The Extension of the Shelf Life of Ready-to-Serve Pizza by a Combination of Modified Atmosphere Packaging and Refrigeration

Preeti SINGH^{1*}, Ali Abas WANI² and Gyanendra Kumar GOYAL³

¹ Chair of Food Packaging Technology, Technical University of Munich, Weihenstephaner Berg, 85354 Freising, Germany

² Department of Food Technology, Islamic University of Science and Technology, Awantipora, Jammu and Kashmir, India

³ Food Packaging Lab, Dairy Technology Division, National Dairy Research Institute, Karnal (Haryana), India (132001)

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This study evaluated the effect of modified atmosphere packaging on shelf-life extension of ready-to-serve pizza stored at 7±1°C using microbiological and sensory analysis. The gaseous atmospheres (atm) used were: atm 1: air (control); atm 2: 100% CO₂, atm 3: 100% N₂ and atm 4: 50%CO₂/50%N₂. Total plate count, yeasts/molds, coliforms, lactic acid bacteria, psychrotrophs and anaerobic spore formers were monitored. Sampling was carried out at predetermined time intervals namely 0, 15, 30, 45 and 60 days. Results of the present work show that the limit of sensory acceptability was only reached for the aerobically stored samples somewhat before days 15 of storage. However, a significant shelf life increase of 45 days (300% increase) was achieved under modified atmospheres for baked pizza samples. From the present study it can be concluded that amongst four atmospheres examined, atm 2 (100% CO₂) was best, followed by atm 4 > atm 3 > atm 1 respectively, in descending order.

Keywords: modified atmosphere packaging, pizza, microbiological quality, sensory evaluation, shelf life

Introduction

Fresh foods are increasingly preferred to frozen foods. Changes in distribution patterns and the demand for increased food quality have resulted in a desire to improve the shelf life of food products. 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 marketability 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 *et al.*, 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) 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 (CO₂), nitrogen (N₂), carbon monoxide (CO), sulphur dioxide (SO₂), etc.; the most commonly used and perhaps the most effective being CO₂ with or without other gases. MA packaging has been shown to protect food products against microbiological deterioration and flavour and colour defects (McMillin, 2008).

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). 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). 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 CO₂ extends the lag phase of bacterial growth and slows the propagation of bacteria, while low O₂ favours mesophilic microbes such as *Listeria* and

*To whom correspondence should be addressed.

E-mail: preeti_ndri@rediffmail.com

lactic acid bacteria (Brackett, 1996). It has been observed that 20-30% CO₂ or even 10% CO₂ was sufficient to retard the bacterial growth (Seideman, 1984) and higher levels of CO₂ were found to be more effective in inhibiting the growth of *Staphylococcus aureus*, *Salmonella*, *Escherichia coli*, and *Yersinia enterocolitica*. Wang (1979) treated fresh broccoli with CO₂ and storage at 5°C found a slower loss of chlorophyll and ascorbic acid and a retardation of mold growth. Christopher *et al.* (1980) reported that psychotropic bacterial counts of pork loins stored in 40% CO₂ + 60% N₂ at 13°C were significantly lower than those of vacuum packaged loins.

Foods usually spoil as a result of microbial activity, which causes severe changes in chemical and sensory properties. 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 microbiological 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

Preparation of ready-to-bake 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 (2004) was followed for preparation of mozzarella cheese from mixed milk (buffalo: cow: 60: 40). The vegetables used for the preparation of pizza were first thoroughly washed and then dipped in solution of potassium metabisulphite (2g/L) for 20 min and air-dried. The pizza samples were prepared by adapting the procedure of 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 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 (110µ) (*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/m²/24 h and 36 ml/m²/24 h. The dimensions of the packages used in the study were 32.5 × 35.0 cm (LxB). 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 & 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% CO₂, atm 3: 100% N₂ and atm 4: 50% CO₂ / 50% N₂). Initially the gas headspace to pizza weight ratio was approx. 2 litre of gas per kg of the product. Unless specified, sampling was interrupted whenever pizzas were rejected by sensory panel.

Microbiological analysis The pizza samples for microbiological analyses were prepared (opened the packages aseptically) by following the procedure as recommended by Labuza and Schmidl (1985), i.e. by mixing whole pizza sample (cut randomly into small pieces) followed by transferring to a stomacher bag (Seward Model, UK), containing 90 ml of sterile Ringer's solution, and homogenised using a stomacher (Lab-Blender 400, Seward Medical, UK) for 60s at room temperature to obtain a representative sample (homogenate). Subsequently, for microbial enumeration, homogenates were serially diluted in Ringer's solution. For total plate count (TPC), the plates were prepared, inoculated and incubated at 30°C for 48 h following the pour plate method (APHA, 1985). Yeast and molds (Y&M) 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 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). In the pizza samples, the total presumptive coliforms were counted on violet red bile agar. The plates after inoculation were incubated at 37°C for 24 h (APHA, 1985). For the selective enumeration of total lactic acid bacteria (LAB) count, MRS agar was used in the study. The inoculated plates were incubated at 37°C for 2 days (APHA, 1985). The psychrotrophs were determined on plate count agar in fresh as well as stored pizza samples. The plates were incubated at 4°C for 10 d (APHA, 1985). For determining anaerobic spore form-

ers, the method of Rao (1991) was employed by using plate count agar procured from HIMEDIA, Mumbai, India. After inoculation, the surface of medium was layered with sterile agar to maintain anaerobic conditions and then incubated in anaerobic jars. All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium.

Chemical analysis Relevant chemical analysis was also performed to measure the food quality during storage. pH of the 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). 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 Ranganna (2000).

Sensory evaluation The packed pizza samples were evaluated organoleptically for sensory attributes by a trained panel for appearance, flavour, body & 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 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-flavours in pizza. Prior to sample evaluation, the seven selected panellists participated in orientation sessions to familiarize with the flavour 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, flavour, body & 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. Analyses were run in triplicate for each replicate ($n = 2 \times 3$). Microbiological data were transformed into logarithms of the number of colony forming units (cfu/g) and 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

Microbiological changes The present study focussed on the monitoring of the following species of micro-organisms: TPC, LAB, coliforms, psychrotrophs, Y&M, anaerobic spore formers. The values for TPC, coliforms and Y&M were in the range as reported by Kamel and Manji (1986); Fasano and Gallo (2001); Donnelly (2002); CFS (2007) for baked and refrigerated pizza samples (Fig. 1a, c and e). Initial TPC reached the value of 6.68 cfu/g, which is considered as the unsatisfactory limit for fresh pizza as defined by CFS (2007) ca. on days 30 of storage (air packaged samples). The atm 2, atm 3 and atm 4 packaged samples did not reach this value throughout the 60 days of storage period under refrigeration. After 30 days of storage, the atm 2 contributed to significantly lower ($P < 0.01$) TPC count than the atm 3 and air pizza samples. This is a result of an extension of lag phase of growth, and a decrease in the growth rate during logarithmic phase (Farber, 1991). The results are in agreement with the findings of Scott and Smith (1971), who investigated the effect of CO₂, N₂ and air atmospheres on the shelf life of cottage cheese and concluded that CO₂ slightly decreased the bacterial count, but N₂ 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); Alam (2004), who while working on MAP of mozzarella cheese observed that CO₂ had bactericidal effect

Of the facultative anaerobic bacterial species, LAB constituted part of the natural microflora of baked pizza samples stored in air and under MAP (Fig. 1b) probably originating from pizza ingredients such as cheese (Eliot *et al.*, 1998) and tomato paste (Villari *et al.*, 1994). Coliforms also found in the pizza samples but to a lesser degree may be due to cross contamination and their contribution is significant only for samples stored under aerobic conditions (Fig. 1c). Of all bacterial species, LAB was a dominant bacterial species and constituted a major part of the microbial association of unbaked pizza product irrespective of the packaging conditions (Fig. 1b). Initial (day 0) LAB counts determined were high, 4.09 log cfu/g, decreasing progressively with storage time attaining final counts of ca. 3.54-3.77 log cfu/g for atm 2,

Fig. 1. Changes (log cfu/g) in total plate count (a); lactic acid bacteria (b); coliforms (c); psychrotrops (d); yeasts and molds (e); and anaerobic spore formers (f) of chilled ready-to-serve pizza samples packaged in four different atmospheres. Each point is the mean SE (0.1-0.4) of two replicate experiments with three samples analyzed per replicate ($n=6$).

atm 3 and atm 4 gas packaged samples after 30 d of storage. Air-packaged samples attained final values of 7.81 log cfu/g. Cabo *et al.* (2001) also observed lower LAB counts in pizzas samples under 90% CO₂ compared to 20% CO₂, when stored at 7±1°C. The lactic acid bacteria (LAB) count of pizza samples closely followed the mean sensory score for flavour (data not shown) in the corresponding atmospheres. Interestingly,

slightly higher counts ($P<0.05$) of coliforms were recorded in air-packaged pizza samples as compared to other modified atmospheres throughout the entire storage period (Fig. 1c). The lower coliform counts (1.16 log cfu/g) were observed in samples packaged with CO₂ after 60 days of storage period. 100% CO₂ was found to be the most effective. The possible reasons for decrease in coliform count during storage might

be the effect of CO₂ by alteration of permeability of cell of membrane and enzymatic reaction pathways (Enfors *et al.*, 1978; Kamel and Manji, 1986, Rosenthal *et al.*, 1991) and also may be due to the inherent sensitivity of gram-negative bacteria to extrinsic factors such as a_w , pH, etc. (Holzapfel, 1998) (results not shown).

Of the remaining bacterial species determined, both psychrotrophs and yeasts/molds although strictly aerobic were also found in the microbial association of the baked pizza; however, significantly higher ($P<0.05$) were recorded for pizza samples stored in air (atm 1) than under atm 2, atm 3 and atm 4 samples throughout the entire storage period under refrigeration (Fig. 1d and e). The results confirmed the earlier findings of Cabo *et al.* (2001) who reported lower yeast counts in pizzas when stored at $7\pm1^\circ\text{C}$ under 90% CO₂ compared to 20% CO₂. It is now well established that atmospheres containing CO₂ either alone or in combination with nitrogen delay the growth of typically aerobic spoilage microflora by inhibiting the growth of aerobic bacteria like psychrotrophs and yeast/molds. The inhibitory effect of CO₂ has also been observed by Chen and Hotchkiss (1991); Rosenthal *et al.* (1991); Day (1992); Fedio *et al.* (1994); Alves *et al.* (1996); Alam (2004). Initial (day 0) counts for both these species were low ($< 3 \log \text{cfu/g}$) and increased progressively with storage time for air-packaged pizza samples attaining final counts of 2.47–4.06 $\log \text{cfu/g}$ after 30 days of storage. For psychrotrophs, counts of atm 2, atm 3 and atm 4 samples remained low ($< 4 \log \text{cfu/g}$) throughout 30 days of storage and attained ($< 6 \log \text{cfu/g}$) on final 60th day due to prolonged refrigerated storage (Fig. 1d). The higher the CO₂ concentration in the MAP gases, the higher the inhibition recorded. The suppression of aerobic bacteria i.e. psychrotrophs using MAP can be beneficial in the sense that the end products of LAB are relatively less offensive as compared to the typical spoilage odours produced by psychrotrophs. The work of Eliot *et al.* (1998) on shredded mozzarella cheese under modified atmosphere also indicated that initial psychrotrophic count 4.36 $\log \text{cfu/g}$ increased to 7.00 $\log \text{cfu/g}$ 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 CO₂ atmospheres in case of refrigerated pizza. Finally, the counts of anaerobic spore formers were also low ($< 3 \log \text{cfu/g}$) and show increasing trend in all pizza samples with anaerobic conditions and slight decrease in air packed pizza samples throughout the entire storage period (Fig. 1f). 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 atmo-

spheric pressure 100% CO₂ could delay toxin production by *Cl. sp.* when compared with an atmosphere of 100% N₂. However, Smoot and Pierson (1982) reported that CO₂ had little effect on germination and toxigenesis of spore formers such as *Clostridium botulinum*. Of the microbial associations developed in pizza samples under aerobic and modified atmospheric storage, yeast/molds were found to be a member, and along with LAB, psychrotrophs and to lesser extent coliforms, play a significant role in spoilage of baked pizza.

Chemical analysis The initial pH of untreated pizza on day 0 was 6.19 indicating the freshness of pizza samples. During storage at $7\pm1^\circ\text{C}$, the pH of 6.19 decreased to 5.26 (corresponding to 15.02% decrease) after 30 d 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% N₂ followed by 50%CO₂/50%N₂, respectively in ascending order after 60 days of storage. 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 CO₂ in bakery products and meat products respectively. In contrast, higher TA (% lactic acid) values were recorded for air-packaged samples after 30 d of storage whereas atm 2, atm 3 and atm 4 packaged samples did not reach this value (Fig. 2a) throughout the 60 d of storage period under refrigeration. 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 d. 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% CO₂ might be due to the fact that CO₂ has bactericidal effect.

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). FFA and peroxides contents of pizza samples increased rapidly from 0.85 (% oleic acid) – 1.36 (meq/kg) to 1.27 (% oleic acid) – 2.19 (meq/kg) in air packaged samples after 30 d of storage whereas MAP with 100% CO₂ samples never reached these values even after 60 days of storage (Fig. 2b, c). The increase in FFA and peroxides during storage most probably was due to the enzymatic action in 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. Generally, FFA and peroxide values of air packed samples were significantly higher ($P<0.01$) than those of modified atmosphere packed

Fig. 2. Biochemical changes in titratable acidity (% lactic acid) (a); free fatty acids (% oleic acid) (b); peroxide value (meq/kg) (c) of chilled ready-to-serve pizza samples packaged in four different atmospheres. Each point is the mean SE (0.1-0.4) of two replicate experiments with three samples analyzed per replicate ($n=6$).

samples because of available oxygen. When these values were correlated with results from sensory evaluation, it was observed that the pizza samples with higher FFA and peroxide values had lower flavour scores and overall acceptability scores (Fig. 3).

Sensory analysis The results of the sensory evaluation

Fig. 3. 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 ($n=6$).

(appearance, flavour and body & texture attributes) of the reheated pizza samples are presented as overall acceptability scores (Fig. 3). Combined scores for appearance, flavour and body & 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 d of storage, indicating that the air packed baked (ready-to-serve) pizza samples were acceptable only upto 15 d. This limit (a score of 2.5) coincided with high TPC and LAB counts (>7 log cfu/g) (Fig. 1a and b) and apparent growth of yeasts/molds on the pizza samples. At the end of 60 d 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. 3). All pizza samples received higher scores during the first 15 d, 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 d of the experiment (Fig. 3). Overall acceptability data (Fig. 3) of air and MA-packaged pizza samples correlated rather well with TPC data (Fig. 1a). In general, the results are in agreement with the findings of Maniar *et al.* (1994), Alves *et al.* (1996) and Alam (2004) 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% CO₂, 100% N₂, and 50% CO₂ / 50% N₂) 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% CO₂ (atm 2), 50% CO₂ / 50% N₂ (atm 4) and 100% N₂ (atm 3), compared to conventional air pack (15 days), as judged by both sensory and microbiological analysis.

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