STUDIES ON THE MODIFIED ATMOSPHERE PACKAGING (MAP) OF PIZZA

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
PREETI SINGH

DIVISION OF DAIRY TECHNOLOGY
NATIONAL DAIRY RESEARCH INSTITUTE
(I. C. A. R.)
KARNAL - 132001 (HARYANA), INDIA
2006

Regn. No. 1030204

DEDICATED

TO

MY REVERED

PARENTS & GUIDE,

Dr. G. K. GOYAL

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Approved by:

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This is to certify that the thesis entitled, **"STUDIES ON THE MODIFIED ATMOSPHERE PACKAGING (MAP) OF PIZZA"** submitted by **Ms. PREETI SINGH**, M.Sc. (Food 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 her under my guidance, and no part of the thesis has been submitted for any other degree or diploma.

2.6.06

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Dated: 2nd June, 2006 *Preeti* *(PREETI SINGH)*

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LIST OF ABBREVIATIONS AND SYMBOLS

μ	:	micron
°C	:	Degree Celsius
μg	:	microgram
@	:	at the rate of
atm	:	atmosphere
a_w	:	Water activity
BA	:	Binding Agent
cm	:	centimetre
dia	:	diameter
g	:	gram
h	:	hour
IS	:	Indian Standards
IU	:	International Unit
LDPE	:	Low Density Polyethylene
lit	:	litre
LLD	:	Linear Low Density Polyethylene
LTLT	:	Low Temperature Long Time
MAP	:	Modified Atmosphere Packaging
mg	:	milligram
min	:	minute
ml	:	millilitre
MT	:	million tonnes
nm	:	nanometre
Pa	:	Pascal
ppm	:	parts per million
RDA	:	Recommended Daily Allowances
RH	:	Relative Humidity
s	:	seconds
sq m	:	square metre
α	:	directly proportional

ABSTRACT

The packaging concept is determined by the demand of both the consumer and the product. New technological developments, environmental awareness, and changes in the consumer market force the packaging technologists to consider an increasing number of factors when designing a package. The food industry is innovative and has gone through many changes in the past two decades, mostly due to the development and implementation of new technology to meet growing consumer demands for convenience products. 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 foods by control of moisture loss or gain or **by the exclusion of oxygen or by inclusion of other gases**. Packaging can be best defined as a coordinated system of preparing goods for transport, distribution, storage, retailing and end-use: a means of ensuring safe delivery to the ultimate consumer in sound condition at minimum cost. 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. This preservation technique is being used extensively for shelf life extension of foods in Europe and is fastly gaining acceptance in the U.S. and other western countries. Due to cushioning effect provided by the MAP, it also saves the product from compression during storage and transportation. The changing life patterns and food habits of people and their growing preferences have catapulted the market to grow for convenience and fast foods. The pizzas

market is growing very fast @ 30% per year and amounts to over Rs 102 crores. Mozzarella cheese provides mild flavour, visual appeal and characteristic texture when melted on the surface of a pizza. The melted cheese is very elastic and is very stretchy and stringy, contributing to the sensory appeal and "fun factor". At present the shelf life of pizza is hardly one day. Its low shelf life is mainly due to microbial and physico-chemical changes. Hence, a study was planned to increase the shelf life of pizza by using MAP technique. Two types of pizza samples namely unbaked (ready-to-bake) and baked (ready-to-serve) were packaged in high barrier bags (LLD/BA*/Nylon-6/BA*/LDPE) under four different atmospheres (air: atm 1; 100% CO_2 : atm 2; 100% N_2 : atm 3 and 50% CO_2 / 50% N_2 : atm 4) and stored at 7±1 °C. Periodically, both types of pizza samples were evaluated for changes in physico-chemical, textural, microbiological and sensory characteristics. The experiments confirmed the preservative effect of CO_2 under MAP. Amongst four atmospheres studied, atm 2 > atm 4 > atm 3 > atm 1 respectively, in descending order. The studies further revealed that the shelf life of pizza could be increased upto 15 days (a 300% increase) for ready-to-bake pizza, and 45 days (a 300% increase) for ready-to-serve pizza at 7±1°C by using MAP technique.

सारांश

उपभोक्ता तथा उत्पाद दोनों की मांग के अनुसार पैकेजिंग की धारणा निश्चित की जाती है। नयी तकनीकों का विकास, वातावरण के प्रति चेतना, तथा बाजार के प्रति उपभोक्ता का बदलाव, पैकेजिंग शिल्प वैज्ञानिकों को पैकेज डिजाईन करते समय विभिन्न कारकों पर ध्यान देने के लिए प्रेरित करते हैं। खाद्य उद्योग खोजी है तथा यह मुख्यतः उपभोक्ता की सुलभ खाद्य पदार्थों के प्रति बढ़ती हुई मांग को पूरा करने के लिए नयी तकनीक के विकास तथा इनको लागू करने के लिए खाद्य उद्योग पिछले दो दशकों में कई परिवर्तनों से गुजरा है। उपयुक्त अवरोधक लक्षणों वाली पैकेजिंग तकनीक और पैक का चुनाव खाद्य पदार्थ की प्रकृति के अनुसार, सूक्ष्मजीवों और कीटों के आक्रमण से संदूषण रोकने के अतिरिक्त पोषणिक मान व गुणवता का सरंक्षण, ऑक्सीजन अपवर्जन व नमी क्षय व लब्धि को अवरूद्ध करने के लिए किया जाता है। पैकेजिंग को एक समन्वयी पद्धति के रूप में सबसे अच्छी प्रकार परिभाषित किया जा सकता है। जिसमें वस्तुओं का परिवहन, वितरण, भण्डारण, विक्रय तथा अन्तिम उपभोग, यानि कि उत्पाद को चरम उपभोक्ता तक कम लागत में अच्छी स्थिति में पहुंचाना है। परम्परागत खाद्य पैकेजिंग, खाद्य पदार्थ को बाहरी सूक्ष्मजीवों के संदूषण, ऑक्सीजन, जल-वाष्प तथा प्रकाश से बचाती है। यद्यपि नयी पैकेजिंग तकनीक जैसे कि परिवर्तित वातावरण संवेष्टन (मैप), बाहरी आक्रमणों से बचाव के अतिरिक्त और भी कार्य करती है। यूरोप में यह संवेष्टन पद्धति खाद्य पदार्थों की निधानी आयु बढ़ाने के लिए प्रमुखता से उपयोग की जा रही है और अमेरिका तथा अन्य पश्चिमी देशों में तेजी से अपनाई जा रही है। मैप द्वारा उपलब्ध कराये गये प्रशामक प्रभाव के कारण, यह उत्पाद को भण्डारण तथा वितरण के दौरान संपीडन से बचाता है। लोगों की बदलती हुई जीवन तथा खाद्य शैलियों और बढ़ती हुई प्राथमिकताओं ने सुलभ एवं फास्ट खाद्यों के बाजार को बढ़ोत्तरी दी है। पिज्ज़ा का बाजार 30 प्रतिशत प्रति वर्ष की दर से बढ़ रहा है तथा 102 करोड़ रूपये से अधिक मूल्य का है। मौज़ेरेला चीज़ जब पिज्ज़ा की सतह पर पिघलती है तब मृदु खुशबू, चाक्षुष आकर्षण तथा

विशिष्ट गठन प्रदान करती है। पिघली हुई चीज बहुत ही इलास्टिक, लचकदार एंव तार की तरह होती है जो कि संवेदिक आकर्षण तथा आमोदजनक कारक में योगदान करती है। वर्तमान में पिज़्ज़ा की निधानी आयु मुश्किल से एक दिन होती है। सूक्ष्मजैविक एंव भौतिक-रासायनिक परिवर्तन इसकी कम निधानी आयु के प्रमुख कारण है। इसलिए इसकी निधानी आयु मैप तकनीक द्वारा बढ़ाने हेतु यह अध्ययन नियोजित किया गया था। दो प्रकार के पिज़्ज़ा अर्थात् अभर्जित (भर्जन के लिए तैयार) एवं भर्जित (परोसने के लिए तैयार) उच्च अवरोधक प्लास्टिक थैलियों (एल.एल.डी./बी.ए./नाईलोन-6/बी.ए./एल.डी.पी.ई.) में चार विभिन्न परिवेशों (वायु : परिवेश 1, 100 प्रतिशत कार्बन-डाई-ऑक्साइड : परिवेश 2, 100 प्रतिशत नाईट्रोजन : परिवेश 3 और 50 प्रतिशत कार्बन-डाई-ऑक्साइड/50 प्रतिशत नाईट्रोजन : परिवेश 4) में पैक किये गये संवेष्टित (पैकेज्ड) नमूने 7 ± 1^0 से० के तापमान पर रखे गये। निश्चित अन्तराल के बाद दोनों प्रकार के पिज़्ज़ा नमूनों का भौतिक-रासायनिक, संरचनात्मक, सूक्ष्मजैविक और संवेदिक लक्षणों में बदलाव के लिए परीक्षण किया गया। अनुप्रयोगों से इस बात की पुष्टि हुई है कि पिज़्ज़ा की निधानी आयु बढ़ाने के लिए मैप में कार्बन-डाई -ऑक्साइड का सर्वाधिक प्रभाव होता है। चार परिवेशों के अध्ययन में, अनुकूलतम परिवेशों का वरीयता क्रम इस प्रकार पाया गया : परिवेश 2 > परिवेश 4 > परिवेश 3 > परिवेश 1। शोध से यह भी निष्कर्ष निकला कि मैप से अभर्जित (भर्जन के लिए तैयार) पिज्जा की निधानी आयु 7 ± 1^0 से० पर 15 दिन तक (300 प्रतिशत वृद्धि) तथा भर्जित (परोसने के लिए तैयार) पिज़्ज़ा की निधानी आयु 45 दिन तक (300 प्रतिशत वृद्धि) बढ़ाई जा सकती है।

CHAPTER 1

INTRODUCTION

INTRODUCTION

1.0.0.0

In today's society, packaging is pervasive and essential. It surrounds, enhances and protects the goods we buy, from processing and manufacturing through handling and storage to the final consumer. One of the major objectives of packaging of food is to protect it against spoilage or deterioration attributable to physical damage, chemical changes or microbial growth, thereby delivering food of the same high quality to the consumer. A package constitutes a vital link between the manufacturer and the end user for the safe delivery of the product.

According to U.K. Institute of Packaging "Packaging can be best defined as a coordinated system of preparing goods for transport, distribution, storage, retailing and end-use: a means of ensuring safe delivery to the ultimate consumer in sound condition at minimum cost". Packaging has also been described as a "complex, dynamic, scientific and controversial segment of business". Packaging 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. At its most sophisticated functions, it contains, protects, preserves, and informs besides boost in selling and convenience. Worldwide packaging industry is estimated to be more than $350 billion. In India, around 16,000 units are engaged in packaging industry. Projected growth rate of demand and consumption for packaging in India is 10-12% (Tanweer Alam *et al.*, 2005).

1.1.0.0 Importance of Packaging

The packaging concept is determined by the demand of both the consumer and the product. New technological developments, environmental awareness, and changes in the consumer market force the packaging technologists to consider an increasing number of factors when designing a package (Goyal and Tanweer Alam, 2006). Packages are clearly an integral part of the manufacturing and distribution processes, and package design has a great significance on the success of foodstuffs. Presently packages face difficult challenges and roles. Food must be available wherever there are people, and with modern lifestyle patterns this is seldom where it is grown or manufactured. Food, in good quality, 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 control of moisture loss or gain or by the exclusion of oxygen or by inclusion of other gases. In competitive market where quality of the products is high, the only difference between brands lies in the packaging.

The shelf life of high moisture and high fat food products is very limited mainly due to the chemical effect of atmospheric oxygen and growth of spoilage microorganisms. Both these factors, alone or in conjunction with one another, result in changes in colour, flavour, odour and overall deterioration of the product. Several methods have been used by various workers to slow down or inhibit these deteriorative changes, which included freezing, heat processing, brining, irradiation, and the use of chemical additives and preservatives. However, increased energy cost associated with freezing and heat processing, and growing consumer concerns about preservatives has forced the food industry to look for alternative methods of food preservation. Product compression is unavoidable and makes vacuum packaging unsuitable for many products (Tanweer Alam and Goyal, 2004). Modified Atmosphere Packaging (MAP), a preservation technique used extensively for shelf life extension of foods in Europe, is fastly gaining acceptance in the USA and other western countries. MAP also saves the product from compression during storage and transportation.

1.2.0.0 Modified Atmosphere Packaging (MAP)

A modified atmosphere, as the name implies, is one in which the normal composition of air is changed or "modified" within a high barrier package. The technique of MAP has been developed partly to retard the activity and growth of bacteria and fungi, and partly to delay biochemical reactions such as oxidation. Modified atmosphere packaging (MAP) reduces physiological changes, respiration rates, oxidative deterioration and microbial growth by changing the levels of gas that surrounds the product. The two major spoilage agents, namely aerobic bacteria and oxidative reactions require oxygen. Therefore, its unavailability is expected to inhibit spoilage and thus maximize quality and/or storage life. Some deterioration however, may occur due to anaerobic / microaerophillic organisms and non-oxidative reactions. This is usually minimised by chilled storage. MAP is extensively used to preserve a wide array of food products (Church and Parson, 1995; Phillips, 1996; Farber, 1991). The technology of packaging products in modified atmosphere is the most advanced food preserving technique with many advantages (Floros *et al.*, 2000). This method of packaging replaces the air headspace of package with a gas or mixture of gases like nitrogen and carbon dioxide. MAP is used to maintain the product's initial quality for much longer period and to extend the product's shelf life. It also helps in increasing the wide range of distribution, prevents mechanical damage to the product by adding a 'cushion of gas around the product', and retains appeal to consumers.

1.3.0.0 Need of MAP for Pizza

It's now the pizzas that drive the Dairy Industry in India. The rapid urbanization and changes in traditional family structure, and fast life style have influenced the eating patterns/food habits and food choices in the society. Now people have less time to stay in kitchen, consequently demand for good quality ready-to-serve fast food has increased many folds. In India, the fast food market is estimated at Rs. 400 crores with a growth rate of 10-12% per annum. Earlier Pizza, a fast food was available only at five-star hotels, but now it has almost become a product of mass consumption and a household item. Pizzerias of the world have in fact helped the food industry, by subtly creating a taste for dairy products such as mozzarella cheese in non-traditional markets. As a consequence, greater opportunities have opened up in the dairy-processing segment, buoyed further by increasing urbanization. Reports indicate that the consumption of dairy products as ingredients in other foods is on upright trend, as in case of mozzarella cheese being the ultimate dairy ingredient in pizza (Anon, 2003 a).

Pizza, one of the most commonly purchased items in retail food stores, has maintained its market share through the changing nature of the processed foods industry and even grown in popularity. Americans consume 100 acres of pizza per day. The great pizza boom has arrived in India and the hype it has created is so great that in the last few years innumerable pizzerias have mushroomed all over the country even in small towns. The nationwide market has been taken over by world famous pizza chains like Dominos, Pizza Hut, Pizza Corner, Amul, Nirula's etc (Anon, 2003 b). Recently the Gujarat Co-operative Milk Marketing Federation (GCMMF) better known by its Amul brand has plans to turn pizza into mass consumption product (Anon 2002). In India due to spiralling demand for pizza, its consumption is increasing very fast and there is tremendous potential for its growth. At present its market is growing very fast @ 30% per year and amounts to over Rs 102 crores.

At present the shelf life of pizza is hardly one day. Its low shelf life is mainly due to microbial and physico-chemical changes. Hence, there is an alarming need to enhance the keeping quality of pizza substantially by employing most advanced technique of packaging namely modified atmosphere packaging so that pizza could be made available as ready-to-serve fast food throughout the year not only in metro cities but even in small towns, including its export with enhanced shelf life.

OBJECTIVES:

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

1. To study the influence of MAP conditions on the shelf life of unbaked (ready-to-bake) and baked (ready-to-serve) pizza.
2. To study the changes in physico-chemical and microbial quality of pizza during storage.
3. To evaluate the sensory quality of modified atmosphere packed pizza.

CHAPTER 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.0.0.0

The main function of packaging is to protect the packaged product against any type of deterioration, be it of chemical, microbial, biological or physical nature. It constitutes a vital link between the manufacturer and the end user for the safe delivery of the product. Generally a package performs the following three functions: (i) contains the food product, (ii) protects and preserves the quality of that product, (iii) appeals to or attracts the customer. The World War II exerted a profound effect on food packaging when food packers started using for the first time the new media of pouch packing, waxed paperboards and water proofing (Anderson, 1964).

The food industry is innovative and has gone through many changes in the past two decades, mostly due to the development and implementation of new technology to meet growing consumer demands for convenience products. The changing life patterns and food habits of people and their growing preferences have catapulted the market to grow for convenience and fast foods. It is in this context that the development of modified atmosphere packaging has taken place so rapidly over the past decade.

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

2.1.0.0 Modified Atmosphere Packaging (MAP)

MAP surrounds the food products in high barrier packaging materials, in which gaseous environment is modified (Inns, 1987). MAP reduces microbial growth, and retards enzymatic spoilage with the final effect of increasing the shelf life of the product (Young *et al.,* 1988; Floros *et al.,* 2000). The first practical use of modified atmosphere (MA) in 1930 was applied in preservation of fresh meat in New Zealand and Australia. A big turning point in MAP technology came in 1981, when Marks & Spencer Co in U.K. introduced a wide range of fresh meat products packaged under MAP. The impetus behind these products came mainly from an increased consumer demand for preservative-free, fresh and chilled products (Tanweer Alam and Goyal, 2004). MAP extended the shelf life of meat, poultry and fresh produce by 50-400% and the quality was maintained beyond the expected shelf life inhibiting the growth of aerobic psychrotrophs (Genigeorgis, 1985).

MAP markedly extends the shelf life of many perishable foods (Hotchkiss, 1988) and hence undergone developments to meet the ever-growing consumer demand for fresh, high quality convenience foods (Brody, 1989). Generally N_2, CO_2 and O_2 gases or their mixtures are used in MAP. MAP technology has been successfully used to extend the shelf life of a wide range of food products including fish, meat, sandwiches, salads and vegetables (Church and Parson, 1995; Phillips, 1996; Sahoo and Anjaneyulu, 1999).

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

MAP technology has been more successful in European countries. Currently, the U.K. is the leader in MAP technology, accounting for about half of the European market, followed by France, which has approx. 25% of the MAP packaged food market. It is estimated that between 300-500 companies use MAP technology for shelf life extension and distribution of foods. With respect to the North America market, it is estimated that the demand for MAP foods could reach 11 billion packages by the year 2005 (Smith *et al.*, 2004).

2.1.1.0 Parameters Critical to MAP

The initial organoleptic and microbial quality of raw material together with temperature control throughout the processing chain is the most important factors for MAP food. The other factors that affect the success of MAP are packaging materials, packaging equipments, and gas mixtures (Tanweer Alam, 2004). The whole MAP chain of food product could be divided into 4 stages. The first stage should deliver pre-specified and pre-cooled products at their best quality. Here the maintenance of low temperature is crucial and critical and pathogen contamination should be properly tackled. MAP can be applied at this stage during transportation or short-term bulk storage. In the second stage, the product is formulated and processed as per the consumers need. At this point, cleaning, grading chemical and microbial checks are essentially performed to make sure the wholesomeness and safety of all the ingredients and products under strict hygienic conditions. In other words, the multiple barrier principle, HACCP, ISO and Codex Alimentarius should be used to resist the pathogen attack. The third stage involves gas flushing and packaging followed by transportation and retail storage, which is the fourth stage (Kader, 1985).

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 & consumption

2.1.2.0 Safety Concerns

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% (Lioutas, 1988). The elevated CO_2 extends the lag phase of bacterial growth and slows the propagation of bacteria, while low O_2 favours mesophilic microbes such as *Listeria* and lactic acid bacteria (Brackett, 1996). It has been observed that 20-30% CO_2 or even 10% CO_2 was sufficient to retard the bacterial growth (Seideman and Durland, 1984) and higher levels of CO_2 were found to be more effective in inhibiting the growth of *Staphylococcus aureus*, *Salmonella, Escherichia coli*, and *Yersinia enterocolitica*. The use of MAP for any food product, which is subsequently cooked, is less hazardous because cooking would kill all vegetative pathogens. Leistener (1995) observed that the combination of chill temperature and MAP results in longer shelf life.

2.1.3.0 Characteristics of Packaging Materials

The packaging materials for MAP should have good mechanical strength to withstand machine handling and subsequent storage. The selected packaging material is very important factor in MAP operation. The packaging material for MAP must have a high gas barrier and low water vapour transmission rate (WVTR). Generally, packages used for MAP are based on thermoplastic polymers. The impermeability of packaging film is the most important factor that influences the anti-microbial effect of CO_2. 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 food products. The packaging of non-respiring product includes nylon/PE/nylon/PVdC/PE or nylon/EVOH/PE. Selby (1968) reported that high barrier films had low WVTR, good resistance to grease, high temperature stability and low oxygen transmission rate (OTR). Tanweer Alam (2004) used high barrier bags namely cryovac (70 μ) and LLD/BA*/Nylon-6/BA*/LDPE (110 μ) (*poly binding agent) for storage of MA packed mozzarella cheese.

Characteristics of an ideal film

According to Church (1994), Kader *et al.* (1989) and Smolander *et al.* (1997), the ideal film for MAP must possess the characteristics of 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, and ease of printing for labelling purpose.

Table: Important factors in selection of packaging materials for MAP

(Smith, 1993)

Property	Desirable characteristics
Barrier properties	Permeability to various gases 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

2.1.4.0 Barrier Properties

The choice of packaging material is an important factor in any MAP operation. A low WVTR together with a high gas barrier must generally be achieved in selected package. 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 are used for MAP of food products. The protection against dehydration is achieved by using films with low WVTR like Al, PVC, PVdC, HDPE (Day, 1993). The layer of Al foil provides a complete barrier to gas transmission and could be advantageously used in MAP (Inns, 1987; Sahoo and Anjaneyulu, 1995; Floros *et al.,* 2000).

Zagory and Kader (1988) suggested some laminates (as given in the following table), which could be used for MAP foods:

Table: Laminates for MAP foods

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

2.1.5.0 Packaging Equipments

The MAP technique involves 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 (Smith *et al.* 1995), which could be achieved by using three types of equipments, continuous forming equipment, thermoforming equipment and snorkel or bulk packaging equipment.

The basic MAP formats can be classified as: 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 is 'bag-in-box', 'master pack' and 'mother pack' systems, which allow centralized packaging operations. These can be MA packed by using rigid and semi rigid tray packers; flexible horizontal flow seal packers; and bulk box / drum packers (Church, 1994).

2.1.6.0 Gases for MAP

Several gases are used for MAP of food products depending upon the nature of the product. However, O_2, N_2 and CO_2 or their mixtures are widely used in the MAP for foods. Other gases which are used in MAP (Day, 1992) are: CO (for maintaining the red colour of meat), helium, ethylene oxide, nitrous oxide, ozone, neon, argon, propylene oxide, and ethanol vapour (used for bakery products), hydrogen, sulphur dioxide and chlorine.

2.1.6.1 Carbon Dioxide (CO_2)

Due to its bacteriostatic and fungistatic properties, CO_2 is considered to be the most important gas for MAP of foods. Valley (1928) reported that Pasteur and Joubart observed *Bacillus anthracis* could be killed by using CO_2. Kolbe (1882) published the first article on the preservative effect of CO_2 on foods revealing extended storage life for beef placed inside a cylinder filled with CO_2 atmosphere. Davies (1995) reported that storing apples in atmospheres containing low levels of O_2 and increased level of CO_2 enhanced the shelf life.

Carbon dioxide 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 observation by several investigators that the effect of CO_2 is increased at low temperatures tends to support the theory that CO_2 acts first by dissolving in the liquid phase. Barnett *et al.* (1971) reported this to occur to a minimum temperature of 1°C, below which no additional bactericidal effect was gained. The solubility of CO_2 in water has been reported (Knoche, 1980) as 3.38 g / kg H_2O (179.7 cm^3 / 100 ml) at 0 °C and 1atm. However, the solubility reduced to 1.73 g / kg H_2O at 20 °C and thus readily absorbed by high moisture refrigerated foods. 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 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 (Tanweer Alam, 2004).

Jennifer (1998) observed that CO_2 directly inhibited the growth of bacteria and moulds, i.e. bacteriostatic and mycostatic effect, but the cell-inhibiting spectrum and preservation of CO_2 was not well understood. However, 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. The work of Wolfe (1980), Daniels *et al.* (1985), Dixon and Kell (1989) and Farber (1991) on the influence of CO_2 on bacterial cell revealed 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 and displacement of O_2.

It has been noted by Dixon and Kell (1989) that CO_2 may inhibit cell division and cause alterations in cell morphology, glucose uptake rates and amino acid absorption, it would be reasonable to suggest that such effects of CO_2 may be associated with changes in the function of the biological membrane. It was suggested that CO_2 interacts with lipids of the cell membrane, decreasing the ability of the cell to uptake various ions. The inhibitory and stimulatory effects of CO_2 on microorganisms in culture medium or food system are dependent on 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). Several reports 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). The inhibitory effect of CO_2 on the growth rate of *Pseudomonas aeruginosa* showed a linear correlation between the growth inhibition of *Pseudomonas aeruginosa* and CO_2 concentration (King and Nagel, 1967; Moir *et al.,* 1993).

2.1.6.2 Nitrogen (N_2)

Nitrogen is the most abundant component in air (~ 78%) and can be used in either gaseous or liquid form. It is physiologically inert and tasteless, and is used in packaging primarily as filler and to exclude other more reactive gases. It is sparingly soluble in water (2.33 ml/100 ml at 0 ºC). N_2 by displacing O_2 in the pack delays oxidative rancidity and also inhibits the growth of aerobic microorganisms indirectly (Sahoo and Anjaneyulu, 1995). It is commonly used as a cushion, ballast, and balance gas-replacing oxygen either as an alternative to vacuum packaging when the product is fragile, or to limit pack collapse caused by absorption of CO_2 (Church and Parson, 1995).

2.1.6.3 Oxygen (O_2)

Most of the reactions with food constituents involving O_2 are degradation reactions resulting in the oxidative breakdown of foods into their constitutive parts. O_2 combines readily with fats and oils and causes rancidity. In addition, most spoilage microorganisms require O_2 to grow and cause off-odours in the presence of sufficient O_2. Oxygen is implicated in staling of bakery goods and pasta. O_2 permeates through plastic polymers at rates depending on the polymer, but it generally permeates more slowly than CO_2. It is very soluble in water, especially in cold water, (179.7 ml/100 ml at 0 ºC), and is readily absorbed by high moisture, refrigerated foods.

Most aerobic bacteria need O_2 for cell division and growth. Also, fruits and vegetables require O_2 for respiration. For retaining the attractive bloom of red meats, the presence of O_2 is essential. The disadvantage of the presence of O_2 includes the oxidation of unsaturated fatty acids in lipids, which lead to rancidity and eventual spoilage (Robertson, 1993; Brody and Marsh, 1997), while lowering the O_2 level in MAP environment help in extending the shelf-life of the product by reducing metabolic and chemical oxidation rates but the outgrowth of anaerobic pathogens might be stimulated (Jones, 1989).

2.2.0.0 Dairy Products

2.2.1.0 Milk

Robertson (1993) successfully improved the keeping quality of cooled milk by CO_2 treatment prior to refrigeration. CO_2 infusion into milk caused decrease of coliform and psychrotrophic bacterial growth. The effect of CO_2 was more significant when the concentrations of CO_2 were increased and the storage temperatures were decreased. The benefit of CO_2 treatment is also dependent on the initial contamination of the raw milk. Hotchkiss *et al.* (1999), King and Mabbit (1982) and Rashed *et al.* (1986) were of the view that the addition of CO_2 could control the growth of psychrotrophic bacteria in both raw and pasteurised milks at refrigeration temperatures. Ganguli (2001) opined that carbonation could extend the storage life of bulk-chilled milk without affecting the stability of vitamin A and E.

According to Hoffman (1906), the bacterial count of milk could be reduced by 50 atm of CO_2, but sterilization was still never obtained. Milk kept under 10 atm of CO_2 remained in good condition for 72 h, whereas untreated milk curdled in 24 h. Increased pressures of CO_2 delayed lactic fermentation. Hotchkiss (1995) reported that MA packed milk lasted more than two months in a refrigerator, tasted fresh and contained no dangerous bacteria. Hotchkiss and Lee (1996) conducted experiments to study the effect of CO_2 addition on the shelf life of milk inoculated with spoilage microorganisms, and observed that the shelf life of milk enhanced by 50-100% and these were dependent on flushing of CO_2, storage temperature and high barrier package.

2.2.2.0 Danedar *Khoa*

Sharma *et al.* (2001) extended the shelf life of Danedar k*hoa* (the main ingredient of khoa-based sweets) up to 60 days at 11 °C by packaging under nitrogen / vacuum in a 3-ply package which consisted of poster paper/ aluminium foil / LDPE.

2.2.3.0 Butter

The studies undertaken by Hunziker (1924) on the effect of CO_2 on butter indicated that carbonation could not be relied upon as a means of destroying bacteria present in cream and rendering such cream, or the butter made from this cream, safe for human consumption. Prucha *et al.* (1925) also observed that carbonation could not be relied upon to improve the keeping quality or to prevent flavour deterioration of the resulting butter. If it was made from unpasteurized cream, carbonated butter developed the usual bacterial flavour defects. It was concluded that if any appreciable benefit was to be obtained from the carbonation of butter, it would be necessary to store the butter in a CO_2 atmosphere, although in time butter stored in this way also developed undesirable flavours.

2.2.4.0 Ice Cream

Carbonation of ice cream was found to have no appreciable effect on the bacteria within the ice cream (Prucha *et al.*, 1922; Rettger *et al.*, 1922). It was further demonstrated that CO_2 at atmospheric pressure had no bacteriostatic or bactericidal effect on organisms originally present in the ice cream.

2.2.5.0 Milk Powder

De (2000) observed that fat decomposition resulting in a tallowy flavour is a major storage defect in whole milk powder, and suggested inhibitory measures by removing O_2 from the headspace of the package followed by flushing either by nitrogen or mixture of N_2 and CO_2, the latter being restricted to 5-20%. This helps in reducing the oxidation of fat.

2.2.6.0 Yoghurt

The gas flushing of CO_2 or N_2 was reported to be a viable preservation method for extending the shelf life of fruit-flavoured yoghurt by inhibiting the growth of yeast and mould (Tamime and Deeth, 1980). The use of carbonation process has been recommended by Fairbairn and Law (1986) for the reasons that it is cheap and safe and apparently did not have any negative impact on cultured dairy products. Blakistone (1990) packed plain yoghurt by aseptically filling in high barrier cups, which were then sealed with a foil lidding and flushing with N_2. Karagul-Yuceer *et al.* (2001) observed that carbonation of yoghurt declined the contaminating bacteria.

2.2.7.0 Cheese

Mozzarella Cheese

Sarantopoulos *et al.* (1993), Sarantopoulos and Sorey (1995) reported an increase in shelf life to about 240% when mozzarella cheese was packed under MAP. Tanweer Alam (2004) observed that the shelf life of mozzarella cheese stored at 7±1 °C significantly increased (a 300% increase) for the product packaged under 100% CO_2 in high barrier bags, while at deep freeze storage (-10 to -15 °C), the shelf life increased to 120%. Alves *et al.* (1996) reported a significant increase (a 385% increase) in the shelf life of sliced mozzarella cheese when packed under 100% CO_2, and stored at 7±1 °C, while the increase had been 246% for product packed under 50% CO_2 / 50% N_2. As per the observations of Alves *et al.* (1996) the inert atmosphere of 100% N_2 was not effective for controlling the microbiological deterioration of sliced mozzarella cheese, whereas the role of CO_2 in controlling microbiological deterioration was demonstrated in MA packed samples with CO_2.

Eliot *et al.* (1998) studied the stability of shredded mozzarella cheese under 8 modified atmospheres (air, vacuum, CO_2, N_2 and mixtures of CO_2 / N_2 in different proportions) and reported that modified atmosphere containing CO_2 efficiently stabilized lactic acid and mesophilic flora, while inhibited staphylococci, yeast and moulds. Psychrotrophs grew in all samples but were less numerous in high CO_2 atmospheres. They further observed that CO_2 was effective in depressing undesirable microorganisms such as staphylococci, yeast and moulds during storage. CO_2 was found to be not as effective in repressing psychrotrophic bacteria but reduced growth of lactics and mesophilics. Higher CO_2 concentrations were observed to be more effective than nitrogen to control mesophilics, and were also more effective than vacuum packaging in inhibiting yeast and moulds. CO_2 levels $\geq$ 75% were found to be the most appropriate for maintaining microbiological quality and safety of shredded mozzarella cheese.

Cottage cheese

Kosikowski and Brown (1973) demonstrated the effectiveness of storing creamed cottage cheese in a CO_2 atmosphere or in a N_2 atmosphere, which suppressed spoilage organisms (psychrotrophic bacteria, yeast and moulds). Through experiments, Honer (1988) demonstrated that quality was maintained in cottage cheese packaged under MA for much longer period compared with air packaging. Maniar *et al.* (1994) was able to extend the quality of cottage cheese upto 28 days by using MAP technique. Fedio *et al.* (1994) observed 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 yeast and moulds was most significantly affected by modifying the storage atmospheres. In air-packed cottage cheese considerable growth of yeast and moulds was observed, while growth was suppressed in samples packaged with N_2. 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.

2.3.0.0 Pizza

Pizza is one of the world-famous Italian dishes because of its simplicity, taste, and nutritional value. Pizza is a round oven-baked pie made with crisp yeast dough, which in the past was covered with tomato sauce, mozzarella cheese and a variety of other ingredients. Now, it can be covered with a variety of savoury ingredients that may include sauces, meats, vegetables and cheeses. The word "pie" neither refers to the crust, nor even to the shape or position of the crust. The word "pie" relates to the Magpie, a bird with feathers splotched in two colours, a bird called "Pica" by the Romans, whence the English "Pie" and the alteration of "Pica" to "Pizza". The name relates to the bird's double colour and its habit of gathering odds and ends as does a Pizza, or Pie, gathers and consists of varied ingredients.

In recent years, fresh refrigerated pizza has become one of the fast growing products in the prepared food category. Sales of refrigerated pizza in U.S. supermarkets increased by an average of 5.1% annually during the 10 year period from 1986 to 1995 to a level of $370 million in 1995. In the past, refrigerated pizzas were usually assembled, packaged, and sold on site at the supermarkets. However, expanding markets have favoured a trend toward producing fresh pizzas at central manufacturing facilities.

The pizzas are either distributed under refrigeration to retail outlets or are distributed frozen, thawed at the retail outlet, and sold as a refrigerated product (Anon, 1996). Refrigerated pizzas produced in centralized manufacturing plants ideally require a 2 to 4 weeks shelf life to accommodate the time needed for distribution and sales. However, such a shelf-life requirement presents a challenge for the pizza industry (Nickols, 1994). Undesirable changes may occur in the cheese during refrigerated storage, including loss of meltability and stretchability and the onset of a weak, mushy consistency of the cheese ingredient. Sales in U.S. keep rising and rising as quality and packaging improve. Innovations like rising crust pizza and **atmospheric packaging bring taste of pizzeria pizza** to the microwave oven (Pierce, 2006).

2.3.1.0 Growth of Pizza Industry

The fast food segment of the food industry in the U.S. represents about one-fifth of the total food service sales. Although the growth of the fast food industry has slowed somewhat in recent years, pizza products remained an exception. The introduction of rising crust pizzas under Kraft's DiGiorno brand in early 1996 and Schwan's Freschetta line in the same year had sparked renewed growth in this market. The impacts of these introductions were that market sales of $1.9 billion in 1996 and $2.2 billion in 1997, resulted in a 12% growth each of these years (U.S. Dept. of Commerce, 1997).

Americans eat approx. 100 acres of pizza each day, or about 350 slices per second. Pizza is a $32+ billion per year industry. There are approx. 61,269 pizzerias in the United States. Approx. 3 billion pizzas are sold in the U.S. each year. Italian food ranks as the most popular ethnic food in America. 94% of the population of the U.S. eat pizza. Pizza restaurants in the U.S. had annual sales of $16.2 billion in 1994 (Siebert, 1997). Frozen pizza sales increased 40% in a six-year period between 1989 and 1995 to $1.6 billion in sales (Saulnier, Schatzberg and Wishnow, 1995). Pizza represents the fastest growing segment of the food-away-from-home industry, enjoying a 12% annual growth rate (Lemki and Ferris, 2001).

According to Anon (2003 b) pizza is a relatively new item to Indian dietary system and gaining wide popularity. In India the demand of pizza has increased tremendously due to the proliferation of pizza parlours and fast food chains. Pizza chains are consolidating their position in the major metros and are also entering small towns of India in a big way. One of the contributing factors to the growth of pizza as fast food has been the Indian flavours which pizzas acquired in India. The pizza industry has been growing at a rate of 30%. Amul has launched an inexpensive pizza in the various cities and now sells 4000 pizzas per day (Anon, 2002).

2.3.2.0 Medicinal, Therapeutic and Nutritive Value of Pizza

In recent years, consumer interest in the nutritive value and wholesomeness of food has steadily increased (FDA, 1973). According to

USDA (2005), cheese vegetable pizza should contain 46.28% moisture, 10.36% protein, 12.28% total fat, 2.06% ash and 29.02% carbohydrates (by difference). Apart from this, it should contain calcium, Iron, magnesium, phosphorus, potassium as 179, 2.27, 23, 179, 152 mg/100 g respectively and lycopene 239 µg/100 g.

Kamel and Manji (1986) evaluated the proximate analysis of fresh pizza (comprising 80% mozzarella cheese + 10% provolone + 10% cheddar) and reported the values as: 48.8% (moisture), 12.2% (protein), 9.2% (fat), 2.6% (ash), 27.2% (carbohydrates), 1.69 mg/100 g (Iron), 288.94 mg/100 g (calcium) and 40.57 mg/100 g (magnesium), 0.26 mg/100 g (thiamine), 0.19 mg/100 g (riboflavin), 1.18 mg/100 g (ascorbic acid) and food energy to be 240 cal/100 g.

Anon (1974) reported that pizza supplies 30% of the U.S. Recommended Daily Allowance (RDA) of vitamins A, C, B_2, calcium, protein and contains 50% of the RDA of Vitamin B_1 and 35% of Iron. Martin (1977) observed that pizza ranks extremely high in wide spectrum of nutrients; rich in protein and calcium (fulfil 127% daily needs); an excellent source of Iron (supplies 50-55% of daily requirements); provides 30% needed niacin; contains 26-42% adult vitamin A quota; provides 900-1150 calories/pizza.

Lycopene is the pigment principally responsible for the characteristic deep-red colour of ripe tomato fruits and tomato products. It has attracted attention due to its biological and physicochemical properties, especially related to its effects as a natural antioxidant (John, 2000), which can be further boosted with health-protecting antioxidants by piling on garlic, green pepper, onions, broccoli, herbs, mushrooms, and spinach. Zimmerman (2002) observed that cooked tomato products (tomato products even pizza) pack more bioavailable lycopene than the raw fruit and reported 4.5 mg lycopene per 100 g of frozen pizza. Silvano *et al.* (2006) studied the favourable role of pizza consumption on the risk of breast, ovarian and prostate cancers using data from three hospital-based case-control studies conducted in Italy between 1991 and 2002. Tomatoes have also been shown to be helpful in reducing the risk of colorectal cancer, prostate cancer and pancreatic cancer (Erhardt *et al.*, 2003; Boileau *et al.*, 2003; Visioli *et al.*, 2003; Etminan *et al.*, 2004; Nkondjock, 2005).

2.3.3.0 Pizza Styles

Pizzas are available in various styles. Pizza crusts topped with apples or cherries, or corn meal crusts topped with picante sauce and Mexican flavour toppings, different types of vegetables, sauces, cheese, and other stuffs are commonly available at pizza chains. The most popular styles of pizza are (www.aboutpizzastyles.com):

Traditional Crust Pizza: The crust is neither too thick nor too thin. It is prepared by rolling the dough into a round circle then topping it with sauce and cheese.

Deep Dish Pizza: A thin crust and dense toppings usually characterize deep-dish pizza, also known as pan pizza. Baking tends to take longer time.

New York Style Pizza: Pizzas from pizza parlours in New York tend to be much thinner than its Chicago cousin. Typically the thin crust allows faster cooking but less dense toppings. The crust is also usually prepared by tossing it up in the air and twirling it.

Calzone: This is basically a stuffed pizza crust. The uncooked crust is filled with traditional toppings then folded and baked. These have gained in popularity across the U.S. in the last few years.

Pizza Bread: The French bread loaf is usually sliced down the centre into two halves. The sauce, cheese and toppings are then placed on the flat, sliced surface.

Bagel Pizza: Made much like the French bread pizza but with bagel slices instead.

Frozen Pizza: It is popular because of its ease to prepare. This is perfect food for the person that hates to cook but wants a cheap and inexpensive pizza. Open the box and put it in the oven. Toppings are usually a matter of preference, but typically consist of tomato sauce and mozzarella cheese.

Montgomery (1969) quoted that frozen pizza has the greatest penetration among all frozen prepared foods. Benner (1983) reported USDA standards for frozen pizza that it must contain at least 12% cheese, and meat pizzas contain at least 12% cooked meat. Bone and Manoski (1981) described a frozen pizza product for microwave cooking, in which the crust comprised a 1^{st} layer of cracker type dough material with moisture content less than 5% and a 2^{nd} layer of baked bread dough type material with moisture content 20-40%; the cracker crust absorbed the vapour phase moisture created during cooking.

Wilmes (1987) studied the frozen pizza product with a special baked pastry shell produced from a dough containing a specified mixture of high and low protein wheat flours. The dough also contained 10-17% solid fat having melting point in the range 48-53 °C, and mixed in such a way that the fat remained in solid pieces prior to baking. After baking and topping with pizza sauce the product is frozen. Subsequently, final re-baking by the consumer gives a crust, which is tender inside and crisp on the bottom. The market for frozen pizza accounts for approx. 19% of the sales for all prepared foods.

2.3.4.0 Basic Ingredients of Pizza

2.3.4.1 Crust (Pizza Base)

Pizza is a flat pie made from bread dough. Generally the crust is wheat flour based but can be either whole wheat or bleached flour, which can be baked in an oven either before toppings are placed on it or can be baked with the toppings placed on it (Labensky, Ingram, Labensky, 1997). Pizza dough can be chemically (Lehman, 1997) or yeasts leavened (Coppola *et al.*, 1998). The overall quality of a pizza depends mostly on the leavening, the flour used, and the preparation procedure (Coppola *et al.*, 1998). The formulation of crust has been reported by several workers as presented in the following table:

Table: Formulation of Crust

Ingredients	Federico (1974)	Westover (1980)	Ghosh & Kanawjia (1986)	www.abc of pizza.com
Flour	100 lb	60.65%	60.0%	5 lb
Water	78.4 lb	36.04%	34.0%	6 cups
Yeast	4.5 lb	1.28%	3.0%	3 packets
Salt	7.0 oz	0.93%	1.0%	5 teaspoons
Sugar	9.0 lb	-	1.0%	2 tablespoons
Vegetable oil	-	-	1.0%	-
Soya oil	-	0.80%	-	-
Olive oil	-	-	-	12 tablespoons
Emulsifier	-	0.30%	-	-
Shortening	4.5 lb	-	-	-
Dried whey	1.5 lb	-	-	-
Eggs	2.0 lb	-	-	-

Anon (1984) reported the method for production of pizza crust, suitable for freezing. The flat dough-piece, formed after proofing, was partly baked at greater than 120 °C by surface contact with a baking substance. This ensured, apart from long and gentle dough treatment, that the bakery products had a crusty surface after crisping up and an inner juicy crumb, having slightly larger pores. These sensory properties were regarded as novel, regarding the crustiness alone and also the combination of crustiness and crumb structure.

Hempenius and Yantis (1981) in a U.S. patent described a method for making pizza crust. Appleby *et al.* (2002) obtained U.K. patent for describing a method for producing a frozen pizza base: forming a dough disc to greater than or equal to 50% of the final surface area of the pizza; adding the dough disc to a pan; allowing the dough disc to proof or relax in the pan; baking the disc in the pan to form a pizza base; and freezing the baked pizza base. Lemki and Ferris (2001) described a method for the preparation of sourdough pizza crusts by using sourdough starter cultures. Hasan (1997) prepared pizza crusts from all purpose flour, dry instant yeast, sugar, canola oil, salt, and amylase (Novamyl), followed by baking for 5 min at 177 ºC in a convection oven.

2.3.4.2 Tomato Sauce

Nutrition experts believe that the functional components of food that may reduce the risk of cancer include traditional nutrients such as lycopene in tomatoes, which has antioxidant properties to protect cells against damage from oxidation (Anon, 2003 c). Stahl and Sies (1992) hypothesized that the heating of tomato products in oil enhanced intestinal absorption of lycopene. The physico-chemical composition of tomato sauce has been reported by McGlasson (1993): moisture 78.4%, total soluble solids (TSS) 21.0 brix, total solids 21.6%, acidity 0.92 (% acetic acid), pH 3.88, salt 3.71%, viscosity 5750 Cp, ascorbic acid 3.39 (mg/100g) and lycopene 3.30 (mg/100 g).

2.3.4.3 Mozzarella Cheese

Mozzarella cheese belongs to 'Pasta Filata' variety of cheese, which involves skilful stretching, pulling and kneading the curd under hot water to arrive desirable texture in cheese. Mann (1997) reported that one of the best parts of the pizza is mozzarella, which is an integral part of pizza toppings. It should be grated and not sliced so that it would melt more evenly.

The mozzarella cheese production in U.S. represents 32.7% of total cheese production (8.6 billion pounds) that accounts 2.81 billion pounds. The demand for mozzarella cheese in the U.S. is in direct relation to the rise in popularity of pizza (USDA, 1996). Mozzarella cheese enjoys an increasing popularity among the younger generation consumers throughout the world, mainly as pizza topping (Sherkat and Walker, 2002).

Govindaswamy *et al.* (2006) investigated the influence of condensed sweet cream buttermilk on the manufacture, yield, and functionality of pizza cheese and observed a significant increase in cheese moisture while lowering of free oil was also noted. Wood *et al.* (2006) developed a delicious, pizza-ready mozzarella that contained < 10% fat - yet was still stretchy and tasted very much like its full-fat counterpart (23%). They believed that high-quality, low-fat cheese could be manufactured using ordinary cheese - making procedures. Other cheeses gaining popularity as pizza toppings include processed, cheddar, feta, parmesan.

2.3.4.4 Cheddar Cheese

Cheddar cheese is the ripened variety of cheese that is most important dairy product in world market, and is regarded as a delightful food contributing to the flavour to a variety of food items. According to PFA (1976), cheddar cheese is the product obtained by coagulation of milk with coagulating agents. It shall not contain any ingredients not found in milk except coagulating agents, sodium chloride, calcium chloride not exceeding 0.02% by weight, annatto or carotene colour and may contain stabilizers or emulsifiers not exceeding 0.02% by weight. According to Prateek (1995), the typical composition of cheddar cheese is: moisture 36%, fat 32%, protein 25%, lactose 2.1%, salt 1.5%, ash 3.7% and FDM 50.79%.

2.3.4.5 Processed Cheese

Processed cheese may be defined as a modified form of natural cheese prepared by blending one or lots of cheese with water, salt, colour and emulsifier. The process involves heating a mixture at 66-75 °C for 2-5 min into homogenous plastic mass (De, 2000). According to the PFA (1976), processed cheese refers to the product obtained by heating cheese with permitted emulsifiers and /or stabilizers, namely citric acid, sodium citrate, sodium salts of orthophosphoric acid and polyphosphoric acid, with or without added condiments, and acidifying agents, viz., vinegar, lactic acid, acetic acid, citric acid and phosphoric acid. Processed cheese may contain not more than 4.0% of anhydrous permitted emulsifiers and / stabilizers, provided that the content of anhydrous inorganic salts in no case exceeds 3.0% of the finished product. It should not contain more than 47.0% moisture. The milk fat content should not be less than 40.0% of dry matter. Processed cheese may contain 0.1% sorbic acid or its sodium, potassium or calcium salts or 0.1% of nisin, either singly or in combination.

2.3.4.6 Parmesan Cheese

Named after an area in Italy, Parma, Parmesan is one of the world's most popular and widely enjoyed cheeses. The 'grana' cheeses (Parmigiano Reggiano and Grana Padano) are the most important characteristic group of Italian cheese from a technological point of view. Particularly, Parmigiano Reggiano is the best known (in the world as 'Parmesan') and is produced according to a traditional technology from raw milk in a restricted region of the Po valley. The average composition of ripened Parmesan cheese (g/100g) is moisture 30.8; total protein 33.0; fat 28.4; ash 4.6; calcium 1.15; phosphorus 0.7; magnesium 0.04; salt 1.4 (Battistotti and Corradini, 1999).

2.3.4.7 **Tomato** *(Lycoperscion esculatum)*

Tomato belongs to the genus *Lycoperscion* of the family *Solanaceae*. On the fresh weight basis, vitamin C content averages about 25 mg/100 g, it also contains folic acid, pantothenic acid, biotin, and vitamin K, in addition to nicotinic acid, riboflavin, and thiamine (Hobson and Davis, 1971). The pulp and juice of the fruit is easily digestible, appetiser, promoter of gastric secretion and a blood purifier (Madhavi and Salunkhe, 1998).

2.3.4.8 **Onion (*Allium Cepa*)**

Onion belongs to the genus *Allium,* which includes several cultivated crops such as onion, garlic. It is used in both immature and mature bulb stages as a vegetable and spice. They are the main ingredient in many pickles and chutneys. Its spice value is due to sulphur containing compounds (Thomas and Parkin, 1994; Randle *et al.*, 1994). Onion is used to treat stomach ulcers, eye disorders, gastrointestinal disturbances, high blood pressure, malarial fevers, and intestinal worms. The onion is known to possess insecticidal, antifungal, antibacterial, antitumour, hypoglycaemic, hypolipidemic and antiatherosclerotic properties (Augusti, 1990). Fresh onion juice has antibacterial properties due to allicin, disulphide and cysteine compounds and their interactions (Mittal *et al.*, 1974; Baghurst *et al.*, 1977). The antioxidant activity of onion has been studied in lipid oxidation models (Al-Saikhan *et al.*, 1995; Ramarathnam *et al.*, 1997; Kahkonen *et al.*, 1999) and in radical scavenging assays (Cao *et al.*, 1996; Vinson *et al.*, 1998).

According to Mac Gillivary (1953), onion contains 87.5% moisture, and the food value per 100 g of edible portion is: energy 49 calories, protein 1.4 g, calcium 32 mg, vitamin A 20 IU, ascorbic acid 12 mg, thiamine 0.03 mg, riboflavin 0.12 mg and niacin 0.1 mg. Brahmachari and Augusti (1967) reported that fresh onions contained 86.8% moisture, 11.6% carbohydrates, 1.2% protein, 0.1% fat, 0.2-0.5% calcium and traces of Fe, Al, Cu, Zn, Mn, thiamine, riboflavin.

2.3.4.9 Capsicum (*Capsicum annum var. grossum*)

Capsicum also known as simla mirch, sweet pepper, bell pepper, is widely cultivated vegetable crop worldwide (FAO, 1994). The larger bell-shaped fruits with thick pericarp are less pungent or non-pungent and used in the fresh condition as a vegetable, in salads and in pickles. They are used in salads, in stews for imparting flavour, pizza, meatloaf, dehydrated processed meat, and for canning (Rajput and Parulekar, 1998). Bell or sweet peppers are very rich in vitamins A and C and are also good source of β-carotene. Bell pepper contains 48 kcal energy, 2.0 g protein, 0.8 g fat, 10 g carbohydrates, 2.6 g fibre, 29 mg calcium, 61 mg phosphorus, 2.6 mg iron, 0.12 mg thiamine, 0.15 mg riboflavin, 2.2 mg niacin, 140 mg ascorbic acid per 100 g edible portion (Bernal *et al.*, 1993).

2.3.4.10 Chillies (*Capsicum annum*)

Chilli, also known as hot pepper, is an important vegetable and condiment. Due to their medicinal properties, vitamin contents, the demand for chillies has been increasing all over the world. The pungency in pepper is due to the oleoresin *capsicin,* which is a volatile alkaloid. According to Aykroyd (1941), green chillies contain 82.6% moisture, 6.1% carbohydrates, 2.9% protein, 0.6% fat, 6.7% fibre, 0.91% mineral matters (0.03% calcium, 0.08% phosphorus and 0.0012% iron). In India it is used as a principal ingredient of various curries and chutneys. It stimulates the taste buds that result in increased flow of salivary amylase helping the digestion of starchy foods. Green chillies are good source of vitamin A and C. With asafoetida and camphor, it is used in the form of pills in cases of cholera (Rajput and Parulekar, 1998).

2.3.4.11 Ginger (*Zingiber officinale*)

Plant derived products have been used for medicinal purposes for centuries. In traditional Indian medicine or Ayurveda, ginger and many other spices have been used as medicine (ICMR, 2003).

Ginger belongs to family *Zingiberaceae* of genus *Zingiber.* Fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fibre and 12.3% carbohydrates. The minerals present in ginger are iron, calcium and phosphorous. It also contains vitamins such as thiamine, riboflavin, niacin and vitamin C (Govindrajan, 1982). Green ginger has low fibre content, and has mild aroma and pungency. It is used as a fresh flavouring material in curries, sauces, pickles or salads and can be further processed into ginger syrup, candied ginger and ginger paste. Mature ginger is used for production of essential oils and oleoresins, which contain gingerols, that have been found to possess cardiotonic, antipyretic, analgesic, hepatoprotective, and sedative properties (Salzer, 1982). Besides culinary uses, ginger is also known for its medicinal and antibacterial properties. Studies have shown that it may assist digestive absorption by up to 200% (Negbenebor *et al.*, 1995).

2.3.5.0 Potassium Metabisulphite (KMS)

Sulphur dioxide has been used in foods for long as a preservative. It is used in the treatment of fruits and vegetables before and after dehydration to extend the storage life and prevent the growth of undesirable microorganisms. Forms in which sulphur dioxide is employed as a preservative include the gas (SO_2), sodium and potassium bisulphites ($NaHSO_3$ or $KHSO_3$), sulphites (Na_2SO_3 or K_2SO_3) and metabisulphites ($Na_2S_2O_5$ or $K_2S_2O_5$) (Ranganna, 2000).

Balasubramaniam and Poole (1995) suggested possible mechanism of KMS action on berries (fruits): KMS in solution produces sulphur dioxide, which acts as a fumigant, and this fumigant action might be instrumental in killing the *Botrytis* spores, and in preventing infection of healthy berries. For the chemical buffs, when KMS (which is either a white powder or a crystalline solid) is dissolved in water, a mildly acidic solution is produced, with a strong sulphur dioxide odour. This is caused by chemical reactions with water, which can be represented as:

$K_2S_2O_5$	+	$2H_2O$	=	$2K^+$	+	$2HSO_3$		(A)
(KMS)		(water)		(potassium)		(hydrogen sulphite)		
H^+	+	HSO_3	=	H_2SO_3				(B)
(Hydrogen)				(sulphurous acid)				
H_2SO_3	+	H_2O	=	SO_2	+	$2H_2O$		(C)
				(sulphur dioxide)				

Reaction B occurs to a great extent in more acid or low pH conditions. Since the product of reaction C is volatile, SO_2 is not very soluble in water; it is emitted by the solution. This is more pronounced at higher temperatures, and under conditions of free air circulation through or over the solution. SO_2 and related compounds are readily oxidized to sulphates by oxygen. The most likely fate of KMS applied to the outside of the fruit is that it will be washed off or oxidised on the skin to potassium sulphate (K_2SO_4). Other reactions, for example with the aldehyde groups that appear in sugars or in aroma compounds are theoretically possible if the treatment materials enter the fruit, but the extent to which they might occur is not known. If unoxidised salt remains on the fruit, or if any enters the berry, it could theoretically raise the SO_2 content of the must.

2.3.6.0 Preparation of Pizza

Lamberg (1971) suggested a method for the preparation of long shelf life pizza, which could be easily reheated in a domestic oven for 5 min at 200-250 °C. The method involved rolling and shaping the usual type of dough and adding peeled tomatoes, cheese containing 30% fat, oregano, anchovies and chilli peppers. The pizza was heated at 350 - 400 °C for 2-2.15 min in an electric oven; the exact time depended on the moisture level of the dough. The cooked pizza was then removed, cooled for 10 min to room temperature and sealed in a plastic package. The packed, sealed pizza was then heated to 100 °C for another 10 min to sterilize it, and cooled to below freezing point for 10 min in a freezer. West *et al.* (1979) prepared pizza (30.5 cm dia) using crust and tomato sauce.

Ghosh and Kanawjia (1986) recommended that 85, 125 and 170 g 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 g 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 total cheese followed by baking the pizza in oven at 220-230 °C for about 15-20 min. Kamel and Manji (1986) studied six pizza formulations varying in kind and level of added protein concentrates in a commercial pizza outlet by standard operating procedures. All the pizzas processed were 19 cm in dia and weighed approx. 200±15 g. Of this, 80±5 g was crust, 35±2 g was tomato sauce, 50±2 g was cheese, and 35±5 g was ground beef, which included α-tocopherol and spices. Each assembled pizza was placed on an aluminium plate, inserted into a polyethylene bag and sealed, and immediately put in freezing chambers maintained at -10, -20 and -30 °C.

For preparing pizza, Matz (1996) suggested to pour generous layer of pizza sauce made from tomato sauce, paste, puree or combination thereof. Typical spices for pizza sauce viz. oregano, basil, black pepper, garlic powder were spread uniformly on pizza crust. The amount of sauce used varied with the size of crust. Pizza made for sale as a frozen product was typically prepared by baking or frying the crust separately and then applying topping just before freezing. Rudan and Barbano (1998) prepared pizza by placing 150 g of tomato sauce and 100 g shredded mozzarella cheese on 30 cm pizza crust. Approx. 1 g of vegetable oil (100% canola oil) was sprayed on the surface of the cheese shreds after they were placed in the pie pan. The pizza was baked in commercial food service pizza ovens at 232 °C for 5 min.

Lemki and Ferris (2001) produced sourdough frozen pizza, which had a high acceptance level with 95.4% of the respondents indicating they liked the product. However, consumers liked the pizza but not the dough by itself. Tanweer Alam (2004) prepared pizza by smearing the crust with tomato sauce (40 g). Then two chopped green chillies and approx. 5 g crushed ginger were sprinkled all over the crust. The grated mozzarella and cheddar cheese in the ratio of 80:20 (approx 120 g per pizza) were topped on crust followed by vegetable toppings, which included approx. 80 g chopped capsicum, approx. 80 g sliced tomatoes, and approx. 50 g chopped onion. After topping, the pizza was baked in conventional oven at 220-230 °C for 15-20 min.

Microwave Cooking of Frozen Pizza

In a Developing Foods report on Microwave Foods, Bertrand (2005) remarked that "microwavable foods satisfy need for speed and palatability", and in turn food processors, ingredient vendor, and packaging suppliers are teaming up to meet the demands of on-the-go consumers who want quick food preparation and superior taste and texture. Peleg (1993) described a method and appliance for microwave cooking of frozen pizza. The frozen pizza consisted of an uncooked dough layer covered with a topping, except for an outer sauce-free ring of dough and a microwave susceptor component, which enabled the pizza to be cooked in a microwave oven so that the outer ring became crisp and brown.

Blisters on pizza

One of the main ingredients of pizza is mozzarella cheese. When pizza is baked, the cheese develops brownish spots due to maillard reaction between milk proteins and carbohydrates. Since pizza makers compete in speed at which they can fill an order, they increase the temperature of ovens, consequently the spots turn darker and dark blisters develop. The factors, which affect the browning of mozzarella cheese are: sugar concentration, amino acid concentration, pH, and temperature during baking, and/or culture (http://www.anka.livstek.lth.com).

2.3.7.0 Packaging of Pizza

Bakery products, like many processed foods, are subject to physical, chemical and microbiological spoilage. While physical and chemical spoilage limit the shelf life of low and intermediate moisture bakery products, microbiological spoilage by bacteria, yeast and moulds is the concern in high moisture products, i.e. products with $a_w > 0.85$. Classification of products on the basis of their pH and a_w is helpful in recognizing the spoilage and safety potential of bakery products. Smith and Simpson (1995) also classified bakery products on the basis of their a_w as (i) low moisture bakery products with $a_w < 0.6$, (ii) intermediate moisture bakery products with a_w between 0.6 and 0.85, and (iii) high moisture bakery products with $a_w > 0.85$ and generally between 0.95 and 0.99. The a_w of pizza crust has been reported to vary from 0.94-0.95, while that of pizza as 0.99.

Pizza packaging probably began in the 1940s after World War II. With the advent of carryout pizza, the first package was most likely a combination of paper bag and a chipboard or corrugated square. With this, the pizza was placed on the square and the entire unit was slid into the bag, which was taped or stapled shut. Subsequently, a circle replaced the square shape, making it easier to insert into the bag. This package was convenient (no pre-folding needed) and highly economical, but it lacked stacking strength, heat retention, and product protection capability (www.pizzapackaging.com). Consequently, the paperboard pizza box was introduced, which resembled the structure of bakery cake cartons of the time, in that the four corners of the box were formed by inserting a tab projecting from one wall into a slot in an adjacent wall. Due to the thinness of the material, it required a piece of aluminium foil or a chipboard or corrugated pad in the bottom. This was slightly less convenient than the circle and bag package, but it imparted a measure of heat retention and product protection.

Circa 1960, the corrugated pizza carton, provided substantial stacking strength, improved heat retention, and great product protection over the paperboard box. Tom Monaghan, founder of Domino's Pizza, had stated that adopting the corrugated pizza box (*Circa 1960)* was one of the fundamental innovations responsible for the growth of the Domino's delivery system. Although a pizza carton can be made of any material such as plastic and moulded paper pulp, but paper has generally been the material of choice for three reasons: economical, has substantial stacking strength, or crush-resistance and resists condensation build-up on the interior surfaces.

Cabo *et al.* (2003) packed various types of pizza into thermo-moulded laminates and subsequently the gas mixture was injected, and immediately another laminate was sealed on top by using a vacuum compensated heat sealer. Daifas *et al.* (1999) packed pizza under modified atmospheres (CO_2: N_2 :: 60: 40) in 210 x 210 mm high gas barrier cryovac bags (O_2 transmission rate: 3-6 ml/m^2/24h, 1atm at 4.4 °C, 0% RH) using a Multivac chamber heat - seal packaging machine to obtain the desired mix of CO_2 and N_2 and all packaged samples were stored at 25 °C. Smith *et al.* (1990) recommended gas composition (%) for MAP pizza as CO_2 : O_2 : N_2 :: 40-60 : 0-10 : 40-60 for increased shelf life.

2.3.8.0 Storage of Pizza

The handling, storage, and preservation of a food product often cause changes in its nutritive value, most of which are undesirable. The freezing process (prefreezing, freezing, frozen storage and thawing) can affect the retention of sensory qualities, retention of nutrients, and microbiological contamination (Fennema, 1975; Kramer *et al.*, 1976). The most important factor that determines the shelf life is storage temperature, which can lower nutritional and sensory quality. The effects of storage temperature can occur during all stages of processing, in trade, in distribution, and in the home (Richardson, 1976). Kamel and Manji (1986) studied six pizza formulations varying in kind and level of added protein concentrates in a commercial pizza outlet by standard operating procedures, and assembled pizza was inserted into a polyethylene bag and sealed immediately in freezing chambers of temperature -10, -20 and -30 °C. Kramer and Kamel (1980) reported the losses in the sensory quality and nutritive value of frozen pizza as influenced by time (6 months) and temperature (-10, -20, -30 °C).

2.3.9.0 Physico-Chemical Characteristics of Pizza

Donnelly (2002) observed that the development of a standardized protocol for shelf life determination of refrigerated foods is an important step in assuring the quality and safety of these products. Fu and Labuza (1993) were of the opinion that chemical or instrumental analysis, such as moisture, nutrient loss, free-fatty acids or colour measurement that closely correlate to sensory attributes, could supplement sensory techniques. They are usually less expensive and less time-consuming than sensory approaches. A correlation between a physical or chemical test can increase the confidence level of the sensory results. Most sensory experts agree that analytical methods should complement the sensory tests.

Labuza (1986) was of the view that following constituents or properties could be considered for monitoring chemical changes of pizza quality during frozen storage: total free fatty acids, specific volatile free fatty acids by HPLC, peroxides, oxidative volatiles (e.g., hexanal) by Gas Chromatography (GC), spice volatiles by GC, lysine, colour, in addition to sensory evaluation of taste and flavour.

2.3.9.1 Headspace volume

In order to obtain a gas headspace to cheese weight ratio of 1-3 lit of gas per kg of the product for packaging under MAP, Day (1992) used vacuum chamber Selovac (Sao Paulo, Brazil) CV-18 machine, with gas injection condition. Tanweer Alam (2004) accomplished the packaging of mozzarella cheese under modified atmospheres by using a vacuum chamber Quick 2000 machine (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), and optimised the injection conditions of the gases to obtain required gas headspace.

Alves *et al.* (1996) demonstrated that the packages with 100% CO_2 and 50% CO_2 had an average of 2.5 lit of gas per kg of cheese. During the storage the average volume of headspace in packages under 100% CO_2 decreased to 2.0 lit of CO_2 per kg of cheese. Tanweer Alam (2004) studied the changes in the headspace volume of MAP mozzarella cheese stored at 7±1 °C and -10 to -15 °C, and observed that the initial headspace volume decreased in all the samples packed under CO_2 / N_2 during storage.

2.3.9.2 Moisture content

As per the recommendation of USDA (2005), the cheese vegetable pizza should contain 46.28% moisture. Kamel and Manji (1986) observed that the moisture content of frozen pizza decreased from 48.8% to 44.2% after 12 months of storage at -10, -20, and -30 °C. They further reported that the storage temperatures had no significant effect on moisture loss in frozen pizza, but storage time decreased the moisture content.

Tanweer Alam (2004) analysed the moisture content of mozzarella cheese samples packed in high barrier bags (LLD/BA*/Nylon-6/BA*/LDPE) under 5 modified atmospheres, and stored at 7±1 °C. The studies revealed that the initial moisture content of 51.88% in air packed mozzarella cheese decreased to 51.70% (0.35% moisture loss) after 5 weeks of storage. After 12 weeks of storage, the moisture loss increased to 2.95%. The initial moisture content of 51.88% in the packages with 100% CO_2, 100% N_2 and 50% CO_2 / 50% N_2 respectively, decreased to 50.93; 50.67; and 50.75% suggesting that the maximum decrease in the value had been in the case of samples packed under 100% N_2 followed by 50% CO_2 / 50% N_2 and 100% CO_2. Ghosh (1987) reported higher moisture loss (1.80%) in mozzarella cheese when air packed in cryovac and stored for 49 days at 8 -10 °C.

2.3.9.3 Fat content

Kamel and Manji (1986) reported 9.2% fat in frozen pizza, while the USDA (2005) recommended it to be 12.28% total fat. According to www.nutritionanalyser.com, fresh pizza should contain 8.78% total fat.

2.3.9.4 Protein content

Kamel and Manji (1986) reported 12.2% protein content in frozen pizza, while the USDA (2005) suggested it to be 10.36%. According to www.nutritionanalyser.com, fresh pizza should have 12.37% protein.

2.3.9.5 Ash content

Kamel and Manji (1986) reported 2.6% ash content, 1.69 mg/100 g (Iron), 288.94 mg/100 g (calcium) and 40.57 mg/100 g (magnesium) in frozen pizza, while the USDA (2005) reported it to be 2.06%. Apart from this, it should contain calcium, Iron, magnesium, phosphorus, potassium as 179, 2.27, 23, 179, 152 mg/100 g respectively. Grajeta *et al.* (2002) conducted study on the Iron, calcium and magnesium contents in selected fast food products (including pizza) in Poland and reported that among the above minerals, calcium was found to be highest (192.2 mg/100 g) for pizza and concluded that highest percentage of the recommended calcium intake could be covered by one serving of pizza (17-24% RDA).

Kota *et al.* (2005) determined the effect of baking on the retention of the folic acid and the total folate in pizza and observed that the percent retentions for folic acid and total folate of pizza (on dry weight basis) upon baking were ≥ 90.

2.3.9.6 Water activity (a_w)

According to Smith and Simpson (1995), pizza is a high moisture bakery product having a_w 0.99. Fu and Labuza (1993) stated that frozen foods such as frozen pizzas might present problems with moisture migration. The moisture may diffuse from the pizza sauce, which has a higher a_w, into the crust containing a lower a_w, creating a pizza crust that is limp and soggy.

2.3.9.7 pH

In order to identify the changes in sauces and toppings, which might influence the overall flavour of pizza, and thus beneficial for determining the shelf life of pizza, Childers and Kayfus (1982) suggested testing of frozen pizza for pH. Cabo *et al.* (2001) investigated the spoilage of ham pizza comprising of tomato paste, cheese, ham, mushrooms and olives, in terms of pH resulted from the fermentative activity of yeasts and LAB by including the joint effect of MAP (CO_2 ranged from 30-90%, and N_2 was added as a filler gas) and nisin (10^6 IU/g). He concluded that acidification was detected only in ham, where pH decreased from 6.5 to 4.9 after 33 days of storage.

Daifas *et al.* (1999) reported decrease in pH of pizza from 5.62 to 5.48, when packaged under modified atmosphere comprising CO_2: N_2 ::

60:40 and stored for 42 days at 25 °C. The slight decrease in pH might be attributed to lactic acid production by spoilage organisms or dissolution of CO_2 in the product.

The work of Tanweer Alam (2004) on the changes in pH of mozzarella cheese samples packed under modified atmospheres and stored at 7±1 °C showed that the initial pH of 5.61 in air packed mozzarella cheese samples decreased to 5.15 after 5 weeks of storage. The pH of samples in the packages with 100% CO_2, 100% N_2 and 50% CO_2 / 50% N_2 decreased to 5.21; 5.16; and 5.20 respectively indicating that the maximum decrease in the value had been in case of samples packed under 100% N_2. Asperger (1982) and Matteo *et al.* (1982) also reported the consistent decrease in pH of mozzarella cheese during storage. However, Alves *et al.* (1996) reported that the pH of sliced mozzarella cheese samples increased from 5.2 to 5.6 in all the treatments (modified atmospheres) studied.

2.3.9.8 Rancidity

Smith *et al.* (2004), while reviewing the shelf life and safety concerns of bakery products, identified two types of rancidity problems in bakery products, especially those with a high fat content, which is characterized by lipid degradation resulting in off-odours and off-flavours, rendering products unpalatable and decrease shelf life; oxidative rancidity results in the breakdown of unsaturated fatty acids by oxygen through an autolytic free-radical mechanism. Consequently, malodorous aldehydes, ketones, and short chain fatty acids are formed. These free radicals and peroxides formed during lipid oxidation might lead to even more detrimental effects on food quality like bleaching pigments (e.g., loss of lycopene in tomato paste in pizza), destroying certain vitamins, such as vitamin A and E, and protein degradation; hydrolytic rancidity, unlike oxidative, occurs in the absence of O_2 and results in the hydrolysis of triglycerides, and the subsequent release of glycerol and malodorous fatty acids, which is further enhanced by the presence of moisture and endogenous enzymes, such as lipases and lipoxygenases, commonly found in vegetables, wheat flour, spices and cheese, which catalyse the oxidation of unsaturated fats, producing peroxides and heat stable compounds that survive the baking process.

Labuza and Schmidl (1985) observed that enzymes of greatest potential concern in pizza are lipases and lipoxygenases. Lipases exist in natural cheese and might produce sufficient amounts of fatty acids, which cause a hydrolytic rancid flavour, especially if butyric acid is released. Lipases are also present in vegetables (green peppers) and in the pizza crust. They further reported that in addition to causing off-flavours, oxidative rancidity could lead to bleaching of the deep-red tomato colour, giving rise to an orange colour, and suggested test for colour changes either with a colorimeter or by extracting the pigments and measuring them in a spectrophotometer, and recommended vacuum packaging or nitrogen flushing (MAP) for maintaining the stability of pizza.

Tanweer Alam (2004) reported FFA of mozzarella cheese samples packed in 5 modified atmospheres and stored at 7±1 °C. The initial FFA value of 4.52 (meq/g) of mozzarella cheese packed under atmospheric air increased to 15.39 after 5 weeks of storage. The FFA of mozzarella cheese samples in the packages with 100% CO_2, 100% N_2 and 50% CO_2 / 50% N_2 increased to 12.49; 13.29 and 12.67 indicating that the minimum increase in the value had been with the samples packed in 100% CO_2 and maximum in case of 100% N_2, establishing a very significant influence of MAP on the lipolysis of mozzarella cheese during storage.

2.3.9.9 Thiobarbituric Acid (TBA) value

Komolprasert *et al.* (1998) studied the effect of temperature, light, freeze-thaw cycling, and package material on the flavour stability of frozen pizza. Pork sausage and pepperoni from pizzas were evaluated monthly for 5 months, and estimated the extent of lipid oxidation by TBA value, which was correlated with the results from sensory evaluation. They also observed that packaging pizza in a good O_2 barrier material (nylon/polyethylene laminate) and sealing under vacuum substantially improved stability of pork sausage compared with pizzas in a poor barrier material.

2.3.9.10 Proteolysis

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). The index utilized by Alves *et al.* (1996) to evaluate the extent of proteolysis in mozzarella cheese varied from 5.46% initially to 5.87% (14^{th} day) in 100% N_2, 10.45% (58^{th} day) in 100% CO_2, and 8.43% (50^{th} day) in 50% CO_2 / 50% N_2, indicating that the proteolysis that occurred in mozzarella cheese submitted to the 3 modified atmospheres was small during storage period.

Creamer (1976) and Addeo *et al.* (1983) observed casein degradation of mozzarella cheese during storage. 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). Tanweer Alam (2004) reported that the initial value for soluble nitrogen content (%) of air packed mozzarella cheese gradually increased from 0.41 to 0.89 after 5 weeks of storage at 7±1 °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 100% CO_2 followed by 50% CO_2 / 50% N_2, 100% N_2, vacuum, and air respectively, in ascending order, suggesting occurrence of consistent proteolysis in the product. Minimum proteolysis was observed in samples packaged under CO_2 atmosphere. White and Marshal (1972), Micketts and Olson (1974) reasoned that the increase in soluble N_2 might be due to residual coagulating enzyme or the enzymes produced by microorganisms.

2.3.9.11 Lycopene content

The red colour of tomato is due to the pigment lycopene ($C_{40}H_{56}$). Estimation of lycopene is considered to be a good index of the quality of fruit used in the manufacture of tomato products. Lycopene has absorption maxima at 473 nm and 503 nm. The molecular extinction coefficient for all trans-lycopene at 473 nm is 18.6×10^4 and at 503 nm, 17.2×10^4. A rapid method for the estimation of lycopene in tomato products is based on the measurement of absorption of the petroleum ether extract of the total carotenoids at 503 nm (Ranganna, 2000). Nguyen and Schwartz (1999) suggested that the extraction of lycopene and analysis should be carried out under controlled environmental factors to minimize oxidative degradation. Nguyen and Schwartz (1999) gave a list of common food sources of lycopene, which are presented in the following table:

Table: Common food sources of lycopene

Food	Type	Amount (mg/100 g wet weight)
Chilli	Processed	1.08 - 2.62
Ketchup	Processed	16.60
Pizza sauce	Canned	12.71
Pizza sauce	From pizza	32.89
Tomatoes	Red, fresh	3.1 - 7.74
Tomatoes	Whole, peeled, processed	11.21
Tomato juice	Processed	7.83
Tomato soup	Canned, condensed	3.99
Tomato paste	Canned	30.07

USDA (2005) recommended that cheese vegetable pizza should contain lycopene 239 µg/100 g. Garcia and Barrett (2006) observed that there were significant differences in percentage of peeled tomatoes, peel index, and yields of whole peeled and diced tomatoes between two consecutive growing seasons. Heat, light, oxygen, and different food matrices have been reported to be the factors that have an effect on lycopene isomerization and auto-oxidation. Lycopene may isomerize to mono- or poly-*cis* forms with the presence of heat or oil or during dehydration. Reisomerization takes place during storage. After oxidation, the lycopene molecule split, which causes loss of colour and off-flavour (Xianquan *et al.*, 2005). Goula *et al.* (2006) stored freeze-and oven-dried tomato samples in closed containers at room temperature; the lycopene loss was found to be higher in the freeze - dried samples (97%) than in the oven-dried ones (73.3 - 78.9%) after 4 months of storage.

2.3.9.12 Colour Estimation

L*, a*, b* is an international standard for colour measurements adopted by the Commission Internationale d' Eclairage (CIE) in 1976. L* is the luminance or lightness component, which ranges from 0 to 100, and a* (from green to red) and b* (from blue to yellow) are the two chromatic components, which range from -120 to +120.

Papadakis *et al.* (2000) developed a versatile and inexpensive technique (colour model) for measuring colour of pizza, by the combination of digital camera, and graphics software. They evaluated the colour profile of microwaved whole pizza (ready-to-cook frozen pizza, microwaved at 100% power for 3 and 3.5 min) because the colour of the bottom surface of cooked pizza is not only important to visual perception, but also related to crispiness. The mean L* values for pizzas microwaved on the susceptor were higher than those microwaved on plain paperboard. The darkening achieved was more pronounced in the outer section than in the middle section and the inner section, because of the edge effect (Buffler, 1993). Increasing the microwaving time from 3 min to 3.5 min decreased the L* values only for the samples microwaved on the susceptor. Similar observations could also be made for a* and b* values. Fu and Labuza (1993) observed that frozen storage of pizza resulted in decrease in red colour or increase in brown colour.

Kaya and Aksu (2005) studied the effect of modified atmosphere and vacuum packaging on the quality characteristics of sliced *sucuk* (dry fermented sausage) produced using probiotics culture and observed that addition of probiotics and the storage time resulted in a significant decrease of colour values (L*, a*, b*).

2.3.10.0 Textural Characteristics of Pizza

The textural quality is an overall physical sensation perceived about a food during mastication. The heating performance of a food system in a microwave oven is governed by oven parameters, food parameters and type of packaging (Schiffmann, 1990). The textural properties of pizza are very important as the difficulties of time-temperature-moisture relations associated with the microwave heating of bakery products often culminate in the development of inferior textural product characteristics subsequent to microwave heating (Schiffmann, 1993).

2.3.10.1 Hardness

Clarke and Farrell (2000) determined the effect of selected test ingredients namely water binding agents (potato starch, pea fibre, oat fibre and locust bean gum), emulsifying agents, ingredients blends and proteolytic enzymes on the textural profile analysis of microwave-reheated pizza. Instrumental textural evaluation was carried out using Universal Testing machine TAXT2*i* (Stable micro system, Godalming, Surrey, U.K.) textural analyser equipped with a 25 kg load cell and 36 mm aluminium blunt cylindrical probe. A crosshead speed of 5.0 mm s^{-1} with a trigger force of 20 g was used to compress the central area of pizza samples to 80% of their original height. Each sample was compressed twice in a reciprocating motion to give a two-bite texture profile curve from which values of textural attributes, viz. hardness, springiness, cohesiveness, chewiness and gumminess were obtained. The hardness values (g), i.e. the force necessary to deform the food between the molar teeth, were 12571, 14461 and 19989 for control pizza samples reheated for 120 s, 150 s and 180 s respectively. Hardness increased with increased reheating times.

Tanweer Alam (2004) observed that the hardness of mozzarella cheese (packed under 5 atmospheres and stored at 7±1 °C) exhibited a decreasing trend in hardness (N) throughout the storage period. At the end of 12 weeks, the hardness was minimum for the samples packed under 100% CO_2 followed by 50% CO_2 / 50% N_2, 100% N_2 and vacuum, respectively.

2.3.10.2 Cohesiveness

Cohesiveness values may be defined as the strength of the internal bonds making up the body of the food. Clarke and Farrell (2000) reported that the cohesiveness values for the control pizza samples increased as the time of reheating of microwave-pizza was increased (0.481 for 120 s, 0.500 for 150 s, 0.554 for 180 s).

Tanweer Alam (2004) observed that the cohesiveness of mozzarella cheese (packed under 5 atmospheres and stored at 7±1 °C) decreased throughout the storage period. The initial cohesiveness value of 0.38 (air packed samples) decreased to 0.15 after 6 weeks of storage. The minimum decrease in cohesiveness was observed for the samples packed under 100% CO_2 (0.19) followed by 50% CO_2 / 50% N_2 (0.18), 100% N_2 (0.17) and vacuum (0.14) after 12 weeks of storage.

2.3.10.3 Springiness

Springiness is defined as the rate at which the deformed food goes back to its undeformed state once the deforming force has been removed. The experiments of Clarke and Farrell (2000) revealed that the springiness scores for control pizza samples were 0.916, 0.914 and 0.947 reheated for 120, 150 and 180 s respectively. Tanweer Alam (2004) noted that the initial mean springiness value for fresh mozzarella cheese sample was 0.50 mm, which decreased to 0.20 at the end of 6 weeks of storage at 7±1 °C in air packed samples. After 12 weeks, the values were found to be 0.19 in vacuum packed samples, 0.25 in 100% CO_2, 0.22 in 100% N_2 and 0.24 in 50% CO_2 / 50% N_2.

Ghosh (1987) determined the springiness of mozzarella cheese samples prepared by using buffalo milk, and observed that the springiness decreased with increased storage period, regardless of packing material and storage temperature. The research work of Tunick *et al.* (1991), who compared mozzarella cheese of various ages and composition, showed that the reduced moisture levels in mozzarella cheese result in higher values for springiness.

2.3.10.4 Gumminess

Gumminess may be defined as the energy needed to disintegrate a semi-solid food to make it ready for swallowing. Clarke and Farrell (2000) reported gumminess values as 6079, 7230 and 11097 for control pizza samples reheated for 120, 150 and 180 s respectively, indicating that gumminess values increased with increased reheating times.

Tanweer Alam (2004) reported that the value of gumminess continued to decrease during storage in all the cheese samples (packed under 5 atmospheres and stored at 7±1 °C). At the end of 6 weeks, the values for gumminess in air packed samples decreased to 15.52 N. Further storage of samples under other four modified atmospheres for 12 weeks showed that the rate of decrease was minimum for the samples of 100% CO_2 (22.10), and maximum for vacuum packed samples (17.32) and inferred that the gumminess decreased consistently. Ghosh (1987) and Malhotra (1991) also reported decrease in gumminess values of cheese samples during storage.

2.3.10.5 Chewiness

Chewiness may be defined as a measure of the length of time required to masticate a solid food until it is ready for swallowing. Chewiness values (g x mm) obtained by Clarke and Farrell (2000) were 5596, 6604 and 10527 for control pizza samples reheated for 120, 150 and 180 s respectively, revealing that chewiness values increased as the reheating times increased.

Tanweer Alam (2004) also observed decreasing trend in the values for chewiness of mozzarella cheese (packed under 5 atmospheres and stored at 7±1 °C) during storage. The initial chewiness value of 25.15 (N x mm) of mozzarella cheese decreased to 4.98 in air packed samples after 6 weeks of storage. The chewiness of samples in the packages with 100% CO_2, 50% CO_2 / 50% N_2 and 100% N_2, decreased to 7.64, 7.45 and 5.02 respectively.

2.3.11.0 Microbiological Quality of Pizza

Foods usually spoil as a result of microbial activity, which causes severe changes in chemical and sensory properties. Cabo *et al.* (2003) developed a model to describe the kinetics of gas release in various kinds of pizzas packaged under different gas mixtures in the presence and absence of Nisaplin by considering the complex microbial ecology of highly heterogeneous foods, such as pizzas, where different microorganisms contribute to spoilage.

2.3.11.1 Total Plate count (TPC)

Scott and Smith (1971) investigated the effect of CO_2, N_2 and air atmospheres on the shelf life of cottage cheese and concluded that CO_2 slightly decreased the bacterial counts but produced an acid or tart cheese, while N_2 neither significantly decrease the bacterial counts nor affected the taste of the cheese. Alves *et al.* (1996), Fedio *et al.* (1994), Eliot *et al.* (1998) and Tanweer Alam (2004), who worked on the MAP of mozzarella cheese also observed that CO_2 had bactericidal effect. Sinell *et al.* (1988) studied 'in plant' quality monitoring scheme of deep frozen pizza, and found that the total bacterial count (aerobes) was 106 cfu/g, and the statistical analyses of the results revealed significant influences due to the composition of the product topping, the season of the year and the type of sanitation programme, all of which showed the importance of in-plant hygiene control.

Kamel and Manji (1986) determined the microbial quality of frozen pizza (comprising 80% mozzarella + 10% provolone + 10% cheddar) at different time (0, 3, 6, 9 and 12 months) and temperatures (-10, -20 and -30 °C), both constant and fluctuating, in terms of total plate count (10^4/g) and reported 150 (initial count), which decreased to 18, 26, 27 at constant temperatures of -10, -20 and -30 °C respectively, while at studied fluctuating temperatures, the counts were 9.4, 33, 26, respectively. Fluctuating temperatures resulted in lower counts than those for constant temperatures, probably due to thermal shock.

Fasano and Gallo (2001) gave the proposed limits of acceptability during the 15-day shelf life of fresh pizza packaged by modified atmosphere and stored at 6-8 °C as: aerobic plate count (APC) < 10^3 cfu/g; lactobacilli < 10^2 cfu/g; yeast and moulds < 10 cfu/g; Listeria monocytogenes absent in 25 g and Salmonella absent in 25 g. According to Donnelly (2002), the APC in refrigerated pizza should not exceed 5 x 10^4 cfu/g upon initial sampling and total increase of ≤ 3 log over product shelf life.

2.3.11.2 Yeast and Moulds (Y & M) count

No growth of yeast and moulds was detected by Alves *et al.* (1996) in the mozzarella cheese samples stored under 100% CO_2 during 58 days at 7±1 °C, but with regard to the proliferation of Y&M, only the development of yeast was detected in the sliced mozzarella cheese packed in air and under atmospheres of 100% N_2 and 50% CO_2 / 50% N_2. The experiments of Eliot *et al.* (1998) showed that the growth of staphylococci, Y&M 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. 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 established a destructive effect of CO_2. The inhibitory effect of CO_2 has also been noted by Kosikowski and Brown (1973), Chen and Hotchkiss (1991), Rosenthal *et al.* (1991), Day (1992), Fedio *et al.* (1994) and Alves *et al.* (1996).

Villari *et al.* (1994), while studying the effectiveness of CO_2 and nisaplin on increasing shelf life of fresh pizza, identified the yeast and lactic acid bacteria (LAB) as the main microflora components in tomato paste, and in mozzarella cheese (Eliot *et al.*, 1998). Jones and Greenfield (1982) and Kuriyama *et al.* (1993) observed that the growth of yeast was inhibited under highly CO_2 -enriched atmosphere. Donnelly (2002) suggested that the Y & M count in refrigerated pizza should not exceed 1 x 10^2 cfu/g upon initial sampling and total increase of ≤ 3 log over product shelf life. Tanweer Alam (2004) also reported the inhibitory effect of MAP on the Y & M count of mozzarella cheese when stored at 7±1 °C.

2.3.11.3 Coliform count

Khalafalla *et al.* (1993) conducted analysis concerning microbiological quality of pizza sold in Egypt by taking 73 samples of ready-to-eat pizza from retail bakery shops, and observed that all samples contained relatively high total viable counts and most samples contained yeasts and acid-forming, spore-forming, lipolytic and proteolytic bacterial groups. Pizza toppings generally had higher levels of contamination (particularly for faecal coliforms and staphylococci) than pizza dough. Storage of pizza at 5 °C for 4 weeks resulted in a decrease in counts of most microbial groups investigated over the 1st 72 h followed by an increase; storage at 20 or 30 °C for 1st week showed an immediate increase in most groups for the 1st 2 days then remaining fairly constant. The exception was coliform and enterococci, which decreased at all storage temperatures to undetectable levels after the 1st week of storage. According to Donnelly (2002), in refrigerated pizza, the coliform count should not exceed 1 x 10^2 cfu/g upon initial sampling and total increase of $\leq$ 3 log over product shelf life. Sinell *et al.* (1988) studied 'in plant' quality monitoring scheme of deep frozen pizza, and noted that the coliform averaged 2 x 10^2 cfu/g.

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

Tanweer Alam (2004) reported that the initial coliform count of 2.62 (log cfu/g) in air packed mozzarella cheese samples increased to 3.01 (log cfu/g) after 5 weeks of storage at 7$\pm$1 °C, while the samples stored for 12 weeks, the coliform counts were found to be minimum in 100% CO_2 (0.63) followed by 50% CO_2 : 50% N_2 (0.73), 100% N_2 (1.84) and vacuum (1.70), respectively. Further, the coliform counts were found to decrease in all the packages with the increase of storage periods irrespective of the atmosphere inside the package. According to Rosenthal *et al.* (1991), the decrease in coliform count during storage is most likely due to bacteria killed by membrane destructive plamolysis

2.3.11.4 Total Lactic acid bacteria (LAB) count

Acidification and CO_2 production are typically ascribed to fermentative metabolism. Ham, cheese and tomato paste are ingredients of ham pizza. Therefore, lactic acid bacteria (LAB) and yeasts are presumably responsible for such effects (Cabo *et al.*, 2001). LAB is the major bacterial group associated with the spoilage of refrigerated meat products packed under a vacuum or modified atmosphere (Mol *et al.*, 1971; Borch and Molin, 1989). With respect to fresh refrigerated pizza, maintaining the quality of cheese during storage requires protection from dehydration and reduction of undesirable microorganisms including pathogens (Eliot *et al.*, 1998). These undesirable organisms can cause odour and flavour changes (Kornaki and Gabis, 1990) and may also modify cheese texture and appearance (Fedio *et al.*, 1994). According to Donnelly (2002), the lactobacilli count in refrigerated pizza should not exceed 1 x 10^2 cfu/g upon initial sampling and total increase of ≤ 3 log over product shelf life.

Cabo *et al.* (2001) investigated the spoilage due to fermentative activity of LAB by carrying out the joint effect of MAP (CO_2 ranged from 30-90%, and N_2 was added as a filler gas) and Nisaplin (10^6 IU/g), added either by mixing in the tomato paste and spraying on top of the pizza, on the quality of ham pizza comprising of tomato paste, cheese, ham, mushrooms and olives. The packaging system enabled some residual oxygen (~1%) to be left in the headspace, which prevented the risk of growth of anaerobic pathogens

(Church and Parson, 1995). Packed pizzas were stored at 7±1°C. The results showed lower LAB and yeast counts in pizza with 1000 IU/g of Nisaplin stored under 90% CO_2 than in those stored under commercial conditions (20% CO_2). Molin (1983) while studying the resistance of CO_2 of some food related bacteria observed that some of the lactobacilli especially the homofermenters were inhibited by 100% CO_2.

2.3.11.5 Psychrotrophic count

The work on sliced mozzarella cheese in MAP revealed that in the beginning microbiological growth was retarded when the atmosphere inside the package contained higher concentration of CO_2 (Alves *et al.* 1996). The psychrotrophic organisms were found to be less numerous when stored in high CO_2 atmospheres in case of refrigerated pizza (Villari *et al.*, 1994) and mozzarella cheese (Eliot *et al.*, 1998). Scott and Smith (1971), Rosenthal *et al.* (1991), Fedio *et al.* (1994) and Maniar *et al.* (1994) also demonstrated the inhibitory effect of CO_2 on psychrotrophs.

Tanweer Alam (2004) reported that the psychrotrophic count of mozzarella cheese exhibited increasing trend throughout the entire period of storage under all the studied atmospheres at 7±1 °C. The initial psychrotrophic count 4.23 (log cfu/g) increased to 8.99 after 5 weeks of storage. At the end of 12 weeks, the samples packed under 100% CO_2 (6.92) showed minimum increase in psychrotrophs followed by 50% CO_2 / 50% N_2 (7.48), 100 % N_2 (7.75) and vacuum (8.28) respectively, revealing that the growth was retarded when the atmosphere inside the package contained 100% CO_2.

2.3.11.6 Anaerobic spore formers

Killeffer (1930) studied the possible preservation effect of CO_2 by conducting experiments on meat and fish and confirmed that CO_2 atmospheres inhibited anaerobic bacteria. Callow (1932) confirmed these findings by replacing the bacterial growth atmosphere with 100% N_2. Smoot and Pierson (1982) found that CO_2 inhibited, stimulated or had little effect on germination and toxigenesis of spore formers such as *Clostridium botulinum*.

Johannsen (1965) suggested that those bacterial species which predominate in CO_2 atmospheres, such as lactobacilli, form peroxides and acids that may inhibit growth of *Clostridium*. According to Enfors and Molin (1978), germination of *Cl. sporogenes* spores was inhibited slightly at pressure of 4 atm of CO_2 and inhibited almost completely at 10 atm. In addition to decreasing the rate of spores germination, CO_2 has also been shown to be lethal to certain clostridia (Hays *et al.* 1959). Farber (1991) reported that at atmospheric pressure 100% CO_2 can delay toxin production by *Cl. sp.* when compared with an atmosphere of 100% N_2. Nissen *et al.* (2002) stored vacuum packed Salmon, Herb sauce and Chicken at 20 °C, and observed that the initial anaerobic count of <100 in all the three products increased respectively to 6.2×10^6, 3.7×10^6 and 1.7×10^7.

2.3.12.0 Sensory Quality of Pizza

The best method currently available for testing pizza is sensory evaluation. Organoleptic evaluations are a good method of testing product quality and are inexpensive. However, they are somewhat time consuming and subject to human variation unless trained testers or taste panels are used (Childers and Kayfus, 1982). Kamel and Manji (1986) got frozen pizzas tested organoleptically by ten panellists who were partially trained in sensory evaluation on a 5-point scale. A score of 5 represented very well; 4, good; 3, satisfactory; 2, poor but acceptable; and 1, unacceptable.

Lemki and Ferris (2001) prepared frozen sourdough pizza and conducted Consumer Acceptance Tests along with the three national brands of self-rising pizza for characteristics like appearance, flavour, and texture using 9-point hedonic scale. The sourdough pizza had a high acceptance level with 95.4% of the respondents indicating they liked the product.

The studies of Tanweer Alam (2004) revealed that the initial sensory score (5.0) of pizza made from air packed mozzarella cheese stored at 7±1 °C for 5 weeks, decreased to 1.8. When the stored (12 weeks) mozzarella cheese packed under vacuum was used for pizza making, the initial pizza score decreased to 2.3; and the sensory score decreased to 3.8; 3.4, and 2.5 respectively for samples made by using stored MAP mozzarella cheese from the packages with 100% CO_2, 50% CO_2 / 50% N_2 and 100% N_2. When pizzas were made from stored (12 months at -10 to -15 °C) MAP mozzarella cheese, the initial sensory score (5.0) of pizza decreased to 2.6, while the score decreased to 3.0, respectively for air and vacuum packed samples. However, the use of product packaged under 100% CO_2, 50% CO_2 / 50% N_2 and 100% N_2 resulted in decrease of sensory score of pizza, respectively to 3.0, 3.2 and 3.0.

2.3.13.0 Shelf life of Pizza

The shelf life of refrigerated food products is dependent upon the interactive effects of several factors including the raw material quality, formulation of product, sanitary conditions during production, processes applied, storage temperature, the numbers and types of cold tolerant spoilage bacteria, the packaging materials used and the gaseous environment of the product (Mead, 1990; Walker, 2000). Smith *et al.* (1987) reported that in general MAP food products stored at 4 °C, have a shelf life 2 to 3 times greater than air-packed products.

Childers and Kayfus (1982) have reviewed the shelf life of frozen foods, with special reference to pizza and reported that microbiological tests were of limited application, but chemical tests could be a direct measure of shelf life. Sensory evaluation was considered for determining the shelf life of frozen pizza. Time-temperature tolerance plays an important role in maintaining product quality and longer shelf life in pizza.

Ahvenainen *et al.* (1990) experimented with commercially prepared ham pizza packaged in plastic laminates using the different gas atmospheres: 100% N_2, 20% CO_2 + 80% N_2, 40% CO_2 + 60% N_2, 60% CO_2 + 40% N_2, air, and stored the product for 36 days at 5±1 °C. Samples were withdrawn at intervals and studies were conducted on gas composition, pH, microbial and sensory quality. Results established the major advantages of gas packaging over air packaging in terms of retardation of mould growth and delay in discolouration. Farber (1991) found the shelf life of pizza product under different gas mixtures (*Pizza sub,* marketed in Canada) as 17 days under 100% CO_2; 30 days under 20% CO_2 + 80% N_2; and 30 days under 50% CO_2 + 50% air.

Blakistone (1998) reported the shelf life of fresh pizza as 6 days and 21 days under air and MAP conditions (40-60% CO_2 and 50-60% N_2), respectively under refrigeration storage. Daifas *et al.* (1999) carried out sensory analysis of modified atmosphere packaged pizza by a 3-member untrained panel. Pizza was assessed for texture, colour and odour using 6-point hedonic scale. For texture, colour and odour, a score of 2.5 was taken as the cut-off for acceptability and termination of shelf life and reported that pizzas packaged in air with or without O_2 absorbent, were sensorially unacceptable after 42 days at ambient temperature. Pizza had a crumbly texture and a strong "fruity" odour. Furthermore, mould growth was observed on all air-packaged pizza. However, pizza packaged in 60% CO_2 was marginally acceptable since both texture and odour scores approached the cut-off score for acceptability (>2.5).

Cabo *et al.* (2001) investigated the spoilage resulted from the fermentative activity of yeasts and LAB by carrying out the joint effect of MAP

(CO_2 ranged from 30-90% and N_2 was added as a filler gas) and Nisaplin (10^6 IU/g) on the quality of ham pizza stored at 7±1 °C, and found that scores for odour and flavour dropped notably in commercial samples (20% CO_2), which were rejected for consumption after 21 days of storage. The development of off-flavour and off-odours was much slower in pizzas with 1000 IU/g of Nisaplin stored under 90% CO_2, which were still acceptable after 44 days of storage. However, CO_2 enriched atmosphere was highly effective in slowing down the spoilage of ham pizza during storage. Fasano and Gallo (2001) could get the shelf life of 15 days for fresh pizza packaged under modified atmosphere and stored at 6 °C - 8 °C.

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 pizza in the preceding chapter that the presently available information on the technology for packaging of pizza under modified atmosphere is very scanty. Very little work on modified atmosphere packaging (MAP) of unbaked pizza has been done. **However, no work has so far been reported on the influence of MAP conditions on the shelf life of unbaked (ready-to-bake) and baked (ready-to-serve) pizza using indigenously available packaging materials.**

3.1.0.0 Scope

For the systematic study on the modified atmosphere packaging of two types of pizza samples, i.e. ready-to-bake and ready-to-serve 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 pizza in different atmospheres and

3.1.2.0 The interaction of the product and atmospheres during various stages of storage under preset condition of temperature.

3.2.0.0 Plan of work

The plan of work was set to be as follows:

3.2.1.0 Preparation of pizza samples by following standard procedure with modifications.

3.2.2.0 Packaging of two types of pizza samples in 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 pizza was also packaged under atmospheric (air) condition in order to study the benefits of packaging of pizza under MAP.

3.2.3.0 Packaged pizza samples as indicated in section 3.2.2.0 were stored at refrigeration temperature (7±1 °C).

3.2.4.0 Ready-to-bake pizza samples stored as above (section 3.2.3.0) were examined at intervals of 0, 5, 10, 15 and 20 days, while ready-to-serve pizza samples were evaluated at intervals of 0, 15, 30, 45 and 60 days.

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

3.2.5.1 Physico-Chemical Characteristics

The fresh pizza samples were tested for percent contents of moisture, fat, protein, ash, carbohydrates, food energy (cal/100g), titratable acidity, pH, water activity, free fatty acids (FFA), peroxide value (meq/kg fat), TBA value, tyrosine besides lycopene content (mg/100g) and colour estimation by Hunter Colour Lab. The stored samples of pizza were analysed for moisture (%), tyrosine (mg/100g), water activity, pH, titratable acidity (% lactic acid), FFA (% oleic acid), peroxide value, lycopene content, colour estimation by Hunter Colour Lab, total colour difference and relative yellowness. The headspace volume in the package of stored pizza samples was also determined.

3.2.5.2 Textural Characteristics

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

3.2.5.3 Microbiological Characteristics

In order to assess the extent of microbiological deterioration, the pizza samples were examined for the counts of total plate, yeast & moulds, total lactic acid bacteria (LAB), coliform, psychrotrophs and anaerobic spore formers.

3.2.5.4 Sensory Characteristics

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

3.2.5.5 Since the period of storage and the type of gas are concerned with the quality of pizza during storage; interaction between 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 and pizza. In the second section are described the techniques of packaging and storage of pizza in different modified atmospheres. The third section deals with the methods employed for quality assessment of pizza during storage, which included headspace volume, physico-chemical changes, textural characteristics, microbiological analysis and sensory evaluation. The fourth section is concerned with the procedure adopted for evaluation of modified atmosphere (MA) packed pizza with conventionally packed pizza. 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 and Pizza

4.1.1.0 Mozzarella Cheese

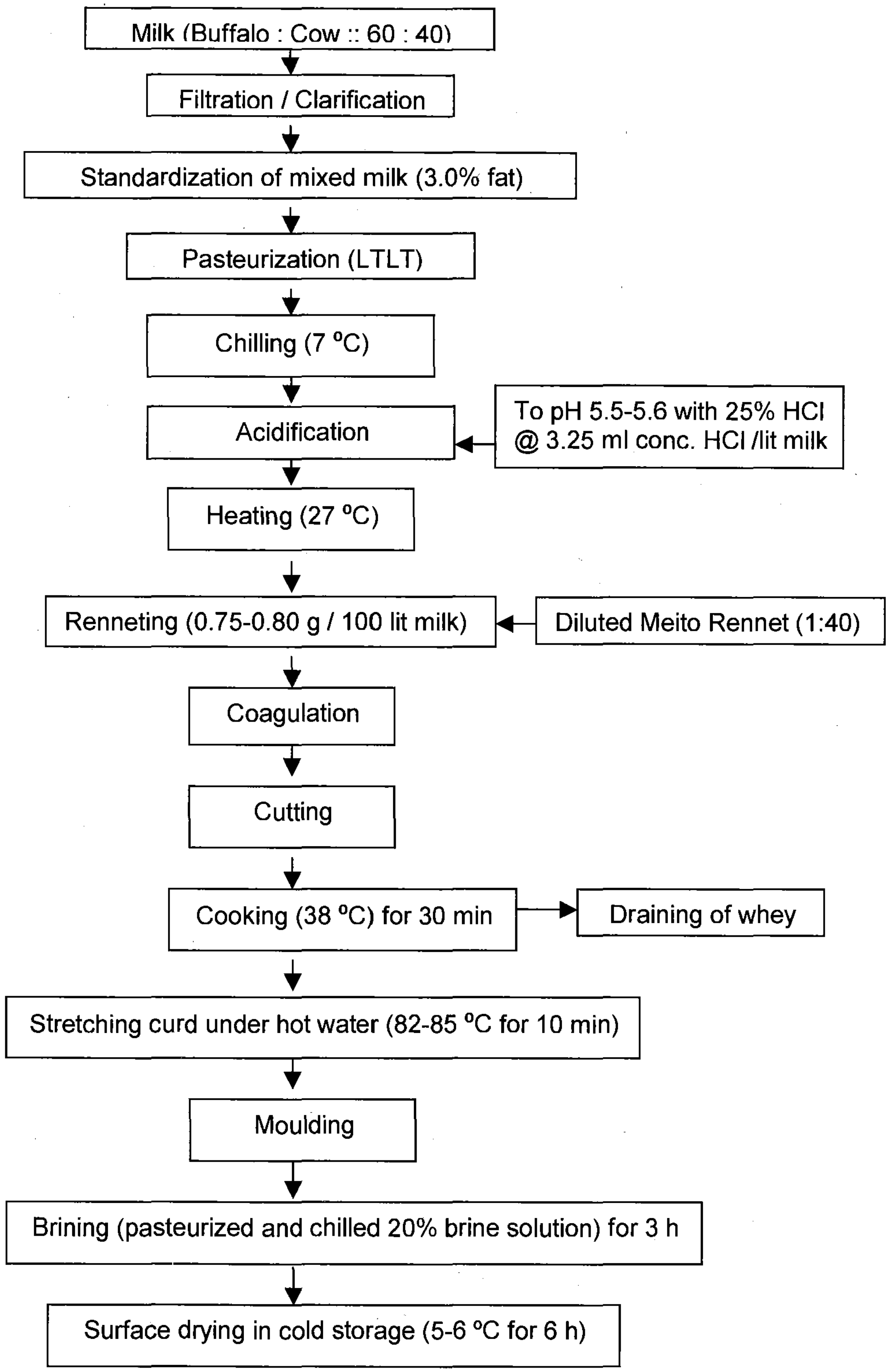
Technique of Mozzarella Cheese manufacture

In the present study, the method standardized by Tanweer Alam (2004) was followed for preparation of mozzarella cheese from mixed milk. 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.

The two types of milk were first filtered/clarified and then standardized as buffalo: cow :: 60: 40, and fat was adjusted to 3%. The standardized milk was pasteurized by LTLT method and cooled to 5 - 6 °C. The chilled milk was acidified to 5.5-5.6 pH with 25% HCl @ 3.25 ml conc. HCl / lit milk. The HCl used in the experiments was obtained from M/s s. d- Fine- CHEM Limited, Mumbai. The temperature of the acidified milk was raised to 27 °C. Then diluted microbial rennet, i.e. Meito (1:40 in distilled water) was added @ 0.75 - 0.80 g /100 lit milk. The renneted milk was left undisturbed for 15 min for setting of curd. The microbial rennet was obtained in powder form from M/s Meito Sangyo Co. Limited, Tokyo, Japan which was produced from *Mucor pusillus* var. *lindt* .

The properly set curd was cut with sterilized cheese knives, and then allowed to stand in whey for 5 min. Thereafter, the temperature of curd was raised @ 1°C / 5 min till the temperature reached 38 °C and maintained for 30 min with continuous stirring in order to achieve uniform cooking and to avoid lump formation. Then the whey was drained to aggregate the curd particles followed by stretching and moulding. The stretching was achieved in hot water (82 - 85 °C) for 10 min. Then it was moulded in ball shape, each ball weighing approx. 300 g. Mozzarella cheese balls were then immersed in pasteurised chilled (8 - 10 °C) brine solution (20% w/v) for 4 h. The commercial grade fine grain salt of reputed brand procured from the local market was used for brining. After surface drying of mozzarella cheese (in a cold storage maintained at 5 - 6 °C for 6 h), the cheese balls were packaged in LDPE bags (100 μ) and stored at -10 to -15 °C for using them in MAP Pizza studies.

FLOW DIAGRAM FOR THE MANUFACTURE OF MOZZARELLA CHEESE (DIRECT ACIDIFICATION METHOD)



4.1.2.0 Pizza

4.1.2.1 Materials used

The following materials were used during the present investigation:

Mozzarella cheese

The mozzarella cheese as prepared under section 4.1.1.0 was used in the preparation of pizza samples.

Cheddar cheese

For preparing the pizza samples, cheddar cheese was collected from the Experimental Dairy, National Dairy Research Institute (NDRI), Karnal (Haryana).

Pizza Base

Pizza bases each of 20 cm in dia and weighing approx. 120±10 g were used for the preparation of pizza samples, which were procured from a reputed Bakery Shop located in Karnal.

Vegetables and Spices

The fresh vegetables namely tomatoes, capsicum and green chillies of superior quality were procured from the local market. Onion, ginger and black pepper powder (having brand value) were procured from the local market.

Table Butter

The table butter used during the preparation of pizza samples was obtained from the Experimental Dairy of National Dairy Research Institute, Karnal.

Tomato Sauce

Tomato sauce of a popular brand used in the experiments was procured from the local market.

Microwave oven

The microwave oven having power output 900 W with internal dimensions of 36 x 37 x 23 cm^3 and 32 Lit capacity from Samsung, South Korea; Model Bio ceramic, CE118KF was used during the study.

4.1.2.2 Preparation of Ready-to-bake & Ready-to-serve Pizza

Dipping of vegetables in potassium metabisulphite (KMS)

The tomatoes, capsicum and green chillies used for the preparation of pizza samples were first thoroughly washed in clean tap water and then dipped in solution of potassium metabisulphite (2g / lit) for 20 min and air dried. The onions and ginger were, however peeled before dipping in KMS solution.

Cutting of vegetables

The air-dried tomatoes, capsicum, and onions were sliced in round shape (approx. 0.5 cm thick) by using 'Philips' make Food Processor (Essence HR7754). The green chillies were also cut in round shape (approx. 0.5 cm thick), while ginger was grated using clean stainless steel grater.

Topping of Pizza

The pizza samples were prepared by adapting the procedure of Tanweer Alam (2004) with slight modification. Firstly the lower side of each pizza base was grilled (heated) for a minute in a microwave oven on a rotating table, and then the upper side of the pizza base was smeared with approx. 5 g table butter followed by spreading of tomato sauce (approx. 40 g) over the butter smeared pizza surface. Then approx. 4 g chopped green chillies (*Capsicum annum var. acuminatum*) and approx. 10 g grated ginger (*Zingiber officinale*) were evenly spread all over the pizza base. Then grated mozzarella and cheddar cheese in the ratio of 80:20 (approx 120 g per pizza) was topped on pizza base followed by vegetable toppings which included approx. 50 g sliced onion (*Allium cepa*), approx. 70 g sliced tomatoes

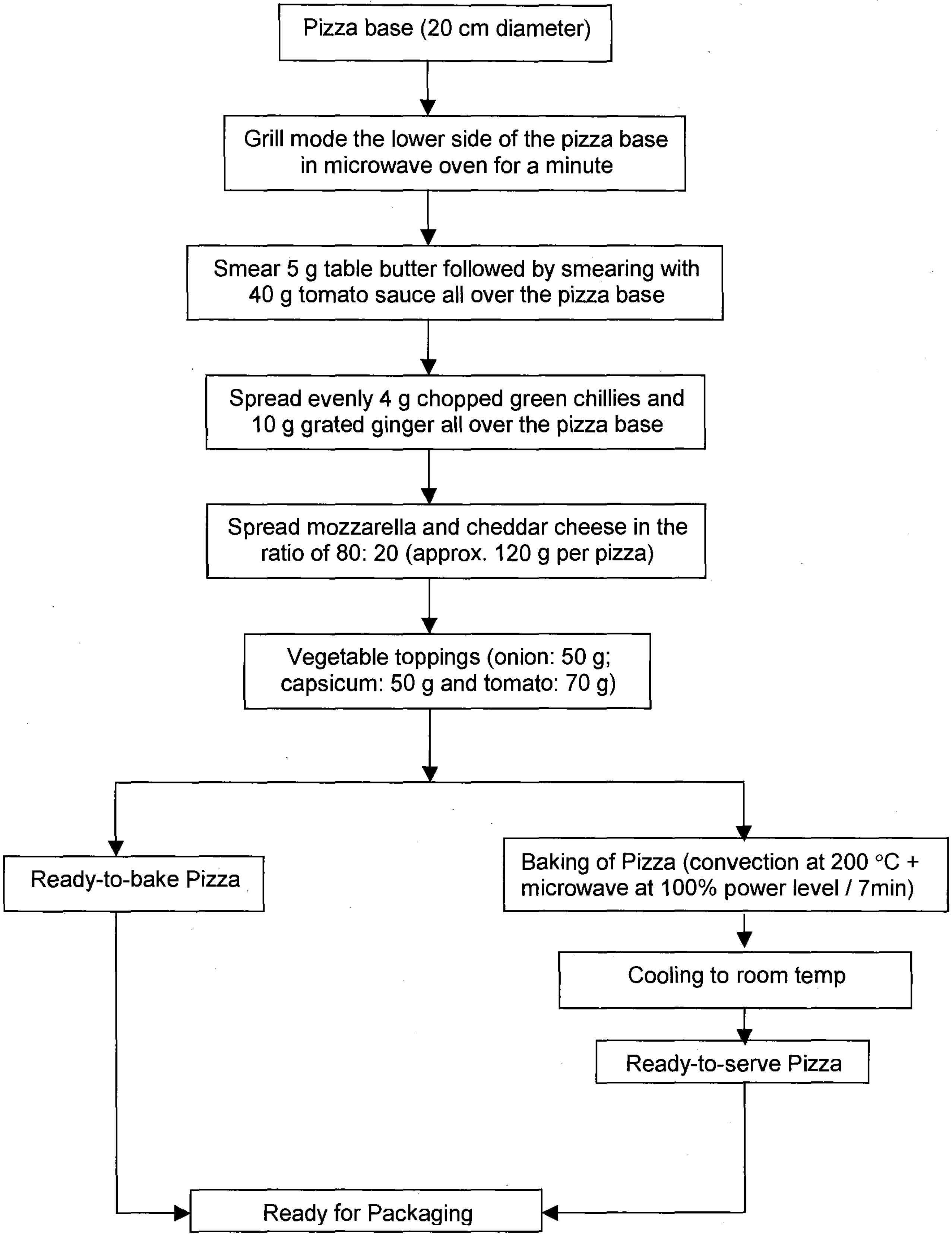
(*Lycoperscion esculatum*), and approx. 50 g sliced capsicum (*Capsicum annum var. grossum*). The ready-to-bake (unbaked) pizza samples were then used for MAP studies.

For preparation of pizza, the mozzarella cheese as prepared under Section 4.1.1.0 was used (moisture 52.25%; fat 22.03%; protein 21.12%; salt 1.65% and ash 1.70%), while the cheddar cheese (as obtained under Section 4.1.2.1) having chemical composition: moisture 35.72%; fat 33.96%; protein 23.98%; salt 1.58% and ash 3.61% was used.

Baking of Ready-to-serve Pizza

The baking of pizza was done in a preheated (220 °C) microwave oven. The pizza was placed on a stainless steel, elevated susceptor, and baked at combination mode (convection at 200 °C + microwave at 100% power level) for 7 min. The pizza samples were then taken out from microwave oven, which after cooling at room temperature, were used for modified atmosphere packaging (MAP) studies.

FLOW DIAGRAM FOR THE PREPARATION OF READY-TO-BAKE & READY-TO-SERVE PIZZA



4.2.0.0 Packaging and Storage of Ready-to-bake and Ready-to-serve Pizza in Different Atmospheres

4.2.1.0 Packaging Equipments:

4.2.1.1 Quick 2000 for MAP

Packaging of pizza samples under modified atmospheres was accomplished by following the method of Day (1992) and adopted by Tanweer Alam (2004), 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) (Plate 1). The injection conditions of the gases were optimised in order to obtain gas headspace.

4.2.1.2 Vertical Heat Sealing Machine

Packaging of pizza samples 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.

4.2.2.0 Packaging Materials used

For packaging and storage of pizza samples (both ready-to-bake and ready-to-serve), high barrier bags namely LLD / BA */ Nylon-6 / BA */ LDPE (110 μ) (*poly binding agent) were used. The dimensions of the packages used in the study were 32.5 x 35.0 cm (L x B). The packages were obtained from the reputed packaging film producer and converter of India.

4.2.2.1 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

Plate 1: Modified Atmosphere Packaging (MAP) System

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

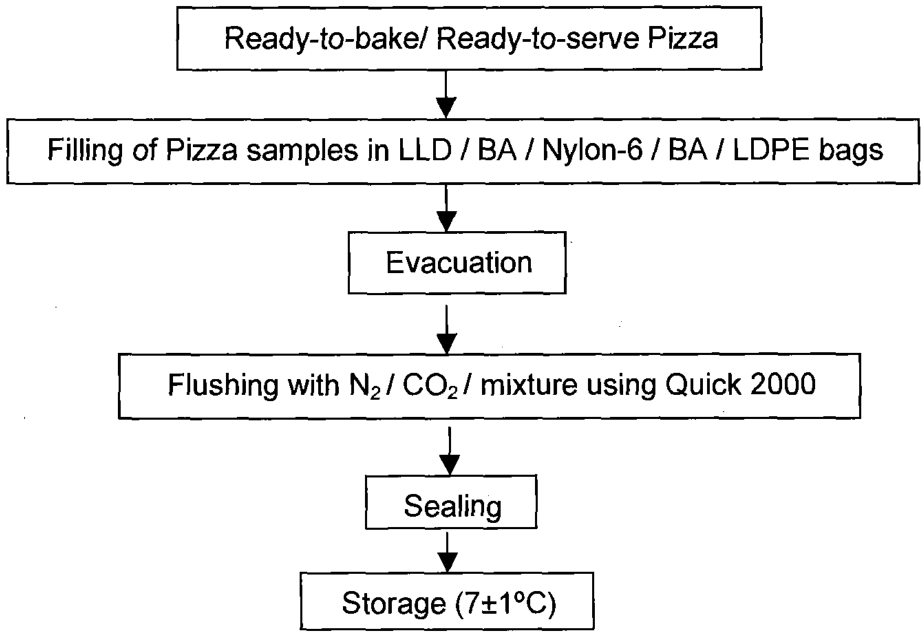
4.2.2.2 Sterilization of Packages

The empty packages used for pizza samples were sterilized under UV - light for 30 min immediately before packaging of the pizza samples.

4.2.3.0 Packaging of Pizza samples

The prepared pizza samples both ready-to-bake (Plates 2, 3, 4 and 5) and ready-to-serve (Plates 6, 7, 8 and 9) were individually packed in sterilized packages under different atmospheres (atm), i.e. air (atm 1), 100% CO_2 (atm 2), 100% N_2 (atm 3) and 50% CO_2 / 50% N_2 (atm 4) by using packaging machines as described under 4.2.1.1. & 4.2.1.2. Initially the gas headspace to pizza weight ratio was approx. 2 lit of gas per kg of the product. The packaged samples were then stored at 7±1 °C. The gases used were of industrial grade procured from the reputed supplier.

FLOW DIAGRAM FOR MODIFIED ATMOSPHERE PACKAGING (MAP) OF READY-TO-BAKE & READY-TO-SERVE PIZZA



4.3.0.0 Quality Assessment of Pizza samples during storage

4.3.1.0 Headspace Volume

The headspace volume of the packaged pizza samples was determined by following the method of Alves *et al.* (1996) and Tanweer Alam (2004) 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 Physico-Chemical Characteristics

Technique of Sampling

The samples of ready-to-bake and ready-to-serve pizza for chemical and microbiological analyses were prepared (opened the packages aseptically) by following the procedure as recommended by Labuza and Schmidl (1985), i.e. by mixing whole sample followed by grinding to obtain a representative sample (homogenate). The grinding of pizza samples was performed in 'Philips' make Food Processor.

Plate 2: Unbaked Pizza (Air packed) : ready for storage

Plate 3: MAP Unbaked Pizza (100% CO_2) : ready for storage

Plate 4: MAP Unbaked Pizza (100% N_2) : ready for storage

Plate 5: MAP Unbaked Pizza (50% CO_2: 50% N_2): ready for storage

Plate 6: Baked Pizza (Air packed) : ready for storage

Plate 7: MAP Baked Pizza (100% CO_2) : ready for storage

Plate 8: MAP Baked Pizza (100% N_2) : ready for storage

Plate 9: MAP Baked Pizza (50% CO_2 : 50% N_2) : ready for storage

4.3.2.1 Moisture

The moisture contents of mozzarella cheese, cheddar cheese and pizza samples were analysed by the method as described in IS: SP: 18 (Part XI), 1981.

4.3.2.2 Fat

The fat contents of mozzarella cheese, cheddar cheese and pizza samples were analysed using the method as given in IS: SP: 18 (Part XI), 1981.

4.3.2.3 Protein

The total protein contents of mozzarella cheese, cheddar cheese and pizza samples were estimated by micro-Kjeldahl method (Meneffee and Overman, 1940) using Kjeltec automatic digester and distillation equipment (Tecator make, Sweden).

4.3.2.4 Carbohydrates

Carbohydrates in pizza samples were obtained by difference method.

4.3.2.5 Ash

The ash contents of mozzarella cheese, cheddar cheese and pizza samples were estimated in accordance with procedure of AOAC (1984).

4.3.2.6 Salt

The salt contents in mozzarella cheese and cheddar cheese samples was determined by following the method used by Tanweer Alam (2004): 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 °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 filter paper Whatman No. 1. 25 ml of filtrate were taken in a 150 ml Erlenmeyer flask. 2 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 $AgNO_3$ / lit 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.

4.3.2.7 Food Energy value

The Food Energy value of pizza samples was calculated from the proximate composition by multiplying the protein and carbohydrates by 4 calorie and fat by 9 calorie by following the method as recommended by Kamel and Manji (1986).

4.3.2.8 Water Activity (a_w)

Water activity (a_w) determination of the pizza samples was carried out by using Aqua Lab water activity meter, Model number: series 3TE (Decagon Devices Inc., Washington, USA) by following the procedure as detailed in the instruction manual supplied by the manufacturer of the instrument. The a_w of the prepared samples was determined at 25 ºC.

4.3.2.9 Titratable Acidity

Ten g of the grounded sample of pizza as prepared under 4.3.2.0 were dissolved in 100 ml of distilled water and filtered through filter paper Whatman No.1.Ten ml of the aliquot was titrated against 0.1 N NaOH, using phenolphthalein as indicator. The acidity was expressed as percent lactic acid (% LA). Titratable acidity of pizza samples was determined by the method recommended by the Ranganna (2000). The total titratable acidity (% lactic acid) was calculated by following the formula:

$$\text{Acidity (\% LA)} = \frac{\text{Titre value x Normality x vol. made up x Eq. wt of acid x 100}}{\text{Volume of sample for estimation x wt of sample x 1000}}$$

4.3.2.10 pH

The 20 ml of filtrate as obtained under 4.3.2.9 was taken in a clean beaker and the pH was determined by using a pH meter, Model No. 420 A Plus Bench Top pH/ MV/ORP/Temperature Meter, Thermo Orion, supplied by M/s Thermo Electrone Corporation, Beverly, MA, USA.

4.3.2.11 Free Fatty Acids (FFA)

The fat breakdown in pizza samples was determined by estimating free fatty acids (FFA) (% oleic acid) adopting the procedure of Thomas *et al.* (1954).

4.3.2.12 Peroxide Value

The peroxide value of the pizza sample was determined on the lines detailed in IS: 3508 (1966). The prepared sample was extracted with 50: 50 mixture of diethyl ether and petroleum ether (40-60 °C B.P) in Soxhlet apparatus for 1½ hr in order to obtain sufficient fat. One gm of extracted fat was accurately and quickly weighed into test tube. Immediately 1 g of powdered potassium iodide and 20 ml of solvent mixture (a mixture prepared by adding 2 volumes of glacial acetic acid and 1 volume of chloroform) were added into the test tube. The contents of the tube were then heated to boiling within 30 s in a steam bath and then allowed to boil vigorously for another 30 s. The contents were then transferred into a 250 ml conical flask containing 20 ml of 5% aqueous solution of potassium iodide. The test tube was twice washed with 30 ml of distilled water and the washings were transferred into the flask. This solution was then titrated with 0.002 N standard solution of sodium thiosulphate using 1% starch indicator. The blank test was also performed in the same way.

The peroxide value (as milli - equivalents of peroxides per kg of fat) was calculated by using the following formula:

$$\frac{2T}{W}$$

where,

T = volume in ml of 0.002 N sodium thiosulphate solution used

W = weight in gram of sample taken

4.3.2.13 Thiobarbituric Acid (TBA) Value

The TBA value of the pizza sample was determined by the method prescribed by (Pokorny and Dieffenbacher, 1989).

4.3.2.14 Tyrosine Content

Extent of proteolysis during storage was expressed as tyrosine content. Proteolytic changes in sample were determined by method described by Hull (1947) with slight modification.

Accurately weighed 3 g sample as prepared under section 4.3.2.0 was thoroughly mixed with 10 ml of warm (45 °C) distilled water. 5 ml of the suspension was pipetted into a clean and dry 50 ml conical flask. Then 10 ml of 0.72 N Trichloroacetic acid (TCA) and 1 ml of distilled water were added. The mixture was then thoroughly shaken and allowed to stand for 30 min followed by filtration, using filter paper Whatman No. 42. Five ml of this filtrate was pipetted into a clean and dry 50 ml conical flask. To this were added 10 ml mixture of sodium carbonate and sodium hexametaphosphate solution (this solution was prepared by dissolving 75 g of sodium carbonate, and 10 g of sodium hexametaphosphate in 500 ml of distilled water). The mixture was then gently shaken and 3 ml of diluted Folin - Ciocalteau's reagent (1 part of Folin - Ciocalteau's reagent and 2 parts of distilled water v/v) was added with continuous shaking. A reagent control was also prepared. The readings were taken at 650 nm after the lapse of 5 min in Spectrophotometer model number GENESYS10 series supplied by Thermo Spectronic, Rochester, N.Y., USA.

Preparation of Standard Curve for Tyrosine:

100 mg of L (-) tyrosine was dissolved in glass double distilled water and the volume was made up to 100 ml. One ml of this stock solution (Solution - I) contained 1 mg of tyrosine.

10 ml of Solution-I was diluted to 100 ml by using glass distilled water. One ml of this solution (Solution-II) contained 0.1 mg of tyrosine. Now 20 ml of Solution-II was further diluted to 100 ml using glass double distilled water. One ml of this solution (Solution-III) contained 0.02 mg of tyrosine.

A known volume (corresponding to 0.02 mg to 0.42 mg of tyrosine) of Solutions-II and / or III was transferred into a series of 50 ml clean and dry conical flasks. The final volume in each flask was made to 6 ml using glass double distilled water. Then 10 ml of 0.72 N TCA was added to each flask.

5 ml aliquots of the above mixture were taken out from every flask for colour development and the readings were taken as described earlier. A linear curve was obtained by plotting absorbance against concentration of tyrosine (Appendix LIII).

4.3.2.15 Lycopene content

For estimation of lycopene content in pizza samples, the method suggested by Ranganna (2000) was followed with slight modifications. 5-10 g of the prepared sample (as under section 4.3.2.0) of the pizza was weighed. It was repeatedly extracted with acetone in a 100 ml capacity Erlenmeyer flask until the residue became colourless. The acetone extract was then transferred to a separating funnel containing 10-15 ml of petroleum ether and mixed gently. The carotenoid pigments were taken up into the petroleum ether by diluting the acetone (lower phase) with water containing 5% Na_2SO_4. The lower phase was transferred to another separating funnel and the petroleum ether containing the carotenoid pigments to an amber coloured bottle. Repeated extraction of the acetone phase similarly with petroleum ether until it became colourless. The acetone phase was discarded. To the petroleum ether extract, added 50 mg of anhydrous Na_2SO_4 and the contents were transferred to a 50 ml volumetric flask and diluted to mark with petroleum ether. The colour was measured in a 1 cm cell at 503 nm in a Spectrophotometer model number GENESYS10 series supplied by Thermo Spectronic, Rochester, N.Y., USA using petroleum ether as blank. The lycopene content of the sample was calculated as per the following formula using the relationship that an optical density (OD) of 1.0 = 3.1206 μg of lycopene / ml.

$$\text{Lycopene (mg/100g)} = \frac{3.1206 \times \text{OD of sample} \times \text{vol. made up} \times \text{dilution} \times 100}{1 \times \text{weight of sample} \times 1000}$$

4.3.2.16 Colour Estimation

Hunter Lab Colour Values (L*, a*, b*)

The colour of the prepared pizza samples (as under section 4.3.2.0) was measured by taking multiple readings and then calculating the average value, using a Colourflex Model 45°/0° (HunterLab, Reston, Virginia, USA) along with the universal software (version 4.10). Before the test, instrument was calibrated with standard black glass and white tile as specified by the manufacturer. The light source was dual beam xenon flash lamp. Data was received through the software in terms of L* [lightness, which ranges from zero (black) to 100 (white)], a* [redness, ranges from +60 (red) to -60 (green)] and b* [yellowness, ranges from +60 (yellow) to -60 (blue)] in values of the international colour system, when presenting the samples to the instrument in a standard, repeatable manner.

Hunter Lab Total Colour Difference (ΔE)

The Hunter Lab Total Colour Difference (ΔE) of pizza samples was calculated by the following formula as given by Liu *et al.* (2003):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

where,

$\Delta L = L_{sample} - L_{standard}$ (if +ΔL, sample is lighter than standard; if -ΔL, sample is darker than standard)

$\Delta a = a_{sample} - a_{standard}$ (if +Δa, sample is redder than standard; if -Δa, sample is greener than standard)

$\Delta b = b_{sample} - b_{standard}$ (if +Δb, sample is yellower than standard; if -Δb, sample is bluer than standard)

Relative Yellowness (E*)

Hunter Lab Total Colour Difference represents the comprehensive contributions from the variations of three colour indexes. To enhance the fraction of yellowness relative to those of lightness and redness, Relative Yellowness (E*) of pizza samples was calculated by the following formula (Liu *et al.*, 2003):

$$E^* = b^* / L^* + b^* / a^*$$

4.3.3.0 Textural Profile Analysis (TPA)

Instrumental textural evaluation was carried out (Clarke and Farrell, 2000) using Universal Testing machine TAXT*2i* (Stable Micro Systems, Godalming, Surrey, UK) Texture Analyser fitted with a 5 kg load cell and 75 mm aluminium blunt cylindrical probe (P75 mm compression platen). The pizza samples were cut in the round standard size cores (diameter 1.9 cm and height 1.1 cm) by using sampler provided with the instrument, and were placed centrally beneath the probe. A crosshead speed of 5.0 mm s^{-1} with a trigger force of 20 g was used to compress the cores to 80% of their original height. Each sample was compressed twice in a reciprocating motion to give a two-bite texture profile curve as shown in figure given below:

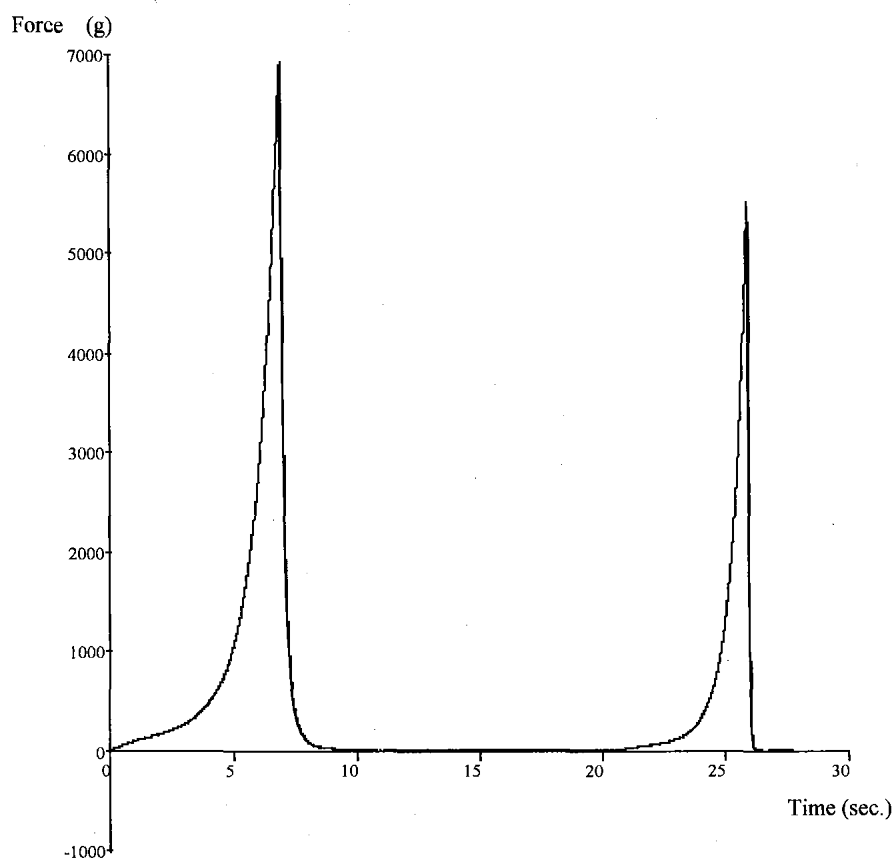


Fig: Texture Profile Curve of Pizza

Textural calculation

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

The graphs obtained were analysed for hardness, springiness, cohesiveness, gumminess, and chewiness using Texture Expert Exceed Software supplied along with the instrument.

4.3.4.0 Microbiological Analysis

The microbiological quality (TPC, yeast and moulds, coliform, total LAB count, psychotrophs and anaerobic spore formers) of fresh and stored unbaked and baked pizza samples were analysed by following the standard procedures as referred to under sections 4.3.4.1 to 4.3.4.7.

Preparation of Dilution Blanks

The dilution blank consisted of 99 ml and 9 ml portions of sterile peptone water (AR Grade) in screw capped dilution bottles and culture tubes, respectively. These were autoclaved at 15 lb pressure (121°C) for 20 min. The dilution blanks were warmed to 45 °C before use for preparation of samples.

4.3.4.1 Sampling of Pizza

The 11 g of the prepared sample (as under section 4.3.2.0) were thoroughly mixed in dilution bottles using 99 ml dilution blank. Further serial dilutions were prepared with 9 ml dilution blanks.

4.3.4.2 Total Plate Count (TPC)

In order to determine the total count of viable bacteria in pizza samples nutrient agar was used as medium. 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 °C) for 15 min. The plates were prepared, inoculated and incubated at 30 °C for 48 h (APHA, 1985). The nutrient agar used in the experiments was procured from HIMEDIA, Mumbai.

4.3.4.3 Yeast and Moulds (Y & M) 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, containing 10 ppm each of chloramphenicol and chlorotetracycline to suppress bacterial growth (Smith *et al.*, 1983), 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 °C) for 15 min. 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 °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 experiments was obtained from HIMEDIA, Mumbai.

4.3.4.4 Coliform Count

In the pizza samples, 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 min to dissolve the medium completely. It was then cooled to 45 °C. The plates after inoculation were incubated at 37 °C for 24 h (APHA, 1978). The violet red bile agar used was procured from HIMEDIA, Mumbai.

4.3.4.5 Total Lactic Acid Bacteria (LAB) Count

For the selective enumeration of total lactic acid bacteria count, MRS agar was used in the study. To rehydrate the medium, 65.25 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 °C) for 15 min. The plates after inoculation were incubated at 37 °C for 48 h (APHA, 1978). The presence of creamish coloured colonies indicated the presence of lactic acid bacteria.

4.3.4.6 Psychrotrophic Count

In fresh as well as stored pizza samples, the 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 °C) for 15 min. The plates were incubated at 4 °C for 10 days (APHA, 1978). The plate count agar used in the trials was procured from HIMEDIA, Mumbai.

4.3.4.7 Anaerobic Spore formers

For determining anaerobic spore formers, the method of Rao (1991) was employed by using plate count agar procured from HIMEDIA, Mumbai. The media was rehydrated and prepared as per the procedure described for TPC under section 4.3.4.2. After inoculation, the surface of medium was layered with sterile agar to maintain anaerobic conditions.

4.3.5.0 Sensory Evaluation of Pizza

The samples of pizza packed under four different atmospheres and stored at 7±1 °C (as given under section 4.2.3.0) in the high barrier bags were evaluated organoleptically for sensory attributes by a panel of five judges for appearance, body & texture, flavour and overall acceptability.

Before presenting the pizza samples to judges, the stored ready-to-serve test samples were reheated in microwave oven for 2 min at 100% power level. To minimise variability during reheating, single samples were placed in the central position on the turntable. The reheating time was based on the amount of time necessary for the product to yield an adequately reheated appearance, i.e. 2 min, while the ready-to-bake pizza samples were baked in microwave oven in combination mode (convection at 200 °C + microwave at 100% power level) for 7 min. After baking, the pizza sample was taken out of microwave oven and allowed to stand for a minute before cutting to make it crisp, then presented to the judges under code numbers. The pizza samples were evaluated using 5-point Hedonic Scale. A score of 5 represented excellent; 4, very good; 3, good; 2, fair; and 1, poor (Appendix LII).

4.4.0.0 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 pizza when fresh and after different intervals of storage, which included changes in headspace volume, chemical, microbiological, textural, and sensory properties are presented in Tables 1-11 and Appendices I to LIII. 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 period are included and discussed. The Tables on statistical analysis are also included in the body of the text to facilitate discussion.

5.1.0.0 PHYSICO-CHEMICAL, MICROBIOLOGICAL, AND TEXTURAL CHARACTERISTICS OF FRESHLY PREPARED UNBAKED (READY-TO-BAKE) AND BAKED (READY-TO-SERVE) PIZZA USED FOR THE PACKAGE AND STORAGE STUDY

The method for the preparation of ready-to-bake and ready-to-serve pizza used for the experiments has been given in section 4.1.2.2. The data, concerning unbaked (ready-to-bake) and baked (ready-to-serve) pizza, on moisture, protein, fat, ash, carbohydrates and food energy value are presented in Table 1, and the microbiological quality namely total plate count (TPC), yeast and moulds (Y & M) count, total lactic acid bacteria (LAB) count, coliform count, psychrotrophic count and anaerobic spore formers are depicted in Table 2.

The textural characteristics of ready-to-serve pizza are reflected in Table 3, which includes hardness, cohesiveness, springiness, gumminess and chewiness.

5.1.1.0 Physico-Chemical Characteristics

The proximate composition of freshly prepared unbaked (ready-to-bake) and baked (ready-to-serve) pizza samples, which includes the minimum, maximum and average values for moisture, protein, fat, ash and carbohydrates contents besides food energy value are presented in Table 1.

Table 1: Proximate Composition of Fresh Unbaked (ready-to-bake) and Baked (ready-to-serve) Pizza*

Attributes	Unbaked Pizza			Baked Pizza		
	Min.	**Max.**	**Avg.**	**Min.**	**Max.**	**Avg.**
Moisture (%)	55.11	56.26	55.68	42.73	44.68	43.71
Protein (%)	11.45	11.85	11.65	14.44	14.82	14.63
Fat (%)	9.45	9.61	9.53	11.84	12.12	11.98
Ash (%)	2.51	2.63	2.57	2.88	3.08	2.98
Carbohydrates**	19.65	21.48	20.57	25.30	28.11	26.71
Food Energy (cal/100 g)***	209.45	219.81	214.65	265.52	280.80	273.18

* based on three trials

** Carbohydrates were obtained by difference

*** Food energy values were obtained from the proximate composition by multiplying protein and carbohydrate by 4 calories, and fat by 9 calories

5.1.1.1 Table 1 on the chemical quality of samples of freshly prepared pizza indicates that (i) the average moisture content (%) was 55.68 (min. 55.11; max. 56.26) for unbaked pizza and (ii) 43.71 (min. 42.73; max. 44.68) for baked pizza. The difference in the values of moisture content in the two types of pizza samples is obviously due to the fact that during baking process a portion of the moisture had evaporated. Kamel and Manji (1986) reported the moisture content in unbaked pizza as 48.8%. The difference in moisture contents of laboratory made pizza samples might be due to many variables such as: initial moisture content in the crust, mozzarella cheese, cheddar cheese and vegetables used during the preparation of pizza samples. USDA (2005) recommended the moisture content in baked cheese vegetable pizza to be 46.28%, which is close to the values obtained for baked pizza (Table 1).

5.1.1.2 The minimum and maximum values for protein content (%) in unbaked pizza samples were 11.45 and 11.85% with an average of 11.65%, while the values were 14.44, 14.82 with an average of 14.63, respectively in case of baked pizza (Table 1). These values had been much higher than the value (10.36%) recommended by USDA (2005) for protein. These figures agree with the earlier findings: protein content 12.2% in the unbaked pizza samples (Kamel and Manji, 1986) and 12.37% protein in baked samples (www.nutritionanalyser.com).

5.1.1.3 The average fat content (%) in unbaked pizza samples was observed to be 9.53 with a range of 9.45 to 9.61. These values are almost similar to the findings of Kamel and Manji (1986) who found the fat content to be 9.2% in unbaked pizza. Table 1 also indicates that the fat content in baked pizza samples varied from 11.84 to 12.12 with an average of 11.98. These values are much above the recommended value of 8.78% by www.nutritionanalyser.com, but the values had been lower than the recommendations of USDA (2005), according to whom total fat content in pizza samples should be 12.28%.

5.1.1.4 The ash content (%) of the samples of pizza used in the studies on modified atmosphere packaging (MAP) averaged 2.57 for unbaked samples and 2.98 for baked samples. These values are in agreement with the findings of Kamel and Manji (1986), who reported ash content as 2.6% in unbaked pizza samples. However, ash content in all the samples is higher than the one (2.06%) recommended by USDA (2005).

5.1.1.5 The average values for the carbohydrates content (%) of the samples of unbaked and baked pizza were 20.57 and 26.71 respectively. It looks as the unbaked pizza samples with higher moisture content had a lower carbohydrates compared to baked samples. The figure of 26.71 for baked pizza samples is lower than the value of 29.02 recommended by USDA (2005) for cheese vegetable pizza.

5.1.1.6 The food energy values (cal/100g) averaged 214.65 and 273.18 for unbaked and baked pizza samples respectively. Since food energy values are obtained from the proximate composition by multiplying the protein and carbohydrates by 4 calories, and fat by 9 calories, the baked pizza samples, which had more protein, carbohydrates and fat contents, had higher food energy values than unbaked pizza samples. The food energy reported by Kamel and Manji (1986) of fresh pizza was 240 cal/100g.

5.1.2.0 Microbiological Characteristics

The observations relating to the microbiological quality (counts in log cfu/g) of fresh samples of unbaked and baked pizza revealed (Table 2) that the various average microbiological counts were → total plate count (TPC): 6.65 log cfu/g (unbaked), 5.58 log cfu/g (baked); yeast & moulds (Y & M): 2.78 log cfu/g (unbaked), 2.13 log cfu/g (baked); coliform: 2.57 log cfu/g (unbaked), 1.39 log cfu/g (baked); total lactic acid (LAB) bacteria: 5.02 log cfu/g (unbaked), 4.09 log cfu/g (baked); psychrotrophs: 2.39 log cfu/g (unbaked), 2.04 log cfu/g (baked); anaerobic spore formers: 2.67 log cfu/g (unbaked), 1.91 log cfu/g (baked).

The values for TPC, Y & M and coliforms were slightly higher than those reported by Kamel and Manji (1986), Fasano and Gallo (2001) and Donnelly (2002) for unbaked and refrigerated pizza samples.

Table 2: Microbiological quality of Fresh Unbaked (ready-to-bake) and Baked (ready-to-serve) Pizza*

Characteristics	Unbaked Pizza (log cfu/g)			Baked Pizza (log cfu/g)		
	Min.	Max.	Avg.	Min.	Max.	Avg.
Total Plate Count	6.48	6.81	6.65	5.37	5.78	5.58
Y & M Count	2.56	3.00	2.78	1.89	2.37	2.13
Coliform Count	2.23	2.90	2.57	1.25	1.53	1.39
LAB Count	4.69	5.34	5.02	3.92	4.27	4.09
Psychrotrophic Count	2.18	2.59	2.39	1.97	2.11	2.04
Anaerobic Spore formers	2.56	2.78	2.67	1.76	2.05	1.91

* based on three trials

5.1.3.0 Textural Characteristics

Instrumental textural evaluation of fresh baked (ready-to-serve) pizza samples (Table 3) show that the hardness (g) averaged 6621.87, cohesiveness averaged 0.461, springiness (mm) averaged 0.786, gumminess (g) averaged 3000.01 and chewiness (g x mm) averaged 2330.81.

Table 3: Textural properties of Fresh Baked (ready-to-serve) Pizza*

Characteristics	Min.	Max.	Average
Hardness (g)	6305.92	6937.82	6621.87
Cohesiveness (A2/A1) **	0.450	0.472	0.461
Springiness (mm)	0.779	0.793	0.786
Gumminess (g)	2728.705	3271.315	3000.01
Chewiness (g x mm)	2113.37	2548.25	2330.81

* based on three trials

** A2: +ve area under 2nd peak; A1: +ve area under 1st peak

However, Clarke and Farrell (2000) reported the following values for textural characteristics of microwave-reheated pizza → hardness (g): 12571, 14461 and 19989 for samples reheated for 120 s, 150 s and 180 s respectively; cohesiveness: 0.481 for 120 s, 0.500 for 150 s and 0.554 for 180 s; springiness (mm): 0.916, 0.914 and 0.947 for samples reheated for 120, 150 and 180 s respectively; gumminess (g): 6079, 7230 and 11097 for samples reheated for 120, 150 and 180 s respectively; chewiness (g x mm): 5596, 6604 and 10527 for pizza samples reheated for 120, 150 and 180 s, respectively.

5.2.0.0 CHANGES IN QUALITY OF MAP UNBAKED (READY-TO-BAKE) AND BAKED (READY-TO-SERVE) PIZZA DURING STORAGE

Freshly prepared unbaked (ready-to-bake) and baked (ready-to-serve) pizza samples were examined for their degree of deterioration during storage when packed under 4 atmospheres in the high barrier bags (LLD/BA*/Nylon-6/BA*/LDPE) and stored under the preset conditions of temperature (7±1 °C). The parameters adopted for the evaluation of the degree of deterioration in different samples under different atmospheric conditions are discussed in this section under the heads: changes in headspace volume, chemical changes, microbiological changes, textural changes, and sensory characteristics of pizza samples.

5.2.1.0 Changes in Headspace volume

The observations on the headspace volume of unbaked pizza samples packed under different atmospheres and stored at 7±1 °C are presented in Appendix I and illustrated in Figure 1. The statistical analysis of the data on headspace volume is given in Table 4. From Appendix I and Figure 1, it can be seen that the initial headspace volume of 76 ml in air packed (atm 1) unbaked pizza decreased to 75 ml (1.32% decrease) after 10 days of storage at 7±1 °C. The initial headspace volume of 748 ml in the

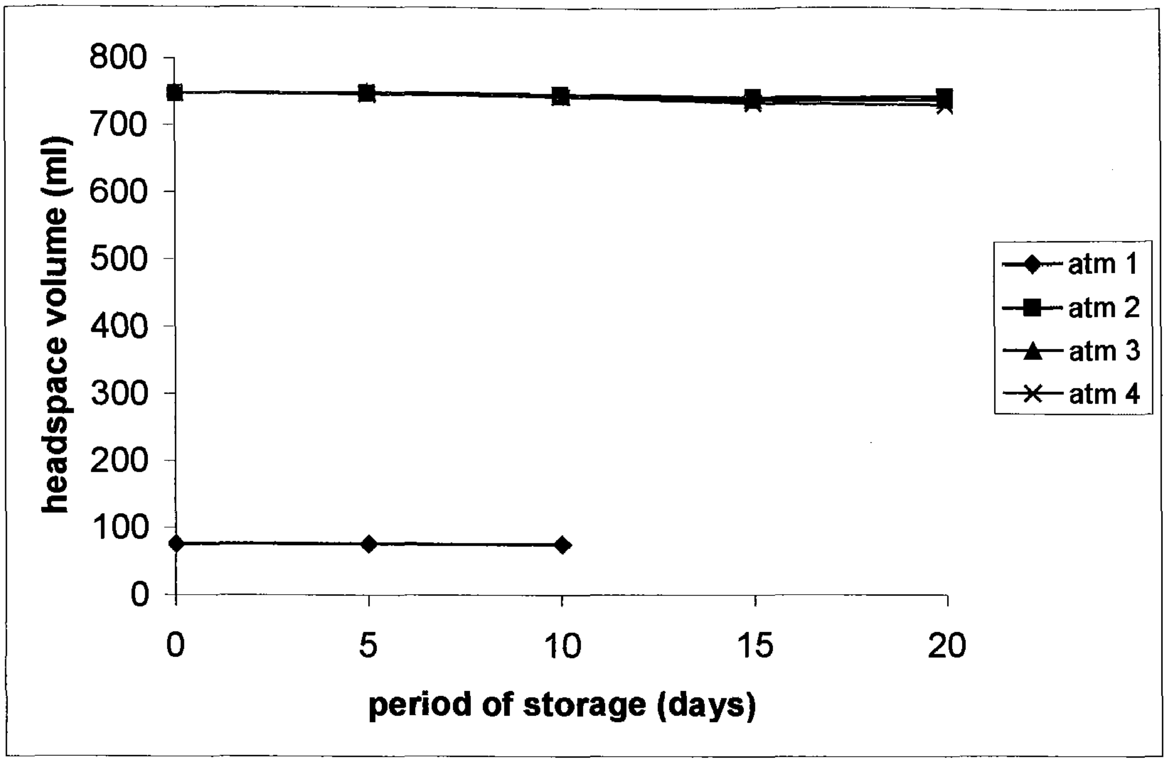


Fig 1: Effect of MAP on the Headspace volume (ml) of unbaked pizza stored at 7 ± 1 °C

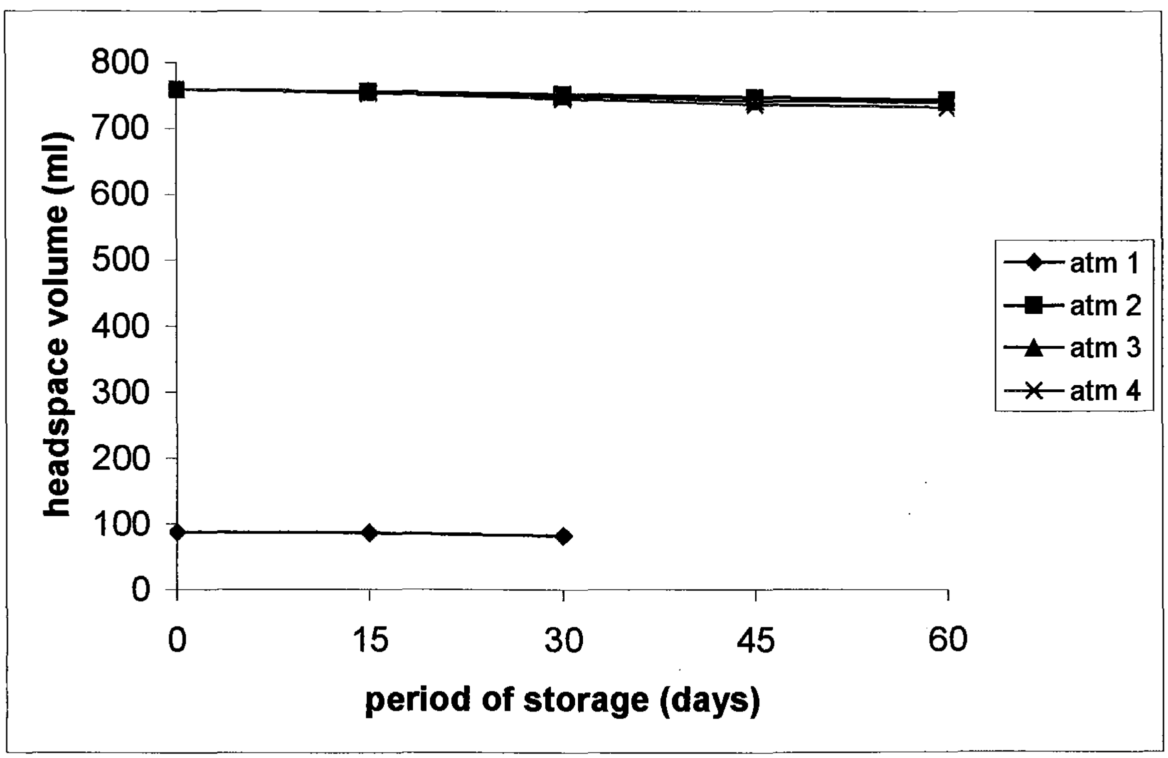


Fig 2: Effect of MAP on the Headspace volume (ml) of baked pizza stored at 7 ± 1 °C

Table 4. Analysis of variance for Headspace volume of MAP Pizza stored at 7±1 °C

Source of variation	d. f.	Mean sum of square	
		Unbaked	Baked
Among Intervals of storage	4	0.0182**	0.0715**
Among atmospheres	3	101.2769**	100.8114**
Error	46	0.0003	0.0004
Interaction [Intervals x atmospheres (atm 2, atm 3, atm 4)]	8	18.4499**	20.2000**
Error	30	0.4333	0.3333

**** Significant at 1% level of probability**
atm 2: 100% CO_2
atm 3: 100% N_2
atm 4: 50% CO_2 : 50% N_2

packages with 100% CO_2 (atm 2), 100% N_2 (atm 3) and 50% CO_2 / 50% N_2 (atm 4) decreased to 745 ml (0.40% decrease), 741 ml (0.94% decrease) and 737 ml (1.47% decrease) respectively after 15 days of storage, indicating that maximum decrease in the value had been in the case of atm 4 and minimum in atm 2.

Data on the headspace volume of baked (ready-to-serve) pizza packed under 4 atmospheres and stored at 7±1 °C (Appendix II, Fig. 2) revealed that after 30 days, the initial value of 87 ml decreased to 82 ml (5.75% decrease) in case of samples packed under air (atm 1). However, for the product packaged under atm 2, atm 3 and atm 4, the initial headspace volume of 759 ml decreased to 743 ml (2.11% decrease), 740 ml (2.50% decrease) and 732 ml (3.55% decrease) respectively, indicating that the maximum decrease had been in case of atm 4 followed by atm 3 and atm 2, in ascending order. The variation in the headspace volume of the pizza samples packaged under different modified atmospheres was most likely due to varied permeability rates of gases or mixture of gasses through packaging materials (Selby, 1968; Sachrow and Griffin, 1970). The results in general agree with the findings of Tanweer Alam (2004) who also observed that initial headspace volume of mozzarella cheese packed in the same high barrier bags decreased in all the samples packed under 100% CO_2, 100% N_2 and 50 % CO_2 / 50 % N_2 during storage.

Analysis of variance showed highly significant ($P<0.01$) difference towards headspace volume among intervals of storage, among the four types of modified atmospheres, and the interaction intervals x atmospheres (atm 2, atm 3, atm 4) for both types (unbaked and baked) of pizza samples.

5.2.2.0 Chemical changes

5.2.2.1 Moisture content

The observations relating to the moisture content of unbaked pizza samples packed under 4 atmospheres, and stored at 7±1 °C are presented in Appendix III and illustrated in Figure 3. The statistical analysis of the data on moisture content is given as part of Table 5. From Figure 3 and Appendix III, it can be observed that the initial moisture content of 55.68% in air packed (atm 1) pizza decreased to 54.77 (1.63% moisture loss) after 10 days of storage. In case of pizza samples packed under 100% CO_2 (atm 2), 100% N_2 (atm 3) and 50% CO_2 / 50% N_2 (atm 4), the initial moisture content of 55.68% decreased to 54.02 (2.98% decrease) for atm 2, 54.17 (2.71% decrease) for atm 3, and 53.76 (3.44% decrease) for atm 4 respectively, after 20 days of storage at 7±1 °C, indicating that the minimum decrease was for the samples packed under atm 3 followed by atm 2 and atm 4 respectively, in ascending order.

In case of baked pizza samples stored at 7±1 °C (Appendix IV, Fig. 4), the initial moisture value of 43.71% decreased to 43.31 (0.92% moisture loss) for atm 2, 43.29% (0.96% moisture loss) for atm 3 and 43.35 (0.82% moisture loss) for atm 4, respectively. From the Appendices III and IV, it is interesting to note that the moisture loss had been much more in case of unbaked pizza samples compared to baked pizza samples. In general, the results match with the findings of Kamel and Manji (1986) who observed that the moisture content of pizza decreased during storage after 12 months of storage at subzero temperatures. They further observed that the storage temperature had no significant effect on the moisture loss in frozen pizza, but the storage time decreased the moisture content. Tanweer Alam (2004) also reported loss in moisture content of mozzarella cheese samples packed under 5 different atmospheres and stored at 7±1 °C.

Table 5. Analysis of variance for Chemical characteristics of MAP Pizza stored at 7±1 °C

Source of variation	d. f.	Mean sum of square							
		Moisture		Water Activity		pH		Titratable Acidity	
		Unbaked	Baked	Unbaked	Baked	Unbaked	Baked	Unbaked	Baked
Among Intervals of storage	4	4.4870**	0.1964**	0.0002**	0.0001**	0.0759**	0.3221**	0.0504**	0.1443**
Among atmospheres	3	0.0683**	0.0617**	0.0007**	0.0004**	0.0341**	0.2316**	0.0987**	0.0573**
Error	46	0.0082	0.0030	0.0001	0.0001	0.0012	0.0015	0.0021	0.0027
Interaction [Intervals x atmospheres (atm 2, atm 3, atm 4)]	8	0.0405	0.0148	0.0001	0.0001	0.0020**	0.0048**	0.0082**	0.0006**
Error	30	0.0015	0.0004	0.0001	0.0001	0.0004	0.0005	0.0003	0.0002

**** Significant at 1% level of probability**
atm 2: 100% CO_2
atm 3: 100% N_2
atm 4: 50% CO_2 : 50% N_2

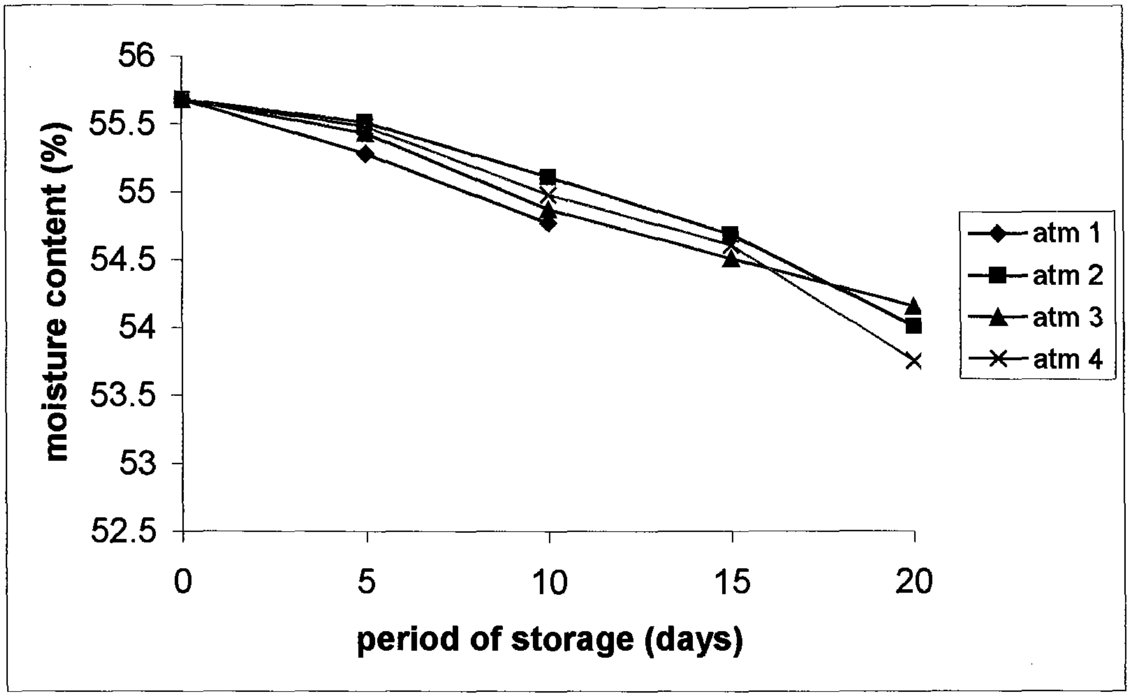


Fig 3: Effect of MAP on the Moisture content (%) of unbaked pizza stored at 7 ± 1 °C

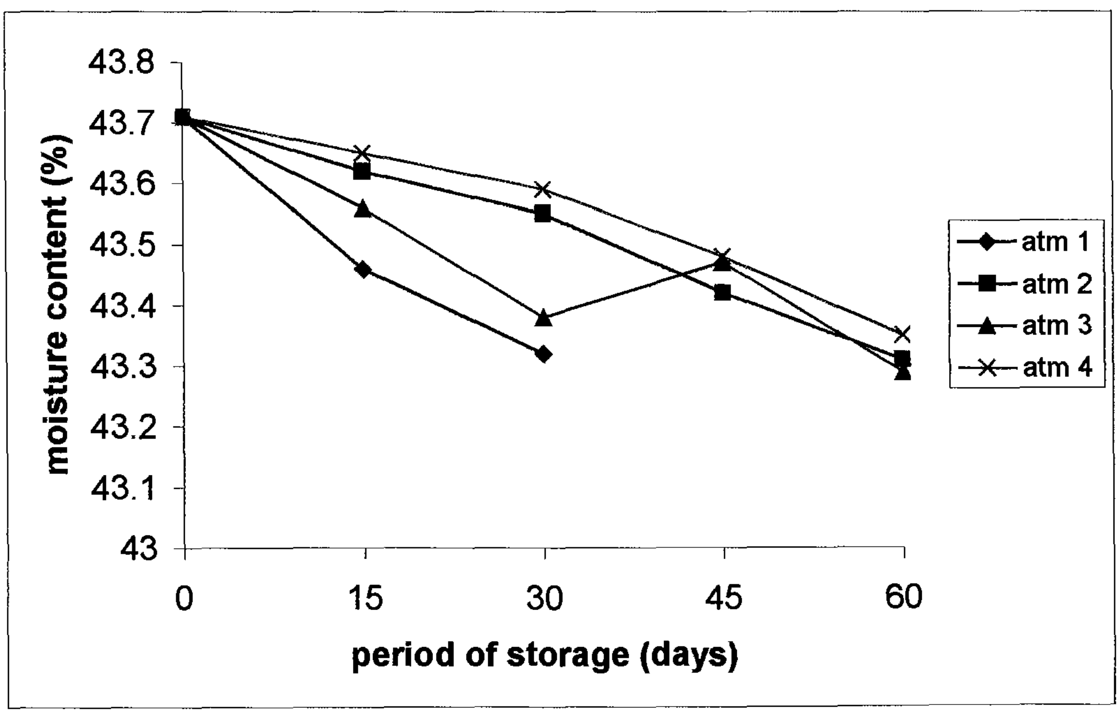


Fig 4: Effect of MAP on the Moisture content (%) of baked pizza stored at 7 ± 1 °C

Intervals of storage and the type of atmosphere, both individually, significantly ($P<0.01$) affected the moisture content in both the types of pizza samples. However, the interaction intervals x atmospheres (atm 2, atm 3, atm 4) was found to be not significant for both types of pizza samples (Table 5).

5.2.2.2 Water activity (a_w)

Appendices V and VI, Figures 5 and 6 represent the changes in water activity of unbaked and baked pizza samples during storage. The mean initial value of 0.975 in unbaked pizza was found to decrease during storage in all the samples packed under 4 different atmospheres. It can be seen from Appendix V that initial a_w 0.975 decreased to 0.963 in atm 2, 0.965 in atm 3 and 0.966 in atm 4, suggesting that the maximum decease was for the samples packed under 100% CO_2 followed by 100% N_2 (a_w 0.965), and 50% CO_2 / 50% N_2 (a_w 0.966) respectively. Since water activity is the index of moisture content in the product, the changes in a_w of pizza samples during storage correspond to the moisture content of the samples during storage (Appendix III).

The initial water activity of baked pizza samples used for storage studies was found to be 0.959, while Smith and Simpson (1995) reported that pizza being high moisture bakery product had a_w 0.99. The a_w decreased from 0.959 to 0.956 in case of product packed under atm 2, 0.954 for product packed under atm 3, and 0.957 for atm 4; thus revealing maximum decease (0.52%) for atm 3 followed by atm 2 (0.31% decrease) and atm 4 (0.21% decrease) (Appendix VI).

Analysis of variance of the data on water activity (Table 5) revealed that the five intervals of storage and the four types of atmospheres, each individually, were highly significant ($P<0.01$) for both types of pizza samples. However, no significant interaction was observed in case of intervals x atmospheres (atm 2, atm 3, atm 4) for the two types of pizza samples.

5.2.2.3 pH

In Figure 7 and Appendix VII are given the values for pH of MAP pizza samples packed under 4 atmospheres stored at 7±1 °C. Anova is given as a part of Table 5. The data showed that the initial pH of 5.80 in air packed (atm 1) pizza samples decreased to 5.63 and 5.52 respectively, after 5 and 10 days of storage. The decrease in pH might be attributed to lactic acid production by spoilage organisms (Daifas *et al.*, 1999). The initial value of pH (5.80) decreased to 5.67 (2.24% decrease), 5.54 (4.48% decrease) and 5.61 (3.27% decrease) respectively for pizza samples packed under 100% CO_2 (atm 2) followed by 100% N_2 (atm 3) and 50% CO_2 / 50% N_2 (atm 4), showing that the least decrease in pH values was for the samples packed under atm 2 followed by atm 4 and atm 3.

The changes in pH values of baked pizza samples packaged under 4 atmospheres and stored at 7±1 °C (Appendix VIII and Fig. 8) revealed that the initial pH of 6.19 decreased to 5.26, (corresponding to 15.02% decrease) after 30 days of storage for air packed samples, while the initial pH of 6.19 decreased to 5.78 (atm 3), 5.83 (atm 4) and 5.96 (atm 2), registering the maximum decrease for the samples packed under 100% N_2 followed by 50% CO_2 / 50% N_2 and 100% CO_2, respectively in ascending order. The observations agree with the findings of Daifas *et al.* (1999) who reported decrease in pH of pizza from 5.62 to 5.48 when packaged under modified atmosphere comprising CO_2 : N_2 :: 60 : 40 and stored for 42 days at 25 °C, and ascribed the reason for decrease in pH to be the dissolution of CO_2 in the product. The results also match with the work of Tanweer Alam (2004) who found that the initial pH of 5.61 in air packed mozzarella cheese samples decreased to 5.15 after 5 weeks of storage and the pH of samples in the packages with 100% CO_2, 100% N_2 and 50% CO_2 / 50% N_2 decreased, and

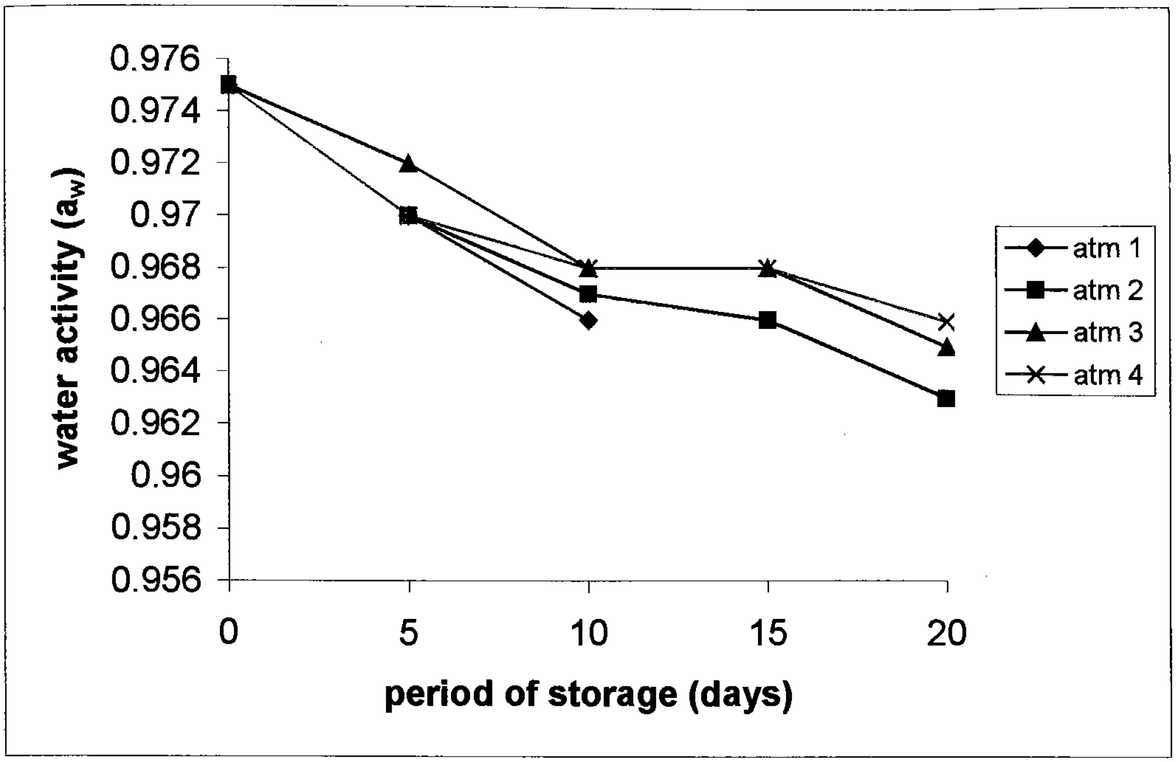


Fig 5: Effect of MAP on the water activity (a_w) of unbaked pizza stored at 7 ± 1 ºC

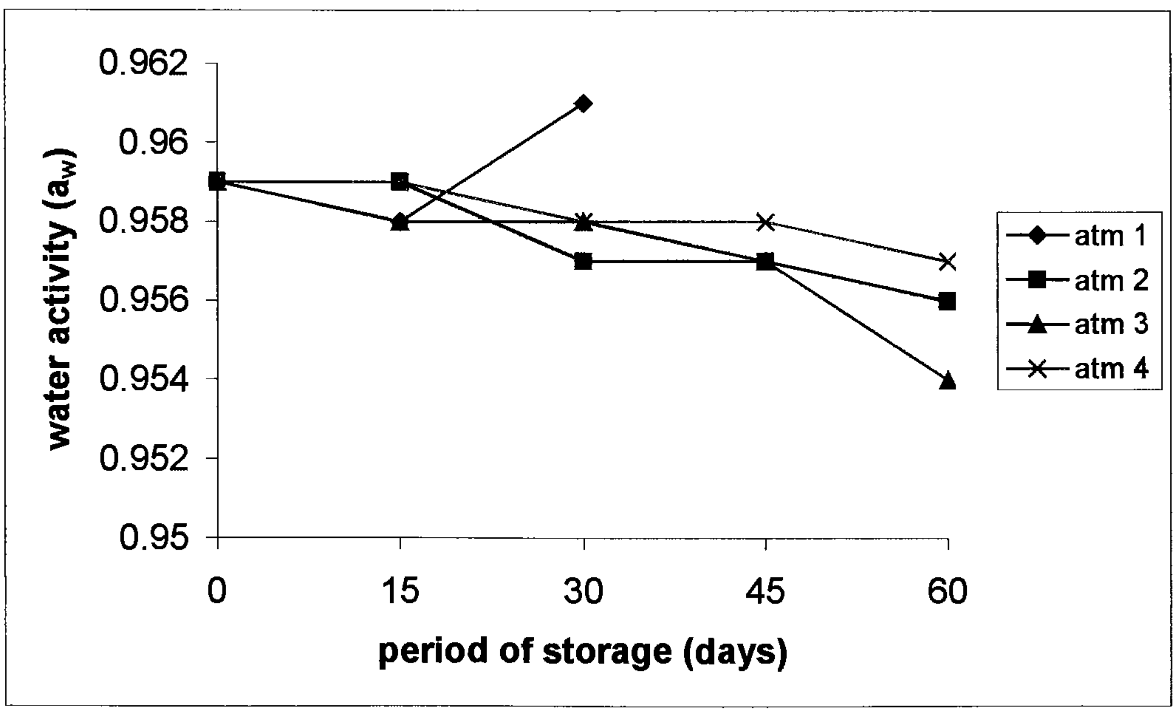


Fig 6: Effect of MAP on the water activity (a_w) of baked pizza stored at 7 ± 1 ºC

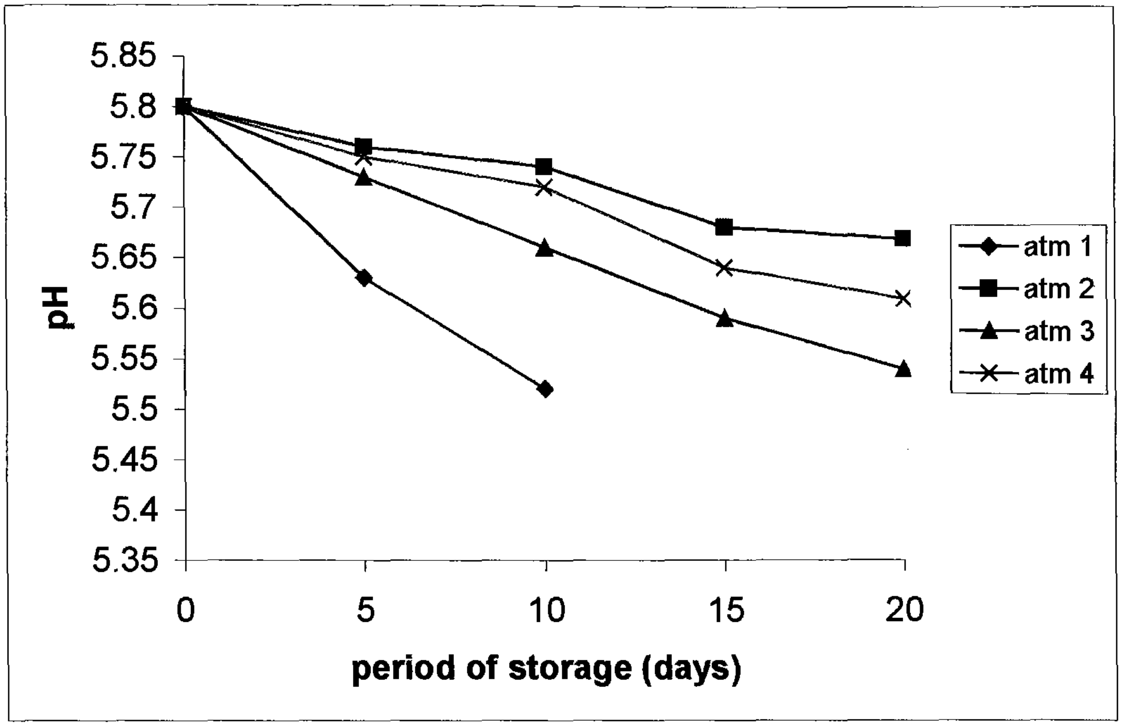


Fig 7: Effect of MAP on the pH of unbaked pizza stored at 7 ± 1 °C

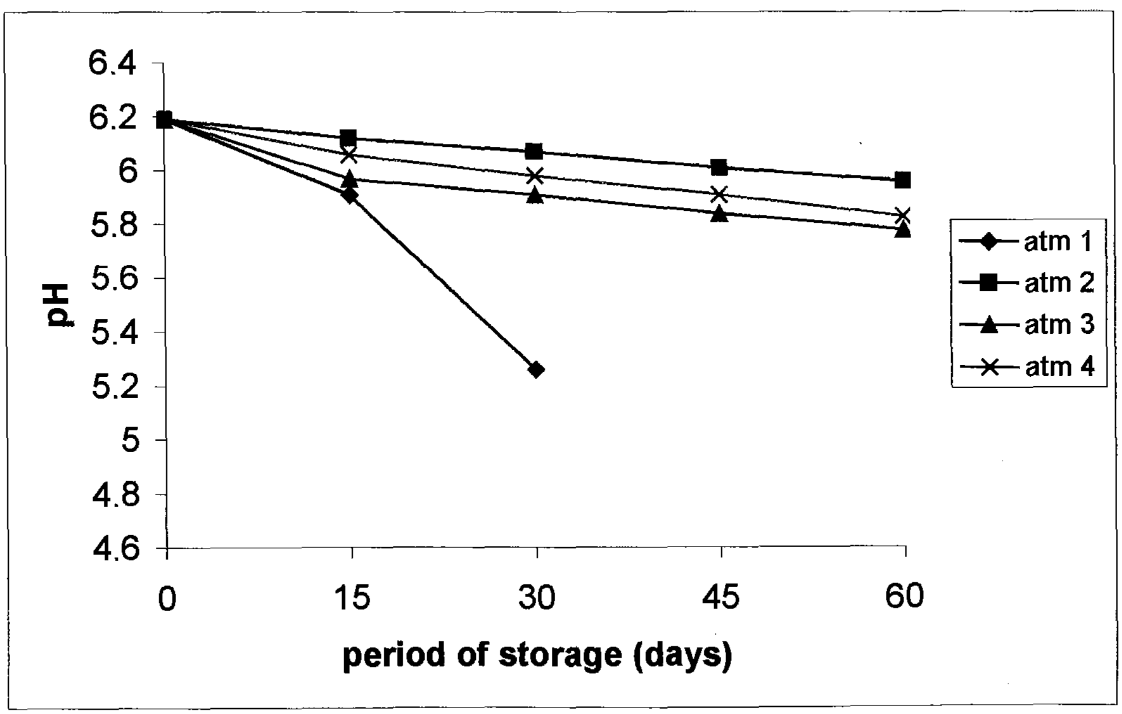


Fig 8: Effect of MAP on the pH of baked pizza stored at 7 ± 1 °C

the maximum decrease in the value was in case of samples packed under 100% N_2.

The analysis of variance established very significant ($P<0.01$) influence on the pH of both the types of pizza samples, due to the intervals of storage, the four types of atmospheres (atm 2, atm 3, atm 4) (Table 5).

5.2.2.4 Titratable acidity

The data pertaining to titratable acidity (TA) of unbaked pizza samples packed under 4 modified atmospheres and stored at 7±1 °C are presented in Appendix IX and illustrated in Figure 9. The anova of the data is recorded as a part of Table 5. The initial titratable acidity (% lactic acid) of 0.52 in air packed (atm 1) unbaked pizza increased to 0.72 and 0.78 after 5 and 10 days of storage, respectively. In case of samples stored for 20 days, the data indicate that the TA had been minimum (0.58) in samples packed under atm 2 followed by atm 4 (0.62) and atm 3 (0.81) respectively, in ascending order. The least increase in TA in samples packed under atm 2 (100% CO_2) might be due to the fact that CO_2 has bactericidal effect (Alves *et al.,* 1986; Eliot *et al.,* 1998; Tanweer Alam, 2004).

Appendix X and Figure 10 reflect the effect of MAP on the titratable acidity (% lactic acid) of baked pizza packaged under 4 different atmospheres and stored at 7±1 °C. The data indicate that the initial TA value of 0.35 of baked pizza packed under air (atm 1) increased to 0.82 after 30 days of storage, while the initial value of 0.35 increased to 0.57 (atm 2), 0.64 (atm 3) and 0.61 (atm 4) after 60 days of storage, revealing that the minimum increase was for the samples packed under 100% CO_2 followed by 50% CO_2 / 50% N_2, and100% N_2 respectively, in ascending order (Appendix X). The results are in harmony with the findings of Tanweer Alam (2004) who found minimum increase in TA of mozzarella cheese samples packed under 100% CO_2 followed by samples packed under 50% CO_2 / 50% N_2 and 100% N_2, when stored at 7±1 °C for 5 weeks. The trend in change of TA coincides well with the change in the values of pH of unbaked and baked pizza (Appendix VII and VIII).

From the consideration of TA, the intervals of storage, the four types of atmospheres and the interaction intervals x atmospheres (atm 2, atm 3, atm 4), all individually, were found to be highly significant ($P<0.01$) for both the types (unbaked and baked) of pizza samples (Table 5).

5.2.2.5 Free Fatty acids

Figure 11 and Appendix XI give the values for free fatty acids (FFA) of the unbaked pizza samples packaged under 4 modified atmospheres and stored at 7±1 °C. Analysis of variance is presented as a part of Table 6. The initial FFA value of 0.98 (% oleic acid) of unbaked pizza increased to 1.26 (atm 1), 1.09 (atm 2), 1.19 (atm 3) and 1.17 (atm 4) respectively after 10 days of storage. More increase in FFA was noted for the samples packed under atm 1, i.e. air (28.57% increase) compared to atm 3 (21.42% increase), atm 4 (19.38% increase) and atm 2 (11.22% increase). On further storage of pizza samples for 20 days, the FFA values increased least (1.31) in atm 2 followed by atm 4 (1.40) and atm 3 (1.44) respectively, in ascending order, thus proving that from the consideration of fat cleavage, atm 2, i.e. 100% CO_2 is the best. Similar observations were also reported by Tanweer Alam (2004) that MAP plays a very significant influence on the lipolysis of mozzarella cheese during storage.

The observations on the FFA of MAP baked pizza stored at 7±1 °C showed that after 60 days of storage, the initial value of 0.85 (% oleic acid) increased to 1.17, 1.38 and 1.26, respectively in case of atm 2, atm 3 and atm 4 (Appendix XII, Fig. 12). The results indicate that the minimum

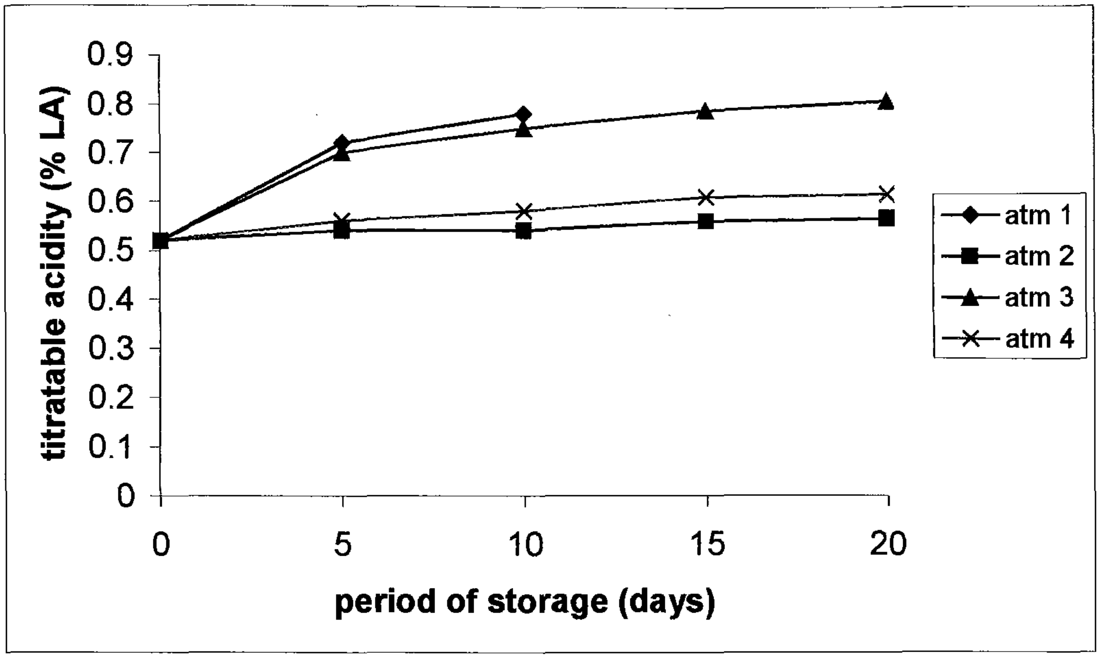


Fig 9: Effect of MAP on the Titratable Acidity (% lactic acid) of unbaked pizza stored at 7 ± 1 °C

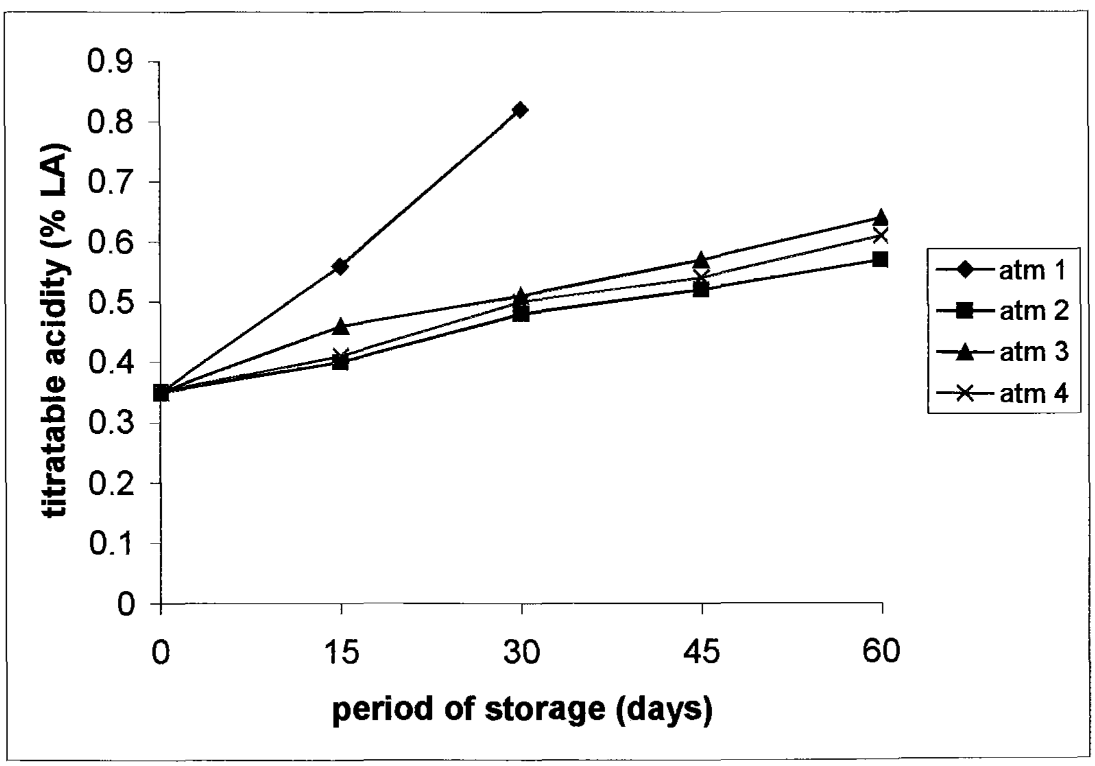


Fig 10: Effect of MAP on the Titratable Acidity (% lactic acid) of baked pizza stored at 7 ± 1 °C

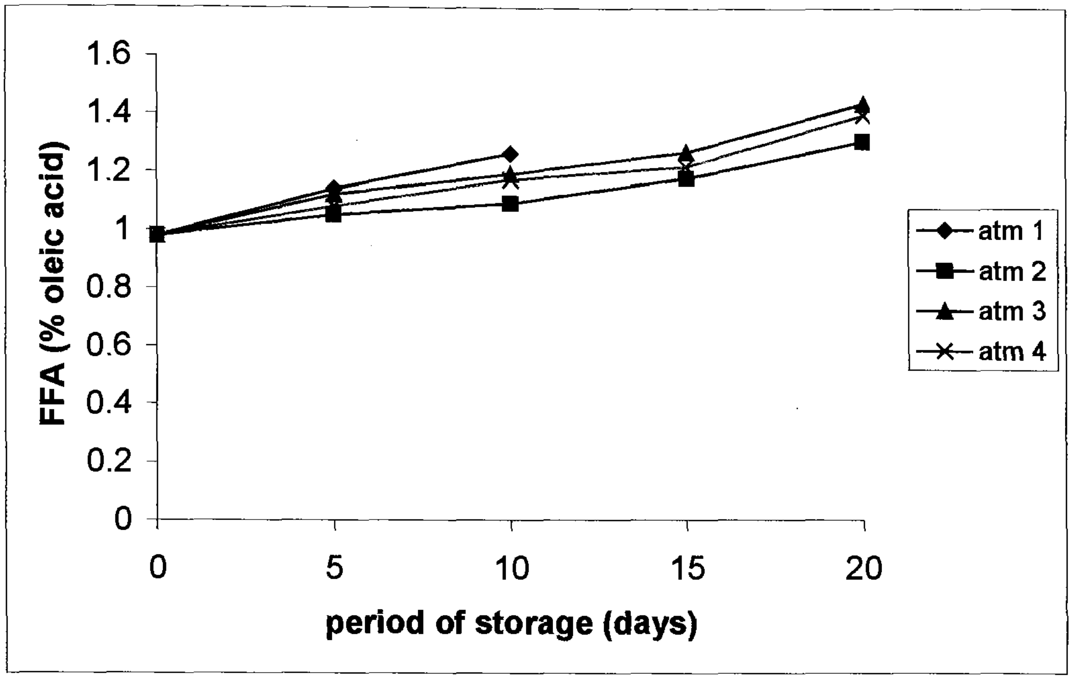


Fig 11: Effect of MAP on the Free Fatty Acids (% oleic acid) of unbaked pizza stored at 7 ± 1 °C

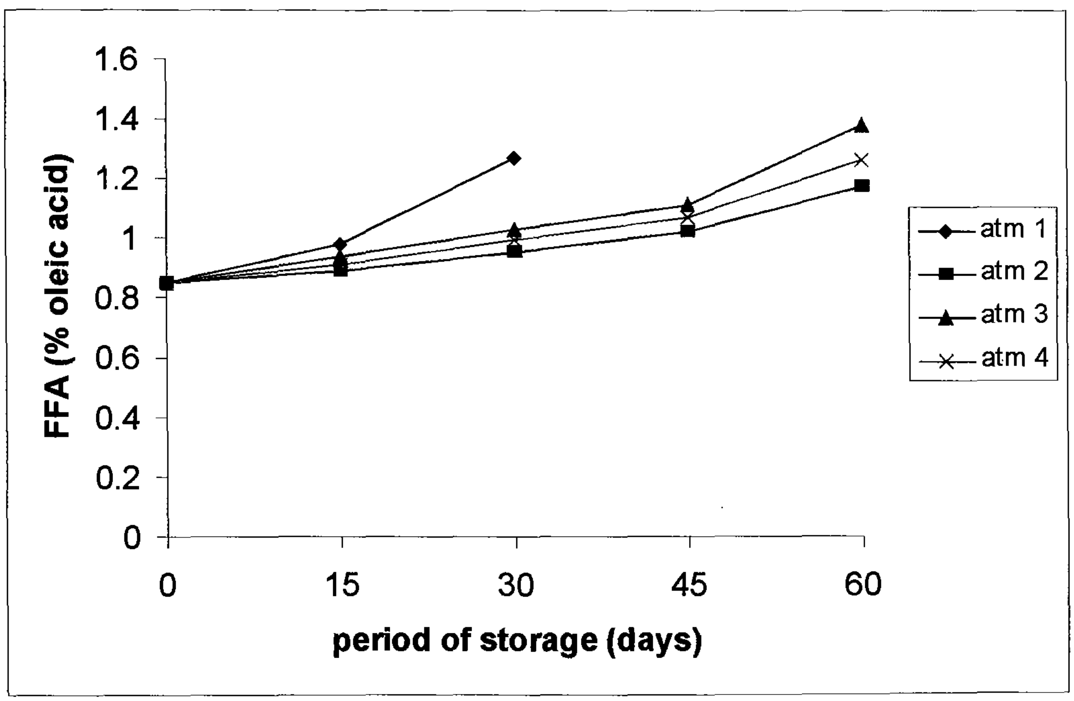


Fig 12: Effect of MAP on the Free Fatty Acids (% oleic acid) of baked pizza stored at 7 ± 1 °C

***Table 6.* Analysis of variance for Chemical characteristics of MAP Pizza stored at 7±1 °C**

Source of variation	d. f.	Mean sum of square							
		Free Fatty acids		Peroxide value		TBA		Tyrosine	
		Unbaked	Baked	Unbaked	Baked	Unbaked	Baked	Unbaked	Baked
Among Intervals of storage	4	0.2336**	0.2831**	1.3514**	1.7946**	0.0007**	0.0011**	705.2833**	893.6351**
Among atmospheres	3	0.0224**	0.0485**	0.0616**	0.2342**	0.0007**	0.0002**	110.7007**	116.7153**
Error	46	0.0006	0.0029	0.0020	0.0166	0.0003	0.0001	3.3066	3.0598
Interaction [Intervals x atmospheres (atm 2, atm 3, atm 4)]	8	0.0020**	0.0045**	0.0042**	0.0578**	0.0001**	0.0002**	6.9299**	6.1543**
Error	30	0.0003	0.0004	0.0003	0.0003	0.0001	0.0001	0.0008	0.0007

**** Significant at 1% level of probability**
atm 2: 100% CO_2
atm 3: 100% N_2
atm 4: 50% CO_2 : 50% N_2

increase in FFA was observed in case of samples packed under 100% CO_2 followed by 50% CO_2 / 50% N_2 and 100% N_2, respectively. 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 pizza samples, packed in different modified atmospheres.

Analysis of variance for the data on FFA revealed highly significant ($P<0.01$) differences among the intervals of storage, among the atmospheres and the interaction intervals x atmospheres (atm 2, atm 3, atm 4) for both the types (unbaked and baked) of pizza samples (Table 6).

5.2.2.6 Peroxide value

Figure 13 and Appendix XIII show the peroxide value (PV) for unbaked pizza samples packaged under 4 atmospheres and stored for various time intervals at 7±1 °C. The initial PV (meq/kg of fat) for the sample increased from 1.36 to 1.87 (atm 1), 1.59 (atm 2), 1.72 (atm 3) and 1.66 (atm 4) after 10 days of storage, revealing that the minimum increase in PV was observed in pizza samples packed under 100% CO_2 followed by 50% CO_2 / 50% N_2, 100% N_2 and air respectively, in ascending order. The trend even continued during further storage of samples upto 20 days confirming the beneficial effect of MAP from the consideration of lower PV in stored pizza samples.

A perusal of Figure 14 and Appendix XIV indicate that the initial peroxide value in case of baked pizza samples, packaged under 4 atmospheres and stored for 30 days at 7±1 °C, increased to 1.62 (atm 2), 1.68 (atm 4), 1.73 (atm 3) and 2.19 (atm 1) showing that the maximum increase was for the samples packaged under air (atm 1) followed by 100% N_2 (atm 3), 50% CO_2 / 50% N_2 (atm 4) and 100% CO_2 (atm 2) respectively, in descending order. Similar trend was observed during further storage of MAP baked pizza (Appendix XIV). The higher peroxide value in case of air packed

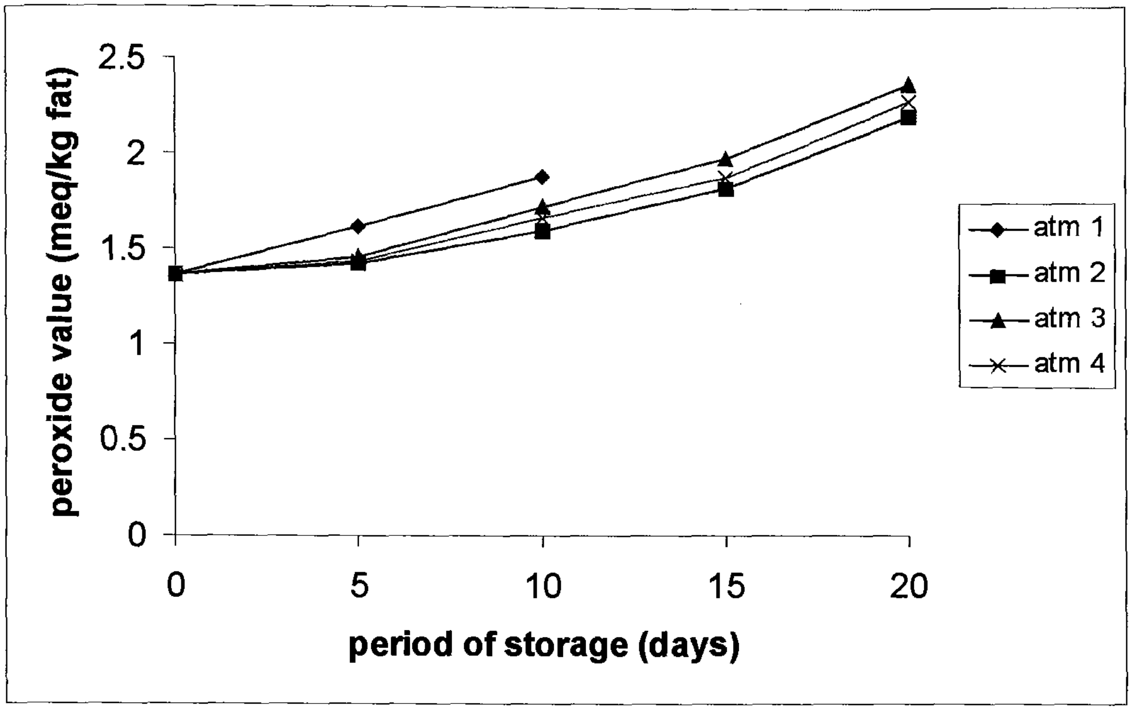


Fig 13: Effect of MAP on the Peroxide Value (meq/kg fat) of unbaked pizza stored at 7 ± 1 °C

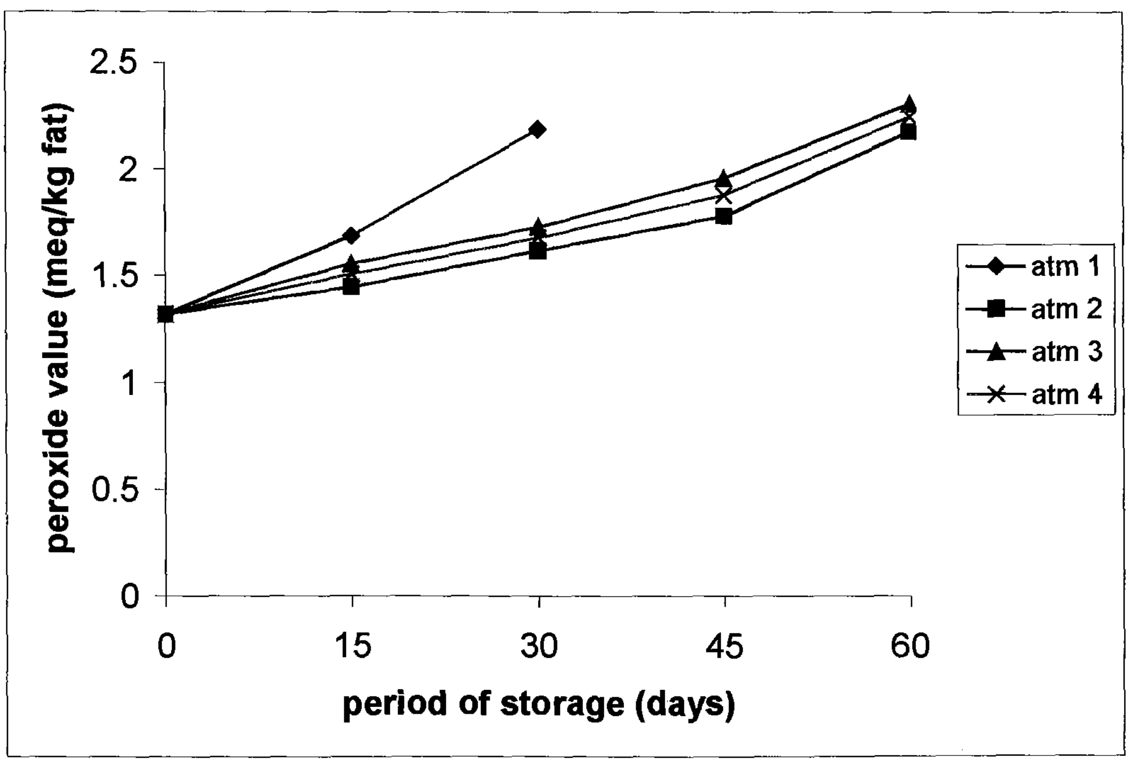


Fig 14: Effect of MAP on the Peroxide Value (meq/kg fat) of baked pizza stored at 7 ± 1 °C

pizza samples was obviously due to the fact that oxygen, which is responsible for the oxidative changes, was available in the package.

Statistical analysis of the data on PV (Table 6) showed highly significant ($P<0.01$) differences among the intervals of storage, among the four types of atmospheres and the interaction intervals x atmospheres (atm 2, atm 3, atm 4) for both types of pizza samples.

5.2.2.7 Thiobarbituric acid (TBA) value

Figure 15 and Appendix XV give the values for TBA of the unbaked pizza samples packaged in high barrier bags under 4 atmospheres and stored at 7±1 °C. Analysis of variance is presented as a part of Table 6.

The initial value for TBA (OD at 530 nm) increased from 0.023 to 0.047 and 0.066 after 5 and 10 days of storage respectively for air packed (atm 1) pizza samples, while the minimum increase (0.029) in TBA value was observed in samples packed under atm 2 (100% CO_2) after 10 days of storage, followed by atm 4 (50% CO_2 / 50% N_2) (0.035) and atm 3 (100% N_2) (0.035), respectively. On further storage of pizza samples for 20 days, it was observed that the TBA value increased least in case of atm 2 followed by atm 4 and atm 3 respectively, in ascending order.

The data showing the effect of MAP on the TBA value (OD at 530 nm) of baked pizza samples stored at 7±1 °C is included in Appendix XVI and shown graphically in Figure 16. The initial TBA value of 0.012 increased to 0.037 (atm 1), 0.022 (atm 2), 0.028 (atm 3) and 0.024 (atm 4) after 30 days of storage, revealing that the extent of lipid oxidation had been maximum for air packed samples and was minimum for the samples packed under atm 2 (100% CO_2). The maximum lipid oxidation was expected for samples packed under atm 1 because the oxygen was available for the oxidation of fat present in the samples. Similar trend continued till the product was stored for 60 days, establishing the favourable influence of CO_2

over N_2, where the TBA value was lower (0.033) compared to 0.043 (100% N_2).

The lower TBA values in baked and unbaked pizza samples packaged under 100% CO_2 were found to be minimum during storage, which may perhaps be also due to the higher lycopene content in these samples (Appendix XIX, Appendix XX), as lycopene has been identified as natural antioxidant (John, 2000). When the TBA values of baked and unbaked pizza samples packed under 4 different atmospheres were correlated with the results from sensory evaluation, it was observed that the pizza samples (both unbaked and baked) with higher TBA values had lower flavour scores (Appendix XLVI and XLVII) and overall acceptability scores (Appendix L and LI).

Table 6 indicates that the intervals of storage, the four types of atmospheres and the interaction intervals x atmospheres (atm 2, atm 3, atm 4) had highly significant influence ($P<0.01$) on the TBA values of both the types of pizza samples.

5.2.2.8 Tyrosine content

The values for tyrosine content (mg/100g) of the unbaked pizza samples packaged under 4 different atmospheres for different periods and stored at 7±1 °C are contained in Appendix XVII and represented in Fig. 17.

The results indicate that the rate of protein breakdown was slower in pizza samples packaged under modified atmospheres, i.e. 100% CO_2 (atm 2), 100% N_2 (atm 3), 50% CO_2 / 50% N_2 (atm 4), compared to samples packaged under air (atm 1). After 10 days of storage, the extent of proteolysis in pizza samples varied from 13.40 to 35.80 (atm 1), 22.71 (atm 2), 29.60 (atm 3) and 27.34 (atm 4), indicating that the proteolysis occurred in all the samples submitted to air and the three modified atmospheres (Fig. 17). However, the tyrosine content, which is the index of proteolysis, were

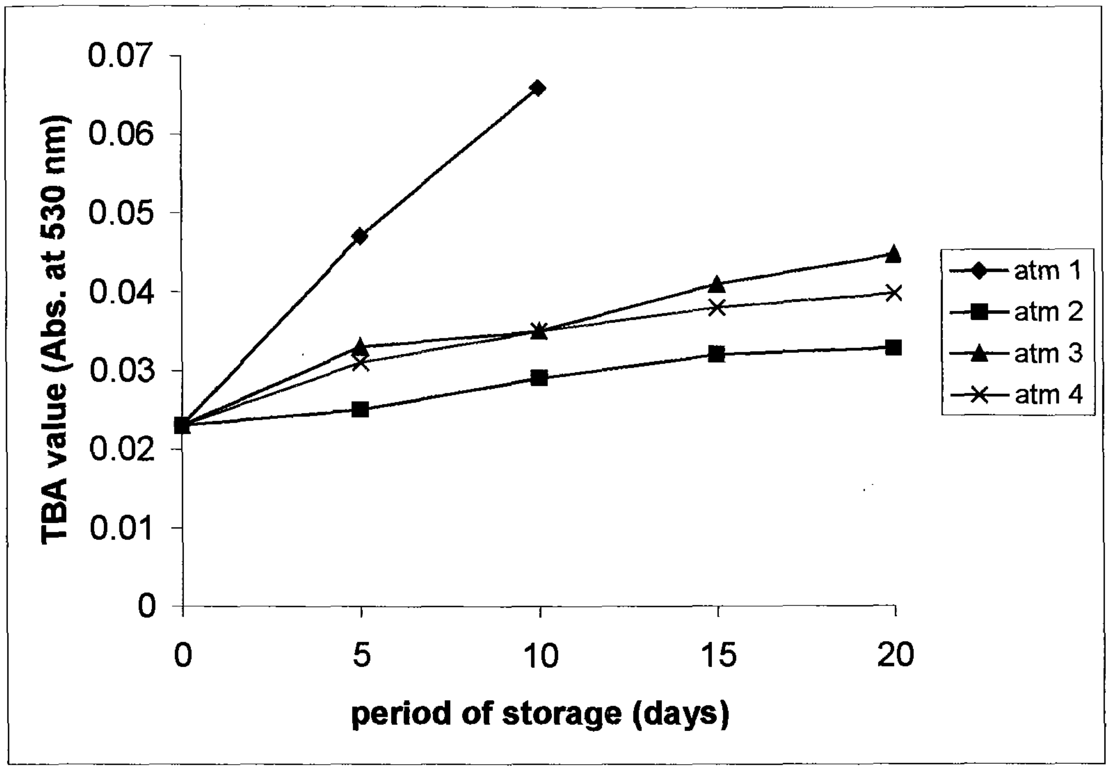


Fig 15: Effect of MAP on the TBA Value (Absorbance at 530 nm) of unbaked pizza stored at 7 ± 1 °C

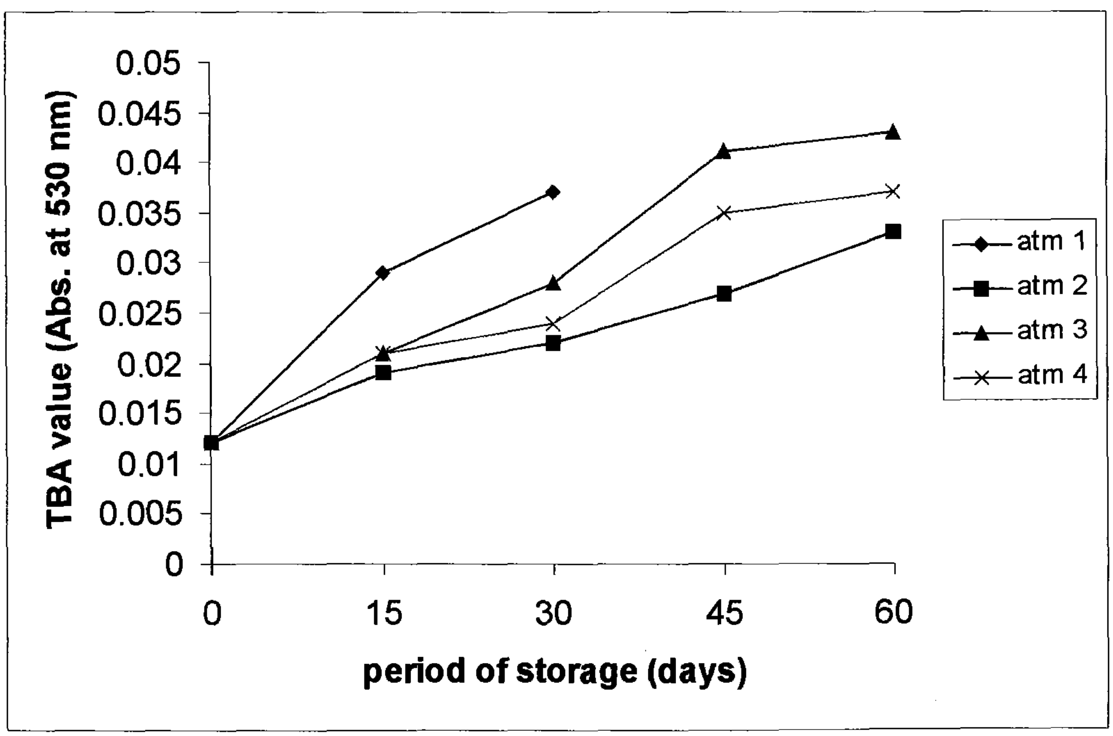


Fig 16: Effect of MAP on the TBA Value (Absorbance at 530 nm) of baked pizza stored at 7 ± 1 °C

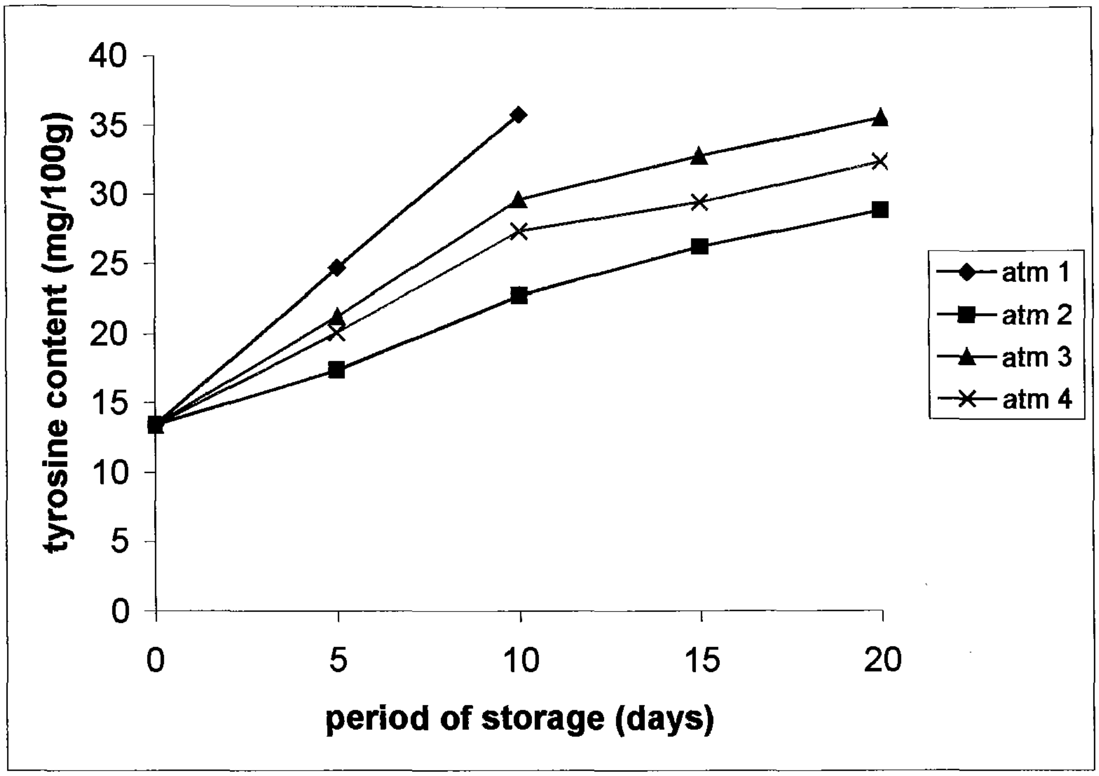


Fig 17: Effect of MAP on the Tyrosine content (mg/100g) of unbaked pizza stored at 7 ± 1 °C

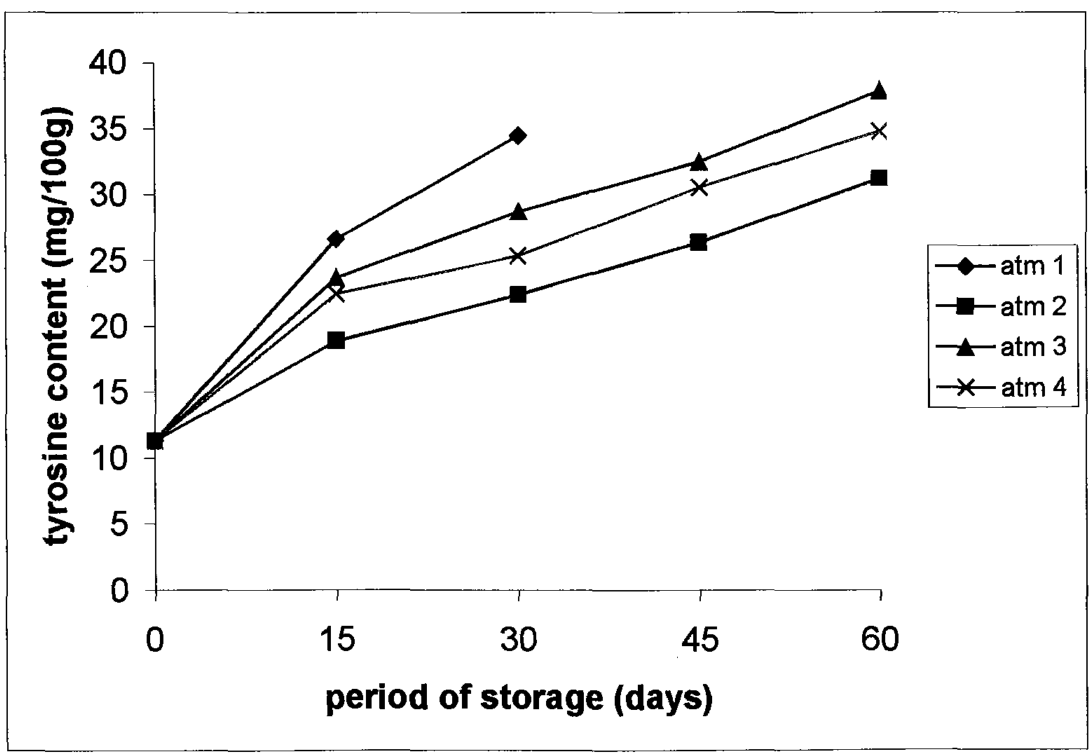


Fig 18: Effect of MAP on the Tyrosine content (mg/100g) of baked pizza stored at 7 ± 1 °C

found to be minimum for the samples packaged under 100% CO_2 and maximum for the air packed samples (Appendix XVII).

The tyrosine content (mg/100g) increased from 11.30 to 34.50 (atm 1), 22.41 (atm 2), 28.72 (atm 3) and 25.36 (atm 4), revealing that the minimum proteolysis occurred in the samples packaged under 100% CO_2, and the maximum proteolysis was in the air packed samples. The further storage of the samples for 60 days followed the similar trend (Appendix XVIII, Figure 18). Most probably the proteolysis occurred in all the samples due to presence of enzymes or microorganisms might have produced the enzymes during storage (Micketts and Olson, 1974). The results agree with the observations of Tanweer Alam (2004) who observed that the proteolysis occurred least in case of mozzarella cheese samples packed under 100% CO_2 followed by 50% CO_2 / 50% N_2 and 100% N_2, in ascending order.

Statistical analysis of the data on tyrosine content in the two types of pizza samples (Table 6) indicate highly significant ($P<0.01$) differences due to the intervals of storage, the four types of atmospheres and the interaction intervals x atmospheres (atm 2, atm 3, atm 4).

5.2.2.9 Lycopene content

Lycopene content, the pigment mainly responsible for the characteristic deep red colour of ripe tomato fruits and tomato products, in unbaked fresh pizza samples was found to be 4.84 mg/100g (Appendix XIX, Fig. 19). The value was much above than the recommendations of USDA (2005) for cheese vegetable pizza that lycopene content should be 239 µg/100g. On storage, the initial value of 4.84 of lycopene content (mg/100 g) of unbaked pizza stored for 10 days at 7±1 °C decreased to 4.42 (atm 1), 4.73 (atm 2), 4.68 (atm 3) and 4.71 (atm 4) respectively, revealing that the minimum decrease had been in the samples packaged under 100% CO_2 (atm 2), and the maximum decrease was noted in the air packed (atm 1) samples. After 20 days of storage, the lycopene content further reduced to 4.37 (atm 3), 4.48 (atm 4) and 4.59 (atm 2), indicating that the minimum decrease in lycopene content was for the samples packaged under 100% CO_2. It is worth mentioning here that the pattern of lycopene content in the samples packaged under 4 atmospheres were directly proportional with the redness (a*) values for the unbaked pizza samples packaged under the four types of atmospheres (Appendix XXI).

The data pertaining to effect of MAP on the lycopene content (mg/100g) of baked pizza samples packaged under 4 atmospheres and stored at 7±1 °C are presented in Appendix XX and Figure 20.

The value of lycopene content in fresh baked pizza samples was 5.04 mg/100g while in unbaked pizza samples the lycopene content averaged 4.84 mg/100g. The difference in lycopene content of the two types of pizza samples may perhaps be also due to the fact that cooked tomato products like pizza pack more bioavailable lycopene than raw fruit (Zimmerman, 2002). On storage the initial value of lycopene content 5.04 decreased to 4.53 (atm 1), 4.93 (atm 2), 4.85 (atm 3) and 4.89 (atm 4), showing that the minimum decrease (2.18% decrease) in lycopene content was in the samples packaged under 100% CO_2, and the maximum decrease (10.12% decrease) was noted in the samples packaged under air. From the data contained in Appendix XX, it can also be seen that after 60 days of storage, the minimum decrease (6.54% decrease) occurred in samples packaged under 100% CO_2 followed by samples under 50% CO_2 / 50% N_2 (7.54% decrease) and 100% N_2 (9.12% decrease), suggesting preservative role of CO_2 towards lycopene content in pizza samples. Here also, like unbaked pizza samples, the values of lycopene content match with the changes in red colour (a*) (Appendix XXII) of the baked pizza samples packed under 4 atmospheres during storage, i.e. lycopene content α a*.

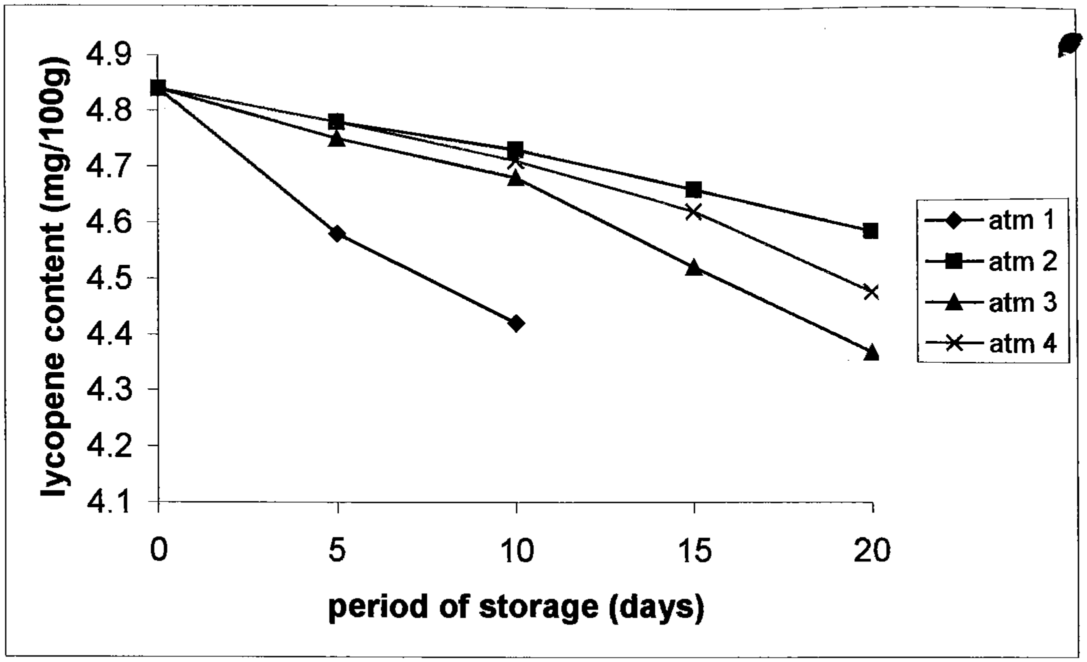


Fig 19: Effect of MAP on the Lycopene content (mg/100g) of unbaked pizza stored at 7 ± 1 °C

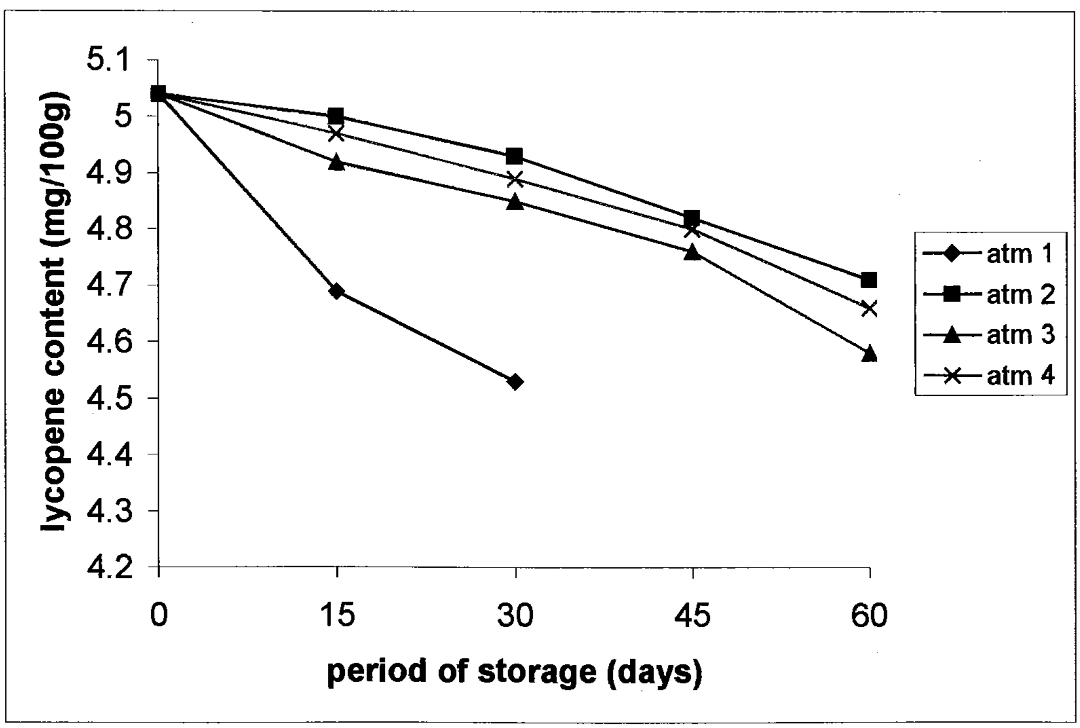


Fig 20: Effect of MAP on the Lycopene content (mg/100g) of baked pizza stored at 7 ± 1 °C

The analysis of variance of the data pertaining to unbaked and baked pizza samples packaged under 4 atmospheres and stored at 7±1 °C indicated (Table 7) that the influence of different intervals of storage, the four different packaging atmospheres, and the interaction intervals x atmospheres (atm 2, atm 3, atm 4), each individually, were highly significant ($P<0.01$) towards the changes in lycopene content.

5.2.2.10 Colour Profile (L*, a*, b*)

The observations relating to effect of MAP on the changes in colour values in terms of L*, a*, b* of unbaked and baked pizza samples packaged under 4 atmospheres and stored at 7±1 °C are recorded in Appendix XXI (unbaked samples) and Appendix XXII (baked samples) and illustrated for unbaked pizza in Fig. 21 (atm 1), Fig. 22 (atm 2), Fig. 23 (atm 3), Fig. 24 (atm 4), and for baked pizza in Fig. 25 (atm 1), Fig. 26 (atm 2), Fig. 27 (atm 3), Fig. 28 (atm 4). The initial data in Appendix XXI show that the initial value 62.87 of L* (lightness) decreased to 58.36, 62.26, 62.06 and 62.19 in samples packaged under atm 1, atm 2, atm 3 and atm 4 respectively, after 10 days of storage, revealing that the maximum decrease had been in case of atm 1 (air) and the minimum decrease in atm 2 (100% CO_2). Similar pattern was also noted for the values of a* (redness), suggesting maximum retention of red colour in pizza samples packed under 100% CO_2 and minimum retention of red colour in air packed samples. However, a quite opposite pattern was noticed for the values of b* (yellowness), where the initial value of 35.23 increased to 42.16 (atm 1), 35.89 (atm 2), 37.56 (atm 3) and 36.12 (atm 4). This trend of decrease in L* values and a* values, and increase in b* values persisted even during subsequent storage of unbaked pizza upto 20 days, and also in baked pizza

Table 7. Analysis of variance for Chemical characteristics of MAP Pizza stored at 7±1 °C

Source of variation	d. f.	Mean sum of square					
		Lycopene		Total colour difference (ΔE)		Relative yellowness (E*)	
		Unbaked	Baked	Unbaked	Baked	Unbaked	Baked
Among Intervals of storage	4	0.2252**	0.2688**	2.0357	2.9791*	0.6299	0.3977*
Among atmospheres	3	0.0764**	0.1127**	0.8221	0.9949*	0.9158	0.3127*
Error	46	0.0032	0.0037	0.0290	0.0299	0.0315	0.0119
Interaction [Intervals x atmospheres (atm 2, atm 3, atm 4)]	8	0.0063**	0.0017**	0.0182	0.0381	0.0275	0.0106
Error	30	0.0001	0.0002	0.0027	0.0003	0.0001	0.0002

**** Significant at 1% level of probability**
*** Significant at 5% level of probability**
atm 2: 100% CO_2
atm 3: 100% N_2
atm 4: 50% CO_2 : 50% N_2

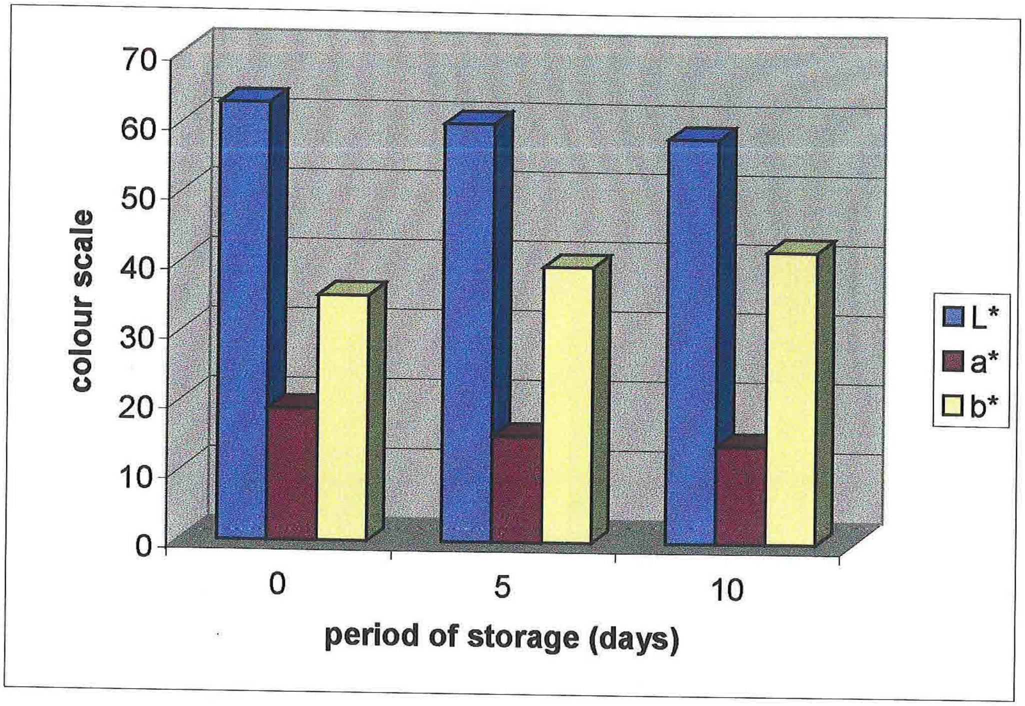


Fig 21: Effect of MAP on the Hunter Colour values (L*, a*, b*) of air packed (atm 1) unbaked pizza stored at 7 ± 1 ºC

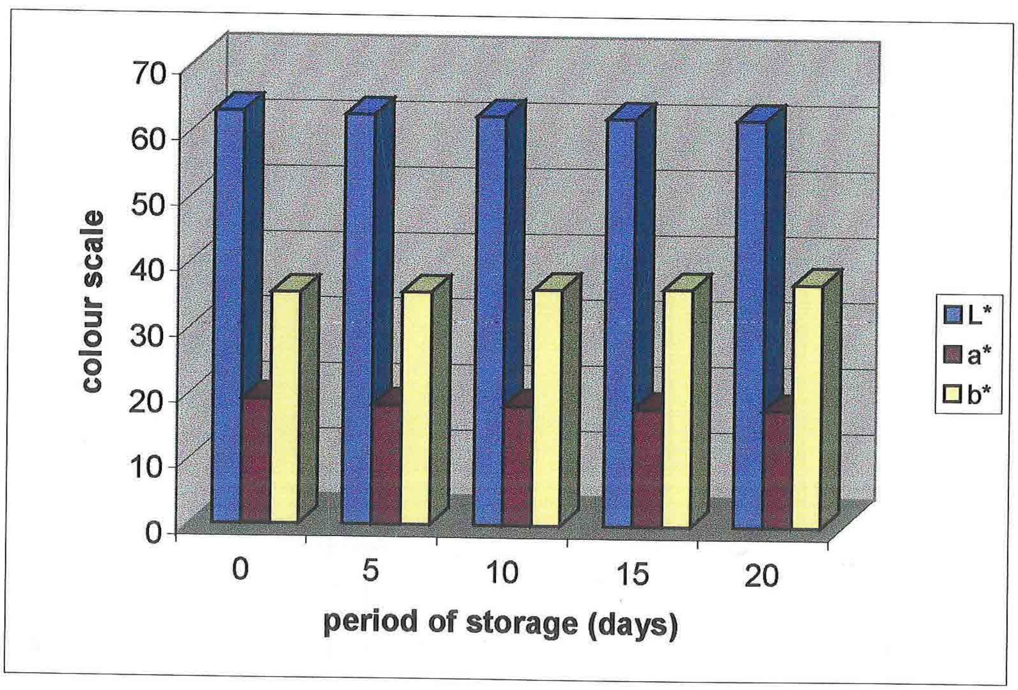


Fig 22: Effect of MAP on the Hunter Colour values (L*, a*, b*) of 100% CO_2 packed (atm 2) unbaked pizza stored at 7 ± 1 ºC

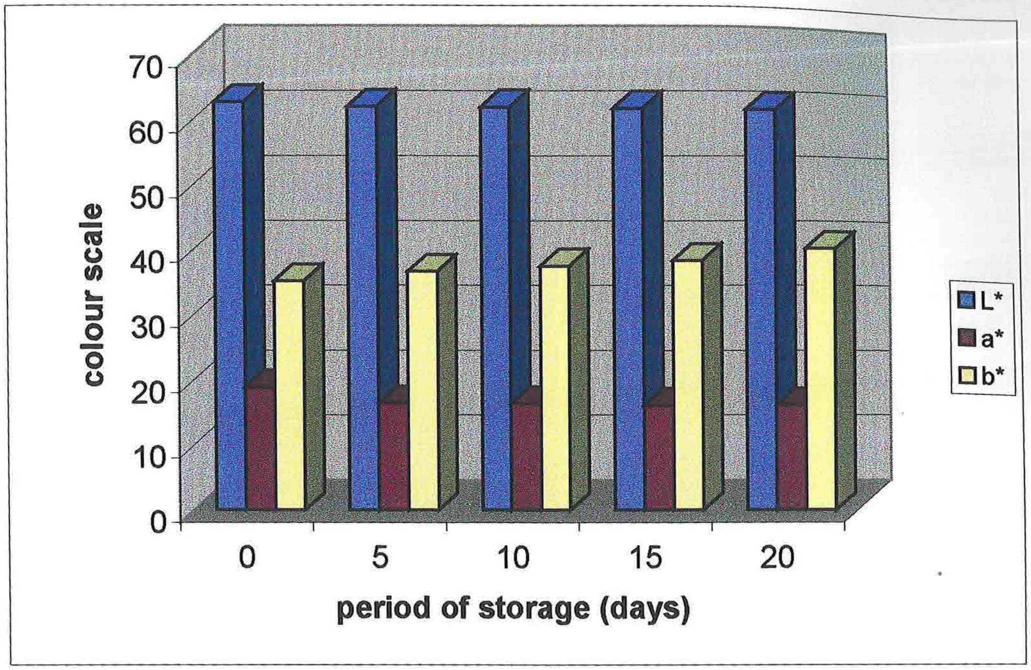


Fig 23: Effect of MAP on the Hunter Colour values (L*, a*, b*) of 100% N_2 packed (atm 3) unbaked pizza stored at 7 ± 1 ºC

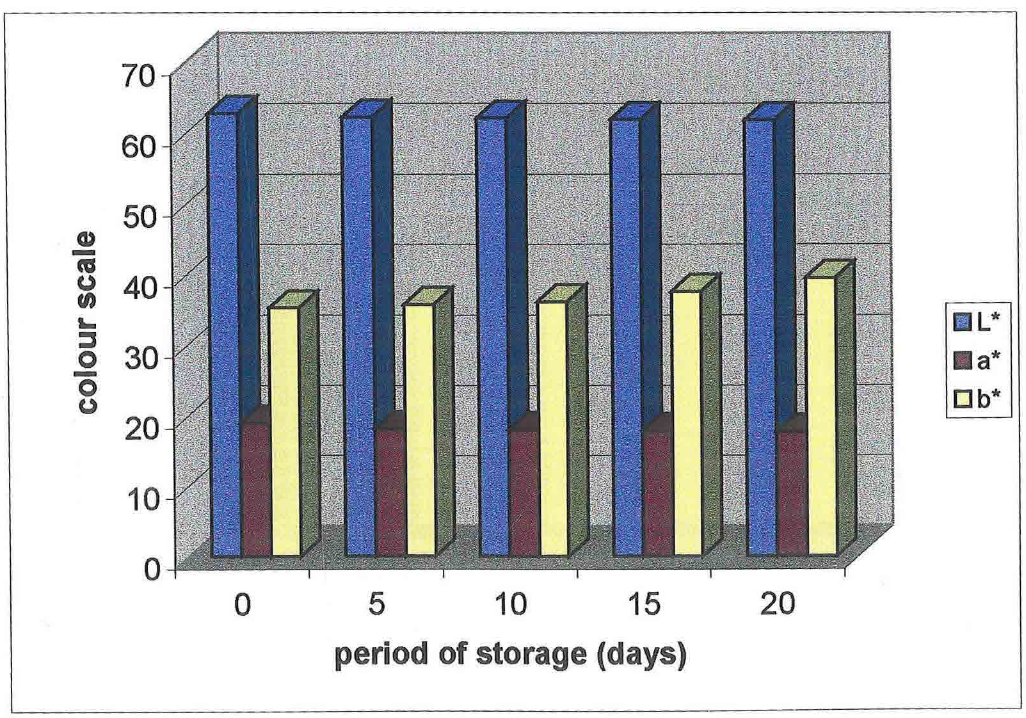


Fig 24: Effect of MAP on the Hunter Colour values (L*, a*, b*) of 50% CO_2 : 50% N_2 packed (atm 4) unbaked pizza stored at 7 ± 1 ºC

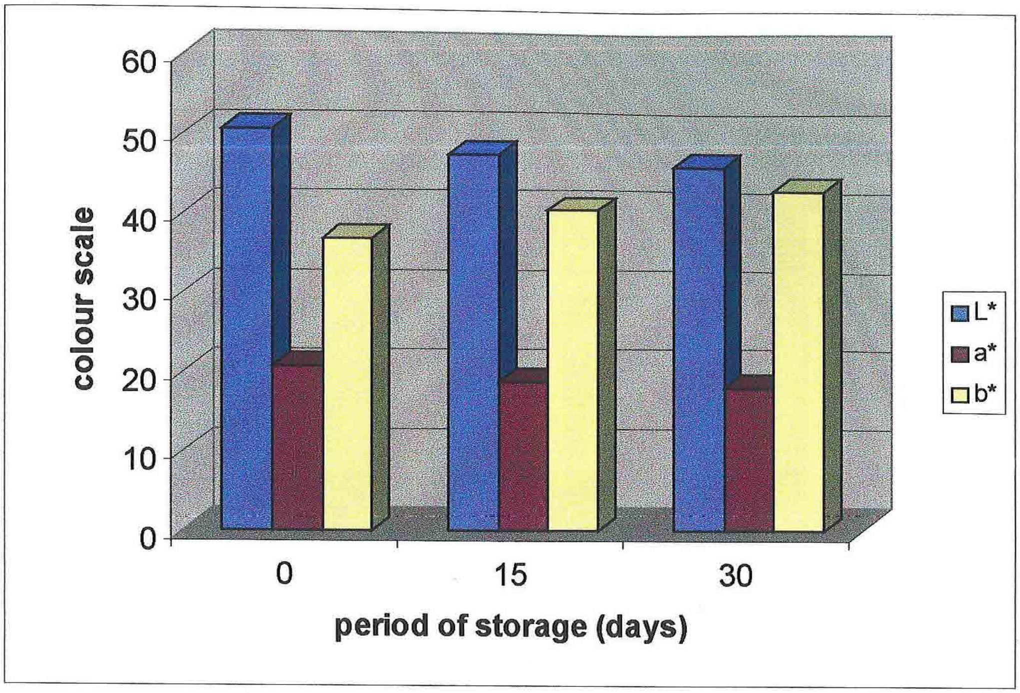


Fig 25: Effect of MAP on the Hunter Colour values (L*, a*, b*) of air packed (atm 1) baked pizza stored at 7 ± 1 ºC

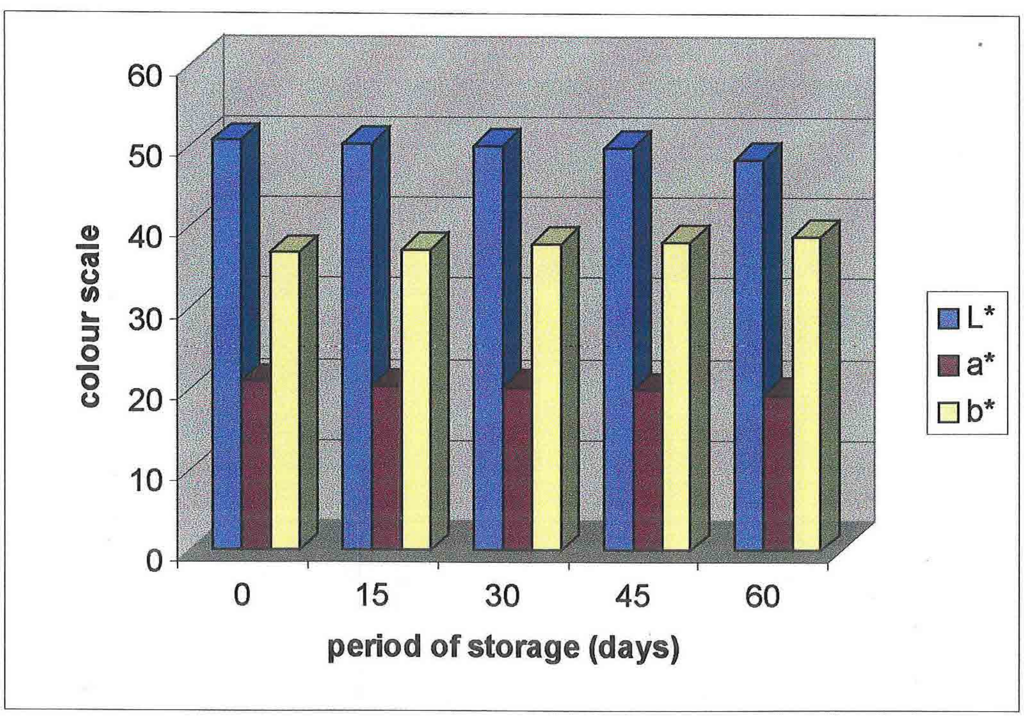


Fig 26: Effect of MAP on the Hunter Colour values (L*, a*, b*) of 100% CO_2 packed (atm 2) baked pizza stored at 7 ± 1 ºC

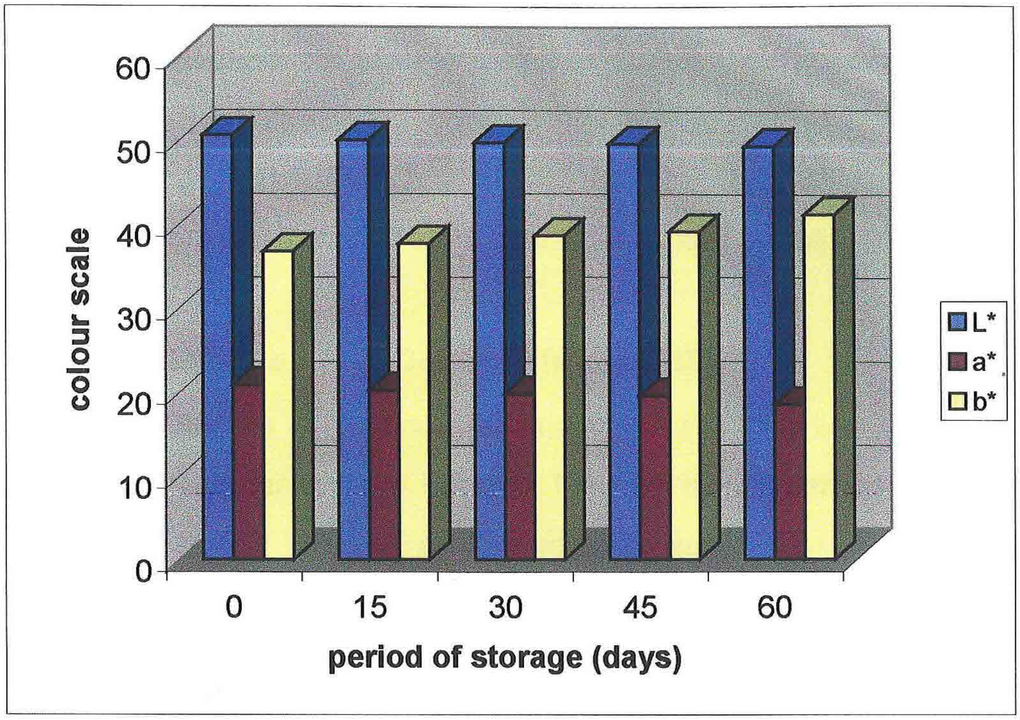


Fig 27: Effect of MAP on the Hunter Colour values (L*, a*, b*) of 100% N_2 packed (atm 3) baked pizza stored at 7 ± 1 ºC

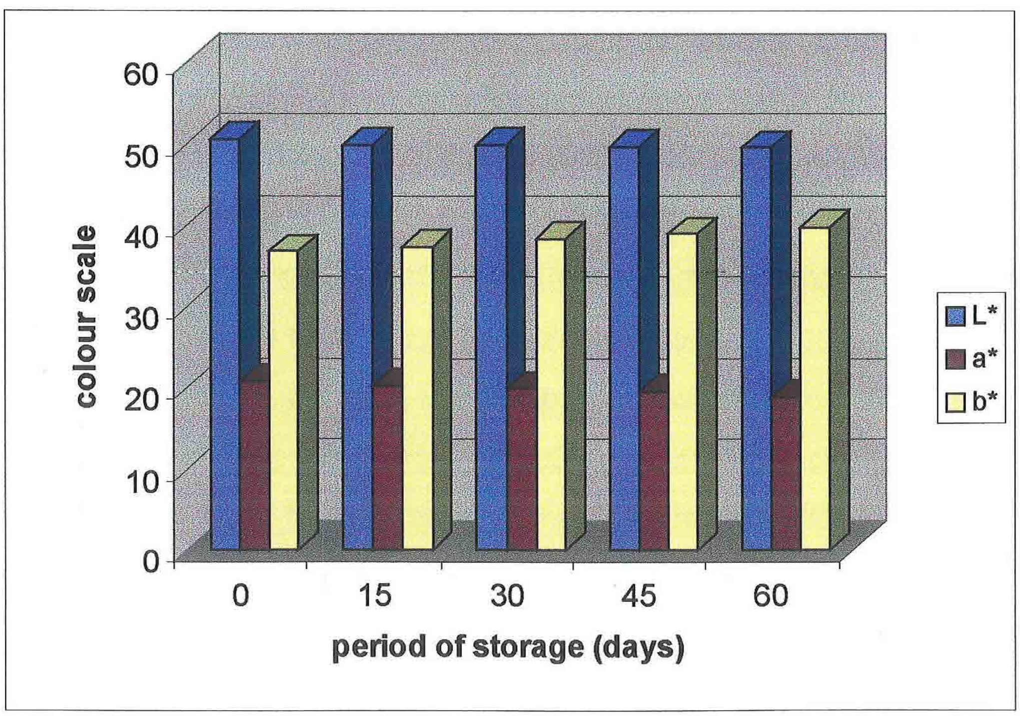


Fig 28: Effect of MAP on the Hunter Colour values (L*, a*, b*) of 50% CO_2 : 50% N_2 packed (atm 4) baked pizza stored at 7 ± 1 ºC

samples throughout the storage period of 60 days, for the 4 atmospheres (Appendix XXII). The results, in general, are in agreement with the findings of Fu & Labuza (1993) and Kaya & Aksu (2005) who observed decrease in red colour during storage of frozen pizza and sliced *sucuk* (dry fermented sausage).

5.2.2.11 Hunter Lab Total Colour Difference (ΔE)

Since the Appearance, which also includes the colour, is one of the primary sensory factors, the effect of MAP on the Hunter lab total colour difference (ΔE) of unbaked and baked pizza samples packaged under 4 different atmospheres and stored at 7±1 °C for various time intervals was determined, and the values are depicted in Appendix XXIII and Appendix XXIV and illustrated in Figures 29 and 30, respectively. The Hunter lab values varied from 52.16 to 53.52 (atm 1), 52.36 (atm 2), 52.72 (atm 3) and 52.47 (atm 4) after 10 days in case of unbaked pizza samples, indicating minimum colour difference in pizza samples packaged under 100% CO_2 (0.38% change) and maximum difference in samples packed under air (2.61% change). When the unbaked samples packed under atm 2, atm 3 and atm 4 were stored for 20 days, the colour difference was maximum (2.28% change) for the product packed under 100% N_2 (atm 3) followed by 50% CO_2 / 50% N_2 (atm 4) (1.92% change), and 100% CO_2 (atm 2) (1.65% change).

When the baked pizza samples were stored for 60 days under atm 2, atm 3 and atm 4, the initial Hunter lab value increased from 53.07 to 54.08 in case of samples packed under 100% CO_2, 54.59 for samples packed under 100% N_2 and 54.18 for the product packaged under 50% CO_2 / 50% N_2, thus revealing the minimum change in Hunter lab values for the samples packaged under atm 2 followed by atm 4 and atm 3, in ascending order (Appendix XXIV). The results, in principle, are in harmony with the observations of

Nanke *et al.* (1998, 1999) who reported the colour changes due to package environment.

The analysis of variance of data pertaining to total colour difference (ΔE) indicate that intervals of storage, and the four different atmospheres, both individually, had significant ($P<0.05$) influence on the changes in Hunter lab colour values for baked pizza samples only. However, the interaction intervals x atmospheres (atm 2, atm 3, atm 4) were observed to be not significant for both the types of pizza samples (Table 7).

5.2.2.12 Relative Yellowness (E*)

Appendix XXV, Fig. 31, Appendix XXVI and Figure 32 represent the values of the relative yellowness (E*) as affected by MAP of unbaked and baked pizza samples stored for various time intervals at 7±1 °C.

The data in Appendix XXV and XXVI indicate that the values for E* (the fraction of yellowness relative to those of redness and lightness) increased in all the unbaked and baked MAP samples, and the increase had been maximum in case of samples packed under 100% N_2 followed by 50% CO_2 / 50% N_2, and 100% CO_2 respectively. The analysis of variance show that the intervals of storage, and the four modified atmospheres, both individually, had significant influence ($P<0.05$) on E* for baked pizza samples only. However, the interaction intervals x atmospheres (atm 2, atm 3, atm 4) was found to be not significant for both the types of pizza samples studied (Table 7).

5.2.3.0 Microbiological changes

5.2.3.1 Total Plate count (TPC)

TPC of the unbaked pizza samples packaged in high barrier bags under 4 different atmospheres and stored at 7±1 °C are presented in Appendix XXVII and Figure 33. The initial mean value of 6.65 (log cfu/g) for TPC increased to 7.10 for the samples packed under air (atm 1), 4.81 (atm 2),

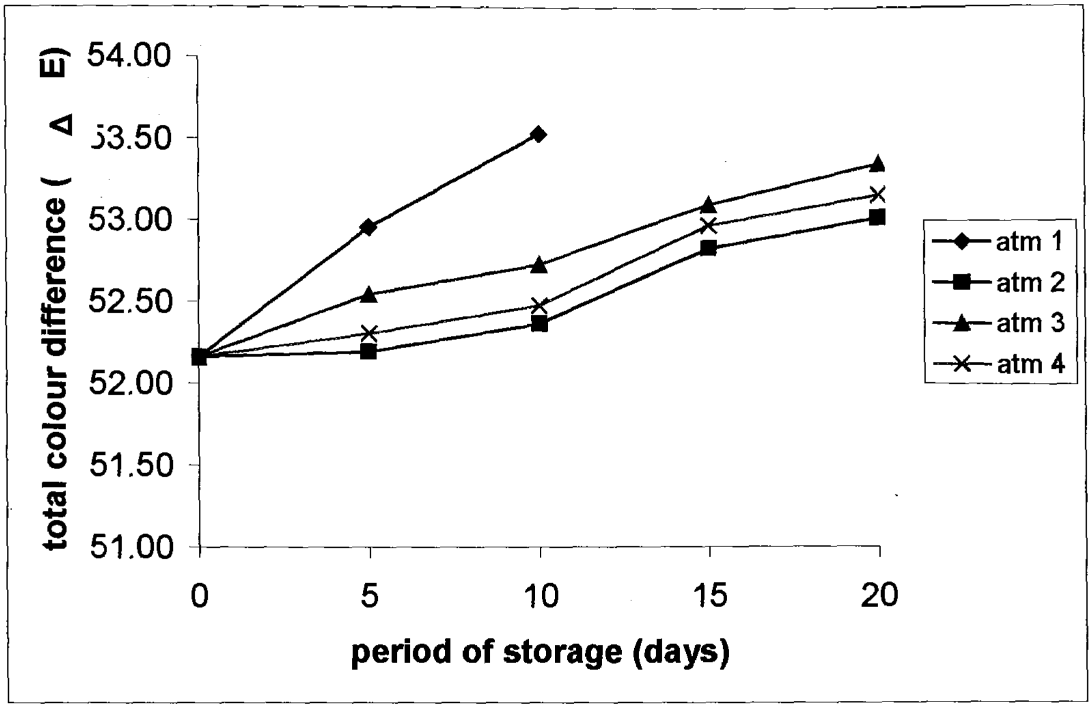


Fig 29: Effect of MAP on the Total Colour Difference (ΔE) of unbaked pizza stored at 7 ± 1 ºC

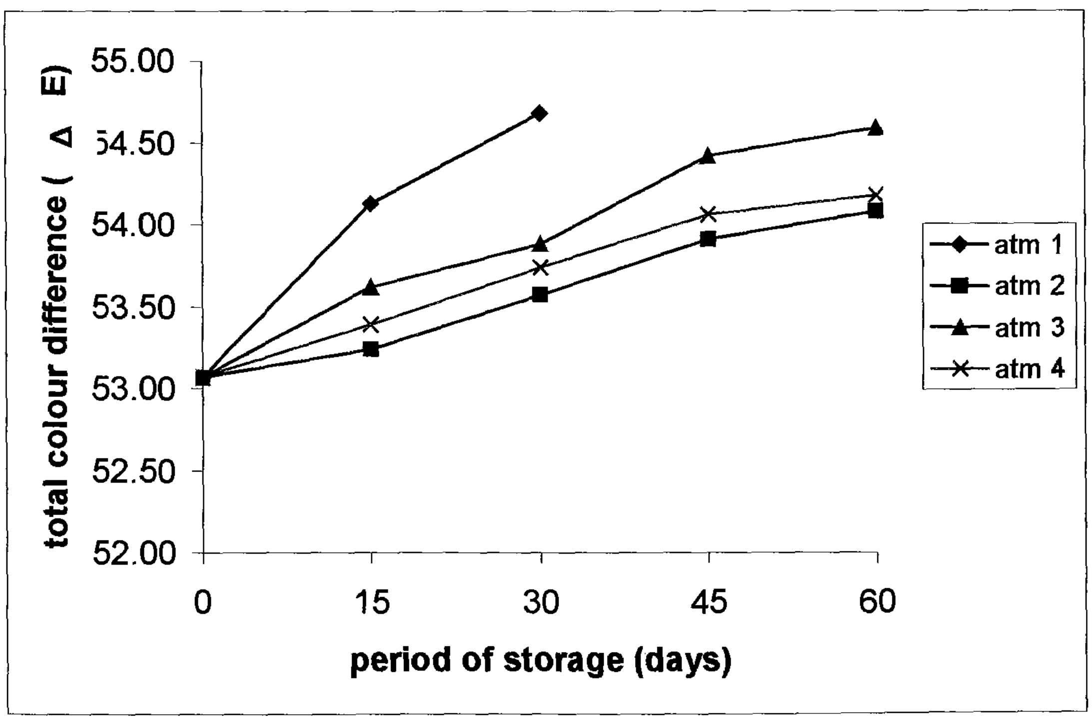


Fig 30: Effect of MAP on the Total Colour Difference (ΔE) of baked pizza stored at 7 ± 1 ºC

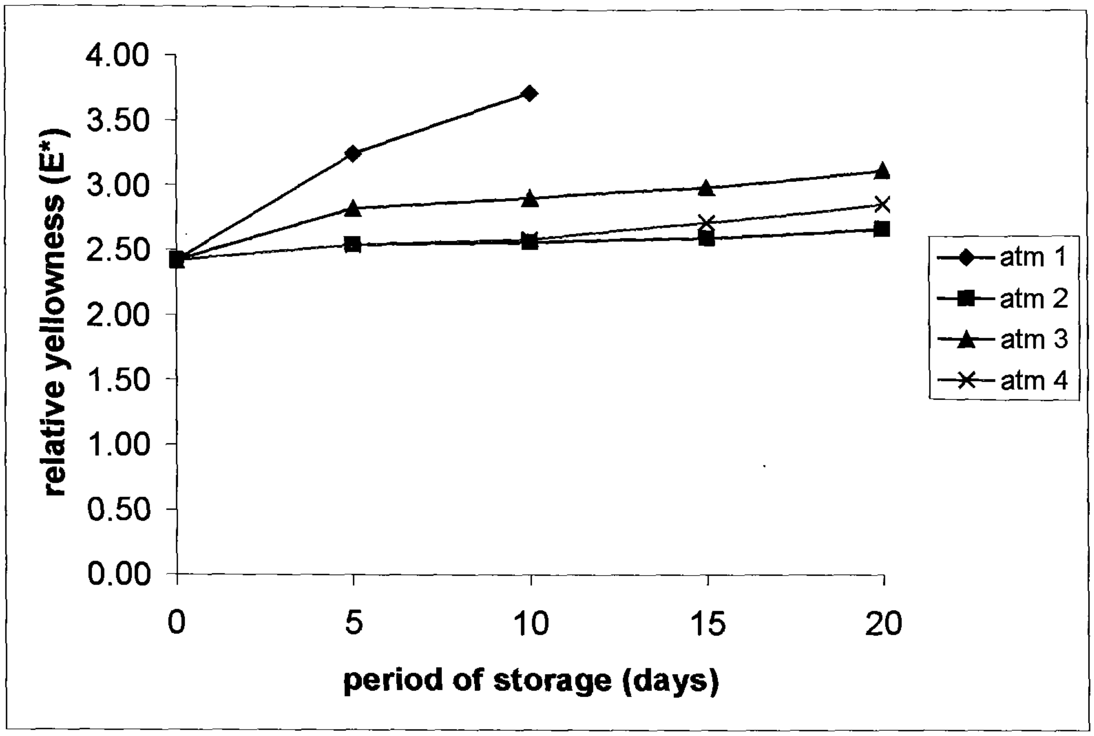


Fig 31: Effect of MAP on the Relative Yellowness (E*) of unbaked pizza stored at 7 ± 1 °C

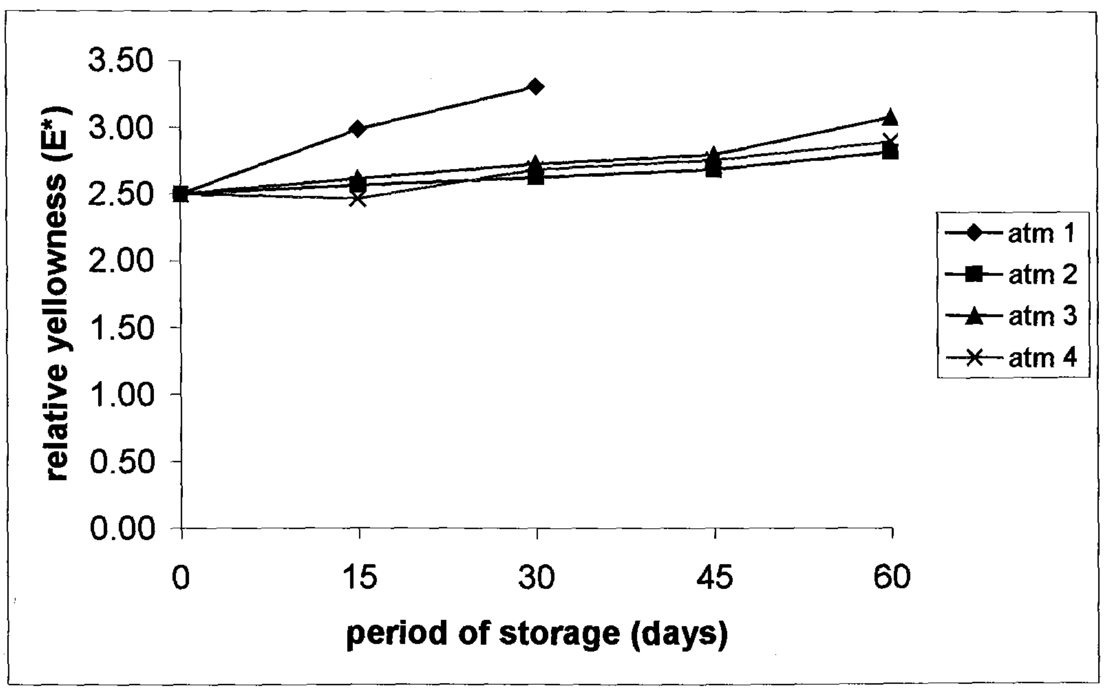


Fig 32: Effect of MAP on the Relative Yellowness (E*) of baked pizza stored at 7 ± 1 °C

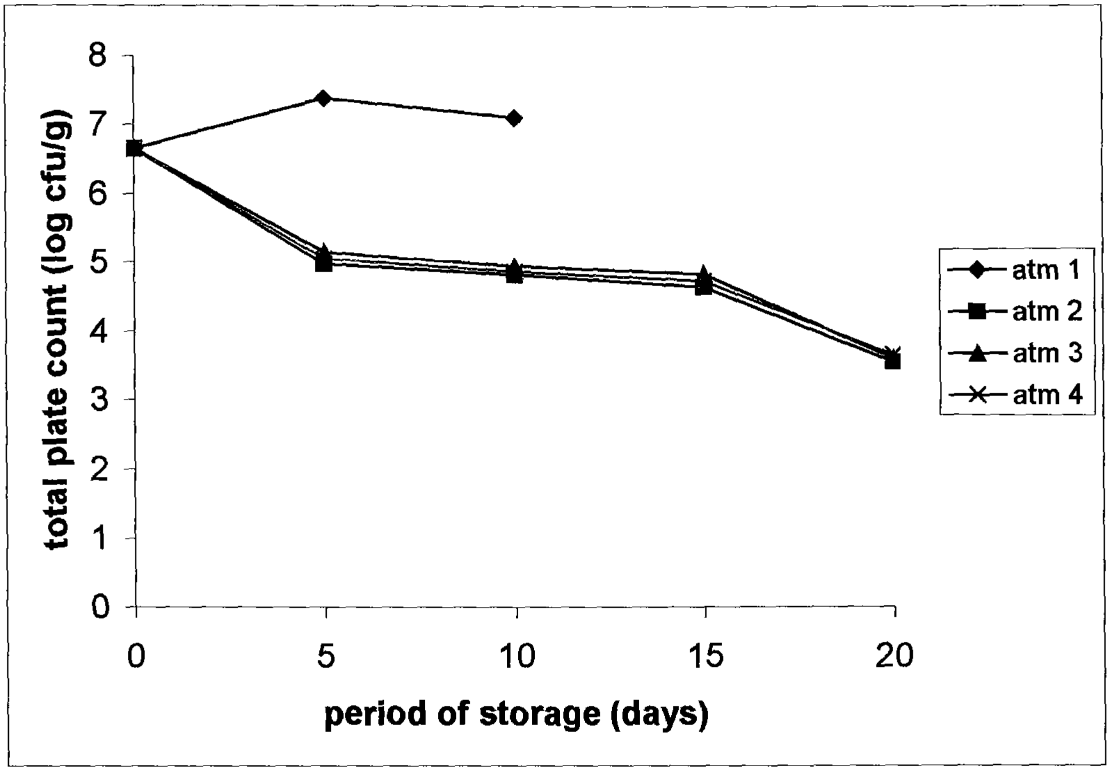


Fig 33: Effect of MAP on the Total Plate count (log cfu/g) of unbaked pizza stored at 7 ± 1 ºC

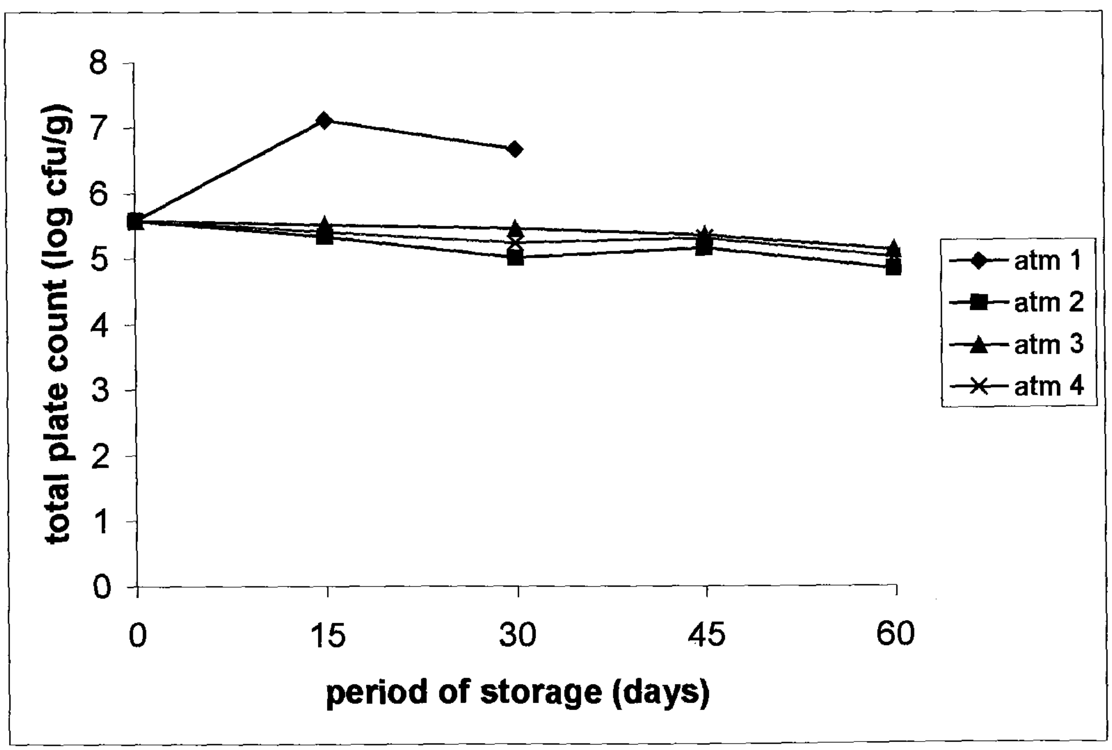


Fig 34: Effect of MAP on the Total Plate count (log cfu/g) of baked pizza stored at 7 ± 1 ºC

***Table 8.* Analysis of variance for Microbiological characteristics of MAP Pizza stored at 7±1 °C**

Source of variation	d. f.	Mean sum of square					
		Total Plate count		Yeast & moulds		Coliform	
		Unbaked	Baked	Unbaked	Baked	Unbaked	Baked
Among Intervals of storage	4	8.9422**	0.4171**	3.0882**	0.3221**	0.1058**	0.0668**
Among atmospheres	3	5.2277**	2.5753**	2.1596**	0.2316**	2.2611**	0.2111**
Error	46	0.1708	0.0852	0.0707	0.0075	0.0738	0.0086
Interaction [Intervals x atmospheres (atm 2, atm 3, atm 4)]	8	0.0065**	0.0210**	0.0492	0.0182	0.0029**	0.0021**
Error	30	0.0001	0.0003	0.0001	0.0002	0.0003	0.0003

**** Significant at 1% level of probability**
atm 2: 100% CO_2
atm 3: 100% N_2
atm 4: 50% CO_2 : 50% N_2

4.93 (atm 3) and 4.85 (atm 4) after 10 days of storage revealing maximum increase for the product packed under atm 1 followed by atm 3, atm 4 and atm 2 respectively, in descending order. On further storage upto 20 days, the value decreased to 3.56 (atm 2), 3.61 (atm 3) and 3.66 (atm 4), indicating that modified atmospheres had been effective towards lower microbial growth.

The observations pertaining to TPC of baked pizza samples packed under 4 atmospheres and stored at 7±1 °C are presented in Appendix XXVIII and Figure 34. After 30 days of storage, the mean value for TPC was maximum for the samples packed under air (atm 1) followed by 100% N_2 (atm 3), 50% CO_2 / 50% N_2 (atm 4) and 100% CO_2 (atm 2) respectively (Appendix XXVIII). The TPC further decreased to 4.85 (atm 2), 5.14 (atm 3) and 5.03 (atm 4) after 60 days of storage, establishing bactericidal effect of CO_2. The results are in agreement with the findings of Scott and Smith (1971), who investigated the effect of CO_2, N_2 and air atmospheres on the shelf life of cottage cheese and concluded that CO_2 slightly decreased the bacterial count, but N_2 did not significantly decrease the count. The results also confirm the earlier findings of Alves *et al.* (1996), Fedio *et al.* (1994), Eliot *et al.* (1998) and Tanweer Alam (2004), who while working on MAP of mozzarella cheese observed that CO_2 had bactericidal effect.

Table 8 reveals that from the consideration of TPC, the duration of storage, the four types of atmospheres, and the interaction intervals x atmospheres (atm 2, atm 3, atm 4), each individually, were highly significant ($P<0.01$) for both types (unbaked and baked) of pizza samples.

5.2.3.2 Yeast & Moulds (Y & M) count

The changes in yeast and moulds count of unbaked pizza samples packaged under modified atmospheres and stored at 7±1 °C are depicted in Figure 35 and Appendix XXIX. The Y & M count of pizza samples packed under air (atm 1) exhibited increasing trend throughout the entire period of

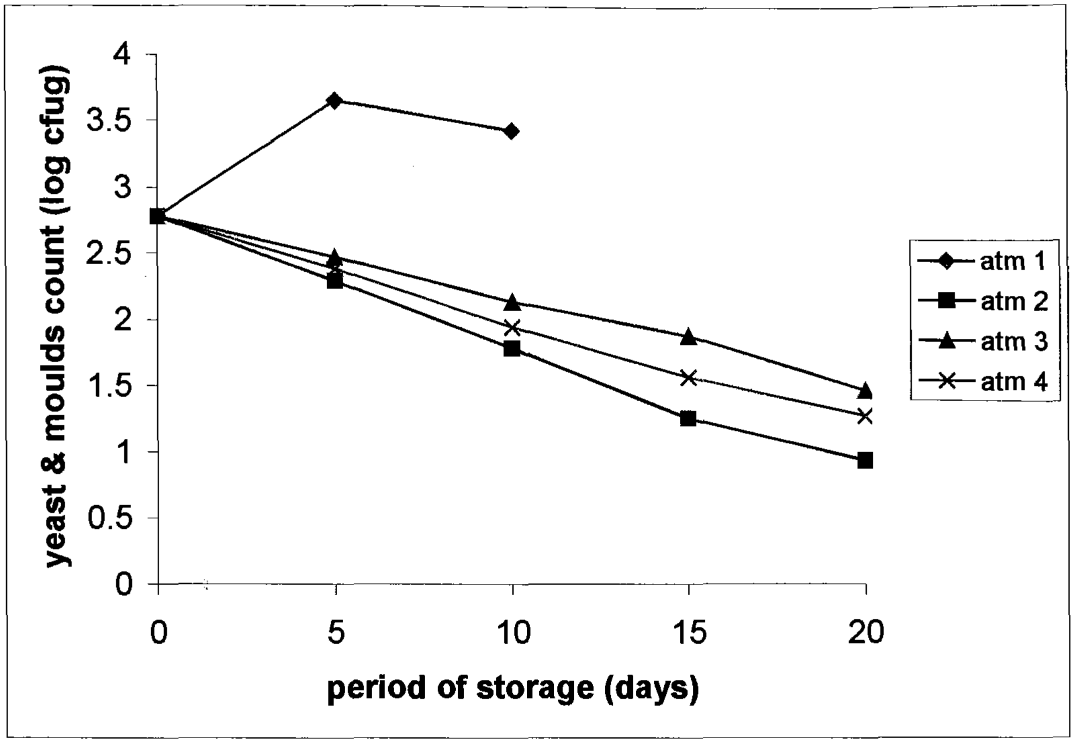


Fig 35: Effect of MAP on the Yeast & moulds count (log cfu/g) of unbaked pizza stored at 7 ± 1 °C

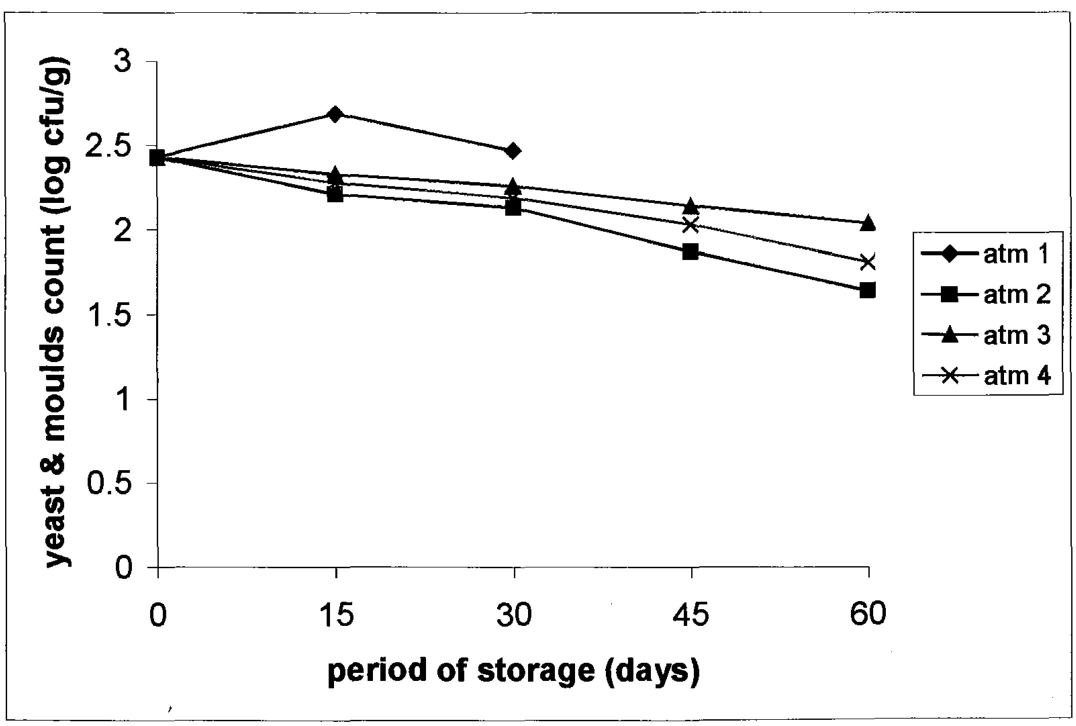


Fig 36: Effect of MAP on the Yeast & moulds count (log cfu/g) of baked pizza stored at 7 ± 1 °C

storage, while the initial Y & M count 2.78 (log cfu/g) decreased to 0.93 (atm 2), 1.27 (atm 4) and 1.47 (atm 3) respectively after 20 days of storage, revealing that the growth was retarded when the atmosphere inside the package contained higher concentration of CO_2. The results confirmed the earlier findings of Cabo *et al.* (2001) who reported lower yeast counts in pizzas when stored at 7±1 °C under 90% CO_2 compared to 20% CO_2.

The data showing the effect of MAP on the Y & M count (log cfu/g) of baked pizza samples packed under 4 atmospheres and stored at 7±1 °C are included in Appendix XXX and graphically presented in Figure 36.

The initial mean value of Y & M count (log cfu/g) of baked pizza samples increased from 2.43 to 2.69 (atm 1) after 15 days of storage, while the value decreased to 2.21 (atm 2), 2.33 (atm 3) and 2.28 (atm 4) respectively. More decrease in Y & M count was noted after 60 days of storage; the maximum decrease being with the samples packed under 100% CO_2 (atm 2) followed by 50% CO_2 / 50% N_2 (atm 4) and 100% N_2 (atm 3) respectively, in ascending order, establishing a destructive effect of CO_2 on Y & M. The inhibitory effect of CO_2 has also been observed by Kosikowski and Brown (1973), Chen and Hotchkiss (1991), Rosenthal *et al.* (1991), Day (1992), Fedio *et al.* (1994), Alves *et al.* (1996) and Tanweer Alam (2004).

Analysis of variance for the data on Y & M counts indicated that the intervals of storage, and the atmospheres of packaging, both individually, were highly significant ($P<0.01$) for both the types (unbaked and baked) of pizza samples. However, the interaction intervals x atmospheres (atm 2, atm 3, atm 4) was not significant for any of the pizza samples (Table 8).

5.2.3.3 Coliform count

The coliform counts for the unbaked pizza samples packed under 4 atmospheres and stored at 7±1 °C are given in Figure 37 and Appendix XXXI. The initial coliform count of 2.57 in air packed (atm 1) pizza samples increased to 3.94, but decreased to 2.34 (atm 2), 2.45 (atm 3) and 2.37 (atm 4) after 5 days of storage. Thereafter, the decreasing trend continued till the end of the storage period of 20 days irrespective of the atmosphere inside the package (Fig. 37).

The observations pertaining to baked pizza samples (Appendix XXXII and Fig. 38) reveal that the type of atmosphere played a very significant role in controlling the coliform count. 100% CO_2 was found to be the most effective. The initial coliform count (1.39 log cfu/g) decreased maximum in case of atm 2 (1.16) followed by atm 4 (1.25) and atm 3 (1.29), in ascending order. Tanweer Alam (2004) also observed decrease in coliform counts of mozzarella cheese samples when packed under CO_2, N_2 and their mixtures, during storage at 7±1 °C. The possible reasons for decrease in coliform count during storage might be the effect of CO_2 by alteration of permeability of cell of membrane and enzymatic reaction pathways (Enfors and Molin, 1981; King and Nagel, 1975; Rosenthal *et al.*, 1991).

Anova of the data on coliform counts revealed highly significant ($P<0.01$) differences among the intervals of storage, amongst the atmospheres and the interaction intervals x atmospheres (atm 2, atm 3, atm 4) for unbaked and baked pizza samples (Table 8).

5.2.3.4 Total Lactic acid bacteria (LAB) count

In Figure 39 and Appendix XXXIII are presented the total lactic acid bacteria (LAB) count of the unbaked pizza samples packed under 4 different atmospheres and stored at 7±1 °C.

It is apparent from Appendix XXXIII that the atmosphere of 100% CO_2 (atm 2) proved to be the best (as maximum decrease for LAB was observed) followed by 50% CO_2 / 50% N_2 (atm 4), and 100% N_2 (atm 3). However, the initial total lactic acid bacteria count increased from 5.02 (log cfu/g) to 8.14 after 10 days of storage under atm 1 (air). Cabo *et al.* (2001)

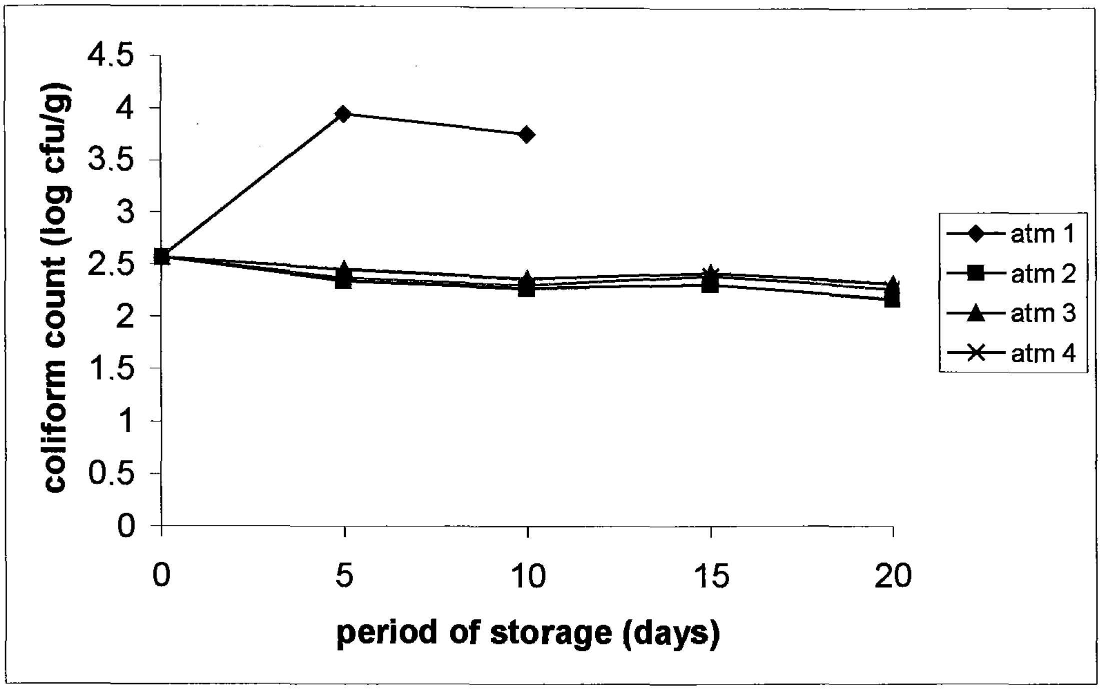


Fig 37: Effect of MAP on the Coliform count (log cfu/g) of unbaked pizza stored at 7 ± 1 °C

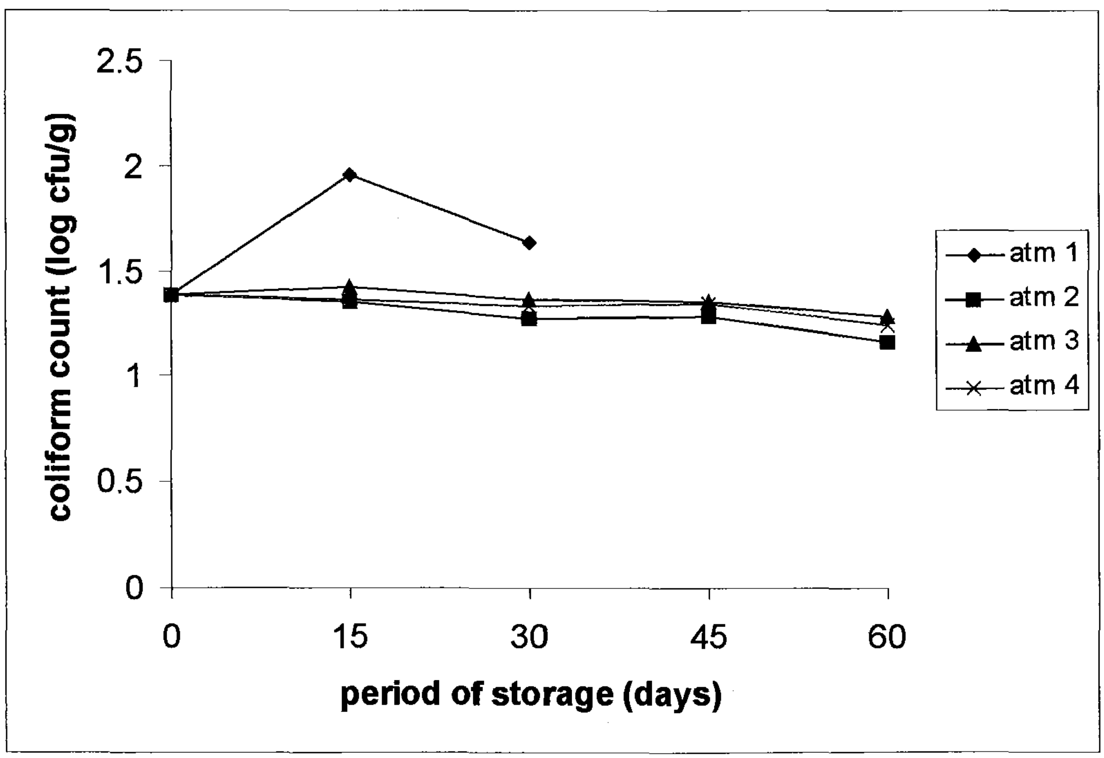


Fig 38: Effect of MAP on the Coliform count (log cfu/g) of baked pizza stored at 7 ± 1 °C

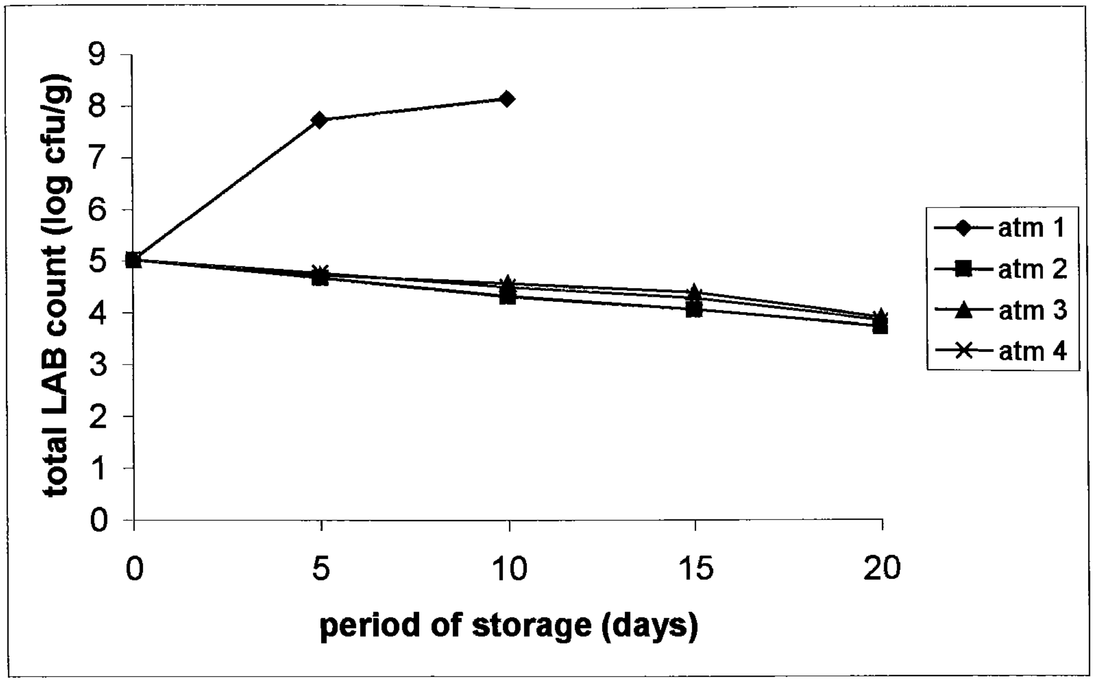


Fig 39: Effect of MAP on the Total Lactic Acid Bacteria (LAB) count (log cfu/g) of unbaked pizza stored at 7 ± 1 °C

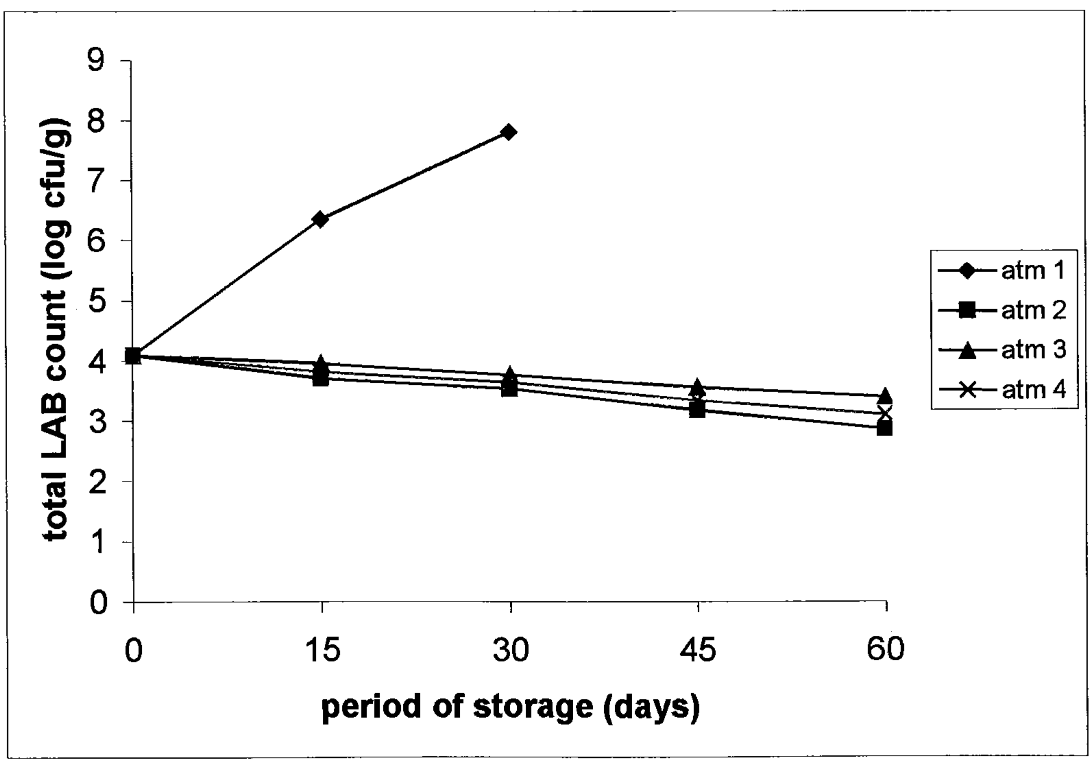


Fig 40: Effect of MAP on the Total Lactic Acid Bacteria (LAB) count (log cfu/g) of baked pizza stored at 7 ± 1 °C

also observed lower LAB in pizzas samples under 90% CO_2 compared to 20% CO_2, when stored at 7±1 °C.

The data relating to effect of MAP on the total lactic acid bacteria count of baked pizza samples stored at 7±1 °C are presented in Appendix XXXIV and Figure 40.

The initial LAB value of 4.09 (log cfu/g) increased to 7.81 after 30 days of storage under air (atm 1), while the value decreased to 2.87 (atm 2), 3.11 (atm 4) and 3.41 (atm 3) after 60 days of storage at 7±1 °C (Appendix XXXIV). From these results, it can be concluded that 100% CO_2 (atm 2) was most suited followed by 50% CO_2 / 50% N_2 (atm 4), and 100% N_2 (atm 3) among the modified atmospheres tried for the storage of pizza samples. The lactic acid bacteria (LAB) count of both the types of pizza samples (unbaked and baked) closely followed the mean sensory score for flavour in the corresponding atmospheres (Appendix XLVI and XLVII).

There was significant ($P<0.01$) influence on total lactic acid bacteria count due to the 4 types of atmospheres and the interaction intervals x atmospheres (atm 2, atm 3, atm 4). However, the influence of intervals of storage on LAB was less significant ($P<0.05$) for baked pizza samples compared to unbaked pizza samples ($P<0.01$) (Table 9).

5.2.3.5 Psychrotrophic count

Appendix XXXV and Figure 41 depict the changes in psychrotrophic count of unbaked pizza samples packaged under 4 different atmospheres and stored at 7±1 °C.

The psychrotrophic count of unbaked pizza samples showed increasing trend throughout the entire period of storage in all the 4 studied atmospheres. The initial psychrotrophic count 2.39 (log cfu/g) increased to 5.21 after 10 days of storage when packed under air (atm 1), while the increase was 3.59 (atm 2), 3.67 (atm 4) and 3.92 (atm 3), indicating that

Table 9. Analysis of variance for Microbiological characteristics of MAP Pizza stored at 7±1 °C

Source of variation	d. f.	Mean sum of square					
		LAB		Psychrotrophs		Anaerobic spore formers	
		Unbaked	Baked	Unbaked	Baked	Unbaked	Baked
Among Intervals of storage	4	1.7723**	1.6280*	19.4759**	11.9856**	0.3908	0.5053
Among atmospheres	3	11.2937**	11.3747**	1.6691**	1.0108**	0.4043	0.0317
Error	46	0.3796	0.4344	0.0739	0.0320	0.0163	0.0027
Interaction [Intervals x atmospheres (atm 2, atm 3, atm 4)]	8	0.0147**	0.0300**	0.1141**	0.1243**	0.0028	0.0029
Error	30	0.0003	0.0002	0.0002	0.0001	0.0001	0.0001

**** Significant at 1% level of probability**
*** Significant at 5% level of probability**
atm 2: 100% CO_2
atm 3: 100% N_2
atm 4: 50% CO_2 : 50% N_2

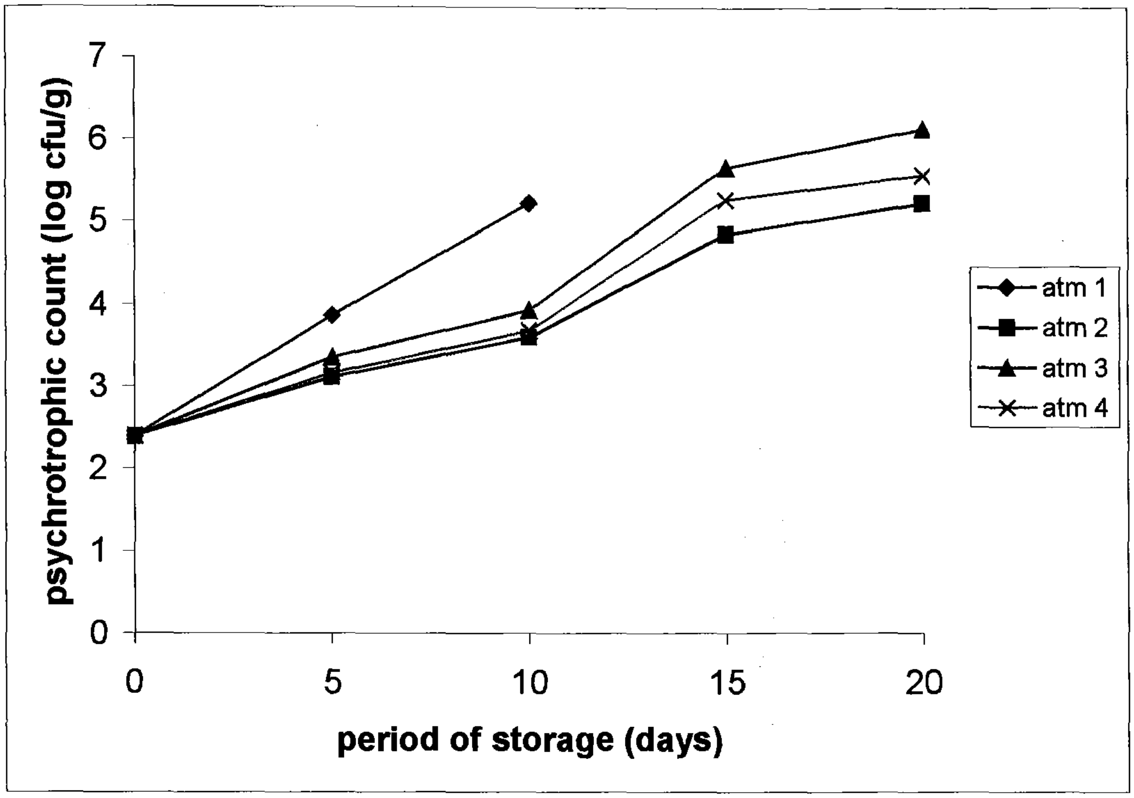


Fig 41: Effect of MAP on the Psychrotrophic count (log cfu/g) of unbaked pizza stored at 7 ± 1 °C

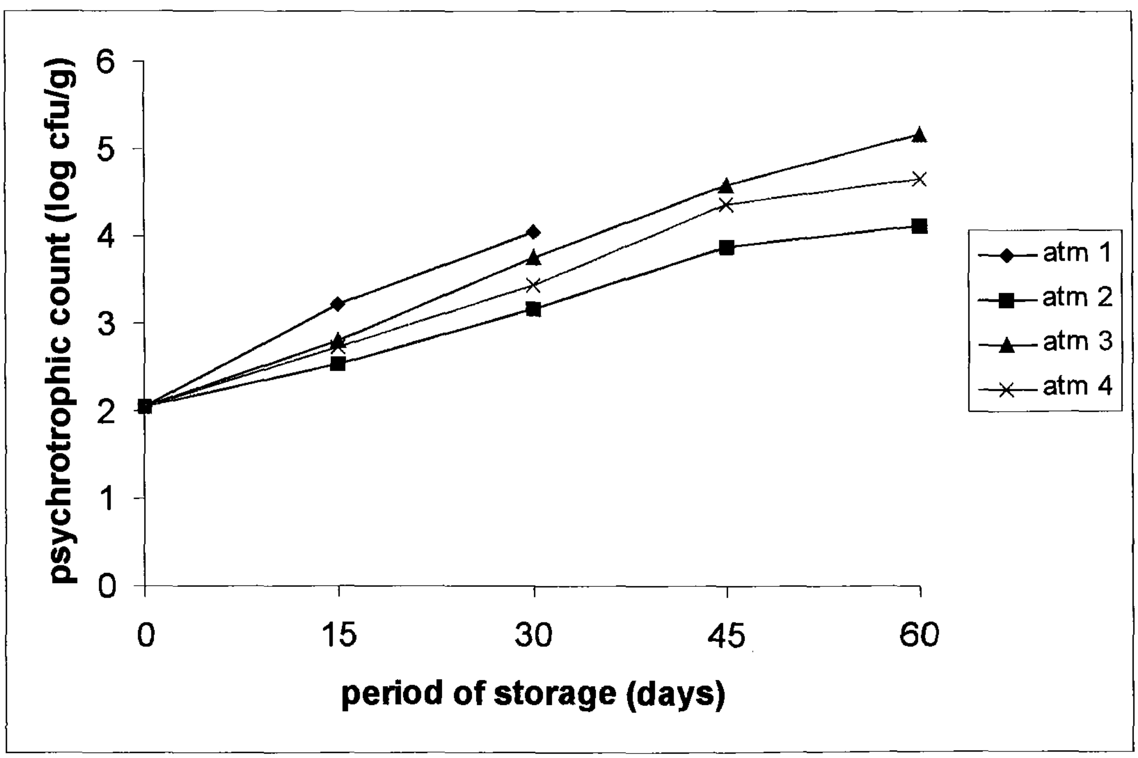


Fig 42: Effect of MAP on the Psychrotrophic count (log cfu/g) of baked pizza stored at 7 ± 1 °C

minimum increase occurred in pizza samples packed by using 100% CO_2 atmosphere.

In case of baked pizza samples, the initial psychrotrophic count of 2.04 (log cfu/g) increased to 4.06 (atm 1), 3.16 (atm 2), 3.76 (atm 3) and 3.45 (atm 4) after 30 days of storage (Appendix XXXVI). It can be seen from Figure 42 that the minimum growth of psychrotrophs was observed for the samples packed under 100% CO_2 (atm 2) followed by 50% CO_2 / 50% N_2 (atm 4) and 100% N_2 (atm 3) respectively, in ascending order, revealing that the growth was retarded when the atmosphere inside the package contained 100% CO_2. The work of Eliot *et al.* (1998) on shredded mozzarella cheese under modified atmosphere also indicated that initial psychrotrophic count 4.36 log cfu/g increased to 7.00 log cfu/g after 3 weeks of storage. The results match with the findings of Villari *et al.* (1994) who observed that the psychrotrophic organisms were less numerous when stored in high CO_2 atmospheres in case of refrigerated pizza. The results are also in agreement with the findings of Tanweer Alam (2004) who reported the increasing trend (throughout the storage period) in the growth of psychrotrophs of mozzarella cheese packed under modified atmospheres, and concluded that the samples packed under 100% CO_2 showed minimum increase in psychrotrophs followed by 50% CO_2 / 50% N_2 and 100% N_2, respectively.

The analysis of variance of the data concerning psychrotrophic count for baked and unbaked samples revealed that the intervals of storage, the 4 types of atmospheres, and the interaction intervals x atmospheres (atm 2, atm 3, atm 4) had highly significant ($P<0.01$) influence on the growth of psychrotrophs (Table 9).

5.2.3.6 Anaerobic Spore formers

In Figure 43 and Appendix XXXVII are presented the anaerobic spore formers (log cfu/g) counts for unbaked pizza samples packaged under 4 atmospheres and stored for indicated periods at 7±1 °C.

On storage the initial anaerobic spore formers count increased from 2.67 to 3.41 (atm 1), 2.95 (atm 2), 2.96 (atm 4) and 2.99 (atm 3) after 10 days of storage. The counts were observed to increase further to 2.98 in case of atm 2, 3.06 for atm 4 and 3.18 with respect to atm 3 revealing that there had been consistent increase in the counts for all the samples, and the minimum increase was for the product packed under 100% CO_2 followed by 50% CO_2 / 50% N_2 and 100% N_2 respectively, in ascending order (Appendix XXXVII).

The effect of MAP on the anaerobic spore formers (log cfu/g) of baked pizza samples packaged under 4 atmospheres and stored at 7±1 °C is contained in Appendix XXXVIII and presented in Figure 44.

The observations revealed that the initial anaerobic spore formers count 1.91 increased to 2.21 after 30 days of storage in case of air packed samples, while the count was found to be 2.41 (atm 2), 2.47 (atm 4) and 2.55 (atm 3) after 60 days of storage indicating that the minimum increase was in case of samples packed under 100% CO_2 followed by 50% CO_2 / 50% N_2 and 100% N_2, respectively (Appendix XXXVIII). Nissen *et al.* (2002) stored vacuum packed Salmon, Herb sauce and Chicken, at 20 °C, and observed that the initial anaerobic count of <100 in all the three products increased respectively to 6.2×10^6, 3.7×10^6 and 1.7×10^7. Farber (1991) also reported that at atmospheric pressure 100% CO_2 could delay toxin production by *Cl. sp.* when compared with an atmosphere of 100% N_2. However, Smoot and Pierson (1982) reported that CO_2 had little effect on germination and toxigenesis of spore formers such as *Clostridium botulinum*.

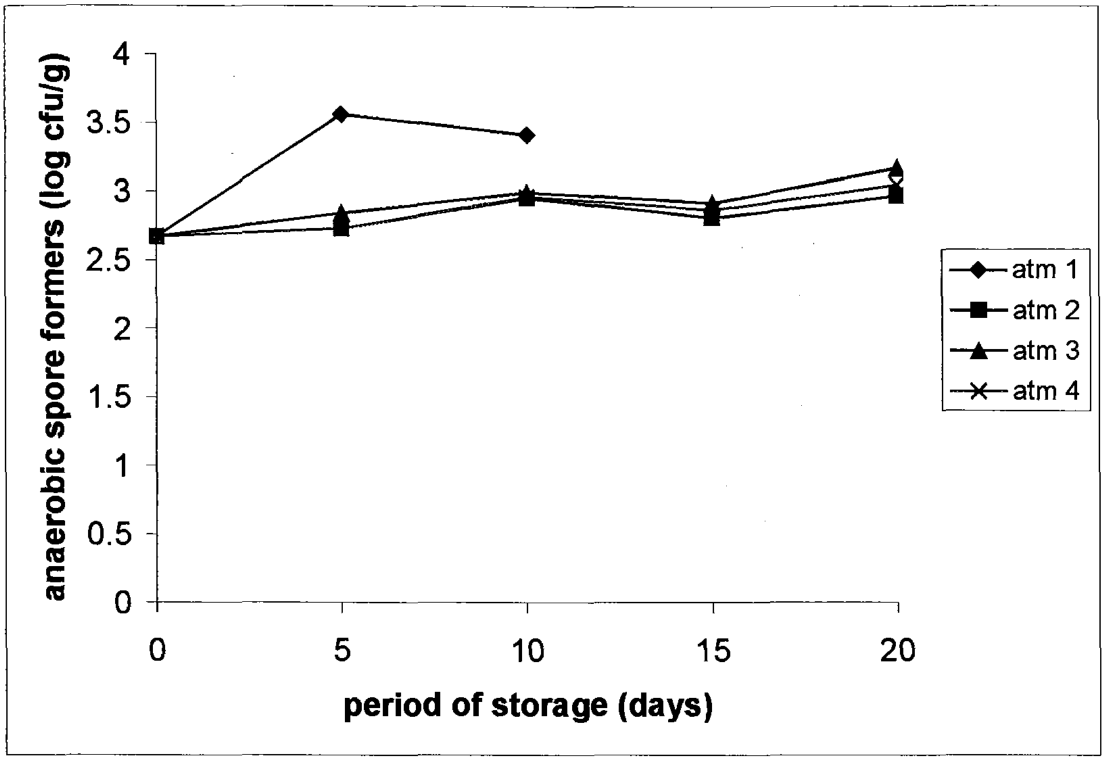


Fig 43: Effect of MAP on the Anaerobic Spore formers (log cfu/g) of unbaked pizza stored at 7 ± 1 °C

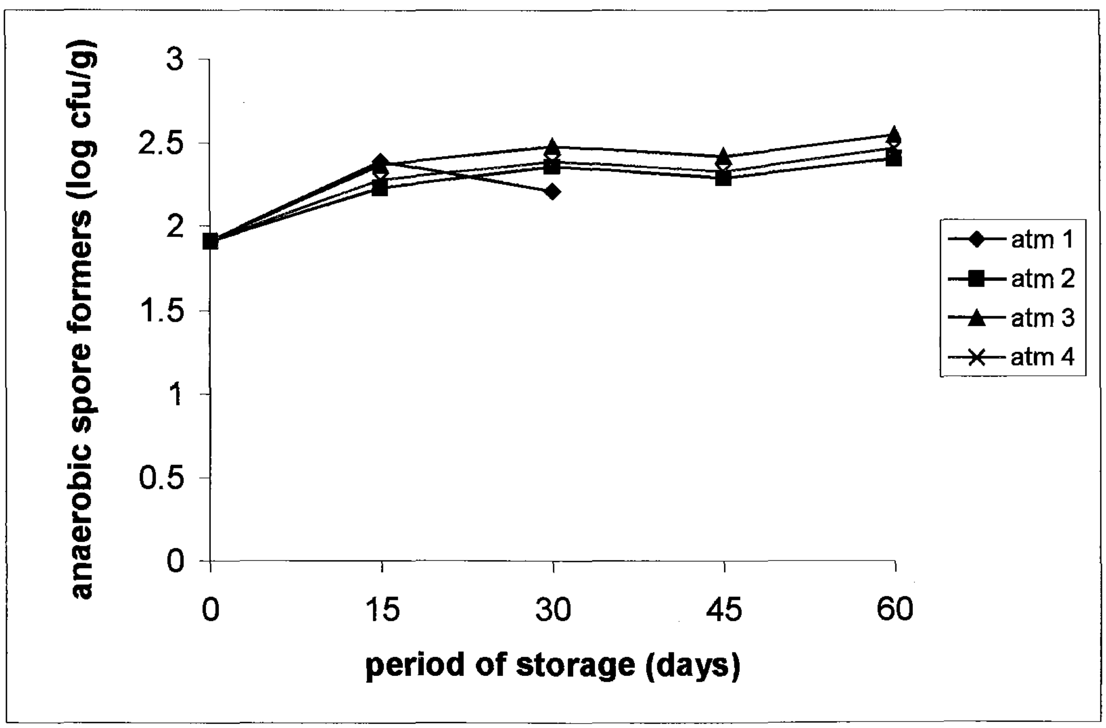


Fig 44: Effect of MAP on the Anaerobic Spore formers (log cfu/g) of baked pizza stored at 7 ± 1 °C

The analysis of variance (Table 9) revealed that the various intervals of storage, different atmospheres of packaging the product and the interaction intervals x atmospheres (atm 2, atm 3, atm 4) had no significant influence on the anaerobic spore formers for both types of samples, i.e. unbaked and baked pizza.

5.2.4.0 Changes in Textural Properties

5.2.4.1 Hardness

The changes in hardness (the force necessary to deform the food between the molar teeth) of baked pizza samples packaged under 4 different atmospheres and stored at 7±1 °C are illustrated in Figure 45 and recorded in Appendix IXL.

The hardness of baked pizza samples exhibited an increasing trend throughout the storage period for all the atmospheres. The initial hardness value (g) of 6621.87 increased to 16602.76 (atm 1), 8606.27 (atm 2), 13744.69 (atm 3) and 12859.69 (atm 4) after 30 days of storage revealing that the hardness was minimum for the samples packed under 100% CO_2 (atm 2) followed by 50% CO_2 / 50% N_2 (atm 4), 100% N_2 (atm 3) and air (atm 1) respectively, in ascending order. Our results pertaining to proteolysis of samples during storage (Appendix XVIII) also showed that the tyrosine content was lowest in case of baked pizza samples packed under atm 2 followed by atm 4, atm 3 and atm 1, respectively. Clarke and Farrell (2000) reported the hardness values (g) for control pizza samples reheated in microwave for 120 s, 150 s and 180 s as 12571, 14461 and 19989 respectively, and concluded that hardness increased with increased reheating times. In general, our results (Appendix IXL) are in harmony with the observations of Tanweer Alam (2004) who reported that at the end of 12 weeks (stored at 7±1 °C), the hardness of mozzarella cheese was minimum for the samples packed under 100% CO_2 followed by 50% CO_2 / 50% N_2, and 100% N_2, respectively.

Anova of the data on hardness (Table 10) revealed that the intervals of storage, the 4 types of atmospheres and the interaction intervals x atmospheres (atm 1, atm 2, atm 3, atm 4) contributed significantly ($P<0.01$) towards the changes in hardness of baked pizza samples.

5.2.4.2 Cohesiveness

Cohesiveness is molecular attraction by which the particles of the body are bonded throughout the mass. The changes in cohesiveness (A2/A1) of baked pizza samples packaged under 4 different atmospheres and stored at 7±1 °C are presented in Appendix XL and depicted in Figure 46.

The cohesiveness of baked pizza samples showed a decreasing trend throughout the entire storage period for all the atmospheres (Fig. 46). The initial cohesiveness value of 0.461 decreased to 0.231 (atm 1), 0.388 (atm 2), 0.314 (atm 3) and 0.327 (atm 4) respectively, after 30 days of storage revealing that the minimum decrease in cohesiveness was observed for the samples packed under atm 2 (100% CO_2) followed by atm 4 (50% CO_2 / 50% N_2), atm 3 (100% N_2) and atm 1 (air), in ascending order (Appendix XL). Clarke and Farrell (2000) observed that cohesiveness values for control pizza samples increased as the time of reheating of microwave pizza increased: 0.481 for 120 s, 0.500 for 150 s, 0.554 for 180 s.

Analysis of variance of the data on cohesiveness reveals that the intervals of storage, the 4 types of atmospheres and the interaction intervals x atmospheres (atm 1, atm 2, atm 3, atm 4) were not significant towards the changes in cohesiveness of baked pizza samples (Table 10).

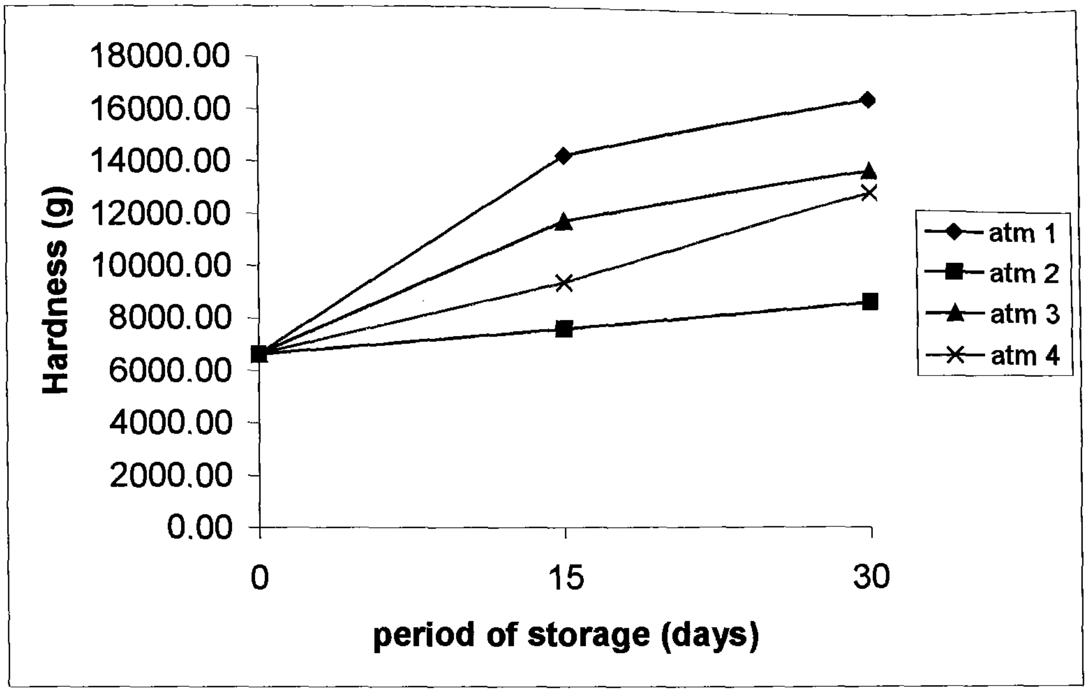


Fig 45: Effect of MAP on the Hardness (g) of baked pizza stored at 7 ± 1 °C

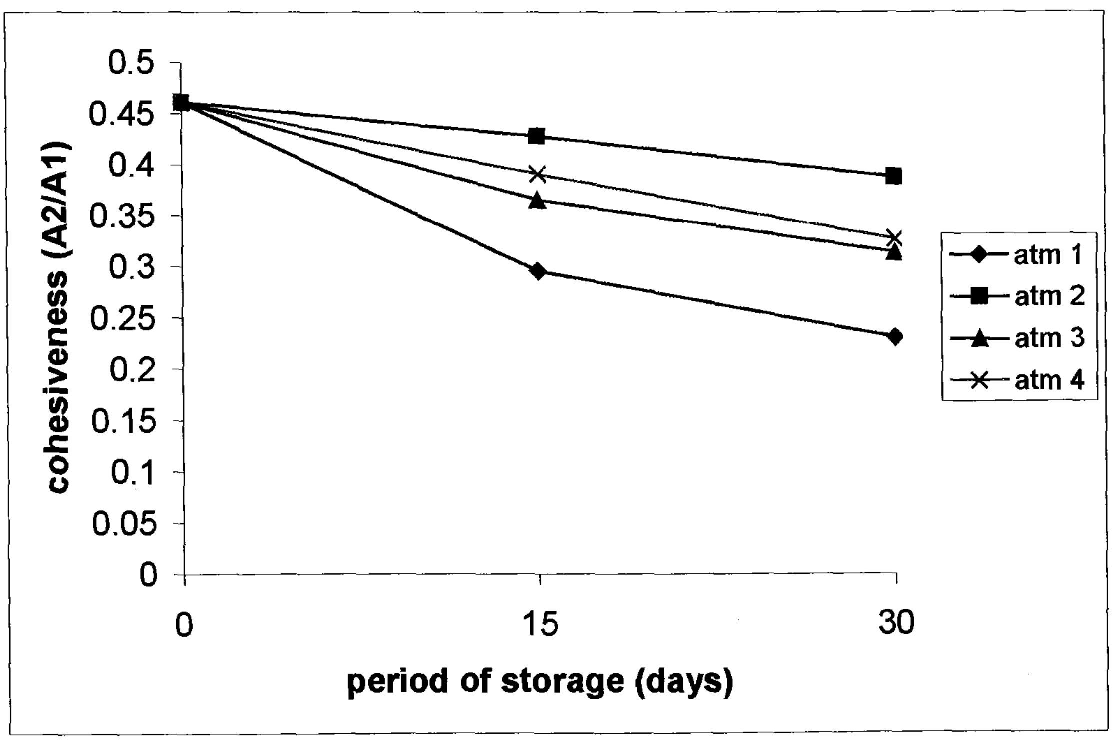


Fig 46: Effect of MAP on the Cohesiveness (A2/A1) of baked pizza stored at 7 ± 1 °C

Table 10. Analysis of variance for Textural characteristics of MAP Baked Pizza stored at 7±1 °C

Source of variation	d. f.	Mean sum of square				
		Hardness	Cohesiveness	Springiness	Gumminess	Chewiness
Among Intervals of storage	2	12365037.12**	0.0652	0.0900	18227122**	997642.0**
Among atmospheres	3	37405896**	0.0144	0.0142	3394017.2**	1708583.9**
Error	30	2028596.9	0.0007	0.0008	217751.56	15655.36
Interaction [Intervals x atmospheres (atm 1, atm 2, atm 3, atm 4)]	6	10142991**	0.0037	0.0042	1088747.1**	782787.06**
Error	24	12.8174	0.0002	0.0001	2.7823	0.0016

**** Significant at 1% level of probability**
atm 1: air
atm 2: 100% CO_2
atm 3: 100% N_2
atm 4: 50% CO_2 : 50% N_2

5.2.4.3 Springiness

The influence of MAP on the springiness of baked pizza samples packed under 4 atmospheres and stored at 7±1 °C is given in Appendix XLI and depicted in Figure 47.

The initial mean springiness value (mm) for fresh baked pizza samples was 0.786 which decreased to 0.524 for samples packed under atm 1, while the value decreased to 0.688 for atm 2, 0.590 for atm 3, and 0.656 for atm 4 indicating lowest value for air packed samples, and highest for samples packed under 100% CO_2. Perhaps higher degree of proteolysis (Lawrence *et al.*, 1987) might be the reason for maximum decrease in springiness in case of samples packaged under atm 1, and least proteolysis in samples packaged under atm 2 (Appendix XVIII). The results are in agreement with the findings of Tanweer Alam (2004) who reported more decrease in springiness for air packed mozzarella cheese samples as compared to samples packaged under 100% N_2, 50% CO_2 / 50% N_2 and 100% CO_2 respectively, in ascending order. Ghosh (1987) also observed that springiness of mozzarella cheese samples decreased with the increase in storage period, regardless of packing material and storage temperature.

Statistically, the influence of intervals of storage, the 4 types of atmospheres, and the interaction intervals x atmospheres (atm 1, atm 2, atm 3, atm 4), on the springiness of baked pizza samples was found to be not significant (Table 10).

5.2.4.4 Gumminess

The changes in gumminess of baked pizza samples packaged under 4 atmospheres and stored at 7±1 °C for various time periods are presented in Appendix XLII and Figure 48.

The mean initial value of gumminess (g) was found to be 3000.01, which continued to increase in all the pizza samples during storage. At the

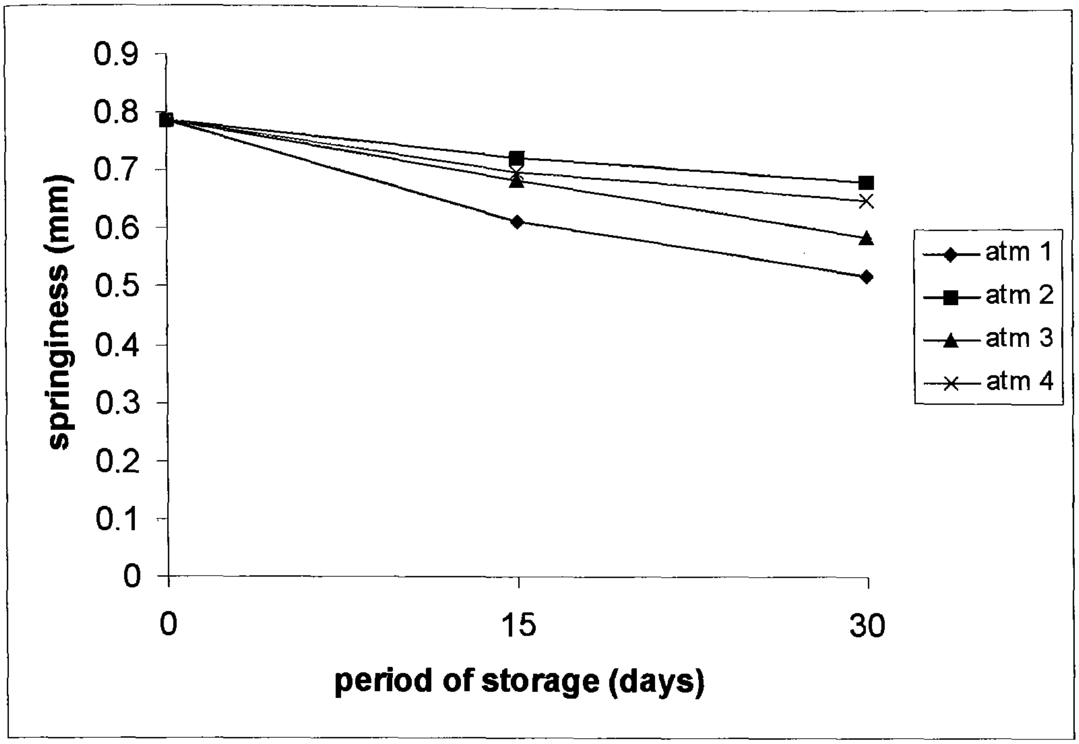


Fig 47: Effect of MAP on the Springiness (mm) of baked pizza stored at 7 ± 1 °C

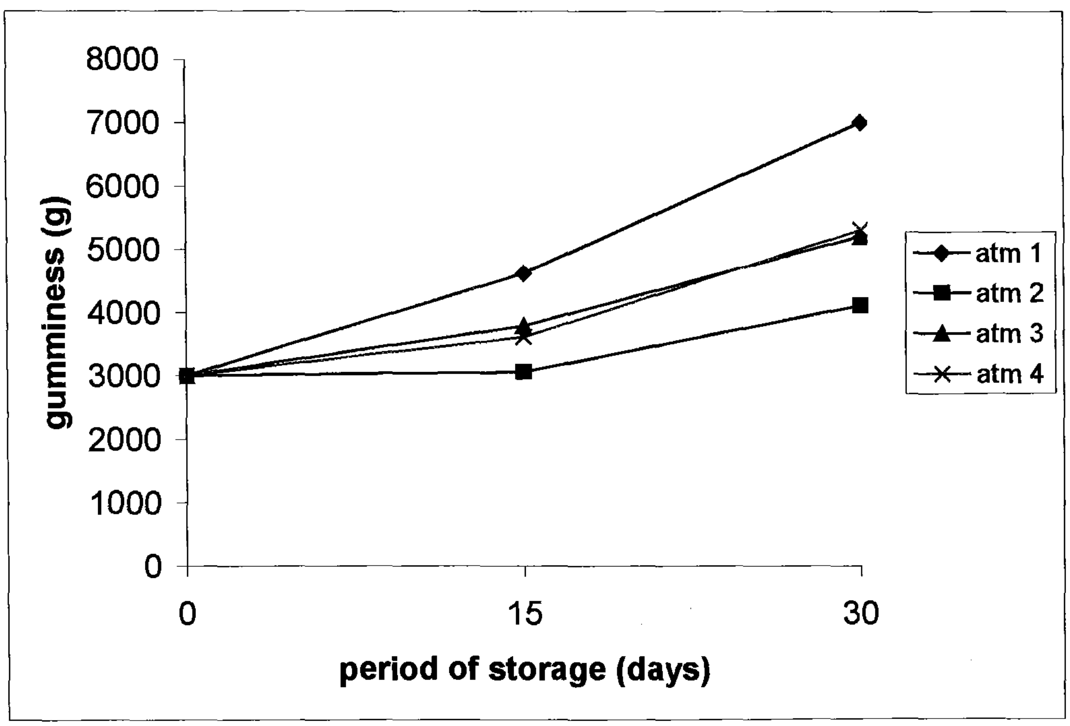


Fig 48: Effect of MAP on the Gumminess (g) of baked pizza stored at 7 ± 1 °C

end of 30 days storage, the values for gumminess increased to 7016.00 g (atm 1), 4112.81 g (atm 2), 5212.13 g (atm 3) and 5312.74 g (atm 4), respectively. From these results, it can be inferred that the gumminess increased maximum for the samples packed under air followed by 50% CO_2 / 50% N_2, 100% N_2 and 100% CO_2, in descending order (Appendix XLII). Clarke and Farrell (2000) also reported increase in gumminess values (g) when the control pizza samples were reheated for increased times, and the values reported by them were 6079 (120 s), 7230 (150 s) and 11097 (180 s). However, these results are at variance with the findings of Ghosh (1987), Malhotra (1991) and Tanweer Alam (2004) who reported consistent decrease in gumminess of cheese samples during storage.

The analysis of variance of the data concerning gumminess of baked pizza samples is included as a part of Table 10, which indicates that the intervals of storage, the 4 types of atmospheres, and the interaction intervals x atmospheres (atm 1, atm 2, atm 3, atm 4), all individually, were highly significant ($P<0.01$).

5.2.4.5 Chewiness

Appendix XLIII and Figure 49 represent the changes in chewiness of baked pizza samples packed under 4 different atmospheres and stored at 7±1 °C for various time intervals.

The mean initial value of chewiness (g x mm) was found to be 2330.81, which increased to 3280.75 (atm 1), 2526.33 (atm 2), 2833.35 (atm 3) and 2778.08 (atm 4) respectively after 15 days of storage. This trend persisted even during further storage of 30 days. However, the minimum increase in chewiness was observed for pizza samples packed under 100% CO_2 (32.89% increase), followed by 50% CO_2 / 50% N_2 (56.96% increase), 100% N_2 (81.44% increase), and air (132.85% increase) respectively (Appendix XLIII). Clarke and Farrell (2000) also reported increase in chewiness values when the control pizza samples were reheated for longer time. However, Ghosh (1987), Malhotra (1991) and Tanweer Alam (2004) reported decrease in chewiness values during storage of cheese samples.

The analysis of variance of the data on chewiness revealed that the various time intervals of storage, the 4 types of atmospheres, and the interaction intervals x atmospheres (atm 1, atm 2, atm 3, atm 4), all individually, played a very significant ($P<0.01$) role in influencing the chewiness of the product (Table 10).

5.2.5.0 Sensory changes

5.2.5.1 Appearance

The changes in Appearance score of pizza prepared from unbaked samples packaged under 4 different atmospheres and stored at 7±1 °C are illustrated in Figure 50 and Appendix XLIV.

The initial mean Appearance value of 5 of fresh unbaked pizza samples decreased to 2.6 in case of atm 1 (air), 3.5 (atm 3), 3.9 (atm 2) and 4.1 (atm 4), indicating that the lowest value had been for the samples packaged under air. On further storage, the value decreased to 2.1 (atm 3), 2.5 (atm 4), and 2.8 (atm 2) revealing highest Appearance score for pizza samples packed under 100% CO_2 followed by 50% CO_2 / 50% N_2 and 100% N_2 respectively (Appendix XLIV). The results confirm the observations of Floros *et al.* (2000) that modified atmosphere packaging (MAP) is effective in retaining appeal of the product to consumers.

The Appearance scores of MAP baked pizza samples stored at 7±1 °C are given in Appendix XLV and reflected in Figure 51. The data showed that the packaging of pizza samples under 100% CO_2 had been most effective from the consideration of higher Appearance score, followed by 50% CO_2 / 50% N_2, 100% N_2 and air, respectively, in descending order. In general, these findings agree with the observations of Farber (1991), Davies (1995),

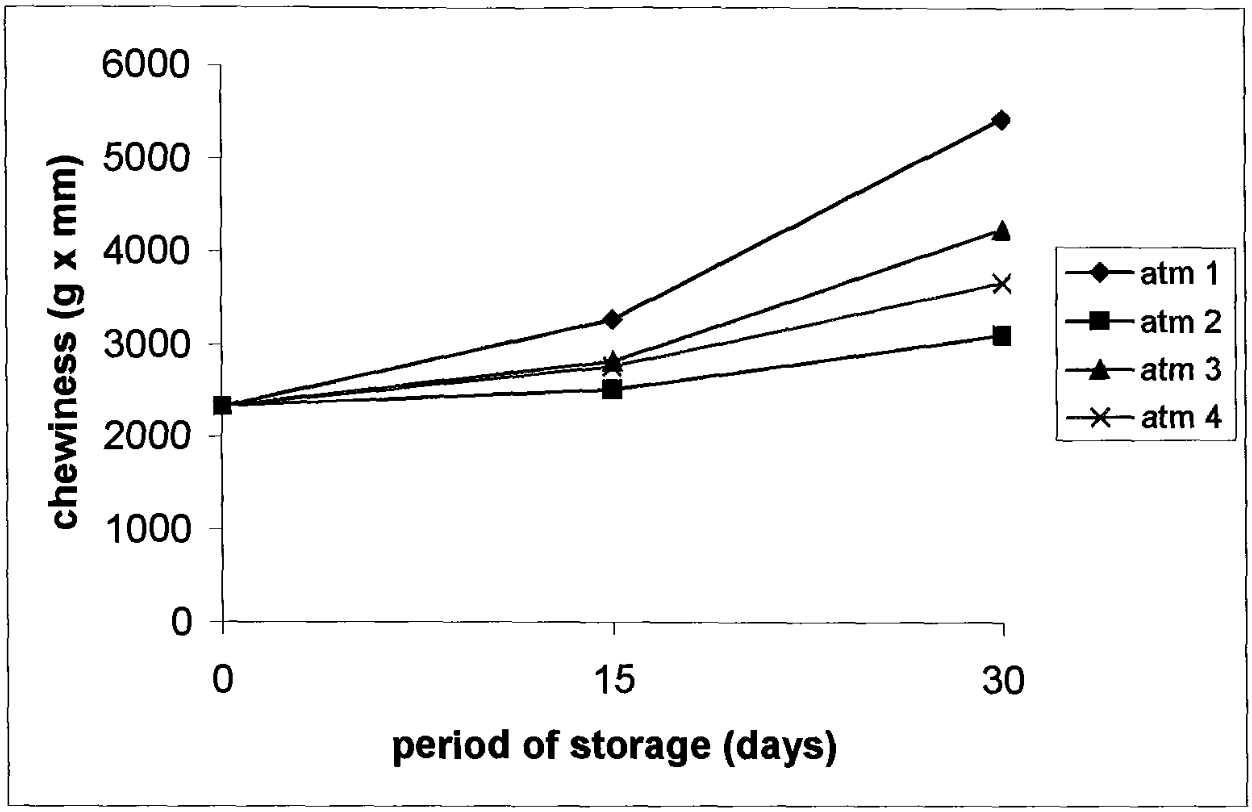


Fig 49: Effect of MAP on the Chewiness (g x mm) of baked pizza stored at 7 ± 1 °C

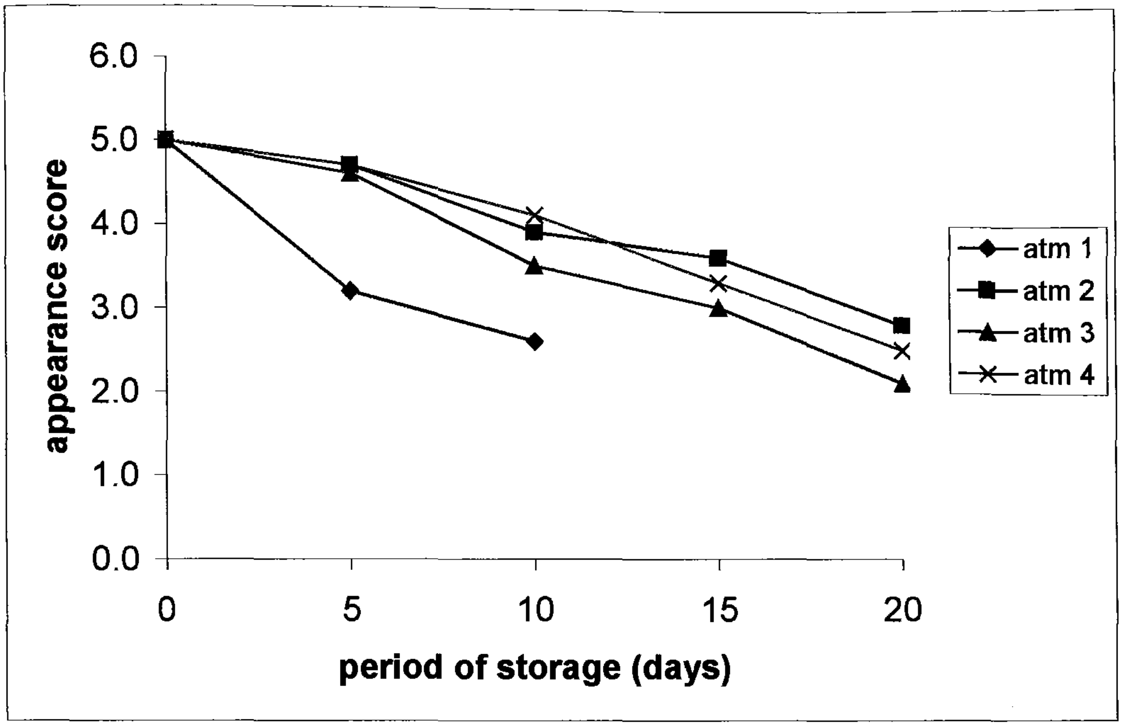


Fig 50: Effect of MAP on the Appearance of unbaked pizza stored at 7 ± 1 ºC

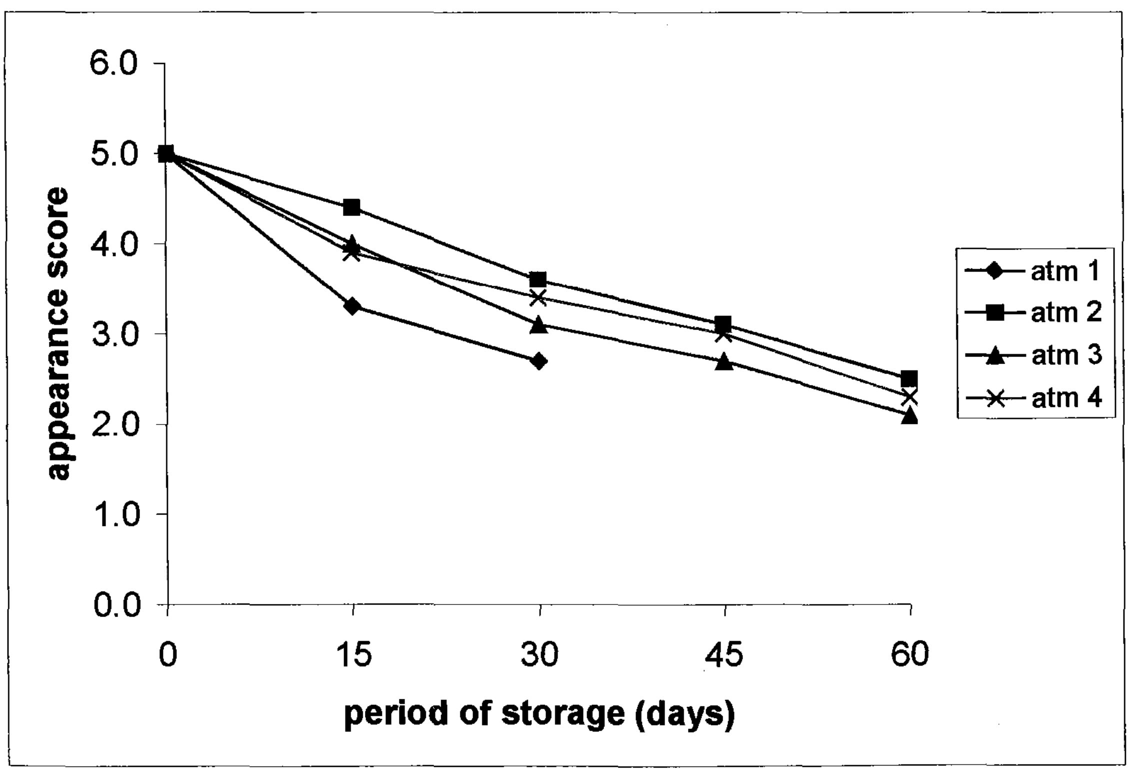


Fig 51: Effect of MAP on the Appearance of baked pizza stored at 7 ± 1 ºC

Sivertsvik (1995) and Floros *et al.* (2000) that modified atmosphere packaging retain the appearance of the product for longer time.

Statistically, the influence of intervals of storage, the 4 types of atmospheres, and the interaction intervals x atmospheres (atm 2, atm 3, atm 4), each individually, were highly significant ($P<0.01$) for changes in Appearance of both unbaked and baked pizza samples (Table 11).

5.2.5.2 Flavour

The mean hedonic sensory scores for Flavour of pizza prepared from unbaked samples packed under 4 atmospheres and stored at 7±1 °C reveal that the initial value 4.8 decreased to 1.3 (atm 1), 2.3 (atm 3), 2.8 (atm 4) and 3.1 (atm 2) respectively, after 10 days of storage (Appendix XLVI, Fig. 52). Based on the Flavour scores, the product was acceptable upto 5 days when packed under air (score 2.7) and upto 15 days for atm 2 (score 2.6), atm 3 (score 2.3) and atm 4 (score 2.8), indicating that the maximum Flavour score was for the samples packaged under 100% CO_2 followed by 50% CO_2 / 50% N_2 and 100% N_2, in descending order.

The data concerning the Flavour score of MAP baked pizza samples packaged under 4 different atmospheres and stored at 7±1 °C are presented in Appendix XLVII and graphically shown in Figure 53. After 15 days of storage, the Flavour score was found to be maximum (3.9 out of 5.0) for the samples packed under 100% CO_2 and minimum in the samples packed under air (2.8 out of 5.0). On further storage for 15 days, the samples packed under 100% N_2 scored minimum (2.2) followed by 50% CO_2 / 50% N_2 (2.6) and 100% CO_2 (3.0) respectively, in ascending order, establishing beneficial role of CO_2 in inhibiting the deterioration of the product. The results agree with the findings of Maniar *et al.* (1994), Alves *et al.* (1996) and Tanweer Alam (2004) who also observed less development of off-flavour in cheese samples packaged under 100% CO_2 than by 50% CO_2 / 50% N_2 and

Table 11. Analysis of variance for Sensory characteristics of MAP Pizza stored at 7±1 °C

Source of variation	d. f.	Mean sum of square							
		Appearance		Flavour		Body & Texture		Overall Acceptability	
		Unbaked	Baked	Unbaked	Baked	Unbaked	Baked	Unbaked	Baked
Among Intervals of storage	4	11.1042**	12.0735**	20.2670**	19.7781**	18.8333**	15.7745**	18.8100**	18.8465**
Among atmospheres	3	2.1798**	0.8297**	1.6669**	2.7318**	2.0877**	1.6451**	2.5150**	2.7762**
Error	46	0.0805	0.0292	0.0719	0.1194	0.1109	0.0510	0.1086	0.1172
Interaction [Intervals x atmospheres (atm 2, atm 3, atm 4)]	8	0.0985**	0.0529**	0.1074**	0.0715**	0.1194**	0.0685**	0.0674**	0.0764**
Error	30	0.0033	0.0033	0.0033	0.0033	0.0032	0.0035	0.0039	0.0038

**** Significant at 1% level of probability**
atm 2: 100% CO_2
atm 3: 100% N_2
atm 4: 50% CO_2 : 50% N_2

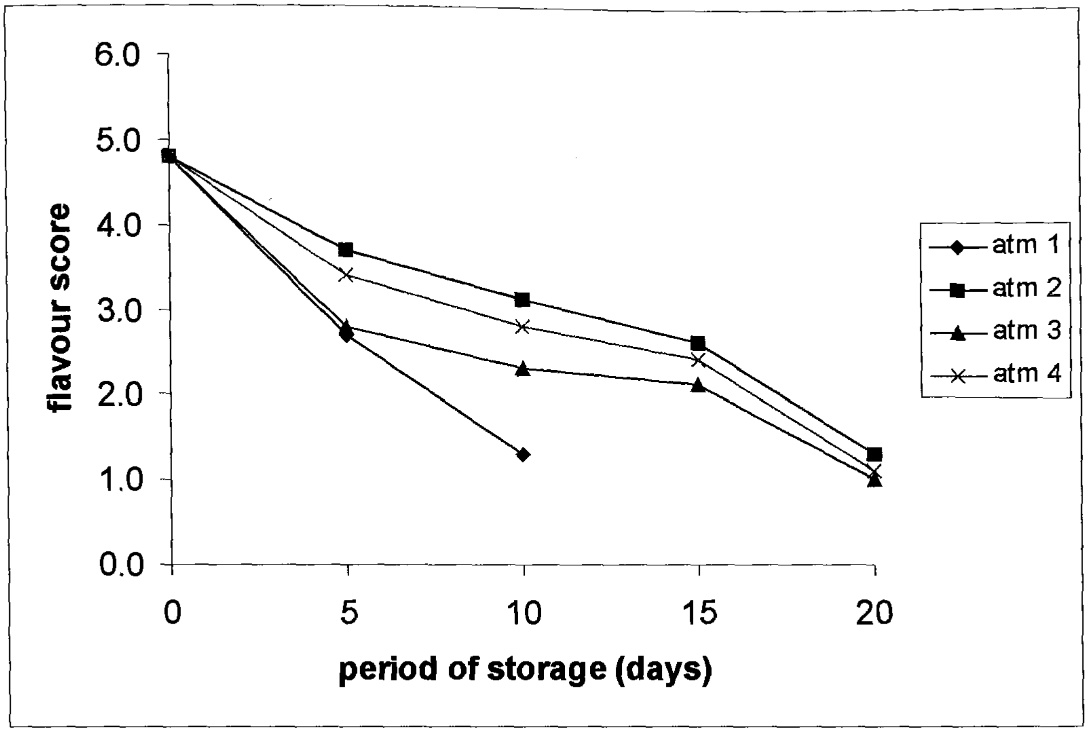


Fig 52: Effect of MAP on the Flavour of unbaked pizza stored at 7 ± 1 °C

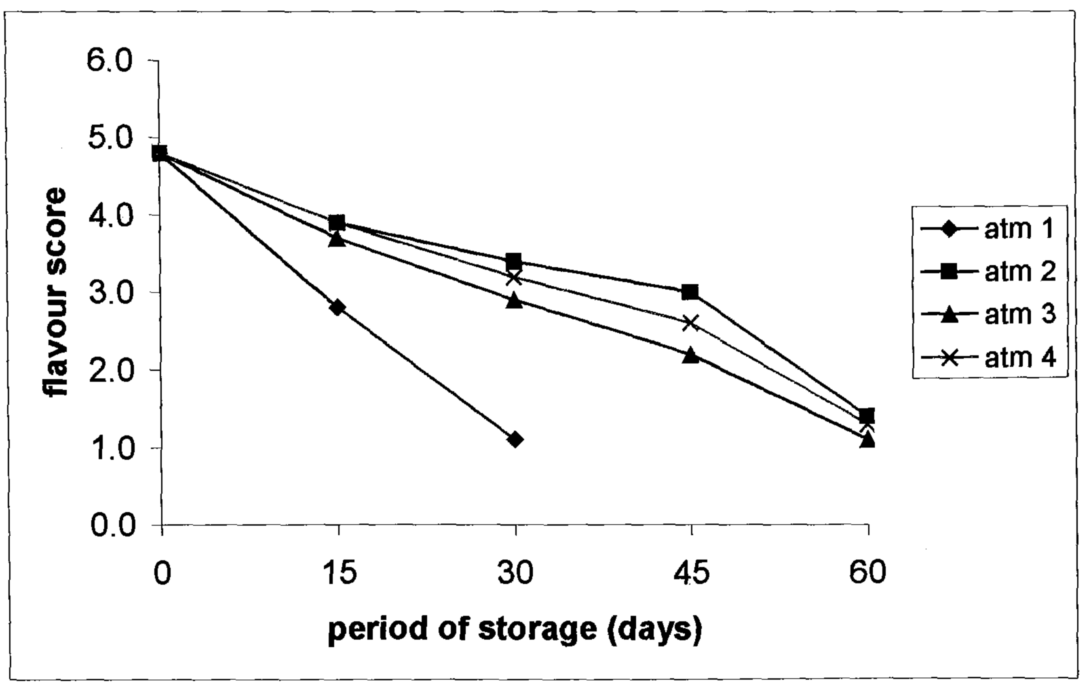


Fig 53: Effect of MAP on the Flavour of baked pizza stored at 7 ± 1 °C

100% N_2. On correlating these figures with the data on the TBA value (Appendix XVI), it can be seen that the TBA value had been minimum for the samples packed under 100% CO_2 followed by 50% CO_2 / 50% N_2 and 100% N_2, respectively. However, all the samples packed under modified atmospheres were found to be not acceptable after 60 days of storage, as the Flavour score values dipped below 2, which was the cut off point for acceptance (Appendix XLVII).

The analysis of variance for Flavour scores of baked and unbaked pizza samples packaged under 4 different atmospheres and stored at 7±1 °C shown as a part of Table 11 indicate that the intervals of storage, the 4 types of atmospheres used during packaging, and the interaction intervals x atmospheres (atm 2, atm 3, atm 4), each individually, were highly significant ($P<0.01$).

5.2.5.3 Body & Texture

The data for the changes in Body & Texture in pizza prepared from unbaked samples packaged under 4 atmospheres and stored at 7±1 °C are illustrated in Appendix XLVIII and Figure 54. The results revealed decreasing trend in the values for Body & Texture of pizza samples during storage (Fig. 54). The initial value of 4.6 for Body & Texture of pizza decreased to 1.3 in case of atm 1, 3.5 (atm 2), 2.9 (atm 3) and 3.1 (atm 4), respectively after 10 days of storage, indicating least changes in Body & Texture for samples packed under 100% CO_2, and maximum changes in case of pizza samples packaged under air, while the values had been intermediary for the samples packed under atm 3 and atm 4, and the trend persisted till the product was stored for 20 days (Fig. 54).

On storage at 7±1 °C for 60 days, the maximum Body & Texture score of baked pizza was observed in samples of atm 2 followed by atm 4 and atm 3, in descending order (Fig. 55), 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 100% N_2, and the samples packed under air (atm 1) were acceptable to the panelist upto 30 days with respect to Body & Texture, while the samples packed under 3 other atmospheres (atm 2, atm 3, atm 4) were acceptable upto 45 days (Appendix XLIX). A perusal of Appendix XXXIX, which deals with the changes in the values of hardness for stored baked pizza samples, indicates that the values for hardness (g) had been directly proportional to the values of Body & Texture (Appendix XLIX), i.e. lower hardness value resulted in higher Body & Texture score.

The results, in general, are in harmony with the findings of Tanweer Alam (2004) who also observed that the values for Body & Texture decreased in the similar fashion when mozzarella cheese was packaged under different modified atmospheres.

Anova of the data on Body & Texture of stored unbaked and baked pizza samples revealed (Table 11) that intervals of storage, types of atmospheres, and the interaction intervals x atmospheres (atm 2, atm 3, atm 4) were highly significant ($P<0.01$) with regard to Body & Texture characteristics for both types of pizza samples.

5.2.5.4 Overall Acceptability

The changes in the values for Overall Acceptability of pizza prepared from unbaked samples packed under 4 different atmospheres and stored at 7 ± 1 °C are recorded in Appendix L and illustrated in Figure 56.

The overall acceptability of pizza samples exhibited a decreasing trend throughout the storage period under all studied atmospheres. The initial Overall Acceptability score 4.7 (atm 1) decreased to 2.5 and 1.0 respectively, after 5 and 10 days of storage, indicating that the air packed unbaked (ready-to-bake) pizza samples were acceptable only upto 5 days. Appendix L shows that at the end of 20 days of storage, none of the sample was acceptable

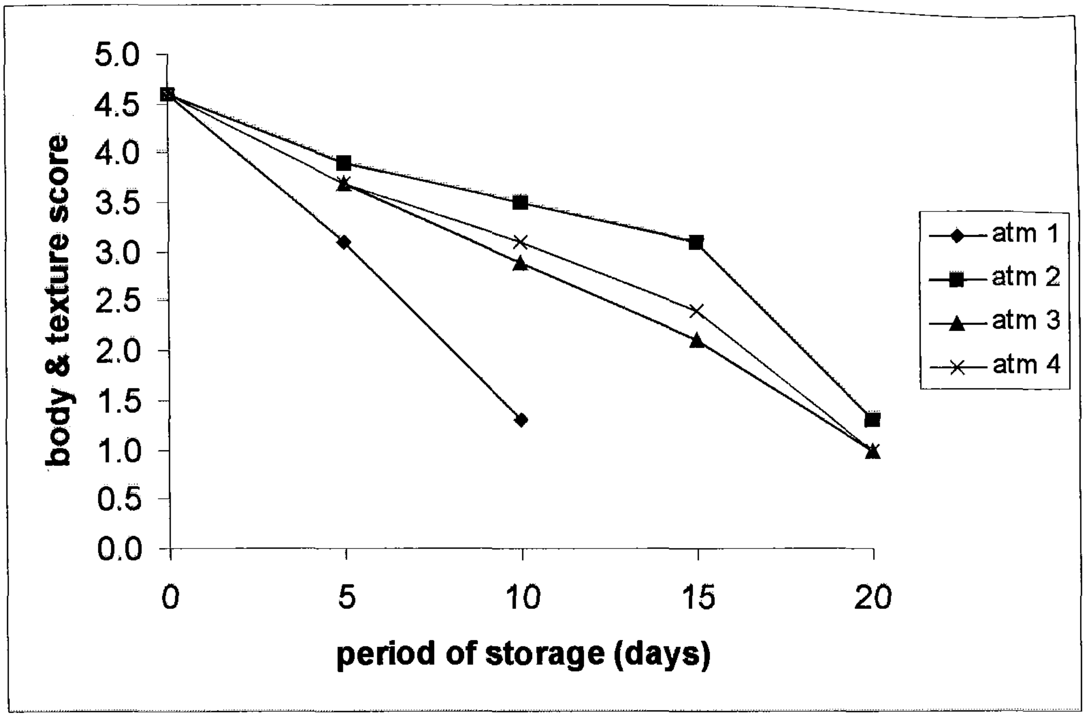


Fig 54: Effect of MAP on the Body & Texture of unbaked pizza stored at 7 ± 1 °C

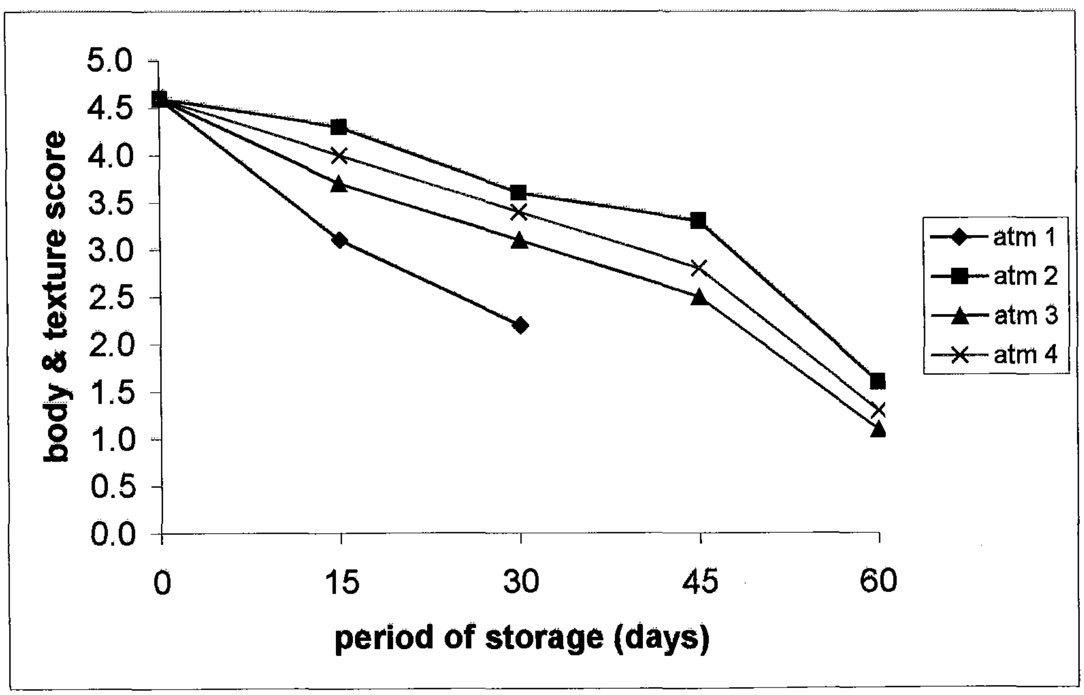


Fig 55: Effect of MAP on the Body & Texture of baked pizza stored at 7 ± 1 °C

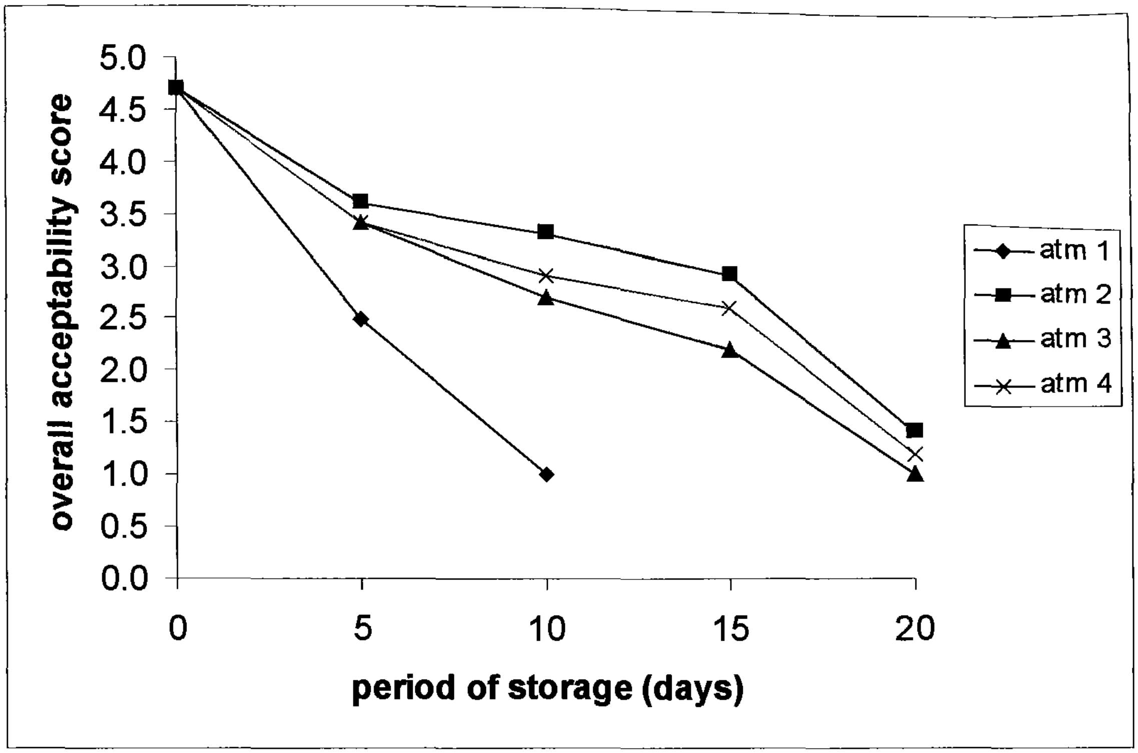


Fig 56: Effect of MAP on the Overall Acceptability of unbaked pizza stored at 7 ± 1 °C

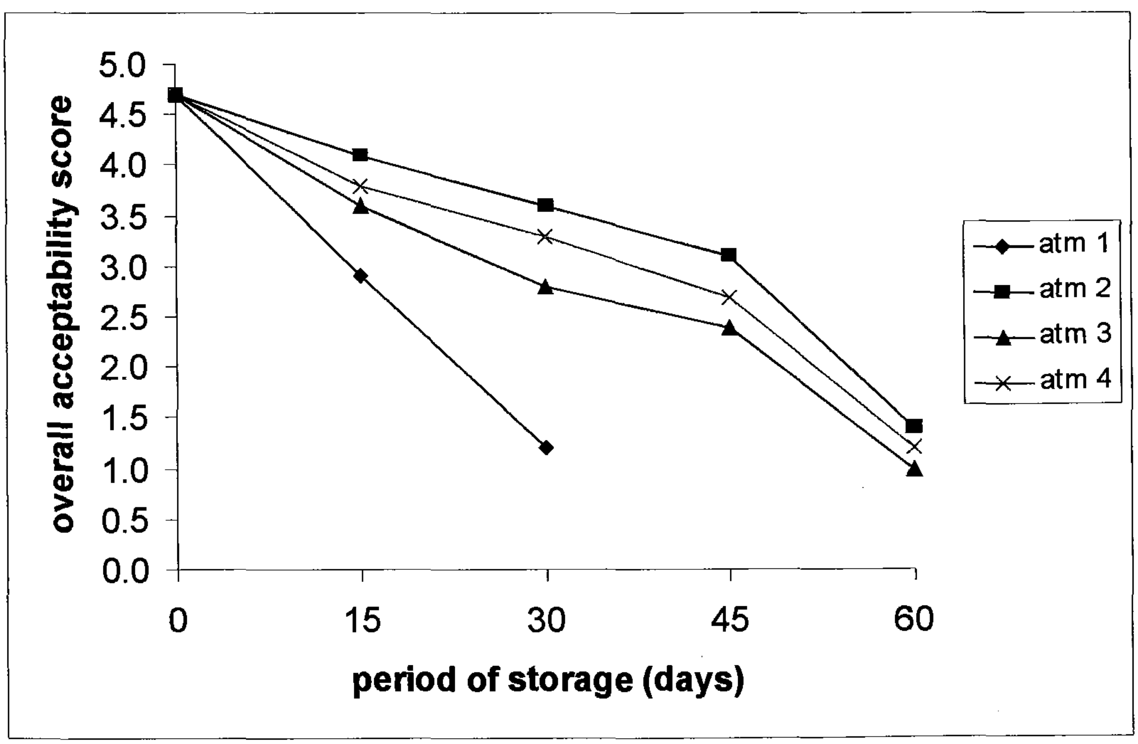


Fig 57: Effect of MAP on the Overall Acceptability of baked pizza stored at 7 ± 1 °C

under all the 3 modified atmospheres (atm 2, atm 3, atm 4), but were acceptable only upto 15 days. The samples packed under 100% CO_2 (atm 2) were liked most followed by 50% CO_2 / 50% N_2 and 100% N_2 respectively, in descending order (Fig. 56).

The storage of baked pizza samples at 7±1 °C for varied time intervals resulted in decrease of Overall Acceptability score for the samples packaged under 4 atmospheres (Fig. 57). The observations (Appendix LI) revealed that the type of atmosphere played a very significant role in affecting the Overall Acceptability of the product. The pizza samples packaged under 100% CO_2 (atm 2) were rated best, followed by samples of atm 4, atm 3 and atm 1 respectively, in descending order. A perusal of Appendix LI indicates that the baked pizza samples (ready-to-serve) packed under air (atm 1) were of acceptable quality only upto 15 days, while the samples packed under other 3 modified atmospheres, i.e. atm 2, atm 3 and atm 4 were found to be acceptable upto 45 days, by securing the Overall Acceptability score 3.1 (atm 2), 2.4 (atm 3) and 2.7 (atm 4) respectively, establishing the superior role of 100% CO_2, which resulted in highest Acceptability scores for the pizza samples. In general, the results are in agreement with the findings of Maniar *et al.* (1994), Alves *et al.* (1996) and Tanweer Alam (2004) who also observed that 100% CO_2 atmosphere best maintained the sensorial characteristics of the product.

Anova for the data on Overall Acceptability (Table 11) revealed that different intervals of storage, the 4 types of atmospheres, and the interaction intervals x atmospheres (atm 2, atm 3, atm 4), each individually, contributed very significantly ($P<0.01$) towards the changes in Overall Acceptability of unbaked (ready-to-bake) and baked (ready-to-serve) pizza samples.

5.2.6.0 Shelf life of MAP Pizza

In order to determine the shelf life of MAP unbaked (ready-to-bake) and baked (ready-to-serve) pizza, the samples were subjected to 4 types of atmospheres (air, 100% CO_2, 100% N_2, and 50% CO_2 / 50% N_2) and stored for various time intervals at 7±1 °C. The data obtained for the Overall Acceptability were used to establish the product's shelf life. The shelf life of ready-to-bake (unbaked) pizza significantly increased upto 15 days (a 300% increase) for the samples packaged under 100% CO_2 (atm 2), 50% CO_2 / 50% N_2 (atm 4) and 100% N_2 (atm 3), compared to conventional air pack (5 days) (Plates 10, 11, 12 and 13) but the samples packaged under atm 2 > atm 4 > atm 3.

Ahvenainen *et al.* (1990) could enhance the shelf life of ham pizza upto 36 days at 5 ± 1°C by using the different gas atmospheres. Farber (1991) reported the shelf life of pizza product under different gas mixtures as 17 days under 100% CO_2; 30 days under 20% CO_2 + 80% N_2; and 30 days under 50% CO_2 + 50% air. Blakistone (1998) could increase the shelf life of pizza upto 21 days (a 350% increase) by using MAP conditions, while Fasano and Gallo (2001) obtained the shelf life of 15 days for fresh pizza packaged under modified atmospheres and stored at 7±1 °C.

In case of ready-to-serve (baked) pizza samples, a 300% increase in the shelf life was achieved due to the 3 different modified atmospheres, i.e. 100% CO_2 (atm 2), 100% N_2 (atm 3), and 50% CO_2 / 50% N_2 (atm 4) used for the packaging of pizza samples. The samples under these modified atmospheres were rated as acceptable upto 45 days compared to 15 days for the pizza samples packed under air (atm 1) (Plates 14, 15, 16, 17).

However, amongst the four atmospheres studied, atm 2 (100% CO_2) was observed to be the most effective followed by atm 4 (50% CO_2 / 50% N_2), atm 3 (100% N_2) and atm 1 (air) respectively, in descending order

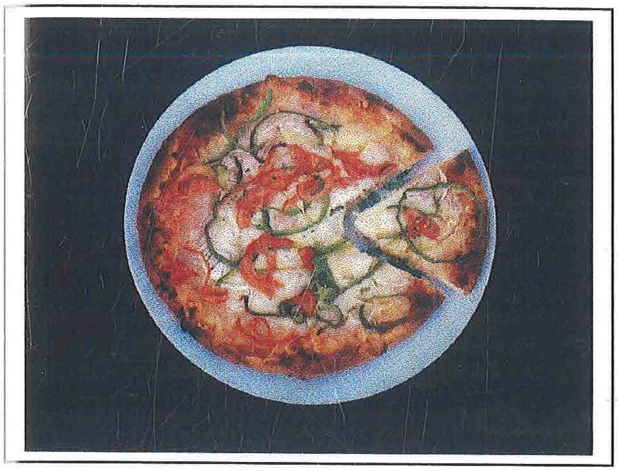


Plate 10: Pizza prepared from Unbaked sample stored at 7±1 ºC for 5 days (air → atm 1)

Plate 11: Pizza prepared from Unbaked sample stored at 7±1 ºC for 15 days (100% CO_2 → atm 2)

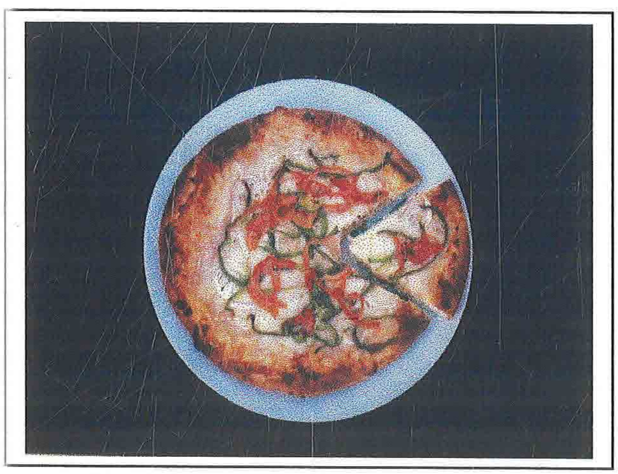


Plate 12: Pizza prepared from Unbaked sample stored at 7±1 ºC for 15 days (100% N_2 → atm 3)

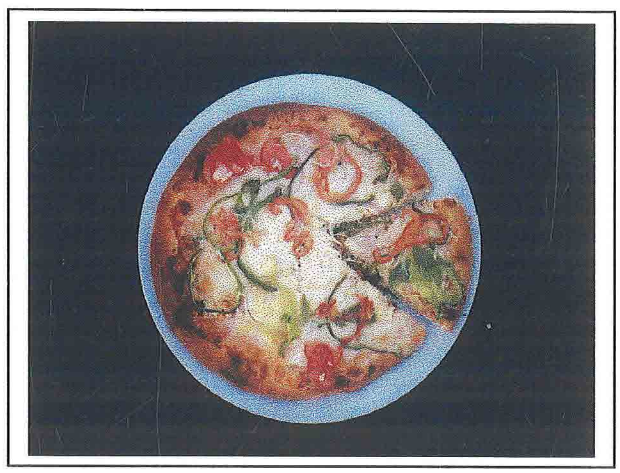


Plate 13: Pizza prepared from Unbaked sample stored at 7±1 ºC for 15 days (50% CO_2 : 50% N_2 → atm 4)

Plate 14: Pizza prepared from Baked sample stored at 7±1 ºC for 15 days (air → atm 1)

Plate 15: Pizza prepared from Baked sample stored at 7±1 ºC for 45 days (100% CO_2 → atm 2)

Plate 16: Pizza prepared from Baked sample stored at 7±1 ºC for 45 days (100% N_2 → atm 3)

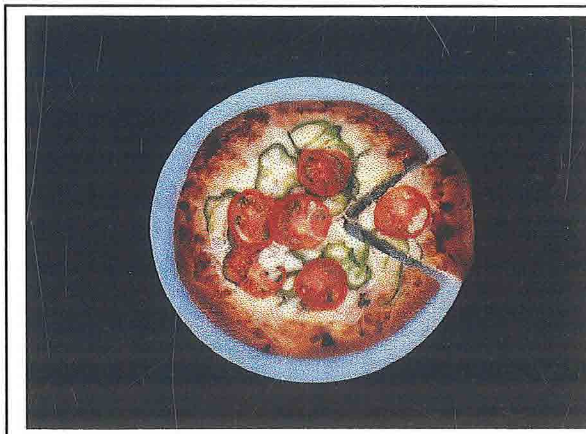


Plate 17: Pizza prepared from Baked sample stored at 7±1 ºC for 45 days (50%CO_2 : 50% N_2 → atm 4)

(atm 2 > atm 4 > atm 3 > atm 1) from the consideration of packaging and storage (at 7±1 ºC) of ready-to-bake (unbaked) and ready-to-serve (baked) pizza samples.

CHAPTER 6

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSIONS

6.0.0.0 The materials presented in the foregoing pages of this thesis relate to studies on the modified atmosphere packaging (MAP) of pizza. Under modified atmospheres, four types of atmospheres namely air, 100% CO_2, 100% N_2, and 50% CO_2 / 50% N_2 have been studied by using high barrier bags.

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

6.2.0.0 A comprehensive review of the available literature on modified atmosphere packaging, which includes the parameters critical to MAP, characteristics of packaging materials, equipments and gases used for MAP is presented. This review also includes the information concerning the modified atmosphere packaging of various dairy products namely milk, danedar khoa, butter, ice cream, milk powder, yoghurt and cheese. The published information on preparation, packaging and storage of pizza, physico-chemical and textural characteristics of pizza, microbiological quality, including the shelf life of pizza is also referred to.

6.3.0.0 Since no systematic approach to study the modified atmosphere packaging of the two types of pizza, i.e. ready-to-bake (unbaked) and ready-to-serve (baked) 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 pizza, its packaging under different modified atmospheres and influence of storage at 7±1 °C on various properties viz. physico-chemical and microbiological quality, textural and sensory characteristics. The experimental techniques employed during the course of present studies have also been described.

6.4.0.0 The data related to proximate, microbiological and textural properties of freshly prepared pizza samples used for the package and storage studies are presented.

6.5.0.0 Determination of changes in the headspace volume of two types of pizza samples packaged under four different types of atmospheres and stored for various time intervals also formed a part of the present study. The data for physico-chemical, microbiological, textural and sensory changes in two types of pizza samples during the course of storage are recorded.

6.6.0.0 The data collected during the investigations are discussed with the help of their mean values presented in LI table of details (Appendices), 11 Tables of consolidated data, 57 Figures and Plates 1-17. From the results obtained, following conclusions are indicated:

6.6.1.0 The proximate composition of freshly prepared ready-to-bake (unbaked) pizza indicated that the average values for various characteristics were: moisture 55.68%, protein 11.65%, fat 9.53%, ash 2.57%, carbohydrates 20.57%, and the food energy 214.65 cal/100 g, while the values for ready-to-serve (baked) pizza were: moisture 43.71%, protein 14.63%, fat 11.98%, ash 2.98%, carbohydrates 26.71%, and food energy 273.18 cal/100g, revealing that the ready-to-serve pizza had more fat and protein contents with higher food energy. The microbiological quality of the two types of pizza samples revealed that the average counts for TPC, Y&M, coliforms, LAB, psychrotrophs and anaerobic spore formers were lower in case of ready-to-serve samples compared to ready-to-bake pizza. The instrumental textural evaluation of fresh baked (ready-to-serve) pizza samples showed that the hardness (g) averaged 6621.87, cohesiveness averaged 0.461, springiness (mm) averaged 0.786, gumminess (g) averaged 3000.01 and chewiness (g x mm) averaged 2330.81.

6.6.2.0 Analysis of variance established significant ($P<0.01$) difference towards headspace volume due to intervals of storage, the 4 types of modified atmospheres, and the interaction between intervals and atmospheres for both the types of pizza samples (unbaked and baked).

6.6.3.0 The intervals of storage, and different modified atmospheres were found to have significant ($P<0.01$) effect on the moisture content of MAP unbaked and baked pizza samples, while the influence of interaction between intervals and atmospheres (atm 2, atm 3, atm 4) was found to be not significant for any of the pizza samples.

6.6.4.0 The four types of modified atmospheres, and the intervals of storage, each individually, significantly ($P<0.01$) influenced the water activity (a_w) of the two types of pizza samples, but the interaction between intervals and atmospheres (atm 2, atm 3, atm 4) did not affect the a_w of any of the pizza samples.

6.6.5.0 The values for pH of the two types of pizza samples were significantly affected ($P<0.01$) by the intervals of storage, the 4 types of modified atmospheres, and the interaction between intervals and atmospheres (atm 2, atm 3, atm 4).

6.6.6.0 Analysis of variance confirmed highly significant (P<0.0.1) effect on titratable acidity due to the difference among the intervals of storage, among the atmospheres, and the interaction between storage intervals and atmospheres (atm 2, atm 3, atm 4) for both the types of pizza samples.

6.6.7.0 The values for free fatty acids (FFA) of baked and unbaked pizza samples were significantly influenced (P<0.0.1) by the four types of modified atmospheres, different intervals of storage, and the interaction between intervals of storage and atmospheres (atm 2, atm 3, atm 4).

6.6.8.0 The intervals of storage, the 4 types of modified atmospheres, and the interaction between intervals and atmospheres (atm 2, atm, 3, atm 4) were found to have significant (P<0.01) effect on peroxide value for both types of pizza samples.

6.6.9.0 Analysis of variance showed significant (P<0.01) influence on TBA value due to the intervals of storage, the different types of atmospheres, and the interaction between intervals and atmospheres (atm 2, atm 3, atm 4) for the two types of pizza samples.

6.6.10.0 The tyrosine content of two types of pizza samples were significantly (P<0.01) affected due to the variation among 4 types of modified atmospheres, among intervals of storage, and the interaction between intervals and atmospheres (atm 2, atm 3, atm 4).

6.6.11.0 The four types of modified atmospheres, intervals of storage, and the interaction between intervals and atmospheres (atm 2, atm 3, atm 4), all individually, significantly (P<0.01) influenced the lycopene content of both types of pizza samples.

6.6.12.0 The analysis of variance of the data revealed that the intervals of storage and the four types of atmospheres, each individually, significantly (P<0.05) affected the total colour difference (ΔE) in case of baked pizza samples only, while the interaction between intervals and atmospheres (atm 2, atm 3, atm 4) was found to be not significant for any of the pizza samples studied.

6.6.13.0 The intervals of storage, and the four types of atmospheres significantly influenced (P<0.05) the relative yellowness (E*) for baked pizza samples only. The interaction between intervals and atmospheres (atm 2, atm 3, atm 4) was observed to be not significant for both the types (unbaked and baked) of pizza samples.

6.6.14.0 Anova of the data revealed highly significant ($P<0.01$) differences among the intervals of storage, amongst atmospheres, and the interaction between intervals and atmospheres with regard to changes in total plate count for both types of pizza samples.

6.6.15.0 The effects of intervals of storage and also that of four types of atmospheres on the values for Y & M were observed to be highly significant ($P<0.01$), while the interaction between intervals and atmospheres (atm 2, atm 3, atm 4) was found to be not significant for any of pizza samples studied.

6.6.16.0 The coliform counts of the stored pizza samples were significantly ($P< 0.01$) affected by the intervals of storage, the four types of atmospheres, and the interaction between intervals and atmospheres (atm 2, atm 3, atm4) for both the types of pizza samples.

6.6.17.0 Analysis of variance for the data on lactic acid bacteria (LAB) confirmed that the four types of atmospheres and the interaction between intervals and atmospheres (atm 2, atm 3, atm 4), each individually, were highly significant ($P<0.01$) for unbaked and baked pizza samples. However, the influence of intervals of storage on LAB was less significant ($P<0.05$) for baked pizza samples compared to unbaked samples ($P<0.01$).

6.6.18.0 There were found to be highly significant ($P<0.01$) differences in the psychrotrophic count due to intervals of storage, the four types of atmospheres, and the interaction between intervals and atmospheres (atm 2, atm 3, atm 4) for both types of pizza samples.

6.6.19.0 Statistically, the influence of intervals of storage, the types of atmospheres, and the interaction between intervals and atmospheres (atm 2, atm 3, atm 4) did not influence the anaerobic spore formers count for both baked and unbaked pizza samples.

6.6.20.0 The textural analysis of the stored MAP baked pizza revealed that statistically the hardness, gumminess and chewiness were significantly ($P<0.01$) affected by the intervals of storage, the four types of atmospheres, and the interaction between intervals and atmospheres, while cohesiveness and springiness were not affected.

6.6.21.0 The four types of modified atmospheres, intervals of storage, and the interaction between intervals and atmospheres (atm 2, atm 3, atm 4), each individually, were found to be highly significant ($P<0.01$) from the consideration of scores for appearance, flavour, body & texture, and overall acceptability for both, i.e. unbaked (ready-to-bake) and baked (ready-to-serve) pizza samples.

6.6.22.0 With the aim to determine the shelf life under MAP, the unbaked (ready-to-bake) and baked (ready-to-serve) pizza samples were submitted to 4 types of atmospheres (air, 100% CO_2, 100% N_2, and 50% CO_2 / 50% N_2). The samples were packaged in high barrier bags (LLD/BA/Nylon-6/BA/LDPE) and stored at 7±1 ^oC. The data of the overall acceptability were used as an index to define the product shelf life. It was observed that the shelf life of unbaked (ready-to-bake) pizza samples significantly increased upto 15 days (a 300% increase) for the product packaged under 100% CO_2, 100% N_2 and 50% CO_2 / 50% N_2 compared to air pack (5 days). Similar increase of 300% (45 days) was observed in case of baked (ready-to-serve) pizza samples when packaged under 100% CO_2, 100% N_2 and 50% CO_2 / 50% N_2 compared to conventional air pack (15 days)

6.7.0.0 From the present study it can be concluded that amongst four atmospheres, namely atm 1 (air), atm 2 (100% CO_2), atm 3 (100% N_2) and atm 4 (50% CO_2 / 50% N_2) used for the packaging of two types of pizza samples, i.e. unbaked and baked, atm 2 (100% CO_2) was best, followed by atm 4 > atm 3 > atm 1 respectively, in descending order, and may be used for packaging and storage of ready-to-bake and ready-to-serve pizza samples at 7±1 °C.

6.8.0.0 An exhaustive bibliography of the references included 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 Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	76	748	748	748
5	76	748	748	746
10	75	746	744	749
15	■	745	741	737
20	■	743	738	731

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix II: Effect of MAP on the Headspace volume (ml) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	87	759	759	759
15	86	756	756	754
30	82	752	749	745
45	■	747	742	736
60	■	743	740	732

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix III: Effect of MAP on the Moisture content (%) of Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	55.68	55.68	55.68	55.68
5	55.28	55.51	55.43	55.48
10	54.77	55.11	54.87	54.98
15	■	54.69	54.51	54.61
20	■	54.02	54.17	53.76

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix IV: Effect of MAP on the Moisture content (%) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	43.71	43.71	43.71	43.71
15	43.46	43.62	43.56	43.65
30	43.32	43.55	43.38	43.59
45	■	43.42	43.47	43.48
60	■	43.31	43.29	43.35

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix V: Effect of MAP on the Water activity (a_w) of Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	0.975	0.975	0.975	0.975
5	0.970	0.970	0.972	0.970
10	0.966	0.967	0.968	0.968
15	■	0.966	0.968	0.968
20	■	0.963	0.965	0.966

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix VI: Effect of MAP on the Water activity (a_w) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	0.959	0.959	0.959	0.959
15	0.958	0.959	0.958	0.959
30	0.961	0.957	0.958	0.958
45	■	0.957	0.957	0.958
60	■	0.956	0.954	0.957

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix VII: Effect of MAP on the pH of Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	5.80	5.80	5.80	5.80
5	5.63	5.76	5.73	5.75
10	5.52	5.74	5.66	5.72
15	■	5.68	5.59	5.64
20	■	5.67	5.54	5.61

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix VIII: Effect of MAP on pH of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	6.19	6.19	6.19	6.19
15	5.91	6.12	5.97	6.06
30	5.26	6.07	5.91	5.98
45	■	6.01	5.84	5.91
60	■	5.96	5.78	5.83

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix IX: Effect of MAP on the Titratable acidity (% lactic acid) of Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	0.52	0.52	0.52	0.52
5	0.72	0.54	0.70	0.56
10	0.78	0.54	0.75	0.58
15	■	0.56	0.79	0.61
20	■	0.58	0.81	0.62

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix X: Effect of MAP on the Titratable acidity (% lactic acid) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	0.35	0.35	0.35	0.35
15	0.56	0.40	0.46	0.41
30	0.82	0.48	0.51	0.50
45	■	0.52	0.57	0.54
60	■	0.57	0.64	0.61

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XI: Effect of MAP on the Free Fatty acids (% oleic acid) of Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	0.98	0.98	0.98	0.98
5	1.14	1.05	1.12	1.08
10	1.26	1.09	1.19	1.17
15	■	1.18	1.27	1.22
20	■	1.31	1.44	1.40

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XII: Effect of MAP on the Free Fatty acids (% oleic acid) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	0.85	0.85	0.85	0.85
15	0.98	0.89	0.94	0.91
30	1.27	0.95	1.03	0.99
45	■	1.02	1.11	1.07
60	■	1.17	1.38	1.26

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XIII: Effect of MAP on the Peroxide Value (meq/kg of fat) of Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	1.36	1.36	1.36	1.36
5	1.61	1.42	1.46	1.43
10	1.87	1.59	1.72	1.66
15	■	1.82	1.98	1.87
20	■	2.21	2.37	2.28

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XIV: Effect of MAP on the Peroxide Value (meq/kg of fat) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	1.32	1.32	1.32	1.32
15	1.69	1.45	1.56	1.51
30	2.19	1.62	1.73	1.68
45	■	1.78	1.96	1.88
60	■	2.18	2.31	2.25

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XV: Effect of MAP on the Thiobarbituric Acid (TBA) value (Absorbance at 530 nm) of Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	0.023	0.023	0.023	0.023
5	0.047	0.025	0.033	0.031
10	0.066	0.029	0.035	0.035
15	■	0.032	0.041	0.038
20	■	0.034	0.045	0.040

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XVI: Effect of MAP on the Thiobarbituric Acid (TBA) value (Absorbance at 530 nm) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	0.012	0.012	0.012	0.012
15	0.029	0.019	0.021	0.021
30	0.037	0.022	0.028	0.024
45	■	0.027	0.041	0.035
60	■	0.033	0.043	0.037

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XVII: Effect of MAP on the Tyrosine content (mg/100g) of Unbaked Pizza stored at 7±1 ^0C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	13.40	13.40	13.40	13.40
5	24.70	17.35	21.23	20.05
10	35.80	22.71	29.60	27.34
15	■	26.25	32.91	29.50
20	■	29.05	35.76	32.55

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XVIII: Effect of MAP on the Tyrosine content (mg/100g) of Baked Pizza stored at 7±1 ^0C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	11.30	11.30	11.30	11.30
15	26.65	18.90	23.69	22.48
30	34.50	22.41	28.72	25.36
45	■	26.35	32.51	30.55
60	■	31.24	37.90	34.85

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XIX: Effect of MAP on the Lycopene content (mg/100 g) of Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	4.84	4.84	4.84	4.84
5	4.58	4.78	4.75	4.78
10	4.42	4.73	4.68	4.71
15	■	4.66	4.52	4.62
20	■	4.59	4.37	4.48

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XX: Effect of MAP on the Lycopene content (mg/100 g) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	5.04	5.04	5.04	5.04
15	4.69	5.00	4.92	4.97
30	4.53	4.93	4.85	4.89
45	■	4.82	4.76	4.80
60	■	4.71	4.58	4.66

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXI: Effect of MAP on the Colour Profile (L*, a*, b*) of Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package											
	atm 1 air			atm 2 100% CO_2			atm 3 100% N_2			atm 4 50% CO_2 : 50% N_2		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
0	62.87	18.92	35.23	62.87	18.92	35.23	62.87	18.92	35.23	62.87	18.92	35.23
5	60.12	15.29	39.52	62.45	18.13	35.64	62.18	16.46	36.69	62.38	18.12	35.74
10	58.36	14.02	42.16	62.26	18.09	35.89	62.06	16.35	37.56	62.19	18.06	36.12
15	■	■	■	62.10	17.85	36.12	61.90	16.12	38.43	62.03	17.81	37.61
20	■	■	■	62.01	17.77	37.06	61.78	16.15	40.27	61.92	17.65	39.53

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXII: Effect of MAP on the Colour Profile (L*, a*, b*) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package											
	atm 1 air			atm 2 100% CO_2			atm 3 100% N_2			atm 4 50% CO_2 : 50% N_2		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
0	50.59	20.79	36.76	50.59	20.79	36.76	50.59	20.79	36.76	50.59	20.79	36.76
15	47.37	18.85	40.39	50.18	20.34	37.18	50.04	20.18	37.67	49.92	20.21	37.32
30	46.16	18.12	43.15	50.02	20.19	37.85	49.72	19.73	38.56	49.83	19.88	38.23
45	■	■	■	49.76	19.86	38.17	49.63	19.46	39.12	49.69	19.61	38.90
60	■	■	■	48.35	19.24	38.88	49.38	18.45	41.27	49.51	18.89	39.76

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXIII: Effect of MAP on the Hunter Lab Total Colour Difference (ΔE) of Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	52.16	52.16	52.16	52.16
5	52.95	52.19	52.54	52.30
10	53.52	52.36	52.72	52.47
15	■	52.82	53.09	52.96
20	■	53.02	53.35	53.16

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXIV: Effect of MAP on the Hunter Lab Total Colour Difference (ΔE) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	53.07	53.07	53.07	53.07
15	54.13	53.24	53.62	53.39
30	54.68	53.57	53.88	53.74
45	■	53.91	54.42	54.06
60	■	54.08	54.59	54.18

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXV: Effect of MAP on the Relative Yellowness (E*) of Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	2.42	2.42	2.42	2.42
5	3.24	2.54	2.82	2.54
10	3.72	2.56	2.90	2.58
15	■	2.60	3.00	2.72
20	■	2.69	3.14	2.88

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXVI: Effect of MAP on the Relative Yellowness (E*) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	2.50	2.50	2.50	2.50
15	2.99	2.57	2.62	2.60
30	3.31	2.63	2.73	2.69
45	■	2.69	2.80	2.76
60	■	2.82	3.08	2.90

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXVII: Effect of MAP on the Total Plate count (log cfu/g) of Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	6.65	6.65	6.65	6.65
5	7.38	4.97	5.15	5.05
10	7.10	4.81	4.93	4.85
15	■	4.63	4.82	4.72
20	■	3.56	3.61	3.66

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXVIII: Effect of MAP on the Total Plate count (log cfu/g) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	5.58	5.58	5.58	5.58
15	7.12	5.34	5.52	5.41
30	6.68	5.02	5.47	5.25
45	■	5.16	5.36	5.31
60	■	4.85	5.14	5.03

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXIX: Effect of MAP on the Yeast & moulds count (log cfu/g) of Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	2.78	2.788	2.78	2.78
5	3.65	2.29	2.47	2.38
10	3.42	1.78	2.13	1.94
15	■	1.25	1.87	1.56
20	■	0.93	1.47	1.27

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXX: Effect of MAP on the Yeast & moulds count (log cfu/g) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	2.43	2.43	2.43	2.43
15	2.69	2.21	2.33	2.28
30	2.47	2.13	2.26	2.19
45	■	1.87	2.14	2.03
60	■	1.64	2.04	1.81

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXXI: Effect of MAP on the Coliform count (log cfu/g) of Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	2.57	2.57	2.57	2.57
5	3.94	2.34	2.45	2.37
10	3.75	2.27	2.36	2.30
15	■	2.31	2.42	2.39
20	■	2.18	2.33	2.28

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXXII: Effect of MAP on the Coliform count (log cfu/g) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	1.39	1.39	1.39	1.39
15	1.96	1.36	1.43	1.37
30	1.64	1.28	1.37	1.34
45	■	1.29	1.36	1.35
60	■	1.16	1.29	1.25

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXXIII: Effect of MAP on the Total Lactic Acid Bacteria (LAB) count (log cfu/g) of Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	5.02	5.02	5.02	5.02
5	7.73	4.67	4.72	4.76
10	8.14	4.31	4.56	4.49
15	■	4.06	4.39	4.28
20	■	3.75	3.93	3.87

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXXIV: Effect of MAP on the Total Lactic Acid Bacteria (LAB) count (log cfu/g) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	4.09	4.09	4.09	4.09
15	6.36	3.71	3.96	3.82
30	7.81	3.54	3.77	3.65
45	■	3.18	3.56	3.34
60	■	2.87	3.41	3.11

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXXV: Effect of MAP on the Psychrotrophic count (log cfu/g) of Unbaked Pizza stored at 7±1 ^0C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	2.39	2.39	2.39	2.39
5	3.86	3.11	3.35	3.16
10	5.21	3.59	3.92	3.67
15	■	4.85	5.66	5.27
20	■	5.24	6.14	5.58

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXXVI: Effect of MAP on the Psychrotrophic count (log cfu/g) of Baked Pizza stored at 7±1 ^0C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	2.04	2.04	2.04	2.04
15	3.21	2.53	2.81	2.74
30	4.06	3.16	3.76	3.45
45	■	3.89	4.59	4.36
60	■	4.12	5.17	4.67

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXXVII: Effect of MAP on the Anaerobic Spore formers (log cfu/g) of Unbaked Pizza stored at 7±1 ^0C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	2.67	2.67	2.67	2.67
5	3.56	2.73	2.84	2.73
10	3.41	2.95	2.99	2.96
15	■	2.81	2.92	2.87
20	■	2.98	3.18	3.06

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix XXXVIII: Effect of MAP on the Anaerobic Spore formers (log cfu/g) of Baked Pizza stored at 7±1 ^0C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	1.91	1.91	1.91	1.91
15	2.39	2.23	2.37	2.28
30	2.21	2.36	2.48	2.39
45	■	2.29	2.42	2.33
60	■	2.41	2.55	2.47

atm: atmosphere

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

* Poly binding agent

■ Samples spoiled, hence analysis discontinued

Appendix IXL: Effect of MAP on the Hardness (g) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	6621.87	6621.87	6621.87	6621.87
15	14205.82	7582.50	11698.06	9344.95
30	16602.76	8606.27	13744.69	12859.69

atm: atmosphere

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

* Poly binding agent

Appendix XL: Effect of MAP on the Cohesiveness (A2/A1) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)**

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	0.461	0.461	0.461	0.461
15	0.296	0.428	0.366	0.391
30	0.231	0.388	0.314	0.327

atm: atmosphere

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

* Poly binding agent

** A2: +ve area under 2nd peak; A1: +ve area under 1st peak

Appendix XLI: Effect of MAP on the Springiness (mm) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	0.786	0.786	0.786	0.786
15	0.613	0.723	0.683	0.696
30	0.524	0.688	0.590	0.656

atm: atmosphere

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

* Poly binding agent

Appendix XLII: Effect of MAP on the Gumminess (g) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	3000.01	3000.01	3000.01	3000.01
15	4625.36	3060.10	3793.36	3612.12
30	7016.00	4112.81	5212.13	5312.74

atm: atmosphere

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

* Poly binding agent

Appendix XLIII: Effect of MAP on the Chewiness (g x mm) of Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	2330.81	2330.81	2330.81	2330.81
15	3280.75	2526.33	2833.35	2778.08
30	5427.35	3097.48	4228.95	3658.42

atm: atmosphere

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

* Poly binding agent

Appendix XLIV: Mean Hedonic sensory score (based on 5 point scale) for Appearance of MAP Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)**

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	5.0	5.0	5.0	5.0
5	3.2	4.7	4.6	4.7
10	2.6	3.9	3.5	4.1
15	■	3.6	3.0	3.3
20	■	2.8	2.1	2.5

atm: atmosphere
Package used: LLD /BA*/ Nylon-6/ BA*/ LDPE (110 μ)
* Poly binding agent
** Excellent 5; Very Good 4; Good 3; Fair 2; Poor 1
■ Samples spoiled, hence analysis discontinued

Appendix XLV: Mean Hedonic sensory score (based on 5 point scale) for Appearance of MAP Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)**

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	5.0	5.0	5.0	5.0
15	3.3	4.4	4.0	3.9
30	2.7	3.6	3.1	3.4
45	■	3.1	2.7	3.0
60	■	2.5	2.1	2.3

atm: atmosphere
Package used: LLD /BA*/ Nylon-6/ BA*/ LDPE (110 μ)
* Poly binding agent
** Excellent 5; Very Good 4; Good 3; Fair 2; Poor 1
■ Samples spoiled, hence analysis discontinued

Appendix XLVI: Mean Hedonic sensory score (based on 5 point scale) for Flavour of MAP Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)**

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	4.8	4.8	4.8	4.8
5	2.7	3.7	2.8	3.4
10	1.3	3.1	2.3	2.8
15	■	2.6	2.1	2.4
20	■	1.3	1.0	1.1

atm: atmosphere
Package used: LLD /BA*/ Nylon-6/ BA*/ LDPE (110 μ)
* Poly binding agent
** Excellent 5; Very Good 4; Good 3; Fair 2; Poor 1
■ Samples spoiled, hence analysis discontinued

Appendix XLVII: Mean Hedonic sensory score (based on 5 point scale) for Flavour of MAP Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)**

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	4.8	4.8	4.8	4.8
15	2.8	3.9	3.7	3.9
30	1.1	3.4	2.9	3.2
45	■	3.0	2.2	2.6
60	■	1.4	1.1	1.3

atm: atmosphere
Package used: LLD /BA*/ Nylon-6/ BA*/ LDPE (110 μ)
* Poly binding agent
** Excellent 5; Very Good 4; Good 3; Fair 2; Poor 1
■ Samples spoiled, hence analysis discontinued

Appendix XLVIII: Mean Hedonic sensory score (based on 5 point scale) for Body & Texture of MAP Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)**

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	4.6	4.6	4.6	4.6
5	3.1	3.9	3.7	3.7
10	1.3	3.5	2.9	3.1
15	■	3.1	2.1	2.4
20	■	1.3	1.0	1.0

atm: atmosphere
Package used: LLD /BA*/ Nylon-6/ BA*/ LDPE (110 μ)
* Poly binding agent
** Excellent 5; Very Good 4; Good 3; Fair 2; Poor 1
■ Samples spoiled, hence analysis discontinued

Appendix XLIX: Mean Hedonic sensory score (based on 5 point scale) for Body & Texture of MAP Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)**

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	4.6	4.6	4.6	4.6
15	3.1	4.3	3.7	4.0
30	2.2	3.6	3.1	3.4
45	■	3.3	2.5	2.8
60	■	1.6	1.1	1.3

atm: atmosphere
Package used: LLD /BA*/ Nylon-6/ BA*/ LDPE (110 μ)
* Poly binding agent
** Excellent 5; Very Good 4; Good 3; Fair 2; Poor 1
■ Samples spoiled, hence analysis discontinued

Appendix L: Mean Hedonic sensory score (based on 5 point scale) for Overall Acceptability of MAP Unbaked Pizza stored at 7±1 ^{0}C (mean of 3 trials)**

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	4.7	4.7	4.7	4.7
5	2.5	3.6	3.4	3.4
10	1.0	3.3	2.7	2.9
15	■	2.9	2.2	2.6
20	■	1.4	1.0	1.2

atm: atmosphere
Package used: LLD /BA*/ Nylon-6/ BA*/ LDPE (110 μ)
* Poly binding agent
** Excellent 5; Very Good 4; Good 3; Fair 2; Poor 1
■ Samples spoiled, hence analysis discontinued

Appendix LI: Mean Hedonic sensory score (based on 5 point scale) for Overall Acceptability of MAP Baked Pizza stored at 7±1 ^{0}C (mean of 3 trials)**

Period of storage (days)	Treatment given to package			
	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	4.7	4.7	4.7	4.7
15	2.9	4.1	3.6	3.8
30	1.2	3.6	2.8	3.3
45	■	3.1	2.4	2.7
60	■	1.4	1.0	1.2

atm: atmosphere
Package used: LLD /BA*/ Nylon-6/ BA*/ LDPE (110 μ)
* Poly binding agent
** Excellent 5; Very Good 4; Good 3; Fair 2; Poor 1
■ Samples spoiled, hence analysis discontinued

Appendix LII: **SCORE CARD FOR PIZZA**

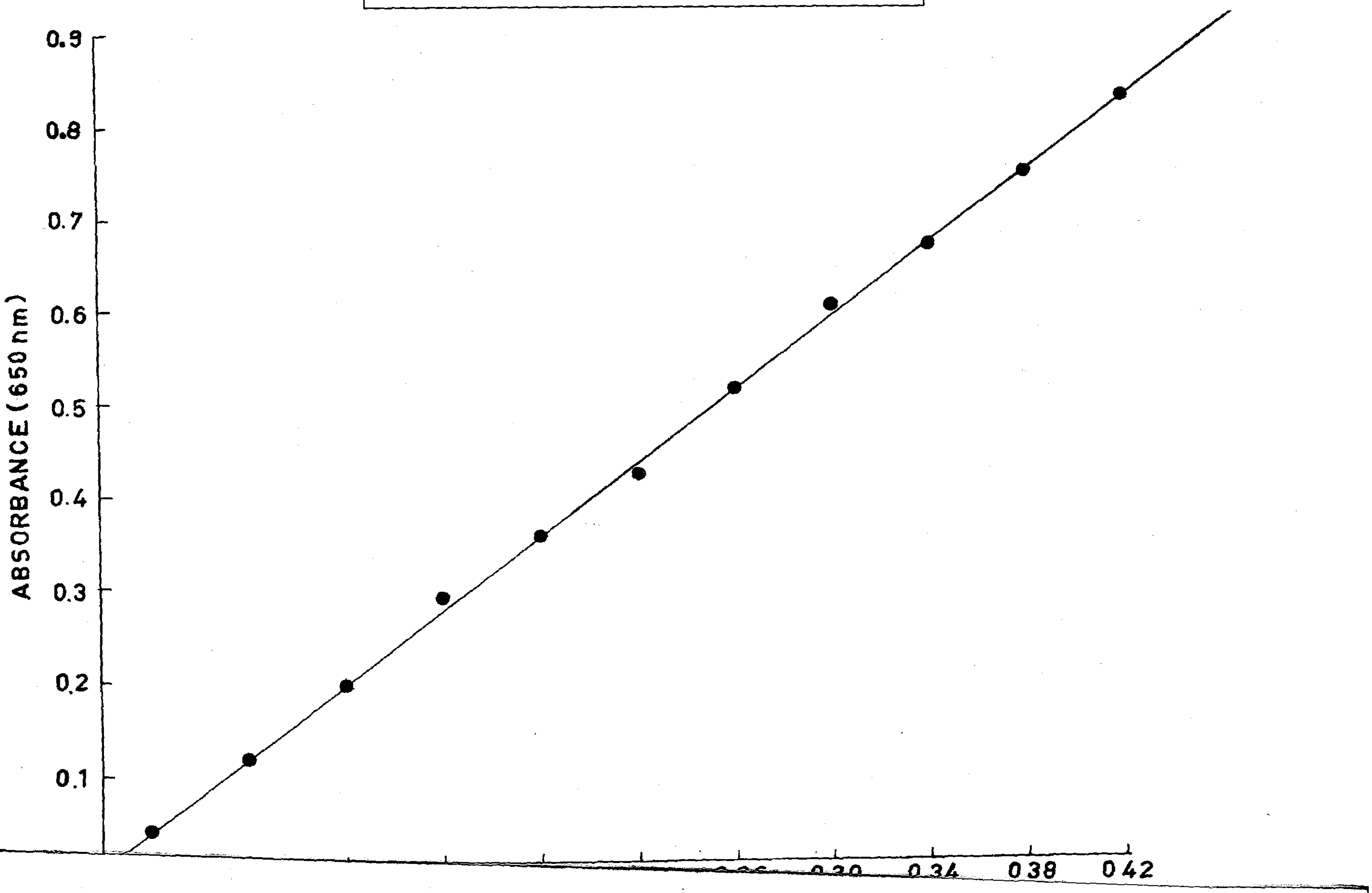
Kindly evaluate the samples of pizza on the basis of scale given below:

Excellent	5
Very Good	4
Good	3
Fair	2
Poor	1

	Appearance	Flavour	Body & Texture	Overall Acceptability
Sample 1				
Sample 2				
Sample 3				
Sample 4				

Signature with Date

Appendix LIII: Standard Curve for Tyrosine



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