J. Agric. Sci. Mansoura Univ., 31 (11): 7149 - 7155, 2006

EFFECT OF DIFFERENT CONCENTRATIONS OF JIBEN *(Solanum dubium)* SEED EXTRACTS ON THE PHYSICOCHEMICAL AND ORGANOLEPTIC PROPERTIES OF SOFT CHEESE

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ABSTRACT

Studies were conducted to determine the enzymatic and coagulating properties on aqueous extracts of Jiben seeds. Different levels (5, 10, 15 and 20g) of the seeds were soaked in water containing different concentrations (0, 2, 4, 6 and 8g/100) of sodium chloride (NaCl) and the enzymes were extracted with both water and 0.1N citrate phosphate buffer. Physicochemical and organoleptic properties of soft cheese were also evaluated. The results showed that Jiben seeds extracted in water had less milk clotting activity than those extracted using NaCl. Increasing Jiben seeds and NaCl concentrations increased the effectiveness of milk clotting enzymes thereby decreasing the coagulation time of the milk ($p< 0.001$). The maximum yield of the cheese (12%) obtained when 15-20g Jiben seeds were used with 8g/100ml NaCl solution was significantly ($p< 0.01$) different from the minimum yield of 9% obtained on 5-10g Jiben seeds. The enzymatic and organoleptic assessments of soft cheeses produced using Jiben seed extracts showed that 15g seeds soaked in 100ml water containing 8g NaCl for two days at room temperature was the optimum. Extraction of the seeds with 0.1 citrate phosphate buffer for three hours at pH 4.6 was found to be very effective. Also results showed that the rate of loss in activity of Jiben seed extracts increased with advancing storage.

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INTRODUCTION

Rennet is a general term that describes a variety of enzymes of animal (especially calves) plant or microbial origin used to coagulate milk during cheese making (O'Connor,1995). The gastric enzyme of calf rennet, is the most desirable enzyme for coagulating milk in the manufacture of cheese, and is the standard for evaluation of other milk clotting enzymes. Rennet is used in cheese making and is important in the formation of the casein network during coagulation. It is also known to contribute to proteolysis in pickled cheese (Gouda, 1990).

Use of calf rennet as milk clotting enzyme in the manufacture of cheese has been predominant in the industry for years. Lately, a shortage of this enzyme has occurred because of the decrease in general availability of sucking calves as they are left for beef and not killed for veal. Since 1960s there has been a decline in calf slaughter worldwide, thus decreasing the

rennet availability and increasing its cost. The limited supply of rennet and its resulting high price have necessitated research, for many decades, to come up with an alternative milk coagulant (Craw, 1983).Calf rennet is no longer available in sufficient amounts to cover the needs of the cheese industry.

Ibiama and Griffiths (1987) reported that milk coagulating enzyme from the latex, stem and leaves of Sodom Apple *(Calotropis procera)* has been used traditionally in Nigeria for the manufacture of soft bodied cheese called *Warankashi.* Yousif *et al.* (1996) tested extracts from different parts of the plant *Solanum dubium* for their milk-clotting activity. Extracts from the seeds has the greatest activity followed by extracts from the whole berries, the ground whole berries and the berries coates.

The current study was designed to extract a milk-clotting enzyme from Jiben seed extracts by either water or buffer and to use this extract as a substitute for calf rennet in cheese production.

MATRIALS AND METHODS

Source of Milk:

Milk was obtained from the herds of white Fulani and Red Bororo in N'djamena, Chad and Bauchi, Nigeria. The milk was collected using 20 liter capacity plastic containers and transported to the laboratory.

Source of Jiben:

The dried seeds of Jiben were collected from the surrounding bushes of N'djamena, during the month of January, threshed, decorticated manually and stored in the refrigerator until required.

Preparation of Jiben Seed Extracts for the Production of Soft Cheese:

The Jiben seeds were weighed at four units of 5, 10, 15, and 20g. Each unit weight was soaked in 100ml of water containing 0, 2, 4, 6 and 8g of NaCl for 48 hours at room temperature. This was done to obtain four different concentration levels of Jiben seeds, at five different salt concentrations. Each of the 20 solutions was filtered through muslin cloth into brown glass jar and kept in the refrigerator (6 ±1°C) until required. Three milliliters of each of the 20 combinations of the Jiben seeds and sodium chloride solutions were used to coagulate 1 liter of milk to produce soft cheese.

Processing of Milk for Soft Cheese Production

The cheese was produced according to the following procedure:

Twenty kilograms of fresh cow milk was pasteurized at 63°C for 30 minutes and then cooled to 45°C in a 5-litre bucket. Starter culture of *Lactobacillus lactis* was added at the rate of 0.2% w/v and left for 30 minutes to develop acidity. Extracts of fifteen grammes Jiben seeds in 100ml of water containing 8g NaCl were added to the milk at the rate of 3ml/kilogram. Rennet was used as a control. The milk was thoroughly mixed and left to curdle for 45 minutes. The curd was cut vertically and horizontally into $1cm^3$ with a sharp knife. The whey obtained from the cheese curd was drained and curd was laddled into small cylindrical moulds lined with cloth and pressed lightly overnight. The curd was cut into cubes of 3 x 3 x $3cm^3$ and placed in glass jar containers, then filled with whey containing 15% NaCl (w/v) and sealed. Samples of cheese were stored at room temperature and in the refrigerator for 5 months. Cheese yield expressed as percentage of the

amount of milk used, was determined by weighing the product after pressing for 24 hours.

Assay of Milk Clotting Time and Milk Clotting Activity:

Some 10 ml of fresh milk (pH 6.5) were placed in 40 ml capacity beaker and the contents heated up to 40°C using a constant temperature water bath. Then 1ml of the Jiben seed solution was added. Curd formation was observed by manually rotating the beaker continuously so as to observe formation of a thin film on the milk surface. The end point was taken instantly when discrete milk particles appeared. A stopwatch (Chronometer) was used to record the clotting time in seconds (Metwalli *et al.* 1982).

The milk clotting activity was calculated (Ibiama and Griffiths, 1987) as follows

$$X=100D/T$$

Where:

X= Milk clotting activity (unit/ml)

D= Dilution or quantity of milk containing 1ml of crude enzyme

T= Clotting time in seconds

Extraction of Crude Enzyme from Jiben Seeds:

Extraction with water:

The best out of the 20 combinations in terms of clotting time, coagulation time and organoleptic properties (15g of *Solanum dubium* seeds and 8g NaCl) was selected. This was dipped in a 250ml flask containing 100ml distilled water for two weeks at room temperature. Clotting time and pH of the solution were recorded every two days to determine-the maximum activity of the enzyme according to the method described by Metwalli *et al.* (1982).

Extraction with buffer:

The 15g of *Solanum dubium* seeds were extracted in 100ml of 0.1 N citrate phosphate buffer (pH 3.6, 4.6, 5.6 and 6.6) containing 8g NaCl (w/v) extracts were stirred using continuous automatic shaker at 25°C with speed of 150 rotator per minute for three hours. The solutions were then centrifuged at 3000 rpm for five minutes and the precipitate removed, the solutions were then divided into 50ml portion in dark brown bottles and stored in the refrigerator (6 ±1°C). Clotting time was determined according to the method described by Metwalli *et al.* (1982).

Physicochemical Analyses of Milk:

The milk was mixed thoroughly before sampling and analysed for physicochemical properties (titratable acidity, pH, fat, total protein, total solids and specific gravity as suggested by O'Connor 1995).

Physicochemical Analyses of Cheese:

Cheese samples were analysed for physicochemical properties (titratable acidity, pH and yield) as described by O'Connor (1995). Cheese yield was expressed as percentage of the amount of milk used, was determined by weighing the product after pressing for 24 hours.

Organoleptic Properties of Cheese:

Organoleptic properties of cheese samples were determined using ten member trained test panel as described by Williams (1982) considering the

total score of 50 points, flavor 10 points, body and texture 10 points, colour 10 points, appearance 10 points and taste 10 points. Ascore below 4 was considered not acceptable.

Statistical Analysis:

The data were subjected to analysis of variance and significantly different means were separated using LSD.

RESULTS AND DISCUSSION

Table (1) shows that Jiben seed extracts extracted in water gave less milk clotting activity than extracts obtained with different concentrations of NaCl. The results indicate that 20g of Jiben seeds soaked in 100ml water containing 8g NaCl had more milk clotting activity than the other treatments .Cheese produced using 20% Jiben seeds with 8g NaCl had a bitter taste and lower sensory evaluation scores than cheese produced with 15% Jiben seed extracts containing 8% NaCl which was found to be the most suitable concentration for cheese production. Increasing Jiben seeds and NaCl concentrations increased the effectiveness of milk clotting enzyme and reduced coagulation time . The current results are in accordance with those of Yousif *et al.* (1996), who stated that using water during extraction was not as effective as using NaCl.

The result of pH and titratable acidity table(1) of cheese demonstrated a gradual increase in acidity accompanied by a gradual decrease in pH as the concentration of Jiben seed extracts and NaCl decreased. The development of acidity is caused by the production of lactic acid, free fatty acids and amino acids as a result of break down of carbohydrates, fats and proteins in the cheese .The results, of the present study agreed with Prasad and Alvarez (1999) who stated that higher salt concentration tends to produce cheese with a higher pH.

Table (I) show that the yield of cheeses made with 5 and 10% Jiben seed extracts, with different concentrations of NaCl was low (9%) compared to cheese made with 15 and 20% Jiben seed extracts (12%). These suggest that as the concentrations of Jiben seed extracts increased, the yield of the cheese also increased. The maximum yield of 12% was obtained with 15% and 20% Jiben. O'connor (1995) reported the cheese yield of 1Kg from 9 Kg of milk during the production of white pickled cheese.

Organoleptic assessment table (2) revealed that the cheeses were scored high in colour (5.04 to 7.0), texture (5.3 to 6.5), flavour (2.26 to 7.94), taste (4.14 to 6.34) and appearance (5.64 to 7.48). Cheese produced with high concentration (20%) of Jiben seeds was scored least in overall acceptance by the panelists ,because of the bitter taste and nutty flavour associated with it. The development of bitterness is not understood, but there is evidence that it may be due to unspecific proteolytic activity of enzymes obtained from the berries, it may be possible to reduce bitterness by using either a purified form of the extract or optimum extract concentration (Yousif *et al.* 1996).

Activity of crude enzyme as affected by varying the pH of 0.1N citrate phosphate buffer as extractant was shown in table (3).

Table 1 : Effect of Different Concentrations of Jiben Seed Extracts and Sodium Chloride on the Physicochemical Properties of Soft Cheese.

	Jiben seed extract concentrations (%)																				LS	SE	LSD
	5					10					15					20							
NaCl(g)	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8			
CT (sec)	60	40	33	30	27	55	38	31	25	20	47	34	26	20	14	43	25	20	14	10	ns	0.32	-
C (min)	184	157	138	120	99	132	127	105	82	65	128	120	97	77	60	84	67	60	52	41	***	0.534	3.376
TA	0.16	0.11	0.11	0.11	0.11	0.13	0.11	0.10	0.10	0.10	0.13	0.11	0.10	0.10	0.10	0.11	0.11	0.10	0.10	0.10	ns	0.018	-
pH	6.04	6.18	6.22	6.23	6.25	6.13	6.20	6.24	6.32	6.40	6.15	6.20	6.27	6.30	6.42	6.16	6.23	6.30	6.33	6.45	ns	0.017	-
CF	Jelly					Jelly					Firm					Firm							
Yield(%)	9					9					12					12					**	0.127	1.794

S = level of significance; ** = p < 0.01; *** = p < 0.001;
ns = not significant SD = least significant difference, CT = clotting time,
TA = titratable acidity as % lactic acid. E = standard error, CF = curd firmness

Table 2: Average Ascore of Organoleptic Properties of Soft Chesse Made with Different Concentrations of Jiben Seed Extracts.

Property (point scale)	Jiben extract concentrations (%)			
	5	10	15	20
Colour (10)	5.04	7.00	6.40	6.00
Body and texture (10)	5.30	6.36	6.50	5.96
Appearance (10)	5.64	6.42	7.48	5.68
Flavour (10)	5.90	6.24	7.94	2.26
Taste (10)	5.60	6.00	6.34	4.14
Total (50)	27.48	32.02	32.80	27.04
Percentage	54.96	64.04	65.60	54.08

The results demonstrated that the optimum pH for the extraction was 4.6 and 5.6. Increasing the pH over these ranges was found to decrease the enzyme activity. The optimum pH for the extraction was 4.6 .The trend was also observed by Husek *et al.* (1982), they reported that the highest yield and enzyme activities of rennet from abomasa were obtained using counter current extraction with 8% NaCl at pH 2 for two to five hours.

The clotting activity of 15g dry berries soaked in 100 ml distilled water containing 8% NaCl for 14 days is shown in table (4). Results indicated that as the soaking period progressed, the enzyme solubility increased up to 2 days, followed by slight decrease in clotting activity after 4 days. The optimum period for obtaining high milk clotting activity was 2days of soaking at room temperature.

Table 3: Effect of Extraction with O.N Citrate Phosphate Buffer at Different pH Levels on Milk Clotting Activity of Crude Enzyme for 3 Hours.

pH (buffer)	Clotting time (sec)	Clotting activity unit/ml
3.6	13	77
4.6	14	71
5.6	16	62
6.6	20	50

Table 4: Effect of Extraction with Water on Milk Clotting Activity of Crude Enzyme.

Days of extraction	pH	Clotting time (sec)	Clotting activity unit/ml
1	4.90	27	37
2	3.80	13	77
4	4.14	14	71
6	4.17	15	66
8	4.33	18	55
10	6.00	20	50
12	6.55	22	45
14	6.60	25	40

Conclusion

Based on the results of the study the following conclusions were made:

- Jiben seeds extracted in water had less milk clotting activity than extracts obtained with different concentrations of NaCl and increasing the NaCl concentration increased the solubility of milk clotting enzyme .

- Soaking 15g Jiben in 100ml water containing 8g NaCl was found to be the optimum for the extraction of enzymes for white pickled cheese production.
- An inclusion level of 3ml Jiben seed extracts per Kilogram milk at 40°C should be used for the production of white pickled cheese.

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تأثير استخدام تركيزات مختلفة من بذور الجبين على الصفات الفيزيوكيميائية والحسية للجبن الطرى

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تم دراسة الصفات الإنزيمية والتخثرية لمستخلص بذور نبات الجنين. فى هذه التجربة تم غمر ٥ ، ١٠ ، ١٥ ، ٢٠ جرام من البذور فى ١٠٠ مل من الماء المحتوى على تركيزات مختلفة ٠ ، ٢ ، ٤ ، ٦ ، ٨ جرام من ملح الطعام لمدة ٤٨ ساعة على حرارة الغرفة. وفى تجربة أخرى تم الاستخلاص أيضا بالمحلول المنظم سترات الفوسفات لمدة ٣ ساعات باستخدام Shaker.

دلت النتائج أن استخلاص البذور بالماء والملح يؤدى إلى زيادة النشاط الإنزيمى مقارنة بالاستخلاص بالماء فقط. وأدت زيادة تركيز البذور وملح الطعام إلى تقليل زمن التخثر ($P<0.001$) وقد أبرز كل من التقييم الحسى ودراسة الصفات الإنزيمية للبذور المستخلصة أن نقع ١٥ جرام من البذور فى ١٠٠ مل يحتوى على ٨ جرام ملح الطعام لمدة ٤٨ ساعة على حرارة الغرفة كان التركيز الأمثل لصناعة الجبن الطرى الذى بلغت كميته ١٢% ($P<0.01$) وأظهرت الدراسة أن النشاط الإنزيمى يقل كلما زادت فترة التخزين للمحلول ، وإذا يمكن استخدام بذور هذا النبات كبديل للمنفحة المستوردة.