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Labneh with probiotic properties produced from kefir: development and sensory evaluation

Daniela Mayumi Usuda Prado ROCHA[1]*, Joice de Fátima Laureano MARTINS[2], Thanise Sabrina Souza SANTOS[1], Ana Vládia Bandeira MOREIRA[1]

Abstract

The labneh or labaneh is a popular fermented milk in the Middle East. Another fermented product that deserves special mention is kefir since it has probiotic activity and unique sensory, nutritional, and therapeutic properties. The aim of the present study was to develop a functional probiotic labneh using kefir as a fermenting agent and to perform a sensory analysis of the obtained product. Kefir was obtained by growing grains in pasteurized milk. Samples of skimmed and whole labneh were prepared from the inoculation of 5% kefir milk (skimmed/whole) at 28 °C for 24h, followed by cooling (12-18h) and whey drainage (12-24h), both at 4 °C. Sensory analysis was performed with 70 untrained panelists using a 9-point scale hedonic in the acceptance tests. The paired t-test was used to compare the differences between the means of the scores obtained, with the significance level of 5%. The labneh prepared showed good acceptance by the judges, and the whole labneh samples had the highest scores in the acceptance test. Further studies on the analysis of microbiological viability, nutritional composition, and determination of shelf life, also to improve acceptability of the low-fat version of the product, are needed.

Keywords: strained kefir; Greek yogurt; fermented milk; functional foods; taste; texture.

1 Introduction

1.1 Labneh

Labaneh or labneh (concentrated yogurt) is a popular fermented milk product in the Middle East, which has a significant role in family nutrition (Abd El-Salam et al., 2011). It is also known as skyr (Iceland), shrikhand (India), and Greek yogurt (Greece and other countries) (Ramos et al., 2009).

Labneh has a cream or white color, a soft and smooth body, good spreadability with little syneresis, a clean flavor, and slight acidity (Nsabimana et al., 2005). It is considered an intermediate product between fermented milks and immature cheeses with high water content, such as quark, boursin, and petit suisse, with physical characteristics similar to those of these products due to the syneresis process (Ramos et al., 2009).

Nutritional and therapeutic properties of labneh are considered similar to or even better than those of yogurt. Labneh has 2.5 times higher protein content, 50% more minerals, and a considerably larger number of viable microorganisms than common yoghurt (Nsabimana et al., 2005). In addition, the lactose concentration of labneh is low (approximately 6%) due to its fermentation into lactic acid, which makes it more suitable for use by lactose intolerant individuals (Nsabimana et al., 2005; Özer & Robinson, 1999).

Due to its high total solids content, labneh may be considered a suitable matrix for probiotics since it offers protection when added to them (Abd El-Salam et al., 2011).

1.2 Probiotics

The term probiotic is used to designate live microorganisms, which when administered in adequate amounts, exert positive influences on the host's health (Guarner et al., 2012). The beneficial effects of probiotic use on health are associated with the maintenance of a healthy intestinal microbiota by its regular consumption (Yerlikaya, 2014).

When used in foods, probiotic organisms must be able to survive passage in the gastrointestinal tract, resisting the action of the gastric juice and bile exposure. They are then able to proliferate and colonize the digestive tract to display their beneficial effects. Moreover, probiotics should be safe and effective, and also maintain their effectiveness during the shelf life of the product (Food and Agriculture Organization of the United Nations & World Health Organization, 2006).

The main mechanisms of action of probiotics include: an improved intestinal mucosal barrier (Persborn et al., 2013; Zeng et al., 2008); increased adhesion to the intestinal lumen interface and concomitant inhibition of the adhesion of pathogens (Balgir et al., 2013; Lara-Villoslada et al., 2007); competition with pathogenic microorganisms for binding sites, nutrients, and colonization (Hojo et al., 2007; Mehling & Busjahn, 2013); production of antimicrobial metabolites including the synthesis of bacteriocins, hydrogen peroxide (H_2O_2), and organic acids such as lactic acid responsible for the acidification of the environment (Madureira et al., 2013; Messaoudi et al., 2013; Tomás et al., 2003); promotion of innate

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[1]Departamento de Nutrição e Saúde, Universidade Federal de Viçosa – UFV, Viçosa, MG, Brasil
[2]Departamento de Tecnologia de Alimentos, Universidade Federal de Viçosa – UFV, Viçosa, MG, Brasil
*Corresponding author: dmuprocha@gmail.com

and adaptive immune responses (Ganguli et al., 2013; Yan & Polk, 2011); and many other unknown modes of action.

These mechanisms lead to the modulation of the microbiota and/or inhibition of colonization by potential pathogens, and bacterial translocation across the intestinal wall (Eizaguirre et al., 2011; Wine et al., 2009; Wong et al., 2013).

A minority of bacterial species meet the definition of a probiotic microorganism, but among them are those of the genera *Lactobacillus* and *Bifidobacterium*, commonly found in the human intestine and widely used in functional foods and dietary supplements (Bermudez-Brito et al., 2012; Howarth & Wang, 2013; Wohlgemuth et al., 2010).

These probiotic bacteria may contribute to the human health in various ways including xenobiotic detoxification, biosynthesis of vitamin K, metabolic effects of the fermentation of nondigestible fibers (Resta, 2009), effect on gastrointestinal transit on intestinal peristalsis (Matsumoto et al., 2012), competition with pathogenic microorganisms, and modulation of the immune response.

Moreover, *Lactococcus*, *Streptococcus*, *Enterococcus*, nonpathogenic strains of *Escherichia coli*, and certain bacilli and yeast strains such as *Saccharomyces boulardii* can also have probiotic activity; thus, they are considered probiotic-like microorganism (Howarth & Wang, 2013; Iannitti & Palmieri, 2010; Magalhães et al., 2011a). They are different from probiotics in that they are not found in the gastrointestinal tract of the host (Magalhães et al., 2011a).

Foods presenting these microorganisms may be considered functional foods, i.e., besides nourishing the body, these products have biologically active components that contribute to the maintenance of good health and wellness, while reducing the risk of diseases (Mohammadi & Mortazavian, 2011; Saad et al., 2013).

Dairy products, especially fermented products, are the main and most popular vehicles of probiotics due to their compatibility with probiotic microorganisms, pleasant sensory profile, and high consumption around the world (Mohammadi & Mortazavian, 2011).

1.3 Kefir

Kefir, a product with probiotic action that deserves special attention, is a fermented beverage originating from the Caucasus mountains (Grishina et al., 2011) which provides natural probiotic microorganisms in large amount, especially *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, lactic acid bacteria, and yeasts (Guzel-Seydim et al., 2011).

Traditionally, kefir is produced by inoculating kefir grains in milk for 18 to 24h at a temperature of 20 to 25 °C (Leite et al., 2013; Sarkar, 2008). At the end of the fermentation process, the kefir grains are recovered and reused, a step which is different from those in the production of other fermented milk products. Moreover, kefir does not result from the metabolic activity of a single or a few microbial species (Guzel-Seydim et al., 2011; Leite et al., 2013).

The fermentation agent of kefir known as the "kefir grain" is a gelatinous mass of protein and polysaccharide structure containing a variety of species of lactic acid bacteria, acetic acid bacteria, and yeasts, which make up a natural microbial ecosystem (Farnworth, 2005; Guzel-Seydim et al., 2011; Montanuci et al., 2012; Sarkar, 2008). Due to the large quantity and complex nature of the associations between the species involved, the microflora of kefir grains has not been fully elucidated yet (Pogačić et al., 2013).

Kefir has a distinct flavor due to the presence of various compounds produced during the fermentation process (Farnworth, 2005). Lactic acid is the major metabolite produced; other important metabolites produced are carbon dioxide and ethanol at low concentrations and flavor components, such as acetaldehyde and acetoin (Guzel-Seydim et al., 2011; Pogačić et al., 2013); bioactive peptides, vitamins, exopolysaccharides, and bacteriocins (Bergmann et al., 2010; Pogačić et al., 2013). These compounds can act independently or in combination to provide the many beneficial health effects attributed to the consumption of kefir (Farnworth, 2005).

1.4 Benefits of kefir

The name kefir is derived from the Turkish word *keyif* meaning "good food". In addition to this designation, kefir is also known by several other names, such as kefer, kefyr, kephir, kepi, kiaphur, kipi, and knapon (Yerlikaya, 2014).

Traditionally consumed in Eastern European countries, Russia, and Southeast Asia, kefir has also become popular in many countries due to its probiotic activity, unique sensory characteristics, and nutritional and therapeutic properties (Leite et al., 2013).

Among the health benefits associated with kefir are: antimicrobial property and cicatrizing activity (Rodrigues et al., 2005), antimutagenic and antioxidant effect (Liu et al., 2005), hypocholesterolemic properties (Huang et al., 2013), beta-galactosidase activity (Leite et al., 2013), anti-allergenic properties, anti-inflammatory activity, and stimulation of the immune system (Lee et al., 2007; Vinderola et al., 2006).

1.5 Development of new probiotic products

There is a growing trend for the consumption of healthy and attractive foods by many segments of the population that increasingly seek pleasure of eating combined with health benefits and life quality. Accordingly, various dairy products with probiotic activity, especially fermented products, have been developed (Komatsu et al., 2008; Mohammadi & Mortazavian, 2011).

Yogurt is perhaps the most common and familiar fermented product to consumers in different countries; kefir, however, is less well-known, but it has bioactive compounds that result in its unique health benefits (Farnworth, 2005).

Based on this information, the present study was conducted to develop a functional labneh, made from skim and whole milk, with probiotic activity using kefir as the fermenting agent, and to evaluate its acceptance by sensory analysis.

2. Materials and methods

2.1 Origin of kefir grains

The kefir grains used in this study were obtained in the city of Viçosa, Minas Gerais, Brazil, and were of artisanal origin.

2.2 Production of kefir

Kefir grains were inoculated into different types of fluid pasteurized milk (whole and skimmed milk, with 3.0% and 0.5% of total fat, respectively), previously heat treated at 28 °C. The total amount of 5% of grains were used for fermentation, a percentage considered ideal for adequate production of ethanol and volatile acids (Sarkar, 2008). The inoculate was maintained at 28 °C ± 2 °C, room temperature, for 24 hours without agitation (Santos et al., 2013). At the end of this process, the grains were recovered using a sieve, washed with distilled water, and used for a subsequent fermentation run in another nutrient substrate. This process was performed three consecutive times for activation of the grains and to ensure equilibrium of the microbiota (Santos et al., 2013). At the end of this stage, the kefir obtained was separated for use in later stages of the pilot study in physicochemical analyses and development and production of labneh.

2.3 Pilot study: physicochemical analysis of the kefir

Labneh was initially prepared in a pilot study to identify the fermentation time that did not exceed the acidity of 1.0g of lactic acid per 100g of the product, as recommended for this type of fermented milk (Brasil, 2007).

Acidity was determined by titrating 10mL of kefir with 0.1N of NaOH and 1% phenolphthalein (Instituto Adolfo Lutz, 2008) for kefir prepared with different fermentation times.

2.4 Labneh preparation

Labneh preparation from skimmed and whole milk was performed experimentally, as shown in Figure 1.

Skimmed and whole labneh were supplemented with previously ground dried tomatoes (30%, w/w), garlic (0.2%, w/w), and oregano (0.4%, w/w). The ingredients were mixed until homogenization of the product, and the mixture was then stored refrigerated overnight until sensory analysis.

2.5 Sensory analysis

Sensory analysis was performed in individual partitioned booths with neutral colored walls under white lightening.

Untrained panelists, chosen by convenience, received randomly 2 coded samples (each containing approximately 15g of skimmed and whole labneh/portion), water to rinse their mouth, the evaluation questionnaire, and a pencil and an eraser. The parameters evaluated were appearance, texture, flavor, and overall impression. A structured 9-point hedonic scale ranging from 1 (extremely disliked) to 9 (extremely liked) was used in the preference tests (Peryam & Pilgrim, 1957). The panelists were also asked about their intent to purchase the products.

Criteria for inclusion in the study were: being 18 or older, agreed to voluntarily participate in the study, and signed the Informed Consent Form. Those who had any kind of allergy or intolerance to any component of the product (milk, dried tomatoes and spices) were not allowed to participate, and the potential participants were informed about this restriction.

The present study was approved by the Ethics Committee on Human Research of the Federal University of Viçosa (case number 339.319), on July 5, 2013, as required by Resolution 466/12, which deals with ethics in research involving humans (Brasil, 2012).

2.6 Statistical analysis

Samples characterization and purchase intent were expressed as mean ± standard deviation or frequency (%). The paired t-test was used to compare the statistically significant differences between the means of the samples (Lawless & Heymann, 2010), with the significance level of 5%. The software

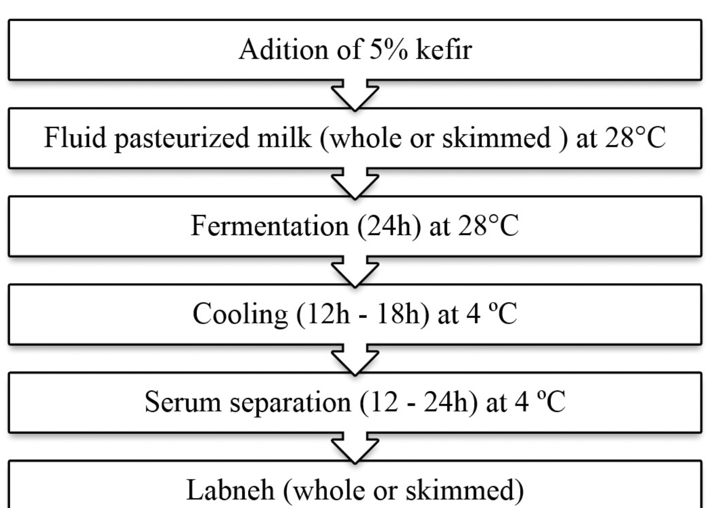

Figure 1. Flowchart of kefir labneh preparation.

SPSS (Statistical Package for the Social Sciences version 20.0 for Windows) was used in the statistical analysis.

3. Results and discussion

3.1 Physicochemical analysis of kefir

In the pilot study, there was an increase in acidity during the fermentation of kefir (Figure 2). This result is of great importance because lactic acid is a fermentation product that not only adds pleasant taste to kefir but also inhibits the growth of undesirable microorganisms or pathogens due to increased acidity of the kefir (Magalhães et al., 2011b).

According to the standards of identity and quality of fermented milks, the lactic acid concentration in kefir should be less than 1.0g of lactic acid per 100g of the final product (Brasil, 2007). The values found after 24 hours of fermentation are within the range established: whole and skimmed kefir showed maximum acidity values of 0.75 and 0.91% lactic acid, respectively.

3.2 Labneh

Preparation of labneh from kefir can be easily done at home. However, it showed low yield due to the syneresis process in which there was a loss of approximately 60% and 75% of the initial volume in the kefir made from whole and skimmed milk, respectively.

At the industrial scale, this procedure can be performed using modern techniques of centrifugation, recombination technology, and ultrafiltration methods (Kaaki et al., 2012), minimizing protein losses from the product (Santos et al., 2012).

The price of 30g of the product ranges from US$ 0.31 to US$0.23 for the skimmed and whole versions both with dried tomatoes and spices, respectively, considering the price of the ingredients purchased from local supermarkets.

3.3 Characterization of the judges

A total of 70 untrained panelists (52 women (74.3%) and 18 men (25.7%)) participated in the study. The age of the participants ranged from 18 to 65 years, with an average of 26.33 ± 9.45 years. Regarding their level of education, only one individual (1.4%) reported having only the elementary level of education, 41 (58.6%) completed high school, and 28 (40%) completed higher education.

3.4 Acceptance test

The panelists gave higher scores to the whole labneh than to skimmed labneh for appearance (7.87 vs 7.43; $p<0.001$), flavor (7.86 vs. 7.13; $p=0.001$), texture (7.86 vs 7.46; $p=0.001$), and overall impression (7.99 vs 7.37; $p<0.001$). Differences in the average scores were considered significant.

Greater frequency of scores above 5, corresponding to the term "indifferent", for the attributes (appearance, texture, flavor, and overall impression) was observed for the whole labneh. Additionally, both products received a high percentage of scores above 5 for all attributes evaluated (Figure 3).

As for purchase intent, if the products were marketed, 62 judges (88.6%) said they would buy the whole labneh, and 36 (51.4%) would buy the skimmed labneh. Approximately 41.4% of the judges (n=29) would buy both products, and only 1.4% (n=1) of the participants said that they would not buy either product.

It may be considered that both products were well accepted by the judges, and the whole labneh was the product of greater acceptance, with higher score in all attributes evaluated.

The taste of the product appears to be related to the lower score attributed to skimmed labneh. This version had a more pronounced acidity, resulting from the production of lactic acid during fermentation of the product, as remarked by the judges

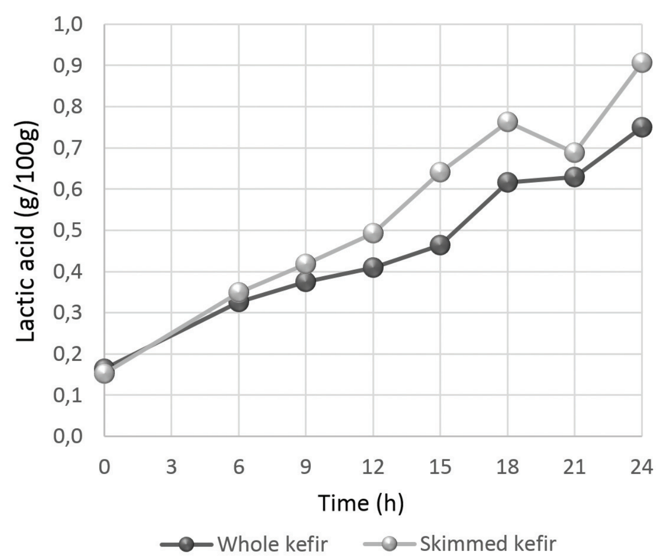

Figure 2. Production of lactic acid during fermentation of kefir at 28 °C.

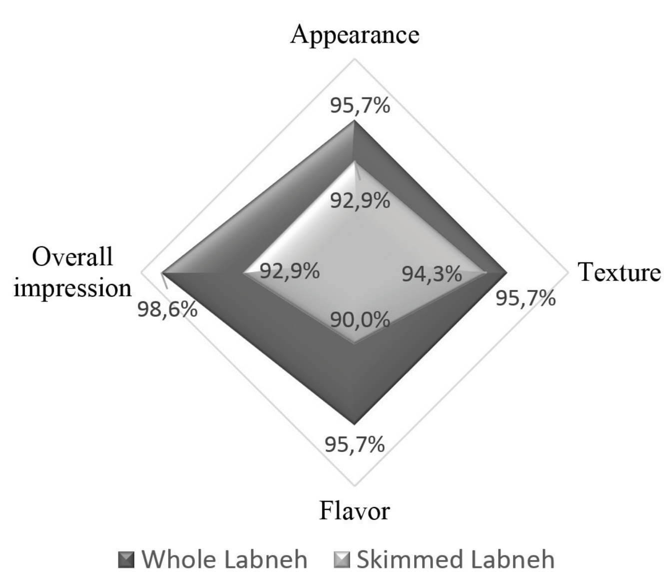

Figure 3. Frequency of ratings above "Indifferent" for Labneh samples.

in the evaluation sheets. In another sensory analysis of cocoa-based kefir, the samples with lower acidity also showed better acceptance (Puerari et al., 2012).

A similar study of comparative sensory evaluation of buffalo milk yogurt indicated preference for whole milk yogurt (3.0% fat) over skimmed milk yogurt (0.5%) (Cunha Neto et al., 2005). Moreover, a comparison of different types of commercial labneh in Lebanon, showed higher rating for whole labneh in terms of appearance, texture, flavor, and overall impression with decreased acceptability as the fat percentage was reduced (Kaaki et al., 2012). This fact reinforces the need for alternatives to improve acceptance of the low-fat versions of these products.

Daily consumption of probiotics containing 1-10 billion CFU/day (Guarner et al., 2012) is recommended to confer the associated health benefits. Thus, quantification of microorganisms present in the final product and the analyses of microbiological viability, shelf life, and nutritional composition are essential for an effective guidance on its consumption and commercialization.

4 Conclusions

Considering the high acceptability of the elaborated labneh, it can be inferred that kefir can be used for its preparation. It is a product with interesting probiotic activity for artisan preparation since it is a low-cost product and is relatively easy to prepare. Therefore, its use should be recommended to encourage consumption, especially among the low-income population with little access to products with probiotic activity. The analysis of microbiological content and viability, nutritional composition, and determination of the shelf life and also improvement of the acceptability of a low-fat version of the product are topics for future studies.

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