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Microbiological Quality, Fatty Acid and Amino Acid Profiles of Kefir Produced from Combination of Goat and Soy Milk

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Abstract: The microbiological quality, fatty acid and amino acid profiles were studied in kefir prepared from combination of goat and soy milk (100:0), (50:50) and (0:100). Total counts, total of lactic acid bacteria (LAB) and total yeast of kefir were counted with Standard Plate Count (SPC). Fatty acids composition was analyzed by gas chromatography (GC), whereas amino acids composition was analyzed by high performance liquid chromatography (HPLC). The results showed that there were no significant difference in total counts, LAB and yeast of all kefir. The acidity of kefir made from soy milk only was lower ($p<0.05$) than kefir made from goat milk or combination of goat and soy milk, whereas there were no significant difference in pH. Caproic (C6:0), heptadecanoic (C17:0), behenic (C22:0) and pentadecanoic (C15:1) acid of kefir made from 50% goat milk and 50% soy milk mixture (50:50) were lower ($p<0.05$) than goat milk kefir (100:0), but the oleic acid (C18:1) of kefir made from combination of 50:50 was higher ($p<0.05$) than 100:0. The amino acids composition of kefir made from goat milk only (100:0) and combination 50:50 was not significantly different. Therefore, this study suggests that soy milk can substitute 50% of goat milk to produce kefir without changing the microbiological quality, acidity, pH value, amino acids profile and even increase the oleic acid.

Key words: Microbiological quality, acidity, pH, fatty acid, amino acid, Kefir

INTRODUCTION

Kefir distinguishes itself from fermented milk products, yoghurt, in that during its production the milk undergoes a dual fermentation process under the action of both lactic acid bacteria and yeast. While yoghurt can readily be made from the lactic acid bacteria present in fresh yoghurt, kefir can be produced by fermenting milk with commercial freeze dried kefir starter cultures or kefir grains as well as the product obtained after the removal of grains. It can be made of any type of milk: cow, goat, sheep, coconut, rice and soy, but cow milk is commonly used (Bensmira and Jiang, 2012). In general, the overall properties of yoghurt, such as acidity level, free fatty acid content, the production of aroma compounds (diacetyl, acetaldehyde, acetoin) as well as the sensory profile and nutritional value, are important traits of the product. These aspects are influenced by the chemical composition of the milk base, processing conditions, the activity of starter culture during the incubation period (Bonczar *et al.*, 2002).The composition of kefir depends on the source and the fat content of milk, the composition of grains or cultures and the technological process of kefir (Otles and Cagindi, 2003).

Due its good nutritional value, good digestibility and acceptability and a low allergenic potential, the goat's milk has been recommended for children, elderly and convalescent persons. However, it cannot be recommended to every child allergic to cow's milk, because in some cases, serious threat to life can also occur with goat's milk (Ribeiro and Ribeiro, 2001). The goat milk fat is composed of several hundred fatty acids, the share of which in the total fatty acid (FA) pool is considerably differentiated. The share of five of those acids (C10:0, C14:0, C16:0, C18:0, C:18-1 cis) comprises over 75% of total FA in milk (Park *et al.*, 2007).

In the Far East, soybean had the reputation of therapeutic food from ancient time. When the composition of soybean is well described, it is known that soybean is cheap foodstuff, rich in valuable proteins, unsaturated fatty acids, soluble and insoluble fibers and isoflavones, which are very important in daily nutrition (Bozanic *et al.*, 2008). Soybean can be use as alternative sources of protein, particularly for the developing countries where malnutrition exists. Since soybean is plentiful, relatively inexpensive and rich in protein, some effort has been devoted to exploiting it for the manufacture of more acceptable and palatable food products. However, the main objections to soybean products by consumer are associated with the beany flavor. This problem may be overcome by processing

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techniques and fermentation (Tamime and Robinson, 1985). In addition, soybeans are the most important food source of isoflavones that exhibit antioxidant activity.

Amino acids of kefir is influenced by the sources of the milk. The previous study by Bensmira and Jiang (2012), showed amino acid composition was different between peanut milk kefir and both of 70% peanut milk kefir and whole milk kefir (Bensmira and Jiang, 2012). In addition, variation in free fatty acids (FFA) content gives rise to changes in the organoleptic and nutritional qualities of milk products, it is important to evaluate the level of these compounds in fermented milk beverages (Regula, 2007).

Although several authors have examined the composition, nutritional and therapeutic aspects (Otles and Cagindi, 2003), little information about LAB and yeast contents, fatty acids and amino acids profile of kefir produced from goat and soy milk combination. Therefore, the present study aimed to evaluating the microbiological quality, fatty acid and amino acid profile of kefir produced from goat and soy milk combination.

MATERIALS AND METHODS

Preparation of soymilk: Whole soybeans was washed and soaked overnight in distilled water. After decanting the water, the soaked soybeans were mixed with 3 times their weight of distilled water for 3 min, blended and filtered (Kasenkas *et al*., 2011 with slight modifications).

Preparation of kefir: Goat milk and soy milk were pasteurized for 10 min at 90°C. The pasteurized milk were devided into 5 groups of kefir combination from goat and soy milk (100:0, 75:25, 50:50, 25:75 and 0:100) and cooled at room temperature. Kefir grains was inoculated into pasteurized milk as much as 2% and incubated at room temperature for 18 hours (Kesenkas *et al*., 2011) with slight modifications. After separating the grains and stirring, kefir samples were analyzed for microbiological, titratable acidity, pH, fatty acid and amino acid.

Microbiological analysis: Each kefir samples were taken 1 ml and diluted with 9 mL physiological NaCl and the procedure was continued to obtain a final dilution of 10^6. About 0.1 mL of 10^5 and 10^6 dilutions (appropriate decimal dilutions) were spread into each a sterile Petri dish on each medium. This was subjected to incubation at 37°C for 24 h for total counts and 48 h for LAB and yeast. To determine the total counts, LAB and yeast populations, the colonies formed were counted and expressed in log CFU/mL (Roostita *et al*., 2011). Plate count agar (PCA) (Merck) medium was used for determination of total counts (Chen *et al*., 1983). Lactic acid bacteria were determined on modified deMan, Rogosa and Sharpe (MRS) agar (Merck) containing 100 ppm NaN_3 (Mundt *et al*., 1967) and 100 ppm $CaCO_3$ (Hwanhlem *et al*., 2011). Lactic acid is strong enough to dissolve calcium carbonate and that can be seen as a clear area in the agar. Thus, in the isolation process the clear areas indicate lactic acid bacteria (Endo *et al*., 2009). Malt extract agar (MEA) (Oxoid) containing 100 ppm chloramphenicol was used for determination of yeasts (Eissa *et al*., 2011).

Titratable acidity and measuring of pH: The pH of kefir was measured using a pH-meter (HANNA-HI 98103) and acidity was anayzed by titratable acidity according to Hashim *et al*. (2009) with slight modification. Titratable acidity is expressed as percentage of lactic acid and determined by titrating 9 g of kefir with 0.1 N NaOH using phenolphthalein as an indicator to an end-point of faint pink color. The measurements were done in duplicate:

$$\text{Acidity (\%)} = \frac{\text{(mL NaOH)x(N NaOH)(MW of lactic acid)}}{\text{sample weightx1000 mg}} \text{x100}$$

Analysis of fatty acid: Fatty acid profile of goat milk, soy milk and kefir made from goat and soy milk was analyzed by Gas Chromatography. This procedure is based on AOAC Official Method 969.33/963.22 (AOAC International, 2002) and analytical method validation according to Gonzalez and Herrador (2007). Sample preparation: 350 mg sample was placed into 50 ml volumetric flask and added 6 ml metanolic NaOH 0.5 M and boiling stones. Cooler was connected and reflux until removed of oil globule (5-10 min) and added 7 mL of boron trifluoride (BF_3) through cooler and continue to reflux for 2 h. Heptana 2-5 mL was added through cooler and reflux for 1 min. Heater and cooler was removed and then added 15 mL saturated NaCl and shaken the mixture for 15 sec. NaCl was added again until the heptane layer is in the neck of the bottle, take 1 mL of heptane layer and put in a tube. Na_2SO_4 was added into the tube and then filtered and injected into Gas Chromatography (Shimadzu GC-2010).

GC condition: Rtx-5 column (oven temperature 180°C, hold for 2 min; increase to 270°C at 10°C/min, hold for 4 min and total of running time 15 min. Carrier gas He 2.43 mL/min, flow rate of air 190 mL/min and flow rate H_2 80 mL/min. Injector temperature is 290ºC and temperature of flame ionization detector (FID) is 290ºC. Methyl laurat (10% in heptana) as a standard with injection volume 0.10 µL. The peak of sample chromatogram having the same retention time with the retention time of standard is the peak of fatty acid.

Analysis of amino acids: Amino acids were determined by HPLC (Knauer) according to Marino *et al*. (2010) with some modification. Sample preparation: 5 g sample was weighed and put into a tube and then covered. Hydrochloric acid 6N was added as much as 10 mL and vortex until homogen. Hydrolysis of sample by using autoclave at 110°C for 8 h. Sample was cooled at room temperature and neutralized by NaOH 6 N. Solution of 40% Pb (CH_3COO)4 and 15% oxalic acid were added and put in vial and diluted with aquadest until 50 mL. Three ml of sample was taken and filtered with filter 0.45 µm. Sample was diluted by 10x and incubated for 3 minutes in o-phthaldehyde (OPA). Sample was injected as much as 30 µL to HPLC.

HPLC condition: Eurospher100-5 C18 column, 250x4.6 mm with precolumn P/N: 1115Y535. Eluent: A) 0.01M acetic-buffer pH 5.9 and B) MeOH: 0.01 M acetic- buffer pH 5.9: Tetrahydrofolic (THF) (80:15:5). Detector: λ Fluorescence (excitation: 340 nm, emission: 450 nm).The elution of samples was performed at a flow rate of 1.5 mL/min by gradient elution and the total running time was 25.02 min. The mixed standards was prepared by 50 ppm of standard solution made from standard stock solution. This standard was diluted 2x with aquadest, put 50 µL and added 950 µL OPA. Solution was mixed, incubated for 3 minutes and injected 30 µL to HPLC.

Statistical analysis: Data of microbiological quality, acidity, pH, fatty acid and amino acid profile of kefir with three replications were analyzed by One Way ANOVA using Statistical Product and Service Solutions (SPSS) version 12.0 (2003).

RESULTS AND DISCUSSION

The average of LAB and yeast in kefir made from goat milk, goat milk and soy milk combination and soy milk showed no significant differences (Table 1). Total amount of LAB in kefir was higher than yeast. Kefir contains many species of bacteria, including *lactobacilli, lactococci, Leuconostoc* (*homofermentatif* and *heterofermentatif*), acetic acid bacteria, *Streptococcus thermophilus* and yeast (which ferment or not ferment lactose). The composition of the microflora in the kefir grains around 65-80% lactobacilli, yeasts 10-15% and 5-25% *Lactococci* and *Leuconostoc* spp. Conversely the proportion of kefir products approximately 80% *lactococci* and *Leuconostoc* spp., 10-15% yeasts and 5-10% *lactobacilli* (Rattray *et al*., 2011).

Total microbia in kefir grains ranged *Lactobacilli*: 10^8-10^9 CFU/ g; *lactococci* and *Leuconostoc* spp: 10^8-10^9 CFU/g and Yeast: 10^6-10^8 CFU/g. Estimation of the number of *lactobacilli* in the kefir product ranges from 10^7-10^8, 10^8-10^9 *lactococci* and *Leuconostoc* and yeast 10^5-10^6 CFU/g. The ratio of BAL and yeast in kefir grains and kefir products vary widely, depending on several factors such as fermentation time, temperature, agitation and type of milk used (Rattray *et al*., 2011). The total population of kefir bacteria in this study (Table 1) showed a larger number than the number of LAB and yeast, because the bacteria in kefir not only LAB but also acetic acid bacteria. Range of kefir bacteria in this study was 8.89 - 9.91 log CFU/mL correspond to a range of bacteria according to HadiNezhad *et al*. (2013), which is ranged from 7.85 to 9.53 log CFU/mL, after 28 days of storage at 4°C. According to Dadkhah *et al*. (2011), soy milk kefir with 2% kefir grain starter that fermented at a temperature of 25°C for 20 h containing *lactobacilli* 9.59±0:07, 9.49±0:07 *lactococci* and yeast 9.02±0.14 CFU/mL. Other study showed that amount of kefir microbes after fermentation for 24 hours, i.e., LAB reach of 4.3×10^8 (log CFU/mL) and 6.6×10^5 yeast CFU/mL (log CFU/mL) (Jascolka *et al*., 2013).

In this study, Kefir grain is used for milk fermentation was able to grow on soy milk. Dadkhad *et al*. (2011), found that LAB from kefir grains grow well in soy milk even though no extra carbohydrates are added. In contrast, Liu and Lin (2000) reported that LAB grew more slowly in soy milk even though there was an additional carbohydrate. That means, microbia in the kefir grain able to use carbohydrates present in soy milk. Soybean milk, which serves as a base for a variety of beverages, contains raffinose, stachyose, pentanal and n-hexanal, the former two may be responsible for flatulence after fermentation, whilst the latter two for a beany flavor. Bifidobacteria can be used for biotechnological processes that employ soymilk as the substrate (Scalabrini *et al*., 1998). The other study showed that *L. rhamnosus* 6013 can favourably utilize soy oligosaccharides as carbon sources (Liu *et al*., 2006).

Acidity and pH: The results of this study showed that titratable acidity of soy milk kefir was lower ($p<0.05$) than goat milk kefir and kefir combination of goat milk and soy milk. This is understandable since soy milk does not contain lactose as a substrate of LAB, resulting in a rather slow growth and the low production of lactic acid. Although the pH of soymilk kefir tends highest among other kefir, but not significantly different. The pH value of kefir is not only affected by lactic acid alone, but also other acids. In this study, pH of soy milk kefir was higher than the pH of the soy milk kefir, according to Dadkhah *et al*. (2011), with 2% kefir grains for 20 hours fermentation at a temperature of 25°C.

In the previous study it was suggested that milk kefir with 2 g/L kefir grains and fermented overnight at room temperature had a pH of 4.37 and 0.55% acidity (HadiNezhad *et al*., 2013). These pH values were close to the results of this study, however have a lower acidity than the acidity of goat milk kefir in this study. The other study had shown that kefir pH is 4.17 (±0.6) at the end of the 24 h fermentation period (Jascolka *et al*., 2013). According to Otles and Cagindi (2003), pH value of kefir was ranging from 4.2-4.6. In contrast, Purnomo and Muslim (2012) reported that, goat milk kefir made with 3% kefir grains during 18 h of incubation showed the pH

Table 1: Average of total bacteria, LAB and yeast (log CFU/mL) of kefir made from goat milk and soy milk

Microbia	100:0	75:25	50:50	25:75	0:100	Average
Total counts	9.91	8.74	9.04	9.77	8.89	9.27[ns]
LAB	7.00	6.86	7.75	7.00	7.72	7.27[ns]
Yeast	5.81	5.30	5.39	5.95	4.99	5.49[ns]

[ns]Not significant

Table 2: Acidity and pH value of kefir made from goat milk and soy milk

Chemical quality	100:0	75:25	50:50	25:75	0:100
Acidity	0.88[a]	0.90[a]	0.79[a]	0.63[a]	0.36[b]
pH	4.74[a]	4.46[a]	4.49[a]	4.47[a]	5.04[a]

The different letter in the same row indicates significantly different ($p<0.05$)

Table 3: Fatty acid profile (% of total fatty acids) of goat and soy milk

Fatty acids	Goat milk	Soy milk
Saturated fatty acids		
Caproic, C6:0	1.22	0.34
Caprylic, C8:0	6.58	1.90
Capric, C10:0	0.07	0.00
Lauric, C12:0	4.87	1.15
Myristic, C14:0	12.24	3.19
Palmitic, C16:0	32.42	21.02
Heptadecanoic, C17:0	0.54	0.25
Stearic, C18:0	9.28	7.50
Arachidic, C20:0	0.10	0.04
Heneicosanoic, C21:0	0.029	0.09
Behenic, C22:0	0.05	0.03
Lignoceric, C24:0	0.00	0.06
Unsaturated fatty acids		
Myristoleic, C14:1	0.23	0.02
Pentadecanoic, C15:1	0.74	0.22
Palmitoleic, C16:1	1.30	0.41
Oleic, C18:1	29.67	34.45
Linoleic, C18:2	0.42	0.07
Linolenic, C18:3	0.20	0.33
Arachidonic, C20:4	0.04	0.36
Nevronic, C24:1	0.01	0.15

as 4.92, the acidity was 0.5, 5.16 lactose and 0.86% ethanol. That pH values were higher and lower acidity than goat milk kefir using 2% kefir grains in this study.

Fermentation decreased pH value, while titratable acidity increased it. Acid production in kefir depends on the growth of microorganisms and their ability to ferment carbohydrates in milk and soy milk. The most dominant carbohydrates in soy milk are sucrose, raffinose and stachyose, whereas in milk is lactose (Pinthong *et al*., 1980). The addition of 1% glucose or lactose in soy milk produces lactic acid levels similar to those of milk kefir, so the addition of these carbohydrates can increase the ability of microorganisms in kefir to produce lactic acid in soy milk (Liu and Lin, 2000). Composition and nutritional value of kefir vary widely, depending on the source and content of milk fat, the composition of kefir grains and kefir processing technology. The dominant products formed during fermentation is lactic acid, CO_2 and alcohol. Diacetyl produced by *Streptococcus lactis* and acetaldehyde which is an aromatic component is also present in kefir. Vitamins are produced by yeasts and *Acetobacter* spp. can stimulate LAB, while some yeasts such as *Debaryomyces hansenii* and *Yarrowia lipolytica* assimilate lactic acid produced by LAB thereby increase pH and stimulate further growth of LAB (Rattray *et al*., 2011).

Fatty acids profile: Based on Table 4, the most abundant saturated fatty acids in kefir were palmitic, stearic and myristic, whereas the highest unsaturated fatty acid was oleic acid. These results were consistent with the findings of Marounek *et al*. (2012), the major FAs in goat milk were palmitic and oleic acids, followed by stearic and myristic acids (23.6, 30.3, 13.6, 8.6%). However oleic in this study was lower than palmitic, otherwise according to the study of Marounek *et al*. (2012), the oleic was higher than palmitic. Differences in forage may effect on milk fatty acid composition as stated by Chilliard *et al*. (2007) change the cow/goat diets potentially to modify milk fatty acids composition. In addition, the pasture has major effects by decreasing saturated fatty acid and increasing fatty acids as favorable for human health such as saturated fatty acids, oleic acid, n-6 or n-3-C18 to C22 polyunsaturated fatty acids, *trans* isomers of C18:1 and C18:2 and isomers of conjugated linoleic acid (CLA). The main dietary factors on bovine and caprine milk fatty acid composition, taken into account are the nature of forages, including pasture, the forage:concentrate ratio and diet starch content and the supplementation of dairy rations with crude or processed vegetable oils or oil seeds and vitamin E. A particular emphasis is given to studies on interactions between these dietary factors, which show that there is a considerable plasticity of ruminant milk fatty acid composition (Chilliard and Ferlay, 2004).

In this study, substitution 50% of goat milk with soy milk in kefir fermentation can decrese the concentration of caproic, heptadecanoic and behenic significantly ($p<0.05$), whereas the substitution of 25% goat milk with soy milk in kefir fermentation can decrease the pentadecanoic significantly. Caproic, heptadecanoic, behenic and pentadecanoic acid in soy milk (Table 3) were lower than goat milk kefir (Table 4). Subtitution of 50% goat milk with soy milk in kefir fermentation can increase oleic acid concentration significantly, because oleic acid in soy milk was higher than the goat milk

Table 4: Fatty acid s profile (% of total fatty acids) of kefir made from goat milk and soy milk

Fatty acids	100:0	75:25	50:50
Saturated fatty acids			
Caproic C6:0	1.27±0.207^{a}	1.08±0.052^{a}	0.78±0.144^{b}
Caprylic C8:0	6.63±1.331^{a}	5.78±0.739^{a}	4.65±0.833^{a}
Capric C10:0	0.06±0.008^{a}	0.05±0.007^{a}	0.04±0.006^{a}
Lauric C12:0	3.90±1.278^{a}	3.50±1.047^{a}	2.85±0.852^{a}
Myristic C14:0	10.07±2.214^{a}	9.65±2.239^{a}	8.66±2.159^{a}
Palmitic C16:0	29.95±4.294^{a}	29.40±4.482^{a}	28.65±4.675^{a}
Heptadecanoic C17:0	0.57±0.023^{a}	0.55±0.004^{a}	0.52±0.013^{b}
Stearic C18:0	17.64±7.197^{a}	15.25±5.032^{a}	14.41±4.090^{a}
Arachidic C20:0	0.10±0.019^{a}	0.11±0.025^{a}	0.08±0.017^{a}
Heneicosanoic C21:0	0.03±0.008^{a}	0.04±0.009^{a}	0.04±0.002^{a}
Behenic C22:0	0.04±0.001^{a}	0.04±0.006^{a}	0.03±0.002^{b}
Lignoceric C24:0	0.01±0.011^{a}	0.02±0.004^{a}	0.03±0.006^{a}
Unsaturated fatty acids			
Myristoleic C14:1	0.07±0.009^{a}	0.10±0.099^{a}	0.05±0.030^{a}
Pentadecanoic C15:1	0.74±0.023^{a}	0.69±0.290^{b}	0.63±0.053^{b}
Palmitoleic C16:1	0.79±0.419^{a}	0.65±0.532^{a}	0.56±0.076^{a}
Oleic C18:1	27.16±4.006^{a}	31.29±3.106^{a}	36.49±3.525^{b}
Linoleic C18:2	0.44±0.143^{a}	0.24±0.095^{a}	0.23±0.079^{a}
Linolenic C18:3	0.25±0.025^{a}	0.26±0.043^{a}	0.28±0.020^{a}
Arachidonic C20:4	0.07±0.022^{a}	0.09±0.018^{a}	0.10±0.064^{a}
Nevronic C24:1	0.02±0.004^{a}	0.02±0.015^{a}	0.03±0.023^{a}

Means in the same row followed by different letters are significantly different ($p<0.05$)

(Table 3). Belewu and Belewu (2007) reported that, oleic in soymilk was 23.15%, palmitic was 12.30% and no capric. These palmitic and oleic acid values of soy milk were lower than soy milk in this study, probably due to differences in source of soybean. Fatty acid composition of soybean oil of 18 important varieties from 43 locations in 16 states of the United States ranged from about 5 to 11% in linolenic, 43 to 56% in linoleic, 15 to 33% in oleic and 11 to 26% in saturated acids. Oil of all 18 varieties had wide ranges in composition at different locations in two crop years. Within each group varieties tended to maintain the same relative order of fatty acid compositions of oil at all locations in the two years (Collins and Sedgwick, 1959).

Epidemiological study showed that dietary monounsaturated fatty acid (MUFA): oleic acid have a beneficial effect on the risk of coronary heart disease (CHD). Compared with saturated fatty acid (SFA), MUFA has lower total and LDL cholesterol levels and relative to carbohydrate, they increase HDL cholesterol levels and decrease plasma triglyceride levels. Additional research is needed in humans and appropriate animal models to gain a better understanding of the effects of high-MUFA diets on atherogenesis. A diet, high in MUFA (versus a high carbohydrate diet) improves glycemic control in individuals with non-insulin-dependent diabetes mellitus (NIDDM) who maintain body weight. Individuals with elevated triglycerides or insulin levels may also get benefit from a high-MUFA diet (Kris-Etherton, 1999).

Lactic acid bacteria possess enzymes that are able to hydrolyze mono-, di- and triacylglycerols. The activity of the lipases depends strongly on the genera and bacteria species, as well as on the temperature and presence of calcium and magnesium ions. However, the activity of bacterial enzymes is crucial and it is not only the level of milk fat degradation that is affected by this phenomenon: also determined by this are the kind of milk, fat content and access of enzymes to the substrate. Also very important is the preliminary treatment of the raw material, because all technological treatments (cooling,

Table 5: Amino acids composition (ppm) of goat and soy milk

Amino acids	Goat milk	Soy milk
Aspartate	3,705.84	3,793.96
Glutamic acid	11,078.76	6,675.05
Serine	2,736.57	1,775.62
Histidine	1,721.95	1,070.01
Glycine+Threonine	3,239.29	2,497.28
Arginine+Alanine	4,795.20	3,555.79
Tyrosine	1,698.17	1,716.17
Methionine	908.53	321.79
Tryptophan+Valine	3,553.99	1,532.09
Phenylalanine	2,368.93	1,572.51
Isoleusine	1,945.13	1,074.37
Leucine	4,547.63	2,262.86
Lysine	5,714.91	2,817.48

Table 6: Amino acids composition (ppm) of kefir made from goat milk and mixture of goat milk and soy milk

Amino acids	100:0^{a}	50:50^{a}
Aspartate	2,953.13±538.52	3,316.81±260.87
Glutamic acid	8,971.35±1515.99	8,040.75±313.72
Serine	2,151.92±425.37	1,961.48±81.09
Histidine	1,406.85±266.64	1,261.99±66.36
Glycine+Threonine	2,609.92±464.94	2,670.79±191.43
Arginine+Alanine	3,796.89±721.57	3,861.43±221.58
Tyrosine	1,323.97±292.89	1,533.53±97.69
Methionine	807.15±198.91	567.52±32.83
Tryptophan+Valine	2,843.97±480.18	2,204.88±64.79
Phenylalanine	1,870.43±343.19	1,732.56±84.62
Isoleusine	1,523.33±274.50	1,299.20±41.54
Leucine	3,621.59±634.08	3,001.77±86.15
Lysine	4,654.70±911.09	3,748.35±211.93

^{a}Same letter indicated that amino acids profile in kefir made from goat milk (100:0) and combination of goat and soy milk (50:50) was not significantly different

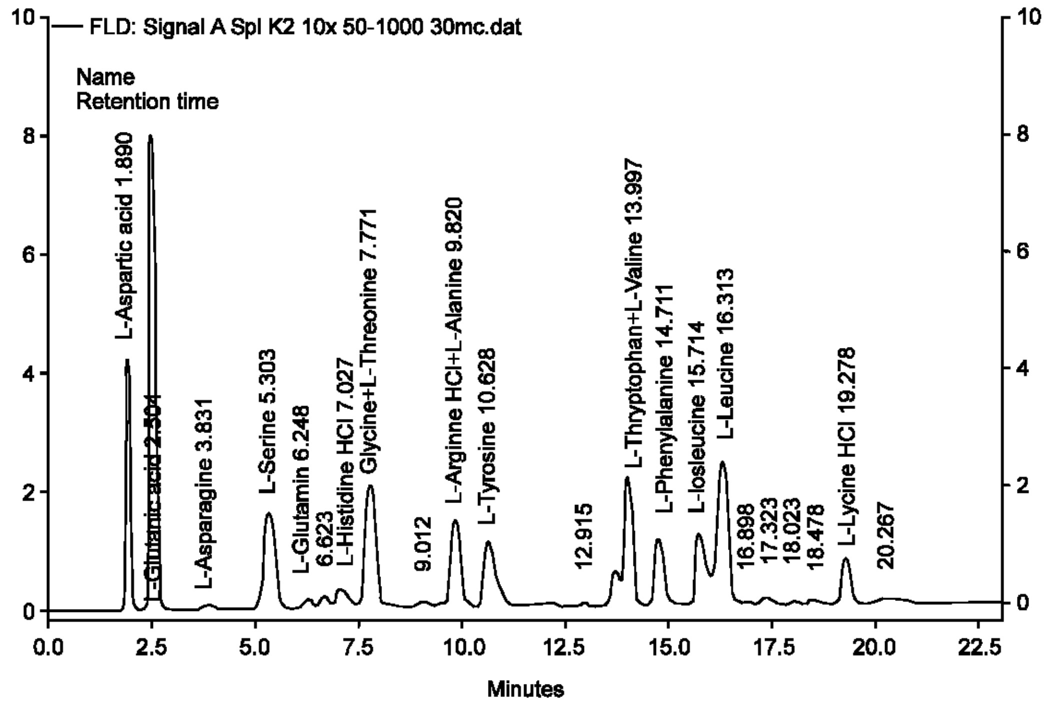

Fig. 1: Amino acids profile of goat milk

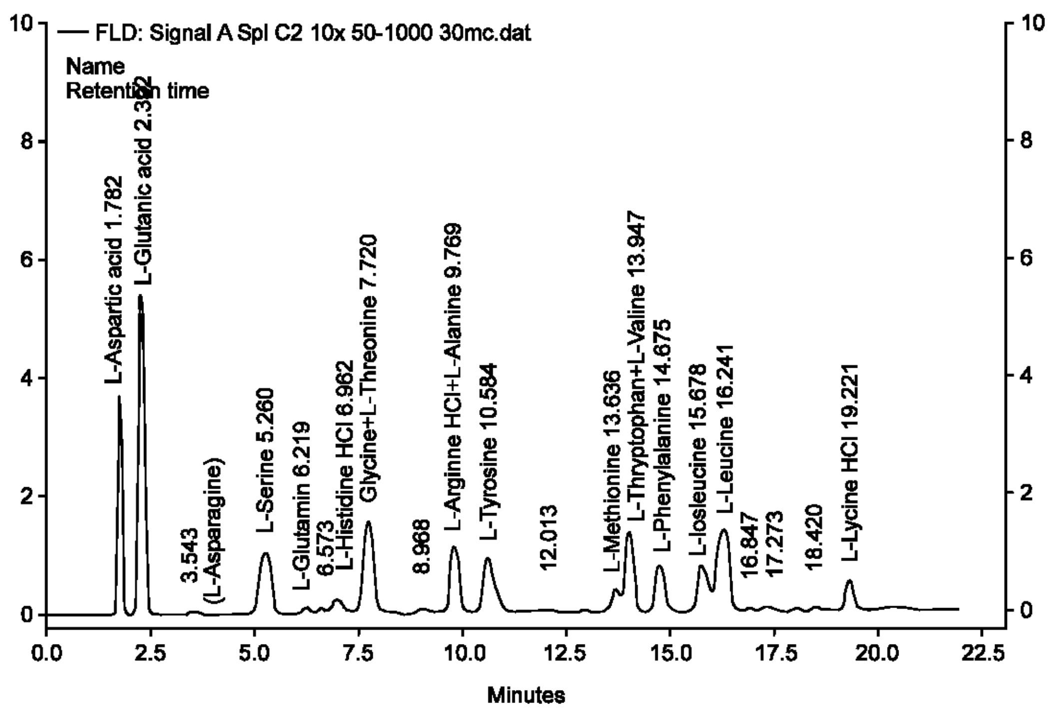

Fig. 2: Amino acids profile of kefir made from 50% goat milk and 50% soy milk mixture

homogenization and stirring) that cause the destruction of the milk fat globule membrane can lead to the risk of excessive lipolysis (Regula, 2007). Related to this study, stearic acid of kefir (Table 4) produced from goat milk or combination of goat and soy milk was higher than goat or soy milk (Table 3). This result was similar to the previous study using fermented beverages made from ewe's milk, showed that stearic acid (C18) and also

C12, C14, C16, C18:1 differed significantly in the level of free fatty acids. In addition, thermophilic bacteria cause more intensive liberation of those acids than mesophilic microorganisms and during the storage period the intensity and direction of changes in FFA level differed depending on the starter culture (Regula, 2007). Besides LAB, kefir grain also contains yeast such as *Candida* and *Torullopsis* species posses extracellular lipase activity that contribute to the flavor of he product, because of their hydrolytic activity on the milk constituents (Tamime, 2006).

Amino acids profile: Goat milk, soy milk and kefir samples contain different amounts of amino acids (Table 2). However, goat milk kefir and kefir made from 50% goat milk and 50% soymilk mixture showed not significantly different in amino acid composition. Actually, goat milk was more superior in amounts of amino acid, especially in glutamic acid, tryptopane+Valine, Leucine and Lysine. According to Wong and Watson (1995), whey protein has a substantial of glutamylcystein that supplies precursor of amino acid to formation of glutathion. This glutathion is thyol three peptides that it important in lysosomal and the other membrane stability, cell protections from radiation effect and free radicals.

Soy milk could substitute 50% of goat milk in kefir preparation to obtained the similar to goat milk kefir in amino acid composition. Goat milk kefir and kefir made from 50% goat milk and 50% soy milk mixture were composed of a good protein quality. In the previous study by Bensmira and Jiang (2012), the 70% peanut milk and 30% whole milk kefir mixture and whole milk kefir seem to have similar quantity of some amino acids like Threonine, Isoleucine and Leucine. Peanut- milk kefir and kefir from mixture of 70% peanut and milk with 30% reconstituted skimmed-milk powder at 12%, were richer in some amino acids such as Aspartate, Glutamate, Serine, Glycine, Arginine, and Phenylalanine than whole milk kefir. According to Zaman and Lin (2010), proteins can only be made when all the necessary amino acids are simultaneously available in the right proportions.

Chromatograph of amino acids profile of goat milk and kefir made from 50% goat milk+50% soymilk showed in Fig. 1 and Fig. 2.

Based on Table 5, 6 and Fig. 1-Fig. 2, amino acid concentration of goat milk was higher than amino acid in goat milk kefir or kefir prepared from 50% goat milk and 50% soy milk mixture. This may be during kefir fermentation the free amino acid was metabolized to another compounds, so tha reduced the free amino acid concentration. According to Tamime (2006), yeast in fermented milk kefir are able to degrade casein to small peptides and free amino acids, the latter are converted to alcohols, aldehydes, volatile acids, ester and sulphur-containing compounds (especially methionin, which is the precursor for volatile aroma compounds). In addition, branced-chain amino acids are precursors of aroma compounds such as isobutyrate, isovalerate, 3-metylbutanol, 2-metthylbutanol and 2-methylpropanal. The previous study showed that the highest degree of protein hydrolysis was in kefir (Wszolek *et al*., 2006). Beside the yeast, lactic acid bacteria also contributes to the aroma and flavor, frequently exert proteolityc activities and produce aromatic compounds (Hati *et al*., 2013). The other study showed that extracellular proteolytic activity was demonstrated for 49 Lactobacillis strains from kefir grain (Kabadjova-Hristova *et al*., 2006).

Conclusion: This study demonstrates that kefir made from combination of 50% goat milk and 50% soy milk has the similar in a total counts, LAB and yeast, acidity and pH, amino acid profile and even higher in oleic acid than kefir made from goat milk only. However, substitution of 50% goat milk with soy milk in kefir fermentation slightly lower of caproic, heptadecanoic, behenic and pentadecanoic acid.

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