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ACCELERATION RIPENING OF EDAM CHEESE USING BACTERIAL PROTEASE AND LIPASE

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ABSTRACT

Edam cheese was made from pasteurized cow's milk . Liquid crude bacterial protease from *Lactobacillus lactis subsp. Cremoris* and liquid crude lipase from *Lactobacillus delbrueckii subsp. Bulgaricus* were used to accelerate cheese ripening with concentration of 0.75 and 1.5 ml/kg milk for protease and 0.1 ml/kg milk for lipase and combination of 1.5 ml protease and 0.1 ml lipase/kg milk. Cheese was ripened for 3 months. Samples were taken monthly intervals from cheese. The samples were analysed for moisture, fat, acidity, nitrtogen fractions, total volatile fatty acids, formol number, tyrosine and tryptophan. Organoleptic qualities were also assessed during ripening period.

The best results were obtained from cheese treated with combination of 1.5 ml protease and 0.1 ml lipase/kg milk. , this treatment showed the best organoleptic quality. This treatment can be successfully use in the manufacture of Edam cheese.
Keywords: Acceleration ripening, Edam cheese, Protease, Lipase

INTRODUCTION

Cheese ripening involves a complex series of biochemical and probably some chemical events, that leads to the characteristic taste, aroma and texture of each cheese variety, Sousa *et al.*, (2001).

One of the most significant challenges facing the cheese industry is the length of the ripening period of most cheese varirties. Several techniques have been undertaken to accelerate cheese ripening, the adoption of any of these techniques is based on the reduction of the ripening time without any negative effects on flavour or texture quality of cheese, Osman., (2003).

Microbial enzyme preparations were introduce to cheese milk or cheese curds to speed up chesse ripening (Hassan *et al.*, (1996). They showed that various combinations of protease and lipase preparations integrated into cheese curds hastened ripening and increased flavour.

Proteolytic enzymes as agent for acceleration of cheese maturation have been studied extensively, Law and Wigmore (1982) found that the neutral proteinase-Neutrase accelerated development of typical Cheddar cheese flavour. However, in the highest concentration of Neutrase they defected a bitter taste.

The evaluation of lipolysis is concerned, most investigators have delt with the determination of free fatty acids, which are the last product of hydrolyic reactions, (Downey, 1980).

The acceleration of the flavour in ripened Edam cheese was the objective of some investigators, Nasr *et al.*, (1991). Our objective was to show the influence of *Lactobacillus lactis subsp. Cremoris* and *Lactobacillus delbrueckii subsp. Bulgaricus* on physicochemical and flavour compounds of Edam cheese in relation to acceleration of ripening.

MATERIALS AND METHODS

Materials:

Fresh cow's milk was obtained from the herd of Faculty of Agriculture, Cairo University, which had the following composition:

Total solids: 11.94 %
Proteins: 3.10 %
Fat : 3.20
pH: 6.64
Lactose: 4.84

Rennet powder (Ha-La) from Chr. Hansen, Denmark. *Lactobacillus lactis subsp. Cremoris*, ATCC 19435 and *Lactobacillus delbrueckii subsp. Bulgaricus* DSM 20011 were obtained from the Egyptian Microbial Culture Collection (EMCC), Cairo Mercen, Ain-Shams University, Egypt.

Culture preparation: Liquid crude bacterial protease from *Lactobacillus lactis subsp. Cremoris* and liquid crude bacterial lipase from *Lactobacillus delbrueckii subsp. Bulgaricus* were prepared by incubation at 2% (v/v) into sterile 10% (w/v) reconstituted skim milk powder. They were subcultured at least twice for 18 hr. at 23°C as described by EL-Tanboly *et al.*, (2000).

Edam cheese manufacture:

Edam cheese was manufactured using pasteurized milk as described by Fox (1987). Five treatments were carried out using 20 kg of each one as follows:

T.1: Cheese treated with 0.7ml/kg milk of protease
T.2: Cheese treated with 1.5ml/kg milk of protease
T.3: Cheese treated with 0.1ml/kg milk of lipase
T.4: Cheese treated with combination of 1.5 ml protease and 0.1 ml lipase/kg milk
Control: Cheese made without addition any enzymes. All cheeses were ripened at 12±1°C till 90 days.

Methods of analysis:

Preparation of protease from *Lactobacillus lactis subsp. Cremoris*, ATCC 19435:

Proteolytic activity (PA) = 7.5 units (µg tyrosine/min/ml)
Protein content (PC) = 0.1 mg protein/ml
Specifiv activity (SP) = PA/PC = 75 units/mg protein/ml

Preparation of lipase from *Lactobacillus delbrueckii subsp. Bulgaricus* DSM 20011:

Lipoolytic activity (LA) = 20 units (µlipase/min/ml)
Protein content (PC) = 0.26 mg protein/ml
Specifiv activity (SP) = LA/PC = 76.9 units/mg protein/ml

The protease and lipase enzymes production and determination of its activities was performas described by Hassan *et al.*, (1996). Edam cheese samples were analysed for moisture, fat and titratable acidity according to Scotte (1981). Total nitrogen, soluble nitrogen and non-protein nitrogen were

determined according to Kjeldahl method as described by Ling (1963). Total volatile fatty acids (TVFA) was determined according to Kosikowski (1978). Formol number was determined according to Abd EL-Tawab and Hofi (1966). Soluble tyrosine and tryptophan contents were measured as described by Vakelris and Price (1959). The organoleptic properties of cheese samples were assessed by a taste panel of 5 persons The panelists were asked to score the cheese for flavour (out of 60 points), body (15 pointa), texture (15 points) and appearance (10 points). Three replicates were made from each treatment.

RESULTS AND DISCUSSION

Gross composition of cheese:

Table (1) shows the chemical composition of Edam cheese made from different treatments. Moisture content of all treatments decreased continuously during the ripening of three months. All samples had moisture content within the range of 45.30 to 45.20% when fresh while, from 41.74 to 41.85 after three months of ripening. These results were in agreement with Nasr *et al.*, (1991). However, the fat/DM basis increased slightly in all treatments with the decrease of moisture content, the same trend was observed for protein content. Acidity of cheese treated with 1.5 ml/kg milk of protease was slightly higher than other treatments during ripening period and reached 2.72. These increase in acidity of all treated cheeses may be attributed to proteolysis and increase in nitrogen fractions during ripening as a result of the action of protease and lipase enzymes added. These results are in agreement with Hassan *et al.*, (1996).

Table (2) shows the soluble nitrogen and non-protein nitrogen and their ratios to total nitrogen of Edam cheese. Both determinations increased with progressive ripening. The highest SN/TN and NPN/TN were obtained with T 2, cheese treated with 1.5ml/kg milk of protease followed by T 1, cheese treated with 0.75 ml/kg milk of protease. Similar results were obtained by Abdel-Baky *et al.*, (1982) and Hassan *et al.*, (1996).

Fig (1) shows that the addition of bacterial lipase preparation to cheese milk stimulate the accumulation of volatile fatty acids. This was more obvious in Edam cheese made with mixture of protease and lipase, 1.5 ml portease and 0.1 ml lipase/kg milk (T 4). Similar results were obtained by Ismail *et al.*, (1977) and Abbas (1988).

Fig (2) shows that the formol number increases continuously during ripening for all treatments. The addition of 1.5 ml protease/kg milk (T 2) gave the highest formol number. The same trend was observed by Hassan *et al.*, (1996).

Fig (3 and 4) shows that the quantity of tyrosine and tryptophan liberated increases with the increase of protease addition. Generally, addition of lipase did not clearly affect the tyrosine and tryptophan content. The same trend was observed by Hassan *et al.*, (1996).

Fig 1: Total volatile fatty acids (TVFA) contents of Edam cheese during ripening (average of three replicates).

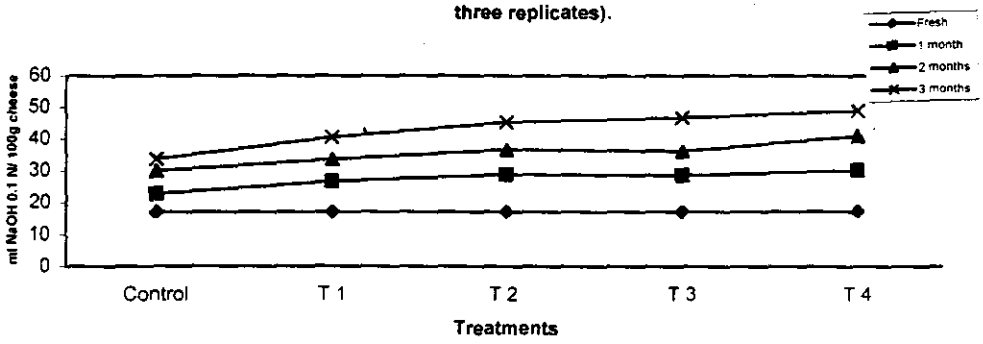

Fig 2: Formol number of Edam cheese during ripening (average of three replicates).

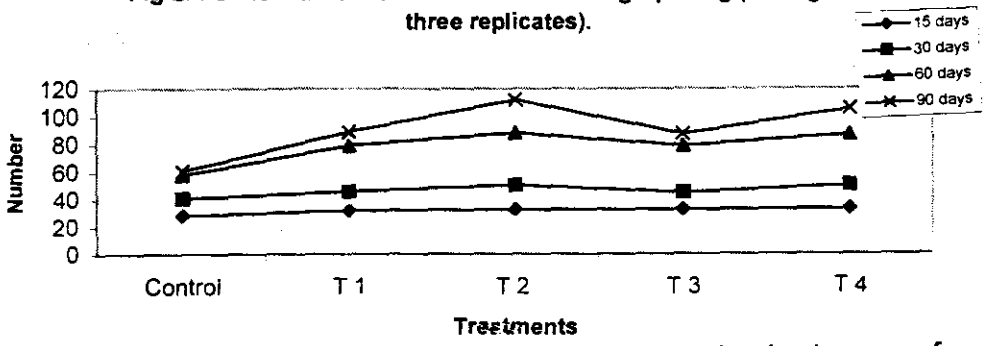

Fig 3: Tyrosine contentof of Edam cheese during ripening (average of three replicates).

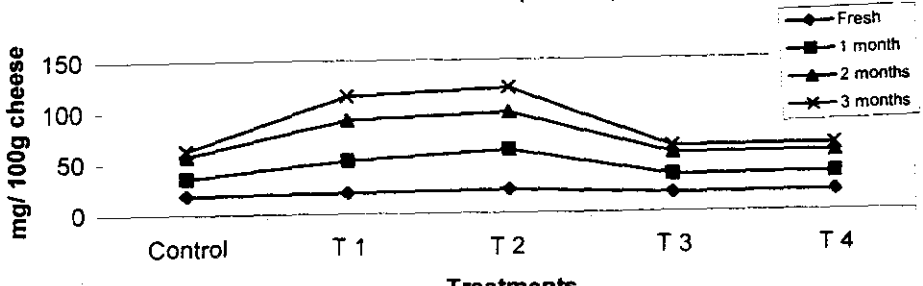

Fig 4: Tryptophan content of Edam cheese ripening (average of three replicates).

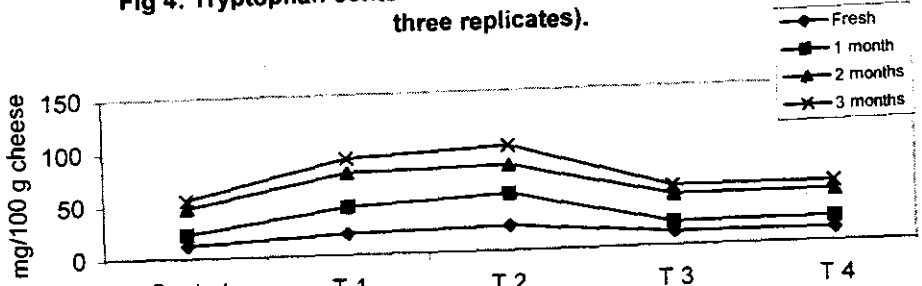

Table (1): Gross composition of Edam cheese during ripening (average of 3 replicates)

%	Moisture	Fat	Fat/DM	Protein	Acidity
Fresh					
Control	45.36	23.22	42.50	22.37	0.90
T 1	45.35	23.21	22.47	22.43	0.98
T 2	45.42	23.15	42.41	22.48	1.10
T 3	45.40	23.18	42.46	22.38	0.96
T 3	45.30	23.19	42.40	22.40	1.08
1 month					
Control	44.20	24.18	43.33	22.88	1.80
T 1	44.16	24.18	43.30	22.93	1.88
T 2	44.15	24.17	43.28	23.05	1.96
T 3	44.08	23.63	42.26	22.95	1.85
T 4	44.18	23.56	42.21	22.89	1.92
2 months					
Control	42.34	25.14	43.60	23.67	2.11
T 1	42.27	25.10	43.48	23.77	2.25
T 2	42.32	25.10	43.50	23.78	2.41
T 3	42.26	25.05	43.38	23.72	2.28
T 4	42.26	25.00	43.31	23.76	2.38
3 months					
Control	41.81	25.50	43.81	23.97	2.48
T 1	41.85	25.47	43.80	23.91	2.65
T 2	41.76	25.46	43.72	24.12	2.72
T 3	41.76	25.50	43.77	23.93	2.60
T 4	41.74	25.46	43.70	23.97	2.68

T.1: Cheese treated with 0.7ml/kg milk of protease
T.2: Cheese treated with 1.5ml/kg milk of protease
T.3: Cheese treated with 0.1ml/kg milk of lipase
T.4: Cheese treated with combination of 1.5 ml protease and 0.1 ml lipase/kg milk

Table (2): Nitrogen fractions of Edam cheese during ripening (average of 3 replicates)

%	TN	SN	SN/TN	NPN	NPN/TN
Fresh					
Control	3.506	0.266	7.59	0.069	1.97
T 1	3.516	0.271	7.71	0.070	1.99
T 2	3.524	0.277	7.86	0.074	2.10
T 3	3.508	0.267	7.61	0.064	1.82
T 3	3.511	0.270	7.69	0.70	1.99
1 month					
Control	3.586	0.326	9.09	0.90	2.51
T 1	3.594	0.471	13.11	0.115	3.20
T 2	3.613	0.535	14.81	0.116	3.21
T 3	3.597	0.331	9.20	0.089	2.47
T 4	3.588	0.335	9.34	0.093	2.59
2 months					
Control	3.710	0.364	9.81	0.127	3.43
T 1	3.726	0.706	18.95	0.181	4.86
T 2	3.727	0.706	20.42	0.186	4.99
T 3	3.718	0.404	10.87	0.123	3.31
T 4	3.724	0.421	11.31	0.133	3.57
3 months					
Control	3.757	0.518	13.79	0.160	4.26
T 1	3.748	0.796	21.42	0.233	6.22
T 2	3.781	0.843	22.30	0.256	6.77
T 3	3.751	0.524	13.97	0.176	4.69
T 4	3.757	0.534	14.21	0.181	4.82

T.1: Cheese treated with 0.7ml/kg milk of protease
T.2: Cheese treated with 1.5ml/kg milk of protease
T.3: Cheese treated with 0.1ml/kg milk of lipase
T.4: Cheese treated with combination of 1.5 ml protease and 0.1 ml lipase/kg milk

Organoleptic assessment:

Table (3) shows the organoleptic evaluation of the different treatments of Edam cheese during 90 days of ripening. The cheese samples showed no significant differences in appearance. Body and texture also flavour scores for all samples increased during ripening up to 90 days. The treatment, (T 4) cheese made with adding 1.5 ml protease and 0.1 ml lipase/kg milkgained the highest score with 92 points followed by treatments (T 2 and T 3) with 88 points after 90 days of ripening period. These results showed the same trend as those of EL-Tanboly *et al.*, (2000).

Table (3): Organoleptic properties of Edeam cheese during ripening period (average of three replicates).

	Flavour 60 points	Body & texture 30 points	Appearance 10 points	Total 100 points
1 month				
Control	36	20	7	63
T 1	40	21	7	68
T 2	40	23	8	71
T 3	41	20	8	69
T 4	42	24	8	74
2 months				
Control	40	22	8	70
T 1	41	23	8	72
T 2	44	25	8	77
T 3	42	24	8	74
T 4	46	26	8	80
3 months				
Control	47	24	8	79
T 1	50	25	9	84
T 2	52	27	9	88
T 3	53	26	9	88
T 4	55	28	9	92

T.1: Cheese treated with 0.7ml/kg milk of protease
T.2: Cheese treated with 1.5ml/kg milk of protease
T.3: Cheese treated with 0.1ml/kg milk of lipase
T.4: Cheese treated with combination of 1.5 ml protease and 0.1 ml lipase/kg milk

CONCLUSION

It can be concluded that it is possible to successively use bacterial protease and lipase in concentration of 1.5 ml protease and 0.1 ml lipase/kg milk for accelerate ripening of Edam cheese with good flavour and a high acceptability when tested after 90 days compared to cheese with other treatments.

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اسراع تسوية جبن الأيدام باستخدام بروتييز وليبيز بكتيري

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قسم الألبان – المركز القومي للبحوث – الدقي

تم صناعة جبن الأيدام من لبن بقري مبستر مع اضافة انزيم بروتييز محضر باستخدام بكتيريا *Lactobacillus lactis subsp. Cremoris* وانزيم الليبيز باستخدام بكتيريا *Lactobacillus delbrueckii supsp. Bulgaricus* وذلك بغرض اسراع تسوية الجبن وذلك بتركيزات ٠,٧٥ مل ، ١,٥ مل/كجم لبن بالنسبة للبروتييز وبتركيز ٠,١ مل/كجم لبن بالنسبة لليبيز ثم خليط من ١,٥ مل/كجم لبن من البروتييز مع ٠,١ مل/كجم لبن ليبيز. تم تسوية الجبن لمدة ثلاث شهور مع اخذ عينات شهريا لتحليل محتواها من الرطوبة ، الدهن ، شقوق النتروجين ، الحموضة ، الأحماض الدهنية الطيارة ، رقم الفرمول ، التيروسين والتربتوفان كذلك تم تقييم الخواص الحسية للجبن خلال فترة التسوية.

أظهرت افضل النتائج بالنسبة الي عوملت بخليط من ١,٥ مل/كجم لبن من البروتييز مع ٠,١ مل/كجم لبن ليبيز ، حيث حصلت هذه المعاملة على اعلا الدرجات في التقييم الحسي.