

**Use of Modified Atmosphere Technology to Maintain Quality  
of Direct-set Cottage Cheese**

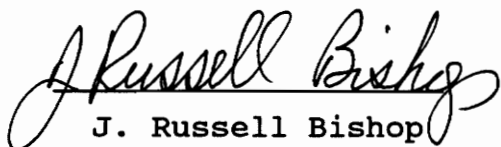
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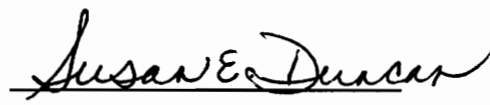
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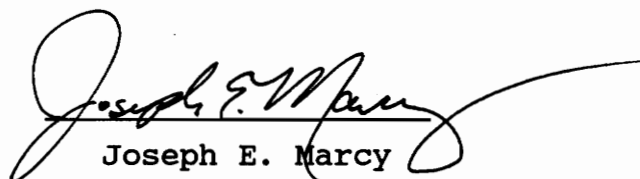
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Approved:

  
J. Russell Bishop

  
Susan E. Duncan

  
Joseph E. Marcy

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**Use of Modified Atmosphere Packaging to Maintain  
Quality of Direct-set Cottage Cheese**

by

Amruta Maniar

Committee Chairman: Joseph E. Marcy

Food Science and Technology

(ABSTRACT)

Sales of cottage cheese have been on the decline since 1972. Several factors have contributed towards this decline, including limited shelf-life. Cottage cheese shelf-life is estimated to be 10-21 days, in standard, non-barrier containers held at refrigeration temperatures. Shelf-life is shortened when aerobic, psychrotrophic microorganisms grow at refrigeration temperatures, producing changes which are undesirable.

Previous studies have demonstrated that modified atmosphere packaging (MAP) is able to maintain cottage cheese quality and extend shelf-life over air packaging.

The objectives of our study were to evaluate the ability of MAP to maintain cottage cheese quality, while establishing the proper atmosphere to be used. Further, we wanted to determine the potential for discoloration and development of undesirable acid flavors in cottage cheese by elevated CO<sub>2</sub> levels.

Direct-set cottage cheese was packaged in barrier containers and flushed with 100% CO<sub>2</sub>, 75% CO<sub>2</sub>:25% N<sub>2</sub>, 100% N<sub>2</sub>, and air, and stored at 4°C for 28 days. Product quality was assessed by sensory evaluation. Microbiological and chemical tests were conducted to obtain a better understanding of the effects of MAP on cottage cheese. Results obtained demonstrated that there was no change during storage for headspace gas composition. Psychrotrophic and lactic acid bacteria increased for air treated samples. Counts for MAP cottage cheese remained unchanged. In contradiction to previous studies, elevated CO<sub>2</sub> levels did not cause product discoloration. Acidity increased over storage life; however, the increase in acidity was not perceived organoleptically. These results contradicted previous studies which demonstrated that elevated CO<sub>2</sub> levels imparted a sharp acid flavor to the food product. Lactic acid did not contribute towards increased acidity. Sensory evaluation demonstrated that air treatment was inadequate in maintaining product quality past day 19. Cottage cheese packaged under 100% CO<sub>2</sub> was judged most acceptable, followed by 75% CO<sub>2</sub>-25% N<sub>2</sub>, and 100% N<sub>2</sub> treatments.

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I would like to dedicate this thesis to my parents for their immeasurable support, and for providing me with the opportunity to study in the United States.

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## I. INTRODUCTION

Cottage cheese is a fresh, unripened cheese made from pasteurized, skimmed cow's milk. Sales of cottage cheese have been on the decline for a decade, as evidenced by annual per capita consumption of 5.3 pounds in 1972 to 4 pounds in 1986 (Lieb, 1987), and down to 3.4 pounds by 1990 (Rieter, 1991). Bland image, lack of advertising support, competition from yogurt, dull flavor varieties, and limited shelf-life contribute to declining sales. Several processors have taken active steps to improve sales by use of creative marketing and promotion of new products, varieties, uses, and eating occasions (Honer, 1988; Reiter, 1991).

Shelf-life of cottage cheese is estimated to be 10-21 days, in standard, non-barrier, plastic containers held at refrigeration temperature (Bishop and White, 1985). Shelf-life is shortened when aerobic, psychrotrophic microorganisms grow at storage temperature, especially bacteria belonging to the genus Pseudomonas, producing changes which are undesirable. Extra-cellular heat stable proteases and lipases secreted by psychrotrophic bacteria result in off-flavors and off-odors; Pseudomonas species produce polysaccharide levans that result in a slimy, discolored curd (Marth, 1970; Patel, 1983).

Modified atmosphere packaging (MAP) is the enclosure of food products in barrier materials in which the atmosphere has been established at closure and then allowed to change with time as a result of dynamic interactions between the packaged atmosphere, the product and the environment outside the package (Brody, 1989). Previous studies conducted by the dairy industry and by private organizations have demonstrated that MAP is able to maintain cottage cheese quality and extend shelf-life over air packaging (Honer, 1988; Dixon and Kelly, 1988).

The objective of this study was to evaluate the ability of MAP in maintaining cottage cheese quality over the expected shelf-life of 21 days while establishing the proper atmosphere to be used. Further, we wanted to determine the potential for discoloration and development of acid off-flavors in cottage cheese by elevated CO<sub>2</sub> levels.

## II. LITERATURE REVIEW

### A. Cottage Cheese

Cottage Cheese is a soft, unripened cheese that is usually made by adding lactic cultures or acidulants to skimmed cows milk, with or without the addition of minute quantities of milk coagulating enzymes (Bodyfelt et al, 1988; Kosikowski, 1977).

Federal Standards of Identity for cottage cheese state that the maximum moisture content should be 80%. For creamed cottage cheese, besides the stipulation for maximum moisture content as in uncreamed cottage cheese, the minimum milk fat content must be 4%. Stabilizers, such as alginic acid, calcium and sodium alginates, carrageenan, edible gums and starches, and coloring agents such as carotene are permitted additives. Sorbic acid at 1000 ppm may be used as a preservative (Food and Drug Administration, 1989).

The majority of the American population think of cottage cheese as a "diet food". An image of this sort indicates a serious problem for one of the dairy industry's most important cultured product. Sales of cottage cheese have been on the decline for a decade, as evidenced by annual per capita consumption of 5.3 pounds in 1972 to an annual per capita consumption of 4.3 pounds in 1986, and 3.4 pounds in 1990. Bland product image, lack of advertising

support, competition from yogurt, dull flavor varieties, and limited shelf-life contribute to declining sales (Lieb, 1987; Reiter, 1991).

A number of processors have taken active steps towards improving sales by creative advertising, and promotion of new varieties, uses, and eating occasions. Since October, 1990 a variety of low fat (0.5-2% milkfat) cottage cheese flavors such as Black Pepper and Herbs, Pineapple and Cherry have been in the market. Extended use of cottage cheese for pasta salad, dips, spreads, and toppings for hamburgers and spaghetti have been suggested. Six oz. single serve containers have also been introduced (Reiter, 1991). The dominant trend in cottage cheese today is the emergence of non-fat (milkfat <0.5%) products. Several dairies have already introduced non-fat cottage cheese or plan to by April, 1991. The list includes Knudsen, Borden, Meadow Gold, Morning Glory Farms, Bancroft Dairy, Kroger, Kraft General Foods, and H.P. Hood (Reiter, 1991).

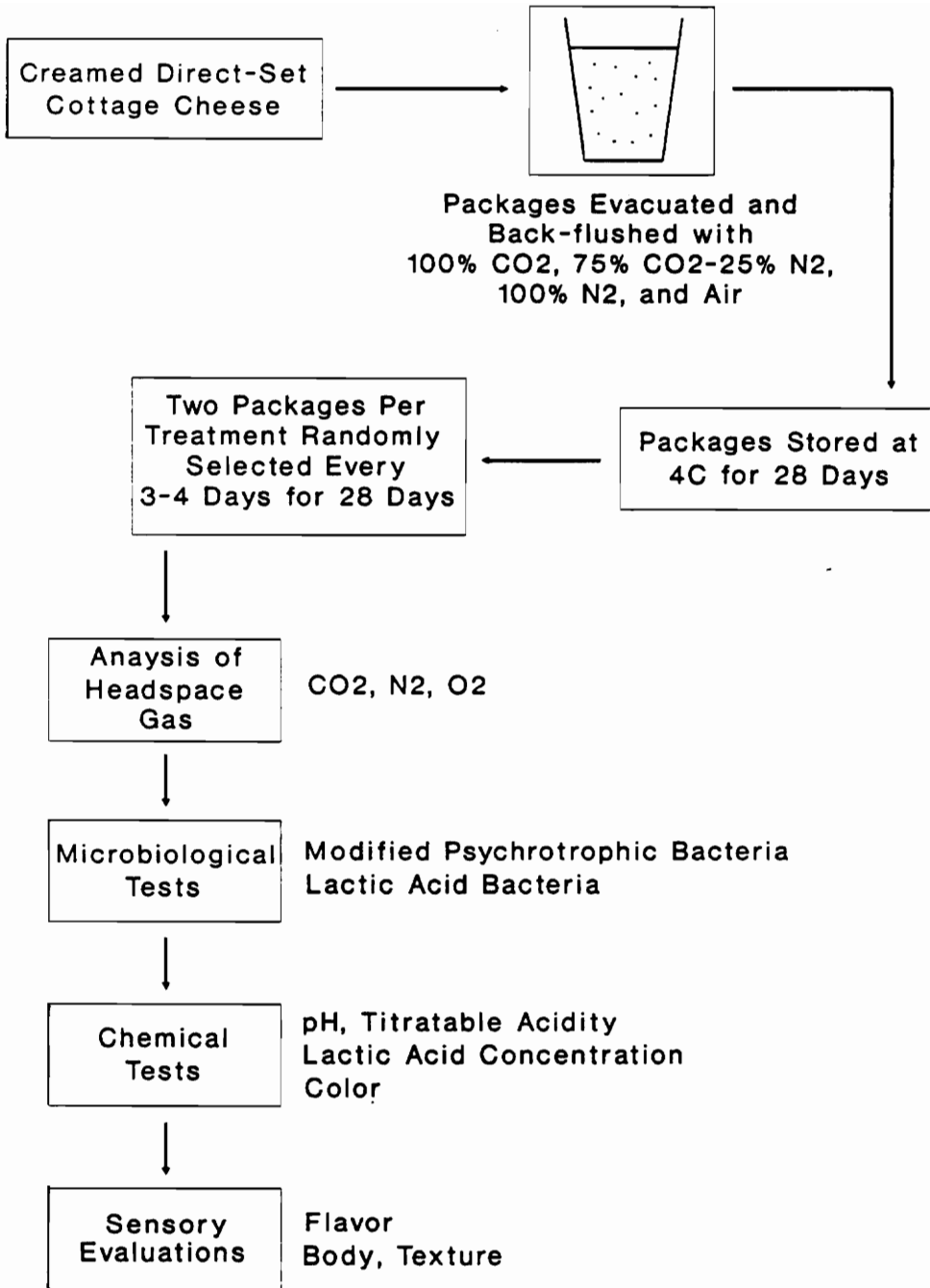
Cottage cheese is far from dead, but it is clear that, to improve sales, creative marketing is needed to shake up the "diet food" image and show that cottage cheese can be much more. Consistent product quality with improved shelf-life are also necessary to improve sales.

## 1. Manufacture of cottage cheese

Manufacturing procedures for cottage cheese vary between processors and producers. Three most common methods used are long-set, short-set, and direct-acid set. Both long-set and short-set require the addition of active acid starter cultures to coagulate the pasteurized skim milk. In the direct-set method an acidulant is used for the coagulation process (Banwart, 1989).

Figure 1 illustrates the manufacturing procedure used to manufacture creamed cottage cheese. Skim-milk, free of antibiotics, is heat treated slightly above 72°C. for 15 seconds. The milk may be fortified with skim-milk powder if required to provide a total solids content of 9.5-10.5%. After the milk is heated, it is delivered to vats, where it is coagulated either by active starter cultures or an acidulant. The active starter culture used is a blend of Lactococcus cremoris and Lactococcus lactis. Cultures are selected are on the basis of rapid acid production and freedom from agglutination sensitivity. Agglutinin sensitive strains lead to clumping of Lactococcal cells. Clumps fall to the bottom of the vat and fail to produce sufficient acid (Bodyfelt et al, 1988). Acidulants used for the production of direct-set cottage cheese may be lactic acid, acetic acid or food grade phosphoric acid (Banwart, 1989).





**FIGURE 1. Flow diagram of the manufacture of creamed cottage cheese.**

The long-set method uses a 1% inoculation of the starter into the milk held at 21-22°C to produce coagulation in 12-13 hours; the short-set method uses an inoculation of 4-5% of the starter into the milk held at 31-32°C to bring about coagulation in 4-5 hours. The latter is the generally adopted system. A little rennet is added (0.1-0.2 ml per 100 liters for the long-set, and 0.2-0.3 ml per 100 liters for the short-set process), to firm the coagulum at cutting, and to facilitate matting of the curd during cooling. When a pH of 4.65-4.75 is obtained, the coagulum is cut into cubes and cooked, with temperature being raised gradually over a period of 1 hour to 53-57°C (Kosikowski, 1977). Acid production ceases at about 39°C, and rate of heating can be increased to 1.5-2.0°C every 5 minutes. The final cook temperature should be at least 53°C to control spoilage organisms and may be as high 65°C if a very dry, firm curd is desired. Whey is then drained off to curd level, and the curd is washed three times at approximately 20 minute intervals. First warm water (22-24°C), is used to prevent curd damage. The next wash uses water at a temperature of 10°C and the final wash is with chilled water at 3-4°C. The curd is then rested for about an hour at 1°C and then packaged.

A cream dressing, made separately is often added to enhance the flavor and aroma of the final product (Chapman

and Sharpe, 1985). Cream is pasteurized (80°C for 15 sec), homogenized (2500-3000 psi), and ripened by live cultures of Streptococcus lactis sub species diacetylis and Leuconostoc cremoris. Skim-milk powder may be added to cream to increase viscosity and ensure a minimum solids content in the product. Live cultures enhance the "cultured aroma" of cottage cheese by producing flavor compounds such as diacetyl and acetoin. The enhanced flavor and aroma is especially required in direct-set cottage cheese, as flavor metabolites produced during natural coagulation by starter cultures are absent. Live cultures in creaming mixtures also aid in inhibiting the growth of spoilage bacteria by producing antimicrobial metabolites such as nisin and hydrogen peroxide. Shelf-life is thereby extended. Cottage cheese producers in the United States recently have begun to incorporate "superpasteurized" cultures of Propionibacterium shermani and Streptococcus cremoris into the cream dressing. These bacteria, like lactic acid bacteria produce anti-microbial compounds, which inhibit growth of microorganisms which potentially spoil cottage cheese, thereby extending shelf-life by 4-5 weeks (Chapman and Sharpe, 1985). Creaming mixtures also add to minimum fat requirement of 4% of finished product. The proportion of dressing to curd varies from 25-40% of the final product and depends largely on the fat content required (Chapman and Sharpe, 1985).

Stabilizers (0.1-0.3%) and salt (1% of the final product) may also be added to the creaming mixture.

## 2. Spoilage of cottage cheese

Cottage cheese is a perishable product with a shelf-life ranging from 10-21 days in standard, non-barrier containers held at refrigeration (Bishop and White, 1985; Lieb, 1987). Limited shelf-life is due to spoilage caused by psychrotrophic bacteria, yeasts, and molds (Patel et al, 1983; Adams et al, 1975; Bonner and Harmon, 1957). Usually, psychrotrophic bacteria, yeasts, and molds do not survive pasteurization. Contamination usually occurs post-pasteurization as a result of contact between raw and pasteurized milk lines, contaminated wash waters, unsanitized or poorly sanitized equipment, or poor sanitation during creaming and packaging (Fitz-Gerald and Deeth, 1983).

Most psychrotrophic bacteria belong to the Pseudomonas, Achromobacter, Alcalagenes, Aeromonas, Proteus, or Flavobacterium genera (Bonner and Harmon, 1957). The psychrotrophs usually are gram-negative, aerobic, polarly flagellated rods which grow at temperatures within a range of 0-35°C. The optimum growth temperature has been found to be 20-25°C. Biochemical examination of psychrotrophic bacteria demonstrate the micro-organisms are capable of

hydrolyzing gelatin, casein, and lipids but not starch. Psychrotrophic micro-organisms are oxidase and catalase positive. Majority of the psychrotrophs are resistant to penicillin, but susceptible to terramycin, streptomycin, and chloramphenicol. A pH range between 5-9 has been found to support growth, although pH between 6-7 has been found to be optimal (Patel et al, 1983). The ability of spoilage organisms to grow at low temperatures poses a threat to foods stored at refrigeration (Banwart, 1989; Adams et al, 1975; Bonner and Harmon, 1957).

Psychrotrophic spoilage bacteria produce heat stable, extra-cellular proteases and lipases. Proteases hydrolyse casein and lactalbumin present in cottage cheese, resulting in the formation of amino acids and peptides. Resultant amino acids and peptides less than 6000 daltons produce bitter off-flavors and odors. Lipases hydrolyze milk fats forming free fatty acids and glycerol. Low molecular weight fatty acids such as butyric and caproic produce off-odors in cottage cheese. Medium molecular weight fatty acids such as capric and lauric produce off-flavors like bitter, fruity, and rancid (Fitz-Gerald and Deeth, 1983; Patel et al, 1983). Optimum temperature for protease and lipase activity has been found to be about 40°C, while optimum pH range is 7.2-7.4 for protease and 7.5-9.0 for lipase activity. Proteases and lipases are inhibited by divalent cations such as  $\text{Cu}^{2+}$ ,

Zn<sup>2+</sup>, Co<sup>2+</sup>, Hg<sup>2+</sup>, whereas Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Mn<sup>2+</sup> caused little effect on rate of enzyme activity (Patel et al, 1983).

Pseudomonas species also produce polysaccharide levans which result in a slimy curd. Slime may be thin and watery, or thick and even ropey. Colors vary from white to yellow to brown. Slime may be putrid or even fruity in odor (Chapman and Sharpe, 1985; Bonner and Harmon, 1957). Pigmented Flavobacterium spp. may also result in surface discoloration. Cottage cheese also provides an ideal substrate for film yeasts, especially Geotrichum, Rhodotorula, and Torulopsis. Rhodotorula produces pink spots on the surface of cottage cheese which eventually become a pink slime. Torulopsis produces a yellow slime, while Geotrichum produces off-white, tan, or yellow surface discolorations. Cottage cheese is usually spoiled by other organisms before mold growth is evident. Molds such as Penicillium, Mucor, and Alternaria do appear on the surface of cottage cheese. Characteristic off-flavors and off-odors produced as a result of yeast and mold growth are yeasty and musty (Bodyfelt et al, 1988; Fitz-Gerald and Deeth, 1983).

Extra-cellular proteases and lipases secreted by psychrotrophic spoilage bacteria are extremely heat resistant and can survive pasteurization, retaining partial activity. Though not the primary source of spoilage, extra-cellular enzymes can also cause spoilage of milk and dairy

products. Resultant off-flavors and off-odors produced are similar to those produced by live psychrotrophic spoilage bacteria (Fitz-Gerald and Deeth, 1983; Marth, 1970).

## **B. Modified Atmosphere Packaging**

### **1. Definition**

Modified atmosphere packaging (MAP) is the enclosure of food products in barrier materials in which the atmosphere has been established at closure and allowed to change with time as a result of dynamic interactions between the packaged atmosphere, the product and the environment outside the package (Brody, 1989).

MAP contrasts with controlled atmosphere packaging (CAP) which involves maintaining a constant atmosphere throughout storage within the package. CAP compensates continuously for atmospheric changes caused by product and microbial respiration or container permeability (Koski, 1988).

### **2. History**

The use of controlled and modified atmospheres to preserve food is well established. In the 1930's chilled fresh beef, stored under carbon dioxide, was being shipped from Australia and New Zealand to the United Kingdom. Until the late 1980's, the use of CO<sub>2</sub> to preserve food was

confined to bulk supplies of meat and fruit. In the late 1950's individual portion packs rather than bulk supplies were vacuum packaged for meat, fish and coffee. In the 1960's, experimentation was extended to gas flushing with nitrogen. Since the early 1970's, there has been an increasing demand by consumers for "fresh" foods with longer shelf-lives. Distributors and retailers too have been demanding the latter. Modified atmosphere packaging offered the possibilities of achieving a significant increase in shelf-life without losing the description of "fresh food" (Brody, 1989; Genigeorgis, 1985). Commercialization of MAP technology was slow until 1981, when a breakthrough occurred. The supermarket chain of Marks and Spencer, introduced MAP for its entire range of fresh meats. Since then, MAP has had a tremendous impact on European markets but has been slow in achieving commercialization in the United States, although revived interest for the use of MAP technology has been demonstrated since the past 6 years (Lioutas, 1988; Genigeorgis, 1985).

### **3. Elements of modified atmosphere packaging**

Several elements work in combination to make MAP a success and to ensure the safety of packaged food products.

#### **i. Nature of the product**

Product nature determines to a great extent the number



and type of micro-organisms potentially capable of growth and subsequent toxin production within the food. Criteria controlling the above mentioned potential are the products water activity ( $A_w$ ), pH, and salt content (Banwart, 1989).

$A_w$  is an index of water available for chemical reactions and microbial growth within the product. Water activity is defined as the ratio of vapor pressure of water above a food to vapor pressure of pure water at the same temperature (Banwart, 1989). Microorganisms have a maximum, optimum, and minimum  $A_w$  for growth and toxin production. Bacteria require a higher  $A_w$  as compared to yeasts, which in turn have a higher  $A_w$  requirement than molds.

Microorganisms can grow up to the maximum  $A_w$  of 1.00, but most will not grow or produce toxin below a  $A_w$  of 0.7. Produce have an  $A_w$  range of 0.97-1.00 and are therefore capable of supporting growth of a wide variety of microorganisms, including those that may cause spoilage and/or pose a hazard for human health. On the other hand, dried pasta would make for a poor growth substrate as it has an  $A_w$  range of 0.0-0.5 (Banwart, 1989).

The pH of a substance is defined as the negative logarithm of the hydrogen ion concentration. Microorganisms have a minimum, optimum, and maximum pH for growth and toxin production. Most bacteria show optimum growth and toxin production at pH values near 7.0. Acid-forming bacteria

such as Lactobacillus and Streptococcus can tolerate acid conditions, while proteolytic bacteria like Pseudomonas can grow under alkaline conditions. Molds are capable of growing at a lower pH compared to yeasts, which in turn can grow at a lower pH than bacteria. Foods are categorized according to their pH values into high acid foods (pH < 4.6) and low acid foods (pH > 4.6). This demarkation is based upon the fact that Clostridium botulinum will not grow or produce toxin below a pH of 4.6. Low acid foods therefore are of greater concern, as they potentially allow the growth of Clostridium botulinum and subsequent toxin production (Banwart, 1989). Fruits are high acid foods while meat, poultry, seafood, vegetables and dairy products are low in acid.

Salt concentration needed to prevent microbial growth is related to food pH,  $A_w$ , temperature, chemical composition of substrate, and type of organism. The reason for inhibition of bacteria by salt is not clear. It is primarily though to be a plasmolysis effect, although dehydration,  $O_2$  removal, interference with enzymes, alteration of pH, or toxicity of sodium or chloride at high concentrations have been proposed (Banwart, 1989). Microorganisms vary in their tolerance to salt. Generally, microorganisms are inhibited by 4-10% salt. Micrococci, Staphylococci, and spore-forming bacteria are halotolerant.

Halotolerants grow well at low salt concentrations, but can tolerate salt concentrations up to 16% (Banwart, 1989). Yeasts vary in tolerance to salt. Some yeasts have been able to grow in salt concentrations up to 22% (Banwart, 1989).

Food source also determine type of inherent microorganisms. A fresh lettuce head picked from the ground would obviously have high levels of microorganisms, especially those growing in soil, such as Erwina spp. while ocean fish would have higher counts of micro-organisms naturally present in ocean waters such as Vibrio spp.

Anti-microbial factors, present naturally or artificially in the food, also determine the ability of microorganisms to grow and produce toxins in food. Processed meats, for example, contain nitrites and are less likely to provide a growth substrate for microorganisms, than is seafood.

## **ii. Atmosphere of storage**

The principal gases used for CAP and MAP are carbon dioxide (CO<sub>2</sub>), nitrogen (N<sub>2</sub>), and oxygen (O<sub>2</sub>). Gases may be used individually, or more commonly, in various combinations. There is no one specific atmosphere that can optimally extend shelf-life and maintain quality of all foods. An optimal combination must be worked out for each

food type. Carbon monoxide, nitrous oxide, and sulfur dioxide are also used in Europe although the FDA has not permitted their use in the United States (Brody, 1989).

**a. Carbon dioxide**

Carbon dioxide has an inhibitory effect on bacterial and mold growth and respiration of fresh produce. The gas has been found to be effective at concentrations above 20%, and is especially effective in inhibiting growth of gram negative bacteria. Inhibitory effect of CO<sub>2</sub> on bacterial cell growth is attributed to the dissolution of CO<sub>2</sub> in the fatty or aqueous phase of food to form carbonic acid. Most spoilage bacteria are gram negative in nature. The ability of CO<sub>2</sub> to inhibit their growth leads to an extension in food shelf-life. Gram positive microorganisms like lactic acid bacteria are more resistant to CO<sub>2</sub>, although organoleptic changes associated with growth are less noticeable as compared to those associated with the growth of gram negative bacteria (Dixon and Kelly, 1988; Gill and Tan, 1979; King and Nagel, 1975). At concentrations above 75% the gas has been said to cause discoloration in meats and impart a sharp acid taste in some foods (Young et al, 1988). The acid taste is produced due to the dissolution of CO<sub>2</sub> in the fatty and aqueous phase of food resulting in the formation of carbonic acid (Dixon and Kelly, 1986; Gill and

Tan, 1979). Dissolution of CO<sub>2</sub> in the fatty or aqueous phase of the food leads to package collapse (Silliker and Wolfe, 1980).

**i. Theories of bacterial cell growth inhibition by CO<sub>2</sub>**

The exact mechanism for the inhibitory effect of CO<sub>2</sub> on bacterial cell growth is not clear. The net result is an extension in the lag phase of growth and an increase in generation time of bacterial cell division (Dixon and Kelly, 1988). Inhibitory effect has been found to be negligible once the bacterial cells have reached the exponential phase of growth. The reason for this effect is unclear (Dixon and Kelly, 1988). Several theories explaining inhibitory action of CO<sub>2</sub> on bacterial cell division have been propounded and can be summarized as follows:

Alteration of bacterial cell membrane permeability

Carbonic acid formed as a result of CO<sub>2</sub> dissolution in the foods fatty or aqueous phase is transported into the bacterial cell. Higher internal pH within the cell results in dissociation of carbonic acid. Hydrogen ions produced by dissociation of carbonic acid increase fluidity of membrane fatty acids and cause carbamination of bacterial cell membrane proteins. This results in a change in permeability characters of cell membranes (King and Nagel,

1975; Gill and Tan, 1979; Dixon and Kelly, 1988).

### Inhibition of bacterial enzymes

Direct inhibition of bacterial enzymes involved in metabolism may be caused by a change in pH within bacterial cell. Carbonic acid present in the fatty or aqueous phase of the food is transported across the bacterial cell membrane. Once within the cell, the acid, which is extremely unstable, ionizes as a result of higher internal pH, giving rise to  $H^+$  ions. Hydrogen ions decrease internal pH of the cell thereby disrupting the activity of metabolic enzymes (King and Nagel, 1975; Dixon and Kelly, 1988). Hydrogen ions formed as a result of carbonic acid dissociation could form electrostatic bonds with  $NH_2$  terminals of amino acids constituting the enzymes forming a carbamate. The carbamate could alter enzyme configuration as a result of electrostatic forces of attraction and repulsion. The altered configuration would result in a change of marker amino acids at the substrate binding site of the enzyme. The substrate is unable to recognize the altered sequence of amino acids at the binding site, and would not bind. Bacterial metabolism and therefore growth would thereby be inhibited (King and Nagel, 1975; Dixon and Kelly, 1988).

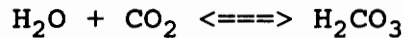
Elevated  $CO_2$  levels may inhibit enzymes involved in

decarboxylation reactions as a result of feed-back inhibition. Equilibrium of decarboxylating enzymes may also be shifted in the reverse direction, thereby limiting growth (Genigeorgis, 1985). Experiments conducted by King and Nagel (1975), using Pseudomonas aeruginosa showed that cells grown on glucose, acetate, fumarate, or succinate had a longer lag time in substrate uptake when subjected to an atmosphere of CO<sub>2</sub> as compared to air grown controls. Assays to determine enzymatic activity between CO<sub>2</sub> and air treatments showed no difference in cytochrome oxidase, fumarase, oxaloacetate decarboxylase, or succinate dehydrogenase activities. However, assays of isocitrate dehydrogenase and malate dehydrogenase showed rate inhibition caused by CO<sub>2</sub>. Although oxaloacetate decarboxylase was not affected by CO<sub>2</sub> in Pseudomonas aeruginosa, it has been reported to be affected in Rhizopus nigricans (King and Nagel, 1975).

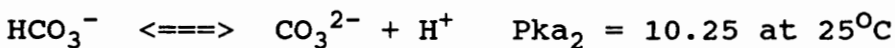
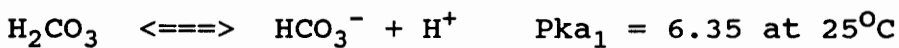
Enzymes inherent to food systems involved in spoilage are also inhibited by the ionization of carbonic acid. Decrease in pH disrupts enzyme systems inherent to the food, thus slowing the rate of catabolic changes (Enfors and Molin, 1980).

Although theories proposed so far to explain the

mechanism of bacterial cell growth inhibition by CO<sub>2</sub> refer to carbonic acid as being the key to preservative effects, the hydration of CO<sub>2</sub> to carbonic acid is estimated to be only 1-2% (Lampila, 1990). Hydration equilibrium for CO<sub>2</sub> is given by (Butler, 1982):



The carbonic acid formed (H<sub>2</sub>CO<sub>3</sub>) is extremely unstable and ionizes to form carbonate (CO<sub>3</sub><sup>2-</sup>) and hydrogen (H<sup>+</sup>) ions, within 10<sup>-6</sup> of a second, with bicarbonate (HCO<sub>3</sub><sup>-</sup>) as the intermediate. The ionization equilibria for carbonic acid are given by (Butler, 1982):



As concentration of H<sub>2</sub>CO<sub>3</sub> is a fraction of the concentration of CO<sub>2</sub>, independent of pH and concentration, it is usually neglected from calculations (Butler, 1982). The final hydration equilibrium for CO<sub>2</sub> is given by (Butler, 1982):



On the basis of the above discussion, it may be proposed that either CO<sub>2</sub> itself or its ions are responsible for the inhibitory effect. CO<sub>2</sub>, being a byproduct of metabolism, could inhibit enzymes involved in metabolism by a feedback mechanism, resulting in absence or slow growth of bacterial cells (King and Nagel, 1975). Bicarbonate (HCO<sub>3</sub><sup>-</sup>), being a monomeric acid, could be toxic in itself to



bacterial and food enzymes. Carbon dioxide or its ions may also alter bacterial cell permeability by a mechanism similar to that proposed for carbonic acid (Lampila, 1990).

**ii. Treatment of dairy products with CO<sub>2</sub>**

Research to examine the inhibitory effect of CO<sub>2</sub> for preservation of dairy products has been studied since the early 1900's (Dixon and Kelly, 1988; Gill and Tan, 1979). Van Slyke and Bosworth (1907) observed that increased pressures of CO<sub>2</sub> delayed lactic acid fermentation of milk, but no noticeable effect was observed at atmospheric pressures (Dixon and Kelly, 1988). Prucha et al (1922) demonstrated that CO<sub>2</sub> at atmospheric pressure had no bacteriostatic or bacteriocidal effect on micro-organisms present in ice-cream, butter, and sour cream, or on Lactococcus lactis, Escherichia coli, or Bacillus cereus added to the food. Prucha et al (1922) observed that milk held under 60 psi CO<sub>2</sub> pressure did not sour for 9 days while untreated milk became sour within 30 hours. Law and Mabbit (1983) showed that when milk was stirred under a headspace of 1 atm CO<sub>2</sub> shelf-life was extended by 3 days at 4°C for "poor" quality milk and longer for "good" quality milk. Further research by Law and Mabbit (1983) indicated that, to inhibit growth and activity of spoilage micro-organisms in dairy products, pressures greater than 1 atmosphere of CO<sub>2</sub>

were required (Dixon and Kelly, 1988).

Research studies conducted by the dairy industry and private individuals support evidence that packaging dairy products under CO<sub>2</sub> benefits shelf-life and quality. Processors of dairy products have remained reluctant to package dairy products under modified atmospheres of elevated CO<sub>2</sub> on a commercial scale. This reluctance could probably be due to the potential ability of CO<sub>2</sub> to cause discoloration (Dixon and Kelly, 1988) and increase acidity as a result of carbonic acid formation (Honer, 1987). Several dairies in the United States in the early 1970's used the "Vitagen" process to transfer creamed cottage cheese under vacuum from the mixer to the filler. In Germany, cottage cheese is commercially packaged under CO<sub>2</sub>. Containers are flushed with CO<sub>2</sub> when empty and again after the cottage cheese has been added. Containers are heat sealed with aluminum foil and lidded. This method has been able to extend the shelf-life of cottage cheese by 7 days (Honer, 1988).

#### **b. Oxygen**

Presence of oxygen is generally avoided in most products as it is responsible for oxidation and enhances growth of aerobic spoilage organisms (Brody, 1989). In fruits and vegetables presence of O<sub>2</sub> is desired to sustain

respiration (Labuza and Breene, 1989). In red meat and fish  $O_2$  is responsible for "bloomed" color of oxymyoglobin pigment (Young et al, 1988). In complete absence of  $O_2$ , anaerobic pathogens such as Clostridium botulinum can grow and produce toxin. To avoid such an undesirable situation,  $O_2$  is usually maintained at 5-10% concentrations. In produce, red meat and fish where  $O_2$  sustains respiration and contributes to desirable appearances,  $O_2$  is maintained at 50-80% concentrations. Although concentrations as low as 5% (Brody, 1989) are sufficient to sustain respiration and allow blooming of myoglobin, concentrations up to 40%  $O_2$  support growth of aerobic spoilage microorganisms. Concentrations above 50% exceed optimum  $O_2$  concentration required for growth and limit growth of spoilage bacteria (Zagory and Kader, 1988).

### **c. Nitrogen**

Nitrogen is an inert, tasteless gas, virtually insoluble in water. Nitrogen is used to replace  $O_2$  to prevent lipid oxidation and mold growth. Being insoluble in water,  $N_2$  is used as a filler to prevent package collapse (Brody, 1989; Foegeding and Busta, 1983).

### **iii. Temperature**

Temperature is a critical factor in MAP. It relates to

the entire distribution process, from the point of harvest or slaughter to the point of sale and all stages between. Lowering temperature of a food product extends shelf-life by direct inhibition of metabolism and respiration and also by increasing solubility of CO<sub>2</sub>. Solubility of gases increases with decreasing temperatures. The effectiveness of CO<sub>2</sub> has been found to be maximum around 0°C. No inhibitory effect has been observed at temperatures above 20°C. Use of MAP to extend product shelf-life is not a substitute for proper temperature control. Good Manufacturing Practices demand that refrigerated MAP foods be maintained at a temperature below 3°C to ensure the inhibition of non-proteolytic strains of Clostridium botulinum (Lammerding and Foster, 1989; Lioutas, 1988; Farber, 1991).

#### **iv. The package**

The primary factors contributing towards a successful package are headspace, permeability, and seal integrity. Larger package headspace increases the volume of gas in contact with food. Most packages are shallow and often have ridges at the bottom to increase surface area of contact between the gaseous atmosphere and the food (Anonymous, 1988).

Gas permeation rate through a package depends upon the polymer used, thickness of polymer, the surface area of the

package, partial pressures gradients within and outside the package, relative humidity, and package storage temperature (Lee, 1986).

Foods may be divided into respiring and non-respiring based upon whether biochemical metabolic activity is present or not. Respiring foods include fresh fruits and vegetables that use oxygen to catabolize carbohydrates present, to form carbon dioxide, water vapor, and heat. In absence of oxygen, undesirable aldehydes and alcohols are produced via anaerobic respiration. Non-respiring foods include meat, poultry, seafood, and bakery goods. Respiration by inherent microorganisms does not significantly alter surrounding atmosphere (Zagory and Kader, 1988; Labuza and Breene, 1989). The polymer is chosen on the basis of permeation rate desired of it. High permeation rates are required for respiring food to allow  $O_2$  to permeate into the package, and to allow the  $CO_2$  to permeate out. Permeation rate should be such that  $O_2$  concentration within the package is sufficient to sustain respiration but insufficient to enhance the growth of spoilage microorganisms. Carbon dioxide concentration within the package should be able to inhibit growth of spoilage microorganisms but should not be inhibitory to respiration (Lioutas, 1988; Mannheim, 1986). For red meat, the polymer should allow  $O_2$  to permeate into the package at a rate sufficient to maintain the bloomed

color of oxymyoglobin (Young et al, 1988). For non-respiring products, a medium to high barrier polymer is required to maintain the initial intended atmosphere within the package. This may also be true of red meats, where the initial altered atmosphere is high in O<sub>2</sub>. Polymers commonly used are polyethylene and/or polypropylene where low-barrier properties are required, while polyesters, metalized polyesters, and polyamides are used for purposes where medium to high-barrier properties are required.

Seal integrity is extremely important in preventing contamination of the packaged food. In most packages there is a trade-off between the tightness of the seal and the ability of a consumer to easily open the package.

#### **4. Advantages and Disadvantages of MAP**

Besides the obvious advantage of extending the shelf-life of food products without altering the products quality or losing its freshness, MAP has other associated advantages, namely (Lioutas, 1988):

- 1) reduction of waste through distribution;
- 2) expanded radius of distribution;
- 3) easier separation of sliced meats.

Despite several advantages, MAP does have certain limitations (Lioutas, 1988; Hintlian and Hotchkiss, 1986):

- 1) strict requirement to use high quality raw materials;
- 2) strict temperature control required;
- 3) increased packaging costs due to the use of high barrier films necessary upgrade of existent packaging machines;
- 4) safety of foods packaged under modified atmospheres.

Safety of MAP foods is of major concern. Conflicting views regarding potential for human health hazards arising from foods packaged under modified atmospheres are responsible for limited commercial use of this technology. MAP uses elevated CO<sub>2</sub> levels to inhibit growth of aerobic spoilage microorganisms. Food spoilage renders food inedible due to undesirable organoleptic changes. The focus of concern is the potential ability of anaerobic food borne pathogens to grow and produce toxins within the packaged food without the consumer being warned of spoilage. In the absence of competition from aerobic spoilage organisms and their metabolites, an environment fostering the growth of anaerobic pathogens and subsequent potential of toxin production is created (Hotchkiss, 1988; Genigeorgis, 1985; Farber, 1990; Silliker and Wolfe, 1980).

Until recent times, major emphasis was placed on the potential growth of the obligate anaerobe Clostridium

botulinum, specifically the non-proteolytic strains. Non-proteolytic strains of Clostridium botulinum include types E, F and non-proteolytic B. Non-proteolytic types are capable of growth at temperatures as low as 3.3°C and do not produce offensive smelling metabolites associated with proteolysis to warn the consumer of growth and presence of potential toxin produced by the microorganisms (Farber, 1990). With the recent emergence of psychrotrophic pathogens such as Listeria monocytogenes, Aeromonas hydrophila, and Yersinia enterocolitica, further concerns for safety have arisen (Farber, 1991; Genigeorgis, 1985; Hintlian and Hotchkiss, 1986). Since MAP foods have extended shelf-lives, pathogens can grow within the food over the prolonged shelf-life to reach levels potentially dangerous to human health. Besides psychrotrophic pathogenic bacteria, Salmonella species, Staphylococcus aureus, Bacillus cereus, enterotoxigenic Escherichia coli, and Campylobacter jejuni also pose a threat to food safety (Farber, 1990; Hintlian and Hotchkiss, 1986).

Research experiments conducted using beef, turkey, and fish to estimate the potential for growth and toxin production of Clostridium botulinum prior to the onset of spoilage at refrigeration temperatures are contradictory. Post et al (1985) and Stier et al (1981) concluded that spoilage precedes toxin formation when the product is held



below 4°C. At temperatures above 4°C, Post et al (1985) concluded that spoilage precedes toxin formation, while Stier et al (1981) concluded that toxin production occurs prior to spoilage. Neither study clearly defined the criteria used for sensory evaluations.

Only four cases of illnesses have been traced back to foods packaged under MAP. In 1987, four circus performers in Florida suffered botulism symptoms after eating coleslaw prepared from cabbage packaged under MAP. The packaged cabbage had been stored at room temperature. The duration of storage at room temperature was unknown (Solomon et al, 1990). Research studies conducted to study the incidence of Clostridium botulinum in foods showed that, though serious the incidence was extremely low and was always associated with temperature abuse as in the above cited case (Taclindo et al, 1967; Abrahamson and Rieman, 1971; Holley, 1981). Use of good quality products, good hygiene from slaughter or harvest on, selection of correct packaging material, appropriate gas composition for the product, and maintenance of temperature below 3°C would greatly minimize health hazards. Additional hurdles or barriers, such as acidity, water activity, competitive flora, redox potential, and preservatives may be incorporated into the food (Lioutas, 1988; Farber, 1991). Microorganisms have minimum, optimum, and maximum conditions for growth. The "hurdle/barrier"

concept of microorganism growth inhibition utilizes sub-optimum conditions of the above mentioned factors to restrict growth of micro-organisms (Lioutas, 1988; Farber, 1990). These hurdles, including modified atmospheres, could interact either directly or synergistically to secure microbial stability of a particular food product.

### III. MATERIALS AND METHODS

A preliminary study was conducted to establish an atmosphere best able to maintain cottage cheese quality over a 60 day shelf-life. The following atmospheres were used to package cottage cheese:

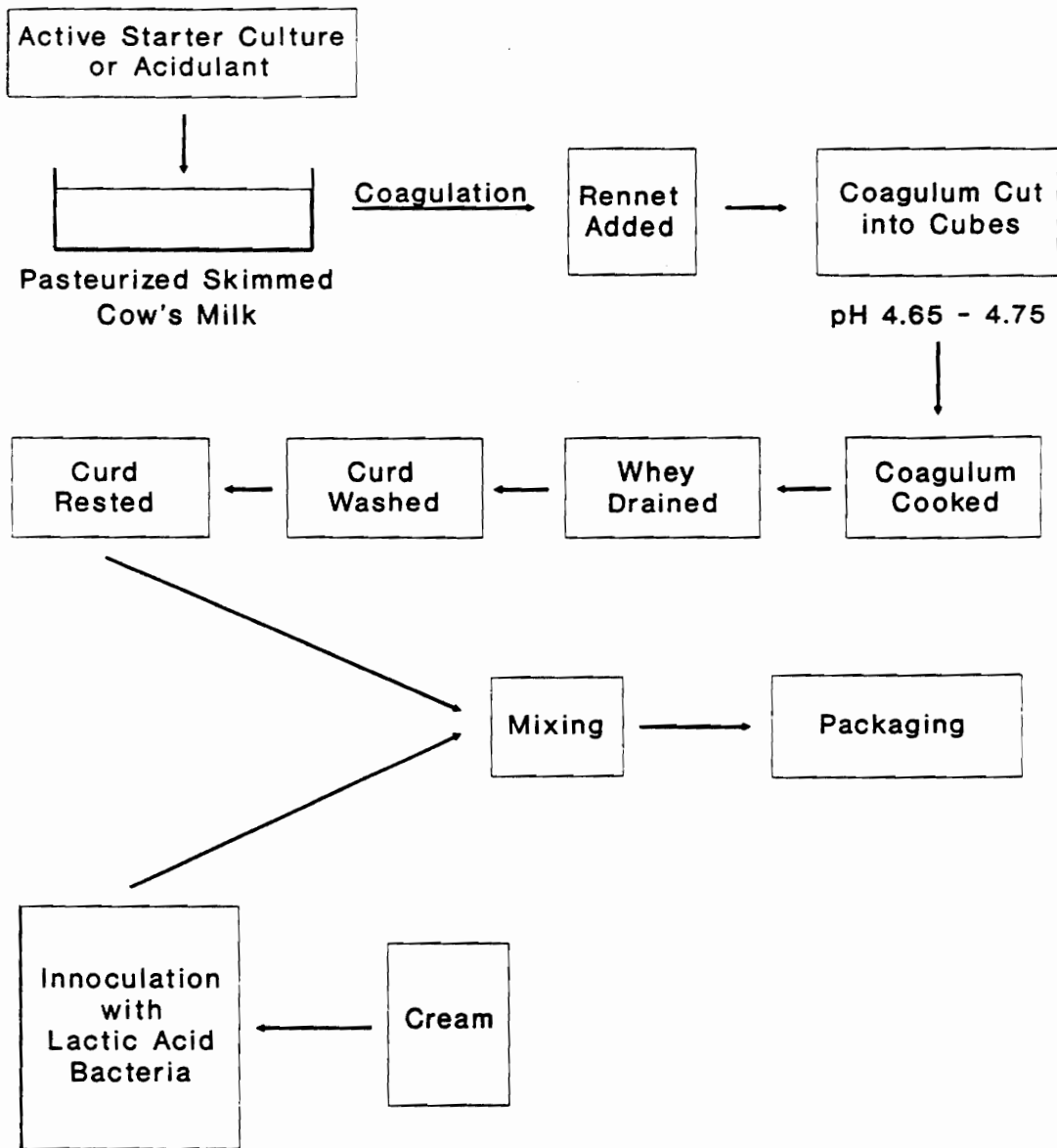
1. 100% CO<sub>2</sub>
2. 75% CO<sub>2</sub>:25% N<sub>2</sub>
3. 50% CO<sub>2</sub>:50% N<sub>2</sub>
4. 25% CO<sub>2</sub>:75% CO<sub>2</sub>
5. 100% N<sub>2</sub>

The above mentioned atmospheres were studied based upon work conducted by researchers on meat and poultry products (Brody, 1989). Cottage cheese quality was evaluated every 7 days by microbiological and chemical analyses.

Specifications on the method used to package and evaluate quality of cottage cheese have been explained in sections A-D.

On completion of the preliminary study the main study was undertaken. Figure 2 describes the sequence of packaging, microbiological and chemical tests, and sensory evaluation of samples. Cottage cheese was packaged under the following atmospheres:

1. 100% CO<sub>2</sub>
2. 75% CO<sub>2</sub>:25% N<sub>2</sub>



**FIGURE 2.** Flow diagram for the methodology used to study the use of MAP to maintain quality of direct-set cottage cheese.

3. 100% N<sub>2</sub>

4. Air

The study was conducted in three replications for a duration of 28 days each. A duration of 28 days was chosen to surpass the normal shelf-life of cottage of 21 days (Bishop and White, 1985). Thus, any extension in the shelf-life or quality maintenance over the storage period would be directly attributed to MAP. Cottage cheese quality was evaluated by sensory evaluations on the fresh, unpackaged product and every 3-4 days for 28 days. Microbiological and chemical tests were conducted to obtain a better understanding of the effects of the atmospheres studied on cottage cheese. In addition to the above mentioned tests, lactic acid was enzymatically quantified.

Packaging material for this project was obtained from commercial sources. Composition of the packaging film used was from the inside, 3 ml low density polyethylene (LDPE), 0.6 ml biaxially oriented nylon coated with polyvinylidene chloride (PVDC). The oxygen permeation rate of the packaging film was predetermined using a Mocon Analyzer (Minneapolis, MN) and the ASTM D-3985 procedure. The oxygen permeation rate was found to be 15-30 cc of O<sub>2</sub>/m<sup>2</sup>/24 hours under 1 atmosphere pressure at 23°C.

Creamed cottage cheese was obtained from Westover Dairy, Lynchburg, VA. The cottage cheese used was direct-

set with phosphoric acid.

## **A. Packaging of cottage cheese**

### **1. Equipment**

Gas Partitioner Model 29 (Fisher Hamilton, Pittsburg, PA)

Integrator HP3396A (Hewlett-Packard, Avondale, PA)

Proportional Gas Blender (Smith Equipment, Waterfront, SD)

Sealer Multivac A300 (Koch Supplies Inc., Kansas City, MO)

Standard Gas Mixture (Airco Industrial Gases, Murray Hill, NJ)

Oxygen (Airco Industrial Gases, Murray Hill, NJ)

Nitrogen (Airco Industrial Gases, Murray Hill, NJ)

Carbon Dioxide (Airco Industrial Gases, Murray Hill, NJ)

### **2. Procedure**

Portions of creamed cottage cheese 141.74 g (5 oz) were filled into containers. Twenty packages per treatment were evacuated, flushed with the required gases and sealed using a Multivac sealer. The seal on each of the containers was checked for leakers by submerging the packages under water. Containers were stored at 4°C in the dark for 28 days.

Gas composition was regulated during packaging by sampling the first and every 10 tenth container packed under each atmosphere. An error of 1 % was permitted for headspace gas composition between the desired and the

obtained headspace. The method of monitoring headspace gas composition of packages was the same as the method used to determine the change in the headspace gas composition.

**B. Analysis of headspace gas composition of cottage cheese packages flushed with gas.**

**1. Equipment**

Gas Partitioner HP3396A (Hewlett-Packard, Avondale, PA)

Proportional Gas Blender (Smith Equipment, Waterfront, SD)

Sealer Multivac A300 (Koch Supplies Inc., Kansas City, MO)

Standard Gas Mixture (Airco Industrial Gases, Murray Hill, NJ)

**2. Procedure**

Headspace gases were analyzed for composition using a Fisher-Hamilton gas partitioner. Quantification of gases was done using a Hewlett Packard Integrator. The integrator calculated gas composition on a percent area basis.

Quantification of gas was possible as the integrator was calibrated in response to a standard gas mixture. Standard gas mixture had a composition of 24.67% CO<sub>2</sub>, 24.89% O<sub>2</sub> and 50.44% N<sub>2</sub>. Two packages of the 20 were randomly chosen on each sampling day and a sample size of 1000 μl of the headspace gas was injected into the gas partitioner.

Subsequent chemical and microbiological tests, and sensory

evaluation was conducted using the cottage cheese from these two containers.

## **C. Microbiological analyses of the cottage cheese**

### **1. Media**

Standard Methods Agar (SMA), (Difco Laboratories, Detroit, MI)

DeMan, Ragosa, Sharpe Agar (MRS), (Difco Laboratories, Detroit, MI)

Peptone Blanks (Difco Laboratories, Detroit, MI)

### **2. Procedure**

Cottage cheese was analyzed for psychrotrophic and lactic acid bacteria counts. Psychrotrophic bacteria were enumerated using the modified Psychrotrophic Bacteria Count method (mPBC) (Richardson, 1985). Lactic acid bacteria were also enumerated using MRS agar (Richardson, 1985).

Dilutions were prepared as seen in Figure 3, and plated in duplicate for each container. Plates were incubated at 21°C for 48 hours to enumerate psychrotrophic bacteria and 32°C for 48 hours to enumerate lactic acid bacteria.

## **D. Chemical analyses of cottage cheese**

Chemical analyses were performed on the cottage cheese in conjunction with microbiological analyses and sensory



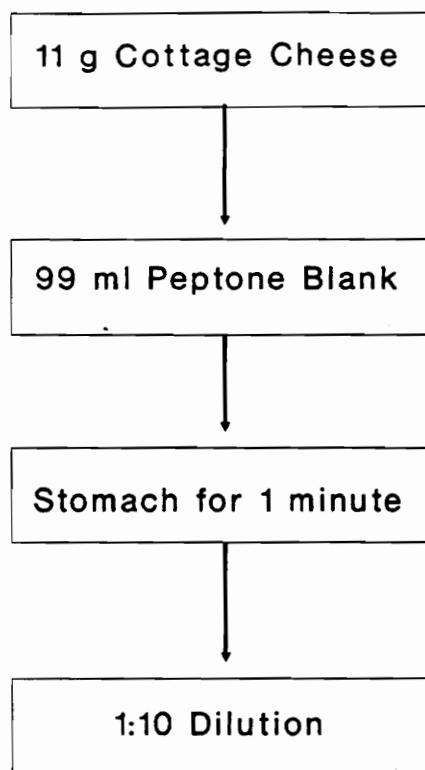


Plate  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  dilutions onto Standard Methods Agar and MRS Agar

**FIGURE 3.** Dilution scheme for enumeration of psychrotrophic and lactic acid bacteria in cottage cheese.

evaluation.

### **1. Measurement of pH**

The pH was determined using an Accumet Model 925 pH meter (Fisher Scientific, Pittsburg, PA)

### **2. Color change determination**

Initial color and potential discoloration of cottage cheese over storage-life was determined using a Minolta Chroma Meter CR-200 color difference meter (Minolta Corporation, Ramsey, NJ). Color was measured in the CIE  $L^* a^* b^*$  system after calibration of the color meter with Calibration Plate CR-A43 (Minolta Corporation, Ramsey, NJ). A  $45^\circ$  angle of illumination was used with a CIE standard illuminant C source.

### **3. Measurement of titratable acidity**

Titratable acidity in terms of percent phosphoric acid was determined by titrating 9 g of cottage cheese against 0.1 N NaOH. Curd particles were first reduced to less than 1 mm in diameter by blending the cottage cheese with 18 ml distilled water for 45 seconds. A Model HS40 hand-held blender (Black and Decker, Shelton, CT) was used. Phenolphthalein (0.5 ml) was used to indicate the end point (Richardson, 1985).

#### **4. Enzymatic quantification of lactic acid**

##### **a. Reagents**

Lactate Dehydrogenase Catalog No. 826-UV (Sigma Chemical CO., St. Louis MO)

Glycine Buffer Catalog No. 826-UV (Sigma Chemical Co., St. Louis, MO)

NAD Catalog No. 826-UV (Sigma Chemical CO., St. Louis, MO)

Trichloroacetic Acid (TCA) Catalog No. 60-7 (Sigma Chemical CO. St.Louis, MO)

##### **b. Equipment**

Chemical Resistance Filters 0.45  $\mu$ M Acrodisc (Gelman Company, Ann Arbor, MI)

Syringes 10cc. (Becton, Dickinson and CO., Shelton, CT)

Centrifuge Dynac (Becton, Dickenson and Co., Shelton, CT)

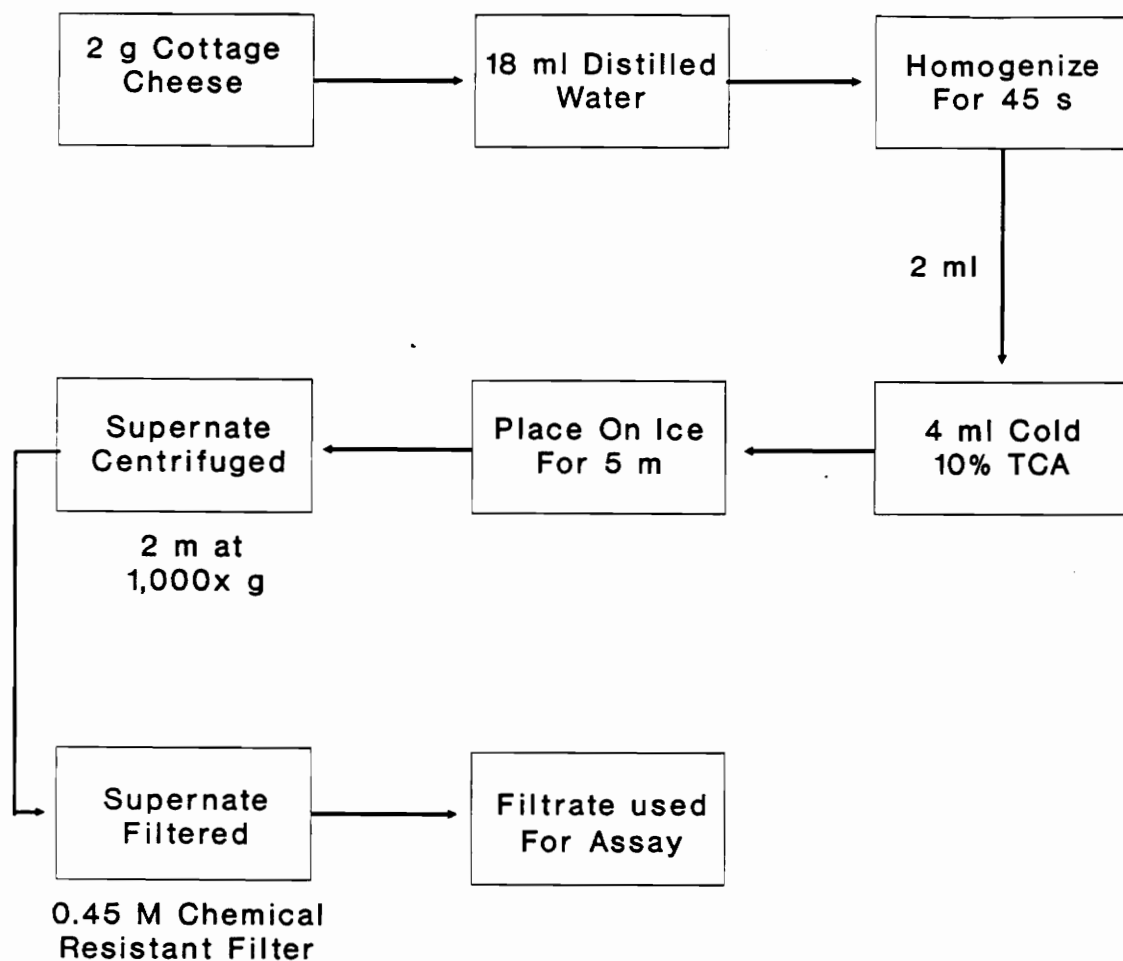
Spectrophotometer Lambda 3 UV/Vis Spectrophotometer (Perkin-Elmer, Norwalk, CT)

Blender Model HS40 (Black and Decker, Shelton, CT)

##### **c. Procedure**

Cottage cheese samples were prepared for quantification of lactic acid as schematized in Figure 4. Lactic acid was enzymatically quantified using SIGMA kit 826-UV.

Instructions in the kit were followed with the following exceptions:



**FIGURE 4. Preparation of cottage cheese sample for the enzymatic quantification of lactic acid.**

1. Reaction volumes were decreased by 50% (to economize on NAD vials required).
2. Both ultraviolet and visible lamps were turned on simultaneously to read the absorbance at 340 nm.

A standard curve was used to determine the concentration of lactic acid. These results were compared to the concentrations calculated from the absorbance values of the samples (equations given in the kit booklet).

#### d. Calculations

For narrow bandwidth instruments, percent lactic acid is calculated from mmol/L lactic acid (calculations as per kit) using the following equation:

$$\frac{A_{340} \times 14.5 \times 20 \times 90 \times 100}{1000 \times 1000 \times 2} = A_{340} \times 1.3$$

Where:  $A_{340}$  = final maximum absorbance at 340 nm  
 14.5 = conversion factor to obtain lactic acid in mmol/L (Sigma kit 826/UV)  
 20 = final volume of cottage cheese extract  
 90 = molecular weight of lactic acid in mg/mmol  
 100 = conversion of g/100 g lactic acid to percent lactic acid  
 1000 = conversion of ml to L of 20 ml cottage

cheese extract  
1000 = conversion of mg/mmol to g/mmol lactic  
acid  
2 = initial weight of cottage cheese used in g

#### **E. Sensory evaluation of cottage cheese**

A panel of 10 experienced judges was used to evaluate cottage cheese quality. Experience was provided by acquainting judges with selected flavor descriptors and presenting market samples characterizing each flavor descriptor. A six point interval scale, with generalized anchor descriptors of "not at all" and "very much so", was used for measuring intensity of flavor characteristics (acid, bitter, sweet, salty, fruity, and unidentified off-flavor) and acceptability of body and texture, flavor, and overall product. Panelists were provided with an identified standard of fresh cottage cheese, identical to that used for packaging, at each sampling session.

#### **F. Statistical analysis**

Data was analyzed by employing a split plot experimental design using Statistical Analysis Systems (SAS Institute, Inc., Cary, NC).

Cottage cheese was subdivided into samples which were evaluated on days 5, 8, 12, 15, 19, 23, and 28. The MAP

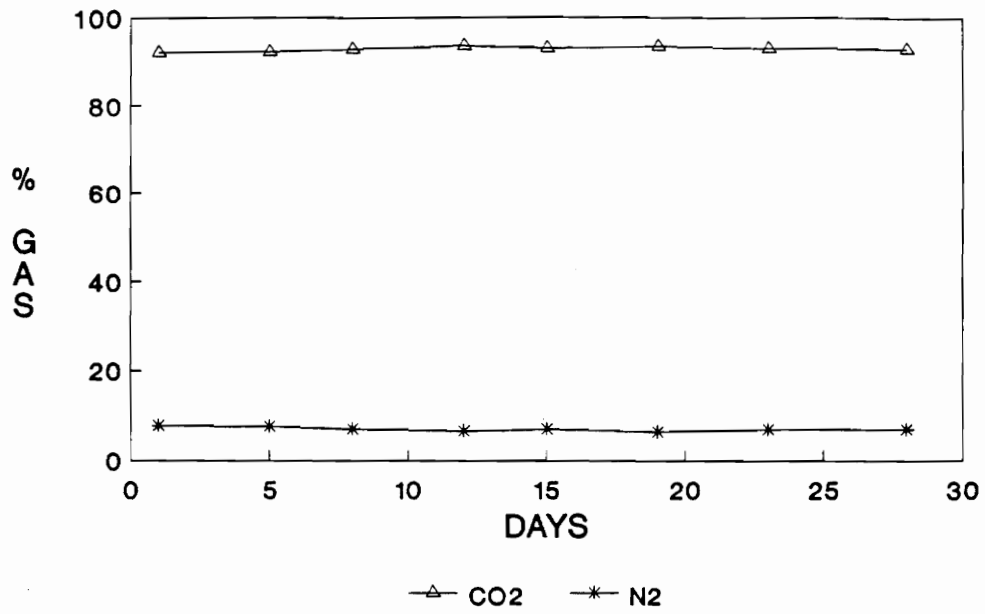
treatment for each batch was considered the whole plot. Individual samples were chosen at random on the above specified days and analyzed as independent split plots. Dependent variables used were psychrotrophic and lactic acid bacteria counts, pH, titratable acidity, lactic acid concentration, cottage cheese color in terms of the CIE  $L^*$   $a^*$   $b^*$  values, and sensory scores associated with overall product appeal, and acceptability of flavor, body and texture as affected by MAP treatment and storage time.

#### IV. RESULTS AND DISCUSSION

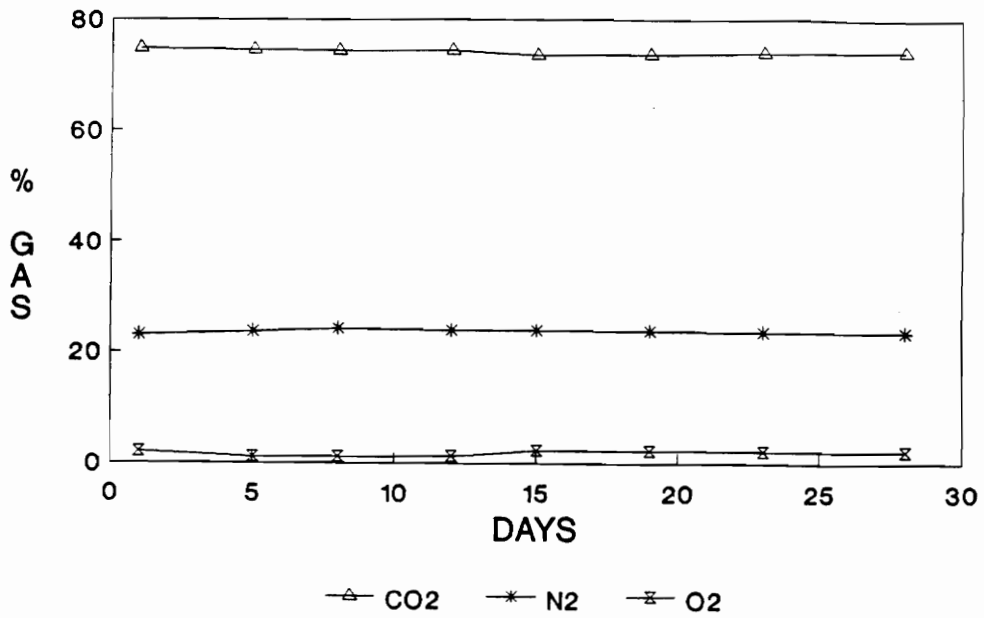
##### A. Analysis of headspace gas composition of cottage cheese packages over storage time.

Intended modified atmospheres for packaging were not completely achieved due to incomplete evacuation of the package. Initial headspace gas compositions obtained instead of 100% CO<sub>2</sub>, 75% CO<sub>2</sub>-25% N<sub>2</sub>, and 100% N<sub>2</sub> were 92.26% CO<sub>2</sub>-7.74% N<sub>2</sub>, 74.78% CO<sub>2</sub>-23.14% N<sub>2</sub>-2.08% O<sub>2</sub>, and 98.16% N<sub>2</sub>-1.84% O<sub>2</sub> respectively. Atmospheres introduced into packages remained unchanged over storage life, as seen in Figures 5-8. Oxygen permeation rate of the film used was found to be between 15-30 cc/m<sup>2</sup>/24 hours under 1 atmosphere pressure at 23°C. At 4°C, O<sub>2</sub> permeation rate was 3-6 cc/m<sup>2</sup>/24 under 1 atmosphere pressure, subsequently minimal gaseous exchange occurred across the barrier film during the storage period. The small amount of O<sub>2</sub> that would permeate through the film was metabolized by the inherent microflora of cottage cheese at a rate equal to or greater than the rate of permeation. Under normal conditions cottage cheese would not be considered a respiring food, and would not contribute towards modification of headspace gases (Labuza and Breene, 1989).

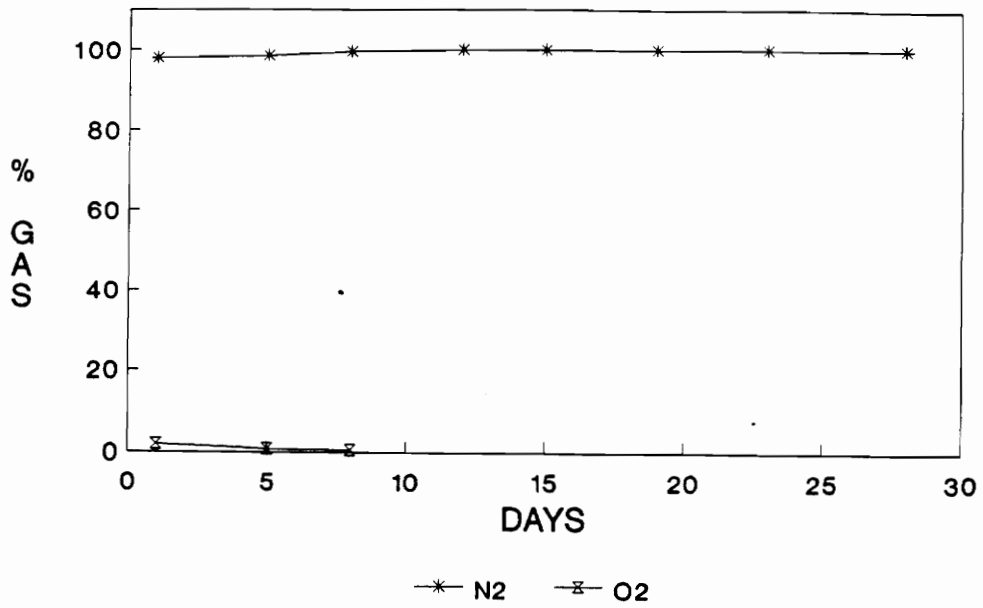




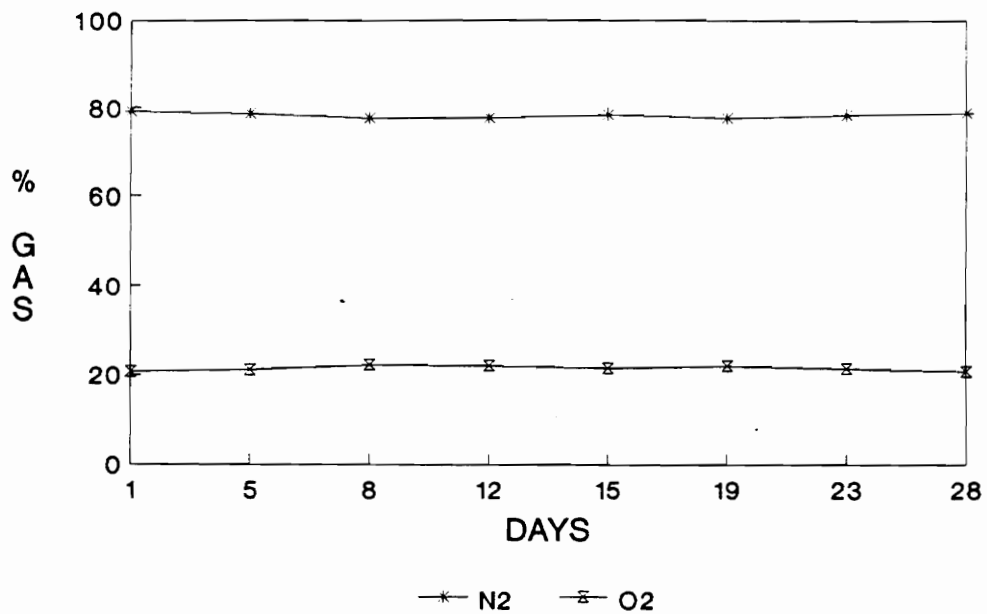
**FIGURE 5. Headspace gas composition of cottage cheese packages flushed with 100% CO<sub>2</sub> over storage time.**



**FIGURE 6. Headspace gas composition of cottage cheese packages flushed with 75% CO<sub>2</sub>:25% N<sub>2</sub> over storage time.**



**FIGURE 7. Headspace gas composition of cottage cheese packages flushed with 100% N<sub>2</sub> over storage time.**

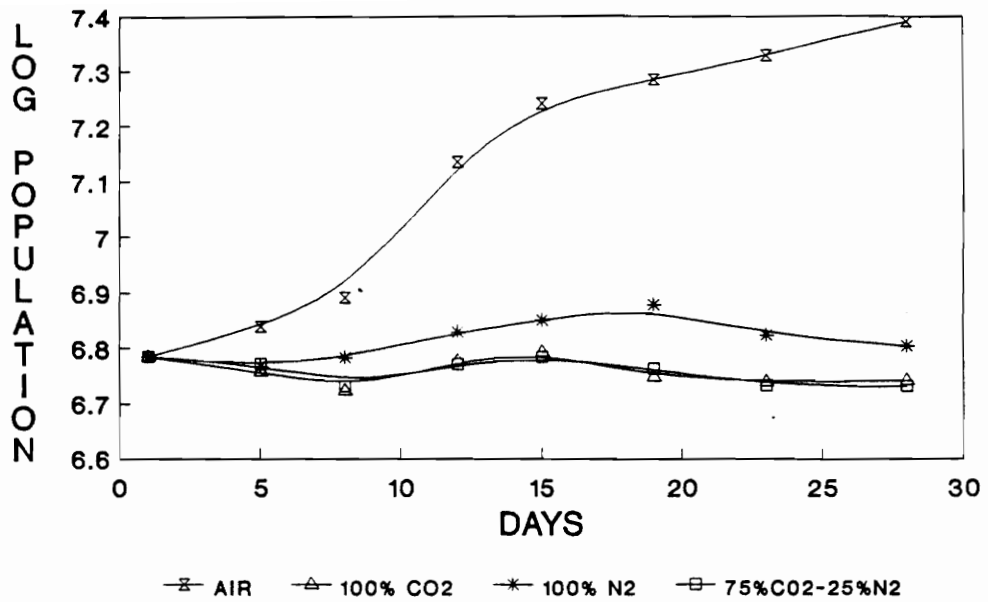


**FIGURE 8. Headspace gas composition of cottage cheese packages flushed with air over storage time.**

**B. Microbiological analysis of cottage cheese over storage time.**

**1. Enumeration of psychrotrophic spoilage bacteria.**

Psychrotrophic bacteria were enumerated by the modified Psychrotrophic Bacteria Count (mPBC) method. The interaction between storage time (day) and MAP treatment was significant ( $p < 0.05$ ) for growth of psychrotrophic microorganisms. As seen in Figure 9, atmosphere used for packaging influenced growth of psychrotrophic bacteria, as cottage cheese flushed with air had higher counts as compared to cottage cheese flushed with 100% CO<sub>2</sub>, 100% N<sub>2</sub>, or 75% CO<sub>2</sub>:25% N<sub>2</sub>. The initial mPBC was  $6.03 \times 10^6$  CFU/g cottage cheese. Counts for cottage cheese flushed with air increased to  $7.8 \times 10^6$  CFU/g by day 8. By day 12 counts increased to  $1.36 \times 10^7$  CFU/g reaching a final count of  $2.45 \times 10^7$  CFU/g by day 28. Cottage cheese flushed with 100% N<sub>2</sub> demonstrated an increase in the mPBC to  $1.57 \times 10^6$  CFU/g by day 19, which then decreased by approximately the same amount by day 28. Carbon dioxide treated samples demonstrated a decrease in mPBC by  $0.78 \times 10^6$  CFU/g by day 8. Between day 8-19, counts increased by  $0.92 \times 10^6$  CFU/g which then decreased by  $0.67 \times 10^6$  CFU/g by day 28. Although interaction between storage time and MAP treatment had a significant ( $p < 0.05$ ) effect on the growth of psychrotrophic bacteria, counts obtained from cottage cheese treated with



**FIGURE 9. Enumeration of psychrotrophic bacteria in air packaged and MAP cottage cheese over storage time.**

CO<sub>2</sub> and N<sub>2</sub> remained unchanged over 28 days, indicating that MAP treatment had a greater effect than storage time.

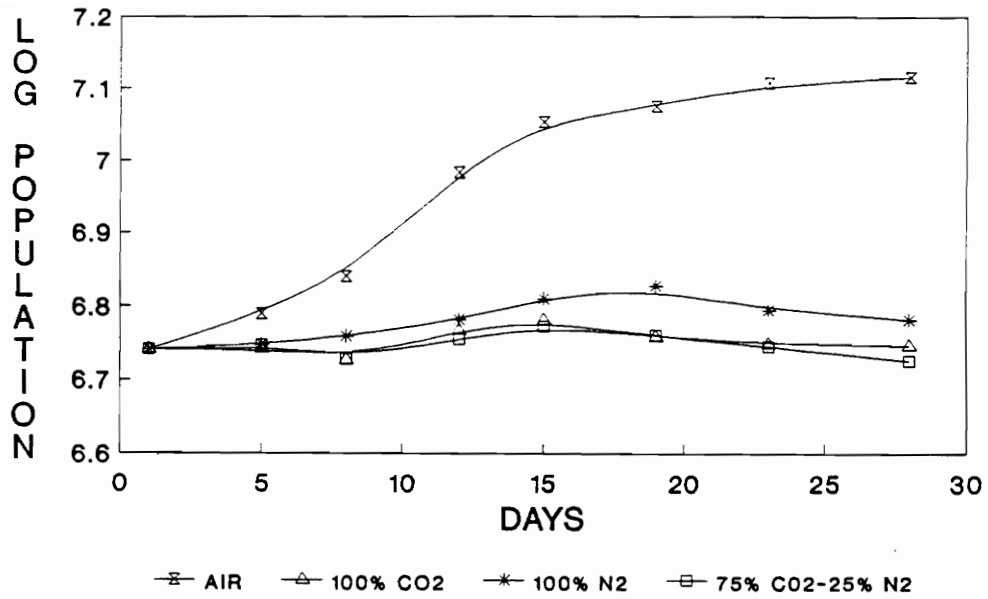
Most psychrotrophic bacteria are aerobes (Banwart, 1989). Growth was therefore not expected in cottage cheese flushed with elevated CO<sub>2</sub> or 100% N<sub>2</sub> atmospheres as O<sub>2</sub> was absent from package headspace. Results further demonstrated that elevated CO<sub>2</sub> was able to inhibit growth of psychrotrophic bacteria to a better extent than elevated N<sub>2</sub>. These results were attributed to not only an absence of O<sub>2</sub> from package headspace but to the bacteriostatic properties of CO<sub>2</sub> (Brody, 1989). Lack of rapid growth of psychrotrophs in air treated samples was attributed to the low temperature of storage. Although, psychrotrophs can grow below 7°C, growth is slow below 5°C (Banwart, 1989). Good quality, fresh direct-set cottage cheese has a population of gram-negative psychrotrophic bacteria within a range of 10<sup>2</sup>-10<sup>4</sup> CFU/g. A population of 10<sup>6</sup> psychrotrophic bacteria/g was obtained during the study. It was believed that some of these colonies were gram-positive lactic acid bacteria. Creaming mixtures for direct-set cottage cheese are often inoculated, as in this case, with lactic acid bacteria, usually Lactococcus lactis sub species diacetylus, and Leuconostoc cremoris. The above mentioned bacteria produce flavor and aroma compounds which enhance flavor and aroma of direct-set cottage cheese (Bodyfelt et al, 1988). To

challenge the theory that the lactic acid bacteria contributed to modified psychrotrophic counts, colonies on crystal violet tetrazolium (CVT) agar were enumerated. Plates were incubated at 21°C for 48 hours. On both occasions of enumeration, counts of  $10^4$  gram-negative bacteria/g. The difference of  $10^2$  CFU/g was attributed to the growth of gram-positive lactic acid bacteria. Counts for lactic acid bacteria on standard methods agar (SMA) and DeMan, Ragosa, Sharpe (MRS) agar did not correspond. This was due to the fact that lactic acid bacteria are extremely fastidious and grow best at around 30°C (Gilliland et al, 1986).

## **2. Enumeration of lactic acid bacteria.**

Interaction between storage time and MAP treatment was significant ( $p < 0.05$ ) for growth of lactic acid bacteria. As seen in Figure 10, the atmosphere used for packaging influenced lactic acid bacteria count, as cottage cheese flushed with air had a higher count when compared to cottage cheese flushed with 100% CO<sub>2</sub>, 100% N<sub>2</sub>, or 75% CO<sub>2</sub>:25% N<sub>2</sub>. The initial lactic acid bacteria count was  $5.5 \times 10^6$  CFU/g cottage cheese increasing to  $1.32 \times 10^6$  CFU/g by day 28 for air treated samples. A typical growth curve was obtained. Cottage cheese flushed with 100% N<sub>2</sub> demonstrated an increase in lactic acid bacteria counts by  $1.0 \times 10^6$  CFU/g by day 19,





**FIGURE 10. Enumeration of lactic acid bacteria in air packaged and MAP cottage cheese over storage time.**

which decreased by  $0.58 \times 10^6$  CFU/g over the next 9 days. For carbon dioxide treated samples lactic acid bacteria counts remained the same over the 28 day period. Although the interaction of MAP treatment and storage time was significant ( $p < 0.05$ ) for growth of lactic acid bacteria, counts obtained from cottage cheese treated with  $\text{CO}_2$ , and  $\text{N}_2$  remained practically the same over 28 days, indicating that MAP treatment had a greater effect on the growth of lactic acid bacteria than storage time.

Results demonstrate that elevated  $\text{CO}_2$  levels were able to inhibit growth of lactic acid bacteria to a greater extent than elevated  $\text{N}_2$ . These results are attributed to the bacteriostatic property of  $\text{CO}_2$  (Brody, 1989). Although lactic acid bacteria are facultative anaerobes, growth is slower under anaerobic conditions than in the presence of  $\text{O}_2$  (Banwart, 1989). This fact has been demonstrated by lower counts obtained from cottage cheese flushed with 100%  $\text{N}_2$  as compared to air treated samples. Limited growth was observed even in air treated samples due to the low temperatures of storage.

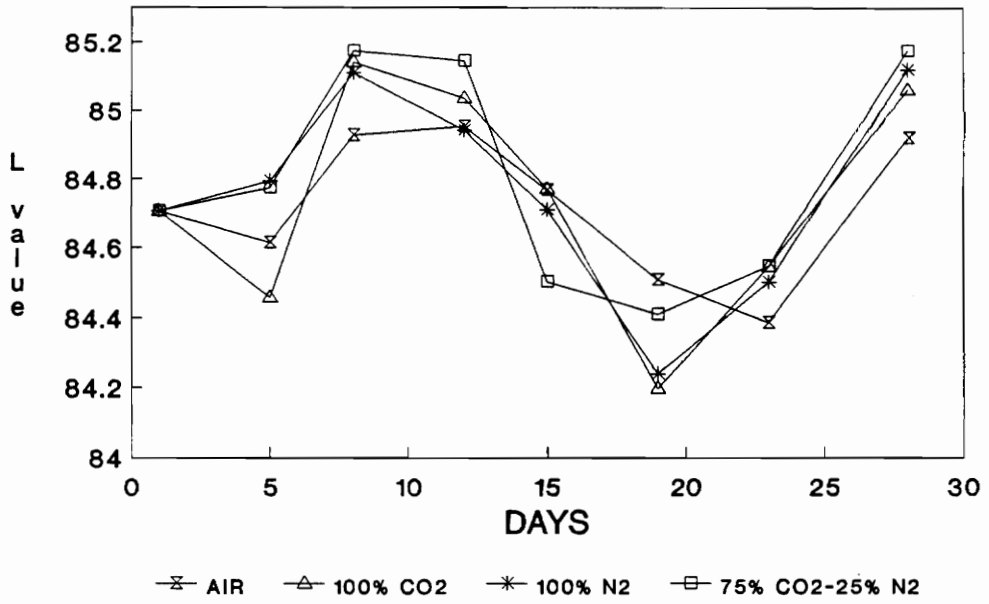
### **C. Color change measurements of cottage cheese over storage time.**

Color was measured in the CIE  $L^* a^* b^*$  system. The CIE  $L^*$  value denotes the lightness; the CIE  $a^*$  value, redness to

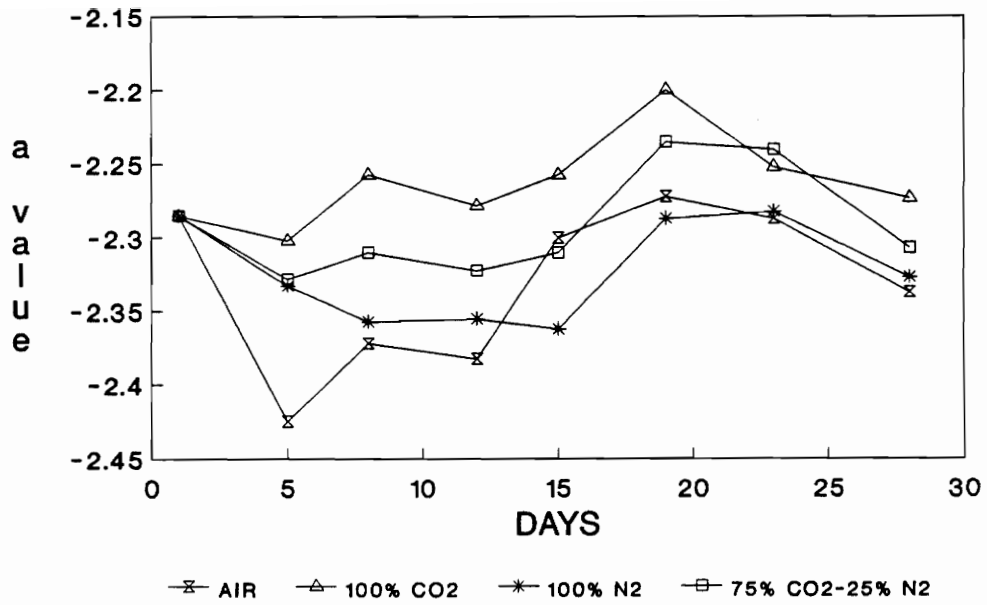
greenness; and the CIE  $b^*$  value, yellowness to blueness of the compound. CIE  $a^*$  and CIE  $b^*$  values represent the hue and chroma of the compound.

Variations in color found to be statistically significant ( $p < 0.05$ ) were not perceived by the panelists. Variations in CIE  $L^*$   $a^*$   $b^*$  values perceived by the color difference meter were attributed to instrument noise and variations in sample presentation (Clydsdale, 1969; Little and Mackenny, 1962).

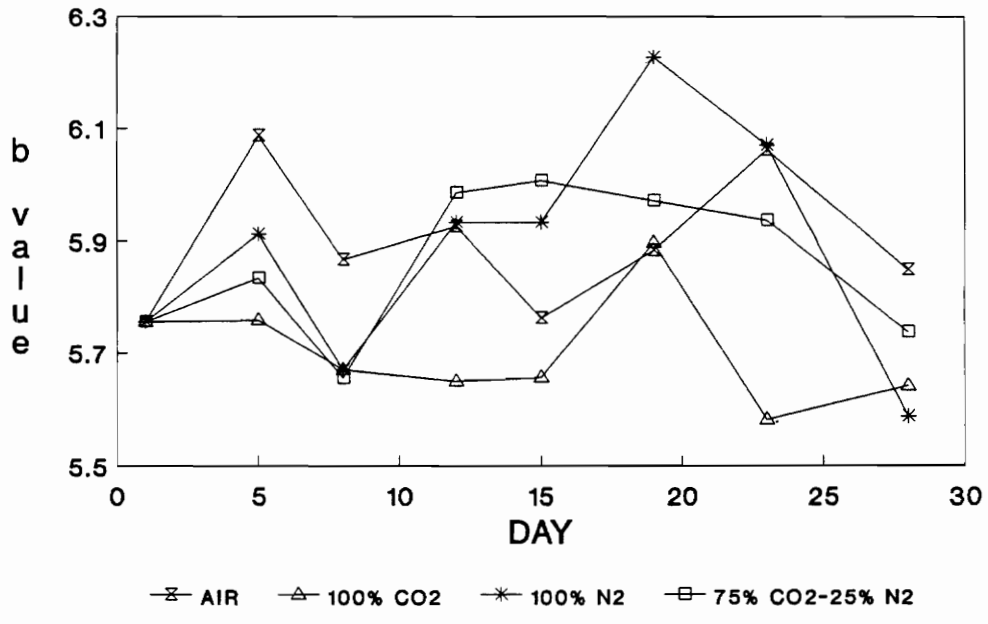
Lightness ( $L^*$ ) is measured on a scale of 0-100; 100 considered as white; 0 as black, with shades of grey in between. Storage time was significant ( $p < 0.05$ ) for cottage cheese lightness. As seen in Figure 11, the trend for lightness was the same for all 4 MAP treatments. CIE  $L^*$  values observed for cottage cheese ranged between 84.2 to 85.2. CIE  $a^*$  values are measured on a scale of +100 to -80, +100 considered red and -80 as green. As seen in Figure 12, the CIE  $a^*$  values observed for cottage cheese ranged between -2.425 and -2.200, indicating that cottage cheese was slightly towards the green side of the scale than the red. MAP treatment had a significant ( $p < 0.05$ ) effect on the CIE  $a^*$  value. Cottage cheese treated with  $CO_2$  was more red than cottage cheese treated with 100%  $N_2$  or air. CIE  $b^*$  values are measured on a scale of +70 to -50 with +70 considered as yellow and -50 as blue. Storage time was



**FIGURE 11. Change in CIE L\* values of MAP cottage cheese over storage time.**



**FIGURE 12. Change in CIE a\* values of MAP cottage cheese over storage time.**



**FIGURE 13. Change in CIE  $b^*$  values of MAP cottage cheese over storage time.**

significant ( $p < 0.05$ ) for CIE  $b^*$  values of cottage cheese. Due to variation within samples over storage-life no conclusion could be drawn regarding the effect of MAP treatment on CIE  $b^*$  values obtained for cottage cheese (Figure 13). The hue and chroma of cottage cheese was more towards the yellow end of the scale than blue.

**D. Measurement of pH, titratable acidity, and lactic acid concentration of cottage cheese over storage time.**

In a previous study conducted by Marcy et al (1990), on cottage cheese, pH decreased with a corresponding increase in titratable acidity. The increased acidity was perceived by panelists and was attributed to formation of lactic acid. As a result, lactic acid was quantified during this study. It should be noted that cottage cheese used by Marcy et al (1990) was set using live cultures of Lactococcus lactis and Lactococcus cremoris.

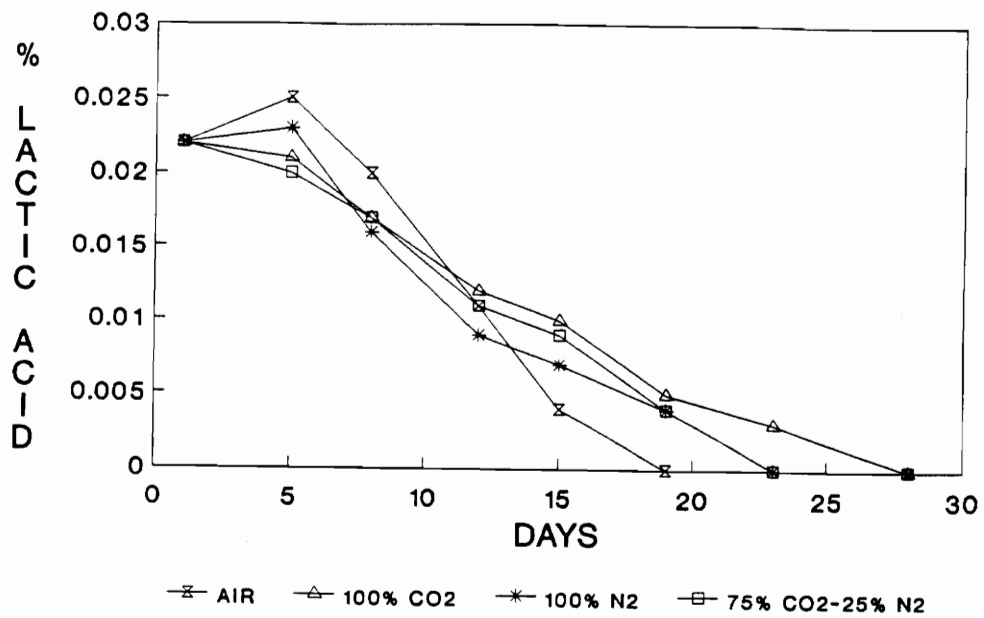
A standard curve (Appendix) was prepared to determine the concentration of lactic acid, the results of which were compared to the concentrations calculated from the absorbance values of the samples. Concentrations determined using the standard curve were comparable to those determined by calculation. Although, the kit (Sigma 826-UV) was intended for determining the amount of lactate in blood, there was no problem in using it to determine the

concentration of lactate in cottage cheese.

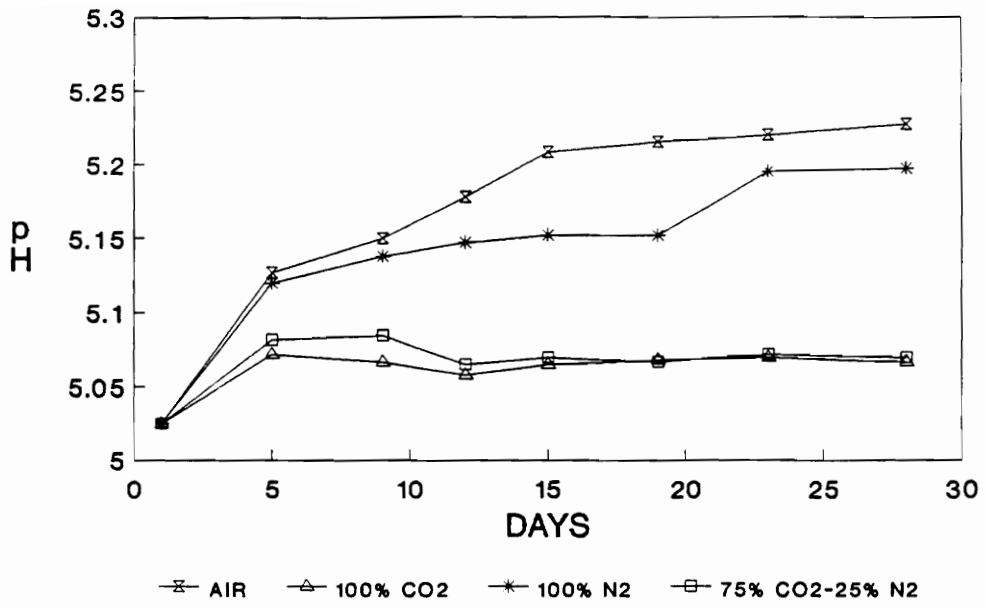
Cottage cheese used in this research study was direct-set using food grade phosphoric acid. Storage time was significant ( $p < 0.05$ ) for lactic acid concentration. The initial average lactic acid concentration was quantified at only 0.02 % by weight of the total titratable acidity as seen in Figure 14. Lactic acid concentrations of samples flushed with air was higher upto day 11 as compared to samples flushed with modified atmospheres. Rate of decrease in acid concentration for cottage cheese flushed with air was more rapid as compared to cottage cheese flushed with modified atmospheres. Lactic acid completely disappeared from all samples by day 28. Disappearance of lactic acid from the cottage cheese samples was attributed to the utilization of lactic acid as a source of carbon by microorganisms present in cottage cheese. Lactic acid was therefore not a significant contributor towards pH or titratable acidity.

The interaction of storage time and MAP treatment was significant ( $p < 0.05$ ) for cottage cheese pH and titratable acidity. Although this interaction was significant, in terms of the true value in this study, the significance has no value. Figure 15 demonstrated that pH increased marginally over storage time. Increase in pH was unexpected and did not conform with increase in titratable acidity

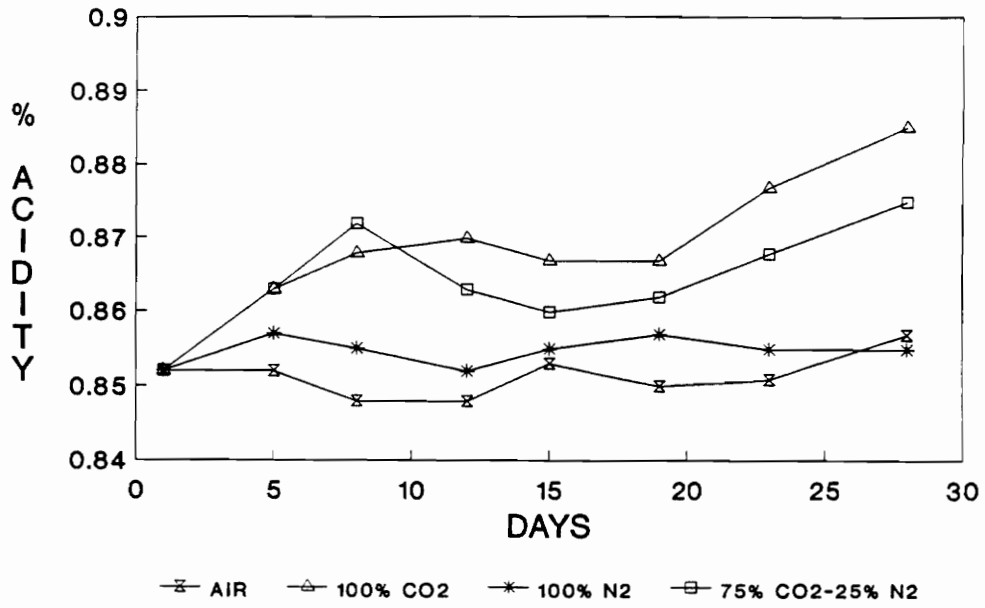




**FIGURE 14. Enzymatic quantification of lactic acid in air packaged and MAP cottage cheese over storage time.**



**FIGURE 15. pH measurements of air packaged and MAP cottage cheese over storage time.**



**FIGURE 16. Titratable acidity measurements of air packaged and MAP cottage cheese flushed over storage time.**

(Figure 16). This discrepancy was attributed to the difference in the method used to measure pH and titratable acidity. Titratable acidity was measured on homogenized cottage cheese while pH was measured on free curd. It was believed that pH increased due to absorption of acid from the cream into the curd. Titratable acidity would not be affected as homogenation of curd particles during measurement of titratable acidity would release acid present within curd particles. To verify the hypothesis that methodology used to measure pH and titratable acidity was responsible for discrepancy in results, cottage cheese samples were homogenized and the pH was measured. Readings indicated that pH of homogenized cottage cheese was indeed lower than non-homogenized samples (Table 1). Samples were also gently squeezed through a cheese cloth, and the filtrate measured for titratable acidity. Homogenized samples of cottage cheese had higher titratable acidity (Table 1). It should be noted that pH and titratable acidity of samples treated with CO<sub>2</sub> were more acidic than N<sub>2</sub> and air treated samples. Carbon dioxide present in the package headspace would result in the formation of carbonic acid in the aqueous phase of cottage cheese (Dixon and Kelly, 1988). Carbonic acid is unstable and ionizes within 10<sup>-6</sup> seconds to form HCO<sub>3</sub><sup>-</sup> and H<sup>+</sup> ions (Butler, 1982). The H<sup>+</sup> formed would contribute to acidity. It should be noted

**Table 1. Comparison of pH and titratable acidity of homogenized and non-homogenized cottage cheese samples.**

	100% CO <sub>2</sub>	75% CO <sub>2</sub> :25% N <sub>2</sub>	100% N <sub>2</sub>	AIR
pH <sup>1</sup>	5.18	5.18	5.25	5.26
pH <sup>2</sup>	4.87	4.88	4.91	4.90
titratable acidity <sup>2</sup>	0.90	0.88	0.85	0.86
titratable acidity <sup>3</sup>	0.75	0.74	0.64	0.67

Samples were 30 days old

Samples taken from replication # 3

- 1 non-homogenized sample
- 2 homogenized sample
- 3 sample passed through cheese cloth and the filtrate homogenized

that hydration of CO<sub>2</sub> to carbonic acid is only 1-2% (Lampila, 1990), therefore contributing minimally towards acidity. Increase in acidity during storage life was not perceived organoleptically. Increase in acidity was attributed to the formation of intermediate acids involved in metabolism such as pyruvic or some fatty acid (Stryer, 1983).

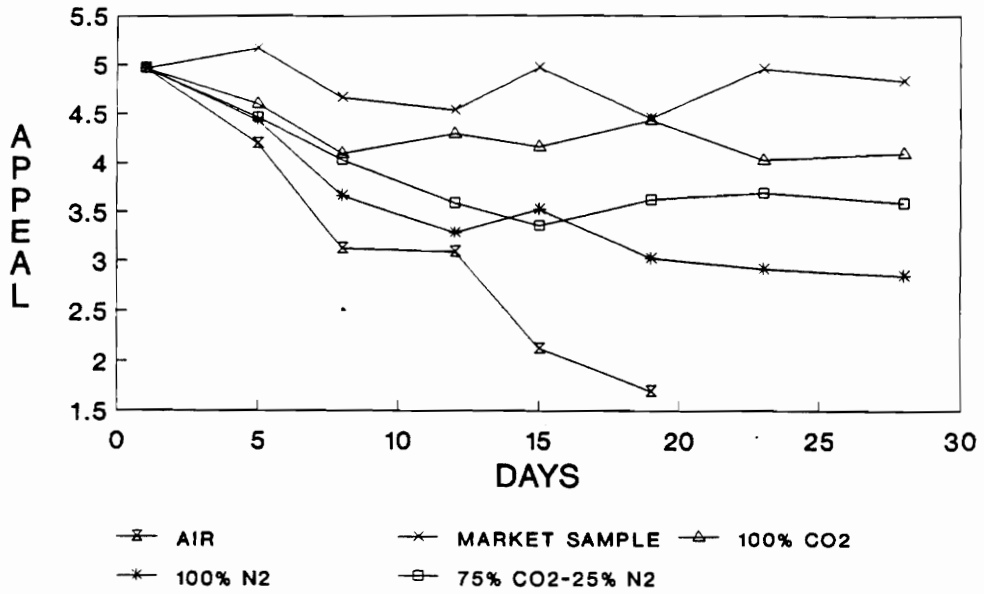
#### **E. Sensory evaluation of cottage cheese over storage life.**

Sensory evaluation was conducted to determine cottage cheese quality over storage-life. Panelists used a six point unstructured interval scale to evaluate cottage cheese. The anchor descriptors were generalized for all evaluations as "not at all" and "very much so". A score of 1 was considered best for unidentified off-flavors, acidity, sweetness, saltiness, and bitterness. For overall product appeal, flavor, and body and texture acceptability a score of 6 was considered best.

Samples treated with air were discontinued after day 19 for replication #1, and from day 15 for replications #2 and #3, as samples appeared gel-like and tasted tart.

##### **1. Evaluation of overall product appeal.**

Interaction between storage time and MAP treatment was significant ( $p < 0.05$ ) for overall product appeal. As



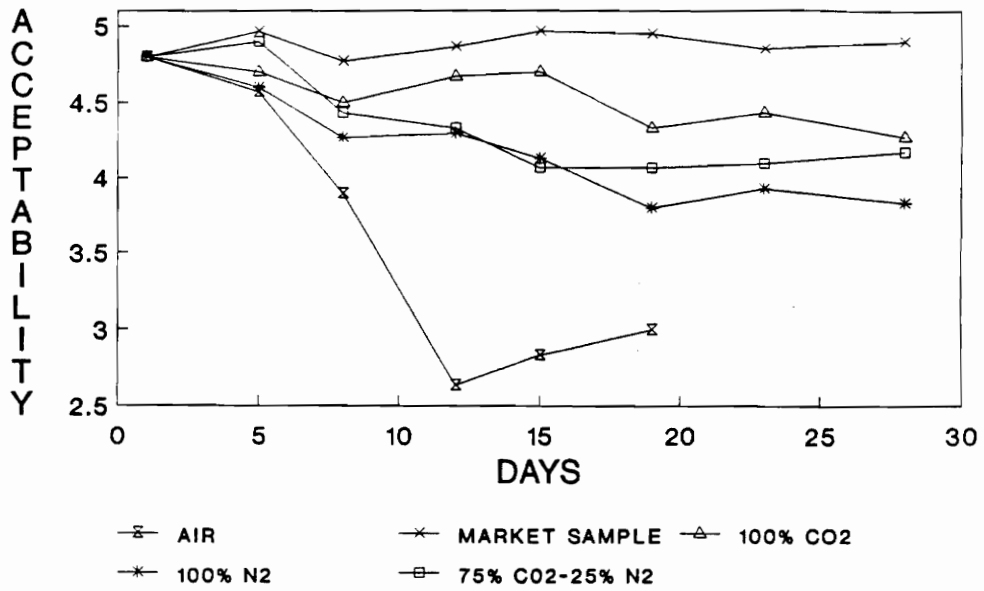
**FIGURE 17. Evaluation of overall product appeal for air packaged and MAP cottage cheese over storage time.**

observed in Figure 17, MAP treatment appeared to have a greater effect on overall product appeal than storage time. Replications also had a significant ( $p < 0.05$ ) effect on overall product appeal, indicating that variations between replications was due to initial product quality. Fresh product quality varied in appeal as seen from the varying score obtained for the market sample (4.5-5.2) over the period of study. Appeal for air treated samples decreased over storage life, with 1.7 as the average score on day 19. Cottage cheese flushed with 100% CO<sub>2</sub> decreased in appeal from an average initial score of 5.0 to 4.0 by day 8. Samples packaged under 75% CO<sub>2</sub>:25% N<sub>2</sub> and 100% N<sub>2</sub> deteriorated in quality to a greater extent than samples treated with 100% CO<sub>2</sub>. Samples treated with 75% CO<sub>2</sub>:25% N<sub>2</sub> were given a score of 3.5 on day 15, while samples treated with 100% N<sub>2</sub> were given a score of 3.0 on day 19. The overall trend appeared to be a greater appeal for cottage cheese flushed with 100% CO<sub>2</sub>, followed by a 75% CO<sub>2</sub>:25% N<sub>2</sub>, and 100% N<sub>2</sub> treatments respectively, with air treatments being the least acceptable.

## **2. Evaluation of body and texture acceptability.**

Interaction between storage time and MAP treatment was significant ( $p < 0.05$ ) for body and texture appeal. Figure 18, clearly depicts that treatment had a greater effect on





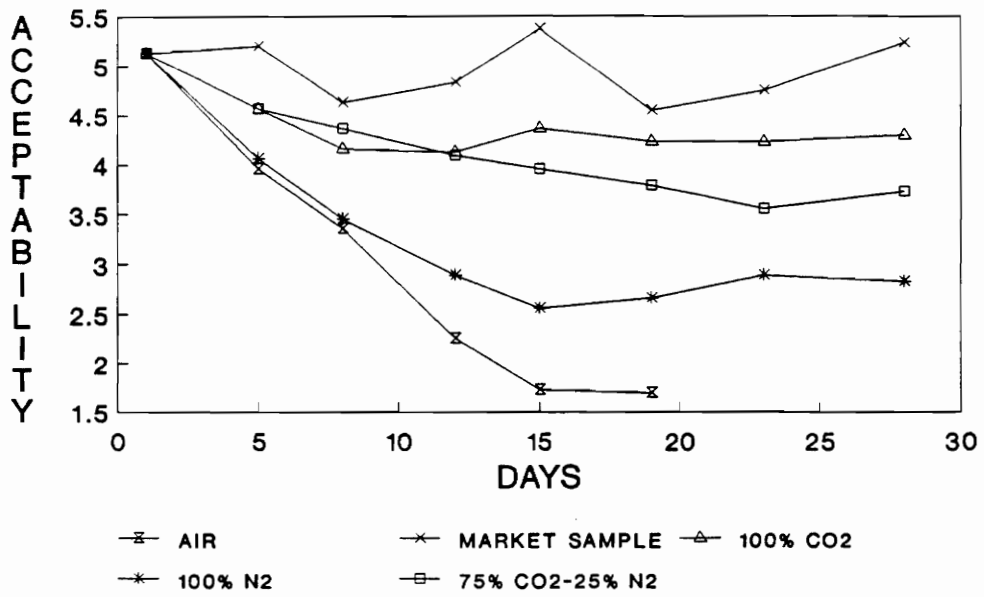
**FIGURE 18. Evaluation of body and texture acceptability for air packaged and MAP cottage cheese over storage time.**

body and texture acceptability than day. Variation within the market sample was narrow over the period of study. Air treated samples decreased in acceptability rapidly and were given an average score of 3.0 on day 19. Panelists indicated that air treated samples appeared gel-like. Samples packaged under 100% N<sub>2</sub> decreased in acceptability from an average 4.8 to 3.8 by day 19. Samples treated with 100% CO<sub>2</sub> decreased in acceptability by approximately 0.5 points over 28 days, while samples treated with 75% CO<sub>2</sub>:25% N<sub>2</sub> decreased in acceptability by 0.6 points. The overall trend appeared to be a greater acceptability for cottage cheese flushed with 100% CO<sub>2</sub>, followed 75% CO<sub>2</sub>:25% N<sub>2</sub>, and 100% N<sub>2</sub> treatments respectively, with air treatments being the least acceptable.

Panelists observed that the cottage cheese got drier over time, with cream being absorbed by curd particles. These observations correspond to increase in pH of free cream. As cream was absorbed by the curd, acid in the cream got absorbed into the curd resulting in an increase in pH of free curd.

### **3. Evaluation of flavor acceptability.**

Interaction between storage time and MAP treatment was significant ( $p < 0.05$ ) for flavor acceptability. As seen in Figure 19, MAP treatment had a greater effect on flavor than



**FIGURE 19. Evaluation of flavor acceptability for air packaged and MAP cottage cheese over storage time.**

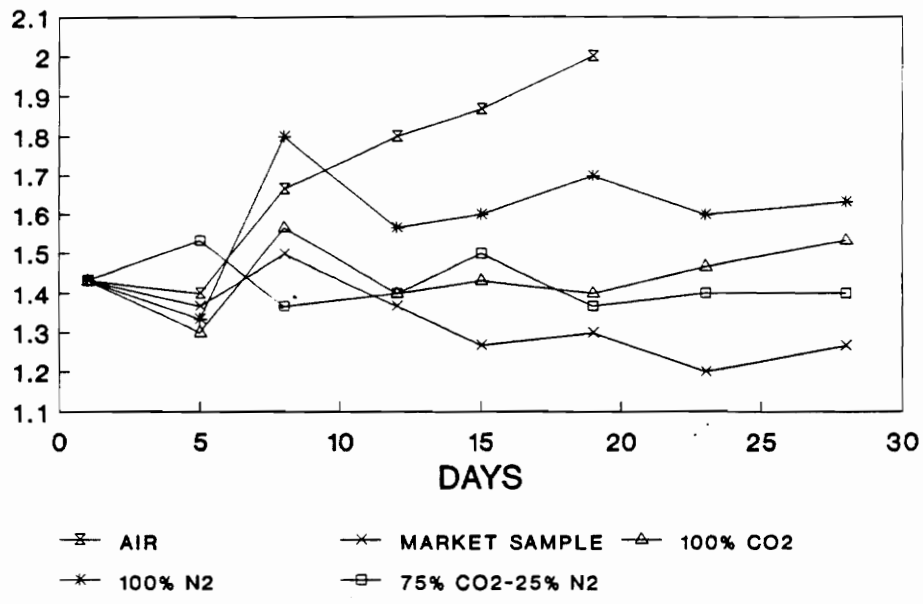
storage time. The market sample varied in the acceptability of flavor between scores of 4.5-5.4. Cottage cheese flushed with 100% CO<sub>2</sub> deteriorated in flavor acceptability from an initial score of 5.1 to 4.1 by day 12. Samples flushed with 75% CO<sub>2</sub>:25% N<sub>2</sub> decreased in flavor acceptability up to day 23 by 1.2 points. Between days 23-28 there was a marginal increase in quality. Samples packaged under 100% N<sub>2</sub> decreased in quality by 2.6 points between days 1-15. Over the next 8 days product quality increased from 2.5 to 3 points. Air treated samples decreased rapidly in quality by 3.7 points over 19 days. Panelists reported that air treated samples tasted tart and puckered the mouth, but were unable to identify a specific off-flavor. Tart flavor was attributed to the formation of excess acetaldehyde from citrate.

The overall trend appeared to be a greater acceptability for cottage cheese flushed with 100% CO<sub>2</sub>, followed by 75% CO<sub>2</sub>:25% N<sub>2</sub>, and 100% N<sub>2</sub> treatments respectively, with air treatments being the least acceptable.

#### **4. Evaluation of the level of unidentified off-flavors.**

MAP treatment was significant ( $p < 0.05$ ) for the level of unidentified off-flavors present in cottage cheese. The overall trend demonstrated lower levels of unidentified off-flavors for cottage cheese flushed with 100% CO<sub>2</sub>, followed

UNIDENTIFIED FLAVORS



**Figure 20. Evaluation of the level of unidentified off-flavors in air and MAP cottage cheese over storage time.**

by cottage cheese flushed with 75% CO<sub>2</sub>:25% N<sub>2</sub>, and 100% N<sub>2</sub> respectively. Cottage cheese treated with air supported the highest levels of unidentified off-flavors as compared to other treatments over the duration of study. As seen in Figure 20, the market sample varied in the level of unidentified off-flavors between scores of 1.2-1.5 points over the period of study. Cottage cheese treated with air, 100% CO<sub>2</sub>, and 100% N<sub>2</sub> decreased in levels for unidentified off-flavors between days 1-5, while there was a increase in the level of unidentified off-flavors for samples packaged under 75% CO<sub>2</sub>:25% N<sub>2</sub>. Between days 5-19, cottage cheese packaged under air increased in the level of unidentified off-flavors by 0.6 points. Air treated samples had maximum levels of unidentified off-flavors as compared to other treatments. Level of unidentified off-flavors increased by 0.5 points for samples treated with 100% N<sub>2</sub> between days 5-8, after which the level decreased by 0.2 points by day 12. The level of unidentified off-flavors for cottage cheese flushed with 100% CO<sub>2</sub> increased by 0.3 points between days 5-8. Over the next 11 days the level decreased by 0.2 points and again increased by 0.3 points between days 19-28. Levels of unidentified off-flavors decreased by 0.15 points for samples flushed with 75% CO<sub>2</sub>:25% N<sub>2</sub> between days 5-8.

The overall product appeal, and acceptability of flavor, body and texture for the fresh, identified market

standard on each day of evaluation was higher as compared to treated cottage cheese samples over storage time.

**5. Evaluation of the level of fruitiness, saltiness, sweetness, bitterness, and acidity.**

To identify specific off-flavors contributing to quality deterioration of cottage cheese, panelists were asked to evaluate levels of fruitiness, bitterness, saltiness, sweetness, and acidity. Storage time, MAP treatment, and replications independent of one another were significant ( $p < 0.05$ ) for the level of bitterness. No interaction, nor independent factor was significant ( $p < 0.05$ ) for the level of saltiness. Interaction between storage time and MAP treatment was significant for levels of acidity, fruitiness, and sweetness. Due to considerable variations within samples, no conclusion could be drawn regarding the effect of MAP treatments on levels of fruitiness, bitterness, sweetness, saltiness, and acidity.

## V. SUMMARY AND CONCLUSIONS

The objectives of this research were to evaluate the ability of modified atmosphere packaging in maintaining cottage cheese quality over the expected shelf-life of 21 days while establishing the proper atmosphere to be used. Further, the study intended to determine the potential for discoloration and development of acid off-flavors in cottage cheese by elevated CO<sub>2</sub> levels.

Cottage cheese quality was determined by sensory evaluations. Microbiological and chemical tests were conducted to achieve a better understanding of the effects of atmospheres on cottage cheese.

Statistically, no change in the headspace gas composition occurred over storage time. MAP treatment was significant ( $p < 0.05$ ) for cottage cheese pH and titratable acidity. Cottage cheese packaged under CO<sub>2</sub>, was more acidic than cottage cheese packaged under air or N<sub>2</sub>. Organoleptically, difference in acidity between MAP treatments was not observed. This observation was in contrast to anticipated results. A sharp acid taste was expected in cottage cheese packaged under CO<sub>2</sub>. According to literature sources (Dixon and Kelly, 1988), elevated CO<sub>2</sub> levels impart a sharp acid taste to foods as a result of carbonic acid formation. The pH of cottage cheese packaged



under air and N<sub>2</sub> increased from an initial pH of 5.00 to 5.223 and 5.2 respectively over the 28 days. Carbon dioxide treated samples increased in pH by only 0.07 units over the 28 days. Titratable acidity was measured in terms of percent phosphoric acid which remained unchanged for cottage cheese packaged under air and N<sub>2</sub>, while cottage cheese packaged under 100% CO<sub>2</sub> and 75%CO<sub>2</sub>:25% N<sub>2</sub> increased from an initial 0.852% to 0.886% and 0.876% respectively. This discrepancy of increase in both pH and titratable acidity was attributed to the method used to measure pH and titratable acidity. Titratable acidity was measured on homogenized cottage cheese, while pH was measured on free cream. The pH of free cream increased as a result of acid absorption by curd particles from the cream.

Direct-set cottage cheese is often ripened with flavor and aroma producing cultures of Lactococcus lactis sub-sp. diacetylus, and Leuconostoc citrovorus, to compensate for the absence of flavor and aroma metabolites produced as a result of fermentation. Lactic acid was produced in minute quantities by the above mentioned cultures, and contributed by 0.02% by weight of the total titratable acidity on day one. Over storage-life of 28 days, concentration of lactic acid decreased to zero percent, with the rate of decrease being higher for air treated samples. Disappearance of lactic acid was due to its utilization as a carbon source by

microorganisms inherent to cottage cheese.

Statistically, storage time was significant ( $p < 0.05$ ) for lightness, hue, and chroma of cottage cheese. However, the variations were marginal and were attributed to instrument noise and variation in sample presentation. No visible discoloration was observed for any treatment throughout the study. This result contradicted previous research which demonstrated that elevated  $\text{CO}_2$  levels cause greying or browning of food products (Dixon and Kelly, 1988).

Treatment of cottage cheese with air was unable to control growth of psychrotrophic or lactic acid bacteria. Counts increased over storage-life from  $6.03 \times 10^6$  to  $2.45 \times 10^7$  CFU/g for psychrotrophic and from  $5.50 \times 10^6$  to  $1.32 \times 10^7$  CFU/g for lactic acid bacteria by day 28, while counts for cottage cheese treated with  $\text{N}_2$  and  $\text{CO}_2$  remained unchanged. The limited growth observed in air treated samples was most probably due to the low ( $4^\circ\text{C}$ ) temperatures used for storing cottage cheese packages.

Sensory evaluation demonstrated that air was able to maintain cottage cheese quality for a maximum of 19 days. Air treated cottage cheese on day 19 appeared gel-like and tasted tart. Cottage cheese packaged under air subsequently had to be discontinued from sensory evaluation. Panelists were unable to identify a specific off-flavor contributing

to the tart taste, although it is possible that acetaldehyde was responsible (Bodyfelt, 1988). Scores for overall product appeal, flavor, body and texture acceptability illustrated that panelists demonstrated greater acceptability for cottage cheese packaged under 100% CO<sub>2</sub> followed by cottage cheese packaged under 75% CO<sub>2</sub>:25% N<sub>2</sub> over 100% N<sub>2</sub> treatments. To identify which off-flavor contributed to quality deterioration, panelists were asked to rate acidity, bitterness, fruitiness, sweetness, and saltiness. Due to considerable variation within treatments over time, no conclusion could be drawn regarding the effect of MAP treatment on the above mentioned off-flavors. Maximum levels of unidentified off-flavors were found in air treated cottage cheese, while CO<sub>2</sub> treatments supporting the least.

The following conclusions may be drawn as a result of this study:

1. Modified atmosphere packaging (MAP) was able to maintain cottage cheese quality to a better extent than air packaging over expected shelf-life.
2. Modified atmospheres with CO<sub>2</sub> levels above 75% were more effective in maintaining quality of cottage cheese than elevated N<sub>2</sub>.
3. Carbon dioxide levels above 75% did not cause visual discoloration of cottage cheese.
4. Carbon dioxide levels above 75% did not impart an

acid off-flavor to cottage cheese.

In continuation of this research, the duration of shelf-life extension should be determined. In order for MAP of cottage cheese under elevated levels of CO<sub>2</sub> to be commercialized, the potential for growth of anaerobic pathogens and possible toxin production in cottage cheese must be established.

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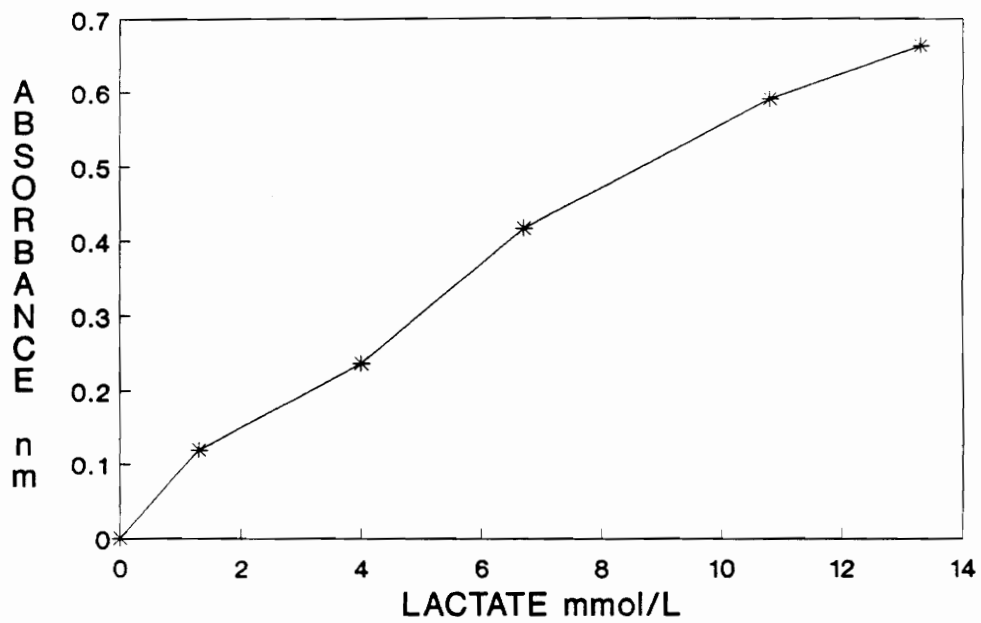
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**VII. APPENDIX**



**FIGURE 21.** Standard curve for the enzymatic quantification of lactic acid using Sigma kit 826-UV.

**PROFILE SCORE CARD**

Panelist \_\_\_\_\_

Date \_\_\_\_\_

Product: Cottage Cheese

You will be given 5 samples. Evaluate the market sample first. Then evaluate the experimental samples in the order on the score card. Mark the score card by placing a check on the line corresponding to the degree to which the characteristic is present in the sample. Please rinse between samples.

Market sample

	NOT AT ALL				VERY MUCH SO
BODY AND TEXTURE ACCEPTABILITY	_____	_____	_____	_____	_____
FLAVOR					
Acid	_____	_____	_____	_____	_____
Bitter	_____	_____	_____	_____	_____
Sweet	_____	_____	_____	_____	_____
Salty	_____	_____	_____	_____	_____
Fruity	_____	_____	_____	_____	_____
Unidentified off-flavor	_____	_____	_____	_____	_____
FLAVOR ACCEPTABILITY	_____	_____	_____	_____	_____
OVERALL PRODUCT ACCEPTABILITY	_____	_____	_____	_____	_____

Sample code \_\_\_\_\_

	NOT AT ALL				VERY MUCH SO
BODY AND TEXTURE ACCEPTABILITY	_____	_____	_____	_____	_____
FLAVOR					
Acid	_____	_____	_____	_____	_____



Bitter	_____	_____	_____	_____	_____	_____
Sweet	_____	_____	_____	_____	_____	_____
Salty	_____	_____	_____	_____	_____	_____
Fruity	_____	_____	_____	_____	_____	_____
Unidentified off-flavor	_____	_____	_____	_____	_____	_____
FLAVOR ACCEPTABILITY	_____	_____	_____	_____	_____	_____
OVERALL PRODUCT ACCEPTABILITY	_____	_____	_____	_____	_____	_____

---

Sample code \_\_\_\_\_

		NOT AT ALL			VERY MUCH SO	
BODY AND TEXTURE ACCEPTABILITY	_____	_____	_____	_____	_____	_____
FLAVOR						
Acid	_____	_____	_____	_____	_____	_____
Bitter	_____	_____	_____	_____	_____	_____
Sweet	_____	_____	_____	_____	_____	_____
Salty	_____	_____	_____	_____	_____	_____
Fruity	_____	_____	_____	_____	_____	_____
Unidentified off-flavor	_____	_____	_____	_____	_____	_____
FLAVOR ACCEPTABILITY	_____	_____	_____	_____	_____	_____
OVERALL PRODUCT ACCEPTABILITY	_____	_____	_____	_____	_____	_____

Sample code \_\_\_\_\_

NOT  
AT ALL

VERY  
MUCH SO

BODY AND TEXTURE  
ACCEPTABILITY

\_\_\_\_\_

FLAVOR

Acid

\_\_\_\_\_

Bitter

\_\_\_\_\_

Sweet

Salty

Fruity

Unidentified off-flavor

FLAVOR ACCEPTABILITY

\_\_\_\_\_

OVERALL PRODUCT  
ACCEPTABILITY

\_\_\_\_\_

---

Sample code \_\_\_\_\_

NOT  
AT ALL

VERY  
MUCH SO

BODY AND TEXTURE  
ACCEPTABILITY

\_\_\_\_\_

FLAVOR

Acid

\_\_\_\_\_

Bitter

\_\_\_\_\_

Sweet

Salty

Fruity

Unidentified off-flavor

FLAVOR ACCEPTABILITY

\_\_\_\_\_

OVERALL PRODUCT  
ACCEPTABILITY

\_\_\_\_\_

---

Thank you for evaluating these samples. Our next cottage  
cheese panel is on \_\_\_\_\_ at \_\_\_\_\_  
Your continued participation is greatly appreciated.

**Table 2. ANOVA for the enumeration of psychrotrophic bacteria in air packaged and MAP cottage cheese over storage time.**

Dependent Variable: log of psychrotrophic bacteria

Source	DF	Mean Squares	F Value	Pr > F
Model	53	0.06387171	135.69	0.0001
Corrected Total	95			
REP	2	0.00697855	1.92	0.2270
TRT	3	0.68485436	188.22	0.0001
Whole Plot Error	6	0.00363864		
DAY	7	0.05140295	109.20	0.0001
TRT*DAY	21	0.04265372	90.61	0.0001
Split Plot Error	42	0.00047072		

**Table 3. ANOVA for the enumeration of lactic acid bacteria in air packaged and MAP cottage cheese over storage time.**

Dependent Variable: log of lactic acid bacteria

Source	DF	Mean Squares	F Value	Pr > F
Model	53	0.02441326	134.72	0.0001
Corrected Total				
REP	2	0.00013377	0.13	0.8795
TRT	3	0.25273831	247.88	0.0001
Whole Plot Error	6	0.00101962		
DAY	7	0.02877226	158.78	0.0001
TRT*DAY	21	0.01468399	81.03	0.0001
DAY*REP	14	0.00139521	7.70	0.0001
Split Plot Error	42	0.00018121		

**Table 4. ANOVA for the change in CIE L\* values of air packaged and MAP cottage cheese over storage time.**

Dependent Variable: CIE L\*

Source	DF	Mean Squares	F Value	Pr > F
Model	53	0.45715072	11.14	0.0001
Corrected Total	95			
REP	2	2.12272110	56.85	0.0001
TRT	3	0.03081285	0.83	0.5262
Whole Plot Error	6	0.03733707		
DAY	7	0.91675402	22.35	0.0001
TRT*DAY	21	0.03897852	0.95	0.5365
DAY*REP	14	0.88794699	21.64	0.0001
Split Plot Error	42	0.04102447		

**Table 5. ANOVA for the change in the CIE a\* value of air packaged and MAP cottage cheese over storage time.**

Dependent Variable: CIE a\*

Source	DF	Mean Squares	F Value	Pr > F
Model	53	0.01432122	6.45	0.0001
Corrected Total	95			
REP	2	0.17632813	45.82	0.0002
TRT	3	0.02397457	6.23	0.0284
Whole Plot Error	6	0.00384826		
DAY	7	0.01402080	6.31	0.0001
TRT*DAY	21	0.00181881	0.82	0.6833
DAY*REP	14	0.01250104	5.63	0.0001
Split Plot Error	42	0.00222197		

**Table 6. ANOVA for the change in the CIE b\* value of air packaged and MAP cottage cheese over storage time.**

Dependent Variable: CIE b\*

Source	DF	Mean Squares	F Value	Pr > F
Model	53	0.18145372	4.88	0.0001
Corrected Total	95			
REP	2	2.48856745	50.58	0.0002
TRT	3	0.14514340	2.95	0.1203
Whole Plot Error	6	0.04920252		
DAY	7	0.14092723	3.79	0.0028
TRT*DAY	21	0.03213348	0.86	0.6315
DAY*REP	14	0.16056953	4.32	0.0001
Split Plot Error	42	0.03715778		

**Table 7. Anova for the enzymatic quantification of lactic acid for air packaged and MAP cottage cheese over storage time.**

Dependent Variable: lactic acid

Source	DF	Mean Squares	F Value	Pr > F
Model	53	0.00015250	35.26	0.0001
Corrected Total	95			
REP	2	0.00020278	7.96	0.0205
TRT	3	0.00000627	0.25	0.8613
Whole Plot Error	6	0.00002547		
DAY	7	0.00098343	227.39	0.0001
TRT*DAY	21	0.00000462	1.07	0.4136
DAY*REP	14	0.00003744	8.66	0.0001
Split Plot Error	42	0.00000432		

**Table 8. Anova for pH measurements of air packaged and MAP cottage cheese over storage time.**

Dependent Variable: pH

Source	DF	Mean Squares	F Value	Pr > F
Model	53	0.01998415	48.99	0.0001
Corrected Total	95			
REP	2	0.30425729	141.52	0.0001
TRT	3	0.06883359	32.02	0.0004
Whole Plot Error	6	0.00008273		
DAY	7	0.00025413	12.69	0.0001
TRT*DAY	21	0.00007367	3.68	0.0002
DAY*REP	14	0.00005391	2.69	0.0067
Split Plot Error	42	0.00040794		

**Table 9. ANOVA for titratable acidity measurements of air packaged and MAP cottage cheese over storage time.**

Dependent Variable: titratable acidity

Source	DF	Mean Squares	F Value	Pr > F
Model	53	0.01998415	48.99	0.0001
Corrected Total	95			
REP	2	0.00052057	6.29	0.0336
TRT	3	0.00155304	18.77	0.0019
Whole Plot Error	6	0.00008273		
DAY	7	0.00025413	12.69	0.0002
TRT*DAY	21	0.00007367	3.68	0.0002
DAY*REP	14	0.00005391	2.69	0.0067
Split Plot Error	42	0.00040794		

**Table 10. ANOVA for the evaluation of overall product appeal for air and MAP cottage cheese over storage time.**

Dependent Variable: product appeal

Source	DF	Mean Squares	F Value	Pr > F
Model	57	1.05028944	9.71	0.0001
Corrected Total	97			
REP	2	0.08812599	1.05	0.3943
TRT	4	8.29036035	98.58	0.0001
Whole Plot Error	8	0.08409425		
DAY	7	1.53664308	14.21	0.0001
TRT*DAY	22	0.31903685	2.95	0.0014
DAY*REP	14	0.22473399	2.08	0.0356
Split Plot Error	40	0.10813601		

**Table 11. ANOVA for the evaluation of body and texture acceptability for air and MAP cottage cheese over storage time.**

Dependent Variable: body and texture acceptability

Source	DF	Mean Squares	F Value	Pr > F
Model	57	0.56809068	7.95	0.0001
Corrected Total	97			
REP	4	0.36755430	5.24	0.0227
Whole Plot Error	8	0.07019246		
DAY	7	0.05280626	4.47	0.0009
TRT*DAY	22	0.04024527	3.41	0.0004
DAY*REP	14	0.02225694	1.89	0.0587
Split Plot Error	40	0.01180456		



**Table 12. ANOVA for the evaluation of flavor acceptability  
for air and MAP cottage cheese over storage time.**

Dependent Variable: flavor acceptability

Source	DF	Mean Squares	F Value	Pr > F
Model	57	1.49259855	35.78	0.0001
Corrected Total				
REP	2	0.11851786	1.19	0.3521
TRT	4	13.59801106	136.83	0.0001
Whole Plot Error	8	0.09937649		
DAY	7	1.40344929	33.64	0.0001
TRT*DAY	22	0.43987668	10.54	0.0001
DAY*REP	14	0.09605357	2.30	0.0198
Split Plot Error	40	0.04172054		

**Table 14. ANOVA for the evaluation of the level of  
unidentified off-flavors in air and MAP cottage  
cheese over storage time.**

Dependent Variable: unidentified off-flavors

Source	DF	Mean Squares	F Value	Pr > F
Model	57	0.06913285	5.86	0.0001
Corrected Total	97			
REP	2	0.13344228	1.90	0.2111
TRT	4	0.36755430	5.24	0.0227
Whole Plot Error	8	0.07019246		
DAY	7	0.05280626	4.47	0.0009
TRT*DAY	22	0.04024527	3.41	0.0004
DAY*REP	14	0.02225694	1.89	0.0587
Split Plot Error	40	0.01180456		

**Table 14. Means (log counts) and standard errors for the enumeration of psychrotrophic bacteria in air packaged and MAP cottage cheese over storage time.**

DAY	100% CO <sub>2</sub>		75% CO <sub>2</sub> -25% N <sub>2</sub>		100% N <sub>2</sub>		AIR	
	mean		mean		mean		mean	
5	6.76 ± 0.007		6.77 ± 0.107		6.77 ± 0.004		6.83 ± 0.021	
8	6.72 ± 0.051		6.73 ± 0.047		6.78 ± 0.025		6.89 ± 0.054	
12	6.78 ± 0.022		6.77 ± 0.019		6.83 ± 0.023		7.13 ± 0.019	
15	6.79 ± 0.022		6.78 ± 0.078		6.85 ± 0.040		7.24 ± 0.006	
19	6.75 ± 0.007		6.76 ± 0.010		6.88 ± 0.037		7.28 ± 0.007	
23	6.74 ± 0.001		6.73 ± 0.008		6.82 ± 0.018		7.33 ± 0.017	
28	6.74 ± 0.005		6.73 ± 0.009		6.80 ± 0.010		7.38 ± 0.002	

Initial mean (log count) and standard error for fresh cottage cheese was 6.78 ± 0.013

N=3

**Table 15. Means (log counts) and standard errors for the enumeration of lactic acid bacteria in air packaged and MAP cottage cheese over storage time.**

DAY	100% CO <sub>2</sub>	75% CO <sub>2</sub> -25% N <sub>2</sub>	100% N <sub>2</sub>	AIR
	means	means	means	means
5	6.74 ± 0.005	6.75 ± 0.007	6.75 ± 0.002	6.79 ± 0.006
8	6.73 ± 0.023	6.73 ± 0.031	6.76 ± 0.008	6.84 ± 0.023
12	6.77 ± 0.012	6.76 ± 0.015	6.78 ± 0.012	6.98 ± 0.009
15	6.78 ± 0.010	6.77 ± 0.015	6.81 ± 0.030	7.05 ± 0.007
19	6.76 ± 0.004	6.76 ± 0.007	6.83 ± 0.022	7.07 ± 0.004
23	6.75 ± 0.006	6.75 ± 0.013	6.80 ± 0.010	7.11 ± 0.007
28	6.75 ± 0.000	6.73 ± 0.005	6.78 ± 0.002	7.12 ± 0.010

Initial mean (log count) and standard error for fresh cottage cheese

was 6.74 ± 0.010

N=3

**Table 16. Means and standard errors for the evaluation of overall product appeal of air packaged and MAP cottage cheese over storage time.**

DAY	MARKET <sup>a</sup> SAMPLE	100% CO <sub>2</sub> <sup>a</sup>	75% CO <sub>2</sub> -25% N <sub>2</sub> <sup>a</sup>	100% N <sub>2</sub> <sup>a</sup>	AIR <sup>b</sup>	
		mean	mean	mean	mean	
5		5.17 ± 0.133	4.60 ± 0.058	4.46 ± 0.260	4.43 ± 0.284	4.20 ± 0.404
8		4.67 ± 0.367	4.10 ± 0.153	4.03 ± 0.067	3.67 ± 0.033	3.13 ± 0.033
12		4.53 ± 0.133	4.30 ± 0.001	3.60 ± 0.200	3.30 ± 0.305	3.10 ± 0.351
15		4.96 ± 0.267	4.17 ± 0.067	3.36 ± 0.233	3.53 ± 0.089	2.13 ± 0.333
19		4.45 ± 0.267	4.43 ± 0.033	3.63 ± 0.088	3.03 ± 0.133	1.70
23		4.95 ± 0.150	4.03 ± 0.352	3.70 ± 0.288	2.93 ± 0.133	—
28		4.83 ± 0.133	4.10 ± 0.289	3.60 ± 0.208	2.87 ± 0.067	—

Initial mean and standard error for fresh cottage cheese was 4.96 ± 0.333

a N=3

b N=3 on days 5-15; N=1 on day 19

**Table 17. Means and standard errors for the evaluation of body and texture acceptability in air packaged and MAP cottage cheese over storage time.**

DAY	MARKET <sup>a</sup> SAMPLE	100% CO <sub>2</sub> <sup>a</sup>		75% CO <sub>2</sub> -25% N <sub>2</sub> <sup>a</sup>		100% N <sub>2</sub> <sup>a</sup>		AIR <sup>b</sup>	
		mean	mean	mean	mean	mean	mean	mean	mean
5		4.97 ± 0.033	4.70 ± 0.305	4.90 ± 0.100	4.60 ± 0.200	4.57 ± 0.033			
8		4.77 ± 0.318	4.50 ± 0.100	4.43 ± 0.240	4.26 ± 0.267	3.90 ± 0.351			
12		4.86 ± 0.067	4.67 ± 0.033	4.33 ± 0.067	4.30 ± 0.058	2.63 ± 0.067			
15		4.97 ± 0.167	4.70 ± 0.058	4.07 ± 0.067	4.13 ± 0.145	2.83 ± 0.285			
19		4.95 ± 0.050	4.33 ± 0.233	4.06 ± 0.033	3.80 ± 0.115	3.00			
23		4.85 ± 0.250	4.43 ± 0.088	4.10 ± 0.003	3.93 ± 0.120				
28		4.90 ± 0.100	4.26 ± 0.088	4.17 ± 0.011	3.83 ± 0.167				

Initial mean and standard error for fresh cottage cheese was 4.80 ± 0.033

a N=3

b N=3 on days 5-15; N=1 on day 19

**Table 18. Means and standard errors for the evaluation of flavor acceptability of air packaged and MAP cottage cheese over storage time.**

DAY	MARKET <sup>a</sup> SAMPLE	100% CO <sub>2</sub> <sup>a</sup>	75% CO <sub>2</sub> -25% N <sub>2</sub> <sup>a</sup>	100% N <sub>2</sub> <sup>a</sup>	AIR <sup>b</sup>
		mean	mean	mean	mean
5		5.20 ± 0.200	4.57 ± 0.186	4.07 ± 0.219	3.96 ± 0.283
8		4.63 ± 0.233	4.17 ± 0.120	3.47 ± 0.145	3.37 ± 0.186
12		4.83 ± 0.033	4.13 ± 0.089	2.90 ± 0.231	2.27 ± 0.133
15		5.37 ± 0.067	4.37 ± 0.067	2.57 ± 0.167	1.73 ± 0.033
19		4.55 ± 0.250	4.23 ± 0.120	2.67 ± 0.067	1.70
23		4.75 ± 0.350	4.23 ± 0.067	2.90 ± 0.058	—
28		5.23 ± 0.067	4.30 ± 0.010	2.83 ± 0.003	—

Initial mean and standard error for fresh cottage cheese was 5.13 ± 0.267

a N=3

b N=3 on days 5-15; N=1 on day 19

**Table 19. Means and standard errors for the evaluation of the level of unidentified off-flavors in air packaged and MAP cottage cheese.**

DAY	MARKET <sup>a</sup> SAMPLE	100% CO <sub>2</sub> <sup>a</sup>		75% CO <sub>2</sub> -25% N <sub>2</sub> <sup>a</sup>		100% N <sub>2</sub> <sup>a</sup>		AIR <sup>b</sup>	
		mean	mean	mean	mean	mean	mean	mean	mean
5		1.36 ± 0.033	1.30 ± 0.100	1.53 ± 0.145	1.33 ± 0.088	1.40 ± 0.153			
8		1.50 ± 0.200	1.56 ± 0.067	1.37 ± 0.120	1.80 ± 0.114	1.67 ± 0.219			
12		1.36 ± 0.033	1.40 ± 0.057	1.40 ± 0.058	1.56 ± 0.033	1.80 ± 0.057			
15		1.26 ± 0.133	1.43 ± 0.033	1.50 ± 0.000	1.60 ± 0.020	1.87 ± 0.067			
19		1.30 ± 0.100	1.40 ± 0.002	1.36 ± 0.033	1.70 ± 0.000	2.00			
23		1.20 ± 0.200	1.46 ± 0.088	1.40 ± 0.058	1.60 ± 0.057	—			
28		1.27 ± 0.133	1.53 ± 0.067	1.40 ± 0.058	1.63 ± 0.033	—			

Initial mean and standard error for fresh cottage cheese was 1.43 ± 0.033

a N=3

b N=3 on days 5-15; N=1 on day 19

## VITA

Amruta Maniar, the author of this thesis, was born September 23, 1967 in Bombay, India. She received her high school certificate in May, 1984 from The Cathedral and John Connon School in Bombay, India. She received her Bachelor of Science degree in Life Sciences in June, 1989 from St. Xavier's College in Bombay, India.

In the Fall of 1989, she began working towards a Master of Science degree in Food Science and Technology at Virginia Polytechnic Institute and State University.

Amruta is a member of Institute of Food Technologists, Carolina-Virginia Institute of Food Technologists, and the Indian Institute of Food Technology.

*Amruta Maniar*