinal bacterial overgrowth syndrome (SIBO) and intestinal methanogen overgrowth (IMO) are disorders involving excessive colonization of the small intestine by bacteria (in the case of SIBO) or methanogenic archaeons (in the case of IMO). These disorders are increasingly being diagnosed in the general population, and their symptoms, such as bloating, a feeling of fullness, abdominal pain, diarrhea or constipation, significantly reduce patients' quality of life. This study assesses the knowledge of patients diagnosed with SIBO or IMO after a hydrogen or hydrogen-methane test about nutrition and supplementation during and after antibiotic therapy.

A survey conducted from February to May 2024 involved 210 people aged 19−68 years. Among respondents, the most common forms of SIBO were hydrogen-methane and hydrogen-methane and IMO (32.4%, 30.5% and 32.9% of respondents, respectively). The most commonly reported symptoms were bloating (97.1%), abdominal pain (71.9%) and excessive gas (55.7%). Irritable bowel syndrome (IBS) was the most common comorbidity in respondents (24.3%). The high percentage of respondents (77.6%) declaring current or past use of the low-FODMAP diet indicates its popularity in the nutritional management of people with SIBO/IMO. In the context of supplementation, respondents most often declared the use of probiotics (92.4%), sodium butyrate (80%), B vitamins (45.7%) and digestive enzymes (30.5%). Knowledge of natural substances that exhibit antimicrobial activity was also high, with oregano indicated by 64.3% of respondents and berberine by 61%. Statistical analysis showed significant differences in the level of knowledge - patients referred for diagnosis by a nutritionist showed higher nutritional awareness than those referred by physicians ($p < 0.05$). There was no correlation between time since diagnosis and patients' level of knowledge. The survey proved that there is a need to intensify nutrition education in this group of patients, especially during the period of active antibiotic therapy. It is reasonable to conduct organized educational activities and to include specialized diet therapy more widely in the standards of management of patients with SIBO/IMO.

Keywords: Small intestinal bacterial overgrowth, intestinal methanogen overgrowth, hydrogen-methane breath test, hydrogen breath test

1. Introduction

The human gastrointestinal tract provides a habitat for a complex and diverse microbiota, including bacteria, fungi and archaeons. Different sections of the gastrointestinal tract, due to their prevailing conditions and varied functions, are characterized by a different quantitative and qualitative composition of the microorganisms inhabiting them. One clinically significant microbial imbalance is small intestinal bacterial overgrowth (SIBO) syndrome, which involves excessive proliferation in the small intestine of bacteria typical of the large intestine [Skrzydło-Radomanska and Cukrowska, 2022].

Under physiological conditions, the highest density of microbiota is found in the colon and reaches 10^{11}-10^{12} colony-forming units per milliliter (CFU/ml), while in the small intestine we can find less than 10^3 CFU/ml. The small intestine is populated primarily by Gram-positive and aerobic bacteria, while the large intestine is dominated by Gram-negative and anaerobic bacteria. The main types of bacteria colonizing the gastrointestinal tract are *Bacteroidetes* and *Firmicutes* [Achufusi et al., 2020]. In the case of SIBO, we can distinguish two main types of bacterial overgrowth. The first in the upper gastrointestinal tract, associated with bacteria from the oral cavity: *Streptococcus viridans* and *Prevotella spp*. The second involves the lower gastrointestinal tract and is associated with intestinal bacteria: *Escherichia coli, Enterococcus spp., Klebsiella pneumoniae, Clostridium spp.* and *Proteus mirabilis* [Czarnik, 2022].

A similar disorder is intestinal methanogen overgrowth (IMO), but in its course, instead of bacterial proliferation in the small intestine, there is an excess of archeons (methanogens), microorganisms that have the ability to produce methane. *Methanobrevibacter smithii* is the main methanogen in the human gut [Pimentel et al, 2020; Bartuzi-Lepczyńska et al, 2024]. Although SIBO and IMO differ in etiology, their clinical presentation and diagnostic methods are similar, which can make it difficult to distinguish between them.

One method for diagnosing SIBO is to aspirate the small bowel fluid and determine the number of bacteria in a culture of the small bowel contents. This test is time-consuming, expensive, invasive and has some limitations. Ordinary endoscopes are unable to reach the middle and distal sections of the small intestine through which aspirates from the proximal small intestine may give a false-negative result. In contrast, the microbiota of the oral cavity and esophagus can lead to an increased number of false positives [Villanueva-Millan et al., 2022].

Due to the invasive nature of direct aspiration and culture techniques, indirect tests have been developed and are now widely used. Hydrogen and methane breath tests, during which the levels of microbial fermentation products in exhaled air are determined, are considered the most popular [Okunkiewicz et al., 2021]. Substrates commonly used in the diagnosis of SIBO/IMO are lactulose and/or glucose, which provide nutrients for both bacteria and archaeons. Microbial fermentation produces hydrogen and/or methane, as well as hydrogen sulfide, which enter the bloodstream and are excreted with exhaled air, which allows their measurement [Okunkiewicz et al., 2021]. An increase in the concentration of hydrogen in the exhaled air by a minimum of 20 ppm and, in the case of methane breath tests, an increase in the level of methane in the exhaled air by a minimum of 10 ppm is considered a positive result. Increased concentrations of hydrogen or methane in exhaled air after oral ingestion of lactulose or glucose indicate that the substrate was not properly absorbed or came into contact with saccharilytic bacteria that proliferated uncontrollably in the small intestine [Bartuzi-Lepczyńska et al., 2024].

Treatment of SIBO is primarily based on alleviating symptoms by reducing excessive bacterial growth. This does not involve complete eradication of the bacteria, but does involve some modulation of the microbiota [Litwiniuk et al., 2023]. It is always important to pay attention to the causes that led to the development of SIBO/IMO, as simply reducing bacterial overgrowth may not give satisfactory long-term results if the root cause has not been effectively treated. Treatments that may yield a positive outcome include: antibiotic or phytotherapy, treatment of concomitant diseases and disorders, and prevention of recurrence [Rao and Bhagatwala, 2019].

Currently, rifaximin is the most commonly used drug due to its broad spectrum of action and low gastrointestinal absorption rate, while maintaining good antimicrobial activity. In addition, it has been shown to prevent mucosal inflammation and reduce mucosal permeability. It is referred to as a eubiotic that restores the normal composition of the intestinal microbiota [Rezaie et al., 2019]. For patients with hydrogen-predominant SIBO, rifaximin at a dose of 1650 mg/day for two weeks is an effective therapy [O'Mahony et al., 2005]. For patients with methane-predominant SIBO, an effective therapy is a combination of neomycin 1000 mg/day and rifaximin 1650 mg/day for two weeks [Leite et al., 2019].

In addition, in recent years there has been growing interest in alternative methods, such as the use of plant extracts (oregano, berberine, licorice, thyme, among others), which have shown comparable efficacy to drug therapy in studies [Redond-Cuevas, 2024]. The nutritional treatment of SIBO also often uses a low-FODMAP diet, which restricts fermentable oligo-, di- and monosaccharides and polyols. The goal of such a diet is to eliminate products that are a breeding ground for bacteria, which consequently leads to a decrease in bacterial proliferation in the small intestine and a reduction in the severity of symptoms [Staudacher and Whelan, 2017]. Most of the studies verifying the effectiveness of the low-FODMAP diet, are based on IBS, which significantly overlaps clinically with SIBO [Ghoshal et al., 2017; Litwiniuk et al., 2023].

Despite the growing number of publications on the pathophysiology, diagnosis and treatment of SIBO and IMO, there is still a lack of data on patient awareness of the importance of diet and supplementation, even though these aspects are an important part of therapy and relapse prevention. Given the chronic nature of these disorders and the high rate of recurrence after antibiotic therapy, proper patient understanding of dietary and supplementation recommendations may be crucial to long-term treatment outcomes. The aim of the study was to assess the knowledge of patients diagnosed with SIBO or IMO after hydrogen or hydrogen-methane testing on nutrition and supplementation during and after antibiotic therapy.

2. Materials and Methods

The research method used was a survey conducted from February to May 2024. The survey questionnaire consisted of 25 questions and was made available electronically through social media (Facebook topic groups and online forums for SIBO and IMO patients). The study enrolled 210 adults (age: 19-68) who had a confirmed diagnosis of SIBO or IMO, based on the results of a breath test (hydrogen or hydrogen-methane). Microsoft Excel 2013 was used to compile the results of the survey, and Statsoft Statistica 13 was used to perform statistical analysis. Pearson's chi-square test was used to test for statistical significance between the study variables, and $p < 0.05$ was used as the level of significance.

3. Results and Discussion

In the study group of 210 people, 88.6% were women. The average age of the participants was 32.6 ± 7.8 years. The largest group was 25-34 years old (45.2%). The majority of respondents had a university degree (72%), and 66% lived in cities with more than 100,000 residents.

Among the respondents, the most common forms of SIBO were hydrogen and hydrogen-methane SIBO, as well as IMO (Figure 1). Hydrogen sulfide SIBO was declared by only 4.3% of the respondents.

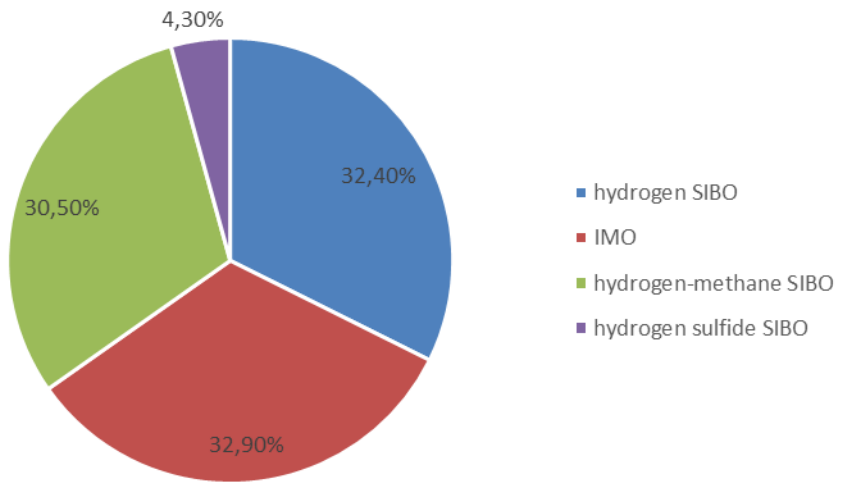

Figure 1. Types of diagnosed SIBO/IMO among respondents.

Bacterial proliferative syndrome and intestinal methanogen overgrowth are characterized by a variety of symptoms, depending on the type of overgrowth. For this reason, respondents were asked to indicate the complaints they observe in themselves. The predominant symptoms reported were bloating (97.1%), abdominal pain (71.9%) and excessive gas production (55.7%). Respondents also reported non-specific symptoms, such as fatigue, food intolerances and loose stools.

Flatulence is the most commonly reported symptom of SIBO/IMO and can result from excessive fermentation of carbohydrates by methane-producing bacteria or archaeons [Quigley, 2014; Pimentel et al., 2020]. This fermentation leads to increased production of hydrogen, methane or hydrogen sulfide, depending on the predominant microbial type. In hydrogen SIBO, diarrhea is more often observed, while in IMO, constipation is the predominant symptom [Rezaie et al., 2017]. Excessive gas production (mainly hydrogen and methane) also leads to stretching of the intestinal walls, which explains the presence of abdominal pain in 71.9% of subjects [Rao and Bhagatwala, 2019].

In the context of treatment and diet therapy, the co-occurrence of SIBO or IMO with other concomitant diseases is important. Therefore, respondents were asked about additional conditions diagnosed in them. The largest group among respondents were those diagnosed with irritable bowel syndrome (IBS) - 24.3%. Among other comorbidities, the most common were *Helicobacter pylori* infection (15.7%), fungal overgrowth (14.8%), thyroid disease (12.9%) and endometriosis (11.9%). Only 6.7% of respondents reported having no other diagnosed diseases.

Irritable bowel syndrome (IBS) is the most common comorbidity in patients diagnosed with small intestinal bacterial overgrowth syndrome (SIBO). A key component of this interaction appears to be a disruption of the gut-brain axis, resulting from dysbiosis of the gut microbiota [Patel and Young, 2022]. According to Pimentel et al. (2020), SIBO can affect up to 80% of patients diagnosed with IBS-D, who show elevated hydrogen and hydrogen sulfide levels in a breath test. A predominance of *Fusobacterium* and *Desulfovibrio spp*. bacteria is also observed in this group. In contrast, patients with the constipated subtype of IBS (IBS-C) have been shown to have methane in their exhaled air, which correlates with increased colonization of the gut by methanogenic archaeons, mainly *Methanobrevibacter smithii* [Villanueva-Millán et al., 2022].

In terms of knowledge of pharmacological and supportive treatment, 91.9% of respondents correctly indicated the definition of prokinetics, the use of which positively affects the effectiveness of treatment and supports it in the long term. The most commonly identified agent with such effects was Iberogast (69%), followed by ginger (42.9%). Triphala (6.2%) and Prukalopride (3.8%) were indicated much less frequently, suggesting their lower recognition and popularity. It is also interesting to note that sodium butyrate, despite its lack of prokinetic effects, was identified by 19% of survey participants as a prokinetic - likely due to its growing popularity in the treatment of gastrointestinal disorders. Lack of knowledge about prokinetics in the context of SIBO/IMO treatment was declared by 10.5% of patients.

In order to achieve a sustained therapeutic effect in the treatment of SIBO and IMO, it is crucial to identify the cause of their onset. In this regard, respondents were asked whether they thought SIBO or IMO could recur if the etiological agent was not identified. The vast majority of respondents (97.6%) answered in the affirmative, indicating relatively high patient awareness in line with current literature reports. According to Attar et al. [2014], the recurrence rate of SIBO after treatment can range from 30% to 60% within 3-9 months after the end of therapy. These most often involve patients in whom the etiology has not been established or the causative treatment (e.g., *Helicobacter pylori* eradication) has not been instituted.

The low-FODMAP diet appears to be the most commonly used form of dietary support in the treatment of SIBO and IMO, as reflected in respondents' answers. As many as 77.6% of respondents reported current or past use of the low-FODMAP diet. Other diets used included a lactose-free diet (26.2%) and a gluten-free diet (11.4%). Less frequently chosen were the elemental diet (2.9%) and the specific carbohydrate diet (SCD) - 1.4%. No diet of any kind was declared by 3.8% of participants. Low glycemic index diet (1%), ketogenic diet (1%) and anti-inflammatory diet (1%) also appeared among additional responses.

The high percentage of patients reporting use of the low-FODMAP diet indicates its popularity in the dietary management of patients with SIBO/IMO. The diet was originally developed for patients with irritable bowel syndrome (IBS), but due to the similarity of symptoms - particularly bloating, abdominal pain and diarrhea - it is increasingly used in the symptomatic management of SIBO [Gibson and Shepherd, 2010; McIntosh et al., 2017]. According to Monash University's recommendation, the elimination phase of the diet should last a maximum of 6-8 weeks, followed by a reintroduction and personalization phase of the diet [Eswaran et al., 2017]. Studies show that many people (including respondents) introduce a low FODMAP diet too early - already during treatment - which can reduce the effectiveness of antimicrobial therapy [Szpunar-Radkowska et al., 2023].

In the context of supplementation (Figure 2), respondents most often declared the use of probiotics (92.4%), sodium butyrate (80%), B vitamins (45.7%) and digestive enzymes (30.5%). Knowledge of natural substances that exhibit antimicrobial activity was also high, with oregano indicated by 64.3% of respondents and berberine by 61%.

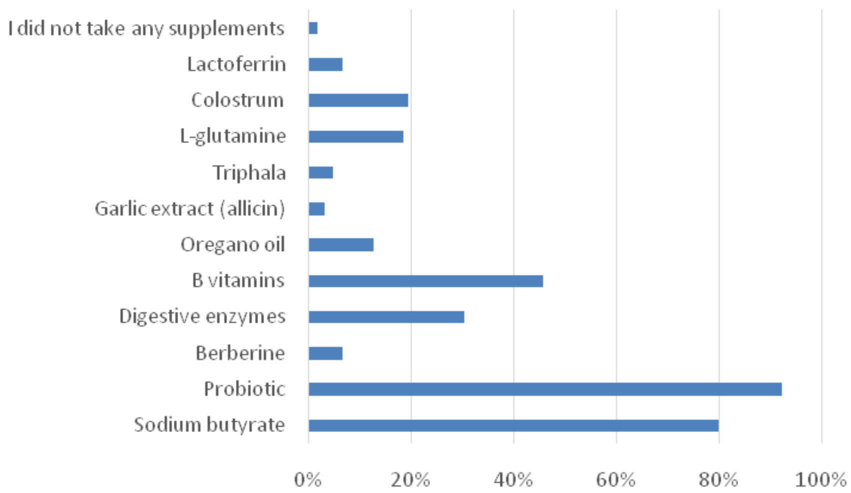

Figure 2. Use of dietary supplements by respondents.

The high popularity of probiotics among respondents is supported by literature data confirming their beneficial effects on microbiota restoration after antibiotic therapy [Suez et al., 2019; Achufusi et al. 2020]. In addition, as reported by Zhong et al. [2017], probiotic supplementation can effectively decontaminate SIBO, reduce hydatidosis and relieve abdominal pain, but is ineffective in preventing SIBO.

In our study, we evaluated whether there was a relationship between the respondents' level of knowledge about diet and supplementation in SIBO/IMO and who (doctor, nutritionist) referred them for a breath test for the diagnosis of these conditions. For this purpose, statistical analysis was performed using a chi-square test based on which it was found that patients referred for diagnosis by a dietitian showed higher nutritional awareness compared to those referred by a doctor. These patients knew that no specific restrictive diet was mandatory during antibiotic/herbal therapy for SIBO/IMO, but it is worthwhile to take care of the quality of the meals consumed ($\chi^2=7.85$, $p<0.05$). Respondents referred for diagnosis by a dietitian were also aware that the use of prokinetics is important for the long-term positive effect of SIBO treatment ($\chi^2=8.98$, $p<0.05$). During the course of antibiotic therapy for SIBO/IMO, there is no specific elimination diet. This is important because during this time it is essential that the bacteria present in the overgrowth are well nourished and thus more susceptible to the effects of specific antibiotics. This will allow the bacteria to be reduced more quickly. During the period of antibiotic therapy, it is worth paying attention to variety and lack of monotony in the type of meals consumed, choosing the least processed products possible.

Bacterial overgrowth syndrome should be considered holistically, and when introducing nutritional therapy, the presence of the underlying cause that contributed to SIBO/IMO should not be overlooked. Therefore, when adjusting the diet both during and after antibiotic therapy, the key is to focus on the current state of the digestive system and the body as a whole, as well as on concomitant diseases. Collaboration with a dietitian is then often multi-step and can change over time, so it is worth focusing on the fact that it must be modifiable.

The present study also examined whether the time elapsed since the diagnosis of SIBO/IMO affected respondents' level of knowledge about diet and supplementation in these conditions. No statistical association was noted between the time that had elapsed since the diagnosis of SIBO/IMO and knowledge of whether an appropriate diet should be used after the end of antibiotic therapy (χ^2=0.11, p>0.05) and the relevance of the use of prokinetics to the long-term positive effect of SIBO treatment (χ^2=0.55, p>0.05).
The survey also asked respondents about sources of knowledge on SIBO/IMO treatment and diet therapy. Websites (87.6%) and thematic groups on social media (83.3%) were cited as the main sources of knowledge, probably due to their wide availability. However, it should be remembered that the information posted there may be wrong, so it is important to consult specialists and use different sources of knowledge. In our own study, however, only 28.1% of respondents reported using scientific literature, 35.2% consulting a nutritionist, and only 17.1% contacting a specialist as their main source of knowledge.
The results suggest the need to increase public meetings, e.g. in lecture form, on the topic of gastrointestinal disorders, highlighting the causes that can lead to SIBO and IMO. Such activities would increase patient knowledge and treatment efficiency while minimizing symptoms, which often accompany patients even for many years.

4. Conclusions

1. Those referred for respiratory tests diagnosing SIBO/IMO by dietitians show a higher level of awareness compared to those referred by physicians.
2. The length of time since diagnosis does not affect the level of knowledge of respondents.
3. There is a need to intensify nutrition education in this group of patients, especially during the period of active antibiotic therapy.
4. It is reasonable to conduct organized educational activities and more extensive inclusion of specialized diet therapy in the standards of management of patients with SIBO/IMO.

5. References

[1] Achufusi O.G.T., Sharma A., Zamora E.A., Manocha D.: Small intestinal bacterial overgrowth: Comprehensive review of diagnosis, prevention and treatment methods. Cureus, 2020.

[2] Attar A., et al.: Small intestinal bacterial overgrowth: Diagnosis and management. Gastroenterol. Clin. Biol., 2014, 38(4), 253–259.

[3] Bartuzi-Lepczyńska M., Ukleja-Sokołowska N.: Zespół rozrostu bakteryjnego jelita cienkiego – kontrowersje i zalecenia. Alergia Astma Immunologia, 2024, 29(2).

[4] Czarnik A.: SIBO – objawy i leczenie – zespół rozrostu bakteryjnego – jak leczyć w POZ?. Lekarz POZ, 2022, 8(1), 24–36.

[5] Eswaran S., Farida J.P., Green J., Miller J.D., Chey W.D.: Nutrition in the management of gastrointestinal diseases and disorders: The evidence for the low FODMAP diet. Curr Opin Pharmacol 2017, 37, 151-157.

[6] Ghoshal U.C., Shukla R., Ghoshal U.: Small intestinal bacterial overgrowth and irritable bowel syndrome: A bridge between functional organic dichotomy. Gut Liver, 2017, 11(2), 196–208.

[7] Gibson P.R., Shepherd S.J.: Evidence-based dietary management of functional gastrointestinal symptoms: The FODMAP approach. J. Gastroenterol. Hepatol., 2010, 25(2), 252–258.

[8] Leite G.G.S., Morales W., Weitsman S., Celly S., Parodi G., Mathur R., Sedighi R., Barlow G.M., Rezaie A., Pimentel M.: Optimizing microbiome sequencing for small intestinal aspirates: Validation of novel techniques through the REIMAGINE study. BMC Microbiol., 2019, 1, 239.

[9] Litwiniuk M., Zaniuk M., Hurkała K., Antonik D., Denys B., Góra K., Zdziennicki W., Zimnicki P., Lato M., Iberszer K.: Leczenie przerostu bakteryjnego jelita cienkiego: Konwencjonalna terapia antybiotykowa oraz terapia alternatywna – probiotyki i dieta low FODMAP. J. Educ. Health Sport, 2023, 41(1), 57–69.

[10] McIntosh K., Reed D.E., Schneider T., Dang F., Keshteli A.H., De Palma G., Madsen K., Bercik P., Vanner S.: FODMAPs alter symptoms and the metabolome of patients with IBS: A randomised controlled trial. Gut, 2017, 66(7), 1241–1251.

[11] O'Mahony L., McCarthy J., Kelly P., Hurley G., Luo F., Chen K., O'Sullivan G.C., Kiely B., Collins J.K., Shanahan F., Quigley E.M.: Lactobacillus and bifidobacterium in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles. Gastroenterology, 2005, 128, 541–551.

[12] Okuniewicz R., Moos Ł., Brzoza Z.: Zespół przerostu bakteryjnego jelita cienkiego. Adv. Microbiol., 2021, 60(3).

[13] Patel S.M., Young C.M.: The identification and management of small intestinal bacterial overgrowth: A functional medicine approach. Phys. Med. Rehabil. Clin. N. Am., 2022, 33(3), 587-603.

[14] Pimentel M., Saad R.J., Long M.D., Rao S.S.C.: ACG clinical guideline: Small intestinal bacterial overgrowth. Am. J. Gastroenterol., 2020, 115(2), 165–178.

[15] Quigley E.M.: Small intestinal bacterial overgrowth: What it is and what it is not. Curr. Opin. Gastroenterol., 2014, 30(2), 141–146.

[16] Rao S.S.C., Bhagatwala J.: Small intestinal bacterial overgrowth: Clinical features and therapeutic management. Clin. Transl. Gastroenterol., 2019, 10(10), e00078.

[17] Redondo-Cuevas L., Belloch L., Martini-Carbonell V., Nicolas A., Alexandra I., Sanchis L., Ynfante M., Colmenares M., Mora M., Reyes Liebana A., et al.: Do herbal supplements and probiotics complement antibiotics and diet in the management of SIBO? A randomized clinical trial. Nutrients, 2024, 7, 16(7), 1083.

[18] Rezaie A., Buresi M., Lembo A., Lin H., McCallum R., Rao S., Schmulson M., Valdovinos M., Zakko S., Pimentel M.: Hydrogen and methane-based breath testing in gastrointestinal disorders: The North American consensus. Am. J. Gastroenterol., 2017, 112(5), 775-784.

[19] Rezaie A., Heimanson Z., McCallum R., Pimentel M.: Lactulose breath testing as a predictor of response to rifaximin in patients with irritable bowel syndrome with diarrhea. Am. J. Gastroenterol., 2019, 114(12), 1886-1893.

[20] Staudacher H.M., Whelan K.: The low FODMAP diet: Recent advances in understanding its mechanisms and efficacy in IBS. Gut, 2017, 66(8), 1517–1527.
[21] Skrzydło-Radomańska B., Cukrowska B.: How to recognize and treat small intestinal bacterial overgrowth?. J. Clin. Med., 2022, 11(20), 6017.
[22] Suez J., Zmora N., Zilberman-Schapira G., Mor U., Dori-Bachash M., Bashiardes S., Zur M., Regev-Lehavi D., Ben-Zeev Brik R., et al.: Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous FMT. Cell, 2018, 174(6), 1406–1423.e16.
[23] Szpunar-Radkowska H.: Żywienie, diagnostyka i leczenie w SIBO. Wyd. Lekarskie PZWL, 2023.
[24] Villanueva-Millan M.J., Leite G., Wang J., Morales W., Parodi G., Pimentel M.L., Barlow G.M., Mathur R., Rezaie A., et al.: Methanogens and hydrogen sulfide producing bacteria guide distinct gut microbe profiles and irritable bowel syndrome subtypes. Am. J. Gastroenterol., 2022, 117(12), 2055–2066.
[25] Zhong C., Qu C., Wang B., Liang S., Zeng B.: Probiotics for preventing and treating small intestinal bacterial overgrowth: A meta-analysis and systematic review of current evidence. J. Clin. Gastroenterol., 2017, 51(4), 300–311.

Dietary Fiber Content and Its Role in the Nutritional Therapy of Gastrointestinal Diseases – A Review of Recent Studies

Monika Sachadyn-Król*, Małgorzata Kostecka, Monika Bojanowska, Marzena Pabich

Faculty of Food Science and Biotechnology,
University of Life Sciences in Lublin
**corresponding author: monika.sachadyn-krol@up.lublin.pl*

Abstract

Dietary fiber is a critical component of nutritional therapy for gastrointestinal (GI) disorders and is increasingly recognized as a therapeutic adjunct. This review summarizes recent evidence (2020–2024) on fiber intake in inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and diverticular disease, based on a literature review of clinical studies, meta-analyses, and guidelines. In IBD, high-fiber diets have shown beneficial effects with differences between Crohn's disease (CD) and ulcerative colitis (UC). A recent meta-analysis in CD found that high fiber intake is safe and associated with prolonged remission, although some CD patients report fiber-related symptom exacerbation. In UC, fiber-rich diets (especially soluble fiber) have been linked to reduced inflammation and sustained remission (for example, a low-fat, high-fiber diet improved quality of life in UC patients in remission). In IBS, soluble fiber (e.g., psyllium) significantly alleviates symptoms, particularly in constipation-predominant IBS, whereas insoluble fiber is often ineffective or poorly tolerated. For diverticular disease, high fiber intake remains a mainstay of therapy: guidelines recommend gradually increasing fiber to reduce symptoms and prevent diverticulitis, and recent studies associate high fiber diets with lower diverticulitis risk. In conclusion, dietary fiber is a valuable component of nutritional therapy across these gastrointestinal conditions, but its role is nuanced. Tailoring the type and amount of fiber to the specific disease (e.g., CD vs UC) and individual tolerance is crucial to maximize benefits and minimize adverse effects.

Keywords: fiber, gastrointestinal diseases, IBD, IBS, diverticular disease

1. Introduction

Dietary fiber (DF) encompasses a diverse group of plant-derived carbohydrates that are resistant to digestion in the human small intestine and impart various physiological benefits to the human gut. Evidence suggests that higher dietary fiber intake exerts protective effects across a range of also non-gastrointestinal diseases diseases, including cardiovascular, metabolic, neurological, and immunological conditions [Zhang et al. 2025]. Fiber appears to contribute to disease prevention and slower progression through multiple mechanisms: improving metabolic profiles (cholesterol, glucose, body weight), nurturing a beneficial gut microbiota that produces bioactive metabolites, and dampening chronic inflammation [Alahmari 2024].

Fibers are broadly categorized by their solubility, fermentability, and viscosity. Soluble fibers dissolve in water and are often fermentable by colonic bacteria, yielding short-chain fatty acids (SCFAs) like butyrate. Insoluble fibers do not dissolve in water, provide stool bulk, and are less readily fermented [Di Rosa et al. 2022]. These distinctions are clinically important because different fiber types can have markedly different effects on the gastrointestinal tract, especially in disease states. Traditionally, fiber has been lauded for normalizing bowel habits, supporting colonic mucosal health, and modulating the gut microbiota. However, its role in gastrointestinal disease (GD) therapy has been nuanced and evolving. Patients with conditions such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and diverticular disease have often received mixed dietary advice regarding fiber intake. For decades, low-fiber or "low-residue" diets were routinely recommended during disease flares or even in remission, based on concerns that fiber could exacerbate symptoms or complications [Loy et al. 2024]. Recent evidence from 2020–2024 has prompted a paradigm shift in this perspective. Emerging clinical trials, meta-analyses, and updated guidelines suggest that adequate fiber intake may be not only safe for many patients with these conditions, but potentially beneficial as part of therapeutic nutritional management. This chapter provides a comprehensive review of recent human studies on dietary fiber in IBD, IBS, and diverticular disease, focusing on fiber types, effectiveness, and safety in nutritional therapy. Key distinctions between fiber types are highlighted, as these characteristics influence clinical outcomes. Accordingly, the aim of this review is to evaluate current evidence on the role of dietary fiber in the nutritional management of gastrointestinal diseases, with particular emphasis on tailoring fiber type and intake to specific disease entities and individual tolerance. By synthesizing recent findings, this work aims to support clinicians and dietitians in optimizing fiber-based interventions to improve patient outcomes.

This narrative review is based on a structured literature search conducted in electronic databases including PubMed, Scopus, and Google Scholar. In the general background section, selected high-quality sources published prior to 2020 were included to provide theoretical context and define key concepts related to dietary fiber and gastrointestinal physiology. However, the core analysis focused on studies published between January 2020 and mid-2025. The search strategy used combinations of keywords such as “dietary fiber”, “inflammatory bowel disease”, “IBD”, “Crohn’s disease”, “ulcerative colitis”, “irritable bowel syndrome”, “IBS”, “diverticulitis”, “diverticular disease”, “nutrition therapy” and “clinical guidelines”. Priority was given to systematic reviews, meta-analyses, randomized controlled trials (RCTs), and official guidelines. Only human studies published in English were included. Articles were selected based on their methodological quality and relevance to the therapeutic use of fiber in gastrointestinal diseases.

2. Types and Characteristics of Dietary Fiber

Dietary fibers are non-digestible carbohydrate polymers that reach the colon where they are partially or fully fermented by gut bacteria. They can be classified by several overlapping criteria, including their solubility in water, fermentability by colonic microbiota, viscosity (gel-forming capacity), and whether they are intrinsic to plant foods or isolated (functional fiber) [McRorie and McKeown 2017; Alahmari 2024]. These properties determine fiber’s behavior in the gastrointestinal tract and its physiological effects.

Soluble fibers dissolve in water to form viscous gels. Examples include psyllium husk, β-glucans (from oats and barley), pectins (from fruits), and gums. In the gut, soluble fiber slows gastric emptying and intestinal transit, and the gel matrix can bind substances like bile acids. Soluble fibers are often fermentable, serving as substrates for colonic bacteria. Insoluble fibers, by contrast, do not dissolve in water and typically are not gel-forming. Examples include wheat bran (rich in cellulose and hemicellulose), lignin (in woody plant parts), and certain resistant starches. Insoluble fiber adds bulk to stool and accelerates transit through a laxative effect. It is less readily fermented, although large bowel bacteria may still partially act on it [Arayici et al. 2021].

Fermentable fibers are those that colonic microbes readily digest to produce short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate. Many soluble fibers (e.g. inulin, fructo-oligosaccharides, pectins) are highly fermentable. Fermentation yields SCFAs, which have recognized beneficial effects on colonic health – for example, butyrate is a key energy source for colonocytes and exhibits anti-inflammatory properties in the gut [Alahmari 2024]. Fermentable fibers also tend to produce gas (carbon dioxide, hydrogen, methane) as a by-product, which can lead to bloating or discomfort in some people. Non-fermentable fibers (often a subset of insoluble fibers like wheat bran) pass largely unchanged, primarily aiding stool bulking. Notably, even insoluble fibers are partially fermented by the microbiota, but to a lesser extent than soluble fibers. The term prebiotic refers to fermentable fibers that selectively stimulate growth of beneficial bacteria (such as *Bifidobacterium* and *Lactobacillus*). Many fermentable fibers (e.g. inulin, galacto-oligosaccharides) are considered prebiotics and can favorably modulate the gut microbiome [Slavin 2013].

Intrinsic fibers are those naturally present in whole plant foods (fruits, vegetables, whole grains, legumes, nuts). Functional fiber refers to isolated fiber (obtained from natural or synthetic sources) that is added to foods or used as a supplement due to its beneficial physiological effects. Examples include purified psyllium husk, wheat dextrin, methylcellulose, and isolated inulin.

Clinically, these differences are important: soluble fibers tend to be more effective at normalizing stool consistency (helpful in diarrhea or constipation) and are generally better tolerated in IBS, whereas insoluble fibers can increase stool bulk and speed but may exacerbate gas and bloating in sensitive individuals [Radziszewska et al. 2023]. Fiber supplements (functional fibers) are often used to achieve a therapeutic intake of specific fiber types. For instance, psyllium (isolated from *Plantago ovata* seeds) is a functional soluble fiber frequently given to treat constipation or IBS symptoms. Functional fibers can be useful for patients who do not tolerate high-fiber foods or who require a concentrated dose of a particular fiber [McRorie and McKeown 2017.] In summary, the heterogeneity of dietary fibers means their effects on the gastrointestinal system are not uniform. Soluble, viscous fibers can soften or solidify stools and are fermented to health-promoting SCFAs, while insoluble fibers primarily increase stool bulk and speed transit. Fermentable fibers influence the composition and metabolic activity of the gut microbiota, an effect increasingly recognized as relevant in diseases like IBD and IBS. Selecting the appropriate type of fiber (or fiber-rich foods) is crucial for effective and safe nutritional therapy tailored to each gastrointestinal disease. An overview of the main types of dietary fiber, their common food sources, and their influence on the gastrointestinal diseases is presented in Table 1.

Table 1. Dietary fiber types, sources, and gastrointestinal (GI) effects.

Fiber Type	Common Sources	Fermentability & Viscosity	GI Effects
Soluble (viscous)	Psyllium husk, oats (beta-glucan), pectin (fruits), legumes	Mostly moderately fermentable; forms viscous gels	Increases stool moisture and viscosity, regulating bowel movements (softens hard stool, firms loose stool) [Gibb et al. 2023]. Slows nutrient absorption (helps glycemic control). Generally well-tolerated; psyllium minimally gas-producing (poorly fermented) [Gunn 2022].
Soluble (highly fermentable)	Inulin (chicory root, onions), FOS (artichoke, garlic), resistant starch (cooled potatoes, green bananas)	Highly fermentable; low viscosity	Produces SCFAs and gas upon fermentation; prebiotic effect [Holscher 2017]. Can cause bloating/gas, especially in IBS; excessive intake may exacerbate symptoms due to fermentation.
Insoluble	Wheat bran, whole grains (cellulose, hemicellulose), vegetables (insoluble fiber fraction)	Poorly fermentable (some fermentation of cellulose); non-viscous	Increases stool bulk and transit speed. Can help prevent constipation, but offers minimal SCFA production. In IBS, coarse insoluble fiber (e.g. bran) may worsen bloating and pain and is not effective for symptom relief [Moayyedi 2014].
Functional/ Isolated	Psyllium; Wheat dextrin; Methylcellulose; Partially hydrolyzed guar gum	Varies by fiber (psyllium is viscous and gel-forming; others less so)	Added to diet via supplements or fortification for specific benefits. Psyllium: proven to improve IBS-C and IBS-D symptoms and lipid/glucose levels [Gibb et al. 2023]. Guar gum (PHGG): soluble, partially fermentable, may aid constipation with less bloating. These fibers are used therapeutically at measured doses [Atzler et al. 2021].

3. Dietary Fiber in Inflammatory Bowel Disease (IBD)

IBD, comprising Crohn's disease (CD) and ulcerative colitis (UC), is characterized by chronic relapsing inflammation of the GI tract. Historically, patients with IBD were often advised to follow low-fiber ("low-residue") diets, especially during flares, to minimize trauma to an inflamed gut and reduce risk of obstruction in stricturing Crohn's disease. Patients also commonly self-restrict fiber, avoiding fruits, vegetables, and whole grains to prevent triggering symptoms or relapses. Surveys indicate IBD patients consume significantly less fiber than healthy individuals and less than recommended amounts, regardless of disease activity [Day et al. 2021]. This long-standing fear of fiber is increasingly being challenged by emerging evidence that adequate fiber intake can be beneficial and is tolerated in IBD, particularly during remission [Loy 2024]. Recent international dietary guidelines for IBD now encourage consumption of fiber-rich foods during periods of remission for both CD and UC, instead of blanket fiber avoidance [Levine et al. 2020; Bischoff et al. 2023]. Dietary fiber may benefit IBD by influencing the gut microbiota, immune system, and producing fermentation metabolites such as SCFAs. Butyrate, a key SCFA, supports epithelial health and has anti-inflammatory effects; in ulcerative colitis, higher fecal butyrate is linked to reduced inflammation [Shin et al. 2023]. Fiber also promotes microbial diversity and beneficial commensals, while low-fiber Western diets contribute to dysbiosis and inflammation. Slowly fermentable soluble fibers (e.g. psyllium, pectin, oats) may reduce inflammation with fewer gastrointestinal side effects compared to rapidly fermentable fibers. Prebiotic fibers are being explored as adjunct therapy in IBD, particularly for enhancing butyrate-producing bacteria such as *Faecalibacterium prausnitzii*. Some fibers may also upregulate regulatory metabolites that suppress inflammation. However, fiber responses in IBD vary between individuals depending on their gut microbiota. A recent study showed consistent responses to prebiotics within individuals, but wide variation between them, highlighting the potential of personalized nutrition strategies [Holmes et al. 2022]. According to the International Organization for the Study of IBD (IOIBD) consensus (2020) and the European ESPEN nutrition guideline (2023), IBD patients in remission should follow a healthy, balanced diet that includes fruits, vegetables, and whole grains – essentially a high-fiber diet – as long as they do not have high risk of strictures [Levine et al. 2020; Bischoff et al. 2023]. These guidelines note that there is no evidence that fiber from whole grain products causes inflammation or relapse in quiescent IBD; on the contrary, fiber may support a more anti-inflammatory gut environment. The British Dietetic Association (BDA) 2022 guidelines similarly emphasize individualized nutrition plans but generally advise against unnecessary long-term restriction of fiber in IBD [Lomer et al. 2023]. Only in certain circumstances – for example, a patient with a known stricture or during an acute severe flare – might a temporary low-fiber diet (to reduce gut residue) be reasonable. Even then, soluble fiber with a soft texture (e.g. oats, psyllium, mashed fruits without skins) may be included cautiously rather than complete fiber elimination [Levine et al. 2020]. The ESPEN 2023 practical guideline echoes this, suggesting "adapted texture" fiber-rich foods (soft cooked produce, smoothies, etc.) for patients with stenosing Crohn's disease [Bischoff et al. 2023]. Importantly, in the absence of strictures or acute inflammation, there is no valid justification to limit fiber in IBD, as concluded by a 2022 systematic review [Serrano Fernandez et al. 2023]. Clinicians are increasingly moving away from broad fiber avoidance in IBD. Despite these recommendations, studies show many IBD patients continue to consume suboptimal fiber quantities due to ingrained beliefs that "fiber is bad" for IBD [Day et al. 2021]. This highlights an educational gap for both patients and providers.

Several intervention trials, albeit small, indicate that adding fiber can improve gastrointestinal symptoms in IBD and enhance quality of life. For instance, controlled reintroduction of fiber-rich foods has been shown to be tolerated in Crohn's patients and not exacerbate symptoms. Similarly, a multicenter randomized controlled trial conducted by Lewis et al. [2021] demonstrated that increasing dietary fiber intake from fruits and vegetables was well tolerated by CD patients with mild to moderate symptoms, regardless of whether they followed a Mediterranean diet (MD) or a specific carbohydrate diet (SCD). The comparable outcomes in terms of symptom improvement, clinical remission, and reduction in inflammatory biomarkers may be attributed to the shared emphasis on fresh fruits and vegetables in both dietary approaches. Another trial in Japan reported that a semi-vegetarian diet (rich in natural fibers) led to higher one-year remission rates in Crohn's disease compared to a regular omnivorous diet [Chiba et al. 2010]. These findings suggest fiber-containing diets are not only safe but may confer benefit in maintaining remission. Patients often subjectively report better bowel regularity when they include moderate fiber, and some note reduced abdominal pain due to the stool-softening effect of soluble fiber. In one study, supplementing psyllium (a soluble fiber) in UC patients led to increased fecal butyrate and was associated with anti-inflammatory effects [Aune et al. 2020]. Perhaps the most compelling evidence comes from a recent systematic review and meta-analysis focused on Crohn's disease. Serrano Fernandez et al. [2023] analyzed 11 studies (including both interventional trials and observational cohorts) involving 2,389 Crohn's patients, examining diets rich in fiber with or without other treatments. They found that a high-fiber diet significantly improved remission rates in Crohn's disease compared to lower fiber intake. Patients receiving abundant dietary fiber had fewer disease relapses, whether fiber was used alone or as an adjunct to standard therapy. Notably, no increase in adverse effects was reported with higher fiber consumption in these patients. This meta-analysis supports the idea that fiber can be incorporated into maintenance therapy for Crohn's disease to help prolong remission. For ulcerative colitis, comparable meta-analytic data are sparse, but smaller trials have suggested that adding soluble fiber to maintenance medication can improve outcomes.

Recent evidence highlights that in patients with IBD who are not experiencing acute inflammation or do not have strictures, dietary fiber is generally both safe and well-tolerated. Many individuals even report improvements in stool consistency and bowel regularity when fiber intake is increased. However, misconceptions persist: a 2014 review revealed that more than 70% of websites inaccurately advise IBD patients to avoid high-fiber foods [Hou et al. 2014], pointing to a pressing need for better dissemination of current, evidence-based guidelines. It should be noted that the online nutritional landscape may have changed considerably over the past decade. An updated analysis of current online dietary recommendations for IBD patients would be warranted to assess whether the accuracy and quality of such content have improved in light of evolving clinical guidelines. Overall, fiber recommendations in IBD should now be personalized. While fiber restriction remains important during active flare-ups or when strictures are present, increasing fiber intake during remission is encouraged to support symptom management and potentially influence disease progression positively.

4. Dietary Fiber in Irritable Bowel Syndrome (IBS)

Irritable bowel syndrome is a functional gastrointestinal disorder characterized by chronic abdominal pain associated with altered bowel habits (diarrhea, constipation, or both). Unlike IBD, IBS involves no structural inflammation but rather gut-brain dysregulation, visceral hypersensitivity, and often dietary triggers of symptoms. Dietary fiber has a long history in IBS management, albeit with mixed results, making it a focus of many clinical studies. Because IBS can manifest with constipation (IBS-C), diarrhea (IBS-D), or a mix (IBS-M), fiber's role must be considered in a subtype-specific manner [Dai et al. 2019]. Recent evidence and guidelines have clarified which types of fiber are beneficial in IBS and how to use them effectively. For decades, increasing dietary fiber was a standard recommendation for IBS, based on fiber's stool-bulking and laxative effects to relieve constipation, and an assumption that fiber could normalize bowel function. Many patients were told to add bran or high-fiber cereals. However, clinical experience revealed a dichotomy: while some IBS patients (particularly those with constipation) improved on fiber, others (especially those with diarrhea or bloating) felt worse. Insoluble wheat bran, in particular, often aggravated abdominal pain and bloating in IBS patients [Ünsal et al. 2020]. This led to research dissecting fiber types in IBS, and by the 2000s, studies suggested that soluble fiber improves overall IBS symptoms, whereas insoluble fiber has little benefit or may even exacerbate symptoms [El Salhy et al. 2017]. Soluble fiber's viscous gel action increases stool bulk in a gentler way than coarse insoluble fiber. In IBS with constipation, it can soften hard stools by retaining water, easing passage; in IBS with diarrhea, the gel can firm loose stools by absorbing excess fluid. Thus, soluble fiber tends to normalize stool form in both IBS-C and IBS-D [Galica et al. 2022]. Unlike poorly fermentable insoluble fiber, which can mechanically irritate, the fermentation of soluble fiber yields metabolic by-products that can promote a healthy mucosa and possibly anti-inflammatory effects. There is preliminary evidence that psyllium supplementation favorably alters the gut microbiota in IBS, increasing beneficial bacterial genera [Garg et al. 2023]. A Garg et al. recent trial even indicated that psyllium can decrease gut markers of low-grade inflammation in IBS and enhance microbial diversity, suggesting a microbiome-modulating role. Soluble fiber can also reduce visceral hypersensitivity indirectly. By forming softer, bulkier stools, fiber prevents the extreme distension of colon segments that might trigger pain in IBS. Patients often report that psyllium leads to more predictable bowel movements and less cramping during defecation. Some studies document reductions in abdominal pain and bloating scores with soluble fiber therapy [Galica et al. 2022].

In the 2010s, dietary management of IBS began focusing on FODMAPs—fermentable sugars and fibers that can trigger symptoms. Many high-fiber foods (e.g., wheat, legumes, some fruits) are high in FODMAPs, creating a paradox: while fiber can help IBS, highly fermentable types may worsen bloating. The solution was to promote low-FODMAP, soluble fiber sources. Standard care involved basic dietary adjustments and adding soluble fiber; if symptoms persisted, a 2–6 week low-FODMAP diet under dietitian supervision was recommended—modifying both fiber type and amount. The years 2020–2025 have seen a solidification and fine-tuning of fiber recommendations in IBS, supported by high-level evidence and formal guidelines. Notably, the American College of Gastroenterology (ACG) released its first comprehensive clinical guideline on IBS in 2021, and the British Society of Gastroenterology (BSG) published updated IBS guidelines in 2021 – both of which echo the primacy of soluble fiber. The BSG 2021 guideline explicitly states: *“Soluble fiber is effective for global IBS symptoms and abdominal pain, but insoluble fiber should be avoided”* [Vasant et al. 2021]. This is a strong recommendation (Grade A evidence) rooted in multiple RCTs and meta-analyses. Similarly, an American Gastroenterological Association (AGA) Clinical Practice

Update in 2022 on diet in IBS underscores that soluble fiber (e.g. psyllium, oats, pectin) is a beneficial first-line nutritional therapy, improving stool consistency and symptoms, whereas insoluble fiber like wheat bran can exacerbate bloating [Chey et al. 2022]. In these recent guidelines, fiber is typically recommended at a dose of about 20–30 g per day, achieved through diet or supplements, with advice to introduce fiber gradually (to minimize gas) and to evaluate response over ~1–2 months. Psyllium husk has the best evidence and is often specifically mentioned as the fiber of choice for IBS (for both constipation and diarrhea predominant IBS, psyllium can help regulate stool form) [Lacy et al. 2021]. The ACG and BSG guidelines list a low-FODMAP diet as a second-line or complementary approach for IBS management, given evidence that ~50–70% of IBS patients have symptom improvement on this diet [Varney and Muir 2021]. This is relevant to fiber because many FODMAPs are short-chain fermentable carbohydrates, some of which are classified as fibers (fructans, galacto-oligosaccharides). The low-FODMAP diet often reduces intake of certain fermentable fibers (e.g. inulin in wheat/garlic, galacto-oligosaccharides in legumes) while still allowing soluble fiber from low-FODMAP sources (e.g. fiber from oats, quinoa, berries). Recent clinical trials (2020–2023) have clarified that a low-FODMAP diet can coexist with adequate fiber intake – patients are usually coached to substitute high-FODMAP fiber foods with low-FODMAP but fiber-rich alternatives (such as kiwifruit or chia seeds for constipation, or oat bran, which is moderate in FODMAP, in small doses). A 2022 network meta-analysis comparing dietary interventions in IBS found that the low-FODMAP diet and soluble fiber were both effective in improving IBS symptom severity, with the low-FODMAP diet having a slight edge in global symptom control [Haghbin et al. 2024]. However, the difference was not huge, and many patients did well with just fiber and general diet advice. Thus, current best practice often is: advise soluble fiber supplementation as a simple, low-cost initial step, and if symptoms persist (particularly bloating and pain), consider a trial of the low-FODMAP diet for further relief, ideally under a dietitian's supervision [Chey et al. 2022].

5. Dietary Fiber in Diverticular Disease

Diverticula are sac-like outpouchings in the wall of the large intestine and represent the most common structural abnormality of the human colon. The term colonic diverticulosis (hereafter: diverticulosis) denotes the condition characterized by the presence of these diverticula in the colon [Tursi et al. 2020]. Dietary fiber has been central to diverticular disease management for decades, ever since observational studies in the 20th century noted that populations with low fiber diets had a higher prevalence of diverticulosis. The classic hypothesis by Burkitt and Painter (1970s) was that a low-fiber Western diet leads to small, firm stools and increased intracolonic pressures, promoting diverticula formation [Kishnani et al. 2022]. Hence, a high-fiber diet was postulated to prevent diverticular disease by producing bulkier stools that exert less pressure on the colon wall. This fiber-diverticulosis theory became widely accepted and still underpins modern dietary advice for diverticular disease [Ma et al. 2019]. However, recent research has added some complexity, questioning fiber's role in diverticula formation while still affirming its importance in preventing diverticulitis and managing symptoms of diverticular disease.

Epidemiological studies support a protective role of fiber: for example, in a large prospective cohort of 46,295 men, those in the highest quintile of a prudent dietary pattern—characterized by high intake of fruits, vegetables, and whole grains—had a 26% lower risk of incident diverticulitis compared to those in the lowest quintile. Conversely, those in the highest quintile of a Western dietary pattern—high in red meat, refined grains, and high-fat dairy—had a 55% higher risk of diverticulitis relative to the lowest quintile [Strate et al. 2017]. Regarding acute diverticulitis management, the traditional approach involved bowel rest or low-residue diet during the acute phase. Patients diagnosed with diverticulitis were frequently placed on clear liquids or minimal fiber until their acute pain resolved, based on the idea that reducing fecal matter in the colon could lessen irritation of the inflamed diverticula. After recovery, a gradual return to a high-fiber diet was advised to prevent future episodes. Patients were also commonly told to avoid certain foods like nuts, seeds, corn, and popcorn – the thinking (now known to be a myth) was that these small, hard particles could lodge in diverticula and precipitate diverticulitis. This advice was widespread for decades. However, even before 2020, evidence emerged debunking it: a large prospective study in 2008 showed that nut, corn, and popcorn consumption did not increase diverticulitis or bleeding risk – in fact, those eating more of these foods had no higher risk than those who avoided them. By the 2010s, most guidelines had quietly dropped the prohibition on nuts and seeds. For example, the 2015 American Gastroenterological Association guideline on diverticulitis explicitly advised against telling patients to avoid seeds, nuts, and popcorn, given no evidence of harm [Peery et al. 2021]. A key point is that by 2019, it was acknowledged that while a high-fiber diet is associated with lower first-time diverticulitis incidence, it was not yet proven to prevent recurrent diverticulitis after an initial episode [Aune et al. 2020].

Recent large prospective studies have provided more granular insight into how fiber (and diet in general) influences diverticulitis risk. An analysis from the Nurses' Health Study by Ma et al. [2019] found that women with the highest total fiber intake had a 14% lower risk of developing diverticulitis compared to those with the lowest intake (hazard ratio [HR] = 0.86; 95% confidence interval [CI]: 0.78–0.95; P-trend = 0.002). Among different fiber sources, fruit fiber showed the strongest association with reduced risk (HR = 0.83; 95% CI: 0.75–0.92), followed by cereal fiber (HR = 0.89; 95% CI: 0.81–0.99), while vegetable fiber was not significantly associated with diverticulitis risk (HR = 0.91; 95% CI: 0.83–1.01). Additionally, each additional daily serving of whole fruit was linked to a 5% reduction in risk (HR = 0.95; 95% CI: 0.92–0.98; P-trend < 0.001). This nuanced finding suggests that not all sources of fiber confer equal benefit – fruit fiber often comes with antioxidants and a favorable microbiome impact, which might contribute to its protective effect. Additionally, these studies emphasized an overall healthy dietary pattern (sometimes labeled as a "prudent" or Mediterranean diet) rather than fiber in isolation. For instance, men following a diet high in fiber-rich foods, and low in red meat and refined grains, had much lower diverticulitis rates than those on a typical Western diet. These findings have reinforced guideline recommendations to not only increase fiber but also improve overall diet quality. The American Gastroenterological Association (AGA) in a 2021 Clinical Update stated that a high-quality diet with ample fruits, vegetables, whole grains, and legumes (i.e., fiber-rich foods) is associated with reduced diverticulitis and should be encouraged for prevention [Peery et al. 2021; Hashash et al. 2024]. In short, the advice remains: maintain a high-fiber diet to lower diverticulitis risk – and recent evidence makes this case even stronger. The period 2020–2025 has not produced a large definitive randomized controlled trial proving that fiber prevents recurrent diverticulitis, but it has seen more acknowledgment of the uncertainty. For example, a 2022 American Family Physician review stated that a high-fiber diet is associated with lower incidence of diverticular disease, but evidence that it prevents recurrence of diverticulitis is lacking [Bailey et al. 2022]. The 2020 ASCRS (surgeons') guidelines note that while fiber supplementation is commonly advised after diverticulitis, the utility of fiber in secondary prevention remains unclear based on available studies [Hanna and Kaiser 2021]. Despite this, in practice fiber is still usually recommended given its safety profile.

Some cases of diverticulitis may result from a dysbiotic microbiota that incites mucosal inflammation in diverticular pockets. Fiber, by restoring healthy fermentation and SCFA production, might mitigate this. SCFAs like butyrate have anti-inflammatory effects on the colonic mucosa and support the integrity of the colonic epithelium. In diverticular patients, butyrate deficiency or a thinner mucus layer (due to low fiber intake) might predispose to microperforations and inflammation. Supporting this, a 2021 study found that patients with recurrent diverticulitis had lower dietary fiber and a distinctly different gut microbiome compared to those without recurrence [Carabotti et al. 2021]. Increasing fiber can enrich beneficial bacteria (like *Bifidobacteria* and *Lactobacillus*) that ferment fiber to SCFAs, theoretically reducing inflammation in the diverticula. These mechanistic insights reinforce the advice for fiber-rich, plant-based diets – which overlap with diets known to reduce systemic inflammation as well. Another consideration is that fiber may help manage Symptomatic Uncomplicated Diverticular Disease (SUDD) – a condition where patients with diverticulosis have IBS-like gastrointestinal symptoms (bloating, pain) without acute diverticulitis. Fiber is often utilized in SUDD to regulate bowel movements and possibly reduce intracolonic pressure, thereby alleviating pain. Psyllium or bran can help treat underlying constipation in SUDD, improving symptoms [Carabotti et al. 2017].

Table 2. Summary of Fiber Recommendations in IBD (UC and Crohn's), IBS, and Diverticular Disease.

Condition	Fiber Type	Intake (g/day)	Phase-specific Recommendations	Sources
IBD (Crohn's disease, UC)	Emphasize fiber-rich, plant-based diet (Mediterranean pattern) with fruits, vegetables, whole grains. Encourage soluble/fermentable fibers (e.g. psyllium, oats, pectin) that promote SCFA production; avoid coarse insoluble fiber (e.g. raw skins, seeds) in stricturing disease. Prebiotic fibers (e.g. inulin) may also be beneficial.	In remission, aim for normal fiber intake (~25–30 g/day) (general guideline). No formal supplement target specified; emphasis on fiber from foods.	Active flare/strictures: Use a temporary low-residue/low-fiber diet (clear liquids/soft foods) to reduce obstruction risk. Chew thoroughly and cook vegetables until very soft to include some fiber. Remission: Resume a high-fiber diet (25–30 g/d) for maintenance and gut health.	Guidelines (AGA, ECCO); Med diet with fruits/veg [Hashash et al.2024]
Irritable Bowel Syndrome (IBS)	Soluble, viscous fibers (e.g. psyllium/ispaghula husk, oat beta-glucan) are recommended to improve global symptoms and stool consistency. Avoid insoluble fibers (e.g. wheat bran) as they may worsen bloating/pain. Some fermentable fiber (e.g. partially fermentable psyllium) is acceptable; low-FODMAP diet also advised for sensitive patients (per AGA CPU).	General fiber intake 25–35 g/d is advised for health. For symptom management, start low: begin ~3–4 g soluble fiber/day and gradually increase to ~20–30 g/day as tolerated.	IBS is chronic (no remission "phase"). Subtypes: IBS-C (constipation): soluble fiber can relieve constipation (improves stool water and transit). IBS-D (diarrhea): avoid insoluble fibers; soluble fiber may still help bulk stools. Adjust fiber gradually to minimize bloating.	IBS guidelines (BSG 2021, ACG 2020, AGA CPU 2022)
Diverticular Disease	High-fiber diet (whole grains, fruits, vegetables, legumes) is recommended for diverticulosis/diverticular disease. Fiber from foods is preferred (supplements only if intake inadequate). Nuts, seeds, corn, popcorn are *allowed* (no need to avoid).	No specific amount given in guidelines; typically target ~25–30 g/d (per general recommendations). The UK recommends "whole grains, fruit, vegetables" without firm g/d.	Diverticulosis/chronic: Encourage life-long high-fiber diet for prevention/maintenance; increase fiber gradually if starting from low intake. Acute diverticulitis: Begin with clear liquids or low-residue diet, then advance diet as symptoms improve. After recovery, resume high-fiber diet to reduce recurrence risk.	NICE NG147 (2019): high-fiber diet lifelong. AGA CPU (2021)

UC = ulcerative colitis; IBS-C/D = constipation-/diarrhea-predominant IBS; SCFA = short-chain fatty acids.

European literature [Tursi et al. 2020] has documented that psyllium therapy leads to symptom relief in many SUDD patients, supporting fiber's use in this context. This is not a new concept, but recent years have seen continued endorsement of fiber for SUDD as part of a conservative management strategy.In diverticular disease, fiber's value lies largely in prevention: lifelong high fiber consumption clearly associates with lower diverticulosis and diverticulitis incidence. For those with diverticula, maintaining a high-fiber diet is a sensible strategy to minimize symptomatic episodes, even if rigorous proof for recurrence prevention is lacking. Fiber supports larger, softer stools and less intra-colonic pressure, which is intuitively protective in diverticular disease. Moreover, high-fiber diets are part of overall healthy eating patterns that confer multiple benefits beyond the gut.

6. Summary

Dietary fiber is a fundamental component of a healthy diet and, as reviewed in this chapter, it holds significant therapeutic value in gastrointestinal diseases when used judiciously. Recent clinical studies and updated guidelines have reshaped the approach to fiber in conditions like IBD, IBS, and diverticular disease. No longer is fiber uniformly restricted in patients with GI disorders; instead, a tailored strategy is adopted.

In IBD, fiber is now encouraged during remission for its role in symptom relief, maintaining remission, and supporting gut health. Rather than avoiding fiber altogether, patients are advised to include well-tolerated sources - mainly soluble, fermentable fibers - except during flares or in cases of strictures, when temporary reduction may be needed. A fiber-rich diet complements medical therapy and supports long-term management. In IBS, fiber can help or harm depending on type. Soluble fiber (e.g. psyllium) is beneficial, improving symptoms with few side effects, while insoluble fiber (like wheat bran) often worsens bloating and discomfort. Current guidance favors soluble fiber for symptom relief, especially in IBS-C, while minimizing insoluble sources. A simple intervention like daily psyllium is safe and effective when introduced gradually. In diverticular disease, a high-fiber diet is key for prevention and long-term management. Patients with diverticulosis should consume plenty of fiber to reduce complications, and after diverticulitis recovery, fiber intake should be resumed to prevent recurrence. While fiber is limited during acute flares, overall evidence supports its ongoing use to maintain bowel regularity and lower disease risk.

Across all these conditions, one unifying message is the importance of fiber type and individualization. Soluble vs insoluble, fermentable vs non-fermentable - these features determine whether fiber will alleviate or aggravate a given patient's symptoms. Healthcare providers should educate patients on reading food labels, identifying high-soluble-fiber choices, and understanding their own tolerances. It is also essential to integrate fiber advice with other dietary and lifestyle modifications: fiber works best in conjunction with adequate fluid intake, regular physical activity, and, particularly for IBS, as part of a comprehensive diet plan that might include FODMAP modulation or probiotics.

In conclusion, dietary fiber, once a divisive topic in GI disease management, is now recognized as a valuable therapeutic ally. The latest studies reinforce that fiber can and should be utilized in nutritional therapy for IBD, IBS, and diverticular disease – with careful attention to type and timing – to improve patient outcomes. Ongoing research is expected to yield increasingly refined recommendations regarding the therapeutic use of specific fiber types—potentially tailored to individual microbiota profiles or genetic predispositions—for the management and prevention of gastrointestinal diseases. At present, a pragmatic and evidence-based approach should prevail: fiber should not be indiscriminately avoided, but rather selected and implemented thoughtfully. Such a strategy allows clinicians to utilize the well-documented benefits of dietary fiber in promoting gastrointestinal health and improving patient outcomes in a safe and sustainable manner.

7. References

[1] Alahmari L.A.: Dietary fiber influence on overall health, with an emphasis on CVD, diabetes, obesity, colon cancer, and inflammation. Front. Nutr., 2024, 11, 1510564.

[2] Arayici M.E., Mert-Ozupek N., Yalcin F., Basbinar Y., Ellidokuz H.: Soluble and insoluble dietary-fiber consumption and colorectal-cancer risk: a systematic review and meta-analysis. Nutr. Cancer, 2021, 74 (7), 2412–2425.

[3] Atzler J.J., Sahin A.W., Gallagher E., Zannini E., Arendt E.K.: Characteristics and properties of fibres suitable for a low-FODMAP diet—an overview. Trends Food Sci. Technol., 2021, 112, 823–836.

[4] Bailey J., Dattani S., Jennings A.: Diverticular disease: rapid evidence review. Am. Fam. Physician, 2022, 106 (2), 150–156.

[5] Bischoff S.C., Bager P., Escher J., et al.: ESPEN guideline on clinical nutrition in inflammatory bowel disease. Clin. Nutr., 2023, 42 (3), 352–379.

[6] Carabotti M., Falangone F., Cuomo R., Annibale B.: Role of dietary habits in the prevention of diverticular-disease complications: a systematic review. Nutrients, 2021, 13 (4), 1288.

[7] Carabotti M., Annibale B., Severi C., Lahner E.: Role of fiber in symptomatic uncomplicated diverticular disease: a systematic review. Nutrients, 2017, 9 (2), 161.

[8] Chey W.D., Hashash J.G., Manning L., Chang L.: AGA clinical practice update on the role of diet in irritable bowel syndrome: expert review. Gastroenterology, 2022, 162 (6), 1737–1745.

[9] Chiba M., Abe T., Tsuda H., Sugawara T., Tsuda S., Tozawa H., Fujiwara K., Imai H.: Lifestyle-related disease in Crohn's disease: relapse prevention by a semi-vegetarian diet. World J. Gastroenterol., 2010, 16, 2484–2495.

[10] Dai L., Zhong L.L., Ji G.: Irritable-bowel-syndrome and functional-constipation management with integrative medicine: a systematic review. World J. Clin. Cases, 2019, 7 (21), 3486–3504.

[11] Day A.S., Davis R., Costello S.P., et al.: The adequacy of habitual dietary-fiber intake in individuals with inflammatory bowel disease: a systematic review. J. Acad. Nutr. Diet., 2021, 121, 688–708.

[12] Di Rosa C., Altomare A., Imperia E., Spiezia C., Khazrai Y.M., Guarino M.P.L.: The role of dietary fibers in the management of IBD symptoms. Nutrients, 2022, 14 (22), 4775.

[13] El-Salhy M., Ystad S.O., Mazzawi T., Gundersen D.: Dietary fiber in irritable bowel syndrome: review. Int. J. Mol. Med., 2017, 40, 607–613.

[14] Galica A.N., Galica R., Dumitrașcu D.L.: Diet, fibers, and probiotics for irritable bowel syndrome. J. Med. Life, 2022, 15, 174–179.

[15] Garg P., Garg P.K., Bhattacharya K.: Psyllium husk positively alters gut microbiota, decreases inflammation, and has bowel-regulatory action, paving the way for physiologic management of irritable bowel syndrome. Gastroenterology, 2024, 166 (3), 545–546.

[16] Gibb R.D., Sloan K.J., McRorie J.W. Jr.: Psyllium is a natural non-fermented gel-forming fiber that is effective for weight loss: a comprehensive review and meta-analysis. J. Am. Assoc. Nurse Pract., 2023, 35 (8), 468–476.

[17] Gunn D., Abbas Z., Harris H.C., et al.: Psyllium reduces inulin-induced colonic gas production in IBS: MRI and in-vitro fermentation studies. Gut, 2022, 71 (5), 919–927.

[18] Haghbin H., Hasan F., Gangwani M.K., et al.: Efficacy of dietary interventions for irritable bowel syndrome: a systematic review and network meta-analysis. J. Clin. Med., 2024, 13 (24), 7531.

[19] Hanna M.H., Kaiser A.M.: Update on the management of sigmoid diverticulitis. World J. Gastroenterol., 2021, 27 (9), 760–781.

[20] Hashash J.G., Elkins J., Lewis J.D., Binion D.G.: AGA clinical practice update on diet and nutritional therapies in patients with inflammatory bowel disease: expert review. Gastroenterology, 2024, 166 (3), 521–532.
[21] Holscher H.D.: Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes, 2017, 8 (2), 172–184.
[22] Holmes Z.C., Villa M.M., Durand H.K., et al.: Microbiota responses to different prebiotics are conserved within individuals and associated with habitual fiber intake. Microbiome, 2022, 10, 114.
[23] Hou J.K., Lee D., Lewis J.: Diet and inflammatory bowel disease: review of patient-targeted recommendations. Clin. Gastroenterol. Hepatol., 2014, 12, 1592–1600.
[24] Kishnani S., Ottaviano K., Rosenberg L., et al.: Diverticular disease—an updated management review. Gastroenterology Insights, 2022, 13 (4), 326–339.
[25] Lacy B.E., Pimentel M., Brenner D.M., et al.: ACG clinical guideline: management of irritable bowel syndrome. Am. J. Gastroenterol., 2021, 116 (1), 17–44.
[26] Levine A., Rhodes J.M., Lindsay J.O., et al.: Dietary guidance from the International Organization for the Study of Inflammatory Bowel Diseases. Clin. Gastroenterol. Hepatol., 2020, 18 (6), 1381–1392.
[27] Lewis J.D., Sandler R.S., Brotherton C., et al.: A randomized trial comparing the specific carbohydrate diet to a Mediterranean diet in adults with Crohn's disease. Gastroenterology, 2021, 161, 837–852.
[28] Lomer M.C.E., Wilson B., Wall C.L.: British Dietetic Association consensus guidelines on the nutritional assessment and dietary management of patients with inflammatory bowel disease. J. Hum. Nutr. Diet., 2023, 36 (1), 336–377.
[29] Ma W., Nguyen L.H., Song M., et al.: Intake of dietary fiber, fruits, and vegetables and risk of diverticulitis. Am. J. Gastroenterol., 2019, 114 (9), 1531–1538.
[30] McRorie J.W. Jr., McKeown N.M.: Understanding the physics of functional fibers in the gastrointestinal tract: an evidence-based approach to resolving enduring misconceptions about insoluble and soluble fiber. J. Acad. Nutr. Diet., 2017, 117 (2), 251–264.
[31] Moayyedi P., Quigley E.M.M., Lacy B.E., et al.: The effect of fiber supplementation on irritable bowel syndrome: a systematic review and meta-analysis. Am. J. Gastroenterol., 2014, 109 (9), 1367–1374.
[32] Peery A.F., Shaukat A., Strate L.L.: AGA clinical practice update on medical management of colonic diverticulitis: expert review. Gastroenterology, 2021, 160 (3), 906–911.
[33] Radziszewska M., Smarkusz-Zarzecka J., Ostrowska L.: Nutrition, physical activity and supplementation in irritable bowel syndrome. Nutrients, 2023, 15 (16), 3662.
[34] Serrano Fernandez V., Seldas-Palomino M., Laredo-Aguilera J.A., Pozuelo-Carrascosa D.P., Carmona-Torres J.M.: High-fiber diet and Crohn's disease: systematic review and meta-analysis. Nutrients, 2023, 15, 3114.
[35] Shin Y., Han S., Kwon J., et al.: Roles of short-chain fatty acids in inflammatory bowel disease. Nutrients, 2023, 15 (20), 4466.
[36] Slavin J.: Fiber and prebiotics: mechanisms and health benefits. Nutrients, 2013, 5 (4), 1417–1435.
[37] Strate L.L., Keeley B.R., Cao Y., Wu K., Giovannucci E.L., Chan A.T.: Western dietary pattern increases, and prudent dietary pattern decreases, risk of incident diverticulitis in a prospective cohort study. Gastroenterology, 2017, 152 (5), 1023–1030.
[38] Tursi A., Scarpignato C., Strate L.L., et al.: Colonic diverticular disease. Nat. Rev. Dis. Primers, 2020, 6, 20.
[39] Ünsal E.N., Akbulut G., Erdal H.: Different nutritional approaches in the treatment of irritable bowel syndrome. Gülhane Med. J., 2020, 62 (3), 139–144.
[40] Varney J., Muir J.: Fiber in the management of irritable bowel syndrome (IBS). Int. Found. Gastrointest. Disord., 2021, n.p.
[41] Vasant D.H., Paine P.A., Black C.J., et al.: British Society of Gastroenterology guidelines on the management of irritable bowel syndrome. Gut, 2021, 70 (7), 1214–1240.
[42] Zhang L., Chen Y., Yang Q., et al.: The impact of dietary fiber on cardiovascular diseases: a scoping review. Nutrients, 2025, 17 (3), 444.

Dietary interventions in Alzheimer's disease: a review of preventive and therapeutic potential

Alicja Skrzypek*, Joanna Matysiak

Faculty of Food Science and Biotechnology,
University of Life Sciences in Lublin,
13 Akademicka Street, 20-950 Lublin
corresponding author: alicja.skrzypek@up.lublin.pl

Abstract

Alzheimer's disease (AD) is one of the greatest health and social challenges of the 21st century. Due to the limited effectiveness of current pharmacotherapies, interest in diet and nutrition is growing. This article aims to critically review and synthesise the latest scientific research on the effectiveness of specific dietary approaches and key nutrients in preventing and treating AD. A literature review was conducted focusing on meta-analyses, randomised controlled trials (RCTs) and large prospective cohort studies from the PubMed, Scopus and Web of Science databases. Analysis of the evidence indicates that dietary patterns such as the Mediterranean diet (MeDi), the DASH diet and, in particular, the hybrid MIND (Mediterranean-DASH Intervention for Neurodegenerative Delay) diet are strongly associated with a lower risk of AD and slower cognitive decline. The mechanisms underlying these benefits are multifaceted and include the reduction of oxidative stress and neuroinflammation, the improvement of vascular function and the modulation of amyloid-beta (Aβ) metabolism. Furthermore, recent studies provide evidence that molecules of microbial origin stimulate the immune response and directly participate in the aggregation of the Aβ peptide, as well as modulating the phenotype of glial cells. There is less clear evidence for the therapeutic potential of diet in people who have already been diagnosed, although the ketogenic diet shows promise. Dietary interventions based on holistic, plant-rich patterns appear to be a safe, cost-effective, evidence-based strategy for preventing AD. Further research is needed to establish its therapeutic role and develop personalised recommendations.

.

Keywords: Alzheimer's disease, dietary intervention, MIND diet, Mediterranean diet, neuroprotection, gut-brain axis.

1. Introduction

Alzheimer's disease is a progressive neurodegenerative disorder that is the most common cause of dementia worldwide. According to reports, more than 55 million people suffer from dementia. The number of such cases - due to an ageing population - is expected to rise to almost 140 million by 2050 [Alzheimer's Disease International 2023]. The enormous economic and social costs associated with Alzheimer's disease make it a global public health priority. Several factors underlie the pathophysiology of Alzheimer's disease. Among the most important of these are extracellular deposits of amyloid-beta (Aβ) peptide, forming so-called senile plaques, and intracellular aggregates of hyperphosphorylated tau protein, forming neurofibrillary tangles. These processes lead to synaptic dysfunction, neuronal death and progressive brain atrophy. Chronic inflammation of the nervous system and oxidative stress also play an important role. Increasing evidence also points to the importance of vascular dysfunction and metabolic disorders of the brain. Despite intensive research, current pharmacotherapies mainly focus on symptom relief. In the case of new-generation drugs, their mechanism focuses on slowing progression by targeting amyloid. Unfortunately, their efficacy is limited. This prompts the search for preventive strategies. It is estimated that up to 40% of dementia cases could be prevented or delayed by modifying risk factors such as diet, education levels, physical activity, cardiovascular disease control [Livingston et al. 2020].

Dietary style, as a foundation of metabolic health and a source of bioactive compounds, is one of the most important and easily modifiable factors influencing brain health throughout the life course. The aim of this paper is to review and critically evaluate the available scientific evidence on the impact of major dietary patterns and individual nutrients on the risk of development and progression of Alzheimer's disease, distinguishing between their preventive and therapeutic potential.

2. Dietary models in the prevention and treatment of AD

Epidemiological studies consistently show that dietary patterns, rather than individual components, have the greatest impact on health. Analysis of the diet as a synergistic system is more biologically relevant, as nutrients interact in complex ways. One of the most widely studied models is the Mediterranean diet (MeDi), based on a high intake of vegetables, fruits, pulses, nuts, whole grains and olive oil. It is characterised by a moderate intake of fish and poultry and a low intake of red meat. Meta-analyses of studies have shown that strict adherence to the

Mediterranean diet is associated with significantly slower cognitive decline and a lower risk of AD [Nucci et al. 2024]. Another important model is the DASH diet, originally developed to control hypertension. It emphasises the consumption of fruit, vegetables and low-fat dairy, and limits sodium and saturated fat. The link between vascular and brain health is well established, and by providing better blood flow to the brain, the DASH diet has been shown to have neuroprotective effects [Arnoldy et al. 2025; Wang et al. 2025].

Of particular note in the literature is the MIND diet, which is an innovative hybrid of the MeDi and DASH diets, specifically modified from research on neurodegeneration. Its uniqueness lies in its singling out of specific food groups considered "brain-healthy", such as green leafy vegetables, berries, nuts, fish and olive oil, while recommending the restriction of "unhealthy" foods, including red meat, cheese, sweets and fried foods. Studies have shown that even moderate adherence to the MIND diet was associated with a significant slowing of cognitive decline and a reduction in the risk of developing AD by up to 53% [Charisis et al. 2025; Timlin et al. 2025].

A separate approach is the ketogenic diet, which involves drastically reducing carbohydrates in instead of fats, leading to a state of ketosis. In this state, the brain uses ketone bodies as an alternative energy source, which may be beneficial in AD where glucose metabolism is impaired. Clinical trials have shown some cognitive benefits in patients with mild AD, but this diet is difficult to maintain and requires further long-term studies to confirm its safety and efficacy [Al-Kuraishy et al. 2024; Oliveira et al. 2023].

3. The role of selected nutrients and bioactive compounds in neuroprotection

Analysis of dietary patterns is crucial, but understanding their neuroprotective potential requires a detailed study of the molecular action of their individual components. The omega-3 polyunsaturated fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are fundamental regulators of brain homeostasis.

DHA is the predominant fatty acid in the phospholipids of neuronal membranes, conditioning their fluidity, integrity and proper receptor function. This is very important for synaptic plasticity and memory. In addition, DHA is a precursor of specialised pro-synaptic mediators (SPMs), such as neuroprotectin D1, which exhibits potent anti-inflammatory and anti-apoptotic effects. EPA, on the other hand, by competing with the pro-inflammatory arachidonic acid, leads to the formation of eicosanoids with a much lower pro-inflammatory potential [Zhou et al. 2022; Serini et al. 2012].

Exogenous antioxidants and modulators of the endogenous response also play a key role. Vitamin E, mainly as α-tocopherol, is a key lipophilic antioxidant that interrupts the lipid peroxidation chain reaction in cell membranes. Vitamin C, as the main hydrophilic antioxidant, acts synergistically to regenerate the oxidised form of vitamin E. Polyphenols, such as flavanols, anthocyanins or resveratrol, have a multidirectional effect - in addition to directly "scavenging" free radicals, they modulate intracellular signalling pathways. They can also activate the transcription factor Nrf2, a "master regulator" of the antioxidant response, which stimulates the expression of genes encoding detoxification enzymes, enhancing the endogenous defence of the cell. A long-term cohort study confirmed that a higher dietary intake of flavonoids is associated with a lower risk of subjective cognitive decline [Arlt et al 2022; Weinstock 2025].

The B vitamins - pyridoxine (B6), folic acid (B9) and cobalamin (B12) - are essential cofactors in the methionine cycle, a central point of one-carbon metabolism. Their deficiency leads to the accumulation of neurotoxic homocysteine, which induces oxidative stress. Groundbreaking clinical studies have shown that supplementation with B vitamins, by lowering homocysteine levels, can slow the rate of brain atrophy in patients with mild cognitive impairment [Ye et al. 2025]. The vitamin D receptor (VDR) is present in key brain areas and has been shown to have neuroprotective effects by inhibiting the production of pro-inflammatory cytokines, stimulating Aβ clearance methods and regulating neurotrophins. Mention should also be made of choline, which is a key precursor for the memory neurotransmitter acetylcholine and an integral component of neuronal cell membranes [Jeong et al. 2024].

4. Key food groups in neuroprotection

Regular consumption of selected nutrients and whole food groups form the foundation of a neuroprotective diet. Green leafy vegetables such as spinach, kale and romaine lettuce are particularly recommended due to their high nutrient density. They are a rich source of folate (vitamin B9), vitamin K, lutein, β-carotene and nitric oxide(I) precursors. Studies have shown that consumption of one serving of these vegetables per day results in a significant slowing of cognitive decline. This effect has been attributed to, among other things, lutein, which accumulates in brain tissue, acting as an antioxidant, and vitamin K, involved in the metabolism of sphingolipids - key components of neuronal membranes [Chu et al. 2022; Duplantier and Gardner 2021].

An important dietary component is berries, including strawberries, blueberries, raspberries and blackberries, which are rich sources of flavonoids, particularly anthocyanins, which give intense colour to these fruits. Anthocyanins have the ability to cross the blood-brain barrier, where they exert powerful anti-inflammatory and antioxidant effects, protecting neurons from damage. Long-term studies have shown that a high intake of flavonols, present in berries among others, is associated with a reduced risk of developing dementia [Li et al. 2023; Ali et al. 2022].

The PREDIMED study demonstrated that supplementation of the Mediterranean diet with nuts was associated with improved working memory in older people. Nuts and seeds are a concentrated source of monounsaturated and polyunsaturated fatty acids, vitamin E (α-tocopherol), polyphenols and fibre. Of particular note are walnuts, which are one of the few to contain significant amounts of alpha-linolenic acid (ALA), a plant precursor of omega-3 fatty acids [Sánchez-Villegas et al. 2013]. Another important component of a health-promoting dietary pattern is oily marine fish (salmon, mackerel, herring, sardines), which are the main source of long-chain omega-3 fatty acids: DHA and EPA. As mentioned earlier, their role in maintaining the structural integrity of neurons and modulating inflammatory processes is very important [Ferreira et al. 2022]. In addition, virgin olive oil should be mentioned. As a cornerstone of the Mediterranean diet, it owes its neuroprotective properties not only to its high oleic acid content, but also to the presence of phenolic compounds (oleocanthal). This compound shows anti-inflammatory effects similar to ibuprofen, and in vitro studies suggest that it may also promote the removal of Aβ peptide from the brain by enhancing the function of transport proteins at the blood-brain barrier [Silva et al. 2023].

An important, neuroprotective part of the diet is whole grains and pulses. They provide complex carbohydrates with a low glycaemic index, which provides a steady supply of energy to the brain and helps maintain insulin sensitivity. They are also a rich source of B vitamins and dietary fibre, which plays a key role in gut-brain axis communication. Regular consumption of whole grains has been linked to improved cognitive function (while pulses, rich in folate and flavonoids, support metabolic and vascular health, which indirectly translates into brain protection [Bouchard et al. 2022].

5. Molecular mechanisms of action of dietary interventions

The synergy of nutrients contained in diets such as MIND or Mediterranean translates into an integrated, multifaceted effect on AD disease pathophysiology. One of the main mechanisms is the modulation of neuroinflammation and the glial response. Microglia adopt a pro-inflammatory M1 phenotype, damaging neurons. Omega-3 fatty acids and polyphenols actively promote the polarisation of microglia towards an anti-inflammatory and phagocytic M2 phenotype, responsible for clearing Aβ aggregates. Some compounds can also inhibit the activation of the NLRP3 inflamasome, a key protein complex that triggers the production of the highly pro-inflammatory interleukin IL-1β [Singh 2022; Ajala et al. 2023].

Another mechanism is the restoration of redox balance and mitochondrial protection. Mitochondrial dysfunction, leading to an energy crisis and overproduction of ROS, is one of the earliest phenomena in AD. Diets rich in antioxidants provide twofold protection: direct, by neutralising reactive oxygen species (ROS), and indirect, by activating nuclear factor erythroid-2 (Nrf2) and enhancing endogenous defence systems. Equally important is the protection of the blood-brain barrier (BBB) and the improvement of cerebral perfusion. In AD, tight junctions between endothelial cells are impaired, allowing pro-inflammatory molecules to infiltrate the brain. The integrity of the blood-brain barrier (BBB), a dynamic and highly selective structure that separates the systemic circulation from the central nervous system, is a fundamental condition for maintaining neuronal homeostasis and protecting the brain from neurotoxic agents. A key role in maintaining the integrity of the BBB and in regulating cerebral blood flow is played by biochemical balance, which is significantly influenced by endogenous factors such as nitric oxide (NO) and homocysteine [Tian et al. 2025].

Nitric oxide activates the guanylyl cyclase pathway and leads to relaxation of the smooth muscle of the blood vessels, which increases their lumen and ensures optimal blood flow, adapted to the metabolic needs of the nervous tissue. In addition, nitric oxide exhibits anti-aggregation and anti-inflammatory properties. In opposition to the protective effect of NO stands homocysteine, a sulphur amino acid whose elevated plasma concentration (hyperhomocysteinemia) is a recognised risk factor for vascular and neurodegenerative diseases. The pathomechanism of homocysteine's action is multifactorial and primarily involves the induction of oxidative stress. ROS generated in excess lead to inactivation of nitric oxide through its conversion to the highly reactive peroxynitrite, resulting in endothelial dysfunction, impaired normal vasodilation (vasodilation) and chronic inflammation. Furthermore, hyperhomocysteinemia directly damages endothelial cells and promotes neurodegenerative processes [Carey and Fossati 2023].

The possibility of dietary modulation of these metabolic pathways represents a promising neuroprotective strategy. A diet aimed at increasing the bioavailability of nitric oxide is based on providing the substrates and cofactors necessary for its synthesis and protecting against its degradation. The richest sources of NO precursors in the diet are leafy vegetables such as spinach, arugula, lettuce, and beetroot. Antioxidants, especially polyphenols present in berries, dark chocolate or green tea, play an equally important role [Doroszkiewicz et al. 2023]. In parallel, it is crucial to keep homocysteine levels low by ensuring an adequate supply of B vitamins, which are cofactors in its metabolic pathways. The proper remethylation of homocysteine to methionine depends on the availability of folic acid (vitamin B9) and vitamin B12, while its transsulfuration to cysteine requires the presence of vitamin B6. Green leafy vegetables and pulses are rich sources of folate. Vitamin B12 is found almost exclusively in animal products, making supplementation necessary for vegan diets. Vitamin B6, on the other hand, is widely found in fish, poultry and whole grain products, among others. Consciously composing a diet rich in these components is therefore the foundation for neutralising the pro-inflammatory and neurotoxic potential of homocysteine.

Diet also affects amyloid-β proteostasis and tau protein. Certain compounds, such as epigallocatechin gallate (EGCG) from green tea, can promote the non-amyloidogenic pathway of amyloid precursor protein processing, preventing the formation of Aβ. Low glycaemic load diets, on the other hand, may inhibit the activity of GSK-3β kinase, responsible for the pathological hyperphosphorylation of *tau* protein [Karati et al. 2025; Valverde-Salazar et al. 2023].

6. Impact of the gut microbiota on the treatment of Alzheimer's disease

Analysis to date has shown strong links between gut dysbiosis and increased neuroinflammation, a key element in the pathogenesis of Alzheimer's disease. The gut microbiota communicates with the brain through neural, endocrine and immunological pathways. A fibre-rich diet stimulates the production of short-chain fatty acids (SCFAs) by the gut microbiota. SCFAs, especially butyrate, can cross the BBB and act in the brain as inhibitors of histone deacetylases (HDACs), promoting the expression of neuroprotective genes [Fock and Parnova 2023; Chen et al. 2022]. Recent studies provide evidence that microbial-derived molecules not only stimulate the immune response, but are also directly involved in amyloid-β (Aβ) peptide aggregation. One of the best characterised bacterial agents is lipopolysaccharide (LPS), an endotoxin derived from the outer cell membrane of Gram-negative bacteria. Numerous studies in vitro and in animal models have shown that LPS acts as a powerful pro-aggregation factor for the Aβ peptide. LPS molecules can bind to Aβ monomers, acting as a pathological scaffold that significantly accelerates the nucleation and formation of amyloid fibrils [Kim et al. 2021; Zhan et al. 2018]. Studies have confirmed that LPS co-localises with amyloid deposits in regions such as the hippocampus and new cortex, suggesting its physical presence and potential involvement in plaque formation [Scheff and Pice 2006]. Furthermore, LPS is the canonical ligand for Toll-like receptor 4 (TLR4) on the surface of microglia. Activation of this receptor leads to the mass production of pro-inflammatory cytokines such as TNF-α and IL-1β [Calvo-Rodriguez et al. 2020].

Mention should also be made of the cross-seeding hypothesis, which involves amyloids produced by bacteria themselves. Many bacterial species, including the common gut bacterium Escherichia coli, produce extracellular amyloid fibrils (*e.g.* curli protein) that have functions in adhesion and biofilm formation. Structurally, these bacterial amyloids show similarity to the human Aβ peptide. It has been postulated that fragments of these amyloids, once they enter the systemic circulation and cross the compromised blood-brain barrier, may act in the brain as elements that initiate or accelerate the process of abnormal folding and aggregation of human Aβ [Subedi et al. 2022; Friedland and Chapman 2013].

Another important mechanism is the phenomenon of microglia priming. Long-term exposure of the brain to low levels of pro-inflammatory signals from leaky gut (*e.g.* LPS) does not necessarily lead to an immediate, rapid response, but puts microglia cells into a state of chronic readiness, or “priming”. Such “primed” cells are characterised by an altered transcriptional and metabolic profile. When they then encounter a second stimulus, such as endogenous Aβ oligomers or damaged neurons, their response is disproportionately strong, rapid and often neurotoxic. Thus, gut dysbiosis may not only provide inflammatory stimuli, but also “calibrate” the reactivity threshold of the brain's innate immunity, making it more vulnerable to damage [Bivona et al. 2023].

Communication along the gut-brain axis is not exclusively pathological. Metabolites produced by symbiotic bacteria provide an important counterbalance to pro-inflammatory processes. Short-chain fatty acids, especially butyrate, show strong neuroprotective effects. Butyrate is a known inhibitor of histone deacetylases (HDACs). By inhibiting the activity of HDACs in microglia, butyrate promotes the acetylation state of histones, which facilitates the transcription of anti-inflammatory and phagocytosis-related genes, improving the ability of microglia to remove Aβ in a non-inflammatory manner [Rubio-Perez and Morillas-Ruiz, 2012].

An effective strategy for the prevention and supportive treatment of AD must be an integrated strategy. The overarching goal becomes quieting systemic inflammation by nurturing intestinal eubiosis, achieved with a diet rich in diverse prebiotic fibre. At the same time, at the molecular level, this strategy must ensure the provision of key neuroprotective nutrients that act directly in the CNS. This requires a supply of omega-3 fatty acids, as well as other compounds e.g. polyphenols that inhibit inflammatory pathways and oxidative stress [Belloy et al. 2022].

7. Summary

The topic of the role of diet in AD cannot be limited to single components or mechanisms, this should be considered synergistically. A diet rich in fibre supports the microbiota, which produces protective butyrate. The same diet, if it contains oily fish, provides DHA, which protects neurons. If it also contains green vegetables, it provides polyphenols that inhibit the nuclear factor NF-κB - which plays a key role in the immune response and inflammatory processes. The combination of all these elements creates a robust, multi-level prevention programme and tool to help prevent and treat Alzheimer's disease. Investing in nutrition education and promoting health-promoting habits is a key component of a global strategy to combat the dementia epidemic.

8. References:

[1] Ajala A., Uzairu A., Shallangwa G.A., Abechi S.E. Virtual screening, molecular docking simulation and ADMET prediction of some selected natural products as potential inhibitors of NLRP3 inflammasomes as drug candidates for Alzheimer disease. *Biocatal. Agric. Biotechnol.* 2023, 48, 102615-102634.

[2] Ali A., Cottrell J.J., Dunshea F.R. Identification and characterization of anthocyanins and non-anthocyanin phenolics from Australian native fruits and their antioxidant, antidiabetic, and anti-Alzheimer potential. *Food Res. Int.* 2022, 162, 111951-111964.

[3] Al-Kuraishy H.M., Jabir M.S., Albuhadily A.K., Al-Gareeb A.I., Jawad S.F., Swelum A.A., Hadi N.R. Role of ketogenic diet in neurodegenerative diseases focusing on Alzheimer diseases, *Ageing Res. Rev.*, 2024, 95, 102233-102242.

[4] Arlt S., Müller-Thomsen T., Beisiegel U. Use of vitamin C and E in the treatment of Alzheimer's disease. *Drug Dev. Res.*2002, 56(3), 452-457.

[5] Arnoldy L., Gauci S., Young L.M., Macpherson H., Civier O., Scholey A., White D.J. Assessing the association between the Mediterranean, Dietary Approaches to Stop Hypertension and Mediterranean-DASH Intervention for Neurodegenerative Delay dietary patterns, structural connectivity and cognitive function. *Br. J. Nutr.*, 2025, 133(7), 1-17.

[6] Belloy M.E., Le Guen Y., Eger S.J., Napolioni V., Greicius M.D., He Z. (2022). A fast and robust strategy to remove variant-level artifacts in Alzheimer disease sequencing project data. *Neurol.Gen.* 2022, 8(5), 200012-200028.

[7] Bivona G., Iemmolo M., Agnello L., Lo Sasso B., Gambino C.M., Giglio R.V., Ciaccio M. Microglial activation and priming in Alzheimer's disease: state of the art and future perspectives. *Int. J.Mol. Sci.* 2023, 24(1), 884-901.

[8] Bouchard J., Malalgoda M., Storsley J., Malunga L., Netticadan T., Thandapilly S.J. Health benefits of cereal grain-and pulse-derived proteins. *Mol.*, 2022, 27(12), 3746-3772.

[9] Calvo-Rodriguez M., García-Rodríguez C., Villalobos C., Núñez L. Role of toll like receptor 4 in Alzheimer's disease. *Frontiers in immunology*, 2020, 11, 1588-1597.

[10] Carey A., Fossati S. Hypertension and hyperhomocysteinemia as modifiable risk factors for Alzheimer's disease and dementia: New evidence, potential therapeutic strategies, and biomarkers. *Alzheimer's Dement.* 2023, 19(2), 671-695.

[11] Charisis S., Yannakoulia M., Scarmeas N. Diets to promote healthy brain ageing. *Nat. Rev. Neurol.* 2025, 21(1), 5-16.

[12] Chen H., Meng L., Shen L. Multiple roles of short-chain fatty acids in Alzheimer disease. *Nutr.* 2022, 93, 111499-111511.

[13] Chu C.Q., Yu L.L., Qi G.Y., Mi Y.S., Wu W.Q., Lee Y.K., Chen W. Can dietary patterns prevent cognitive impairment and reduce Alzheimer's disease risk: Exploring the underlying mechanisms of effects. *Neurosci. Biobehav. Rev.* 2022, 135, 104556-104572.

[14] Doroszkiewicz J., Mroczko J., Rutkowski P., Mroczko B. Molecular Aspects of a Diet as a New Pathway in the Prevention and Treatment of Alzheimer's Disease. *Int. J. Mol. Sci.* 2023, 24(13), 10751-10772.
[15] Duplantier S.C., Gardner C.D. A critical review of the study of neuroprotective diets to reduce cognitive decline. *Nutr.* 2021, 13(7), 2264-2289.
[16] Ferreira I., Rauter A.P., Bandarra N.M. Marine sources of DHA-rich phospholipids with anti-Alzheimer effect. *Marine Drugs*, 2022, 20(11), 662-682.
[17] Fock E., Parnova R. Mechanisms of blood-brain barrier protection by microbiota-derived short-chain fatty acids. *Cells*, 2023, 12(4), 657-686.
[18] Friedland R.P., Chapman M.R. The role of microbial amyloid in neurodegeneration. *PLoS pathogens*, 2017, 13(12), 1006654-1006667.
[19] Jeong S.P., Sharma N., An S.S.A. Role of calcitriol and vitamin D receptor (VDR) gene polymorphisms in Alzheimer's disease. *Int. J. Mol. Sci.* 2024, 25(9), 4806-4824.
[20] Karati D., Meur S., Roy S., Mukherjee S., Debnath B., Jha S. K., Ghosh P. Glycogen synthase kinase 3 (GSK3) inhibition: a potential therapeutic strategy for Alzheimer's disease. *Naunyn-Schmiedebergs Arch. Pharmacol.* 2025, 398, 2319-2342.
[21] Kim H.S., Kim S., Shin S.J., Park Y.H., Nam Y., Kim C.W., Moon M. Gram-negative bacteria and their lipopolysaccharides in Alzheimer's disease: pathologic roles and therapeutic implications. *Transl. Neurodegener.* 2021, 10, 1-23.
[22] Li J., Lyu X., Wang P., Zhu B.T. Bilberry anthocyanins attenuate mitochondrial dysfunction via β-catenin/TCF pathway in Alzheimer's disease. *J. Funct. Food.* 2023, 110, 105827-105838.
[23] Livingston G., Huntley J., Sommerlad A., Ames D., Ballard C., Banerjee S., Brayne C., Corbett A., Firbank M., Gusterson B., Juszczak E., Kasper S., Lewis G., Robert P., Mukadam N. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *Lancet*, 2020, 396(10248), 413-446.
[24] Nucci D., Sommariva A., Degoni L. M., Gallo G., Mancarella M., Natarelli F., Gianfredi V. Association between Mediterranean diet and dementia and Alzheimer disease: a systematic review with meta-analysis. *Aging. Clin. Exp. Res.,* 2024, 36(1), 77-92.
[25] Oliveira T.P., Morais A.L., Dos Reis P.L., Palotás A., Vieira, L.B. A potential role for the ketogenic diet in alzheimer's disease treatment: exploring pre-clinical and clinical evidence. *Metabol.* 2023, 14(1), 25-47.
[26] Rubio-Perez J.M., Morillas-Ruiz J.M. A review: inflammatory process in Alzheimer′ s disease, role of cytokines. *Sci. World J.*, 2012, 1, 756357-756362.
[27] Sánchez-Villegas A., Martínez-González M.A., Estruch R., Salas-Salvadó J., Corella D., Covas M.I., Serra-Majem L. Mediterranean dietary pattern and depression: the PREDIMED randomized trial. BMC *Med.* 2013, 11, 1-12.
[28] Scheff S.W., Price D.A. Alzheimer's disease-related alterations in synaptic density: neocortex and hippocampus. *J. Alzheimers Dis.*, 2006, 9(3), 101-115.
[29] Serini S., Bizzarro A., Piccioni E., Fasano E., Rossi C., Lauria A., Calviello G. (2012). EPA and DHA differentially affect in vitro inflammatory cytokine release by peripheral blood mononuclear cells from Alzheimer's patients. *Curr. Alzheimer Res.* 2012, 9(8), 913-923.
[30] Silva P., Rodríguez-Pérez M., Gómez-Torres Ó., Burgos-Ramos E. Olive oil and wine as source of multi-target agents in the prevention of Alzheimer disease. *Nutr. Res. Rev*. 2023, 36(1), 140-154.
[31] Singh D. Astrocytic and microglial cells as the modulators of neuroinflammation in Alzheimer's disease. *J. Neuroinf.* 2022, 19(1), 206-221.
[32] Subedi S., Sasidharan S., Nag N., Saudagar P., Tripathi T., Amyloid cross-seeding: mechanism, implication, and inhibition, *Mol.* 2022, 27, 1776-1802.
[33] Tian W., Ju J., Guan B., Wang T., Zhang J., Song L., Xu H. Role of hyperhomocysteinemia in atherosclerosis: from bench to bedside. *Ann. Med.* 2025, 57(1), 2457527-2457565.

[34] Timlin D., McCormack J.M., Kerr M., Keaver L., Simpson E.E. The MIND diet, cognitive function, and well-being among healthy adults at midlife: a randomised feasibility trial. *BMC Nut.*, 2025, 11(1), 59-78.

[35] Valverde-Salazar V., Ruiz-Gabarre D., García-Escudero V. Alzheimer's disease and green tea: epigallocatechin-3-gallate as a modulator of inflammation and oxidative stress. *Antioxidants*, 2023, 12(7), 1460-1464.

[36] Wang B., Ma J., Kraus V.B., Gao X., Jiang B., Guo W., Yin Z. Associations of Mediterranean-DASH intervention for neurodegenerative delay diet with blood pressure and hypertension among older adults China, 2019-2022. *China CDC Weekly*, 2025, 7(20), 695-700.

[37] Weinstock M. Role of Oxidative Stress and Neuroinflammation in the Etiology of Alzheimer's Disease: Therapeutic Options. *Antioxid.* 2025, 14(7), 769-785.

[38] Ye M., Yang X., Yan J., Yao Y., Lv H., Yue Z., Liu Z. Causal relationship between B vitamins and neuropsychiatric disorders: a systematic review and meta-analysis. *Neurosci. Biobehav. Rev.* 2025, 170, 106068-106076.

[39] Zhan X., Stamova B., Sharp F.R. Lipopolysaccharide associates with amyloid plaques, neurons and oligodendrocytes in Alzheimer's disease brain: a review. *Front. Aging Neurosci.*, 2018, 10, 42-56.

[40] Zhou L., Xiong J.Y., Chai Y.Q., Huang L., Tang Z.Y., Zhang X.F., Zhang J.T. Possible antidepressant mechanisms of omega-3 polyunsaturated fatty acids acting on the central nervous system, *Front. Psychiatry.* 2022, 13, 933704-933719.

The Use of Kefir Grains for the Production of Non-milk Fermented Beverages

Bożena Sosnowska*, Katarzyna Czerwińska

University of Life Sciences in Lublin,
Department of Biotechnology,
Microbiology and Human Nutrition.
**corresponding author: bozena.sosnowska@up.lublin.pl*

Abstract

The aim of the work was to check the possibility of preparing non-dairy fermented products using kefir grains.The material consisted of commercial beverages: almond and coconut. Both beverages were fermented using kefir grains (48 h, temperature 20 °C). After this time, microbiological, physicochemical and organoleptic analyses were performed. The total number of mesophilic bacteria, lactic bacilli and streptococci, yeast and moulds was determined using plate methods. Active and titratable acidity and lactic acid content were also determined in the beverages. 15 people participated in the organoleptic assessment using the point method. All the parameters tested met the PN requirements for the equivalent of such a fermented product from cow's milk - kefir. Both beverages received positive notes in the organoleptic evaluation, so they can be recommended as beverages enriching beneficial intestinal microflora, which can be prepared even at home.

Keywords: non-dairy beverage, fermented beverage, functional beverage, kefir grains.

1. Introduction

Kefir is a traditional fermented dairy product obtained through mixed lactic and alcoholic fermentation of cow's milk using kefir grains. It is a biologically active beverage valued not only for its nutritional composition but also for its health-promoting properties. As a rich source of easily digestible proteins, calcium, and bioavailable vitamins, kefir significantly contributes to consumers' daily nutritional intake. During fermentation, a complex community of microorganisms, including lactic acid bacteria (LAB) and yeasts, actively metabolizes milk components to produce lactic acid, ethanol, carbon dioxide, and a variety of flavor compounds. These metabolic byproducts not only improve the shelf-life and safety of the product but also enhance its sensory characteristics and potential health benefits.

Lactic acid bacteria play a particularly important role in human health. They have been demonstrated to positively influence intestinal microflora, improve digestion, support immune function, and contribute to the prevention of certain gastrointestinal disorders [Chłopicka et al., 2016]. Despite these benefits, a growing number of consumers are unable to consume dairy-based kefir due to health-related reasons. Milk proteins, particularly casein and β-lactoglobulin, are common allergens. Furthermore, lactose—the primary sugar in milk—is increasingly recognized as a problematic component, especially among individuals with lactose intolerance [Mojka, 2013]. These health conditions have led to a notable demand for non-dairy alternatives. Plant-based beverages, often derived from nuts, grains, legumes, or seeds, offer an attractive solution. While they differ markedly from cow's milk in terms of protein structure, fatty acid profile, and micronutrient composition, they are often enriched with valuable bioactive compounds, such as polyphenols, dietary fiber, and phytosterols, which confer additional health benefits [Hoffmann and Kostyra, 2015]. Their lower allergenic potential and absence of lactose make them a suitable choice for individuals with lactose intolerance or other dietary restrictions. In addition to medical concerns, ideological and environmental motivations also drive the growing popularity of plant-based alternatives. Many consumers now consciously avoid animal products due to ethical or ecological considerations. For these individuals, fermented plant-based beverages can provide functional and nutritional diversity, helping to maintain gut health without compromising dietary principles. A promising method for producing such beverages involves fermenting plant-based substrates using kefir grains, also known as Tibetan mushrooms. These natural starter cultures, originating from the Tibetan region of China, are composed of a stable and symbiotic community of microorganisms embedded in a polysaccharide matrix called kefiran. Kefir grains are known for their ability to adapt to different fermentation environments and substrates, which allows for their repeated use in the production of probiotic beverages [Santos et al., 2019]. Compared to commercial starter cultures, which are typically composed of a limited number of defined microbial strains, kefir grains represent a more complex and dynamic microbial ecosystem.

This includes multiple species of lactic acid bacteria such as *Lactobacillus kefiri*, *L. kefiranofaciens*, *L. helveticus*, *L. plantarum*, *L. casei*, *L. paracasei*, and *Lactococcus lactis*, as well as yeast species capable of producing ethanol and aromatic compounds [Gao et al., 2012]. The microbial diversity present in kefir grains contributes not only to the functional qualities of the final product but also to its distinctive organoleptic properties—namely, its slightly effervescent texture, sour taste, and characteristic aroma. The matrix of kefir grains is gelatinous and irregular in shape, often resembling small cauliflower florets with diameters ranging from 0.3 to 3.5 cm. Their color varies from white to light yellow, depending on the fermentation medium and storage conditions. This biological system can be described as a natural model of immobilized microbial cells, in which microbial communities are embedded in an exopolysaccharide framework. Under favorable fermentation conditions—appropriate temperature, pH, and substrate availability—these grains actively ferment plant-based liquids, converting carbohydrates into lactic acid, CO_2, trace amounts of alcohol, and other metabolites that enhance nutritional and sensory quality [Conde-Islas et al., 2019]. Recent research confirms the ability of kefir grains to successfully ferment non-dairy substrates, including those derived from coconut, almond, oat, or soy. Despite the compositional differences between plant-based beverages and cow's milk, the microbial populations within kefir grains show a high degree of adaptability and metabolic flexibility, enabling consistent fermentation outcomes [Tu et al., 2019]. These findings open up possibilities for developing novel, plant-based kefir alternatives with probiotic characteristics. The aim of the present study was to develop and analyze a fermented plant-based beverage using traditional kefir grains. By employing coconut and almond beverages as substrates, the study sought to evaluate the microbiological safety, physicochemical parameters, and sensory quality of the resulting products. This type of beverage may offer a valuable alternative to conventional dairy kefir for individuals with milk allergies, lactose intolerance, or dietary preferences that exclude animal products. As interest in functional foods and gut-health-promoting products continues to grow, such innovations respond to an important consumer need for safe, natural, and nutritious alternatives.

2. Materials and Methods

2.1. Materials

The research material consisted of two types of plant-based beverages – almond and coconut, available in retail outlets, from one brand (Table 1). Kefir grains were used to ferment them.

Table1. Composition of beverages used for fermentation.

Type of beverage	Composition
Almond beverage	water, almonds (2.3%), calcium, sea salt, stabilizers (locust bean gum, gellan gum), emulsifier (lecithins), vitamins B2, B12, E, D2
Coconut beverage	water, coconut filling (8.8%) (water, coconut cream 4%), rice (3.3%), calcium, stabilizers (guar gum, gellan gum, xanthan gum), sea salt, flavorings, vitamins B12, D2

The beverage (0.7 dm^3) was placed in a 0.9 dm^3 glass jar, 35 g of kefir grains were added, and fermented for 48 hours at 20°C. After this process, the beverage was strained from the grains and subjected to microbiological, physicochemical, and organoleptic analysis.

2.2. Microbiological analysis

The number of lactic acid bacteria (LAB) and streptococci, aerobic mesophilic bacteria, and yeasts and molds was determined using the submerged plating method. Mesophilic bacteria were cultured in aerobic conditions on nutrient agar (BTL) (30°C, 48 h), *Lactobacillus* and *Lactococcus* were cultured on MRS (BTL) and M-17 (Biocorp) agar media (37°C, 48 h), respectively, while yeasts and molds were cultured on DRBC agar (Biocorp) (28°C, 2–5 days). Results are expressed as CFU/cm^3.

2.3. Chemical analysis

Chemical tests determined titratable acidity, including lactic acid content calculations according to PN-A-86061:2002, and active acidity using a pH meter. The analyses were performed in triplicate.

2.4. Organoleptic analysis

The organoleptic evaluation involved sensory evaluation of the color, consistency, taste, and aroma of both beverages. Samples were taken at room temperature. The evaluation was performed using the point method (Table 2) [Wichrowska and Wojdyła 2014]. Fifteen participants participated in the study.

Table 2. Criteria for organoleptic evaluation using the point method.

Parameter	Weighting coefficient	Rating scale 5	4	3	2	1
Color	0,15	typical color, characteristic of kefir	typical or less characteristic	not typical, slightly modified	not typical, clearly changed	unusual, heavily changed
Consistency	0,20	uniform, dense, slightly stringy	uniform, less dense, slightly stringy	uniform, too thin, stringy, visible lumps and slight creases	uniform, too thin, stringy, clearly lumpy	liquid, thin, clearly lumpy
Smell	0,25	intense, characteristic of kefir	slightly noticeable, characteristic of kefir	unclean, not very aromatic	unclean, changed	untypical, unclean, changed
Taste	0,40	pure, characteristic of kefir	clean, slightly sour, characteristic of kefir	sour, slightly bitter	changed, bitter, impure	clearly changed, impure, very bitter

2.5 Statistical analysis of results

Results were statistically analyzed using Statistica 13.0. One-way analysis of variance (ANOVA) was performed to test for significant differences ($p \leq 0.05$). Group homogeneity was verified using Tukey's test at a significance level of $p=0.05$. All data are expressed as means ± standard errors.

3. Results

The results of the microbiological analysis of the beverages are presented in Table 3. The number of mesophilic bacteria ranged from $4.9x10^4$ to $4.86x10^6$ CFU/cm³. The coconut beverage was characterized by a lower content of all microbial groups compared to the almond beverage. Regardless of the beverage type, lactobacilli outnumbered lactic acid streptococci in each beverage ($1.95x10^4$ to $1.4x10^6$ CFU/cm³ and $6.7x10^3$ to $9.7x10^4$ CFU/cm³, respectively). No mold was detected in any of the beverages.

Table 3. Counts of mesophilic bacteria, lactobacilli, lactic acid streptococci, yeasts, and molds in the tested fermented products.

Product	Number of microorganisms (CFU/cm³)				
	Mesophilic bacteria	*Lactobacillus* sp.	*Streptococcus* sp.	Yeasts	Molds
Fermented almond beverage	$4.86x10^6$	$1.4x10^6$	$9.7x10^4$	$9.54x10^4$	none found
Fermented coconut beverage	$4.9x10^4$	$1.95x10^4$	$6.7x10^3$	$8.23x10^3$	none found

In chemical analyses, the coconut beverage was characterized by higher values for all analyzed parameters (titratable and active acidity, and lactic acid content), and these differences were statistically significant. The results are presented in Table 4.

Table 4. Titratable acidity, lactic acid content and active acidity in fermented beverages.

Determinations	Fermented almond beverage	Fermented coconut beverage
Titratable acidity (°SH)	37.4±0.2b	44±0.4a
Lactic acid content per 100 cm³	0.84±0.005b	0.98±0.005a
Active acidity of beverages (pH)	3.86±0.025b	4.48±0.025a

Mean ± standard error; letters a and b indicate significant differences ($p<0.05$).

The final consumer rating results ranged from 3.82±0.13 to 4.21±0.06. The fermented coconut beverage received a higher rating. All analyzed parameters were better for the coconut beverage, but the differences in consistency and taste were not statistically significant.

Table 5. Results of the organoleptic evaluation of the tested beverages.

Parameter	Fermented almond beverage	Fermented coconut beverage
Color	4.53±0.13a	3.53±0.16b
Consistency	3.6±0.16a	3.47±0.19a
Smell	4.6±0.13a	4.0±0.16b
Taste	4.27±0.15a	4.07±0.18a
Final Rating	4.21±0.06a	3.82±0.13b

Mean ± standard error; letters a and b indicate significant differences ($p<0.05$).

4. Discussion

This study provides evidence supporting the beneficial role of kefir grains in the fermentation of plant-based beverages. Microbiological analysis confirmed the presence of viable lactic acid bacteria (LAB), specifically *Lactobacillus* sp. and *Streptococcus* sp., as well as yeast cells, which are both essential for the functional and sensory characteristics of fermented products. Notably, no mold growth was detected in any of the samples, which is crucial from a food safety perspective, considering the potential risk of mycotoxin production associated with mold contamination [Pławińska-Czarnak and Zarzyńska 2010].

The mesophilic bacteria isolated from the beverages were predominantly lactic acid bacteria, as indicated by the microbiological profile and enumeration of specific microbial groups. Both LAB and yeast were present at levels consistent with regulatory and scientific standards for probiotic and fermented products. Specifically, the total viable counts of lactobacilli and lactic acid streptococci reached the recommended threshold of 10^6–10^7 CFU/g or mL, which is considered sufficient to exert beneficial effects on the host's microbiota after passage through the gastrointestinal tract [Valero-Cases et al., 2020]. Moreover, yeast counts exceeded 10^2 CFU/g, aligning with the requirements of the PN-A-86061 standard for kefir products. Interestingly, higher concentrations of LAB and yeast were observed in the fermented almond beverage compared to the coconut-based counterpart. This increased microbial activity may be attributed to the addition of sucrose in the homemade almond formulation, which likely enhanced the growth and metabolic activity of fermentative microorganisms, particularly yeasts. In terms of physicochemical properties, the titratable acidity of the beverages ranged from 37.4 to 44°SH. The fermented almond beverage exhibited a lower acidity (37.4°SH), whereas the coconut beverage demonstrated a higher value (44°SH). Correspondingly, lactic acid concentrations varied between 0.84% and 0.98%, with the coconut beverage again showing higher acidity. These values comply with the PN-A-86061 standard, which requires a minimum lactic acid content of 0.6% in fermented beverages, confirming successful lactic fermentation. The active acidity (pH) of the samples ranged from 3.86 to 4.48. The fermented almond beverage had a slightly higher pH, indicating lower acidity, while the fermented coconut beverage presented with lower pH, reflecting greater acidification. Both titratable and active acidity are closely linked to fermentation conditions such as temperature, duration, and microbial load—especially the abundance of LAB, which produce organic acids during metabolism [Sri et al., 2021; Armistice et al., 2022]. An organoleptic assessment using a 5-point hedonic scale was conducted, evaluating parameters such as color, aroma, taste, and consistency. The fermented coconut beverage consistently received higher scores across all categories compared to the almond-based variant. It was described as having a characteristic, slightly opaque appearance, a mild and pleasant aroma, and a clean, slightly tart taste typical of kefir-like products.

Overall, the findings of this study indicate that plant-based beverages fermented with kefir grains meet both microbiological and chemical safety standards. The presence of live LAB and yeast at functional levels, along with favorable sensory properties, positions these beverages as promising candidates for consumers seeking natural, health-promoting, non-dairy alternatives. In addition to their probiotic potential, their light acidity and effervescent profile make them particularly appealing as refreshing beverages during the summer months. The growing consumer interest in minimally processed, allergen-free, and plant-based products further underscores the relevance and market potential of such fermented beverages.

5. Conclusions

The conducted experiment led to the successful development of a high-quality fermented non-dairy beverage, which demonstrates significant potential for consumer acceptance and market applicability. The use of kefir grains in the fermentation of plant-based substrates, specifically coconut and almond beverages, proved to be effective and reliable. This confirms the adaptability of traditional kefir grains—typically used in dairy fermentation—for application in plant-based alternatives. From a microbiological standpoint, the fermented beverages met the expected standards for viable counts of beneficial microorganisms. Both lactic acid bacteria and yeast were present in quantities consistent with those required for fermented functional beverages, ensuring the potential for positive impacts on the human gastrointestinal microbiota. The presence of these live microorganisms, known for their probiotic properties, positions the resulting beverage within the category of functional foods, offering not only nutritional value but also health-promoting benefits.

Chemical analyses indicated that the titratable acidity of the final products was within the acceptable range for fermented beverages, indicating successful fermentation and microbial activity without over-acidification, which could compromise taste or safety. Organoleptic evaluation further supported the success of the product. Among the tested variants, the fermented coconut beverage was consistently rated higher than its almond-based counterpart. Panelists appreciated its characteristic, slightly opaque white color, subtle and pleasant aroma, and mildly sour taste typical of traditional kefir products. These sensory attributes contributed positively to the overall acceptance of the beverage. In conclusion, the study demonstrates that kefir grains can be effectively used to ferment plant-based beverages, resulting in a microbiologically safe, sensorially acceptable, and nutritionally beneficial product. The findings contribute valuable knowledge to the growing field of non-dairy fermented foods and offer a promising direction for the development of functional beverages aimed at health-conscious consumers, including those following vegan or lactose-free diets.

6. References

[1] Armistice C., Talknice J., Tafadzawa M.: Effect of Lacticaseibacillus rhamnosus Yoba Fermentation on Physicochemical Properties, Amino Acids, and Antioxidant Activity of Cowpea-Peanut Milk. J. Food Qual.., 2022, Feb, DOI: 10.1155/2022/3192061.

[2] Chłopicka J., Foltyn K., Miąsik M., Bartoń H.: Porównanie właściwości antyoksydacyjnych i zawartości kwasów organicznych w napojach fermentowanych otrzymanych przy pomocy grzybka tybetańskiego i herbacianego. Bromatologia Chem. Toksykol., 2016 3, 248-251.

[3] Conde-Islas A.A., Jimenez-Fernandez M., Cantu-Lozano D., Urrea-Garcia G.R., Luna-Solano G.: Effect of the Yreeze-Drying Process on the Physicochemical and Microbiological Properties of Mexican Kefir Grains. Processes, 2019, 7, 127, DOI:10.3390/pr7030127.

[4] Gao J., Cu F., Abdella N.H., Ruan H., He G.: Investigation on Culturable Microflora in Tibetan Kefir Grains from Different Areas of China. Journal of Food Science, 2012, 77, 8, DOI: 10.1111/j.1750-3841.2012.02805.

[5] Hoffmann M., Kostyra E.: Jakość sensoryczna i wartość odżywcza wegańskich substytutów mleka krowiego. Postępy Techniki Przetwórstwa Spożywczego, 2015, 1, 52.

[6] Mojka K.: Charakterystyka mlecznych napojów fermentowanych. Probl Hig Epidemiol., 2013, 94, 4, 722-729.

[7] Pławińska-Czarnak J., Zarzyńska J.: Mikotoksyny w żywności pochodzenia zwierzęcego. Mikologia Lekarska, 2010, 17, 2, 128-133, ISSN1232-986X.

[8] PN-A-86061:2002 Mleko i przetwory mleczne – Mleko fermentowane.

[9] Santos D.C., Goncalves de Oliveira Filho J., Santana A.C.A., Morais de Freitas B.S., Silva F.G., Takeuchi K.P., Egea M.C.: Optimization of soymilk fermentation with kefir and the addition of inulin: Physicochemical, sensory and technological characteristics. LWT - Food Science and Technology, 2019, 104, 30–37, DOI: 10.1016/j.lwt.2019.01.030.

[10] Sri M., Indri J., Yulianti F.K., Yudha E.P., Huriya A.: Examination of titratable acidity, pH, total lactic acid bacteria and sensory properties in whey fermented with probiotic Pediococcus acidilactic BK01. Advances in Animal and Veterinary Sciences, 2022, 10 (1), DOI: 10.17582/journal.aavs/2022/10.1.114.119.

[11] Tu C., Azi F., Huang J., Xu X., Xing G., Dong G., Dong M.: Quality and metagenomic evaluation of a novel functional beverage produced from soy whey using water kefir grains. LWT - Food Science and Technology, 2019, 113, DOI:10.1016/j.lwt.2019.108258.

[12] Wichrowska D., Wojdyła T.: Ocena sensoryczna i fizykochemiczna wybranych jogurtów naturalnych i ekologicznych. Inż. Ap. Chem., 2014, 53, 6, 421 - 423.

[13] Valero-Cases E., Cedra-Bernard D., Pastor J.J., Frutos M.J.:Non-Dairy Fermented Beverages as Potential Carriers to Ensure Probiotics, Prebiotics, and Bioactive Compounds Arrival to the Gut and Their Health Benefits. Nutrients, 2020, 12, 1666; DOI:10.3390/nu12061666.

The influence of spice plants on the prevention of non-communicable diseases development

Jessica Świerc*

Faculty of Production Engineering and Logistics,
Department of Biosystem Engineering and Chemical Processes.
Opole University of Technology,
**corresponding author:j.swierc@po.edu.pl*

Abstract

Non-communicable diseases (NCDs), including cardiovascular diseases, diabetes, and metabolic disorders, are a leading global health concern. Dietary strategies rich in bioactive plant compounds are gaining recognition for their preventive potential. This chapter presents an integrative literature review (2015–2025) on the role of selected culinary spices—oregano, basil, savory, lovage, mustard, thyme, marjoram, and fennel—in the prevention of NCDs. The review includes studies sourced from PubMed, Scopus, Web of Science, and grey literature databases, focusing on phytochemical composition, antioxidant capacity, and biological mechanisms of action.
Spices such as marjoram and fennel exhibit a rich profile of polyphenols, flavonoids, and essential oils with anti-inflammatory, antioxidant, and metabolic-modulating properties. For example, marjoram infusion has been shown to improve insulin sensitivity and hormonal balance in women with PCOS, while fennel contains compounds that may support glucose metabolism and lipid regulation. The biological effects of spices depend heavily on processing methods, with freeze-dried forms demonstrating superior preservation of active compounds compared to conventional drying. However, market analyses reveal substantial variation in the chemical quality and microbiological safety of commercially available dried spices.
The evidence indicates that regular inclusion of high-quality spices in the diet may contribute to NCD prevention through modulation of oxidative stress and metabolic pathways. Ensuring quality control and appropriate preservation methods is essential to maximize their health-promoting potential.

Keywords: culinary spices, non-communicable diseases, spice plants, chronic-disease prevention, antioxidant activity, spice quality

1. Introduction

Non-communicable diseases (NCDs), such as cardiovascular disorders, type 2 diabetes, obesity, and neurodegenerative conditions, represent a major public health challenge globally. These chronic conditions are closely associated with modifiable lifestyle factors, including diet, physical inactivity, and exposure to oxidative stress and chronic inflammation. As conventional therapeutic approaches often focus on symptom management rather than prevention, increasing attention has been directed toward the role of bioactive food components in disease modulation.

Spices and culinary herbs, traditionally valued for their sensory properties, are increasingly recognized as functional food ingredients due to their richness in phytochemicals such as polyphenols, flavonoids, terpenes, and essential oils. These compounds exhibit a wide spectrum of biological activities, including antioxidant, anti-inflammatory, hypoglycemic, hypolipidemic, and neuroprotective effects. Evidence from in vitro, in vivo, and limited clinical studies suggests that regular consumption of certain spices may support the prevention of metabolic and cardiovascular diseases by influencing molecular pathways involved in oxidative stress, lipid metabolism, glucose regulation, and inflammation.

This chapter aims to review the preventive potential of eight selected spices—oregano, basil, savory, lovage, mustard, thyme, marjoram, and fennel—in the context of NCDs. Particular emphasis is placed on their chemical composition, mechanisms of biological action, and findings from preclinical and clinical studies. Additionally, quality and safety concerns associated with the use of dried spices on the consumer market are discussed, highlighting the importance of product standardization and proper processing methods in maximizing health benefits.

2. Methodology

An integrative literature review was conducted on studies between 2015 and 2025, examing eight culinary spices in the prevention of non-communicable diseases, using PubMed, Scopus, Web of Science, Google Scholar and EU grey-literature sources. Articles in English reporting metabolic, cardiovascular, inflammatory or antioxidant outcomes for a single spice were included.

3. Health-promoting properties in the context of NCD prevention

Supplementation with an aqueous basil extract in rats fed a high-cholesterol diet significantly lowered total cholesterol (TC) and triglycerides levels (TG). In the hippocampus, the expression of SOD (superoxide-dismutase) increased and the level of iNOS (inducible nitric oxide synthase) decreased, which confirms the strong antioxidant activity of basil extract. Additionally, supplementation reduced hippocampal amyloid-Aβ deposition (playing a key role in the pathogenesis of Alzheimer's disease) and improved learning and memory performance. Basil may therefore be a valuable element of dietary prevention of many civilization diseases [Mohammadali et al., 2020].

Basil extract also reduces fasting blood glucose, improves oral-glucose-tolerance profiles, partialy regenerates pancreatic β-cells, and inhibits α-amylase and α-glucosidase. These findings, obtained after 21 days of supplementation in diabetic rats, indicate that basil can likewise be considered as a dietary aid in the prevention of type 2 diabetes [Chaudhary et al., 2016]

Taken together, these studies show that basil targets dyslipidaemia, hyperglycaemia, oxidative stress and neurodegeneration—the converging mechanisms that drive cardiovascular, metabolic and neurocognitive disorders. Although the current data are limited to rodent models, they justify considering standardised basil preparations as complementary, food-based interventions for the primary prevention of type 2 diabetes, atherosclerosis and Alzheimer's disease, provided that phytochemical content and dosage are adequately controlled in future clinical trials.

Supplementation with aqueous extract of fennel leaves in rats after 15 days resulted in lower fasting blood glucose and improved glucose tolerance. Additionally, fennel extract showed strong antioxidant activity. These properties of fennel support its preventive effect in the context of non-communicable diseases, mainly type 2 diabetes [El-Ouady et al., 2020]

Supplementation with fennel-seed extract in rats with metabolic syndrome normalized the symptoms typical of this condition and more: cardiac hypertrophy, hypertension, ECG irregularities, hyperglycaemia, dyslipidaemia, hyperuricaemia, insulin resistance.

These findings support the use of fennel as a preventive agent against metabolic syndrome and other lifestyle-related diseases [El- Wakf et al., 2024].

Together, these findings suggest that the anethole- and polyphenol-rich matrices of fennel leaves and seeds target multiple drivers of type 2 diabetes and cardiometabolic disorders, justifying further clinical evaluation of standardised fennel preparations as food-based preventive agents.

The main active ingredients present in lovage include: chlorogenic, rosmarinic and caffeic acids; quercetin; luteolin. Lovage lowers fasting blood glucose, improves the lipid profile (lowers TC, LDL-C (low-density lipoprotein cholesterol), triglycerides and increases HDL-C (high-density lipoprotein cholesterol) in rats. It affects the functioning of glucose transporters and has an impact on the process of carbohydrate absorption. Additionally, it supports the endogenous antioxidant defences and regulates the activity of digestive enzymes. Together, these mechanisms position lovage as a valuable dietary ingredient for preventing type 2 diabetes, dyslipidemia and related metabolic disorders [Ghaedi et al., 2020].

These findings support the inclusion of lovage in dietary strategies aimed at preventing and managing chronic metabolic diseases.

In a study conducted on women with polycystic-ovary syndrome (PCOS), after drinking two cups of marjoram infusion a day, a statistically significant reduction in androgen concentration was observed compared to the placebo group, a reduction in fasting insulin and a significant reduction in the HOMA-IR index. PCOS, in addition to its characteristic symptoms, is a factor that increases the risk of developing insulin resistance or dyslipidemia. The observed results in improving insulin sensitivity and hormonal balance indicate that introducing marjoram in the form of an infusion to the diet of women with PCOS may be a helpful tool in therapy but also act prophylactically in the group of women at increased risk of developing insulin resistance or endocrine disorders [Haj-Husein et al., 2016].

The findings position marjoram tea as a low-risk, food-based adjunct that may aid both therapy and primary prevention of NCDs in women at elevated cardiometabolic risk.

Confirmation in longer, adequately powered trials with standardized preparations and dosing is warranted to translate these benefits into guidance.

Supplementation with mustard-seed-extract in rats with induced type 2 diabetes after 15 days led to a decrease in fasting blood glucose (an effect comparable to the effect of metformin), improved the HOMA-IR index (improved insulin sensitivity), strengthened the internal antioxidant defences and normalized the activity of enzymes involved in carbohydrate metabolism (e.g. glucose-6-phosphatase). These findings suggest that mustard extract may be an alternative to drugs with hypoglycaemic effects and may be incorporated into dietary strategies for preventing or managing carbohydrate-metabolism disorders [Paul et al., 2021].

Similar results were obtained in a study on mice given mustard oil – an improvement in the insulin-resistance index. Additionally, supplementation showed a reduction in inflammation, inhibition of the adipogenesis process and, interestingly, an increase in bone mineralization was noted [Takahashi et al., 2021].

Collectively, these findings support the use of standardised mustard preparations as food-based adjuncts for preventing or managing type 2 diabetes and related cardiometabolic disorders, while future human trials - and attention to oil composition (e.g., erucic-acid content) - are needed for safe translation to practice.

The main component of oregano oil is carvacrol. Studies conducted on rats with induced hypertension confirm the antioxidant effect of this compound. Additionally, it mobilizes endothelial progenitor cells, thereby weakening endothelial dysfunction, which is important in the prevention of cardiovascular diseases [Gonçalves et al., 2023].

Other compounds present in oregano - luteolin-7-O-diglucuronide, lithospermic acid and oreganol A can prevent the pathological process of insulin aggregation. This process affects the hormone's biological activity and reduces its therapeutic effectiveness. The compounds contained in oregano therefore show preventive action in the context of diabetes, its complications and other diseases related to carbohydrate metabolism disorders [Fialová et al., 2025].

Taken together, these findings suggest that well-standardized oregano preparations could complement dietary strategies for cardiovascular risk reduction and glycaemic complication prevention in type 2 diabetes. Robust, longer-term clinical trials with standardized formulations and transparent quantification of active constituents are needed to inform evidence-based dosing and clinical use.

Savory shows multifaceted cardioprotective activity, producing effects on the cardiovascular system comparable to those of metoprolol. Additionally, it improves the lipid profile (lowers TC, LDL-C and triglycerides) and strengthens endogenous antioxidant defences. These findings support the use of savory as a dietary component in cardiovascular-disease prevention strategies [Muthukumar et al., 2024].

Savory also has a favorable fatty-acid profile - high content of α-linolenic acid. Studies indicate that fatty acids obtained from savory have antioxidant and anti-inflammatory effects. Additionally, they inhibit digestive enzymes - α-amylase and lipase. Therefore, they can be used in the prevention of obesity, diabetes and other inflammation-related diseases [Obeidnejad et al., 2024].

Given these effects, standardized savory preparations with verified essential-oil and fatty-acid profiles should be clinically tested to confirm their role in cardiovascular and metabolic risk reduction.

Eight-week supplementation with thyme-seed extract improved insulin sensitivity in rats fed a high-fat diet. Supplementation additionally strengthened the internal antioxidant defences and reduced hepatic steatosis and inflammation. These findings suggest that thyme-seed extract may serve as a dietary strategy for preventing obesity, non-alcoholic fatty-liver disease and type 2 diabetes [Abdelmottaleb Moussa et al., 2025]. Strong anti-inflammatory and antioxidant effects were also demonstrated in a study on mice given thyme essential oil. Thyme therefore has the potential to be a natural ingredient supporting the prevention of diseases that arise from chronic inflammation – metabolic disorders, cardiovascular diseases, etc. [Warman et al., 2023]. Standardized thyme formulations - clearly defining chemotype (thymol/carvacrol), dose and safety - should be evaluated in robust human trials to validate benefits for insulin resistance, NAFLD (nonalcoholic fatty liver disease) and other inflammation-driven NCDs.

4. Quality and safety aspects of dried spices available to consumers

The health-promoting potential of culinary herbs and spices depends on the way they are processed, stored and distributed. The most common form of herbs among consumers is their dried, pre-packaged form, which also has the wildest market availability. However, their antioxidant activity and the contentration of bioactive compounds may differ significantly from those in fresh or minimally processed herbs.

A study of commercially available herbs sold in Poland showed that conventionally air-dried samples contained substantially fewer polyphenols than their freeze-dried counterparts. The findings indicate that prolonged exposure to elevated temperature, oxygen and light during drying and storage markedly depletes these bioactive compounds. The same trend was observed for antioxidant capacity – air-dried herb samples achieved significantly lower results compared to freeze-dried and fresh herbs [Bieżanowska-Kopeć and Piątkowska, 2022].

In another study comparing popular herbs available on the European market, herb samples were tested, among other things, for water activity and its content in the product. Some of the herbs tested exceeded the recommended limits (thyme and fennel). Increased water content exposes the product to the development of unfavorable microbes. The same study assessed essential-oil levels - only 2 types of herbs out of 8 tested met the requirements for their content (basil and oregano). Moreover, oil concentrations varied markedly among suppliers. It is the content of essential oils in herbs that is related to their antioxidant or antibacterial activity, etc. As a result, using herbs with a minimal content of essential oils may not have any beneficial effect on human health [Polańczyk et al., 2024].

In a study conducted on the Irish market, 855 samples of packaged, dried herbs and spices were tested. Some of the samples tested were found to contain Salmonella spp., *Bacillus cereus* spores and *Escherichia coli*, which demonstrates that even in the developed control systems used in the European Union, cases of food products being contaminated with pathogenic bacteria do occur [Food Safety Authority of Ireland, 2020]. Dried, pre-packaged herbs often deliver fewer bioactives and weaker antioxidant effects than fresh or freeze-dried forms, largely due to processing and storage conditions. Market variability—especially excess moisture/activity and sub-standard essential-oil levels—can further erode functional value and raise spoilage or safety risks. These realities argue for tighter standardization (moisture/aw control, verified essential-oil content) and routine microbiological testing to ensure dried herbs reliably confer health benefits.

5. Summary

The reviewed literature indicates that selected culinary spices—oregano, basil, savory, lovage, mustard, thyme, marjoram, and fennel—possess significant health-promoting properties relevant to the prevention of non-communicable diseases (NCDs). These spices are rich in bioactive compounds, including polyphenols, flavonoids, and essential oils, which exert antioxidant, anti-inflammatory, hypoglycemic, hypolipidemic, and metabolic-regulatory effects. Preclinical and clinical findings confirm their role in modulating key mechanisms underlying metabolic disorders, cardiovascular diseases, and neurodegenerative conditions. A summary of the most important compounds contained in the described herbs and their properties is summarized in Table 1.

Spices such as marjoram, basil, and fennel have demonstrated beneficial effects on glucose metabolism and insulin sensitivity. Others, like oregano and savory, contribute to cardiovascular protection through endothelial support and lipid-lowering actions. However, the biological efficacy of these spices is significantly influenced by their form and quality. Studies show that freeze-dried spices retain a higher concentration of active compounds compared to traditionally dried forms. Moreover, variation in essential oil content and microbial contamination in commercial products pose concerns regarding consumer safety and effectiveness.

In conclusion, the integration of high-quality spices into everyday nutrition may offer a natural and accessible strategy for reducing the risk of lifestyle-related diseases. Further standardization of processing methods and quality control is essential to ensure the consistency and efficacy of these botanicals in preventive health care.

Table 1. The most important active compounds and the chemical composition of each herb described in the article

Herb	Key Active Compounds	Notable Chemical Groups	Functional Relevance
Basil (*Ocimum basilicum*)	Polyphenols, flavonoids, essential oils; noted antioxidant enzymes upregulation	Linalool, eugenol, rosmarinic acid (implied by basil profile)	Antioxidant, anti-inflammatory, hypolipidaemic, hypoglycaemic, neuroprotective
Fennel (*Foeniculum vulgare*)	Anethole, polyphenols, flavonoids, essential oils	Phenylpropanoids, flavonoid glycosides	Antioxidant, glucose- and lipid-metabolism regulation, cardiometabolic support
Lovage (*Levisticum officinale*)	Chlorogenic acid, rosmarinic acid, caffeic acid, quercetin, luteolin	Phenolic acids, flavonoids	Hypoglycaemic, hypolipidaemic, antioxidant, enzyme-regulatory
Marjoram (*Origanum majorana*)	Polyphenols, flavonoids, essential oil (e.g., terpinen-4-ol, carvacrol – typical for marjoram)	Terpenoids, phenolic acids	Hormonal modulation, insulin sensitivity improvement, anti-inflammatory
Mustard (*Sinapis alba / Brassica spp.*)	Allyl isothiocyanate, phenolic compounds, omega-3 fatty acids, erucic acid (in oil), sinapine	Glucosinolates, phenolic acids, fatty acids	Hypoglycaemic, anti-inflammatory, anti-adipogenic, bone-supportive
Oregano (*Origanum vulgare*)	Carvacrol, luteolin-7-O-diglucuronide, lithospermic acid, oreganol A	Terpenoids, flavonoids, phenolic acids	Antioxidant, endothelial-protective, anti-diabetic (prevents insulin aggregation)
Savory (*Satureja hortensis*)	α-Linolenic acid, phenolic compounds, essential oils (carvacrol, thymol – typical), flavonoids	Fatty acids, phenolic acids, terpenoids	Cardioprotective, hypolipidaemic, antioxidant, enzyme-inhibitory
Thyme (*Thymus vulgaris*)	Thymol, carvacrol, flavonoids, polyphenols	Monoterpenes, phenolic acids	Antioxidant, anti-inflammatory, insulin-sensitising, hepatoprotective

6. References

[1] Abdelmottaleb Moussa, S.A., Ibrahim, F.A.A., Elbaset, M.A., Morsy, F.A., Aziz, S.W., Abd El-Latif, N.A., Afifi, S.M., Esatbeyoglu, T., El Toumy, S.A., Salib, J.Y., Bashandy, S.A.E., 2025. *Thymus vulgaris* seed extract hampered hepatic oxidative burden and improved insulin sensitivity in obese male rats. Endocr. Metab. Sci. 18, 100235.

[2] Bieżanowska-Kopeć, R., Piątkowska, E., 2022. Total Polyphenols and Antioxidant Properties of Selected Fresh and Dried Herbs and Spices. Appl. Sci. 12, 4876.

[3] Chaudhary, S., Semwal, A., Kumar, H., Verma, H.C., Kumar, A., 2016. In-vivo study for anti-hyperglycemic potential of aqueous extract of Basil seeds (*Ocimum basilicum* Linn) and its influence on biochemical parameters, serum electrolytes and haematological indices. Biomed. Pharmacother. 84, 2008–2013

[4] El- Wakf, A.M., El-Sawi, M.R., El-Nigomy, H.M., El- Nashar, E.M., Al-Zahrani, N.S., Alqahtani, N.G., Aldahhan, R.A., Eldken, Z.H., 2024. Fennel seeds extract prevents fructose-induced cardiac dysfunction in a rat model of metabolic syndrome *via* targeting abdominal obesity, hyperuricemia and NF-κβ inflammatory pathway. Tissue Cell 88, 102385.

[5] El-Ouady, F., Lahrach, N., Ajebli, M., Haidani, A.E., Eddouks, M., 2020. Antihyperglycemic Effect of the Aqueous Extract of Foeniculum vulgare in Normal and Streptozotocin-induced Diabetic Rats. Cardiovasc. Hematol. Disord. Drug Targets. 20, 54–63.

[6] Fialová, S.B., Gančár, M., Kurin, E., Dokupilová, S., Gažová, Z., Nagy, M., 2025. Oregano polyphenols reduce human insulin amyloid aggregation. Biomed. Pharmacother. 184, 117904.

[7] Food Safety Authority of Ireland, 2020. Survey of the microbiological safety of pre-packaged dried herbs and spices (17NS3). Food Safety Authority of Ireland, Dublin.

[8] Ghaedi, N., Pouraboli, I., Askari, N., 2020. Antidiabetic Properties of Hydroalcoholic Leaf and Stem Extract of Levisticum officinale: An implication for α-amylase Inhibitory Activity of Extract Ingredients through Molecular Docking. Iran. J. Pharm. Res. IJPR 19, 231–250.

[9] Gonçalves, T.A.F., Lima, V.S., de Almeida, A.J.P.O., de Arruda, A.V., Veras, A.C.M.F., Lima, T.T., Soares, E.M.C., Santos, A.C. dos, Vasconcelos, M.E.C. de, de Almeida Feitosa, M.S., Veras, R.C., de Medeiros, I.A., 2023. Carvacrol Improves Vascular Function in Hypertensive Animals by Modulating Endothelial Progenitor Cells. Nutrients 15, 3032.

[10] Haj-Husein, I., Tukan, S., Alkazaleh, F., 2016. The effect of marjoram (Origanum majorana) tea on the hormonal profile of women with polycystic ovary syndrome: a randomised controlled pilot study. J. Hum. Nutr. Diet. 29, 105–111.

[11] Mohammadali, S., Heshami, N., Komaki, A., Tayebinia, H., Abbasi Oshaghi, E., Karimi, J., Hashemnia, M., Khodadadi, I., 2020. Dill tablet and Ocimum basilicum aqueous extract: Promising therapeutic agents for improving cognitive deficit in hypercholesterolemic rats. J. Food Biochem. 44, e13485.

[12] Muthukumar, A., Mittal, S., Choezom, T., Bhavani, K., Das, K., Joyce, N., Almuqbil, M., Almadani, M.E., Ahmad, F., Yasmin, F., 2024. Evaluation of the cardioprotective activity of summer savory (*Satureja hortensis* L.) extract in experimental rats with Isoproterenol-induced myocardial infarction. J. King Saud Univ. - Sci. 36, 103236.

[13] Obeidnejad, E., Kavoosi, G., Saharkhiz, M.J., 2024. Antioxidant, anti-amylase, anti-lipase, and efficiency of Satureja fatty acid on the anti-inflammatory parameters in lipopolysaccharide-stimulated macrophage through Nrf2/NF-kB/NADH oxidase pathway. Sci. Rep. 14, 12490.

[14] Paul, K., Chakraborty, S., Mallick, P., Bhattacharjee, P., Pal, T.K., Chatterjee, N., Chakrabarti, S., 2021. Supercritical carbon dioxide extracts of small cardamom and yellow mustard seeds have fasting hypoglycaemic effects: diabetic rat, predictive iHOMA2 models and molecular docking study. Br. J. Nutr. 125, 377–388.

[15] Polańczyk, E., Matuszek, D., Hys, K., Pasek, M., Spalik, M., Rotkegel, A., 2024. Analysis of selected quality parameters of dried herbs available on the European market. Prod. Eng. Arch. 30, 182–190.

[16] Takahashi, A., Ishizaki, M., Kimira, Y., Egashira, Y., Hirai, S., 2021. Erucic Acid-Rich Yellow Mustard Oil Improves Insulin Resistance in KK-Ay Mice. Mol. Basel Switz. 26, 546.

[17] Warman, D.J., Jia, H., Kato, H., 2023. Effects of Thyme (Thymus vulgaris L.) Essential Oil on Aging-Induced Brain Inflammation and Blood Telomere Attrition in Chronologically Aged C57BL/6J Mice. Antioxidants 12, 1178.

Nutritional Deficiencies in Celiac Disease: Insights from a Decade of Research (2015–2025)

Dorota Teterycz*

Department of Engineering and Cereals Technology,
University of Life Sciences in Lublin,
Skromna 8, 20-704 Lublin, Poland
**corresponding author: dorota.teterycz@up.lublin.pl*

Abstract

Celiac disease is a chronic autoimmune condition triggered by gluten ingestion in genetically predisposed individuals. The only effective treatment is strict, lifelong adherence to a gluten-free diet (GFD). While the GFD facilitates mucosal recovery and symptom resolution, ongoing complaints may persist even in patients who strictly comply with dietary guidelines. This review analyzes scientific literature published between 2015 and 2025 concerning selected nutrient deficiencies in individuals with celiac disease adhering to a GFD.

Evidence indicates that individuals with celiac disease are at increased risk of deficiencies in dietary fiber, iron, zinc, magnesium, vitamin D, and B vitamins, especially folate and vitamin B12. These deficits are caused by multiple factors, including villous atrophy, impaired nutrient absorption, and the nutritional limitations of many processed gluten-free products. In some cases, deficiencies persist despite mucosal recovery and normalized antibody levels, which suggests ongoing subclinical malabsorption or inadequate dietary quality.

Improving the nutritional adequacy of a GFD requires both education and strategic food selection. Naturally gluten-free foods—such as legumes, fruits, vegetables, and nuts—as well as fortified gluten-free products. Regular monitoring of nutritional status, including laboratory testing, is essential to prevent long-term complications. Supplementation may be necessary in clinically significant cases. Individualized, evidence-based dietary management remains key to optimizing health outcomes in individuals with celiac disease.

Keywords: gluten free diet, celiac disease, nutritional deficiencies, gluten free products

1. Introduction

Celiac disease (CD) is a chronic autoimmune condition involving the small intestine, triggered by gluten consumption in individuals with a genetic predisposition [Husby et al., 2020; Jivraj et al., 2022]. It affects roughly 1% of the global population, and epidemiological data indicate that its incidence has been increasing over the past decades. In response to gluten, the immune system initiates pathological changes within the intestinal mucosa, most notably villous atrophy accompanied by crypt hyperplasia.

Such alterations impair the absorptive function of the small intestine, often leading to a range of clinical manifestations. In young children, typical symptoms include chronic diarrhea, abdominal bloating, growth retardation, developmental delay, and in more severe cases, malnutrition [Lebwohl & Rubio-Tapia, 2021]. In contrast, older patients frequently present with subtler gastrointestinal complaints — such as recurrent abdominal discomfort—which can hinder timely diagnosis. Malabsorption may also give rise to extraintestinal consequences, including iron deficiency anemia, reduced bone mineral density (osteopenia), and growth impairment [Husby et al., 2020; Jivraj et al., 2022].

Gluten, a protein complex found in wheat, rye, and barley, is considered the main environmental factor involved in the development of celiac disease in genetically susceptible individuals [Sharma et al., 2020]. Among the different protein fractions in gluten, gliadins are considered the most toxic in celiac disease, especially certain peptide fragments that are highly resistant to digestion and can trigger strong immune responses. The presence of HLA-DQ2 or HLA-DQ8 alleles is required for the disease to occur, since they help the immune system recognize and respond to gluten. Still, these alleles only explain about 40% of the genetic risk, while the rest seems to involve many other non-HLA genes, each with a relatively small effect [Aboulaghras et al., 2022].

Besides genetics and gluten, other factors like increased intestinal permeability are also believed to be involved. Increased intestinal permeability allows undigested gluten peptides to pass into the lamina propria, where they can trigger immune responses [Aleman et al., 2023]. Gut dysbiosis — an imbalance in the intestinal microbiota — has been suggested to influence disease progression by exacerbating inflammation, although its causal role in celiac disease remains under investigation. Even though the full mechanism behind celiac disease is still not fully worked out, it is generally accepted that the condition results from a combination of genetic, environmental, immune-related, and microbial factors [Aboulaghras et al., 2022].

Due to impaired absorption caused by villous atrophy in the small intestine, the nutritional status of untreated patients is often compromised. As a result, their diet may be deficient despite adequate intake. Currently, the only recognized effective treatment is strict and lifelong adherence to a gluten-free diet, which allows for mucosal healing and the restoration of nutrient absorption [Cárdenas-Torres et al., 2021]. However, novel therapeutic approaches — including enzyme supplementation, modulation of intestinal permeability, microbiota-targeted therapies, and immunomodulation—are under investigation to reduce dietary burden and improve long-term disease outcomes. However, the gluten-free diet may also be nutritionally inadequate, particularly if it lacks variety or relies heavily on processed gluten-free products that are often lower in fiber, vitamins, and minerals than their gluten-containing equivalents [Krawczyk et al., 2022].

The aim of this review is to analyze literature published between 2015 and 2025 to identify the selected nutritional deficiencies observed in individuals with celiac disease who follow a gluten-free diet.

2. Dietary fiber deficiency

By excluding cereal products such as wheat, barley, rye, and non-certified oats, individuals with celiac disease significantly reduce their intake of dietary fiber—both soluble and insoluble fractions. Since cereal products are among the primary sources of fiber in the Western diet, their elimination increases the risk of fiber deficiency. To ensure adequate fiber intake, it becomes necessary to incorporate alternative, naturally gluten-free sources such as fruits, vegetables, legumes, and nuts. Furthermore, long-term adherence to a gluten-free diet—especially one based on refined, low-fiber products — has been linked to reduced gut microbiota diversity, which may negatively affect gut health and immune function [Vici et al., 2016].

In the study by Taetzsch et al. [2018], two assessments were conducted to compare the nutritional quality of gluten-free and gluten-containing diets. The first involved evaluating nutrient profiles in healthy meal plans based on the Dietary Guidelines for Americans. These were modeled using MyPlate examples prepared by the USDA in both gluten-containing and gluten-free versions. Both dietary modeling and meta-analysis of existing literature indicated that gluten-free diets consistently provide less dietary fiber. The lower fiber intake was mainly due to reduced consumption of whole grain cereals, which are a primary fiber source in Western diets. Gluten-free products are often produced from refined flours and starches that are low in fiber and micronutrients. Consequently, a frequent issue observed with gluten-free diets is a lower fiber intake compared to traditional diets [Demirkesen & Ozkaya, 2022].

Similar findings were reported by Babio et al. [2017]. Their matched case-control cross-sectional study, conducted in Spain between 2012 and 2014 as part of the Celiac Disease Eating Attitudes project, included 98 individuals with celiac disease and 98 matched healthy controls aged 10–23 years. Dietary intake was assessed using 3-day food diaries completed at home and reviewed by trained dietitians. Nutrient content was calculated using Spanish food composition tables, and fiber intake was compared with national recommendations. Results showed that individuals on a gluten-free diet consumed less dietary fiber than healthy controls, although the difference was not statistically significant. Importantly, both groups failed to meet the recommended intake levels. The lower fiber consumption in the celiac group was attributed to reduced intake of whole grains, fruits, and vegetables, as well as the lower fiber content of many gluten-free products.

According to Larretxi et al. [2020], although gluten-free products (GFPs) have long been considered lower in fiber, technological advancements have led to improvements. For example, some specially formulated gluten-free breads may contain more fiber than standard white wheat bread (9.8% vs. 3.9%, respectively), although this is not representative of all gluten-free products. However, despite these improvements, total fiber intake in individuals with celiac disease remained lower than that of individuals on a regular diet. This was primarily due to lower consumption of grains, fruits, and vegetables. In the celiac group, fiber intake was modestly reduced and was largely derived from processed products, particularly bread, which accounted for over 50% of total fiber intake in both children and adults. The authors noted discrepancies between labeled and actual fiber content in gluten-free products, which may lead to errors in dietary assessment. They emphasize the need to update food composition databases and encourage the inclusion of naturally fiber-rich foods—such as legumes, fruits, vegetables, and nuts—in the gluten-free diet to improve its nutritional quality.

3. Minerals deficiences

Iron

One of the most common mineral deficiency disorders diagnosed in individuals with celiac disease is iron deficiency anemia (IDA). Often, IDA is the only symptom of subclinical/atypical forms of celiac disease. According to Paez et al. [2017], it can affect between 11.5% of patients with celiac disease without gastrointestinal symptoms and 69.4% of individuals without these symptoms. IDA results from the disappearance of intestinal villi, which prevents iron from being properly absorbed.

Among children diagnosed with iron deficiency anemia, 8.4–21.3% of patients tested positive for celiac disease. In adult patients, this result ranged from 2.9% to 6%. It is worth noting that iron deficiency is more common in women due to menstrual bleeding [Talarico et al., 2021].

In some individuals with celiac disease, iron deficiency anemia may persist despite following a gluten-free diet (GFD). Possible causes include: incomplete elimination of gluten from the diet, accidental consumption of gluten-contaminated products, insufficient iron content in the diet, prolonged intestinal villous atrophy, or physiological factors such as heavy menstruation or occult gastrointestinal bleeding. Some patients cannot tolerate oral iron supplements due to side effects and require periodic intravenous treatment. In situations where anemia persists despite normal intestinal histology and supplementation, the presence of ultrastructural changes in enterocytes that may limit iron absorption should be considered [Stefanelli et al., 2021].

A gluten-free diet helps to reduce inflammation in the small intestine, which over time improves the absorption of minerals. Despite the regeneration of the mucous membrane, it can take 6 to 12 months to rebuild the body's iron reserves. Therefore, it is important for patients with celiac disease to regularly consume naturally gluten-free foods high in iron, especially meat and vegetables, and to pay attention to the labels of gluten-free products in order to consciously choose those with a higher content of this element [Vici et al., 2016].

A cross-sectional study by Marco et al. [2025] involved 311 patients with biopsy-confirmed celiac disease (184 adults and 127 children) diagnosed and followed up at the University Hospital of L'Aquila. Clinical and laboratory data, including hemoglobin concentration and iron levels, were collected at the time of diagnosis and after 3–5 and 8–10 years of following a gluten-free diet to assess the incidence and persistence of anemia over time. The results show that at the time of diagnosis, anemia was present in 24% of adults and 5.6% of children. In adults, it was more common in women, probably due to iron loss during menstruation. In children, it was associated with younger age and more severe disease. After 3–5 years of a gluten-free diet, anemia was still present in about 20% of adults, despite normal mucosal histology. After 8–10 years, it dropped to 17.8% in adults and 4.4% in children. The results suggest that normalization of the intestinal picture does not always mean improved iron absorption. This may be due to permanent ultrastructural changes in enterocytes or disturbances in the expression of iron transport proteins. The need for long-term monitoring of blood parameters in patients with celiac disease, regardless of diet compliance, is emphasized. In the study by Kreutz et al. [2023], it was shown that after one year on a gluten-free diet, iron deficiency persisted in 45% of patients whose antibody levels had normalized, compared to 36% in the entire study group.

Zinc

Individuals with celiac disease often experience zinc deficiency (ZD), both in the active phase of the disease and during clinical remission while following a gluten-free diet (GFD). Reduced plasma zinc levels have been found in up to 67% of adults and 64% of children newly diagnosed with celiac disease. This deficiency is associated with the severity of intestinal villous atrophy — reported in 60% of patients with partial atrophy, 80% with subtotal atrophy, and 92% with total villous atrophy. Zinc deficiency in the intestinal mucosa may lead to activation of transglutaminase-2 (TG2), which is normally inhibited by zinc. The activated enzyme forms a complex with deamidated gliadin, acting as a neoantigen that triggers an autoimmune response and worsens villous damage [Chao, 2023].

Children with celiac disease may lack not only zinc but also magnesium, selenium, folic acid, calcium, vitamin D, iron, and fiber [Sue et al., 2018]. Even on a gluten-free diet, such deficiencies may persist. In children with celiac enteropathy, zinc levels typically normalize after starting a GFD, but additional supplementation does not always lead to further improvement [Chao, 2023].

ZD can still occur in up to 40% of individuals who have followed a GFD for over two years [Rondanelli et al., 2019]. Impaired zinc homeostasis, such as a reduced exchangeable zinc pool, can lead to deficiency even when gastrointestinal absorption is normal.

Lower zinc concentrations are also seen in children with chronic diarrhea or short stature, which may result from undiagnosed celiac disease. In children with ZD, 4-week zinc supplementation (20 mg) does not always provide a greater benefit than the GFD alone, as zinc levels tend to improve in both cases [Chao, 2023]. However, combined zinc and iron supplementation may be more effective than GFD alone in restoring normal levels of both minerals [Negi et al., 2018].

According to Rondanelli et al. [2019], it is recommended to monitor serum zinc levels from the time of diagnosis, repeating the test every 3 months until normalization, and then every 1–2 years if symptoms persist. If deficiency is confirmed, daily zinc supplementation of 25–40 mg should be continued until levels return to the normal range.

Magnesium

Magnesium is the most important divalent cation inside cells, playing a vital role in numerous biological processes. It is essential for the proper function of many enzymes involved in DNA transcription and replication, mRNA translation, as well as ion pumps and calcium channels. It also participates in protein, nucleic acid, glucose, and fat metabolism, and in membrane transport mechanisms [Vici et al., 2016].

In celiac patients, magnesium deficiency may persist despite adherence to a gluten-free diet, likely due to the naturally low magnesium content in gluten-free cereal products [Di Nardo et al., 2019]. Intake differences have been observed among individuals with celiac disease, particularly between sexes. Men with CD consumed less magnesium than both men in the control group and women with CD. Less than 80% of them met two-thirds of the daily recommended intake, indicating a potential deficiency [González et al., 2018]. In contrast, women with CD were more likely to meet magnesium recommendations, possibly due to different dietary patterns, despite their lower overall energy intake [Churruca et al., 2015].

Low magnesium intake among celiac patients seems to reflect a broader problem of micronutrient deficiencies in the general population, not just a consequence of the gluten-free diet. Considering magnesium's importance in nerve conduction and bone metabolism, inadequate intake may increase the risk of health complications [González et al., 2018].

Micronutrient intake analysis showed that both celiac patients on GFD and healthy controls failed to meet magnesium recommendations. Mean intake reached only 50% of the recommended amount in the CD group and 63.9% in the control group ($P < 0.001$), highlighting the widespread nature of this issue [Babio et al., 2017].

4. Vitamin deficiences

Vitamin D

Vitamin D, as a key factor regulating calcium-phosphate balance and supporting the immune system, plays an important role in the prevention of bone disorders. Its deficiency may result in osteomalacia and decreased bone mineral density (BMD). In celiac patients, the recommended marker for assessing and monitoring vitamin D status is serum 25(OH)D concentration [Zingone & Ciacci, 2018].

Low BMD is often observed in individuals with celiac disease, particularly in children. The underlying causes are multifactorial – including low intake of calcium and vitamins D and K, impaired absorption, and intestinal inflammation. Lifestyle factors like limited sun exposure, low physical activity, and medication use also contribute [Di Nardo et al., 2019].

A gluten-free diet (GFD) generally improves BMD, and bone mineralization may improve within a year in some patients. However, eliminating gluten alone does not always normalize bone status, especially when the diet is deficient in calcium and vitamin D. In such cases, vitamin D supplementation may be beneficial – it can help prevent further bone loss, reduce symptoms of osteomalacia, and improve calcium metabolism. Still, the evidence on its effectiveness is inconsistent. Recent guidelines from the American College of Physicians highlight that the benefit of vitamin D supplementation (even combined with calcium) for fracture prevention remains unclear and contradictory [Zingone & Ciacci, 2018; Di Nardo et al., 2019].

Disturbances in calcium and vitamin D metabolism are common in celiac disease, including vitamin D deficiency, hypocalcemia, and secondary hyperparathyroidism. GFD often leads to normalization, but supplementation may be necessary depending on the patient's baseline status and dietary compliance. Regular monitoring of vitamin D and calcium is important to assess supplementation needs [Di Nardo et al., 2019].

Nonetheless, some reports highlight concerns regarding the excessive or inappropriate use of vitamin D supplements, even among individuals with celiac disease, despite limited evidence of added benefit over a well-balanced gluten-free diet [Taylor & Davies, 2018].

B vitamins

B vitamins are essential for the proper functioning of the body – they participate, among other things, in DNA synthesis, cellular metabolism, and nervous system function. In patients with celiac disease, their deficiencies most often occur in the course of untreated disease, when damage to the intestinal villi leads to malabsorption. However, even individuals who strictly follow a gluten-free diet (GFD) may still experience deficiencies, which emphasizes the need to ensure a balanced diet and the quality of food consumed. Folic acid is mainly absorbed in the jejunum, a section of the intestine that is particularly susceptible to damage in celiac disease, which explains its frequent deficiencies. Vitamin B12, on the other hand, although absorbed in the terminal ileum, may be poorly absorbed, especially in cases of advanced villous atrophy.

The most common deficiencies associated with a gluten-free diet are folate and vitamin B12. In patients who have been on a GFD for a long time – even more than 10 years – reduced concentrations of these vitamins and elevated homocysteine levels have been observed compared to the general population [Vici et al., 2016]. In turn, Kreutz et al. [2023] showed that after 3 months of GFD, vitamin B12 deficiency occurred in 1.4% of patients, and after one year – in 3.5%. In the case of folate, these percentages were 3–5%, respectively. Importantly, some of these deficiencies developed after the diet was implemented, despite previous normal values – this was the case in 38% of B12 cases and 50% of folic acid cases. Deficiencies were also observed in patients with normal IgA anti-TG2 antibody levels. This highlights that serological remission reflects immune response but does not necessarily equate to full nutritional recovery or mucosal healing. Although the terminal ileum is usually unaffected in CD, studies have shown that up to 41% of individuals — particularly in select cohorts — may experience B12 deficiency despite adherence to a gluten-free diet. These may be related to inflammation, impaired digestion of B12-transporting proteins, and deficiencies in other nutrients.

In the event of a deficiency, especially if accompanied by elevated homocysteine levels, vitamin B12 supplementation is recommended. Both oral (1000–2000 µg per day) and intramuscular administration are effective, but oral administration is less invasive and better tolerated. Once B12 levels have normalized, it is advisable to use multivitamin preparations containing 500 µg of this vitamin. B12 levels should be monitored at the time of CD diagnosis and every 1–2 years, especially in patients with persistent symptoms [Rondanelli et al., 2019].

Individuals with celiac disease may continue to have folic acid deficiency even if they follow a gluten-free diet. This applies to approximately 3–5% of patients after one year of GFD, with as many as half of the cases developing after the start of dietary treatment [Kreutz et al., 2023]. These deficiencies may result not only from previous damage to the intestinal mucosa, but also from a deficient elimination diet, low in natural sources of folate, such as green leafy vegetables or fortified products. Since folic acid plays a key role in one-carbon metabolism and homocysteine detoxification, its deficiency can lead to an increase in the level of this amino acid, increasing the risk of cardiovascular disease. Regular assessment of folate levels and possible supplementation should be part of long-term care for patients with celiac disease.

However, a study by González et al. [2018] showed that none of the male celiac disease patients in the study population had low intakes of vitamins B6, B12, niacin, riboflavin, or thiamine. A study by van Megen et al. [2023] showed that women with celiac disease also met the dietary recommendations for vitamins B6, B12, and folate, suggesting that their diet was adequately balanced. In contrast, the study by Valente et al. [2016] showed that patients with celiac disease had lower serum folate concentrations compared to the control group – an average of 7.7 ± 3.5 ng/mL ($P < 0.05$). Furthermore, all participants with celiac disease consumed less folate than the average requirement (EAR), with an average intake of 130.8 ± 53.6 µg/day. Despite these deficiencies, only a small proportion of patients with CD had elevated homocysteine levels (hyperhomocysteinemia), suggesting that some individuals may have mechanisms to compensate for folate deficiency or that the deficiency was not yet advanced enough to affect homocysteine metabolism.

5. Summary

Celiac disease is a chronic autoimmune disorder, and the only effective treatment is strict adherence to a gluten-free diet. Despite the benefits of eliminating gluten, this diet carries a significant risk of nutritional deficiencies, even with high adherence. A review of the literature from 2015–2025 reveals that individuals with celiac disease are particularly at risk of deficiencies in dietary fiber, iron, zinc, magnesium, vitamin D, and B vitamins, especially folic acid and vitamin B12. These deficiencies are caused by both damage to the small intestine mucosa and the low nutritional value of many ready-made gluten-free products. Furthermore, even after intestinal villi regeneration and normalization of serological markers, deficiencies may persist due to permanent changes in the structure and function of enterocytes.

A gluten-free diet requires careful planning and nutritional education. To improve its quality, it is recommended to increase the consumption of natural, fiber-rich foods—such as legumes, vegetables, fruits, and nuts - and to choose nutritionally balanced, gluten-free products.. At the same time, it is necessary to systematically monitor nutritional status and implement supplementation when clinically significant deficiencies occur. Long-term follow-up, including regular laboratory assessments and nutritional counselling, should be a standard part of care, even in asymptomatic patients with good dietary compliance. This is in line with current ESPGHAN (European Society for Paediatric Gastroenterology, Hepatology and Nutrition) recommendations, which emphasize the importance of comprehensive, multidisciplinary management in patients with celiac disease.

An individualized approach, including both clinical and biochemical assessment of the patient, is key to effectively preventing complications and improving the quality of life of individuals with celiac disease. Further research is warranted to better understand the long-term nutritional implications of different gluten-free dietary patterns and their effects on gut microbiota, in order to optimize dietary strategies and improve clinical outcomes in this population.

6. References

[1] [Aboulaghras S., Piancatelli D., Oumhani K., Balahbib A., Bouyahya A., Taghzouti K.: Pathophysiology and immunogenetics of celiac disease. Clin. Chim. Acta, 2022, 528, 74–83.

[2] Aleman R.S., Moncada M., Aryana K.J.: Leaky gut and the ingredients that help treat it. Molecules, 2023, 28(2), 619.

[3] Cárdenas-Torres F.I., Cabrera-Chávez F., Figueroa-Salcido O.G., Ontiveros N.: Non-celiac gluten sensitivity: an update. Medicina, 2021, 57(6), 526.

[4] Babio N., Alcázar M., Castillejo G., Recasens M., Martínez-Cerezo F., Gutiérrez-Pensado V., Salas-Salvadó J.: Patients with celiac disease reported higher consumption of added sugar and total fat than healthy individuals. J. Pediatr. Gastroenterol. Nutr., 2017, 64(1), 63–69.

[5] Buriánek F., Gege C., Marinković P.: New developments in celiac disease treatments. Drug Discov. Today, 2024, 29 (9), 104113.

[6] Chao H.C.: Zinc deficiency and therapeutic value of zinc supplementation in pediatric gastrointestinal diseases. Nutrients, 2023, 15(19), 4093.

[7] Churruca I., Miranda J., Lasa A., Bustamante M.Á., Larretxi I., Simon E.: Analysis of body composition and food habits of Spanish celiac women. Nutrients, 2015, 7(7), 5515–5531.

[8] Demirkesen I., Ozkaya B.: Recent strategies for tackling the problems in gluten-free diet and products. Crit. Rev. Food Sci. Nutr., 2022, 62(3), 571–597.

[9] Di Nardo G., Villa M.P., Conti L., Ranucci G., Pacchiarotti C., Principessa L., Parisi P.: Nutritional deficiencies in children with celiac disease resulting from a gluten-free diet. Nutrients, 2019, 11(7), 1588.

[10] González T., Larretxi I., Vitoria J.C., Castaño L., Simón E., Churruca I., Lasa A.: Celiac male's gluten-free diet profile: Comparison to that of the control population and celiac women. Nutrients, 2018, 10(11), 1713.

[11] Husby S., Koletzko S., Korponay-Szabó I., Kurppa K., Mearin M.L., Ribes-Koninckx C., Wessels M.: European Society Paediatric Gastroenterology, Hepatology and Nutrition guidelines for diagnosing celiac disease 2020. J. Pediatr. Gastroenterol. Nutr., 2020, 70(1), 141–156.

[12] Jivraj A., Hutchinson J.M., Ching E., Marwaha A., Verdu E.F., Armstrong D., Pinto-Sanchez M.I.: Micronutrient deficiencies are frequent in adult patients with and without celiac disease on a gluten-free diet, regardless of duration and adherence to the diet. Nutrition, 2022, 103, 111809.

[13] Krawczyk A., Kretek A., Pluta D., Kowalczyk K., Czech I., Radosz P., Madej P.: Gluten-free diet—remedy for infertility or dangerous trend?. Ginekol. Pol., 2022, 93(5), 422–426.

[14] Kreutz J.M., Heynen L., Vreugdenhil A.C.E.: Nutrient deficiencies in children with celiac disease during long term follow-up. Clin. Nutr., 2023, 42(7), 1175–1180.

[15] Larretxi I., Churruca I., Navarro V., Miranda J., Lasa A., Bustamante M.Á., Simon E.: Effect of analytically measured fiber and resistant starch from gluten-free products on the diets of individuals with celiac disease. Nutrition, 2020, 70, 110586.

[16] Lebwohl B., Rubio-Tapia A.: Epidemiology, presentation, and diagnosis of celiac disease. Gastroenterology, 2021, 160(1), 63–75.

[17] Marco V., Chiara G., Antonio V., Massimo M., Stefano F., Gianpiero S., Giovanni L.: Persistence of anemia in patients with Celiac disease despite a gluten free diet: a retrospective study. BMC Gastroenterol., 2025, 25(1), 128.

[18] Negi K., Kumar R., Sharma L., Datta S.P., Choudhury M., Kumar P.: Serum zinc, copper and iron status of children with celiac disease on three months of gluten-free diet with or without four weeks of zinc supplements: A randomised controlled trial. Trop. Doctor, 2018, 48(2), 112–116.

[19] Paez M.A., Gramelspacher A.M., Sinacore J., Winterfield L., Venu M.: Delay in diagnosis of celiac disease in patients without gastrointestinal complaints. Am. J. Med., 2017, 130(11), 1318–1323.
[20] Rondanelli M., Faliva M.A., Gasparri C., Peroni G., Naso M., Picciotto G., Perna S.: Micronutrients dietary supplementation advices for celiac patients on long-term gluten-free diet with good compliance. Medicina, 2019, 55(7), 337.
[21] Sharma N., Bhatia S., Chunduri V., Kaur S., Sharma S., Kapoor P., Garg M.: Pathogenesis of celiac disease and other gluten related disorders in wheat and strategies for mitigating them. Front. Nutr., 2020, 7, 6.
[22] Stefanelli G., Navisse S., Valvano M., Vernia F., Ciccone A., Melideo D., et al.: Serum transglutaminase antibodies do not always detect the persistent villous atrophy in patients with Celiac disease on a gluten-free diet. Eur. J. Gastroenterol. Hepatol., 2021, 33(1S), e650–e655.
[23] Sue A., Dehlsen K., Ooi C.Y.: Paediatric patients with celiac disease on a gluten-free diet: nutritional adequacy and macro- and micronutrient imbalances. Curr. Gastroenterol. Rep., 2018, 20, 1–12.
[24] Taetzsch A., Das S.K., Brown C., Krauss A., Silver R.E., Roberts S.B.: Are gluten-free diets more nutritious? An evaluation of self-selected and recommended gluten-free and gluten-containing dietary patterns. Nutrients, 2018, 10(12), 1881.
[25] Talarico V., Giancotti L., Mazza G.A., Miniero R., Bertini M.: Iron deficiency anemia in celiac disease. Nutrients, 2021, 13(5), 1695.
[26] Taylor P.N., Davies J.S.: A review of the growing risk of vitamin D toxicity from inappropriate practice. Br. J. Clin. Pharmacol., 2018, 84(6), 1121–1127.
[27] Valente F.X., Campos T.D.N., Moraes L.F.D.S., Hermsdorff H.H.M., Cardoso L.D.M., Pinheiro-Sant'Ana H.M., Peluzio M.D.C.G.: B vitamins related to homocysteine metabolism in adults celiac disease patients: a cross-sectional study. Nutr. J., 2016, 14, 1–7.
[28] van Megen F., Fossli M., Skodje G.I., Carlsen M.H., Andersen L.F., Veierød M.B., Henriksen C.: Nutritional assessment of women with celiac disease compared to the general population. Clin. Nutr. ESPEN, 2023, 54, 251–257.
[29] Vici G., Belli L., Biondi M., Polzonetti V.: Gluten free diet and nutrient deficiencies. Clin. Nutr., 2016, 35(6), 1236–1241.
[30] Zingone F., Ciacci C.: The value and significance of 25(OH) and 1,25(OH) vitamin D serum levels in adult celiac patients. Dig. Liver Dis., 2018, 50(8), 757–760.

Authors' Index

Barabosz Krzysztof 6, 18
Biłos Łukasz 6, 18,
Bojanowska Monika 31, 125, 172, 184,
Byczyński Łukasz 50,
Ciołek Anna 77,
Czeczko Renata 91,
Czerwińska Katarzyna 216,
Iłowiecka Katarzyna 153,
Kostecka Małgorzata 31, 125, 172, 184,
Kowalska Grażyna 105,
Kowalski Radosław 105,
Krzepiłko Anna 140,
Krzysiak-Warzała Barbara 18,
Kulesza Renata 18,
Leszkowicz Dawid 77,
Libera Justyna 153, 164,
Łysakowska Paulina 153
Malik Agnieszka 164
Matuszek Dominika 6, 18,
Matysiak Joanna 203,
Mazurek Artur 105,
Nowosad Karolina 153, 164,
Owsiak Julia 18,
Pabich Marzena 31, 125, 172, 184,
Sachadyn-Król Monika 31, 125, 172, 184,
Skrzypek Alicja 77, 203,
Sosnowska Bożena 216,
Święciło Agata 140,
Świerc Jessica 227,
Sykulska Zuzanna 18,
Szkutnik Krzysztof 18,
Teterycz Dorota 153, 238,
Włodarczyk-Stasiak Marzena 105,
Worku Karolina 91.

ISBN 978-83-953897-7-1