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Effect of initial headspace oxygen level on growth and volatile metabolite production by the specific spoilage microorganisms of fresh-cut pineapple

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ABSTRACT

The effect of initial headspace (IH) O_2 level on the shelf-life of fresh-cut pineapple was evaluated in this study. The results showed that although the IH O_2 level had a minor effect on the growth of *Candida argentea*, *Candida sake* and *Meyerozyma caribbica* on pineapple agar, the quantities of the volatile organic metabolites produced by these yeasts was generally smaller the lower the IH O_2 level. The only exception was the production of ethyl acetate by *C. argentea*, which was higher at low IH O_2 levels. In triangle tests performed with trained panellists, pineapple cubes packaged in an IH of 5% O_2 were determined to be significantly different ($P < 0.05$) to those packaged in 21% O_2 from day 5 of storage. Preference was shown for the pineapple cubes packaged in an IH of 5% O_2. The results imply that packaging in an IH O_2 level of 5% could be used to extend the shelf-life of fresh-cut pineapple.

1. Introduction

Over the last decade fresh-cut fruit have become very popular as they are perceived as being healthy, nutritious and convenient to use and consume. They have a rather limited shelf-life of 5–7 days at 1–7 °C (Rico, Martin-Diana, Barat, & Barry-Ryan, 2007). The most frequent causes of quality loss in fresh-cut products are the results of both physiological and microbial spoilage processes, such as browning, softening, off-flavour and off-odour development (Bierhals, Chiumarelli, & Hubinger, 2011; Rojas-Grau, Oms-Oliu, Soliva-Fortuny, & Martin-Belloso, 2009).

Spoilage by yeasts is characterized by the fermentation of carbohydrates to produce CO_2, alcohol, flavour and off-flavour compounds such as acids, esters and higher alcohols (Heard, 1999; Heard, 2002). Nevertheless, different fresh-cut produce seem to exhibit different spoilage patterns related to the characteristics of the raw materials (Ahvenainen, 2002; Huxsoll & Bolin, 1989). With the exception of Spanier et al. (1998) who reported that alcohols were derived from fermentation by native yeasts during the storage of fresh-cut pineapple, no further literature could be found about the volatile organic compounds (VOCs) produced by spoilage microorganisms during the storage of fresh-cut pineapple.

Low O_2 modified atmospheres (MAs) (2–6%) may increase the shelf-life of packaged produce by reducing respiration rates, delaying senescence of living tissues, and inhibiting the growth of spoilage microorganisms (Al-Ati & Hotchkiss, 2003). Despite this potential, only a few studies have been done to evaluate the effect of low O_2 MAs on the microbial flora, shelf-life, physical and physiological aspects of fresh-cut pineapple (Liu, Hsu, & Hsu, 2007; Singh, Chonhenchob, Chantarasomboon, & Singh, 2007). Among the studies that have been performed on fresh-cut pineapple, none has evaluated the relationship between the growth of yeasts and volatile metabolite production associated with pineapple spoilage. The production of certain VOCs may have a negative effect on the sensory quality of fruit (Amaro, Beaulieu, Grimm, Stein, & Almeida, 2012).

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The aim of this study was to investigate the effect of initial headspace O_2 (IH O_2) level on the microbial and sensory quality of fresh-cut pineapple. This study is the first part of a larger study with the major aim of optimizing the shelf-life of fresh-cut fruit *via* MAP.

2. Materials and methods

2.1. Isolates

Candida argentea (FF 581), *Candida sake* (FF 641) and *Meyerozyma caribbica* (FF 604), previously isolated from spoiled commercial fresh-cut pineapple, were used in this study. These yeasts are maintained in the culture collection of the Laboratory of Food Microbiology and Food Preservation (Ghent University, Gent, Belgium). The identification of these spoilage yeasts performed at MUCL (B-1348 Louvain-la-Neuve, Belgium) was based on morphological, physiological and molecular analysis (sequencing of the large-subunit rDNA D1/D2 domain and the internal transcribed spacer, or ITS rDNA).

2.2. Preparation and inoculation of pineapple agar

Pineapple agar was used as a fresh-cut pineapple simulant. Pure pineapple juice (Materne, Belgium) was supplemented with 1.5% Bacteriological Agar [Oxoid (Hampshire, UK)] and boiled in Schott bottles over a Bunsen burner flame for two minutes. Thereafter it was cooled in a water-bath at 48 °C. Subsequently, 20 ± 0.2 g of pineapple agar was poured into petri plates.

To prepare the inoculum, each yeast was individually subcultured in 10 ml of sterile Sabouraud Broth [SB, Oxoid (Hampshire, UK)] and incubated at 22 ± 1 °C for two days. Second subcultures were prepared in SB and incubated as described above after which they were incubated at 7 °C for 7 h to adapt the yeasts to the storage temperature used in the experiments. 100 μl aliquots of an appropriate dilution of the temperature adapted yeasts (with *ca.* 10^5 CFU/ml) were separately inoculated and spread on pineapple agar plates resulting in an initial inoculation level of 10^{2-3} CFU/cm^2 of agar. The inoculated plates were then individually packaged in a high O_2 barrier film (NX90, EuralPack, Wommelgem, Belgium) in IH O_2 levels of 0, 1, 3, 5 and 21%, balance N_2. This barrier film has an oxygen transmission rate (OTR) of 2 cm^3/m^2 d at 23 °C, 1 bar of O_2 partial pressure and 85% relative humidity (RH). For each time of analysis, six plates were packaged per yeast per condition. The packaging machine consisted of an MULTIVAC A300/42 packaging machine (Sepp. Haggenmüller KG, Wolfertschwenden, Germany) combined with a gas mixing unit (WITT, Vilvoorde, Belgium). A pineapple agar to gas ratio of 1/4 (v/v) was used. The packaged plates were then incubated at 7 °C.

Sample analysis was performed on days 0, 2, 4, 6 and 8 for *C. argentea* and *C. sake*, and days 2, 4, 6, 9, 12 and 15 for *M. caribbica*. Two of the six plates were used for identification and quantification of the VOCs as described in Section 2.3. The remaining two plates were used for measurement of the pH, headspace O_2 and CO_2 levels and enumeration of yeasts. The headspace O_2 and CO_2 levels were measured by a headspace analyser (CheckMate 9900 O_2, PBI - Dansensor, Denmark).

2.3. Identification and quantification of volatile metabolites

The headspace VOCs were identified according to the GC–MS method developed by Ragaert et al. (2006). Quantification of the identified VOCs by Selected Ion Flow Tube - Mass Spectrometry (SIFT-MS) (Syft Technologies Ltd, New Zealand) was done according to the method described by Noseda et al. (2010).

2.4. Growth assessment

The headspace O_2 and CO_2 levels in two packaged plates (duplicates) were first measured. Thereafter, one half of the agar in each plate was used for determination of the pH whilst the other half was used for enumeration of the yeasts. The yeasts were enumerated by aseptically transferring the agar into a sterile stomacher bags after which primary decimal dilutions of each sample were prepared by in physiological peptone saline solution (PPS, 8.5 g NaCl; 1 g peptone per litre). The primary dilutions were then homogenized in a stomacher. Subsequent decimal dilutions were then prepared and spread plated on Yeast Glucose Chloramphenicol agar [YGC, Bio-Rad (Marnes-la-Coquette, France)]. The YGC plates were incubated at 22 ± 1 °C until the resultant colonies were large enough to be enumerated (*ca.* two days).

2.5. Effect of IH O_2 level on the quality of fresh-cut pineapple

2.5.1. Packaging

Fresh-cut pineapple cubes (each 1 ± 0.2 cm thick and 7–9 g) were prepared by a commercial processor. Whole pineapples were first washed by cold non-chlorinated water (7–8 °C) for *ca.* 5 min. After peeling and cutting, the pineapple cubes were dipped in a cold 1% ascorbic acid solution for *ca.* 2 min. The pineapple cubes were then delivered to our laboratory within three hours of processing. 120 ± 1 g of pineapple cubes were aseptically transferred into PP/EVA trays (DECAPAC NV, Herentals, Belgium) with an OTR of 0.5–13 cm^3/m^2 d at 23 °C, 1 bar of O_2 partial pressure and 0% RH. Thereafter the trays were sealed in the desired atmospheres: air, 1% O_2 and 5% O_2 (balance N_2). A high barrier film (BEMIS EUROPE, Monceau-sur-Sambre, Belgium) made of PA/EVA/PE with an OTR of 5 cm^3/m^2 d at 23 °C, 1 bar of O_2 partial pressure and 50% RH, was used to seal the trays by means of a Tray sealer (MECA 900, DECAPAC NV, Belgium). The trays were then stored at 7 °C for up to 5 days.

2.5.2. Microbial quality of fresh-cut pineapple

The yeasts and lactic acid bacteria (LAB) counts of the packaged pineapple cubes were determined in duplicate on days 0, 1, 3, 5 of storage. The sampling procedure was the same as the one used in the experiment performed on pineapple agar (see Section 2.4), however, larger sample sizes were collected for determination of the pH (*ca.* 15 g) and for microbiological analysis (*ca.* 30 g). LAB were enumerated by pour plating the decimal dilutions on de Man Rogosa Sharpe agar [MRS agar, Oxoid (Hampshire, UK)] whose pH had been adjusted to pH 5.7 with sorbic acid. The plates were incubated at 22 ± 1 °C until the resultant colonies were sufficiently large for enumeration (*ca.* three days).

2.5.3. Volatile metabolite production on fresh-cut pineapple

On each day of analysis, 20 g of pineapple were aseptically collected from each tray and put in a 60 ml plastic container and stored at −18 °C until analysis. Quantification of the VOCs by SIFT-MS was performed as described previously in Section 2.3.

2.5.4. Sensory quality of fresh-cut pineapple

Triangle tests were used to determine the effect of the IH O_2 level on the sensory quality of pineapple cubes during storage. The tests compared samples packaged in 21% O_2 (the reference condition) to those packaged in 1 and 5% O_2 (balance N_2). The sensory evaluation was performed at Lavetan NV (Turnhout, Belgium) by 11–13 trained panellists.