Effect of Bifidobacterium Lactis on Free Fatty Acids of Lighvan Cheese during Ripening

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Abstract—**Lighvan cheese a well known cheese produced from ewe's milk was subject of this study. The aim of study was to characterize lipolysis of probiotic Lighvan cheese at 5, 25, 45 and 60 days of ripening. We evaluated the level of lipolysis of cheese by measuring: ADV (%) and FFA (%).The effect of storage time on lipolysis of probiotic Lighvan cheese was significant ($p<0.05$). ADV (%) and FFA (%) values increased continuously until the end of the ripening period. The addition of B. lactis had significant ($p<0.05$) effect on further development of lipolysis characteristics of the Lighvan cheese, but the rate of it comparing with some products using different methods of production was slow.**

Index Terms—**Lighvan, cheese, probiotic, lipolysis.**

I. Introduction

Food containing probiotic bacteria belongs to the category of functional foods described as "foods claimed to have a positive effect on health" (Stanton et al. 1998). Cheese could offer certain advantages in delivering live probiotics to the gastrointestinal tract, the target organ. Cheeses having a higher pH than fermented milks and providing a more stable milieu could support long-term survival of probiotic bacteria. Furthermore, the matrix and high fat content of cheese may protect the organisms during passage through the gastrointestinal tract (Stanton et al. 1998, Gomes et al. 1995). The incorporation of cultures with beneficial effects into a functional food is successful when the cultures maintain viability until being consumed, and if the added cultures do not adversely affect the product's composition, texture or sensory features. Traditional Lighvan is a semi-hard cheese with a large market demand, is one of the most popular traditional cheeses in Iran. It is mostly produced from ewe's or goat's milk, or a mixture of them. Traditional Lighvan cheese which ripened in Brine is a daily diet component especially in the north-west of the country some efforts have been done to produce Lighvan in cheese plant using pasteurized milk. The ripening period of this type of product is about 90 days but the cheeses made by using raw milks in small, rural production units may be ripened for six to eight months.Cheese ripening is a complex process whose main characteristic is proteolysis that involves different enzymes such as chymosin from rennet, plasmin and the microbial enzymes, which in turn depend on changes in the microbial population in the curd. Lipolysis is one of the major biochemical changes that occur during cheese ripening. The free fatty acids (FFA) released during lipolysis contribute, together with the volatile compounds and the proteolysis products, directly to cheese flavor (Georgala et al. 2005). The level of lipolysis varies considerably among the different cheese types from low, in Dutch-type cheeses (Walstra et al. 1993). to extensive in the mould ripened, surface-bacterially ripened and Italian hard cheeses (Gripon 1993, Reps 1993). Furthermore, analysis of the short and medium-chain FFA profile has been suggested as an index for characterizing cheeses over the ripening period (Woo et al. 1984, Woo and Lindsay 1984).

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II. Materials and Methods

A. Cheese Manufacture

Ewe's milk was supplied from an animal husbandry in Varamin region from the Zandy breed. Experimental cheese samples were made in three replications at the Tehran Pegah dairy plant (Tehran, Iran). The composition of cheese milk was as follow: dry matter 17.86% (w/w), protein 5.60% (w/w), fat 6.50% (w/w), lactose 4.70% (w/w), ash 0.88% (w/w). Conversion coefficient of milk to cheese was 4.86. The raw milk was inoculated with B. lactis to reach a concentration of 10^9/ml, then milk was coagulated with fungal rennet (DSM, Food Specialties, France)(rennet force =1/100000), after curdling, the curd was cut into small cubes, approximately 1 cm^3, and left to rest (15 minutes). The slab curd was placed on a mesh table and weighted for draining, after which the curd was cut into large cubes, approximately 10×10×7 cm. These cubes were immersed into brine with 22% concentration for about 7 hours at room temperature, then placed into tin-plate containers with brine at about 12% concentration. The containers were sealed and stored for 60 days at 8°C. The characterizations of probiotic Lighvan cheese were studied. Each experiment was done in triplicate.

B. Lipolysis

The level of lipolysis was measured in cheese samples on 5, 25, 45 and 60 days old by determining the acid degree value (ADV) and free fatty acids (FFA) content.

C. Acid Degree Value (ADV)

The ADV was determined as described by Deeth and Fitz-Gerald 1976. Samples were prepared by mixing 5 g of cheese with 37.5 ml of 2% sodium citrate at 50 °C in a Sorvall Omni-mixer at setting 3 for 1 minute, and then at setting 7 for 2 minutes. The ADV was determined on 35 ml samples of this extract.

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D. Sample Preparation

Extraction of cheese lipids and isolation of the FFA were executed by GC as described by De Jong and Badings 1990.

E. Statistical Analysis

The data were statistically analysed using a completely randomized design (CRD) with three replications. Data were subjected to analysis of variance using the SAS statistical software package (SAS Institute 1988). Mean comparison was performed with LSD's test at the $P<0.05$ level of significance.

III. Results and Discussion

A. Lipolysis of Cheese

The ADV of probiotic and traditional Lighvan cheeses is shown in Table I. The ADV of probiotic Lighvan cheese was 1.49 mg KOH $100g^{-1}$ fat at the day 5, and 1.66mg KOH $100g^{-1}$ fat on the day 60 of storage. ADV of probiotic Lighvan cheese showed significant ($p<0.05$) differences during ripening period. ADV increased continuously during this process. This increase was particularly striking at the 60th day. Similar to our results, Kensenkas and Akbulut (2008) reported that the ADV of Turkish white brined cheese increased during ripening. These results are in accordance with findings reported by (Gürsoy 2005) in white brined cheese, and by (Katsiari et al. 2000) in feta cheese. A slow rate of lipolysis was attributed to salt content, which has inhibitory effect on lipase. ADV increased during ripening; this increase was due to proteolysis and post-acidification. Environmental conditions such as salt and lactic acid concentration can affect the lipolytic and proteolytic activity of Lighvan cheese's indigenous microbial enzymes.

TABLE I: Changes in ADV During Ripening of ProbioticLighvan Cheese[A,B]

Ripening period (Days)	5	25	45	60
ADV(mg KOH/100 g fat)	1.49[a]±0.02	1.55[b]±0.006	1.60[c]±0.01	1.66[d]±0.006

[a]Means of each parameter in the same column with a superscript differ significantly ($p<0.05$).

[b]Mean values ± standard deviation of three trials.

This study's results indicated that native milk lipase was for a part responsible for the hydrolysis of the lipid in traditional Lighvan cheese; and cheese lipase originating from probiotic microorganism was responsible for development of hydrolysis of the lipid in probiotic Lighvan cheese. The hydrolisation of lipids in cheese during ripening is catalysed by indigeneous lipase of the milk and by microbial lipases (Franco et al. 2001). LPL (Lipoprotein lipase) activity is of most significance in raw-milk cheeses, as the enzyme is largely inactivated by pasteurisation (Mc Sweeney 2004). Moreover, native milk lipase is optimally active at a pH range between 8.0 and 9.0, and is inhibited by NaCl to a great extent (Franco et al. 2001, Vlaemynck 1992). The values for salt content and pH in traditional Lighvan cheese were far from the values indicated for optimum activity of milk lipases (pH 4.98 to 5.3, salt content 3.24 to 3.69%).

B. Free Fatty Acids Profile

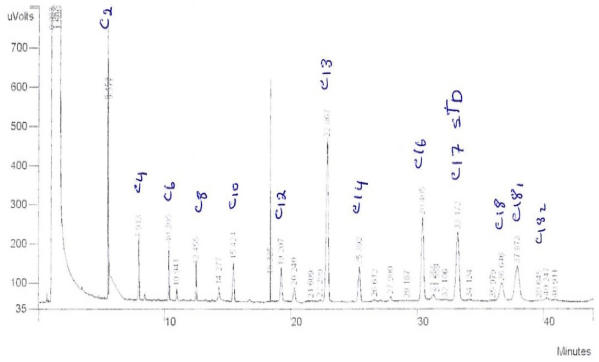

Fig. 2. Gas chromatogram of FFA extracted from a probiotic Lighvan cheese (60-d-old) spiked with internal FFAstandards $C_{13:0}$ and $C_{17:0}$

Chromatographic separation of underivatized FFA (Figure 2.) allowed the quantitation of all major fatty acids in one run. The concentration of acetic acid and total $C_{4:0}$ to $C_{18:2}$ increased throughout ripening. Showing the significant effect ($p<0.05$) of the ripening stage on cheese lipolysis (Table II). The mean concentration of acetic acid and individual FFAs of probiotic Lighvan cheese increased throughout ripening. Acetic acid contributes greatly to the final flavour of traditional Lighvan cheese and is the major volatile acid extracted with FFAs. It is not produced from lipolysis by lipase but from several biochemical pathways. It is formed during the early stages of ripening and is probably a product of citrate or lactate fermentation or of amino acid catabolism by bacteria (Abd El-Salam et al. 1993, McSweeney and Sousa 2000). The increase of acetic acid up to 60 d may be due to lactate fermentation, since lactose can be present even in mature cheese (Abd El-Salam et al. 1993). The content of acetic acid was 28.91 mg/100 g of cheese on the day 5 of storage and 29.85 mg/100 g of cheese at the end of storage. Acetic acid made up 25% of total FFA present in probiotic Lighvan cheese. Butyric acid is also an important component of cheese, which contributes greatly to its flavour and piquant taste. Butyric acid was the main FFA in SCFFA experimental cheese samples, ranging from6.01 mg 100 g^{-1} cheese on the day 5 of storage time to6.20 mg 100 g^{-1} cheese on the day 60 of storage time. The relatively higher increase was viewed in the concentration of SCFFA ($C_{4:0}$ to $C_{8:0}$), which has a significant impact on the development of characteristic aroma of cheese, during ripening than medium chain free fatty acids (MCFFA) ($C_{10:0}$ to $C_{14:0}$) and long chain free fatty acids (LCFFA) ($C_{16:0}$ to $C_{18:2}$) (Table II). This could mainly be due to specificity of milk lipoprotein lipase towards FFA located at the positions sn-1 and sn-3 of the triglyceride. Despite the quantitative importance of medium and long-chain FFA, they are not the main contributors to cheese flavour (Rahmat and Richter 1996, Freitas and Malcata 1998). The predominant FFAs was myristic acid in MCFFA and its value ranging from 8.85 mg 100 g^{-1}cheese on the day 5 of storage time to 8.97 mg 100 g^{-1} cheese on the day60 of storage time, and palmitic acid was dominant FFA among LCFFA in probiotic Lighvan cheese, and its value ranging

from 23 mg 100 g^{-1} cheese on the day 5 of storage time to 23.20 mg 100 g^{-1} cheese on the day 60 of storage time. Researchers revealed that palmitic and oleic acids, which do not intrinsically contribute to cheese flavour quite as much as short-chain FFAs do.

IV. Conclusions

The production of functional cheeses was recently proposed as a suitable and promising alternative to fermented milks (Stanton et al. 1998), because cheese could offer certain advantages as a carrier of probiotic microorganisms. Semi-hard Lighvan cheese has intrinsic features (pH, moisture and aw) that may characterise this ecosystem as hostile for microorganisms. However, the results of this study demonstrated that Lighvan cheese made with added B. lactis seemed to be an effective way to produce a semi-hard ewe's cheese with a considerable number of viable bifidobacterium cells. In particular, B. lactis cells survived in cheese at concentrations up to 6.84 $\log10^{cfu/g}$ for at least 60 days of ripening. B. lactis affected lipolysis, characteristic of the traditional Lighvan cheese. Besides meeting precise consumer demand, the production of functional or probiotic cheeses may be useful for differentiating and increasing the market popularity of various Iranian cheeses such as traditional Lighvan, which still have a strict regional tradition. If eaten daily, probiotic Lighvan cheese can be considered as a probiotic vector or as an additional variety supporting other probiotic foods that are eaten daily but we can conclude that in cheeses ripened in brine, a significant parts of ripening products are transferred into brine and their effects on the sensory properties of final product is limited.

TABLE II: Free Fatty Acids (mg 100g-1) of Probiotic Lighvan Cheese[A,B]

Fatty acid	Times of ripening (Days)			
	5	25	45	60
$C_{2:0}$	28.91[a]±0.01	28.97[b]±0.01	29[c]±0.01	29.85[d]±0.01
$C_{4:0}$	6.01 [a]±0.01	6.12[b]±0.01	6.18[c]±0.01	6.20[d]±0.01
$C_{6:0}$	3.90[a]±0.02	3.95[b]±0.01	3.98[c]±0.01	4.01[d]±0.01
$C_{8:0}$	3.02[a]±0.01	3.17[b]±0.01	3.22[c]±0.01	3.25[d]±0.01
$C_{10:0}$	7.21[a]±0.01	7.29[b]±0.01	7.34[c]±0.01	7.37[d]±0.01
$C_{12:0}$	6.78[a]±0.01	6.85[b]±0.01	6.90[c]±0.01	6.92[c]±0.01
$C_{14:0}$	8.85[a]±0.01	8.90[b]±0.01	8.93[c]±0.01	8.97[d]±0.02
$C_{16:0}$	23[a]±0.02	23.10[b]±0.02	23.15[c]±0.01	23.20[d]±0.01
$C_{18:0}$	9.01[a]±0.01	9.10[b]±0.02	9.14[c]±0.01	9.18[d]±0.01
$C_{18:1}$	19.85[a]±0.005 8	19.90[b]±0.011 5	19.95[c]±0.011 5	19.98[d]±0.005 8
$C_{18:2}$	1.01[a]±0.02	1.20[b]±0.01	1.24[c]±0.01	1.28[d]±0.01
Total	117.55[a]±0.02	118.55[b]±0.01	119.03[c]±0.02	120.21[d]±0.02

[a]Means of each parameter in the same column with a superscript differ significantly (p<0.05)

[b]Mean values ± standard deviation of three trials

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References

[1] M. H. Abd El-Salam, E. Alichanidis, and G. K. Zerfiridis, "Domiati and Feta-type cheeses. In: Fox, PF (ed) Cheese: Chemistry," *Physics and Microbiology*, Chapman and Hall, London, pp 301-335, 1993

[2] H. C. Deeth and C. H. Fitz-Gerald, "Lipolysis in dairy products: A review," *Aust J Dairy Technol* , vol. 31, pp. 53-64, 1976

[3] C. D. Jong and H. T. Badings, "Determination of free fatty acids in milk and cheese," *Procedures for Extraction, Clean Up and Capillary Gas Chromatographic Analysis*. J High Reso Chrom vol. 13, pp. 94-98, 1990

[4] I. Franco, B. Prieto, R. Urdiales, J. M. Fresno, and J. Carballo, "Study of the biochemical changes during ripening of Ahumado de Aliva cheese: A Spanish traditional variety," *J Food Chem*, vol. 74, pp. 463-469, 2001

[5] A. C. Freitas and F. X. Malcata, "Lipolysis in Picante cheese: influence of milk type and ripening time on free fatty acid profile," *Lait*, vol. 78, pp. 251-258,1998

[6] A. K. Georgala, I. G. Kandarakis, S. E. Kaminaridis, and E. M. Anifantakis, "Volatile free fatty acid content of Feta and white-brined cheeses," *Aus J Dairy Technol* vol. 54, pp. 5-8, 1999

[7] A. M. P. Gomes, F. X. Malcata, F. A. M. Klaver, and H. J. Grande, "Incorporation and survival of Bifidobacterium sp. Strain Bo and Lactobacillus acidophilus strain Ki in a cheese product," *Neth Milk Dairy J*, vol. 49, pp. 71-95,1995

[8] O. Gürsoy, "The use of probiotic bacteria as adjunct culture in the production of white cheese," Ph.D. Dissertation, Ege University, Izmir, Turkey, 2005.

[9] M. C. Katsiari, L. P. Voutsinas, E. Alichanidis, and I. G. Roussis, "Lipolysis in reduced-sodium feta cheese made by partial substitution of NaCl by KCl" *Int Dairy J*, vol. 10, pp. 369-373, 2000

[10] H. Kesenkas and N. Akbulut, "Yeasts as ripening adjunct cultures in Turkish white brined cheese production," *Turk J Vet Ani Sci* vol. 32, no. 5, pp. 327-333, 2008

[11] P. L. H. McSweeney, "Biochemistry of cheese ripening," *Int J Dairy Technol* vol. 2, no. 3, pp. 127-144, 2004

[12] P. L. H. McSweeney and M. J. Sousa, "Biochemical pathways for the production of flavour compounds in cheese during ripening," *Lait* vol. 80, pp. 293-324. 2000

[13] A. Rahmat and R. Richter, "Formation of volatile free fatty acids during ripening of Cheddar-like goat cheese," *J Dairy Sci* vol. 79, pp. 717-724. 1996

[14] A. Reps, "Bacterial surface-ripened cheese. In: Fox PF (ed) Cheese: Chemistry," *Physics and Microbiology*; Cheese, Chapman & Hall, London, pp. 137-172, 1993

[15] SAS Institute (1988) SAS/STAT User's Guide Cary. NC, SAS Institute

[16] C. Stanton, G. Gardiner, P. B. Lynch, J. K. Collins, G. Fitzgerald, and R. P. Ross, "Probiotic cheese," *Int Dairy J* vol. 8, pp. 491-496, 1998

[17] G. Vlaemynck, "Study of lipolytic of the lipoprotein lipase in lunch cheese of the Gouda type," *Milch* vol. 47, pp. 164-166, 1992

[18] P. Walstra, A. Noomen, and T. J. Geurts, "Dutch-type varieties. In: Fox, PF (ed) Cheese: Chemistry," *Physics and Microbiology*, Chapman & Hall, London, pp. 39-82, 1993

[19] A. H. Woo, S. Kollodge, and R. C. Lindsay, "Quantification of major free fatty acids in several cheese varieties," *J Dairy Sci* vol. 67, pp. 874-878, 1984

[20] A. H. Woo and R. C. Lindsay, "Concentration of major free fatty acids and flavor development in Italian cheese varieties," *J Dairy Sci* vol. 67, pp. 960-968,1984