

Flavor Enhancement, by Lipase Addition, of Ras Cheese Made from Reconstituted Milk

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ABSTRACT

Lipase enzyme was added to the curd of Ras cheese during its manufacture from fresh and/or reconstituted milk. The gross composition and free fatty acid content of cheese were analyzed and the organoleptic properties were assessed at 1, 2, 4 and 6 months.

The development of free fatty acids was greater in cheese made from fresh milk than that made from reconstituted milk, and in lipase-treated than in untreated, cheese; lipase addition had little effect on the gross composition of either cheese.

The flavour of cheese made from reconstituted milk and treated with lipase was similar to or a little better than, that made from fresh milk only, due to the higher free fatty acid content (especially more volatile free fatty acids) in the former than in the latter.

INTRODUCTION

Curd produced from reconstituted milk is usually soft and characterized by a weak body and texture which results in inferior flavour, body and texture (Peters, 1959).

The addition of mineral salts before renneting was suggested by Hofi *et al.* (1983) to obtain a suitable curd from reconstituted milk for a national hard cheese type (Ras) production, but flavour studies indicated that ripening was slow compared with cheese made from fresh milk (El-Ghandour *et al.*, 1983). The addition of lipases during the manufacture of Ras cheese from pasteurized mixed buffalo's and cow's milk (1:1) had no effect on the gross composition, improved the quality of cheese with little or no flavour defects (Abd-El-Salam *et al.*, 1978; 1979) and increased the free volatile fatty acids during storage (Mervet *et al.*, 1980). Also, the addition of lipase enhanced the development of the flavour of pickled soft cheese made from 70% whole milk powder and 30% skim milk powder after 30 days' storage (Abd-El-Salam *et al.*, 1981).

The work described in this paper was carried out to study the effect of lipase addition on the flavour of Ras cheese made from reconstituted milk in a trial to improve the quality of the resultant cheese.

MATERIALS AND METHODS

Source of milk

Medium heat spray-dried non-fat milk (European Economic Community), fresh cream (from Brown Swiss cow milk) and fresh cow's milk (Brown Swiss) were kindly supplied by the Misr Milk and Food Company, Ismailia Dairy Plant, Egypt.

Cheesemaking

Ras cheese was made from pasteurized (72°C/15 sec) fresh cow's milk according to the method of Hofi *et al.* (1973). The starter culture (*Streptococcus lactis*, 11454 ATTC) at a ratio of 1% was added to the milk at 35°C, mixed well and left to develop up to 0.19% acidity. Rennet was added at a concentration of 100 ml/100 kg of milk. After coagulation, the curd was cut with cheese knives and the temperature raised to 45°C in 15 min. The curd was held at this temperature for 50 min and the whey was later drained to the level of the curd (acidity, 0.14%). Salt was then added at a rate of 2 kg/100 kg of milk used; the curd was stirred in the weak brine solution for 15 min. Finally, the remaining whey was drained completely and the curd was cooled.

Lipase enzyme (Divitello, eslrace, calf Ca Glio Italiano CHR, Hansen SpA) was diluted with cool water and added to the curd at a rate of 15 g/100 kg milk.

The curd was moulded and pressed with 160 psi after the first 2 h. Pressing was continued overnight after increasing the weight to 1000 psi. The cheese was then dried and resalted by rubbing dry salt on both sides. This process was repeated daily for 12 days and the cheese was waxed thereafter and ripened at a temperature of $10 \pm 2^\circ\text{C}$ and a relative humidity of 80–90% for 6 months.

In another set of experiments, the dried milk was reconstituted in warm water and standardized to give a final fat content of about 3% by the addition of freshly separated cream; then Ras cheese was made by the same method. Each set of experiments was repeated three times.

Chemical analysis of cheese

The moisture, fat, pH, total nitrogen (TN), soluble nitrogen (SN), non-protein nitrogen (NPN) and amino acid nitrogen were determined as described by Ling (1963).

Free fatty acid composition

Sodium soaps of the free fatty acids were released from cheese by the method of Kuzdzal & Kuzdzal-Savoie (1966). Volatile (C_2 – C_8) fatty acids were prepared as described by Ross *et al.* (1963). The methyl esters of C_{10} to C_{18} free fatty acids were prepared by the method of Kuzdzal-Savoie & Kuzdzal (1967). Free fatty acids were separated on a Pye Unicam Series 104 gas-liquid chromatograph using a 1.5 m glass column, inner diameter 3 mm, packed with 10% dimethylglycol succinate on Chromosorb AW/80/100, with 2% H_3PO_4 added. The carrier gas was argon, at 40 ml/min, the column was at 150°C and the detector at 250°C . The internal standard was pelargonic acid.

The cheeses were scored for finish and appearance (20 points), body and texture (30 points) and also for flavour (50 points) when they were young (2, 4 and 6 months) by ten members of the staff of the Food Science Department, Suez Canal University, according to the method of Bruncke (1968).

RESULTS AND DISCUSSION

Cheese quality

In this work, the organoleptic properties of the resultant cheese were studied to ensure reasonable consumer preference and to determine the rôle of lipase addition on the development of cheese flavour.

Table 1 shows that cheese made from fresh milk and treated with lipase achieved the highest score, particularly with regard to aroma and taste, while cheese made from recombined milk scored the lowest. The main differences in organoleptic properties between cheese made from fresh milk and that made from recombined milks were correlated with the colour, consistency and flavour.

TABLE 1
Sensory Evaluation of Ras Cheese During Ageing

Sample	Age (months)	Finish and appearance (20)	Body and texture (30)					Total (100)	
			Flavour (50)						
			Holes (5)	Colour (10)	Consistency (15)	Aroma (25)	Taste (25)		
Cheese made from fresh milk	Control	< 1	20	5	10	13	11	10	69
		2	20	5	10	14	14	13	76
		4	20	5	10	14	16	15	80
		6	20	5	10	15	18	19	87
	Treated with lipase	< 1	20	5	10	11	13	12	71
		2	20	5	10	14	16	18	83
		4	20	5	10	15	20	21	81
		6	20	5	10	14	23	24	96
Cheese made from recombined milk	Control	< 1	20	5	9	9	10	9	62
		2	20	5	9	11	11	10	66
		4	20	5	9	11	13	13	71
		6	20	5	9	12	16	17	79
	Treated with lipase	< 1	20	5	9	11	10	10	65
		2	20	5	9	12	13	14	73
		4	20	5	9	12	16	18	80
		6	20	5	9	13	20	21	88

TABLE 2(A)
Free Fatty Acids in Control Ras Cheese

Fatty acid	Fresh milk cheese						Reconstituted milk cheese									
	Age (months)						Age (months)									
	<1	2	4	6	<1	6	<1	2	4	6	<1	2	4	6		
A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
C ₂	0.0	0.0	3.2	6.5	7.5	8.0	9.5	6.6	0.0	0.0	0.0	0.0	3.3	6.2	4.8	5.0
C ₃	0.0	0.0	4.8	9.7	8.6	9.2	10.8	7.5	0.0	0.0	0.0	2.1	7.7	4.5	5.3	5.9
C ₄	2.6	12.8	9.5	19.2	16.6	17.8	21.6	15.0	1.6	11.0	6.3	23.8	9.3	17.4	14.0	15.7
C ₅	0.5	2.5	1.1	2.2	3.2	3.4	6.3	4.4	0.0	0.0	0.0	0.0	2.0	3.7	3.6	4.0
C ₆	3.2	15.7	6.6	13.3	9.8	16.5	18.6	12.9	2.1	14.5	3.9	14.3	8.1	15.1	11.6	13.0
C ₈	4.5	22.6	7.5	15.2	11.8	12.6	27.5	19.0	3.6	24.8	5.9	21.6	8.9	16.5	15.0	16.8
C ₁₀	9.6	47.1	16.8	33.9	36.0	38.5	50.1	34.7	7.2	49.7	9.1	33.3	17.6	32.9	35.2	39.5
Total	20.4	107.7	49.5	93.5	93.5	144	144	14.5	27.3	53.7	89.5	89.5	89.5	89.5	89.5	89.5
C ₂ -C ₁₀	24.6	10.7	36.2	8.7	53.9	6.5	84.2	5.2	14.1	9.0	20.1	9.2	41.4	7.5	63.9	6.2
C ₁₂	30.8	14.4	61.1	14.7	184	22.3	306	18.9	22.4	14.4	43.2	19.8	111	20.2	163	15.7
C ₁₄	3.6	1.6	6.2	1.5	13.8	1.7	26.3	1.6	2.9	1.9	4.6	2.1	9.3	1.7	17.9	1.7
C ₁₅	72.3	31.7	165	39.6	232	28.2	391	24.1	48.3	30.9	67.5	32.0	192	34.9	268	25.8
C ₁₆	3.6	1.6	4.8	1.2	15.6	1.9	29.1	1.8	2.1	1.4	3.6	1.7	8.9	1.6	15.6	1.5
C ₁₇	93.3	40.9	143	34.4	326	39.5	785	48.4	66.3	42.5	79.1	36.3	188	34.1	509	49.1
C ₁₈																
Total	228	249	416	825	825	1622	1622	156	218	551	1038	1038	551	604	1127	1127
TFFA	249	249	453	879	879	1766	1766	171	245	604	1127	1127	604	1127	1127	1127

TFFA, Total free fatty acids.

A, µg per gram of cheese.

B, Percent of total free volatile or non-volatile fatty acids.

TABLE 2(B)
Free Fatty Acids in Lipase-Treated Ras Cheese

Fatty acid	Fresh milk cheese						Reconstituted milk cheese									
	Age (months)		Age (months)		Age (months)		Age (months)		Age (months)		Age (months)					
	<1	2	4	6	<1	2	4	6	<1	2	4	6				
A	B	A	B	A	B	A	B	A	B	A	B	A	B			
C ₂	1.3	2.3	4.8	3.8	8.2	4.0	1.8	5.4	0.6	1.7	1.9	2.6	4.1	2.9	6.9	2.6
C ₃	2.2	4.0	3.8	3.0	6.3	3.1	9.6	2.8	0.4	1.1	2.9	4.0	6.1	4.3	8.5	3.2
C ₄	10.3	18.5	36.3	29	54.0	26.3	78.3	23.1	3.2	8.8	18.1	25.1	36.4	25.8	64.8	24.2
C ₅	3.3	5.9	6.9	5.5	7.3	3.6	10.8	3.2	2.6	7.2	5.3	7.3	7.1	5.0	9.9	3.7
C ₆	8.6	15.5	13.8	11	26.3	12.8	48.9	14.4	5.3	14.6	9.6	13.3	18.9	13.4	37.1	13.9
C ₈	6.3	11.3	18.3	14.6	29.6	14.4	51.8	15.3	7.2	19.9	11.3	15.7	23.3	16.5	39.5	14.1
C ₁₀	23.6	24.5	41.5	33.1	73.5	35.8	122	35.8	16.9	46.7	23.1	32	45.2	32.0	101	37.8
Total	55.6		125.4		205.2		323.3		36.2		72.2		141.1		267.7	
C ₂ -C ₁₀	29.6	10.8	65.4	10.5	103	5.9	185	6.3	16.3	9.3	43.8	10.2	86.9	8.3	154	7.8
C ₁₂	51.9	18.9	82.3	13.2	263	15.0	532	18.3	29.6	16.9	64.3	15.0	175	16.7	324	16.4
C ₁₄	5.9	2.2	13.4	2.2	54	3.1	98.1	3.4	3.4	2.0	7.4	1.7	18.6	1.8	58.1	2.9
C ₁₅	81.1	29.6	214	34.4	294	28.2	712	24.5	50.1	28.7	144	33.4	266	25.4	509	25.7
C ₁₆	4.6	1.7	13.0	2.1	35.2	2.0	63.4	2.2	3.9	2.2	6.7	1.6	18.3	1.8	41.1	2.1
C ₁₇	101	36.9	234	37.6	802	45.8	1322	45.4	71.4	40.9	164	38.2	481	46.0	892	45.1
Total	C ₁₂ -C ₁₈	274	622	1751	2913	430	1046	1978								
TFFA	330	748	1956	3236	211	502	1187	2246								

TFFA, Total free fatty acids.

A, μg per gram of cheese.

B, Percent of total free volatile or non-volatile fatty acids.

The addition of lipase enhanced the organoleptic properties of the resultant cheese, especially those of that made from recombined milk. The main improvements were in aroma and taste. These findings can be explained by the positive action of lipase on the development of free fatty acids (Table 2), which play an important rôle in the formation of the characteristic cheese flavour, as suggested by Kosikowski & Mocquot (1958). These results are in agreement with those of Abd-El-Salam *et al.* (1978) on the development of flavour in Ras cheese made from fresh milk by lipase addition.

Chemical composition

It is clear from the data of Table 3 that the fat content of cheese increased during ripening as a result of moisture loss. The pH value increased during ripening as a result of protein degradation and ammonia formation (Abd-El-Salam *et al.*, 1978), being higher by 0.04–0.05 units in enzyme-treated than in untreated cheese.

The soluble nitrogen, non-soluble protein nitrogen and amino acid nitrogen contents of cheese increased during ripening, as a result of protein degradation, at a higher rate in cheese made from fresh milk than in that made from reconstituted milk because of the changes of protein during the drying process (Peters & Knoop, 1974; 1975). These findings correspond with those reported by Peters & Williams (1961), Czulak & Hammon (1974) and Omar & Buchheim (1983).

The addition of a lipase preparation caused little increase in the contents of soluble nitrogen and non-protein nitrogen but did cause a noticeable increase in the amount of amino acid nitrogen in both cheese made from fresh milk and that made from reconstituted milk. These results can be attributed to the contamination of such a lipase preparation with proteases, as suggested by El-Neshawy *et al.* (1981) during their study on the improvement of the flavour of Domiati cheese with lipase preparations. Also, these results are in agreement with those obtained by Jolly & Kosikowski (1978) on the development of higher amounts of free amino acids in Blue cheese treated with fungal lipases. Therefore, the chemical composition of Ras cheese made from fresh or reconstituted milk was little affected by the addition of lipase preparations.

Free fatty acids

Ras cheese made from fresh or reconstituted milk gave the same patterns of free fatty acids (Table 2). The main free fatty acids were C_{12} , C_{14} , C_{16}

TABLE 3
Chemical Analysis of Ras Cheese During Ageing

<i>Sample</i>	<i>Age (months)</i>	<i>pH</i>	<i>Moisture (%)</i>	<i>Fat (%)</i>	<i>Soluble N of TN (%)</i>	<i>Non-protein N of TN (%)</i>	<i>Amino acid N of TN (%)</i>	
Cheese made from fresh milk	Control	<1	4.96	40.50	21.5	7.4	4.11	0.63
		2	5.08	38.00	22.8	10.5	5.86	1.96
		4	5.19	35.46	24.0	13.9	8.95	3.15
		6	5.31	33.12	25.2	19.5	11.53	4.70
	Treated with lipase	<1	4.85	41.60	21.0	8.0	4.32	0.75
		2	5.05	39.85	22.2	11.3	6.02	2.16
		4	5.20	36.55	23.5	14.3	9.05	3.95
		6	5.35	34.50	24.6	20.5	12.3	5.75
Cheese made from recombined milk	Control	<1	4.90	46.80	19.5	5.9	3.25	0.50
		2	5.00	42.45	20.1	8.5	4.65	1.08
		4	5.20	39.42	21.5	11.3	6.12	2.25
		6	5.25	36.25	23.5	16.4	8.39	3.45
	Treated with lipase	<1	4.90	47.85	19.5	6.6	3.54	0.55
		2	5.05	42.55	20.8	9.4	5.65	1.45
		4	5.15	40.01	22.0	12.5	7.74	2.59
		6	5.30	37.44	23.5	16.9	9.55	4.20

and C_{18} , while the more volatile free fatty acids (C_2 – C_{10}) which are more responsible for the formation of cheese flavour (Kosikowski and Mocquot, 1958) were present at small concentrations. In addition, Omar (1984) found that C_{14} , C_{16} and $C_{18:1}$ were the main free fatty acids in Ras cheese made from fresh cow's milk.

The development of free fatty acids increased gradually during the ripening period at a higher rate in cheese made from fresh milk than that made from reconstituted milk and in the enzyme-treated than in untreated cheese (Fig. 1).

The addition of lipase during the production of Ras cheese from reconstituted milk increased the formation of free fatty acids and raised the ratio of volatile free fatty acids/non volatile free fatty acids until it was higher than that of cheese made from fresh milk without lipase addition. These results may explain the higher scores, shown in Table 1, of Ras cheese made from reconstituted milk and treated with a lipase preparation. Mervet *et al.* (1980) reported an increase in the amount of volatile free fatty acids during the ripening of Ras cheese made from

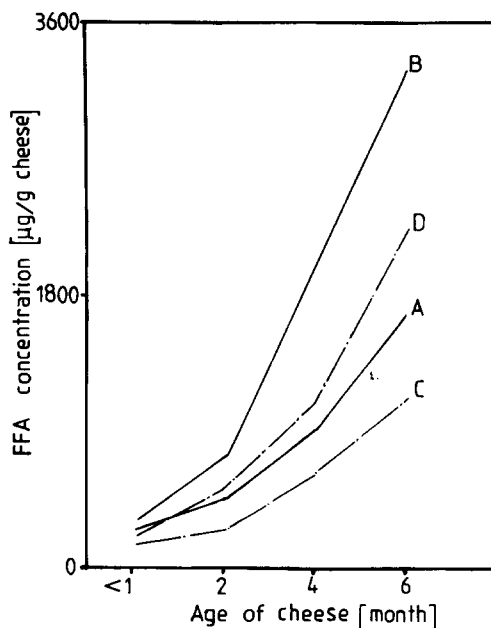


Fig. 1. Free fatty acids (FFA) in Ras cheese during ageing. A, Cheese made from fresh milk; B, cheese treated with lipase; C, cheese made from recombined milk; D, cheese made from recombined milk and treated with lipase.

mixed fresh buffalo's and cow's milk treated by proteolytic and lipolytic enzymes.

Thus it is possible, by lipase addition, to produce, from reconstituted milk, Ras cheese with a flavour similar to that of cheese produced from fresh milk.

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