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A STUDY ON THE PREPARATION AND CLARIFICATION OF RENNET FROM MINCED AND SLICED FRESH VELLS

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ABSTRACT

Calf rennet extract was prepared from minced (A) and sliced (B) fresh vells. Yield of the rennin units in the crude extract was slightly higher in case of using the sliced vells. Adding potassium aluminum sulphate (alum) at the rate0.4% caused great decrease in pH, and rennet units /ml, whereas the sequence addition of di-sodium phosphate (0.8%) decreased the loss of rennin units from 44.18 to 1.32% in case of (A) and from 50.60 to zero% in case of (B).

The microbiological analysis of (A) and (B) extracts revealed that the total bacterial count was higher before clarification and in (A) than in (B). In all cases the extracts were free-from coliforms and molds & yeasts.

During storage at room temperature (20-35 ^{o}C) for 3 months a slight decrease in pH of A and B extracts was recorded, whereas activity of rennet was gradually decreased in both cases. The recorded loss (%) was 29.73 and 27.72 in case of A and B respectively at the end of storage period.

Keywords: Calf rennet, clarification, minced and sliced vells.

INTRODUCTION

The rennet deficiency, as general observed in the world, has partly or completely necessitated a substitution by other enzymes which would have similar proteolytic properties and coagulating activity that rennet has but callf rennet is still considered as the main and most favorable milk clotting enzyme traditionally used for cheese making in most world. However, it is well known that this rennet is a crude extract of gastric enzyme containing 85-95% chymosin and 10-15 pepsin (Ustunol and Zeckzer, 1996). The supply of rennet fluctuates with the number of beef cattle slaughtered and increase in world cheese production require larger amount of rennet especially it is well known that in the course of cheese manufacture, only 30% of the enzyme preparation used for milk coagulation are transferred to the coagulum, whereas approximately 70% remaining enzyme is passed to whey and not recuperated. Treating the prepared rennet extract for clarification with potassium aluminum sulphate (alum) at the rate of 0.4% followed by 0.8% di-sodium phosphate caused significant changes. Clarification of rennet from sliced fresh vells improved recovery of rennet units comparing with minced fresh vells. The fourth stomach of suckling calves is still considered to be the most of suitable but expensive source of rennet. This encourage some investigators to evaluate fresh and dried vells as a source of calf rennet and to improve stability of liquid rennet during storage (Abd El-Salam et al., 1989).

The present study provides some information on the importance of clarification process and efficiency of preparing rennet and improving its recovery from fresh vells during clarification. The changes-on storage-in prepared extracts were also taken into concideration.

MATERIALS AND METHODS

Preparation of fresh vells :-

The collected fresh fourth stomachs were carefully rinsed with tap water to remove superfluous and fat residues. After cleaning the fresh vells were divided into two equal portions. The first on was minced whereas the second portion was sliced into slices of 2x1 cm to be ready for extraction.

Preparation of the extracting solution :-

The extracting solution was prepared mainly according to Fahmi and Amer (1962). It contained 3% boric acid, 5% soduim chloride, 0.2% soduim benzoate and water (pH 5.2).

Extraction condition :-

The extracting solution (pH 5.2) of both sliced and minced vells was treated by using 10 grams vells per 100 ml solution and kept at room temperature (20-35 ^{o}C) with continuous stirring for six days, until the complete recovery of enzyme was achieved.

Preparation of the standard milk :-

This was carried out according to Fahmi and Amer (1962) using American spray dried skim milk, calcium chloride and water.

Determination of milk clotting activity :-

Milk clotting expressed as clotting time in seconds was determined according to Fahmi and Amer (1962).

Calculation of rennin units :-

The number of rennin units (RU) was calculated using the clotting time in seconds following the equation given by Fahmi and Amer (1962).

Determination of pH :-

It was carried out by means of JENWAY 3020 pH meter (England) fortified with glass electrode.

Microbiological analysis :-

The rennet extracts were microbiologically analysed for total bacterial count (Harrigan and Mc Cance , 1976) and coliforms (APHA , 1967).

Statistical analysis :-

The attained data were statistically analyzed according to Steel and Torrie (1984).

RESULTS AND DISCUSSION

Data in Table (1) reveal that the pH values were nearly similar for both extracts prepared from minced (A) and sliced (B) vells. Recovery of rennin units was slightly less in case of A. The recorded values for rennin units (RU / ml) were 25.67 and 27.67 for (A) and (B), respectively. These values were recorded for the crud extract. After clarification, a great loss was recorded in rennin units. Thus, after adding alum the values of RU / ml values were 14.33 and 13.67 in case of (A) and (B) respectively. This was accompanied by a corresponding decrease in pH.

In this respect, **Berridge (**1955) attributed such loss in rennin units to the precipitation of a fine material formed from alminium hydroxide which

adsorbs most of the enzyme extract. So, addition of di-sodium phosphate (DSP) is quite important, since it helps in increasing the pH and the liberation of the adsorbed enzyme.

However, adding DSP to alum-treated extract (in case of A) greatly increased the pH from 4.6 to 5.3 and the RU / ml from 14.33 to 25.33, this was associated with decrease the loss in RU / ml from 44.18 to 1.32%, respectively, but in the extract from sliced vells (B), the pH decreased from 5.36 to 4.70% by adding alum and increased to 5.29 with adding DSP giving 100% recovery for the rennin units. This suggests importance of preparation of rennet extract from sliced vells since the minced one may prevent the complete collection of the enzyme from the vells. However, it is well known that enzyme is concentrated in the inner tissues of the vells and by it is turn no need for minising the vells.

Table (1): Yield and recovery of rennin units (RU / ml) of the extract prepared from minced (A) and sliced (B) vells before (curde extract) and after clarification using potassium aluminum sulphate (alum) and di-sodium phosphate (DSP) (Average of 3 replicates)

Item	(A)			(B)		
	pH	RU/ml	Loss%	pH	RU/ml	Loss%
Crude extract	5.37	25.67	0.0	5.36	27.67	0.0
After adding alum	4.60	14.33	44.18	4.70	13.67	50.60
After adding DSP	5.30	25.33	1.32	5.29	27.67	0.0

Table (2) reveals that the prepared extracts were free from coliforms, modules and yeasts and staphylococus.The total bacterial count was greatly decreased after clarification in both cases being 65 and 61.67 cfu / ml in case of the clarified A and B extracts, respectively. This suggests importance of carrying out the chemical clarification. The microbiological quality of the rennet is considered as the main factor in stability of rennets during storage .

Table (2): Microbiologically quality of rennet extract prepared from minced (A) and sliced (B) vells before and after clarification (Average of 3 replictes)

	Before clarification				After clarification			
	TBC	CBC	M&Y	Staph.	TBC	CBC	M&Y	Staph.
A	75.67	Nil	Nil	Nil	65.00	Nil	Nil	Nil
B	53.33	Nil	Nil	Nil	61.67	Nil	Nil	Nil

TBC: Total bacterial count **CBC: Coliforms bacterial count.**
M&Y: Mould and yeast **Staph : staphylococcus auries.**

During storage at room temperature (20-35 ^{o}C) for 3 months, pronounced changes were recorded in pH values, whereas the percentage of loss in RU/ ml gradually increased (Table 3) reached 29.73 and 27.72% in case of A and B, respectively. The differences in this respect between A and B values were statistically insignificant. In the literature a slight but continuous increase in pH during storage of liquid rennet was reported by Naguib *et al.* (1980).The decrease in RU agrees with the results given by Naguib *et al.* (1980).

Table (3): Changes in pH and stability of rennet extract prepared from minced (A) and sliced (B) vells during storage at room temperature (20-35 °C) for three months (average of 3 replicates)

Storage period (month)	A			B		
	pH	RU/ml	Loss%	pH	RU/ml	Loss%
Zero	5.30	25.33	0.0	5.29	27.67	0.0
1	5.26	20.25	20.06	5.30	27.60	18.32
2	5.28	18.60	26.60	5.29	20.90	24.47
3	5.25	17.80	29.73	5.26	20.00	27.72

RU/ml: rennet units/ ml.

The loss in rennet activity during storage may be attributed to the autolysis caused by action of microflora which is more pronounced at high storage temperature (Naguib *et al*.,1980) as well as to photochemical inactivation (Hill and Laing,1965).

In conclusion, a great attention should be taken into consideration with respect to preparation of rennet in Egypt, since most – or may be all – of the quantity of rennet is still produced using the traditional procedure in small plants and no modern factories are established until now in Egypt for the hygiene preparation of rennet from calf vells.

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دراسة علي تحضير و ترويق المنفحة السائلة باستخدام مفروم و شرائح الاباوات الطازج

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اهتم البحث بمقارنة و تحضير و ترويق مستخلص المنفحة السائلة من مفروم و شرائح الاوباوات الطازجة و تم ذلك باستخدام محلول استخلاص يتكون من 3 % حمض بوريك ، 0.5 % كلوريد صوديوم ، 0.2 % بنزوات صوديوم0

وأوضحت نتائج الدراسة ما يلي :-

1- كان محصول وحدات المنفحة أعلي في حالة مستخلص المنفحة غير المروق المحضر من الشرائح مقارنة بالمحضر من مفروم الأباوات0

2- أدت المعاملة بكبريتات البوتاسيوم والألومنيوم (الشبه) بمعدل 0.4% الي انخفاض الرقم الأيدروجيني والي فقد كبير في نشاط إنزيم الرنين مقدرا بعدد الوحدات لكل مل في كل من مستخلص المفروم و مستخلص الشرائح مقارنة بالمستخلص غير المعامل0 و كان الفقد أعلي قليلا في حالة مستخلص الشرائح مقارنة بالمفروم و لقد عزي ذلك الي ادمصاص الأنزيم علي أسطح المواد المترسبة0 بينما عند اضافة فوسفات ثنائي الصوديوم بنسبة 0.8% بعد المعاملة بالشبة أدي الي زيادة الرقم الأيدروجيني و زيادة كبيرة في عدد وحدات الإنزيم في كل الأحوال و كانت الزيادة الأعلى في حالة مستخلص الشرائح مقارنة بمستخلص المفروم0

3- أوضح التحليل الميكروبيولوجي خلو كلا المستخلصين من بكتيريا الكوليفورم و الفطريات و الخمائر و كذلك بكتيريا استافيلوكوكس ايوريوس0

4- أدت فترة التخزين لمدة ثلاثة شهور علي درجة حرارة الغرفة (20-35 ^{5}م) الي فقد في قوة المستخلص الأنزيمي حيث وصلت وحدات الأنزيم الي 29.73 & 27.72 وحدة لكل مل في حالة مستخلص الشرائح ومستخلص مفروم الأباوات علي التوالي0