

Accelerated Domiati Cheese Ripening with Plant Enzymes Crude Extract

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Abstract – In our previous study we found that the crude enzymes extract prepared from the composite weed *cichorium pumilum* (CECE) had satisfactory proteolytic activities. In this study we investigated the ability of this extract on accelerating ripening of Domiati cheese. Every ml of (CECE) was prepared from 0.2gm dried *cichorium*. Levels of crud extract added to milk which used in cheese manufacturing were 1 and 2 %. The concentration of 1% was satisfactory for accelerated cheese ripening, the higher concentration resulted in access ripening lead to soluble cheese body. Soluble N in cheese was more influenced by *cichorium pomatum* enzymes crud extract at room temp than at refrigerator. Frequency of bitter taste did not appear in cheese at either room or refrigerator temperatures with the use of two concentrations of CECE.

Addition of 1 % (CECE) would reduce ripening time by 6weeks at either room or refrigerator temperature. Therefore, Domiati cheese made from milk to which 1% *cichorium pumilum crud* enzymes extract was added would develop in 2weeks at room temp the same flavor intensity as control Domiati cheese held for 8 weeks, and the corresponding times at refrigerator temp were 3 weeks via 10weeks for control.

Keywords – Ripening Acceleration, Domiati Cheese, *Cichorium Pumilum*, *Crud* Enzymes Extract.

I. INTRODUCTION

Cheese ripening has been the object of considerable scientific research over recent decades. The ripening of cheese is a slow and consequently an expensive process. The economic advantage of rapid development of more intense cheese flavor in shorter periods of time would be substantial. Accelerated ripening of cheeses was achieved by addition of microbial proteinases but microbial proteinases are expensive as well as bitterness was reported as the most common flavor defect in cheeses from milk or curd treated with microbial proteinases (Czulak and Shimmin. 1961, Lowrie and Lawrence. 1972. Kailasapathy, and Lam, (2005), Kilcawley et al., (2012) and Alice Beebyaanda Nongonierma et al, (2013).

Domiati cheese is the most popular soft cheese in Egypt. It could be consume fresh or after pickling. The fresh cheese is mild, but at 8 weeks or older the cheese has a pungent flavor due to the proteolytic and lipolytic activities.

The objective of this research was to accelerate ripening of Domiati cheese with plant enzymes crude extract produced at the laboratory scale from *cichorium pumilum* plant.

II. MATERIAL AND METHODS

Preparation of Crude Enzymes:

Dried plant stems were cut into small pieces, then the plant crude enzymes extract was prepared as described by Silva et al (2006) via maceration 10 gm of stem powder in 50 ml water using an electric mixer . The extract filtered and centrifuged at 30000 rpm for 30min.. The supernatant was referred as *cichorium enzymes crude extract*(CECE)

Manufacture of Domiati Cheese:

The mixture of buffalo -cow milk(1:1) used in cheese making, the same milk was used for the manufacture of the 2 types (control cheese without addition, cheese with *cichorium pumilum* enzymes crud extract at a level of 1and 2%.

Cheese making was carried out on 3 consecutive days (total of 3 batches for each type of cheese) using cheese milk from daily milking. Cheese milk was heated to 75 c for 15 sec immediately cooled to 40 c, salt then added at level of 5%and calcium chloride 0.02%followed by *the addition of cichorium pumilum* enzymes crud extract CECE. Domiati cheese was made by the method of (Nadia 2008) .Samples were taken for analysis from fresh cheese and cheese stored for 8 weeks at room and 10 weeks at refrigerator temp and analysis weekly.

Cheese analysis :

Cheese samples were analyzed for moisture content, pH, fat total nitrogen, soluble nitrogen, non protein nitrogen according to ling 1963.The total volatile fatty acids were determined as given by Kosikowaski (1978). Method of Vakaleris and Price 1959 was applied for determination of soluble tyrosine and tryptophan content Schilovishindicies were estimated as described by Tawab and Hofi (1966) The Determination of free amino acid was carried out as described by Free amino acids were determined according to Biock et al.(1958), using automatic amino acid analyzer, AAA 400, ingosltd.

III. RESULTS AND DISCUSSION

Chemical properties :

Data in Table(1, 2) illustrate the chemical properties of Domiati cheese made with adding different levels of enzymes crude extract produced at the laboratory scale from *cichorium pumilum* plant. The data affected by storage at ambient and refrigerator temps .Slight decreases were observed in pH and moisture content values among all cheese samples as storage period advanced. Fat/DM and Total nitrogen /DM did not considerably affected by

storage period in all samples. However statistical analysis proved that the addition of *cichorium pumilum* enzymes crude extract had no significant effect on pH, moisture, Fat/IDM and Total nitrogen /DM. These results were in agreement with what found BY Mashaly et al1986, Abou –Dawood et al1978and Hassan and abouzeid 1988 when they used other accelerating agents.

Change in the ripening indices:

The changes in soluble nitrogen, soluble tyrosine, soluble tryptophan and Schilovich number during ripening of Domiati cheese are shown in table 3 and 4 .Fresh cheeses from different treatments and control had very close ripening indices . All ripening indices in all cheese samples were increased as ripening storage advanced, however addition of enzymes crude extracted from *cichorium pumilum* plant enhanced protein degradations SN,, N.P.N, S.tyr, S.trp and, SI being significant higher in treatments than in control. Similar trends were obtained by Hussein 1985, Abou –zied and zaki 1988, Badwi and Kebary 1996 and El –sheikh et. al., 2001when they used other accelerating agents . It is obvious from tables 3and 4 that cheese of 0.002*cichoriumpumilum* enzymes crude extract and stored at room temp had the highest ripening indices. Domiati cheeses containing the *cichorium pumilum* enzymes crude extract showed higher free volatile fatty acids than cheeses without adding it. Free volatile fatty acids in cheese increased with increasing *cichorium pumilum* enzymes crude extract levels or storage time and storage temperature this could be explained on the basis that increasing rate of accumulation of free amino acids which act as precursors for free fatty acids through deamination reaction Azzat et al., 2008. Results also showed that Proteolysis and lipolysis were more intense in cheeses ripened at room than at refrigerator temp Similar results were found by Salama1982.

Total Free Amino Acids:

Acids generally increased during ripening and were significantly higher in the enzyme-treated cheeses than in the control cheeses, and they were higher inExp2 cheeses than in Exp1 cheese. Lysineproline, phenyl- alanine and tyrosine, were in large quantities in all cheeses whereas glycine, Cysteic acid, Iso- leucine and threonine existed only in small amount..

Cheese Organoleptic Properties.

At room storage temp

Data presented in Table (6) show the different scores given to various Domiati cheese samples during storage period at room temp. For flavor evaluation, Data obtained showed that sample of(Exp1)was more superior and

attained the highest score after 15 days of storage. The difference between Exp2and Exp1 was not significant up to 15days of storage, while there were significant differences between them as storage period advanced longer than 15 days. Exp1ripened developed no flavor defects when held at room temp. up to 21 days. Conversely Exp2 developed flavor and body &texture defects 15days storage. It showed undesirable excess sharp flavor and weakness, excess soft body & texture. At room temp. 0.001was the most desirable concentration of *cichorium pumilum* enzymes crude extract for producing such cheeses of desired structure and taste at a relatively earlier stage of ripening (15 days)

At refrigerator storage temp

Data of enzyme-treated cheeses sensory analysis of flavor intensity and Body & texture data revealed a significant difference between control and treated cheeses. However there was no significant difference between treatments Exp1, Exp2 up to15. There was gradual improvement in (Exp1) up to21 days and there were gradual decrease in Exp2 quality after 15 days .This treatment showed pronounced accelerated ripening up and then began to show pronounced sour flavor and structure defects such as weak structure and body loose, It is probably due to the more breakdown and hydrolysis of protein. we suggested that Cheese of 0.002 *cichoriumpumilum* crud extract (Exp2) can be repined at refrigerator for a period of 15 days only Conversely, ripening period of (Exp1) Domiati cheese could be reduced from 75 days of control to 21.

Generally the results obtained showed that addition of 0.001 *cichoriumpumilum* crude enzymes would achieve the ripening process of Domiati cheese through 21 days without any defects in its properties while the concentration of 0.002 resulted in quickly destroy of cheese body after 15 days .

IV. CONCLUSION

Generally the results obtained may lead to the conclusion that addition of 0.001 would accelerate the ripening process of Domiati cheese through15 and21 days at room and refrigerator temp respectively without any defects in its properties. While 0.002concentration can be used at refrigerator temp. only for a ripening period not longer than 15 days. In other words, it could be concluded that *cichorium pumilum* could be used as a cheap source of enzymes; used to accelerate cheese ripening, this reduces manufacturing costs for both producers and consumers.

Table (I): Chemical properties of Dodmiati cheese during ripening at room temp. as affected by *cichoriumpumilum* enzymes crude extract

Item	control <i>cichoriumpumilum</i> enzymes crude extract											
	.001				, 002							
	0 d	15 d	21 d	30 d	0 d	15 d	21d	30 d	0 d	15 d	21 d	30 d
pH	6.2	6.0	5.9	5.7	5.0	4.8	4.7	4.6	5.2	5.1	4.9	4.8
Moisture (%)	62.6.	60.2	59.3	57.5	62.6.	60.4	59.0.	58.2.	62.6	59.9	58.3	57.1
Fat/IDM (%)	46.9	46.5	46.1	45.7	46.9	46.4	46.2	45.5	46.9.	46.4	45.6	45.5
Total nitrogen /DM(%)	6.1	6.0.	5.8.	5.7	6.1	5.8	5.7	5.6	6.1.	5.7.	5.5	5.4

Table (II): Chemical properties of Domiati cheese during ripening at refrigerator temp. as affected by *cichoriumpumilum* enzymes crude extract

Item	control <i>cichoriumpumilum</i> crud proteinase											
	.001				, 002							
	0 d	21 d	30 d	45 d	0 d	21 d	30 d	45 d	0 d	21 d	30 d	45 d
pH	6.2	6.0	5.9	5.7	6.0	5.8	5.7	5.6	6.1	5.9	5.8	5.7
Moisture (%)	62.6.	60.3	59.3	57.2	62.6.	60.2	59.2	58.8.	62.6	59.9	58.6	57.9
Fat/IDM (%)	46.9	46.7	46.3	46.1	46.9	46.6	46.3	45.9	46.9.	46.6	45.8	45.8
Total nitrogen /DM(%)	6.1	6.0.	5.9.	5.8	6.1	5.9	5.9	5.8	6.1.	5.7.	5.6	6.6

Table (III): Ripening properties of Domiati cheese during ripening at room temp. as affected by *cichoriumpumilum* enzymes crude extract.

Item	control <i>cichoriumpumilum</i> crude enzymes extract											
	1				2							
	0 d	15 d	30 d	60 d	0 d	15 d	30 d	60 d	0 d	15 d	30 d	60 d
SN	0.231	0,262	0.290	0.301	0.278	0.451	0.599	0.740	0.316	0.502	0,781	0.8930
NPN (%)	0.078.	0,078	0,084	0,097	0,086	0.198	0.259	0.363.	0.102	0.368	0.499	0.568
FN	11.1	11.1	22.2	33.3	11.1	44.2	66.4	77.7	11.1.	66.6	77.7	88.8
SI	10	10	20	30	10	20	40	60	10	30	60	100
T .V.F.A	11.86	14.62	20.66	25.87	12.86	15.44	22.36	26,66	12,86	14,82	21.22	25.44

FN Frmol number

SI Schilovich Index

T .V.F.A Total volatile fatty acids

Table (IV): Ripening properties of Domiati cheese during ripening at refrigerator temp. as affected by *cichoriumpumilum* enzymes crude extract

Item	control <i>cichoriumpumilum</i> crude enzymes extract											
	1				2							
	0 d	15 d	21 d	75 d	0 d	15 d	21 d	75 d	0 d	15 d	21 d	75 d
SN	0.231	0,242	0.260	0.282	0.268	0.288	0.322	0.442	0.286	0.3225	0,491	0.564
NPN (%)	0.078.	0,078	0,080	0,088	0,080	0.091	0.102	0.143.	0.098	0.148	0.169	0.196
FN	11.1	11.1	11.1	22.2	11.1	22.2	33.3	44.4	11.1.	33.3	44.4	66.6
SI	10	10	10	20	10	20	30	50	10	30	50	80
T .V.F.A	11.86	13.62	17.66	20.87	11.916	13.84	17.32	20,66	11,22	13,85	17.52	20.74

FN Frmol number

SI Schilovich Index

T .V.F.A Total volatile fatty acids

Table (V): Mean values¹ for the level of free AA (mg/kg) found in Domiati cheese during ripening at room temp. as affected by *cichoriumpumulum* enzymes crude extract

AA (mg/kg)	Control cheese		Cheese with, 001		Cheese with, 002	
	0 d	15 d	0 d	15 d	0 d	15 d
Cysteic acid	11.51	12.91	8.70	19.85	9.66	25.23
Asp	14.37	16.89	124.61	56.94	15.03	67.28
Thr	12.67	8.74	12.67	99.18	17.06	194.21
Ser	25.06	75.73	36.41	593.03	61.48	1, 090.68
Glu	18.49	28.43	19.02	20088	24.34	1, 363.52
Gly	9.24	22.05	9.82	134.30	10.47	227.67
Ala	29.23	68.21	46.86	311.79	33.40	440.82
Cys	14.24	25.66	8.00	42.05	17.68	85.93
Val	34.17	103.26	42.62	351.19	36.11	526.92
Met	23.75	51.43	28.83	142.98	22.40	209.25
Ile	11.55	28.46	14.56	127.10	21.91	249.01
Leu	48.92	213.90	68.40	1, 291.89	49.15	1, 676.14
Tyr	49.68	76.87	28.74	186.65	40.61	279.65
Phe	52.62	225.81	38.01	710.42	61.05	954.52
His	46.00	186.60	19.07	140.76	36.91	214.59
Trp	15.84	63.81	9.51	43.75	11.15	50.85
Orn	16.78	172.85	28.49	102.57	19.10	139.31
Lys	87.86	153.38	88.74	522.48	97.94	935.24
Arg	37.49	41.10	24.89	145.27	15.99	402.51
Pro	74.67	190.02	58.05	626.46	80.13	818.80
Total	633.8 ± 22 ^d	1, 644.2 ± 56 ^c	667.82 ± 29 ^d	5, 860.5 ± 18 ^b	682.6 ± 29 ^d	9, 950.1 ± 380 ^a

a–dMeans within a row with different superscript letters are significantly different ($P < 0.05$).

1Mean values ± SD for 3 batches of each type of cheese analyzed in duplicate.

Table (VI): Organoleptic properties of Domiati cheese during ripening at room temp. as affected by *cichoriumpumilum* crude extract

Item	control <i>cichoriumpumilum</i> enzymes crud extract											
	.001				, 002							
	0 d	15 d	21d	60d	0 d	15 d	21d	60d	0 d	15 d	21d	60 d
Flavour 50 point	28	33	34	49	32	50	41	45	34	39	35	26
Body and texture 40 points	30	32	35	39	30	40	35	30	30	35	30	26
Appearance 10 p0ints	7	7	7	10	7	10	8	7	8	9	5	4
Total 100 points	65	72	76	98	70	100	84	82	74	85	75	56

Table (VII): Organoleptic properties of Domiati cheese during ripening at refrigerator temp. as affected by *cichoriumpumilum* enzymes crude extract

control <i>cichoriumpumilum</i> enzymes crude extract												Item
.001				, 002								
75 d	21 d	15 d	0 d	75 d	21 d	15 d	0 d	75 d	21 d	15 d	0 d	
25	38	45	28	30	50	40	29	48	32	30	28	Flavour 50 point
16	32	35	30	27	40	38	30	39	35	32	30	Body and texture 40 pointss
4	6	9	8	7	10	8	7	10	7	7	7	Appearance 10 p0ints
45	76	89	66	64	100	88	66	97	76	72	65	Total 100 points

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