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THE EFFECT OF MARINADE INGREDIENTS AND STORAGE CONDITIONS ON THE VARIABILITY OF SELECTED QUALITY PARAMETERS OF RAW AND GRILLED SALMON (*SALMO SALAR* L.)

S u m m a r y

Background. This study aimed to evaluate the effect of marinade ingredients and storage conditions on the selected quality parameters of raw and grilled salmon (*Salmo salar L.*). The basic ingredients of each marinade were lemon juice, salt and black pepper, the samples differed in the yogurt used and the addition of garlic. The marinated samples were stored under different conditions (24 or 48 hours at 2 °C or 8 °C), subsequently grilled (170 °C for 3 minutes) and analyzed at regular intervals.

Results and conclusions. The results showed that coliform bacteria, MFF and yeast were not present in any sample, or their number was <1.10^1 CFU in 1 g. The variability of TVC count between the samples was diverse, which was influenced by the presence of individual components of the marinades. Samples C and D, which contained garlic, had a higher initial TVC count (5.13 and 5.62 log CFU/g, respectively) compared to samples A and B (3.47 and 3.64 log CFU/g, respectively). However, the inhibitory effect of garlic became evident after 24 hours of storage at 4 °C. After grilling, TVC was further reduced to <1.10^1 CFU/g in all samples. The highest pH (6.71) was in raw salmon. Garlic slightly increased pH from 6.40 (A) to 6.48 (C) and from 6.42 (B) to 6.54 (D). Grilling reduced pH in all samples. The comparison of samples A, B, C and D indicates that a higher temperature (8 °C) results in a more rapid pH decrease and a greater reduction in water activity. For example, sample B at 8 °C exhibits a more pronounced pH drop (from 6.42 to 5.68) and a_w decrease (from 0.980 to 0.985) after 48 hours. The average salt content in individual samples ranged from 0.13 to 1.70 g/100 g. The results showed a marginal decrease in salt concentration post-grilling. The fat content in the grilled samples was significantly higher compared to the raw samples. The protein content averages from about 15.05 g/100 g (sample AG) to 21.41 g/100 g (sample BG).

Keywords: salmon, marinade, quality, grilling, food safety

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Introduction

The Atlantic salmon (*Salmo salar* L.), often referred to as the "king of fish “, is an anadromous migratory species that begins its life in freshwater rivers, where it is born and matures. It then migrates to the ocean, where it feeds and grows, before returning to fresh water to spawn. This species is renowned for its ability to leap, which enables it to overcome obstacles along its migratory route. Unlike Pacific salmon, which typically dies after spawning, the Atlantic salmon is remarkable in its ability to survive and spawn multiple times throughout its life [9].

The top five countries engaged in Atlantic salmon farming are Norway, Chile, Scotland, Canada and the Faroe Islands, with Norway being the largest producer, accounting for 55 % of global production. It is currently estimated that 14 million meals of Norwegian farmed Atlantic salmon are consumed worldwide each day [13]. The Atlantic salmon is considered an indicator species, meaning its condition can reflect the overall health of its ecosystem. In clean and well-connected river ecosystems, salmon populations are typically robust and thriving. Conversely, in polluted or poorly connected systems, these populations often decline [8].

The proportion of salmon that is consumed depends on the yield at slaughter, as well as subsequent processing and the utilization of trimmings. Fillet yield (as a percentage of the whole body) is often regarded as the equivalent of the edible portion of salmon. Several other parts of the salmon are also used for human consumption. Heads, backbones and belly trimmings are utilized as protein concentrates. Trimmings are primarily processed into oil capsules, which are marketed as dietary supplements. Gutted salmon accounts for approximately 87 % of its live weight [1].

Salmon is considered an excellent source of nutrients; however, it is important to note that its nutritional composition is largely influenced by its origin, making it essential to know the source when selecting salmon. Farmed and wild salmon differ in their nutritional value, each of them having their own advantages and disadvantages. Farmed salmon contains more fat, including omega-3 fatty acids, due to its nutrient-rich feed. In contrast, wild salmon, which naturally moves and forages, is leaner but contains slightly higher levels of minerals such as iron and zinc [7, 11, 12]. The nutritional value is also influenced by the method of thermal preparation, such as grilling, boiling, baking, or sous-vide, to which the fish is often subjected prior to consumption. Key factors in these processes include temperature, duration and the specific cooking method. These factors not only affect nutritional value but also the sensory and microbiological quality of fish [10].

Microbiological risks represent one of the major challenges to food safety and quality in aquaculture, particularly in salmon farming. In recent years, with the increasing demand for salmon as a highly valued food source, significant attention has been directed towards identifying and managing potential microbiological pathogens. These pathogens, including bacteria, viruses and parasites, may be present in water or feed and can be transmitted to salmon, thereby posing risks to consumer health [17]. The identification, monitoring and control of these microbiological threats are essential to ensuring the safety of salmon intended for human consumption, requiring coordinated efforts from researchers, feed manufacturers, farmers and regulatory authorities. Developing effective microbiological guidelines is crucial for accurate risk assessment, whether for imported or domestically produced products [21]. The risk can be mitigated through the implementation of the Hazard Analysis and Critical Control Points (HACCP) system. It is a system designed to identify, evaluate and control hazards during the production, processing and preparation of food [15, 20, 25]. It is recognized as a key component of food safety management practices in the global food industry and can be applied at any stage of the food supply chain [32]. Proper implementation of the HACCP system enables companies to produce and distribute products of higher quality with a reduced level of risk [18].

The aim of this research was to assess the impact of marinade ingredients and storage conditions on the variability of selected quality parameters of raw and grilled salmon (*Salmo salar L.*).

Material and methodology

In line with the stated objective, this research focused on analyzing the microbiological and physicochemical properties of raw salmon, as well as salmon marinated under various conditions and subsequently grilled.

Photo 1. Grilled salmon
Fotografia 1. Grilowany łosoś

Experimental material

The primary materials for all analyses were chilled salmon fillets without skin and bones (country of origin Norway) and marinades prepared from defined ingredients (Tab. 1). Salmon fillets intended for hot and cold cuisine preparations were purchased in a 500 g package from a retail network. Basic nutritional information about fillets: energy 398 kJ/94 kcal, fats 1.7 g, of which saturated fatty acids 0.4 g, carbohydrates < 0.5 g, of which sugars < 0.5 g, proteins 20 g, salt 0.13 g per 100 g of the product.

Marinade composition:

- full-fat yogurt (energy 290 kJ/69 kcal, fats 3.6 g, of which saturated fatty acids 2.4 g, carbohydrates 4.7 g, of which sugars 4.7 g, proteins 3.9 g, salt 0.14 g per 100 g of the product);
- fat-free yogurt (energy 177 kJ/42.3 kcal, fats 0.1 g, carbohydrates 4 g, of which sugars 2.7 g, proteins 5.7 g, salt 0.1 g per 100 g of the product);
- garlic, salt, black pepper, fresh lemon juice.

Design of experiment:

Salmon fillets were divided into five equal portions: a control sample (CS) without any ingredients and four samples marinated with predefined ingredients (Tab. 1).

The base of each marinade consisted of lemon juice, salt and black pepper, with variations in the type of yogurt and the addition of garlic. Sample A was enriched with full-fat yogurt, sample B with fat-free yogurt, sample C contained full-fat yogurt and garlic, and sample D included fat-free yogurt and garlic. The samples were weighed on analytical balances (weighing range up to 200 g, accuracy 0.001 g). The marinated samples were stored under different conditions (24 or 48 hours at 2 °C or 8 °C), subsequently grilled (170 °C for 3 minutes – Photo 1) and analyzed. The entire experiment was repeated five times, with each sample analyzed three times for its physicochemical properties. A total of 210 samples were analyzed.

According to the Ministry of Health of the Slovak Republic Decree No. 125/2017, marinated meats are classified as semi-finished products and may only be stored for up to 48 hours at a temperature from 0 to 4 °C. The decree prohibits storing marinated meats at higher temperatures or freezing them at -18 °C prior to heat treatment. These regulations were considered in our analyses and complemented with additional storage conditions based on the habits of Slovak households (more than 24 hours, temperature higher than 4 °C).

Table 1. Designation and description of salmon samples
Tabela 1. Oznaczenia i opis próbek łososia

Designation / Oznaczenie	Sample description / Opis próbek
CS/CSG	Salmon (50 g) - analysis in raw state without marinade/after grilling / Łosoś (50 g) - analiza w stanie surowym bez marynaty/po grillowaniu
A/AG	Salmon (50 g), full-fat yogurt (10 g), lemon juice (1 ml), salt (0.5 g), black pepper (0.5 g) - analysis immediately after marination/after grilling / Łosoś (50 g), jogurt pełnotłusty (10 g), sok z cytryny (1 ml), sól (0,5 g), czarny pieprz (0,5 g) - analiza bezpośrednio po marynowaniu/po grillowaniu
A2a/A2aG	Analysis after 24 hours of storage at 2 °C/after grilling / Analiza po 24 godzinach przechowywania w temp. 2 °C/po grillowaniu
A2b/A2bG	Analysis after 48 hours of storage at 2 °C/after grilling / Analiza po 48 godzinach przechowywania w temp. 2 °C/po grillowaniu
A8a/ A8aG	Analysis after 24 hours of storage at 8 °C/after grilling / Analiza po 24 godzinach przechowywania w temp. 8 °C/po grillowaniu
A8b/ A8bG	Analysis after 48 hours of storage at 8 °C/after grilling / Analiza po 48 godzinach przechowywania w temp. 8 °C/po grillowaniu
B/BG	Salmon (50 g), fat-free yogurt (10 g), lemon juice (1 ml), salt (0.5 g), black pepper (0.5 g) - analysis immediately after marination/after grilling / Łosoś (50 g), jogurt beztłuszczowy (10 g), sok z cytryny (1 ml), sól (0,5 g), czarny pieprz (0,5 g) - analiza bezpośrednio po marynowaniu/po grillowaniu
B2a/ B2aG	Analysis after 24 hours of storage at 2 °C/after grilling / Analiza po 24 godzinach przechowywania w temp. 2 °C/po grillowaniu
B2b/ B2bG	Analysis after 48 hours of storage at 2 °C/after grilling / Analiza po 48 godzinach przechowywania w temp. 2 °C/po grillowaniu
B8a/ B8aG	Analysis after 24 hours of storage at 8 °C/after grilling / Analiza po 24 godzinach przechowywania w temp. 8 °C/po grillowani
B8b/ B8bG	Analysis after 48 hours of storage at 8 °C/after grilling / Analiza po 48 godzinach przechowywania w temp. 8 °C/po grillowaniu
C/CG	Salmon (50 g), full-fat yogurt (10 g), lemon juice (1 ml), salt (0.5 g), black pepper (0.5 g), crushed garlic (1 g) - analysis immediately after marination/after grilling / Łosoś (50 g), jogurt pełnotłusty (10 g), sok z cytryny (1 ml), sól (0,5 g), czarny pieprz (0,5 g), zmiażdżony czosnek (1 g) - analiza bezpośrednio po marynowaniu/po grillowaniu
C2a/ C2aG	Analysis after 24 hours of storage at 2 °C/after grilling / Analiza po 24 godzinach przechowywania w temp. 2 °C/po grillowaniu
C2b/ C2bG	Analysis after 48 hours of storage at 2 °C/after grilling / Analiza po 48 godzinach przechowywania w temp. 2 °C/po grillowaniu
C8a/ C8aG	Analysis after 24 hours of storage at 8 °C/after grilling / Analiza po 24 godzinach przechowywania w temp. 8 °C/po grillowaniu
C8b/ C8bG	Analysis after 48 hours of storage at 8 °C/after grilling / Analiza po 48 godzinach przechowywania w temp. 8 °C/po grillowaniu
D/DG	Salmon (50 g), fat-free yogurt (10 g), lemon juice (1 ml), salt (0.5 g), black pepper (0.5 g), crushed garlic (1 g) - analysis immediately after marination/after grilling / Łosoś (50 g), jogurt beztłuszczowy (10 g), sok z cytryny (1 ml), sól (0,5 g), czarny pieprz (0,5 g), zmiażdżony czosnek (1 g) - analiza bezpośrednio po marynowaniu/po grillowaniu
D2a/ D2aG	Analysis after 24 hours of storage at 2 °C/after grilling / Analiza po 24 godzinach przechowywania w temp. 2 °C/po grillowaniu
D2b/ D2bG	Analysis after 48 hours of storage at 2 °C/after grilling / Analiza po 48 godzinach przechowywania w temp. 2 °C/po grillowaniu
D8a/ D8aG	Analysis after 24 hours of storage at 8 °C/after grilling / Analiza po 24 godzinach przechowywania w temp. 8 °C/po grillowaniu
D8b/ D8bG	Analysis after 48 hours of storage at 8 °C/after grilling / Analiza po 48 godzinach przechowywania w temp. 8 °C/po grillowaniu

Analytical methods

The research involved conducting microbiological and physicochemical analyses of salmon meat marinated under various conditions, both before and after grilling.

Microbiological analyses: total viable cells (TVC), microscopic filamentous fungi (MFF) and coliform bacteria (CB). The determination of these microbiological quality indicators (except TVC) for salmon was based on the valid legislation of the Slovak Republic – the Decree of the Ministry of Agriculture and the Ministry of Health of the Slovak Republic No. 06267/2006‒SL, part of the process hygiene criteria. The process hygiene criterion means a criterion that demonstrates the acceptable functioning of the production process. It sets a reference value for contamination, and if this value is exceeded, corrective measures are required to maintain process hygiene in accordance with food law. The plate dilution method was employed to identify individual microorganisms, with samples prepared either by embedding or smearing. A summary of the microbiological determination methods is provided in Tab. 2.

Table 2. Characteristic microbiological analysis
Tabela 2. Charakterystyczna analiza mikrobiologiczna

Microorganism / Mikroorganizm	Dilution / Rozcieńczenie	Volume / Objętość	Culture medium / Medium hodowlane	Cultivation / Hodowla	Slovak technical norm/ISO
Coliforms / Bakterie Coli	10^{-1}, 10^{-2}, 10^{-3}	1 cm^3 embedding / osadzenie	VRBL agar	30±1 °C, 24 hours / godz.	STN EN ISO 4832
TVC	10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}	1 cm^3 embedding / osadzenie	PCA agar	30±1 °C, 72±3 hours / godz.	ISO STN 4833
Yeast and MFF	10^{-1}, 10^{-2}	0.1 cm^3 smear / rozmaz	DRBC agar	25±1 °C, 5 days / dni	ISO 7954

Explanations / Objaśnienia: total viable cells (TVC) / całkowita liczba żywych komórek, microscopic filamentous fungi (MFF) / mikroskopijne grzyby strzępkowe

- Determination of Total Viable Count of Microorganisms – PCA agar (Plate Count Agar). Purpose: Non-selective medium used to monitor the total bacterial growth in a sample. Components: Agar, glucose, enzymatic casein hydrolysate, yeast extract.
- Determination of Coliforms – VRBL agar (Violet Red Bile Lactose). Purpose: For the reproduction and isolation of growth-demanding bacteria, particularly intestinal pathogens. Components: Agar, crystal violet, lactose, sodium chloride, neutral red, yeast extract, bile salts, meat peptone.
- Determination of Microscopic Filamentous Fungi (MFF) and yeast – DRBC agar (Dichloran Rose-Bengal Chloramphenicol Agar). Purpose: Selective medium for isolating and counting yeasts and filamentous fungi in foods with water activity

(aw) above 0.95. Components: Agar, dextrose, potassium dihydrophosphate, magnesium sulfate, dichlorane, Bengal red, meat peptone.

Individual culture mediums were prepared by weighing the corresponding amount of dehydrated nutrient soil and subsequently mixing in distilled water. Culture medium (except the VRBL agar) was subsequently sterilized in an autoclave at the temperature of 121 °C, pressure of 120 kPa, for 15 minutes.

Physicochemical analyses: pH, aw, salt, fat and protein content. Fat and protein content was not determined in the samples of groups C and D (the limiting factor was only garlic). The measurements of individual parameters were repeated three times in each sample.

- Determination of pH – penetration pH meter (Testo 206-pH2, Germany). It is a device with a combined electrode, in which both the indicator (glass) and reference (calomel) electrodes are built in. When both electrodes are used, a galvanic cell is formed. Before each measurement, the probe was cleaned with a disposable cloth and rinsed with distilled water.
- Determination of aw – LabMaster-aw neo (Switzerland). The device operates on the electrolytic principle and uses a surface infrared sensor. Measuring range is 0.0300 – 1.000.
- Determination of salt content – Chloride analyzer 926 M device (Sherwood, UK). The devise measures chloride ions in a water solution. A sample volume of 0.5 cm^3 is required for the measurement and the result is shown in mg/dm^3 Cl^- or mg % (mg/100 cm^3) NaCl.

Table 3. The formulas for calculating the content of salt, fat and protein
Tabela 3. Wzory do obliczania zawartości soli, tłuszczu i białka

Calculation of salt content in % / Obliczenie zawartości soli w %:	*Calculation of fat content in % / Obliczenie zawartości tłuszczu w %:*	*Calculation of protein content in % / Obliczenie zawartości białka w %:*
x = A x 0.0084 x = NaCl (%) A = value from device / wartość z urządzenia (mg/dm^3)	$x = \frac{100 \times a}{b}$ x = fat content / zawartość tłuszczu (%) a = weight of extracted fat / masa ekstraktu tłuszcz (g) b = weighing of the original sample before drying / masa próbki przed suszeniem (g)	$N = \frac{HCl \times 0.14 - 5.6}{\text{sample weight / masa próbki}}$ N = % of nitrogenous substances / % substancji azotowych HCl = volume of hydrochloric acid, consumed (cm^3) / objętość kwasu solnego, 0.14 = molar mass of nitrogen / masa molowa azotu (g/mol), 5.6 = conversion coefficient for fish sample weight = weight of the sample / współczynnik przeliczeniowy dla masy próbki ryby = masa próbki (g)

- Determination of fat content – Soxhlet devise (J.P. Selecta, s.a., Spain). The analysis itself was preceded by drying the meat sample for 12 hours at the temperature of 105 °C.
- Determination of protein content – Kjeltec^{TM} 8200 (FOSS, Denmark). The analyses of individual samples consisted of processes such as precipitation, drying, mineralization, distillation and titration.

While determinations of pH and aw were made directly by measuring on the device, the salt, fat and protein content were recalculated according to the formulas (Tab. 3).

Statistical evaluation

The collected data was statistically analyzed using descriptive characteristics, including the arithmetic mean ($\overline{x}$) and standard deviation (SD), to assess measurement accuracy. The analysis of variance (ANOVA) was employed to compare groups, with the variance agreement verified through the F test. Differences between storage conditions were statistically compared using Scheffé's test. Statistical significance was determined at a significance level of $\alpha = 0.05$. The linear relationship between two variables was assessed using Pearson's correlation coefficient (r), which ranges from +1 to -1. A value of 0 indicates no linear relationship [6].
$r < 0.1$: Trivial dependence;
$0.1 \leq r < 0.3$: Weak dependence;
$0.3 \leq r < 0.5$: Medium dependence;
$r > 0.5$: Strong dependence;

The correlation results were tested for significance at levels $\alpha = 0.05$, $\alpha = 0.01$ and $\alpha = 0.001$. The statistical analysis was performed using the SAS program package (ver. 8.2).

Results and discussion

Presence of microorganisms in salmon meat before and after grilling

Although the determination of TVC is not strictly legislatively required for fishery products (Commission Regulation (EC) No. 1441/2007) , it is an important indicator of the hygiene of the production process, and therefore, it was included in this study as part of the analyzed microorganisms. The presence of TVC also indicates poor control of the cooling process or excessive storage time. Manufacturers of some food products set their own limits for TVC as part of self-monitoring. An example is meat processing plants, which, when assessing the microbiological quality of ham, have set the TVC value at 5.6 log CFU/g during the shelf life of this meat product, provided the packaging remains intact.

Table 4. Mathematical and statistical indicators for evaluating TVC in salmon meat
Tabela 4. Wskaźniki matematyczne i statystyczne do oceny TVC w mięsie łososia

Sample / Próbka	TVC (log CFU/g)					
	x̄	x min	median	x max	sx	v (%)
CS	<4/d	<4/d	<4/d	<4/d	<4/d	0
A	3.47	3.20	3.50	3.70	0.21	6.05
A2a	2.22	2.09	2.20	2.35	0.11	4.95
A2b	3.29	2.97	3.30	3.60	0.26	7.90
A8a	4.02	3.94	4.00	4.10	0.07	1.74
A8b	4.37	4.29	4.30	4.52	0.11	2.52
B	3.64	3.10	3.80	4.00	0.39	10.71
B2a	3.37	3.60	3.74	2.75	0.44	13.05
B2b	4.12	4.21	3.63	4.50	0.36	8.73
B8a	5.33	5.16	5.33	5.50	0.14	2.63
B8b	6.94	6.79	6.93	7.20	0.13	1.87
C	5.13	4.75	5.13	5.50	0.31	6.04
C2a	1.08	1.00	1.09	1.15	0.06	5.55
C2b	3.40	3.15	3.43	3.60	0.19	5.58
C8a	3.74	3.65	3.76	3.80	0.06	1.60
C8b	5.40	5.28	5.33	5.60	0.14	2.59
D	5.62	5.31	5.55	6.00	0.29	5.16
D2a	1.22	1.10	1.26	1.29	0.08	6.56
D2b	4.66	4.32	4.75	4.90	0.25	5.36
D8a	6.06	5.97	6.01	6.20	0.10	1.65
D8b	7.07	6.99	7.02	7.10	0.09	1.27
After grilling (all samples marked with the letter G) < 1/d Po grillowaniu (wszystkie próbki oznaczone literą G) < 1/d						

Explanations / Objaśnienia: < 4/d = < 4.10^1 CFU/g < 1/d = < 1.10^1 CFU/g

The variability of TVC count between the salmon samples was diverse, which was influenced by the presence of individual components of marinades (Tab. 4). Samples C and D, which contained garlic, had a higher initial TVC count (5.13 and 5.62 log CFU/g, respectively) compared to samples A and B (3.47 and 3.64 log CFU/g). However, the inhibitory effect of garlic became evident after 24 hours of storage at 2 °C, reducing the TVC to 1.08 and 1.22 CFU/g, respectively. In the control sample without marinade, the TVC count was < 4.10^1 CFU/g. After grilling, it was further reduced to < 1.10^1 CFU/g in all samples. Tab. 4 also shows that in the basic samples of marinades (A, C) containing full-fat yogurt, the total microbial count was lower compared to those containing low-fat yogurt.

The fat content in yogurt and its relationship to the reproduction of microorganisms is complex and influenced by multiple factors. Fat generally provides microorganisms with protection against adverse temperature effects, which explains why higher temperatures are required for pasteurizing cream compared to milk. This process is essential for minimizing the microbial load in raw materials, such as milk and cream, provided they are properly treated. In the case of raw material contamination, the likelihood of faster microbial growth is higher in products with lower fat content. This is because microorganisms preferentially utilize sugars and proteins as energy sources, while fats are metabolized to a lesser extent. Fat not only acts as a physical barrier but also affects the availability of nutrients to microorganisms, which can slow their growth in fat-rich environments. This mechanism highlights the importance of proper hygiene and adequate technological processing of dairy products, particularly regarding their fat content and the risk of contamination [24, 27, 28].

The meat, muscles and organs of fish are typically sterile, but the skin and digestive tract contain a variety of bacteria. The number of bacteria on the skin ranges from 10^2 to 10^7 CFU/g, while in the gills and GIT, it ranges from 10^3 to 10^9 CFU/g. The bacterial contamination of fish meat is related to the microflora of the digestive tract. The spoilage process of meat is associated with proteolytic enzymes in the intestine, which have a natural origin and may come from either the digestive tract of fish or the external environment. In raw fish meat, aerobic microorganisms are most common, with *Pseudomonas* and *Shewanella* spp. accounting for up to 84 % of them [16].The results for yeasts and MFF were evaluated separately. The results indicated that these microorganisms were not present in any of the analyzed samples or their number was lower than 10 CFU (< 1.10^1 CFU/g).

Yeast requires an acidic pH environment (ideally between 4.2 and 5.5) for growth, while a slightly alkaline environment (around pH 7.5) inhibits growth. However, in a non-acidic environment, yeast can quickly adapt to its optimal pH. Most microscopic fungi prefer neutral pH, but they can reproduce across a wide range of pH values (from 1.2 to 11.0) [12]. Garlic has yeast-inhibiting properties, which are linked to allicin. This sulfur-containing compound is produced in raw garlic cloves at a concentration of 3 to 5 mg per gram. Filamentous fungi and yeast are not the dominant components of the microbial population in meat [30].

In the analyzed salmon samples, the presence of coliform bacteria was not detected, which may indicate good hygienic conditions during their preparation and processing. Monitoring of *Escherichia coli* and coliform bacteria is conducted as a basic indicator of water hygiene or as a primary indicator of sanitary conditions in the environments where food is processed. The method for identifying these microorganisms is a common standard in the food industry [34].

Determination of pH, aw and salt in salmon meat before and after grilling

A pH is a key indicator of the quality and safety of food, including fish. As shown in Fig. 1, the highest pH value was found in raw salmon, specifically 6.71. There was only a minimal difference in pH variability between full-fat (A) and fat-free (B) yogurt, which were used as components of the marinade. The addition of garlic to the marinades caused a slight increase in pH values, rising from 6.40 (A) to 6.48 (C), and from 6.42 (B) to 6.54 (D), respectively. Grilling reduced pH in all samples. A statistical analysis using the Scheffe´s test showed that there was not a statistically significant difference ($p > 0.05$) between all groups of the salmon samples.

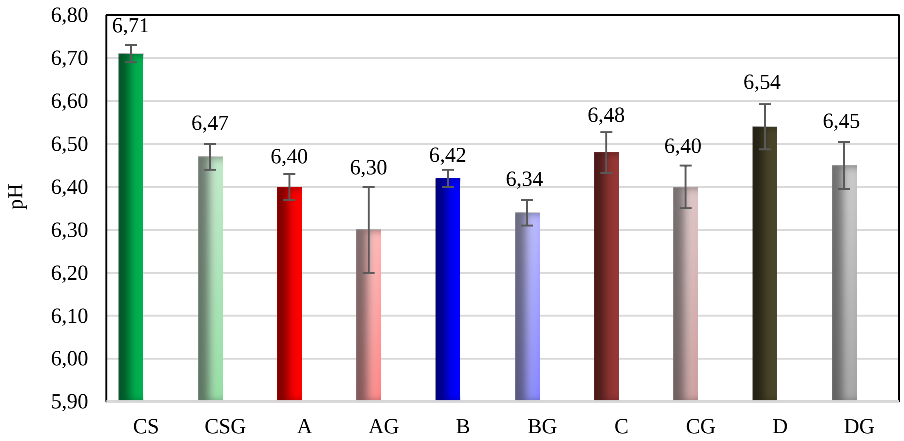


Figure 1. Average value of pH in raw and marinated salmon meat before and after grilling
Rycina1. Średnia wartość pH surowego i marynowanego mięsa łososia przed i po grillowaniu

Grilling meat often results in a decrease in pH, which is the outcome of several chemical and biological processes. One of the primary factors is the formation of organic acids. These acids can contribute to lowering the pH of the meat surface layer. Another factor is water loss. During thermal processing, water evaporates, leading to an increase in the concentration of hydrogen ions in meat juices, which subsequently lowers pH. Protein denaturation also plays a significant role. Heat causes the denaturation of muscle proteins, which can release certain acidic groups, further reducing pH. The decrease in pH during grilling is the most easily seen on the meat surface, where direct contact with high heat and air occurs. The interior of meat, particularly in thicker cuts, may retain a pH closer to its original value if it does not reach high temperatures. This reduction in pH can have a positive impact on the flavor of grilled meat, as a lower pH enhances certain flavors and promotes the formation of aromatic compounds [29, 33].

As shown in Fig. 2, the water activity (a_w) of salmon slightly decreased after grilling. It is hypothesized that this reduction is primarily caused by water evaporation due to high temperatures. In general, fresh fish muscle exhibits very high water activity, indicating a substantial amount of free water available for microbial growth. Grilling leads to partial dehydration, resulting in a decrease in water activity, although the change may not be drastic, as observed in the control sample CS (from 0.990 to 0.982).

If the dehydration process was to continue (e.g. through drying or prolonged grilling at lower temperatures), water activity could decrease further, thereby limiting the growth potential of many microorganisms [4, 23].

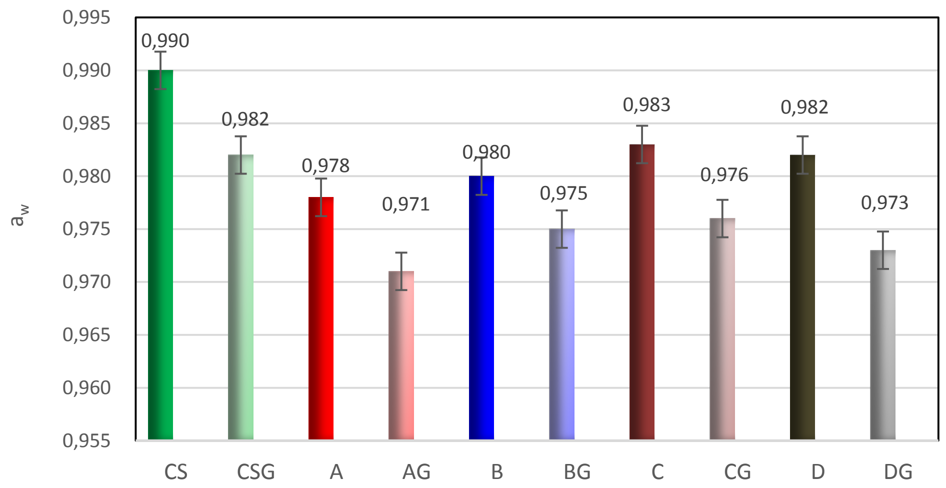


Figure 2. Average value of a_w in raw and marinated salmon meat before and after grilling
Rycina 2. Średnia wartość aw w surowym i marynowanym mięsie łososia przed i po grillowaniu

Several interesting scientific insights regarding the changes in pH and aw in salmon meat during marination under different conditions (time and temperature) can be derived from Tab. 5. The CS sample was not subjected to marination (pH and a_w values for 24 h and 48 h are not provided), indicating that it serves as a reference sample before marination. In all marinated samples (A, B, C, D), a decrease in pH is observed between the "before marination" stage and after 24 h of storage in the marinade. This decrease is more pronounced at a higher temperature (8 °C), particularly in samples A and B (without garlic). Over 48 h, a further pH decline occurs, with 8 °C showing a more significant reduction compared to 2 °C.

This pH decrease may indicate chemical reactions such as protein hydrolysis or acid degradation in the marinade, which are influenced by temperature and marination duration [19, 22].

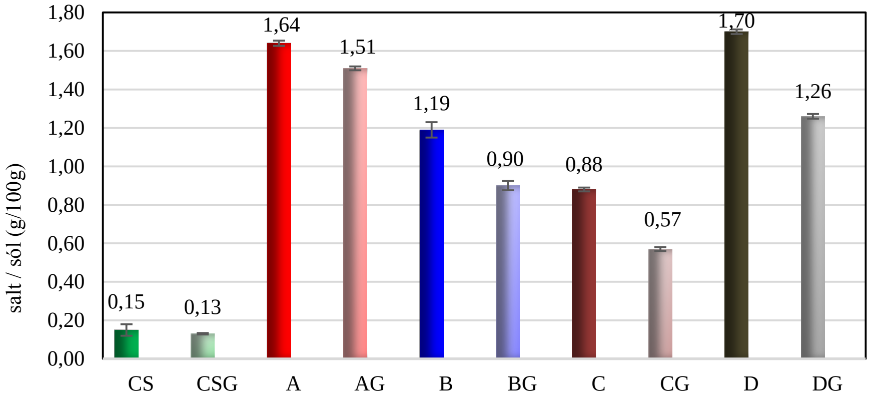
Tab. 5 also indicates that water activity slightly decreases during marination, which is characteristic of many processes involving water extraction from meat or its binding by marinade components. The lowest aw value is observed in the samples marinated for 48 hours at 2 °C. In most cases, aw is higher at 8 °C than at 2 °C after 48 hours, which may suggest that less water binding occurs in meat at a higher temperature. The rate and extent of pH and aw changes may be influenced by various factors, including marinade composition, temperature, duration and interactions between marinade components and meat. A decrease in pH can affect meat texture, as an acidic environment may lead to protein denaturation and structural changes in muscle fibers, resulting in meat tenderization. The reduction in aw is significant from a preservation perspective, as lower water activity can slow microbial growth and enhance the shelf life of marinated meat [3, 5, 34].

Table 5. Change in pH and a_w in salmon meat during marination under different conditions
Tabela 5. Zmiana pH i aw w mięsie łososia podczas marynowania w różnych warunkach

Parameter / Parametr	Conditions / Warunki		CS	A	B	C	D
pH	Before marination / Przed marynowaniem		6.71	6.40	6.42	6.48	6.54
	24 h	2 °C	-	6.42	6.36	6.41	6.43
		8 °C	-	6.32	6.29	6.32	6.36
	48 h	2 °C	-	6.23	6.49	6.47	6.33
		8 °C	-	5.93	5.68	5.87	5.90
a_w	Before marination / Przed marynowaniem		0.990	0.978	0.980	0.983	0.982
	24 h	2 °C	-	0.981	0.979	0.980	0.985
		8 °C	-	0.987	0.981	0.990	0.990
	48 h	2 °C	-	0.973	0.982	0.986	0.986
		8 °C	-	0.978	0.985	0.988	0.990

Salt plays a crucial role in marinades, as it increases the solubility of myofibrillar proteins and enhances the binding properties of meat. Additionally, the penetration of salt into meat during marination causes changes in a protein structure, leading to alterations in a meat texture. Studies have also highlighted that salt concentration and marination time are critical parameters influencing the quality and weight yield of marinated fish [31].

Fig. 3 emphasizes the role of both the initial salt addition and subsequent grilling in shaping the salt content of the samples. The average salt content in individual samples ranged from 0.13 to 1.70 g/100g. The results show a marginal decrease in salt concentration after grilling. The salt content in grilled meat can decrease, primarily due to the loss of moisture during grilling. When meat is cooked, water and juices containing dissolved salt can drip away, leading to a reduction in the overall salt concentration. The composition of marinade plays a significant role in this process. Marinade with high water content or acidic ingredients can increase moisture retention in meat, potentially reducing salt loss during grilling. The balance of ingredients in marinade can thus influence both the salt content and the final taste of grilled meat.



Rysunek 3.
Figure 3. Average value of salt content in raw and marinated salmon meat before and after grilling

According to the Decree of the Ministry of Health of the Slovak Republic No. S08975-OL-2014, the maximum salt content in ready-to-eat meals is set at 1.3 g/100 g. As shown in Fig. 3, this limit was exceeded in samples A, AG and D. Further statistical analysis confirmed the trends and quantified the effect of grilling on salt reduction (Tab. 6).

Table 6. Statistical evaluation of differences in salt content between salmon samples
Tabela 6. Statystyczna ocena różnic w zawartości soli pomiędzy próbkami łososia

F test	1675,37^{+}								
Indicator	CSG	A	AG	B	BG	C	CG	D	DG
CS	-	+	+	+	+	+	+	+	+
CSG		+	+	+	+	+	+	+	+
A			+	+	+	+	+	-	+
AG				+	+	+	+	+	+
B					+	+	+	+	-
BG						-	+	+	+
C							+	+	+
CG								+	+
D									+

Explanations / Objaśnienia: - Statistically insignificant difference according to Scheffe's test / Statystycznie nieistotna różnica według testu Scheffe'a ($p > 0.05$); + Statistically significant difference according to Scheffe's test / Statystycznie istotna różnica według testu Scheffe'a ($p < 0.05$)

Determination of fat and protein content in salmon meat before and after grilling

Fish are an excellent source of protein, rich in essential amino acids, and therefore have high nutritional value. Various physical, chemical and thermal processes used in food processing can alter the nutritional qualities and functional characteristics of these proteins [2]. Grilling and oven baking are identified as the best methods for preparing salmon. Fats in salmon are a key quality parameter that affects not only flavor and texture but also the quality of processed products [26]. The fat content can significantly influence properties such as taste—fillets with lower fat content are often considered "dry" and have a weaker flavor, while fillets with higher fat content are rated as "richer" and "fuller" [35].

From both a scientific and statistical perspective, the data in the Tab. 7 provides insights into the content of fat and protein in the samples (CS, CSG, A, AG, B, BG), as well as variability measures that help assess consistency in the nutritional content of samples. Marinated samples C and D were not analyzed for fat or protein content. These samples differed from samples A and B only in the addition of garlic, which does not directly affect the fat and protein content.

Table 7. Mathematical and statistical indicators for evaluating fat and protein content in salmon meat
Tabela 7. Wskaźniki matematyczne i statystyczne służące do oceny zawartości tłuszczu i białka w mięsie łososia

Indicator	Fat content (g/100 g)						Protein content (g/100 g)					
	CS	CSG	A	AG	B	BG	CS	CSG	A	AG	B	BG
$\bar{x}$	16.71	19.88	16.74	21.56	16.36	21.23	16.93	16.70	17.45	15.05	15.76	21.41
s_x	0.012	0.17	0.042	0.05	0.18	0.12	0.042	0.052	0.049	0.045	015	0.12
x_{min}	16.70	19.65	16.68	21.50	16.12	21.10	16.89	16.63	17.39	14.99	15.55	21.23
x_{max}	16.73	20.07	16.78	21.62	16.56	21.40	16.99	16.75	17.51	15.10	15.90	21.50
median	16.71	19.92	16.75	21.57	16.40	21.20	16.92	16.73	17.46	15.06	15.82	21.49
v (%)	0.08	0.87	0.25	0.22	1.11	0.59	0.25	0.31	0.28	0.30	0.95	0.58

The average fat content across samples ranges from approximately 16.36 g/100 g (sample B) to 21.56 g/100 g (sample AG). This shows that some samples, particularly AG, have a noticeably higher fat content. The protein content averages from about 15.05 g/100 g (sample AG) to 21.41 g/100 g (sample BG). The samples vary significantly in protein content, with BG and AG at opposite ends of the spectrum. There is significant variation in fat and protein content between the samples, especially for the protein content in sample B, which is less consistent compared to the other samples. This variability could be influenced by sample preparation methods, the type of ingredients used or the degree of marination and grilling.

Fig. 4 provides correlation coefficients (r_{xy}) between various variables (e.g. salt content, proteins, pH, fat) across six samples (A, AG, B, BG, CS, CSG). The results are also characterized based on their significance (Tab. 8). The overall analysis suggests that chemical parameters influence each other, but the intensity and direction of this relationship depend on a sample. Samples AG and BG exhibit strong and significant positive correlations between pH and fat. Samples CS and CSG demonstrate varying behavior of salt depending on other variables. Some combinations show a strong positive correlation (e.g. r_{xy}= 0.99 between salt and pH in sample AG). Others are negative, indicating an inverse relationship (e.g. between salt and proteins in sample A). Despite the diversity of correlation relationships among the samples, it can be concluded that statistical significance was not confirmed in any case ($p > 0.05$).

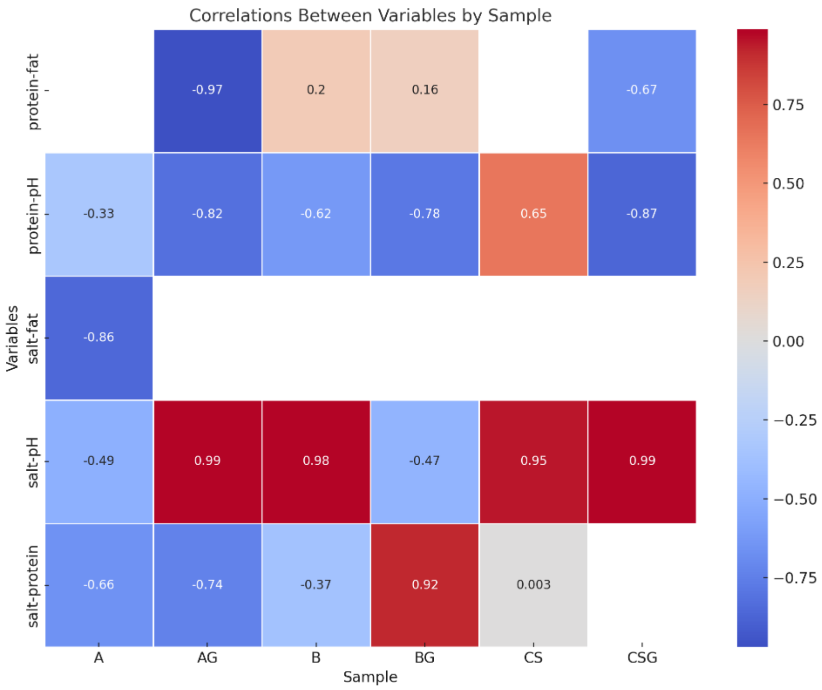


Figure 4. Correlation relationships between salt, pH, protein and fat in salmon samples CS, CSG, A, AG, B, BG

Rycina 4. Zależności korelacyjne pomiędzy solą, pH, białkiem i tłuszczem w próbkach łososia CS, CSG, A, AG, B, BG,

The analysis of the visualization of correlations between different chemical parameters for individual samples (Fig. 4):

– positive correlations (red shades) indicate a strong relationship where an increase in one variable corresponds to an increase in the other;
– negative correlations (blue shades) indicate an inverse relationship, where an increase in one variable leads to a decrease in the other;
– neutral values (closer to white) suggest weak or insignificant relationships.

Table 8. Correlations and statistical significance between parameters in salmon samples CS, CSG, A, AG, B, BG

Tabela 8. Korelacje i istotność statystyczna pomiędzy parametrami w próbkach łososia CS, CSG, A, AG, B, BG

CS				A				B			
	protein / białko	pH	fat / tłuszcz		protein / białko	pH	salt / sól		protein / białko	pH	fat / tłuszcz
salt / sól	0,003-	0,95-	0,84-	salt / sól	- 0,66-	- 0,49-	- 0,86-	salt / sól	- 0,37-	0,98-	0,95-
protein / białko		0,29-	- 0,53-	protein / białko		- 0,33-	0,20-	protein / białko		- 0,52-	- 0,62-
pH			0,65-	pH			0,85-	pH			0,99-
CSG				AG				BG			
	protein / białko	pH	fat / tłuszcz		protein / białko	pH	fat / tłuszcz		protein / białko	pH	fat / tłuszcz
salt / sól	0,99-	- 0,78-	- 0,77-	salt / sól	- 0,74-	0,99-	0,55-	salt / sól	0,92-	- 0,47-	- 0,24-
protein / białko		- 0,87-	- 0,67-	protein / białko		- 0,82-	- 0,97-	protein / białko		- 0,78-	0,16-
pH			0,21-	pH			0,65-	pH			- 0,73-

Explanations / Objaśnienia: +++: Statistically very highly significant difference between variables / Statystycznie bardzo wysoka istotna różnica między zmiennymi ($p < 0.001$); ++: Statistically highly significant difference between variables / Statystycznie wysoce istotna różnica między zmiennymi ($p < 0.01$) / +: Statistically significant difference between variables / Statystycznie istotna różnica między zmiennymi ($p < 0.05$); -: Statistically insignificant difference between variables / Statystycznie nieistotna różnica między zmiennymi ($p > 0.05$)

Conclusions

1. The study evaluated the microbiological, chemical and nutritional properties of salmon meat before and after grilling, focusing on the influence of marinade and processing methods. According to Decree No. 125/2017 of the Ministry of Health of the Slovak Republic, which sets requirements for catering facilities, meat intended for grilling must be stored at a temperature from 0 to 4 °C for a maximum of 48 hours.
2. Microbiological analyses of salmon confirmed that this temperature range is indeed optimal and complies with the storage requirements for raw meat. However, our results indicate that storing meat for 24 hours yields better microbiological quality compared to longer storage times. The results have also shown that the combination of marination and grilling is among the most effective methods of inactivating microorganisms.
3. The results indicate that the composition of marinads and storage conditions should be carefully considered to optimize not only flavor, but also food safety and quality.

4. While low-fat yogurts are recommended for consumption from a nutritional and health perspective, the findings revealed that higher-fat yogurts exhibit better antimicrobial properties, helping to reduce the risk of microbial contamination. Increasing the amount of lemon juice in marinade can effectively lower pH, providing stronger preservative effects against pathogens.
5. It is crucial to monitor and control the amount of added salt to prevent excessive salting and ensure compliance with legislative limits.
6. Incorporating antimicrobial herbs and spices, such as garlic, rosemary, thyme or oregano, not only enhances the flavor profile, but also boosts the protective effects of marinade. Finally, optimizing the temperature and duration of marination reduces the risk of microbial growth, which is essential for maintaining food safety and quality.
7. This study highlights the interplay between marinade composition, grilling and the nutritional and microbiological quality of salmon meat, offering insights for optimizing flavor, safety and compliance with regulatory standards. The study of marinade ingredients is crucial for the food industry for several reasons. It influences sensory properties and ensures consistent product quality. Technological processes can be optimized to enhance ingredient absorption and structural integrity. Food safety depends on controlling preservative effects and microbial risks. The efficient use of raw materials minimizes waste and improves production cost-effectiveness. Product innovation allows for adapting formulations to consumer trends.

Acknowledgments

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Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.

WPŁYW SKŁADNIKÓW MARYNATY I WARUNKÓW PRZECHOWYWANIA NA ZMIENNOŚĆ WYBRANYCH PARAMETRÓW JAKOŚCIOWYCH SUROWEGO I GRILLOWANEGO ŁOSOSIA (*SALMO SALAR* L.)

S u m m a r y

Wprowadzenie. Celem badania była ocena wpływu składników marynaty i warunków przechowywania na wybrane parametry jakościowe surowego i grillowanego łososia (*Salmo salar* L.). Podstawowymi składnikami marynaty były sok z cytryny, sól i czarny pieprz, próbki różniły się użytym jogurtem i dodatkiem czosnku. Zamarynowane próbki przechowywano w różnych warunkach (24 lub 48 godzin w temperaturze 2 °C lub 8 °C), a następnie grillowano (170 °C przez 3 minuty) i analizowano w regularnych odstępach czasu.

Wyniki i wnioski. Wyniki wykazały, że bakterie coli, MFF i drożdże nie były obecne w żadnej próbce lub ich liczba wynosiła $< 1,10^{1}$ CFU w 1 g. Zmienność liczby TVC między próbkami była różnorodna, na co miała wpływ obecność poszczególnych składników marynat. Próbki C i D, które zawierały czosnek, miały wyższą początkową liczbę TVC (odpowiednio 5,13 i 5,62 log CFU/g) w porównaniu z próbkami A i B (odpowiednio 3,47 i 3,64 log CFU/g). Jednak hamujący wpływ czosnku stał się widoczny po 24 godzinach przechowywania w temperaturze 4 °C. Po grillowaniu TVC dalej spadło do $<1,10^{1}$ CFU/g we wszystkich próbkach. Najwyższe pH (6,71) było w surowym łososiu. Czosnek nieznacznie zwiększył pH z 6,40 (A) do 6,48 (C) i z 6,42 (B) do 6,54 (D). Grillowanie obniżyło pH we wszystkich próbkach. Porównanie próbek A, B, C i D wskazuje, że wyższa temperatura (8 °C) powoduje szybszy spadek pH i większą redukcję aktywności wody. Na przykład próbka B w temperaturze 8 °C wykazuje wyraźniejszy spadek pH (z 6,42 do 5,68) i spadek aw (z 0,980 do 0,985) po 48 godzinach. Średnia zawartość soli w poszczególnych próbkach wahała się od 0,13 do 1,70 g/100 g. Wyniki wykazały marginalny spadek stężenia soli po grillowaniu. Zawartość tłuszczu w próbkach grillowanych była znacznie wyższa w porównaniu z próbkami surowymi. Średnia zawartość białka wynosi od około 15,05 g/100 g (próbka AG) do 21,41 g/100 g (próbka BG).

Słowa kluczowe: łosoś, marynata, jakość, grillowanie, bezpieczeństwo żywności