

A STUDY OF MILK CLOTTING ACTIVITY OF CRUDE GASTRIC RENNIN EXTRACTED FROM CAMELS' ABOMASUM AT DIFFERENT AGES

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Abstract

Camel rennet was extracted from camel calf stomachs by the method used for bovine rennet. Coagulation of camel milk for the production of cheese has been proved to be difficult by the use of the available commercial rennet. Therefore that study was focused on the use of crude gastric enzymes extracted from different parts stomach at different ages (1, 3 and 5 years old). The non-purified gastric enzymes extracts from camels (GEC) different parts of stomach and at different ages were characterized by their rennin units (RU/g), milk clotting time (Sec.) and proteolytic activities. The conditions of milk clotting by the crude gastric enzymes extracted were optimized at different pH 5.5, 4.5, 4.0, 3.5 and rennin units (RU/g). The results concluded that the extracting solution from the behind part of the stomach was the greatest rennin units (RU/g), milk clotting time (Sec.) and proteolytic activities compared with the extracting solution from the front part of the stomach pH4.5. The good substitute for calves' rennet can be obtained from the dried vells of camel and can be named adult camel rennet.

Keywords: Camels rennin, Camels milk, Different extracting solutions.

INTRODUCTION

For centuries, calf rennet has been used as a milk coagulant in the production of all varieties of cheese. Rennet is used in medicinal products as well as for the manufacture of lactose. In Egypt and in the rest of the Arabic region the majority of rennet used is from animal sources. Commercial calf rennet consists mainly of two enzymes chymosin and pepsin. The relative proportion of the two enzymes varies with the age of the animal. The major, milk-clotting component of standard rennet is chymosin (88 to 94%), although mature animal rennet may contain up to 90 to 94% of pepsin and only 6 to 10% of chymosin (Broome and Limsowtin, 1998). Compared to chymosin, pepsin has number of disadvantages such as longer clotting time, forming a soft curd, and an undesirable taste. Another important factor with respect to cheese technology is the clotting power of proteolytic enzymes. The clotting activity affects the properties of the curd, such as firmness or softness, during processing (Dejmek and Walstra, 2004, Walstra, *et al.*, 2005). Imperfect rennet manufacturing, defects in packing and improper storage conditions may result in changes in the

clotting activity of these enzymes. Good quality rennet should possess a constant clotting activity and contain no other enzymes than chymosin. In addition, rennet should not contain any microorganisms that produce gas and acid since these might cause serious problems in the final product, such as defects in taste & flavor, putrefaction, disintegration and blowing (Beresford, 2003, Upadhyay, *et al.*, 2004). Camel milk requires more calf rennet than cow milk to coagulate and the relative amount of rennet needed varies widely (Ramet, 2001). Extracts of adult camel abomasums have been used to coagulate cow milk with success (Fox *et al.*, 2000). However, these enzymes have not been tried on camel milk. Rennet extracts from lamb and cow calves were found to be more effective with the milk of the respective species (Kappeler,*et al.*,2006), while pig chymosin and pepsin respectively, were found to have a higher milk clotting activity in pig milk than in cow milk (Bansal, *et al.*,2009). Accordingly, it would not be surprising if camel rennet is more effective on camel milk than calf rennet.

The average activity loss per month is relatively high as compared with imported rennet. When rennet is kept at high temperatures, pepsin activity increases in whey proteins. As a result, some defects in taste, flavor and melting problems in the cheese structure may occur (Hooydonk& Van Den Berg, 1988). Therefore, the ratio of chymosin to pepsin and the microorganism load in rennet are of great importance in cheese technology. Microorganism activity in rennet may cause decrease in the activity and a variety of defects in the cheese (Guinee and Wilkinson, 1992,). Questions are still raised as to their hygienic quality (Ec, 2002). Research is needed for assessing the safety of rennet obtained from calves and adult cattle in Egypt with regard to contamination risks including those resulting from the method of harvesting, contamination with feed, feed bans, the risk from cross-contaminated feed, and geographical sourcing. This work was therefore aimed for extracting camel rennet and testing its ability to coagulate camel milk and proteolytic activity at different ages during storage period.

MATERIALS AND METHODS

Experimental procedure:

The camel stomach tissues were obtained from Butcher in Kafr El-Sheikh, The abomasums were obtained from camels at different ages 1, 3 and 5 years. The stomach tissues were cleaned with running water, (average weight of fresh stomach was 1200g), blowed salted, dried at room temperature, which varied from 20-25°C for 25 to 30 days (average weight of dried vells was 400 g with drying ratio of 32.91%), then cut into small pieces as reported by Fahmi and Amer (1962). The extracting solution was prepared to contain 3% boric acid, 0.5% sodium benzoate and 8% sodium chloride at pH about 5.5. The obtained solution was divided into 4 parts. To

each part, HCL was added to give pH value of 5.5, 4.5, 4.0 and 3.5. Dried fourth stomachs pieces were then added to each part at the rate of 20% (w/v). The extraction was carried out for a period of nine days with daily stirring to mix the tissues in the mixture.

During the extraction period, milk clotting activity (MCA) and proteolytic activity (PA) were determined and the pH was adjusted daily 5.5, 4.5, 4.0 and 3.5 for different treatments. After the end of the extraction period, other steps of the preparation were continued as reported by Fahmi *et al.*, (1979). Each one of the obtained extracts was divided into two parts. One was kept in the refrigerator at $7 \pm 1^\circ\text{C}$ and the other at room temperature ($25 \pm 2^\circ\text{C}$).

The MCA, PA, and pH values were determined every month up to four months.

Methods of analysis:

Milk clotting activity expressed as rennin units per g (RU/g) was determined using reconstituted skim milk according to Fahmi and Amer (1962).

Proteolytic activity of the extracts was measured by the method of Davis and Smith (1955). It was expressed as micrograms of tyrosine released from casein per one minute by 1 ml of enzyme solution. The colour developed was measured at 500nm.

The pH values were measured using a pH meter (HANNA instruments model 8417, USA).

Statistical analysis:

All experiments were performed with three replicates each. All data were reported as means with standard error. An analysis of variance (ANOVA) was applied to assess differences among the "Gastric Enzyme Extract from Camels" (GEC) at different ages by using SPSS (Statistical Package for the Social Sciences) SPSS (1998).

RESULTS AND DISCUSSION

Data given in Table 1 showed the milk clotting time (Sec.) and rennin units (RU/g) of the extracts from dried camel abomasums were affected by days and pH of extracting solution and all data were significantly different ($p \leq 0.05$). Milk clotting time (MCT) throughout the extraction, period was the lowest at pH 4.5, greatest at pH 5.5 compared with pH 4 and 3.5. On the other hand, the extracting solution from the behind part of the stomach had the greatest rennin units (RU/g) compared with the extracting solution from the front part of the stomach at pH 4.5 at three days of extracting solution. While the milk clotting time (Sec.) and rennin units (RU/g) increased after nine days of extracting solution from the other parts of the stomach.

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Table 1. Effect of days extracting solution pH of dried camel abomasums on the milk clotting time Sec. (MCT) and rennin units (RU/g).

pH values	Variance	3 days				5 days				7 days				9 days			
		Front part		Behind part		Front part		Behind part		Front part		Behind part		Front part		Behind part	
		MCT	RU/g														
5.5	Max	675	15.2	190	55.6	570	17.8	140	74.1	520	20.0	134	76.9	490	20.8	130	78.1
	Min.	660	14.8	180	52.6	560	17.5	135	71.4	500	19.2	130	74.1	485	20.4	128	76.9
4.5	Mean. ± SE	668 ± 3.21 a	14.9 ± 0.51b	185 ± 1.21 b	54.1 ± 1.54 a	567 ± 2.35 a	17.6 ± 0.52 b	137 ± 0.62 b	64.2 ± 1.42 a	510 ± 3.54 a	19.1 ± 0.65 b	133 ± 0.54 b	75.2 ± 1.58 a	485 ± 2.11 a	20.6 ± 0.23 b	129 ± 0.68 b	77.2 ± 0.96 a
	Ma. Min.	570 555	18.0 17.5	155 148	67.6 64.5	540 512	20.4 19.2	132 128	78.7 78.1	480 455	21.9 20.8	108 104	95.2 89.3	450 430	23.7 22.2	107 100	95.2 90.9
4.0	Mean. ± SE	562 ± 2.63 a	17.8 ± 0.23 b	151 ± 0.98 b	66.3 ± 1.23 a	524 ± 1.97 a	19.7 ± 0.89 b	130 ± 0.54 b	77.8 ± 1.26 a	465 ± 2.89 a	21.5 ± 0.68 b	106 ± 0.42 b	92.4 ± 1.25 a	440 ± 1.96 a	22.8 ± 1.02 b	104 ± 0.42 b	93.2 ± 1.21 a
	Max. Min.	635 625	16.0 15.7	168 162	62.5 60.6	550 540	18.3 18.2	136 132	76.9 74.1	490 480	20.8 20.4	128 121	81.9 79.4	485 475	21.5 20.6	125 120	83.3 80.0
3.5	Mean. ± SE	628 ± 2.58 a	15.8 ± 0.21 b	165 ± 0.65 b	61.6 ± 0.96 a	544 ± 2.05 a	18.1 ± 0.67 b	134 ± 0.62 b	75.0 ± 1.25 a	485 ± 2.97 a	20.6 ± 0.85 b	125 ± 0.53 b	80.5 ± 1.34 a	480 ± 2.05 a	20.9 ± 0.68 b	123 ± 0.23 b	81.3 ± 1.32 a
	Max. Min.	650 640	15.6 15.4	182 175	57.1 54.9	560 542	18.0 18.3	146 142	71.3 68.9	520 510	20.0 19.2	138 121	78.1 74.1	490 475	21.0 20.8	135 125	80.0 74.1
3.5	Mean. ± SE	645 ± 2.97 a	15.5 ± 0.24 b	179 ± 0.75 b	55.9 ± 0.87 a	584 ± 1.89 a	18.1 ± 0.56 b	144 ± 0.75 b	69.8 ± 1.34 a	510 ± 2.98 a	19.7 ± 0.72 b	132 ± 0.49 b	75.9 ± 1.23 a	483 ± 2.21 a	20.9 ± 0.76 b	130 ± 0.33 b	77.0 ± 1.45 a

a, b, c Means within the same row with different letters are significantly different ($P \leq 0.05$).

Table 2 showed the effect of the extracting solution pH and age of dried camel abomasums the front part and behind part on the milk clotting time Sec. (MCT) and rennin units (RU/g) significantly ($P \leq 0.05$). The milk clotting time was the highest from the front part compared with the behind part at the age one year. Whereas, the rennin unit (RU/g) was the lowest from the front part compared with the behind part in all the extracting solution pH at 5.5, 4.5, 4.0 and 3.5. However, gastric enzyme extract at the age 5-years-old camel from the behind part of stomach showed a lower of the milk clotting time and highest of the rennin unit compared with gastric enzyme extract 1 and 3-years-old camel from the behind part of the stomach extracting solution at pH 4.5. On the other hand, gastric enzyme extract at 5-years-old camel from the front part of stomach showed the highest milk clotting time and the lowest of the rennin unit compared with gastric enzyme extract at 5-years-old camel from the behind part of the stomach extracting solution at pH 4.5. These results were agreed with those obtained by Boudjenah *et al.*, (2012) who reported that the now-purified enzyme preparations gastric enzyme coagulation obtained from the old camel's showed better coagulation activity.

Table 2. Effect of the extracting solution pH and age of dried camel abomasums on the milk clotting time Sec. (MCT) and rennin units (RU/g).

pH values	Variance	1 year				3 years				5 years			
		Front part		Behind part		Front part		Behind part		Front part		Behind part	
		MCT	RU/g										
5.5	Max.	675	15.8	185	55.6	530	19.6	162	64.5	490	20.8	140	78.1
	Min.	635	14.8	180	54.1	510	19.2	155	61.7	485	20.0	130	76.3
Mean. \pm SE		660 \pm 2.31 a	15.2 \pm 0.54 b	183 \pm 1.32 b	54.6 \pm 0.98 a	520 \pm 1.67 a	19.4 \pm 0.68 b	159 \pm 1.05 b	62.9 \pm 0.96 a	485 \pm 1.36 a	20.6 \pm 0.75 b	133 \pm 0.98 b	77.1 \pm 0.88 a
	Max.	560	18.2	175	60.6	465	21.9	148	71.3	455	23.3	110	95.2
4.5	Min.	550	17.9	165	57.1	455	21.5	140	67.6	435	22.2	105	90.9
	Mean. \pm SE	555 \pm 1.98 a	18.0 \pm 0.42 b	168 \pm 1.23 b	59.5 \pm 0.86 a	460 \pm 1.54 a	15.4 \pm 0.52 b	144 \pm 1.08 b	69.3 \pm 0.86 a	445 \pm 1.24 a	22.7 \pm 0.62 b	107 \pm 0.56 b	93.2 \pm 0.97 a
4.0	Max.	640	16.0	190	54.1	540	19.6	182	57.1	485	21.5	125	83.3
	Min.	625	15.6	185	52.6	515	18.3	175	54.9	465	20.6	120	80.0
Mean. \pm SE		633 \pm 2.12 a	15.8 \pm 0.65 b	188 \pm 1.54 b	53.1 \pm 0.75 a	525 \pm 1.63 a	19.0 \pm 0.57 b	179 \pm 1.24 b	55.9 \pm 0.56 a	475 \pm 1.25 a	20.9 \pm 0.74 b	123 \pm 0.68 b	81.3 \pm 0.96 a
	Max.	650	15.6	185	55.6	545	19.2	165	62.5	495	21.0	135	80.0
3.5	Min.	645	15.4	180	54.1	520	18.3	160	60.6	475	20.6	125	74.1
	Mean. \pm SE	647 \pm 2.05 a	15.5 \pm 0.52 b	183 \pm 1.42 b	54.6 \pm 0.65 a	535 \pm 1.45 a	18.6 \pm 0.62 b	163 \pm 1.32 b	61.2 \pm 0.64 a	485 \pm 1.38 a	20.8 \pm 0.68 b	130 \pm 0.74 b	77.0 \pm 0.87 a

a, b, c Means within the same row with different letters are significantly different ($P \leq 0.05$).

Losses percentage of rennin units (Ru/g, %) of dried camel abomasums as affected by storage period in the refrigerator and at room temperature significantly ($P \leq 0.05$) showed in Table (3). The losses of rennin units were observed during the storage period (79.37 Ru/g). However, the losses percentage increased during the storage period (11.90% in 4 months) in the refrigerator. On the other hand, the losses percentage of rennin units at room temperature were the highest compared with the refrigerator sample during the storage period in the extracting solution at pH 4.5.

Table 3. Effect of storage period in the refrigerator (7 ± 1 °C) and at room temperature (25 ± 2 °C) on the loss of rennin units (Ru/g, %) of dried camel abomasums.

Variance	In the Refrigerator									
	Zero time		1 Month		2 Months		3 Months		4 Months	
	Ru/g	Loss %	Ru/g	Loss%	Ru/g	Loss%	Ru/g	Loss%	Ru/g	Loss %
Max.	95.2	Nil.	89.9	6.2	83.4	13.0	80.9	17.8	79.4	19.2
Min.	90.9	Nil.	85.3	4.5	82.2	9.6	78.3	10.9	76.9	11.9
Mean.	93.2	Nil.	88.1	5.4	82.8	11.1	79.7	14.3	77.7	16.3
±	±		±	±	±	±	±	±	±	±
SE	1.89 a		1.65 b	0.21 d	1.42 c	0.31c	1.32 d	0.42 b	1.21 e	0.75 a
Variance	At room temperature									
	Zero time		1 Month		2 Months		3 Months		4 Months	
	Ru/g	Loss %	Ru/g	Loss%	Ru/g	Loss%	Ru/g	Loss%	Ru/g	Loss %
Max.	95.2	Nil.	83.9	13.2	76.3	19.9	70.5	26.0	65.8	33.9
Min.	90.9	Nil.	80.9	10.3	75.1	15.5	68.8	24.6	60.5	29.6
Mean.	93.2	Nil.	81.9	11.8	75.9	18.4	69.7	25.3	63.1	32.4
±	±		±	±	±	±	±	±	±	±
SE	1.89 a		1.23 b	0.35d	1.02 c	0.45c	1.11 d	0.68 b	1.12 e	0.96 a

a, b, c Means within the same row with different letters are significantly different ($P \leq 0.05$).

Data given in Table 4 showed that the milk clotting activity (MCA) of the extracting solution significantly ($P \leq 0.05$) was highest at pH 4.5 and the lowest at pH 5.5 compared with the extracting solution at pH 4.0 and 3.5. Those results suggested that the most suitable pH for extracting was activated enzymes at 4.5. The lowest milk clotting activity of the extracts were obtained at pH values (4.0 and 3.5) might be resulted from the unfavorable effect of the prolonged activation of the enzyme at the lower ranges of pH. The lower milk clotting activity of the enzyme extract at pH5.5 was probably due to the lack of sufficient H⁺ for the activation of the proenzyme. Those results are in agreement with those obtained by Boudjenah *et al.*, (2011) who reported that the non- purified enzyme preparations of gastric enzyme coagulation showed better coagulation activity.

The Proteolytic activity generally increased gradually during the extracting period in all treatments up to the 9th day and then it was almost constant afterward. The Proteolytic activity significantly ($P \leq 0.05$) was the highest at pH4.5 while at the pH 5.5 it gave the lowest one. It can be stated that the Proteolytic activity of camel rennet was almost equal to that of sheep's rennet as reported by Girgis *et al.*, (1983).

Table 4. Milk clotting activity in RU/g and Proteolytic activity ((Ug tyrosine/min./ml) of the extracting solution pH of dried camel abomasums during extraction period (9 days).

pH values of treatments	Days of extraction			
	3	5	7	9
	Milk clotting activity in RU/g			
5.5	0.30±0.01 d	0.35±0.02 d	0.40±0.02 d	0.45±0.03 d
4.5	4.98±0.08 a	5.66±0.12 a	7.32±0.16 a	8.68±0.19 a
4.0	4.45±0.05 b	5.23±0.11 b	6.35±0.14 b	7.56±0.17 b
3.5	3.30±0.03 c	3.95±0.04 c	4.40±0.05 c	4.45±0.06 c
	Proteolytic activity (Ug tyrosine/min./ml)			
5.5	26.32±0.32 d	30.25±0.36 d	36.54±0.39 d	42.68±0.42 d
4.5	53.48±0.75 a	68.85±0.88 a	81.23±0.96 a	88.29±1.02 a
4.0	40.25±0.52 b	48.23±0.67 b	58.25±0.78 b	66.28±0.89 b
3.5	36.54±0.45 c	48.25±0.56 c	55.32±0.69 c	68.24±0.96 c

a, b, c Means within the same column with different letters are significantly different ($P \leq 0.05$).

Table 5 showed the milk clotting time of camel rennet at different pH as affected by prolonged storage in the refrigerator and at room temperature.

It may be clearly observed that the milk clotting time of pH 4.5, significantly ($P \leq 0.05$) extracts stored either under the refrigerator or at the room temperature was decreased as the storage period progressed. The extent of the decrease varied with both the pH of extraction and the storage temperature which significantly ($P \leq 0.05$) was at a lower rate in refrigerator than at room temperature and the descending order was at the pH 4.0 and 3.5 extracts.

Sample extracted at pH 5.5 show increased milk clotting time during storage at both refrigerator and at room temperature. The increase being greater at the latter than the former temperature. This phenomenon might be due to the continued activation of the proenzyme with prolonged storage. The higher range at room temperature which helping the activation process.

Table 5. Milk clotting activity of camel's rennet extracts during storage as affected by pH values of extraction solution.

pH values of treatments	Milk clotting activity in RU/g				
	Zero time	1 Month	2 Month	3 Month	4 Month
In the refrigerator (7 ± 1 °C)					
5.5	0.42±0.01 d	0.54±0.02 d	0.56±0.03 d	0.60±0.05 d	0.62±0.05 d
4.5	7.68±0.62 a	7.45±0.52 a	7.10±0.43 a	6.58±0.32 a	6.46±0.25 a
4.0	4.32±0.32 b	4.10±0.31 b	3.86±0.24 b	3.45±0.21 b	3.25±0.05 b
3.5	4.00±0.22 c	3.86±0.21 c	3.71±0.15 c	3.62±0.14 c	3.45±0.11 c
At room temperature (25 ± 2 °C)					
5.5	0.42±0.01 d	0.58±0.05 d	0.62±0.06 d	0.65±0.06 d	0.68±0.08 d
4.5	7.68±0.62 a	7.63±0.63 a	7.25±0.56 a	6.78±0.48 a	6.66±0.36 a
4.0	4.32±0.32 b	4.35±0.35 b	4.00±0.28 b	3.67±0.26 b	3.58±0.23 b
3.5	4.00±0.22 c	4.10±0.31 c	3.89±0.26 c	3.73±0.21 c	3.55±0.11 c

a, b, c Means within the same column with different letters are significantly different ($P \leq 0.05$).

Data given in Table 6 showed the decreases in proteolytic activity of the extracts obtained at 5.5, 4.5, 4.0 and 3.5. The extent of decrease in proteolytic activity was higher at room temperature than in refrigerator.

On the other hand, storage of pH5.5 extracts, showed proteolytic activity in an opposite trend to that of milk clotting time. At that pH, the proteolytic activity extended lower in refrigerators compared to at room temperature.

Table 6. proteolytic activities of camel rennet extracts during storage as affected by extraction solution pH.

pH values of treatments	Proteolytic activity (Ug tyrosine/min./ml)				
	Zero time	1 Month	2 Month	3 Month	4 Month
In the refrigerator (7 ± 1 °C)					
5.5	54.23±1.12 d	42.58±1.05 d	35.65±1.02 d	28.21±0.96 d	17.89±0.53 d
4.5	98.21±1.56 a	81.89±1.43 a	71.65±1.35 a	52.36±1.21 a	41.87±1.05 a
4.0	78.32±1.36 b	68.25±1.25 b	52.12±1.16 b	43.36±1.08 b	32.89±0.98 b
3.5	63.25±1.25 c	52.98±1.11 c	42.58±0.99 c	35.74±0.75 c	25.78±0.54 c
At room temperature (25 ± 2 °C)					
5.5	54.23±1.12 d	45.36±1.04 d	36.35±0.96 d	29.34±0.56 d	19.87±0.25 d
4.5	98.21±1.56 a	83.56±1.35 a	73.69±1.26 a	54.25±1.12 a	43.87±0.89 a
4.0	78.32±1.36 b	71.12±1.26 b	54.54±1.18 b	46.21±1.05 b	35.62±0.68 b
3.5	63.25±1.25 c	55.21±1.15 c	45.64±0.89 c	37.25±0.67 c	27.58±0.32 c

a, b, c Means within the same column with different letters are significantly different ($P \leq 0.05$).

CONCLUSION

The attained results concluded that the extracting solution from the behind part of stomach was the greatest rennin units (RU/g) compared with the extracting solution from the front part of the stomach at pH4.5. The good substitute for calves' rennet can be obtained from the dried vells of camel and can be named adult camel rennet.

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دراسة النشاط التخثري للمنفحة المستخرجه من المعده الخام للنوق فى اعمار مختلفه

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نظرا لصعوبه تخثر حليب النوق باستخدام الإنزيمات المستخلصة من الغشاء المبطن لمعدة العجل المتوفرة تجاريا والمكونة من الرنين و الكيموسين و الببسين فى انتاج الجبن. تم اجراء هذا البحث لتحسين عمليه استخلاص منفحه النوق بصوره جيده وتقليل التكاليفه الاقتصاديه حيث ان معده النوق تباع لدى الجزارين بالوزن وليست بالقطعه كما فى الانفحه الخاصه بالماشيه.

لذلك تم تقسيم معده النوق الى جزئين الجزء الامامى و الجزء الخلفى اى بدايه المعده و نهايتها و كان متوسط الوزن لكل جزء 1.200 كيلو جرام و بعد التجفيف لمدته شهر كان الوزن 400 جرام. وتمت عمليه الاستخلاص فى محلول يحتوى على 3% حمض بوريك و 0.5% بنزوات صوديوم و 8% كلوريد صوديوم و تم تعديل الاس الهيدروجينى باستخدام حامض الهيدروكلوريك.

وتم الاستخلاص خلال 9 ايام للحصول على اعلى عدد من الوحدات لانزيم الرنين ثم بعد ذلك التخزين لمدته 4 اشهر فى الثلجه و على درجه حراره الغرفه. و اظهرت النتائج ان الجزء الخلفى لمعده النوق كان افضل حيث انه اعطى اكبر عدد من وحدات الانزيم مقارنة بالجزء اللامامى للمعده. وان افضل اس هيدروجينى لمحلول الاستخلاص هو 4.5. وان ال 9 ايام كانت كافيه للاستخلاص.

كذلك فان القدره التجبنيه لمنافح النوق كبيره السن افضل من الصغيره. وان فتره التخزين فى الثلجه قلت من معدل الفقد فى الانزيم خلال فتره التخزين ال 4 اشهر.