Survey of the Microbiological Quality of Adult Bovine Rennet Extracts

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ABSTRACT

Sixty-nine samples of liquid bovine rennet extract from several cheese-making plants were examined for microbiological quality. Wide differences were observed in the microbiological results, as well as in the pH, which ranged from 4.0 to 6.5, reflecting the manufacturing practices and sanitary conditions. The highest level of contamination was always caused by sporulated bacteria, both aerobic and anaerobic. Coliforms, considered to be enteric indicator bacteria were not detected, although halotolerant bacteria were found.

Bovine rennet extracts are largely employed as clotting agents in cheese manufacture. Formerly, development of contaminants could not be prevented by purification. At present, preservative practices and sanitary improvements during extraction have made it possible to successfully destroy or injure bacteria (6,16,18). Because microorganisms may diminish the activity of rennet extracts and cause texture and flavor defects in cheese (77), their microbiological quality has been investigated (10,15).

Argentina’s food regulations require that pasteurized milk be used in the manufacture of cheese (1). Cheese failures, such as holes or putrid odors caused by gas production or proteolysis, must therefore be attributed to ingredients of low bacteriological quality, such as rennet extracts and/or poor sanitary conditions. Rennet extracts should not provide a source of microorganisms that are pathogens or that produce defects in cheese. This survey was carried out to determine the microbiological quality of liquid bovine rennet extracts, and a basis for the future development of microbiological specifications.

MATERIALS AND METHODS

Sampling

Liquid bovine rennet extracts were aseptically collected in several cheese manufacturing plants located in the Province of Córdoba, kept refrigerated at 5°C and analyzed 24 h later. Extracts had been produced by four local manufacturers, one of which is the biggest in the country.

Sample preparation

Undiluted rennet extracts were not tested because of their low pH and the inclusion of preservatives. The pH of the first decimal dilution in 0.1% sterile peptone was raised to 6.8 with NaOH 0.1 N; additional 10-fold dilutions were then made.

Aerobic plate count (AC)

Mesophilic bacteria were enumerated in pour-plates of Plate Count Agar (PCA) incubated at 32°C± 1°C for 72 h (14).

Aerobic spore-forming bacteria counts (SC)

Tubes with 10 ml of diluted rennet extract were heated in a waterbath at 80°C for 10 min, cooled, inoculated in PCA pour-plates and incubated as indicated for AC. The bath temperature was checked in a test tube of rennet extract.

Most Probable Number (MPN) of total coliforms

Brilliant-green lactose bile broth, 2%, was inoculated and incubated at 32°C for 48 h for the three-tube MPN test (13). The same procedure was followed with lactose broth.

Molds and yeasts

Rennet extracts were inoculated into pour plates of oxytetracycline glucose agar (14) and incubated at 21°C for 5 d. Molds and yeasts are reported separately.

Halophilic bacteria

Modified Gibbon’s agar plates with 15% NaCl (14) were surface-inoculated and incubated at 30°C for 7 d.

Most Probable Number of sugar fermenting clostridia (SFC)

Decimal dilutions from 10⁻¹ to 10⁻³ g of rennet extract per ml were heated in a waterbath at 75°C for 10 min (5), cooled and inoculated into tubes with reinforced clostridial liquid medium (12) for the 5-tube MPN procedure. Tubes were plugged with 2% agar and incubated at 35°C for 5 d.

Lactate-fermenting clostridia (butyric-acid bacteria) (LFC)

Decimal dilutions of rennet extracts from 10⁻¹ to 10⁻³ g per ml were heated as above and inoculated into lactate broth with inverted Durham tubes (2). Tubes were plugged with vaspar,
incubated at 35°C for 10 d, and the 5-tube MPN was calculated.

**Sulfite-reducing bacteria (SRC)**
Anaerobic pouch plastic bags (3) were inoculated with 10⁻¹ to 10⁻³ dilutions, filled with sulfite-polymyxin sulfadiazine agar (13), sealed, and incubated at 35°C for 24 h. Black colonies were submitted to *Clostridium perfringens* identification tests (11).

**Bacillus cereus counts**
Phenol-red egg-yolk polymyxin agar plates were surface-streaked with 0.1 ml of 1:10 rennet extract dilution and incubated at 32°C for 24 h. Representative colonies were confirmed by biochemical tests (21).

**Determination of pH**
This assay was carried out with an Orion Research Ionalyzer/model 407A, standardized at pH 4 and pH 7 against phosphate pH buffer.

**Preservatives**
Presence of preservatives in 1:10 and 1:100 dilutions of rennet extracts was determined by the method described by Moseel (14).

**RESULTS AND DISCUSSION**
A total of 69 samples of liquid bovine rennet extracts from four manufacturers were analyzed. The pH of the rennet extract samples ranged from 4.0 to 6.5; most were between 5.6 and 6.0. The pH profile is shown in Table 1. It would seem that rennet extract “B” had been buffered (standardized) by the manufacturer. Samples of brands C and D showed a wide pH range, suggesting heterogeneous manufacturing practices or inadequate control during production.

Distribution of bacterial counts is shown in Table 2. Aerobic plate counts were above 10³ CFU/ml in 60.8% of the samples. Halophilic bacteria counts above 5 × 10² CFU/ml were found in 16.4% of the samples.

Mold counts were below 10 CFU/ml in 76.5% of the samples. Five samples from brand C showed counts above 10² CFU/ml. Yeasts counts were below 10 CFU/ml in almost 70% of the samples, and above 10² CFU/ml in 30.4%. Highest yeast counts were obtained during the winter season in samples from brands C and D and decreased thereafter. This type of contamination should be investigated further.

Clostridia counts were below 10 MPN/ml in 36.3% of the samples and between 10⁻¹⁻¹⁻² MPN/ml in 27.2%. Counts above 10⁻¹⁻¹⁻²/ml were found in 36.3%. However, the level of lactate fermenting clostridia was lower than expected, as 91% samples had counts below 10/ml and only six had detectable levels of butyric acid bacteria (above 10/ml). Although *Clostridium butyricum* and *C. tyrobutyricum* were not detected, *C. acetobutylicum* (4) was isolated.

Sulfite-reducing clostridia counts were performed on 37 samples. Counts were below 10 CFU/ml in 36.3%, while 10⁻¹⁻¹⁻² CFU/ml were found in 62.5%. Counts above 10⁻¹⁻¹⁻²/ml were found in 36.3%. However, the level of lactate fermenting clostridia was lower than expected, as 91% samples had counts below 10/ml and only six had detectable levels of butyric acid bacteria (above 10/ml). Although *Clostridium butyricum* and *C. tyrobutyricum* were not detected, *C. acetobutylicum* (4) was isolated.

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microorganism in cheese (19). B. cereus counts were below 10^2, between 10^2-10^3 and above 10^3 CFU/ml in 62.2, 33.3 and 4.3% of the samples, respectively. Their salt-tolerant capacity allowed their growth in halophilic media. They also grew in lactose enrichment broth.

Preservatives were found in samples of brands C and D at the 1:10 dilution and in brands A and B at the 1:10 and 1:100 dilution, but not at 1:1000. A low pH, a high concentration of salt, and preservatives should be protective factors against growth of potentially pathogenic and saprophytic bacteria in rennet extracts. However, large quantities of injured Staphylococcus aureus cells, not obtainable by commonly used selective media, were detected by pyruvate reactivation techniques (20).

The highest level of contamination in rennet extracts was caused by both aerobic and anaerobic spore-forming bacteria. Even though lactate-fermenting clostridia counts found in this survey were low, studies carried out in cheese (8,9) demonstrated that a few spores per ml of milk may cause blooming during the ripening of cheese. Therefore, it is advisable that the manufacturer produce rennet extract with the lowest level of butyric acid bacteria.

The microbiological quality of the rennet extracts analyzed differed widely according to the brand, a fact that probably reflects different manufacturing techniques and hygiene conditions in the processing plants. The distribution of Clostridium (SC) in liquid bovine rennet extracts is shown in Table 3. A high number of subsamples from brands C and D showed more than 100 MPN Clostridium/ml. Samples of the same brands C and D were also highly contaminated with the remaining microorganisms tested. Samples of low quality rennet extracts had a white precipitate, whereas samples from brands A and B remained clear after several months, indicating higher stability.

The results of the present survey suggest the need for improvement in sanitation and standardization practices in the manufacturing of rennet extracts. Rennet manufacturers and cheese-makers will profit from realistic microbiological standards for their products, which should be developed accordingly.

### TABLE 3. Distribution of Clostridium in 66 samples of liquid bovine rennet extracts from four manufacturers.

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<thead>
<tr>
<th>Manufacturer</th>
<th>MPN/ml</th>
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<tr>
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<td>10</td>
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<tr>
<td>A</td>
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<td>B</td>
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<td>C</td>
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REFERENCES