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Effect of superatmospheric oxygen storage on the content of phytonutrients in 'Sanguinello Comune' blood orange

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ABSTRACT

The effect of cold storage under oxygen-enriched atmosphere on nutritional quality of 'Sanguinello Comune' blood oranges was investigated. The fruit were kept for 40 days at 10 °C in hermetically closed chambers continuously ventilated with atmospheric air (control) or with oxygen-enriched air containing 76 kPa O_2 ($EnrO_2$). Superatmospheric oxygen caused a remarkable enhancement of anthocyanin accumulation in the fruit juice. By the end of storage, total anthocyanin content in the juice of the $EnrO_2$ oranges increased almost tenfold compared with the initial level (from 2.6 to 24.0 mg 100 mL^{-1}) while only threefold increase was observed in the control. The concentrations of the major anthocyaninins cyanidin 3-glucoside and cyanidin 3-(6″-malonylglucoside) in the $EnrO_2$ juice and in the control increased during the storage period twentyfold and sixfold, respectively. The dramatic enhancement of anthocyanin accumulation was accompanied by significant increase in total content of phenolic compounds and in total antioxidant activity in the $EnrO_2$ oranges while in the control these parameters did not change significantly. The phenomena observed might be related to protective response of the fruit toward oxidative stress caused by the oxygen-enriched atmosphere. At the same time, superatmospheric oxygen storage caused a significant decline in acidity, total content of soluble solids, contents of sucrose, glucose, fructose and ascorbic acid in the juice of blood oranges although these changes were relatively minor compared to the enhancement of anthocyanin accumulation. Such trends could stem from the increased respiratory activity of the fruit in the presence of high oxygen concentration; in normal air they were less pronounced or insignificant. Data obtained could have implications for processing industry as a natural way of enhancing health value, stability and color of the juice and reducing the dependence on antioxidant additives and food colorants.

1. Introduction

Health awareness has linked diet and nutrition habits with disease prevention and treatment. This brings about fruit and vegetable quality evaluation and trade to depend more and more on their nutraceutical properties (Liu, 2003). Red-fleshed varieties of sweet oranges [*Citrus sinensis* (L.) Osbeck] known as 'blood oranges' (Saunt, 2000) have been listed as one of the promising new "superfoods" due to their outstanding health properties (Sloan, 2008). In particular, drinking juice of blood oranges, but not of regular (so-called 'blond') varieties, prevented obesity symptoms in mice fed with high-fat diet (Titta et al., 2009). The blood oranges are distinguished from blond varieties by high accumulation of anthocyanins and superior antioxidant activity of the juice. Anthocyanins belong to the group of flavonoids where the aglycon moiety (anthocyanidin) is based on the flavilium ion structure. The phenolic structure allows these molecules to behave as strong antioxidant and free radical scavengers while, at the same time, the chromophore properties of the flavilium ion make anthocyanins the most common natural pigments (Treutter, 2006). They perform an array of biological functions in plant organism and play an increasingly important role in medicine as pharmacologically active compounds (Amorini et al., 2001; Wang and Stoner, 2008; Cooke et al., 2009) and in the food industry as natural colorants. However, health-promoting properties of blood oranges cannot be

Abbreviations: $EnrO_2$, oxygen-enriched air containing 76 kPa O_2; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TA, titratable acidity; TSS, total soluble solids; GAE, gallic acid equivalents; TAA, total antioxidant activity; DAD, diode array detection; UV, ultraviolet; Vis, visible; PCA, principal component analysis.

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attributed exclusively to anthocyanins. L-ascorbic acid (vitamin C) is another important phytonutrient of blood oranges accounting at harvest for about 70% of their total antioxidant activity (Arena et al., 2001). The anti-obesity effect of blood oranges was not reproduced by isolated anthocyanins implying that this phenomenon might be related to synergistic interaction of multiple juice components (Titta et al., 2009).

The blood oranges are predominantly grown in Sicily (Italy) and among them 'Tarocco', 'Moro' and 'Sanguinello' are most important commercially (Rapisarda et al., 2001). Rapisarda et al. (1998) studied the antocyanin biosynthesis throughout the harvesting period and found the highest accumulation in 'Moro' oranges followed by 'Tarocco' and 'Sanguinello'. Further studies of the anthocyanin fraction of these three varieties evidenced a similar qualitative composition with great variation of quantitative profile of the components (Dugo et al., 2003). The level of anthocyanins in blood oranges depends on preharvest (climate, cultural practices, harvesting time) and postharvest factors because the genes for their biosynthesis are regulated on transcriptional level (Lo Piero et al., 2005). As a result of unfavourable growing conditions, 'Tarocco' and 'Sanguinello' oranges are often poorly pigmented at harvest, causing significant economic losses. Cold storage stimulates anthocyanins accumulation after harvest. In 'Tarocco' oranges total anthocyanins content after 75 days at 4 °C was 8 times higher than in fruit stored at 22 °C (Rapisarda et al., 2001). The antioxidant activity in blood oranges increased after 65 days at 6 °C due to the rise in the content of all phenolic fractions (anthocyanins, flavanones and hydroxycinnamic acids) in spite of a certain decline of vitamin C. In contrast, in blond oranges the increase in total antioxidant capacity under similar storage conditions was due to the enhanced vitamin C accumulation accompanied by the decline in flavanones content (Rapisarda et al., 2008). However, as in many other *Citrus* fruits, extended storage of blood oranges at temperatures below 8 °C causes rind disorders (Schirra et al., 1998). Aharoni and Houck (1982, 1980) found that storage in oxygen-enriched atmospheres of 40 or 80 kPa O_2 for 30 days at a non chilling temperature (15 °C) caused a significant colour enhancement in the endocarp and juice of both blond and blood varieties that became deep-orange and dark-red, respectively. However, the chemical basis of these phenomena has not been investigated so far.

High-O_2 atmospheres may stimulate, reduce or have no effect on the nutraceutical content of fruit, depending on either the commodity or the storage conditions. The beneficial effect of superatmospheric oxygen storage on bioactive compounds has been demonstrated for blueberry (Zheng et al., 2003) and strawberry (Zheng et al., 2007; Ayala-Zavala et al., 2007). Conversely Allende et al. (2007) reported the decrease of polyphenols content in strawberry stored under enriched oxygen atmospheres, whilst Maghoumi et al. (2014) showed no effect of high O_2 concentration on pomegranate anthocyanins content.

The aim of the present work has been the study of the chemical changes in the composition of blood oranges caused by the superatmospheric oxygen storage.

2. Materials and methods

2.1. Chemicals

All reagents and solvents were of analytical grade and used without further purification, except for those used for instrumental analyses that were of HPLC grade. Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picryhydrazyl (DPPH), L-ascorbic acid, cyanidin 3-glucoside chloride were purchased from Sigma–Aldrich, Inc. (St. Louis, MO). Gallic acid (3,4,5-trihydroxybenzoic acid) was from Carlo Erba Reagenti SpA (Rodano, MI-Italy). Water was purified with a milli-Q system (Millipore Corporation, Billerica, MA, USA). Commercially available imazalil fungicide preparation (Deccozil 50, 44,66% a.i., Elf Atochem, Paris, France) was used for pre-storage fruit treatment after dilution in deionized water.

2.2. Fruit

'Sanguinello Comune' oranges were harvested in late March from an organically farmed grove located in north Sardinia (Italy). Once in the laboratory, the fruit were immersed in 0.2% NaOCl solution for 2 min, rinsed with distilled water and dipped in 1000 mg L^{-1} of aqueous imazalil mixture for 1 min. Then, oranges were allowed to dry at room temperature, graded and divided into two groups each of 300 fruit (5 replicate boxes of 60 fruit).

2.3. Storage trial

Boxes with fruit of each group were put into hermetically closed chambers (500 L). One chamber was continuously ventilated with atmospheric air (control) and the second one with oxygen-enriched (76 kPa O_2) air ($EnrO_2$). The oxygen partial pressure of 76 kPa was chosen as the closest value to the efficient level reported by Aharoni and Houck (1982) achievable in our system.

The oxygen-enriched gas mix was generated by an oxygen concentrator (100 DeVilbiss Healthcare, PA 15501 U.S.A.) and stable atmosphere composition was reached within 24 h. The two gas media were humidified (90% RH) and flow rates kept at 5 L h^{-1}. The storage was performed at 10 °C for 40 days and the gas composition was daily monitored using a digital O_2/CO_2 analyzer (Combi Check 9800-1, PBI-Dansensor A/S, Denmarck).

2.4. Chemical analyses

All chemical analyses were performed on centrifuged and filtered juice obtained by squeezing the fruit with a domestic juice extractor. The analyses were carried out in three replicates of fifteen fruit each at harvest and after 40 days storage. The following juice parameters were measured: pH, titratable acidity (TA), total soluble solids content (TSS), sugars composition, vitamin C content, total phenolics content, total anthocyanins content, antocyanins profile, antioxidant activity.

TSS content was determined using a digital refractometer Atago PR-101 (Atago, Tokyo, Japan) at 20 °C and results expressed in Brix degrees (°Brix). Total acidity was quantified by potentiometric titration (pH meter ORION 420A) with 0.1 N NaOH up to pH 8.2, using 5 mL of juice diluted in 50 mL distilled water. The results were expressed as percent citric acid equivalent. The pH was measured by dipping the pH-meter probe (Horion Polyplast) into the juice.

2.5. Total phenolic concentrations and total antioxidant activity

Total phenolics content was determined using the Folin–Ciocalteu assay (Singleton and Rossi, 1965) on 1 mL of juice purified on a Sep-Pak cartridge (Strata C-18-E, 500 mg–6 mL, Phenomenex) in order to remove polar and hydrophilic substances that could cause interferences. Sample purification was performed as described by Rigo et al. (2000) with some modifications: 1 mL of orange juice was loaded into the cartridge previously conditioned with 2 mL of methanol followed by 5 mL of 5 mM H_2SO_4. After washing with 5 mL of 5 mM H_2SO_4, the phenolic compounds were eluted with 5 mL of MeOH followed by 5 mL of milli-Q water into a 10 mL calibrated flask. One milliliter of the methanolic extract was added with 1 mL of Folin–Ciocalteu reagent, 10 mL of