African Journal of Microbiology Research Vol. 5(22), pp. 3737-3746, 16 October, 2011
Available online http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.579
ISSN 1996-0808 ©2011 Academic Journals

*Full Length Research Paper*

# Physicochemical, microbiological and sensory characteristics of Soymilk Kefir

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Accepted 30 June, 2011

**The physicochemical, microbiological and sensory characterization of kefir samples produced from cow/soy milk mixtures was carried out during storage at refrigerated conditions. Gross composition of samples was very closely related except lactose contents. Tyrosine levels of kefirs were also very similar. Leucine contents were increased with the raised soymilk ratio. Serum separation increased during storage for all samples. The lowest viscosity value was obtained when the soymilk were mixed to cow milk in a ratio of 50:50. Lactic acid was the highest one among the other organic acids. Microbiological population was not affected with addition of soymilk significantly. Generally sensory scores decreased with increasing soymilk ratio.**

**Key words:** Fermentation, LAB, organic acid, soymilk kefir, yeast.

## INTRODUCTION

Kefir is produced by diverse spectrum of microbial species present. Lactobacilli are present as the largest portion of the microbial population with lactoccoci, acetic acid bacteria and yeasts making up the remaining portion of the microorganisms present in the kefir grains or cultures (Wsolek et al., 2001; Witthuhn et al., 2004, 2005; Irigoyen et al., 2005). The end products of fermentation are lactic acid, acetaldehyde, acetoin, diacetyl, ethanol and $CO_2$ (Guzel-Seydim et al., 2000; Irigoyen et al., 2005). Besides, vitamin $B_1$, $B_{12}$, vitamin K, folic acid, calcium and amino acid contents increase in kefir during fermentation (Otles and Cagındi, 2003; Irigoyen et al., 2005). The benefits of consuming kefir in the diet are numerous, as it is reported to possess the antibacterial, immunological, antitumoral and hypercholesterolemic effects (Farnworth, 2005; Irigoyen et al., 2005).

The potential of soymilk which is the water extract of soybean as a substitute for cow or human milk has been emphasized over the years especially in the case of infants or children allergic to cow milk or adults with low level of lactase in their intestine. It is rich in high quality proteins, contains no cholesterol and only a small quantity of saturated fatty acid (Garro et al., 1999; Tsangalis et al., 2003; Garro et al., 2004). Moreover, soy products are now considered to have potential role in prevention of chronic diseases such as atherosclerosis, cancer, osteoporosis, obesity, menopausal disorder and diabetes (Anderson et al., 1999; Messina, 1999; Bhathena and Velasquez, 2002; Shimakawa et al., 2003; Chien et al., 2006).

The range of soy-based products is still very limited mostly because many consumers find the taste of soymilk undesirable; nevertheless, it is highly probable that their chemical and nutritional benefits, flavored soymilk and novel functional soy-containing foods will become more popular in the future. A significant improvement in the marketing soy-based foods could especially improve to the optimization of industrial processing and the introduction of products with similar sensory and nutritional traits to cow milk (Wilson, 1995; Canganella et al., 2000).

Consumer awareness regarding health and marketing trends are made for development of probiotic foods using milk and soy based products as raw material. Today supermarket shelves in US, Europe and Japan carry a range of functional dairy beverages with probiotics, prebiotics, omega-3, plant sterols and many other components (Sharma, 2005).

So, the objective of present study was to produce a functional dairy beverage (kefir) which carries the

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beneficial properties of both probiotics and soymilk. For this purpose, the physicochemical, microbiological and sensory attributes of kefirs manufactured with cow:soy milk mixtures at different ratios using two different starter type were assessed.

## MATERIALS AND METHODS

### Preparation of soymilk

Whole soybeans were washed and soaked overnight in distilled water containing sodium bicarbonate (0.8%). After decanting the water, the soaked soybeans were comminuted to get soymilk in a lab type soymilk machine (Kamaly, 1997).

### Production of Kefir

Soymilk and cow milk (both containing 3% fat and 8.5% total solids) were dispensed into containers and pasteurized for 10 min. at 90°C. Then bulk samples of cow milk and soymilk were divided into 5 different portions (A: 100% cow milk, B: 75% cow milk – 25% soymilk, C: 50% cow milk – 50% soymilk, D: 25% cow milk – 75% soymilk and E: 100% soymilk) and cooled to 25°C. Afterward, ten different milk portions were obtained ($A_G$, $B_G$, $C_G$, $D_G$, $E_G$, $A_C$, $B_C$, $C_C$, $D_C$, $E_C$) by inoculation of 3% kefir grains (G) and 3% kefir culture (C) (Danisco-Biolacta, Poland), respectively. All inoculated samples were incubated overnight at 25°C until pH reached 4.7. After separating the grains, stirring and glass bottling, all kefir samples were stored for 28 days at 4±1°C.

### Physical and chemical analyses

Total solids, fat, protein, lactose contents and titratable acidity were determined according to methods reported by Oysun (2001). The pH values were measured by Hanna 210 pH-meter. Tyrosine and leucine contents were observed by spectrophotometric methods explained by Hou et al. (2000). Viscosity measurements were carried out using a coaxial cylinder viscosimeter (Brookfield DV-II+Pro) Serum separation was determined by using measuring cylinders (250 ml) according to Paraskevopoulou et al. (2003)

### Determination of organic acids

The organic acid analyses were performed according to modification method of Bevilacqua and Califano (1989) by using a perkin Elmer Series 200 Model HPLC apparatus equipped with a UV absorbance detector set at 214 nm. Chromatographic separation was performed on a Shodex RSpak KC-118 model ion-exchange organic column (8 × 300 mm). The mobile phase was 0.1% (w/v) of phosphoric acid and flow rate was 0.8 ml/min.

### Microbiological analyses

Trinatrium citrate at a concentration of 2 g/L was used to prepare dilutions for the microbiological analyses. Lactobacilli counts were performed on MRS Agar (pH=6.5±0.2) at an incubation temperature of 30°C under anaerobic conditions for 72 h. Lactococci counts were carried out on M17 Agar (pH=7.2±0.2) at an incubation temperature of 30°C under anaerobic conditions for 48 h. Also cycloheximide (200 mg/L) was added to above mentioned mediums to inhibit yeast growth (Irigoyen et al., 2005). Yeast and mould were counted on OGYE Agar (pH=7.0±0.2) containing 1% oxtetracyline and incubated at 25°C for 5 days. Acetic acid bacterial counts were carried out on a medium containing 5% glucose, 1% yeasts extract, 2% agar, 100 mg/L pimaricin (to inhibit yeast growth) and 3 mg/ml penicillin (to inhibit lactic acid bacterial growth). Anaerobic incubation at 25°C for 2 days was applied for this group of bacteria.

### Sensory analysis

The sensory qualities of the experimental kefirs were assessed by numerically anchored line intensity scales. Six trained judges analyzed the sensory profile of kefirs. The intensity of each characteristic was evaluated in a ten point score (Bodyfelt et al., 1998).

### Statistical analysis

Analysis of variance (ANOVA) using 95% confidence intervals was run on each of the physicochemical, microbiological and sensory variables to disclose possible differences among the samples for three factors "the type of kefir culture, storage time and the rate of soymilk". All analyses were carried out using SPSS© 15.0 statistical package (SPSS, 1994).

## RESULTS AND DISCUSSION

### Physical and chemical properties

Table 1 shows the means of chemical composition of kefir samples produced with grain or culture at first day of storage. The chemical properties of kefir samples are quite similar with each other except the lactose content. As expected, the lactose contents of kefirs were decreased while the soymilk amount was increased. The obtained values were similar to those recorded for other fermented milks and kefir by Ching and Ching (1999), Muir et al. (1999), Irigoyen et al. (2005) and Tratnik (2006). The pH values of all kefir samples were found so close to each other and a slight decrease was obtained by the soymilk addition but it was not significant ($p>0.05$).

The tyrosine based spectrophotometric assay detects released α-amino groups which result from the proteolysis of milk proteins thus giving a direct measurement of proteolytic activity. As seen from Table 2, tyrosine content of kefir samples were changed between 0.004 and 0.016 mg/g. Increase in tyrosine content of fermented milk products dependent on storage time, culture type and protein structure (Tamime and Marshall, 1997; Chou and Hou, 2000). Contrarily both culture and milk types were not significantly affected the tyrosine content in this study ($p>0.05$). However the amount of tyrosine slightly increased in all tested kefirs during the storage except sample $D_G$. Also when tyrosine content of fermented milk products exceeds 0.5 mg/ml bitterness occurs (Asperger and Brandl, 1983). The tyrosine contents of all samples were lower than threshold level of bitterness during the storage. Generally it can be said that proteolytic activity of culture added

**Table 1.** Chemical composition and acidity of kefir samples at first day of storage.

| Samples | Total solids (%) | Fat (%) | Protein (%) | Lactose (%) | pH |
|---|---|---|---|---|---|
| $A_G$ | 11.70 | 3.05 | 4.10 | 3.89 | 4.60 |
| $B_G$ | 11.43 | 3.10 | 4.12 | 3.05 | 4.65 |
| $C_G$ | 11.38 | 3.00 | 4.03 | 2.12 | 4.63 |
| $D_G$ | 11.51 | 3.05 | 4.16 | 1.04 | 4.58 |
| $E_G$ | 11.27 | 3.05 | 4.01 | nd. | 4.58 |
| $A_C$ | 11.62 | 3.10 | 4.16 | 3.92 | 4.60 |
| $B_C$ | 11.54 | 3.05 | 4.15 | 3.12 | 4.65 |
| $C_C$ | 11.51 | 3.10 | 4.09 | 2.09 | 4.63 |
| $D_C$ | 11.39 | 3.05 | 4.09 | 1.02 | 4.58 |
| $E_C$ | 11.32 | 3.00 | 4.08 | nd. | 4.58 |

$A_G$: 100% cow milk- grain inoculated; $B_G$: 75% cow milk-25% soymilk- grain inoculated; $C_G$: 50% cow milk-50% soymilk- grain inoculated; $D_G$: 25% cow milk-75% soymilk- grain inoculated; $E_G$: 100% soymilk- grain inoculated, $A_C$: 100% cow milk- culture inoculated; $B_C$: 75% cow milk-25% soymilk- culture inoculated; $C_C$: 50% cow milk-50% soymilk- culture inoculated; $D_C$: 25% cow milk-75% soymilk- culture inoculated; $E_C$: 100% soymilk- culture inoculated.

**Table 2.** Proteolytic activity, serum separation and viscosity in kefir samples.

| Products | Samples | Storage days | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 21 | 28 |
| Tyrosine (mg/g) | $A_G$ | 0.004 | 0.004 | 0.005 | 0.007 | 0.010 |
| | $B_G$ | 0.005 | 0.006 | 0.007 | 0.008 | 0.011 |
| | $C_G$ | 0.004 | 0.005 | 0.005 | 0.007 | 0.009 |
| | $D_G$ | 0.012 | 0.005 | 0.007 | 0.007 | 0.011 |
| | $E_G$ | 0.006 | 0.005 | 0.009 | 0.010 | 0.014 |
| | $A_C$ | 0.003 | 0.005 | 0.005 | 0.007 | 0.009 |
| | $B_C$ | 0.004 | 0.006 | 0.007 | 0.009 | 0.010 |
| | $C_C$ | 0.004 | 0.006 | 0.009 | 0.010 | 0.012 |
| | $D_C$ | 0.004 | 0.006 | 0.009 | 0.011 | 0.013 |
| | $E_C$ | 0.006 | 0.007 | 0.009 | 0.011 | 0.016 |
| Leucine (mmol/L) | $A_G$ | 0.99 | 1.25 | 1.59 | 1.80 | 1.97 |
| | $B_G$ | 1.89 | 2.32 | 2.79 | 3.05 | 3.29 |
| | $C_G$ | 3.89 | 4.25 | 4.80 | 5.25 | 5.89 |
| | $D_G$ | 4.74 | 5.11 | 5.68 | 6.14 | 8.52 |
| | $E_G$ | 5.45 | 7.49 | 7.99 | 8.32 | 9.21 |
| | $A_C$ | 0.87 | 1.09 | 1.27 | 1.76 | 1.89 |
| | $B_C$ | 1.78 | 2.30 | 2.71 | 2.90 | 3.22 |
| | $C_C$ | 3.71 | 3.84 | 4.02 | 4.75 | 5.32 |
| | $D_C$ | 4.45 | 4.94 | 5.36 | 5.98 | 6.05 |
| | $E_C$ | 6.85 | 7.50 | 8.21 | 8.90 | 9.56 |
| Serum (ml/250 ml kefir) | $A_G$ | nd | 21 | 40 | 69 | 95 |
| | $B_G$ | 18 | 27 | 54 | 83 | 102 |
| | $C_G$ | 23 | 32 | 65 | 96 | 120 |
| | $D_G$ | 15 | 23 | 49 | 75 | 96 |
| | $E_G$ | nd | 24 | 52 | 78 | 84 |
| | $A_C$ | nd | 15 | 24 | 48 | 73 |
| | $B_C$ | 10 | 19 | 41 | 62 | 80 |
| | $C_C$ | 13 | 24 | 51 | 79 | 103 |
| | $D_C$ | 9 | 20 | 34 | 62 | 81 |

**Table 2** Contd.

| | | | | | | |
|---|---|---|---|---|---|---|
| | $E_C$ | 5 | 17 | 33 | 64 | 78 |
| Apparent viscosity (MPas) | $A_G$ | 460 | 368 | 315 | 189 | 198 |
| | $B_G$ | 400 | 321 | 289 | 175 | 190 |
| | $C_G$ | 320 | 284 | 255 | 160 | 184 |
| | $D_G$ | 540 | 458 | 394 | 232 | 255 |
| | $E_G$ | 560 | 439 | 386 | 260 | 271 |
| | $A_C$ | 435 | 349 | 290 | 180 | 194 |
| | $B_C$ | 369 | 318 | 266 | 160 | 197 |
| | $C_C$ | 308 | 256 | 203 | 141 | 190 |
| | $D_C$ | 450 | 394 | 305 | 223 | 270 |
| | $E_C$ | 500 | 414 | 353 | 228 | 279 |

$A_G$: 100% cow milk- grain inoculated; $B_G$: 75% cow milk-25% soymilk- grain inoculated; $C_G$: 50% cow milk-50% soymilk- grain inoculated; $D_G$: 25% cow milk-75% soymilk- grain inoculated; $E_G$: 100% soymilk- grain inoculated, $A_C$: 100% cow milk- culture inoculated; $B_C$: 75% cow milk-25% soymilk- culture inoculated; $C_C$: 50% cow milk-50% soymilk- culture inoculated; $D_C$: 25% cow milk-75% soymilk- culture inoculated; $E_C$: 100% soymilk- culture inoculated

samples was higher than grain culture added samples.

The increase in leucine equivalent during incubation and storage period indicates that some of the milk protein was hydrolyzed to a soluble form in lactic acid by lactic acid bacteria as reported to possess proteolytic activity (Rasic and Kurmann, 1983; Tamime and Marshall, 1997; Chou and Hou, 2000; Rekha and Vijayalakshmi, 2008). The degree of protein hydrolysis expressed as leucine equivalent in kefir samples (Table 2) was increased as the soymilk ratio was raised and the storage period was also effective on this parameter ($p<0.05$). In contrast, fermentation with culture or grain was not significantly effective ($p>0.05$). These results indicate that the protein source is more important rather than variety of microorganism from proteolysis point of view.

Typical serum separation and flow characteristics for kefirs with containing different concentration of soymilk are also shown in Table 2.

The different factors such as stabilizers, acidity, total solids, milk kind, and culture type affected to serum separation in fermented milk beverages (Lucey et al., 1999; Köksoy and Kılıc, 2003). When the soymilk were added to cow milk in a ratio of 25:75 ($B_G$-$B_C$) and 50:50 ($C_G$-$C_C$) serum separation was increased compared to other samples ($p<0.05$). Initially the serum separation was not detected in samples produced from cow milk ($A_G$-$A_C$) but also in soymilk kefir inoculated with grains ($E_G$). As expected serum separation was gradually increased throughout the storage in all kefir samples ($p<0.05$). It is clear that serum separation occurs in fermented milk products due to the aggregation of protein particles during storage and sedimentation of them under gravity.

The data shown in Table 2 illustrate that kefir samples produced with the ratio of 50:50 ($C_G$, $C_C$) had significantly lower viscosity than the other kefirs ($p<0.05$). the viscosity of all samples ($p<0.05$). It is also seen that $D_G$-$D_C$ and $E_G$-$E_C$ samples were more viscous during the Furthermore the storage period was negatively affected storage period than the samples of $A_G$-$A_C$ and $B_G$-$B_C$. So, although some contrary results were obtained in the literature soymilk was effectively stabilized in kefir as well as cow milk (Liu and Lin, 2000).

## Organic acids

Organic acids may occur in dairy products as a result of hydrolysis of fat, biochemical and metabolic processes or bacterial metabolism. Also organic acids are very important for the flavor properties of fermented dairy products. The main organic acids in kefir samples throughout storage were lactic, citric, pyruvic and acetic acids (Table 3). The lactic acid is extremely important for producing high-quality fermented milk and appropriate concentrations are needed to ensure proper flavor with minimum syneresis during storage (Liu and Lin, 2000). As seen it was the most abundant one in all kefir samples. Although the lactic acid concentrations were slightly higher in grain inoculated samples the differences were not statistically significant. Some previous studies indicated that usually lactic cultures cannot produce adequate level of lactic acid in soymilk (Lee et al., 1990; Liu and Lin, 2000). However, mixing cow milk with soymilk resulted in lactic acid concentrations similar to those of milk kefir, showing that the addition of different ratio cow milk improves the ability of microorganisms in kefir grains or culture to produce lactic acid in soymilk (Liu and Lin, 2000).

Citric acid in fermented milks is metabolized by lactic acid bacteria into flavor components such as acetic acid, acetaldehyde and diacetyl. Citric acid content was higher in kefirs made from cow:soymilk with the ratio of 75:25 ($B_G$, $B_C$) ($p<0.05$). The level of citric acid also changed

**Table 3.** Concentration of organic acids during storage of kefir samples (mg/kg).

| Organic acids | Samples | Storage days | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 21 | 28 |
| Lactic acid | $A_G$ | 296.63 | 286.58 | 233.11 | 281.15 | 260.51 |
| | $B_G$ | 146.00 | 153.20 | 171.25 | 130.21 | 107.80 |
| | $C_G$ | 300.14 | 271.52 | 269.79 | 288.74 | 282.40 |
| | $D_G$ | 232.15 | 229.87 | 277.94 | 227.96 | 249.61 |
| | $E_G$ | 256.57 | 246.30 | 218.22 | 275.23 | 228.15 |
| | $A_C$ | 265.22 | 254.11 | 232.20 | 259.22 | 261.22 |
| | $B_C$ | 142.86 | 149.32 | 165.21 | 141.14 | 110.24 |
| | $C_C$ | 265.23 | 240.33 | 239.14 | 244.32 | 253.36 |
| | $D_C$ | 223.31 | 221.84 | 243.23 | 221.36 | 228.26 |
| | $E_C$ | 244.31 | 241.32 | 223.18 | 230.41 | 219.32 |
| Citric acid | $A_G$ | 1.02 | 1.69 | 2.00 | 2.26 | 2.29 |
| | $B_G$ | 9.42 | 6.83 | 4.21 | 3.00 | 2.11 |
| | $C_G$ | 1.46 | 1.58 | 1.72 | 1.33 | 1.86 |
| | $D_G$ | 2.24 | 1.65 | 1.53 | 1.67 | 1.79 |
| | $E_G$ | 2.40 | 2.73 | 2.97 | 2.33 | 2.60 |
| | $A_C$ | 2.33 | 2.58 | 2.15 | 2.69 | 2.73 |
| | $B_C$ | 13.24 | 12.84 | 9.42 | 6.71 | 4.33 |
| | $C_C$ | 3.27 | 3.61 | 3.89 | 3.41 | 3.57 |
| | $D_C$ | 4.21 | 3.89 | 3.67 | 3.51 | 3.29 |
| | $E_C$ | 4.54 | 4.78 | 5.32 | 4.81 | 5.08 |
| Pyruvic acid | $A_G$ | 0.40 | 0.37 | 0.35 | 0.41 | 0.44 |
| | $B_G$ | 0.41 | 0.33 | 0.21 | 0.23 | 0.19 |
| | $C_G$ | 0.43 | 0.41 | 0.39 | 0.36 | 0.44 |
| | $D_G$ | 0.32 | 0.35 | 0.38 | 0.37 | 0.39 |
| | $E_G$ | 0.43 | 0.40 | 0.47 | 0.47 | 0.32 |
| | $A_C$ | 0.38 | 0.40 | 0.41 | 0.50 | 0.45 |
| | $B_C$ | 0.39 | 0.36 | 0.25 | 0.26 | 0.17 |
| | $C_C$ | 0.37 | 0.41 | 0.36 | 0.30 | 0.37 |
| | $D_C$ | 0.29 | 0.31 | 0.34 | 0.37 | 0.35 |
| | $E_C$ | 0.37 | 0.38 | 0.44 | 0.51 | 0.29 |
| Acetic acid | $A_G$ | 0.74 | 0.63 | 0.55 | 0.64 | 0.62 |
| | $B_G$ | 0.54 | 0.51 | 0.32 | 0.59 | 0.38 |
| | $C_G$ | 0.63 | 0.61 | 0.51 | 0.58 | 0.65 |
| | $D_G$ | 0.49 | 0.45 | 0.47 | 0.47 | 0.50 |
| | $E_G$ | 0.47 | 0.49 | 0.69 | 0.52 | 0.43 |
| | $A_C$ | 0.60 | 0.54 | 0.40 | 0.51 | 0.50 |
| | $B_C$ | 0.55 | 0.59 | 0.40 | 0.63 | 0.42 |
| | $C_C$ | 0.54 | 0.50 | 0.53 | 0.62 | 0.66 |
| | $D_C$ | 0.40 | 0.47 | 0.51 | 0.53 | 0.59 |
| | $E_C$ | 0.51 | 0.56 | 0.62 | 0.51 | 0.45 |

$A_G$: 100% cow milk- grain inoculated; $B_G$: 75% cow milk-25% soymilk- grain inoculated; $C_G$: 50% cow milk-50% soymilk- grain inoculated; $D_G$: 25% cow milk-75% soymilk- grain inoculated; $E_G$: 100% soymilk- grain inoculated, $A_C$: 100% cow milk- culture inoculated; $B_C$: 75% cow milk-25% soymilk- culture inoculated; $C_C$: 50% cow milk-50% soymilk- culture inoculated; $D_C$: 25% cow milk-75% soymilk- culture inoculated; $E_C$: 100% soymilk- culture inoculated.

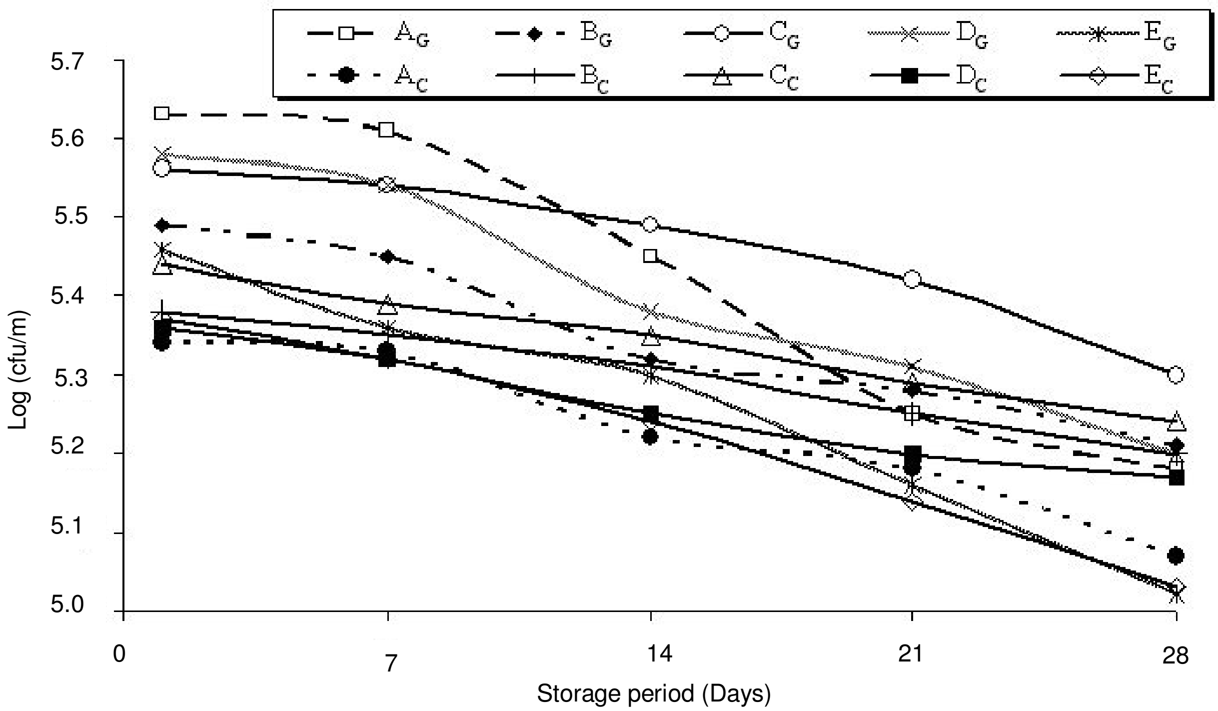


**Figure 1.** Changes in the lactobacilli counts of kefir samples during storage. $A_G$: 100% cow milk- grain inoculated; $B_G$: 75% cow milk-25% soymilk- grain inoculated; $C_G$: 50% cow milk-50% soymilk- grain inoculated; $D_G$: 25% cow milk-75% soymilk- grain inoculated; $E_G$: 100% soymilk- grain inoculated, $A_C$: 100% cow milk- culture inoculated; $B_C$: 75% cow milk-25% soymilk- culture inoculated; $C_C$: 50% cow milk-50% soymilk- culture inoculated; $D_C$: 25% cow milk-75% soymilk- culture inoculated; $E_C$: 100% soymilk- culture inoculated.

significantly during the storage period ($p<0.05$). Citric acid is known to be utilized during the fermentation process but negligible utilization of this acid was observed during storage which is in accordance with other studies (Guzel-Seydim et al., 2000; Adhikari et al., 2002; Gueimonde et al., 2003; Serra et al., 2009).

Formation of pyruvic acid was also detected in all kefir samples (Table 3). Pyruvic acid content was not changed during storage and the samples had close values. *Lactococcus lactis*, a common component of the kefir culture, uses the EMP pathway to produce pyruvic acid. Pyruvic acid is preferentially converted to lactate and residual pyruvic acid is converted to acetaldehyde and diacetyl. Contrarily, the concentration of pyruvic acid in kefir has been reported to increase during fermentation (Guzel-Seydim et al., 2000).

Acetic acid can be produced from citrate, lactose and amino acids. It is desirable that fermented products contain low quantities of acetic acid because of its objectionable vinegary taste. The type of kefir culture used and storage time were not significantly affected by the acetic acid contents. In disagreement Scalabrini et al. (1998), Hou et al. (2000), Tsangalis and Shah (2004) showed that starter cultures especially *Bifidobacterium* species generally produced higher quantities of acetic acid in fermented soymilk products with considerable variation in lactic and acetic acid production between different strains.

## Microbiological characteristics

The populations of lactobacilli were decreased during storage for all samples as illustrated in Figure 1. The lactobacilli counts in both culture and grain inoculated samples were never declined less than 5.0 log cfu/mL throughout the storage ($p>0.05$). Although grain inoculated samples had slightly higher lactobacilli counts, differences were insignificant. However results are considerably lower than the counts observed by Kiliç et al. (1999) and Guzel-Seydim et al. (2005) in Turkish kefirs. Initially the lactococci levels nearly 10 log cfu/mL for all kefir samples (Figure 2). There was a sharp decrease in the number of lactoccoci after 14 days ($p<0.05$). Guzel-Seydim et al. (2005) also reported declines in lactococci counts towards the end of cold storage for 21 days.

Yeasts are important in kefir fermentation because of the production of ethanol and carbon dioxide, which give the kefir drink its unique taste. The counts of yeasts were increased progressively during the first week of storage and then remained relatively constant (Figure 3) until the

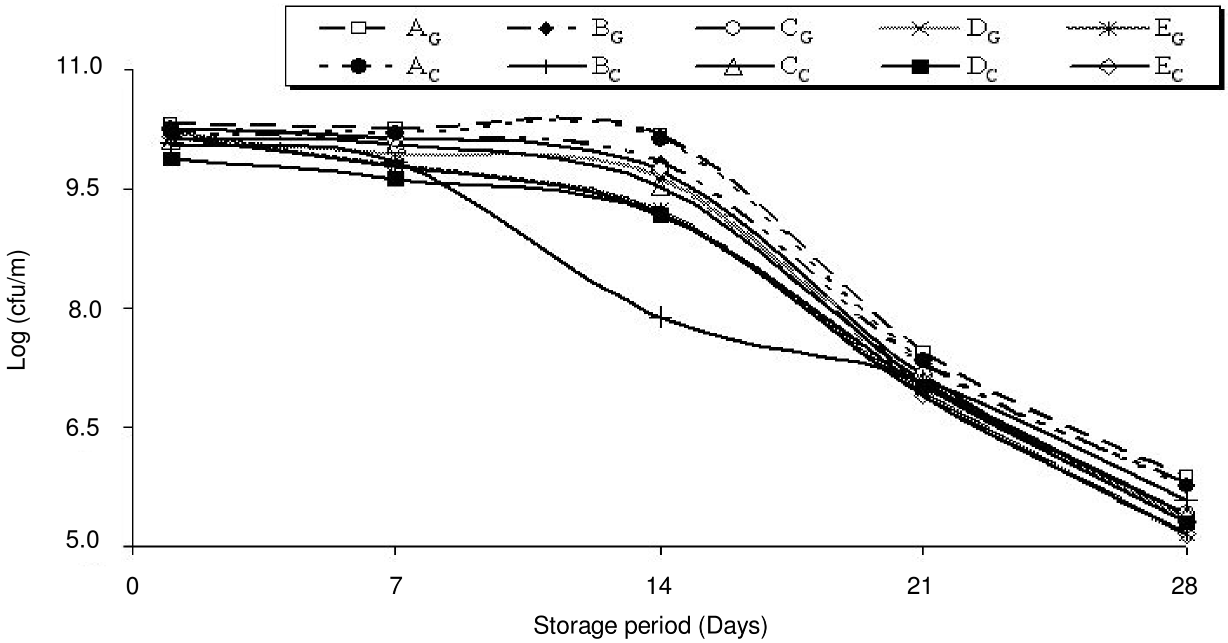


**Figure 2.** Changes in the lactococcus counts of kefir samples during storage. $A_G$: 100% cow milk- grain inoculated; $B_G$: 75% cow milk-25% soymilk- grain inoculated; $C_G$: 50% cow milk-50% soymilk- grain inoculated; $D_G$: 25% cow milk-75% soymilk- grain inoculated; $E_G$: 100% soymilk- grain inoculated, $A_C$: 100% cow milk- culture inoculated; $B_C$: 75% cow milk-25% soymilk- culture inoculated; $C_C$: 50% cow milk-50% soymilk- culture inoculated; $D_C$: 25% cow milk-75% soymilk- culture inoculated; $E_C$: 100% soymilk- culture inoculated.

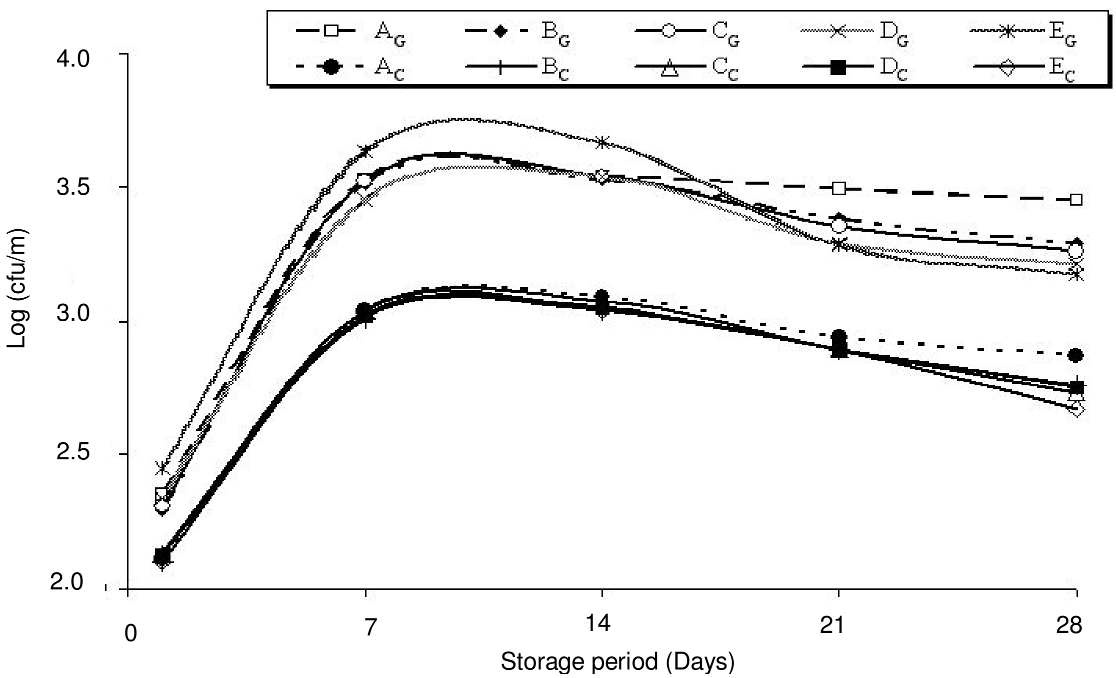


**Figure 3.** Changes in the yeast counts of kefir samples during storage. $A_G$: 100% cow milk- grain inoculated; $B_G$: 75% cow milk-25% soymilk- grain inoculated; $C_G$: 50% cow milk-50% soymilk- grain inoculated; $D_G$: 25% cow milk-75% soymilk- grain inoculated; $E_G$: 100% soymilk- grain inoculated, $A_C$: 100% cow milk- culture inoculated; $B_C$: 75% cow milk-25% soymilk- culture inoculated; $C_C$: 50% cow milk-50% soymilk- culture inoculated; $D_C$: 25% cow milk-75% soymilk- culture inoculated; $E_C$: 100% soymilk- culture inoculated.

**Table 4.** Sensory scores of kefir samples during storage.

| Sensory properties | Samples | Storage days | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 21 | 28 |
| Taste | $A_G$ | 7.83 | 7.80 | 8.10 | 7.85 | 7.05 |
| | $B_G$ | 7.05 | 7.00 | 6.96 | 6.89 | 6.60 |
| | $C_G$ | 5.30 | 5.22 | 5.00 | 4.89 | 4.30 |
| | $D_G$ | 4.00 | 3.90 | 3.60 | 3.50 | 2.90 |
| | $E_G$ | 3.23 | 3.00 | 2.50 | 2.00 | 1.50 |
| | $A_C$ | 8.00 | 8.25 | 8.40 | 8.10 | 7.96 |
| | $B_C$ | 7.34 | 7.25 | 7.19 | 7.20 | 7.09 |
| | $C_C$ | 5.75 | 5.64 | 5.39 | 5.21 | 5.40 |
| | $D_C$ | 4.51 | 4.44 | 4.19 | 4.22 | 3.50 |
| | $E_C$ | 3.75 | 3.50 | 3.30 | 3.40 | 3.29 |
| Consistency | $A_G$ | 7.75 | 7.24 | 7.50 | 7.43 | 7.08 |
| | $B_G$ | 7.50 | 7.30 | 7.20 | 7.45 | 7.20 |
| | $C_G$ | 6.89 | 6.80 | 6.75 | 6.60 | 6.56 |
| | $D_G$ | 6.54 | 6.40 | 6.55 | 6.70 | 6.43 |
| | $E_G$ | 6.38 | 6.74 | 6.40 | 6.27 | 6.35 |
| | $A_C$ | 8.10 | 8.30 | 8.25 | 8.10 | 8.00 |
| | $B_C$ | 7.74 | 7.80 | 7.54 | 7.25 | 7.30 |
| | $C_C$ | 7.00 | 7.10 | 6.80 | 6.79 | 6.70 |
| | $D_C$ | 6.70 | 6.49 | 6.25 | 6.19 | 6.25 |
| | $E_C$ | 6.51 | 6.69 | 6.53 | 6.40 | 6.50 |
| General acceptance | $A_G$ | 7.05 | 7.00 | 6.84 | 6.50 | 6.24 |
| | $B_G$ | 7.10 | 6.89 | 6.80 | 6.75 | 6.00 |
| | $C_G$ | 6.30 | 6.22 | 5.70 | 5.40 | 4.98 |
| | $D_G$ | 5.24 | 5.00 | 4.47 | 4.30 | 3.96 |
| | $E_G$ | 4.56 | 4.40 | 4.20 | 4.00 | 3.98 |
| | $A_C$ | 7.84 | 7.33 | 7.55 | 7.48 | 7.30 |
| | $B_C$ | 7.15 | 7.00 | 7.22 | 7.09 | 6.90 |
| | $C_C$ | 6.70 | 6.78 | 6.40 | 6.10 | 5.45 |
| | $D_C$ | 6.00 | 5.54 | 5.40 | 5.00 | 4.39 |
| | $E_C$ | 5.73 | 5.58 | 5.20 | 4.80 | 4.18 |

$A_G$: 100% cow milk- grain inoculated; $B_G$: 75% cow milk-25% soymilk- grain inoculated; $C_G$: 50% cow milk-50% soymilk- grain inoculated; $D_G$: 25% cow milk-75% soymilk- grain inoculated; $E_G$: 100% soymilk- grain inoculated, $A_C$: 100% cow milk- culture inoculated; $B_C$: 75% cow milk-25% soymilk- culture inoculated; $C_C$: 50% cow milk-50% soymilk- culture inoculated; $D_C$: 25% cow milk-75% soymilk- culture inoculated; $E_C$: 100% soymilk- culture inoculated

end of the 28 days ($p<0.05$). The yeast counts of kefir samples inoculated with grains (≈3.20 log cfu/mL) was significantly higher than the yeast counts of samples inoculated with kefir culture (≈2.70 log cfu/mL) ($p<0.05$). The yeast population level in our study were lower than the enumerations reported by Kılıc et al. (1999), Wszolek et al. (2003), Witthuhn et al. (2004) and Irigoyen et al. (2005). Similarly with lactobacilli and lactococci counts, the differences between yeast numbers of samples were insignificant ($p>0.05$). So, it is clear that addition of soymilk was not affected by these microbial populations negatively. The major carbohydrates present in soymilk are sucrose, raffinose and stachyose whereas in milk it is lactose. The fact that, lactic acid bacteria and yeasts from kefir grain or culture grew well in soymilk, even when no extra carbohydrate was added, means that these organisms can utilize soymilk carbohydrates for growth (Liu and Lin, 2000).

## Sensory properties

The results of sensory evaluation were shown in Table 4. The highest sensory scores were awarded to the kefir samples made from 100% cow milk ($A_G$-$A_C$) and scores were decreased as the soymilk ratio was raised ($p<0.05$). Moreover kefir samples inoculated with kefir culture had slightly higher sensory scores than grain inoculated ones and the storage period significantly affected the sensory scores. According to preference of panelists the more important factor for kefir quality was taste than consistency because kefir samples containing 100% soymilk ($E_G$-$E_C$) had approximately 4 times lower taste scores than 100% cow milk kefirs ($A_G$-$A_C$). It may be due to the objectionable beany flavor and taste of soymilk which remained in kefirs.

## Conclusions

Functional dairy beverages were successfully produced from different cow:soy milk mixtures and commercial kefir culture or grains. The gross chemical compositions were similar, but the lactose contents were decreased while the soymilk contents were increased. Although the tyrosine values were so close to each other the degree of protein hydrolysis expressed as leucine equivalent in kefir samples was increased as the soymilk ratio was raised. The serum separation was gradually increased throughout the storage while the viscosity was decreased. Lactic acid was found to be the most abundant organic acid in all samples. During refrigerated storage usually yeast counts increased during the first week while the lactic acid bacteria counts decreased. The sensory characters of the samples were mainly influenced by the type of milk used and storage period.

Today food and beverage manufacturers have targeted functionality as an extremely important marketing tool. Therefore the results of the present study show that production of kefir with soymilk addition carries great competitive advantages for them in the market place.

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