Studies on Acceleration of Ras Cheese Ripening by Aminopeptidase Enzyme from Buffaloes' Pancreas. II- Utilization of Buffaloes' pancreas aminopeptidase in acceleration of Ras cheese ripening

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Abstract: Aminopeptidases are an exopeptidase that catalyzes the hydrolysis of amino acid residues from the N-terminus of peptide or protein substrates, these are believed to act in concert to completely degrade the products of proteolysis into amino acids. The presence of free amino acids in Cheddar cheese and their contribution to aged flavor clearly and debittering effect in cheese indicates the importance of aminopeptidases in the ripening process. Fresh mixture of buffaloes' and cows' milk (1:1) was divided into five portions. The first portion was left without treatment and served as a control (C). The aminopeptidase was added at levels of 0.03 (T1), 0.06 (T2), 0.09 (T3) and 0.15 (T4) units / kg milk. All five milk samples were manufactured into ras cheese. Ras cheese samples were taken periodically when fresh and after 15, 30, 45, 60, 75, 90, 105 and 120 days of storage at 14 ± 1°C for analysis. After 60 days of ripening, sample T2 was more superior as it had an acceptable acidity and pH value and the difference between it and control was significant. The moisture and total nitrogen contents of sample T2 were 36.65 and 4.643% and there is no significant difference between T2 and control. The level of soluble nitrogen in the control cheese was lower than those of treated cheeses and the difference between sample T2 and control was significant. The use of buffaloes' pancreas aminopeptidase increased soluble tyrosine and tryptophan contents in the resultant cheeses as compared with the control. Specific assessment of proteolysis by measuring decrease in the intensity of protein bands by urea-PAGE showed considerable decreases in intact α_{s1} and β-casein in sample T2 compared to the control. Organolyptically treatment 2 had the highest total score among treatments followed by sample T1 then T3, T4 and finally control cheese. Therefore addition of 0.06 units of buffaloes' pancreas aminopeptidase / kg milk would accelerate the ripening process of Ras cheese through 60 days without any defects in its properties. [Journal of American Science 2010; 6(9):575-581]. (ISSN: 1545-1003).

Keywords: Buffaloes' pancreas, Aminopeptidase, Ras cheese, Cheese ripening.

1. Introduction

Pancreas is a complex organ of both exocrine tissue and endocrine tissue that performs several functions. The exocrine part consists of acini, which produce digestive enzymes (pancreatic juice) and duct system which carries pancreatic juice to the small intestine. The endocrine part consists of pancreatic islets (islets of langerhans), which form less than 2% of the pancreatic tissues (Seeley *et al.*, 1998).

Aminopeptidases are an exopeptidase that catalyzes the hydrolysis of amino acid residues from the N-terminus of peptide or protein substrates, these are believed to act in concert to completely degrade the products of proteolysis into amino acids. Leucine aminopeptidase (EC. 3.4.11.1) was preferentially releases leucine amino acid (Kilcawley *et al*., 2002 and Jankiewicz and Bielawski, 2003). Aminopeptidase has been used to accelerate the ripening process of Italian caciotta cheese and Cheddar cheese as reported by Gobbetti *et al*., (1995) and Raksakulthai *et al*., (2002).

Ras cheese requires along period of time to develop the full flavour and texture of ripened cheese. Thus the operating and capital costs for aging cheese represent a significant portion of the production cost for manufacture. Shortening the ripening time through a low cost method will reduce the cost of cheese for the producer and the consumer. So aminopeptidase (EC. 3.4.11.1) enzyme of buffaloes' pancreas was isolated, purified and its charcteristics were studied (Abd Rabo *et al.,* 2008). Utilization of that purified aminopeptidase to accelerate Ras cheese ripening in order to reduce the cost of Ras cheese production was the aim of this study.

2. Materials and Methods

2.1. Materials:

a. Buffaloes' pancreas

Fresh buffaloes' pancreas was obtained from El-Monib slaughterhouse and then directly transported in crushed ice to the laboratory. Aminopeptidase (EC. 3.4.11.1) enzyme of

buffaloes' pancreas was isolated, purified and its charcteristics were studied (Abd Rabo *et al.,* 2008).

b. Cheese milk

Fresh whole Cows' and buffaloes' milk were obtained from the herd of the Faculty of Agriculture, Cairo University.

c. Rennet

Valirren 150-microbial cheese rennet was purchased from Valley Research, Inc., USA.

d. Cheese starter culture

Streptococcus salivarious subsp. *thermophilus* and *Lactococcus lactis* subsp. *lactis* were purchased from Chr. Hansen Labortories, Copenhagen, Denmark.

2.2. Experimental procedure:

2.2.1. Ras cheese manufacture:

Ras cheese was made by the conventional method descibed by Hofi *et al*., (1970). The aminopeptidase extracted from buffaloes' pancreas were added during the salting stage at levels of 0.03 (T1), 0.06 (T2), 0.09 (T3) and 0.15 (T4) unit/kg milk. Cheese moulds were ripenied at 14 ± 1°C for 4 months. Two batches were prepared for each treatment.

2.2.2. Methods of analysis

a. Milk analysis

Milk samples were analyzed for pH values, titratable acidity, protein and fat contents according to Ling (1963).

b. Cheese analysis

Cheese samples were analyzed fresh and after 15, 30, 45, 60, 75, 90, 105, 120 days during cheese ripening period at 14 ± 1ºC.

Moisture, titratable acidity, total and soluble nitrogen and non protein nitrogen contents were determined according to Ling (1963).

The pH measurements of cheese samples were carried out using pH-meter with glass electrodes, Ingold, Knick, Germany. The soluble tyrosine and tryptophan contents were measured according to Vakaleris and Price (1959).

Electrophoretic patterns of cheese samples were investigated by one dimensional Urea gel electrophoresis to monitor casein proteolysis at zero and 60 days of ripening period according to Fafikye *et al*. (1991).

The organoleptic properties of the cheese samples were organoleptically evaluated by a regular score panels chosen from the staff members of the National Research Centre according to the method of Hofi *et al*. (1970). Score points for flavour, body & texture, and appearance were 50, 40 and 10 respectively.

c. Statistical analysis

The values of all experiments are presented as the means of triplicate analysis. Statistical analysis was carried out using COSTAT, PC statistical software (Costat, 2000). Data were tested by analysis of variance (ANOVA). The differences between means were tested for significant using least significant differences (LSD) test at ($p< 0.05$).

3. Results and Discussion

Fresh mixture of buffaloes' and cows' milk (1:1) was divided into five portions. The first portion was left without treatment and served as a control (C). Aminopeptidase was added at levels of 0.03 (T1), 0.06 (T2), 0.09 (T3) and 0.15 (T4) unit/kg milk. All five milk samples were manufactured into Ras cheese. Ras cheese samples were taken periodically when fresh and after 15, 30, 45, 60, 75, 90, 105 and 120 days of storage at 14 ± 1ºC for analysis.

3.1. Cheese chemical composition

Results presented in Figures (1-10) indicate that the cheese chemical composition was affected by the aminopeotidase and/or the ripening period. This may be due to the production of some acidic compounds as a result of the enzyme action, and also to the probable stimulatory action of some compounds produced by protein hydrolysis. This means that the protein degradation and formation of soluble nitrogenous components, tyrosine and tryptophan contents of Ras cheese were further enhanced by the addition of different concentrations of aminopeptidase

After 60 days of ripening sample T2 was more superior as it had an acceptable taste and flavour (acceptable acidity) and the difference between it and each of control, T3 and T4 was significant. The moisture and total nitrogen (TN) contents were 36.65% and 4.643% which agreed with the Egyptain standards and there is no significant difference between sample T2 and control or sample T1. The level of soluble nitrogen (SN), non protein nitrogen (NPN) or (NPN/TN) in the control cheese were lower than those of the treated cheeses and the difference between sample T2 and control was significant. The used buffaloes' pancreas aminopeptidase increased soluble tyrosine and tryptophan contents in the resultant cheese.

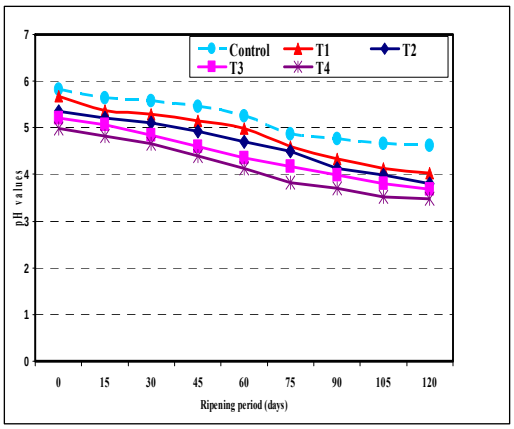


Figure. 1. Titratable acidity (TA) of control and aminopeptidase treated cheeses during ripening period. (LSD $_{0.05}$: 0.138 ^{ns})

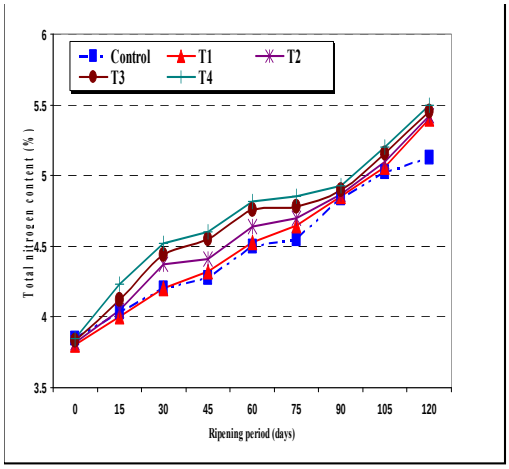


Figure. 2. pH values of control and aminopeptidase treated cheeses during ripening period. (LSD $_{0.05}$: 0.142 ^{ns})

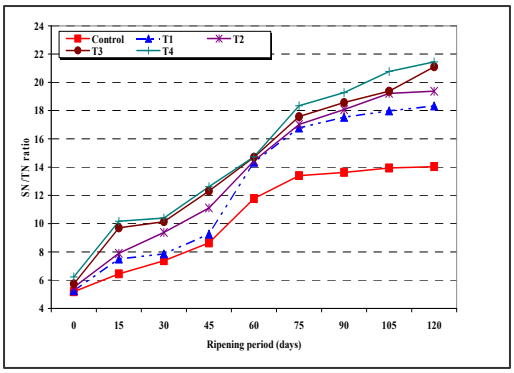


Figure. 3. Moisture content of control and aminopeptidase treated cheeses during ripening period. (LSD $_{0.05}$: 1.691 ^{ns})

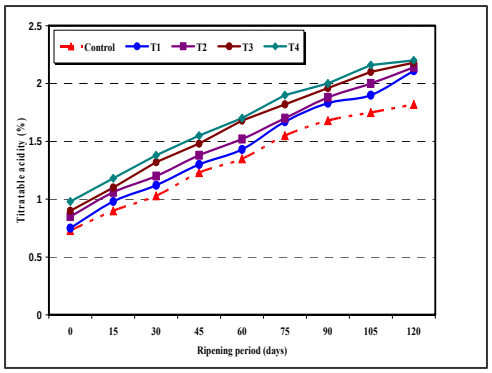


Figure. 4. Total nitrogen (TN) content of control and aminopeptidase treated cheeses during ripening period. (LSD $_{0.05}$: 0.181 ^{ns})

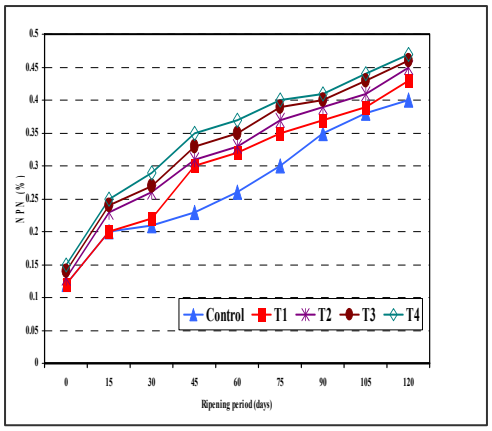


Figure. 5. Soluble nitrogen (SN) content of control and aminopeptidase treated cheeses during ripening period. (LSD $_{0.05}$: 0.126 ^{ns})

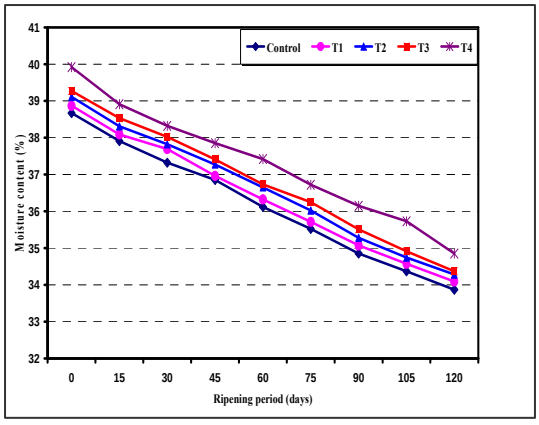


Figure. 6. Soluble nitrogen as a percentage of total nitrogen (SN/TN) content of control and aminopeptidase treated cheeses during ripening period. (LSD $_{0.05}$: 2.839 ^{ns})

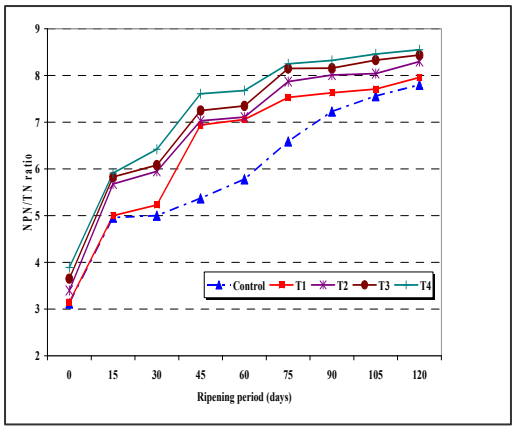


Figure. 7. Non protein nitrogen (NPN) content of control and aminopeptidase treated cheeses during ripening period. (LSD $_{0.05}$: 0.094 ^{ns})

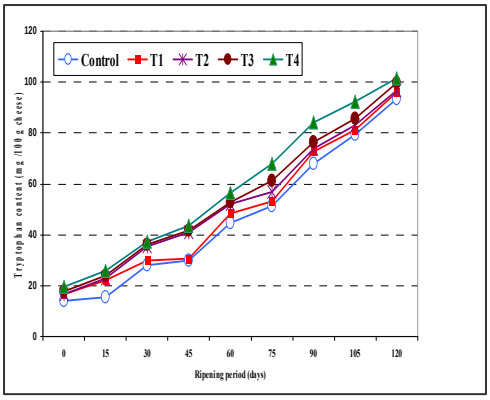


Figure. 8. Non protein nitrogen as a percentage of total nitrogen (NPN/TN) content of control and aminopeptidase treated cheeses during ripening period. (LSD $_{0.05}$: 2.070 ^{ns})

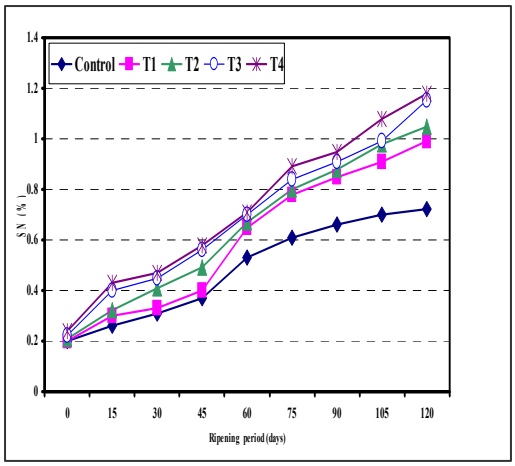
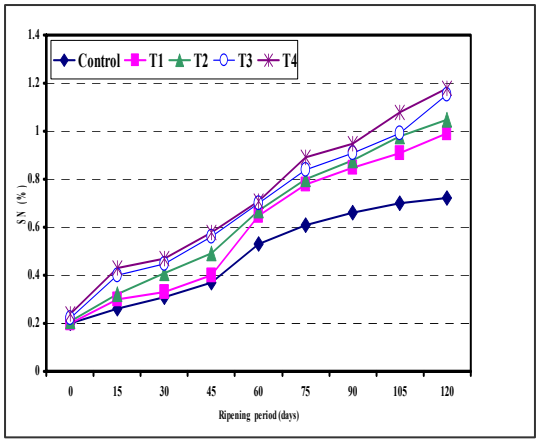


Figure. 9. Tyrosine content of control and aminopeptidase treated cheeses during ripening period. (LSD $_{0.05}$: 29.360 ^{ns})



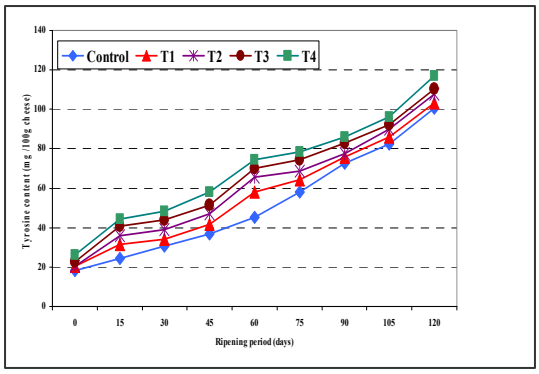


Figure. 10. Tryptophan content of control and aminopeptidase treated cheeses during ripening period. (LSD $_{0.05}$: 9.866 ^{ns})

3.2. Elecrophoretic patterns of control and aminopeptidase treated cheeses

Figure (11) revealed a considerable decrease in intact α_{s1} and β-casein in the samples. The decrease in the intensity of the band corresponding to α_{s1}-casein were accompanied by concomitant increases in the intensity of the band corresponding to α_{s1-1} peptide. The percentage of increase in the intensity of α_{s1-1} of samples T1 and T2 compared to control was 54.95 and 22.52 % after 60 days of storage as shown in Table (1). Data obtained also show that the decreases in the intensity of the band corresponding to β-casein after 60 days of storage were accompanied by increases in the intensity of the γ-casein bands. The percent decrease in β-casein band density was 20.86 and 25.16 % in samples T1 and T2 compared to the control as shown in the same Table. These results are in agreement with the results of Farkye *et al*. (1991) and Grudden *et al*. (2005). They mentioned that the hydrolysis of β-casein is attributable in part at least, to the activity of proteinase or plasmin which hydrolysis

β-casein to γ-casein and proteose peptones. Grudden *et al*. (2005) on the other hand, reported that the temperature of storage had a significant effect on the composition of the casein micelles.

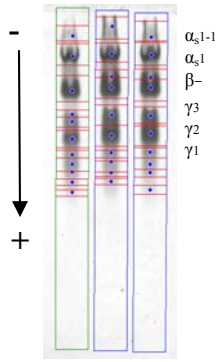


Fig. 11. Elecrophoretic patterns of control and aminopeptidase treated cheeses after 60 days of storage at 14ºC. (C: Control. T1, T2: Cheeses with added aminopeptidase at levels of 0.03 and 0.06 units of aminopeptidase/kg milk, respectively.

3.3. Cheese organoleptic properties

Data presented in Table (2) show the different scores given to various Ras cheese samples during storage period up to 120 days at 14 ± 1ºC.

For flavour evaluation data obtained showed that sample T2 was more superior and attained the highest score than the others after 60 days of storage. The difference between T2 and T1 was non significant, while there were significant differences between treatments T2 and C, T2 and T3 and between T2 and T4 after this period. Sensory analysis of flavour intensity suggested that the maturation period of Ras cheese could be reduced from 4-6 months to 2 months by adding buffaloes' pancreas aminopeptidase at level of 0.06 units/kg milk (T2). The improvement in cheese flavour during storage may be due to the rate of proteolysis and lipolysis in cheese.

Table 1. Elecrophoretic patterns of control and aminopeptidase treated cheeses after 60 days of storage at 14ºC.

Treatments	α_{s1-1}		α_{s1}		β		γ_3		γ_2		γ_1	
	%	Decrease or increase %	%	Decrease or increase %	%	Decrease or increase %	%	Decrease or increase %	%	Decrease or increase %	%	Decrease or increase %
C	6.66	-	19.66	-	26.99	-	7.85	-	14.87	-	4.70	-
T1	10.32	54.95 +	16.66	15.26 -	21.36	20.86 -	15.08	92.10 +	18.12	21.85 +	3.69	21.49 -
T2	8.16	22.52 +	18.59	5.44 -	20.20	25.16 -	12.12	54.39 +	17.16	15.40 +	5.38	14.47 +

C: Control. T1, T2: Cheeses with added aminopeptidase at levels of 0.03 and 0.06 units of aminopeptidase/kg milk, respectively.

Table 2. Organoleptic properties of control and aminopeptidase treated cheeses during ripening period.

Organoleptic properties	ripening period (days)	C	T1	T2	T3	T4
Flavor (50)	0	35	36	38	36	37
	15	39	43	44	40	38
	30	40	44	43	42	40
	45	41	45	45	42	40
	60	41	46	47	43	42
	75	41	46	46	44	41
	90	40	45	46	41	40
	105	41	44	45	40	39
	120	41	44	45	40	39
Body and texture (40)	0	27	28	30	29	27
	15	33	37	36	34	33
	30	32	36	37	34	34
	45	32	36	37	35	34
	60	32	36	37	36	33
	75	32	37	37	35	33
	90	32	37	36	36	32

	105	32	37	37	35	32
	120	31	38	38	34	32
	0	7	6	7	7	6
	15	8	8	9	8	7
	30	8	9	9	8	6
	45	8	9	9	8	7
Appearance (10)	60	8	9	9	8	7
	75	8	9	9	8	7
	90	7	9	9	8	6
	105	8	9	9	8	7
	120	8	8	8	7	6
	0	69	70	75	72	70
	15	80	88	89	82	78
	30	80	89	89	84	80
	45	81	90	91	85	81
Total (100)	60	81	91	93	87	82
	75	81	92	92	87	81
	90	79	91	91	85	78
	105	81	90	91	83	78
	120	80	90	91	81	77
LSD $_{0.05}$	**Flavor**		**Body and texture**		**Appearance**	
	3.192		2.982		1.076	

C: Control. T1, T2, T3, T4: Cheeses with added aminopeptidase at levels of 0.03, 0.06, 0.09 and 0.15 units of aminopeptidase/kg milk, respectively.

Body and texture data revealed that there was gradual improvement in body and texture properties of treated cheeses through the storage period. It is probably due to the breakdown and hydrolysis of protein. A significant difference between control and treated cheeses T1, T2 and T3 after 60 days storage was detected. However there was no significant difference between treatments T1 and T2 after this period. Sample T2 again had the highest score among treatments after 60 days of storage.

From the same Table, it appears that after 60 days storage, samples T1 and T2 attained higher scores for appearance than control, T3 and T4. Statistical analysis on the other hand, showed significant differences among them and sample T4. Treatment 2 as shown had the highest total score compared to the other treatments and rather with the control after 60 days storage followed by T1 then T3, T4 and finally control cheese.

Generally the results obtained showed that addition of 0.06 units/kg milk (T2) would accelerate the ripening process of Ras cheese through 60 days without any defects in its properties.

4. CONCLUSION

Generally the results obtained may lead to the conclusion that addition of 0.06 unit of buffaloes' pancreas aminopeptidase/kg milk (T2) would accelerate the ripening process of Ras cheese through 60 days without any defects in its properties. In other words, it could be concluded that slaughterhouse wastes could be used as a cheap source of the aminopeptidase enzyme; used to accelerate Ras cheese ripening, this reduces manufacturing costs for both producers and consumers, in addition to preserving the environment.

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