

*Вивчено динаміку заморожування напівфабрикату для приготування напою смузі. За допомогою низькотемпературного калориметру встановлені діапазони кристалізації та кількість вимороженої вологи досліджуваного напівфабрикату. Наведено результати мікробіологічних досліджень, встановлено кількісну зміну мікрофлори у процесі низькотемпературного зберігання. Отримані дані можуть бути використані для визначення раціональних режимів заморожування та розморожування напівфабрикату і дадуть можливість розширити асортимент замороженої продукції*

*Ключові слова: виморожена волога, мікрофлора, заморожена продукція, температура кристалізації, кінетика заморожування, дефростація*

*Изучена динамика замораживания полуфабриката для приготовления напитка смузи. С помощью низкотемпературного калориметра установлены диапазоны кристаллизации и количество вимороженой влаги исследуемого полуфабриката. Приведены результаты микробиологических исследований, выявлено количественное изменение микрофлоры в процессе низкотемпературного хранения. Полученные данные могут быть использованы для определения рациональных режимов замораживания и размораживания полуфабриката и дадут возможность расширить ассортимент замороженной продукции*

*Ключевые слова: вимороженная влага, микрофлора, замороженная продукція, температура кристаллизации, кінетика замораживания, дефростація*

# CRYOSCOPIC AND MICROBIOLOGICAL STUDY OF THE SEMI-FINISHED PRODUCT FOR MAKING A SMOOTHIE DRINK

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## 1. Introduction

A major role in strengthening health of people belongs to providing their full and healthy nutrition. A steady growth in the production of high-quality food products and beverages is required in order to accomplish this goal. Healthy nutrition is achieved by a balanced consumption of nourishing nutrients. The human body cannot receive a balanced diet that contains the entire set of essential substances because of the technological treatment of raw materials [1]. To ensure the proper and healthy diet, it is necessary to develop and create new products with high quality. Such products must be balanced in their composition, as well as enriched with different functional properties [2]. When applying plant raw materials, there occurs the enrichment of products with natural nutrients, creating thereby the products with preventive and health-improving properties.

The utilization of Ukrainian plant raw materials and the implementation of developments related to new technologies in food production is a priority direction in the technology of food industry and public catering.

Fruits and berries have a short shelf life, which determines the need to study processing techniques for the year-round provision of people with such products [3]. One of the unique techniques to preserve the nutritional and biological value of fruits and berries is the low-temperature refrigeration.

Refrigeration of fruit and berry semi-finished products makes it possible to obtain marketable products, which are sold to people, and then processed into drinks, juices, compotes, jams in the off-season period [4]. Freezing is based on a change in temperature below the cryoscopic, which terminates or suspends most physical, biochemical, and microbiological processes. Quick frozen fruits and berries are almost not inferior to fresh ones for their nutritional value and content of water-soluble vitamins [5].

Development and wide implementation of effective technologies for freezing, low-temperature storage and processing of fruits and berries, will contribute to solving the task on the balanced nutrition of population, on bringing down the level of diseases, on improving quality of life. In addition, the implementation of such technologies will significantly enlarge the base of local processing industry, the development of which, in turn, would promote the development of agricultural production in a region. The potential of the use of fruits and berries for producing frozen semi-finished products remains inexhaustible given the commodity-related indicators and functional-technological properties. Extending the assortment of semi-finished products made from fruits and berries, as well as maintaining useful properties and taste quality, is an important and relevant scientific-technical task.

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## 2. Literature review and problem statement

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Frozen fruit and vegetable semi-finished products are intended for fast preparation, they play an important role in modern society with people leading a rapid pace of life. Refrigerated canning is considered to be the most appropriate technique for preserving fruits and vegetables [6]. The laws of change in the thermophysical properties of components are important for the process of freezing and low-temperature storage of fruit and vegetable products. Paper [7] explored changes in heat capacity, heat conductivity, and fraction of frozen moisture. The calculation is based on a physical model of the process of moisture crystallization in the solutions of glucose, sucrose, and fructose in water. Dependences of thermophysical properties on a change in temperature during freezing were obtained. The study found that peaks of heat generation during crystallization of the components of a fruit and vegetable mixture occur at cryoscopic temperatures, such as the crystallization temperature of fructose solutions (minus 21 °C), sucrose (minus 8.5 °C), and glucose (minus 5.3 °C). It was established that the most favorable temperature at which a fruit and vegetable mixture should be stored, in order to exclude the development of microorganisms and extend shelf-life, is minus 22 °C.

The proper quality of a ready fruit semi-finished product is one of the main, and basic, problems at each stage of freezing of berries and fruits. Paper [8] studied the process of freezing and storing of fresh raspberry, red and black currants, grown in Latvia. To examine the process of freezing and storing, a layer of volumetric frozen berries with a thickness of 10.0 cm was chosen. The dynamics of freezing the berries is characterized by measuring the temperature in the layer and at its surface. Based on the results of research, the authors tested the developed equipment for measuring a heat flow. The study demonstrated that the effect of temperature had to be differentiated; the obtained thermal-physical parameters of frozen berries would be subsequently applied to mathematically model both the process of storing and the process of thawing the frozen berries. The study provided additional information on the kinetics of the process of freezing berries and fruits, which could more accurately predict the change in quality. The work does not indicate the reversibility or irreversibility of the freezing-thawing process of berries and fruits.

It was found in paper [9] that the amount of ascorbic acid in frozen strawberry remains unchanged over a year, if strawberry is kept below  $-18$  °C. These data were fundamental for setting the temperature of  $-18$  °C as the upper limit during storage of frozen foods, and the use of biologically active ascorbic acid as an indicator of the deterioration in the state of raw materials.

Study [9] explored two different methods of freezing (slow at  $-20$  °C, and quick at  $-50$  to  $-100$  °C) of Iranian strawberry (the variety Cordestan). Over three months of storing, the authors registered changes in color, in the content of anthocyanin, ascorbic acid and pH. For the method of slow freezing, only the pH level at all three temperatures was a statistically significant difference. It was established that the storage temperature of  $-18$  and  $-24$  °C was optimal for maintaining qualitative characteristics (color, texture, taste, and integrity) of strawberry. There is no data on the amount of a mass fraction of the frozen-out moisture at different temperatures.

In paper [10], much attention is paid to the effect of the freezing process on a change in the bioactive compounds in fruits; the results, however, are often contradictory. Two

varieties of strawberry, Dorit and Selva, were investigated in study [11], at the research and applied gardening center in Isparta; they were stored at a temperature of 0 °C and a relative humidity of 90–95 % for 10 days. The authors noted a reduced content of malic, oxalic and fumaric acids during storage. It was described in paper [12] that the texture of cherry and strawberry, when canned cold, depended on the composition of pectin fractions, rather than on the amount of pectin in fruits.

In work [13], the purpose of study was to examine effects of the conditions of freezing, thawing, and storing on the physical-chemical properties of fruit semi-finished products. The study revealed that the selection of varieties and maturation stages are important factors contributing to the process of freezing and maintaining quality of a ready fruit semi-finished product. The results of research would allow the transportation of products to distant markets that do not have access to fresh fruit, and would allow using seasonal fruits throughout the year. However, there is no data in the paper on the rate and the process of ice formation when freezing a semi-finished product.

Much attention is paid to studying microbiological parameters in the process of production and storage of frozen semi-finished products with the addition of plant raw materials [14]. It was found experimentally that the process of prolonged refrigerated storage of a frozen semi-finished product did not lead to any changes in the microbial composition; all microorganisms cease to multiply, some of them die [15].

Scientists conducted a study into microbiological quality indicators of frozen semi-finished products made from large-fruit cranberry and ordinary viburnum during 9 months of refrigeration at a temperature of  $-18$  °C. Compared to fresh frozen berry semi-finished products, the amount of mesophilic-aerobic and optionally anaerobic microorganisms after 1, 2, 3, 6, 9 months of refrigeration storage reduces considerably. The results obtained indicate that over the specified period of storage the microbiological parameters of semi-finished products met the standards [16].

Thus, an analysis of the scientific literature has revealed that it is not always that high quality indicators of food products during storage are ensured by low temperature and elevated freezing rate. The process of refrigeration depends on the properties of raw materials, the ratio of components and techniques for preliminary preparation. There is a need in a scientific substantiation of conditions of freezing for raw material components, which, within the limits of techniques for cold preservation, and technical characteristics of devices, vary in a rather wide range.

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## 3. The aim and objectives of the study

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The aim of present study is to examine the kinetics of freezing of a semi-finished product for making a smoothie drink, and to explore the microbiological quality indicators over 270 days of storage at a temperature of  $-18 \pm 2$  °C.

To accomplish the aim, the following tasks have been set:

- to conduct cryoscopic study of a semi-finished product for making a smoothie drink;
- to determine the ranges of temperatures for crystallization and the mass fraction of frozen moisture;
- to conduct microbiological study into quality of a frozen semi-finished product for making a smoothie drink, which was stored for 270 days at a temperature of  $-18 \pm 2$  °C.

#### 4. Materials and methods of research

The object of the study was a semi-finished product for making a smoothie drink, made from strawberry, dried apple, and the oat flakes “Hercules”. Strawberry of the medium-early variety “Ducat”, cultivated in Ukraine, was used. A given strawberry variety is suitable for processing and storing at low temperatures. Dried apple of the variety “Borovinka” was selected because it differs from other varieties in the elevated resistance to low temperatures. The fruits can be transported, they are well preserved until mid-winter. Apple of this variety is consumed both fresh and after any processing.

The methods applied in this study are described in detail in paper [17].

#### 5. Results of research into basic parameters of the freezing process and microbiological indicators of a semi-finished product for making a smoothie drink

A general diagram of the thermogram of freezing (to a temperature of  $-20\text{ }^{\circ}\text{C}$ ) and heating (to the ambient temperature) of the examined sample is shown in Fig. 1.

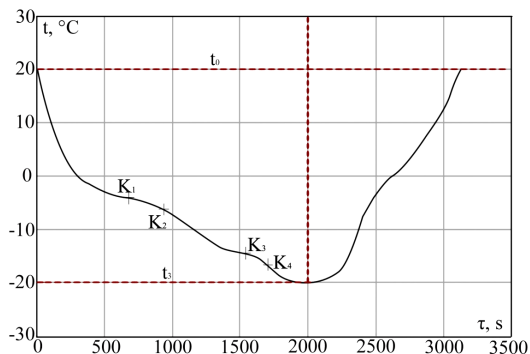


Fig. 1. Mean temperature of the sample at freezing (to  $t = -20\text{ }^{\circ}\text{C}$ ) and defrosting (to the room temperature):

- $K_1$  – cooling the sample until ice formation;
- $K_2$  – crystallization process of part of the frozen water;
- $K_3$  – cooling the sample and crystallization of the non-frozen water;  $K_4$  – cooling the sample to the freezing temperature  $-20\text{ }^{\circ}\text{C}$

The thermogram in Fig. 1 is divided into two sections. The left part of the thermogram is the freezing section for a temperature of freezing ( $-20\text{ }^{\circ}\text{C}$ ), which is constant, the right part is the defrosting. It should be noted that the duration of the freezing process is larger than that of defrosting.

Characteristic four regions are observed along all curves based on critical points:

1. From the beginning of freezing to point  $K_1$  – cooling the sample until formation of ice.
2. From point  $K_1$  to point  $K_2$ , there is a process of crystallization of part of the water (frozen).
3. From point  $K_2$  to point  $K_3$  – cooling the sample and the beginning of crystallization of water (non-frozen).
4. From point  $K_4$ , the sample is cooled directly to the freezing temperature of  $-20\text{ }^{\circ}\text{C}$ .

Table 1 gives an analysis of the thermograms of the freezing process of the examined sample.

Thus, it was experimentally established that the studied sample had two ranges of crystallization and recrystallization of the frozen moisture. Freezing at  $-20\pm 2\text{ }^{\circ}\text{C}$  contrib-

utes to the complete preservation of products, and further keeping them at the temperatures within such a range ensures storage for a long period of time.

Table 1

Results of analysis of freezing curves of the examined sample

| Freezing $t$ , $^{\circ}\text{C}$ | Range 1 $t_{\text{crystallization}}$ of frozen moisture, $^{\circ}\text{C}$ | Range 2 $t_{\text{crystallization}}$ of frozen moisture, $^{\circ}\text{C}$ | Range 1 $t_{\text{melting}}$ of frozen moisture, $^{\circ}\text{C}$ | Range 2 $t_{\text{melting}}$ of frozen moisture, $^{\circ}\text{C}$ | Mass fraction of frozen moisture, % |
|-----------------------------------|---|---|---|---|-------------------------------------|
| $-20$                             | $-2.5...-5.1$   | $-13.85...-17.2$  | $-11.8...-10.2$   | $-5.1...-2.0$   | 68.6                                |

Fig. 2 shows a typical curve in the coordinates  $\Delta t-t$  of freezing the sample at a temperature of  $-20\text{ }^{\circ}\text{C}$ .

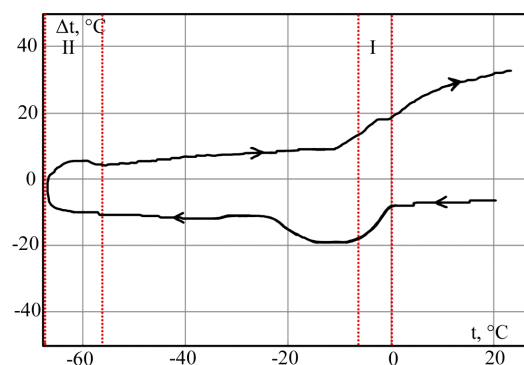


Fig. 2. Difference in temperatures output-input due to the temperature freezing-defrosting of the examined sample: region I – water crystallization; region II – water recrystallization in a sample

The upper region of the Figure relative to  $\Delta t = 0\text{ }^{\circ}\text{C}$  corresponds to the cooling and freezing of a semi-finished product, the lower region – heating. In a given coordinate system, curves  $\Delta t = f(t)$  are rather sensitive to the processes of crystallization and recrystallization of water in the samples (regions I and II).

The freezing curve varies depending on the technique of freezing, size, shape, chemical composition, and physical properties of products. As can be seen in Fig. 2, the freezing and defrosting curves of the semi-finished product sample do not coincide, that is, the character of the temperature dependence during freezing and defrosting is different. This indicates the irreversibility, during ice formation and thawing, of the plant fiber. The regions corresponding to critical points (I and II) along the defrosting curves are less pronounced. This is explained by a disruption in the system of hydrogen bonds between water molecules and hydroxyl groups of sugars and proteins, which is accompanied by a partial destruction of the colloidal system from the protein gel that was formed when the temperature decreased. As a result, the thermal conductivity of the sample increases during thawing.

Destruction of the colloidal plant tissue system is the reason for the disruption in permeability of the cell membranes, which determines the effect of the irreversibility of freezing.

When producing a frozen semi-finished product, much attention is paid to the level of microbiological contamination of plant raw materials and compliance to the sanitary and hygienic conditions during production.

In accordance with the acting hygienic standards for the frozen semi-finished products, the following groups of microorganisms are defined: mesophilic aerobic and optional anaerobic microorganisms, bacteria of *E. coli*; conditionally pathogenic microorganisms of bacteria of the genus *Salmonella*, yeast, and mold [18]. Table 2 gives microbiological data on strawberry, dried apple, and oat flakes, used for the production of a semi-finished product.

Table 2

Microbiological indicators of ingredients used for the production of a semi-finished product

| Indicator title  | Strawberry          | Dried apple         | Oat flakes          |
|--|---------------------|---------------------|---------------------|
| Mesophilic-Aerobic and Optionally Anaerobic Microorganisms             | 1.5×10 <sup>3</sup> | 9.7×10 <sup>2</sup> | 1.1×10 <sup>2</sup> |
| Bacteria of <i>E. coli</i>   | Not found           | Not found           | Not found           |
| Pathogenic microorganisms, incl. <i>Salmonella</i> per 25 g of product | Not found           | Not found           | Not found           |
| Yeast  | 1.5×10 <sup>2</sup> | 0.9×10 <sup>2</sup> | –                   |
| Mold fungi   | 9.1×10              | 6.8×10              | 8.7×10              |

The data given in Table 2 show that strawberry has the largest microbiological contamination while the oat flakes have the lowest value. Strawberry has the highest indicator of mesophilic-aerobic and optionally anaerobic microorganisms – 1.5×10<sup>3</sup>. The high value of mesophilic-aerobic and optionally anaerobic microorganisms indicates that there were improper conditions of cultivation, hygienic conditions of production were compromised, no proper storage conditions were maintained.

Food safety is predetermined by the environment non-contaminated with industrial waste, keeping the standards of cultivation without the use of chemicals, as well as the high-quality treatment and storage conditions.

To determine the microbiological parameters of changes during storage, the study was carried out immediately after production, followed by studies in 30, 60, 90, 180, and 270 days of refrigeration storage at a temperature of –18±2 °C. Results of research are given in Table 3.

Table 3

Microbiological indicators of a frozen semi-finished product during refrigeration storage at a temperature of –18 °C

| Indicator title   |   |  |   |  |
|---|---|--|---|--|
| Mesophilic-aerobic and optionally anaerobic microorganisms, CFU/1 g of product, not exceeding 5×10 <sup>4</sup> | Bacteria of <i>E. coli</i> per 0.1 g of product | Pathogenic microorganisms, incl. <i>Salmonella</i> per 25 g of product | Yeast CFU/per 1 g of product, not exceeding 5×10 <sup>3</sup> | 1×10 <sup>3</sup> Mold fungi CFU/1 g of product, not exceeding 1×10 <sup>3</sup> |
| <i>Freshly prepared (before freezing)</i>   |   |  |   |  |
| 1.2×10 <sup>3</sup>   | Not found                                       | Not found  | 1.3×10 <sup>2</sup>   | 8×10   |
| <i>In 30 days of refrigerator storage</i>   |   |  |   |  |
| 8.7×10 <sup>2</sup>   | Not found                                       | Not found  | 9.3×10  | 7.1×10   |
| <i>In 60 days of refrigerator storage</i>   |   |  |   |  |
| 7.5×10 <sup>2</sup>   | Not found                                       | Not found  | 9.1×10  | 6.8×10   |
| <i>In 90 days of refrigerator storage</i>   |   |  |   |  |
| 7.1×10 <sup>2</sup>   | Not found                                       | Not found  | 9.0×10  | 6.7×10   |
| <i>In 180 days of refrigerator storage</i>  |   |  |   |  |
| 6.5×10 <sup>2</sup>   | Not found                                       | Not found  | 8.7×10  | 6.5×10   |
| <i>In 270 days of refrigerator storage</i>  |   |  |   |  |
| 6.3×10 <sup>2</sup>   | Not found                                       | Not found  | 8.5×10  | 6.4×10   |

According to the requirements of normative documents, the number of mesophilic-aerobic and optionally anaerobic microorganisms (CFU per 1 g) should not exceed 5.0×10<sup>4</sup>, bacteria of *E. coli* and pathogenic microorganisms are not allowed. Yeast CFU per 1 g of product should not exceed 5×10<sup>3</sup>; mold fungi CFU per 1 g of product – not exceeding 1×10<sup>3</sup>. The study has shown that the sample of a frozen semi-finished product did not contain the gram-negative microflora – bacteria of *E. coli*, pathogenic microorganisms, including bacteria of the genus *Salmonella*.

**6. Discussion of results of the conducted study of the semi-finished product for making a smoothie drink**

It was established that the studied sample had two ranges of crystallization and recrystallization of frozen moisture. Freezing at –20±2 °C contributes to the full preservation of products and keeping them further at the temperature within such limits ensures storing them for a long period of time. A mass fraction of the frozen water in the examined sample is calculated, which is 68.6 %.

We have noted a reduction in the total microbiological contamination during long-term refrigerated storage, which indicates the negative effect of cold on the viability of microorganisms. The number of mesophilic-aerobic and optionally anaerobic microorganisms in 30, 60, 90, 180, and 270 days of refrigeration storage at a temperature of –18±2 °C slightly reduces compared to freshly prepared. The amount of yeast and mild fungi also decreases during storage. It is important to comply with sanitary and hygienic standards during production, packaging, storing, and selling since the complete extinction of the microflora does not occur.

The microbiological safety of frozen semi-finished products mainly depends on the contamination of raw materials, the sanitary-hygienic conditions of production and the speed of performing preparatory operations. The results obtained indicate that over the specified period of storage the microbiological parameters of a semi-finished product met the standards.

The experimental data obtained could be used to determine the rational freezing and defrosting modes of a semi-finished product in order to improve nutritional properties of frozen semi-finished products of functional purpose. Thus, the applied aspect of the obtained scientific result is the possibility to improve the technological process for producing a semi-finished product for making a smoothie drink. Research results could be used at the enterprises in food industry and in trade organizations.

Freezing is one of the safest and most effective techniques for canning, which significantly slows down deterioration of food products. However, many physical and biochemical reactions continue in frozen foods, albeit with less intensity. The disadvantages of the proposed method include the need for adjusting the modes and features of preliminary preparation to freezing depending on the type of a raw material and its chemical composition. Further direction of present research is determining the safety of a semi-finished product, specifically the level of the content of toxic elements, nitrates, pesticides, mycotoxins, and radionuclides.

**7. Conclusions**

1. In the course of research we determined the points of the beginning and the end of the process of crystallization – melting of the frozen moisture, as well as the its actual



amount in the examined sample. Freezing at  $-20 \pm 2$  °C contributes to the full preservation of products and keeping them further at the temperature within such limits ensures storing over a long period of time.

2. It was established that the freezing and defrosting curves of a semi-finished product sample do not coincide, that is, the character of the temperature dependence during freezing and defrosting is different. The temperature of the first and the second crystallization range is  $-2.5...-5.1$  °C, and  $-13.85...-17.2$  °C; the temperature of the first and the second melting range is  $-11.8...-10.2$  °C, and

$-5.1...-2.0$  °C. This testifies to the irreversibility of plant tissue during ice formation and thawing.

3. Based on the results obtained in the course of a microbiological study, the frozen semi-finished product for making a smoothie drink meets the established requirements of normative documents. This confirms the safety and quality of the semi-finished product consumption over 270 days.

4. The use of a frozen semi-finished product for making a smoothie drink would make it possible to extend the assortment of frozen products, to reduce expenditures of labor and time for the preparation of drinks at home and in public catering.

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