Center of Strategic Initiatives and Progressive Development (Ukraine)
Institute of Global Strategies of the National University «Ostroh Academy» (Ukraine)
Varna Free University «Chernorizets Hrabar» (Bulgaria)

INNOVATIONS IN SCIENCE AND EDUCATION:

CHALLENGES OF OUR TIME

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INNOVATIONS IN SCIENCE AND EDUCATION: CHALLENGES OF OUR TIME

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EFFECTIVENESS OF DISINFECTANTS IN SUGAR PRODUCTION

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This article presents the results of studies on the effectiveness of the new generation of disinfectants based on dichloroisocyanuric acid sodium, polyhexamethyleneguanidine, cytrocide, monenzyne sodium and natural hydroxy acids on contaminating microflora of sugar beet - active gray rot causative agents, which are the main cause of loss in beet mass and sucrose, as well as a sharp decline in the quality of raw materials.

Keywords: *sugar beet storage, gray rot, disinfectant*

Ensuring the quality of sugar beet during storage is an important task, because during processing of sugar beets affected by gray rot or mucous bacteriosis, the technological parameters of juices and products are being significantly deteriorated, and there are associated negative consequences - namely, gas formation in the diffusion machine, foaming during saturation, significant difficulties in filtering juices, slow massecuite boiling [1]. As a result of the above-mentioned technological problems sugar plant capacity is reduced, sucrose losses are increased due to decomposition to some organic acids, including through the course of microbiological processes, which leads to a decrease in overall sugar yield and deterioration of its quality.

Having a rotten mass in addition to direct loss of sucrose, leads to a deterioration of a number of technological parameters during beets processing. The results of our research show that in the case of significant microbiological processes, beets become unsuitable for processing, which is also consistent with the findings of other researchers [2, 3, 4].

We found that the contaminating microflora of sugar beet includes a number of Micromycetes of *Fusarium, Botrytis, Mucor, Penicillium, Aspergillus, Trichotecium, Verticillium* genus [5]. In addition, under adverse conditions of roots storage in the heap, active lesions of sugar beet with slimy bacteriosis is possible due to bacteria of the *Leuconostoc* genus, and due to the combined action of ammonifying bacteria *Bacillus subtilis and B. megatherium* and others. The research of the effectiveness of these disinfectants was performed on pure museum or isolated cultures of microorganisms from the heaps of Nabutivsky sugar plant.

According to modern requirements [3] applicable to disinfectants, the chemicals used as an active substance should be characterized by a wide spectrum of biocidal action and maintain its activity for a long time, should not make a negative impact on the quality of products, as well as belong to class III-IV of moderately hazardous substances under the parameters of acute toxicity. Considering the above factors, the research applied selection of the following disinfectants: based on dichloroisocyanuric acid sodium salt - "Sanitarin", "Javel-Kleyd"; on polyhexamethyleneguanidine (PHMG) - "Biodez", "Hembar"; on cytrocide - "Nobak", "Nobak-enzyme"; monenzyne sodium - "Kamoran" and natural hydroxy acids - "Betastab."

To determine the sensitivity of microorganisms to antiseptic preparations, the "holes in the thick agar" method was used. Cultivation of microorganisms was performed on the following nutrient medium: a) MIA(meat infusion agar) + saccharose and beetroot agar with inclusion of pure culture of Leuconostoc mesenteroides; b) Czapek medium with inclusion of pure cultures of Micromycetes - Rhizopus nigricans, Mucor mucedo, Aspergillus niger, Penicillium, Botrytis cinerea Pers, Fuzarium culmorum, Gliocladium roseum. Nutrient medium with corresponding culture of microorganisms were poured into sterile Petri dishes. After solidification of nutrient medium the holes were made within each 1.8 ... 2.2 cm from the edge of the dish, using a sterile drill. The appropriate disinfectant solutions of various concentrations were inserted into the holes.

Conclusions about the effectiveness of disinfectants at a certain solution concentration were made according to the availability of areas of stunted growth of microorganisms. No areas of stunted growth indicates that the studied culture is insensitive to the action of the product at the specified concentration. With the zone diameter of 15 mm we believe that microorganisms have a small degree of sensitivity to the corresponding concentration of the product, with the zone diameter of 15 to 25 mm the average degree of sensitivity is indicated. Availability of the zone with diameter greater than 25 mm indicates a high degree of sensitivity of microorganisms to the concentration of the antimicrobial agent.

Results of the research on the effectiveness of the above-mentioned disinfectants for certain types of microorganisms are given in Table 1.

Tab. 1.

Results of the research on the effectiveness of antimicrobial action of some disinfectants on pure cultures of micromycetes and slime-forming bacteria by "holes in the thick agar" method

Concentration of working solution, %	The diameter of the activity zone of antimicrobial agents on microorganisms, mm							
	Rhizopus nigricans	*Mucor mucedo*	*Penicillium rugulosum*	*Botrytis cinerea Pens*	*Fuzarium culmorum*	*Gliocladium roseum*	*Aspergillus niger*	*Leuconostoc mesenteroides*
1	2	3	4	5	6	7	8	9
Sanitarin								
0,02	27	38	32	14	39	38	10	14
0,04	38	No growth	40	24	No growth	No growth	14	20

0,06	No growth			36	No growth	No growth	19	28
0,08	No growth						25	32
Javel-kleyd								
0,02	24	35	28	12	37	32	Solid growth	12
0,04	32	40	34	22	No growth	40	8	20
0,06	38	No growth	38	32	No growth	No growth	16	26
0,08	No growth						22	30
Biodez								
0,05	11	12	8	12	19	24	Solid growth	12
0,1	25	22	16	24	26	30	7	16
0,2	29	28	30	30	32	38	13	23
0,3	37	38	35	36	37	No growth	22	28
Hembar								
0,05	14	14	10	15	20	25	4	13
0,1	26	24	17	26	28	30	8	18
0,2	32	30	32	33	34	39	14	24
0,3	No growth	40	38	40	No growth	No growth	23	29
Nobak								
0,025	Solid growth							19
0,05	Depressed growth the areaof the cup					–	Solid growth	24
Nobak-enzyme								
0,025	28	26	22	22	23	–	20	22
0,05	No growth					–	32	34
Betastab								
0,25	Solid growth							32
0,5	Solid growth							38
Kamoran								
0,1	20	15	8	Depress ed growth	25	–	12	24
0,2	23	16	14	16	24	–	14	36
0,4	28	22	16	16	28	–	17	40

Analysis of research results (Table 1) demonstrates the high efficiency of “Sanitarin”, “Javel-Kleyd”, “Biodez” and “Hembar” on mycelial fungi of different genus. The “Nobak-enzyme” should also be noted which compared to “Nobak” has showed high antimicrobial effect to a wider range of microorganisms.

As for the “Betastab”, which is an environmentally safe product, derived from hydroxy acids of hops, it shows high efficacy in slime-forming bacteria, including the Leuconostoc genus, at the same time, at these values it is not effective on Micromycetes.

Disinfectant “Kamoran” is active on different groups of microorganisms, including Micromycetes and slime-forming bacteria.

The high efficiency of the presented products on slime-forming bacteria (Table 1., Fig. 1.) should be noted. Thus, at concentration of 0,1 – 0,2 % working solution of disinfectant “Kamoran” the zone of stunting growth of slime-forming bacteria *Leuconostoc mesenteroides* is 24 ... 26 mm, which demonstrates the high efficiency of the product.

Analysis of experimental research results on *Mucor mucedo* species has shown the high efficiency of disinfectant “Kamoran” at concentration of 0,4 % working solution.

One of the most active gray rot causative agents are the *Fuzarium* genus. The results of the above products on Micromycetes of *Fuzarium culmorum* genus are presented in Fig. 1 and indicate the high efficiency of it actions at concentration of 0,2 – 0,4 % working solution.

Disinfectant “Kamoran” has slightly lower efficiency on *Botrytis sinerea Pers* and *Penicillium rugulosum* species.

Therefore, “Kamoran” disinfectant has high antimicrobial activity against slime-forming bacteria and various micromycetes species presented in the raw materials, process water and the intermediates of sugar production.

Given the results of the research, there is a proved need of the further study on the effectiveness of products based on chlorine (“Sanitarin”, “Javel-Kleyd”), based on PHMG (“Hembar”, “Biodez”) and based on monenzyne sodium (“Kamoran”) for processing of sugar beet before entering into heap storage.

In order to establish the range of necessary expenditures for further processing of roots there was conducted an additional research to determine the effectiveness of their action on certain types of bacteria and yeast that characterize contaminating microflora of sugar beet.

The following bacteria were selected as objects of research: *Bacillus subtilis*, *B. megatherium* (gram positive spore-forming) ammonifying bacteria *Pseydomonas spp*, yeast Sacharomyces *cerevisiae*, *Rhodotorula glutinis*, *Endomyces lactis*. Cultivation of microorganisms was performed on the following nutrient mediums: a) MIA (meat infusion agar) and beetroot agar with inclusion of pure cultures of microorganisms of *Bacillus subtilis*, *B. megatherium*; b) wort-agar with inclusion of pure cultures of microorganisms *Sacharomyces cerevisiae*, *Rhodotorula glutinis*, *Endomyces lactis.*

Results of the research on the effectiveness of the above-mentioned disinfectants for certain types of microorganisms are given in Table 2.

Analysis of the research results (Table 2) shows the high efficiency of selected products on bacterial microflora of sugar beet production. The nature of the toxic action of chlorine–based chemicals is associated with oxidative processes in the cytoplasm of microbial cells, leading to its death. [3] Thus, in the case of using disinfectant “Sanitarin” in the range of working solution concentrations of 0,02 ... 0,04 % there was observed the loss of vegetative forms of spore-forming mesophilic bacteria *B. subtilis*, *B. megatherium*, and the yeast *Rhodotorula glutinis*, *Endomyces lactis.*

Tab. 2.

Results of the research on the effectiveness of antimicrobial action of some disinfectants on pure cultures of microorganisms by "holes in the thick agar" method

Concentration of working solution, %	*B.subtilis*	*B.megatherium*	*Psevdomonas*	*Sacharomyces cerevisea*	*Rhodotorula glutinis*	*Endomyces lactis*
Sanitarin						
0,02	25	24	28	17	32	34
0,04	33	32	40	22	No growth	No growth
Javel-Kleyd						
0,02	20	20	25	15	28	26
0,04	32	32	36	19	35	33
0,06	No growth	No growth	No growth	26	No growth	No growth
Biodez						
0,1	18	18	34	20	22	–
0,2	26	26	38	28	30	32
0,4	30	32	No growth	33	35	36
Hembar						
0,2	22	30	36	36	40	34
0,4	29	36	42	39	в.р	38

Results of the research on the effectiveness of the disinfectant "Kamoran" for certain types of microorganisms are given in Table 3.

Tab.3.

Results of the research on the effectiveness of antimicrobial action of the disinfectant «Kamoran» on pure cultures of microorganisms by «holes in the thick agar» method

Concentration of working solution, %	*B. cereus*	*B.subtilis*	*B.megatherium*	*B.mesentericus*	*Psevdomonas*	*E. coli*
0,025	30	12	35	13	15	16
0,05	34	24	39	18	20	18
0,1	36	38	40	25	23	24
0,2	44	42	42	27	26	29
0,4	46	46	43	35	42	38

Analysis of the research results demonstrates high efficiency of "Kamoran" disinfectant against sugar production microflora. Its especially high efficiency against the following bacteria species should be noted: *Bacillus subtilis, B. cerreus, B. megatherium, V.mesentericus, Pseudomonas, E. coli* with concentration of 0,05 – 0,2 % working solution.

An important indicator in determining the optimal cost of disinfectant is the effect of the decontamination. According to research methods to the original juice added certain amount of disinfectant "Kamoran." Then the source and diffusion juice with antiseptic analyzed to determine the total content of microorganisms, including mesophilic bacteria content, the micromycetes and yeast. Determination was carried out by known methods by dilution and seeding tests on solid nutrient media in Petri dishes. After counting the colonies grown on appropriate nutrient media calculated effect disinfecting the formula:

$$E_{\text{д}} = \frac{(B_1 - B_2)}{B_1} \cdot 100,$$

where B_1 - 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 the appropriate treatment.

Because most problems in manufacturing the formation of " accumulation of dextran " (slime) as the object of study was chosen as the diffusion medium quality juice and juice with added slime-forming bacteria isolated from the factory " accumulation of dextran". As mentioned earlier, slime-forming bacteria are *Leuconostoc mesenteroides*, *Leuconostoc dextranicum* and others.

Analysis of the research results (Table 4, 5) demonstrates a high bactericidal effect of "Kamoran" disinfectant against microorganisms present in the diffusion juice.

Tab. 4.

Results of the research on the effectiveness of antimicrobial action of the disinfectant "Kamoran" against microorganisms present in the diffusion juice.

Indicators	Concentration of working solution, %			
	0	0,01	0,02	0,04
The total content of microorganisms, CFU ml. / cm^3	2,4	1,46	0,97	0,7
The effect of disinfection%	–	39,2	59,6	70,8
Content of slime-forming bacteria, CFU ml. / cm^3	1,1	0,68	0,51	0,4
The effect of disinfection%	–	38,2	53,6	63,6

Tab. 5.

Results of the research on the effectiveness of antimicrobial action of the disinfectant «Kamoran» on slime-forming bacteria isolated from the factory «accumulation of dextran».

Indicators	Concentration of working solution, %			
	0	0,01	0,02	0,04
The total content of microorganisms, CFU ml. / cm^3	7,8	4,02	3,14	1,9
The effect of disinfection%	–	48,5	59,7	75,6
Content of slime-forming bacteria, CFU ml. / cm^3	6,5	3,8	2,9	1,95
The effect of disinfection%	–	41,6	55,4	68,5

Therefore, the results obtained by research disinfectant «Kamoran» is highly effective against most microorganisms present in raw materials and intermediates of sugar beet production.

Thus, the research results have shown that the products"Sanitarin", "Javel-Kleyd", "Hembar", "Biodez", "Nobak-enzyme", "Kamoran" have stable fungicidal and fungistatic effect against a broad spectrum of Micromycetes which are gray rot causative agents and lead to poor technological quality of sugar beet. In addition, these agents are also effective in inhibiting the development of slime-forming bacteria.

According to the results, there should be noted the high efficiency of selected products -"Sanitarin", "Javel-Kleyd", "Hembar", "Biodez", "Kamoran" on a wide range of microorganisms.

According to results of the experimental research, we can conclude on the feasibility of the aforementioned means for sugar beet processing for the purpose of disinfection.

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STRUCTURE OF NITROGEN FRACTIONS ORGANIC AND CONVENTIONAL COW'S MILK

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Raw organic and conventional cow's milk samples were analyzed for total nitrogen, non-protein nitrogen and milk urea content with the aim of evaluation of the different diets effect. Conventional milk contained higher level of total milk urea nitrogen as well as higher proportion in total nitrogen and non-protein nitrogen fraction. We detected that ratio of milk urea nitrogen to non-protein nitrogen is the most significant criterion for assessment of diet differences.

Keywords: *organic, conventional milk, diet, nitrogen, urea.*

Balanced cattle diet is an essential element of dairy farming. The optimum ratio of rumen degradable protein and energy for its digestion achieves the desired level of the metabolize protein concentration in the cow's small intestine and provides maintenance and the production of milk. If diet is unbalanced, the surplus of protein will deaminated in cow's liver and urea level will increase in blood plasma and milk. With the aim of evaluation of the effect of different diets at certified organic and conventional farms on the structure of the nitrogenous fractions of milk, we analyzed raw organic (n = 24) and conventional milk samples (n = 24) in the period

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