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POTENTIALITIES OF SOME OILSEED PROTEIN PRODUCTS AS NITROGEN SOURCES IN CULTURE MEDIA FOR THE PRODUCTION OF RENNIN-LIKE ENZYME FROM *Rhizomucor pusillus 1653*

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ABSTRACT

A comparative study on different culture media for the production of a rennin-like enzyme from *Rhizomucor pusillus 1653* was carried out for preparation of an economic medium to be used for production of rennin like enzyme from *Rhizomucor pusillus 1653*. The investigated media were prepared mainly from whey permeate supplemented with oilseed meals as sources of nitrogen. The oilseed meals included: sesame meal, sunflower meal, peanut meal, cottonseed meal, as well as two degossypolized cottonseed meals.

Results, revealed that the ratio of milk clotting activity (MCA) to proteolytic activity (PA) was taken as an index for the selection between different media. On this basis results showed that the milk clotting enzyme from *Rhizomucor pusillus 1653* was produced by all the media supplemented with oilseed meals in the following order: Sesame meal > sunflower meal > cottonseed meal (A:H:W)>cottonseed meal (acetic: ether)>cottonseed meal > peanut meal.

The activity of milk clotting enzyme resulting from whey permeate supplemented with sesame meal decreased rapidly as heat treatment increased over 55°C and pH 6.0. Furthermore, the enzyme activity decreased gradually as Nacl concentration increased but was improved greatly by increasing the calcium ion concentration. Utilization of the *Rhizomucor pusillus* rennet proved to be compatible with calf rennet for the preparation of Ras cheese slurry.

KeyWords: Oilseeds, culture media, *Rhizomucor pusillus*, rennin-like enzyme.

INTRODUCTION

The coagulant traditionally used for cheese-making throughout the world is the rennet extracted from abomasums of 10-30 days old calves (Otani,*et al*.,1991). However, interest in finding calf rennet substitutes, continues because of the worldwide shortage of calf rennet resulting from the increase in cheese making combined with a decline in the number of slaughtered calves (Green,1977). Milk clotting enzymes have been prepared from a great variety of sources, mainly microorganisms, such as: fungi, bacteria, actinomycetes, streptomyces (Abdel-Fattah *et al*.,1974 ;Foda et al.,1985; Selim *et al*.,1991 and Salem *et al* .,1998). The microbial enzyme from *Mucor pusillus* has been so far regarded as calf rennet substitute (Sternberg, 1977).

Introduction of reverse osmosis and ultrafiltration technology to the dairy industry has resulted in the reuse and recycling of whey to produce whey protein concentrate, leaving behind the whey permeate as the final dairy by-product.

Oilseed meals resulting from the oilseed industry in Egypt are not properly utilized, they go to the fodder industry. Oilseed meals are considered rich sources of proteins. The use of vegetable proteins as nitrogen sources in culture media for microorganisms is documented. Baerwald *et al*.,1972 used media containing soy meal or corn steep liquor as nitrogen sources for the production of alkaline protease by *Asp, ochraceus* .Various media were suggested and used for fungal protease production by (Fujishima & Suzuki,1970; Lee & Kim,1975 and Matsushima,1980). Most of the suggested media consisted of different carbon and nitrogen sources including molasses, sucrose, skim milk, modified wheat bran, soybean products and malt sprouts. Soybean extracts were used as culture media for the growth and toxin production of *Corynebacterium diphtheria*.(Taha and El-kholy, 1985).

The aim of this study was to prepare an economic medium for the production of a rennin-like enzyme using the fungi *Rhizomucor pusillus* 1653. To achieve this goal the nonutilized whey permeate was supplemented with different oilseed meal proteins as a cheap and available source of nitrogen, also the utilization of the whey permeate contributes decreasing to environmental pollution. The prepared media were investigated for the cultivation of *Rhizomucor pusillu*s and the rennin-like enzyme produced was tested for its milk clotting activity (MCA) and proteolytic activity (PA). The enzyme with the highest MCA/PA was chosen for further investigation. The effect of pH, temperature, salt concentration, and calcium concentration on the activity of the purified enzyme was carried. The prepared rennin-like enzyme from *Rhizomucor pusillus* cultivated on whey permeat supplemented with sesame meal, was used for the preparation of Ras cheese slurry, and the curd synersis was examined as a function of the rennet.

MATERIALS

1- Microorganism: *Rhizomucor pusillus* 1653 was obtained from CSIRO Food Research Center (FRR) Australia in the slant from.
2- Animal rennet: Calf. rennet (HA-LA) powder was obtained from Chr. Hansens Lab Denmark A/S,
3- Whey activation medium: Fresh sweet whey medium (T.S. 5%, pH 5.5) was obtained from Misr Food & Milk Co., Cairo, Egypt. Fungal strain was inoculated into the fresh whey medium and incubated at 30°C for 24 hours on a rotary shaker incubator 150 rpm.
4- Fresh whole cow milk (for preparation of Ras cheese slurry) was obtained from the local market.
5- Oilseed Meals

5.1- Sesame meal (SM): Was Prepared by first grinding the sesame seeds, then extraction with n-hexane, the hulls were then removed by floatation. The resulting sesame cake was again extracted with n-hexane in a Waring blender, and filtered. The filtered cake re-extracted several times in same manner until the residual oil in the meal did not exceed 1%.

The meal was spread to dry ground and sieved to pass an 80-mesh screen.

5.2- Sunflower meal (SFM): Sunflower seeds were dehulled, and kernels ground then extracted with n-hexane as in 5.1. resulting in sunflower meal.

5.3- Peanut meal (PM): Peanut were deshelled, blanched and ground then extracted with n-hexane as in 5.1 and 5.2. to give peanut meal.

5.4- Cottonseed meal: (CSM I): Supplied by El-Menya Ginning Co.., Egypt.

5.5- Cottonseed meal (CSM II): Prepared in the laboratory by dehulling, grinding of the kernels then preparation of the meal same as SM, SFM, and PM.

5.6- Cottonseed meal (CSM III): Cottonseed was dehulled, the kernels ground then subjected to azeotropic extraction using acetone hexane : water (53 : 44 : 3, v/v). Extraction was carried in a soxhlet extraction unit. The resulting meal was spread to dry, ground and sieved to pass 80 –mesh screen. This treatment was carried for the removal of gossypol.

5.7- Cottonseed meal (CSM IV): The cottonseed meal was prepared as before, then extracted three times with acetic acid : ether mixture 50 : 50 v/v, the residual acetic acid then removed by extraction three more times with ether saturated with water, then twice with anhydrous ether to remove last traces of water, meal was then spread to dry ground and sieved to pass 80 mesh screen. This treatment was carried for the reduction of gossypol.

METHODS

1- Media and production condition:

The following media were tested for their ability to support the production of rennin-like enzyme by *Rhizomucor pusillus* 1653. The tested media were composed principally of whey permeate supplemented with 25 g/L from meals of sesame, sunflower, peanut, and four cottonseed products as nitrogen sources. Conical flasks containing 75 ml of whey permeate together with nitrogen sources adjusted to pH 5.5 were sterilized then inoculated with 5% (v/v) from active medium of *Rhizomucor pusillus* 1653. The enzyme activity was detected in the cultures after four days, and reached its maximal value after five days under aerobic incubation conditions at 30°C on a rotary shaker at 150 rpm.

2- Assay for Rennin-like Enzyme Activity

The activity of the rennin-like enzyme was determined according to the method of (Berridge,1957 and Kawai & Mukai 1970). The milk clotting activity was calculated as soxhlet unit (SU) using the following equation.

$$MCA(SU) = \frac{2400 \text{ X } amount \text{ of milk}}{\text{Clot time (sec) X amount of enzyme}}$$

3- Assay of proteolytic Activity

The proteolytic activity was determined according to the method described by (Otani,*et al*.,1991) using 1% casein as the substrate.

4- Protein Content Determination

Protein content was measured colormeterically at 650 nm according to (Ohnishi and Barr,1978).

5- Purification of Enzyme

The culture supernatant was treated with ammonium sulfate (50-80% saturation). The precipitates were collected by centrifugation at 3000 rpm for 15 min. in a cooling centrifuge and dissolved in a minimum amount of distilled water. The precipitates were dialyzed against distilled water for 24 h. For further purification the precipitated enzyme was chromatographed on a column (45 x 2.5 cm) filled with sephadex G-100 (pharmacia, Uppsala, Sweden), eluted with 0.2 M citrate phosphate buffer pH 5. Fractions of 5 ml were collected at a flow rate of 0.8 ml/min and analyzed for protein and rennin-like enzyme (Salem,1995), enzyme containing fractions were pooled and stored at 4°C.

6- Optimum pH

Phosphate and citrate phosphate buffers ranging between 5.0 to 7.0 pH were used lower pH values were excluded because the coagulating effect on casein, the isoelectric pH of casein is 4.6 (Ismail, *et al* .,1978).

7- Effect of Calcium Chloride

To equal portions of reconstituted milk, anhydrous $CaCl_2$ was added in the percentage of 0, 200, 370, 700, 1100 and 1600 P.P.M. The milk clotting activity was measured.

8- Effect of Sodium Chloride

Various concentration of NaCl namely 0, 3, 5, 7.5, 10 and 15% were incorporated in the reconstituted skim milk to be tested for milk clotting activity.

9- Effect Of Heat Treatment On Purified Enzyme:

The purified enzyme was heated for 15 min at different temperature 40°, 45°, 50°, 55°, 60 and 65°C, then cooled to 35°C before being added to the milk at the renneting temperature.

10- Preparation of Rass Cheese Slurry:

Ras cheese slurry prepared once from fresh milk and the fungal rennet, then from fresh milk and calf rennet (control) were carried according to the method described by (Kristoffersen *et al* .,1967) with some modifications carried according to (Abd El-hamid *et al* .,1991).

The two salted unpressed, curds resulting from the aforementioned preparation after 24 hours, were mixed with 1 part of 4% sterilized sodium chloride solution (warmed to 45°C), then potassium sorbate 0.1% was added. The mixture was well blended in an electric blender for 2-3 min. The blended slurry was transferred into glass beakers, covered with aluminum foil. All treatments were incubated at 37°C for 7 days with daily agitation. The slurry was periodically sampled for analysis.

11- Analysis of Ras Cheese Slurry

11.1- Chemical Analysis

Moisture content and titratable acidity were determined according to (Ling,1963) and pH values measured using a digital pH meter.

11.2- Microbiological Analysis

Total viable count, proteolytic and lipolytic bacteria count mould and yeast count were estimated according to (Harrigan & McConce, 1966) coliforms were counted on MacConkey agar (Difco, 1966).

12- Water Holding Capacity Of Curd

The water holding capacity of curd was determined according to (Lawrence, 1959).

13- Analysis of Oilseed Meals

Moisture, oil, protein, ash, and fiber were determined according to (AOAC 1980)standard methods of analysis. Phytates were measured according to (Wheeler & Ferrel, 1971). Free gossypol measured as described by (Pons, *et al* ., 1958).

RESULTS AND DISCUSSION

1- Effect of Supplementation of Whey Permeate with Oilseed meal Protein

The chemical composition of the seven oilseed meals used to enrich the whey permeate with nitrogen is given in Table 1. It is clear that all oilseed meals are rich sources of protein. Protein content ranges from 44.6 to 57.21%, residual oil values are negligible. Ash content ranges between 3.35 to 9.20%, fiber from 0.85 to 8.2%, and nitrogen free extract 24.84 to 43.88%. Phytate content is high for all investigated meals except for peanut meal which is 5.63 mg phytate P/g meal.

Table (1): Chemical composition of oil seed meals used as nitrogen sources in culture media for the cultivation of *Rhizomucor pusillus* 1653

Meal	Protein %	Oil %	Ash %	Fibre %	NFE %	Phytate mg. Phytate P	Gossypol %
Seasame meal (SM)	54.01	1.05	3.35	3.2	38.84	14.5	none
Sunflower meal (SFM)	57.21	0.98	8.7	8.22	24.82	10.63	none
Peanut meal (PM)	51.13	0.86	4.9	4.63	38.48	5.63	none
Cottonseed meal (CSMI)	44.6	3.52	8	1.05	43.83	10	1.91
Cottonseed meal (CSMII)	46.02	1.11	9.2	1.01	42.66	10	1.91
Cottonseed meal (CSMIII)	46.56	0.89	8.91	0.85	42.79	9.85	0.03
Cottonseed meal (CSMIV)	52	0.99	9	0.91	37.1	9.91	0.08

All values are given on moisture free basis
NFE=nitrogen free extract
Gossypol measured as free gossypol
phytate calculated as mg phytate P/g meal

CSMI= Cottonseed meal (factory)
CSMII= Cottonseed meal (laboratory)
CSMIII= Cottonseed meal (A:H:W)
CSMIV= Cottonseed meal (acetic:ether)

Table 2. shows the effect of supplementation of whey permeate with different oilseed meals as nitrogen sources on the production of rennin-like enzyme from *Rhizomucor pusillus 1653*. It can be observed that all media used supported the production of rennin-like enzyme with different milk clotting activities (MCA). Highest MCA was recorded for the medium containing sesame meal SM (420 SU/ml). Following sesame meal came the sunflower meal SFM supplemented medium with a MCA value 192 SU/ml., lowest MCA was recorded for CSM II, CSM I, and PM supplemented media showing values for MCA 137, 144, 144 SU/ml, respectively. The two degossypolized meals showed higher MCA than the meals containing gossypol.

Table (2): Effect of oilseed meals as nitrogen surceases for the the production of rennin-like Enzyme from *Rhizomucor pusillus* 1653.

Culture medium	MCA Su/ml	PA Unit	PC mg/ml	Sp. MCA	Sp. PA
Whey	82	1.28	2.34	34.19	0.55
Permeate + SM	420	2.35	3.67	65.4	0.64
Permeate + SMF	192	2.57	3.75	51.2	0.69
Permeate + PM	144	2.47	4.08	35.29	0.61
Permeate + CSMI	144	2.26	3.37	42.73	0.67
Permeate + CSMII	137	2.35	3.18	43.08	0.74
Permeate + CSMIII	160	1.87	3.05	52.46	0.61
Permeate + CSMIV	169	2.3	3.89	43.45	0.59

Permeate Whey permeate
SM=Sesame meal
PM= Peanut meal
SFM=Sunflower meal
CSM=Cottonseed meal
MCA=Milk Clotting Activity
CSMI = Factory
CSMII = Laboratory
CSMIII=A:H:W
CSMIV = acetic:ether
PA=Proteolytic Activity

On the other hand, categorizing the media supplemented with oilseed meal as to their efficiency for the production of rennin-like enzyme with clotting activity was done according to their MCA /PA index which is represented in Figure 1. It is clear that the meals can be categorized in the following order SM>SFM> CSM III> CSM IV> CSM I> CSM II> PM.

In an attempt to verify these results the following postulations are made.

Both sesame meal and sunflower meal are rich sources of sulphur amino acids, while cottonseed and peanut meals are limiting in the sulphur amino acids. Then perheps the sulphur amino acids played a role in the high production of enzyme by sesame and sanflower media. Rowe and Gilmour (1983) reported that amino acids are responsible for the induction of extracellular enzyme production.

Another attempt might be based on the phytate content and mineral content of SM, SFM, and PM. Phytate or phytic acid is the inositol

hexaphosphate, which is known to form complexes with minerals and proteins, thus rendering them unavailable for the body. Phytate ion complexes with metallic ion and in many cases form insoluble compounds. Complexes with Ca, Fe, Zn are insoluble and a combination of Ca and Zn forms an even less soluble compound. When proteins are complexed with phytates they are less subject to proteolytic digestion. (O'Dell & Boland, 1976 and Gifford & Clydesdale,1993). Most of the phosphorous content of oilseeds is in the form of phytin P (Altschul,1958,).

Sesame meal is reported to 2.29% Ca and its phosphorous content 1.39% (Altschul,1958,) which suggests that part of the Ca is still available. Sunflower meal contains 0.57% Ca and 0.58% P, but it is reported that sunflower contains more Ca than phytin phosphate and thus the assimilation of Ca is not impeded (Altschul,1958,). The same author reported peanut kernel to contain 0.12% Ca and 0.53% P, (Altschul,1958,) although the phytate content of peanut meal is only 5.93 mg phytin P/g meal yet the ratio of Ca: P suggests that all the Ca might be bound. Phytate content of the four cottonseed products is more or less the same but is relatively high (Altschul,1958,) reported cottonseed meal to contain in average 0.22% Ca and 1.655% P. this suggests that all Ca has been probably complexed with the phytate. (Laan *et al* .,1989 and Gomes *et al* ., 1992) recorded that the proteinase activity of *L lactis* are stimulated by Ca ions. Gomaa, 1981, proved that salts of Ca, Mg, Na, and phosphates to be among activators to increase the activity of protease. Other investigators found other activators resulting in increase in the activity of proteases, such as, phytin, oleic acid phospholipids and other (Fujishima & Suzuki,1970 and Bhunibhaman,1982).

Looking at the results of the cottonseed separately, another factor that could affect its use as nitrogen source for culture media of microorganisms, must be considered and that is the gossypol content. Gossypol is a bright yellow pigment that occurs in cottonseed and its products and its toxicity to monogastric animals is well-documented (El-Nockrashy, *et al* ., 1963). MCA and MCA/PA for rennin-like enzyme produced by *Rhizomucor pusillus 1653* cultivated on permeate media supplemented with nitrogen from CSM I, CSM II, CSM III, CSM IV, are 144, 137, 160, 169 SU/ml, respectively, and 63.70, 58.22, 74.20 and 73.64, respectively. These results show that the two cottonseed products with the reduced gossypol content CSM III and CSM IV exhibited higher enzyme activity which suggests that gossypol could act as an inhibitor to enzyme activity.

Kawai & Mukai, 1970 reported the MCA/PA of three commercial rennets, one from animal source and two from microbial sources. The MCA/PA of the *Rhizomucor pusillus* rennet was 62% that of the animal rennet (MCA/PA of animal rennet 167) and superior to the two microbial rennets. Other sources of nitrogen investigated compares well with commercial microbial source.

Therefore the rennin-like enzyme produced by *Rhizomucor pusillus 1653* cultivated on a medium consisting of whey permeate supplemented with sesame meal was chosen for purification and further investigation.

2- Rennin-like Enzyme Purification

The ammosulphate precipitated enzyme (50-80%). On dialysis yielded 53.85% recovery and 7.18 fold purification of the enzyme. When the ammonium sulphate precipitated enzyme was chromatographed on a column of Sephadex G-100, the enzyme was eluted as a single peak as shown in Figure 2, Results from a typical purification procedure show that the enzyme was purified 12.26 fold with a total yield of 16.99% of the original activity (Table3)

Table (3): Purification of extracellular Rinnen-like enzyme from *Rhizomucor pucillus* 1653

Purification steps	Volume ml	MCA su/ml	P.C Mg/ml	Sp. MCA	Total activity unit	Recovery %	Purification factor
Crude culture filtrate	100	240	3.67	65.4	24000	100	1
Ammonium sulph. 50-80%	7	1846.2	3.93	469.77	12923.4	53.85	7.18
Sephadex G100	45	90.6	0.113	801.77	4077	16.99	12.26

Table (4) Effect of *Rhizomocur pusillus* 1653 rennet and calf rennet on the total solids (TS), Acidity, and pH of Ras Cheese Slurry during incucbation at 37 C for 7 days

Rennet	Incubation period (days)	T.S%	Acidity%	PH
Calf Rennet	0	36.77	0.54	6.2
	3	37.16	0.63	6.0
	5	37.79	0.79	5.8
	7	38.18	0.92	5.4
Fungal Rennet	0	35.65	0.55	6.2
	3	36.29	0.67	5.9
	5	36.89	0.84	5.6
	7	37.61	0.97	5.2

3- Optimum pH

Figure 3 shows the changes in milk clotting activity as affected by the pH of the milk. It is obvious from the figure that there was a proportional decrease in the clotting activity of the fungal enzyme and animal rennet used as control, with the gradual increase in pH of milk. Where the fungal enzyme exhibited RMCA against the pH change as compared with control. These results agree with those reported by (Salem,1995).

4- Effect of Calcium Chloride Concentration

As illustrated in Figure 4, it can be observed that there is a positive proportional relationship between RMCA of the purified enzyme and the

$CaCl_2$ within the range tested from 0 to 1000 P.P.M. These results agree with (Salem *et al*., 1998).

5- Effect of Sodium Chloride Concentration

The data in Figure 5 indicate that the RMCA of the purified enzyme decreased as the concentration of NaCl increased. The RMCA decreased from 100% to 74% with the addition of 3% while the RMCA decreased from 100% to 54% in case of calf rennet as control. However, the RMCA declined at higher concentration of NaCl to reach 26% and 20% at 15% salt for fungal purified enzyme and calf rennet, respectively. These results agree with (El-Tanbouly & Selim,1995 and Salem *et al*., 1998).

6- Effect of Heat Treatment

Figure 6 demonstrates the effect of heat treatment at different temperature, on the purified enzyme activity. It is obvious that the rise in temperature has a negative effect on the enzyme activity. The decrease was slight and gradual up to 55°C, after which a sharp decrease is recorder. Heat treatment at 65°C for 15 min caused complete inactivation of the enzyme.
These results are in agreement with (Otani *et al*.,1991; El-Tanbouly & Selim,1995; Salem,1995 and Salem *et al*., 1998).

7- Utilization of *Rhizomucor pusillus* rennet in the preparation of Ras Cheese Slurry.

Previous result revealed that the rennet resulting from the fungus *Rhizomucor pusillus 1653* showed promising results as a calf rennet substitute, therefore, the actual efficiency of this rennet substitute was examined through its utilization in the preparation of Ras cheese slurry.
Analysis of Ras cheese slurry prepared from fresh milk and the rennet substitute resulting from *Rhizomucor pusillus* together with that prepared from fresh milk and calf rennet (used for comparison) are represented in Table 4.
Total solid content increased with increasing period of incubation for both the tested slurry control. Total solid content of control is generally slightly higher than that of tested slurry. The lowest value was recorded at zero time for the slurry with the rennet-substitute. Fresh slurries of the tested curd and control had same pH values and acidity. Generally, pH values decreased while acidity increased during the incubation period. This might be due to the conversion of residual lactose into lactic acid. These results agree with those of (Abd El-Hamid *et al*.,1991 and El-Sayed & Abbas,1992).

Table 5 gives the results of the microbiological analysis carried on the two Ras cheese slurries including the tested slurry and the control. The total number of bacteria increased throughout the incubation period to reach their maximum value after five days, and decreased thereafter. Similar results were reported by (Kristoffersen *et al*.,1967 and El-Sayed & Abbas,1992). Coliforms were not detected in all slurries either when fresh or during incubation. Both the lipolytic and proteolytic bacterial counts of the two slurries increased gradually during the incubation period, probably due to the

suitable conditions for their growth during the incubation period. Counts on yeast extract agar media increased during incubation to reach the maximum at the end of the incubation period for the control and the test rennet-substitute slurry. Based on the color and shape of colonies, and on microscopic examination of organisms, yeast appeared to be the only microorganism present, and no moulds were detected throughout the incubation period. This is due to the antifungal effect of potassium sorbate used as a preservative. This results are in agreement with those (El-Sayed & Abbas,1992 and Salem, 1995) .

By investigating the curd synersis as a function of the rennet sources, it was noted that the synersis rate of the *Rhizomucor pusillus* rennet was about 90% or more that of the control (Figure 7). This indicates that one of the important curd properties of the resultant microbial rennet is comparable to that of the animal rennet, and thus could be used successfully for cheese production. Similar results were recorded by (Aboel Naga,1984 and Salem, 1995).

In the meantime the difference in the whey drained from both curds is not so great, which proves that the *Rhizomucor pusillus 1653* rennet can compete with the calf rennet in the production of cheese.

Table (5): Effect of *Rhizomocur pusillus* 1653 rennet and calf rennet on the total viable counts, coliforms, lipolytic, yeast and mold counts in Ras Cheese Slurry during incubation at 37 C for 7 days

Rennet	Incubation period (days)	Microbiological Count				
		CFU/g				
		TVC/g	CC/g	PC/g	LC/g	Y&MC/g
Calf rennet	0	9x10^6	ND	14x10^4	15x10^3	8x10
	3	36x10^6	ND	65x10^4	43x10^3	32x10
	5	88x10^6	ND	83x10^4	177x10^3	84x10^2
	7	78x10^6	ND	12x10^5	38x10^4	86x10^2
Fungal Rennet	0	15x10^6	ND	16x10^4	11x10^3	9x10
	3	53x10^6	ND	90x10^4	59x10^3	45x10
	5	96x10^6	ND	13x10^5	23x10^4	93x10^2
	7	86x10^6	ND	23x10^5	45x10^4	96x10^2

TVC = total viable count **PC = proteolytic count**
CC = coliform count **LC = lipolytic count**
Y&MC=yeast & mold count **ND = not detection**

REFERENCES

Abdel-Fathah, A.F., El-Hawary, N.M. and Amr, A.s. (1974) Milk-clotting enzymes of some streptomyces species, Acta Microbiol, Pol. 6, 27-32.

Abdel-Hamid, L.B., Mahran, G.A., Abbas, H.A. and Hofi, A.A. (1991) Ample production conditions for Ras cheese slurry, Egyptian J. Dairy Sci. 19, 327.

Aboel-Naga, F.M. (1984) Direct acidification in hard cheese making. ph. D. Thesis. Faculty of Agric. Ain Shams Univ. Egypt.

Altschul, A.M. (1958) Processed plant protein foodstuff, Academic press New York 441, 442, 499, 547, 567, PP.

AOAC "Association of official Agricultural chemist" (1980) Official Methods of Analysis, 13th Ed. Washington D.C.

Baerwald, G., Jahn, G. and Metzner, B. (1972) Fermentative preparation of an alkaline protease., Brit. 1, 286, 880 (C.F. Chem. Abst. 77 (19), 124798 a (1972).

Berridge, N.J. (1957) In " Methods in Enzymology" Vol. 2. Academic Press, New York P. 69-77.

Bhunibhaman, O. (1982) Precipitation and characteristics of acid protease of *Asperagillus Pheonicia* produced with and without surfactant in the culture medium, Thai J. Agric. 15, 157. (C.F. chem. Abst. 97 (25) 21115 z, 1982)

Difco (1966) Mannual of dehydrated culture media and reagents for microbiological and clinical Laboratory procedures 9th ed Difco Lab. Michigan U.S.A.

El- Sayed, N.H. and Abbas, H.M. (1992) Effect of milk type on the properties of Ras cheese slurries, Egyptian J. Dairy Sci., 21, 163-170.

El-Nockrashy, A.S., Lyman, C.M. and Dollahite, J.W. (1963) The acureorol toxicity of cottonseed pigmient glouds and intraglauduloo pigmeint, J. Am. Chemists Soc, 40, 14-17.

El-Tanboly, E.E. and Selim, M.H. (1995) Studies on milk-clotting enzymes produced by streptomyces II purification and properties of milk-clotting enzymes from *streptomyces ramoseus* NRRL 12907, J. Agric Sci. Mansura Univ, 20, 4731.

Foda, M.S., Salama, H.S., Farid, M. A. and Shaker, H.M. (1985) Formation, physiology and characterization of extracellular protease produced by *Bacillus thuringiensis* var *galleriae*, Egypt J. Microbiol 20, 129.

Fujishima, T. and Suzuki, M. (1970) Production of protease. Japan patent 7009931 (CF. Chem. Abst. 73 (5) 23903 t, (1970).

Gifford, S.R.S. and Clydesdole, F.M. (1993) Effect of Varying concentrations of phytate, calcium and zinc on the solubility of protein, calcium, zinc and phytate in soy protein concentrate, Journal of Food Protection 56, 42-46.

Gomaa, E.G. (1981) Production of protease and mycelium by propagation of edible mashrooms as food wastes Ph.D Thesis. Faculty of Agriculture, Alex. Univ. Egypt.

Gomez, R.R.L. and Pelaez, C. (1992) Caseinolytic activity of *Lactococcus lacts* sub. Sp *lactis* study of different culture media. 2 Lebensm Unters Forsd, 195, 569-571.

Green, M.L. (1977) Review of the progress of dairy science: Milk coagulants, J. Dairy Res. 44, 159-188.

Harrigan, W.F. and McConce, M.E. (1966) Laboratory methods in Microbiology, Academic Press, London and New York.

Ismail, A.A., Foda, M.S. and Khorshid, M.A. (1978) Purification and properties of Rennin lilk enzyme from *Aspergillus ochraceus*, Zbl. Bakt. II. Abt., Bd, 133, 321-328.

Kawai, M. and Mukai, N. (1970) Studies on milk clotting enzyme produced by *Basidiomycetes*. I. Screening tests of *Basidiomycetes* for the production of milk-clotting, enzymes, Agric. Biol. Chem. 34, 159-163.

Kristoffersen, T., Mikdajcik, E.M. and Gould, I.D. (1967) Chedder cheese flavour, IV. Directed and accelerated ripening process, J. Dairy Sci, 50, 292.

Laan, H., Smid, E.J., Paris STT and Konings, W.N. (1989) Enzymes involved in the degradation and utilization of casein in *lactococcus lactis*, Neth. Milk Dairy. J. 43, 327-345.

Lawrence, A.J. (1959) Syneresis of rennet curd. Aust. J. Dairy Technol. 14, 166.

Lee, J. H. and kim, K.W. (1975) Study on meat tenderizer part II. Tenderizating ability of enzyme from *Asperigelleus oryzae*, Hauguk Sikpium Kwahakhoe Chi 7, 229-237 (C.F. Chem. Abst. 84 (17), 120016r 1976).

Ling, E.K. (1963) A text Book of Dairy Chemistry vol. II. Charmam and Hall Ltd-London.

Matsushima, K. (1980) Ecological study on the proteolytic enzyme of microorganisms part I. Features of proteolytic enzyme system of penicillum. Hippon kagaku kaishi 54 (10) 871-3 (C.F. Chem. Abst. 94 (13) 99347e, 1981).

O'Dell, B.L. and Boland, Anade (1976) Complexation of phytate with proteins and cotions in corn Green and oilseeds, J. Agric. Food Chem. 24, 804-808.

Ohnishi, S.T. and Barr, J.K. (1978) A simplified method of quantitating protein. The buiret and phenal reagents, Anal Biochem. 86, 193-200.

Otani, H. Iwagaki, M. and Hosono, A. (1991) The screening of trees having milk clotting activity, Anim. Sc. Technol. (Japan) 62, 417.

Pons, W.A. Jo., Piiman R.A. and Holfpauir, C.L. (1958) 3- Amino –1 propanol as a complexing agent in the determination of gosypol., J. An. Oil Chemist. Soc. 35, 93-97.

Rowe, M.T. and Gilmour, A. (1983) Nutritional factors affecting extracellular enzyme production by *pseudomon* as *fluorescens* B 52, Milchwisswn – schaft 38, 705-707.

Salem, M.M (1995) Studies on the production of microbiel rennet. Ph.D. Thesis. Faculty of Agreic Ain Shams Univ. Egypt.

Salem, M.M., Murad, H.A. and El-Tanboly, E.E. (1998) Asalt tolerant strain of Mucor spp. For production of milk-clotting enzyme from salted whey, Egyptian J. Dairy Sci., 26, 39-50.

Selim, M.H., El-Shafei, A.M., Tawfek, N.F and Sharaf, O.M. (1991) Milk-clotting enzyme from *Bacillus stearothermophiluss*. Egyptian J. of Microbiology 26, 403-413.

Sternberg, M.Z. (1977) Microbial Rennets, Adv. In Applied Microbiology 20, 135-157.

Taha, F.S and El- Kholy, S. (1985) Soybean extracts as culture media for the growth toyui production of *Corynebacterium dephtheriae*, J. of the Egyptian public Health Association. Vol Lx, No 1,2.

Wheeler, E.L. and Ferrel, R.E. (1971) A method for phytic Acid Determination in wheat and wheat fractions, Cereal Chem. 48, 312-320.

الإستفادة من المنتجات البروتينية لبعض البذور الزيتية كمصدر نتروجينى فى البيئة لإنتاج إنزيم مشابه للرنين بإستخدام فطر *Rhizomucor pusilus 1653*

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أجريت تلك الدراسة للتعرف على تأثير اختلاف تركيب البيئات على انتاج الانزيمات المجبنة للبن بواسطة فطر *Rhizomucor pusillus 1653* وذلك للحصول على افضل بيئة من بينهم لاستخدامها فى انتاج الانزيمات المجبنة للبن .

حيث تم تنمية الميكروب على بيئة الشرش كبيئة اساسية للانتاج ثم استخدم البرمييت المضاف اليه كسب البذور الزيتية والغنية فى البروتين مثل كسب السمسم ، عباد الشمس ، الفول السودانى ، والقطن المحتوى على الجوسيبول والمعامل لازالة الجوسبيول .

ولقد اوضحت النتائج أن النسبة بين النشاط النوعى للانزيمات المجبنة للبن والمحللة للبروتين التى استخدمت كاساس للتفرقة بين البيئات المختلفة اوضحت أن هذه النسبة كانت اعلى فى حالة البيئة المدعمة ببروتين السمسم ثم عباد الشمس وكانت اقلها البيئة المدعمة ببروتين الفول السودانى .

هذا ولقد اظهرت النتائج أن النشاط النسبى للانزيم الناتج المنقى تناقص بسرعة عند معاملة الانزيم عند درجة حرارة اعلى من ٥٥ ْم وكذلك عند درجة pH اعلى من pH 6 كما لوحظ ايضا أن النشاط النسبى تناقص تدريجيا بزيادة نسبة ملح كلوريد الصوديوم المضاف وعلى العكس من ذلك تزايد النشاط النسبى بزيادة نسبة كلوريد الكالسيوم المضاف .

وقد استخدم الانزيم الناتج فى تصنيع الـ slurry وذلك بالمقارنة مع المنفحة الحيوانية.