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THE RATIONALIZATION OF THE PARAMETERS OF MILK PROTEINS' THERMO ACID COAGULATION BY BERRY COAGULANTS

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Abstract: *This paper presents the results related to the influence of berry coagulant amount, its proactive acidity and duration of thermo acid coagulation on the process of milk proteins' sedimentation. In the present work, the regression equations and response surface analysis were used to design and optimize an industrial bioprocess. Increase in the berry coagulant amount to 11 % and reduction of active acidity to 2.4 units were determined. pH up to 3 minutes is characterized by the highest processes of destabilization. Moreover, it improves the organoleptic properties and has the biggest impact on the yield of protein-berry clot (to 25 %) and active acidity.*

Keywords: thermo acid coagulation, milk proteins, berry coagulant, protein-berry clots, rationalization of parameters

1. Introduction

Insufficient amount of dairy raw materials and seasonality of milk manufacturing affect the volumes of dairy-protein products manufacturing (cottage cheese and dairy products on its basis, hard and soft cheeses, etc.) during the year [1]. The acidic, rennet, acid-rennet, thermo calcium, thermo acid coagulation and ultrafiltrational concentration of milk are used in the manufacturing technology of these products. The chemical nature of milk proteins' coagulation is complicated and not studied in detail up to now. According to some researchers, fundamental changes of protein macrostructure are occurring at denaturational transformations. These changes are related to the weakening of the interaction forces between the protein chains of amino acid residues [2-3]. The first stage of denaturation consists in change of the original type of polypeptide chains. This change is caused by different methods, but it has a common pattern – the gap of the minimal amount of intramolecular ties for globule deploying. All typical manifestations of denaturation are explained by the release of single radicals, which in the native state of the protein are closely grouped among them. Secondary effects after denaturation - association of deployed globules and their chemical change lead to the allotment of proteins. Denatured whey proteins, in the form of aggregates with irregular shape, surround changed casein particles and probably partially connect with it. Together with the temperature increase, the degree of whey proteins' denaturation increases and a complex of denatured whey proteins with casein amplify. The influence of high temperature cannot lead to coagulation at the pH of whole milk. At the acidification whey proteins coagulate as casein [4-5].

From all existing ways of proteins allotment only the thermo calcium, thermo acid coagulation and ultrafiltrational concentration of milk provide comprehensive selection of proteins that allows receiving dairy-protein product with increased biological value. The expansion of protein products manufacturing made by intensive technologies is actual. In particular thermo acid method of milk processing, which is based on the simultaneous coagulation of milk casein and whey proteins under the influence of acid and high temperature deserves attention. The degree of proteins application in this method is up to 95...97 %, while about 90 % at acid coagulation, and about 85 % at rennet [6-8]. Food acids (hydrochloric, acetic, lactic, rarer citric) or acidic whey are used as coagulants [9]. The innovative developments where berry raw materials are used as a coagulant of milk protein are known. Berries are a source of vitamins, minerals and food fibers [10-11].

Berry raw materials coagulants have been used more in dairy technology, especially in cheese-making. Modern principles of high quality products manufacturing are based on selection and justification of appropriate raw materials kinds in proportions which would ensure expected quality, consumer and functional properties and maximal food components balance in chemical composition with increased nutritional and biological value.

The aim of research is to study the rationalization of the parameters of milk proteins' thermo acid coagulation with the help of mathematical modeling methods. Beside this, to study the effect of these parameters on the yield and quality indicators of protein-berry clot (PBC).

The object of research is thermo acid coagulation of milk proteins by berry coagulant.

Subject of research - skimmed milk, blackcurrant puree produced by LiQberry (*Ukraine Technical Conditions 15.3-24110704-003:2011*), the parameters of thermo acid coagulation process, quality indicators of protein-berry clots.

Moreover, selecting an optimum amount of berry coagulant with different values of active acidity for thermo acid coagulation processing and to achieve maximum efficiency protein-berry clot has been evaluated.

2. Matherials and methods

To achieve the goal the full factorial experiment (FFE) was used. Skimmed milk with solid mass fraction – 11.2 %, protein mass fraction – 3.7 % active acidity – 6.7 units was used. pH was chosen as a basis for the thermo acid coagulation. Blackcurrant puree with active acidity 2.8...2.4 units was used. pH was used as coagulant. The amount of puree is 3...11 % of milk weight. Homogenized blackcurrant puree was produced in industrial conditions by advanced technology, using hydrodynamic (cavitation) raw materials, processed by devices of TEK-SM. The berries were treated in the said above setup according to the designed regime until a necessary level of homogenization and industrial sterility was achieved [12-13].

The determination of physico-chemical indicators of skimmed milk, protein-berry clot and whey was made. Moisture mass fraction was determined by drying the sample to the constant weight. The active acidity of all samples was measured by potentiometric pH meter PB-20 Sartorius; the protein mass fraction of skimmed milk by formalin titration method, which is based on previous amino groups of the protein neutralization by formalin. Formed in this conditions metilenamino acid is titrated by 0.1 N of sodium hydroxide solution. Water-retaining capacity PBC was determined by the Grau-Hamm method in modification of A. A. Alekseev, which is based on the determination of the water amount (mass that is allocated from the product at its mild pressing) and is absorbed by filter paper.

At the department of milk and dairy products technology of the National University of Food Technologies the technology of protein-berry clots (PBC) obtaining, with a pH of clots 5.15...5.25 was developed. This technology provides berry coagulant introduction (pH 2.6±0.2) . to the milk with the temperature (75±1) °C for the process of thermo acid coagulation implementation. The qualitative indicators of the ready product depend on the realization of conditions of the above mentioned process. A parameter scheme of the process of milk proteins thermo acid coagulation by berry coagulants (Fig. 1) was prepared; input (controlling) and outbound (operated) factors were defined; the rationalization criteria were chosen and substantiate.

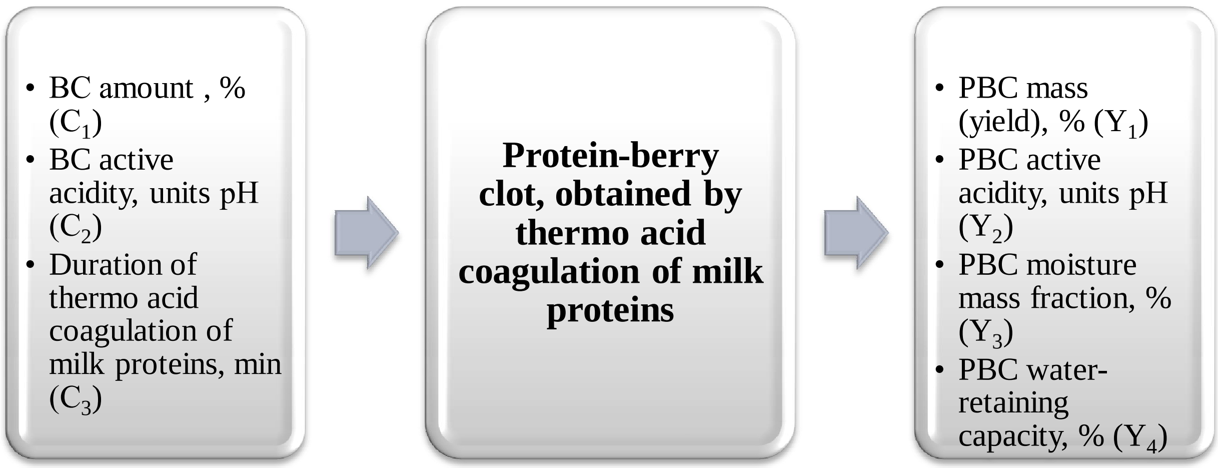

Fig. 1. Parameter scheme of the process of milk proteins thermo acid coagulation by berry coagulants

For parameters of rationalization, zero level of factors was defined. It is based on prior information and the interval of factors variation in such a way that it was outside of measurement error but not too wide.

The Box-Uilson on cube method of the mathematical modeling was used in the rationalization of the thermo acid coagulation process. Determination of the functional dependencies was made by the least square method. This method is based on the determination of the regression equation coefficients which provides a minimum sum of squared deviations of experimental data (Ye) from the values calculated by regression equation [14-16].

For the role of controlling factors which have a significant effect on PBC quality indicators were chosen: C_1 – amount of berry coagulant, %, C_2 – active acidity of berry coagulant, units. pH, C_3 – duration of thermo acid coagulation of milk proteins, min. Outbound (operated) parameters that mostly significantly characterize the thermo acid coagulation and have influence on the formation of protein berry clot quality indicators are: Y_1 – mass (yield), %, Y_2 – active acidity units. pH, Y_3 – moisture mass fraction, %, Y4 – water-retaining capacity, %.

The amount of berry coagulant varied in the range from 3 to 11 % with discrete 4 %; active acidity of berry coagulant – from 2.4 to 2.8 units. pH; step of varying - 0.2 units; duration of thermo acid coagulation – from 1 min to 3 min with the 1 min interval. The coefficients of regression equation were calculated, whose significance analysis was defined by the Fisher's criterion (Fr). The essence of criterion consists in decomposition of total variance of statistical complex on constituent elements. Their next evaluation gives the opportunity to determine the proportion of effective sign change from the effect of factor signs, in other words to narrow the area of research. That proves the adequacy of resulting equations. Experimental data were processed by the method of mathematical statistics - STATISTIKA (StatSoft) [17]. For determination of functional dependence which recreates the most accurately the change of indicators, the coefficient of approximation reliability (R^2) of each function was found. The accuracy of the results obtained is provided by three or five time's repeatability of experiments.

3. Results and discussion

Processing of results allows getting the analytical dependencies in the form of regression equations to calculate the effect of the active acidity, amount of berry coagulant and duration of milk proteins' thermo acid coagulation on yield (1) and quality indicators of protein-berry clots (PBC) – active acidity (2), moisture mass fraction (3), and water–retaining capacity (4):

$$Y_{1\,(m)} = 193,500 + 6,250 \cdot X_1 + 3,500 \cdot X_1^2 + 27,000 \cdot X_2 + 3,500 \cdot X_2^2 - 13,600 \cdot X_3 - 3,500 \cdot X_3^2 + 7,750 \cdot X_1 \cdot X_2 + 13,500 \cdot X_1 \cdot X_3 + 12,250 \cdot X_2 \cdot X_3 \quad (1)$$

$B_{кр\ i} = 0,530$; $B_{кр\ ii} = 1,068$; $F_r = 0,187$

$$Y_{2\,(pH)} = 5,229 - 0,309 \cdot X_1 - 0,0492 \cdot X_2 - 0,066 \cdot X_1 \cdot X_2 \quad (2)$$

$B_{кр\ i} = 0,149$; $B_{кр\ ij} = 0,054$; $B_{кр\ ii} = 0,098$; $F_r = 1,105$

$$Y_{3\,(W)} = 70,254 - 0,647 \cdot X_1 - 0,379 \cdot X_1^2 - 0,863 \cdot X_2 - 0,379 \cdot X_2^2 - 0,401 \cdot X_3 + 0,378 \cdot X_3^2 + 0,825 \cdot X_1 \cdot X_2 + 0,557 \cdot X_1 \cdot X_3 + 0,096 \cdot X_2 \cdot X_3 \quad (3)$$

$B_{кр\ i} = 0,1$; $B_{кр\ ii} = 0,1$; $F_r = 0,031$

$$Y_{4\,(ВУЗ)} = 43,383 + 2,552 \cdot X_1 + 3,182 \cdot X_1^2 - 7,349 \cdot X_2 + 3,187 \cdot X_2^2 + 5,278 \cdot X_3 - 3,187 \cdot X_3^2 + 1,964 \cdot X_1 \cdot X_2 - 8,414 \cdot X_1 \cdot X_3 - 2,504 \cdot X_2 \cdot X_3 \quad (4)$$

$B_{кр\ i} = 0,095$; $B_{кр\ ii} = 0,191$; $F_r = 0,001$

The graphical interpretation (of the response surface) of the obtained data is shown in the form of ternary charts (Fig. 2), which are commonly used in experimental research of the response dependencies from the relative content of three options (in this case, amount of BC, % (C_1) active acidity BC, units. pH (C_2), duration of milk proteins' thermo acid coagulation, min (C_3)). The correlation of these parameters is changed in order to determine rational values of initial parameters (yield (Y_1) and quality indicators of protein-berry clots ($Y_{2\text{-}4}$)).

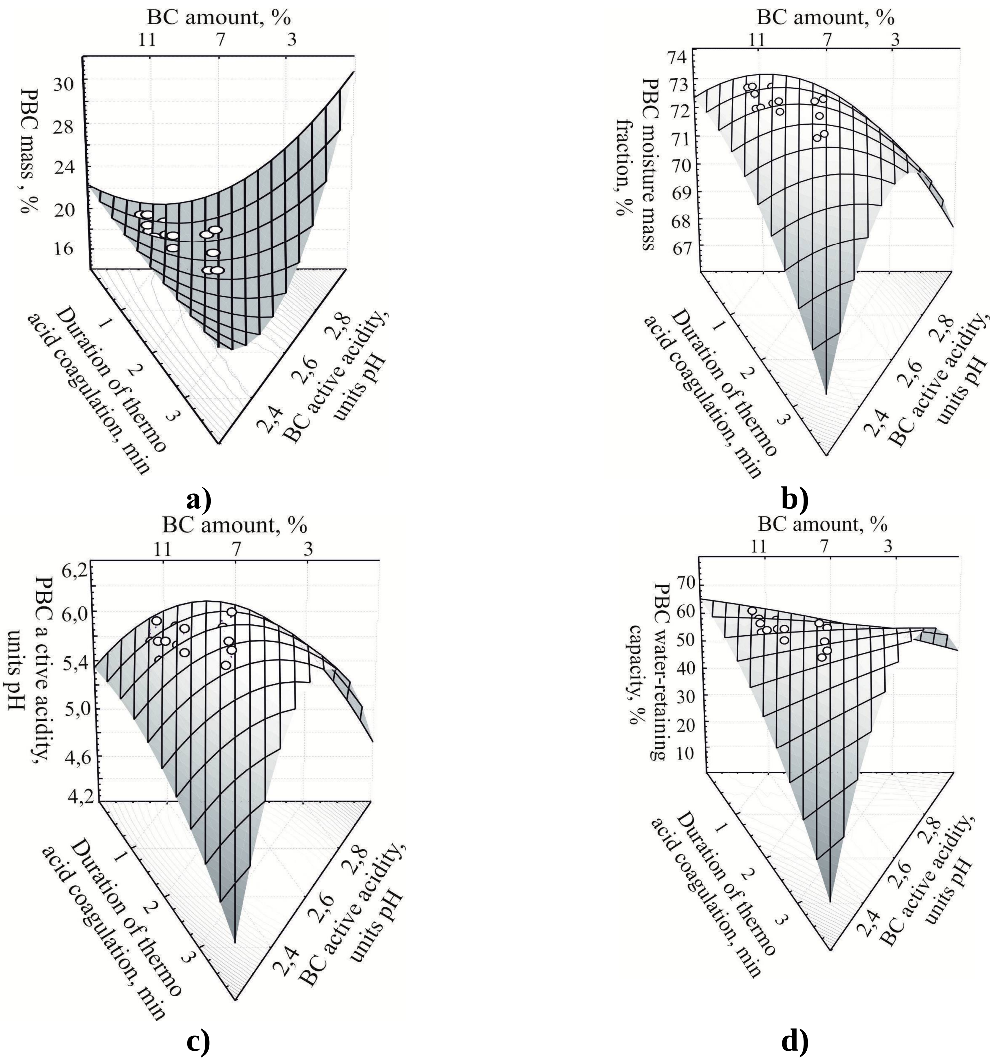

Fig. 2. The response surfaces of protein–berry clot mass change (a), of moisture mass fraction (b), of active acidity (c), of water-retaining capacity (d) of protein–berry clot depending on the parameters of milk proteins' thermo acid coagulation

The conducted research has shown that at the thermo acid coagulation the increasing of berry coagulant (BC) amount decreases its pH level and at the increasing of process duration, the PBC yield growth (Fig. 2a) and the moisture mass fraction decrease (Fig. 2b). These changes are characterized by the transition of casein and whey fraction into the clot. At the heating of skimmed milk, whey proteins denature in the presence of acid, deploying their polypeptide chains. The complexes of whey protein and casein, obtained at denaturation, capture dry matters (pectin matters) of berry coagulant – they are the main components that make up the structure of the protein–berry clot which was obtained by thermo acid coagulation. The coefficient of complexes transition was about 25 % (duration of exposure - 3 min and adding 11 % of berry coagulant with pH 2.4). Such parameters are 8.2–10.8 % higher than those at the adding to skimmed milk during the berry coagulant processing in amount of 3 % and with the active acidity of 2.8...2.6.

As a result of PBC active acidity analysis, the general tendency that PBC pH decreased linearly at the increasing of berry coagulant amount and decrease of its pH level was set (Fig. 2c). It was determined, that by adding 3 % of BC with pH 2.8 and 1 min duration of coagulation, the process provided the highest value of PBC active acidity at the level 6.02. Perhaps it is connected with a shift of the isoelectric point of the «casein micelles – whey proteins» complex. Coagulation begins at higher values of pH – (4.7...4.6). After coagulation of denatured whey proteins, isoelectric point of micelles is changed to the level of pH – 5.2, while coagulation of milk proteins at a temperature of 75 °C, amount of berry coagulant 11 % being introduced, thus providing the acidity of coagulation environment (skimmed milk + BC) at the level of about 6 units of pH.

The PBC samples which were obtained by coagulation with 3 % berry coagulant with pH 2.8 and exposure time 3 min (Fig. 2d) were characterized by the maximum value of water–retaining capacity. This is due to the fact that the value of the ionic adsorption of proteins varies with the change of medium reaction. In the isoelectric point, when the protein's degree of molecules dissociation is minimal and the charge of the protein molecule is close to zero then the ability of the protein to bind water is the smallest.

With displacement of pH medium in one side or another from the isoelectric point, the dissociation of basic or acid groups of protein is enhanced, the charge of protein molecules and protein hydration increases. The interaction between the κ-casein and whey proteins (β-laktoglobulin) by using remnants H-groups and other hydrophobic relations occurs.

Conducted researches have shown that the amount and pH of berry coagulant have influence on the yield of protein–berry clot. The increasing of hydrogen ions concentration shifts the equilibrium between dissociated carboxyl and phosphate groups at the surface of casein micelles and hydrogen ions to the side of oversaturated carboxyl and phosphate groups. Tht is why the decrease in the protein particles negative charge occurs. Exception makes the negatively charged groups of amino groups (–NH_3) which are situated on the surface of the protein particles. At a certain concentration of hydrogen ions (pH 4.6...4.7) the alignment of negative and positive charges occurs, in other words the isoelectric state comes (forces of electrostatic repulsion between the particles of protein become weaker and the forces of intermolecular interaction begin to dominate) in which the conformational changes of macromolecules protein happens. They lose solubility and connect among themselves forming a meshy three-dimensional structure of clot or come in the form of flakes. Under the action of berry coagulant acids the structure of caseinate calcium phosphate complex is disturbed: phosphate and organic calcium in the form of lactate calcium split off from the complex and go into plasma. Calcium citrate goes in a more soluble calcium lactate as well. The smallest yield – 14.2 % was observed at the adding of 3 % of BC with the active acidity 2.8 and 1 min endurance duration. Thus, the process occurs due to the initial denaturation of serum proteins which installs itself at the surface of casein micelles, increases their hydrophilic, thus reducing the degree of coagulation. The highest values of active acidity – 4.35 were obtained at the adding of 11 % of BC with pH 2.4. The smallest impact on the PBC proteins water–retaining capacity (23.51 %) was noticed under the following conditions of thermo acid coagulation: 3 % of BC with pH 2.8 and 1 min endurance duration.

4. Conclusions

1. This study presents the findings of an experimental investigation into the effect

of quality indicators of protein–berry clots in obtaining optimal operating conditions.

2. According to the above mentioned findings, the thermo acid coagulation during 1 min by berry coagulant which is added in amount of 3 % (pH 2.8) is characterized by minimal destabilization processes, which results in whey proteins not settling-down on the casein micelles and transfer in serum. While the increasing of the amount to 11 % decreases the active acidity to 2.4 units. pH and endurance duration to 3 min increase the degree of casein and whey proteins transition into protein-berry clot.

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