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Quality Assessment of Locally Produced Egyptian Liquid Rennet

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Abstract

Most tradition milk cheeses are coagulated with locally produced Egyptian liquid rennet; questions are still raised as to their hygienic quality. Safety, microbiological, physical, chemical, technological characteristics of rennet was evaluated. 19 Samples were collected from different regions in Egypt. The quality of the various rennet samples varied widely between factories.The total bacterial count ranged from 0.44x104 to 1.7 x 10 7 cfu/ml, the yeast and mold count was <1-50 cfu/ml and the spore forming anaerobe count was <1-140 cfu/ml. only 37% of the samples contained coliform bacteria.. pH values, and milk clotting activity of the samples ranged between 5.03 & 5.85 and 177 & 310 u /ml respectively . Relative density ranged from 1.13 ± 0.02. The average activity loss is 20 %per month. It can be concluded that most of the commercial liquid rennet samples characterized with low activity, stability, in addition to variations in terms of their microbiological, physical, chemical characteristics. To ensure rennet safety, the manufactures and trade are advised to pay attention on some key areas from the preparation to application of rennet. The use of artisan-produced rennet of questionable hygienic quality for the manufacture of raw milk cheeses yields products of good hygienic quality.

Key words: rennet, activity, stability, safety.

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 probably 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 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 &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 proteolytic enzymes. The clotting activity affects the properties of the curd, such as firmness or softness, during processing (Rampilli, & Barzaghi 1995; Fox & Mcsweeney, 1997; Dejmek & 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 but 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 (Fox et al., 2000, Beresford, 2003, Upadhyay, et al., 2004).

Rennet is used in cheese manufacturing primarily as a milk coagulant. Other enzymes present in rennet also play an important role in cheese production, especially in cheese ripening and may be a cause of the development of

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bitterness during storage (Mara &Kelly, 1998; Sohal et al., 1988; Zakrewski et al., 1991, Harboe & Budtz, 1999). An important factor affecting cheese quality is the rennet dose in production. In Egypt new widely spread low cost recombined soft cheese is made using high fat level, low quality skim milk powder and high salt content, in order to obtain a workable curd from this type of milk, a higher incubation and more chymosin is usually required.

Because of the lack of a standardized preparation procedure yielding rennet of consistent quality; their use has been gradually avoided by cheese manufacturers .Most local rennet producer does not have purification facilities, Such as chromatography or membrane separation, to ensure a sparkling clear solution with much lower mucoprotein content. It contains many deodorants or reodorants. 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 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 decreases in activity and a variety of defects in the cheese (Guinee & Wilkinson, 1992, and Fox & Mcsweeney, 1997).

Questions are still raised as to their hygienic quality (Ec, 2002). Research are needed for assessment 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, risk from cross-contaminated feed, and geographical sourcing. The aim of our study was to evaluate the present status of the rennet's locally produced in Egypt by assessment some of their sensorial, microbiological, physical and chemical properties.

Material and Methods

Materials

19 commercial liquid rennet samples were collected from various locations in Egypt. Samples were obtained from cheese producing plants. Duplicate (i.e., two identical) samples were collected from each type of product at each sampling session .Approximately 50 ml of each sample was transferred to sterile glass jars for microbiological analysis while about 100 ml was put into clean, dry jars for physical, chemical and sensory analyses. The samples were transported in an ice chest and stored at 5° c before analyses.

Methods

a. Microbiological analysis

Sampling and dilutions were performed in accordance with International Dairy Federation standards (1985). Duplicate plates were used for all of the microbiological analyses .Count of total viable microorganisms in plate count agar (Oxoid), after incubation for 72 h at 30°C, was determined by the procedure previously used by Gómez et al., (1989). Violet Red Bile Agar (Merck) was used for coliform bacteria (Ira, 1984) and Potato Dextrose Agar (Merck) for yeasts and molds (Koburger, & Marth, 1984). The plates were incubated at 32 ± 1 C for 48 ± 3 h for TAMB counts, 37 ± 2 ℃ for 24 h for coliforms, and 25± 2 C for 5 days for yeast and mold counts. For the determination of anaerobe spores, the appropriate dilutions were heated at 80 ℃ for 10s and then transferred onto duplicate plates of Differential Reinforced Clostridium Medium (DRCM) (Merck) containing 1.5% agar-agar, and the plates were placed into anaerobic jars (Anaerocult A, Merck) and incubated at 30 ℃ for 72 h. After the incubation period, the black colonies were counted (Baumgart et al., 1986).

b. Sensorial, Physical and Chemical Analysis

The rennet samples were evaluated for several sensory properties such as color, odor and appearance. Rennet clotting activity was determined according to El-Bendary et al., (2007). pH values were measured using a pH meter (HANNA instruments model 8417 , USA). Relative density and rennet strengths were determined using the procedure described in FAO, 1977. The strength of rennet is defined as the amount of milk, expressed as ml of milk which one ml rennet can clot in 40 minutes at 35 ℃. Proteolytic activity was determined according to Iwasaki et al., (1967).One Proteolytic unit (PU) was defined as the amount of enzyme which release the equivalent of 1mg tyrosine under the assay conditions.

Results and Discussion

A. Microbiological properties:

One of the most critical concerns with the use of rennet in cheese making is their poor microbiological quality. Evaluation of microbial flora present in collected sample indicated that (Table 1) , there was no TAMB growth (<1 cfu/ml) in 2 samples, but that growth was observed in the other 17 samples, up to 1.7 x 10 7 cfu/ml TAMB. 13 of the samples analyzed in this research did not contained coliform bacteria (<1 cfu/ml). The results showed that 4 samples did not contain yeast or mold, while 4 samples analyzed had 10 cfu/ml and the rest had over 20 cfu/ml of yeast and mold. No anaerobe spores were found in 7 rennet samples, but that growth was observed in the other 12 samples, up to 140 cfu/ml.

It has been suggested that due to the poor quality of raw materials and defects in production processes, rennet might contain high levels of TAMB (Davis, 1965). For example, in Turkey Cakmaki & Boroúlu, (2004) reported that, growth was observed in the 20 from 25 total samples., up to 2.5 x 106 cfu/ml . No anaerobe spores were found in 21 rennet samples.

Our results indicated that the majority of the samples contained more TAMB than allowed in the standard few samples conformed to the standard. Rennet must not contain any coliform bacteria. Since the anaerobe spores cannot be completely destroyed during pasteurization their presence in milk for cheese or contamination via rennet is a serious problem. According to the obtained results rennet can be source of non-starter lactobacilli (NSLAB) in cheese. Many of the studies concluded that non-starter lactobacilli have a role to play in cheese quality. Control of NSLAB has proved more difficult. The fact that , non-starter lactobacilli are ubiquitous microorganisms and can readily grow in cheese. The approaches involved sterilization of all cheese making ingredients and utensils prior to cheese manufacture, more stringent milk pasteurization procedures and attempts to avoid contamination from the environment during manufacture (Schlesser, et al .,2006, and Beresford, 2003).

Table 1: Microbiological properties of rennet samples

Samples	Total bacterial count (cfu/ml)	Coliform bacteria (cfu/ml)	Yeast & molds (cfu/ml)	Anaerobic spores (cfu/ml)
1	2x10 ^{6}	n.d.	20	110
2	1.9 x 10 ^{6}	n.d.	10	20
3	1 x 10 ^{6}	n.d.	20	60
4	0.41 x 10 ^{6}	100	3o	70
5	2x10 ^{4}	n.d.	10	35
6	0.32x 10 ^{5}	< 1	<1	<1
7	< 1	< 1	< 1	<1
8	0.44x 10 ^{4}	n.d.	40	70
9	3x 10 ^{5}	n.d.	30	< 1
10	4 x10 ^{6}	n.d.	20	50
11	0.61 x 10 ^{5}	100	20	< 1
12	8 x10 ^{5}	< 1	10	20
13	2 x 10 ^{6}	30	50	70
14	1.7 X10 ^{5}	< 1	< 1	< 1
15	2x 10 ^{6}	< 1	< 1	< 1
16	.52 x10 ^{6}	40	10	< 1
17	< 1	10	20	30
18	1.7 x 10 ^{7}	200	30	120
19	1x 10 ^{5}	< 1	50	140

*n.d : not detected

b. Sensorial properties:

The results of the sensorial analysis showed considerable differences among the rennet samples collected from different locations in Egypt with respect to color, appearance and odor, probably due to differences in raw material used processing technology applied. Four samples were of a light brown and the remaining 16 samples were brown (like caramel color). Nine samples had an odor unique to rennet, while the remaining 11 samples had an unpleasant odor. While 14 rennet samples were clear and sediment free, 2 samples were opaque and another sample contained sediments. Rennet must be free of sediments, have a clear, caramel color and a specific odor. Liquid rennet's originating from animals should be translucent and clear without an unpleasant flavor. Nine of the rennet samples analyzed in our research possessed those properties.

c. Physical properties:

Physical quality of the various rennet samples varied widely between factories .pH values of the rennet samples ranged from 5.03 to 5.85 with an average of 5.43.All of the rennet samples had pH values between 5 and 6. The pH values of 3 samples were above 5.75 (Table 2). pH plays a significant role in the stability of chymosin, and that its maximum pH stability is 5.8 (Davis, 1965).

As seen from the same table, relative density range was 1.13 ± 0.02 kg/l. eight samples had relative density values between 1.12 and 1.14 kg/l.

Table 2: Physical and technological properties of rennet samples.

Samples	pH	Clotting Time (sec.)	Relative density (kg/l)	Milk Clotting Activity (u /ml)	Strength (ml)*	Proteolytic activity (PU)	MCA /PA**
1	5.03	178	1.13	270	13.5	1.85	146
2	5.51	155	1.14	310	15.5	1.1	282
3	5.85	265	1.13	181	9.1	1.5	121
4	5.22	205	1.15	234	11.7	2.05	114
5	5.67	188	1.11	255	12.8	3.1	82
6	5.09	253	1.12	189	9.5	1.86	102
7	5.11	196	1.11	245	12.2	3.7	66
8	5.65	271	1.10	177	8.8	.95	186
9	5.46	225	1.14	213	10.6	1.75	122
10	5.75	249	1.11	193	9.6	2.5	77
11	5.25	169	1.13	284	14.2	2.65	107
12	5.65	158	1.11	304	15.1	1.8	169
13	5.33	262	1.15	183	9.2	2.09	88
14	5.78	181	1.14	265	13.3	3.02	278
15	5.08	236	1.15	203	10.2	2.3	88
16	5.44	241	1.12	199	10.1	1.1	180
17	5.64	175	1.12	274	13.7	2.41	114
18	5.05	196	1.13	245	12.2	.98	250
19	5.66	258	1.10	186	9.30	1.5	124

* Ml of milk which one ml rennet can clot in 40 minutes at 35 ^{o}c

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

D. Technological properties:

The milk-clotting activity (MCA) of rennet relies on its ability to degrade casein micelles, and this action depends on the chymosin content of the complex. Chymosin proportion may differ by source (cow, lamb, goat, chicken, camel, or no animal sources) and age, accompanied by a concomitant alteration of pepsin content (Guinee & Wilkinson, 1992). The proportions normally present in commercial rennet's are 70% chymosin and 30% pepsin.

The standard ration is approximately 80% chymosin and 20% pepsin (Irigoyen, 2001). As seen from table (2), rennet samples illustrated varying degrees of activity, MCA of the samples ranged from 177 to 310(u /ml). However, none of the samples ranked as high MCA.

Rennet strength recorded similar results. Strength of the rennet samples ranged from 8.8to 15.5 ml, with considerable differences among the samples. Strength in 6 out of the 19 samples was 13.3 or higher, and these samples may therefore be classified as medium class rennet: the 13 others were weak rennet, with the strength of the lowest being 9.1.

Variation MCA and strength might be related to the processing of the raw material used for rennet, or be due to differences in raw materials, variations in processing technology, differences in storage conditions (packaging, temperature, etc.) and differences in production and usage time. According to carefully monitored for physical, chemical and microbiological properties as well as strict production process and standardization procedures result in a finished product that meets every prevailing quality requirement.

Proteolytic activity

As seen from obtained results (Table, 2), variations in proteolytic activity (PA) among collected sample has bee recorded, PA values of the rennet samples ranged from 0.95 to 3.02 with an average of 2.01.6 samples had PA values between0.95 and1.5, and the PA values of 5 samples were above 2.5.

MCA/PA ratios for the commercial rennet were shown in the same table. From the data we could find that the commercial rennet had the highest clotting activity and lowest proteolytic activity, its MCA/PA ratio ranged from 66 to 282. This finding was the same as the result of Bustamante, et al., (2000). Proteolytic activity reflect variation in raw material used in manufacture, in particular the age of animal from which rennet are extracted .The rennet from the young milk-fed calf is rich in rennin (chymosin), while rennet from the older bovine is rich in pepsin (Bustamante, et al., 2000). Each of these enzymes has different clotting as well as proteolytic activities and affects milk proteins in different ways during cheese ripening .Generally milk clotting enzymes, which showed greater proteolytic activity, will lead to produce lower yields of cheese. Enzymes with lower MCA/PA ratio used as clotting agent for cheese making will produce the cheese of lower yields, soft body and biter taste (Sardinas, 1972). Clotting activity should be calculated precisely to avoid possible failures in curd formation.

E. Rennet stability

Rennet stability expressed as changes in strength of the rennet stored at 4°c.Strength loss values reported as a percentage of the original strength. The graphs below (fig, 1, 2), illustrated the fluctuation in rennet activity of selected samples in relation to storage temperatures during a specific period. The average activity loss is 20 % per month. High recorded loss may be related to primitive way of manufacture and lack in purification processes. As reported by De Caro et al., (1995), the average activity loss is 0.5% per month; rennet can be kept for 12 months without any loss of activity provided it is stored in the closed original packaging, in a cool and dark place between 0 and 6°C.

Davis, (1965) attributed the stability of chymosin to the microorganisms and other enzymes that accelerate the destruction. He also noted that pH plays a significant role in the stability of chymosin, and that its maximum pH stability is 5.8. Two samples fulfilled this criterion given by Davis. On the other hand he stated that the optimum pH range of liquid rennet was 5.5-5.7, and 4 of the samples analyzed in this research were within that range.

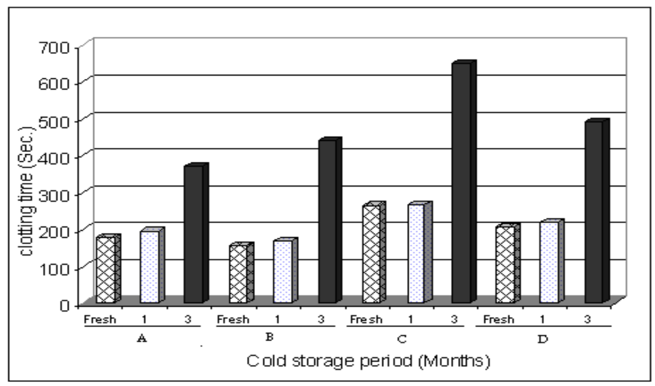

Fig.1: Changes in collecting time during storage at 4 °c of selected rennet samples (A,B,C,D)

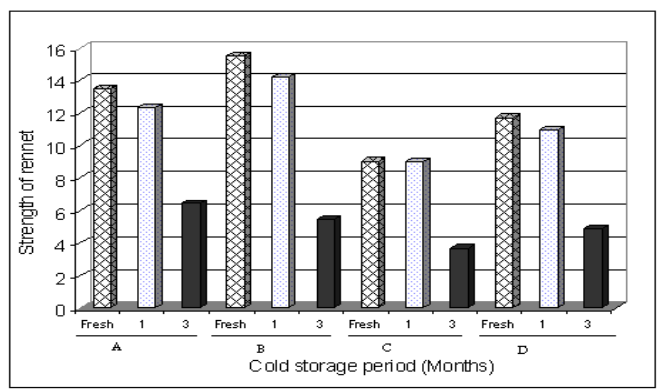

Fig.2 :Changes in rennet strength during storage at 4 °c of selected samples (A,B,C,D)

The results of this study indicated that the rennet's 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 modern equipment and technology should be used in the production of commercial rennet's. In addition packaging and storage conditions should be improved

Conclusion

The obtained results contribute to a better knowledge of the locally produced rennet properties and safety. It can be concluded that, locally produced liquid rennet characterized with low activity, stability and shelf life. Variations in terms of their microbiological, physical, chemical characteristics were investigated. Because of the lack of standardized preparation procedure yielding rennet of inconsistent quality, their use has been gradually avoided by cheese manufacturers. It can be recommended that, a simple and reproducible technological process must be developed and applied in order to produce alternative one to those now available in the market, as it not only exhibited an improved microbial quality but also retained the peculiarities of the rennet elaborated in artisanal manner. The use of artisan-produced rennet of questionable hygienic quality for the manufacture of raw or pasteurized milk cheeses yields products of good hygienic quality.

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