MAXIMIZATION OF PRODUCTION AND STABILITY OF RENNIN DURING PREPARATION AND STORAGE OF LIQUID RENNET

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SUMMARY

The objective of the present study was to maximize production of rennin units (RU) from calf vells and to study impact of clarification and storage on the final yield of RU. In this respect, three extracting solutions A, B and C were used. Solution A consisted of 3% boric acid, 7% sodium chloride, 0.3% calcium chloride and 0.3% sodium benzoate (pH 5.2-5.3), solution B consisted of 2% boric acid, 10% sodium chloride and 0.2% sodium benzoate (pH 6.4-6.5) whereas solution C consisted of 4 % boric acid and 0.2%sodium benzoate (pH 4.6).Extraction was carried out twice during ten days of soaking. The attained results revealed that the total RU/g of 206.32 RU/g was obtained when solution A was used before clarification which gave the total RU/g of 310.78 after clarification. Milk clotting activity (MCA) was the highest when solutions A was used (pH 5.2-5.3) whereas the minimum values were at pH 6.4-6.5 (solution B). The opposite was recorded with respect to proteoltic activity (PA). On the other hand, the total loss of RU due to clarification was the maximum in case of solution B (3.13%), whereas the minimum one (2.90%) was in case of solution A. Storage period had a pronounced effect on the loss of RU being the maximum effect was recorded when solution B was used. Much higher losses were recorded with all extracting solutions when rennen extract was stored at room temperatures.

Keywords: Rennin, Different extracting solutions, storage.

INTRODUCTION

Calf rennet has been used as a milk coagulant in the production of most of cheese varieties. Rennet is used in medicinal products as well as for the manufacture of lactose. In Egypt and probably in the rest of the Arabic countries, 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 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 a number of disadvantages such as a longer clotting time, forming a soft curd and an undesirable taste. Another important factor with respect to cheese technology is the clotting power of the

proteolytic enzymes. The clotting activity affects the properties of the curd, such as firmness or softness during processing (Walstra, et al., 2005).The standardized preparation procedures' yielding rennet of consistent quality. Most of local rennet producers have no purification facilities, Such as chromatography or membrane separation, to ensure a sparkling clear solution with much lower mucoprotein content. It contains many deodorants so the strength of natural calf rennet is not maintained at constant level. The average activity loss per month is relatively high as compared with imported rennet. When rennet is kept at high temperatures, pepsin activity increases and causes as a result, some defects in taste, flavor and melting problems in the cheese structure (Hooydonk and Van Den Berg, 1988). Clotting activity should be calculated precisely to avoid possible failures in curd formation. Imperfect rennet manufacturing defects in packing and improper storage conditions may result in changes in the clotting activity of such enzymes. Sometimes, there is a discrepancy between the declared value on the label and the experimentally determined clotting activity (Tabayenejad, et al.2012). Good quality rennet should possess a constant clotting activity and contain no other enzymes but mainly chymosin. In addition, rennet should be free from any microorganisms that produce gas and acid since these might cause serious problems in the final product, such as defects in taste and flavour, putrefaction, disintegration and blowing (Najera, et al. 2003).Calf rennet as a traditional milk-clotting enzyme is very important in the production of cheese in Egypt. The present study was conducted to achieve maximum recovery of rennin from different extraction solutions. The successive extraction using different solutions and improving such extraction during the extraction period were taken also in mind.

MATERIALS AND METHODS

Calves vells were cleaned, blown, dried and then cut into pieces as demonstrated by Fahmi and Amer (1962). The extracting solution was prepared to contain 3% boric acid, 7% sodium chloride, 0.3% calcium chloride and 0.3% sodium benzoate at pH 5.2-5.3(extracting solution A). Extracting solution B was prepared to contain 2%boric acid, 10% sodium chloride and 0.2%sodium benzoate at pH 6.4-6.5. Extracting solution C was prepared to contain 4% boric acid and 0.2 sodium benzoate at pH 4.6. The extraction was carried out for ten days; other steps of preparation were followed as reported by Fahmi and Amer (1962). The pH values were measured using a pH meter (HANNA instruments model 8417, USA). pH values, milk clotting and proteolytic activities were determined every 2 days during the ten days of the extraction. The strength of rennet is defined as ml of milk which

one ml rennet can clot in 40 minutes at 35 ºC. Proteolytic activity was determined according to Irigoyen, et al., (2001).

RESULTS AND DISCUSSION

Table (1) shows milk clotting time and the corresponding calculated rennin units (RU) as affected by extracting solutions A, B and C and time of extraction being I and II. In extracting solution A the average of RU/g was 206.32 ± 0.24 and represented 66.03±0.03 % of the total RU, whereas in the second extraction, the RU/g were 104.46 ±0.03 and represented 31.97±0.03 % of the total. Much lower values were recorded in extracting solution B and C comparing with the values of extracting solution A. In case of extracting solution B the RU/g was 184.96±0.26 and 92.82±0.121 in case of first and second extractions which represented 66.19±0.02 and 32.81±0.021 % of the total RU respectively. In case of extracting solution C the average of RU/g was 196.83± 0.24 and 98.40±0.26 after the first and second extraction respectively which represented the percentages of 67.21 and 32.79 of the total recovery RU in order. On the other hand, the highest total RU value was recorded in extracting solution A (310.78± 0.25) compared with extracting solution B and C (277.81 ±0.24 and 295.20 ±0.06 respectively).

Table 1: Effect of extracting solutions (ES) on the recovery of rennin units (RU/g) after extraction (EX) for tow times (EXI and EX II) (Average ± SE of three replicates)

ES		Extraction 1			Extraction 11			Total RU /g
		MCT (Sec.)	RU /g	% of the total	MCT (Sec.)	RU /g	% of the total	
A pH (5.2-5.3)	I	100.00	200.00	66.10	195.00	102.26	33.90	302. 26
	II	97.00	206.19	64.98	190.00	111.11	35. 25	317.30
	III	94.00	212.77	68.03	200.00	100.00	31.97	312.77
	AV. ±SE	97.00 ±0.01	206.32 ±0.24	66.03 ±0.03	195.00 ± 0.02	104. 46 ± 0.03	31.97 ±0.03	310.78 ±0.25
B pH (6.4-6.5)	I	105.00	190.48	66.67	210.00	95.24	33.33	285.82
	II	115.00	173.91	65.85	220.00	90.19	34.15	264.10
	III	105.00	190.48	67.19	215.00	93.02	32.81	283.50
	AV. ±SE	105.00 ±0.12	184.96 ±0.26	66.19 ±0.02	215.00 ±0.32	92.82 ±0.12	32.81 ±0.02	277.81 ±0.24
C pH (4.6)	I	100.00	200.00	66.67	200.00	100.00	33.33	300.00
	II	105.00	190.48	66.13	205.00	97.56	33.87	288.04
	III	100.00	200.00	67.21	205.00	97.56	32.78	297.56
	AV .±SE	102.00 ±0.12	196.83 ±0.24	67.21 ±0.21	201.00 ±0.13	98.40 ±0.26	32.79 ±0.01	295.20 ±0.06

MCT= Milk clotting time.
After the two extractions applied.

Table (2) shows milk clotting activity and the proteolytic activity as affected by extracting solutions A, B and C and number of extraction being I and II. In extracting solution (A), milk clotting activity was 200.33 ± 2.04 RU/g in the first extraction, whereas in the second one, it was 182.07 ±1.02. Much lower values were given in extracting solution. B and C comparing with the values of extracting solution A. In case of extracting solution B milk clotting activities were 179.18±1.25 and 171±1.25 RU/g in case of first and second extractions respectively. In case of extracting solution C, milk clotting activities were 190.77± 1.56 and 174.13±1.74 after the first and second extraction respectively.

The proteolytic activity increased as the pH of the extracting solution increased. The recorded values in case of solutions B, A and C were 2.05± 0.02, 1.91±0.01 and 1.85± 0.06 respectively in extraction I. The same observation was recorded with extraction II. On the other hand, the highest value was recorded from MCA/PA in extracting solution A (200.33± 2.04) compared with extracting solution B and C (179.18 ±1.25 and 190.77 ±1.56**)** respectively in extraction I. Whereas, MCA/PA from extraction II the lower value was recorded 68.60±1.01 from extracting B.

Generally milk clotting enzymes, which showed greater proteolytic activity, will lead to produce lower cheese yields. Enzymes with lower MCA/PA ratio when used as clotting agent for cheese making will produce the cheese of lower yield, soft body and bitter taste (Sardinas, 1972).

Table 2: Milk clotting activity and proteolytic activities as affected by extracting solutions (ES) after extraction (EX) for tow times (EXI and EX II) (Average ± SE of three replicates)

ES	Extraction I			Extraction II		
	MCA RU/g	PA Ug Tyrosine/g	MCA/PA	MCA RU/g	PA Ug Tyrosine/g	MCA/PA
A pH (5.2-5.3)	200.33 ±2.04	1.91 ±0.01	104.88 ±1.21	182.07 ±1.02	1.95 ±0.12	93.37 ±1.56
B pH (6.4-6.5)	179.18 ±1.25	2.05 ±0.02	87.40 ±1.16	171.50 ±1.25	2.50 ±0.32	68.60 ±1.01
C pH (4.5)	190.77 ±1.56	1.85 ±0.06	103.12 ±1.26	174.13 ±1.74	1.86 ±0.45	93.62 ±1.25

MCA: Milk clotting activity.
PA: Proteolytic activity**.**

Table (3) shows impact of clarification on the total RU/g when solutions A, B and C were used in the extraction process. Clarification of the extracted NO. I and II cased the total RU/g before clarification 206.32, 184.96 and 196.83 when solution A, B and C were used in order. The corresponding total RU/g values after clarification were 200.33, 179.18 and 190.77 respectively.

The total RU/g was expected since precipitate formed after clarification contained some of the RU which was collected during extraction. Clarification of the extracted NO. I cased percentages of loss 2.90, 3.13 and 3.08 when solutions A, B and C were used in order. The corresponding increasing values after adding the precipitate formed of extraction I to clarification of second extraction were 74.30, 84.77 and 76.96% respectively.

This loss was expected since precipitate formed after clarification contained some of the RU which was collected during extraction. Whereas, increasing RU/g after adding the precipitate formed of extraction I to clarification of second extraction.

Table 3:-Impact of clarification of the liquid rennet prepared using different extraction solution (ES) on the loss (%) of rennet units (RU) after the first and second (EXI and EXII) as well as the total RU/g (Average of three replicates)

ES	EXI			EXII		
	BC	AC	Loss (%)	BC	AC	Increasing (%)
A	206.32 ±1.09	200.33 ±2.04	2.90 ±0.02	104.46 ±0.98	182.07 ±1.02	74.30 ±0.69
B	184.96 ±1.56	179.18 ±1.25	3.13 ±0.21	92.82 ±0.36	171.50 ±1.25	84.77 ±1.21
C	196.83 ±1.58	190.77 ±1.56	3.08 ±1.14	98.40 ±0.98	174.13 ±1.74	76.96 ±1.02

BC= before clarification.
AC = after clarification.

As can be seen from data given in Table (4) that the effect of storage period in refrigerator on rennin units was more pronounced after 4 months. In extracting solutions A, B and C, the losses in RU/g increased with advancing storage period. On the other hand, the lower losses of RU/g were in extracting solution (C) at the end of storage period compared to the other extracting solutions. Whereas, the highest losses of RU/g was occurred from extracting solution (B) at the end of storage period. However, the highly value of RU/g was recorded in extracting solution (A) 317.46±0.685 compared to

extracting solution B and C (235.16±0.587 and 298.51±0.658 respectively) at the end of storage period.

Results in Table (5), generally revealed that the loss in RU/g increased with advancing storage period at room temperature. On the other hand, the lower loss of RU/g was observed in extracting solution. (A) at the end of storage period compared to the other extracting solutions. The highest losses of RU/g were from extracting solution. (B) at the end of storage period. Results were accompanied with such RU/g values as averages of 270.27, 222.22 and 250 at the end of storage of rennet's prepared using solutions A, B and C respectively.

Table 4:-Effect of storage period in refrigerator on loss of rennin units (%) from the liquid rennet prepared using different extracting solutions (EX) (Average ± SE of three replicates)

ES		Storage period (mon.)									
		Zero		1		2		3		4	
		Ru/ g	Loss	Ru/ g	Loss)	Ru/ g	Loss)	Ru/ g	Loss	Ru/ g	Loss)
A	I	372.30	0	357.14	4.07	344.82	7.38	327.87	11.93	312.50	16.06
	II	390.48	0	370.37	5.15	357.14	8.54	333.33	14.64	317.46	18.72
	III	384.43	0	363.37	5.48	353.03	7.67	333.33	13.29	315.81	17.42
	AV. ±SE	382.40±0.87	0	363.63±0.48	4.90 ±0.012	357.14±0.69	13.29 ±0.02	331.51±0.54	13.29 ±0.23	317.46 ±0.69	17.40 ±0.01
B	I	355.73	0	327.87	7.83	307.69	13.50	263.16	26.02	250.00	29.72
	II	355.73	0	317.46	10.76	298.51	16.09	270.27	24.02	253.16	28.83
	III	340.28	0	312.50	7.24	285.71	16.40	259.74	23.74	240.96	29.25
	AV. ±SE	350.58 ±0.75	0	319.28±0.69	8.61 ±0.01	297.30±0.65	15.33 ±0.12	264.39±0.26	24.59 ±0.23	248.04 ±0.59	29.27 ±0.06
C	I	381.82	0	357.14	6.46	344.82	9.70	327.87	14.13	307.46	19.48
	II	355.73	0	344.82	3.07	327.87	7.83	312.50	12.15	285.71	19.68
	III	357.15	0	333.33	6.67	327.87	8.20	312.50	12.50	298.51	16.24
	AV. ±SE	364.90 ±0.79	0	345.10±0.69	5.40 ±0.01	333.52±0.76	8.58 ±0.02	317.62±0.96	12.93 ±0.21	297.23 ±0.656	18.47 ±0.13

Table 5:- Effect of storage period at room temperature on loss of rennin units (%) from the liquid rennet prepared using different extracting solutions (EX) (Average ± SE of three replicates)

ES		Storage period (mon.)									
		Zero		1		2		3		4	
		Ru/ g	Loss	Ru/ g	Loss	Ru/ g	Loss	Ru/ g	Loss	Ru/ g	Loss
A	I	372.32	0	344.82	7.36	333.33	10.47	298.51	19.82	263.16	29.31
	II	390.48	0	363.37	6.94	344.82	11.69	327.87	16.03	285.71	26.83
	III	384.43	0	363.37	5.48	340.58	11.41	312.50	18.71	270.27	29.70
	AV. ±SE	382.41 ±0.65	0	357.19 ±0.45	6.59 ±0.01	339.58±0.55	11.19 ±0.01	312.96 ±0.85	18.19 ±0.012	273.05±0.95	28.61 ±0.05
B	I	355.73	0	333.33	6.30	285.71	19.68	253.16	28.83	222.22	37.53
	II	340.58	0	312.50	8.24	263.16	22.73	240.96	29.25	212.77	37.52
	III	355.73	0	327.87	7.83	270.27	24.02	259.74	26.98	222.22	37.53
	AV. ±SE	350.68 ±0.85	0	324.57 ±0.35	7.46 ±0.01	273.05±0.65	22.14 ±0.06	251.29 ±0.55	28.35 ±0.06	219.07±0.65	37.53 ±0.06
C	I	381.82	0	363.37	4.83	344.82	9.69	285.71	25.17	259.74	31.97
	II	355.73	0	327.87	7.83	312.77	12.08	263.16	26.02	240.96	32.26
	III	357.15	0	333.33	6.67	307.69	13.85	270.27	24.33	250.00	30.00
	AV. ±SE	364.90 ±0.75	0	341.52 ±0.55	6.44 ±0.01	312.76±0.45	11.87 ±0.01	273.05 ±0.75	25.17 ±0.06	250.23 ±0.85	31.41 ±0.06

CONCLUSION

The attained results concluded that extracting solution A was the best one compared with the extracting solutions B and C. The lowest value of the proteolytic activity was also recorded in extracting solution (A) compared B and C. The loss was expected since precipitate formed after clarification contained some of the RU which was collected during extraction. Whereas, increasing RU/g after adding the precipitate formed of extraction I to clarification of second extraction.During the storage period, the result recorded the lowest loss in the rennin units when storage was carried out in the refrigerator.

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الملخص العربي

تعظيم انتاج وثبات انزيم الرنيين اثناءاعداد و تخزين المنفحه السائله

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تهدف الدراسة الى زيادة عدد وحدات الرنين وتقليل الفاقد أثناء الاستخلاص لوحدات الرنين حيث تم الاستخلاص بالمحاليل الاتيـة:- المحلول (أ) 3% حمض بوريك +7% كلوريد صوديوم+3و % كلوريد كالسيوم+2و% بنزوات صوديوم على pH 5.2-5.3 و المحلول (ب) 2% حمض بوريك +10% كلوريد صوديوم+2و% بنزوات صوديوم على pH6.4-6.5 و المحلول (ج) 4% حمض بوريك +2و% بنزوات صوديوم على pH4.6 و تقدير زمن التجبن و نشاط التجبن و القدرة التحليلية وكذلك الفقد أثناء التخزين. أظهرت النتائج أن أفضل المحا ليل هو المحلول (أ) من حيث الحصول على أكثر عدد من الوحدات بعد الترويق حيث كانت عدد الوحدات قبل الترويق 206.32 بينما كانت عدد الوحدات بعد الترويق 200.33 بنسبه فقد قدرها2.90% بينما فى نفس المحلول (أ) وفى الاستخلاص الثانى بعد اضافه راسب الاستخلاص الاول الى المستخلص الثانى لترويقه كان عدد الوحدات قبل الترويق 104.46 و بعد الترويق 182.07 بنسبه زياده فى عدد الوحدات قدرها 74.30% قدرها مقارنه بالمحلول (ب)– (ج) على التوالى. وكذلك اقل زمن للتجبن مقارنـه بـالمحلول (ب)– (ج) علـى التـوالي . ولكـن كـان اجمـالى عـدد الوحـدات أكثـر فـي المحلـول . وسجلت أعلـى قيمـة النشـاط التجبنـى (200 ± 2.04) فـي المحلـول(أ) مقارنـة مـع (ب)– (ج) 179.18 ± 1.25و 190 ± 1.56 على التوالي. في حين سجلت اقل قيم التحلل البروتينى1.68±0.45 في المحلول (ج). في حين كانت نسبة النشاط التجبنى على التحلل البروتينى أعلى قيمة في الحلول (ب) (2.50 ± 0.32) مقارنة مع (ب) - (ج) وخلال فترة التخزين فقد سجلت النتائج اقل فقد في عدد الوحدات عند التخزين فى الثلاجة مقارنه بنتائج التخزين لنفس المده على درجة حراره الغرفه.