

Determination of moisture connection forms of protein-herbal clots

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Abstract

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Introduction. The interest is research by the moisture bonding forms in the modified milk-protein concentrates, especially, in protein-herbal clots (PHC) which are obtained by thermo acid coagulation of milk proteins.

Materials and methods. Protein-herbal clot (control) and protein-cereal mixture on its basis with rice extrudate.

Determination of moisture content was carried out by thermogravimetric method. Research of the moisture content of various forms of bonding in protein-herbal clots and mixtures based on rice extrudate – protein-cereal mixture was carried out on a derivative of the Paulik-Erdey Q-1000 system in the temperature range of 20–250 °C at a heating rate of samples of 1000 mg – 2.5 °C per minute.

Results and discussion. The removal of the main part of the moisture (free) from the specimen PHC without rice extrudate occurs faster – for 6.0–6.5 min, and with the addition of rice extrudate – slower and this figure is within 7.5–8.0 min

Extrudate's hydrophilic substances interact with PHC moisture due to the formation of H-bound polyasociates with the participation of water molecules and H-bound functional groups of hydrophilic substances. Hydrophobic groups are aggregated at the expense of dispersion forces.

The interaction of the rice extrudate with the milk proteins, after coagulation, leads to the formation of conglomerates that do not differ in the strength of the bonds, but are quite sufficient to bind the free moisture contained in the PHC in the form of whey.

For examples of protein-herbal clots, protein-cereal mix and the mixtures based on its and rice extrudate are characterized by the presence of four critical temperatures (T_I 49–58 °C, T_{II} 91–98 °C, T_{III} 131 °C, T_{IV} 179–186 °C), which removes moisture of different types, differing in the strength of the bond. As a result the temperature rises from T_I 49–58 °C to T_{II} 91–98 °C. All free (mechanically bound) moisture, which appears as a result compaction of protein-grain mixture's structure, is removed and is in layers between the protein-herbal base and rice extrudate.

Conclusions. The results are confirmed the expediency of adding rice extrudate to the protein-herbal clots for the free moisture binding which will provide steel quality indicators in protein-herbal clots.

Introduction

The quality of dairy raw materials, the conditions and the storage of finished products, the prescription composition features and technological operations are factors affecting on production volumes and product-line expansion.

The priority direction of research in the milk processing industry is extension of the storage at low temperatures of milk-protein concentrates (MPC) obtained by different methods with a limited storage. At a temperature from 4 to 8 °C in MPC slowly continues to evolve the extraneous microflora resistant to acidic environment. There is a process of syneresis conditioned by low water-retaining capacity of milk protein [1]. The most common ways of extending storage life are: the use of stabilizers and preservatives; thermisation of fermented products; creation of aseptic conditions for production; freezing, drying; storage in an atmosphere of gases, etc. [2, 3]. *Among them, a special place is taken by the methods of refrigerated processing: cooling and freezing. In the case for prolonged storage of dairy products the advantage is given to the latter one.* [4, 5].

During the storage water in foods plays an important role in maintaining texture, structure and storage stability. The total moisture indicates the quantity and characterizes its relation to chemical, biochemical and microbiological changes in milk-protein products. The ratio of free and bound moisture plays an important role in ensuring stability of MPC during the storage. Chemically bound water in the form of hydroxyl ions or crystalline hydrates is the strongest and can be removed from the MPC only due to chemical interaction or high temperatures. Physical-chemically bound water is divided into adsorption-bound and osmotically absorbed. Adsorption-bound water is contained by a force field on the external and internal micelles surface of casein-calcium phosphate complex (CCPC). Due the large internal surface, milk proteins have free superficial energy thanks to which adsorption binding of water happens. Adsorption heat is allocated at adsorption binding of the first monomolecular layer of water with proteins. Moreover, there is volume compression; herewith volume of hydrated protein appears to be less than the volumes sum of the protein itself and the absorbed water.

Mainly, osmotically absorbed water binds with milk proteins and keeps them firmly. When gel is formed, part of the water is absorbed inside the CCPC and is contained as in a semi-permeable capsule. The other part of the osmotically absorbed water penetrates inside the gel of CCPC through the cell walls from the environment due to osmosis. Concentration of dissolved fraction of substances is greater inside the CCPC gel than in the external.

The physically and mechanically bound water is contained in indefinite ratios and is usually freely secreted from MPC by drying or pressing. Physically and mechanically bound water can be divided into bounded by macro- and micro-capillaries with an average radius greater than 10^5 cm and less than 10^5 cm accordingly. Capillary moisture can be considered as free, because it moves in the capillaries of MPC in the form of milk whey.

The analysis of existing technologies of milk – protein concentrates has shown that the properties' stabilization is achieved through the use of technological ingredients most often. The use of stabilizers, thickeners, emulsifiers, etc. ensures the formation of necessary structure and stability in the technological flow. The lack of scientifically grounded storage recommendations of modified MPC dictates the necessity conducting additional researches. The introduction of cereal products processed in different ways, including extruding, for the bonding of free moisture in dairy products is actual. Cereal extrudates contain in large quantities easily digestible polysaccharides, proteins, food fibers (cellulose, hemicellulose, lignin, fiber), vitamins, minerals and others.

The interest is research by the moisture bonding forms in the modified MPC, especially, in protein-herbal clots (PHC) which are obtained by thermo acid coagulation of milk proteins. As coagulant is used sorrel (*Rumex*) which belongs to the genus of one-, two- and perennial herbaceous plants of buckwheat family, the order of polygonales [6, 7].

In the received protein-herbal clot is a small amount of compounds with pronounced colloidal properties – nondenaturing milk proteins which are capable of absorbing large quantities of water during swelling. Adding extruded cereal products which include high molecular weight carbohydrates – starch, pectin and other substances will contribute the bonding of free water. Swelling speed and maximum absorption of water depend on the nature of colloids, their individual hydrophilicity, concentration, the presence of various substances. As you know, extruding of cereals provides a change in the properties of starch macromolecules – decreasing of the crystalline phase by 52–62%, destruction of starch polysaccharides and the formation of dextrin the number of which increases in 7–18 times. [8]. Changes of starch complex in the extrusion process are accompanied by gelatinization with the formation of high concentration starch pastes; thermal and mechanical destruction of polysaccharide chains, consequence of which is increase of water-soluble substances.

During the hydrothermal processing of grain raw materials the amount of water-soluble proteins is decreases by 20–30%, but salt-, meadow-, and alcohol-soluble – increases. Decrease solubility can be explained by non-covalent interactions between polypeptide chains and other constituents, the formation of new amide and disulphide bonds due to exchange reactions and the additional formation of cystine from cysteine.

Moisture-thermal processing and mechanical influence cause a partial structural deployment of the protein with the break of some weak ties. The thermal motion of peptide chains causes the discontinuity of hydrogen bonds between the chains, and the connections between the hydrophobic groups begin to "melt". Simultaneously with the structural deployment of proteins, their aggregation is also taking place.

The amount of proteins' low-molecular fraction decreases and the amount of high-molecular fraction increases as a result of extrusion processing. This is explained by the fact that as a result of denaturation is formation of disulfide bonds from sulfhydryl groups. The intermolecular interaction of reactive protein groups contributes to the emergence of a significant amount of covalent, hydrogen and other forms of communication electrostatic origin and leads to the formation of sufficiently stable high-molecular protein substances.

Obviously, the changes caused by these processes will find a reflection in the formation of physico-chemical indicators of protein-herbal clots (PHC) with the adding extrudate.

The reaction course and the integral group characteristics of the obtained protein-herbal clots should be explored by the method of differential thermal analysis and thermogravimetric method. The study of the swelling mechanism, the bonds' identification that arise in the interaction of carbohydrate extrudates with PHC moisture were conducted by using the above method.

The aim of research is to determine the moisture bonding forms in protein-herbal clots and mixtures based on them with rice extrudate.

Materials and methods

Materials

Objects of research – protein-herbal clot (control) and protein-cereal mixture on its basis with rice extrudate (RE), with the following chemical composition (Figure 1) [9].

Total protein content 7.0%, including, %	Total of the main components in rice extrudate, %
Albumin – 7.8	Carbohydrates – 63.1
Globulin – 8.4	Starch – 73.7
Prolamin – 5.7	Cellulose – 9.0
Glutelin – 54.8	Water – 14.0
Insoluble residue – 20.8	Ash – 4.6

Fig. 1. Composition of rice extrudate

Method of obtaining protein-herbal clot

For thermo acid coagulation, juice with dry solids weight ratio 3,8% is obtained from the leaves of Rumex and introduce in the prepared milk under appropriate regimes. Aerial part is sorted, inspected from contaminations and mechanical impurities, washed, dried and crushed to a homogeneous state for 2–3 minutes on the DEX DHB-572 device with a power of 750 W. Rumex juice is introduced into the milk heated to a temperature of 93–95 °C in the amount of 7–8%, moderately mix up and kept for 3–5 min before clot formation. The complex effect of high temperatures and acid coagulant on milk proteins leads to fullest coagulation. Coagulation process is fixed visually for intense formation of a strong protein clot and whey detachment.

The research is directed at determining the moisture bonding forms in the protein-cereal mixture (PCM) on basis of protein-herbal clot obtained for the modes by thermo acid coagulation of milk proteins with Rumex juice. The obtained PHC has the following quality indicators: moisture mass fraction at the level (64±2)%, titrated acidity (80±1) °T, color – light pistachio, non-uniform, consistency – soft, mastic, to a degree dense, taste – milk-protein, cheesy, without foreign smells, with a slight herbal flavor. Introduction interest of rice extrudate in the PHC is 3%.

Thermogravimetric method

Determination of moisture content in protein-herbal clots (PHC) and mixtures based on rice extrudate – protein- cereal mixture (PCM) was carried out by thermogravimetric method on the laboratory electronic moisture meters ADGS 50 (production of the company "Axis"). This method is to determine the mass of the test sample before and after it is dried by heating to a temperature not higher than 160 °C. Moisture scales are general purpose laboratory weights of grade 3 of precision with a built-in drying device and have the following characteristics: the largest / the smallest weighing limit is 50 / 0.02 g; discreteness of reading of mass values – 0,5 mg; limit of permissible error of determination of mass – 0,5 mg; RMS deviation of no more than 0.16 mg; the limit of the permissible error of determination of moisture content – 0,3%.

The moisture meter is equipped with an RS-232C interface, which allows information on the results of weighing and determining the moisture content to be printed on the printer or on a computer.

The evaporation of the moisture from the sample during heating leads to a decrease in its mass, which allows, based on the mass measurement data, to calculate the content in the sample of moisture which was before the process of drying the sample.

Determination of moisture content in the same specimen can be realized with the same accuracy at significantly different values of the drying temperature of the sample (the difference will be solely in the time of the procedure).

Moisture scales consist of laboratory weights and a device for drying the samples (hereinafter – a dryer). The principle of determining the moisture content with a weight-moisturizer is to automatically measure the mass before, during and after drying of the prototype (BTS and BZS with rice eczema) with a drying agent. The results of weighing determine the moisture content of the sample that was in it before the start of the drying procedure. The moisture content calculation is carried out automatically by the moisture meter according to the pre-selected formula (1), which is displayed on the moisture meter indicator. The automatic termination of the drying procedure takes place provided that the difference between several consecutive measurements of the sample mass is not more than 20 mg.

$$W = \frac{m_0 - m}{m_0} \times 100\% \quad (1)$$

The moisture content display on the humidity indicator allows you to monitor the moisture evaporation process and, if necessary, correct the drying parameters.

Differential-thermal analysis

Research of the moisture content of various forms of bonding in protein-herbal clots (PHC) and mixtures based on rice extrudate – protein-cereal mixture (PCM) was carried out on a derivative of the Paulik-Erdey Q-1000 system in the temperature range of 20–250 °C at a heating rate of samples of 1000 mg – 2.5 °C per minute. The sensitivity of thermogravimetric analysis (TG) was 2,5 mv/mg, and the differential-thermogravimetric research (DTG) – 2,5 mv·s/mg, the sensitivity of differential-thermal analysis (DTA) – 200 mv·g/j.

This device is an automatic installation for complex thermal analysis: differential-thermal and thermogravimetric. The temperature and differential weight loss curves can be received actually in one format. The essence of method is experimental samples (PHC and PCM with rice extrudate) samples weighing 1000 mg are placed in the working volume of device and heated at a constant rate 2.5 °C / min in the temperature range 20–250 °C. At this, the sample temperature (curve TA) is measured and the temperature difference (DTA curve) is continuously recorded using a differential thermocouple. In parallel with the temperature measurement, the PCM is weighed. During the heating, moisture is removed and this leads to PCM weight decrease. The change in samples' mass (TG curve) and the mass difference of PCM (DTG curve) during the heating is recorded in parallel with the curves TA and DTA. Four charts are recorded on thermogram: TA, DTA, TG, and DTG.

The change in samples' mass of PHC and PCM (TG), differential rate of mass change (DTG), heat conductivity (DTA), temperature (T) were fixed during the heating process. The differential rate of mass change which characterizes the different rate of its loss throughout the experiment, was calculated by the formula (2):

$$DTG = \frac{\Delta m}{\Delta t} = \frac{m_1 - m_0}{t_1 - t_0} \quad (2)$$

where, t_0 – initial heating time of the sample; t_1 – duration of sample heating at a certain temperature; m_0 – primary mass of the sample; m_1 – the sample mass at a certain time t_1 .

Results and discussion

The thermoanalytical method was used in the study of the forms of bonding of moisture in the protein-herbal clots and protein-cereals mixtures based on it using laboratory electronic scales-moisture and derivative. This is traditional in the research of chemical reactions and physical transformations under thermal activity in multicomponent systems between individual compounds. Thermal processes are always accompanied by a change in the internal heat content of the system.

According to the disperse systems classification, the protein-herbal clot has a thixotropic structure of the coagulation type in which the particles are held by intermolecular forces. The presence of liquid (whey) causes less structure strength which gives it plasticity and elasticity. The thicker layers the less structure strength. Synaeresis and thixotropy are characteristic for coagulation structures. Milk whey contains on average 95,8–92,6% moisture, which is a dispersion medium for swelling and partial dissolution of rice extrudate. Moisture is bound in a protein-herbal clot by physical-mechanical and physical-chemical bonds. Moisture affects the structural and mechanical properties of mixture and the technological parameters during prolonged storage.

When a protein-herbal base with RE is combined, moisture bonding forms are redistributed – increase in the amount of bound water that does not freeze at low temperatures does not dissolve the electrolytes, has twice the density that a free water density. This is due to bonds that arise when combined with carbohydrate and protein complexes of RE with whey.

The dynamics of evaporation of moisture from the prototype were fixed on electronic scales-moisture. The results are presented in Figure 2.

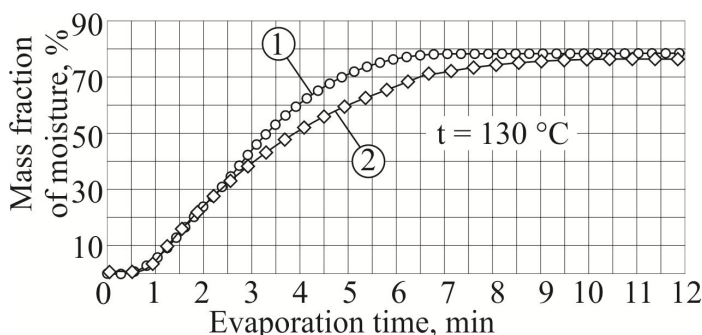


Figure 2. Dynamics of evaporation of moisture from protein-grass clusters:
1 – without extrudate rice (control), 2 – with rice extrudate.

According to the results of the measurements, the removal of the main part of the moisture (free) from the specimen PHC without rice extrudate occurs faster – for 6.0–6.5 min, and with the addition of rice extrudate – slower and this figure is within 7.5–8.0 min. Extrudate's hydrophilic substances interact with PHC moisture due to the formation of H-bound polyasociates with the participation of water molecules and H-bound functional groups of hydrophilic substances. The molecules of hydrophobic groups become more orderly as evidenced by decreasing entropy. Hydrophobic groups are aggregated at the expense of dispersion forces.

In the case of milk proteins, when the acid & heat coagulation occurs, irreversible deposition reactions with loss of primitive properties occur. This is accompanied by the deployment of polypeptide chains of proteins that have been folded in the native protein molecule. As a result of such transformations of chains (with the destruction of tertiary and secondary structures), hydrophobic groups "emerge" on the surface of protein molecules. In this casein and whey proteins lose solubility, aggregation and fall in the precipitate.

The interaction of the rice extrudate with the milk proteins, after coagulation, leads to the formation of conglomerates that do not differ in the strength of the bonds, but are quite sufficient to bind the free moisture contained in the PHC in the form of whey.

Research results of protein-cereal mixture with EP is presented in the form of derivatograms in Figure 3.

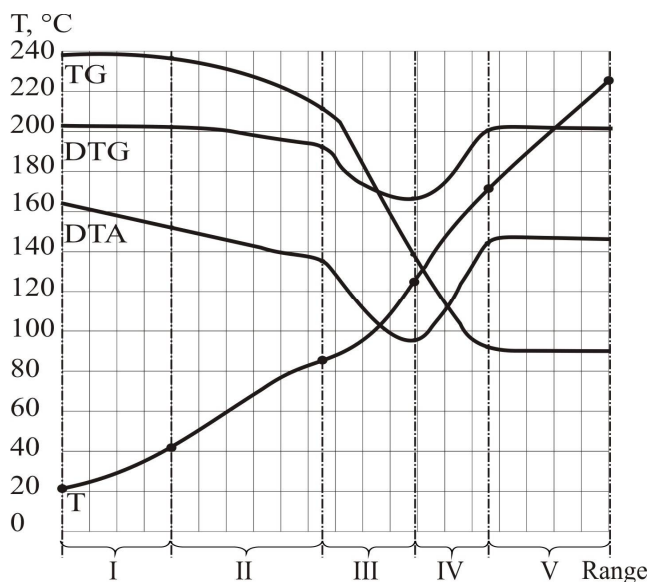


Figure 3. Derivatogram of PCM on the protein-herbal clot basis with rice extrudate: where TA – temperature curve, DTA – differential-thermal analysis curve of enthalpy, DTG – differential-thermal analysis curve of weight loss, TG – mass loss curve.

The curve fixes the changes in the temperature of research samples and the thermogravimetric curves of TG show a change in mass as the temperature function. The curves of the differential-thermogravimetric DTG research and the differential-thermal DTA analysis characterize the rate of moisture mass evaporation and the heat content change in protein-cereal mixture with RE.

The analysis of obtained derivatograms allows to reveal some regularities for PHC and PCM samples. Characteristic is presence four critical temperatures which the moisture various types, differing in the bonding strength, is removed.

DTA and DTG curves go to the negative side. Moisture evaporation from the surface of protein-cereal mixtures at a slight speed is in I and II ranges on all curves. The heat content

increases more intensively in I range on the DTA curve (to a value T_I 49–58 °C) than in II range on the same curve. This is due not only to the increase in temperature (as in the entire area OT_{II}), but also due to an increase in the water heat capacity to the maximum value. Areas I and II range are characterized by increasing speed of mass transfer (II range, DTG curve) and heat content. As a result the temperature rises from T_I 49–58 °C to T_{II} 91–98 °C. All free (mechanically bound) moisture, which appears as a result compaction of protein-grain mixture's structure, is removed and is in layers between the protein-herbal base and rice extrudate.

The nature of the DTA and DTG curves makes it possible to assert that the process is endothermic. The maxima of these curves coincide. This means at these temperatures there are changes that are not related to chemical or physical transformations – the rupture of physical-mechanical and physical-chemical moisture bonds.

After reaching the phase transformation temperature to 131 °C, the mass reduction process (DTG curve, III range) is intensified, under which removal the capillary (free) liquid and vapor phase from the bioscience occurs.

The heat content is predicted to fall due to the intense moisture mass evaporation as evidenced by the DTA course curve in the III range. The rate reduction of moisture removal due to the completion the molar removal residues vapor phase of capillary moisture from the protein-cereal mixture is observed on the TG and DTG curves in the IV range.

In the same period evaporation of the physical-mechanical capillary-bound liquid phase the temperature in cells of PCM with RE spatial structure, formed as a result of the formation connecting bridges between the PHC protein globules and the RE carbohydrate complex, rises from T_{III} from 131 °C to T_{IV} 179–186 °C and pressure inside the structure reaches strength limit. An increase in the evaporation rate of the internal liquid phase, by the formed capillary-structural channels, occurs on this site. The DTA curve in the IV range determines in this respect a rate decrease in enthalpy increase. There are thermal processes characterizing the removal of moisture which are bound by PCM adsorption centers. They can be hydrophilic groups that are on the PHC protein globules surface and in carbohydrate RE macromolecules. When the samples reached a temperature to 179–186 °C, the removal of all of the physical-mechanical bound intracellular moisture is complete and the rate of weight reduction sharply falls.

As evidenced by the IV range of the DTG curve.

At temperatures above 189 °C (V range) begin the decomposition processes of organic and mineral components – pyrolysis, i.e. chemically bound moisture is evaporated.

Designing the DTG curve minimum for the TG curve (mass loss curve), the amount of free and bound moisture was determined. For PHC and PCM with RE the amount of bonded and free moisture is respectively: 27,08% and 71,26%; 33,65% and 68,32%. Taking into account the research results, we can conclude the addition of rice extrudate to PHC can increase the amount of bound moisture by 36,07% compared to the control.

Conclusions

1. The moisture bonding forms in protein-cereal mixtures with rice extrudate on the basis of protein-herbal clot has investigated by thermogravimetric method and differential-thermal analysis.
2. The amount of bonded moisture PCM samples with EP is 33.65% which is 36.07% higher than in PHC. The results are confirmed the expediency of adding rice extrudate to the free moisture binding which will provide steel quality indicators in protein-herbal clots.

References

1. Erlihman V.N., Fatyihov Yu.A. (2004), *Konservirovanie i pererabotka pischevyyih produktov pri otritsatelnyih temperaturah*, Kaliningrad.
2. Temesgen M., Yetneberk S. (2015), Effect of Application of Stabilizers on Gelation and Syneresis in Yoghurt, *Food Science and Quality Management*, 4, pp. 90–102.
3. Nurgin R. Memiši, Slavica M. Vesković Moračanin, Mirela D. Iličić, Mira Đ. Ač. (2014), *Storage temperature: a factor of shelf life of dairy products*, *Acta Periodica Technologica*, 45, pp. 55–66.
4. Nathdanai Harnkarnsujarit, Kiyoshi Kawai, Manabu Watanabe, Toru Suzuki (2016), Effects of freezing on microstructure and rehydration properties of freeze-dried soybean curd, *Journal of Food Engineering*, 184, pp. 10–20.
5. Agarwal S., Prasad R. (2013), Effect of Stabilizer on Sensory Characteristics and Microbial Analysis of Low-fat Frozen Yoghurt Incorporated with Carrot Pulp, *International Journal of Agriculture and Food Science Technology*, 4(8), pp. 797–806.
6. Rao K., Sunitha C., Banji D. (2011), A study on the nutraceuticals from the genus *Rumex*, *Hygeia journal for drugs and medicines*, pp. 76–88.
7. Pereira C., Barros L., Carvalho A., Ferreira I. (2013), Use of UFLC-PDA for the Analysis of Organic Acids in Thirty-Five Species of Food and Medicinal Plants, *Food Anal. Methods*, 6, pp. 1337–1344
8. Mank V.V., Kobylinskaya E.V., Kovbasa V.N. (1999), Sostoyanie vodyi v krahmale i ego ekstrudatah po dannym YaMR, *Pischevyie ingredientyi, syire i dobavki*, 2, pp. 14–15.
9. Magomedov G.O., Shatnyuk L.N., Okulich-Kazarin E.G. (2004), Produktiyi funktsionalnogo pitaniya i ekstruziya, *Pischevaya promyshlennost*, 5, pp. 36–38.