Acta Biotechnol. 6 (1986) 2, 167—174

Production, Characterization and Use of Expendable Kefir Starter Culture in the Kefir Production Process

KRAMKOWSKA, A., FESNAK, D., KORNACKI, K., BAUMAN, B.

Central Administration of Dairy Industry
Production Laboratory of Dairy Biopreparations
ul. Warszawska 111, 10-957 Olsztyn, P.O.B. 1, Poland

Summary

Upon sterilization, freeze-dried kefir grains are to be supplemented with yeast preparation, yeast showing the lowest survival in the course of freeze drying.
A mixture of 20% sucrose solution and starch appeared to be the most efficient dispersing agent to protect yeast in the freeze-drying process.
A starter which is produced with the kefir starter culture, does not differ in its microbiological, biochemical and organoleptic properties from a starter as obtained with kefir grains in the same time, such starter shows a better consistency.
The preparation of such starters is less laborious and less complicated than the preparation of starters using kefir grains.

Introduction

The interest for fermented milks is connected with the theory proposed by METCHNIKOV that the growth of the putrefactive bacteria is inhibited in the intestines by lactic acid bacteria. The recent findings of science have confirmed that those products are characterized by valuable dietetic properties which are reflected in their chemical, microbiological and vitamin composition, favourable for the human organism. The most utilized fermented milks are yoghurt and kefir [16, 17].
Kefir is obtained by the complexe lactic and alcoholic fermentation in milk. In its production, kefir grains are involved, which have the appearance of rice grains cooked to rags, of irregular shape, elastic to the touch [5, 8, 16].
The kefir grain microflora is no uniform culture, but it may contain various kinds of microorganisms, according to the cultural conditions. In general, three groups of microorganisms are found to occur: streptococci, bacilli and yeasts. They involve mesophilic acidifying bacteria, *Str. lactis, Str. cremoris*; aromaforming bacteria, *Str. diacetilactis* and *Leuconostoc*; *Lactobacilli, Lbc. caucasicus* and acetic bacteria [9]. Of yeast, spore-forming species are present, such as *Saccharomyces, Schizosaccharomyces*, which utilize lactose directly, as well as non-spore forming and non-lactose-fermenting yeasts of *Torula* species [8, 16].
In kefir production, the greatest difficulties are provided by the kefir grain cultivation. To obtain a product of proper quality, the following condition are to be observed: optimum temperature of grain cultivation must be maintained; milk must be changed

in grains every day, to maintain milk-grain ratio 1 : 15 to 1 : 20; overacidity is to be avoided and extreme caution is to be applied to prevent infection. The studies on troubles of kefir grain cultivation permitted the cases of their occurance to be partially determined. A cause of a strong gas formation was found to be the overgrowth of *Leuconostoc dextranicum* bacteria and yeasts. The presence of foreign yeast strains may result in fruit flavour and an excessive growth of acetic bacteria may lead to slime in grains. Slimy grains lose their characteristic elasticity and a mealy bloom is formed on their surface. That defect may be caused by the mould *Geotrichum candidum*. Grains are deacidified by the mould growing on them and they become suspectible to the action of putrefactive bacteria. On industrial conditions, the Coli bacteria infection may be caused by ineffective pasteurization of starter milk or by reinfection, the growth of those bacteria cause an unclear flavour and excessive gas formation. Such troubles as poor acidification or whey secretion are often caused by chemical and physical changes in milk [1].

The observation of all principles for kefir grain cultivation requires diligence, some length of time, appropriate laboratory conditions and a working staff properly skilled.

In addition to the above inconveniences, the use of kefir grains appear to be troublesome because of the short time of expiration. In viable form, the kefir grains are active when just produced and they conserve their keeping quality for 5 days at ambient temperature. Unfavourable transport conditions or exceeded expiration time (5 days) cause the kefir grains to lose their activity [7].

The above inconveniences in kefir grains cultivation impelled the studies to be undertaken, intending to produce a starter culture which would eliminate the kefir grains cultivation in dairy plants. These attempts resulted in a freeze-dried kefir preparation being obtained which would replace the kefir grain starter, as prepared by traditional procedure.

The first satisfactory results enabled the continuation of studies on the freeze drying possibility of kefir starter cultures [10]. The greatest difficulties were provided by a low survival of yeasts in the course of stabilization [4]. Hence it appeared to be necessary for yeasts, to be propagated and stabilized separately and then to be added in such form, to the freeze-dried kefir grains in which a high percentage of yeast had been removed.

Experimental and Methods

Preparation of Kefir Grains for Stabilization

The principle of kefir grains preparation consists in combining the duly prepared kefir with viable kefir grains. Kefir was obtained from sterile milk, inoculated with 3% of kefir starter, as produced from a kefir grain culture, and then incubated at 23 °C for 20 hours. Twice during incubation, the kefir was neutralized with sodium hydroxide up to the acidity of 8—10 °SH. Viable kefir grains were mixed with kefir and then 18% lactose solution was added. When preparing the mixture, the following ratio of grains, starter and lactose was observed: 1 : 1 : 0.5. The final acidity of ready-made preparation was 16—20 °SH.

Preparation of Kefir Yeasts

A superficial cultivation of yeasts, as isolated from kefir grains was run on a wort-agar medium at 23 °C for 48—72 hours. Superficially grown yeasts were rinsed with sterile 20% sucrose solution and mixed with sterilized starch.

Control of Preparations before Stabilization

In the kefir grain mixture, quantitative percentage of the following groups of microorganisms was determined:

- acidifying mesophile streptococci, on the medium according DEMETER [8],
- aroma-forming mesophile streptococci, on the NICKELS-LEESMENT (N-L) medium [14],
- lactobacilli, on MRS medium [3],
- yeasts, on the wort-agar medium [8].

In kefir yeasts preparation, counts of yeast germs in 1 ml were determined [8].

Stabilization of Preparations Obtained

Kefir starter culture obtained was stabilized using two procedures, freezing and freeze-drying:

- freezing of the preparation in an alcohol bath, type CD-165, of the Heto Company (Denmark) at temperature −50 °C, and then hardening at −40 °C for 6—8 hours,
- freeze-drying — frozen preparations were freeze-dried using a Type CD-155 freeze-dryer, produced by the Heto Company (Denmark), in vacuum of the order of 10^{-2} for about 24 hours. Final drying temperature did not exceed 40 °C.

Control of Dry Preparation

Dried kefir grain and yeast preparations were desintegrated in a grinder and then the following determinations were made:

- microbiological purity — presence of foreign microflora, including Coli-bacteria [8],
- presence of various groups of microorganisms as under „Control of Preparations before Stabilization",
- milk coagulation time, when inoculated with kefir grain preparation at 23 °C,
- organoleptic properties of milk, pasteurized at 95 °C/30 min. inoculated with the kefir grain preparation and incubated at 23 °C for 20—24 hours.

Standardization of Ready-Made Product

For producing kefir starter culture, to be used for inoculation of 3 l milk of kefir (mother) starter:

300 mg of kefir grain preparation,
300—600 mg of yeast preparation,
300—600 mg of complement

were used, depending on counts of microorganisms.

Quality and Keeping Quality Testing of Freeze Dried Kefir Starter Culture

The quality of starter cultures was checked twice during storage. Counts of microorganisms were tested on media, as under „Control of Preparations before Stabilization". Simultaneously, milk was inoculated with a freeze-dried starter culture, previously pasteurized at 95 °C for 30 min. and incubated at 23 °C for 20—24 hours. Parallelly, milk was inoculated with a traditional kefir starter. In starters obtained, fermentation and biochemical properties were compared. The assays involved:

- milk coagulation time at 23 °C,
- titrable and active acidity,
- fermentation activity, using TTT-titrator of the Radiometer Company (Denmark), pH changes of milk being tape-recorded during 24 hours incubation at 23 °C,

— organoleptic examination,
— acetyl producing ability, according to the PIEN's method [15],
— joint acetoin and diacetyl producing ability, by the method of BRANDL [2],
— protein degrading ability, up to N-alpha-amino acids, by the method of LEE and TAKAHASHI [12],
— volatile fatty acid content (VFT), by the method of HEMPENIUS and LISKA [6],
— acetaldehyde production, by the method of LINDSAY and DAY [13],
— ethanol and acetone production, by gas chromatography, using a gas chromatograph of Pay-Unicam Company [England).

Results and Discussion

The studies were intended to obtain an expendable kefir starter culture which could be substituted for the traditional kefir grain cultivation, and then to compare its properties with those of kefir grains. The manufacturing conditions were chosen in that manner, that, on vivifying a kefir starter preparation, a starter could be obtained, comparable with kefir starter, as produced from the traditional kefir grain cultivation. To this end, the quantitative composition of individual groups of microorganisms was determined in the preparation before and after freeze-drying, as well as in starters obtained from the dried preparation. Based on the results of these assays, the survival of acidifying bacteria, aroma forming bacteria, lactobacilli and yeasts was found to be 50%, 44.7%, 16% and 5%, respectively (Table 1).

Table 1. Survival of the microflora in the kefir grain preparation and yeast preparation in stabilization process

Stabilization procedure	Acidifying bacteria germs in 1 ml	Survival [%]	Aroma forming bacteria germs in 1 ml	Survival [%]	Lacto-bacilli germs in 1 ml	Survival [%]	Yeast germs in 1 ml	Survival [%]
Liquid	4.6×10^7	—	2.9×10^7	—	6.9×10^7	—	8.1×10^4	—
Frozen	3.3×10^7	73.9	2.3×10^7	79.3	1.2×10^7	17.4	1.2×10^4	14.8
Freeze-dried	2.3×10^7	50.0	1.3×10^7	44.7	1.1×10^7	16.0	4.2×10^3	5.1

Good survival results were obtained for mesophile bacteria strains in the course of freezing process. The survival of acidifying bacteria and aroma forming bacteria was 73.9% and 79.3%, respectively. The highest losses during freezing were observed for yeasts (85.2%) and *lactobacilli* (82.6%). A low survival of yeasts during stabilization is probably caused by the fact that yeast germs are larger than those of bacteria, and therefore they are more exposed to low temperature in the course of freezing. Ice crystals are formed which destroy cell walls of yeasts [4].
In this connection, it appeared to be necessary, to supplement the kefir grain preparation with yeasts, in the amount which would be sufficient to compensate losses occurring in the course of stabilization. Satisfactory results were obtained in the course of freezing and storage, when rinsing the surface growth of yeasts on wort-agar with 20% sucrose solution. The yeast rinsings were combined with sterilized starch to obtain the consistency of thick pulp. Kefir grain and yeast preparations were produced in triplicate, to prepare 3 variants of kefir starter cultures. The starter cultures were stored at +5 °C for a period of 3 months. Results of assays, just produced and after 6 weeks of storage, are presented in Table 2.

Table 2. Changes of microbial counts in the kefir starter culture during 6 weeks of storage at +5 °C

Starter cultures (variants)	Acidifying bacteria germs in 1 g of d.m.		Aroma forming bacteria germs in 1 g of d. m.		Lactobacilli germs in 1 g of d. m.		Yeasts germs in 1 g of d. m.	
	a	b	a	b	a	b	a	b
I	3.2×10^7	1.3×10^7	1.0×10^7	6.0×10^6	2.5×10^8	1.5×10^8	9.8×10^4	4.8×10^4
II	3.8×10^7	7.2×10^6	4.0×10^6	2.9×10^6	7.5×10^8	7.9×10^7	3.6×10^5	9.6×10^4
III	2.6×10^7	9.0×10^6	5.2×10^6	2.5×10^6	6.4×10^7	1.5×10^7	2.5×10^4	4.0×10^3

a — Microbial counts after production
b — Microbial counts after 6 weeks storage

As it appears from the counts obtained, the average germ counts of organisms decreased, in all groups, by one power during storage, but they were still comparable to the respective microbial counts, as present in the microflora of viable kefir grains.
In the same time, biochemical and fermentation properties as well as organoleptic characteristics of kefir starter cultures were estimated and compared with those of the control starter (Table 3).

Table 3. Estimation of biochemical properties of starters as produced with starter cultures

Starter culture (variants)	pH		Acetoin [mg/l]		Diacetyl. [mg/l]		Amino acid nitrogen [mg/l]		Acetaldehyde [mg/l]		VFA — ml 0.1n NaOH/100 ml sample	
	a	b	a	b	a	b	a	b	a	b	a	b
I	4.71	4.53	8.96	244.4	1.74	0.595	43.26	42.5	0.34	0.21	7.8	7.8
II	4.73	4.44	18.20	259.4	0.95	0.863	32.05	44.2	0.39	0.29	9.4	9.4
III	4.72	4.45	16.10	245.6	1.23	0.943	46.29	41.8	0.28	0.26	9.6	8.8
IV*	4.41	4.35	38.5	211.0	0.56	0.949	63.49	45.7	0.33	0.34	8.6	8.4

a — just produced
b — after 6 weeks of storage
* — control starter, as produced with a traditional kefir grain culture

The analysis of biochemical properties, made in duplicate, showed only slight differences between starters, as produced from starter cultures, and those prepared by traditional procedure.
The presence of volatile fatty acids (VFA) is the most apparent of biochemical characteristics of kefir studies [11]. When just prepared, the VFA content of starter cultures ranged between 7.8—9.6 ml 0.1 n NaOH/100 ml in test samples and 8.6 ml 0.1 n NaOH in a control sample. After storage, the respective amounts were 7.8—9.4 ml 0.1 n NaOH/100 ml and 8.4 ml 0.1 n NaOH/100 of sample.
The test samples were characterized by producing aroma substances at the middle level. Upon producing, the acetoin content of starter cultures was low and it ranged from 8.96 to 18.2 mg/l in test samples, as compared to 38.5 mg/l in control sample. After storage, those amounts increased to 244.4—259.4 mg/l and 211 mg/l, respectively. The diacetyl content was higher upon production and it was at the level of 0.95 to 1.74 mg/l in test samples and 0.56 mg/l in a control sample. After storage, the amount

of that compound decreased to 0.595—0.943 mg/l in test samples, and it was 0.949 mg/l in the control sample. These changes may be only apparent, because assays were carried out with fresh bulk milk, the chemical composition of which varies depending on the way of feeding and the season [9]. It may be stated that both after production and after storage, there are no essential differences between test samples and the control sample in producing aroma substances.

In samples assayed, a low but even content of acetaldehyde was found, at the level of 0.28—0.34 mg/l, and 0.21—0.34 mg/l after storage. That amount provides a proper test of kefir.

The kefir starter culture may be used in a frozen or freezedried form. In view of more convenient storage and lower transport costs, freeze-dried starter cultures are exclusively used in the dairy industry.

Biochemical properties of starters and kefir, as produced with freeze-dried and frozen starter cultures are compared in Table 4.

Table 4. Characterization of biochemical properties of kefir starter cultures and kefir as produced with them

Starter culture (variant)	pH	Acetoin [mg/l]	Diacetyl [mg/l]	VFA — ml 0.1 n NaOH/100 ml sample	Ethanol [mg/l]	Acetone [mg/l]
I — freeze-dried	4.44	259.0	0.863	9.4	17.5	6.5
II* — freeze-dried	4.30	310.0	0.675	9.8	11.8	3.7
II — frozen	4.49	254.0	0.280	11.8	18.8	4.3
II* — frozen	4.31	266.0	0.650	11.6	23.8	3.3
III — control	4.35	211.0	0.949	7.6	45.2	7.4
III* — control	4.33	245.0	0.836	14.2	17.9	3.4

* — kefir produced on the first starter — kefir

The alcoholic fermentation is a characteristic property of kefir grain microflora. When obtained with a freeze-dried starter culture, the kefir starter was characterized by the ethanol content of 0.17%, while the ready-made kefir contained 0.11% of that compound. When obtained with a frozen starter culture, the starter and kefir contained 0.18% and 0.23% of ethanol, respectively.

The highest ethanol content was shown in the starter as produced with the control sample — 0.45%, while the kefir produced by means of that starter contained 0.17% ethanol. These results were in agreement with the findings of KOROVKINA [11] who reported, following other authors, that the ethanol content of one day kefir was 0.12%. She informs, too, that the best organoleptic characteristics were found in kefir and kefir starter which contained 0.16—0.21% and 0.18—0.40% ethanol, respectively.

In these studies, kefir starters were obtained in which free fatty acid contents were 9.4 and 11.8 ml 0.1 n NaOH/100 ml of a sample, with dry and frozen preparation, respectively, as compared with 7.6 ml 0.1 n NaOH/100 ml for a control sample. For kefir, as obtained with those starters, the respective amounts were: 9.8, 11.6, and 14.2 ml 0.1 n NaOH/100 ml of sample, respectively. The product studies showed diacetyl and acetoin content which ranged between 0.280—0.949 ml/l and 211.04—310 mg/l, respectively.

The results of these studies showed, that the kefir starter culture, both in frozen and freeze-dried form, enables to obtain a kefir which does not differ, by its test properties, biochemical characteristics and composition of individual groups of microflora, from the product, as obtained by traditional method (Table 5).

Table 5. Characterization of fermentation properties and organoleptic features of kefir

Starter culture (variant)		Coagulation time [h]	VP test	Organoleptic characteristics	Microscopic picture
I	— freeze-dried	4.90	22	+	Moderately acid, clean, cream consistency, effervescent, sl. refreshing, flavour of kefir grains	Short chains of streptococci, 2—3 germs of yeast per several fields of view, few lactobacilli
I*	— freeze-dried	4.78	16	+	Acid, clean, cream consistency, effervescent, sl. refreshing, aromatic flavour of kefir grains	
II	— frozen	4.49	16	+	Moderately acid, cream consistency, sl. refreshing, effervescent, flavour of kefir grains	Short chains of streptococci, diplococci, 2—3 germs of yeast per several fields of view, 1—2 lactobacilli per several fields of view
II*	— frozen	4.31	16	+	Acid, clean, cream consistency, effervescent, sl. refreshing flavour of kefir grains	
III	— control	3.68	20	+	Acid, thick cream consistency, effervescent, refreshing, flavour of kefir grains	Streptococci in form of diplococci or short chains, 2—3 germs of yeast per several fields of view
III*	— control	3.90	16		Acid, clean, regular consistency, less viscid, sl. effervescent, flavour of kefir grains	

* — kefir produced on the first starter — kefir

On commercial conditions, the use of kefir starter in frozen form would be possible, provided that convenient containers would be available for production and low temperature could be assured during transport and storage. Starter cultures in dry form do not require any special caution or protection, no changes occurring in their propertie, transport or storage.

When produced with the expendable starter culture, the kefir shows a great reproducibility of properties. It is much more appreciated for its homogeneous, viscid consistency, no tendency for delamination and more pronounced, refreshing taste. When following instructions, the starter culture ensures an active bulk starter to be obtained in commercial conditions.

Because of being less laborious and less complicated, the bulk starter preparation using the expendable starter culture is decidedly preferable to the traditional starter, and those advantages speak for using those starter culture for kefir production.

The Use of Starter Cultures

From one portion of starter culture, 3 litres of mother starter may beprepared. That starter may be used to inoculate milk for kefir preparation, or to prepare a bulk starter in dairy plants in which larger amounts of kefir are produced.
To inoculate milk for making kefir or bulk starter, 3% of inoculum is used.

Received May 3, 1984

References

[1] Bawina, N. A., Rozkowa, J.: Mołocznaja Prom. 4 (1974), 18.
[2] Brandl, E.: Milchwissenschaft Ber. 4 (1960), 289.
[3] Burbianka, M., Pliszka, A.: Mikrobiologia żywności (1971).
[4] Górzyńska, J.: Przemysł fermentacyjny i rolny. 11 (1971) 7.
[5] Hattowska, H.: Mikrobiologia mleczarstwa (1964).
[6] Hempenius, W., Liska, B.: J. Dairy Sci. 51 (1966), 221.
[7] Instrukcja stosowania szczepionki jednorazowego użycia (1980).
[8] Karnicka, H., Knaut, T.: Mikrobiologia ogólna (1964).
[9] Karolewa, N. S.: Techniczeskaja mikrobiołogia celnomołocznych produktow. 126 (1975).
[10] Kornacki, K., Kramkowska, A., Fesnak, D., Bauman, B.: Przegląd mleczarski. 4 (1980),13.
[11] Korowkina, Ł. N., Patkul, T. M.: Mołocznaja Prom. 6 (1976), 18.
[12] Lee, P., Takahashi: Annal. Biochem. 14 (1966), 71.
[13] Lindsay, B., Day, A.: J. Dairy Sci. 48 (1965), 665.
[14] Nickels, W. E., Leesment, H.: Milchwissenschaft. 19 (1964), 374.
[15] Pien, J.: Lait. 27 (1975), 119.
[16] Pijanowski, E.: Zarys chemii i technologii mleczarstwa (1980).
[17] Smetański, R.: Sfermentowane napoje mleczne (1948).