



SUBLIMED PLANT BIOLOGICALLY ACTIVE ADDITIVES WITH RADIOPROTECTING ACTION

Halyna Simakhina, Natalia Stetsenko, Natalia Naumenko

*Department of Technology of Healthy Products, Faculty of Technology of Healthy Products and
Food Expertise, National University of Food Technologies, P. O. Box 01033,
68 Volodymyrska Str., Kyiv, Ukraine*

Abstract

The low-temperature (cryogenous) technology of processing the plant raw materials into biologically active food additives with increased content of vitamins, mineral elements and other precious components important for human organism functioning was designed by the scientists of NUFT, and thereto presented in this article. The dry products obtained with such a technology were outstanding due to their high quality, positive influence on human organism's organs and functions, the ability to avoid accumulating radionuclides and other toxic substances and, otherwise, to remove those present in the organism. Sublimed products made possible to widen the range of healthy food products, and, consequently, had got the real perspective to be realized on domestic and foreign markets. This could be achieved just thanks to the composition of several indices, including high quality, absolute safety, and moderate prices, in a product.

Keywords: cryogenous technology, biological activity, freezing, cooling agents, powders, radionuclides, quality, safety.

Introduction

During the last decade, health of Ukrainian population had sharply worsened by a number of indices. The birth rate had got lower (7.8 percent per 1,000 people), and the death rate had got higher (14.8 percent). It resulted in the negative natural increase of population (–7.0 percent).

One of the main causes of these conditions is the sharp worsening of ecological environment after Chernobyl disaster. More than 90 percent of Ukrainian territory was polluted by radionuclides, and about 2,000,000 people have been living in the area of radiological control. The small doses of ionizing radiation influenced the inhabitants of the large area of Ukraine.

The most realistic, safe, and reliable way to keep good health on the proper degree in such complicated conditions, and to provide the social and intellectual activity is the usage of the new-generation foodstuff with natural radioprotectors and biocomponents that would help increase the immunity level of human organism and its adaptive possibilities.

That is why the work trending on production of such foodstuff from the local cheap plant raw material was extremely actual, socially necessary, and economically expedient.

The scientific innovation of this work was based on the usage of temperatures below zero (by Celsius) to dehydrate the plant raw material. Now the mentioned technology is the only one that makes possible to keep the whole natural biologically-active complex of all of the necessary substances undamaged. Cryogenous technologies were widespread in the developed countries of the world, like

the USA, England, Japan, France. Generally, there was established the principally new way to preserve the agricultural raw material, which, according to the scientists, would soon replace the traditional ways (like sterilization, high-temperature drying etc.). Talking about Ukraine, the usage of cryogenous technologies in food industry has just begun developing. That is why each way of work in such a trend fills the knowledge sum about the special features of freezing and sublimation of plant materials.

The main tasks of researches in the present article were defined as confirming the expedience of low temperatures' usage to preserve the plant raw materials (based on the wide range of fruit and berry raw); studying the specificities of phase transitions "water – ice" and "ice – water" in freezing the biological objects; elucidating the mechanism of water crystallization during freezing the carbohydrate-containing raw materials; establishing the optimal technological parameters of raw's sublimation dehydration; carrying out the biological experiments in the live testing objects to research the radionuclides' de-corporation by sublimed plant powders.

Materials and methods

Fruit (apples, citrus, apricots, peaches), berries (strawberries, raspberries, grapes, black currant), vegetables (potatoes, topinambour, carrot, red beet, sugar beet, sweet pepper), and seeds (parsley seeds, fennel seeds). All of the mentioned materials were grown in Ukraine (except of citrus which was imported from Spain). Selection of those raw materials was



based, first of all, on their physical and biological value. Total carbohydrates were determined by a colorimetric method based on Phenol reaction with glucose [2].

Vitamin C content was determined by potentiometric titration by sodium 2,6-dichlorophenolindophenolate according to USSR State Standard No. 24556-89. Micro and macro elements' contents were determined by SPECORD UV VS spectrophotometer [1].

Kinetics of the processes that take place in thawing the biological objects was investigated by the method of differential scanning microcalorimetry [6].

The experiments were carried out in white non-breed male rats (with average body weight of 140...160 gr.). The biologists had studied 105 animals in ten groups (15 in each group). One group of animals got usual vivary diet, others got sublimed products (400 gr. per capita). The influence of those products on removal of incorporated strontium and cesium radionuclides was studied in dynamics within 30-day experiment. In a week after animals got adapted to the given diets, they received one-time dose (with food) of radionuclides (in proportion of 1306 Bc per capita of ^{90}Sr and 343 Bc per capita of ^{137}Cs). The content of radionuclides in animal's organism was measured by gamma-radiation of ^{137}Cs and ^{90}Sr on "Ortec" portable device with 4,000-channel impulse analyzer, and on "Bicron" scintillation detector once in three days.

All the experiments planned within the framework of our researches were accomplished in threefold sequence. The experimental results were subsequently processed by 'least squares' statistic method [4]. Experimental results were calculated by the methods of statistic modeling based on Microsoft Excel 2003 and MathCad 13 problem-oriented software.

Results and discussion

There was well-known that transformation of water into solid phase, accompanied by temperature decrease in the entire system and ice-making warm excretion, was the main physical process characterizing the freezing of any plant raw materials. So, from this viewpoint, the selected research objects allowed the authors of this article setting up the temperature intervals for water crystallization and ice melting in the wide range of indices of materials' initial humidity (from 90 to 20 percent). That was why the obtained data got universal.

The obtained data are presented in table 1. According to the table figures, crystallization of free water during researched samples' freezing began at

the significant overcooling, and its initial temperature got lower along with samples' initial humidity decrease.

The comparative analysis of the tables showed that the adaptability to overcooling depended on the kind of a material, the grade of its maturity, its chemical composition, and initial humidity. High-molecular compositions and hydrophilic colloids, which are inclined to swelling and water constraining, play the significant role in this process.

There is well-known [5] that the presence of stable embryos is necessary for development of crystallization process in the solution. Embryos got created in the certain grade of overcooling in the system; as in our experiments, this grade varied from $-7\text{ }^{\circ}\text{C}$ to $-32\text{ }^{\circ}\text{C}$ (particularly, for black currant this index was $-14\text{ }^{\circ}\text{C}$). The subsequent growing of ice crystals depended not on temperature, but on time; ice "grew" in the entire volume of liquid.

Table 1
Experimental data of crystallization / melting
of apple water

Relative humidity	Freezing water	Non-freezing (constrained) water (percent to the main mass of water)	Starting crystallization temperature, $^{\circ}\text{C}$	Starting melting temperature, $^{\circ}\text{C}$
80.77	76.74	23.26	-23.0	-6.5
80.28	80.28	74.05	-23.0	-11.5
77.32	70.67	29.33	-26.5	-13.5
76.79	65.22	34.78	-24.5	-12.5
68.82	62.12	37.88	-26.5	-9.5
62.28	54.23	44.60	-26.5	-11.5
58.07	46.17	53.83	-26.5	-8.5
57.09	40.99	59.01	-26.5	-11.5
54.58	35.33	64.67	-26.5	-17.5
38.44	-	100.00	-	-

In thawing the samples with velocity of $4\text{ }^{\circ}\text{C}$ per minute, starting melting temperature of crystallized water got also decreased. The consequence in temperature changes got observed in the moment of endothermic peak. The absence of first-grade phase transition on the thermograms of samples with low initial



humidity was evidence that all water contained by the researched object was constrained.

Setting up the optimal conditions for carbohydrate-containing raw materials' freezing based on the studies of the main processes (overcooling, crystallization's start and finish, intensive crystallization).

The choice of optimal freezing temperature was based on the fact that the minimal melting temperature for crystallized water, which could be defined experimentally, was significantly higher than the maximal solid-phase temperature. This could be connected with overcooling the transitive eutectic mixtures during the freezing process, which delayed the subsequent crystallization [3], so that the plant raw material was to be cooled to lower temperatures. The index of marginal temperature was determined by the properties of the cooled object and the characteristics of any solid matter which was present at the same environment.

Subliming dehydration of fruit and vegetable raw materials can be presented as the sequence of complicated one-direction processes of mass and warm transfer in capillary and porous matters to which the objects of our researches belong [7]. Sublimation drying was carried out according to the optimal parameters which were set up previously (particularly, upon reaching the pressure of 13.3 Pa at the sublimator, we used the regulator on power control device to adjust so high voltage that would allow obtaining the specific potency of warm stream within 800 W per square meter).

Upon reaching the product temperature of 273 K (according to the data of the "tray – products" resistance thermometer), we decreased the specific potency of warm stream to 400 W per square meter, and then the process was carried out until the temperature fell to 298 K. That temperature was kept constant by the voltage regulator with precision of ± 5 K until the pressure in the chamber reached 1.33 Pa.

Sublimation drying was carried out in the following regimes:

- The raw material got frozen by liquid nitrogen irrigation with the velocity of 16...32 K per minute;
- Drying was going on with the initial warm stream of 800 W per square meter;
- The sample temperature in the sublimation period was kept at 268 K;
- The warm stream in 25 minutes was weakened to 400 W per square meter and kept constant for a long time;
- The additional drying temperature was kept at 287 ± 1 K.

The next regime was similar to the previous one; however, it differed from it by the following features:

- The specific warm stream got gradually weakened to 800 to 100 W per square meter;
- The additional drying temperature was 300 ± 3 K;
- The material got self-frozen to 248 K;
- Drying was carried out at the initial warm stream of 800 W per square meter;
- Temperature of the product in sublimation was 268 K;
- The warm stream got gradually weakened;
- The additional drying temperature was 293 ± 3 K.

Sublimation drying was accomplished in direct-radiation warming conduction until the final humidity in the products within 8...10 percent.

There was confirmed that the periods of constant and decreasing energy conduction were expedient to organize the process of sublimation drying, independently of the kind of raw.

Generally, the process of sublimation drying consists of several simple processes:

- Sublimation from mono-molecular layer of the surface whose zone is moving up to down;
- Sublimed particles of migrating water were in the state of diffusion through the capillary and porous skeleton of the partial raw layer from the conditional surface to the free one;
- Water got removed from the free surface to the entire volume of sublimation chamber.

Process of sublimation drying finished with removal of all crystalline water (except constrained) from the raw layer.

The sublimed products that were obtained in the mentioned technological regime practically do not differ from initial raw by their qualitative indices. I.e., the dry products can keep the whole biological compound (for example, the carbohydrate complex).

The necessity to work out the technologies that would help obtaining the food products with increased content of biologically active substances has eventually grown up at the beginning of the 21st century. After Chernobyl disaster, the natural environment got critically contaminated by the products of nuclear fuel decay. Upon eating radioactively polluted food, the people in Ukraine got exposed to the permanent internal radiation of human organism. The gradual accumulation of incorporated radionuclides in some organs is the specific feature of their biological influence, which would cause destruction and loss of various proteins, enzymes, and other extra molecular structures



[8]. It was necessary to talk about the process of formation of free radicals that had high chemical activity, being able to set up the reactions with different tissue substances, causing their destruction.

The relatively long-term cesium and strontium isotopes are great interest for research, because of their ability to imitate behavior of elements that are necessary for human organism (potassium and calcium). If potassium and cesium are simultaneously present in the organism in the shape of low-hydrated ions, their metabolism goes relatively fast; otherwise, strontium transforms into non-soluble state and hereinafter gets accumulated in bone tissue.

To prove the possibility to remove the radionuclides by plant materials experimentally, the specialists from laboratory of endogenous radiation prophylactic (All-Union Radiation Medicine Scientific Center of USSR Medical Sciences Academy) set up and conducted the long range of researches. Their goal was to study the influence of cryopowders made of apples, beets, currant, carrot, topinambour, amaranth, and citrus peel on the main biochemical indices of laboratory animals' organism functioning (on the background of long-term contamination by cesium and strontium radionuclides, heavy metals, and pesticides in previously calculated concentration).

The results of researching the dynamics of cesium and strontium removal (in control and in experiment) were presented on Figure 1.

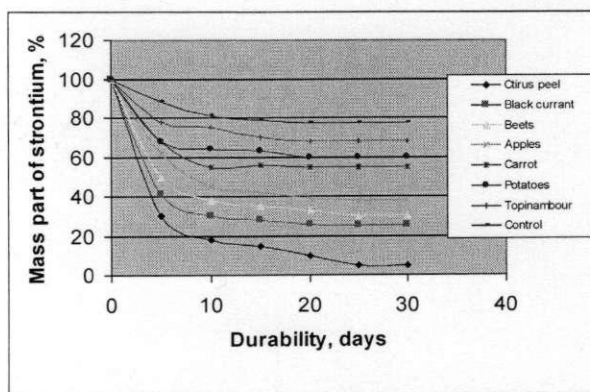


Fig. 1. Dynamics of strontium radionuclides' removal in control group and in experiments with sublimed powders after monodic radiation of laboratory animals

The figure showed that the radionuclides incorporated into the organism have the certain removal velocity. Cesium nuclides are especially labile. The kinetics of cesium and strontium removal from rats' organism could be approximately described by two exponents. Cesium got partially removed during 2...3 days, strontium did so during

4...5 days; the remained quantity got removed more slowly.

The sublimed materials also had the antioxidant action on live organism. This was the main factor of decrease and neutralization of great amount of toxic substances. This action got expressed in the fall of the level of lipid peroxide oxidation and, correspondingly, of the content of lipid and Malone di-aldehyde dien conjugates.

The everyday consumption of cryopowders in the amount of 8...12 gr. also caused some probable positive changes in blood protein spectrum (particularly, they provide increasing the relative content of albumin faction and decreasing the gamma-globulin faction). Generally this was evidence that the powders' biocomponents were able to resist the pathogenous influences and to lower significantly the heavy metals' and pesticides' toxic effect on the live organism. For some biocomponents of sublimed powders (particularly for organic acids), we studied the mechanism of strontium ion conjugation. The results are presented in Table 2.

Table 2
Strontium ion conjugation by organic acids of sublimed cryopowders

Cryopowder	Complex making acids, %	[Sr ²⁺] concentration, mol per liter	[Sr ²⁺] balanced concentration, percent to the general
Carrot	0.20	0.001	6.41
		0.005	6.56
		0.0010	6.87
Apples	0.30	0.001	4.30
		0.005	4.41
		0.0010	4.62
Oranges	1.80	0.001	0.75
		0.005	0.75
		0.0010	0.75
Beets	0.30	0.001	4.30
		0.005	4.41
		0.0010	4.62
Topinambour	0.12	0.001	10.33
		0.005	10.79
		0.0010	11.28

The presented data are evidence that each of sublimed powders, thanks to the organic acid content, helps conjugating the certain amount of strontium and thus avoids its accumulation in the live organism.



Conclusions

Based on the results of conducted researches of low-temperature procession of carbohydrate-containing raw materials and on the new scientific notions of quality and ecology of foodstuff, we proved, designed, and realized some of the newest decisions in the technology of production of plant raw materials into sublimed biological additives with high nutritional quality. Those technologies would increase the output of high-quality products; provide the growth of production efficiency; make possible to use the fruit and vegetable raw on the new level as the source of important biocomponents synthesized by nature. Our technologies would allow widening the new generation foodstuff spectrum, and implement some radical changes into Ukrainians' nutrition structure. The mentioned thesis describes the main factor of healthy lifestyle, prevention and medication of different diseases.

Systematic consumption of sublimed food products, made of carbohydrate-containing raw material, helps rising the physical and mental workability, stimulates the work of blood-making organs, strengthens the organism's resistance to destructive environmental factors, and thereto decreases the risk of oncology, heart and vessel diseases, and so on.

Significant decreasing the losses of plant raw materials during harvesting and storage, high quality of final products and their absolute safety for consumer, possibility to widen the range of half-fabricates and final products with increased content of essential and biologically active components through the year, creation of competitive and competition-oriented foodstuff with healthy and preventive destination were the cardinal advantages of using the low temperatures to process and to store fruit and vegetable raw and the products made of it.

References

- [1] Bulatov, M.I., Kalinkin, I.P. (2002) *Prakticheskoe rukovodstvo po fotokolorimetriceskim i spektrofotometriceskim metodam analiza* (The Practical Guide to Photocolorimetric and Spectrophotometric Analysis). Saint Petersburg, Chemistry Publishers, 408 p.
- [2] Daniels, L., Hanson, R., & Phyllips, J.A. (1994). Chemical analysis. In: P. Gerhardt, R.G.E. Murray, W.A. Wood, & N.R. Krieg (Eds.), *Methods for General and Molecule Bacteriology* (pp. 518-519). Washington DC: American Society for Microbiology.
- [3] Gordiyenko, E.A., Pushkar, N.C. (1994) *Fizicheskiye osnovy nizkotemperaturnogo konservirovaniya kletochnykh suspenzij* (Physical Fundamentals for Low-Temperature Preservation of Cellular Suspensions). Kyiv, Naukova Dumka Publishers, 141 p.
- [4] Guter, R.S., Ovchynsky, B.V. (1990). *Elementy chislennogo analiza i matematicheskoy obrabotki rezul'tatov opyta* (The Elements of Numeral Analysis and Mathematical Procession of Experimental Results). Moscow, Nauka Publishers, 432 p.
- [5] Kaatz, U., Dietrich, A., Gopel, K.D., Pottel, R. (1984). Dielectric studies on water in solutions of purified lecithine vesicles. *Chemistry and Physics of Lipid*, 35 (3), 279-290.
- [6] Mychaylik, V.A., Davydova, E.O. (2000). O lozhnykh teplovykh effektach v skaniruyushchej kalorimetrii vlagosoderzhashchych objektov (To the False Warm Effects in Scanning Calorimetry of Water-Containing Objects). *Kriobiologiya*, 1, 17-20.
- [7] Simakhina, G.A. (2011). Biologicheskaya tsennost' i funktsional'noye deystviye komponentov krioporoshkov sakharnoy svekly (Biological Value and Functional Activity of Sugar Beet Cryopowders' Components). *Tsukor Ukrayiny*, 6-7, 66-72.