International Journal of Food Properties

ISSN: 1094-2912 (Print) 1532-2386 (Online) Journal homepage: http://www.tandfonline.com/loi/ljfp20

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Angelo Maria Giuffrè, Clotilde Zappia & Marco Capocasale

To cite this article: Angelo Maria Giuffrè, Clotilde Zappia & Marco Capocasale (2017) Physicochemical stability of blood orange juice during frozen storage, International Journal of Food Properties, 20:sup2, 1930-1943

To link to this article: https://doi.org/10.1080/10942912.2017.1359184

Accepted author version posted online: 08 Aug 2017.
Published online: 27 Dec 2017.

Blood Orange Juice: the Effect of Concentration and Frozen Storage on its Physicochemical Properties.

Angelo Maria Giuffrè*, Clotilde Zappia, Marco Capocasale.

Dipartimento di AGRARIA. Università degli Studi Mediterranea di Reggio Calabria, Italia.

Corresponding author: Dr. Angelo M. Giuffrè, Dipartimento di Agraria, Università degli Studi "Mediterranea" di Reggio Calabria, Salita Melissari, (89124 - Reggio Calabria, Italia). E.mail: amgiuffre@unirc.it.

Phone +39 (0) 965.324077.

Fax +39 (0) 965.311092

Keywords: Anthocyanins, Citrus, European Fruit Juice Association, Flavonoids, Freezing, Nutraceuticals, Orange juice, Phenolic acids.

Summary

Blood orange juice from *Citrus sinensis* (L) Osbek cv Moro was compared to a concentrated blood orange juice and both juices were studied during frozen storage at -20 °C. Analyses were conducted on pH (3.33 – 3.81) and titratable acidity as citric acid (11.2 – 13.16 g/L). Vitamin C and formol number decreased in both blood orange juice and concentrated blood orange juice during frozen storage, whereas ash content showed an inverse trend. Scavenging abilities of the juices for the DPPH· radical ranged from 35.16% to 75.28% and showed a decreasing trend during the initial 3-4 months and an increase in the last analysed sample. Four phenolic acids were detected: gallic, chlorogenic (the highest quantity, 13-27 mg/L), caffeic and ferulic, the latter showed the lowest content. Ten flavonoids were identified, 2 flavonols (rutin and quercetin) and 8 flavanones: narirutin (the second highest flavonoid), naringin, hesperidin (the highest quantity), neoeriocitrin, didymin, eriocitrin, neohesperidin, and hesperitin. Concentration and duration of frozen storage were found to influence the physicochemical properties of blood orange juice in different ways.

INTRODUCTION

South Italy has a tradition of citrus cultivation. In 2011-2012, Italy produced 2,260 tons of orange fruit whereas the largest producers were the USA (7,954.4 tons) and China (6,600 tons) (1). In 2007, Calabria (South Italy) produced 1,213 tons *(2)* and 1,468 tons in 2011 (3). Citrus juice is normally recognized by consumers for its beneficial effects on human health for its vitamin C content. Orange juice from *Citrus sinensis* (L.) is rich in vitamin C (ascorbic acid) and in other antioxidant compounds. It is one of the most widely recognised functional foods and has been widely studied. Citrus juice induces changes in the human metabolome linked to the steroid biosynthesis pathway, therefore, a dietary dose of citrus juice was able to produce short-term effects on endogenous metabolites without requiring its chronic intake (4). Drinking orange juice (500 mL/day) increases plasma concentrations of vitamin C and reduces concentrations of 8-epi-prostaglandin F (2 alpha) (8-epi-PGF (2 alpha)) in humans, these effects were significantly more pronounced in smokers (5). The administration of citrus juice has shown beneficial effects on certain parameters of the lipid profile with a beneficial reduction in plasmatic cholesterol, HDL-c and LDL-c in metabolic syndrome compared to the baseline values. Also improved values of ox-LDL, C-reactive protein and homocysteine levels were found after six months of consumption (6).

The need to sell orange juice on the world market, the continuous evolution of the food industry, the evolution of consumer choice which requires food products to appear as natural as possible, all determine the necessity to study minimally processed systems to store the juice to maintain its physicochemical properties.

For orange juice producers it is very important to extend all positive properties of blood orange juice (as far as possible) during storage. In order to prevent misleading advertising, the manufacturer should evaluate the shelf life of the product depending on

Comment [LMP1]: Is there more recent statistics? We are in 2017!!!

the ingredients used, their concentration and their durability (7). Studies have conducted to improve or extend citrus juice shelf-life by pulsed electric field (8), by ultrafiltration (9), by reverse osmosis and osmotic distillation (10). Beltrán *et al* (11) studied the vitamin C content in mandarin orange *(Citrus reticulata)* juice packed in two different non-transparent cartons made of polyethylene and at different temperatures, and found a common decreasing trend in all samples but with a different rate according to the duration of storage, packaging and temperature.

The aim of this work was to study the influence of freezing and frozen storage on the physicochemical properties of blood orange juice (BOJ) and concentrated blood orange juice (CBOJ).

MATERIALS AND METHODS

Fruit and juice material and analytical criterion

Fruits were sampled in Sicily (South Italy). Commercial maturity of fruits was assessed at 10.50 ± 0.80 °Brix (this value was calculated on 50 fruits). The juice used for the experiment was an aliquot randomly sampled from 10 tons of BOJ of *Citrus sinensis* (L.) cv. Moro: a fruit widely grown in Sicily. BOJ was obtained by the following procedure: i, orange collection; ii, orange washing in a tank with water; iii, orange transportation by a conveyor-belt; iv, cutting in two halves; v, squeezing; vi, juice-pulp centrifugation; vii, juice pasteurisation; viii, juice concentration. A SPECIALE equipment model SP40 (Catania, Italy), for citrus juice extraction was used. After squeezing, the obtained juice-pulp mixture was introduced into a horizontal decanter to reduce the pulp content to 2-3% of the total extract. Orange juice was pasteurised at 92 °C for 32 sec by a plate pasteuriser. Following this, a SANTORO five-effect falling film evaporator equipment (Catania, Italy) was used to concentrate juice from 10.50 to 55 °Brix. The concentration temperature was managed under vacuum from 95 °C in the

Comment [LMP2]: The storage of the juice depends of the fruit quality and the explanation is very weak in this section

Comment [LMP3]: More details are needed about the orchard. Is it from a commercial orchard? The roostock? The climatic conditions? When was the harvest?

first effect to 30 °C in the fifth effect, and the pressure changed from 0.74 to 0.30 atm from the first to the fifth effect.

Two types of blood orange juice were prepared for the experiment: a BOJ and a concentrated (55 °Brix) blood orange juice (CBOJ). In the laboratory, both BOJ and CBOJ were diluted to 11.2 °Brix as required by AIJN and immediately analysed (T_0). Other aliquots of BOJ and CBOJ (11.2 °Brix) were frozen at -20 °C for 5 months in 100 mL polyethylene flasks and analysed monthly (T_1, T_2, T_3, T_4 and T_5). Three samples of each juice were analysed at for each month of storage. In this work, the analyses performed were chosen on the basis of the relevant European regulations, as well as the parameters required by the orange juice industry.

Comment [LMP4]: What does it mean? European Fruit Juice Association

Chemicals

All reagents (analytical and HPLC grade) were purchased from VWR International (Milan, Italy). Analytical standards (purity > 97%) were from Extrasynthese (Lyon, France).

pH

This potentiometric analysis was conducted by a pH meter model Crison basic 20 (city, country).

°Brix

°Brix was determined by a digital refractometer Atago PR-101α (city, country).

Titratable Acidity

The TA was determined by the IFUMA03 method (12). Two grams of juice and 50 mL of deionized water were placed into a glass beaker and the solution was titrated to a pH of 8.1 with a 0.25 N NaOH solution. The result was expressed as % of citric acid monohydrate.

Vitamin C

Vitamin C quantification was carried out using an iodometric titration. Ten mL of juice

and 5 mL of deionised water were placed into a beaker. The solution was titrated with a 0.01N iodine solution using a 2% starch solution as an indicator. The result is expressed as mg/L of …….. The colour of the orange juice, even if diluted with water, interferes with the iodine-starch system making it difficult to detect the exact point of toning. For this reason the mixture was exposed to an intense light during titration to help distinguish the red colour of the juice from the purple colour of the titrated solution.

Formol number

The formol number was determined with the method IFUMA30 (12). Ten mL of juice and 10 mL of 40% by volume formaldehyde solution were placed in a beaker and phenolphthalein as the indicator was added. The mixture was titrated with a 0.1 NaOH solution until pH 8.2. The formol number was calculated with the following formula: mL NaOH used for titration/2.

Ash

An aliquot of sample was evaporated to dryness and ignited at 550 °C to constant weight. Ash was calculated as g/L of juice.

DPPH·

DPPH· methodology is based on the reduction of the stable free radical 2.2-diphenyl-1-picrylhydrazyl (DPPH·) that strongly absorbs at 515 nm on the corresponding hydrazine by the free radical scavenging activity of the antiradical (13).

The antiradical activity of orange juice was carried out using a solution of $6 * 10^{-5}$ M DPPH· in methanol and reading in a spectrophotometer Agilent spectrophotometer model 89090A at 515nm (14).

A 2.5 mL aliquot of $6 * 10^{-5}$ M DPPH· in methanol was placed in a quartz cuvette and the absorbance was read at t0 min. At this point 10 µL of juice was placed in the cuvette maintaining a constant agitation for 5 min and the absorbance was read again (t5 min).

Comment [LMP5]: Why not FRAP or ABTS?

The result is expressed as % reduction of DPPH· by the following formula:

% DPPH· = 100 * (1- (t5 / t0)).

Total phenols

The orange juice was centrifuged at 10,000 rpm for 5 minutes (15) and the supernatant was used to determine the total phenolic content and the antioxidant activity by DPPH· assay. The analysis of total phenols was performed following the colourimetric method of Folin-Ciocalteu. Into a 25 mL glass flask were placed: 0.2 mL juice, 5 mL deionised water and 1 mL Folin-Ciocalteu reagent. After 8 minutes, 10 mL of 20% Na_2CO_3 solution was added and the mixture was made up to volume with deionised water. At the same time a reference solution (blank) was prepared using the same amount of reagents but without the sample. The mixtures were left in the dark for two hours before reading the absorbance at 765nm in a Agilent spectrophotometer model 89090A (city, country). The total phenol content was expressed as mg of gallic acid /L of juice.

Total anthocyanic content.

Anthocyanins were analysed using the pH differential method No 2 suggested by Rapisarda et al (16). Results were expressed as mg/L of cyaniding 3-glucoside chloride. Spectrophotometric analysis was conducted using a Perkin Elmer instrument model Lambda 2 (city, country).

Phenolic acids and Flavonoids

The orange juice was centrifuged at 10,000 rpm (5 min) and filtered through a 0.45 µM particle size and 25 mm diameter filter (Chromafil RC-45/25, Macherey-Nagel GmbH & Co. KG) before injection (20 µL) directly into the HPLC (15-17).

The HPLC analysis was carried out using a Knauer instrument equipped with a DAD detector (model 2600) (city, country).; the selected wavelengths were 254 nm for quercetin and rutin; 280 nm for gallic acid, eriocitrin, neoeriocitrin, narirutin, naringin,

hesperidin, neohesperidin, didymin and hesperetin; and 305 nm for chlorogenic acid, caffeic acid and ferulic acid. For quercetin and rutin, preliminary tests were conducted at 254, 280, 305 and 360 nm, and the maximum absorbance was found at 254 nm, this was in accordance with studies of other authors (18, 19, 20, 21, 22). Flavonoids have a maximum absorbance at 254 nm and at 360 nm. HPLC-DAD is commonly used in the study of antioxidants in other matrices as grape berry skin (23).

Elution was with a binary gradient: bi-deionized water (mobile phase A) and acetonitrile (mobile phase B), both mobile phases were acidified (pH 3) with formic acid. Analyses were performed using the following gradient: 95% A and 5% B in isocratic (20 min); eluent B increased from 5 to 40% from 20 min to 50 min; from 40% to 95% from 50 to 60 min; and then decreased from 95% to 5% from 60 to 65 min and finally 95% A and 5% B in isocratic from 65 to 70 min at 1 mL/min flow rate. The separation column was a Knauer C18 Eurosphere II with pre-column (250 mm length x 4.6 mm internal diameter x 5 μm particle size). Phenolic compounds were quantified with the external standard technique and using a standard solution containing all the detected phenols.

HPLC method validation

Selectivity. Each standard compound was singly injected and the retention time was noted. Afterwards a standard solution combining all the different standards was injected and the retention times were compared between each single injections and the combined standards solution injection. No co-eluting peaks were detected.

Linearity. The combined standard solutions were prepared at five different concentrations and injected into the chromatographic system. The correlation coefficient was calculated in the range of 0.9970-0.9981 for phenolic acids and in the range of 0.9984-0.9989 for flavonoids.

Limits of detection and quantification. The limit of detection (LOD) varied from 0.01 to

185 0.03 mg/L for phenolic acids and between 0.01 and 0.02 mg/L for flavonoids. The limit
186 of quantification (LOQ) varied from 0.03 to 0.05 mg/L for both phenolic acids and
187 flavonoids.

188 Statistical analysis

189 Analyses were conducted in triplicate; means and standard deviations were calculated
190 by the Excel software (2007 version). One way ANOVA was applied to the different
191 storing date using SPSS version 15.0 for Windows (SPSS Inc., Chicago IL, U.S.A.);
192 differences between sample means were analysed by Tukey's method of multiple
193 comparison at $p < 0.05$.

194 RESULTS AND DISCUSSION

195 pH

196 pH showed a slight tendency to decrease for both BOJ and CBOJ during frozen storage
197 (Table 2). The highest values were found in T_0 (3.74 and 3.81 respectively for BOJ and
198 CBOJ). After 5 months' storage, pH values decreased to 3.34 (BOJ) and 3.50 (CBOJ),
199 (Table 2). Similar data were found in cv. Navel (3.7), in Tarocco cv. (3.6), in Moro cv.
200 (3.6) and in Sanguinello cv (3.6) from Sicily (24). Other Authors found similar values
201 with a slight increase from 3.34 to 3.43 in the pH value in non-processed orange juice
202 during 12 days storage at 4 °C (25).

203 °Brix

204 This measure represents the level of total soluble solids in a given sample, the system
205 previously having been calibrated with sucrose solutions at known concentration.

206 °Brix was similar in both BOJ and CBOJ during five months of storage and ranged from
207 10.7 to 11.5 (Table 1). In all cases Brix values were above 10.0 (Table 1): the minimum
208 indicated by the European Fruit Juice Association (26). Destani *et al* (27) found 11.0
209 °Brix in squeezed, depectinized and filtered blood orange juice cv. Tarocco from

Calabria (South Italy). In squeezed and centrifuged orange juices, Ingallinera *et al* (24) found a 13.4 °Brix in cv. Navel, 13.5 °Brix in cv. Tarocco, 11.6 °Brix in cv. Moro and 12.6 °Brix in cv. Sanguinello from Sicily (South Italy). Other Authors found lower °Brix values in non-sonicated (8.9 °Brix) and sonicated (8.8-8.9 °Brix) fresh filtered orange juice of *Citrus sinensis* cv. Valencia from Spain (28).

Titratable Acidity

Acidity increased in the BOJ over the five months' storage, from 12.66 in T_0 to 13.16 g/L in T_5 (0.5 acidity more, i.e. 3.94% increase). Similar results were found in CBOJ, from 11.17 g/L in T_0 to 11.60 g/L in T_5 (0.7 g/L acidity more, i.e. 6.25% increase), (Table 2). All values were in the range indicated by the AIJN (26), 5.8-15.4 g/L (Table 1). The titratable acidity evolution found in the studied samples confirmed the pH decrease, the higher the acidity, the lower the pH value. Tiwari *et al* (28), in squeezed, filtered and sonicated juice of *Citrus sinensis* cv. Valencia from Spain, found 6.3 g/L as a titratable acidity, half of that in our samples. Kelebek *et al* (29), in squeezed and centrifuged blood orange juice, found 11.3 and 13.4 g/L as TA after freezing at -18 °C (cvs Moro and Sanguinello respectively). Palma *et al* (30) found 13.8 g/L in Tarocco cv juice from South Sardinia (Italy); they also found a slight decrease in TA content after hot water postharvest treatments (from 20 °C to 59 °C) and after simulated quarantine conditions for fruit disinfestation. In Moro cv juice, Lo Scalzo *et al*. (31) found an effect in TA content caused by thermal treatments, they found 106 meq/100g in untreated orange juice; 80.1 meq/100g in sqeezed segments previously blanched fror 6 min at 80 °C; 101 meq/100g in squeezed segments pasteurised at 80 °C for 1 min.

Vitamin C

Vitamin C is found almost exclusively in foods of plant origin. Its absorption in human body occurs in the buccal mucosa, stomach and small intestine. Buccal absorption is

thought to be by way of passive diffusion through the membrane of the buccal mucosal cells. Gastrointestinal absorption of the vitamin is rapid and efficient, and an active carrier-mediated transport system has been suggested, especially at low concentrations (32). Vitamin C is one of the most important parameters used by consumers when a nutraceutical is chosen. In oranges the predominant form of vitamin C is ascorbic acid whereas dehydroascorbic acid is less than 10% of total vitamin C (33). It is a water-soluble molecule that cannot be produced by humans but has to be ingested in the diet. The National Institute of Health recommends a daily intake of 15 mg/day for 1-3 year old children up to 120 mg/day for breastfeeding women (34). The AIJN (*26*) requires a vitamin C content of at least 200 mg/L in orange juice. This value was largely below the vitamin C content found in all samples during frozen storage (Table 2). The vinyl alcohol group present in the ascorbic acid molecule determines its fast-kinetic activity. The findings of this work showed that the vitamin C content was always higher than 560 mg/L during four months storage for both BOJ and CBOJ, but a fall was found in the fifth month in both BOJ and CBOJ. The initial value of both BOJ (679.8 mg/L) and CBOJ (621.3 mg/L) in the present work were higher than that found by other authors in the juice of Belladonna: a blond cv growing in Calabria (South Italy) (606.7 mg/L) (35). Rapisarda *et al* (33), in four of five studied cultivars, found a slight tendency in vitamin C to decrease during 65 days storage of orange fruits at 6 °C. In those five cultivars, the vitamin C content ranged from 550 to 674 mg/L, (from 551 to 489 mg/L in cv Moro), after HPLC analysis. Burdurlu *et al* (36), after spectrophotometric analysis found a decreasing trend in vitamin C content in orange juice during 8 weeks' storage; in addition they found a negative relation between vitamin C content and increasing storage temperature. Cortés *et al* (37), with a polarographic system found that the nutritional quality of orange juice (ascorbic acid) from Navel cv is maintained longer in

pulsed electric field-treated juice than in juice preserved by means of conventional pasteurization treatments.

Formol number

The formol number is useful to determine the total aminoacidic content in a fruit juice. This parameter is also influenced by the compounds able to fix formaldehyde and at the same time to increase the acidity of a juice fruit.

During frozen storage the formol number for both BOJ and CBOJ was always in the range (15-26) indicated by the AIJN (26) and showed a decreasing trend. After 5 months frozen it was borderline i.e. 15.2 for BOJ and 15.1 for CBOJ (Table 2) but above the minimum value indicated by AIJN (26). Esteve *et al* (38) found a formol number ranging from 19.0 to 22. 3 in orange juices after mild pasteurization at 77 °C for 20 seconds and after rapid cooling at 0-2 °C. Ramos and Ibarz (39) found 1.90 as formol number in concentrated orange juice (60 °Brix).

Ash

Ash content increased during frozen storage. In BOJ the ash content was 1.78 g/L at T_0 and it was 5.16 g/L at T_5 (i.e. 2.90 times higher). In CBOJ the ash content was 1.81 at T_0 and it was 3.89 g/L (i.e. 2.20 times higher) after 5 months' storage. A lower ash content (1.2 g/L) was found by Chaudhary and Verma in an orange juice (40). Corpas *et al* (41) found 3.33 g/L ash in fresh and untreated orange juice obtained from squeezed oranges from a Romanian hypermarket. Grigelmo-Miguel and Martín-Belloso (42) found 2.8%, 2.6% and 3.1% ash content in dietary fibers from *Citrus sinensis* cv Navel, Salustiana and Valencia late, respectively.

DPPH·

Comment [LMP6]: Why is higer? Could you explain, please?

283 Many components contained in orange juice show a quenching activity. As a function of
284 their reaction kinetics with DPPH· they can be divided into three general groups: fast-
285 kinetics, fast + slow-kinetics and slow-kinetics (13).
286 DPPH· value decreased for three months during frozen storage and increased in the
287 fourth and fifth months' storage. In BOJ the DPPH· value decreased from 53.39% in T_0
288 to 43.61% in T_2 and increased to 75.28% and 73.21% in T_4 and T_5 respectively. A
289 similar trend was found in CBOJ in which the DPPH· value decreased from 39.16% in
290 T_0 to 35.16% in T_2 and increased to 69.84% and 65.08% in T_4 and T_5. A similar trend
291 was found in the total phenol content. Findings of other Authors were 31.4% in
292 centrifuged and filtered Tarocco cv juice (30). Roussos (43) studied the orange juice
293 (*Citrus sinensis*) from fruits cultivated on two adjacent Greek commercial organic and
294 integrated orange groves and found 36.8% (organic) and 36.4% (integrated) as DPPH·
295 value in the methanol extract 1:1.
296 Total phenols
297 The trend in the total phenol content was similar to that of DPPH·, with an initial
298 decrease and a subsequent increase (Table 2). In the BOJ the phenolic content was
299 249.5 mg/L at T_0 and decreased to 241.5 mg/L and 230.9 mg/L at T_3 and T_4 respectively
300 but reached a maximum at T_5 (265.7 mg/L). In the CBOJ the phenolic content was
301 247.5 mg/L at T_0 and decreased to 235.3 mg/L at T_3 with a subsequent increase to 252.
302 mg/L and 258.4 mg/L at T_4 and T_5. Higher phenolic content was found in Sicilian
303 orange juice: 696 mg/L in cv Moro, 524 mg/L in cv Tarocco, 507 mg/L in cv Tarocco
304 and 571 mg/L in cv Valencia (33).
305 Total anthocyanins
306 Freezing scarcely influenced the total anthocyans in BOJ which remained constant
307 throughout all five months of storage (143 mg/L), whereas a significant decrease was

found in CBOJ if compared with BOJ. The freezing storage of CBOJ significantly influenced its total anthocyanic content and a constant decrease was found from 129.7 to 122.2 mg/L from T_0 to T_5 (Table 2). This means that the BOJ concentration process caused both a decrease of these precious antioxidants and a juice instability during storage.

Phenolic acids (HPLC)

Gallic acid (from the hydroxybenzoic group) was significantly higher in the CBOJ and in this orange juice formulation it showed a linear increase during frozen storage from 1.73 mg/L to 1.91 mg/L at T_5 (Table 3).

Three hydroxycinnamic acids were found: chlorogenic, caffeic and ferulic. Chlorogenic acid is classified for its fast + slow antiradical activity due to their isolated *p*-catechol group (44). Chlorogenic acid showed a tendency to increase in both BOJ and CBOJ from T_0 to T_5: from 15.31 to 19.66 mg/L in BOJ and from 12.85 to 27.05 mg/L in CBOJ. Caffeic acid constantly increased up to the fourth month (52.30% more in BOJ and 39.21% more in CBOJ) and dropped in the last sampling (Table 3). Ferulic acid content increased for the first three months' frozen storage in both BOJ (0.12 – 0.27 mg/L) and CBOJ (0.13 – 0.26 mg/L) and dropped in the fourth and fifth month. Roussos (43) found a similar caffeic acid content in orange juices (*Citrus sinensis*, cv Salustiana) from Greek commercial organic (3.8 mg/mL) and integrated (4.7 mg/mL) orange groves. Arena *et al*. (44) found a similar ferulic acid content (less than 0.50 mg/L) in blood orange juice before and after concentration and in orange juice from the market, whereas Destani *et al* (27) found 3.61 to 4.28 mg/L ferulic acid in ultra filtrate blood orange juice.

Flavonoids (HPLC)

Ten flavonoids were identified: 2 flavonols (rutin and quercetin) and 8 flavanones: narirutin, naringin, hesperidin, neoeriocitrin, didymin, eriocitrin, neohesperidin, and hesperitin (Table 4).

The rutin content was higher than 1 mg/L only in the first sampling of BOJ, whereas in all other samplings its content ranged from 0.63 to 0.93 mg/L.

The quercetin content was highly significantly different in the twelve samplings (6 for BOJ and 6 for CBOJ) all values were below 0.40 mg/L, with a constant tendency to decrease found in CBOJ. Quercetin is a flavonol commonly present also in other vegetable matrixes: 2.25-15.71 mg/kg in glucuronide form and 4.57-27.26 mg/kg in glucoside form in red wine grape skin (23); 9.8-90.5 mg/g in different Opuntia species (45).

Narirutin has slow antiradical activity kinetics (46); it was the second- major flavonoid in the studied samples. CBOJ showed a higher narirutin content during the storage period, in fact, the lowest narirutin content in CBOJ was in T_2 (45.4 mg/L), whereas the highest narirutin content in BOJ was found in T_0 (43.0 mg/L), in a lower amount compared to the narirutin content from frozen orange juices found by Roussos (43) from hand squeezed fruits, and in a similar content to findings of Galaverna *et al* (10) in fresh blood orange juice or treated in different conditions.

Naringin showed a decreasing trend in both BOJ and CBOJ, also this flavanone was higher in the concentrated juice.

Findings on *Citrus sinensis* (L.) Osbeck emphasise the potential protective effect of hesperidin against ulcers which is associated with antioxidant and free radical scavenging ability in the stomach mucosal tissue (47). Under normal conditions, hesperidin molecules are almost insoluble in aqueous solution (15 mg/L), and tend to crystallize quickly. The size of the crystals increases to a certain critical point when they

357 precipitate (48). In our work, hesperidin was the major flavonoid detected in the studied
358 orange juices. Freezing caused it to decrease, in particular after 5 months' storage, the
359 hesperidin content dropped by 53.3% in BOJ and 13.20% in CBOJ, even if the lowest
360 hesperidin content of CBOJ (68.7 mg/L) was in T_2 and T_3, with a calculated 31% drop
361 from the initial content. Roussos (43) found 236 and 261 mg/L hesperidin content in
362 orange juices respectively from organic and integrated orange groves (*Citrus sinensis* cv
363 Salustiana), in this case the oranges were squeezed and the juice was frozen at -25 °C.
364 Galaverna *et al* (10) studied a blood orange juice of cv Tarocco and found a lower
365 hesperidin content: 45.1 mg/L in fresh juice; 45.5 mg/mL after ultrafiltration permeate
366 treatment; 46.6 mg/mL after reverse osmosis and 35.2 mg/mL after thermal
367 concentration.
368 Neoeriocitrin increased remarkably during freezing, from 0.40 to 3.15 mg/L after 5
369 storage months in BOJ and from 0.70 to 2.25 mg/L after 5 storage months in CBOJ.
370 Didymin was subjected to a constant decrease but with a different trend, in fact, the
371 original didymin content was highest in the CBOJ and after a 35.48% fall at T_2, it
372 increased from 3.09 to 3.56 mg/L from T_3 to T_5. In the BOJ the didymin content fell by
373 83.83% at T_2 and remained between 0.52 and 0.86 mg/L.
374 Eriocitrin content decreased during storage in both BOJ and CBOJ and always remained
375 below 0.33 mg/L, in a slightly by higher quantity than in frozen, centrifuged and filtered
376 *Citrus aurantium* L. juice (49).
377 Neohesperidin was always less than 1% but with a two-fold higher content in CBOJ.
378 The hesperetin content was always less than 0.52 mg/L and it was very highly
379 significantly different in the 12 samplings. BOJ and CBOJ showed a neohesperidin
380 content at least 10 times higher than in other citrus juice as *Poncirus trifoliata* (50).
381 CONCLUSIONS

The findings of this work showed that concentration and duration of frozen storage very highly significantly influenced the physicochemical properties of the blood orange juice. Vitamin C, formol number, naringin, hesperidin, and didymin significantly decreased during frozen storage, whereas chlorogenic and caffeic acid showed an increasing trend in both BOJ and CBOJ. Ten flavonoids were detected: 2 flavonols (rutin and quercetin) and 8 flavanones. Among the latter, hesperidin and narirutin were respectively the first and the second most represented. Narirutin, naringin and neohesperidin were higher in CBOJ than in BOJ. The total anthocyanic content was stable in the frozen blood orange juice and decreased in the concentrated blood orange juice after the concentration process, caused anthocyanin instability in the juice.

Comment [LMP7]: And the color? One of the most important parameters for consumers is the juice color

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