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RESEARCH ARTICLE

Plant latex as vegetable source for milk clotting enzymes and their use in cheese preparation

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Abstract

Evaluation is carried on milk clotting activity of the crude proteolytic enzymes present in the lattices of plants belonging to Euphorbiaceae family along with their use in cheese production. The results showed that milk clotting activity of crude enzyme of latex of four plants are in order of *Euphorbia tirucalli* followed by *Euphorbia nerifolia*, *Euphorbia nivulia* and *Pedilanthus tithymaloides*. Milk coagulation is the basic step in cheese making, therefore these crude enzymes were subjected to the production of cheese. Comparative account on percentage yield, total solid, moisture content and chemical composition of resultant cheese samples were also analyzed. The results revealed that, nutritional quality of cheese prepared from milk clotting enzymes of plant lattices of Euphorbiaceae family is not fully matched with Amul cheese; however it is comparable with cheese prepared using purified papain.

INTRODUCTION

Cheese is one of the numerous products from the processing of milk. Cheese is used as a form of preserving essential nutrients in milk and is an excellent source of nutrients such as protein, fat, minerals and vitamins. Cheese manufacture is essentially a dehydration process in which the fat and casein of milk are concentrated 6-10-fold (Omotosho et al., 2011). Since long the animal rennin (or rennet) is employed in making cheese. The enzyme rennet is obtained on a commercial scale from the fourth or true stomach of the unweaned calves which are specifically slaughtered for this purpose. One calf produces only 5 to 10 gm of rennet. The enzyme helps in coagulating the casein of milk. The milk clotting property of enzyme is important with regard to the quality and yield of cheese. It has been suggested that a firmer curd at cutting is positively correlated to yield of cheese (Wedholm et al., 2006).

Much research interest has been directed towards discovering a milk-clotting enzyme which would satisfactorily replace calf rennet produced by genetically engineered bacteria have proven suitable substitutes for animal rennet but increasing attention has been directed towards natural rennet extract of plant origin (Mahajan and Badgujar, 2010). Plant proteases employed for cheese production in various areas of the world include papain, bromelin, ficin, oryzasin, cucumisin, sodom apple and *Jacaratia corumbensis* (Duarte et al.2009). In Sudan dairy farmers used the berries of *Solanum dubium* to make white soft cheese using goat and sheep milk (Ahmed et al., 2009). Another commonly used vegetable rennet is an extract from the shrub, *Calotropis procera* commonly known as Dead Sea Apple. It contains the enzyme, calotropin, which is more active at pH 6.4 (Omotosho et al., 2011). Recently, Mahami et al., (2012) demonstrated that *Moringa oleifera* seed extract enrichment resulted in significant increases in the yield, protein content and mineral content of cottage cheese. The crude enzyme of plant lattices used to prepare white cheese was suitable as compared with rennet extract in addition, it is less expensive than other coagulants to manufacture white cheese Therefore, in the present study comparative account on milk clotting enzyme of lattices of four members of Euphorbiaceae family is given along with their application in cheese production.

2. Material and Methods

2.1 Plant collection

The lattices of latex bearing medicinal plants of Euphorbiaceae family were collected early in the morning by superficial incisions of stem, fruit or trunk of healthy plant and allowing the milky latex to drain in clean glass vials separately, brought to the laboratory and stored at 4^0C.

2.2 Preparation of crude enzyme

All operations were carried out at 0 – 5 ^{0}C. The plant latex was homogenized in homogenizer under chilled condition and filtered successively through four folds of muslin cloth and Whatmann filter paper No. 1. Filtrate was centrifuged at 10,000g for 30 min and supernatant labelled as "Crude enzyme", and used for further investigation of milk clotting activity.

2.3 Milk clotting activity

The milk clotting activity of proteases was performed as described by (Badgujar and Mahajan, 2009). The enzyme source (0.2 mL) was added to 2 mL of substrate solution (12% skim milk powder in 0.01M $CaCl_2$). The time necessary for the formation of curd fragment was measured. Milk clotting activity is expressed in term of Soxhlet unit.

2.4 Total Protein

Protein concentration in the enzyme extract was determined using Folin Ciocalteu reagent as per the procedure of Lowry *et al*. (1951), Crystalline Bovine Serum Albumin used as standard protein for preparation of standard curve. The absorption of the blue color developed was measured at 660 nm using spectrophotometer.

2.5 Cheese preparation

Fresh morning 200 mL raw Caw milk is usually used and warm to a temperature of about 50^{o}C. The milk is stirred gently during the initial and subsequent heating and cooling. Then 2 mL of the crude enzyme was added to the warmed milk and kept for 10 minutes. It was heated slowly with intermittent stirring until it reaches boiling. The milk was kept at heating until it coagulates and there is visible separation of curds and whey then the pot was removed from the fire and poured the curd and whey into baskets placed over a container for whey collection. The basket or mould facilitates whey drainage and also gave the cheese its characteristic shape and size. When the cheese was firmed enough to retain its shape, it was removed from the basket and placed it in a container of cool water. Afterwards cheese is soaked in brine (20% NaCl) for 12–15 hours.

2.6 Cheese analysis

Biochemical analysis of prepared cheese were carried out according to the Official Methods of Analysis (AOAC, 1990) for food with respect to yield, total solid, moisture content, total carbohydrate, total protein and total fat.

2.6.1 Determination of yield

The yield of cheese was calculated from the following equation: Yield (g/100 g of milk) = (W1 × 100) / (W_2 + W_3) Where, W1 was the weight of the cheese prepared, W2 was the weight of the milk and W3 was the weight of the enzyme used

2.6.2 Total Solids and Moisture content

A known weight of grated cheese is dried at a constant temperature (102±2°C) to a constant weight. The weight after drying is the weight of total solids and is expressed as % by weight. Moisture (% cheese) = 100 - TS
Moisture content of cheese samples was determined in triplicate using nutritional analysis methods as described above for food. Three grams of samples were weighed into pre-dried and weighed moisture dish with tight-fit cover. Samples were partially dried and weighed on a steam bath prior to oven combustion at 105ºC for 8 hrs. Moisture content was determined by difference and expressed as a percentage of the initial weight of cheese product.

2.6.3 Total fat

One gram of cheese sample was first hydrolyzed with 4M hydrochloric acid and placed in a water bath (70- 80ºC) with stirring them frequently for about 30 - 40 minutes. After cooling to room temperature it is subjected for extraction with 25 mL of petroleum ether. During extraction, upper ether layer was taken into clean dried weighed flasks and dried them in a water bath at 80 º C until a constant weight obtained.
% of Total fat = (Wt. of Fat / Wt. of sample) * 100

2.6.4 Total Carbohydrate

Total carbohydrate was estimated by the anthrone method described in the book written by Sadasivam and Manickam (2008). Briefly, 1 mL aliquot of appropriately diluted sample or standard solutions of glucose (0-100 µg/ml) was taken to which 4 mL of the anthrone reagent was added and kept them in boiling water bath for 10 minutes. Cool to room temperature and measured the optical density of blue colour at 620 nm against blank.

2.6.5 Protein Estimation

This method is described in section 2.4

3. Results and Discussion

In our earlier communication, we have reported 21 plants bearing latex as source of milk clotting enzymes (Badgujar and Mahajan, 2009). In this work, we have focused our study taking advantage of milk clotting nature of enzymes for cheese production.

Table 1 List of rennet sources reported in literature for cheese production.

Sr. No.	Source	Rennet	Reference
1	Animal	Calf rennate	O'Connor, 1993.
		Goat	
		Sheep	
2	Micro-organisms	*Mucor miehei*	Thakur et al., 1990.
		Mucor pucillus	Richardson et al., 1967.
		Cryphonectria parasitica	
		Endothia parasitica	Ustunol and Hicks, 1990.
		Bcillus subtilis	Rao and Mathur, 1979.
		Rhizomucor miehei	Sai et al., 2012.
3	Vegetable	*Ananas comosus,*	Roseiro et al., 2003.
		Albizia julibrissin	
		Calotropis procera	Aworths and Muller, 1987; Adetunji and Saawu, 2008.
		Carica papaya	Adetunji and Saawu, 2008; Diouf et al., 2012.
		Centaurea calcitrapa	Reis et al., 2000.
		Cynara cardunculus, C. humilis, C. scolymus	Roseiro et al., 2003; Vioque et al., 2000.
		Euphorbia amygdaloides	Demir et al., 2005.
		Ficus carica, F. glomerata, F. religiosa	Oner and Akar, 1993; Faccia et al., 2012; Krishnaswamy and Johar, 1960; Nouani et al., 2009.
		Moringa oleifera	Mahami et al., 2012.
		Solanum dubium	Talib et al., 2009; Kheir et al., 2011.
		Taraxacum officinale	Akuzawa and Yokoyama,1988.
		Withania coagulans	Pezeshki et al., 2011.

Table 1 summarizes biological source of rennet used for cheese production in literature. From the table it was clear that to replace animal rennet researcher have tried microbial and vegetable rennet for cheese preparation, In this regard, presently use of vegetable rennet in cheese preparation is receiving importance. Near about 16 plant species have been used for this purpose. The 12 plant species belongs to Indian origin and remaining 4 species are exotic. Only a single plant species of Euphorbiaceae has reported for above said property. The purpose of present

investigation to search milk clotting activity of proteases of Euphorbian plants and its related properties for cheese production.

Table 2 List of selected latex bearing plants of Euphorbiaceae family

Sr. No.	Botanical Name	Common name	Nature	Habitat
1	*Euphorbia heterophylla*	Milk weed	W	H
2	*Euphorbia hirta*	Asthma plant	W	H
3	*Euphorbia milli*	Crown of Thorns	O	H
4	*Euphorbia nivulia*	Leafy Milk Hedge	M	T
5	*Euphorbia nerifolia*	Indian Spurge Tree	M	T
6	*Euphorbia tirculli*	Pencil plant	O	S
7	*Jatropha curcas*	Physic Nut	M	S
8	*Jatropha gossipifolia*	Bellyache Bush	O	S
9	*Pedilanthus tithymaloides*	Devil's Backbone	O	S
10	*Synandenium granti*	Chameleon Plant	O	S

O = Ornamental, W = Weed and M = Medicinal plant
H = Herb, S = Shrub and T = Tree

Details of identified plants of Euphorbiaceae family with botanical name, abbreviation used, Common name, nature, habitat and part used is summarized in table 1. Among selected members of Euphorbiaceae family 50% are ornamental, 20% weed and 30% medicinal plants. And in the case of habitat of these plants, 30% are herb, 50% shrub and tree 20%.

Table 3 Comparative milk clotting activity of latex of some members of Euphorbiaceae family

Sr. No.	Botanical name	Total activity (U)	Protein (mg)	Specific activity (U/mg)
1	*Euphorbia heterophylla*	216.6±28.2	5.8±0.20	37.3±4.8
2	*Euphorbia hirta*	162.0±12.6	3.8±0.09	42.6±3.3
3	*Euphorbia milli*	157.3±13.9	4.5±0.06	34.6±3.0
4	*Euphorbia nerifolia*	290.1±21.0	5.3±0.20	54.7±3.9
5	*Euphorbia nivulia*	315.0±16.7	5.2±0.30	60.5±3.2
6	*Euphorbia tirculli*	148.2±17.1	1.9±0.10	78.4±9.0
7	*Jatropha curcas*	80.9±10.8	2.0±0.04	40.4±5.4
8	*Jatropha gossipifolia*	88.1±1.3	2.1±0.03	41.9±0.6
9	*Pedilanthus tithymaloides*	80.9±10.8	5.7±0.08	41.3±2.2
10	*Synandenium granti*	88.1±1.3	3.8±0.06	38.8±3.5

Milk clotting potential of crude enzyme of lattices of plants of Euphorbiaceae family was found to be highest in *E. tirucalli* followed by *E. nivulia* > *E. nerifolia* > *P. tithymaloides*. The crude enzyme of other plants possesses moderate milk clotting activity. The results are comparable with the observations reported by Badgujar and Mahajan (2012).

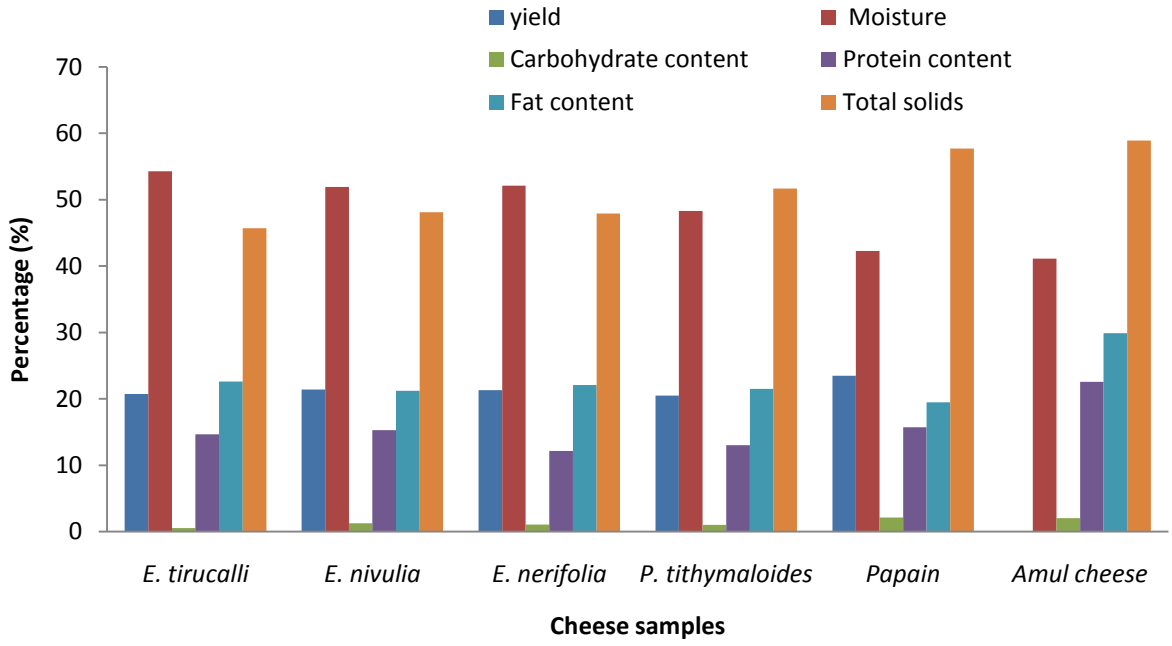
Figure 1 Photo plate of cheese prepared using crude enzyme of plant latex

Table 4 Nutritional analysis of cheese sample prepared using Cow's raw milk

Sr. No.	Cheese	Yield	Moisture	Total solids	Carbohydrate	Protein	Fat
1	*E. tirucalli*	20.73	54.3±5.3	45.7±5.3	0.52±0.14	14.66±0.62	22.6±3.4
2	*E. nivulia*	21.41	51.9±2.4	48.1±2.4	1.26±0.15	15.29±0.52	21.2±2.7
3	*E. nerifolia*	21.30	52.1±5.7	47.9±5.7	1.05±0.39	12.14±0.18	22.1±1.9
4	*P. tithymaloides*	20.49	48.3±3.6	51.7±3.6	1.0±0.76	13.04±0.92	21.5±1.7
5	Papain	23.49	42.3±1.8	57.7±1.8	2.11±0.68	15.71±0.68	19.5±1.3
6	Amul cheese		41.1±1.3	58.9±1.3	2.01±0.27	22.56±0.53	29.9±1.8

All values are expressed in percentage. Values are expressed as mean ± SEM.

Figure 2 Nutritional analysis of cheese sample prepared using Cow's raw milk



3.1 Yield

The yield of cheese products was determined on the basis of weight of coagulated milk product. Cheese yield produced from each of the crude enzyme of latex of four plants of Euphorbiaceae was expressed in percentage as shown in table 3. However, papain gave the highest amount of cheese (23.4%) followed by *E. nivulia* (21.4%), *E. nerifolia* (21.3%), *E. tirucalli (20.7%)* and *P. tithymaloides* (20.4%).

3.2 Total solids and Moisture content

Loss of weight and moisture in cheese during storage was observed. The weight loss in cheese during ripening has been attributed mainly to the loss of moisture (Buffa et al., 2003). The uptake of salt also affects the loss of moisture (Melilli, 2006). When cheese is salted in brine, it responsible for inward migration of salt into the cheese and the accompanying transport of water to the outside of the cheese. Cheese samples during ripening in brine conform to the 'Donnan equilibrium' which controls the partition of ions between the curd and the brine (Guinee, 2004; Fox et al., 2004). Total solids was found to increase and moisture content was found to decrease in the following order Amul cheese > papain > *P. tithymaloides* > *E. nivulia* > *E. nerifolia* > *E. tirucalli* (Figure 2). Highest total solids and minimum moisture content was present in Amul cheese.

3.3 Total protein

The ripening of cheese is accompanied by partial protein degradation (Gaya et al., 2005). Proteolytic enzymes such as rennin are responsible for the formation of nitrogenous products of intermediate size, such as proteoses, peptones, polypeptides, peptides and free amino acids. Enzymes of micro-organisms act on these and other substances to form products like amino acids, amines, fatty acids, esters, aldehydes, alcohols and ketones (Fox and McSweeney, 1996). Cheese serves as a storehouse of essential amino acids, having similar proportion of essential amino acids that is present in milk except the methionine and cysteine. During the ripening of cheese, part of the water-insoluble casein is converted into water-soluble nitrogenous compounds including the intermediate products of protein hydrolysis and free amino acids. (Cross and Overby, 1988). According to Guinee, (2004), salt has a retarding influence on protein breakdown. Studies by Barac et al., (2013) have shown that during ripening protein content of cheese decreases. Wolf et al., (1983) reported that salt has selective effect against proteolytic micro-organisms. The minimum decline recorded for salted samples may be due to the inhibition of micro-organisms and enzyme activity. Highest protein content was found in Amul cheese (22.5**%**) followed by papain (15.7**%**), *E. nivulia* (15.2**%**) , *E. tirucalli* (14.6**%**), *P. tithymaloides* (13.0**%**) and *E. nerifolia* (12.1**%**).

3.4 Total fat content

According to Talib et al., (2009) during ripening, gradual decrease in fat content due to breakdown of fat, salt uptake and continuous loss of degraded components of cheese was observed. Previous studies have indicated that sodium chloride may inhibit lipolysis in cheese (Wolf et al., 1983). However, within the range 0.5% - 3.0% (w/w) of NaCl investigated no statistical influence of NaCl on lipolysis was found (Rulikowska et al., 2013). Highest fat content was found in Amul cheese (29.9%) followed by *E. tirucalli* (22.6%), *E. nerifolia* (22.1%), *P. tithymaloides* (21.5%), *E. nivulia* (21.2%), and papain (19.5%).

3.5 Total carbohydrate content

Lactose is one of the basic nutrients consumed by lactic acid producing micro-organisms. Lactose remaining in the curd is converted into lactic acid. Lactic acid inhibits the growth of undesirable micro-organisms. It is very important in production of acid flavour in the cheese. It determines the smoothness of the body of the cheese (Van et al., 2002). Lactose, the major carbohydrate of milk. In addition to lactose, milk contains small amounts of glucose, galactose, and other saccharides (Jenness, 1988). When milk is coagulated, greater percentage of the lactose is present in the whey and the remaining in the curd. For this reason, cheese that is prepared from the curd is low in carbohydrates (Penfield and Campbell, 1990). Highest carbohydrate content was found in papain (2.1%) followed by Amul cheese (2.0%), *E. nivulia* (1.2%) *E. nerifolia* and *P. tithymaloides have similar* (1.0%), and *E. tirucalli have minimum* (0.5%).

4. Conclusion

Milk coagulation is the primary step in the development of texture and flavour of cheeses depend on specific enzymatic proteolytic degradation of milk compounds a specially proteins. In order to improve textural properties and the nutritional value of cheeses and in addition to decreasing in the number of young animals has lead the producers to the investigate alternative milk clothing enzymes of different origins. These include especially microbial and plant enzymes are commonly accepted for lacto vegetarians. The results showed that milk clotting enzymes of plant lattices of Euphorbiaceae family have cheese making property. It could be useful in the dairy

industry as a rennet substitute. The results revealed that, nutritional quality of cheese prepared from milk clotting enzymes of plant lattices of Euphorbiaceae family is not fully matched with Amul cheese but it is comparable with cheese prepared using purified papain. However further investigation and attention for nutritional status as well as quality produced by plant enzymes is essential for matching with Amul cheese. This is possible by enriching cheese samples with essential nutrients such as mineral, vitamins, amino acids, probiotics and flavoring agents etc.

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References

1. Adetunji, V.O. and Salawu, O.T. (2008): West African soft cheese 'wara'processed with *Calotropis procera* and *Carica papaya*: A comparative assessment of nutritional values. African journal of Biotechnology, 7(18): 3360-3362.
2. Ahmed, I.A.M., Morishima, I., Babiker, E.E. and Mori, N. (2009): Characterisation of partially purified milk-clotting enzyme from *Solanum dubium* Fresen seeds. Food chemistry, 116(2): 395-400.
3. Akuzawa, R. and Yokoyama, K. (1988): Trial cheese prepared with dandelion (*Taraxacum officinale*) extract. Japanese Journal of Dairy and Food Science, 37(2):39-44.
4. Ardo, Y. and Polychroniadou, A. (1999): Laboratory manual for chemical analysis of cheese: improvement of the quality of the production of raw milk cheeses. Office for Official Publications of the European Communities.
5. Aworth, O.C. and Muller H.G. (1987) Cheese-making properties of vegetable rennet from sodom apple (*Calotropis procera*). Food Chemistry, 26(1): 71-79.
6. Badgujar, S.B. and Mahajan, R.T. (2009): Proteolytic Enzymes of Some Laticiferous Plants Belonging to Khandesh Region of Maharashtra, India. Journal of Pharmacy Research, 2(9): 1434-1437.
7. Badgujar, S.B. and Mahajan, R.T. (2012): Comparison of cysteine proteases of four laticiferous plants and characterization of *Euphorbia nivulia* Buch.-Ham. latex glycosylated cysteine peptidase. Indian Journal of Natural Products and Resources, 3(2): 152-160.
8. Barac, B.M., Smiljanić, M., Pesic, B.M., Stanojevic, P.S., Jovanovic, T.S. and Macej, D.O. (2013): Primary proteolysis of white brined goat cheese monitored by high molarity Tris buffer SDS-PAGE system. Mljekarstvo, 63(3): 122-131.
9. Buffa, M., Guamis, B., Saldo, J. and Trujillo, A.J. (2003): Changes in water binding during ripening of cheeses made from raw, pasteurized or high-pressure-treated goat milk. Le Lait, 83(1): 89-96.
10. Cross, H.R. and Overby, A.J. (1988): Meat science, milk science and technology. Elsevier Science Publishers BV.
11. Davies, F.L. and Law, B.A. (1984): Advances in the microbiology and biochemistry of cheese and fermented milk. Elsevier Applied Science Publishers Ltd.
12. Demir, Y., Alayli, A., Yildirim, S. and Demir, N. (2005): Identification of protease from *Euphorbia amygdaloides* latex and its use in cheese production. Preparative biochemistry & biotechnology, 35(4): 291-299.
13. Diouf, L., Mallaye, A., Mbengue, M., Kane, A. and Diop, A. (2012). *Carica papaya* leaves: a substitute for animal rennet in cheese-making tradition. Journal of Natural Product & Plant Resources, 2(4): 517-523.
14. Duarte, A.R., Duarte, D.M.R., Moreira, K.A., Cavalcanti, M.T.H., Lima-Filho, J.L.D. and Porto, A.L F. (2009): *Jacaratia corumbensis* O. Kuntze a new vegetable source for milk-clotting enzymes. Brazilian Archives of Biology and Technology, 52(1): 1-9.
15. Faccia, M., Picariello, G., Trani, A., Loizzo, P., Gambacorta, G., Lamacchia, C. and Luccia, A. (2012): Proteolysis of Cacioricotta cheese made from goat milk coagulated with caprifig (*Ficus carica sylvestris*)

or calf rennet. European Food Research and Technology, 234(3): 527-533.

16. Fox, P.F. and McSweeney, P.L.H. (1996): Proteolysis in cheese during ripening. Food Reviews International, 12(4): 457-509.
17. Fox, P.F., McSweeney, P.L., Cogan, T.M. and Guinee, T.P. (2004): Cheese: Chemistry, Physics and Microbiology: General Aspects. (Vol. 1). Academic Press.
18. Gaya, P., Sanchez, C., Nuñez, M. and Fernandez-Garcia, E. (2005): Proteolysis during ripening of Manchego cheese made from raw or pasteurized ewes' milk. Seasonal variation. Journal of dairy research, 72(03): 287-295.
19. Guinee, T.P. (2004): Salting and the role of salt in cheese. International Journal of Dairy Technology, 57(2-3): 99-109.
20. Helrich, K.C. (1990): Official methods of Analysis of the AOAC. Volume 2. Ed. 15, Association of Official Analytical Chemists Inc.
21. Kheir, S.E.O., Owni, E., Osman, O.A. and Abdalla, M.O.M. (2011): Comparison of Quality of Sudanese White Cheese (Gibna bayda) Manufactured with *Solanum dubium* Fruit Extract and Rennet. Pakistan Journal of Nutrition, 10(2):106-111.
22. Krishnaswamy, M.A. and Johar, D.S. (1960): Preparation of cheddar and processed cheese using the milk clotting enzyme from *Ficus carica* L. Proceedings of the symposium on Proteins, Mysore, August, pp. 302-306.
23. Lowry, O.H., Roserbrough, N.J., Farr, A.L. and Randall, N.J. (1951): Estimation of total protein. Journal of Biological Chemistry, 193: 265-275.
24. Mahami, T., Ocloo, F.C.K., Odonkor, S.T., Owulah, C. and Coffie, S.A. (2012): Preliminary study on the influence of moringa seed extracts supplementation on the yield and quality of cottage cheese. 2(1):4-8.
25. Mahajan R. T., and Badgujar, S. B. (2010): Biological aspects of proteolytic enzymes: A Review. *J*ournal of Pharmacy Research, *3*(9).
26. Metwalli, N.H., Shalabi, S.I., Zahran, A.S. and El-Demerdash, O. (1982): The use of soybean milk in soft cheese making. International Journal of Food Science & Technology, 17(3): 297-305.
27. Melilli, C., Barbano, D.M., Caccamo, M., Tuminello, L., Carpino, S. and Licitra, G. (2006): Interaction of brine concentration, brine temperature, and presalting on salt penetration in Ragusano cheese. Journal of dairy science, 89(5): 1420-1438.
28. Nouani, A., Dako, E., Morsli, A., Belhamiche, N., Belbraouet, S., Bellal, M.M. and Dadie, A. (2009): Characterization of the purified coagulant extracts derived from Artichoke Flowers (*Cynara scolymus*) and from the Fig Tree Latex (*Ficus carica*) in light of their use in the manufacture of traditional cheeses in Algeria. Journal of Food Technology, 7(1): 20-29.
29. O'Connor, C. (1993): *Traditional cheesemaking manual*. ILRI (aka ILCA and ILRAD).
30. Omotosho, O.E., Oboh, G. and Iweala, E.E.J. (2011): Comparative Effects of Local Coagulants on the Nutritive Value, in vitro Multienzyme Protein Digestibilit y and Sensory Properties of Wara Cheese. International Journal of Dairy Science, DOI: 10.3923/ijds.2011.
31. Oner, M.D. and Akar, B. (1993): Separation of the proteolytic enzymes from fig tree latex and its utilization in gaziantep cheese production. LWT-Food Science and Technology, 26(4): 318-321.
32. Penfield, M.P. and Campbell, A.M (1990): Experimental Food Science. Academic Press.
33. Pezeshki, A., Hesari, J., Ahmadi Zonoz, A. and Ghambarzadeh, B. (2011): Influence of *Withania coagulans* Protease as a Vegetable Rennet on Proteolysis of Iranian UF White Cheese. Journal of Agricultural Science and Technology, 13:567-576.
34. Prieto, B., Franco, I., Fresno, J. M., Bernardo, A. and Carballo, J. (2000): Picon Bejes-Tresviso blue cheese: an overall biochemical survey throughout the ripening process. International dairy journal, 10(3): 159-167.

35. Rao, K.L. and Mathur, D.K. (1979): Assessment of Purified Bacterial Milk Clotting Enzyme from *Bacillus subtilis* K-26 for Cheddar Cheese Making. Journal of Dairy Science, 62(3): 378-383.
36. Reis, P.M., Lourenço, P.L., Domingos, A., Clemente, A.F., Salomé, P.M. and Malcata, X. (2000): Applicability of extracts from *Centaurea calcitrapa* in ripening of bovine cheese. International dairy journal, 10(11): 775-780.
37. Richardson, G.H., Nelson, J.H., Lubnow, R.E. and Schwarberg, R.L. (1967): Rennin-like Enzyme from *Mucor pusillus* for Cheese Manufacture. Journal of Dairy Science, 50(7): 1066-1072.
38. Roseiro, L.B., Barbosa, M., Ames, J.M. and Wilbey, R.A. (2003): Cheesemaking with vegetable coagulants—the use of Cynara L. for the production of ovine milk cheeses. International Journal of Dairy Technology, 56(2): 76-85.
39. Rulikowska, A., Kilcawley, K.N., Doolan, I.A., Alonso-Gomez, M., Nongonierma, A.B., Hannon, J.A. and Wilkinson, M.G. (2013): The impact of reduced sodium chloride content on Cheddar cheese quality. International Dairy Journal, 28(2): 45-55.
40. Sai, M.J., Shalini, N.N., Yamuna V.L. and Shailaja R.M. (2012): Production of Cheese from Rennet enzyme using *Rhizomucor miehei* Isolated from cow dung. Helix 3:169-172.
41. Sadashivam, S., and Manickam, A. (2008): Biochemical methods. New Age International (P) Limited.
42. Talib, M.A., Abubakar, M.M., Jideani, I.A. and Hassan, A. (2009): Use of Jiben Seeds Extract to Manufacture Soft White Cheese. American Journal of Applied Sciences, 6(4): 551-554.
43. Thakur, M.S., Karanth, N.G. and Nand, K. (1990): Production of fungal rennet by *Mucor miehei* using solid state fermentation. Applied microbiology and biotechnology, 32(4): 409 -413.
44. Ustunol, Z. and Hicks, C.L. (1990): Effect of Calcium Addition on Yield of Cheese Manufactured with *Endothia parasitica* Protease. Journal of dairy science, 73(1): 17-25.
45. Van den Berg, J.C.T. (1988): Dairy technology in the tropics and subtropics. Pudoc.
46. Van Kranenburg, R., Kleerebezem, M., van Hylckama Vlieg, J., Ursing, B.M., Boekhorst, J., Smit, B.A. and Siezen, R.J. (2002): Flavour formation from amino acids by lactic acid bacteria: predictions from genome sequence analysis. International Dairy Journal, 12(2): 111-121.
47. Vioque, M., Gómez, R., Sánchez, E., Mata, C., Tejada, L. and Fernández, S.J. (2000): Chemical and microbiological characteristics of ewes' milk cheese manufactured with extracts from flowers of *Cynara cardunculus* and *Cynara humilis* as coagulants. Journal of agricultural and food chemistry, 48(2): 451-456.
48. Wedholm, A., Hallén, E., Bach Larsen, L., Lindmark-Månsson, H., Hans Karlsson, A. and Allmere, T. (2006): Comparison of milk protein composition in a Swedish and a Danish dairy herd using reversed phase HPLC. Acta Agriculturae Scand Section A, 56(1): 8-15.
49. Wolf, I.D., Raper, N.R. and Rosenthal, J.C. (1983): USDA activities in relation to the sodium issue: 1981-83. Food Technology, 37.