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CHARACTERISTICS OF THE COURSE OF PROTEOLYTIC PROCESSES IN BRYNZA WITH REDUCED COMMON SALT CONTENT

S u m m a r y

Background. Cheese is the main component of the daily ration of the vast majority of the population as a biologically complete and functional product. Therefore, the formation of its competitive production is one of the most important tasks for solving the country's food security and the successful development of the agrarian sector of the economy. In the world, there is a steady increase in the level of consumption of cheeses, which stimulates an increase in the volume of their production, which, in turn, requires a significant improvement in the quality of cheeses, the creation of new types and innovative technologies, and their scientific substantiation. Salted cheeses are cheeses that are matured and stored in brine – in barrels or containers, where they are tightly packed after forming and filled with a 16 ÷ 20 % solution of common salt. They are made according to the technology of soft, hard or semi-hard cheeses. They have a sharp, salty, sour taste, the dough is rough and brittle. Salted cheeses have no rind and are white in color.

Results and conclusions. Six samples of sheep's milk brynza with 20 and 30 % substitution of kitchen common salt with potassium chloride were made. It was found that the substitution does not have a negative influence on the organoleptic and physicochemical indices of the cheese. The use of the Fresh-Q in parallel with 20 and 30 % substitution with potassium chloride has a positive influence on the course of proteolytic processes, which is evidenced by an increase in casein fragments with a lower molecular weight, an increase in the area of absorption of acetonitrile by water-soluble peptides, and an increase in the concentration of free amino groups and free amino acids. This is also accompanied by a corresponding increase in total soluble nitrogen and nitrogen in soluble protein compounds.

Keywords: salted cheeses, brynza, sodium chloride, potassium chloride, proteolysis, amino acids, cheese technology

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Introduction

Natural cheeses are especially important for providing the human ration with complete nutrients. They have a high biological value due to the concentration and modification of milk components. Cheeses have a wide range of flavors, and their production is characterized by high profitability and, as a result, the growth of annual volumes [6, 12, 22, 25]. Recently, there has been an increase in consumer interest in salted cheeses. This is one of the most dynamically developing segments and occupies a special niche in the cheese industry. The environment (brine of various concentrations) in which cheeses are ripened and further stored determines their specific properties, a peculiar spicy-salty taste and determines the characteristic dense consistency.

Brine cheeses of the traditional assortment include: Chanach, Tushyn, Ossetian, Kobiy, Yerevan, Georgian, Suluguni, Brynza, Mozzarella, Chechil and local (national – Bulgarian *Sirene*, Turkish *Beyaz Peynir*, Romanian and Ukrainian *Brynza*; Iranian *Ligvan*, Lebanese *Nabulsi*) types [8, 10, 11]. The international annual consumption of brine cheeses reaches 650 ÷ 700 thousand tons. The profitability of the cheese business reaches 30 ÷ 40 %, which is a strong incentive for increasing the production of brine cheeses [6, 19, 22, 23].

The range of brine cheeses consists of more than 30 items. Brynza is traditionally made from sheep's milk. A raw material for the production of brine cheese can be not only sheep’s, but also cow's and goat's milk or their mixture, which is usually used for the production of this type of cheese. The milk composition of different species of mammals is determined by the influence of genetic factors and environmental conditions [6, 25]. Sheep's milk is characterized by a higher content of all its dry matter components compared to cow's and goat's milk [2, 9], the calorie content of sheep's milk is almost twice as high as that of cow's and goat's milk [4, 24].

One of the most important indicators of the suitability of milk for cheese production is the fractional composition of caseins. It is known that individual fractions of casein react differently to the action of rennet enzyme. For processing into brynza, milk needs to have a high casein content of α-, ß-, κ-fractions and a small amount of proteose-peptone fraction [15, 18, 27, 28]. Sheep's milk contains the entire complex of vitamins of group B and more than 50 mineral elements [12, 27].

The salt content in brynza is 4 ÷ 7 %. Common salt is an essential ingredient in the production of cheeses. A special feature of the brine cheese technology is ripening in a solution of common salt of a certain concentration. Physico-chemical, biochemical and microbiological processes in cheese and their intensity depend on the concentration of salt in brine. Salt is involved in maintaining and regulating the water-salt balance in the organism, sodium-potassium ion exchange [1, 3]. The recommended dose of NaCl consumption is 6 g (2.4 g Na) [10]. Since the organism does not need salt as such, but sodium ions and chloride ions, the need for salt is influenced by the con-

sumption of other sodium and chlorine salts [8]. The excessive consumption of common salt and the associated risks of diseases cause concern and determine the need to reduce the NaCl content in food products [3, 28]. There is a worldwide tendency to reduce the salt content in food products, including dairy products. At the expense of cheeses, a person consumes 11 ÷ 20 % of the daily need for salt. Therefore, there is a growing interest in reducing the salt concentration in cheeses. Average daily sodium consumption in Hungary is decreased by 60 %, in Poland – by 49 %, in Italy – by 41 %, in Ukraine – by 28 %. In Australia, Canada, the EU countries and Japan, voluntary campaigns for reducing salt consumption have been held [3].

One of the ways to reduce the concentration of sodium chloride and at the same time to prevent the deterioration of brynza quality, the reduction of its storage period, is to partially replace it with potassium chloride. The positive results of this replacement are evidenced by data of authors of Australia and the USA [1, 2, 3]. The studies were conducted on hard cheeses, however, it is brine cheese that has the highest sodium chloride content (4 ÷ 7 %), and there is no literature data on the investigation of such a replacement.

The organism of an adult contains from 160 to 250 g of potassium, 98 % of which is inside the cells. The main role of intracellular potassium is to ensure the normal functioning of cell membranes. This happens due to harmonious interaction with sodium. All tissues are characterized by a certain concentration ratio between potassium and sodium. Potassium in the human organism also performs the following functions: it maintains the constant composition of cellular and intercellular fluid; supports acid-base balance; participates in the work of nerve and muscle cells; improves oxygen supply to the brain; participates in the nervous regulation of heart contractions; helps lower blood pressure. The daily need for potassium for a child is 16 ÷ 30 mg per 1 kg of body weight, for an adult – 1.5 ÷ 2.5 g, while the required minimum is 1 g. A toxic dose of potassium for a person is 6 g, and a lethal dose is 14 g [1, 3]. The human organism's need for this macroelement necessitates its use in the technology of food production, namely in cheese making. Today, the world's leading scientists are engaged in the introduction of various types of crude potassium chloride into technology [1, 2, 3, 11].

One of the ways to extend the shelf life of brynza by reducing the concentration of sodium chloride in it is the use of protective cultures, the use of which in brynza technology is also lacking in scientific reports. To extend the shelf life of brynza, the bacterial preparation Fresh-Q is used, which is the composition of cultures of traditional lactic acid bacteria, which include *Lactobacillus rhamnosus*. The preparation has a harmful action on yeast and molds.

Materials and methods

The experimental part of the work was performed in the laboratory of the department of technology of milk and dairy products of the Stepan Gzhytsky Lviv National University of Veterinary Medicine and Biotechnologies; the laboratories of the Institute of Animal Biology of the National Academy of Sciences of Ukraine; the laboratories of the Department of Livestock Product Technology and Quality Management of the Wroclaw University of Natural Sciences of the Republic of Poland. Dairy raw materials were selected from the "Vivcharyk" farm, Nadvirnyan district, Ivano-Frankivsk region, Ukraine by Ukrainian mountain carpathian sheep breed.

The CHY-MAX enzyme preparation manufactured by Chr. Hansen (Denmark). CHY-MAX is a recombinant chymosin produced by fermentation with *Aspergillus niger varietas Awamori*. It does not contain enzymes capable of breaking down starch. The preparation contains lactic enzymes with a high specific action of splitting k-casein, which as a result ensures very good clot formation. CHY-MAX meets the requirements of the World Health Organization of the United Nations and the FCC regarding the degree of purity of enzymes [29]. RSF-742 (Chr. Hansen, Denmark) preparation of direct introduction as a leavening culture was used, which contains the following strains of lactic acid bacteria: *Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis, Streptococcus thermophilus, Lactobacillus helveticus*. To ensure the long-term storage of manufactured cheese, the bacterial preparation Fresh-Q (Chr. Hansen, Denmark) was used, which inhibits yeast and mold in fermented dairy products. This preparation is composed of cultures of traditional lactic acid bacteria, which include *Lactobacillus rhamnosus*.

Six samples of sheep cheese brynza were made: K – control sample using sodium chloride; D1 – brynza made with 20 % replacement of sodium chloride with potassium chloride; D2 – brynza made with 30 % replacement of sodium chloride with potassium chloride; KF – brynza, which is made with the use of sodium chloride and Fresh-Q; DF1 – brynza made with 20 % replacement of sodium chloride with potassium chloride and the use of Fresh-Q; DF2 is brynza made with 30 % replacement of sodium chloride with potassium chloride and the use of Fresh-Q.

The normalization of the milk mixture of sheep's milk was carried out according to the mass fraction of fat, taking into account the mass fraction of protein in milk. The mass fraction of fat in the dry matter of brynza was 45 %. The pasteurization of the normalized mixture was carried out at (73 ± 2) ºC with a holding time of 15 seconds. CHY-MAX enzyme preparation, calcium chloride, RSF-742 bacterial starter and Fresh-Q were added to milk cooled to 33 ± 1 ºC, thoroughly mixed for 10 ÷ 12 minutes. After mixing, the coagulation of caseins was carried out, the pH was adjusted to 5.3. Next, the clot was cut into $1 \times 1 \times 1$ cm^3 cubes and left alone for 12 ÷ 15 minutes to separate the serum. The finished curd, which after cutting fell to the bottom

of the cheese-making tub, was submitted for self-pressing, which lasted 12 hours, and pressing for 2 ÷ 3 hours. The pressed layer of cheese, having a thickness of 4 ÷ 5 cm, was cut into 5 ÷ 7 cm^2 squares and filled with previously prepared brine (aqueous solution of common salt) with a salt concentration of 18 %. The salt content was controlled during the salting period. The salt content in the finished product was 4 %. After salting, the brine was replaced with an isotonic common salt solution, the salt concentration of which corresponded to the salt concentration in the product (4 %). In terms of 1 dm^3 of brine, 40 g of common salt was used for the control sample; for samples D1 and DF1, 20 % of sodium chloride was replaced by potassium chloride (32 g of sodium chloride + 8 g of potassium chloride); for samples D2 and DF2, 30 % of sodium chloride was replaced by potassium chloride (28 g of sodium chloride+12 g of potassium chloride). This will ensure that the necessary salt content is maintained in the product throughout the ripening and storage period. Brynza ripening took place at a temperature of 6 ÷ 8 ºC for 20 days.

The organoleptic indices of the brynza were investigated – by the organoleptic method; mass fraction of fat – butyrometrically forthe 10^{th} and 20^{th} day of ripening (mature cheese); mass fraction of moisture – by the drying method to the 10^{th} and 20^{th} day of ripening; mass fraction of salt – by reaction with silver nitrate during salting, as well as on the 10^{th} and 20^{th} day of ripening; active acidity - by the potentiometric method using the ARN-9 pH meter; titrated acidity – titrometrically (in degrees Turner); mass fraction of protein using the method of Lowry [23], total nitrogen, total soluble nitrogen, nitrogen of non-protein soluble nitrogen-containing compounds, nitrogen of soluble protein compounds – by the Kjeldahl method for the 10^{th} and 20^{th} day of ripening; electrophoretic separation of proteins was carried out by the method of Andrews [19, 20] for the 10^{th} and 20^{th} day of ripening; the protein-peptide content of brynza was investigated using the Chromatograf 1220 Infinity LC Agilent Technologies device; the isolation and identification of free amino acids was carried out with the "Biotronik" LC-2000 amino acid analyzer on the 12^{th} day of maturation; the concentration of free amino groups was determined using the method of Snydera and Sobocinski, Kuchroo and Ramilly [18, 20, 23] in Chrzanowska's modification for the 10^{th} and 20^{th} day of ripening; the determination of the digestibility of mature brynza proteins was carried out using the basic method of Pokrovsky and Yertanov [18], which was improved by Lipatov, Yudina and Lisitsyn [20]; the structural and mechanical parameters of brynza were investigated using the Zwick/Roel Z010 apparatus in mature brynza.

The Statistica©5.XX for Windows program (StatSoft Inc., USA) was used to process the results. The research results were evaluated according to the level of significance R. The research was carried out with five repetitions. The reliability of the re-

sults was accepted at $P<0.05$. The graphic processing of the results was carried out in the program Microsoft Excel 7.0 Harvard Chart XL for Windows XP Version 2.0.

Results and discussion

In the production of cheese, special requirements are placed on the quality of raw milk. Analyzing the data in Table 1, onecan conclude that sheep's milk is characterized by a chemical composition appropriate for this species of milk and provides for the possibility of its use for brynza production. As showed by the results of the study of bacterial contamination of sheep's milk, it belongs to the 1st grade.

The organoleptic indicators of the finished product are formed as a result of the splitting of milk sugar, proteins and fats, during which a whole range of compounds is formed: lactic acid, fermentation products of milk sugar, citrates and citric acid, free amino acids, carbonyl compounds (acetoin, diacetyl), fatty acids, etc.

Table 1. Physico-chemical indices and chemical composition of sheep's milk (n = 5)
Tabela 1. Wskaźniki fizykochemiczne i skład chemiczny mleka owczego (n = 5)

Properties / Właściwość	Sheep's milk / Mleko owcze
Protein / Białko [%]	5.10 ± 0.11
Fat / Tłuszcz, [%]	5.70 ± 0.12
Lactose / Laktoza [%]	4.63 ± 0.11
Mass fraction of dry substances / Sucha masa, [%]	17.00 ± 0.12
Density / Gęstość [kg/m^3]	1035.2 ± 0.12
pH	6.38 ± 0.11
Titrated acidity / Kwasowość miareczkowa [°T]	24 ± 12

The replacement of table salt and the use of Fresh-Q ensures high quality of the product according to organoleptic indicators: such samples have a more pronounced creamy taste. The smell, appearance and consistency of all cheese samples were similar. During the ripening of cheeses, complex biochemical transformations take place, as a result of which, under certain conditions, taste and aromatic compounds accumulate, determining the specific taste and aroma of cheese [2, 29]. Biochemical and microbiological processes occurring during cheese ripening lead to significant changes in all constituent components of fresh cheese, which is reflected in the formation of organoleptic properties.

Tables 2 ÷ 3 show the changes in the physical and chemical indices of brynza during the fresh ripening period. Attention is given to the change in active acidity: samples with the use of Fresh-Q are marked by lower acidity of the curd than similar samples without the preparation. In addition, the mass fraction of moisture is decreasing to-

wards the end of ripening in all experimental samples of cheese. The control sample at the end of ripening has the highest moisture content (53.1 %), and the sample with 20 % replacement of sodium chloride with potassium chloride has the lowest. Evaluating the mass fraction of fat, one can see an increase in this indicator until the end of ripening, which is consistent with an increase in the mass fraction of dry substances.

Table 2. Physico-chemical indices of brynza from sheep's milk with partial replacement of sodium chloride with potassium chloride on the 10th day of ripening (n = 5)

Tabela 2. Wskaźniki fizykochemiczne bryndzy z mleka owczego z częściowym zastąpieniem chlorku sodu chlorkiem potasu w 10 dniu dojrzewania (n = 5)

Samples of brynza / Próbki bryndzy	Indices / Wskaźniki				
	Fat in dry substances / Tłuszcz w suchej masie [%]	Moisture / Wilgotność [%]	Mass fraction of NaCl+KCl / NaCl+KCl [%]	Mass fraction of NaCl / NaCl [%]	pH
K	42.0 ± 0,3	66.0 ± 0,2	4.10 ± 0,1	4.10 ± 0,1	4.30 ± 0,03
D1	41.0 ±0 ,3	64.8 ± 0,3*	4.25 ± 0,2	3.40 ± 0,2*	4.27 ± 0,02
D2	42.0 ± 0,2	63.2 ± 0,3*	4.08 ± 0,1	3.26 ± 0,1	4.31 ± 0,03*
KF	43.0 ± 0,4**	65.0 ± 0,2	4.26 ± 0,1	4.26 ± 0,1	4.22 ± 0,02
DF1	41.0 ± 0,3*	58.0 ± 0,3*	4.12 ± 0,2*	3.29 ± 0,2**	4.13 ± 0,02
DF2	42.6 ± 0,4**	58.2 ± 0,2	4.23 ± 0,2	3.38 ± 0,2	4.10 ± 0,03*

Explanation notes / Objaśnienia: K – control sample using sodium chloride / próbka kontrolna z użyciem chlorku sodu; D1 – brynza made with 20 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 20 % zastąpienia chlorku sodu chlorkiem potasu; D2 – brynza made with 30 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 30 % zastąpienia chlorku sodu chlorkiem potasu; KF – brynza made using sodium chloride and the Fresh-Q / bryndza wytwarzana z użyciem chlorku sodu i Fresh-Q; DF1 – brynza made with 20 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza wyprodukowana z 20 % zastąpienia chlorku sodu chlorkiem potasu i zastosowaniem Fresh-Q; DF2 – brynza made with 30 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza powstała w wyniku zastąpienia chlorku sodu w 30 % chlorkiem potasu oraz przy użyciu Fresh-Q

Note: statistically significant differences were taken into account compared to the control group: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$. Samples were compared in-lines /

Uwaga: uwzględniono różnice istotne statystycznie w porównaniu z grupą kontrolną: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$. Próbki porównano w wierszach.

Analyzing the salt content in cheese, it should be noted that its value increases during the entire ripening period. Naturally, the highest content of NaCl in mature cheese was found in the control samples, while it was 0.82 ÷ 0.97 % lower in the experimental samples. This ensures a reduction in the consumption of table salt at the daily rate of cheese consumption (70 g) [3].

Table 3. Physico-chemical indices of brynza from sheep's milk with partial replacement of sodium chloride with potassium chloride on the 20th day of ripening (mature cheese) (n = 5)

Tabela 3. Parametry fizykochemiczne bryndzy z mleka owczego z częściowym zastąpieniem chlorku sodu chlorkiem potasu w 20 dniu dojrzewania (ser dojrzały) (n = 5)

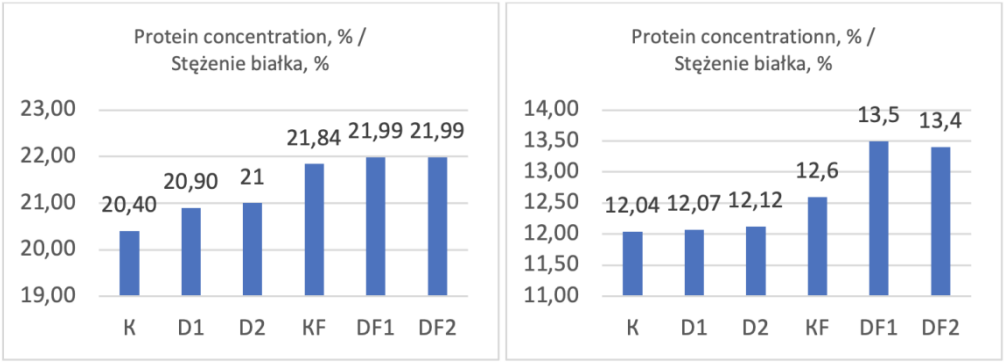
Samples of brynza / Próbki bryndzy	Indices / Wskaźniki				
	Fat in dry Substances / Tłuszcz w suchej masie [%]	Moisture / Wilgotność [%]	Mass fraction of NaCl+KCl / NaCl+KCl [%]	Mass fraction of NaCl / NaCl [%]	pH
K	45.9 ± 0.3	53.1 ± 0.2	4.2 ± 0.1	4.2 ± 0.1	4.23 ± 0.03
D1	44.3 ± 0.3	52.2 ± 0.3**	4.3 ± 0.2	3.44 ± 0.2	4.22 ± 0.02
D2	45.8 ± 0.2	52.4 ± 0.3*	4.1 ± 0.1	3.28 ± 0.1*	4.33 ± 0.03*
KF	45.1 ± 0.4	51.7 ± 0.2	4.3 ± 0.1	4.3 ± 0.1	4.12 ± 0.02
DF1	43.9 ± 0.3	51.0 ± 0.3*	4.2 ± 0.2	3.36 ± 0.2*	4.10 ± 0.02**
DF2	44.8 ± 0.4	51.3 ± 0.2	4.3 ± 0.2**	3.44 ± 0.2	4.08 ± 0.03

Explanation notes / Objaśnienia: K – control sample using sodium chloride / próbka kontrolna z użyciem chlorku sodu; D1 – brynza made with 20 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 20 % zastąpienia chlorku sodu chlorkiem potasu; D2 – brynza made with 30 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 30 % zastąpienia chlorku sodu chlorkiem potasu; KF – brynza made using sodium chloride and the Fresh-Q / bryndza wytwarzana z użyciem chlorku sodu i Fresh-Q; DF1 – brynza made with 20 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza wyprodukowana z 20 % zastąpienia chlorku sodu chlorkiem potasu i zastosowaniem Fresh-Q; DF2 – brynza made with 30 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza powstała w wyniku zastąpienia chlorku sodu w 30 % chlorkiem potasu oraz przy użyciu Fresh-Q

Note: statistically significant differences were taken into account compared to the control group: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$. Samples were compared in-lines /

Uwaga: uwzględniono różnice istotne statystycznie w porównaniu z grupą kontrolną: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$. Próbki porównano w wierszach.

The content of protein compounds determined by the Lowry method is presented in the form of a diagram (Fig. 1 a, b). Brynza made with Fresh-Q has the highest protein content, the replacement of common salt causes a tendency to increase its content. Comparing the content of protein on the 10th and 20th day of ripening, a significant decrease in its content by the end of the ripening period is noticeable, which is a consequence of the redistribution of nitrogenous compounds, namely, an increase in the share of non-protein peptides, amino acids and their transformation products.



a) b)

Figure 1. The protein content of brynza made from sheep's milk with 20 and 30 % replacement of common salt with potassium chloride (a – on the 10th day of ripening, b – in mature cheese (on the 20th day of ripening))

Rycina 1. Zawartość białka w bryndzie z mleka owczego z 20 i 30 % zastąpieniem soli kuchennej chlorkiem potasu (a – w 10 dniu dojrzewania, b – w serze dojrzałym (w 20 dniu dojrzewania))

Explanation notes / Objaśnienia:
K – control sample using sodium chloride / próbka kontrolna z użyciem chlorku sodu; D1 – brynza made with 20 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 20 % zastąpienia chlorku sodu chlorkiem potasu; D2 – brynza made with 30 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 30 % zastąpienia chlorku sodu chlorkiem potasu; KF – brynza made using sodium chloride and the Fresh-Q / bryndza wytwarzana z użyciem chlorku sodu i Fresh-Q; DF1 – brynza made with 20 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza wyprodukowana z 20 % zastąpienia chlorku sodu chlorkiem potasu i zastosowaniem Fresh-Q; DF2 – brynza made with 30 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza powstała w wyniku zastąpienia chlorku sodu w 30 % chlorkiem potasu oraz przy użyciu Fresh-Q

Table 4. The content of nitrogen-containing compounds of brynza on the 12th day of ripening (n = 5)
Tabela 4. Zawartość związków azotowych w bryndzie w 12 dniu dojrzewania (n = 5)

Samples of brynza / Próbki bryndzy	Total nitrogen / Azot ogółem [mg/g]	Total soluble nitrogen / Azot rozpuszczalny ogółem [mg/g]	Soluble nitrogen of non-protein substances / Rozpuszczalny azot niebiałkowy [mg/g]	Soluble nitrogen protein substances, / Rozpuszczalny azot białkowe [mg/g]
K	28.80±0.12	14.96±0.12	2.32±0.12	12.64±0.11
D1	30.50±0.14**	18.54±0.13**	2.50±0.15**	16.04±0.12**
D2	29.66±0.13**	17.84±0.16**	2.43±0.13**	15.41±0.12**
KF	30.80±0.11	20.02±0.11	2.38±0.14	17.64±0.13
DF1	31.38±0.15**	26.50±0.15**	2.69±0.13**	23.81±0.13
DF2	30.94±0.15**	26.40±0.14**	2.58±0.12**	23.82±0.11**

Explanation notes / Objaśnienia:
K – control sample using sodium chloride / próbka kontrolna z użyciem chlorku sodu; D1 – brynza made with 20 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 20 %

zastąpienia chlorku sodu chlorkiem potasu; D2 – brynza made with 30 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 30 % zastąpienia chlorku sodu chlorkiem potasu; KF – brynza made using sodium chloride and the Fresh-Q / bryndza wytwarzana z użyciem chlorku sodu i Fresh-Q; DF1 – brynza made with 20 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza wyprodukowana z 20 % zastąpienia chlorku sodu chlorkiem potasu i zastosowaniem Fresh-Q; DF2 – brynza made with 30 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza powstała w wyniku zastąpienia chlorku sodu w 30 % chlorkiem potasu oraz przy użyciu Fresh-Q

Note: statistically significant differences were taken into account compared to the control group: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$. Samples were compared in-lines /

Uwaga: uwzględniono różnice istotne statystycznie w porównaniu z grupą kontrolną: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$. Próbki porównano w wierszach.

Enzymes of bacterial preparations and microbial cultures play an important role in the hydrolysis of proteins, the formation of low-molecular peptides and amino acids, which serve as precursors for the formation of the taste composition of cheeses and their biological value [14, 20]. The processes of protein cleavage and amino acid conversion can occur both uncontrolled in raw whole and skimmed milk and secondary products, and controlled in the curd mass during cheese ripening, where the deepest hydrolysis of proteins takes place under the action of lactic enzymes and proteolytic enzymes of fermenting microflora. The content of nitrogen-containing compounds of brynza during ripening is shown in Tables 4 ÷ 5. There is a tendency to increase the content of total and total soluble nitrogen in samples with 20 and 30 % replacement of NaCl with KCl compared to the corresponding control on the 12th day of ripening. The use of the Fresh-Q has an influence on the content of both total and soluble nitrogen during this period: it increases compared to brynza produced without Fresh-Q (the content of total nitrogen in DF2 samples is 30.94 against 28.80 mg/g in the control; the content of soluble Nitrogen in DF2 samples is 26.40 against 14.96 mg/g in the control).

Table 5. Nitrogen-containing compounds of brynza on the 20th day of ripening (mature cheese) (n = 5)
Tabela 5. Związki azotowe bryndzy w 20 dniu dojrzewania (ser dojrzały) (n = 5)

Samples of brynza / Próbki bryndzy	Total Nitrogen / Ogólny azot [mg/g]	General soluble nitrogen / Ogólny rozpuszczalny azot [mg/g]	Nitrogen of non-protein soluble nitrogen-containing compounds / Azot związków nierozpuszczalnych w białkach [mg/g]	Soluble nitrogen protein substances / Rozpuszczalny azot substancje białkowe [mg/g]
K	33.48 ± 0.12	28.50 ± 0.13	7.30 ± 0.12	21.2 ± 0.12
D1	36.16 ± 0.14**	30.02 ± 0.13	8.70 ± 0.14**	21.32 ± 0.14**
D2	35.54 ± 0.11**	29.28 ± 0.11**	8.10 ± 0.11**	21.18 ± 0.1**
KF	36.5 8± 0.12	31.00 ± 0.11	8.28 ± 0.12	22.72 ± 0.12
DF1	37.96 ± 0.15**	32.72 ± 0.12**	9.10 ± 0.15**	23.62 ± 0.15**
DF2	37.66 ± 0.12**	32.14 ± 0.12**	9.06 ± 0.12	23.08 ± 0.12

Explanation notes / Objaśnienia:
K – control sample using sodium chloride / próbka kontrolna z użyciem chlorku sodu; D1 – brynza made with 20 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 20 % zastąpienia chlorku sodu chlorkiem potasu; D2 – brynza made with 30 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 30 % zastąpienia chlorku sodu chlorkiem potasu; KF – brynza made using sodium chloride and the Fresh-Q / bryndza wytwarzana z użyciem chlorku sodu i Fresh-Q; DF1 – brynza made with 20 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza wyprodukowana z 20 % zastąpienia chlorku sodu chlorkiem potasu i zastosowaniem Fresh-Q; DF2 – brynza made with 30 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza powstała w wyniku zastąpienia chlorku sodu w 30 % chlorkiem potasu oraz przy użyciu Fresh-Q
Note: statistically significant differences were taken into account compared to the control group: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$. Samples were compared in-lines /
Uwaga: uwzględniono różnice istotne statystycznie w porównaniu z grupą kontrolną: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$. Próbki porównano w wierszach.

In mature brynza, the highest content of total soluble nitrogen was registered for samples DF1 (32.72 mg/g) and D1 (30.02 mg/g), the lowest indicator was characterized by the control sample (28.50 mg/g). As regards the nitrogen of soluble protein compounds, which determines the dietary properties of cheese, it should be emphasized that the highest values for brynza with 20 % replacement of NaCl with KCl and Fresh-Q are 23.62 mg/g. Thus, the partial replacement of sodium ions with potassium ions and the use of protective cultures influence proteolytic processes [13, 17, 20].

The processes of proteolysis in salted cheeses are more intensive compared to cheeses of other groups, which is associated with a shorter ripening period [5, 17, 21]. They are closely related to the action of rennet enzyme [16], as well as the concentration of common salt [7]. The products of proteolytic processes are a number of compounds: high molecular weight insoluble fragments of casein fractions, water-soluble peptides, free amino acids, as well as amino acid conversion products as a result of deamination, decarboxylation and peramination. The degree of decomposition of protein fractions occurs specifically for each type of cheese. It is known that the maturity of cheeses can be determined by the depth of hydrolysis of these fractions, and their cleavage products, in turn, have a certain correlation with the intensity of taste, aroma and consistency of the product [5, 14]. Rennet enzyme breaks down α_{s1}-casein to a greater extent than β-casein because the former has more chymosin-sensitive bonds. At the same time, a shallow breakdown of casein occurs with the formation of long-chain and medium-chain peptides and peptones, and further breakdown of β-casein is carried out by microbial proteases [1, 5, 13, 15].

Analyzing the proteolytic processes in brynza on the 10^{th} day of ripening (Fig. 2), it should be noted that they are quite active, as evidenced by the presence of fragments of protein fractions, which are shown in electrophoretic images. The fraction with the highest molecular weight β CN (f 1–*) was placed the highest. It is significant that the

control samples K and KF have similar electrophoretic patterns. Brynza with 20 and 30 % replacement is characterized by a deeper breakdown of proteins with a significantly wider fractional content. In particular, the presence of β CN (f1–189/192)(β-1) fragments is evident in all samples with the replacement of sodium chloride with potassium chloride and the Fresh-Q.

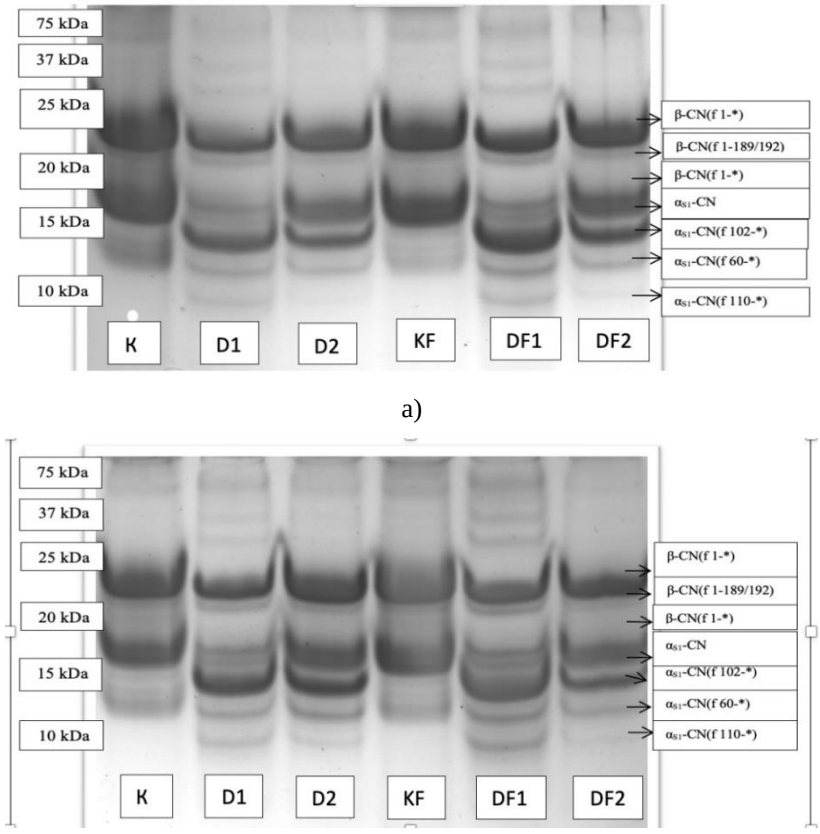


Figure 2. Electrophoretic separation of brynza proteins with 20 and 30 % substitution of common salt with potassium chloride for the 10th (a) and 20th (b) day of ripening

Rycina 2. Elektroforetyczna separacja białek bryndzy z 20 i 30 % podstawieniem soli kuchennej chlorkiem potasu na 10 (a) i 20 (b) dni dojrzewania

Explanation notes / Objaśnienia:
K – control sample using sodium chloride / próbka kontrolna z użyciem chlorku sodu; D1 – brynza made with 20 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 20 % zastąpienia chlorku sodu chlorkiem potasu; D2 – brynza made with 30 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 30 % zastąpienia chlorku sodu chlorkiem potasu; KF – brynza

made using sodium chloride and the Fresh-Q / bryndza wytwarzana z użyciem chlorku sodu i Fresh-Q; DF1 – brynza made with 20 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza wyprodukowana z 20 % zastąpienia chlorku sodu chlorkiem potasu i zastosowaniem Fresh-Q; DF2 – brynza made with 30 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza powstała w wyniku zastąpienia chlorku sodu w 30 % chlorkiem potasu oraz przy użyciu Fresh-Q

Electrophoregrams of caseins of mature brynza demonstrate that similar intergroup features regarding individual fractions and their fragments are preserved compared to those in the samples on the 10^{th} day of ripening. However, differences in the profiles of casein fractions in brynza samples on the 10^{th} and 20^{th} day of ripening were not set, which is obviously due to the fact that the rennet enzyme, whose action has already been completed, is responsible for the formation of these large peptides.

Only sharper profiles of lower molecular weight peptides can be noted in samples with sodium chloride replacement and with Fresh-Q on the 20^{th} day of maturation. It is obvious that the replacement of sodium chloride with potassium chloride causes a different profile, which is expressed by an increase in the fractions of peptides of lower molecular weight.

Great attention today is focused on the research of water-soluble casein peptides and their biological role. We chromatographically separated the water-soluble peptides of brynza samples, the duration of the separation was 60 minutes, but the most active phase was between the 30^{th} and 40^{th} minute of retention. Comparing the chromatograms obtained by us with the data of other authors using this method, we identified the received peaks as peptides of β-CN and α_{s1}-CN fractions.

The summary results of the chromatograms of the protein-peptide content of brynza samples, which characterize their quantity, are presented in Table 6. Comparing the chromatograms of the protein-peptide content for samples without the Fresh-Q, one can note the presence of similar fragments of β-CN f fractions (60-63) and α_{s1} -CN (f 102 -*), however, there are differences regarding the initial fragments of the α_{s1}-CN fraction. In the sample K, the fragment α_{s1}-CN f (4–13) was isolated, and for experimental samples D1 and D2 α_{s1}-CN f (1–5) and α_{s1}-CN f (–*). Regarding the protein-peptide content of the samples using the Fresh-Q, a fragment of the β-CN f fraction (60-63) is present in the KF and DF2 samples, while another β-CN fragment (f 81-86) was identified in the DF1 sample. These fragments of the β-CN fraction (60-63 and 81-86) have opioid properties [24]. As for the identified fragments of the α_{s1}-CN fraction, its final fragments are similar, and the initial fragments are decreasing sequentially from control to DF2 by 1 amino acid residue. As showed in literature data, these fragments of the α_{s1}-CN fraction have antioxidant and immunomodulatory properties [24].

Table 6. Protein-peptide composition of brynza from sheep's milk for 20 and 30 % replacement of sodium chloride with potassium chloride

Tabela 6. Skład białkowo-peptydowy bryndzy z mleka owczego dla 20 i 30 % zastąpienia chlorku sodu chlorkiem potasu

Samples of brynza / Próbki bryndzy	Peak / Faction / Szczyt / Frakcja	Fragment / Fragment	Coverage area [relative units] / Obszar zasięgu [jednostki względne]	Coverage sequence / Pokrycie sekwencji [%]
K	Pik 1: β-CN	f 60 – 63	560.6	15
	Pik 2: α_{S1}-CN	f 4 – 13	1453.2	17
	Pik 3: α_{S1}-CN	f 102 – *	2221.6	30
D1	Pik 1: β-CN	f 60 – 63	545.1	5
	Pik 2: α_{S1}-CN	f 1 – 5	653.8	4
	Pik 3: α_{S1}-CN	f 102 – *	1980.6	20
D2	Pik 1: β-CN	f 60 – 63	567.7	11
	Pik 2: α_{S1}-CN	f 1 – *	845.9	17
	Pik 3: α_{S1}-CN	f 102 – *	2267.2	35
KF	Pik 1: β-CN	f 60 – 63	564.9	11
	Pik 2: α_{S1}-CN	f 1 – 8	912.9	17
	Pik 3: α_{S1}-CN	f 102 – *	2100	25
DF1	Pik 1: β-CN	f 81 – 86	605.9	11
	Pik 2: α_{S1}-CN	f 1 – 7	868.1	15
	Pik 3: α_{S1}-CN	f 102 – *	2304.6	30
DF2	Pik 1: β-CN	f 60 – 63	561,6	11
	Pik 2: α_{S1}-CN	f 1 – 6	786.3	18
	Pik 3: α_{S1}-CN	f 102 – *	2301.2	32

Explanation notes / Objaśnienia:
K – control sample using sodium chloride / próbka kontrolna z użyciem chlorku sodu; D1 – brynza made with 20 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 20 % zastąpienia chlorku sodu chlorkiem potasu; D2 – brynza made with 30 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 30 % zastąpienia chlorku sodu chlorkiem potasu; KF – brynza made using sodium chloride and the Fresh-Q / bryndza wytwarzana z użyciem chlorku sodu i Fresh-Q; DF1 – brynza made with 20 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza wyprodukowana z 20 % zastąpienia chlorku sodu chlorkiem potasu i zastosowaniem Fresh-Q; DF2 – brynza made with 30 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza powstała w wyniku zastąpienia chlorku sodu w 30 % chlorkiem potasu oraz przy użyciu Fresh-Q
Note: * – undefined remainder / Uwaga: * – reszta niezdefiniowana

The largest coverage area of the acetonitrile fragment of the β-CN fraction in the sample K in the group of cheeses without Fresh-Q is noteworthy, while the coverage area of the fragment from 102 amino acid residues is the largest in the sample with 30 % replacement. In the group with the Fresh-Q, the coverage area of this fragment is

greater in all experimental samples. Considering the similarity of the previous fragments, it can be assumed that the replacement of sodium chloride and the use of the Fresh-Q result in higher antioxidant properties of the cheese.The content of free amino acids (AA) in the cheese samples is presented in Table 7. It should be noted that the combination of RSF and Fresh-Q has an influence on the content of free amino acids. As for their total content, the same dependence remains as when replacing NaCl with KCl without Fresh-Q – their total amount increases significantly in samples with the 30 % replacement of NaCl with KCl [7, 19].

We analyzed the difference in the amount of free amino acids, it can be stated that K was characterized by a lower content of them by 3.75 % compared to D1 and by 50.2 % compared to D2. Similar results were observed in the samples DF1 and DF2 compared to the sample KF. It should be noted that the use of the Fresh-Q increased the content of free amino acids by 66.7 % compared to K. As for the content of the sum of essential amino acids, a significant difference in concentrations was registered for DF2 samples – 133.6 μg/g against 89.97 μg/g in the K control. Given the sum of the content of glutamic acid, proline, valine and leucine, which are responsible for the formation of a pleasant taste, the samples prepared with the replacement of sodium chloride with potassium chloride and the use of a microbial preparation of direct input in combination with Fresh-Q significantly prevailed over K and KF, which is confirmed by the results of the organoleptic evaluation of taste saturation. The content of tyrosine and arginine, which influence the taste, in the control sample K was 9.59 μg/g, and in DF2 – 25.34 μg/g.

As is known, the formation of the bitter taste of cheese is influenced by the concentration of such amino acids as methionine, histidine and lysine [1]. The lowest concentration of these amino acids was in D1, which proves the positive influence of partial replacement of common salt with potassium chloride in the amount of 20 %. When using the Fresh-Q, the content of almost all individual free amino acids increases significantly (by 2 ÷ 2.5 times), but the content of sulfur-containing amino acids almost does not change. Thus, replacing 20 and 30 % of sodium chloride with potassium chloride in the production of brynza has an influence on both the protein content and the course of proteolytic processes. At the same time, the taste and textural properties of cheese do not deteriorate.

The course of proteolytic processes can also be judged by the change of free amino groups using the method of Snyder and Sobocinski and Kuchroo and Ramilly [18, 19], as modified by Chrzanowska, which is shown in the form of a diagram (Fig. 3 a, b). On the 10^{th} day of ripening, the samples prepared using the Fresh-Q and the replacement of common salt had significantly higher results, but no significant intragroup differences were registered. In mature sheep brynza, a different pattern was

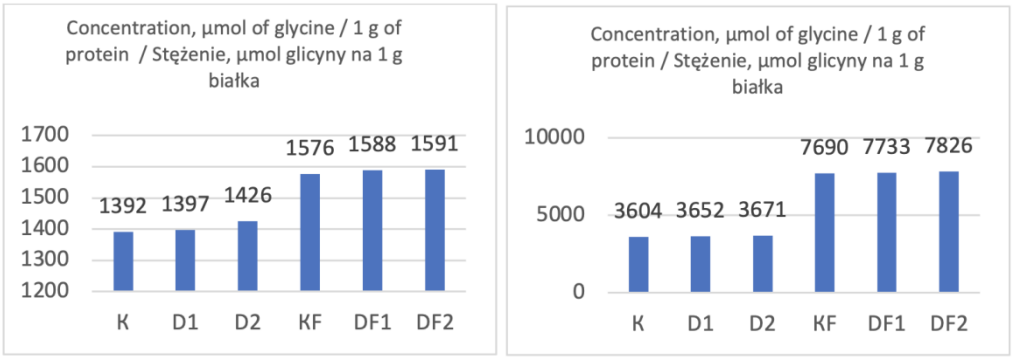
Table 7. The content of free amino acids in brynza on the 10th day of ripening [μg/g] (n = 5)
Tabela 7. Zawartość wolnych aminokwasów w bryndzie w 10 dniu dojrzewania [μg/g] (n = 5)

Amino acid / Aminokwas	Samples of brynza / Próbki bryndzy					
	K	D1	D2	KF	DF1	DF2
Aspartic acid / Kwas asparaginowy	5.81±0.22	5.25±0.07	10.07±0.11**	14.48±0.12	14.48±0.12	14.23±0.12
Threonine / Treonina	0.97±0.15	1.00±0.01	1.26±0.11	1.58±0.12	2.91±0.12**	1.34±0.12
Serin / Seryna	5.36±0.12	4.75±0.24	12.69±0.13***	11.54±0.12	15.73±0.12***	15.81±0.12***
Glutamic acid / Kwas glutaminowy	13.33±0.14	9.79±0.14***	20.61±0.12***	33.41±0.12	33.77±0.12***	26.33±0.12
Proline / Prolina	7.80±0.13	26.05±0.08***	17.35±0.12***	18.42±0.12	15.95±0.12	16.39±0.12***
Glycine / Glicyna	3.01±0.12	1.87±0.08**	5.88±0.12***	9.09±0.09	8.38±0.12**	5.16±0.12***
Alanine / Alanina	7.41±0.11	4.87±0.12***	11.59±0.14***	10.03±0.12	10.16±0.17*	11.59±0.12***
Cysteine / Cysteina	5.41±0.14	2.59±0.09***	6.01±0.12*	2.93±0.12	3.88±0.12**	6.15±0.17***
Valin / Walina	11.21±0.13	10.26±0.16**	17.45±0.12***	12.25±0.12	15.18±0.12**	18.38±0.12***
Methionine / Metionina	5.20±0.14	2.74±0.14***	6.18±0.10	5.12±0.13	6.43±0.12**	5.29±0.12
Isoleucine / Izoleucyna	0.61±0.13	1.24±0.11*	0.86±0.12	0.47±0.12	1.14±0.12	1.32±0.12**
Leucine / Leucyna	10.74 ±0.13	14.73±0.12***	16.72±0.12***	17.24±0.12	14.58±0.12***	18.39±0.12**
Tyrosine / Tyrozyna	6.74±0.13	10.62±0.12***	11.10±0.05***	8.93±0.12	8.78±0.12	14.84±0.12***
Phenylalanine / Fenyloalanina	22.84±0.11	29.56±0.13***	33.73±0.13***	30.13±0.14	31.09±0.12**	38.42±0.12***
Histidine / Histydyna	29.22±0.12	21.49±0.13***	32.19±0.10***	27.67±0.12	23.31±0.12***	29.09±0.14**
Lysine / Lizyna	2.44±0.12	1.77±0.12*	5.72±0.12***	2.20±0.12	3.00±0.12**	6.53±0.12***
Arginine / Arginina	2.85±0.12	1.77±0.13**	6.42±0.12***	5.10±0.12	4.07±0.12**	10.50±0.12***
Ammonia / Amoniak	7.32±0.09	3.49±0.14***	6.84±0.21	6.72±0.12	6.90±0.12**	7.43±0.12*
The sum of essential amino acids / Suma niezbędnych aminokwasów	89.97±0.13	93.41±0.12	125.21±0.12	105.59±0.12	106.42±0.12	133.60±0.12
The sum of free amino acids / Suma wolnych aminokwasów	148.27±0.13	153.84±0.12	222.67±0,12	217.31±0.12	219.74±0.12	247.19±0.12

Explanantial notes / Objaśnienia:
K – control sample using sodium chloride / próbka kontrolna z użyciem chlorku sodu; D1 – brynza made with 20 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 20 % zastąpienia chlorku sodu chlorkiem potasu; D2 – brynza made with 30 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 30 % zastąpienia chlorku sodu chlorkiem potasu; KF – brynza made using sodium chloride and the Fresh-Q / bryndza wytwarzana z użyciem chlorku sodu i Fresh-Q; DF1 – brynza made with 20% replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza wyprodukowana z 20 % zastąpienia chlorku sodu chlorkiem potasu i zastosowaniem Fresh-Q; DF2 – brynza made with 30 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza powstała w wyniku zastąpienia chlorku sodu w 30 % chlorkiem potasu oraz przy użyciu Fresh-Q
Note: statistically significant differences were taken into account compared to the control group: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$. Samples were compared in-lines / Uwaga: uwzględniono różnice istotne statystycznie w porównaniu z grupą kontrolną: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$. Próbki porównano w wierszach.

registered: deeper proteolysis occurred in the samples where part of sodium chloride was replaced, the level of its growth was correlated with an increase in the percentage of replacement. This was the case with the group of samples without and with Fresh-Q. Also, the number of free amino groups in mature brynza increases compared to unripe cheese, which, in fact, is consistent with the data of previous investigations of the content of free amino acids.

Thus, the Fresh-Q and the use of a partial replacement of common salt intensify deep proteolysis. This is confirmed by the data in Fig. 3. Biological value is an important indicator of quality, as it determines the degree of availability and, accordingly, assimilation of nutrients. The most important indicator of protein quality is its digestibility, that is, the ability to be hydrolyzed by enzymes of the gastrointestinal tract. This property is investigated by in vitro and in vivo methods, which have a high degree of correlation [23].



a) b)

Figure 3. The concentration of free amino groups in sheep brynza with 20 and 30 % replacement of table salt with potassium chloride (a – on the 10th day of ripening, b – in mature cheese)

Rycina 3. Stężenie wolnych grup aminowych w bryndzie owczej przy 20 i 30 % zastąpieniu soli kuchennej chlorkiem potasu (a – w 10 dniu dojrzewania, b – w serze dojrzałym).

Explanantion notes / Objaśnienia:
K – control sample using sodium chloride / próbka kontrolna z użyciem chlorku sodu; D1 – brynza made with 20 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 20 % zastąpienia chlorku sodu chlorkiem potasu; D2 – brynza made with 30 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 30 % zastąpienia chlorku sodu chlorkiem potasu; KF – brynza made using sodium chloride and the Fresh-Q / bryndza wytwarzana z użyciem chlorku sodu i Fresh-Q; DF1 – brynza made with 20% replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza wyprodukowana z 20 % zastąpienia chlorku sodu chlorkiem potasu i zastosowaniem Fresh-Q; DF2 – brynza made with 30 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza powstała w wyniku zastąpienia chlorku sodu w 30 % chlorkiem potasu oraz przy użyciu Fresh-Q

Data on the digestibility of brynza proteins are shown in Table 8. It should be noted that the degree of digestibility of proteins by pepsin and trypsin and the dynamics of this process had their own characteristics for different samples of brynza. Based on the results of the research on the digestibility of brynza proteins, it can be stated that the proteins of the sample DF1 (82.48 %) and D1 (80.35 %) have the best digestibility; the difference with respect to the corresponding controls is 30.2 and 26.9 %. In addition, the curve of dynamics of protein digestibility of the samples D1 and DF1 is characterized by the highest indicators, which is confirmed by data on the general digestibility of brynza samples.

Table 8. Digestibility of brynza proteins (under in vitro conditions) (n = 5)
Tabela 8. Strawność białek bryndzy (w warunkach in vitro) (n = 5)

Samples of brynza / Próbki bryndzy	Mass fraction of tyrosine [g/ 100 g of protein] / Ułamek masowy tyrozyny [g/100 g białka]	Protein digestibility / Strawność białka [mg tyrosine/g protein] / [mg tyrozyny/g białka]			[%]
		Pepsin / Pepsyna	Trypsin / Trypsyna	Summarized / Streszczony	
K	4.45 ± 0.15	6.75 ± 0.12	20.71 ± 0.20	27.46 ± 0.22	61.71
D1	4.45 ± 0.19**	9.91 ± 0.12	25.84 ± 0.25*	35.75 ± 0.30*	80.35
D2	4.45 ± 0.15	8.12 ± 0.11	22.65 ± 0.19**	30.77 ± 0.34*	69.15
KF	4.45 ± 0.14	8.85 ± 0.11	20.07 ± 0.21	28.92 ± 0.20	64.99
DF1	4.45 ± 0.22*	11.24 ± 0.15**	25.46 ± 0.26*	36.70 ± 0.38*	82.48
DF2	4.45 ± 0.15**	10.34 ± 0.11	23.33 ± 0.17**	33.67 ± 0.29*	75.65

Explanation notes / Objaśnienia:
K – control sample using sodium chloride / próbka kontrolna z użyciem chlorku sodu; D1 – brynza made with 20 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 20 % zastąpienia chlorku sodu chlorkiem potasu; D2 – brynza made with 30 % replacement of sodium chloride with potassium chloride / bryndza wytwarzana z 30 % zastąpienia chlorku sodu chlorkiem potasu; KF – brynza made using sodium chloride and the Fresh-Q / bryndza wytwarzana z użyciem chlorku sodu i Fresh-Q; DF1 – brynza made with 20 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza wyprodukowana z 20 % zastąpienia chlorku sodu chlorkiem potasu i zastosowaniem Fresh-Q; DF2 – brynza made with 30 % replacement of sodium chloride with potassium chloride and using Fresh-Q / bryndza powstała w wyniku zastąpienia chlorku sodu w 30 % chlorkiem potasu oraz przy użyciu Fresh-Q
Note: statistically significant differences were taken into account compared to the control group: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$. Samples were compared in-lines /
Uwaga: uwzględniono różnice istotne statystycznie w porównaniu z grupą kontrolną: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$. Próbki porównano w wierszach.

Analyzing the investigations conducted, it is possible to distinguish the positive influence of 20 % replacement of sodium chloride with potassium chloride in combination with Fresh-Q in brynza on the digestibility of brynza proteins, because the degree of digestibility of proteins is the highest in these samples. The data is confirmed by the results of proteolysis, which are given above.

Conclusions

1. The common salt in the brine was partially replaced with potassium chloride in the technology of making brynza from sheep's milk, which did not have an influence on the organoleptic properties of the brynza.
2. A positive influence of 20 and 30 % replacement of common salt with potassium chloride and the use of Fresh-Q on the course of proteolytic processes was found, which is evidenced by an increase in casein fragments with a lower molecular weight, an increase in the area of absorption of acetonitrile by water-soluble peptides and an increase in the concentration of free amino groups and free amino acids. This is also accompanied by a corresponding increase in total soluble nitrogen and nitrogen in soluble protein compounds.
3. The degree of digestibility of brynza protein from sheep's milk is the highest with the use of 20 % replacement of sodium chloride with potassium chloride and the Fresh-Q (82.48 %).
4. Due to the partial replacement of table salt with potassium chloride, the salt content in cheese is reduced to a minimum value (4 %), which will allow to reduce the level of table salt consumption. This will have a positive effect on the functioning of the human cardiovascular and musculoskeletal system.

References

[1] Ayyash M. M., Shah N. P.: Proteolysis of low-moisture Mozzarella cheese as affected by substitution of NaCl with KCl. Journal of Dairy Science, 2011, 94, 3769-3777.

[2] Ayyash M. M., Shah N. P.: The effect of substituting NaCl with KCl on Nabulsi cheese: chemical composition, total viable count, and texture profile. Journal of Dairy Science, 2011, 94, 2741-2751.

[3] Ayyash M. M., Sherkat F., Shah N. P.: The effect of NaCl substitution with KCl on Akawi cheese: Chemical composition, proteolysis, angiotensin-converting enzyme-inhibitory activity, probiotic survival, texture profile, and sensory properties. Journal of Dairy Science, 2012, 95(9), 4747-4759.

[4] Burda L. R., Stapai P. V.: Physico-chemical parameters of sheep's milk of the Ukrainian Mountain Carpathian breed under different conditions of keeping. Scientific and technical bulletin, 2008, 9(4), 13-17.

[5] Dugat-Bony E., Sarthou A.-S., Perello M.-C., G. de Revel, Bonnarme P., Helinck S.: The effect of reduced sodium chloride content on the microbiological and biochemical properties of a soft surface-ripened cheese. Journal of Dairy Science, 2016, 99, 2502-2511.

[6] Farrell H. M., Jimenez-Flores R., Bleck G. T., Brown E. M., Butler J. E., Creamer L. K., Hicks C. L., Hollar C. M., Ng-Kwai-Hang K. F., Swaisgood H.E.: Nomenclature of the proteins of cow`s milk. Sixth revision. Journal of Dairy Science, 2004, 87(6), 1641-1674.

[7] Fox P. F., Uniacke-Lowe T., McSweeney P. L. N., O`Mahony J. A.: Dairy Chemistry and Biochemistry (Second Edition). New York: Springer, 2015, 585 p.

[8] Grummer J., Bobowski N., Karalus M., Vickers Z. and Schoenfuss T.: Use of potassium chloride and flavor enhancersin low sodium Cheddar cheese. Journal of Dairy Science, 2013, 96, 1401-1418.

[9] Holt C., Carver J. A., Ecroyd H., Thorn D.C.: Invited review: Cfseins and the casein micelle: their biological function, strectures, and behavior in foods. Journal of Dairy Science, 2013, 96(10), 6127-6146.

[10] Innocente N., Munari M., Biasutti M.: Characterization by solid-phase microextraction-gas chromatography of the volatile profile of protected designation of origin Montasio cheese during ripening. Journal of Dairy Science, 2013, 96(1), 26-32.

[11] Kamleh R., Olabi A., Toufeili I., Najm N. E. O., Younis T. and Ajib R.: The effect of substitution of sodium chloride with potassium chloride on thephysicochemical, microbiological, and sensory properties of Halloumi cheese. Journal of Dairy Science, 2012, 95, 1140-1151.

[12] Kushnir I., Tsisaryk, O., Kushnir, I., Semen, I., Slyvka, I., Musiy, L.: Properties of formed compositions of probiotic strains isolated from Carpathian bryndza. Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies named after S.Z. Gzhytskyj. Series: Agricultural Sciences, 2020, 22(93), 119-125.

[13] Kushnir I. I., Tsisaryk O. Y., Kushnir I. M., Skulska, I. V.: The technology of manufacturing cheese from cow's milk using a starter culture Enteroplan. Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies. Series: Agricultural sciences, 24(96), 9-15.

[14] Lu Y., McMahon D. J.: Effects of sodium chloride salting and substitution with potassium chloride on whey expulsion of Cheddar cheese. Journal of Dairy Science, 2015, 98, 78-88.

[15] Pastorino A., Hansen C., McMahon D.: Effect of Salt on Structure-Function Relationships of Cheese. Journal of Dairy Science, 2003, 86(1), 60-69.

[16] Rasmusson K.: Bioactive peptides in long-time ripened open texture semi-hard cheese. Swedish University of Agricultural Sciences, Department of Food Science, 2012, N.338.

[17] Saldo J. , McSweeney P. L. H. , Sendra E. et al. Proteolysis in caprine milk cheese treated by high pressure to accelerate cheese ripening. International Dairy Journal, 2002, 12(1), 35-44.

[18] Skulska I. V., Tsisaryk O. Y.: Changes in protein substances of brynza cheese under the influence of partial replacement of salt with potassium chloride. Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies. Series: Food Technologies, 2020, 22(93), 49-53.

[19] Skulska I. V., Tsisaryk O. Y.: Effect of partial replacement of sodium chloride on proteolysis in cheese production. Eastern-European Journal of Enterprise Technologies, 2014, 5/11(71), 12-17.

[20] Skulska I. V., Tsisaryk O. Y.: Changes in the protein substances of cheese under the influence of partial replacement of table salt with potassium chloride. Bulletin of Mogilev State University of Food, 2016, 1(20), 40-45.

[21] Skulska I. V., Tsisaryk O. I., Slyvka N. B.: Formation of qualitative indicators of the cheese made at partial replacement of sodium chloride. Tavriya National University Vernadsky. Technical sciences, 2020, 31(6), 95-100.

[22] Slyvka I. M., Tsisaryk O. Y., Dronyk G. V., Musiy L. Y.: Strains of lactic acid bacteria isolated from traditional Carpathian cheeses. Regulatory Mechanisms in Biosystems, 2018. 9(1), 62-68.

[23] Tsisaryk O. Y., Skulska I. V.: Improvement of technology brine cheese by reducing the sodium chloride. Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies named after S.Z. Gzhytskyj. Series: Food Technologies, 2013, 15(57), 126-131.
[24] Voigt D. D., Chevalier F., Donaghy J. A., Patterson M. F., Qian M. C. and Kelly A. L.: Effect of high-pressure treatment of milk for cheese manufacture on proteolysis, lipolysis, texture and functionality of Cheddar cheese during ripening. Innovative Food Sci. Emerg. Technol., 2012, 13, 23-30.
[25] Yukalo V., Datsyshyn K., Krupa O., Storozh L.: Adaptation of Stadier's apparatus for electrophoresis of main milk proteins. Eastern-European Journal of Enterprise Technologies, 2024, 1/11(127), 73-80.
[26] Yukalo V., Datsyshyn K., Storozh L.Comparison of products of whey proteins concentrate proteolysis, obtained by different proteolytic preparations. Eastern-European Journal of Enterprise Technologies, 2019, 5/11(101), 40-47.
[27] Yukalo V., Datsyshyn K., Storozh L. Electrophoretic system for express analysis of whey protein fractions. Eastern-European Journal of Enterprise Technologies, 2019, 2/11(98), 37-44.
[28] Yukalo V., Storozh L.: Isolation of κ-CN-1P and β-CN-5P fractions from native casein micelles. Ukrainian Biochemical Journal, 2018, 90(4), 74-79.
[29] https://www.chr-hansen.com/en/search?term=CHY-MAX

CHARAKTERYSTYKA PRZEBIEGU PROCESÓW PROTEOLITYCZNYCH W BRYNDZY O OBNIŻONEJ ZAWARTOŚCI SOLI KUCHENNEJ

S t r e s z c z e n i e

Wprowadzenie. Sery stanowią główny składnik codziennej diety zdecydowanej większości społeczeństwa jako produkt biologicznie kompletny i funkcjonalny. Dlatego kształtowanie jego konkurencyjnej produkcji jest jednym z najważniejszych zadań dotyczących rozwiązania bezpieczeństwa żywnościowego kraju i pomyślnego rozwoju sektora rolnego gospodarki. Na świecie obserwuje się systematyczny wzrost spożycia serów, co stymuluje wzrost wolumenu ich produkcji, co z kolei wymaga znacznej poprawy jakości serów, tworzenia nowych typów i innowacyjnych technologii oraz ich naukowe uzasadnienie. Sery solone to sery dojrzewające i przechowywane w solance - w beczkach lub pojemnikach, gdzie po uformowaniu są szczelnie pakowane i napełniane 16 ÷ 20 % roztworem soli kuchennej. Produkowane są według technologii serów miękkich, twardych i półtwardych. Mają ostry, słono-kwaśny smak, ciasto jest szorstkie i kruche. Sery solone nie mają skórki, ich kolor jest biały.

Wyniki i wnioski. Przygotowano 6 próbek bryndzy owczej z 20 i 30 % substytucją soli kuchennej chlorkiem potasu. Stwierdzono, że substytucja nie ma negatywnego wpływu na parametry organoleptyczne i fizykochemiczne sera. Stosowanie preparatu Fresh-Q równolegle z 20 i 30 % zamiennikiem chlorkiem potasu wpływa pozytywnie na przebieg procesów proteolitycznych, czego dowodem jest wzrost fragmentów kazeiny o niższej masie cząsteczkowej, zwiększenie powierzchni wchłaniania acetonitrylu przez peptydy rozpuszczalne w wodzie i wzrost stężenia wolnych grup aminowych i wolnych aminokwasów. Towarzyszy temu również odpowiedni wzrost całkowitego rozpuszczalnego azotu i azotu w rozpuszczalnych związkach białkowych.

Słowa kluczowe: sery marynowane, bryndza, chlorek sodu, chlorek potasu, proteoliza, aminokwasy, technologia sera