STUDIES ON THE MODIFIED ATMOSPHERE PACKAGING (MAP) OF MOZZARELLA CHEESE

THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN
DAIRYING
(DAIRY TECHNOLOGY)

BY
MD. TANWEER ALAM

DIVISION OF DAIRY TECHNOLOGY
NATIONAL DAIRY RESEARCH INSTITUTE
(I. C. A. R.)
KARNAL - 132001 (HARYANA), INDIA

2004

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Approved by:

(M. K. Salooja)
EXTERNAL EXAMINER

(G. K. GOYAL)
MAJOR ADVISOR & CHAIRMAN
(GUIDE)

Members of Advisory Committee

1. **Dr. G. R. Patil**
   PS & Head, DT Division
2. **Dr. S. K. Kanawjia**
   Principal Scientist, DT Division
3. **Dr. D. N. Gandhi**
   Principal Scientist, DM Division
4. **Dr. (Ms) B. K. Wadhwa**
   Principal Scientist, DC Division
5. **Dr. S. K. Anand**
   Senior Scientist, DM Division

DIVISION OF DAIRY TECHNOLOGY
NATIONAL DAIRY RESEARCH INSTITUTE
(DEEMED UNIVERSITY)
KARNAL-132001 (HARYANA), INDIA

ICAR

Dr. G. K. GOYAL, Ph.D.
Adv. Trg. in Packaging
School of Packaging
Michigan State University, U.S.A.
Principal Scientist (Dairy Technology)

Date: 29th **May, 2004**

CERTIFICATE

This is to certify that the thesis entitled, " **STUDIES ON THE MODIFIED ATMOSPHERE PACKAGING (MAP) OF MOZZARELLA CHEESE"** submitted by **MD. TANWEER ALAM**, M.Sc. (Dairy Technology) towards the partial fulfilment for the award of the degree of **DOCTOR OF PHILOSOPHY** in **DAIRYING (DAIRY TECHNOLOGY)** of the **NATIONAL DAIRY RESEARCH INSTITUTE (DEEMED UNIVERSITY)**, Karnal (Haryana), India, is a bonafide research work carried out by him under my guidance, and no part of the thesis has been submitted for any other degree or diploma.

(G. K. GOYAL) 29.5.04
Major Advisor & Chairman
(Guide)

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(***Md. Tanweer Alam***)

CONTENTS

LIST OF FIGURES

LIST OF PHOTOPLATES

LIST OF PHOTOPLATES

LIST OF APPENDICES

Appendix No.	Title
XIX	Effect of MAP on the Hardness (N) of mozzarella cheese stored in different packages at 7± 1^{0}C (mean of three trials)
XX	Effect of MAP on the Hardness (N) of mozzarella cheese in different packages stored at –10 to–15 ^{0}C (mean of 3 trials)
XXII	Effect of MAP on the Cohesiveness (A2/A1) of mozzarella cheese stored in different packages at 7± 1^{0}C (mean of three trials)
XXII	Effect of MAP on the Cohesiveness (A2/A1) of mozzarella cheese in different packages stored at –10 to–15 ^{0}C (mean of 3 trials)
XXIII	Effect of MAP on the Springiness (mm) of mozzarella cheese stored in different packages at 7± 1^{0}C (mean of three trials)
XXIV	Effect of MAP on the Springiness (mm) of mozzarella cheese in different packages stored at –10 to– 15 ^{0}C (mean of 3 trials)
XXV	Effect of MAP on the Gumminess (N) of mozzarella cheese stored in different packages at 7± 1^{0}C (mean of three trials)
XXVI	Effect of MAP on the Gumminess (N) of mozzarella cheese in different packages stored at –10 to–15 ^{0}C (mean of 3 trials)
XXVII	Effect of MAP on the Chewiness (N x mm) of mozzarella cheese stored in different packages at 7± 1^{0}C (mean of three trials)
XXVIII	Effect of MAP on the Chewiness (N x mm) of mozzarella cheese in different packages stored at –10 to–15 ^{0}C (mean of 3 trials)
XXIX	Effect of MAP on the Standard plate count (log cfu/g) of mozzarella cheese stored in different packages at 7±1 ^{0}C (mean of three trials)
XXX	Effect of MAP on the Standard plate count (log cfu/g) of mozzarella cheese stored in different packages at -10 to-15 ^{0}C (mean of 3 trials)
XXXI	Effect of MAP on the Yeast and mould count (log cfu/g) of mozzarella cheese stored in different packages at 7±1 ^{0}C (mean of three trials)
XXXII	Effect of MAP on the Yeast and mould count (log cfu/g) of mozzarella cheese stored in different packages at –10 to–15 ^{0}C (mean of three trials)
XXXIII	Effect of MAP on the Psychrotrophic count (log cfu/g) of mozzarella cheese stored in different packages at 7± 1^{0}C (mean of three trials)
XXXIV	Effect of MAP on the Psychrotrophic count (log cfu/g) of mozzarella cheese in different packages stored at –10 to–15 ^{0}C (mean of 3 trials)
XXXV	Effect of MAP on the Coliform count (log cfu/g) of mozzarella cheese stored in different packages at 7± 1^{0}C (mean of three trials)
XXXVI	Effect of MAP on the Coliform count (log cfu/g) of mozzarella cheese in different packages stored at –10 to–15 ^{0}C (mean of 3 trials)
XXXVII	Mean sensory score (based on 3 point scale) for Appearance of MAP mozzarella cheese stored in different packages at 7±1 ^{0}C (mean of three trials)

ABSTRACT

Consumer's demand for fresh naturally preserved food products has grown dramatically in recent years. Food must be available wherever there are people, and with modern population patterns this is seldom where it is grown or manufactured. Food, in interesting variety, must be available all the year round, irrespective of the growing season. It must be presented in a way that is convenient to purchase and use, and in most instances this means that it must be packaged. The packaging technique and choice of a pack with appropriate barrier properties is designed to prevent spoilage of food by microbial or insect attack, depending upon its physical nature, and also to **preserve quality and nutritive value of many foods by the exclusion of oxygen** and the control of moisture loss or gain. Packaging is described as a "complex, dynamic scientific and controversial segment of business." Packaging is certainly dynamic and is constantly changing. New materials need new methods, new methods demand new machinery, new machinery results in better quality, and better quality opens up new markets, which require changes in packaging. The cycle then starts again. Thus, at its most fundamental packaging contains, protects, preserves, and informs. Also it provides two more functions - those of selling and convenience. In situations where quality of products is high, in many instances almost the only difference between competitive brands lies in the packaging, and only the packaging influences the selling operation. Traditional food packaging protects food from external microbial contamination, oxygen, water vapour and light. However, newer packaging technologies such as Modified Atmosphere Packaging (MAP) do more than just provide protection from outside influences. In Europe, the technique is being used extensively for retail packaging of food items and seen as vulnerable growth during past few years. MAP is finding wide application. Generally, the shelf life of mozzarella cheese is approximately 14-15 days at 7 ± 1°C. Hence, a study was planned to increase the shelf life of mozzarella cheese by using MAP technique. Three hundred gms of mozzarella

cheese was packaged in two types of high barrier bags under five different atmospheres (air: atm 1; vacuum: atm 2; 100% CO_2: atm 3; 100 % N_2: atm 4; and 50% CO_2 / 50 % N_2: atm 5). The packaged samples were then stored at 7±1 ^{0}C and - 10 to-15^0 C. Periodically, the product was evaluated for changes in sensory, microbiological, physico-chemical and textural characteristics. The experiments confirmed the preservative effect of CO_2 under MAP. Amongst the five atmospheres studied, atm 3> atm 5> atm 4> atm 2> atm 1 respectively, in descending order. The studies further revealed that from the present study it can be concluded that the shelf life of mozzarella cheese can be increased upto 12 weeks (300% increase) at 7 ±1 ^{0}C storage, and upto 12 months (120%) at deep freeze conditions by using MAP technique.

सारांश

पिछले कुछ वर्षों में ताजे व प्राकृतिक रूप में संरक्षित खाद्य उत्पादों की मांग असाधारण दर से बढ़ रही है। खाद्य सामग्री वहां उपलब्ध होनी चाहिए, जहां लोग रहते हैं। खाद्य पदार्थ बारहों महीने उपलब्ध होने चाहिए और इनकी उपलब्धता इस प्रकार होनी चाहिए कि ये खरीदने व प्रयोग करने में सरल हो, या दूसरे शब्दों में ये पैकेज्ड हों। उपर्युक्त अवरोधक लक्षणों वाली पैकेजिंग प्राविधि और पैक का चुनाव खाद्य पदार्थ की प्राकृति के अनुसार, सूक्ष्मजीवों और कीटों के आक्रमण में संदूषण को रोकने के अतिरिक्त पोषणिक मान व गुणवत्ता का संरक्षण ऑक्सीजन अपवर्जन व नमी क्षय व लब्धि को अवरुद्ध करने के लिए किया जाता है। संवेष्टिकरण व्यवसाय का एक जटिल, गतिशील, वैज्ञानिक व विवादास्पद भाग है। पैकेजिंग वास्तव में गतिशील है और निरन्तर परिवर्तित हो रही है। नये पैकेजिंग पदार्थों को नए तरीकों की, नए तरीकों को नई मशीनरी की, नयी मशीनरी से बेहतर गुणवत्ता और बेहतर गुणवत्ता से नये बाजारों का रास्ता खुलता है, जिसके लिए पैकेजिंग पद्धति में परिवर्तन आवश्यक है। तथा चक्र पुनः आरम्भ हो जाता है। इस प्रकार पैकेजिंग मूलभूत कार्यों: रखना, सुरक्षा करना, संरक्षण करना तथा सूचित करने के अलावा, यह दो अतिरिक्त कार्यों का भी निर्वहन करती है: विक्रय में मदद व उपभोक्ताओं को सुविधा प्रदान करना। जहां कहीं उत्पादों की गुणवत्ता उच्च होती है, वहां अन्तर केवल प्रतिस्पर्द्धात्मिक ब्राण्ड में अन्तर पैकेजिंग के कारण होता है, और अकेले पैकेजिंग ही सारी विक्रय गतिविधियों को प्रभावित करती है। परम्परागत खाद्य संवेष्टन (पैकेजिंग) खाद्य पदार्थ को बाह्य सूक्ष्मजीवों के संदूषण, ऑक्सीजन, जल-वाष्प और प्रकाश से बचाए रखती है। जबकि, नयी संवेष्टन पद्धतियाँ जैसे परिवर्तित वातावरण संवेष्टन (मैप), बाहरी आक्रमणों से बचाव के अतिरिक्त और भी कार्य करती है। यूरोप में यह तकनीक खाद्य पदार्थों के खुदरा संवेष्टन के लिए कुछ वर्षों में इसमें अच्छी बढ़ोत्तरी दर्ज की गई है। मैप का उपयोग अब विविध अनुप्रयोगों में किया जा रहा है। सामान्यतया, मौजेरेला चीज की आयु $7\pm1°$ सें. पर लगभग 14-15 दिन होती है। चूंकि यह कम है; इस लिए इसकी आयु मैप तकनीक द्वारा बढ़ाने हेतु यह अध्ययन नियोजित किया

गया था। 300 ग्राम मौजेरेला चीज दो प्रकार के उच्च अवरोधक प्लास्टिक थैलियों में पाँच विभिन्न परिवेशों (वायु: परिवेश-1; निर्वात: परिवेश-2; 100 प्रतिशत कार्बन डाई ऑक्साइड: परिवेश-3; 100 प्रतिशत नाईट्रोजन: परिवेश-4, और 50 प्रतिशत कार्बन डाइ ऑकसाइड/ 50 प्रतिशत नाईट्रोजन: परिवेश-5) में पैक की गई संवेष्टित (पैकेज्ड) नमूने 7±1° सें. और -10 से -15° सें. के तापमानों पर रखे गए। निश्चित अन्तराल के बाद उत्पाद संवेदिक, सूक्ष्मजैविक, भौतिक-रासायनिक और संरचनात्मक लक्षणों में बदलाव के लिए परीक्षण किया गया। अनुप्रयोगों से इस बात की पुष्टि हुई है कि कार्बन डाइ ऑक्साइड का सर्वाधिक प्रभाव मैप में देखने को मिलता है। पाँच परिवेशों के अध्ययन में, अनुकूलतम परिवेशों का वरीयता क्रम इस प्रकार पाया गया: परिवेश-3 > परिवेश-5 > परिवेश-4 > परिवेश-2 > परिवेश-1 और अधिक शोध से निष्कर्षत: यह ज्ञात हुआ कि मैप से मौजेरेला चीज की आयु 7±1° सें. पर 12 सप्ताह तक (300 प्रतिशत वृद्धि) और अतिहिमीयकरण परिस्थितिओं में 12 महीनों तक (120 प्रतिशत वृद्धि) बढ़ाई जा सकती है।

CHAPTER 1

INTRODUCTION

INTRODUCTION

1.0.0.0

Man has many competitors for the food he produces, animals particularly rodents, insects and microorganisms (moulds, yeasts, and bacteria), all cause spoilage at various stages in the processing, storage, transport and sale of food. If microorganisms are permitted to flourish in food, they make it unattractive, and it gets spoiled by putrefaction, fermentation, or mould growth. These organisms particularly bacteria, can affect food and render it poisonous to man, thereby causing sickness and even death. Packaging plays a decisive role in achieving the objectives of spoilage prevention, safety and preservation.

Packaging is described as a "complex, dynamic scientific and controversial segment of business." Packaging is certainly dynamic and is constantly changing. New materials need new methods, new methods demand new machinery, new machinery results in better quality, and better quality opens up new markets, which require changes in packaging. The cycle then starts again.

Thus, at its most fundamental packaging **contains**, **protects**, **preserves**, and **informs**. Also it provides two more functions - those of selling and convenience. In situations where quality of products is high, in many instances almost the only difference between competitive brands lies in the packaging, and only the packaging influences the selling operation.

1.1.0.0 Importance of Packaging

Food must be available wherever there are people, and with modern population patterns this is seldom where it is grown or manufactured. Food, in interesting variety, must be available all the year round, irrespective of the growing season. It must be presented in a way that is convenient to purchase and use, and in most instances this means that it must be packaged. The packaging technique and choice of a pack with appropriate barrier properties is designed to prevent spoilage of food by microbial or insect attack, depending upon its physical nature, and also to **preserve quality and nutritive value of many foods by the exclusion of oxygen** and the control of moisture loss or gain.

The preservation of food product packed in plastic film mainly depends on maintaining its original quality by protecting it against external deteriorative influences. This is achieved through the barrier properties of the packaging material. The required protection of the foodstuff may be achieved with a single layer of polymer or necessitates the use of multi-layered films of different polymers, coating and /or metal foils. The barrier properties of a package mainly originate from its permeability to gases and vapours that are noxious to the quality of the product during storage. More harmful than moisture is oxygen. Its fixation to the product is irreversible. It causes lipid oxidation and provokes rancidity. The other requirements for the preservation of qualities (physical, chemical, organoleptic) of the food are to prevent changes in taste, colour and odour and if a **modified atmosphere** is applied inside the package, the quality of the product can be maintained for considerable length of time.

1.2.0.0 Modified Atmosphere Packaging (MAP)

The technology of packaging products in modified atmosphere is the most advanced food preserving technique with many advantages. MAP is a process by which the shelf life of food product is significantly increased (Floros *et al.*, 2000). This method of packaging replaces the air headspace of package with a gas or mixture of gases like N_2 and CO_2. MAP is used to extend the product's shelf life and to maintain the product's initial quality for much longer period. It helps in increasing the wide range of distribution, retains appeal to consumers, and retards the growth of mould and bacteria besides reducing the oxidative deterioration and preventing mechanical damage to the product by adding a 'cushion of gas around the product'.

1.3.0.0 Need of MAP for Mozzarella Cheese

Cheese is appreciated by consumers with great interest. It adds variety to the eating experience. It has an excellent image, being perceived as healthy, natural and nutritious. Cheese is an important dairy ingredient, which is used in many food products such as pizza, processed cheese, cheese dips and spreads, bakery goods, snack foods and canned foods and so on (Timothy, 2002). The volume of cheese used in cooking applications has increased dramatically in recent years. As the use of cheese has increased, so have the demands on the functionality. When looking at pizzas topping; mozzarella cheese is ultimate ingredient.

Mozzarella cheese belongs to 'Pasta Filata' variety of cheese, which involves skillful stretching, pulling and kneading of curd under hot water, which imparts to the finished cheese its characteristic fibrous structure, melting and stretching properties. The presence of such unique functional characteristics (stretching and melting) of mozzarella cheese enables it to use on pizza topping (Bhaskararcharya and Shah, 2002).

Pizza cheese is perhaps the prime example of a market driven product. There are no standards to identity for pizza cheese and so the customer dictates the standards to which it is made. The changing food habit and use of newer appliances for cooking and processing demand specific tailor-made functionalities. The type of milk and ingredients used, the method of manufacture adopted and the storage conditions affect the functionality of the cheese when used as a pizza topping. There is little doubt that mozzarella cheese has earned world fame. Thanks to **its extensive use as a major ingredient of pizza throughout the world** (Mann, 1997; Sherkat and Walker, 2002).

The past decade has witnessed extraordinary worldwide growth in the production of mozzarella cheese, fuelled by a spiralling demand for pizza. Americans consume – 100 acres of pizza per day. This translates into an average of 7.7 pounds of mozzarella cheese topping per person per year.

About 2.25 billion pounds of mozzarella cheese are made annually in US and 75% of it is used for pizza making (Market Tracking International Ltd., 1998).

In India cheese production has been increasing quite steadily, being 1000 tonnes in the year 1980 to 12000 tonnes in 2001 against world production of 13.5 million tonnes in 2000 (Anon, 2000; Sorenson, 2001). The demand for cheese in India in the year 2000 was estimated to be 30,000 tonnes (Singh, 1998). The global cheese market is also beckoning the Indian producers. Mozzarella cheese is one such variety, which is gaining momentum and is very widely used for pizza making. Pizza as well as mozzarella cheese are relatively new introduction to the Indian dietary system and are gaining wide popularity. In India, the consumption of pizza is increasing very fast @ 20 %per year and 700-800 tonnes of mozzarella cheese are being used for pizza making. Pizza market in India is growing at an annual rate of 15 - 20 per cent (Anon, 2003 b). Some Indian companies like Amul, Domino, Nirula's etc. are using mozzarella cheese on large scale for pizza preparation.

Pizza players, big pitch for Indian hinterland with its desi transformation almost compete the much celebrated Italian pizza is all set to invade the Indian hinterland with the industry now making a play for second rung cities in the country. In the current year, the Gujarat Co-operative Milk Marketing Federation (GCMMF) better known by its Amul brand is planning to open 3000 pizza outlets across 300 cities in the country (Anon, 2002). For 14 - inch size pizza, 150 g mozzarella cheese is required. Amul expects the pizza to boost its sale of cheese, which currently stands at 3500 metric tonnes, by another 1000 metric tonnes annually, which would boost the annual sales of mozzarella cheese to 4000 tonnes (Anon, 2001). At present the shelf-life of mozzarella cheese is approximately 14-15 days at 7 ± 1°C. Its low shelf-life is mainly due to microbial deterioration and fat oxidation. It is expected that several thousands of pizza retail counters would open throughout the country including small towns in very near future. Mozzarella cheese being essential ingredient of pizza, it must be available wherever there are pizza counters, and with available mozzarella cheese manufacturing facilities this is seldom where it is manufactured. Hence, there is an alarming need to enhance the keeping quality of mozzarella cheese substantially by employing recently introduced technique of packaging namely modified atmosphere packaging so that mozzarella cheese could be available even at far off places irrespective of the manufacturing place.

In view of the above, present investigation has been proposed with the following objectives:

OBJECTIVES:

1. To study the influence of different packaging materials & MAP condition on the shelf life of mozzarella cheese.
2. To study the changes in physico-chemical, microbial, and functional properties of MA packed mozzarella cheese during storage under different conditions.
3. To compare the quality of pizza made from MA packed mozzarella cheese with conventionally packaged mozzarella cheese.

CHAPTER 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.0.0.0

The most important function of packaging is to deliver to the consumer, food of the same high quality, he is used to getting in fresh food or freshly manufactured foods. Packaging protects the packaged product against any type of deterioration, be it of chemical, microbial, biological or physical nature. A package constitutes a vital link between the manufacturer and the end user for the safe delivery of the product. A package generally performs the following functions: (i) contains the food product, (ii) protects and preserves the quality of the product, (iii) appeals to or attracts and informs the customer.

The choice of suitable packaging involves a number of considerations. For most food products, the main objective is that the package must provide the optimum protective properties to keep the product it contains in good condition for its anticipated shelf life.

The information has been collected under four broad headings namely modified atmosphere packaging (MAP), MAP of dairy products, mozzarella cheese and pizza.

2.1.0.0 Modified Atmosphere Packaging (MAP)

According to Genigeorgis (1985) MAP extends the shelf life of meat, poultry and fresh produce by 50-400% and the quality is maintained beyond the expected shelf life inhibiting the growth of aerobic psychrotrophs.

MAP is an enclosure of food products in high barrier materials, in which gaseous environment has been modified (Inns, 1987). MAP slows respiration rate, reduces microbial growth, and retards enzymatic spoilage with the final effect of lengthening the shelf life of the product (Young *et al.*, 1988). MAP is capable of substantially extending the shelf life of many perishable foods (Hotchkiss, 1988) and has accordingly undergone developments to meet the growing consumer demand for fresh, high quality convenience foods. Brody

(1989) defined MAP as the enclosure of food products in barrier materials in which the atmosphere changes as a result of dynamic interaction between atmosphere and product. Generally three types of gases or their mixtures namely N_2, CO_2 and O_2 are used in MAP. The use of appropriate gas mixtures for MAP offer an alternative to vacuum packaging (Parry, 1993). MAP technology has been successfully used to extend the shelf life of a wide range of commodities including fish, meat, sandwiches, salads and vegetables (Church and Parson, 1995; Phillips, 1996; Sahoo and Anjaneyulu, 1999). MAP is a group of techniques in which the package is actively involved with the food product or interacts with the internal atmosphere to extend shelf life, while maintaining quality and safety (Floros *et al.,* 1997). In order to control the internal atmosphere of a package, substances that absorb (scavengers) or release (emitters) a specific gas are required (Farkas, 1998).

Table: Advantages and disadvantages of MAP

(Farber, 1991; Davies, 1995; Sivertsvik, 1995)

Advantages	Disadvantages
Fresh appearance	Visible added cost
Potential shelf life increase of 50-400%	Temperature control necessary
Reduced economic loss due to longer shelf life	Different gas formulation needed for each product type
Product can be distributed over long distances and increase market area	Special equipment and training required.
Provides a high quality product	Product safety to be established
Easier separation of slices (Vacuum vs MAP)	Loss of benefits once the pack is opened or leaks
Easy to open, i.e. user friendly	CO_2 if dissolved into the food, could lead to pack collapse

In most MAP applications, it is desirable to maintain the atmosphere initially introduced into package for a long period as far as possible and to maintain the gas ratio unchanged. MAP reduces the rate of oxidation reactions and microbial growth by modifying the level of gases (CO_2, N_2 and O_2) that surround the product and improve the shelf life to a great extent

(Floros *et al.,* 2000). MAP is intended to create an appropriate gaseous atmosphere around a commodity packed in a package to enhance shelf-life and to conserve the quality of product (Deepak *et al.*, 2002; Sivertsvik *et al.*, 2002a; b).

2.1.1.0 Critical Parameters of MAP Product

The initial quality (organoleptic and microbial) of raw produce coupled with proper temperature control throughout the processing chain are the foremost important factors for MAP food and account for 50- 60 % synergy. The packaging materials, packaging equipments, and gas mixtures are the other parameters that account for 40 % of synergistic effect, are critical to maintain overall quality of MAP product. The whole MAP chain of food product could be divided into 4 stages. The first stage is pre-processing stage. It should deliver pre-specified and pre-cooled products at their best quality. During this stage maintenance of low temperature is crucial and critical and pathogen contamination should be addressed and resolved at this stage (Kader, 1985). MAP could be applied at this stage during transportation or short-term bulk storage. The second stage is that the product is formulated and processed according to consumer needs. During this stage, cleaning, grading chemical and microbial checks are done to assure the wholesomeness and safety of all the ingredients and products under strict hygienic conditions. At this point, the multiple barrier principle, HACCP, ISO and Codex alimentarious should be used to formulate a product, which would resist the pathogen attack. The third stage starts with gas flushing and packaging. The fourth stage is transportation and retail storage.

MAP packages are of two general types, i.e. breathing and non-breathing types. Milk and dairy types come under non-breathing types. The breathing packages allow the gaseous exchange from and into the package, and the final atmosphere is the result of a dynamic equilibrium which is a function of parameters such as film gas permeability, temperature, respiration rates (biological or microbial), relative humidity, surface area etc. On the other hand non-respiring food does not involve any kind of gaseous exchange and thus it is suitable only for non-respiring foods, except some variety of cheeses.

Table: Product Optimisation Parameters and Critical Points in MAP System

(Kader, 1985)

Product parameters	Critical points
Organoleptic & microbiological quality of raw materials	Microbiological safety and shelf life of product
Temperature throughout the chain	Food chain (distribution and safety system)
Packaging equipment	Temperature control at the point of sale.
Appropriate gas mixture	Consumer handling between sale and consumption

2.1.2.0 Safety Aspect of MAP on Microbial and Pathogenic Organisms

Lioutas (1988) reported that the microbial load in the packaged food has a significant effect on the quality of final product. A high microbial load, and temperatures higher than recommended for particular food can reduce the shelf life of a product by 50-70%. There is ample evidence that elevated CO_2 extends the lag phase of bacterial growth and can slow the propagation of bacteria. Low O_2 is likely to favour mesophillic microbes such as listeria and lactic acid bacteria. Elevated CO_2 may favour gram-positive bacteria over gram-negative bacteria, especially coliforms and lactic acid bacteria (Brackett, 1996). A great deal of work has been done on the effect of MAP relating to the growth of human pathogens particularly *Listeria monocytogenes* on fresh–cut produce (Sizmur and Walker 1988). Pathogenic bacteria have been isolated from many different commodities including fruits and vegetables such as *Listeria monocytogens* and those are able to multiply at very low levels of O_2 such as Psychrotrophic, *Clostridium botulinum* which are of particular concern (Beuchat, 1996). *Clostridium botulinum* is anaerobic in nature and causes severe illness. Studies suggested that the overall incidence of *Clostridium botulinum* might increase the potential health risk to consumer (Lilly *et al.*, 1996).

Seideman and Durland (1984) studied that 20- 30 % CO_2 or even 10 % CO_2 might be sufficient to retard the bacterial growth. High levels of CO_2 have generally been found to have an inhibitory effect on *Staphylococcus aureus*, *Salmonella sp. Escherichia coli*, and *Yersinia enterocolitica*. Smith *et al.* (1987) reported MAP stored products at 4 °C, had a shelf life 2 to 3 times greater than air-packed products. Hintalian and Hotchkiss (1987) reported that the growth *S. typhimurium* was inhibited by MA environment. Mycotoxins, which are secondary metabolites of fungi and their presence in any amount was undesirable. Several studies on MA's effects on *A. flavus* and aflatoxin accumulation have been reported (Landers *et al.,* 1979; Shih and Marth, 1973). In general, 20 % or higher of CO_2 concentration might be suppressive for the growth of *A. flavus* and aflatoxin accumulation considerably, but much less suppression was observed at 10% or less CO_2.

An important factor in determining the microbiological safety of MAP food and dairy products is whether the food is sold as "ready-to-eat' or raw. The use of MAP for any raw produce, which is subsequently cooked, is considered less hazardous because cooking would kill all vegetative pathogens (Hotchkiss, 1988). The safety and stability of foods largely depend upon the initial microbial load. Modification of the atmosphere surrounding the food may provide one condition or hurdle that helps restrict the growth of microorganisms. Another hurdle can be provided by storage at< 4^0 C. The combination of chill temperature and MAP generally results in more effective safer storage regime and longer shelf life (Leistener, 1995).

2.1.3.0 Packaging Materials for MAP

The choice of packaging material is an important factor in any MAP operation. A low water vapour transmission rate (WVTR) together with a high gas barrier must generally be achieved. Generally, all MA packages are based on thermoplastic polymers. The packaging materials need to have mechanical strength to withstand machine handling and subsequent storage, distribution and retailing. Largely the shelf life of a product is dependent on its packaging. One of the most important parameters to be considered for MAP studies is the need of proper packaging materials. The most important factor influencing the anti-microbial effect of CO_2 is impermeability of packaging film. The success or failure of MAP for respiring and non- respiring foods depends on both the O_2 and CO_2 impermeability of packaging materials in order to maintain the correct gas mixture in the package headspace. In addition, film used in gas packaging should also have low WVTR to prevent moisture loss or moisture gain. Since all the desired characteristics of a packaging film, i.e. strength, impermeability and heat sealability are seldom found in one polymer, individual polymers are laminated to one another to produce films of desired characteristics for gas packaging of both respiring and non respiring products. The packaging of non-respiring product include nylon /PE /nylon /PVdC /PE or nylon / EVOH / PE. These composite structures have all the desired characteristics for gas packaging of non- respiring product, specially strength, provided by the outer most layer of nylon, gas and moisture vapour impermeability, provided by EVOH or PVdC, and heat sealability provided by PE (Goodburn and Halligan, 1988). Selby (1968) and Sacharow & Griffin (1970) observed that high barrier films had low WVTR, good resistance to grease, high temperature stability and low oxygen transmission rate (OTR).

Characteristics of an ideal film

The ideal film must possess the following characteristics:

- Ability to change the gas permeability properties in the case of rise in temperature.
- Controllable moisture vapour transmission rate (MVTR) in order to prevent supersaturated vapour accumulation and condensation problem
- Ability to warn the consumer that the quality is not at its best.
- Good thermal and ozone resistance
- Commercial suitability and ease of handling and application
- Non reactant with produce and non-toxic
- Ease of printing for labelling purpose.

(Church, 1994; Kader *et al.,* 1989; Smolander *et al.,* 1997)

Table : Factors to be considered in selection of packaging materials for MAP

(Smith, 1993)

Barrier properties	Permeability to various gasses and water vapour
Machine capability	Capacity to trouble free operation
Sealing reliability	Ability to seal to itself and to the container
Anti-fog properties	Good product visibility
Special characteristics	Temperature sensitive, quality & freshness indicator

2.1.4.0 Barrier Properties

Packages for MAP in use having barrier properties are generally laminates or co-extruded materials of polyethylene with polyester or nylon with or without the addition of a high barrier layer of vinylidenechloride co-polymer or ethylene-vinyl alcohol co-polymer, depending on the properties required. The protection against dehydration could be achieved by using films with low WVTR like semi-barrier (PP and LDPE) or barrier films (Al, PVC, PVdC, HDPE) Day (1993). The layer of aluminium foil provides a complete barrier to gas transmission and was well used in MAP and controlled atmosphere packaging (CAP) systems (Inns., 1987; Sahoo and Anjaneyulu, 1995; Floros *et al.,* 2000). For packaging of cottage cheese under MA, Maniar *et al.* (1994) used the material having an inside layer of 3 mil LDPE layered with 0.6 mil biaxially oriented nylon coated with an outside layer of PVdC. Mozzarella cheese was packaged using MAP into high gas-barrier packages composed of ethylene-vinyl acetate (EVA), copolymer sandwiched with a centre layer of vinylidene chloride copolymer (EVA / PVdC / EVA).

Table: Examples of laminates used in MAP foods

(Zagory and Kader, 1988)

Laminate	**Gauge*/ microns****	**Permeability (ml /m^2/24h/ 1atm)**		
		O_2	CO_2	N_2
PET/PVdC/PE	12** /3** /50*	8-10	30	8
PVC/LDPE	400*/ 75*	15	30	4
Nylon/PVdC/PE	60*/ 5**/ 100*	9	-	-
PVdC-coated PET/PE	15**/ 60*	2-4	-	-
PVC/PE	400*/75*	15	-	-
Nylon/EVAL/Nylon/PE	25**/10**/25**/ 100*	20	20	1

These packages consisted of bags measuring 18 by 28 cm with O_2 and CO_2 permeability rates of 26.53 and 79.90 ml / m^2 / day respectively at 1 atm and 24 °C under dry condition (Alves *et al.,* 1996). Hard cheeses such as cheddar are commonly packed in 100% CO_2 using horizontal form-fill-seal (FFS) pillow pack machines. The packaging materials used include cellophane or polyester/ polyethylene (Damske, 1990), 15 μm oriented polyster / 50 μm LDPE with 4 % EVA (Addington, 1991) and PP (Hampton, 1982). Cheese packed in PP film had a shelf life of up to 4 weeks using MAP, compared with only 14-15 days when packaged under normal conditions (Hampton, 1982). Addington (1991) and Subramaniam (1993) reported that for mould ripened cheese appropriate permeability and gas flushing combination was required for controlled growth of mould. According to Nordmark (2000) a good barrier film should have oxygen permeability of 2- 8 cm^3/ m^2 / day and WVTR of 5 g / m^2 / day.

Kosikowski (1982) advocated that the cheese should be dried for sometime and wrapped in parchment, saran or vacuum packaged in cryovac, polyethylene or cellophane pouches, followed by refrigerated storage. The experiments of Ghosh (1987) showed that mozzarella cheese packaged in polyethylene bag without vacuum, polyethylene bags with vacuum and cryovac had shelf life of 18 days, 21- 42 days and 42 days, respectively at refrigerated temperatures; and 90 days at frozen condition (deep freeze) regardless of packaging material. Scott and Smith (1971) suggested that mozzarella cheese should be packaged in saran or multilayer film and stored at low temp (4 °C) until used. Cottage cheese was packaged into high barrier multilayer poly bags (Pack-all packaging Inc., Mississauga, Ontario, Canada) with an oxygen transmission rate of 7.7 c.c / m^2/ 24 h at 18 °C and 0 % relative humidity. The pouches (15.24 x 22.86 cm^2) were vacuum packaged in multivac (Model AG - 500, Sepp Haggenmuller KG, Germany) packaging machine with double seal. Karagul-Yuceer *et al.* (2001) packed Yoghurt in 340 gm tightly capped PET bottles and stored at 4 ^{0}C.

2.1.5.0 Packaging Equipment for MAP

Smith *et al.* (1995) cited that the gas packaging technique involved removing air from the pack and replacing it with gas or mixture of gases, the pressure of gas inside the package usually reaching about one atmosphere, i.e. equal to the external pressure. This could be achieved by using three types of equipments: (1) continuous forming equipment (2) thermoforming equipment and (3) snorkel or bulk packaging equipment. In the continuous forming or gas flushing technique the machine creates a tube of film, which encloses the product. Then the appropriate gas mixture is introduced in a continuous flow into the package, followed by sealing and cutting. In the thermoforming technique, a compensated vacuum method is used to introduce the gas mixture. In this case, product is placed into a thermoformed tray and a vacuum is drawn to remove most of the air. The advantages of thermoforming method of gas flushing is that the high efficiency of removing O_2 to residual level is <1% and its high production rate with as many as 120 packages per minute . The third type of equipment is the snorkel or bulk package equipment. In this method machine holds the bag and inserts probes or snorkels, which remove the air from inside the bag. The vacuum is broken by the addition of the appropriate gas mixture, the probes are removed and the package is sealed. These types of equipment have been used for bulk packaging and retain the product in the gas-packaged bag throughout storage and distribution. The basic MAP formats are: the semi-rigid tray (e.g. for fresh salads); and the flow pack format (e.g., for cheeses and bakery products). In addition to these retail formats, there are 'bag-in-box', 'master pack' and 'mother pack' systems, which allow centralized packaging operations. The machinery used to produce these packs could be divided into four types: rigid and semi rigid tray packers; flexible horizontal flow seal packers; and bulk box / drum packers (Church, 1994). The FFS operations are involved: forming the packaging material around the product; gas flushing; cross sealing; and cutting to give individual packs. Depending on the size of the blocks and other factors, the packaging speeds vary from

42- 180 packs per minute and vertical FFS machines with packaging speeds of 45-75 bags per minute were used (Damske, 1990).

2.1.6.0 Gases for MAP

Generally three major gases namely O_2, N_2 and CO_2 or their mixture are used in the MAP for foods. Several other gases such as CO (maintains the red colour of meat), ozone, ethylene oxide, nitrous oxide, helium, neon, argon, (increases the shelf life for some fruits and vegetables), propylene oxide, and ethanol vapour (used for some bakery products), hydrogen, sulphur dioxide and chlorine have been used experimentally or commercially to extend the shelf-life of a number of food products (Day, 1992).

2.1.6.1 Effect of CO_2

CO_2 is the most important gas for MAP of foods, due to its bacteriostatic and fungistatic properties. In 1877, Pasteur and Joubart observed that *Bacillus anthracis* could be killed by using CO_2 (Valley, 1928), and five years later the first article on the preservative effect of CO_2 on foods was published by Kolbe (1882), showing extended storage life for beef placed inside a cylinder filled with CO_2 atmosphere. In the 1920s work at the Low Temperature Research Station in Cambridge, UK showed that storing apples in atmospheres containing lowered levels of O_2 and increased level of CO_2 enhanced the shelf life. In the 1930s beef carcases containing CO_2 approximately doubled the storage life (Davies, 1995).

It is only in the last two decades that MAP has become widely commercially used technology for storage and distribution of foods. CO_2 is present in the atmosphere at low level (0.03%). It is highly soluble in water and fat, and the solubility increases greatly with decreased temperature. The solubility of CO_2 in water is 3.38 g / kg H_2O (179.7 cm^3 / 100 ml) at 0 ^0C and 1atm.However, the solubility reduced to 1.73 g / kg H_2O at 20 ^0C and thus readily absorbed by high moisture refrigerated foods (Knoche, 1980). Therefore, effectiveness of the gas is always conditioned by the storage temperature, resulting in increased inhibition of bacterial growth as the temperature is decreased (Ogrydziak and Brown, 1982; Gill and Tan, 1980).

CO_2 is soluble in water as well as fat and oils and permeates most packaging materials more rapidly than other atmospheric gases. CO_2 extends both the lag phase and generation time of spoilage microorganisms and enhance the shelf life of the perishable foods. When CO_2 dissolves in water it has an acidifying effect (Hammann and Marth, 1984). This acidification as well as direct antimicrobial and antifungal effect of CO_2 can suppress the growth of many spoilage microorganisms.

CO_2 is an important component of MAP (Zagory, 1997). As per the observation of Jennifer (1998) CO_2 directly inhibited the growth of bacteria and mould, i.e. bacteriostatic and mycostatic effect. Smoot and Pierson (1982) showed that CO_2 inhibited, stimulated or had little effect on germination and toxigenesis of spore formers such as *Clostridium botulinum*. However, the cell-inhibiting spectrum and preservation of CO_2 is not well understood. In general, gram-negative bacteria were observed to be very sensitive to CO_2 while, the count and growth rates of lactic acid bacteria were much less affected (Enfors and Molin, 1981). Wolfe (1980); Daniels *et al.* (1985); Dixon and Kell, (1989) and Farber (1991) summarized the influence of CO_2 on bacterial cell which suggested:

- Alteration of cell membrane function including effect on nutrients uptake and absorption.
- Direct inhibition of enzyme or decrease in the rate of enzyme reactions.
- Penetration of bacterial membranes, leading to intracellular pH changes.
- Direct changes to the physico-chemical properties of cell proteins.
- Displacement of O_2.

Effect of CO_2 on growth of microorganisms

The inhibitory and stimulatory effects of CO_2 on microorganisms in culture medium or food system are dependent on many factors. These include the partial pressure, concentration and temperature of CO_2 (Blickstad *et al.*, 1981), volume of headspace gas, acidity, water activity, the type of microorganisms (Dainty, 1971; Davidson and Juneja, 1990), type of product

(Ogden and Inventor 1997), the microbial growth phase, and the growth of medium used for maximum antimicrobial effect. The storage temperature of MAP product should be kept as low as possible because the solubility of CO_2 decreases dramatically with increasing temperature. Dissolved CO_2 could increase the lag phase and generation time of microorganisms (Daniels *et al.,* 1985). Investigations have shown that CO_2 is effective in lengthening the lag phase and slowing the growth of primarily gram-negative bacteria and vegetative bacterial cells (Hendrick and Hotchkiss, 1997; King and Mabbit, 1982; Roberts and Torry, 1988). King and Nagel (1975) and Moir & Darey (1993) studied the inhibitory effect of CO_2 on the growth rate of *Pseudomonas aeruginosa*. They observed a linear correlation between the growth inhibition of *Pseudomonas aeruginosa* and CO_2 concentration.

2.1.6.2 Effect of Nitrogen

Nitrogen gas is generally considered a neutral filler gas; it neither influences the colour of the product nor its microbiological quality. It is an inert gas. N_2 by displacing O_2 in the pack can delay oxidative rancidity and also inhibits the growth of aerobic microorganisms indirectly (Sahoo and Anjaneyulu, 1995), and could be used as an alternative to vacuum packaging to inhibit the aerobic microorganism (Silvertsvik *et al.*, 2002 b).

2.1.6.3 Effect of Oxygen

Oxygen is essential for respiration of fruits and vegetables, cell division and growth of most aerobic bacteria. Benefit of its oxidizing effect covers the maintenance of the oxidized state of haemoglobin, which retains the attractive bloom of red meats; disadvantages of some characteristics include the oxidation of unsaturated fatty acids in lipids, which lead to rancidity and eventual spoilage (Robertson, 1993; Brody and Marsh, 1997). Jones (1989) reported that lowering the oxygen level in MAP environment helped in extending the shelf - life of this product by reducing metabolic and chemical oxidation rates but the outgrowth of anaerobic pathogens might be stimulated. The use of O_2 in MAP is normally set as low as possible to inhibit the growth of aerobic spoilage bacteria (ACMSF, 1992). However, high levels of O_2

(30 %) are used in red meats to maintain its red colour (Stiles, 1991; Gill, 1996).

2.1.7.0 MAP of Dairy Products

The spoilage of dairy products is mainly due to microbial growth and oxidative rancidity. MAP can significantly extend the shelf life of dairy products by retarding the microbial growth and reducing the oxidative rancidity.

2.1.7.1 Milk

High partial pressure of CO_2 has long been known to be inhibitory to many microorganisms and not surprisingly, the effect of CO_2 microclimate on quality of milk and milk products has also been studied. Thus, the keeping quality of cooled milk could be improved by CO_2 treatment prior to refrigeration. CO_2 infusion into milk resulted in a decrease of coliforms and psychrotrophic bacterial growth. The effect was enhanced with increasing concentrations of CO_2 and decreasing storage temperature. However, it seems that the CO_2 prolonged the lag phase but did not influence cells in logarithmic growth when used to preserve milk and therefore the final effect depended upon the growth phase of the cells at the time of treatment. The benefit of CO_2 treatment might also be a function of the initial contamination of the raw milk and eventually of the processing routine. The application of CO_2 to raw milk with a standard plate count (SPC) of 10^5 cfu/ml showed signs of benefits after 24 h storage at 7 °C (Robertson, 1993). The addition of CO_2 could control the growth of psychrotrophic bacteria in both raw and pasteurised milks at refrigeration temperatures (Hotchkiss *et al.,* 1999; King and Mabbit, 1982; Rashed *et al.,* 1986).

Hotchkiss (1995) believed that certain dairy products could be kept fresh for several months by carbonation. The safety of refrigerated, pasteurised milk was observed to be further enhanced with the use of carbonation by inhibiting the growth of pathogenic/ spoilage bacteria. He also reported that MA packed milk could last more than two months in a refrigerator, tasted fresh and contained no dangerous bacteria. Hotchkiss and Lee (1996) studied the effect of CO_2 addition on the shelf life of milk inoculated with spoilage microorganisms. The shelf life of milk was enhanced by 50- 100% and these were dependent on flushing of CO_2, storage temperature and high barrier package. Though, MAP is not currently being used in the retail packaging of milk, but N_2 flushing might be useful in preserving the quality of milk. According to Addington (1991) N_2 could be used as the headspace in UHT milk packs, which helps in retaining the flavour during latter half of the storage-life of the product, possibly by reducing oxidative changes. Ganguli (2001) suggested that carbonation could extend the storage life of bulk-chilled milk without affecting the stability of vitamin A and vitamin E.

Impact of CO_2 addition to milk on selected analytical testing methods

Ma *et al.* (2001) observed that addition of 1000 ppm CO_2 to milk did not affect the performance of antibiotic testing (β-lactams) methods namely, IDEXXSNAP, Charm II Sequential tablet, and Delvo- Ampule. Milk freezing point decreased linearly with increasing concentration of dissolved CO_2. Increase in carbonation from 0 to 1000 ppm decreased milk pH (King and Mabbit, 1997) at 38 ^{0}C from 6.61 (control) to 6.15 (1000 ppm).

Effect of CO_2 on growth of *Bacillus cereus* spores in milk during storage

Werner and Hotchkiss (2002) suggested that addition of moderate levels of CO_2 didn't show either stimulatory or inhibitory effect on the initiation of germination and subsequent outgrowth of *B. cereus spores* over long-term storage and didn't increase the risk of food borne illness due to the organism. The number of viable organism declined in all samples. No visual defects were detected in any of the milk samples. The addition of CO_2 on the growth of *B. cereus* spores inoculated into sterile homogenized whole milk at 10^1 and 10^6 spores/ ml and stored at 6.1^{0}C was examined weekly for 35 days. They reported that added CO_2 reduced the pH of the milk from average value of 6.61 to 6.31. They also reported that moderate levels of CO_2 had no effect on spoilage and risk of food poisoning due to germination of spores of *B. cereus* over long storage. Similar conclusions were also made regarding *Clostridium botulinum* (Glass *et al.*,1999).

2.1.7.2 Danedar *Khoa*

Danedar *khoa*, the main ingredient of khoa based sweets, is highly perishable. Its shelf life could be extended up to 60 days at 11 ^{0}C by packaging under nitrogen / vacuum in a flexible pouch of poster paper/ aluminium foil / LDPE (Sharma *et al.,* 2001).

2.1.7.3 Milk Powder

Nitrogen flushing of whole milk powder helps in reducing the oxidation of fat. Milk powder in retail and bulk packs, particularly for export to tropical countries, is nitrogen flushed to retain quality. Examples of retail packaging used are bag-in-box with barrier lining and form-fill-seal pouches (Addington, 1991).

2.1.7.4 Yoghurt

Yoghurt is cultured dairy product that has shelf life of up to 60 days under refrigeration. The storage life depends on the degree of sanitation during processing and packaging (Karagul- Yuceer *et al.,* 2001). Tamime and Deeth (1980) stated that gas flushing of CO_2 or N_2 was a viable alternate preservation method to extend the shelf life of fruit- flavoured yoghurt by inhibiting the growth of yeast and mould. Fairbairn and Law (1986) recommended the use of carbonation process as it is cheap and safe and apparently doesn't have any negative impact on cultured dairy products. Blakistone (1990) studied plain yoghurt by aseptically filling in high barrier cups, which were then sealed with a foil lidding and flushing with N_2 at 40 ^{0} F with residual O_2 level in headspaces 0.1-0.2 % after 8 weeks of storage. Some dairies have also flushed CO_2 into the headspace of yoghurt and sour cream packages to increase the shelf life (Fierheller, 1991). Several methods have been reported for adding CO_2 to a product, like addition of carbonated water, production of liquid drinkable yoghurt by commercial carbonation process, and the addition of metal carbonates. However, these methods were found be not suitable for yoghurt, because it is a highly viscous product.

However, flushing of gaseous CO_2 into the product has been suggested as a way to overcome this problem (Ogden and Inventor, 1997), which inhibits the growth of yeast and moulds in yoghurt. Karagul-Yuceer *et al.*, (2001) reported that carbonation of yoghurt declined the contaminating bacteria; *E. coli* and *L. monocytogenes* count. They also reported that carbonation did not significantly affect the growth of typical yoghurt bacterial strain and probiotics in yoghurt.

2.1.7.5 Cheeses

MAP and active packaging (AP) can be applied to dairy products to extend shelf life and to control some of the fungal problems. The various fungi respond differently to the altered gaseous environment produced by MAP and AP (Haasum and Nielsen 1998 a; Haasum and Nielsen 1998 b). For example, the growth of *P. roqueforti* is essentially unaffected by the varying amounts of O_2 and CO_2 in the package, while that of *P. verrucosum* is significantly reduced under high CO_2 levels. Floros *et al.* (2000) reported that when O_2 ranged from 4- 21%, it did not affect the fungal growth to great extent, and even at low level CO_2, 0-2% slowed the fungal growth. This finding could be of interest to dairy industry that even at low level of CO_2 and by application of MAP and AP, significantly extends the shelf life of some cheeses.

Soft Cheese

Owing to soft texture, these products are not suitable for vacuum packaging, but can be successfully packaged under MAP because of the cushioning effect of the gas. Horizontal form-fill-seal (FFS) machines can be used for gas packaging. Half fat soft cheese packaged in the metal films gets benefited from gas flushing of the headspace (Addington, 1991).

Cottage cheese

Kosikowski and Brown (1973) demonstrated the effectiveness of storing creamed cottage cheese in a CO_2 atmosphere or in a nitrogen atmosphere which suppressed spoilage organisms (psychrotrophic bacteria, yeast and moulds). Storage was led to deterioration in flavour after 11-18 days and in texture after 32-45 days. The shelf life of cottage cheese in standard barrier plastic container held at refrigeration temperature was estimated to be 10-21 days (Bishop and White, 1985). Honer (1988) demonstrated that quality was maintained in cottage cheese packaged under MA for much longer period compared with air packaging. Chen and Hotchkiss (1991 a) studied that dissolved CO_2 inhibited the growth of gram-negative bacteria and improved the keeping quality of creamed cottage cheese. The quality of cottage cheese was extended upto 28 days by using MAP technique (Maniar *et al.,* 1994). Fedio *et al.* (1994) studied the effect of MAP on the growth of microorganisms in cottage cheese. They reported that cottage cheese inoculated with listeria showed growth in packages containing air and packages containing 100% N_2, but not in packages containing elevated CO_2 levels. The growth of other pseudomonas was observed in samples packed in air. The growth of yeast and moulds was most strongly affected by modifying the storage atmosphere. In air-packed cottage cheese considerable growth of yeast and moulds was observed, while growth was suppressed in samples packaged with nitrogen. The yeast and mould count of cottage cheese packaged in 100% CO_2 and storage presumably due to the combined effects of CO_2 and the acidic environment of the cheese. The use of CO_2 has been found to be beneficial in preserving cottage cheese commercially in Germany (Anon, 1987 a). In this process cups were flushed with CO_2 and as well as headspace was filled with CO_2, after that sealed with aluminium foil and then cap was placed over the foil (Anon, 1987 b).

Whey cheese

MAP with CO_2 under refrigeration temperatures was effective in extending the shelf life of Requeijao, a Portuguese whey cheese. Pintado and Malcata (2000) studied the effect of MAP on the microbial ecology in Requeijao cheese. They reported that the viable numbers of enterobacteria, staphylococci, yeast and spore-forming bacteria in the experimental whey cheeses did not increase within 15 days when stored at 4 °C under 100% CO_2, those of enterococci increased significantly after 6 days under similar conditions, and a similar inhibiting effect was observed against *Bacillus*, P*seudomonas*, *Lactobacillus*, and *Streptococcus sp.* It was also observed that 100 % N_2 at 4 °C was able to completely inhibit the growth of staphylococci and lactobacilli for 2 days.

Quarg cheese

Rosenthal *et al.* (1991) studied the effect of CO_2 on the shelf life of quarg cheese by monitoring flavour, pH value, yeast & mould and pseudomonas count. No growth of active yeast and moulds or changes in the pH value for the entire length of experiment (67 days of storage at 4°C) were reported in cheeses stored under CO_2 enriched atmosphere (67.1 % CO_2, 26.3% N_2 and 6.6 % O_2). The growth of gram-negative bacteria was also inhibited. The flavour of cheese was preserved. They concluded that the effect of CO_2 was fungistatic and bacteriostatic.

Other cheeses

MAP is particularly effective for crumbly cheeses such as Cheshire and grated cheese, where vacuum packaging would cause undesirable compression (Fierheller, 1991). MAP for parmesan grated cheese allowed a shelf life of upto 98 days, whereas in air, the shelf life of this product was only few days (Sarantopoulos and Sorev, 1995). Piergiovanni *et al.* (1993) compared Tallegiio cheese packaged under four modified atmospheres, and stored at 6°C, to traditional paper wrapping and found that samples packaged in MAP had satisfactory quality. MAP caused significant differences in sensory, chemical and colour properties, but not in microbiological analysis. Westall and Filternborg (1998) studied the influence of yeast on the spoilage of decorated soft cheese packed in MAP and found that the increase in concentration of CO_2 affected the growth of spoilage yeast.

Table: Cheese types and their special MAP requirements

(Floros *et al.*, 2000)

Cheese types	Examples	Optimal gas conditions	Optimal package
Stabilized cheese	Cream cheese, Processed cheese, Decorated cheese, Feta cheese.	Low O_2, high CO_2	High barrier; O_2 absorbing film, anti-microbial film
Bacterial ripened cheese	Semi-soft and hard cheeses (whole, sliced and shredded)	Low O_2, CO_2 control	Medium barrier; CO_2 absorbing film, anti-microbial film
Mould ripened cheese	White cheese Camembert, Blue-veined cheese	Low O_2 (1-2%), CO_2 control	Medium to low barrier; advanced absorbers

MAP Mozzarella Cheese

Modified atmosphere packaging is capable of substantially extending the shelf life of food products. The shelf life of mozzarella cheese increased to about 240% when packed under MAP (Sarantopoulos *et al.,* 1993; Sarantopoulos and Sorev, 1995).

Sliced Mozzarella cheese

Alves *et al.* (1996) studied the stability of sliced mozzarella cheese in MAP and reported a significant increase in shelf life upto 63 days (a 385 % increase) for product under 100 % CO_2, and up to 45 days (a 246 % increase) for product under 50 % CO_2 / 50 % N_2, compared to conventional air pack (13 days). The shelf life for sliced mozzarella cheese in the four different treatments, stored at 7 $\pm$ 1°C, can be predicted as air, 13 days; 100 % N_2, 16 days; 50 % CO_2 / 50 N_2, 44 days; and 100 % CO_2, 64 day.

Alves *et al.* (1996) concluded that the inert atmosphere of 100 % N_2 was not effective for controlling the microbiological deterioration of sliced mozzarella cheese, probably because the residual oxygen determined after the equilibrium of the gases in the headspace was around 3 to 4%. Such residual oxygen came from the air left between the cheese slices and on the tray and could hardly be reduced to levels below 1%, especially in industrial equipment, unless oxygen absorbents were used.

The efficacy of carbon dioxide in controlling microbiological deterioration was demonstrated in systems under modified atmosphere with CO_2 (Alves *et al.,* 1996). The experiments conducted by them proved that the increment of the relation from 0.8 to 2.0 litres of CO_2 per kg of cheese led to an increase in the efficacy of this gas. They also observed that in cheeses under an atmosphere with 0.8 litres of CO_2 per kg of cheese (50 % CO_2 / 50 % N_2), there was a reduction in the rate of development of aerobic psychrotrophs and moulds and yeasts. They also observed that in cheeses under an atmosphere with 2.0 litres of CO_2 per kg of cheese (100 % CO_2), the beginning of the growth of aerobic psychrotrophs was retarded and their growth rate was diminished. Also, the growth of moulds and yeasts in the cheeses was totally inhibited. Atmospheres with high percentage of CO_2 did not cause undesirable changes in the mozzarella flavour.

Alves *et al.* (1996) reported the shelf life of sliced mozzarella cheese at 7 ± 1^oC, based on the overall quality evaluation, for the four different packaging systems as: under conventional air, 13 days; under 100 % N_2, 16 days; under 50 % CO_2 /50 % N_2, 44 days; and under 100 % CO_2, 64 days. They inferred that of the four systems evaluated, the atmosphere with 100% CO_2 constituted an interesting MAP alternative for commercialising sliced mozzarella cheese, making possible a shelf life comparable to the vacuum-packed product.

Shredded Mozzarella cheese

The stability of shredded mozzarella cheese was studied by Eliot *et al.* (1998) under 8 modified atmospheres (air, vacuum, CO_2, N_2 and mixtures of CO_2 / N_2 in different proportions) for eight weeks. They reported that modified atmosphere containing CO_2 efficiently stabilized lactic acid and mesophillic flora, while inhibited staphylococci, yeast and mould. Psychrotrophs grew in all samples but were less numerous in high CO_2 atmospheres. Levels of 75 % CO_2 were found to repress undesirable organisms and reduced gas formation.

Eliot *et al.* (1998) concluded that CO_2 was effective in depressing undesirable microorganisms such as staphylococci, yeasts and moulds in shredded mozzarella cheese during storage. CO_2 was not as effective in repressing psychrotrophic bacteria but it reduced growth of lactics and mesophillics. According to them, two hypotheses might explain the small effect of carbon dioxide on psychrotrophic flora, a high temperature of storage, or a reduced sensitivity to CO_2 in mozzarella cheese. Higher CO_2 concentrations were observed to be more effective than nitrogen to control mesophillics, and were also more effective than vacuum packaging in inhibiting yeasts and moulds. CO_2 levels $\geq$ 75% were found to be the most appropriate for maintaining microbiological quality and safety of shredded mozzarella cheese during 8 weeks and for reducing carbon dioxide production inside package, hence minimizing package distension.

2.1.8.0 Headspace gas composition and volume

Day (1992) accomplished packaging under MAP by using vacuum chamber Selovac (Sao Paulo, Brazil) CV – 18 machine, with gas injection condition in order to obtain a gas headspace to cheese weight ratio of 1 - 3 litres of gas per kg of the product. The study of Alves *et al.* (1996) showed that the packages with 100 % CO_2 and 50 % CO_2 had an average of 2.5 litres of gas per kg of cheese. During the storage the average volume of headspace in packages under 100 % CO_2 was 340 ml; therefore, there were 2.0 litres of CO_2 per kg of cheese. In the packages into which 50 % CO_2 and 50% N_2, the average volume of CO_2 was 143 ml. Thus, there was 0.8 litres of CO_2 per kg of cheese. The initial N_2 concentration was 99.0 % and between 4^{th} and the 18^{th} storage days it varied in the range of 97.1 to 94.4%. The initial O_2 concentration in the packages with 100% CO_2 was 1.4% and during 58 days of storage at 7 $\pm$ 1°C it ranged from 3.3% to 4.1%. This O_2 probably came from the residual air left between the slices and absorbed on the expanded polystyrene (EPS) tray. Another fact that confirmed the existence of residual air left in the packages was the reduction of the initial concentration of CO_2 from 97 % to 76 - 81 % range during storage and the increase of the percentage of N_2 from 2 initially, to the 16 to 20 % range (Alves *et al.,* 1996). In studies of other kinds of cheeses in MAP, the efficiency of CO_2 in inhibiting or retarding microbiological development, thus prolonging the shelf life of the cheeses, has also been verified by other workers (Chen and Hotchkiss, 1991a, b; Maniar *et al.,* 1994; Sarantopoulos *et al.,* 1993; Sarantopoulos and Sorev, 1995).

According to Eliot *et al.* (1998) oxygen consumption and CO_2 production in samples of atmosphere (air) were probably due to respiration by aerobic microflora (Fedio *et al.,* 1994) or due to oxidative and enzymatic reactions involving oxygen. CO_2 concentration was observed to increase after 8 weeks by 17 % in packages of 100 % N_2. The growth of aerobic and anaerobic microorganisms was probably responsible for this increase (Alves *et al.,* 1996). This CO_2 production might have been caused by hetero fermentative lactobacilli (Lee *et al.,* 1990).

2.2.0.0 Manufacture of Mozzarella cheese

Mozzarella cheese belongs to 'Pasta Filata' variety of cheese, which involves skilful stretching and moulding of curd under hot water to achieve smooth texture . An ideal mozzarella cheese has smooth, moist surface with a perfect sheen and an elastic, stringy body free from mechanical opening (Apostolopolous, 1994; Cavella, *et al.*, 1992).

Mozzarella cheese possesses mild flavour, visual appeal and characteristic texture when melted on the surface of a pizza. Mozzarella cheese melts smoothly and browns nicely when baked. The melted cheese is very elastic and is very stretchy and stringy, contributing to the sensory appeal and "fun factor" (Kosikowski, 1982). Mozzarella cheese should be white in colour have a blend, slight nutlike flavour but never sour; low melting point; should never by slimy nor coarse or rough; the rind should be very thin, soft and edible and easy to peel off from the main piece and the cheese should be elastic.

A pizza is not a pizza without a large handful of grated cheese topped and melted onto it. The cheese of choice is mozzarella, which is stringy and white. Mozzarella cheese enjoys an increasing popularity among the younger generation consumers throughout the world, mainly as pizza topping (Sherkat and Walker, 2002). There are two types of mozzarella that are acceptable for pizza: low moisture, which has a moisture content less than 52%, and high moisture, which has a moisture content of more than 52%. The low moisture version tends to have a longer shelf life. The latter is more popular for the pizza and restaurant industry. In order to top the pizza, the cheese should be grated, and not sliced. This allows for more even - cooking and better distribution. Overcooking of cheese should be avoided. The mozzarella has a soft elastic consistency (Sigsard, 1994). In order to obtain such quality cheese, the adherence to certain critical points in the manufacture of cheese are essential so that it has the requisite functional properties.

2.2.1.0. Methods of manufacture

Generally four methods for the manufacture of mozzarella cheese are followed. They are (i) starter culture (SC) method (ii) direct acidification (DA) method (iii) continuous method and (iv) ultrafiltraton (UF) method. The choice of the method depends on the individual cheese maker. Variations in the cheese making parameters can affect the compositional and functional properties of mozzarella cheese. Direct acidified mozzarella is functional on the day of manufacture due to its unique soft body caused in part by its high moisture and low calcium content (Fox, 2000).

2.2.1.1 Starter culture (SC) method

According to Jana and Upadhyay, (1991 a; b) in traditional (SC) method, the lowering of pH, which leads to the formation of the pliable monocalcium paracaseinate may be brought about by the fermentation of lactose to lactic acid by lactic bacteria. This imparts a fresh flavour to cheese, assists in the formation of rennet coagulum, and causes shrinkage of the curd and moisture expulsion, hence promotes characteristic texture formation during cheese making. Reinbold (1963) advocated the use of 1.5 per cent or more starter culture, depending on initial acidity of milk. Christensen (1966) and Hutkins *et al.* (1986) suggested the addition of starter culture in mixture, containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Kosikowski (1982) suggested the addition of 1.5 per cent *L. bulgaricus* or 0.75 per cent *S. thermophilus* for the manufacture of low moisture mozzarella cheese from pasteurised milk. Scott (1981) observed that 0.05 to 0.5 per cent active starter culture (*S. thermophilus* and *L. bulgaricus*) is desirable to produce mozzarella cheese by increasing the acidity by 0.02 per cent at a temperature of 31 to 32°C for renneting. Martley (1983) observed that *S. thermophilus* exhibited maximum rate of acid production between 39.3 to 46.1°C by addition of 1- 1.5 per cent starter culture, usually in mixture consisting of *S. thermophilus* and *L. bulgaricus* in the ratio of 1:1 or 2:1. Ghosh (1987) manufactured mozzarella cheese of acceptable quality from buffalo milk using *S. thermophilus* and *L. bulgaricus* in the ratio of 1:1.

Parente *et al.* (1989) reported that best results in terms of acid production and species composition were obtained when thermophilic multiple strain starter cultures were incubated at 37 °C and mesophillic multiple strain species at 22 ^{0}C or 30 °C. Rottigini and Batello (1989) concluded that freeze dried *S. thermophilus* starters for direct inoculation into the mozzarella cheese vat allowed more precise control of acidification and cheese quality, besides increasing the yield by 0.1- 0.2 per cent. Oberg *et al.* (1989) reported that cultured cheeses had better stretch, better melt and more browning than direct acidified cheese.

Starter cultures can have an impact on the functionality of mozzarella cheese by affecting the pH of cheese (Barbano, 1994), through their proteolytic activity (Oberg *et al.,* 1991 b) and through the metabolism of lactose to galactose (Johnson and Olson, 1985). Yun *et al.* (1995) reported that it was difficult to separate the effects of starter culture on the initial formation of curd and the subsequent changes that occur during ageing. A rod-to-coccus ratio of 9:1 was found to significantly increase the rate of proteolysis in mozzarella cheese during storage compared to using rod-to-coccus ratios 5:5 and 1:9. Hassen, (2000) observed that 10:1 ratio of *Streptococcus thermophilus* and *Lactobacillus helveticus* gave highest meltability and oiling- off when fresh and during cold storage for 4 weeks compared to lower coccus : rod ratios.

2.2.1.2 Direct Acidification (DA) method

Use of direct acidification of milk with acids, instead of starter cultures, has been suggested as a means of eliminating the variability of acid production by bacteria. The use of acid provides additional advantages such as shorter manufacturing time, absence of problems with bacteriophage or slow starters, and better control over pH variations in cheese manufacture.

The normal method of mozzarella cheese manufacture has been described by Kosikowski (1982). As a bland, unripened cheese, mozzarella is particularly amenable to direct acidification method. Mozzarella has been manufactured without starter culture from milk acidified with organic and inorganic acids (Breene *et al.,* 1964; Keller *et al.,* 1974; Paulson *et al.,* 1998), and these methods have been used to develop patented processes (Barz and Creamer, 1995). Keller *et al.* (1974) opined that care needs to be taken with the choice of acids, as some can be strong calcium chelating agents and cause curd demineralisation. Kindstedt and Guo, (1997 a) observed that the first weeks after manufacture, mozzarella made by using direct acidification generally had a softer body and greater melt characteristics than cheese of similar age made with starter cultures. The mozzarella made by using DA method may require less ageing (or no ageing) to attain the desirable functional properties. Higher yields might also be attainable as higher moisture contents could be achieved with mozzarella cheese made by direct acidification. Lactic acid tends to give high moisture cheese while hydrochloric and phosphoric acids yield cheese, which are of almost equal satisfactory quality. Phosphoric acid gives better retention of calcium in the curd with consequently higher yields (Shehata *et al.*, 1967).

Quarne *et al.* (1968) observed that procine pepsin or fungal rennet gave better cheese than calf rennet. They also noted that reduced amount of enzymes retarded development of bitter flavour, and they also compared with traditional cheese and observed that bitterness developed more rapidly in cheese made by direct acidification because of the absence of lactic acid bacteria. The experiments of Micketts and Olson (1974) suggested that bitterness might be a problem in cheese at low pH but could be overcome by reducing the amount of rennet used. Demott (1983) reported that yield of mozzarella cheese by culture method was more than that of prepared by direct acidification method. Schafer and Olson (1975) found that greater recovery of milk protein resulted in increased yield (3.4 per cent).

2.2.1. 3 Continuous method

Mechanization of cheese making has been an ongoing process over many years and has proceeded step by step. Automation and mechanization of the collection and storage of the cheese milk has reached a high degree of efficiency in dairying. The kneading part of mozzarella cheese manufacture, which produces the "pasta filata" type of cheese (mozzarella) is the most important and laborious part of the complete process. To get rid of this manual labour, Nilson (1977) introduced a highly mechanized, large capacity mixer-cooker and moulding machine, which produced a smooth, uniform product at a high rate. Mechanical brining, automatic vacuum and gas packaging was also followed.

An installation for continuous salting of mozzarella cheese has been described by Nilson and LaClair (1975) in relation to possibilities of an automation of mozzarella cheese manufacture. Saal (1975) illustrated a mechanical process which involved mixing and stretching the curd in hot water and forcing it into the moulding machine where it shaped into 5 lb loaves which were then placed in plastic trays and immersed in cold water for 8 h. Dzenis (1976) suggested a mechanized method for the production of soft uniform mozzarella cheese at high speed where the curd at 130 °F was cooled (with water at 45 °F) to an outside temperature of 60 °F to 65 °F and an inside temperature of 80 °F to 85 °F in 6 to 7 min. The machine converted cheese mass into exact rectangular or round portion and simultaneously cooled them sufficiently to preserve their shape. Muzzarelli (1977) reported a fully mechanized and patented line for continuous production, hardening, salting and packaging of traditional mozzarella cheese which had a plasticizing and moulding capacity of 200 to 1200 kg / h. Mongiello (1979) described a moulder system to produce mozzarella cheese in block sizes of 8 oz to 20 lbs at capacities of 2000, 4000, and 8000 lb/h. An improvement in moisture and fat retention in the cheese was claimed for this system. Nilson *et al.* (1979) produced natural mozzarella cheese from pasteurised milk with added rennet and culture (1 %) in a continuous system. The milk was kept for more than 4 h at 8 °C, heated to 32 °C and then lactic acid was added to bring the pH to 5.8 to 5.9. The entire system was automatic up to bringing of the cheese. Thalmann (1981) described continuous mixing, cooking, draining, moulding and packaging machine for mozzarella cheese production by traditional (culture) as well as direct acidification method.

2.2.1.4 Ultrafiltration (UF) method

Ultrafiltration is a sieving process. It uses highly permeable membranes with variable pore size that allows the passage of water and low molecular solutes while being impermeable to macromolecules. Research on ultrafiltration of milk for mozzarella cheese at the pilot plant level has shown a great future potential. Kosikowski (1977) produced mozzarella cheese from UF concentrate which did not stretch in hot water but more promising results were obtained with dialysis ultrafiltration coupled with simultaneous fermentation which had the effect of removing more insoluble Ca^{++} salts, increasing the protein content and reducing buffer capacity. The resulting cheese had a good appearance and very good stretching qualities with low melt down properties as compared to commercial product. Morris (1984) described a computerized mozzarella cheese plant where skim milk was ultrafiltered and the retentates were blended with 80 per cent cream for further processing of mozzarella cheese production.

2.2.2.0 Processing of Milk for the Manufacture of Mozzarella cheese

2.2.2.1 Admixing and Standardisation of Milk

Milk meant for cheese making is standardized to ensure compositional standards and also to improve the quality and the yield. The process is carried out by the addition of a calculated quantity of skim milk, skim milk powder or cream or by the removal of a known quantity of fat from the whole milk. Standardisation of milk for cheese making has been reviewed by Chapman (1981).

Both cow as well as buffalo milk give good quality mozzarella cheese. However, cow milk cheese exhibits higher melting and fat leakage but has a slight yellow colour (not desirable), while buffalo mozzarella is pure white in colour (hence higher appearance score) with slightly superior stringiness (Rowney *et al.*, 1999).

Nilson *et al.* (1979) recommended milk standardization to 3.2 per cent fat with different protein levels ranging from 2.53 to 3.71 per cent (mean 3.2 per cent) for low moisture mozzarella cheese. Partridge and Nilson (1981) made mozzarella cheese from milk standardized to 3.2 per cent fat, pasteurised and stored at 4 °C. They observed no considerable effect on the constituents, flavour, body & texture or appearance of the finished cheese as compared to fresh milk.

Mozzarella cheese was traditionally manufactured from high fat buffalo milk. Though, there are no established standards for the casein / fat ratio for preparing mozzarella cheese. Satisfactory quality cheese was prepared from cow milk having 2 or 3 per cent fat (Flanagan *et al.,*1978; Kosikowski, 1982 and Honer, 1983). Shukla (1986) made mozzarella cheese of satisfactory quality by direct acidification method from buffalo milk standardized to 3 to 4 % fat. Ghosh (1987) reported that mozzarella cheese made from buffalo milk using 4.0 per cent fat exhibited overall good sensory and physico-chemical characteristics. Mozzarella-like cheese made from recombined milk did not have the characteristic stretch and melt behaviour associated with mozzarella cheese (Lelievre *et al.,* 1990). Sabikhi (1991) made mozzarella cheese of satisfactory quality by direct acidification method from buffalo milk and goat milk admixture (1: 1).

2.2.2.3 Homogenization

Homogenization is not a common commercial practice for mozzarella cheese manufacture. Quarne *et al.* (1968) found the highest fat recoveries of 92.68, 88.90 and 89.90 per cent in mozzarella cheese manufactured from homogenized milk (500 psi) by (i) traditional procedure, (ii) direct acidification with subsequent cutting of the curd, and (iii) direct acidification method, with continuous agitation during coagulation of milk, respectively. The recovery of fat was lower for all methods, when unhomogenised milk was used for preparing mozzarella cheese.

Homogenisation of cheese milk at high pressures (670 kPa) has been found to result in a cheese with reduced stretching and melting properties, while homogenisation at a lower pressure of 400 kPa did not affect the melting and stretching properties. Lelievre *et al.* (1990) and Tunick (1994) observed that one of the benefits of homogenizing cheese milk or cream at lower pressure was reduction in free-oil formation. Low-fat Mozzarella cheese has been manufactured using homogenized milk (Tunick *et al.,* 1993 b; Merrill *et al.*, 1994), yielding a cheese with increased meltability. Possible explanations might include a reduction in the rigidity of the casein matrix by smaller fat globules acted as co-polymers with the casein, or increased water retention by the cheese due to transfer of casein from the bulk casein matrix to the newly formed fat globule membrane. This is in contrast to the effect found in full- fat mozzarella cheese, where meltability was reduced by homogenization of the milk before cheese making (Lelievre *et al.,* 1990; Tunick *et al.,* 1995). Fife *et al.* (1996) reported that the increase in moisture content of low- fat mozzarella cheese as a result of a reduction in fat content might also improve its functionality.

2.2.2.4 Pasteurisation of Milk

Mozzarella cheese has been manufactured traditionally from raw buffalo milk. However, the enforcement of legal standards has put a check on the use of raw milk for cheese making. Kosikowski (1982) has recommended pasteurisation of milk at 72 °C for 16 sec and cooling to 32.2 °C for all types of mozzarella cheese. Pasteurisation of milk for the manufacture of mozzarella cheese has been suggested by many workers (Christensen, 1966; Keller *et al.,* 1974; Scott, 1981; Partridge *et al.,* 1982).

2.2.2.5 Acidification of Milk

Breene *et al.* (1964) acidified 2 per cent fat milk at 40 °F to pH 5.6 with lactic, acetic and hydrochloric acids. Shehata *et al.* (1967) reported that calcium levels were higher in pizza cheese made with phosphoric and hydrochloric acids than those made with hydrochloric and lactic acids. Cheese made with phosphoric and hydrochloric acids had lower moisture content and were firmer than cheese made with lactic acid.

Keller *et al.* (1974) prepared mozzarella cheese from 2.0 per cent fat milk, pasteurized at 72 ^{0}C for 15 sec and homogenized at 35.2 kg / cm^2 by direct acidification method. Shukla (1986) reported that acidification of cheese milk with acetic acid resulted in higher yield of mozzarella cheese than that made with hydrochloric acid, probably due to less total solids loss in whey in the former. Sabikhi (1991) observed that HCl was found most suitable acidulant for production of good quality mozzarella cheese.

2.2.2.6 pH levels

Manufacture of mozzarella cheese has been attempted by acidifying milk to different pH levels. Breene *et al.* (1964), Quarne *et al.* (1967) and Micketts & Olson (1974) made mozzarella cheese by acidifying milk to pH 5.6. The same pH level was used for mozzarella cheese manufacture from ultrafiltered whole milk retentates (Fernandez and Kosikowski, 1986 a;

1986 b), from ultra-high-temperature processed milk (Schafer and Olson, 1975) and from reconstituted milk made from non-fat dry milk and cream (Demott, 1983). Keller *et al.* (1974) attempted manufacture of mozzarella cheese by acidifying with several acids to pH levels of 5.6, 5.4 or 5.2. They concluded that moisture content increased with decreasing pH. Bitterness also increased in high moisture cheese made at low pH. In general, meltability decreased with increasing pH level. Shukla (1986) made mozzarella cheese from buffalo milk by acidifying to pH 5.4-5.6 with hydrochloric or acetic acid and concluded that the cheese was of acceptable quality. Sabikhi, (1991) prepared mozzarella cheese (admixture of buffalo & goat milk) acidified to pH of 5.6 had most satisfactory quality.

2.2.2.7 Coagulation

The unique characteristic step in the manufacture of mozzarella cheese is the enzymatically-induced coagulation of the casein micelles. The reaction involves the specific rapid hydrolysis of k-casein by rennet, causing it to lose its ability to stabilize the calcium sensitive caseins (α and β) and gel formation.

In the primary phase of the enzymatic action, the phe_{105}- met_{106} bond of the k-casein is cleaved, thereby forming paracasein and soluble peptides. The secondary action involves the coagulation of paracasein by Ca^{++} ions, above 20 °C. Phelan *et al.* (1973), Green and Foster (1974) suggested that the ideal coagulant for cheese manufacture is calf rennet. Rennin, the major enzyme of rennet, brings about coagulation of milk and causes slow proteolysis of casein during curing. Coagulation of milk occurs in two phases (i) a primary; enzymatic phase in which rennin alters phenyl- alanine-methionine peptide bond of k-casein releasing glycomacro-peptide (Jolles *et al.,* 1968) and (ii) secondary; non enzymatic phase in which the para -casein system clots in the presence of calcium ions (Fox, 1970). Quarne *et al.* (1968) used 7.5 ml of pepsin and 10 ml of fungal rennet in 45.4 kg milk to achieve a clotting time equivalent to veal rennet for the preparation of pizza cheese by direct acidification method.

Naudts (1969), Martens and Naudts (1973) observed that cheese made with microbial enzymes often ripened differently from those made with calf rennet and had bitter flavour as well as body defects. Sandoval *et al.* (1972) compared the mozzarella cheese made from Meito rennet (*Mucor pusillus*) and calf rennet and recommended the use of 2.5 g Meito rennet powder / 100 lit milk. Micketts and Olson (1974) reported that mean yield, composition and properties of cheese were not affected by the amount of enzyme, except for a limited number of characteristics. Single strength rennet extract of 100 ml for 45.4 kg of milk when pH was 5.6 in direct acidification method has been reported by Keller *et al.* (1974).

Di-Matteo *et al.* (1982) reported that the degradation of casein in mozzarella cheese could be minimized by adding less quantity of rennet in cheese milk. Mozzarella cheese was made by the direct acidification, continuous agitation method using varying amounts of commercial single strength rennet (2.5, 5.0, 7.5, 10.0 or 15 ml) or pepsin (1.7, 3.4, 5.0 and 6.7, equivalent to 10 ml rennet) for 45.4 kg milk. Kosikowski (1982) observed that addition of 60 to 85 ml of single strength rennet extract per 100 lbs milk just after the starter inoculation was optimum. He further recommended that the calculated amount of rennet extract should be diluted 1: 40 with pure cold water just before addition to milk. Ghosh (1987) found that the body of mozzarella cheese made from microbial rennet was slightly softer and hence, its melting property was superior, as also were the yield and moisture retention in cheese. However, fat and TS recovery were comparatively lower than those of calf rennet. Shukla and Ladkani (1989) made mozzarella cheese by direct acidification method from buffalo milk, using calf rennet @ 0.9-1.0 g or Meito rennet @ 0.4-0.5 g / 100 l milk, while, Sabikhi (1991) prepared mozzarella cheese using HCl and Meito rennet @ 0.75 g / 100 l milk.

2.2.2.8 Cutting

The coagulum is cut when it reaches the proper firmness, so that the surface area from which whey escapes is increased and also to facilitate uniform heating of the curd mass. Scott (1981) recommended curd cube size of 1 to 1.5 cm (walnut size) whereas Kosikowski (1982) suggested the use of 5 / 8 or 3/4 inch knives for cutting of set curd.

2.2.2.9 Cooking

Cooking of the cheese curd is started about 10 to 15 min after cutting (Reinbold, 1963; Christensen, 1966 and Kosikowski, 1982). The cooking temperatures are governed by the desired moisture level and acidity development in the curd. Breene *et al.* (1964) employed renneting at 27 °C, holded at this temperature for 8 minutes and then heated to 49 °C in 4 minutes for making direct acid mozzarella cheese. Christensen (1966) recommended cooking between 110-114 °F for 35 - 45 min for low moisture mozzarella cheese. Demott (1983) manufactured a mozzarella-cheese- like product from non-fat dry milk and cream by employing cooking temperature of 36°C to 38°C. Fernandez and Kosikowski (1986 b) reported 44 °C as the cooking temperature for preparing direct acid mozzarella cheese from ultrafiltered whole milk retentates. Ghosh (1987) found that cooking temperature of 41°C was optimum for getting good quality product.

Tunick *et al.* (1993 a) compared the effect of a wider cooking temperature range from 32- 46^0 C and found that meltability decreased and hardness increased with increasing cooking temperature, most likely as a result of the lower moisture content and decreased proteolysis during storage at higher temperatures. Meltability and free-oil formation were not significantly affected over the cooking temperature range of 38- 41^oC, even though the moisture contents were significantly lower as the cooking temperature increased (Yun *et al.,* 1993 c).

2.2.2.10 Draining of whey

Altering the pH at the time of whey draining affects the colloidal calcium phosphate concentration and in turn the casein micelle structure. Kiely *et al.* (1992) reported that whey drained at pH 5.9 had 17 % less calcium than whey drained at pH 6.4, even though both were cheddared to pH 5.2. In addition, as the pH was lowered, more casein dissociated from the micelle, especially in the pH range of 5.6 to 5.2. Kindstedt and Guo (1998) observed that the pH at which the whey drained affected the amount of lactose retained in the cheese curd and hence influenced the rate of acid development during cheddaring or dry stirring.

2.2.2.11 Stretching and Moulding

Stretching is one of the most important physical properties of mozzarella cheese. Scott (1981) reported that the curd (pH 5.1 to 5.4) in hot water at 70 °C to 82 °C should stretch out to one meter long. Kosikowski (1982) placed milled raw acidified curd in hot water at 180 °F (82.2 °C) for few minutes. The curd was then stretched by using a wooden paddle or revolving blender until a smooth, long grain, white plastic mass resulted. Webb *et al.* (1983) observed that the curd of mozzarella cheese was usually heated to between 50 ^{0}C - 60 ^{0}C after it had matted, and was worked while, hot until it became very elastic, shiny, stringy and free from mechanical openings. Hutkins *et al.* (1986) stretched the curd in hot water of 77 °C temperature.

Apostolopoulos (1994) observed that the functionality of mozzarella cheese might also be affected by the method used to stretch the curd. Compared to cheese from a conventional cooker / stretcher, mozzarella cheese produced from a high pressure, twin-screw extruder resulted in a cheese with lower meltability and no detectable free-oil.

2.2.2.12 Salting

Salting is an essential step, which serves the following purposes: (i) controls the growth of undesirable microorganisms, (ii) retards acidity development by inhibiting the growth of lactic acid organisms, and (iii) gives cheese an appealing taste.

Reinbold (1963) suggested brining of cheese at a temperature of 45 to 50 °F for a period of 1- 3 days, depending upon the size of the cheese block so that the salt content in finished cheese is approximately 1.6 per cent. Christensen (1966) observed that salt content in mozzarella cheese samples ranged from 1.5 to 1.7 per cent, and too high a salt content gave a cheese with poor melting characteristics. Nilson (1968) developed a system in which the salt is directly sprinkled over the hot stretched curd followed by shaping it into five pound loaves. The loaves were then placed in plastic moulds for 20-30 min and finally cooled in either cold water or refrigerated brine. Cooling in cold water resulted in 1.46 per cent salt in cheese loaves while cooling in refrigerated brine resulted in 1.5 per cent salt. Leake and Nilson (1969) noticed that brining temperature affected moisture content of mozzarella cheese, but had little effect on the salt penetration. Scott (1981) suggested that 0.75 per cent salt could be mixed into the plastic curd, or cooled cheese of definite shape immersed in 16 to 20 per cent brine at 8 °C to 10 °C for sufficient time (5 min to 24 h) to allow 1.6 per cent salt in the cheese.

Kosikowski (1982) recommended that after cooling and washing in chilled water, the firmed curd blocks should be dipped in saturated brine (about 23 %) at 45 °F, and the satisfactory salting depended on the size and shape of the cheese. Ghosh (1987) obtained salt content of 1.65 per cent in half kg rectangular block of mozzarella cheese after 4 h of brining in 20 per cent (w / v) solution at 8 °C to 10 °C. In order to attain similar levels of salts in 1 kg round ball, it required 8 to 9 h of brining.

2.2.2.13 Yield

Schafer and Olson (1975) observed that there was greater recovery of milk proteins in mozzarella cheese made by direct acidification, resulting in an increase of 3.4 per cent of the cheese yield, made from UHT milk. Nilson *et al.* (1979) reported a rise in yield to the tune of 0.2 to 0.36 per cent for every increase of 0.1 per cent protein in milk. Kosikowski (1982) cited the average yield of commercial mozzarella cheese as 11.5 per cent containing 53.6 per cent moisture made from cow milk of 3.0 per cent fat. Cheese yield and fat content were significantly higher when buffalo milk alone was used (Bonassi *et al.*, 1982).

The research conducted by Ghosh (1987) showed that the yield was 14.98 per cent and 12.4 per cent when mozzarella cheese was prepared from buffalo milk and cow milk, respectively. The cow milk cheese contained slightly higher levels of moisture, fat and salt, whereas buffalo milk cheese had higher protein and ash content. Altero *et al.* (1989) suggested a regression equation for predicting the yield of mozzarella cheese from buffalo milk on the basis of fat and protein content in milk. Rottigni and Batello (1989) observed that the yield of mozzarella increased by 0.1 to 0.2 per cent when freeze-dried *S. thermophilus* culture was used for direct inoculation into the vat. McMahon *et al.* (1996) used fat replacers to manufacture low-fat mozzarella cheese with limited success. Fat replacers that yielded an increase in cheese moisture also reduced cheese meltability. Rudan *et al.* (1998 a; b) observed that the use of Salatrim (Pfizer Inc., Milwaukee, WI) as a fat replacer neither improved nor adversely affected the functional properties of mozzarella cheese.

2.2.2.14 Defects

According to Reinbold (1963) the smooth surface with good sheen is the desired quality of mozzarella cheese after thawing. "Marbling" in the finished product might be associated with incomplete stretching or mixing, too low water temperature, low acidity of the curd or a combination of these defects. Christensen (1966) reported that a too high salt content might result in poor meltability, which is necessary for a good quality of cheese. Use of *S. thermophilus* (which do not use galactose) culture alone might be the cause of browning defects in mozzarella cheese when used as a pizza topping and with processed cheese. The defective mozzarella cheese with superficial reddish brown marks, putrid smell and distinct bitter flavour due to the presence of *Pseudomonas putida, P. fluroscences and P. palloroni* with an optimum growth at 17 °C to 32 °C has been highlighted by Cabrini and Neriane (1983). A soft body defect in commercial mozzarella resulted in cheese that didnot slice or 'melt down' satisfactorily, and high levels of non-starter bacteria characterized as *L. casei* sub sp. casei were associated with the defect (Hull *et al.,* 1983).

2.2.2.15 Preservation

A report suggested the use of Delvocid (1000 ppm) as preservative for mozzarella cheese, which resists the growth of yeast and mould on the surface of the blocks (Anonymous, 1982). Another report recommended the application of dielectric technique including microwave process for mozzarella cheese production and preservation (Demeczky, 1985).

2.2.2.16 Packaging

Kosikowski (1958) observed that the spoilage of mozzarella is mainly caused by the mould growth because of high initial count and absence of natural inhibitors such as propionic acid. However, vacuum packaging helped in reducing the mould growth in packaged cheese, but with sliced mozzarella a high vacuum of 29 inch must be maintained for effective control. Kosikowski (1982) suggested that after brining, the cheese should be dried briefly and wrapped in parchment, saran, or vacuum packed in barrier films, polyethylene or cellophane pouches followed by storage at refrigeration temperature. Scott (1981) recommended that both mozzarella and pizza cheese should be packaged in saran or multilayer films and should be stored at low temperature (4 °C) until used. Saal (1975) packed 5 lb loaves of brined cheese in vacuum-sealed plastic bags. Beck (1983) described a transparent package for mozzarella cheese floating in whey for storage in tightly sealed plastic containers.

2.2.2.17 Storage

Storage of mozzarella cheese after manufacture is an important aspect in the manufacturing process because of the major changes that occur during storage. According to Oberg *et al.* (1992 b) freezing, storage, and thawing significantly affect the stretching and melting of mozzarella cheese but do not affect cook colour. Kindstedt *and* Guo (1997 a) stated that generally 1 to 3 week aging period in cold storage is required to develop the desired melt, stretch, and mouthfeel.

Di- Matteo *et al.* (1982) studied the storage effect on mozzarella cheese made form 100% cow milk, 100 % buffalo milk and mixed milk of different proportions. The cheese samples were stored at 10 to 11 ^{0}C. Proteolysis of mozzarella cheese during 50 days of refrigeration storage was affected by coagulant. Yun *et al.* (1993 a) reported that the αs- casein decreased significantly during storage at 4 ^{0}C irrespective of coagulants, but β- casein significantly decreased during 50 days of refrigeration storage.

Fennema (1972) suggested that high moisture cheeses, such as mozzarella, which have short storage lives, are potential candidates for freezing. Kielsmeier *et al.* (1988) patented a method of rapid freezing to maintain the moisture content of the frozen shreds of cheese, which could be added frozen directly onto a pizza for cooking with no detrimental effects. This rapid freezing method has been used in subsequent patented applications to produce cheese with a combined fat plus moisture content above 70% (Barz and Creamer, 1995). These cheeses exhibited good melting and low blistering properties, without the need for ageing, and retained this functionality for up to 12 months after manufacture.

Studies have shown that freezing increased hardness (Diefes *et al.,* 1993), decreased meltability and increased stretchability (Oberg *et al.,* 1992 a) and caused less free-oil formation (Bertola *et al.*, 1998). Allowing frozen cheese to ripen for 14 to 21 days at 4 °C, after freezing at – 20°C, resulted in no loss of quality, as measured by apparent viscosity, free-oil formation and meltability, compared to unfrozen control samples (Bertola *et al.,* 1998). Shredding of the cheese before freezing was also found to increase stretchability and decrease meltability (Oberg *et al.,* 1992 a).

2.2.3.0 Thawing

Dahlstorm (1978) used freezing and thawing conditions in such a way that times to transverse from –67 °C to –1.1°C were approximately 12- 60 hrs. He also concluded that none of these freezing and thawing rates caused differences in detrimental effects of the freeze thaw cycle on texture of mozzarella cheese. However, freeze-thawing rates used in this study were approximately 6 and 5 hrs respectively for 2.5 kg loaves. Cervantes *et al.* (1983) studied the effect of freezing, thawing, and texture of low moisture part-skim mozzarella cheese that had been frozen at – 15 ^{0}C and thawed at 5.6 ^{0}C. Shredded and un- shredded cheese samples were thawed at either

4.4 ^{0}C for 3 hours, 12- 8 ^{0}C for 2 hrs or 25 ^{0}C for 45 minutes (Oberg *et al.*, 1992 b).

2.2.4.0 Physico-Chemical Characteristics of Mozzarella Cheese

2.2.4.1 Moisture Content

The moisture content of mozzarella cheese is economically important as increased moisture content results in increased cheese yield. However, variation in moisture content may adversely affect the functionality of cheese, especially meltability. Tunick *et al* (1991) observed that increase in moisture content of mozzarella cheese from 47 % to 52 % resulted in a softer texture and significantly increased meltability. The moisture content of mozzarella cheese is affected by many variables; higher cooking temperatures have been

Table: Composition of low moisture and high moisture mozzarella cheese

(Kosikowski, 1982)

Attributes	Low moisture mozzarella	High moisture mozzarella
Moisture (%)	50.50	57.40
Fat (%)	15.22	13.36
Protein (%)	24.84	21.80
FDM (%)	30.75	31.36
Total Solids (%)	50.50	53.0
Lactose (%)	2.30	2.48
Salt (%)	1.58	1.70
Ash (%)	3.76	3.48
pH	5.66	5.62
TA (% lactic)	0.38	0.36

shown to result in lower moisture content (Yun *et al.,* 1993 b); increased cheese manufacturing time (as a result of slow acid production) resulted in lower moisture (Renda *et al.,* 1997); and salt levels were found to be inversely proportional to moisture levels (Kindstedt *et al.,* 1992). Cheeses are frequently classified according to their moisture in the non-fat substance (MNFS), which is equal to the percentage of moisture divided by 100 – percentage of fat. Variations in MNFS, which is basically a ratio of water to protein can lead to differences in textural quality (Olson & Johnson, 1990). In both high fat (HF) and low fat (LF) cheeses, hardness, springiness decreased significantly with increasing MNFS, whereas meltability increased significantly. Increasing the moisture content in cheese resulted in greater hydration of the casein network, which in turn decreased the hardness (Taranto *et al.*, 1979).

Table: Composition of mozzarella cheese as reported by various workers

(Sabikhi, 1991)

Moisture (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	Salt (%)	FDM (%)	Reference
47.3	-	-	-	-	2.1	41.7	Nilson & LaClair (1976)
53.0	22.0	22.0	0.3	-	1.0	38.3	Webb et al. (1983)
53.6	18.0	22.1	-	-	1.6	-	Singh (1984)
54.0	18.0	22.1	-	2.3	0.7	-	Kosikowski (1982)
49.81*	18.0	22.35	0.67	2.96	1.56	-	Ghosh & Singh (1996)
50.30	23.0	20.31	0.73	2.76	1.72		Ghosh & Singh (1996)

* from buffalo milk

2.2.4.2 pH

The pH of cheese may also affect the reactivity of binding sites on the casein molecule, and thus influence the structure of the casein matrix. Therefore, small changes in pH at different stages during manufacturing can affect the structure of the casein matrix and ultimately that of the curd.

Kosikowski (1977) observed as the pH was lowered to pH 4.8, almost all calcium phosphate solubilized, and at the iso-electric point of pH 4.6, the solubility of casein was negligible. Lower pH values resulted in dissolution of colloidal calcium phosphate and dissociation of casein micelles, thus affecting the stability of micelles in milk. As pH drops towards 5.2, the zeta potential of casein micelles rises from negative values to about zero and most of the colloidal calcium phosphate dissolves (Swaisgood,1992). Alves *et al.* (1996) reported that the pH of sliced mozzarella cheese sample increased from 5.2 to 5.6 in different modified atmospheres studied.

2.2.4.3 Fat Content

Kindstedt and Rippe (1990) reported that when FDM exceeded 37 %, excessive free-oil was formed during melting, texture in particular, was noticeably affected. However, there are some commercially acceptable low-fat mozzarella cheeses now being manufactured (Tunick *et al.,* 1993 b).As the fat content of mozzarella cheese increases, the softness of the cheese increases, the cheese becomes difficult to shred (McMahon *et al.,* 1993), and meltability increases (Tunick *et al.,* 1991). Decreasing the fat content of cheese usually results in physical and flavour changes that lead to poor quality products (McMahon *et al.,* 1993). Jha (1984) reported that the FFA increased tremendously in samples made from microbial enzymes and further accelerated at higher ripening temperatures. Tiwari (1982) reported that FFA of cow milk cheddar cheese manufactured by using bacterial rennet increased to 19.54 μ meq /g fat in 90 days during ripening from an initial value of 12-09 μ meq /g fat at 0 day.

2.2.4.4 Protein Content

Ganguli and Bhalerao (1964) reported that all the three casein components of buffalo milk had slow mobility. The relative concentrations for these fractions, namely α-, β- and γ- caseins in whole casein were 44.4, 52.4 and 3.1 per cent in buffalo milk and 54.4, 39.1 and 6.4 per cent in cow milk, respectively. Ganguli and Majumder (1967) found that cow and buffalo milks differed in their quantity and quality of protein. The level of casein in buffalo milk was higher (2.62 to 3.38 %) than that of cow milk (2.42 to 2.67 %). Most of the casein in buffalo milk is present in the miceller form unlike cow casein, which is also present in soluble form. The ratio of miceller and soluble casein for buffalo milk was 91 and that for cow milk 21. The casein content is slightly lower in goat milk, with a very low proportion or absence of α_{s1} – casein and a high proportion of β-casein (Junarez and Ramos, 1986).

Walsh *et al.* (1998) investigated the impact of κ-casein genetic variants in mozzarella cheese and found no significant differences in cheese functionality, but significant differences in cheese yield and curd formation. The experiments revealed that the changes in total protein were small, varying from 21.8 to 23.7%; however, there was an increase in soluble nitrogen during storage, which indicates the occurrence of proteolysis in the product. This was more accentuated in cheeses under 100 % CO_2 (2.44 % of Ns x 6.38 on the 58^{th} storage day) and under 50 % CO_2 / 50 % N_2 (1.83 of Ns x 6.38 on the 50^{th} storage day), because they were stored for longer period of time.

The index utilized to evaluate the extent of proteolysis varied from 5.46% initially to 5.87 % (14^{th} day) in 100 % N_s, 10.45 % (58^{th} day) in 100 % CO_2, and 8.43 % (50^{th} day) in 50 % CO_2 / 50 % N_2. This indicates that the proteolysis that occurred in mozzarella cheese submitted to the three MAPs was small during storage period (Alves *et al.,* 1996).

Proteolysis in cheese is affected by various factors including activities of residual coagulant, indigenous activities of proteases, pH, casein, moisture, salt, moisture ratio, and ripening temperature (Farkye *et al.*, 1991). Tunick *et al.* (1995) increased the degree of proteolysis prior to pizza baking by storing lower fat mozzarella cheese (17 % fat) for 70 days, that is about 5 times longer than the normal aging period of about 2 weeks (Kinsdtedt, 1991).

2.2.4.5 Lactose Content

Johnson and Olson (1985) reported that the accumulation of galactose in mozzarella cheese could be a significant problem because of the role that it played in Maillard browning. According to Oberg *et al.*(1991 b) when mozzarella is made by direct acidification, less proteolysis takes place and the cheese remains white during cooking, even though lactose is present. Galactose-fermenting and galactose-non-releasing strains have also been used to manufacture low-browning mozzarella cheese without being detrimental to melt behaviour and free-oil formation (Matzdorf *et al.,* 1994; Mukherjee and Hutkins, 1994).

2.2.4.6 Salt Content

The salt content of cheese can affect the functionality of mozzarella cheese. Olson (1982) observed that mozzarella cheese with a higher salt concentration of 1.78 % was less meltable and less stringy than cheese with a lower salt concentration of 1.06 %. The viscosity, as measured by compressional analysis, of higher salt (2.4 %) mozzarella cheese compared to lower salt (0.3 %) was also found to change less rapidly during ageing (Cervantes *et al.,* 1983), most likely because of the inhibition of growth of micro-organisms and the slower rate of proteolysis. Salt in the serum phase is thought to cause micro-structural swelling of the casein matrix, which increases both the water-binding capacity and solubilization of the casein from the matrix (Guo and Kindstedt, 1997). Paulson *et al.* (1998) compared the micro-structure, using scanning electron microscopy (SEM) of salted and unsalted non-fat mozzarella cheese and concluded that the cheese samples containing salt had a more uniform casein matrix, while the unsalted cheese had pockets within the matrix.

2.2.5.0 Functional Characteristics of Mozzarella Cheese

The ranges of functional characteristics attributable to melted mozzarella cheese are wide and varied, and are largely responsible for consumer perception. Generally, melting, stretching, free oil formation, elasticity and browning properties are considered most important. There are many methods for assessing the functional properties of mozzarella cheese, ranging from subjective to objective and from basic to complex. According to McMahon *et al.* (1993) the analysis of the cooked cheese is commonly used for measuring functional properties of mozzarella by cooking cheese on top of a pizza with sauce, while stringiness, meltability, browning, blistering and free oil formation are subjectively assessed.

2.2.5.1 Meltability

The meltability of mozzarella cheese is also commonly measured by the distance cheese flows down a glass tube upon melting (McMahon *et al.,* 1993) or the time taken for cheese shreds to coalesce and form a molten mass in an oven (Guinee and O'Callaghan, 1997). The Arnott and Schreiber tests as described by Arnott *et al.* (1957) and Kosikowski (1982) are the most common tests for assessing meltability. These tests are based on the heating of a standard cylindrical specimen of cheese under specified conditions of oven temperature and time, followed by measuring the specimen's diameter increase (Schreiber) or height decrease (Arnott). These procedures have been used by many workers with different specimen dimensions and heating conditions (Breene *et al.,* 1964; Keller *et al.,* 1974; Schafer and Olsen, 1975; Chang, 1976; Kosikowski, 1982; Nilson and LaClair, 1976; Rayan *et al.,* 1980).

Breene *et al.* (1964) determined the melting quality of pizza cheese and reported that the fat leakage could be reduced by decreasing the fat content of the cheese milk or by low pressure (500 psi) homogenization of the whole milk before standardization. Keller *et al.* (1974) determined the meltability of direct acidified mozzarella cheese by taking discs of 5 mm thickness and 15 mm diameter in Mojonnier dishes covered with cheese cloth, and heating them in a boiling water bath. They reported that the meltability of cheese was affected significantly by the type of acid used and the pH of coagulation. Cheese made at pH 5.6 with phosphoric acid was found to be less meltable than cheese made either with citric acid (at 5.2, 5.4 and 5.6 pH) or with malic or acetic acid at pH 5.2. Smith *et al.* (1980) evaluated the flowability of melted mozzarella cheese by capillary rheometry. Park *et al.* (1984) compared the Schreiber test, the Arnott test and two other microwave modification tests and concluded that there was a marked lack of correlation between the first two tests. Kindstedt *et al.* (1989) described a method for measurement of mozzarella cheese melting by helical viscometry and stated that cheese composition affected the melting properties. A quantitative test for free-oil formation in melted mozzarella cheese was suggested by Kindstedt and Rippe (1990) and they found that free-oil increased with increase in cheese fat on dry matter basis.

Fennema (1972) observed that mozzarella cheese frozen at –20 °C melted more than cheese frozen at –196 °C, which suggested that faster freezing (–196 °C) resulted in large ice crystals and greater breakdown of cheese. Tunick *et al.* (1991) froze mozzarella cheese at –20 °C for 8 weeks and found that frozen cheese had greater meltability than unfrozen. Dahlstrom (1978) showed that mozzarella cheese had poor melt down, acid flavour, bleached discolouration, and poor cohesivness, immediately after thawing, but regained normal characteristics after the thawed cheeses were aged for 1-3 weeks. Oberg *et al.* (1992 a) reported that meltability of mozzarella cheese samples decreased when stored at –20 °C for 42 days.

2.2.5.2 Fat Leakage

Studies conducted by Bertola *et al.* (1998) showed that the apparent viscosity and free oil formation in mozzarella cheese increased for first 2 weeks of storage and then remained constant. The mean free oil values of frozen mozzarella cheese (-20 °C) samples were 3.2 ± 0.2 cm and 5.8 ± 0.2 cm for 6 and 21 days, respectively. Kindstedt and Rippe (1990) observed that refrigerated storage of mozzarella cheese increased the amount of free oil.

Free-oil formation, also called 'oiling off or 'fat leakage' is the tendency of liquid fat to separate from melted cheese and accumulate in pockets or pools, particularly at the cheese surface. In mozzarella cheese, both excessive free-oil and limited free-oil are considered serious defects. Free-oil formation can be measured by using a modified Babcock test (Kindstedt and Rippe, 1990). Kindstedt and Fox (1991) also described a method, which could be used with Gerber apparatus.

2.2.5.3 Stretchability

Tunick *et al.* (1991) observed rapid decrease in stretching of refrigerated mozzarella cheese during storage followed by gradual decline.

Oberg *et al.* (1991) noticed that stretch of refrigerated mozzarella cheese rapidly decreased from 7-14 days, after 14 days, the stretch of un-shredded cheese slowly decreased, whereas the stretch of shredded cheese continued to increase till day 42 followed by rapid decrease in stretch, thereby gradual decline. Mozzarella cheese under tension should form fibrous strands that elongate without breaking. The ability of mozzarella cheese to form these fibrous strands is an index of stretchability. The traditional method for measuring the stretchability of mozzarella cheese is the 'fork' test, where cooked cheese on top of the pizza base is lifted up with a fork and the stretch and tensile strength are subjectively assessed. However, the most popular technique employed is helical viscometry (Kindstedt *et al.,* 1989; Oberg *et al.*, 1991 ; Kindstedt and Kiely 1992 b).

2.2.6.0 Textural Characteristics of Mozzarella Cheese

The textural quality is an overall physical sensation perceived about a food during mastication. The textural properties of mozzarella cheese are very important, especially when they are incorporated into a more complicated food system such as pizza. Texture profile analysis (TPA) technique, which involves the use of Instron Universal Testing Machine has been developed by Szcesniak (1968). Another machine which has been used to test the textural parameters of cheeses is TAXT2i (Stable micro system, UK).

The Instron Universal Testing Machine has been commonly used to measure rheological properties of unmelted mozzarella cheese (Diefes *et al.,* 1993; Yun *et al.,* 1993e; Apostolopoulos, 1994; Tunick *et al.,* 1995). Tests performed using this instrument included stress relaxation and compression tests (Yun *et al.,* 1993 e), hardness and springiness (Tunick *et al.,* 1995), hardness, springiness, cohesiveness, chewiness, and gumminess (Lucisano, *et al.*, 1987; Halmos, 1997). Bhaskaracharya and Shah (2002) concluded that the composition of the cheeses influenced the textural characteristics. The increased moisture content decreased hardness. These results are comparable to the findings of Olson and Johnson (1990) and Tunick *et al.* (1991) that the increase in fat content increased springiness. The increase in protein content increased cohesiveness. Lawrence et al.(1987) reported that proteolysis was significantly negatively correlated with cheese springiness. The hardness was reported to have significant negative correlation with proteolysis and moisture content (Fedrick and Dulley, 1984).

Ghosh (1987) reported that hardness, springiness, gumminess and chewiness of mozzarella cheese from buffalo milk decreased with increased storage period, regardless of packing material and storage temperature. Lawrence *et al.* (1987) inferred that the values for hardness and springiness decreased significantly with time. The casein matrix in cheese became softer and less elastic during storage because of the breakdown of αs_1-casein, which provided the major contribution to the structure of casein in the curd. However, if the αs_1-casein in mozzarella cheese is degraded by proteolytic cleavage, it loses the ability to link with other casein, causing the protein matrix to lose strength and elasticity.

Tunick *et al.* (1991) compared mozzarella cheese of various ages and composition, and observed that the reduced moisture levels in mozzarella cheese resulted in higher values for hardness and springiness, and lower values for cohesiveness and meltability. Decreasing the fat levels to less than 30 % FDM, increased the values of each texture profile analysis (TPA) parameter and decreased meltability. In fresh samples, hardness and springiness were found to be higher and meltability lower than in samples stored for 6 weeks at 4 °C. Tunick *et al.* (1993 a) concluded that textural properties of mozzarella cheeses were affected by moisture and fat content and by the age. Hardness, springiness and viscosity decreased with increasing MNFS but springiness decreased with increasing fat. Refrigerating mozzarella at 4 ^{0}C for 6 weeks produced lower values for hardness and springiness. The cohesiveness of high fat mozzarella increased marginally with storage, from 0.39 $\pm$ 0.04 at 1 week to 0.43 $\pm$ 0.06 at 6 weeks. The cohesiveness of low fat mozzarella also showed a slight increase, from 0.43 ± 0.04 at 1 week to 0.47 ± 0.09 at 6 weeks.

2.2.7.0 Microbial Quality of Mozzarella Cheese

2.2.7.1 Standard Plate count

Costamagna (1976) analyzed the microbiological and chemical quality of mozzarella cheese to understand the effects of freeze-drying and storage without oxygen for 90 days at ambient temperature. The results of Asperger (1982) suggested that the total bacterial count (TBC) in mozzarella cheese stored at 4 °C increased, and was greater than 10^7 cfu / g after one week of storage. When mozzarella cheese was stored at 20 °C for 24 h, the TBC increased by about 2 log unit, and coliform from 0 to 10^6 cfu / g, and within 14 days at 9 °C yeast and moulds were also detected (Asperger and Brandl, 1982).

2.2.7.2 Yeast and Moulds

Alves *et al.* (1996) reported that with regard to the proliferation of yeast and moulds, only the development of yeasts was detected in the sliced mozzarella cheese packed in air and under atmosphere of 100 % N_2 and 50 % CO_2 / 50 % N_2. No growth of yeasts and moulds was detected in the mozzarella cheese stored under 100 % CO_2 during 58 days at 7 ± 1°C.

The growth of yeasts in the mozzarella cheese in air and under 100 % N_2 was observed after the 8^{th} storage day. From the 14^{th} day, the counts of yeasts stabilized at 8.4 logs CFU/g for the product in air. The cheese under 100% N_2 presented lower counts of yeasts than cheese stored in air throughout the study. On the 18^{th} day of storage, when cheese under 100 % N_2 was sensorially rejected, the counts of yeasts reached levels of 7.6 log CFU/g. The population of yeasts in the cheese under 50% CO_2 and 50% N_2 showed some growth from the 9^{th} to 29^{th} storage day, when it stabilized at levels of about 7.0 log CFU/g. The growth in the product under 50% CO_2 / 50% N_2 occurred at a slower speed then the growth in the product in air and under 100% N_2. That could also be credited to the action of CO_2 i.e. that what really inhibited the growth of yeasts was the presence of CO_2. In the product under 50% CO_2 / 50% N_2, the effect of the CO_2 was smaller than in the product under 100% CO_2, because there were 2.5 times less CO_2 per kg of the product.

According to Eliot *et al.* (1998) the growth of staphylococci, yeasts and mould in shredded mozzarella cheese samples was completely inhibited under modified atmospheres containing CO_2, except on week 4 in 10 / 90 and 25 / 75, CO_2 / N_2 for moulds. The counts of staphylococci were < 1 log CFU/ g. High concentrations of CO_2 ($\geq$ 50 % CO_2) also maintained yeast counts $\leq$ 1 log CFU / g and were more efficient than vacuum to inhibit yeast growth. The decrease of staphylococci and yeast counts in atmospheres containing high level of CO_2 suggested a destructive effect of CO_2. Final counts on week 8 were lower than initial counts in most cases, and equal to initial counts in the others (atm 5: 50 / 50 CO_2 / N_2 for yeast). Moulds were not detected in the majority of samples containing CO_2 (10-100 CO_2). The inhibitory properties of CO_2 have also been demonstrated by others (Kosikowski and Brown, 1973; Chen & Hotchkiss, 1991; Rosenthal *et al.,* 1991; Day, 1992; Fedio *et al.,* 1994; Alves *et al.,* 1996).

2.2.7.3 Psychrotrophic Count

The work of Alves *et al.* (1996) on sliced mozzarella cheese in MAP showed the following observations: from the 4^{th} to 14^{th} storage day, the counts of aerobic psychrotrophs in the mozzarella cheese in air and under 100% increased. Then the counts stabilized at levels of 8.3 log CFU / g and 7.5 log CFU / g, respectively. In the product under 50% CO_2/50% N_2 the growth of aerobic psychrotrophs occurred from the 9^{th} to 25^{th} storage day, when they stabilized around 7.1 log CFU / g. That growth occurred at lower rate than the one verified from the 29^{th} storage day on and reached counts higher than 6.5 log CFU / g on the 58^{th} storage day. The results revealed that the beginning of microbiological growth was retarded when the atmosphere inside the package contained higher concentration of CO_2.

The experiments of Eliot *et al.* (1998) on shredded mozzarella cheese under modified atmosphere indicated the following: The initial psychrotrophic count (4.36 log CFU/ g) reached 7 log CFU/ g after 3 weeks and then stabilized around 7.2 log CFU/g. Mean of MAP were also similar between 6.68 and 6.82 log CFU/g in 100% CO_2 and vacuum respectively. However, these results did not confirm the inhibitory effect of CO_2 on psychrotrophs, which had been demonstrated by earlier workers (Scott and Smith, 1971; Rosenthal *et al.,* 1991; Fedio *et al.,* 1994; Maniar *et al.,* 1994). They confirmed the results of Alves *et al.* (1996) that growth of psychrotrophs also occurred in presence of CO_2 in sliced mozzarella cheese. Further, Eliot *et al.* (1998) demonstrated that the beginning of growth was retarded with higher CO_2 and their count stabilized around 7.1 log CFU/ g after 25 days in the 50 % CO_2 / N_2 mixture (vs 7.2 log CFU / g on week 4), and they reached more than 6.5 log CFU/ g on the 58^{th} storage day under 100 % CO_2 (Vs 7 log CFU/ g on week 8). The psychrotrophic organisms were less numerous in samples of atm 1(i.e. air), with mean count of 6.45 log CFU/ g.

2.2.7.4 Coliform Count

Alves *et al.* (1996) reported that coliforms were not detected in any of the sliced mozzarella cheese samples throughout the shelf-life study, the reason being the use of adequate sanitary practices during the product packaging. The results of Asperger (1982) suggested that when mozzarella cheese was stored at 20 °C for 24 h, the coliforms increased from 0 to 10^6 CFU/ g.

2.2.8.0 Sensory Quality of Mozzarella Cheese

Mozzarella cheese stored at 20 °C for 24 h did not affect the sensory properties but when stored at 4°C showed noticeable flavour deterioration after 3- 4 weeks, and at 9°C flavour deteriorated significantly (Asperger, 1982). According to Cervantes *et al.* (1983) there was no statistically significant effect or consistent trend on compression and sensory evaluations of mozzarella cheese that had been frozen as compared to unfrozen cheese.

The development of off flavour in mozzarella cheese under 50% CO_2 / 50% N_2 was significantly more intense than that observed in the product under 100% CO_2 as of the 43rd storage day. In both atmospheres with CO_2, the development of off flavour was slower than in the other treatments. From the 11th storage day, the cheeses in air presented overall quality significantly inferior to that of the product under 100% CO_2 (Alves *et al.,* 1996).

The total inhibition of yeast development in the sliced mozzarella cheese under 100 % CO_2 was reported to be responsible for the better preservation of the sensorial qualities of the product (Alves *et al.,* 1996).

2.3.0.0 Shelf life of Mozzarella cheese

The shelf life of mozzarella cheese increased to about 240% when packed under MAP (Sarantopoulos *et al.,* 1993; Sarantopoulos and Sorev, 1995). The shelf life of sliced MAP mozzarella cheese increased to 238 % and 392 % for the samples packed under 50 % CO_2 / 50 % N_2 and 100 % CO_2, respectively, and the shelf life under four different treatments & stored at $7 \pm 1^{\circ}C$ could be predicted as air, 13 days; 100 % N_2, 16 days; 50 % CO_2 / 50 N_2, 44 days; and 100 % CO_2, 64 day (Alves *et al.,* 1996).

2.4.0.0 Pizza

The changing life pattern and food habits of people and their growing preference have catapulted the market to grow for convenience and fast foods. Pizza has become most favourite fast food. Pizza is relatively a new item to Indian dietary system and gaining popularity very fast. One of the contributing factors to the growth of pizza as fast food has been the Indian flavours, which pizzas acquired (Anon, 2003 a; b).

Anon (1974) reported that the pizza supplies 30% of the US recommended daily allowance (RDA) of vitamins A, C, B_2, and Ca and protein. It contains 50 % of the RDA of Vitamin B1 and 35 % of Fe. Martin (1977) observed that pizza ranked extremely high in wide spectrum of nutrients, has more protein and rich in Ca (fulfils 127% daily needs); an excellent source of Fe (supplies 50 - 55% of daily requirements); provides 30 % needed niacin; contains 26- 42 % adult vitamin A quota; provides 900-1150 calories / pizza.

Scientists in Italy (Anon, 2003 c) have reported that regular consumption of pizza might reduce the risks of cancer to the digestive tract. According to the results, of a study conducted by the Mario Negri Research Institute, Milan published in the International Journal of Cancer, patients with cancers of the oral cavity, pharynx, oesophagus, larynx, colon and rectum included a higher percentage of non-pizza eaters compared to non-cancer patients. The findings suggested "consuming pizza reduces the risk of cancer of the digestive tract". The previous studies have linked refined carbohydrates with colorectal cancer, and since pizza does contain such carbohydrates, the authors suggested that its favourable influence might be related to tomatoes or olive oil, which have been shown to be inversely associated to the risk of various cancers.

2.4.1.0 Pizza preparation

Matz (1996) suggested a method for the preparation of pizza that included pouring a generous layer of pizza sauce made from tomato sauce, paste, puree or combination thereof. Typical spices for pizza are oregano, basil, black pepper, garlic powder etc. which are spread uniformly on pizza base. The amount of sauce used varied with the size of base. Ghosh and Kanawjia (1986) recommended that 85, 125 and 170 gm of sauce should be used for 12, 14, and 16 inch round pizza base, respectively. Further, shredded mozzarella cheese should be added in the following proportion: 110, 150 and 210 gm for 12, 14 and 16 inch round pizza base respectively. For better flavour processed cheese or Parmesan cheese could be added up to 20% of the total cheese. Finally, pizza should be topped with materials like onion, tomato, capsicum, mushroom etc.

For the preparation of pizza, mozzarella cheese was shredded using a food processor, and 125 g of low-browning cheese were spread on the one-half of each pizza, and 125 g of high-browning cheese were spread on other half to ensure that temperature would not affect browning, crust, or other variables. Each pizza was baked at 296 ^{0}C in a Lincoiln Impinger III conveyer oven (Lincoln Food Service Products, Inc., Fort Wayne, IN) with a residence time of 2.7 min (Matzdorf *et al.*, 1994). Rudan and Barbano (1998) prepared pizza by placing 150 gm of tomato sauce on 30 cm pizza crust. The 100 gm shredded mozzarella cheese were spread on pizza crust. About 1 gm of vegetable oil (100% canola oil) was sprayed on the surface of the cheese shreds after they were placed in the pie pan, and aluminium foil was placed over cheese. The pizza was baked in commercial food service pizza ovens at 232°C for 5 min. West *et al.* (1979) prepared pizza (30.5 cm diameter) using crust and tomato sauce.

CHAPTER 3

SCOPE AND PLAN OF WORK

SCOPE AND PLAN OF WORK

3.0.0.0

It could be inferred from the detailed review of literature on the packaging of mozzarella cheese in the preceding chapter that the presently available information on the technology for packaging of mozzarella cheese under modified atmosphere is very scanty. Very little work on modified atmosphere packaging of sliced mozzarella cheese packaged in expanded polystyrene trays, and some work on the modified atmosphere packaging of shredded mozzarella cheese in multilayers of polyolefins with polyvinylidene chloride barrier layer has been done. However, no work has so far been reported on the modified atmosphere packaging of mozzarella cheese using indigenously available packaging materials.

3.1.0.0 Scope

For the systematic study on the modified atmosphere packaging of mozzarella cheese in indigenously available high barrier packaging material, the presently reported studies have the scope of:

3.1.1.0 Study on the packaging and storage of mozzarella cheese in different atmospheres and

3.1.2.0 The interaction of the product and package during various stages of storage under preset condition of temperatures.

3.2.0.0 Plan of work

The plan of work was set to be as follows:

3.2.1.0 Preparation of mozzarella cheese using mixed milk (buffalo milk : cow milk :: 60: 40) by direct acidification method.

3.2.2.0 Packaging of mozzarella cheese in Cryovac, and LLD / BA / Nylon- 6 / BA / LDPE employing MAP technique, which included the use of gases namely CO_2 (100 %), N_2 (100 %), and mixture of gases (50 % CO_2 and 50 % N_2). Alongside with these gases, the mozzarella cheese was also packaged under vacuum and atmospheric (air) condition in order to study the benefits of packaging of mozzarella cheese under MAP.

3.2.3.0 Packaged mozzarella cheese samples as indicated in section 3.2.2.0 were stored under the following conditions.

3.2.3.1 Refrigerator Temperature (7 ± 1 ^{0}C)

3.2.3.2 Deep Freeze Temperature (– 10 to – 15 ^{0}C)

3.2.4.0 Mozzarella cheese samples stored as above (section, 3.2.3.0) were examined at intervals of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 weeks for samples under refrigerator temperature, and after 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months for samples stored at – 10 to – 15 ^{0}C.

3.2.5.0 Fresh as well as stored samples of mozzarella cheese were examined for the following characteristics:

3.2.5.1 Sensory Characteristics:

Acceptability of product on the basis of appearance, body & texture and flavour.

3.2.5.2 Physico-chemical Quality:

The fresh mozzarella cheese samples were tested for percent contents of yield, moisture, fat, protein, salt, ash, lactose, water soluble nitrogen and titratable acidity, besides pH and free fatty acids (FFA). The stored samples of mozzarella cheese were analysed for moisture (%), water soluble nitrogen (%), pH, titratable acidity (% LA) and FFA (meq / g). The meltability, fat leakage, stretchability and electrophoretic behaviour of casein hydrolysis were also estimated for all the samples, both when fresh and after storage in the different packs under the two different temperature conditions. The headspace volume of the package and stored mozzarella cheese samples was also determined.

3.2.5.3 Textural Properties:

The changes in textural properties, i.e. hardness, cohesiveness, springiness, gumminess, and chewiness of fresh as well as stored mozzarella cheese samples were determined.

3.2.5.4 Microbiological Quality:

In order to assess the extent of microbiological deterioration, the mozzarella cheese samples were examined for the counts of standard plate, yeast & moulds, coliform and psychrotrophs.

3.2.5.5 Since the type of package, period of storage and the type of gas are concerned with the quality of mozzarella cheese during storage, interaction between packages, among intervals of storage and among types of gases was also studied.

3.2.5.6 Statistical Analysis:

The data obtained during the experiments were subjected to statistical analysis using Systat.

CHAPTER 4

MATERIALS AND METHODS

MATERIALS AND METHODS

4.0.0.0

The materials used and the experimental procedures adopted to study the various aspects referred to in the scope are described in this chapter. The first section is concerned with the preparation of mozzarella cheese. In the second section are described the techniques of packaging and storage of mozzarella cheese in different modified atmospheres. The third section deals with the methods employed for quality assessment of mozzarella cheese during storage, which included headspace volume, physico-chemical changes, functional properties, textural characteristics, microbiological analysis and sensory evaluation. The fourth section is concerned with the procedure adopted for evaluation of pizza made from modified atmosphere (MA) packed mozzarella cheese with conventionally packed mozzarella cheese. The last section includes the procedure followed for statistical analyses of the data.

4.1.0.0 Techniques followed in the Preparation of Mozzarella Cheese

4.1.1.0 Preparation of Mozzarella Cheese

4.1.1.1 Collection of Milk

For preparing the mozzarella cheese from mixed milk of buffalo and cow, the two types of milk were collected from the receiving platform of the Experimental Dairy, National Dairy Research Institute, Karnal. The cow milk was from the herd of crossbred and Indian cows, while buffalo milk was from Murrah herd.

4.1.1.2 Technique of Mozzarella Cheese manufacture

In the present study, the method standardised by Sabikhi (1991) was followed with slight modification for preparation of mozzarella cheese samples. For production of mozzarella cheese from mixed milk, the milks were first filtered / clarified and then standardised as buffalo: cow :: 60:40, and fat was adjusted to 3%.

4.1.1.3 Pasteurization: The standardized milk was pasteurized by LTLT method and cooled to 5-6 ^{0} C.

4.1.1.4 Acidification: The chilled milk was acidified to 5.5 -5.6 pH with 25 % HCl @3.25 ml conc. HCl/ l milk. The HCl used in the trials was obtained from M/s s.d-fiNE- CHEM LiMiTED Mumbai-40025.

4.1.1.5 Renneting: The temperature of the acidified milk was raised to 27 ^{0}C. Then diluted microbial rennet, i.e. Meito (1:40 in distilled water) was added @ 0.75- 0.80 g /100 l milk. The renneted milk was left undisturbed for 15 minutes for setting of curd. Microbial rennet produced by *Mucor pusillus* var.*lindt* was obtained in the powder form from M/s Meito Sangyo Co. Ltd., Tokyo, Japan.

4.1.1.6 Cutting of Curd: The properly set curd was cut with sterilized cheese knives, and then allowed to stand in whey for 5 minutes.

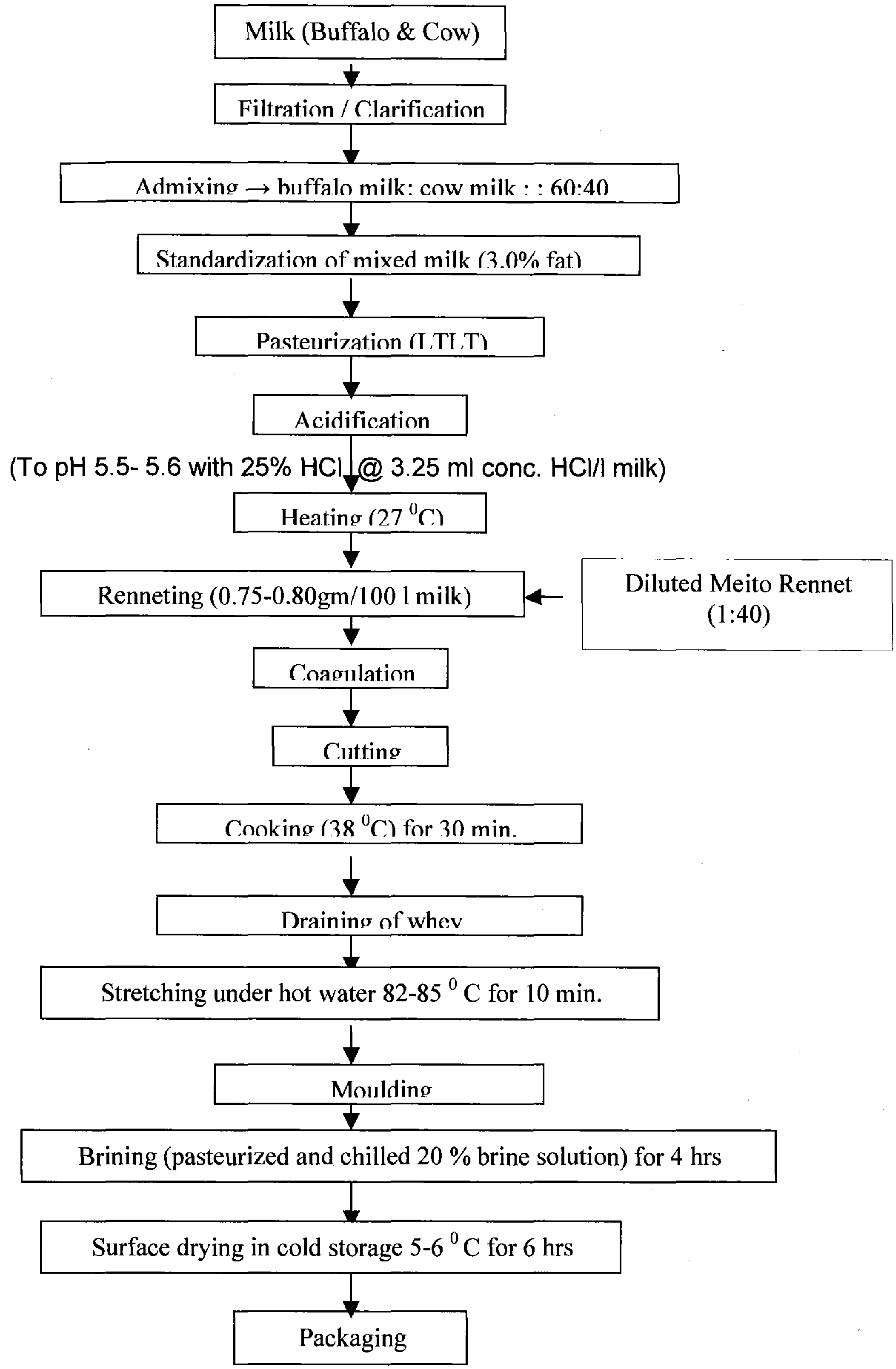
4.1.1.7 Cooking, Stirring and Draining: The temperature of curd was raised @1^{0}C /5 minute till the temperature reached 38 ^{0}C and maintained for 30 minutes with continuous stirring in order to achieve uniform cooking and to avoid lump formation. Then the whey was drained to aggregate the curd particles.

4.1.1.8 Stretching and Moulding: For proper stretching and elasticity, mozzarella cheese was stretched in hot water (82-85 ^{0} C) for 10 minutes. After that it was moulded in ball shape, each ball weighing approximately 300 g.

4.1.1.9 Brining: Mozzarella cheese balls were then immersed in pasteurised chilled (8-10 ^{0}C) brine solution (20 % w/v) for 4 hours. The commercial grade fine grain (Tata salt) was procured from the local market.

4.1.1.10 Surface Drying: After brining, the surface drying of mozzarella cheese was done in a cold storage maintained at 5-6 ^{0} C for 6 hours.

Flow Diagram for the manufacture of mozzarella cheese by direct acidification method



4.2.0.0 Packaging and Storage of Mozzarella Cheese in different Atmospheres

4.2.1.0 Packaging Materials Used

For packaging and storage of mozzarella cheese, high barrier bags namely Cryovac (70 μ) and LLD / BA */Nylon-6 / BA */LDPE (110 μ) (* poly binding agent) were used. The dimensions of the packages used were 19.0 by 22.5 cm for conventional (atmosphere) packaging, 16.5 by 18.0 cm for vacuum packaging, and 22.5 by 32.5 cm for MAP. The packages were obtained from the reputed firms of the country.

4.2.1.1 Cryovac Package:

The firm which supplied the Cryovac packages, claimed the following properties for the bags.

Total thickness : 70 μ

Water Vapour Transmission Rate : 0.5-0.6 g / sq in./24 hrs

Oxygen Transmission Rate : 3-6 ml/ sq m/24 hrs

Properties	Unit	Values (range)
Impact strength	kg/cm	25-45
Elongation	%	100-135
Tear propagation	g	20-30
Tensile Strength	kg/ sq cm	6,500-9,000

4.2.1.2 Characteristics of LLD/BA /Nylon-6 / BA/ LDPE Package:

The firm claimed the following properties for the packaging material (LLD/ BA / Nylon-6 / BA/ LDPE) :

Total thickness : 110 μ

Water Vapour Transmission Rate : 3.96 g/sq m/ 24 hrs

Oxygen Transmission Rate : 36 ml /sq m /24 hrs

Properties	Unit	MD	TD
Tensile at break	PSI	6227	5731
Elongation	%	494	469
Tensile Strength	Kg / sq cm	200	150
Yield Strength	PSI	2314	2251
Elongation at yield	%	25	17

4.2.2.0 Packaging Equipments:

4.2.2.1 Quick 2000 for MAP

Packaging under modified atmospheres was accomplished (Day, 1992) by using a vacuum chamber Quick 2000 machine (α ALFA– LAVAL, KRAMER GREBE, GmbH & Co. KG Maschinefabrik, 3560 Biedenkopf-Wallau, Germany), with gas injection after establishing a vacuum of 25 inches Hg (ca.85 Pa). The injection conditions of the gases were optimised in order to obtain gas headspace.

4.2.2.2 Vertical Heat Sealing Machine

Packaging under atmosphere (air) was done by using vertical heat-sealing machine model QS-300 FE, procured from M/s Sevana Traders Pvt Limited, Kizhakkmbalam, Kerala.

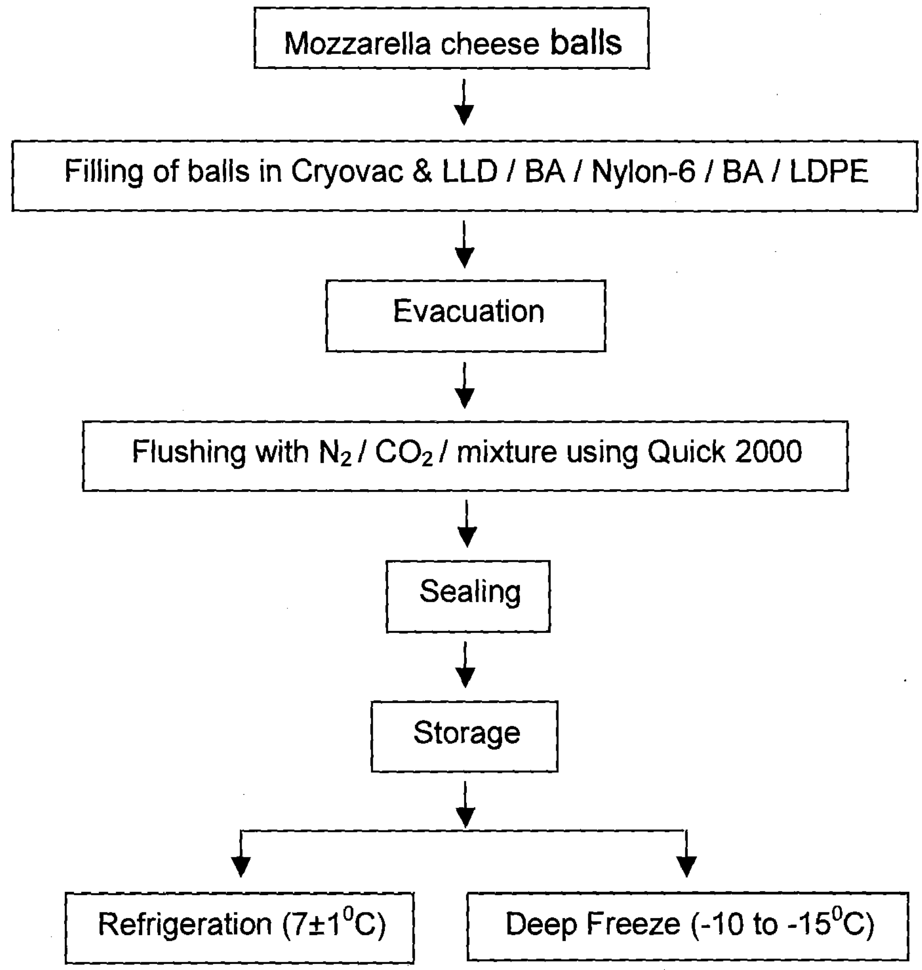
4.2.2.3 Sterilization of Packages

The empty packages were sterilized under UV- light for 30 minutes immediately before packaging of the cheese samples.

4.2.2.4 Packaging of Mozzarella Cheese

The freshly prepared mozzarella cheese balls (300 g each) were individually packaged in sterilized packages under different atmospheres (atm), i.e air (atm 1), vacuum (atm 2), 100% CO_2 (atm 3), 100% N_2 (atm 4) and 50% CO_2 / 50% N_2 (atm 5) by using packaging machines as described under **4.2.2.1. & 4.2.2.2**. Initially the gas headspace to cheese weight ratio was approximately 2 litres of gas per kg of the product. The packaged samples were then stored at 7 ± 1^0 C and – 10 to – 15 ^{0} C. The gases used were of industrial grade procured from the reputed supplier.

Flow Diagram for modified atmosphere packaging (MAP) of mozzarella cheese



4.3.0.0 Quality Assessment of Mozzarella Cheese during Storage

4.3.1.0 Headspace Volume

The headspace volume was determined by following the method of Alves *et al.* (1996) with slight modification, as the difference between the displacement of volume of water from the water filled container by the bag with product, flushed gas, package, and the water displaced by product and package.

4.3.2.0 Thawing of cheese

Mozzarella cheese samples were removed from frozen storage and thawed at 7 $\pm$ 1^{0}C for 8 hours before analyses.

PLATE 6: Preparing the MAP machine for packaging of mozzarella cheese

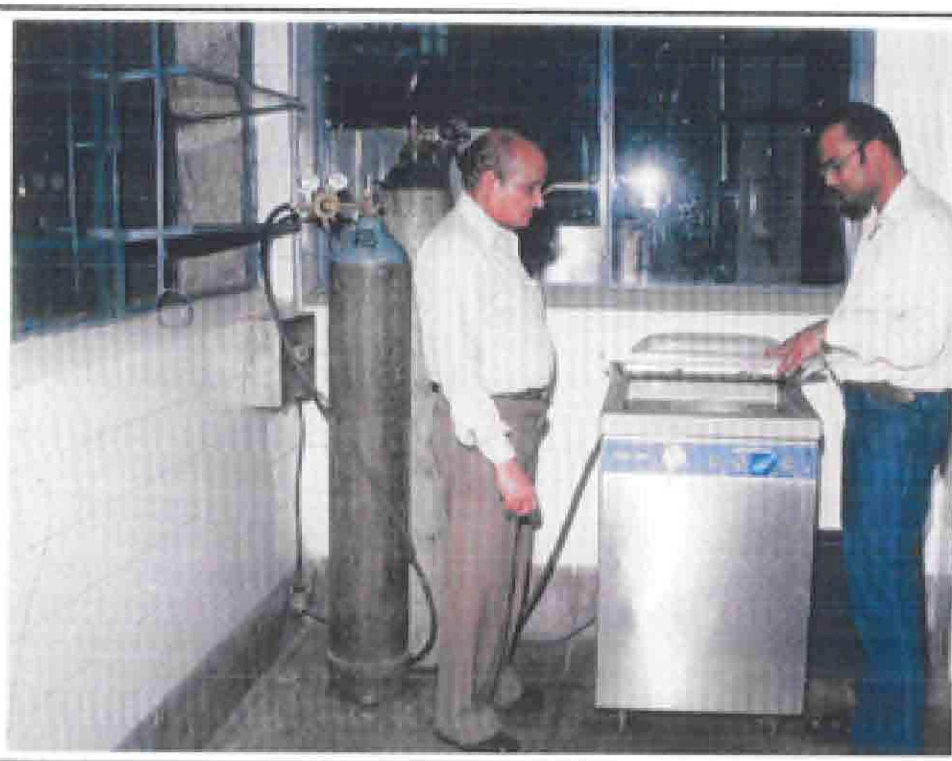


PLATE 7: Packaging of mozzarella cheese by using MAP machine

4.3.3.0 Physico-Chemical Analysis

4.3.3.1 Total Solids

The TS content of mozzarella cheese was determined by gravimetric method as given in IS: SP: 18 (Part XI), 1981.

4.3.3.2 Titratable Acidity

Titratable acidity of mozzarella cheese samples was determined by the method recommended by the Association of Official Analytical Chemists (AOAC, 1984).

4.3.3.3 Moisture

The moisture content in cheese was analysed by the method as described in IS: SP: 18 (Part XI), 1981.

4.3.3.4 pH

Ten g of the grated mozzarella cheese sample was mixed with 10 ml of glass-distilled water and slurry was prepared thereof in a mortar. The pH of the slurry was determined by using double junction electrode pH scan 3 +, Eutech Instruments Pte Limited, Singapore.

4.3.3.5 Fat

The fat contents in milk and cheese samples were analysed using the methods as described in IS: SP: 18 (Part XI), 1981.

4.3.3.6 Lactose

The lactose content in cheese samples was determined using method as given in IS: SP: 18 (Part XI), 1981.

4.3.3.7 Protein

The total protein content of cheese was estimated by micro-Kjeldahl method (Meneffee and Overman, 1940) using Kjeltec automatic digester and distillation equipment (Tecator make, Sweden).

Accurately weighed 200 mg of the cheese sample was transferred to a Kjeltec tube and digested after adding one digestion tablet (1.5 g K_2SO_4 and

0.0075 g Se) and 5 ml of conc. sulphuric acid till the content became clear. After digestion, the flask was cooled in air. The contents of the flask were cooled. Then the content was distilled with 20 ml of 50 % (w / v) sodium hydroxide, and liberated ammonia was entrapped in 25 ml saturated boric acid containing 4 drops of mixed indicator (prepared by dissolving 100 mg methyl red and 30 mg methylene blue in 60 ml of 90 % ethyl alcohol, and then making the volume to 100 ml with distilled water). Approximately 65-70 ml of distillate was collected in 100 ml conical flask. The contents of the flask were titrated against 0.02 N HCl. The blank determination using distilled water in place of sample was also carried out. The total nitrogen and per cent protein were calculated as follows:

$$\text{Percent total nitrogen} = \frac{14.007 \times (X-Y) \times N \times 100}{W}$$

where,

X = ml of HCl used for the sample, Y = ml of HCl used for the blank

N = normality of HCl used, and

W = weight of the sample in mg

Per cent total protein = per cent total nitrogen x 6.38.

4.3.3.8 Soluble Nitrogen

The soluble protein of cheese samples was determined following the procedure delineated by Kosikowski (1982) with slight modification. Three gm of cheese was accurately weighed and mixed with a small amount of Sharpe's extraction solution (tempered to 50°C in a mortar). The contents were ground to a paste and transferred to a 100 ml volumetric flask. The volume was made up to the mark with Sharpe's extraction solution. The flask was placed in a water bath at 50 to 55°C for one hour with occasional shaking. It was then filtered through Whatman filter paper No. 42. Fifty ml of filtrate was transferred into a Kjeldahl flask. Thereafter, digestion and distillation were carried out as described under section **4.3.3.7**.

Calculation:

$$\text{Per cent soluble nitrogen} = \frac{14.007 \times (X-Y) \times N \times 100}{W}$$

where,

X = ml of HCl used for the sample
Y = ml of HCl used for the blank
N = normality of HCl used, and
W = weight of the sample in mg

Sharpe's extraction solution:

Stock solution

5.75 ml glacial acetic acid
136.10 g sodium acetate
47.00 g sodium chloride
8.90 g calcium chloride (anhydrous)
+ distilled water to make the volume upto 1000 ml

Bench solution

250 ml of the Stock solution was taken and made the volume up to 1000 ml with distilled water.

4.3.3.9 Salt

The salt content of cheese samples was determined following the procedure delineated by Sabikhi (1991) with slight modification Ten grams of cheese samples were taken in a mortar and ground with a pestle to make a paste with warm distilled water (40- 45 ^{0}C). It was then transferred to 250 ml volumetric flask and made the volume up to the mark with glass-distilled hot water through washing the pestle and mortar 2-3 times. The contents of the flask were mixed thoroughly and filtered through Whatman filter paper No. 1. Twenty-five ml of filtrate were taken in a 150 ml Erlenmeyer flask. Two ml of 2% solution of potassium chromate indicator were added and the mixture was titrated against 0.1711 N solution of silver nitrate (29.06 g $AgNO_3$ / litre of glass distilled water) to the first discernible colour change. By this method 1 ml of silver nitrate solution used for titration was equivalent to 1 % of salt content in the cheese sample.

4.3.3.10 Ash

The ash content in cheese samples was estimated using the method as described in IS: SP: 18 (part XI), 1981. Three gram grated cheese sample was taken in a previously weighed crucible. The sample was completely charred on an electric heater and then kept in muffle furnace (550°C ± 20°C) for 2 to 3 hr, (till grey ash resulted). The crucible containing ash was cooled in a desiccator and weighed accurately. The ash content was determined using the following formula:

$$\text{Per cent ash} = \frac{(W_1 - W) \times 100}{(Ws - W)}$$

where,

W = weight in g of the empty crucible

Ws = weight in g of the crucible with the cheese sample taken for the test

W_1 = weight in g of the crucible containing the ash

4.3.3.11 Free Fatty Acids (FFA)

The total free fatty acids of the cheese sample were estimated by the method delineated by Deeth and Fitz-Gerald (1976) with modification. The method is based on the principle that involves the extraction of fat from a known quantity of cheese and estimation of the FFA of the extracted fat by titrating against standard alkali.

Three grams of grated cheese sample was taken in a 60 ml stoppered test tube. Five ml of distilled water was added to this to make it into a paste. This was followed by the addition of 10 ml of extraction mixture containing iso-propanol (2-propanol), petroleum ether and 4 N H_2SO_4 in proportion of 40:10:1. An additional 6 ml of petroleum ether was then added. The test tubes were stoppered and tempered at 40°C for 10 min. The contents were vigorously shaken for 20 seconds. The two layers were allowed to separate (5-10 min), and an aliquot of upper layer (5 ml) was withdrawn and transferred to 50 ml conical flask. After addition of 2 drops of methanolic alpha-naphtholphthalein indicator (1 g alpha- naphtholphthalein in 100 ml methanol), the contents were titrated against 0.02 N methanolic KOH solutions. The reagent blank was used to obtain background titration. The normality of methanolic KOH used for titration was determined using standard oxalic acid solution. The FFA content of cheese was determined using the following formula:

$$\text{FFA} (\mu \text{ eq /g}) = \frac{\text{Titration Volume (ml)} \times \text{N} \times 10^3}{\text{P} \times \text{W}}$$

Where,

N = normality of methanolic KOH solution

P = proportion of upper layer of aliquot withdrawn / total volume of upper layer, and

W = weight of the cheese sample taken for analysis

4.3.3.12 Electrophoretic behaviour of Casein Hydrolysis of Mozzarella Cheese during Storage

The hydrolysis of casein of mozzarella cheese was determined by following the method described by Creamer (1991). The instrument used was Mini Gel electrophoresis unit based on low molecular weight (LMW) SDS-PAGE.

Freeze-drying of Mozzarella Cheese:

The cheese samples were freeze-dried in Universal Christ freeze drier (laboratory bench top unit), Model No. ALPHA 2-4 basic unit with controller LDC–1M and Acrylic Cover, supplied by M/s Martin Christ Gefriertrocknungsanlagen, GmbH, Germany, having following specifications:

Specification of Freeze drier:

Technical Data	ALPHA2-4
Ice condenser	
Capacity (max.kg)	4
Performance (max.kg/24 h)	3
Temperature (approx. ^{0}C)	-85
Refrigeration capacity	
With–60 ^{0}C	820
Cooling bath	
Temperature (approx. ^{0}C)	-40 / - 50
Shelf temperature control	
Process A- pre freezing & drying inside ice condenser chamber (^{0}C)	- 50 / + 99
Process B- drying external to the ice condenser chamber (^{0}C)	Upto + 99
Drying modes	
Shelf without sealing device (max.m^2; 10 x ϕ 200 mm), heatable	0.31
Shelf with sealing device (max. m^2; 4 x ϕ 250 mm), heatable	0.18
Number of vacuum valves individually controllable	1- 24
Control panel	
1=L-1, 2=LDC-1M	1/ 2
Refrigerator	
Number x HP	2 x 1/ 2
Type (A=air-cooled)	Cascade- A
Dimensions/connection data/weight	
Width x depth x height	370 x 470 x 405
V/Hz/KVA (Other voltages for 60 Hz available)	220 /50 /1.2
Weight without vacuum pump (kg)	65

For freeze-drying, 0.5 g grated cheese was placed in a petridish. Thereafter, the dish was kept in freeze-drying unit. The vacuum was maintained at 10 mbar at 25 ^{0}C.

Sample Buffer:

10.0 ml of 10% (w/v) SDS solution, 15 ml glycerol, 0.23 g dithiothreitol (DTT), 12.5 ml of "0.5 M, pH 6.8" buffer (0.075 g Tris base, 3.85 g Tris HCl made up to volume 50 ml).

Preparation of Sample:

Freeze dried sample of mozzarella cheese (0.5 g) was transferred to centrifuge tube containing 25 ml sample buffer. The contents of centrifuge tube were then mixed thoroughly by using vortex mixture. The tube was then centrifuged at 10,000 g for 10 minutes and 2 ml of the middle portion was taken, heated in a boiling water bath for 90 seconds and bromophenol blue solution was added.

Resolving Gel Solution:

The resolving gel solution was made using 9.1 ml of resolving gel stock acryl amide solution (37.5 g acrylamide, 0.1875 g bis- acrylamide made up to 100 ml with water 1.7 ml of glycerol, 4.25 ml of 3.0 M pH 9.3 buffer solution (16.1 g Tris base, 2.7 g Tris HCl made to volume 50 ml), 0.17 ml of a 10% (w/v) SDS solution made up to 17 ml with water.

Stacking Gel:

Stacking gel was made from 2.5 ml of a stacking gel stock acrylamide solution (37.5 gm acrylamide, 1.875 g bis- acrylamide made up to 100 ml), 1.0 ml glycerol, 1.0 ml of "0.5 M, pH 6.8" buffer, 0.1 ml 10% SDS solution and made to 8.5 ml with water.

Preparation of Gel Plates:

The resolving gel solution was mixed with 24 µl of TEMED, warmed (or cooled) to 20 ^{0}C and then 48 µl of 10% (w/v) of freshly prepared ammonium per sulphate solution was quickly dispersed into the gel solution. The gel solution was poured into the Mini-Slab apparatus and overlaid with about 0.5 ml water. After the gel had set, the water was removed using tissue paper. The stacking gel was mixed with 24 µl of TEMED, adjusted to 20 ^{0}C, and 30 µl of ammonium per sulphate solution was added. It was then poured into the former and the comb inserted. After setting, excess gel and the comb were removed and 5 µl of the 2 % cheese solutions carefully loaded into each slot.

Chamber Buffer:

The chamber buffer was prepared by using 5.0 g SDS, 142.7 g glycine, 25.66 g Tris base and 6.14 g Tris HCl made up to 5 litres, and 300 ml of chamber buffer was then put into the apparatus.

Electrophoretic Run:

The samples were electrophoresed using a mini gel electrophoresis unit (Techware, M/s Sigma Chemical company, USA) power supply set at 210 volt (maximum) and 70 mA (maximum).

Staining and Destaining:

The gel was stained with 50 ml Coomassie brilliant blue R solution for one hour and destained for an hour each with two 100 ml changes of destaining solution, taking care not to remove too much of the dye at the first change. The gel was then photographed.

4.3.4.0 Functional properties

4.3.4.1 Meltability

Meltability of the cheese samples was determined by the method of Nilson and LaClair (1976) with slight modification. A cylindrical sample of cheese was taken from the cheese ball with the help of cork borer, whose area was 3.35 cm^2. It was sliced into disc of 10 mm height. The disc was then placed on Whatman No.42 filter paper in a petridish. The petridish was then transferred to a preheated oven maintained at 140 ^{0}C. After 15 minutes, the area of the melted cheese was traced on to a paper using a carbon paper and pencil and the area was measured. The meltability was determined using formula:

Meltability = B / A, where

B = area of melted disc (cm^2)

A = area of original disc (cm^2)

4.3.4.2 Fat Leakage

Fat leakage in cheese samples was measured with the help of same discs that were used for the meltability test as under section 4.3.4.1. The free fat of cheese leaked, when cheese melted. This fat was soaked by the filter paper and formed a ring around the cheese disc. The area of fat ring was traced and measured. The Fat leakage in the cheese samples was calculated using formula:

Fat leakage = B / A, where

B = area of melted disc (cm^2)

A = area of original disc (cm^2)

4.3.4.3 Stretchability Test

The stretch quality of mozzarella cheese samples was evaluated by following the procedure as described by Sabikhi (1991):

A 250 ml beaker was filled to 3/4 th of its volume with hot water at 85 ^{0} C. About 10 g of cheese was placed in a beaker and allowed to remain immersed for a minute. A glass rod was immersed in the centre of the molten mass of cheese and the cheese lifted with rod. The length of the thread formed was measured. Longer threads indicated better stretching characteristics. The stretchability was graded on a 5- point arbitrary scale where 5 represented best product.

4.3.5.0 Textural Profile Analysis (TPA)

The textural analysis tests were performed using TAXT2*i* (Stable micro system, UK) textural analyser fitted with a 25 kg load cell. The cubes of mozzarella cheese samples 2 x 2 x 2 cm^3 were subjected to mono-axial compression of 80 % succession on the textural analyser. The test conditions maintained were as given in Fig. :

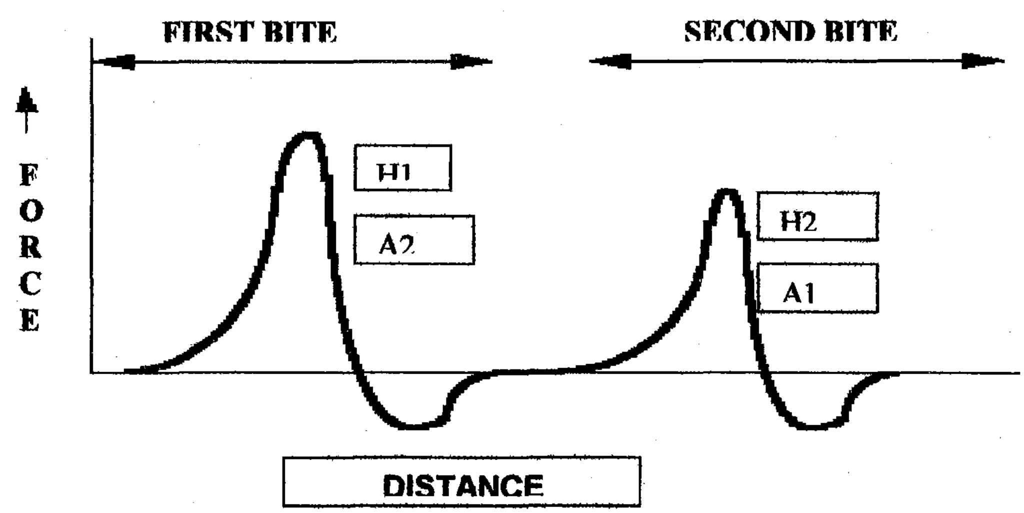


Fig. Texture profile curve of Mozzarella cheese

Textural calculation

Textural variables	Definition	Unit
Hardness	Height of the peak during the first compression (H1)	N
Cohesiveness	Ratio of positive area under second peak to that of the first peak (A2/A1)	
Springiness	Distance that the cheese covered during the time that elapsed between the first bite and the start of second bite (s)	mm
Gumminess	Product of hardness and cohesiveness	N
Chewiness	Product of gumminess and springiness	Nmm

4.3.6.0 Microbiological Analysis

The microbiological quality (SPC, yeast and moulds, coliform count and psychotrophic count) of freshly prepared mozzarella cheese and stored cheese samples were analysed by following the standard procedures as referred to under sections **4.3.6.1 to 4.3.6.6.**

4.3.6.1 Preparation of Dilution Blanks

The dilution blank consisted of 2% (w/v) sterile sodium citrate solution (AR Grade, procured from M/s s.d.- fiNE- CHEM LiMiTED Mumbai-40025) in 99 ml and 9 ml portions in screw capped dilution bottles and culture tubes, respectively. These were autoclaved at 15 lb pressure (121^{0}C) for 20 minutes. The dilution blanks were warmed to 45^0 C before use for preparation of samples.

4.3.6.2 Sampling of Cheese

The packages were aseptically opened and samples of mozzarella cheese (11 g) were weighed and transferred to a sterile mortar. The samples were thoroughly mixed using 99 ml dilution blank. Further serial dilutions were prepared with 9 ml dilution blanks.

4.3.6.3 Standard Plate Count (SPC)

Nutrient agar was used as medium to determine the total count of viable bacteria in cheese. To rehydrate the medium, 23.5 g of the dry media was suspended in 1000 ml distilled water and boiled for 2-3 min to dissolve the medium completely. It was then filled in a flask and sterilised by autoclaving at 15 lb pressure (121^0C) for 15 minutes. The plates were prepared, inoculated and incubated at 30 ^{0}C for 48 h (APHA, 1985). The nutrient agar used in the experiments was procured from HIMEDIA, HiMedia Laboratories Pvt.Limited, Mumbai.

4.3.6.4 Yeast and Moulds Count

Yeast and moulds count was enumerated on potato dextrose agar (PDA). To rehydrate the medium, 39 g of the dry media was suspended in 1000 ml distilled water and boiled for 2-3 min to dissolve the medium completely. It was then filled in a flask and sterilised by autoclaving at 15 lb pressure (121^0C) for 15 minutes. The pH of the media was adjusted to 3.5 at the time of plating by using 10% tartaric acid solution. The plates were incubated at 22 ^{0}C for 3- 5 days (APHA, 1992). Yeast formed yellowish circular curved opaque colonies and moulds formed white or green downy colonies. PDA used in the study was procured from HIMEDIA, HiMedia Laboratories Pvt. Limited, Mumbai.

4.3.6.5 Psychrotrophic Count

Psychrotrophs were determined on plate count agar. To rehydrate the medium, 30 g of the dry media was suspended in 1000 ml distilled water and boiled for 2-3 min to dissolve the medium completely. It was then filled in a flask and sterilised by autoclaving at 15 lb pressure (121^0C) for 15 minutes. The plates were incubated at 4 ^{0}C for 10 days (APHA, 1978). The plate count agar used in trials was procured from HIMEDIA, HiMedia Laboratories Pvt. Limited, Mumbai.

4.3.6.6 Coliform Count

The total presumptive coliforms were counted on violet red bile agar. To rehydrate the medium, 41.53 g of the dry media was suspended in 1000 ml distilled water and boiled for 2-3 minutes to dissolve the medium completely. It was then cooled to 45^0 C without autoclaving. The plates after inoculation were incubated at 37 ^{0}C after 24 h (APHA, 1978). The violet red bile agar used for plating was procured from HIMEDIA, HiMedia Laboratories Pvt.Limited, Mumbai.

4.3.7.0 Sensory Evaluation

The mozzarella cheese samples were evaluated organoleptically for different quality attributes such as appearance, body and texture, flavour and overall acceptability. The score card developed by Duthie *et al.* (1980) based on 18-point scale was used for the purpose (Appendix-XLVII).

4.4.0.0 Evaluation of Pizza made from MA packed Mozzarella Cheese with conventionally packed Mozzarella Cheese

4.4.1.0 Pizza Base

The freshly prepared Pizza base (8 inch size) were procured from the local market.

4.4.1.1 Topping of Pizza

Pizza was prepared by the procedure of Ghosh and Kanawjia (1986) with modification. Firstly the pizza base was smeared with tomato sauce (40 gm). Then two chopped green chillies (*Capsicum annum var. acuminatum*) and approx. 5 gm crushed ginger (*Zingiber officinale*) were sprinkled all over the pizza base. The grated mozzarella and cheddar cheese in the ratio of 80:20 (approx 120 gm per pizza) was topped on pizza base followed by vegetable toppings which included approx. 80 gm chopped bell pepper / simla mirch (*Capsicum annum var. grossum*), approx. 80 gm sliced tomatoes (*Lycopercicum esculatum*), and approx. 50 gm.chopped onion (*Allium cepa*).

4.4.1.2 Baking of Pizza

After topping the pizza was baked in conventional oven adjusted at a temperature of 220-230 ^{0}C for about 15- 20 minutes by following the method as described by Sabikhi (1991). The pizza was taken out from the oven when the cheese browned slightly.

4.4.1.3 Evaluation of Pizza

A panel of five experienced judges evaluated the pizza samples made from modified atmosphere (MA) packed mozzarella cheese with conventionally packed mozzarella cheese. The pizza samples were evaluated using 5-point Hedonic Scale score card (Appendix XLVIII).

4.5.0.0 Statistical Analysis

The data obtained during the experiments were subjected to Systat (Systat version 6.0.1, Inc., SPSS, 1996) for statistical analysis.

CHAPTER 5

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

5.0.0.0

The results obtained during the present studies on the preparation, packaging in different atmospheres, and the quality assessment of mozzarella cheese when fresh and after different intervals of storage, which included changes in headspace volume, chemical, textural, microbiological and sensory properties are presented in Tables 1-11 and Appendices I to XLVI. In the body of the text, the Figures (graphs) for such changes for the values at the beginning and at the end of the storage are included and discussed. The tables concerning the statistical analysis are also included in the body of the text to facilitate discussion.

5.1.0.0 PHYSICO–CHEMICAL, MICROBIOLOGICAL, TEXTURAL AND FUNCTIONAL CHARACTERISTICS OF FRESHLY MADE MOZZARELLA CHEESE USED FOR THE PACKAGE AND STORAGE STUDY

The method for the preparation of mozzarella cheese used for the experiments has been outlined in section 4.1.1.0.The data on yield, moisture, fat, protein, salt, ash, lactose, soluble nitrogen, pH, titratable acidity, free fatty acids and FDM are given in table 1. The functional properties namely meltability, fat leakage and stretchability are depicted in table 2.

The textural properties of mozzarella cheese are detailed in table 3 and deals with particulars such as hardness, cohesiveness, springiness, gumminess and chewiness. The microbiological quality of mozzarella cheese in terms of standard plate count (SPC), yeast and moulds count, coliforms and psychrotrophs is presented in table 4.

5.1.1.0 Physico-chemical characteristics

5.1.1.1 Table 1 pertaining to physico- chemical quality of fresh mozzarella cheese prepared by using mixed milk (buffalo: cow:: 60:40) of 3 % fat indicates that the yield (%) varied from 14.45 to 14.85 with an average of

14.65. The results are almost similar to the findings of Ghosh (1987), who reported the yield of mozzarella cheese made from buffalo milk of 3 % fat employing culture method as 14.20 %. However, Sabikhi (1991) reported higher values for the yield (15.44%) for the samples made from buffalo milk.

Table 1: Physico-chemical characteristics of fresh mozzarella cheese*

Attributes	Min.	Max.	Average
Yield (%)	14.45	14.85	14.65
Moisture (%)	51.78	51.98	51.88
Fat (%)	22.65	22.81	22.73
Protein (%)	20.78	20.92	20.85
Salt (%)	1.66	1.72	1.69
Ash (%)	1.69	1.75	1.72
Lactose (%)	0.91	0.93	0.92
Soluble nitrogen (%)	0.40	0.42	0.41
PH	5.61	5.61	5.61
Titratable acidity (% LA)	0.53	0.55	0.54
FFA meq/g	4.49	4.55	4.52
FDM (%)	47.18	47.30	47.24

* based on three trials

5.1.1.2 The moisture content (%) of samples averaged 51.88 with the range of 51.78 to 51.98. These results agree with the findings of Sabikhi (1991) and Ghosh (1987) who observed the moisture content in buffalo mozzarella cheese to be 51.46 % and 52.26 %, respectively. The moisture content in commercial mozzarella cheese was found to be 53.60 % (Kosokawski, 1982). Rudan *et al.* (1998) reported the moisture content in mozzarella cheese, made from reduced fat (0.8%) unhomogenized milk, as 52.81% . The values for moisture content in mozzarella cheese samples prepared by using standardized milk were observed to be 50.20, 47.74 46.6 and 48.49, respectively by Micketts and Olson (1974), Thaker *et al.* (1991), Patel and Upadhaya (1999) and Rudan *et al.* (1997). The moisture content of mozzarella cheese is an economically important aspect, as increased moisture content results in increased cheese yield. However, variation in moisture content may adversely affect the functionality of cheese, especially meltability. Tunick *et al.* (1991) observed that increase in moisture content of mozzarella cheese from 47 to 52 % resulted in a softer texture and significantly increased meltability. The moisture content of mozzarella cheese is affected by many variables; higher cooking temperatures have been shown to result in lower moisture content (Yun *et al.*, 1993 b).

5.1.1.3 The minimum and maximum values for fat content (%) in mozzarella cheese samples were 22.65 and 22.81 % with an average of 22.73 % (Table 1). Sabikhi (1991) also observed similar value (22.92 %) for fresh mozzarella cheese samples prepared by using 3 % buffalo cheese milk and employing direct acidification method. When mozzarella cheese was prepared by using reduced fat or partially skimmed milk, lower values for fat content were reported, being 9.22 % (Rudan *et al.*, 1998) and 20.0% (Breene *et al.*, 1964).

5.1.1.4 The protein content (%) of mozzarella cheese samples varied from 20.78 to 20.92 % with an average of 20.85. These figures agree with the findings of Sabikhi (1991) who reported the protein content to be 19.96 % in the samples made from 3 % buffalo cheese milk employing direct acidification method. Rudan *et al.* (1991) observed the protein content as 31.10 % in mozzarella cheese samples prepared by using reduced fat standardized milk. It may be pointed out that as the fat content in cheese milk decreases, the protein content increases.

5.1.1.5 The average salt content (%) in mozzarella cheese samples was observed to be 1.89 with a range of 1.66 to 1.72. These values are similar with the findings of Sabikhi (1991). Alves *et al.* (1996) noted the salt content in commercial mozzarella cheese samples to be 1.52 %, while Patel and Upadhaya (1999) reported the salt content as 0.9 % when standardised buffalo cheese milk was used for preparing the product.

5.1.1.6 The ash content (%) of freshly made mozzarella cheese samples ranged from 1.69 to 1.75 % with an average of 1.72. The values for ash content reported by other workers were 1.54 (Sabikhi, 1991) and 1.94

(Paulson *et al.*, 1998). The difference in values may be attributed to the type of milk used and the process employed for the manufacture of mozzarella cheese.

5.1.1.7 The lactose content (%) in mozzarella cheese samples averaged 0.92 with a range of 0.91 to 0.93. Breene *et al.* (1964) observed the lactose content as 2.89 % when the mozzarella cheese samples were prepared by using partially skimmed milk.

5.1.1.8 The soluble nitrogen, which is the index of proteolysis, was found to range from 0.40 to 0.42 % with an average of 0.41%. Vakaleris and Price (1959) found 0.30 % soluble nitrogen in freshly made mozzarella cheese. The initial soluble nitrogen content may be due to the enzymatic action in cheese milk during manufacture of the product.

5.1.1.9 The pH of fresh mozzarella cheese samples was observed to be 5.61, which is similar to the findings of Breene *et al.* (1964), who reported the value as 5.58 for samples prepared by direct acidification method. The values were reported to be lower in case of samples prepared by using culture methods, i.e. 5.11 (Jana and Upadhaya 1991) and 5.36 (Ghosh, 1987).

5.1.1.10 The titratable acidity (% lactic) of mozzarella cheese samples varied from 0.53 to 0.55 with an average of 0.54, which is in agreement with the findings of Gangopadhay and Thaker (1991), Yun *et al.* (1993), and Ghosh (1987) who reported the values as 0.50 - 0.52, 0.53 and 0.64, respectively. However, Sabikhi (1991) reported slightly lower value (0.63) for tittratable acidity.

5.1.1.11 The FFA content (meq/g), which is an index of lipolysis, in freshly prepared mozzarella cheese samples averaged 4.52 with a minimum value of 4.49 and maximum 4.55.

5.1.1.12 The fat on dry matter basis (%) varied from 47.18 to 47.32 with a mean value of 47.24. The values are in agreement with the observation of Sabikhi (1991) who reported the FDM as 47.28 % in cheese samples prepared by direct acidification method. Patel and Upadhaya (1999) also observed 51.8 % FDM in mozzarella cheese samples prepared from standardized buffalo milk. However, Rudan *et al.* (1998 b) reported the FDM in mozzarella cheese samples as 9.22 %. This low value was obviously due to the fact that standardized milk having 0.8 % fat was used for the preparation of mozzarella cheese.

5.1.2.0 Functional Properties

Table 2 Functional properties of fresh mozzarella cheese*

Functional properties	Min.	Max.	Average
Meltability	2.87	3.09	2.98
Fat leakage	4.68	4.76	4.72
Stretchability (arbitrary scale)	5	5	5

* based on three trials

5.1.2.1 The meltability, which is the ratio of area of melted disc of cheese samples and the area of original disc, ranged from 2.87 to 3.09 with an average of 2.98 (table 2). The results are almost similar to the findings of Ghosh (1987) who observed the meltability of fresh mozzarella cheese sample to be 3.12. However, Sabikhi (1991) reported the meltability of cheese samples as 1.96.

5.1.2.2 The table 2 indicates that the fat leakage, which is the ratio of area of melted cheese sample and the area of original disc, varied from 4.68 to 4.76 with a mean value of 4.72. The values are in agreement with the observation of Ghosh (1987) who reported the value to be 5.07 for the mozzarella cheese samples prepared by using 3 % fat buffalo cheese milk. Sabikhi (1991) noted the value for fat leakage as 3.85 for the mozzarella cheese samples prepared from 3 % fat buffalo cheese milk and employing direct acidification method.

5.1.2.3 The stretchability, an unique desirable characteristic of mozzarella cheese, which was graded on 5 – point arbitrary scale, scored 5 points (Table 2) in fresh cheese samples prepared by using mixed milk. Sabikhi (1991) who used 3 % fat buffalo cheese milk for preparing mozzarella cheese reported the stretchability value as 4.07.

5.1.3.0 Textural Characteristics

The textural analysis of fresh mozzarella cheese samples (Table 3) showed that the hardness (N) averaged 138.89, cohesiveness averaged 0.38, springiness (mm) averaged 0.50, gumminess (N) averaged 50.34 and chewiness averaged 25.17.

Table 3: Textural properties of fresh mozzarella cheese*

Characteristics	Min.	Max.	Average
Hardness (N)	136.14	141.64	138.89
Cohesiveness (A2/A1) **	0.37	0.39	0.38
Springiness (mm)	0.50	0.50	0.50
Gumminess (N)	49.10	51.58	50.34
Chewiness (N x mm)	24.55	25.79	25.17

* based on three trials; ** A2: +ve area under 2nd peak; A1: +ve area under 1st peak

5.1.4.0 Microbiological Quality

The microbiological analysis of freshly prepared mozzarella cheese samples (Table 4) revealed that the various average microbiological counts were → standard plate count (SPC): 6.10 log cfu/g. yeast & moulds : 2.15 log cfu/g, coliform : 2.63 log cfu/g, and psychrotrophs : 4.23 log cfu/g. Eliot *et al.* (1998) also reported the similar values for psychrotrophic count (4.36 log cfu/g) for fresh mozzarella cheese.

Table 4: Microbiological quality of fresh mozzarella cheese*

Characteristics	Min.	Max.	Average
SPC (log CFU/g)	5.92	6.28	6.10
Y & M (log CFU/g)	2.41	2.61	2.51
Coliform Count (log CFU/g)	2.57	2.67	2.62
Psychrotrophic Count (log CFU/g)	4.18	4.28	4.23

* based on three trials

5.2.0.0 CHANGES IN QUALITY OF MAP MOZZARELLA CHEESE STORED IN DIFFERENT PACKAGES UNDER DIFFERENT TEMPERATURE CONDITIONS

Freshly prepared mozzarella cheese samples were examined for their degree of deterioration during storage when packed under 5 modified atmospheres in the selected packages P_1 & P_2, and stored under the preset conditions of temperature. The parameters adopted for the evaluation of samples in different packages under different conditions are discussed in this section under the heads: changes in headspace volume, chemical changes, changes in functional properties, textural changes, microbiological changes, sensory characteristics of mozzarella cheese and pizza.

5.2.1.0 Changes in Headspace volume

Data on the headspace volume of mozzarella cheese samples packed in different atmospheres and stored in different packages at 7±1 ^{0}C are given in Appendix I and illustrated in Figure 1. The statistical analysis of the data on headspace volume is presented in table 5. From Figure 1 and Appendix I, it can be observed that the initial headspace volume of 65 ml in air packed (atm 1) mozzarella cheese decreased to 62 and 60 ml, respectively in case of P_1 & P_2 after 5 weeks of storage at 7 ±1 ^{0}C. More decrease in headspace volume was noted in P_2 compared to P_1, obviously because P_2 was more permeable to gases. Interestingly in case of mozzarella cheese packed under vacuum (atm 2) showed reverse trend where the initial headspace volume of 0 ml increased to 4 & 5 ml respectively, in P_1 & P_2. This trend is most likely due to the fact that some of the gases might have transmitted from the outer atmosphere to the inside of the package due to difference in partial gaseous pressure gradient. The initial headspace volume of 625 ml in the packages with 100% CO_2 (atm 3), 100% N_2 (atm 4) and 50 % CO_2 / 50 % N_2 (atm 5) decreased to 607 ml (P_1), 601 ml (P_2); 613 ml (P_1), 609 ml (P_2); 602 ml (P_1) and 600 ml (P_2) respectively, indicating that maximum decrease in the value had been in the case of P_2 (atm 5) and minimum for P_1 (atm 4). It was clearly due to the difference in the gas transmission rate of P_1 and P_2.

Table 5. Analysis of variance for Headspace volume of MAP Mozzarella cheese stored in different packages at 7± 1 ^{0}C & -10 to -15 ^{0}C

Source of variation	d.f.	Mean sum of square	
		Headspace volume	
		7± 1 ^{0}C	-10 to -15 ^{0}C
Among intervals of storage	12	2761.769	1178.658
Among atmospheres	4	2193687.818**	2094398.949**
Between packages	1	14.423	109.869
Interaction Intervals x atmospheres	48	157.586	27.500
Interaction Intervals x packages	12	0.323	2.053
Interaction atmospheres x packages	4	3130228.244 **	2996701.472**
Interaction Intervals x atmospheres x packages	48	285.012	49.662
Error	260	48035.133	46319.328

**** Significant at 1 % level of probability**

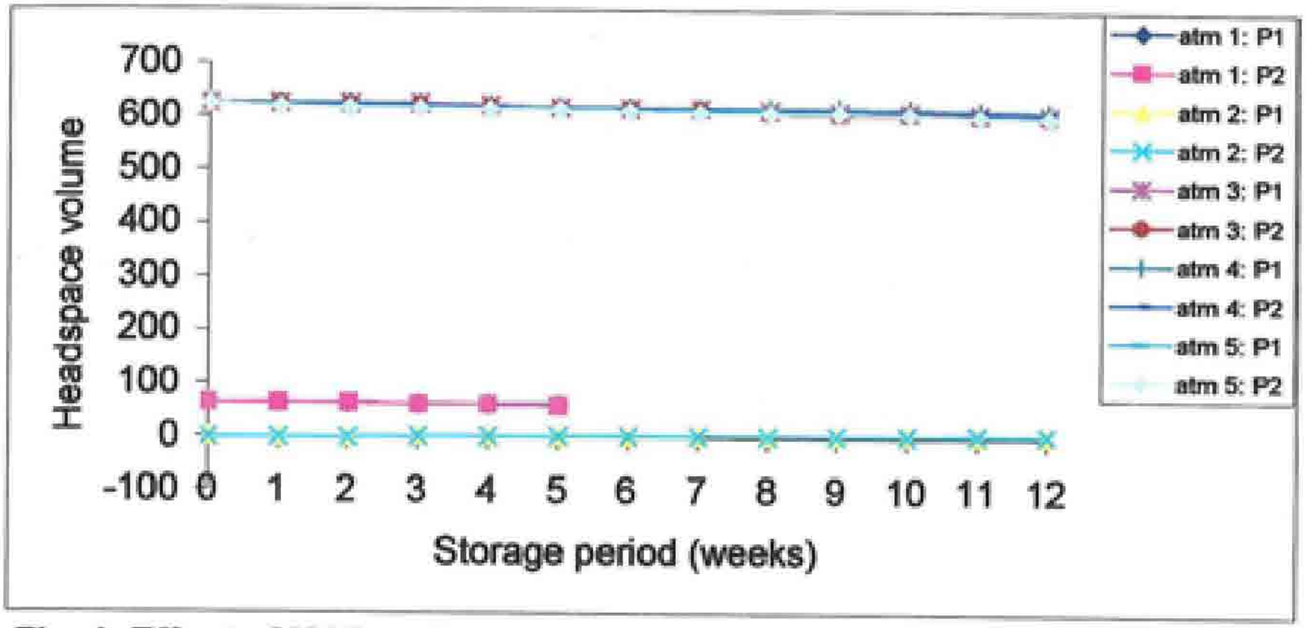


Fig. 1. Effect of MAP on headspace volume of mozzarella cheese stored in different packages at 7±1 ^{0}C

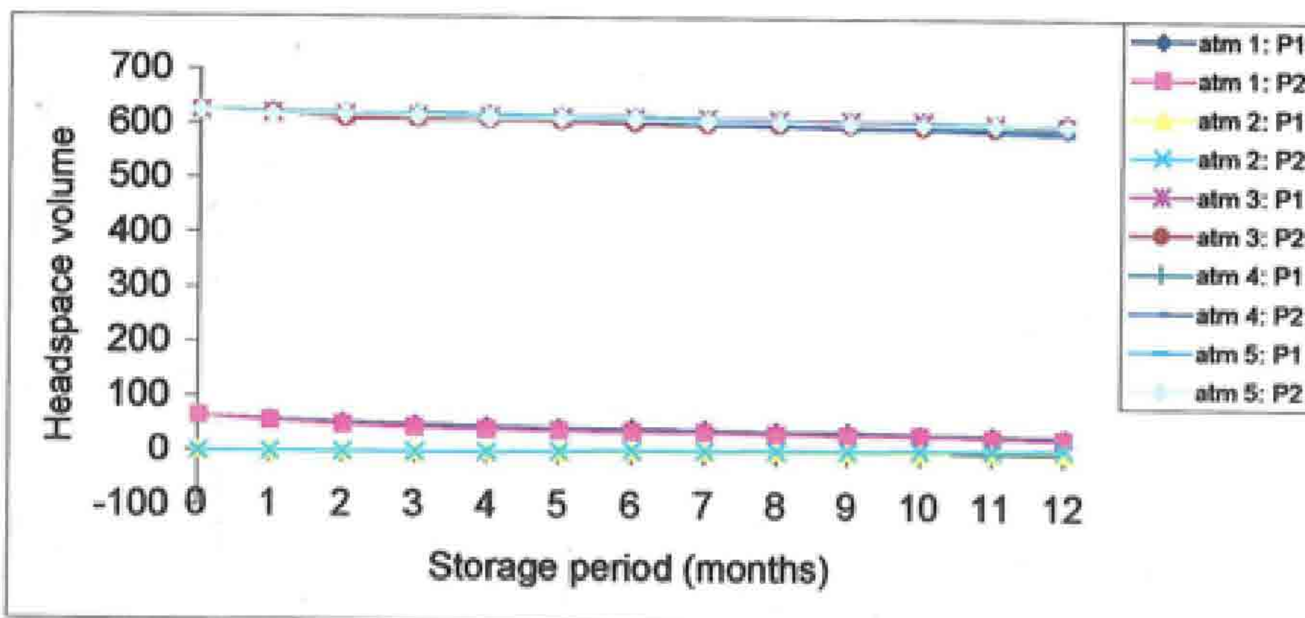


Fig. 2. Effect of MAP on headspace volume of mozzarella cheese stored in different packages at -10 to -15 ^{0}C

The observations on the headspace volume of MAP mozzarella cheese packaged under 5 atmospheres and stored at -10 to -15 ^{0} C (Appendix II and Figure 2) showed that after 12 months the initial value of 65 ml decreased to 32 and 29 ml respectively, in packages P_1 and P_2 (atm 1), while the headspace volume increased to 4 and 9 ml, respectively for the samples packed in P_1 & P_2 (atm 2). However, for the product packaged under atm 3, atm 4, and atm 5, the initial headspace volume of 625 ml decreased to a range of 587 to 625 ml. Variation in the headspace volume of the product packaged under different modified atmospheres was most probably due to varied permeability rates of gases or mixture of gases through packaging materials (Selby, 1968; Sacharow and Griffin, 1970). It can be seen from the Appendix II that in all cases the headspace volume was lower in case of P_2 than P_1. It was most likely due to the fact that the gas barrier properties of P_1 were better than P_2.

Analysis of variance established significant ($P< 0.01$) difference towards headspace volume among the 5 types of modified atmospheres, and interaction atmospheres x packages under both the storage conditions. The influence of intervals of storage and types of packaging material, both individually, were not significant. Interactions intervals x atmospheres, intervals x packages, and intervals x atmospheres x packages were found to be not significant for headspace volume in the context of the two storage conditions studied.

5.2.2.0 Chemical changes

5.2.2.1 Moisture content

Data on the moisture content of mozzarella cheese samples packed under 5 modified atmospheres, and stored in different packages at 7 ± 1^{0}C and -10 to -15 ^{0}C are given in Appendices in III & IV and illustrated in Figures 3 and 4. The statistical analysis of the data on moisture content is presented as part of Table 6. From Figure 3 and Appendix III, it can be observed that the initial moisture content of 51.88 % in air packed (atm 1) mozzarella cheese decreased to 51.72 (0.32 % moisture loss) and 51.70 % (0.35 % moisture

Table 6. **Analysis of variance for Chemical characteristics of MAP Mozzarella cheese stored in different packages at 7± 1 ^{0}C & -10 to -15 ^{0}C**

Source of variation	d.f.	Mean sum of square									
		Moisture		Titratable acidity		pH		Soluble N_2		FFA	
		7±1^{0}C	-10 to -15 ^{0}C	7±1^{0}C	-10 to -15 ^{0}C	7± 1^{0}C	-10 to -15 ^{0}C	7±1^{0}C	-10 to -15 ^{0}C	7±1^{0}C	-10 to -15 ^{0}C
Among intervals of storage	12	941.565**	18.251**	0.089*	0.209**	13.182**	0.323**	4.887**	0.658**	107.781**	285.111**
Among atmospheres	4	1980.375**	0.207*	0.298**	0.017**	22.017**	0.005**	1.303**	0.011**	45.457**	10.538**
Between packages	1	0.311	0.854**	0.004	0.005*	0.014	0.008**	0.039	0.014**	0.098*	0.414
Interaction intervals x atmospheres	48	141.661	0.008	0.038	0.001	1.465	0.001	0.134	0.001	10.100	0.523
Interaction Intervals x packages	12	0.015	0.040	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.007
Interaction atmospheres x packages	4	3293.721**	0.394**	0.524**	0.028**	36.673**	0.009**	2.222**	0.018**	79.153**	17.414**
Interaction intervals x atmospheres x packages	48	235.525*	0.019	0.065*	0.002**	2.452*	0.001	0.225*	0.001**	17.270*	0.846*
Error	260	188.486	0.065	0.043	0.002	2.029	0.001	0.177	0.001	9.407	0.780

*** Significant at 5 % level of probability**
**** Significant at 1 % level of probability**

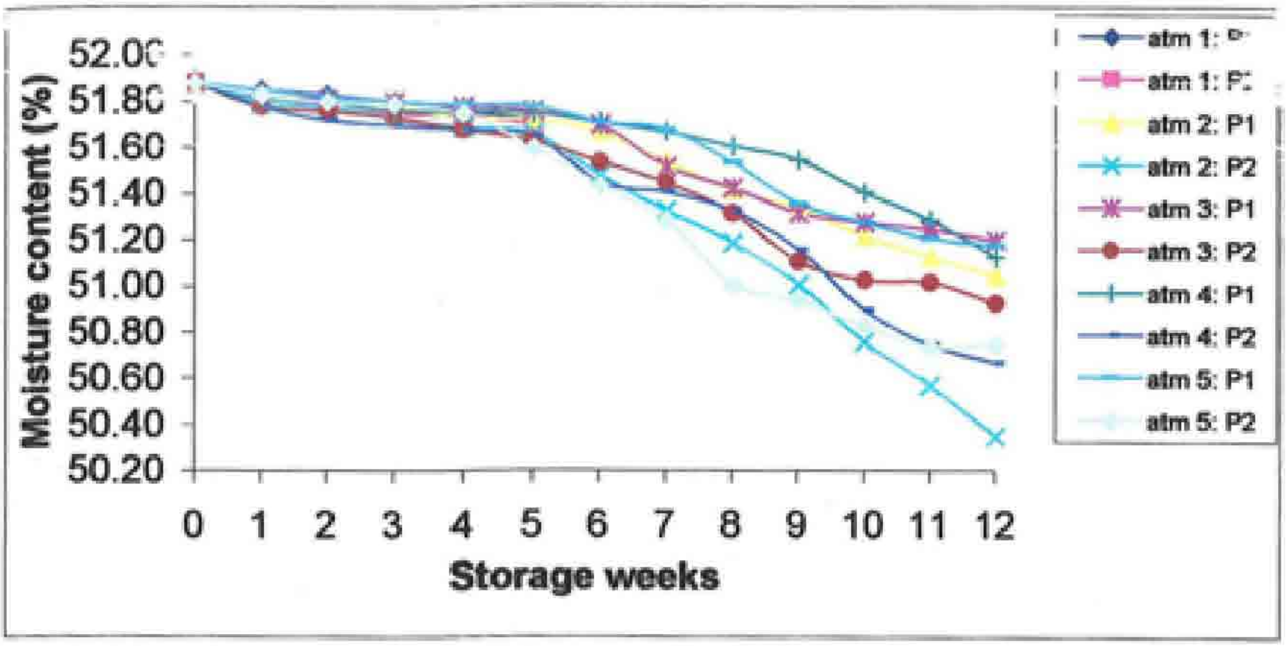


Fig. 3. Effect of MAP on moisture content of mozzarella cheese stored in different packages at 7±1 ^{0}C

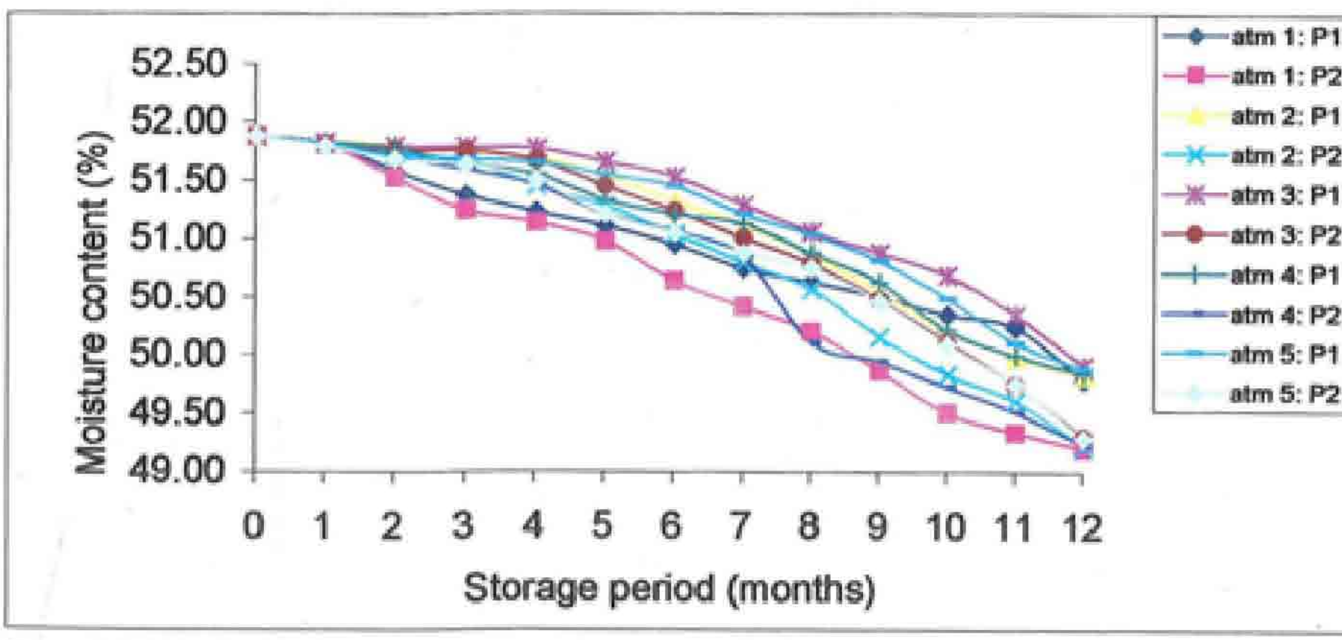


Fig. 4. Effect of MAP on moisture content of mozzarella cheese stored in different packages at -10 to -15 ^{0}C

loss) respectively, in case of P_1 & P_2 after 5 weeks of storage at 7 ±1 ^{0}C. More decrease in moisture content was noted in P_2 compared to P_1, and the reason for it can be ascribed to the difference in the value of WVTR of P_1 and P_2. After 12 weeks of storage P_1 being less permeable to WVTR, resulted in less moisture loss (1.40%) than in P_2 (2.95 %). In case of mozzarella cheese packed under vacuum (atm 2), the initial moisture content decreased to 51.05 & 50.35 % in P_1 & P_2 respectively. Ghosh (1987) reported higher moisture loss (1.80%) in mozzarella cheese when mozzarella cheese was air packed in cryovac and stored for 49 days at 8 -10 ^{0}C. The initial moisture content of 51.88% in the packages with 100% CO_2 (atm 3), 100% N_2 (atm 4) and 50 % CO_2 / 50 % N_2 (atm 5) respectively, decreased to 51.20 (P_1), 50.93 (P_2); 51.13 (P_1), 50.67 (P_2); 51.18 (P_1) and 50.75% (P_2) indicating that the maximum decrease in the value had been in the case of P_2 (atm 2) and minimum for P_1 (atm 3). However, the effect of P_1 and P_2 on the moisture content of the samples was statistically not significant (Table 6) because both the packaging materials had high barrier properties towards WVTR.

At -10 to -15 ^{0} C storage, the initial moisture value of 51.88 % decreased to 49.79 (4.02 % moisture loss) and 49.20 % (5.17% moisture loss) respectively, in packages P_1 and P_2 (atm 1), while the moisture content decreased to 49.83 (3.95% moisture loss) and 49.22% (4.40% moisture loss) respectively, in P_1 & P_2 (atm 2). However, for the product packaged under atm 3, atm 4, and atm 5, the initial moisture content decreased to 49.91 (P_1) (3.80 % moisture loss); 49.30(P_2) (4.13 % moisture loss); 49.85 (P_1) (3.91% moisture loss); 49.85 (P_2) (5.11% moisture loss); 49.23 (P_1)(3.85 % moisture loss) and 49.29% (P_2) (4.35 % moisture loss), respectively. It can be seen from the Appendix IV that in all cases the moisture content was lower for the samples packed in P_2 than P_1 for the reasons explained earlier.

Analysis of variance showed highly significant ($P< 0.01$) difference for moisture content among intervals of storage, and interaction atmospheres x packages under both storage conditions, while the effect of type of packages was significant ($P< 0.01$) only for –10 to –15 ^{0}C storage. The influence of different modified atmospheres on moisture content was more significant ($P<0.01$) for 7± 1 ^{0}C than –10 to –15 ^{0}C ($P< 0.05$). However, interactions intervals x atmospheres, and intervals x packages were found to be not significant under any of the storage condition.

5.2.2.2 Titratable acidity (% lactic)

The observations relating to titratable acidity (TA) of mozzarella cheese samples packed under 5 modified atmospheres and stored in different packages at 7±1^{0}C and -10 to -15 ^{0}C are given in Appendices in V & VI and illustrated in Figures 5 and 6. The statistical analysis of the data is recorded in Table 6. The initial titratable acidity (TA, % lactic) of 0.54 in air packed (atm 1) mozzarella cheese increased to 0.93 and 0.96, respectively in P_1 & P_2 after 5 weeks of storage at 7 ±1 ^{0}C (Fig.5 and Appendix V). Slightly more increase (statistically not significant) in TA was noticed in P_2 compared to P_1. In case of samples stored for 12 weeks, the data revealed that the TA had been minimum (0.75:P_1; 0.79:P_2) in samples packed under atm 3 followed by atm 5 (0.80: P_1; 0.86 P_2); atm 4 (0.84 P_1; 0.90 P_2) and atm 2 (0.91 P_1: 0.92 P_2) respectively, in ascending order. It has been established by other workers (Alves *et al.*, 1986; Eliot *et al.*, 1998) that CO_2 (atm 3) has bactericidal effect also and this might have resulted in least TA in samples packed under atm 3 (100% CO_2) and maximum in samples packed under atm 2 (vacuum), which had neither CO_2 nor N_2. The TA was found to be slightly higher (statistically not significant) for samples packed in P_2 than P_1 irrespective of the atmospheric variation.

The data revealed that after 12 months of storage at -10 to -15 ^{0} C, the initial TA value of 0.54 increased to 0.95 and 0.97 respectively, in packages P_1 and P_2 (atm 1), and increased to 0.76 and 0.79 respectively, in P_1 & P_2 (atm 2). However, for the product packaged in atm 3, atm 4, and atm 5, the initial TA increased to 0.72 (P_1), 0.74(P_2), 0.74 (P_1), 0.77(P_2), 0.74 (P_1), 0.75 (P_2), respectively. The results agree with the findings of Ghosh (1987) who also observed increase in TA during storage at 8-10 ^{0}C and –10 to –15 ^{0}C. It

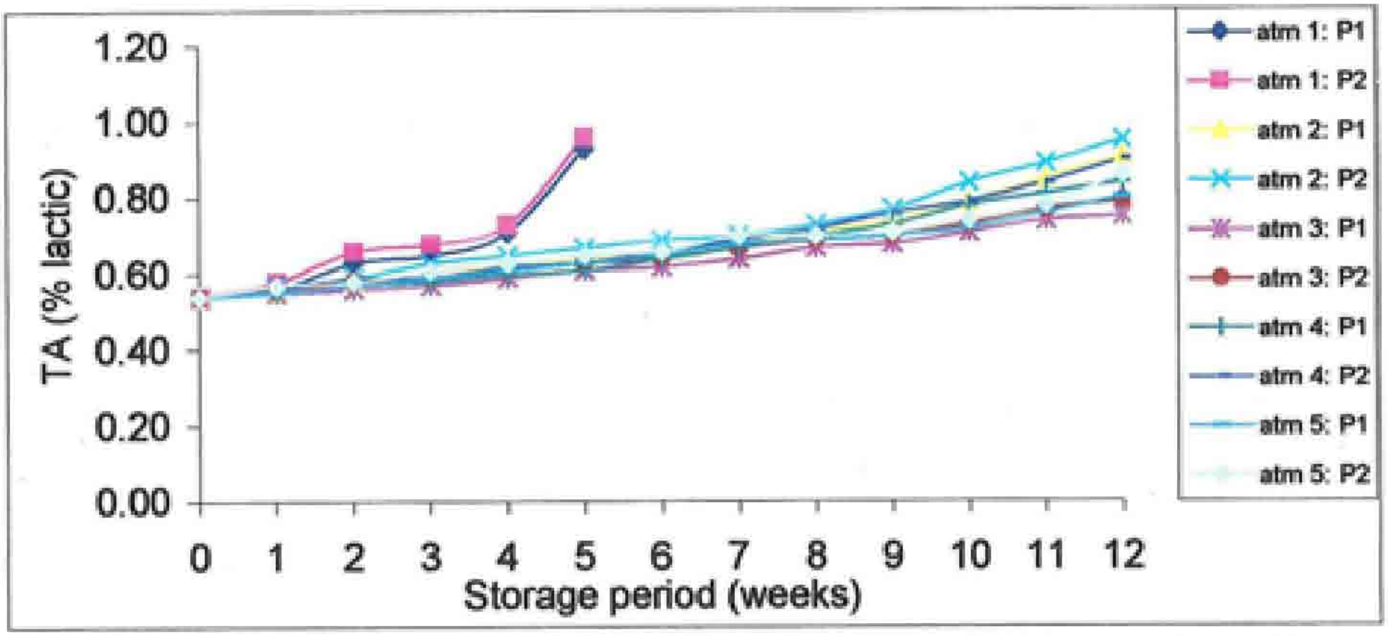


Fig. 5. Effect of MAP on titratable acidity of mozzarella cheese stored in different packages at 7±1 ^{0}C

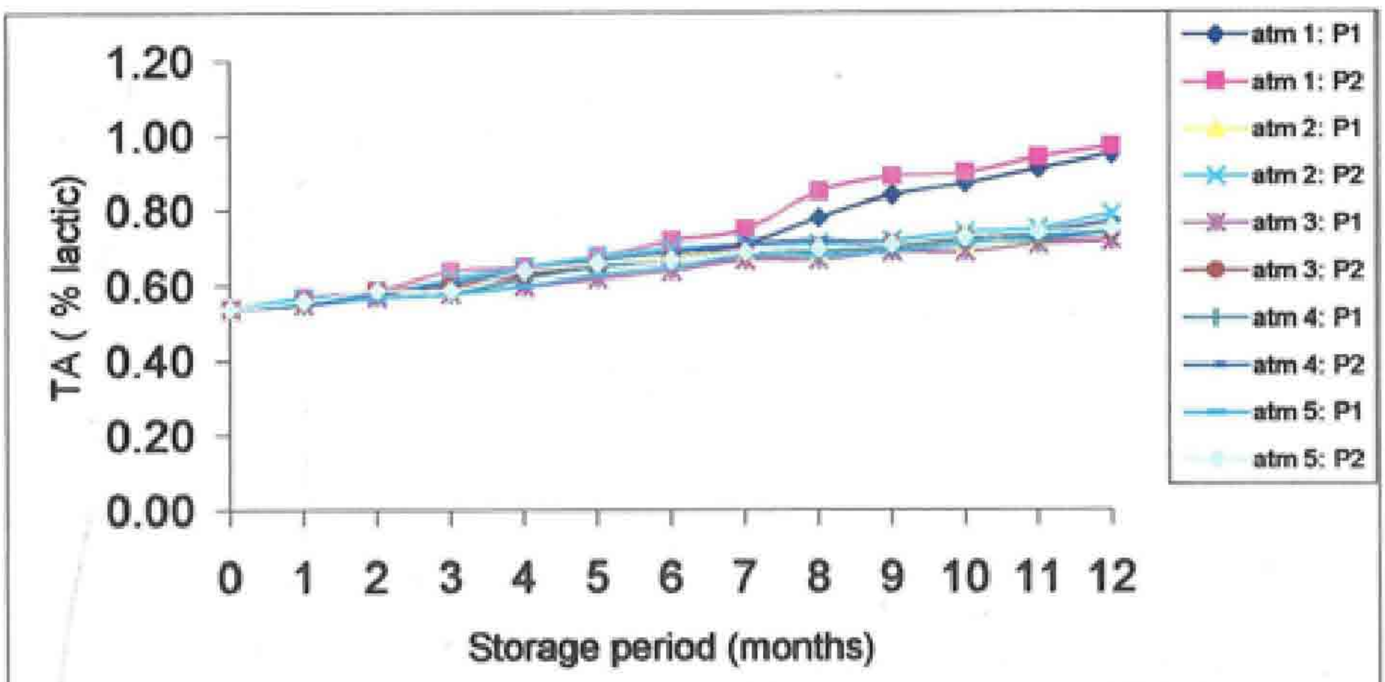


Fig. 6. Effect of MAP on titratable acidity of mozzarella cheese stored in different packages at –10 to -15 ^{0}C

can be seen from the Appendix VI that in all cases the TA had been higher ($P< 0.05$) in case of P_2 than P_1.

From the consideration of TA, the 5 types of modified atmospheres and interaction atmospheres x packages, both individually, were found to be significant ($P<0.01$) under both the conditions of storage, while the effect of intervals of storage and interaction intervals x atmospheres x packages was more significant ($P< 0.01$) for -10 to -15 ^{0} C storage compared to 7±1 ^{0} C storage ($P< 0.05$). Interactions intervals x atmospheres and intervals x packages were observed to be not significant under any of the two storage conditions (Table 6).

5.2.2.3 pH

Data on the pH of mozzarella cheese samples packed under modified atmospheres and stored in different packages at 7±1^{0}C and -10 to -15 ^{0}C are given in Appendices in VII & VIII, and illustrated in Figures 7 and 8. The statistical analysis of the data on pH is presented as part of Table 6. The studies showed that the initial pH of 5.61 in air packed (atm 1) mozzarella cheese samples decreased to 5.20 and 5.15 respectively, in case of P_1 & P_2 after 5 weeks of storage at 7 ±1 ^{0}C. Slightly more decrease in pH was noted (statistically not significant) in P_2 compared to P_1. The mozzarella cheese packed under vacuum (atm 2) showed that the initial pH decreased to 5.22 and 5.17 in P_1 & P_2, respectively. The pH of samples in the packages with 100% CO_2 (atm 3), 100% N_2 (atm 4) and 50 % CO_2 / 50 % N_2 (atm 5) decreased to 5.29 (P_1), 5.21 (P_2); 5.21 (P_1), 5.16 (P_2); 5.25 (P_1) and 5.20 (P_2), respectively indicating that the maximum decrease in the value had been in case of P_2 (atm 2) and minimum for P_1 (atm 3). Asperger (1982) and Matteo *et al.* (1982) also reported the consistent decrease in pH during storage. These results match with the observations recorded in Appendix V depicting corresponding increase in TA.

After 12 months of storage at –10 to –15 ^{0}C, the initial pH value of 5.61 decreased to 5.25 and 5.24 respectively, in packages P_1 and P_2 (atm 1), while the pH decreased to 5.32 and 5.31, respectively in P_1 & P_2 (atm 2). However, for the product packaged under atm 3, atm 4, and atm 5, the pH decreased to 5.34 (P_1), 5.33(P_2), 5.29 (P_1), 5.28(P_2), 5.33 (P_1), 5.32 (P_2), respectively. The results, in general are in contrast with the findings of Alves *et al.* (1996) who reported that the pH of sliced mozzarella cheese samples increased from 5.2 to 5.6 in all the treatments (modified atmospheres) studied. However, the data (Appendix VII & VIII) agree with the experiments of Ghosh (1987), Asperger (1982) and Matteo *et al.* (1982) who noted consistent decrease in pH of mozzarella cheese samples during storage under the two studied conditions.

The 5 types of modified atmospheres, intervals of storage, and interaction atmospheres x packages, all individually, were found to be highly significant ($P<0.01$) from the consideration of pH under both the storage conditions. The effect of types of package was highly significant ($P< 0.01$) for -10-15 ^{0}C storage condition only. Interaction intervals x atmospheres x packages was significant ($P<0.05$) for 7±1 ^{0}C storage condition. Interactions intervals x atmospheres and intervals x packages were not significant under any of the two storage conditions studied.

5.2.2.4 Soluble N_2

In Figures 9, 10 and Appendices IX and X are given the values for soluble nitrogen of MAP mozzarella cheese packed in different materials under 5 modified atmospheres stored at 7±1 ^{0}C and –10 to –15 ^{0} C for indicated periods. Analysis of variance is shown as part of Table 6.

The initials value for soluble nitrogen content (%) of mozzarella cheese packed in atm 1 gradually increased from 0.41 to 0.79 and 0.89 respectively, in P_1 & P_2, , after 5 weeks of storage at 7 ±1 ^{0}C. The further storage upto 12 weeks showed that soluble N_2 varied from 1.83 to 2.15 % and minimum increase in soluble N_2 had been in case of atm 3 followed by atm 5, atm 4, atm 2, and atm 1, respectively in ascending order (Fig. 9), suggesting occurrence of consistent proteolysis in the product. Minimum proteolysis was observed in samples packaged under CO_2 atmosphere. The increase in soluble N_2 may be due to residual coagulating enzyme or the enzymes produced by microorganisms (White & Marshal, 1972; Micketts and Olson,

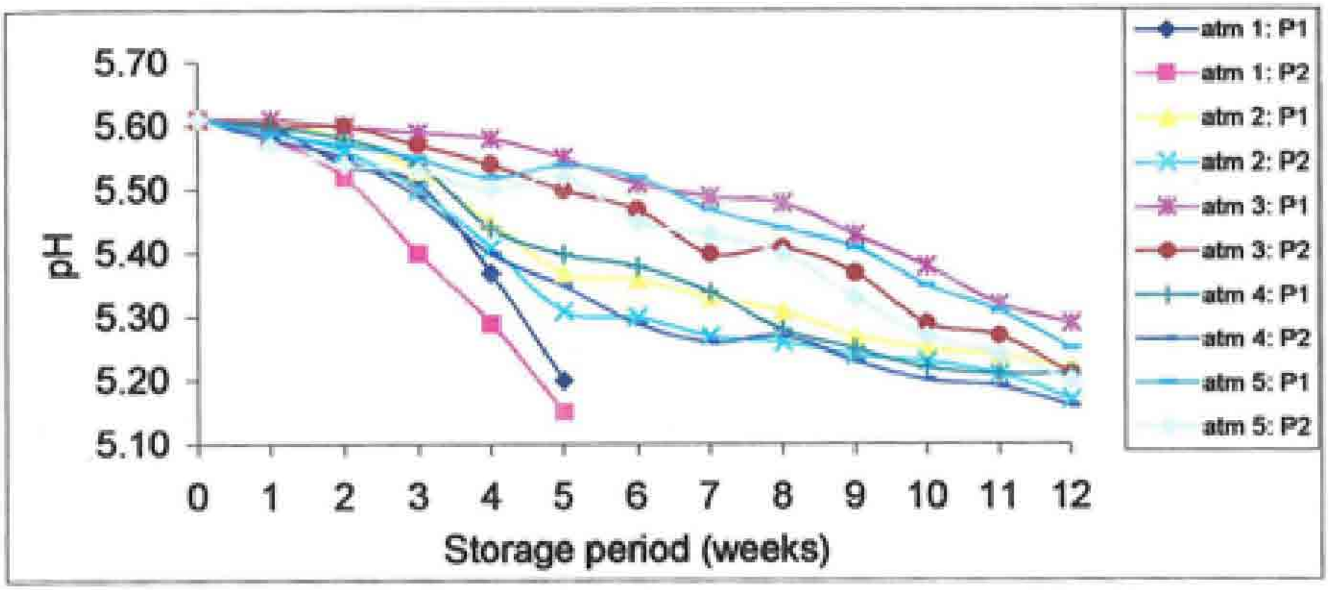


Fig. 7. Effect of MAP on pH of mozzarella cheese stored in different packages at 7±1 ^{0}C

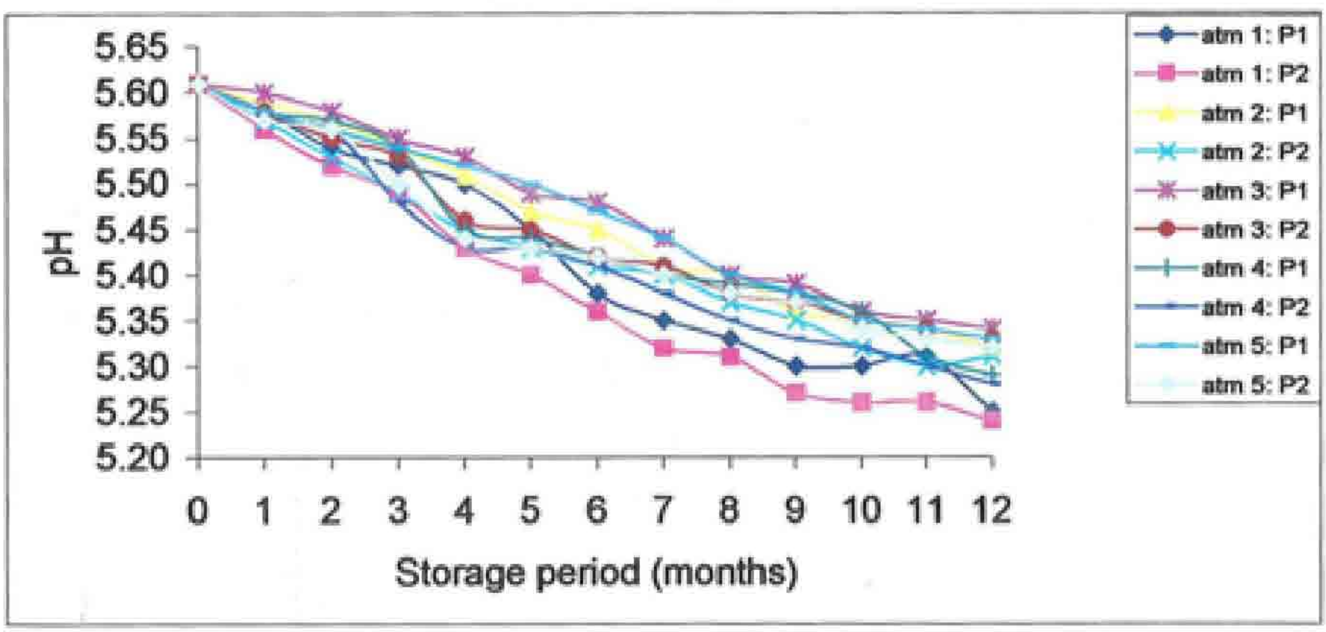


Fig. 8. Effect of MAP on pH of mozzarella cheese stored in different packages at -10 to -15 ^{0}C

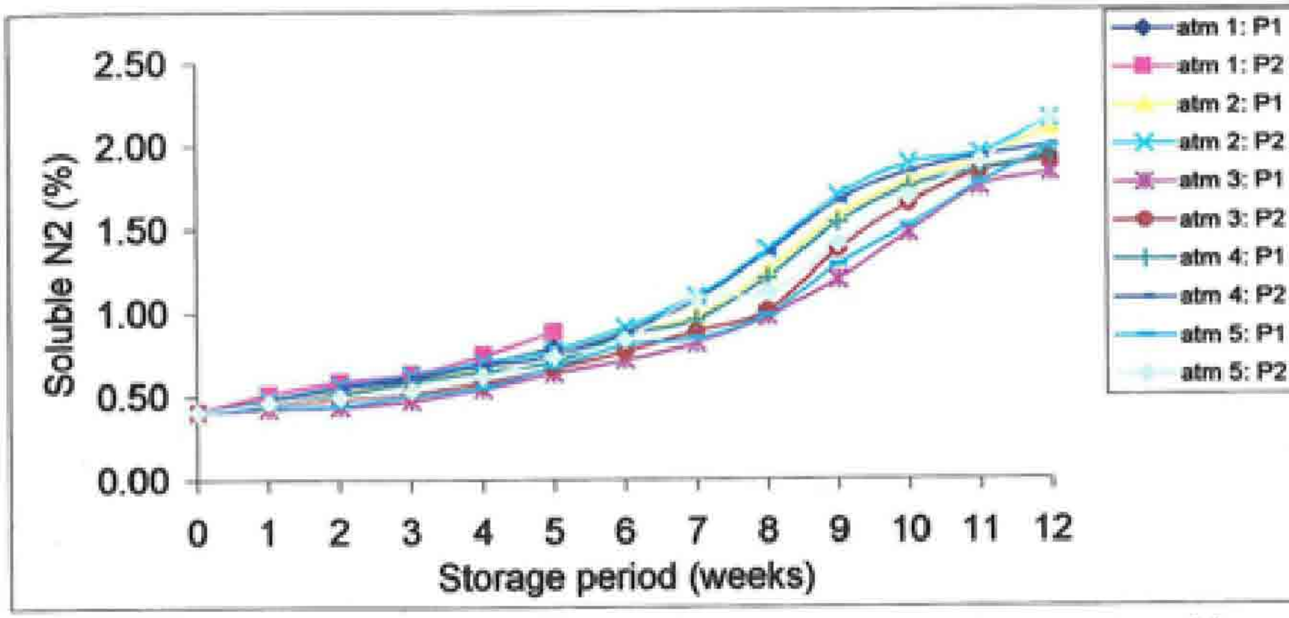


Fig. 9. Effect of MAP on soluble N_2 of mozzarella cheese stored in different packages at 7±1 ^{0}C

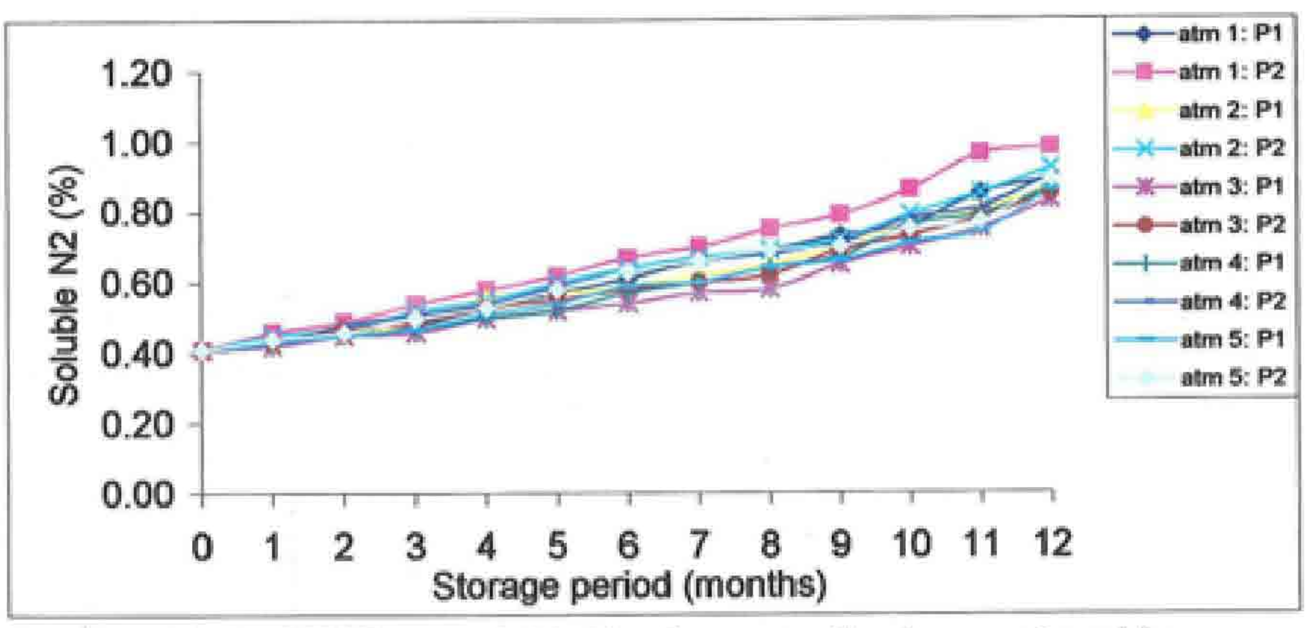


Fig. 10. Effect of MAP on soluble N_2 of mozzarella cheese stored in different packages at -10 to -15 ^{0}C

1974). Creamer (1976) and Addeo *et al.* (1983) also reported casein degradation of mozzarella cheese during storage. Alves *et al.* (1996) also reported that the proteolysis that occurred in mozzarella cheese submitted to the three MAPs was small during storage period

On storage under -10 to - 15 ^{0} C for 12 months the minimum proteolysis of mozzarella cheese was observed in atm 3 followed by atm 5, atm 4, atm 2 and atm 1 in ascending order (Fig.10), suggesting that CO_2 in (atm 3) and 50% CO_2 / 50% N_2 (atm 5) had played a vital role in checking the proteolysis of the product compared to other 3 studied atmospheres. This trend was observed for both types of packaging materials. The degree of proteolysis was observed to be more in P_2 ($P< 0.01$) compared to P_1. Ghosh (1987) also reported increase in soluble nitrogen in mozzarella cheese samples during storage at –10 to –15 ^{0}C.

Significant ($P< 0.01$) differences among the 5 types of modified atmospheres, among intervals of storage and interaction atmospheres x packages towards the soluble N_2 content of mozzarella cheese were noticed for both the storage conditions. Interaction intervals x atmospheres x packages was more significant in case of P_2 ($P<0.01$) than P_1 (P2 < 0.05). However, the influence of type of packages at 7 $\pm$1 ^{0}C storage, and interactions intervals x atmospheres, and intervals x packages for both the storage conditions were observed to be not significant.

5.2.2.5 FFA

Data on the FFA of mozzarella cheese samples packed in 5 modified atmospheres and stored in different packages at 7$\pm$1^{0}C and -10 to -15 ^{0}C are given in Appendices XI & XII and illustrated in Figures 11 and 12. The statistical analysis of the data on FFA is presented as a part of Table 6. The initial FFA value of 4.52 (meq / g) of mozzarella cheese increased (atm1) to 15.15 and 15.39 respectively, in P_1 & P_2 after 5 weeks of storage at 7 $\pm$1 ^{0}C. More increase in FFA was noted in P_2 compared to P_1. In case of mozzarella cheese packed under vacuum (atm 2), the initial FFA (4.52 meq / g) increased to 14.56 and 14.78 (meq / g) in P_1 & P_2, respectively. The effect of storage on the FFA content of cheddar cheese has been studied by several workers (Bhat *et al.* 1978; Tiwari, 1982; Jha, 1984 and Khamrui, 1996) and they also reported consistent increase in FFA. The FFA of mozzarella cheese samples in the packages with 100% CO_2 (atm 3), 100% N_2 (atm 4) and 50 % CO_2 / 50 % N_2 (atm 5) respectively, increased to 12.38 (P_1), 12.49 (P_2); 13.04 (P_1), 13.29 (P_2); 12.56 (P_1) and 12.67 (P_2), indicating that the minimum increase in the value had been with the samples packed in P_1 (atm 3), and maximum for P_2 (atm 2) establishing a very significant influence of MAP on the lipolysis of mozzarella cheese during storage.

The observations on the FFA of MAP mozzarella cheese stored at deep freeze conditions (-10 to –15 ^{0}C) showed that after 12 months of storage, the initial value of 4.52 (meq /g) increased to 15.64 and 15.77 respectively, in packages P_1 and P_2 (atm 1), while the FFA increased to 13.54 and 13.65 respectively in P_1 & P_2 (atm 2). However, for the product packaged under atm 3, atm 4, and atm 5, the FFA increased to 10.69 (P_1), 10.88(P_2); 12.45 (P_1), 12.78 (P_2) and 12.04 (P_1), 12.28 (P_2), respectively (Appendix XII). The increase in FFA value during storage most probably was due to the bacterial/ enzymatic action, which might have caused varied degree of lipolysis in mozzarella cheese samples packed in different modified atmospheres.

Analysis of variance for the data on FFA revealed highly significant (P<0.0.1) differences among the atmospheres, and interaction atmospheres x packages for both the conditions. Differences in the values for FFA of mozzarella cheese due to the types of packages and interaction intervals x atmospheres (Table 6) were found to be significant (P<0.05). The effect of packages on FFA at –10 to –15 ^{0}C; and interactions intervals x atmospheres, and intervals x packages were however found to be not significant under both the storage conditions.

5.2.2.6 Electrophoretic behaviour of casein hydrolysis of MAP mozzarella cheese

The proteolysis in cheese during storage was studied using electrophoresis. The degree of casein hydrolysis of stored MAP mozzarella

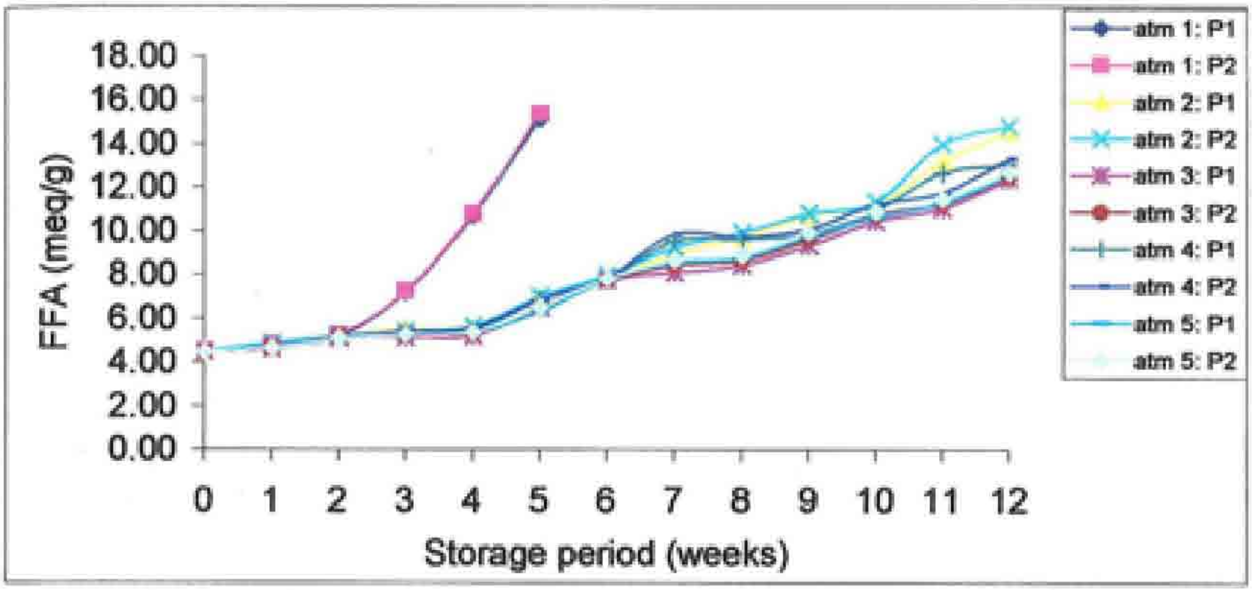


Fig. 11. Effect of MAP on FFA of mozzarella cheese stored in different packages at 7±1 ^{0}C

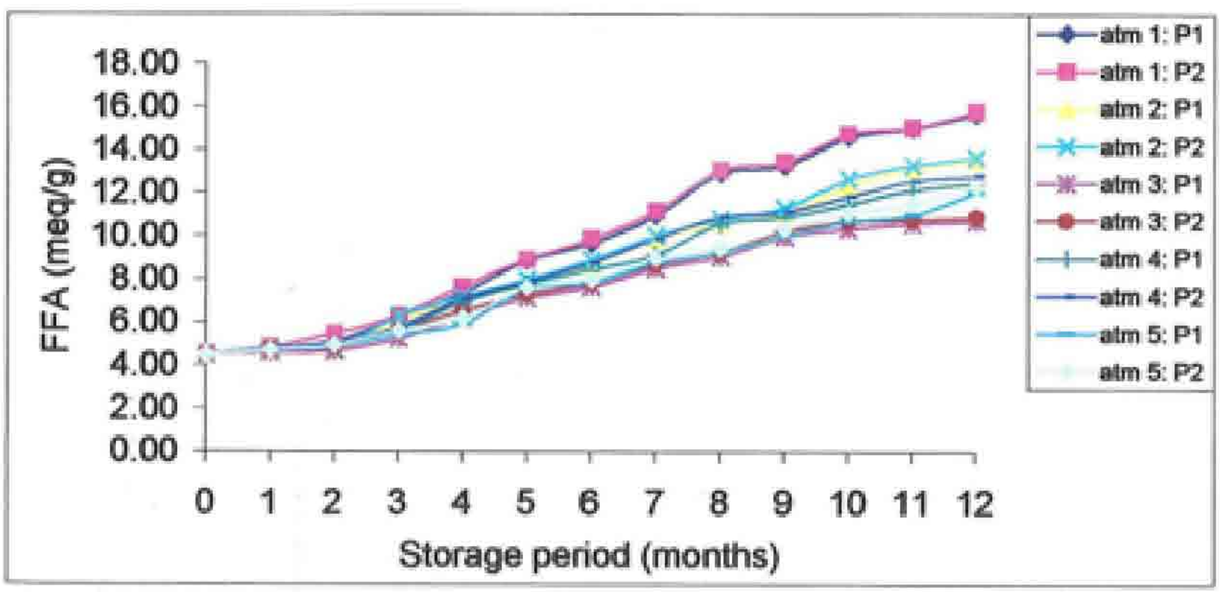


Fig. 12. Effect of MAP on FFA of mozzarella cheese stored in different packages at -10 to -15 ^{0}C

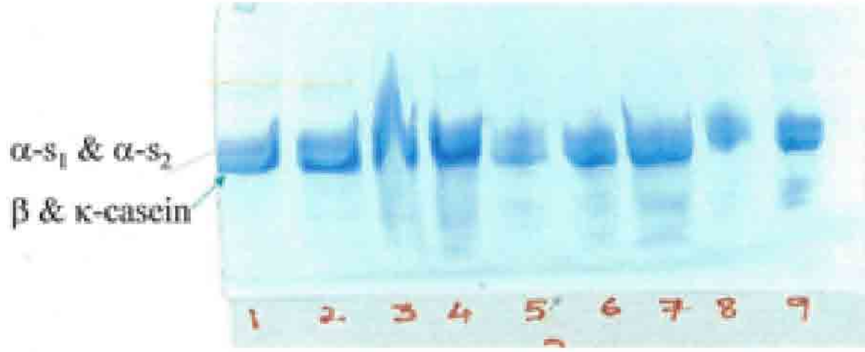


Plate 1: Electrophorogram of fresh mozzarella cheese

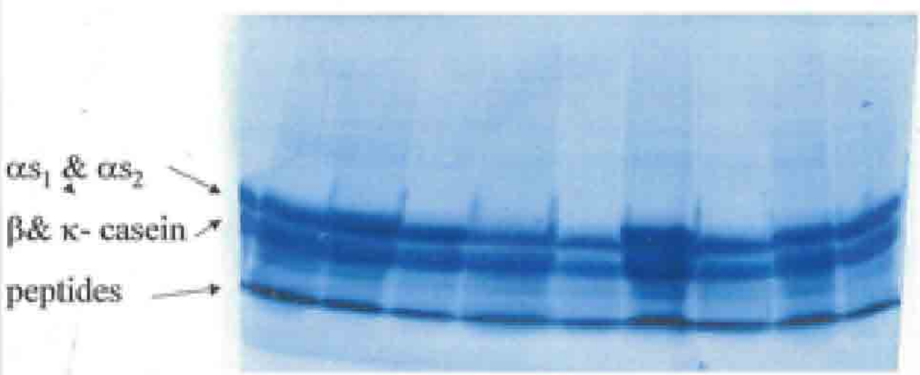


Plate 2 : Electrophorogram of mozzarella cheese after 4 weeks (T_1)

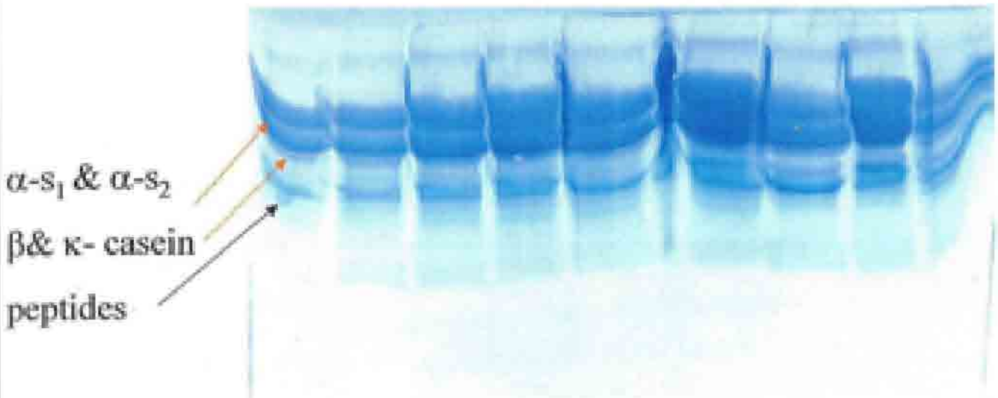


Plate 3 : Electrophorogram of mozzarella cheese after 12 weeks (T_1)

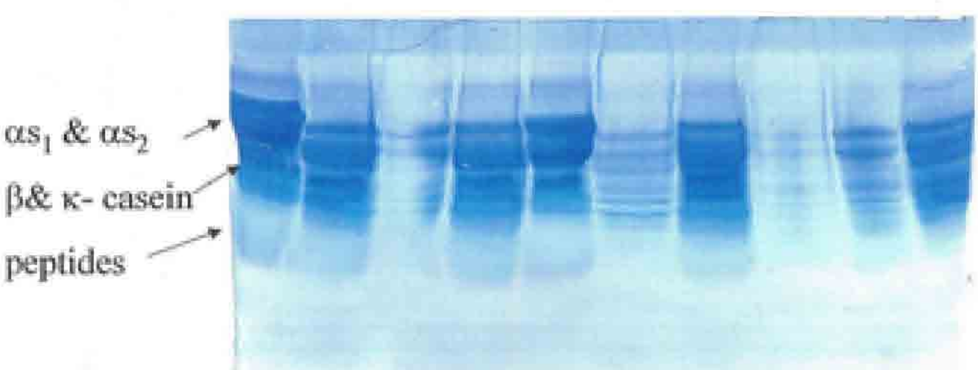


Plate 4: Electrophorogram of 6 months of MAP mozzarella cheese (T_2)

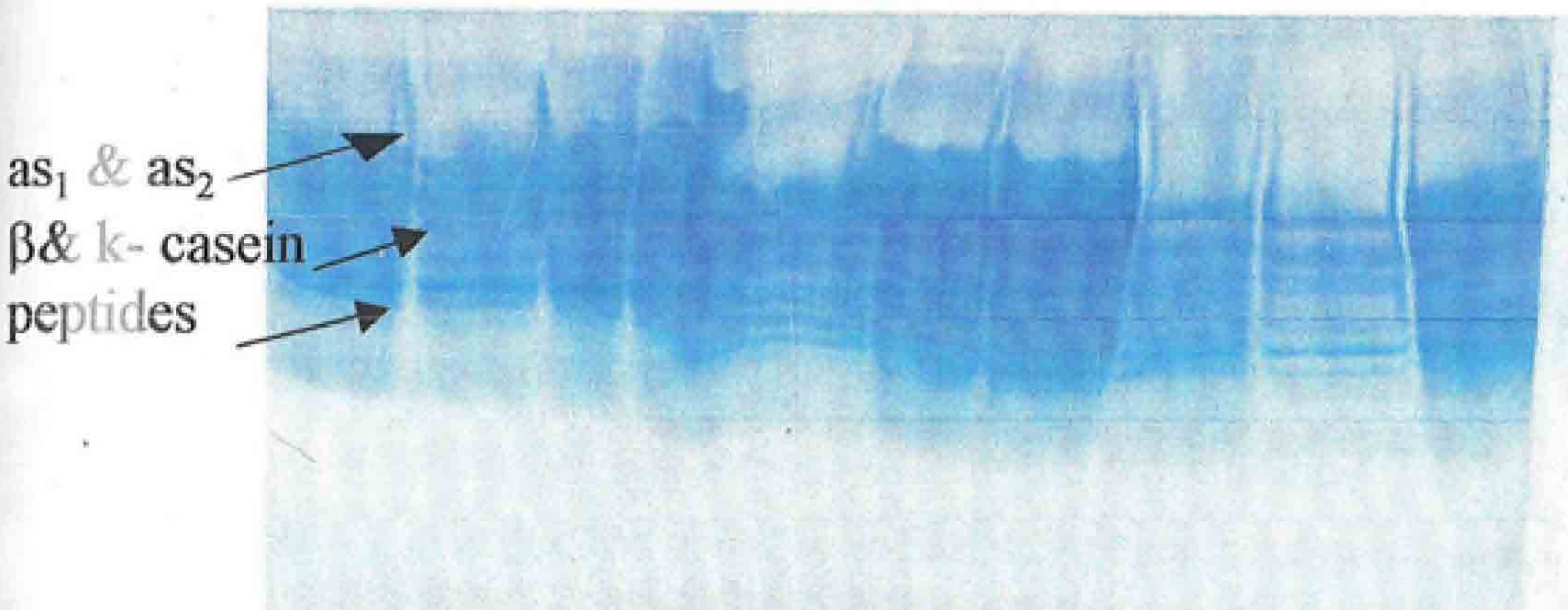


Plate 5: Electrophorogram of 12 months of MAP mozzarella cheese(T2)

cheese was examined by using LMW SDS- PAGE for determining the proteolysis and formation of smaller peptides. A perusal of Photoplate I (elctrophorogram prepared by using fresh mozzarella cheese sample) revealed two bands for acid casein sample (lane 1) and two bands of fresh MC samples (lane 2). During refrigeration storage the degradation of protein started after four weeks with two major bands (Plate II). However, there was no difference in the pattern of casein fractions of MAP MC during storage at 7±1 ^{0}C under 5 atmospheres upto 4 weeks, irrespective of the type of package. After 8 weeks of storage the intensity of bands decreased and lower molecular weight peptides increased. After 12 weeks of storage the intensity of bands and lower molecular weight peptides increased as shown in elctrophorogram (Plate III). It is clear from the Photo plate III that degradation of β - casein was not much, whereas α - casein degraded faster (atm 1) upto 6 weeks of storage. These results are in agreement with the findings of other workers (Farkye *et al.*, 1991; Di-Matteo *et al.*, 1982; Ghosh and Singh 1991).

At deep freeze storage condition casein of mozzarella cheese samples did not degrade upto 6 months. The degradation of a_S – casein started after 6 months (Photo plate IV) and increased with the storage time upto 12 months (Photo plate V), irrespective of the type of atmosphere or type of package.

5.2.3.0 Changes in Functional properties

5.2.3.1 Meltability

In Figures 13, 14 and Appendices XIII & XIV are given the values of meltability of mozzarella cheese stored in two types of packaging materials at 7± 1^{0}C and –10 to –15 ^{0}C for indicated periods. Analysis of variance is shown as a part of table 7. The initial meltability of 2.98 in air packed (atm 1) mozzarella cheese sample increased to 5.06 and 5.05 respectively, in P_1 & P_2 after 5 weeks of storage at 7 ±1 ^{0}C. More increase in meltability was noted in P_1 compared to P_2. The mozzarella cheese stored for 12 weeks revealed that the meltability had been minimum (5.88:P_2; 5.92:P_1) in samples packed under atm 2, followed by atm 4 (5.92 P_2; 5.99 P_1); atm 5 (6.71 P_2; 6.78 P_1) and atm 3 (6.83 P_2; 6.90 P_1) respectively, in ascending order. The meltability was

Table 7. **Analysis of variance for Functional properties of MAP Mozzarella cheese stored in different packages at 7± 1 ^{0}C & -10 to –15 ^{0}C**

Source of variation	**d.f.**	**Mean sum of square**					
		Meltability		**Fat leakage**		**Stretchability**	
		7±1^{0}C	-10 to -15^{0}C	7±1^{0}C	-10 to -15 ^{0}C	7± 1^{0}C	-10 to -15 ^{0}C
Among intervals of storage	12	11.910**	26.160**	10.200**	59.056**	25.919**	19.787**
Among atmospheres	4	28.456**	0.806**	27.430**	1.941**	11.931**	0.142**
Between packages	1	0.019	0.041	0.036	0.049	0.640	0.172**
Interaction intervals x atmospheres	48	1.888	0.037	3.437	0.074	0.658	0.020
Interaction intervals x packages	12	0.001	0.001	0.001**	0.001	0.113	0.006
Interaction atmospheres x packages	4	41.891**	1.307**	46.750*	3.179**	20.317*	0.230**
Interaction intervals x atmospheres x packages	48	2.789*	0.060**	5.748*	0.120	1.118	0.037*
Error	260	2.387	0.059	3.928	0.139	0.918	0.026

*** Significant at 5 % level of probability**
**** Significant at 1 % level of probability**

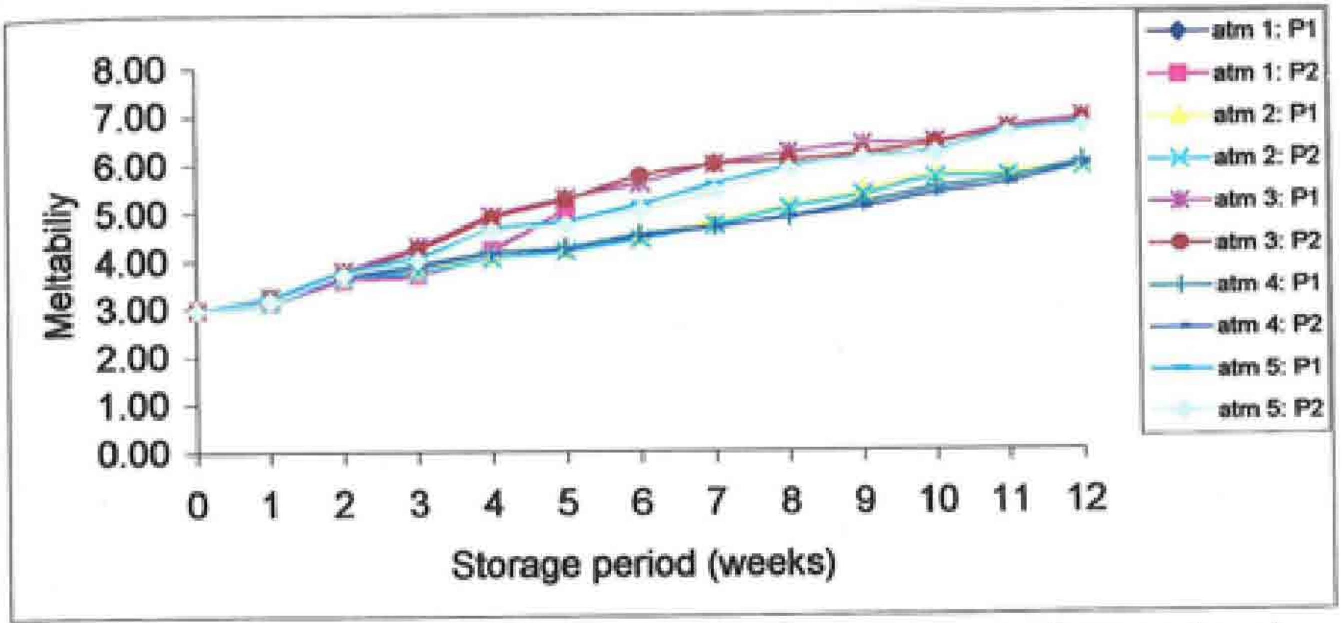


Fig. 13. Effect of MAP on meltability of mozzarella cheese stored in different packages at 7±1 °C

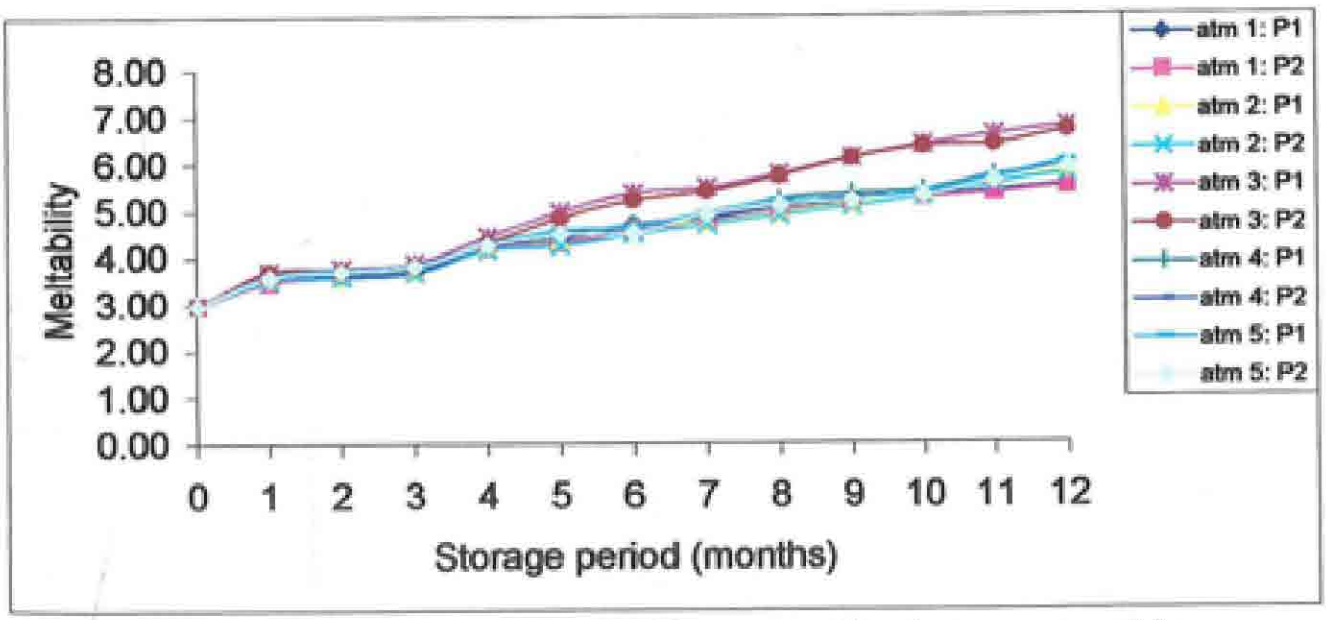


Fig. 14. Effect of MAP on meltability of mozzarella cheese stored in different packages at –10 to -15 °C

found to increase in all the packages with the increase of storage periods (Fig. 13). This trend was also noted by Ghosh (1987). The increase in meltability might be due to the retention of higher moisture, higher TA and probably higher solublization of proteins during cooking (Hutkins *et al.*, 1986; Christenson, 1966; Nilson and LaClair, 1976). The maximum meltability in samples packed under atm 3 was most likely because the moisture retention was highest and soluble nitrogen (which decreases meltability) was lowest.

The data revealed that after 12 months of storage at –10 to –15 ^{0}C, the initial meltability increased to 5.52 and 5.49 respectively, in packages P_1 and P_2 (atm 1), and increased to 5.81 and 5.75 respectively, indicating that the meltability increased with the storage period irrespective of the packaging material or atmosphere. However, our results do not agree with the findings of Oberg *et al.* (1992) who reported that meltability of mozzarella cheese samples decreased when stored at –20 ^{0}C,but agree with the observations of Ghosh (1987) who reported increase in meltability with storage period at –10 to –15 ^{0}C in all the packages studied. Kindstedt *et al.* (1997b) recommended the ageing of mozzarella cheese for 1 to 3 weeks in cold storage for developing the desired meltability in the product.

From the consideration of meltability, the anova (Table 7) indicated that the intervals of storage, 5 types of atmospheres and the interaction atmospheres x packages, each individually, were highly significant ($P<0.01$) for both storage conditions. Interaction intervals x atmospheres x packages was more significant ($P<0.01$) for deep freeze storage. The influence of type of packages, interactions intervals x atmospheres, and intervals x packages were observed to be not significant for both the storage conditions.

5.2.3.2 Fat leakage

Free-oil formation, also called 'oiling off or 'fat leakage' is the tendency of liquid fat to separate from melted cheese and accumulate in pockets or pools, particularly at the surface of cheese. In mozzarella, both excessive free-oil and limited free-oil are considered serious defects. Figures 15 & 16 and Appendices XV and XVI depict the trend and values for fat leakage of MAP mozzarella cheese packed in two types of materials under modified atmospheres and stored at 7±1 ^{0}C and –10 to –15 ^{0} C for different periods. Analysis of variance is included as part of Table 7.

The initial value for fat leakage of mozzarella cheese packed in atm 1 gradually increased from 4.72 to 7.89 and 8.05 respectively, in P_1 & P_2 after 5 weeks of storage at 7 ±1 ^{0}C. The further storage of samples packaged under modified atmospheres upto 12 weeks showed that the fat leakage consistently increased and varied from 7.58 to 7.69. The minimum increase in fat leakage had been with the samples packed under atm 3 followed by atm 5, atm 4 and atm 2 , respectively in ascending order (Fig. 15), suggesting that fat leakage increases with storage period. Similar observations were also recorded by Kindstedt and Rippe (1990) and Ghosh (1987) who observed that refrigerated storage of mozzarella cheese increased the amount of free oil.

Minimum fat leakage was observed in samples packaged under 100 %CO_2. Perhaps, it was due to least FFA content in the stored samples packaged under 100 % CO_2, which in all probably might be ascribed to the bactericidal effect of CO_2 (Alves *et al.*, 1996; Eliot *et al.*, 1998).

On storage at –10 to –15 ^{0} C for 12 months, the maximum fat leakage in mozzarella cheese samples was observed in case of atm 1 (10.12P_1; 10.21 P_2), followed by atm 2(8.77P_1; 8.80 P_2), atm 4(8.63P_1; 8.69 P_2), atm 5 (8.40P_1; 8.45 P_2), and atm 3 (8.25P_1; 8.31P_2), (Fig. 16). This trend was observed for all the samples packed in the two types of packaging materials. Ghosh (1987) also reported increase in fat leakage in mozzarella cheese samples during storage at –10 to –15 ^{0}C irrespective of the packages used.

Intervals of storage and type of atmospheres, both individually, significantly ($P<0.01$) affected the fat leakage in mozzarella cheese samples under both the storage conditions. Effects of types of package and interaction intervals x atmospheres were found to be not significant under any of the storage conditions, but interaction intervals x packages was highly significant for 7± 1 ^{0}C storage. Interaction atmospheres x packages was more significant ($P< 0.01$) in case of –10 to –15 ^{0}C storage, while interaction intervals x atmospheres x packages was significant ($P<0.05$) for 7± 1 ^{0}C for storage only (Table 7).

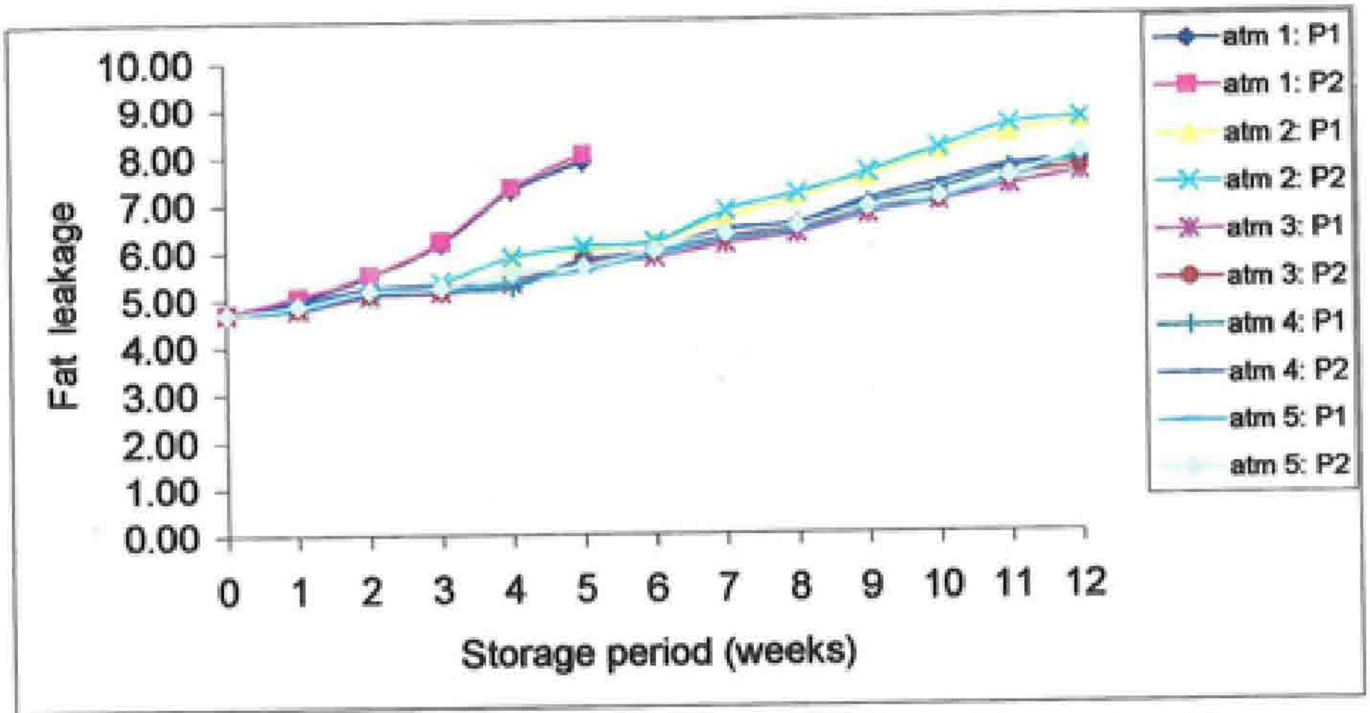


Fig. 15. Effect of MAP on fat leakage of mozzarella cheese stored in different packages at 7±1 ^{0}C

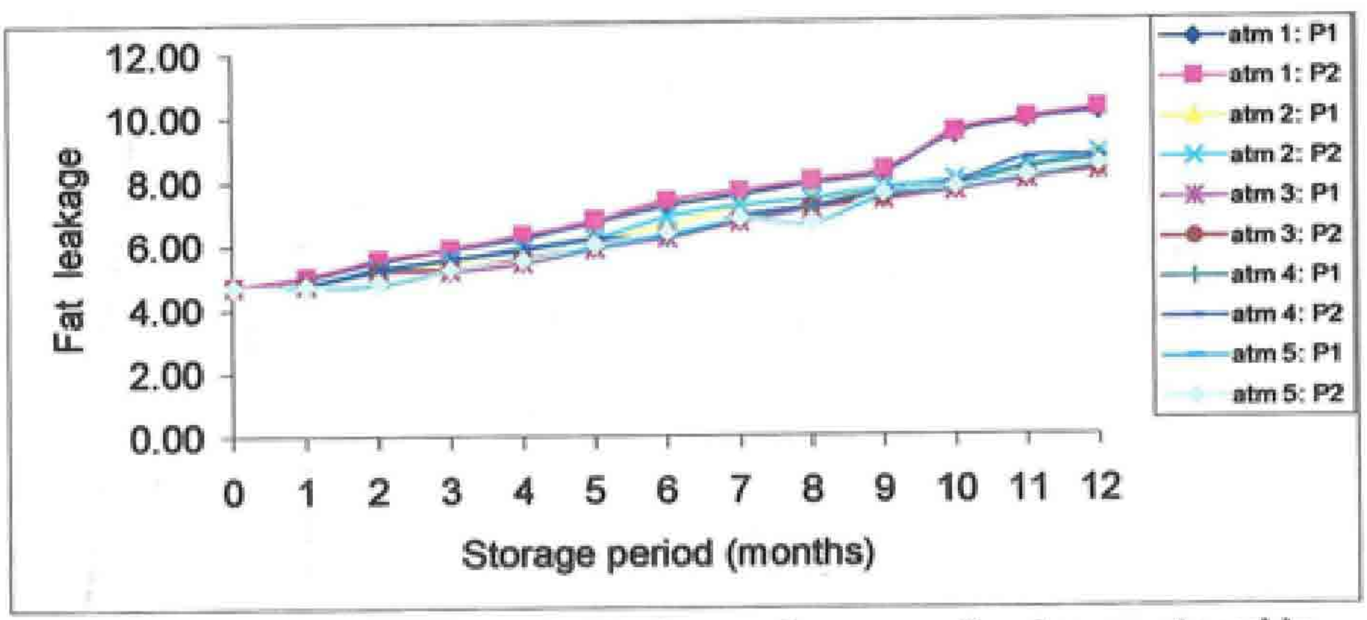


Fig. 16. Effect of MAP on fat leakage of mozzarella cheese stored in different packages at -10 to -15 ^{0}C

5.2.3.3 Stretchability

Data on the stretchability of mozzarella cheese samples packed under modified atmospheres in different packages and stored at 7±1^{0}C and -10 to -15 ^{0}C are presented in Appendices in XVII & XVIII, and graphically shown in Figures 17 and 18. The statistical analysis of the data on stretchability is presented as a part in Table 7. The experiments revealed that the initial stretchability of 5 (arbitarary scale) of air packed (atm 1) mozzarella cheese samples decreased to 3.4 (32 % decrease) and 3.1 (38% decreased) respectively, in case of P_1 & P_2 after 5 weeks of storage at 7 ±1 ^{0}C. These results are in harmony with the findings of Ghosh (1987) who also reported decrease in stretchability of stored mozzarella cheese. In case of mozzarella cheese packed under vacuum (atm 2), the initial stretchability decreased to 2.5 (50 % decrease) and 2.4 (52 % decrease) in P_1 & P_2, respectively. The results are in agreement with the findings of Tunick *et al.* (1991) and Oberg *et al.* (1991) who also observed decrease in stretching of refrigerated mozzarella cheese during storage followed by gradual decline; however, stretch of refrigerated mozzarella rapidly decreased from 7-14 days, but after 14 days, the stretch of un-shredded cheese slowly decreased, whereas the stretch of shredded cheese continued to increase till day 42 followed by rapid decrease in stretch, thereby gradual decline. The initial stretchability of 5 for samples in the packages with 100% CO_2 (atm 3), 50 % CO_2 / 50 % N_2 (atm 5) and 100% N_2 (atm 4) decreased respectively to 3.6 (28 % decrease) (P_1); 3.6, (28 % decrease) (P_2); 2.9, (42 % decrease), (P_1) and 2.8, (44 % decrease), (P_2) and 2.6 (48 % decrease) (P_1); 2.5, (50 % decrease) (P_2), indicating that modifying the atmosphere for packaging of mozzarella cheese helps in checking the loss of stretchability.

After 12 months of storage at –10 to –15 ^{0}C, the initial stretchability value of 5 decreased to 2.5 and 2.3 respectively, in packages P_1 and P_2 (atm 1), while the stretchability decreased to 2.5 and 2.4, respectively in P_1 & P_2 (atm 2). However, for the product packaged under atm 3, atm 4, and atm 5, the initial stretchability decreased to 2.8 (P_1), 2.7(P_2); 2.6 (P_1), 2.5 (P_2); 2.7 (P_1), 2.6 (P_2), respectively. Our observations agree with the experiments of Ghosh (1987) who noted consistent decrease in stretchability during storage of mozzarella cheese samples at –10 to –15 ^{0}C.

Analysis of variance of the data pertaining to stretchability showed highly significant ($P< 0.01$) differences among intervals of storage, and among atmospheres for both the storage conditions, while the influence of packages was significant ($P<0.01$) for –10 to –15 ^{0}C storage only. Interaction atmospheres x packages was more significant ($P<0.01$) at –10 to –15 ^{0}C than 7 ± 1^0 C storage ($P< 0.05$). The interaction intervals x atmospheres x packages was significant ($P< 0.05$) for –10 to –15 ^{0} C storage only, while other studied interactions were observed to be not significant under the two storage conditions. Oberg *et al.* (1992 b) reported that meltability of mozzarella cheese samples decreased when stored at –20 ^{0}C for 42 days.

5.2.4.0 Changes in Textural Properties

5.2.4.1 Hardness

The changes in hardness of mozzarella cheese samples packaged in two different materials under modified atmospheres, and stored at 7 ± 1 ^{0}C and -10 to -15 ^{0}C are depicted in Figures 19, 20 and Appendices XIX & XX. The analysis of variance of the data is given in Table 8.

The hardness of mozzarella cheese exhibited a decreasing trend throughout the entire period of storage in both the packaging materials, and at all the atmospheres for 7 ± 1^0C (Fig. 19). The initial hardness value of 138.89 (N) decreased (atm 1) to 100.63 and 94.11 respectively, in P_1 and P_2 after 6 weeks of storage. Appendix XIX indicates that at the end of storage period of 12 weeks the hardness was minimum for the samples packed under atm 3 (100% CO_2) (P_1: 98.15; P_2: 91.08) followed by atm 5 (50% CO_2 / 50% N_2) (P_1: 94.88; P_2 87.71), atm 4 (100 % N_2) (P1: 93.00; P_2 84.95) and atm$_2$ (vacuum) (P_1: 78.00; P_2 71.24) respectively. Our results pertaining to proteolysis of samples during storage (Appendix IX) also showed that the soluble N_2 content was lowest in case of product packed under atm 3 followed by atm 5, atm 4,

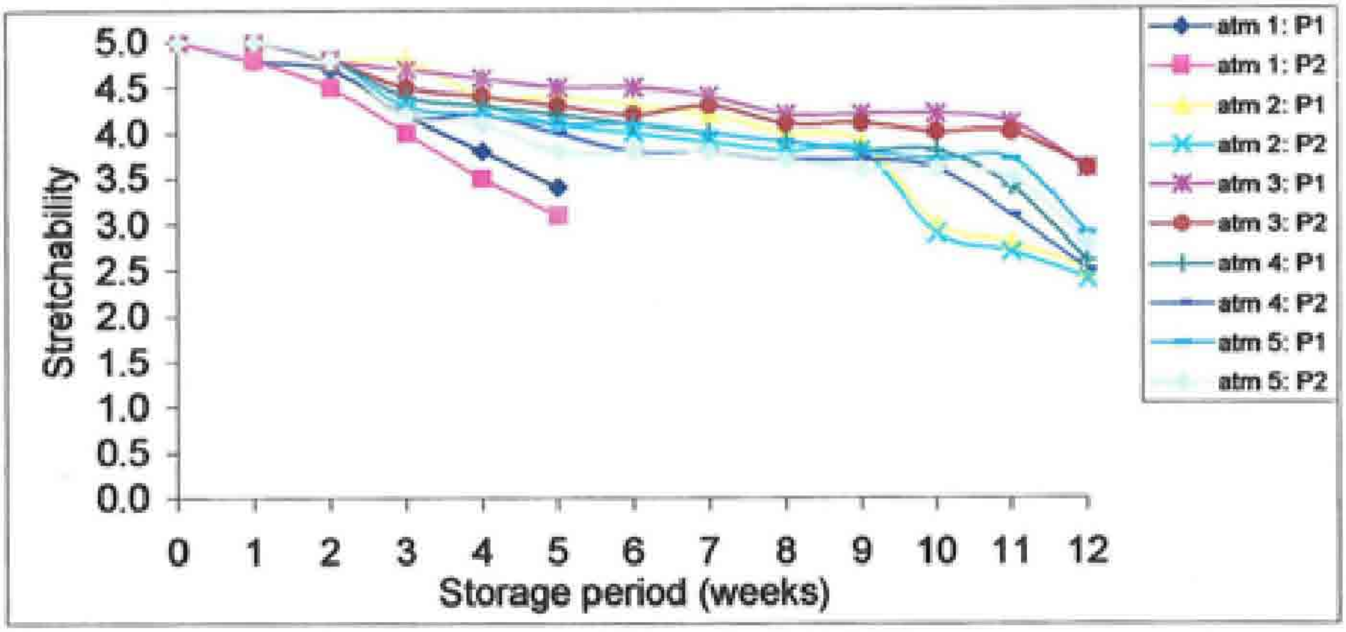


Fig. 17. Effect of MAP on stretchability of mozzarella cheese stored in different packages at 7±1 ^{0}C

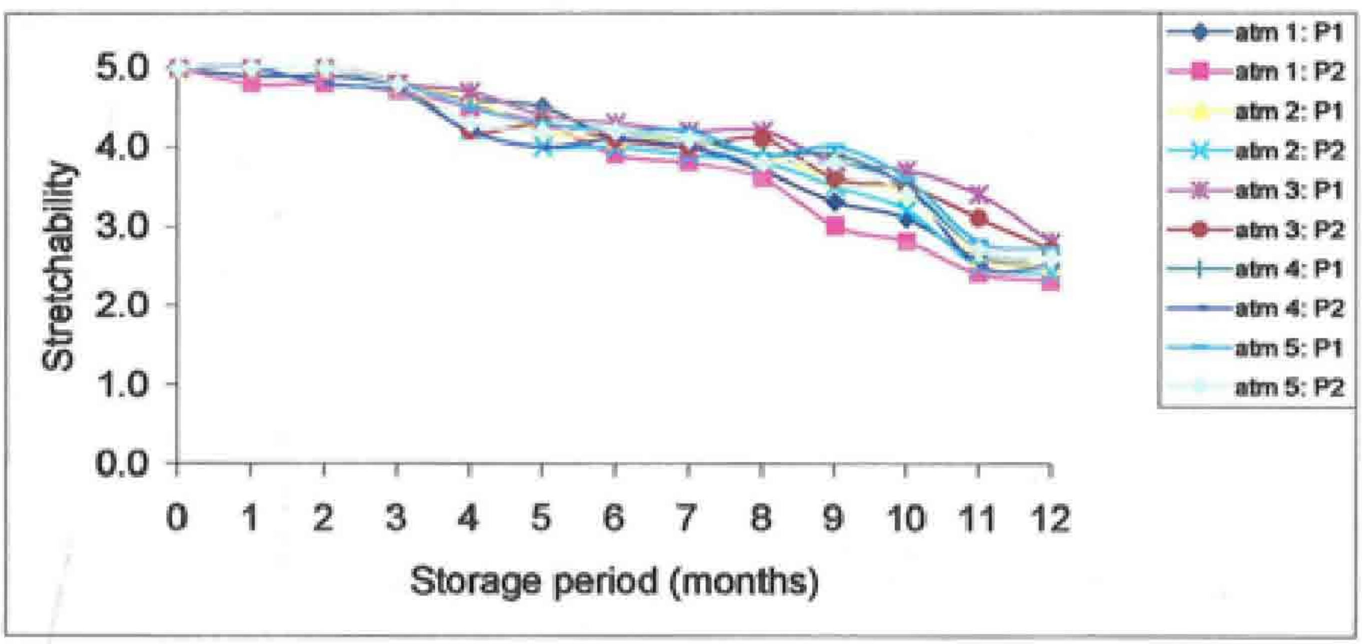


Fig. 18. Effect of MAP on stretchability of mozzarella cheese stored in different packages at -10 to -15 ^{0}C

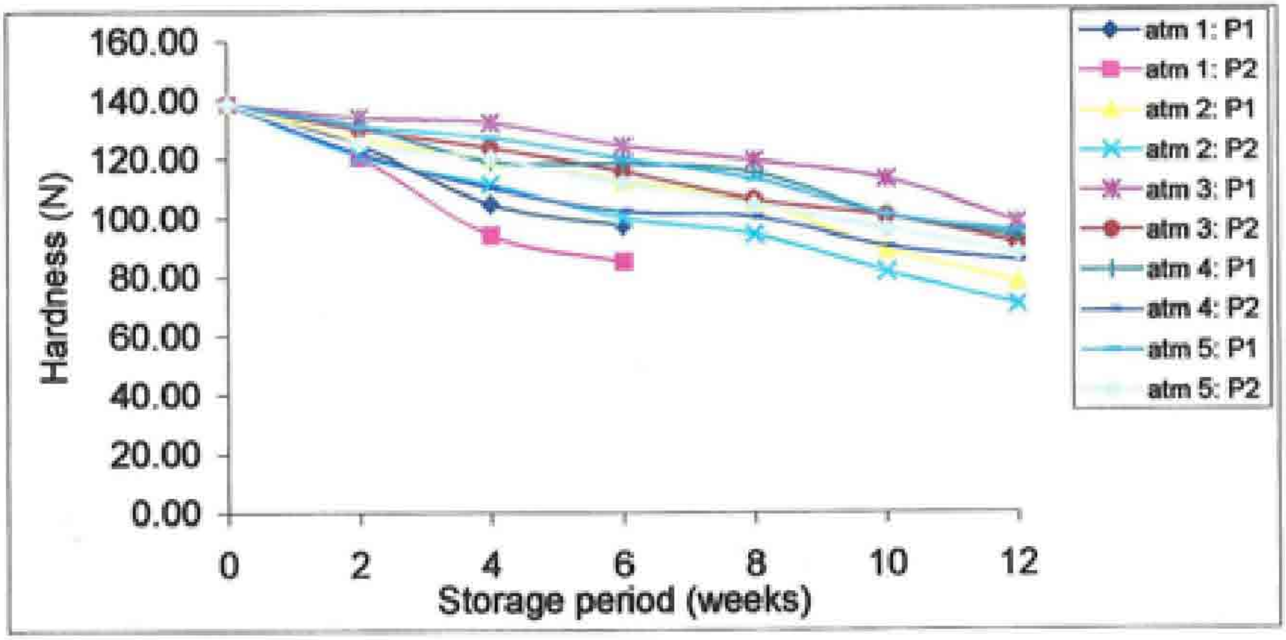


Fig. 19. Effect of MAP on hardness of mozzarella cheese stored in different packages at 7±1 °C

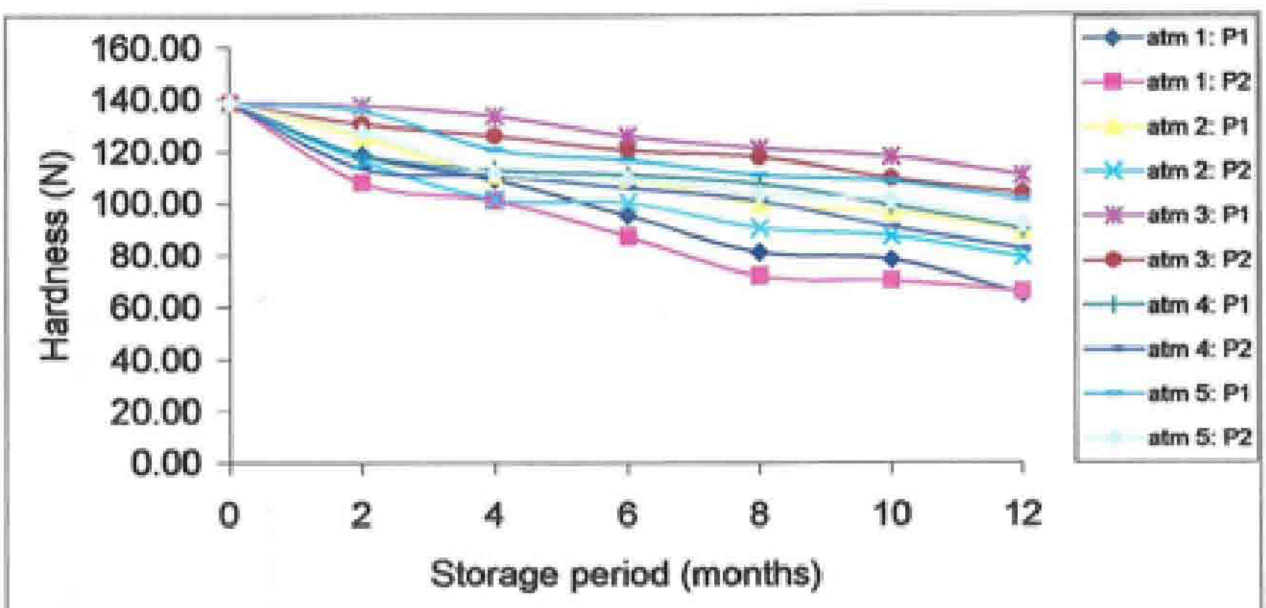


Fig. 20. Effect of MAP on hardness of mozzarella cheese stored in different packages at -10 to -15 °C

Table 8. Analysis of variance for Textural properties of MAP Mozzarella cheese stored in different packages at 7± 1 ^{0}C & -10 to -15 ^{0}C

Source of variation	d.f.	Mean sum of square									
		Hardness		Cohesiveness		Springiness		Gumminess		Chewiness	
		7±1^{0}C	-10 to -15^{0}C	7±1^{0}C	-10 to -15 ^{0}C	7± 1^{0}C	-10 to -15 ^{0}C	7±1^{0}C	-10 to -15 ^{0}C	7±1^{0}C	-10 to -15 ^{0}C
Among intervals of storage	6	20658.288**	9142.442**	0.226**	0.129**	0.365**	0.252**	4402.364**	3360.922**	1497.609**	1288.904**
Among atmospheres	4	3916.217**	836.708**	0.022**	0.001*	0.034*	0.004*	429.745**	59.560**	94.717**	13.527*
Between packages	1	285.162	232.008	0.003*	0.003**	0.006*	0.004*	39.868	22.836	41.274	37.313**
Interaction intervals x atmospheres	24	488.184	41.948	0.002	0.002	0.003	0.001	24.931	9.244	3.533	0.495
Interaction intervals x packages	6	11.351	6.737	0.002	0.001	0.001	0.001	2.489	1.500	1.585	1.765
Interaction atmospheres x packages	4	6096.301**	1318.685**	0.033**	0.001**	0.053**	0.007	677.262**	155.082	142.268**	20.284**
Interaction intervals x atmospheres x packages	24	778.111**	65.685	0.003	0.001*	0.005	0.001	38.877	17.785**	5.763	0.870*
Error	140	565.345	114.691	0.003	0.001	0.005	0.001	48.582	16.719	13.201	4.469

*** Significant at 5 % level of probability**
**** Significant at 1 % level of probability**

atm 2 and atm 1, respectively. The findings coincide with the observation of Federick and Dulley (1984) that lesser degree of proteolysis significantly contributes to the higher hardness in cheese samples.

The storage of mozzarella cheese samples at -10 to -15 ^{0}C for 12 months resulted in the gradual decrease of hardness throughout the period in both the packaging materials and atmospheres (Fig. 20). The observations (Appendix XX) reveal that the type of atmospheres had played a very significant role in controlling the hardness characteristic of mozzarella. The Cheese samples packaged under 100% CO_2 (atm 3) were found to be least affected towards the changes in hardness. The initial hardness value 138.89 N decreased minimum in case of atm 3 (P_1: 110.45; P_2: 103.23) followed by atm 5 (P_1: 100.78; P_2: 91.63), atm 4 (P_1: 89.54; P_2: 82.37), atm 2 (P_1: 88.94; P_2: 78.84) and atm 1 (P_1: 64.89; P_2: 65.76) respectively. The results in general are in accordance with the findings of Ghosh (1987), Olson and Johnson (1990) and Lawrence *et al.* (1987), who inferred that values for hardness decreased significantly with time.

However, the results are contradictory with the observations of Tunick *et al.* (1991) who compared mozzarella cheese of various ages and found that the reduced moisture levels in mozzarella cheese resulted in higher values for hardness.

Anova of the data on hardness (Table 8) reveals that intervals of storage, types of atmospheres and interaction atmospheres x packages contributed significantly ($P<0.01$) towards the changes in hardness during storage under both the conditions, whereas interaction intervals x atmospheres x packages was significant ($P<0.01$) for 7$\pm$1 ^{0} C storage only. However, effect of types of package, and interaction intervals x packages were not significant for both the storage conditions.

5.2.4.2 Cohesiveness

Cohesiveness is molecular attraction by which the particles of a body are bonded throughout the mass. The changes in cohesiveness (A2/A1) of mozzarella cheese samples packaged in two different materials under

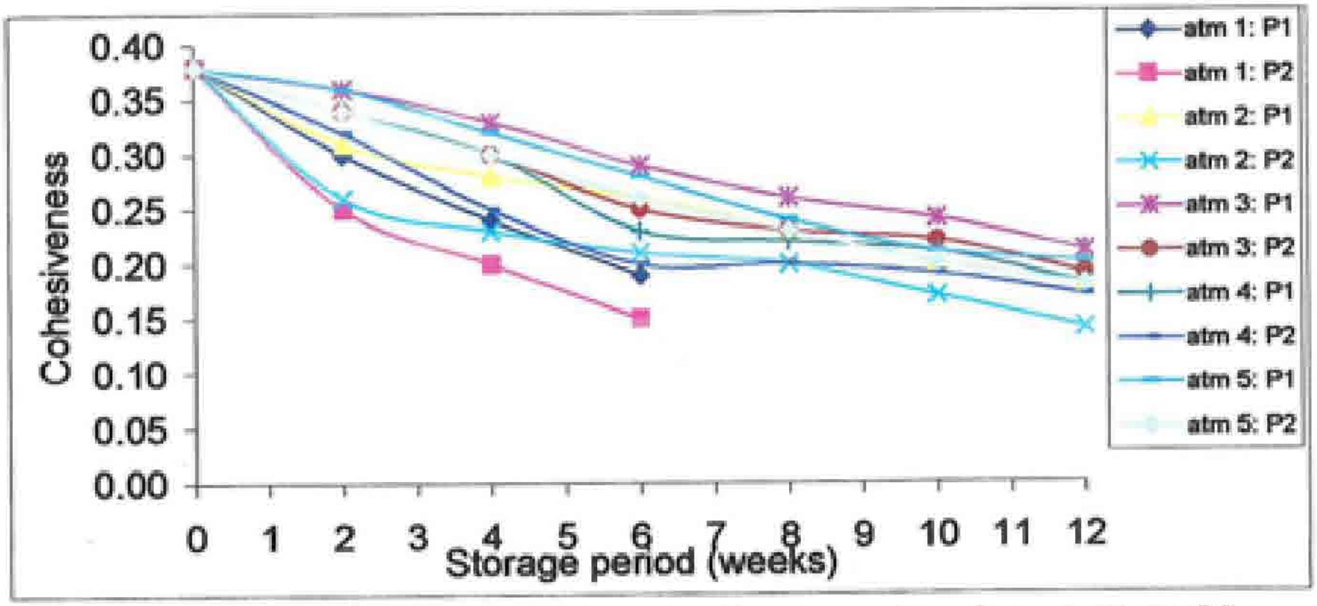


Fig. 21. Effect of MAP on cohesiveness of mozzarella cheese stored in different packages at 7±1 ^{0}C

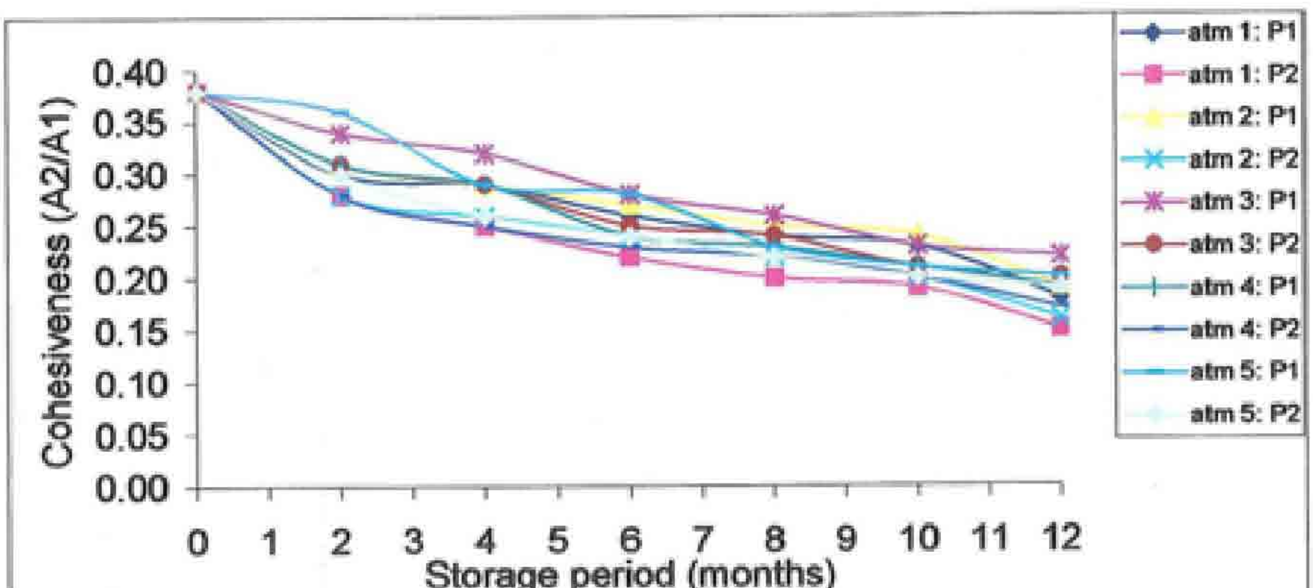


Fig. 22. Effect of MAP on cohesiveness of mozzarella cheese stored in different packages at -10 to -15 ^{0}C

modified atmospheres, and stored at 7±1 ^{0}C and -10 to -15 ^{0}C are presented in Figures 21, 22 and Appendices XXI & XXII. The analysis of variance of the data is given in Table 8.

The cohesiveness of mozzarella cheese showed a decreasing trend throughout the entire period of storage in both the packaging materials, and for all the atmospheres at 7±1^{0}C (Fig. 21). The initial cohesiveness value of 0.38 (atm 1) decreased to 0.19 and 0.15 respectively, in P_1 and P_2 after 6 weeks of storage. The minimum decrease in cohesiveness was observed for the samples packed under atm 3 (100% CO_2) (P_1: 0.21; P_2: 0.19) followed by atm 5 (50% CO_2 / 50% N_2) (P_1: 0.20; P_2 0.18), atm 4 (100 % N_2) (P_1: 0.18; P_2 0.17) and atm 2 (vacuum) (P_1: 0.18; P_2 0.14) respectively after 12 weeks of storage (Appendix 21). Our results pertaining to proteolysis of samples during storage (Appendix IX) also showed that the soluble N_2 content was lowest in case of product packed under atm 3 followed by atm 5, atm 4, atm 2 and atm 1 respectively, indicating comparatively more intact protein network in mozzarella cheese samples of atm 3 > atm 4> atm 2> atm 1 resulting in increased cohesiveness in respective order (Bhaskaracharya and Shah 1999).

The storage of mozzarella cheese samples at -10 to -15 ^{0}C for 12 months resulted in decrease of cohesiveness in both the packaging materials and all atmospheres (Fig. 22). The observations (Appendix XXII) revealed that the type of atmospheres played a very significant role in controlling the cohesiveness characteristic of mozzarella cheese. The Cheese samples packaged under 100% CO_2 (atm 3) were found to be least affected towards the changes in cohesiveness. The initial cohesiveness value 0.38 decreased minimum in case of atm 3 (P_1: 0.22; P_2: 0.20) followed by atm 5 (P_1: 0.20; P_2: 0.19), atm 4 (P_1: 0.19; P_2: 0.17), atm 2 (P_1: 0.19; P_2: 0.16) and atm1 (P_1: 0.18; P_2: 0.15), suggesting more intact protein network in samples under atm 3. The results in general are in accordance with the findings of Ghosh (1987) who inferred that the values for cohesiveness of mozzarella cheese decreased significantly with time.

Anova of the data on cohesiveness (Table 8) revealed that the intervals of storage, and interaction atmospheres x packages contributed significantly ($P<0.01$) towards the changes in cohesiveness for both the storage conditions, whereas interaction intervals x atmospheres x packages was significant ($P<0.05$) for -10 to -15 ^{0} C storage only. The effect of types of package was more significant ($P<0.01$) for deep freeze storage condition, while influence of atmospheres was highly significant ($P<0.01$) in case of 7±1 ^{0}C storage. Other studied interactions were found to be not significant for any of the storage conditions.

5.2.4.3 Springiness

The changes in springiness of mozzarella cheese samples packaged in two different materials under modified atmospheres and stored at 7±1 ^{0}C and -10 to -15 ^{0}C are depicted in Figures 23, 24 and Appendices XXIII & XXIV. The analysis of variance of the data is given in Table 8.

The initial mean springiness value for fresh mozzarella cheese sample was 0.50 mm, which decreased to 0.27 and 0.20 in P_1 and P_2, respectively at the end of 6 weeks of storage at 7±1 ^{0}C in atm1. After 12 weeks the values were found to be atm 2 (P1 : 0.24; P2 : 0.19), atm 3 (P1 : 0.28; P2 : 0.25), atm 4 (P_1: 0.26; P_2 : 0.22) and atm 5 (P_1: 0.27, 0.24 P_2) (Appendix XXIII). Fig. 23 indicates that least change in springiness occurred in samples packaged under atm 3 followed by atm 5, atm 4 and atm 2 respectively, in ascending order. Perhaps higher degree of proteolysis (Lawrance *et al.* 1987) might be the reason for medium decrease in springiness in case of samples packaged under atm 2.

On storage under -10 to - 15 ^{0} C for 12 months the minimum decrease in springiness (mm) of mozzarella cheese was observed in samples of atm 3 followed by atm 5, atm 4, atm 2 and atm 1 in ascending order (Fig.24), suggesting that CO_2 (atm 3) and 50% CO_2 / 50% N_2 (atm 5) had played a vital role towards springiness of the product compared to other 3 studied atmospheres. This trend was observed for both types of packaging materials. The results are similar to the findings of Ghosh (1987) and Lawrence *et al.*

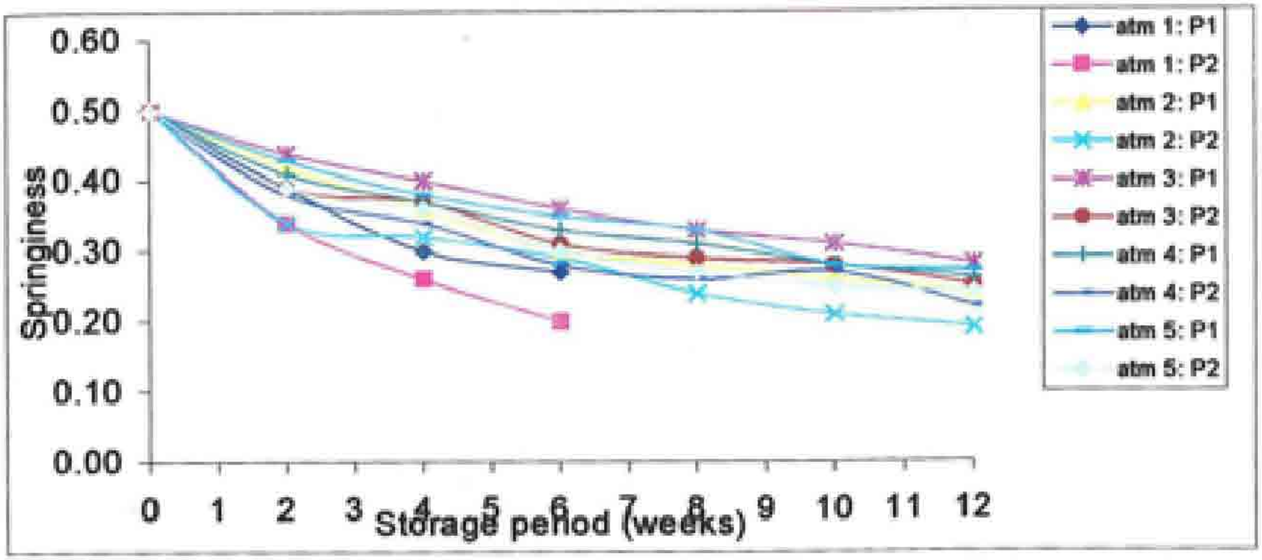


Fig. 23. Effect of MAP on springiness of mozzarella cheese stored in different packages at 7±1 ^{0}C

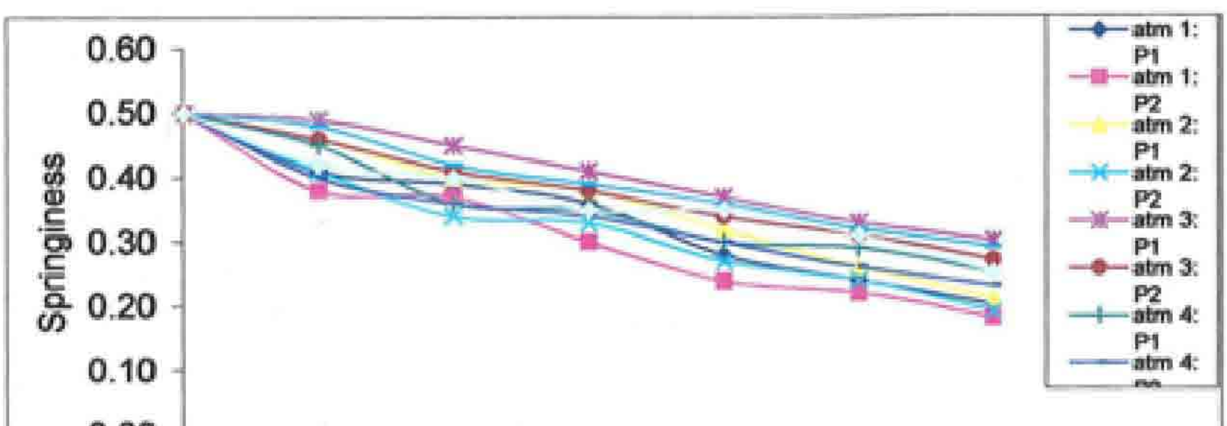


Fig. 24. Effect of MAP on springiness of mozzarella cheese stored in different packages at -10 to -15 ^{0}C

(1987) that values for springiness decrease significantly with time. However, the results are at variance with the observations of Tunick *et al.* (1991) who compared mozzarella cheese of various ages and found that the reduced moisture levels in mozzarella cheese resulted in higher values for springiness.

Statisticallly, the influence of intervals of storage on the springiness of mozzarella cheese samples was highly significant ($P<0.01$) for both the conditions of storage, while interaction atmospheres x packages was significant ($P<0.01$) for 7±1 ^{0}C storage only (Table 8). The variation among atmospheres and between packages, both individually, was observed to be significant ($P<0.05$) for both the storage conditions. Other studied interactions were, however, found to be not significant under any of the studied storage condition.

5.2.4.4 Gumminess

Figures 25, 26 and Appendices XXV and XXVI represent the changes in gumminess of mozzarella cheese during storage. The mean initial value of gumminess was found to be 50.34 N and it continued to decrease in all the cheese samples during storage. At the end of 6 weeks storage (7±1 ^{0}C), the values for gumminess (atm 1) decreased to 23.83 N in case of P_1 and 15.52 N for P_2. Further storage of samples under other four modified atmospheres for 12 weeks at 7±1 ^{0} C showed (Fig. 25) that the rate of decrease was minimum for the samples of atm 3(25.41 P_1, 22.10 P_2),, and maximum for atm 2 (19.55 P_1, 17.32 P_2), while the decrease had been moderate with the samples packed under atm 4 (22.31 P_1, 18.96 P_2) and atm 5 (23.29 P_1, 20.89 P_2). From these results, it can be inferred that the gumminess decreased consistently in all packages during storage. The results are in agreement with the findings of Ghosh (1987) and Malhotra (1991) who also reported decrease in gumminess of cheese samples during storage. Fig. 26 indicates that the storage at –10 to –15 ^{0}C for 12 months resulted in decrease of gumminess of mozzarella cheese samples for both the packaging materials and under all the studied atmospheres. The observations (Appendix XXVI) also revealed that the type of atmospheres played a very significant role in controlling the gumminess of cheese samples. The samples packaged under 100% CO_2 (atm 3) were found to be least affected towards the changes in gumminess. The initial gumminess value of 0.38 decreased minimum in case of atm 3 (P_1: 24.89; P_2: 24.77) followed by atm 5 (P_1: 23.61; P_2: 14.14), atm 4 (P_1: 24.54; P_2: 24.44), atm 2 (P_1: 18.99; P_2: 18.84) and atm1 (P_1: 14.14; P_2: 14.01) respectively, in consistent manner throughout the storage period. Ghosh (1987) also reported that values for gumminess of stored mozzarella cheese decreased significantly with time.

Analysis of variance of the data concerning gumminess of the product stored at 7 ± 1 ^{0}C revealed that the influence of different intervals of storage and effect of different atmospheres was highly significant ($P<0.01$) for both storage conditions, while interaction atmospheres x packages was significant ($P<0.01$) for 7 ± 1 ^{0} C storage only. Interaction intervals x atmospheres x packages was significant ($P< 0.01$) for deep freeze storage condition. However, interactions intervals x atmospheres, intervals x packages, and also between packages were not significant for both the storage conditions.

5.2.4.5 Chewiness

Data on the chewiness of mozzarella cheese samples packed in 5 modified atmospheres and stored in different packages at 7 ± 1^0C and -10 to -15 ^{0}C are given in Appendices XXVII & XXVIII and illustrated in Figures 27 and 28. The statistical analysis of the data on chewiness is presented as a part of Table 8. Perusal of the Fig. 27, 28 and Appendices XXVII & XXVIII, indicate decreasing trend in the values for chewiness of mozzarella cheese during storage. The pattern was almost similar to gumminess and springiness. This was expected as the chewiness is a secondary parameter derived from springiness and gumminess. The initial chewiness value of 25.15 (Nmm) of mozzarella cheese decreased to 5.20 and 4.98 respectively, in P_1 & P_2 (atm1) after 6 weeks of storage at 7 ±1 ^{0}C. More decrease in chewiness was noted in P_2 compared to P_1. In case of mozzarella cheese packed under vacuum (atm 2), the initial chewiness (25.15) decreased to 7.25 and 4.00 (Nmm) in P_1 & P_2, respectively.

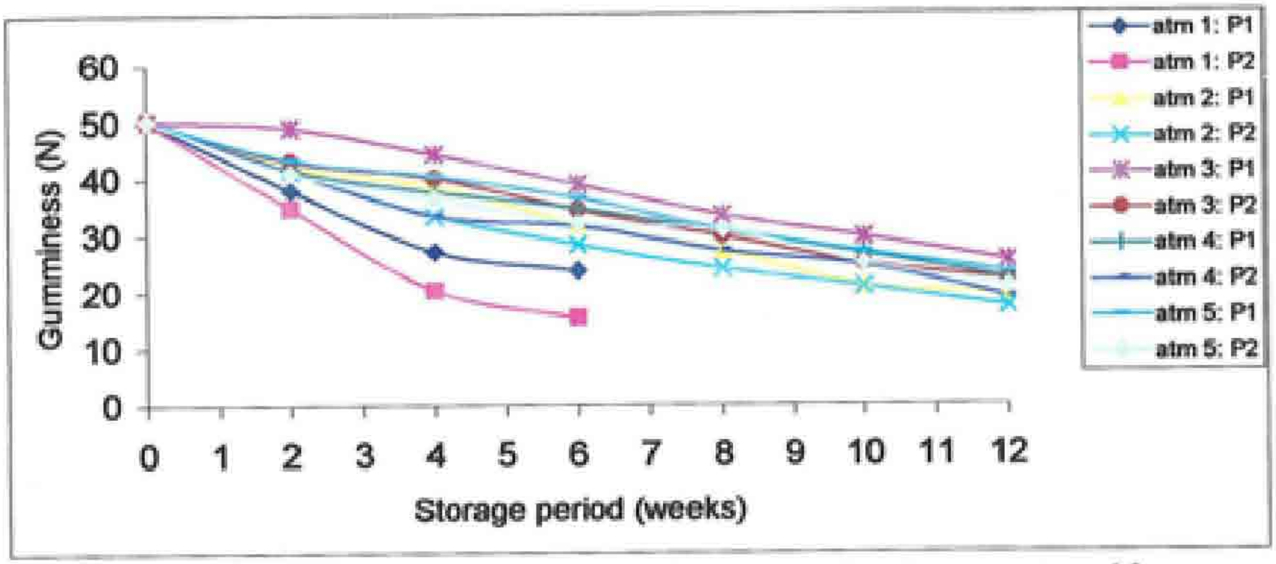


Fig. 25. Effect of MAP on gumminess of mozzarella cheese stored in different packages at 7±1 ^{0}C

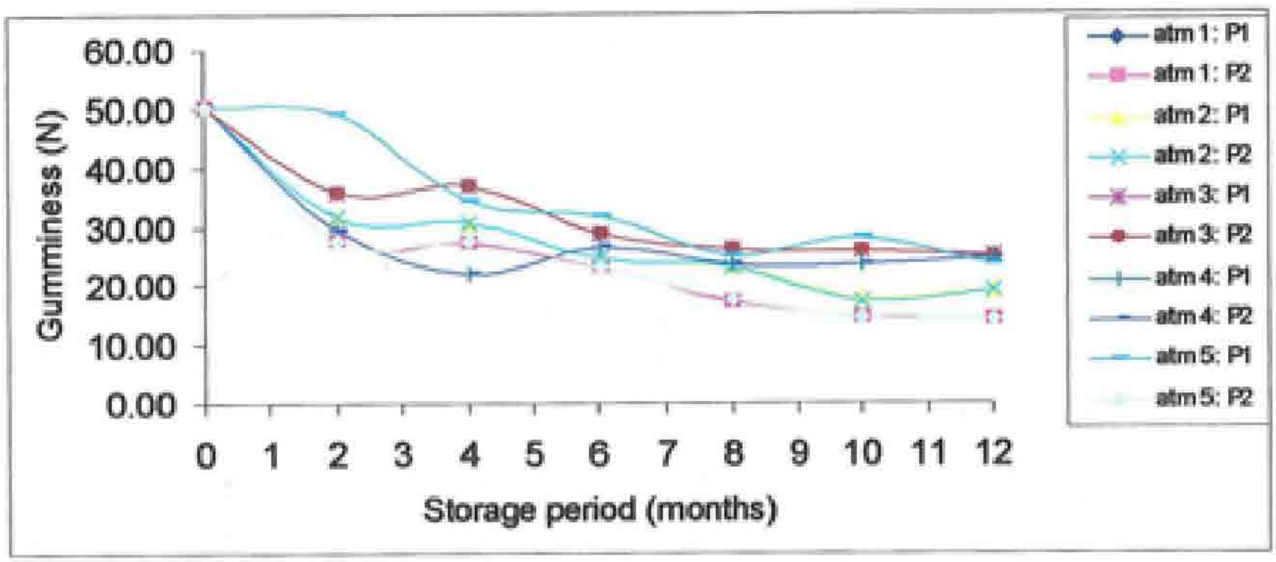


Fig. 26. Effect of MAP on gumminess of mozzarella cheese stored in different packages at -10 to -15 ^{0}C

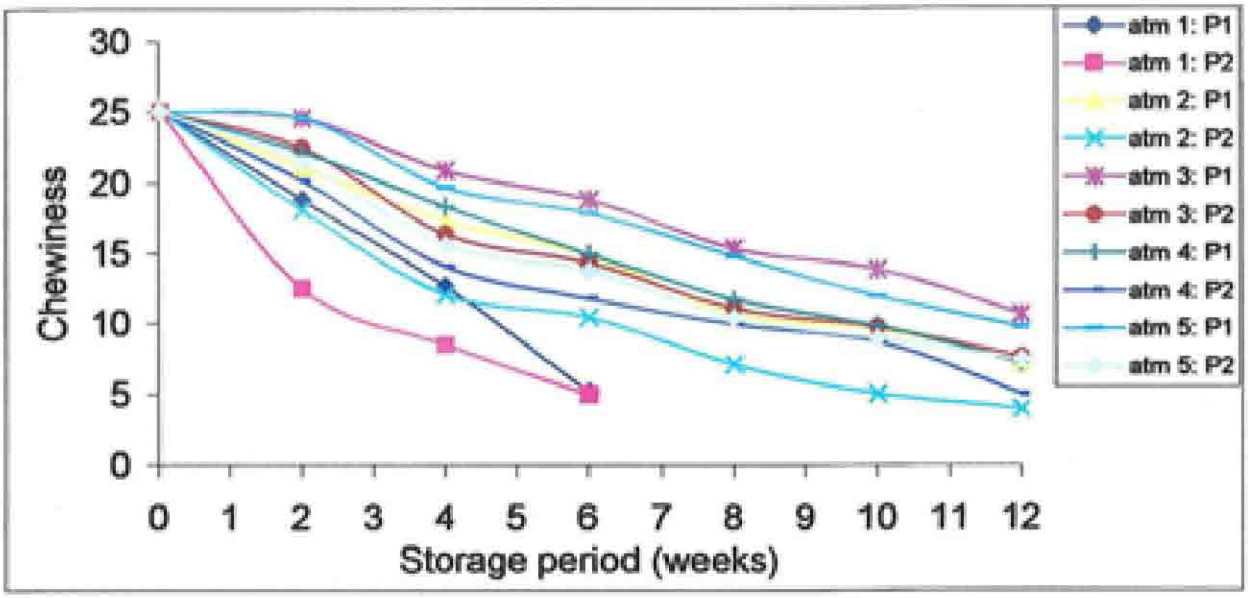


Fig. 27. Effect of MAP on chewiness of mozzarella cheese stored in different packages at 7±1 °C

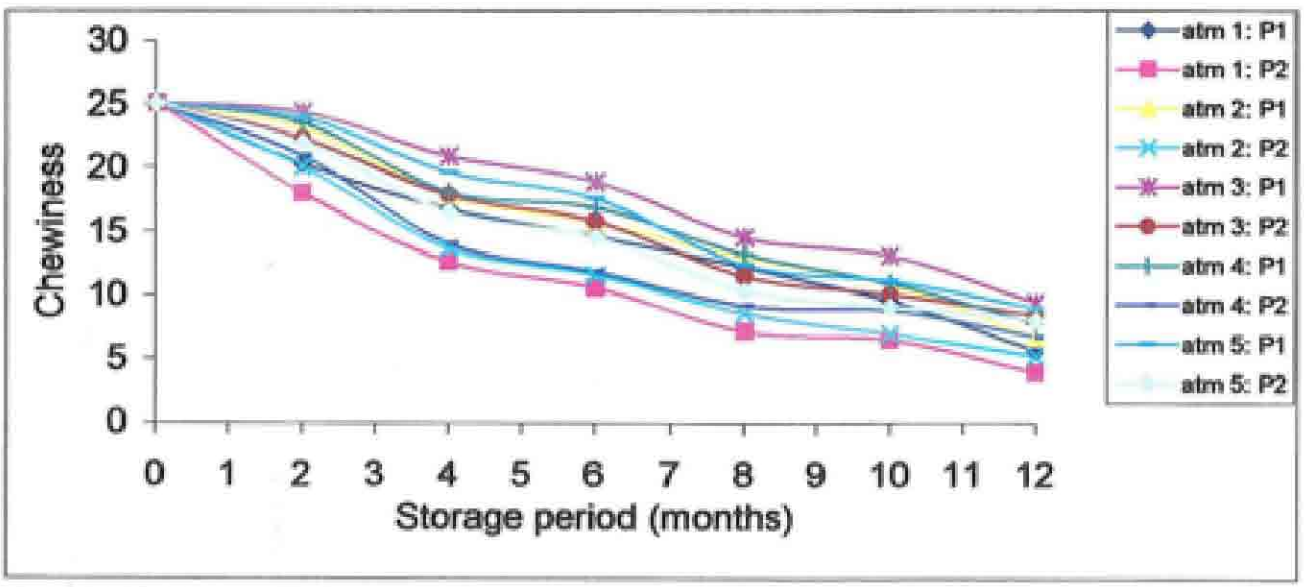


Fig. 28. Effect of MAP on chewiness of mozzarella cheese stored in different packages at -10 to -15 °C

The chewiness of mozzarella cheese samples in the packages with 100% CO_2 (atm 3), 50 % CO_2 / 50 % N_2 (atm 5) and 100% N_2 (atm 4) respectively, decreased to 10.67 (P_1), 7.64 (P_2); 9.78 (P_1) and 7.45 (P_2) and 7.23 (P_1), 5.02 (P_2); indicating that the minimum increase in the value had been with the samples packed in P_1 (atm 3), and maximum for P_2 (atm 2) establishing a very significant influence of MAP on the chewiness characteristic of mozzarella cheese during storage.

The observations on the chewiness of MAP mozzarella cheese stored at deep freeze conditions showed that after 12 months of storage, the initial value of 25.15 (N mm) increased to 5.66 and 3.92 respectively, in packages P_1 and P_2 (atm 1), while the chewiness decreased to 6.67 and 5.23 respectively in P_1 & P_2 (atm 2). However, for the product packaged in atm 3, atm 4, and atm 5, the chewiness decreased to 9.43 (P_1), 8.43 (P_2); 7.80 (P_1), 6.75 (P_2) and 8.96 (P_1), 8.05 (P_2), respectively (Appendix XXVIII). Most probably the decrease in chewiness during storage was due to bacterial/ enzymatic action, which might have caused varied degree of proteolysis in mozzarella cheese samples packed in different modified atmospheres.

Anova of the data on chewiness of the stored mozzarella cheese samples revealed (Table 8) that the intervals of storage and interaction atmospheres x packages were highly significant ($P< 0.01$) with regard to changes in chewiness characteristic. The influence of different atmospheres was more significant ($P<0.01$) for $7\pm1\ ^0C$ storage than -10 to $-15\ ^0C$ ($P<0.05$), while variation between packages ($P<0.01$) and interaction intervals x atmospheres x packages ($P<0.01$) affected the chewiness significantly for deep freeze storage only. Other studied interactions were not significant.

5.2.5.0 Microbiological analysis

5.2.5.1 Standard plate count (SPC)

The observations relating to standard plate count (SPC) of mozzarella cheese samples packed under 5 modified atmospheres and stored in different packages at 7 ± 1^0C and -10 to -15 ^0C are given in Appendices in XXIX & XXX

Table 9. **Analysis of variance for Microbiological parameters of MAP Mozzarella cheese stored in different packages at 7± 1 ^{0}C & -10 to –15 ^{0}C**

Source of variation	d.f.	Mean sum of square							
		SPC		Yeast & mould count		Psychrotrophic count		Coliform count	
		7±1^{0}C	-10 to -15^{0}C	7±1^{0}C	-10 to -15 ^{0}C	7± 1^{0}C	-10 to -15 ^{0}C	7±1^{0}C	-10 to -15 ^{0}C
Among intervals of storage	12	39.048**	41.542**	10.703**	8.775**	21.559**	22.580**	6.573**	14.936**
Among atmospheres	4	7.274*	0.140**	4.465**	0.094**	26.619**	0.481**	1.155*	0.088**
Between packages	1	0.253	0.012	0.060	0.002	0.045	0.012	0.380	0.013
Interaction intervals x atmospheres	48	1.782	0.007	1.120	0.008	3.514	0.016	0.388	0.003
Interaction intervals x packages	12	0.012**	0.001	0.001	0.001	0.002	0.001	0.005	0.001
Interaction atmospheres x packages	4	17.799**	0.255**	8.840**	0.165**	45.608**	0.968**	3.333**	0.159**
Interaction intervals x atmospheres x packages	48	3.040*	0.012	1.860**	0.015**	5.917**	0.035	0.670	0.004
Error	260	2.573	0.015	1.172	0.014	3.767	0.064	0.630	0.008

*** Significant at 5 % level of probability**
**** Significant at 1 % level of probability**

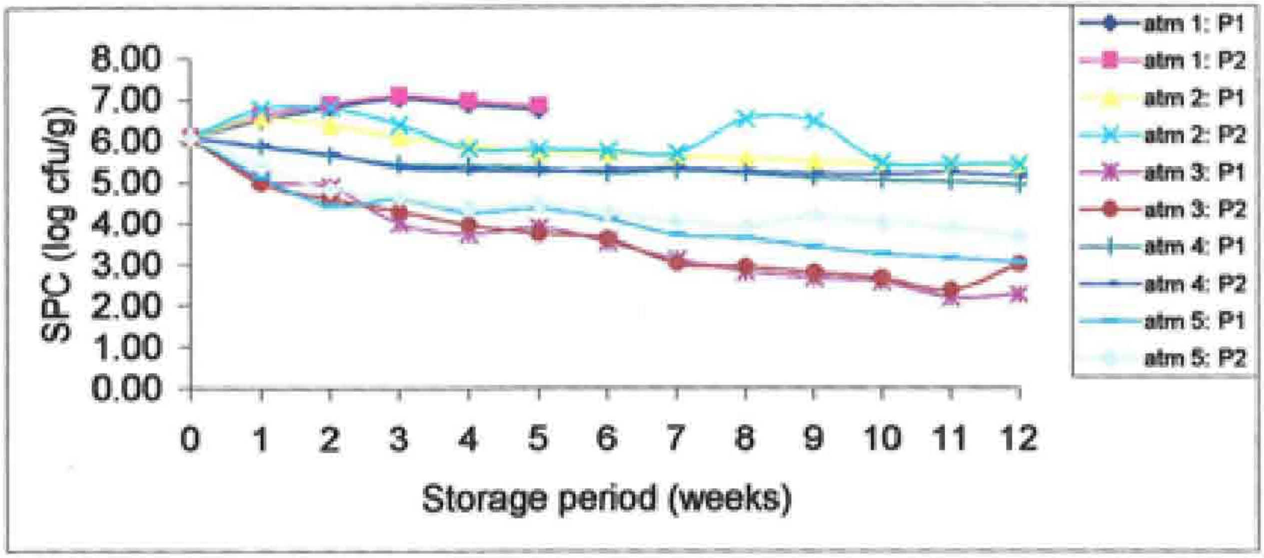


Fig. 29. Effect of MAP on SPC of mozzarella cheese stored in different packages at 7±1 ^{0}C

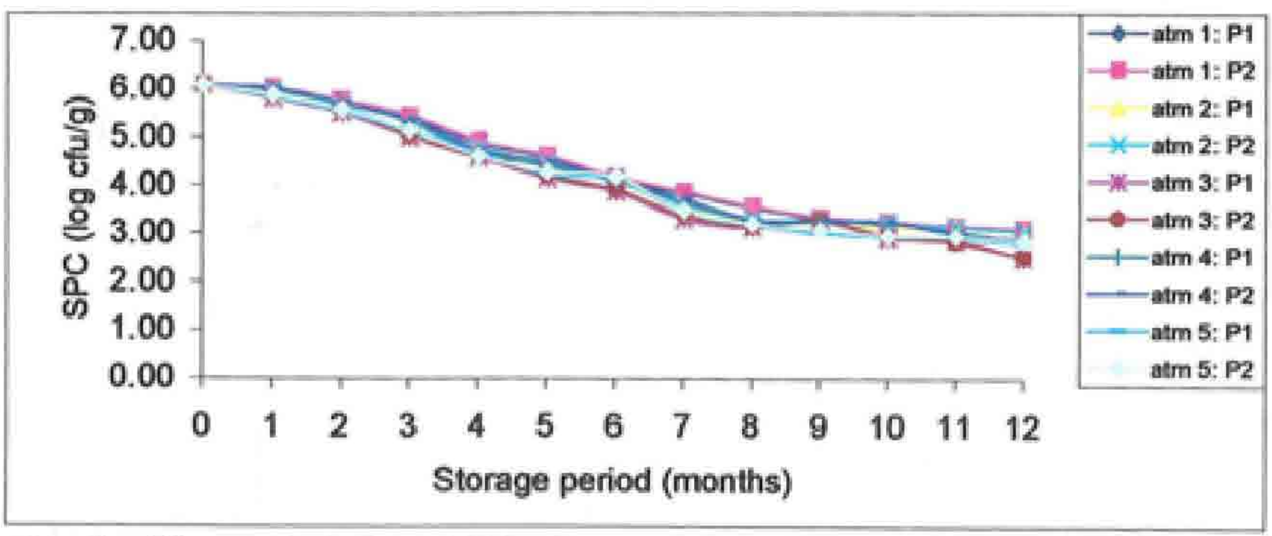


Fig. 30. Effect of MAP on SPC of mozzarella cheese stored in different packages at -10 to -15 ^{0}C

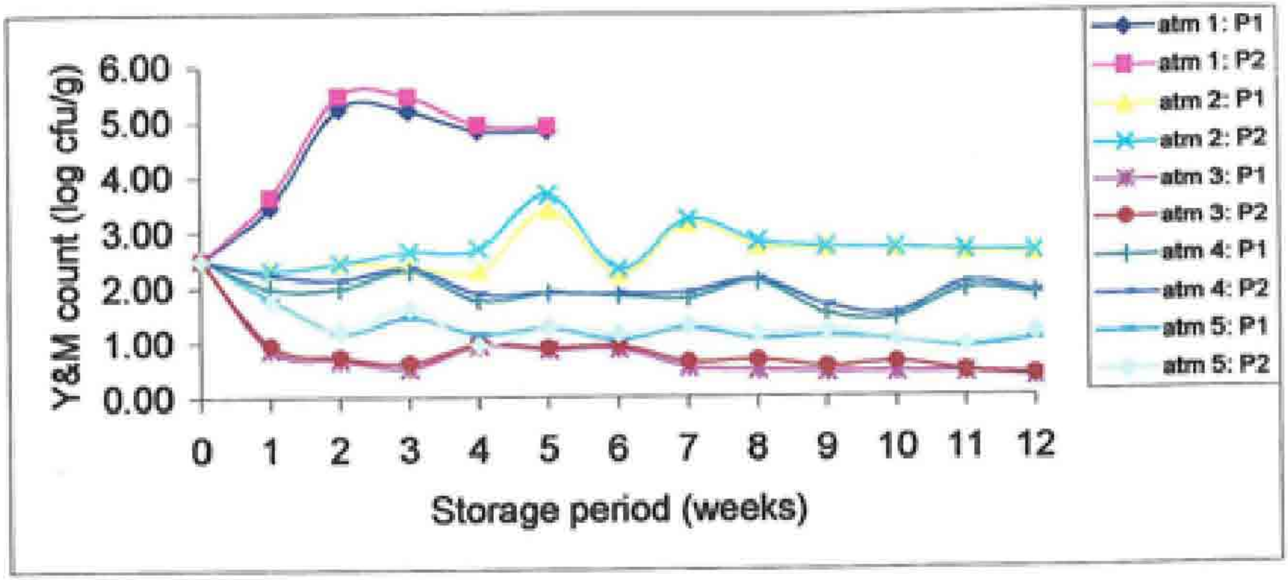


Fig. 31. Effect of MAP on Y & M count of mozzarella cheese stored in different packages at 7±1 ^{0}C

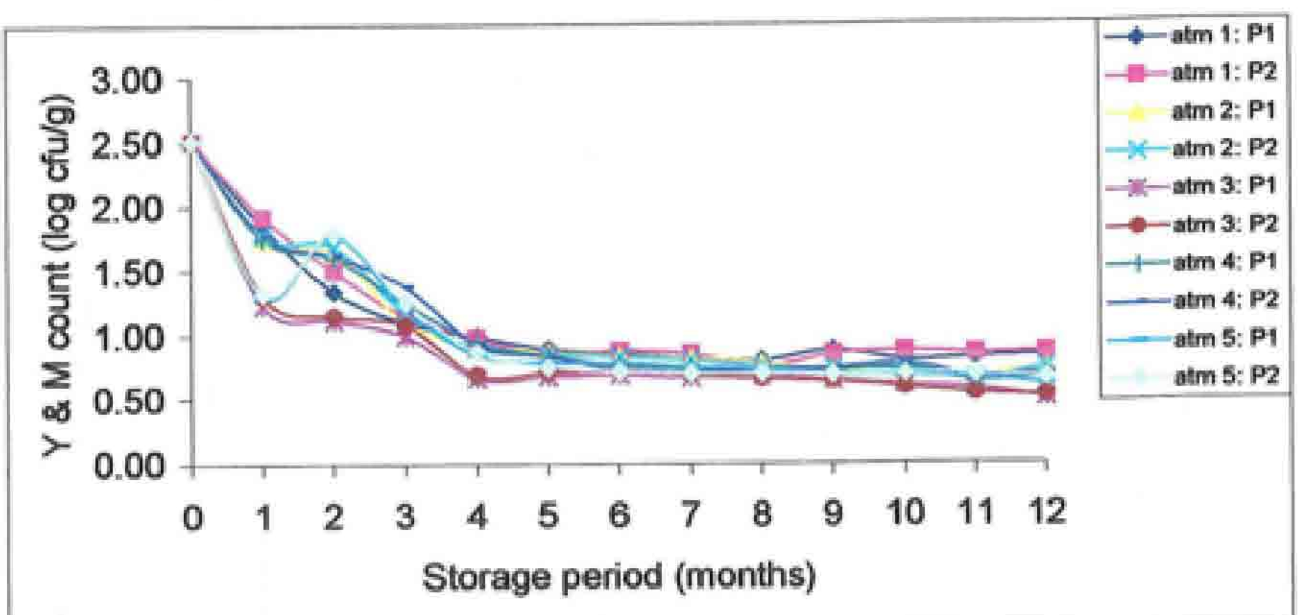


Fig. 32. Effect of MAP on Y & M count of mozzarella cheese stored in different packages at –10 to -15 ^{0}C

and illustrated in Figures 29 and 30. The statistical analysis of the data is recorded in Table 9. The initial standard plate count (SPC) was 6.10 (log CFU / g) in air packed (atm 1) mozzarella cheese and increased to 6.75 and 6.85 (log CFU / g) respectively, in P_1 & P_2 after 5 weeks of storage at 7 $\pm$1 ^{0}C. The results of Asperger (1982) suggested that the total bacterial count (TBC) in mozzarella cheese stored at 4 °C increased, and was greater than 10^7 CFU / g after one week of storage. When mozzarella cheese was stored at 20 °C for 24 h, the TBC increased by about 2 log unit (Asperger and Brandl, 1982). The results are in agreement with the experiments of Asperger (1988) who also noted increase in bacterial count in mozzarella cheese samples stored at 4 ^{0}C. Slightly more increase (statistically not significant) in SPC was noted in P_2 compared to P_1. In case of samples stored for 12 weeks, the data revealed that the standard plate count (SPC) was minimum (2.26:P_1; 2.99:P_2) in samples packed under atm 3 (100 % CO_2) followed by atm 5 (50% CO_2 / 50 % N_2) (3.03: P_1; 3.66 P_2); atm 4 (100 % N_2) (4.91 P_1; 5.12 P_2) and atm 2 (Vacuum) (5.36 P_1: 5.39 P_2) respectively, in ascending order (Appendix XXIX), confirming the findings of other workers (Alves *et al.*, 1996: Fedio *et al.*, 1994: Eliot *et al.*, 1998) that CO_2 has bactericidal effect, and maximum in samples packed under atm 2 (vacuum), which had neither CO_2 nor N_2.

The data revealed that after 12 months of storage of mozzarella cheese samples at -10 to -15 ^{0} C, the initial standard plate count of 6.10 (log CFU/ g) decreased to 3.06 (P_1) and 3.10 (P_2) under atm 1; and decreased to 3.02 (P_1) and 3.05 (P_2) under atm 2, respectively. The decrease in SPC was substantial in case of product packaged under atm 3 followed by atm 5 and atm 4 respectively, establishing the bactericidal role of CO_2.

A perusal of Table 9 indicates that the effect of intervals of storage and also of the interaction atmospheres x packages on the values for SPC for both the storage conditions was significant (P<0.01). The difference in the values for SPC due to the type of atmospheres was found to be more significant (P<0.01) for deep freeze storage condition, while interactions intervals x packages (P<0.01), and intervals x atmospheres x packages (P< 0.05) were significant for 7±1 ^{0}C storage only. The difference in the values of SPC due to the type of package, and interaction intervals x atmospheres were found to be not significant under any of the two storage conditions.

5.2.5.2 Yeast and moulds count

The effect of storage of mozzarella cheese samples packaged under different atmospheres and stored at two conditions for stipulated period on the yeast & moulds count is shown in Figures 31, 32 and Appendices XXXI & XXXII. Analysis of variance is presented in Table 9.

The initial mean value of Y & M count (log CFU /g) of mozzarella cheese samples increased from 2.51 to 5.20 and 5.46 respectively, in P_1 and P_2 after 3 weeks of storage at 7±1 ^{0}C. However, the count decreased to 4.84 and 4.92 (log CFU / g) in respect of these two packages after 5 weeks of storage (atm 1). Slightly more increase in Y& M count was noted in P_2 compared to P_1 (statistically not significant). In case of samples stored for 12 weeks the data revealed that the Y& M counts were minimum (0.35: P_1; 0.39 P_2) for samples packed under atm 3 followed by atm 5 (1.03 P_1; 1.15 P_2); atm 4 (1.86 P_1; 1.91 P_2) and atm 2 (2.58: P_1; 2.62: P_2) respectively, in ascending order. This trend confirms the findings of other workers (Alves *et al.*, 1996: Fedio *et al.*, 1994: Eliot *et al.*, 1998). However, Alves *et al.* (1996) reported that with regard to the proliferation of yeast and moulds, only the development of yeasts was detected in the sliced mozzarella cheese packed in air and under atmosphere of 100 % N_2 and 50 % CO_2 / 50 % N_2. No growth of yeast and moulds was detected in the mozzarella cheese stored under 100 % CO_2 during 58 days at 7 ± 1°C, and the growth of yeasts in the mozzarella cheese in air and under 100 % N_2 was observed after the 8th storage day. From the 14th day, the counts of yeasts stabilized at 8.4 log CFU/g for the product in air. The cheese under 100% N_2 presented lower counts of yeasts than cheese stored in air throughout the study. Our results in general agree with the findings of Alves *et al.* (1996) who observed that the population of yeasts in the cheese under 50% CO_2 and 50% N_2 showed some growth. The growth in the product under 50% CO_2 / 50% N_2 occurred at a slower speed than the growth in the product in air and under 100% N_2. That could also be credited to the action of CO_2, i.e. what really inhibited the growth of yeasts was the presence of CO_2. In the product under 50% CO_2 / 50% N_2, the effect of the CO_2 was smaller than in the product under 100% CO_2, because there were 2 times less CO_2 per kg of the product. But our findings do not match with the observations of Eliot *et al.* (1998) that the growth of yeasts and mould in shredded mozzarella cheese samples was completely inhibited under modified atmospheres containing CO_2, except the findings that concentrations of CO_2 ($\geq$ 50 % CO_2) maintained yeast counts $\leq$ 1 log CFU / g and were more efficient than vacuum to inhibit yeast growth. The decrease in yeast counts in atmospheres containing high level of CO_2 suggested a destructive effect of CO_2. Final counts on week 12 were lower than initial counts in most cases, and equal to initial counts in the others (50 / 50 CO_2 / N_2 for yeast). Moulds were not detected in the majority of samples containing CO_2 (10-100 CO_2). The inhibitory properties of CO_2 have also been demonstrated by others (Kosikowski and Brown, 1973; Chen & Hotchkiss, 1991; Rosenthal *et al.,* 1991; Day, 1992; Fedio *et al.,* 1994; Alves *et al.,* 1996),i.e. CO_2 has fungicidal effect ; and in all probability this might have resulted in minimum Y& M count in our samples packed under atm 3 (100% CO_2) and maximum in samples packed under atm 2 (vacuum), which had neither CO_2 nor N_2.

In respect of the packages stored at -10 to - 15 ^{0}C for 12 months, the initial Y & M count (2.51 log CFU / g) decreased in all the samples irrespective of the atmosphere (Fig. 32). The decrease had been maximum in case of product packed under 100% CO_2 (atm 3) followed by 50 % CO_2 / 50 % N_2 (atm 5), 100 % N_2 (atm 4), Vacuum (atm 2) and air (atm 1) respectively, in descending order (Appendix XXXII).

Analysis of variance for the data on Y& M counts indicated that the intervals of storage, two types of packages, interactions atmospheres x packages, and intervals x atmospheres, each individually, were highly significant under both the storage conditions (P<0.01). Other studied interactions were not significant for any of the two storage conditions.

5.2.5.3 Psychrotrophic count

The changes in psychrotrophic count of mozzarella cheese samples packaged in two different materials under modified atmospheres, and stored at 7±1 ^{0}C and -10 to -15 ^{0}C are depicted in Figures 33, 34 and Appendices XXXIII & XXXIV. The analysis of variance of the data is given in Table 9.

The psychrotrophic count of mozzarella cheese exhibited increasing trend throughout the entire period of storage in both the packaging materials, under all the atmospheres at 7± 1^{0}C (Fig.33). The initial psychrotrophic count 4.23 (log cfu /g) increased to 8.88 and 8.99 respectively, in P_1 and P_2 after 5 weeks of storage. At the end of 12 weeks, the samples packed under atm 3 (100% CO_2) (P_1: 6.90; P_2: 6.92) showed minimum increase in psychrotrophs followed by atm 5 (50% CO_2 / 50% N_2) (P_1: 7.35; P_2 7.48), atm 4 (100 % N_2) (P_1: 7.52; P_2 7.75) and atm 2 (vacuum) (P_1: 8.05; P_2 8.28) respectively, revealing that the growth was retarded when the atmosphere inside the package contained higher concentration of CO_2.

The work of Alves *et al.* (1996) on sliced mozzarella cheese in MAP showed that the counts of aerobic psychrotrophs in the mozzarella cheese in air and under 100% N_2 increased. Then the counts stabilized at levels of 8.3 log CFU / g and 7.5 log CFU / g, respectively. In the product under 50% CO_2/50% N_2 the growth of aerobic psychrotrophs occurred from the 9th to 25th storage day, when they stabilized around 7.1 log CFU / g. The results revealed that the beginning of microbiological growth was retarded when the atmosphere inside the package contained higher concentration of CO_2. The experiments of Eliot *et al.* (1998) on shredded mozzarella cheese under modified atmosphere indicated that the initial psychrotrophic count (4.36 log CFU/ g) reached 7 log CFU/ g after 3 weeks and then stabilized around 7.2 log CFU/g.

The observations (Appendix XXXIV) pertaining to deep freeze storage of MAP mozzarella cheese revealed that the counts decreased in all the samples irrespective of the type of atmosphere inside the package, but the maximum decrease in count was observed in samples packed under 100 %

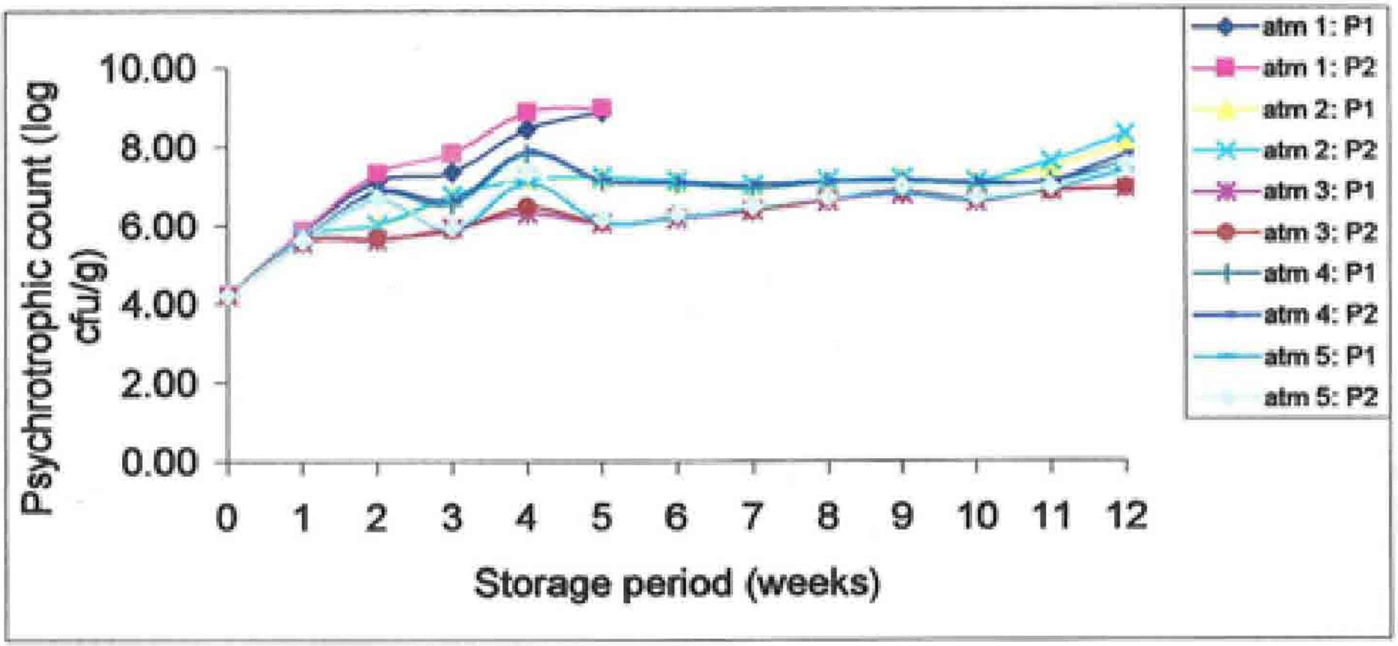


Fig. 33. Effect of MAP on Psychrotrophic count of mozzarella cheese stored in different packages at 7±1 ^{0}C

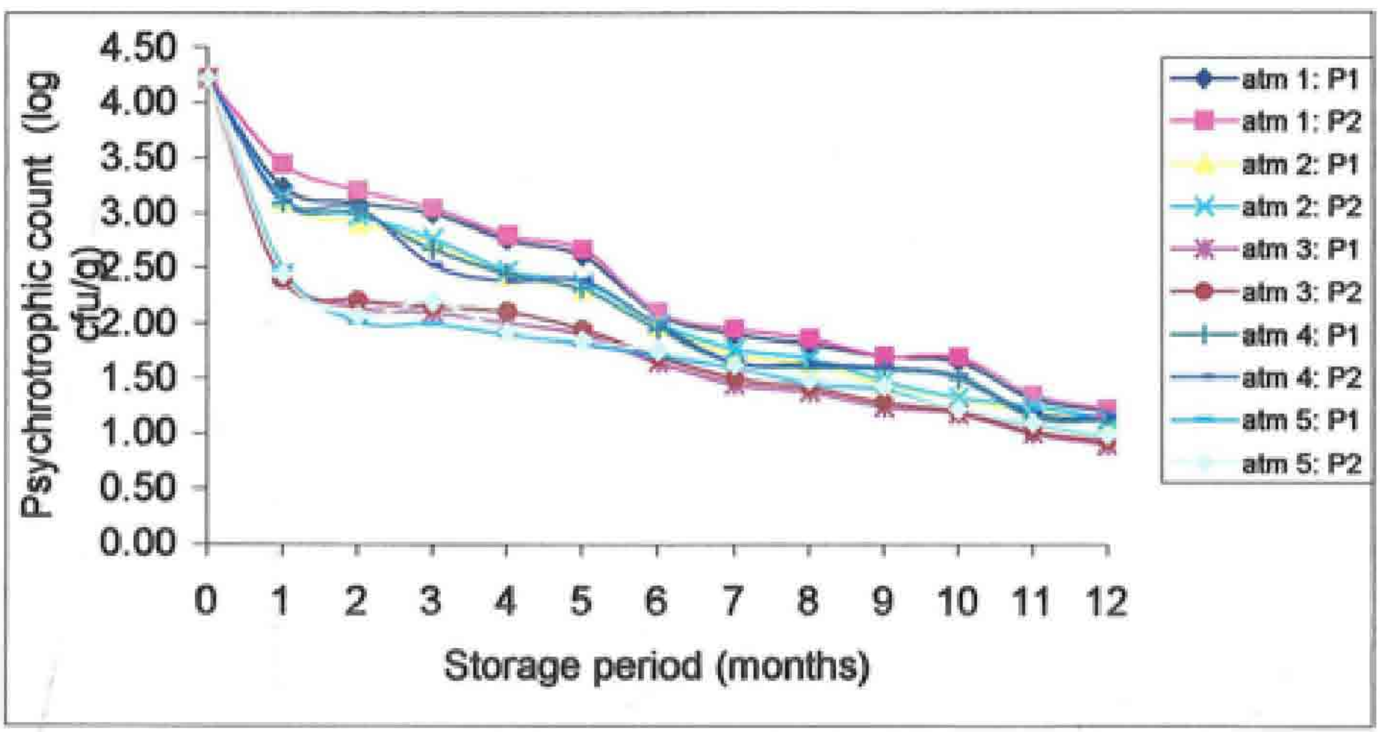


Fig. 34. Effect of MAP on Psychrotrophic count of mozzarella cheese stored in different packages at -10 to -15 ^{0}C

CO_2 (atm 3; P_1: 0.90, P_2: 0.93) followed by 50% CO_2 / 50% (atm 5; P_1: 0.98; P_2: 0.99), N_2 (atm 4; P_1: 1.12; P_2: 1.15), vacuum (atm 2; P_1: 1.11; P_2: 1.13) and air (atm 1; P_1: 1.20; P_2: 1.22), respectively. The decrease in counts in all the cases was most likely due to deep freeze conditions.

Anova of the data on psychrotrophic counts revealed highly significant ($P<0.01$) differences among the intervals of storage, amongst atmospheres and interaction atmospheres x packages for both the storage conditions. Interaction intervals x atmospheres was significant ($P<0.01$) for refrigeration storage conditions only. The difference due to type of package and other studied interactions were found to be not significant for any of the two storage conditions (Table 9).

5.2.5.4 Coliform count

In Figures 35, 36 and Appendices XXXV & XXXVI are given the values for coliform count of MAP mozzarella cheese stored in two types of packaging materials at 7± 1°C and -10 to -15 °C for indicated periods. Analysis of variance is presented in table 9. The initial coliform count of 2.62 (log CFU / g) in air packed (atm 1) mozzarella cheese samples increased to 2.98 and 3.01 (log CFU/ g) respectively, in P_1 & P_2 after 5 weeks of storage at 7 ±1 °C. More increase in coliform count was noted in P_2 compared to P_1. Asperger and Brandl (1982) also observed that when mozzarella cheese was stored at 20 °C for 24 h, the coliform increased from 0 to 10^6 CFU / g. The mozzarella cheese stored for 12 weeks at refrigeration temperature revealed that the coliform count had been minimum in atm 3 (0.45, P_2; 0.63 P_1) followed by atm 5 (0.89, P_2; 0.73 ,P_1), atm 4 (1.88, P_2; 1.84, P_1) and atm 2 (1.86, P_2; 1.70, P_1) respectively, in ascending order. The coliform count was found to decrease in all the packages with the increase of storage periods irrespective of the atmosphere inside the package (Fig. 35). The decrease in coliform count was most likely due to bacteria killed by membrane destructive plamolysis (Rosenthal *et al.*, 1991).

The observations (Appendix XXXVI) revealed that the type of atmospheres played a very significant role in controlling the coliform count.

100% CO_2 was found to be most effective. The initial coliform count (2.62 log CFU/g) decreased maximum in case of atm 3 (P_1: 0.35; P_2: 0.38) followed by atm 5 (P_1: 0.40; P_2: 0.46), atm 4 (P_1: 0.52; P_2: 0.56), atm 2 (P_1: 0.63; P_2: 0.68) and atm1 (P_1: 0.76; P_2: 0.79), in ascending order. The possible reasons for such decline might be the effect of CO_2 by alteration of permeability of cell membrane and enzymatic reaction pathway (Enfors and Molin, 1981; King and Nagel, 1975).

There were found to be highly significant ($P<0.01$) differences in the coliform count due to intervals of storage and interaction intervals x package under both the storage conditions. The influence of atmospheres on coliform count was more significant ($P<0.01$) at deep freeze condition. The effect of type of package and other studied interactions were not significant for any of the two storage conditions (Table 9).

5.2.6.0 Sensory changes

5.2.6.1 Appearance

The data pertaining to the appearance of MAP mozzarella cheese samples packed under modified atmospheres and stored in different packages at 7 ± 1^0C and -10 to -15 ^{0}C are depicted in Appendices in XXXVII & XXXVIII, and illustrated in Figures 37 and 38. The statistical analysis of the data on appearance is presented as part of Table 10. The studies revealed that the initial appearance score of 3.0 in air packed (atm1) mozzarella cheese samples decreased to 1.9 and 1.7 respectively, in case of P_1 & P_2 after 5 weeks of storage at 7 $\pm$1 ^{0}C (Fig. 37). Slightly more decrease in appearance score was noted (statistically not significant) in P_2 compared to P_1. The mozzarella cheese packed under vacuum (atm 2) showed that the initial appearance score decreased to 1.7 and 1.6 in P_1 & P_2, respectively, after 12 weeks of storage. The value for appearance score of samples in the packages with 100% CO_2 (atm 3), 50 % CO_2 / 50 % N_2 (atm 5) and 100% N_2 (atm 4) decreased to 2.0 (P_1), 1.9 (P_2); 1.9 (P_1), 1.8 (P_2); 1.7 (P_1) and 1.6 (P_2), respectively indicating that the minimum decrease in the appearance score

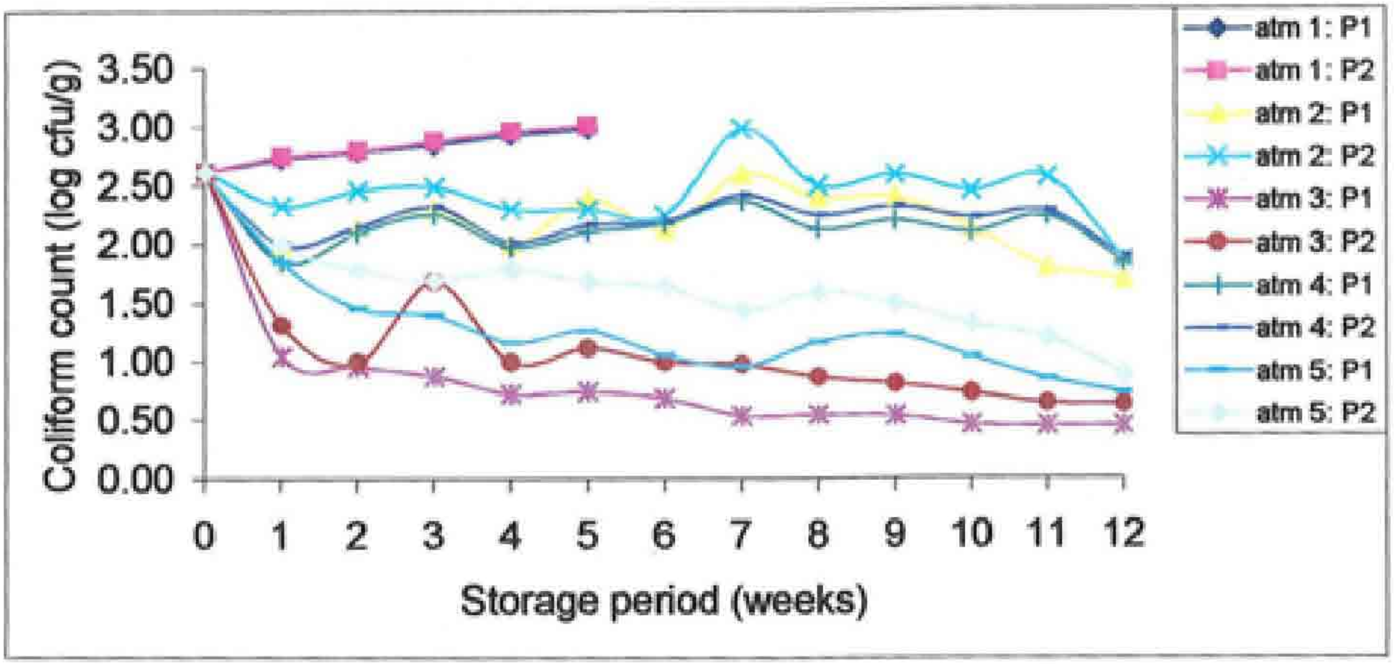


Fig. 35. Effect of MAP on Coliform count of mozzarella cheese stored in different packages at 7±1 ^{0}C

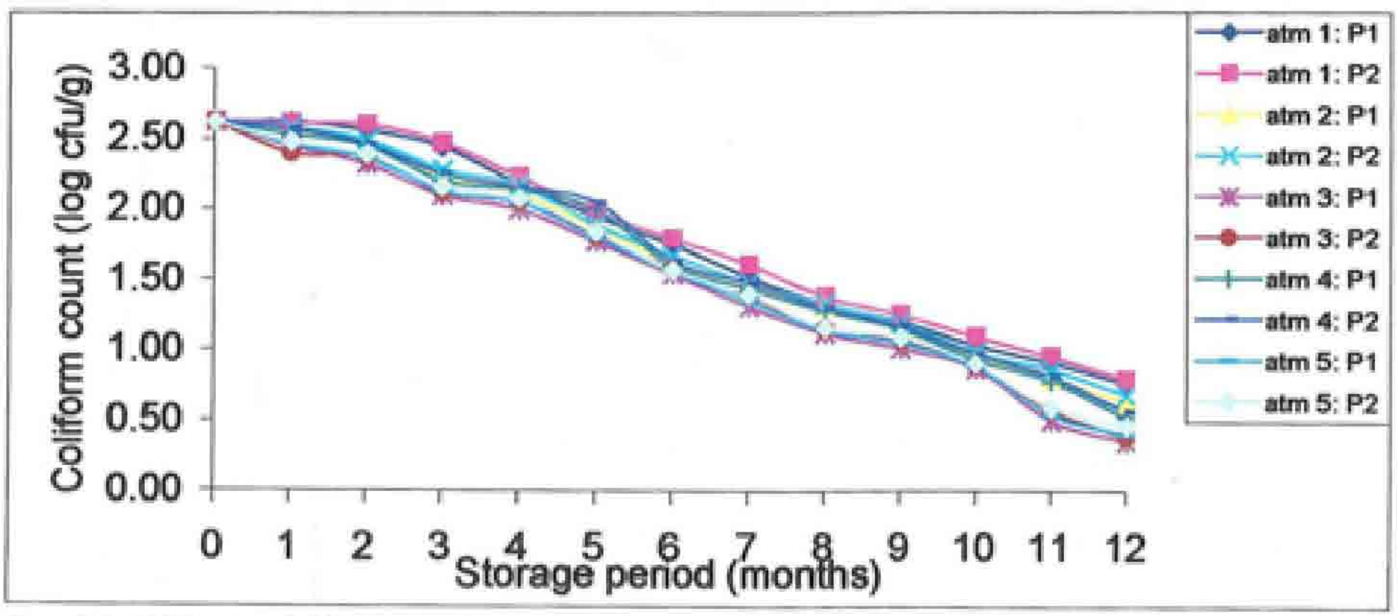


Fig. 36. Effect of MAP on Coliform count of mozzarella cheese stored in different packages at –10 to -15 ^{0}C

Table 10. **Analysis of variance for Sensory characteristics of MAP Mozzarella cheese stored in different packages at 7± 1 ^{0}C & -10 to -15 ^{0}C**

Source of variation	d.f.	Mean sum of square							
		Appearance		Body & Texture		Flavour		Overall acceptability	
		7±1^{0}C	-10 to -15 ^{0}C	7±1^{0}C	-10 to -15 ^{0}C	7± 1^{0}C	-10 to - 15 ^{0}C	7±1^{0}C	-10 to -15 ^{0}C
Among intervals of storage	12	9.741**	4.811**	30.430**	17.865**	76.833**	29.574**	325.903**	126.909**
Among atmospheres	4	3.740**	0.301**	9.670**	0.074**	50.169**	2.407**	147.099**	4.816**
Between packages	1	0.108	0.072*	0.532	0.112*	0.724	0.785*	2.593	2.448*
Interaction intervals x atmospheres	48	0.201	0.008	0.346	0.008	1.878	0.046	5.505	0.072
Interaction intervals x packages	12	0.003	0.002	0.092	0.004	0.018	0.011	0.050	0.027
Interaction atmospheres x packages	4	6.230**	0.491**	18.014**	0.121**	82.240**	3.875**	242.907**	8.156**
Interaction intervals x atmospheres x packages	48	0.336**	0.014	0.579	0.014	3.146	0.074	9.187	0.120
Error	260	0.297	0.023	0.702	0.018	3.539	0.165	10.308	0.386

*** Significant at 5 % level of probability**
**** Significant at 1 % level of probability**

had been with the samples packed under atm 3 (P_1: 2.0; P_2: 1.9) followed by atm 5 (P_1: 1.8; P_2: 1.7) and atm 4 (P_1: 1.7; P_2: 1.6) respectively, in ascending order (Appendix XXXVII). No visible mould growth was observed in any of the samples during entire storage. The results are contrary to the findings of Ghosh (1987) who reported the mould growth after 21 to 30 days in vacuum-packed samples. The decrease in appearance score was mainly due to dull yellowish colour on the surface of the samples. The results in general are similar to the findings of Asperger (1982) and Matteo *et al.* (1982) who reported the consistent decrease in appearance score of cheese during storage.

After 12 months of storage at -10 to -15 ^{0}C, the initial appearance score of 3.0 decreased to 1.5, in both the packages P_1 and P_2 (atm 1), while the appearance score decreased to 1.9 and 1.7 respectively, in P_1 & P_2 (atm 2). However, for the product packaged under atm 3, atm 5 and atm 4, the appearance score respectively decreased to 2.2 (P_1), 2.1(P_2); 2.0 (P_1), 1.9 (P_2); and 1.8 (P_1), 1.7(P_2), showing consistent gradual decrease in appearance score during storage in all the packages under different atmospheres (Fig. 38). No visible mould growth was observed in any of the samples upto 12 months. However, samples packed under air (atm 1) and vacuum (atm 2) showed bleached white patches on the surface of cheese samples with the increase in storage period. Ghosh (1987) also reported similar observations with respect to mozzarella cheese stored under deep freeze conditions. Appearance-wise, the samples under 100% CO_2, and 50 % CO_2 / 50% N_2 were acceptable upto 12 months.

The 5 types of modified atmospheres, intervals of storage, and interaction atmospheres x packages, each individually score, were found to be highly significant (P<0.01) from the consideration of appearance score of mozzarella cheese under both the storage conditions. The effect of types of package was significant (P< 0.01) for -10-15 ^{0}C storage condition only. Interaction intervals x atmospheres x packages was significant (P<0.01) for refrigeration storage only. Interactions intervals x atmospheres, and intervals

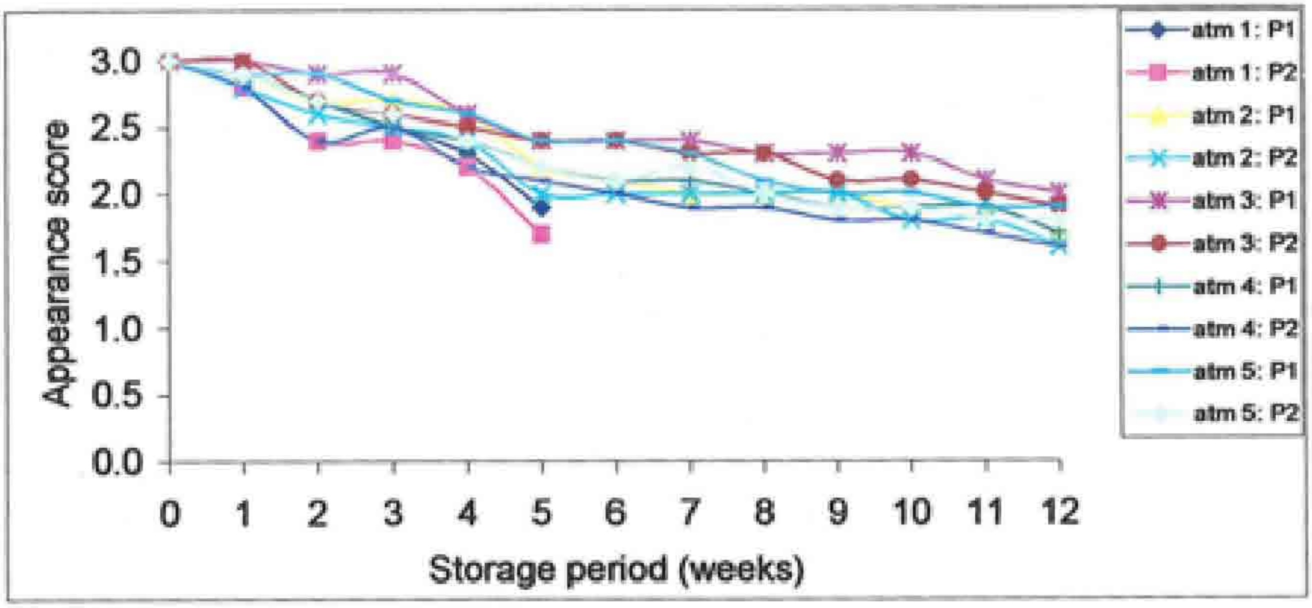


Fig. 37. Effect of MAP on Appearance of mozzarella cheese stored in different packages at 7±1 ^{0}C

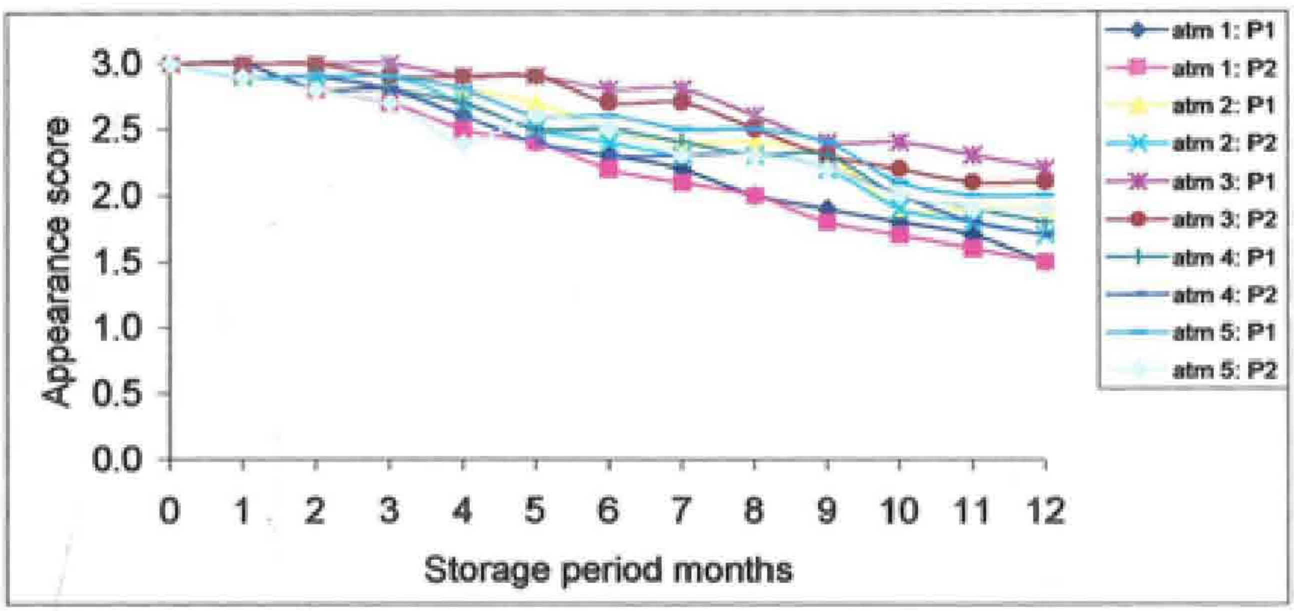


Fig. 38. Effect of MAP on Appearance of mozzarella cheese stored in different packages at –10 to -15 ^{0}C

x packages were not significant under any of the two storage conditions studied.

5.2.6.2 Body & Texture

The changes in body & texture of mozzarella cheese samples packaged in two different materials under modified atmospheres, and stored at 7±1 ^{0}C and -10 to -15 ^{0}C are illustrated in Figures 39, 40 and Appendices XXXIX & XL. The analysis of variance of the data is given in Table 10.

The initial mean body & texture value of 4.9 of fresh mozzarella cheese samples decreased to 2.8 in case of atm 1 (air) and 2.6, in P_1 and P_2, respectively at the end of 5 weeks of storage at 7±1 ^{0}C, and the samples were observed to be slightly pasty. After 12 weeks, the values were found to be P_1: 2.6; P_2: 2.5 (atm 2); P_1: 2.7; P_2: 2.6 (atm 3); P_1: 2.6; P_2: 2.5 (atm 4); and P_1: 2.5, 2.2 P_2 (atm 5). The results revealed (Fig. 39) that the changes in body & texture in samples packaged under atm 3 followed by atm 5, atm 4 and atm 2 respectively were in ascending order. The body and texture of mozzarella cheese samples packed under atm 2 (vacuum) was noted to be more weak and softer compared to samples packed under 100 % CO_2, 50 % CO_2 / 50% N_2 and 100% N_2. These changes were most likely due to varied degree of proteolysis of samples during storage (Table 9). From the viewpoint of body & texture, it was acceptable upto 4 weeks (atm 1), 7 weeks (atm 2), 10 weeks (atm 3), 7 weeks (atm 4) and 9 weeks (atm 5), revealing that CO_2 played a significant role towards body and texture characteristic of stored mozzarella cheese samples.

On storage under -10 to - 15 ^{0} C for 12 months, the maximum body & texture score of mozzarella cheese was observed in samples of atm 3 followed by atm 5, atm 4, atm 2 and atm 1, in descending order (Fig.40) suggesting that 100% CO_2 and 50% CO_2 / 50% N_2 had played a vital role in checking the reduction of body & texture score of the product compared to the other 3 studied atmospheres, and the samples were acceptable to the panellist upto 12 months with respect to body & texture irrespective of the type of package and atmosphere (Appendix XXXIX). The results in general are similar to the findings of Ghosh (1987) who also observed that values for body & texture decreased significantly with time.

Statistically, the influence of intervals of storage, types of atmospheres and interaction atmospheres x packages, each individually, were highly significant ($P<0.01$) for the changes in body and texture of mozzarella cheese samples, while the effect of type of package was significant ($P<0.05$) for deep freeze storage only (Table 10). Other studied interactions were found to be not significant for any of the two storage conditions.

5.2.6.3 Flavour

Data on the flavour of mozzarella cheese samples packed in 5 modified atmospheres and stored in different packages at 7±1 ^{0}C and -10 to -15 ^{0}C are given in Appendices XLI & XLII, and illustrated in Figures 41 and 42. The statistical analysis of the data on flavour is presented in Table 10. The results revealed decreasing trend in the values for flavour of mozzarella cheese during storage (Fig. 41). The initial value of 9.7 for flavour of mozzarella cheese decreased to 5.6 and 5.4 respectively, in P_1 & P_2 (atm 1) after 5 weeks of storage at 7 ±1 ^{0}C. More decrease in flavour score was noted in P_2 compared to P_1. In case of mozzarella cheese packed under vacuum (atm 2), the initial flavour score decreased to 5.4 and 5.2, respectively in P_1 & P_2, .

The flavour of mozzarella cheese samples in the packages with 100% CO_2 (atm 3), 50 % CO_2 / 50 % N_2 (atm 5) and 100% N_2 (atm 4), respectively decreased to 7.2 (P_1), 6.6 (P_2); 6.6 (P_1) and 6.3 (P_2) and 6.0 (P_1), 5.8 (P_2); indicating that the highest score for flavour had been with the samples packed in P_1 (atm 3), and minimum for P_2 (atm 2) establishing a very significant influence of MAP on the flavour characteristic of mozzarella cheese during storage. The results are in agreement with the findings of Alves *et al.* (1996) and Maniar *et al.* (1994) who also observed less development of off-flavour in samples under 100% CO_2 than 50% / CO_2 / 50 % N_2.

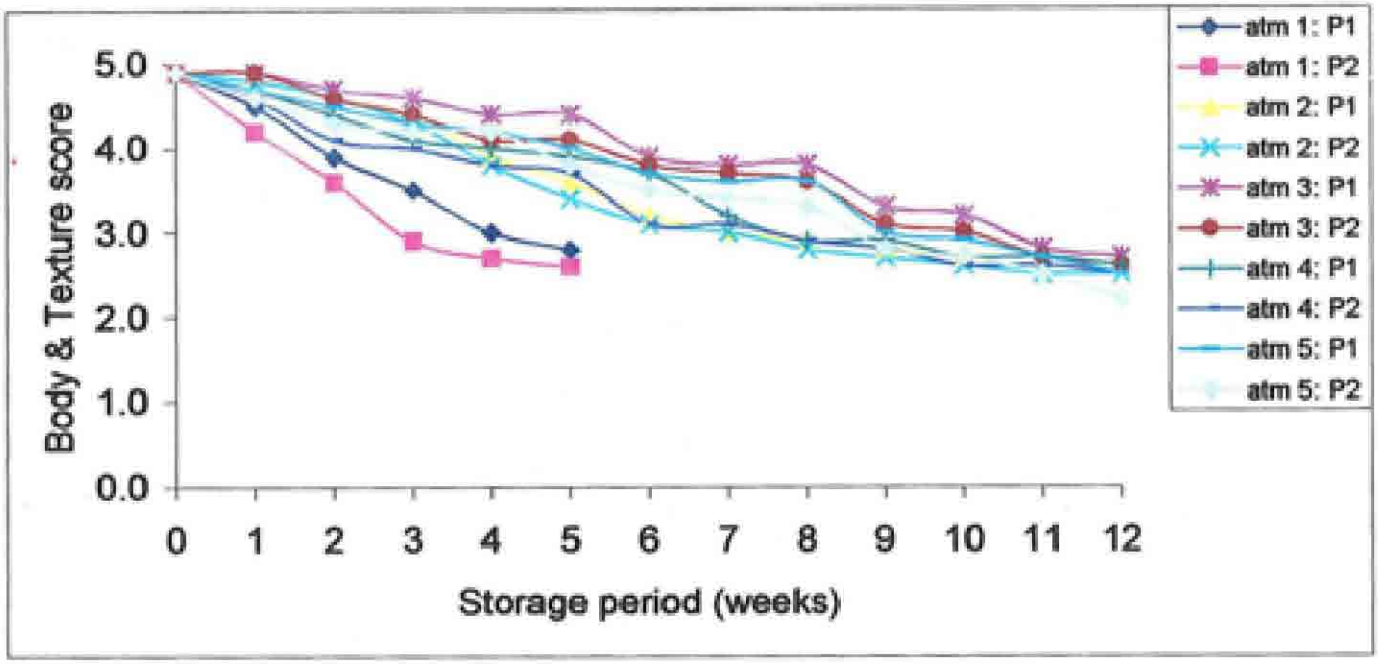


Fig. 39. Effect of MAP on Body & Texture of mozzarella cheese stored in different packages at 7±1 ^{0}C

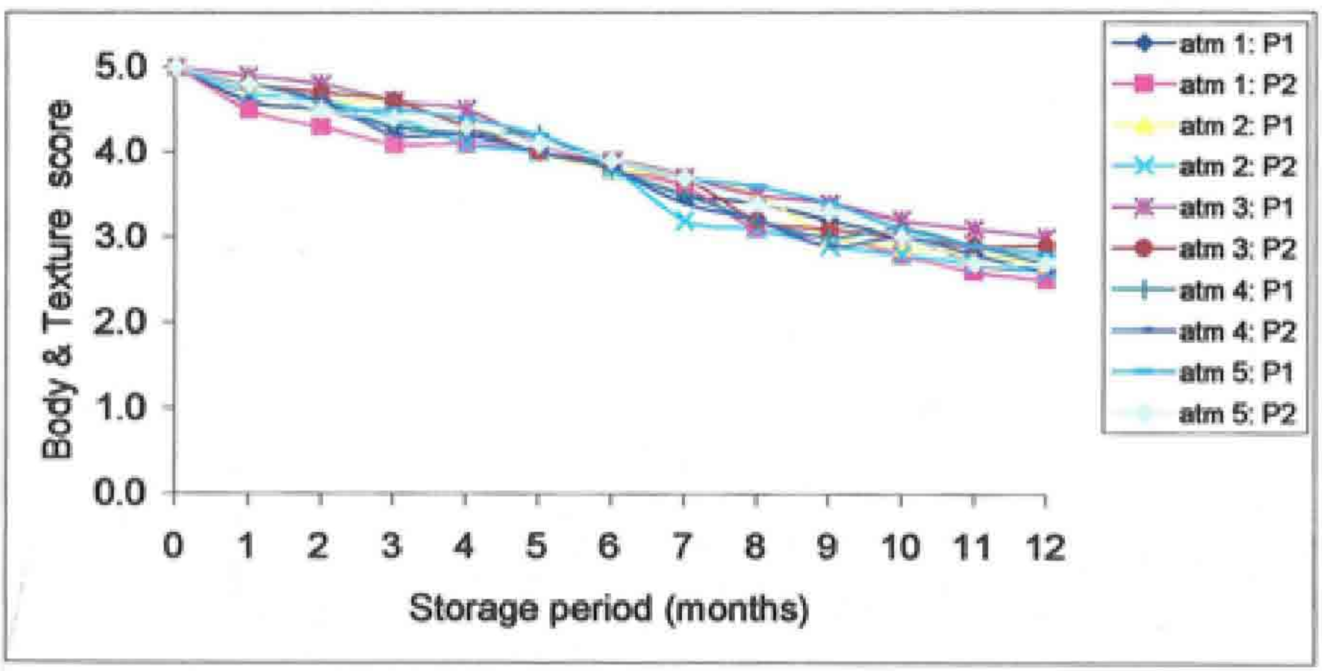


Fig. 40. Effect of MAP on Body & Texture of mozzarella cheese stored in different packages at –10 to -15 ^{0}C

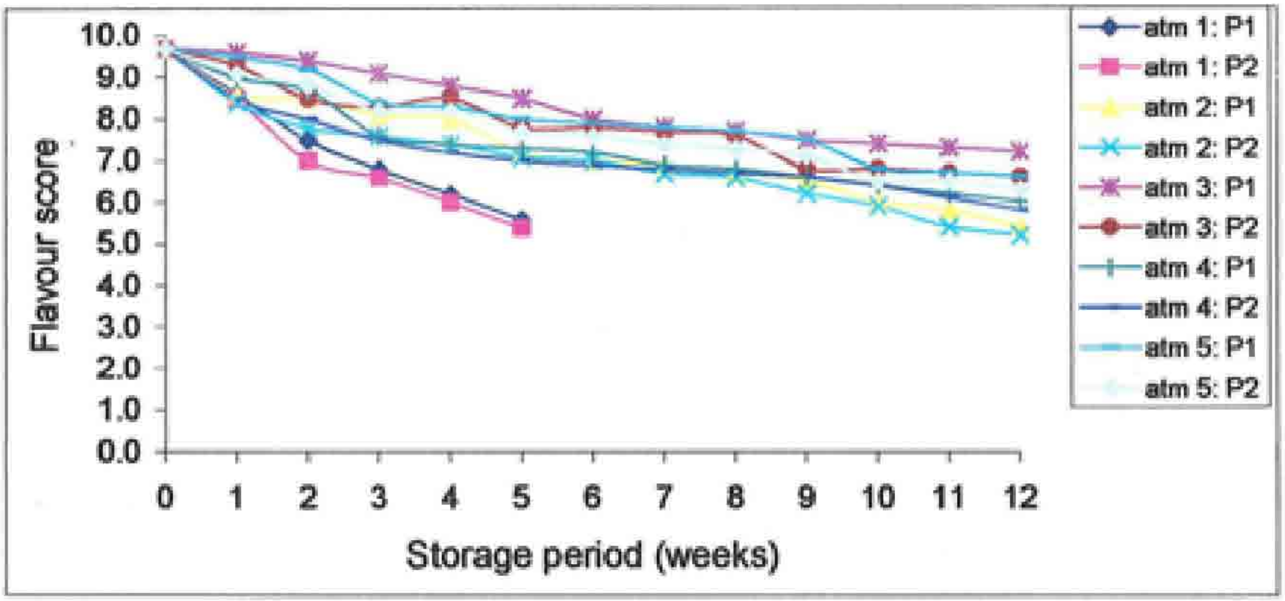


Fig. 41. Effect of MAP on flavour of mozzarella cheese stored in different packages at 7±1 ^{0}C

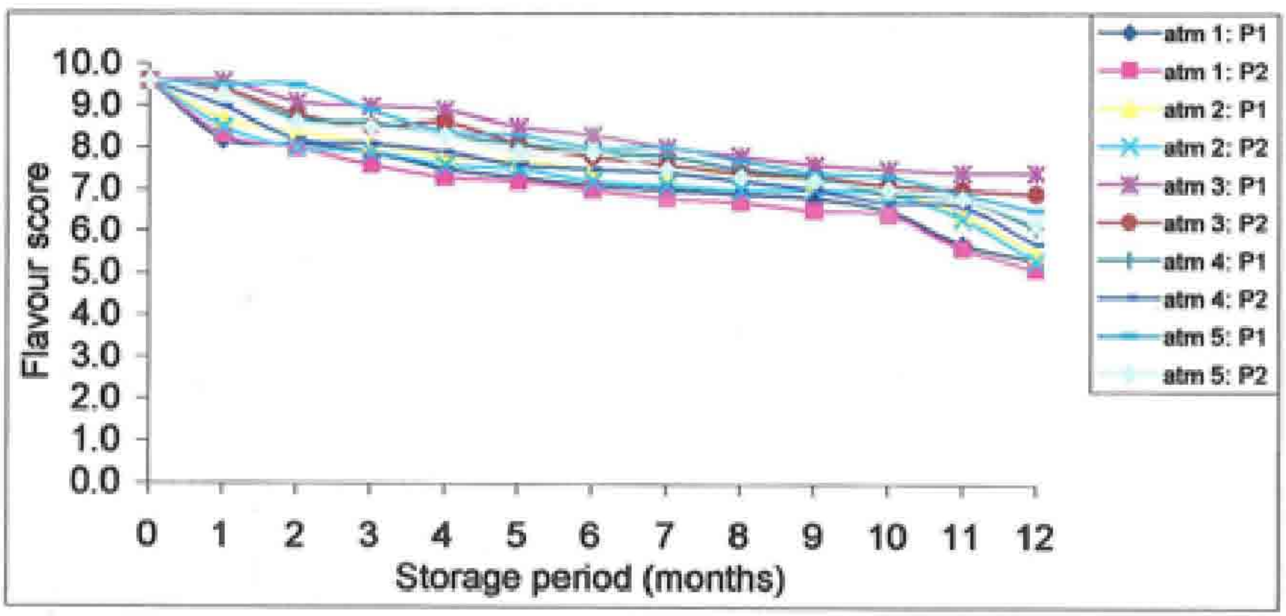


Fig. 42. Effect of MAP on flavour of mozzarella cheese stored in different packages at -10 to -15 ^{0}C

The observations on the flavour of MAP mozzarella cheese stored at deep freeze conditions (-10 to –15 ^{0}C) showed that after 12 months of storage, the initial flavour value of 9.7 decreased to 5.3 and 5.1 respectively, in packages P_1 and P_2 (atm 1), while the flavour score decreased to 5.5 and 5.3 respectively, in P_1 & P_2 (atm 2). However, for the product packaged under atm 3, atm 5 and atm 4, the flavour score decreased to 7.4 (P_1), 6.9 (P_2); 6.5 (P_1), 6.2 (P_2) and 6.1 (P_1), 5.7 (P_2), respectively (Appendix XLII) suggesting that CO_2 and combination of CO_2 & N_2 had been very effective in checking the deterioration of flavour in mozzarella cheese samples during storage. The similar observations were noted by Alves *et al.* (1996) and Maniar *et al.* (1994).

Anova of the data on flavour of stored mozzarella cheese samples revealed (Table 10) that intervals of storage, types of atmospheres and interaction atmospheres x packages were highly significant ($P< 0.01$) with regard to changes in flavour characteristics for both the storage conditions. The influence of types of packages was significant ($P<0.05$) for -10 to -15 ^{0}C storage only. Other studied interactions were found to be not significant under any of the studied storage conditions.

5.2.6.4 Overall acceptability

The changes in the values for overall acceptability of mozzarella cheese samples packaged in two different materials under modified atmospheres, and stored at 7±1 ^{0}C and -10 to -15 ^{0}C are illustrated in Figures 43, 44 and Appendices XLIII & XLIV. The analysis of variance of the data is given in Table 10.

The overall acceptability of mozzarella cheese exhibited a decreasing trend throughout the storage period at 7± 1^{0}C (Fig. 43) in both the packaging materials under all atmospheres. The initial overall acceptability score 17.6 (atm 1) decreased to 10.3 and 9.7 respectively, in P_1 and P_2 after 5 weeks of storage. Appendix XLIII indicates that at the end of 12 weeks of storage, the overall acceptability decreased consistently for the samples packed under atm 3 (100% CO_2) (P_1 : 10.7; P_2 : 9.8) followed by atm 5 (50% CO_2 / 50% N_2)

(P_1: 10.4; P_2 9.8), atm 4 (100 % N_2) (P_1: 9.3; P_2 9.2) and atm 2 (vacuum) (P_1: 8.05; P_2 8.28) respectively. The overall acceptability of the stored mozzarella cheese samples depends upon several factors like degree of proteolysis, lipolysis, flavour changes and microbial activity. The overall acceptability was found to be better for the samples in which the proteolysis, lipolysis, flavour changes and microbial growth had been lesser (atm 3> atm 5> atm4 > atm 2).

The storage of mozzarella cheese at -10 to -15 ^{0}C for 12 months resulted in decrease of acceptability score for the samples packaged under 5 atmospheres in two types of packages (Fig. 44). The observations (Appendix XLIV) revealed that the type of atmospheres played a very significant role in affecting the overall acceptability of the product. The cheese samples packaged under 100% CO_2 (atm 3) were rated best for overall acceptability, followed by samples of atm 5, atm 4, atm 2 and atm 1 respectively, in descending order. The results are in accordance with the findings of Manair *et al.* (1994) and Alves *et al.* (1996) who also observed that 100% CO_2 atmospheres best maintained the sensorial characteristics.

Anova of the data on overall acceptability (Table 10) revealed that intervals of storage, types of atmospheres, and interaction atmospheres x packages contributed significantly ($P<0.01$) towards the changes in overall acceptability during storage under both the conditions, whereas the effect of type of package was significant (P< 0.0.5) for deep freeze condition only. Other studied interactions were, however, not significant under any of the storage conditions.

5.2.7.0 Shelf life of MAP Mozzarella cheese

In order to determine the shelf-life of MAP mozzarella cheese, the samples were submitted to 5 types of atmospheres (air, vacuum, 100% CO_2, 100% N_2 , and 50% CO_2 / 50% N_2) and stored for various time intervals at 7 $\pm$ 1°C and –10 to –15 ° C. The data obtained for the overall acceptability were used to determine the product's shelf-life. The shelf life of mozzarella cheese significantly increased upto 12 weeks (a 300 % increase) for the product

PLATE 8 : Vacuum packed mozzarella cheese

PLATE 9 : MAP mozzarella cheese ($100\ \%\ CO_2$)

PLATE 10 : MAP mozzarella cheese ($100\%\ N_2$)

PLATE 11 : MAP mozzarella cheese ($50\%\ CO_2$ / $50\%\ N_2$)

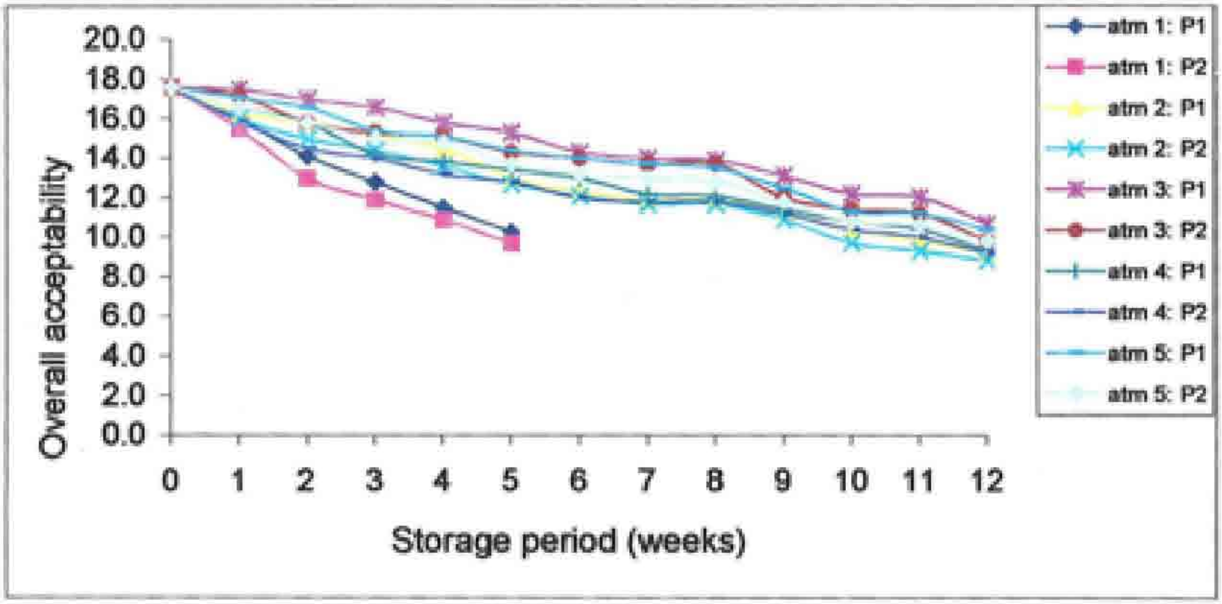


Fig. 43. Effect of MAP on overall acceptability of mozzarella cheese stored in different packages at 7±1 ^{0}C

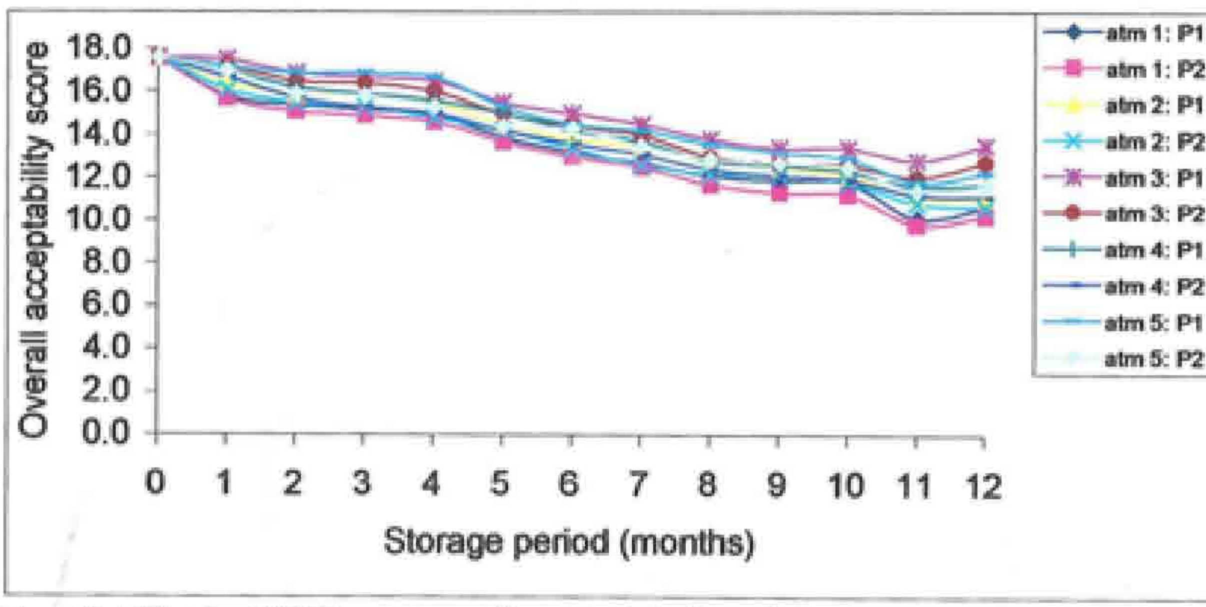


Fig. 44. Effect of MAP on overall acceptability of mozzarella cheese stored in different packages at -10 to -15 ^{0}C

packaged under 100 % CO_2 in case of P_1 , and 11 weeks(a 275 % increase) in case of P_2. Under 50 % CO_2 / 50 % for 11 weeks (a 275 % increase) in case of P_1,and 10 weeks(a 250 % increase) in case of P_2. Under 100% N_2, 250 % increase in shelf life was achieved in case of P_1, and 9 weeks (a 225% increase) in case of P_2. For the product packaged under vacuum, the shelf life was found to be 9 weeks (a 225 % increase) compared to conventional air pack (4weeks).

Alves *et al.* (1996) reported a significant increase in the shelf life upto 63 days (a 385 % increase) for sliced mozzarella cheese under 100 % CO_2, and upto 45 days (a 246 % increase) for product under 50 % CO_2 / 50 % N_2, compared to conventional air pack (13 days). Similar results were attained by Sarantopoulos (1993) for round buffalo milk mozzarella cheese, but with a shelf life increase of 240 %. Hampton (1982) could increase the shelf life of cheese (packed in PP film) upto 4 weeks using MAP, compared with only 14-15 days when packaged under normal conditions.

The shelf life of MAP mozzarella cheese packaged in two types of materials under 5 different atmospheres and stored at deep freeze conditions, increased upto 12 months (a 120 % increase) in all the studied MAP conditions irrespective of the packaging material, compared to conventional air pack (10 months).

5.2.8.0 Sensory Evaluation of pizza made from MAP Mozzarella cheese

The data pertaining to the pizza made from MAP mozzarella cheese (packed under five modified atmospheres and stored in different packages at 7 ± 1^0C and -10 to -15 ^0C) are recorded in Appendices in XLV, XLVI and illustrated in Figures 45 and 46. The statistical analysis of the data on pizza is presented in Table 11. The studies revealed that the initial sensory score (5.0) of pizza made from air packed (atm 1) stored (P_1 & P_2 after 5 weeks at 7 ± 1 ^0C) mozzarella cheese decreased to 2.2 and 1.8 respectively, Slightly more decrease in pizza score was noted in P_2 compared to P_1. When the stored (12 weeks) mozzarella cheese packed under vacuum (atm 2) was used for pizza making, the initial pizza score decreased to 2.5 and 2.3 for P_1 & P_2,

Table 11. Analysis of variance for Sensory characteristics of Pizza made from MAP mozzarella cheese stored in different packages at 7± 1 ^{0}C & -10 to -15 ^{0}C

Source of variation	d.f.	Mean sum of square	
		Pizza	
		7± 1 ^{0}C	-10 to -15 ^{0}C
Among intervals of storage	12	24.725**	13.056**
Among atmospheres	4	15.362**	0.181**
Between packages	1	0.172	0.236*
Interaction Intervals x atmospheres	48	0.646	0.016
Interaction intervals x packages	12	0.003	0.006
Interaction atmospheres x packages	4	24.551**	0.363**
Interaction intervals x atmospheres x packages	48	1.059**	0.031
Error	260	0.999	0.037

*** Significant at 5 % level of probability**
**** Significant at 1 % level of probability**

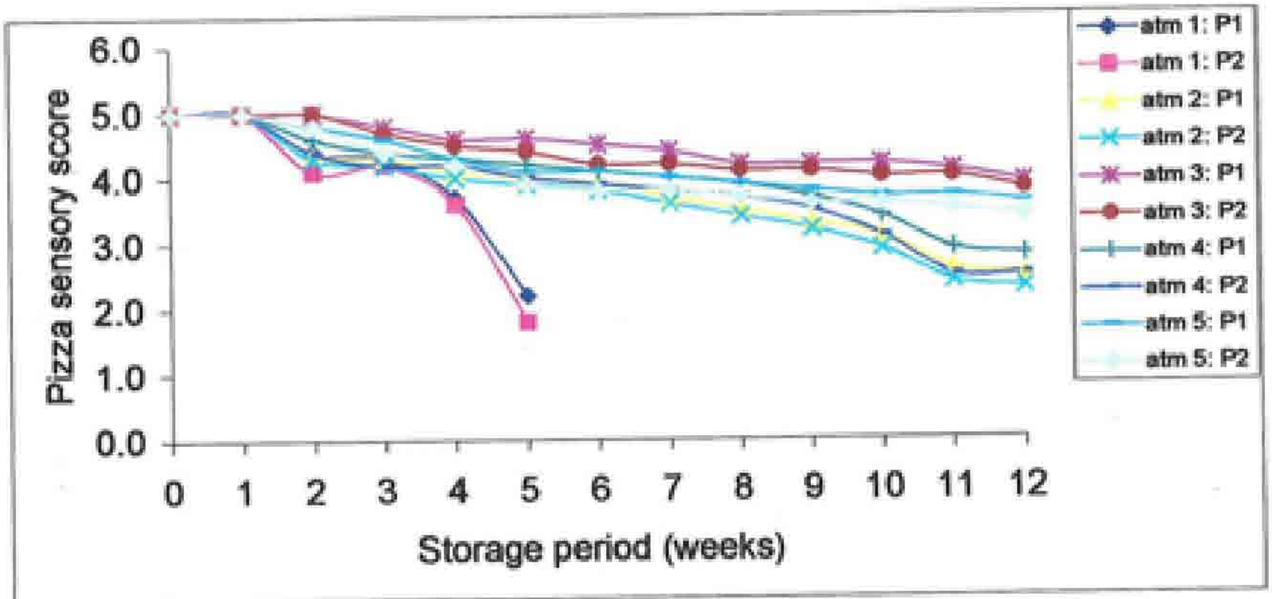


Fig. 45. Effect of MAP on sensory score of pizza made from mozzarella cheese stored in different packages at 7±1 °C

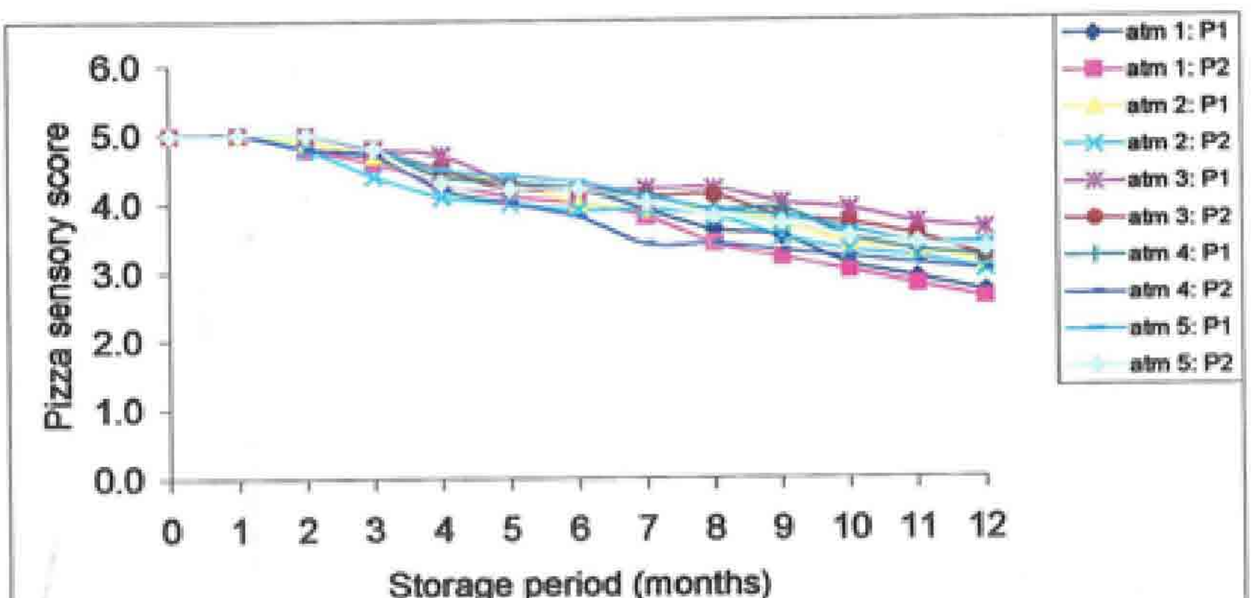


Fig. 46. Effect of MAP on sensory score of pizza made from mozzarella cheese stored in different packages at -10 to -15 °C.

respectively; and the sensory score decreased to 3.9 (P_1), 3.8 (P_2); 3.6 (P_1), 3.4 (P_2), 2.8 (P_1) and 2.5 (P_2), respectively for samples made by using stored MAP mozzarella cheese from the packages with 100% CO_2 (atm 3), 50 % CO_2 / 50 % N_2 (atm 5) and 100% N_2 (atm 4) indicating that minimum decrease in pizza score had been with the samples prepared by using mozzarella cheese of atm 3 (P_1: 3.9; P_2:3.8) followed by atm 5 (P_1: 3.6; P_2: 3.4) and atm 4 (P_1: 2.8; P_2: 2.5) respectively, in ascending order (Appendix XLV).

When pizzas were made from stored (12 months at -10 to -15 ^{0}C) MAP mozzarella cheese, the initial sensory score (5.0) of pizza decreased to 2.7 (P_1) and 2.6 (P_2) (atm 1), while the score decreased to 3.1 and 3.0, respectively for pizza made from mozzarella cheese of P_1 & P_2 (atm 2). However, the use of product packaged under atm 3, atm 5 and atm 4 resulted in decrease of sensory score of pizza, respectively to 3.6 (P_1), 3.2 (P_2), 3.4 (P_1), 3.3 (P_2) and 3.2 (P_1), 3.0(P_2), showing consistent gradual decrease in sensory score of pizza (Fig. 46).

The anova of the data relating to the sensory score of pizza made from stored mozzarella cheese samples revealed that the 5 types of modified atmospheres, intervals of storage, and interaction atmospheres x packages significantly ($P<0.01$) influenced the sensory score of pizza, under both the storage conditions. The effect of types of package was significant ($P< 0.05$) for -10-15 ^{0}C storage condition only. Interaction intervals x atmospheres x packages was significant ($P<0.01$) for 7$\pm$1 ^{0}C storage only. Interactions intervals x atmospheres, and intervals x packages were observed to be not significant under any of the two studied storage conditions (Table 11).

CHAPTER 6

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSIONS

6.0.0.0 The material presented in the foregoing pages of this thesis relates to studies on modified atmosphere packaging (MAP) of mozzarella cheese. Under modified atmospheres, five types of atmospheres namely air, vacuum, 100% CO_2, 100% N_2 and 50% CO_2 / 50 % N_2 have been studied by using two types of high barrier bags.

6.1.0.0 In the introduction the importance of packaging in general, modified atmosphere packaging (MAP) in particular and need of MAP for mozzarella cheese has been pointed out. The need to substantially enhance the shelf life of mozzarella cheese is stressed.

6.2.0.0 A comprehensive review of the available literature on mozzarella cheese including its methods of manufacture like starter culture method, direct acidification method, continuous method and ultra-filtration method and physico-chemical characteristics is presented. This review also includes the information on the functional characteristics, textural characteristics, microbiological & sensory quality of mozzarella cheese. Published information on the modified atmosphere packaging (MAP), packaging materials and equipment for MAP, and the MAP of various dairy products namely milk, milk powder, danedar khoa, yoghurt and various types of cheeses including mozzarella is also referred to.

6.3.0.0 Since no approach to study the modified atmosphere packaging of mozzarella cheese made from mixed milk (buffalo milk: cow milk:: 60:40) of 3% fat in high barrier packaging materials has so far been made, the scope and plan of work of the present study were drawn up to include the observations on the preparation of mozzarella cheese using mixed milk by direct acidification method, packaging of mozzarella cheese under different modified atmospheres and effect of storage at 7±1 ^{0}C and –10 to –15 ^{0} C on various properties viz. physico-chemical & microbiological quality, textural and sensory characteristics. The experimental techniques adopted in the course of present studies have also been described.

6.4.0.0 The data collected for physico-chemical, microbiological, textural, functional and sensory properties of freshly made mozzarella cheese used for the package and storage studies are given.

6.5.0.0 Determination of changes in the headspace volume of mozzarella cheese samples packaged in two different materials under five different atmospheres and stored in two different conditions for various time intervals also formed a part of the present study. The data for chemical, functional, textural, microbiological and sensory changes in mozzarella cheese during the course of storage are recorded. Also, the effect of storage of mozzarella cheese on the sensory quality of pizza is included.

6.6.0.0 The data collected during the investigations are discussed with the help of their mean values presented in XLVIII table of details (Appendices), 11 Tables of consolidated data, 46 figures and Plates 1-15. From the results obtained, following conclusions are indicated:

6.6.1.0 The quality of freshly prepared mozzarella cheese made from mixed milk by direct acidification method indicated that the average values for various characteristics were: moisture 51.88%, fat 22.73 %, protein 20.85 %, salt 1.69 %, ash 1.72%, lactose 0.92 %, soluble N_2 0.41 %, pH 5.61, titratable acidity 0.54 (% LA), FFA 4.52 (m eq/g) and FDM 47.24 %. The meltability, fat leakage and stretchability of fresh samples were 2.98 (ratio, hence no units), 4.72 (ratio) and 5 (arbitrary scale), respectively. The values for hardness (N) varied from 136.14 to 141.64, and that of cohesiveness (ratio) from 0.37 to 0.39. The springiness (mm), gumminess (N) and chewiness (N x mm) averaged 0.50, 50.34 and 25.17, respectively. The microbiological quality of fresh mozzarella cheese samples revealed that the average counts (log CFU/ g) for SPC, Y& M, coliforms and psychrotrophs were 6.10, 2.51, 2.62, and 4.23 respectively, indicating that the various counts were not abnormally high.

6.6.2.0 Analysis of variance established significant ($P< 0.01$) difference towards headspace volume among the 5 types of modified atmospheres, and interaction between atmospheres and packages under both the storage conditions. The influence of intervals of storage and types of packaging material, both individually, were not significant. Interactions between intervals and atmospheres, between intervals and packages, and among intervals, atmospheres and packages were found to be not significant for headspace volume in the context of two storage conditions.

6.6.3.0 The intervals of storage, and interaction between atmospheres and packages under both storage conditions were found to have significant ($P< 0.01$) effect on moisture content of MAP mozzarella cheese, while the influence of type of package was significant ($P< 0.01$) only for -10 to -15 ^{0}C storage. The effect of different modified atmospheres on the moisture content was more significant ($P<0.01$) for 7± 1 ^{0}C than –10 to –15 ^{0}C ($P< 0.05$). However, interaction between intervals and atmospheres, and between intervals and packages did not affect the moisture content of samples under any of the storage conditions.

6.6.4.0 The values for titratable acidity of mozzarella cheese were significantly affected ($P<0.01$) by the 5 types of modified atmospheres, and interaction between atmospheres and packages, both individually, under both the storage conditions; while the effect of intervals of storage, and interaction among intervals, atmospheres and packages was more significant ($P< 0.01$) for -10 to -15 ^{0} C storage compared to 7±1 ^{0} C storage ($P< 0.05$). Interaction between intervals and atmospheres, and between intervals and packages were observed to be not significant under any of the two storage conditions with regard to changes in titratable acidity.

6.6.5.0 The 5 types of modified atmospheres, intervals of storage, and interaction between atmospheres and packages, all individually, significantly ($P<0.01$) influenced the pH of mozzarella cheese samples under both the storage conditions. The types of package affected the pH ($P< 0.01$) in case of -10 to -15 ^{0}C storage condition only. Interaction among intervals, atmospheres and packages was significant ($P<0.05$) for 7 ± 1 ^{0}C storage condition. Interaction between intervals and atmospheres, and between intervals and packages were not significant for changes in pH under any of the two storage conditions studied.

6.6.6.0 The soluble N_2 content of mozzarella cheese samples were significantly ($P< 0.01$) affected due to the variation among 5 types of modified atmospheres, among intervals of storage and interaction between atmospheres and packages. Interaction among intervals, atmospheres and packages was more significant ($P<0.01$) in case of deep freeze storage than refrigeration storage ($P<0.05$). However, the influence of type of packages at 7 ± 1 ^{0}C storage, and interaction between intervals and atmospheres, and between intervals and packages, were observed to be not significant for both the storage conditions.

6.6.7.0 Analysis of variance confirmed highly significant ($P<0.0.1$) effect on FFA due to the difference among the atmospheres, and interaction between atmospheres and packages, for both the storage conditions. Differences in the values for FFA of mozzarella cheese due to the types of packages and interaction between intervals and atmospheres significantly ($P<0.05$) affected the FFA content of samples. The effect of packages on FFA at -10 to -15 ^{0}C and interaction between intervals and atmospheres, and between intervals and packages was, however, found to be not significant under any of the storage conditions.

6.6.8.0 The degree of casein hydrolysis of stored MAP mozzarella cheese was examined by employing polyacrylamide gel electrophoresis. Photo plate reveals that there was no difference in the pattern of casein fractions of MAP mozzarella cheese during storage at 7 ± 1 ^{0}C packages in 5 modified atmospheres upto 4 weeks irrespective of packaging. It is clear that degradation of β - casein was not much, where as α - casein degraded faster with the case of storage period upto 6 weeks (atm 1). Degradation of α_{s1} – casein. Proportion of α_{s1} -I casein degraded to α_{s1} - II fraction was more prominent on 8 weeks in atm 2 and 4. This additional α_{s1} bands (α_{s1} –I and α_{s1} -I) appeared as a result of degradation by microbial load.

The deep frozen frozen sample (Photo plate) casein was not degraded upto 4 months of storage at -10 ^{0}C to -15 ^{0}C packaged in P_1 and P_2. The slight degradation in α_s –casein started after 6 months of storage but the degradation started from 7 months onwards. It increased with the storage time.

6.6.9.0 The anova indicated that the intervals of storage, 5 types of modified atmospheres and the interaction between atmospheres and packages, each individually, significantly ($P<0.01$) affected the meltability of mozzarella cheese samples under both the storage conditions. Interaction among intervals, atmospheres and packages was more significant ($P<0.01$) for deep freeze storage. The influence of type of packages, interaction between intervals and atmospheres, and between intervals and packages did not affect the meltability under any of the storage conditions.

6.6.10.0 Intervals of storage and different modified atmospheres, both individually, significantly ($P<0.01$) affected the fat leakage in mozzarella cheese samples under both the storage conditions. Effect of types of package, and interaction between intervals and atmospheres were found to be not significant under any of the storage conditions, but interaction between intervals and packages was highly significant ($P< 0.01$) for 7± 1 ^{0}C storage only. Interaction between atmospheres and packages was more significant ($P< 0.01$) in case of -10 to -15 ^{0}C storage, while interaction intervals between atmospheres and packages was significant ($P<0.05$) for 7± 1 ^{0}C for storage only.

6.6.11.0 Analysis of variance of the data pertaining to stretchability showed highly significant ($P< 0.01$) differences among intervals of storage and among atmospheres for both the storage conditions, while the influence of packages was significant ($P<0.01$) for –10 to –15 ^{0}C storage only. Interaction atmospheres x packages was more significant ($P<0.01$) at –10 to –15 ^{0}C than 7±1^0 C storage ($P< 0.05$). The interaction intervals x atmospheres x packages was significant ($P < 0.05$) for –10 to –15 ^{0} C storage only, while other studied interactions were observed to be not significant under the two storage conditions. Oberg *et al.* (1992) reported that meltability of mozzarella cheese samples decreased when stored at –20 ^{0}C for 42 days.

6.6.12.0 Anova of the data on hardness revealed that intervals of storage, types of atmospheres, and interaction between atmospheres and packages contributed significantly ($P<0.01$) towards the changes in hardness during storage under both the conditions, whereas interaction among intervals, atmospheres and packages was significant ($P<0.01$) for 7±1 ^{0} C storage only. However, effect of type of package, interaction between intervals and packages were not significant under the two storage conditions.

6.6.13.0 The intervals of storage, and interaction between atmospheres and packages significantly ($P<0.01$) influenced the changes in cohesiveness for both the storage conditions, while the interaction among intervals, atmospheres and packages was significant ($P<0.05$) for -10 to -15 ^{0} C storage only. The type of package affected the cohesiveness more significantly ($P<0.01$) for deep freeze storage condition. The influence of different modified atmospheres was highly significant ($P<0.01$) in case of 7±1 ^{0}C storage.

6.6.14.0 The influence of intervals of storage on the springiness of mozzarella cheese samples was highly significant ($P<0.01$) for both the conditions of storage, while interaction between atmospheres and packages was significant ($P<0.01$) for 7±1 ^{0}C storage only. The different modified atmospheres and types of packages, both individually, significantly ($P<0.05$) affected the springiness of mozzarella cheese for both the storage conditions.

6.6.15.0 The samples packaged in two different materials, under different atmospheres and stored at 7±1 ^{0}C revealed that the influence of different intervals of storage, and effect of different atmospheres were highly significant ($P<0.01$) with regard to gumminess for both storage conditions, while interaction between atmospheres and packages was significant ($P<0.01$) for 7±1 ^{0} C storage only. Interaction among intervals, atmospheres and packages was found to be significant (P< 0.01) for deep freeze storage condition. However, interactions between intervals and atmospheres, intervals and packages and also effect of types of packages were not significant for changes in gumminess for both the storage conditions.

6.6.16.0 The chewiness of the stored mozzarella cheese was significantly (P<0.01) affected by the intervals of storage and interaction between atmospheres and packages; and the influence of different modified atmospheres was seen to be more significant (P<0.01) for 7±1 ^{0}C storage than -10 to -15 ^{0}C (P<0.05). Also, variation between packages (P<0.01) and interaction among intervals, atmospheres and packages affected the chewiness significantly (P<0.01) in case of deep freeze storage only.

6.6.17.0 The effects of intervals of storage and also that of interaction between atmospheres and packages on the values for SPC were observed to be highly significant (P<0.01) for both the storage conditions. The difference in the values for SPC due to different modified atmospheres was found to be more significant (P<0.01) for deep freeze storage conditions, while interactions between intervals and packages (P<0.01) and among intervals, atmospheres and packages (P< 0.05) were significant for only 7±1 ^{0} C storage. The variation in the values of SPC due to type of package and interaction between intervals and atmospheres were found to be not significant for both the two storage conditions studied.

6.6.18.0 Analysis of variance for the data on yeast and mould counts confirmed that the intervals of storage, two types of packages, interactions between atmospheres and packages, and between intervals and atmospheres, each individually, were highly significant in affecting the growth of Y & M under both the storage condition (P<0.01).

6.6.19.0 Anova of the data revealed highly significant (P<0.01) differences among the intervals of storage, amongst atmospheres, and interaction between atmospheres and packages with regard to changes in psychrotrophic count for both the storage conditions. Interaction between intervals and atmospheres was significant (P<0.01) for refrigeration storage condition only.

The difference in psychrotrophic count due to type of package was found to be not significant for any of the two storage conditions.

6.6.20.0 There were found to be highly significant ($P<0.01$) differences in the coliform count due to intervals of storage, and interaction between intervals and packages under both the storage conditions. The influence of different modified atmospheres on coliform count was more significant ($P<0.01$) at deep freeze condition. The effect of type of package was not significant for any of the two storage conditions.

6.6.21.0 The 5 types of modified atmospheres, intervals of storage, and the interaction between atmospheres and packages, each individually, were found to be highly significant ($P<0.01$) from the consideration of appearance score of mozzarella cheese under both the storage conditions. The effect of type of package was significant ($P< 0.01$) for -10-15 ^{0}C storage condition only. Interaction among intervals, atmospheres and packages was significant ($P<0.01$) for refrigeration storage only. Interactions between intervals and atmospheres, and between intervals and packages were not significant for appearance score under any of the two storage conditions studied.

6.6.22.0 Statistically, the influence of intervals of storage, types of atmospheres and interaction between atmospheres and packages, each individually, significantly ($P<0.01$) affected the body and texture score of mozzarella cheese samples, while the effect of type of packages was significant ($P <0.05$) for deep freeze storage only.

6.6.23.0 The intervals of storage, types of modified atmospheres, and interaction between atmospheres and packages significantly ($P< 0.01$) contributed towards the changes in sensory flavour score of mozzarella cheese samples for both the storage conditions. The influence of types of packages was significant ($P<0.05$) in case of -10 to -15 ^{0}C only.

6.6.24.0 The data on overall acceptability revealed that intervals of storage, types of atmospheres, and interaction between atmospheres and packages contributed significantly ($P<0.01$) towards the changes in overall acceptability during storage under both the conditions, whereas the effect of type of package was significant (P< 0.0.5) for deep freeze condition only.

6.6.25.0 In order to determine the shelf-life of MAP mozzarella cheeses samples were submitted to 5 types of atmospheres (air, vacuum, 100% CO_2, 100% N_2 and 50% CO_2 / 50% N_2). The samples were stored at 7 ± 1°C and at deep freeze storage conditions. The results of the overall quality were used to define the product shelf life. It was observed that the shelf life of mozzarella cheese significantly increased upto 12 weeks (a 300 % increase) for the product packaged under 100 % CO_2 in case of P_1 , and 11 weeks(a 275 % increase) in case of P_2. Under 50 % CO_2 / 50 % for 11 weeks (a 275 % increase) in case of P_1, and 10 weeks (a 250 % increase) in case of P_2. Under 100% N_2, 250 % increase in shelf life was achieved in case of P_1, and 9 weeks (a 225% increase) in case of P_2. For the product packaged under vacuum, the shelf life was found to be 9 weeks (a 225 % increase) compared to conventional air pack (4weeks).

During the deep freeze storage, it was found that modified atmosphere packaging of mozzarella cheese resulted in significant increase in shelf life, i.e. upto 12 months (120 % increase) in all studied modified atmospheric conditions irrespective of type of packaging material, compared to conventional air pack (10 months).

6.6.26.0 The sensory score of pizza made from stored mozzarella cheese confirmed the chemical and microbiological results of the stored mozzarella cheese under different atmospheres and storage conditions that the 5 types of modified atmospheres, intervals of storage, and interaction between atmospheres and packages, for mozzarella cheese significantly (P<0.01) influenced the sensory score of pizza, under both the storage conditions. The effect of types of package was significant (P< 0.05) for -10-15 ^{0}C storage condition only. Interaction among intervals, atmospheres and packages was significant (P<0.01) for 7±1 ^{0}C storage only. Interactions between intervals and atmospheres; and between intervals and packages were observed to be not significant under any of the two studied storage.

6.7.0.0 From the present study it can be concluded that amongst five atmospheres, namely atm1 (air), atm 2 (vacuum), atm 3 (100% CO_2), atm 4(100% N_2) and atm 5 (50% CO_2 / 50% N_2) employed for the packaging of mozzarella cheese in two types of packages viz. P_1 (Cryovac) and P_2 (LLD /BA / Nylon-6 / BA/ LDPE), atm 3(100 % CO_2) was best, followed by atm 5> atm 4> atm 2> atm 1 respectively, in descending order, and may be used for packaging and storage of mozzarella cheese at 7 $\pm 1^0$C and –10 to –15 ^{0}C. Between the two types of packages used in the study, cryovac was found to be better than LLD/ BA/ Nylon-6/ BA/LDPE for packaging and storage of MAP mozzarella cheese.

6.8.0.0 An exhaustive bibliography of the references reported in the review and elsewhere is also separately attached immediately following this summary of results.

6.9.0.0 Appendices

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BIBLIOGRAPHY

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APPENDICES

Appendix I: Effect of MAP on the Headspace volume (ml) of Mozzarella cheese stored in different packages at 7±1 ^{0}C (mean of 3 trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	65	65	0	0	625	625	625	625	625	625
1	65	65	0	0	625	624	625	623	620	620
2	64	64	0	0	625	623	625	624	619	617
3	63	62	2	2	625	623	623	622	618	616
4	63	62	2	2	623	620	620	620	617	615
5	62	60	2	3	619	617	620	619	616	615
6	■	■	2	3	618	617	619	618	614	613
7	■	■	3	3	618	616	619	617	612	610
8	■	■	3	3	615	610	618	615	612	609
9	■	■	3	4	612	607	618	615	610	609
10	■	■	3	4	611	607	617	614	607	607
11	■	■	3	5	608	604	615	612	604	603
12	■	■	4	5	607	601	613	609	602	600

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix II: Effect of MAP on the Headspace volume (ml) of Mozzarella cheese stored in different packages at –10 to–15 ^0C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	65	65	0	0	625	625	625	625	625	625
1	58	56	0	0	621	620	622	621	620	618
2	53	49	0	0	618	612	620	619	620	618
3	50	45	0	0	616	611	620	615	619	615
4	47	42	0	0	616	610	618	613	618	613
5	45	40	0	2	614	608	615	611	616	612
6	45	39	2	4	614	604	615	609	613	612
7	43	38	2	4	613	602	612	603	611	608
8	40	36	2	5	611	602	609	601	610	608
9	40	35	3	5	610	601	605	597	607	605
10	37	34	3	7	609	598	602	596	607	604
11	35	31	4	7	605	596	598	591	606	604
12	32	29	4	9	602	605	594	587	605	603

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix III: Effect of MAP on the Moisture content (%) of Mozzarella cheese stored in different packages at 7± 1 ^{0}C (mean of 3 trials)

Period of storage (weeks)	Treatment given to packages									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P1
0	51.88	51.88	51.88	51.88	51.88	51.88	51.88	51.88	51.88	51.88
1	51.85	51.82	51.85	51.83	51.81	51.79	51.82	51.78	51.83	51.78
2	51.83	51.78	51.82	51.80	51.80	51.78	51.81	51.76	51.81	51.72
3	51.80	51.75	51.80	51.79	51.76	51.73	51.80	51.73	51.78	51.70
4	51.76	51.74	51.79	51.75	51.76	51.69	51.78	51.68	51.73	51.68
5	51.72	51.70	51.78	51.60	51.75	51.66	51.76	51.63	51.72	51.65
6	■	■	51.71	51.44	51.71	51.48	51.70	51.54	51.67	51.45
7	■	■	51.68	51.29	51.67	51.33	51.52	51.45	51.54	51.41
8	■	■	51.54	51.01	51.61	51.19	51.43	51.32	51.42	51.33
9	■	■	51.36	50.94	51.55	51.01	51.32	51.11	51.34	51.16
10	■	■	51.28	50.83	51.41	50.76	51.28	51.03	51.22	50.90
11	■	■	51.21	50.74	51.29	50.57	51.25	51.02	51.13	50.75
12	■	■	51.13	50.75	51.15	50.35	51.20	50.93	51.05	50.67

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD/BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix IV: Effect of MAP on the Moisture content (%) of Mozzarella cheese stored in different packages at –10 to –15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to packages									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	51.88	51.88	51.88	51.88	51.88	51.88	51.88	51.88	51.88	51.88
1	51.83	51.82	51.84	51.82	51.83	51.81	51.83	51.82	51.82	51.80
2	51.59	51.53	51.81	51.74	51.80	51.77	51.78	51.72	51.72	51.69
3	51.38	51.25	51.75	51.67	51.79	51.76	51.64	51.60	51.69	51.64
4	51.23	51.14	51.69	51.45	51.77	51.67	51.55	51.46	51.65	51.50
5	51.11	50.98	51.54	51.28	51.66	51.45	51.33	51.21	51.55	51.21
6	50.95	50.64	51.32	51.04	51.53	51.24	51.21	51.06	51.45	51.05
7	50.75	50.42	51.12	50.80	51.29	51.01	51.13	50.85	51.21	50.88
8	50.63	50.21	50.86	50.57	51.06	50.79	50.88	50.11	51.04	50.75
9	50.51	49.87	50.55	50.16	50.88	50.48	50.63	49.95	50.81	50.47
10	50.35	49.51	50.21	49.84	50.69	50.12	50.22	49.73	50.49	50.10
11	50.24	49.34	49.99	49.61	50.35	49.75	50.00	49.52	50.11	49.75
12	49.79	49.20	49.83	49.22	49.91	49.30	49.85	49.23	49.88	49.29

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix V: Effect of MAP on the Titratable acidity (% lactic acid) of Mozzarella cheese stored in different packages at 7 ± 1^{0}C (mean of 3 trials)

Period of storage (weeks)	Treatment given to packages									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
1	0.56	0.58	0.55	0.57	0.55	0.56	0.55	0.56	0.55	0.57
2	0.63	0.66	0.58	0.59	0.56	0.58	0.57	0.59	0.57	0.58
3	0.65	0.68	0.61	0.63	0.57	0.59	0.58	0.59	0.59	0.61
4	0.71	0.73	0.62	0.65	0.59	0.61	0.60	0.62	0.61	0.63
5	0.93	0.96	0.64	0.67	0.61	0.63	0.61	0.63	0.63	0.65
6	■	■	0.65	0.69	0.62	0.64	0.64	0.66	0.65	0.66
7	■	■	0.68	0.70	0.64	0.67	0.67	0.69	0.68	0.70
8	■	■	0.71	0.73	0.67	0.69	0.70	0.72	0.69	0.70
9	■	■	0.74	0.77	0.68	0.70	0.73	0.76	0.70	0.71
10	■	■	0.79	0.84	0.71	0.73	0.78	0.79	0.72	0.74
11	■	■	0.85	0.89	0.74	0.77	0.81	0.84	0.76	0.78
12	■	■	0.91	0.95	0.75	0.79	0.84	0.90	0.80	0.86

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix VI: Effect of MAP on the Titratable acidity (% lactic acid) of Mozzarella cheese stored in different packages at –10 to –15 ^{0}C (mean of 3 trials)

Period of storage (weeks)	Treatment given to packages									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
1	0.56	0.57	0.55	0.57	0.55	0.56	0.55	0.55	0.56	0.56
2	0.58	0.59	0.58	0.58	0.57	0.59	0.57	0.58	0.57	0.59
3	0.61	0.64	0.61	0.62	0.58	0.60	0.58	0.61	0.58	0.59
4	0.63	0.65	0.63	0.65	0.60	0.63	0.62	0.64	0.60	0.64
5	0.65	0.68	0.65	0.68	0.62	0.65	0.65	0.67	0.63	0.66
6	0.68	0.72	0.68	0.70	0.64	0.67	0.67	0.69	0.65	0.67
7	0.71	0.75	0.69	0.71	0.67	0.69	0.69	0.71	0.68	0.69
8	0.78	0.85	0.69	0.71	0.67	0.70	0.69	0.72	0.68	0.70
9	0.84	0.89	0.70	0.72	0.69	0.71	0.70	0.71	0.69	0.71
10	0.87	0.90	0.71	0.74	0.69	0.73	0.72	0.73	0.71	0.73
11	0.91	0.94	0.72	0.75	0.71	0.73	0.72	0.74	0.73	0.74
12	0.95	0.97	0.76	0.79	0.72	0.74	0.74	0.77	0.74	0.75

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix VII: Effect of MAP on the pH of Mozzarella cheese stored in different packages at 7± 1^0 C (mean of 3 trials)

Period of storage (weeks)	Treatment given to packages									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	5.61	5.61	5.61	5.61	5.61	5.61	5.61	5.61	5.61	5.61
1	5.60	5.58	5.61	5.59	5.61	5.60	5.60	5.58	5.59	5.57
2	5.54	5.52	5.58	5.56	5.60	5.60	5.58	5.55	5.57	5.54
3	5.51	5.40	5.53	5.50	5.59	5.57	5.54	5.49	5.55	5.53
4	5.37	5.29	5.45	5.41	5.58	5.54	5.44	5.40	5.52	5.50
5	5.20	5.15	5.37	5.31	5.55	5.50	5.40	5.35	5.54	5.52
6	■	■	5.36	5.30	5.51	5.47	5.38	5.29	5.52	5.45
7	■	■	5.33	5.27	5.49	5. 40	5.34	5.26	5.47	5. 43
8	■	■	5.31	5.26	5.48	5.41	5.28	5.27	5.44	5.40
9	■	■	5.27	5.24	5.43	5.37	5.25	5.23	5.41	5.33
10	■	■	5.25	5.23	5.38	5.29	5.22	5.20	5.35	5.27
11	■	■	5.24	5.21	5.32	5.27	5.21	5.19	5.31	5.24
12	■	■	5.22	5.17	5.29	5.21	5.21	5.16	5.25	5.20

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix VIII: Effect of MAP on the pH of Mozzarella cheese stored in different packages at–10 to -15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to packages									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	5.61	5.61	5.61	5.61	5.61	5.61	5.61	5.61	5.61	5.61
1	5.58	5.56	5.59	5.57	5.60	5.58	5.58	5.57	5.58	5.57
2	5.54	5.52	5.57	5.53	5.58	5.55	5.57	5.56	5.56	5.56
3	5.52	5.49	5.54	5.49	5.55	5.53	5.54	5.48	5.54	5.50
4	5.50	5.43	5.51	5.45	5.53	5.46	5.45	5.43	5.52	5.44
5	5.45	5.40	5.47	5.43	5.49	5.45	5.44	5.43	5.50	5.43
6	5.38	5.36	5.45	5.41	5.48	5.42	5.42	5.41	5.47	5.42
7	5.35	5.32	5.41	5.40	5.44	5.41	5.40	5.38	5.44	5.40
8	5.33	5.31	5.40	5.37	5.40	5.38	5.39	5.35	5.40	5.38
9	5.30	5.27	5.36	5.35	5.39	5.37	5.38	5.33	5.38	5.37
10	5.30	5.26	5.35	5.32	5.36	5.35	5.36	5.32	5.35	5.34
11	5.31	5.26	5.34	5.30	5.35	5.34	5.31	5.30	5.34	5.33
12	5.25	5.24	5.32	5.31	5.34	5.33	5.29	5.28	5.33	5.32

atm: atmosphere

P1: Cryovac (70 µ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 µ)

* Poly binding agent

Appendix IX: Effect of MAP on the Soluble nitrogen content (%) of Mozzarella cheese stored in different packages at 7±1 ^{0}C (mean of 3 trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
1	0.49	0.52	0.46	0.49	0.43	0.46	0.47	0.49	0.44	0.47
2	0.56	0.59	0.53	0.57	0.44	0.49	0.52	0.56	0.45	0.50
3	0.61	0.64	0.59	0.63	0.48	0.53	0.59	0.62	0.51	0.54
4	0.70	0.75	0.68	0.71	0.55	0.58	0.65	0.69	0.56	0.62
5	0.79	0.89	0.75	0.78	0.64	0.67	0.71	0.75	0.68	0.74
6	■	■	0.88	0.92	0.72	0.77	0.87	0.89	0.81	0.84
7	■	■	0.98	1.10	0.82	0.89	0.96	1.08	0.85	1.09
8	■	■	1.25	1.38	0.98	1.01	1.21	1.36	0.99	1.12
9	■	■	1.58	1.70	1.20	1.38	1.54	1.67	1.29	1.41
10	■	■	1.78	1.89	1.48	1.65	1.75	1.84	1.52	1.69
11	■	■	1.91	1.95	1.75	1.84	1.85	1.93	1.77	1.90
12	■	■	2.10	2.15	1.83	1.91	1.93	1.99	1.98	2.15

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

*   Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix X: Effect of MAP on the Soluble nitrogen content (%) of Mozzarella cheese stored in different packages at –10 to–15 ^{0}C in (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
1	0.44	0.46	0.43	0.45	0.42	0.43	0.43	0.43	0.43	0.44
2	0.46	0.49	0.45	0.47	0.45	0.47	0.45	0.48	0.45	0.46
3	0.48	0.54	0.49	0.52	0.46	0.49	0.47	0.51	0.47	0.50
4	0.53	0.58	0.56	0.55	0.50	0.53	0.50	0.54	0.51	0.53
5	0.57	0.62	0.57	0.60	0.52	0.55	0.52	0.59	0.54	0.58
6	0.61	0.67	0.59	0.64	0.54	0.58	0.57	0.63	0.59	0.63
7	0.66	0.70	0.62	0.67	0.57	0.60	0.60	0.66	0.60	0.66
8	0.69	0.75	0.65	0.69	0.58	0.62	0.64	0.68	0.64	0.69
9	0.73	0.79	0.70	0.72	0.65	0.69	0.67	0.71	0.66	0.70
10	0.76	0.86	0.76	0.79	0.70	0.73	0.76	0.78	0.71	0.75
11	0.85	0.96	0.80	0.85	0.75	0.78	0.79	0.81	0.74	0.77
12	0.89	0.98	0.87	0.92	0.83	0.85	0.86	0.90	0.86	0.89

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix XI: Effect of MAP on the FFA content (m eq/g) of Mozzarella cheese stored in different packages at 7±1 ^{0}C (mean of 3 trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	4.52	4.52	4.52	4.52	4.52	4.52	4.52	4.52	4.52	4.52
1	4.78	4.81	4.69	4.88	4.65	4.68	4.67	4.70	4.66	4.67
2	5.19	5.27	5.17	5.19	5.12	5.14	5.12	5.15	5.08	5.10
3	7.22	7.26	5.54	5.41	5.18	5.24	5.39	5.40	5.26	5.27
4	10.74	10.82	5.58	5.65	5.23	5.34	5.48	5.59	5.37	5.38
5	15.15	15.39	6.99	7.01	6.53	6.58	6.86	6.88	6.33	6.55
6	■	■	7.88	7.99	7.77	7.78	7.81	7.89	7.81	7.85
7	■	■	8.95	9.28	8.12	8.45	9.56	9.88	8.55	8.68
8	■	■	9.74	9.97	8.45	8.68	9.65	9.76	8.77	8.91
9	■	■	10.74	10.82	9.35	9.61	9.88	10.12	9.75	9.96
10	■	■	11.22	11.34	10.46	10.75	10.97	11.23	10.65	10.89
11	■	■	13.18	13.99	11.04	11.25	12.65	11.77	11.28	11.49
12	■	■	14.56	14.78	12.38	12.49	13.04	13.29	12.56	12.67

atm: atmosphere

P1: Cryovac (70 µ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 µ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XII: Effect of MAP on the FFA content (m eq/g) of Mozzarella cheese stored in different packages at –10 to–15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	4.52	4.52	4.52	4.52	4.52	4.52	4.52	4.52	4.52	4.52
1	4.78	4.83	4.66	4.68	4.61	4.73	4.73	4.81	4.69	4.71
2	5.10	5.48	4.72	4.78	4.69	4.87	4.99	5.01	4.88	4.95
3	6.18	6.29	6.13	6.28	5.24	5.65	5.65	5.78	5.41	5.63
4	7.35	7.65	6.99	7.18	6.21	6.52	6.93	7.08	5.92	6.18
5	8.86	8.91	7.71	7.96	7.13	7.28	7.77	7.89	7.54	7.62
6	9.64	9.87	8.65	8.89	7.65	7.84	8.44	8.76	7.85	7.99
7	10.95	11.15	9.88	10.02	8.50	8.68	9.12	9.89	8.76	8.98
8	12.89	13.08	10.58	10.78	9.04	9.23	10.56	10.87	9.25	9.44
9	13.25	13.44	11.07	11.31	9.98	10.23	10.88	11.12	10.01	10.36
10	14.58	14.74	12.33	12.65	10.34	10.65	11.48	11.83	10.67	11.08
11	15.01	15.04	13.12	13.27	10.58	10.75	12.14	12.55	10.98	11.52
12	15.64	15.77	13.54	13.65	10.69	10.88	12.45	12.78	12.04	12.28

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix XIII: Effect of MAP on the Meltability of Mozzarella cheese stored in different packages at 7±1 ^{0}C (mean of 3 trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98
1	3.17	3.14	3.19	3.15	3.27	3.25	3.25	3.20	3.26	3.17
2	3.63	3.59	3.66	3.65	3.80	3.75	3.70	3.65	3.80	3.72
3	3.75	3.70	3.82	3.78	4.31	4.25	3.92	3.88	4.09	4.01
4	4.25	4.22	4.10	4.05	4.93	4.88	4.16	4.15	4.65	4.61
5	5.06	5.05	4.23	4.20	5.31	5.27	4.28	4.25	4.82	4.74
6	■	■	4.51	4.45	5.61	5.75	4.55	4.52	5.18	5.05
7	■	■	4.79	4.73	6.00	5.99	4.70	4.69	5.59	5.42
8	■	■	5.11	5.08	6.22	6.07	4.89	4.88	5.93	5.88
9	■	■	5.41	5.34	6.38	6.19	5.18	5.09	6.12	6.09
10	■	■	5.73	5.68	6.42	6.38	5.45	5.35	6.22	6.18
11	■	■	5.76	5.70	6.71	6.66	5.64	5.55	6.63	6.56
12	■	■	5.92	5.88	6.90	6.83	5.99	5.92	6.78	6.71

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XIV: Effect of MAP on the Meltability of Mozzarella cheese stored in different packages at –10 to– 15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98
1	3.51	3.48	3.56	3.52	3.71	3.70	3.61	3.57	3.62	3.58
2	3.60	3.59	3.61	3.59	3.78	3.72	3.65	3.62	3.75	3.71
3	3.75	3.70	3.71	3.67	3.89	3.83	3.76	3.70	3.85	3.82
4	4.28	4.20	4.25	4.16	4.46	4.32	4.29	4.24	4.30	4.27
5	4.35	4.30	4.36	4.25	4.97	4.85	4.49	4.40	4.58	4.50
6	4.70	4.56	4.68	4.50	5.38	5.22	4.70	4.64	4.65	4.52
7	4.81	4.72	4.73	4.69	5.49	5.41	4.89	4.85	4.95	4.92
8	5.06	4.97	4.93	4.89	5.77	5.73	5.23	5.12	5.18	5.10
9	5.14	5.09	5.11	5.06	6.12	6.10	5.35	5.25	5.20	5.18
10	5.28	5.25	5.32	5.26	6.38	6.34	5.41	5.33	5.38	5.29
11	5.39	5.35	5.62	5.55	6.58	6.39	5.71	5.68	5.67	5.60
12	5.52	5.49	5.81	5.75	6.77	6.68	5.90	5.88	6.02	5.89

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix XV: Effect of MAP on the Fat leakage of Mozzarella cheese stored in different packages at 7±1 ^{0}C (mean of 3 trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	4.72	4.72	4.72	4.72	4.72	4.72	4.72	4.72	4.72	4.72
1	5.01	5.09	4.83	4.87	4.80	4.83	4.91	4.95	4.82	4.89
2	5.51	5.53	5.10	5.19	5.09	5.13	5.12	5.25	5.15	5.20
3	6.18	6.22	5.35	5.38	5.15	5.17	5.20	5.29	5.20	5.25
4	7.29	7.35	5.87	5.89	5.35	5.38	5.26	5.33	5.38	5.55
5	7.89	8.05	6.05	6.11	5.75	5.79	5.81	5.83	5.60	5.69
6	■	■	6.18	6.21	5.88	5.97	5.99	6.01	5.95	6.03
7	■	■	6.71	6.85	6.16	6.28	6.39	6.45	6.25	6.38
8	■	■	7.09	7.20	6.35	6.49	6.55	6.59	6.42	6.52
9	■	■	7.51	7.62	6.75	6.84	6.97	7.05	6.79	6.89
10	■	■	8.02	8.11	6.99	7.01	7.25	7.39	7.02	7.09
11	■	■	8.39	8.58	7.31	7.48	7.66	7.72	7.44	7.50
12	■	■	8.65	8.71	7.58	7.69	7.80	7.83	7.92	7.99

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XVI: Effect of MAP on the Fat leakage of Mozzarella cheese stored in different packages at –10 to– 15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	4.72	4.72	4.72	4.72	4.72	4.72	4.72	4.72	4.72	4.72
1	5.01	5.05	4.80	4.82	4.78	4.79	4.80	4.81	4.75	4.76
2	5.51	5.58	5.23	5.31	5.19	5.21	5.22	5.28	4.78	4.85
3	5.89	5.92	5.51	5.58	5.21	5.29	5.58	5.59	5.25	5.27
4	6.23	6.33	5.87	5.91	5.45	5.58	5.85	5.91	5.54	5.55
5	6.71	6.79	6.19	6.21	5.89	5.99	6.15	6.20	5.91	6.02
6	7.22	7.38	6.62	6.88	6.22	6.37	6.25	6.29	6.31	6.41
7	7.55	7.69	7.17	7.20	6.70	6.78	6.77	6.89	6.80	6.82
8	7.91	7.98	7.38	7.42	7.10	7.15	7.14	7.17	6.67	6.70
9	8.23	8.32	7.65	7.75	7.37	7.41	7.58	7.63	7.45	7.59
10	9.41	9.48	7.82	7.98	7.65	7.73	7.80	7.88	7.72	7.78
11	9.85	9.91	8.31	8.38	7.98	8.05	8.34	8.66	8.06	8.12
12	10.12	10.21	8.77	8.80	8.25	8.31	8.63	8.69	8.40	8.45

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XVII: Effect of MAP on the Stretchability (5-point arbitrary scale) of Mozzarella cheese stored in different packages at 7±1 ^{0}C (mean of 3 trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
1	4.8	4.8	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
2	4.7	4.5	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8
3	4.2	4.0	4.8	4.4	4.7	4.5	4.4	4.2	4.3	4.2
4	3.8	3.5	4.4	4.3	4.6	4.4	4.3	4.2	4.2	4.1
5	3.4	3.1	4.4	4.1	4.5	4.3	4.2	4.0	4.1	3.8
6	■	■	4.3	4.0	4.5	4.2	4.1	3.8	4.1	3.8
7	■	■	4.2	3.9	4.4	4.3	4.0	3.8	4.0	3.8
8	■	■	4.0	3.8	4.2	4.1	3.9	3.7	3.9	3.7
9	■	■	3.9	3.8	4.2	4.1	3.8	3.7	3.8	3.6
10	■	■	3.0	2.9	4.2	4.0	3.8	3.6	3.7	3.6
11	■	■	2.8	2.7	4.1	4.0	3.4	3.1	3.7	3.5
12	■	■	2.5	2.4	3.6	3.6	2.6	2.5	2.9	2.8

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XVIII: Effect of MAP on the Stretchability (5-point arbitrary scale) of Mozzarella cheese stored in different packages at –10 to–15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
1	4.9	4.8	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
2	4.9	4.8	5.0	5.0	5.0	5.0	5.0	4.8	5.0	5.0
3	4.8	4.7	4.8	4.8	4.8	4.8	4.8	4.7	4.8	4.8
4	4.6	4.5	4.6	4.2	4.7	4.2	4.5	4.2	4.5	4.3
5	4.5	4.3	4.2	4.0	4.4	4.3	4.3	4.0	4.3	4.2
6	4.1	3.9	4.1	4.0	4.3	4.1	4.2	4.1	4.2	4.2
7	4.0	3.8	4.1	3.9	4.2	4.0	4.1	4.0	4.2	4.1
8	3.7	3.6	3.9	3.8	4.2	4.1	3.9	3.8	3.9	3.8
9	3.3	3.0	3.6	3.5	3.8	3.6	3.9	3.8	4.0	3.8
10	3.1	2.8	3.4	3.2	3.7	3.5	3.5	3.5	3.6	3.4
11	2.6	2.4	2.6	2.5	3.4	3.1	2.7	2.5	2.8	2.7
12	2.5	2.3	2.5	2.4	2.8	2.7	2.6	2.5	2.7	2.6

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XIX: Effect of MAP on the Hardness (N) of Mozzarella cheese stored in different packages at 7± 1^0C (mean of three trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	138.89	138.89	138.89	138.89	138.89	138.89	138.89	138.89	138.89	138.89
2	124.81	120.76	127.28	122.16	134.27	130.28	132.28	122.11	131.47	125.25
4	95.63	91.12	125.34	116.33	132.31	123.82	119.30	117.21	127.349	120.32
6	100.63	94.11	112.70	100.14	124.58	116.01	118.55	107.41	120.20	110.10
8	■	■	105.38	94.42	119.47	106.32	115.56	103.43	113.27	104.52
10	■	■	88.83	81.55	112.97	100.44	100.92	90.18	100.76	95.92
12	■	■	78.00	71.24	98.15	91.08	93.00	84.95	94.88	87.71

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XX: Effect of MAP on the Hardness (N) of Mozzarella cheese in different packages stored at –10 to–15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	138.89	138.89	138.89	138.89	138.89	138.89	138.89	138.89	138.89	138.89
2	118.50	107.98	125.76	116.56	137.78	130.65	118.77	113.60	135.79	127.48
4	109.11	100.93	110.69	101.56	133.68	126.19	112.79	110.66	120.75	111.50
6	95.21	87.16	109.10	100.06	125.89	120.35	110.88	105.73	116.53	109.34
8	81.06	72.05	99.45	90.32	121.08	117.65	107.07	100.70	110.67	103.20
10	78.11	70.09	96.21	87.19	117.55	109.21	98.83	90.41	107.99	100.72
12	64.89	65.76	88.94	78.84	110.45	103.23	89.54	82.37	100.78	91.63

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix XXI: Effect of MAP on the Cohesiveness (A2/A1) of Mozzarella cheese stored in different packages at 7± 1^{0}C (mean of three trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
2	0.30	0.25	0.31	0.26	0.36	0.34	0.34	0.32	0.36	0.34
4	0.24	0.20	0.28	0.23	0.33	0.30	0.30	0.25	0.32	0.30
6	0.19	0.15	0.26	0.21	0.29	0.25	0.23	0.20	0.28	0.26
8	■	■	0.23	0.20	0.26	0.23	0.22	0.20	0.24	0.23
10	■	■	0.20	0.17	0.24	0.22	0.21	0.19	0.21	0.20
12	■	■	0.18	0.14	0.21	0.19	0.18	0.17	0.20	0.18

A2: + ve area under 2nd peak
A1: + ve area under 1st peak
atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XXII: Effect of MAP on the Cohesiveness (A2/A1) of Mozzarella cheese in different packages stored at –10 to–15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
2	0.30	0.28	0.31	0.28	0.34	0.31	0.31	0.28	0.36	0.30
4	0.29	0.25	0.29	0.26	0.32	0.29	0.29	0.25	0.29	0.26
6	0.26	0.22	0.27	0.24	0.28	0.25	0.24	0.23	0.28	0.24
8	0.24	0.20	0.25	0.22	0.26	0.24	0.23	0.22	0.23	0.22
10	0.23	0.19	0.24	0.20	0.23	0.21	0.21	0.20	0.21	0.20
12	0.18	0.15	0.19	0.16	0.22	0.20	0.19	0.17	0.20	0.19

A2: + ve area under 2nd peak
A1: + ve area under 1st peak
atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix XXIII: Effect of MAP on the Springiness (mm) of Mozzarella cheese stored in different packages at 7± 1^0C (mean of three trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
2	0.39	0.34	0.42	0.34	0.44	0.39	0.41	0.38	0.43	0.39
4	0.30	0.26	0.36	0.32	0.40	0.37	0.37	0.34	0.38	0.36
6	0.27	0.20	0.30	0.29	0.36	0.31	0.33	0.28	0.35	0.30
8	■	■	0.28	0.24	0.33	0.29	0.31	0.26	0.33	0.27
10	■	■	0.26	0.21	0.31	0.28	0.28	0.27	0.28	0.25
12	■	■	0.24	0.19	0.28	0.25	0.26	0.22	0.27	0.24

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XXIV: Effect of MAP on the Springiness (mm) of Mozzarella cheese in different packages stored at –10 to– 15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
2	0.41	0.38	0.46	0.41	0.49	0.46	0.45	0.40	0.48	0.42
4	0.39	0.37	0.40	0.34	0.45	0.41	0.36	0.36	0.42	0.40
6	0.36	0.30	0.38	0.33	0.41	0.38	0.35	0.34	0.39	0.35
8	0.28	0.24	0.32	0.27	0.37	0.34	0.30	0.30	0.36	0.35
10	0.24	0.22	0.26	0.24	0.33	0.31	0.29	0.26	0.32	0.31
12	0.20	0.18	0.21	0.19	0.30	0.27	0.25	0.23	0.29	0.25

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix XXV: Effect of MAP on the Gumminess (N) of Mozzarella cheese stored in different packages at 7± 1^{0}C (mean of three trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	50.34	50.34	50.34	50.34	50.34	50.34	50.34	50.34	50.34	50.34
2	38.07	34.85	42.33	41.53	49.19	43.25	41.69	41.54	43.51	41.20
4	27.17	20.35	38.76	33.48	44.43	40.23	37.78	33.56	40.60	36.65
6	23.83	15.52	32.12	28.51	39.12	34.58	34.83	31.77	36.71	32.23
8	■	■	27.32	24.16	33.60	29.88	30.72	27.35	31.05	30.98
10	■	■	21.12	20.94	29.67	24.96	26.71	24.62	27.02	25.00
12	■	■	19.55	17.32	25.41	22.10	22.31	18.96	23.29	20.89

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XXVI: Effect of MAP on the Gumminess (N) of Mozzarella cheese in different packages stored at –10 to–15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	50.34	50.34	50.34	50.34	50.34	50.34	50.34	50.34	50.34	50.34
2	27.67	27.50	31.83	31.71	35.80	35.78	29.35	29.32	49.19	27.67
4	27.71	27.37	30.60	30.52	36.72	36.71	21.96	21.81	34.38	27.71
6	23.29	23.28	24.46	24.41	28.86	28.80	26.60	26.49	31.83	23.29
8	17.45	17.32	23.10	23.1	26.18	26.11	23.45	23.44	24.83	17.45
10	14.60	14.55	17.56	17.22	25.72	25.62	23.39	23.30	27.83	14.60
12	14.14	14.01	18.99	18.84	24.89	24.77	24.54	24.44	23.61	14.14

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix XXVII: Effect of MAP on the Chewiness (N x mm) of Mozzarella cheese stored in different packages at 7± 1^0C (mean of three trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	25.15	25.15	25.15	25.15	25.15	25.15	25.15	25.15	25.15	25.15
2	18.86	12.52	21.14	18.11	24.65	22.51	22.23	20.21	24.61	21.44
4	12.72	8.54	17.38	12.11	20.92	16.45	18.38	14.12	19.73	15.65
6	5.20	4.98	14.92	10.51	18.85	14.38	15.03	11.84	17.92	13.82
8	■	■	10.78	7.15	15.44	11.13	11.79	10.00	14.86	10.46
10	■	■	9.68	5.08	13.89	9.81	9.88	8.75	12.00	9.01
12	■	■	7.25	4.00	10.67	7.64	7.23	5.02	9.78	7.45

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XXVIII: Effect of MAP on the Chewiness (N x mm) of Mozzarella cheese in different packages stored at –10 to–15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	25.15	25.15	25.15	25.15	25.15	25.15	25.15	25.15	25.15	25.15
2	20.31	18.04	23.11	20.09	24.41	22.31	23.58	20.95	24.00	22.01
4	16.78	12.64	17.78	13.71	20.96	17.88	18.10	14.09	19.66	16.56
6	14.67	10.61	15.78	11.62	18.92	15.85	16.91	11.88	17.63	14.61
8	12.25	7.21	13.02	8.63	14.60	11.54	13.27	9.22	12.41	10.38
10	09.55	6.51	10.81	6.99	13.12	10.10	10.96	8.81	11.17	9.14
12	5.66	3.92	6.67	5.23	9.43	8.43	7.80	6.75	8.96	8.05

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XXIX: Effect of MAP on the Standard plate count (log cfu/g) of Mozzarella cheese stored in different packages at 7±1 ^{0}C (mean of three trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	6.10	6.10	6.10	6.10	6.10	6.10	6.10	6.10	6.10	6.10
1	6.52	6.63	6.55	6.80	5.09	5.00	5.88	5.90	5.14	5.40
2	6.84	6.92	6.40	6.82	4.91	4.60	5.66	5.69	4.46	4.83
3	7.05	7.11	6.11	6.41	4.01	4.32	5.44	5.38	4.60	4.65
4	6.90	6.97	5.92	5.82	3.76	3.97	5.40	5.31	4.29	4.38
5	6.75	6.85	5.71	5.80	3.90	3.76	5.34	5.27	4.37	4.48
6	■	■	5.68	5.76	3.55	3.63	5.22	5.30	4.11	4.26
7	■	■	5.61	5.70	3.13	3.03	5.29	5.32	3.72	4.05
8	■	■	5.56	6.51	2.83	2.94	5.21	5.26	3.64	3.91
9	■	■	5.47	6.45	2.68	2.80	5.10	5.20	3.43	4.18
10	■	■	5.41	5.44	2.58	2.66	5.03	5.18	3.25	4.00
11	■	■	5.37	5.41	2.19	2.38	4.99	5.21	3.15	3.88
12	■	■	5.36	5.39	2.26	2.99	4.91	5.12	3.03	3.66

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XXX: Effect of MAP on the Standard plate count (log cfu/g) of Mozzarella cheese stored in different packages at -10 to-15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	6.10	6.10	6.10	6.10	6.10	6.10	6.10	6.10	6.10	6.10
1	6.01	6.05	5.92	5.98	5.82	5.88	5.99	6.03	5.85	5.89
2	5.75	5.78	5.61	5.65	5.52	5.59	5.71	5.73	5.56	5.59
3	5.42	5.47	5.13	5.15	5.01	5.04	5.33	5.38	5.11	5.15
4	4.87	4.92	4.74	4.77	4.58	4.61	4.70	4.75	4.69	4.62
5	4.58	4.62	4.38	4.45	4.15	4.19	4.40	4.49	4.22	4.28
6	4.11	4.18	4.08	4.10	3.87	3.92	4.08	4.13	4.14	4.18
7	3.84	3.88	3.55	3.59	3.30	3.37	3.66	3.72	3.47	3.49
8	3.54	3.57	3.25	3.29	3.13	3.15	3.27	3.29	3.18	3.20`
9	3.31	3.34	3.21	3.28	3.27	3.29	3.24	3.28	3.01	3.08
10	3.20	3.25	3.14	3.21	2.91	2.95	3.22	3.25	2.95	2.96
11	3.15	3.16	3.11	3.14	2.86	2.82	3.04	3.00	2.92	2.95
12	3.06	3.10	3.02	3.05	2.50	2.52	2.84	2.89	2.81	2.85

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix XXXI: Effect of MAP on the Yeast and mould count (log cfu/g) of Mozzarella cheese stored in different packages at 7±1 ^{0}C (mean of three trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51
1	3.47	3.65	2.33	2.33	0.88	0.97	2.00	2.24	1.81	1.75
2	5.25	5.48	2.46	2.46	0.69	0.74	2.00	2.15	1.20	1.22
3	5.20	5.46	2.40	2.66	0.54	0.63	2.30	2.36	1.45	1.63
4	4.84	4.95	2.30	2.71	0.94	0.98	1.78	1.90	1.17	0.97
5	4.84	4.92	3.41	3.70	0.87	0.91	1.91	1.92	1.27	1.29
6	■	■	2.25	2.35	0.88	0.94	1.87	1.90	1.06	1.16
7	■	■	3.15	3.24	0.55	0.65	1.82	1.93	1.27	1.32
8	■	■	2.75	2.84	0.50	0.68	2.11	2.15	1.08	1.16
9	■	■	2.71	2.74	0.46	0.55	1.52	1.68	1.13	1.23
10	■	■	2.69	2.70	0.45	0.62	1.44	1.52	1.04	1.05
11	■	■	2.60	2.65	0.43	0.46	1.95	2.05	0.90	0.95
12	■	■	2.58	2.62	0.35	0.39	1.86	1.91	1.03	1.15

atm: atmosphere

P1: Cryovac (70 µ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 µ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XXXII: Effect of MAP on the Yeast and mould count (log cfu/g) of Mozzarella cheese stored in different packages at –10 to–15 ^{0}C (mean of three trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51
1	1.85	1.92	1.75	1.78	1.23	1.30	1.75	1.78	1.30	1.32
2	1.34	1.50	1.65	1.69	1.12	1.15	1.60	1.62	1.78	1.79
3	1.10	1.15	1.10	1.18	1.00	1.08	1.25	1.38	1.20	1.25
4	0.99	0.98	0.93	0.90	0.67	0.69	0.95	0.93	0.85	0.87
5	0.89	0.85	0.87	0.85	0.68	0.71	0.84	0.83	0.78	0.75
6	0.86	0.88	0.84	0.83	0.69	0.72	0.76	0.73	0.78	0.72
7	0.82	0.85	0.82	0.80	0.67	0.69	0.74	0.71	0.75	0.70
8	0.80	0.75	0.78	0.75	0.68	0.66	0.72	0.73	0.73	0.70
9	0.88	0.85	0.71	0.73	0.65	0.64	0.72	0.74	0.69	0.69
10	0.80	0.87	0.69	0.72	0.61	0.59	0.74	0.77	0.68	0.69
11	0.82	0.85	0.68	0.65	0.58	0.54	0.64	0.67	0.66	0.69
12	0.84	0.86	0.73	0.74	0.50	0.52	0.67	0.69	0.61	0.66

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix XXXIII: Effect of MAP on the Psychrotrophic count (log cfu/g) of Mozzarella cheese stored in different packages at 7± 1^{0}C (mean of three trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	4.23	4.23	4.23	4.23	4.23	4.23	4.23	4.23	4.23	4.23
1	5.85	5.88	5.70	5.72	5.58	5.59	5.65	5.66	5.61	5.64
2	7.15	7.34	6.01	6.04	5.63	5.68	6.85	6.89	6.68	6.71
3	7.36	7.85	6.75	6.78	5.95	5.89	6.55	6.67	5.90	5.95
4	8.45	8.89	7.18	7.20	6.32	6.48	7.83	7.88	7.10	7.35
5	8.88	8.99	7.20	7.23	6.08	6.10	7.15	7.18	6.10	6.18
6	■	■	7.10	7.15	6.18	6.27	7.08	7.10	6.20	6.31
7	■	■	7.01	7.05	6.38	6.40	6.95	7.05	6.45	6.49
8	■	■	7.11	7.14	6.62	6.68	7.09	7.10	6.69	6.70
9	■	■	7.15	7.17	6.77	6.83	7.11	7.14	6.78	6.98
10	■	■	7.08	7.10	6.58	6.66	7.03	7.07	6.62	6.69
11	■	■	7.43	7.59	6.85	6.83	7.10	7.11	6.90	6.95
12	■	■	8.05	8.28	6.90	6.92	7.52	7.75	7.35	7.48

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XXXIV: Effect of MAP on the Psychrotrophic count (log cfu/g) of Mozzarella cheese in different packages stored at –10 to–15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	4.23	4.23	4.23	4.23	4.23	4.23	4.23	4.23	4.23	4.23
1	3.24	3.45	3.11	3.15	2.45	2.40	3.10	3.12	2.50	2.45
2	3.10	3.21	2.91	2.98	2.15	2.21	3.00	3.05	2.02	2.08
3	3.00	3.05	2.74	2.77	2.10	2.15	2.67	2.53	2.00	2.20
4	2.76	2.80	2.44	2.48	2.01	2.11	2.45	2.39	1.90	1.93
5	2.61	2.67	2.31	2.37	1.90	1.95	2.31	2.39	1.82	1.85
6	2.08	2.10	1.98	2.00	1.65	1.70	1.95	1.99	1.71	1.75
7	1.90	1.95	1.72	1.77	1.45	1.50	1.64	1.65	1.60	1.62
8	1.81	1.86	1.63	1.68	1.38	1.42	1.60	1.63	1.45	1.49
9	1.70	1.70	1.45	1.48	1.24	1.28	1.58	1.60	1.40	1.43
10	1.65	1.69	1.32	1.33	1.18	1.20	1.50	1.52	1.22	1.22
11	1.32	1.35	1.20	1.25	1.00	1.02	1.15	1.18	1.09	1.10
12	1.20	1.22	1.11	1.13	0.90	0.93	1.12	1.15	0.98	0.99

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix XXXV: Effect of MAP on the Coliform count (log cfu/g) of Mozzarella cheese stored in different packages at 7± 1^{0}C (mean of three trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	2.62	2.62	2.62	2.62	2.62	2.62	2.62	2.62	2.62	2.62
1	2.73	2.75	2.00	2.33	1.05	1.32	1.85	2.00	1.85	2.00
2	2.79	2.80	2.15	2.46	0.96	1.00	2.10	2.15	1.46	1.78
3	2.85	2.88	2.30	2.49	0.88	1.69	2.25	2.32	1.40	1.70
4	2.93	2.96	2.00	2.30	0.73	1.00	1.98	2.02	1.17	1.79
5	2.98	3.01	2.39	2.30	0.75	1.12	2.11	2.17	1.27	1.69
6	■	■	2.13	2.25	0.69	1.00	2.18	2.20	1.06	1.65
7	■	■	2.60	2.98	0.54	0.98	2.37	2.42	0.96	1.44
8	■	■	2.40	2.50	0.55	0.87	2.13	2.25	1.17	1.60
9	■	■	2.40	2.59	0.54	0.81	2.20	2.32	1.23	1.50
10	■	■	2.13	2.45	0.46	0.73	2.10	2.22	1.04	1.32
11	■	■	1.80	2.56	0.45	0.64	2.23	2.27	0.85	1.20
12	■	■	1.70	1.86	0.45	0.63	1.84	1.88	0.73	0.89

atm: atmosphere

P1: Cryovac (70 µ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 µ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XXXVI: Effect of MAP on the Coliform count (log cfu/g) of Mozzarella cheese in different packages stored at –10 to–15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	2.62	2.62	2.62	2.62	2.62	2.62	2.62	2.62	2.62	2.62
1	2.62	2.61	2.54	2.58	2.45	2.40	2.53	2.57	2.46	2.48
2	2.56	2.60	2.46	2.49	2.32	2.37	2.45	2.46	2.36	2.39
3	2.44	2.47	2.23	2.28	2.10	2.12	2.20	2.25	2.13	2.16
4	2.18	2.23	2.11	2.17	2.00	2.05	2.14	2.16	2.05	2.08
5	1.95	1.98	1.87	1.89	1.77	1.81	2.00	2.05	1.80	1.84
6	1.75	1.79	1.62	1.67	1.54	1.58	1.60	1.63	1.56	1.57
7	1.52	1.61	1.45	1.48	1.31	1.39	1.44	1.48	1.35	1.40
8	1.33	1.38	1.31	1.33	1.12	1.15	1.28	1.30	1.14	1.16
9	1.20	1.25	1.14	1.19	1.01	1.06	1.15	1.17	1.05	1.09
10	1.03	1.09	0.95	0.98	0.87	0.89	0.93	0.97	0.88	0.90
11	0.91	0.95	0.80	0.86	0.50	0.57	0.78	0.81	0.54	0.59
12	0.76	0.79	0.63	0.68	0.35	0.38	0.52	.056	0.40	0.46

atm: atmosphere

P1: Cryovac (70 µ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 µ)

* Poly binding agent

Appendix XXXVII: Mean sensory score (based on 3 point scale) for Appearance of MAP Mozzarella cheese stored in different packages at 7±1 ^{0}C (mean of three trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
1	2.9	2.8	2.9	2.8	3.0	3.0	2.9	2.8	2.9	2.9
2	2.7	2.4	2.7	2.6	2.9	2.7	2.7	2.4	2.9	2.7
3	2.5	2.4	2.7	2.5	2.9	2.6	2.5	2.5	2.7	2.6
4	2.3	2.2	2.6	2.4	2.6	2.5	2.4	2.2	2.6	2.4
5	1.9	1.7	2.2	2.0	2.4	2.4	2.2	2.1	2.4	2.2
6	■	■	2.1	2.0	2.4	2.4	2.1	2.0	2.4	2.1
7	■	■	2.0	2.0	2.4	2.3	2.1	1.9	2.3	2.2
8	■	■	2.0	2.0	2.3	2.3	2.0	1.9	2.1	2.0
9	■	■	2.0	2.0	2.3	2.1	1.9	1.8	2.0	1.9
10	■	■	1.9	1.8	2.3	2.1	1.9	1.8	2.0	1.9
11	■	■	1.9	1.8	2.1	2.0	1.9	1.7	1.9	1.8
12	■	■	1.7	1.6	2.0	1.9	1.7	1.6	1.9	1.8

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XXXVIII: Mean sensory score (based on 3 point scale) for Appearance of MAP Mozzarella cheese stored in different packages at –10 to–15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
1	3.0	2.9	2.9	2.9	3.0	3.0	2.9	2.9	2.9	2.9
2	2.8	2.8	2.9	2.9	3.0	3.0	2.9	2.9	2.9	2.8
3	2.8	2.7	2.9	2.9	3.0	2.9	2.8	2.8	2.9	2.7
4	2.6	2.5	2.8	2.7	2.9	2.9	2.7	2.6	2.8	2.4
5	2.4	2.4	2.7	2.5	2.9	2.9	2.5	2.4	2.6	2.6
6	2.3	2.2	2.5	2.4	2.8	2.7	2.5	2.3	2.6	2.5
7	2.2	2.1	2.4	2.3	2.8	2.7	2.4	2.3	2.5	2.3
8	2.0	2.0	2.4	2.3	2.6	2.5	2.3	2.3	2.5	2.3
9	1.9	1.8	2.3	2.2	2.4	2.3	2.3	2.2	2.4	2.2
10	1.8	1.7	1.9	1.9	2.4	2.2	2.0	2.0	2.1	2.0
11	1.7	1.6	1.9	1.8	2.3	2.1	1.9	1.8	2.0	1.9
12	1.5	1.5	1.9	1.7	2.2	2.1	1.8	1.7	2.0	1.9

atm: atmosphere

P1: Cryovac (70 µ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 µ)

* Poly binding agent

Appendix IXL: Mean sensory score (based on 5 point scale) for Body & Texture of MAP Mozzarella cheese stored in different packages at 7±1⁰C (mean of three trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
1	4.5	4.2	4.8	4.8	4.9	4.9	4.7	4.6	4.7	4.6
2	3.9	3.6	4.6	4.6	4.7	4.6	4.4	4.1	4.5	4.3
3	3.5	2.9	4.4	4.3	4.6	4.4	4.1	4.0	4.3	4.2
4	3.0	2.7	3.9	3.8	4.4	4.1	4.0	3.8	4.2	4.2
5	2.8	2.6	3.6	3.4	4.4	4.1	3.9	3.7	4.0	3.8
6	■	■	3.2	3.1	3.9	3.8	3.7	3.1	3.7	3.5
7	■	■	3.0	3.0	3.8	3.7	3.2	3.1	3.6	3.4
8	■	■	2.9	2.8	3.8	3.6	2.9	2.9	3.6	3.3
9	■	■	2.8	2.7	3.3	3.1	2.9	2.8	3.0	2.8
10	■	■	2.7	2.6	3.2	3.0	2.7	2.6	2.9	2.7
11	■	■	2.7	2.5	2.8	2.7	2.7	2.6	2.7	2.5
12	■	■	2.6	2.5	2.7	2.6	2.6	2.5	2.5	2.2

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XL: Mean sensory score (based on 5 point scale) for Body & Texture of MAP Mozzarella cheese stored in different packages at –10 to –15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
1	4.6	4.5	4.8	4.7	4.9	4.8	4.8	4.8	4.8	4.8
2	4.5	4.3	4.6	4.6	4.8	4.7	4.6	4.6	4.5	4.5
3	4.3	4.1	4.6	4.4	4.6	4.6	4.3	4.2	4.5	4.4
4	4.2	4.1	4.3	4.1	4.5	4.3	4.2	4.2	4.4	4.3
5	4.0	4.0	4.0	4.0	4.1	4.0	4.2	4.0	4.2	4.1
6	3.8	3.8	3.8	3.8	3.9	3.9	3.8	3.8	3.9	3.9
7	3.5	3.6	3.7	3.2	3.7	3.7	3.5	3.4	3.7	3.7
8	3.4	3.1	3.5	3.1	3.5	3.2	3.2	3.2	3.6	3.4
9	3.2	3.0	3.0	2.9	3.4	3.1	3.0	2.9	3.4	3.3
10	3.0	2.8	2.9	2.8	3.2	3.0	3.1	3.0	3.1	3.0
11	2.7	2.6	2.8	2.7	3.1	2.9	2.9	2.8	2.9	2.7
12	2.6	2.5	2.7	2.6	3.0	2.9	2.7	2.6	2.8	2.7

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix XLI: Mean sensory score (based on 10 point scale) for Flavour of MAP Mozzarella cheese stored in different packages at 7±1 ^{0}C (mean of three trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7
1	8.6	8.5	8.6	8.4	9.6	9.3	9.0	8.5	9.5	9.1
2	7.5	7.0	8.5	7.8	9.4	8.5	8.7	8.0	9.2	8.8
3	6.8	6.6	8.1	7.6	9.1	8.3	7.6	7.5	8.4	8.2
4	6.2	6.0	8.0	7.4	8.8	8.5	7.4	7.2	8.3	8.2
5	5.6	5.4	7.2	7.1	8.5	7.8	7.3	7.0	8.0	7.7
6	■	■	7.0	7.0	8.0	7.8	7.2	6.9	7.9	7.6
7	■	■	6.9	6.7	7.8	7.7	6.9	6.8	7.8	7.4
8	■	■	6.7	6.6	7.7	7.6	6.8	6.7	7.7	7.3
9	■	■	6.5	6.2	7.5	6.8	6.6	6.6	7.5	7.1
10	■	■	6.0	5.9	7.4	6.8	6.4	6.4	6.8	6.5
11	■	■	5.8	5.4	7.3	6.7	6.2	6.1	6.7	6.5
12	■	■	5.4	5.2	7.2	6.6	6.0	5.8	6.6	6.3

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XLII: Mean sensory score (based on 10 point scale) for Flavour of MAP Mozzarella cheese stored in different packages at –10 to–15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	9.7	9.7	9.7	9.7	9.7	9.6	9.7	9.7	9.7	9.7
1	8.2	8.3	8.7	8.5	9.6	9.4	9.3	9.0	9.5	9.3
2	8.1	8.0	8.4	8.0	9.1	8.8	8.7	8.2	9.5	8.6
3	7.9	7.6	8.1	7.9	9.0	8.5	8.6	8.1	8.9	8.5
4	7.5	7.3	7.8	7.6	8.9	8.6	8.3	7.9	8.4	8.3
5	7.3	7.2	7.7	7.5	8.5	8.1	8.1	7.6	8.3	7.9
6	7.1	7.0	7.5	7.2	8.3	7.8	7.9	7.5	8.0	7.9
7	7.0	6.8	7.4	7.1	8.0	7.6	7.8	7.4	8.0	7.5
8	6.9	6.7	7.4	7.0	7.8	7.4	7.5	7.2	7.7	7.3
9	6.8	6.5	7.2	7.0	7.6	7.3	7.3	7.0	7.4	7.2
10	6.5	6.4	7.0	6.9	7.5	7.1	6.9	6.7	7.3	7.0
11	5.7	5.6	6.4	6.3	7.4	7.0	6.8	6.6	6.9	6.8
12	5.3	5.1	5.5	5.3	7.4	6.9	6.1	5.7	6.5	6.2

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix XLIII: Mean sensory score (based on 18 point scale) for Overall Acceptability of MAP Mozzarella cheese stored in different packages at 7±1 ^0C (mean of three trials)

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	17.6	17.6	17.6	17.6	17.6	17.6	17.6	17.6	17.6	17.6
1	16.0	15.5	16.3	16.0	17.5	17.2	16.6	15.9	17.1	16.6
2	14.1	13.0	15.8	15.0	17.0	15.8	15.8	14.5	16.6	15.8
3	12.8	11.9	15.2	14.4	16.6	15.3	14.2	14.0	15.4	15.0
4	11.5	10.9	14.5	13.6	15.8	15.1	13.8	13.2	15.1	14.8
5	10.3	9.7	13.0	12.7	15.3	14.3	13.4	12.8	14.4	13.7
6	■	■	12.3	12.1	14.3	14.0	13.0	12.0	14.0	13.2
7	■	■	11.9	11.7	14.0	13.7	12.2	11.8	13.7	13.0
8	■	■	11.9	11.7	13.9	13.7	12.1	11.8	13.5	12.8
9	■	■	11.3	10.9	13.1	12.0	11.4	11.2	12.5	11.8
10	■	■	10.4	9.7	12.2	11.4	10.8	10.4	11.3	10.8
11	■	■	9.8	9.3	12.0	11.2	10.4	10.0	11.2	10.6
12	■	■	9.2	8.8	10.7	9.8	9.3	9.2	10.4	9.8

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

■ Sample spoiled hence analysis discontinued

Appendix XLIV: Mean sensory score (based on 18 point scale) for Overall Acceptability of MAP Mozzarella cheese stored in different packages at –10 to–15 ^{0}C (mean of 3 trials)

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	17.6	17.6	17.6	17.6	17.6	17.6	17.6	17.6	17.6	17.6
1	15.8	15.7	16.4	16.1	17.5	17.2	17.0	16.7	17.2	17.0
2	15.4	15.1	15.9	15.5	16.9	16.5	16.2	15.7	16.9	15.9
3	15.2	14.9	15.9	15.3	16.7	16.4	15.9	15.3	16.9	15.8
4	14.9	14.6	15.5	14.8	16.5	16.0	15.6	15.0	16.7	15.3
5	13.9	13.7	14.5	14.2	15.5	15.0	15.0	14.2	15.3	14.4
6	13.2	13.0	13.8	13.4	15.0	14.4	14.2	13.6	14.5	14.3
7	12.7	12.5	13.5	12.6	14.5	14.0	13.7	13.1	14.2	13.5
8	12.1	11.7	12.8	12.2	13.8	13.0	12.8	12.4	13.6	12.9
9	11.9	11.3	12.5	12.1	13.4	12.7	12.6	12.1	13.2	12.7
10	11.8	11.2	12.1	11.8	13.4	12.6	12.3	11.9	12.9	12.5
11	10.1	9.8	11.1	10.8	12.8	12	11.6	11.2	11.8	11.4
12	10.6	10.2	11.0	10.6	13.5	12.7	11.7	11.1	12.2	11.6

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

Appendix XLV:Mean Hedonic sensory score (based on 5 point scale) for Pizza made from MAP Mozzarella cheese stored in different packages at 7±1 ^{0}C (mean of three trials)**

Period of storage (weeks)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
1	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
2	4.4	4.1	4.4	4.3	5.0	5.0	4.6	4.4	4.8	4.8
3	4.3	4.2	4.3	4.2	4.8	4.7	4.4	4.2	4.6	4.4
4	3.7	3.6	4.1	4.0	4.6	4.5	4.3	4.2	4.3	4.2
5	2.2	1.8	4.0	3.9	4.6	4.4	4.2	4.0	4.1	3.9
6	■	■	3.9	3.8	4.5	4.2	4.1	3.9	4.1	3.8
7	■	■	3.7	3.6	4.4	4.2	4.0	3.8	4.0	3.8
8	■	■	3.5	3.4	4.2	4.1	3.9	3.7	3.9	3.7
9	■	■	3.3	3.2	4.2	4.1	3.7	3.5	3.8	3.6
10	■	■	3.1	2.9	4.2	4.0	3.4	3.1	3.7	3.6
11	■	■	2.6	2.4	4.1	4.0	2.9	2.5	3.7	3.5
12	■	■	2.5	2.3	3.9	3.8	2.8	2.5	3.6	3.4

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

** Excellent 5; Very good 4; Good 3; Fair 2; Poor 1.

■ Sample spoiled hence analysis discontinued

Appendix XLVI: Mean Hedonic sensory score (based on 5 point scale) for Pizza made from MAP Mozzarella cheese stored in different packages at –10 to–15 ^{0}C (mean of three trials)**

Period of storage (months)	Treatment given to package									
	atm 1 air		atm 2 vacuum		atm 3 100% CO_2		atm 4 100% N_2		atm 5 50% CO_2 : 50% N_2	
	Type of package									
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
1	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
2	4.8	4.8	4.9	4.8	5.0	5.0	5.0	4.8	5.0	5.0
3	4.7	4.6	4.7	4.4	4.8	4.8	4.8	4.7	4.8	4.8
4	4.5	4.3	4.5	4.1	4.7	4.5	4.4	4.2	4.5	4.3
5	4.3	4.1	4.3	4.0	4.3	4.2	4.3	4.0	4.4	4.2
6	4.2	4.0	4.0	3.9	4.2	4.2	4.2	3.8	4.3	4.2
7	3.9	3.8	3.9	3.9	4.2	4.1	4.1	3.4	4.1	4.0
8	3.6	3.4	3.9	3.8	4.2	4.1	3.9	3.4	3.9	3.8
9	3.5	3.2	3.7	3.5	4.0	3.8	3.9	3.3	3.8	3.7
10	3.1	3.0	3.4	3.3	3.9	3.7	3.5	3.2	3.6	3.5
11	2.9	2.8	3.3	3.2	3.7	3.5	3.3	3.1	3.4	3.4
12	2.7	2.6	3.1	3.0	3.6	3.2	3.2	3.0	3.4	3.3

atm: atmosphere

P1: Cryovac (70 μ)

P2: LLD /BA*/Nylon-6/ BA*/ LDPE (110 μ)

* Poly binding agent

** Excellent 5; Very good 4; Good 3; Fair 2; Poor 1.

Appendix XLVII. Score card for Mozzarella cheese

Date…………Batch no………….. **Code…………………...**

Appearance Excellent Score = 3

Panel list	Code number							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Acid cut								
Mold								
Mottled								
Rough surface								
Soiled surface								
Wavy								
Slimy								
Unclean								
Score for appearance								

Body / Texture Excellent score = 5

Panel list	Code number							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Coarse								
Gassy								
Lack of flexibility								
Mealy								
Pasty								
Sweet holes								
Weak								
Score for body/texture								

Flavour Excellent score = 10

Panel list	Code number							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Acid								
Bitter								
Flat								
Fruity								
Lipolysed								
Musty								
Sour								
Yeasty								
Panelist Score for flavour								
Total score for each sample (excellent score = 18)								
Placement of each sample in the group								

Name of Judge **Signature of Judge** **Date…………**

Appendix XLVIII. SCORE CARD FOR PIZZA

Kindly evaluate the samples of pizza on the basis of scale given below:

Excellent	5
Very Good	4
Good	3
Fair	2
Poor	1

Sample 1 ☐

Sample 2 ☐

Sample 3 ☐

Sample 4 ☐

Sample 5 ☐

Comments, if any ____________________

____

Name of the Judge ____________________

Signature with Date