

QUALITY OF EDAM CHEESE WITH USING RENNIN-LIKE FUNGAL ENZYME

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ABSTRACT

Edam cheese was made from cow's milk. Rennin-like enzyme from *Rhizomucor miehei* a rennet substitute was used as milk clotting enzyme in manufacture of Edam cheese. The cheese results showed that, the breakdown of protein content(SN/TN, NPN/TN), and TVFA were higher in Edam cheese made with rennin-like enzyme or its mixture with rennet enzyme than in control cheese throughout the ripening period. Moreover experimental cheese gained acceptable body with a good flavour during the ripening period.

From the obtained data, it is concluded that using rennin-like enzyme of *Rhizomucor miehei* for Edam cheese manufacture is acceptable, enhanced flavour development, and improved cheese quality during ripening.

Keywords: Edam cheese, fungal enzyme, *Rhizomucor miehei*

INTRODUCTION

The rennet normally employed in the manufacture of cheese is derived from the abomasum of unwarmed calves. This source of supply varies seasonally and is becoming progressively more scanty as a result of increasing cheese production, decreasing slaughter of calves and thereby less availability of calf rennets (Ismail et al. 2004). This situation encourages to find other sources of rennet substitutes from bacterial or fungal sources.

Milk-clotting enzymes have been obtained from several fungal organisms like, *Endothia parasitica* (Joseph L., 1968), *Mucor pusillus* (Richardson et al. 1967; Somkuti & Babel, 1968, and Sternbeg, 1971. *Aspergillus niger* Foda, 1982, and Mostafa et al. 1988); *Mucor miehei* (Ibrahim M.K. et al, 1973, Abdo et al. 1976, El-Shibiny & Abdel-Salam et al. 1977; El-Safy & El-Shibiny, 1980, and Mashaly et al. 1983, and El-Soda, 1986, El-Tanboly et al. 2003). *R. miehei* was studied by Fadel et al. (1992). This organism had no toxin production and no biological effect appeared on *B. megaterium*. Moreover, Fadel (1993) studied some properties of the enzyme extracted from *P. funiculosum*. The extracted enzyme preparation had a milk clotting time MCT (min) of 7.30, milk clotting activity (MCA) of 5.31 SU/ml. and proteolytic activity (P.A) of 0.076 U/ml at optimum pH 5.8 the MCA/P.A was 69.86 after six days in aerobic incubation at 32-35°C in cheese whey containing 5% sodium chloride (El-Sisey 2002; El-Batawy et al., 2004).

The objective of this research was aimed at evaluating the use of a milk-clotting enzyme produced from *Rhizomucor miehei* in the manufacture of Edam cheese.

MATERIALS AND METHODS

- Fresh cow's milk was obtained from Animal Production Research Institute, Ministry of Agriculture, Cairo, Egypt.

Animal rennet powder and Rennin-like enzyme from *Rhizomucor miehei* were obtained from Hansen's laboratory Copenhagen, Denmark.

3- Edam cheese manufacture:

Fresh cow's milk was standardized to $3.2 \pm 0.1\%$ fat. Three lots of 20 kg. each were set up and Edam cheese was manufactured as described by Scott (1998) with 0.5% of mixed starter of *Str. lactis* and *Str. cremoris* at rate of (1:1). The control Edam cheese was made with animal rennet powder. Two treatments of Edam cheese were made, the first by using a mixture of rennet and fungal enzyme of *R. miehei* (1:1)(Treatment 1), while the second was prepared by fungal enzyme of *R. miehei* only(Treatment 2).Edam cheese ripened at $12^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and analyzed when fresh and at 15, 30, 60 and 90 days of ripening. Three replicates of each treatments were carried out.

Analysis of Edam cheese:

Cheese samples were analyzed for moisture%, acidity%, fat % , total nitrogen%, soluble nitrogen% (SN), non-protein nitrogen% (NPN)and amino acid nitrogen according to A.O.A.C.(1990). Soluble tyrosine and tryptophane content were measured according to Voakeleris and Price(1959). Total volatile fatty acids (TVFA) were estimated according to Kosikowski (1978). In addition, Edam cheese samples were organoleptically examined as described by ADSA,(1987).

RESULTS AND DISCUSSION

Table (1) shows that the moisture contents of Edam cheese made with rennet or coagulating enzyme of *Rhizomucor miehei* were not the same throughout the ripening period. Edam cheese made with enzyme of *R. miehei* had slightly higher moisture content than the control cheese. Similar result were reported by Foda *et al.* (1993). The titratable acidity of the control Edam cheese sample and the two experimental cheeses generally increased with progressing of storage time. It is clear, that using enzyme of *R. miehei* alone or in mixture with rennet for Edam cheese making had no effect on the titratable acidity during cheese ripening. The fat content in dry matter of Edam cheese is also shown in Table (1). In general fat in dry matter gradually increased in both control and treated samples with increasing of ripening periods and showing different levels, depending on the differences in moisture content of cheese during ripening periods. The results were in agreement with those obtained by El-Safty and El-Shibiny, 1980 , El Abd *et al.* (1982); El-Shabrawy (1985)and Ismail *et al.*.(2004).

Ripening indices of Edam cheese made with *R. miehei* enzyme and rennet enzyme is shown in Table (2). The obtained data indicated that, SN/TN ratio of microbial enzyme treated Edam cheese were higher than those of control cheese during all ripening periods. At the end of ripening (90 days) these values were 16.10, and 16.88% for cheese made with mixed enzyme of *R. miehei* and rennet (1:1) and for Edam cheese made with microbial enzyme only as compared to only 16.02% for the control Edam cheese. This finding obviously indicated that the *R. miehei* enzyme had a more powerful proteolytic activity than rennet enzyme. Also NPN/TN ratios of the cheese samples showed a similar trend as SN/TN %.

Table (1):Effect of using rennin-like enzyme from *R. miehei* and rennet on some chemical composition of Edam cheese during ripening.

Treatments	Ripening period (days)	Moisture %	Acidity %	Fat /DM %	TN %
Control	Fresh	44.20	0.36	46.71	4.12
	15	41.60	0.54	47.34	4.18
	30	40.18	0.76	48.54	4.21
	60	40.10	0.98	49.08	4.31
	90	39.16	1.24	49.30	4.42
T 1	Fresh	44.23	0.37	46.72	4.16
	15	41.71	0.50	47.31	4.22
	30	40.15	0.76	48.50	4.32
	60	40.15	0.95	49.06	4.41
	90	39.71	1.25	49.20	4.58
T 2	Fresh	44.31	0.36	46.70	4.17
	15	41.86	0.51	47.28	4.22
	30	40.54	0.73	48.45	4.33
	60	40.84	0.91	48.81	4.46
	90	39.82	1.20	49.04	4.52

Control: animal rennet powder

T 1 : mixture of rennet and fungal enzyme (1:1)

T 2 : fungal enzyme

Moreover, the accumulation of amino acid nitrogen/TN% in Edam cheese made with *R. miehei* enzyme only or its mixture with rennet was more remarkable than that in control cheese throughout the ripening period. From the obtained data it could be concluded that, the proteolytic activity of *R. miehei* enzyme is greater than that of the rennet enzyme in Edam cheese during ripening. Similar results are reported by Richardson *et al.* 1967 and Somkuti and Babel, (1968) who remarked that, using fungal rennet of *M.pusillus* for making cheddar or parmesan cheese lead to increase in non protein-nitrogen content and total volatile fatty acid (TVFA) during ripening as compare with rennet enzyme cheeses. Similar findings were obtained in Domaiti cheese by El-Safty & El-Shibiny (1980)and El-Sheikh *et al.*,(1999).

Table (2) shows the total volatile fatty acids (TVFA) of Edam cheese made with enzyme of *R. miehei* and rennet during ripening. From this Table, it could be seen that the TVFA is gradually increased with higher rate in cheese treated with microbial enzyme than that in control one, throughout ripening period. This finding indicate that, the enzyme of *R. miehei* has a noticeable effect on the lipolysis cheese fat and accumulated more TVFA during cheese ripening, that the rennet. Foda *et al.* (1993) also observed that the TVFA values were increased in Domiati cheese treated with microbial rennet than in cheese made with animal rennet. Moreover, they reported that the increase in the rate of liberation of free amino acids which act as precursors for free fatty acids through domination reaction could explain this findings.

The scores gained from the resultant cheese of all treatments are given in Table (3). It is clear from these results that, the score of flavour,

body & texture and appearance increased gradually during ripening period, furthermore, the treatment 2 with rennin-like enzyme showed the highest scoring values.

From the previous results, it could be concluded that, the rennin-like enzyme from of *R. miehei*, could be recommended for Edam cheese manufacture as a substitute of calf rennet. Moreover, fungal enzyme enhanced flavour development and improved the quality of cheese during ripening.

Table (2): Effect of using rennin-like enzyme from *R. miehei* and rennet on protein breakdown of Edam cheese during ripening.

Treatments	Ripening period (days)	SN/TN %	NPN/TN %	Amino acid N/TN%	Tyrosine	Tryptophane
Control	Fresh	6.20	2.27	0.20	13.62	4.65
	15	8.91	4.18	0.31	14.22	24.54
	30	12.34	5.30	0.40	48.17	51.70
	60	14.81	6.15	0.66	122.61	82.19
	90	15.02	6.81	0.75	146.11	90.28
T 1	Fresh	6.20	2.31	0.22	14.78	5.42
	15	9.30	4.71	0.38	16.12	26.12
	30	12.87	5.43	0.54	61.62	54.18
	60	15.68	6.68	0.72	138.14	84.71
	90	16.10	7.03	0.86	169.83	96.40
T 2	Fresh	6.23	2.33	0.23	14.82	6.12
	15	9.48	4.51	0.42	17.26	28.10
	30	13.05	5.80	0.61	98.88	58.26
	60	15.77	6.11	0.80	144.43	89.50
	90	16.88	7.45	0.88	211.85	104.37

Table (3): Average score of organoleptic properties of Edam cheese made with *R. miehei* enzyme and rennet during ripening.

Treatments	Ripening period (days)	Flavour (50)	Body & Texture (35)	Appearance (15)	Total (100)
C	30	32	29	11.8	72.8
	60	37	29	11.8	77.8
	90	43	31	12.3	86.1
T1	30	33	29	11.8	73.8
	60	38	29	11.8	78.8
	90	43	32	12.6	87.6
T2	30	33	29	11	77.0
	60	38	29	12	79.8
	90	44	32	13	89.0

REFERENCES

A.D.S.A.(1987). American Dairy Science Association. Score card for cheese, Champaign TL. P.84.

- A.O.A.C.(1990). Official Methods of Analysis. 15th Ed. Association of Official Analytical Chemists, Inc. Arlington Virginia, USA.
- Abdo, S. Ghita, I., and El-Shibiny, S. (1976). The use of microbial rennet and pepsin in the manufacture of Domiati cheese. *Egypt. J. Dairy Sci.*, 4:147.
- El-Abd, M.M., Elein S. Girgis, M.R. Nagmouh, T.K. Fahmy, and S.A. Fikry (1982). Effect of some pre-treatments on the chemical, bacteriological and organoleptic properties of baby Edam cheese. *Egypt. J. Dairy Sci.*, 10:87-94.
- El-Batawy, M.A.; W.A. Metry; M.A. Zedan and H.S.M. El-Tawei.(2004). Influence of salting method on the quality of Edam cheese. *Proc. The 9th Egypt. Conf. for Dairy Sci. & Tech.* 493-508.
- El-Safty, M.S. and El-Shibiny, S. (1980). The use of *Mucor pusillus* protease in the manufacture of Domiati cheese. *Egypt. J. Dairy Sci.*, 8:41-48.
- El-Shabrawy, S.A. (1985). Comparison of proteolysis in Edam cheese manufactured by standard and by ultrafiltration techniques. *Egypt. J. Dairy Sci.*, 13:9-17.
- El-Sheikh, M.M.; Farrag, A.F.; Al-Khany, A.F. and El-Shibiny, S. (1999) Edam cheese from whey protein milk retentate. *Egypt. J. Dairy Sci.*, 27: 317-329.
- El-Shibiny, S., and Abdel-Salam, M.H. (1977). Action of milk clotting enzymes on α -casein from buffalo's and cow's milk. *J. Dairy Res.* 17:312.
- El-Sisey, S.A.(2002). Studies on semi- hard cheese. Ph.D. Thesis, Menofiya Univ., Egypt.
- El-Soda M. (1986). Accelerated ripening of Egyptian cheese varieties (A review). *Egypt. J. Dairy Sci.*, 14:115-126.
- EL -Tanboly, E.; M.A. El-Hofi and A. Ismail. (2003). Changes of proteolytic and lipolytic activities during ripening of Gouda cheese prepared with fungal rennet substitute of *Mucor miehei* NRRL 3169. *Michwissenschaft* 58:27 - 30
- Fadel M. (1993). Production of milk-clotting by *Penicillium funiculosum* during growth in whey permeate. *Egypt. J. Food Sci.*, 21:205
- Fadel, M. Amra H.A., Murad, H.A., and El-Shenawi, K. (1992). Production of fungal protein from wheat straw by *R. miehei*. *Egypt. J. Food. Sci.*, 20, suppl., 1-13.
- Foda, M.S. (1982). Characterization of rennin-like enzyme produced in submerged culture of *A.niger*. *Egypt. J. Microbiol.*, 17:105-114.
- Foda, M.S., Saleh Y., Hayam M. Abbas, and Layla A.Rizkallah (1993). Application of *B.thuringiensis* milk clotting enzyme in manufacture of Domiati cheese. *Egypt. J. Dairy Sci.*, 21:35-43.
- Ibrahim, M.K.E., Amer, S.N., and El-Abd, M.M.(1973). Study on a microbial rennet produced by *Mucor mieheu*. *Egypt. J. Dairy Sci.*, 1:127-149.
- Innocente, N.; Pittia, P.; Stefanuto, O. and Corradini, C. (2002). Correlation among instrumental texture, chemical composition and presence of characteristic holes in a semi-hard Italian cheese. *Milchwissenschaft*, 57:204-208.

- Ismail, A.M.; M.M. Ismail and M.I. Hashem.(2004).Effect of some heat treatments on the quality of Edam cheese made from buffale's milk. The 9th Egypt.Conf. for Dairy Sci. & Tech.477-491.
- Joseph L.Sardinas, (1968). Rennin enzyme of *Endothia parasitica*. Applied microbiology, 16 :248-255.
- Kosikowski, F.V. (1978). Cheese and fermented milk foods, 2nd ed., Edward, Brothers. Inc., Ann Arbor, Michigan, U.S.A.
- Mashaly, R.I., Ramadan,B.I., Tahon , M.K., El-Soda, M., and Ismail, A.A. (1983). Factors affecting the production of milk clotting protease from *Mucor miehei*. Egypt. J. Dairy Sci., 11 : 255.
- Mostafa, M.E., Ab El-Hamid, A. and Saleh A.E. (1988). Optimization of protein production from *Aspergillus niger*. Egypt. Microbial, 23: 301.
- Richardson, G.H. Nelson, J.H., Lubnow, R.E. and Shwarberg, R.L. (1967). Rennet like enzyme from *Mucor pusillus* for cheese manufactrue. J. Dairy Sci., 50: 1066.
- Sciancalepore, V. Alviti, F.S. (1987). A dialysis method for measuring cheese ripening. Milchwissenschaft, 42: 220-221.
- Scott, R. (1998). Cheese making practice. Third edition. Applied science Publ. LTD London.
- Somkuti G.A., and Babel, F.J. (1968). Purification and properties of *Mucor pusillus* acid protease. J. of Bacteriology, 95: 1407-1414.
- Sternberg, M.Z. (1971). Crystalline milk-clotting protease from *Mucor miehei* and some of its properties. J. Dairy Sci., 54:159-167.
- Visser, S.(1993). Proteolytic enzymes and their relation to cheeses ripening and flavour. J. Dairy Sci., 76: 329-350.
- Vakaleris, D. G. and W.V. Price. (1959). A rapid spectrophotometric method for measuring cheese ripening. J. Dairy Sci. 42:261.
- Zaki, N. and Salem, S.A. (1992). Effect of proteolytic enzymes or accelerated ripening of Edam cheese. Indian J. Dairy Sci., 45:6.

دراسات على جودة الجبن الايدام باستخدام المنفحة الميكروبية

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تم استخدام انزيم المنفحة المنتج من فطر *Rhizomucor miehei* في انتاج الجبن الايدام من اللبن البقري. ومن اهم النتائج المتحصل عليها انه لوحظ زيادة في نسبة اللنتروجين الذائب واللنتروجين الغير بروتيني الى نسبة البروتين الكلي وكذلك زيادة في قيم التيروزين والترتوفان كنواتج تحلل للبروتين ودلائل علي تسوية الجبن . كما حدث زيادة في الاحماض الدهنية الطيارة في الجبن المصنع من المنفحة الميكروبية بالمقارنة بالجبن المصنع من المنفحة الحيوانية.

كما تميزت الجبن الناتج من المنفحة الميكروبية بجودة النكهه، كما حصل على درجات تحكيم اعلى خلال فترة التخزين. وقد دلت النتائج على ان استخدام المنفحة الميكروبية اعطى جبن ذو صفات مقبولة وجيدة بالمقارنة بالمنفحة الحيوانية.