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THE USE OF *PECT-KAO®* SUSPENSION AND *QUESTRAN®* POWDER AS CLARIFIERS OF THE COMMERCIAL LIQUID RENNET EXTRACT

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ABSTRACT

The effect of using *pect-kao®* suspension and *questran®* powder as clarifying agents with different concentrations (6, 10, 20, 30%) and pH values on chemical, clarity and microbiological properties of commercial liquid rennet extract (CLRE) was studied. Both clarifying agents caused changes, *i.e.* an increase in rennet coagulation time (RCT), loss in clotting activity (CA) and decrease in the ratio of CA to proteolytic activity (PA) irrespective of concentration of clarifying agent and pH values. On the other hand, using of *questran®* powder with any concentration at pH 5.5 was less effective in the changes of clarified rennets. However, adding tri-sodium phosphate (TSP) at concentration 0.8 and 0.4% to *pect-kao®* suspension and *questran®* powder, respectively, minimized the loss in CA during clarification process at pH 5.5.

The clarified rennets had maximum clarity, low total bacterial count (TBC) with the absence of coliforms as compared with control.

INTRODUCTION

Rennet is one of the important ingredients used in cheese industry. The variability in the rennet strength used and its microbial content would affect coagulation process and the properties of the final product.

Nowadays, however, there was shortage and increase price of veal calves and insufficient of young calf stomachs to meet the needs of cheesemaker, and this induced investigators to search for other animals and microbial origin *(Fox, 1968; Fahmi et al, 1979; Amer et al., 1979; Anis et al., 1983; Girgis et al., 1983; El-Abbassy and Wahba, 1986; El-Batawy et al., 1987 and Madkor, et al., 1990).*

In Egypt, rennet is usually produced in small factories in the form liquid rennet, which shows inferior stability and poor microbiological quality *(Naguib et al, 1975).* Although successful attempts have been made for the production of both liquid and powdered rennet extracts of good quality by *Fahmi and Amer (1962)* and *Amer, (1963)* from calves, however, great loss in activity of rennin may occur during clarification. Therefore, *Abd El-Salam., (1989), Rashed* and *Saleh., (1992), Mehanna et al, (1998),* demonstrated the importance of clarification process (i.e. centrifugation, chemicals and adding some preservatives) of the crude rennet extract for better keeping quality on storage. On the other hand, the reduction of activity losses in dilute solution of chymosin and pepsin or rennet extract can be prevented with polyethylene glycol *(Friedenthal and Visser, 1985)* or with potassium aluminium sulphate and tri-sodium phosphate (TSP) or di-sodium phosphate (DSP), (Rashed and Saleh, 1992 and Mehanna, *et al.*, 1998).

Other technique,through an anionexchange chromatography column, is utilized to separate the chymosin and bovine pepsin components of a rennet extract and producing of rennet containing 100%chymosin possible *(Pszczola, 1989)*. In this paper, work was directed towards the possible application of *pect-kao®* suspension as cationic exchange and *questran®* powder during clarification the commercial liquid rennet extract, and to investigate its effect on the chemical, clarity and microbiological properties of the resultant rennet with minimum loss in clotting activity (CA) during clarification process.

MATERIALS AND METHODS

1. Materials:

The commercial liquid rennet extract (CLRE) was obtained from local market and stored in the refrigerator in brown glass bottles. *Pect-kao®* suspension is a pharmaceutical suspension which is used as antidiarrhoeal. It consists of pectin as polysaccharide and light kaolin as natural cationic exchange at ratio of 0.4: 19.0g/100 ml. It was obtained from Egyptian International Pharmaceutical Industries Co. Tenth of Ramadan city. Cairo. *Questran®* powder is the chloride salt of a basic anion-exchange resin processed by Mead. Johnson Bristol Myers Souibb Co., U.S.A.

2. Experimental procedure:

The CLRE was divided into 25 portions (200 ml). The first served as a control, 8 portions were divided into two parts and mixed with *pect-kao®* suspension at ratio 6, 10, 20, 30% v/v and the pH adjusted to 4.5 and 5.5 respectively. The others 8 portions, were mixed with *questran®* powder (w/v) with the above same procedure. The rest 8 portions, were divided into two parts, mixed with *pect-kao®* suspension and *questran®* powder at ratio 6, 10, 20, 30 (v/v, w/v) in the presence, 0.8, 0.4% TSP, respectively, and adjusted the pH to 5.5. All treatments were centrifuged at 800 r.p.m for 10 min, then the supernatants or clarified rennets were stored in the refrigerator.

3. Methods of analysis:

The rennet coagulation time (RCT) and clotting activity (CA) as rennin units (Ru/ml) was determined using reconstituted low heat treatment skim milk powder according to Fahmi and Amer (1962). The proteolytic activity (PA) was measured as optical density at 520nm according to the casein digestion method as described by *Kuntiz (1947)*. As an indication of the efficiency of clarification process, the optical density (OD) of the clarified rennet was measured by spectrophotometer (*Spectronic 20, BAUSCH & LOMB*, USA) at 600nm comparing with unclarified rennet. The total bacterial count (TBC) and coliforms were determined according to the procedures described in "standard methods for examination of dairy products" *(APHA, 1992)*.

Three trails were carried out for each experiment and each analysis in triplicate and the mean values were tabulated. Average results were recorded.

RESULTS AND DISCUSSION

1- Effect of different kind of clarifiers on the chemical properties of commercial liquid rennet extract (CLRE):-

Table (1) shows the chemical properties of CLRE e.g pH, rennet coagulation time (RCT) and clotting and proteolytic activity (CA, PA) as affected by *pect-kao®* suspension and *questran®* powder at different concentration of clarifier and pH values.

In a preliminary study, it is found that the chemical properties of CLRE were not affected with *pect-kao®* suspension and *questran®* powder at concentration below 6% at pH 4.5 and 5.5.

The pH of CLRE decreased from 5.43 to 5.08 and 3.82 at 6% of *pect-kao®* suspension and *questran®* respectively. A continuous decrease in pH resulted in with the progressive concentration of clarifiers until it reached to pH 4.72 and 3.32 upon the addition of 30% of *pect-kao®* suspension and *questran®* powder respectively. As can be seen from data given in Table (1), the RCT of clarified rennets was prolonged with increasing concentration of *pect-kao®* suspension, or *questran®* powder at pH 4.5 or 5.5 and consequent loss of its CA as compared with unclarified rennets. This might be attributed to binding or interface adsorption of the enzyme molecules by clarifying agents.

Rashed and Saleh (1992) show that the clarification of liquid rennet extract with potassium aluminium sulphate (alum) at the rate 0.8% caused significant changes, being decrease in pH, increase in rennet coagulation time and loss in rennet activity (91.67%). This action of alum can be explained on the basis of forming a gelatinous precipitate of aluminum hydroxide, with all precipitated proteins and rennin being adsorbed on the surface of the precipitate *(Berridge, 1945 and 1955, Fahmi and Amer, 1962).* Data in the same table show that loss of CA of clarified rennets was lower at pH 5.5 than 4.5 at all concentrations of clarifying agents. Therefore, the most suitable pH for clarification of CLRE by above clarifiers was 5.5, since, it helped in continuation activatien of the pro-enzyme *(Fahmi et al., 1979).* On the other hand, it was observed that clarification process with *questran®* powder caused low loss in CA being 11.95 and 22.55% at pH 5.5 with 20 and 30% of clarifier compared with *pect-kao®* suspension being 25.13% and 41.81% respectively, where the later clarifier was much bind of the rennet enzyme than the former clarifier.

The difference in this respect could be explained on the basis of the heterogeneity of the commercial liquid rennet enzyme, on the other hand, kind of clarifier being cationic or anionic-exchange and pH of clarification process medium.

As regards to the PA, it is clear that clarified rennets with *pect-kao®* suspension had higher PA than that with *questran®* powder at all concentration and pH values. However, the PA at pH 4.5 was higher than that at pH 5.5 as a result of the higher pepsin content of *pect-kao®* rennets. This is in accordance with *Pszczola (1989),* who stated that crude rennet extract is adjusted to pH 4.5 and passed through an anion-exchange chromatography

Table (1): Effect of adding *pect-kao*® suspension and *questran*® powder on the rennet coagulation time (RCT), clotting (CA) and proteolytic activates (PA) of the commercial liquid rennet extract (CLRE) at pHs 4.5 and 5.5.

Treatment	Concentration of clarifier %	pH*	pH values									
			4.5					5.5				
			RCT (sec)	CA (Ru/ml)	Loss** %	PA O.D (520nm)	CA/PA (Ratio)	RCT (sec)	CA (Ru/ml)	Loss** %	PA O.D (520nm)	CA/PA (Ratio)
Control	-	5.43	98	9.71	-	0.036	265.5	98	9.71	-	0.036	265.5
Pect-kao® sus.	6.0	5.08	130	8.15	16.07	0.047	173.4	123	8.62	11.23	0.039	221.0
	10.0	4.96	152	7.24	25.43	0.049	147.7	129	8.53	12.15	0.040	213.2
	20.0	4.81	236	5.09	74.58	0.049	103.8	165	7.27	25.13	0.040	181.7
	30.0	4.72	334	3.89	59.94	0.051	76.2	230	5.65	41.81	0.043	131.4
Questran® powd.	6.0	3.82	119	8.40	13.49	0.044	190.9	115	8.70	10.40	0.036	241.7
	10.0	3.70	130	7.69	20.80	0.047	163.6	116	8.62	11.23	0.039	221.0
	20.0	3.46	139	7.19	25.95	0.049	146.7	117	8.55	11.95	0.039	219.2
	30.0	3.32	142	7.04	27.50	0.049	143.6	133	7.52	22.55	0.042	179.1

* Initial pH

** % loss in CA = $\frac{\text{CA of control - CA of treatment}}{\text{CA of control}} \times 100$

column, the negative charged bovine pepsin will be bound by the positively charged anion exchange resin; the neutral or uncharged chymosin will pass directly through the anion-exchange column. On the other hand, the pH 4.5 was permitted more suitable environment for the proteolytic enzymes *(Amer et al., 1983)*. It was also observed (Table 1) that the PA of all treatments increased with increasing of clarifying agents and the ratio CA/PA was opposite trende.

From the foregoing results it may be observed that loss in clotting activity (CA) of clarified rennets and the best pH to minimize this loss problem is 5.5. So, the amount o tri-sodium phosphate (TSP) added during clarification is quite important, since it helps in increasing the pH and in the liberation of the adsorbed or bound (binded) enzyme *(Rashed and Saleh, 1992)*. Therefore, TSP was choised to further studies.

2- Effect of adding TSP on the chemical properties of CLRE during clarification with *pect-kao*® suspension and *questran*® powder at pH 5.5:-

Table (2) illustrates the pH, RCT, CA, PA and CA/ PA ratio of clarified rennets as affected by TSP during clarification.

It is clear that addition of 0.8, and 0.4% TSP during elarification with *pect-kao*® suspension and *questran*® powder, respectively, increased the pH and decreased the RCT, loss CA and PA, compared with treatments without TSP (Table 1). On the other hand, the presence of TSP during clarification process with *questran*® powder improved the chemical properties of CLRE than those with *pect-kao*® suspension. The RCT was approached to control and loss of CA being 6.28, 6.28, 8.86 and 11.23% at 6, 10, 20 and 30% of the former clarifier, the corresponding values were 8.24, 10.81, 14.21 and 30.59% of the latter clarifier.

Table (2): Effect of adding tri-sodium phosphate (TSP) on the recovery of rennin units (Ru/ml) of the commercial liquied rennet extract (CLRE) treated with *pect-kao*® suspension and *questran*® powder at pH 5.5.

Treatment	Concentration of clarifier %	pH*	RCT (sec)	CA (Ru/ml)	Loss ** %	PA O.D (520nm)	CA/PA (Ratio)
Control	-	5.43	98	9.71	-	0.036	265.5
Pect-kao®	6.0	7.92	119	8.91	8.24	0.036	245.9
sus.	10.0	7.70	127	8.66	10.81	0.037	233.3
+	20.0	7.27	144	8.33	14.21	0.037	226.9
TSP, 0.8%	30.0	7.00	193	6.74	30.59	0.039	172.8
Questran ®	6.0	6.92	111	9.1	6.28	0.035	258.5
powd.	10.0	6.98	111	9.1	6.28	0.036	252.7
+	20.0	6.11	113	8.85	8.86	0.036	245.8
TSP, 0.4%	30.0	5.80	116	8.62	11.23	0.037	232.9

* Tnitial pH

** % loss in CA = $\frac{\text{CA of control - CA of treatment}}{\text{CA of control}} \times 100$

Amer *et al., (1983)* stated that the best clotting and proteolytic activities were obtained when the propionic acid and potassium sorbate were added to the extracting solution at 0.15 and 0.1% for calves remnet and 0.30 and 0.15% for adult bovine rennet, respectively. On the other hand, extracts treated with propionic acid gave better results then both the control and potassium sorbate treatments.

Rashed and Saleh *(*1992*)* added alum (0.8%) to the crude extract followed by adding tri-sodium phosphate (1.6%) to minimize the losses in rennet activity. However, *Mehanna et al., (*1998*)* showed that the maximum recovery of rennin was achieved when clarification was done by adding alum followed by di-sodium phosphate at the rates 0.4% and 0.8%, in order. *Friedenthal and Visser, (1985)* showed that the addition of 0.1-0.3% of polyethylene glycol in dilute solutions of chymosin and pepsin in order to prevent the loss of activity.

3- Effect of clarifying agents on the clarity and micriobiological properties of CLRE at pH 5.5:-

Data presented in Table (3) indicate that the use of *pect-kao®* suspension and *questran®* powder during clarifications process of CLRE improved the clarity and microbiological properties compared to unclaifiried rennet. However, the *questran®* powder had higher rate of clarification than that *pect-kao®* suspension. Concerning micribiological quality, the percentage of reduction in total bacterial count (TBC) of the *pect-kao®* suspension rennets was reached to 93-99% followed by the *questran®* powder rennets. This is due to the antibacterial effect of *pect-kao® suspension (E.I.P.I. Co, 2000).* However, all treated rennets were free from coliforms. In this respect, *Rashed and* Saleh, *(*1992*)* noted the TBC increased during storage of untreated and centrifuged rennet extracts, where the changes in the chemically-treated one were negligible. While *Mehanna et al., (1998)* used that sodium benzoate and thymol as preservatives to improve stability of the clarified rennet during storage. However, *Pszczola, (1989)* showed that the rennet produced through anion-exchange chromatography process had superior micriobiological purity, standard plate count values to < 1000/ml.

Table (3): Effect of adding *pect-kao*® suspension and *questran*® powder on clarity properties and bacteriological quality of the commercial liquid rennet extract (CLRE) at pH 5.5.

Treatment	Concentration of clarifier %	Clarity properties		Bacteriological quality	
		O.D (600 nm)	Removal* %	TBC (CFU/ml)	Coliform (CFU/ml)
Control	-	0.428	-	355×10^3	19×10^2
Pect-kao® sus. + TSP, 0.8%	6.0	0.095	77.8	$25.3\times10^{3(92.87)}$	Nil
	10.0	0.087	79.6	$18.4\times10^{3(94.81)}$	Nil
	20.0	0.080	81.3	$4.7\times10^{3(98.67)}$	Nil
	30.0	0.074	82.7	$3.6\times10^{3(98.98)}$	Nil
Questran ® powd. + TSP, 0.4%	6.0	0.047	89.0	$88\times10^{3(75.21)}$	Nil
	10.0	0.042	90.1	$51\times10^{3(75.63)}$	Nil
	20.0	0.032	92.5	$24.7\times10^{3(93.04)}$	Nil
	30.0	0.029	93.2	$10.5\times10^{3(97.04)}$	Nil

* % Removal = $\frac{\text{O.D of control - O.D. of treatment}}{\text{O.D of control}}$

From the foregoing results, it could be recommend to use *pect-kao*® suspension and *questeran*® powder (i.e. cationic and anionic exchange respectively) as clarifying agents with TSP at pH 5.5 to obtain clear, minimum in loss of CA and good micriobiological psoperties of CLRE to be used for cheese industry. In comparison with other processes, the present clarification method did not require expensive and complex requirements, beside *pect-kao*® suspension and *questran*® powder are a readily available, inexpensive and safety products *(E.I.P.I.Co., 2000* and *M.J.B.M.S. Co., 2002)* while, contrifugation process was insufficient to clarify the rennet extract *(Rashed and Saleh, 1992)*. Moreover the treated rennet with anion exchange process had several advantages over conventional veal rennet preparations, it provides consistency of cheese yield and quality, has improved functionality. It is less affected by higher milk pH, maximizes cheese yield, reduces the amount of rennet required, is no expensive than veal rennet with 10% bovine pepsin and lower levels of nonspecific proteolysis (allowing consequently its use in traditicnally aged cheeses without development of bitter flavours) than microbial rennet (Pszczola, 1989).

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استخدام *pect-kao*® suspension و *questran*® powder كمروقات لمستخلص المنفحة التجارية السائلة

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يعاب على المنفحة الحيوانية السائلة المعروضة بالأسواق المظهر السيئ والعكارة الشديدة و ارتفاع حملها الميكروبي مما يترتب عليه ظهور عيوب عديدة بالجبن الناتج لذلك تهدف هذه الدراسة إلى محاولة استخدام *pect-kao*® suspension ، questran® powder كمروقات لها و دراسة بعض الخواص الكيمائية ودرجة ترويق والجودة الميكروبية للمنفحة المعاملة بتلك المروقات. وقد أوضحت النتائج المتحصل عليها ما يلي:

١- كان لاستخدام كل من *pect-kao*® suspension و *questran*® powder بتركيز ٦، ١٠، ٢٠، ٣٠% على التوالي عند pH ٤,٥، ٥.٥ في ترويق المنفحة التجارية السائلة أثرا واضحا في إطالة مدة تجبن اللبن الفرز وخفض النشاط التجبنى للمنفحة الرائقة بالمقارنة بالمنفحة الكونترول
٢- يفضل ضبط وسط الترويق على درجة pH ٥.٥ حيث ساعدت على استمرار نشاط الإنزيم أثناء الترويق
٣- كان لإضافة مادة تراى صوديوم فوسفات بتركيز ٠,٨% للمنفحة المعاملة بـ *pect-kao*® suspension او بتركيز ٠,٤% للمعاملة بـ *questran*® powder أثرا واضحا في خفض طول مدة تجبن اللبن الفرز مع زيادة النشاط التجبنى لهذه المنافح
٤- أظهرت كل من المروقات السابقة كفاءة عالية في درجة ترويق المنفحة التجارية السائلة.
٥- حدوث انخفاض في المحتوى الميكروبي للمنفحة التجارية السائلة المعاملة بتلك المروقات حيث بلغ معدل الانخفاض اكثر من ٩٠% بالمقارنة بالمنفحة الكنترول علاوة على اختفاء تام لبكتريا القولون

لذلك نوصى باستخدام تلك المروقات على نطاق تجارى لتحسين جودة الجبن الناتج.