Effect of Dissolved Carbon Dioxide on the Growth of Psychrotrophic Organisms in Cottage Cheese

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ABSTRACT

The effect of dissolved CO₂ on the growth of psychrotrophic spoilage bacteria in 2% fat creamed cottage cheese was investigated. Cottage cheese was inoculated with 10³ cfu/g of a mixture of three Gram-negative psychrotrophic spoilage bacteria and stored in sealed glass containers at 4 and 7°C for 80 d. Carbon dioxide was added by dissolution into the cream dressing prior to the addition of curd. No growth, including yeasts and molds, was detected in the cheese containing CO₂ during 70 d of storage at 4°C or 30 d at 7°C. Fresh appearance of the cheese containing CO₂ was maintained for 80 d at 4°C or 60 d at 7°C. During the first 10 d of storage at 7°C or 17 d at 4°C, cottage cheese packaged without dissolved CO₂ had 10⁴-fold more colony-forming units (predominantly Gram-negative psychrotrophs) than that packaged with dissolved CO₂. Gram-negative organisms made up a smaller proportion of the total bacterial counts in CO₂-treated cheese than in the control product. These data indicate that dissolved CO₂ can effectively inhibit the growth of Gram-negative bacteria in creamed cottage cheese packaged in high barrier containers.

(Key words: cottage cheese, carbon dioxide)

INTRODUCTION

The US production of cottage cheese in 1988 was 422 million kg (10). Shelf-life of properly refrigerated cottage cheese is approximately 21 to 28 d. There is an interest in extending the refrigerated shelf-life of cottage cheese to 45 to 60 d without detracting from its quality attributes, including its perception as "natural." Deterioration of cottage cheese is primarily caused by microbial growth of psychrotrophic Gram-negative bacteria species such as Pseudomonas, Alcaligenes, Proteus, Aerobacter, or Aeromonas, which can result in undesirable flavors and slimy curd (1, 2, 7). Growth of yeasts and molds (e.g., Geotrichum, Penicillium, Mucor, and Alternaria) may also cause flavor, textural, and visual spoilage.

Methods to inhibit the growth of spoilage organisms are available. Sorbate is added at a concentration of .25% to extend cottage cheese shelf-life by inhibiting yeasts and molds and certain spore-forming organisms (9, 14). Unfortunately, sorbate imparts bitterness at a level as low as .1% (4, 5). Control of spoilage by pasteurization before or after packaging extends shelf-life but has an adverse effect on texture (16). For this reason, the "hot-packed" cottage cheese has not been widely adopted.

Atmospheres containing CO₂, coupled with high barrier packaging, are effective at inhibiting psychrotrophic organisms, particularly Gram-negative ones, in a broad range of refrigerated products (3, 6, 11, 13). However, few reports on the effectiveness of CO₂ in extending the shelf-life of dairy foods such as cottage cheese have been made. In one of the few reports, Kosikowski and Brown (8) investigated the effects of flushing the headspace of rigid plastic containers of unspecified construction with CO₂ or N₂. Inhibition of yeasts, molds, and psychrotrophic bacteria was equally demonstrated with both gases. However, CO₂ is biostatic for certain organisms even
in the presence of 20% O₂ (6). Gas flush systems would require substantial package headspace to act as a CO₂ reservoir to be effective. The major effect of gas flushing may be the simple removal of O₂.

Carbon dioxide is antimicrobial but must be dispersed throughout the product. The most effective way of ensuring uniform dispersion of the CO₂ into the product would be to dissolve the CO₂ in the product prior to packaging. Our objective was to determine whether dissolving CO₂ directly into the cream prior to curd addition was effective in controlling spoilage microorganisms.

MATERIALS AND METHODS

Curd and Cream Dressing

Fat-free small curd cottage cheese was obtained from a local commercial plant. The curd was made by addition of 1.5% active starter (Chr. Hansen’s Laboratory, Inc., Milwaukee, WI), setting for 4 and .5 h at 32.2°C, and cooking to final temperature at 54.4°C. The curd was refrigerated (4°C) until use the following day. Six percent fat cream dressing (pH 6.45, 20% total solids) was prepared by mixing one part pasteurized light cream with two parts pasteurized skim milk and adding 5% (wt/vol) sweet whey powder. No further pasteurization was made.

Inoculum

The cream dressing was inoculated by adding phosphate buffer (.3 mM, pH 7.1) containing cells harvested by centrifugation and two washes from a 48-h mixed culture of Pseudomonas fluorescens, Pseudomonas aeruginosa, and Pseudomonas marginata, which were obtained from the Food Research Laboratory at Cornell University. Harvested cells were diluted to 10⁸ cfu/ml prior to inoculation.

Packaging

The cheese was maintained at 2 to 4°C during inoculation, mixing, and packaging. The cream dressing was inoculated at a level that would result in approximately 10³ cfu/g of finished product. After inoculation, the dressing was divided into two portions; one was carbonated, and the other served as an uncarbonated control. Curd and cream dressing were mixed (2:1 wt/wt) with gentle stirring in a sanitized mixer. In the case of the product containing CO₂, the mixing was done under slight positive CO₂ pressure in an enclosed vessel. The creamed cottage cheese was filled into 227-g (8-oz) glass jars leaving approximately 5 mm of headspace, sealed with metal dome lids and screw bands, and stored at 4 or 7°C.

Carbonation

A modified Zahm beverage carbonation tank (Zahm & Nagel Co., Inc., Buffalo, NY) was used to carbonate the cream dressing. The 19-L tank was modified by attaching a 2.5-cm diameter outlet at the bottom side for transferring of the contents. Cream dressing was introduced into the tank, pressurized with CO₂, and held refrigerated for 2 h at a pressure of 2.8 kg/cm². The amount of CO₂ dissolved was not directly measured. The cream was then drained and mixed with curd as described.

Analysis

Duplicate test and control packages from the same batch were analyzed for the CO₂ content of the small headspace, microbial enumeration, and pH. All results are the average of duplicates.

CO₂ Analyses

The CO₂ content of the headspace was determined by gas chromatography (Aerograph 200, Wilkens Instrument Co., Walnut Creek, CA, with thermal conductivity detectors; Chromosorb 102, 2 M column, oven temperature 70°C, injector 115°C, and detector 135°C). Jars were sampled (100 µl) through rubber septum added to the metal lid using a gas tight syringe. Peak areas were compared against a commercial standard (Scotty II Analyzed Gases, Scott Specialty Gases, Scott Environmental Technology, Inc., Plumsteadville, PA).

Microbial Counts

Approximately one-half of the contents of each jar was aseptically transferred to presteri-
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Figure 2. Standard plate counts (colony-forming units per gram) for creamed style cottage cheese packaged with (solid figures) or without (open figures) added dissolved CO₂ and stored at 4°C (○, △) and 7°C (■, □)

Figure 1. Standard plate counts (colony-forming units per gram) for creamed style cottage cheese packaged with (solid figures) or without (open figures) added dissolved CO₂ and stored at 4°C (○, △) and 7°C (■, □).

RESULTS

Initial headspace concentrations of CO₂ ranged from 35 to 45% (percentage volume of headspace; n = 70; average = 40%) in the samples to which CO₂ had been added. The balance of the gas was air remaining from the filling process. Initial (time zero) colony-forming units for SPC and Gram-negative bacteria were $3.6 \times 10^3$ and $2.4 \times 10^3$ cfu/g, respectively. The SPC in the cottage cheese not packaged with dissolved CO₂ increased rapidly to >7 × 10⁷ cfu/g within 10 and 17 d at 7°C and 4°C, respectively (Figure 1). No growth was detected in any samples containing CO₂ until 35 d of storage, at which time the samples stored at 7°C showed an increase in colony-forming units (Figure 1). During the first 30 d of storage at 7°C, colony-forming units for SPC in the cheese packaged with dissolved CO₂ averaged $2.5 \times 10^3$ cfu/g. This was a slight decrease compared with the initial counts. The samples containing CO₂ that were stored at 7°C increased to 10 cfu/g between 35 and 50 d of storage.

No increase in microbial counts was detected in the samples containing dissolved CO₂ and stored at 4°C until 80 d of storage. Total colony-forming units during the first 70 d of storage ranged from $<1.0 \times 10^3$ to $3.5 \times 10^3$ cfu/g with an average $2.3 \times 10^3$ cfu/g (Figure 1). The Gram-negative colony-forming units in cottage cheese packaged with dissolved CO₂ showed a slight decline during the 80 d of storage at 4°C (Table 1) at 7°C, Gram-negative organisms began to increase after 30 d. However, the Gram-negative bacteria represented a smaller proportion of the bacteria present in samples containing dissolved CO₂ than in the controls (Table 1).

There was a build up of CO₂ in the headspace of jars to which CO₂ had not been added (Figure 2). The initial CO₂ in the control was <2% but increased with the growth of the bacteria (Figure 2), reaching 12% when the
TABLE 1. Gram-negative (G–) colony-forming units (log_{10}) and their percentage of standard plate count (SPC) for the inoculated cottage cheese stored with or without the addition of dissolved CO₂.

<table>
<thead>
<tr>
<th>Day</th>
<th>Stored at 4°C</th>
<th>Stored at 7°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With CO₂</td>
<td>Without CO₂</td>
</tr>
<tr>
<td>G–</td>
<td>% of SPC</td>
<td>G–</td>
</tr>
<tr>
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<td>3.38</td>
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bacterial counts rose to 10⁷ cfu/g. As expected, this increase in CO₂ was more rapid in jars stored at 7°C and occurred in the first 10 d compared with 20 d for samples stored at 4°C. This CO₂ accumulation likely affected the growth of Gram-negative bacteria in the latter days of storage (Table 1). Microscopic examination revealed that a large proportion of the colony-forming units from cheese containing CO₂ after 40 d storage were Gram-positive bacilli and cocci.

Yeasts and molds were not detected in any of the samples plated out at a 1:10 dilution; however, the control samples stored at both temperatures yielded counts from 10 to 30 cfu/g throughout the study period. The numbers did not increase over time of storage. The pH of the creamed cottage cheese was 5.20 to 5.25, which was not affected by the addition of CO₂.

**DISCUSSION**

Currently, a combination of sanitation, refrigeration, and acidification is used to give cottage cheese a 21- to 28-d shelf-life. Sorbate or pasteurization can increase this to 40 d but not without adversely affecting quality. Kosinski and Brown (8) investigated low oxygen content packaging to control spoilage. Commercial creamed cottage cheese was filled into thermoplastic containers, evacuated to 73.6 cm, flushed with CO₂ or N₂, and heat-sealed with lids containing aluminum foil (8). They reported that the product preserved with CO₂ maintained excellent flavor for 45 d at 4°C but noted the development of flavor and texture deterioration after 45 d. They suggested that this might be overcome by using a mixture of CO₂ and N₂.

In the present work, the colony-forming units of the creamed cottage cheese packaged without dissolved CO₂ increased, both by SPC and Gram-negative bacteria count, 10⁴-fold in 10 d at 7°C or in 17 d at 4°C. Although not directly tested, observation suggested that the control cottage cheese probably spoiled in the first 2 or 3 wk of storage. Numbers of yeasts and molds recovered in the control were irregular, but no increases were noted. No yeasts and molds were recovered from samples.
treated with CO₂ (1:10 dilution) throughout the study period.

Incorporation of CO₂ directly into the cream dressing prior to mixing with curd was a unique and effective way of uniformly distributing the gas through the matrix. By this method, the microbial counts in the inoculated creamed cottage cheese were suppressed for up to 80 d at 4°C. The high level of spoilage organism inoculation (>10³ cfu/g) represented a severe test. However, glass jars do not allow the permeation of CO₂ out of the container as would plastic-based containers. This means that cottage cheese packaged in plastic containers with dissolved CO₂ would likely have a shorter shelf-life due to the escape of the gas from the container. We are currently studying the effect of dissolved CO₂ on microbial counts in cottage cheese packaged in more practical systems such as high barrier tubs as well as overwrapped low barrier tubs.

We have not rigorously investigated the effects of dissolved CO₂ on the sensory attributes of creamed cottage cheese. Preliminary blind sensory tests by experienced cottage cheese eaters, however, indicated that the levels of CO₂ used in this study did not produce detectable "carbonation" flavor in the product.

ACKNOWLEDGMENTS

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REFERENCES