# PERIÓDICO TCHÊ QUÍMICA

ARTIGO ORIGINAL

# ESTUDO DAS FORMAS DE CONEXÃO À UMIDADE DE COMPONENTES EM CONCENTRADOS DE PROTEÍNA-CARBOIDRATO DE SORO DE QUEIJO

# THE RESEARCH OF THE TYPES OF MOISTURE BONDS IN PROTEIN-CARBOHYDRATE CONCENTRATES OF CHEESE WHEY

# ИЗУЧЕНИЕ ФОРМ СВЯЗИ ВЛАГИ КОМПОНЕНТОВ В БЕЛКОВО-УГЛЕВОДНЫХ КОНЦЕНТРАТАХ ПОДСЫРНОЙ СЫВОРОТКИ

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#### RESUMO

O objetivo da pesquisa é melhorar a tecnologia de produção de concentrados de proteína-carboidrato de soro de queijo, em termos de ligação da umidade com os principais componentes. Os objetos da pesquisa foram soro de queijo natural obtido na produção de queijos Rossiyskii e soro de ultrafiltração com percentual de 35 e 55% de matéria seca de proteínas, produzido com o uso da unidade industrial de ultrafiltração MMS Swissflow UF com membranas cerâmicas. A composição química e as propriedades físico-químicas das matérias-primas e do produto acabado foram estudadas usando métodos padrão. Foram identificados três estágios de desidratação da amostra. Eles estão em conformidade com a liberação de umidade com diferentes ligações e energia. O estágio I é a área de produção em que a umidade não ligada é aquecida e removida, e as moléculas de água são mantidas por forças capilares fraças. O estágio II é a área de produção para remoção de umidade imobilizada. O estágio III é a área de produção para remoção de água guimicamente ligada, gue se adapta à umidade residual após a secagem da amostra. Um aumento na água menos móvel e uma mudança na proporção entre os diferentes tipos de ligação à umidade foram observados com um aumento na fração de massa protéica na matéria seca das amostras. Verificou-se que a reação de Maillard ocorreu em concentrados de soro de queijo com porcentagem de matéria seca de proteína de 35 e 55% nas temperaturas acima de 78 e 70 °C, respectivamente. A faixa de temperatura da liberação de umidade não ligada estava aumentando durante o processo de secagem devido ao aumento do teor de proteínas nos concentrados, bem como à taxa de interação entre proteínas e lactose, levando ao escurecimento não enzimático dos produtos secos. Foi estabelecido que a secagem de proteína-carboidrato de soro de queijo concentrada com porcentagem de 35% de matéria seca de proteína é inconveniente a temperaturas superiores a 130 e 173 °C, respectivamente.

**Palavras-chave**: matérias-primas lácteas secundárias, características de secagem, concentrado de proteínacarboidrato.

#### ABSTRACT

The purpose of the research is to improve the technology of producing cheese whey proteincarbohydrate concentrates, in terms of binding moisture with the main components. Objects of the research were natural cheese whey obtained in the production of *Rossiyskii* cheese and ultrafiltration cheese whey concentrates with protein dry matter percentage of 35 and 55%, produced with the use of MMS Swissflow UF industrial ultrafiltration unit with ceramic membranes. The chemical composition and Physico-chemical properties of raw materials and the finished product have been studied using standard methods. Three stages of sample dehydration have been identified. They conform to moisture release with different bonds and energy. Stage I is the production area where unbound moisture is heated and removed, and water molecules are held by weak capillary forces. Stage II is the production area for immobilized moisture removal. Stage III is the production area for chemically bound water removal, which conforms to the residual moisture after drying the sample. An increase in the least mobile water and a change in the ratio between different types of moisturebinding have been observed with an increase in the protein mass fraction in the dry matter of the samples. It has been found that the Maillard reaction occurred in cheese whey concentrates with protein dry matter percentage of 35 and 55% at the temperatures above 78 and 70 °C, respectively. The temperature range of the unbound moisture release was increasing during the drying process due to an increase of the protein content in concentrates as well as the rate of interaction between proteins and lactose leading to non-enzymatic browning of dry products. It has been established that drying cheese whey protein-carbohydrate concentrates with protein dry matter percentage of 35, and 55% is inexpedient at temperatures exciding 130 and 173 °C, respectively.

Keywords: secondary dairy raw materials, drying features, protein-carbohydrate concentrate

#### АННОТАЦИЯ

Цель исследований – совершенствование технологии получения белково-углеводных концентратов подсырной сыворотки с учетом форм связи в них влаги с основными компонентами. Объекты исследований: натуральная подсырная сыворотка, полученная при производстве сыра «Российский», ультрафильтраци-онные концентраты подсырной сыворотки с массовой долей белка в сухом веществе 35 и 55%, выработанные на промышленной ультрафильтрационной установке MMS Swissflow UF с керамическими мембранами. Химический состав и физико-химические показатели сырья и готового продукта изучены по стандартным методикам. По результатам исследований установлены три ступени дегидратации образцов, которые соответствовали высвобождению влаги с различной формой и энергией: І ступень – участок, на котором происходил нагрев и удаление свободно связанной влаги, а также молекул воды, удерживаемых слабыми капиллярными силами; II ступень – участок, где удалялась иммобилизованная влага; III ступень - участок, на котором удалялась химически связанная вода, что соответствовало остаточной влаге после высушивания навески. Наблюдалось повышение количества наименее подвижной воды и изменение соотношения между различными формами связи влаги с увеличением массовой доли белка в сухом веществе образцов. Установлено, что реакция Майяра протекала в концентратах подсырной сыворотки с массовой долей белка в сухом веществе 35 и 55% при температурах более 78 и 70 °C соответственно. С увеличением массовой доли белка в концентратах повышался температурный диапазон высвобождения удаляемой влаги в процессе сушки и увеличивалась скорость взаимодействия между белками лактозой, приводившая И к неферментативному покоричневению сухих продуктов. Установлено, что сушка белково-углеводных концентратов подсырной сыворотки с массовой долей белка в сухом веществе 35 и 55% при температуре более 130 и 173 °С соответственно нецелесообразна.

Ключевые слова: вторичное молочное сырьё, особенности сушки, белково-углеводные концентраты

#### **1. INTRODUCTION**

Current market trends show an increase in the intensive production of milk protein is products, the technology of which accompanied by the production of large volumes of whey (Tihomirova, 2017). The problem of complete and rational processing of secondary raw materials in the dairy industry, including whey, is taking a special value in the conditions of the whole milk shortage. It contains about 50% of milk solids, more than 80% of whey proteins, which determine its high biological value (Ponomarev et al., 2018).

The most common technologies of whey processing include the usage of all its components. These are the technologies of dry whey production, including demineralized ones, which are implemented in Russia. Thus, 129,000 tons of these products were produced in the

Russian Federation in 2017 (Kharitonov, 2019). Nevertheless, the domestic production of whey powder does not satisfy the needs due to the weak development of secondary raw milk processing. Most of the whey produced in the country (59%) is used to feed farm animals, 20% is drained into fields or wastewater, and only 21% is used to further processing (Khramtsov, 2018).

The fractionation of whey proteincarbohydrate-mineral complex is a prospective technique. This technological method enables to obtain of food ingredients with high added value, for example, concentrates and isolates of whey proteins, microparticulate, lactose, dry permeate, mineral salts, including lactates and calcium phosphates, which are in high demand. World production of the ingredients manufactured by such technologies is characterized by high average annual growth rates, at the level of 5 – 10% (Božanic *et al.*, 2014; Topalov *et al.*, 2016; Barukcic, 2018). They are in high demand in the Russian market but are hardly ever produced in our country. The demand for these products is satisfied mainly by imports.

The implementation of this direction requires the use of membrane technologies, which allow fractionation of whey components according to the size of their molecules. The obtained semi-finished products can be processed using traditional lines, including filmtype vacuum evaporators, characterized by a minimal thermal effect on the raw materials, and modern drying units, carrying out multi-stage drying using efficient systems of preparation and purification of the drying agent.

Import volumes of whey protein concentrates and isolates are increasing steadily every year. This is determined by their essential physical and chemical properties, such as fatand moisture retention, emulsifying capacity, and a number of technological functions in food systems, which depend on the degree of their hydration, surface activity and type of proteinprotein interactions in partially unfolded structures (degree of denaturation) (Kassem, 2015). The functional properties of whey proteins are induced by peculiarities of the tertiary structure formation and its flexibility, in particular, the presence of a hydrophobic pocket inside the  $\beta$ cylinder of the  $\beta$ -lactoglobulin tertiary structure, as well as the activity of surface areas and the presence of disulfide bonds and partially buried sulfhydryl group. Moreover, the ability of whey proteins to exhibit amphiphilic properties makes a significant contribution to the stabilization of their structure (Damodaran et al., 2012).

Whey protein concentrates and isolates do not have the status of food additives and, as a consequence, lack the "E" indexed letter, providing a "clean" label of the finished food product. Whey proteins are the source of essential amino acids and branched-chain amino acids (valine, leucine, and isoleucine) in terms of physiological effects on the human body. Their amino acid profile is very similar to the composition of human muscle tissue; therefore, these nutrients are characterized by a high rate of cleavage in comparison with other proteins. The usage of these components in the production of various food products makes it possible to increase their nutritional and biological value (Damodaran et al., 2012).

The use of whey protein concentrates is determined by the protein mass fraction content. Russian standard GOST R 53456-2009 "Whey

protein concentrate powders. Specifications" provides the production of concentrates with dry protein matter (PDM) percentage not less than 35.0 and 55.0%. Such products are widely used in baby food, gerodietetic food, sports nutrition, and confectionery production for the enrichment and as stabilizers in the production of meat and dairy products.

Since irreversible denaturation of whey proteins reduces their solubility and affects adversely the technological properties, which require high surface activity, a number of research on observation of the stability factors of whey proteins, as well as the features of the individual components interaction of cheese whey during heat treatment have been conducted by Russian and foreign scholars (Bannikova and Evdokimov, 2016; Budanina et al., 2017; Bull et al., 2017; Cabral et al., 2019; Henriques et al., 2017; Kumar et al., 2013; Meena et al., 2017; Savadkoohi et al., 2014; da Silva et al., 2015; Schön et al., 2017; Torres et al., 2017; Wijayant et al., 2014). However, the problem of obtaining ultrafiltration concentrates (UF-concentrates) and justification from cheese whev of technological modes of their processing are still urgent issues. Thus, the purpose of this research was to improve the technology of UFconcentrates obtained from cheese whey and to study the forms of binding moisture with main components. Based on this data, it is possible to prevent irreversible structural changes in the whey protein molecules during subsequent evaporation and drying process to ensure their maximum structural stability and preservation of fat- and moisture retention and emulsification.

In the furtherance of this goal, the following tasks have been defined:

- to justify the modes of technological processing of cheese whey for production of UF-concentrates with various protein mass fractions;

- to study the forms of moisture-binding in UF-concentrates of cheese whey with various protein mass fractions;

- to determine the chemical composition, properties, and quality characteristics of the obtained UF-concentrates of cheese whey.

### 2. MATERIALS AND METHODS

Natural cheese whey obtained in the production of *Rossiyskii* cheese at the PSC Dairy Plant "Voronezhskii" (Kalacheevskii Cheese Factory, Kalach, Voronezh Region) and UF- concentrates of cheese whey with various protein mass fractions, produced using MMS Swissflow UF industrial ultrafiltration unit with ceramic membranes were the objects of the study. The research was carried out in the laboratories of the Core Facility Center of the Voronezh State University of Engineering Technologies and Mollab Ltd.

The chemical composition, physical and chemical properties of raw materials and the finished product were studied in compliance with the standard and generally used methods, as well as modified and improved techniques.

The nephelometric method, based on the removal of denatured whey proteins from the system by precipitation with a saturated solution of sodium chloride, was used to determine the whey protein nitrogen index (WPNI) (Sikand et al., 2008). The precipitate was separated; a saturated solution of sodium chloride (10 cm<sup>3</sup>) and 2 drops of hydrochloric acid solution (10% wt) were added into a test tube containing 1 cm<sup>3</sup> of the filtrate. The optical density of the test sample was measured at 420 nm using the PE-5400 UF spectrophotometer (Russia) and compared to a standard curve obtained by analyzing the samples with a known WPNI value. The whey protein nitrogen index characterizes the content of non-denatured whey proteins in the sample.

The types of moisture-binding in UFconcentrates of cheese whey were investigated using the STA 449 F3 Jupiter simultaneous thermal analyzer (Germany) in the laboratory of the Core Facility Center "Control and Management of Energy-efficient Projects" at the Voronezh State University of Engineering Technologies under the state order No 10.8678.2017/7.8. This device combines the advantages of a highly sensitive thermobalance and a differential scanning calorimeter. The analysis consisted of the curves plotting for temperature changes in the sample with a weight of 10 ± 0.5 g during drying. The method of differential scanning calorimetry (DSC) is based on the registration of thermal effects of physicochemical and structural transformations occurring in the sample during a programmed change in temperature regime. The software, such as NETZSCH Proteus and MS Excel, was used for processing the obtained data of DSC and thermos-gravimetric (TG) curves. Subsequently, dDSC and dTG differential curves were plotted. The experiments were carried out by heating from 25 to 300 °C at a speed of 5 K/min in an oxidized aluminum pan in a gaseous

nitrogen medium of class 5 with a purge gas flow rate of 60 cm<sup>3</sup>/min. The accuracy of temperature measurement by DSC and TG methods was 0.1 °C.

The content of 5-hydroxymethylfurfural was determined using a photometric method with a PE 5400 UF spectrophotometer (Russia) in accordance with GOST 29032-91. The optical density of the samples was measured at a wavelength of 540 nm, using vessels having a working length of 30 mm (Zhang *et al.*, 2017).

Statistical analysis of the results was carried out using methods of mathematical statistics according to the data obtained from 5 to 10 experiments in three replications. The MathCad 16.0 application package was used for information processing and graphical interpretations of the results.

#### 3. RESULTS AND DISCUSSION:

Whey protein concentrates (WPC) were produced by ultrafiltration according to the process flow chart in Figure 1. Cheese whey samples with the active acidity of 6.4 - 6.8 and the protein content of 0.8% were subjected to preliminary purification in a clarifying centrifuge at a temperature of 43 - 45 °C to remove the protein dust and fat. The fat content in skimmed whey should not exceed 0.05%. Then pasteurization was carried out at a temperature of  $76\pm2$ °C for 15 sec. Pasteurized cheese whey was collected in a storage tank. Microfiltration was included in the manufacturing cycle due to the necessity of having a low-fat content in UF WPC.

The cheese whey ultrafiltration was carried out at t = (10 - 12) °C under pressure of 0.3 - 0.6 MPa in order to obtain WPC with various protein content. The concentration factor for protein was 4.4 - 4.5 and 8.8 - 8.9 to obtain concentrates with a PDM percentage of 35 and 55%, respectively. The choice of the ultrafiltration mode was justified by the necessity to minimize the effect on the native protein structure to prevent bacterial growth in the retentate and calcium phosphate precipitation in the pores of the membrane modules.

Since high content of mineral substances **UF-concentrates** in can contribute to causing solubilization of lactose poor diafiltration dehydration, is а compulsory technological operation, which allows reduction of the mass fractions of lactose and mineral salts (Nesterenko et al., 2014).

Including lactose pre-crystallization into the process flow chart in the form of condensed mixture enables to prevent dry product clumping during storage. The concentrate should be cooled to 30 - 32 °C quickly at the end of evaporation. Crystallization of lactose is advisable to carry out by subsequent slow cooling to 20 °C with finecrystalline lactose seed in an amount of 0.02 - 0.1%. Then the condensed mixture should be mixed at the agitator speed rate of 10 rpm for 30 sec. clockwise and 30s. counter-clockwise with intermediate switching off periods for 30 sec. between them (Nesterenko *et al.*, 2014).

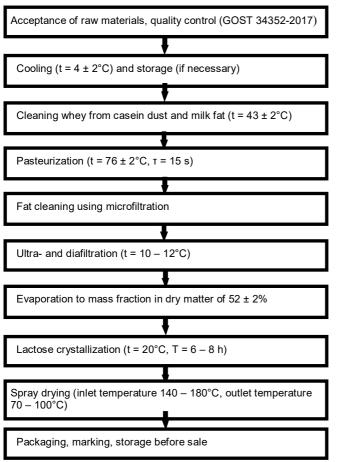


Figure 1. Process flow chart for production of whey protein UF-concentrates

The chemical composition and properties of cheese whey and the obtained UFconcentrates are given in Table 1.

The amphiphilicity of whey proteins, being an integral part of UF-concentrates, explains their ability to bind water molecules in solutions. The water is known to have a more structured form in concentrated systems due to the changes in protein conformation. These changes depend on whether the moisture content is critical or can be caused by the influence of lactose crystallization on the specific heat capacity (Kramer, 2012). Hydrophobic group assemblage and hydrophobic interaction have an adverse effect on thermodynamics in such solutions. It is the mainspring of protein folding, forcing many hydrophobic residues to occupy positions that are deep inside the protein. However, non-polar groups of globular proteins usually cover about 40-50% of their surface area, and with the decrease temperature, in hydrophobic interactions weaken, whereas hydrogen bonds strengthen (Damodaran et al., 2012).

Differential thermal analysis (DTA) and thermogravimetry (TG) were used to study the free and bound moisture ratio in cheese whey and its UF-concentrates. Thermograms (heating curves) are the result of thermal analysis; these depend mainly the curves on chemical composition and the structure of the investigated material. The thermogram has a number of critical points (Figure 2). The peaks on the DTG curve characterize temperature limits for the removal of free moisture and bound moisture of various types. Capillary moisture is removed at a constant temperature at the initial stage of drying. The inflection of curve characterizes moisture removal in cable and abutting phases. At the initial stage, the linear section is determined by the removal of multi-molecular adsorption moisture, and then monomolecular adsorption moisture (Karam et al., 2017). There is one endothermic effect observed on the DSC curves, which is accompanied by heat absorption and mass changes of the samples on the TG-curves. The dDSC peak area is proportional to the change of the reaction enthalpy and to the mass of the samples and inversely proportional to their thermal conductivity (Niftaliev et al., 2018; Pozhidaeva et al., 2018; Rodionova et al., 2018), see Table 2.

The analysis of the DTG curves made it possible to assume that at the temperature above 129 °C, the peaks determine lactose melting and proteins thermolysis in the samples. Moreover, endo-effect peaks grow up with an increase in PDM percentage in the studied systems. Dehydration of UF WPC was carried out at higher temperatures than for the initial cheese whey, which proved a larger degree of moisture-binding in them. Whereas the hydrophobic effect is caused only by entropy at room temperature, with rising temperature, the energy of hydrogen split bonds with non-polar groups on the surface of globular proteins is of increasing importance (Finkel'stein and Ptitsyn, 2012). In addition, the dependence of reducing energy costs for moisture removal from the samples on the

increase in PDM percentage in UF WPC has been identified.

The primary hydration for most proteins is 0.2-0.5 mol of water/100 g of polymer, and it is an integral part of its structure. There are several hypothesis concerning the nature of these primary binding sites, such as monomolecular adsorption on the polar sites (arg, his, lys, asp, gly, cys, met, ser, thr, tyr, trp) and multimolecular adsorption on the specific sites, where water molecules can bind with two, three or four hydrophilic molecular groups simultaneously. The existence of such binding sites depends on the arrangements specific geometric in the macromolecules. A hydrogen bond donator or an acceptor can act as a hydrophilic group.

Probably, sites that provide only singular hydrogen bonds with water molecules do not serve as primary sites for the formation of any bond with a certain free energy. Thus, depending on the geometry of the hydrophilic groups in the vicinity, a particular group may or may not form the primary binding site. Weak bonds, such as cysteine or methionine, are hardly considered as hydrogen bonds in this respect; they can only serve as weak additional stabilizing interactions (Damodaran et al., 2012).

Using TG curves, the transformation degree of  $\alpha$  (ratio of mass at the time *t* to the total mass change at the end of the process) has been calculated depending on the temperature (Figure 3). The dependencies obtained were S-shaped, which indicated the complex nature of the interaction between water molecules and whey proteins and between low molecular weight components of cheese whey and its UF-concentrates and suggested a different water release rate at the dehydration stages.

Figure 4 shows the dependencies of the logarithm of the degree of substance conversion (*a*) on the temperature  $lg \ \alpha = f(1/T)$ , which were used for quantitative evaluation of the types of moisture bonds in the samples.

Three stages of sample dehydration have been established, corresponding to various forms of moisture release and energy (Figure 3). Stage I is the production area, where free-bound moisture was heated and removed, and water molecules were held by weak capillary forces. Stage II is the production area where immobilized moisture (water molecules forming more distant adsorption layers) was removed. Stage III is the production area, where chemically bound water was removed, which characterized the monomolecular water layer associated with polar

groups, and the water-dipole interaction took place, which corresponded to the residual moisture after drying the sample (Niftaliev *et al.*, 2018; Andiç-Çakir *et al.*, 2014).

Thus. with an increase in PDM percentage in UF WPC, the amount of least mobile water increased, and the ratio between different types of moisture changed. The shape of the curve for a sample with a PDM percentage of 55% (Figure 4) indicates the maximum monolayer in it, which is formed only over the easily accessible components of the dry matter with a pronounced polarity.

Lactose contributed to an increase in protein hydration through non-covalent interaction with water and protein molecules by hydrogen bonds (Aalaei et al., 2018). This additionally makes it possible to increase the stability of whey proteins against denaturation and to inhibit lactose crystallization during subsequent concentration and drying (Yazdanpanah and Langrish, 2011; Amdadul Haque et al., 2018; Lisitsyn et al., 2016; Liu et al., 2016; Norwood et al., 2017; Abd El-Salam and El-Shibiny, 2018; Lin et al., 2018; Swarnalatha and Mor, 2019). However, increased protein content in UFconcentrates can lead to the faster flow of the Maillard reaction.

A complex peak on the DSC curves (Figure 2) takes into account the conversions that occurred with lactose during the drying process of studied samples: glass transition. the crystallization, and the course of the Maillard reaction. To isolate non-enzymatic browning on dDSK curves upon reaching effect temperatures, the presence of 5-hydroxymethylfurfural was determined in the samples as an indicator of the Maillard reaction onset. It was established that non-enzymatic the reaction of browning proceeded in UF WPCs with PDM percentage of 35 and 55% at temperatures above 78 and 70°C, respectively. Numerous peaks on the dDSK curve for a sample of UF WPCs with PDM percentage of 55% are likely to characterize different stages of the Maillard reaction. However, their speed is low, which is associated with a low mass fraction of lactose in this sample.

### 4. CONCLUSIONS:

Thus, the moisture content of various bonds in protein-carbohydrate concentrates of cheese whey is influenced by the protein dry matter percentage, including minerals, in the protein-lactose ratio. With a change in this ratio when concentrating cheese whey proteins, the temperature range of moisture release in the process of drying grows, and the rate of interaction between proteins and lactose increases, leading to non-enzymatic browning of the products. According to the results of the conducted research, it has been established that drying of cheese whey protein-carbohydrate concentrates with PDM percentage of 35 and 55% at the temperatures over 130 and 173 °C, respectively, is inexpedient, since it may result in the occurrence of the defects in color, taste, and odor in the finished product.

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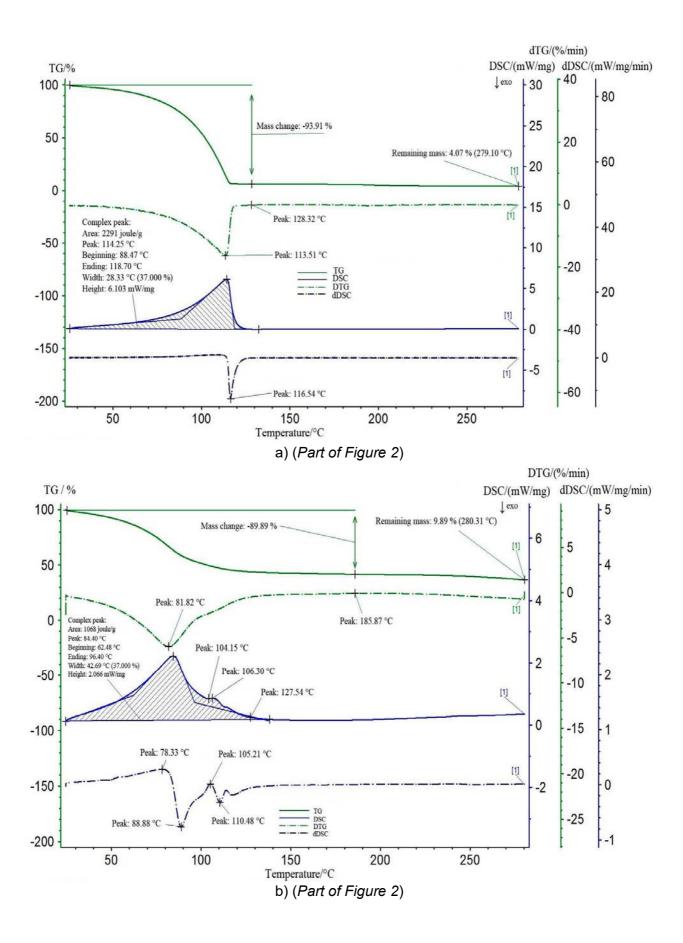
Indicator	Cheese whey	UF-WPC with PDM percentage of 35%	UF-WPC with PDM percentage of 55%
Milk in dry matter, %	6.09	10.11	12.37
Fat in dry matter, %	0.05	0.40	0.65
Total protein in dry matter, %	0.74	3.55	6.83
Lactose in dry matter, %	4.54	5.17	3.14
Protein-lactose ratio	1:6.1	1:1.4	2.2 : 1
Acidity, °T	16	21	28
WPNI, mg of nitrogen/g	5.5	5.3	5.2

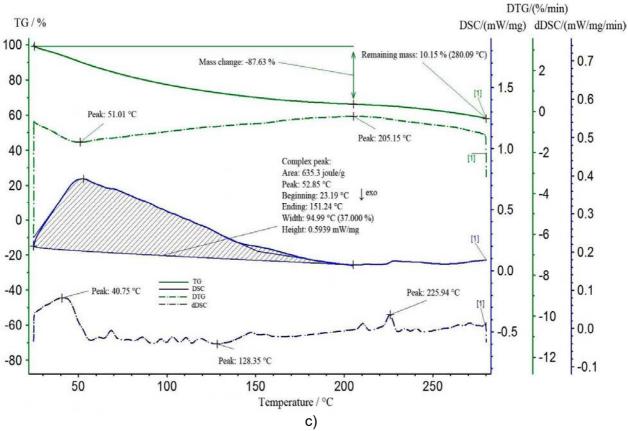
Table 1. Chemical composition and properties of the samples

**Table 2.** Enthalpies and mass changes during the process of heating cheese whey and its UF-<br/>concentrates

Sample	Temperature range ΔT, °C	Enthalpy ΔH, joule/kg (DSC curve)	Sample mass change, % (TG curve)
Cheese whey	30 – 132	2.291	93.91
UF-WPC with PDM percentage of 35%	25 – 140	1.068	89.89
UF-WPC with PDM percentage of 55%	25 – 205	0.635	87.63

Dehydration degree	ΔΤ, Κ	∆t, °C	Δα	Mass fraction of free moisture, %
		Cheese whey		
I	298 – 346	25 – 73	0 – 0.135	13.491
II	346 – 397	73 – 124	0.135 – 0.999	86.406
	397 – 402	124 – 129	0.999 – 1.000	0.104
	UF-WP0	C with PDM percen	tage of 35%	
	298 – 323	25 – 50	0 - 0.130	13.047
II	323 – 402	50 – 129	0.130 – 0.970	83.537
	402 – 458	129 – 185	0.970 – 1.000	3.416
	UF-WP(	C with PDM percen	tage of 55%	•
	298 – 313	25 – 40	0 - 0.170	12.778
II	313 – 445	40 – 172	0.170 – 0.950	81.878
	445 – 478	172 – 205	0.950 – 1.000	5.344





a) cheese whey; b) UF-WPC with PDM percentage of 35%;c) UF-WPC with PDM percentage of 55%

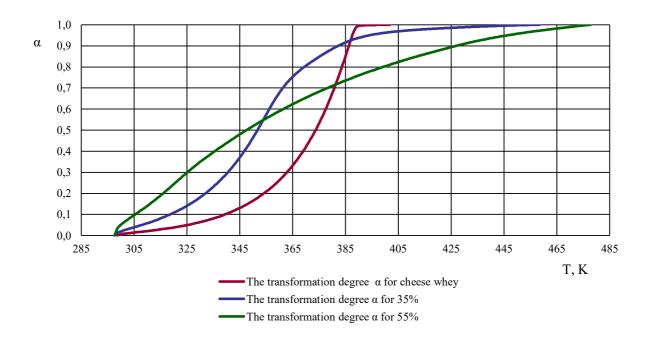
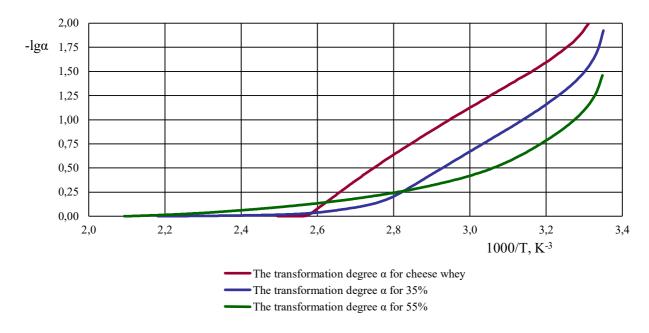


Figure 3. The dependence of the transformation of substance  $\alpha$  on temperature



**Figure 4.** The dependence of the transformation of a substance (-lg  $\alpha$ ) on the value of 1000/T when heated

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