

Extraction and Characterization of *Solanum Dubium* (Gubbain) Fruit Extract

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Abstract: The objective of the present investigation was to extract, partially purify and characterize the enzyme from *Solanum dubium* for cheese making. The enzyme was extracted by distilled water, its clotting activity was determined and the effect of temperature, preheating of the extract, incubation temperature and enzyme concentration on coagulation time were studied. The results showed that *Solanum dubium* fruit extract reached its maximum activity when the fruits were dark yellow in colour and completely dry. *Solanum dubium* fruits crushed by laboratory mortar and soaked in distilled water for 24 hours had a highest activity. The milk clotting time increased with increasing temperature at which milk has been kept before addition of *Solanum dubium* extract. The higher the temperature of milk when *Solanum dubium* extract was added the shorter the coagulation time of milk. The results showed that with increasing crude *Solanum dubium* extract concentration, the time of clotting decreased. Keeping the extract at 70°C up to 10 minutes showed no detrimental effect on its milk coagulating power. The milk activity was completely destroyed when kept at 80°C for 20 minutes.

Key words: *Solanum dubium*, extraction, clotting activity.

INTRODUCTION

In the latter part of the last century, cheese consumption increased while the availability of calf rennet decreased, which led to rennet shortages and subsequent price increase. In addition, more restrictive ethical concerns associated with production of such animal rennet led to a search for suitable rennet substitutes for cheese making. Several proteases from animal, microbial and plant sources were investigated as likely substitutes and have been reviewed (Guinee and Wilkinson, 1992; Broome and Limsowtin, 1998; Fox *et al.* 2000).

Some plants of the family solanaceae such as *Solanum dubium* "Gubbain", which is a well known wild plant, grow wildly in many regions in Sudan during the rainy season. They had been tried for the extraction of milk-clotting enzymes (Ahmed *et al.*, 2009a; Ahmed *et al.*, 2009b; Habbani, 1992; Mohamed and Habbani, 1996; Osman, 1996; Osman, 2001; Talib *et al.*, 2009; Yousif *et al.*, 1996). Their research showed positive results in using this extract for manufacture of Sudanese white soft cheese. This research aims to study the extraction and characterization of milk clotting activity from *Solanum dubium* fruit.

MATERIALS AND METHODS

Plant Material:

The plant material used in this study was collected from Abu-Naama area, Sinnar State, Sudan. The *Solanum dubium* fruits were collected at different stages of maturity; from first stage in September when the fruits were small, green with white seeds till the last stage of maturity in April when the fruits were yellow, with black and completely dry seeds.

The *Solanum dubium* fruits were carefully cleaned and powdered using finger, laboratory mortar and electric grinder. The dry powder (5gm) was macerated with distilled water (30ml) and kept at 5°C for 15 minutes, 24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours and 168 hours, then filtered through filter paper.

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Determination of Crude Solanum Extracts Activity:

The activity of *Solanum dubium* fruit extract was determined according to the method described by Mohamed and Habbani (1996). One ml of the *Solanum* extract was pipetted into a glass tube containing 10 ml skim milk, placed in a water bath at 37°C, and continuously examined for the first onset of coagulation. Clotting activity was determined according to the following equation:

$$\text{Activity (U)} = \frac{\text{Volume of extract}}{\text{Clotting time (seconds)}} \times 100$$

The unit of activity (U) is defined as the number of ml of extract required to clot 10 ml of skim milk solution in 100 seconds at 37°C.

Properties of Solanum Dubium Extract (Raw):

Effect of Milk Temperature on Time of Coagulation:

The milk was brought to different temperatures starting from 35°C to 80°C, then the extract was added and the time taken for the coagulation of milk at such temperature was recorded. In all cases, 1 ml of the extract was added to 10 ml of reconstituted skim milk.

Effect of Preheating of Milk on Solanum Dubium Activity:

The same volumes of milk were heated to different temperatures; 35°C, 40°C, 50°C, 60°C, 70°C and 80°C, kept at those temperatures for 10 minutes and then cooled. One ml of *Solanum* extract was added to 10 ml of the milk, and the time of coagulation was recorded at each temperature.

Effect of Incubation Temperature on Activity of Solanum Dubium Extract:

The activity of *Solanum dubium* extract was measured at various temperatures by adding 1 ml of *Solanum* extract to 10 ml of reconstituted skim milk and kept at different temperatures, and the time of coagulation at each temperature was recorded.

Effect of Heating the Solanum Dubium Extract:

In order to ascertain the change in activity of enzyme kept at different temperatures, the following experiment was performed. A series of small test-tubes each containing about 1.5 ml of *Solanum dubium* extract in water were immersed in a water-bath at the desired temperature. The tubes were gently shaken for half a minute and then stoppered so that no water could evaporate. After varying times of heating, each tube was plunged into an ice-bath which quickly stopped further destruction of the enzyme. The tubes were tilted and rotated horizontally to collect condensed moisture on the walls of the tube. The residual clotting activity was determined and compared to that of unheated solution.

Effect of Solanum Dubium Extract Concentration on its Activity:

Effect of concentration of *Solanum dubium* extract on activity was studied by two different methods. In the first experiment, different volumes (50 ml, 100 ml, 150 ml, 200 ml, 250 ml, 300 ml, 350 ml and 400 ml) of reconstituted skim milk were used for clotting study using the same concentration (1 ml) of *Solanum* extract. In the other experiment, to the same volume of milk (10 ml) different quantities (0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, 0.6 ml, 0.7 ml, 0.8 ml, 0.9 ml, 1 ml) of *Solanum dubium* extract were added for the curdling study.

RESULTS AND DISCUSSION

The highest activity ($P>0.05$) was obtained when *Solanum dubium* fruit was crushed using laboratory mortar (1.27 ± 0.23 U/ml), followed by fingers (0.90 ± 0.09 U/ml) and electric grinder (0.57 ± 0.08 U/ml). The slight loss of activity from the latter extract may be attributed to the combined effect of the mechanical process and the heat generated by the grinder. Mohamed and Habbani (1996) found that a milk coagulating substance was obtained from dry, finger crushed "Gubbain" berries showed no detectable enzymatic activity.

Figure 1 show the milk-clotting activity of *Solanum dubium* fruit soaked in distilled water for 15 minutes to 168 hours. The maximum milk-clotting activities were 2.26 ± 0.05 , 1.84 ± 0.04 , and 1.27 ± 0.10 obtained in 24, 48, and 72 hours of soaking the crushed plant materials. Analysis of variance showed significant differences ($P>0.001$) among time intervals. Similar results were found by Osman (1996) and Osman (2001).

Our results disagree with Habbani (1992), Mohamed and Habbani (1996) and Yousif *et al.* (1996) who found that maximum milk-clotting activity was obtained after 7 days of soaking the whole berries of *Solanum dubium*.

Effect of Stage of Maturity of *Solanum Dubium* Fruit on the Enzyme Activity:

Table 1 shows the relationship between stage of maturity of *Solanum dubium* fruit and the milk clotting activity. Activity significantly ($P>0.001$) increased from 0.03 ± 0.01 at stage 1 to 1.87 ± 0.38 U/ml at stage 8. The *Solanum dubium* fruit reached its maximum activity after the plant was completely dry and the fruit is dark yellow in color and dry (approximately 8 months after growing). These results are in agreement with Rifaat *et al.* (1970) who found that, in general the milk-clotting activity of the latex of the different varieties was affected by the stage of ripening. These activities seemed to be correlated with the degree of maturity of the fruits. However, the results are in disagreement with Whitaker (1958) who reported that the enzyme activity of the fruits (*Ficus carica*) was at its highest level when they were unripened green.

Effect of Milk Temperature on Coagulation Time:

Figure 2 illustrates the effect of milk temperature on coagulation time. The coagulation time gradually decreased from 51.28 ± 0.08 seconds at 30°C to 10 ± 0.00 seconds at 80°C . It may be observed that, the higher the temperature of milk when the *Solanum dubium* extract is added, the shorter the coagulation time.



Solanum dubium bush

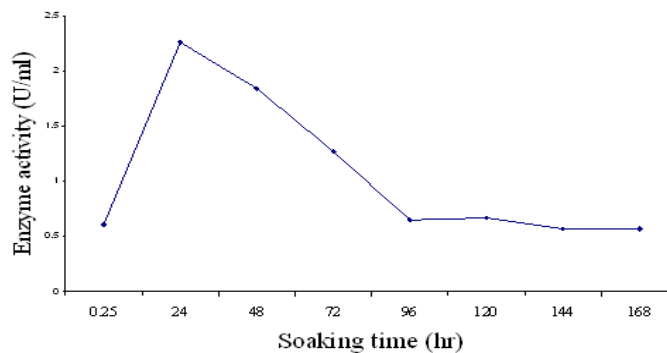


Fig. 1: Effect of soaking time of crushed *Solanum dubium* fruit in distilled water on milk clotting activity.

Effect of Preheating of Milk on Coagulation Time:

Temperature at which milk has been kept before addition of *Solanum dubium* extract also shows a considerable effect on coagulation time. As has been seen from the results in Figure 3 the coagulation time (seconds) increased with an increase in temperature at which the milk is preheated. Heating probably denatures the protein and consequently the coagulation time is lengthened. Krishnamurti and Subrahmanyam (1948) found the same results when using milk coagulant enzyme of *Ficus carica* Linn. The effect seems to be more pronounced in the case of animal rennet which is not able to coagulate cow milk heated to $70-80^{\circ}\text{C}$.

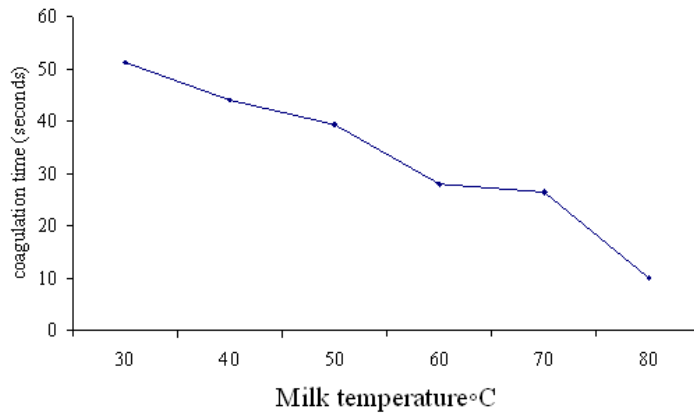


Fig. 2: Coagulation time as affected by temperature of milk treated with *Solanum dubium* extract.

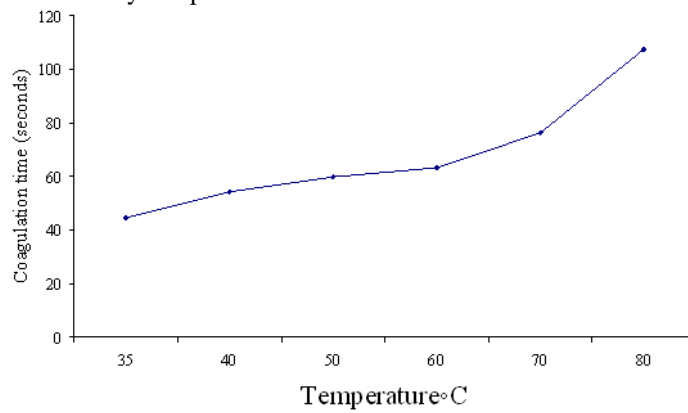


Fig. 3: Coagulation time as affected by preheated milk treated with *Solanum dubium* extract.

Table 1: Effect of maturity stage of *Solanum dubium* fruit on enzyme activity.

Stage of maturity	Activity U/ml
Stage 1	0.03 ^a ±0.01
Stage 2	0.04 ^a ±0.01
Stage 3	0.13 ^a ±0.08
Stage 4	0.25 ^b ±0.09
Stage 5	0.30 ^b ±0.09
Stage 6	0.53 ^c ±0.14
Stage 7	0.71 ^d ±0.11
Stage 8	1.87 ^e ±0.38
L.S	***

Means in the same column with same superscripts are not significantly different (P<0.05).

*** = (p> 0.001)

L.S= Level of significance

Effect of Incubation Temperature on Coagulation Time:

Results from Figure 4 show that the milk-clotting time decreased with increasing incubation temperature (P>0.05), with a weak curd at 80°C. The findings are similar to Walde *et al.* (1984) who reported that when temperature decreased, the clotting time of a crude extract of sunflower seeds (*Helianthus annus* L.) increased. However, Macedo *et al.* (1993) pointed that; the best coagulation temperature is 27 to 29°C for extraction of cardoon flower (*Cynara cardunculus* L.).

Heat Stability of the *Solanum Dubium* Extract:

It may be seen from the results in Table 2 that keeping the extract at 70°C up to 10 minutes did not have any detrimental effect on milk coagulation power. The milk coagulum was weakened at 80°C when kept for 20 minutes, and the activity of *Solanum dubium* fruit extract was completely destroyed at 90 and 100°C.

Compared with animal rennet, the enzyme is more stable and resistant to heat, the former being completely destroyed even at 55°C. This result is in accord with Osman (1996) who found that milk-clotting activity was lost after wet-heating of *Solanum dubium* fruit extract at 80°C for 10 minutes, and after dry-heating *Solanum dubium* fruits extract at 100°C for 24 hours, the present activity remained decreasing until it reached a very low value at 100°C. Both calf and microbial rennets from a culture of *Bacillus cereus* showed a high degree of inactivation after heating for three minutes above 55°C and 65°C, respectively (Melachouris and Tuckey 1967). Pascaline and Daniel (2006) found that *Mucor miehei* and *Mucor pusillus* proteases are much more stable at 53°C when heated for 100 minutes, while *Endothia parasitica* protease is rapidly inactivated at 53°C in 10 minutes. Nouani *et al.* (2009) found the enzyme extracted from *Mucor pusillus* to be stable at 30-50°C and it was completely inactivated by heating for 30 minutes at 65°C. The enzyme from glutinous rice wine mash liquor was completely inactivated by heating for 20 min at 60 °C (Wang *et al.*, 2009).

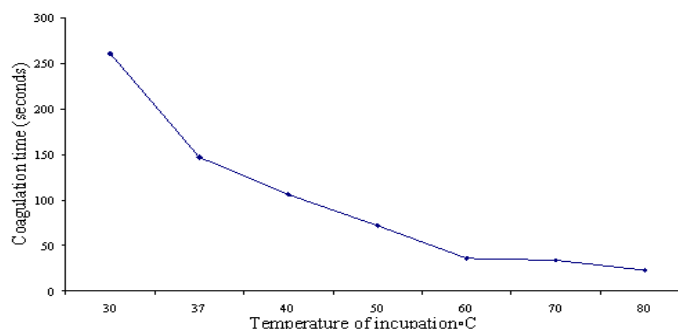


Fig. 4: Effect of incubation temperature on milk coagulation time.

Table 2: Effect of thermal treatment on *Solanum dubium* extract activity (U/ml).

Temperature (°C)	Time (minutes)			
	0	10	20	30
30	1.03±0.00	1.03±0.00	1.03±0.00	1.03±0.00
40	0.80±0.05	0.70±0.05	0.61±0.03	1.03±0.02
50	0.75±0.04	0.69±0.06	0.53±0.06	0.59±0.00
60	0.67±0.01	0.65±0.01	0.63±0.03	0.56±0.03
70	0.62±0.01	0.49±0.02	0.32±0.01	0.50±0.03
80	0.33±0.00	0.1±0.00	0.01±0.00	0.26±0.03
90	0.004±0.00	0.004±0.00	0.004±0.00	0.004±0.03
100	0.004±0.00	0.004±0.00	0.004±0.00	0.004±0.03
L.S	***	***	***	***

*** = (P >0.001)

L.S = Level of significance

Enzyme Concentration:

Effect of increasing enzyme concentration on time taken to coagulate the same volume of milk (10 ml) is shown in Figure 5. The results showed that with increasing crude *Solanum dubium* extract concentration, the time of clotting decreased from 18.53±0.76 to 1.05±0.05 minutes. Similar results were obtained by Ahmed *et al.* (2009b), Osman (1996) and Habbani (1992).

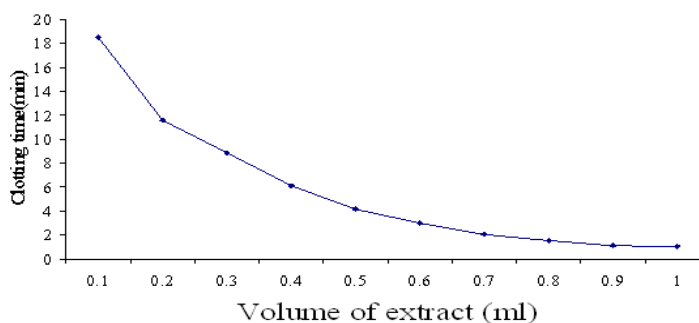


Fig. 5: Effect of *Solanum dubium* extract concentration on milk coagulation time.

Conclusions:

The extraction of the enzyme by distilled water produced higher activity, and increase in soaking time reduced the activity. As the fruit changes to dry yellow the activity increases. While milk temperature (up to 80°C) increases activity, preheating temperature (up to 80°C) decreases the activity, and the incubation temperature (up to 80°C) and volume of extract (up to 1 ml) increases the activity.

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