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EFFECT OF STORAGE TEMPERATURES ON CHEMICAL, MICROBIOLOGICAL AND SENSORY PROPERTIES OF RENNET EXTRACTED BY SOAKING AND MECHANICAL PROCESSES

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ABSTRACT

A comparison was done among rennet samples extracted from different forms of calves vells (fresh and dried) (sliced or minced) by using soaking and mechanical processes, during storage at room temperature (25-30 ^{o} C) and refrigeration +- 5^{o} C) for 90 days. The resultant rennet solutions were tested for milk clotting time (MCT), rennet activity (total rennin units/gram (RU/g), rat of loss % of rennet activity or rennet stability, microbiological assessments and sensory properties. Results show that storage temperature had a highly significant effect on all treatments and led to a continuous decrease in RU/g, yield and the keeping quality of all forms of vells at both temperatures. After 90 days of storage, protective effect and stability of rennet activity were superior when storage was carried out in refrigerator, almost double clotting actively of rennet and an increase in the shelf-life of. The average of rennet actively and rat of activity loss were 107.25 RU/g and 14.96 % respectively, with the storage at refrigerator, whereas the average of the other ones stored at room temp. were 94.04 RU/g and 25.41 % in order. T1 with of dried sliced vells kept at refrigerator had the highest rate of stability (119.62 RU/g), while T3 of fresh minced vells stored at room temperature had the lowest one (95.84 RU/g). T3 of fresh sliced vells stored at room temperatures had the highest rate of activity loss (36.51 %), while C soaking treatment of dried sliced vells stored at room temperature recorded the lowest rate of activity loss (10.28 %). Results show also that the mechanical process was superior than the soaking one and stirring at 100 rpm.(T1) was the perfect treatment for extraction. Microbiologically results revealed that initial bacterial counts (TBC) in all treatments, ranged between 10^4 to 10^6. C of all treatments had the lowest TBC followed by T1, T2 and T3 in order. Dried sliced vells recorded the lowest TBC followed by dried minced, fresh sliced and fresh minced vells respectively. No growth of coliform bacteria was detected in all treatments, along the storage period. Yeasts & moulds were absent in C and T1 treatments while appeared in case of T2 and T3treatments. Results of sensory evaluation show considerable differences among treatments. C and T1 were found the best ones, characterized by brown, unique odor, clear and free from sediments.

Keywords: Storage temperature, chemical, sensorial, microbiological properties, rennet extract, soaking and mechanical processes.

INTRODUCTION

The fourth stomach obtained from 10 to 30 days old milk-fed of suckling calves still considered the main and the most suitable and efficient source of milk clotting agents used in the production of most varieties of cheese. In Egypt, and in the rest of the Arabic region, the majority of rennet used is prepared by using animal sources. Calf rennet, a crude extract of gastric enzyme, consists mainly of two enzymes (85 to 95%) chymosin, being responsible for milk clotting and (10-15%) bovine pepsin (proteolytic enzyme). The relative proportion of the two enzymes varies with the age of animal (Ustunol and Zeckzer, 1996 and Broome &Limsowtin, 1998). Liquid rennet is more commonly prepared and used in variable quality. Successful attempts have been made for the production of liquid rennet of good quality by Fahmi and Amer (1962).

Most traditional of the traditional cheese-milks are coagulated by locally produced in Egyptian liquid rennet. Rennet available on the Egyptian market are not of a standard quality in terms of sensorial, microbiological, physical and chemical properties. In addition, some samples failed to comply with the requirements of the International Food Standards and to the specifications of the Joint FAO/WHO Expert Committee on Food Additives. Therefore, developed equipment and improved technology should be used in preparing of commercial rennet. Furthermore, packaging and storage conditions should be improved (M. Hofi *et. al*, 2011). Changes in the quality of rennet and its clotting activity may be affected by imperfect manufacturing defects in packaging and improper storage condition.

Rennet of proper quality should possess constant clotting activity, and must be free from other enzymes except chymosin. However, rennet should not contain any deteriorating microorganisms, which result in serious defects in taste & flavor, putrefaction, disintegration and blowing in the final product (Fox *et al.*, 2000, Beresford, 2003, Upadhyay, *et al.*, 2004). The average of loss in the rennet activity per month is relatively high, as compared with imported dry rennet. Pepsin activity increased when kept at high temperatures. (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. Decrease in the rennet activity and some defects in the resulting cheese might be due to microorganism activity in it (Guinee & Wilkinson, 1992, and Fox & Mcsweeney, 1997). The progressive increase in cheese production locally or universally may led to critical and shortages in rennet supply and this may be happened also in some traditional producing countries like Denmark, New Zealand and the Netherlands.

The aim of this work was directed to compare the effect of rennet extraction method and storage period on some

chemical, microbiological and sensory properties of extracted rennet solutions stored at room temperature or in the refrigerator, for 90 days.

MATERIALS AND METHODS

- Fresh young calf stomachs: were obtained from local Slaughtering House of Tanta city.
- Low heat skim milk powder (LHSMP) imported from USA and obtained from the local market Cairo, Egypt. Clean food grade salt was bought from El-Nasr Co. Boric acid, Na-benzoate and calcium chloride were obtained from Misr Food Additives Company, Cairo, Egypt.

Preparation of fresh and dried vells: The collected fourth stomachs were carefully rinsed with tap water to remove superfluous and fat residues. After cleaning the vells were divided into two equal portions to be ready for extraction (fresh or dried and every portion was minced or sliced to 2x1 cm).

Treatments were carried out as follows:

1- Minced (FM)

a- Soaking method for 7 days, control treatment (C).

b- Mechanical method

- At 100 rpm (tratment1 T1)
- At 800 rpm (tratment2 T2)
- At 1500 rpm (tratment3 T3)

2- Sliced (FS): was done as the previous fresh minced vells.

Dried vells: treatment of dried vells were done as fresh vells extract of replacing FM abbreviation by DM and FS abbreviation by DS. Clarification of liquid rennet extracts was carried out as given by Fahmi et al, (1979).

The extracting solution was prepared mainly according to Fahmi and Amer (1962), to contain: water, 3% boric acid, 5% sodium chloride, 0.2% sodium benzoate with adjusting pH to 5.2.

Both sliced or minced vells were added to the prepared extracting solution at a rate of 100 grams vells / L solution, and kept at room temperature (25 - 30^{o}C) with manual or mechanical stirring until the complete recovery of enzyme was achieved. The rennet extracts were stored at room or refrigerator temperature (5 +-1 ^{o}C) for 90 days.

Preparation of standard milk was done according to Fahmi and Amer (1962) using LHSMP, calcium chloride and distilled water.

Milk clotting activity: (expressed as Rennin units per gram (RU/gm.) were determined at zero time, 45 day and 90 days during storage using reconstituted skim milk according to El-Bendary *et al.*, (2007).

Milk clotting time (MCT): was estimated according to Fahmi and Amer (1962)

pH values were measured directly in the rennet samples using digital pH meter model JENWAY 3020, ENGLAND.

Microbiological analysis: Rennet samples were analysed for total bacterial count (T.B.C.) using standard plate count medium (SPC), mould & yeast using acidified malt dextrose agar medium and coliform bacteria using macconcky agar as described by APHA (2004).

Sensory analysis: Rennet samples were evaluated for sensory properties such as color, odor and appearance … etc.

Means and Standard error (SE) were estimated using SPSS computer program SPSS, (1999). All experiments were carried out in triplicates and each analysis in duplicates and the average values ± SE were tabulated.

RESULTS AND DISCUSSION

Milk clotting time MCT of all treatments has a negative relationship with rennet activity RU/g, as shown in Fig. (1), the total recovery of RU/g of different forms of vells was affected by treatments and reached reaching maximum level at the end of extraction process. There were no significant differences in MCT and RU/g between T1 and C of all treatments. T2 and T3 were found approximately near. T1 of dried sliced vells (DS) recorded the lowest MCT (152 sec.) and highest RU/g (138.13), while the highest MCT (198.33sec.) and lowest RU/g (113.70) were noticed in C of (FS). these findings were confirmed by the previous results given by Rao and Dutta (1981), Girgis *el al.*(1983), El-Batawy *el al.* (1987), Amer *et al.* (1992) and Mehanna et.al. (1998 b). On the other hand, an opposite results were given by Abd El-Salam *et al.* (1987), who reported that RU/g extracted from fresh vells were higher than that obtained from dried vells.

Data also show that the rate of extraction RU/g in T1 of all treatments, except of fresh minced treatment FM was higher than that found in the rest of both soaking and mechanical methods. Moreover, the rate of stirring in the mechanical method of all treatments was found to have the same effect on MCT, except that of (FM) treatment T1 which was high. Generally, it was concluded that the mechanical method for rennet extraction was superior than traditional (soaking) method, especially when stirring was at 100 rpm

Effect of storage period after 45 days:

Rennet stability expressed as the changes in strength of rennet activity, and the rate of reduction of rennet strength, as the percentage of the original strength, stored at room temperature and in refrigerator temperature are shown in Fig. (2). Data revealed that storage temperature plays a significant role in the stability and keeping quality of extracted rennet of all treatments. As storage time advanced the strength of rennet gradually decreased and the rate of decrease in RU/g were always higher at room temperature than that at refrigerator temperatures. The average of rennet activity at room temperature was 108.46 RU/g, while at refrigerator was 115.94 RU/g.

The average of reduction in rennet activity at room temperature was 14.00 %, while at refrigerator was 9.38 %. DS (T1) stored at refrigerator temperatures recorded the highest rate of stability (129.16 RU/g) while the lowest one (95.84 RU/g) was to FM (T3) stored at room temperature. Data, moreover, show that the highest rate of reduction was to FM (T3). This may be due to the high rate of stirring at (1500 rpm) during extraction which increase the amount of oxygen

incorporated, causing oxidation of chymosin and change in pH value, resulting in decreasing the rennet stability and increasing the rate of reduction. As reported by De Caro *et al.*, (1995), rennet can be kept for 12 months without any loss of activity when stored in the closed packaging, at 0 to 5°C in a cool and dark place. Davis, (1965), attributed the reduction of rennet stability of chymosin to the action of spoilage microorganisms and other enzymes which accelerate the destruction and increasing the rate of activity loss.

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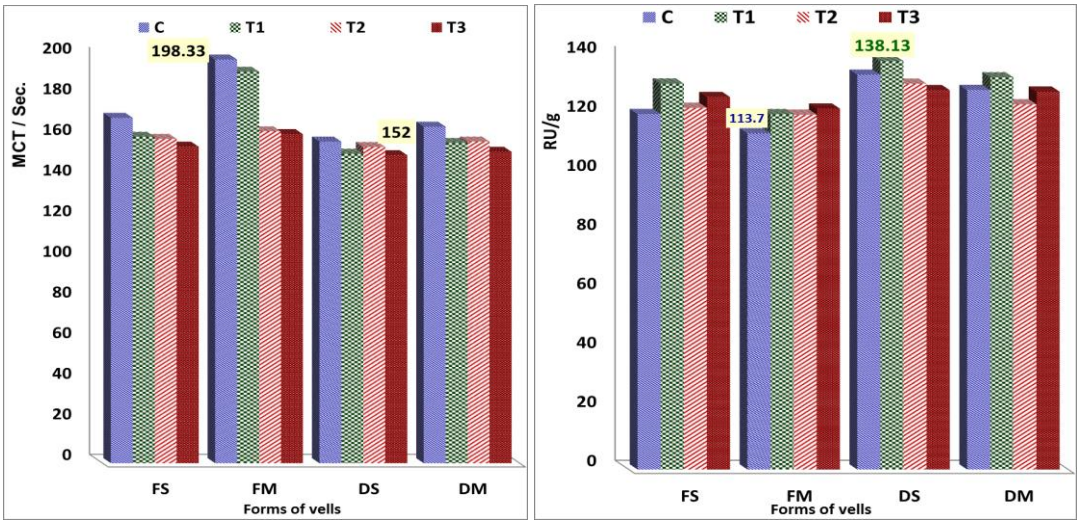

Fig. (1): MCT (sec.) and RU/g of extracted rennet solutions from soaking and mechanical processes at the end of extraction process.

FS: Fresh sliced vells FM: Fresh minced vells
DS: Dried sliced vells DM: Dried minced vells

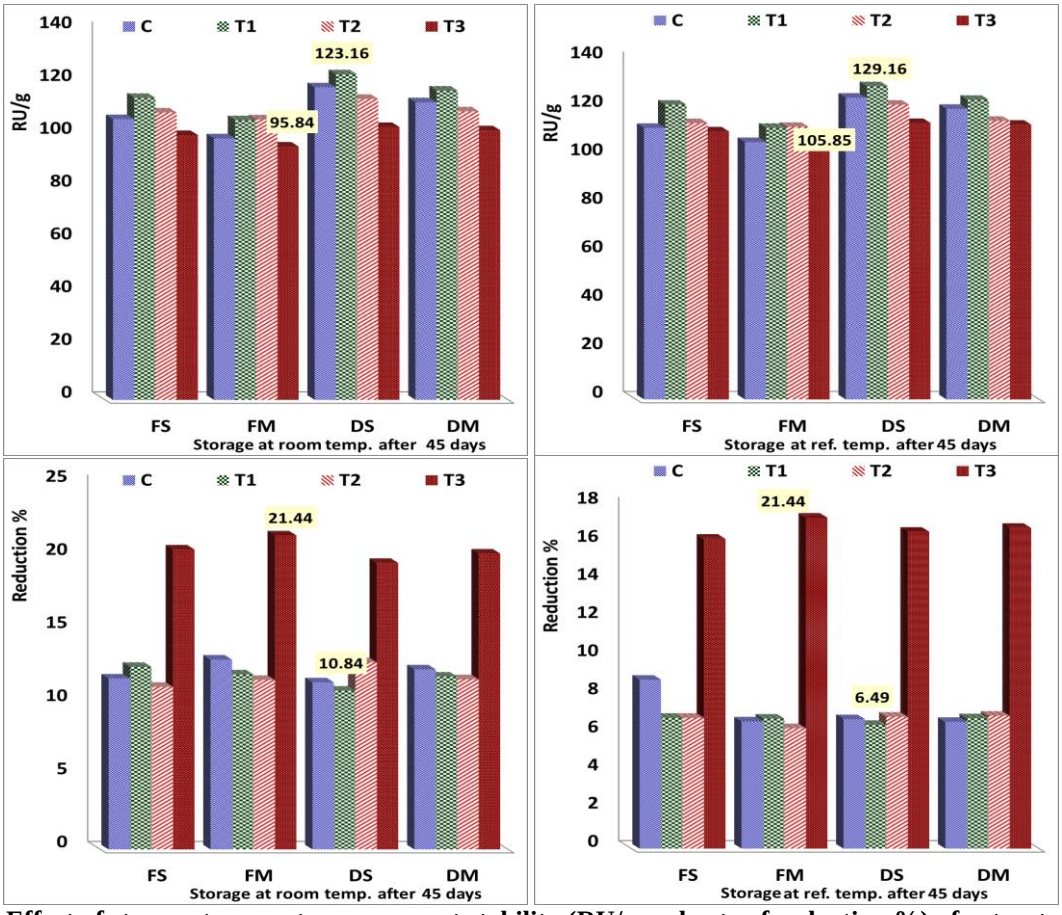

Fig. (2): Effect of storage temperature on rennet stability (RU/g and rate of reduction %) of extracted rennet solutions after 45 days. See legend to Fig. (1) for details.

Effect of storage period after 90 days:

It could be noticed from data given in Fig. (3) and the statistical analysis that, storage temperature to 90 days had a highly significant effect on the strength of rennet (RU/g) and the keeping quality of the all forms of vells. Better stability was observed at refrigerator temperature, almost double the shelf-life of rennet. The average of rennet activity at refrigerator (107.25 RU/g.) was higher than that at room temperature (94.04 RU/g). T1 of DS at refrigerator temperatures had the highest stability rate in RU/g (119.62), while T3 of FM at room temperature had the lowest one in RU/g (92.6). On the other hand the average of activity loss at room temperature (25.41 %) was higher than that at refrigerator (14.96 %). T3 of FS at room temperatures had the highest rate of activity loss (36.51 %), while C of DS recorded the lowest one (10.28 %). These results are in agreement with those given by Naguib et.al. (1980) and Mehanna et.al. (1998 b). Generally, it was noticed that storing at refrigerator was preferred that the traditional method of extraction(soaking) and treatment T1 (stirring at 100 rpm) of all treatments, stored at refrigerator, was found the best method for extraction

Microbiological analysis:

One of the most critical concerns with the use of rennet in cheese making is their poor microbiological quality. Results given in Table (1) indicate that the rennet samples had different initial total bacterial count (TBC), ranged between 10^4 to 10^6 org./ml. C had the lowest TBC followed by T1, T2 and T3in order. This may be due to the preservative effect of the extraction solution during long extracting time for C. Concerning forms of vells, DS form had the lowest TBC followed by DM, FS and FM respectively. This may be due to the increase of the surface area in minced form than sliced one. No growth of coliforms in all treatment along the storage period. Yeast & mould were absent in case of C and T1, while appeared in case of T2 and T3. Similar results were obtained by Naguib *et al.*(1980), Mehanna et.al. (1998 a) Bakr (1996) and El-Ghandour *et al.* (2007). According to the obtained results, storage temperature and extraction time had a highly significant effect on microbiological quality of rennet. During storage, standard plate count and Yeast & mould counts were continuously decreased during storage at both room and refrigerator temperatures but at variable rates and this may be due to the differences in processing technology and differences in storage temperature. The rate of decrease was high and faster at room temperature than at refrigerator. T2 and T3 had the highest contents of TBC and yeast & mould which were approximately near, while C and T1 had the lowest ones and had the same TBC and yeast & mould. Similar results were obtained by El-Hawary, M.Y; *et al.*, (1983): Beresford, (2003), Schlesser, *et al.*, (2006), Genina, (2008) and Bakry (2013)

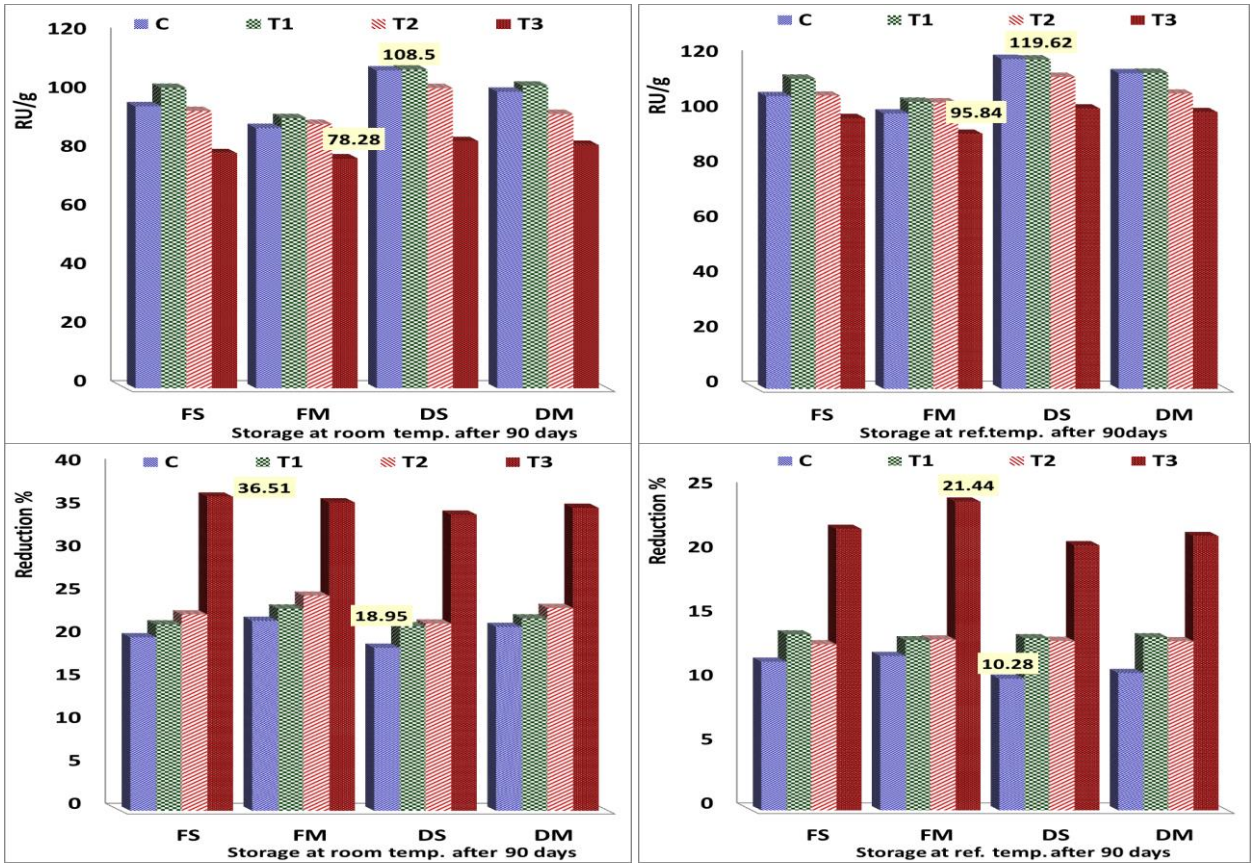

Fig. (3): Effect of storage temperature on rennet stability (RU/g and rate of reduction %) of extracted rennet solutions after 90 days.

Table 1: Microbiological analysis of extracted rennet samples during storage period for 90 days at room temperature.

Treatment	* Vells Form	Zero Time			45 days			90 days		
		TBC (cfu/ml) $x10^6$	Coliforms (cfu/ml) $x10^2$	Yeast & Mould (cfu/ml) $x10^4$	TBC (cfu/ml) $x10^6$	Coliforms (cfu/ml) $x10^2$	Yeast & Mould (cfu/ml) $x10^4$	TBC (cfu/ml) $x10^6$	Coliforms (cfu/ml) $x10^2$	Yeast & Mould (cfu/ml) $x10^4$
C (Soaking for 7 days)	FS	0.68	n.d.	<1	0.30	n.d.	<1	0.27	n.d.	<1
	FM	1.00	n.d.	<1	0.50	n.d.	<1	0.48	n.d.	<1
	DS	0.40	n.d.	<1	0.21	n.d.	<1	0.20	n.d.	<1
	DM	0.53	n.d.	<1	0.28	n.d.	<1	0.27	n.d.	<1
T1 (100 rpm)	FS	7.58	n.d.	<1	3.45	n.d.	<1	2.82	n.d.	<1
	FM	8.26	n.d.	<1	4.12	n.d.	<1	3.18	n.d.	<1
	DS	0.50	n.d.	<1	0.30	n.d.	<1	0.26	n.d.	<1
	DM	0.60	n.d.	<1	0.29	n.d.	<1	0.28	n.d.	<1
T2 (800 rpm)	FS	14.28	n.d.	95	10.15	n.d.	43	6.41	n.d.	20
	FM	20.86	n.d.	100	14.24	n.d.	56	8.56	n.d.	28
	DS	2.57	n.d.	40	1.95	n.d.	21	1.00	n.d.	14
	DM	4.66	n.d.	70	2.69	n.d.	28	1.75	n.d.	16
T3 (1500 rpm)	FS	20.00	n.d.	79	13.95	n.d.	32	10.22	n.d.	18
	FM	40.00	n.d.	100	28.19	n.d.	58	20.18	n.d.	26
	DS	10.00	n.d.	30	6.24	n.d.	18	4.67	n.d.	12
	DM	10.00	n.d.	50	5.92	n.d.	27	4.12	n.d.	16

*** See legend to Fig. (1&3) for details. * n.d : not detected**
TBC: total bacterial count.

Table 2:Microbiological analysis of extracted rennet samples during storage period for 90 days at refrigerator temperature.

Treatment	Vells Form	Zero Time			45 days			90 days		
		TPC (cfu/ml) $x10^6$	Coliforms (cfu/ml) $x10^2$	Yeast & Mould (cfu/ml) $x10^4$	TPC (cfu/ml) $x10^6$	Coliforms (cfu/ml) $x10^2$	Yeast & Mould (cfu/ml) $x10^4$	TPC (cfu/ml) $x10^6$	Coliforms (cfu/ml) $x10^2$	Yeast & Mould (cfu/ml) $x10^4$
C (Soaking for 7 days)	FS	0.68	n.d.	<1	0.27	n.d.	<1	0.16	n.d.	<1
	FM	1.00	n.d.	<1	0.45	n.d.	<1	0.40	n.d.	<1
	DS	0.40	n.d.	<1	0.18	n.d.	<1	0.16	n.d.	<1
	DM	0.53	n.d.	<1	0.20	n.d.	<1	0.20	n.d.	<1
T1 (100 rpm)	FS	7.58	n.d.	<1	3.00	n.d.	<1	2.11	n.d.	<1
	FM	8.26	n.d.	<1	3.60	n.d.	<1	2.77	n.d.	<1
	DS	0.50	n.d.	<1	0.29	n.d.	<1	0.18	n.d.	<1
	DM	0.60	n.d.	<1	0.22	n.d.	<1	0.20	n.d.	<1
T2 (800 rpm)	FS	14.28	n.d.	95	9.12	n.d.	32	5.86	n.d.	16
	FM	20.86	n.d.	00	13.26	n.d.	52	7.29	n.d.	20
	DS	2.57	n.d.	40	1.14	n.d.	19	0.90	n.d.	10
	DM	4.66	n.d.	70	2.12	n.d.	25	1.12	n.d.	12
T3 (1500 rpm)	FS	20.00	n.d.	79	12.50	n.d.	24	9.16	n.d.	12
	FM	40.00	n.d.	100	27.56	n.d.	49	19.40	n.d.	22
	DS	10.00	n.d.	30	5.38	n.d.	14	4.00	n.d.	09
	DM	10.00	n.d.	50	5.00	n.d.	24	3.96	n.d.	13

*** See legend to Fig. (1&3) for details. * n.d : not detected TBC: total bacterial count.**

IV. Quality and sensory properties:

Liquid rennet must be free of sediments, have a clear, caramel color and a specific odor. And free from unpleasant flavor. Results show considerable differences among the treatments with concerning color, appearance and odor, probably due to the differences in processing technology applied and the extraction time. T2 and T3 for FM and DM treatments had a light brown color, an unpleasant odor and contained sediments. The remaining samples C and T1 for FS and DS treatments were brown (like caramel)color, unique odor near to traditional rennet, clear and sediment free. C and T1 for FS and DS samples possessed those properties.

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تأثير حرارة التخزين علي الخواص الكيميائية والميكروبية والحسية لمستخلص المنفحة الناتجة بطريقة النقع أو باستخدام التقليب الميكانيكي

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تم عمل مقارنة بين عينات المنحة المستخلصة من أبوات العجول الصغيرة الطازجة (شرائح أو مفرومة) والجافة (شرائح أو مفرومة) باستخدام طريقة النقع التقليدية (في محلول استخلاص لمدة ٧ أيام) أو الطرق الميكانيكية (التقليب الألى المستمر) على سرعات ١٠٠ (معاملة ١), ٨٠٠ (معاملة ٢) , ١٥٠٠ (معاملة ٣). وذلك أثناء التخزين على درجة حرارة الغرفة (٢٥-٣٠ ^{0}م) أو في الثلاجة ٥ +- ١^{0}م) لمدة ٩٠ يوم. تم تقدير زمن التجبن بالمنفحة, نشاط أو قوة المنفحة (عدد وحدات الانزيم/جم), نسبة الفقد أو الانخفاض في قوة المنفحة, الصفات المكروبيولوجية والحسية للمستخلصات الناتجة خلال مدة التخزين. أظهرت النتائج ما يلى:

أ- أثر طريقة الاستخلاص:- كانت طريقة التقليب الميكانيكية افضل من طريقة النقع وكان التقليب على سرعة ١٠٠ لفة/ دقيقة افضل طرق الاستخلاص.

ب- أثر درجة حرارة ومدة التخزين:- كان لدرجة الحرارة ومدة التخزين تأثيرا معنويا كبيرا على كل ن قوة ونشاط المنفحة والتصافى وقوة الحفظ. بعد ٩٠ يوم وجد ان نشاط المنفحة وقوة حفظها كن افضل عند التخزين على درجة حرارة الثلاجة مقارنة بنظيرتها المخزنة على درجة حرارة الغرفة. كان متوسط قوة المنفحة ١٠٧.٧٣ وحدة انزيم/جم ومعدل الانخفاض في نشاطها ١٤.٩٦ % (عند التخزين في الثلاجة), في حين كانت القيم المقابلة على درجة حرارة الغرفة ٩٤.٠٤, ٢٥.٤١ % . سجلت المعاملة (١) لشرائح الابوات المجففة المخزنة في الثلاجة أعلى قيم لنشاط المنفحة ١١٩.٦٢ بينما كانت المعاملة (٣) للأبوات المفرومة الطازجة المخزنة في الغرفة أقل القيم ٧٨.٢٨. كان أعلى معدل انخفاض في قوة المنفحة (٣٦.٥١ %) للمعاملة (٣) لشرائح الابوات المخزنة في الغرفة بينما سجلت طريقة النفع (الكنترول) لشرائح الابوات المجففة والمخزنة في الغرفة أقل معدل انخفاض ١٠.٢٨ %.

جـ - التقييم الميكروبيولوجي:- تراوح العدد الكلى للبكتيريا بين جميع المعاملات بين ١٠^4 – ١٠^6. كان العدد البكتيري فى طريقة النقع أقل من باقي المعاملات يليها المعاملة (١) و (٢) واخيرا المعاملة (٣). سجلت شرائح الابوات الجافة اقل عدد بكتيري يليها مفروم الابوات المجففة و الشرائح الطازجة وأخيرا الشرائح المجففة. ظهرت الخمائر والفطريات في المعاملة (٢) والمعاملة (٣) في حين اختفت من المعاملة (C) والمعاملة (١).

د – التقييم الحسى:- ظهرت فروق معنوية بين جميع المعاملات خلال مدة التخزين. كانت المعاملة (C) والمعاملة (١) افضل المعاملات حسيا وتميزت المنفحة الناتجة منهما باللون البنى (لون الكرامل و الرائحة المميزة للمنفحة و النقاء والخلو من الرواسب.