The Influence of Honey Addition on Microbiological, Physicochemical and Sensory Characteristics of a Kefir-Type Product During Shelf-Life

Adriana PĂUCEAN, Elena MUDURA, Mirela Ana Maria JIMBOREAN, Simona MAN

University of Agricultural Sciences and Veterinary Medicine, Faculty of Agriculture, Mănăstur Street 3-5, 400372, Cluj-Napoca, Romania
Department of Food Science and Technology, apauecan@yahoo.com

Abstract. In order to obtain a kefir-type product, a mesophilic starter culture, containing Lactococcus lactis ssp. and Leuconostoc ssp., and kefir yeast, Debaryomyces hansenii was used to inoculated 1.8% skimmed, pasteurized milk which was fortified with acacia honey at different levels: 1%, 2.5%, 4%. The addition of honey to kefir-type products did not significantly influence (p>0.05) the growth and survival of lactococcus bacteria. In any of the studied levels 1%, 2.5%, 4%, the honey addition increased the microbial density of lactococi at all the sampling intervals and probiotic bacteria were present at a high level during the product shelf-life. Honey had no effect on pH and lactic acid levels of the final products. Honey at 2.5% and 4% significantly decreased the syneresis (p<0.01) and increase the kefir’s consistency. The sensory evaluation indicates that 2.5% honey added is the optimum level for the kefir-type product.

Keywords: Lactococcus, fermented milk, honey, physicochemical and sensory properties.

INTRODUCTION

Fermented milk is one of the most popular fermented foods and has been traditionally consumed for a long time in many countries. World-wide there are many types of dairy products. Generally, these different fermented products are classified by the fermentation and processing methods that depend on the microorganisms used. Each type of product is obtained with specific microorganisms but there are similarities between manufacturing technologies. A fermented dairy product is made from milk by the action of specific microorganisms that cause clotting and reducing the pH.

Kefir is an acid-alcoholic fermented milk. Kefir and products manufactured similar to kefir are an important part of the fermented milks market. Thousands of people, all over the world, consume kefir-type products every day. The characteristic flavour of kefir products is a result of a complex interaction between the milk matrix and compounds formed during the metabolic activity of the applied bacteria culture and especially the yeast culture. Kefir typically contains both ethanol and CO₂. The content of CO₂ and ethanol give kefir products a refreshing and sparkling sensation. The typical flavour of kefir is developed by the yeast strains in particular. Mesophilic cultures are composed of several species of lactic acid bacteria of which each has a specific role in the formation of the final taste, texture and flavour. (Tamine, 1999).

According CODEX STAN 243-2003, kefir must contain an abundant and viable microflora of starter origin at the time of consumption: minimum 10⁷ cfu/mL for lactic acid bacteria and minimum 10⁴ cfu/mL for yeasts.

Flavoured fermented milks are composite milk products, which contain a maximum of 50% (w/w) of non-dairy ingredients (i.e. nutritive and non-nutritive sweeteners etc.) The non-dairy ingredients can be mixed in prior to/or after fermentation. The microbiological
requirements which are verified for plain fermented milk are also valid for flavoured fermented milks up to the date of minimum durability (CODEX STAN 243-2003).

Plain dairy products have sour taste, therefore flavouring or sweeteners have been added to improve the flavour balance (Sert et al., 2011; Varga, 2006). Because of their taste and flavour, kefir-type products may be rejected by some consumers, especially by children (Dogan, 2010). The addition of flavor generally increases the sensory acceptance of the yogurt. Honey, sucrose, corn syrup are some of the flavors that are quite acceptable.

Due to its functional properties, honey has been gaining interest as a substitute sweetener in foods such as yoghurt. (Roumyan et al., 1996; Chick, 2001). Honey in combination with milk provides excellent nutritional value and it is recommended for children (Garanis-Papadatos and Katsas, 1999). Honey is a rich source of carbohydrates (fructose, glucose, maltose, sucrose etc.). Its low pH value, due to a variety of organic acids, makes honey compatible with much food (Varga, 2005). Some authors reported a good antimicrobial activity against pathogens such as Bacillus cereus, Listeria monocytogenes, Escherichia coli, Mycobacterium tuberculosis, Salmonella ssp., Shigella ssp., Staphylococcus aureus etc. Antibacterial factors which are responsible for the antibacterial action are: the osmotic effect of sugars, pH and honey acids, hydrogen peroxide and other compounds: phenolics, carbohydrates, proteins, methylglyoxal. (Molan, 1992 a, b; Molan, 1997; Bogdanov, 1997)

Honey enhances the growth of dairy starter cultures in milk and milk products. Especially species with week growth rates in milk such as bifidobacteria are usually fortified by growth enhancers or by honey. Honey supported the growth of some bacterial strains (Lactobacillus, Streptococcus, Bifidobacterium) the authors conclude that various oligosaccharides found in honey may be responsible for the enhanced lactic acid production by bifidobacteria (Bogdanov, 2008). The application of honey as a food additive is based on its manifold properties. The antibacterial effect of honey counteracts microbial spoilage of food. The antioxidant effect of honey prevents oxidation of food during storage. Other physical and sensory properties make honey a good candidate for an additive to a wide variety of food: good sensory and rheological properties. A study conducted at University of Georgia reported the effect of honey addition on the four basic tastes (sweet, sour, bitter and salty taste). Due to its property to decrease the sourness of solutions and to improve consumer acceptability of sour products, honey can be incorporated into fermented dairy products.

The aim of this study was to assess the capacity of a mesophilic starter culture, containing Lactococcus lactis ssp. and Leuconostoc ssp., to grow on skimmed milk supplemented with acacia honey at different concentrations 1%, 2.5%, 4% (w/v) in order to obtain a kefir-type product with good physicochemical properties and to improve its sensory characteristics.

MATERIALS AND METHODS

Microorganisms

The microorganisms used to form the inoculum were represented by a bacterial starter culture and a yeast cultures. We used a mesophilic bacterial culture FD-DVS CHN-22 (provided by Chr. Hansen) of Lactococcus lactis (ssp.cremoris, ssp. lactis and ssp lactis biovar diacetylactis), Leuconostoc mesenteroides subsp. cremoris, and containing $10^{10}$ colony forming units per mL (cfu/mL). The yeast culture consisted on $10^{10}$ cfu/ml Debaryomyces hansenii kefir yeasts LAF 3, provided by Chr. Hansen. Both of these starter cultures were freeze-dried powders and Direct Vat Set (DVS). The density of microorganisms was determined by direct counting in the Thoma Chamber.
**Acacia (Robinia pseudo-acacia) honey origin**
The acacia honey used in this study was obtained from a local beekeeper (Transylvania region). The honey was stored in an air-tight jar in dark place at room temperature for 9 months. The microbial quality was acceptable: total microorganisms, yeast and mould, did not exceed $10^2$ cfu/g. Coliforms and aerobic spores were negative in 10 g. The pH value of acacia honey was 3.9. Acacia honey had a pale yellow colour and a delicate flavour of acacia blossoms.

**Manufacture and storage of Kefir-type products**
In the pilot station of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania, 1.8% skimmed, pasteurized cow milk (4 L) was cooled at 30°C. The milk quantity was divided in four equal portions (1 L) and each portion was fortified with pasteurized acacia honey (60°C/30 min) at levels of 0% (control sample) 1%, 2.5% and 4% (w/v). Next the milk with and without honey (control sample) was inoculated with the inoculum previously presented. The volumetric ratio, expressed in mL, between milk and microorganisms, milk/mesophilic lactic culture/kefir yeast was 1000:1:2. After inoculation, the manufacturing process operations were: incubation at 29-30°C for 12 hr, pre-cooling at 18-20°C for 1hr, cooling again at 4-6°C for 10 hr. The product was stored up to 21 days at 0-4°C. Three replications of all batches and samples were performed.

**Microbiological analysis**
In order to monitor the density and viability of the lactic bacteria, a tryptone-water (Difco) mixture (1 g/L) was used to prepare the dilutions for the microbiological analysis. The standard pour plate method was employed to determine the counts of microorganisms. The results were expressed as a logarithm of colony forming units (log cfu/mL) (IDF 1992). *Lactococcus* bacteria were counted in M17 medium (Difco), a selective medium for lactococci (Terzaghi & Sandine, 1975), at pH 7.2 ± 0.2, after incubation under anaerobic conditions, 5% (v/v) CO$_2$, at 30 °C for 18–24 hr. The *Lactococcus* bacteria identified on the basis of colonial type were confirmed by microscopic examination using a Zeiss Axio Observer microscope, examined at 40X magnification. Members of species *L. lactis* are G-positive, mesophilic, facultative anaerobes, non-spore-forming and non-motile. They have a spherical or ovoid morphology and occur in pairs or chains. Three replications of all measurements were carried out for each sample.

**Physicochemical analyses**
Three replications of all measurements (pH, acidity and syneresis) were carried out for each sample.

**pH measurement**
The pH values of samples were determined at room temperature using an electronic pH-meter (Hanna Instruments Inc.). Standard buffer solutions (pH 4.01 and 7.01, Merck Kga, Germany) were used.

**Measurement of acidity**
The acidity was determined by titrating with 0.1 N NaOH, using phenolphtalein as an indicator. In order to express the acidity as % lactic acid, the following correspondence was used: 1 mL NaOH 0.1 N= 0.009008 g lactic acid.

**Syneresis measurement**
Syneresis of the kefir-type products was determined by centrifugation using and adapting a method describe by Li and Guo (2006).Samples of 20 g of product (P) was centrifugated for 10 min at 2500 rpm at 4°C. We used a MIKRO 220R centrifuge, produced by Hettich. The whey expelled (W) was removed and weighed. The syneresis was calculated as:

\[
\text{Syneresis} (\%) = \frac{(W/P) \times 100}{100}
\]
Sensory evaluation
The sensory analysis of the samples was performed by 15 panellists, specially trained or this study, which were dairy products consumers with ages between 20 and 45 years; they had a great focusing capacity, without being cold or tired and without being under any treatment which might affect their sensorial perception. Panellists were instructed to cleanse the palate between each sample with the drinking water provided. We used the hedonic scale for evaluating the appearance, odour, taste, flavour, colour and consistency of samples by according 5 points for very pleasant to 1 point for unpleasant.

Statistical analysis
In order to study significant differences between the different sampling points during the fermentation process and the shelf-life, a variance analysis (ANOVA, using the software Graph Pad Prism 5.00) was performed, with a confidence interval of 95% (p<0.05).

RESULTS AND DISCUSSION

The microbial density of the mesophilic lactic bacteria was monitored during the storage period (the shelf-life, 1 to 21 days) at 0-4°C, at 7 days intervals.

The microbial density of the mesophilic lactic bacteria in the control sample (without added honey) confirmed their high growth ability in milk. The addition of honey to kefir-type products did not significantly influence (p>0.05) the growth and survival of lactococcus bacteria. In any of the studied levels 1%, 2.5%, 4%, the honey addition increased the microbial density of lactococi at all the sampling intervals (Fig.1) and probiotic bacteria were present at sufficiently high level during the product shelf-life. The lactococical count appeared to be more affected by the level of 2.5% and 4% honey added. At 2.5% honey added we founded that the lactococical count was $10^9$ cfu/mL in the first day of storage and decreased to $10^8$ cfu/mL in the 7th and 14th days of storage. In the 21th days of storage the microbial density for lactococcus bacteria was $10^7$ cfu/mL for the sample with 2.5% honey added and this was the highest level determined from all the studied samples. Honey was no inhibitory to lactococcus bacteria when we added in skimmed milk at levels of 1%, 2.5% and 4% (w/v).

These results are consistent with those of Curda and Plockova, 1994, who reported that mesophilic starter, was affected by honey concentrations as low as 3% and levels below 10% added honey did no inhibit the growth of mesophilic starters.

The initial pH values of samples with honey were lower than for the control. (Fig.2). In the presence of honey, variation pH during storage at 4°C was not significantly different (p>0.05) for samples with added honey. The decrease in pH values was 0.22 units and 0.19 units for the samples with 1% respectively 2.5% added honey. These falls in pH units were lower than the falls for the control sample (0.32) and the sample with 4% honey (0.36). The pH values were more stabile for these samples from the 7th day of storage to the end of studied period. Similar findings were reported by Varga, 2006; Dogan, 2010 for yoghurt and kefir products.
Fig. 1. Microbial density, log cfu/mL, for the mesophilic lactic bacteria at different honey levels addition during storage period (1 to 21 days at 0-4°C)

Fig. 2. The pH variation for samples with different levels of added honey and for control sample (without honey) during storage period (1 to 21 days at 0-4°C)

These results are supported by the evolution of acidity for the analyzed samples. We founded a decrease of the % of lactic acid for all samples (0%, 1%, 2.5% and 4% added honey) in the first 7th days of storage period. For the remaining studied period the acidity remained almost constant (data not shown). No significant difference (p>0.05) was noted in lactic acid production in all honey-supplemented products.

Whey separation can be defined as the appearance of whey on the curd surface. Syneresis is the shrinkage of the curd, which then leads to whey separation. (Li and Guo, 2006). The yogurt industry is now dominated by the stirred-type product, which allows manufacturers to add various stabilizers to try to prevent whey’s separation. (Lucey, 2002). Syneresis can be reduced by increasing the casein content of the milk or by adding stabilizers that interact with the casein network.
Honey at 2.5% and 4% significantly decreased the syneresis (p<0.01). The syneresis for the sample with 1% honey was no significantly different to the control sample. The samples with 2.5% and 4% honey appeared more stable in the second half of storage period. These results are consistent with those reported by Sert *et al.*, 2011, who funded that honey, by its fructose content, has high water-binding capacity.

At the beginning of storage, taste and flavour intensity of kefir-type product with honey has increased with the honey’s level addition. It was founded a significant variation (p<0.01). Panelists founded that kefir-type product with 1% (w/v) honey was weak in taste and flavour but the 4% (w/v) honey level was founded too sweet. The flavour intensity of the sample with 2.5% (w/v) added honey was considered optimum. The odour, the colour and the appearance values were not significantly affected (p>0.01) by honey addition. The consistency of samples with added honey was higher than the consistency of the control sample. The acceptability score indicated that 2.5% honey added is the optimum level for the kefir-type product (Fig.4).

**CONCLUSIONS**

Honey was no inhibitory to mesophilic lactic starter when was added at levels 1%, 2.5%, 4%. The pH and lactic acid production was not significantly affected by the honey.
addition. An improvement of syneresis, consistency, taste and flavour were obtained in kefir-type product with 2.5% honey added.

The production of kefir with the addition of honey may be alternative for desired taste and nutrition for new fermented dairy beverages to the children and new taste of kefir-type products.

REFERENCES


