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Research Article

Some Quality Characteristics of Commercial Liquid Rennet Samples

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Abstract: The aim was to investigate some of the microbiological, physical, chemical and sensorial properties of commercial rennet samples collected from different regions in Turkey. A total of 25 liquid rennet samples were tested. The total aerobic mesophilic bacteria count was <1-2.5 x 10^6 cfu/ml, the yeast and/or mold count was <1-30 cfu/ml and the spore forming anaerobe count was <1-35 cfu/ml. None of the samples contained coliform bacteria. Sensorial analysis showed that 7 out of the 25 samples conformed to the Turkish Rennet Standard (TS-3844), which states that rennets are to be clear and caramel colored, carry no sediments and have a specific smell. The other 18 samples possessed none of the properties set out in the standard. pH values, salt levels and milk clotting activity of the samples ranged between 5.08 and 5.82, 4.83 and 14.05% and 1/5670 and 1/45450, respectively. Our results suggested that most of the commercial liquid rennet samples showed variations in terms of their microbiological, physical, chemical and sensorial properties and that the values were not within the limits of TS-3844.

Key Words: Chymosin, rennet, clotting, cheese.

Ticari Sıvı Peynir Mayalarının Bazı Kalite Özellikleri

Özet: Bu araştırmada Türkiye'nin değişik bölgelerinden temin edilen peynir mayalarının (rennet) mikrobiyolojik, fiziksel, kimyasal ve duyusal özellikleri araştırılmıştır. İncelenen 25 adet sıvı peynir mayası örneğinde <1-2,5 x 10^6 kob/ml arasında total aerobik mezofilik bakteri, <1-30 kob/ml arasında maya ve/veya küf, <1-35 kob/ml arasında ise anaerob spor olduğu saptanmıştır. Örneklerin hiçbirinde koliform grubu bakteriye rastlanmamıştır. Duyusal analiz sonuçlarına göre 7 adet örnek karamel renkte, tortusuz, berrak ve kendine özgü kokuya sahip olup Peynir Mayası Standardı (TS-3844)'na uygun, diğerleri ise uygun bulunmamıştır. 25 adet örneğin pH değerlerinin 5,08-5,82 arasında, NaCl miktarının % 4,83-14,05 arasında ve maya kuvvetinin 1/5670- 1/45450 arasında değiştiği belirlenmiştir. Araştırma sonuçlarına göre, ticari sıvı rennet örneklerinin çok değişik mikrobiyolojik, fiziksel, kimyasal ve duyusal özelliklere sahip olduğu ve bazı örneklerin Peynir Mayası Standardı'na uymadığı belirlenmiştir.

Anahtar Sözcükler: kimozin, ticari rennet, pıhtılaşma, peynir.

Introduction

An important factor affecting cheese quality is the rennet used in production. Today, nearly all types of cheese are manufactured by clotting the milk with a milk-clotting enzyme and then processing the curd in various ways. For this purpose, proteolytic enzymes originating from plants, animal tissues and microorganisms are widely used. These types of cheese have several advantages compared to those produced by acid clotting methods. Since they are ripened under controlled conditions for a specific time, these cheeses may have a longer shelf life and be more popular with consumers due to their unique characteristics such as texture, color, taste and aroma (1-4).

Rennets originating from young ruminants are produced from the fourth stomach (abomasum) of milk-fed calves or other young ruminants. The rennet from the young milk-fed calf is rich in rennin (chymosin), while rennet from the older bovine is rich in pepsin (5,6-10). In addition to the calf many other animals including the lamb, goat, pig, bovine, rabbit and hen contain the enzyme chymosin in their digestive systems (11,12).

Due to the acidic features of protease enzymes, chymosin and pepsin coagulate milk by splitting specific peptide bonds between the phe- (105) and met (106) residues of κ-casein (1,5,13,14). Each of these enzymes has different clotting activities and affects milk proteins in different ways during cheese ripening (7-14).

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 (15).

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 these enzymes. Sometimes, there is a discrepancy between the declared value on the label and the experimentally determined clotting activity (16).

A 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 and flavor, putrefaction, disintegration and blowing (17-18).

In Turkey, the milk used for making white pickled cheese is subjected to severe heat treatment resulting in the coagulation of whey proteins. To obtain a workable curd from this type of milk, a higher incubation temperature, higher amounts of culture and more chymosin are usually required. 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 (3,9,17). 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 (9,17,19).

Uraz (15) investigated 19 rennet samples, and reported that these were contaminated with total aerobic mesophilic bacteria (TAMB), yeast and mold, and anaerobic spore forming bacteria, but that none contained coliform. Koçak (9) reported that plastic packaging materials and ambient temperatures were not appropriate for the storage of rennet.

The aim of this research was to determine the present status of the rennets commercially marketed in Turkey, by evaluating some of their sensorial, microbiological, physical and chemical properties and comparing these with the Turkish Rennet Standard (TS-3844) (20).

Materials and Methods

Materials

A total of 25 commercial liquid rennet samples were obtained from various locations in Turkey; 6 from the Marmara region (2 Tekirdağ, 2 Edirne, 1 Balıkesir, 1 Bursa); 6 from the East Anatolia region (3 Erzurum, 2 Kars, 1 Elazığ); 4 from the central Anatolia region (2 Ankara, 2 Konya); 3 from the Aegean region (3 İzmir); 2 from the Black Sea region (2 Trabzon); 2 from the Southeast Anatolia region (2 Urfa); and 2 from the Mediterranean region (1 Mersin, 1 Antalya). The rennet samples were obtained from cheese producing plants and small scale cheeseries. Approximately 150 ml of each sample was transferred to sterile glass jars from larger bottles (at least 1 liter) for microbiological analysis while about 100 ml was put into clean, dry jars for physical, chemical and sensory analyses. All of the sample jars were covered with aluminum foil and brought to the laboratory in an ice box. They were kept in a refrigerator until they were analyzed.

Methods

a. Microbiological analysis

All samples were vigorously shaken, and 1 ml of each sample was transferred into tubes containing a sterile physiological saline solution (9 ml 0.85% NaCl) and serial dilutions were then prepared. Duplicate plates were used for all of the microbiological analyses.

Plate Count Agar (Merck) was used for TAMB, Violet Red Bile Agar (Merck) for coliform bacteria (20-21), and Potato Dextrose Agar (Merck) for yeasts and molds (22). The plates were incubated at 32 ± 1 °C for 48 ± 3 h for TAMB counts, 37 ± 2 °C for 24 h for coliforms, and 21 ± 2 °C for 5 days for yeast and mold counts.

For the determination of anaerobe spores, the appropriate dilutions were heated at 80 °C for 10 s 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 °C for 72 h. After the incubation period, the black colonies were counted (23).

b. Sensorial, Physical and Chemical Analysis

The rennet samples were evaluated for several sensory properties such as color, odor and appearance.

Total salt and rennet clotting activity were determined according to TS-3844 (20). pH measurements were performed directly using a Perkin Elmer, Coleman 28 C Metrion IV pH meter.

Results

a. Microbiological properties

Table 1 shows that there was no TAMB growth (<1 cfu/ml) in 5 samples, but that growth was observed in the other 20 samples, up to 2.5 x 10^6 cfu/ml TAMB. None of the samples analyzed in this research contained coliform bacteria (<1 cfu/ml). The results showed that 21 samples did not contain yeast or mold, while 3 samples analyzed had 10 cfu/ml, and another sample had 30 cfu/ml of yeast and/or mold. No anaerobe spores were found in 21 rennet samples.

b. Sensorial properties

The results of the sensorial analysis showed considerable differences among the rennet samples collected from nationwide markets in Turkey with respect to color, appearance and odor, probably due to differences in raw material processing technology. Eleven samples were of a caramel-like color and the remaining 14 samples were pale yellow. Seventeen samples had an odor unique to rennet, while the remaining 8 samples had an unpleasant odor. While 23 rennet samples were clear and sediment free, 1 sample was opaque and another sample contained sediments.

c. Physical and chemical properties

pH values of the rennet samples ranged from 5.08 to 5.82 with an average of 5.46. All of the rennet samples had pH values between 5 and 6. Fourteen samples had pH values between 5.5 and 5.7, and the pH values of 2 samples were above 5.8 (Table 2). The salt content of the rennet samples ranged between 4.83% and 14.05%, with an average value of 10.62%. The clotting activity of the rennet samples, both as declared on the labels and as determined in the laboratory, is shown in Table 2. The clotting activity of the samples ranged from 1/5670 to 1/45450, with considerable differences among the samples. None of the clotting activity determined by laboratory analysis conformed to the values declared on the labels, and 11 samples had higher clotting powers than those indicated on the labels (Table 2).

Discussion

TS-3844 permits a maximum 10^3 cfu/ml TAMB count in rennet (20). The TAMB counts of the results showed that 4 samples (16%) contained more TAMB than allowed in the standard. The remaining samples conformed to the standard (Table 1). 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 (24). For example, Koçak (9) reported that 13 liquid rennet samples contained a range of 41-65000 cfu/ml TAMB, while Uraz (8) determined 20-30000 cfu/ml TAMB in 19 rennet samples.

Table 1. Microbiological properties of rennet samples.

Samples	TAMB (cfu/ml)	Coliform Bacteria (cfu/ml)	Yeasts and / or Molds (cfu/ml)	Anaerobe Spores (cfu/ml)	Samples	TAMB (cfu/ml)	Coliform Bacteria (cfu/ml)	Yeasts and / or Molds (cfu/ml)	Anaerobe Spores (cfu/ml)
1	<1	<1	30	<1	14	1.0 x 10^1	<1	<1	<1
2	<1	<1	<1	<1	15	2.2 x 10^1	<1	<1	<1
3	1.0 x 10^1	<1	<1	<1	16	4.8 x 10^1	<1	<1	<1
4	1.2 x 10^1	<1	10	13	17	5.8 x 10^2	<1	10	<1
5	1.6 x 10^3	<1	<1	<1	18	2.0 x 10^2	<1	<1	<1
6	<1	<1	<1	<1	19	<1	<1	<1	30
7	1.1 x 10^1	<1	<1	<1	20	4.0 x 10^1	<1	<1	<1
8	1.3 x 10^3	<1	<1	35	21	3.2 x 10^1	<1	<1	<1
9	2.5 x 10^6	<1	10	<1	22	1.2 x 10^1	<1	<1	<1
10	1.0 x 10^1	<1	<1	<1	23	1.5 x 10^2	<1	<1	<1
11	1.0 x 10^1	<1	<1	7	24	<1	<1	<1	<1
12	6.2 x 10^2	<1	<1	<1	25	5.0 x 10^1	<1	<1	<1
13	1.8 x 10^3	<1	<1	<1					

None of the samples analyzed in this research contained coliform bacteria (<1 cfu/ml) (Table 1). TS-3844 stipulates that rennet must not contain any coliform bacteria (20). Mold and/or yeast were detected in 4 out of the 25 samples; nevertheless, these microorganisms were within the limits permitted by TS-3844. Özer (25) studied undesired microorganisms in rennet, and reported no yeast or mold in 60 samples. However, Uraz (8) and Koçak (9) determined yeast and/or mold in 7 of 19 rennet samples and 5 of 13 rennet samples, respectively.

Since the anaerobe spores cannot be completely destroyed during pasteurization their presence in milk for cheese or contamination via rennet is a serious problem (9). Therefore, TS-3844 (20) allows no anaerobe spores, although 4 samples analyzed in this research did contain such spores.

According to TS-3844 (20), rennets must be free of sediments, have a clear, caramel color and a specific odor. However, in this study, 8 samples had an unpleasant odor, 14 samples were yellow, one sample had sediments and another was opaque. Thus only 7 rennet samples completely met the requirements of TS-3844 (20). Eralp (6) noted that liquid rennets originating from animals should be translucent and clear without an unpleasant flavor. Fifteen of the rennet samples analyzed in our research possessed those properties.

Unfortunately, TS 3844 contains no provisions regarding the pH of rennet. Davis (24) 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 Koçak (9) stated that the optimum pH range of liquid rennet was 5.5-5.7, and 14 of the samples analyzed in this research were within that range.

The salt content results indicated a great difference among the rennet samples, resulting from the addition of salt without considering a standard level. It has been stated that various additives were used to extract the enzyme easily and to increase stability, and that of these NaCl was the most important. TS-3844 states that cheese chymosin from animals must contain at least 15% NaCl. However, no rennet samples analyzed in this research complied with the standard regarding NaCl content (Table 2).

When compared with the clotting activities reported by Koçak (9), Özer (25), and Uraz (8) the clotting activity of the rennet samples in this study was rather high. With regard to TS-3844 (20), clotting activity in 13 out of the 25 samples was 1/10,000 or higher, and these samples may therefore be classified as first class rennet: the 12 others were second class rennet, with the clotting activity of the lowest being 1/5000. However, none of the samples ranked as low in the range 1/3000-1/5000 (Table 2). This might be related to the processing of the raw material used for rennet, or be due to differences in

Table 2. Physical and chemical properties of rennet samples.

			Clotting activity					Clotting activity	
Samples	pH	Salt (%)	On label	Determined	Samples	pH	Salt (%)	On label	Determined
1	5.58	10.93	12,000	9520	14	5.82	4.83	15,000	10,000
2	5.11	9.94	15,000	14,280	15	5.12	14.05	15,000	14,600
3	5.57	11.50	50,000	45,450	16	5.39	11.07	50,000	41,670
4	5.59	12.21	12,000	14,590	17	5.45	12.21	15,000	12,600
5	5.53	11.99	12,000	15,270	18	5.50	12.50	12,000	9760
6	5.44	11.64	12,000	9850	19	5.11	9.23	12,000	12,820
7	5.21	9.37	6000	6450	20	5.50	12.35	5000	8690
8	5.53	9.08	10,000	9850	21	5.53	11.07	12,000	9660
9	5.63	4.90	12,000	19,610	22	5.67	9.51	3500	5670
10	5.54	10.79	12,000	15,500	23	5.09	11.60	5000	5850
11	5.80	10.79	12,000	15,750	24	5.62	10.94	12,000	6600
12	5.08	10.77	12,000	9760	25	5.53	11.07	5000	6040
13	5.55	11.22	50,000	44,440					

raw materials, variations in processing technology, differences in storage conditions (packaging, temperature, etc.) and differences in production and usage time. It should be emphasized that it is not good practice for cheese producers to use rennet without determining its activity, since this may give rise to problems in cheese quality.

The results of this study indicated that the rennets available on the Turkish market are not of a standard quality in terms of sensorial, microbiological, physical and chemical properties. In addition, some samples failed to comply with TS-3844. Therefore modern equipment and technology should be used in the production of commercial rennets. In addition packaging and storage conditions should be improved. The most important stipulation is that cheese makers should not use excessive amounts of rennet.

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