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RESEARCH OF QUALITY INDICATORS IN PROTEIN-BLUEBERRY CONCENTRATES

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ABSTRACT

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The effect of blueberry coagulant in the form of specially processed blueberry paste on the building-up process of proteinblueberry concentrates has been considered in the article and the change in their physical-chemical indicators during storage has been determined. A study of blueberry coagulant application in the amount of up to 12% provides the effective running to process of thermo acid coagulation of milk proteins with a maximum clot yield (excluding limiting factors – organoleptic indicators and active acidity). It has been found that with an increase in the amount of blueberry paste adding from 2% to 10%, the yield of protein-blueberry concentrates increases from 5% to 40%, and the moisture mass fraction in clots decreases, on the contrary, from 73.4% to 67.1%. Other quality indicators of protein-blueberry concentrates were recorded: active acidity (from 5.0 pH at the beginning to 4.7 pH at the end of the storage life), and the water-retaining capacity at the level of (75.44 \pm 0.5%). Based on chromatographic studies, the degree of polyphenolic compounds transition (including anthocyanins) to protein-blueberry concentrates at the level of 52.26% has been determined by the calculation method and analyzed compared with control sample (blueberry paste) and colored whey. Based on the researches, proteinblueberry concentrates obtained by thermo acid coagulation of milk proteins are suggested to be used as basis in the cheese products recipes.

Keywords: coagulant; coagulation; concentrate; milk; protein

INTRODUCTION

Full use of all milk protein components during its processing is a condition for increasing the efficiency of obtaining concentrates. There are different methods of milk proteins coagulation: acid, acid rennet, rennet, thermo acid and thermo calcium. The most common methods include acid and rennet coagulation - the basics in the production of fermented milk product (Bittante, Penasa and Cecchinato, 2012). According to the authors (Kalmykova, 2013; Dabija and Sion, 2012), the production of protein products by acid milk coagulation is a popular and common practice. Enzymatic coagulation lasts until 10 hours. There is a need for the use of capacitive equipment. The technology of producing products by thermo acid coagulation of milk proteins involves less time, production space and equipment, but has several disadvantages (Lyalin and Fedotov, 2009; Bittante, 2011). However, the degree of protein extraction during thermo acid coagulation is up to 95 - 97%, while for acid coagulation about 90%, and for rennet - 85% (Osintsev et al., 2013; Ostroumova et al., 2009).

There is a problem of losing valuable proteins with whey in the production of milk protein clots. The use of various technological methods for the conversion of whey proteins to concentrate is actual. This approach will not only increase the yield, but also increase the biological value of the clots (Abeykoon et al., 2016).

Complex precipitation of milk proteins can be achieved by thermo acid coagulation with various coagulants: acid whey (higher 150 °T) or food acids (hydrochloric, acetic, lactic, less often citric) (Chinprahast, Subhimaros and Pattorn, 2015). Without limiting the foregoing, another task for the dairy industry is to increase the dairy products production with a high content of biologically active substances – using berry raw materials.

One of the qualitative characteristics of raw berry is acidity. Blueberries are different of variety and high content of organic acids at the level of (1.25 - 2.3 %). Organic acids at (93 - 95 %) represented by citric, malic, and in smaller quantities succinic, salicylic, and phosphoric (Simakhina, 2018).

The biochemical composition of blueberries indicates not only high nutritional value, but also the pharmacological properties of this crop. It is expected that the therapeutic effect of blueberries is largely due to the content of phenolic compounds in it (Vikul and Khomich, 2011). Berries have radioprotective properties, contribute to the neutralization of radionuclides in the body (Van Breda, Briedé and de Kok, 2019).

For mitigate seasonal fluctuations in the use of berries in the dairy products production, including for coagulation, it is advisable to attract raw materials, which are stable during storage. Because berries are products that quickly deteriorate, there is a need in their preservation to regulate biochemical processes (Korotkiy, Korotkaya and Ibragimova, 2010).

A modern method of conservation is the processing of raw berry using cavitation devices and those that operate in the mode of developed turbulence (**Pakhomova**, **Dashkovsky and Stoyanova**, **2012**). Technologies of various homogenized berry pastes (blueberry, blackcurrant, lingonberry, etc.) of long-term storage with increased biological value have been deveped by using hydrodynamic cavitation processing of berry raw materials (**Bessarab et al., 2014**).

Sterile pastes are industrially produced from blueberries with stable indicators, which excludes the introduction of extraneous microflora and, as a result, the production of a product safe by microbiological parameters during the storage period. Specially processed blueberry paste has an active acidity at 3.0 pH and the following chemical composition: dry soluble substances – 11.0%, polyphenols 457 mg.100g⁻¹, sugars – 7.92%, fiber – 1.57%, pectin – 0.27%.

Known technologies where berry raw materials are used as a filler in the production of cheese products, as well as a coagulant for the acid coagulation of milk proteins Koltyugina and Kosynkina, (Shchetinin, 2011; Shchetinin et al., 2010). The development of milk-based products with berry raw materials is actual for using the functional and technological properties of berries and optimizing the composition of products in matter of vitamins and minerals. The use of berries as natural coagulants for thermo acid coagulation with the proteinberry concentrates production - the basis for cheese products, has not been enough investigated. The use of colored products as the basis for different dairy products will provide appropriate quality indicators with the exception of food colors and artificial flavorings, which corresponds to the concept of a healthy diet.

Scientific hypothesis

Scientific hypothesis is formulated, which is based on the assumption that it is possible to use blueberries as a coagulant, which will provide a clot yield, a natural color and the corresponding physicochemical parameters of the concentrates.

The aim of the work is to study the quality indicators of protein-blueberry concentrates obtained by thermo acid coagulation of milk proteins using blueberries in the form of a specially treated paste as a coagulant.

MATERIAL AND METHODOLOGY

The object of research is the quality indicators of proteinblueberry concentrates (PBC) obtained by thermo acid coagulation of milk proteins using blueberry paste as a coagulant.

Subjects of research – whole milk, blueberry paste (TU 10.8-2789021380-001 2012), yield, organoleptic, physicalchemical indicators (moisture mass fraction, active acidity, water-retaining capacity, polyphenol compounds content) of protein-blueberry concentrates.

The technology of protein-blueberry concentrates provides for thermo acid coagulation of milk proteins using berry raw materials as a coagulant according to the classical condition – coagulation temperature (93 - 95 °C)with duration of 5 min. To obtain a control sample (milkprotein concentrate), acid whey with titratable acidity of 160 °T in the amount of 8 - 10% of milk mass was used (Grek and Skorchenko, 2009). The complex effect on milk proteins of high temperatures and acid reagents leads to the most complete coagulation of them. The coagulation process was established visually by the intensive formation of protein flakes and whey excretion. The obtained protein-blueberry concentrates were formed and selfpressed duration of $(30 \pm 2 \text{ min})$. Pressing was carried out to a moisture mass fraction of 65 - 75%, depending on the further use of PBC for the manufacture of various cheese products.

In order to modify the method of thermo acid coagulation and rationalize the dose of blueberry coagulant (pH 2.6 \pm 0.2), a range from 2% to 12% with a variation step of 2% has determined. That particular amount in a different extent changed the active acidity in the mixture to ensure a balanced isoelectric state of milk proteins at a pH level (4.1 – 4.5) in the entire volume and led to their active coagulation (Grek, Onopriichuk and Pshenychna, 2017). The products of this technological operation are protein-blueberry concentrates and the colored whey with a touch of blueberry coagulant added.

The yield (mass) of protein-blueberry concentrates was calculated by the gravimetric method based on the mass obtained from 4 dm³ of milk and converted in percentage terms (Pytel et al., 2017).

The active acidity was determined potentiometrically on Sartorius PB-20 universal pH meter (**Pytel et al., 2016**).

The moisture mass fraction was investigated by the accelerated method on a KVARTS-21M-33 moisture meter and by the thermogravimetric method on an ADS series laboratory electronic moisture meter, manufactured by AXIS (Poland).

The water-retaining capacity (WRC) of proteinblueberry concentrates was determined by the Grau-Hamm method by A. A. Alekseev modification based on the determination of the water amount that is released from the product by light pressing, which is absorbed by filter paper (Grek et al., 2015).

The polyphenol composition of protein-blueberry concentrates was determined by high-performance liquid chromatography using the spectrophotometer of Prominence LC-20 Shimadzu system (Japan). The substances identification in the extracts was determined by comparing the retention time and spectral characteristics of the investigated substances with similar characteristics of the standards in accordance with the method of polyphenols identification (MIček et al., 2019). Chromatography was performed at a wave-length of 225, 255, 286 and 350 nm (Khodakov and Makarenko, 2010; Huang et al., 2012; Giovanelli and Buratti, 2009).

The titratable acidity of concentrates was determined according to **GOST 3624-92** and measured in degrees Turner (°T). Turner degrees mean the amount of alkali solution milliliters of 0.1 mol.dm⁻³ that spent on neutralization of 5 g concentrate (**Grek et al., 2015**).

Statistical analysis

The studies were repeated three times and processed mathematically using Microsoft Excel 2007 to provide accuracy of the obtained results.

RESULTS AND DISCUSSION

The obtained concentrates are a polydisperse colloidal system in which whey is the dispersion medium and milk proteins and dry substances of the blueberry coagulant are the dispersed phase. The yield and change in the moisture mass fraction of protein-blueberry concentrates depending on the amount of blueberry coagulant is shown in Figue 1.

The obtained yield results of the concentrates were corrected depending on the amount of dry substance of added blueberry coagulant. The research results (Figure 1) showed that under the same conditions of the thermo acid coagulation process with an increase in the amount of blueberry paste adding from 2% to 12%, the yield of PBC increases from 5% to 42%. The moisture mass fraction in PBC decreases, on the contrary, from 73.4% to 66.0%. Generally, the process is characterized by an increase in the transition degree of casein and the maximum amount of whey proteins into concentrates.

It has been established that with the addition of 12% blueberry coagulant, the yield of PBC increased by 2% compared with a sample containing 10% coagulant. The difference was in a margin of error, and the concentrate was characterized by too pronounced (berry) organoleptic indicators. Accordingly, for the further studies, the amount of blueberry coagulant has been selected at a level from 2% to 10%.

The next step of research was the study of changes in active acidity and water-retaining capacity of proteinblueberry concentrates for 72 hours at a temperature of $(4 \pm 2 \text{ °C})$. The results are presented in the diagrams in Figure 2 and Figure 3.

Research has shown, that the active acidity of proteinblueberry concentrates depends on the pH of the blueberry coagulant and the amount of it added. So, the CBF sample, which was obtained by thermo acid coagulation of milk proteins with a blueberry coagulant in the amount of 10% (PBC10), has the lowest active pH value of 5.0 pH at the beginning and 4.7 pH at the end of the storage life. For control sample and all other PBC samples, the decrease in active acidity took place at (0.15 - 0.23 pH). This makes it possible to assert that the use of different amounts of blueberry coagulant for the production of PBC almost does not affect on this indicator of concentrates during storage. Similar results were reported by several authors (**Perreault et al., 2017**).

The more amount of blueberry coagulant (10%) used in thermo acid coagulation of milk proteins, the lower value of the water-retaining capacity in model samples of protein-blueberry concentrates – 71.36 $\pm 0.5\%$. With a decrease in the amount of blueberry coagulant from 10% to 2%, the WRC of PBC increases to 8.14%. The average value of the water-retaining capacity of the obtained concentrates is (75.44 $\pm 0.5\%$). During storage, a sharp decrease of the water-retaining capacity in all PBC is observed, and in the end the WRC ranged from 43.82% to 56.75%, which is on average 14% higher than in the control sample. The dynamics of moisture evaporation from model samples on an Axis electronic moisture meter has determined to study the effect of blueberry coagulant on the qualitative and quantitative moisture state in concentrates. PBC sample obtained by thermo acid coagulation of milk proteins with a blueberry coagulant in amount of $6 \pm 1\%$ and a milk-protein concentrate obtained by classical technology were used for research **(Ondrušíková et al., 2019)**. The dynamics of moisture evaporation from concentrates is presented in Figure 4.

According to the measurement results, the main part of the moisture (free) has removed from the milk-protein concentrate (control) sample faster – in 18.0 ± 0.5 min, and from protein-blueberry concentrate – more slowly and the indicator was within 21 ± 0.5 min. There are differences in the speed of processes. During thermo acid coagulation, irreversible whey protein precipitation reactions occur with the loss of native properties, which is accompanied by the unfolding of the polypeptide chain of the protein molecule (Donato and Guyomarc'h, 2009). As a result of such chain transformations and destruction of the tertiary and secondary structures, hydrophobic groups are "released" on the surface of the protein molecule. In this case, whey protein loses its solubility, aggregates and precipitates. It is likely that the interaction of milk proteins with carbohydrates of blueberry coagulant leads to the formation of additional complexes, which differ by the presence of strong bonds between pectins, dietary fiber and plasma proteins (Chevalier et. al., 2019; Jakobek, 2015; Han et. al., 2011). So blueberries are carriers of vitamins, including vitamin C $(14.1 - 26.4 \text{ mg}.100\text{g}^{-1})$, pectin substances (0.32 - 0.45%), phenolic substances $(339 - 364 \text{ mg}.100\text{g}^{-1})$ major mineral – and trace elements and other substances indispensable for the normal functioning of the body, with the ability to improve the consumer properties of products (Toshev, Chaplinsky and Vakhitov, 2012; Li et. al., 2017). From a practical perspective, that is how you can justify using blueberry paste for thermo acid coagulation of milk proteins to bind free moisture and prevent syneresis.

PBC (presented in Figure 5) were characterized by the presence of the natural violet color inherent in blueberries, which contain anthocyanins, it was appropriate to study the degree of coloring substances transition to concentrates (Mendelová et al., 2013). Coloring substances of raw berry are low molecular phenolic compounds, relate to bioflavonoids, in particular anthocyanins, which in plants are in the form of glycosides (Medvecký et al., 2015). It is known that the complex of blueberry phenolic compounds is represented by chlorogenic acid, kempferol and quercetin glycosides; free, condensed catechins and proanthocyanidins (Aly, Maraei and El-Leel, 2019). The blueberry anthocyanin complex is determined by the set of main components: 3-glycosides and 3-rutinoside of dolphinidin and cyanidine, which is unchanged for berries of all varieties with black color (Goldina, Safronova and Gaidul, 2015). In addition, the berries contain flavones, flavonols, catechins, hydroxycinnamic acids, which determine the natural violet color of the product (Le Pham, 2020). The use of colored PBC as a basis for cheese products will ensure the exclusion of food grade dyes and artificial flavorings (Slozhenkina et al., 2019).

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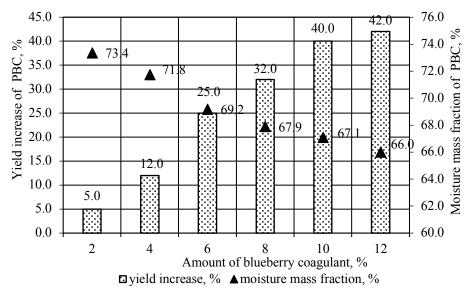


Figure 1 Yield and change in the moisture mass fraction of protein-blueberry concentrates on the amount of blueberry coagulant.

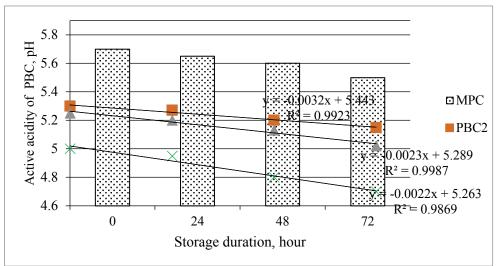


Figure 2 Change in active acidity of protein-blueberry concentrates.

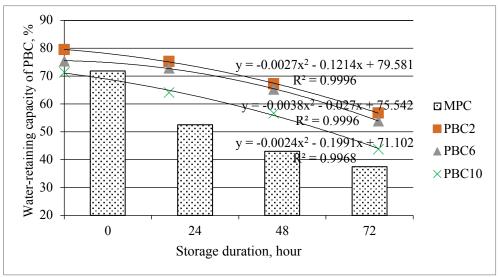


Figure 3 Change in the water-retaining capacity of protein-blueberry concentrates.

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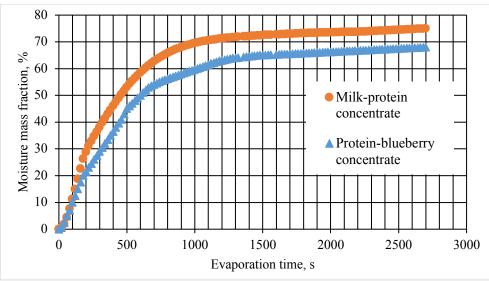


Figure 4 Dynamics of moisture evaporation from concentrates: milk-protein (control sample) and protein-blueberry.

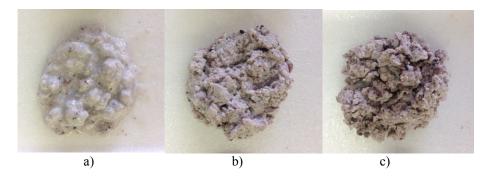


Figure 5 Protein-blueberry concentrates obtained by thermo-acid coagulation of milk proteins by berry coagulant. Note: The amount of milk proteins by berry coagulant, %: a) 2; b) 6; c) 10.

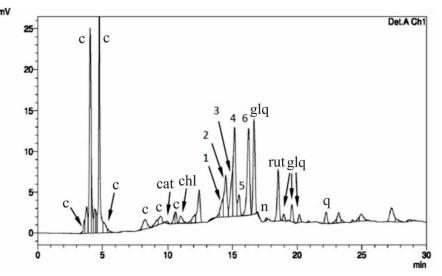


Figure 6 Chromatogram of protein-blueberry concentrates at 255 nm. Note: c - catechins, cat - catechin, chl - chlorogenic acid, n - naringin, rut - rutin, glq - quercetin glycosides, q - quercetin, 1 - delphinidin-galactoside, 2 - delphinidin-glucoside, 3 - cyanidin-galactoside, 4 - delphinide-arabinoside, 5 - cyanidin-glucoside, 6 - petunidine-galactoside + cyanidine-arabinoside + petunidine-glucoside + peonidin-galactoside.

The polyphenolic composition of blueberry paste (control sample), protein-blueberry concentrates and colored whey have been analyzed to determine the degree of coloring compounds transition. The chromatogram of protein-blueberry concentrates is presented in Figure 6.

Research results were analyzed in comparison with the control sample and the degree of their transition to separation products has been determined. The content of polyphenolic substances in protein-blueberry concentrates and colored whey is 281.10 and 146.19 mg.100g⁻¹, respectively. For comparison, the content of polyphenols in blackcurrant paste was on average 457 mg.100g⁻¹. The degree of polyphenolic compounds transition to PBC is 61.51% of their total number. About 31.99% of polyphenolic compounds, including anthocyanins, stay in colored whey. This effect is due to the weight loss correlation of the concentrate during technological operations, such as pressing and forming (Cipolat-Gotet, et al., 2018).

Research results are fully sufficient to develop the technology of protein-berry concentrates obtained by thermo acid coagulation of milk proteins with a blueberry coagulant and subsequent use in the recipes of cheese products.

CONCLUSION

The quality indicators of protein-blueberry concentrates depending on the amount of blueberry paste added during the process of thermo-acid deposition of milk proteins have been studied. It has been found that with an increase in the amount of blueberry paste adding from 2% to 10%, the yield of protein-blueberry concentrates increases from 5% to 40%, and the moisture mass fraction in clots decreases, on the contrary, from 73.4% to 67.1%. Changes occur due to the complex coagulation and transition of milk proteins into a clot, as well as the hydrocarbon components of blueberry paste. This leads to the formation of additional complexes with tightly bound free moisture. The active acidity of protein-blueberry concentrates was in the range of 5.0 - 5.25 pH at the beginning of the storage life and decreased by (0.15 - 0.23 pH) for 72 hours. The average value of the water-retaining capacity of the obtained concentrates is $(75.44 \pm 0.5\%)$.

The degree of polyphenolic compounds transition (including anthocyanins) from berry raw materials in PBC at the level of 61.51% was determined by high performance liquid chromatography. About 31.99% of polyphenolic compounds, including anthocyanins, stay in colored whey of their total amount in blueberry paste.

Protein-blueberry concentrates can be used in the recipes of cheese products with a regulated moisture mass fraction, active acidity, water-retaining capacity and natural color.

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