

Shelf life extension of minimally processed vegetables using combinations of bacterial bioprotection and modified atmosphere packaging

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Abstract. The objective of the work: to study the effect of combination of bacterial bioprotective cultures and modified atmosphere packaging for prolonging the refrigerated storage period of minimally processed vegetables. Sweet pepper, zucchini, eggplant, celery stalks were used for preparation of minimally processed vegetables. SafePro® bio-products from Chr. Hansen (Denmark) containing strains of *Lactobacillus sakei*, *Pediococcus acidilactici*, *Lactobacillus curvatus*, *Leuconostoc carnosum* were used as bioconservatives. For packaging minimally processed vegetables, the bags made of flat multilayer PA/adhesive/PE films and composite PET/A1/PE film material were used. The bags were filled with gas mixtures including nitrogen and carbon dioxide. Storage of packaged minimally processed vegetables was carried out in a refrigerator at a temperature of 4 ± 2 °C for 16 days. The viability of cultures *Lactobacillus sakei*, *Pediococcus acidilactici*, *Lactobacillus curvatus*, *Leuconostoc carnosum* in modified atmosphere packaging was studied. It was revealed that the gas mixture of 60% nitrogen and 40% carbon dioxide and the culture of *Lactobacillus sakei* contribute to the preservation of the quality of fresh-cut sweet pepper, eggplant and zucchini, and *Leuconostoc carnosum* is the more effective for celery storing. In the process of refrigerated storage for 14 days, the solids content in the experimental samples increased 1.3–2.1 times, the loss of organic substances was 26–50%, depending on the type of vegetables. The developed technology for the refrigeration preservation of minimally processed vegetables using bio-products treatment and in modified atmosphere packaging made it possible to increase the shelf life of fresh-cut vegetables by 2 times.

Key words: minimally processed vegetables, bacterial bioprotection, *Lactobacillus sakei*, modified atmosphere packaging, shelf life extension, refrigerated storage, vegetable microbiota.

INTRODUCTION

Nowadays the HoReCa segment (Hotel, Restaurant, Cafe/Catering) is rapidly developing around the world. The HoReCa trading channel is an association of enterprises of food industry, services, suppliers of B2B (Business to Business) products, which are targeted for resale at HoReCa sale outlets, as well as representatives of information and marketing services.

In high demand are convenient food sets including meat, fish and minimally processed vegetables (MPV) packaged using combined technologies, including vacuum and modified atmosphere packaging (MAP) (Preeti et al., 2011; Bouletis et al., 2017; Bazarnova et al., 2018b) for fast cooking at home.

In food industry, the production of MPV is the most labor-intensive. Moreover, the methods used for vegetables processing should not only prevent their spoilage, but also contribute to the preservation of vitamins, mineral and biologically active substances in them (Hounsoume et al., 2008; Sun & Holley, 2012; Evstigneeva et al., 2016; Eliseeva et al., 2018; Pankina et al., 2019).

A promising technology for preserving minimally processed vegetables is the combined usage of various biotechnological techniques, which gives a pronounced synergistic effect and allows prolonging the shelf life of culinary products without the use of chemical preservation agents (Sun & Holley, 2012; Oluwafemi et al., 2013).

Many bacteria synthesize protein-peptide antibiotic substances, which suppress affined species or strains that inhibit their growth, or have a wider spectrum of antibacterial action (Durango et al., 2006). These substances with a very specific action are called bacteriocins, their biosynthesis is encoded by specific plasmids and in most cases occurs on ribosomes. In food industry bacteriocinogenic strains have found application as biological preservatives. The main advantage of using bio-preservatives is safety. Some of the microorganisms used as bio conservatives are probiotic for the human intestinal microbiota, including *Lactobacillus*, *Bifidobacterium*, *Propionibacterium*, *Streptococcus thermophilus*, *Lactococcus* (Vinderola & Reinheimer, 2000).

The main cultures used in food technology are lactic acid bacteria (*Lactobacillus* and *Pediococcus*) (Axelsson, 2004), which reduce pH, so that the development of pathogenic microbiota in acidified media slows down (Timoshenkova et al., 2019). Micrococaceae, homofermentative lactic acid bacteria and *Pediococcus*, yeast and atypical lactic acid bacteria mostly in the form of pure cultures are also used as bioprotective ones (Lucera et al., 2012).

The MAP technology for long keeping products is successfully used for products in portion consumer packaging. It is the most modern way to maintain the quality and freshness of food products and increase their shelf life for several times without freezing and also to exclude of use of chemical additives and preservatives (Oluwafemi et al., 2013; Moor et al., 2014; Kirse et al., 2017). In MAP packaging, air is replaced by a mixture of inert gases, which eliminates or slows down the oxidation (spoilage) of food. The presence of carbon dioxide prevents the development and reproduction of fungi, bacteria and other microorganisms. Oxygen (O₂), carbon dioxide (CO₂) and nitrogen (N₂) are the three main gases used for MAP. The gas choice depends on the type of product being packaged (Speranza et al., 2009; Ahmed et al., 2011). These gases are used individually or in combination with each other to achieve a balance between the product shelf life and its optimal organoleptic properties (Brandenburg & Zagory, 2009).

To determine the efficiency of bioprotective cultures' impact on the microbiota caused by biodeterioration of vegetables, it is necessary to study the tolerance of bio protective cultures in inert gas mixtures based on nitrogen and carbon dioxide. The other task is to develop a technology for product processing by these cultures in order to obtain a synergistic effect from the combined usage of several biological barriers (Gialamas et al., 2010; Bartolini & Ducci, 2017; Bazarnova et al., 2018b), as well as to assess the

dynamics of changes in biochemical and microbiological quality indicators of minimally processed vegetables during cold storage.

The objective of the work: to study the effect of combination of bacterial bioprotective cultures and modified atmosphere packaging for prolonging the refrigerated storage period of minimally processed vegetables.

MATERIALS AND METHODS

SafePro® microorganism specimen (Chr. Hansen, Denmark) containing strains of *Lactobacillus sakei*, *Pediococcus acidilactici*, *Lactobacillus curvatus*, *Leuconostoc carnosum* (Table 1) were used as bio protective cultures.

There were used red and yellow sweet pepper, zucchini, eggplant, celery stalks for preparation of MPV.

For packaging MPV prepared from fresh vegetables there were used bags made from flat multilayer PA/adhesive/PE films and composite PET/A1/PE film material (Lion Company LLC, Russia), as well as food gas mixtures: Biogon ®NC20 (80% nitrogen, 20% carbon dioxide by volume) and Biogon ®NC40 (60% nitrogen, 40% carbon dioxide by volume) from Linde Gas, Russia. For spraying of bio-products' suspension onto the surface of MPV, the 4000-B1 finely dispersed spray nozzle was used (VKT, Russia).

Table 1. Characteristics of bio products*

Characteristics	Name			
	SafePro B-SF-43	B-2 SafePro	B-LC-48 SafePro	B-LC-20 SafePro
Content	<i>Leuconostoc carnosum</i>	<i>Lactobacillus sakei</i>	<i>Lactobacillus curvatus</i>	<i>Pediococcus acidilactici</i>
Type	<i>Microaerophilic</i>	<i>Facultative anaerobic</i>	<i>Microaerophilic</i>	<i>Microaerophilic</i>
Total amount of cells, CFU g ⁻¹	1×10 ⁹	1×10 ⁹	1×10 ⁹	1×10 ⁹
Produced substances	<i>D(-)-lactic acid</i>	<i>L (+)-lactic acid</i>	<i>L (+)-lactic acid</i>	<i>DL (+/-)-lactic acid</i>
Produced bacteriocins	<i>Leukacin C</i>	<i>Sakacin A</i>	<i>Kurvacin</i>	<i>Pediocin</i>
Temperature Opt/Max/Min, °C	20/30/-4	25/40/-4	37/40/4	43/52/15

* Data according to Vinderola & Reinheimer (2000).

For microscopic analysis of cultures' activity, there were lyophilized preparations with addition of nutrient substratum activated in 1 l of warm water (37 °C). To determine the activity of the studied cultures, there were activated biological products, microfilmed and counted the number of rods and coccus of cultures with using statistical methods. For microscopic analysis of cultures, there were samples activated with nutrient substratum in 1 L of warm water (37 °C). There was estimated degree of development of cocci bacteria by the number of cocci in staphylococci and streptococci (Belyaev, 2016).

There was determined influence of the modified gas mixture on the cultures' vitality with the microscopy method by staining with methylene blue to detect dead cells.

The Eclipse binocular microscope with the TC-100 camera (100× magnification), the IS-500 digital camera, and photo microanalysis program were used to study the microscopic image of cultures during storage.

To study the tolerance of cultures in MAP packaging during cold storage, the foil substrate was sprayed with suspensions of activated cultures. Further the substrates were placed in a gas barrier film with mixtures of Biogon® NC20, Biogon® NC40 and stored at a temperature of 4 ± 2 °C. The control sample was packaged in a film with atmospheric air and stored under similar conditions until the microorganisms stopped functioning.

To study the combined effect of biological products and inert gas mixture on the dynamic of shelf life of MPV, the samples of raw vegetables were treated with suspensions of activated bio cultures. To prepare a suspension of a biological product the sample weighing 2 g was set into a container and diluted by filtered water (0.2 L) with a temperature of 4 ± 2 °C. After thoroughly shaking the container, there was another 0.8 L of water with a temperature of 4 ± 2 °C added. The lyophilized biological product was placed in a spray container.

The raw materials are subjected to primary processing and sorting, i.e. removal of spoiled and damaged vegetables. After that vegetables are washed in cold water to remove contaminants, after which they are laid out in perforated containers to remove excess moisture from the surface of vegetables. Further the vegetables are cut into pieces of the required size depending on their type and put on sheets.

The prepared vegetables were sprayed with the suspension 4 ± 2 °C. Spraying is carried out at a nozzle operating pressure of 7 bar. After spraying the suspension, the fresh-cut vegetables are dried or centrifuged at 300–600 rpm, depending on the type of vegetables, to remove droplet-liquid moisture. The processed vegetables are packaged by polypropylene gas-barrier bags using Biogon®NC20, Biogon®NC40 gas mixtures. There were packed control samples of MPV in the film with use MAP packaging without any treatment by bio products.

Packed experimental and control samples of MPV were stored in a refrigerator at a temperature of 4 ± 2 °C for 16 days.

During storage, organoleptic, biochemical, physicochemical and sanitary-significant microbiological indicators of MPV were determined.

To analyze fresh-cut vegetables for sensory characteristics there was used standardized method (ISO 13299: 2003). Sensory analyzes (5-point grading scale) were carried out over 14 days of storage by the 5 member sensory panel. Sensory profiles were developed for each sensory characteristic of fresh-cut vegetables, and the main sensory differences were assessed using a standardized technique. Sensory characteristics included assessment of appearance, color, texture, taste and smell.

The mass fraction of solids in MPV was determined by drying to a constant mass at a temperature of (135 ± 1) °C (Nilova et al., 2017).

To determine the sum of phenolic compounds, the Folin-Ciocalteu reagent was used (Bazarnova et al., 2018a). The content of organic acids in vegetables was determined by titration (Bazarnova & Ivanchenko, 2016).

The barrier properties of bio protective cultures relative to the microbiota of vegetables were determined by analyzing the sanitary-indicative microbiota of fresh-cut vegetables using official methods of analysis (AOAC, 2015). There was investigated yeast and mold count (AOAC method 997.02), *Escherichia coli* count (E-coli) (AOAC

method 991.14), Aerobic plate count (APC kfu g⁻¹) (AOAC method 986.32), *Staphylococcus aureus* count (AOAC method 975.55).

To assess the microbiological parameters, there were used experimental and control samples from fresh-cut vegetables and unprocessed ones samples before their storage set and on the 7th and 14th days of refrigerated keeping.

The each sample was undergone researched in triplicate. The accuracy of the experimental data was evaluated by using mathematical statistical methods in Microsoft Excel. The data gained through this process are presented with a confidence coefficient of 0.95.

RESULTS AND DISCUSSION

Fig. 1, (a–d) shows influence of MAP-packaging on the vitality of bio-barrier microorganisms at 4 ± 2 °C. The microscopic examination showed that the culture of *Lactobacillus sakei* (B-2 SafePro preparation) had the greatest growth potential. The 0.05 mL of its suspension had the highest concentration of microorganisms from streptobacilli, consisting on average from 20 to 40 rods, which explains their active development. A good development of the *Leuconostoc carnosum* culture (SafePro B-SF-43 preparation) is also noticed which has about 20 rods in 0.05 mL of its suspension. Cultures of *Lactobacillus curvatus* (B-LC-48 SafePro), *Pediococcus acidilactici* (B-LC-20 SafePro) (c and d) showed low activity, which is expressed by low concentration of microorganisms in suspension. In addition, it is worth noting that the storage temperatures of minimally processed vegetables are outside the range of active growth of *Pediococcus acidilactici*.

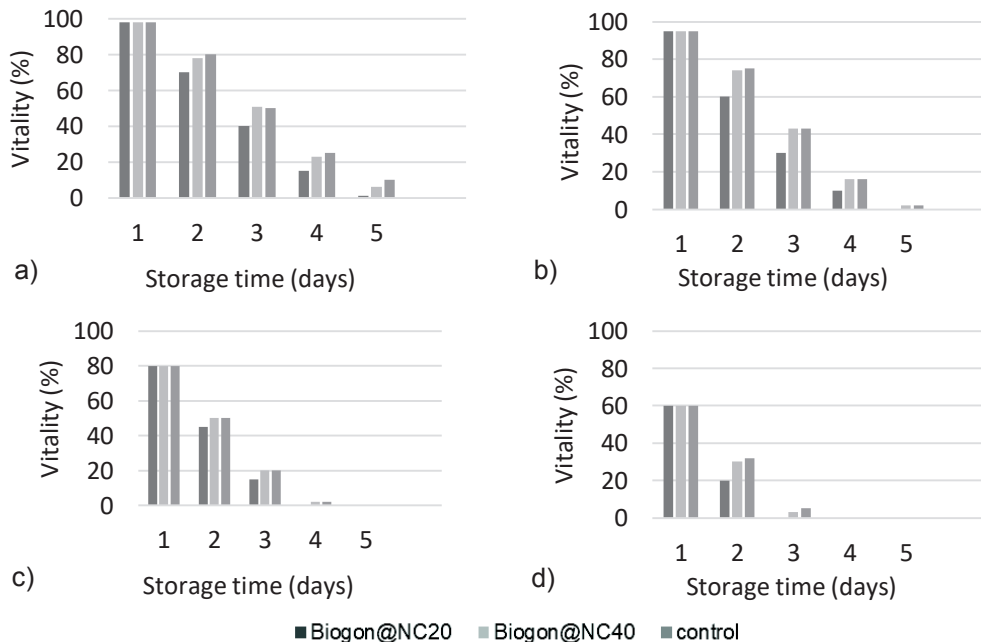


Figure 1. Influence of MAP-packaging on the vitality of bio-barrier microorganisms at 4 ± 2 °C: a – B-2 SafePro; b – SafePro B-SF-43; c – B-LC-48 SafePro; d – B-LC-20 SafePro.

The results presented in Fig. 1 show that *Lactobacillus sakei* culture (B-2 SafePro), remained viable up to 5 days, has the highest vitality in the Biogon ®NC40 gas medium. The same culture showed less viability in Biogon ®NC20 gas medium.

The other cultures show low tolerance in Biogon ®NC20 and Biogon ®NC40 gas media. This is due to a lack of oxygen in gas mixtures and a significant content of carbon dioxide.

Table 2. The sanitary-indicative microbiota of minimally processed vegetables during cold storage for 14 days; 4 ± 2 °C

Minimally processed vegetables	Bioprotective cultures	The sanitary-indicative microbiota				
		APC, CFU g ⁻¹	Yeast, CFU g ⁻¹	Mold, CFU g ⁻¹	<i>E. coli</i>	<i>S. aureus</i>
		Limits				
		no more than 1·10 ⁴	no more than 500	no more than 500	absence in 1 g	absence in 1 g
		Values after 14 days of storage				
Red sweet pepper	Control	5·10 ⁵	120	300		
	<i>Lactobacillus sakei</i>	2·10 ²	8	20		
	<i>Leuconostoc carnosum</i>	2·10 ²	12	22	absence	absence
	<i>Lactobacillus curvatus</i>	2·10 ²	14	20		
	<i>Pediococcus acidilactici</i>	2·10 ²	16	20		
Yellow sweet pepper	Control	1·10 ⁵	140	510		
	<i>Lactobacillus sakei</i>	2·10 ²	10	20		
	<i>Leuconostoc carnosum</i>	2·10 ²	15	22	absence	absence
	<i>Lactobacillus curvatus</i>	2·10 ²	14	20		
	<i>Pediococcus acidilactici</i>	2·10 ²	16	20		
Zucchini	Control	1·10 ⁵	80	502		
	<i>Lactobacillus sakei</i>	6·10 ²	10	36		
	<i>Leuconostoc carnosum</i>	6·10 ²	14	40	absence	absence
	<i>Lactobacillus curvatus</i>	6·10 ²	14	44		
	<i>Pediococcus acidilactici</i>	6·10 ²	18	50		
Eggplant	Control	2·10 ⁵	120	500		
	<i>Lactobacillus sakei</i>	3·10 ²	12	44		
	<i>Leuconostoc carnosum</i>	3·10 ²	14	56	absence	absence
	<i>Lactobacillus curvatus</i>	3·10 ²	18	56		
	<i>Pediococcus acidilactici</i>	3·10 ²	18	56		
Celery stalks	Control	1·10 ⁵	140	80		
	<i>Lactobacillus sakei</i>	3·10 ²	22	60		
	<i>Leuconostoc carnosum</i>	3·10 ²	18	38	absence	absence
	<i>Lactobacillus curvatus</i>	3·10 ²	18	68		
	<i>Pediococcus acidilactici</i>	3·10 ²	30	70		

Table 2 shows the results of studies of sanitary-indicative microbiota of the experimental and the control samples of fresh-cut vegetables after their refrigerated storage in MAP packaging (Biogon ®NC40). The results were compared with the limits set by the Commission Regulation (EC) on microbiological criteria for foodstuffs (2005). It was established all processed samples of fresh-cut vegetables had a safe level

of studied microbiological parameters, in contrast to untreated control samples, the APC level of which was exceeded already for 7 days of refrigerated storage. Proper peeling and washing of vegetables before treatment by bio protective cultures, as well as the absence of risks of recontamination during their packaging, determined their microbiological safety in relation to *Escherichia coli* and *Staphylococcus aureus*.

The deterioration of organoleptic properties goes slower in the samples treated by bio protective cultures in contrast to the control samples (Fig. 2).

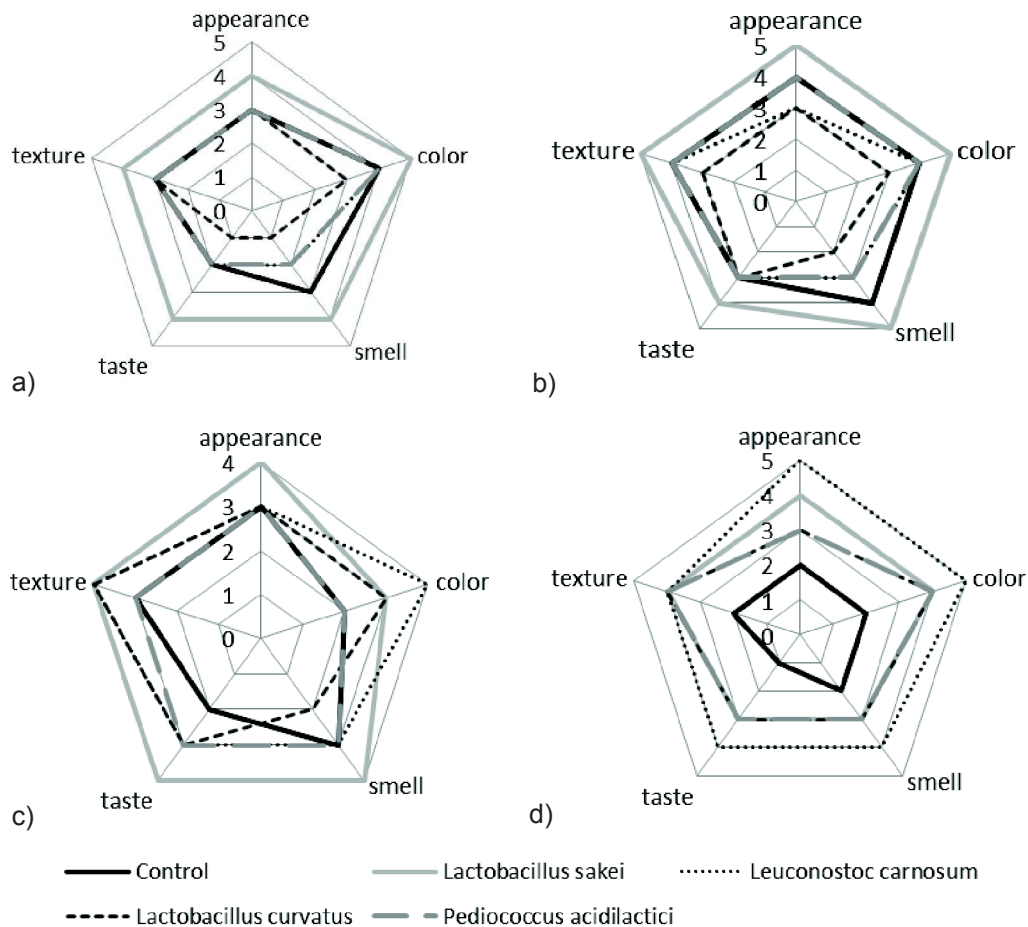


Figure 2. Sensory characteristics of minimally processed vegetables during cold storage for 14 days; 4 ± 2 °C: a – red sweet pepper; b – zucchini; c – eggplant; d – celery stalks.

The total quality level of the minimally processed vegetables from red pepper and zucchini treated by *Lactobacillus sakei* during 14 days of refrigerating storage can be described as ‘good’, and for eggplant and celery as ‘satisfactory’. At the same time, unprocessed fresh-cut vegetables from pepper, eggplant and celery were rated as ‘unsatisfactory’, since at least one of the signs they had was rated at 2 marks.

It was established the culture of *Lactobacillus sakei* (B-2 SafePro preparation) has the greatest barrier effect during the refrigerated storage of fresh-cut red and yellow

pepper, eggplant, zucchini in Biogon ®NC40 medium. This allows preserving the organoleptic and microbiological parameters of the studied MPV up to 14 days at a temperature of 4 ± 2 °C at the required level. The most effective culture for celery was *Leuconostoc carnosum* (SafePro B-SF-43). The shelf life of untreated (control) samples of MPV under the same conditions was 7 days, which is 2 times less.

There were presented results of studies of dynamics of content of dry substances and organic acids of fresh-cut vegetables from eggplant, red pepper, zucchini and celery during refrigerated storage in the Table 3.

Table 3. Dynamics of the mass fraction of solids and organic acids in test MPV during the refrigeration storage

Minimally processed vegetables	Duration of storage, days	Mass fraction of solid, %		Content of organic acids, mg g ⁻¹	
		control	test	control	test
Eggplant	1	7.11 ± 0.35	7.11 ± 0.35	0.70 ± 0.05	0.80 ± 0.05
	7	7.23 ± 0.35	7.12 ± 0.30	0.70 ± 0.05	0.80 ± 0.05
	10	8.84 ± 0.41	7.73 ± 0.35	0.50 ± 0.04	0.60 ± 0.04
	14	16.29 ± 0.80	9.88 ± 0.51	0.30 ± 0.02	0.40 ± 0.02
	16	18.21 ± 0.91	11.78 ± 0.55	0.20 ± 0.01	0.30 ± 0.02
Red sweet pepper	1	4.12 ± 0.08	4.11 ± 0.08	2.10 ± 0.20	2.00 ± 0.20
	7	5.24 ± 0.06	4.79 ± 0.08	1.60 ± 0.15	1.70 ± 0.15
	10	5.87 ± 0.19	5.13 ± 0.15	1.30 ± 0.15	1.40 ± 0.15
	14	8.63 ± 0.10	5.33 ± 0.11	0.90 ± 0.10	1.20 ± 0.10
	16	10.81 ± 0.20	6.44 ± 0.22	0.80 ± 0.10	1.10 ± 0.10
Celery stalks	1	2.24 ± 0.09	2.24 ± 0.09	0.20 ± 0.05	0.20 ± 0.05
	7	3.83 ± 0.07	3.22 ± 0.08	0.20 ± 0.05	0.20 ± 0.05
	10	5.18 ± 0.12	3.47 ± 0.09	0.10 ± 0.02	0.10 ± 0.02
	14	6.34 ± 0.06	4.01 ± 0.13	0.10 ± 0.02	0.10 ± 0.02
	16	7.32 ± 0.12	4.84 ± 0.08	not determined	not determined
Zucchini	1	5.61 ± 0.08	5.61 ± 0.09	4.00 ± 0.50	4.20 ± 0.50
	7	7.82 ± 0.07	6.81 ± 0.09	3.80 ± 0.50	5.10 ± 0.60
	10	12.49 ± 0.28	7.89 ± 0.09	2.90 ± 0.30	4.60 ± 0.50
	14	16.32 ± 0.11	12.32 ± 0.12	2.10 ± 0.20	3.10 ± 0.40
	16	20.91 ± 0.17	15.82 ± 0.10	1.60 ± 0.15	2.60 ± 0.30

It was registered that in control samples of MPV the solids content increased by 2.5–3 times over the 16 days, which is explained by moisture loss due to shrinking of vegetables (Barsukova et al., 2019). This process proceeded 1.5 times slower in the experimental samples.

In this case, processed fresh-cut red pepper and eggplant were characterized by the lowest degree of shrinkage. The solids content in them on the 16th day of storage increased by 56 and 65%, respectively.

The highest content of organic acids before storage was registered in red pepper and zucchini: 2.1 and 4.2 mg g⁻¹, respectively. During refrigerated storage, their amount decreases. On the 7th day, there is a noticeable difference between the content of organic acids in control samples and samples treated by bio-products, which ranges from 5 to 10%, depending on the vegetables type. This difference increases with long time storage and reaches 20% on the 14th day.

Fig. 3 presents the results of studies of the content of phenolic compounds in minimally processed eggplant during storage.

Loss of phenolic compounds over the entire period of refrigerated storage for the experimental samples is about 30%, in comparison with 50% for the control samples. In experimental samples, the dynamics of phenolic substances slows down by about 15%.

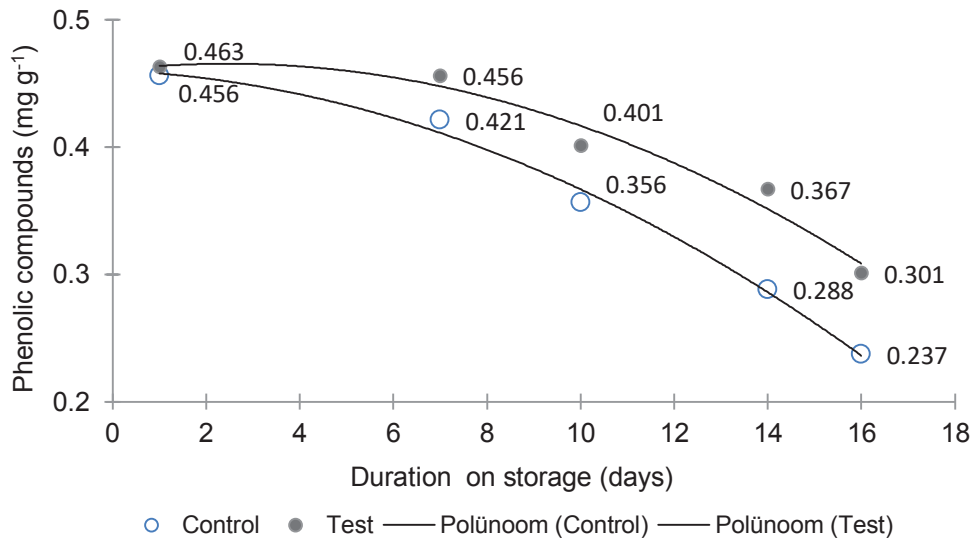


Figure 3. Dynamics of phenolic compounds of eggplant during the refrigeration storage.

The determining factor in the duration of the heat treatment of vegetables is their texture – one of the important indicators of culinary readiness. It was experimentally established that the control samples of vegetables reached culinary readiness faster than the experimental samples subjected processed by the biological products. Table 4 shows the estimated duration of frying of the experimental and the control samples of fresh-cut vegetable at $t = 180 \pm 2$ °C at the end of their shelf life in MAP packaging. The criterion of a paired comparative test between the test samples and the control were the duration of frying vegetables until culinary readiness and the desired texture of ready vegetables.

Table 4. Duration of frying vegetables until culinary readiness

Vegetables	Duration of frying, sec	
	control	test
Eggplant	290 ± 10	360 ± 10
Red sweet pepper	220 ± 10	260 ± 10
Celery stalks	390 ± 15	440 ± 15
Zucchini	150 ± 10	210 ± 10

The obtained experimental data formed the basis for the development of technological recommendations for the culinary processing of fresh-cut vegetables treated by bio-protective cultures, which we recommend to use as part of sets for preparing first and second courses, from which consumers can prepare hot dishes according to the developed instructions enclosed in consumer packaging.

The technological scheme for production of sets with indicated critical control points (CCP) at which it is necessary to carry out safety control at the factory is presented in Fig. 4.

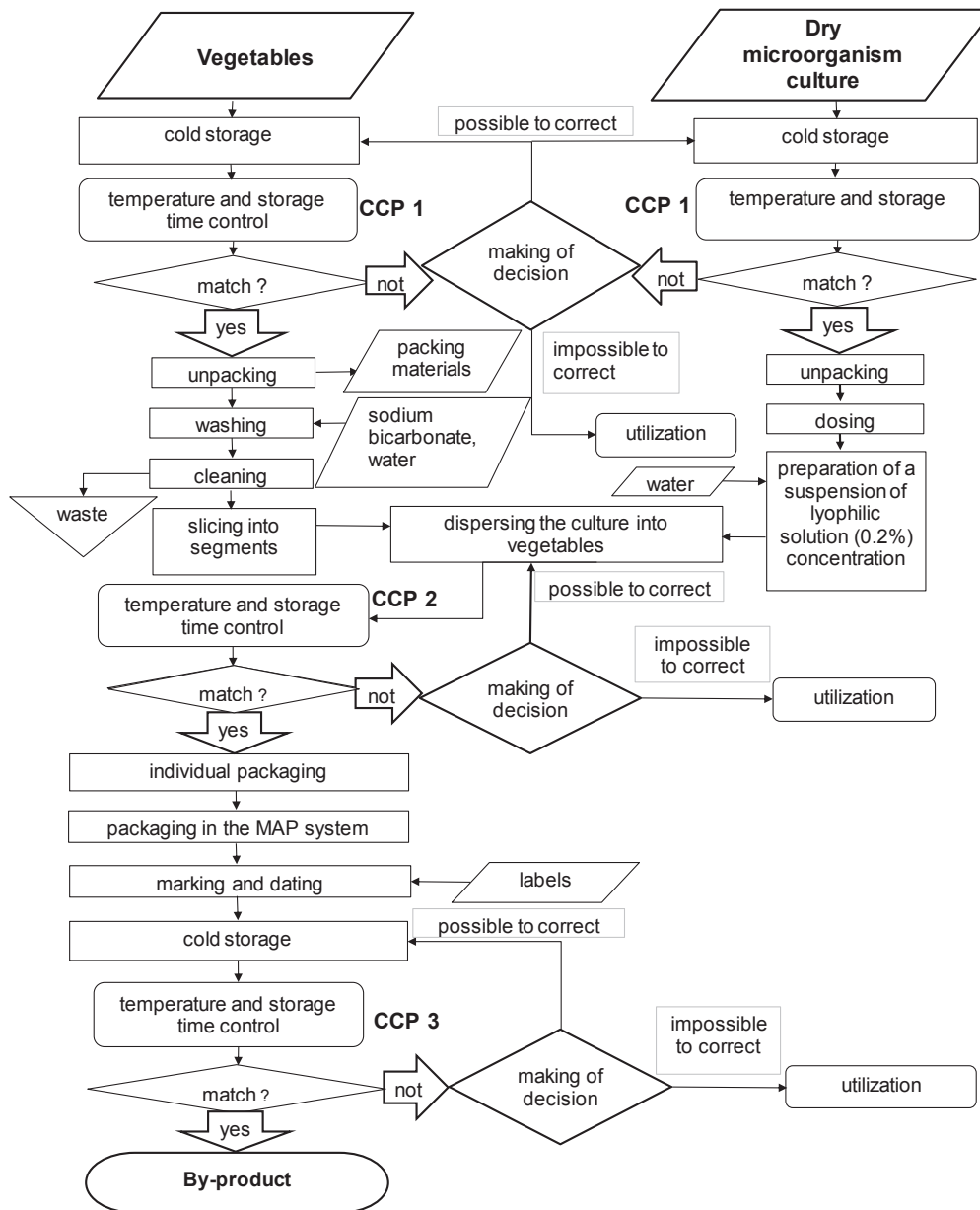


Figure 4. Technological scheme for production of fresh-cut vegetables with indicated critical control points.

CONCLUSIONS

There was studied impact of modified gas mixtures Biogon ®NC40 and Biogon ®NC20 on the vitality of cultures of *Lactobacillus sakei*, *Pediococcus acidilactici*, *Lactobacillus curvatus*, *Leuconostoc carnosum*. It was set that culture of *Lactobacillus sakei* (B-2 SafePro) was able to active growth in the Biogon ®NC40 gas environment up to 5 days including. The other studied cultures showed low resistance in gas mixtures Biogon ®NC20 and Biogon ®NC40. It can be due to the lack of oxygen and a significant content of carbon dioxide in the tested gas mixtures.

It was found that minimally processed zucchini, eggplants, red pepper treated with *Lactobacillus sakei* culture and celery stems treated with *Leuconostoc carnosum* culture are preserved under the temperature 2–4 °C for up to 14 days, which is 2 times longer than the minimally processed vegetables untreated by cultures under the same conditions.

There has been developed method of processing minimally processed vegetables with a suspension of bio-protective cultures before packaging in a polymer film using a gas mixture, including 60% nitrogen and 40% carbon dioxide by volume (Gnilitskij et al., 2019).

Treated with bio-protective cultures fresh-cut vegetables retain organoleptic, biochemical and microbiological parameters in accordance to the regulated norms for 14 days in MAP packaging and after this storage period do not lose their suitability for further heat treatment.

The technology of minimally processed vegetables using of combination of biological products and MAP packaging for refrigerated storage indicating critical control points in the technological cycle of their production has been introduced into the production of the food company 'Velikoross' (St. Petersburg).

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