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Original Research Article

Biochemical markers defining growing area and ripening stage of imported avocado fruit cv. Hass

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ABSTRACT

Intra-varietal differences in avocado fruit composition were investigated with regard to fruit growing area, maturity, ripening stage and storage conditioning. In particular, mesocarp nutrients such as fatty acids and C7 sugars were investigated as they relate to fruit origin and ripening stage, respectively. The effect of storage temperature on nutrient level was also assessed. Fruit from Chile, Peru and Spain and harvested in the respective early, middle and late season were ripened for seven days at 18 or 23 °C. At specific intervals, mesocarp fatty acids and sugar profiles were identified. The oil composition differed according to origin and harvest-time, suggesting oleic acid as a potential marker in distinguishing fruit origin. Chilean fruit had higher oleic content (57–61%) follow by Spanish (54–60%) and Peruvian (40–47%) fruit. In early season fruit D-mannoheptulose content decreased during shelf life from 128 to 23.5 mg g^{-1} (Chile), from 115 to 33.6 mg g^{-1} (Peru), from 65.2 to 23.5 mg g^{-1} (Spain). A similar trend was noted in middle and late season fruit and from the three origins. For the first time a relationship between C7 sugar content and mesocarp softening, fruit maturity and origin was identified.

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1. Introduction

Avocado (*Persea americana* Mill.) is a tropical-subtropical fruit highly appreciated in European countries. Particular in the UK, year round consumption is supported by a wide range of suppliers (McGrath et al., 2008). This leads to increased intra-varietal differences in fruit composition as well as difficulties in controlling and forecasting avocado fruit ripening, which are major causes of concern among consumers and importers (Adam Shaw, private communication).

Avocado fruit is unique in its nutritional value due to the high oil content mainly represented by unsaturated fatty acids. Generally, the oil fraction can be up to 70% of the mesocarp dry matter (Landahl et al., 2009) and is mainly composed of the monounsaturated oleic acid (from 50% to 60% of the fatty acid content), saturated palmitic acid (15–20%), unsaturated palmitoleic (6–10%), polyunsaturated linoleic (11–15%) and linolenic acid (*ca.* 1%) (Ozdemir and Topuz, 2004; Meyer and Terry, 2008; Landahl et al., 2009). Variations in oil content and composition have been observed across growing regions (Landahl et al., 2009), cultivar (Gomez-Lopez, 1999), harvest time (Ozdemir and Topuz, 2004) and also can differ spatially within the fruit (Landahl et al., 2009). Changes in the fatty acid content have been found to be affected by postharvest handling; however, the influence on oil quality is minor (Ozdemir and Topuz, 2004). Moreover, late harvest fruit, defined by higher oil content (Ozdemir and Topuz, 2004), tends to soften faster (Cutting and Wolstenholme, 1992) and be more susceptible to physiological disorders (Cutting et al., 1992). No specific relationship has been found between the lesser fruit quality and the higher mesocarp dry matter or oil content in late season fruit (Hofman et al., 2000).

Other compounds present in avocado mesocarp which have been reported to have bioactive properties are the non-structural carbohydrates with a seven carbon structure, D-mannoheptulose and perseitol (Meyer and Terry, 2010). These uncommon sugars

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(Liu et al., 1999) along with an unidentified 'tree factor' (Adato and Gatiz, 1974) have been hypothesized as being responsible for the inhibition of ripening in avocado fruit while attached to the tree. Avocado is one of the most sensitive fruits to ethylene after harvest (Pesis et al., 1978), yet the regulatory mechanisms which prevent fruit from ripening while still on the tree are still not completely understood. It has been hypothesised that the carbon seven (C7) sugars are the main energy source in avocado fruit (Bertling and Bower, 2005), are possible ripening regulators (Liu et al., 1999) and may contribute to the antioxidant potential of the mesocarp (Tesfay et al., 2010). Variation in the sugar content has been reported for fruit from different harvest season, cultivar, tissue type and tissues region (Bertling and Bower, 2005; Landahl et al., 2009). In contrast, the involvement of the principal non-structural carbohydrates, mannoheptulose and perseitol, in fruit shelf life is better established and can be influenced by storage conditions (Liu et al., 1999, 2002).

Besides the management of the postharvest environment with the intent to extend fruit life (Woolf et al., 2005; Zhang et al., 2011), there has been increased scientific awareness of the influence of preharvest factors on the determination of postharvest avocado fruit quality (Ferguson et al., 1999; Arpaia et al., 2004).

There is still a lack of literature on the effect that pre-and postharvest factors have on imported avocado fruit bioactive compounds and consequently often poor control of the ripening process. This is due to the unique nature of avocado fruit and to the limited number of studies comparing fruit across different growing conditions. Nevertheless, the presence of different suppliers and the characteristic poor predictability of avocado fruit ripening present a problem to importers in the European market. As previously stated, many preharvest factors can determine the composition of this fruit, which in turn influence postharvest ripening behaviour and final fruit quality. Moreover, it is not clear yet as to the role that C7 sugars have on fruit metabolism during ripening or the influence of fruit origin on oil content and composition (Woolf et al., 2003; Landahl et al., 2009).

The aim of this work was to compare the effect of different preharvest conditions (such as growing area and harvest time) on the main bioactive compounds changes during ripening of imported avocado cv. Hass fruit. Accordingly, fruits imported into the UK in 2008–09 from the main supplier areas *viz.* Chile, Peru and Spain harvested in each of the three main commercial periods (early, middle and late season) were investigated for differences in fatty acid and sugar profiles during ripening. The relationship between growing areas, maturity at harvest and ripening stage, and the mesocarp oil composition and sugar levels are investigated herein variability within each origin was not studied.

2. Materials and methods

2.1. Plant material

Avocado fruit cv. Hass ($n = 720$) of commercial size 16 (236–265 g) were sourced in 2008 from Malaga, Spain, in February, March and April; from La Libertad, Peru, in May, June and July 2008 and from Chile, in August, October and January 2009 from the Quillota Province. All fruits were imported by Mack Multiples (Paddock Wood, Kent, UK) and were held at 5–6 °C until they reached the UK. Due to the different geographical locations of the suppliers, fruit had different transit times; Spanish fruit had a transit time of < 10 days, Peruvian 33–39 days and Chilean fruit 35–37 days. Once in the laboratory, the fruit were held at 5 °C overnight until experiments commenced. Fruits were not pre-treated with 1-methylcyclopropene.

2.2. Experimental design

A total number of nine experiments, one experiment for each harvest time (3) for each country (3), were carried out during one year (February 2008–January 2009). For each experiment, 80 fruit were ripened at either 18 or 23 °C in two Sanyo incubators (model MLR-350HT, Sanyo Ltd, Osaka, Japan) for a total of 240 fruits from each origin. For each experiment, fruits were randomly used for the assessment of ripeness, analysis of fatty acids and sugar content throughout storage. At day 0 (after cold storage) 16 fruit were randomly chosen and measured. The rest of the fruit ($n = 32$) were arranged into two separated test chambers with an internal temperature of 18 or 23 ± 0.5 °C and sampled at specific intervals (day 1, 2, 4 and 7) of shelf life ($n = 4 \times 8 = 32$).

2.3. Ripening assessment

Softening of the mesocarp was measured as the decrease in firmness with an Instron Universal Testing Machine (Model 5542, High Wycombe, Bucks., UK) with a 500 N load cell as previously described by Meyer and Terry (2010) with a flat-head 8 mm probe and a crosshead speed at 20 mm min^{-1}. Firmness was expressed as the maximum force required for mesocarp tissue failure and recorded as maximum load (N). Data were analysed with Bluehill 2, version 2.11, Instron. Measurements were taken on two opposite sides located in the equatorial zone of each fruit. A small part of the skin was removed just before the measurement was taken to allow direct contact of the probe with the mesocarp tissue.

2.4. Biochemical analysis

After ripening measurement, fruits were cut vertically in half. The stone and the peel were removed manually and the mesocarp was immediately chopped into small chunks, mixed and then pooled. Approximately 30 g of the pooled sample was snap-frozen in liquid nitrogen and held at −40 °C before being freeze-dried for 7 days in Edwards Modulyo (Crawley, West Sussex, UK). Dry matter content (DM) was recorded and samples stored at −40 °C until analysed.

All chemicals used were of analytical grade. Hexane and methanol were purchased from Fisher Scientific Chemicals (Loughborough, Leics, UK); methyl palmitate, methyl palmitoleate, methyl oleate, methyl linoleate, methyl linolenate, sucrose, glucose, fructose and mannoheptulose external standards were purchased from Sigma (Gillingham, Dorset, UK). Perseitol (D-glycero-D-galacto-heptitol) was provided by Industrial Research Ltd. (IRL – Fine Chemical, Lower Hutton, New Zealand).

2.5. Oil extraction and non-structural carbohydrate extraction

Oil was extracted from the fruit mesocarp according to Meyer and Terry (2008) from ground lyophilized mesocarp tissue (1 g dry weight) and homogenized repeatedly in hexane and filtered. The method used to analyse fatty acids and oil content was previously compared with standard methods from Meyer and Terry (2008). The oil was stored in amber vials under nitrogen at −40 °C until use. The powder recovered after oil extraction was stored at −40 °C for the sugar analysis as previously described (Meyer and Terry, 2008) with modification. Briefly, 100 mg of powder residue was combined with 3 mL of 62.5% aqueous methanol (v/v) in a water bath (55 °C for 15 min) (Terry et al., 2007). Samples were filtered (syringe filters 0.2 μm pore diameter; Millipore Corp., Bedford, MA, USA) and stored at −40 °C until needed. Before analysis extracts were diluted 1:10 with water (HPLC grade).

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