# CRYOBIOLOGICAL STUDIES AND FREEZE DRYING OF COW'S MILK AND CURD

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## ABSTRACT

*An analysis of the specific technological conditions for lyophilization of dairy products (cow milk and curd), ensuring the preservation of product quality was carried out. Differential scanning calorimetry was used to study phase transitions in 3 variants of fresh cow's milk and 3 variants of low-fat curd at temperatures from -80°C to 50°C. An endothermic phenomenon in the interval (-5°C to 40°C) was observed on all thermograms, which corresponds to the melting of the samples. The initial temperature of melting ($T_{im}$) was around -5°C. The melting point peak ($T_{mp}$) in milk samples varies from 9,83 ± 0,76°C to 12,33 ± 2,43°C, while in crud samples – from 6,96 ± 2,27°C to 9,73 ± 1,31°C. Samples with a higher moisture content have higher values of $T_{mp}$. The obtained results made it possible to substantiate thermodynamically the temperature regimes of lyophilization. Properly programmed temperature regime of lyophilization provided optimal drying speed and good quality of the final products.*

Keywords: *lyophilization, differential scanning calorimetry, cow milk, curd.*

## INTRODUCTION

Freeze drying (lyophilization) is a method of separating water from the frozen matrix of materials, in which water passes directly from solid to vapor phase [1]. As a method of dehydrating materials, freeze-drying produces the highest quality final product, compared to conventional drying methods. One of the main advantages is that the solid matrix of the frozen product does not allow the material to shrink during drying, thus preserving its structure and shape to the maximum extent possible. The result is a porous structure that rehydrates relatively quickly and almost completely when water is added to the dry product. Another major advantages of lyophilization is the low drying temperature, the relatively low percentage of unfrozen water and the rapid passage of the product to a dehydrated state at a negative temperature. This abrupt transition minimizes destructive processes, such as non-enzymatic browning, protein denaturation and various enzymatic reactions during drying. The low operating temperature of drying inhibits the transport reactions that cause the loss of volatile and aromatic substances. In addition, the resulting lyophilized products are reduced in weight and stable at a room temperature [2].

The presence of unfrozen water is inevitable in materials subjected to freeze-drying [3]. However, there is a sufficiently low temperature at which enough of the water content is frozen for the process to proceed properly and to obtain satisfactory quality of the final product [4].

As a rule, the process of freeze drying involves three phases - freezing, primary drying and secondary drying [5]. Initially, the treated material is frozen, and in this state is subjected to a reduced pressure (vacuum). When a certain amount of heat equal to or greater than the latent heat of sublimation (mainly dependent on the binding energy of the water and the eutectic temperature) is supplied to the material, the water in solid phase is converted into a gaseous (vapor) phase [6]. The vapor, released during sublimation condenses on a cooled surface – desublimator (condenser) while non-condensing gases are pumped into the atmosphere.

Food lyophilization requires specific conditions that reduce damage and maintain product quality. In many cases, this leads to a prolonged process cycle, which necessitates its optimization. Therefore, the primary goal of research on freeze drying is to improve process economics by reducing processing time [5]. Optimization of the freeze-drying process needs to characterize the physical state of frozen and dried product [7]. Food products represent complex solutions, emulsions, suspensions or structural systems. Their composition and structure also affect their behavior during freezing and subsequent drying, and thus their further properties (storage stability, rehydration, etc.) [2]. There is a significant correlation between the process parameters and the quality of the final product [8]. This also applies to a great extent to milk and dairy products.

Milk is a diluted emulsion consisting of a dispersed oil phase and an aqueous colloidal continuous phase. The physical properties of milk are similar to those of water, but are altered by the presence of dissolved proteins, lactose and salts in the continuous phase and by the degree of dispersion of the emulsified and colloidal components. Lyophilization of milk is considered to be the best way to extend the shelf life while preserving its nutritional properties and flavor. After rehydration, the temperature stability of proteins is similar to that of fresh milk [9].

Curd is derived from whey - a by-product of the production of white brine cheese. The liquid obtained after precipitation of casein in the cheese production process contains approximately 55 % of the nutrients present in milk [10]. Among the nutrients found in the whey, the major part corresponds to lactose 39 - 60 g $L^{-1}$, proteins 6 - 8 g $L^{-1}$, lipids 4 - 5 g $L^{-1}$ and mineral salts (8 - 10 % of dried extract) [11]. The production of whey curd including methods such as ultrafiltration, evaporation or drying and a final protein concentration vary from 34 % to 85 % [12, 13]. The high nutrient content of curd makes it a valuable product with a variety of applications in the food industry, but like milk, it has a limited shelf life. This problem could be solved by freeze drying, which is one of the methods for significantly extending the shelf life of foods and food concentrates.

The aim of the study is to analyze the specific technological conditions for lyophilization of dairy products (cow's milk and curd), in which to ensure the preservation of product quality and reduce the damage caused by low temperature treatment.

## EXPERIMENTAL

The analysis was performed with samples of dairy products, purchased from a local supermarket in the region of Sofia, Bulgaria - 3 batches of skimmed cow's milk (variant 1, variant 2, variant 3) and 3 batches of low-fat curd (variant 1, variant 2, variant 3). Low fat curd is obtained by coagulation of whey proteins after the production of white brined cheese. All the chemicals used were of analytical grade.

**Differential scanning calorimetry**

The sample thermograms were obtained on the SETARAM 141 Differential Scanning Calorimeter (France) under the following conditions: the dairy samples were first cooled to (-80°C) with liquid nitrogen and then heated to 50°C at a rate of 5°C/min. The analysis was done 3 times for each sample.

**Lyophilization**

The freeze drying was carried out in vacuum sublimating installation TG 16.50 (Hochvacuum", Germany), with contact heating of the shelves.

**Physico-chemical analysis of lyophilized dairy products**

The analyses of the products have been carried out as follows:

*Determination of residual moisture after lyophilization*: The moisture content of the lyophilized samples was measured with Sartorius Thermo Control YTC 01L balances with infrared heating of the sample;

*Total protein content* - Kjeldahl method by ISO 8968-1:2014 [14];

*Total fats* - by extraction with hexane in "Soxtec 2005" apparatus;

*Total ash* - by sample mineralization in muffle oven by AOAC Official Method 930.30 [15].

**Statistical analyses**

The analysis of each sample was performed in triplicate. Data are presented as mean ± standard deviation (SD) and were considered significant at $P < 0.05$.

## RESULTS AND DISCUSSION

The results of the differential scanning calorimetry for each sample of dairy products are shown in Figs. 1 and 2. The thermal curve analysis provides information on enthalpy, melting points and crystallization. All six thermograms look similar – an endothermic phenomenon between -5°C and 40°C is observed, which corresponds to the melting of the samples. $T_{im}$ was observed at about -5°C. Up to -80°C there is no change in the heat capacity and thus a glass transition. This allows

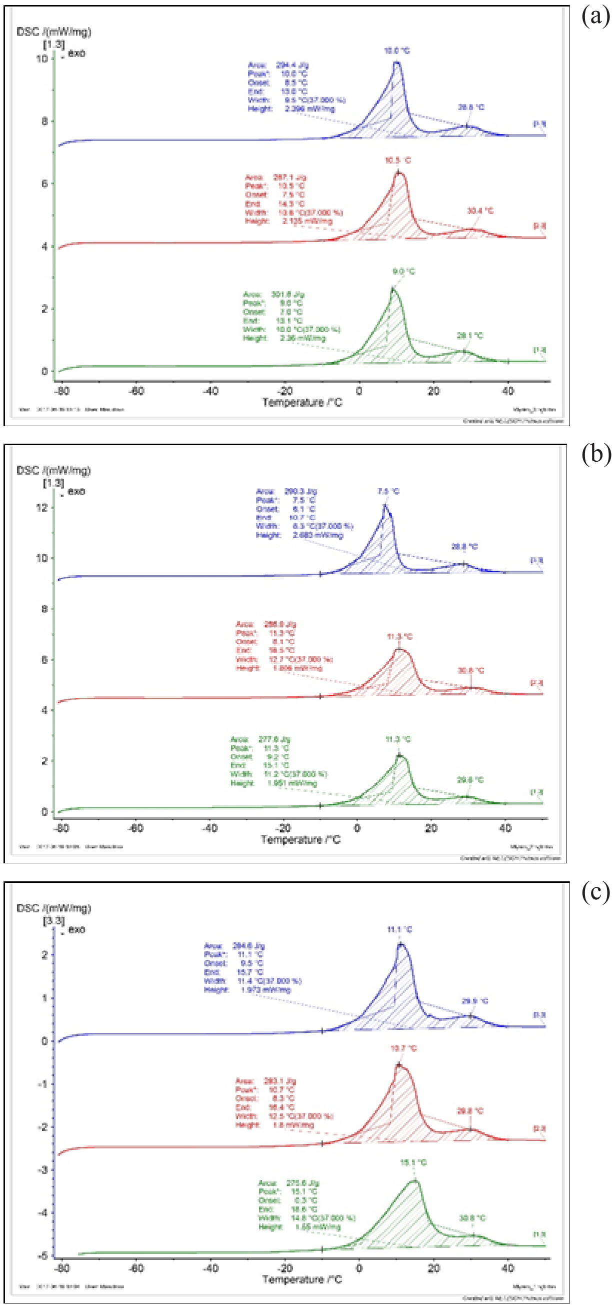


Fig. 1. DSC thermograms of cow milk samples: (a) variant 1; (b) variant 2; (c) variant 3.

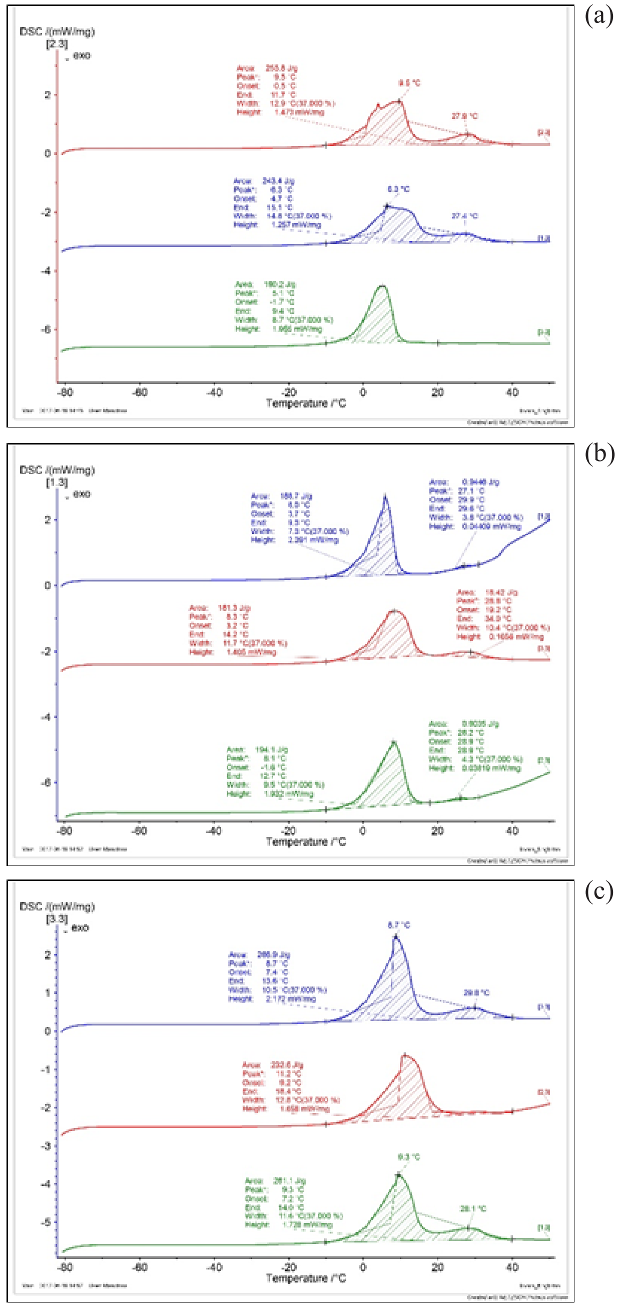


Fig. 2. DSC thermograms of curd samples: (a) variant 1; (b) variant 2; (c) variant 3.

the samples to be frozen under standard conditions in a freezer and no lower temperature treatment is required.

Table 1 shows the values of water content, dry matter content and $T_{mp}$ of the experimental milk samples. Higher moisture content is found to increase the $T_{mp}$ value.

The temperature regime of freeze drying must be chosen in such a way as to obtain the optimum ratio between the speed of drying and the quality of the product, and in order to obtain quality products 75 % - 90 % of the water must be removed at temperatures below 0ºC.

The phenomena of ice formation are more complex in the presence of organic substances in the system: crystallization inhibitors - carbohydrates, organic acids, vitamins, and various stabilizers. These substances form a wide eutectic zone, with the initial melting accompanied by a weak endothermic effect [5].

Table 1. Water content, dry content and $T_{mp}$ in samples of cow milk and curd. Data are presented as means ± SD.

| Samples | | Water content g/100g | Dry content g/100g | $T_{mp}$ (°C) |
|---|---|---|---|---|
| Cow milk | Variant 1 | 87,50±1,25 | 12,50±0,63 | 9,83±0,76a |
| | Variant 2 | 88,12±0,68 | 11,88±0,41 | 10,03±2,19a |
| | Variant 3 | 88,75±0,98 | 11,25±0,73 | 12,33±2,43b |
| Whey curd | Variant 1 | 78,05±2,35 | 21,95±0,56 | 6,96±2,27a |
| | Variant 2 | 80,24±1,41 | 19,76±0,91 | 7,46±1,27a |
| | Variant 3 | 83,35±1,65 | 16,65±0,72 | 9,73±1,31b |

For lyophilization of food products, sublimation should be carried out at a temperature not higher than $T_{im}$. This is also the optimal theoretical sublimation temperature at which the initial properties of the product are preserved in terms of lyophilization.

**Lyophilization of the tested milk products**

In order to introduce optimal parameters of lyophilization of the tested milk products, the process was carried out in a pilot scale freeze-dryer with contact heating of the shelves. For provision the production of a quality lyophilized product, the following factors are of great importance: temperature and freezing rate, initial sample composition, secondary drying final temperature, packaging and storage conditions.

During the lyophilization, the process was monitored in all phases, mainly sublimation (primary drying) and secondary drying. The specific technological regimes

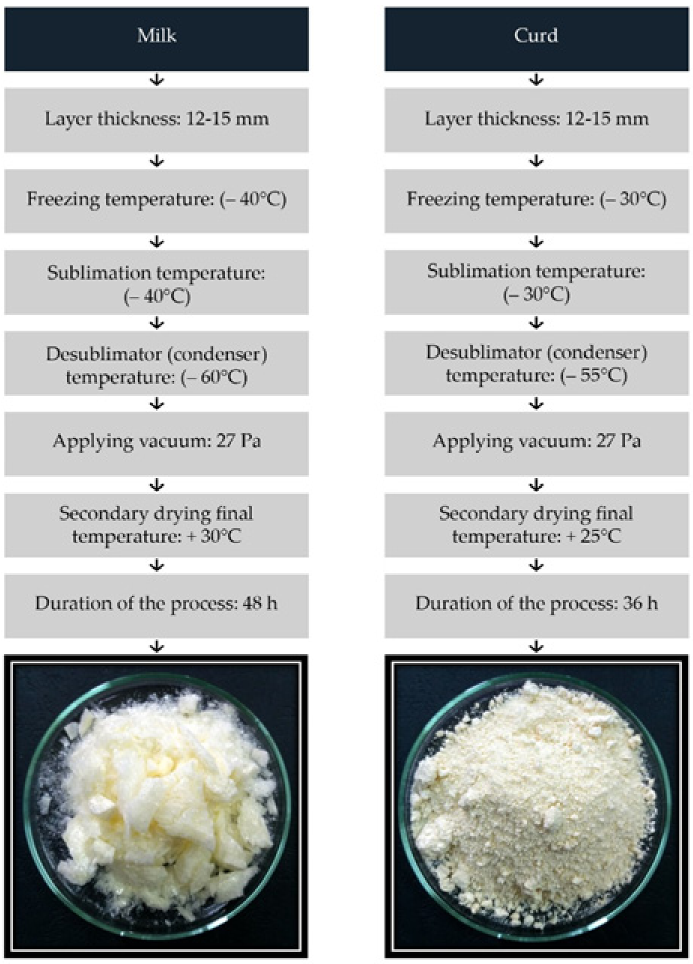


Fig. 3. Technological parameters of the freeze-drying process.

Table 2. Composition of samples of lyophilized milk and curd. Data represented means ±SD.

| Samples | | Residual moisture (%) | Fat (%) | Protein (%) |
|---|---|---|---|---|
| Lyophilized milk | Variant 1 | 3.14±0.32 | 4.80 ± 0,15 | 33.58 ± 0.78 |
| | Variant 2 | 3.58 ± 0.47 | 4.23 ± 0.52 | 32.95 ± 0.84 |
| | Variant 3 | 4.20 ± 0.76 | 4.30 ± 0.42 | 32.43 ± 1.06 |
| Lyophilized curd | Variant 1 | 2.96 ± 0.24 | 5.20 ± 0.56 | 43.58 ± 0.89 |
| | Variant 2 | 2.80 ± 0.58 | 5.08 ± 0.78 | 43.25 ± 1.31 |
| | Variant 3 | 3.20 ± 0.41 | 5.10 ± 0.56 | 42.90 ± 1.08 |

for sublimation drying of cow milk and curd are summarized in a diagram on Fig. 3.

The temperature regime of sublimation drying was programmed to obtain the optimum drying rate for good quality of the final products. At the beginning of the process, the sublimation installation is manually entered into operation mode, with the predefined mode parameters being set. When the set parameters are reached, the installation switches to automatic mode.

After freezing the samples to the appropriate temperature, the desublimator (condenser) is cooled to a temperature of (-55°C) - (-60°C), which ensures a sufficiently low partial pressure of water vapor above the desublimator and creates favorable conditions for mass transfer. After reaching the operating temperature of the desublimator, the sublimator switches to operating mode.

Sublimation of ice is only possible if the equilibrium between the partial pressure of the vapor in the material and in the volume of the drying chamber is disturbed. Therefore, after reaching the required vacuum, which corresponds to a certain negative temperature, energy is supplied to the material, through which the freeze-drying process is performed. The sublimation energy used to remove the ice in the main drying process - $\Delta H_S$, is brought to the product through the shelves in the form of heat energy – dQ.

The primary drying process is completed when the material temperature reaches positive values. This is followed by the separation of the bound water in the product - secondary drying, whereby the temperature rises slowly to a certain limit depending on the properties of the dried material.

The qualitative changes of the surface layers of the samples determine the permissible values of the temperature of the treated material in the secondary drying. The secondary drying stage involves the removal of bound water by desorption. Usually, the amount of bound water remained is about 10 % - 35 % of the total moisture content [16]. The temperature of the tested samples in this stage is in the range of 25°C to 30°C, and the residual moisture content in final product is below 4.5 %. The proper determination of the temperature and duration of drying resulted in the quality end products.

The results of the physico-chemical analysis of each sample of the obtained lyophilized dairy products are shown in Table 2. All samples are with low residual moisture: from 2,96 % to 4.20 %. The low water content guarantees the long term storage at room temperature without any changes in the main components.

## CONCLUSIONS

The analysis of the phase transitions of cow's milk and curd at temperatures from (-80°C) to 50°C, allows to determine the optimal temperature regimes at different stages of freeze drying and the establishment of the operating temperature zone. The thermograms of all samples have a similar appearance with a clear peak in the range (-5°C - 40°C), which corresponds to the melting of the samples. Up to (-80°C) there is no change in the heat capacity and respectively no vitreous transition. This allows the samples to be frozen under standard conditions in a freezer with no requirement for lower temperature treatment. Important for the obtaining of a quality lyophilized product are the composition of the material (and especially the water content), the temperature and the freezing rate as well as the duration of the primary drying and the final secondary drying temperature. It is necessary to separate 75 % - 90 % of the moisture at negative temperatures. In the sublimation drying mode, the duration of the primary drying of the milk samples is from 8 to 10 hours. The final secondary drying temperature is in the range of 25°C - 30°C. The obtained lyophilized milk and curd have a porous structure which rehydrates easily and low residual moisture content which. guarantees the long term storage at room temperature.

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