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STUDY OF THE SURVIVAL OF *BACILLUS CEREUS* IN LOW-ACID CANNED VEGETABLES

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ABSTRACT

The species *B. cereus* is widely distributed in food products and is often the cause of food poisoning. Therefore, data on the ability of this microorganism to develop in low-acid canned products are of scientific and practical interest. The aim of the study was to determine the survival of *B. cereus* in low-acid canned vegetables stored at different temperatures. The objects of the study were industrial samples of aseptic canned carrot puree (pH = 5.11, a_w = 0.912) and sliced beets sterilized in vacuumized polymer bags (pH = 5.43, aw = 0.887). The *B. cereus* 11778 strain was used. The listed products were contaminated daily culture of the test-strain, with a concentration of 10^4–10^5 CFU/ml, and incubated at 6 and 30°C for 26 days. Sampling of products was carried out every 3 days to identify viable microorganisms. The generally accepted methods of experimental microbiology were used. It was found that in the canned "Beet sliced" *B. cereus* died after 10 days of storing the product at a temperature of 30°C. At a storage temperature of the contaminated product equal to 6°C, the test-microorganism did not die during the whole experiment and was detected in the amount of tens CFU in 1 ml of the product. In aseptic canned carrot puree, regardless of the storage temperature, *B. cereus* remained viable throughout the experiment, i. e. 26 days. The approximation of the obtained numerical values showed acceptable convergence of the curves with experimental data (R^2 = 0.9186 ÷ 0.985). Microscopic examination of Gram-stained *B. cereus* 11778 preparations isolated from contaminated samples of canned products during the experiment showed abundant sporulation of the test-strain. The research results can be used to predict the activity of *B. cereus* in canned vegetable products under various environmental conditions.

Keywords: *Canned vegetables, Bacillus cereus, Survival curves, Environmental conditions*

INTRODUCTION

Among the pathogens that are traditionally found in food, *Bacillus cereus*, aerobic, spore-forming gram and catalase-positive bacteria (Bergey, 1994), are able to survive in sterilized canned food. They are associated with food poisoning in Europe, at least since 1906 (Jay, 1996). The *B. cereus* group includes seven closely related species: *B. cereus sensustricto* (here called *B. cereus*), *B. anthracis*, *B.*

thuringiensis, *B. mycoides*, *B. pseudomycoides*, *B. weihenstephanensis* and *B. cytotoxicus* (Guinebretière *et al*., 2013). Numerous studies have shown that a large variability of properties is characteristic of this microorganism, therefore, according to aberrant and deviant strains are often encountered (Goeffert, Spira, Kim, 1972). The following data on the prevalence of *B. cereus* are given in studies describing studies of the contamination of food products with this microorganism.

Kjellander and Nygren (1957) examined 514 food samples and found that 26% of meat samples, 77% of milk samples, and 51% of fruits, nuts, and vegetables contained *B. cereus*. Andersson and Storgards (1959) reported that 129 of 486 samples of pasteurized milk, 147 of 161 samples of cream and 157 of 161 samples of whipped cream contained viable *B. cereus* cells. Nygren (1962) examined 3888 food samples and found that *B. cereus* contained in 51.6% of 1546 samples of food ingredients, in 43.8% of 1911 samples of cream and pudding, and in 52.2% of 431 samples of meat and vegetable products. In most cases, the level of contamination was less than 1×10^2 CFU/g (Goeffert *et al*., 1972). A study of selected dry products purchased at retail outlets in Madison, Wisconsin, revealed *B. cereus* in 25.3% of the products. Most often, this type of bacteria was found in spices, flavoring, dried potatoes, powdered milk and spaghetti sauces (Blackburn, 2008). So, studies of 20 species of spices showed that 71% of samples were contaminated with *B. cereus* (Merchina, 2003). There is evidence of the presence of *B. cereus* in canned food, sausage and confectionery (Vasiliev *et al*., 2013). It is believed that the optimum temperature for the development of this microorganism is 30–32°C, the maximum is 37–48°C, and the minimum is 10°C. However, in the literature there is evidence of the ability of *B. cereus* to multiply at 49°C and grow at 50°C. Studies performed by Halvorson *et al*. and Knaysi showed a difference in temperature limits during spore germination and vegetative growth of *B. cereus*. So, the temperature limits for spore germination were minus 1ºC (minimum), 30ºC (optimal) and 59ºC (maximum). It should be noted that this range is established in nutrient media containing mineral acids and alkalis as pH regulators (Jay, 1996). The type of acid affects the vital activity of *B. cereus*. Acetic acid has the greatest bacteriostatic effect. Growth delay of *B. cereus* with this acid is observed at pH = 4.5 and even 6.0. Acidification of products with other acids retards growth only at pH = 4.0 (Lindsay *et al.*, 2000). A microbe can develop at a concentration of table salt in an environment of up to 10–15%, sugar up to 30–60% (Blackburn, 2008).

Of great practical interest is the thermal stability of *B. cereus* spores (Burgos *et al*., 1972, Ordonez and Burgos, 1976). Ingram (1969), referring to data from various sources, determined the value of D_{100} in food products with low acidity (pH> 4.5), equal to 5 minutes. There were published D values at 85ºC, 90ºC, 95ºC and 100ºC in phosphate buffer (pH = 7.0), namely: 220, 71, 13 and 8 min, respectively, the values of D_{121} in soybean and olive oil were 30 and 17.5 min, accordingly (Guinebretière *et al*., 2013). Concluding the brief review, it can be noted that, despite a certain scientific and practical groundwork in the field of predicting the activity of the *B. cereus* in various foods, there are still insufficiently studied questions of its survival in low acid canned foods from vegetables. Thus, the

objective of this work was to determine the survival of *B. cereus* in low-acid canned vegetables stored at different temperatures.

MATERIAL AND METHODS

The objects of study were food products (Table 1) and *Bacillus cereus* 11778 strain, kindly provided to us by the Department of Microbiology of the RSPC of Hygiene (Minsk, Belarus).

Table 1. Summary description of food products.

Food	Composition	Indicators				
		pH	Mass fraction,%:		a_w	Eh, mV
			soluble solids	titrated acids		
Sliced beets sterilized in vacuumized polymer bags	Beet roots, prepared water, citric acid	5.43	12.3	0.125	0.887	77.75
Aseptic canned carrot puree	Carrot roots, citric acid	5.11	7.47	0.07	0.912	95.2

In the experiments, daily culture *B. cereus* 11778 was used. For this purpose, for 18...20 h before testing, the test strain was sown on sloped nutrient agar. A suspension of the microorganism culture grown overnight was prepared on the day of testing. Determination of the survival of *B. cereus* in different food environments was carried out according to the plan given in Table 2. The results of microbiological studies were processed using methods of mathematical statistics (Garnayev, 1999, Alekseev *et al*., 2008). Additionally, we studied the change in the morphological properties of isolates of *B. cereus* during the experiment. For this purpose, the isolated cultures of the test microorganism were Gram-stained and microscopy.

Table 2. Experiment plan for determining the survival curves of *B. cereus* in different food environments.

Food and storage temperature, ºC*	The initial titer in product, CFU/cm^3	Sampling frequency	Thermostating conditions
Sliced beets sterilized in vacuumized polymer bags, 6 30	 1.1×10^5 1.1×10^5	Every 3 days during the 26 days	Nutrient agar, 30ºC, 48 h
Aseptic canned carrot puree 6 30	 8.6×10^4 1.7×10^4		

* Control (noncontaminated) food samples were stored in parallel.

Organoleptic (color, texture, odor) and physical and chemical (pH, mass fractions of soluble solids and titrated acids, water activity and redox potential) characteristics of control and experimental food samples were investigated at the end of the experiment. The pH value was measured with an accuracy of ± 0.01 using a pH-meter Hanna Instruments HI 2211-02 (GOST 26188). Mass fraction of titrated acids was determined by a potentiometric method using a pH-meter "Hanna Instruments HI 2211-02" and a glass electrode with a spherical membrane and a ceramic diaphragm with an accuracy of ± 1% (GOST ISO 750). Mass fraction of soluble solids was measured with an accuracy of ± 0.1% (GOST ISO 2173) using the refractometric method (Atago NAR-1T refractometer). The determination of water activity was carried out using a Roremeter RM-10 type water activity analyzer, the measurement accuracy was ± 0.2 (GOST ISO 21807). To determine the redox potential, the following measuring systems were used: an ionomer I–160 M, a platinum high-temperature electrode EVP-1 and a silver chloride reference electrode EVL-1M3.1 (accuracy class (error Δ (Eh) = ± 1mV); pH meter pH 210 manufactured by HANNA Instruments with a HI 3131 P combined electrode (accuracy class (error Δ (Eh) = ± 3mV).

RESULTS AND DISCUSSION

The results of studies of the survival of *B. cereus* in canned vegetables thermostatted at different temperatures are shown in Figures 1 and 2. As can be seen from the data (Figure 1), the death of the test-microorganism occurred after 10 days into canned sliced beet which was thermostated at 30°C, while at a temperature of 6°C, this test-microorganism was detected in the amount of tens of CFU in contaminated product after 26 days of storage. Other results were obtained in an experiment with samples of carrot mashed aseptic canning (Figure 2). Regardless of the storage temperature of samples of contaminated products, *B. cereus* did not lose viability throughout the experiment. However, unlike storage in canned sliced beets, the lowered temperature (6°C) had an inhibitory effect on the growth of the test-microorganism. So at the end of the experiment, the content of *B. cereus* in a contaminated carrot puree stored at 6°C did not exceed 10 CFU. In contaminated samples of carrot puree stored at 30°C for 26 days, the content of *B. cereus* increased by 3 orders of magnitude compared with the original titer.

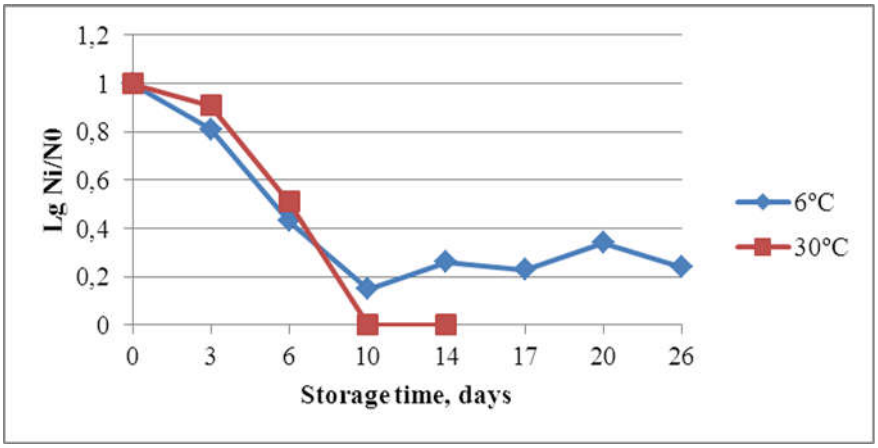

Figure 1. The survival of *B. cereus* 11778 into canned sliced beets, stored at different temperatures

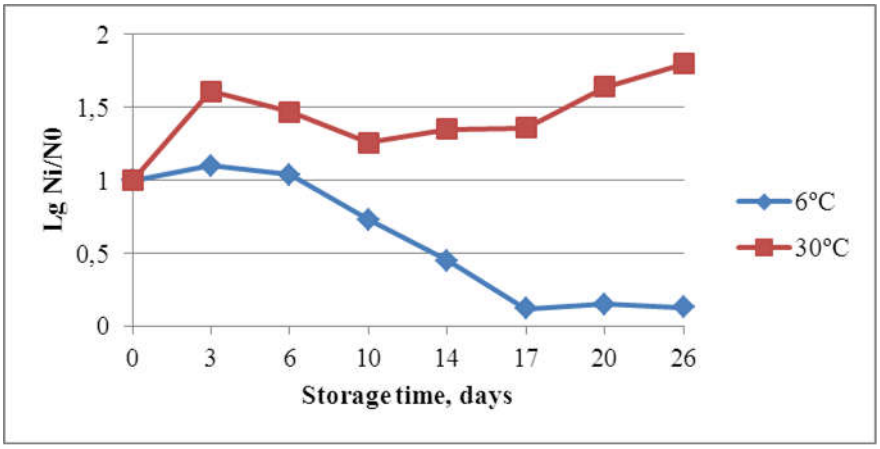

Figure 2. The survival of *B. cereus* 11778 into aseptic canned carrot puree, stored at different temperatures

The approximation of the experimental data on the change in the number of the test-strain we studied in two types of canned products stored at different temperatures allowed us to obtain equations describing the survival curves with different degrees of accuracy (Table 3).

Table 3. Prediction equations for *B. cereus* in low-acid canned vegetables thermostatted at different temperatures

Food	Temperature conditions, °C	Prediction equation	R^2 value
Sliced beets sterilized	6	$y = -1E\text{-}04x^4 + 0.0041\ x^3 - 0.0313x^2 - 0.4117x + 5.1565$	0.9695
	30	$y = -0.4162x + 5.1866$	0.9186
Carrot puree	6	$y = 0.0014x^3 - 0.0535x^2 + 0.2653x + 5.0146$	0.985
	30	$y = -1E\text{-}05x^5 - 0.001x^4 + 0.0295x^3 - 0.36x^2 + 1.6533x + 4.2586$	0.969

The results of changes in the physical and chemical parameters of the control and experimental samples of food products are given in Table 4 and indicate the following. The presence of *B. cereus* in canned sliced beets, stored in at temperature 6°C, caused a decrease in pH (1.2 times) and an increase in titrated acidity (one and a half times) and redox potential (almost 2 times). Analysis of the data obtained in the study of the physical and chemical parameters of the control and experimental samples of carrot puree showed that the greatest changes occurred in the value of the redox potential (decreased by 1.12–1.16 times) and water activity (decreased by approximately 0.03). At the same time, the consistency, color and smell of experimental samples of canned sliced beets and canned carrot puree remained unchanged.

Table 4. Physical and chemical parameters of the control and experimental samples of food products after 26 days of storage at different temperatures

Food	Kind	Temperature conditions, °C	Indicators				
			pH	Mass fraction,%:		a_w	Eh, mV
				soluble solids	titrated acids		
Canned sliced beets*	Control	6	5.49	11.54	0.22	0.9457	74.2
	Experimental	6	4.55	11.28	0.34	0.9457	127.3
Aseptic canned carrot puree	Control	6	5.2	7.09	0.106	0.973	90.2
		30	5.1	7.43	0.101	0.959	97.9
	Experimental	6	5.38	7.18	0.0744	0.945	80.4
		30	5.34	7.60	0.113	0.933	84.3

* Physical and chemical indicators in the control and experimental samples of sliced beets, stored at a temperature of 30°C, were not determined due to the premature termination of the experiment.

CONCLUSIONS

Based on the analysis of the sources of literature and the results of own experimental studies, we can create the following conclusions. At a temperature that is optimal for the development of *Bacillus cereus* (30ºC), its survival in low-acid canned vegetables (canned sliced beets and aseptic canned carrot puree) depended on the composition of the nutrient medium. The temperature of thermostating equal to 6 ° C led to the death of *Bacillus cereus* 11778 in canned sliced beets after 10 days of storage and gradual dying off in aseptic canned carrot puree after 26 days of storage (the number of test-microorganism did not exceed a few CFU/g).

The obtained data on the survival of Bacillus cereus 11778 into canned sliced beets and aseptic canned carrot puree can be used to risk assessment of their growth at different stages of the production process and use, as well as to develop appropriate control measures.

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