Food Chemistry **14** (1984) 215–225

β-Galactosidase in the Acceleration of Ras Cheese Ripening

S. M. Farahat, A. M. Rabie, A. A. Abdel Baky, A. A. El-Neshawy & S. Mobasher

Food Science Department, Faculty of Agriculture, Zagazig University, Egypt

(Received: 11 January, 1984)

ABSTRACT

An attempt has been made to accelerate Ras cheese ripening by pre-treatment of cheese milk with β-galactosidase. Milk was treated with a β-galactosidase enzyme preparation, namely lactozym (1 ml/kg milk), at 33°C for 1 h or at 4°C for 18 h, and then used for Ras cheese making. Flavour intensity, formation of soluble nitrogen compounds and free amino acids, and liberation of free fatty acids were enhanced in cheese made from β-galactosidase-treated milk. In addition, the ripening period was reduced to 2 months compared with the 4 months required for the control cheese. Treatment of cheese milk with β-galactosidase at 4°C or 33°C showed similar effects on the properties of cheese.

INTRODUCTION

Cheese maturation is a complex process involving the breakdown of the curd by proteolysis, lipolysis and other enzyme-catalysed reactions to give rise to the flavour and texture changes typical of the different varieties. There is increasing interest in methods which may enable cheese makers to produce mature cheese in a short time (Law, 1981).

Several attempts have been made to accelerate the ripening of Ras cheese (a popular hard cheese in Egypt) by the incorporation into cheese milk or curd of certain additives, such as trace elements (Hofi *et al.*, 1973*a*), protein hydrolysate (Hofi *et al.*, 1973*b*), autolysed starters

(Nassib, 1974), commercial protease and lipase preparations (Abdel Salam *et al.*, 1978, 1979) and ripened cheese slurry (Abdel Baky *et al.*, 1982).

There has been considerable interest over the last 15 years in the pre-treatment of cheese milk with β-galactosidase and the use of hydrolysed lactose milk (HLM) in cheese making. The treatment appears to be useful in shortening both making and ripening times of many cheese varieties, e.g. Cheddar, Mozarella, Camembert, Blue and Domiati cheese (Thompson & Brower, 1976; Marscke *et al.*, 1980; Hassan *et al.*, 1983).

No research work appears to have been carried out on the use of HLM in Ras cheese making. Therefore, the present investigation was planned to assess information on the quality and ripening changes of Ras cheese made from β-galactosidase-treated milk.

MATERIALS AND METHODS

Milk

Fresh cows' milk was obtained from the herd of the Faculty of Agriculture, Zagazig University, Egypt.

Rennet

A rennet powder (1:100 000) was obtained from L. C. Glad Co. A/S, Copenhagen, Denmark.

Starter culture

A single strain starter culture of *Streptococcus lactis* was obtained from Chr. Hansen's Laboratorium A/S, Denmark and used for ripening of cheese milk.

Lactozym preparation

Lactozym (a liquid commercial β-galactosidase preparation containing 3000 LAU/ml) derived from a yeast (*Kluyveromyces fragilis*) was donated by Novo Industries, Novo, Alle, Dk-2880 Bagsvaerd, Denmark.

Cheese making

The following treatments were carried out:

1. Lactozym preparation was added to raw milk (at a level of 1 ml/kg), held at 4 °C for 18 h, heated to 72 °C for 15 s, cooled to 33 °C and then converted into Ras cheese (Treatment A).
2. Cheese milk was heated to 72 °C for 15 s, cooled to 33 °C, treated with lactozym at a level of 1 ml/kg milk, held at the same temperature for 1 h and made into Ras cheese (Treatment B).
3. Ras cheese was made from untreated milk and served as a control.

The cheese for all treatments was made according to the method described by Abdel Tawab (1963).

The addition level and time required for treating cheese milk with lactozym were chosen according to the results of preliminary studies, which indicated that 60·4 % and 66·9 % of milk lactose were hydrolysed when lactozym was added to cheese milk at a level of 1 ml/kg and held at 4 °C for 18 h and at 33 °C for 1 h, respectively. Thompson & Brower (1976) used β-galactosidase-treated milk in which 65–70 % of its lactose was hydrolysed in hard cheese making. Resultant cheeses were ripened at 12 ± 2 °C for 4 months.

Chemical analysis

Lactose and its hydrolysed products were determined in cheese milk as described by Nickerson *et al.* (1976).

Cheese samples were analysed, when fresh, after 2 months and after 4 months of ripening, for moisture, fat, salt, total nitrogen (TN), soluble nitrogen (SN), non-protein nitrogen (NPN) and titratable acidity, as described by Ling (1963). Amino acid nitrogen (AN) was estimated as described by Stadhouders (1959).

Determination of free amino acids

Free amino acids were extracted according to the method described by Ismail & Hansen (1972). The fraction containing amino acids was evaporated and dissolved in citrate buffer, pH 2–2·2.

The separation was carried out by an automatic amino acid analyser, model Unicrom, type CRS 110 A, Vien. The results were calculated and expressed as mg/100 g protein.

Determination of free fatty acids

The sodium soaps of the free fatty acids were prepared from the 2- and 4-month-old cheese samples according to the method of Kuzdzal & Kuzdzal-Savoie (1966). Methyl esters of the free fatty acids were prepared as described by Kuzdzal-Savoie & Kuzdzal (1967). Methyl esters were separated in a Pye Unicam series 104 gas chromatograph (Pye Unicam, Cambridge, Great Britain) equipped with a dual flame ionisation detector. Columns 3·6 m long and of 2 mm inner diameter were used with 80–100 mesh silanised Chromosorb W carrier, coated with 10 % polyethylene glycol adipate as a stationary phase. Temperature programming at a rate of 5 °C/min was applied in the range of 130–180 °C. The temperature of the injection port was 200 °C and that of the detector, 300 °C. Carrier gas flow (He) was adjusted to 35 ml/min. Chart speed was 5 mm/min. Peak areas were calculated by multiplying the peak height at the maximum by the width of the peak at half its height. Results were expressed as mg/100 g of cheese.

Organoleptic properties

The organoleptic properties of Ras cheese were examined by a test panel of five staff members, as described by Abdou *et al.* (1977), with maximum score points of 10, 50 and 40 for appearance, flavour, and body and texture, respectively.

RESULTS AND DISCUSSION

Cheese making time as affected by using HLM

Table 1 shows that pre-treatment of cheese milk with lactozym shortened the time required for milk ripening, coagulation and curd scalding by 24, 33·3 and 22·2 %, respectively when cheese milk was treated with lactozym at 4 °C for 18 h. The corresponding values when milk was treated at 33 °C for 1 h were 30, 42·2 and 37·8 %, respectively. These results could be explained on the basis that β-galactosidase acts in milk by cleaving lactose to free glucose and galactose. The glucose is used preferentially and more rapidly than either lactose or galactose and starter growth is stimulated (Thompson & Gyuricsek, 1974). Thompson & Brower (1976) found that

TABLE 1
Effect of Pre-treatment of Cheese Milk with Lactozym on some Technological Steps of Cheese Making

Treatments[a]	*Milk ripening time*		*Coagulation time*		*Scalding time*	
	(min)	*(% of time saved)*	*(min)*	*(% of time saved)*	*(min)*	*(% of time saved)*
Control	50	—	45	—	45	—
A	38	24	30	33·3	35	22·2
B	35	30	26	42·2	28	37·8

[a] A: Milk treated at 4 °C for 18 h. B: Milk treated at 33 °C for 1 h.

the addition of commercial lactase (from *Saccharomyces lactis*) to raw milk at 4 °C and held for 24 h achieved about 65–70 % lactose hydrolysis. The authors also observed that milk ripening and cheddaring times were reduced by 25–30 % and 20–25 %, respectively.

Gross chemical composition of cheese

Data given in Table 2 show that pre-treatment of cheese milk with lactozym did not greatly affect the moisture, fat, and salt contents of cheese. However, the rate of acidity development in HLM-cheese was higher than that of the control cheese. This could be explained on the basis that β-galactosidase action provides an immediate source of simple sugars leading to growth stimulation of the starter used in cheese making. Similar results were reported by Thompson & Gyuricsek (1974).

Ripening indices of cheese

Cheese ripening was assessed by the determination of SN, NPN, AN, free amino acids and free fatty acids.

Soluble nitrogen compounds

Table 3 shows that pre-treatment of cheese milk with lactozym at 33 °C for 1 h or at 4 °C for 18 h enhanced the formation of SN, NPN, and AN during ripening. Addition of lactozym to cheese milk at 33 °C for 1 h was slightly more effective in this respect. The level of soluble nitrogen compounds in 2-month-old HLM-cheese approached that of control cheese

TABLE 2
Gross Chemical Composition of Ras Cheese made from Hydrolysed Lactose Milk (HLM)

Properties	*Ripening period (months)*	*Control cheese*	*Cheese made from HLM*[a]	
			A	*B*
Moisture %	Fresh	39·6	39·2	38·9
	2	26·6	36·4	36·2
	4	35·1	34·9	34·8
Fat % (DM)	Fresh	48·2	48·5	48·7
	2	48·9	49·1	49·0
	4	49·2	49·8	49·8
Salt % (DM)	Fresh	3·96	3·98	3·98
	2	4·00	4·01	4·20
	4	4·10	4·11	4·10
Acidity %	Fresh	0·50	0·62	0·60
	2	1·10	1·42	1·46
	4	1·65	1·98	2·00

[a] A: Milk treated at 4°C for 18 h. B: Milk treated at 33°C for 1 h.

TABLE 3
Nitrogen Fractions (% of TN) of Ras Cheese made from β-Galactosidase-treated Milk

Nitrogen fractions	*Ripening period (months)*	*Control cheese*	*Cheese made from HLM*[a]	
			A	*B*
SN	Fresh	10·6	11·0	11·2
	2	21·7	25·6	25·9
	4	25·3	34·1	35·2
NPN	Fresh	2·00	2·00	2·00
	2	4·60	5·70	5·90
	4	6·20	9·20	9·60
AN	Fresh	0·68	0·70	0·74
	2	1·50	2·46	2·50
	4	2·70	3·90	4·05

[a] A: Milk treated at 4°C for 18 h. B: Milk treated at 33°C for 1 h.

after 4 months ripening. These results could be explained on the basis that HLM stimulated the growth of lactic acid bacteria which are considered to be essential contributors to protein degradation in cheese (Desmazeaud & Gripon, 1977). Thompson & Brower (1976), and Marscke *et al.* (1980) attributed the high proteolysis observed in HLM-cheese to the contamination of β-galactosidase preparations with proteinases.

Free amino acids

Table 4 shows that total free amino acids in HLM-cheese were higher than the control cheese. The concentrations of free amino acids in cheese made from milk treated with lactozym at 33 °C for 1 h or at 4 °C for 18 h and in

TABLE 4
Free Amino Acids (mg/100 g protein) of Ras Cheese made from Hydrolysed Lactose Milk (HLM)

Amino acids	*Control cheese (Ripening period in months)*		*Cheese from HLM*[a]			
			A		*B*	
			(Ripening period in months)			
	2	*4*	*2*	*4*	*2*	*4*
Phe	9·6	5·6	12·4	20·6	10·0	24·8
Tyr	2·6	3·8	3·2	19·2	4·6	6·6
Leu	34·5	68·5	42·6	100·8	40·6	115·6
Ileu	9·8	11·5	15·7	20·6	10·8	11·8
Met	11·1	14·3	12·6	12·8	15·6	18·7
Val	13·5	19·5	14·6	26·5	20·8	21·6
Ala	45·5	15·8	44·8	26·6	56·8	18·2
Gly	2·8	10·6	6·0	16·0	3·6	21·8
Glu	26·0	52·6	36·0	98·1	30·6	100·8
Ser	4·5	15·6	16·0	36·3	16·8	20·8
Thr	11·5	20·8	22·8	32·8	16·6	25·6
Asp	5·5	10·6	9·5	17·6	9·8	10·6
His	6·0	11·0	9·6	14·6	16·6	26·6
Lys	4·2	15·6	10·6	17·6	12·5	16·6
Pro	10·3	14·8	20·0	28·2	15·8	17·9
Total	197·4	290·6	276·4	488·3	281·5	458·0

[a] A: Milk treated at 4 °C for 18 h. B: Milk treated at 33 °C for 1 h.

that made from untreated milk were 282, 276 and 197 mg/100 g protein, respectively, after 2 months of ripening. These values increased to 458, 488 and 291 mg/100 g protein after 4 months.

The concentrations of the individual amino acids in HLM-cheese were also higher than in the control cheese. The results obtained were in agreement with those reported by Thompson & Brower (1976) and Weaver & Kroger (1978) in cheddar cheese.

TABLE 5
Free Fatty Acids (mg/100 g) of Ras Cheese made from Hydrolysed Lactose Milk (HLM)

Free fatty acids	*Control cheese (Ripening period in months)*		*Cheese from HLM*[a]			
			A (Ripening period in months)		*B (Ripening period in months)*	
	2	*4*	*2*	*4*	*2*	*4*
C_2	4·3	5·7	4·2	6·2	5·0	6·0
C_4	0·8	1·2	1·0	1·5	1·2	1·7
C_6	0·6	1·0	0·8	1·4	1·0	1·6
C_8	0·9	1·7	1·1	2·1	1·2	1·8
C_{10}	3·9	6·2	4·4	7·2	5·0	8·1
C_{12}	6·8	7·4	6·9	7·5	7·0	7·6
C_{14}	9·5	10·4	11·7	15·7	12·1	16·0
C_{16}	11·1	14·7	13·8	21·6	14·2	23·0
C_{18}	2·1	2·4	2·4	3·9	2·9	4·0
$C_{18:1}$	6·7	9·9	8·1	13·8	9·9	13·9
$C_{18:2}$	1·0	1·3	1·9	2·2	2·0	2·6
$C_{18:3}$	Trace	1·0	1·0	1·6	1·1	1·9
Total	47·7	62·9	57·3	84·7	62·6	88·2

[a] A: Milk treated at 4°C for 18 h. B: Milk treated at 33°C for 1 h.

Free fatty acids

As shown in Table 5 addition of lactozym to cheese milk accelerated the formation of free fatty acids during cheese ripening. The pattern of the individual free fatty acids of 2- and 4-month-old HLM-cheese and of the control was found to be similar. However, HLM-cheese contained higher levels of free fatty acids compared with the control cheese. These results agree with those obtained by Thompson & Brower (1974).

Organoleptic properties

Table 6 shows that pre-treatment of cheese milk with lactozym enhanced the intensity of flavour and improved the body and texture compared with the control. In addition, 2-month-old HLM-cheese showed flavour intensity and body characteristics similar to that of the 4-month-old control cheese. Similar trends were observed by Thompson & Brower

TABLE 6
Organoleptic Properties of Ras Cheese made from Hydrolysed Lactose Milk (HLM)

Ripening period (months)	*Properties*[a]	*Control*	*Cheese from HLM*[b]	
			A	*B*
1	Appearance	8	8	8
	Body texture	24	26	26
	Flavour	32	34	34
2	Appearance	8	8	8
	Body texture	30	32	33
	Flavour	36	39	40
3	Appearance	8	8	8
	Body texture	32	34	34
	Flavour	38	41	41
4	Appearance	8	8	8
	Body texture	33	35	36
	Flavour	40	44	44

[a] Maximum score points: appearance, 10; body texture, 40; flavour, 50.
[b] A: Milk treated at 4 °C for 18 h. B: Milk treated at 33 °C for 1 h.

(1976) and Weaver & Kroger (1978). They obtained results that could be explained on the basis that HLM-cheese contained higher levels of SN compounds and fatty acids than the control cheese (Tables 4 and 5). Thompson & Brower (1976) showed that the flavour intensity of 2- to 4-month-old HLM-cheese was found to be similar to that of 4- to 6-month-old cheese. Also, Marchke *et al.* (1980) found that the flavour of 21-week-old HLM-cheese was similar to that of the 13- to 19-week-old control cheese.

REFERENCES

Abdel Baky, A. A., El Fak, A. M., Rabie, A. M. & El Neshawy, A. A. (1982) Cheese slurry in the acceleration of Cephalotyre Ras cheese ripening. *J. Food Protection*, **45**, 894.

Abdel-Salam, M. H., El-Shibiny, S., El-Bagoury, E., Ayad, E. & Fahmy, N. (1978). Effect of lipase on the ripening of Egyptian Cephalotyre Ras cheese. *J. Dairy Res.*, **45**, 491.

Abdel-Salam, M. H., Mohamed, A. A., Ayad, E., Famy, N. & El-Shibiny, S. (1979). Changes in the quality and chemical composition of Ras cheese by some commercial enzyme preparations. *Egypt. J. Dairy Sci.*, **7**, 63.

Abdel-Tawab, G. (1963). Manufacturing Ras cheese from pasteurized milk, cited in Youseff, A. M. (1966). MSc Thesis, Ain Shams University, Egypt.

Abdou, S. N., Abdel Hamid, A. B. Dawood, A. H. M., Youseff, A. M. & Mahran, G. A. (1977). Studies on Cephalotyre Ras cheese coating. II. Effect on ripening. *Egypt. J. Dairy Sci.*, **5**, 191.

Desmazeaud, M. J. & Gripon, J. C. (1977). General mechanism of protein breakdown during cheese ripening. *Milchwissenschaft*, **32**, 731.

Hassan, H. N., Mehanna, N. M., El-Deeb, S. E. & Mashaly, R. I. (1983). Manufacture of white soft cheese from hydrolysed-lactose milk. *Egypt. J. Dairy Sci.*, **11**, 137.

Hofi, A. A. Mahran, G. A., Abdel-Salam, M. H. & Rifaat, I. D. (1973*a*). Acceleration of Cephalotyre Ras cheese ripening by using trace elements. II. Optimum conditions. *Egypt. J. Dairy Sci.*, **1**, 45.

Hofi, A. A., Mahran, G. A., Ashour, M. Z., Khorshid, M. A. & Farahat, S. M. (1973*b*). The use of casein and whey protein hydrolyzates in Ras cheese making. *Egypt. J. Dairy Sci.*, **1**, 79.

Ismail, A. A. & Hansen, K. (1972). Accumulation of free amino acids during cheese ripening of some types of Danish cheese. *Milchwissenschaft*, **27**, 556.

Kuzdzal, W. & Kuzdzal-Savoie, S. (1966). Etude comparee des acides gras non volatiles libres et ester fies les fromages. *XVII International Dairy Congress*, **D**, 335.

Kuzdzal-Savoie, S. & Kuzdzal, W. (1967). Les acides gras libres du fromage. *Lait*, **47**, 9.

Law, B. A. (1981). Accelerated ripening of Cheddar cheese with microbial proteinases. *Neth. Milk Dairy J.*, **35**, 313.

Ling, E. R. (1963). *A text book of dairy chemistry*, Vol. II. Chapman and Hall Ltd, London.

Marscke, R. J., Nickerson, D. E. J., Jarret, W. D. & Dully, J. R. (1980). A cause of increased proteolysis in Cheddar cheese manufactured from milk containing added maxilact. *Australian. J. Dairy Tech.*, 35–84.

Nassib, T. A. (1974). Acceleration of El-Ras cheese ripening by using autolysed starters. *Assiut J. Agric. Sci.*, **5**, 123.

Nickerson, T. A., Vujicic, I. F. & Lin, A. Y. (1976). Colorimetric estimation of lactose and its hydrolytic products. *J. Dairy Sci.*, **59**, 386.

Stadhouders, J. (1959). Hydrolysis of protein during the ripening of Dutch cheese. *XV International Dairy Congress*, **2**, 703.

Thompson, M. P. & Brower, D. P. (1974). Manufacture of cheddar cheese from hydrolysed lactose milks. *J. Dairy Sci.*, **57**, 598.

Thompson, M. P. & Brower, D. P. (1976). Hydrolysed lactose cultured dairy products. 1—Manufacture of cheddar cheese. *Cultured Dairy Products. J.*, **11**, 22.

Thompson, M. P. & Gyuricsek, D. M. (1974). Manufacture of yoghurt, butter milk and cottage cheese from hydrolysed lactose milks. *J. Dairy Sci.*, **57**, 584.

Weaver, J. C. & Kroger, M. (1978). Free amino acid and rheological measurements on hydrolysed lactose cheddar cheese during ripening. *J. Food Sci.*, **43**, 579.