ISSN 2308-4057. Foods and Raw Materials, 2016, vol. 4, no. 1

THE PHYSICAL AND CHEMICAL CHANGES OF WATER AND THE HYDRATION OF THE PROTEIN COMPLEX IN CHEESE DURING FREEZING

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Received March 16, 2015; Accepted in revised form November 27, 2015; Published June 27, 2016

Abstract: The use of low temperatures is one of the available factors to inhibit the over-ripening of cheeses and the preservation of their quality. This study reveals the patterns and physical and chemical nature of the phase transition of water into ice, and the state of bound water, when freezing semi-hard cheeses in the range of ultra-low temperatures (-20 ... -50°C). The authors research the cheese's resistance to freezing based on the water retention capacity of the proteins. They study the factors of product stability during storage in the frozen state conditioned by a change in state of tightly bound water in the protein complex during freezing to different temperatures. The paper examines three main subclasses of semi-hard cheeses with a high, low temperature second heating which, based on fat content in dry matter, are considered fat and semi-fat cheeses. The research conducted tests to obtain the basic patterns of rapid freezing at different air conditions. The air velocity in the cooling zone was up to 10 m/s. Samples of the finely packaged cheeses weighing up to 0.2 kg were being frozen at a given volume-average temperature of -20°C. The tests allowed to obtain the data about water phase transformation into ice, depending on the values of the low temperature. The kinetics of the process has shown a gradual transition of heterogeneous water into ice in accordance with its binding energy in descending order. Based on the analysis of the experimental data, the phase diagram of water states, depending on the final volume-average temperature of frozen cheese, has been created, and the data on the degree of hydration of the protein complex in the temperature range of -20 to -70°C has been obtained.

Keywords: freezing, tightly bound moisture, low temperatures, cheese, casein, hydrophilicity, frozen water

DOI 10.21179/2308-4057-2016-1-13-18 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 13–18.

INTRODUCTION

Modern innovative technologies in the dairy industry allow to extend dairy products' shelf life and to resolve urgent challenges of the competitive production of dairy products with the highest quality and the lowest cost. The domestic and international practice has accumulated experience and has the means to prolong the shelf life of products in the technological chain, providing minimal changes in their quality parameters [1, 2]. It is important to maintain a valuable set of consumer properties in food products, their high organoleptic properties and satisfactory appearance. The use of low temperatures is one of the practically available factors to inhibit the over-ripening of cheeses and the preservation of their quality. Storage efficiency is determined by how effectively it slows down the micro-biological, biochemical and physical-chemical processes in cheeses.

The peculiarity of Russian cheese-making is that most companies produce semi-hard rennet cheeses with a low temperature second heating, mainly in the framework of the "low-cost" market segment. The most popular cheeses in the Russian market are "Rossyisky", "Gollandsky", "Poshehonsky", etc. One of the factors limiting their consumption, is brief shelf life, generally not exceeding 3–4 months.

Freezing is a solution for extension of the long-term storage of semi-hard rennet cheeses. The methods of refrigerated processing allow the creation of a long-term reserve of cheeses and to transform the supply system, thus solving the problem of preserving their quality and satisfying the needs of the Russian dairy market [2, 3, 4].

Storage at low positive temperatures does not not ensure the quality of the cheese during long-term preservation, because it does not sufficiently slow down the microbiological processes in dairy products. As of today, freezing is the best method in many respects and a promising way of extending the shelf life of food products. Ultra-low temperatures can significantly slow down the rate of the microbiological and biochemical processes that lead to product quality deterioration. Freezing has a number of benefits for the conservation of the original, natural properties of the product; it is also beneficial from an economic and energy consumption viewpoint. The low-temperature processing and storage ensure longer food preservation up to a year or more.

The results of extensive use of low positive temperatures in the food technology have become the basis of studies on the effect of negative temperatures on possible qualitative changes in cheeses and their storage in the frozen conditions. Researchers' opinions differ about it; however, the idea of long-term storage of ripened cheeses in frozen conditions prevails as the possibility of the recovery of their structure after thawing is satisfactory [1, 2].

A number of Russian scientists in the 30–50's years of the twentieth century attempted to freeze semi-hard cheeses, but it had not become a basis for the development of the detailed methods and the technology of the low-temperature food storage. In some countries of the southern regions (Greece, Turkey, Italy, Spain) the research succeeded to develop the technology of the deep freezing and the storage of their traditional cheeses and curd made from cow, goat and sheep milk [3].

The analysis of the research on the rapid freezing and storage of frozen dairy products showed that the possibility of long-term storage of frozen semi-hard cheeses is least studied. A number of Russian researchers today (Moscow, Uglich) froze hard rennet cheese, and their research findings allowed them to infer the rational regime parameters of its freezing and thawing [6].

Kemerovo Institute of Food Science and Technology (University) has been conducting a study on the low-temperature storage of dairy products, in particular cheeses of different types, over the past 25 years.

When freezing, the modes and methods of refrigerated treatment are crucial in preserving the original properties of the products. The phase transition of water into ice can damage the original internal structure of the cheese, and thus reduce its qualitative characteristics.

The purpose of this study is to develop the theoretical foundations of the physical and chemical nature of the freezing of semi-hard rennet cheeses in order to increase their shelf life.

OBJECTS AND METHODS OF STUDY

We studied three main subclasses of semi-hard cheeses with a high, low-temperature second heating and cheddaring curd of a fat and semi-fat type, depending on the mass fraction of the fat in the dry matter. The geometrical dimensions met the standard to ripen cheeses of different shapes. To get finely packaged cheeses, we cut ripened cheese bars and heads into portions weighing 0.1–0.2 kg before testing.

To study the freezing process, a test bench was set up, designed to change and to maintain the air temperature in the chamber to minus 100°C and a flow rate of 10 m/s. The monitoring of the temperature in the chamber, the tunnel and the tested samples during freezing was carried out according to the indications of the automatic electronic potentiometer (PCB-4 with a scale of 40 to -200°C, accuracy class 0.5). As the sensor, we used a Chromel-Copel thermocouple junction with a diameter of 0.3×10^{-3} m.

The cheese samples were packaged in the plastic wraps and bags of a new generation of Cryovac VV3U type.

The freezing was carried out at various ambient air conditions in the range -20 ... -50°C. The air velocity in the cooling zone was measured by a hot-wire anemometer (Testo 405-v1) with a measuring range of 0..15 m/s, and a scale of division of 0.1 m/s. The test samples were placed onto freezer shelves simulating a commercial freezer. The samples were frozen down from an initial temperature of 20°C to a pre-set volume-average temperature of -20°C and -12°C. Thermal images of the freezing were the main testing instruments during the heat-exchange experiments. They helped to determine the basic indicators of the process – the duration and the average freezing rate. The air temperature at 0 to -3°C in the refrigerator served as control storage conditions.

We have been examining the quality of the tested samples before the freezing and during the refrigerated storage over 18 months with a sampling frequency of every 3 months. Prior to sampling, the samples were thawed out at a room temperature in air of 0 ... 3°C.

To evaluate the properties of the original product at all stages of its low-temperature storage, we determined a set of quality indicators. We used the conventional and original research methods, including the physical-chemical, microbiological, biochemical and other methods.

RESULTS AND DISCUSSION

When considering the factors of product stability during its storage in a frozen form, it is necessary to single out the content and the properties of the protein fraction in cheeses, as the most significant component. A high proportion of milk protein in their composition (from 23 to 29%) leads to the high water absorption and water-binding properties of the curd [5]. The hydrophilic properties of the casein determine the steadiness of the protein particles to freezing. Unwanted loss of water upon freezing and during the storage can lead to protein aggregation at low temperatures.

The water absorption properties affect the structural and mechanical characteristics of the product's consistency; in this connection, the degree of hydration of the protein complex is one of the most important physical and chemical factors in assessing the impact of the cold on the frozen product. Low protein hydration is one of the causes of the texture's defects: not elastic enough, crumbly, powdery [5]. For frozen products, the preservation of the degree of the hydration of the curd and consequently, of its satisfactory texture, is a matter of paramount importance.

The amount of bound water is a criterion of the changes in the cheese protein complex. The polar groups of the protein molecules -COOH, -OH, >CO-NH<, NH_2-, -SH, and others are laid in several layers around the hydrophilic centers of the protein molecules, forming a so-called hydration (water) shell (Fig. 1). This tightly-bound portion of the water is strongly compressed at the surface of the protein, and therefore it is very difficult to remove. The cold destroys the adsorbed layers of water, especially those that are at a greater distance from the surface of the molecule. Thus, casein micelle stability in frozen cheese will be determined by the strength of the hydration shell.

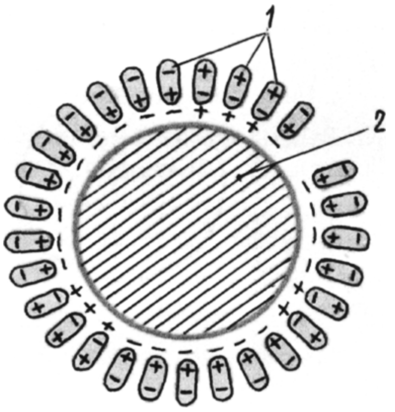

Fig. 1. The chart of the hydration shell: 1 – the water molecules; 2 – protein particles.

In determining the hydration characteristics of a combining form of the casein complex, we used P.A. Rehbinder's classification scheme, R.I. Ramanauskas' methods and recommendations, as well as the graphic differentiation of the thermal images of drying [7, 8]. We determined the equilibrium binding energy (E) in the dependence on the temperature of the frozen cheese. For the calculations, we used the D.G. Ryutov's formula. It is known that the kinetics of the freezing process is a gradual transition of the heterogeneous water into ice in accordance with its binding energy in descending order of [1].

The data analysis showed that due to the temperature lowering in cheeses in the frozen system, a portion of the water remains unfrozen, and it has a high binding energy with the dry substances (Table 1).

Table 1. The binding energy of water in frozen cheeses

Temperature of the product, ºC	Parameters	
	L, кJ/кg	E, кJ/кg
-5	323.5	5.92
-10	313.4	11.40
-20	300.5	21.80
-30	271.0	29.76
-40	256.1	37.13
-50	229.2	41.95
-60	222.3	48.80
-70	206.4	55.00
-80	191.5	58.60
-90	177.4	68.30
-100	163.2	76.00

The process of water freezing goes from low to high-energy forms of the water bond. Low temperatures do not break the chemical bond between the product and the moisture, and so it is hard to completely remove the moisture. At temperatures below minus 70÷80°C the chemical bond between the moisture and the proteins is the strongest, and thus it does not turn into ice. We studied the resistance of a combining form of casein complex to freezing by its ability to bind moisture. While the proportion of bound water in the protein matrices structure remains the same, the stability of the protein molecules in the frozen cheese increases. We considered the degree of water freezing based on its unavailability for chemical reactions and on the preservation of the monomolecular layer around the protein macromolecules [9]. The results of the research on the change in the water states in the cheeses at low temperatures were used to design the phase diagram of the water solutions in cheeses.

Fig. 2 shows the effect of the freeze modes on the change of the amount of the most tightly bound forms of the water with proteins, i.e., on the hydration of the cheeses' combining form of casein complexes. The amount of moisture of the mono- and multilayer adsorption in the non-freezing water phase served as a criterion of change in the cheeses' protein complex. The kinetics of the bound water's slow freezing is shown as the product's temperature decreases. The freezing mechanism is such that at -10°C the amount of bound moisture has not changed due to the removal from the cheese of unbound moisture, loosely kept together by proteins (capillary moisture). Thus, the water modifies its physical state (transition into ice) in a relatively loose form (micro capillaries and coarse cheese pores) with a low binding energy (E = 11.4 kJ/kg) with the dry cheese matter.

At the stage of the cheese freezing to -20°C, the moisture of the various binding forms transforms into the crystalline form, except for the tightly-bound moisture of the mono- and multi molecular adsorption. Under these conditions, the freezing energy of water binding with the polar groups of proteins is higher than the energy released during the crystallization and turning into ice.

For example, in the cheese "Sovetsky", 18.2% of the liquid phase was recorded at that temperature, and this quantitatively corresponds to the degree of the cheeses' hydration in the form of bound by absorption moisture. However, we noticed the beginning of the decrease in the amount of that water, which could be due to the crystallization of the diffusion layer of the water of multilayer adsorption.

Based on the energy images of cheese "Sovetsky", P.F. Krasheninin, N.I. Gamayunov and V.P. Tabachnikov noted that 15% of the moisture in the cheese mass corresponds to the moisture's maximum binding energy at which the water molecules are unable to separate from the protein mass [7]. Apparently, in this connection, when the temperature in the centre of the frozen product reached -30°C, 13.2% of the water remained unfrozen, and it retained the hydrate shell around the micelles of casein (Fig. 2). In the subsequent phases of the freezing, the water tightly bound by the proteins in the form of MMA (moisture-monomolecular adsorption) was gradually freezing out, turning into ice. Further lowering of the product temperature below -30°C (-40°C, -50°C, etc.) leads to a quantitative reduction of the liquid phase of the bound water (VMA) and thus dehydrates the cheese protein complex, especially in the semi-hard cheeses with a low temperature of second heating.

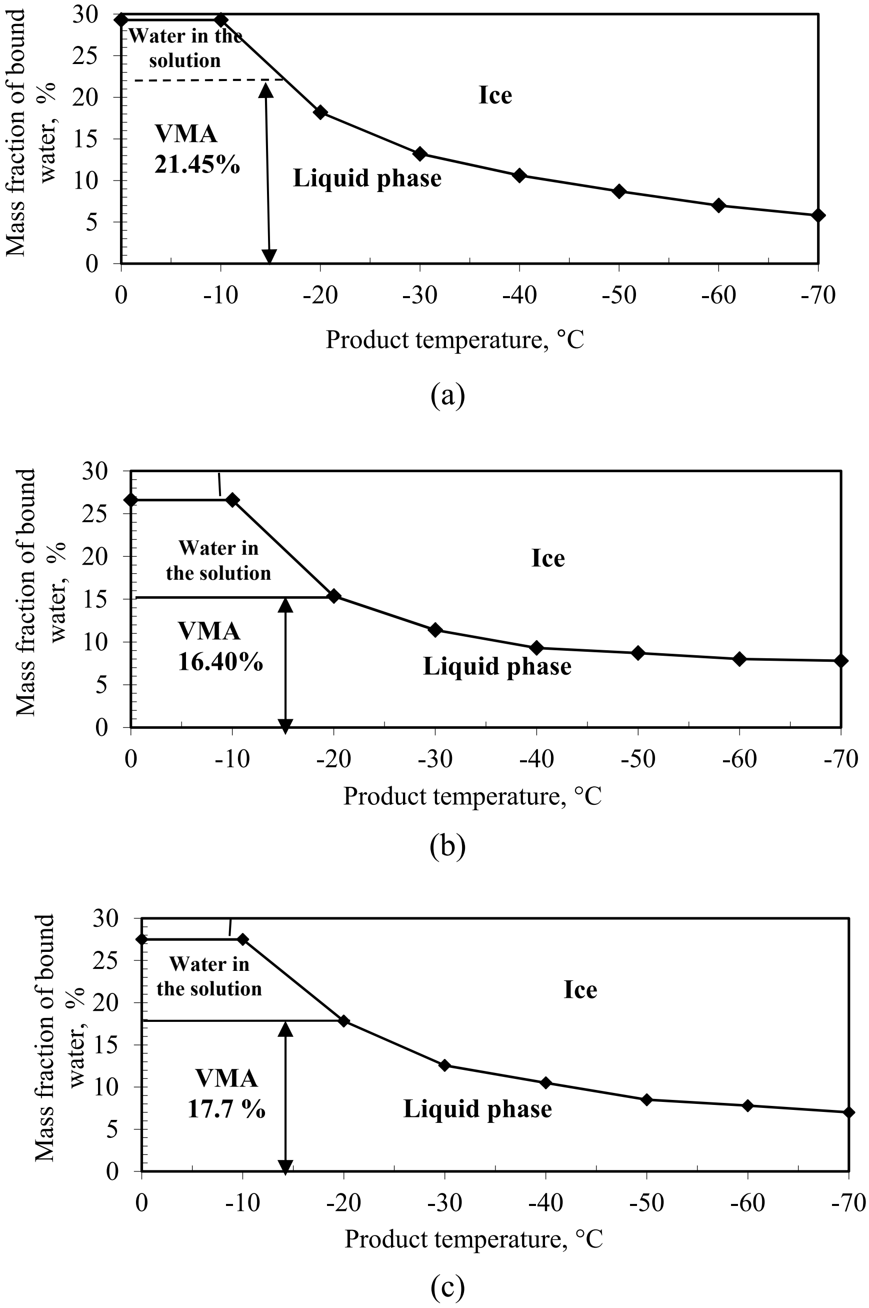

Fig. 2. Change in the amount and physical condition of the bound water in the cheese depending on the freezing temperature (BMA – related moisture adsorption): (a) Group of "Sovetsky" cheeses, (b) Group of "Rossyisky" cheeses, and (c) Group of "Gollandsky" cheeses.

In these cases, the protein macromolecules lost retained water, and they were willing to participate in the new intermolecular bonds. In such a state intermolecular protein aggregation is possible. It seems like this happened in our experiments, when at low temperatures (below -40°C) we observed structural changes in cheese consistency. Thus, freezing the product at the average volume temperature of -18 ÷ -20°C allowed for the best preservation of the hydration of the combined casein complex. The values of the ultimate temperatures for the freezing of the product are sufficient at -20 (± 2)°C, to maintain the hydration of the cheese's proteins without affecting its structure. We observed the same results when we analyzed the changes in the bound water state during the freezing of the cheeses of the "Gollandsky" type. Figure 2 shows that the amount of moisture, tightly bound by the curd proteins, remained unchanged during the freezing down to -20°C. In the other freeze modes the proteins were slowly losing a small amount of water due to its tendency to crystallize. In this case, the amount of bound water or its degree of hydration served as the criterion for evaluating changes in the protein complex of the frozen cheese. Figure 2 shows that the bound by proteins moisture does not crystallize at -20°C, and its physical properties remain unchanged. This temperature suits the maximum level of the conservation of the proteins' structure. The amount of adsorption-bound moisture (BMA) under these conditions is consistent with the maximum hydration of the protein complex and is equal to: 21.45% – in "Sovetsky" cheese, 12.76% – in "Rossyisky" cheese, 17.7% – in "Gollandsky" cheese.

Research results show that the degree of the crystallization of the water regulates changes in the product. Thus, at the average volume temperature of average -20°C, the proteins' water-holding capacity and the curd's hydrophilic properties were preserved. When freezing to a lower temperature, resulting in an unwanted transition of the micelle-bound water into ice, there were structural changes that led to the appearance of an extra elastic and crumbly consistency of the cheese. An analysis of the data on the cheese's durability in storage showed that products stored at a temperature of -20 (± 2)°C, had the longest shelf life and the most satisfactory consistency. In this freezing condition there were no factors damaging the protein complex (Fig. 3).

The analysis of the graphs in Fig. 3 shows that in this mode the speed of the chemical reactions is significantly reduced. The main factors in this case are: 1) the presence of unfrozen water (18.3%) in the form of a layer of the polarized water molecules in the form of a monolayer adsorption (BMA), which has a low activity and is not available for the microorganisms' activity; 2) the lack of water which is a solvent with a high concentrations of solutes, that causes the denaturation changes in the proteins. In this freezing mode (volume average -20°C) the hydration of the proteins and hence the water-holding ability of the curd is retained in full. We found that the cheeses had defects in the consistency, caused by the dehydration of the protein complex during the freezing to the lower ultimate temperatures (-30 ... -40°C). The cheeses frozen to a temperature of minus 20°C had the best quality. In this mode, the unfrozen water (18.3%) with the 31.2% concentration in the form of the moisture of the physical and chemical bond, was the most tightly-held by the cheese's protein complex.

Because this type of moisture can't be a solvent, we can assume that the freezing conditions, the physical and chemical changes of the aqueous phase (the viscosity, the degree of mobility of the solutes) will not affect the native structure of the proteins during freezing and storage, unlike in other freezing modes. The preserved hydrophilicity of the proteins frozen to -20°C resulted in a cheese with good texture and water binding capacity. It should be noted that this allows for a fairly low water activity of Aw = 0.67–0.70, which is below the minimum required for microbial activity [10]. Strong energy of water interaction with the protein macromolecules characterizes a low mobility of the adsorbed water molecules in the non-crystallized low-temperature phase. Therefore, due to the lack of a source of enzymatic decay during storage the cheeses' durability will increase significantly.

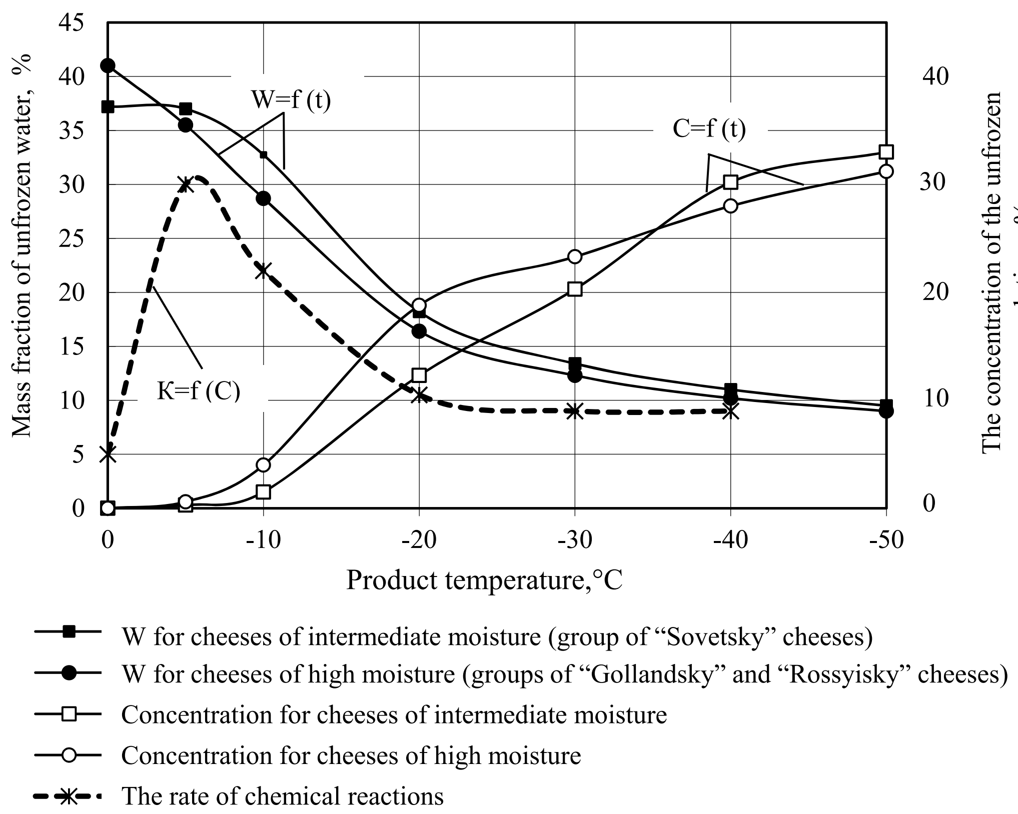

Fig. 3. Factors of stability of deep-frozen cheeses during storage.

Moreover, the changing of the pH to a level of 5.3–5.5 corresponds to a zone of maximum swelling of the proteins, which suggests saving of the water-binding capacity in the curd's proteins. Other freezing modes resulted in poorer chesses' properties due to the chemical reactions caused by a high concentration of the unfrozen solution and by the freezing out of the bound water. Thus, as the proportion of the bound water in the protein matrix structure remains unchanged, the stability of the protein molecules in the frozen cheeses increases. Therefore, by freezing to -20°C the hydration of the protein complex remains unchanged, and the frozen cheeses have high organoleptic properties.

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Please cite this article in press as: Buaynov O.N. and Buaynova I.V. The physical and chemical changes of water and the hydration of the protein complex in cheese during freezing. *Foods and Raw Materials,* 2016, vol. 4, no. 1, pp. 13–18. doi: 10.21179/2308-4057-2016-1-13-18.