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Electrophoretic Patterns and Thin-layer Chromatography of Common Cheeses in Egypt: Comparison and Quantification

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ABSTRACT

The extent of cheese ripening and the type of proteolysis and lipolysis of common cheeses in Egypt were measured by concentration of each of soluble tyrosine; soluble trypophan; amino N; soluble N/total N; total volatile fatty acids and free fatty acids, and by quantitative gel electrophoresis and thin-layer chromatography. The effects of concentration-related factors (e.g. moisture, salt and pH) on cheese protein and fat hydrolysis were also studied.

The results showed that, as a heterogeneous group of cheese, differences were marked in gross chemical composition and both the extent of cheese ripening and the relative proportions of protein, fat and their degradation products. Among the selected cheeses, ras cheese has higher values of ripening indices, while kariesh cheese has lower values. Increasing the salt content of mish cheese caused an inhibition in degradation of its protein and fat.

The principal protein regions in electrophoretic patterns and fractions of fat in TLC patterns were similar in number and relative mobility. In most of the cheeses, α_s-casein was degraded more extensively than β-casein, while the whole of the γ-caseins were resistant to further hydrolysis. Also, there was close correlation between α_s-casein and its degradation products. In spite of the absence of significant relationships between the soluble nitrogen and the

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relative amounts of unattached α_s-, β-, and γ-caseins, the amino nitrogen and soluble tyrosine and tryptophan were in close correlation with α_s- and β-caseins and their degradation products.

A positive relationship was noted between pH (from 4·40 to 5·85) and both protein and fat hydrolysis. The fat of roquefort cheese was more hydrolysed than other cheeses; however, the fat of the soft cheeses was less hydrolysed. Moreover, negative and highly significant correlations between triglycerides and their degradation by both TLC and chemical analysis were obtained.

INTRODUCTION

Enzymes of coagulants or from starter bacteria, milk proteases and lipases, contribute to proteolysis and lipolysis in cheese during ripening. These agents decompose the protein and fat of cheese to smaller compounds which are responsible for cheese flavour (Law, 1981).

The degree of proteolysis in cheese was measured by various methods such as: soluble nitrogen, amino nitrogen, soluble tyrosine and soluble tryptophan (Vakaleris & Price, 1959). Also, the degree of lipolysis in cheese was estimated by total volatile fatty acids (Kosikowski, 1966) and free fatty acids (Lynes, 1964).

Polyacrylamide gel electrophoresis and thin-layer chromatography have been used increasingly as the methods of choice to study quantitatively protein and fat breakdown, respectively. This study was carried out to compare the extent of ripening and the type of proteolysis and lipolysis in common cheeses in Egypt by combining the chemical methods and electrophoretic and thin-layer chromatography methods.

MATERIALS AND METHODS

Cheese samples

Samples of kariesh and mish cheeses were purchased from Alexandria market. The other samples of ras, kashkawal, provolone, gouda, processed ras cheese, domiati and roquefort cheeses were collected from Siclam dairy plant and the National dairy training centre in Alexandria, Mansoura dairy plant and Misr Milk and Food Company of Egypt.

Cheese analysis

Chemical analysis

All cheese samples were analysed for moisture, salt, total nitrogen (TN) and soluble nitrogen (SN) according to Ling (1963). The pH was measured by a

radiometer (type M28 pH-meter) using both calomel and glass electrodes. Soluble tyrosine (S. tyr.) and soluble tryptophan (S. trp.) were determined by the procedure outlined by Vakaleris & Price (1959). Amino nitrogen (AN) was tested as outlined by Shokry (1982). Total volatile fatty acids (TVFA) were determined following the method developed by Kosikowski (1966). Free fatty acids (FFA) were measured as outlined by Lynes (1964).

Gel electrophoresis

Polyacrylamide gel electrophoresis was carried out as described by Caric *et al.* (1978) on amido black-dyed gel scanned with a Fujiox FD-AIV integrating scanner.

Thin-layer chromatography

TLC analysis for extracted fat was carried out as described by Alexander *et al.* (1985). The fractions were determined by a double wavelength TLC scanner model CS-910, made by the Shimatzu Co., Japan. The data obtained were statistically anlaysed by the correlation method.

RESULTS AND DISCUSSION

Chemical analysis of the cheeses

The moisture and salt contents and the pH of common cheeses in Egypt are illustrated in Table 1. As expected, the cheeses divide into three groups according to their moisture contents; ras, kashkawal and provolone as hard

TABLE 1
Some Chemical Indices of Ripening of Cheese Samples

Sample	*Moisture* (%)	*Salt/ moisture* (%)	*pH*	*SN/TN* (%)	*S. tyr.* (*mg/100 g of cheese*)	*S. trp.*	*AN* (%)	*TVFA*[a]	*FFA*[b]
Ras	35·39	12·2	5·00	31·7	145	169	0·71	27·0	8·80
Kashkawal	35·82	10·7	4·90	21·4	143	149	0·65	23·8	3·70
Provolone	36·42	10·3	4·95	19·5	155	182	0·63	23·9	3·80
Gouda	52·41	5·91	5·15	14·8	59	84	0·64	24·3	4·20
Roquefort	48·64	7·03	5·00	21·9	86·1	75·7	0·72	19·9	2·10
Processed	58·39	4·28	5·85	17·9	120	68	0·58	14·8	4·10
Domiati	59·39	8·27	4·46	26·5	45	61	0·48	18·5	2·80
Kariesh	69·52	4·60	4·43	18·8	35	52	0·41	8·7	2·05
Mish	65·85	14·9	4·48	23·4	41	59	0·49	14·8	3·14

[a] Total volatile fatty acids (ml 0·1 M NaOH/100 g of cheese).
[b] Free fatty acids (ml 0·1 M NaOH/3 g of cheese fat).

cheeses (35·39% to 36·42%), gouda, roquefort and processed-ras cheese as semi-hard cheeses (48·64 to 58·39%), domiati, kariesh and mish as soft cheeses (59·82% to 69·52%).

As can be seen also in Table 1, the salt as a percentage in the moisture content of the cheese is considerably higher in hard and mish cheeses than in other cheeses, with roquefort and domiati intermediate.

Table 1 also shows that only a slight difference was observed between the pH of the hard (4·9 to 5·0) and semi-hard (5·0 to 5·15) cheeses, while the pH of processed cheese was as high as 5·85. The pH of the soft cheese was much lower (4·34 to 4·48) than any of the others.

Concerning the ripening indices, Table 1 indicates that the hard cheeses are always highly ripened as all the S. tyr., S. trp., AN, TVFA, and FFA contents are comparatively high in hard cheese > in semi-hard cheese > in soft and mish cheeses, while the ras cheese is the highest in S. tyr. and S. trp. followed by the kashkawal. These differences in different ripening indices could correspond clearly to the differences in the rates of protein and fat breakdown during ripening of the different cheeses. The soft cheeses are always the lowest in the ripening indices except that the domiati cheese contains TVFA as high as the roquefort (18·5 and 19·9, respectively), both cheeses being considerably higher in TVFA than kariesh (8·7) but comparatively lower than gouda and the two hard cheeses, kashkawal and provolone. The ripening indices in mish cheese are comparatively low, as in soft cheeses, except in the TVFA (14·8). The high salt/moisture ratio in mish cheese (14·9%) for pickling could decrease the degradation rate of the cheese protein (Abou-Donia & El-Soda, 1986).

Electrophoretic patterns of the cheeses

The electrophoretic patterns of cheeses at alkaline pH are presented in Fig. 1. As expected, differences were marked both in the extent of cheese ripening as measured by protein degradation and in the relative proportions of the major casein fractions and their degradation products. However, the principal protein zones were similar in number and relative mobilities of the main components so most cheeses showed common protein patterns which divided into several bands (Marcos *et al.*, 1979), those of the fast moving components, α_s-casein and β-casein, and the slow moving bands of γ_1-, γ_2- and γ_3-caseins. The α_s-casein has many bonds susceptible to the action of rennin. The formed peptides were more acidic; therefore, their electrophoretic mobilities were slightly higher. β-casein is also attacked by rennin to give *N*-terminal peptides, which are faster moving in polyacrylamide gel at alkaline pH. Moreover, milk proteases and microbial enzymes are more responsible for these peptides in cheese (Creamer, 1975), while the slow

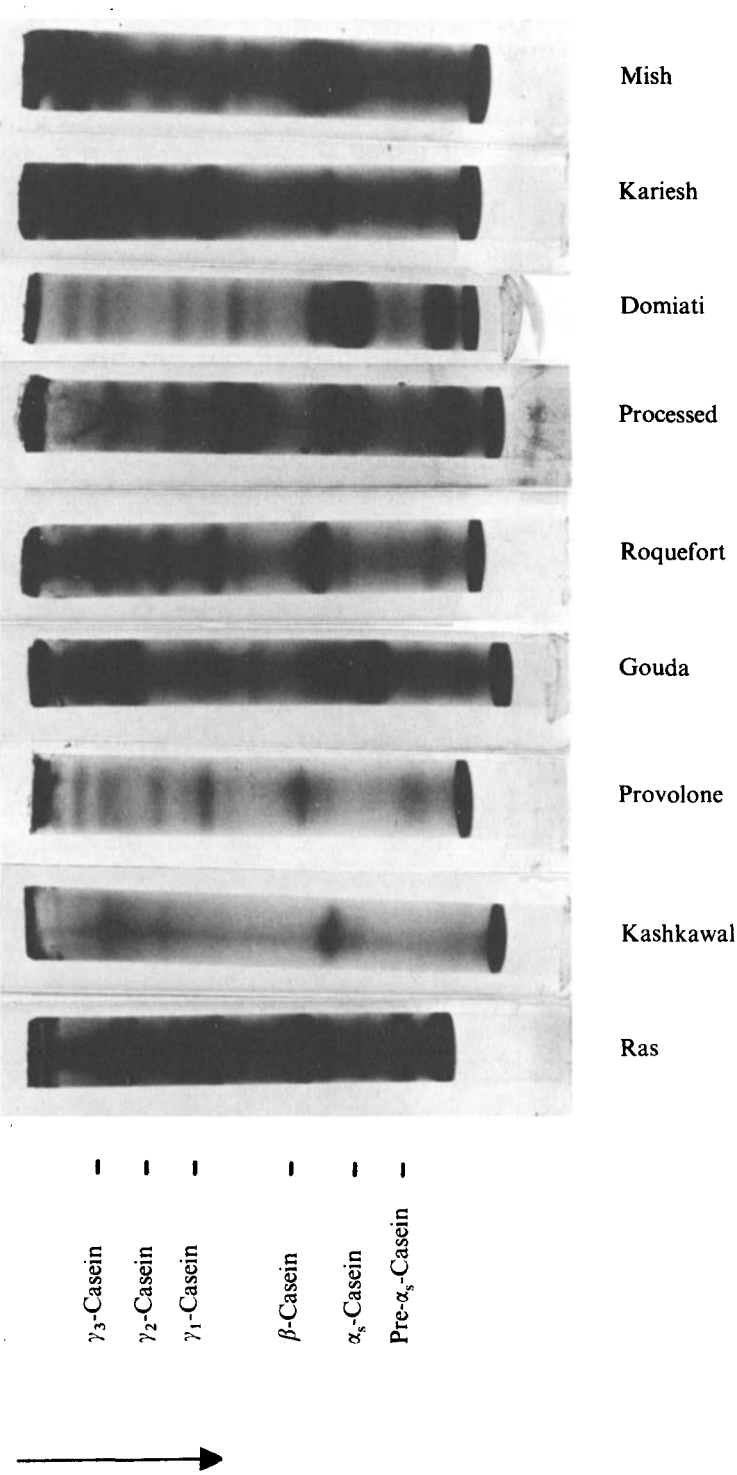

Fig. 1. Polyacrylamide gel electrophoretograms of the different cheese varieties on the market. The positions of the tentatively identified components are shown (according to Marcos *et al.*, 1979).

mobility of γ-casein is due to the lack of *N*-terminal peptides (Marcos *et al.*, 1979).

From electrophoretograms presented in Fig. 1, it can be seen that the proteins of kashkawal and provolone cheeses were degraded more extensively than those of the other cheese samples. It can also be seen that kashkawal, provolone and roquefort contain lower concentrations of β-casein. On the other hand, roquefort, processed-ras cheese, kariesh and mish cheeses contain substantially higher concentrations of low mobility peptides than the remaining cheese varieties. The results are in agreement with the findings of Creamer (1975) who reported that, as cheese matures, β-casein concentrations decrease while γ_1- and γ_2-casein remain almost constant. Later, when the β-casein was depleted, γ_1-casein concentration decreased, with a rise in the concentration of γ_2- and γ_3- casein.

Table 2 shows the results of scanning densitometry of casein electrophoretic patterns. The results indicate that, in most cheese varieties in Egypt α_s-casein was degraded (to γ-caseins) more extensively than β-casein. Table 3 lists the glyceride fractions in the different cheeses. These results are confirmed by the correlation coefficients (in Table 4) between the percentages of α_s-casein and its degradation products (γ_1-, γ_2- and γ_3-caseins) which were negative and highly significant. At the same time, there was negative correlation between β-casein and its degradation products (γ-caseins) but this relationship was not significant, with the exception of the correlation coefficient between β-casein and γ_3-casein ($r = -0{\cdot}66$) which was highly significant. These results are in agreement with the findings of Ledford *et al.* (1966).

Table 4 also shows that there was an absence of significant relationships

TABLE 2

The Integration of Scanning Densitometric or Electrophoretic Patterns of the Cheese Varieties on the Egyptian Market

Cheese sample	*Casein (% of total scanning density)*					
	Pre-α_s-	α_s-	β-	γ_1-	γ_2-	γ_3-
Ras	27·2	15·2	15·3	15·8	13·5	13·0
Kashkawal	35·3	13·8	12·1	13·5	11·4	13·9
Provolone	26·1	14·6	14·8	13·6	10·8	19·9
Gouda	34·2	12·8	14·5	17·1	9·2	12·2
Roquefort	18·8	21·3	11·5	13·4	16·4	19·2
Processed	30·5	14·8	15·1	17·5	12·3	11·8
Domiati	32·7	18·4	16·1	8·6	7·3	16·9
Kariesh	37·1	18·2	17·1	11·8	13·4	2·4
Mish	28·5	17·9	17·5	15·7	14·9	5·5

TABLE 3
The Integration of Scanning Densitometric Measurements for the TLC of Fat Degradation Fractions of the Cheese Varieties on the Egyptian Market

Cheese sample	*Fat fractions (% of total scanning density)*			
	Triglycerides	*Free fatty acids*	*Diglycerides-1,3*	*Diglycerides-1,2*
Ras	51·2	19·3	15·7	13·8
Kashkawal	51·7	17·5	16·2	14·6
Provolone	53·3	18·9	15·8	12·0
Gouda	53·4	17·3	14·3	14·5
Roquefort	48·3	12·1	17·2	22·4
Processed	53·1	20·2	13·5	13·3
Domiati	65·8	11·2	10·1	12·9
Kariesh	62·1	14·5	11·5	11·4
Mish	64·3	13·2	10·6	11·9

between the soluble nitrogen and the relative amounts of unattached α_s- and β-casein and their degradation products (γ_1-, γ_2- and γ_3-casein) while there were significant relationships between the other ripening indices (S. tyr., S. trp. and AN) and α_s-, and β-casein and their degradation products. On the other hand, it was observed that there were positive relationships between the relative concentration of each of γ_1-, γ_2-, and γ_3-caseins and each ripening index. This means that all the γ-casein is resistant to further hydrolysis and the major degradation products arise from α_s- and β-casein. Marcos *et al.* (1979) reported similar results.

Moisture, salt content and pH of cheese and their relationships with casein hydrolysis

As Creamer (1970) reported, the extent of hydrolysis of β-casein in cheese increased with increasing moisture content and was inhibited at higher NaCl concentration, while α_s-casein was not affected significantly by changes in moisture and salt contents. Our data in Table 4 show the correlation coefficients between casein fractions, ripening indices and moisture, salt content and pH of the cheeses in Egypt. The results indicate that there is a highly significant and positive relation between moisture content and residual β-casein. However, highly significant and negative relationships also appear between moisture content and each of γ_3-casein, S. tyrosine, S. tryptophan and S. nitrogen. At the same time, the salt contents of cheeses are not related significantly to the extent of casein hydrolysis. These results were unexpected and suggest an inverse influence of moisture and salt contents on the hydrolysis of casein, which is different from that reported by Creamer (1975). This difference might be attributed to (a) the age of the cheeses not being the

TABLE 4

The Correlation Coefficients (and Significance) between Casein Fractions, Ripening Indices and some Compositional Factors

	Variable	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
1	Pre-α_s-Casein	−0·47*	0·39	−0·14	−0·56**	−0·57**	−0·29	−0·19	−0·29	−0·58**	0·32	−0·25	−0·20
2	α_s-Casein		0·04	−0·52*	0·49*	−0·02	−0·50*	−0·52*	0·29	−0·25	0·45*	−0·03	−0·46*
3	β-Casein			−0·07*	−0·14	−0·66**	−0·51*	−0·35	0·13	−0·79	0·64**	0·14	−0·40
4	γ_1-Casein				0·32	−0·18	0·31	−0·29	0·34	0·43	−0·16	0·04	0·69**
5	γ_2-Casein					−0·26	0·04	−0·09	0·13	0·21	0·06	0·19	0·03
6	γ_3-Casein						0·54*	0·49*	0·14	0·66**	−0·67**	0·03	0·29
7	Soluble tyrosine (mg/100 g of cheese)							0·88**	0·17	0·70**	−0·27	0·21	0·54*
8	Soluble tryptophan (mg/100 g of cheese)								0·29	0·63**	−0·88**	0·44	0·17
9	Soluble nitrogen/ total nitrogen (%)									0·15	−0·93**	0·60**	−0·33
10	Amino nitrogen (%)										−0·83**	0·14	0·53*
11	Moisture (%) (from 35·8 to 69·5)											−0·34	−0·30
12	Salt/moisture (%) (from 4·20 to 14·9)												−0·40
13	pH-value (from 4·40 to 5·85)												

* Significant at the 0·05% level of probability.
** Significant at the 0·01% level of probability.

TABLE 5

The Correlation Coefficients (and Significance) between Fat Fractions, TVFA, FFA and some Compositional Factors

	Variable	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
1	Triglycerides (%)	−0·56*	−0·96**	−0·62**	−0·61**	−0·37	0·74**	0·14	−0·68**
2	Free fatty acids (%)		0·46*	−0·29	0·63**	0·63**	0·53*	−0·06	0·70**
3	Diglycerides-1,3 (%)			0·62**	0·67**	0·32	−0·83**	−0·001	0·49*
4	Diglycerides-1,2 (%)				0·26	−0·16	−0·26	−0·18	0·23
5	TVFA (ml 0·1M NaOH/100 g of cheese)					0·63**	−0·89**	0·37	0·25
6	FFA (ml 0·1M NaOH/3 g of fat)						−0·57**	0·36	0·31
7	Moisture (%) (from 35·8 to 69·5)							−0·34	−0·30
8	Salt/moisture (%) (from 4·2 to 14·9)								—
9	pH value (from 4·40 to 5·85)								

* Significant at the 0·05% level of probability.

** Significant at the 0·01% level of probability.

same, (b) kariesh cheese having a high moisture content and being consumed without ripening, (c) in spite of mish cheese having a high moisture content, its higher salt content inhibiting the rate of protein hydrolysis.

Table 4 also shows the correlation coefficients between casein hydrolysis and pH values of cheeses (ranged from 4·40 to 5·85). Most of these correlations are not highly significant, but there are positive relationships between them. It means that the activity of most of the microorganisms and enzymes which are responsible for cheese protein hydrolysis increased with increasing pH values.

Thin-layer chromatography (TLC) of cheese lipids

The number and relative mobility of the main components of cheese lipids by TLC patterns were similar (Table 3), but there were marked differences in the relative proportions of triglycerides and their degradation products. They sub-divide into four groups: triglycerides, the largest, and three smaller groups, free fatty acids, diglyceride-1,3 and giclyceride-1,2.

It is clear from the results of Table 3 that the fat of roquefort cheese was more hydrolysed than that of other cheese samples. This could be attributed to the growth of internal moulds which produce more lipases. However, the lipids of soft cheeses were less hydrolysed.

Table 5 shows the correlation coefficients between triglycerides and their degradation products in both TLC patterns and as chemically determined. As expected, the relationships were significant and negative. However, there is no correlation between salt concentrations and the extent of triglyceride lipolysis, although the extent of hydrolysis of cheese lipids decreased with increasing moisture content. These results suggest an inverse influence as expected, but this difference could be explained in terms of difference in age; kariesh cheese has a higher moisture content and is consumed without ripening; mish cheese has a higher moisture content but its salt content is high, which partially inhibits the fat hydrolysis.

As can be seen also from Table 5, there are positive and significant correlation coefficients between pH and some degradation products of cheese lipids. More lipolysis occurred at higher pH, which is more suitable for microbial growth and lipase activity.

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