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

Maria Giovanna Molinu[a,*], Antonio Dore[a], Amedeo Palma[a], Salvatore D'Aquino[a], Emanuela Azara[b], Victor Rodov[c], Guy D'hallewin[a]

[a] Consiglio Nazionale delle Ricerche, Istituto di Scienze delle Produzioni Alimentari, Traversa La Crucca, 3 Loc. Baldinca, 07040 Li Punti, Sassari, Italy
[b] Consiglio Nazionale delle Ricerche, Istituto di Chimica Biomolecolare, Traversa La Crucca, 3 Loc. Baldinca, 07040 Li Punti, Sassari, Italy
[c] Agricultural Research Organization, The Volcani Center, Department of Postharvest Science, Bet-Dagan 50250, Israel

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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.

* Corresponding author. Fax: +39 79 2841799.
E-mail address: mariagiovanna.molinu@ispa.cnr.it (M.G. Molinu).

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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

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7.5% sodium carbonate solution and brought to a volume of a 25 mL with milli-Q water. The reaction mixture was incubated for 120 min in the dark at room temperature and the absorbance of the resulting blue solution measured at 750 nm with a UV–vis spectrophotometer (Agilent 8453, Agilent Technologies, Palo Alto, CA, USA). The results were expressed as mg 100 mL^{-1} of gallic acid equivalents (GAE) by means of a calibration curve ($R^2 = 0.99$) prepared using five gallic acid concentrations (10, 20, 40, 80 and 100 mg L^{-1} in duplicate) processed as the samples. Total antioxidant activity (TAA) was determined with the DPPH assay according to Bondet et al. (1997) and expressed as mmol Trolox equivalents per 100 mL juice.

2.6. Anthocyanins analysis

Quantitative determination of total anthocyanins was performed spectrophotometrically by the pH differential method according to Rapisarda et al. (2000) and concentration expressed as mg 100 mL^{-1} of cyanidin 3-glucoside. Individual anthocyanins were determined in the orange juice purified as decribed for the Folin–Ciocalteu assay. One milliliter of methanolic extract, obtained from orange juice purified on Sep-Pak cartridge as previously reported, was diluted with 1 mL of trifluoroacetic acid 0.1% and analysed by HPLC. Chromatographic separation was performed with an Agilent 1100 LC System equipped with a binary pump, degaser, column thermostat, autosampler (G1313A) and a diode-array detector (DAD) (Agilent Technologies, Palo Alto, CA, USA) operating at 270 and 520 nm. The column was a Luna C18 (150 × 2.1 mm, 3 μm) from Phenomenex (Torrance, CA, USA) with a security guard cartridge (4 × 2 mm). The flow rate was set at 0.250 mL min^{-1} and the column temperature was 35 °C. Elution was carried out with a binary gradient combining solvent A (water, 0.2 % acetic acid and 0.1% trifluoroacetic acid) and solvent B (acetonitrile) as follows: at 0 min 90:10 (A:B), from 90:10 to 80:20 (A:B) in 20 min, from 80:20 (A:B) to 68:32(A:B) in 18 min, held for 7 min. Total run time was 45 min and the injection volume was 10 μL. The concentration of the single anthocyanins was calculated according to external standard method curve of cyanidin 3-glucoside (six concentrations in duplicate between 0.25-50 mg L^{-1}, $R^2 = 0.99$) and expressed as mg L^{-1} of cyanidin 3-glucoside equivalent. Identification and peak assignment of anthocyanins was based on comparison of their HPLC retention times and mass spectra with data reported in literature (Dugo et al., 2003; Hillebrand et al., 2004). Mass spectra were recorded with an Agilent G19 instrument was interfaced with an e46 (MSD 1100, Agilent Technologies, Palo Alto, CA, USA) single stage quadrupole. Thelectrospray atmospheric pressure ionization (ES-API) source used in the positive ion mode. The mass spectrometer was programmed to admit protonated molecules at mass range 270–800 *m/z*. The positive ion spray voltage was 3200 mV and the fragmentor was 85 eV. After optimization, heated nebulizer parameters were set as following: drying gas (nitrogen) heated at 350 °C and flow rate of drying gas 9.8 L min^{-1}, nebulizer gas (nitrogen) at a pressure of 289,4 Pa. Analytical data were acquired by Agilent ChemStation HP A.10.02.

2.7. Sugars and ascorbic acid analysis

HPLC analyses were performed to quantify sugars and ascorbic acid. Fructose, glucose, and sucrose concentrations in the juice were calculated according to Yang and Ming-Yu (2000). A LaChrom Merck-Hitachi liquid chromatograph (Hitachi Ltd., Tokyo, Japan) equipped with a L-7100 pump, a L-7200 autosampler, and evaporative light scattering detector (ELSD SEDEX 60LT, France) was used. Column was a carbohydrate ES column (250 × 4.6 mm, 5 μm, Alltech Italy srl, Milan, Italy) with a guard column (7.5 × 4.6 mm i.d.) thermostated at 30 °C. The mobile phase was a mixture of acetonitrile/milli Q water (75:25, v:v) at a flow rate of 1 mL min^{-1} and the injection volume was 20 μL. The ELSD detector was set as following: drift tube temperature 45 °C; nebulizer gas (air) pressure, 2.5 bar. The concentrations of fructose, glucose, and sucrose were calculated with calibration curves ($R^2 = 0.99$) using 5 standard concentrations (10, 20, 40, 80 and 100 mg L^{-1}) in duplicate.

L-ascorbic acid was quantified as described by Choi et al. (2002) with a slight modification. HPLC system was the same used for anthocynins quantification. A sample of 20 μL of diluted orange juice was injected in a liquid chromatograph (the same apparatus as for the anthocyanins determination) equipped with a Zorbax C18 column (Agilent 250 × 4.6 mm, particle size 5 μm). A 2% KH_2PO_4 solution was used as mobile phase (adjusted to pH 2.8 with phosphoric acid) at a flow rate of 0.7 mL min^{-1}. DAD was set at 254 nm. Quantification was made by a calibration curve ($R^2 = 0.99$) using L-ascorbic acid standard solutions at six concentrations between 100 and 10 mg L^{-1} in duplicate.

2.8. Statistical analysis

Analysis of variance (ANOVA) of all data was performed using the MSTAT-C software (Michigan State Univ., East Lansing, 1995) and when appropriate means separation was performed according to the Duncan's multiple range test at $P < 0.05$ or 0.01. Principal Component Analysis (PCA) was performed by an R-Based Chemometric Software (http://gruppochemiometria.it, 2014).

Table 1
Chemical analysis of 'Sanguinello Comune' orange juice extracted at harvest and after 40 days of fruit storage at 10 °C and 90% RH in regular (Control) or oxygen-enriched ($EnrO_2$) air. Q5

	At harvest	After 40 days of fruit storage	
		Control	$EnrO_2$
pH	3.30 ± 0.02[c]	3.67 ± 0.06[b]	3.94 ± 0.06[a]
Acidity(%)[d]	1.62 ± 0.06[a]	1.07 ± 0.32[b]	0.74 ± 0.15[c]
TSS (°Brix)	13.62 ± 0.36[a]	13.10 ± 0.10[b]	12.37 ± 0.06[c]
Sucrose (g 100 mL^{-1})	6.81 ± 0.08[a]	5.75 ± 0.08[b]	5.46 ± 015[c]
Glucose (g 100 mL^{-1})	2.43 ± 0.04[a]	2.36 ± 0.09[a]	1.94 ± 0.09[b]
Fructose (g 100 mL^{-1})	2.71 ± 0.03[a]	2.70 ± 0.04[a]	2.33 ± 0.05[b]
Ascorbic Acid (100 mL^{-1})	99.55 ± 2.88[a]	94.36 ± 5.70[a]	80.79 ± 2.82[b]
Total Phenols (100 mL^{-1} of GAE)	13.85 ± 1.74[a]	15.36 ± 0.82[a]	29.89 ± 1.02[b]
Total Anthocyanins (mg 100 mL^{-1})[e]	2.56 ± 0.85[c]	7.50 ± 0.34[b]	24.01 ± 0.93[a]
TAA (mmol 100 mL^{-1} of Trolox equiv)	5.99 ± 0.01[a]	5.98 ± 0.01[a]	6.76 ± 0.01[b]

Each result represents a mean ± standard deviation of three replicates, each replicate comprising pooled juice of 15 oranges. Values within a row followed by the same letter are not significantly different ($P < 0.05$) according to the Duncan's multiple range test at $P < 0.05$.
[d] As percentage of citric acid equivalents.
[e] As mg 100 mL^{-1} of cyanidin-3-glucoside.

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3. Results and discussion

3.1. Juice pH, acidity and total soluble solids content

In agreement with previous results reported by Rapisarda et al. (2001) with blood oranges kept at 8 °C, an overall increase in pH alongside with a concomitant decrease of titratable acidity occurred during storage in both treatments. These changes were accompanied by certain decline in TSS content and were more significant in fruit stored under O_2-enriched atmosphere than in regular air (Table 1). In contrast, Aharoni and Houck (1982) observed no change in pH, TA and TSS of blood oranges stored for 4 weeks at 15 °C under 80 kPa O_2 atmosphere, but this difference might be attributed to different storage conditions.

3.2. Phenolic compounds and antioxidant activity

The most striking effect of $EnrO_2$ atmosphere was the marked stimulation of anthocyanins production. By the end of storage, total anthocyanin content expressed as cyanidin 3-glucoside increased almost tenfold, from 2.6 to 24.0 mg 100 mL^{-1} of juice, a value about three times higher than that in the control (Table 1). The enhancement of anthocyanin concentration in fruit stored under $EnrO_2$ conditions was also visually detectable both in juice and in the flesh which at the end of storage exhibited a deeper red colour than the control fruit (Fig. 1).

Not surprisingly, the dramatic enhancement of anthocyanin accumulation in the $EnrO_2$ oranges was accompanied by significant increase in total antioxidant activity and in the total content of phenolic compounds. Antocyaninins accounted for at least the major part of the total phenolics content added. However, additional $EnrO_2$ effect on other phenolic compounds cannot be excluded. No significant changes of antioxidant activity and of total phenolic content were revealed in the control (Table 1).

The increase of anthocyanin content and antioxidant activity in red oranges by exposure to superatmospheric O_2 may be associated with a physiological response to oxidative stress. The involvement of the anthocyanins in plant tissue protection against oxidative damage in vivo was demonstrated by Gould et al. (2002).

It is known that anthocyanin concentration in fruit can be enhanced by different postharvest abiotic stresses induced by physical (Zhang et al., 2012; Rodov et al., 2012; Lia et al., 2014) or chemical elicitors (Baenas et al., 2014). Aharoni and Houck (1982) reported an enhanced pigmentation in oranges following storage in an oxygen-enriched atmosphere. They supposedly related it to an augmented anthocyanins synthesis, but did not show any analytical evidence of this suggestion.

The positive effect of high O_2 concentrations on nutraceutical properties has also been reported for other species. Oxygen-enriched atmosphere increased antioxidant capacity, total anthocyanins and total phenolic contents and controlled decay in cold-stored blueberries (Zheng et al., 2003) and strawberries (Zheng et al., 2007; Ayala-Zavala et al., 2007). On the other hand, the worth of applying this approach to strawberries was questioned by its negative effect on the fruit sensory characteristics (Wszelaki and Mitcham, 2000; Perez and Sanz, 2001).

3.3. Anthocyanins profile

Table 2 presents the anthocyanins identified in the juice of 'Sanguinello Comune' blood oranges while the effect of fruit storage conditions on the anthocyanin profiles is shown in Table 3. Cyanidin 3-glucoside, cyanidin 3-(6″-malonylglucoside) and peonidin 3-(6″-malonylglucoside) were the main anthocyanins accounting for 85% of the total amount at harvest. Similar to the

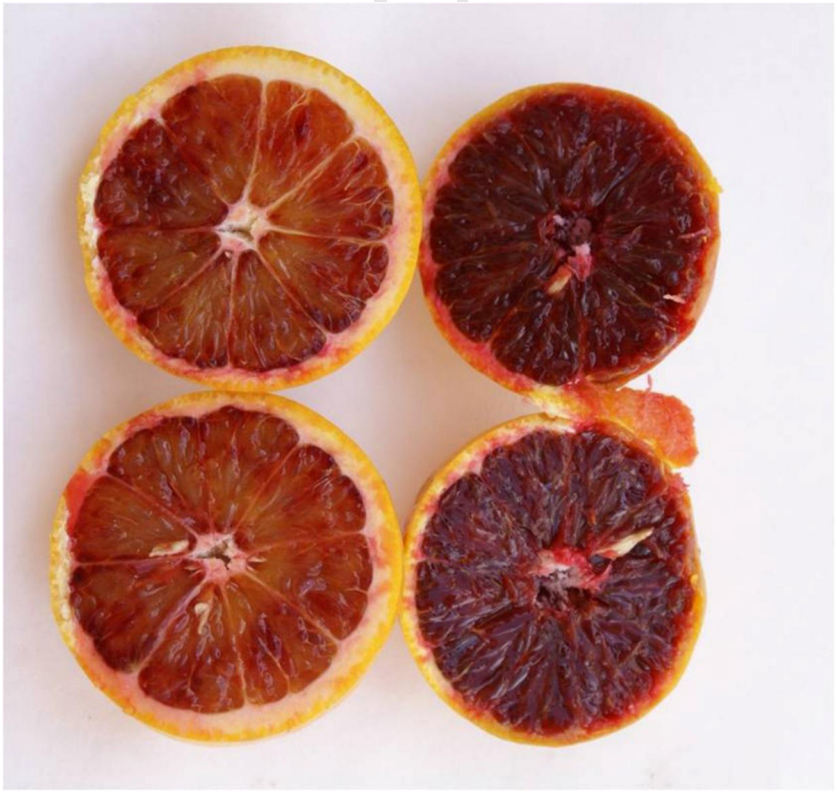

Fig. 1. Color difference in 'Sanguinello Comune' orange after 40 days of storage at 10 °C and 90% RH between Control (to the left) and oxygen-enriched (76 kPa O_2) air (to the right). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Table 2
Identification of anthocyanins in the juice of 'Sanguinello Comune' fruit.

Peak	Compound	t_R (min)	$[M+H]^+$	Fragment ions (m/z)
1	Delphinidin 3-glucoside	7.33	465	303
2	Cyanidin 3,5-diglucoside	8.1	611	449; 287
3	Cyanidin 3-galactoside	9.2	449	287
4	Cyanidin 3-glucoside	10.44	449	287
5	Delphinidin 3-(6″-malonylglucoside)	13.9	551	465; 303
6	Cyanidin 3-(3″-malonylglucoside)	14.7	535	449; 287
7	Cyanidin 3-(6″-malonylglucoside)	17.1	535	449; 287
8	Cyanidin 3-(6″-dioxalylglucoside)	18.69	593	449; 287
9	Cyanidin	19.98	287	–
10	Pelargonidin 3,5-di-(6-acetylglucoside)	21.29	679	463
11	Peonidin 3-(6″-malonylglucoside)	22.6	549	301; 463

Table 3
Anthocyanins content in the juice of 'Sanguinello Comune' orange extracted at harvest and after 40 days of fruit storage at 10 °C and 90% RH in regular (Control) or oxygen-enriched ($EnrO_2$) air[d].

Compound	At harvest	After 40 days of fruit storage	
		Control	$EnrO_2$
Delphinidin 3-glucoside	0.13 ± 0.0^c	0.80 ± 0.19^b	5.13 ± 0.72^a
Cyanidin 3,5-diglucoside	0.35 ± 0.04^c	2.05 ± 0.16^b	11.29 ± 0.37^a
Cyanidin 3-galactoside	0.20 ± 0.02^c	1.01 ± 0.04^b	4.27 ± 0.21^a
Cyanidin 3-glucoside	4.04 ± 0.61^c	26.47 ± 1.56^b	88.63 ± 2.03^a
Delphinidin 3-(6″-malonylglucoside)	0.12 ± 0.01^c	0.56 ± 0.09^b	3.75 ± 0.50^a
Cyanidin 3-(3″-malonylglucoside)	0.35 ± 0.03^c	1.67 ± 0.09^b	6.00 ± 0.21^a
Cyanidin 3-(6″-malonylglucoside)	4.62 ± 0.41^c	$33{,}64 \pm 1.07^b$	115.94 ± 1.06^a
Cyanidin 3-(6″-dioxalylglucoside)	0.53 ± 0.08^c	2.62 ± 0.16^b	11.23 ± 0.59^a
Cyanidin	0.13 ± 0.01^c	0.31 ± 0.08^b	1.67 ± 0.20^a
Pelargonidin 3,5-di-(6 -acetylglucoside)	0.15 ± 0.01^c	0.37 ± 0.03^b	1.17 ± 0.07^a
Peonidin 3-(6″ malonylglucoside)	1.10 ± 0.16^c	2.67 ± 0.27^b	9.95 ± 0.51^a
Total	11.69	72.17	259.05

[d]The values are expressed as cyanidin 3-glucoside equivalents (mg L^{-1}). Each result represents a mean ± standard deviation of three replicates, each replicate comprising pooled juice of 15 oranges. Values within a row followed by the same letter are not significantly different ($P < 0.05$) according to the Duncan's multiple range test at $P < 0.05$.

trends observed with the total anthocyanin content, the concentrations of cyanidin 3-glucoside and cyanidin 3-(6″-malonylglucoside) increased during 40 days of superamospheric oxygen storage over 20-fold, reaching 88.6 and 115.9 mg L^{-1} respectively, more than triple those in the control. Even higher increase rates were observed with some minor constituents, e.g., delphinidin 3-glucoside, cyanidin 3,5-diglucoside and delphinidin 3-(6″-malonylglucoside).

3.4. Sugars and L-ascorbic acid

Oranges stored in regular air showed no significant changes in the contents of glucose and fructose and certain decrease in sucrose content. On the other hand, superatmospheric oxygen storage resulted in decline in the contents of the three sugar constituents (Table 1). Enhanced decrease of sugars and organic acids under superatmospheric O_2 was reported for other species e.g., blueberry (Zheng et al., 2003) and strawberry (Wszelaki and Mitcham, 2000) and associated with higher tissue respiration rates although their magnitude was commodity dependent (Kader and Ben-Yehoshua, 2000).

Storage in oxygen-enriched atmosphere caused a decrease of L-ascorbic acid content by 20% while in regular air its change was not statistically significant. Similar results were reported for table grapes (Deng et al., 2005) and fresh-cut tomatoes (Odriozola-Serrano et al., 2009) stored in 80 kPa of O_2. It is noteworthy that, despite the remakable reduction in vitamin C, juice total antioxidant activity-increased during storage under $EnrO_2$ atmosphere, showing the important role played by the polyphenols and anthocyanins fraction in the overall juice antioxidant activity.

3.5. Principal component analysis

Principal component analysis (PCA) was performed on the correlation matrix produced from twenty-one attributes of blood orange juice and storage conditions (Fig. 2). The factor loadings for the different nutri-functional ratings were plotted based on the first two principal components (PC1, 2) which covered 98% of the total variance. PC1 accounted for the maximal amount of total variance (94%) while, PC2 for only 4.3%. This meant that PC1 correlated with a high number of the observed variables which were principally responsible for the separation of the objects. After the analysis of the cold storage and of air composition effects on the nutri-functional attributes the samples stored under $EnrO_2$ were situated in the right part of the score plot (Figs. 2 and 3). A close relationship occured between antixidant activty, total phenolic compounds, total and single anthocyanin values, and the $EnrO_2$ stored samples. Therefore, increase of the antioxidant capacity under $EnrO_2$ conditions was related to phenolic compounds rather than to vitamin C. Moreover, Fig. 2 highlighted that all single anthocyanins were grouped together providing a similar information. This result is important for further research showing that analysing total anthocyanins or total polyphenols may be sufficient for receiving valuable information on this topic.

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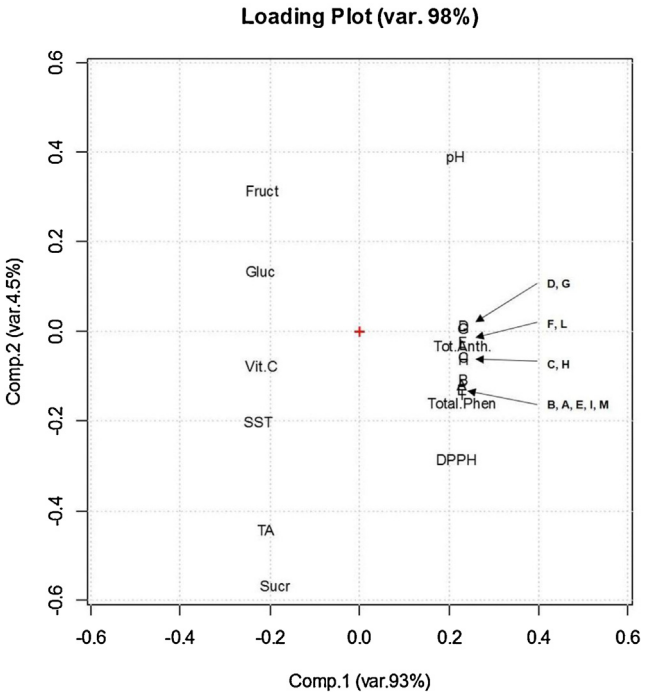

Fig. 2. PCA plot of 'Sanguinello Comune' oranges at harvest and after 40 days of storage at 10 °C and 90% RH in atmospheric (Control) or oxygen-enriched ($EnrO_2$) air. The labels A–M represent the individual anthocyanis: (A) Delphinidin 3-glucoside, (B) Cyanidin 3,5-diglucoside, (C) Cyanidin 3-galactoside, (D) Cyanidin 3-glucoside, (E) Delphinidin 3-(6″-malonylglucoside), (F) Cyanidin 3-(3″-malonylglucoside), (G) Cyanidin 3-(6″-malonylglucoside), (H) Cyanidin 3-(6″-dioxalylglucoside), (I) Cyanidin, (L) Pelargonidin 3,5-di-(6 -acetylglucoside), (M) Peonidin 3-(6″ malonylglucoside).

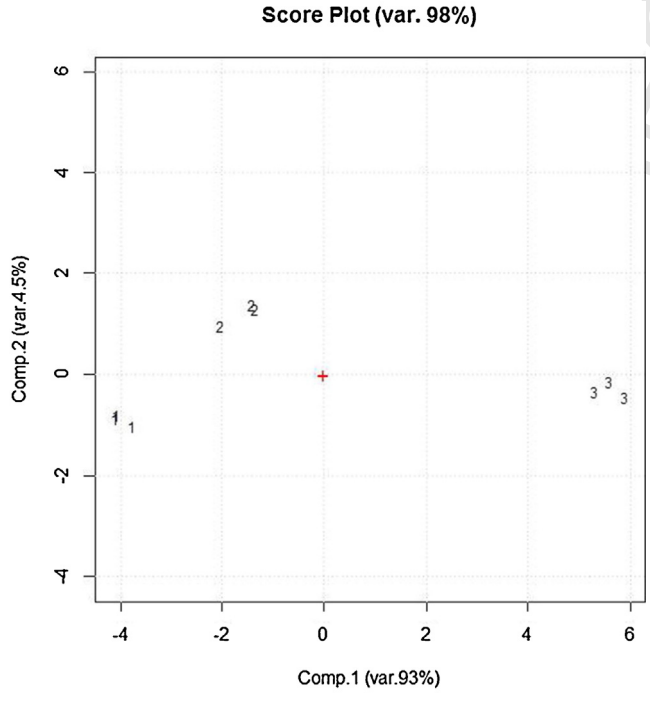

Fig. 3. Score plot of PC1 versus PC2 of all 'Sanguinello comune' samples: 1 Harvest; 2 Control; 3 $EnrO_2$.

4. Conclusion

The research has demonstrated a strategy for obtaining fruits with a higher content of antioxidant anthocyanins by postharvest stimulation of their synthesis under oxidative stress conditions. Oxygen-enriched atmosphere can fit this purpose despite the enhanced losses of sugars, vitamin C and acids. Keeping in mind that these losses were relatively minor compared with the dramatic increase in anthocyanin content, the overall effect on nutraceutical juice value was positive and could be further improved by blending the juice from $EnrO_2$-stored oranges with one from non-stored or normal air-stored fruit. In addition, further optimiztion of the storage conditions is needed in order to reach the anthocyanins stimulation with minimal undesirable side effects. Besides their health importance, the results of this study may have implications for the processing industry reducing its dependence on potentially allergenic antioxidant additives and food colorants such as carminic acid.

References

Aharoni, Y., Houck, L.G., 1980. Improvement of internal color of oranges stored in oxygen-enriched atmospheres. Sci. Hortic. 13, 331–338.

Aharoni, Y., Houck, L.G., 1982. Changes in rind, flesh, and juice color of blood oranges stored in air supplemented with ethylene or oxygen-enriched atmospheres. J. Food Sci. 47, 2091–2092.

Allende, A., Marín, A., Buendía, B., Tomás-Barberán, F., Gil, M.I., 2007. Impact of combined postharvest treatments (UV-C light, gaseous O_3, superatmospheric O_2 and high CO_2) on health promoting compounds and shelf-life of strawberries. Postharvest Biol. Technol. 46, 201–211.

Amorini, A.M., Fazzina, G., Lazzarino, G., Tavazzi, B., Di Pierro, D., Santucci, R., Sinibaldi, F., Galvano, F., Galvano, G., 2001. Activity and mechanism of the antioxidant properties of cyanidin-3-*O*-beta-glucopyranoside. Free Radic. Res. 35, 953–966.

Arena, E., Fallico, B., Maccarone, E., 2001. Evaluation of antioxidant capacity of blood orange juices as influenced by constituents, concentration process and storage. Food Chem. 74, 423–427.

Ayala-Zavala, J.F., Wang, S.Y., Wang, C.Y., González-Aguilar, G.A., 2007. High oxygen treatment increases antioxidant capacity and postharvest life of strawberry fruit. Food Technol. Biotechnol. 45, 166–173.

Baenas, N., García-Viguera, C., Moreno, D.A., 2014. Elicitation: a tool for enriching the bioactive composition of foods. Molecules 19, 13541–13563.

Bondet, V., Brand-Williams, W., Berset, C., 1997. Kinetics and mechanisms of antioxidant activity using the DPPH:free radical method. LWT-Food Sci. Technol. 30, 609–615.

Choi, M.H., Kim, G.H., Lee, H.S., 2002. Effect of ascorbic acid retention on juice color of pigment stability in blood orange (*Citrus sinensis*) juice during refrigerated storage. Food Res. Int. 35, 753–759.

Cooke, D., Brown, K., Marczylo, T.H., Cooke, D., Brown, K., Steward, W.P., Gescher, A.J., 2009. Pharmacokinetics and metabolism of the putative cancer chemopreventive agent cyanidin-3-glucoside in mice. Cancer Chemother. Pharmacol. 64, 1261–1268.

Deng, Y., Wu, Y., Li, Y., 2005. Effects of high O_2 levels on post-harvest quality and shelf life of table grapes during long-term storage. Eur. Food Res. Technol. 221, 392–397.

Dugo, P., Mondello, P., Morabito, D., Dugo, G., 2003. Characterisation of the anthocyanin fraction of Sicilian blood orange juice by micro-HPLC–ESI–MS. J. Agric. Food Chem. 51, 1173–1176.

Gould, K.S., Mckelvie, J., Markham, K.R., 2002. Do anthocyanins function as antioxidants in leaves? Imaging of H_2O_2 in red and green leaves after mechanical injury. Plant Cell Environ. 25, 1261–1269.

Hillebrand, S., Schwarz, M., Winterhalter, P., 2004. Characterization of anthocyanins and pyranoanthocyanins from blood orange [*Citrus sinensis* (L.) Osbeck] juice. J. Agric. Food Chem. 52, 7331–7338.

Kader, A.A., Ben-Yehoshua, S., 2000. Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. Postharvest Biol. Technol. 20, 1–13.

Lia, D., Luoa, Z., Moua, W., Wanga, Y., Yinga, T., Mao, L., 2014. ABA and UV-C effects on quality, antioxidant capacity and anthocyanin contents of strawberry fruit (*Fragaria ananassa* Duch.). Postharvest Biol. Technol. 90, 56–62.

Liu, R.H., 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am. J. Clin. Nutr. 78, 517S–520S.

Lo Piero, A.R., Pugliesi, I., Rapisarda, P., Petrone, G., 2005. Anthocyanins accumulation and related gene expression in red orange fruit induced by low temperature storage. J. Agric. Food Chem. 53, 9083–9088.

Maghoumi, M., Mostofi, Y., Zamani, Z., Talaie, A., Boojar, M., Gómez, P.A., 2014. Influence of hot-air treatment: superatmospheric O_2 and elevated CO_2 on bioactive compounds and storage properties of fresh-cut pomegranate arils. Int. J. Food Sci. Technol. 49, 153–159.

Odriozola-Serrano, I., Oms-Oliu, G., Soliva-Fortuny, R., Martín-Belloso, O., 2009. Effect of high-oxygen atmospheres on the antioxidant potential of fresh-cut tomatoes. J. Agric. Food Chem. 57, 6603–6610.
Perez, A.G., Sanz, C., 2001. Effect of high-oxygen and high-carbon-dioxide atmospheres on strawberry flavour and other quality traits. J. Agric. Food Chem. 49, 2370–2375.
Rapisarda, P., Bellomo, S.E., Intelisano, S., 2001. Storage temperature effects on blood orange fruit quality. J. Agric. Food Chem. 49, 3230–3235.
Rapisarda, P., Carollo, G., Fallico, B., Tomaselli, F., Maccarone, E., 1998. Hydroxycinnamic acids as markers of Italian blood orange juices. J. Agric. Food Chem. 46, 464–470.
Rapisarda, P., Fanella, F., Maccarone, E., 2000. Reliability of analytical methods for determining anthocyanins in blood orange juices. J. Agric. Food Chem. 48, 2249–2252.
Rapisarda, P., Lo Bianco, M., Pannuzzo, P., Timpanaro, N., 2008. Effect of cold storage on vitamin C, phenolics and antioxidant activity of five orange genotypes [*Citrus sinensis* (L.) Osbeck]. Postharvest Biol. Technol. 49, 348–354.
Rigo, A., Vianello, F., Clementi, G., Rossetto, M., Scarpa, M., Vrhovšek, U., Mattivi, F., 2000. Contribution of proanthocyanidins to the peroxy radical scavenging capacity of some italian red wines. J. Agric. Food Chem. 48, 1996–2002.
Rodov, V., Vinokur, Y., Horev, B., 2012. Brief postharvest exposure to pulsed light stimulates coloration and anthocyanin accumulation in fig fruit (*Ficus carica* L.). Postharvest Biol. Technol. 68, 43–46.
Saunt, J., 2000. The sweet oranges: Pigment oranges, In: Saunt, J. (Ed.), Citrus Varieties of the World—An Illustrated Guide. 2nd Ed. Sinclair International Limited, Norwich, England, pp. 35–40.
Schirra, M., D'hallewin, G., Cabras, P., Angioni, A., Garau, V.L., 1998. Seasonal susceptibility of Tarocco oranges to chilling injury as affected by hot water and thiabendazole postharvest dip treatments. J. Agric. Food Chem. 46, 1177–1180.
Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. Am. J. Enol. Viticult. 16, 144–158.
Sloan, A.E., 2008. The top 10 functional food trends. Food Technol. 62, 25–44.
Titta, L., Trinei, M., Stendardo, M., Berniakovich, I., Petroni, K., Tonelli, C., Riso, P., Porrini, M., Minucci, S., Pelicci, P.G., Rapisarda, P., Reforgiato Recupero, G., Giorgio, M., 2009. Blood orange juice inhibits fat accumulation in mice. Int. J. Obesity 34, 578–588.
Treutter, D., 2006. Significance of flavonoids in plant resistance: a review. Environ. Chem. Lett. 4, 147–157.
Wang, L., Stoner, G.D., 2008. Anthocyanins and their role in cancer prevention. Cancer Lett. 269, 281–290.
Wszelaki, A.L., Mitcham, E.J., 2000. Effects of superatmospheric oxygen on strawberry fruit quality and decay. Postharvest Biol. Technol. 20, 125–133.
Yang, W., Ming-Yu, D., 2000. Analysis of carbohydrates in drinks by high-performance liquid chromatography with a dynamically modified amino column and evaporative light scattering detection. J. Chromatogr. A 904, 113–117.
Zhang, Z., Li, X., Chu, Y., Zhang, M., Wen, Y., Duan, C., Pan, Q., 2012. Three types of ultraviolet irradiation differentially promote expression of shikimate pathway genes and production of anthocyanins in grape berries. Plant Physiol. Biochem. 57, 74–83.
Zheng, Y., Wang, C.Y., Wang, S.Y., Zheng, W., 2003. Effect of high-oxygen atmospheres on blueberry phenolics, anthocyanins, and antioxidant capacity. J. Agric. Food Chem. 51, 7162–7169.
Zheng, Y., Wang, S.Y., Wang, C.Y., Zheng, W., 2007. Changes in strawberry phenolics, anthocyanins, and antioxidant capacity in response to high oxygen treatments. LWT-Food Sci. Technol. 40, 49–57.

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