Making of pasteurized Lfrik (fermented Moroccan camel milk) by selected lactic starters

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(Reçu le 31/06/2017; Accepté le 19/09/2017)

Abstract

Traditional Moroccan Lfrik is processed by spontaneous fermentation of whole raw camel milk at ambient temperature. In this study, pasteurized Lfrik was made using pasteurized camel milk inoculated with strains of *Lactococcus* and *Leuconostoc* obtained from selected commercial starters (Flora Danica and CHN11) according to characteristics of the fermented milk. Biochemical and microbial changes during fermentation were studied. The acidification behavior of the fermented camel milk and cow milk were compared in eight samples of each one. Fermentation rate was slower in camel milk than in cow milk in which the acidity was twice higher. However, the Non Protein Nitrogen levels increase was faster during camel milk fermentation than cow milk fermentation. Natural benzoic acid was found at higher concentration in final fermented camel milk products. In addition, organoleptic quality of the pasteurized Lfrik has been tested and was found to be similar to that of the traditional Lfrik.

Keywords: Lfrik, camel milk, characteristics, controlled fermentation, sensory quality

Préparation de Lfrik pasteurisé (lait camelin fermenté) par des levains lactiques sélectionnés

Résumé

Lfrik marocain traditionnel est produit par fermentation spontanée du lait camelin cru et entier à la température ambiante. Dans ce travail, Lfrik pasteurisé a été élaboré en utilisant du lait camelin pasteurisé et inoculé avec des souches de *Lactococcus* et de *Leuconostoc* obtenues à partir des levains lactiques du commerce (Flora Danica et CHN11) selon les caractéristiques du lait fermenté. L'évolution de quelques caractéristiques biochimiques et microbiologiques au cours de la fermentation a été étudiée. L'acidification du lait camelin fermenté et du lait bovin a été comparée dans huit échantillons pour chacun. La fermentation était plus lente dans le lait camelin que dans le lait bovin dont l'acidité était deux fois plus élevée. Cependant, l'augmentation de l'azote non protéique a été plus rapide pendant la fermentation du lait camelin et la concentration en acide benzoïque naturel après fermentation a été plus élevée dans le lait de chamelle. De plus, la qualité organoleptique de Lfrik pasteurisé a été testée et s'est révélée similaire à celle de Lfrik traditionnel.

Mots-clés: Lfrik, lait de chamelle, caractéristiques, fermentation contrôlée, qualité sensorielle

INTRODUCTION

Milk fermentation is a process that extends its shelf life, improves its taste and enhances its digestibility (Shiby and Mishra, 2013).

In the traditional fermented milk process, raw milk is allowed to ferment naturally at ambient temperature and without prior heat treatment until it turns sour. Due to the spontaneous nature of this fermentation, this traditional method results in a product with varying taste and flavor (Farah *et al*., 1989). Thus, nowadays milk fermentation is a carefully controlled microbial process for which selected cultures have been developed (Shiby and Mishra, 2013). These starter cultures have been formulated by using micro-organisms which impart special desired characteristics to the fermented product (Marshall and Law, 1984). In addition, they must be able to multiply in a few hours in order to produce enough lactic acid and aroma compounds for the complete conversion of milk to fermented dairy products, that depends not only on nutritional factors but also on other environmental parameters such as temperature (Breheny *et al*., 1975; Lawrence and Tomas, 1979; Ross 1980) and associative growth (Driessen *et al*., 1982). In the case of camel milk, few studies were previously published worldwide on its controlled fermentation process (Farah *et al*., 1990; Attia, 2001; Abdel Rahman, 2009).

In Morocco, fermented camel milk "Lfrik " is traditionally made by the herders and/or small dairy shops "Mahlabas" using raw camel milk without prior heat treatment and allowing it to spontaneously ferment in goat skin bags. In a recent study, our team reported that raw camel milk was contaminated with high levels of coliforms bacteria presenting, therefore, a potential health risk for its consumers (Ismaili *et al*., 2016). To our knowledge, no previous published work was done on controlled fermentation of camel milk produced in Morocco. Therefore, the purpose of the present study was to produce a fermented product similar to traditional Lfrik but with improved hygienic quality using pasteurized camel milk and selected commercial starter cultures. Biochemical and microbiological properties during fermentation and in fermented products as well as the sensory characteristics were studied and compared to those of fermented cow milk produced in the same conditions.

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MATERIAL AND METHODS

Milk and Lfrik samples

Eight samples of raw camel milk, raw cow milk and traditional Lfrik were obtained from traditional dairy shops in Laayoune city and at private farm located in Ain Aouda, close to Rabat. Milk samples were transported refrigerated in coolers to laboratory and kept refrigerated until use for analysis, within 1 hour to ONSSA (Office Nationale de Sécurité Sanitaire des Aliments) laboratory in Laayoune and within 1 day to IAV laboratory in Rabat.

Upon arrival, raw milk samples and Lfrik were analyzed for their pH, acidity and total aerobic flora (TFA).

Pasteurization and fermentation

Raw camel milk and cow milk samples were batch pasteurized at 90°C for 15 min in a water bath and immediately cooled to about 30°C.

Pasteurized camel milk samples were then directly inoculated using one of two commercial freeze-dried mesophilic lactic cultures (Flora Danica and CHN-11, CHR Hansen, Denmark). The inoculation rate was as suggested by the producer of the culture package. The two commercial cultures are composed of a mixture of *Leuconostoc, Lactococcus lactis biovar diacetylactis* and *Lactococcus lactis subsp. cremoris.*

Incubation of inoculated samples was at 20°C or 30°C for 24 h. During the fermentation process, monitoring of pH and titratable acidity (TA) was carried out every 3 hours and TFA every 6 hours. Samples inoculated with "Flora Danica" were used to monitor content changes of lactose, benzoic acid and Non Protein Nitrogen (NPN) at different intervals during incubation.

Pasteurized cow milk samples were inoculated with "Flora Danica" under the same conditions as camel milk. These samples were used for comparison purpose.

Biochemical and microbial Analyses

The pH of milk samples was measured using a digital pH meter (CyberScan pH 1500, Eutech Instruments) and their TA was determined based on the AOAC method (1990).

TFA counts were determined using PCA medium (Plate Count Agar, Difco laboratories, Detroit, Michigan, USA) incubated at 30°C for 3 days (IDF, 1987a).

The AOAC method 991.21(AOAC, 2010) as described by Barbano *et al*. (1991) was used to determine NPN content in fermented milk samples. Protein precipitation was done by the addition of trichloroacetic acid (TCA) solution. Precipitated milk protein was removed by filtration and NPN in the filtrate was determined by Kjeldahl method.

Benzoic acid extraction and quantification were done according to the IDF method (1987b). A volume of 20 µl of extracts and standard solution were injected in a High-Performance Liquid Chromatography (HPLC) system with a UV detector (Agilent 1100 Series, Germany). A C18 column (250 x 4.6 mm, i.d.) with 5 microns particle size (IBM Instrument Inc.) was used. The mobile phase was composed of 20 % acetonitrile and 80 % sodium acetate solution (0.005 M adjusted to pH 4.4 with acetic acid). The flow rate of the mobile phase was set at 0.8 ml/min and the benzoic acid detection at 227 nm.

Determination of lactose in camel and cow milk samples at different intervals during the fermentation process was done by HPLC according to the method reported by Xinmin *et al*. (2008). Separation was performed using water–acetonitrile (20/80, v/v) mobile phase maintained at a flow rate of 1 ml/min.

Sensory evaluation

Sensory evaluation of fermented milk samples was done in two trials. A first trial was conducted in Laayoune city using a panel of 63 consumers of traditional Lfrik and a set of 4 samples: fermented camel milk with "Flora Danica", Fermented camel milk with CHN-11, fermented cow milk with "Flora Danica" and traditional Lfrik as a control. The Lfrik sample was purchased the same day of the trial from a "Mahlaba".

The second trial was carried out in the city of Rabat with a panel of 52 non-consumers of camel milk and Lfrik. Samples used in this trial were: Fermented camel milk with "Flora Danica", fermented camel milk with "CHN-11" and fermented cow milk with "Flora Danica".

The fermented milk samples used in both trials were incubated at room temperature (18 ± 2**°C**) for about 12 h. In both trials, Samples were coded with three digit numbers and served at room temperature (20 ± 2°C) in white plastic cups. The order of samples presentation was balanced to account for first order and carry-defects.

Hedonic evaluation of samples was done using a 9 points balanced verbal hedonic scale (dislike extremely to like extremely) (Meilgaard *et al*., 1999). The panelists were also asked to rate the intensity of acidity, bitterness, viscosity and fat sensation in fermented camel and cow milk samples using a 5 points rating scale (very low "1", low "2", medium "3", high "4", very high "5"). Mineral water, at ambient temperature, was provided to panelists for rinsing out the mouth between samples.

The instructions given to participants during the sensory evaluation session were done in writing and orally in Arabic and French.

Statistical analyses

Statistical analysis of data was performed with Minitab program using analysis of variance (ANOVA) and statistically significant differences between means were determined using Tukey test at a level of significance $\alpha = 0.05$.

The analysis of overall sensory liking and sensory attributes intensity scores was performed with excel using XLSTAT statistical software.

RESULTS AND DISCUSSION

Milk samples fermentation monitoring

Figures 1 and 2 show the pH and TA values at different intervals during the incubation of inoculated camel milk and cow milk samples at 20 and 30°C. At both temperatures, camel milk samples inoculated with "Flora Danica" showed faster pH decrease and higher increase in TA values compared to those inoculated with "CHN-11". In addition, the fermentation rate was faster and more intense in inoculated cow milk than camel milk samples. In cow milk samples, TA values after 12 h incubation were approximately twice higher than those of camel milk either at 20°C or 30°C. TA values reached respectively 0.81 and 0.86 % lactic acid at 20°C and 30°C while that of camel milk inoculated either with "Flora Danica" or "CHN-11" did not exceed 0.42 % lactic acid at 20°C and 0.51 % lactic acid at 30°C. Similar findings were reported by Attia *et al*. (2001).

This slow camel milk fermentation rate can be explained by the presence of natural microbial inhibitors (lysosyme, lactoferrin, lactoperoxydase and immunoglobulins) that are in higher levels in camel milk than in cow milk (Yagil, 1982; Barbour *et al*., 1984; El-Agamy *et al*., 1992; Attia *et al*., 2001). This is supported by the microbial growth rate observed during the incubation of milk samples (Figure 3). After 12 h of incubation, TFA in fermented cow milk reached values of 1.0 10^8 Cfu.mL^{-1}, while those of fermented camel milk were 1.0 10^3 Cfu.mL^{-1}.

In addition, more lactose use during the fermentation process was observed in cow milk than in camel milk samples. After 12 h incubation at 20°C, lactose in cow and camel products were respectively about 80 % and 60 % less than initial values (Figure 4).

Evolution of NPN and benzoic acid contents during fermentation

Evolution during incubation of NPN and benzoic acid contents were carried out in camel and cow milks inoculated with the lactic starter "Flora Danica" at 20 and 30°C.

The increase of NPN content during fermentation in camel milk was faster than in cow milk (Figure 5a). After 12 h incubation, NPN content of fermented camel milk was 1.74 and 1.94 mg.Kg^{-1} at 20 and 30°C, respectively. However, NPN content in cow milk after 12 h incubation was around 1.0 mg.Kg^{-1}. The observed larger content of

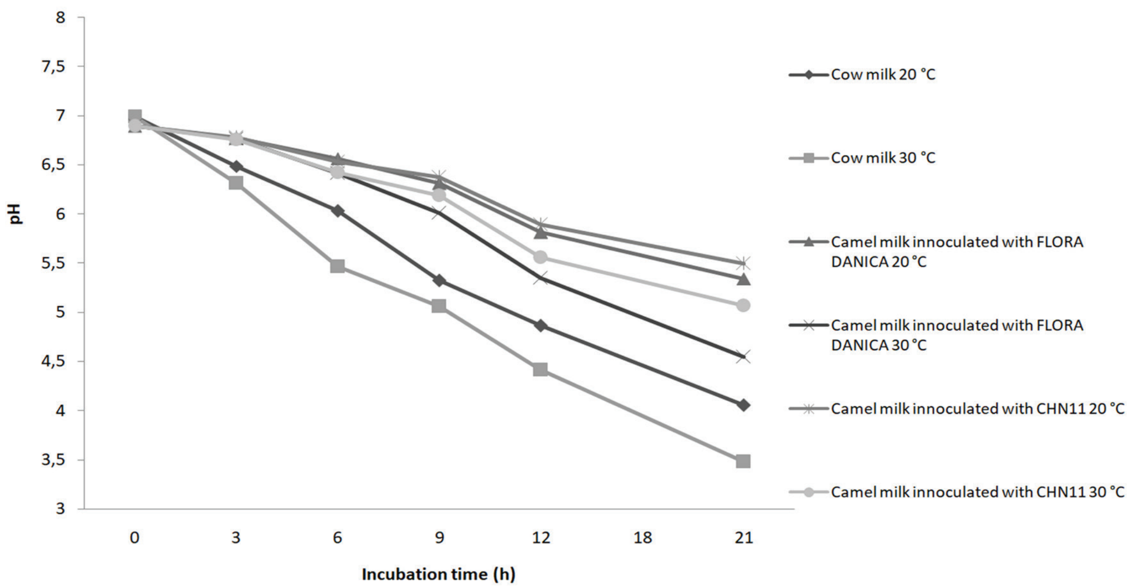

Figure 1: pH values during fermentation of the inoculated milk at 20°C and 30°C

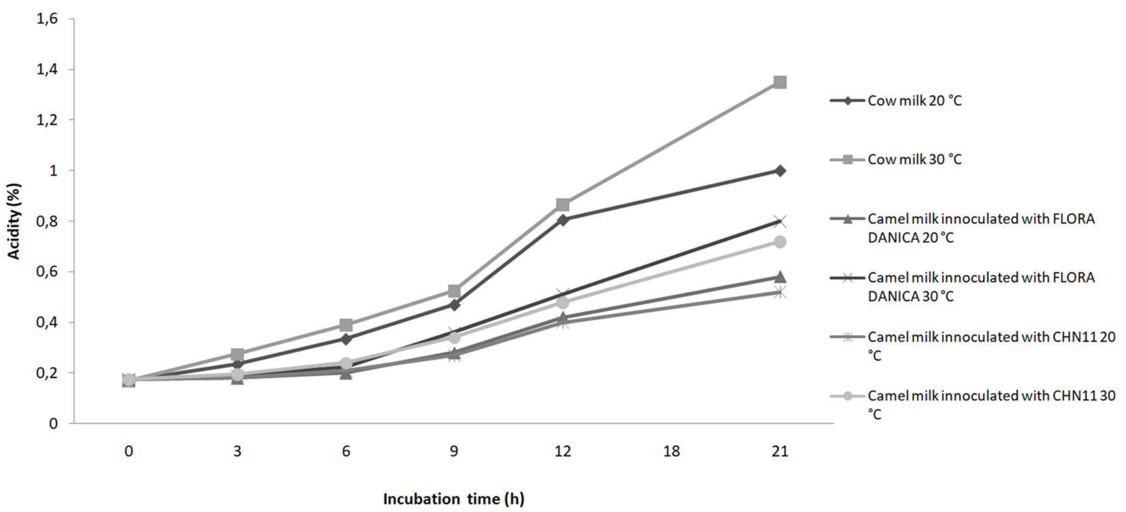

Figure 2: Acidity values (%) during fermentation of the inoculated milk 20°C and 30°C

NPN in fermented camel milk than in fermented cow milk is probably due to more proteolytic activity in camel milk. Several authors explained this increase in NPN during milk fermentation by an increase in free amino acids (IDF, 1983; Alm, 1983; Saidi and Warthesen, 1993).

Benzoic acid production during fermentation was low and had identical trend in milk samples throughout the fermentation up to 9 h and then increased significantly from about 2 ppm to reach respectively values of 11 and 15 ppm in cow and camel milk samples at 12 h of fermentation (Figure 5b). Similar results of benzoic acid of fermented cow milk were obtained by Nishimoto *et al*. (1969) and Saidi and Faid (2001) who reported respectively an average of about 11 ppm and a range of 5-18. It should be noticed that benzoic acid plays an important role as an antifungal agent in fermented products (Chipley, 1993; Davidson, 2001).

Sensory evaluation

In the first sensory trial carried out in the city of Laayoune and where a panel of regular consumers of Lfrik was used, overall liking results (Table 1) showed that the

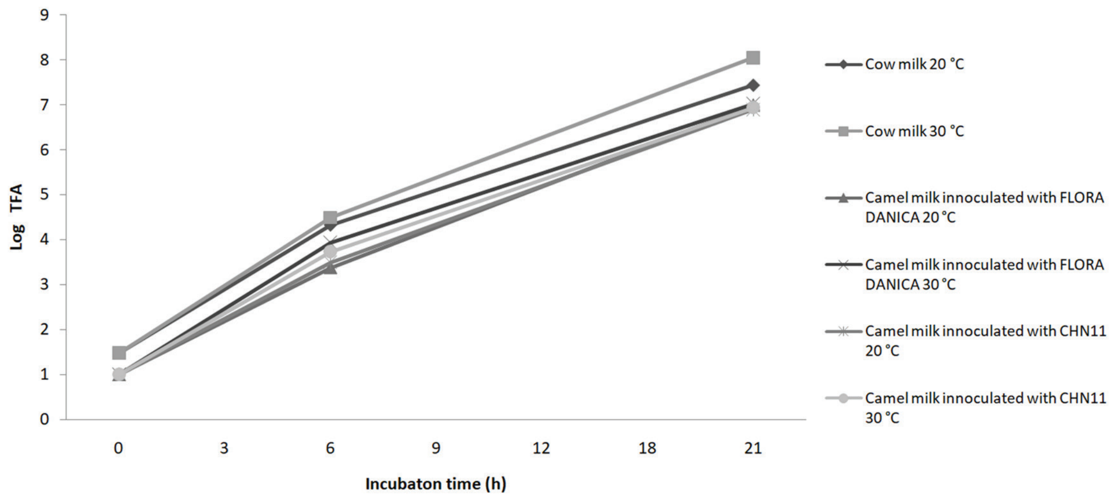

Figure 3: TFA values (Log cfu.mL^{-1}) of inoculated camel and cow milk during fermentation at 20°C and 30°C

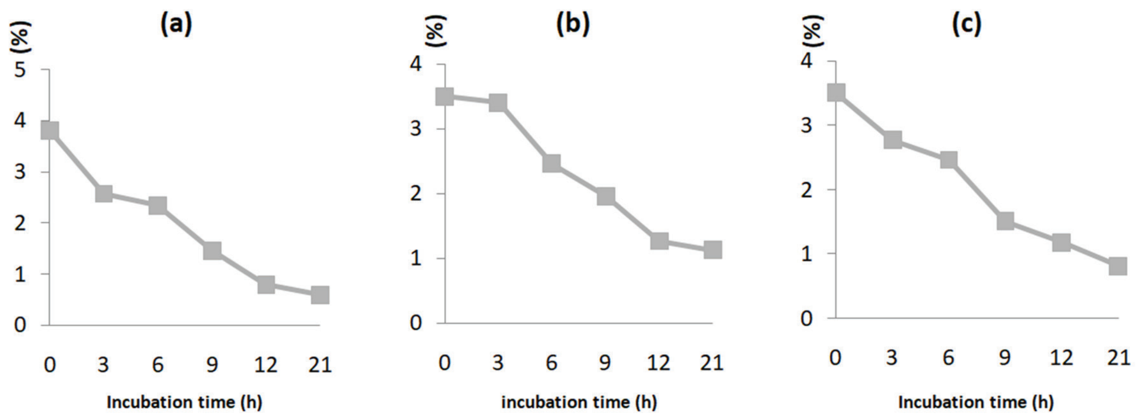

Figure 4: Lactose values (%w/w) during fermentation of (a) cow milk and (b) camel milk inoculated with Flora Danica at 20°C and (c) 30°C

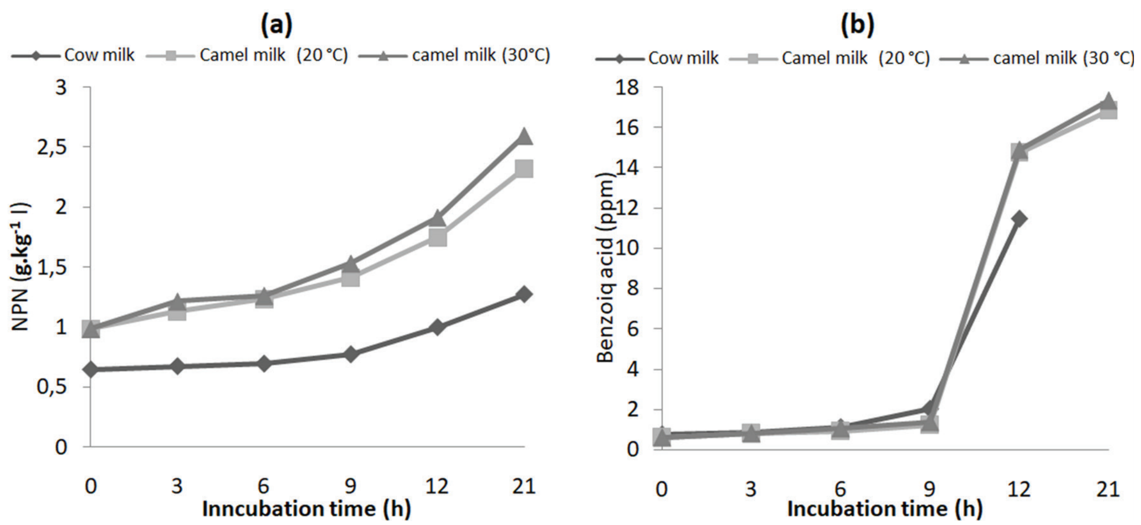

Figure 5: (a) NPN (g.kg^{-1}) values and (b) benzoic acid (ppm) values during fermentation of the inoculated milk at 20°C and 30°C

fermented camel milk samples prepared using the commercial starters had similar scores (p>0.05) as the control (traditional fermented camel milk). Average scores in a scale of 9 points were respectively 7.6, 7.5 and 7.2 for Lfrik, camel milk fermented with "Flora Danica" and camel milk fermented with "CHN-11". In this trial, cow milk fermented with "Flora Danica" had the lowest overall liking score and was significantly less liked (p<0.05) than the fermented camel milk.

Concerning the other sensory attributes (acidity, viscosity, bitterness and sensation of fat) of fermented milk samples, a significant difference (p<0.05) was observed for the viscosity attribute. However, Tukey test analysis showed no significant difference between traditional Lfrik and fermented camel milk with "Flora Danica" (Table 1).

Organoleptic quality of the pasteurized Lfrik tested by regular consumers of Lfrik was found to be similar to that of the traditional Lfrik.

In the second trial done in the city of Rabat and using a panel of non-consumers of camel milk and Lfrik, overall liking scores showed that fermented cow milk was more liked than fermented camel milk samples (Table 2). However, the difference of overall liking scores was not significant (p > 0.05) between fermented cow milk and fermented camel milk prepared using "Flora Danica". In this trial, a significant difference (p < 0.05) was observed only for the acidity.

It should also be noted that overall liking scores were higher with the first panel than in the second one. This can be explained by the difference in the raw milk samples used for the preparation of different fermented batches and the extent of fermentation process between the two trials. This can be supported by the higher values of acidity in the first trial than in the second trial.

CONCLUSION

Preparation of Lfrik from pasteurized camel milk using commercial lactic starters and incubated at room temperatures (20-30°C) for about 12 h gave products with similar pH and acidity as the traditional Lfrik.

The fermentation process was slower in camel milk than in cow milk. After 12 h of fermentation, the acidity reached respectively about 0.4 and 0.8% lactic acid in camel and cow milks. In addition, NPN and benzoic acid contents were higher in obtained fermented camel milks than in fermented cow milk.

Sensory evaluation showed that fermented camel milk using commercial lactic starters had similar characteristics and overall liking scores than the traditional Lfrik. Fermented camel milk samples prepared using "Flora Danica" were more liked than those prepared using "CHN-11".

The use of pasteurized camel milk in the preparation of its fermented products allowed a significant improvement of their hygienic quality.

Acknowledgments

This work was financially supported by the Ministry of Agriculture and Maritime fisheries of Morocco. The authors would like to thank "Direction Régionale d'Agriculture" and "(Office National de Sécurité Sanitaire des Aliments" in the city of Laayoune for their precious support.

Table 1: Mean values (n = 63) of overall liking and sensory attributes intensity scores by the panel of accustomed consumers of 'Lfrik ' in the first trial

Fermented sample*	Chemical characteristics		Overall liking**	Sensory attributes intensity***			
	pH	TA % lactic acid		Acid	Viscous	Bitter	Fat sensation
Traditional Lfrik	5.2	0.45	7.60 ^{a}	3.19	2.00 ^{a}	*1.81*	2.29
C.M.FLORA	5.1	0.43	7.54 ^{ab}	3.33	2.21 ^{ab}	2.05	2.19
C.M.CHN 11	5.1	0.42	7.22 ^{ab}	3.21	2.41 ^{a}	1.89	2.30
Cow.M.	4.1	0.82	7.08 ^{b}	3.19	2.05 ^{b}	1.90	2.09

* Traditional Lfrik; C.M.FLORA: camel milk inoculated with "Flora Danica"; C.M.CHN11: camel milk inoculated with "CHN-11"; Cow.M.: cow milk inoculated with "Flora Danica"
**9 point hedonic rating scale
***5 point intensity rating scale
^{abc} Means bearing different letters within the same column differ significantly (p < 0.05)

Table 2: Mean values of overall sensory liking and sensory attributes intensity scores by the panel of non-consumer subject in the 2nd trial and Tukey test results

Fermented sample*	Chemical characteristics		Overall liking**	Sensory attributes intensity***			
	pH	TA % lactic acid		Acid	Viscous	Bitter	Fat sensation
C.M.CHN 11	5.6	0.40	5.06	2.30 ^{b}	2.87	2.17	2.42
Cow.M.	4.1	0.80	5.58	2.61 ^{ab}	3.08	2.23	2.50
C.M. FLORA	5.8	0.42	5.25	2.80 ^{a}	3.13	2.47	2.60

*C.M.CHN11: camel milk inoculated with "CHN-11"; Cow. M.: cow milk inoculated with "Flora Danica"; C.M.FLORA: camel milk inoculated with "Flora Danica"
**9 point hedonic rating scale
***5 point intensity rating scale
^{abc} Means bearing different letters within the same column differ significantly (p < 0.05)

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