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Effects of modified atmosphere packaging on physico-chemical characteristics and sensory evaluation of bitter orange (*Citrus aurantium*)

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ABSTRACT

The present study was undertaken to evaluate the effect of modified atmosphere packaging (MAP) during storage on some quality parameters of bitter orange. Physical, chemical, and sensory properties were monitored at 4 ºC for 20 days. Bitter oranges were sanitized, peeled, segmented and packed under normal (21%O_2 +79% N_2 as passive MAP) and two modified atmospheres (20% O_2+20% CO_2+60% N_2 as active MAP1 and 60% O_2+30% CO_2 +10% N_2 as active MAP2) in polyethylene (PE) trays. In general, there was no significant effect of MAP observed in chemical properties ($P > 0.05$). The firmness values increased at all applications ($P = 0.05$). Bitter orange segments remained viable for 20 days under enriched oxygen (60%) and less than 20 days under active (20% oxygen) and passive MAP applications considering quality parameters. We reported that bitter oranges could be prepared as ready to eat fruits with considerable shelf-life, quality and convenience with using MAP2. The longest shelf-life was obtained with MAP containing 30% of carbon dioxide (MAP2).

Key words: Bitter orange, Modified atmosphere packaging, Polyethylene packaging, Shelf-life, Storage

Fruits are attractive and nutritional foods, because of their colour, shape, unique taste and smell, enriched minerals, vitamin and other beneficial components (Cassano *et al.* 2003). Bitter orange (*Citrus aurantium*) is a plant that belongs to the Rutaceae family. It also called Seville orange, is known botanically as *Citrus* x *aurantium* L. and sometimes by its taxonomic synonyms, *C. aurantium* L. subsp. *aurantium* and *C. aurantium* subsp. *amara* (L.) Engler (Blumenthal 2005). It is presently commercially cultivated in southern Europe and other sub-tropical areas, particularly Spain, Portugal, Israel, and the various islands of the Caribbean (Bisset and Wichtl 1994).

In the food industry, bitter orange oil, which is usually expressed from the fresh peels, is widely used as a flavouring agent. Bitter orange oil is used as flavourings for beverages, particularly liqueurs and to intensify the orange character of soft drinks (Colombo *et al.* 2002).The Food and Drug Administration (FDA) has approved bitter orange (in small amounts) as a flavouring agent. In manufacturing, bitter orange is used in cosmetics and soaps (Jeff 2002). Even though Iran has been among the oldest citrus-producing countries of the world, unfortunately, there has not been much progress achieved either in its industrial processing or in its export qualities, causing it not to be of a favourable standing in the international markets. Fruit packaging installations have been founded in five northern cities of Ramsar, Shahsavar, Noshahr, Chaloos and Amol to process and pack citrus in advanced modern ways, but packaging is not yet done in the most suitable and proper way. Grading and sizing of fruit is a pre-requisite to proper packaging, but not much importance has been attached to its study (Anonymous 2005). There is no suitable set of standards for grading and sorting of fruits.There does not exist any suitable set of standards for grading and sorting of the fruit in Iran (Sharifi *et al*. 2007).

Modified atmosphere packaging techniques constitute one of the fresh food preservation methods with no or only minimal use of additives and are accepted now as the technology of the future (Tas and Ayhan 2005). In modified atmosphere (MA) applications, the O_2 and CO_2 concentrations are modified initially and then change dynamically depending on respiration rate of product and the permeability of the film surrounding the produce (Erkan and Wang 2006). Reduced O_2 and/or enriched CO_2 levels reduce respiration and decrease ethylene production, inhibit or delay enzymatic reactions, alleviate physiological disorders, and preserve the product from quality losses (Day 1994, Soliva- Fortuny and Martin-Belloso 2003). However, exposure to O_2 and CO_2 levels outside the limits of tolerance may lead to anaerobic respiration and the production of undesirable metabolites

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and other physiological disorders (Zagory and Kader 1988). The benefits of modified atmosphere packaging have been extensively studied in extending shelf-life of many fruits and vegetables. However, there are only limited studies regarding ready-to-eat citrus fruits.

The objective of this work is to evaluate the potential of modified atmosphere packaging in preserving the quality parameters of bitter orange samples and to determine the quality losses for 20 days at 4°C. In this research, three different atmospheres (21%O_2 +79%N_2 as passive MAP, 20% O_2+ 20% CO_2+ 50% N_2 as active MAP1 and 60% O_2+ 30% CO_2+ 10% N_2 as active MAP2) were applied.

MATERIALS AND METHODS

Ripe fresh bitter orange fruits (*Citrus aurantium*) were used for all the experiment in this study. The fruits were collected from Shahsavar province, Iran in September 2009. The bitter oranges (*Citrus aurantium*) harvested and forced air cooled before the experiment was set up. Polyethylene (PE) packaging rolls were used for preparation of packages of 30 cm ×20 cm.

All equipment was sanitized using 300 ppm chlorine solution prior to processing. Fruits were selected for uniformity, washed with tap water and immersed in 200 ppm NaOCl for 3 min., then dried and peeled manually. In this study three different gas compositions such as normal atmosphere (normal), MAP1 (20% O_2+ 20% CO_2+ 60% N_2), and MAP2 (60% O_2+ 30% CO_2+ 10% N_2) were applied with the modified atmosphere packaging machine (200A), combined with a triple gas mixer (B20-010). After the experiment was set up, fruits were stored in a cold room maintained at 4°C, for 20 days. Quality parameters were evaluated after 0, 5th, 10th and 20th days. Sensory analysis (Tas 2007) was performed at 0, 10th and 20th days. All analyses were performed in the laboratory of Tehran University, Department of Food Science.

Fruit flesh firmness was assessed using a texture analyzer. Speed of 20 mm/s and penetration distance of 13 mm was used to cut the segments at the centre, and the firmness was expressed as maximum cutting force (N). The segments similar in thickness were selected for firmness measurement. Six segments from two parallel packages (10 segments/ package) were measured for each treatment on each sampling day (Tas 2007). The readings were recorded in N force. The storage duration was determined for each treatment.

All chemical properties of the bitter orange were investigated on randomly selected 50 fruit samples. The nutritional composition of the orange fruits juice was studied as explained below: Total dry matter, water soluble dry matter, and *p*H of the samples were determined according to the methods of AOAC (1990). Each sample (about 25g from) was blended for 3 min. and filtered by using cheese cloth. The prepared juice of the sample was used for chemical analysis. The acidity was measured by titration with 0.1 N NaOH (AOAC 1984). Results were expressed as per cent citric acid. Total soluble solid contents (TSS) were determined by extracting and mixing two drops of juice from the two cut ends of each fruit into a digital refractometer at 23°C and the result expressed as Brix (AOAC 1984). The juice of the sample was centrifuged at 3 000 rpm for 10 min. and the supernatant was filtered by using a 0.45-μm-pore size filter to determine sugar content (fructose, glucose, sucrose, and total sugar) of bitter orange segments by using a HPLC equipped with refractive index detector (Escalona *et al.* 2005). Chemical analysis was repeated twice for each package and the average of four measurements of two parallel packages were calculated (Tas 2007).

Bitter orange segments packaged with different atmospheres were evaluated for overall appearance, firmness, aroma and acidity using a 5-point rating scale with six trained panelists. The attributes and product acceptability were expressed as follows:

Visual appearance 4: excellent/fresh; 4: good; 3: acceptable; 2: poor; 1: very poor. Aroma 5: natural; 4: loss of aroma; 3: no aroma; 2: light strange aroma; 1: strong strange aroma. Firmness 5: excellent firmness/juiciness; 4: firm/ juicy; 3: acceptable firmness/juiciness; 2: hard; 1: very hard. Acidity 5: very good; 4: good; 3: acceptable; 2: acidic; 1: too much acidity. (Karacay and Ayhan 2009)

The analysis of variance (ANOVA) was carried out to test the possibility of significance of treatment effect. LSD, as described by Ott (1984) was used to perform all possible pair comparisons between means of different treatments. Significance of differences was represented at 5% (P = 0.05).

RESULTS AND DISCUSSION

Physical quality of bitter orange segments

Firmness: Firmness was not influenced by the two different atmosphere packaging (Table 1). In general there was no significant difference in segment firmness among three MAP conditions (P =0.0879). However, storage time affected segment firmness significantly (P < 0.0001). Firmness values were 18.01, 11.59 and 12.96 for bitter orange samples in Normal MAP package, MAP1 and MAP2 respectively (day0). Increases in firmness values were observed in the first five days of storage and tended to reverse for the rest of the storage at all applications. Increase in firmness may be attributed to surface drying of the segments due to water loss by respiration and transpiration as stated by Martin-Belloso and Soliva-Fortuny (2006) and Erkan and Wang (2006). However, our sensory panel did not find significant changes in firmness for all treatments during storage.

Chemical quality of bitter orange segments

Important chemical properties such as titratable acidity, *p*H, soluble solids and sugar content of the bitter orange segments are shown in Table 2. No significant changes were

Table 1 Effect of modified atmosphere packaging on firmness (N) of bitter orange segments stored at 4ºC

Treatment/time	Normal MAP	MAP1	MAP2
Day 0	18.01±9.878 b	11.59±4.012 c	12.96±3.535 c
Day 5	18.21±6.334 b	17.91±4.665 b	18.19±5.221 b
Day 10	20.49±6.956 ab	22.32±5.734 ab	21.71±7.450 ab
Day 20	24.45±4.211 a	20.48±5.988 ab	21.40±5.892 ab

Mean values followed by different letter for a given parameter are significantly different (P = 0.05)

observed in titratable acidity in terms of citric acid, *p*H and soluble solids among Normal and active MAP applications ($P > 0.05$). The storage time also did not have significant effect on titratable acidity ($P = 0.8361$) and *p*H ($P = 0.052$). However, there was significant effect of storage time on total soluble solids ($P < 0.001$). The effects of storage became significant for active MAP1 application after day 5 and for high oxygen application after day 10. MAP application with different gas compositions did not have any significant effect on the sugar content of bitter orange segments as shown in Table 2 ($P > 0.05$). The sugar content (fructose, glucose and total sugar) of bitter orange segments did also not change significantly during storage ($P > 0.05$). Studies with fresh-cut pears (Senesi *et al.* 1999) and kiwifruit (Agar *et al.* 1999) showed that sugar content did not significantly change under refrigerated storage, as expected because sugar content is not much influenced by the atmospheric conditions (Soliva-Fortuny and Martin-Belloso 2003). Our results are in agreement with the results by Pretel *et al.* (1998) who reported no significant changes in chemical attributes (*p*H, titratable acidity, and soluble solids) during 11 days of storage of minimally processed oranges (Pretel *et al.* 1998). After 20 days of storage, vitamin C losses were of ~30 and ~39% for (MAP 1) and (MAP 2), respectively. When compared with 20 days of normal MAP storage, losses measured in bitter orange stored were by 32% lower. Thus limitation of oxygen diffusion by PE packaging would have had a limited positive effect. These results show that use of modified atmospheres and PE packages with good oxygen barrier properties had impact against vitamin C losses.

Sensory quality of bitter orange segments

The results of the sensory evaluation are presented in Table 3. There was no significant difference in sensory quality of bitter orange segments between normal (air) and MAP1 ($P > 0.05$) for 20 days. There were no significant effect of MAP treatment storage time interaction on any of the attributes tested ($P > 0.05$). However, MAP treatment had significant effect on appearance ($P < 0.001$). Although bitter orange segments packaged under air or active MAP with 20% oxygen were found acceptable for 20 days in terms of sensory quality, the atmosphere was anaerobic after the 15th day which could be risky for consumption.

The scores for bitter orange segments treated with high oxygen were below acceptability limit (score 3) at the 20 days of storage for most of the sensory attributes and the product acceptability. That's why the viability of bitter orange segments packaged with high oxygen was limited to 15 days. This might be due to negative effects of CO_2 on the sensory attributes after the 20th day of storage under MAP2.

Storage atmosphere and temperature have great importance on safety of the produce. The bitter orange segments were viable for 20 days under high oxygen (60% oxygen) due to sensory attributes and acceptability scores, and less than 10 days for passive and active MAP with 20% oxygen due the internal atmosphere at 4 ºC. Although the package atmosphere was aerobic during 20 days at high

Table 2 Effect of modified atmospheres on chemical parameters (*p*H, soluble solids, sucrose, titratable acidity and vitamin C) of bitter orange segments stored at 4ºC

Treatment / storage time		*p*H	TSS (%)	Sucrose (%)	Titratable acidity (% citric acid)	Vitamin C (mg/L ±10 mg/L)
Normal MAP	Day0	3.14±0.064abc	7.00±0.000a	5.50±0.343a	1.6&±0.132c	258
	Day5	3.15±0.071a	6.70±0.000b	6.02±0.556a	1.87±0.019a	205
	Day10	3.13±0.025abcd	7.00±0.000a	5.85±0.297a	1.70±0.160abc	181
	Day20	3.06±0.042cd	7.00±0.000a	5.97±0.189a	1.70±0.070abc	175
MAP1	Day0	3.14±0.055abc	7.00±0.000a	5.49±0.344a	1.80±0.052ab	258
	Day5	3.15±0.048a	7.00±0.000a	6.12±0.122a	1.73±0.012abc	200
	Day10	3.14±0.012ab	7.00±0.000a	6.32±0.233a	1.79±0.031ab	193
	Day20	3.07±0.087bcd	6.80±0.000b	5.98±0.765a	1.72±0.102abc	180
MAP2	Day0	3.14±0.034abc	7.00±0.000a	5.51±0.240a	1.75±0.054abc	258
	Day5	3.09±0.017a	7.00±0.000a	5.74±0.283a	1.72±0.092abc	191
	Day10	3.06±0.045abcd	7.00±0.000a	5.87±0.445a	1.81±0.082ab	172
	Day20	3.10±0.061d	7.00±0.000a	6.26±0.651a	1.79±0.113ab	145

Mean values followed by different letter for a given parameter are significantly different (P = 0.05)

Table 3 Effect of modified atmosphere packaging on the sensory attributes of bitter orange segments stored at 4ºC

Treatment/storage	time		Appearance	Firmness	Aroma acidity
Normal MAP	Day0	4.5± 0.354[a]	4.7±0.78[a]	4.6±0.323[a]	4.0±1.415[ab]
	Day5	4.5± 0.711[a]	4.7±0.13[a]	4.0±1.546[ab]	4.5±0.343[a]
	Day10	4.2± 0.112[ab]	4.4±1.64[ab]	4.1±0.491[ab]	4.3±1.717 [a]
	Day20	3.9± 0.564[ab]	3.6±0.20[ab]	4.0±0.720[ab]	3.0±1.031[abc]
MAP1	Day0	4.5± 0.989[a]	4.7±0.12[a]	4.7±0.551[a]	4.1±1.113[ab]
	Day5	4.5± 0.222[a]	3.9±0.21[ab]	4.7±0.718 [a]	4.2±0.432[ab]
	Day10	4.0± 0.334 [ab]	4.1±0.43[ab]	4.3±0.211[ab]	3.5±1.878[ab]
	Day20	3.5± 1.460[ab]	4.0±0.18[ab]	4.1±0.327[ab]	2.5±1.95[bc]
MAP2	Day0	4.5± 0.444[a]	4.8±0.39[a]	4.9±0.818[a]	4.0±0.849[ab]
	Day5	3.5± 1.378[ab]	4.4±0.87[ab]	5.0±0.910[a]	4.5±0.233[a]
	Day10	3.0± 0.657[bc]	3.7±0.34[ab]	4.5±0.561[ab]	3.9±1.009[ab]
	Day20	2.5± 0.816[c]	3.4±0.70[b]	3.8±1.456[ab]	2.5±1.334[c]

Mean values followed by the different letter for a given parameter are significantly different (P = 0.05)

oxygen application, the segments were found not acceptable by the sensory panel after 15 days possibly due to negative effects of high amount of carbon dioxide on the sensory attributes. High oxygen application provided further advantages over normal MAP or MAP1 after 20 days of storage. Bitter orange segments can be prepared as ready-to-eat fruits with considerable shelf-life of 15 days, good quality and convenience to the consumer using enriched oxygen MAP using PE trays. Modified atmospheres and PE packages with good oxygen barrier properties had impact against vitamin C losses.

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