INFLUENCE OF FREEZING ON CHANGES IN THE STRUCTURE OF BLACK CURRANT FRUITS

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ABSTRACT

Object of research: frozen fruits of black currant varieties Bilorus Sweet, Minai Shmyrev.

Investigated problem: substantiation of the influence of the method, the duration of freezing on changes in the structure of black currant fruits of different degrees of maturity and pomological varieties.

The main scientific results: the advantages of fast freezing of black currant fruits in a fast freezing chamber with forced air circulation at a speed of 1.5–2.5 m/s at a temperature of –30…–32 °C compared to slow freezing in " Calex " freezers were established at a temperature of –20…–22 °C. Rapid freezing forces cooling, freezing and freezing due to a decrease in the temperature of initiation of ice formation, an increase in the rate of heat extraction and an increase in the temperature of freezing.

The area of practical use of the research results: food industry enterprises specializing in the freezing of plant products.

An innovative technological product: the technology of freezing black currant fruits, which allows to extend the period of consumption of high-quality fruits with a preserved structure.

Scope of application of an innovative technological product: nutrition of the population.

1. Introduction

1. 1. The object of research

The object of research is the black currant fruits of Bilorus Sweet, Minai Shmyrev varieties of varying degrees of ripeness.

1. 2. Problem description

One of the most powerful and practically real ways of storing products is freezing. The range of frozen fruits and berries is wide enough. But there is an increased demand from the population for such fruits as strawberries (67 %), cherries (20 %), black currants (9 %), apricots, cherries, plums (4 %) [1, 2].

Freezing is accompanied by a deep decrease in the temperature of the fruit and the transformation of moisture into ice, that is, dehydration. The formation of ice crystals causes changes in the tissue structure of the product, the extent of which depends on the temperature and the speed of freezing. The speed of movement of the front of the freezing area for fast freezing of products is 0.05–0.10 m/h, with normal (average) – 0.01–0.05 m/h, with slow freezing, less than 0.01 m/h. Cryogenic freezing – ultrafast freezing (crystallization rate – more than 0.01 m/h.), which is carried out in cryogenic liquids (liquid nitrogen, carbon dioxide, freon) by irrigation or immersion [3].

According to the definition of the International Academy of Refrigeration and the FAO/WHO Committee, the term "fast freezing" should be used in the case when during freezing there is a passage through the zone of maximum formation of ice crystals, that is, the temperature range, which in most products lies in the range from –1 to –5 °C. Freezing is considered complete when the equilibrium temperature reaches –18 °C. At the same temperature and below with minimal fluctuations, food is stored and transported. The quality of the fruit is associated with the transformation of more than 80 % of water into ice [2].

In the process of freezing, three periods are distinguished: preliminary freezing – a decrease in temperature from the initial to cryoscopic; freezing – a period during which the temperature in some places of the product is almost constant, since the release of heat is accompanied by the transition of most of the water into ice; freezing – the period during which the temperature of the product is reduced to a predetermined final [4].

1. 3. Suggested solution to the problem

Freezing is carried out, as a rule, at temperatures of –30 °C and below in an air stream with a speed of 7 m/s or more. Under such conditions, the duration of the process is from several minutes to 1.3 hours. and more depending on the initial temperature, mass, layer thickness, heat capacity, temperature and thermal conductivity, type of packing and other factors [5].

An increase in the freezing rate occurs due to a decrease in temperature. It is important that the lower the temperature, the better the quality of the food. But on the other hand, moisture concentration occurs when exposed to extreme temperatures, negatively affects the reversibility of cell colloids, and this reduces the quality of thawed products. Too high speeds can lead to cracking of the fruit. For example, for plums in less than 22 minutes. [2].

The aim of research is to scientifically substantiate the influence of the method, the duration of freezing on changes in the structure of black currant fruits of various pomological varieties.

2. Materials and Methods

The fruits of black currant varieties Bilorus Sweet, Minai Shmyrev, which were used in the study, were grown in the experimental field of the educational and production department of the Uman National University of Horticulture (Ukraine).

The studies were carried out during 2008-2016. Under the conditions of the laboratory of the Department of Storage and Processing Technologies of Plant Growing Products and the Quick Freezing Shop of the Uman Cannery (Ukraine) according to the "methodological specified for conducting research with frozen fruits, berries and vegetables" [6].

The materials and methods for studying the effect of freezing on the process of ice formation in black currant fruits are described in more detail in [7].

3. Results

Establishing the mechanisms underlying the damaging effect of low temperatures is important for the development of the theory and practice of freezing black currant fruits. Research in this direction in black currant fruits has not been previously conducted.

The fruit of the black currant is a simple multi-seeded false berry. The fertilization of the fetus develops from the walls of the ovary, which merges with the receptacle.

The anatomical structure of the pericarp of black currant in a fresh and frozen state are investigated. So, **Fig. 1, *a*** shows that black currant fertilization consists of exocarp (epidermis and hypodermis), mesocarp (the layer lines the fetal cavity and internal epidermis) and endocarp.

The epidermis is the primary integumentary tissue that protects the fetus from environmental influences, excessive transpiration, mechanical damage, damage by microorganisms, that is, it performs a protective function.

Pores are retained on the outer epidermis of the pericarp. They can be seen in **Fig. 1, *c*** in the form of strokes of light color on the black shiny surface of the epidermis. It is characteristic that they diverge from the place of attachment of the stalk like beams of rays and wrap around the entire surface of the fruit. The largest number of pores is in the peduncle, which justifies the greatest loss of moisture during storage in this particular place and the manifestation of the phenomenon of drying out.

In **Fig. 1, *b*,** it can be seen that the black shiny surface of the epidermis is not uniform, smooth, as it seems to us with the naked eye. This is because in the epidermis there are developed original multicellular glandular hairs of a mushroom-like, almost sitting, shape. Oil accumulates in their cavities, which leads to a specific smell of black currant. Most of these hairs are in the epidermis of the ovary [8, 9]. With the ripening of the fruits, they are somewhat destroyed.

The outer epidermis is clearly visible in a continuous layer. In its juicy cells, anthocyanins are localized, which are most of all in this layer, providing the pericarp with a purple-blue, up to black, color (**Fig. 1, *a***). The hypodermis has an uneven thickness of the cell membrane in the tangential plane. The nature of the thickening of the membranes gives grounds to attribute the hypodermis to lamellar collenchyma. Obviously, the size and shape of collenchyma cells are different, but they are elongated and somewhat similar to fibers, which are comparatively less dense than those of the outer epidermis. The accumulation of anthocyanins is well expressed in the cells of the hypodermis.

Collenchyma is located under the integumentary tissue and performs a mechanical function. It is formed from the cells of the primary cortex after differentiation of the tissues of the primary meristem. In this case, the cells of the collenchyma gradually mix with the cells of the primary cortex and lose their independent expression. The cells of the hypodermis are connected with each other and with the cells of the epidermis by wide simple pores.

In cross section (**Fig. 1, *a***), the inner epidermis looks like a layer consisting of cells of various shapes and sizes. It is well-defined, because its cells are predominantly elongated, thickened as a result, probably stiffening and form a tissue of the sclereid type. Perhaps this is why the well-defined palisade epidermal layer separates the endocarp.

The mesocarp of the pericarp of black currant consists of small parenchymal and large puffy cells filled with juice. Anthocyanins are localized in them, however, in a much smaller amount than in exocarp, which can be seen in the intensity of color. The color of the mesocarp is light pink. **Fig. 1, *a*** shows that the mesocarp has a heterogeneous structure. Closer to the internal exocarp, there are cells containing calcium oxalate drusen [9]. Part of the dark inclusions of the mesocarp are parenchymal cells (idioblasts – specialized cells) with tannins, as well as conductive bundles, the membranes of which make up the sclerenchyma fibers, which makes it possible to store their structure.

The juicy part of the fruit, in which the seeds are immersed, is the endocarp of the pericarp of black currant (**Fig. 1, *d–f***). It is pierced with branching vascular-conducting bundles. Seeds are attached to the latter. It is elongated, sometimes prismatic. In the cross section, the black currant seed (**Fig. 2**) is represented by the skin, endosperm and embryo. The skin of the seed consists of the epidermis, the cells of which are rounded and do not stand out in a solid line. Under the epidermis there are sclerenchyma cells with yellow-brown membranes. Further, the endosperm is located, consisting of rounded, oval and mainly 4, 5, 6-gonal cells filled with storage substances.

The juicy part of the fruit is mainly formed by the arillus. It is a formation that arises on the seed during its development (**Fig. 3**). Arillus develop on the basal (lower) part of the seed peduncle. Arillus cells cover the seeds along the ribs in the form of reeds. Over time, the arillus of opposite placentas grow together and each seed becomes surrounded on all sides by their cells. Arillus to some extent surround the seed, but do not grow together with the seed coat [9]. Observations show that in mature fruits, the cell membranes of the arillus become mucous, become thinner, intermittent, and are cords of mucus.

Taking into account the analysis of the anatomical structure of the blackcurrant pericarp, important technological points: are established: only the mesocarp with plump cells is filled with liquid cell sap, and the endocarp, due to the mucousness of the arilles cells, has a mucus-like mass. On the one hand, this has a positive effect on the structure of frozen fruits and the high gelling properties of raw materials in the production of jam, confiture, jelly, chopped or mashed currants with sugar. On the other hand, there are great difficulties in the extraction of juice, wiping, the production of products such as jams, fruit desserts.

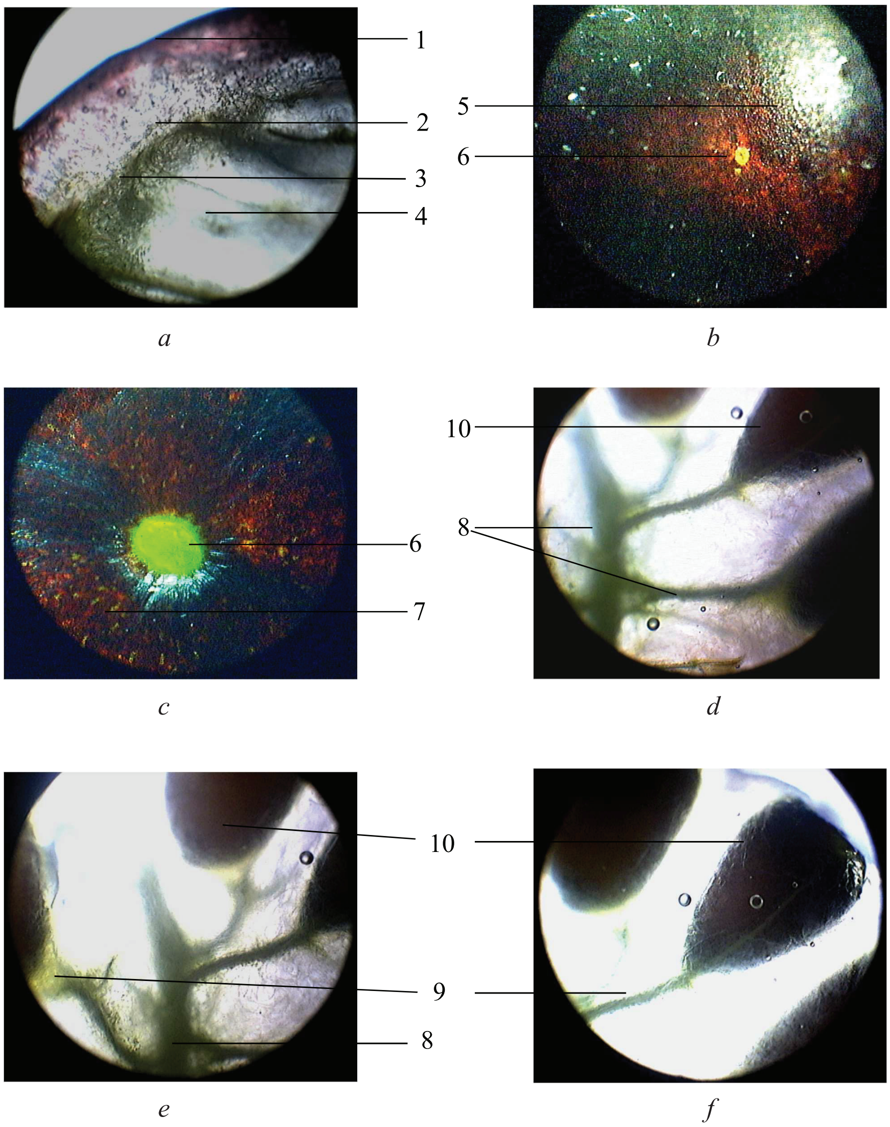


Fig. 1. The structure of the pericarp of Bilorus Sweet black currant varieties:
a, b, d, e, f – an increase of 38; *c* – an increase of 140; 1 – exocarp; 2 – mesocarp; 3 – internal epidermis; 4 – endocarp; 5 – glandular hairs; 6 – trace of the peduncle; 7 – pores; 8 – vascular-conducting bundles; 9 – arillus, 10 – seeds

The intensity and nature of changes in black currant fruits during freezing, of course, depend on the conditions and parameters of the process. But the qualitative characteristics of fruits, the specificity of their structure, the peculiarities and interrelationships of physicochemical and biochemical processes that take place have a significant impact on the preservation of their properties when frozen.

In our opinion, the state of cell membranes, their permeability, the concentration of soluble substances of individual structural formations of tissues, the degree of hydration of the components determine the regularities in the development of the ice formation process in black currant fruits.

The study [9] of ice formation in dilute solutions made it possible to establish that the features of the formation of ice crystals are determined by the properties of the solution (concentration and diffusion capacity of the components) and the rate of movement of the phase boundary.

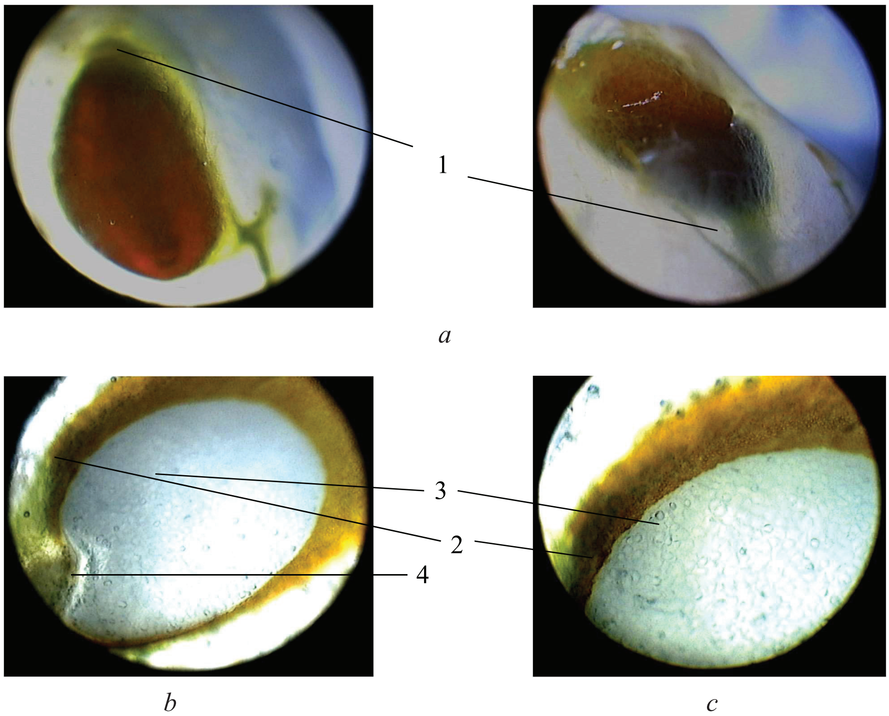


Fig. 2. Seeds of the black currant fruit of Bilorus Sweet varieties: *a* – an increase of 38; *b* – an increase of 100; *c* – an increase of 140; 1– arillus, 2 – peel, 3 – endosperm, 4 – embryo

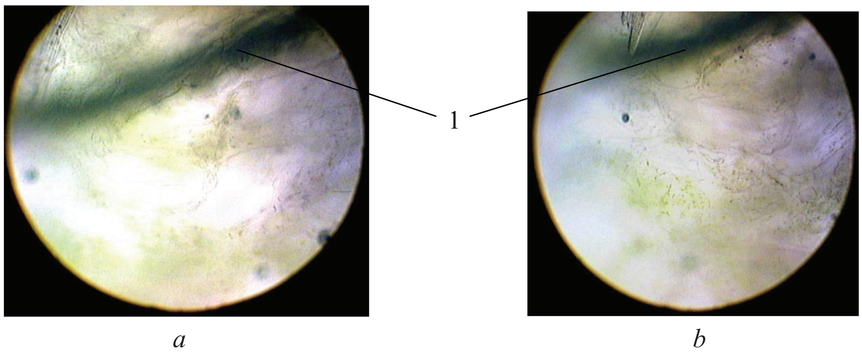


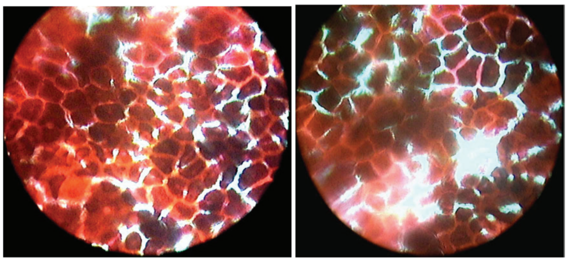
Fig. 3. The pulp of the fruit of black currant, depending on the variety (magnification 400): *a* – Minai Shmyrev variety; *b* – Bilorus Sweet variety; 1 – vascular leading bundle

However, in the tissue juice of fruits, it has a polydisperse solution containing, along with highly dispersed and coarsely dispersed components, the formation of ice is determined by the joint influence of the components on the process. In addition, the structure of the fetal tissue significantly affects the development of the process, limiting it to the framework of the microchip, in which ice formation takes place.

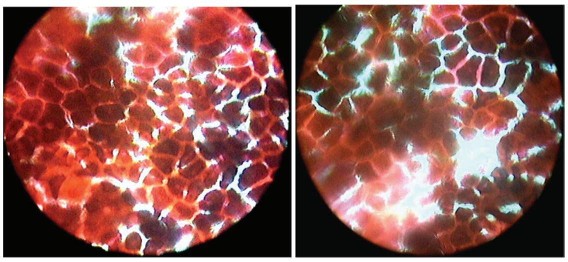
First, let's made a comparative assessment of the structural tissues of fresh fruits of Bilorus Sweet and Minai Shmyrev black currant varieties. It turned out that at the molecular level, cells of the single-layer epidermis of black currant fruits have an irregular polygon cross-section, somewhat elongated or spherical. In the fruits of the Bilorus Sweet variety, the corners of the polyhedron can be rounded. The epidermal cells contain anthocyanins. Moreover, the intensity of their color indicates an unequal amount of pigments in individual cells.

Fig. 4 shows a photo of the peel of black currant fruits of various varieties before and after freezing. The formation of intercellular spaces is clearly visible. This is due to the fact that with the transition of cells to a mature state, the middle lamina is destroyed, mainly at the corners of the cells. In these places, as a result of the turgor pressure of neighboring cells, the intercellular spaces are rounded and formed. They are triangular in cross section. With the growth of the membranes of neighboring cells, the intercellular spaces increase and merge, forming an interconnected branched network, which is filled with water vapor and gases.

At a magnification of 400 times, it can be seen that the cells of the epidermis of the rind of the Minai Shmyrev variety are compactly contained among themselves. In practice, the intercellular spaces only locally separate the cells. At the same time, in the epidermis of fruits of the Bilorus Sweet variety, in some areas, cell separation is observed, and the thickness of the branching of the intercellular spaces is greater.



a



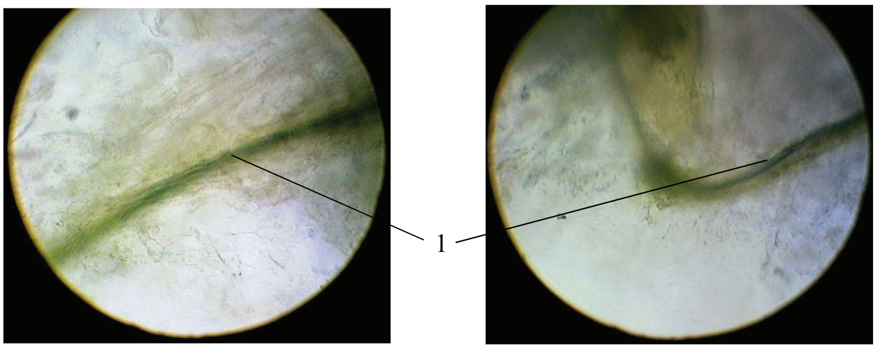
b

Fig. 4. Peel of black currant fruits of different varieties: left – before freezing, right – after freezing (magnification 400); *a* – Bilorus sweet variety; *b* – Minai Shmyrev variety

The result of the above was reflected in the structure of the epidermis of the peel after freezing. With an increase of 400 times in the epidermis of the peel of the Belorussian sweet fruit, the intercellular spaces are well expressed. They are filled with ice. This is explained by the fact that a lower concentration of soluble substances in the intercellular space determines the difference in the values of cryoscopic temperatures of structural elements. As a result, ice crystals form primarily in the intercellular fluid. This is because at temperatures below the freezing point, water vapor in the large intercellular space begins to condense in the form of moisture droplets on the adjacent cell walls. It is this water that turns into microscopic ice crystals. The latter spread along the intercellular spaces and seem to envelop the cell walls. Crystals grow between the cells of the epidermis. As the authors of [10–12] point out, the process is accompanied by an increase in osmotic pressure due to an increase in the concentration of salts soluble in the liquid, which in turn causes the migration of moisture from the cells. Further growth of crystals occurs due to the moisture contained in the cells. This is explained by the difference in vapor pressure on the surface of different crystals. With a decrease in temperature, a state of hypothermia occurs in the cells, and then crystallization centers are formed in them, leading to the formation of intracellular ice.

The size, shape and distribution of ice crystals in the structure of the fruit are of interest. In the literature [12] it is stated that crystals can be of various shapes: branched in the form of dendrites (resembling tree branches) are formed when individual crystals grow on top of each other; in the form of peaks – when not all crystals develop freely, therefore, not all have equally developed faces; in the form of lenses – spherical crystals.

The study of the juicy part of the fruit (pulp) before freezing (**Fig. 3**) and after freezing (**Fig. 5**) showed that it is impossible to find the difference between the preparations. In our opinion, after freezing, the pulp juice acquires a glassy (amorphous) state. This situation differs from the crystalline one in that the molecules of the substance are distributed randomly, and not according to a certain spatial plan, as occurs during crystallization. Glass formation in the protoplasm of cells is called water vitrification. According to the vitreous state, the tissue acquires the properties of a solid. Let's believe that, in addition to glassy (amorphous) ice, ice crystals are also formed, but they can be so small that they are elusive with such optical research methods. It should be borne in mind that in black currant fruits the total amount of bound water (78 **%**) is colloid-bound – 32 **%** and osmotically bound – 46 **%** [8]. According to some data [12], colloid-bound (hydration water) crystallizes at a much lower temperature than usual, therefore ice crystals are not formed at low temperatures, which can damage the cell structure.



a *b*

Fig. 5. Black currant fruit pulp, depending on the variety, after freezing (magnification 400): *a* – Minai Shmyrev; *b* – Bilorus Sweet; 1 – vascular-leading bundle

The authors of [13] associate the vitreous state of the fruit pulp with an increase in the amount of intracellular colloids as a result of keeping the fruit in a sucrose solution. That is, this is a manifestation of cryoprotective properties.

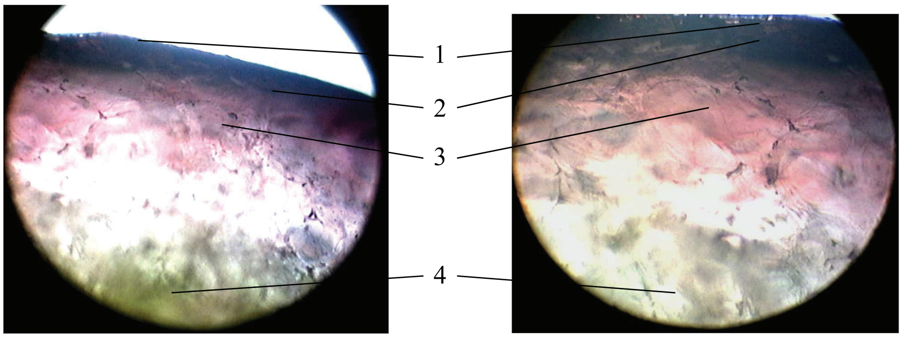
Let's associate a similar cryoprotective role in black currant fruits with pectin substances. It is established that the pulp of the fruit is gelatinous, therefore it is represented by strands of pectin mucus. If to assume that some of the bound water migrates to the ice-forming region, then the pectin particles can approach so closely that the sol turns into a gel. In pectin gels, water does not freeze completely, part of it remains in its entirety.

In addition, pectin substances have high hydrophilic properties. They bind a significant amount of water and contribute to the formation of a gel-like structure. Hydrophilic colloids are particularly resistant to low temperatures. The water shell of such gels is under high pressure, which makes it difficult for water to freeze. Therefore, hydrophilic colloids often coagulate when frozen. Thus, the solidification of a liquid in a glassy state contributes to an increase in its viscosity at low temperatures, which complicates the rearrangement of molecules into crystal structures.

By rapid heating, the glassy state can turn into a liquid, bypassing the crystalline one. This eliminates the structural schedule following intracellular crystallization, prevents cell death and achieves the return of the freezing process, on which the maximum preservation of fruit quality depends. On preparations of frozen pulp of currant fruit, the integrity of the phase boundary is not violated, a heterogeneous structure is visible. But concentration thickening was noted on the vascular-conducting bundles. It is possible that during ice formation, the components of the polydisperse phase, due to the limited diffusion capacity, accumulate at the interface and form a layer of concentration compaction. This worsens the access of the solvent (water) to the surface, largely determines the features of ice formation and, specifically, the complexity of its pattern.

The regularities of ice formation in black currant fruits established by us are of practical importance. It is clear that when freezing black currant fruits, the damaging effect of low temperatures on the structure of the pulp is minimal. Moreover, with a decrease in temperature and an increase in the rate of heat extraction, the damaging mechanical pressure on the cell decreases, since moisture hardens without its distribution, reduces destructive changes. However, the theory of salt denaturation should be rejected, which explains the damage to cells during freezing due to the hyperconcentration of salts both inside and outside the cell [13, 14].

Considering the positive cryoprotective role of the pulp composition on the marketable state of frozen fruits, practically the most structural changes in black currant fruits occur in the exo and mesocarp of the pericarp. This is evidenced by **Fig. 6.**



a *b*

Fig. 6. Black currant pericarp, depending on the variety after defrosting (increase 140): *a* – Bilorus Sweet; *b* – Minai Shmyrev; 1 – epidermis, 2 – hypodermis, 3 – parenchyma (mesocarp), 4 – endocardium

In the cross section of the pericarp of thawed currants (**Fig. 6**), exo and mesocarp detachment from the endocarp is observed. A well-defined endocarp exfoliates locally. The inner epidermis is completely invisible. Cavities are visible between the mesocarp and the endocarp, the separation of cells from each other. This confirms that we have established a significant increase in the thickness of the intercellular spaces and the peculiarities of the anatomical structure of the epidermis, hypodermis. It is likely that the water-holding capacity of currant fruit will depend mainly on these structural changes.

4. Discussion

The advantages of the study are the determination of recommendations for the storage of frozen black currant fruits at a temperature not higher than minus 21 °C after fast and minus 24 °C after slow freezing.

Due to a decrease in the temperature of initiation of ice formation, an increase in the rate of heat extraction and an increase in the temperature of freezing, rapid freezing forces cooling, freezing and freezing. The state of the cell membranes of the peel of the fruit, their permeability, the concentration of soluble substances of individual structural formations of tissues, the degree of hydration of the components are varietal features (**Fig. 3**) and determine the patterns of development of the process of ice formation in black currant fruits.

As a result of the fact that after freezing the fruits of black currant, the molecules of the substance are distributed randomly (**Fig. 5**), the pulp juice acquires an amorphous state, and the tissue – the properties of a solid. In fruits of black currant, hydration water is quantitatively significantly inferior to osmotically bound water and crystallizes at a lower temperature than usual. That is why ice crystals do not form at low temperatures, which can damage the cell structure of black currant fruits.

The study of the juicy part of the fruit (pulp) before freezing (**Fig. 3**) and after freezing (**Fig. 5**) showed that it is impossible to find the difference between the preparations. This situation differs from the crystalline one in that, and not according to a certain spatial plan, as occurs during crystallization. Glass formation in the protoplasm of cells is called water vitrification. By the vitreous state. Let's believe that, in addition to glassy (amorphous) ice, ice crystals are also formed, but they can be so small that they are elusive with such optical research methods. It should be borne in mind that in black currant fruits the total amount of bound water (78 %) is colloid-bound – 32 % and osmotically bound – 46 % [8].

Temperature storage conditions have been established for frozen fruits of Beilorus Sweet, Minai Shmyrev black currant varieties of technical and consumer degrees of maturity.

The issues of the influence of the established conditions of freezing and storage on weight loss, indicators of cryoresistance (tendency to cracking, loss of juice after defrosting, etc.), changes in the commodity and quality indicators of black currant fruits during the year, as well as the study of the effect of freezing on changes in the structure of fruits and berries of other crops.

5. Conclusions

So, studies of fresh and frozen fruits of black currant have established technological features of the anatomical structure of the pericarp of black currant, characterized by the presence of thickened membranes of cells of the epidermis and hypodermis (exocarp) of small parenchymal and large plump cells filled with liquid juice (mesocarp epidermis) of the internal scleroid layer; the largest gelatinous layer – the actual pulp (endocarp). Anthocyanins in fruits are localized mainly in the exocarp, a small amount in the mesocarp, absent in the endocarp.

There is a significant difference in the thickness of the intercellular spaces, the network of their branches in the structure of exocarp by pomological varieties. In the frozen endocarp of the pericarp, the juice acquires a glassy (amorphous) state, has advantages over the crystalline one. This is a consequence of the cryoprotective properties associated with strands of pectin mucus.

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