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Short Communication: *Kefir* production in Iran

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Kefir grains were prepared in a goat-hide bag using pasteurized milk inoculated with sheep intestinal flora, followed by culture of the surface layer in milk. From the grain, 11 strains of lactic acid bacteria, non-lactic acid bacteria and yeasts were isolated and identified. Six samples of *kefir* were prepared by fermenting pasteurized milk for different lengths of time. Sensory evaluation identified the sample prepared by 24 h fermentation as the best product.

Key words: Kefir grain, microorganisms.

Kefir, a traditional popular middle eastern drink originates from the Caucasus in Central Asia. It is produced by adding activated kefir grain to cow or goat milk under mesophilic conditions. The resulting fermentation produces an acidic alcoholic beverage that has become increasingly popular in many countries during the last decade. *Kefir* has frequently been claimed to be effective against a variety of complaints and diseases (Honsono *et al*. 1990). There is evidence to support the antitumour activity of polysaccharides from kefir grain (Shiomi *et al*. 1982). The microbial flora of the kefir grains seems to differ according to the place of the origin. The microbiological and product quality of isolated kefir grain in Iran has been investigated.

Materials and Methods

A goat-hide bag (4-l capacity) obtained from Pariz and Babak villages in Kerman (Southwest Iran) was washed several times with sterile water, filled with pasteurized milk and intestinal flora from sheep. It was kept at 24 to 26 °C for 48 h and shaken hourly. When the milk was coagulated, 75% was replaced with fresh milk. This procedure was repeated for 12 weeks. Gradually a polysaccharide layer (spongy form) appeared on the surface of the hide. The layer was removed aseptically from the hides and propagated in pasteurized cow's milk.

Kefir grains of variable size (0.5–3.2 cm in diameter) were added several times to the fresh cow's milk.

Isolation and Identification of Yeasts

Each of the grain samples was cut so that a portion of the grains' interior could be obtained. These pieces were thoroughly washed with sterile water and ground in 1% (w/v) sterile saline (10 ml). Portions (0.1 ml) of a known dilution were plated in duplicate on different culture media similar to the method of Angulo *et al*. (1993).

The isolation of yeasts was carried out by surface spreading on plates of malt extract agar and yeast extract–malt extract agar. After incubation at 28 °C for 3–6 days colonies of different morphology were obtained. The methods of Barnett *et al*. (1990) were used for their identification.

Isolation and Identification of Bacteria

For isolation of lactic acid bacteria (LAB), 0.1 ml portions were plated on MRS agar and Rogosa agar (Difco). Cycloheximide (0.05% w/v) was added to inhibit yeast growth. The plates were incubated at 30 °C for 7–12 days in aerobic and anaerobic (10% CO_2) atmospheres. For the isolation of lactic streptococci, azide agar medium (Difco) was used aerobically. The isolation medium for *Acetobacter* contained 0.5% Bacto yeast extract (Difco), 1.5% ethanol and 2.5% agar. *Leuconostoc mesenteroides* was isolated on whey agar and tomato juice agar with an initial pH below 4.5. The colonies were provisionally considered LAB on the basis of their cellular morphology, Gram-positive and catalase-negative reactions. The criteria applied in the taxonomic characterization were those described by Garvie (1986), Hardie (1986) and Kandler & Weiss (1986). All the cultures were verified and deposited in the Persian Type Culture Collection (Tehran MIRCEN).

Kefir Manufacturing Process

Kefir grain (5%) was added to pasteurized (85 °C for 25 min) homogenized milk and incubated at 25 °C for 12, 24, 36, 48, 60 and 72 h. Each tested *kefir* was mixed with 1% sucrose. After through mixing, the *kefir* was diluted with sterile water and distributed in 200-ml glass bottles (196 g per bottle) capable of withstanding a pressure of atmosphere closed with a crown cap and incubated at room temperature.

Titrable Acidity, pH, Alcohol

Titrable acidity was determined using a potentiometer: 10 ml of *kefir* was transferred to a beaker containing 100 ml of distilled water, a magnetic stirrer used to expel the carbon dioxide when the contents were heated to 45 °C, cooled to 20 °C, and titrated

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with 0.1 N NaOH to pH 8.2. The acidity was expressed as percent lactic acid. For other products heating was omitted. A Radiometer model 26 pH meter (Copenhagen) was used for the determination of pH and titrable acidity.

Alcohol was determined using the enzymatic method of Boehringer Mannheim.

Viscosity, Carbon dioxide and Fat Content of Kefir

Viscosity was determined according to Duitschaever *et al*. (1987). Carbon dioxide was measured manometricaly according to the method of Sandine *et al*. (1957). The fat content was estimated by Plummer's method (1989).

Sensory Evaluation

The *kefirs* were evaluated by a trained panel of judges, for the characteristics acidity, viscosity, flavour, effervescence and degree of liking, using standard evaluation forms. The *kefirs* were stored at 4–5 °C for 10 days before being evaluated.

Results and Discussion

Kefir differs from other fermented milk products in that it is not produced by the metabolic activity of an evenly distributed microflora. It is made by fermentation with a mixed microflora which is confined to discrete kefir grains which contain several types of LAB, non-lactic acid bacteria and yeasts and which are recoverable after fermentation (Table 1).

Among the yeasts, *Saccharomyces cerevisiae*, *S. fragilis*, *S. lactis* and *Candida kefir* were isolated. The predominace of isolated yeasts in the grains was consistent with the studies carried out by Rossi & Govvetti (1991) and Angulo *et al*. (1993).

A total of 11 lactic acid and non-lactic acid bacteria and yeast species were isolated and identified. Among the isolated bacteria *L. brevis*, *L. Kefir*, *Leuconostoc mesenteroides*, and *Acetobacter aceti* were predominant over the rest of the bacteria. Duitschaever *et al.* (1988), Hon-

Table 1. The Microflora of kefir grain.

Microflora	Number per g kefir grain
Yeast	
Candida kefir	1×10^5
Saccharomyces lactis	1×10^4
S. cerevisiae	1×10^6
S. fragilis	1×10^5
Bacteria	
Lactobacillus kefir	1×10^9
L. brevis	1×10^6
L. casei	1×10^6
L. Plantarum	1×10^6
Streptococus lactis	1×10^4
Leuconostoc mesenteroides	1×10^5
Acetobacter aceti	1×10^6

Table 2. The characteristics of *kefir* produced in different time intervals.

Kefir	time (h)	pH	Acidity (g/100g)	Fat	Viscosity (cm/10s)	CO_2 (%)	Alcohol (%w/w)
A	12	4.0	1.18	2.1	7.1	3.0	0.10
B	24	3.89	1.47	1.95	7.0	3.0	0.15
C	36	3.01	2.35	1.93	6.83	3.5	0.15
D	48	3.0	2.40	1.90	8.0	3.5	0.18
E	60	2.98	2.41	1.87	6.75	3.0	0.20
F	72	2.98	2.45	1.90	6.75	3.0	0.20

sono *et al*. (1990), Rossi & Govvetti (1991) and Angulo *et al*. (1993) also noted the presence of most of these isolated bacteria and yeast from kefir grain of different countries. *Candida kefir* and *Saccharomyces cerevisiae* are the most commonly isolated species as compared to the rest or kefir isolated yeast.

As a result of fermentation of milk with Iranian kefir grain a foamy, effervescent drink has been achieved by varying the first step fermentation incubation time (12, 24, 36, 48, 60 and 72 h). There was no significant difference in pH, acidity, fat, sugar, CO_2 and alcohol content between the *kefir* samples A and B whereas in *kefirs* C, D and F the acidity increased (Table 2). Reduction in sugar content was observed as a function of incubation time. There was no significant difference in alcohol contents of all the tested *kefir* samples.

Duitschever *et al*. (1987) found similar results in pH, total acidity and viscosity between the three tested *kefirs*. The trained panel of judges accepted *kefir* B although there was no significant effect as compared to the rest of the tested *kefirs*.

Kefir B was kept for one month in order to check the shelf-life at room temperature. Fortunately there was a negligible increase in acidity and CO_2 in the beverages with this duration of storage. The sensory qualities was still judged to be excellent after 30 days.

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