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Investigation of chemical, sensory, and rheological properties of Kyrgyz ethnic fermented beverage from cereals during cold storage

Janyl Iskakova[1], Jamila Smanalieva[2]

[1] Environmental Engineering Department, Engineering Faculty, Kyrgyz-Turkish Manas University, Bishkek, Kyrgyzstan. ORCID: 0000-0002-1614-3984

[2] Department of Food Production Technology, Technology Faculty, Kyrgyz State Technical University after I. Razzakov, Bishkek, Kyrgyzstan. ORCID: 0000-0002-3929-4291

ABSTRACT

Maksym is a Kyrgyz ethnic beverage made from milled cereals such as wheat, barley, or maize, boiled in water, and then fermented using yeast and lactic acid bacteria as a starter culture. The aim of this study was to investigate the changes in the consistency and sensory properties of Maksym after industrial production during refrigerated storage. The acidity of the beverage increases and the total soluble solids decrease after 21 days. The amount of lactic acid bacteria (LAB) and yeast after production were 9.36 and 9.46 log cfu/mL, respectively. After 21 days it is indicated that the amount of both starter cultures decreased by 2 log units. In terms of acceptability, the sensory properties after 7 days of storage received the highest rating. However, the acidic taste after 21 days of storage received the lowest rating. Rheological measurements carried out at 5, 10, 20, and 30°C also revealed these changes. Four rheological models (Newtonian, Herschel-Bulkley, Ostwald De-Waele, and Casson) were used to describe the flow behaviour of the beverage. The Casson model was superior to other rheological models. The effective viscosity of Maksym was decreased from 2.66 to 1.96 mPa·s after 21 days of cold storage. The activation energy was decreased from 23.10 and 14.94 kJ/mol and indicated the thinning of viscosity of the beverage. The obtained parameters can be used to predict the shelf-life model and product labelling by its expiry period, as well as to control the quality of Maksym.

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**Corresponding Author*

1. Introduction

Cereal-based fermented beverages are widespread in various countries of Asia and Africa [1]. According to [2], fermented products have a lot of benefits such as eliminating unwanted factors arising from raw materials, providing a safer product with a longer shelf life. Fermentation helps to improve the organoleptic qualities of the product, facilitating digestion due to its probiotic properties compared to unfermented foods. Probiotics are products containing live microorganisms, which contribute to endoecology of the intestines of human beings as well as other animals [3]. Other functional ingredients are prebiotics, which are indigestible substances that improve the growth of probiotic microorganisms [4]. Since prebiotics support the growth and vital functions of the probiotic microbial community, there is great potential for the synergetic impact of prebiotics and probiotics. The use of prebiotic ingredients provides not only nutritional but also techno-functional benefits that can advance the texture parameters of the final products [5].

Figure 1. *Traditional beverage Maksym.*

The nomadic lifestyle of Kyrgyz required the use of food products with a long shelf life. The lactic acid fermentation process is widely used for the production of cereal beverages such as Jarma, Maksym and Bozo. For the production of Maksym, roasted and shredded cereals were used, usually wheat, barley and corn. It is characterized as a colloidal fluid with small grain particles, which has a slightly sour smell and brownish colour (Fig.1). Maksym was traditionally prepared in every household during the summer season and consumed chilled to relieve thirst and hunger. At least two decades before the company "Shoro" that first introduced Maksym as a commercial product was established, after which it became widely available all over the country. The company also entered China, in Kashgar, around the restaurant selling this beverage, local Kyrgyz and those who are in China only temporarily to work are joining [6, 7]. Currently, in Kyrgyzstan, Maksym is industrially produced by many enterprises. Methods and recipes of Maksym production differ from the region of Kyrgyzstan.

The traditional production method and recipe of Maksym are shown in Fig. 2. The general step is boiling the roasted and milled cereals, called "Talkan" in water (1:30 w/w) until starch gelatinization. For the preparation of "Talkan", cereals (wheat and barley) are peeled, cleaned from the husk, washed and dried. The roasting of cereals is carried out at 170–180°C until the brownish colour of the grain. Then they are cooled down to 30–40°C and ground in a millstone and sieved through a sieve with a pore diameter of 1 mm. To give the beverages a viscous consistency, roasted wheat flour with mutton fat is used. Water is added to the roasted wheat flour in small portions and boiled. After boiling, "Talkan" is added to the water-flour suspension thoroughly and boiled for about 50 minutes. After cooling to 25-30°C, 2% (w/v) of a previous batch of the beverage is added to the water-flour-talkan fluid. The fermentation is usually conducted at room temperature (about 25-30°C) for 12-14 hours and then cooled and stored at fridge temperature [7]. According to the Kyrgyz Standard Maksym contains 0.9 g/100 mL of proteins, 4.38 g/100 mL of carbohydrates, 0.08 mg/100 mL of vitamin B_1, 0.1 mg/100mL of vitamin B_2, 0.4 mg/100mL of vitamin PP, 0.59 g/100mL of ascorbic acid and 27 kcal/100 mL energy value [8].

The main problem of fermented alcohol-free beverages is the growth of microflora within the storage period. Fermented beverages have a limited shelf-life due to the growth of their own starter culture, unpleasant odours, browning, and bitterness, which, in turn negatively impact consumer perception [9]. Therefore, to track undesirable changes in fermented beverages, it is necessary to study some quality indicators during storage. In addition, it is important to determine the recommended maximum storage time during which a given product quality remains acceptable. Rheological parameters are a good indicator of structural changes in products containing starch and live microorganisms [10]. Consequently, an investigation of the rheological and sensory behaviour of the Kyrgyz ethnic beverage Maksym during storage is necessary. The producer guaranteed 30 days of storage, however, there is no information on the shelf-life of the opened Maksym in the refrigerator. Therefore, the aim of this study was to study changes in the consistency, microbiological and sensory properties of fermented beverages after opening the industrial-produced bottle. The research question was: How many days does the quality of the opened Maksym remain acceptable for consumers? The results will help predict the model for the shelf-life of Maksym and label the product by expiration date after the opening of the bottle.

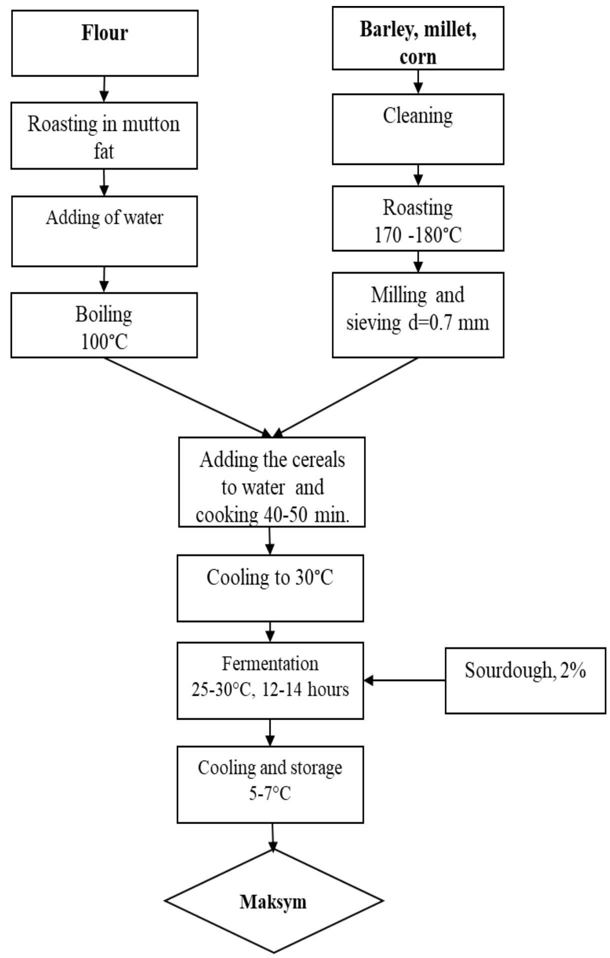

Figure 2. *Production scheme of Maksym.*

1. Materials and methods

1.1. Materials and Sample Preparation

Industrially produced fresh Maksym samples were obtained from a local market in Bishkek, Kyrgyzstan, and were refrigerated at 5°C.

pH meter (Mettler Toledo, Greifensee, Switzerland) was used for the determination of pH and titratable acidity of samples (AOAC method 981.12, 2019). The titratable acidity was given as the percentage of lactic acid in the beverage [11]. The determination of total soluble solids content was carried out using a refractometer (Reichert Abbe Mark II Plus Refractometer, Reichert, Inc., NY, USA) at 20°C.

1.2. Microbiological analyses

For microbiological analyses 10 ml sample was taken under aseptic conditions and transferred into 90 ml of 0.9 % NaCl solution. After making serial dilutions, the samples were plated on selective media for both yeasts and lactic acid bacteria. For the isolation of yeasts Wort Agar (Merck, Germany) was used and plates were incubated at 27 °C for 5 days. Lactic acid bacteria were isolated using MRS Agar (Merck, Germany). Incubation was carried out at 30 °C for 5 days using the anaerobic incubation system Anaerocult® A (Merck, Germany) in an anaerobic jar [12]. Colonies were counted and the results were expressed as cfu/mL of the beverage. Three replicate counts were performed for each tray.

1.3. Rheological Measurements

The rheological properties of Kyrgyz ethnic beverage Maksym were measured at 5, 10, 20 and 30°C using rotational measurements using an MCR 302 rheometer (Anton Paar, Graz, Austria). The concentric cylinder geometry (CC27) was used as the working tool. The rheological data were analysed using the software Rheoplus 32 Multi 6 version 3.40. The flow curves were measured in the modes of increasing, holding and decreasing the shear rate from 0.1 to 100 1/s, for the measurement of time-dependent properties. To describe the sample's flow behaviour, the flow curves obtained from the 3rd interval were modelled using equations such as Newtonian, Ostwald-De-Waele, Herschel-Bulkley, and Casson [13].

1) Newtonian:

$$\tau = \eta\dot{\gamma} \quad (1)$$

2) Ostwald–de Waele (or Power-law):

$$\tau = k\dot{\gamma}^n \quad (2)$$

where k is the consistency index ($Pa\cdot s^n$) and n is the flow behaviour index, $\dot{\gamma}$ is shear rate.

3) Herschel – Bulkley:

$$\tau = \tau_0 + k\dot{\gamma}^n \quad (3)$$

where τ_0 is yield stress, that is the stress at which the sample begins to flow or deform plastically.

4) Casson:

$$\tau^{0,5} = \tau_0^{0,5} + \eta_{Ca}\dot{\gamma}^{0,5} \quad (4)$$

where η_{Ca} is Casson's coefficient of viscosity.

The temperature dependence of Maksym's viscosity was expressed with the activation energy E_a and was calculated using the Arrhenius-type equation (Eq. 5, 6):

$$\eta(T) = A\exp\left(-\frac{E_a}{R\cdot T}\right) \quad (5)$$

$$\ln\eta = \ln A + \left(-\frac{E_a}{R}\right)\cdot\left(\frac{1}{T}\right) \quad (6)$$

Where *A* is the pre-exponential factor, *R* is the ideal gas constant (8.31 J/mol·K), *T* is the absolute temperature (K). E_a is the activation energy (J/mol) [14].

1.4. Evaluation of sensory properties

The evaluation of sensory properties changes during the storage period was conducted using an evaluation test covering mouthfeel, aroma, taste, acidic taste, and overall acceptability according to [10]. A five-point hedonic scale, from like extremely (5), like moderately (4), like a little (3), not sure (2), dislike a little (1) to dislike extremely (0) was used to assess the acceptance test and judged by a sensory group of 10 trained panellists [10, 15].

1.5. Statistical analysis

Each analysis was performed three times. Obtained data were analysed by the IBM statistical software SPSS 22 (SPSS Inc., Chicago, IL) using Tukey's post hoc tests and one-way analysis of variance (ANOVA) with a 95% confidence interval. A p-value < 0.05, was considered statistically significant.

2. Results and discussion

2.1. Chemical changes during storage

The pH of samples decreased with increasing storage time; with a starting value of 3.68, it decreased in 21 days to 3.62 (Fig. 3). After 21 days of cold storage, the total acidity of the samples slightly increased from 4.2 to 4.7 g/100 mL (Fig. 3), which indicates the continuance of acid maintenance during cold storage. According to previous studies, this is due to the prolongation of fermentation with lactic acid bacteria (LAB) during the storage period; a pH range of 3.6 to 4.9 allows LAB, which include probiotic microorganisms, to grow under usual conditions without damaging the product [16-18]. For mathematical modelling and prediction for 10 days of the change in solid content and acidity of the beverage during storage days, the polynomial regression analysis, and for pH - a linear regression analysis were carried out using the Excel MS Office program. The results are shown in Fig. 3.

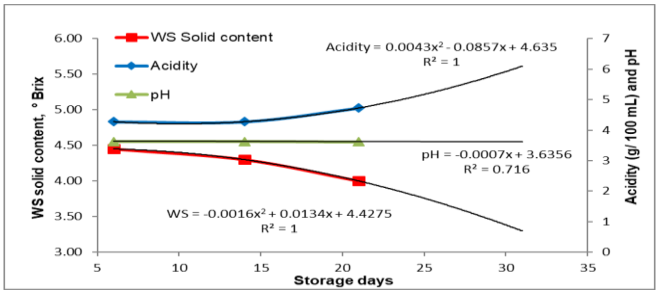

Figure 3. *Dependence of acidity, pH, and water-soluble solids content on storage days.*

The total soluble solids showed a slight decrease from 4.5 to 4.0 °Brix throughout the shelf life, and this was inversely proportional to the total acidity (Fig. 3). This change is associated with the metabolism of LAB and yeast cells. Lactic acid bacteria produce enzymes such as lipases, proteases, amylases, peptidases, esterases, ureases, polysaccharide degrading enzymes, and phenol oxidases [19]. The amylase enzymes produced by LAB break down insoluble starch molecules, making them soluble and subsequently usable by the same fermenting microorganisms such as *Saccharomyces cerevisiae* [17, 20].

2.2. Lactic acid bacteria enumeration

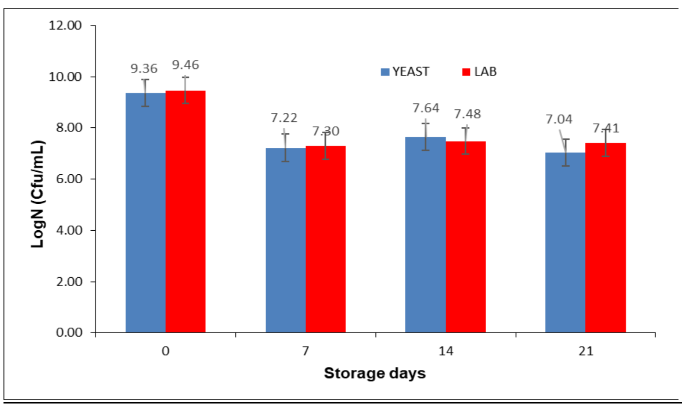

Figure 4. *Change in lactic acid bacteria (LAB) and yeast counts (cfu/mL) during the storage of fermented beverage Maksym.*

The microbiological characteristics of the final fermented product can be influenced by various factors, such as the ratio of starter cultures to food products, incubation time and temperature, agitation, and storage conditions [21]. The enumeration of lactic acid bacteria (LAB) and yeast number (cfu/mL) during the refrigerated storage of fermented beverage "Maksym" is shown in Fig.4. The amount of lactic acid bacteria (LAB) and yeast after production were 9.46 and 9.36 log cfu/mL, respectively. After 21 days the numbers of both starter cultures decreased to 7.41 and 7.04 log cfu/mL for LAB and yeast, respectively. The viability of yeast cells at the end of the shelf life period was slightly higher (78 %), compared to LAB (75%). In general, fermented cereal products contained LAB and mesophilic aerobic bacteria in the range of 5 to 9 log cfu/g [21]. The amount of LAB in some acidic beer products from Belgium, such as lambic and gueuze, ranged from 2 to 5 log cfu/g [22, 23]. For comparison, fermented dairy products contain LAB in the range of 1 to 10 log cfu/g [21]. According to [24] in Brazilian kefir containing yeast and LAB cells, the count of yeast was approximately 6 log cfu/mL and LAB group count was approximately 10 cfu/mL. They remained constant until the end of the storage period of 28 days. The amount of LAB cfu in khainak milk fermented by mesophilic starter cultures (*Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*) immediately after fermentation was also high at 9.42 log cfu/mL and decreased after 3 days of storage to 8.22 log cfu/mL [25].

2.3. Flow behaviour of the beverage Maksym

The flow curves: shear stress versus shear rate and viscosity curves: shear rate ($\dot{\gamma}$) versus dynamic viscosity (η) at 5°C for various storage periods: 7, 14, 21 days are shown in Fig. 5.

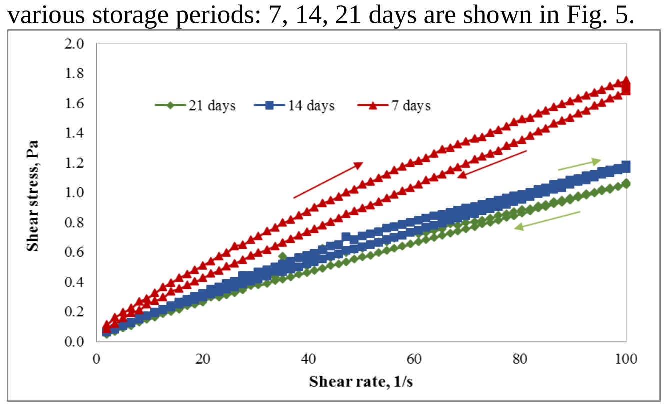

Figure 5. *Flow curves: shear-thinning and thixotropic behaviour of Maksym at 5°C.*

All shear stress vs. shear rate curves of Maksym represent shear-thinning behavior: a plot in which the apparent viscosity decreases with increasing shear rate. The viscosity of other Kyrgyz fermented cereal beverage Boza decreased as shear rate increased [10]. The two curves up (by increasing the shear rate) and down (decreasing shear rate) of Maksym samples did not overlap and led to the formation of a hysteresis loop (area). The downward curves were lower than the upward curves, indicating a thixotropic behaviour of the sample. The hysteresis area of Maksym at 5°C decreases with increasing storage time. This is the typical flow behaviour of starch-containing food and yogurts [15]. The shear stress will destroy the original structure, which will not recover as it moves down the curve, resulting in lower yield stress values [13]. The downward curves were used for regression analysis with the Newtonian, Herschel-Bulkley, Ostwald De-Waele, and Casson models to obtain parameters for inputting engineering calculations. The most suitable rheological model for describing Maksym flow was the Casson model. The calculated rheological model constants are presented in Table 1.

Table 1. *The rheological constants of Maksym according to Casson model (n=0.5)*

Days	T (°C)	τ_0 (Pa)	η_{Ca} Pa ·s	R^2	SD	A (Pa/s)	at shear rate of 50 s^{-1}
7	5	0.134	0.115	0.9996	0.01	11.30	0.028
	10	0.110	0.103	0.9999	0.01	4.63	0.022
	20	0.085	0.087	0.9999	0.00	2.54	0.016
	30	0.080	0.071	0.9988	0.00	0.45	0.016
14	5	0.113	0.096	0.9999	0.00	3.96	0.021
	10	0.077	0.090	1.0000	0.00	2.21	0.017
	20	0.058	0.077	1.0000	0.00	0.81	0.012
	30	0.049	0.068	0.9999	0.00	1.08	0.010
21	5	0.089	0.094	0.9995	0.00	3.30	0.019
	10	0.068	0.087	0.9999	0.00	0.39	0.015
	20	0.057	0.076	0.9999	0.00	0.29	0.012
	30	0.054	0.071	0.9989	0.00	1.95	0.011

* Mean values are given of three independent measurements; standard deviation is SD < 0.01.

Since the investigated samples have a small but measurable yield stress, defined as the minimum shear stress that must be applied to the material to initiate flow, mathematical models such as the Casson and Herschel-Bulkley models can be used and in terms of the correlation coefficient R^2, as well as the most suitable models of the dependence of shear stress on shear rate, the Casson and Herschel-Bulkley models outperformed other rheological models. However, the Casson model was superior to the Herschel-Bulkley model.

For the calculation of effective viscosity, the shear rate value was taken to be 50 s^{-1} as a mean of typical shear rate ranges under processing conditions such as stirring and swallowing beverages.

The values of effective viscosity and the yield stress (τ_0) measured at 5°C decreased from 0.028 to 0.019 Pa·s and 0.13 to 0.09 Pa, respectively. as the storage time increased from 7 to 21 days of storage. The pattern of decreasing rheological parameters with increasing storage time suggests complex carbohydrates such as starch. dextrin, etc., may hydrolyse as the yeast grows. Thus, the storage stability of the colloidal suspension will decrease. However, ANOVA analysis revealed that there is no significant difference in Casson's viscosity coefficient (η_{Ca}), yield stress values (τ_0), and effective viscosity (η_{eff}) of beverage Maksym within the storage time.

The values of yield stress (τ_0), effective viscosity (η_{eff}) and hysteresis area (A) of the samples decreased significantly with increasing temperature. ANOVA analysis also showed that these values of Maksym samples were significantly different ($p < 0.05$) when comparing temperatures at 5 and 30°C and differences are not statistically significant at 10 and 20°C. An Arrhenius-type equation (Eq. 4 and 5) was used to calculate flow activation energies for Maksym. The results of the calculation revealed that the activation energies of samples were 23.10; 19.92 and 14.94 kJ/mol at 7, 14, 21 days of storage, respectively. Correlation coefficients ranged from 0.973 to 0.992. The activation energy Ea is the energy barrier that must be overcome before the flow process is initiated [14]. Therefore, the decrease of activation energy during storage indicated the thinning of viscosity of the beverages Maksym. The activation energy of another Kyrgyz ethnic fermented beverage Bozo was found as 9.50 and 12.62 kJ/mol at 7 and 21 days of storage, respectively [10]. The activation energies of ghee samples stored at room and refrigerator temperatures were 26.3 kJ/mol and 29.9 kJ/mol [27], respectively, and the activation energy of Süzmö was 61.66 kJ/mol [28]. Lower activation energies (3.03 – 10.53 kJ/mol) at 5 – 65°C and 4 – 14°Brix were obtained for Josapine pineapple juice by [29]. Similar results for a malt wort of 21.17 kJ/mol and higher values for a hopped wort of 42.8 kJ/mol at 5 – 40°C were reported [30].

2.4. Sensory properties of beverage Maksym

Fig. 6 shows the sensory evaluation of the Maksym on different days of storage. In terms of mouthfeel, aroma, flavour, acidic taste, and overall acceptability after 7 days of storage, the panellists gave the highest scores. However, after 14 and 21 days of storage, the beverage was scored much lower in all parameters. Acidic taste after 21 days of storage received the lowest rating. This observation is also consistent with the titratable acidity and pH analysis. These changes are due to the continuation of lactic acid and alcoholic fermentation, resulting in an increase in the content of lactic acid and ethyl alcohol, as well as carbon dioxide [31]. Since storage at refrigerator temperature does not stop the microbiological activity, the accumulation of metabolic by-products will continue.

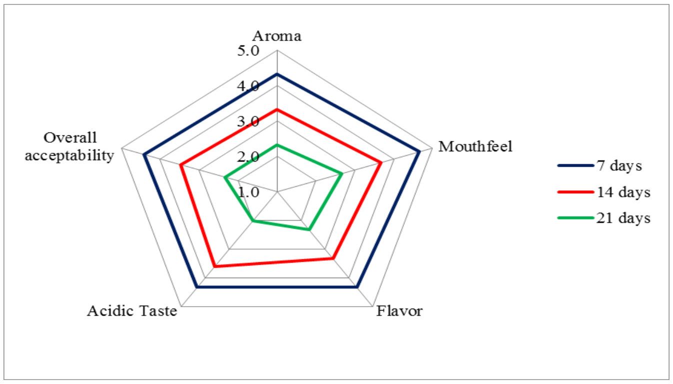

Figure 6. *Sensory evaluation of Maksym at different storage days.*

3. Conclusion

In this study, for the first time, the chemical, rheological and sensory properties of Kyrgyz ethnic cereal beverage Maksym were studied during storage. Research shows that there is an increase in acidity and a decrease in the total amount of soluble solids. The flow characteristics of Maksym during storage in the refrigerator also changed, which may be due to the hydrolysis of polysaccharides by LAB and yeast cells in the sample. Namely, Maksym's effective viscosity, the yield stress (τ_0), and Casson's coefficient of viscosity (η_{Ca}) decreased after 14 days of storage. However, these changes are statistically insignificant. Sensory properties investigated after 21 days of storage received the lowest score. Microbiological investigations have shown, that the number of lactic acid bacteria (LAB) and yeast after production were 9.46 and 9.36 log cfu/mL, respectively. After 21 days the viability of yeast cells at the end of the shelf life period was slightly higher (78 %), compared to LAB (75%). Thus, the shelf life of beverage Maksym after opening can be stated as 14 days. These rheological and sensory parameters provide food manufacturers with useful information on post-production storage parameters. Also, consumers can use the information to declare the quality of Maksym during refrigerated storage.

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