EFFECT OF THE BLENDING METHOD OF PLANT RAW MATERIALS ON THE STATE OF PARENCHYMAL TISSUES OF RED BEETROOT AND PRESERVATION OF BETANINE IN THE DEHYDRATION PROCESS

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Abstract. *When drying red beetroot the main problem is high energy consumption and the destruction of the natural pigment - betanine, which gives this vegetable antioxidant properties. The article presents a new method of preparing plant raw materials for drying in order to preserve betanine - blending. Microstructural studies and IR-spectra studies confirm the influence of organic acids of vegetable raw materials (lemon, rhubarb, tomato), which were used in blending, on the cell membrane of red beetroot, which as a result becomes semi-permeable for the release of moisture during dehydration and reduces the duration of the process during drying. In addition, organic acids of vegetable raw materials partially stabilize betanine. The IR spectra obtained as a result of experimental studies made it possible to identify the fluctuations of the main groups of the main components of plant tissues (water, fibers, hemicellulose, sugars, proteins, acids) and to monitor more subtle chemical changes in red beetroot tissues under the influence of lemon blending.*

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Introduction. The processing of agricultural raw materials into food products should ensure the maximum preservation of functionality of original raw materials with minimal energy consumption. One of the most effective methods of technological processing of plant raw materials is drying. Due to this, the duration of plant product storage is significantly increased, the need for storage facilities and costs for packaging and transportation are reduced. However, dehydration processes are energy-consuming. Therefore, it is important to investigate the preliminary preparation of raw materials for dehydration and correctly choose the technical parameters of drying, which can ensure the preservation of biochemical properties and initial nutritional value of plants and reduce energy costs in comparison with existing technologies. That is, the further development and increase in the efficiency of technologies for drying plants, in particular red beetroot, is important. Dried red beetroot are used as a functional powder (a source of betanine, natural vitamins and minerals), and for the production of food dye E-162 for use in the meat, dairy, confectionery, and medical industries.

In most cases, the basis of research is the use of red beetroot to obtain food coloring from its juice. In this case, energy-consuming methods of drying (spraying, sublimation, vacuum) and such auxiliary materials as maltodextrin, xanthan gum are used. The influence of vacuum drying on the preservation of biologically active substances of red beetroot was studied (Sz´ekely D. et al., 2016). Spray and freeze drying and the influence of pH on the stability of microcapsules of red beet extract were investigated (Duenha J. et al., 2018). The influence of preliminary osmotic dehydration on

convective drying of red beetroot was studied (Kowalski S.J. et al., 2015). In Malaysia, the method of drying with foaming was used (Ling M. et al., 2018).

Water-soluble red beetroot pigments can be used as a food coloring are betalains found in cell vacuoles, which are structurally and chemically different from anthocyanins, because they contain nitrogen in their structure. Betalains are aromatic indole compounds whose biosynthesis precursor is the amino acid tyrosine. Each of the betalains is a glycoside, that contains sugar and a dye part (Robinson et al., 1963). Synthesis of betalains in aerial parts of plants is stimulated by light. Betalains contain two groups of pigments: red betacyanins and yellow betaxanthins. Their ratio determines the difference in the color of red beetroot. Betanine is the best studied pigment (Gonçalves L. et al., 2012; Guesmi A. et al., 2012), which is also called red beetroot. Preservation of betanine, i.e. the natural color of red beetroot, is the main technological problem, since during long-term storage, red beetroot lose up to 70 % of coloring substances, and under the influence of high temperatures, up to 85 %.

The loss of red beetroot color is due to the hydrolysis of betanine into glucose and betanidin at the site of the double bond near C-11 (Wegler R., 1982). It is known, that even a small change in the acidity of the environment can lead to changes in biochemical processes. This is true not only for reactions involving ions H^+, and for many others, since most biomolecules contain groups capable of ionization. The color of the betadine food coloring is sensitive to the environment pH, therefore, to stabilize red beetroot pigments, it is recommended to change the acidity of the environment to pH = 3 - 5 (Dubinina A.A. et al., 2013) by adding ascorbic, sorbic, citric, acetic, lactic acid, or apple, rowan, sauerkraut juice. Caramel molasses, phosphate and sodium chloride can also be added as stabilizing additives. The stabilizing effect is provided by extracts of grape, cherry and dogwood seeds, tea, decoction of oak acorns. All these methods increase the stability of betaninw, but they have not been widely used in the food and canning industry. Therefore, further search for storage methods of red beetroot pigments during processing is relevant.

The purpose of this study is to determine how exactly the mixing method affects the state of red beetroot parenchymal tissues and the preservation of betanine in the process of drying with the help of microscopy, infrared spectroscopy and spectrophotometry.

Materials and Methods. Parenchymal tissues of red beetroot, tomato, rhubarb and lemon were selected for research. Preparation of samples was done according to the following stages: washing and cleaning of plants; mechanical grinding; mixing red beetroot in certain proportions with plants, that contain organic acids; exposure of the composition for a time equivalent to the time of convective heat drying of the selected plant material (~ 1 hour).

To determine the pH of the samples, the prepared plant parenchyma tissues and their compositions were transformed into a liquid porridge-like mass with the help of a blender, which was filtered through filter paper. The acidity of the filtered solution was measured according to the method (Nelson D.L. et al., 2008), using a pH-meter, that has glass electrodes, that are extremely sensitive to H+ ions but not to other cations.

The Light microscope Axio Imager Z1M (Carl Zeiss, Germany) transmission microscope equipped with a micro photography camera was used to study the effect of organic acids on the cell membrane of red beetroot.

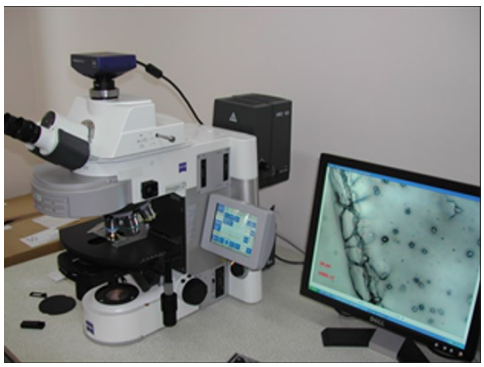


Fig. 1. Light microscope Axio Imager Z1M (Carl Zeiss, Germany)

The device was put into operation by the Institute of Engineering Thermophysics of the National Academy of Sciences of Ukraine in 2008 under the program of centralized acquisition of imported scientific instruments and equipment at the expense of budget funds of the National Academy of Sciences of Ukraine. In 2019, the device was modernized.

Axio Imager Z1M - universal motorized light microscope, which equipped with lenses with a flat field EC image «Plan-Neofluar» 5x/0,16 M27; 10x/0,3 M27; 20x/0,5 M27; 40x/0,75 M27; 100x/1,30 Oil M27; IC2S- optics of ultra-high resolution and contrast; a set of fluorescent light filters.

The study of internal chemical changes in red beetroot tissues was carried out by IR spectroscopy of disturbed total internal reflection on an IR Fourier spectrometer with standard grading capabilities. Experiments were conducted in transmission format, in the wave range 4000…400 см$^{-1}$. Infrared spectra were recorded on a spectrometer Vertex 70 firm Bruker with KBr beam refractor and RT-DLaTGS detector at room temperature (20 ± 1°C). 16 scans of each spectrum with a resolution of 2 cm^{-1} were averaged. Bruker Optics software version 6.0 was used for data processing.

The betanine content in parenchymical tissues of untreated and blended red beetroot was determined by direct spectrophotometry (Florian C. et al, 2006). Betanine, a water-soluble pigment, therefore it was extracted from red beetroot parenchymical tissues with water until the weight was completely decolorized. On a spectrophotometer SF-26, in a cuvette with a layer thickness of 10 mm, the optical density of the obtained solution was determined at a wavelength of 530-536 nm, which was converted to the extinction coefficient for 1% of the solution under study according to the formula:

$$D_{1sm}^{1\%} = \frac{D \cdot v}{a \cdot 100}, \tag{1}$$

where D - indicator of the optical density of the investigated solution at a wavelength of 530-536 nm; a - weight, g; v - volume of aqueous extract after complete decolorization of the sample, ml; 100 in the denominator - conversion to 1% solution.

Knowing the extinction coefficient for a standard aqueous solution of pure betanine and the test solution, the betanine content C in the experimental samples was calculated in percent, according to the formula:

$$C = \frac{D_{1sm}^{1\%} \cdot 100}{D_{st-1sm}^{1\%}} = \frac{D \cdot v \cdot 100}{a \cdot 100 \cdot D_{st-1sm}^{1\%}} = \frac{D \cdot v}{a \cdot 1100}, \tag{2}$$

where $D^{1\%}_{\text{st-1sm}} = 1100$ - extinction coefficient for a standard solution of pure betanine; 100 in the numeral - recalculation in %.

This technique makes it possible to estimate the content of dyes in red beetroot in objective units, in terms of betanine, in contrast to previously used methods that helped to estimate the content of dyes in conditional cobalt units of enine, cyanide or amaranth.

Results and discussion. Difficulties in drying plant raw materials are related to the fact that under the influence of heat treatment, light, oxygen in the air, pH of the environment, losses of biologically active substances (betanine of red beetroot) occur, therefore, to determine the most promising ways, the development and improvement of new methods of preparing raw materials for drying. The environment pH is important and should be in the range of 3.7-4.2 (Pavliuk R. Yu. et al., 2008; Petrova et al., 2018).

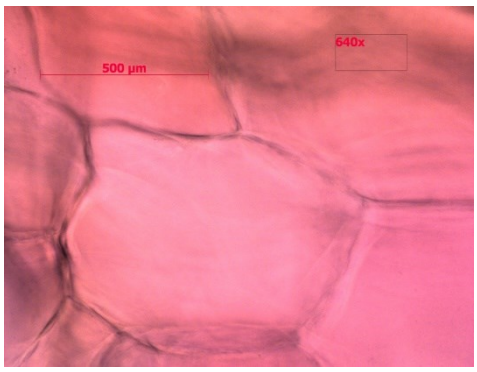
As it was indicated above, scientists are constantly engaged in research on the processing of red beetroot into a dry product in order to obtain a useful food powder with a high content of betanine (Telezhenko L. N., 2004; Pavliuk R. Yu. et al., 2008; Kowalski S.J. et al., 2015; Sz´ekely D. et al., 2016; Duenha J. et al., 2018; Ling M. et al., 2018; Kerr L. W. et al., 2020; Szadzińska Ju., et al., 2020). In Ukraine, scientists (Telezhenko L.N., 2004) developed a method of reducing the pH of red beetroot at the stage of preparation for drying by adding whey, citric acid and lemon balm (for flavor). Red beetroot juice is most of the researches.

In previous studies, scientists of Institute of Engineering Thermophysics developed and investigated the preliminary preparation of red beetroot for drying, namely hydrothermal treatment in an acidified environment (Petrova et al., 2018). This method allows to preservation of betanine at the level of almost 96 % with a fairly long shelf life, but it is energy-consuming and takes a lot of time. Also, this method is energetically impractical to use on an industrial scale.

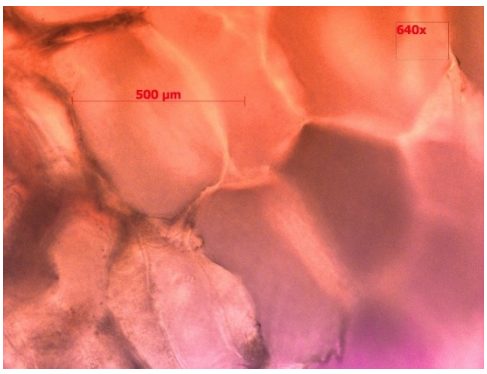
Therefore, at the stage of preparation for drying, it was proposed to create functional compositions based on red beetroot with the addition of plants, that contain organic acid (Petrova et al., 2018). When reviewing the literature, no such method was found.

After that it was investigated the effect of such mixing on the drying process, heat of dehydration and thermal stability of components and plant composition investigated. The conducted experiments showed that: with the correct selection of compositions and the use of a graded drying mode (100/60ºC), the rate of dehydration of a mixture of red beetroot and rhubarb (2:1) is 10 %, and red beetroot and tomato (3:1) is 40 % higher, than the average rate of dehydration of red beetroot tissues; specific heat consumption for water evaporation from red beetroot-based compositions with the addition of tomato (3:1), rhubarb (2:1) and lemon (3:1) decreased by 4-5 % compared to only red beetroot; mixing red beetroot with rhubarb (2:1) leads to the almost complete disappearance of the peak of thermal destruction of beet tissues at 210 °C (Sniezhkin Yu.F. et al., 2013; Sniezhkin Yu.F. et al., 2022). These methods confirmed the interaction of organic acids on the permeability of the red beetroot cell membrane.

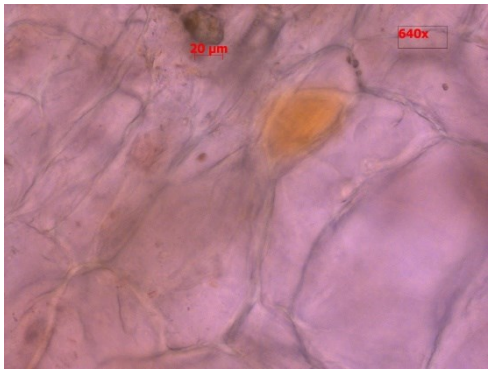
In fig. 2 presents electron-microscopic photographs of sections of parenchymical tissue of red beetroot untreated and blended with tomato and rhubarb in different proportions. In photo 2*a*, shows the rounded convex cells of red beetroot, which are surrounded by a clearly visible cell membrane. In the photos of red beetroot tissues, that were mixed with tomato and rhubarb, observed different degrees of damage to cell membranes. These damages are more significant, when the direction of pH movement is towards an acidic environment.



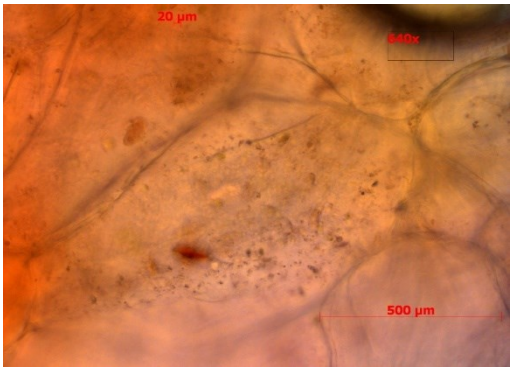
a) pH=6.26



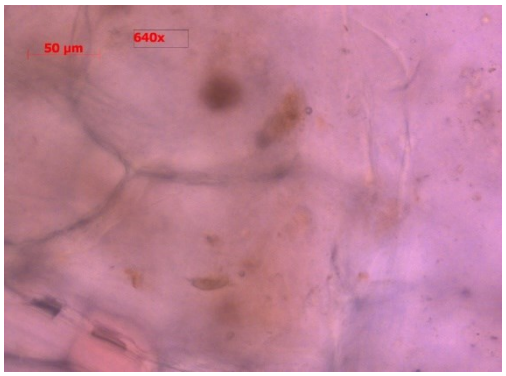
e) 4:1, pH=5.2



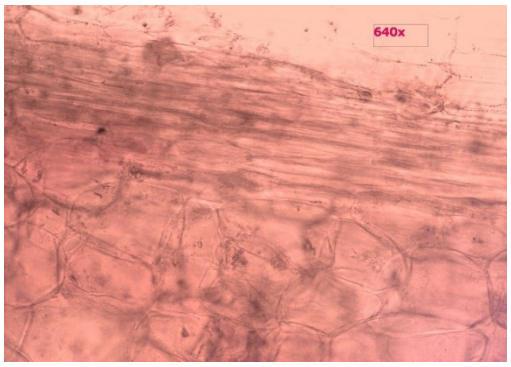
b) 3:1, pH=3.9



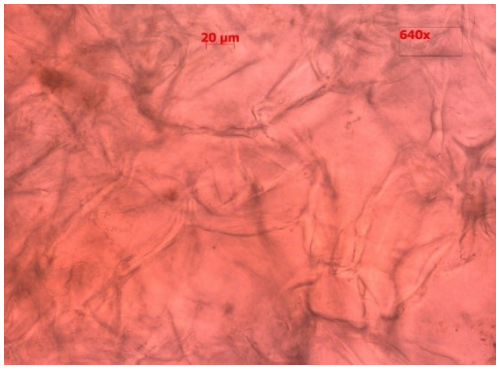
f) 3:1, pH=4.9



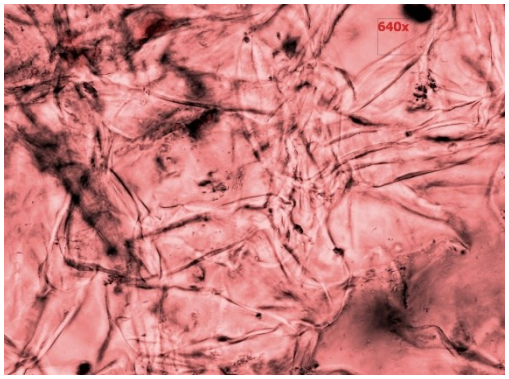
c) 2:1, pH=2.5



g) 2:1, pH=3.75



d) 1:1, pH=2.3



h) 1:1, pH=2.6

Fig. 2. Microphotographs (x 640) of parenchymical tissues of the fresh red beetroot *(a)* and red beetroot which was blending to tomato *(b, c, d)* and rhubarb *(e, f, g, h)* in different ratios

One of the properties of the cell membrane is its selective permeability. The membrane is ultra microporous, the holes in it are so small that molecules of small sizes, for example, water molecules, can pass through them (albeit with some effort). Larger molecules, such as sugar, cannot pass through the pores of the shell. Thus, it is, as it is customary to say, semi-permeable. The semi-permeability of the cell membrane complicates diffusion and physical processes in food production and must be taken into account when developing technologies.

To stabilize betanine during red beetroot processing, it is nesessary to change the pH of its cell environment to acidic, to pH=3.7 - 4.2 (Telezhenko L. N., 2004). To increase the permeability of the cell membrane of red beetroot tissues and change the cytoplasm pH, a certain irritating action is required. No matter, what stimulus is applied to the cell (mechanical damage, high temperature, electric current, etc.), the reaction of its shell is always the same: the orientation of the lipid layer is destroyed, colloidal micelles stick together into large aggregates, between which large passages are formed, which leads to increase in membrane permeability.

To irritate red beetroot cell membranes, it was used other plant materials with a sufficient content of organic acids: rhubarb (pH=4.5), tomato (pH=4.4), lemon (pH=3.0), which were mixed in different proportions with red beetroot (pH=6.26). Organic acids of the selected plants became an irritating factor for red beetroot cell membranes and, at the same time, changed the pH of its cellular environment to acidic. As we can see from fig. 2*e*, when creating a composition of 4 parts of red beetroot and 1 part of rhubarb, the effect of rhubarb juice on the cell membranes of red beetroot parenchymical tissues is insignificant. With a decrease in the proportion of red beetroot to 3:1, the acidity of the mixture changed insignificantly (pH=4.9), but the cell membranes of the red beetroot had already begun to break down (Fig. 2*f*). At a ratio of 2:1 (Fig. 2*g*), there was already an obvious effect of rhubarb oxalic acid on the membranes of red beetroot cells, which led to an increase in the marginal permeability of the membranes and a decrease in their barrier function. Substances were able to diffuse more freely inside the plant tissue and interact in a variety of ways, significantly changing the acidity of the intracellular environment (pH=3.75). When the ratio of components is 1:1 (Fig. 2*h*), completely destroyed cell membranes. This destruction enabled a number of chemical reactions, that interfered with the membranes and led to an increase in the acidity of the environment (pH=2.6) far beyond the level of acidity of the individual components of the mixture. In addition, due to the ruptured membranes, all substances were able to flow out of the tissues together with cell juice. And this leads to the loss of biologically active substances, including betanine, and significantly worsens the quality of finished products. So, from the conducted experiment on determination of pH and those shown in fig. 2 photo shows, that the partial increase in the permeability of red beetroot cell membranes, need to increase its acidity to pH=3.75 can be achieved by creating a red beetroot-rhubarb composition in the ratio 2:1.

A similar pattern is observed for red beetroot tissues blended with tomato. But here the cell membranes of the red beet are partially damaged already at the ratio of the components of the composition of 3 parts of red beetroot to 1 part of tomato (Fig. 2*b*). There is an obvious effect on the membranes of tomato organic acids, which already at this ratio increased the acidity of the medium to pH=3,9. When combining 2 parts of red beetroot and 1 part of tomatoes, there was already noticeable destruction of cell membranes (Fig. 2*c*). When the ratio of plant tissues is 1:1, then the destruction of almost all cell membranes, and complete mixing of intracellular components (Fig. 2*d*). Therefore, it is possible to damage the red beetroot cell membranes only partially and increase its acidity to the required pH 3.7 - 4.2 by creating a beet-tomato composition in the ratio 3:1.

The obtained IR spectra (Fig. 3) made it possible to identify the fluctuations of the main groups of the main components of plant tissues (water, fiber, hemicellulose, sugars, proteins, acids) and to monitor more subtle chemical changes in red beetroot tissues under the influence of lemon blending.

Spectra of parenchymical tissues of lemon, red beetroot and red beetroot which were kept in a mixture with lemon in a ratio of 5:1 (pH=4.6), 3:1 (pH=3.8) and 1:1 (pH=2.1), are generally similar.

The influence of the blending process is evident in the details. As part of a wide high-frequency band of reduced transmission with a maximum in the region of 3310 cm^{-1}, there are bands of valence vibrations of NHn and OH groups of all major biocomponents, with a preference for valence vibrations of OH groups of water. The band in the region of 1750...1540 cm^{-1} belongs to the vibrations of carbonyl C=O groups and indicates the presence of free carboxylic acids and protein components in the samples.

This band in the spectrum of lemon has practically no shoulder corresponding to vibrations of protein components. In the spectra of red beetroot this shoulder is clearly expressed. With an increase in the amount of crushed lemon in which the red beetroot tissue was immersed before the study, this shoulder decreases, which indicates the degradation of the protein components of the red beetroot tissue, i.e. destruction of intracellular protein membranes in it.

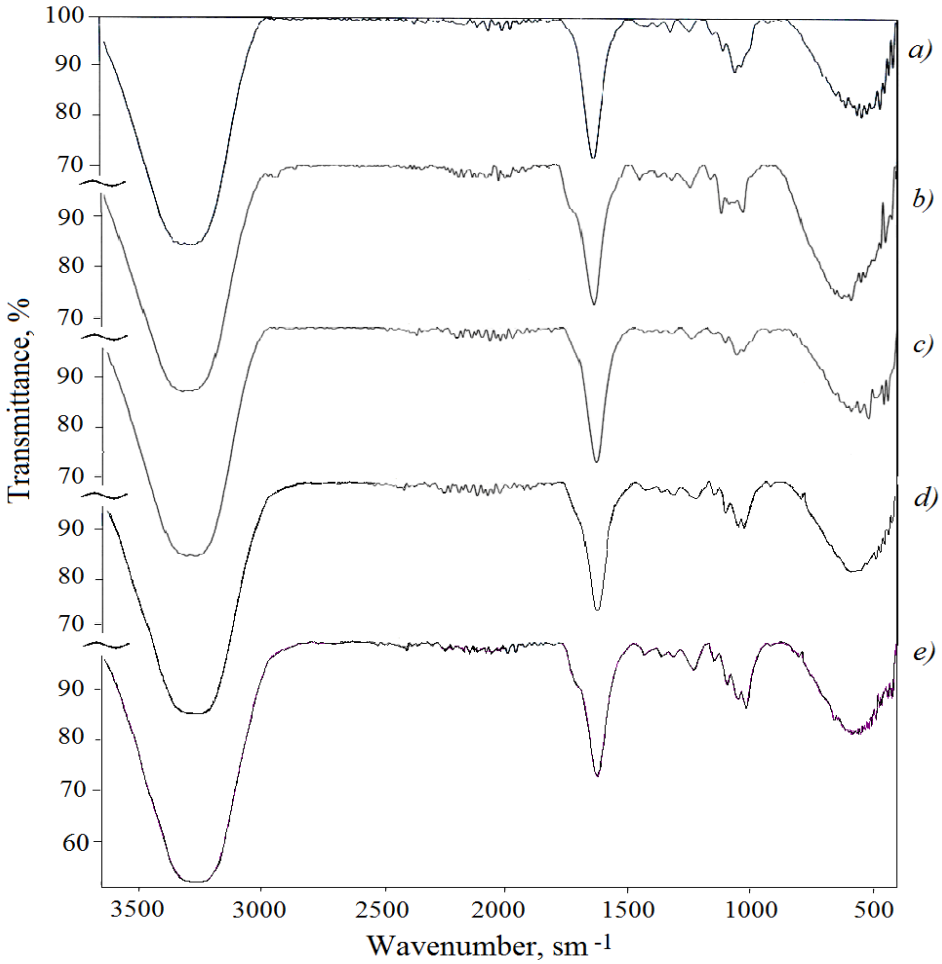
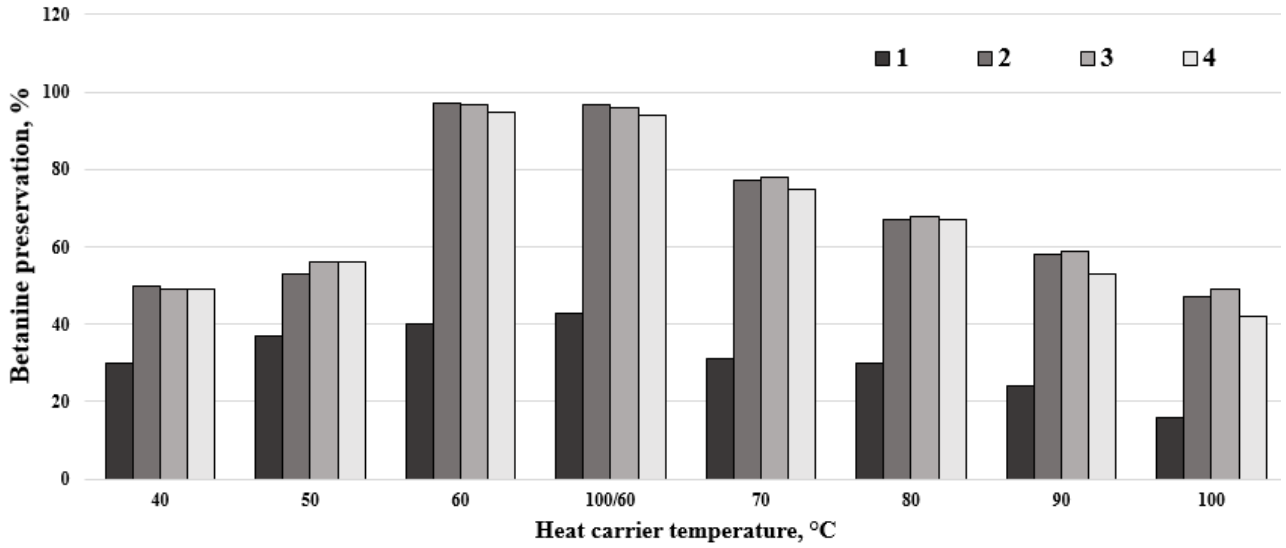


Fig. 3. Infrared spectra of parenchymical tissues of lemon *(a)*, beetroot *(b)* and beetroot, which was blending with a lemon in a ratio of 5:1 *(c)*, 3:1 *(d)*, 1:1 *(e)*

The complex band at 1400...990 cm^{-1} corresponds to vibrations of the C–O bonds of mono-, oligo- and polysaccharides. It is already very different in shape in the spectra of lemon and beet due to the different composition of their saccharides. From Fig. 3*b* and 3*c* it is observed, that after blending the shape of this band in the spectrum of red beetroot has changed significantly. This can be explained by the fact that some of the C–O bonds in the red beetroot polysaccharides were destroyed, which caused the polysaccharides to disintegrate into smaller fragments, i.e. changes in the qualitative composition of saccharides in beet tissue. With an increase in the amount of crushed lemon in which the red beetroot tissue was immersed before the study (from 1:1 to 5:1), this band also significantly (almost 3 times) decreased in area and intensity (Fig. 3 c, d, e). This indicates a more significant destruction of C–O bonds, i.e. significant destruction of the polysaccharide cellular framework of red beetroot tissues. This result is quite consistent with the results of our microscopic study.

Destruction of the polysaccharide framework and degradation of protein shells naturally leads to a decrease in the number of centers capable of retaining moisture inside beet tissue. Water partially passes from a bound state to a free state. This is evidenced by a change in shape and a significant decrease in the intensity and area of the complexly structured band 900 - 400 cm^{-1} in the spectrum of red beetroot after blending (compare fig. 3 b and fig 3 c, d, e). It is in this band that deformation vibrations of various water associates manifest themselves.

The effect of acid blending on the preservation of betanine was investigated depending on the temperature of the heat carrier, after thermal convective drying of red beetroot and compositions based on it (Fig. 4).



1 – red beetroot are not processing, 2 – red beetroot-lemon (3:1),

3 – red beetroot-rhubarb (2:1), 4 – red beetroot-tomato (3:1)

Fig. 4. Dependence of betanine preservation on heat carrier temperature for drying

Fig. 3 shows, when drying unprepared red beetroot, betanine is kept at the level of 30 - 40 % in all the investigated drying modes. The production of compositions allows you to increase the preservation of betanine in the dry product by 1.5 - 2 times at any temperatures of the heat carrier during drying. At a coolant temperature of 40 - 50 °C and its high moisture content, even blended red beetroot spoils, and betanine after drying is stored for no more, than 50 %. The same results are

obtained by drying at temperatures of 90 - 100 °C, in this case, significant thermal decomposition of betanine occurs. The maximum, 96 – 97 % betanine preservation was achieved after drying the composition at a heat carrier temperature of 60 °C and a step mode of changing the heat carrier temperature 100/60 °C (). Separately, it should be noted, that the composition of red beetroot with tomato made it possible not only to preserve betanine in the dry product at a high level, but also to enhance its red color due to the chemical interaction of the components of this mixture.

Conclusions. The conducted research made it possible to track changes in red beetroot parenchymical tissues under the influence of mixing with crushed rhubarb, tomato, and lemon parenchymical tissues: disruption of cell membranes due to the degradation of protein and polysaccharide structures and complex changes in water associates due to the degradation of water-retaining centers.

We consider the most likely reason for the obtained results to be a change in the structure and composition of the components of the mixture under the conditions of blending with plants that contain organic acids. The proposed method of changing the pH of the environment led not only to a change in the permeability of the cell membranes of red beetroot tissues and a decrease in the number of water-retaining centers, but also to the stabilization of betanine.

The developed vegetable compositions based on red beetroot are a fundamentally new material that stabilizes and protects red beetroot betanin at the biochemical level from the effects of high temperatures during convective heat drying. Compared to the components of the compositions, this material has a lower heat of vaporization and increased thermal stability. That is, the creation of plant compositions at the stage of preparing plants for drying made it possible to reduce energy costs for the process of their dehydration and preserve the maximum amount of biologically active substances.

The studied compositions can be used in the mass production of functional powders, which are an available source not only of food pigments, but also of natural vitamins and minerals to strengthen human immunity during epidemics and exacerbations of viral diseases.

As a result of these developments and research, when it was possible to preserve the betanine of red beetroot as much as possible, a quick-cooking dry borscht was developed, that does not require a cooking process and is actively used by the soldiers of the Armed Forces at the front.

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