Inhibition of microbiological processes in sucrose extraction

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Abstract

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Introduction. Due to the intensive development of microbiological processes in the diffusion installation there is an additional loss of sucrose as a result of decomposition, as well as deteriorating qualitative parameters of diffusion juice, which adversely affects all subsequent stages of sugar production.

Materials and methods. The objects of research were: diffusion juice; pure cultures of bacteria *Leuconostoc mesenteroides*, *Bacillus subtilis*, *Pseudomonas spp.*, *Escherichia coli*, which cause significant loss of sucrose in the production of sugar from sugar beet; a new generation of disinfectants.

Results and discussion. The greatest losses of sucrose in diffusion juice were caused by bacterias of the *Pseudomonas spp.* genus, because within 24 hours of incubation these samples of the diffusion juice, the content of sucrose decreased in 2 times. In samples of juice from *B. subtilis* and *E. coli*, a significant increase in nitrite was observed throughout the term of the incubation. In fact, the final content of nitrite in the samples after 24 h incubation increased 10-15 times. In the sample of juice from *L. mesenteroides* there was a significant increase in the level of lactic acid, compared with the initial value. After incubation at 37 °C for 2 h, the content of lactic acid was 10.63 mg/100 cm³, compared to the initial content of lactic acid – 3.07 mg/100 cm³. In the case of the development of slime-forming bacteria in diffusion juice, intensive accumulation of dextran is observed, which correlates with the increase of the number of cells of test culture *L. mesenteroides*.

Disinfectants on the basis of polyhexamethylenebiguanidine hydrochloride; quaternary ammonium compounds; dichloroisocyanuric acid sodium salt; peracetic acid and hydrogen peroxide showed high efficacy in most microorganisms that cause loss of sucrose in the process of its extraction from beetroot shavings and lead to a deterioration of the technological quality of intermediates beet sugar production. In addition, these means are also effective concerning slime-forming bacteria.

Conclusions. The investigated means are characterized by high bactericidal action against the microorganisms present in diffusion juice. These agents are also effective in suppressing the development of slime-forming bacteria.

Introduction

During extraction of sucrose from beetroot shavings, processes occur leading to the decomposition of sucrose due to the action of beet cellular invertase, acid catalysis and microorganism activity.

The activity of the enzyme invertase, which is part of the beet juice, may increase with the prolonged storage of root crops. During extraction of sucrose from beetroot shavings at a temperature of 70–75 °C, the enzyme is inhibited. Consequently, decomposition of sucrose under the influence of cellular invertase of beets can occur only at the initial stage of extraction. The issue of decomposition of sucrose as a result of inversion is devoted to a series of studies A.R. Sapronov and S.E. Harina [1]. According to their conclusions, decomposition of sucrose occurs as a result of auto-catalytic and consecutively reactions. The intensity of the autocatalytic reaction is influenced by the temperature and pH₂₀ of the medium.

Research of the influence of microbiological processes on sucrose loss during extraction is devoted to a large number of scientific works in Ukraine and abroad. It was established that, subject to the optimum regimen of the extraction process, the loss of sucrose from the abovementioned factors does not exceed 0.1% to the mass of beets. In the event of a violation of the technological process and the processing of beet affected by rot or mucous bacteriosis, the loss of sucrose from decomposition can reach 0.6–0.9% and above [2]. The microflora of diffusion juice is represented by bacteria, yeast and micelial fungi, the total amount of which in 1 centimeter of cubic juice ranges from tens of thousands to tens of millions. This is due to the technological quality and microbiological contamination of shavings and feed water entering the diffusion installing [3, 4].

Thus, microbiological processes cause the decomposition of sucrose with the formation of various metabolites, which leads to deterioration of the quality of white sugar. In particular, bacteria of the *Clostridium* genus are strong gas generators, which leads to increased foam ability of juice. During the active development of butyric bacteria there is formation of ammonia, acetone, acetic aldehyde, organic acids, which are the products of the decomposition of protein substances of diffusion juice [5, 6]. Accumulation of ammonia in juices inhibits the decomposition of amides on defecation, causing in juice of II saturation the alkalinity in the form of NH₄ON, which decreases during condensation at the evaporation station. Ammonia is a catalyst for increasing the color of the syrup boiling and subsequent crystallisation. When microbial decomposition of protein substances of sugar beet, amides are also formed, the main part of which in products of sugar production is glutamin and asparagine. Established [7], that the data of amino acids are involved in the processes of formation of melanodynes and increased the molasses forming coefficient.

Under the influence of denitrification microorganisms, in particular bacteria of *Bacillus*, *Pseudomonas* genus, sugar beet nitrates are transformed into nitrites [5], and then potassium imidosulfonate is formed during sulfitation. As a result of this conversion, the ash content of sugar increases [7].

Accumulation of organic acids (milk, oily, acetic) leads to acid hydrolysis of sucrose, increasing the content of calcium salts. In the case of microbiological decomposition of sucrose, lactic acid is up to 96% of the total content of organic acids. There is correlation dependence between the amount of decomposed sucrose and the lactic acid formed. The content of lactic acid in beet juice from conditioned raw materials is 1-3 mg per 100 g of juice. As a result of the microbiological damage of sugar beet, the content of lactic acid in beet juice can be increased to 10–12 mg per 100 g of juice and above. Accordingly, when processing beets of high quality and adhering to the technological regime of the extraction

process, the content of lactic acid in diffusion juice does not exceed 2-5 mg per 100 g of juice. When processing microbiologically damaged raw materials or non-compliance with the technological regime, the content of lactic acid is increased to 10-20 mg per 100 g of juice and above [8]. Therefore, it is recommended when calculating sucrose losses because of microbiological decomposition take into account the increase in the content of lactic acid in diffusion juice compared to beet juice [9, 10].

Due to the activity of bacteria of the *Leuconostoc* genus (*L. mesenteroides*, *L. dextranicum*) and spore-forming bacteria of the *Bacillus* genus (*B. betaevicosum*, *V. mesentericus*, *V. subtilis*), in the diffusion juice are formed polysaccharides of glucose and fructose – dextran and levan [11, 12]. These substances are well dissolved in water with the formation of viscous solutions. The negative influence of life products of slime-forming bacteria is manifested in: reducing the quality of intermediate products of sugar beet production; increase in sucrose losses in production (due to increased content of sucrose in molasses); deterioration of filtration properties of juices in the process of purification; distortion of the indexes of sucrose content in beets at the polarimetric method of determination and, accordingly, purity of cell and diffusion juices [11, 13–15].

The greatest effect of suppression of vital activity of microorganisms can be achieved by maintaining a temperature of about 75 °C throughout the extraction process. However, from a technological point of view, such a temperature regime leads to an increase in the content of soluble pectin substances in diffusion juice, which negatively affects the filtration process and leads to deterioration of the quality of the purified juice. Therefore, at the sugar factories, in order to inhibit microbiological processes, disinfectants are introduced at the extraction stage [8].

Accordingly, our research objectives was to study the microbiological and acid decomposition of sucrose and investigate the effectiveness of a new generation of disinfectants on inhibiting the activity of microorganisms which cause significant loss of sucrose in the production of sugar from sugar beet.

Materials and methods

As for the objects of research, we selected pure cultures of bacteria *Bacillus subtilis*, *Pseudomonas spp.*, *Escherihia coli* and slime-forming bacteria *Leuconostoc mesenteroides* [16], which cause significant loss of sucrose in the production of sugar from sugar beet [8, 15, 17].

The research applied the use of laboratory diffusion juice of average technological quality and factory diffusion juice with additionally introduced culture *L. mesenteroides*.

In accordance with the current requirements [18], which put forward to for disinfectants, chemical compounds used as active substances should be characterized by a wide range of biocidal action, maintain their activity for a long time, should not have a negative impact on the quality of products, according to the parameters of acute Toxicity belongs to the 3rd–4th grade of moderately hazardous substances. Taking into account the above-mentioned factors, the following disinfectants were selected for research: on the basis of polyhexamethylenebiguanidine hydrochloride – "Antykam® CID-LEUCO 20"; quaternary ammonium compounds – "XSG des 3"; dichloroisocyanuric acid sodium salt – "XSG des 4"; peracetic acid and hydrogen peroxide – "XSG des 5".

Determination the influence of microbiological processes on the accumulation of metabolism products in diffusion juice

In samples sterile diffusion juice made a certain number of vegetative cells of a certain type of bacteria (*Leuconostoc mesenteroides*, *Bacillus subtilis*, *Pseudomonas spp.*, *Escherichia coli*). Incubation was performed at 37 °C for 24 hours. After 2, 5 and 24 hours indicator determined by the change in pH, purity, the content of lactic acid, nitrites, dextran, microorganisms and compared with control values of the original diffusion juice. Determination of lactic acid, nitrites and dextran was carried out by colorimetric method [13, 19]. The pH value of the medium was monitored using a pH meter. The total number of microorganisms was determined by sowing on the medium of MIA (meat infusion agar) and MIA + 10% sucrose.

Determination of the effectiveness of disinfectants activity

For research, disinfectants were used with the following expenditures: "Antykam® CID-LEUCO 20" – 0.002%; "XSG Des 3" – 0,0006%; "XSG Des 4" – 0.002%; "XSG Des 5" – 0.004%, which were added to the initial diffusion juice. The factory diffusion juice with additionally introduced culture *L. mesenteroides* was used for research. The samples were thermostated at 37 ° C. for 24 hours. Thermostatic the sample of diffusion juice without disinfectants was also performed. Microbiological examination of the output diffusion juice and juices with the means was carried out after 30 minutes. Determination was conducted by seeding the samples using the Koch method for dense nutrient media: MIA – for determining the total content of microorganisms QMAFAnM – Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms), MIA + 10% sucrose – for the determination of slime-forming bacteria; indicator beetroot agar – for acid-forming bacteria; wort agar – for micelial fungi and yeast.

After counting the colonies grown on the appropriate nutrient medium, the effect of disinfection calculated according to the formula:

$$E_d = \frac{(B_1 - B_2)}{B_1} \cdot 100$$

where B_1 is the initial content of microorganisms in 1 g (cm^3) of the product; B_2 – the content of microorganisms in 1 g (cm^3) of the product after appropriate treatment.

Determination of microbiological contamination of diffusion juice by spontaneous fermentation. The laboratory diffusion juice of average technological quality was used for research. Disinfectants were used with the following expenditures: "Antykam® CID-LEUCO 20" – 0.002%; "XSG Des 3" – 0,0006%; "XSG Des 4" – 0.002%; "XSG Des 5" – 0.004%, which were added to the initial diffusion juice. The samples were thermostated at 37 ° C. for 24 hours. Thermostatic the sample of diffusion juice without disinfectants was also performed. In the samples at the beginning of the experiment and at certain intervals, the value of pH indicator of the juice was measured. On the basis of the obtained indicators, the control of microbiological processes in diffusion juice was carried out and conclusions were made about the intensity of microorganisms in it.

Results and discussion

An important task for extraction of sucrose is to minimize losses due to decomposition. We consider the microbiological and acid decomposition of sucrose. In addition, comparative studies of the effectiveness of modern disinfectants at the sucrose extraction stage have been carried out.

Determination the influence of microbiological processes on the accumulation of metabolism products in diffusion juice

The purpose of this study was to determine the dependence of the growth of metabolism products of microorganisms in diffusion juice on the temperature, duration of the process and the nature of the contaminating microflora.

Table 1 shows the results of studies using pure cultures *L. mesenteroides*, *B. subtilis*, *Pseudomonas spp.*, *E. coli*.

Table 1
Incubation of diffusion juice with clean cultures of bacteria

Technological indices of diffusion juice	pH value	Mass fraction of dry substances, %	Mass fraction of sucrose, %	Cleanliness, %	Lactic acid content, mg/ 100 cm ³	Nitrite content, mg/dm ³
Output juice (sterile)	6,6	10,3	8,25	80	3,07	2,38
2 hours later						
<i>L. mesenteroides</i>	6,0	10,2	8,0	78,4	6,69	2,46
<i>B. subtilis</i>	6,02	10,3	8,2	79,6	3,13	3,6
<i>Pseudomonas sp.</i>	5,99	10,2	7,6	74,5	3,2	3,74
<i>E. coli</i>	6,06	10,3	8,1	78,6	3,45	3,48
5 hours later						
<i>L. mesenteroides</i>	5,8	10,2	7,8	76,5	7,29	2,8
<i>B. subtilis</i>	6,14	10,3	7,9	76,7	3,18	8,06
<i>Pseudomonas sp.</i>	6,03	10,0	7,1	69,6	4,42	7,59
<i>E. coli</i>	5,9	10,3	7,4	71,8	3,6	20,3
24 hours later						
<i>L. mesenteroides</i>	5,36	10,4	6,7	64	10,63	2,92
<i>B. subtilis</i>	6,22	10,0	7,0	70	3,21	27,08
<i>Pseudomonas sp.</i>	6,21	10,3	4,5	43,7	9,14	36,1
<i>E. coli</i>	5,48	10,4	6,9	66,3	6,08	43,76

On the basis of the analysis of the results of the conducted studies, there was a decrease in the content of sucrose and the purity of the diffusion juice in all the analyzed samples. It

has been established that the greatest losses of sucrose in diffusion juice are caused by bacteria of the *Pseudomonas spp.* genus. So, after incubation these samples of diffusion juice for 24 hours, the sucrose content decreased 2-fold. In samples of juice from *B. subtilis* and *E. coli*, a significant increase in nitrite was observed throughout the term of the incubation. In the sample juice from *L. mesenteroides* after incubation at 37 °C for 2 h, the content of lactic acid increased by 50%, after 18 h. – by 60%, and after one day – by 3.5 times and was 10.63 mg / 100 cm³ compared with the initial content of lactic acid – 3.07 mg / 100 cm³.

The most dangerous type of microbial damage to sugar beets is mucous bacteriosis. As a result of affection of roots in beet juice, dextran is formed, which causes not only direct loss of sucrose, but also creates significant problems in production. The most common species among the slime-forming bacteria found in sugar factories is *Leuconostoc mesenteroides*. The formation of dextran by the bacteria of the *Leuconostoc mesenteroides* genus occurs as a result of the decomposition of sucrose, similar to the enzymatic inversion, except that glucose is polymerized in dextran, and fructose is used to feed bacteria [11].

We have established the dependence (Figure 1) accumulation of dextran at incubation a diffusion juice samples infected with *L. mesenteroides* culture.

Figure 1. Accumulation of dextran at incubation of diffusion juice samples infected with *L. mesenteroides*:

1 – Viable count of *L. mesenteroides* cells; 2 – Dextran content.

* Note: the initial content of *L. mesenteroides* cells is 1 × 10⁵ CFU/cm³

It should be noted that at incubation samples of diffusion juice, the number of test cultures cell increased by almost two orders of magnitude compared with the initial content and amounted to 4.8×10^7 CFU/cm³ after 24 hours of cultivation, and the content of dextran in 24 hours increased 20 times compared with initial meaning.

According to the conducted studies, it can be concluded that with significant infection of diffusion juice in the event of untimely response and failure to comply with appropriate disinfection measures increases the amount of unaccounted loss of sucrose due to the livelihoods of microorganisms. In addition, products of microbial metabolism accumulate, which also cause sucrose loss in the subsequent stages of production.

Determination of the effectiveness of disinfectants activity

Consequently, in the process of extraction, undesired processes of decomposition of sucrose are possible. In this case, the most effective way of preventing is the use of modern disinfectants. Among the wide range of modern disinfectants we have chosen to study the means on the basis of active substances that have found the most widely used in the sugar industry of Ukraine.

Intensive development of microorganisms and, consequently, decomposition of sucrose occurs in the processing of bad quality beet and in the event of significant infection of the diffusion juice. Therefore, the factory diffusion juice with additionally introduced culture *L. mesenteroides* was used for research.

The results of experimental studies of the microflora of diffusion juice during the use of disinfectants are given in table 2, indicate that the selected means have a high bactericidal effect on the microorganisms present in the diffusion juice.

Table 2

Microbiological parameters of diffusion juice when administering disinfectants

Indicators	Control diffusion juice	Investigated disinfectants			
		Antykam	XSG des 3	XSG des 4	XSG des 5
		Expense of means, % by weight of juice			
		0,002	0,002	0,0006	0,004
QMAFAnM, CFU $\times 10^8$ / cm ³	6,0	1,05	0,5	0,6	0,7
The effect of disinfection, %	-	82,6	91,6	90,0	88,3
The content of slime-forming bacteria, CFU $\times 10^7$ / cm ³	61,2	12	4,6	6,0	2,3
The effect of disinfection, %	-	80,4	92,5	90,2	96,2
The content of acid-forming bacteria, CFU $\times 10^7$ / cm ³	98	21	12	3	4
The effect of disinfection, %	-	78,6	87,7	96,9	95,9
Content of mold fungi and yeast, CFU $\times 10^6$ / cm ³	8,6	1,5	1,0	0,8	4,8
The effect of disinfection, %	-	82,5	88,4	90,6	41,2

Determination of microbiological contamination of diffusion juice by spontaneous fermentation

One of the indirect methods for determining the microbiological contamination of diffusion juice is the method of spontaneous fermentation. It should also be noted that along with microbial decomposition of sucrose there is a decomposition of sucrose due to acid catalysis. Since disinfectants are different in terms of the chemical nature of the substance, it is interesting to research their effect on the technological quality of diffusion juice. To this end, has been carried out the determination of the change in the meaning of pH₂₀ of diffusion juice by introducing of different expenditure of disinfectants and thermostating of diffusion juice at 37°C for 24 hours.

Changing the value of pH₂₀ of diffusion juice at incubation treated with disinfectants is given in Table 3.

Table 3
Dynamics of change of pH₂₀ at incubation of diffusion juice treated with disinfectants

Duration of incubation, hour	Control diffusion juice	Investigated disinfectants			
		Antykam	XSG des 3	XSG des 4	XSG des 5
		Expense of means,% by weight of juice			
		0,002	0,002	0,0006	0,004
Immediately after entering, 0 hours	6,40	6,28	6,31	6,30	6,02
1 hour	5,89	6,00	6,01	6,25	6,01
2 hours	5,70	5,91	5,93	6,17	5,76
6 hours	5,30	5,60	5,32	6,08	5,40
24 hours	3,60	4,90	3,75	5,90	4,16

The results of the studies presented in tables 2-3 show an intensive course of microbiological processes in the control sample of diffusion juice (QMAFAnM – 6.0×10^8 CFU / cm³), resulting in a decrease in the pH of the juice for 24 hours incubation at 37 °C from 6.4 to 3.6 units.

At the same time, in the case of the use of the tool "XSG Des 4" for the expenses of 0,0006% to the mass of the product, the development of microbiological processes is significantly suppressed, as evidenced by the meanings of pH of the juice during its incubation at 37 °C and a duration of 24 hours, as well the effect of disinfecting diffusion juice on different groups of microorganisms.

It should be noted that the meanings of pH of the diffusion juice samples with the means "XSG Des 5" has decreased immediately after its introduction. This is due to the chemical nature of the active substance of the product (peroxyethane acid) and, consequently, the low meaning of pH of the working solutions of the means.

Therefore, when choosing a disinfectant, the meaning of pH of the working solutions should be taken into account, as well as the change of the pH of the diffusion juice after the application of the disinfectant.

Conclusions

As a result of experimental studies, it has been established that the development of microbiological processes at the stage of extraction of sucrose from beetroot shavings causes significant loss of sucrose.

Researches have shown that in the case of the development of slime-forming bacteria in diffusion juice there is an intense accumulation of dextran, which correlates with an increase in the number of cells of the test culture *L. mesenteroides*.

The results of the research show that the means of "XSG des 3", "XSG des 4", "XSG des 5", "Antykam® CID-LEUCO 20" have high efficiency in relation to the majority microorganisms that cause loss of sucrose in the process of its extraction from beetroot shavings and lead to a deterioration of the technological quality of intermediate products of sugar beet production.

It can be concluded that it is expedient to use the means of "XSG Des 4" and "Antykam® CID-LEUCO 20" for processing of nourishing water and a juice-shavings (juicer) mixture in order to disinfect them and prevent the development of microbiological processes. At that, the range of concentrations of working solutions for root crop processing is: for the means on the basis of active chlorine ("XSG des 4") 0,02–0,006%, based on polyhexamethylenebiguanidine hydrochloride (Antykam® CID-LEUCO 20) – 0,1–0,2%.

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