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Chemical changes during myrtle (*Myrtus communis* L.) fruit development and ripening

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ABSTRACT

The chemical composition of fruit belonging to 'Barbara' and 'Daniela' myrtle cultivars was monitored during development from fruit-set to an over-ripe stage (July–January), with the aim to identify a reliable maturity index. Acidity, pH, reducing and total sugars, phenols, tannins, anthocyans, carbon dioxide and ethylene production rates were monitored over two different year seasons. Titratable acidity decreased during maturation, with significant differences due to cultivar and year of observation. Reducing sugars increased in both cultivars approximately sevenfold from fruit set to complete maturation. Total sugar content increased similarly ranging from 1.43% and 1.41% at fruit set to 8.28% and 7.56% at maturation for 'Barbara' and 'Daniela', respectively. Total phenols and tannins occurred at high levels after fruit set and declined during development. Anthocyans levels increased, in both cultivars, according to a sigmoid curve. The pattern of respiration rate showed a gradual decline in both cultivars ranging from 365.81 and 396.42 mg kg^{-1} h^{-1} to 79.98 and 52.27 mg kg^{-1} h^{-1}, respectively for 'Barbara' and 'Daniela' in 2006. A peak of variable size was observed in October–November period. Small increases in ethylene production have been detected during fruit development ranging from 130.57 and 269.14 μL kg^{-1} h^{-1} measured at the onset of development to 13.04 and 19.36 μL kg^{-1} h^{-1} measured at harvest for 'Barbara' and 'Daniela', respectively.

1. Introduction

Myrtle (*Myrtus communis* L.) is an evergreen shrub 1.5–3 m tall which grows spontaneously in the Mediterranean area. Its aromatic properties have long been known. Several studies have focused on the antioxidant (Alamanni and Cossu, 2004), antifungal and antiviral (Zolfaghari et al., 1997) properties of myrtle extracts. Recently medical literature have confirmed the antimicrobial properties (Deriu et al., 2007) and assessed anticancer activity of some myrtle compounds (Tretiakova et al., 2008). Despite its pharmaceutical properties, myrtle importance has recently grown in association with the increasing success of the liqueurs produced by its fruits and leaves. Liqueur production accounts for more than three million litres per year and its success is still increasing (Mulas and Cani, 1999; Mulas et al., 2002a). In the last 10 years a cultivar selection programme was carried out in order to provide raw material to industry (Mulas et al., 1999). At present several cultivars are available and myrtle industry is increasingly supported by crop yields (Mulas and Melis, 2008).

The commercial success has driven the attention on quality aspects of myrtle liqueur. Investigations are mainly focused on the effect of different technological techniques applied during maceration (Tuberoso et al., 2007) and on preservation of myrtle quality and stability. Phenols and anthocyanidins have been identified as key compounds responsible for myrtle liqueurs quality and organoleptic properties (Montoro et al., 2006; Tuberoso et al., 2007). Liqueurs with higher amounts of anthocyanine had better colour parameters and maintained superior organoleptic properties. Trials on myrtle hydroalcoholic extracts revealed the influence of different myrtle selections on total amount of anthocyanine content, while no differences were observed in the qualitative composition (Tuberoso et al., 2007). Phenolic compounds and anthocyanins, in particular, are the most important phytochemicals in myrtle berries as well (Moyer et al., 2002).

Myrtle berry chemical composition is well documented (Aydin and Öczan, 2007) but less is known about the evolution, during fruit development and ripening, of compounds responsible of myrtle antioxidant and organoleptic properties, (tannins, phenols, anthocyanins) and of other quality parameters (pH, acidity and sugar content).

The maturity index, for a horticultural commodity, is a measurement or a set of measurements that can be used to define whether a particular commodity is mature and implies the definition of mea-

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surable points during commodity development that can fit well with maturity (Crisosto, 1994). The parameters often used as indicators of maturity, like fruit size, titratable acidity, soluble solids content, peel colour, starch and sugar content are affected by several factors. Fruit size, titratable acidity and soluble solids contents depends on the cultivar and vary in relation to crop load, environmental conditions and cultural practices (Kingstone, 1992). Fruit weight, colour, firmness and fruit calcium content vary in relation to cultivation sites and soil characteristics (Guerra and Casquero, 2009).

In this context the use of a single parameter to define fruit maturity is not recommended but more than one parameter should be used (Kingstone, 1992). Moreover the choice of a proper harvest index should reflect the quality requirements, of the harvested product, needed for consumption or transformation.

Based on these considerations, the aim of this work was to identify, through the study of the evolution of fruit chemical composition during development, reliable maturity indices, useful to establish the optimal harvest period for industrial purposes. The influence of some main cultural variables such as year and cultivar has been also evaluated.

2. Materials and methods

2.1. Plant material

The investigation was carried out over two harvesting seasons, during 2005 and 2006 on 'Daniela' and 'Barbara' myrtle cultivars (Mulas et al., 2002b) grown in an experimental orchard located in Alghero (Italy, 40° 39' N, 8° 21' E, 39 m a.s.l.).

Plants were 7 years old, planting distance was 1 m × 3 m and plants were trained as a free bush of 1.5 (height) × 1 (width) m size. The orchard was irrigated with two drip water supplies of about 200 m^3 per hectare each. No soil tillage, fertilization and pest treatments were provided. Only weed cutting was performed. The rainfalls and the air temperature were recorded at a nearby station and reported in Fig. 1. During fruit development, from fruit-set in August (30 days after full blossom) to January (over-ripe fruit stage), 1 kg of berries were monthly harvested from 20 plants selected among a total of one hundred and arranged in five replications of four plants per cultivar each, and transferred to the laboratory. Each replication was divided into two 100 g sub-samples. Fruits of the first sub-sample were immediately used to measure fresh weight, respiration rate and ethylene production, while fruits of the second group were frozen and stored at −20 °C until analysis.

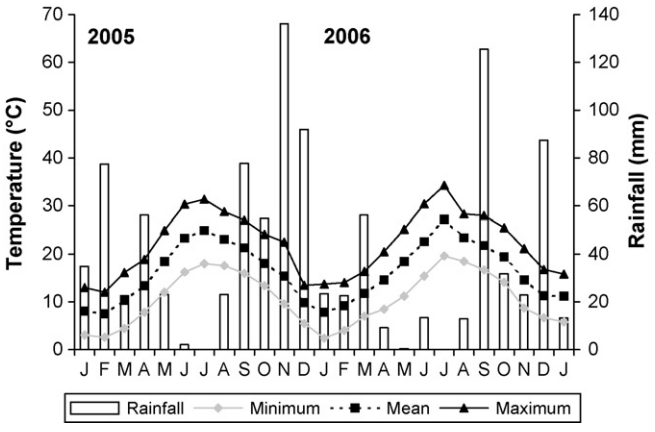

Fig. 1. Mean of maximum, minimum and average temperatures and total rainfall as recorded monthly at Alghero in the North West of Sardinia (Italy) in 2005 and 2006.

2.2. Fruit weight and moisture content

For moisture content, fruits were arranged into five replicates of 50 fruits each, weighted for fresh weight, kept at 105 °C and measured after 24 and 48 h of dehydration. Dry weight was expressed as the mean of 50 dried fruit weight, while the fruit dry matter was the percentage of the fruit dry weight over fresh weight.

2.3. Chemical composition during fruit development

2.3.1. Titratable acidity, reducing and total sugars

Five replicates of 100 g fruits were grinded and used for analysis.

Titratable acidity was measured by titrating, with 0.1 mol/L NaOH to pH 8.2, 10 g of grinded fruits blended with 40 mL of distilled water. The results were expressed as percentage of malic acid.

Sugars were analysed by diluting 20 g of grinded fruits with 50 mL of a saturated solution of calcium carbonate and left overnight. Clarification of the solution was performed by adding 10 mL of lead acetate and subsequently 10 mL of sodium oxalate. The solution was then filtered and used to determine reducing and total sugars. Reducing sugars were assessed according to the Fehling method. Briefly a standard solution with 5 mL of Fehling A, 5 mL of Fehling B and 40 mL of distilled water was titrated by adding the sugar solution previously prepared, until the complete colour turned. Reducing sugars were expressed as percentage of fresh weight.

Total sugars content was analysed according to the Fehling method after inversion in acid environment of non-reducing sugars. Inversion was performed by adding 5 mL of hydrochloric acid and heating the sugar solution for 15 min in a thermostatic bath at 67–70 °C. After rapid cooling some drops of phenolphthalein and NaOH were added until the solution reached a pink colour. Before the determination with Fehling solutions, acetic acid was added. Total sugars were expressed as percentage of fresh weight. The total sugar/acidity ratio was calculated from data collected throughout fruit development.

2.3.2. Total Phenols, tannins and anthocyanins

Phenolic compounds were extracted according to Franco et al. (2002) and determined with a Cary 1E spectrophotometer (Varian, Palo Alto, CA, USA).

Frozen fruit samples of 10 g each were grinded, transferred in a flask and diluted in 100 mL of acid methanol (0.1% hydrochloric acid). The flask was stored for 1 h in the dark. The extract was then filtered and the volume was adjusted to 100 mL with acid methanol and stored at 4 °C in the dark until analysis. For anthocyanin content determination 1 mL of the diluted methanolic extract was added to a reaction solution containing 1 mL of acidified ethanol and 10 mL of hydrochloric acid 1 N. After 30 min of incubation, the absorbance was read at 525 nm against a blank prepared with 1 mL of the extract, 1 mL of ethanol acidified and 10 mL of a buffer solution at pH 3.5. Anthocyanin content was calculated using the calibration curve designed reading the absorbance of malvidine solutions at six different concentrations and expressed as mg/100 g of fresh fruit.

Tannins were assayed in a reaction solution containing 4 mL of the diluted (1/25) methanolic extract, 2 mL ethanol, 4 mL vanillin solution (1% vanillin in 70% sulphuric acid). Samples were compared to a control with 4 mL of sulphuric acid instead of vanillin solution and absorbance was detected at $\lambda = 500$ nm. Tannin concentration was calculated following a calibration curve with pure catechine solution of known concentrations.

Total phenolic content was measured following the Folin-Ciocalteu colorimetric method (Georgea et al., 2005). Absorbance