Potravinarstvo Slovak Journal of Food Sciences

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 15, 2021, p. 732-740
https://doi.org/10.5219/1679
Received: 37 July 2021. Accepted: 25 August 2021.
Available online: 28 September 2021 at www.potravinarstvo.com

ISSN 1337-0960 (online)

CHANGES IN ORGANOLEPTIC, MICROBIOLOGICAL AND BIOCHEMICAL PROPERTIES OF KEFIR WITH IODINE ADDITION DURING THE STORAGE

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ABSTRACT
Iodine is a vital trace element that must be constantly and daily supplied with food to the organism. Currently, the amount of food that can provide the organism with the required amount of iodine is insufficient. The purpose of the study was to investigate changes in organoleptic, microbiological, and biochemical parameters of kefir made with the addition of iodine during its refrigeration storage. It was found that during the storage of samples of kefir with iodine there is a slowdown in the reproduction of lactic acid bacteria, compared with the control sample. In particular, the number of lactic acid bacteria during the first two days of storage increased 1.3 times in the experimental sample and 1.5 times in the control sample of kefir. After 12 days of storage, the number of lactobacilli in the test sample of kefir increased 2.5 times, and in the control 3.2 times, compared with the amount in fresh kefir. Similar patterns were observed in determining the development of yeast. In particular, the reproduction rate of yeast in the control sample of kefir was, on average, 1.3 times faster (8 – 12 days), compared with yeast in the experimental sample. It was also found that during the 12-day storage period at a temperature of +6 °C, the titrated acidity in kefir with iodine increased 1.4 times, and in the control 1.6 times and was 130.5 °T and 154.1 °T, respectively. At such acidity values, the test sample of kefir still met the requirements of the standard, and the control was 24.1 °T higher. In this case, kefir containing iodide had better organoleptic characteristics during 12 days of storage. Produced kefir with the addition of iodine can be considered a functional product to provide the population with sufficient iodine.

Keywords: kefir; iodide concentrate; titrated acidity; lactic acid microorganisms; storage temperature

INTRODUCTION
Iodine is a vital trace element that must enter the organism constantly and daily with food **(Hynes, et al., 2013; Abel, et al., 2017)**. Iodine is the most important component in the formation of thyroid hormones: thyroxine and triiodothyronine, which in turn regulate metabolism and several vital functions of the organism **(Kopchak, et al., 2018a)**. At insignificant earnings of iodine in an organism, adaptive processes directed on effective use of this element work are triggered. However, prolonged lack of iodine leads to iodine deficiency diseases, accompanied by various pathologies **(Abel, et al., 2017; Kopchak and Pokotylo, 2018b)**. The problem of iodine deficiency is especially relevant for regions that are far from the sea and have mountainous relief **(Ryzhkova, et al., 2017)**. Ukraine is an iodine-deficient country with varying degrees of deficit, depending on the region.

The main natural source of iodine is seafood. However, according to research **(Nerhus, et al., 2018)**, the iodine content in marine fish ranged significantly from 18 μg/100g of wet mass (Atlantic halibut) to 1210 μg/100g (pollock). There were significant differences between individuals within one species and between fish of one species from different geographical areas. For example, Atlantic cod from the North Sea had lower concentrations of iodine than cod from the Barents or Norwegian seas. It is reported that even in such developed marine countries as Norway, Finland, Iceland, England, fish an,d fish products provide only 20-30% of the daily requirement of iodine **(Dahl, et al., 2004; Bath, et al., 2013; Nystrom, et al., 2016)**. The main products in these countries that provide the organism with iodine include dairy - 20 – 64% of demand and iodized salt **(Rasmussen, et al., 2008; Van der Reijden, et al., 2017; Nerhus, et al., 2018)**.

Researchers **(Nerhus, et al., 2018)** indicate that in Norway, the average concentration of iodine in milk ranged from 12 to 19 μg/100 g, and the concentration of iodine in eggs ranged from 23 to 43 μg/100 g. In Ukraine, the average iodine content in milk was 9.3 µg/100 g **(Mikláš, et al., 2021)**. In determining iodine in dairy products **(Haldimann, et al., 2005)** was found that the highest concentrations were in serum with a concentration of 100 to 450 µg/100 g. It was also found that during cheese production, approximately 75% of iodine from milk enters the whey, about 25% remains in the cheese **(Van der Reijden, et al., 2019)**. At the same time, it was found that heat processing does not affect the concentration of iodine in milk **(Wheeler, et al., 1983; Norouzian, et al., 2011; Soriguer, et al., 2011)** whereas defatting increased the amount of iodine in milk by only 1 – 2 µg/dm^3 **(Jahreis, et al., 2007; Rasmussen, et al., 2014)**. In semi-hard and soft cheeses, iodine was detected in the range of 83 – 101 µg/kg, in yogurt – 156 – 169 µg/kg **(Haldimann, et al., 2005; FCN, 2013)**. However, researchers believe that the concentration of iodine in milk can reach up to 300 µg/dm^3 with an additional introduction into the ratio of cows **(Van der Reijden, et al., 2019)**. Even though dairy products supplement the human ratio with iodine, its concentration is rarely determined and is not always taken into account for the prevention of iodine deficiency.

For a long time, many countries around the world have tried to solve the problem of iodine in the daily ratio by providing the population with iodized salt containing the usual 25 g of potassium iodide per 1 ton of salt. However, potassium iodide (KJ) has now been found to be unstable to light and moisture **(WHO, 2007)**. In addition, the use of inorganic compounds (KJ, KJO_3) in iodine enrichment of rations, from the point of view of the theory of functional nutrition is not perspective, because they are a chemical unnatural compound and cannot be considered a source of functional ingredients. The best way to provide the population with stable iodine is the use in iodine-deficient regions of organic iodine compounds – iodine-casein, iodine-active, "Iodis concentrate", seafood – natural sources of iodine, in particular, sea fish, mussels, shrimp, squid and, especially, brown seaweed and products of their processing (salads, jams, pasta).

One of the well-recommended products with biologically active iodine is "Iodis-concentrate" **(Story, et al., 2016; Mikláš, et al., 2021)**. "Jodis-concentrate" is mineral artesian water with iodine, stable during storage and heat processing, recommended for use in the food industry for iodine enrichment of water, bakery products, meat, dairy, and sour-milk products **(Kopchak, et al., 2017; Mikláš, et al., 2021)**.

Because Ukraine does not have enough functional food products that can provide consumers with the necessary level of iodine. The use of fermented milk products as a basis for the introduction of biological iodine-containing preparations is quite real and perspective. In addition, such products. as kefir is considered to be often used because, in addition to specific taste qualities, they provide the organism with useful lactic acid microflora **(Kukhtyn, et al., 2018a)**.

Therefore, given the urgency of the problem and the literature, it is currently promising to create functional dairy products with biologically active iodine, which would allow its use for prophylactic purposes to prevent thyroid dysfunction.

The purpose of the work was to investigate changes in organoleptic, microbiological, and biochemical parameters of kefir produced with the addition of iodine during its refrigeration storage.

Scientific hypothesis. It consists of the possible production of iodized fermented milk products, which are enriched with the help of water "Iodis-concentrate".

MATERIAL AND METHODOLOGY

The work was carried out at the Ternopil Ivan Puliuj National Technical University in the laboratories of the Department of Food Biotechnology and Chemistry.

Samples

10 samples of kefir with the content of "Jodis-concentrate" were investigated - experimental samples and 5 samples of kefir were in control (without "Jodis-concentrate").

Chemicals

Kefir leaven (Good food, Italia) was used to make kefir, and Jodis-Concentrate solution (Yark-Kyiv, Ukraine) was used as a source of iodine.

Artesian mineral water "Jodis-concentrate" of the following chemical content (Table 1) **(TC, 2001)** was used in the experiment.

Before the conducting of the study the kefir samples had been mixed for one minute with a sterile spatula, heated in a water bath at +37 ±1 ° C, and prepared ten-fold dilutions according to the standard (**DSTU, 2003**).

Determination of the number of lactic acid microorganisms in experimental and control samples of kefir was carried out on *MRS-agar* medium (HiMedia, India) for this purpose, the sowings were incubated at a temperature of 37 ±1 °C for 48 hours. Yeast and molds were determined on Saburo medium (HiMedia, India) at a temperature of 25 ±1 °C for 3-5 days. The titer of coliform bacteria was determined in Kessler medium (Pharmactive, Ukraine) incubation of crops at a temperature of 37 ±1 °C for 24 hours. Determination of Staphylococcus aureus in kefir was carried out on Baird-Parker agar medium (Merck KGaA, Germany).

Laboratory Methods

The titrated acidity of milk and kefir was determined by the classical titrimetric method (**IS 2004**). Chemically pure KOH was used for titration (Khimreaktiv, Ukraine).

Description of the Experiment

Sample preparation: The process of making kefir with iodine included the following technological operations: 1) assessment of quality and safety of raw materials (microbiological and physicochemical research); 2) normalization and homogenization of the mixture for fat content up to 2.5% on the laboratory homogenizer (Microtron MB 800, Kinematica, Germany); 3) pasteurization at a temperature of 80 – 85 °C for 15 s on a mini pasteurizer (SP12, Motor Sich, Ukraine); 4) cooling to a temperature of 25 ±1 °C, adding "Jodis concentrate" in the amount of 7.5 mL per 1 liter of raw material; 5) making of kefir leaven and fermentation for 6 h to a titratable acidity of 85 – 90 °T; 6) cooling to +4 to +6 °C; 7) packing and storage.

Number of samples analyzed: This technology was used to make 10 samples of kefir with adding of "Iodis-concentrate" and 5 samples of kefir without adding of iodine (control). Each sample was divided into 5 parts and placed in a sterile container with a lid and stored in a refrigerator at

+4 – +6 °C for 12 days. The first part (freshly made kefir) was analyzed immediately after cooking, the second part was examined after 2 days of storage, the third – after 5 days of storage, the fourth – after 8 days, the fifth – after 12 days.

Number of experiment replication: Each portion of kefir was analyzed three times and subjected to statistical processing.

Number of experiment replication: A total of 15 samples of kefir were analyzed for microbiological, biochemical and organoleptic parame.

Statistical analysis. Statistical processing of the results was carried out using methods of variation statistics using the program Statistica 9.0 (StatSoft Inc., USA). Non-parametric methods of research were used (Wilcoxon-Mann-Whitney test). The arithmetic mean (x) and the standard error of the mean (SE) were determined. The difference between the comparable values was considered to be significant for $p < 0.05$.

RESULTS

At the first stage of the research, the chemical content of "Iodis-concentrate" was analyzed (Table 1) and it was found that 1 mL of solution contains 40.0 µg/mL of iodine. That is, to ensure the minimum daily requirement of an adult for iodine (200-300 mcg recommendation) (**WHO, 2007**), it is necessary to consume an average of 7.5 mL of "Iodis concentrate". This amount was calculated and kefir was made from cow's milk so that 1 liter contained at least 300 mcg of iodine.

Organoleptic, physicochemical, and microbiological indices were determined in freshly made kefir with iodine (experimental sample) and without it (control) for compliance with the requirements of the normative document (**DSTU, 2005**). According to organoleptic indices, the experimental and control samples of kefir had a homogeneous, viscous, with an intact clot, a semi-liquid without gas formation, appearance and consistency, and a clean, sour-milk taste and smell. According to physicochemical indices in the experimental sample, the value of titrated and active acidity was 93.2 ±0.2 °T and pH 4.6 ±0.03 units. In the control sample, the titrated acidity was 98.0 ±0.2 °T and pH 4.4 ±0.04 units. The results of microbiological research are given in table 2.

It is set (Table 2) that according to microbiological indicators freshly made samples of experimental and control kefir met the requirements provided in the standard. At the same time, it was found that the number of lactic acid bacteria in the experimental sample of kefir was 1.7 times ($p < 0.05$) less, compared with the control sample. The yeast content also in the experimental sample was 2.5 times ($p < 0.05$) less than in the control and was $2.4 \pm 0.1 \times 10^3$ CFU/g and $6.1 \pm 0.2 \times 10^3$ CFU/g, in accordance.

Therefore, freshly made samples of kefir met the requirements of the standard in organoleptic, physicochemical, and microbiological indices, despite the lower titratable acidity and lower content of lactic acid bacteria and yeast in the test sample of kefir.

Further research was sent on determining the influence of added "Jodis" on the dynamics of changes in lactic acid and yeast microflora during the storage of kefir at a temperature of + 6 ±0.1 °C for 12 days. The maximum allowed by the standard (+ 6°C) storage temperature was chosen for the more intensive reproduction of microorganisms. The results of the research of changes in lactic acid bacteria in kefir during storage are shown in Figure 1.

The classical dynamics of change of lactic acid bacteria in kefir samples was revealed (Figure 1), which was characterized by gradual growth of lactic acid microorganisms during the 12-day storage period at a temperature of +6 ±0.1 °C. At the same time, we observe a slowdown in the reproduction of lactic acid bacteria in the experimental sample of kefir, against the control. In particular, the number of lactic acid bacteria during the first two days of storage increased 1.3 times ($p < 0.05$) in the experimental sample and 1.5 times in a control sample of kefir.

After five days of storage, the number of lactic acid bacteria in kefir continued to increase. However, the intensity of reproduction of the microflora in the experimental sample was slightly lower than in the control sample of kefir, that is, the increase was 1.6 and 1.9 times ($p < 0.05$), in accordance, compared to the initial number.

The following definition of the number of lactic acid bacteria after 8 and 12 days of storage revealed a similar trend in the reproduction of the microflora as during the five-day studies. In particular, the number of lactobacilli after 12 days of storage in the test sample of kefir increased 2.5 times ($p < 0.05$), and in the control 3.2 times ($p < 0.05$), comparing with the amount in fresh kefir.

Data on the change of yeast in kefir containing "Jodis", compared with the control sample are shown in Figure 2.

It was found (Figure 2) that the presence of Jodis in the experimental sample of kefir influenced the intensity of yeast reproduction. In particular, during the two days of kefir storage, the amount of yeast increased on average 1.2 times in the experimental sample, against 1.3 times ($p < 0.05$) in the control. That is, there is a similar regularity as for the development of lactic acid bacteria. During further storage, the reproduction rate of yeast in the control sample of kefir was on average 1.3 times ($p < 0.05$) faster (8 – 12 days), compared with yeast in the experimental sample.

Therefore, the research indicates that "Jodis" added to the milk mixture for the production of kefir inhibits the development of lactic acid and yeast microflora in the product during storage.

The results of the research of the change in titratable acidity in kefir samples during storage are shown in Figure 3.

The results (Figure 3) indicate that the growth curve of the values of the titrated acidity had a regularity to the fastest increase in the control sample of kefir. In particular, during eight days of storage, the titrated acidity in the control sample of kefir increased 1.5 times ($p < 0.05$) and was 142.9 ±0.3 °T. This value of titrated acidity by 12.9 °T exceeded the permissible level (130 °T), which is normalized by the standard (**DSTU, 2005**). At the same time, in the experimental sample of kefir with "Jodis" the value of titrated acidity reached the level of 130 °T on the 12th day of storage.

Thus, in kefir with "Jodis" the dynamics of enzymatic processes were slower compared to the control sample. This gives reason to believe that it can be stored for a longer period without violating the requirements of the standard in terms of titrated acidity of the product.

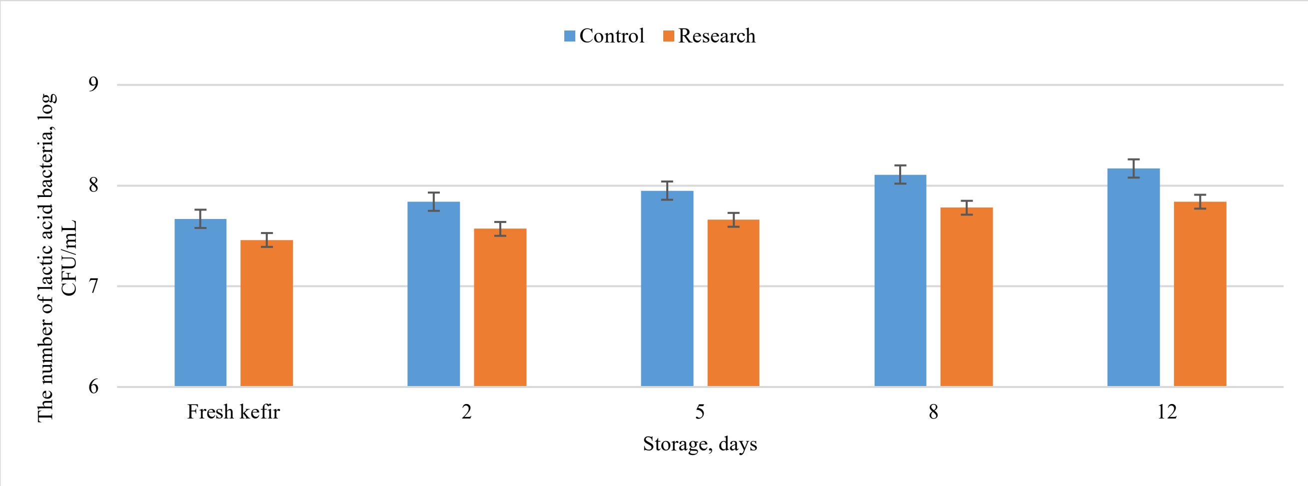

Figure 1 Dynamics of changes in the number of lactic acid bacteria in kefir containing "Iodis" during storage at a temperature of +6 ±0.1 °C.

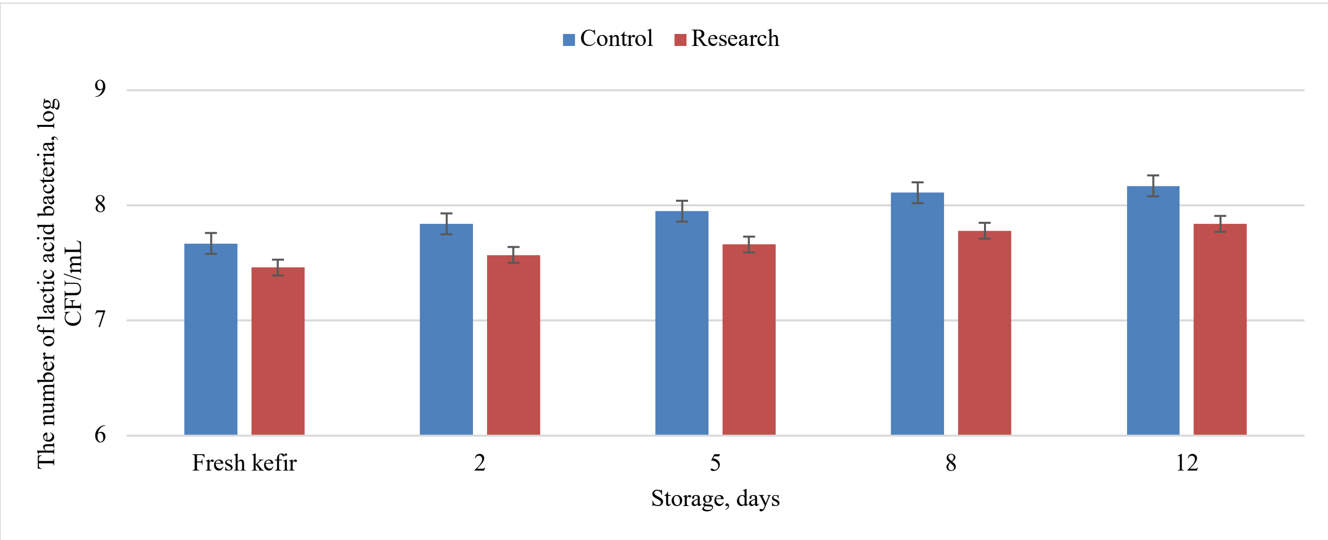

Figure 2 Dynamics of change in the amount of yeast in kefir containing "Jodis" during storage at a temperature of + 6 ±0.1 °C.

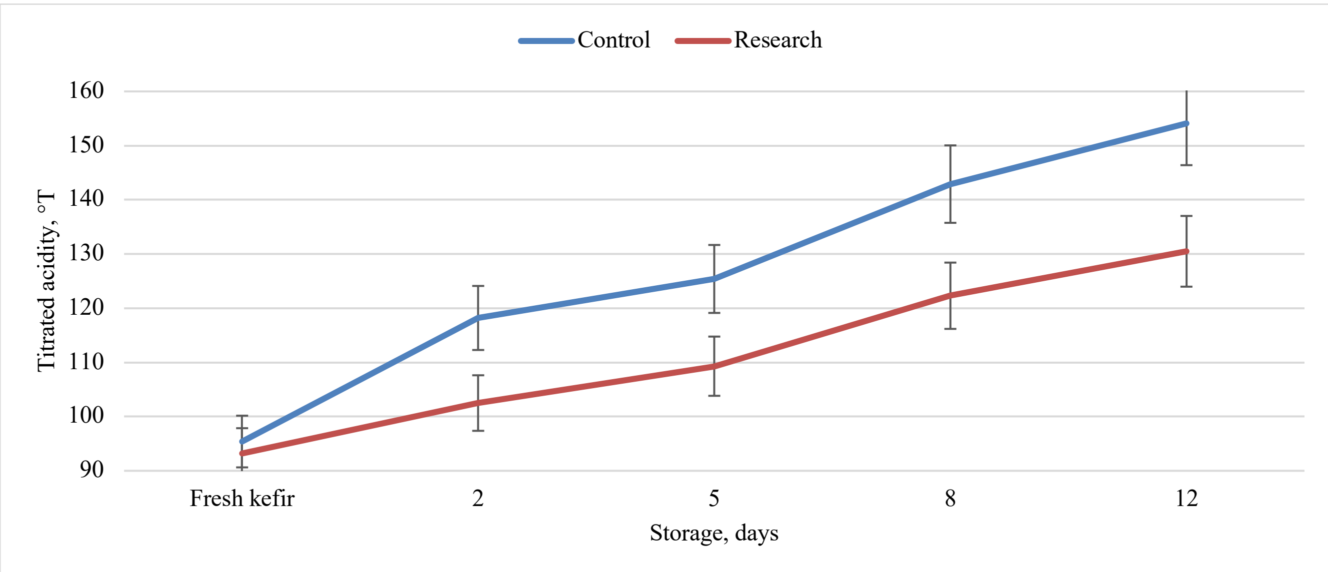

Figure 3 Dynamics of change of titrated acidity in kefir with the content of "Jodis" during storage at a temperature of +6 ±0.1 °C.

Table 1 Chemical composition of "Jodis concentrate".

Indicators	Number
Total mineralization, g/dm^3	0.4 – 0.8
Sodium + Potassium, mg/dm^3	10 – 100
Calcium, mg/dm^3	50 – 150
Magnesium, mg/dm^3	10 – 100
Chlorides, mg/dm^3	<50
Sulfates, mg/dm^3	<50
Hydrocarbonates, mg/dm^3	300 – 600
Iodine, $\mu g/cm^3$	40.0
Organic matter content, mg/dm^3	<30

Table 2 Microbiological indices of freshly made experimental and control samples of kefir.

Indicator	Search sample - kefir with iodine	Control - kefir	Permissible quantity according to DSTU 4417:2005
The number of lactic acid microorganisms, CFU/g	$2.8 \pm 0.2 \times 10^7$	$4.7 \pm 0.2 \times 10^7$	No less 1×10^7
Yeast, CFU/g	$2.4 \pm 0.1 \times 10^3$	$6.1 \pm 0.2 \times 10^3$	No less 1×10^3
The titer of coliform bacteria, g	>1	>1	Is not allowed 0,01
Molds, CFU/g	8.3 ±0.3	12.1 ±0.3	Not more 50
Staphylococcus aureus, g	>1	>1	Is not allowed 1.0
Salmonella spp., 25 g	Not found	Not found	Is not allowed 25 g

Table 3 Scale for organoleptic evaluation of kefir with "Jodis".

The name of the indicator	Characteristics of the indicator	Evaluation in points
Taste and smell (5 points)	Pure, sour milk. Taste without extraneous tastes and smells	5
	Pure, sour milk. The taste is pungent, without foreign tastes and odors	4
	Sour milk. The taste is excessively pungent, without extraneous flavors and odors	3
	Excessively sour milk (sour)	2
	Unexpressed taste or presence of extraneous flavors	1
Appearance and consistency (5 points)	Homogeneous, viscous, with intact clot, semi-liquid, without gas formation	5
	Homogeneous, viscous, with intact clot, with slight serum separation, semi-liquid, without gas formation	4
	Homogeneous, viscous, with some broken clot, with slight serum separation, semi-liquid, with some gas formation	3
	Homogeneous, with a broken clot and serum separation, liquid, with gas formation	2
	Homogeneous, with significant serum separation, liquid, with gas formation	1
Color (2 points)	Milky white, uniform throughout the mass	2
	Milk white, not uniform for the whole mass	1
General perception (3 points)	The harmonious combination of taste and smell	3
Overall maximum score		15

Table 4 Organoleptic evaluation of kefir containing "Jodis" during storage at a temperature of + 6 ±0.1 °C.

Product sample	Indicators	Storage for, days				
		fresh yogurt	2	5	8	12
Experimental kefir with "Jodis"	Taste and smell (5)	5.0	5.0	4.8	4.6	4.4
	Appearance and consistency (5)	5.0	5.0	5.0	5.0	5.0
	Color (2)	2.0	2.0	2.0	2.0	2.0
	General perception (3)	3.0	3.0	3.0	2.8	2.8
	The total number of points (15)	15.0	5.0	14.8	14.4	14.2
Control (kefir)	Taste and smell (5)	5.0	5.0	4.6	4.2	4.0
	Appearance and consistency (5)	5.0	5.0	5.0	5.0	4.8
	Color (2)	2.0	2.0	2.0	2.0	2.0
	General perception (3)	3.0	3.0	3.0	2.8	2.8
	Total Points (15)	15	15.0	14.4	14.0	13.6

It is known that the organoleptic properties of fermented milk products primarily depend on the quality of milk raw materials which undergo fermentation, introduced food additives, and the activity of fermentation crops. Therefore, the next part of the work was to conduct an organoleptic evaluation of kefir samples. However, before the tasting, a scale was developed to evaluate kefir samples (Table 3).

The scale for evaluating test samples of yogurt included the determination of taste and smell, which was evaluated with a maximum of 5 points; appearance and consistency - 5 points; color was evaluated at 2 points and general perception – 3 points.

According to the results of the tasting commission of kefir samples (Table 5), it was found that the shelf life of the product deteriorates its organoleptic properties. The decrease in the overall score of the experimental and control sample of kefir is mainly due to a defect in taste, in particular the appearance of a pungent sour-milk taste, which develops due to lactic acid fermentation. No exterior taste or odor was detected in the research sample. At the same time, in the research sample of kefir, a decrease in the total score by 0.2 points, compared with the initial amount was observed on the 5th day of storage. At the same time, in the control sample for this storage period, the decrease was 0.6 points. On the 12th day of kefir storage, the difference between the experimental and control samples was 0.6 points, with an overall score of 14.2 and 13.6 points, respectively.

Thus, the addition of 7.5 mL of "Iodis concentrate" per 1 liter of milk mixture for fermentation of kefir does not reduce the organoleptic characteristics of the produced product. In this case, kefir containing iodide had better organoleptic characteristics during 12 days of storage, compared with the control sample.

DISCUSSION

Iodine is a trace element whose daily requirement ranges from 100 to 300 μg/day, depending on age **(Hynes et al., 2013; Abel et al., 2017; Walther et al., 2018)**. Many countries, including Ukraine, are in the zone of endemic iodine deficiency **(Dahl et al., 2004; Bath et al., 2013; Nystrom et al., 2016; Ahvanooei, et al., 2021)**. Unfortunately, in Ukraine, there is a lack of food that can provide the organism with the required amount of iodine **(Ryzhkova et al., 2017; Kopchak and Pokotylo, 2018b)**. On the other hand, dairy products are a good basis for the production of many functional products **(Dal Bello et al., 2017; Kukhtyn et al., 2021; Albracht-Schulte et al., 2018; Yukalo et al., 2019; Lialyk et al., 2020)**. This research shows that the problem of iodine deficiency can be solved by producing high-quality kefir enriched with biological iodine - a solution of "Iodis-concentrate". In particular, it was found that freshly made kefir with iodide in organoleptic, physicochemical, and microbiological indicators met the requirements of the standard **(DSTU, 2005)**, despite 2.3 °T lower titratable acidity and 1.7 times lower content of lactic acid bacteria than in the control sample of kefir.

During the storage of samples of kefir with Jodis, a slowdown in the reproduction of lactic acid bacteria was detected compared to the control sample. In particular, the number of lactic acid bacteria during the first two days of storage increased 1.3 times in the experimental sample and 1.5 times in the control sample of kefir. After 12 days of storage, the number of lactobacilli in the test sample of kefir increased 2.5 times, and in the control 3.2 times, compared with the amount in fresh kefir. Analogical patterns were observed in determining the development of yeast. In particular, the rate of reproduction of yeast in the control sample of kefir was, on average, 1.3 times faster (8 – 12 days), comparing with yeast in the experimental sample. This gives reason to consider that "Jodis" added to the milk mixture for the production of kefir inhibits the development of lactic acid and yeast microflora during production technology and in the finished product during its storage. The presence of antibacterial action in iodine concentrate has been reported by other researchers **(Mikláš, et al., 2021)**. Despite the influence of added Jodis concentrate on the development of microflora in the technology of production and storage of kefir, the researchers **(TC, 2001; Mikláš, et al., 2021)** report that overdose and negative action on consumers from this preparation is not possible. It was also found that during the 12-day storage period at a temperature of +6 °C, the titrated acidity in kefir with iodine increased 1.4 times, and in the control 1.6 times and was 130.5 °T and 154.1 °T, respectively. For such values of acidity, the test sample of kefir still met the requirements of the standard **(DSTU, 2005)**, and the control was 24.1 °T higher. In general, we can confirm that in this case, iodine in kefir acts in part as a biological preservative, due to which it is extended the expiration date of the product. Thus kefir with iodine on organoleptic indicators exceeded 0.6 points of the test sample, generally because of less sour taste.

Thus, we agree with the data of researchers **(Haldimann, et al., 2005; Rasmussen, et al., 2008; Norouzian et al., 2011; Nerhus et al., 2018; Van der Reijden, et al., 2019)**, who recommend increasing the concentration of iodine in dairy products by introducing iodine into the ration of cows or immersion of teats in iodine-containing drugs (**Reijden et al., 2017; Walther et al., 2018; Ahvanooei, et al., 2021**). At the same time, we consider that the priority direction of overcoming iodine deficiency is the production of traditional fermented milk products enriched with biological iodine from natural sources. It is Jodis concentrate that belongs to such food additives **(Stori, et al., 2016; Kopchak & Pokotylo, 2018b; Mikláš, et al., 2021)**.

CONCLUSION

It was found that freshly produced kefir with iodide in organoleptic, physicochemical, and microbiological indicators met the requirements of the standard, despite 2.3 °T lower titratable acidity and 1.7 times lower content of lactic acid bacteria than in the control sample of kefir.

Therefore, the produced kefir with the addition of iodine was characterized by good microbiological, biochemical indicators, and organoleptic properties and can be considered a functional product to normalize the intake of stable iodine.

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