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MILK CLOTTING ENZYME BY USING *SOLANUM DOBIUM* PLANT AS RENNET SUBSTITUTE 1- PRELIMINARY STUDIES ON THE ENZYME EXTRACT FROM *SOLONUM DOBIUM* PLANT ON MILK CLOTTING

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ABSTRACT: ***Solanum dobium*** **seeds extract (S.D.E.) was investigated as a substitute for traditonal animal rennet used in milk clotting. Preliminary studies was carried out to evaluate the effect of different conditions being pH, setting temperature, enzyme concentration, as well as sodium or calcium chloride levels on the clotting activity as well as curd tension and proteolytic activity of resultant extract. Calf rennet (C.R.) was used as control. Clotting time of milk by *Solanum dobium* extract (S.D.E.) is less sensitive to high pH than calf rennet (C.R.). On the other hand *Solanum dobium* extract (S.D.E.) was more heat tolerant than calf rennet (C.R.) . Coagulation time decreased by increasing enzyme concentration of both C.R. and S.D.E. But this was more noticed for S.D.E. comparing with C.R.**

Addition of calcium chloride significantly ($P \leq 0.05$) increased the clotting activity and decreased the clotting time of S.D.E. in compare with C.R. Also S.D.E. was less sensitive than C.R. for increasing the sodium chloride level. S.D.E. significantly ($P \leq 0.05$) decreased curd tension and increased proteolysis than C.R.

Key word: **Curd tension, proteolytic activity, calf rennet, *Solanum dobium* extract , coagulation time.**

INTRODUCTION

Milk clotting is a complex process, involving a primary enzymic phase in which K-casein altered and losses its ability to stabilize the remainder of the caseinate complex, a secondary non–enzymic phase in which aggregation of the caseinate micelles forms a firm gel structure and a possibly separate fourth step where the curd structure tightens and syneresis occurs (McMahon and Brown 1984).

The purpose of adding milk clotting enzymes to milk is to cleave K-casein and thereby initionate the coagulation of the milk. In addition to this action, all milk–clotting enzymes have gerenal proteolysis capability (Berridge 1954).

It is well known that main rennet is the conversion of liquid milk to gel form under specific conditions. Calf rennet, the conventional enzyme which is extracted from the calf fourth stomach, is used widely in cheese-making all over the world. Although many proteinases from different sorces have been found suitable to colt milk in cheese–making.

But calf rennet (and more recently, genetically engineered chymosin rennet) is the most desirable because it contains chymosin which exhibits specific and limited proteolysis of the phe105- Met.106 bond in K-casein (Tam and Whitaker 1973). However, in many countries, shortages and high prices of calf rennet have encouraged the search for rennet substitutes, some sources including micro-organisms and plants (Green, 1972 and 1977; Foda *et al.*, 1978 and Alichanidis *et al.*, 1984).

Isolation and chemical properties of some rennet–like enzyme from different plant sources have been studied by several workers, such as Hamdy *et al.* (1976), Fouda *et al.* (1976), Gupta and Eskin (1977) and Aworth and Nakai (1986).

Also several investigators have been used vegetable rennet, successfully in manufacturing deferent type of cheese, i.e. Domiate cheese by El-Shibiny *et al.* (1973) and cheeder cheese by Gupta and Eskin (1977).

La serena cheese (is a semi – hard spinach variety manufacture from ewe's milk with vegetable rennet from *Cynara cardunculus* L. as coagulant. Nunez *et al.* (1989).

A milk clotting enzyme found in the flowers of cardo (*Cynara cardunculus*) was investigated as to its suitability as substitute for

animal rennet used in Edam cheese – making, Vieira and Barbosa, (1972). Leaf extracts from *Calotropis procera* showed high milk clotting activities and relatively low protealytic activities over a wide temperature range. In addition *C. procera* extract has been used as milk coagulation in some part of Africa to produce unripend soft cheese, Mohamed and O'Conner, (1999). Therefore, it is not surprising that milk clotting enzymes have obtained from plant sources. The world literature on this subject is already vary large. However, very little references about *Solanum dobium* as source of vegetable rennet was found. In the Sudan, the berries of *Solanum dobium* are used to manufacture of white soft cheese. *Solanum dobium* a major problem for many farmers in Sudan, is a moxious weed belonging to the plant family solanaceae that grows in vast areas of sudan Yousif *et al.* (1996).

Therefore, this work is aimed to extract a milk clotting enzyme from the seeds of *Solanum dobium* and use this extract as substitute of calf rennet in cheese making. Milk clotting activity of the resultant extract as affected by some factors have been also studied during this work.

MATERIALS AND METHODS

Reconstituted skim Milk:

Skim milk powder imported from U.S.A. extra grade (A), supplied by Misr Dairy and Food Company, Cairo , Egypt. Standard milk consisting of 12% (w/v) of a skim milk powder solution was used. The reconstituted milk was pasteurized at 65°C for 30 min. then, cooled to 10°C, kept in refrigerator over might. Then anhydrous calcium chloride at rate of 0.03% was added.

Enzymes:

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

Vegetable rennet:

The seeds of *Solanum dobium* were obtained from Sudan Republic, kept at room temperature at about 20°C ± 2, till they were used.

***Solanum dobium* extract:**

Rennet–like enzyme was extracted from the seeds of *Solanum dobium* by using the method of Singh *et al.* (1973),

except that the centrifugation was not used. The seeds were grinded, then 10g of ground seeds were immersed in phosphate buffer solution at pH 6.9 for 24 hours, then 5% of sodium chloride and 2% boric acid were added. The extracted solution was thoroughly mixed for 3-5 days, then filtrated by muslin cloth. The extract was then filtered again using watman No. 4 filter papers (Yousif *et al.*, 1996). The filtrate solution is kept at refrigerator for about 5°C ± 2 till it was used.

Effect of pH on the clotting activity:

Portions of reconstituted skim milk [12% (w/v) and 0.03% $Cacl_2$] were adjusted to pH 5.8, 6, 6.2, 6.4, 6.6, 6.8 and 7.0. One ml of the stock extract enzymes solution preparation from *Salonum dobium* seeds was added to 10ml of reconstituted skim milk at 40°C and calf rennet was used as control.

Effect of milk temperature on the clotting activity:

The effect of temperature of reconstituted skim milk [12% (w/v) and 0.03% $Cacl_2$] on clotting properties of the S.D.E. and C.R. were determined by heating milk from 30 to 56°C at 2°C intervals and then performing the clotting test by addition one ml of extract enzyme to 10 ml of each of these substrates at pH 6.5.

Effect of NaCl concentration on milk clotting time:

Sodium chloride in concentration of 0 to 12% to 0.5% intervals were added at pH 6.5 to reconstituted skim milk (12% w/v, 0.03% $Cacl_2$) before renneting. *Solanum dobium* (S.D.E.) extract was added to milk resulted at 40°C. Control treatment with calf rennet was done.

Effect of enzyme concentration:

The experiment was performed at 40°C using 10ml of reconstituted skim milk powder [12% (w/v) 0.03 $Cacl_2$] and 1 ml of aliquot of the enzymes concentration mad by dilution up 0.1 to 1 ml intervals 0.1 for 10 ml reconstituted skim milk at pH 6.5 .

Effect of $CaCl_2$ concentration:

Various concentration of anhydrous $Cacl_2$ (0.000, 0.005, 0.010, 0.015, 0.020, 0.025, 0.030, 0.055 and 0.040 %) were added to reconstituted skim milk (12% w/v) at pH 6.5. Stock solution of the *Solanum dobium* (S.D.E.) and calf rennet separately assayed in milk at 40°C.

Clotting activity:

The concentration of the *Solanum dobium* extract solution was adjusted to give the same

clotting time as that of the calf rennet solution with standard milk at 40°C, as described by Vieira, de SA and Barbosa, (1972).

The proteolytic activity:

It was expressed as non protein nitrogen (N.P.N)/ total nitrogen (TN)% as follows :

One ml of enzyme extract was added to 10 ml of 3% casein solution (pH 6.5) in a test tube and incubated at 40 °C for 0, 20, 40, 60, 90 and 120 min. The N. P. N. and T. N.% were determined by semimicro kjeldahl method given by Rowland (1938).

Curd tension and synersis:

It was determined using the method of Chandrasekhara *et al.* (1957) the results were expressed as weights in grams would be required directly proportional to the curd to get the knife cut the curd. Thus the weights required would be directly proportional to the curd tension.

Synersis :

Synersis of the resultant curd was calculated as a present of the volume of drained whey and calculated as a present of the milk volume as described by Lowrence (1959).

Statical analysis:

Factorial design 2 factors x 3 replicates and the completely randomized design was used to analyze all the data and Newman keuls test was followed to make the multiple comparisons (Stell and Torrie, 1980) using costat program significant differences were determined at $P \leq 0.5$.

RESULTS AND DISCUSSION

Effect of pH on the clotting time:

The effect of pH on the clotting time for both C. R. or S.D. E. ranging from pH 5.5 to 7.0 is shown in Table (1). The results indicate that clotting activity of C.R. was more significantly ($P \leq 0.05$)influenced by the decrease of pH less than pH 6.5 in comparison with *Solanum dobium* extract (S.D.E.). Above pH 6.5 the clotting time was significantly ($P \leq 0.05$) increased in both C. R. or S. D. E. However, above pH 6.8 the clotting activity of C. R. completely disappeared. But S.D.E. showed clotting activity till pH 7.0. These results are in agreement with those reported by Storry and Ford (1982) and El-Abasy (1988) who found that rennet clotting time was decreased by the reduced pH of milk. Also, Zeidan and El-Abasy (1986) found that clotting time for goat's milk, bouffaloe's milk and cow's milk decreased as pH was decreased

from pH 7.2 to 5.8 with pepsin or calf rennet as clotting agent.

Effect of temperature on the clotting time:

The results in Table (2) illustrated the effect of setting temperature on the clotting time of C. R. or S.D. E. The clotting time is significantly ($P \leq 0.05$) affected by the temperature.

As the temperature of setting was increased above 30°C the clotting time was significantly ($P \leq 0.05$) decreased in case of C.R. up to 42°C and up to 56°C for *Solanum dobium* extract. But no clotting was observed after 20 min. at 54 °C in case of calf rennet (C. R.) The calf rennet reached it maximum activity at 42°C. However, under similar conditions S.D.E. is significantly stable at high temperature and shows optimum clotting activity at 46°C, above this temperature clotting time was very sharp decreased. This results are in agreement with those reported by several investigations, Garnat and Molle (1987) who pointed out that the chymosin inactivated in gelled milk at 53°C. Ibrahim *et al.* (1973) found that the enzyme of M. miehi (Rennilase) was inactive at temperature higher than 75°C, while the animal rennet failed to coagulate milk at 60°C. Also, Vieira and Barbosoa (1972) found that vegetable rennet from cardo (*Cynara cardunculus*) was stable at high temperature and shows an increasing clotting activity up to 70°C. Moreover, Ogugua and Nakai (1986) found that the extract of milk clotting enzyme from sodom apple (*Calotropis procera*) was more active at 65°C than at 35°C. These results revealed that *Solanum dobium* extract was more heat tolerant than calf rennet.

Effect of enzyme concentra-tion:

The general rule stating that the time of coagulation (T) in inversely proportion to the amount of the enzyme (E) as expressed by equation (E.x T.= K) holds only true over a narrow range of the enzyme concentration from 0.1 to 1.0 / 10 ml of substrate .The data in Table (3) show that the equation (E.T.=K) is only theoretical but on the practice it was found that (K) is nearly constant in calf rennet and *Solanum dobium* extract from 0.3 to 0.6 and 0.2 to 0.6 (ml/10ml of milk) enzyme concentration respectively. The results also indicated that *Solanum dobium* extract was more significantly ($P \leq 0.05$) influenced by enzyme concentration than calf rennet.

It could be also noticed that increasing the enzyme concentration significantly ($P \leq 0.05$) reduced the clotting time for both C.R. and S.D.E. But this was more noticed in case of S.D. E. than C. R., these results agreed with those reported by many authors, who found that rennet clotting time was decreased by increasing concentration of rennet Magdoub *et al.* (1984) and El-Abbassy (1977) .

Effect of calcium chloride ($CaCl_2$) concentration on the clotting time:

It is obvious from the results given in Table (4) that addition of calcium chloride to milk resulted in a significant ($P \leq 0.05$) decrease in the clotting time with both (C.R.) and S. D. E.

These results also indicated that the percentage of activity of calf rennet and S. D. E. was 211 and 157% respectively at 0.040% calcium chloride concentration. The results also showed that S.D.E. significantly ($P \leq 0.05$) influenced by $Cacl_2$ addition than calf rennet. These results agree with Amer *et al.* (1979), Storry and Ford (1982) and Magdoub *et al.* (1984).

Effect of sodium chloride concentration on the clotting time:

From the data presented in Table (5) it could be noticed that the relative activity of both C. R. and S. D. E. was significantly ($P \leq 0.05$) decreased as the concentration of sodium chloride in the milk was increased up to 12%. But the decrease was significantly ($P \leq 0.05$) greater in case of C. R. than S. D. E. It could be also noticed that S. D. E. is more stable than C. R. in the present of higher concentration of sodium chloride. So, it could be used in the manufacturer of white salted cheese successfully.

Proteolytic activity of calf rennet and *solanum dobium* extracts during incubation time at 40°C:

The effect of incubation time on the soluble nitrogen liberation from casein in 4% trichlorractic acid (T.C.A.), filterate by C. R. or S. D. E. is sumarized in Table (6). Data showed the average of N. P. N.% of the filtrate increased gradually during the incubation period. The highest rate of proteolyties was noticed when casein solution was treated with S.D.E. than of C.R.

These results indicate that proteolysis rate of casein significantly ($P \leq 0.05$) increased with the advance of incubation of both C. R. or, S. D. E. But S. D. E. showed the significant higher proteolytic activity compared with C. R.

These results are in agreement with these reported by Vieira de SA and Barbos (1972) who found that cardo extract shows a stronger proteolytic activity than calf rennet. Also, Nunez. *et al.* (1991) reported that proteolysis of La serena cheese was found to be at higher rate when it was made using vegetable rennet than that made using calf rennet .

Also, these results are in agreement with those reported by Sousa and Malcata (1998) who found that primary proteolysis of ovine and caprine casainates by proteinases from extracts of *C. cardunculus* using experimental conditions that mimic milk (pH 6.5), fresh cheese (pH 5.5) and cheese at the beginning of ripening (pH 5.2 with 5% Nacl), they found that Caprine caseinate underwent more extensive proteolysis than the ovine one, under similar conditions (i.e. pH 6.5 and pH 5.5).

Curd tension and synersis :

Results presented in Table (7) showed that S. D. E. significantly ($P \leq 0.05$) reduced both curd tension and whey synersis than C. R. Also, it could be noticed that increasing enzyme concentration significantly ($P \leq 0.05$) increased curd tension and whey synersis in both C. R. and S. D. E. The decrease in curd tension in case of S. D. E. could be due to the higher proteolytic activity of S. D. E. than that of C. R. as shown in Table (7).

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Table (1): Effect of pH on the milk clotting activity

pH	Calf rennet			*Solanum dobium* extract				
	C.T. (sec.)	Ru/ml	R.A.%	C.T. (sec.)	Ru/ml	R.A.%	L.S.D.	Significant
5.8	$34^{b}{}_{12}$◄	11.76	1059.45	$75^{a}{}_{12}$◄	5.33	459.48	9.068	***
5.9	$42^{b}{}_{12}$	9.52	857.66	$94^{a}{}_{11}$	4.26	367.24	9.068	***
6.0	$58^{b}{}_{11}$	6.90	621.62	$116^{a}{}_{10}$	3.45	297.41	9.881	***
6.1	$75^{b}{}_{10}$	5.33	480.18	$123^{a}{}_{9,10}$	3.25	280.17	9.843	***
6.2	$91^{b}{}_{9}$	4.40	396.40	$131^{a}{}_{9}$	3.05	262.93	10.989	***
6.3	$133^{b}{}_{8}$	3.00	270.27	$145^{a}{}_{8}$	2.76	237.93	11.335	*
6.4	$250^{a}{}_{7}$	1.60	144.14	$260^{a}{}_{7}$	1.54	132.76	11.221	non
6.5	$360^{a}{}_{6}$	1.11	100.00	$346^{b}{}_{6}$	1.16	100.00	10.264	*
6.6	$620^{a}{}_{5}$	0.65	58.59	$375^{b}{}_{5}$	1.07	91.90	14.066	***
6.7	$927^{a}{}_{4}$	0.43	38.74	$465^{b}{}_{4}$	0.86	74.14	13.975	***
6.8	$1190^{a}{}_{3}$	0.34	30.63	$598^{b}{}_{3}$	0.67	57.76	15.624	***
6.9	$1482^{a}{}_{2}$	0.27	24.32	$867^{b}{}_{2}$	0.60	51.72	11.335	***
7.0	$1715^{a}{}_{1}$	0.23	20.72	$735^{b}{}_{1}$	0.54	46.55	10.138	***
L.S.D.	8.905			8.009				
Sign.	***			***				

C.T. : Clotting time (Sec.) ***: Very high significant Sign. : Significant
R.A.: Relative activity: was calculated as 100% at pH and increase or decrease according to clotting time (Sec.)
a , b : Means having different letters in the same raw significantly differed at $P \leq 0.05$
◄ : Means having different small number in same column significantly differed at $P \leq 0.05$

Table (2): Effect of temperature on the milk clotting activity

Temperature °C	Calf rennet.			Solanum dobium Extract				
	C.T. (sec.)	Ru/ml	R.A.%	C.T. (sec.)	Ru/ml	R.A.%	L.S.D.	Significant
30	$505^{b}{}_{14}$◄	0.79	68.70	$926^{a}{}_{1}$◄	0.43	35.53	7.169	***
32	$415^{b}{}_{13}$	0.96	83.48	$756^{a}{}_{2}$	0.53	43.80	9.347	***
34	$382^{b}{}_{12}$	1.05	91.30	$646^{a}{}_{3}$	0.62	51.24	5.998	***
36	$374^{b}{}_{11}$	1.07	93.04	$416^{a}{}_{4}$	0.96	79.34	9.347	***
38	$360^{a}{}_{10}$	1.11	96.52	$350^{a}{}_{5}$	1.14	94.21	10.138	non
40	$347^{a}{}_{9}$	1.15	100.00	$330^{b}{}_{6}$	1.21	100.00	6.412	**
42	$320^{a}{}_{8}$	1.25	108.70	$268^{b}{}_{7}$	1.49	123.14	8.632	***
44	$439^{a}{}_{7}$	0.91	79.13	$185^{b}{}_{8}$	2.16	178.51	8.482	***
46	$465^{a}{}_{6}$	0.86	74.78	$150^{b}{}_{9}$	2.67	220.66	12.102	***
48	$610^{a}{}_{5}$	0.66	57.39	$145^{b}{}_{9}$	2.76	228.10	9.618	***
50	$808^{a}{}_{4}$	0.50	43.48	$118^{b}{}_{10}$	3.39	280.17	7.169	***
52	$1194^{a}{}_{3}$	0.34	29.57	$78^{b}{}_{11}$	4.59	379.34	9.068	***
54	$1310^{a}{}_{2}$	0.31	26.95	$67^{b}{}_{12}$	5.97	493.39	9.347	***
56	$1562^{a}{}_{1}$	0.26	22.61	$58^{b}{}_{13}$	6.90	570.25	8.925	***
L.S.D.	6.096			6.910				
Sign.	***			***				

C.T. : Clotting time (Sec.) *** : Very high significant Sign. : Significant

R.A. : Relative activity: was calculated as 100% at 40°C and increase or decrease according to clotting time (Sec.)

a , b : Means having different letters in the same raw significantly differed at $P \leq 0.05$

◄ : Means having different small number in same column significantly differed at $P \leq 0.05$

Table (3): Effect of enzyme concentration on the milk clotting activity

E. conc. ml/10 ml	Calf rennet.			*Solanum dobium* extract				
	C. T. (sec.)	E. x T =K	$^1/_t \times 10^3$	C.T. (sec.)	E. x T =K	$^1/_t \times 10^3$	L.S.D.	Significant
0.1	$1863^{a}{}_{1}$◄	186.3	0.536	$1372^{b}{}_{1}$◄	137	0.728	7.169	***
0.2	$1540^{a}{}_{2}$	308.0	0.649	$1167^{b}{}_{2}$	233	0.856	14.338	***
0.3	$1110^{a}{}_{3}$	333.0	0.900	$790^{b}{}_{3}$	273	1.270	19.763	***
0.4	$882^{a}{}_{4}$	353.0	1.130	$594^{b}{}_{4}$	238	1.680	17.850	***
0.5	$676^{a}{}_{5}$	338.0	1.470	$476^{b}{}_{5}$	238	2.100	11.221	***
0.6	$566^{a}{}_{6}$	340.0	1.770	$396^{b}{}_{6}$	238	2.530	11.559	***
0.7	$518^{a}{}_{7}$	363.0	1.930	$332^{b}{}_{7}$	232	3.010	10.138	***
0.8	$473^{a}{}_{8}$	378.0	2.110	$251^{b}{}_{8}$	200	3.980	12.208	***
0.9	$422^{a}{}_{9}$	380.0	2.370	$225^{b}{}_{9}$	198	4.550	9.347	***
1.0	$385^{a}{}_{10}$	385.0	2.600	$195^{b}{}_{0}$	195	5.130	10.138	***
L.S.D.	8.448			10.812				
Sign.	***			***				

E. : Enzyme concentration C.T. : Clotting time (Sec.) ***: Very high significant Sign. : Significant

a , b : Means having different letters in the same raw significantly differed at $P \leq 0.05$

◄ : Means having different small number in same column significantly differed at $P \leq 0.05$

Table (4): Effect of $Cacl_2$ concentration on the milk clotting activity

$CaCl_2$ %	Calf rennet.			*Solanum dobium* extract				
	C.T. (sec.)	Ru/ml	R.A.%	C.T. (sec.)	Ru/ml	R.A.%	L.S.D.	Significant
0.000	$631^{a}{}_{1}$◄	0.63	100.00	$400^{b}{}_{1}$◄	1.00	100	16.347	***
0.005	$456^{a}{}_{2}$	0.88	139.70	$343^{b}{}_{2}$	1.17	117	10.989	***
0.010	$447^{a}{}_{3}$	0.89	141.27	$321^{b}{}_{3}$	1.25	125	10.264	***
0.015	$421^{a}{}_{4}$	0.95	150.79	$316^{b}{}_{3}$	1.27	127	11.335	***
0.020	$412^{a}{}_{5}$	0.97	153.96	$312^{b}{}_{8}$	1.28	128	9.068	***
0.025	$327^{a}{}_{6}$	1.22	193.65	$294^{b}{}_{4}$	1.36	136	12.313	***
0.030	$318^{a}{}_{7}$	1.26	200.00	$587^{b}{}_{4}$	1.39	139	5.998	***
0.035	$308^{a}{}_{8}$	1.30	206.34	$260^{b}{}_{5}$	1.54	154	16.5037	***
0.040	$300^{a}{}_{8}$	1.33	211.11	$255^{b}{}_{5}$	1.57	157	12.3127	***
L.S.D.	8.227			9.986				
Sign.	***			***				

C.T. : Clotting time (Sec.) ***: Very high significant Sign. : Significant

R.A.: Relative activity: was calculated as 100% without added $CaCl_2$ and increase or decrease according to clotting time (Sec.)

a , b : Means having different letters in the same raw significantly differed at $P \leq 0.05$

◄ : Means having different small number in same column significantly differed at $P \leq 0.05$

Table (5): Effect of NaCl concentration on the milk clotting activity

NaCl %	Calf rennet.			*Solanum dobium* extract				
	C.T. (sec.)	Ru/ml	R.A.%	C.T. (sec.)	Ru/ml	R.A.%	L.S.D.	Significant
0.0	358^{a}_{23}	1.12	100.0	358^{a}_{18}	1.12	100.00	7.169	non
0.5	$363^{a}_{22,23}$	1.10	98.21	365^{a}_{17}	1.09	97.77	8.174	non
1.0	369^{a}_{22}	1.08	96.43	372^{a}_{16}	1.07	95.98	9.483	non
1.5	377^{a}_{21}	1.06	94.62	381^{a}_{15}	1.05	93.66	8.925	non
2.0	440^{a}_{20}	0.91	81.25	397^{b}_{14}	1.01	89.91	11.335	***
2.5	462^{a}_{19}	0.87	77.68	411^{b}_{13}	0.97	86.61	7.519	***
3.0	555^{a}_{18}	0.72	64.29	421^{b}_{12}	0.95	84.82	10.333	***
3.5	647^{a}_{17}	0.62	55.36	432^{b}_{11}	0.93	83.04	8.015	***
4.0	737^{a}_{16}	0.54	48.21	446^{b}_{10}	0.90	80.36	5.998	***
4.5	758^{a}_{15}	0.53	47.32	453^{b}_{9}	0.88	78.57	8.925	***
5.0	798^{a}_{14}	0.50	44.64	$457^{b}_{8,9}$	0.88	78.57	5.317	***
5.5	869^{a}_{13}	0.46	41.07	455^{b}_{9}	0.88	78.57	8.482	***
6.0	952^{a}_{12}	0.43	38.39	454^{b}_{9}	0.88	78.57	6.987	***
6.5	956^{a}_{11}	0.42	37.50	456^{b}_{9}	0.88	78.57	8.174	***
7.0	964^{a}_{10}	0.41	36.60	$460^{b}_{8,9}$	0.87	77.77	5.317	***
7.5	985^{a}_{9}	0.41	36.60	$462^{b}_{8,9}$	0.87	77.77	8.482	***
8.0	996^{a}_{8}	0.40	35.71	460^{b}_{8}	0.87	77.77	8.174	***
8.5	1007^{a}_{7}	0.40	35.71	$465^{b}_{6,7}$	0.86	76.79	8.015	***
9.0	1024^{a}_{6}	0.39	34.82	$470^{b}_{5,6}$	0.85	75.89	9.068	***
9.5	1043^{a}_{5}	0.38	33.93	476^{b}_{5}	0.84	75.00	8.482	***
10.0	1078^{a}_{5}	0.37	33.04	483^{b}_{4}	0.83	74.1	8.174	***
10.5	1146^{a}_{4}	0.35	31.25	491^{b}_{3}	0.81	72.32	11.106	***
11.0	1195^{a}_{3}	0.33	29.46	502^{b}_{2}	0.80	71.43	9.347	***
11.5	1260^{a}_{2}	0.32	28.57	$508^{b}_{1,2}$	0.79	70.53	9.068	***
12.0	1350^{a}_{1}	0.30	26.79	512^{b}_{1}	0.78	69.64	7.169	***
L.S.D.	6.154			6.048				
Sign.	***			***				

C.T. : Clotting time (Sec.) ***: Very high significant Sign. : Significant

R.A.: Relative activity: was calculated as 100% without added $CaCl_2$ and increase or decrease according to clotting time (Sec.)

a , b: Means having different letters in the same raw significantly differed at $P \leq 0.05$

◄ : Means having different small number in same column significantly differed at $P \leq 0.05$

Table (6): Proteolytic activity of calf rennet and *Solanum dobium* extracts during incubation time at 40°C.

Incubation time (min.) at 40°C	Calf rennet.		*Solanum dobium* Extract		L.S.D.	Significant
	N.P.N. %	N.P.N./T.N. %	N.P.N. %	N.P.N./T.N. %		
0	0.035	$5.942^{a}{}_{6}$◄	0.040	$5.602^{b}{}_{6}$◄	0.285	*
20	0.041	$6.961^{b}{}_{5}$	0.094	$13.165^{a}{}_{5}$	0.267	***
40	0.054	$9.168^{b}{}_{4}$	0.127	$17.787^{a}{}_{4}$	1.075	***
60	0.096	$16.299^{b}{}_{3}$	0.143	$20.028^{a}{}_{3}$	0.481	***
90	0.102	$17.318^{b}{}_{2}$	0.197	$27.591^{a}{}_{2}$	0.390	***
120	0.108	$18.336^{b}{}_{1}$	0.229	$32.073^{a}{}_{1}$	0.724	***
L.S.D.		0.545		0.397		
Sign.		***		***		

N.P.N.% : Non protein nitrogen % T.N.% : Total nitrogen % ***: Very high significant
a , b : Means having different letters in the same raw significantly differed at P≤0.05 Sign. : Significant
◄ : Means having different small number in same column significantly differed at P≤0.05

Table (7): Effect of enzyme concentration on curd tension and synersis

A-Curd tension

Enzyme conc. ml/100ml of milk	Calf rennet.	*Solanum dobium* extract	L.S.D.	Significant
	Curd tension g/100ml	Curd tension g/100ml		
1	$14.92^{a}{}_{5\blacktriangleleft}$	$12.40^{b}{}_{4\blacktriangleleft}$	1.428	**
2	$43.84^{a}{}_{4}$	$15.92^{b}{}_{3}$	2.353	***
3	$47.03^{a}{}_{3}$	$25.49^{b}{}_{2}$	1.967	***
4	$52.59^{a}{}_{2}$	$29.75^{b}{}_{1}$	1.570	***
5	$55.42^{a}{}_{1}$	$30.72^{b}{}_{1}$	1.604	***
L.S.D.	1.632	1.258		
Sign.	***	***		

B-Synersis

Enzyme conc. ml/100ml of milk	Calf rennet.	*Solanum dobium* extract	L.S.D.	Significant
	Wheying off (ml/100ml)	Wheying off (ml/100ml)		
1	$31^{a}{}_{4\blacktriangleleft}$	$23^{b}{}_{3\blacktriangleleft}$	5.317	*
2	$35^{a}{}_{3,4}$	$27^{b}{}_{2,3}$	5.317	*
3	$38^{a}{}_{2,3}$	$30^{b}{}_{2}$	7.519	*
4	$43^{a}{}_{1,2}$	$37^{b}{}_{1}$	5.069	*
5	$45^{a}{}_{1}$	$40^{b}{}_{1}$	7.346	non
L.S.D.	5.335	4.602		
Sign.	***	***		

*** : Very high significant

a , b : Means having different letters in the same raw significantly differed at $P \leq 0.05$ Sign. : Significant

$\blacktriangleleft$: Means having different small number in same column significantly differed at $P \leq 0.05$

التجبن الإنزيمى للبن باستخدام نبات *Solanum dobium* كبديل للمنفحة

١- دراسات أولية على المستخلص الإنزيمى من نبات *Solanum dobium* فى تجبن اللبن

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المستخلص الإنزيمى لبذور نبات *Solanum dobium* (S.D.E.) تم اختياره كبديل للمنفحة الحيوانية Calf rennet (C.R.) وقد تم إجراء بعض الدراسات الأولية لتقييم تأثير بعض العوامل المختلفة مثل درجات pH ودرجات الحرارة وتركيز الإنزيم وتركيزات مختلفة من كل من كلوريد الكالسيوم وكلوريد الصوديوم وذلك على النشاط التجبنى للإنزيم وكذلك تم دراسة صلابة الخثرة والتحلل البروتينى كما تم استخدام المنفحة الحيوانية للمقارنة. وكانت أهم النتائج المتحصل عليها هو أن المستخلص الإنزيمى لنبات *Solanum dobium* كان أقل حساسية لدرجات pH المرتفعة مقارنة بالمنفحة الحيوانية ومن ناحية أخرى فإن مستخلص (S.D.E.) كان أكثر تحملاً لدرجات الحرارة عن المنفحة الحيوانية (C.R.) كما إتضح من الدراسة إنخفاض زمن التجبن بزيادة تركيز كلاً من (S.D.E.) وأيضاً (C.R.) ولكن الإنخفاض فى زمن التجبن كان بدرجة أكبر فى حالة المستخلص الإنزيمى (S.D.E.) مقارنة بـ (C.R.).

كذلك عند إضافة كلوريد الكالسيوم بتركيزات مختلفة كان الانخفاض فى زمن التجبن أكثر وضوحاً فى حالة المستخلص الإنزيمى (S.D.E.) والذى اظهر أيضاً مقاومة للتركيز العالى من كلوريد الصويوم بمقارنة بالمنفحة الحيوانية (C.R.). كما أوضحت النتائج أيضاً إنخفاض معنوى فى صلابة الخثرة وزيادة معنوية فى معدل تحلل البروتين عند استخدام مستخلص (S.D.E.) مقارنة بالمنفحة الحيوانية (C.R.).