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Effect of atmospheres combining high oxygen and carbon dioxide levels on microbial spoilage and sensory quality of fresh-cut pineapple

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ABSTRACT

Fresh-cut fruit such as pineapple have a very limited shelf-life. The study aims at prolonging the shelf-life of fresh-cut pineapple by means of modified atmospheres (MAs). The effect of MAs combining high O_2 (21–70%) and CO_2 (21–50%) levels on microbial spoilage and sensory quality of fresh-cut pineapple was therefore evaluated. In the first part of the study, the behaviour of two spoilage yeasts (*Candida sake* and *Candida argentea*) and one lactic acid bacterium (*Leuconostoc citreum*), which had previously been isolated from spoiled commercial fresh-cut pineapple cubes, were monitored on pineapple agar separately. In the second part of the study, the shelf-life of commercial fresh-cut pineapple cubes packaged in selected MAs was evaluated at 7 °C. The results showed that MAs combining high O_2 and high CO_2 levels had a large inhibitory effect on the growth and volatile metabolite production of *C. sake* and *C. argentea* on pineapple agar. A MA with 50% O_2 and 50% CO_2 was in both cases the most inhibitive. Although MAs induced the production of ethyl acetate by the yeasts, the quantity of ethyl acetate was much lower in high O_2 and high CO_2 than that in air due to lower yeast population density in MAs. With regards to growth, *L. citreum* was not sensitive to high O_2 and CO_2 levels. The fresh-cut pineapple packaged in air had deteriorated and were not acceptable any more by day 7, while those packaged in 50% O_2 combined with 50% CO_2, which also retarded the growth of aerobes and yeasts on pineapple cubes during storage, were still acceptable. It can be concluded that a MA with 50% O_2 and 50% CO_2 shows the best potential for extension of the shelf-life of fresh-cut pineapple.

1. Introduction

With unprecedented consumer demand for fresh-cut produce, this has become the fastest-growing segment in the food industry consistently achieving double digit growth rates (Gorny, 2005). Fresh-cut pineapple has become more and more popular as it is considered to be convenient to use compared to whole pineapple (Bierhals et al., 2011). However, the minimal processing employed may increase microbial spoilage of fruit through transfer of skin (surface) microflora to the fruit flesh where they can grow rapidly upon exposure to nutrient laden juices (O'Connor-Shaw et al., 1994). Current commercial fresh-cut pineapple products have a shelf-life of 5–7 days at 1–7 °C, limited largely by the development of off-flavours and off-odours from physiological processes and microbiological spoilage (Liu et al., 2007; Montero-Calderon et al., 2010a).

Modified atmosphere packaging (MAP), typically consisting of a combination of a lowered level of O_2 (2–6%) and an elevated level of CO_2 (7–15%), is often used for extending the shelf-life of fresh-cut pineapple by inhibiting fast-growing aerobes and slowing the respiration of living tissues (Martinez-Ferrer et al., 2002; Sandhya, 2010). However, the low O_2 levels may stimulate the proliferation of anaerobic psychrotrophic microorganisms (Rojas-Grau et al., 2009). High O_2 MAP has recently been suggested as an alternative for those using low O_2 levels to extend the shelf-life by inhibiting the growth of naturally occurring spoilage microorganisms, preventing undesired anoxic respirative processes and decay of fresh and fresh-cut produce (Wszelaki and Mitcham, 2000; Day, 2001; Jacxsens et al., 2001; Oms-Oliu et al., 2008). However, the sensitivity of different organisms to O_2 may vary greatly. Additionally, high MAP with about 99% O_2 cannot prevent the growth of *Pseudomonas fragi*, *Aeromonas hydrophila*, *Yersinia enterocolitica*, and *Listeria monocytogenes* (Kader and Ben-Yehoshua, 2000). To date, only a handful of studies have been performed in which the positive

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effect of atmospheres combining high O_2 and CO_2 on the shelf-life of fresh-cut vegetables has been reported (Amanatidou et al., 2000; Conesa et al., 2007). No studies have yet been published regarding the effect of atmospheres combining elevated O_2 and CO_2 levels on the shelf-life of fresh-cut fruit.

Volatile compounds are primarily responsible for the unique flavour characteristics that distinguish different fruit and vegetables and determine their desirability to the consumer (Forney et al., 2009). Spoilage microorganisms may produce volatile metabolites which can have an effect on the sensory quality of fresh-cut produce during storage (Ragaert et al., 2006; Amaro et al., 2012). However, as far as we know, little has been reported on the effect of atmospheres combining elevated O_2 and CO_2 levels on the behaviour of spoilage microorganisms and their volatile organic metabolite production and the sensory quality of fresh-cut pineapple during storage. Earlier research (Spanier et al., 1998) reported that fresh-cut pineapple chunks in the lower portion of containers (where the availability of O_2 may be limited) developed off-flavours associated with microbial fermentation after seven to 10 days of storage.

The objective of this study was to investigate the effect of modified atmospheres (MAs) consisting of various levels of O_2 (21–70%) and CO_2 (21–50%) on the growth and volatile organic compound (VOC) production of specific spoilage organisms (SSOs) on pineapple agar. Additionally, the effects of the headspace O_2 and CO_2 levels on the shelf-life of fresh-cut pineapple cubes were evaluated.

2. Materials and methods

This study was performed in two parts. The first part of the study evaluated the effect of initial headspace (IH) air and MAs comprising of different combinations of O_2 (21–70%) and CO_2 (21–50%) levels on the growth and volatile metabolite production of SSOs on pineapple agar at 7 °C. The second part of the study evaluated the effect of air and selected MAs on the microbial and sensory quality of commercial fresh-cut pineapple cubes stored at 7 °C. The atmospheres applied in the second part were selected on the basis of the results of the experiments on pineapple agar. The methods used in each part of this study are described in detail below.

2.1. Part 1 – effect of MAs on growth and volatile metabolite production on pineapple agar of the specific spoilage organisms of fresh-cut pineapple

2.1.1. Isolates

Two yeasts (*Candida argentea* and *Candida sake*) and one lactic acid bacterium (*Leuconostoc citreum*), previously isolated from spoiled commercial fresh-cut pineapple, were used in this study. 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 the yeasts was performed at BCCM/MUCL (BCCM/MUCL Agro (Industrial) Fungi and Yeasts Collection, Louvain-la-Neuve, Belgium) based on morphological, physiological and molecular analysis (sequencing of the large-subunit rDNA D1/D2 domain and the internal transcribed spacer, or ITS rDNA). The identification of lactic acid bacteria (LAB) was done at BCCM/LMG (Laboratorium voor Microbiologie, Faculty of Sciences, Ghent University, Gent, Belgium) based on amplified fragment length polymorphism (AFLP) analysis.

2.1.2. Preparation and inoculation of pineapple agar

Pineapple agar was used as a simulation medium for fresh-cut pineapple. Pure pineapple juice (Materne, Belgium) supplemented with 1.5% Bacteriological Agar [Oxoid (Hampshire, UK)] was boiled over a Bunsen burner flame for 2 min in Schott bottles after which the bottles were placed in a water bath at 48 ± 1 °C. Thereafter, when the agar had cooled to 48 °C, 71 ± 0.2 g of pineapple agar were poured into trays (volume = 269 mL, O_2 transmission rate (OTR) = 0.5–13 cm^3/m^2 day bar 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 pineapple agar were then measured in three duplicates by means of a_w-kryometer (NAGY, Gaeufelden, Germany) and a SevenEasy pH metre (Mettler Toledo GmbH, Schwerzenbach, Switzerland), respectively. The a_w (0.9903 ± 0.0003) and pH (3.7 ± 0.1) of the simulation agar did not differ from that of the pineapple juice.

To prepare the inoculum, the yeasts were individually sub-cultured in 10 mL of sterile Sabouraud Broth [SB; Oxoid (Hampshire, UK)] whilst the LAB was 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 in the same media as the first sub-cultures and incubated for the same duration at the same temperature. Thereafter, the second sub-cultures were transferred to a refrigerator at 7 ± 1 °C for 7 h to adapt the SSOs to the final incubation temperature used in the experiments. The temperature adapted SSOs were inoculated on the pineapple agar trays. Inoculated trays were individually sealed by a tray sealer (MECA 900, DECAPAC NV, Herentals, Belgium) using a high O_2 barrier film (OTR = 5 cm^3/m^2 day bar at 23 °C, 50% RH. OPAEVOH (polyamide ethyl vinyl alcohol)/PE (polyethylene)/PP, BEMIS EUROPE Flexible Packaging, Monceau-sur-Sambre, Belgium) in the following initial 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), all balanced with N_2 and stored at 7 ± 1 °C.

Two sealed trays per condition per SSOs were prepared for microbial growth, headspace gas composition and volatile organic compounds (VOCs), pH and sugar determination. The headspace O_2 and CO_2 levels in the tray were first measured by a headspace analyzer (CheckMate 9900 O_2, O_2/CO_2 Headspace Analyzer, PBI – Dansensor, Denmark) before the packages were opened. Subsequently, the packages were opened aseptically and a quantity of 20 ± 0.1 g of pineapple 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, sugars and microbial analysis. The analysis of these parameters was performed over a 12- or 14-day incubation period.

2.1.3. Microbial analysis

Microbial analysis was performed on days 0, 2, 4, 6, 8, 10 and 12 for yeast growth, while it was on days 0, 2, 4, 6, 9, 12 and 14 for LAB of incubation. On each day of analysis a piece of inoculated agar (*ca.* 10 g) was aseptically transferred to a sterile stomacher bag. Primary decimal dilutions of each sample were prepared by adding an appropriate volume of peptone saline solution [PSS, 8.5 g NaCl; 1 g peptone per litter, 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 PSS. The decimal dilutions were then spread plated on Yeast Glucose Chloramphenicol agar [YGC, Bio-Rad (Marnes-la-Coquette, France)] for the spoilage yeasts whilst the LAB was determined by pour plating on de Man Rogosa Sharpe agar [MRS agar, Oxoid (Hampshire, UK)]. The plates were then incubated at 22 ± 1 °C until the colonies were sufficiently large for enumeration (2–3 days).

2.1.4. Quantification of volatile organic compounds

The VOCs produced on pineapple agar by *C. argentea* and *C. sake* were previously identified by GC–MS (see Table 1). The identified VOCs were then used to develop a quantification method in the SIFT-MS. The underlying principle of SIFT-MS is well described by