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Combined Effect of Refrigeration and Modified Atmosphere Packaging on the Shelf Life of Ready-to-Serve Pizza: Biochemical and Sensory Attributes

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ABSTRACT

This study evaluated the combined effect of Modified Atmosphere Packaging (MAP) and refrigeration (7±1°C) on shelf-life extension of ready-to-serve pizza. Quality assessment was based on sensory and biochemical indices determination. The gaseous atmospheres used were: atm1: air (control); atm2: 100% CO_2, atm3: 100% N_2 and atm4: 50% CO_2/50% N_2. Moisture content, water activity, pH, titratable acidity, free fatty acids, peroxide value, thiobarbituric acid, tyrosine content, lycopene content, color profile (L* = Lightness of the product; a* = Redness of the product; b* = Yellowness of the product; total color difference; relative yellowness) were monitored. Sampling was carried out at predetermined time intervals namely 0, 15, 30, 45 and 60 days. The results indicated that MAP with 100% CO_2 more significantly inhibited the lipolytic oxidation, reduced proteolysis, effectively prevented total acidity (less decrease in pH) and have preservative effect on color indices and hence extended shelf life than did other treatments. Results of the present study show that the limit of sensory acceptability was only reached for the aerobically stored samples somewhat before days 15 of storage. The shelf life of baked pizza samples in conventional air packages was 15 days. However, a significant shelf life increase of 45 days (300% increase) was achieved under modified atmospheres for baked pizza samples.

Key words: Sensory evaluation, chemical quality, lycopene, lipolytic oxidation, water activity

INTRODUCTION

Bakery products are widely consumed and therefore specifically defined requirements for their quality characteristics have been established. Bakery products such as bread, pizza, buns, are characterized by specific water activity (a_w) values, which allow their market ability for a short period of time. Their shelf life is mainly limited by microbial spoilage and staling. After baking, these products are free of viable moulds and bacteria, but some bacterial spores can survive the baking process or contamination can occur before packaging is completed (He and Hoseney, 1990; Pfeiffer *et al.*, 1999; Risch, 1999). Recently, in order to achieve longer shelf life for bakery products, refrigerated conditions were employed to prebaked or not baked doughs, as well as new technologies packaging were investigated (Byrne, 2000; Kohn, 2000).

Modified Atmosphere Packaging (MAP), along with refrigeration, of food to extend its shelf life has been the subject of many investigations in recent years. The technique involves packaging of the product under the atmosphere of various combinations of gases such as carbon dioxide (CO_2), nitrogen (N_2), carbon monoxide (CO), sulphur dioxide (SO_2) etc., the most commonly used and perhaps the most effective being CO_2 with or without other gases. The MAP is used to maintain the product's initial quality for much longer periods and to extend the product's shelf life and retain appeal to consumers (Church and Parson, 1995; Phillips, 1996; Farber, 1991). The MAP technology has been particularly effective in chilled, short shelf life low-acid foods, especially minimally processed and highly perishable or semi-perishable foods (Fabiano *et al*., 2000) and the shelf life of such products is limited in the presence of normal air by the chemical effects of atmospheric oxygen and the growth of aerobic spoilage microorganisms (Parry, 1993; McMillin, 2008).

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. Foods usually spoil as a result of microbial activity, which causes severe changes in chemical and sensory properties (McMillin, 2008). A suitable combination of different gas mixtures is needed for investigation by considering the complex microbial ecology of highly heterogeneous foods, such as pizzas, where different microorganisms contribute to spoilage. The low shelf life of pizza is mainly due to microbial and physico-chemical changes; hence, the aim of this study was to observe the biochemical quality and shelf life of the product (pizza) and the interaction of the product and modified atmospheres after storage under refrigerated conditions.

MATERIALS AND METHODS

Materials

Preparation of ready-to-serve pizza samples and storage conditions: Each experimental unit consisted of one single baked pizza. Pizza bases each of 20 cm in diameter weighing approx. 120±10 g were used for the preparation of pizza samples. The method standardized by Alam and Goyal (2007) was followed for preparation of mozzarella cheese from mixed milk (buffalo: cow:: 60: 40). The cheddar cheese and table butter were collected, in 2005, from the Experimental Dairy, NDRI, Karnal (Haryana). The microwave oven having power output 900 W with internal dimensions of 36×37×23 cm^3 and 32 L capacity from Samsung, South Korea; Model Bio ceramic, CE118KF was used during the study. The tomatoes, capsicum and green chillies used for the preparation of pizza were first thoroughly washed and then dipped in solution of potassium metabisulphite (KMS) (2 g L^{-1}) for 20 min and air-dried. The onions and ginger were, however peeled before dipping in the prepared KMS solution of the same concentration. The pizza samples were prepared by adapting the procedure of Alam and Goyal (2007) 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 green chillies, grated ginger were evenly spread all over the pizza base followed by grated mozzarella and cheddar cheese in the ratio of 80:20 (approx. 120 g per pizza) was topped on pizza base concluded by vegetable toppings (sliced onion, tomatoes and capsicum). 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. For packaging of pizza samples, high barrier bags namely LLD/BA*/Nylon-6/BA*/LDPE (110 µ) (*poly binding agent) were used. The Water Vapour Transmission Rate (WVTR) and Oxygen Transmission Rate (OTR) of the packaging material used was 3.96 g/m^2/24 h and 36 mL/m^2/24 h. The dimensions of the packages used in the study were 32.5×35.0 cm (L×B). Packaging under modified atmospheres was accomplished following the method of Day (1992) by using a vacuum chamber Quick 2000 machine (Alfa-Laval, Kramer, Grebe GmbH and Co. KG Maschinenfabrik, 3560 Biedenkopf-Wallau, Germany), with gas injection after establishing a vacuum of 25"Hg (ca.85 Pa). Packaging under atmosphere (air) was done by using vertical heat-sealing machine, model QS-300 FE. The prepared pizza samples (baked) were individually packed in sterilized (under UV-light for 30 min) packages under different atmospheres (atm 1: air, atm 2: 100% CO_2, atm 3: 100% N_2 and atm 4: 50% CO_2/50% N_2). Initially the gas headspace to pizza weight ratio was approx. Two liter of gas per kg of the product and stored at 7±1°C. This temperature was chosen to stimulate real conditions during distribution and storage at retail outlets. Triplicate samples of each treatment were subjected to a number of analyses at different periods of storage. Unless specified, sampling was interrupted whenever pizzas were rejected by sensory panel. Sampling was carried out at predetermined time intervals namely 0, 15, 30, 45 and 60 days.

Methods

Sampling technique: The samples of ready-to-serve pizza for chemical analyses were prepared 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).

Proximate analysis: Moisture content and total fat were estimated by using the method of IS: SP: 18.Total protein and ash content in pizza samples were determined by AOAC (1995). Carbohydrates were obtained by difference method and food energy value of pizza was calculated from proximate composition by the method recommended by Kamel and Manji (1986).

Determination of water activity (a_w): Water activity determination of the pizza samples at 25°C 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.

Determination of pH and titratable acidity: The pH of prepared sample (filtrate from homogenate) 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, US. Titratable acidity of pizza samples was determined by the method recommended by the Ranganna (2000). The acidity was expressed as percent lactic acid (% LA).

Determination of free fatty acids, peroxide value and thiobarbituric acid (TBA) value: The fat breakdown in pizza samples was determined by estimating Free Fatty Acids (FFA) (% oleic acid) adopting the procedure of Thomas *et al.* (1954). The peroxide value of the pizza sample was determined on the lines detailed in IS: 3508 (1966). The TBA value of the pizza sample was determined by the method prescribed by Pokorny and Dieffenbacher (1989).

Determination of 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. Three gram prepared sample was thoroughly mixed with 10 mL of warm (45°C) distilled water. Five milliliter of the suspension was pipetted in 50 mL conical flask. Then, 10 mL of 0.72 N Trichloroacetic acid and 1 mL of distilled water were added. The mixture was then thoroughly shaken and allowed to stand for 30 min followed by filtration. Five milliliter of this filtrate was added to 10 mL mixture of sodium carbonate and sodium hexametaphosphate solution and 3 mL of diluted Folin-Ciocalteau's reagent (1:2) in Spectrophotometer model number GENESYS10 series supplied by Thermo Spectronic, Rochester, N.Y., USA. The concentration of tyrosine was calculated from the standard curve using L (-) Tyrosine as the standard component.

Determination of lycopene content: For estimation of lycopene content in pizza samples, the method suggested by Ranganna (2000) was followed with slight modifications. Five to ten gram sample was repeatedly extracted with acetone in 100 mL Erlenmeyer flask until the residue became colorless. The acetone extract was then transferred to separating funnel containing 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 colored bottle. Repeated extraction of the acetone phase similarly with petroleum ether until it became colorless. 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 color was measured at 503 nm in a Spectrophotometer model number GENESYS10 series supplied by Thermo Spectronic, Rochester, N.Y., USA using petroleum ether as blank.

Hunter Lab., color values (L* a* b*): The color of the prepared pizza samples was measured by taking multiple readings (to minimize variability) and then calculating the average value, using a Colorflex Model 45°/0° (Hunter Lab., Reston, Virginia, USA) along with the universal software (version 4.10). In a description of instrument geometry, the first number is the angle or method of illumination and the second number is the angle or method of viewing. These are both relative to the perpendicular to the surface of the sample being measured. In an instrument with 45°/0° geometry, the illumination (light surface) shines on the sample at an angle 45° from the perpendicular to the sample surface. The viewer (detector) receives the reflected light at a location of 0° from (in line with) the perpendicular to the sample.

Total color difference (ΔE) and relative yellowness (E*): The total color difference (ΔE) of pizza samples was calculated by the formula as given by Liu *et al.* (2003). Total color difference represents the comprehensive contributions from the variations of three color indices. To enhance the fraction of yellowness relative to those of lightness and redness, relative yellowness (E*) of pizza samples was calculated by the formula recommended by Liu *et al.* (2003).

Sensory analysis: The packed pizza samples were evaluated organoleptically for sensory attributes by a trained panel for appearance, flavor, body and texture and overall acceptability. A quality evaluation was carried out by monitoring inflation of packages, examination for presence of yeasts and molds and changes in the color of pizza components. Before presenting the pizza samples to judges, the stored test samples were reheated in microwave oven for 2 min at 100% power level. The reheating time was based on the amount of time necessary for the product to yield an adequately reheated appearance. A panel of seven judges experienced in baked products evaluation was used for sensory analysis. Panelists were trained for a period of 3 months in 1 h sessions three times a week (36 h total) (Winger and Pope, 1976). Triangle tests were performed in order to select seven panelists who could detect off-flavors in pizza. Prior to sample evaluation, the seven selected panellists participated in orientation sessions to familiarize with the flavor (off-odour, off-taste) and textural attributes of pizza samples. Along with the test pizza samples, fresh baked pizza was used as the reference sample. Overall acceptability as a composite of all sensory attributes (appearance, flavor, body and texture) was estimated using a 5 point hedonic scale ranging from 1-5, where: A score of 5 represented excellent; 4, very good; 3, good; 2, fair and 1, poor. A mean score of 2.5 or above indicates an acceptable product. A mean score below 2.5 marks the end of refrigerated pizza shelf life (Cabo *et al*., 2001).

Statistical analysis: Experiments were replicated twice on different occasions with different ready-to-serve pizza samples. Different packages were sampled on predetermined time intervals. Analysis were run in triplicate for each replicate (n = 2×3). Biochemical data were subjected to Analysis of Variance (ANOVA). Means and standard deviations were calculated and when F-values were significant at the $p<0.05$ level, mean differences were separated by the Least Significant Difference (LSD) procedure (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Proximate analysis: Proximate analysis results (day 0) are shown in Table 1. The results are in close proximity with the values reported by Kamel and Manji (1986) and U.S. Department of Agriculture (2005). 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 100 g^{-1}, revealing that the ready-to-serve pizza had more fat and protein contents with higher food energy.

Effects of different atmospheres on moisture content and water activity (a_w): Moisture content in fresh baked pizza was 43.71% which significantly ($p<0.01$) 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 after 60 days of storage period (Table 2). In general, the results match with

Table 1: Proximate analysis (%) * of fresh ready-to-serve pizza

Composition	Mean value (%)
Moisture	43.71±0.2
Protein	14.63±0.6
Fat	11.98±0.5
Ash	2.98±0.1
Carbohydrates	26.71±0.2
Food energy (cal/100 g)	273.18±0.3

*Based on three trials and represent the mean of six determinations±SD

Table 2: Moisture content* (%) of ready-to-serve pizza stored at 7±1°C

	Treatment given to package			
Period of storage (days)	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	43.71±0.1	43.71±0.1	43.71±0.1	43.71±0.1
15	43.46±0.2	43.62±0.3	43.56±0.1	43.65±0.1
30	43.32±0.3	43.55±0.1	43.38±0.2	43.59±0.4
45	■	43.42±0.1	43.47±0.2	43.48±0.2
60	■	43.31±0.2	43.29±0.3	43.35±0.3

■: Samples spoiled, hence analysis discontinued, *Values represent the mean of six determinations (n = 2×3)±SD (0.1-0.4)

Table 3: Water Activity (a_w) * of ready-to-serve pizza stored at 7±1°C

	Treatment given to package			
Period of storage (days)	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	0.959±0.2	0.959±0.2	0.959±0.2	0.959±0.2
15	0.958±0.3	0.959±0.1	0.958±0.3	0.959±0.1
30	0.961±0.2	0.957±0.1	0.958±0.1	0.958±0.2
45	▪	0.957±0.2	0.957±0.2	0.958±0.3
60	▪	0.956±0.4	0.954±0.5	0.957±0.4

▪: Samples spoiled, hence analysis discontinued, *Values represent the mean of six determinations (n = 2×3)±SD (0.1-0.5)

Table 4: The pH * of ready-to-serve pizza stored at 7±1°C

	Treatment given to package			
Period of storage (days)	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	6.19±0.1	6.19±0.1	6.19±0.1	6.19±0.1
15	5.91±0.2	6.12±0.1	5.97±0.1	6.06±0.2
30	5.26±0.2	6.07±0.2	5.91±0.2	5.98±0.1
45	▪	6.01±0.2	5.84±0.1	5.91±0.1
60	▪	5.96±0.3	5.78±0.2	5.83±0.2

▪: Samples spoiled, hence analysis discontinued, *Values represent the mean of six determinations (n = 2×3)±SD (0.1-0.3)

the findings of Kamel and Manji (1986), who observed that the moisture content of pizza decreased during storage after 12 months at subzero temperatures. Alam and Goyal (2006) also reported loss in moisture content of mozzarella cheese samples packaged under modified atmospheres stored at 7±1°C.

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 those samples. 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 of 0.99. The a_w decreased significantly ($p<0.01$) from 0.959 to 0.956 in case of product packaged under atm 2, 0.954 for atm 3 and 0.957 for atm 4; thus, revealing maximum decrease (0.52%) for atm 3 followed by atm 2 (0.31%) and atm 4 (0.21%) (Table 3). Dharmaveer *et al.* (2007) also reported that storage period significantly decreased the water activity of vacuum packed sausages.

Effects of different atmospheres on pH and Titratable Acidity (TA): The initial pH of untreated pizza on day 0 was 6.19 indicating the freshness of pizza samples (Table 4). During storage at 7±1°C, the pH of 6.19 decreased to 5.26 (corresponding to 15.02% decrease) after 30 days of storage for air-packed samples, while it significantly ($p<0.01$) 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, respectively in ascending order. The observations, agree with the findings of Daifas *et al.* (1999) and Rajkumar *et al.* (2007) that ascribed the reason for decrease in pH to be the dissolution of CO_2 in bakery products and meat products respectively.

In contrast, higher TA (% lactic acid) values were recorded for air-packaged samples after 30 days of storage whereas atm 2, atm 3 and atm 4 packaged samples did not reach this value (Table 5) throughout the 60 days of storage period under refrigeration. After 30 days of storage, atm 2 contributed to significantly lower ($p<0.01$) TA than the atm 3 and air packed pizza samples.

Table 5: Titratable acidity (% lactic acid) * of ready-to-serve pizza stored at 7±1°C

	Treatment given to package			
Period of storage (days)	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	0.35±0.2	0.35±0.2	0.35±0.2	0.35±0.2
15	0.56±0.1	0.40±0.1	0.46±0.1	0.41±0.1
30	0.82±0.1	0.48±0.1	0.51±0.3	0.50±0.1
45	■	0.52±0.2	0.57±0.1	0.54±0.2
60	■	0.57±0.3	0.64±0.1	0.61±0.3

■: Samples spoiled, hence analysis discontinued, *Values represent the mean of six determinations (n = 2×3)±SD (0.1-0.3)

Table 6: Free fatty acids (% oleic acid) * of ready-to-serve pizza stored at 7±1°C

	Treatment given to package			
Period of storage (days)	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	0.85±0.1	0.85±0.1	0.85±0.1	0.85±0.1
15	0.98±0.3	0.89±0.1	0.94±0.2	0.91±0.2
30	1.27±0.1	0.95±0.2	1.03±0.2	0.99±0.1
45	■	1.02±0.1	1.11±0.1	1.07±0.1
60	■	1.17±0.3	1.38±0.3	1.26±0.2

■: Samples spoiled, hence analysis discontinued, *Values represent the mean of six determinations (n = 2×3)±SD (0.1-0.3)

Table 7: Peroxide value (meq kg^{-1} of fat) * of ready-to-serve pizza stored at 7±1°C

	Treatment given to package			
Period of storage (days)	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	1.32±0.2	1.32±0.2	1.32±0.2	1.32±0.2
15	1.69±0.1	1.45±0.1	1.56±0.1	1.51±0.1
30	2.19±0.1	1.62±0.2	1.73±0.2	1.68±0.2
45	■	1.78±0.1	1.96±0.2	1.88±0.3
60	■	2.18±0.2	2.31±0.4	2.25±0.2

■: Samples spoiled, hence analysis discontinued, *Values represent the mean of six determinations (n = 2×3)±SD (0.1-0.4)

The trend in change of TA coincides well with the changes in the value of pH of baked pizza (Table 4, 5). It can be well correlated that the pH decreased from 6.19 to 5.26 (corresponds to TA-0.82), 6.07 (TA-0.48), 5.91 (TA-0.51) and 5.98 (TA-0.50) for atm 1, atm 2, atm 3 and atm 4, respectively after 30 days. As the pH decreased, the total acidity of the product also decreased vice-versa throughout the storage period. Alves *et al.* (1996), Eliot *et al.* (1998) and Alam and Goyal (2006) also concluded that the least increase in TA in samples packed with 100% CO_2 might be due to the fact that CO_2 has bactericidal effect.

Effects of different atmospheres on free fatty acids, peroxide value and thiobarbituric acid value: The content of Free Fatty Acids (FFA), peroxides and hydroperoxides are often used as an indicator of lipid peroxidation, resulting from oxidative stress (Smirnoff, 1995). The FFA and peroxides contents of pizza samples increased rapidly from 0.85 (% oleic acid) 1.36 (meq kg^{-1}) to 1.27 (% oleic acid) 2.19 (meq kg^{-1}) in air packaged samples after 30 days of storage whereas MAP with 100% CO_2 samples never reached these values even after 60 days of storage (Table 6, 7). The increase in FFA and peroxides during storage most probably was due to the enzymatic action in

Table 8: Thiobarbituric acid (TBA) value (Absorbance at 530 nm) * of ready-to-serve pizza stored at 7±1°C

	Treatment given to package			
Period of storage (days)	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2:50% N_2
0	0.012±0.1	0.012±0.1	0.012±0.1	0.012±0.1
15	0.029±0.1	0.019±0.1	0.021±0.1	0.021±0.1
30	0.037±0.2	0.022±0.3	0.028±0.2	0.024±0.3
45	▪	0.027±0.2	0.041±0.2	0.035±0.2
60	▪	0.033±0.1	0.043±0.3	0.037±0.1

▪: Samples spoiled, hence analysis discontinued, *Values represent the mean of six determinations (n = 2×3)±SD (0.1-0.3)

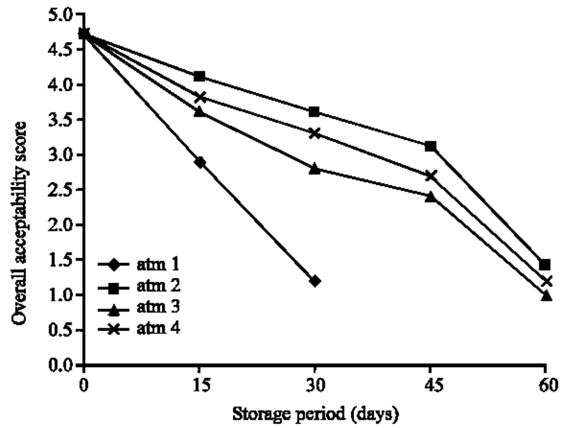

Fig. 1: Changes in overall acceptability scores of chilled ready-to-serve pizza samples packaged in four different atmospheres. Each point is the mean SE (0.1-0.3) of two replicate experiments with three samples analyzed per replicate (n = 6)

presence of oxygen (Labuza and Schmidl, 1985) which might have caused varied degree of lipolysis in pizza samples. Analysis of variance revealed highly significant ($p<0.01$) differences among the atmospheres studied for baked pizza samples.

The thiobarbituric acid (TBA) value is an index of lipid oxidation measuring malondialdehyde (MDA) content. MDA formed through hydroperoxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen (Fernandez *et al.*, 1997). The TBA values for pizza samples are presented graphically in Table 8. Generally, TBA values of air packed samples were significantly higher ($p<0.01$) than those of modified atmosphere packed samples because of available oxygen. The lower TBA values was found to be minimum under 100% CO_2 during storage, which may perhaps be also due to the higher lycopene content in these samples (Table 10) as lycopene has been identified as natural antioxidant (John, 2000; Singh and Goyal, 2008). When TBA values were correlated with results from sensory evaluation, it was observed that the pizza samples with higher TBA values had lower flavour scores (data not shown) and overall acceptability scores (Fig. 1).

Effects of different atmospheres on tyrosine content: When stored at 7±1°C, the concentration of tyrosine, which is the index of proteolysis, was found maximum in air packed pizza samples and minimum for samples packaged with 100% CO_2. The tyrosine content (mg/100 g) differ significantly ($p<0.01$) among all the atmospheres studied after 30 days of storage. The further

Table 9: Tyrosine content (mg 100 g^{-1}) * of ready-to-serve pizza stored at 7±1°C

	Treatment given to package			
Period of storage (days)	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	11.30±0.1	11.30±0.1	11.30±0.1	11.30±0.1
15	26.65±0.2	18.90±0.2	23.69±0.1	22.48±0.3
30	34.50±0.1	22.41±0.2	28.72±0.2	25.36±0.1
45	▪	26.35±0.1	32.51±0.1	30.55±0.2
60	▪	31.24±0.2	37.90±0.4	34.85±0.4

▪: Samples spoiled, hence analysis discontinued, *Values represent the mean of six determinations (n = 2×3)±SD (0.1-0.4)

Table 10: Lycopene content (mg 100 g^{-1}) * of ready-to-serve pizza stored at 7±1°C

	Treatment given to package			
Period of storage (days)	atm 1 air	atm 2 100% CO_2	atm 3 100% N_2	atm 4 50% CO_2 : 50% N_2
0	5.04±0.2	5.04±0.2	5.04±0.2	5.04±0.2
15	4.69±0.1	5.00±0.1	4.92±0.1	4.97±0.1
30	4.53±0.1	4.93±0.4	4.85±0.3	4.89±0.3
45	▪	4.82±0.1	4.76±0.2	4.80±0.4
60	▪	4.71±0.3	4.58±0.5	4.66±0.2

▪: Samples spoiled, hence analysis discontinued, *Values represent the mean of six determinations (n = 2×3)±SD (0.1-0.5)

storage of the samples for 60 days followed the similar trend (Table 9). Most probably 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 are in agreement with the findings of Alam and Goyal (2006), who observed that the proteolysis occurred least in case of cheese samples packed under 100% CO_2.

Effects of different atmospheres on lycopene content and color indices: Lycopene content, the pigment mainly responsible for the characteristic deep red color of ripe tomato fruits and tomato products, in baked fresh pizza samples was found to be 5.04 mg 100 g^{-1} (Table 10) which is higher as compared to fresh unbaked pizza (4.84 mg 100 g^{-1}). The difference in lycopene content of two types of pizza samples may perhaps be due to the fact that cooked tomato products like pizza pack more bioavailable lycopene than raw fruit (Zimmerman, 2002; Singh and Goyal, 2008). After storage of 30 days, the initial value of lycopene content 5.04 decreased significantly ($p<0.01$) 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%) was in samples packaged under 100% CO_2 and the maximum decrease (10.12%) was noted in air-packaged samples, suggesting preservative role of CO_2 towards lycopene content in pizza samples. It is worth mentioning here that the pattern of lycopene content in samples were directly proportional with the redness values (a*) (Table 11) under four types of atmospheres. However, a quite opposite pattern was noticed for the values of b* (yellowness) which increased significantly ($p<0.01$) in all the atmospheres (Table 11). The initial value of L* and a* decreased significantly ($p<0.01$) from 50.59 to 46.16 and 20.79 to 18.12 while b* increased from 36.76 to 43.15 in air-packaged samples (data not shown). The results, in general, match with the observations of Fu and Labuza (1993) and Kaya and Aksu (2005), who observed decrease in red color during storage of frozen pizza and sliced sucuk (dry fermented sausage).

Table 11: Hunter lab., color values (L* a* b*) * of ready-to-serve pizza stored at 7±1°C

Storage period (days)	Treatment given to package								
	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*
0	50.59±0.2	20.79±0.3	36.76±0.3	50.59±0.2	20.79±0.3	36.76±0.3	50.59±0.2	20.79±0.3	36.76±0.3
15	50.18±0.3	20.34±0.2	37.18±0.6	50.04±0.2	20.18±0.5	37.67±0.6	49.92±0.1	20.21±0.5	37.32±0.6
30	50.02±0.6	20.19±0.3	37.85±0.2	49.72±0.3	19.73±0.3	38.56±0.5	49.83±0.2	19.88±0.6	38.23±0.2
45	49.76±0.2	19.86±0.1	38.17±0.5	49.63±0.2	19.46±0.2	39.12±0.6	49.69±0.6	19.61±0.2	38.90±0.4
60	48.35±0.5	19.24±0.5	38.88±0.3	49.38±0.2	18.45±0.6	41.27±0.4	49.51±0.6	18.89±0.1	39.76±0.3

*Values represent the mean of six determinations (n = 2×3)±SD (0.1-0.6)

Table 12: Hunter lab., total color difference (ΔE) * of ready-to-serve pizza stored at 7±1°C

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±0.1	53.07±0.1	53.07±0.1	53.07±0.1
15	54.13±0.3	53.24±0.2	53.62±0.2	53.39±0.3
30	54.68±0.1	53.57±0.3	53.88±0.3	53.74±0.5
45	▪	53.91±0.1	54.42±0.1	54.06±0.1
60	▪	54.08±0.4	54.59±0.2	54.18±0.4

▪: Samples spoiled, hence analysis discontinued, *Values represent the mean of six determinations (n = 2×3)±SD (0.1-0.5)

Table 13: Relative yellowness (E*) * of ready-to-serve pizza stored at 7±1°C

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±0.1	2.50±0.1	2.50±0.1	2.50±0.1
15	2.99±0.1	2.57±0.2	2.62±0.1	2.60±0.2
30	3.31±0.2	2.63±0.1	2.73±0.3	2.69±0.1
45	▪	2.69±0.1	2.80±0.2	2.76±0.3
60	▪	2.82±0.2	3.08±0.2	2.90±0.4

▪: Samples spoiled, hence analysis discontinued, *Values represent the mean of six determinations (n = 2×3)±SD (0.1-0.4)

Since, the appearance, which also includes the color, is one of the primary sensory factors, the effect of MAP on total color difference (ΔE) on pizza samples was calculated (Table 12) as the cumulative sum of all three color indices (L* a* b*). When the baked pizza samples were stored for 60 days under atm 2, atm 3, atm 4, the initial ΔE value increased significantly ($p<0.05$) from 53.07 to 54.08 in atm 2, 54.59 in atm 3 and 54.18 for atm 4, thus, revealing the beneficial effects of CO_2. The results, in principle, are in harmony with the observations of Nanke *et al*. (1998, 1999), who reported the color changes due to package environment. Similar pattern was observed for value of relative yellowness (E*) (the fraction of yellowness relative to those of redness and lightness) in gas-packed samples (Table 13) and the increase had been maximum in case of samples followed by 50% CO_2/50% N_2 and 100% CO_2 respectively. The analysis of variance show a significant influence ($p<0.05$) of different atmospheres on value of E* for baked pizza samples.

Effects of different atmospheres on sensory analysis: The results of the sensory evaluation (appearance, flavour and body and texture attributes) of the reheated pizza samples are presented as overall acceptability scores (Fig. 1). Combined scores for appearance, flavour and body and texture showed a similar pattern of decreasing acceptability (individual results not shown). The overall acceptability of reheated 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 2.9 and 1.2, respectively, after 15 and 30 days of storage, indicating that the air packed baked (ready-to-serve) pizza samples were acceptable only upto 15 days. At the end of 60 days of storage, none of the sample was acceptable under all the 3 modified atmospheres (atm 2, atm 3, atm 4), but were acceptable only upto 45 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. 1). All pizza samples received higher scores during the first 15 days, while after this period significant differences ($p<0.01$) were observed in sensory scores between air and modified atmosphere packed samples. The limit of overall acceptability (score 2.5) was reached somewhat around day 15 (air samples) and day 45 (atm 3 samples), while atm 2 and atm 4 samples never reached this limit within 45 days of the experiment (Fig. 1). Overall acceptability data (Fig. 1) of air and MA-packaged pizza samples correlated rather well with TPC data (data not shown). In general, the results are in agreement with the findings of Maniar *et al.* (1994), Alves *et al.* (1996) and Alam and Goyal (2007), who also observed that 100% CO_2 atmosphere best maintained the sensorial characteristics of the product. Baked pizza samples were better preserved under atm 2 and atm 4 maintaining acceptable odour/taste attributes even on final day of storage.

CONCLUSION

In order to determine the shelf life of MAP 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-serve pizza significantly increased upto 45 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 (15 days), as judged by both sensory and biochemical analysis.

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