INFLUENCE OF STORAGE TEMPERATURE ON THE DEVELOPMENT OF YEASTS IN FRUIT JAM WITH POTASSIUM SORBATE

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Abstract

The aim of our work was to study the change in the quantity of yeast in unsterilized fruit jam with potassium sorbate, packaged in soft packs of the "Doy Pack" type, at different storage temperatures to establish shelf life. The objects of research were samples of blueberry jam, canned by hot bottling using potassium sorbate and packaged in soft packs of the "Doy Pack" type. The *Saccharomyces cerevisiae* and *Candida scottii* strains were used. Jam samples infected with test microorganisms were placed in a refrigerator (6±1.0°C) and in a thermostat (24±0.5°C). The control samples of jam in the original packaging were also stored at the same temperatures. Sampling of contaminated products was carried out every 3-4 days for 28 days, then every 2 weeks. Control samples of jam were examined monthly. In addition, organoleptic and physicochemical parameters of control and infected samples were determined. As a result of studies, it was found that the industrial sterility of the control samples of jam remained during 6 months of storage at temperatures of 6°C and 24°C. Organoleptic and physicochemical parameters of control samples of product during storage did not change compared to their initial value. The storage of yeast contaminated samples at different temperatures indicated the absence of favorable conditions for the development of microorganisms. At a storage temperature of 24°C, all test microorganisms died after 25 days of storage. Visible changes in the organoleptic characteristics of contaminated samples were not detected. After the 4 months of storage at a temperature of 6°C, contaminated samples contained yeast in the amount of some tens of CFU/cm^3. The data obtained suggest that canned blueberry jam by hot bottling using potassium sorbate, packaged in a soft pack "Doy Pack" type, is resistant to microbiological spoilage and can be stored for at least 6 months under standard conditions.

Keywords: *Jam, Storage, Yeast, Survival*

Introduction

Yeast is usually a minor component of the microbiota and cannot compete with bacteria in most food systems. But in the presence of certain factors (low pH, low values of water activity, high sugar content, etc.) yeast can become the main cause of food spoilage [Blackburn, 2008]. Fruit jams are a good breeding ground for yeast that can cause spoilage. Such yeasts include: *Saccharomyces cerevisiae* and some types of yeast of the genus *Candida* [Blackburn, 2008]. According to available data [Blackburn, 2008], yeast of the genus *Saccharomyces* grows well at temperatures between 15 and 30°C. At temperatures below 10°C, the growth rate is significantly reduced, and the lag phase becomes longer. The lowest temperature at which the growth of these yeasts stops is not exactly known, but there is evidence of the growth of *S. cerevisiae*, *S. bayanus*, *S. unisporus*, *S. dairienensis* and *S. exiguus* at 4–7°C for 1–3 weeks [Deak, Beuchat, 1993; Drocklenhurst, White, Dennis, 1983; Jermini, Schmidt-Lorenz, 1987; Savard *et al.,* 2002]. The minimum growth temperature depends on factors such as pH value, sugar and salt concentration [Betts, Linton, Betteridge,

2000; Fleet, 1992]. The yeast of the genus *Saccharomyces* ceases to develop at a_w below 0.85–0.88 [Tokuoka, 1993; Lagos, Silva-Graca, Lucas, 1999]. The minimum a_w for yeast growth depends not only on the sugar and salt content, but also on the type of solute. In addition, the upper limits of growth in the presence of other stress factors (low pH value, low temperature, the presence of antibacterial drugs) are significantly reduced [Tibury, 1980]. There are few systemic comparative studies on the effect of pH values, weak organic and inorganic acids, often used as preservatives, on the growth of yeast of the genus *Saccharomyces* [Blackburn, 2008]. The optimal conditions for the growth of yeast of the genus *Saccharomyces* are acidic conditions (pH 3.0–7.0), and the limiting conditions are pH 1.5–2.5 and pH 8.0–8.5 [Betts, Linton, Betteridge, 2000; Praphailong, Fleet, 1997]. Some minimum concentrations that inhibit *S. cerevisiae* at pH 3.5 are given in [Fleet, 1992; Praphailong, Fleet, 1997]. According to these authors, the minimum inhibitory concentration of sorbic acid ranges from 200 to 600 mg/l. According to the literature [Blackburn, 2008], yeasts of the genus *Candida* make up almost 25% of all known yeasts, therefore, the influence of environmental factors and their stress effect, as well as the resistance of this yeast group to the effects of these factors, is the same as for the entire group yeast. Temperature ranges for the growth of yeast of the genus *Candida* are in the range from 0 to 40°C. Many species are mesophilic and grow best at temperatures between 25 and 35°C. The growth temperature may be less than 37°C (e.g. *C. zeylanoides*, *C. vini*) or may exceed 45°C (e.g. *C. albicans*, *C. glabrata*). The lower temperature limit for growth can be several degrees below zero, provided that the solutes are in an unfrozen state, as in many food systems. Another factor is the availability of free water, i.e. the value of a_w. Most *Candida* yeasts grow well at a_w from 0.90 to 0.95, but survive at lower values (about 0.80) due to the increased concentration of solutions. Factors such as the type of solution, temperature, pH value, and other factors influence the resistance of *Candida* yeast to low water activity values [Tokouka, 1993].
The factor limiting the growth of yeast, including the genus *Candida*, is the pH value. *Candida* generally prefer a slightly acidic environment and grow optimally between pH 4.5 and 5.5. At the same time, the facts of growth of yeast of the genus *Candida* (*C. krusei* and *C. valida*) are known even at pH 1.5. Weak organic (benzoic and sorbic) acids used as preservatives can effectively inhibit yeast growth if applied in appropriate concentrations and if the pH values are low enough to slow down the dissociation of these weak acids [Blackburn, 2008]. Concluding a brief review of the properties and role of yeasts of the genus *Saccharomyces* and *Candida* in food spoilage, it can be noted that, despite numerous studies, the issues of their survival in unsterilized fruit jams with a preservative remain insufficiently studied, which was the purpose of this work.

Material and Methods

The object of the study was pilot samples of non-sterilized hot bottling blueberry jam with potassium sorbate (Tables 1 and 2). The subject of research is the strains of *Saccharomyces cerevisiae* and *Candida scottii*, kindly provided to us from the working collection of microorganisms of the Department of Biotechnology of the Belarusian State Technological University. For experimental studies, selected samples of blueberry jam were divided into several parts. The first and second parts were stored at a temperature of 6±1.0°C (refrigerator) and 24±0.5°C (thermostat XT-3/70-1). The third part in the form of a combined sample was poured into four sterile glass jars (type III-82-450) and inoculated with a suspension of cells of test yeast strains (1 cm^3 each), than mixed thoroughly to uniformly distribute the yeast over the product volume. Contaminated jams (2 jars with each microorganism) were placed in a refrigerator (T = 6±1.0°C) and in a thermostat (T = 24±0.5°C). The indicated temperature modes were selected based on the following considerations: 6±1.0°C is the temperature at

which the typical microbiota of fruit jams does not develop; 24±0.5°C is optimum temperature for yeast growth.

Table 1 – Identification signs of the research object

Identification signs	Description
Designation of the documentation for which the products are manufactured	TC BY 291416503.004-2019 (draft of specification)
Manufacturing date	14.11.2019
Type of packaging	Soft packaging «Doy Pack»
Net weight of packing unit, kg	0,3
Component composition	Frozen or fresh blueberries, sugar, citric acid, pectin, potassium sorbate

Table 2 – Summary characteristics of the research object

Research object	Physical and chemical indicators			
	pH	Mass fraction of soluble solids, %	a_w	Eh, mV
Unsterilized hot bottling blueberry jam with potassium sorbate	2,80	69,84	0,685	255,0

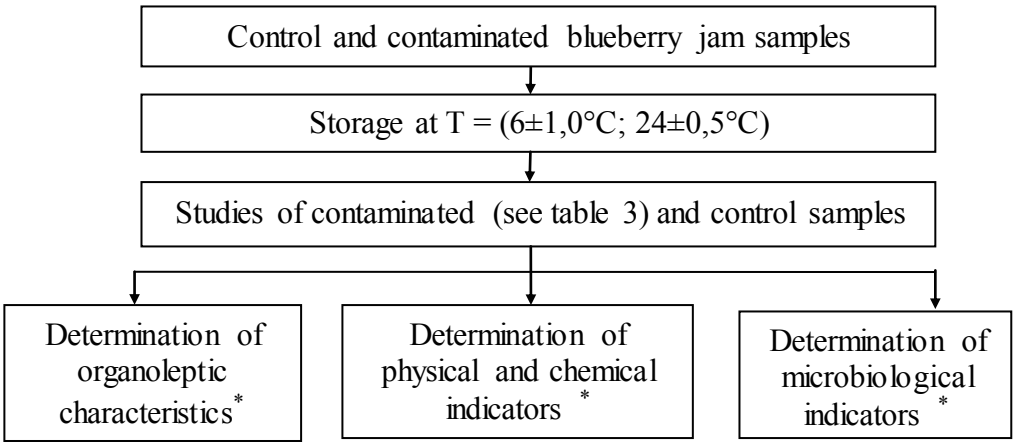
In the experiments, we used 25-day-old cultures of the studied test strains grown on slant Sabouraud agar at a temperature of 24±0.5°C. The research scheme and the experiment plan are shown in Figure 1 and Table 3, respectively.

Table 3 – Experimental plan for determining the survival of test microorganisms in unsterilized blueberry hot bottling jam with potassium sorbate

Storage temperature, ºC	Test strain designation and initial content in the contaminated product, CFU / cm^3	Frequency of product sampling	Thermostating conditions
6	*Saccharomyces cerevisiae,* $2,5\times10^5$ *Candida scottii,* $1,7\times10^5$	Every 3–4 days for 28 days, then every 2 weeks for 5 months	Sabouraud agar, 24±0,5°C, 120 h
24	*Saccharomyces cerevisiae,* $4,3\times10^5$ *Candida scottii,* $1,7\times10^5$		

The following indicators were determined in the contaminated and control samples of jam using standardized methods or by the instrument manual:
– appearance and consistency (according to GOST 8756.1-79);
– water activity (according to GOST ISO 21807-2015 using the water activity analyzer «Roremeter RM-10», the measurement error is ± 0.02);
– hydrogen indicator (pH) (according to GOST 26188-84 using a pH meter ionomer «Hanna Instruments HI 2211-02», the measurement error is ± 0.01);

– the content of soluble solids (by the refractometric method according to GOST ISO 2173-2013 using the refractometer «Atago NAR-1T», the measurement error is ± 0.1%);
– redox potential (Eh) (the following measuring systems were used: ionomer I-160 M, high-temperature platinum electrode EVP-1 and silver chloride reference electrode EVL-1M3.1, error Δ (Eh) ± 3 mV).



Note. * Organoleptic and physicochemical indicators of contaminated jam samples were determined at the end of the experiment and organoleptic, physicochemical and microbiological indicators of control samples were detected monthly.

Figure 1 – Scheme of studies to determine the dynamics of the number of test strains of yeast in blueberry jam unsterilized hot bottling with potassium sorbate during storage at different temperature conditions

In control samples of jams, the following microbiological parameters were determined:
– the presence and quantity of mesophilic aerobic, facultative anaerobic and anaerobic microorganisms (according to GOST 30425-97);
– the amount of yeast and molds (according to GOST 10444.12-2013);
– the presence of bacteria of the group of Escherichia coli (coliform bacteria) in 1 cm^3 of the product (according to GOST 31747-2012).
The processing of the results of microbiological studies was carried out using the methods of mathematical statistics [Garnayev, 1999; Alekseev, Chesnokova, Rudchenko, 2008].

Results and Discussion

The results of studies of changes in the amount of yeasts of the species *S. cerevisia* and *C. scottii* in blueberry jam during storage at different temperatures are shown in Figures 2–5, from which it can be seen that during storage of contaminated samples of blueberry jam, a gradual dying off of test microorganisms occurred. The rate of death of the yeast depended on the temperature conditions of product storage. Despite the fact that the temperature of 24±0.5°C is optimal for the development of these types of microorganisms, the test strains of *S. cerevisiae* and *C. scottii* died in blueberry jam on the 25th day of storage (Figures 2 and 4).
The treatment of experimental data (see the equations shown in Figures 2 and 4) using polynomial approximation indicated that the obtained dependences of the number of test microorganisms on the storage duration showed good convergence of curves and experimental data (R^2 were equal to 0.971 and 0, 9658, respectively) and can be used to

predict the activity of *S. cerevisiae* and *C. scottii* yeasts in fruit jams with a high sugar content and with sorbic acid under normal storage conditions.

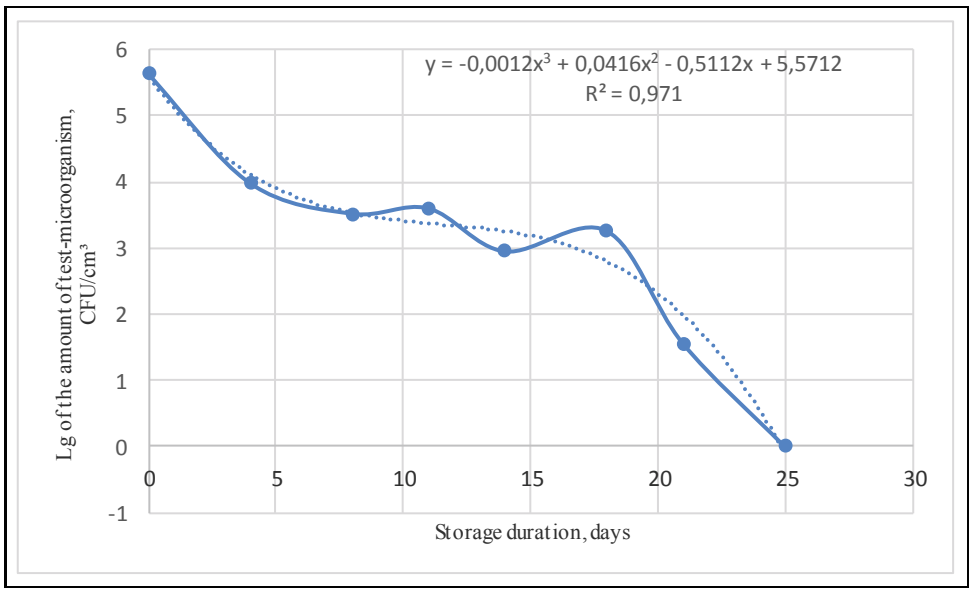


Figure 2 – Survival curve of *S. cerevisiae* in blueberry jam stored at 24±0.5°C

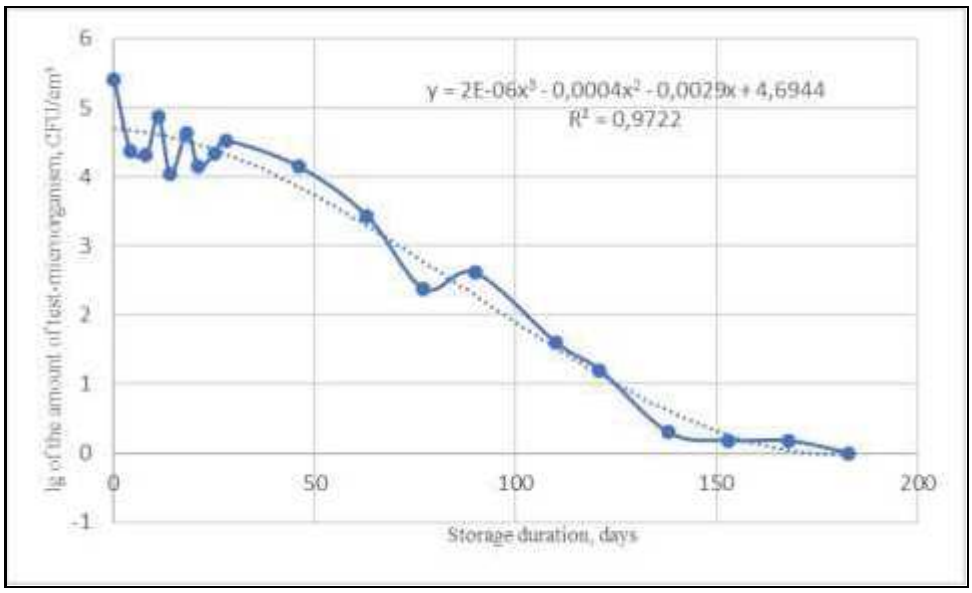


Figure 3 - Survival curve of *S. cerevisiae* in blueberry jam stored at 6±1.0°C

Other results were obtained when examining samples of blueberry jam with sorbic acid, contaminated with yeast test strains and stored in a refrigerator (Figures 3 and 5). First, the survival time of both strains in blueberry jam was six months. Second, when comparing the dynamics of the number of test strains during the first month of storage at different temperatures, a significant difference in the rate of die-off is obvious: in a refrigerator, the number of test microorganisms decreased on average by only 1 order of magnitude. The use of polynomial approximation for treatment data on the survival of test yeast strains in blueberry jam stored at 6±1°C (see equations in Figures 3 and 5) allowed to obtain an acceptable coincidence of the curves with the experimental data (R^2 = 0.9722 and R^2 = 0 ,

9487). Thus, our data confirm the fact that yeast can adapt to a certain extent and survive in unfavorable environmental conditions.

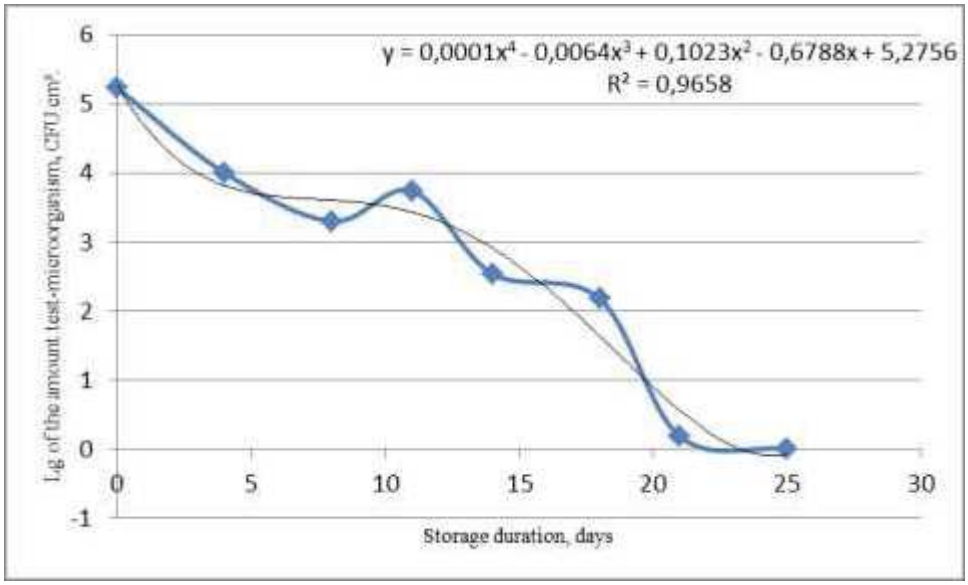


Figure 4 – Survival curve of *C. scottii* in blueberry jam stored at 24±0.5°C

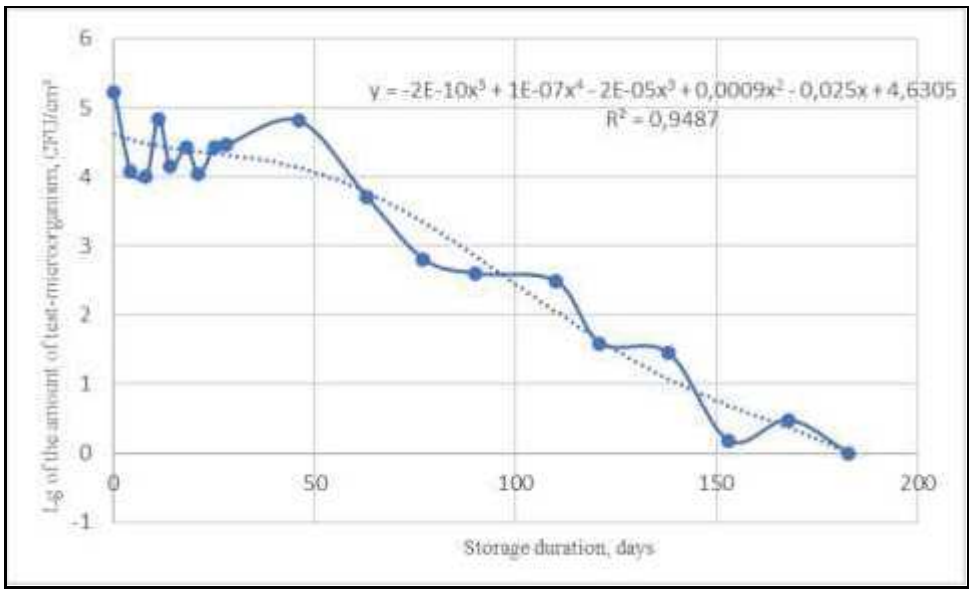


Figure 5 – Survival curve of *C. scottii* in blueberry jam stored at 6±1.0°C

The results of physicochemical tests of contaminated jam samples and their comparative analysis are shown in Tables 4 and 5. The data given in tables 4 and 5 indicate some influence of yeast on the physicochemical parameters of contaminated samples of blueberry jam with potassium sorbate. At the same time, the *C. scottii* strain exhibited greater enzymatic activity compared to the *S. cerevisiae* strain during short-term storage at a temperature of 24±0.5°C. It should also be noted that the presence of test yeast strains had a greater effect on such indicators of blueberry jam as water activity (all storage temperatures) and Eh (at a storage temperature of 24±0.5°C). However, no visible signs of spoilage, i.e. changes in organoleptic indicators (appearance and consistency) of contaminated samples of blueberry jam, were found.

Table 4 – Physicochemical indicators of control and contaminated samples of blueberry jam after the end of the experiment

Test microorganism	Storage temperature, °C	Storage duration, days	Mass fraction of soluble solids, %	pH	a_w	Eh, mV
S. cerevisiae	6±1,0	183	67,60	3,02	0,666	220,2
C. scottii			67,20	3,03	0,699	220,8
Control			68,90	2,98	0,722	223,1
S. cerevisiae	24±0,5	25	68,95	2,86	0,633	245,5
C. scottii			68,35	2,98	0,624	242,5
Control			69,50	2,86	0,660	258,5

Table 5 – Comparative analysis of physicochemical parameters of control and contaminated samples of blueberry jam after completion of the experiment

Test microorganism	Storage temperature, °C	Storage duration, days	Change (in %) to control			
			Mass fraction of soluble solids	pH	a_w	Eh
S. cerevisiae	6±1,0	183	–1,89	+1,34	–7,76	–1,30
C. scottii			–1,72	+1,68	–3,19	–1,04
S. cerevisiae	24±0,5	25	–0,80	0	–4,10	–5,03
C. scottii			–1,66	+4,20	–5,46	–6,19

To confirm that the control samples of blueberry jam are resistant to spoilage, we conducted microbiological studies indicating the absence of mesophilic aerobic, facultative anaerobic and anaerobic bacteria, yeast, molds and coliform bacteria in 1 cm^3 of jam samples.

Conclusions

Our research results allow us to formulate the following conclusions. Unsterilized hot bottling blueberry jam with potassium sorbate according to physicochemical parameters refers to products with a minimal risk of yeast development: the value of water activity is below 0.7; pH value does not exceed 3.0; the content of soluble solids is more than 65%. Storage of samples of blueberry jam contaminated with *S. cerevisiae* and *C. scottii* at different temperatures indicated the absence of favorable conditions for yeast development. At storage temperatures equal to 24±0.5°C and 6±1.0°C, all test microorganisms died after 25 and 183 days of storage, respectively. No visible signs of spoilage (change in consistency, appearance and color, gassing) of contaminated blueberry jam samples stored at different temperatures were found. Thus, the use of preservatives and short-term heat treatment (hot bottling) in the production of fruit jams allows, to a greater extent, in comparison with classical technology, to preserve natural properties without loss of functional qualities, ensuring a long shelf life, stability, safety and organoleptic acceptability. However, because food ecosystems are rarely static [Blackburn, 2008], there is opportunity for adaptation and survival of certain yeast strains in unfavourable environments, such as fruit jams with potassium sorbate.

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