Визначено вміст нітратів у овочах та вивчено особливості перебігу мікробіологічного і денітрифікуючого процесів під час соління томатів з різним вмістом нітратів. Встановлено, що в процесі соління розмножуються молочнокислі мікроорганізми і кількість нітратів у томатах зменшується до норми. Отже, обґрунтовано доцільність використання овочів з наднормативним вмістом нітратів на виробництво солених продуктів

Ключові слова: нітрати, овочева продукція, денітрифікуючий процес, мікробіологічний процес, молочнокисла мікрофлора

Определено содержание нитратов в овощах и изучены особенности течения микробиологического и денитрифицирующего процессов при солении томатов с различным содержанием нитратов. Установлено, что в процессе соления размножаются молочнокислые микроорганизмы и количество нитратов в томатах уменьшается до нормы. Обоснована целесообразность использования овощей со сверхнормативным содержанием нитратов на производство соленых продуктов

Ключевые слова: нитраты, овощная продукция, денитрифицирующий процесс, микробиологический процесс, молочнокислая микрофлора

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EFFECT OF LACTIC ACID MICROORGANISMS ON THE CONTENT OF NITRATES IN TOMATO IN THE PROCESS OF PICKLING

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1. Introduction

Contamination of the environment with toxic substances of anthropogenic origin (salts of heavy metals, nitrates, nitrites, pesticides, radionuclides, etc.) is an acute problem in Ukraine [1]. Along with food, these compounds penetrate human body where they subsequently accumulate or are converted into more toxic substances. Nitrates cause acute and chronic poisoning; along with other xenobiotics they provoke the occurrence of serious endoecological diseases that impact the health of descendants [2, 3]. Nitrates quickly convert hemoglobin into met- and sulfhemoglobin, which contributes to the development of hemic hypoxia [4]. In addition, nitrates block enzyme systems of the cells, which causes disruption of oxidative phosphorylation [5]. At present, nitrates are considered to be the predecessors of highly cancerogenic nitroso compounds [5, 6]. For an adult, a safe dosage of nitrates is considered to be 200–300 mg per day, maximum permissible is up to 500 mg, and a dose of 600 mg can be toxic [7].

When analyzing the content of nitrates in food products, as well as factors that affect the amount, most attention is paid to plant food. They are the main source of nitrates that penetrate the human body [7, 8]. Therefore, conducting monitoring studies on the content of nitrates in raw materials and food products, as well as development of scientifically-justified ways to reduce their content in finished products during processing, is a relevant and important issue. However, this requires the research and substantiation of the application of technology, which would predetermine denitrification changes in raw materials and would be feasible for industrial implementation.

2. Literature review and problem statement

Nitrates enter plant produce from soil, which is excessively fed with nitrogenous fertilizers [7]. However, data in the scientific literature on the main sources of nitrates that penetrate the human organism are rather controversial. In the opinion of some authors, the main risk is in the consumption of water with high nitrate content [1]. Other scientific studies indicate that nitrates get into the human organism from vegetables and comprise 60–70 % of the total intake of nitrates. 15–20 % of nitrates are supplied with drinking water, while fish and meat products account for not more than 10–15 % [7, 9].

According to the scientific literature, almost 20 % of all plant products that are sold now contain the amount of nitrates that exceeds the norm [10, 11]. Thus, researchers found that the content of nitrates in vegetable produce grown in the open ground exceeded MPC (maximum permissible concentration) by 6 % on average [12]. In spring, the early vegetable produce grown in greenhouses contained nitrates in the amount that exceeded MPC by 2–5 times. In this case, the highest concentration of nitrates was found in table beet (from 3,546 mg/kg to 8.050 mg/kg against MPC of 1,400 mg/kg), as well as in salad vegetables and early cabbage. Very high nitrate content was revealed in potato from Artzizskiy district of Odesa oblast (Ukraine), specifically 881±13.5 mg/kg against MPC of 250 mg/kg [12]. By having thoroughly examined the content of nitrates in Odesa oblast, scientists devised a zoned map of risk in terms of contamination of vegetable products with nitrates. When studying the content of nitrates in the plant produce from Zhytomyr oblast (Ukraine), it was detected that their concentration in such food products as table beet, tomato, radish, onion, cabbage, exceeded MPC by 2–4 times [13].

Monitoring study of nitrate content in vegetables in Estonia found their significant amount in spinach, salad, beet. Average concentrations amounted to 2,936, 2,508 and 1,446 mg/kg, respectively, which significantly exceeds MPC [14]. Korean researchers also detected large amounts of nitrates in vegetables (cabbage, radish, beetroot), from 1,740 to 4,259 mg/kg [15]. In Spain, a study into nitrate content in vegetables and baby food based on vegetables found that MPC was exceeded only in salad and spinach [16].

Thus, the literary sources indicate that vegetables and plant produce can be a source of excessive amounts of nitrates that penetrate the organism of consumer. Moreover, control over vegetable produce for nitrate content is not always fully ensured.

When studying effect of the techniques for treating vegetable produce on the nitrate content, it was found that usual washing and mechanical cleaning of vegetable produce (potato, table beet, carrot, cabbage, etc.) reduces the amount of nitrates by 10 % on average [17]. Washing potato, carrot, table beet, cabbage for 1 hour decreases the level of nitrates by 25–30 %; greenery (parsley, celery, spinach, dill, green onion) – by 20 % [18, 19]. However, water-soluble nutrients are also partially lost during treatment. It was established that in the process of boiling the content of nitrates is reduced by 60–70 % because they pass into the broth [20]. Moreover, such a method of treatment of raw materials significantly reduces their biological value. The scientific literature also lacks data on the canning of vegetables with different content of nitrates and about the amount of nitrates in prepared canned products.

Thus, there are almost no studies at present that highlight the issue of the influence of lactic acid microflora on the nitrate content when applying technology of vegetable pickling or souring. The research to be conducted in this field will reveal the concentrations of nitrates in raw materials at which the denitrification process is still active. This, in turn, will ensure the production of safe finished products. Therefore, given the presence of vegetables that are on sale and whose nitrate content exceeds the norm, it is a promising task to explore methods for lowering the content of nitrates in vegetable produce at which the biological and nutritional value is preserved.

3. The aim and objectives of the study

The aim of present study was to conduct a monitoring study into nitrate content in vegetable produce sold in the open markets of the Western regions of Ukraine, and to examine special features in the course of denitrification and microbiological processes in the technology for pickling tomato with different content of nitrates.

To accomplish this goal, the following tasks were set:

– to determine the actual content of nitrates in vegetables, which are sold in the open markets;

– to explore the dynamics of composition of the microflora and the amount of nitrates during tomato pickling for the nitrate content within MPC (137±10 mg/kg);

– to explore the dynamics of composition of the microflora and the amount of nitrates during pickling tomatoes whose nitrate content is twice as large than MPC (619±±32 mg/kg);

– to explore the dynamics of composition of the microflora and the amount of nitrates during pickling tomatoes whose nitrate content is five times larger than MPC (1,576±114 mg/kg).

4. Materials and methods for exploring the content of nitrate and the microflora of pickled tomato

The research was conducted in the scientific laboratory of the Department of Food Biotechnology and Chemistry at the I. Puluy Ternopil National Technical University (Ukraine). The first part of the research was to monitor the content of nitrates in vegetables that are sold in the agrifood markets in Ternopil, Khmelnytsky, Chernivtsi and Lviv oblasts (Ukraine).

Materials and methods for examining the content of nitrates and the microflora of pickled tomato are described in detail in [21].

5. Results of the study of nitrates in vegetables, and microbiological and denitrification processes during tomato pickling

Table 1 gives results of exploring the nitrate content in vegetables sold in the agrifood markets of the Western region of Ukraine.

Data in Table 1 show that the agrifood markets sold vegetables with a different content of nitrates. On average, 15 % of the samples of vegetables grown in open ground contained nitrates in the amount that exceeded MPC. For the vegetables grown in greenhouses, the number of samples with a nitrate content above the norm was on average twice larger in comparison to the produce grown in open ground. It should be noted that some samples of greenhouse vegetables contained nitrates in the amount that exceeded MPC by more than 1.9 times. The produce with such a nitrate content is dangerous for the health of consumers and may cause food poisoning [6, 7].

Table 1

Nitrate content in vegetables sold in the open markets, %, *n*=320

Vegetable produce	MPC, mg/kg	Number of samples that contain nitrates in vegetable food products			
		open ground		greenhouses	
		within MPC	above MPC	within MPC	above MPC
Cucumber	200/400	85	15	70	30
Tomato	150/300	80	20	65	35
White cabbage	500/900	85	15	70	30
Carrot	250/400	85	15	70	30
Table beet	1,400	90	10	75	25
Onion	90	95	5	80	20
Green onion	600/800	85	15	75	25
Potato	250	90	10	70	30

Note: MPC in numerator – for open ground; in denominator – for greenhouses

Given this situation, the problem of using vegetables with a nitrate content above the norm requires scientific solution. Thus, understanding the mechanisms of denitrification for various technological methods of processing, it is possible to obtain a finished product with the amount of nitrates safe for the health of consumers. For this purpose, it is possible to employ such a biological way of vegetable processing as pickling (souring). Pickling is a method of treatment of vegetables and fruits, at which, as a result of the action of lactic acid bacteria, sugar contained in the raw materials is fermented into lactic acid.

That is why, as an example, we studied effect of the technology for pickling tomatoes with different nitrate content on the microbiological and denitrification processes.

Results of research into change in the composition of microflora during pickling of tomatoes with a nitrate content within MPC (137±10 mg/kg) are given in Table 2.

Table 2 shows that over a period of active fermentation during one day at a temperature of 25±°C, a significant increase in the amount of microflora occurred, specifically, bifidobacteria – by 490 times, lactobacillus – by 475 times, fungi – by 12 times, and bacilli – by 48 times.

Over a 10 day-period of fermentation at a temperature of 9±1 °C, lactobacillus reproduced most intensively, the number of which increased by 163 times. Bifidobacteria and fungi reproduced somewhat slower, the number of which increased over this period by 57 and 44 times, accordingly. Spore forms of bacteria increased by 2–3 times, while clostridia were not observed at all.

Table 2

Change in the composition of microflora in the pickling solution in the process of pickling tomatoes with the initial amount of nitrates at 137±10 mg/kg

Hours, days of pickling	Content of microorganisms in pickling solution, CFU/cm^3				
	bifidobacteria	lactobacillus	yeast, fungi	bacilli	clostridia
Freshly prepared pickle at a temperature of 25±1 °C	2.0±±0.1×10^2	1.2±±0.1×10^3	1.1±±0.1×10^2	0.7±±0.1×10^1	0
In 24 h. of active fermentation at a temperature of 25±1 °C	9.8±±0.6×10^4	5.7±±0.3×10^5	1.3±±0.1×10^3	3.4±±0.2×10^2	0
In 10 days at a temperature of 9±1 °C	5.6±±0.3×10^6	9.3±±0.6×10^7	5.7±±0.3×10^4	8.1±±0.5×10^2	0
In 20 days at a temperature of 9±1 °C	5.1±±0.3×10^8	9.0±±0.7×10^8	7.5±±0.5×10^4	8.0±±0.6×10^2	0
In 30 days at a temperature of 9±1 °C	8.0±±0.6×10^9	9.2±±0.7×10^9	7.9±±0.5×10^4	8.1±±0.5×10^2	0

From the tenth day till the twentieth day of tomato fermentation such microorganisms participated intensively in microbiological process as lactobacillus and bifidobacteria. Fungi and bacilli practically stopped to proliferate over a given period and their number did not increase. On day 30, that is, at the time of finishing the technological process of pickling, bifidobacteria and lactobacillus comprised 8–9 billion CFU/cm^3 of the pickle, fungi and bacilli – 80 thousand CFU/cm^3 each on average.

A change in the content of nitrates in tomato during pickling is shown in Fig. 1.

Figure 1 shows that at preliminary fermentation of tomato, which lasts 24 hours, the nitrate content in tomato fruits decreased only by 1.3 times, while in the pickle it increased by 1.7 times. These changes can be explained by the transition of nitrates from fruits and spices to the pickle.

Over the following 10 days of fermentation, we registered intensification of the denitrification process, which is caused by germination of the lactic acid microflora. During this time, the content of nitrates in tomato decreased by 2.6 times, while that in the pickle – by 1.4 times; it was equal to 60 mg/kg and 46 mg/cm^3, respectively.

From the tenth day to the thirtieth day, denitrification properties of the microflora caused a reduction in the nitrate content in tomato and pickle by 2.7 and 2.1 times, respectively. After the process of pickling was finished, the amount of nitrates was 17 mg/kg in tomato, and 28 mg/cm^3 in the pickle.

Table 3 gives results of the study of the change in microflora composition during pickling of tomatoes with the content of nitrate that exceeded MPC by 2 times (619±±32 mg/kg).

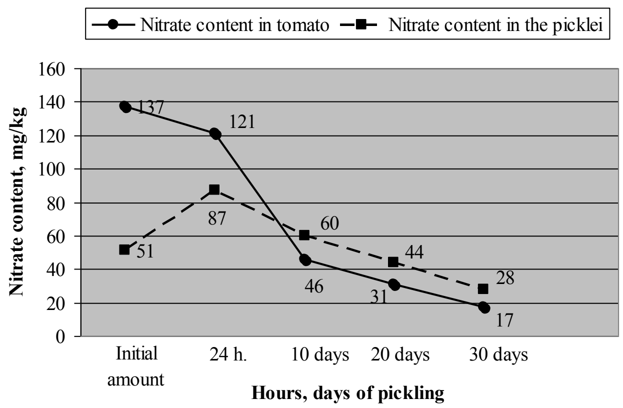

Fig. 1. Dynamics of denitrification process during pickling of tomatoes with an initial amount of nitrates at 137±10 mg/kg

Table 3

A change in the composition of microflora in the pickle in the process of pickling tomatoes with the initial amount of nitrates at 619±32 mg/kg

Hours, days of pickling	Microorganisms content in pickle, CFU/cm3				
	bifidobacteria	lactobacillus	yeast, fungi	bacilli	Clostridia
Freshly prepared pickle at a temperature of 25±1 °C	1.7± ±0.1×10^2	1.0± ±0.1×10^3	1.1± ±0.1×10^2	0.6± ±0.01×10^1	0
In 24 h. of fermentation at a temperature of 25±1 °C	7.0± ±0.3×10^4	9.3± ±0.7×10^4	1.0± ±0.1×10^3	2.9± ±0.2×10^2	0
In 10 days at a temperature of 9±1 °C	1.8± ±0.1×10^6	7.3± ±0.4×10^7	5.2± ±0.3×10^4	7.9± ±0.5×10^2	0
In 20 days at a temperature of 9±1 °C	3.0± ±0.2×10^8	6.4± ±0.4×10^8	6.7± ±0.4×10^4	8.0± ±0.6×10^2	0
In 30 days at a temperature of 9±1 °C	6.1± ±0.4×10^9	7.8± ±0.5×10^9	7.1± ±0.5×10^4	7.9± ±0.5×10^2	0

Data in Table 3 show that the initial composition of the microflora was practically the same as in the case of pickling tomatoes with a nitrate content within MPC. However, microbiological process during the time of preliminary fermentation (24 hours) was a little slower. The amount of microflora was 1.2 times less on average as compared with the amount in the pickled tomatoes with a nitrate content of 137±10 mg/kg (Table 2). Slowdown of the microbiological process is related to a certain bacteriostatic effect of nitrates and nitrites on microflora during pickling.

During subsequent fermentation at a temperature of 9±1 °C over 20–30 days the intensity of microbiological process slightly decreased. Reproduction of lactic acid microflora practically corresponds to the reproduction in the pickle with the normative content of nitrates. The amount of bifid- and lactobacillus at the end of the technological process of pickling was 7.8–7.1 billion CFU/cm^3 of the pickle.

Almost similar trend is observed in the analysis of denitrification process, the results of which are shown in Fig. 2.

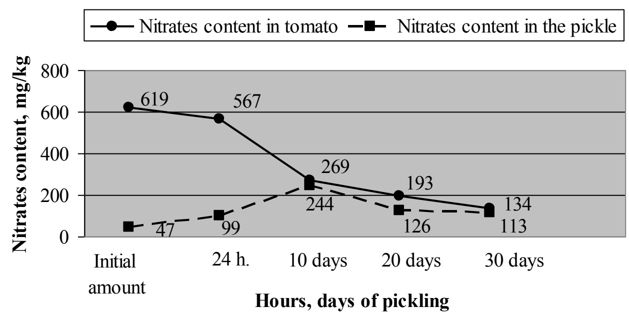

Fig. 2. Dynamics of denitrification process during pickling of tomatoes with the initial amount of nitrates at 619±32 mg/kg

The obtained results shown in Fig. 2 indicate that during preliminary fermentation over 24 hours there is a reduction in the content of nitrates by 1.1 times. This is obviously linked to the transition of nitrates to the pickle as their amount in the pickle grew by 2.1 times. Over the following days of pickling, a reduction in the content of nitrates occurred similar to that of tomatoes with the amount of nitrates at 137±10 mg/kg, that is, intensive denitrification occurred. On the thirtieth day of pickling, the nitrate content was 134 mg/kg in tomato and 113 mg/cm^3 in the pickle.

Fig. 3, 4 show results of the study of change in the amount of lactic acid and the dynamics of denitrification process during pickling of tomatoes with the content of nitrates that is five times larger than MPC (1,576±114 mg/kg).

Results of the studies revealed that the quantitative and qualitative composition of microflora in the pickle during pickling process did not change, that is, the microbiological process slowed down. This is confirmed by data on the study of titration acidity (Fig. 3), which did not change over the entire process of pickling. In other words, the lactic acid microflora did not develop, and the fermentation of sugars did not take place.

The inhibition of reproduction of the lactic acid microflora under the action of high concentration of nitrates caused a lack of denitrification process in tomato (Fig. 4). In this case, the amount of nitrates in tomato remained almost unchanged over the entire period of the experiment.

Therefore, research results shown in Fig. 3–4 indicate that vegetable produce containing nitrates in the amount of 1,576±114 mg/kg, or larger, cannot be used in the technology of pickling (souring) because of the inhibiting effect of nitrates on the microflora. Such produce must be disposed of.

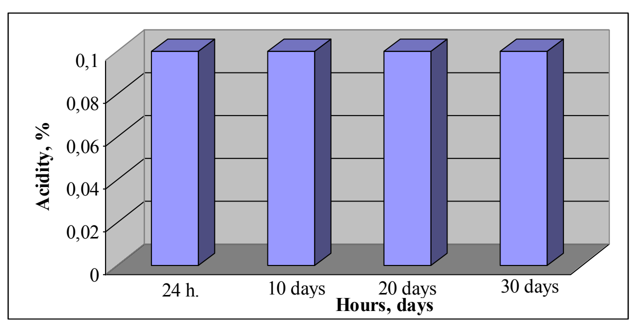

Fig. 3 Change in the titration acidity when pickling tomatoes with the initial amount of nitrates at 1,576±114 mg/kg

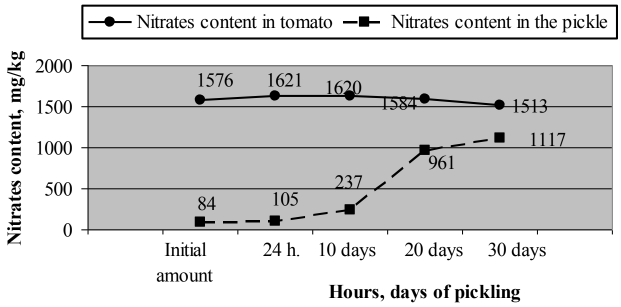

Fig. 4. Dynamics of denitrification process when pickling tomatoes with the initial amount of nitrates at 1,576±114 mg/kg

6. Discussion of results of the study of microbiological and denitrification processes during pickling of tomato

Nitrates that are found in food and drinking water negatively affect the health of consumers. Acute nitrate poisoning that are associated with the use of products with an excessive nitrate content are very rare [7]. However, an important problem at present is the chronic effect of nitrates, which is not so easy to determine. After all, the small amounts of nitrates above the norm in food products does not attract attention. At the same time, the constant exposure to such subtoxic doses of nitrates, together with other harmful substances (pesticides, antibiotics, preservatives, stabilizers), causes different types of oncological diseases [5, 6]. On average, 20 % of all vegetable produce contain the amount of nitrates that exceeds MPC [12]. It was established that of all vegetable produce sold in the Western regions of Ukraine, 15 % of the samples showed the nitrate content exceeding MPC. Therefore, studies confirm results reported by many scientists on that vegetable produce that is on sale contain nitrates in the amounts above the norm [10–12]. Taking this situation into consideration, we studied the effect of technology of pickling on the dynamics of change in the amount of nitrates in tomato. In the biological processes of pickling (souring) an active role belongs to the lactic acid microflora whose separate representatives possess denitrification properties. It was found that when pickling the tomatoes with a nitrate content within MPC (137±10 mg/kg) there occurs intensive microbiological process, which is dominated by lactic – and bifidobacteria. The number of these microorganisms in the pickle at the end of pickling process was $8.0–9.2 \times 10^9$ CFU/cm^3. Reproduction of lactic acid microorganisms and bifidobacteria causes denitrification changes in tomato and in the pickle. As a result of denitrification, the nitrate content in prepared pickled tomato decreases by 5.7–8.0 times in comparison with the initial content.

When studying the process of pickling tomatoes with the nitrate content twice larger than MPC (619±32 mg/kg), it was established that the microbiological process during preliminary fermentation is slower. Subsequent microbiological process is similar to that of pickling the tomatoes with the normative content of nitrates. After thirty days of pickling, the content of lactic acid microorganisms and bifidobacteria in the pickle is $6.1–7.8 \times 10^9$ CFU/cm^3. Denitrification process at the beginning is also a little slower – a change in the nitrate content in tomato is negligible. At the same time, the amount of nitrates in the pickle significantly increased until the tenth day of pickling, obviously because of the migration of nitrates from tomato fruits to the pickle. We believe that a slowdown in the reproduction of microorganisms at the beginning of the process of pickling occurs due to the inhibition effect of nitrates. Studies by scientists testify to the effect of bacteriostatic properties of nitrates on the microflora of food products [20]. The tenth day of pickling saw intensive microbiological and denitrification processes, which led to a reduction in the nitrate content in tomato to 134 mg/kg.

The study into effect of the amount of nitrates exceeding the norm of MPC by 5 times established that at the content of nitrates in tomato of 1,576±114 mg/kg, pickling did not occur at all. This is related to the inhibition effect of nitrates on the microflora, whereupon the growth does not happen. The amount of lactic acid microorganisms and bifidobacteria over the entire process of pickling did not change. The lack of progress in the microbiological process causes termination of the denitrification process. The amount of nitrates over the entire technological process did not change. Thus, our study indicates that the process of tomato pickling will not occur at a nitrate content of 1,576±114 mg/kg, that is, when it is five times larger than MPC for a given produce.

Previous studies were mainly aimed at detecting the influence of mechanical and physical treatment of vegetables and fruits (cleaning, soaking), aa well as heat treatment, on the reduction of the amount of nitrates in finished products [17–20]. In the present study, we established effect of the biological process – pickling – on the nitrate content in tomato, which revealed significantly better dynamics in the reduction of nitrates in vegetables, as compared to the above described findings. In addition, pickled vegetables are characterized by a positive effect on the functioning of the human gastrointestinal tract due to the presence of useful lactic acid microorganisms. That is why pickled vegetables are also considered to be products with functional purpose.

Obtained results of the research point to the possibility of using vegetables with a nitrate content above the norm for preparing pickled products. However, canning factories, as well as open markets, do not always analyze nitrate content, which is why vegetables are used without accounting for this indicator. Our study is of practical interest as the results make it possible to employ a safe technique for processing vegetables with the nitrate content above the norm. In addition, from a scientific point of view, the use of lactic acid fermentation would be also interesting for the processing of other raw materials, such as raw milk with the nitrate content exceeding the norm. Study [22] indicates that the problem of using raw milk with excessive amounts of nitrates is also relevant for dairy enterprises.

Thus, given the negative consequences of the effect of nitrates in food products on the human body, it is necessary to conduct monitoring studies into the quality of plant produce. Based on the data obtained, we recommend using a process of pickling for the processing of tomato and other vegetables with elevated content of nitrates.

7. Conclusions

1. It was established that the agri-food markets of the Western region of Ukraine sell vegetables containing the amount of nitrates that exceeds MPC, in 15–30 % of all

samples. MPC of nitrate content in vegetables was exceeded, on average, by 1.5–1.9 times.

2. It was established that when pickling the tomatoes with a nitrate content of 137±10 mg/kg there occurs an intensive microbiological process, which is dominated by lactic- and bifidobacteria. At the end of the process of pickling, the number of these microorganisms in the pickle was 8.0–9.2×10^9 CFU/cm^3. As a result of reproduction of lactic acid microorganisms there happens the process of denitrification with the amount of nitrates in ready pickled tomatoes decreased by 5.7–8.0 times.

3. It was found that when tomatoes with a nitrate content twice larger than MPC (619±32 mg/kg) are pickled, the microbiological process during preliminary fermentation progresses slower, but it subsequently becomes active. Denitrification changes in tomato at the beginning of the process of pickling are also slower, and at the tenth day of fermentation there occurs an intensive denitrification process with the amount of nitrates reduced to MPC.

4. It was found that in the process of pickling tomatoes whose nitrate content is five times larger than MPC (1,576±114 mg/kg) the denitrification does not occur at all as a result of the inhibiting influence of nitrates on the microflora.

The absence of the microbiological process predetermines the lack of denitrification process, as it depends on the denitrification capability of lactic acid microflora. The nitrate content over the entire process of pickling remains at the initial level.

Therefore, the study into microbiological and denitrification processes in the technology of tomato pickling allows us to recommend the technology of pickling for the processing of tomato and other vegetables that contain the amount of nitrates twice larger than MPC (619±32 mg/kg). Vegetable produce that contain nitrates in the amount of 1,500 mg/kg and above cannot be used in the technology of pickling (souring) because of the inhibitive influence of nitrates on the microflora.

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