ISSN: 0976-3104
REGULAR ISSUE
Abou-zeid.

THE IIOAB JOURNAL

RESEARCH ARTICLE OPEN ACCESS

THE USE OF PLANT ENZYMES FOR RIPENING ACCELERATION OF RAS CHEESE

Nadia A. Abou-zeid
Dairy Science and Technology Department, Faculty of Agriculture, Menoufia University, Shebin El-kom, EGYPT

ABSTRACT

Plant enzymes could be the cheap replacement of the costs microbial enzymes used in acceleration cheese ripening .Purified enzymes extract was prepared from the composite weed Cichorium pumilum. Cichorium enzymes purified extract (CEPE) was added at concentrations of 0.1, 0.2, or 0.3% (V /V) to milk (M treatments) or to cheese curd (P treatments). Proteolytic and lipolytic parameters were higher in treatments than control and in P treatments than in corresponding M ones. Rheological characteristics illustrated that cheese with CEPE had lower hardness, springiness, chewiness, gumminess, and higher adhesiveness and cohesiveness than control. Sensory evaluation proved that both M2 and P1cheese acquired after ripened for 60 days only the typical full flavor and body & texture of control cheese ripened for 150 days.

Received on: 16th-Feb-2015
Revised on: 31st-March-2015
Accepted on: 25th– Apr-2015
Published on: 18th –June-2015

KEY WORDS

Ripening acceleration, Ras cheese, Cichorium pumilum enzymes purified extract

***Corresponding author:** Email: nadia_abou_zeid@yahoo.com **Tel:** +0020482356932; Fax: 002025769495

[I] INTRODUCTION

Ras cheese requires a long period of time to develop the full flavor and texture of ripened cheese. Accelerated ripening of cheese was achieved by addition bacterial strains or by addition of microbial proteases [1-3]. Both bacterial strains and microbial proteases are expensive, also bitterness was reported as the most common flavor defect in cheeses from milk or curd treated with microbial proteases [4-12,2,13].Although plant enzymes could be the cheap replacement of the costs microbial enzymes used in acceleration of cheese ripening, little work has been done in this field. Sadeq et al reported the possibility of using partially purified Calotropis procure leaves protease (Calotropin) to accelerate ripening of Monterey cheese [14]. Nadia and El Sisi used Cichorium enzymes crude extract for acceleration ripening of Domiati cheese [15].

Cichorium is a safe weed; US Food and Drug Administration Poisonous Plant Database, Cornell University Poisonous Plants Informational Database and Provet Poisonous Plants Database do not list any toxic effects of Cichorium. Rasheeduz and Mujahid reported that Cichorium had anti- hepatoxic effects in Wistar strain of Albino rats against carbon tetrachloride induced hepatic damage [16]. Vohra reported that Chicory has been listed as one of the 38 plants that are used to prepare Bach flower remedies, a kind of alternative medicine [17].Thus, in view of fermentation, the objectives of this study are to evaluate the possibility of utilization CEPE to accelerate Ras cheese ripening.

[II] MATERIALS AND METHODS

2. 1. Cichorium enzymes Crude extraction

Plant stems were cut into small pieces, dried, ground then acidic Cichorium enzymes crude extract was prepared as described by Vadde and Ramakrishna [18] with some modifications by stirring 10 grams of stem powder in 100 ml chilled 0.05 M tris-HCl buffer, pH 7.2 in blendor for 1 to 2 minutes The extract was filtered through cheese cloth and centrifuged at 20000 rpm for 15 min. The residue is discarded. The supernatant was referred as Cichorium enzymes crude extract (CECE).

2. 2. Purification of crude extract

Purification was done using the procedure described by Vadde and Ramakrishna [18].

2. 3. Estimation of proteins

Protein content in the Cichorium extract was estimated by the method of Lowry [19].

2. 4. Assay of proteolytic enzymes

Endopeptidase activity was measured using the method described by Sarath et al [20] and modified by Vadde and Ramakrishna [18].

2. 5. Assay of lipoletic enzymes

Lipase activity was measured using the method described by Qixin and Charles [21].

THE IIOAB JOURNAL
ISSN: 0976-3104

2. 6. Ras cheese manufacture

Ras cheese was made by the conventional method described by Nadia [22]. Fresh mixture of buffaloes' and cows' milk (1:1) (obtained from the herds of the Faculty of Agriculture Menoufia University) was divided into seven portions. The first portion was left without treatment and served as a control (C). Cichorium enzymes Purified extract CEPE was added at levels of 0.1, 0.2 or 0.3 % to both of milk prior to renting (M treatments) and cheese curd particles before hoping (P treatments). Cheese was ripened at 14 ± 1°C for 5 months.

2. 6. Methods of cheese analysis

2. 6. 1. Chemical analysis

Moisture, acidity, total & soluble nitrogen and non protein nitrogen contents were determined according to Ling [23]. Schilovish index was estimated as described by Tawab and Hofi [24].

Determination of free amino acid was carried out as described by Bertacco et al [25].Total and individual free fatty acids (FFA) were determined by the method of Deeth et al [26].

2. 6. 2. Rheological analysis

Texture analyzer CNS – (The Farnell, England) was used. The probe was TA17 (30 angle and 25 mm diameter) at speed of 1 mm/ second and 10 mm distance in cheese. Cheese samples were cut into cubs 3cm3 and kept at 12 c for 1 hour before analysis.

2. 6. 3. Electrophoresis

Samples for electrophoresis were prepared as described by Sood et al [27].

2. 6. 4. Organoleptic evaluation

The organoleptic properties of the cheese samples were evaluated by regular score panels chosen from the staff members of Department of Dairy Science , Faculty of Agriculture ,Menoufia University according to the method of Nadia and Wafaa [28].

2. 6. 5. Statistical analysis

Factorial design 1 and 2 factors , 3 replicates was used to analyze all the data Steel and Torrie [29].

[III] RESULTS AND DISCUSSION

3. 1. Purification of protease

The results of the purification are summarized in **Table –1**. The crude extract had specific activity of 32.62 units/mg. The precipitate obtained between 40 – 60% NH2SO4 saturation recovered 1.68 fold by this step. The purified enzyme was about 2.56 fold for application of CG cellulose. In the next step, The purified enzyme was about 4.53 fold by DEAE-cellulose. The final efficient step in the purification procedure is the fractionation on Sephadex G75. The enzyme was highly purified by Sephadex G75 gel filtration chromatography. The specific activity of the protease enzyme is very high (286.48 units/mg). The purified enzyme was about 8.78 fold by this step

.

3. 2. Purification of lipase

The crude extract had lipase specific activity of 48.41units/mg **[Table– 1]**. The precipitate obtained between 40 – 60% NH2SO4 saturation recovered 1.12 fold by this step. In the next step, the purified enzyme was about 2.19 fold. Specific activity was 185.50 of application of DEAE cellulose chromatography for the purification of lipase. The enzyme was highly purified by Sephadex G 75gel filtration chromatography. The specific activity of the lipase enzyme was very high (418.2 units/mg). The purified enzyme was about 8.64 fold by this step.

Table: 1. Summary of purification

TEPS	PROTEIN(MG/ML)	SPECIFIC ACTIVITY Of protease U/mg	SPECIFIC ACTIVITY of lipase U/mg
Crude extract	3.2	32. 62	48.4
NH_2SO_4 fractionation	2.5	54. 70	53.6
CM-cellulose	1.8	83.42	106.12
DEAE-celellulose	0 .9	147.73	185.5
Sephadex G 75	0.3	286.48	418.2

3. 4. Chemical analysis of cheese

3. 4. 1. Ripening parameters

Data in **Table– 2** illustrate that the level of soluble nitrogen (SN/TN), non protein nitrogen (NPN/TN), Schilovish index and TVFA in the control cheese were significantly lower than those of the corresponding treated cheeses.

3. 4. 2. Free amino acid:

Data in **Table–3** shows the amount of free amino acids after 60 days in control, P1 and M1 cheese. Acids generally increased during ripening and were significantly higher in the CEPE treated cheeses than in the control cheeses, and they were higher in P1 than in M1 cheese. From these results, it seems that the

THE IIOAB JOURNAL
ISSN: 0976-3104

CEPE increased liberation of FAA significantly and it was more effective when added to cheese curd than cheese milk.

Table: 2. Ripening properties of Ras cheese during ripening as affected by CEPE

Chemical properties	Ripening period	Treatments							
		C	M1	M2	M3	P1	P2	P3	LSD
Acidity	0	0.85^{a}	0.87^{a}	0.83^{a}	0.86^{a}	0.87^{a}	0.84^{a}	0.83^{a}	±0.05
	30	1.36^{a}	1.42^{b}	1.56^{c}	1.63^{d}	1.56^{c}	1.74^{e}	1.94^{f}	
	60	1.57^{a}	1.77^{b}	1.85^{c}	1.92^{d}	1.89^{c}	1.98^{d}	2.12^{e}	
	120	1.49^{a}	1.37^{b}	1.29^{c}	1.09^{d}	1.34^{b}	1.08^{d}	1.05^{d}	
	150	1.26^{a}	1.13^{b}	1.07^{c}	1.06^{c}	1.15^{b}	1.08^{c}	1.06^{c}	
SN/TN %	0	6.18^{a}	6.22^{a}	6.15^{a}	6.16^{a}	6.21^{a}	6.20^{a}	6.11^{a}	±0.15
	30	8.05^{a}	8.85^{b}	9.95^{c}	10.96^{d}	9.84^{c}	10.73^{d}	12.81^{e}	
	60	11.51^{a}	12.23^{b}	15.01^{c}	17.51^{d}	15.12^{c}	17.67^{d}	18.92^{e}	
	120	14.63^{a}	16.84^{b}	19.63^{c}	21.92^{d}	19.72^{c}	21.73^{d}	22.03^{e}	
	150	17.13^{a}	19.14^{b}	22.41^{c}	25.17^{d}	22.22^{c}	25.24^{d}	27.24^{e}	
N.P.N/TN%	0	4.13^{a}	4.15^{a}	4.18^{a}	4.19^{a}	4.20^{a}	4.21^{a}	4.15^{a}	±0.32
	30	4.32^{a}	4.73^{b}	5.97^{c}	7.96^{d}	5.92^{c}	7.74^{d}	9.17^{e}	
	60	5.65^{a}	6.88^{b}	8.21^{c}	10.67^{d}	8.19^{c}	10.43^{d}	14.16^{e}	
	120	8.87^{a}	9.54^{b}	10.88^{c}	12.08^{e}	11.41^{d}	12.33^{e}	15.12^{f}	
	150	10.43^{a}	10.97^{b}	11.51^{c}	13.44^{e}	12.97^{d}	13.18^{e}	15.71^{f}	
Schilovich index	0	15.92^{a}	16.24^{a}	16.23^{a}	16.31^{a}	15.82^{a}	15.98^{b}	15.94^{a}	±0.78
	30	28.16^{a}	32.92^{b}	38.97^{c}	45.93^{d}	38.77^{c}	45.65^{d}	56.15^{e}	
	60	45.56^{a}	55.82^{b}	67.16^{c}	71.86^{d}	67.22^{c}	73.12^{e}	80.77^{f}	
	120	58.11^{a}	69.33^{b}	82.16^{c}	92.55^{d}	85.52^{e}	95.61^{f}	108.52^{g}	
	150	71.92^{a}	80.66^{b}	96.22^{c}	109.29^{d}	99.13^{e}	113.39^{f}	125.18^{g}	
T.V.F.A	0	18.41^{a}	18.27^{a}	18.14^{a}	18.28^{a}	18.14^{a}	18.38^{a}	18.12^{a}	±0.84
	30	32.19^{a}	37.12^{b}	45.92^{c}	61.74^{d}	45.84^{c}	61.33^{d}	73.11^{e}	
	60	41.83^{a}	46.64^{b}	53.16^{c}	72.92^{d}	53.36^{c}	72.87^{d}	89.62^{e}	
	120	52.11^{a}	57.68^{b}	68.41^{c}	96.49^{e}	69.13^{d}	96.72^{e}	109.52^{f}	
	150	59.82^{a}	67.88^{b}	84.68^{c}	118.6^{f}	86.75^{d}	114.32^{e}	133.67^{g}	

See **Table– 1** for details, a–g Means within a row with different superscript letters are significantly different (P < 0.05)

Lysine, proline, phenylalanine and tyrosine, were in large quantities in all cheeses whereas glycine, Cysteic acid, Iso-leucine and therionine existed only in small amount. Ivana et al reported that in twenty five cheeses commercially available in the Czech Republic glutamic acid, proline, aspartic acid, leucine, valine, and phenylalanine are present in higher concentrations [30]. Other amino acids are present in lower concentrations. Sood et al mentioned that asparagine, glutamine, valine, leucine, phenylalanine, and arginine were in large quantities in all Cheddar cheeses whereas proline and histidine existed initially only in traces but increased later [27].

The determination of free amino acids plays an important role in assessing the nutritional quality of foods [31-33].

THE IIOAB JOURNAL
ISSN: 0976-3104

3. 4. 3. Free fatty acids

The percentage concentration of free fatty acids (FFA) in Ras cheeses are shown in **Table– 4**. In terms of short (C4-C8) and long (C16, C18, C18:1) chain fatty acids, a significant interaction ($P<0.05$) was found between ripening period and cheese samples.

Amounts of butyric, caproic and caprylic acids were higher in samples with added CEPE than in the control cheese sample and in P1 than M1. Amount of free fatty acids were approximately equal in m2 and p1 cheese . From these results, it seems that the CEPE increased liberation of FFA significantly and it was more effective when added to cheese curd than cheese milk.

In control cheese sample the level of butyric, caproic, caprylic acids increased until 60 days, but then did not increase anymore. Adding *Cichorium* enzymes increased the level of butyric, caproic, caprylic acids until 150 days. Concentrations of capric, lauric and myristic acids increased more obviously in the cheese with added CEPE ($P<0.05$). Katsiari *et al* and Collins *et al* reported that the short and medium chain fatty acids contribute more to the flavor formation than the long chain ones [1],[34].

Palmitic, stearic and oleic acids were the most abundant FFA in all the cheeses throughout the ripening especially oleic acid. These results are in accordance with the findings of Guller and Kondyli *et al* [2, 35].

Table: 4. Free fatty acids of total FFAs in Ras cheese during ripening as affected by CEPE

Free fatty acids %	Storage Periods	cheese samples* C	M1	M2	P1
Butyric $C_{4:0}$	0	0.09	0.12	0.15	0.13
	60	0.14	0.17	0.34	0.37
	150	0.18	0.18	0.53	0.56
Caproic $C_{6:0}$	0	0.04	0.06	0.08	0.09
	60	0.06	0.08	0.16	0.18
	150	0.05	0.08	0.37	0.36
Caprylic $C_{8:0}$	0	0. 04	0.05	0.06	0.05
	60	0.05	0.07	0.15	0.18
	150	0.06	0.06	0.32	0.35
Capric $C_{10:0}$	0	0.15	0.18	0.21	0.19
	60	0.19	0.25	0.35	0.38
	150	0.18	0.26	0.52	0.56
Lauric $C_{12:0}$	0	0.43	0.50	0.58	0.52
	60	0.51	0.67	0.75	0.79
	150	0.62	0.78	1.88	1.95
Maristic $C_{14:0}$	0	1.5	1.6	1.8	1.8
	60	2.1	2.3	4.3	4.2
	150	3.7	4.1	6.3	6.5
Palmitic $C_{16:0}$	0	19.1	18.5	18.5	18.5
	60	17.6	16.2	12.0	11.8

*See **Table– 1** for details, Values are means of 3 replicates

3. 4. 4. Textural characteristics of cheese

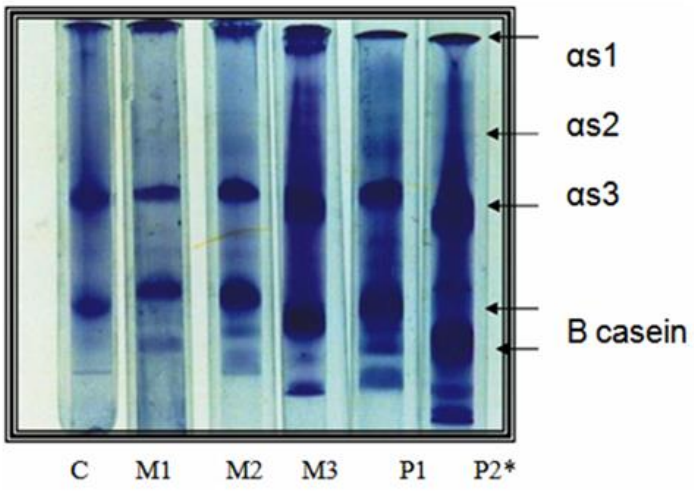
Rheological characteristics **[Table-5]** illustrated that cheese with CEPE had lower hardness, springiness, chewiness, gumminess, and higher adhesiveness and cohesiveness than control. Data also illustrate that there was sharp decrease in gumminess, and chewiness values of all cheese as ripening period advanced. A negative relationship was found between hardness and the values found for gumminess, springiness and chewiness. These results agree with that reviewed by Bryant et al , Fox et al and Katsiari et al [1, 36, 37].

THE IIOAB JOURNAL
ISSN: 0976-3104

Table: 5. Rheological properties of Ras cheese during ripening. as affected by CEPE

Rheological properties	Ripening periods	cheese samples*							
		C	M1	M2	M3	P1	P2	P3	
Hardness (g) C	0	722.2^{a}	718,9^{a}	724.8^{a}	721.3^{a}	720.2^{a}	722.3^{a}	719.4^{a}	±9.2
	60	968.4^{a}	899.5^{b}	855.9^{c}	803.1^{e}	845.3^{d}	804.2^{e}	658.7^{f}	
	150	8112.1^{a}	751.7^{b}	534.8^{c}	411.5^{d}	528.9^{c}	415.9^{d}	332.9^{e}	
Adhesiveness gs^{-1}	0	9.27^{a}	9.18^{a}	9.34^{a}	9.25^{a}	9.32^{a}	9.23^{a}	9.24^{a}	±0.72
	60	15.48^{a}	18.12^{b}	32.25^{c}	22.88^{d}	31.85^{c}	21.65^{e}	14.35^{f}	
	150	39.30^{a}	35.15^{b}	14.67^{c}	5.08^{d}	14.38^{c}	4.88^{d}	4.05^{e}	
Springiness m m	0	8.91^{a}	8.72^{a}	8.73^{a}	8.82^{a}	8.99^{a}	8.88^{a}	8.84^{a}	±0.21
	60	6.16^{a}	6.56^{b}	5.45^{c}	4.67^{d}	5.62^{c}	4.51^{d}	3.22^{e}	
	150	5.85^{a}	4.98^{b}	4.08^{c}	2.88^{d}	4.05^{c}	2.76^{d}	2.05^{e}	
Cohesiveness ratio	0	1.38^{a}	1.32^{a}	1.36^{a}	1.35^{a}	1.30^{a}	1.31^{a}	1.36^{a}	±0.14
	60	2.92^{a}	2.51^{b}	1.58^{d}	0.89^{e}	1.72^{c}	0.92^{e}	0.72^{f}	
	150	0.74^{a}	0.71^{a}	0.62^{b}	0.60^{b}	0.74^{a}	0.66^{b}	0.51^{c}	
Gumminess gs^{-1}	0	1011.2^{a}	1014.5^{a}	1012.1^{a}	1017 .4^{a}	1013.3^{a}	1018.6^{a}	1015.7^{a}	±11.3
	60	943.3^{a}	953.8^{a}	756.7^{b}	542.3^{c}	747.8^{b}	550 .8^{c}	445.4 ^{d}	
	150	740.8^{a}	680.5^{b}	558.6^{c}	465.2^{d}	548.3^{c}	426.1^{e}	278.7^{f}	
Chewiness gs^{-1}	0	11304^{a}	11293^{a}	11298^{a}	11292^{a}	11303^{a}	11292^{a}	11297^{a}	±13.1
	60	3456^{a}	3124^{b}	2541^{c}	1342^{d}	2533^{c}	1326^{d}	819^{e}	
	150	1934^{a}	1740^{b}	1265^{c}	856^{d}	1254^{c}	846^{d}	563^{e}	

*See **Table 1**



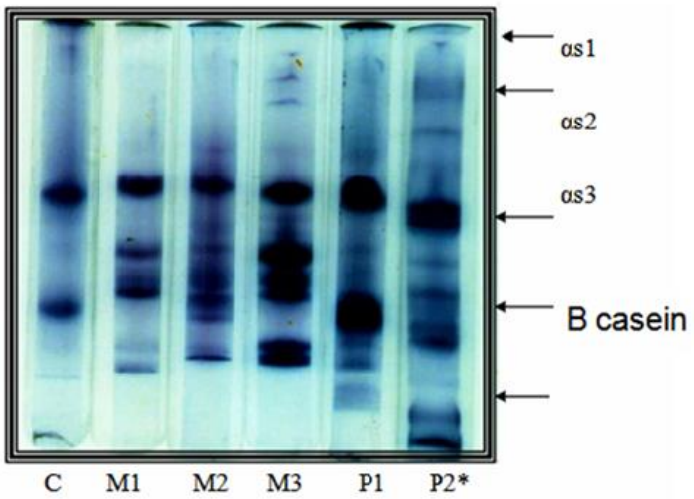


Fig: 1. Gel electrophoretic pattern of 60 days old Ras cheese. Fig: 2. Gel electrophoretic pattern of 150 days old Ras cheese

THE IIOAB JOURNAL
ISSN: 0976-3104

3. 4. 5. Electrophoresis of cheese casein

Hydrolysis of αs1 casein was significantly greater and faster in cheeses treated with CECE than in control cheeses. The αs2 and αs3 caseins also were hydrolyzed but less than αs 1. The proteolysis of B-casein was greater in the Cichorium enzyme treated cheeses than in the control as well as was greater and faster in P treatments than M ones **[Figures– 1 and -2]** . Addition of CECE to Ras cheese milk or curds accelerated casein proteolysis. The B-casein, which remains essentially intact in conventional Ras cheeses was considerably degraded in the enzyme-treated cheeses. The α1- casein rose in all cheeses during ripening with more increase in CECE -treated cheeses. This suggests that added CECE either facilitate the action of rennin on the phenylalanine (24)-valine (25) bond as of casein or that the Cichorium enzymes attacks that particular bond. These results was in agreement with what found by sood *et al* [27].

3. 4. 6. Organoleptic properties of cheese

M1and P2 gained the highest total score after 60 days of storage .After ripening for 60 days only, they acquired the typical full flavor, body and texture of control cheese ripened for 150 days **[Table– 6]**.

[IV] CONCLUTION

Generally the results obtained lead to the conclusion that Cichorium could be used as a cheap source of plant enzymes; used to accelerate Ras cheese ripening. This reduces manufacturing costs for both producers and consumers. The most successful method of CEPE addition is adding 0.1% to cheese curd. It reduces the period required for Ras cheese ripening to 60 days instead of 150 days without any defects in cheese properties

Table: 6. Statistical analysis of total scores of organoleptic properties

		cheese samples*							
	Ripening Period	C	M1	M2	M3	P1	P2	P3	LSD
Total Criticisms (100Point	0	46.1[a]	45.2[a]	45.3[a]	46.3[a]	43.2[a]	44.4[a]	44.4[a]	± 3.8
	60	70.3[a]	83.1[b]	98.3[c]	85.4[b]	99.3[c]	85.2[b]	71.6[a]	
	150	98.5c	97.3[c]	67.1[b]	64.2[b]	67.2[b]	59.5[a]	56.2[a]	

*See **Table– 1**

ABBREVIATIONS: (CEPE) Cichorium pumilum enzymes Purified extract, C control cheese, M1,M2 and M3 cheese treated with 0.1 ,0.2 or 0.3% CEPE added to cheese milk – P1,P2or P3 cheese treated with 0.1 ,0.2 or 0.3% CEPE added added to cheese curd.

CONFLICT OF INTEREST

The Author assures that there is no conflict of interest

FINANCIAL DISCLOSURE

There is no Funding source for this paper

REFERENCES

[1] Katsiari MC, Voutsinas,LP.and Kondyli E. [2002]. Improvement of sensory quality of low-fat Kefalogra-viera-type cheesewith commercial adjunct culltues. *Inter.dairy J* 12:757–764.

[2] Kondyli E, Masouras T, Katsiari MC, Voutsinas LP. [2003] Free fatty acids and volatile compounds in low-fat Kefalograviera-type cheese made with commercial adjunct culture. *International Dairy journal* 13: 47–54.

[3] Wang W, Zhang W, Li Y. [2012] Effect of specialized combined strains on reconstituted milk reduced-fat cheese. *Afr J Biotechnol*. 11(5), 1169-1176.

[4] Richardson BC and Creamer LK. [1973] Caseiproteolysis and bitter peptides in cheddar cheese.*J Dairy Sci. Technol.* 8:45.

[5] Thomas TD, Mills OE. [1981] Proteolytic enzymes of starter bacteria. Neth. *Milk Dairy* J 35:255.

[6] Khan MR, Nasreen K, Perveen Z. [1981] Protease Coalotropisprocera. *Journal of Natural Sciences and Mathematics*, 21(2): 199.

[7] Visser S, Hup G, Exterkate FA, stadhouders J. [1983] a. Bitter flavour in cheese. 2. Model studies on the formation and degradation of bitter peptides by proteolytic enzymes from calf rennet, starter cells and starter cell fractions. Neth. *Milk Dairy J* 37:169.

[8] Visser S, Slangen KJ, Hup G, Stadhouders J. [1983] b. Bitter flavour in cheese. 3- Comparative gel chromatographic analysis of hydrophobic peptide fractions from twelve gouda-type cheeses and Identification of bitter peptides isolated from a cheese made with streptococcus cremoris strain hp. Neth. *Milk Dairy* J37:181.

[9] El-Soda M, Ezzat N, Salam AE, Khamis A. [1990] Acceleration of ras cheese ripening. III. Evalution of

commercial enzyme mixtures. *Egyptian J Dairy Sci*. 18 (2): 273-279.

[10] Ezzat N. [1990] Accelerated ripening of ras cheese with a commercial protease and intracelluar enzymes from Lactobacillus delbrueckiisubspbulgaricus, Propionibacterium freudenreichii and Brevibacterium linens. Lati. 70:459.

[11] Gaya P, Medina M, Rodriguez-Marin MA and Munez M. [1990] Accelerated ripening of ewe`s milk manchego cheese. *J Dairy Sci*.73: 26.

[12] Fox PF, Wallace JM, Morgan S, Lynch CM, Niland EJ, Tobin J. [1996] Acceleration of cheese ripening. *Antonie Van Leeuwenhoek*, 70: 271.

[13] El-Hofi M, Ismail A, Rabo FA; El-Dieb S, Ibrahim O. [2010] Studies on Acceleration of Ras Cheese Ripening by Amino peptidase. *New York Science Journal*, 3(9): 91-96.

[14] Sadeq H, Al-Sheraji, Sabri J Abood, Khalida A Shake. [2009]r Using of Calotropisprocere Protease in Acceleration Ripening of Monterey Cheese Jordan *Journal of Agricultural Sciences*, 5(4): 1169–1176.

[15] Nadia A. Abou-Zeid, A.S.El-Sisi. [2014].Accelerated Domiati Cheese Ripening With Plant Enzymes Crude Extract The8 th International Conference On Technology and Sustainable Development in the Third millenmium 14-16 November 2014 .New planet Association,Euro-Arab Cooperation Center.

[16] Rasheeduz Zafar, S MujahidAli. [1998] Anti-hepatotoxic effects of root and root callus extracts of Cichoriumintybus L.*Journal of Ethnopharmacology.* 63(3): 227–231.

[17] Vohra DS. [2013] Bach Flower Remedies: *A Comprehensive Study. B. Jain Publishers*. pp. 3. ISBN 978-81-7021-271-3.

[18] Vadde Ramakrishna and P .Ramakrishna .[2005] Purification of acidic protease from the cotyledons of germinating Indian bean Doliches Lablab L. VAR (Lignosus) seeds .*African Journal of Biotechnology* 4 (7):703–707.

[19] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. [1951] Protein measurement with the folin reagent. *J Biol Chem* 193: 265–275.

[20] Sarath G, Dela Motte RS, Wagner FW. [1989] In: "Proteolytic enzymes. A Practical approach", IRL Press, Oxford, England Inc. 25-156.

[21] Zhong Q, Glatz CE. [2006] Enzymatic Assay Method for Evaluating the Lipase Activity in Complex Extracts from Transgenic Corn Seed. *J Agric Food Chem*, 54(9):3181-3185.

[22] Nadia A Abou Zeid. [2008] Cheese and its fractions. Inc.,El reyad, Sodia Arabic kingdam. pp. 102–106.

[23] Ling E R. [1963] A Text Book Of Dairy Chemistry. Vol. II. Chapman and Hall, Ltd. London.

[24] Tawab GA, Hofi AA. [1966] Tasting cheese ripening by rapid chemical techniques. *Indian J Dairy Sci*.19: 39-41.

[25] Bertacco G, Boschelle O, Lenker G. [1992] Gas chromatographic determination of free amino acids in cheese. *Milchwissenschaf*, 47: 348-350.

[26] Deeth HC, C.H Fitz-Gerald and AJ Snow. [1983] A gas chromatographic method for the quantitative determination of free fatty acids in milk and milk products. *New Zealand Journal of Dairy Science and Technology* 18: 13-20.

[27] SOOD VK. and KOSIKOWSKI FV.[1979] Accelerated Cheddar Cheese Ripening by Added Microbial Enzyme *J Dairy Sci* 62:1865-1872 .

[28] Nadia A Abou Zeid, Wafaa.H.Mahmoud. [1992] The use of heat shocked Lactobacillus helveticus for ripening accerelation of Ras cheese with special reference to slurry.*Minufiya J. Agric.Res* 17 (2): 645-665.

[29] Steel RGD and JH. Torrie. [1980] Principle and Procedures of Statistics. A biometrical approach. 2nd Ed. McGraw-Hill Book Co., NY.

[30] Ivana Kabelová, Markéta Dvořáková, Hana Čížková, Pavel Dostálek and Karel Melzoc2009 Determination of free amino acids in cheeses from the Czech market 27(3): 143-150.

[31] Erbe T, Thummes K., Knochenhauer J, Gross P, Bruckner H. [1999] Enantioselective amino acid analysis in beers and raw materials for beer manufacture by GC and HPLC. Lebensmittelchemie, 53: 118-121.

[32] ErbeT, Bruckner H. [2000] Chromatographic de-termination of amino acid enantiomers in beers and raw materials used for their manufacture. *Journal of Chromatography*, 881:81-91.

[33] Casella IG, Contursi M.[2003] Isocratic ion chro-matographic determination of un derivatized amino acids by electrochemical detection. *Analytica Chimica Acta* 478: 179-189.

[34] Collins YF, PLH McSweeney and MG Wilkinson. [2003] Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge. *International Dairy Journal* 13: 841-866.

[35] Güler, Z. [2000] A research on relationship between free fatty acids with organoleptic properties (Flavour) in White,Kasar and Tulum cheeses. Ph.D. Thesis, AnkaraUniversity, 95s. Ankara.

[36] Bryant A Z Ustunol, and J Steffe. [1995] Texture of Cheddar cheese as influenced by fat reduction. *J Food Sci.* 60:1216-1219.

[37] Fox PF, T P Guinee, TM Cogan, PLH McSweeney. [2000] Cheese Rheology and texture. Pp. 305–333 in Fundamentals of Cheese Science. Aspen Publishers, Inc., Gaithersburg, MD.

ABOUT AUTHOR

Dr. Nadia A. Abou-zeid works at Dairy Science and Technology Department, Faculty of Agriculture, Menoufia university, Shebin El-kom, Egypt. She have authored fifteen books published in the largest publishing houses and distributed all over Arab countries and they are considered references in the field of food and dairy products.She has published seven researches in international journals and two researches are under publishing.