MODIFIED ATMOSPHERE PACKAGING OF HARD GRATED CHEESES

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(ABSTRACT)

The objective of this study was to use MAP technology to produce safe, shelf-stable, high quality, hard grated cheeses not requiring preservatives or refrigeration during distribution and sale.

Initially, a challenge study with Staphylococcus aureus (S. aureus) was conducted to determine the water activity (Aw) level of high-moisture cheeses necessary to prevent the growth of a food pathogen when packaged under a modified atmosphere (25% CO_2 and 75% N_2). Other microbial analysis included mold and yeast enumerations. Secondly, product quality and shelf-stability were determined biweekly by sensory, microbial, and instrumental analysis to evaluate product safety and changes in the natural aromas and flavors of hard grated cheeses. Instrument color analysis CIE L* a* b* values were determined to measure color changes.

Parmesan cheese with high Aw levels (Aw= 0.90 and 0.88) supported the growth and survival of S. aureus. The microorganism was incapable of surviving at Aw levels of 0.86 and below. S. aureus was not able to survive on Romano

cheese. Mold and yeast proliferated on higher Aw Parmesan cheeses. Visible mold was detected on the Parmesan sample of Aw= 0.90 after 8 weeks of storage. No mold growth was observed on Romano cheese. However, yeast were capable of growing on Romano cheese.

The sensory evaluation study of hard grated cheeses was unable to detect a difference between the fresh cheese sample and the cheeses packaged under MAP.

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I could write another thesis about all the people who have helped me get through my years (six !!) in college.

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I. INTRODUCTION

While the dollar value of hard grated cheeses increased slightly during 1990 to $276.3 million, the total volume of hard grated cheese sold decreased by 2.41%. This is in sharp contrast to natural shredded cheeses which increased by 21% in dollar value and 8.5% in volume sales during 1990. These refrigerated, shredded cheeses include hard cheeses such as Parmesan and Romano, demonstrating that consumers are anxious to have high-quality hard cheeses in a convenient form even at a premium price. Shredded hard cheeses in this form have not been dried, therefore they maintain their full aroma and flavor. Consumer response to these high quality, hard cheeses is clearly positive, but they must be specially packaged and refrigerated which restricts their convenience and use opportunities.

Development of high moisture, full aroma and flavor, hard grated cheeses in convenient, shelf-stable packages would produce new use opportunities for high quality, hard grated cheeses which are clearly favored by consumers to dried, hard grated cheeses. Shelf-stable, high quality, hard grated cheese could be useful in industrial, institutional and retail markets where refrigerated shredded cheeses are not practical.

Shelf-stable, hard grated cheeses in traditional paper, shaker-top cans must be dried before packaging and usually have potassium sorbate (an anti-microbial preservative) added to provide shelf-stability. Hard grated cheese of approximately 30-32% moisture are dried to approximately 18% moisture to enhance shelf-stability. This drying process is detrimental to the aroma, flavor, color and melting quality of the cheeses. Elimination of the drying operation would save the processor the costs of this expensive operation and decrease packaged product loss by approximately 14%.

The objective of this research was to develop safe, shelf-stable, high quality, hard grated cheeses that require minimum drying and do not require preservatives or refrigeration during distribution and sale. In phase I, Parmesan and Romano cheeses were dried to Aw levels of 0.90, 0.88, 0.86, 0.84, and 0.75, inoculated with Staphylococcus aureus and packaged with an atmosphere of 25% CO_2 and 75% N_2 to establish safety limits. In the second phase of the study, sensory and physical characteristics were evaluated for cheeses with Aw levels of 0.90, 0.88 and 0.84 packaged under MAP and stored for 8 to 12 weeks at 23°C to determine quality and shelf-stability.

II. LITERATURE REVIEW

1. Cheese

The conversion of milk into cheese is probably the most effective way of storing milk in a convenient form while at the same time producing a highly nutritious and palatable food. Cheese is of significant value in man's diet because it contributes the same rich source of the vitamins, minerals and proteins found in milk.

1.1 Italian Hard Cheese Industry Trend

In the past decade, the Italian cheese market has grown at a faster rate than the general cheese market. This trend has been attributed to the popularity of Italian ethnic cuisine such as pizza, lasagna, and spaghetti, which have created a demand for Mozzarella and Parmesan cheeses. In 1991, overall production of Italian cheeses increased by 5.5% over 1990's growth trend (National Cheese Industry, 1992).

In 1991, total production of Italian cheeses reached 1 billion kg (NCI, 1992). Of that 1 billion kg, 78% (800 million kg) was Mozzarella cheese. The production of Italian hard cheeses, Parmesan and Romano reached 46.62 million and 12.9 million kg, respectively. Based on the popularity of Italian dishes, the 1991 U. S. per capita consumption of total Italian cheeses was 4.25 kg. Again, Mozzarella cheese exceeded the Italian hard cheeses with 3.25 kg per capita consumption where as per capita consumption of Parmesan and Romano was 0.20 kg and 0.11 kg, respectively. The increasing popularity of Italian foods has caused the production of Italian cheeses to boost annual cheese industry sales.

1.2 History of Italian Cheese

Italian cheese has a history at least 2500 years old. In the era of the Roman Empire, cheese graced the banquet tables of the Caesar and served as rations to the conquering Roman armies (Fox and Guinee, 1987). Today, the escalating popularity of Italian dishes, especially pizza and spaghetti is creating an international demand for Mozzarella-type and Parmesan-type cheeses, respectively (Fox and Guinee, 1987).

Although Italy is not ranked among the leading dairy countries, it is the third largest cheese producing country (Fox and Guinee, 1987). A large portion of the milk produced in Italy is used for cheese making rather than direct consumption, which requires Italy to import milk from neighboring countries for its citizens. Unlike other cheese producing countries, Italy uses milks from four species, cow, sheep, goat and buffalo, for cheese manufacturing.

Of all the Italian cheese varieties, Gorgonzola and Parmesan are ranked among the most famous international cheese varieties (Fox and Guinee, 1987). Parmesan is also recognized as the principal grating cheese. Another group of Italian cheeses which are gaining international popularity are the pasta filata or stretch curd cheeses, i.e., Mozzarella, Provolone.

Many U.S. cheese manufacturers learned the art of cheese making from the large numbers of Italian emigrants, which later made the U.S. the second largest producer of Italian cheeses. Today, over 900 million kg of Italian cheese are annually produced in the U.S.

1.3 Manufacturing Protocol for Italian Hard Cheeses

Parmesan

Parmesan is a hard grating cheese. It is light cream to cream colored. Its characteristic flavor is slightly sweet and nutty (S&R Cheese Corporation, 1993). Parmesan is a versatile cheese that makes any recipe an Italian delight. Compared to other cheese varieties, Parmesan is naturally low in fat. It also makes a tasty topping for pizza, soups, and pasta (S&R Cheese Corporation, 1993).

Traditionally in Italy, Parmesan cheese is only made from the prized milk of the *zona tipica*, a local Italian milk supply that costs twice the price of standard milk (Abrahams, 1990). Italian cheese makers believe that a first class cheese calls for first class raw materials (Abrahams, 1990). In the United States and Canada, Parmesan cheese production is more mechanized than traditional Italian cheese-making practices. This allows the production of a greater amount of Parmesan cheese because the manufacturing protocol is not as selective as the Italian manufacturing practice.

Parmesan is a very hard cheese made from cow's milk. For cheese manufacturing purposes, the milk is standardized to 1.8 - 2.5% fat by either blending whole milk with skim milk or by removing fat with a mechanical separator (Kosikowski, 1982; Scott, 1986; Fox and Guinee, 1987). After standardization, the milk is given a heat treatment of 60-70°C for 16 sec. or pasteurized at 72°C for 16 sec. Then, the milk is cooled to a temperature of 32°C and transferred to a cheese vat.

After the milk has been transferred into a cheese vat, it is inoculated with active starter cultures (<u>Lactobacillus bulgaricus</u> and <u>Lactococcus thermophilus</u>). Approximately 0.75 - 1.0% of each starter culture is stirred into the milk (Kosikowski, 1982; Scott, 1986; Fox, 1987). Kosikowski (1982) also suggests the addition of 0.5% lactic acid starter along with the starter cultures. After allowing the cheese milk to ripen for 15-30 min., rennet is added and the milk is agitated. Scott (1986) suggests the addition of 18-25 ml rennet per 100 liters cheese milk, where as Kosikowski (1982) recommends the addition of 70 ml rennet plus 30 g animal lipase powder (Italase). The cheese milk is agitated 5 minutes longer and then held at 32.2°C until the curd is firm enough to cut, usually a 20-30 minute holding period. The cheese is continuously cut into 3 mm cubes with cheese knives. Using steam, the temperature of the vat is slowly raised to 51-54°C to gradually cook the cheese curd. The curd is held at this temperature and slowly stirred to allow the whey to separate from the curd. Stirring continues until the whey titratable acidity raises to 0.14-0.19 % (Kosikowski, 1982; Scott, 1986; Fox and Guinee, 1987). When a whey titratable acidity of 0.14-0.19% is reached, the vat drainage valve is opened to remove the whey. The cheese curd is continuously stirred to remove any whey remaining trapped in the curd. The curd is removed from the vat and placed in lined circular steel hoops to form a 10-12.7 kg cheese wheel. A pressure of 12 kN/m^2 is applied to cheese for 30-60 minutes at room temperature (21-24°C). Then, the cheese is turn and re-pressed under the same conditions (Kosikowski, 1982; Scott, 1986; Fox and Guinee, 1987).

Before salting, the cheese is dried for 2-3 days. The dried, pressed cheese is placed in a 24% NaCl brine solution for 14-15 days at a temperature of 7-10°C (Kosikowski, 1982; Scott, 1986; Fox and Guinee, 1987). Routinely, the cheese is turned and the surface of the cheese is sprinkled with dry salt.

After brine soaking, the cheese is ripened for 10-24 months. Ripening occurs at a temperature of 15.6°C and a relative humidity of 75%. The cheese is turned daily and rubbed with vegetable oil. Within this period, the Parmesan cheese develops its characteristic aroma, flavor and texture (Kosikowski, 1982; Scott, 1986; Fox and Guinee, 1987).

Romano

Romano is a flavorful grating cheese with a more piquant and zesty flavor than Parmesan cheese (S&R Cheese Corporation, 1993). At least five months aging produces the sharp, piquant flavor of Romano cheese. Romano is light yellow to white in color. In many recipes, Romano is used as a topping for soups and casseroles.

Traditionally, the variety of Romano cheese depends on the type of milk used for manufacturing. Pecorino, caprino and vacchino are Romano varieties manufactured from the milk of a sheep, goat and cow, respectively (Kosikowski, 1982; Scott, 1986; Fox and Guinee, 1987).

The manufacturing protocol for Romano cheese is quite similar to that of Parmesan cheese. Initially, the milk is standardized to 2.0-2.2% fat (Fox and Guinee, 1987; Kosikowski, 1982). Then, the milk is pasteurized at 72°C for 15-20 sec. and cooled to 38-40°C before it is delivered to the cheese vat. Approximately, 1.0-1.5% of a mixed starter culture containing Lactobacillus bulgaricus and Lactococcus thermophilus is stirred into the milk to enhance coagulation (Kosikowski, 1982). The starter culture blend is usually propagated in milk or whey (Kosikowski, 1982). After the addition of starter cultures, rennet paste is added to the milk. Some manufacturers suggest 22.68 g per 453.6 kg milk while others recommend 28.4-56.7 g animal gastric lipase powder per 453.6 kg milk (Kosikowski, 1982). The milk is agitated for 5 min., the vat is covered and held at a warm temperature to allow formation of the curd.

The formation of a smooth, soft curd initiates the cutting operation. The curd is cut with a .95 cm wire knife to the size of a hazelnut. Before the second cutting, time is permitted for the whey to drain. After the second cutting, the curd is the size of a wheat grain. With slight agitation, the whey continues to drain from the curd (Kosikowski, 1982; Scott, 1986; Fox and Guinee, 1987).

The vat temperature is increased to 46.7°C and the curd is cooked for 45 min. with slow agitation. This step continues until the curds are firm enough to withstand deformation by squeezing (Kosikowski, 1982). The curds remain in the whey until the titratable acidity reaches 0.22-0.25%. At the desired whey titratable acidity, the whey is drained from the curd and the curd is packed into a cloth-lined, round, metal hoops (Kosikowski, 1982; Scott, 1986; Fox and Guinee, 1987). The cheese wheels are pressed to drain excess whey deposits.

The cheese is soaked in a 24% brine solution for 2-5 days then the cheese is stored at a temperature of 10°C for 30-60 days and hand salted (Kosikowski, 1982; Fox and Guinee, 1987 and Scott, 1986). For ripening purposes, the cheese wheels are stored at 10-12.7°C and 85% relative humidity for 5-12 months with frequent turning. During a 5 month storage period, the Romano cheese develops its characteristic aroma, flavor and texture.

1.4 Spoilage of Italian Hard Cheeses

At refrigerated temperatures, Italian hard cheeses have an extended shelf-life of 1-2 years. The low moisture, low pH, low storage temperature, and long, slow ripening of Italian hard cheeses restricts the possibility of microbial growth. However, these conditions do not control the growth of molds and yeasts. Surface discoloration is an indication of mold growth, usually blacks spots of Aspergillus niger on the cheese. Extended storage in a moist, refrigerated area results in rind rot. Rind rot is caused by the accumulation of moisture on the cheese surface providing the ideal conditions for growth of proteolytic bacteria, molds and yeasts.

The greatest source of bacterial contamination in Italian hard cheeses is the result of post-processing contamination. This is caused by improper dairy sanitation, which implies unclean equipment and/or personnel hygiene. Contamination from dairy personnel could cause a Staphylococcus aureus food poisoning outbreak. This organism is naturally present on the human body, i.e., hair, nose, skin, etc. Although there are no reported cases of Staphylococcus aureus outbreaks in Parmesan and Romano cheeses the possibility always exists so precautions must be taken to protect the consumer.

2. Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) is a multi-disciplinary technique that uses the basic principle sciences such as chemistry, microbiology, physics, food science, engineering, and polymer chemistry to maintain product freshness and extend product shelf-life (Lioutas, 1988). Within the past 10 years, MAP technology has gained tremendous popularity in the U. S. whereas the European community has utilized MAP technology for the past 25 years with a considerable amount of success. In the U. S., the increasing acceptance of MAP technology enabled food manufacturers to meet consumer demands for fresh, refrigerated foods with an extended shelf-life (Farber, 1991).

2.1 Definition

"Modified atmosphere packaging (MAP) is the enclosure of food products in high gas-barrier materials, in which the gaseous environment has been changed once to slow respiration rates, reduce microbiological growth, and retard enzymatic spoilage - with the final effect of a lengthened shelf-life" (Koski, 1988).

2.2 History

The benefits obtained from the use of modified atmospheres in handling perishable commodities is not a new phenomenon. During the 1930s, a major portion of beef and lamb carcasses shipped from Australia and New Zealand to Great Britain was stored under carbon dioxide (CO_2) (Genigeorgis, 1985). In the same decade, researchers at Cornell University were prolonging the quality of fresh apples with elevated levels of carbon dioxide and reduced levels of oxygen during storage. Around 1970, the meat industry regained an interest in controlled/modified atmosphere/vacuum packaging as an application to extend the shelf-life of fresh meats for retail. In 1981, a turning point in MAP technology emerged when Marks and Spencer, an English grocery chain, introduced a wide variety of fresh meat products package under modified atmospheres (Farber, 1991). Thus, Marks and Spencer introduced the numerous opportunities of MAP technology to the food industry. Increased consumer demand for fresh, chilled products with an extended shelf-life initiated the utilization of MAP technology. Today, MAP is being applied to several different food commodities such as fruits and vegetables, meats, poultry, seafood, dairy products, and more.

2.3 Gases Utilized for MAP

The primary gases utilized in MAP are nitrogen (N_2), oxygen (O_2), and carbon dioxide (CO_2). There is no correct gas mixture for MAP packaging. The MAP environment can be a single gas or a combination of the different gases. The MAP gas composition is usually tailored to the product, the package, and the storage conditions.

2.3.1 Nitrogen

Nitrogen is predominately an inert, filler gas which exhibits little or no antimicrobial activity on its own. By displacing the oxygen within the package, nitrogen has the ability to inhibit microorganisms that require oxygen to proliferate and to minimize oxidation and/or rancidity in MAP foods.

Nitrogen has a low solubility in water. In a MAP atmosphere containing CO_2 and N_2, nitrogen prevents the package from collapsing around the food as CO_2 begins to dissolve into the product. Several investigators suggest a nitrogen concentration of 30-100% for hard cheeses (Farber, 1991; Selman, 1987).

2.3.2 Oxygen

Without the presence of oxygen, MAP meats lose the bright red color that most consumers associate with fresh red meat. The major function of oxygen in MAP meat is to maintain myoglobin in its oxygenated form, oxymyoglobin, which delays the irreversible conversion of myoglobin to metmyoglobin (Genigeorgis, 1985). This leads to an extended shelf-life.

In most MAP products, oxygen is displaced by gas-flushing with CO_2 and/or N_2. An environment containing oxygen stimulates the growth of aerobic and spoilage organisms and can inhibit the growth of anaerobes. Yet, facultative anaerobes (i.e., *Staphylococcus aureus*) tend to proliferate in the presence of oxygen. Therefore, oxygen is usually eliminated from the gas mixture.

2.3.3 Carbon Dioxide

Carbon dioxide is used for its ability to inhibit the growth of common food spoilage microorganisms, molds and yeast, separate from the exclusion of oxygen. Despite numerous reports on the use of CO_2 for MAP application, the mechanism for inhibition remains unknown with little chance of a unitary explanation (Dixon and Kell, 1989). Several possible explanations have been proposed which include:

a) A decrease in the pH of the food product due to adsorption of CO_2 into the product surface. Solubility of CO_2

into product fluids has been thought to cause a drop in pH, which would inhibit microbial growth (Valley and Rettger, 1927; King and Nagel, 1967). The growth inhibitory effects become more pronounced with decreasing storage temperatures (Gill et al., 1990; Brody, 1989; Baker et al., 1986; Gill and Tan, 1979). This is due to the high solubility of CO_2 into fluids at low storage temperatures. However, pH changes reported seem to have no effect on microbial growth in MAP products. Huffman et al. (1975) reported a 0.1 pH unit change over a 27 day storage period. It follows that growth depression in CO_2 stored samples is not the result of lowering pH.

b) The inhibitory effect of CO_2 on bacteria has been attributed to the changes in the function of the biological membrane. Carbonic acid is formed as a result of CO_2 dissolution into the food surface. The concentration of bicarbonate influences the molecular arrangement at the lipid and water interface. High concentrations of bicarbonate cause a decrease in interfacial tension and an increase in hydration. Thus, hydrogen ions produced by dissociation of carbonic acid increase fluidity of membrane fatty acids and cause carbamination of bacterial cell membrane proteins. Sears and Eisenberg (1961) reported that CO_2 and bicarbonate may alter contact between the cell and its external aqueous environment through influence on the structure of the membrane.

c) A third possible mechanism for CO_2 inhibition suggests that a specific enzyme system is blocked by CO_2, which would retard microbial growth. King and Nagel (1975) reported an inhibition of isocitrated dehydrogenase and malate dehydrogenase activity in Pseudomonas aeruginosa caused by CO_2. They proposed that large amounts of CO_2 in the reaction mixture could shift the equilibrium of decarboxylating enzymes, which would cause the enzymatic steps involved in decarboxylation to become rate limiting. The effect of the block would be to slow growth. In their study of the influence of CO_2 upon metabolism of Psedomonas aerginosa, King and Nagel (1975) concluded that 50% CO_2 had a mass action effect upon certain enzymatic decarboxylation rates. Gill and Tan (1980) acknowledge that specific enzymes may be involved in CO_2 inhibition of microbial growth. They observed that respiration as well as growth was inhibited, which suggests that enzymes of oxidative metabolism may be involved in CO_2 inhibition. However, Gill and Tan (1980) found the possibility of inhibition resulting from a mass action effect by CO_2 on decarboxylation enzymes unlikely. In most cases maximum inhibition is not total and occurs at comparatively low CO_2 concentrations. However, the postulated mechanism should result in a decrease in growth rate directly proportional to CO_2 concentration and complete inhibition by CO_2 should be possible. In a study involving Pseudomonas fluorescens, Tan and Gill(1981) observed three decarboxylating enzymes that had different responses to CO_2. They found that pyruvate dehydrogenase was unaffected by CO_2; isocitrate dehydrogenase decreased linearly with increasing CO_2 concentration; and malate dehydrogenase was severely inhibited at low concentration of CO_2. Again, Tan and Gill (1981) found the mechanism proposed by King and Nagel (1975) was untenable, concluding P. flourescens inhibition resulted from non-specific effects on substrate uptake.

No matter which mechanism(s) is involved in CO_2 inhibition of microorganisms, the result is seen as an extension of the lag phase of bacterial growth and an increase in the generation time (Dixon and Kell, 1989).

2.4 The Effect of CO_2 on Microorganisms

Numerous reports have focused attention on the effect of CO_2 on the common spoilage bacteria found on fresh meat, poultry, and seafood. Although results confirm that CO_2 is effective in retarding microbial growth, the optimum level of CO_2 remains uncertain.

Use of high concentrations of CO_2 is inhibitory to many microorganisms, yet the antimicrobial activity/selectivity of CO_2 is not well understood. Gram-negative bacteria are very sensitive while gram-positive lactic acid bacteria are very resistant (Enfors and Molin, 1980) to CO_2 concentration levels.

In MAP packaged meats, the elevated level of CO_2 retards Gram-negative spoilage microflora (Pseudomonas spp.) and allows the less detrimental Gram-positive microflora (Lactobacillus spp.) to dominate. Psychotrophic Gram-negative bacteria are responsible for producing off-flavors and off-odors in raw meat. CO_2 concentration affects microbial development in two ways: 1) the concentration of CO_2 applied determines the dominate microflora and 2) CO_2 concentration has a direct affect on growth rate of organisms.

Gill and Tan (1980) reported respiration and growth inhibition of Pseudomonas, Acinetobacter, and Y. enterocolitica by CO_2. Maximum inhibition was reached at low concentrations of CO_2. Gill and Tan (1980) suggested that inhibition of respiration and growth was due to the effect of CO_2 on enzymes involved in oxidative metabolism.

2.5 Treatment of Dairy Products with CO_2

Over the years, the shelf-life and quality of food products have been extended by treatment with CO_2. Since the early 1900's, researchers have been investigating the benefits of CO_2 as a preservative in dairy products (Dixon and Kell, 1989). Van Slyke and Bosworth (1907) observed that increased pressures of CO_2 delayed lactic fermentation, but at atmospheric pressure there was no noticeable effect of CO_2 (Dixon and Kell, 1989). Experiments with butter indicated that carbonation had no antibacterial effect. Furthermore, the CO_2 treatment was unable to improve the keeping quality or prevent deterioration of flavor in butter. Valley and Rettger (1927) demonstrated that CO_2 at atmospheric pressure had no bacteriostatic or bacteriocidal effect on the natural microflora of ice cream or on Lactococcus lactis, Escherichia coli, and Bacillus cereus. Valley and Rettgers (1927) also observed the diffusion of CO_2 from carbonated ice cream with prolonged storage. CO_2 exerted a selective action, but did not enhance the keeping quality of milk or prevent bacterial proliferation (Valley and Rettger, 1927). Law and Mabbit (1983) showed that under a headspace of 1 atm, CO_2 could extend the shelf-life by 3 days at 4°C for poor quality and longer for quality milk. The CO_2 can be easily removed before pasteurization though it is not necessary if the milk is being converted to cheese or yogurt.

Research studies conducted by the dairy industry support evidence that packaging dairy products under CO_2 benefits shelf-life and quality. Yet, processors of dairy products have been reluctant to package under modified atmospheres of elevated CO_2 on a commercial scale. This reluctance could possibly be due to the potential ability of CO_2 to cause discoloration (Dixon and Kell, 1989) and increase acidity as a result of carbonic acid (Honer, 1988). MAP has slowly gained acceptance among cultured dairy products. Maniar (1991) showed that a modified atmosphere containing CO_2 levels above 75% were able to maintain the quality of cottage cheese more effectively than nitrogen. Maniar also observed that CO_2 levels above 75% did not cause visual discoloration nor impart an acid off-flavor over the expected shelf-life. Before controlled/modified/vacuum packaging, dairy processors in the United States used the "Vitagen" process to transfer creamed cottage cheese under vacuum from mixer to filler. In Germany, cottage cheese is commercially packaged under CO_2, extending the shelf-life by 7 days (Honer, 1988).

2.6 Advantages and Disadvantages of MAP

There are many potential advantages to MAP packaging. The most obvious advantage is extending the shelf-life of a food product without affecting the original freshness or quality of the product. Hotchkiss (1988) reported that MAP can extend the shelf-life of many perishable foods by 50-400%. MAP has other associated advantages, namely:

1 An active inhibitor of bacteria, molds, fungus, and post-harvest respiration

2 Distribution over longer distances

3 Reduced economic losses

(Farber, 1991; Wolfe, 1980).

Despite the advantages, MAP has several limitations:

1 Special equipment and training required

2 Different gas formulations dependent on product

3 Requires temperature regulation

4 Initial cost implementing MAP operation

5 Safety of foods packaged under modified atmospheres

(Farber, 1991; and Wolfe, 1980).

The safety of MAP food is a serious concern to the food industry. The reluctance to implement MAP technology as a widespread commercial application stems from conflicting views on the potential health hazards that may or may not arise. MAP uses elevated levels of CO_2 to retard the growth of aerobic spoilage microorganisms. The undesirable organoleptic characteristics that render food inedible serves as a warning to consumers. The health threat of concern is the potential ability of anaerobic or facultative anaerobic foodborne pathogens to grow and product toxins within the packaged food without the warning of spoilage (Farber, 1991; Hotchkiss, 1988; Hintlian and Hotchkiss, 1986; Genigeorgis, 1985; Wolfe and Silliker, 1980). Therefore, the suppression of aerobic spoilage microorganisms creates an environment, which could nourish the growth of anaerobic pathogens and the subsequent production of toxins.

Until recently, Clostridia botulinum, specifically the non-proteolytic type, were considered a safety threat to MAP applications. The non-proteolytic types of C. botulinum are B, E, and F. These non-proteolytic types can grow at temperatures as low as 3.3°C without producing offensives odors associated with proteolysis to warn the consumer of potential growth and toxin formation by pathogenic microorganisms (Hintlian and Hotchkiss, 1986). The recent emergence of psychrotrophic pathogens such as Listeria monocytogenes, Aeromonas hydrophilia and Yersinia enterocolitica, poses another possible health threat (Farber, 1991; Genigeorgis, 1985; Hintlian and Hotchkiss, 1986). These pathogens are capable of growing at low storage temperatures. Therefore, MAP products with an extended refrigerated shelf-life may pose an additional health hazard. Besides psychrotrophic pathogenic bacteria, Salmonella species, Staphylococcus aureus, Bacillus cereus, enterotoxigenic Escherichia coli, and Camplyobacter jejuni also pose a threat to food safety (Farber, 1991; Hintlian and Hotchkiss, 1986).

Use of high quality products, good hygiene from slaughter on or harvest on, selection of correct packaging material, an appropriate gas formulation for the product and maintenance of temperatures below 3°C creates a MAP environment without potential health hazards. MAP is not a panacea. It was not designed to improve the quality of the product, it simply functions to delay the rate of spoilage (Farber, 1991; Hotchkiss, 1988; Hintlian and Hotchkiss, 1986; Genigeorgis, 1985).

III. MATERIALS AND METHODS

Two Italian hard grated cheese varieties, Parmesan and Romano, were obtained from Sargento Food Service Corporation (Plymouth, WI) as cheese wheels (11 kg) and stored in a 3.3°C cooler until further processing. Aw levels, Aw= .90, .88, .86, .84 and .75, were tested for potentially supporting Staphylococcus aureus (S. aureus) growth on hard grated cheese.

Phase I. Challenge Study with Staphylococcus aureus

1. Chemical Analysis

Chemical analyses were conducted to determine the characteristics of the natural hard grated cheeses and possible changes during the course of the 3 month storage period. All analyses were performed in duplicate. Aw was determined using a water activity meter (Decagon CX-1, Pullman, WA). Salt concentration for hard grated cheeses was determined by the Quantab Chlorine Titrator method (AOAC, 1990). Using the filtrate from the salt concentration assay, the pH of the cheeses was determined using a pH meter (Accumet model 10, Fisher Scientific, Philadelphia, PA). The fat content of hard grated cheeses was determined by the Babcock Method (Marshall, 1993). The moisture content of a 1-2 gram sample of hard grated cheese was determined by a moisture analyzer (Denver Instruments Co. IR-100, Arvada, CO).

2. Preparation of Cheese

Parmesan and Romano cheese wheels (11 kg) were tempered to room temperature (23°C) prior to cutting into small wedges. A food processor (Cuisinart DLC-X, Greenwich, CT) with a fine shredding blade was used to shred cheese wedges. Approximately 2.5 kg portions of shredded cheese were vacuum packaged and stored at 3.3°C until further processing.

A modified rotary dryer was designed to dry cheese samples to their respective water activity levels. A high density polyethylene (HDPE) plastic container (Nalgene, Co., Rochester, NY) was used as the drying chamber. Inlet and outlet openings were cut, allowing heated air to flow through the chamber. The outlet was covered with mesh screen to prevent product loss. Resistance electric coils served as the heated air source. Thermocouples (Omega Engineering Inc., Stamford, CT) were placed at the inlet and outlet openings to monitor temperature. The drying chamber was rotated at a constant rate, 4 rpm, on a rotary tumbler (Globus, Austria).

Each 2.5 kg cheese sample was divided into two equal portions. All cheese samples were tumbled at a constant rate (4 rpm) and temperature (40.5 to 46.1°C) until the desired Aw level (Aw= .88, .86, .84 and .75) was achieved.

3. Inoculation of Cheese

Prior to beginning the study with cheese, the growth characteristics of three different strains of S. aureus (FDA strain #485, USDA strain B and a wild strain) were examined in BHI broth with adjusted salt content. S. aureus were grown in BHI broth with salt concentrations of 16, 18, 22 and 24% NaCl, that corresponding to Aw levels of 0.86, 0.84, 0.83 and 0.82, respectively. The FDA strain of S. aureus was eliminated from the study because no growth was observed at salt concentrations above 16%. The other two strains were able to grow in a medium containing 16-18% salt (Aw equivalent to 0.84).

Two cultures of S. aureus (USDA strain B and a wild strain) were grown in Brain-Heart Infusion broth (Fisher Scientific, Philadelphia, PA) for 24 hours. An optical density measurement (wavelength = 650nm) was obtained using a spectrometer (Spectronic 70, Baush and Lomb, Rochester, NY). The wild strain had an optical density of 1.1 and the USDA strain had an optical density of 0.610. Before mixing the two strains, the wild strain was diluted to the optical density of the USDA strain. The two S. aureus strains were mixed in equal portions and set aside for inoculation into the cheese samples.

Each cheese sample (approximately 2.0 kg after drying) of desired Aw level was placed into a LDPE (Low Density

Polyethylene) plastic bag, inoculated with 0.1 ml S. aureus mixture (10^6 cfu/ml), and vigorously shaken for an even distribution of the organism.

4. Packaging of Inoculated Cheese

Inoculated, shredded cheese was weighed out into 100 g portions and placed in a cheese bag (72 gauge Saran[tm] coated nylon 1.75 mil linear low density polyethylene) provided by Cryovac (W.R. Grace & Co., Cryovac Division, Duncan, SC) and/or Duralam (Appleton, WI). Fifteen packages per treatment per cheese were evacuated and than back flushed with the designated gas mixture (25% CO_2 and 75% N_2) (Airco Industrial Gases, Murray Hill, NJ). A multivac sealer (Model X200, Koch Suppliers Inc., Kansas City MO) was used to seal packages. The sealer was set at 80% vacuum and 50% gassing during packaging to give a pillow pac appearance. Packages were inspected for leaks by submerging packages under water and observing air bubbles and repackaged if necessary. Packages were stored in an incubator at 30°C for a period of 12 weeks.

Gas composition was regulated during packaging by sampling the gas mixture within the blender and by sampling the first and last package in each treatment packed under the atmosphere, as described below.

5. Analysis of Headspace Gas Composition

Each package sampled on test days was analyzed to verify headspace gases. Headspace gases were analyzed for composition using a gas partitioner (Fisher Hamilton model 29, Pittsburg, PA). Gas composition was calculated with an integrator (Hewlett-Packard HP 3396A, Avondale, PA) on a percent area basis. Quantification of gas composition was possible as the integrator was calibrated in response to a standard gas mixture (Airco Industrial Gases, Murray Hill, NJ), which had a composition of 24.67% CO_2, 24.98% O_2 and 50.35% N_2. Samples (1000 ul) of the headspace gas were injected into the gas partitioner. Subsequent microbiological tests were conducted using the cheese from these packages.

6. Microbiological Testing

Cheese samples were analyzed for S. aureus, mold and yeast counts. Each microbiological test was conducted using 11 g of shredded cheese sample, diluted with 99 ml of dairy product standard methods diluent and blended in a stomacher (Model STO-400, Tekmar Company, Cincinnati, OH) for 30 seconds. S. aureus counts were determined using Baird-Parker agar with EY tellurite enrichment (Difco Laboratories, Detroit, MI). Plates were incubated at 32°C for 48 hours after which the number of colony forming units (cfu) per gram were calculated (Lancette, G.A. and Tatini, S.R., 1992). Only black colonies with a zone of inhibition were counted as *S. aureus* colonies.

Mold and yeast counts were determined using plate count agar with 100 ug/ml chloramphenicol (Sigma, St. Louis, MO) added. Plates were incubated at 21°C for 5-6 days after which the number of colony forming units (cfu) per gram were calculated (Mislivec, et al., 1992).

7. Statistical Analysis

Two simultaneous replications were completed over a 3 month duration. Headspace gas composition and microbial data were analyzed for each type of cheese for five treatments (Aw= 0.90, 0.88, 0.86, 0.84, and 0.75) and 13 testing times (weeks 0 thru 12) using a generalized randomized complete block design programmed in SAS (Cary, NC).

Phase II. Sensory and Shelf-life Study of MAP Packaged Hard Grated Cheeses

1. Preparation of Cheese

Hard-grated cheese samples with a specific desired Aw level (0.90, 0.88 and 0.84) were prepared in the same manner as cheese samples in phase I.

2. Packaging of Cheese

Cheese samples were packaged under the same MAP conditions as used in phase I. A total of six packages per treatment per cheese were packaged. Samples were packaged in duplicate to provide adequate samples for both chemical and microbial testing and sensory evaluation. Packages were stored in an environmental chamber (Hotpack, Philadelphia, PA) at 23°C for a period of 12 weeks.

Gas composition was regulated during packaging by sampling the gas mixture within the gas blender and by sampling the first and last package in each treatment packed under the atmosphere.

3. Analysis of Headspace Gas Composition

Headspace gas composition was determined as previously stated in phase I. Sample packaged randomly were drawn from each treatment and analyzed biweekly for headspace gas composition.

4. Microbiological Testing

After analyzing the headspace gas composition, cheese samples were analyzed for S. aureus counts and mold and yeast counts as a safety precaution and quality check for sensory evaluation. Microbial procedures listed in phase I for evaluation of cheese samples were applied.

5. Chemical Analysis

The same procedures from phase I were used for analysis of Aw, salt content, pH, fat content and moisture content.

6. Sensory Evaluation

A panel of nine experienced individuals was used to evaluate characteristics reflecting hard grated cheese quality based on a modification of quantitative descriptive analysis methodology (Stone and Sidel, 1985). Prior to intitiating testing, panelists were trained to evaluate the identified characteristics. Training was performed in a conference-style room which allowed panelists to freely express their opinions on the sensory characteristics of the cheese samples. During the training sessions (1 hour session), a variety of Parmesan and Romano cheese market samples were presented and panelists characterized the natural aromas and flavors in each cheese. Based on these market samples, panelists defined eight different descriptors including six aromatic descriptors (acrid, buttery, butyric, fermented/fruity, musty, yeasty) and two flavor descriptors (flavor impact, and unclean) common to both types of cheese (Table 1). References standards were used to describe aromatic odors of cheeses (Table 2).

In a preliminary sensory evaluation test, panelists evaluated four cheese samples, of these two samples were identical. Comparing the results from a t-test on the two

Table 1. Definition of Sensory Attributes for Italian Cheese

Descriptor	Definition
Acrid	a burning, sharp, acid odor. It causes a puckering of the jaw and flaring of the nostrils. "extreme"= vinegar
Buttery	a warmed, buttered popcorn aroma
Butyric acid	characteristic odor of short-chain fatty acid, ie., Butyric acid
Fermented/fruit	odor attributes that may result with prolonged storage. Fermented as characterized with sour milk and fruity, as in a pineapple or strawberry odor.
Musty	odor attributes that may result with prolonged storage. Smell associated with mold growth or a damp, poorly ventilated basement.
Yeasty	odor attributes that may result with prolonged storage. Smell associated with bakers' yeast, ie. bread dough.
Flavor impact	the overall perception of flavor intensity. "none'= no flavor; "extreme"= high flavor.
Unclean	the degradation of flavor; negative flavor notes, i.e., lingering unpleasant aftertaste or morning mouth

Table 2. Reference Standards for Aromatic Cheese Attributes

Attribute	Reference standard
Acrid	Extra sharp cheddar cheese, vinegar
Butyric acid	Blue and feta cheese, butyric acid
Plastic	Adhesive bandage (Band-aid, Johnson& Johnson)
Buttery	Diacetyl (Fisher Scientific)
Fermented/fruity	Plain and pineapple yogurt
Musty/yeasty	Bakers yeast

identical samples, panelists were able to consistently rate the intensity level of seven of the original eight attributes. The panelists' responses to the fermented/fruity attribute were slightly different. In later training sessions, the plastic descriptor was eliminated and musty/yeasty was separated into two different categories.

Confusion with the original attribute "blue cheese" was reduced when the descriptor, "butyric acid" was used. The butyric acid reference standard helped clear up the confusion. Volatile free fatty acids and nonvolatile free fatty acids (C_4-C_{18}) concentrations are relatively high in Parmesan and Romano cheese (Nath, 1992). During training panelists also learned how to identify the attributes and practiced rating attribute intensity on an unstructured line scale (Table 3).

Table 3. Scorecard for Evaluation of Shelf-stable Grated Cheese

Date ______________
Panelist Name ______________
Sample Number ______________

Please taste the product before you. Evaluate it for the attributes listed. Mark the intensity of each attribute by placing a hash mark at the appropriate location on the line. Continue until all attributes have been scored. You may smell and re-taste the sample as many times as necessary. Pass the sample and scorecard through the hatch and wait for the next sample. Reminder, please chew the sample (1/2 teaspoon) 15 times before evaluating flavor impact and after expectorating the sample evaluate for an unclean attribute.

Acrid

none extreme

Buttery

none extreme

Butyric acid

none extreme

Fermented/fruity

none extreme

Musty

none extreme

Yeasty

none extreme

Flavor impact

none extreme

Unclean

none extreme

Pass the sample and scorecard through the hatch and wait for the next sample. Rinse your palate with water during this time. Please rest at least 1 minute between each sample. You have ____ more samples to taste today.

After clarifying definitions of attributes, the panelists evaluated another set of four cheese samples using the attribute definitions and scorecard (Table 1 and 3). In the next sensory evaluation testing session, panelists were able to consistently rate the intensity level of all eight attributes for the two identical cheese samples presented. A total of nine training sessions and two preliminary testing sessions to evaluate panelist performance were completed prior to beginning the shelf-life study.

The aroma and flavor characteristics of refrigerated, freshly, shredded (reference) and MAP cheeses at three Aw levels were evaluated biweekly by the sensory panel. A twenty gram cheese sample was portioned into 1 oz. capped plastic portion cups and identified with a three digit random code. Panelists evaluated four cheese samples (a fresh cheese and cheeses with Aw= 0.90, 0.88 and 0.84 packaged under MAP) presented in a random order. The aroma and flavor characteristics were evaluated by first sniffing the sample instead of deeply inhaling the aromatic sample, i.e. sniff the sample, as one would a hazardous material in chemistry lab. For evaluation of flavor, approximately 1/2 teaspoon of cheese sample was chewed thoroughly, approximately 15 times. Flavor impact was evaluated with the sample in the mouth. The unclean attribute was evaluated after expectoration of the sample. To minimize fatigue, vanilla was used as a refreshing scent, vanilla, to clear nasal passages between samples. The intensity of each attribute was measured on a 15.2 cm unstructured line scale (Table 3).

7. Color Evaluation

Color of hard grated cheese over storage period were determined using a chroma meter (CR-200 Chroma meter, Minolta, Osaka, Japan). The CIE L* a* b* color system was used. Three readings were taken from each sample and an average calculated. Samples from each treatment were tested for color following the microbial testing.

8. Statistical Analysis

Sensory data was analyzed by a factorial design programmed in SAS. Defined treatments and time significant differences were determined if the p value was ≤ 0.05. Color data was analyzed by the generalized randomized complete block design programmed in SAS. When appropriate, mean separations were completed by least significant differences.

IV. RESULTS AND DISCUSSION

Phase I. Challenge Study with Staphylococcus aureus

1. Preliminary Drying Study

A preliminary study was devised to determine the conditions (sample size, temperature, time, tumbling speed) necessary to lower the water activity (Aw= 0.90) of hard grated cheeses. A modified rotary drier was used to accomplish this task. For research purposes, it was necessary to dry a 2.5 kg sample of cheese. Cheese samples were dried to desired Aw (0.88, 0.86, 0.84, 0.75) at 40.5 to 46.1°C in 10 to 80 minutes.

2. Chemical Properties of Hard Grated Cheeses

The chemical and physical properties of the natural cheeses were determined before altering the Aw levels (Table 4). Both cheeses, Parmesan and Romano, had an initial Aw of 0.90. Romano had a pH value of 5.06 whereas Parmesan had a pH value of 5.42. The salt content of the cheeses was similar (Table 4).

Table 4. Chemical Properties of Fresh Hard Grated Cheeses

	Aw level	pH	%Salt	%Moisture	%Fat
Parmesan	0.90	5.42	3.11	37.55	25.0
Romano	0.90	5.06	3.11	36.46	27.5

n=2

Drying the cheeses, Parmesan and Romano, to various Aw levels affected the salt, moisture, and fat contents (Tables 5 and 6). Increased drying caused the cheese solids to become concentrated with decreased moisture content. As moisture was expelled with extensive drying, the fat and salt contents were expected to increase.

Table 5. Chemical Properties of Parmesan Cheese after Drying to Desired Aw levels

Aw level	pH	%Salt	%Moisture	%Fat
0.90	5.41	3.11	34.88	24.0
0.88	5.38	3.03	31.40	24.0
0.86	5.39	2.43	29.98	25.5
0.84	5.41	3.22	23.92	25.5
0.75	5.37	3.19	21.71	26.5

n=2

Table 6. Chemical Properties of Romano Cheese after Drying to Desired Aw levels

Aw level	pH	%Salt	%Moisture	%Fat
0.90	5.06	3.12	4.46	26.40
0.88	5.06	3.26	28.05	29.10
0.86	5.02	2.76	24.60	29.50
0.84	5.09	4.43	23.20	30.50
0.75	5.06	3.41	17.85	31.00

n=2

3. Headspace Gas Analysis

Prior to microbial analysis, headspace gas composition was monitored for changes from the initial atmosphere (25% CO_2 and 75% N_2) during storage (Figure 1-6). This atmosphere was chosen to mimic the gas mixture used in MAP packaged refrigerated, shredded cheeses. As expected, storage time had a significant effect on the headspace gas composition. After two weeks of storage, oxygen (> 10%) was detected in the headspace gases of the all cheese packages (Figures 1 and 2). To avoid problems, the cheeses were repackaged to remove the unwanted oxygen. In both Parmesan and Romano cheese packages, the carbon dioxide content was depleted from 25% to 10% CO_2 and the nitrogen content increased from 75% to 90% N_2 over storage time (Figures 3, 4, 5 and 6). In the last six weeks of the study, O_2 was detected in the headspace gas composition (Figures 1 and 2).

The package material used in this study is similar to the package used for refrigerated, shredded cheeses. The results from the headspace gas analysis indicates that the packaging material was not able prevent the migration of oxygen through the package.

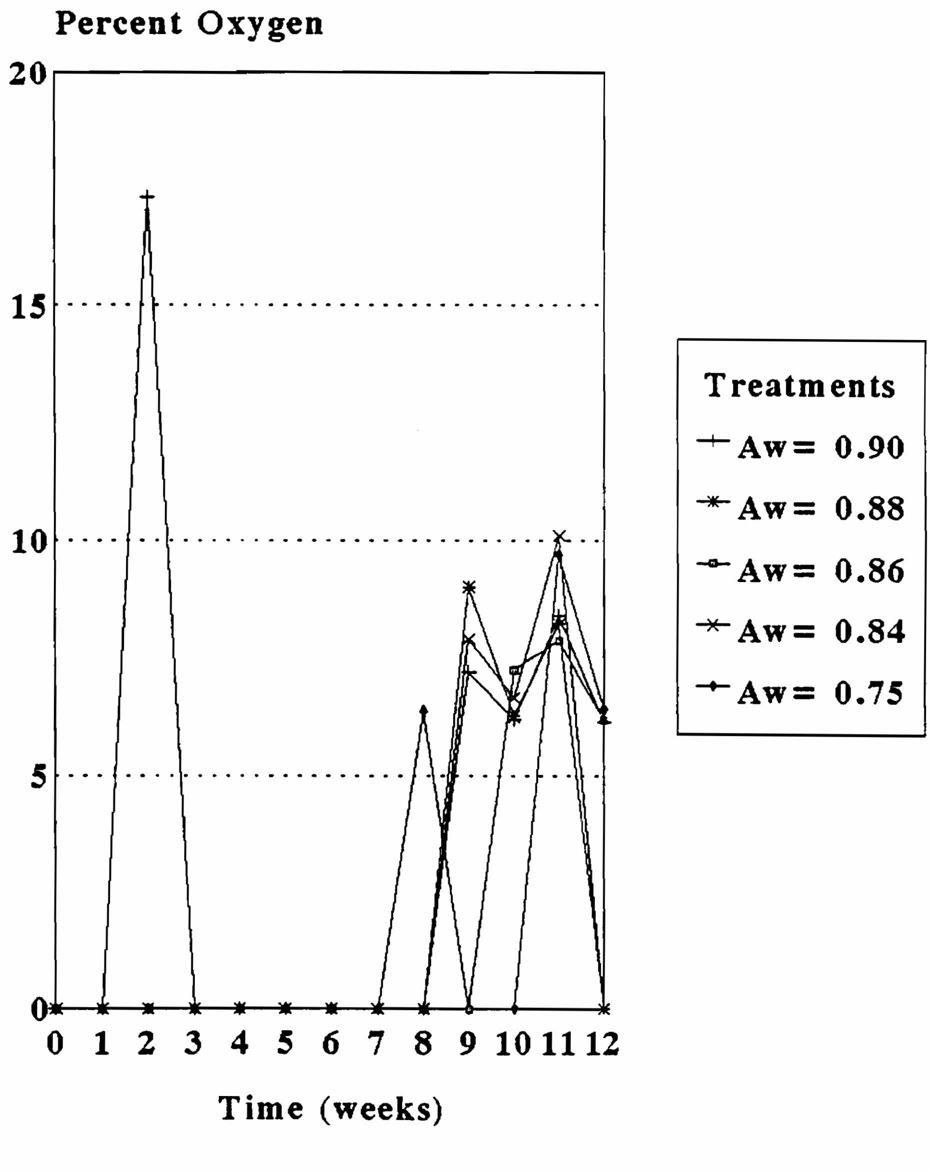

Figure 1: Headspace Oxygen Levels of MAP Parmesan Stored at 35°C.

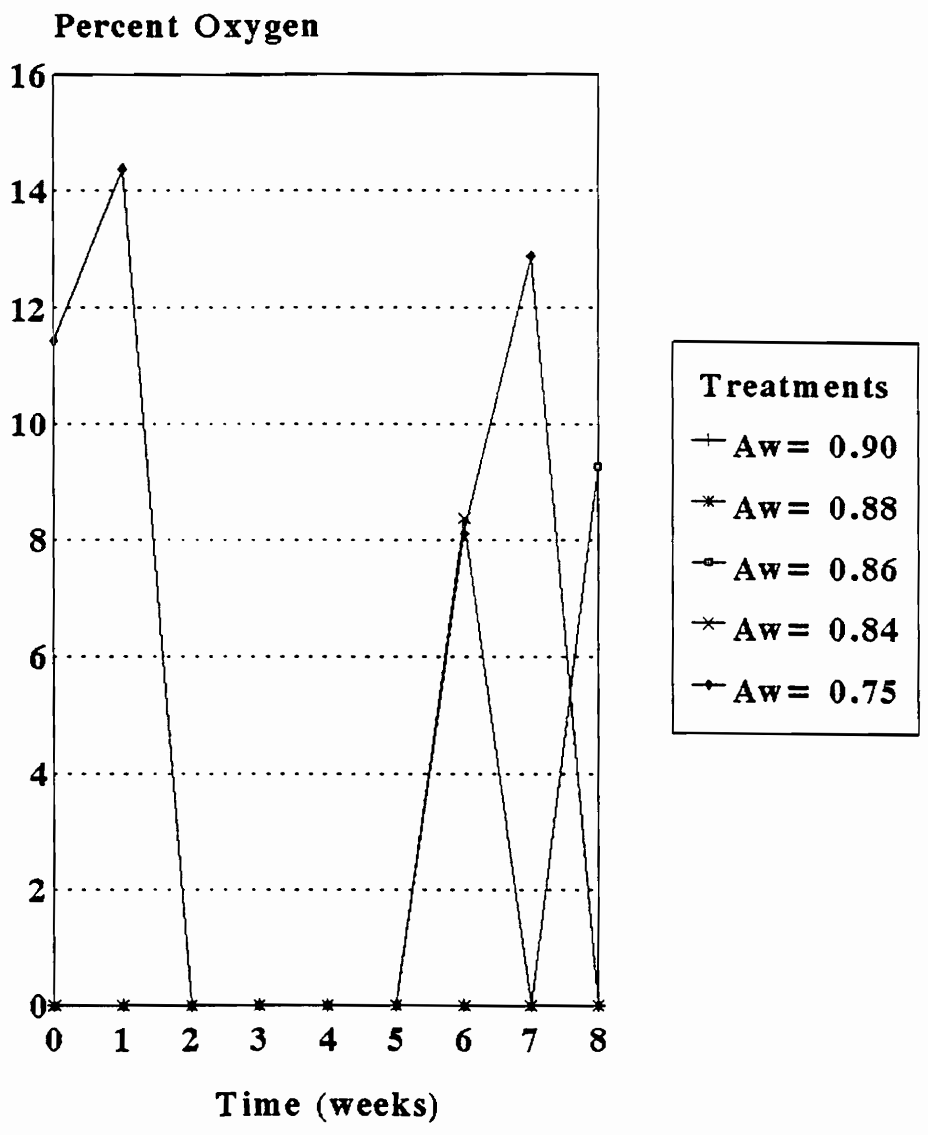

Figure 2: Headspace Oxygen Level of MAP Romano Stored at 35°C.

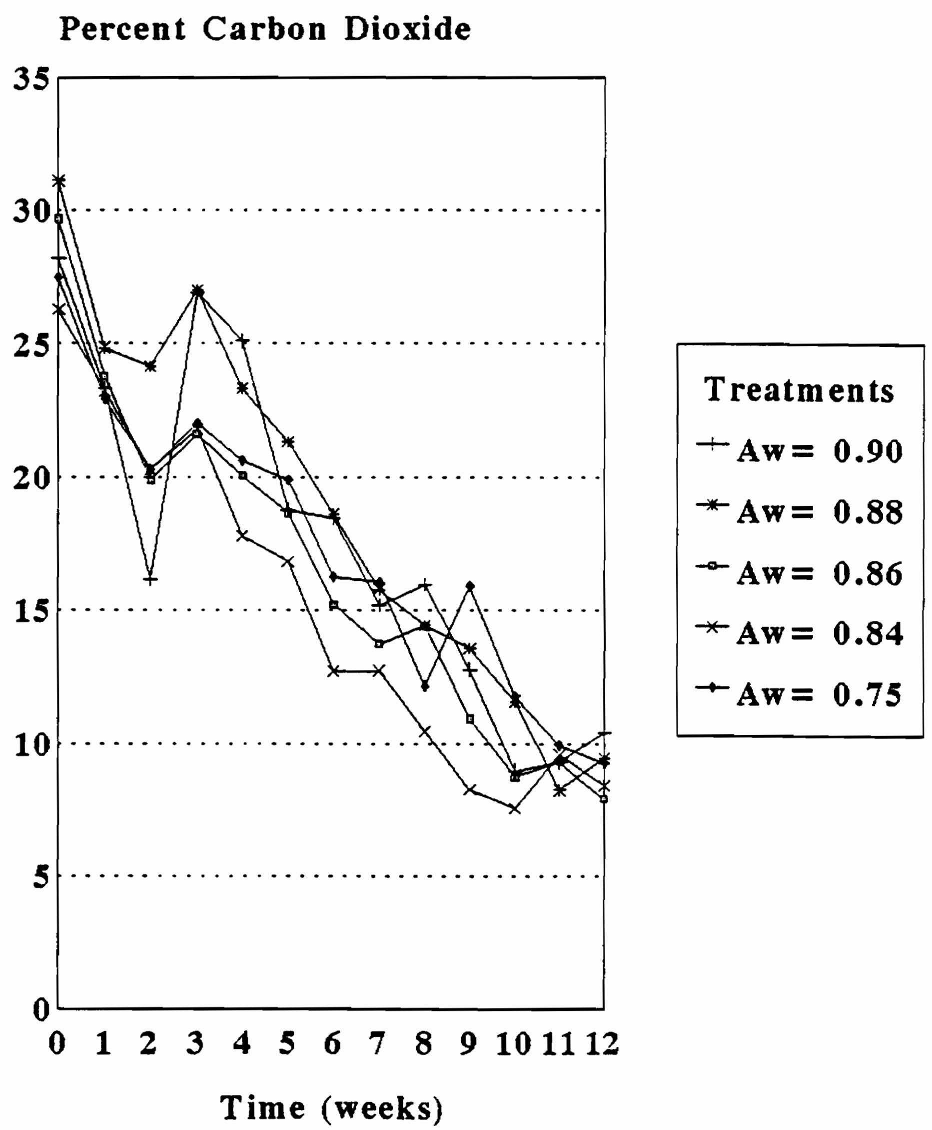

Figure 3: Headspace Carbon Dioxide Levels of MAP Parmesan Stored at 35°C.

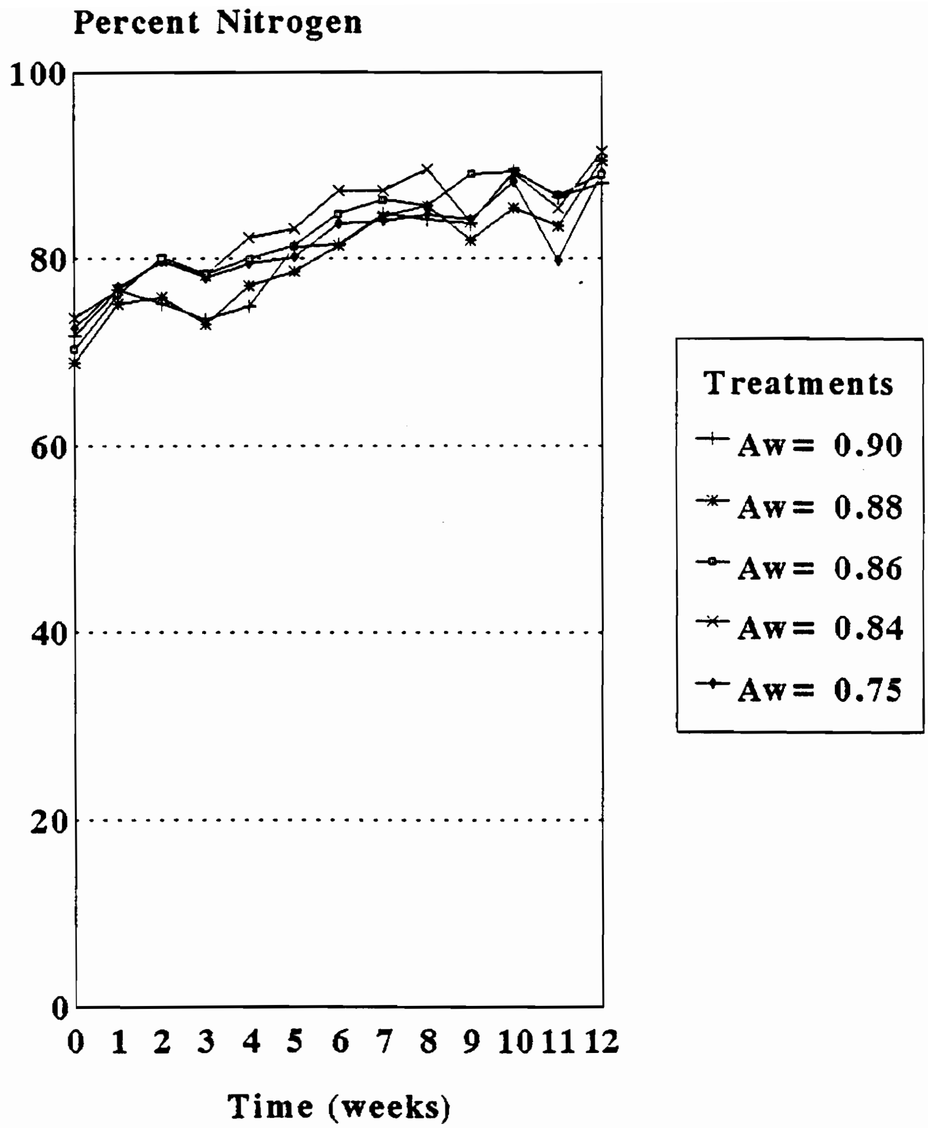

Figure 4: Headspace Nitrogen Levels of MAP Parmesan Stored at 35°C.

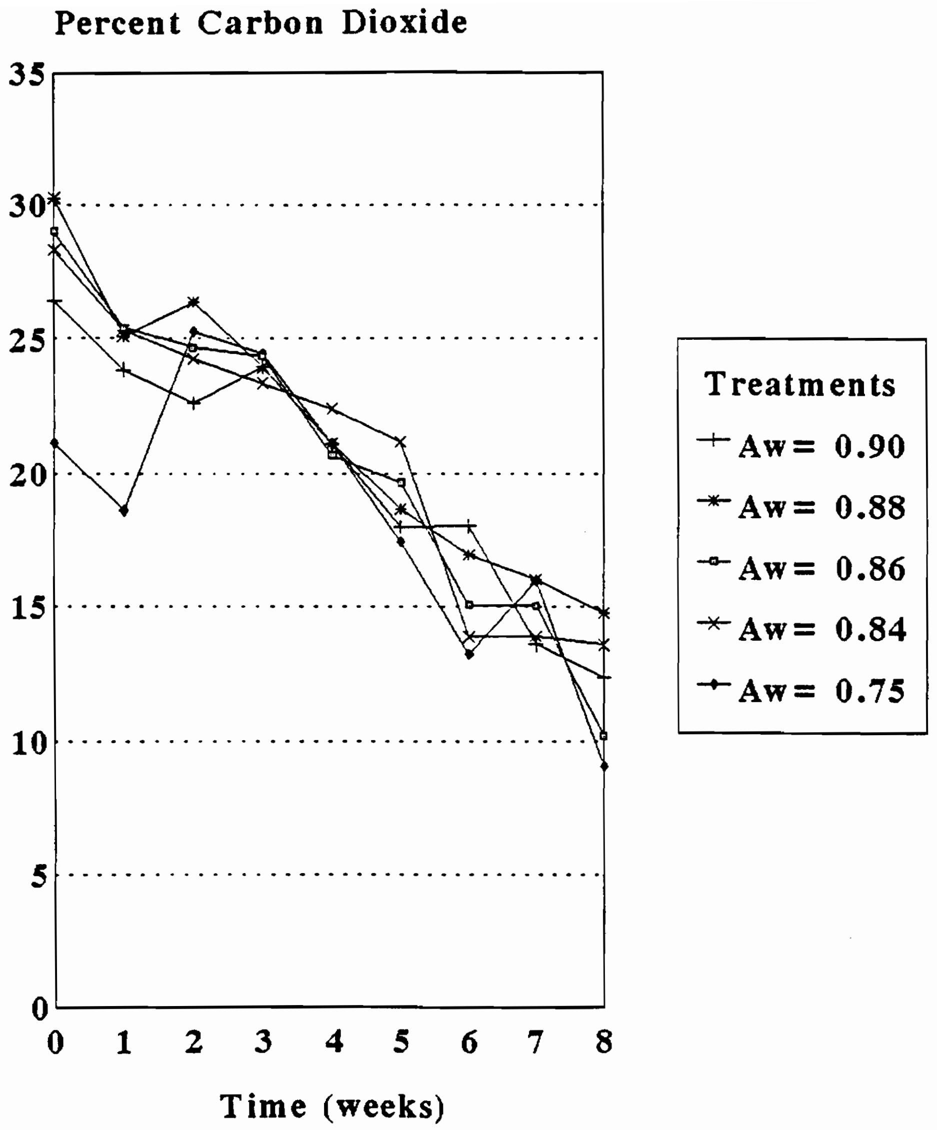

Figure 5: Headspace Carbon Dioxide Levels of MAP Romano Stored at 35°C.

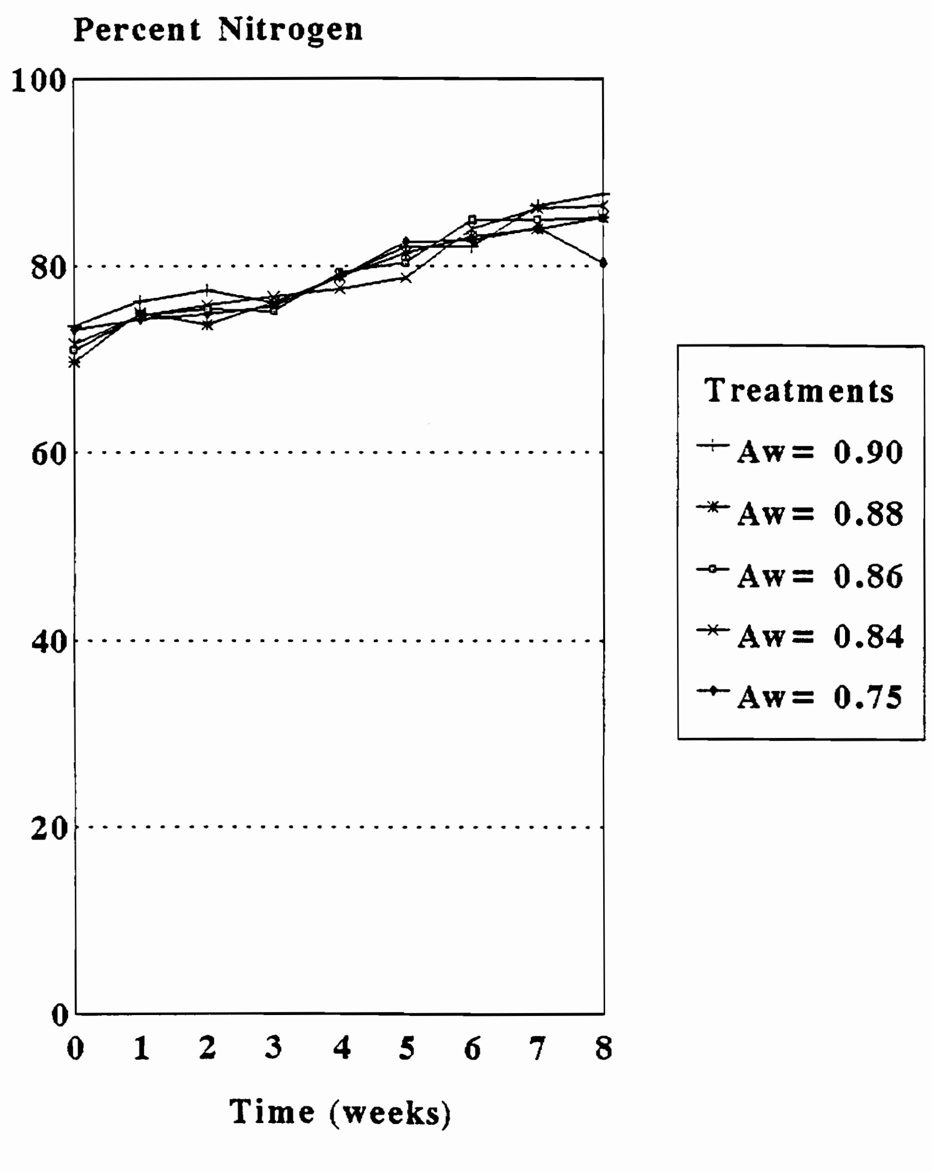

Figure 6: Headspace Nitrogen Levels of MAP Romano Stored at 35°C.

4. Microbial Analysis

4.1 Staphylococcus aureus Growth and Survival

Due to its tolerance of low Aw levels, S. aureus was believed to be the most potentially troublesome pathogen in hard grated cheeses. Parmesan and Romano cheese samples were inoculated with S. aureus to examine the microorganism's growth characteristics at various Aw levels in hard grated cheeses stored under MAP.

All Parmesan cheese samples were inoculated with 10^6 cfu S. aureus/ g cheese. After three weeks, the growth of S. aureus at Aw= 0.86 MAP and below declined in Parmesan cheese (Figure 7). Thus, the low Aw levels were able to suppress growth of the microorganism. The growth of S. aureus reached 10^6 cfu/g in the cheese sample with Aw= 0.90 MAP after three weeks. The population of S. aureus in the sample with Aw= 0.90 MAP declined over the remaining storage period. The S. aureus population in the cheese sample with Aw= 0.88 MAP remained between 10^4-10^5 cfu/g over a six week period, than numbers declined over remaining storage time. The control sample was contaminated with S. aureus and followed the same trend as the cheese with Aw= 0.90 MAP.

Romano cheese samples were inoculated with S. aureus at a level of approximately 10^3 cfu/g. Survival of S. aureus was rapidly decreased after three weeks of storage (Figure 8). Reduction of microbial numbers may be attributed to the

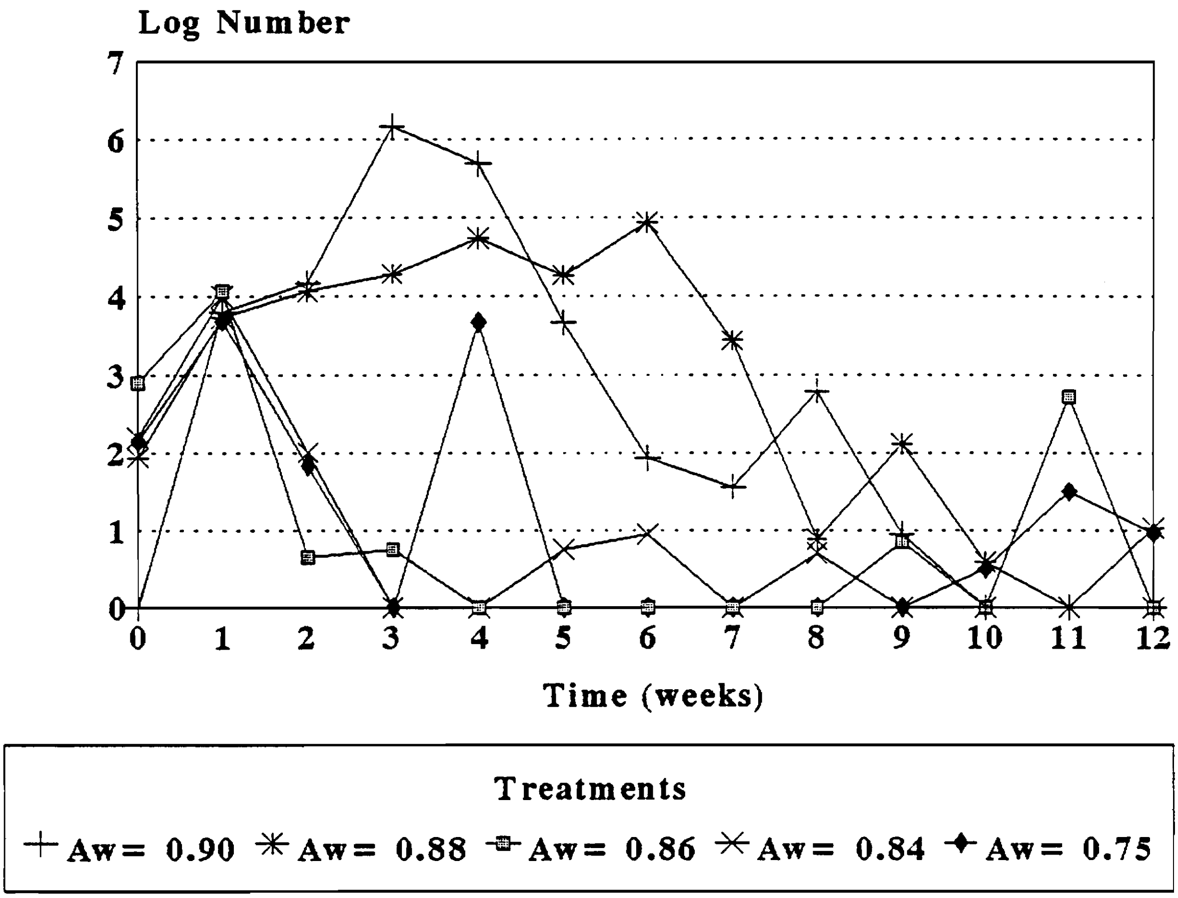

Figure 7: <u>Staphylococcus</u> <u>aureus</u> Growth (cfu/g) on MAP Parmesan Cheese at Various Aw Levels Stored at 35°C.

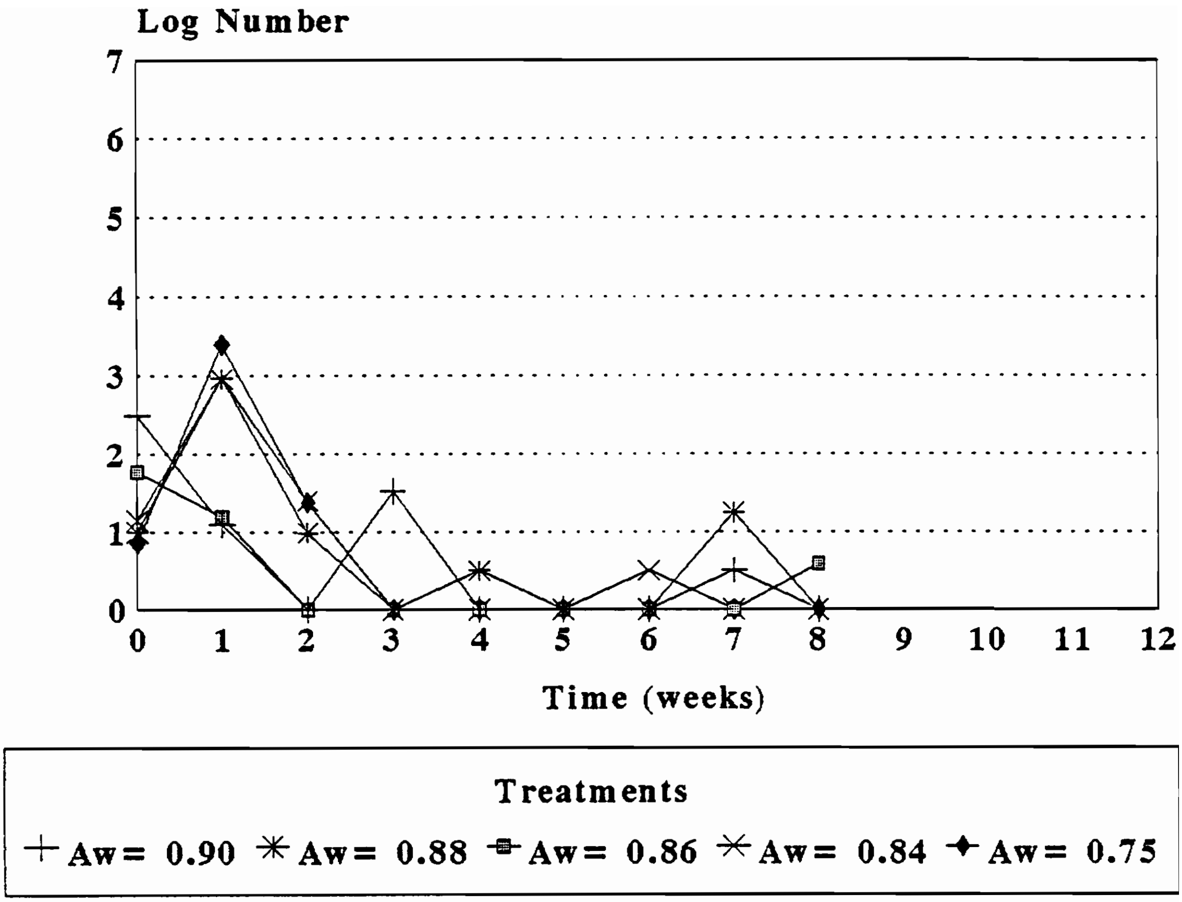

Figure 8: <u>Staphylcoccus</u> <u>aureus</u> Growth (cfu/g) on MAP Romano Cheese at Various Aw Levels Stored at 35°C.

presence of short-chained free fatty acid components and low initial pH of the Romano cheese. There was no contamination observed on the control cheese.

4.2 Mold and Yeast Growth and Survival

Cheese samples were monitored for growth of mold and yeast. For Parmesan cheese, mold and yeast counts increased over storage time for all treatments (Figures 9 and 10). Visible mold was detected on Parmesan cheese with Aw= 0.90 after eight weeks of storage. The detection of O_2 within the headspace gas composition may explain the increasing mold and yeast counts observed on Parmesan cheese. Thus, the different Aw levels were unable to prevent mold and yeast growth on Parmesan cheese samples. Commercial grated Parmesan cheese has a low Aw (0.75) and antimicrobial agent (Potassium sorbate) to prevent growth of mold and yeast. Growth of molds was prevented in Romano cheese (Figure 11). The presence of short-chained free fatty acid, such as propionic acid may explain the inhibition of molds on Romano cheese. However, yeast growth on Romano cheese increased over storage time for all treatments (Figure 12).

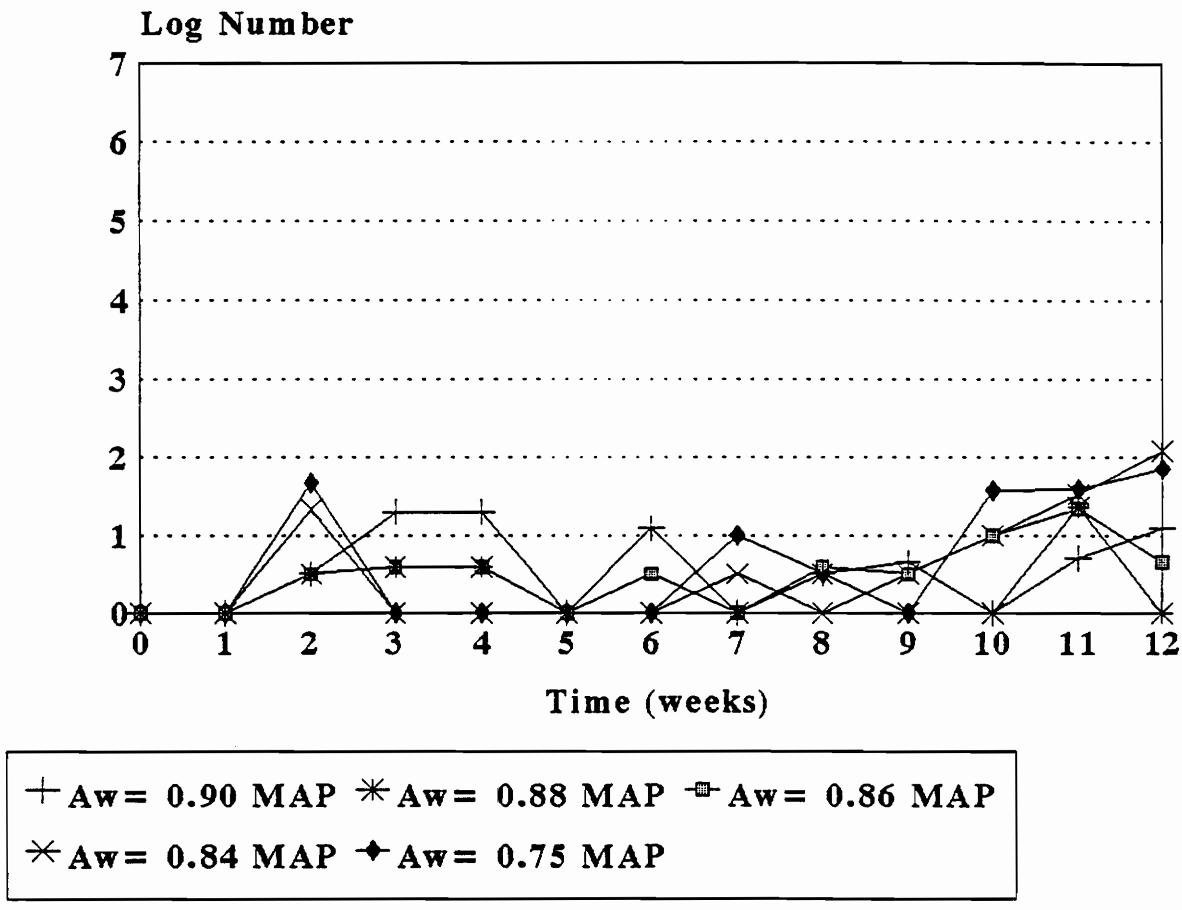

Figure 9: Mold Growth (cfu/g) on MAP Parmesan Cheese at Various Aw Level Stored at 35°C.

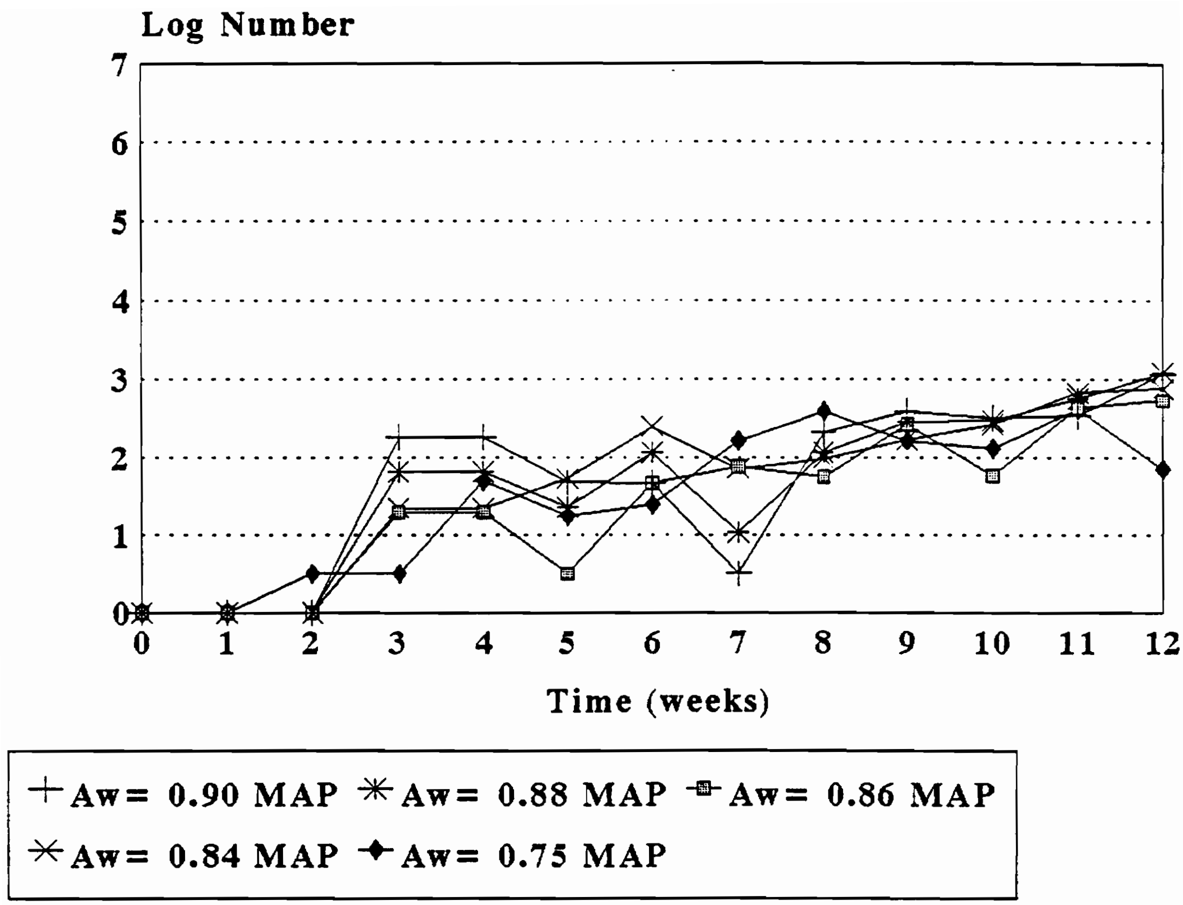

Figure 10: Yeast Growth (cfu/g) on MAP Parmesan Cheese at Various Aw Levels Stored at 35°C.

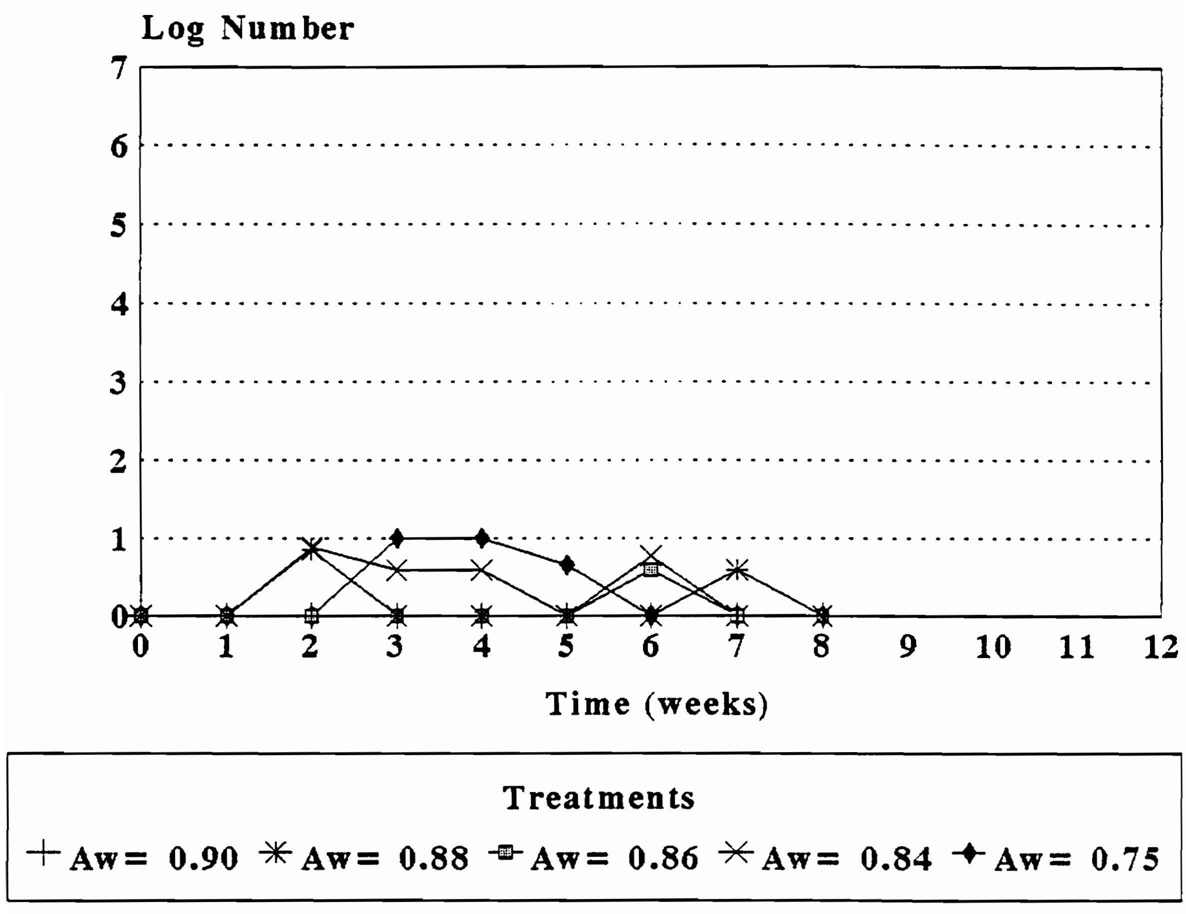

Figure 11: Mold Growth (cfu/g) on MAP Romano Cheese at Various Aw Levels Stored at 35°C.

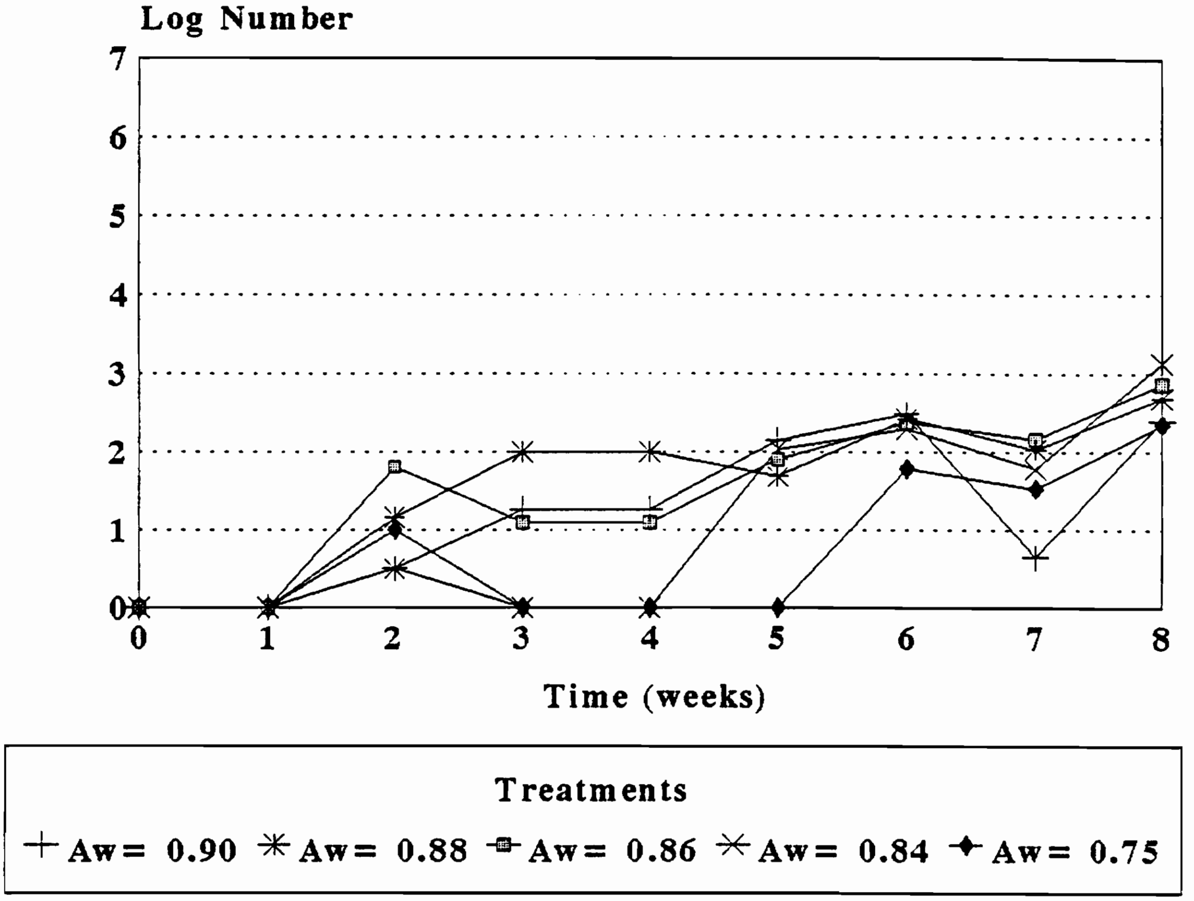

Figure 12: Yeast Growth (cfu/g) on MAP Romano Cheese at Various Aw Levels Stored at 35°C

Phase II. Sensory and Shelf-stability of MAP Packaged Hard Grated Cheeses

1. Chemical Properties

The chemical properties of Parmesan and Romano cheese were determined upon arrival of cheeses (Table 7). The Romano cheese was slightly more acidic, had slightly lower salt concentration and had slightly more moisture and fat than the Parmesan cheese.

Table 7. Chemical Properties of Fresh Hard Grated Cheeses

	Aw level	pH	%Salt	%Moisture	%Fat
Parmesan	0.90	5.31	2.71	33.20	24.90
Romano	0.90	5.09	2.51	35.97	26.40

n=2

Drying the cheese to water activities of 0.88 and 0.84, had no affect on pH. The salt content was expected to increase with the reduction of moisture; however, this was not observed in Parmesan or Romano cheese (Table 8 and 9). The fat content of the cheeses increased as the moisture content decreased (Table 8 and 9).

Table 8. Chemical Properties of Parmesan Cheese Dried to Three Aw Levels

Aw Level	pH	%Salt	%Moisture	%Fat
0.90	5.31	2.71	33.20	24.90
0.88	5.32	2.86	27.97	26.30
0.84	5.35	2.72	25.63	28.50

n=2

Table 9. Chemical Properties of Romano Cheese Dried to Three Aw Levels

Aw Level	pH	%Salt	%Moisture	%Fat
0.90	5.09	2.51	35.97	26.40
0.88	5.12	2.71	29.44	29.10
0.84	5.13	2.16	23.23	30.50

n=2

2. Headspace Gas Analysis

Headspace gas composition was monitored in packages selected for chemical, microbial, and sensory analysis over the 12 week storage period. The headspace gas in the packages did not change with time. This may be attributed to the reduced storage temperature of $23^{0}C$ as observed in the previous study.

3. Microbial Analysis

Cheese samples intended for human consumption were checked biweekly for Staphylococcus aureus and mold and yeast growth. This was a precautionary step as a measure of product safety prior to sensory evaluation. For the sample to present a risk, there must be a minimum of 10 cfu/g of S. aureus. Parmesan and Romano cheese samples had less than 100 cfu/g of S. aureus indicating the product was safe to consume. The Parmesan and Romano cheese samples had less than 100 cfu/g of Staphylococcus aureus over the entire sensory study. For all treatments, mold growth increased over storage time in Parmesan cheese samples. The Parmesan cheese sample with Aw= 0.90 package under MAP had the largest increase in mold growth over time. For Romano cheese, mold growth was suppressed.

Storage time and treatment had no affect on yeast growth in Parmesan cheese. Yeast growth slightly increased over the 12 week storage time on Parmesan cheese. For all treatments, yeast growth on Romano cheese significantly increased over storage time.

4. Sensory Evaluation of Hard Grated Cheeses

Sensory evaluation was conducted to determine the quality and shelf-stability of hard grated cheeses over storage time. Nine panelists participated in a modified Quantitative Descriptive Analysis panel (QDA).

Although evaluation of panelist performance following training indicated consistency in evaluation of all attributes, variability among panelists responses relative to samples evaluated during the study resulted in statistically significant interactions between panelists and time and between panelists and treatment for all attributes. The influence of panelists was accounted for in the statistical model indicating that any statistical differences in attribute means for treatment or time are valid.

4.1 Natural Aroma Descriptors

<u>Parmesan</u>

Acrid, buttery and butyric acid were used to describe the natural aromas present in hard grated cheese by the descriptive sensory panel. The volatile short-chained fatty acids present in hard grated cheese influenced the sensory panel to use the descriptor, butyric acid. Italian hard cheeses contain volatile fatty acids in relatively high concentrations (Manning and Nursten, 1992). In general, aroma intensities for the acrid and butyric acid characteristics for the MAP packaged cheeses at the three Aw levels were similar to those of the fresh, refrigerated control product for both Parmesan and Romano cheeses. Some minor trends were noted over storage time.

A moderate acrid aroma intensity was reported for all treatments. Mean intensities of acrid aroma were not significantly different over storage time for the refrigerated, fresh control treatment and the MAP packaged Parmesan cheese with Aw= 0.90 and 0.88. The perception of the acrid aroma in the MAP packaged Parmesan cheese at an Aw of 0.84 increased slightly on each week of evaluation. The mean acrid intensity was higher on the 12th week of storage than the initial intensity. No differences were observed between treatments (Figure 13). The buttery aroma of Parmesan cheese decreased significantly over storage time for treatments, fresh Parmesan and MAP Parmesan with Aw= 0.84. For fresh Parmesan, no difference in the buttery aroma was detected until the last sampling period (12 wks.). For the MAP Parmesan with Aw= 0.84, the buttery aroma decreased significantly after 2 weeks of storage. No differences were observed between treatments (Figure 14). Storage time and treatment had no affect on the detection of butyric acid for all treatments (Figure 15). However, at one sampling period (week 8) of storage, the MAP Parmesan sample with Aw= 0.90 was significantly different from the other treatments.

Romano

For sensory evaluation of Romano, the detection of acrid significantly increased over storage time for all treatments. However, treatment had no effect on the perception of acrid in Romano (Figure 16). Storage time and treatment had no effect on the detection of the attribute, buttery (Figure 17).

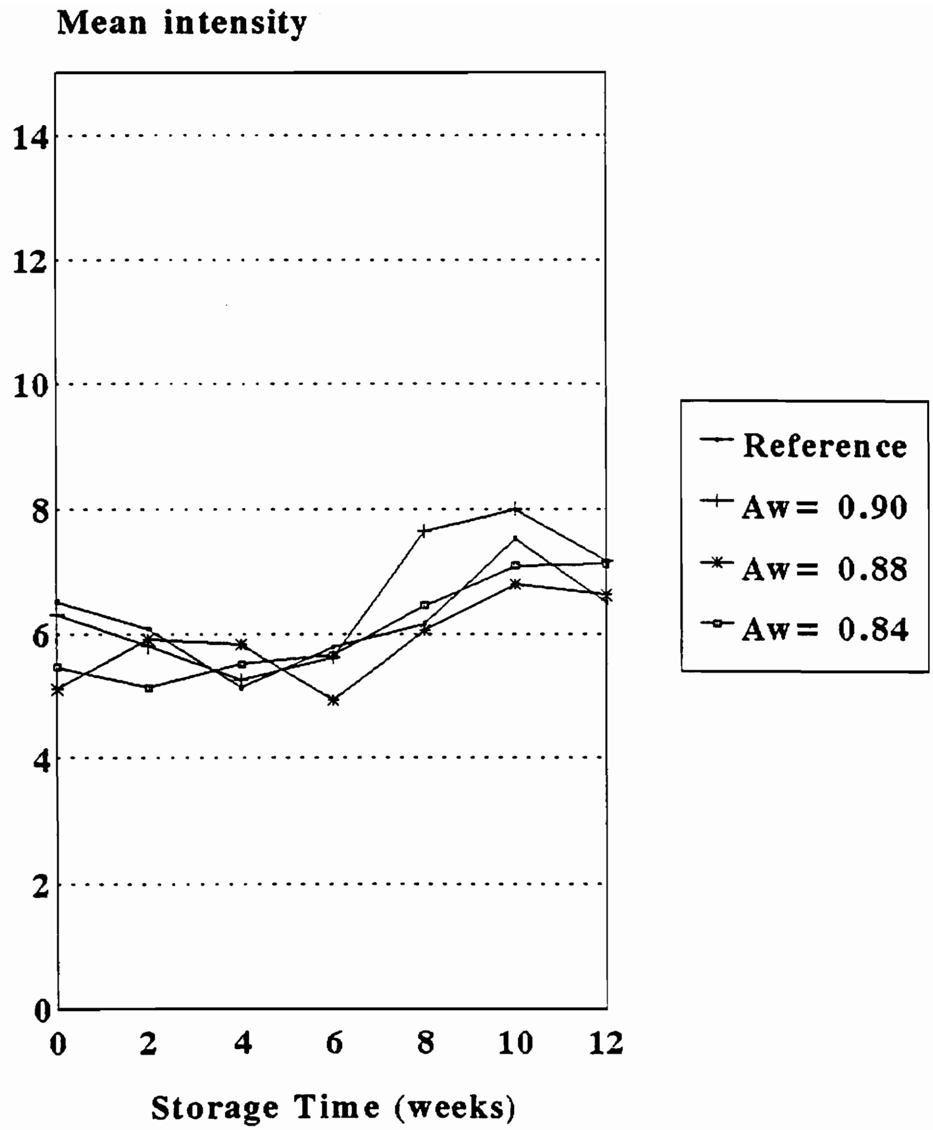

Figure 13: Mean Sensory Scores (n=9) for Acrid Aroma of MAP Parmesan Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

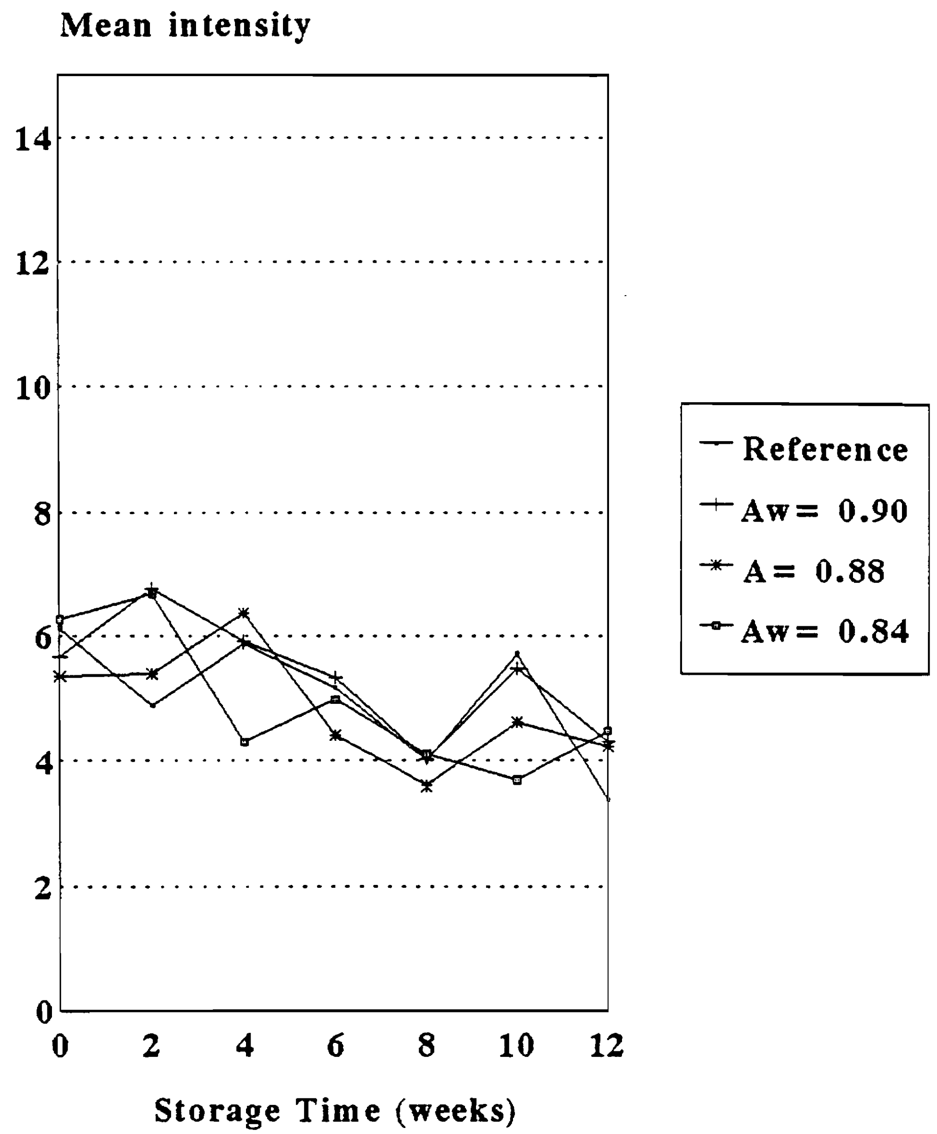

Figure 14: Mean Sensory Score (n=9) for Buttery Aroma of MAP Parmesan Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

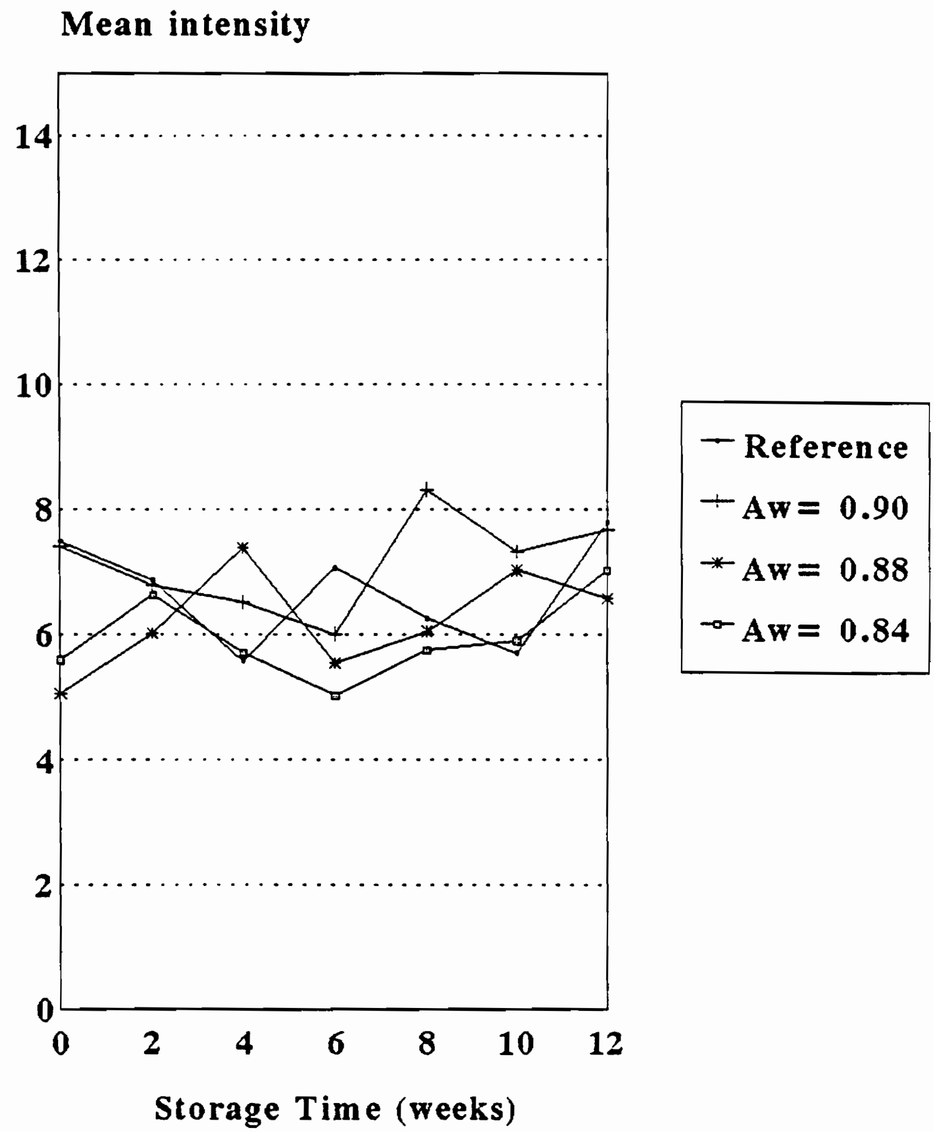

Figure 15: Mean Sensory Score (n=9) for Butyric Acid Aroma of MAP Parmesan Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

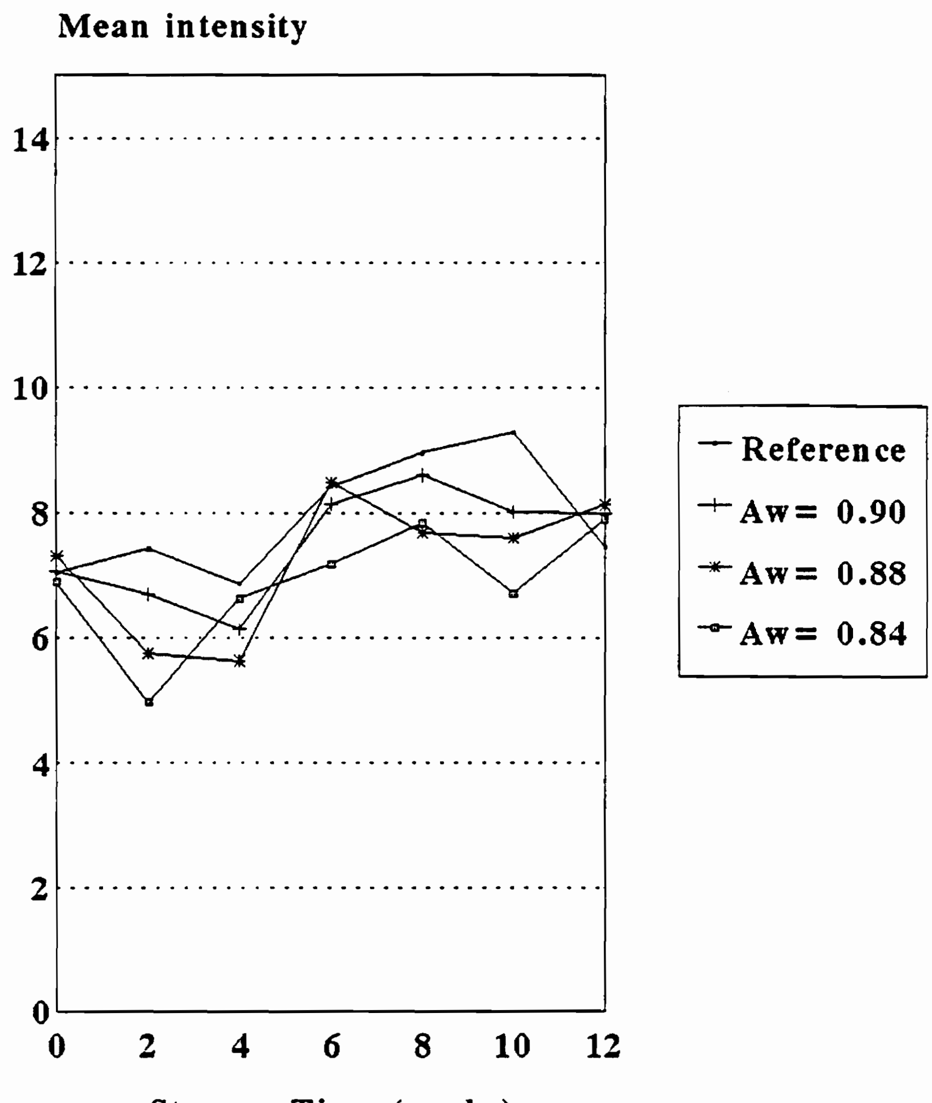

Figure 16: Mean Sensory Score for Acrid Aroma of MAP Romano Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

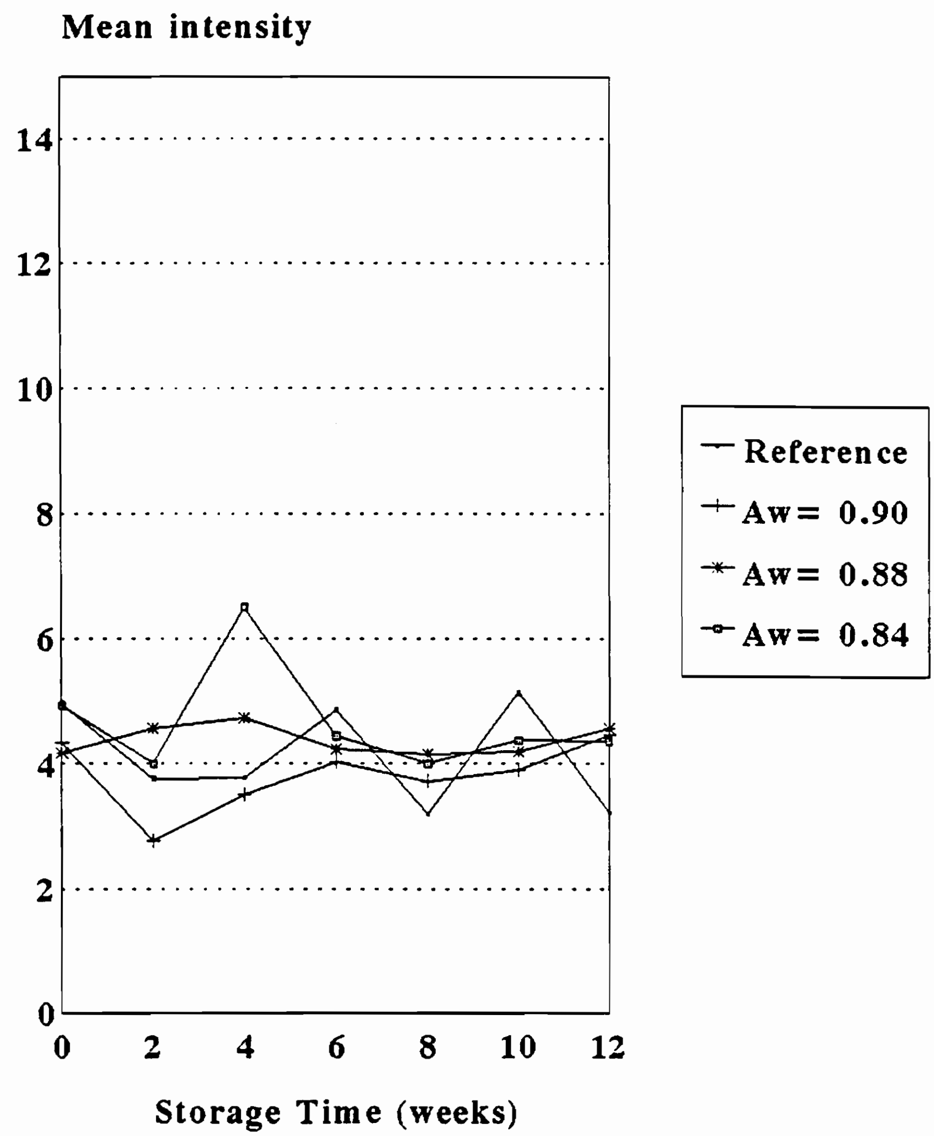

Figure 17: Mean Sensory Score (n=9) for Buttery Aroma of MAP Romano Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

However, detection of the buttery aroma increased significantly at week 4 and resumed the original level at week 6 for the MAP Romano with Aw= 0.84 sample. No difference was observed between treatments. Storage time and treatment had no affect on the detection of butyric acid in Parmesan cheese (Figure 18).

4.2 Odors Resulting from Prolonged Storage

Parmesan

Fermented/fruity, musty and yeasty were developed by the QDA panel to describe the odors which may develop with prolonged storage. Odors associated with sour milk and pineapple defined the attribute, fermented/fruity. Smells of a damp, poorly ventilated basement defined the attribute, musty. The attribute, yeasty was defined by the odors associated with bread dough.

For sensory evaluation of Parmesan cheese, the detection of fermented/fruity remained unchanged over storage time for all treatments. The detection of fermented/fruity in the MAP Parmesan sample with Aw= 0.84 peaked at week 6 (8.06). Therefore, the perception of the aroma at week 6 was significantly different from the first three time periods. Treatment had a no affect on the attribute, fermented/fruity (Figure 19). After 4 weeks of storage, the perception of

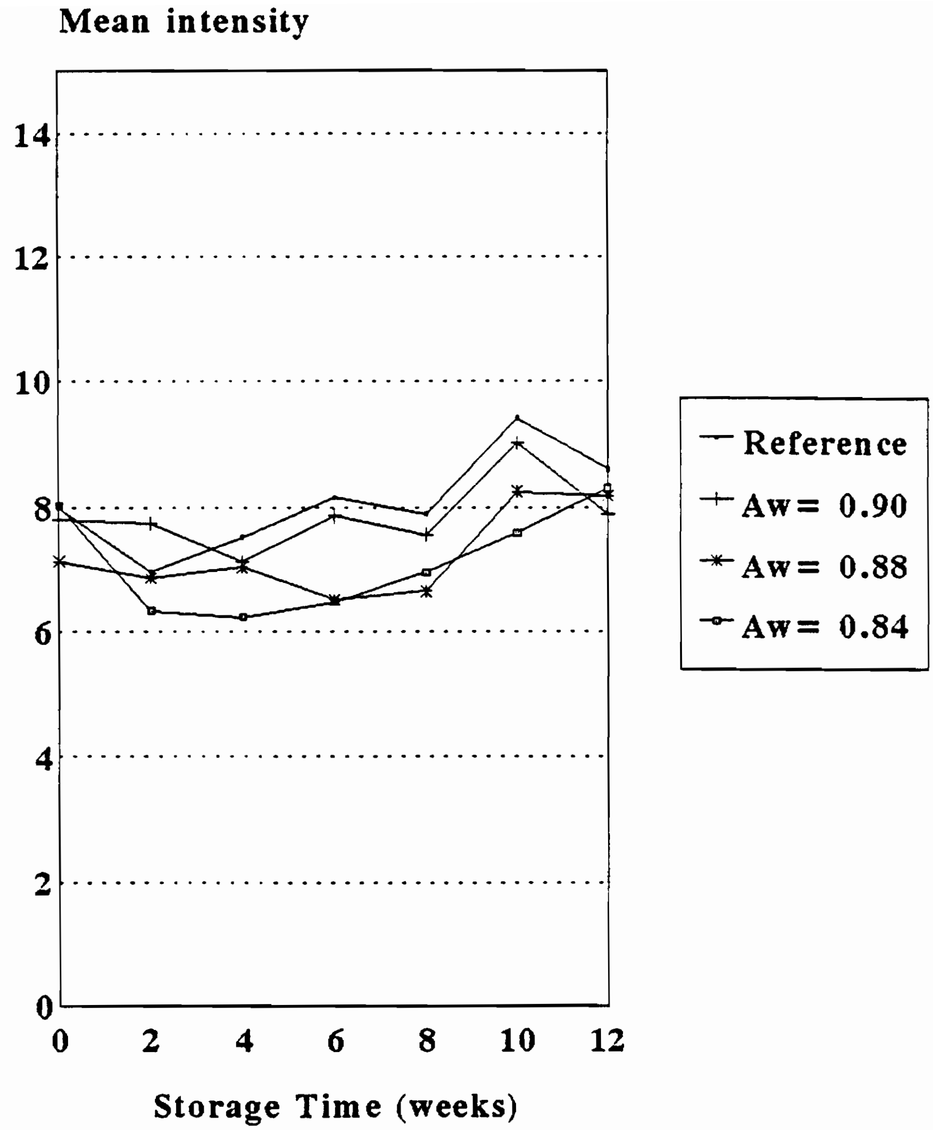

Figure 18: Mean Sensory Score for Butyric Acid Aroma of MAP Romano Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

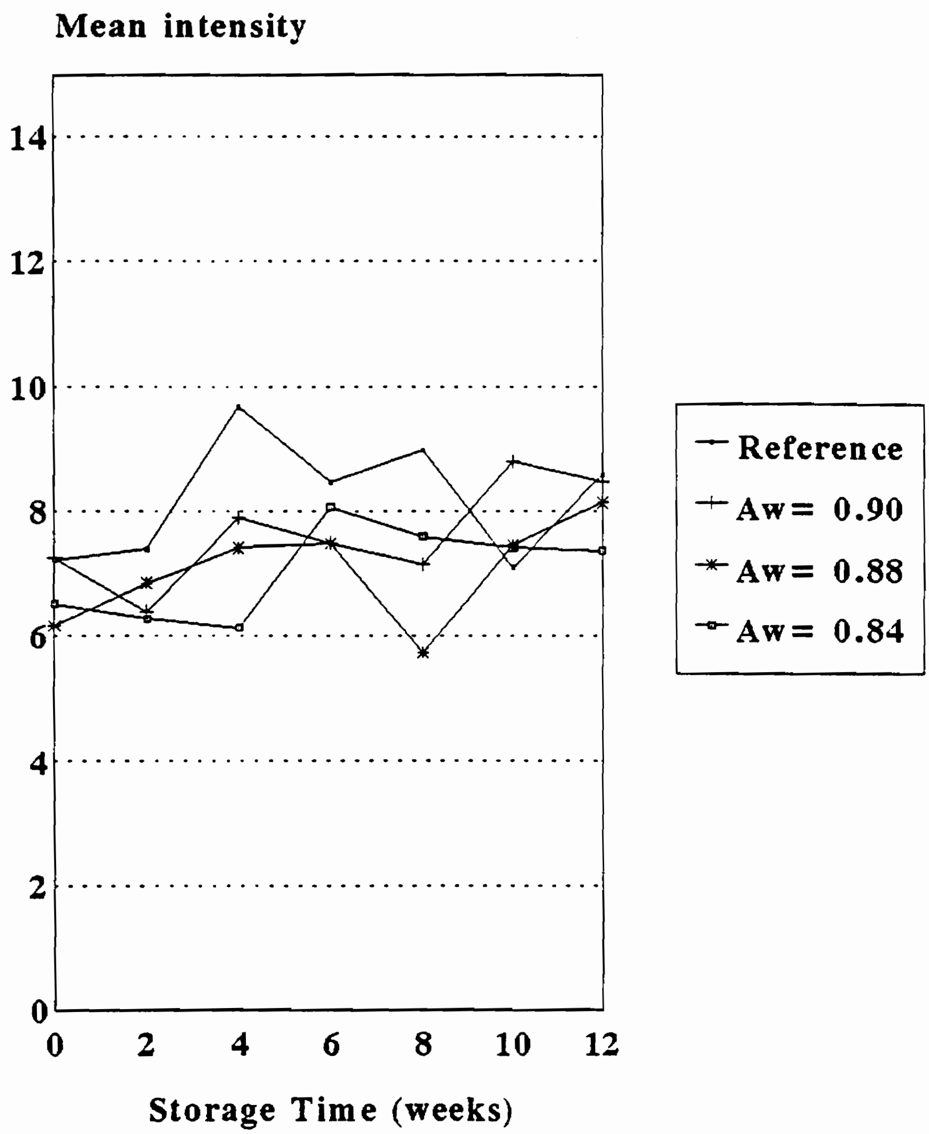

Figure 19: Mean Sensory Score (n=9) for Fermented/fruity Aroma of MAP Parmesan Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

fermented/fruity in the fresh cheese sample was increased significantly in fresh Parmesan cheese compared to the other treatments. The level of musty perceived in Parmesan cheese was unaffected by storage time. Treatment had no influence on the level of musty perceived (Figure 20). The perception of a yeasty odor did not change over storage time (Figure 21). The perception of the odor in MAP Parmesan with Aw= 0.84 peaked after 10 weeks of storage causing a significant increase. The detection of yeasty decreased after 2 weeks of storage, increased after 6 weeks of storage and decreased at the last storage period in fresh Parmesan causing significant differences between storage times. Treatment had no affect on the detection of the attribute.

Romano

For sensory evaluation of Romano cheese, detection of the off-odor attribute, fermented/fruity, was not affected by storage time and treatment (Figure 22). The detection of musty was not affected by storage time or treatment (Figure 23). However, after 12 weeks of storage, the level of musty detected in MAP Parmesan with Aw= 0.90 increased significantly over the other treatments. The detection of yeasty was not affected by storage time or treatment (Figure 24).

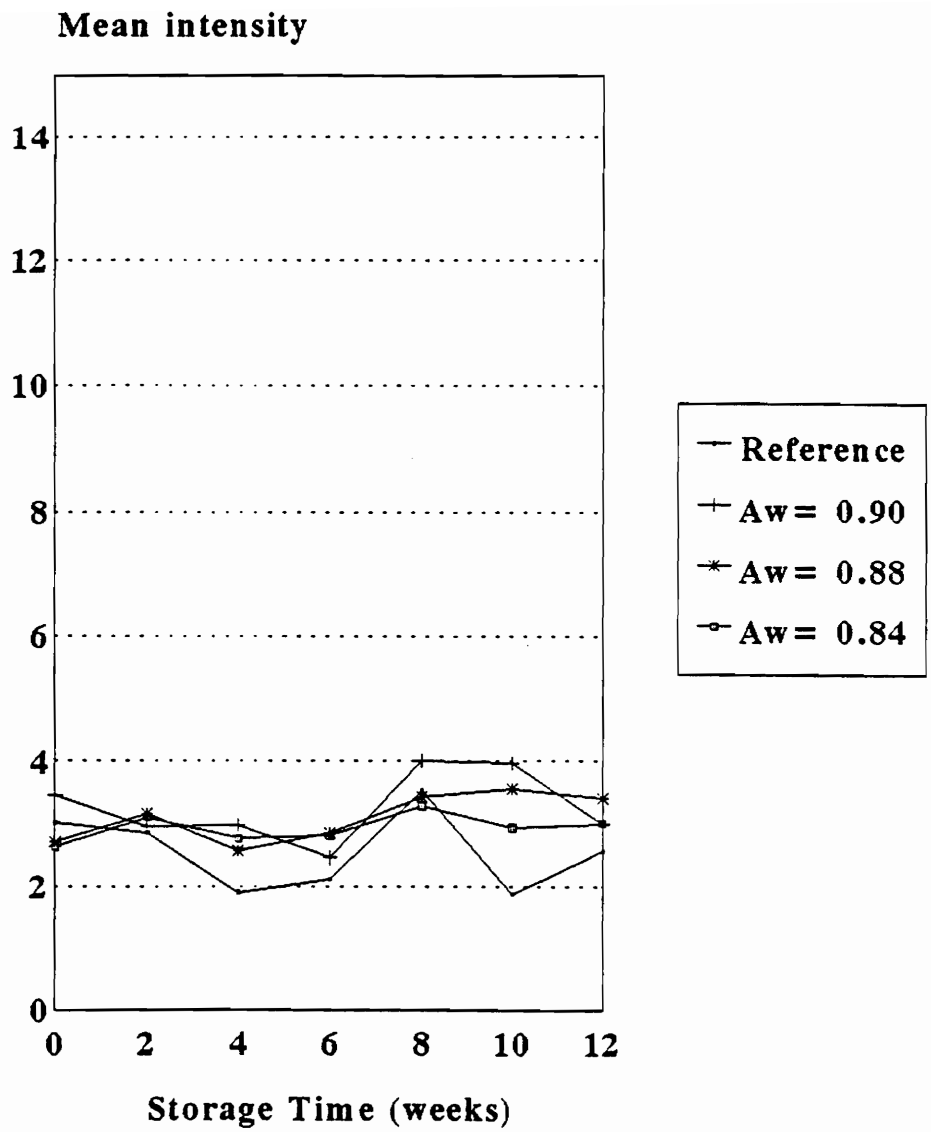

Figure 20: Mean Sensory Score (n=9) for Musty Aroma of MAP Parmesan Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

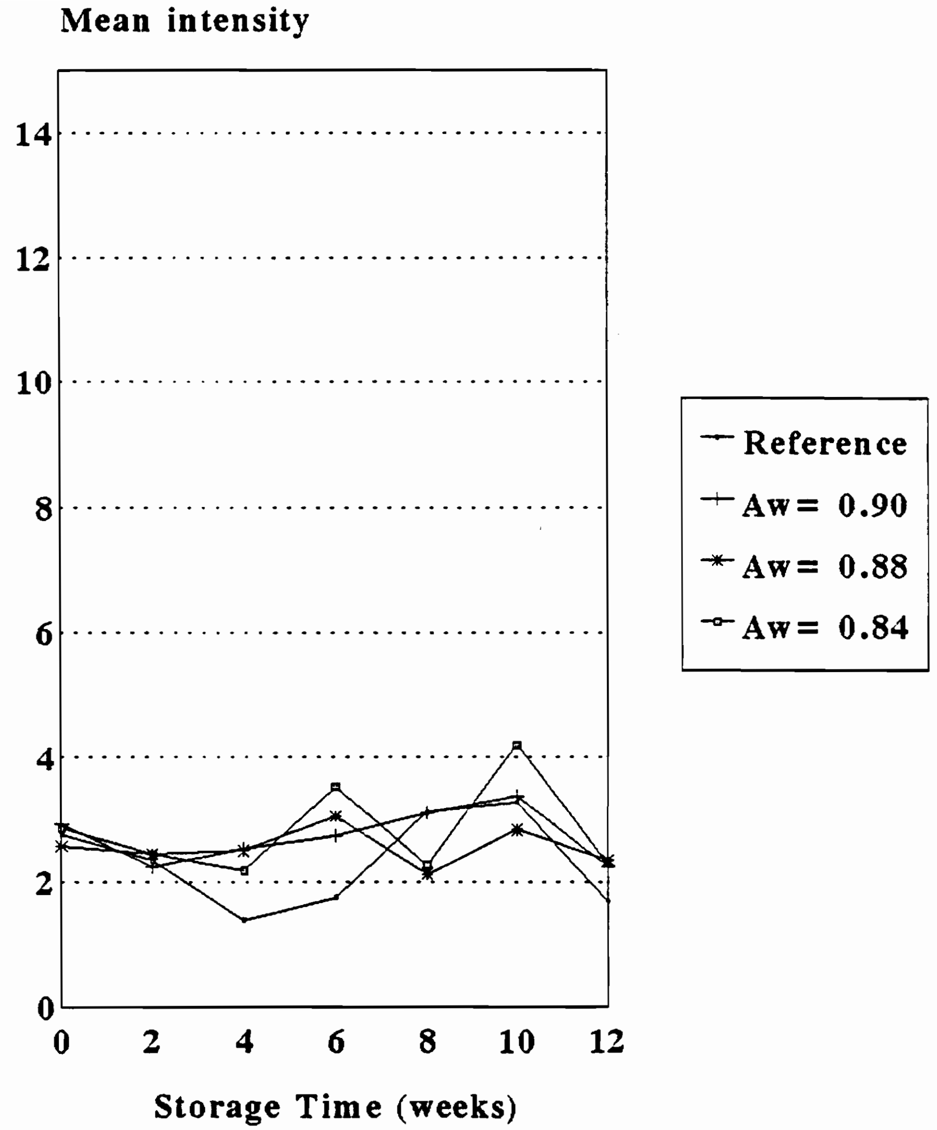

Figure 21: Mean Sensory Score (n=9) for Yeasty Aroma of MAP Parmesan Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

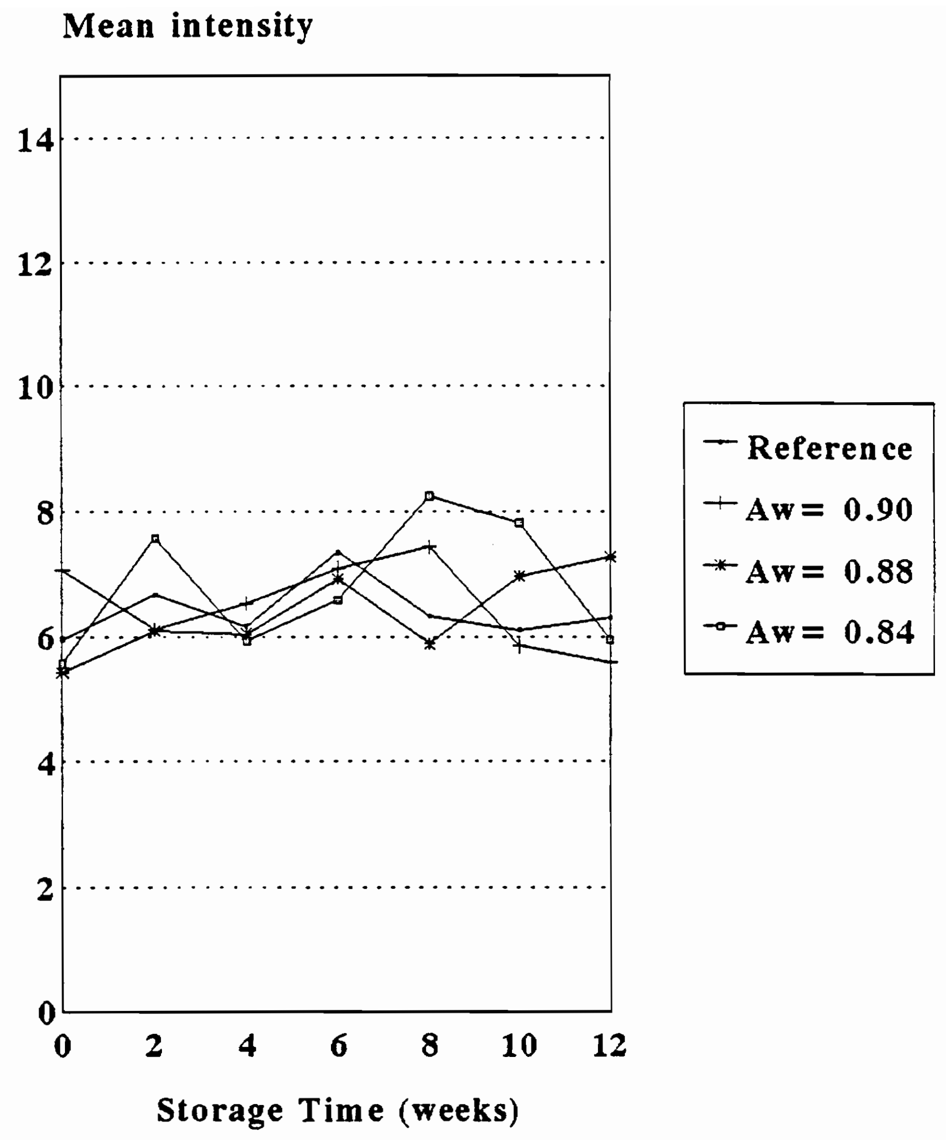

Figure 22: Mean Sensory Score (n=9) for Fermented/fruity Aroma of MAP Romano Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

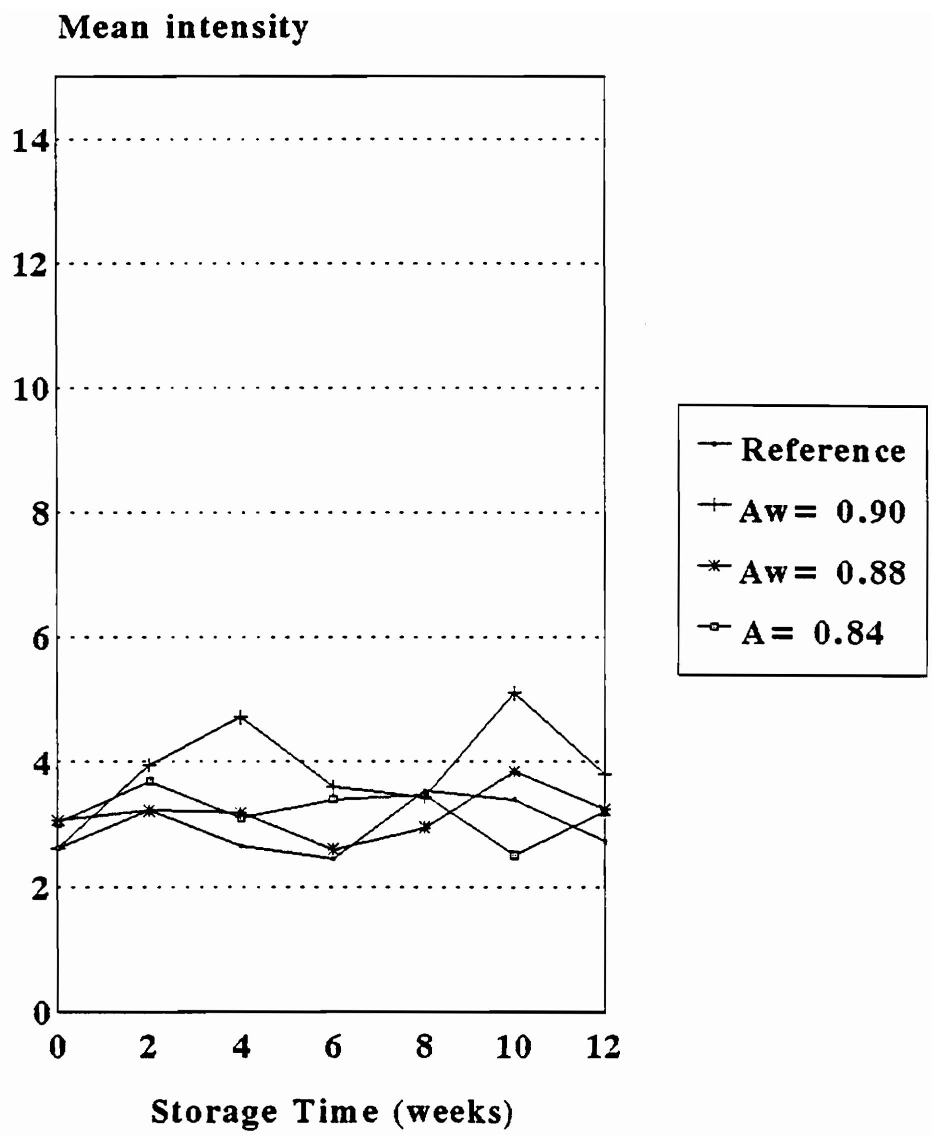

Figure 23: Mean Sensory Score (n=9) for Musty Aroma of MAP Romano Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

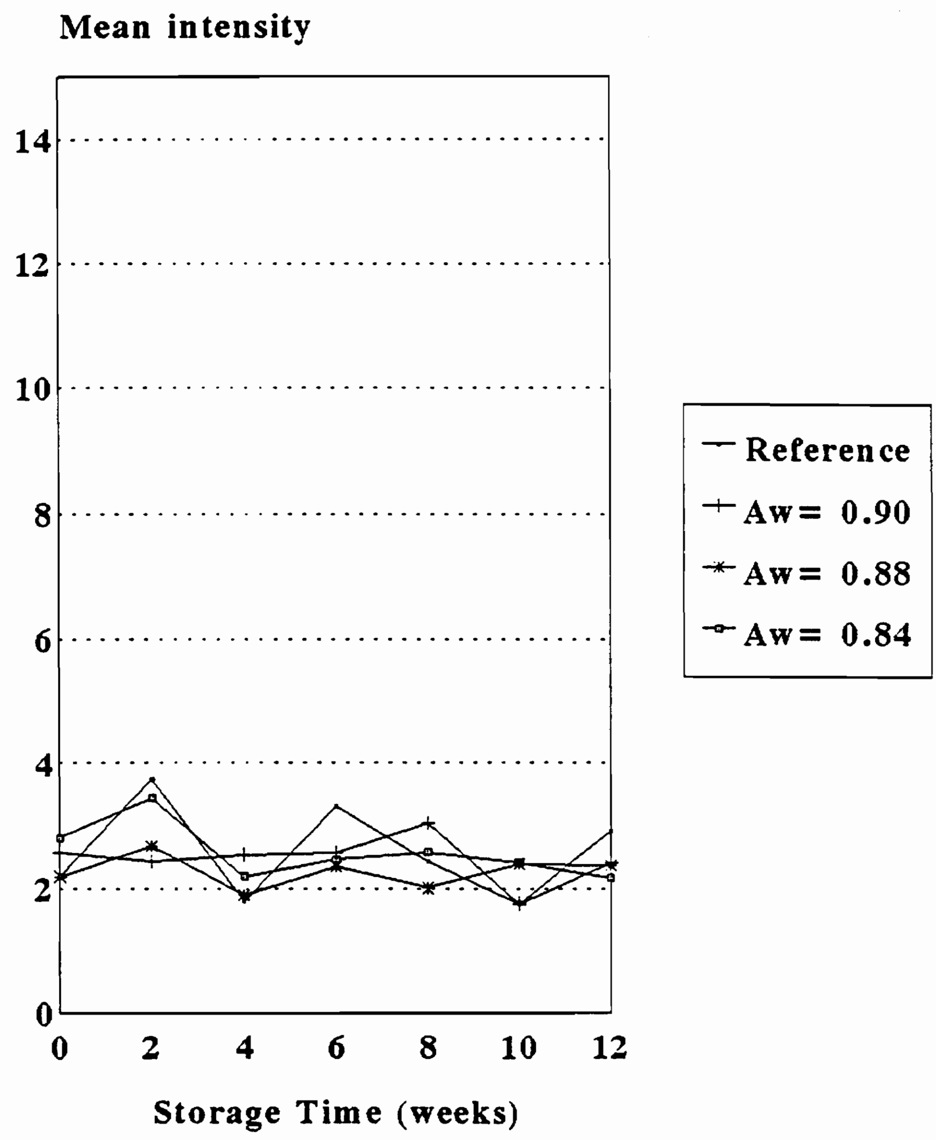

Figure 24: Mean Sensory Score (n=9) for Yeasty Aroma of MAP Romano Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

4.3 Flavor Descriptors

Parmesan

Two descriptors, flavor impact and unclean, describe the flavors present in hard grated cheeses. Flavor impact defined the overall perception of flavor intensity. Butyric acid and minor branched fatty acids that occur in milk appear to contribute to the piquant flavor of Parmesan (Nath, 1992). Unclean defined the degradation of flavor or negative off-flavor, i.e., lingering unpleasant aftertaste. Too high a free fatty acid level in cheese gives a strong, soapy, undesirable flavor (Nath, 1992). Evaluation of flavor descriptors was discontinued after 8 weeks due to high mold and yeast counts.

The flavor intensity of Parmesan cheese did not change with storage time. However, the flavor intensity of the MAP Parmesan with Aw= 0.90 significantly increased after 8 weeks of storage. No differences existed between treatments for the attribute, flavor impact (Figure 25). The perception of an unclean aftertaste did not change over storage time in Parmesan cheese. However, the unclean aftertaste of the MAP Parmesan with Aw= 0.90 significantly increased after 8 weeks of storage (Figure 26). Treatment had no significant affected on the detection of the attribute, unclean.

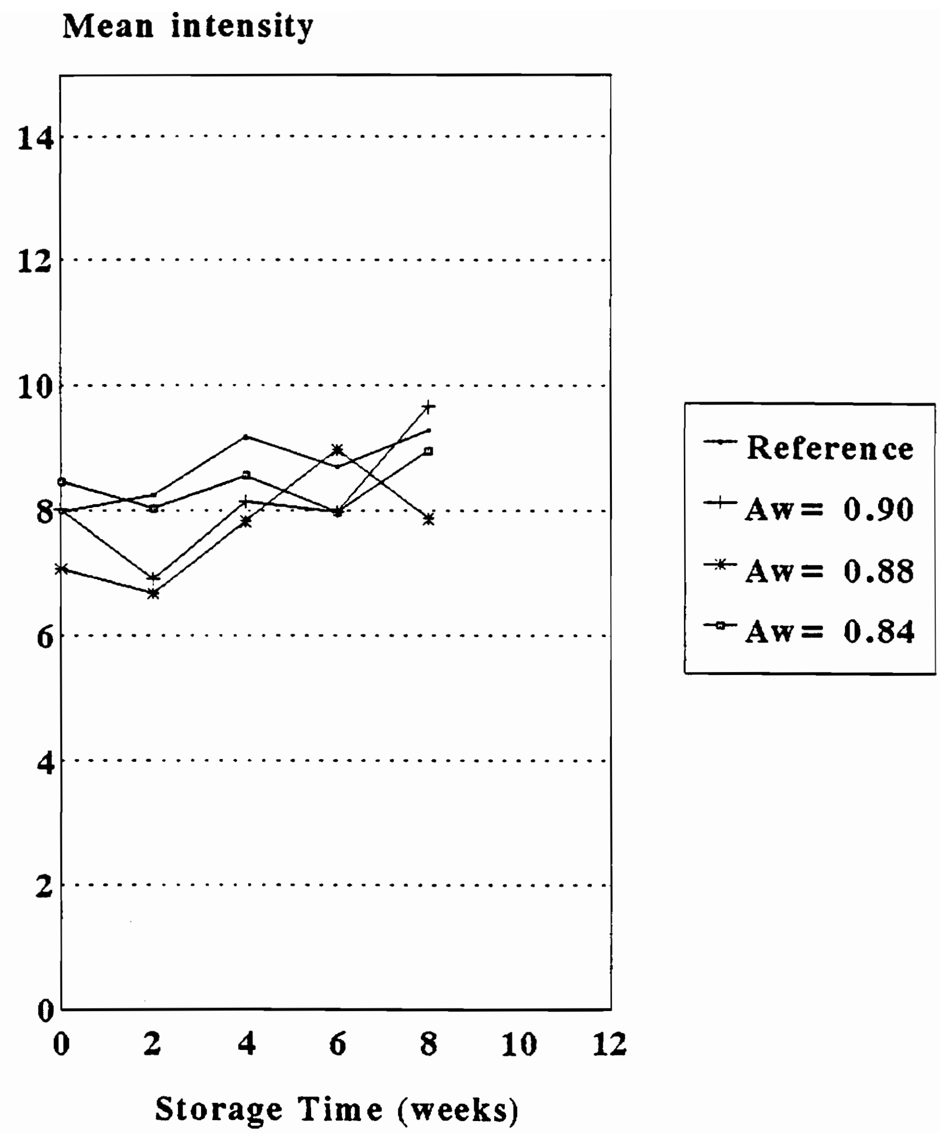

Figure 25: Mean Sensory Score for Flavor Impact of MAP Parmesan Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

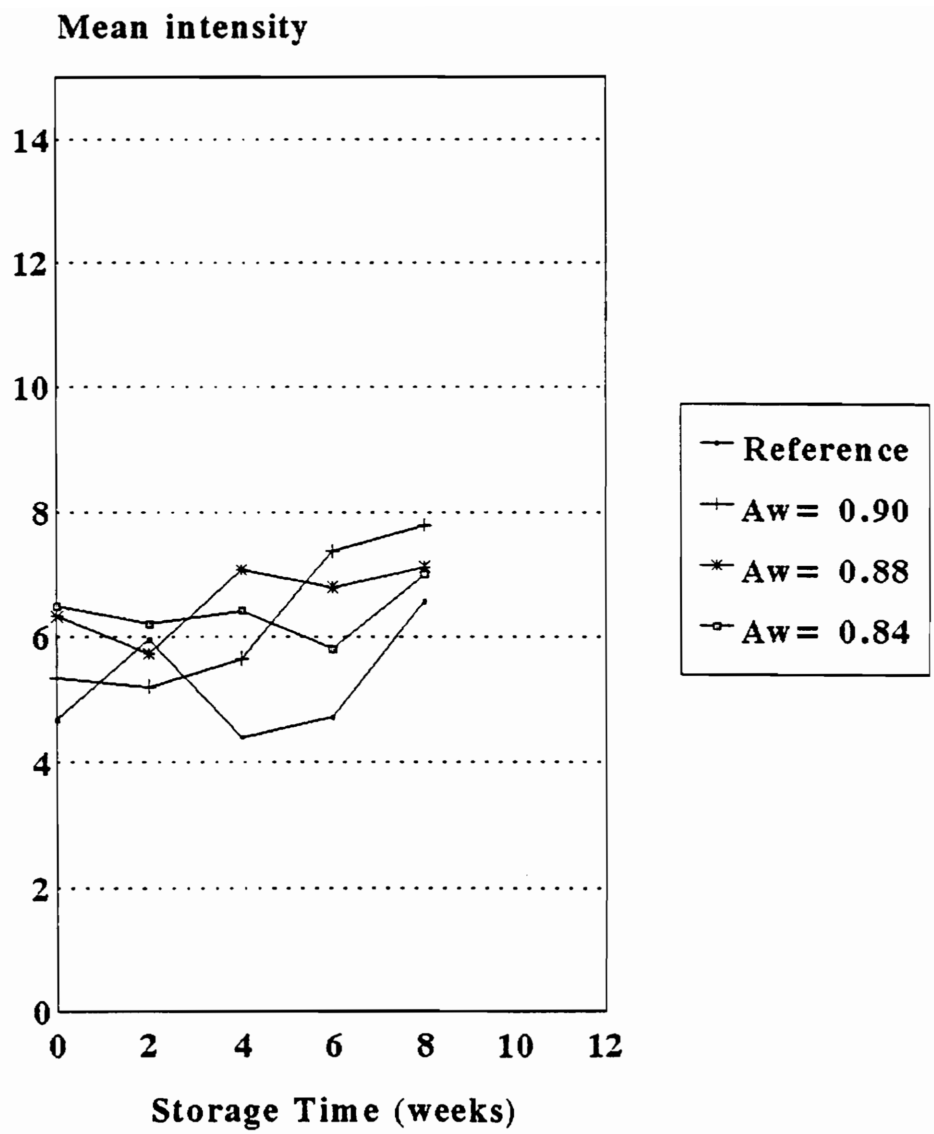

Figure 26: Mean Sensory Score (n=9) for Unclean of MAP Parmesan Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

Romano

The flavor intensity of Romano cheese was not affected by storage time (Figure 27). A peak at week 4 caused significant difference to occur over storage time for MAP Parmesan with Aw= 0.84. For fresh Parmesan, a peak at week 4 caused a significant difference to occur over storage time. Treatment had a significant difference on the perception of the attribute, flavor impact. Storage time had no affect on the detection of the attribute, unclean. However, the perception of the unclean flavor in MAP Parmesan with Aw= 0.90 increased significantly after two weeks of storage. Treatment had caused no affect on the perception of the attribute, unclean (Figure 28).

Parmesan and Romano were very complex cheeses to evaluate. There was no literature was available to assist panelists in characterizing the natural aromas and flavors of the cheeses. Agreement on the intensity of the attributes present in cheese samples was a major problem. More training with references to re-enforce attribute intensity levels would have helped combat this problem. However, there was no time available for extra training sessions. The sensory study consumed an entire semester and required panelists to evaluate cheese twice a week for 12 weeks, causing sever fatigue. The variability among panelists caused interactions between time

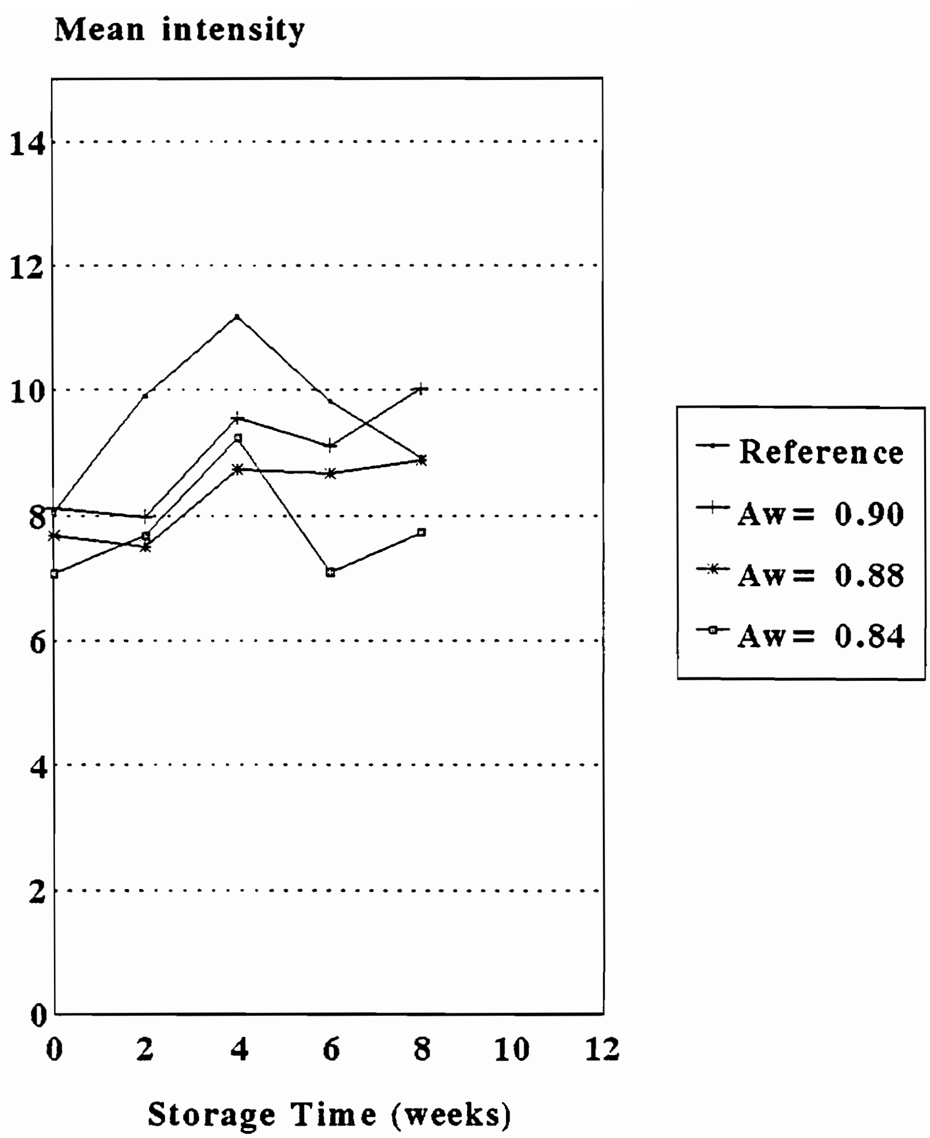

Figure 27: Mean Sensory Score (n=9) for Flavor Impact of MAP Romano Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

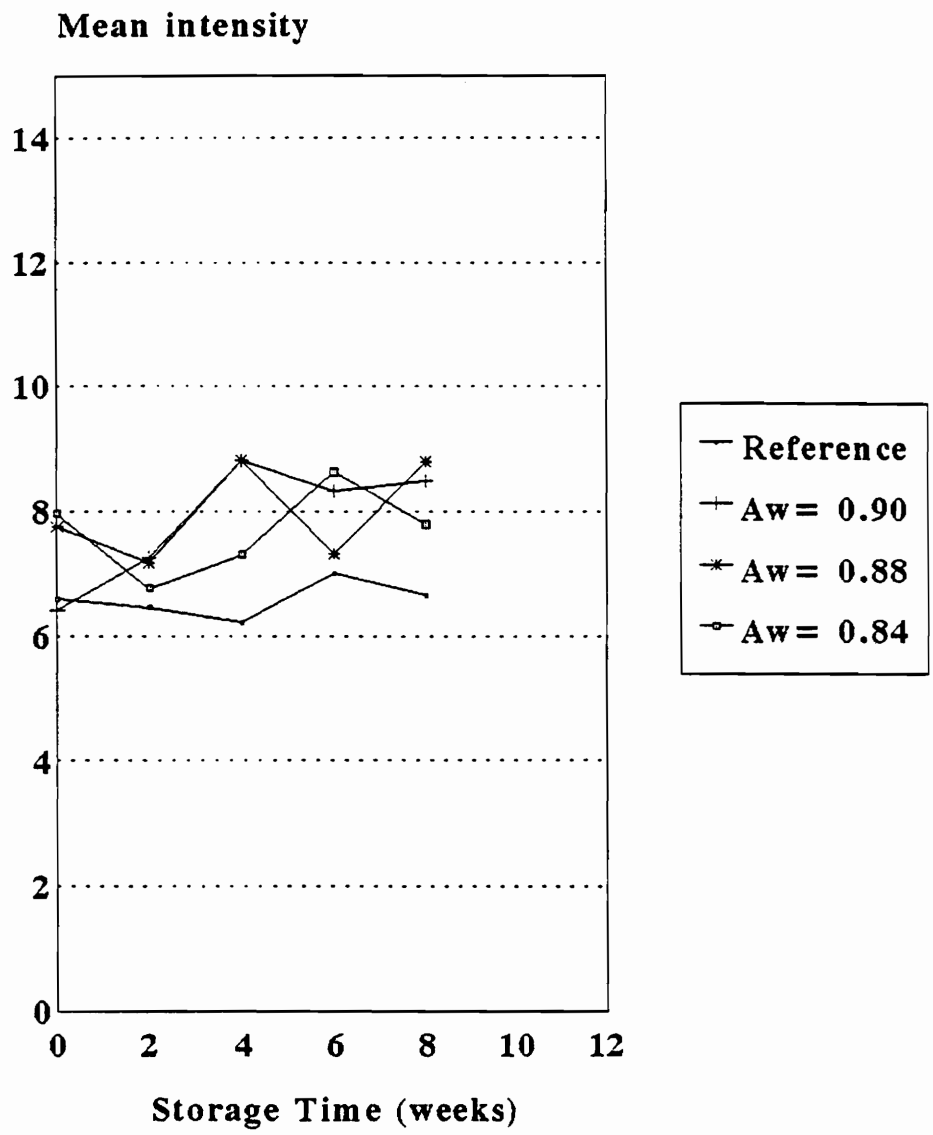

Figure 28: Mean Sensory Score (n=9) for Unclean of MAP Romano Cheese Stored at 23°C. Reference Sample Vacuum Packaged on Day 0 and Stored at Refrigeration, 4°C.

and/or treatment for most attributes. Therefore, panelists could not distinguish any differences between the fresh cheese sample and the cheese samples with Aw levels of 0.90, 0.88 and 0.84 packaged under MAP. Several panelists were able to pick out the cheese with the lowest Aw level from the sample set.

5. Color Analysis of Hard Grated Cheeses

Color analysis was conducted to determine if any differences existed between treatments over storage time. In this CIE color system, L* indicated lightness and a* and b* were the chromaticity coordinates, which represents the hue and chroma of a compound.

Lightness (L*) was measured on a scale of 0-100 (0=black; 100=white); with shades of gray in between. MAP treatment had a significant ($p < 0.01$) effect on Parmesan cheese lightness. As seen in Figure 29, the trend for lightness was the same consistent for each treatment over the storage period. CIE a* values were measured on a scale of +60 to -60, +60 considered red and -60 considered green. Storage time has a significant ($p < 0.01$) effect on the CIE a* values for Parmesan cheese (Figure 30). The CIE a* values observed for MAP packaged Parmesan cheese ranged between -4.60 to -1.81, indicating that Parmesan cheese remained on the green side of the scale rather than the red. CIE b* values were measured on a scale of +60

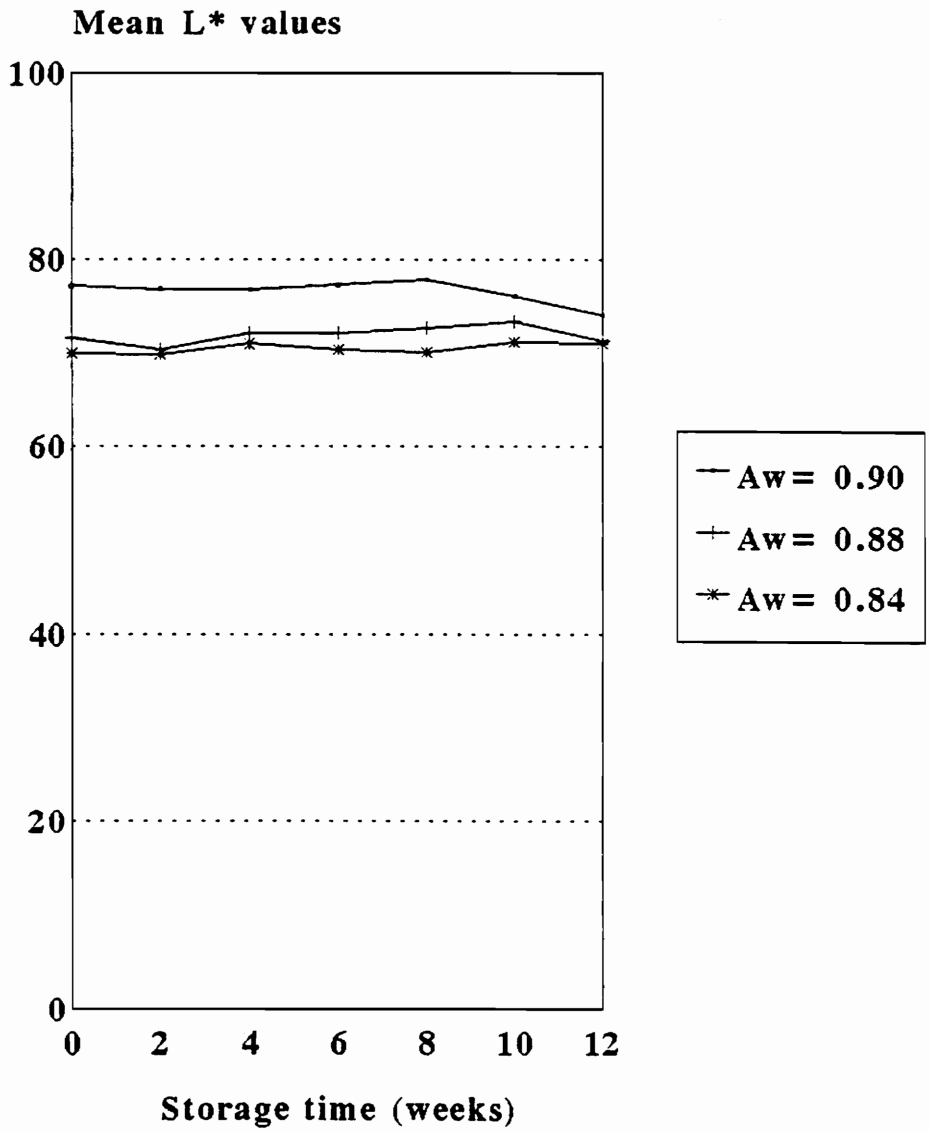

Figure 29: L* Color Values for MAP Parmesan Stored at 23°C.

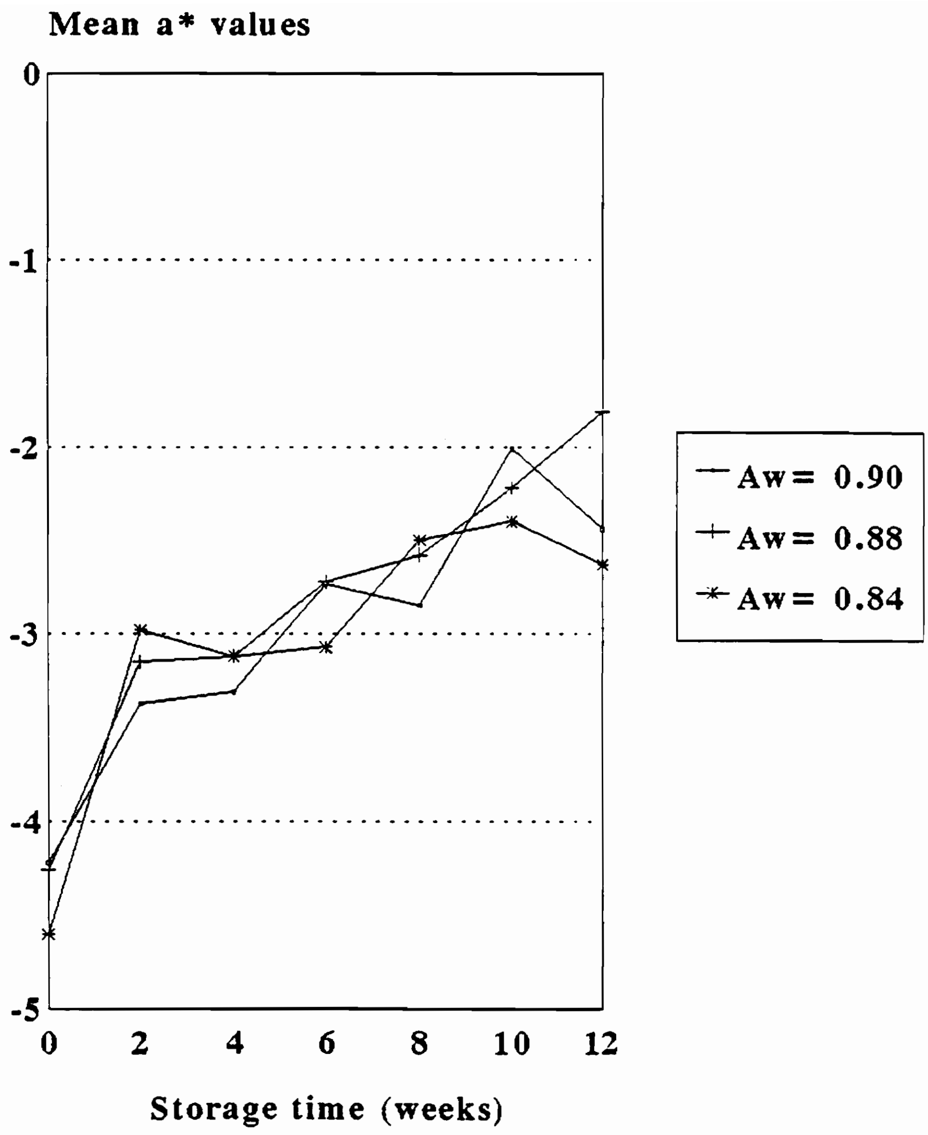

Figure 30: a* Color Values for MAP Parmesan Stored at 23°C.

to -60, +60 considered yellow and -60 considered blue. Storage time and MAP treatment had a significant ($p<0.01$) effect on CIE b* values for Parmesan cheese. The CIE b* values for MAP packaged Parmesan cheese ranged between 16.34 to 22.55, indicating that Parmesan cheese leans towards the yellow side of the scale (Figure 31).

Storage time and MAP treatment had a significant ($p< 0.01$) effect on Romano cheese lightness. As seen in Figure 32, the L* values of MAP treatments increased slightly over storage time. Storage time had a significant ($p< 0.01$) effect on the a* values of Romano cheese. The CIE a* values observed for MAP packaged Romano cheese ranged between -5.43 to -2.75, a* values increased slightly over storage time (Figure 33). Interaction ($p< 0.01$) between storage time and MAP treatment gave no explanation on b* values for Romano cheese (Figure 34).

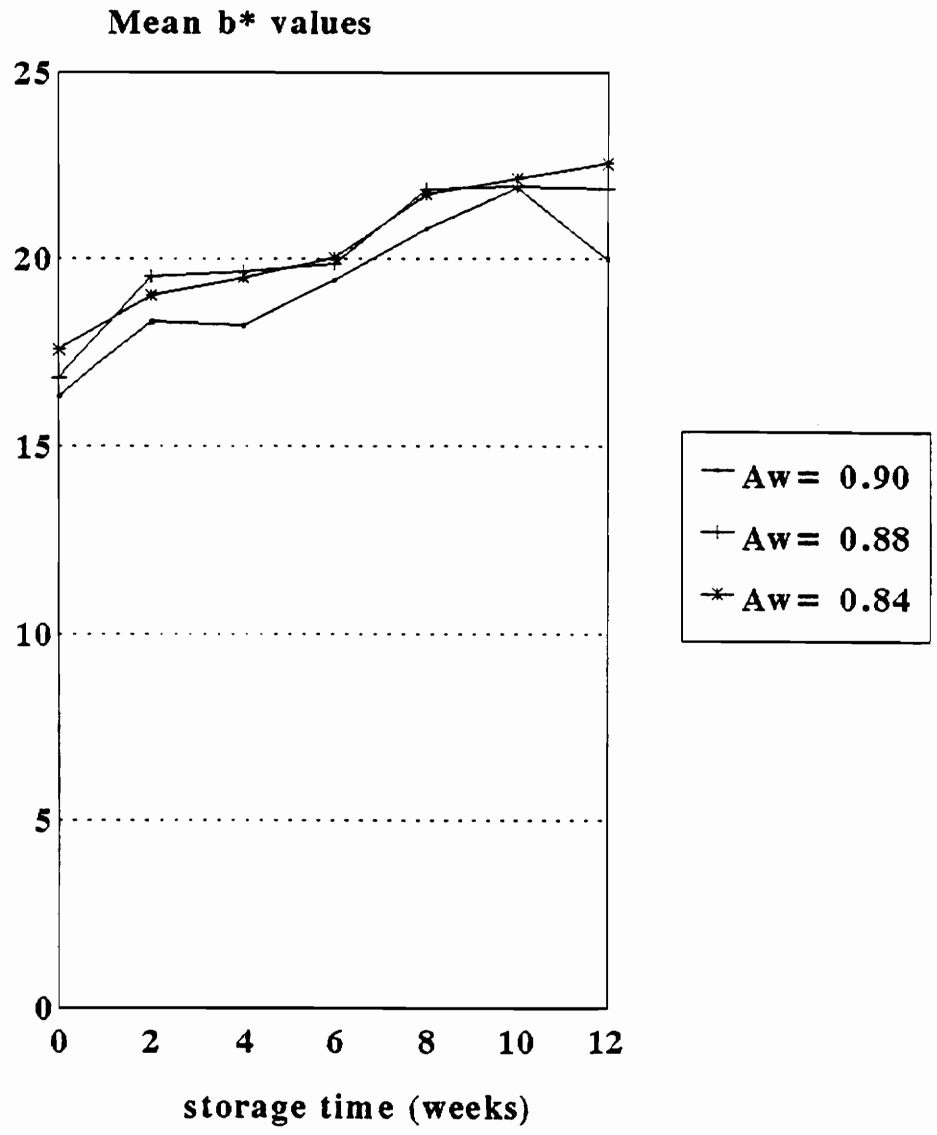

Figure 31: b* Color Values for MAP Parmesan Stored at 23°C.

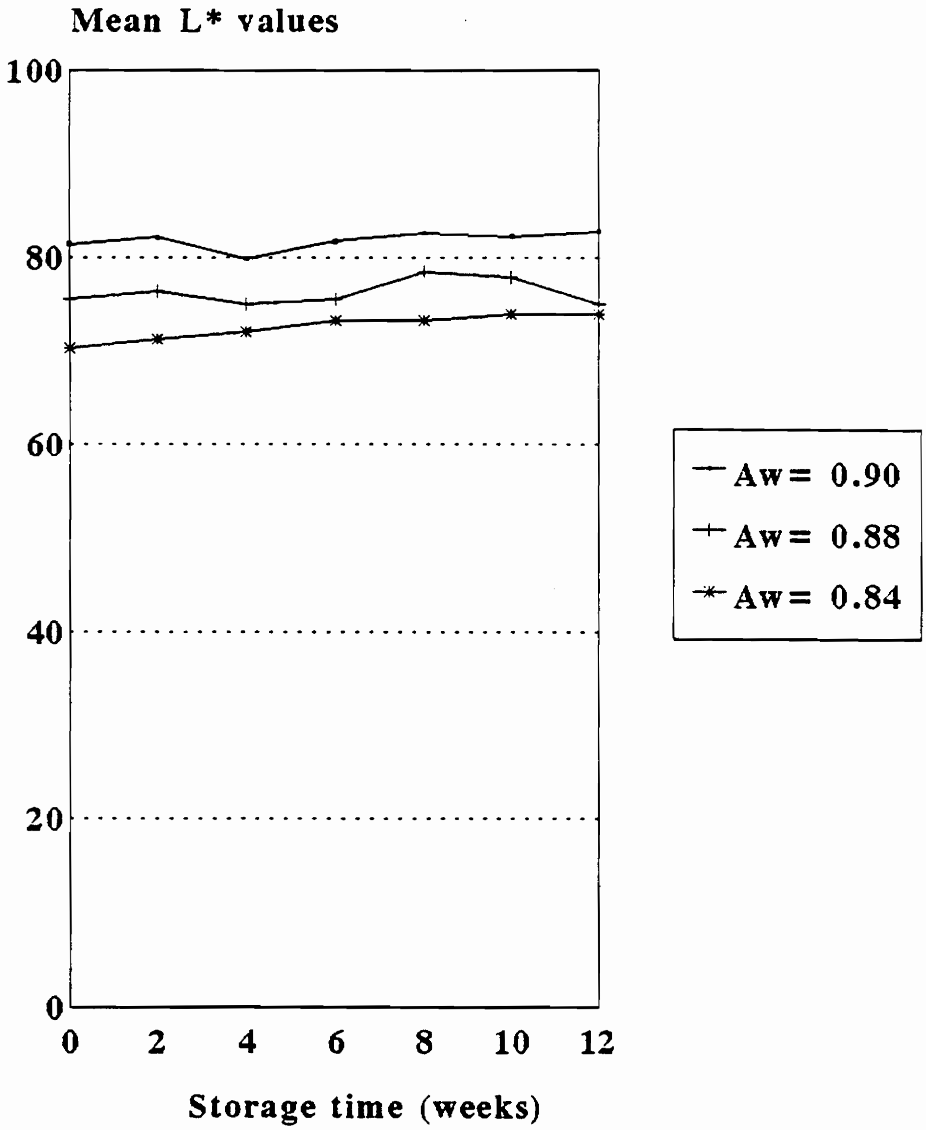

Figure 32: L* Color Values for MAP Romano Stored at 23°C.

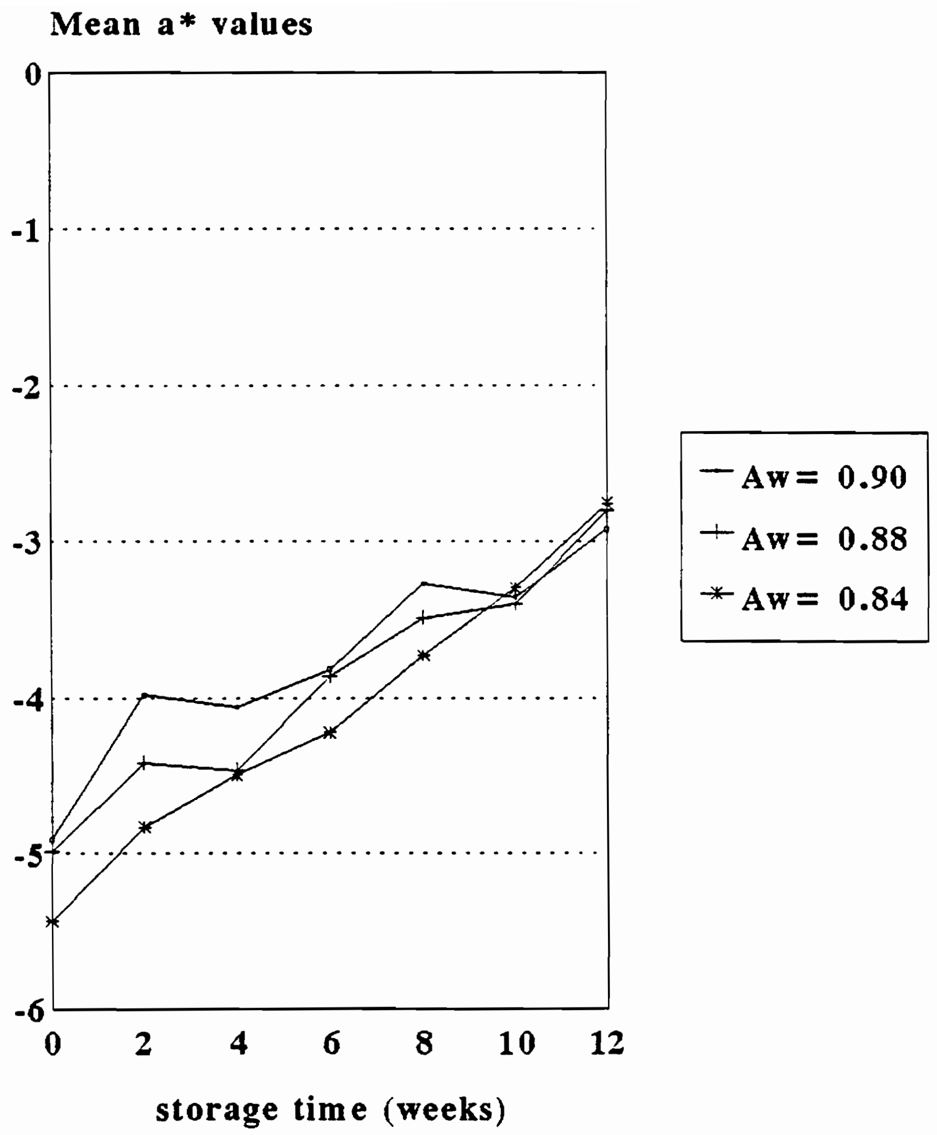

Figure 33: a* Color Values for MAP Romano Stored at 23°C.

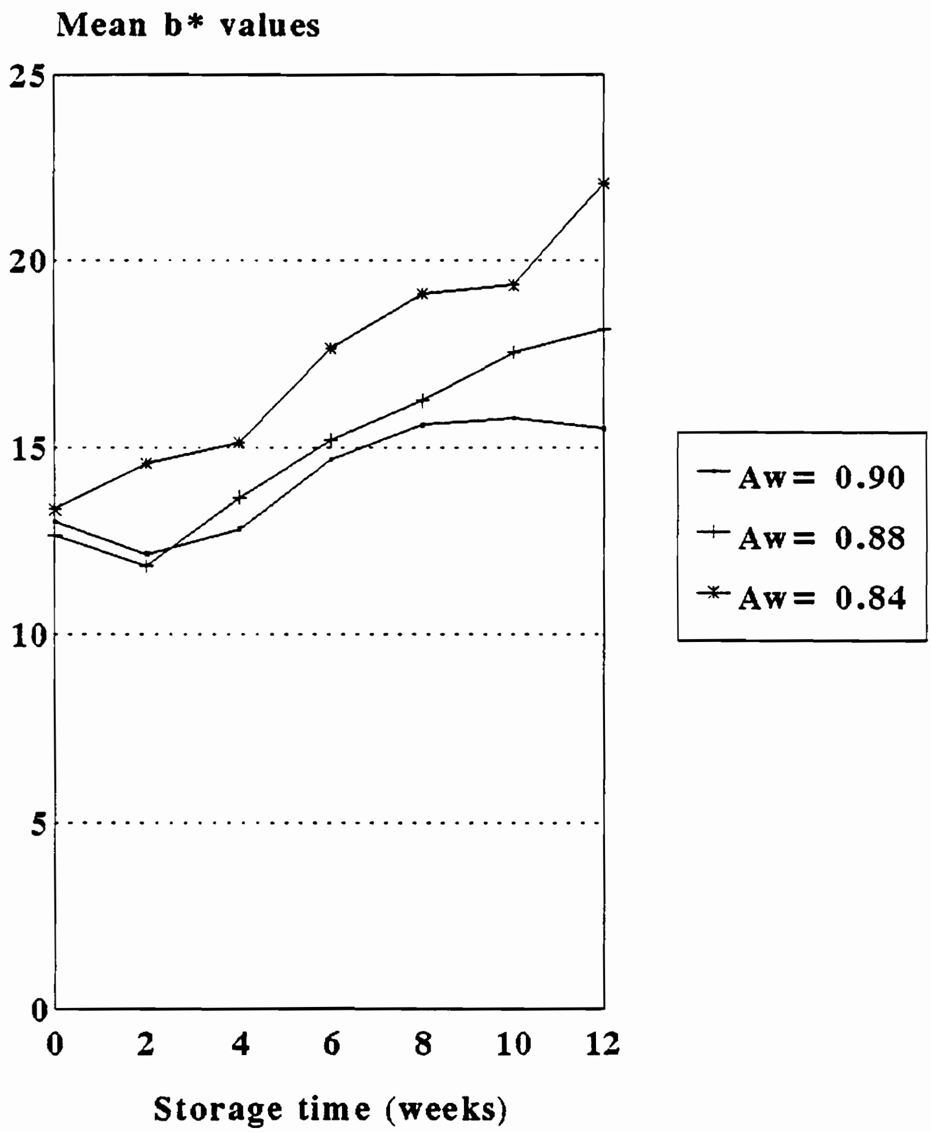

Figure 34: b* Color Values for MAP Romano Stored at 23°C.

V. SUMMARY AND CONCLUSIONS

This research project was conducted to develop a safe, shelf-stable, high quality, hard grated cheese that did not require drying, preservatives or refrigeration during distribution and sale.

In the challenge study with Staphylococcus aureus, the headspace gas composition changed significantly over storage time. The growth of mold and yeast on the cheese samples was the result of O_2 migrating into the packaging.

Parmesan cheese samples with Aw= 0.86 and below were able to suppress the growth of S. aureus. The high Aw cheese samples provided a rich environment for the growth of S. aureus. The level of S. aureus observed in the high Aw cheese posed a health threat because of the production of enterotoxin. However, the risk was reduced as growth of the microbe in high Aw cheese diminished over storage time. Growth of S. aureus was suppressed on Romano cheese. A possible explanation is that high concentration of volatile free fatty acids present in Romano cheese acted as an antimicrobial agent.

Sensory evaluation was conducted to determine the shelf-stability and quality of MAP packaged hard grated cheeses. For Parmesan cheese the following attributes: acrid, yeasty, flavor impact and unclean, tended to increase over storage time. The buttery attribute decreased over storage time for

Parmesan cheese. The treatments caused a significant difference to exist for the attributes, butyric acid and fermented/fruity, in Parmesan cheese. For Romano cheese, the perception of acrid, butyric acid and flavor impact significantly increased over storage time. The detection of musty, flavor impact and unclean varied significantly among treatments. Although the QDA panel was trained, the perception of the attributes varied widely among panelists.

In future sensory evaluation studies involving cheese, more time will be needed to develop a descriptive vocabulary and train panelists with reference standards. Sensory evaluation should be limited to one product. Parmesan and Romano cheese were very different cheeses. QDA may not be the correct sensory methodology for cheese. The complexity of the products confused panelists and caused misjudgments.

Storage time and treatment had a significant effect on the cheese color. The drying procedure caused the cheeses to lose their natural color.

VI. REFERENCES

Anonymous, 1993. S&R makes superior Italian cheese, great reputations and a wonderful partner. Plymouth, WI.

Anonymous, 1992. Cheese facts, 1st ed. National Cheese Institute, Washington, D.C.

Anonymous, 1990. Official Methods of Analysis, 15th ed. Association of Analytical Chemists, Washington, D.C.

Baker, R.C., Qureshi, R.A., and Hotchkiss, J.H. 1986. Effect of an elevated level of carbon dioxide containing atmosphere on the growth of spoilage and pathogenic bacteria at 2^0, 7^0, and 13^0C. Poultry Sci. 65:729.

Brody, A.L. 1989. Microbiological safety of modified/controlled atmosphere/vacuum packaged foods. In Controlled/Modified Atmosphere/Vacuum Packaging of Foods. Aaron L Brody (ed.). Food & Nutrition Press, Inc., Trumbull, CT. pp 159-174.

Case, R.A., Bradley, R.L., Jr., and Williams, R.R. 1985. Chemical and physical method, Ch. 18. In Standard Methods for the Examination of Dairy Products, 15th ed. Richardson, G.H. ed. American Public Health Association, Washington, D.C. pp. 347-351.

Daniels, J.A., Krishnamurthi, R., and Rizvi, S.H. 1985. A review of effects of carbon dioxide on microbial growth and food quality. J. Food Prot. 48:532.

Dixon, N.M. and Kell, D.B. 1989. The inhibition by CO_2 of the growth and metabolism of micro-organisms. J. Appl. Bact. 67:109.

Enfors, S.O. and Molin, G. 1980. Effect of high concentrations of carbon dioxide on growth rate of Pseudomonas fragi, Bacillus cereus, and Streptococcus cremoris. J. Appl. Bacteriol. 48:409.

Farber, J.M. 1991. Microbiological aspects of modified-atmosphere packaging technology- A review. J. Food Prot. 54:58.

Fox P.F. and Guinee, T.P. 1987. Italian cheeses, Ch. 10. In Cheese: chemistry, physics and microbiology. Vol. 2. Fox, P.F. (ed.) Elsevier Applied Science, NY. pp. 221-255.

Genigeorgis, C.A. 1985. Microbial and safety implications of the use of modified atmospheres to extend the storage life of fresh meat and fish. Int. J. Food Microbiol. 1:237.

Gill, C.O. and Tan, K.H. 1979. Effect of carbon dioxide on growth of Pseudomonas fluorescens. Appl. Env. Micro. 38:237.

Gill, C.O. and Tan, K.H. 1980. Effect of carbon dioxide on growth of meat spoilage bacteria. Appl. Env. Micro. 39:317.

Hintlian, C.B. and Hotchkiss, J.H. 1986. The safety of modified atmosphere packaging: A review. Food Tech. 40(12):70.

Hintlian, C.B. and Hotchkiss, J.H. 1987. Comparative growth of spoilage and pathogenic organisms on modified atmosphere-packaged cooked beef. J. Food Prot. 50:218.

Honer, C. 1988. Closing the freshness gap with CAP and MAP. Dairy Foods 89: 53.

Hotchkiss, J.H. 1988. Experimental approaches to determining the safety of food packaged in modified atmospheres. Food Tech. 42(9):55.

Huffman, D.L., Davis, K.A., Marple, D.N., and McGuire, J.A. 1975. Effect of gas atmosphere on microbial growth, color, and pH of beef. J. Food Sci. 40:1229.

King, A.D. and Nagel, C.W. 1967. Growth inhibition of Pseudomonas by carbon dioxide. J. Food Sci. 32:575.

King, A.D. and Nagel, C.W. 1975. Influence of carbon dioxide upon the metabolism of Pseudomonas aeruginosa. J. Food Sci. 40:362.

Kosikowski, F.V. 1982. Hard Italian cheese, Ch. 12. In Cheeses and Fermented Milk Foods. F.V. Kosikowski and Associates, NY. pp. 231-227.

Kosikowski, F.V. and Brown, D.P. 1982. Influence of carbon dioxide and nitrogen on microbial populations and shelf life of cottage cheese and sour cream. J. Dairy Sci. 56:12.

Koski, D.V. 1988. Is current modified/controlled atmosphere packaging technology applicable to the U.S. food market? Food Tech. 42:54.

Lancette, G.A. and Tatini, S.R. 1992. Staphylococcus aureus, Ch. 33. In Compendium of Methods for the Microbiological Examination of Foods. Vanderzant, C. and Splittstoesser, D.F. ed. American Public Health Association, Washington, D.C. pp. 533-592.

Law, B.A. and Mabbit, L.A. 1983. New methods for controlling the spoilage of milk and milk products. In Food Microbiology: Advances and Prospects. Roberts, T.A. and Skinner, F.A. (eds.). pp. 131-150.

Maniar, A. 1991. Use of modified atmosphere technology to maintain quality of direct-set cottage cheese. M.S. thesis. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Manning, D.J. and Nursten, H.E. 1992. In Developments in Dairy Chemistry, Vol. 3. Fox, P.F., ed. Elsevier Applied Science Publishers, NY. pp 234.

Mislivec, P.B., Beuchat, L.R., and Cousin, M.A. 1992. Yeasty and mold, Ch. 16. In Compendium of Methods for the Microbiological Examination of Foods. Vanderzant, C. and Splittstoesser, D.F. ed. American Public Health Association, Washington, D.C. pp. 533-592.

Nath, K.R. 1992. Cheese, Ch. 3. In Dairy Science and Technology Handbook, Vol. 2 Hui, Y.H., ed. VCH Publishers, NY. pp. 228-229.

Scott, R. 1986. Cheesemaking Practice, 2nd ed. Elsevier Applied Science, NY.

Sears, D.F. and Eisenberg, R.M. 1961. A model representing the physiological role of CO_2 at the cell membrane. J. Gen. Phys. 44:869.

Selman, J.N. 1987. New methods of preservation. Food Manuf. 62(7):52.

Silliker, J.H. and Wolfe, S.K. 1980. Microbiological safety considerations in controlled-atmosphere storage meats. Food Tech. 34(3):59.

Valley, G. and Rettger, L.F. 1927. The influence of carbon dioxide on bacteria. J. Bacteriology. 14(2):101.

Wolfe, S.K. Use of Co- and CO_2- enriched atmospheres for meats, fish, and produce. Food Tech. 34(3):55.

VII. APPENDIX

Appendix A. Headspace gas analysis on MAP packaged Parmesan cheese

Means (n=4) and Standard Deviation for Carbon Dioxide Content within MAP Parmesan Packages

Time	Aw= 0.90 Mean± S.D.	Aw= 0.88 Mean± S.D.	Aw =0.86 Mean± S.D.	Aw= 0.84 Mean± S.D.	Aw= 0.75 Mean± S.D.
0	28.2±0.9^{a}	31.1 ±0.9^{a}	29.7 ±1.9^{a}	26.3 ±4.0^{a}	27.5±1.7^{a}
1	23.3±1.3^{b}	24.9 ±0.6^{b}	23.8 ±1.1^{b}	23.3 ±1.8^{b}	23.0±1.1^{b}
2	16.1±13.5^{c}	24.2 ±6.7^{b}	19.9 ±2.9^{cd}	20.3 ±2.9^{cd}	20.3±1.8^{c}
3	26.9±1.7^{d}	27.0 ±2.7^{c}	21.6 ±0.5^{c}	21.8 ±0.8^{bc}	22.1±0.7^{bd}
4	25.1±2.3^{e}	23.3 ±4.7^{b}	20.0 ±0.1^{c}	17.8 ±1.5^{de}	20.6±1.5^{cd}
5	18.8±1.3^{f}	21.3 ±3.2^{d}	18.6 ±0.5^{d}	16.8 ±0.6^{e}	19.9±2.9^{c}
6	18.5±1.1^{f}	18.6 ±0.9^{e}	15.2 ±2.1^{e}	12.7 ±1.1^{f}	16.3±2.9^{e}
7	15.2±0.4^{c}	15.8 ±1.3^{f}	13.7 ±0.6^{e}	12.6 ±0.7^{f}	16.0±1.7^{e}
8	16.0±2.6^{c}	14.4 ±0.4^{g}	14.4 ±2.0^{e}	10.5 ±0.3^{fg}	12.2±4.3^{f}
9	12.8±2.1^{g}	13.6 ±3.1^{g}	10.9 ±0.2^{f}	8.3 ±0.3^{gh}	15.9±1.2^{e}
10	9.0±0.5^{h}	11.6 ±4.2^{h}	8.8 ±1.5^{f}	7.6 ±0.4^{h}	11.7±1.3^{f}
11	9.3±2.3^{h}	8.3 ±1.8^{i}	9.3 ±1.9^{f}	9.6 ±1.6^{gh}	10.4±2.1^{g}
12	10.4±2.1^{i}	9.4 ±0.8^{j}	7.9 ±1.3^{f}	8.5 ±0.2^{gh}	9.2±3.0^{h}

Means within a column with different letters are significantly different ($P < 0.05$) for time.

Means (n=4) and Standard Deviation for Nitrogen Content within MAP Parmesan Packages

Time	Aw= 0.90 Mean± S.D.	Aw= 0.88 Mean± S.D.	Aw =0.86 Mean± S.D.	Aw= 0.84 Mean± S.D.	Aw= 0.75 Mean± S.D.
0	71.8 ±0.9^{a}	68.9 ±0.9^{a}	70.3 ±1.9^{a}	73.7 ±3.9^{a}	72.5±1.7^{a}
1	76.7 ±1.3^{b}	75.1 ±0.6^{b}	76.2 ±1.1^{b}	76.7 ±1.8^{ab}	77.0±1.1^{b}
2	75.2 ±1.2^{bc}	75.8 ±6.7^{b}	80.1 ±2.9^{c}	79.7 ±2.9^{bcd}	79.7±1.8^{cd}
3	73.6 ±2.4^{ac}	73.0 ±2.7^{c}	78.4 ±0.5^{bc}	78.2 ±0.8^{bc}	77.9±0.7^{bc}
4	74.9 ±2.3^{bc}	77.2 ±5.4^{d}	79.9 ±0.1^{c}	82.2 ±1.5^{cde}	79.4±1.5^{ce}
5	81.2 ±1.3^{d}	78.7 ±3.2^{e}	81.3 ±0.5^{cd}	83.2 ±0.6^{de}	80.1±2.9^{de}
6	81.5 ±1.1^{d}	81.4 ±0.9^{f}	84.8 ±2.1^{de}	87.3 ±1.1^{fh}	83.7±2.9^{f}
7	84.8 ±0.4^{e}	84.5 ±1.3^{g}	86.3 ±0.6^{efg}	87.4 ±0.7^{fg}	84.0±1.7^{f}
8	84.0 ±2.6^{ef}	85.6 ±0.4^{h}	85.6 ±2.0^{eg}	89.5 ±0.3^{f}	84.7±0.7^{f}
9	83.6 ±2.1^{de}	81.9 ±2.2^{f}	89.1 ±0.2^{fh}	83.8 ±0.3^{egi}	84.0±1.2^{f}
10	89.5 ±2.7^{g}	85.3 ±0.7^{gh}	89.3 ±3.4^{h}	89.1 ±3.5^{fj}	88.3±1.3^{g}
11	86.5 ±2.8^{ef}	83.5 ±1.7^{i}	86.7 ±3.1^{efh}	85.3 ±4.3^{chi}	79.9±2.8^{e}
12	88.0 ±2.8^{g}	90.6 ±0.8^{j}	89.0 ±3.1^{fh}	91.5 ±0.2^{j}	89.1±2.3^{g}

Means within a column with different letters are significantly different ($P < 0.05$) for time.

Means (n=2) and Standard Deviation for Oxygen Content within MAP Parmesan Packages

Time	Aw= 0.90 Mean±S.D.	Aw= 0.88 Mean±S.D.	Aw =0.86 Mean±S.D.	Aw= 0.84 Mean±S.D.	Aw=0.75 Mean±S.D.
0	.	.	.	.	.
1	.	.	.	.	.
2	17.3 ±.	.	.	.	.
3	.	.	.	.	.
4	.	.	.	.	.
5	.	.	.	.	.
6	.	.	.	.	.
7	.	.	.	.	.
8	.	.	.	.	6.4 ±0.3
9	7.2 ±0.3	9.0 ±0.5	.	7.9 ±0.6	.
10	6.2 ±.	6.3 ±0.2	7.3 ±0.8	6.7 ±0.7	.
11	8.4 ±1.7	8.3 ±0.3	7.0 ±1.4	10.1 ±0.4	9.7 ±1.7
12	6.1 ±.	.	6.2 ±0.5	.	6.4 ±.

Appendix B. Headspace gas analysis on MAP packaged Romano Cheese

Means (n=2) and Standard Deviation for Carbon Dioxide Content within MAP Romano Packages

Time	Aw= 0.90 Mean± S.D.	Aw= 0.88 Mean± S.D.	Aw =0.86 Mean± S.D.	Aw= 0.84 Mean± S.D.	Aw= 0.75 Mean± S.D.
0	26.4 ±6.3^{a}	30.3 ±0.9^{a}	29.0 ±1.6^{a}	28.3 ±1.2^{a}	21.1 ±8.0^{a}
1	23.8 ±2.1^{b}	24.9 ±0.9^{b}	25.4 ±1.1^{b}	25.3 ±0.6^{b}	18.6 ±9.5^{b}
2	23.6 ±1.4^{c}	26.4 ±0.1^{b}	24.7 ±0.4^{b}	24.3 ±1.5^{b}	25.3 ±0.7^{c}
3	24.1 ±0.7^{b}	23.9 ±0.5^{bc}	24.4 ±0.5^{b}	23.4 ±1.1^{b}	24.5 ±1.3^{c}
4	21.5 ±0.8^{d}	21.1 ±1.0^{d}	20.7 ±1.0^{c}	22.4 ±0.4^{b}	21.1 ±0.4^{a}
5	19.5 ±2.1^{e}	18.6 ±0.8^{e}	19.7 ±0.8^{c}	21.2 ±1.1^{c}	17.4±0.2^{bd}
6	19.5 ±1.9^{e}	16.9 ±1.4^{f}	15.1 ±4.8^{d}	13.9 ±2.9^{d}	13.2 ±1.1^{e}
7	15.8 ±2.7^{f}	16.0 ±0.6^{f}	15.0 ±1.5^{d}	13.9 ±2.1^{d}	16.0 ±0.8^{d}
8	15.0 ±2.9^{g}	14.8 ±0.8^{g}	10.2 ±1.6^{e}	13.6 ±0.6^{d}	9.1 ±1.6^{f}

Means within a column with different letters are significantly different ($P < 0.05$) for time.

Means (n=2) and Standard Deviation for Nitrogen Content within MAP Romano Packages

Time	Aw= 0.90 Mean±S.D.	Aw= 0.88 Mean± S.D.	Aw =0.86 Mean± S.D.	Aw= 0.84 Mean± S.D.	Aw= 0.75 Mean± S.D.
0	73.6 ±6.3^{a}	69.7 ±0.9^{a}	71.0 ±1.6^{a}	71.7 ±1.2^{a}	73.2 ±1.8^{a}
1	76.2 ±2.1^{b}	75.1 ±0.9^{b}	74.6± 1.1^{b}	74.7 ±0.6^{ab}	74.2 ±0.7^{ab}
2	76.4 ±1.4^{c}	73.6 ±0.1^{b}	75.5 ±0.4^{b}	75.7 ±1.5^{bc}	74.7 ±0.7^{ab}
3	75.9 ±0.7^{b}	76.0 ±0.5^{bc}	75.1 ±0.5^{b}	76.6 ±1.1^{bd}	75.5 ±1.3^{b}
4	78.5 ±0.8^{d}	78.9 ±1.0^{d}	79.3 ±1.0^{c}	77.6 ±0.4^{be}	78.9 ±0.4^{bc}
5	80.5 ±2.1^{e}	81.4 ±0.8^{e}	80.3 ±0.8^{c}	78.8 ±1.1^{cde}	82.6 ±0.2^{cd}
6	80.5 ±1.9^{e}	83.1 ±1.4^{f}	84.9 ±4.8^{d}	84.0 ±3.5^{f}	82.7 ±4.6^{cd}
7	84.1 ±2.7^{f}	84.0 ±0.6^{f}	85.0 ±1.5^{d}	86.1 ±2.1^{f}	84.0 ±0.8^{d}
8	85.0 ±2.9^{g}	85.2 ±0.8^{g}	85.2 ±3.8^{d}	86.4 ±0.6^{f}	80.3 ±2.3^{cd}

Means within a column with different letters are significantly different (P< 0.05) for time.

Means (n=2) and Standard Deviation for Oxygen Content within MAP Romano Packages

Time	Aw= 0.90 Mean±S.D.	Aw= 0.88 Mean±S.D.	Aw =0.86 Mean± S.D.	Aw= 0.84 Mean± S.D.	Aw= 0.75 Mean± S.D.
0	.	.	.	.	11.4 ±1.5
1	.	.	.	.	14.4 ±.
2	.	.	.	.	.
3	.	.	.	.	.
4	.	.	.	.	.
5	.	.	.	.	.
6	.	.	.	8.4 ±.	8.1 ±1.2
7	.	.	.	.	.
8	.	.	9.3 ±1.4	.	12.9 ±2.7

Appendix C. Staphylococcus aureus Growth and Survival on MAP Parmesan Cheese

Means (n=2) and Standard Deviation for Staphylococus aureus growth and survival on MAP Parmesan and Romano Cheese

Time	Aw= 0.90 Mean± S.D.	Aw= 0.88 Mean± S.D.	Aw =0.86 Mean± S.D.	Aw= 0.84 Mean± S.D.	Aw= 0.75 Mean± S.D.
0	0.0^{a}	1.94 ±0.3^{a}	2.90 ±1.0^{a}	2.20 ±2.0^{a}	2.15 ±0.2^{a}
1	3.81 ±0.2^{b}	3.73 ±0.2^{a}	4.07 ±0.1^{a}	4.00 ±3.9^{b}	3.70 ±0.4^{b}
2	4.17 ±0.0^{bd}	4.07 ±0.0^{b}	0.65 ±0.9^{b}	2.01 ±1.3^{ac}	1.83 ±1.2^{a}
3	6.17 ±0.3^{cd}	4.29 ±0.2^{b}	0.75 ±1.0^{b}	0.0^{d}	0.0^{c}
4	5.69 ±0.6^{cd}	4.74 ±0.5^{b}	0.0^{c}	0.0^{d}	3.67±0.4^{bd}
5	3.66 ±0.4^{bd}	4.27 ±0.0^{ab}	0.0^{c}	0.75 ±1.0^{cd}	0.0^{c}
6	1.93 ±2.7^{abc}	4.94 ±0.8^{b}	0.0^{c}	0.95 ±1.3^{acd}	0.0^{c}
7	1.56 ±2.2^{abc}	3.43 ±1.6^{ab}	0.0^{c}	0.0^{d}	0.0^{c}
8	2.79 ±0.8^{bc}	0.89 ±1.2^{a}	0.0^{c}	0.70 ±1.0^{cd}	0.0^{c}
9	0.95 ±1.3^{a}	2.11 ±0.7^{ab}	0.85 ±1.2^{c}	0.0^{d}	0.0^{c}
10	0.0^{a}	0.59 ±0.8^{a}	0.0^{c}	0.0^{d}	0.50 ±0.7^{d}
11	0.0^{a}	0.0^{a}	2.72 ±2.7^{ab}	0.0^{d}	1.50±0.0^{acd}
12	0.0^{a}	1.03 ±1.4^{a}	0.0^{c}	0.0^{d}	0.97±1.3^{acd}

Means within a column with different letters are significantly different ($P < 0.05$) for time.

Means (n=2) and Standard Deviation for *Staphylococcus aureus* Growth and Survival on MAP Romano Cheese

Time	Aw= 0.90 Mean$\pm$ S.D.	Aw= 0.88 Mean$\pm$ S.D.	Aw =0.86 Mean$\pm$ S.D.	Aw= 0.84 Mean$\pm$ S.D.	Aw= 0.75 Mean$\pm$ S.D.
0	2.49 ± 0.7^{a}	1.00 ± 1.4^{a}	1.77 ± 2.5	1.15 ± 1.6^{a}	0.85 ± 1.2^{a}
1	1.09 ± 1.5^{b}	2.96 ± 0.1^{b}	1.20 ± 1.7	2.96 ± 0.5^{b}	3.39 ± 0.6^{b}
2	0^{b}	1.00 ± 1.4^{a}	0	1.40 ± 0.1^{a}	1.38 ± 1.9^{a}
3	1.53 ± 0.2^{ac}	0^{a}	0	0^{ac}	0^{a}
4	0^{b}	0.50 ± 0.7^{a}	0	0^{ac}	0^{a}
5	0^{b}	0	0	0^{ac}	0a
6	0^{b}	0^{a}	0	0.50 ± 0.7^{a}	0^{a}
7	0.50 ± 0.7^{bc}	1.24 ± 0.0^{a}	0	0^{ac}	0^{a}
8	0^{b}	0^{a}	0.59 ± 0.8	0^{ac}	0^{a}

Means within a column with different letters are significantly different ($P < 0.05$) for time.

Appendix D. Mold and Yeast Growth and Survival on MAP Parmesan and Romano Cheese

Means (n=2) and Standard Deviation for Mold Growth and Survival on MAP Parmesan Cheese

Time	Aw= 0.90 Mean± S.D.	Aw= 0.88 Mean± S.D.	Aw =0.86 Mean± S.D.	Aw= 0.84 Mean± S.D.	Aw= 0.75 Mean± S.D.
0					
1					
2	0.50 ± 0.7	0.50 ± 0.7	0.50 ± 0.7	1.33 ± 0.5^{a}	1.68 ± 0.2^{a}
3	1.30 ± 0.4	0.59 ± 0.8	0.59 ± 0.8	0^{b}	0^{b}
4	1.30 ± 0.4	0.59 ± 0.8	0.59 ± 0.8	0^{b}	0^{b}
5	0	0	0	0^{b}	0^{b}
6	1.09 ± 0.1	0	0.50 ± 0.7	0^{b}	0^{b}
7	0	0	0	0.50 ± 0.7^{b}	1.00 ± 0.0^{bc}
8	0.50 ± 0.7	0.50 ± 0.7	0.59 ± 0.8	0^{bd}	0.50 ± 0.7^{b}
9	0.65 ± 0.9	0	0.50 ± 0.7	0.50 ± 0.7^{bd}	0^{b}
10	0	0	1.00 ± 0.0	1.00 ± 0.0^{ad}	1.58 ± 0.1^{a}
11	0.70 ± 1.0	1.36 ± 0.3	1.35 ± 0.1	1.52 ± 0.0^{a}	1.59 ± 0.3^{a}
12	1.09 ± 0.1	0	0.65 ± 0.9	2.08 ± 0.0^{bc}	1.86 ± 0.3^{a}

Means within a column with different letters are significantly different ($P < 0.05$) for time.

Means (n=2) and Standard Deviation for Yeast Growth and Survival on MAP Parmesan Cheese

Time	Aw= 0.90 Mean$\pm$ S.D.	Aw= 0.88 Mean$\pm$ S.D.	Aw =0.86 Mean$\pm$ S.D.	Aw= 0.84 Mean$\pm$ S.D.	Aw= 0.75 Mean$\pm$ S.D.
0					
1					
2	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
3	2.26 ± 0.0^{b}	1.82 ± 1.2^{bc}	1.30 ± 0.4^{bc}	1.35 ± 0.0^{b}	0.50 ± 0.7^{ac}
4	2.26 ± 0.0^{b}	1.82 ± 1.2^{bc}	1.30 ± 0.4^{bc}	1.35 ± 0.0^{b}	0.50 ± 0.7^{ac}
5	1.68 ± 0.7^{b}	1.36 ± 0.3^{ac}	0.50 ± 0.7^{ac}	1.73 ± 0.2^{b}	1.70 ± 0.3^{b}
6	1.67 ± 0.1^{bcd}	2.07 ± 0.5^{bc}	1.67 ± 0.5^{b}	2.40 ± 0.4^{bc}	1.25 ± 0.4^{bc}
7	0.50 ± 0.7^{ad}	1.03 ± 1.5^{ac}	1.88 ± 0.0^{b}	1.86 ± 0.5^{bc}	1.39 ± 0.6^{bc}
8	2.32 ± 0.0^{b}	2.07 ± 0.0^{bc}	1.76 ± 0.0^{b}	1.99 ± 0.2^{bc}	2.21 ± 0.0^{bd}
9	2.58 ± 0.4^{bc}	2.45 ± 0.2^{bc}	2.42 ± 0.6^{bd}	2.23 ± 0.0^{bc}	2.59 ± 0.1^{bd}
10	2.51 ± 0.2^{bc}	2.48 ± 0.0^{bc}	1.77 ± 0.2^{b}	2.44 ± 0.0^{bc}	2.23 ± 0.3^{bd}
11	2.54 ± 0.1^{bc}	2.75 ± 0.1^{bc}	2.64 ± 0.1^{bd}	2.83 ± 0.3^{bc}	2.11 ± 0.1^{b}
12	3.07 ± 0.3^{bc}	3.09 ± 0.0^{b}	2.73 ± 0.1^{bd}	2.88 ± 0.0^{bc}	2.62 ± 0.5^{bd}

Means within a column with different letters are significantly different ($P < 0.05$) for time.

Means (n=2) and Standard Deviation for Mold_Growth and Survival on MAP Romano Cheese

Time	Aw= 0.90 Mean± S.D.	Aw= 0.88 Mean± S.D.	Aw =0.86 Mean± S.D.	Aw= 0.84 Mean± S.D.	Aw= 0.75 Mean± S.D.
0					
1					
2	0	0.85 ± 1.2	0	0.89 ± 1.3	0^{a}
3	0	0	0	0.59 ± 0.8	1.00 ± 0.0^{bc}
4	0	0	0	0.59 ± 0.8	1.00 ± 0.0^{bd}
5	0	0	0	0	0.65 ± 0.9^{acd}
6	0	0	0.59 ± 0.8	0.77 ± 1.1	0^{a}
7	0.50 ± 0.7	0.59 ± 0.8	0	0	0^{a}
8	0	0	0	0	0^{a}

Means within a column with different letters are significantly different ($P < 0.05$) for time.

Means (n=2) and Standard Deviation for Yeast_Growth and Survival on MAP Romano Cheese

Time	Aw= 0.90 Mean± S.D.	Aw= 0.88 Mean± S.D.	Aw =0.86 Mean± S.D.	Aw= 0.84 Mean± S.D.	Aw= 0.75 Mean± S.D.
0					
1					
2	0.50 ± 0.7	1.16 ± 1.6	1.81 ± 0.9	0.50 ± 0.7^{a}	1.00 ± 0.0^{a}
3	1.27 ± 1.8	2.00 ± 0.6	1.10 ± 1.5	0^{a}	0^{b}
4	1.27 ± 1.8	2.00 ± 0.6	1.10 ± 1.5	0^{a}	0^{b}
5	2.14 ± 0.9	1.68 ± 0.5	1.90 ± 0.4	2.03 ± 0.0^{b}	0^{b}
6	2.48 ± 0.2	2.42 ± 0.1	2.38 ± 0.1	2.28 ± 0.1^{b}	1.79 ± 0.1^{bc}
7	0.65 ± 0.9	2.04 ± 0.2	2.16 ± 0.0	1.78 ± 0.4^{b}	1.53 ± 0.5^{bc}
8	2.39 ± 0.1	2.68 ± 0.1	2.87 ± 0.2	3.13 ± 0.1^{bc}	2.35 ± 0.0^{bcd}

Means within a column with different letters are significantly different ($P < 0.05$) for time.

Appendix E. SAS program for analysis of headspace gas data and microbial data

Headspace gas analysis

```
Enter data
      Data t0r1; time=1; rep=1;
      input sample $ CO2 N2 O2;
            Cards;
      .
      .
      .
      .
Model
      Data all; set t0r1 t1r1 t2r1 t3r1 t4r1 t5r1 t6r1 t7r1 t8r1
            t0r2 t1r2 t2r2 t3r2 t4r2 t5r2 t6r2 t7r2 t8r2;
      Title "Romano headspace gas data";
      Proc glm; classes time aw rep;
      Model CO2 N2 O2 = time aw rep time*aw time*aw*rep;
      Test H=time E=time*aw*rep;
      Test H=aw E=time*aw*rep;
      Test H=time*aw E=time*aw*rep;
```

Microbial Analysis

```
Enter data
      Data t0r1; time=1; rep=1'
      input micro;
            cards;
      .
      .
      .
      .
Model
      Data all; set t0r1 t1r1 t2r1 t3r1 t4r1 t5r1 t6r1 t7r1 t8r1
            t0r2 t1r2 t2r2 t3r2 t4r2 t5r2 t6r2 t7r2 t8r2;
      Title "Staph. aureus growth on MAP Romano";
      Proc glm; micro time aw rep;
      Model micro = time aw rep time*aw time*aw*rep;
      Test H=time E=time*aw*rep;
      Test H=aw E=time*aw*rep;
      Test H=time*aw E=time*aw*rep;
```

Appendix E. Analysis of sensory data on Parmesan cheese

Mean Sensory Scores (n=9) and Standard Deviations for Acrid Aroma of MAP Parmesan Cheese

Time	Reference Mean ± S.D.	Aw= 0.90 Mean ± S.D.	Aw= 0.88 Mean ± S.D.	Aw= 0.84 Mean ± S.D.
0	6.52 ± 2.5	6.30 ± 2.6^{abc}	5.11 ± 2.8	5.47 ± 2.9^{ab}
2	6.08 ± 3.0	5.80 ± 3.3^{ac}	5.92 ± 3.1	5.12 ± 3.1^{a}
4	5.13 ± 2.3	5.26 ± 2.7^{a}	5.83 ± 2.8	5.52 ± 3.5^{ab}
6	5.80 ± 3.5	5.63 ± 2.9^{ac}	4.94 ± 2.8	5.67 ± 2.7^{ab}
8	6.17 ± 3.4	7.64 ± 3.1^{b}	6.07 ± 3.3	6.46 ± 2.5^{abc}
10	7.54 ± 4.0	8.00 ± 2.3^{b}	6.80 ± 3.5	7.10 ± 3.6^{bc}
12	6.51 ± 4.2	7.17 ± 3.7^{bc}	6.63 ± 2.8	7.13 ± 3.2^{c}

Means within a column with different letters are significantly different (P< 0.05) for time.

Mean Sensory Scores (n=9) and Standard Deviations for Buttery Aroma of MAP Parmesan Cheese

Time	Reference Mean ± S.D.	Aw= 0.90 Mean ± S.D.	Aw= 0.88 Mean ± S.D.	Aw= 0.84 Mean ± S.D.
0	6.13 ± 2.8^{a}	5.68 ± 3.5^{ac}	5.37 ± 3.1^{ab}	6.27 ± 3.2^{ab}
2	4.89 ± 3.2^{abc}	6.76 ± 3.9^{a}	5.41 ± 2.9^{ab}	6.68 ± 2.4^{b}
4	5.88 ± 3.2^{a}	5.91 ± 3.7^{a}	6.37 ± 3.3^{ad}	4.31 ± 3.5^{c}
6	5.18 ± 2.9^{ac}	5.35 ± 3.0^{ab}	4.42 ± 3.4^{b}	4.99 ± 2.9^{ac}
8	4.01 ± 3.8^{bc}	4.03 ± 2.5^{b}	3.61 ± 2.3^{bc}	4.11 ± 2.4^{c}
10	5.72 ± 3.6^{ac}	5.49 ± 2.7^{ab}	4.62 ± 3.0^{bd}	3.70 ± 2.7^{c}
12	3.39 ± 3.3^{bd}	4.32 ± 2.8^{bc}	4.24 ± 2.7^{b}	4.49 ± 2.9^{c}

Means within a column with different letters are significantly different (P< 0.05) for time.

Mean Sensory Scores (n=9) and Standard Deviations for Butyric Acid Aroma of MAP Parmesan Cheese

Time	Reference Mean ± S.D.	Aw= 0.90 Mean ± S.D.	Aw= 0.88 Mean ± S.D.	Aw= 0.84 Mean ± S.D.
0	7.48 ± 4.2	7.39 ± 3.9	5.06 ± 3.4	5.59 ± 2.7
2	6.86 ± 3.5	6.79 ± 2.7	6.02 ± 3.0	6.63 ± 3.1
4	5.58 ± 2.6	6.51 ± 4.0	7.38 ± 2.7	5.71 ± 3.4
6	7.06 ± 3.2	6.00 ± 2.8	5.56 ± 2.9	5.03 ± 3.6
8	6.26 ± 3.7^{a}	8.31 ± 3.1^{b}	6.05 ± 3.7^{a}	5.75 ± 3.6^{a}
10	5.69 ± 4.1	7.31 ± 4.2	7.02 ± 4.4	5.90 ± 3.0
12	7.79 ± 3.5	7.66 ± 3.2	6.58 ± 3.1	7.02 ± 2.8

Means within a row with different letters are significantly different (P< 0.05) for time.

Mean Sensory Scores (n=9) and Standard Deviations for Fermented/fruity Aroma of MAP Parmesan Cheese

Time	Reference Mean ± S.D.	Aw= 0.90 Mean ± S.D.	Aw= 0.88 Mean ± S.D.	Aw= 0.84 Mean ± S.D.
0	7.22 ± 3.9	7.25 ± 3.3	6.17 ± 3.4	6.52 ± 3.2
2	7.39 ± 3.8	6.40 ± 3.8	6.85 ± 3.4	6.29 ± 3.6
4	9.69 ± 3.0^{a}	7.89 ± 3.0^{b}	7.41 ± 3.2^{b}	6.13 ± 2.7^{b}
6	8.46 ± 4.2	7.49 ± 3.8	7.48 ± 2.7	8.06 ± 2.5
8	8.98 ± 4.0^{a}	7.15 ± 4.4^{ab}	5.73 ± 3.4^{b}	7.59 ± 2.7^{ab}
10	7.10 ± 4.1	8.80 ± 4.2	7.45 ± 3.2	7.42 ± 3.6
12	8.58 ± 3.7	8.46 ± 4.0	8.13 ± 3.7	7.36 ± 4.0

Means within a row with different letters are significantly different (P< 0.05) for time.

Mean Sensory Scores (n=9) and Standard Deviations for Musty Aroma of MAP Parmesan Cheese

Time	Reference Mean $\pm$ S.D.	Aw= 0.90 Mean $\pm$ S.D.	Aw= 0.88 Mean $\pm$ S.D.	Aw= 0.84 Mean $\pm$ S.D.
0	3.02 $\pm$ 3.4	3.46 $\pm$ 3.8	2.71 $\pm$ 3.4	2.63 $\pm$ 2.9
2	2.86 $\pm$ 2.9	2.97 $\pm$ 4.0	3.17 $\pm$ 3.1	3.11 $\pm$ 4.1
4	1.90 $\pm$ 1.9	2.98 $\pm$ 3.3	2.57 $\pm$ 3.5	2.77 $\pm$ 3.5
6	2.11 $\pm$ 2.9	2.46 $\pm$ 3.9	2.86 $\pm$ 4.4	2.81 $\pm$ 3.7
8	3.54 $\pm$ 3.9	4.01 $\pm$ 4.2	3.44 $\pm$ 3.8	3.29 $\pm$ 4.1
10	1.87 $\pm$ 3.4	3.97 $\pm$ 3.3	3.56 $\pm$ 3.7	2.94 $\pm$ 3.9
12	2.57 $\pm$ 2.9	3.01 $\pm$ 3.6	3.42 $\pm$ 3.8	3.01 $\pm$ 4.0

Mean Sensory Scores (n=9) and Standard Deviations for Yeasty Aroma of MAP Parmesan Cheese

Time	Reference Mean $\pm$ S.D.	Aw= 0.90 Mean $\pm$ S.D.	Aw= 0.88 Mean $\pm$ S.D.	Aw= 0.84 Mean $\pm$ S.D.
0	2.76 $\pm$ 2.8^{a}	2.93 $\pm$ 2.6	2.57 $\pm$ 2.8	2.85 $\pm$ 2.9^{ab}
2	2.34 $\pm$ 2.8^{ab}	2.24 $\pm$ 2.3	2.45 $\pm$ 2.3	2.44 $\pm$ 2.6^{a}
4	1.39 $\pm$ 1.5^{b}	2.53 $\pm$ 3.5	2.50 $\pm$ 3.0	2.18 $\pm$ 3.1^{a}
6	1.76 $\pm$ 3.1^{bx}	2.73 $\pm$ 3.2^{xy}	3.04 $\pm$ 3.8^{y}	3.52 $\pm$ 3.6^{by}
8	3.12 $\pm$ 3.1^{a}	3.11 $\pm$ 3.5	2.13 $\pm$ 3.1	2.27 $\pm$ 2.8^{a}
10	3.29 $\pm$ 3.7^{a}	3.37 $\pm$ 3.6	2.93 $\pm$ 3.3	4.21 $\pm$ 4.0^{b}
12	1.69 $\pm$ 3.2^{b}	2.24 $\pm$ 3.1	2.34 $\pm$ 2.4	2.29 $\pm$ 2.4^{a}

Means within a column with different letters are significantly different ($P < 0.05$) for time.
Means within a row with different letters are significantly different ($P < 0.05$) for time.

Mean Sensory Scores (n=9) and Standard Deviations for Flavor Impact of MAP Parmesan Cheese

Time	Reference Mean ± S.D.	Aw= 0.90 Mean ± S.D.	Aw= 0.88 Mean ± S.D.	Aw= 0.84 Mean ± S.D.
0	7.98 ± 2.9	8.02 ± 2.4^{a}	7.07 ± 3.3	8.45 ± 2.8
2	8.24 ± 2.4	6.90 ± 2.9^{a}	6.68 ± 2.4	8.03 ± 2.8
4	9.18 ± 2.2	8.14 ± 2.7^{a}	7.82 ± 2.9	8.55 ± 2.8
6	8.69 ± 3.0	7.97 ± 2.5^{a}	8.97 ± 2.4	7.97 ± 2.6
8	9.29 ± 2.1	9.67 ± 3.1^{b}	7.86 ± 2.8	8.95 ± 3.0

Means within a column with different letters are significantly different (P< 0.05) for time.

Mean Sensory Scores (n=9) and Standard Deviations for Unclean Flavor of MAP Parmesan Cheese

Time	Reference Mean ± S.D.	Aw= 0.90 Mean ± S.D.	Aw= 0.88 Mean ± S.D.	Aw= 0.84 Mean ± S.D.
0	4.68 ± 3.4	5.34 ± 3.6^{a}	6.32 ± 3.4	6.49 ± 3.9
2	5.95 ± 3.8	5.21 ± 3.6^{a}	5.73 ± 4.0	6.20 ± 4.3
4	4.40 ± 2.8^{x}	5.66 ± 3.1^{axz}	7.07 ± 3.3^{y}	6.42 ± 4.0^{yz}
6	4.72 ± 3.0^{x}	7.37 ± 4.2^{aby}	6.79 ± 3.2^{yz}	5.81 ± 3.3^{xz}
8	6.57 ± 4.5	7.79 ± 3.2^{b}	7.12 ± 3.8	6.99 ± 3.2

Means within a column with different letters are significantly different (P< 0.05) for time.
Means within a row with different letters are significantly different (P< 0.05) for time.

Appendix F. Analysis of sensory data on Romano cheese

Mean Sensory Scores (n=9) and Standard Deviations for Acrid Aroma of MAP Romano Cheese

Time	Reference Mean ± S.D.	Aw= 0.90 Mean ± S.D.	Aw= 0.88 Mean ± S.D.	Aw= 0.84 Mean ± S.D.
0	7.05 ± 2.8	7.07 ± 3.3	7.31 ± 2.7^{ab}	6.89 ± 2.9^{a}
2	7.44 ± 3.2	6.70 ± 3.0	5.75 ± 2.9^{bd}	4.98 ± 2.5^{b}
4	6.86 ± 3.6	6.15 ± 2.8	5.64 ± 3.7^{b}	6.64 ± 3.2^{a}
6	8.43 ± 3.0	8.14 ± 3.5	8.48 ± 2.1^{ac}	7.19 ± 2.7^{a}
8	8.95 ± 2.8	8.60 ± 3.6	7.69 ± 3.7^{acd}	7.85 ± 2.5^{a}
10	9.29 ± 3.5	8.01 ± 3.3	7.60 ± 2.6^{ab}	6.71 ± 2.2^{ab}
12	7.46 ± 4.0	7.99 ± 3.5	8.13 ± 3.5^{ac}	7.91 ± 3.9^{a}

Means within a column with different letters are significantly different ($P < 0.05$) for time.

Mean Sensory Scores (n=9) and Standard Deviations for Buttery Aroma of MAP Romano Cheese

Time	Reference Mean ± S.D.	Aw= 0.90 Mean ± S.D.	Aw= 0.88 Mean ± S.D.	Aw= 0.84 Mean ± S.D.
0	4.96 ± 3.2	4.33 ± 3.0	4.18 ± 2.4	4.93 ± 2.7
2	3.77 ± 2.8	2.78 ± 2.2	4.56 ± 2.5	4.00 ± 2.7_
4	3.78 ± 2.2^{a}	3.51 ± 2.6^{a}	4.73 ± 2.7^{a}	6.50 ± 2.9^{b}
6	4.86 ± 3.6	4.03 ± 2.5	4.22 ± 2.7	4.44 ± 2.3
8	3.22 ± 2.9	3.74 ± 3.2	4.16 ± 2.7	4.00 ± 3.0
10	5.13 ± 2.3	3.91 ± 2.8	4.19 ± 2.9	4.37 ± 3.1
12	3.23 ± 2.7	4.46 ± 3.3	4.56 ± 2.9	4.36 ± 3.6

Means within a row with different letters are significantly different ($P < 0.05$) for time.

Mean Sensory Scores (n=9) and Standard Deviations for Butyric Acid Aroma of MAP Romano Cheese

Time	Reference Mean ± S.D.	Aw= 0.90 Mean ± S.D.	Aw= 0.88 Mean ± S.D.	Aw= 0.84 Mean ± S.D.
0	8.00 ± 3.6	7.80 ± 3.1	7.14 ± 3.3	8.03 ± 3.4
2	6.97 ± 3.3	7.75 ± 3.5	6.88 ± 3.0	6.34 ± 3.6
4	7.53 ± 3.4	6.14 ± 3.5	7.05 ± 3.1	6.23 ± 3.1
6	8.16 ± 3.5	7.86 ± 3.6	6.52 ± 4.1	6.48 ± 3.6
8	7.90 ± 4.0	7.57 ± 4.5	6.65 ± 3.7	6.96 ± 4.0
10	8.42 ± 4.0	9.02 ± 3.6	8.25 ± 3.9	7.60 ± 2.9
12	8.60 ± 3.5	7.89 ± 3.9	8.18 ± 3.3	8.30 ± 3.0

Mean Sensory Scores (n=9) and Standard Deviations for Fermented/fruity Aroma of MAP Romano Cheese

Time	Reference Mean ± S.D.	Aw= 0.90 Mean ± S.D.	Aw= 0.88 Mean ± S.D.	Aw= 0.84 Mean ± S.D.
0	5.97 ± 3.7	7.07 ± 2.7	5.42 ± 3.5	5.57 ± 3.9_
2	6.68 ± 3.2	6.12 ± 3.5	6.10 ± 3.2	7.59 ± 2.7
4	6.17 ± 4.3	6.54 ± 3.6	6.05 ± 3.7	5.94 ± 3.8
6	7.35 ± 3.9	7.10 ± 4.1	6.93 ± 3.2	6.59 ± 3.6
8	6.33 ± 4.0	7.43 ± 4.4	5.89 ± 3.0	8.24 ± 2.5
10	6.11 ± 4.4	5.85 ± 2.6	6.97 ± 2.3	7.81 ± 3.0
12	6.31 ± 3.2	5.60 ± 3.4	7.28 ± 4.7	5.95 ± 3.8

Mean Sensory Scores (n=9) and Standard Deviations for Musty Aroma of MAP Romano Cheese

Time	Reference Mean ± S.D.	Aw= 0.90 Mean ± S.D.	Aw= 0.88 Mean ± S.D.	Aw= 0.84 Mean ± S.D.
0	2.62 ± 2.8	2.62 ± 3.1^{a}	3.07 ± 3.1	3.02 ± 3.8
2	3.22 ± 3.6	3.95 ± 3.5^{bc}	3.22 ± 2.8	3.69 ± 3.5
4	2.65 ± 3.8	4.72 ± 3.9^{b}	3.19 ± 4.0	3.10 ± 4.1
6	2.45 ± 2.7	3.59 ± 3.6^{ac}	2.60 ± 3.6	3.40 ± 3.6
8	3.52 ± 3.5	3.44 ± 2.8^{ac}	2.96 ± 3.0	3.48 ± 3.6
10	3.40 ± 3.4	5.11 ± 5.0^{b}	3.85 ± 4.5	2.51 ± 4.1
12	2.73 ± 3.0^{x}	3.81 ± 3.2^{acy}	3.24 ± 4.0^{x}	3.20 ± 4.2^{x}

Means within a column with different letters are significantly different ($P < 0.05$) for time.
Means within a row with different letters are significantly different ($P < 0.05$) for time.

Mean Sensory Scores (n=9) and Standard Deviations for Yeasty Aroma of MAP Romano Cheese

Time	Reference Mean ± S.D.	Aw= 0.90 Mean ± S.D.	Aw= 0.88 Mean ± S.D.	Aw= 0.84 Mean ± S.D.
0	2.19 ± 2.5	2.57 ± 3.1	2.19 ± 2.4	2.81 ± 3.2
2	3.74 ± 3.3	2.43 ± 2.9	2.67 ± 2.7	3.44 ± 3.1
4	1.79 ± 2.8	2.54 ± 3.2	1.89 ± 3.2	2.19 ± 3.1
6	3.31 ± 3.2	2.57 ± 3.2	2.34 ± 2.8	2.46 ± 3.5
8	2.43 ± 2.7	3.05 ± 3.0	2.01 ± 2.2	2.58 ± 3.0
10	1.76 ± 3.2	1.76 ± 2.9	2.39 ± 1.7	2.41 ± 3.6
12	2.92 ± 3.8	2.40 ± 3.5	2.37 ± 2.7	2.17 ± 2.5

Mean Sensory Scores (n=9) and Standard Deviations for Flavor Impact of MAP Romano Cheese

Time	Reference Mean ± S.D.	Aw= 0.90 Mean ± S.D.	Aw= 0.88 Mean ± S.D.	Aw= 0.84 Mean ± S.D.
0	8.04 ± 2.2^{ac}	8.12 ± 2.3^{a}	7.68 ± 2.4	7.07 ± 1.9^{a}
2	9.90 ± 2.9^{bc}	7.98 ± 3.3^{a}	7.51 ± 2.5	7.68 ± 2.9^{a}
4	11.19 ± 2.7^{b}	9.55 ± 3.0^{ab}	8.73 ± 2.6	9.23 ± 2.8^{b}
6	9.82 ± 2.2^{bcx}	9.11 ± 2.8^{abx}	8.67 ± 2.0^{xy}	7.09 ± 1.7^{ay}
8	9.92 ± 2.6^{c}	10.03 ± 2.7^{b}	8,88 ± 2.0	7.73 ± 2.0^{a}

Means within a column with different letters are significantly different (P< 0.05) for time.
Means within a row with different letters are significantly different (P< 0.05) for time.

Mean Sensory Scores (n=9) and Standard Deviations for Unclean Flavor of MAP Romano Cheese

Time	Reference Mean ± S.D.	Aw= 0.90 Mean ± S.D.	Aw= 0.88 Mean ± S.D.	Aw= 0.84 Mean ± S.D.
0	6.59 ± 2.8	6.41 ± 3.7^{a}	7.75 ± 3.8	7.96 ± 3.8
2	6.46 ± 3.0	7.25 ± 3.2^{a}	7.17 ± 4.1	6.77 ± 4.6
4	6.23 ± 4.0	8.82 ± 4.0^{b}	8.81 ± 3.4	7.30 ± 4.0
6	7.01 ± 3.7	8.33 ± 4.0^{b}	7.32 ± 3.0	8.63 ± 3.5
8	6.65 ± 3.6	8.50 ± 3.0^{b}	8.79 ± 3.4	7.79 ± 3.4

Means within a column with different letters are significantly different (P< 0.05) for time.

Appendix G. SAS program for analysis of sensory data

Enter data

```
      Data time01; time=0; rep=1; flavor;'      ';
      Do flavor='acrid', 'buttery', 'butyric', 'f&f', 'musty',
'yeasty',   'flavor', 'unclean';
      Do n=1 to 4;
      Input waid p1-p9;
      Pave=mean(of p1-p9); drop n;
      If waid=815 then trt= '0.90 F';
      If waid=765 then trt= '0.90 M';
      If waid=360 then trt= '0.88 M';
      If waid=753 then trt= '0.84 M';
      Output;
      End;
      End;
      Cards;
      .
      .
      .
```

Model

```
      Proc print; title 'Jonna Romano Data';
      Data all; set time01 time21 time41 time61 time81 time 101 time121
      time 02 time22    time42 time62 time82 time102 time122;
      Y=p1; panel=1; output;
      Y=p2; panel=2; output;
      Y=p3; panel=3; output;
      Y=p4; panel=4; output;
      Y=p5; panel=5; output;
      Y=p6; panel=6; output;
      Y=p7; panel=7; output;
      Y=p8; panel=8; output;
      Y=p9; panel=9; output;
      Drop p1-p9; pave waid;
      Proc sort; by flavor;
      Proc glm; by flavor; classes time trt panel rep;
            model y=time trt panel rep rep*panel time*trt
panel*trt          panel*time panel*trt*time;
      Test H=panel E=rep*panel;
```

Or

```
      Proc glm; by flavor trt; classes time panel rep;
      model y=time panel rep rep*panel panel*time;
      test H=panel E= rep*panel;
```

Or

```
      Proc glm; by flavor time; classes trt panel rep;
      model y=trt panel rep rep*panel panel*trt;
      test H= panel E=rep*panel;
```

Appendix H. CIE L*a*b* values for Color Analysis of Parmesan Cheese

Means (n=12) and Standard Deviations for CIE L* values for MAP Parmesan

Time	Aw= 0.90 Means ± S.D.	Aw= 0.88 Means ± S.D.	Aw= 0.84 Means ± S.D.
0	77.2 ± 2.0	71.7 ± 1.8	70.0 ± 1.5
2	76.9 ± 1.9	70.5 ± 1.8	69.8 ± 2.0
4	76.8 ± 1.9	72.2 ± 2.1	71.0 ± 2.0
6	77.4 ± 2.4	72.2 ± 1.8	70.4 ± 2.2
8	77.9 ± 1.6	72.7 ± 1.1	70.1 ± 1.7
10	76.2 ± 1.7	73.4 ± 2.1	71.3 ± 2.1
12	74.1 ± 3.9	71.4 ± 1.4	71.1 ± 1.7

Means (n=12) and Standard Deviations for CIE a* values for MAP Parmesan

Time	Aw= 0.90 Means ± S.D.	Aw= 0.88 Means ± S.D.	Aw= 0.84 Means ± S.D.
0	-4.2 ± 0.2	-4.3 ± 0.2	-4.6 ± 0.3
2	-3.4 ± 0.6	-3.1 ± 0.3	-3.0 ± 0.5
4	-3.3 ± 0.6	-3.1 ± 0.4	-3.1 ± 0.4
6	-2.7 ± 0.7	-2.7 ± 0.4	-3.1 ± 0.5
8	-2.8 ± 0.8	-2.6 ± 0.3	-2.4 ± 0.3
10	-2.0 ± 0.4	-2.2 ± 0.3	-2.4 ± 0.3
12	-2.4 ± 0.3	-1.8 ± 1.2	-2.6 ± 0.4

Means (n=12) and Standard Deviations for CIE b* values for MAP Parmesan

Time	Aw= 0.90 Means ± S.D.	Aw= 0.88 Means ± S.D.	Aw= 0.84 Means ± S.D.
0	16.3 ± 2.2	16.8 ± 1.9	17.5 ± 1.7
2	18.3 ± 2.5	19.5 ± 2.0	19.0 ± 2.6
4	18.2 ± 2.6	19.6 ± 1.8	19.5 ± 2.3
6	19.4 ± 1.8	19.9 ± 2.1	20.0 ± 2.2
8	20.8 ± 2.7	21.9 ± 2.1	21.8 ± 2.2
10	21.9 ± 1.6	22.0 ± 1.1	22.1 ± 1.4
12	20.0 ± 1.9	21.9 ± 2.6	22.6 ± 1.8

Appendix I. CIE L*a*b* values for Color Analysis of Romano Cheese

Means (n=12) and Standard Deviations for CIE L* values for MAP Romano

Time	Aw= 0.90 Means ± S.D.	Aw= 0.88 Means ± S.D.	Aw= 0.84 Means ± S.D.
0	81.4 ± 1.1	75.5 ± 2.2^{a}	70.3 ± 1.4^{a}
2	82.1 ± 1.7	76.4 ± 2.3^{ab}	71.2 ± 2.4^{ab}
4	79.9 ± 6.8	75.1 ± 2.7^{a}	72.0 ± 1.8^{bc}
6	81.7 ± 1.9	75.6 ± 1.8^{a}	73.2 ± 1.4^{cd}
8	82.5 ± 1.4	78.5 ± 0.9^{c}	73.3 ± 1.6^{cd}
10	82.2 ± 1.6	77.9 ± 1.6^{bc}	73.9 ± 1.6^{d}
12	82.8 ± 1.3	75.0 ± 2.2^{a}	74.0 ± 2.3^{d}

Means within a column with different letters are significantly different ($P < 0.05$) for time.

Means (n=12) and Standard Deviations for CIE a* values for MAP Romano

Time	Aw= 0.90 Means ± S.D.	Aw= 0.88 Means ± S.D.	Aw= 0.84 Means ± S.D.
0	-4.9 ± 0.2^{a}	-5.0 ± 1.1^{a}	-5.4 ± 0.2^{a}
2	-4.0 ± 0.3^{b}	-4.4 ± 0.2^{b}	-4.8 ± 0.3^{b}
4	-4.1 ± 0.4^{bc}	-4.5 ± 0.2^{b}	-4.5 ± 0.6^{bc}
6	-3.8 ± 0.2^{c}	-3.9 ± 0.3^{c}	-4.2 ± 0.4^{cd}
8	-3.2 ± 0.5^{d}	-3.5 ± 0.6^{c}	-3.7 ± 0.7^{de}
10	-3.4 ± 0.4^{d}	-3.4 ± 0.6^{c}	-3.3 ± 1.3^{e}
12	-2.9 ± 0.1^{e}	-2.8 ± 0.2^{d}	-2.8 ± 0.2^{f}

Means within a column with different letters are significantly different ($P < 0.05$) for time.

Means (n=12) and Standard Deviations for CIE b* values for MAP Romano

Time	Aw= 0.90 Means ± S.D.	Aw= 0.88 Means ± S.D.	Aw= 0.84 Means ± S.D.
0	13.0 ± 1.9^{a}	12.6 ± 2.5^{a}	13.3 ± 2.0^{a}
2	12.1 ± 1.8^{a}	11.8 ± 2.0^{ab}	14.6 ± 1.5^{ab}
4	12.8 ± 1.7^{a}	13.7 ± 1.0^{ac}	15.1 ± 1.2^{b}
6	14.7 ± 1.3^{b}	15.2 ± 1.3^{d}	17.6 ± 2.0^{c}
8	15.6 ± 1.4^{b}	16.2 ± 1.5^{de}	19.1 ± 1.1^{d}
10	15.8 ± 1.8^{b}	17.5 ± 1.2^{ef}	19.3 ± 1.6^{d}
12	15.5 ± 1.3^{b}	18.1 ± 1.9^{f}	22.0 ± 1.6^{e}

Means within a column with different letters are significantly different ($P < 0.05$) for time.

VITA

Jonna D. Yoder was born December 12, 1969 in Baltimore, MD. She was raised on a small dairy farm in Long Green, MD. She graduated from Loch Raven High School, Towson, MD in June 1988.

In the fall of 1988, Ms. Yoder began her undergraduate degree in Food Science and Technology at Virginia Tech. She was very active in the FST department. Jonna was a work-study student for several professors. She completed undergraduate research work under Dr. Susan Duncan evaluating fatty acid and lipolysis in milk. She participated on the Dairy Products Sensory Evaluation Team and placed 2nd in milk evaluation at the 1991 National Contest. She was an active leader in the Food Science Club and Alpha Zeta, as well as, a charter member of Sigma Alpha Professional Sorority.

Ms. Yoder graduated with her B.S. in Food Science and Technology in May 1992 and began working on her M.S. degree in Food Science at Virginia Tech.