

## Prolonging of Shelf Life of Spring Rolls Pastry by Ozone Treatment and Modified Atmosphere Packaging

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### ABSTRACT

Spring roll consists of a remarkably thin pastry which widely consumed by consumers both domestic and abroad after filling. There is a problem left to be solved on quality of stored spring rolls pastry; inhibition of common mold and bacteria species causing spoilage during storage, where spring roll is recognized as a perishable commodity. This study compared three treatments for this target; packaging the rolls in 0.2% sodium metabisulphite (w/w) for pastry, packaging the rolls in modified atmosphere packaging (100% N<sub>2</sub>). Packaging the rolls in ozone gaseous (7.0 ppm), all with respect to air packaging samples. Microbiological analysis, and sensory attributes were checked at 10 days intervals up to 60 days on samples stored at 4°C. Moreover, the effect of water activity  $a_w$  and pH values on Mold & Yeast growth rate at 25°C after one week of storage was studied on air packaging spring roll pastry samples. The results indicated that microbial growth was prevented in ozone – treated samples up to 50 days of cold storage for Molds & Yeasts and for 60 days for coliforms, staphylococci, Psych., An., and ASFB. Also sensory evaluation indicated significant improvement in color, texture, order, freshness. Ozone-treated samples exhibited higher tenacity and whiteness as well as lower yellowness in the same ozone-treated samples which achieved the highest sensory hedonic scores. The results of this study emphasize the importance of combining several hurdles such as  $a_w$  and pH, that have synergistic or additive effects on the inhibition of yeast & mold growth. The optimum growth was found at high pH 6.5 ( $4.8 \times 10^6$  cfu/g) and at low  $a_w$  0.75 ( $2.0 \times 10^6$  cfu/g) in air packaged samples after one week storage at 25°C of fresh samples. Therefore if the  $a_w$  of pastry samples is lower than 0.75 and its pH value higher than 6.5, these products cannot spoiled by fungal for a long time.

**Key words:** Bakery products, spring roll pastry, water activity, spoilage, MAP, ozone gaseous.

### Introduction

Bakery products are a valuable source of nutrients in our diet providing us with most of our food calories and approximately half of our protein requirements. Variety breads and other bakery products have increased in sales volume within the last decades. Bakery industry is the largest of the food industries. Microbiological spoilage, in particular mould growth is the major economic importance that limit the shelf life of both high and intermediate-moisture bakery products (Guynot *et al.*, 2003). Mold spoilage is still a major problem limiting the shelf life of many bakery products losses due to mold spoilage have been resulting in lost revenue to the baking industries. (Saranraj and Geetha, 2012).

Many traditional preservation methods are based on the application of different hurdles that act synergistically to inhibit or retard microbial growth (Leistner, 1992). pH and water activity ( $a_w$ ) are among the most common variables used to ensure the microbial stability of a particular foodstuff. Also, the addition of weak organic acids, such as sorbic, propionic, and benzoic acids, is widely used in the preservation of food products (Chirife and Favetto, 1992). However, it is well known that the antimicrobial activity of these acid depends on their undissociated molecule. (Suhr and Nielsen, 2004).

Propionic acid inhibits moulds and Bacillus spores, but not yeasts to the same extent, and has therefore been the traditional choice for bread preservation. Sorbic acid is considered to be more effective than propionic acid. It inhibits both moulds and yeasts, and is used in a broad variety of food products, including fine bakery products, confectionary and bread. According to the European

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Parliament and Council Directive No. 95/2/EC, propionic and sorbic acid may be added to bakery wares in concentrations up to 3000 and 2000 ppm, respectively. Benzoic acid is allowed in concentrations of up to 1500 ppm (European Union, 1995).

The preservatives are often added as a salt of the acid because salts are more soluble in aqueous solution. The effectiveness of the preservatives is dependent on the pH of the product, as the antimicrobial effect of the undissociated acid is much stronger than the dissociated acid. Maximum pH for activity is around 6.0 – 6.5 for sorbate, 5.0 – 5.5 for propionate and 4.0 – 4.5 for benzoate. (Suhr and Nielsen, 2004).

Consumers today are not in favour of additives as preservatives and an urge to reduce the quantities used exists within the bakery industry (Membre *et al.*, 2001). Reduction of preservatives to sub-inhibitory levels has nevertheless been shown to stimulate growth of spoilage fungi in some cases or/and stimulate mycotoxin production (Marin *et al.*, 1999).

A more recent and increasingly popular way of preserving foods is controlled atmosphere storage or modified atmosphere packaging. These methods take advantage of a combination of the inhibitory effects of low oxygen levels and elevated carbon dioxide levels on many deterioration processes in foods and are effective in preventing microbial spoilage (Wang *et al.*, 2016).

The three major gases used in commercial modified atmosphere packaging (MAP) are oxygen (O<sub>2</sub>), nitrogen (N<sub>2</sub>), and carbon dioxide (CO<sub>2</sub>) (Davies, 1995). Nitrogen is an inert, tasteless gas that displays little or no antimicrobial activity on its own. Because of its low solubility in water, the presence of N<sub>2</sub> in MAP food can prevent packages from collapsing for products that can absorb CO<sub>2</sub>. Anna *et al.*, (2016) showed that the use of 70/30 N<sub>2</sub>/CO<sub>2</sub> ratio for packaging of fresh filled pasta resulted in a shelf life extension up to 42 days and quality was maintained as well during the whole storage period, while air packaged samples spoiled after 14 days of storage. Modified atmosphere (MAP) resulted, thus, in a threefold of the shelf life of fresh filled pasta. CO<sub>2</sub> is the most important gas in the gas mixture; it is both bacteriostatic and fungi static. Its preserving effect varies with concentration, incubation temperature, organism, and the water activity (a<sub>w</sub>) of the medium which the reduction in the a<sub>w</sub> level increased the inhibitory effects of high levels of CO<sub>2</sub> on the germination and mycelium growth of moulds. The interaction between CO<sub>2</sub> and O<sub>2</sub> levels were the most significant factors affecting mould growth, for the attainment of long shelf lives. The levels of residual O<sub>2</sub> must be kept below 1% (Anna *et al.*, 2016).

The beginning use of ozone as an anti-microbial agent and to control biological growth of unwanted organisms in products, is in the early 1900's. The potential utility of ozone to the food industry lies in the fact that ozone is 52% stronger than chlorine and has been shown to be effective over a much wider spectrum of microorganisms than chlorine and other disinfectants. (Nath *et al.*, 2014). Complementing the effectiveness, is the fact that ozone, unlike other disinfectants, leaves no chemical residues and degrades to molecular oxygen upon reaction or natural degradation (Charles *et al.*, 2015).

Ozone has a high oxidizing potential of 2.07 V compared to chlorine (1.36 V) and oxygen (1.23 V) (Pehkonen, 2001). The high oxidizing power of ozone makes it a strong disinfecting agent with high-power germicidal properties that can inactivate a wide variety of pathogenic organisms including bacteria, viruses, and protozoa. Ozone can be used directly in foods to inactivate microorganisms, leading to increase in shelf life (Bermudez and Barbosa, 2013).

A major number of scientific papers has been published on different bakery products, while less can be found on spring rolls and wrappers pastry, only one paper has dealt on improve the quality of processing characteristics of frozen spring roll wrapper (Wang *et al.*, 2014). The authors developed a basic formulation to improve the hardness and tensile properties of spring roll wrappers during the frozen storage without exposure to fungal contamination that limit its shelf life.

Spring rolls are a traditional festive food, consists of a filling surrounded by a remarkably thin pastry that is subjected to tension as it is wound around the filling. Spring roll pastry is made from dough composed of wheat flour, water and salt. However, there is a serious and costly problem left to be solved on short shelf life of stored spring rolls due to the fungal contamination.

Xerophilic fungi are the most common microbiota causing spoilage of bakery products. Eurotium, Aspergillus, and Penicillium are the main spoilage fungi in Spanish bakery products (Guynot *et al.*, 2005). Eurotium species are usually the first fungi to colonize improperly dried, stored commodities, and when they grow, they increase the level of available water allowing other species

(e.g. *Aspergilli* and *Penicillia*) to thrive and spoil bakery products. *Eurotium* spp. do not produce any significant mycotoxins, but several species of *Aspergillus* and *Penicillium* produce mycotoxins (Abellana *et al.*, 2001).

The objective of this study was: (1) to investigate inhibition of spoilage organisms of spring roll pastry for enhance its quality and extend the shelf life. (2) to examine three systems and definition the adequate for this target, included:

- I- Food preservatives.
- II- Modified atmosphere packaging.
- III- Ozone treatment packaging.

(3) to study the effect of water activity ( $a_w$ ) and pH values on the level of fungi and yeasts growth.

## **Materials and Methods**

### *Specimens:*

Fresh automated spring roll pastry samples were prepared through a locally factory. The samples were divided to four groups; Air packaged samples, Sodium metabisulphite (0.2%) packaged samples, modified atmosphere packaged samples (100%N<sub>2</sub>) and 7 ppm (7.0 mg/kg) ozone-treated packaged samples.

### *Preparing modified atmosphere and ozone-treated packaged samples:*

Two openings were done at the two corresponding ends of the package. One of the opening was used an inlet for gas injection (N<sub>2</sub> or O<sub>3</sub>), and the other opening was used an outlet. Flushing was repeated 3– 4 times to make complete replacement of the internal atmosphere, then the outlet hole was sealed and the gases injected in packages before sealing the inlet hole.

### *Production of ozone:*

Ozone generated by bubbling gaseous ozone into packages. Ozone gas was produced using an ozone generator machine (Healthy life AK-103 ozonizer, 69m – equipment designed by Exponent, Egypt).

All refrigerated samples were checked at 10 days intervals up to 60 days for evaluate the quality and shelf life of the samples.

### *Samples analysis:*

#### *Chemical-physical analysis:*

##### *pH values:*

pH was determined with a pH-meter (Orion 710A, Thermo Scientific, Waltham, USA) fitted with an Ag/AgCl and POLISOLVE (reference) electrode for solids and semi-solids (Mod, Double Pore, Hamiltom, Reno, USA).

##### *Water activity ( $a_w$ ):*

$a_w$  was measured with an electronic hygrometer (Aw-Win, equipped with a Karl-Fast probe, Rotronic, Switzerland), calibrated in the rang 0.1–0.95 with solutions of LiCl of known activity (Anna *et al.*, 2016).

##### *Microbiological analysis:*

Ten grams of sample were added to 90 ml. sterile 0.1% peptone solution and homogenized for 2

min in a Stomaker Lab blender 80 (PBI, Milan, Italy). A sterile 0.1% peptone solution was used to obtain decimal dilutions that were spread on specific media to enumerate microbial groups. Total microbial count (TMC) was determined in Plate Count Agar medium (Oxoid, Milan, Italy). After incubation at 32°C for 48 h. Staphylococci were detected with Mannitol Salt Agar following incubation for 48 h at 37°C. Total coliforms were enumerated on Violet Red Bile Agar (Oxoid, Milan, Italy) after incubation at 37°C for 48 h. The Nutrient Agar (Oxoid, Milan, Italy) was used to detect aerobic spore-forming bacteria (ASFB), after incubation at 30°C for 48 h and previously destroying all vegetative forms by pasteurizing sample dilutions at 80°C for 10 min. Mould and yeasts (M&Y) enumerated on Rose Bengal Chloramphenicol Agar incubated at 25°C for 4 days. Anaerobic bacteria (An.) were examined using nutrient agar medium, the plate were put into anaerobic jar and incubated at 37°C for 48 h.

From each previously prepared serial dilution of the samples one ml was inoculated onto duplicates, using sufficient amount of liquefied standard plate count agar medium, both inoculated and control plates were left to dry before being incubated at 7°C for 10 days. Total psychrotrophic count/ml (psych) of examined samples were calculated and recorded. (Saranraj & Geeta, 2012).

All analysis were performed in triplicates.

#### *Sensory analysis:*

Samples were served to 10 judges after 30 and 60 days. The acceptance of the samples were evaluated by using an intensity scale from 1 to 9 (1, extremely dislike; 5, acceptable; 9 extremely like) for the attributes; color, odor, freshness, textural, Firmness and over all acceptability. An average of 10 scores for each attribute was reported. (Amal, 2016).

#### *Statistical Analysis:*

All sensory evaluation data were subjected to analysis of variance. Duncan's multiple range tests at ( $P \leq 0.05$ ) level to compare between means. The analysis was carried out using the PRO ANOVA procedure of SAS Program (1996).

### **Results and Discussion**

The effect of various conditions of roll pastry packaged on the growth of microorganisms at 4°C are shown in Tables 1–4, respectively.

Bacteria and fungi are known to cause spoilage of foodstuffs, not only in their storage on shelf but even during their storage under refrigerated conditions. In fact, bacteria, fungi and other microbes reside, grow, and sporulate on surface of the foods and cause the spoilage in due course when their number and mass is sufficient enough to secrete the enzyme which can break down the complex molecules structure of these food substances (Pundir and Jain, 2011).

In the present endeavour a one of bakery products, roll pastry, was evaluated for microbiological analysis after packaging in different conditions. Table (1) presents the microbiological analysis of air packaged pastry samples during 60 days of storage. As shown in this table, counts of TMC, coliforms, staphylococci, Molds & Yeasts, Psych., An., and ASFB reached after 60 days of cold storage to  $3.15 \times 10^4$  CFU/g,  $2.9 \times 10$  CFU/g,  $4.4 \times 10$  CFU/g,  $38.8 \times 10^2$  CFU/g,  $29.1 \times 10$  CFU/g and  $25.5 \times 10$  CFU/g respectively, while the counts were decreased in sodium metabisulphite packaged samples (Table 2) to less than 50% of the air packaged samples counts which TMC was  $3.36 \times 10^2$  CFU/g, Y&M was  $30 \times 10$  CFU/g, Psych was  $8.2 \times 10$  CFU/g, and ASFB was  $11.3 \times 10$  CFU/g. Moreover, coliforms and Staphylococci were not detected. Suhr and Nielsen (2004) revealed that the inhibitory effect of preservatives such as sodium metabisulphite on organisms in bakery products is much stronger at low pH (4.18– 4.88) and / or low water activity ( $a_w$ ) (0.75– 0.80) of the samples. Saranraj and Geetha (2012) found that sodium metabisulphite and sodium benzoate, the more suitable preservatives to be used in combination with the common levels of pH and  $a_w$  which the antimicrobial activity of these salts depends on their undissociated molecule.

The evolution of microorganisms in-package  $N_2$  gas was shown in Table (3). All  $N_2$  modified packaged samples did not spoiled before 40 days of storage for Yeasts & Moulds, Psych, and ASFB. Moreover, coliforms and staphylococci not detected until the end of storage (60 days). On the other

hand there were a significant differences in TMC between N<sub>2</sub> modified packaged samples and air packaged samples after 60 days of cold storage, while TMC reached a maximum value of 3.15x10<sup>4</sup> CFU/g on air packaged samples. The counts did not increase than 8.8x10 CFU/g at the same period on N<sub>2</sub> packaged samples.

**Table 1:** Microbiological evolution (CFU/g) on spring roll pastries of air packaged samples and during storage at 4°C.

| Storage time/ days | TMC                  | Coliforms | Staphylococci | M&Y                  | Psych.  | An.     | ASFB    |
|--------------------|----------------------|-----------|---------------|----------------------|---------|---------|---------|
| 0                  | 1.27x10 <sup>3</sup> | 1.0x10    | 1.5x10        | 7.0x10               | 19.5x10 | 10.0x10 | 5.0x10  |
| 10                 | 2.22x10 <sup>3</sup> | 1.2x10    | 2.1x10        | 11.7x10              | 25.1x10 | 13.3x10 | 10.4x10 |
| 20                 | 2.94x10 <sup>3</sup> | 1.7x10    | 2.6x10        | 13.6x10              | 33.4x10 | 16.7x10 | 13.7x10 |
| 30                 | 3.41x10 <sup>3</sup> | 1.9x10    | 2.9x10        | 15.0x10              | 50.0x10 | 19.0x10 | 16.9x10 |
| 40                 | 1.92x10 <sup>4</sup> | 2.2x10    | 3.2x10        | 22.2x10 <sup>2</sup> | 61.3x10 | 22.8x10 | 19.4x10 |
| 50                 | 2.07x10 <sup>4</sup> | 2.6x10    | 3.7x10        | 30.2x10 <sup>2</sup> | 72.8x10 | 25.3x10 | 22.3x10 |
| 60                 | 3.15x10 <sup>4</sup> | 2.9x10    | 4.4x10        | 38.8x10 <sup>2</sup> | 83.6x10 | 29.1x10 | 25.5x10 |

**Table 2:** Microbiological evolution (CFU/g) on spring roll pastries of Sodium meta bisulphite (0.2%) packaged samples and during storage at 4°C.

| Storage time/ days | TMC                  | Coliforms | Staphylococci | M&Y     | Psych.  | ASFB    |
|--------------------|----------------------|-----------|---------------|---------|---------|---------|
| 0                  | 1.27x10 <sup>3</sup> | 1.0x10    | 1.5x10        | 7.0x10  | 19.5x10 | 5.0x10  |
| 10                 | 1.10x10              | <10       | ND            | 9.8x10  | ND      | 5.3x10  |
| 20                 | 1.50x10              | <10       | ND            | 11.4x10 | ND      | 6.0x10  |
| 30                 | 1.80x10              | <10       | ND            | 15.1x10 | ND      | 7.1x10  |
| 40                 | 2.0x10               | <10       | ND            | 19.5x10 | 3.3x10  | 8.2x10  |
| 50                 | 2.45x10 <sup>2</sup> | <10       | ND            | 26.2x10 | 4.0x10  | 9.6x10  |
| 60                 | 3.36x10 <sup>2</sup> | <10       | ND            | 30.0x10 | 8.2x10  | 11.3x10 |

ND: Not detected

**Table 3:** Microbiological evolution (CFU/g) on spring roll pastries of modified atmosphere packaged samples and during storage at 4°C.

| Storage time/ days | TMC                  | Coliforms | Staphylococci | M&Y     | Psych.  | An.     | ASFB    |
|--------------------|----------------------|-----------|---------------|---------|---------|---------|---------|
| 0                  | 1.27x10 <sup>3</sup> | 1.0x10    | 1.5x10        | 7.0x10  | 19.5x10 | 10.0x10 | 5.0x10  |
| 10                 | 1.70x10              | <10       | ND            | <10     | <10     | 5.5x10  | <10     |
| 20                 | 2.40x10              | <10       | ND            | <10     | <10     | 7.2x10  | <10     |
| 30                 | 2.70x10              | <10       | ND            | <10     | <10     | 7.4x10  | <10     |
| 40                 | 3.50x10              | <10       | ND            | 1.0x10  | 4.7x10  | 8.3x10  | 10.0x10 |
| 50                 | 6.70x10              | <10       | ND            | 6.0x10  | 8.9x10  | 8.9x10  | 10.0x10 |
| 60                 | 8.80x10              | <10       | ND            | 12.0x10 | 10.0x10 | 9.5x10  | 12.0x10 |

ND: Not detected

**Table 4:** Microbiological evolution (CFU/g) on spring roll pastries of ozone-treated packaged samples and during storage at 4°C.

| Storage time/ days | TMC                  | Coliforms | Staphylococci | M&Y    | Psych.  | An.     | ASFB   |
|--------------------|----------------------|-----------|---------------|--------|---------|---------|--------|
| 0                  | 1.27x10 <sup>3</sup> | 1.0x10    | 1.5x10        | 7.0x10 | 19.5x10 | 10.0x10 | 5.0x10 |
| 10                 | 1.4x10               | <10       | ND            | <10    | ND      | ND      | ND     |
| 20                 | 1.5x10               | <10       | ND            | <10    | ND      | ND      | ND     |
| 30                 | 1.7x10               | <10       | ND            | <10    | ND      | ND      | ND     |
| 40                 | 1.8x10               | <10       | ND            | <10    | ND      | ND      | ND     |
| 50                 | 2.4x10               | <10       | ND            | 1.0x10 | ND      | ND      | ND     |
| 60                 | 2.7x10               | <10       | ND            | 2.0x10 | ND      | ND      | ND     |

ND: Not detected

Sodium metabisulphite packaged samples showed a mold-free shelf life up to 40 days. These results were in agreement with those reported by Sanguinetti *et al.*, (2015). They found that a mold-free shelf life up to 42 days at N<sub>2</sub> concentration in the package higher than 30% of fresh pasta. Moreover, packaging fresh pasta with N<sub>2</sub>/CO<sub>2</sub> ratio 70/30 and a residual O<sub>2</sub> content of 1% resulted in an extension of the lag phase of *Penicillium* spp.

Thus, the concomitant very low O<sub>2</sub> level and the N<sub>2</sub> concentration close to about 100% of our packages were effective in inhibiting the mold growth in roll pastry packaged samples up to 40 days.

As expected the ozone treated packaged samples in gaseous form up to a concentration of 7.0 mg/L can be successfully used as an anti-microbial agent layers of pastries without any changes in physical properties of the rolls (Table 4). As shown in this table samples did not spoiled before 50 days of cold storage for Yeasts & Moulds. At the same time, coliforms, staphylococci, Psych., An., and ASFB not detected until the end of storage (60 days). Nath *et al.*, (2014) revealed that ozone mainly reacts with the surface of the polymers and causes modification of the surface properties of polymers such as polarity and surface tension due to the formation of oxygen containing functional groups and degradation of the polymer chains. Also, there were an extremely decrease in TMC than the counts in air packaged samples during storage period which it reached 2.7x10<sup>4</sup> CFU/g after 60 days of storage, however it reached 3.15x10<sup>4</sup> CFU/g at the same period in air packaged samples. Small quantities of ozone and short contact times are sufficient for the desired effect on the food product, which assist in the preservation of the products, and is also used to sanitize its packaging materials (Senol, 2001).

Tables 5, 6 shows the effect of water activity *a<sub>w</sub>* and pH values on growth rate of Yeasts & Moulds at 25°C on the roll pastry samples. The *a<sub>w</sub>* values studied were 0.75, 0.85, and 0.90, and the experiments were carried out at constant temperature and constant pH value (4.9) after one week of storage. Also pH values studied were 4.5, 5.5, and 6.5 and the experiments were carried out at constant temperature and constant *a<sub>w</sub>* (0.90) after one week of storage. Changing *a<sub>w</sub>* at constant pH & temperature affected the growth rates of fungi and yeasts. The maximum growth (42.5x10<sup>4</sup> CFU/g) over the *a<sub>w</sub>* range tested, was at 0.90 *a<sub>w</sub>*, but the optimum growth (2.0x10<sup>4</sup> CFU/g) was at 0.75 *a<sub>w</sub>*. Also, this study has shown that pH value more effective on growth of these fungi and yeasts which the maximum growth rate over the pH range tested was at pH 4.5 (21.4x10<sup>4</sup> CFU/g), but the optimum growth was found at pH 6.5 (4.8x10<sup>4</sup> CFU/g). Our results show that, if the temperature was suitable, the growth rates dependence on *a<sub>w</sub>* and pH values. Therefore if the *a<sub>w</sub>* of the bakery products is lower than 0.75 and its pH value higher than 6.5, these products cannot be spoiled by the fungal or yeasts. Low pH can serve chemical preservation only. (Abellana *et al.*, 2015).

This study has shown that it is possible to limit growth rates dependence on *a<sub>w</sub>* and pH values. A recent study carried out with five different Spanish bakery products (Abellana *et al.*, 2015) showed that the *a<sub>w</sub>* of these intermediate moisture products ranged from 0.71 to 0.79 with pH values between 4.26 and 6.82, differs and influences fungal growth levels. High *a<sub>w</sub>* increase the level of available water allowing fungi spp. to thrive and at the same time high pH values did not encourage them to grow. Good control of *a<sub>w</sub>* and pH values of the product will give favorably results with fungi contamination problem.

Table 7 represents the sensory evaluation of the four different conditions of packaged spring roll pastry samples. Statistical analysis showed that there were a significant sensory differences between air packaged samples and other packaged samples ( $P \leq 0.05$ ). Air packaged samples received the lowest scores in all sensory aspects and in overall acceptability. Ozone-treated samples achieved the highest sensory hedonic score, especially in color and texture, moreover, exhibited higher tenacity and whiteness as well as lower yellowness and extensibility. Scores indicated significant improvement in color, odor, freshness, texture, and overall scores than the other packaged samples, therefore, we can concluded that ozone treatment more effective than chemical preservative additives and modified atmosphere with nitrogen (100%). Nath *et al.*, (2014) found that the effectiveness of ozone might be due to the significant increase in powerful disinfectant to control biological growth of unwanted organisms while leaving no residues and without affecting the quality of food. Relatively low concentrations of ozone and short contact time are sufficient to inactivate bacteria, molds, yeasts and viruses.

**Table 5:** Effect of pH value on the rate of Yeasts & Moulds growth at constant water activity (0.90) and ambient temperature (25°C) in fresh roll pastry samples after one week storage.

| Temp. °C | a <sub>w</sub> | pH  | Y & M contents (CFU/g) |
|----------|----------------|-----|------------------------|
| 25       | 0.90           | 4.5 | 21.4x10                |
| 25       | 0.90           | 5.5 | 8.5x10                 |
| 25       | 0.90           | 6.5 | 4.8x10                 |

**Table 6:** Effect of water activity on the rate of Yeasts & Moulds growth at constant pH value (4.7) and ambient temperature (25°C) in fresh roll pastry samples after one week storage.

| Temp. °C | pH  | a <sub>w</sub> | Y & M contents (CFU/g) |
|----------|-----|----------------|------------------------|
| 25       | 4.9 | 0.75           | 2.0x10                 |
| 25       | 4.9 | 0.85           | 8.6x10                 |
| 25       | 4.9 | 0.90           | 42.5x10                |

**Table 7:** Sensory evaluation of spring roll pastry samples packaged at different conditions after storage for 30 and 60 days at 4°C.

| Storage per days | Samples | Color                 | Odor                  | Freshness             | Texture               | Firmness              | OAA*                   |
|------------------|---------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|------------------------|
| 30               | A       | 7.58±0.8 <sup>d</sup> | 7.62±1.2 <sup>b</sup> | 7.65±0.5 <sup>b</sup> | 7.39±0.9 <sup>b</sup> | 6.94±1.5 <sup>b</sup> | 7.43±0.9 <sup>bc</sup> |
|                  | B       | 7.61±0.2 <sup>d</sup> | 6.88±1.3 <sup>c</sup> | 7.30±0.7 <sup>c</sup> | 7.24±0.4 <sup>b</sup> | 6.98±1.4 <sup>c</sup> | 7.20±0.1 <sup>b</sup>  |
|                  | C       | 7.70±0.4 <sup>d</sup> | 7.78±1.0 <sup>b</sup> | 8.34±0.2 <sup>a</sup> | 8.25±0.2 <sup>a</sup> | 7.66±1.1 <sup>c</sup> | 7.94±0.4 <sup>b</sup>  |
|                  | D       | 9.82±0.5 <sup>a</sup> | 8.93±0.3 <sup>a</sup> | 8.90±0.2 <sup>a</sup> | 8.72±0.4 <sup>a</sup> | 8.44±0.9 <sup>a</sup> | 8.96±0.3 <sup>a</sup>  |
| 60               | A       | 7.46±0.7 <sup>d</sup> | 7.38±1.3 <sup>b</sup> | 7.47±1.1 <sup>c</sup> | 7.11±0.7 <sup>b</sup> | 6.25±1.4 <sup>d</sup> | 7.13±0.6 <sup>bc</sup> |
|                  | B       | 7.50±0.5 <sup>d</sup> | 6.72±1.1 <sup>c</sup> | 7.18±0.8 <sup>c</sup> | 7.09±0.3 <sup>b</sup> | 6.64±1.0 <sup>d</sup> | 7.02±1.6 <sup>b</sup>  |
|                  | C       | 7.61±0.1 <sup>d</sup> | 7.62±1.2 <sup>b</sup> | 8.21±0.5 <sup>a</sup> | 8.17±0.1 <sup>a</sup> | 7.18±1.4 <sup>c</sup> | 7.76±1.1 <sup>b</sup>  |
|                  | D       | 9.67±0.7 <sup>a</sup> | 8.89±0.8 <sup>a</sup> | 8.83±0.6 <sup>a</sup> | 9.61±0.1 <sup>a</sup> | 7.25±0.8 <sup>b</sup> | 8.85±0.2 <sup>a</sup>  |

A: air packaged samples. B: preservative packaged samples. C: modified packaged samples D: Ozone packaged samples. \*OAA: over all acceptability. Data with different superscript are significantly different at  $P \leq 0.05$ .

## Conclusion

Microbiological spoilage, especially mold spoilage, is still a major problem limiting the shelf life of many high and intermediate moisture bakery products. Losses have been resulting in lost revenue to the baking industries. Therefore, methods to control mold and bacteria growth and to extend the shelf life of bakery products is of great economic importance to the baking industry where an increased demand in global consumption exists. This could lead to development the predominant methods of keeping the quality and extend the shelf life of bakery foods.

Sodium metabisulphite packaged samples, modified atmosphere packaged samples, ozone treated packaged samples were compared to air packaged spring roll pastry samples in term of microbes content and sensory properties and definition of the adequate for control common mold and bacteria species causing spoilage and extend the shelf life.

It may be concluded from the present study that ozone-treated packaged samples achieved the best results and highest sensory hedonic scores. Little doses of ozone gas (7 ppm) had a powerful disinfectant to control growth of unwanted organisms while leaving no residues and without affecting the quality of spring roll pastry samples.

Also, the results of this study emphasizes the importance of combining several barriers, such as modified atmosphere packaging, a<sub>w</sub>, and pH, that have synergistic or additives effects on the inhibition of mold growth. A balanced use of hurdles (pH, a<sub>w</sub>, temperature) should, therefore, be applied, which a<sub>w</sub> levels & pH values are of paramount importance for the efficiency of preservatives in bakery products.

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