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Cheese-making Properties of Vegetable Rennet from Sodom Apple (*Calotropis procera*)

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ABSTRACT

Yield, chemical composition and texture profile of cheese made with vegetable rennet from sodom apple leaves were compared with those of a direct acid cheese made with calf rennet. Yield, moisture, fat and protein contents were 14·47%, 49·70%, 26·15% and 20·0%, respectively, for cheese made with vegetable rennet and 12·45%, 44·80%, 29·84% and 20·4%, respectively, for the direct acid cheese made with calf rennet. Cheese made with vegetable rennet had less soluble nitrogen than that made with calf rennet despite the fact that vegetable rennet was more proteolytic in casein solution than calf rennet. Relative to that made with calf rennet, cheese made with vegetable rennet was harder, less cohesive and more gummy, presumably because of differences in chemical composition and physical characteristics between the cheeses.

INTRODUCTION

Despite the fact that many other proteases capable of coagulating milk have been extracted from a variety of plant sources since the crystallisation of ficin from fig latex (Walti, 1938), only animal rennets and, more recently, microbial rennets derived from *Endothia parasitica*, *Mucor miehei* and *Mucor pusillus* are used for commercial cheese-making. Most plant

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proteases, because of their excessive proteolytic activities, have proved unsuitable for cheese-making (Green, 1977). However, in some countries extracts of higher plants are used traditionally as coagulants for cheese-making. In Portugal, for example, extract of the flowers of cardoon (*Cynara cardunculus*) has been used since ancient times for farm production of various types of sheep-milk cheese (Vieira de Sá & Barbosa, 1972).

In parts of West Africa, including Nigeria and the Republic of Benin, the juice from the leaves of the sodom apple (*Calotropis procera*) is used for traditional cheese-making. Cheese-making with this juice is purely empirical and little is known about the properties of the juice and the mechanism of milk coagulation (Ogundiwin & Oke, 1983). Consequently, the quality of the cheese is highly variable and often very poor, reducing its acceptability in urban areas (Aworh & Egounlety, 1985). Recently, a partially purified milk-clotting enzyme was extracted from sodom apple leaves (Aworh & Nakai, 1986). The present research was undertaken to study the cheese-making properties of this enzyme.

MATERIALS AND METHODS

Milk coagulants

Vegetable rennet was prepared using an extraction procedure previously described (Aworh & Nakai, 1986), except that freshly harvested leaves, without the petioles, from greenhouse-grown sodom apple plants were used instead of freeze-dried leaves. In addition, the supernatant fluid of acidification to pH 3·0 was stabilised with 0·02M cysteine prior to ammonium sulphate fractionation. The vegetable rennet was kept in frozen storage at −18°C and, prior to use, was activated with 0·02M cysteine.

Standard rennet powder from calf stomach containing approximately 80% NaCl and papain (crude powder, type II, partially purified from papaya latex) were obtained from Sigma Chemical Co. (St Louis, MO).

Measurement of enzymatic activities

Milk-clotting and proteolytic activities of vegetable rennet were compared with those of calf rennet and crude papain at pH 6·4, since this closely approximated actual cheese-making conditions. Except where otherwise indicated, milk-clotting activity was determined at 35°C by the method of Whitaker (1959). The substrate was a 20% solution of Carnation instant skim milk powder in 0·34M acetate buffer with a pH of 6·2. One unit of enzyme activity clots 1 ml of milk in 1 min.

Proteolytic activity was determined by measuring the increase in non-protein nitrogen (NPN) during incubation in casein substrate. 1·5 ml of vegetable rennet and of solutions of calf rennet and crude papain of the same coagulating power at 35°C, were added to 15 ml of a 3% solution of purified casein (Sigma Chemical Co.) in 0·2M phosphate buffer, pH 6·4. After incubation, with continuous stirring, for 0, 1, 2, 4 and 6 h at 30°C, the reaction mixture was mixed with 15 ml of 24% trichloroacetic acid (TCA) and filtered. The sample at zero time was obtained by adding 1·5 ml of each coagulant, denatured by boiling for 10 min, to 15 ml of casein solution. The NPN of the filtrate and the total nitrogen of the casein solution were determined by the Kjeldahl method. The increase in NPN, expressed as a % of the total N, and set out as a function of the incubation time, is a measure of the proteolytic activity of the coagulants (Vanderpoorten & Weckx, 1972).

All values reported were the averages of at least duplicate tests.

Cheese-making

In order to have some indication of the cheese-making potentials of the vegetable rennet relative to calf rennet, cheeses were prepared in duplicate in the laboratory with vegetable rennet and calf rennet as coagulants. Owing to the large differences in the properties of the rennets, especially with respect to the effects of temperature and pH on enzyme activity (Ogundiwin & Oke, 1983; Aworh & Nakai, 1986), two cheese-making procedures that are roughly equivalent, except for cooking temperatures and pH, were adopted and modified to create conditions best suitable for each coagulant. A technique based on traditional West African cheese-making (Aworh & Egounlety, 1985) was used for vegetable rennet, whilst a direct acid cheese was prepared with calf rennet using a procedure developed by Freeman & Bucy (1971), with slight modification.

The vegetable rennet cheese was made from milk pasteurised at 72°C for 16 s. Calcium chloride (1·8 mM) was added to the pasteurised milk (4·7 kg) at 40°C in a steam-jacketed kettle and, after mixing, 7 ml of vegetable rennet was added. After stirring for 2 min, the milk was gently heated at the rate of 1·5–2·0°C per min until curd formation was observed at about 75°C. Coagulum formation was allowed to proceed for 30 min, after which the curds were cooked with gentle stirring, by increasing the temperature to 80°C for 10 min. The loose curd pieces were ladled into stainless steel hoops and, after dipping for 1 h, pressed overnight at 120 kPa.

For the preparation of the direct acid cheese, glucono-delta-lactone (11 g) was added to pasteurised milk (4·7 kg) at 31°C. The mixture was stirred until a pH of 6·5 was attained, when calf rennet (1·9 g) was added. Stirring was

continued for 1 min to ensure even distribution of rennet. Coagulum formation was allowed to proceed for 45 min, after which the curd was cut, allowed to heal for 10 min, and then cooked, with gentle stirring, by increasing the temperature to 38°C for 1 h. The whey–curd mixture was ladled into stainless steel hoops and, after dipping for 1 h, pressed overnight at 120 kPa.

Determination of yield and chemical composition

Cheese yield was determined gravimetrically immediately after pressing. Fat content of milk and whey samples was determined by the Röse-Gottlieb method (BS 1741: 1963*a*). Whey samples were taken immediately after dipping. Cheeses, wrapped in plastic film, were analysed after 1 day at 5°C. The Werner-Schmid process was used for the analysis of fat in cheese (Egan *et al.*, 1981). Nitrogen (N) determinations were by the Kjeldahl procedure using a copper catalyst. Protein was estimated as total N × 6·38. Acid-soluble N in cheese was extracted as described by Vakaleris & Price (1959). NPN in milk and whey was extracted with 15% TCA (Shahani & Sommer, 1951). Recovery of protein and fat in cheese was expressed as a percentage of the input of these components in the renneted milk. Moisture was determined by drying to constant weight in an oven at 102°C (BS 770:1963*b*). The pH of cheese, milk and whey samples were measured directly on a Corning digital pH meter.

Texture profile analysis

Texture profiles of the cheeses were performed with the Instron Universal Testing Machine, Model 1112 with a 50 kg load cell using basically the procedure described by Chen *et al.* (1979), with slight modification. Cylindrical samples, 10 mm diameter and 30 mm height, were cut with a cork borer from cheese blocks wrapped in plastic film held at 5°C for 24 h. A plunger, 6·4 mm in diameter, was attached to the Instron crosshead set to cycle at constant speed of 25 mm/min and with a stroke length of 10 mm. The recorder chart speed was 20 cm/min. One bite was one cycle of a downward plus upward motion of the plunger which penetrated into, and was retrieved immediately from, the sample. Two bites were taken, and four measurements were made for each cheese.

Three textural parameters, as defined by Bourne (1968), were derived from the force–deformation curves. Hardness is the maximum force that is exerted on the sample. Cohesiveness is the ratio of the area under the force–deformation curves of the second and first bites, respectively. Gumminess is the product of hardness and cohesiveness.

RESULTS AND DISCUSSION

Milk-clotting and proteolytic activities of coagulants

At 35°C, vegetable rennet had a milk-clotting activity of 1·41 unit/ml which was equivalent to the activity of a 0·13% solution of calf rennet or crude papain. Addition of 0·02M cysteine and 0·025M $CaCl_2$ to the substrate increased the activity to 1·94 unit/ml. Cysteine alone increased the activity by 32%. Enzyme activity increased several-fold at higher temperatures. Milk-clotting activities at 50° and 60°C, without cysteine and $CaCl_2$ in the substrate, were 3·76 and 6·68 unit/ml, respectively. This increase in activity at higher temperatures is in agreement with previous reports (Ogundiwin & Oke, 1983; Aworh & Nakai, 1986).

A comparison of the proteolytic activity of vegetable rennet with solutions of calf rennet and crude papain of the same coagulating power is shown in Fig. 1 and the results follow normal first order reaction curves. The rate of reaction of papain is approximately twice that of the vegetable rennet, but that of calf rennet is very low. It is because of this low proteolytic to clotting activity that calf rennet is considered the ideal coagulant for cheese-making (Green, 1977). The vegetable rennet and papain curves are typical of those enzymes with undesirable proteolytic activities (Richardson *et al.*, 1967; Phelan, 1973). Excessive proteolysis has been associated with high fat and protein losses in whey, lower cheese yield and impaired quality (Vieira de Sá & Barbosa, 1972; Puhan & Irvine, 1973).

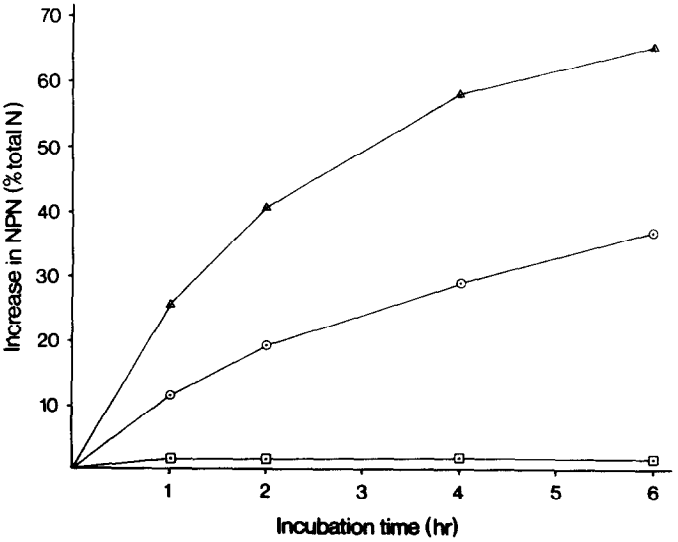

Fig. 1. Increase in non-protein nitrogen due to casein digestion by calf rennet (□), vegetable rennet (○) and crude papain (△).

TABLE 1
A Comparison of Yield and Composition of Cheeses made with Calf and Vegetable Rennets

Coagulant	*Yield* (%)	*pH*	*Moisture* (%)	*Fat* (%)	*Protein* (%)	*Soluble N* (%)
Calf rennet	12·45	5·95	44·80	29·84	20·4	0·373
	(12·50)[a]			(29·73)	(20·4)	(0·372)
Vegetable rennet	14·47	6·25	49·70	26·15	20·0	0·157
	(13·23)			(28·59)	(21·8)	(0·172)

[a] Value in brackets is adjusted to 45% moisture.

Yield, composition and texture profile of cheeses

The yield and composition of cheeses made with vegetable and calf rennets are presented in Table 1. The cheeses were prepared from milk with similar chemical composition (Table 2). Cheese made with vegetable rennet was higher in moisture than the direct acid cheese made with calf rennet. When adjusted to the same moisture content, the yield of cheese made with vegetable rennet was 0·73% higher than that of the direct acid cheese made with calf rennet. Calf rennet cheese was higher in fat but lower in protein than vegetable rennet cheese. Soluble N content of vegetable rennet cheese was less than half that of calf rennet cheese.

The higher cheese yield obtained with vegetable rennet relative to calf rennet is presumably due to differences in the cheese-making procedures, especially the use of high temperatures for the manufacture of vegetable rennet cheese. Whey proteins, denatured by heat, may have been enclosed in the vegetable rennet cheese curd, thus contributing to higher yield. This is consistent with the substantially higher recovery of protein in the cheese, and the lower level of protein in the whey (Table 3). The production of acid-soluble N is used as a measure of the degree of proteolysis in ripening cheese (Vakaleris & Price, 1959; Richardson & Nelson, 1968). The low level of soluble N in vegetable rennet cheese is contrary to results obtained in cheese-making trials with other rennet substitutes with high proteolytic activity

TABLE 2
Composition of Renneted Milk used for Cheese-making

Coagulant	*pH*	*Moisture* (%)	*Fat* (%)	*Protein* (%)	*Non-protein N* (%)
Calf rennet	6·70	87·05	3·96	3·40	0·031
Vegetable rennet	6·70	87·12	3·96	3·38	0·032

TABLE 3
Fat and Protein Recoveries in Cheeses and Residual Levels in Whey from Cheese-making with Calf and Vegetable Rennets

Coagulant	*Recoveries in cheese*		*pH*	*Composition of whey*		*Non-protein N*
	Fat (%)	*Protein* (%)		*Fat* (% *dry matter*)	*Protein*	
Calf rennet	93·82	74·8	5·84	4·30	14·3	0·643
Vegetable rennet	95·55	85·5	6·12	3·66	8·14	0·563

(Puhan & Irvine, 1973; Chen & Zall, 1986). This suggests that, during the course of cheese-making, the vegetable rennet was inactivated soon after the milk was coagulated or that its proteolytic activity was much lower in cheese curd than in casein solution. This view is supported by the low level of NPN in the whey (Table 3). The assay of proteolytic activity in casein solution at 30°C was therefore not an accurate reflection of that obtained under actual cheese-making conditions.

The results of the texture profile analysis of the cheeses are presented in Table 4. The force–deformation curves for the cheeses were similar in shape to those obtained by Chen *et al.* (1979). The results indicate that cheese made with vegetable rennet was harder, less cohesive and more gummy than the direct acid cheese made with calf rennet. These textural characteristics, measured objectively with the Instron, correlated closely with sensory evaluation scores for 11 cheeses with widely different chemical composition (Chen *et al.*, 1979). The differences in texture between cheeses made with calf and vegetable rennets might be due to differences in chemical composition and physical characteristics. The implication of these textural differences for the acceptability of vegetable rennet cheese is not clear, as there are widely differing regional preferences for cheeses.

In conclusion, yield and recovery of milk solids were satisfactory in laboratory cheese-making with vegetable rennet from sodom apple leaves. It

TABLE 4
Texture Profile of Cheese Made with Calf and Vegetable Rennets

Coagulant	*Hardness* (*Force, N*)	*Cohesiveness*	*Gumminess* (*Force, N*)
Calf rennet	3·95	0·54	2·15
Vegetable rennet	6·13	0·48	2·97

would appear that the vegetable rennet could also be used in other cheese-making recipes. The possibility of using it in small-scale industrial cheese-making is being investigated.

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REFERENCES

Aworh, O. C. & Egounlety, M. (1985). Preservation of West African soft cheese by chemical treatment. *J. Dairy Res.*, **52**, 189–95.

Aworh, O. C. & Nakai, S. (1986). Extraction of milk clotting enzyme from sodom apple (*Calotropis procera*). *J. Food Sci.*, **51**, 1569–70.

Bourne, M. C. (1968). Texture profile of ripening pears. *J. Food Sci.*, **33**, 223–6.

British Standard (1963*a*). *Methods for the Chemical Analysis of Liquid Milk and Cream.* BS 1741. British Standards Institution, London.

British Standard (1963*b*). *The Chemical Analysis of Cheese.* BS 770. British Standards Institution, London.

Chen, H. C. & Zall, R. R. (1986). Evaluation of thiol activated proteases from clam viscera as a rennet substitute for cheese-making. *J. Food Sci.*, **51**, 815–20, 825.

Chen, A. H., Larkin, J. W., Clark, C. J. & Irwin, W. E. (1979). Textural analysis of cheese. *J. Dairy Sci.*, **62**, 901–7.

Egan, H., Kirk, R. S. & Sawyer, R. (1981). *Pearson's chemical analysis of foods.* (8th edn), Churchill Livingstone, Edinburgh.

Freeman, T. R. & Bucy, J. L. (1971). Method and apparatus for producing experimental cheese curd. *J. Dairy Sci.*, **54**, 758. (Abstr.)

Green, M. L. (1977). Review of the progress of dairy science: Milk coagulants. *J. Dairy Res.*, **44**, 159–88.

Ogundiwin, J. O. & Oke, O. L. (1983). Factors affecting the processing of Wara-a Nigerian white cheese. *Food Chem.*, **11**, 1–13.

Phelan, J. A. (1973). Laboratory and field tests on new milk coagulants. *Dairy Industries*, **38**, 418–23.

Puhan, Z. & Irvine, D. M. (1973). Proteolysis by proteases of *Bacillus subtilis* used to make Canadian cheddar cheese. *J. Dairy Sci.*, **56**, 317–321.

Richardson, G. H. & Nelson, J. H. (1968). Rapid evaluation of milk coagulating and flavor producing enzymes for cheese manufacture. *J. Dairy Sci.*, **51**, 1502–3.

Richardson, G. H., Nelson, J. H., Lubnow, R. E. & Schwarberg, R. L. (1967). Rennin-like enzyme from *Mucor pusillus* for cheese manufacture. *J. Dairy Sci.*, **50**, 1066–72.

Shahani, K. M. & Sommer, H. H. (1951). The protein and non-protein nitrogen fractions in milk. I. Methods of analysis. *J. Dairy Sci.*, **34**, 1003–9.

Vakaleris, D. G. & Price, W. V. (1959). A rapid spectrophotometric method for measuring cheese ripening. *J. Dairy Sci.*, **42**, 264–76.

Vanderpoorten, R. & Weckx, M. (1972). Breakdown of casein by rennet and microbial milk-clotting enzymes. *Neth. Milk Dairy J.*, **26**, 47–59.
Vieira de Sá, F. & Barbosa, M. (1972). Cheese-making with a vegetable rennet from Cardo (*Cynara cardunculus*). *J. Dairy Res.*, **39**, 335–43.
Walti, A. (1938). Crystalline ficin. *J. Am. Chem. Soc.*, **60**, 493.
Whitaker, J. R. (1959). Properties of the milk-clotting activity of ficin. *Food Technol.*, **13**, 86–92.