

*Розглянуто актуальні питання щодо способу підвищення біологічної цінності овочевого соку з моркви. Обґрунтовано доцільність обробки моркви електрохімічно активованою (ЕХА) водою під час зберігання. Досліджено вплив кислотного-лужних умов овочевого соку на активність ферменту глутаматдекарбоксилази. Встановлено, що рН у межах 5,4...6,0 сприяють виділенню ферменту з максимальною активністю. В дослідженнях показано, що збільшити кількість  $\gamma$ -аміномасляної кислоти у рослинних тканинах можна шляхом змінення обміну речовин в сировині.*

*Досліджено вплив температури та часу витримки сировини на швидкість перетворення глутамінової кислоти в  $\gamma$ -аміномасляну кислоту (ГАМК). Виявлена закономірність підвищення активності глутаматдекарбоксилази при чередуванні аеробних і анаеробних умов витримки сировини протягом 24 годин. Встановлено, що витримка овочів протягом 10...60 хв у розрідженій атмосфері і при відносній вологості 95 % не впливає на зміни сухих речовин.*

*Обґрунтовано вибір режиму подачі тиску для перетворення глутамінової кислоти рослинної сировини на  $\gamma$ -аміномасляну кислоту (ГАМК). Доведено, що спосіб витримки сировини при багатократній зміні циклів підвищення і пониження тиску дозволяє отримати готові продукти (соки, напої тощо) з підвищеним вмістом  $\gamma$ -аміномасляної кислоти.*

*На основі виконаного комплексу аналітичних, експериментальних досліджень та математичних розрахунків запропоновано спосіб обробки сировини для виготовлення овочевих соків овочевих соків і напоїв з підвищеним вмістом  $\gamma$ -аміномасляної кислоти. Проведена робота свідчить про доцільність виготовлення овочевих соків і напоїв функціонального призначення, яка може бути впроваджена на консервних переробних підприємствах*

*Ключові слова:  $\gamma$ -аміномасляна кислота, глутамінова кислота, глутаматдекарбоксилаза, аеробіоз, анаеробіоз, ферментативне перетворення, метаболізм*

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# INFLUENCE OF PRELIMINARY PROCESSING OF VEGETABLES ON INCREASING THE CONTENT OF $\gamma$ -AMINO-BUTYRIC ACID IN JUICES

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## 1. Introduction

Fruit and vegetable can factories store large quantities of plant raw materials in fresh form to ensure rhythmic operation during the off-season. Raw material storage changes both quantity and quality of raw materials. An increase in the storage life of vegetables helps to extend a season of raw materials processing.

It is also necessary to take into account new trends in human nutrition. Topical issue is creation of new products with a specific purpose of their biological and physiological action. We know them as functional products, which are different from the traditional ones by the controlled content of physiologically active substances. The most appropriate products among the range of functional products are the products based on fruit and vegetable juices, because there are different classes of functional nutrients, which function simultaneously [1]. A functional component of such products is  $\gamma$ -aminobutyric acid (GABA). It plays a significant role in many metabolic transformations. The most important of them relate to the exchange of dicarboxylic amino acids and glucose.  $\gamma$ -aminobutyric acid takes part in regulation of a physiological state of the nervous system. It affects activity of neurons and synaptic transmission in them, causing an inhibitory effect, that is, it plays a role of a neurotransmitter.  $\gamma$ -aminobutyric acid helps to improve

language and to restore memory for stroke survivors at doses of 0.5 to 2 g per day. In addition, it reduces the blood sugar content at the same dose, and it is capable of lowering blood pressure and maintenance of cardiac function at doses of 3 g per day. Doses of 1 g to 2 g of GABA per day are usually very helpful to reduce anxiety and irritability. Patients suffering schizophrenia and Alzheimer's disease also have a deficiency of  $\gamma$ -aminobutyric acid.

Melatonin increases the content of  $\gamma$ -aminobutyric acid and serotone in the midbrain and hypothalamus according to studies [2]. Glutamine and valerian essential oils also influence increasing the concentration of  $\gamma$ -aminobutyric acid [3, 4].

The main source of  $\gamma$ -aminobutyric acid may be plant raw material with the mentioned amino acid in the free state. It is possible to increase its amount in plant tissues by changing the metabolism in raw materials. Thus, it is possible to obtain products with the increased content of  $\gamma$ -aminobutyric acid without introduction of it from the outside.

Authors of papers [1–4] applied complex technological solutions and additional equipment. Introduction of glutamic acid from the outside and long treatment time requires considerable cost. Therefore, it is expedient to carry out studies for implementations to optimize the technology of obtaining of products with an increased content of  $\gamma$ -aminobutyric acid in fruit and vegetable canneries.

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## 2. Literature review and problem statement

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Paper [5] presents results from studying methods for obtaining  $\gamma$ -aminobutyric acid applying microbiological synthesis of red beans and barley by strains of TISTR 860 (LB860) *Lactobacillus brevis* and TISTR 868 (LB868) *L. brevis*. It shows that LB860 gives a higher yield of  $\gamma$ -aminobutyric acid than LB868. The reason is the imperfectly selected composition of a nutrient medium, namely different concentrations of glucose and peptones. The solution is to optimize a nutrient medium to increase bacterial activity. Therefore, we can state that it is necessary to adapt conditions of the fermentation process of raw materials for production of vegetable juices.

Work [6] presents the results of a method for obtaining of  $\gamma$ -aminobutyric acid under low pH of lactic acid bacteria extracted from fermented products. It shows a possibility of using of *Lactobacillus buchneri*, *Weissella hellenica*, and *Lactobacillus brevis* for production of  $\gamma$ -aminobutyric acid at low pH values of a medium. The activity of inhibition of the glutamate decarboxylase enzyme in vegetable raw materials is not studied sufficiently. An alternative to resolving the issue is application of bacterial strains in production of new types of fermented vegetable products and beverages. It is possible to produce new functional products using low-pH production sources such as fruit juices, jams, or beverages, which were not sources for LAB fermentation before.

Paper [7] presents results of optimization of the extraction of  $\gamma$ -aminobutyric acid in the fermentation process under conditions of fermentation of wheat sowings. It shows that obtaining of  $\gamma$ -aminobutyric acid by microbiological methods is very advantageous, since the product will be purely optically active. The maximum yield in the studies was in glucose (10.3 g/l), sucrose (8.7 g/l) and fructose (6.8 g/l). The issues of determination of the mechanism of creation of effective fermentation conditions, which depend on statistical optimization studies, remain unresolved. An alternative solution to the issue is additional study to determine an amount of carbon and nitrogen under optimal conditions for achievement of the maximum yield of  $\gamma$ -aminobutyric acid. The approach makes us state that obtaining amino acids by enzymatic methods is very advantageous. The current direction for further research is the search for ways to activate glutamate decarboxylase in vegetable raw materials.

Paper [8] describes the mechanism of obtaining  $\gamma$ -aminobutyric acid by lactic acid bacteria in yoghurt. It shows that there is no  $\gamma$ -aminobutyric acid in yoghurt made by using *S. thermophilus* Lp. However, glutamic acid converted to  $\gamma$ -aminobutyric acid completely at using of *S. thermophilus* Hp. The possible reason is a substrate mismatch. An alternative is to investigate substrate properties additionally. The approach will make it possible to obtain the maximum amount of  $\gamma$ -aminobutyric acid.

Paper [9] presents results from studying a method for increasing the content of  $\gamma$ -aminobutyric acid by fermentation with *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. It shows the possibility of obtaining  $\gamma$ -aminobutyric acid by decarboxylation of L-glutamic acid by cells of *Arthrobacter simplex* bacteria. The issues regarding determination of the biomass growth period and the high cost of production of  $\gamma$ -aminobutyric acid (0.43 g of dry biomass per 1 g of product) remain partially unresolved. The solution may be an increase in the glutamate decarboxylase activity

of the used strain. Therefore, one can say that it is necessary to search for additional ways to increase the activity of glutamate decarboxylase. It is possible to use the studies in functional beverages technology. The approach allows us to state that the obtained products with an increased content of  $\gamma$ -aminobutyric acid have significant benefits for human health.

Work [10] presents results of studies on processes of extraction of *Lactobacillus plantarum* strains from fruits and fermented foods. It shows that strains are characterized in vitro for presence of probiotic features. The strains exhibited antimicrobial activity against indicator strains and produced enzymes useful for human health. The reason may be ability of the strains to produce  $\gamma$ -aminobutyric acid (GABA), which takes part in various metabolic reactions. An option to resolve the issue is to expand food products as a starter crop in production of functional probiotic products. Therefore, one can suggest that the influence of technological processes during storage of raw materials affects formation of  $\gamma$ -aminobutyric acid.

Paper [11] presents results of studies into methods for obtaining electro-activated water (EW), which has a strong bactericidal and fungicidal action. It shows efficiency of the use of electro-activated water as a new wide-range sanitary agent. However, there are questions about limited use in food production. The possible reasons may be low pH ( $\leq 2.7$ ), water has corrosive nature and affects organoleptic properties of some foods. A possible solution is to study the influence of such factors as water temperature, pH, a type of electrolyte, a flow rate of water and electrolyte, a concentration of salts, presence of organic matter in a product, hardness of water and pollutants.

Work [12] presents results from studying the effect of chlorine and pH on the bactericidal activity of electro-activated water (EW) against O157:H7 *Escherichia coli* and *Listeria monocytogenes*. It shows that the bactericidal activity of water increases with a decrease in pH for both pathogens for each residual chlorine level. A possible reason is an inadequate assessment of synergistic effects. An option to solve the issue is to study the bactericidal mechanism by means of atomic-force microscopy and thermal modes for raw materials.

Analysis of literature data reveals that it is necessary to establish a mechanism for increasing the content of  $\gamma$ -aminobutyric acid in vegetable juices by preliminary treatment of raw materials. It simplifies the technology greatly. There is a need for additional studies on the influence of environmental parameters on changes in the metabolism in raw materials without the use of external glutamic acid supplements and without participation of microorganisms.

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## 3. The aim and objectives of the study

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The aim of this study is to determine the influence of preliminary treatment of vegetables on increasing the content of  $\gamma$ -aminobutyric acid in juices. This will increase the nutritional value of vegetable juices.

We solved the following tasks to achieve the objective:

- determination of the influence of carrot treatment on electrochemically activated (ECHA) water during storage;
- establishment of the mechanism, conditions, and parameters of changes in metabolism of glutamic acid under the action of glutamate decarboxylase with formation of

$\gamma$ -aminobutyric acid in vegetable raw materials under the influence of external factors;

- determination of the constant of destruction of  $\gamma$ -aminobutyric acid at the temperature of sterilization of a finished product and different pH values.

**4. Determining the influence of treatment of vegetables with electrochemically activated (ECHA) water during storage**

Raw materials used for production of juices go for washing, inspection, cleaning and rinsing with electrochemically activated water. There is drinking chlorinated water (with the chlorine content of 0.05...0.1 mg/dm<sup>3</sup>) used for washing of raw materials at canning factories. Often, factories carry out additional chlorination of water to achieve a residual chlorine content of 2...10 mg/dm<sup>3</sup>. They obtain chlorine in the form of gas by electrolysis of table salt. Prolonged exposure of fresh fruits at the temperature of 20...25 °C under the action of pulsing pressure can promote development of microorganisms. Therefore, additional processing of fruits to reduce microbiological contamination is necessary.

Some food industries use electro-activated water obtained by electrolysis of water salt solutions in the apparatus, which makes it possible to separate electro-activated water into catholyte and anolyte. A base of water electro-activation is a transfer of ions and electrons through a semipermeable membrane placed in an electrolyte solution while creating a potential difference in liquid on both sides of a membrane. There are catholyte – alkaline (“living”) water – obtained in the cathode chamber in the process of electro-activation, and anolyte – acid (“dead”) water – in the anode one.

Electro-activated water has high energy (redox potential value from minus 800 to plus 1,200 mV), which makes it possible to use it in the food industry for the following purposes: an increase in the storage life of raw materials at raw materials sites, alkaline cleaning of vegetables; an influence on enzyme activity; storage of vegetables in salt solutions [13].

Anolyte serves for disinfection and washing of any type of equipment (reservoirs, tanks, heat exchangers, bottling and packaging lines), pipelines, implements, containers, and surfaces of industrial premises in the food industry.

Therefore, vegetables with an increased content of  $\gamma$ -aminobutyric acid used further to obtain juices are rinsed with electro-activated water, which has a bactericidal action, to prevent propagation of aerobic and anaerobic microorganisms [14].

We took “Carotel” carrot variety as an object for the study. The anolyte and catholyte had the following characteristics – anolyte: pH 3.5–4, a redox potential (RP) +980...+1,100 mV, a content of active chlorine 110...130 mg/dm<sup>3</sup>; catholyte: pH 10–10.4, RP –750...–800 mV. We obtained electro-activated water at a laboratory installation with graphite electrodes from drinking water. It had the following characteristics: pH 7.5, Ca<sup>2+</sup> – 2.8 mg-eq/dm<sup>3</sup>, Mg<sup>2+</sup> – 0.98 mg-eq/dm<sup>3</sup>, HCO<sub>3</sub><sup>-</sup> – 3.8 mg-eq/dm<sup>3</sup>, Cl<sup>-</sup> – 150 mg/dm<sup>3</sup>. We measured pH and RP with I 130 ionometer. We stored carrots at a temperature of +1...+3 °C in open plastic bags with a film thickness of 30  $\mu$ m and a moisture content of

92...95 % in a refrigerator. We performed microbiological control by seeding samples from a surface of carrots on a solid nutrient medium – meat-peptone agar, and holding them in a thermostat at a temperature of 30 °C for 7 days to detect mycelium fungi.

Carrots were divided into two batches. We investigated the effect of electrochemically activated water on contamination of carrots and pathogenic fungi in the first batch. In the second batch, we investigated the effect of the dependence of carrot contamination and a degree of carrot affection by pathogenic microorganisms.

The first batch was split into two parts. We treated the first part with anolyte for 20 min (sample No. 1), the second part – with anolyte for 20 min, and then with catholyte for 5 min (sample No. 2).

The second part was split into two parts as well. We treated the first part with anolyte for 30 min (sample No. 3), the second part – for 10 min (sample No. 4). We left a control sample (a control) for storage together with research samples. We weighed samples, carried out visual inspection of roots, performed microbiological control of carrot surface and determined the vitamin C content each month (Tables 1, 2).

Table 1

Microbiological and visual observations

Sample No. and type of treatment	Microbiological observations	Visual observations
First batch		
Sample No. 1. Carrots treated with anolyte (20 min)	Cocci in the depressed state – less than 10 units in 2–3 fields of view	Almost unchanged initial condition of carrots, smooth surface, wrinkle-free
Sample No. 2. Carrots treated with anolyte (20 min), and then with catholyte (5 min) (Fig. 1)	Cocci, rod-like bacteria, streptococci	Presence of air roots, sprouted leaves, wrinkled surface
Second batch		
Sample No. 3. Carrots treated with anolyte (30 min) (Fig. 2)	Cocci in the depressed state – less than 10 units in 2–3 fields of view	Unchanged initial state of root crops
Sample No. 4. Carrots treated with anolyte (10 min)	Cocci in the depressed state – more than 10 units in 2–3 fields of view	Small number of air roots, smooth surface
Control sample (Fig. 3)	Cells of fungi with a diameter of 1–2 mm. Cocci, rod-like bacteria, streptococci	Air roots sprouted green shoots, cells of fungi affection on roots

Sample No. 1, 2 did not contain mycelium fungi in any of the fields of view immediately after treatment in the first batch. The control sample contained *Botrytis* fungi, there was gray fluffy plaque of *Rhizopus* fungi (mycelium on the substrate partially colored in dark brown) on the agar. Visually, Sample No. 1 had no significant changes throughout the storage life, except only a slight growth of air roots and leaves. We observed growth of air roots and top leaves of carrots (Fig. 1) on Sample No. 2. There were rotting and significant growths of air roots on the control sample (Fig. 3).

Table 2

Physical-and-chemical changes in the studied carrot samples during storage

Storage month	Control	Research													
		Batch 1				Batch 2				Vitamin C, mg/100 g					
	Weight loss, %	Dry matter, %	Vitamin C, mg/100 g	Weight loss, %		Dry matter, %		Vitamin C, mg/100g				Weight loss, %		Dry matter, %	
				Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 3	Sample 4	Sample 3	Sample 4	Sample 3	Sample 4
November	1.2	14.8	6.2	0.5	0.5	14.8	14.8	6.2	6.2	0.5	0.5	14.8	14.8	6.2	6.2
December	0.7	13.2	5.27	0.5	0.5	14.6	14.2	5.87	5.67	0.5	0.6	14.7	14.3	5.89	5.89
January	0.7	11.3	4.58	0.4	0.4	14.3	13.3	5.52	4.98	0.4	0.5	14.5	13.6	5.6	5.47
February	0.6	10.4	3.9	0.4	0.5	14.1	12.6	5.19	4.23	0.4	0.6	14.4	12.9	5.38	5.21
March	0.9	9.54	3.32	0.5	0.6	13.9	11.8	4.93	3.62	0.5	0.7	14.3	12.1	5.27	4.87
April	0.7	8.74	2.82	0.5	0.7	13.8	10.9	4.68	3.12	0.5	0.6	14.2	11.3	5.16	4.58
Total	4.8	6.06	3.38	2.8	3.2	1.0	3.9	1.52	3.08	2.8	3.5	0.6	3.5	1.04	1.62



Fig. 1. Changes during storage of carrots: a – Sample No. 2; b – Sample No. 3; c – control sample

In the second batch, sample No. 3, 4 did not contain pathogenic fungi immediately after treatment with the anolyte also; the control sample contained *Botrytis* fungi, and there was a gray fluffy plaque on the agar. Visually, Sample No. 3 had the smallest growth of air roots and leaves at the end of storage; the surface of roots was almost the same as it was initially (Fig. 2), the second one – a growth of top leaves of carrots. Sample No. 4 had an appearance similar to Sample No. 1, it had some larger leaves and superficial roots, cocci were in a depressed state.

We studied the content of dry matter by drying of the sample to constant weight. We found that dry matter

stores best in carrots treated with anolyte for 30 min. The total loss was 2.8 % for the entire storage period, and the total loss in the control sample was within the normalized losses – 4.8 %.

We also found that the treatment of carrots with ECHA water did not reduce the content of vitamin C, but rather prevented its losses during storage. Thus, the optimal duration of ECHA-water treatment should be within 20–30 min.

Next, we carried out studies to determine a degree of contamination of raw materials by microorganisms at all stages of processing. Table 3 gives data from microbiological studies.

Table 3

Microbiological contamination of raw materials and finished products (n=3; P>0.95)

Objects of the study	QMAFAnM, CFU per g	Bacteria of intestinal bacillus, thousand/g	Spores of mesophilic bacilli and clostridia, thousand/g	Molds, thousand/g	Yeast, thousand/g
Carrot juice an increased GABA content					
Raw material before washing	180.400×10 <sup>3</sup>	0.050	24.000	7.000	8.180
Raw material after washing	9.000×10 <sup>3</sup>	0.012	11.000	3.000	6.480
Raw material before washing with anolyte	1.500×10 <sup>3</sup>	absent	1.300	0.760	0.480
Raw material after treatment with pulsating pressure, wiped	5.600×10 <sup>3</sup>	absent	2.320	1.470	1.980
After sterilization of a product	1.050×10 <sup>3</sup>	absent	0.480	absent	absent

Table 3 shows that washing of raw material with electro-activated water can reduce contamination of vegetables by 2 orders of magnitude at all stages of treatment, which is a very effective way of prevention of microbial spoilage of raw materials.

After treatment of raw material with electro-activated water, it is necessary to expose vegetables under an action of pulsating pressure at a temperature of 24...25 °C and a relative humidity of 95 %. Thermometers and psychrometers control temperature and relative humidity daily. It is possible to create a gas medium, which differs in composition from the surrounding atmosphere, in vacuum apparatus, which gives possibility to obtain the composition of a gas medium required for the specific type of product [15].

### 5. Determining the mechanism, conditions, and parameters of change in metabolism of glutamic acid into $\gamma$ -aminobutyric acid under the influence of external factors

A basis of respiration is the number of patterns, a course of the oxidation reaction, reduction, decarboxylation, deamination and others. A specific enzyme catalyzes each of the reactions. There is a genetic connection between aerobic and anaerobic types of respiration. Causes of anaerobic respiration in fruits and vegetables can be a lack of oxygen, an excess carbon dioxide, tissue damage or a variety of other causes. Different intermediate products of metabolism accumulate under different conditions. The main cause of anaerobic respiration is a lack of oxygen in tissues. The process of anaerobic respiration intensifies at exposure of fruits in an atmosphere with low oxygen content, as a result, oxidizing products, such as acetaldehyde and ethyl alcohol, accumulate in tissues.

There was accumulation of acetaldehyde and ethyl alcohol in small quantities at treatment of vegetables with pulsating pressure. Free amino acids undergo deamination and decarboxylation processes. Decarboxylation of amino acids leads to formation of amines and carbon dioxide. Products formed by decarboxylation of amino acids often have a physiological effect, and there are new amino acids formed in the case of decarboxylation of dicarboxylic acids. Processes of decarboxylation are especially intensive in plant tissue. Thus, there is alanine formed of aspartic acid, and  $\gamma$ -aminobutyric acid of glutamic acid [16].

Protective reactions of plants to adverse effects become adaptive in nature with the possibility of their coordination by different systems of regulation. A complementary restructuring of metabolism, which is necessary for formation of sufficient ATP and intermediates, generation and oxidation of recovered cofactors, detoxification of anaerobic metabolism products, controls enzyme regulation at hypoxia and anoxia. Researchers found that activity of a number of enzymes changes under action of environmental factors on fruits, namely, under conditions of oxygen deficiency. Corresponding reactions of a plant organism change in dependence on duration of hypoxic action and composition of a gas medium. There are significant disturbances in carbohydrate metabolism reflected in the content of organic acids under the influence of relatively short-term effects of anaerobic conditions in a fruit. A rate of protein synthesis decreases, and a need for amino acids decreases due to lack of energy under anaerobic conditions. This is

the main reason for an increase in the concentration of free amino acids in cells.

An effective adaptation mechanism is restructuring of amino acid metabolism directed towards formation of so-called "stress" amino acids. One of such amino acids is  $\gamma$ -aminobutyric acid. Plant tissues accumulate it under adverse conditions in large quantities without damaging cells. It acts as an easily mobilized form of succinate in restoration of normal respiration by blocking of its utilization through reactions of the tricarboxylic acid cycle.

Synthesis of  $\gamma$ -aminobutyric acid occurs by  $\alpha$ -decarboxylation of glutamate, which catalyzes calcium/calcium modulin-dependent glutamate decarboxylase with a sufficiently low pH optimum (5.9). Glutamate decarboxylase of various vegetable raw materials functions in different acid-alkaline conditions with a pH optimum of 3.0 to 6.0 (carrots, pumpkins, tomatoes, beets, etc.). There was extraction of glutamate decarboxylase from fruits having different pH levels performed to determine the optimum pH. The locus of  $\gamma$ -amino butyric acid may be a vacuole, where we can find a significant number of individual amino acids.

We carried out studies of the isolated enzyme glutamate decarboxylase and found that pH values within 5.4...6.0 contribute to the extraction of the enzyme with maximum activity. We also observed an increase in the activity of glutamate decarboxylase at pH values from 3.0 to 5.4. Fig. 2 shows results of the studies.

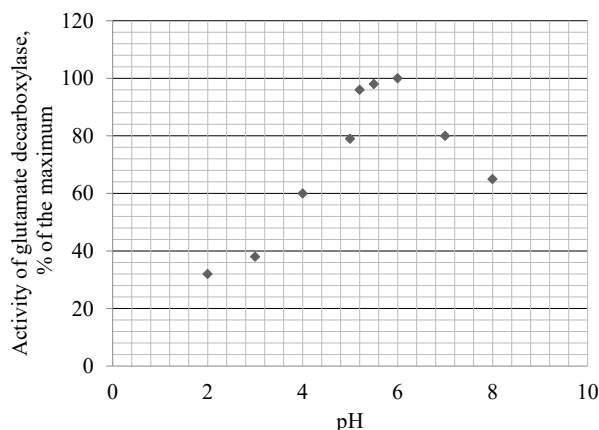


Fig. 2. The influence of pH of a medium on the activity of glutamate decarboxylase in carrot juice

Since vegetable juices and beverages have an active acidity of 3.9...4.5, the activity of glutamate decarboxylase is satisfactory to catalyze conversion of glutamic acid to  $\gamma$ -aminobutyric acid. We studied the influence of temperature and exposure time of raw material to select optimal conditions, which contribute to an increase in the content of  $\gamma$ -aminobutyric acid in raw material during treatment with pulsating pressure (Fig. 3, 4).

Fig. 3 shows the optimum temperature (23...24 °C), which promotes intensification of the activity of glutamate decarboxylase. The highest activity of glutamate decarboxylase was during alternation of aerobic and anaerobic conditions of exposure of raw material for 24 hours.

We investigated the influence of air relative humidity in a preliminary treatment chamber under vacuum on technological parameters of the obtained juice experimentally. Carbon dioxide accumulates faster as the temperature rises. It dissolves better than oxygen in juice. An increase in the

concentration of carbon dioxide in vegetables manifests itself as a regulator of metabolism that affects the redox systems, including oxidative enzymes. We established that the gaseous composition of the atmosphere of vegetables depends on duration of their exposure in a rarefied atmosphere and research temperature.

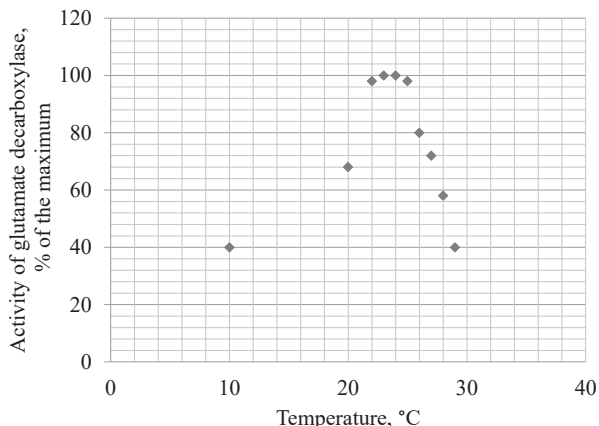


Fig. 3. The influence of exposure temperature of raw material on the activity of glutamate decarboxylase under an action of pulsing pressure (24 cycles, 70 kPa and 101.3 kPa) in carrot juice

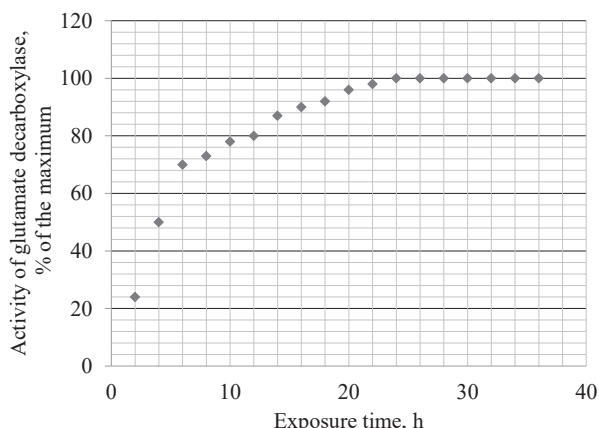


Fig. 4. The influence of exposure time of raw material on the activity of glutamate decarboxylase under an action of pulsating pressure (70 kPa and 101.3 kPa) in carrot juice

According to Henry law, a value of the partial pressure determines the maximum saturation with a gas. It depends on temperature. A decrease in pressure during vacuuming leads to an imbalance, and there are soluble gases released in the form of microbubbles. Generation of the vapor phase joins these processes. A depth of the liquid phase entering the metastable state determines intensity of gas and vapor phase formation. Thus, the process of vacuuming removes not only gases but also moisture from the raw material.

We determined main technological parameters of juice to investigate the influence of relative humidity in a chamber for preliminary treatment of whole fruits for the yield and characteristics of the juice. To do this, we kept vegetables in the rarefied atmosphere (70 kPa) at the relative humidity of 71 %; 80 %; and 95 % within 10 min; 20 mins and 60 mins.

There was a loss of weight at the relative humidity (71 % and 80 %) in the process of vacuuming. It led to an increase in the mass fraction of dry matter in the juice from 0.1 to 0.5 %,

which was 2.7...4.5 % of the total content. It should affect the juice yield.

An increase in duration of the vacuuming of vegetables to 60 min at the relative humidity of 71 % did not change the yield of juice significantly, because of adaptation processes in the cytoplasmic membranes and loss of weight of vegetables during the treatment.

The mass fraction of soluble dry matter did not change relatively to the control under vacuum for 10... 60 min at the relative humidity of 95 %.

Such studies make us suggest that exposure of vegetables in a rarefied atmosphere at high relative humidity does not affect changes in dry matter. The fact there is no weight loss process under such conditions also confirms this.

To study the influence of pulsating vacuum (vacuum violated at regular intervals) on changes in the metabolism of raw materials, we examined a change in the content of  $\gamma$ -aminobutyric acid in vegetables depending on a number of vacuum violations. We placed the prepared vegetables in a chamber and changed the pressure from 70 kPa to 101.3 kPa every hour in it. The temperature was constant throughout the study. It was 24 °C (Fig. 5).

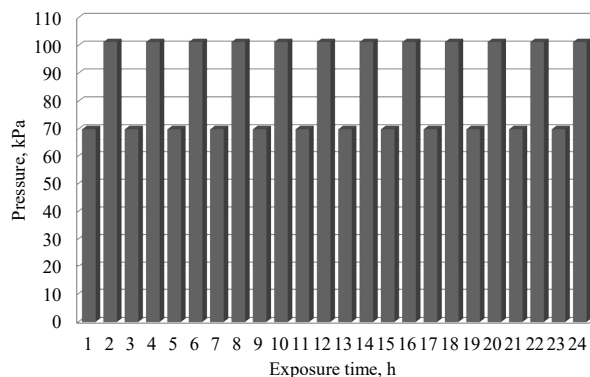


Fig. 5. The cyclic change of pressure depending on the exposure time of raw material

The graph in Fig. 5 shows that eight pressure drops for 8 h increased the content of  $\gamma$ -aminobutyric acid in 4 times, subsequent pressure drops up to 24 hours at the same frequency led to an increase in the content of  $\gamma$ -aminobutyric acid in 8 times (on the example of carrot juice). The results confirmed the effectiveness of preliminary treatment of raw materials within 24 hours. The studies showed that the content of  $\gamma$ -aminobutyric acid did not change after exposure of the raw material under the given conditions, and therefore it is not rational to increase the exposure time.

A number of vacuum disturbances influence changes in the content of  $\gamma$ -aminobutyric acid significantly (Fig. 6).

The proposed method of exposure of raw material at repeatedly changing cycles of raising and lowering of the pressure makes possible to obtain finished products (juices, drinks, etc.) with an increased content of  $\gamma$ -aminobutyric acid. Raw materials undergo conversion processes of free amino acids. Glutamic acid, which is about 40 % of the total free amino acid content of raw materials, forms  $\gamma$ -aminobutyric acid and carbon dioxide under the action of the enzyme glutamate decarboxylase.

We proved that pressure drops have a more effective influence on increasing of the content of  $\gamma$ -aminobutyric acid in raw materials than constant reduced pressure, since, accumulation of acetaldehyde and ethyl alcohol be-

gins at constant exposure of raw material under reduced pressure.

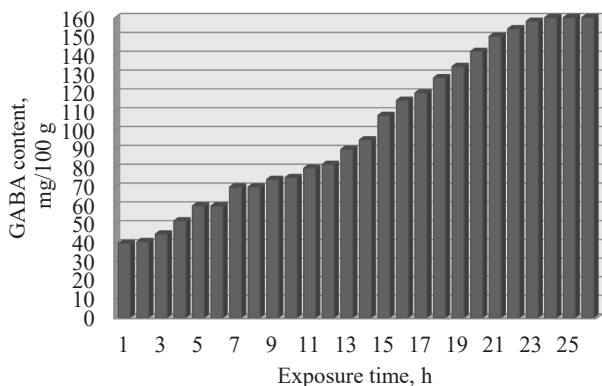


Fig. 6. Changes in the content of  $\gamma$ -aminobutyric acid depending on the exposure time at cyclic pressure changes in carrot juice

The content of  $\gamma$ -aminobutyric acid increased significantly under the action of oxygen deficiency in the tissues of fruits. The optimum treatment time is 24 hours, because the maximum amount of  $\gamma$ -aminobutyric acid accumulates and alcohol fermentation does not begin during this time. Thus, protective reactions of fruits to adverse conditions become adaptive in nature with possibility of coordination of them through different systems of regulation of oxygen deficiency.

It was found that the activity of glutamate decarboxylase increases under influence of environmental factors on vegetables, namely, under conditions of oxygen deficiency.

We determined GABA content using a formal titration method.

A base of determination of amine nitrogen is blocking of amine groups of amino acids with formalin with subsequent titration of free carboxyl groups.

We placed 5 ml of carrot juice in a conical flask and added 20 ml of distilled water. 20 ml of distilled water was poured into the second flask, it was the control one. We added 3 drops of neutral red to each flask and titrated with 0.2 N sodium hydroxide solution to a pale orange color (we did not take into account the amount of alkali).

Then, we added 10 ml of the formaldehyde mixture to each flask. We additionally titrated the control solution with 0.2 N sodium hydroxide solution to a pale pink color, fixing the amount of alkali consumed. We titrated the solution under study with 0.2 N sodium hydroxide to the same color as the control solution, fixing the amount of alkali consumed.

When we determined the amount of amine nitrogen, we took into account a change in the color of the solution. when we neutralized the original solution of amino acids, we added sodium hydroxide by the indicator of neutral red to a pale orange color, thus to a pH close to 7.0.

The formal mixture binds the amine group due to hydrogen ions, which causes crimson color. Sodium hydroxide titration neutralizes carboxyl groups, resulting in a pale orange color.

Addition of excess sodium hydroxide shifts pH of the solution to a pH of 8.0–8.5 and, crimson color appears due to the presence of phenolphthalein.

We calculated the percentage of amine nitrogen from formula:

$$\% \text{ amine nitrogen} = \frac{(V_1 - V_2) \times 2,8}{a} \times 100,$$

where  $V_1$  is the amount of solution of 0.2 N sodium hydroxide spent for titration to the intense red color of the test solution, ml;  $V_2$  is the amount of solution of 0.2 N sodium hydroxide spent for titration to an intense red color of the control solution, ml; a is the sample of the studied sample, ml; 2.8 – a titer of 0.2 N sodium hydroxide solution by nitrogen.

### 6. Determining a constant of the rate of destruction of $\gamma$ -aminobutyric acid

The final stage in production of any type of canned food is thermal sterilization (pasteurization) carried out at different temperatures and different process duration. Since formulations of the developed juices with the increased content of  $\gamma$ -aminobutyric acid belong to group A by pH value and dry matter content for microbiological control, sterilization of these products occurs at the temperature of 120 °C. Therefore, we studied the effect of thermal treatment of model solutions of  $\gamma$ -aminobutyric acid in a temperature field of 120 °C on a degree of destruction of  $\gamma$ -aminobutyric acid.

It is known that most amino acids are stable under conditions of acid hydrolysis of proteins (20 % HCl solution, temperature 105 °C). Such amino acids are serine, threonine, tyrosine, phenylalanine. Alkaline hydrolysis destroys such amino acids as cysteine, cystine, methionine, tryptophan almost completely. There is no information on destruction of  $\gamma$ -aminobutyric acid at different pH values [17].

We took a solution with a mass fraction of  $\gamma$ -aminobutyric acid of 0.2 %, at pH=3.0...7.0 to study the kinetics of destruction of  $\gamma$ -aminobutyric acid as a model solution. We investigated thermal destruction (by analogy with the real conditions of sterilization of canned vegetables) at the temperature of 120 °C and the duration of 20...80 min.

To achieve the desired content of  $\gamma$ -amino butyric acid in the finished product, it is necessary to determine a constant of the rate of destruction of  $\gamma$ -aminobutyric acid (K), which is the inverse of  $D_t$  – time required to reduce the content of  $\gamma$ -aminobutyric acid by 90 % or by 10 times.

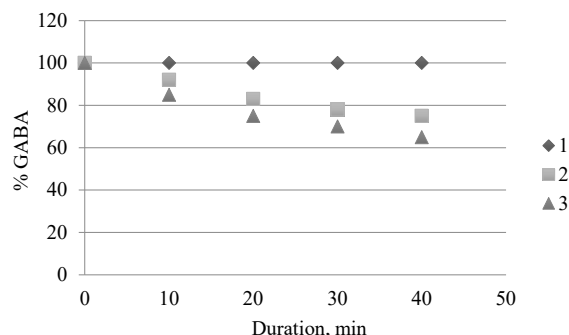


Fig. 7. Changes in the content of  $\gamma$ -aminobutyric acid in the stationary temperature field (120 °C): 1 – pH=3.0; 2 – pH=4.0; 3 – pH=6.5

Fig. 7 shows the destruction of  $\gamma$ -aminobutyric acid in a stationary temperature field (120 °C) at pH values of 3.0, 4.0, and 6.5.

The curve of changes in the content of  $\gamma$ -aminobutyric acid is exponential (except for the curve with pH=3.0), that is it corresponds to the kinetics of the chemical reaction of the first order.

We constructed a corresponding graph in the semilogarithmic coordinate system to determine the constant of the rate of the destruction of  $\gamma$ -aminobutyric acid ( $K$ ). The abscissa axis represents the duration of the process, the ordinate axis –  $\lg \gamma$  (% content of  $\gamma$ -aminobutyric acid, Fig. 8.

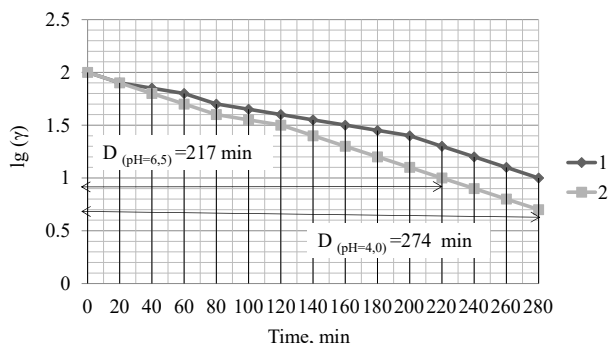


Fig. 8. The curve of changes in the content of  $\gamma$ -aminobutyric acid at temperature 120 °C and pH=4.0, pH=6.5

Fig. 8 shows the curve of changes in the content of  $\gamma$ -aminobutyric acid at temperature 120 °C and the values of pH=4.0, pH=6.5. The points correspond to the results of experimental studies in the range of 0–40 min. The points correspond to the calculations of the mathematical model in the range of 40–280 min, respectively.

We processed the obtained data by methods of mathematical statistics and correlation analysis using Mathcad software. The measurement error of the parameters did not exceed 5 %.

As one can see in Fig. 10, the passage time of the rectified curve of one logarithmic cycle was  $D=217$  min, respectively, the half-life of the  $\gamma$ -aminobutyric acid is  $D_{\tau/2}=108.5$  min. We determined the constant of the destruction rate of  $\gamma$ -aminobutyric acid ( $K$ ) by calculation as  $K=\frac{1}{D}$ ,  $K=4.6 \cdot 10^{-3} \text{ min}^{-1}$ . Therefore, one can conclude that  $\gamma$ -aminobutyric acid is a rather stable amino acid in sterilization of cans with different pH values.

To take into account the spread of points during straightening and to eliminate inaccuracies in the definition of  $D$ , we determine the true value of the curve by the method of least squares by the equation of straight  $\lg \gamma = a + bx$ , where  $x$  is heating time, which corresponds to a given value of  $\lg \gamma$ . Below, there is a definition of  $D$  constant for the temperature of 120 °C and pH=6.5 and pH=4.0 below.

## 7. Discussion of results from studying an increase in the nutritional value of vegetable juice

The results of studies of a method of increasing of the activity of glutamate decarboxylase on the concentration of  $\gamma$ -aminobutyric acid in carrot juice indicate the conversion of glutamic acid into  $\gamma$ -aminobutyric acid under the influence of external factors. The inclusion of vegetable juices with an increased content of  $\gamma$ -aminobutyric acid in the human diet will help to increase nutritional value, because there are many functional nutrient classes at the same time in

vegetable juice-based products. The acceptable daily dose of  $\gamma$ -aminobutyric acid is 2 g for an adult. The investigated method of treatment of carrots gives possibility to obtain carrot juice with the content of  $\gamma$ -aminobutyric acid of 140 mg/100 g of juice.

The feature of the proposed method is a change in the metabolism during storage of carrots treated with electrochemically activated water, which increases the content of  $\gamma$ -aminobutyric acid in the finished carrot juice significantly. The method of treatment of carrots with electrochemically activated (ECHA) water makes possible to reduce contamination of vegetables by 2 orders of magnitude at all stages of processing. Therefore, it is a very effective way to prevent microbial spoilage of raw materials.

We analyzed previous studies and saw that their authors were unable to obtain  $\gamma$ -aminobutyric acid directly in raw material and without participation of microorganisms. In view of the above, we chose a change in conditions and parameters of the gas medium during storage of carrots as a method for obtaining of  $\gamma$ -aminobutyric acid. The activity of glutamate decarboxylase increases under an influence of external factors. Its increase leads to changes in the metabolism of amino acids in carrots. These are the benefits of the study.

Analysis of the method of increasing the activity of glutamate decarboxylase allows us to state the following.

- treatment with ECHA-water makes possible to increase the storage life of raw materials by reduction of the microbial spoilage of raw materials. As well as to reduce losses in dry matter during storage of carrots, to prevent losses of vitamin C. We can recommend application of the method of preliminary treatment before introduction in the technological process of production of vegetable juices (Fig. 1);

- acid-alkaline conditions of vegetable juice affect the activity of the glutamate decarboxylase enzyme. It was established that pH values within 5.4...6.0 contribute to extraction of the enzyme with maximum activity (Fig. 2);

- changes in aerobic and anaerobic conditions have a significant influence on the metabolism in vegetables during storage. Optimization of parameters of multiple changes in pressure cycles from 70 to 101.3 kPa (duration from 8 to 24 hours) leads to an increase in the content of  $\gamma$ -aminobutyric acid (Fig. 3, 4);

- the mechanism and parameters of enzymatic conversion of glutamic acid to  $\gamma$ -aminobutyric acid in carrots depend on the action of pulsating pressure and the number of vacuum disturbances (Fig. 5, 6). Thus, protective reactions of fruits to adverse conditions become adaptive in nature with possibility to coordinate them through various systems of oxygen deficiency regulation;

- thermal sterilization of finished products shows significant influence on the storage of  $\gamma$ -aminobutyric acid. Therefore, the constant of destruction gives possibility to control duration of sterilization in a stationary temperature field at the temperature of 120 °C (Fig. 7, 8).

The performed studies may be expedient from a practical point of view, as they make possible to substantiate the ratio of amino acids in carrot juice. From a theoretical point of view, the expediency of the conclusions lies in determination of the kinetics of the conversion of glutamic acid to GABA. The advantage of the method is the technological scheme of preliminary treatment of raw materials, because it does not require external addition of glutamic acid.

However, one should note that the analysis of technological processes of carrot exposure requires optimization



of parameters of a gas medium during the exposure of raw materials. Based on the experimental data, we need substantiation for the choice of technological equipment for control of the carbon dioxide and oxygen content. Our study did not resolve this ambiguity. This is a task for further research.

One can use the obtained data in production of functional juices in the canning industry.

## 8. Conclusions

1. It was found that treatment of carrots with ECHA water does not reduce the content of vitamin C, on the contrary, it prevents loss of vitamin C during storage. We determined the optimal duration of treatment with ECHA water – within 20–30 min. The total loss of dry matter during the whole storage period was 2.8 %, and it was 4.8 % within the normalized losses in the control sample. Washing of raw materials with electro-activated water can reduce vegetable contamination by 2 orders of magnitude at all stages of processing. Therefore, it is a very effective way of prevention of microbial spoilage of raw materials.

2. We studied the kinetics of enzymatic conversion of glutamic acid to  $\gamma$ -aminobutyric acid under the action of glutamate decarboxylase extracted from carrots: 24 hours

at cyclic pressure changes of 70 and 101.3 kPa, temperature 23...24 °C.

We carried out studies of the extracted glutamate decarboxylase enzyme and found that pH values within 5.4...6.0 contribute to extraction of the enzyme with maximum activity.

It was proven that pressure drops have a more effective influence on the increase in the content of  $\gamma$ -aminobutyric acid in raw materials than constant reduced pressure, since, constant exposure of raw materials at constant reduced pressure leads to the accumulation of acetaldehyde and ethyl alcohol. Eight pressure drops for 8 h increase the content of  $\gamma$ -aminobutyric acid in 4 times, subsequent pressure drops up to 24 hours at the same frequency lead to an increase in the content of  $\gamma$ -aminobutyric acid in 8 times (on the example of carrot juice). The obtained results confirm the effectiveness of preliminary treatment of raw materials within 24 hours. We substantiated that the content of  $\gamma$ -aminobutyric acid does not change after exposure of raw material under the given conditions.

3. It was established that the constant of destruction of  $\gamma$ -aminobutyric acid ( $K$ ) is smallest at the temperature of  $120 \pm 2$  °C, pH=6.5 ( $K=4.6 \cdot 10^{-3} \text{ min}^{-1}$ ). Therefore,  $\gamma$ -aminobutyric acid is a rather stable amino acid under conditions of industrial sterilization at various pH values.

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**Останнім часом в різних галузях харчової промисловості набули широкого використання нетрадиційні способи обробки сировини та напівфабрикатів. Це сприяє інтенсифікації виробництва, подовженню терміну збереження свіжості нових виробів, дозволяє впроваджувати ресурсо- та енергозберігаючі технології.**

**Актуальними слід вважати дослідження можливості використання ультразвуку в технології борошняних кондитерських виробів, а саме бісквітів.**

**Запропоновано технологію збивання меланжу з цукром за допомогою міксерів, чаша якого встановлена в ультразвукову ванну, заповнену водою.**

**Досліджено вплив дії ультразвуку на показники якості піни яєчно-цукрової суміші (піноутворювальну здатність, стійкість піни, її мікроструктуру) та готових бісквітних напівфабрикатів.**

**Встановлено, що піноутворювальна здатність дослідних зразків з використанням ультразвуку збільшувалась на 35 %. Крім того, максимальне значення піноутворювальної здатності зразку з використанням ультразвуку досяглося за майже у двічі коротший час ніж у контрольного. Порівняльний аналіз стійкості піни після 60 хв вистоявання показав, що найбільш стійкою виявилася система меланж-цукор під дією ультразвуку – 90 %. Визначено, що дія ультразвуку на яєчно-цукрову суміш забезпечила отримання піни, майже з рівномірними однаковими бульбашками невеликого розміру, розташованими близько один до одного.**

**Встановлено оптимальні параметри збивання яєчно-цукрової суміші бісквітного тіста в полі ультразвуку: потужність ультразвуку – 0,6 кВт, температура води в ультразвуковій ванні – 26 °С, час збивання меланжу з цукром – 6,5 хв.**

**Доведено, що використання ультразвуку в технології бісквітних напівфабрикатів сприяє інтенсифікації процесу піноутворення яєчно-цукрової суміші; дає змогу збивати всі компоненти одночасно, що значно спрощує сам процес виробництва бісквітів; підвищенню пористості готових виробів та більш рівномірному розподілу пор**

**Ключові слова: ультразвук, яєчно-цукрова суміш, піноутворювальна здатність, стійкість піни, бісквітні напівфабрикати**

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## EXPLORING A POSSIBILITY OF USING ULTRASOUND IN THE TECHNOLOGY OF CONFECTIONERY PRODUCTS

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### 1. Introduction

Flour-based confectionary products (FCPs) are increasingly popular among different age groups of consumers. The range of FCPs is diverse and has more than a hundred titles. A significant proportion of products within a given group belongs to sponge-cake dough products. It is characterized by easy digestibility, pleasant taste and aroma, as well as pleasant physical appearance.

Sponge cake dough is a complex dispersed system consisting of about 45 % of air bubbles, separated from each other by films of the liquid dispersed medium, which includes eggs, sugar, and flour. The structure of a baked semi-finished product depends on the size and quantity of air bubbles in the dough [1].

The formation of a foam-like structure of a sponge cake semi-finished product is primarily affected by the properties

of a base raw material, the duration of whipping process, and the mechanical impact on dough when mixing it.

Ultrasound (US) is quite widely used in the food industry. It was established that ultrasonic vibrations are able to change the aggregate state of a substance, thereby dispersing and emulsifying it, to modify the rate of diffusion, crystallization, and dissolving of substances, to intensify reactions, and to boost technological processes. When examining the influence of US waves on the technological process of making food products, one observes a decrease in energy consumption and labor intensity. It becomes possible to make food products with new consumer properties, improved quality and extended shelf life [2].

The base of sponge cake dough is egg-sugar foam, which represents a dispersed system that easily reacts to any changes in the technological process. At the same time, significant