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Headspace gas analysis and fungal count in parmesan cheese in various packages

Adina Pauline Naiman
San Jose State University

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**HEADSPACE GAS ANALYSIS AND FUNGAL COUNT
IN PARMESAN CHEESE IN VARIOUS PACKAGES**

A Thesis

Presented to

**The Faculty of the Department of Nutrition and Food Science
San Jose State University**

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Adina Pauline Naiman

August 1998

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APPROVED FOR THE DEPARTMENT
OF NUTRITION AND FOOD SCIENCE

Dr. Miriam Saltmarch

Dr. Parfilo Belo

Dr. Lucy McProud

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ABSTRACT

HEADSPACE GAS ANALYSIS AND FUNGAL COUNT IN PARMESAN CHEESE IN VARIOUS PACKAGES

by Adina Pauline Naiman

Headspace gas and fungi of grated Parmesan cheese in various packages were examined during shelf life. Headspace concentrations of both O₂ and CO₂ were compared to fungal culture counts and visible mold detection during the shelf life of various packages. All packages were hand flushed with 25% CO₂: 75% N₂. The package types sampled were polypropylene cups, polypropylene cups plus an enzymatic oxygen scavenger, and a glass jar control group. Each 450 ml plastic cup and 473 ml glass jar contained 5 oz. of cheese aged 1 month. Samples were stored at 19°C to approximate ambient temperature. Visible mold, the shelf life end point characteristic, was detected in all packages on storage day 10. Storage day 10 was also associated with increased mold plate counts, increased fungal plate counts, decreased headspace O₂ concentrations, and increased headspace CO₂ concentrations in all packages. The active packaging did not extend sample shelf life.

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PREFACE

This document contains a publication style thesis. The second chapter is written for submission to and according to the style guidelines for the *Journal of Food Protection*. Both the first and third chapters are written according to the style guidelines stated in the *Publication Manual of the American Psychological Association*, fourth edition 1994.

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CHAPTER 1 INTRODUCTION AND LITERATURE REVIEW

Introduction

Parmesan cheese is a flavorful and complete protein food (Whitney & Rolfes, 1993) that is produced and eaten in many countries, including Italy, the USA, and Argentina (Herbst, 1990). Parmesan cheese is classified as a very hard cheese which is made from partially defatted cow's milk (Herbst, 1990). *Parmigiano-Reggiano* refers to Parmesan style cheese produced in the Italian regions of *Bologna, Mantua, Modena, or Parma*. Parmesan cheese is the same type of cheese made outside of the *Parmigiano-Reggiano* areas (Herbst, 1990). Also, *Parmigiano-Reggiano* cheese is usually aged for at least two years while Parmesan cheese is usually aged for 14 months (Herbst, 1990).

The first recorded date of Parmesan cheese production was in 1579 (Smith, 1995). Basically, Parmesan cheese is made by coagulating pasteurized skimmed milk; cutting, heating, pressing, and salting the curd; adding inoculating microbial cultures to the curd; and finally aging the cheese (21 C.F.R. 133.165, 1997). Common cultures for inoculating Parmesan cheese include *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Early, 1992). According to the U.S. Code of Federal Regulations, Parmesan cheese must be at least 32% moisture, at least 32% milkfat, and aged at least 10 months (21 C.F.R. 133.165, 1997).

Parmesan cheese is approximately (by weight) 18% water, 41% protein, 4% carbohydrate, and 30% fat (Whitney & Rolfes, 1993). Yoder et al. (1995) stated that

Parmesan cheese exhibits a water activity level of approximately 0.90. Based on these conditions, possible deterioration reactions in stored Parmesan cheese include unwanted fungal growth, lipid oxidation, and Maillard browning (Fennema, 1996).

Convenient plastic packages containing grated Parmesan cheese have been available in retail quantities for over a decade (Hartman, 1996). However, since grated Parmesan cheese has increased surface area for reactions such as fungal growth and oxidation it deteriorates faster than blocks of Parmesan cheese (Sarantopoulos et al., 1995).

Two packaging modifications can be used, separately or in combination, to extend the shelf life and quality of grated Parmesan cheese: Modified Atmosphere Packaging (MAP) and active packaging (Labuza & Breene, 1989). MAP refers to changing the gas atmosphere in a package before sealing it (Labuza & Breene, 1989; Parry, 1993; Rooney, 1995). Active packaging refers to adding substances prior to final packaging that actively alter the package environment (Labuza & Breene, 1989; Parry, 1993; Rooney, 1995). Both packaging methods are used to enhance the quality of foods, such as by extending the shelf life of a product.

It is a challenge to determine the quality of packaged grated Parmesan cheese over time. The term “shelf life” is defined by Potter and Hotchkiss (1995) as the time a product remains acceptable or salable. Potter and Hotchkiss (1995) outlined many shelf life study methods based on the proposed decay mechanisms of each food product. For example, the primary limiting shelf life factor for cheese is mold growth according to Subramaniam (1993) and total fungal growth according to Yoder et al. (1995). Microbial shelf life

analysis of mold growth or of total fungal growth can be done by sample culture (White, 1993). Customers and researchers can also use visible mold growth as an end of shelf life indicator. Since fungal growth requires oxygen and carbon dioxide will be respired (McGee, 1984; Potter & Hotchkiss, 1995), the analysis of headspace gases oxygen and carbon dioxide may provide some similar information about the microbial status of packaged grated Parmesan cheese.

Objectives

The main objective of this study was to examine the relationship between headspace gas analysis and fungal count in Parmesan cheese in various package types. An additional objective of this study was to determine the efficacy of combining MAP and active packaging compared to MAP packaging alone for a grated Parmesan cheese product. Visible mold detection, mold counts, yeast counts, total fungal counts, and headspace gas analysis of both oxygen and carbon dioxide with a headspace gas analyzer were studied.

Significance of the Study

Shelf life determination of cheese and other foods provides valuable information to the producer and the consumer about the freshness and wholesomeness of a product. However, shelf life testing often requires considerable time, equipment, materials, and expense. Microbial analysis is a particularly costly albeit important shelf life determination method for the evaluation of dairy products.

The microbial shelf life analysis of dairy products is often determined by sample plate counts (White, 1993). Plate count methods require time for the incubation period,

costly materials and equipment, and they create biohazardous materials. A headspace analysis method that measures oxygen and carbon dioxide as an indication of microbial growth takes only minutes to determine, requires little materials and equipment, and does not create any biohazardous material.

There are no easily accessible published studies available in English about MAP grated Parmesan cheese in plastic polymer cups. Nor are there published data available about using both active packaging and MAP to extend shelf life for grated Parmesan cheese products. However, general information is available about using both MAP and active packaging to increase food product quality (Labuza & Breene, 1989; Parry, 1993; Rooney, 1995; Qualcepts Nutrients Inc, 1997; Sarantopoulos et al., 1995). A study of MAP and active packaging applications for grated Parmesan cheese contributes to the current body of published packaging information about foods.

Literature Review

Packaging

MAP Packaging

Currently MAP is used to extend the shelf life of grated Parmesan cheese in plastic packages (Sarantopoulos et al., 1995). Usually a blend of carbon dioxide and nitrogen is used to modify the atmosphere of a cheese package (Sarantopoulos et al., 1995). The carbon dioxide is used for its fungistatic and bacteriostatic effects (Labuza & Breene, 1989; Parry, 1993; Sarantopoulos et al., 1995; Rooney, 1995). Labuza and Breene (1989) stated that theoretically, a headspace gas change of increased carbon dioxide and decreased oxygen can lead to carbonic acid production on the surface of food which

provides an inhibitory effect. However, Parry (1993) stated that carbon dioxide does not inhibit certain types of yeasts. The nitrogen gas is used as a relatively impermeable inert filler (Parry, 1993; Sarantopoulos et al., 1995). Additionally, the oxygen displacement by nitrogen and carbon dioxide in the packages reduces deteriorative oxidation reactions (Parry, 1993; Sarantopoulos et al., 1995).

Advantages of applying MAP technology to foods include increased shelf life, decreased food waste, and decreased need for chemical preservatives (Parry, 1993). Disadvantages of MAP technology include increased production costs, increased needs for processing space, and the loss of protective MAP packaging once the package is opened (Parry, 1993). Yoder et al. (1995) used 25% carbon dioxide: 75% nitrogen MAP in a study of grated Parmesan cheese quality.

Active Packaging

Active packaging, the use of added active materials to packaging (Rooney, 1995), can be used with MAP to extend the shelf life of fresh refrigerated foods (Labuza & Breene, 1989), including grated Parmesan cheese (Qualcepts Nutrients Inc, 1997). One type of active packaging reduces the oxygen content of the package atmosphere by using enzymatic oxygen scavengers (Labuza & Breene, 1989; Parry, 1993; Rooney, 1995). The reduced oxygen content in the packages retards both mold growth and oxidation and thereby extends the shelf life of packaged grated Parmesan cheese (Labuza & Breene, 1989; Parry, 1993; Sarantopoulos et al., 1995; Rooney, 1995). OXYVACTM, an enzymatic oxygen scavenger developed by Qualcepts Nutrients, Inc. (1997) is one of various active packaging products used to improve the shelf life quality of packaged

grated cheese. Some advantages of active packaging include increased shelf life and decreased food waste. Some disadvantages of active packaging include the increased costs and the additional processing needed.

Polypropylene Packaging

Polypropylene is a commonly used plastic material for packaging MAP foods and can be heat sealed (Parry, 1993). In addition, polypropylene provides a good barrier against gas, water vapor, and grease (Parry, 1993). Grease resistance is an important characteristic for packaging grated Parmesan cheese due to its high fat content. However, polypropylene is gas permeable while glass and metal packaging are gas impermeable. Thus, a study of headspace gas using gas permeable polypropylene packaging can be compared to a gas impermeable control group of glass and metal packaging.

Shelf Life Prediction

Experimental Temperatures

Temperature is one of the most important shelf life prediction factors (Parry, 1993). According to White (1993), 7°C is the most common shelf life prediction temperature used for testing dairy products because 7°C is the approximate refrigeration temperature for cold storage in markets and homes. However, for accelerated shelf life testing of dairy products, higher temperatures can be used. Yoder et al. (1995) and Sarantopoulos et al. (1995) used ambient temperature (approximately 23°C) to study the shelf life of grated Parmesan cheese with MAP. Ideally, two temperatures can be studied over time and plots of quality attributes using the Arrhenius approach can be used for shelf life prediction at various temperatures (Taoukis & Labuza, 1996).

Microbial Shelf Life Determination Methods

There are many shelf life determination methods used to evaluate the quality of dairy products (White, 1993). Four of the many parameters that could be studied during the shelf life of grated Parmesan cheese packages are visible mold detection, fungal count, headspace oxygen content, and headspace carbon dioxide content.

Visible Mold Detection.

Product acceptability is an important shelf life quality. Both consumers and scientists can use visible mold detection as a shelf life quality parameter for grated Parmesan cheese. However, scientists can also obtain sample microbial culture quantitative data.

Fungal Count.

One common method of quality determination is microbial plate counting. The advantages of this method include the direct nature of the analysis, the quantitative results, and its general acceptance in industry. The disadvantages of this method include the expense, the days of waiting for results, the growth of microbial biohazards, and the many possible steps where errors might occur.

According to Subramaniam (1993), the primary factor limiting the shelf life of cheese is mold growth. Yoder et al. (1995) concluded that total fungal growth was the greatest quality concern for hard grated cheeses. Fungal count by microbial plating is a common dairy product shelf life determination method (White, 1993) that could be studied as a control to compare with other experimental methods of microbial quality

determination. Yoder et al. (1995) and Sarantopoulos et al. (1995) used fungal plate counting to study the shelf life of grated Parmesan cheese with MAP.

Today, microbial films can be used instead of handmade microbial culturing plates. Beuchat et al. (1990) concluded that the Petrifilm™ Yeast and Mold product (3M, Microbiology Products, St. Paul, Minnesota, USA) is an acceptable alternative to traditional microbial plates used for hard cheeses like Parmesan. However, the films, disposable pipettes, buffer solutions, and sterile conditions are expensive to purchase and maintain.

Despite the acceptance of plates and films for Parmesan cheese microbial analysis, Thompson and Marth (1986) found that the microflora of Parmesan cheese changes during ripening and that yeasts continue to grow at least during the first 14 months of Parmesan cheese ripening. If inoculating yeasts continue to grow in Parmesan cheese during and after ripening, then fungal plate counts are not an optimal quality determination method for yeast. However, sample culture mold counts are still useful quality indicators.

Headspace Gases: Oxygen and Carbon Dioxide.

Fungi require oxygen (McGee, 1984; Potter & Hotchkiss, 1995). Thus, during their growth, deteriorative fungi may consume enough oxygen and produce enough carbon dioxide during respiration to measurably change the headspace atmosphere in grated Parmesan cheese packages. Sarantopoulos et al. (1995) studied oxygen, nitrogen, and carbon dioxide concentration changes during the shelf life of MAP packaged grated Parmesan cheese.

Headspace analysis of carbon dioxide and oxygen can be determined using an analyzer designed to detect only those two gases (MOCONTM PAC CheckTM 650 Oxygen and Carbon Dioxide Analyzer, MOCONTM, Minneapolis, Minnesota, USA). Although the initial cost of a MOCONTM PAC CheckTM 650 Oxygen and Carbon Dioxide Analyzer is higher than purchasing microbial plate count materials, the subsequent expenses (the calibration gas and the septa) are much lower than those for sample microbial counts.

Published Shelf Life Determination Methods of MAP Cheese

There are few published studies about MAP packages of cheese (Alves et al., 1996; Sarantopoulos et al., 1995; Yoder et al., 1995). Details of two studies concerning grated Parmesan cheese in MAP are available (Yoder et al., 1995; Sarantopoulos et al., 1995). In these two studies the cheese shelf life determination was based on either the presence of visible mold (Yoder et al., 1995) or sensory evaluation of the cheese flavor (Sarantopoulos et al., 1995).

Yoder et al. (1995) reduced the water activity of Parmesan cheese samples to 0.88 and 0.84 from the control of 0.90. They then packaged the cheese samples in 25% carbon dioxide: 75% nitrogen gas MAP, stored the samples at 23°C for 12 weeks, and analyzed them for various quality attributes. Visible mold was detected in the Parmesan control samples by week eight. Yoder et al. (1995) concluded that fungal growth was the greatest quality concern for grated hard cheeses.

Sarantopoulos et al. (1995) studied samples of grated Parmesan cheese packaged in high carbon dioxide MAP polymer bags stored at approximately 25°C for 124 days. The shelf life of the product was determined to be 98 days using sensory evaluation. In the

headspace gas analysis of the samples, the carbon dioxide content slowly increased until approximately day 45 and then slowly decreased for the rest of the study. The oxygen gas content decreased steadily until approximately day 35 and was low for the rest of the study. In addition, the fungal count increased from 1.10×10^2 Colony Forming Units (CFU)/g cheese initially to 4.20×10^5 CFU/g cheese at the end of the study.

Alves et al. (1996) studied sliced Mozzarella cheese in various MAP conditions. The mozzarella cheese was similar to Parmesan cheese in water activity range (0.90 and above). In the study there were four different package atmospheres: 100% nitrogen gas, 100% carbon dioxide, 50% nitrogen gas: 50% carbon dioxide, and an air control group. Alves et al. (1996) found that mold and yeast development was successfully prevented in 100% carbon dioxide MAP samples and inhibited in the 50% nitrogen gas: 50% carbon dioxide MAP samples. Alves et al. (1996) calculated that the estimated shelf lives of the cheese samples were: 64 days for the 100% carbon dioxide MAP samples, 44 days for the 50% nitrogen gas: 50% carbon dioxide MAP samples, 16 days for 100% nitrogen gas MAP samples, and 13 days for the air packaged samples. Alves et al. (1996) stated that 100% carbon dioxide MAP samples exhibited the best sensory and microbial quality in their study. However, one limitation of this method is the possible off flavor that 100% carbon dioxide imparts to MAP packaged foods.

CHAPTER 2 JOURNAL ARTICLE

Authors' Title Page

Running head: HEADSPACE GAS ANALYSIS

HEADSPACE GAS ANALYSIS AND FUNGAL COUNT IN PARMESAN CHEESE IN VARIOUS PACKAGES

Adina Pauline Naiman, Miriam Saltmarch*, Panfilo S. Belo, and Lucy McProud

Department of Nutrition and Food Science

San Jose State University

1 Washington Square

San Jose, CA 95192-0058

Key words: grated Parmesan cheese, headspace analysis, Modified Atmosphere Packaging (MAP), active packaging, fungal count.

*** Address comments and questions to: Dr. Miriam Saltmarch, Department of Nutrition and Food Science, San Jose State University, 1 Washington Square, San Jose, CA 95192-0058, phone (408) 924-3118, Fax (408) 924-3114.**

Abstract

Grated Parmesan cheese in various package types was examined for a relationship between fungal count over time and headspace gas analysis. Both headspace oxygen content and headspace carbon dioxide content were compared to fungal culture counts and visible mold detection during the shelf life of various grated Parmesan cheese package types. All of the packages were hand flushed with 25% carbon dioxide: 75% nitrogen gas to create Modified Atmosphere Packaging (MAP). The package types sampled were polypropylene cups, polypropylene cups plus an enzymatic oxygen scavenger, and a control group of glass cups with metal lids. Each 450 ml plastic cup and 473 ml glass jar contained five oz. of grated Parmesan cheese previously aged one month. All packages were stored at 19°C to approximate ambient temperature during the study. Visible mold, the shelf life end point characteristic, was detected in all packages on day ten of storage. Day ten of storage was associated with an increased mold plate count, an increased total fungal plate count, a decreased headspace oxygen concentration, and an increased headspace carbon dioxide concentration in all of the packages. The enzymatic oxygen scavenger did not alter nor extend the shelf life of the cheese packages.

Introduction

Parmesan cheese is a very hard cow's milk cheese that is produced and eaten in many countries, including Italy, the USA, and Argentina (6). *Parmigiano-Reggiano* refers to Parmesan style cheese produced only in the Italian regions of *Bologna*, *Mantua*, *Modena*, or *Parma* (6). According to the US Code of Federal Regulations (3), Parmesan cheese must be at least 32% moisture, at least 32% milkfat, and aged at least 10 months. Possible deterioration reactions in stored Parmesan cheese include unwanted fungal growth, lipid oxidation, and Maillard browning (4).

Convenient plastic packages containing grated Parmesan cheese have been available in retail quantities for over a decade (5). However, since grated Parmesan cheese has increased surface area for reactions such as fungal growth, oxidation, and volatile flavor loss, it deteriorates faster than blocks of Parmesan cheese (13).

Two packaging modifications can be used, separately or in combination, to extend the shelf life quality of grated Parmesan cheese: Modified Atmosphere Packaging (MAP) and active packaging (7, 10, 12). MAP refers to changing the gas atmosphere in a package before sealing it (7, 10, 12). Active packaging refers to substances added prior to final packaging to actively alter the interior package atmosphere (7, 10, 12).

Currently MAP is used to extend the shelf life of grated Parmesan cheese in plastic packages (13). Usually a blend of fungistatic carbon dioxide gas and inert filler nitrogen gas is used to modify the atmosphere of a package (7, 10, 12, 13). Processing plants may hand flush the packages with these MAP gases, resulting in packages containing leftover air. Active packaging can be used with MAP to extend the shelf life of fresh refrigerated

foods (7) and lessen the effects of incomplete gas flushing. Enzymatic oxygen scavenger active packaging reduces the oxygen content of a package to inhibit both mold growth and oxidation (7, 10, 12).

Polypropylene is a commonly used plastic package for MAP foods and can be heat sealed (10). In addition, polypropylene provides good a barrier against gas, water vapor, and grease (10). However, polypropylene is gas permeable while glass and metal packaging is gas impermeable. Thus, a study of headspace gas using gas permeable polypropylene packaging can be compared to a gas impermeable control group of glass and metal packaging.

It is a challenge to determine the quality of grated Parmesan cheese in packages over time. Potter and Hotchkiss (11) outlined many shelf life prediction methods based on the proposed decay mechanisms of food products. For example, the primary limiting shelf life factor for cheese is generally mold growth according to Subramaniam (14) and total fungal growth for grated Parmesan cheese according to Yoder et al. (18). In either case, microbial shelf life analysis of cheese for yeast and mold growth can be done by sample culture (17). Beuchat et al. (2) concluded that the Petrifilm™ Yeast and Mold product (3M, Microbiology Products, St. Paul, Minnesota, USA) is an acceptable alternative to traditional microbial plates used for hard cheeses like Parmesan. Yoder et al. (18) and Sarantopoulos et al. (13) used fungal plate counting to study the shelf life of grated Parmesan cheese with MAP.

Despite the acceptance of plates and films for Parmesan cheese microbial analysis, Thompson and Marth (16) found that the microflora of Parmesan cheese changes during

ripening and that yeasts continue to grow at least during the first 14 months of Parmesan cheese ripening. If inoculating yeasts continue to grow in Parmesan cheese both during and after ripening, then fungal plate counts are not an optimal quality determination method for yeast. However, sample culture mold counts are still useful quality indicators.

The advantages of fungal plate counting include the direct nature of the analysis, the quantitative results, and its general acceptance in industry. Unfortunately, microbial count testing is expensive, time intensive, and creates biohazards. Fortunately, molds are aerobic and yeasts are either aerobic, microaerophiles, or facultative aerobes (10). Thus, during their growth, deteriorative fungi may consume enough oxygen and respire enough carbon dioxide to measurably change the headspace atmosphere in grated Parmesan cheese packages. Then fungal counts by microbial plating could be studied as a reference standard to compare with oxygen and carbon dioxide headspace gas analysis.

Sarantopoulos et al. (13) studied both fungal counts and headspace gas changes for oxygen, nitrogen, and carbon dioxide during the shelf life of MAP packaged grated Parmesan cheese.

Headspace carbon dioxide and oxygen analysis can be determined using an analyzer designed to detect only those two gases (MOCON™ PAC Check™ 650 Oxygen and Carbon Dioxide Analyzer, MOCON™, Minneapolis, Minnesota, USA). Although the initial cost of a MOCON™ PAC Check™ 650 Oxygen and Carbon Dioxide Analyzer is higher than purchasing microbial plate count materials, the subsequent expenses (the calibration gas and the septa) are much lower than those for microbial plate counts.

Temperature is one of the most important shelf life prediction factors (10).

According to White (17), 7°C is the most common shelf life prediction temperature used for testing dairy products because 7°C is the approximate refrigeration temperature for cold storage in markets and homes. However, for accelerated shelf life testing of dairy products, higher temperatures can be used. Yoder et al. (18) and Sarantopoulos et al. (13) used ambient temperature (approximately 23°C) to study the shelf life of grated Parmesan cheese with MAP. Ideally, two temperatures can be studied over time and plots of quality attributes using the Arrhenius approach can be used for shelf life prediction at various temperatures (15).

In two studies of MAP packaged grated Parmesan cheese, the shelf life determination of the cheese was based on either the presence of visible mold (18) or sensory evaluation of the cheese flavor (13). In a published abstract by Yoder et al. (18), the water activity of Parmesan cheese samples was reduced to 0.88 and 0.84 from the control of 0.90. Then the cheese samples were packaged with 25% carbon dioxide: 75% nitrogen gas MAP flushing, stored at 23°C for 12 weeks, and analyzed for various quality attributes. Yoder et al. (18) concluded that fungal growth was the primary shelf life limiting factor for grated hard cheeses.

Sarantopoulos et al. (13) studied samples of grated Parmesan cheese packaged in high carbon dioxide MAP polymer bags stored at approximately 25°C for 124 days. The shelf life of the packaged cheese was determined to be 98 days using sensory evaluation. Sarantopoulos et al. (13) concluded that flavor, color, and clumping were the primary shelf life limiting factors for the samples.

The main objective of this study was to examine the relationship between headspace gas analysis and fungal count in Parmesan cheese in various packages. An additional objective of this study was to determine the efficacy of combining MAP and active packaging of grated Parmesan cheese compared to MAP packaging alone. Visible mold detection, fungal counts, and headspace gas analysis of oxygen and carbon dioxide with a headspace gas analyzer were studied.

Materials and Methods

Materials

The Parmesan cheese, the plastic packaging materials, and all of the fungal count materials were obtained from Talamo Foods, Inc. (Gilroy, California, USA). The Parmesan cheese initial values were pH = 5.20, wet basis butterfat = 24.4%, and wet basis moisture = 32.1%, with one month of aging before the shredding and testing (The Antigo Cheese Company, Antigo, Wisconsin, USA). The specifications for the polypropylene 450 ml cup packages are listed in Table 1. MAP gases carbon dioxide and nitrogen were used. OXYVAC™ enzymatic oxygen scavenger was used for the active packaging (OXYVAC™, Qualcepts Nutrients, Inc., Minnesota, USA). The polypropylene cups were also heat sealed with a flexible polymer layer beneath the polypropylene lid. The fungal count materials were Petrifilm™ Yeast & Mold Count Plates (3M, Microbiology Products, St. Paul, Minnesota, USA), Butterfield's solution, and disposable pipettes. All microbial counts were performed according to the directions for use.

The additional materials used were glass packages and headspace analysis equipment. The 473 ml gas impermeable glass jars with dome metal lids were Ball™

Wide Mouth Tapered Pint canning jars (Allistra Corp., Muncie, Indiana, USA). Each metal lid was punctured twice with a small nail and sealed with closed cell foam tape (Scotchfoam Single Coated Vinyl Foam 1/8 inch black #4508, Scotch™ 3M, St. Paul, Minnesota, USA) to enable MAP flushing and headspace analysis. A MOCON™ PAC Check™ 650 O₂ and CO₂ Analyzer with MOCON™ Sticky Nickel™ septa (MOCON™, Minneapolis, Minnesota, USA) were used for the headspace gas analysis.

Experimental Design

All grating and packaging occurred at Talamo Foods, Inc. (Gilroy, California, USA) in a manner consistent with their normal processing and packaging methods whenever possible. Five oz. portions of grated Parmesan cheese were packaged in each container. All containers were hand flushed with a MAP gas combination of 25% CO₂: 75% N₂. The plastic cups were MAP flushed and packaged by hand in accordance with normal processing procedures for the plant. The glass jars were also packaged and MAP flushed by hand. All packages were stored at 19°C both to approximate ambient temperature and to accelerate the shelf life testing.

Three types of packages were studied: plastic (polypropylene) cups with MAP (=PM), plastic (polypropylene) cups with MAP and OXYVAC™ (=PMO), and glass jars with MAP (=GM). In the PMO samples, the OXYVAC™ was mixed with the grated cheese to account for 2% of the final product weight. The dependent variables studied were visible mold, mold count (CFU/g cheese), yeast count (CFU/g cheese), total fungal count (CFU/g cheese), headspace oxygen concentration (%v/v), and headspace carbon dioxide concentration (%v/v).

Analysis Method

Three packages of each type were sampled for headspace gas analysis first, visible mold second, and microbial counts third. Samples were analyzed three times each week for 24 days. Since only one temperature was studied, visible mold was chosen to indicate the end of shelf life.

For the headspace gas analysis, each package was sampled separately and the results were averaged for each package type each testing day. A MOCONTM PAC CheckTM 650 O₂ and CO₂ Analyzer with MOCONTM Sticky NickelTM septa (MOCONTM, Minneapolis, Minnesota, USA) was used to perform the headspace gas analysis according to the directions for use. Triplicate samples of each packaging type were tested in each session for headspace concentrations (% v/v) of both O₂ and CO₂. The analyzer was used in automatic mode to extract at least eight cc of headspace from each package for analysis (9). It was stated in the MOCONTM PAC CheckTM 650 manual (9) that the O₂ analysis accuracy is within 0.1% and the CO₂ analysis accuracy is within 2%.

For the visible mold analysis, each package was sampled separately. The cheese was examined both in the package and outside of the package for visible mold. The first day of visible mold for at least one of each package type was considered the last day of shelf life for that package type.

For the microbial count analysis, triplicate packages of each package type were blended and 9.0 g samples were taken from the blends to make the microbial films. Each 9.0 g sample was added to 90 ml Butterfield's solution and mixed. Additional tenfold serial dilutions of each solution were made. The four most dilute solutions were applied in

1.0 ml amounts to Petrifilm™ Yeast & Mold Count Plates (3M, Microbiology Products, St. Paul, Minnesota, USA) according to the instructions for proper use. The plates were incubated at ambient temperature, and the yeast and mold counts were determined on both day three and day five of incubation. The greatest numbers from each count were used for the results. At least four films were prepared for each package type each day. The results for each package type for each day were averaged and outliers were not discarded.

Results and Discussion

Visible Mold

Day ten was the first day of visible mold for all of the package types. The PM and PMO samples had a few small specks of mold visible in each package which were present in the same size and number from day ten onward. The GM samples had larger visible mold colonies. After day ten there was no visible mold change in the plastic samples while the glass sample mold colonies grew larger until by day 24 the glass cheese samples were covered in mold. The PM and PMO samples in the polypropylene cups did have some water vapor transmission which may have helped to eliminate some water from the packages to keep the visible mold content low. The GM samples were gas impermeable and the water vapor that was generated in the packages was available to nourish the larger mold colonies.

Fungal Count

The mold count data are represented in Figure 1. Once the cheese samples were packed, the ripening ceased and the samples began to deteriorate. On the first day of visible mold (day ten), all of the package mold counts had risen from 175 CFU/g cheese

originally to at least 1,337 CFU/g cheese. The mold CFU/g cheese counts peaked between day ten and day 13, and then steadily decreased to numbers similar to those detected originally by day 24. The PMO packages had very high mold count peaks that could be due to contamination of the oxygen scavenger prior to the experiment.

The fungal count results were not the same as the visible mold results. Despite the visible mold detection from day ten onward, the mold CFU/g cheese counts were very low by day 24. In addition, although the GM samples had much more visible mold, the CFU/g cheese counts for the GM samples were similar to the plastic sample counts, especially by day 24. The differing colony growth in each package type may be related to water availability in each package type. It is possible that the water vapor transmission in the plastic samples enabled the growth of many small mold colonies but inhibited large colony growth due to the amount of water remaining in the packages. In the glass packages, there was no water vapor transmission so the initial colonies that formed grew large using all of the remaining water while new colony growth was inhibited.

The mold count data were much lower than the yeast or the total fungal count data. The mold CFU/g cheese range on the first day of visible mold (1,337-15,584 CFU/g cheese) was significantly higher than the original mold count (175 CFU/g cheese). The PMO samples had the highest peak (day 13), followed by the GM samples (day ten), while the PM samples had the lowest peaks (days ten and 20). A count of over 1,000 mold CFU/g cheese could be used to indicate that the product had reached the end of its shelf life.

The yeast count data are represented in Figure 2. The maximum yeast CFU/g cheese counts were found on day six for the PMO samples, day ten for the GM samples, and day 17 for the PM samples. By day 24 all of the package systems were approaching the original low yeast count. The yeast count data are not necessarily meaningful because fluctuating numbers of yeasts have been identified in Parmesan cheese during ripening (16). In addition, the yeast CFU/g cheese numbers do not vary greatly except for a possible outlier on day six for the PMO samples. If the outlier is discarded, then no associations are apparent for the yeast data.

The total fungal CFU/g cheese count data are represented in Figure 3. The total fungal count data were most greatly influenced by the yeast counts that were at least an order of magnitude higher than the mold counts. Thus, no associations are visible.

Limitations of the fungal analysis include not being able to apply descriptive statistics to the sampling technique used and including CFU count estimates in the results. The optimal Petrifilm™ Yeast and Mold film counting range is 15-150 CFU/g cheese and values outside of this range are considered estimates (1). In addition, the OXYVAC™ may have been used improperly. Because the oxygen scavenger was supposed to be added directly to the cheese, even distribution may not have occurred which could have reduced the effectiveness of the OXYVAC™. Additionally, the high yeast and mold count peaks for the PMO packages may have been due to contamination of the OXYVAC™.

Headspace Oxygen and Carbon Dioxide Analysis

The data for headspace O₂ concentration are represented in Figure 4 and the data for CO₂ concentration are represented in Figure 5. There was a notable decrease in O₂ concentration and an increase in CO₂ concentration by day ten, the first day of visible mold, for all of the samples. The O₂ concentration dropped below 1% and the CO₂ data was above 30% for all of the packages by day ten even though the PM and PMO samples showed greater fluctuations than the GM samples for both gases.

The glass packages had very different original O₂ and CO₂ concentrations than the plastic packages. The plastic package concentrations were originally between 15-20% for both O₂ and CO₂; the glass package concentrations were originally less than 5% for O₂ and above 30% for CO₂. The differences may be due to the different types of manual gas flushing used for each the plastic vs. glass packages. It appears that more air was displaced when MAP flushing the glass packages than with the plastic, however, all of the package types reached the last day of shelf life on day ten.

Between days six and ten there was a change in the gas concentrations of the packages. The O₂ concentrations all fell beneath 2% and the CO₂ concentrations all rose above 30%. These results correspond to the first day of visible mold in all of the packages on day ten and an increase in the mold CFU/g cheese of at least sevenfold in all of the packages and may be related to respiration of the deteriorative fungi growing in the packages. Thus, headspace O₂ and CO₂ concentrations were associated with changes in mold growth for all grated Parmesan cheese packages used in this study.

The packaging type also influenced the mold growth in the samples. By day 24 the water vapor transmissible plastic packages were associated with little visible mold and similar numbers of mold colonies as the glass samples. Conversely, by day 24, the gas impermeable glass packages were associated with a lot of visible mold despite similar colony counts as the plastic packages. Thus, the plastic package samples were associated with less visible cheese deterioration due to mold growth than the glass samples.

An objective of this research was to study the relationships between headspace gas and fungal count in various package types. A positive association was found in all of the package types between initial visible mold detection, high mold CFU/g cheese, oxygen concentrations below 2% and carbon dioxide concentrations above 30%. A positive association was also found in all of the package types between visible mold detection, oxygen concentrations below 2%, and carbon dioxide concentrations above 30%. Plastic packaging was associated with less visible deterioration due to mold growth than glass packaging. Further studies can be used to confirm and elaborate on these relationships. Additional studies should include daily statistical sampling of the packages and respiration kinetics measurements.

It is not clear whether the yeasts or the molds are consuming more of the oxygen in the package headspace. Molds are always aerobic while certain yeasts are microaerophiles or facultative aerobes and require very little oxygen (10). However, since the yeast counts were in the six-figure range while the mold counts were often in the four- or five-figure range, the yeasts may have consumed more of the available oxygen. Alternately, because the mold colonies were large enough to be visible by day ten, the

molds may have consumed more oxygen and simply produced fewer colonies. It is also not clear which fungi produced the most carbon dioxide.

After the initial peaks, the mold and yeast counts decreased back to the original fungal counts while the O₂ concentration continued to slightly decrease and the CO₂ concentration continued to slightly increase. The fungi may have depleted their oxygen supply until their growth was first slowed and then inhibited by the low O₂ and high CO₂ environment. Labuza and Breene (7) stated that an additional concern for microbial shelf life quality of MAP foods is that after aerobic microbes consume the available oxygen then anaerobic microbes can grow in the oxygen reduced atmosphere. Anaerobic microbial growth was not measured on the fungal count plates studied and should be examined in future studies.

The PM samples were produced at the processing plant in the same way that this product was produced in the past. The PMO samples were produced at the processing plant in the same way that this product was produced at the time of this study with the oxygen scavenger added to increase the shelf life of the product. However, visible mold was detected in all of the package types on the same day. Based on this fact, it is apparent that the enzymatic oxygen scavenger did not extend the shelf life of these packages.

Table 1. Manufacturer's Specifications for Polypropylene Cups

<u>Characteristic</u>	<u>Value</u>
Oxygen Permeation Rate at 23°C	230 cc/ml/100 in. ² /24h/atm
Carbon Dioxide Permeation Rate at 23°C	610 cc/ml/100 in. ² /24h/atm
Water Permeation Rate at 38°C & 90% RH	0.5 g/ml/100 in. ² /24 h
From Berry Plastics, Inc., Evansville, Indiana, USA.

Figure 1. Mold growth (CFU/g cheese) vs. days of shelf life for grated Parmesan cheese packs stored at 19°C.

Figure 2. Yeast growth (CFU/g cheese) vs. days of shelf life for grated Parmesan cheese packs stored at 19°C.

Figure 3. Total fungal growth (CFU/g cheese) vs. days of shelf life for grated Parmesan cheese packs stored at 19°C.

Figure 4. Percent headspace O₂ concentration vs. days of shelf life for grated Parmesan cheese packs stored at 19°C.

Figure 5. Percent headspace CO₂ concentration vs. days of shelf life for grated Parmesan cheese packs stored at 19°C.

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CHAPTER 3 SUMMARY AND RECOMMENDATIONS

Summary

Grated Parmesan cheese was analyzed in various package types to determine headspace gas analysis relationships with microbial analysis. For the microbial quality determination, commercially processed packages of grated Parmesan cheese were analyzed for visible mold, mold count, yeast count, total fungal count, headspace oxygen concentration, and headspace carbon dioxide concentration. The Parmesan cheese packages were either plastic cups with MAP, plastic cups with MAP and an oxygen scavenger (OXYVAC™, Qualcepts Nutrients, Inc., Urbana, Ohio, USA) or gas impermeable glass jars with metal lids and MAP. The MAP gas mixture was 25% carbon dioxide: 75% nitrogen. A study was also performed to determine any notable changes in moisture content or water activity of the cheese samples over time, and no notable changes were found.

For the microbial analysis, Petrifilm™ Yeast and Mold films (3M, Microbiology Products, St. Paul, Minnesota, USA) were used for the fungal cultures and a MOCON™ PAC Check™ 650 Oxygen and Carbon Dioxide Analyzer (MOCON™, Minneapolis, Minnesota, USA) was used to determine the headspace oxygen and carbon dioxide concentrations in the samples.

The main objective of this study was to examine the relationship between headspace gas analysis and fungal count in Parmesan cheese in various package types. A relationship was found in these results between the headspace oxygen and carbon dioxide data and both the fungal count data and visual observations of mold growth. The first day

of visible mold for all of the packages (day ten of the study) was used to determine the end of the shelf life for the packages. Day ten visible mold results corresponded to a decline in headspace oxygen content (below 2%), a rise in headspace carbon dioxide content (above 30%), and a sharp rise in the mold CFU/g cheese count. Water vapor transmissible plastic packaging was also associated with less visible deteriorative mold growth than gas impermeable glass packaging.

If mold is considered the primary mode of deterioration for this system, and these results could be repeated with an acceptable degree of confidence, then this method could be useful. Headspace oxygen and carbon dioxide determination could be used as a new quality determination method for shelf life studies. The day that both the carbon dioxide values are high and the oxygen values sink below 2% could be used as an end of shelf life indicator. Alternatively, if a single sampling is performed on a package type, low oxygen and high carbon dioxide concentrations could be used as expiration indicators if the manufacturing date of the product is unknown.

An additional objective of this study was to determine the efficacy of combining MAP and active packaging compared to MAP packaging alone for grated Parmesan cheese packages. Both of the plastic package types containing MAP or MAP plus active packaging were actual products on the market. The oxygen scavenger was the active packaging added to the MAP packages by the producer to extend the shelf life of the cheese packages. Based on the results from this study, all of the package types were equal in shelf life duration. Thus, the active packaging did not extend the shelf life of MAP packaged grated Parmesan cheese in this study nor did it provide any notable advantages

over MAP packaging alone. The best indicators of shelf life changes appeared to be visible mold observation and combined headspace oxygen concentration with headspace carbon dioxide concentration. This study contributes to the ongoing research for efficient shelf life quality determination methods.

Recommendations

Although quality determination methods for foods are expensive, labor intensive, and time consuming, it is crucial to understand the shelf life of a food product. Food that has deteriorated in microbial quality can be unsafe, can cost producers money and credibility, and can cost consumers money and their health. The best quality determination methods used in food analysis are still only estimates and do not capture the real shelf life experience. However, useful predictions can be determined with shelf life testing.

Headspace analysis of food products is one of the many newer areas of food science. Headspace analysis methods enable food scientists and food technologists to examine a food product using a minute sample quantity, such as a few milliliters. These minute samples can be increasingly illuminating with advanced technology such as headspace analyzers for oxygen and carbon dioxide.

The methods outlined in this paper provide useful information about a relationship between shelf life microbial quality and headspace gas analysis for different package types. Future microbial analysis research areas include repeating the methods used here and headspace atmosphere kinetic modeling. Future research could also be performed on the products tested in this study to determine if the enzymatic oxygen scavenger is providing any quality benefit in the grated Parmesan cheese packages.

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IMAGE EVALUATION TEST TARGET (QA-3)

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