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Organic Acids Formation During the Production of a Novel Peanut-Milk Kefir Beverage

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Abstract: The aim of this study was to evaluate the organic acids formation, ethanol and Exopolysaccharides (EPS) production during the fermentation of a novel beverage made from peanut-milk using a commercial kefir starter culture. Samples were collected at 0, 18, 22, 26 and 30 h of fermentation (final pH 4.2). Samples were analyzed for lactic, citric, acetic and pyruvic acids by HPLC. Ethanol production was analyzed using GC equipped with headspace autosampler. The results showed that the concentration of lactic and acetic acids increased from 0 to 1011 $\mu\text{g/g}$ and from 2 to 22 $\mu\text{g/g}$, respectively while the citric acid decreased from 2098 to 249 $\mu\text{g/g}$ over the same period. Similarly, peanut-milk kefir pH decreased to 4.21 after 30 h of incubation. The production of ethanol increased sharply reaching a mean value of 14 mg/mL. The level of EPS production reached its high content (525.83 $\mu\text{g/g}$) after 22 h of fermentation.

Key words: Exopolysaccharides (EPS), fermentation time, organic acids, peanut-milk kefir

INTRODUCTION

Kefir is a refreshing, naturally carbonated fermented dairy beverage with a slightly acidic taste, yeasty flavour and creamy consistency (Powell *et al.*, 2007). One of the basic parameters through which starter cultures for fermented milks are characterized is their ability to produce aroma compounds (Yuguchi *et al.*, 1973; Kneifel *et al.*, 1992). According to researchers, the aroma and flavour of yogurt and yogurt-related milks are basically due to the production of non-volatile and volatile acids and carbonyl compounds (Fernandez-Garcia and McGregor, 1994; Tamime and Robinson, 1999). Only one group of the volatile organic compounds (carbonyl) is believed to have a decisive influence on the resulting aroma owing to their comparatively high concentration (Imhof *et al.*, 1994, 1995).

During fermentation, the pH is reduced by cultures which convert milk sugar (lactose) into lactic acid (Tamime and Marshall, 1997).

Several researchers have investigated animal-milk kefir (Güzel-Seydim *et al.*, 2000; Magalhães *et al.*, 2010), but little work has been done on peanut-milk kefir. Therefore, the objective of this work was to determine the production of organic acids and volatile flavor compounds as well as EPS during peanut-milk kefir fermentation.

MATERIALS AND METHODS

Materials: This study was conducted during the period of November to February 2010 at State key laboratory of food science and technology, Jiangnan University, Wuxi, China. Freeze-dried kefir starter culture (BE010) was purchased from Wilderness Family Naturals (Finland, USA). According to the supplier, the starter culture contained lactic and acetic acid bacteria, as well as fungi. Skimmed-milk powder (Guangming, China) and the Spanish red-skinned peanut seeds were purchased from a local supermarket in Wuxi, China. Care was taken to ensure that good quality and mould-free seeds were selected.

Preparation of kefir working-culture: The resuscitation of freeze-dried starter culture and the preparation of working-culture were carried out as described by Bensmira *et al.* (2010).

Milk preparation: Peanut-milk was prepared using a method reported by Isanga and Zhang (2009). Briefly, sorted peanut seeds were roasted (130°C for 20 min), de-skinned, and weighed before being soaked in 0.5 g/100 mL NaHCO_3 for at least 12 h. After washing with water, the kernels were then mixed with water at a ratio of 1:5

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[peanuts (g):water (mL)] and transferred to a blender, wherein they were blended for at least 5 min. Finally, the resultant slurry that formed was filtered using a three-layered cheese cloth in order to yield peanut milk.

Kefir production: A mixture of 60% peanut-milk with 40% reconstituted skimmed-milk powder at 12% was stirred and warmed at 43°C for 30 min. A total of 3% (w/v) of sucrose was added to the sample as a sweetener and homogenised at 25 MPa [Homogeniser, (JHG-Q954)-P (60), Shanghai, China] and then pasteurised at 92°C for 15 min (Beshkova *et al.*, 2002) using a water bath, cooled down to approximately 25°C, inoculated with the working culture at 3% (v/v), and then fermented at 24 (Bensmira *et al.*, 2010) for various periods (18, 22, 26, and 30 h). Kefir samples were matured by storing at 4 for 24 h before further analysis.

Organic acids determination: The concentration of organic acids in kefir samples was measured according to Güzel-Seydim *et al.* (2000). Four grams of each sample were diluted with 25 mL 0.01 N H₂SO₄, vortexed for 1 min, filtrated using 0.45 µm filters (Acrodisc™, Gelman Sciences, Ann Arbor, MI). Filtered samples were then injected into a Agilent 1100 HPLC system equipped with an Diamonsil C₁₈ column (46 mm × 250 mm i.d., 5 mm) maintained at 30°C, and 0.05% CH₃OH was used as mobile phase. Lactic, citric, pyruvic, and acetic acids were detected using UV detection at 275 nm.

Ethanol determination: Samples were analyzed for ethanol production using GC equipped with headspace autosampler according to Güzel-Seydim *et al.* (2000).

Measurement of pH: The pH was monitored in kefir samples, prepared in triplicate for each batch, using a Delta 320 pH-meter (Mettler Toledo, Shanghai, China) previously calibrated with standard buffer solutions at pH 4.0 and 7.0.

Determination of Exopolysaccharides (EPS) production: The EPS production was determined following the method of Purwandari *et al.* (2007). Approximately 30 g of peanut-milk kefir was first centrifuged (Centrifuge 5804 R eppendorf AG 22331, Hamburg, Germany) at 11,000 *x g* at 4 for 4 min. The supernatant was collected and combined with two volumes of chilled ethanol and stored at 4 overnight. Consequently, the precipitate was collected by centrifugation at 2000 *x g* at 4 for 15 min (Centrifuge 5804 R eppendorf AG 22331, Hamburg, Germany). About 10 mL of distilled water was then added to dissolve

the EPS-containing precipitate, followed by the addition of 250 µL 80% trichloroacetic acid to precipitate the remaining proteins. The mixture was stored overnight at 4°C, centrifuged at 2000 *x g* at 4 for 15 min (Centrifuge 5804 R eppendorf AG 22331, Hamburg, Germany) and the supernatant was again collected. The EPS in the supernatant was finally collected using ethanol precipitation and cold storage as described above. The whole procedure for EPS purification using water and TCA was repeated once more. After that, the EPS was vacuum dried at 55 and weighed.

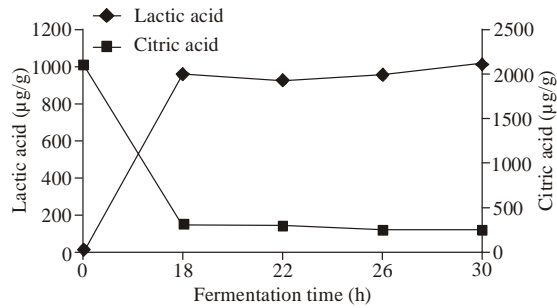
Statistical analysis: Analysis of variance (ANOVA) was carried out using SAS software (The SAS System for Windows, Version 8.1). The Duncan Multiple Range Test (DMRT) was used to determine the differences between data means at 5% significance level.

RESULTS AND DISCUSSION

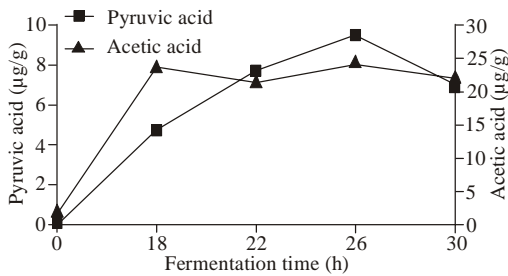
Organic acids: Volatile compounds are important contributors to the flavours of beverages, as they determine different desirable sensory characteristics (Arrizon *et al.*, 2006). Organic acids may occur in dairy products as a result of hydrolysis of butterfat (fatty acids), biochemical metabolic processes, or bacterial metabolism Güzel-Seydim *et al.* (2000). Figure 1 shows the time evolution of lactic, citric, acetic, and pyruvic acids during the fermentation of peanut-milk.

As can be observed, the lactic acid which is a common end product of bacterial fermentation was increasing during peanut-milk kefir fermentation to reach 1011 µg/g after 31 h (Fig. 1a). Similar results were reported earlier by Güzel-Seydim *et al.* (2000) who studied the production the organic acids like lactic acid during kefir fermentation using kefir grains. It has been reported earlier (Fernandez-Garcia and McGregor, 1994) that in plain yogurt which is produced by lactic acid fermentation, lactic acid content was 8760 µg/g. However, kefir has a lower lactic acid content than yogurt. This may be due to the preferential use of a heterofermentative pathway with a resultant production of CO₂.

The level of citric acid during peanut-milk kefir fermentation decreased significantly (*p*<0.05) from 2098 µg/g at 0 h to 249 µg/g after 32 h (Fig. 1a). Similar trends have been reported about kefir prepared from whole-milk (Güzel-Seydim *et al.*, 2000) and traditional fermented milk (Narvhus *et al.*, 1998). According to Güzel-Seydim *et al.* (2000), citric acid is the preferred substrate for acetoin and diacetyl formation by some lactic acid bacteria.



(a)



(b)

Fig. 1: Effect of incubation time on lactic, citrate, pyruvic, and acetic acid concentration

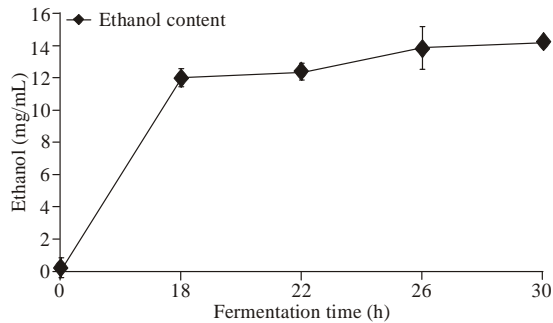


Fig. 2. Effect of incubation time on ethanol concentration

Pyruvic acid level increased significantly ($p < 0.05$) to reach 9.5 µg/g after 26 h of incubation, but decreased slightly upon completion of fermentation (Fig. 1b). Our results are in concordance to some extent with those reported by Güzel-Seydim *et al.* (2000). In addition, the concentration of pyruvate in cultured buttermilk has been reported to increase during fermentation (Marsili *et al.*, 1981).

The concentration of acetic acid was almost zero during the first 18 h of peanut-milk fermentation (Fig. 1b), before it increased reaching a final

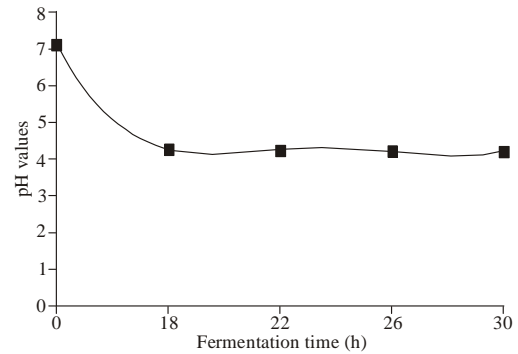


Fig. 3: Change in pH during peanut-milk kefir fermentation

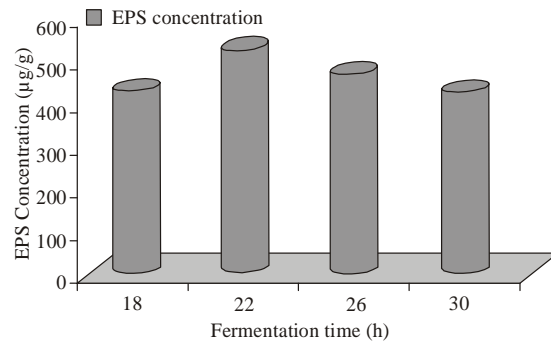


Fig. 4: Effect of incubation time on EPS concentration

concentration of 22 µg/g. Similar observations have been made by other authors (Rea *et al.*, 1996; Magalhães *et al.*, 2011) during whey-based and skim-milk kefir fermentation. Magalhães *et al.* (2010) attributed the presence of acetic acid in kefir beverage to the heterofermentative pathway used by lactic acid and acetic acid kefir culture.

Ethanol content: Yeasts are primarily responsible for the alcohol production in kefir Güzel-Seydim *et al.* (2000). Figure 2 shows the time evolution of ethanol during the fermentation of peanut-milk. It is interesting to notice that ethanol content in peanut-milk kefir increased significantly ($p < 0.05$) by 18 h of fermentation with a final concentration of 14 mg/mL. The final ethanol concentration was within the range of that reported previously by Papapostolou *et al.* (2008) for the production of kefir using lactose and raw cheese whey as substrates. Higher alcohol content may be associated with a yeasty flavor; though authentic kefir does have a very slight yeasty flavour (Vedamuthu, 1977).

pH evolution: The evolution of pH in kefir during fermentation is shown in Fig. 3. The pH decreased first

drastically during the first 18 h and then slightly afterwards. Similar changes in pH were observed for milk gels acidified with glucono- δ -lactone or starter culture (Rea *et al.*, 1996; Lucey *et al.*, 1998; Güzel-Seydim *et al.*, 2000; Haque *et al.*, 2001; García Fontán *et al.*, 2006).

The pH drop in kefir during fermentation might be attributed to a decrease in lactose content and a consequent increase in lactic acid content. Moreover, the pH of kefir prepared by incubating milk at 24°C for 18 h was found to be in the range reported by Odet (1995).

Exopolysaccharides (EPS) production: As can be seen, the lowest (427.66 $\mu\text{g/g}$) and highest (525.83 $\mu\text{g/g}$) amounts of EPS was observed for kefir samples fermented for 30 and 22 h, respectively (Fig. 4). Kefir samples incubated during 22 h had significantly higher ($p < 0.05$) EPS content than other samples. In this study, the decrease in EPS production recorded after 22 h may be due to the presence of glyco-hydrolases capable of hydrolyzing EPS and liberating monomers. This observation coincides with that of Pham *et al.* (2000) and Lin and Chang Chien (2007) as well as Bensmira *et al.* (2010).

CONCLUSION

In conclusion, kefir manufactured using peanut-milk as raw material showed a production of flavor components different than those noted in yogurt but similar to some extent to those reported for kefir produced from animal milk. In addition, Exopolysaccharides (EPS) production and pH evolution were significantly affected by Fermentation time.

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