

ACCELERATION OF DOMIATI CHEESE RIPENING BY
USING PANCREATIC EXTRACT

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ABSTRACT

Domiati cheese manufactured from milk treated with pancreatic extract (1 ml contains 6000 Lipolytic unit and 1800 proteolytic unit) levels of 5, 7.5 and 10 ml/10 kg milk revealed the following points:

Addition of 7.5 ml pancreatic extract was found to be the most suitable concentration for accelerating cheese pickling in 30 days as evaluated by SN/TN, NPN/TN ratios, soluble tyrosine and soluble tryptophan as ripening indices during a period of 120 days.

The electrophoretic pattern showed that the breakdown of proteins during ripening was higher in cheese treated with pancreatic extract untreated one.

Quantitative changes in the free amino and free fatty acids during ripening of treated cheese were clear as affected with the proteolytic and lipolytic enzymes existing in the pancreatic extract.

The organoleptic properties of treated cheese (7.5 ml/10 kg milk) were acceptable in quality at 30 days than the treated cheese without detecting any defects in flavour till the end of the ripening period.

INTRODUCTION

Cheese ripening is a complex process involving many physicochemical changes. This process results in the accumulation of lactic acid, fatty acids and free amino acids. The changes during ripening are catalyzed by enzymes, primarily from microorganisms, that result in the formation of the end products typical for each particular cheese variety or batch within a variety (Weaver and Kroger, 1978).

The chemical and physical changes cause the body of fresh cheese to lose its firm, tough curdy texture and to become soft and mellow. This is related mainly to the progressive breakdown of proteins.

The cheese enzymologist looks at microorganism as another source of enzymes in addition to enzymes from milk, milk clotting preparations and added enzymes such as lipases and lactases (Scott, 1972).

In particular, Kosikowski & Tiwasaki (1975) and Sood & Kosikowski (1979), have shown that controlled additions of very small amounts of proteinases and lipases can produce strang flavour in American chadder cheese in a relatively short time without inducing flavour defects.

In this study different concentrations of cattle pancreatic extract were used in order to select the suitable level which accelerate domiati cheese ripening and improve its quality.

EXPERIMENTAL

Domaiti cheese was manufactured as described by Fahmi and Sharara (1950) using mixture of buffaloes and cows milk (1:1) from the herds of Fac. of Agric. at Moshtohor. The milk was heated to 74°C, immediately cooled to 38°C and salt awns then added with a level of 8%. Salted milk was divided into four parts. The milk of the first part was used as a control without pancreatic extract. To the other three milk parts pancreatic extract prepared from fresh defatted pancreas glands as described by Dawood *et al.* (1988), was added at a level of 5, 7.5 and 10 ml/10 kg milk respectively before renneting (each 1 ml contains 6000 lipolytic unit and 1800 proteolytic unit/ml). The other steps of cheese manufacturing were completed as in the traditional method. The resultant cheese was pickled in its own drained whey, sealed and stored at room temperature for 4 months. Periodical samples were taken when fresh and after 15, 30, 60, 90 and 120 days of storage. All cheese treatments were carried out in triplicates and samples were weighed, scored and chemically analysed.

Methods of Analysis:

Cheese samples were analysed for: moisture, total acidity, fat, total nitrogen, soluble nitrogen, non protein nitrogen according to Ling (1963). The pH of the cheese was measured in the cheese filtrate using a pye unicam

pH meter with glass electrode. The total volatile fatty acids were determined as described by Kosikowski (1970), while the soluble tyrosine and tryptophan were assessed by spectrophotometric method of Vakaleris and Price (1959). The method of El-Shibiny and Abd El-Salam was used to separate cheese proteins by Disc electrophoresis, (1976). Free fatty acids of cheese were determined by liquid gas-chromatography method adapted by Nieuwenhof and Hup (1971). The paper chromatography procedure of Block (1958) and White-head (1964), was used for free amino acids determinations.

RESULTS AND DISCUSSION

Cheese composition:

Table (1) illustrates the changes in the chemical composition and ripening indices of Domiati cheese manufactured with different concentrations of pancreatic extract during ripening period of 4 months.

It is clear that the moisture, content decreased in all treatments during the progressive period of cheese pickling. This decrease is due to the curd contraction and whey expulsion resulting from acid development during the ripening period, which is in agreement with the statistical analysis. The differences due to adding pancreatic extract was insignificant.

In respect of the changes in total acidity and pH values of Domiati cheese with different concentrations of pancreatic extract it was obvious that the acidity developed very rapidly during the first month of storage in all treatments then increased gradually in a low rate in the remaining period of ripening. However, the total acidity was increased by increasing the quantity of added pancreatic extract which contains trypsin, chymotrypsin and lipase. The pH value of the cheese took an opposite trend in variations of the acidity. Analysis of variance for these changes were highly significant (Table 2).

Regarding the fat content of Domiati cheese in the control and treated cheese it increased particularly during the first months parallel with the total solids then it was decreased gradually during the rest of ripening period. This may be due to the partial degradation by lipase and its partial losses in whey.

Table (1) : Effect of different concentrations of pancreatic extract on chemical composition yield and organoleptic properties of Domaiti cheese during ripening

Pancreatic extract concentration (ml/10 kg milk)	Ripening period (days)	Moisture %	TA acidity	pH	per dry matter				NPN %	TVPA ml 0.1N NaOH	Tyrosine mg/100 g	Tryptophan (mg/100)g	Yield %	Organoleptic Properties	
					Pat %	TN %	SN %	Flav-B+T %							
Control 0 ml	Fresh	61.50	0.20	6.54	44.94	5.79	0.52	0.26	6.2	3.21	15.2	23.22	34	36	70
	15	59.70	0.60	5.50	48.88	5.76	0.62	0.36	10.0	41.30	50.9	22.0	39	35	74
	30	58.96	1.10	4.93	54.57	5.70	0.68	0.39	17.6	69.21	55.9	21.16	42	32	74
	60	55.82	1.57	4.52	55.45	5.12	0.77	0.52	21.8	102.49	62.4	20.29	43	33	76
	90	53.76	1.77	4.23	54.71	4.67	0.87	0.74	27.4	122.22	67.0	19.78	45	35	80
	120	53.23	1.82	4.20	53.40	4.45	0.94	1.13	28.0	160.37	74.0	19.45	50	36	84
5 ml/10 kg milk	Fresh	61.57	0.20	6.54	44.77	5.80	0.55	0.29	6.8	4.0	15.82	23.28	36	36	72
	15	59.62	0.85	5.26	48.29	5.72	0.79	0.37	12.4	54.70	56.79	22.0	42	35	77
	30	58.82	1.36	4.60	53.67	5.68	0.87	0.41	18.0	86.02	62.80	21.0	45	35	80
	60	55.61	1.72	4.22	54.07	5.05	0.95	0.59	22.4	125.80	70.00	20.11	48	40	88
	90	53.44	1.93	4.16	53.05	4.64	1.01	0.79	30.0	178.32	78.00	19.46	50	39	89
	120	52.94	1.96	4.14	51.82	4.39	1.10	1.17	31.4	198.70	81.00	19.12	52	39	91

Continued:

Continued Table (1)

7.5 ml/10 kg milk														
Fresh	61.57	0.22	6.50	44.50	5.82	0.55	0.31	7.6	4.68	18.0	23.30	38	38	74
15	59.44	1.0	5.08	47.83	5.67	0.91	0.42	14.8	68.78	65.50	21.79	44	37	81
30	58.57	1.58	4.50	52.62	5.62	1.18	0.51	20.8	98.66	77.20	20.89	49	38	87
60	55.22	1.86	4.14	53.37	4.65	1.25	0.71	29.2	150.70	82.60	19.96	52	40	92
90	52.80	2.05	4.11	51.69	4.43	1.38	0.91	36.4	215.98	84.14	19.28	54	39	93
120	52.46	2.10	4.10	50.89	4.16	1.51	1.22	38.2	234.56	94.00	18.97	54	38	92
10 ml/10 kg milk														
Fresh	61.57	0.25	6.30	44.24	5.82	0.57	0.31	8.0	5.0	18.4	23.31	38	38	76
15	59.44	1.05	5.00	47.58	5.67	0.94	0.43	15.2	63.90	68.00	21.78	44	37	81
30	58.54	1.60	4.46	52.10	5.61	1.18	0.53	22.2	101.0	78.39	20.86	49	38	87
60	55.11	1.88	4.12	53.02	4.92	1.30	0.78	30.0	156.43	85.70	19.85	53	40	93
90	52.80	2.06	4.08	51.27	4.40	1.44	1.02	38.6	210.55	96.20	19.28	52	38	90
120	52.40	2.11	4.05	50.31	4.14	1.55	1.28	40.6	232.81	96.80	18.97	51	38	89

TA = Total acidity & TN = Total nitrogen & SN = Soluble nitrogen & NPN = Non-protein nitrogen

TVFA = Total volatile fatty acids & B + T: Body + Texture

Table (2): F-Values of the chemical composition of Domiati cheese made from milk treated with pancreatic extract during ripening.

Chemical composition	F-Value for source of variance		
	Effect of pancreatic extract concentration	Effect of ripening period	Interaction
Moisture %	0.989	289.00**	0.146
Total acidity	23.17**	411.00**	1.018
pH	9.99**	742.00**	1.570
Fat %	0.92**	558.00**	0.220
TN %	14.27**	511.00**	4.390*
SN %	792.00**	1384.00**	41.000**
NPN %	69.44**	2477.00**	5.390**
TVFA %	13.35**	112.70**	0.750
Tyrosin mg/100 g	23.22**	251.00**	2.110
Tyrptophan mg/100 g	161.00**	114.05**	0.540
Yield	9.55	22.23*	0.020

* Significant (P = 0.05) ** Highly significant (P = 0.01)

It was noticed that increasing the amount of pancreatic extract induced a decrease in the fat content of cheese during ripening. This can be attributed to the high concentration of lipase enzyme resulting in more fat hydrolysis which was confirmed by Abd El-Salam *et al.* (1979), who worked on Ras cheese and El-Neshawy *et al.* (1983), who studied Domiati cheese.

The changes in total nitrogen of Domiati cheese under the present study, cleared that the TN increased slightly in the first month of the ripening according to the increase in dry matter then, decreased as ripening process was advanced as explained by Mogakyan and Stepanyan (1962), where the loss of soluble compounds in whey occurred as a result of proteolytic activity took place in cheese during pickling. The SN increased in cheese from different treatments through the storage period which in accordance with Abu-Dawood (1968). This was emphasized by the statistical analysis (Table 2).

Data of the non protein nitrogen (NPN) of the tested Domiati cheese revealed that it increased gradually to the end of ripening period (4 months). This was related to the presence of proteolytic enzymes from the pancreatic

extract and other natural flora, The results are in accordance with El-Abbassy and Wahba (1986).

The proteolytic breakdown involved during the ripening period expressed as SN/TN and NPN/TN ratios in cheese were calculated. The results indicated an increase along the ripening period as the concentration of pancreatic extract increased. The calculated ratio SN/TN in the control was 21.15 at the end of the ripening period which can be achieved after 30 days by using pancreatic extract with a concentration of 7.5 ml/10 kg milk. This in fact can be considered as an excellent result where the ripening period was shortened to one month instead of four months in the case of control cheese. In respect of using NPN/TN ratio as an index for evaluating the ripening process of Domiati cheese it was found that the treated cheese samples differ greatly than the untreated cheese.

Dealing with soluble tyrosine and tryptophan, the obtained data and the statistical analysis cleared that they increased progressively during the ripening and with increasing the amount of pancreatic extract. These results emphasize the great effect of the proteolytic enzymes existing in the pancreatic extract in splitting these two amino acids. Therefore, when depending on these amino acids as ripening indices, the ripening time was reduced to one month according to soluble tryptophan and to two months as evaluated with soluble tyrosine for treated cheese with 7.5 ml and 10 ml/10 kg milk compared with similar results at the end ripening period (120 day) for control cheese.

The obtained results are in agreement with those given by Jolly and Kosilowski (1978) and Wabba and El-Abbassy (1984).

The total volatile fatty acids (TVFA) of Domiati cheese without and with adding pancreatic extract during storage for cheese ripening increased, but the increase was more pronounced with increasing the amount of pancreatic extract and with advancing the ripening period due to the presence of lipase which enhanced the development of TVFA. This agrees with statistical analysis and the results found by Hofi et al., (1975).

Free amino acids:

Values of free amino acids concentration for pickled Domiati cheese without (control) and with pancreatic extract at a level of 7.5 ml/10 kg milk as found to be the suitable concentration for accelerating Domiati cheese ripening

are presented in Table (3). It is clear that the concentration of free amino acids increased throughout the ripening period giving higher concentration in the treated cheese compared with the control. The addition of pancreatic extract induced a noticeable variations in the concentrations and the individuality of the amino acids where more than twice the value in the treated cheese compared with the control. These variations are due to the effect of the proteolytic enzymes existing in the added pancreatic extract which start their function on protein hydrolysis just after the addition during the manufacturing steps and throughout the ripening period.

Glutamic acid was found to be the highest one at the end of ripening which influenced cheese flavour as stated by Webb and Johnson (1965). Glutamic acid, lysine, isoleucine, phenylalanine and leucine formed about 56% of the total free amino acids of the ripened cheese. This may reflect the characteristic flavour of the matured Domiati cheese produced by the addition of pancreatic extract. The essential amino acids were 85.5 and 137.14 mg/100 g cheese representing 57.8 and 58.2% from the total amino acids for untreated and treated cheese with 7.5 ml/pancreatic extract/10 kg milk in the same order.

The electrophoretic pattern of cheese proteins during ripening:

It was found that the addition of pancreatic extract resulted in excessive protein degradation (Fig. 1) particularly in the α s-casein zone, producing many minor bands weakly stained and fastly migrated towards anods, while those with slower rate of migration would result from B-casein. In all cheeses, the α s-casein disappeared faster than B-casein. Similar results were found by El-Shibiny and Abd El-Salam (1976). Fox and Walley (1971), explained that salt content might therefore be a major factor controlling the extent of B-casein proteolysis, which due to the relatively small decrease in water activity produced by the action of salt.

Free fatty acids (FFA):

The changes in the free fatty acids during ripening of Domiati cheese made from milk without and with 7.5 ml/pancreatic extract/10 kg milk are shown in Table (4).

It could be noticed that oleic acid was the highest in quantity for both cheeses at the beginning then decreased gradually with ripening development. The second major free fatty acid was palmitic acid which showed the same trend

Table (3): Changes in free amino acids in Domiati cheese without and with added pancreatic extract (7.5 ml/10 Kg milk) during ripening (mg/100 g cheese).

Amino acids	Ripening period (days)			
	Commencement		90	
	Untreated	Treated	Untreated	Treated
Lysine	3.1	4.70	7.80	13.00
Arginine	-	1.18	5.20	8.20
Serine	-	-	4.60	6.70
Asparatic	0.68	-	4.60	12.60
Threonine	1.50	1.70	3.19	8.20
Glutamic acid	2.20	1.90	6.30	8.80
Alanine	-	4.50	10.22	18.50
Proline	3.21	-	5.60	7.23
Tyrosine	-	5.10	10.11	17.30
Valine	2.26	2.80	4.80	11.64
Methionine	0.52	5.13	14.00	15.03
Tryptophan	-	0.60	1.60	6.80
Phenylalanine	2.63	-	1.84	4.92
Isoleucine	-	7.18	11.40	18.72
Leucine	2.11	-	3.50	10.14
		4.00	13.50	19.54
Total F.A.A.	18.21	38.79	99.66	177.62
% of T.F.A.A.	12.27	16.45	42.27	76.64
			66.09	100.00
			148.38	235.77
			100.00	100.00



Fig. (1-a) Disc electrophoretic pattern of Domiati cheese proteins (made without pancreatic extract) during ripening.

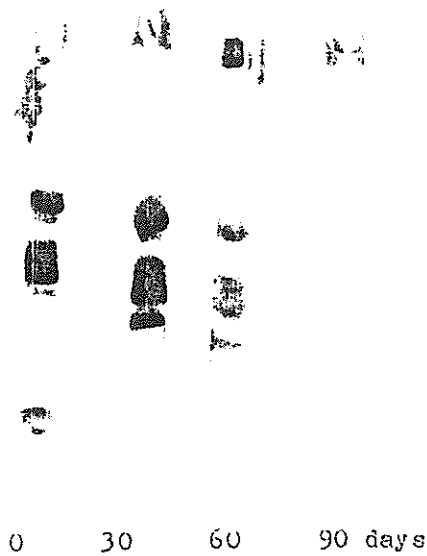


Fig. (1-b) Disc electrophoretic pattern of Domiati cheese proteins (treated with 7.5 ml pancreatic extract /10 Kg milk) during ripening.

Table (4.): Changes in free fatty acids of Domiati cheese without and with added pancreatic extract (7.5 ml/10 Kg milk) during ripening.

Fatty acids	Ripening period (days)							
	Commencement		30		60		90	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
C ₂	-	-	-	-	-	0.24	0.21	0.38
C ₄	-	0.86	-	1.10	1.10	2.26	1.30	2.95
C ₆	1.80	6.50	2.00	3.80	2.20	3.67	2.40	3.60
C ₈	0.60	1.13	1.60	2.77	2.00	3.80	3.00	4.96
C ₉	1.20	1.11	1.50	2.20	1.60	2.60	2.00	2.49
C ₁₀	1.50	3.60	2.84	4.20	2.70	3.90	2.50	3.60
C ₁₁	-	-	1.20	1.75	1.75	1.86	2.11	2.55
C ₁₂	1.80	0.60	2.70	1.10	3.30	1.50	2.95	1.57
C ₁₃	-	1.50	1.40	3.80	2.12	3.64	2.50	2.00
C ₁₄	7.60	7.50	8.55	8.12	8.92	8.80	10.60	9.48
C _{14:1}	0.60	0.80	1.10	1.50	1.32	1.47	0.42	0.65
C ₁₅	0.80	1.75	2.30	3.11	4.80	4.40	6.00	5.50
C ₁₆	31.80	26.1	27.00	23.13	24.00	19.49	23.20	19.28
C _{16:1}	2.40	2.45	2.00	0.77	1.90	0.72	0.80	0.55
C ₁₇	-	0.70	1.70	1.50	2.00	1.75	2.60	2.37
C ₁₈	9.90	8.18	11.70	9.70	10.79	10.23	10.60	12.00
C _{18:1}	32.50	34.00	25.00	27.81	24.00	25.55	21.80	21.24
C _{18:2}	4.30	2.00	4.40	2.14	2.70	2.25	2.22	2.67
C ₂₀	3.20	1.21	3.00	1.50	2.80	1.87	2.60	2.17
Total	100	99.99	99.99	100	100	100	100	100

during ripening as oleic acid. The recorded data cleared that while the above mentioned fatty acids decreased the remaining fatty acids increased during ripening; stearic acid and myristic acid were the highest of this group. Comparing the accumulated free fatty acids in Domiati cheese without (control) and with added pancreatic extract, data obtained from analysis gave a different pattern. It was clear that the total amount of lower fatty acids (Volatile fatty acids C_4-C_{10}) was higher in the treated cheese than the control after manufacture as they were 13.2 and 5.1% at the commencement and increased to be 17.98 and 11.41% after 90 days respectively. This can be attributed to the lipolytic activity of the pancreatic extract which increased the rate of fat hydrolysis and accelerated the flavour development.

Organoleptic properties of cheese:

The cheese samples were evaluated subjectively by a score panel from Staff Members of Food Science Department Faculty of Agric. at Moshtohor.

It could be noticed from Table (1) that the addition of pancreatic extract (which contains proteolytic and lipolytic enzymes) enhanced the development of cheese flavour and the good texture. This was proportional to the quantity added from the pancreatic extract as good flavour and highly acceptable consistency were observed after 30 days. The scoring for the third and Fourth treatments (with 7.5 and 10 ml/10 kg milk pancreatic extract) showed a tendency towards an increase in the score points (Table 1) during the first ripening period reaching its maximum at 60 days of storage then remained constant for the third treatment without detecting any defects in flavour or taste. The fourth treatment scored slightly less at 90 days and showed rancid flavour and bitterness at the end of the ripening period (120 days).

Cheese yield:

The addition of pancreatic extract to milk for manufacturing Domiati cheese with different concentrations had insignificant effect on cheese yield as was clear from the statistical analysis (Tables 1 & 2).

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اسراع تسوية الجبن الـدمياطى باستخدام مستخلص البنكرياس

صلاح مخير عبد الهادى احمد حسن داوود سنية محمود عبده

أظهر الجبن الـدمياطى المصنع من لبن معامـل بمـستخلص البنكرياس بمعدل ٥ ، ٧ر٥ ، ١٠ مل / ١٠ كجم لبن (كل ١ مل يحتوى على ٦٠٠٠ وحدة أنزيم محللة للدهن ، ١٨٠٠ وحدة انزيم محللة للبروتين) النتائج الآتية :

- وجد ان اضافة ٧ر٥ مل مستخلص كانت أنسب كمية لاسراع تسوية الجبن عند تقييمها على أساس النسب بين النتروجين الذائب / النتروجين الكلى والنتروجين غير البروتينى الذائب / النتروجين الكلى وحمى التبروزين الذائب والتربتوفان الذائب كموءشرات للتسوية مقارنة بجبن المقارنة بعد ١٢٠ يوم فترة تسوية .
- أدت اضافة مستخلص البنكرياس الى تحلل اسرع فى بروتينات الجبن فى جميع المعاملات فى جبن المقارنة .
- كانت التخيرات الكمية فى الاحماض الامينية الحرة والاحماض الدهنية الحرة اثناء التخزين واضحة من تأثير الانزيمات المحللة للبروتين والمحللة للدهن المحتوى عليها مستخلص البنكرياس .
- كانت الخواص الحسية للجبن المعامل بـ ٧ر٥ مل / ١٠ كجم لبن ممتازة وصفات الجودة لها عالية بدون ظهور اى عيوب فى النكهة والطعم حتى نهاية فترة التخزين .

