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Combining high oxygen atmospheres with low oxygen modified atmosphere packaging to improve the keeping quality of strawberries and raspberries

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Abstract

The quality of strawberries and raspberries, packaged in different consumer-sized systems was assessed. The following consumer-sized packaging systems for strawberries and raspberries are compared: (1) the conventional method of packaging in a macro-perforated high-barrier film (AIR), (2) low O_2 modified atmosphere (i.e. 3–5 kPa O_2 and 5–10 kPa CO_2-balance N_2), (3) high O_2 modified atmosphere (HOA, i.e. >70 kPa O_2-balance N_2): HOA in a high-barrier film and (4) HOA in a MA film with an adjusted film permeability. The high O_2 atmosphere in the MA film reached steady-state after 5 days at about 3 kPa O_2 and 5 kPa CO_2. The O_2 content in the high-barrier film package remained above 21 kPa O_2 during the first 5 days of storage, but then decreased to anaerobic conditions, resulting in off-flavors and odors. To avoid an accumulation of ethylene inside the high-barrier package, an ethylene adsorbing monolayer was added. Shelf life of strawberries and raspberries given the AIR treatment was limited by growth of moulds, rather than by sensory unacceptance. On the other hand, sensory properties limited the shelf life of the fruit packaged under MA. The initial high O_2 atmospheres retarded the growth of moulds. However, when O_2 was depleted and CO_2 had accumulated in the barrier film, sensory quality (odor, taste and firmness) declined. In the MA film, the inhibitory effect on mould growth was maintained, due to the initial high O_2 levels.

Keywords: Strawberries; Raspberries; High oxygen; Shelf life; Sensory quality; Modified atmosphere packaging

1. Introduction

Strawberry and raspberry fruit have a very short postharvest life, mostly due to their relatively high metabolic activity, and the incidence of microbial moulds and rots (Brown et al., 1984; Joles et al., 1994). The use of low O_2 concentration (1–5 kPa) and high CO_2 concentration (5–10

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kPa), balanced with N_2, in combination with storage temperature at refrigeration levels, is proposed by many researchers as optimal storage conditions for some selected fresh fruit, vegetables and fresh-cut vegetables (Church, 1994; Ohlsson, 1994; Ahvenainen, 1996; Kader et al., 1989; Jacxsens et al., 1999). By matching film permeation rates for O_2 and CO_2 with the respiration rate of the packed fresh-cut produce, steady-state conditions of 3–5 kPa O_2 and 5–10 kPa CO_2 can be established inside the package (Jacxsens et al., 2000). Often the produce is sealed in packaging film of insufficient permeability resulting in development of undesirable fermentation reactions (i.e. < 2 kPa O_2 and > 20 kPa CO_2). Such hypoxic atmospheres have to be avoided in packaging of respiring produce because the shift to fermentation will cause formation of ethanol, acetaldehyde, off-flavors and odors (Jacxsens et al., 2000; Day, 2001).

Elevated O_2 atmospheres (> 70 kPa O_2) have been suggested to overcome the disadvantages of low O_2 MAP. High O_2 MAP was found to be particularly effective at inhibiting enzymatic discoloration, preventing anaerobic fermentation reactions and undesirable moisture and odor losses. In addition, the high O_2 MAP was found to be very effective at inhibiting microbial growth and reducing decay of the fresh produce (Day, 1998). Under high O_2 MAP, it is hypothesized that reactive oxygen species damage vital cellular macromolecules and thereby inhibit microbial growth when oxidative stresses overwhelm cellular antioxidant protection systems (Amanatidou et al., 1999; Kader and Ben-Yehoshua, 2000; Day, 2001). Amanatidou et al. (1999) studied the impact of high O_2 MAP on micro-organisms associated with minimally-processed vegetables. When high O_2 was applied alone, the inhibitory effect on microbial growth was highly variable. Inhibition was more pronounced when the elevated O_2 concentration was combined with an elevated CO_2 concentration (10–20 kPa). Jacxsens et al. (2001a) evaluated the application of high O_2 atmospheres as an alternative for low O_2 modified atmosphere packaging for mushroom slices, grated celeriac and cut chicory endive, at 4 °C. High O_2 MAP inhibited enzymatic browning and yeast growth. Typical spoilage causing micro-organisms (*Pseudomonas fluorescens*, *Candida lambica*, *Aspergillus flavus*) and psychrotrophic pathogens associated with refrigerated minimally processed vegetables, *Aeromonas caviae* (HG4) and *Listeria monocytogenes*, showed an increased lag-phase and decreased maximum specific growth rate during the in vitro tests conducted in high O_2 atmospheres (95 kPa O_2–5 kPa N_2) compared to experiments with low O_2 MAP. Wszelaki and Mitcham (2000) showed an inhibitory effect of high O_2 MAP on *Botrytis* growth.

Safety precautions have to be taken into consideration when applying high oxygen atmosphere packaging on industrial scale, because O_2 concentrations > 21 kPa can be explosive (BCGA, 1998).

The objective of this study was to compare the conventional packaging system of strawberry and raspberry fruit in a macro-perforated film (AIR) with three modified atmosphere packaging systems: low O_2 MA in a film with an adjusted permeability (low O_2 MA), high O_2 in a high-barrier film (HOA), and a combination of both, i.e. high O_2 in a film with an adjusted permeability for low O_2 conditions (HOA-MAP).

As the weather conditions in Belgium do not allow cultivation of strawberries and raspberries during the whole year, import from abroad is necessary for Belgian retailers. The products are shipped to Belgium and are then packaged under MA, regardless of the pretreatment. Preharvest and other conditions and handling of the fruit are part of the logistics and are therefore not taken into account in these experiments.

2. Materials and methods

2.1. Preparation of the fruit

All the fruit were bought from a local wholesale company in Ghent on the day of their arrival for the public auction. After refrigerated transport to the laboratory (15 min), the fruit were immediately weighed, filled in the appropriate bags and packaged. The storage experiments were done with strawberries (*Fragaria elvira*) from Israel and raspberries (*Rubus idaeous*) from Spain. The fruit were sorted to remove injured berries and to obtain berries of uniform color. The strawberries were packaged with 500 g in a package of 23×19 cm^2, the raspberries with 150 g in a package of 18.5×20.5 cm^2 (consumer-sized packages). The sizes of the packages were calculated according Jacxsens et al. (1999), taking into account the O_2 permeability of the packaging film and the steady-state conditions of 3–5 kPa O_2 and 5–10 kPa CO_2 that must be obtained. All fruit were stored at 7 °C.

During storage, the gas atmospheres, sensory quality and microbiological quality were regularly analyzed (day 0, 3, 5, 7, 10, 12 and 14 for raspberries and day 0, 3, 5 and 7, 10 and 12 for strawberries).

2.2. Packaging

For low O_2 MA, the modification of the atmosphere (3 kPa O_2–5 kPa CO_2–balance N_2) in the packages was achieved actively by using a gas packaging unit composed of a gas mixer (WITT M618-3MSO, Gasetechnik, Germany) and a vacuum compensation chamber (Multivac A300/42 Hagenmüller KG, Wolfertschwenden, Germany). In order to maintain steady-state conditions inside the packages, the O_2 (OTR) and the CO_2 (CO_2TR) transmission rates of the packaging film have to be matched to the O_2 consumption rate and the CO_2 production rate of the fruit, respectively (Jacxsens et al., 1999). Therefore the respiration rate of the fruit was measured in air conditions, as described by Jacxsens et al. (1999), with 0.15 kg of fruit in air-tight glass jars (620 ± 0.5 ml) stored at 7 °C. A gas sample of 0.3 ml was taken periodically through an air-tight septum and analyzed by gas chromatography (Catharometer IGC11M-TCD, 2 faze column, Alltech, Laarne, Belgium). The column temperature was 35 °C and helium was used as carrier gas with a flow of 0.33 ml s^{-1}. All determinations were conducted in triplicate.

By following the O_2 depletion and CO_2 accumulation of the produce over time, the respiration rate of the fruit, expressed as O_2 consumption (mol kg^{-1} s^{-1}) or CO_2 production rate (mol kg^{-1} s^{-1}), could be determined at 7 °C and 3 kPa O_2. Using the model described by Jacxsens et al. (1999, 2000), the O_2 transmission rate of the packaging film can be chosen according to the respiration rate, in order to obtain 3–5 kPa O_2 and 5–10 kPa CO_2 in the packages.

The low O_2 MA films, applied during the storage experiments, have a very high permeability for CO_2 ($> 1.08 \times 10^{-10}$ mol m^{-2} s^{-1} Pa^{-1}) at 7 °C and high RH (90%). Consequently, relatively low CO_2 levels (1–4 kPa) accumulated inside the packages, so that the choice of the packaging film was only based on the O_2 transmission rate of the film. The low O_2 MA film for strawberries had an oxygen transmission rate of 2.54×10^{-11} mol m^{-2} s^{-1} Pa^{-1} and an ethylene transmission rate of 2.93×10^{-11} mol m^{-2} s^{-1} Pa^{-1} at 7 °C and 90% RH, 30 μm thickness and a permeability for water vapor of 1.16×10^{-4} g m^{-2} s^{-1} at 23 °C (WA7217-3, Hyplast N.V., Klerck's Group, Hoogstraten, Belgium). The low O_2 MA film for raspberries had the same thickness, an oxygen transmission rate of 1.74×10^{-11} mol m^{-2} s^{-1} Pa^{-1} and an ethylene transmission rate of 2.62×10^{-11} mol m^{-2} s^{-1} Pa^{-1} at 7 °C and 90% RH and a permeability for water vapor of 1.16×10^{-4} g m^{-2} s^{-1} at 23 °C (WA7805-1, Hyplast N.V., Klerck's Group, Hoogstraten, Belgium).

For packaging with high O_2, the packages were flushed for 5 min with 95 kPa O_2–balance N_2, mixed with a gas mixer (WITT M618-3MSO, Gasetechnik, Germany). The high O_2 was applied in a high-barrier film (Euralpack, Wommelgem, Belgium) of 110 μm thickness with an oxygen transmission rate of 1.083×10^{-14} mol m^{-2} s^{-1} Pa^{-1} and an ethylene transmission rate of 2.71×10^{-15} mol m^{-2} s^{-1} Pa^{-1} at 23 °C and 90% RH and a permeability for water vapor of 2.89×10^{-5} g m^{-2} s^{-1} at 23 °C. High O_2 was also applied in the above mentioned low O_2 MA films, with corresponding barrier properties. After flushing, the packages were heat-sealed (Sealboy 321, Audion Elektri, Weesp, the Netherlands) and stored at 7 °C. A realistic gas/product ratio of 2/1 was used in the jars and in the packages for all gas combinations. This ratio remained constant during the storage period.

To avoid accumulation of ethylene inside the high-barrier film, the fruit were wrapped, before packaging in the high-barrier film, in a permeable monolayer ethylene adsorbing film (Schulman Plastics N.V., Bornem, Belgium). The ethylene concentration in the headspace of the jars (same jars as for determining the respiration rate) was measured using gas chromatography, by taking another gas sample of 0.3 ml (FID DI 200, Porapack QS 1/8 stainless steel column, Biorad, Belgium), column temperature at 135 °C and helium as carrier gas (Air Liquide, Aalter, Belgium) with a flow of 30 ml min^{-1}, hydrogen with a flow of 30 ml min^{-1} and air with a flow of 300 ml min^{-1}, flame ionization detector). By following the ethylene accumulation in the headspace of the jars over time, the ethylene production rate of strawberries and raspberries was calculated, in order to determine the configuration of the ethylene adsorbing film.

The traditional commercialized packaging system was used as an air control. Therefore the high-barrier film was perforated with holes of 3 mm diameter (ca. 200 holes m^{-2}) to create air conditions.

2.3. Gas exchange

The gas composition inside the packages was analyzed in each package before opening. The O_2, CO_2 and ethylene concentrations were measured as described above by gas chromatography. The O_2 levels in the headspace of the packages with a permeable film are not corrected for argon.

2.4. Loss of weight and visual quality

During storage, the weight loss due to transpiration and respiration of the fruit was followed (expressed as a percentage of the original weight of the packaged fruit), by weighing the fruit each day of the experiment.

The quality of strawberries and raspberries was also visually assessed. The loss of fruit due to fungal growth (expressed as a percentage of the original weight of the packaged fruit) was measured by weighing fruit with visible mycelium growth. A package of 500 g strawberries or 150 g raspberries was considered unacceptable when 5% of the berries were visibly affected by mycelium growth (Hertog et al., 1999; Sanz et al., 1999).

Beside this, the loss of fruit due to visual decay (mycelium growth not included) was determined by weighing fruit with at least 1/3 damaged surface due to softening and bruising (expressed as a percentage of the original weight of the packaged fruit). When 10% of the fruit was lost due to visible damage, the package was rejected (Sanz et al., 1999).

2.5. Determination of sensory quality

A trained taste panel, with a minimum of six persons, evaluated the sensory quality of the packaged fruit. All sensory tests were performed in a special taste room with separated boxes, and organoleptical properties such as taste, odor and firmness were evaluated under IR light to exclude influence of the visual characteristics. The visual properties (color of the fruit, color of the calyx of the strawberry and general freshness of the fruit, i.e. the first impression the consumer gets about the brightness, the size and the absence of damage) were judged under daylight. A numerical score between 1 and 10 was given for each property to describe the sensory quality of the fruit. For taste, odor, firmness and color, scores 1–4 were associated with properties of unripe fruit, such as no or acid taste, no odor, too hard, light color. Score 5 represented the optimal ripeness of the fruit and scores 6–10 were associated with deterioration of the fruit (earthy taste, rotten odor, very soft, dark color). For general freshness and color of the calyx, on the other hand, scores 1–4 represented a very good and fresh value, score 5 was still acceptable and scores 6–10 were associated with a non-acceptable general freshness or a rotten calyx. The cut-off score was defined at 5. Below this score, the sample was still acceptable (Jacxsens et al., 2001a,b).

2.6. Determination of the microbial levels

After opening the packages, 30 g of each package was aseptically transferred into a stomacher bag and diluted with a Peptone Saline Solution

($8.5\ g\ l^{-1}$ NaCl + $1\ g\ l^{-1}$ peptone). A dilution series was made and the microbial levels of the packaged vegetables determined. Lactic acid bacteria were counted on a plate with a top layer of de Man, Rogosa, Sharpe agar (MRS, OXOID, CM361) after 3 days of incubation at 30 °C. Total psychrotrophic count was enumerated on Plate Count Agar (PCA, OXOID, CM325), incubated at 22 °C for 5 days. Yeasts were quantified on Yeast Glucose Chloramphenicol agar (YGC, Sanofi Diagnostics Pasteur, 64104) after 3 days of incubation at 30 °C. The total anaerobic count was determined on a plate with a top layer of Reinforced Clostridial Agar (RCA, OXOID, CM 151) after 48 h of anaerobic incubation in a closed jar with an AeroGen™ sachet (OXOID, AN025A) at 30 °C. On raspberries, the amount of Enterobacteriaceae was analyzed on day 0 on a plate with a top layer on Violet Red Bile Glucose Agar (VRBGA, OXOID, CM485) for 24 h at 37 °C.

2.7. Statistical analysis

The storage experiment was conducted in duplicate (two packages per measurement). Data were statistically analyzed by a computer software program (Microsoft Office Excel 97). Results of gas concentrations and microbial activity are given by the mean and standard deviation. Results of sensory analysis were analyzed by a Tukey and Duncan test by a computer software program (SPSS 9.0 for Windows 98) in order to determine significant differences ($P < 0.05$) between the applied modified atmospheres. Multivariate statistical analysis using principal component analysis (SPSS 9.0 for Windows 98) was applied to study the interrelation of sensory data.

3. Results and discussion

3.1. Change of headspace gas composition

The low O_2 MA is flushed inside the package before sealing, so that steady-state conditions are immediately reached. The target of 3 kPa O_2 and 5 kPa CO_2 (balance N_2) was maintained relatively constant during the storage period of the raspberries (Table 1). The permeability of the chosen film was matching the respiration rate of the raspberries at 7 °C. At the end of the storage period (from day 14 on), O_2 accumulated and CO_2 depleted due to degradation of the fruit (Kays, 1991). For strawberries, on the contrary, the respiration rate was a little underestimated. This resulted in steady-state conditions of less than 3 kPa O_2 and more than 5 kPa CO_2 inside the

Table 1
O_2 and CO_2 concentrations in the headspace of packaged raspberry and strawberry fruit using low O_2 MA, HOA, and HOA-MAP, and stored at 7 °C; data are accompanied by SDs

	Storage time (days)	Low O_2 MA		HOA		HOA-MAP	
		kPa O_2	kPa CO_2	kPa O_2	kPa CO_2	kPa O_2	kPa CO_2
Raspberries	0	3.60 ± 0	6.70 ± 0	95.60 ± 0	3.70 ± 0	95.60 ± 0	3.70 ± 0
	3	5.49 ± 5.61	4.40 ± 0.72	49.00 ± 0.24	43.86 ± 2.01	17.60 ± 7.04	2.82 ± 2.64
	7	4.70 ± 1.57	6.12 ± 1.07	5.27 ± 7.45	72.27 ± 2.01	6.45 ± 1.69	5.30 ± 0.89
	10	7.65 ± 5.72	5.70 ± 3.25	n.d.	56.25 ± 17.18	n.d.	3.90 ± 2.40
	14	13.50 ± 3.32	3.55 ± 0.92	14.30 ± 17.96	51.45 ± 11.95	5.85 ± 0.35	7.25 ± 0.49
Strawberries	0	3.60 ± 0	8.50 ± 0	94.80 ± 0	2.30 ± 0	94.80 ± 0	2.30 ± 0
	3	2.52 ± 1.82	7.13 ± 0.13	36.47 ± 28.22	21.71 ± 13.64	17.15 ± 5.67	5.14 ± 3.36
	7	n.d.	9.04 ± 1.48	22.04 ± 10.29	75.22 ± 8.79	9.41 ± 12.58	6.64 ± 1.53
	10	0.56 ± 0.39	9.89 ± 1.67	n.d.	51.19 ± 4.79	n.d.	4.36 ± 5.64
	12	0.05 ± 0.07	11.25 ± 0.35	11.95 ± 2.33	13.05 ± 8.41	16.1 ± 0.42	5.70 ± 0.14

Packages with AIR maintained 21%O_2–0.1%CO_2–balance N_2. n.d., no data available.

package (1–2 kPa O_2 and 6–7 kPa CO_2) (Table 1).

For strawberries and raspberries with HOA, the O_2 concentrations inside the packages remained >21 kPa until day 5. The CO_2 concentration in the packages increased quickly and exceeded >20 kPa after day 3 (Table 1). Data of Wszelaki and Mitcham (2000) indicated a stimulated O_2 uptake of the strawberries treated with high O_2 in comparison with the O_2 uptake in air, at 5 °C, explaining the rapid decrease of O_2 inside the packages. The ethylene adsorbing film prevented ethylene accumulation in the strawberry packages of high-barrier film during the whole storage period, as they produce very small amounts of ethylene. Raspberries produced higher amounts of ethylene. The ethylene adsorbing monolayer was not effective enough since there was 80 $\mu l\ l^{-1}$ ethylene after 5 days (data not shown), compared to 160 $\mu l\ l^{-1}$ without the ethylene adsorber (Jacxsens et al., 2001b). This was also predicted based on the mass balance of the ethylene production rate of raspberries in HOA conditions and the measured ethylene adsorbing rate of the applied adsorbing film.

Oxygen concentration in HOA-MAP, where a permeable film was used, decreased faster than in HOA where a high-barrier film was applied. Both for strawberries and for raspberries in HOA-MAP, steady-state conditions of 5–6 kPa CO_2 after 5 days and of 4–7 kPa O_2 after 7 days were established (target gas concentrations). From the ethylene permeability of the low O_2 MA film, the predicted ethylene accumulation inside low O_2 MA and HOA-MA packages was 1 $\mu l\ l^{-1}$ ethylene for strawberries and 35 $\mu l\ l^{-1}$ ethylene for raspberries. These amounts were reached after 5 days of storage and were not exceeded (data not shown).

Inside the macro-perforated packages (AIR), air conditions (21 kPa O_2–0.01 kPa CO_2–balance N_2) were maintained during the whole storage period and no ethylene accumulation occurred.

3.2. Weight loss due to transpiration and respiration

The macro-perforated packages (AIR) exhibited higher fruit weight loss than any non-perforated packaging system, and this was more evident as storage continued. The AIR control of raspberries and strawberries showed the biggest losses of weight due to transpiration and respiration, 20 and 5%, respectively, at the end of the storage period (14 and 10 days, respectively). The low O_2 MA film has a low permeability for water vapor (1.16×10^{-4} g m^{-2} s^{-1} at 23 °C), resulting in a lower loss of weight due to transpiration and respiration. This is also true for the high-barrier film, which has even lower permeability for water vapor (2.89×10^{-5} g m^{-2} s^{-1} at 23 °C).

3.3. Loss of fruit due to visible decay and due to visible fungal growth

An individual berry was considered unacceptable if it was visibly affected by any form of deterioration, visible fungal growth not included, on at least 1/3 of the surface (Sanz et al., 1999), and the limit for acceptance was set at 10% loss of marketable fruit in a package (i.e. two affected strawberries and four raspberries in a consumer-sized package of 500 g and 150 g, respectively). Fruit treated with AIR suffered from the highest visible damage, with rejection of the raspberries after 12 days of storage at 7 °C. The raspberries in HOA, HOA-MAP and low O_2 MA, did not lose more than 10% of their weight due to visible decay, during the 14 days of storage (Fig. 1a). Strawberries in all the AIR packages lost significantly more weight due to visible decay than in the other packaging types, although the limit of 10% was never exceeded during the 10 days of measuring (data not shown).

By day 5, the raspberries packaged in AIR and low O_2 MA had more than 5% loss due to fungal growth (Fig. 1b) and were consequently rejected. The strawberries packaged in AIR and low O_2 MA exceeded the limit of 5% loss due to fungal growth on day 3 and 7, respectively. The moulds growing on the fruit in AIR were of a white filamentous origin, while the low O_2 MA raspberries were damaged by *Botrytis* growth. *Botrytis* infection is a major factor limiting keeping quality of strawberries, with the potential to cause up to 50% loss (Hertog et al., 1999; Wszelaki and Mitcham, 2000).

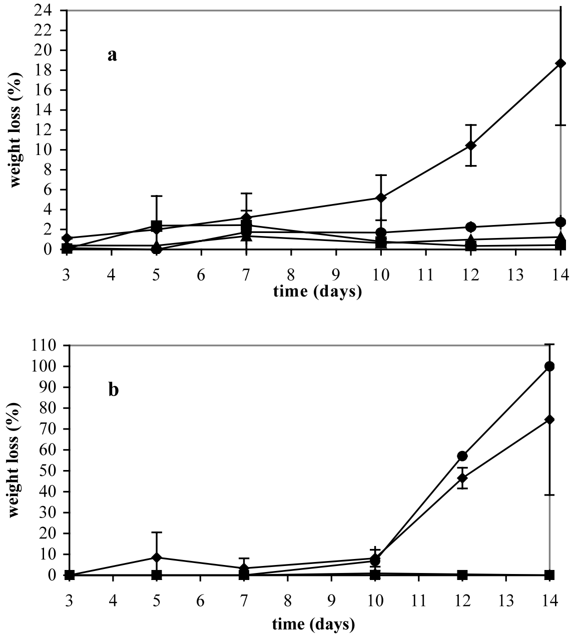

Fig. 1. Loss of weight of raspberries (a) due to visible decay (fungal growth not included) and (b) due to visible fungal growth, treated with AIR (diamonds), HOA (squares), HOA-MAP (triangles) and low O_2 MA (circles), during storage at 7 °C.

The raspberries and strawberries treated with HOA and HOA-MAP were not at all affected by *Botrytis* or by any other type of mould. Experiments of Wszelaki and Mitcham (2000) showed that 100 kPa O_2 treatment of strawberries was more effective in inhibiting fruit decay and mycelium growth than 15 kPa CO_2 or air after 14 days at 5 °C. Development of in vitro mycelium growth and the amount of spores of *A. flavus* decreased significantly with 80 kPa O_2 and 95 kPa O_2 in comparison to 5 kPa O_2 and air conditions while growth of *Rhizopus stolonifer*, *Botrytis cinerea* and *Penicillium echinulatum* var. *discolor* showed a higher degree of inhibition when treated by a combination of 80 kPa O_2 and 20 kPa CO_2 (Hoogerwerf et al., 2000; Jacxsens et al., 2001a).

3.4. Sensory quality

Tasting ceased when growth of moulds was noted. For strawberries this occurred on day 5 for the AIR and low O_2 MA treatments. The strawberries packaged in low O_2 MA and HOA were unacceptable for taste at day 5 (Table 2). Several authors have mentioned that off-flavors may occur when low O_2 and/or high CO_2 are used during storage of strawberries (Brown et al., 1984; Ke et al., 1991; Shamaila et al., 1992). The strawberries treated with HOA exhibited lower firmness and off-flavors compared to low O_2 MA and HOA-MAP, a phenomenon that was linked to the accumulation of CO_2 in the packages (Table 3) (Garcia et al., 1998; Wszelaki and Mitcham, 2000). There were no differences of color between the four types of packaging. The calyx of the strawberries packaged under HOA-MAP got the best scores during storage (data not shown). After day 6 all the strawberries were evaluated as being too ripe.

Table 2
Taste score (average ± 95% confidence interval) of packaged raspberries and strawberries using AIR, low O_2 MA, HOA, and HOA-MAP, stored at 7 °C; values above the cut-off score of 5 are indicated in bold

	Storage time (days)	Air	Low O_2 MA	HOA	HOA-MAP
Raspberries	0	3.89 ± 0.54	3.89 ± 0.54	3.89 ± 0.54	3.89 ± 0.54
	3	**n.a.**	**5.21 ± 1.11**	4.00 ± 0.94	3.85 ± 0.84
	5	**n.a.**	**6.13 ± 0.64**	**5.56 ± 1.05**	3.75 ± 0.99
	7	**n.a.**	**n.a.**	**7.63 ± 1.38**	**5.75 ± 1.27**
Strawberries	0	3.53 ± 0.65	3.53 ± 0.65	3.53 ± 0.65	3.53 ± 0.65
	3	4.50 ± 0.52	4.62 ± 1.04	4.50 ± 0.74	4.62 ± 0.82
	5	**n.a.**	**n.a.**	**5.28 ± 1.33**	**5.28 ± 0.82**
	7	**n.a.**	**n.a.**	**8.33 ± 1.09**	**7.17 ± 1.63**

n.a., not acceptable, >5% loss of weight due to visible moulds.

Table 3
Firmness score (average ± 95% confidence interval) of packaged raspberries and strawberries using AIR, low O_2 MA, HOA, and HOA-MAP, stored at 7 °C; values above the cut-off score of 5 are indicated in bold

	Storage time (days)	AIR	Low O_2 MA	HOA	HOA-MAP
Raspberries	0	3.94 ± 0.54	3.94 ± 0.54	3.94 ± 0.54	3.94 ± 0.54
	3	**n.a.**	4.07 ± 0.88	4.21 ± 0.03	4.64 ± 0.86
	5	**n.a.**	4.81 ± 0.60	**5.81 ± 0.80**	3.50 ± 0.73
	7	**n.a.**	**n.a.**	**6.75 ± 0.81**	**5.25 ± 1.03**
Strawberries	0	4.28 ± 0.52	4.28 ± 0.52	4.28 ± 0.52	4.28 ± 0.52
	3	**5.25 ± 0.49**	**5.12 ± 1.01**	**5.12 ± 0.58**	**5.37 ± 0.73**
	5	**n.a.**	**n.a.**	**4.86 ± 0.51**	**5.28 ± 0.70**
	7	**n.a.**	**n.a.**	**7.33 ± 0.65**	**6.50 ± 1.41**

n.a., not acceptable, > 5% loss of weight due to visible moulds.

The raspberries stored in AIR and low O_2 MA were rejected on day 3 because of fungal growth. There were no significant differences in odor and color between the four packaging systems. The odor generally increased from near optimal (score 3) on day 0 to optimal (score 5) on day 7. The color, in contrast, changed from an optimal red color (score 5) on day 0 to a dark red color associated with an overmature state (score 6). These trends were equal for all four types of packaging. The raspberries treated with AIR and low O_2 MA were by day 5 infected by white filamentous moulds and *Botrytis*, respectively, preventing further sensory analysis. The raspberries treated with high O_2 atmosphere (HOA and HOA-MAP) kept an acceptable taste and firmness until day 5 (Table 3). By day 7 the raspberries stored under HOA developed a strong fermented taste due to anaerobic respiration of the fruit (Table 1). Until day 5, the general freshness of the raspberries packed under HOA got the best score. After day 7 anaerobic conditions were established and as a consequence, the general freshness decreased.

In general, strawberries and raspberries had the highest quality when treated with HOA-MAP during 7 days at 7 °C. The advantage of using a highly permeable packaging film instead of a high-barrier film existed in lower accumulation of CO_2 and depletion of O_2 in the packages. The advantage of HOA-MAP in comparison to low O_2 MA is the inhibiting effect on the growth of moulds.

Principal component analysis showed two principal components (PC) with eigen-values greater than 1 which accounted for 70.7% of the variance for strawberries and 89.4% of the variance for raspberries. Both for strawberries and for raspberries, PC1 was associated with organoleptical attributes (taste, odor and firmness), while PC2 was associated with visual properties, such as color and general freshness (data not shown).

3.5. Microbial count

Lactic acid bacteria were not important in the spoilage of strawberry and raspberry fruit as their number remained very low: about 10^3 cfu g^{-1} at day 0 to about 10^4 cfu g^{-1} at day 14. The total anaerobic count stayed under the detection limit of 10^2 cfu g^{-1}, for both fruit types. Also, the number of Enterobacteriaceae on day 0 did not exceed the detection limit, suggesting hygienic treatments before and after harvesting of the fruit.

Debevere (1996) published microbiological criteria for minimally processed fruit. The limit for yeast is fixed at 10^5 cfu g^{-1}, because above this number spoilage of products due to yeast growth becomes detectable for consumers (Fleet, 1992). On strawberries, this limit was not reached during storage (Table 4). The high O_2 atmosphere had a slight inhibiting effect on yeast growth, although it was not significant due to the variability between the packages. There was a more important growth of yeast on raspberries. The limit of 10^5 cfu g^{-1} was already exceeded at day 5 for all packaging systems (Table 4). The total psychrotrophic count on strawberries and raspberries was dominated by yeast growth and moulds. Jacxsens et al. (2001a) concluded from in vitro experiments that the yeast *C. lambica* was clearly reduced in its growth by the application of high O_2 levels: an increase in the lag phase and a reduction of the maximum specific growth rate were obtained. An O_2 level of 80 kPa alone resulted in a stimulation of both *Candida guillermondii* and *Candida sake* at 8 °C. The combined application of 80 kPa O_2 and 20 kPa CO_2 almost completely inhibited the growth of *C. guillermondii*. Similar growth characteristics were observed for *C. sake*, although to a lesser extent (Amanatidou et al., 1999). It is clear that high O_2 treatments have a reducing effect on yeast growth in vitro, but that this reduction is not that pronounced with in vivo storage experiments of raspberries and strawberries. Jacxsens et al. (2001a) found a clear reducing effect on the in vitro development of mycelium of *A. flavus* and *Botrytis cinerea* starting from 80 kPa O_2 but more pronounced at 95 kPa O_2 (balanced with N_2). The amount of spores decreased by one log through the application of O_2 concentrations above 80 kPa for *A. flavus*.

The limit for yeasts on fruit, 10^5 cfu g^{-1}, defined by Debevere (1996), has to be interpreted carefully. The fruit are not necessarily rejected by the taste panel for reason of off-flavors when yeasts have grown to $> 10^5$ cfu g^{-1}. By determining the quality of minimally processed vegetables and fruit, both microbial and sensory evaluations are important. Moreover, fruit such as raspberries already have a high initial load of yeast (approximately 10^4 cfu g^{-1}).

4. Conclusion

The strawberries and raspberries packaged in AIR or low O_2 MA had a relatively short shelf life, because of the growth of moulds (AIR) or *Botrytis* (low O_2 MA).

Applying a high O_2 atmosphere in a high-barrier film (HOA), had a beneficial effect on the microbial and sensory shelf life of raspberries and strawberries, by inhibiting the development of moulds and by maintaining fresher sensory properties, as long as atmospheric conditions did not induce fermentation. The depletion of O_2 and the accumulation of CO_2, using a high-barrier film, resulted in a deterioration of the sensory quality (odor, taste and firmness). As this was not the case with high O_2 in an low O_2 MA film (HOA-MAP), the latter system can be regarded as a promising packaging configuration for respiring fresh produce. The combination of HOA and low O_2 MA combines the advantages of both systems (i.e. inhibition of moulds and prolongation of the sensory shelf life). Because of the variability be-

Table 4
Yeast count on strawberries treated with AIR, low O_2 MA, HOA and HOA-MAP, and stored at 7 °C; values $>10^5$ cfu g^{-1} are indicated in bold (Debevere, 1996)

	Storage time (days)	AIR ($\log_{10}$ cfu $g^{-1} \pm$ SD)	Low O_2 MA ($\log_{10}$ cfu $g^{-1} \pm$ SD)	HOA ($\log_{10}$ cfu $g^{-1} \pm$ SD)	HOA-MAP ($\log_{10}$ cfu $g^{-1} \pm$ SD)
Raspberries	0	2.49 ± 0.00	2.49 ± 0.00	2.49 ± 0.00	2.49 ± 0.00
	3	3.37 ± 0.16	3.28 ± 0.46	3.14 ± 0.09	3.92 ± 1.30
	5	**5.00 ± 1.62**	**5.07 ± 0.56**	**5.19 ± 0.21**	**5.38 ± 0.25**
	7	**5.24 ± 0.05**	**5.29 ± 1.07**	**4.52 ± 0.19**	**5.89 ± 0.21**
	10	**6.18 ± 1.31**	**7.32 ± 0.20**	**5.13 ± 1.91**	**6.59 ± 0.93**
	14	**7.17 ± 0.47**	**7.68 ± 0.05**	**6.15 ± 1.20**	**7.45 ± 0.05**
Strawberries	0	3.59 ± 0.16	3.59 ± 0.16	3.59 ± 0.16	3.59 ± 0.16
	3	5.44 ± 0.23	4.40 ± 0.14	4.61 ± 0.11	4.05 ± 0.29
	5	4.00 ± 0.00	4.00 ± 0.00	3.71 ± 0.34	4.15 ± 0.64
	7	4.19 ± 1.01	4.76 ± 0.12	2.65 ± 0.92	3.39 ± 0.12
	10	4.63 ± 0.30	4.63 ± 1.45	3.39 ± 0.12	4.31 ± 0.01

tween the packages, the inhibition of yeast growth by high O_2 atmospheres cannot be considered as significant.

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References

Ahvenainen, R., 1996. New approaches in improving the shelf life of minimally processed fruit and vegetables. Trends Food Sci. Technol. 7, 179–187.

Amanatidou, A., Smid, E.J., Gorris, L.G.M., 1999. Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated micro-organisms. J. Appl. Microbiol. 86, 429–438.

BCGA, 1998. The safe application of oxygen enriched atmospheres when packaging food. British Compressed Gases Association Hampshire, UK, pp. 39.

Brown, K.M., Geeson, J.D., Dennis, C., 1984. The effects of harvest date and CO_2-enriched storage atmospheres on the storage and shelf-life of strawberries. J. Hort. Sci. 59, 197–204.

Church, N., 1994. Developments in modified-atmosphere packaging and related technologies. Trends Food Sci. Technol. 5, 345–352.

Day, B.P.F., 1998. Novel MAP—a brand new approach. Food Manufacture 73, 22–24.

Day, B.P.F., 2001. Fresh prepared produce: GMP for high oxygen MAP and non-sulphite dipping. Guideline No.31. Campden & Chorleywood Food Research Association Group, Chipping Campden, Gloshister, UK, p. 76.

Debevere, J., 1996. Criteria en praktische methoden voor de bepaling van de houdbaarheidsdatum in de etikkering. In: Etikettering, houdbaarheid en bewaring (voedingsmiddelen en recht 2). Die Keure, Brugge, Belgium, pp. 37–64.

Fleet, G., 1992. Spoilage yeasts. Crit. Rev. Biotechnol. 12, 1–44.

Garcia, J.M., Medina, R.J., Olias, J.M., 1998. Quality of strawberries automatically packaged in different plastic films. J. Food Sci. 6, 1037–1041.

Hertog, M.L.A.T.M., Boerrigter, H.A.M., van den Boogaard, G.J.P.M., Tijskens, L.M.M., van Schaik, A.C.R., 1999. Predicting keeping quality of strawberries (cv. 'Elsanta') packed under modified atmospheres: an integrated model approach. Postharvest Biol. Technol. 15, 1–12.

Hoogerwerf, S.W., Moezelaar, R., Kets, E., 2000. Effect of novel high oxygen atmospheres on growth of food related associated moulds. In: Second International Symposium on Food Packaging—Ensuring the Safety and Quality of Foods, Vienna, pp. 107.

Jacxsens, L., Devlieghere, F., Debevere, J., 1999. Validation of a systematic approach to design Equilibrium Modified Atmosphere packages for fresh-cut produce. Lebensm.-Wiss. u-Technol. 32, 425–432.

Jacxsens, L., Devlieghere, F., De Rudder, T., Debevere, J., 2000. Designing Equilibrium Modified Atmosphere packages for fresh-cut vegetables subjected to changes in temperature. Lebensm.-Wiss. u-Technol. 33, 178–187.

Jacxsens, L., Devlieghere, F., Van der Steen, C., Debevere, J., 2001a. Effect of High Oxygen Atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce, Int. J. Food Microbiol., Int. J. Food Microbiol. 71, 197–210.

Jacxsens, L., Devlieghere, F., Van der Steen, C., I. Siro, Debevere, J., 2001b. Application of ethylene adsorbers in combination with high oxygen atmospheres for the storage of strawberries and raspberries, in: 8th International Conference of Controlled Atmospheres, Rotterdam, the Netherlands.

Joles, D.W., Cameron, A.C., Shirazi, A., Petracek, P.D., 1994. Modified-atmosphere packaging of 'Heritage' red raspberry fruit: respiratory response to reduced oxygen, enhanced carbon dioxide, and temperature. J. Am. Soc. Hort. Sci. 119, 540–545.

Kader, A.A., Zagory, D., Kerbel, E.L., 1989. Modified atmosphere packaging of fruits and vegetables. Crit. Rev. Food Sci. Nut. 28, 1–27.

Kader, A.A., Ben-Yehoshua, S., 2000. Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. Postharvest Biol. Technol. 20, 1–13.

Kays, S.J., 1991. Postharvest Physiology of Perishable Plant Products. Van Nostrand-Reinhold, New York.

Ke, D., Goldstein, L., O'Mahony, M., Kader, A.A., 1991. Effects of short-term exposure to low O_2 and high CO_2 atmospheres on quality attributes of strawberries. J. Food Sci. 56, 50–54.

Sanz, C., Perez, A.G., Olias, R., Olias, J.M., 1999. Quality of strawberries packed with perforated polypropylene. J. Food Sci. 64, 748–752.

Shamaila, M., Powrie, W.D., Skura, B.J., 1992. Sensory evaluation of strawberry fruit stored under modified atmosphere packaging (MAP) by quantitative descriptive analysis. J. Food Sci. 57, 1168–1176.

Ohlsson, T., 1994. Minimal processing—preservation methods of the future: an overview. Trends Food Sci. Technol. 5, 341–344.

Wszelaki, A.L., Mitcham, E.J., 2000. Effects of superatmospheric oxygen on strawberry fruit quality and decay. Postharvest Biol. Technol. 20, 125–133.