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MILK CLOTTING ENZYME BY USING *Solanum dobium* PLANT AS RENNET SUBSTITUTE:
II – QUALITY AND RIPENING CHARACTERISTICS OF GOUDA CHEESE AS AFFECTED BY REPLACING CALF RENNET WITH AQUEOUS EXTRACT OF SOLANUM DOBIUM SEEDS

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ABSTRACT

Effects of replacing calf rennet (C.R.) with crude aqueous extract of *Solanum dobium* seeds (S.D.E.) at levels 0,25,50,75 and100% on yield, chemical composition, proteolysis and sensory quality of Gouda cheese were evaluated during ripening period (30,60 and 90 days) . Replacing C.R. with S.D.E. significantly increased the fat and protein loss in the whey and cheese weight loss than that of co ıtrol. This was associated with the increase in the replacing level. Cheese made with S D.E. showed higher levels of moisture, salt in moisture and acidity than that of control cheese during ripening .The former cheese showed also lower pH , fat/DM . But TN/DM decrease did not significantly affected. Proteolysis was found to be more marked rapid in cheese containing higher replacing rate of C.R. with S.D.E. than control cheese. Also, free amino acids significantly ($P \leq 0.05$) increased with the increase in the replacing rate of C.R. with S.D.E. compared with control cheese. However, control cheese showed higher levels of total volatile fatty acids (T.V.F.A.) than experimental cheeses. Increasing replacing rate more than 50% of C.R. with S.D.E. the resultant cheeses were more pronounced pasty texture as well as some flavour defects (especially bitterness) compared with control cheese. No flavour defects were observed in S.D.E. cheese with replacing level less than 50%. So, it could be concluded that an acceptable quality Gouda cheese could be obtained when C.R. was replaced by S.D.E. up to 50%.

INTRODUCTION

Rennet produced from calf stomach has been traditionally used since ancient time as a coagulant in most cheese manufacturing. However increasing demand for cheeses and shortage of calf rennet have lead to increasing its price and search for alternative milk coagulants. Milk clotting enzymes suitable for cheese making must have a high specific cleavage capacity toward K-casein at the normal pH of milk. Calf rennet, which contains chymosin as the main enzyme component, has been the most widely used as a milk clotting enzyme having this specification.

Coagulation of milk can be achieved by a number of proteolytic enzymes from various sources , such as different animal species (e.g. pig, bovin and chicken pepsins), microbial proteinases and vegetable rennet extracted from fruits (e.g. pineapple, papaya and Sodom apple) and plants such as wild cardoon (Vioque *et al.,* 2000) . When a potential rennet substitute is

studied, it is of chief importance to adequateiy evaluate ihe degradation patterns of the casein because of their effects on yield, consistency and flavour of the final cheese (Fox , 1989) . *Solanum dobium* is a noxious weed belonging to the plant family solanaceae that grows in vast areas of Sudan. It is a perennial plant that flowers during the rainy season (starting in June remaining – August) and usually bears fruits about January with green fruits which become yellow at fully ripe. Dairy farmers in some parts of Sudan use the berries of *Solanum dobium* to make a type of white soft cheese (Yousif *et al.,* 1996). Some Spanish and protuguese varieties of raw ewes' and goats' milk cheeses are made with aqueous extracts of dried wild thistle flowers from various species of genius Cynara L. (e.g. *C. cardunculus* or *C. humilis*) as a coagulants (Fernandez del Pozo *et al.,* 1988b; Nunez *et al.,* 1991; Macedo *et al.,* 1993; Sousa and Malcata , 1997a; Carmona *et al.,* 1999; Fernandez – Salguero and Sanjuan,1999; Roa *et al.,* 1999; Vioque *et al.,* 2000 and Tavaria *et al.,* 1997 and 2001).

However, no information about hard or semi hard cheese manufactured by aqueous extract of *Solanum dobium* seeds. Therefore, characterization for cheese produced with *Solanum dobium* extract (S.D.E.) must be known in order to design the cheese making process .

Extracting a milk clotting enzyme from *Solanum dobium* have been tried by Abd El – Galeel and El – Zawahry , (2004).Effect of some factors being pH , setting temperature, Ca cl_2 concentration , Na cl concentration and enzyme concentration on the clotting activity of the enzyme extract have been also studied. Attention has been also directed toward the proteolytic activity of the resultant extract in compare with calf rennet. The optimum conditions for obtaining a suitable curd using this extracted was concluded to be used in the manufacture of cheeses .

Therefore , the present work was carried out to evaluate the of effect replacing calf rennet with aqueous extract of Solarium dobium seeds (S.D.E.) on yield ,chemical composition, proteolysis and sensory characteristics of Gouda cheese during ripening period .

MATERIALS AND METHODS

Calf rennet :

Calf rennet (Habo) powder was obtained from Chr. Hansen A/s Denmark. It is dissolved in distilled water at a concentration of 62.5 mg % (w / v) ,calf rennet served as control (Vieira de Sa and Barbosa (1972) .

Vegetable rennet :

The seeds of *Solanum dobium* were obtained from Sudan Republic. The seeds extraction was carried out by using the method of Singh *et al.* (1973) and it is adjusted to give the same clotting time as that of the calf rennet with milk at 40°c according to the method described by Vieira de Sa and Barbosa (1972).

Milk :

Fresh bulk cows' milk (4% fat) was obtained from special Dairy Products Unit at Food Science Department ,Faculty of Agric. Zagazig University .

Salt :

Clean, food grad, salt (Nacl) was used.

Calcium chloride :

Pure food grade calcium chloride used in cheese making, it was purchased from El – Gomhoria Co. Cairo Egypt .

Coating materials :

A wax mixture composed of white soft paraffin wax, pellet honey and medical vaslin at a ratio of 1 : 1 : 0.2 respectively were used, it was purchased from El – Gomhoria Co. Cairo Egypt .

Cheese manufacture :-

The procedure of Gouda cheese manufacture as described by Scott, (1981) was adapted. The milk was heated at 72°C/١٥sec., then cooled at 35°C and calcium chloride was added at the rate of 0.02%. Mesophilic starter (Chr. Hansen, Copenhagen, Denmark) of *Lactococcus lactis* Sub sp. *Cremoris* and *Lactococcus lactis* Sub sp. *diacetylactis* was added at the rate of 1% and thoroughly mixed with the milk. Then milk was divided into 5 portions and left at 35°C for one hour. When acidity reached 0.19 %, Anato (Chr. Hansen, Copenhagen, Denmark) was added at the rat of 1 ml / 20 kg milk, then calf. rennet and *Solanum dobium* extract (S.D.E.) were added as follows; the first portion was renneted using only calf rennet and it was served as a control. The other four portions were renneted by replacing calf rennet with S.D.E. at the rate of 25, 50, 75, and 100% for the second, th[rd, fourth and fifth portions respectively. All portions were left for complete coagulation at 30°C. Resultant curds were cutted into large cubes (2 x 2 x 2 cm).The cheese making process was completed as described by Scott, (1981). Completing the processing Resaltant cheese was then immersed in a 20 % brine solution for 24 hours at 10 °C for salting. After salting, the green cheese was weighted and placed for 2 days in ripening room for surface drying. The cheese was then carefully coated with wax. Resultant cheeses were then kept in the ripening room at 15 °C, and 85-90 % relative humidity for 90 days.

Cheese analysis :

Cheese yield and recovery of milk fat and proteins in the resultant cheese were estimated . The cheese samples were taken when fresh and at the age of 30, 60 and 90 days of ripening. Samples were ground and then stored in the deep freezer at (-18 °C) until chemical analysis . Resultant cheese were analyzed for moisture, pH, titratable acidity, fat and salt. Total nitrogen, soluble nitrogen and non-protein nitrogen as described by Ling (1963) . Amino acids nitrogen was estimated as given by Stadhouders (1959) .

Free amino acids :

Free amino acids (F.A.A.) were determined in cheese samples according to Mondino *et al.* (1972), using a high performance amino acid analyzer (Eppendorf – Germany LC 3000 Amino acid analyzer). Conditions : Flow rate : 0.2 ml / min. Pressure of buffer 0 to 50 bar. Pressure of reagent 0 to 150 bar. Reaction temperature 123 °C.

Total volatile fatty acids (T.V.F.A) :

Total volatile fatty acids were determined by the method described by Kosikowski (1978).

Sensory evaluation :

Cheeses were sensory evaluated for appearance, body & texture and flavour by trained panel of 10 judges as described by Fernandez del Pozo *et al.* (1988 b), results were expressed as a mean score for the whole panel on each cheese.

Statistical analysis :-

Factorial design 2 factors 3x replicates and the completed randomized design was used to analyze . All data and Newman Keuls test was followed to make the multiple comparison (Steel and Torrie, 1980) using Co stat program significant differences were determined at $P \leq 0.05$.

RESULTS AND DISCUSSION

Data presented in Table (1 and 2) showed the effect of replacing calf rennet with S.D.E. at different levels on yield, loss of weight and retention of both fat and total protein in the curd. Cheese yield significantly ($P \leq 0.05$) decreased in case of replacing calf rennet with SDE. particularly at the high replacing rates. During ripening cheese made with calf rennet and that made with replacing rate of 50 % S.D.E. showed relatively similar yield percentage and was higher than all experimental cheeses. On the other hand, highest weight loss was noticed by increasing the replacing rate of calf rennet with S.D.E. for all experimental cheeses .Higher yield for cheese made with animal rennet than that made with vegetable rennet was reported by Vieira de sa and Barbosa, (1972). The general trend of results are in agreement with that reported by Puhan and Irvine (1973) who reported that higher solids, protein and fat contents were found in whey from Cheddar cheese made with *Bacillus subtilis* proteinases as coagulant enzymes. Also Vieira de Sa and Barbosa (1972) showed that the whey from curds made by cardo extracts was more turbid, presumably owing to greter protein breakdown, that reduced the yield. As shown in Table (2) loss of fat and protein in whey was significantly ($P \leq 0.05$) higher for cheese produced by replacing rate of 100 % compared with all treatments. In contrast, lower levels of fat and protein retention in these curds. In control cheese fat and protein which lost in whey could be attributed to curd cutting to small grain size and subsequent mechanical work during cheese making which resulted in cloudy or turbid whey, with considerable content of fine curd , independently of the type of coagulant used Nunez *et al.* (1991). High levels of total nitrogen in whey from milk coagulated with vegetable rennet may ascribed mainly to the strong proteclytic activity of S.D.E. (Abd El-Galeel and El-Zawahry, (2004), with information of soluble nitrogen which was released into whey, this breakdown of the casein network resulted in heavy fat and total solids loss in whey from milk coagulated with vegetable rennet Nunez *et al.* (1991) .

Table (1): Effect of replacing rate of calf rennet with *Solanum dobium* seeds extract on Yield and weight loss of Gouda cheese.

Properties	Ripening period (days)	Control	Replacing rate of calf rennet by S.D.E.				L.S.D.	Signi.
			25 %	50 %	75 %	100%		
Yield %	0	13.95^{b}	13.65^{c}	14.37^{a}	13.84^{bc}	12.95^{d}	0.229	***
	30	12.74^{b}	12.43^{c}	13.09^{a}	12.21^{d}	11.62^{e}	0.133	***
	60	12.54^{b}	12.22^{c}	12.86^{a}	12.00^{d}	11.41^{e}	0.137	***
	90	12.43^{b}	12.11^{c}	12.69^{a}	11.87^{d}	11.32^{e}	0.133	***
Weight loss %	30	8.67^{d}	8.94^{c}	8.91^{c}	9.42^{b}	10.27^{a}	0.071	***
	60	10.11^{d}	10.48^{c}	10.51^{c}	10.98^{b}	11.89^{a}	0.074	***
	90	10.90^{e}	11.28^{d}	11.69^{c}	11.94^{b}	12.59^{a}	0.085	***

Table (2):Effect of replacing rate of calf rennet with *Solanum dobium* seeds extract on the retention of the fat and protein in the curd and loss of both in the whey of Gouda cheese :

Properties		Control	Replacing rate of calf rennet by S.D.E.				L.S.D.	Signi.
			25 %	50 %	75 %	100%		
Fat	Loss in the whey %	5.16^{e}	5.24^{d}	5.51^{c}	6.38^{b}	9.24^{a}	0.090	***
	Retention in the curd %	94.84^{a}	94.76^{a}	94.49^{b}	93.62^{c}	90.76^{e}	0.090	***
Protein	Loss in the whey %	15.41^{e}	16.38^{d}	17.15^{c}	17.85^{b}	18.14^{a}	0.094	***
	Retention in the curd %	84.59^{a}	83.62^{b}	82.85^{c}	82.15^{d}	81.86^{e}	0.094	***

a,b,c,d and e: Means having different letters in the same raw significantly differed at $p \leq 0.05$.

S.D.E.: Aqueous extract of *Solanum dobium* seeds.

Signi. : Significant * ** : High Significant * : Very high Significant**

Compositional characteristics :

Table (3) shows the change in acidity (as % of lactic acid) and pH values of Gouda cheese made from cows' milk coagulated with calf rennet (control) or with replacing calf rennet with *Solanum dobium* extract at different levels . It could be noticed that acidity of all cheeses were significantly ($P \leq 0.05$) increased throughout the ripening period . However , pH values of all cheese significantly decreased during the same period of ripening.This may be attributed to lactose fermentation in the initial period of ripening. These results are in agreement with those reported by Zakai and Salem, (1992). The highest contents of acidity Vs pH values were found to be in cheese made using 100% replacing rate. All experimental cheeses showed higher acidity and lower pH values than control cheese . Similar result were obtained by Roa *et al.* (1999) who, found that pH values of La Serena cheese manufactured from ewes' milk with vegetable rennet decreased gradually till the end of ripening period. Similar results at the same ripening time (90 days) was reported by Vioque *et al.* (2000). The general trend of these results are in agreement with that reported by Fernandez del Pozo *et al.* (1988 a & b); Poulet *et al.* (1991) and Sousa & Malcata, (1997a).

Table (3):Effect of replacing rate of calf rennet with *Solanum dobium* seeds extract on acidity % and pH of Gouda cheese :

Properties	Ripening period (days)	Control	Replacing rate of calf rennet by S.D.E.				L.S.D.	Signi.
			25 %	50 %	75 %	100%		
Acidity %	0	0.89^{b}	0.90^{ab}	0.92^{ab}	0.92^{ab}	0.94^{a}	0.030	*
	30	1.14^{b}	1.15^{b}	1.18^{a}	1.19^{a}	1.21^{a}	0.026	***
	60	1.23^{c}	1.24^{bc}	1.27^{abc}	1.28^{ab}	1.30^{a}	0.033	**
	90	1.33^{c}	1.33^{c}	1.36^{bc}	1.38^{b}	1.41^{a}	0.027	***
pH	0	4.52^{a}	4.49^{ab}	4.47^{bc}	4.46^{bc}	4.43^{c}	0.036	**
	30	4.31^{a}	4.29^{ab}	4.28^{ab}	4.26^{ab}	4.25^{b}	0.037	*
	60	4.23^{a}	4.20^{ab}	4.17^{abc}	4.15^{bc}	4.11^{c}	0.054	**
	90	4.09^{a}	4.08^{a}	4.05^{ab}	4.03^{b}	4.01^{b}	0.037	**

a,b,c,d and e : Means having different letters in the same raw significantly differed at p ≤ 0.05.
S.D.E.: Aqueous extract of *Solanum dobium* seeds.
Signi. : Significant * ** : High Significant * : Very high Significant**

Table (4) shows the average moisture content, fat / D.M., salt, in moisture and T.N. / D.M. during ripening of Gouda cheese made from cows' milk as affected by replacing calf rennet with *Solanum dobium* extracts (S.D.E.) at rates 25, 50, 75 and 100 %. It is clear from these data that the moisture content of cheese from all treatments gradually decreased throughout the ripening period especially during the early stages of ripening. Results also indicated that replacing calf rennet with S.D.E. significantly ($P \leq 0.05$) increased the moisture content of resultant cheese and this was associated with the replacing level (till 30 days). On the other hand, when resultant cheeses were stored more than 30 days, moisture content significantly ($P \leq 0.05$) decreased gradually by increasing replacing calf rennet with S.D.E. till the end of ripening period (90 days). These results are in agreement with those obtained by Sousa & Malcata, (1997), who found that cheese made with plant rennet (*C. cardunculus*) exhibited higher moisture content than cheese with animal rennet. A similar trend was obtained by Fernandez – Salguera and Sanjuan (1999) and Vioque *et al.* (2000).

Changes in fat on dry matter (fat / DM %) of cheese samples during ripening period are presented in Table (4). As indicated in this table replacing calf rennet with S.D.E. significantly ($P \leq 0.05$) decreased the fat / DM % content of resultant cheeses This was associated with the replacing level. This decrease could be due to the increase in fat lost in the whey (Table 2). During the ripening period some slight increase in fat content of all treatments. These results are in agreement with that obtained by Sousa & Malcata (1997a) for Ovine cheese, Freitas & Malcata (1998) for Picante cheese and Vioque *et al.* (2000) for ewes' milk cheese manufactured with vegetable rennet.

Salt in moisture in all treatments significantly ($P \leq 0.05$) increased during ripening period (Table 4). It could be also observed that slight higher salt in moisture of cheese made by higher replacing rate than that obtained with calf rennet . These results are in agreement with those reported by

Fernandez del Pozo *et al.* (1988 a), Nuenez *et al.* (1991) and Vioque *et al.* (2000). The same table also, showed marked gradually increase in total nitrogen on dry matter (T.N./D.M.%) during ripening in cheese of all treatments. This could be attributed to the loss of moisture during ripening period, on the other hand, the obtained results showed that TN / DM values were significantly ($P \leq 0.05$) decreased after 30 days of ripening period. Variations in TN / DM values of cheeses prepared with 25, 50, 75 and 100% replacing rate were generally insignificant compared with control cheese. Also, T.N. / D.M. values decreased during ripening with increasing replacing rate. The general trend of these results are in agreement with those reported by Fernandez – Salguera and Sanjuan, (1999).

Table (4):Effect of replacing rate of calf rennet with *Solanum dobium* seeds extract on chemical composition of Gouda cheese :

Properties	Ripening Period (days)	Control	Replacing rate of calf rennet by S.D.E.				L.S.D.	Signi.
			25 %	50 %	75 %	100%		
Moisture %	0	49.70^{e}	49.96^{d}	50.21^{c}	50.48^{b}	50.82^{a}	0.099	***
	30	44.22^{e}	44.35^{d}	44.87^{c}	45.12^{b}	45.32^{a}	0.062	***
	60	42.86^{a}	42.78^{b}	42.75^{b}	42.61^{c}	42.24^{d}	0.079	***
	90	41.63^{a}	40.39^{b}	40.28^{c}	40.24^{c}	39.98^{d}	0.069	***
Fat / DM %	0	49.25^{a}	49.12^{b}	49.01^{b}	49.53^{d}	48.66^{c}	0.118	***
	30	50.13^{a}	50.05^{a}	49.77^{b}	49.42^{c}	49.01^{d}	0.132	***
	60	51.05^{a}	50.89^{b}	50.64^{c}	50.36^{d}	49.95^{e}	0.100	***
	90	51.88^{a}	51.70^{b}	51.56^{c}	51.21^{d}	50.75^{e}	0.092	***
Salt in Moisture %	0	6.24^{b}	6.25^{b}	6.27^{b}	6.28^{b}	6.32^{a}	0.037	***
	30	7.55^{c}	7.60^{bc}	7.62^{bc}	7.67^{ab}	7.72^{a}	0.060	***
	60	9.15^{c}	9.19^{bc}	9.22^{ab}	9.22^{ab}	9.26^{a}	0.043	***
	90	9.61^{e}	9.98^{d}	10.06^{c}	10.11^{b}	10.21^{a}	0.047	***
TN / DM %	0	6.84^{ab}	6.82^{ab}	6.87^{a}	6.79^{b}	6.77^{b}	0.055	*
	30	6.69^{ab}	6.65^{bc}	6.73^{a}	6.60^{cd}	6.58^{d}	0.053	***
	60	6.63^{a}	6.59^{ab}	6.66^{a}	6.53^{bc}	6.49^{c}	0.069	**
	90	6.58^{a}	6.56^{a}	6.60^{a}	6.51^{a}	6.52^{a}	0.071	ns

a,b,c,d and e : Means having different letters in the same raw significantly differed at $p \leq 0.05$.

Signi. : Significant * ** : High Significant * : Very high Significant**

Proteolysis characteristics :

Proteolysis is very important in cheese maturation . It contributes to the development of characteristics flavour, body and texture of the matured cheese. Proteolysis indices, which included the concentration of water soluble nitrogen (S.N.), non–protein nitrogen (N.P.N), amino acids nitrogen (AAN) and free amino acids (F.A.A.) of resultant cheeses were estimated. Table (5) shows the average values of S.N./T.N., N.P.N./T.N. and A.A.N./T.N. As can be seen from this table the SN/TN values differed significantly ($P \leq 0.05$) between control cheese and all experimental cheeses. After 30 days of ripening period, the SN/TN values for experimental cheeses were significantly higher than that obtained with calf rennet .These values were

significantly (P≤ 0.05) increased for all cheese treatments until 90 days of ripening period. But this increase was significantly (P≤ 0.05) greater in case of increasing the replacing rate of calf rennet with S.D.E. These results are in agreement with that obtained by Nunez *et al.* (1991) who reported that cheese made with vegetable rennet contained more SN/TN values during ripening, than cheese made with animal rennet. Vieira de Sa and Barbosa (1972) reported that the proteolytic activity of cardo extract was markedly stronger at all stages than that of animal rennet. Also, these results are in agreement with that reported by Visser, (1977), Low and Wigmora, (1982), Cardeiro *et al.* (1992) and Sousa and Malcata, (1997a).

Also, table (5) summarize the values for NPN / TN % . The NPN / TN ratio in cheeses manufactured at replacing CR with SDE at rate 25, 50, 75 and 100% significantly (P≤ 0.05) increased during ripening period. But the values were significantly higher (P≤0.05) in a case of replacing rate of calf rennet with S.D.E. up to the end of the ripening period. As it can be seen, that NPN / TN values increased steadily in all cheese batches throughout ripening period. these results are in agreement with those reported by Vieira de Sa and Barbosa (1972); Ordonez *et al.* (1980); Barbosa *et al.* (1981); Fernandez del Pozo *et al.* (1988b); Nunez et al., (1991); Vioque *et al.* (2000) and Abd El -Galeel and El –Zawahry, (2004). On the other hand, Fernandez – Salguero and Sanjuan, (1999) found that NPN content was similar in cheese produced with animal or vegetable rennet.

Table(5):Effect of replacing rate of calf rennet with *Solanum dobium* seeds extract on Ripening indices of Gouda cheese :

Properties	Ripening period (days)	Control	Replacing rate of calf rennet by S.D.E.				L.S.D.	Signi.
			25 %	50 %	75 %	100%		
S.N./T.N. %	0	7.76^{d}	7.92^{c}	7.95^{c}	8.13^{b}	8.23^{a}	0.072	***
	30	11.82^{e}	12.24^{d}	13.59^{c}	15.61^{b}	16.36^{a}	0.100	***
	60	14.57^{e}	16.26^{d}	16.93^{c}	17.47^{b}	18.72^{a}	0.070	***
	90	17.47^{e}	18.67^{d}	19.85^{c}	21.62^{b}	23.91^{a}	0.073	***
N.P.N./T.N. %	0	3.95^{c}	4.14^{b}	4.15^{b}	4.20^{ab}	4.23^{a}	0.056	***
	30	6.86^{e}	6.97^{d}	7.20^{c}	7.29^{b}	7.42^{a}	0.072	***
	60	9.76^{e}	9.97^{d}	10.47^{c}	10.75^{b}	11.47^{a}	0.061	***
	90	12.63^{e}	12.97^{d}	13.60^{c}	14.01^{b}	14.89^{a}	0.079	***
N.P.N./T.N. %	0	2.15^{a}	2.17^{a}	2.19^{a}	2.20^{a}	2.22^{a}	0.061	ns
	30	4.48^{d}	4.54^{d}	4.66^{c}	4.95^{b}	5.25^{a}	0.081	***
	60	7.86^{e}	7.96^{d}	8.19^{c}	8.64^{b}	8.85^{a}	0.072	***
	90	9.43^{e}	9.62^{d}	10.08^{c}	10.46^{b}	10.74^{a}	0.070	***
T.V.F.A. (ml Na oH N/10/100 gm cheese)	0	12.60^{a}	12.52^{a}	12.50^{a}	12.46^{a}	12.42^{a}	0.123	ns
	30	23.81^{a}	23.60^{b}	23.2^{c}	22.83^{d}	22.15^{e}	0.123	***
	60	35.70^{a}	34.61^{b}	34.15^{c}	33.00^{d}	32.76^{e}	0.131	***
	90	40.86^{a}	38.72^{b}	37.23^{c}	36.45^{d}	34.31^{e}	0.136	***

a,b,c,d and e : Means having different letters in the same raw significantly differed at p ≤ 0.05.
S.D.E.: Aqueous extract of *Solanum dobium* seeds.
Signi. : Significant * ** : High Significant * : Very high Significant**

The amino acid nitrogen represented by A.A.N. / TN Table (5) up to 90 days increased during ripening period in all cheeses. Nevertheless, higher significant (P≤ 0.05) A.A.N./T.N. content after 30 days of ripening period were detected in cheeses made with S.D.E. 25, 50, 75 and 100% replacing rate than cheese made with calf rennet. Increasing in the replacing rate and ripening period significantly (P≤ 0.05) influenced the contents of A.A.N. / TN . These results agree with those obtained by other workers using vegetable rennet for the production of different types of cheeses such as La Serena cheese (Fernandez del Pozo *et al.*, 1988b, Roa *et al.*, 1999) and Ovine cheese (Vioque *et al.*, 2000). In contrast, Sousa and Malcata, (1997a) for Seera cheese found that A.A.N. / TN values were much lower in cheese made with vegetable rennet than with animal rennet .

Lipolysis characteristics :

From the data in Table (5), it could be seen that replacing calf rennet with S.D.E. remarkably affected the total volatile fatty acids (T.V.F.A.) of the resultant cheese. These data showed that T.V.F.A. of all experimental cheeses significantly (P≤ 0.05) decreased by increasing the replacing rate. The same results clearly indicated that T.V.F.A. content of cheese treatments gradually increased throughout the ripening period . But control cheese showed the highest content of T.V.F.A. than all experimental cheeses. These results are in agreement with that obtained by Nunez *et al.* (1991), who reported that cheese produced with vegetable rennet was less lipolysis than in the cheese produced with animal rennet. The general trend for these results was reported by Fernandez del Pozo *et al.* (1988b) and Gaya *et al.* (1990).

Free amino acids :

Results presented in Table (6), Fig. (1 & 2) show the free amino acids content of cheese during ripening period as affected by replacing rate of C.R. with S.D.E.. Data showed that glutamic acid was the most abundant amino acid followed by aspartic acid in all cheeses. Differences in amino acid profiles were noticed between experimental and control cheeses during ripening particularly increasing proline, isoleucine, glycine, alanin and tyrosine acids. These increasing associated with increasing ripening period . It could be noticed that cheeses manufactured with replacing rate of 25, 50, 75 and 100 % of calf rennet with S.D.E. exhibited the highest content of total free amino acids compared with control cheese. Also, this was associated with the increasing replacing rate. The concentration of total F.A.A. of all cheese treatments increased gradually during ripening. These results are in agreement with the average of proteolysis (S.N., N.P.N. and A.A.N. values), that largely due to the proteolytic activity which was associated with S.D.E. (Table 5). The higher levels of F.A.A. contents of S.D.E. cheeses could be due to the higher proteolytic activity of S.D.E. in compared with C.R. (Abd El – Galeel and El Zawahry, (2004). Also, the general trend for these results are in agreement with those reported by Cardiero *et al.* (1992); El – Shafie (1994); Roa *et al.* (1999) and Ayad (2004) .

Table(6):Effect of replacing rate of calf rennet with aqueous extract of *Solanum dobium* seeds on amino acids content (mg/100 g cheese) of Gouda cheese during ripening.

Storage period (days) / Amino acids	Control			Replacing rate of calf rennet with S.D.E.											
				25 %			50 %			75 %			100 %		
	0	60	90	0	60	90	0	60	90	0	60	90	0	60	90
Aspartic cid	2.079	7.399	9.803	2.145	7.195	9.523	2.137	7.248	10 125	2.237	7.830	10.377	2.304	7.916	10.195
Thereonine	0.805	2 802	3.506	0.823	2.694	3.606	0.781	2.766	3.863	0.798	2.859	3.946	0.852	2.869	3.639
Serine	1.054	3 758	4.673	1.135	3.562	4.760	1.080	3.606	5.228	1.123	3.880	5.182	1.114	3.790	4.564
Glutamic acid	4.525	15.835	20 642	4.904	15.364	20.870	4.815	15.769	21.878	5.224	16.422	22.823	5.016	16.860	10.417
Proline	1.204	4.348	5.289	1.335	4.149	5.678	1.292	4.133	6.374	1.382	4.581	6.105	1.429	4.766	13.012
Glycine	1.084	3.560	4.672	1 083	3.520	4.691	1.087	3.587	5 465	1.083	3.779	5.012	1.110	3.937	5.084
Alanine	0.952	3.313	4.294	0.972	3.246	4.404	0.971	3.332	4.617	0.987	3.506	4.687	1.044	3.556	3.974
Cystine	0.252	0.793	1.397	0.271	0.842	1.243	0.296	1.047	1.369	0.316	0.883	1.346	0.268	1.182	0.001
Valine	0.754	2.104	2.782	0.647	2.296	3.015	0.636	2.322	2.964	0.652	2.298	3.228	0.825	2.437	5.064
Methionine	0.093	0.001	0.417	0.062	0.547	1.348	0.082	1.031	0.001	0.195	0.165	1.414	0.331	1.127	0.375
Leucine	0.529	1.619	1.970	0.476	1.568	2.112	0.458	1.562	2.236	0.479	1.633	2.273	0.494	1.630	3.541
Isoleucine	1.195	4.151	5.199	1.230	4.031	5.339	1.179	4.086	5.741	1.239	4.213	5.766	1.277	4.254	5.244
Phenylalanine	0.451	1.735	2.392	0.570	1.757	2.366	0.573	1.898	2.668	0.578	1.901	2.572	0.552	1.960	3.249
Tyrosine	0.663	2.183	2.939	0.652	2.202	2.978	0.643	2.267	2.675	0.686	2.355	3.142	0.687	2.343	4.301
Histidine	0.413	1.694	2.150	0.482	1.615	2.056	0.426	1.684	2.289	0.469	1.698	2.300	0.494	1.658	4.499
Lysine	0.404	1 225	1.652	0.418	1.184	1.670	0.336	1.240	1.718	0.339	1.316	1.860	0.386	1.308	6.367
NH_4^+	1.665	4.058	5.945	1 635	50338	6.465	2.256	4.839	7.245	1.544	5.794	7.251	1.436	5.278	13.353
Arginine	1.572	4.997	7.092	1.562	5.244	7.441	1.612	5.604	7.583	1.750	5.837	7.962	1.702	5.815	3.085
Total	19 694	65.575	86.319	20 402	66.354	89.565	20.660	68 021	94.039	21.081	70.950	97.246	21.221	72 686	99.964

S.D.E.: Aqueous extract of *Solanum dobium* seeds.

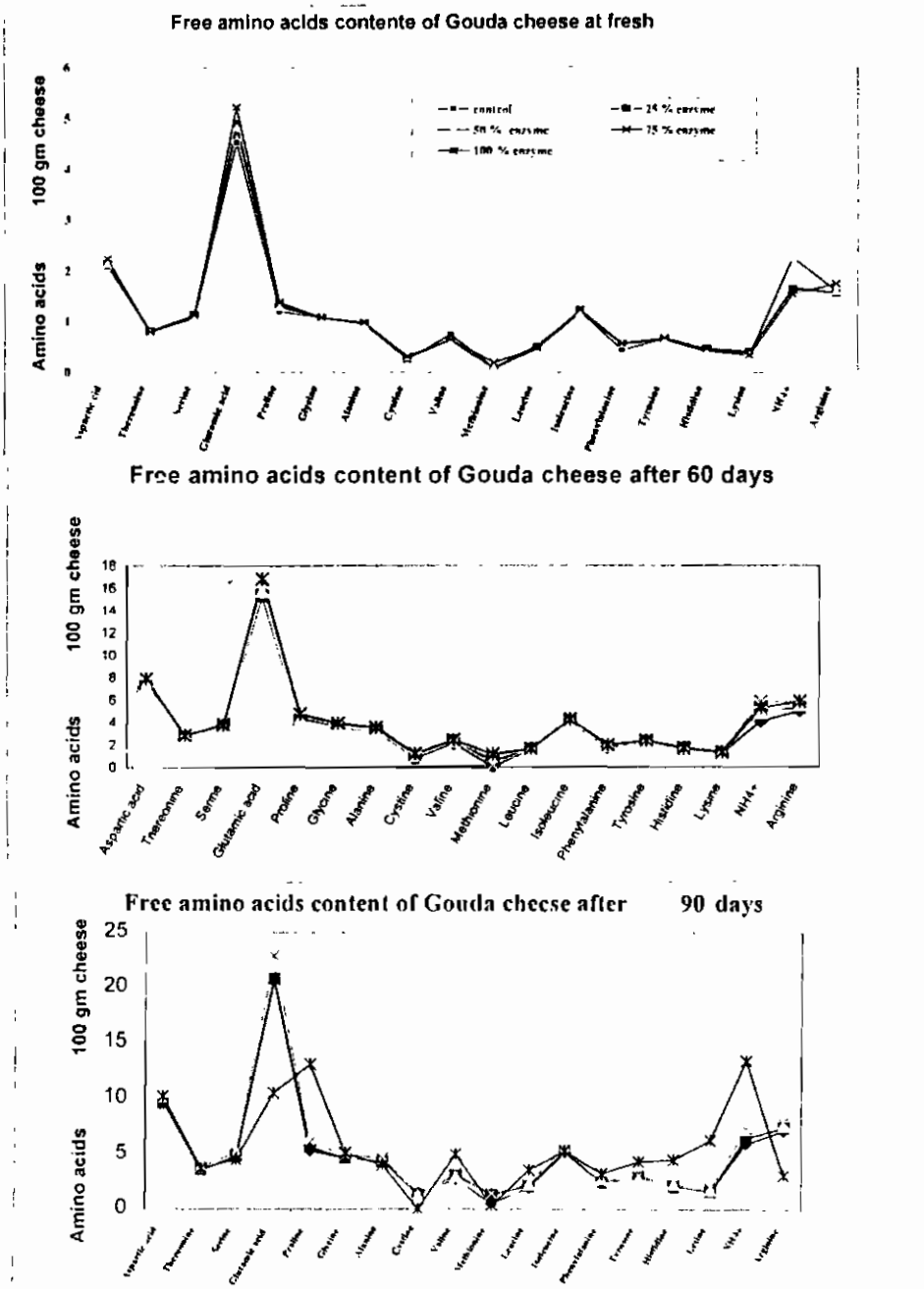

Fig .(1) : Effect of replacing rate of calf rennet with *Solanum dobium* seeds extract on free amino acids content (mg/100 gm cheese) of Gouda cheese:

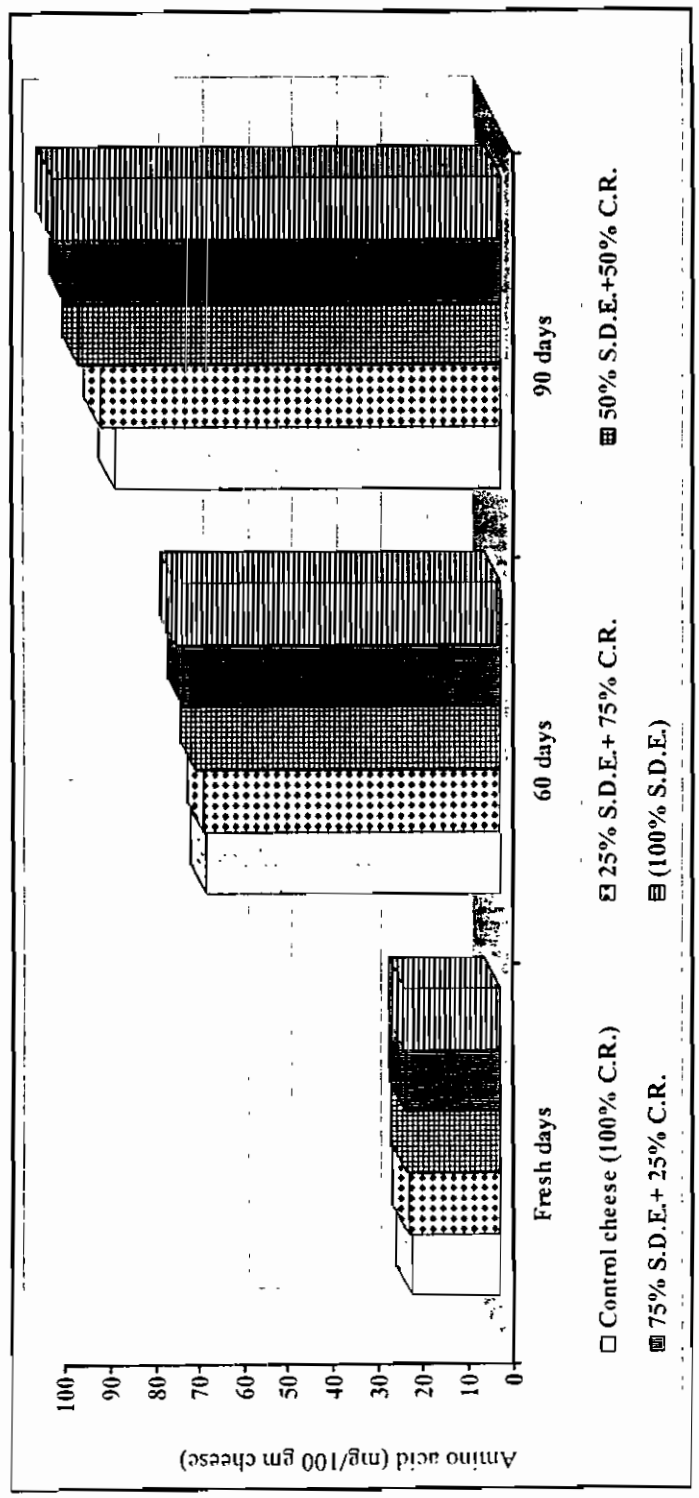

Fig. (2): Effect of replacing rate of calf rennet (C.R.) with *aqueous extract of Solanum dobium* seeds on total amino acids content of Gouda cheese during storage period.

Sensory characteristics :

Results presented in Table (7) summarize the average score given for resultant cheese during ripening period. From these results it could be noticed that replacing calf rennet with S.D.E. at a rate higher than 50 % significantly ($P \leq 0.05$) decreased the score points for resultant cheese in compare with the control cheese, whereas then organoleptic properties of cheese made by replacing calf rennet with S.D.E. at levels of 25 and 50% did not significantly differed in compared with control cheese . But that made with replacing levels of 75 and 100% showed significantly lower scores in compare with the control cheese .The lowest score were shown in case of 100% replacing level. Therefore it could be concluded that replacing calf rennet with S.D.E. up to 50 % enable to produce Gouda type cheese of acceptable quality.

Table (7):Effect of replacing rate of calf rennet with *Solanum dobium* seeds extract on organoleptic properties of Gouda cheese.

Ripening period (days)	Scoring		Control	Replacing rate of calf rennet by S.D.E.				L.S.D.	Signi.
				25 %	50 %	75 %	100%		
0	Appearance	10	8.00	8.00	8.00	8.00	8.00		
	Bod & Texrure	40	34.50	34.50	34.25	34.00	30.35		
	Flavour	50	43.00	41.80	41.50	34.62	32.50		
	Total	100	85.50^{a}	84.30^{a}	83.75^{a}	76.62^{b}	70.85^{c}	2.542	***
30	Appearance	10	9.00	8.50	9.00	8.50	8.00		
	Bod & Texrure	40	35.42	35.43	34.90	33.95	30.39		
	Flavour	50	45.22	44.80	44.60	40.25	35.45		
	Total	100	89.64^{a}	88.73^{a}	88.50^{a}	82.70^{b}	73.84^{c}	4.642	***
60	Appearance	10	9.50	9.00	9.50	9.00	8.50		
	Bod & Texrure	40	38.91	39.34	39.30	33.93	31 84		
	Flavour	50	47.55	48.50	48.66	40.82	36.36		
	Total	100	95.96^{a}	96.84^{a}	97.46^{a}	83.75^{b}	76.70^{c}	3.666	***
90	Appearance	10	9.50	9.50	9.50	9.00	8.50		
	Bod & Texrure	40	39.75	38.48	38.62	38.45	32.20		
	Flavour	50	48.25	49.45	48 78	40.00	38 00		
	Total	100	97 50^{a}	97.43^{a}	96 90^{a}	87.45^{b}	78.70^{c}	2.566	***

a,b,c,d and e : Means having different letters in the same raw significantly differed at $p \leq 0.05$.
S.D.E.: Aqueous extract of *Solanum dobium* seeds.
Signi. : Significant *
** : High Significant
*** : Very high Significant

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التجبن الإنزيمى للبن باستخدام مستخلص بـذور نبـات *Solanum dobium* كبديل للمنفحة:

٢- تأثير استبدال المنفحة الحيوانية بالمستخلص الإنزيمى المائى لبـذور نبـات *Solanum dobium* على جودة وتسوية الجبن الجودا

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تم دراسة تأثير استبدال المنفحة الحيوانيـة (CR) للمسـتخلص الإنزيمـى المـائى لبـذور نبـات *Solanum dobium* بمعدلات صفر ، ٢٥ ، ٥٠ ، ٧٥ ، ١٠٠ ٪ على كل من التصافى والتركيـب الكيماوي والتحلل البروتينى والخواص الحسية لجبن الجودا خلال فترة التخزين لمدة ٣٠، ٦٠، ٩٠ يوم .

وقد أظهرت النتائج زيادة معنوية ($P \leq 0.05$) لكل من الفاقد من الدهن والبـروتين فـى الشـرش وكذلك الفقد فى الوزن مقارنة بجبن المقارنة . وكانت هذه الزيادة مرتبطة بالزيادة فى نسبة الاستبدال . كما لـوحظ زيادة معنوية فى كلا من الرطوبة والملح منسوبا للمحتوى المائى للجبن أكثر من جبن المقارنة خلال فترة التخزين . كما لوحظ انخفاض فى قيم الـ pH و الدهن بالنسبة للمادة الجافة . كما لوحظ انخفـاض غيـر معنـوى فـى النيتروجين الكلى بالنسبة للمادة الجافة . أما بالنسبة للتحلل البروتينى فقد لـوحظ زيـادة معنويـة ($P \leq 0.05$) مقارنة بجبن المقارنة . وهذه الزيادة مرتبطة بزيادة نسبة الاستبدال . كما لوحظ زيادة فـى محتـوى الأحمـاض الأمينية الحرة مع زيادة نسبة الاستبدال خلال فترة التخزين كما وجد أن محتوى جبن المقارنـة مـن الأحمـاض الدهنية الطيارة الكلية (T.V.F.A.) أعلى من باقى المعاملات .

أما بالنسبة للتحكيم الحسى فقد لوحظ ظهور القوام العجينى مع بعض عيوب فى النكهة خاصة ظهـور الطعم المر عند استخدام نسبة استبدال أكثر من ٥٠ ٪ بينما عند استخدام نسبة استبدال حتى ٥٠ ٪ لم يظهـر اى عيوب فى القوام والنكهة .

وبذلك يمكن التوصية بصناعة جبن جودا بخواص جـودة مقبولـة باسـتبدال المنفحـة الحيوانيـة (CR) بالمستخلص الانزيمى المائى لبذور نبات *Solanum dobium* (*S.D.E.*) حتى نسبة استبدال ٥٠ ٪ .