PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 10 (2): 106-111, 2011
ISSN 1680-5194

Comparison of Quality of Sudanese White Cheese (*Gibna bayda*) Manufactured with *Solanum dubium* Fruit Extract and Rennet

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Abstract: This study was carried out to compare white cheese (*Gibna Bayda*) made with partially purified *Solanum dubium* extract and rennet chemically, microbiologically and organoleptically, in addition to evaluating weight loss. Three batches of white cheese were made in duplicate, in two of which partially purified *Solanum dubium* fruit extract was used as coagulant, while in the third batch rennet was used as a coagulant. Results indicated that type of coagulant had no significant effect on weight loss, titratable acidity, protein, salt and ash contents of white cheese. However, there was a significant effect on total solids, fat, soluble protein, tyrosine and tryptophan contents. The interaction between the storage period and type of coagulant did not significantly affect all chemical components of cheese except tyrosine. Storage period had a significant effect on titratable acidity, total solids, protein and ash contents of whey. Total viable bacteria, streptococci and lactobacilli counts (cfu/gm) were significantly affected by storage period, and as the storage period increased their count decreased. No significant differences were observed in sensory characteristics in terms of colour, flavour, texture and saltiness between the two cheeses.

Key words: *Solanum dobium* extract, rennet, white cheese, quality

INTRODUCTION

Cheese manufacture is essentially a dehydration process in which the fat and casein of milk are concentrated 6-10-fold, depending on cheese variety. With the exception of some minor varieties, dehydration is traditionally achieved by coagulating the casein enzymatically, isoelectrically, or by a combination of heat and acid, the fat is occluded in the coagulum, if present. Ultrafiltration offers the possibility of concentrating the total colloidal phase of milk, but the retentate is usually renneted or acidified to provide a characteristic texture. Most, if not all, ripened cheeses are produced by rennet coagulation (Fox, 1989).

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 from plants (Ahmed *et al*., 2009). 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).

Ahmed *et al*. (2009) reported that dairy farmers in some part of the Sudan use the berries of *Solanum dubium* to make white soft cheese using goat and sheep milk. Milk coagulation takes about 2 h and the curd is pressed to remove whey. The cheese obtained has a slight bitter taste and a fragile crumbly texture. It may be possible to reduce bitterness by using a purified enzyme and optimum extract concentration (Yousif *et al*. 1996). Gubbain (*Solanum dubium*) seed extract 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 this work we would like to study the effect of using partial purified *Solanum dubium* fruit extract on weight loss, chemical composition, microbiological properties and sensory characteristics of Sudanese white cheese (*Gibna Bayda*) and compare with that made with animal rennet.

MATERIALS AND METHODS

***Solanum dubium* fruit extract preparation:** Completely dry (yellow) *Solanum dubium* fruits were collected from Abu-Naama area, Sinnar State, Sudan, carefully cleaned and powdered using laboratory mortar. Five grams were soaked in a conical flask at 5°C for 24 h using distilled water (30 ml) with occasional shaking for the first 3 h and solutions were then filtered through filter paper. The crude extract of *Solanum dubium* fruit was precipitated by ammonium sulphate (60%) at 5°C and the precipitate was recovered by centrifugation using a refrigerated centrifuge (5°C) at 3000 rpm. The supernatant was

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discarded and the sediment from each salt concentration was re-suspended in buffer solution (pH 10) and dialyzed against distilled water for 24 h during which the distilled water was changed six times.

Cheese making: Three batches of white cheese (*Gibna Bayda*) were made; in each batch two cheeses were produced. The milk was strained by a clean cloth, heated to 62°C for 30 min and cooled to 40°C. The salt (8%) was dissolved in a small quantity of milk and added to milk. Rennet tablets (1 tablet/50 L milk) were added to the first batch, while to the second and third batches, partially purified *Solanum dubium* extract was added (25 ml/50 L milk) and the milk was stirred for 10 min and left undisturbed for curd formation. The curd from each batch was cut and poured into a wooden mould lined with a clean cloth and pressed by weight (5 kg) overnight. Next day, the curd was removed from the mould and cut into small cubes (2 x 2 x 2 cm). The whey of each batch was collected in a separate container, boiled for five minutes, cooled and used for preservation of the particular cheese. All batches were stored at 35-37°C for 90 days, and cheese from each batch was analyzed at day 1, 15, 30, 45, 60, 75 and 90 for chemical composition, microbiological quality and sensory characteristics, while the whey was only analyzed for chemical composition.

Chemical composition: Titratable acidity, total solids, protein and ash contents of the milk, cheese and whey were determined according to the method described by AOAC (1990), while fat content was determined according to Foley *et al.* (1974). Soluble protein content was determined according to Ling (1963). Salt content was determined by titration according to Breene and Price (1961). Tyrosine and tryptophan contents were determined according to method described by Vakaleris and Price (1958).

Microbiological examination: Total viable bacterial count was determined according to Houghtby *et al.* (1992) using standard plate count agar and the plates were incubated at 32°C for 48 h. M17 agar medium (Merck-15108) was used to determine streptococci count according to Oksuztepe *et al.* (2005). Total lactobacilli were determined according to Frank *et al.* (1992) using MRS agar medium.

Sensory characteristics: A panel of 10 untrained panelists were chosen to judge on the quality of cheese (colour, flavour, texture and saltiness) using a sensory evaluation sheet.

Statistical analyses: Statistical analyses were carried out using SPSS program (ver. 12) using Completely Randomized Design and General linear models. Duncan's multiple range test was used for mean separation at $p \leq 0.05$.

RESULTS AND DISCUSSION

The average chemical composition of milk used for cheese making in this study was as follows: titratable acidity 0.20%, total solids 12.12%, protein 3.26%, fat 3.85% and ash 0.73%.

Table 1: Effect of coagulant type on weight loss and chemical composition of white cheese

Composition	Rennet	*Solanum*	SE	LS
Weight loss (%)	17.70[a]	19.78[a]	0.74	NS
Acidity (%)	0.95[a]	0.96[a]	0.04	NS
Total solids (%)	49.64[b]	52.09[a]	0.54	**
Fat (%)	24.80[b]	27.12[a]	0.53	**
Protein (%)	17.49[a]	17.50[a]	0.32	NS
Soluble protein (%)	0.61[b]	0.80[a]	0.05	**
Salt (%)	4.38[a]	4.45[a]	0.09	NS
Ash (%)	3.07[a]	3.12[a]	0.17	NS
Tyrosine[1]	1.34[b]	1.62[a]	0.05	***
Tryptophan[1]	0.38[b]	0.47[a]	0.02	***

SE = Standard Error; LS = Level of Significance; ** = $p<0.01$; *** = $p<0.001$; NS = Not Significant. [1]mg/100 kg cheese

Effect of coagulant type on weight loss and chemical composition: Data in Table 1 show that cheese weight loss was not significantly ($p>0.05$) affected by type of coagulant, although higher weight loss was found in cheese made by *Solanum dubium* extract (19.78%). This may be due to high proteolytic activity of the *Solanum dubium* extract. Table 1 shows the effect of coagulant type on chemical composition of white cheese. Statistical analysis revealed that type of coagulant had no significant effect ($p>0.05$) on titratable acidity of cheese. However, a slight increase in titratable acidity was observed in cheese produced by *Solanum dubium* extract (0.96%) compared to cheese produced with rennet. Our results are similar to Abu-Zeid (1994) who found higher acidity value in cheese made with vegetable rennet from *Sonchus olerceus* L., which was explained that the longer coagulation time of vegetable rennet possibly favored microbial growth and consequently, a higher acidity was reached in curd from vegetable rennet. However, these results are not similar to Nunez *et al.* (1991) who reported that high whey retention in cheese with animal rennet was obtained which allowed more lactose to be available for microbial fermentation, with a more pronounced increase in acidity than in vegetable rennet cheese. Total solids and fat contents were significantly ($p<0.01$) higher in cheese made with *Solanum dubium* extract compared to the cheese made with rennet. Our findings agreed with Talib *et al.* (2009) who found that the moisture content of cheeses made with Jiben (*Solanum dubium*) seeds

extract are less than that made with rennet extract, Nunez *et al*. (1991) and Abu-Zeid (1994) who reported that total solids and fat contents were higher in cheese from vegetable rennet compared to that from animal rennet. Mohamed and O'Conner (1996) found values of 40-44.32% for fat in cheese made by *Calotropis procera* as a coagulant. Coagulant type had no significant effect on protein content, with the difference in protein content of cheese made with two coagulants being small. These findings are in accord with Talib *et al*. (2009). Soluble protein content of cheese was significantly ($p<0.01$) affected by type of coagulant. Cheese made with *Solanum dubium* extract showed the highest soluble protein content (0.80%) compared to that made with rennet and this result is in line with Nunez *et al*. (1991) who reported 14% protein content for vegetable rennet cheese and 13.3% for animal rennet cheese and Fernandez-Salguero and Sanjuan (1999) who reported that the high levels of soluble nitrogen was found in cheese produced using vegetable rennet. The salt content of cheese was not significantly affected by type of coagulant. However, cheese made with *Solanum dubium* extract secured the highest salt content (3.72%), while that made with rennet had the lowest (3.62%). This result agreed with Nunez *et al*. (1991) who reported that type of coagulant had no significant effect on salt content of cheese. Coagulant type did not significantly affect the ash content, although cheese made with *Solanum dubium* extract was slightly higher. Mohamed and O'Conner (1996) reported 1.97-2.59% ash for white soft cheese made with *Calotropis procera* juices. Tyrosine and tryptophan contents were significantly ($p<0.001$) higher in cheese made with *Solanum dubium* extract (1.62 and 0.47) than cheese made with rennet (1.34 and 0.38). These results are in agreement with Abu-Zeid (1994) and Fernandez-Salguero and Sanjuan (1999).

Effect of coagulant type and storage period on weight loss and chemical composition of white cheese: Results in Table 2 show no significant differences ($p>0.05$) in weight loss, titratable acidity, total solids, fat, protein, soluble protein, salt, ash and tryptophan contents as affected by storage period and coagulant type. The weight loss gradually increased with progress in storage period, the maximum weight loss being reached at the end of storage for both rennet and *Solanum dubium* extract cheeses. The titratable acidity increased to a maximum at day 60 (1.28% and 1.35%) then gradually decreased to 0.92% and 0.99% in cheese made with rennet and *Solanum dubium* extract respectively. The total solids (54.94%) and fat (29.42%) contents were higher in cheese made with *Solanum dubium* extract at days 30 and 90 respectively. The lower total solids and fat contents were 44.35% and 19.83% in cheese made with animal rennet at day 1. Similar results were found by Sousa and Malcata (1997) who reported that type of rennet (aqueous extract of flowers of *Cynara cardunculus*) had no significant effect on total solids and fat contents of the cheese over the ripening period. However, our findings disagreed with El-Shibiny *et al*. (1973) for Domiati cheese made with enzyme preparation from fig latex. Alalade and Adeneye (2006)

Table 2: Effect of interaction between storage period and type of coagulant on weight loss and chemical composition of white cheese

Storage period	Weight loss (%)		Acidity (%)		Total solids (%)		Fat (%)		Protein (%)	
(days)	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*
1	0.00	0.00	0.56	0.57	44.35	48.44	19.83	24.67	15.88	15.19
15	7.59	8.85	0.89	0.79	49.40	51.25	22.83	24.50	19.55	18.47
30	11.21	15.23	0.87	0.89	50.28	54.94	25.17	28.33	17.37	18.57
45	15.47	19.44	1.08	0.97	52.40	52.26	25.83	27.50	18.54	17.17
60	21.48	22.78	1.28	1.35	51.25	52.75	25.17	27.58	15.58	16.37
75	23.56	23.25	1.05	1.13	49.04	51.33	27.00	27.83	17.86	18.85
90	26.53	29.13	0.92	0.99	50.74	53.66	27.75	29.42	17.70	17.86
SE	1.82		0.10		1.44		1.40		0.84	
LS	NS		NS		NS		NS		NS	
Storage period	Soluble protein (%)		Salt (%)		Ash (%)		Tyrosine[1]		Tryptophan[1]	
(days)	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*
1	0.54	0.88	4.77	4.50	3.58	4.26	1.38	1.63	0.42	0.47
15	0.49	0.63	4.22	4.53	2.93	3.64	1.66	2.09	0.45	0.57
30	0.67	0.75	4.33	4.48	3.01	3.31	1.27	1.84	0.40	0.51
45	0.93	1.04	4.40	4.43	3.48	3.41	1.71	1.50	0.44	0.46
60	0.60	0.91	4.36	4.48	2.74	2.28	1.09	1.45	0.34	0.44
75	0.86	0.71	4.26	4.38	2.41	2.31	1.08	1.57	0.29	0.44
90	0.37	0.68	4.35	4.31	3.32	2.62	1.21	1.21	0.34	0.38
SE	0.12		0.25		0.44		0.12		0.04	
LS	NS		NS		NS		*		NS	

SE = Standard Error; LS = Level of Significance for interaction; * = $p<0.05$; NS = Not Significant; [1]mg/100 kg cheese

found that the total solids content of Wara cheese was significantly affected by storage period. The highest protein content was at day 15 for cheese made with rennet and at day 75 for cheese made with *Solanum dubium* extract and lowest content was at day 1 in cheese made with *Solanum dubium* extract and at day 60 in cheese made with rennet. These results agree with those of Talib *et al*. (2009) who reported that the decrease in protein content of soft white cheese manufactured using *Solanum dubium* seeds extract during pickling as a result of protein degradation leading to the formation of water soluble compounds, some of which lost in the pickling solution leading to increase of nitrogen content in whey. Sousa and Malcata (1997) reported that aqueous extract of flowers of *Cynara cardunculus* had no significant effect on protein content of cheese over the ripening period. Slight decrease in protein content was reported by Krishnaswamy *et al*. (1961) in cheddar cheese made with vegetable rennet from *Ficus carica* during ripening. The highest soluble protein content was in cheese made with *Solanum dubium* extract at day 45, compared to lowest soluble protein in rennet cheese at day 90. Similar results were reported by Fernandez-Salguero and Sanjuan (1999) who reported that soluble nitrogen of cheese made with thistles of the genus *Cynara* at 60, 80 and 100 days of ripening was greater than that in cheese produced using animal rennet during ripening period of 100 days. El-Shibiny *et al*. (1973) reported that during storage the soluble nitrogen content of cheese made with the enzyme preparation from fig latex gradually increased reaching a value of 0.57%. The increase in soluble nitrogen content during pickling may be due to the proteolytic action of microbial proteinases on the curd. The salt content of cheese made with rennet and *Solanum dubium* extract started high at the beginning of storage period, then fluctuated decreasing towards the end. The results in this study are in line with the findings of Sousa and Malacta (1997) and Nunez *et al*. (1991). The ash content gradually decreased to the lowest at day 75 in both cheeses, before increasing at the end of storage. Krishnaswamy *et al*. (1961) found that ash content was 3.39% and 3.47% at third and sixth months of storage for vegetable rennet cheese and 3.46% and 3.53% at third and sixth months of storage for animal rennet cheese. The tyrosine content (mg/100 g cheese) was significantly ($p<0.05$) affected by type of coagulant during storage period. However, there is no significant effect on tryptophan content. The highest tyrosine and tryptophan values were 1.84 and 0.57 at day 30 and 15 respectively in cheese made by *Solanum dubium* extract, while the lowest tyrosine and tryptophan contents were 1.08 and 0.34 in rennet cheese. Our findings are in disagreement with those of Chen and Zall (1986), Abu-Zeid (1994) and Fernandez-Salguero and Sanjuan (1999) who reported that the levels of soluble amino acids gradually increased with ripening. The lower tryptophan values compared to tyrosine values throughout ripening may be as a result, not only of the lower original casein content, but also of the fact that this amino acid is more readily hydrolyzed by microorganisms. Higher contents in both soluble amino acids were observed in cheese made with vegetable rennet.

Table 3: Effect of coagulant type on chemical composition of whey

Composition	Rennet	*Solanum*	SE	LS
Acidity (%)	1.26	1.22	0.04	NS
Total solids (%)	14.03	14.68	0.14	**
Fat (%)	0.32	0.36	0.04	NS
Protein (%)	1.83	1.66	0.10	NS
Salt (%)	7.59	8.16	0.09	**
Ash (%)	6.32	6.91	0.17	*

SE = Standard Error; LS = Level of Significance; * = $p<0.05$; ** = $p<0.01$; NS = Not Significant

Effect of coagulant type on chemical composition of whey: Results in Table 3 show the effect of coagulant type on chemical composition of whey from white cheese. The titratable acidity was not significantly affected by coagulant type, although whey from rennet cheese was slightly acidic than that from *Solanum dubium* extract. Total solids content of whey from cheese made by *Solanum dubium* extract was significantly ($p<0.01$) higher than that made by rennet. The results in this study are in agreement with Barbosa *et al*. (1981) and Nunez *et al*. (1991). The increase in total solids of whey from milk coagulated with vegetable coagulant may be attributed to strong proteolytic activity of the coagulant leading to breakdown of the casein network resulting in a higher total solids loss in whey. Fat content was not significantly ($p>0.05$) affected by coagulant type, however, a slight increase (0.36%) was observed in fat content of whey from cheese made with *Solanum* coagulant. This finding disagreed with Nunez *et al*. (1991) and Puhan and Irvine (1973). Protein content of whey was not significantly ($p>0.05$) affected by type of coagulant. The protein content was 1.83% and 1.66% in whey from cheese made with rennet and *Solanum*, respectively. This result is in disagreement with Vieira and Barbosa (1972), Puhan and Irvine (1973) and Nunez *et al*. (1991) who reported that higher levels of total N in whey from milk coagulated with vegetable rennet may be attributed mainly to the storage proteolytic activity of the coagulant with the formation of soluble N which was released into whey. The salt ($p<0.01$) and ash ($p<0.05$) contents were significantly affected by type of coagulant, being higher in whey from cheese made by *Solanum dubium* extract.

Table 4: Effect of coagulant type and storage period on microbiological quality of white cheese

Storage period (days)	TVBC (cfu/gm)		Streptococci count (cfu/gm)		Lactobacilli count (cfu/gm)	
	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*
1	4.60×10^7	2.40×10^7	1.30×10^7	8.10×10^7	1.90×10^7	9.25×10^6
15	8.87×10^6	4.42×10^6	2.00×10^7	4.15×10^6	1.70×10^7	4.58×10^6
30	6.72×10^6	4.87×10^6	6.05×10^6	4.67×10^6	7.95×10^6	5.32×10^6
45	1.60×10^6	3.48×10^5	1.10×10^6	3.44×10^5	2.25×10^6	4.03×10^5
60	4.58×10^6	1.57×10^6	3.40×10^6	1.04×10^6	2.21×10^6	1.69×10^6
75	1.54×10^5	2.55×10^4	9.02×10^4	1.16×10^5	1.40×10^5	3.09×10^4
90	3.00×10^4	1.36×10^4	2.91×10^4	8.85×10^3	3.96×10^4	6.22×10^3
SE	4.39×10^6		1.20×10^7		2.29×10^6	
LS	NS		*		NS	

SE = Standard Error; LS = Level of Significance for interaction; * = $p<0.05$; NS = Not Significant

Effect of coagulant type on microbiological quality of white cheese during storage: Results in Table 4 show the effect of coagulant type on microbiological quality of cheese during storage. TVBC was not significantly ($p>0.05$) affected by type of coagulant. The result was not similar to Nour El Diam and El Zubeir (2006). However, cheese made by rennet had higher TVBC (4.60×10^7) in comparison with that made using *Solanum dubium* extract as coagulant (2.40×10^7) at day 1. The lowest TVBC (1.36×10^4) was in cheese made using *Solanum* as a coagulant at day 90. Streptococci count was significantly ($p<0.05$) affected by coagulant type (Table 4). Cheese samples made using *Solanum* coagulant had higher streptococci count (8.10×10^7) compared to those made with rennet (1.30×10^7) at day 1. As storage period progressed the streptococci count decreased. Coagulant type did not significantly ($p>0.05$) affect lactobacilli count in cheese samples (Table 4). The result show that as storage period increased lactobacilli count decreased. The lactobacilli count was 1.9×10^7 and 9.25×10^6 at day 1 in cheese made with rennet and *Solanum* coagulant, respectively. The lowest count was 6.22×10^3 and 3.09×10^4 at day 75 and 90, respectively in cheese made with *Solanum* extract. Similar results were found by Sousa and Malcata (1997) who reported that lower lactobacilli count was obtained for cheese manufactured with plant rennet until 28 days of the ripening.

Effect of coagulant type on sensory characteristics of white cheese: Results in Table 5 present the effect of coagulant type on sensory characteristics of cheese. The coagulant type did not significantly ($p>0.05$) affect the quality of cheese in terms of colour, flavor, texture and saltiness. However, cheese made with *Solanum dubium* extract scored higher in colour compared to cheese made with rennet. Our findings are similar to those of Prados *et al.* (2007) who reported that the colour of cheese made with a powdered vegetable coagulant from cardoon *Cynara carnculus* scored higher compared to cheese with animal rennet. The flavour of cheese made with rennet was better than that made with *Solanum dubium* extract. These results are in disagreement with Nunez *et al.* (1991) who reported that the use of vegetable rennet resulted in a cheese with a more pleasant and pronounced flavour and the effect of coagulant type on flavour quality and intensity being highly significant ($p<0.001$) and Roserio *et al.* (2003) who reported that plant proteases are considered too proteolytic, leading to bitter flavour. The coagulant type significantly ($p<0.001$) affected the texture, with the highest score being in cheese made with rennet. This result was not similar to Chen *et al.* (2003) and Roserio *et al.* (2003) who reported that plant proteases lead to texture defect in cheese according to proteolytic activity. Cheese made with *Solanum dubium* extract had slightly lower saltiness score (5.63) than that made with rennet (5.75).

Table 5: Effect of coagulant type on sensory characteristics of white cheese

Item	Rennet	*Solanum*	SE	LS
Colour	5.97[a]	6.11[a]	0.11	NS
Flavour	5.95[a]	5.82[a]	0.16	NS
Texture	5.47[a]	5.56[a]	0.15	NS
Saltiness	5.75[a]	5.63[a]	0.15	NS

SE = Standard Error; LS = Level of Significance; NS = Not Significant

Conclusion: White cheese from *Solanum dubium* extract showed better nutritional value than that from rennet and the chemical components increased during storage period. Cheese from *Solanum dubium* extract did not differ in sensory characteristics from that made by rennet.

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