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Effect of high oxygen and high carbon dioxide atmosphere packaging on the microbial spoilage and shelf-life of fresh-cut honeydew melon

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ABSTRACT

This study evaluated the potential of modified atmospheres (MAs) combining high oxygen (O_2) and high carbon dioxide (CO_2) levels to extend the shelf-life of fresh-cut honeydew melon. Firstly, the effect of MA on the growth and volatile organic metabolite production of *Candida sake*, *Leuconostoc mesenteroides* and *Leuconostoc gelidum*, which had all been previously isolated from spoiled commercial fresh-cut honeydew melon, was evaluated separately on honeydew melon agar at 7 °C. Additionally, the effect of selected MAs on the microbial, physico-chemical and sensory quality of commercial fresh-cut honeydew melon cubes was evaluated at 7 °C. The results showed that MAs with high O_2 and high CO_2 levels greatly retarded the growth, CO_2 and volatile metabolite production (i.e. ethanol, 2-methyl-1-butanol, ethyl acetate, phenylacetic acid, nonanal) of *C. sake* on honeydew melon agar; especially MAs consisting of 50% O_2 + 50% CO_2 and 70% O_2 + 30% CO_2. In contrast, the MAs evaluated only had a minor effect on the growth and volatile metabolite production of *L. mesenteroides* and *L. gelidum*. Overall, the effect of MAs on colour, juice leakage, juiciness, and firmness of fresh-cut honeydew melon was small during storage. Sensory preference was generally for fresh-cut honeydew melon cubes packaged in MA of 50% O_2 + 50% CO_2. These were still acceptable on day five of storage and had appreciably lower populations of yeasts and lactic acid bacteria, lower quantities of volatile organic compounds, but slightly stronger colour oxidation compared to honeydew melon that was packaged in air. Additionally, most of the samples packed in air had blown by day five due to the large quantity of CO_2 production during storage. Therefore, 50% O_2 + 50% CO_2 is a potential MA solution for extending the shelf-life of fresh-cut honeydew melon.

1. Introduction

Fresh-cut fruits are very perishable and usually have a shelf-life of 5–7 days at 1–7 °C. The shelf-life of fresh-cut honeydew melon is usually limited by microbial spoilage and physiological processes (Qi et al., 1999). To date, most of the studies found in literature which have evaluated the shelf-life of honeydew melon have investigated the physico-chemical and sensory changes during storage (Amaro et al., 2012; Ezura et al., 2002; Larson and Johnson, 1999; O'Connor-Shaw et al., 1994; Portela and Cantwell, 1998; Qi et al., 1999; Saftner et al., 2006; Supapvanich and Tucker, 2011). Very little is known about the relationship between the shelf-life and microbial growth and their volatile organic metabolite production of fresh-cut honeydew melon. It is well known that fresh-cut fruits are a favourable environment for the proliferation of spoilage organisms (Fleet, 2003; Garcia and Barrett, 2005; Heard, 2002; Ragaert et al., 2006a). Moreover, fresh-cut honeydew melon is particularly susceptible to microbial contamination and spoilage as it has higher pH values (5.2–5.8) in comparison with other fruits (Garcia and Barrett, 2005). Volatile organic compounds (VOCs), which are one of the most important determinants of fruit quality for the consumer (Forney et al., 2009; Perry et al., 2009), produced by spoilage microorganisms may also affect the quality of fresh-cut honeydew melon. Amaro et al. (2012) have observed that changes in the quantities of VOCs were likely to have a stronger impact on the sensory appraisal of fresh-cut melons during storage than other quality attributes such as colour, firmness or soluble solid content.

Modified atmospheres (MAs) combining reduced O_2 (2–6%) and slightly elevated CO_2 (7–15%) levels have been determined to maintain the quality of fresh-cut melons by reducing microbial growth and slowing the respiration of living tissues (Gorny, 2003; Oms-Oliu et al., 2007; Qi et al., 1999). However, low O_2 concentrations may stimulate the proliferation of anaerobic psychrotrophic microorganisms (Rojas-Grau et al., 2009). Oms-Oliu et al. (2008) observed that a 70 kPa O_2 atmosphere prevented fermentation and significantly improved the quality of fresh-cut melon during storage at 5 °C, whilst preserving its microbiological stability. MAs combining high O_2 and CO_2 levels have been shown to not only retard the growth of spoilage microorganisms, but to also have a positive effect on the sensory quality of fresh-cut bell peppers (Conesa et al., 2007) and carrots (Amanatidou et al., 2000).

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The aim of this study was to investigate the effect of various atmospheres combining various O_2 (21–70%) and CO_2 (0–50%) levels on the growth and VOC production of yeasts and lactic acid bacteria associated with the spoilage of honeydew melon cubes on a sterile simulation agar. A shelf-life study on modified atmosphere packaged fresh-cut honeydew melon cubes was also performed in order to evaluate the effect of high O_2 and CO_2 atmospheres on the evolution of microbiological, physico-chemical and sensory quality.

2. Materials and methods

The experiments performed in this study were performed in two stages. In the first part of the study, the spoilage microorganisms of honeydew melon were inoculated on honeydew melon agar which were then packaged in MAs with different combinations of initial headspace (IH) O_2 (21–70%) and CO_2 (0–50%) levels and stored at 7 °C. In the second part of the study, commercial fresh-cut honeydew melon cubes were packaged in air and selected MA and stored at 7 °C. The atmospheres evaluated in this part were selected on the basis of the results from the experiments on honeydew melon agar. The methods used in each part of this study are described in detail below.

2.1. Effect of MA on growth and volatile metabolite production of spoilage microorganisms

2.1.1. Isolates

Candida sake (FF 655) and two lactic acid bacteria (*Leuconostoc mesenteroides* (FF653) and *Leuconostoc gelidum* (HM45)) were used in this study. These isolates were determined in a preceding study to dominate the micro-flora of spoiled commercial honeydew melon cubes (results not shown). All three isolates are maintained in the culture collection of the Laboratory of Food Microbiology and Food Preservation (Ghent University, Gent, Belgium). The identification of *C. sake* was performed at BCCM/MUCL (BCCM/MUCL Agro (Industrial) Fungi and Yeasts Collection, Louvain-la-Neuve, Belgium) based on morphological, physiological and molecular (sequencing of the large-subunit rDNA D1/D2 domain and the internal transcribed spacer, or ITS rDNA) analyses. The identification of the lactic acid bacteria (LAB) was done at BCCM/LMG (Laboratory for Microbiology, Faculty of Sciences, Ghent University, Gent, Belgium) based on amplified fragment length polymorphism (AFLP) analysis.

2.1.2. Preparation and inoculation of honeydew melon agar

Honeydew melon agar was used as a simulant for fresh-cut honeydew melon. Honeydew melon juice was made by blending fresh-cut honeydew melon pieces (Braun Mr530multi Quick Heavy Duty Hand Blender, Braun, Kronberg, Germany) and squeeze-filtering the juice through a towel. The filtered honeydew melon juice was then supplemented with 1.5% Bacteriological Agar [Oxoid (Hampshire, UK)] and boiled over a Bunsen flame for 2 min in Schott bottles, after which the bottles were placed in a water bath at 48 °C. When the agar had cooled to 48 °C, a quantity of 71 ± 0.2 g of honeydew melon agar was poured into plastic packaging trays (volume = 269 ml, O_2 transmission rate (OTR) = $5.787E^{-11}$–$1.505E^{-9}$ $cm^3/m^2 \cdot s \cdot Pa$ at 23 °C, 0% relative humidity (RH), polypropylene (PP)/ethyl vinyl alcohol (EVOH), DECAPAC NV, Herentals, Belgium). The initial water activity (a_w) and pH of the honeydew melon agar were then measured in triplicate by means of a_w-kryometer (NAGY, Gaeufelden, Germany) and a SevenEasy pH meter (Mettler Toledo GmbH, Schwerzenbach, Switzerland), respectively. The a_w (0.9902 ± 0.0003) and pH (5.8 ± 0.1) of the simulation agar did not differ from those of the honeydew melon juice.

To prepare the inoculum, the *C. sake* was sub-cultured in 10 ml of sterile Sabouraud Broth [SB, Oxoid (Hampshire, UK)] whilst the LAB were individually sub-cultured in 10 ml of de Man Rogosa Sharpe broth [MRS broth, Oxoid (Hampshire, UK)] at 22 ± 1 °C for 2 days. Second sub-cultures were prepared as described for the first sub-culture. After ca. 48 h they were transferred to a refrigerator at 7 ± 1 °C for 7 h to adapt to the final incubation temperature used in the experiments. 100 μl aliquots of appropriate dilutions (ca. 10^{5-6} CFU/ml) of the temperature adapted *C. sake*, *L. mesenteroides* and *L. gelidum* were separately inoculated and spread on honeydew melon agar in trays resulting in an initial inoculation level of 10^{2-3} CFU/cm^2 of agar. Inoculated trays were individually sealed by a Traysealer (Traysealer MECA 900, DECAPAC NV, Belgium) in a high O_2 barrier film (OTR = $5.787E^{-10}$ $cm^3/m^2 \cdot s \cdot Pa$ at 23 °C, 50% RH, OPAEVOH (polyamide ethyl vinyl alcohol)/PE (polyethylene)/PP, BEMIS EUROPE Flexible Packaging, Monceau-sur-Sambre, Belgium) in the following conditions: 21% O_2 + 21% CO_2, 50% O_2 + 30% CO_2, 50% O_2 + 50% CO_2, 70% O_2 + 30% CO_2 and 21% O_2 (air), balanced with N_2 and stored at 7 ± 1 °C.

Two trays were prepared per atmosphere per isolate per sampling moment (days 0, 2, 4, 6, 8, and 10 and in some cases day 12) for the purpose of assessing the microbial growth, headspace gas composition, VOCs, and pH and sugar levels. The headspace O_2 and CO_2 levels in the tray were first measured by a headspace analyser (CheckMate 9900 O_2, O_2/CO_2 Headspace Analyser, PBI — Dansensor, Denmark) before the packages were opened. Subsequently, the packages were opened aseptically and 20 ± 0.1 g of honeydew melon agar was immediately transferred to a sterile plastic container (60 ml) and closed quickly. This sample was used for quantification of VOCs by means of SIFT-MS (selected ion flow tube mass spectrometer, Voice 200, Syft Technologies). The rest of the agar in the tray was used for the measurement of the pH and sugars and the assessment of the growth of the isolates.

2.1.3. Microbial analysis

For assessment of the growth of the isolates, ca. 10 g of agar from each sample was aseptically transferred to a sterile Stomacher bag and primary decimal dilutions were prepared by adding an appropriate volume of physiological peptone saline solution [PPS, 8.5 g NaCl; 1 g peptone per litre, Oxoid (Hampshire, UK)]. The samples were homogenized for 30 s in a Stomacher (Stomacher Lab-Blender 400, Led Techno, Eksel, Belgium). Subsequent decimal dilutions were then prepared from the primary decimal dilution in test-tubes containing 9 ml of sterile PPS. The decimal dilutions were then spread plated on Yeast Glucose Chloramphenicol agar [YGC, Bio-Rad (Marnes-la-Coquette, France)] plates for the yeast or pour plated on de Man Rogosa Sharpe agar [MRS agar, Oxoid (Hampshire, UK)] for the LAB. The plates were then incubated at 22 ± 1 °C until the colonies were sufficiently large enough for enumeration.

2.1.4. Identification and quantification of volatile organic compounds

The headspace VOCs were identified according to the GC–MS method developed by Ragaert et al. (2006b). The VOCs produced by *C. sake* and the LAB (*L. mesenteroides* and *L. gelidum*) are shown in Table 1. For those VOCs a quantification method in SIFT-MS was developed. The principle of SIFT-MS is well described by Noseda et al. (2010). Samples intended for the quantification of the VOCs (duplicates per sampling day) were stored in a freezer at −18 ± 1 °C until analysis. On the day of analysis each sample (20 g) was initially repackaged in 0.9 l of N_2 by means of the MULTIVAC packaging machine (Sepp Haggenmüller KG, Wolfertschwenden, Germany) and stored at 4 °C for at least 2 h, to allow the liquid and gas phases to equilibrate (Noseda et al., 2010). Thereafter the VOCs in the thawed samples were measured by the SIFT-MS using the multiple ion monitoring mode (MIM). Quantification of the VOCs occurred by using the reaction rate coefficients (K) and the branching rations (b) of the reaction between the precursor ions (H_3O^+, NO^+ and O_2^+) and the VOCs. The ionized masses used for quantification are presented in Table 1.

2.1.5. Quantification of sugars and acids

The concentration of sucrose, glucose, fructose, lactic acid and acetic acid in honeydew melon agar samples was determined according to the HPLC method developed by Ragaert et al. (2006b). The concentrations