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Effect of Time and Temperature Storage on Orange Beverage Stability

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Abstract

The aim of this study was to monitor antioxidant properties of commercial orange beverage during storage. For that purpose, special attention was paid to ascorbic acid, total flavonoids, total polyphenols, hydrogen peroxide scavenging capacity, ferric reducing capacity and ferric chelating capacity. The results showed that there was a decline as well as in antioxidants and antioxidant activity; however, low temperature storage, allowed stability. Statistical analysis showed a significant positive linear relationship between antioxidant and antioxidant capacity. Data obtained in this study suggested that low temperature storage maintained higher antioxidant content and higher levels of antioxidant capacity.

Keywords: *Antioxidant Properties; Orange Beverage; Storage Conditions*

Introduction

Nowadays, natural antioxidants have attracted considerable interest among nutritionists and food manufacturers because of their presumed safety and potential therapeutic value [1].

Fruits and vegetables consumption have been shown by multiple epidemiology studies to reduce the risk of chronic diseases such as cancer and heart disease [2,3]. Fruits are particularly interesting because they are rich in antioxidants and can be consumed on various occasions either as fresh, dried or processed like jam, jelly, juice, etc [4].

Orange fruit is native from China and are widely adapted to Mediterranean climate; they are consumed around the world due to its pleasant and delightful aroma [5].

The degradation of some bioactive compounds such as ascorbic acid, total phenolic compounds and total flavonoids might be a critical factor for fruit beverages quality, since these substances undergo destruction during storage [6-9].

Long-term stability is often required. It involves chemical and physical stability and includes the prevention of degradation reactions [10]. To our understanding, long-term stability is dependent mainly on the formulation, the knowledge of the degradation ways, and the storage conditions.

The objective of this study was to investigate the effects storage on antioxidant properties of commercial orange beverage.

Materials and Methods

Samples

Commercial orange beverage used in this investigation had shelf life at room temperature of up to 12 months, as declared on the label

by the producer. Three lots of samples (cartons tetra brick of 1 L) were divided into three groups; the first group was stored at 5°C, the second at 25°C and the third at 37°C. The tested parameters were determined after 1, 2, 4, 8 and 16 weeks of storage. The values of assessed parameters before storage were considered as control and represented by one hundred percent.

Chemicals

2,6-dichlorophenolindophenol (DCPIP) were purchased from SigmaChemical (Sigma–Aldrich GmbH, Germany), Folin-Ciocalteu phenol reagent from Biochem, Chemopharma (Montreal. Quebec) and ferrozine from Sigma–Aldrich (Sternheim, Germany). All chemicals and solvents used were of analytical grade.

Bioactive compounds

Ascorbic acid

Ascorbic acid content was determined by the method using DCPIP reagent [11]. One millilitre of sample was added to 9 mL of 2.6-dichloro-indophenol (15 µg/mL). The absorbance was read at 515 nm and result expressed as mg ascorbic acid equivalent (AAE) per 100 mL of beverage.

Total polyphenols

Total polyphenols content was determined according to Singleton and Rossi method [12]. Aliquots (200 µL) of sample was added to 1 mL of Folin–Ciocalteu reagent (10%) and 800 µL of sodium carbonate solution (7.5%). The absorbance was recorded at 765 nm and result expressed as mg gallic acid equivalent (GAE) per 100 mL of beverage.

Total flavonoids

Total flavonoids content was determined according to the method reported by Quettier-Deleu., *et al*. which consist to mix Aluminium chloride reagent to sample (v/v) [13]. The absorbance was recorded at 430 nm after incubation for 10 min and result expressed as mg quercetin equivalent (QE) per 100 mL of beverage.

Antioxidant activities

Ferric reducing capacity

Ferric reducing capacity was assessed according to the method described by Oyaizu [14]. Briefly, 2.5 mL of sample were incubated (20 min at 50°C) after adding 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%); then, 2.5 mL of TCA (10%) were added. After centrifugation, 2.5 mL of the upper layer were diluted with distilled water (v/v) and 500 µL of $AlCl_3$ (0.1%) were added. The absorbance was recorded at 700 nm and result expressed as mg ascorbic acid equivalent (AAE) per 100 mL of beverage.

Ferric chelating capacity

Ferric chelating capacity was assessed according to the method reported by Dinis, Madeira and Almeida [15]. Volume of sample was mixed with same volume solution of $FeCl_2$ (1 mM) and ferrozine (0.25 mM). The absorbance was read at 562 nm after incubation (10 min). The result was calculated using the following equation: $(\%) = [(A_c-A_s)/A_c] \times 100$ (A), where A_c and A_s are the absorbance of the control and the absorbance of the sample, respectively.

Hydrogen peroxide scavenging capacity

Hydrogen peroxide scavenging capacity was assessed according to the method described by Ruch, Cheng and Klaunig [16]. [Volume of 1.5 mL of sample was added to 1 mL of H_2O_2 (40 mM) prepared in phosphate buffer (0.1 M, pH 7.4). The absorbance was measured at 230 nm after incubation (10 min) and result calculated by using the equation (A).

Statistical analysis

The results were submitted to a bi-factorial (time and temperature) analysis of variance (ANOVA). The mean values were compared using the least significant difference test (LSD) at 5% level using infostat software. All the test were performed in triplicates and the results average (n = 3). Finally, Pearson's correlation analysis was performed on the studied parameters.

Results and Discussion

Effect of storage on bioactive compounds

Ascorbic acid

The degradation reactions of ascorbic acid are often responsible for significant quality changes that occur during the storage of foods; hence, the loss of ascorbic acid might be a quality indicator and a critical factor for the shelf life [17]. Titration with 2,6-dichlorophenolindophenol is a rapid, easy and cheap method, so it has been widely used for the determination of ascorbic acid.

Based on the results presented in figure 1a, storage at 5°C showed loss lower than 13% while storage at 25°C induced 37.75% of decrease; however, the marginal decrease was happened at 37°C (> 70%). Moreover, beverage samples kept for 16 weeks showed more than six times loss between 5 and 37°C, and more than three time between 5 and 25°C. At the end of storage, the retention of ascorbic acid content was 87.78, 62.25 and 24.16 % under 5, 25 and 37°C, respectively. In this line, Silveira., *et al.* reported 26 and 35% loss of initial ascorbic acid value at 5°C after 7 days storage for cantaloupe and galia juices, respectively [18]. According to Zulueta., *et al.* ascorbic acid content of juice-milk beverage decreased by 30.56 and 48.02% after 35 day of storage under 5 and 10°C, respectively [19]; while Tiwari., *et al.* reported 23.8 and 47.71% of ascorbic acid loss for strawberry after 10 days of storage at 4 and 20°C, respectively [20]. Likewise, the retention of ascorbic acid content assessed in the present study was higher than that obtained by Klimczak., *et al.* [21]; according to these authors, orange juice ascorbic acid content decreased by 21, 31 and 81% after 6 months of storage at 18, 28 and 38°C, respectively. Different retention potencies with regard to ascorbic acid due depending on the processing techniques, ingredients added and packaging.

Total polyphenols

A number of studies have shown that the presence of phenolics in food and especially in fruit can be particularly important for consumers, because of their beneficial health properties. Besides antioxidant effects, phenolic compounds possess a wide spectrum of biochemical properties and also can have a beneficial effect in preventing the development of diseases such as cancer and cardiovascular diseases [22].

From figure 1b, orange beverage stored at 5 and 25°C exhibited no significant decrease of total polyphenol content (< 4 and 11%, respectively) even at the end of storage; whereas, storage at 37°C induced the highest decrease which occurred after 8 weeks with 19.39%. Furthermore, at the end of storage there was no significant increase of total polyphenol content. This fluctuation might be due to the formation of some compounds during storage that were able to react with the Folin-Ciocalteu and then enhance the amount of this antioxidant. Moreover, beverage kept for 8 weeks showed more four times total polyphenol content loss between 5 and 37°C and more than three times between 5 and 25°C. At the end of storage, the retention of total polyphenol content was 96.07, 89.94 and 83.65% under 5, 25 and 37°C, respectively. Unlike to our findings, Odriozola-Serrano., *et al*. observed that there were significant differences in total polyphenol content of tomato juice during storage at 4°C regardless the applied treatment [23]. Nevertheless, the results presented were in line with those reported by Fischer, Dettmann, Carle, Kammerer who found that total polyphenol content of pomegranate juice decreased significantly after 6 months of storage at 20°C [24].

Total flavonoids

Flavonoids play an important role in normal growth, development, and defense of plants [25]; furthermore, it has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable [26].

From figure 1c which represents the evolution of total flavonoid content of orange beverage during storage, total flavonoid content remained very stable at 5°C even after 16 weeks; while at 25 and 37°C, significant differences were observed after 8 and 1 weeks, respectively. Moreover, beverage samples kept for 16 weeks showed more than five times loss between 5 and 37°C, and less than two times between 5 and 25°C. At the end of storage, the retention of total flavonoid content was 89.47, 81.95 and 47.37% under 5, 25 and 37°C, respectively.

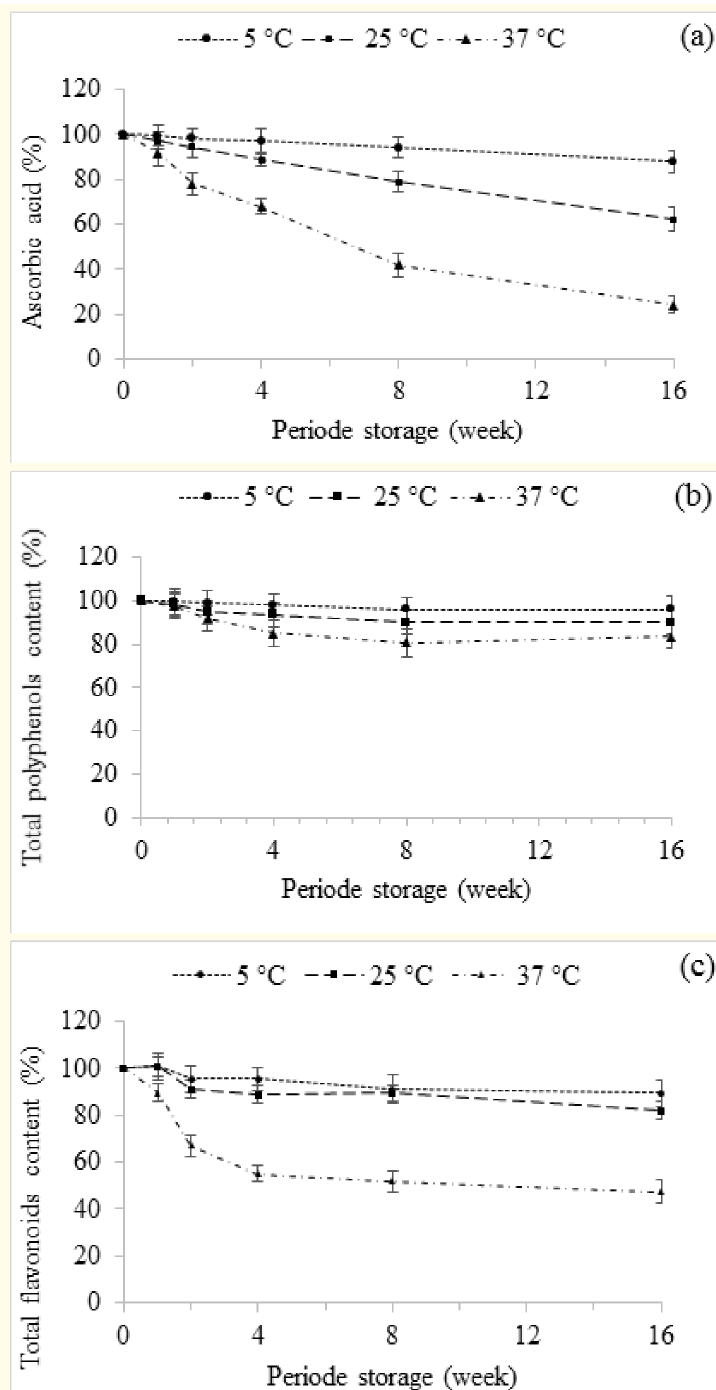

Figure 1: *Acid ascorbic (a), total polyphenols (b) and total flavonoids (c) contents of orange beverage during storage.*

Effect of storage on assessed antioxidant activities

Evaluation of the total antioxidant capacity cannot be performed accurately by any single test due to the complex nature of phytochemicals [27,28]; so many methods have been proposed to evaluate the antioxidant potential of natural antioxidant source. Usually, these methods measure the ability of antioxidants to chelate metal ions, scavenge reactive oxygen species (ROS) or inhibit lipid peroxidation. In the present work, three antioxidant activity assays (FRP, FCC and HPSC) were used to evaluate the antioxidant capacity of orange beverage.

Ferric reducing capacity

One of the three methods used to assess the antioxidant capacity of orange beverage was the reducing capacity. This latter was based on the presence of reductants, which cause the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form; and the Fe2+ was monitored by measuring the formation of Perl's Prussian blue at 700 nm [29].

The evolution of ferric reducing capacity of orange beverage during storage was presented in figure 2a. Orange beverage stored at 5 and 25°C exhibited significant decrease only after 8 weeks of storage while at 37°C it was occurred after 1 week. Moreover, beverage kept for 16 weeks showed more two times loss between 5 and 37°C, and more than one time between 5 and 25°C. At the end of storage, the retention of ferric reducing capacity was 85.13, 72.52 and 37.45 % under 5, 25 and 37°C, respectively.

Ferric chelating capacity

Elemental species such as ferrous iron (Fe^{2+}) can facilitate the production of ROS and lipid peroxidation within animal and human systems; hence, the ability of substances to chelate iron can be a valuable antioxidant capacity. The chelating effect of ferrous ions by the investigated beverage was determined according to the method of Dinis., *et al*. [15]; in the presence of chelating agents the complex formation (Ferrozine - Fe^{2+}) is disrupted resulting in a decrease of the complex colour.

The evolution of chelating capacity of orange beverage during storage was presented in figure 2b. It was observed that storage temperature of 37°C had obviously affected the chelating capacity compared to that stored at 5 and 25°C; the maximum loss of chelating capacity was occurred under storage temperature of 37°C with 62.52%. Moreover, beverage stored for 16 weeks showed more than four times loss between 5 and 37°C, and less than two times between 5 and 25°C. At the end of storage, the retention of ferric chelating capacity was 85.16, 72.38 and 37.48% under 5, 25 and 37°C, respectively.

Hydrogen peroxide scavenging capacity

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly, and inside the cell, hydrogen peroxide probably reacts with Fe^{2+}, and possibly Cu^{2+} ions (Fenton reaction) to form hydroxyl radical which may be the origin of many of its toxic effects [30]. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate.

The evolution of hydrogen peroxide scavenging capacity of orange beverage during storage was presented in figure 2c. It was observed that storage temperature of 37°C had markedly affected the orange beverage scavenging capacity compared to those stored at 5 and 25°C; the maximum loss of hydrogen peroxide scavenging capacity was induced by temperature storage of 37°C with 65.82%. Moreover, beverage samples kept for 16 weeks showed more than four times loss between 5 and 37°C, and less than two times between 5 and 25°C. At the end of storage, the retention of ferric chelating capacity was 85.13, 72.51 and 34.18% under 5, 25 and 37°C, respectively. The decrease in scavenging capacity upon storage was might be resulted by the decrease in antioxidants substances, especially ascorbic acid.

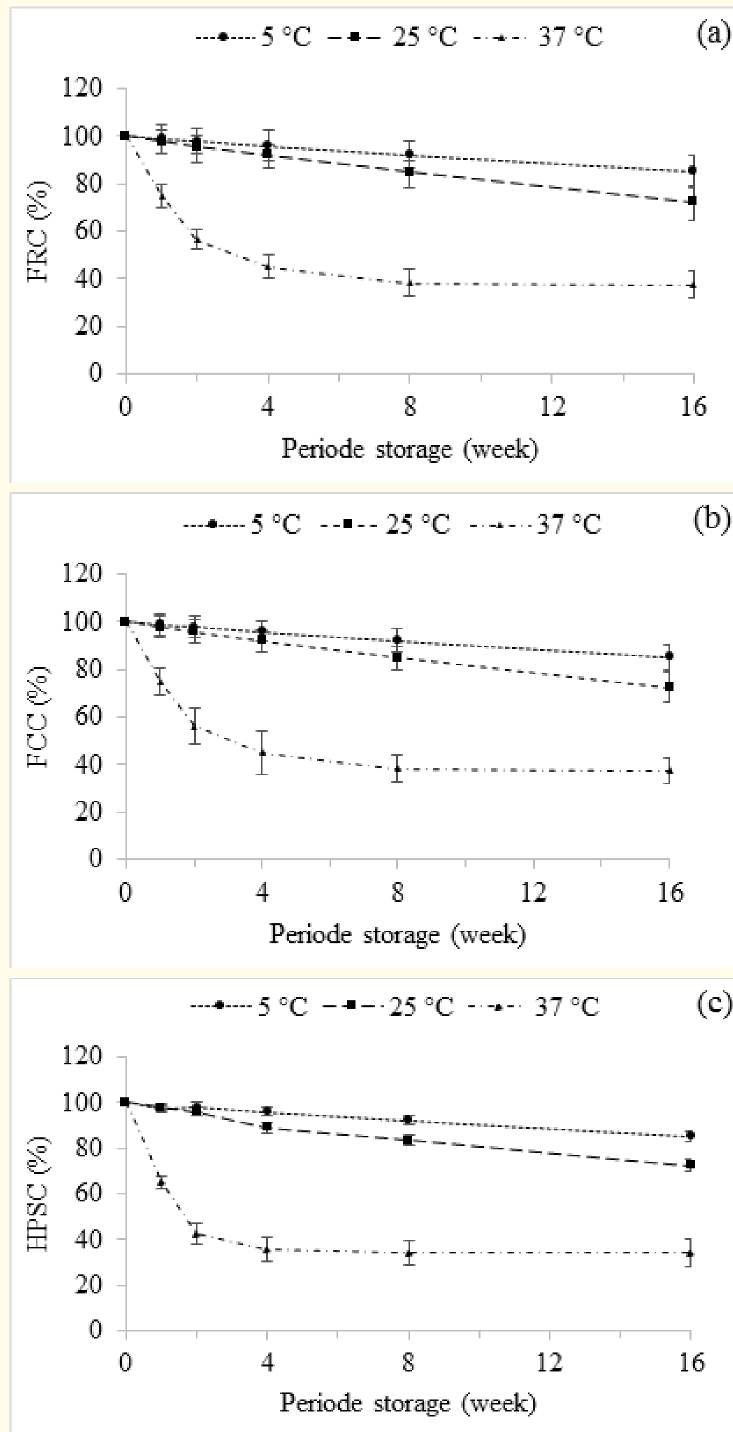

Figure 2: *Ferric reducing capacity (a), ferric chelating capacity (b) and hydrogen peroxide scavenging capacity (c) of orange beverage during storage.*

Correlation

The antioxidant capacity of orange beverage was largely influenced by ascorbic acid and phenolic contents. This fact was confirmed by results of Pearson´s test (Table 1) which showed that there were extremely significant correlations ($p < 0.001$). These results were in agreement with literature [31-34].

	FRC	FCC	HPSC
AA	0.90***	0.90***	0.85***
TP	0.82***	0.80***	0.74***
TF	0.97***	0.96***	0.95***

Table 1: *Correlation matrix between antioxidants and antioxidant activities.*

**** p < 0.001 : extremely significant correlation.*

AA: Acid Ascorbic; TP: Total Polyphenols; TF: Total Flavonoids; FRC: Ferric Reducing Capacity; FCC: Ferric Chelation Capacity; HPSC: Hydrogen Peroxide Scavenging Capacity.

Conclusion

The results of our study supplied detailed information regarding functional stability of orange beverage. A storage at 5°C induced a changes on functional values of beverages, but not as important as a storage at 25 and 37°C. The information obtained in the present study finds its practical application because it showed the behaviour of orange beverage during the period separating the production from the consumption, then allowed us to take necessary measures to remedy the undesirable changes.

Bibliography

1. Vijaya Kumar Reddy C., *et al*. "Antioxidant activity of fresh and dry fruits commonly consumed in India". *Food Research International* 43.1 (2010): 285-288.

2. Agudo A., *et al*. "Vegetable and Fruit Intake and the Risk of Lung Cancer in Women in Barcelona, Spain". *European Journal of Cancer* 33.8 (1997): 1256-1261.

3. Kabat GC., *et al*. "Intake of fruits and vegetables, and risk of endometrial cancer in the NIH-AARP Diet and Health Study". *Cancer Epidemiology* 34.5 (2010): 568-573.

4. Patthamakanokporn O., *et al*. "Changes of antioxidant activity and total phenolic compounds during storage of selected fruits". *Journal of Food Composition and Analysis* 21.3 (2008): 241-248.

5. Gutierrez-Martinez P., *et al*. "Discrimination of eight varieties of apricot (Prunus armeniaca) by electronic nose, LLE and SPME using GC-MS and multivariate analysis". *Sensors and Actuators B* 125.2 (2007): 415-421.

6. Touati N., *et al*. "Effect of storage time and temperature on the quality of fruit nectars: determination of nutritional loss indicators". *Journal of Food Quality* 39.3 (2016): 209-219.

7. Touati N., *et al*. "Effect of storage time and temperature on the physicochemical and sensory characteristics of commercial apricot jam". *Food Chemistry* 145 (2014): 23-27.

8. Barba FJ., *et al*. "Evaluation of quality changes of blueberry juice during refrigerated storage after high-pressure and pulsed electric fields processing". *Innovative Food Science and Emerging Technologies* 14 (2012): 18-24.

9. Trost K., *et al*. "Anthocyanin degradation of blueberry-aronia nectar in glass compared with carton during storage". *Journal of Food Science* 73.8 (2008): 405-411.

10. Abdelwahed W., *et al*. "Freeze-drying of nanoparticles: Formulation, process and storage considerations". *Advanced Drug Delivery Reviews* 58.15 (2006): 1688-1713.

11. Klein BP and Perry AK. "Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States". *Journal of Food Science* 47.3 (1982): 941-948.

12. Singleton VL and Rossi JA. "Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents". *American Journal of Enology and Viticulture* 16 (1965): 144-158.

13. Quettier-Deleu C., *et al*. "Phenolic compounds and antioxi-dant activities of buckweat (Fagopyrum esculentum Moench) hulls and flour". Journal of Ethnopharmacology 72.1-2 (2000): 35-42.

14. Oyaizu M. "Studies on product of browning reaction prepared from glucose amine". *Japanese Journal of Nutrition* 44.6 (1986): 307-315.

15. Dinis TCP., *et al*. "Action of phenolic derivates (acetoaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers". *Archives of Biochemistry and Biophysics* 315.1 (1994): 161-169.

16. Ruch RJ., *et al*. "Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea". *Carcinogenesis* 10.6 (1989): 1003-1008.

17. Plaza L., *et al*. "Effect of refrigerated storage on vitamin C and antioxidant activity of orange juice processed by high-pressure or pulsed electric fields with regard to low pasteurization". *European Food Research and Technology* 223.4 (2006): 487-493.

18. Silveira A., *et al*. "Shelf-life and quality attributes in fresh-cut Galia melon combined with fruit juices". *Food Science and Technology* 50.1 (2013): 343-348.

19. Zulueta A., *et al*. "Ascorbic acid in orange juice-milk beverage treated by high intensity pulsed electric fields and its stability during storage". *Innovative Food Science and Emerging Technologies* 11.1 (2010): 84-90.

20. Tiwari BK., *et al*. "Stability of anthocyanins and ascorbic acid in sonicated strawberry juice during storage". *European Food Research and Technology* 228.5 (2009): 717-724.

21. Klimczak I., *et al*. "Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices". *Journal of Food Composition and Analysis* 20.3-4 (2007): 313-322.

22. Lattanzio v., *et al*. "Low temperature metabolism of apple phenolics and quiescence of Phlyctaena vagabunda". *Journal of Agricultural and Food Chemistry* 49.12 (2001): 5817-5821.

23. Odriozola-Serrano I., *et al*. "Carotenoid and phenolic profile of tomato juices processed by high intensity pulsed electric fields compared with conventional thermal treatments". *Food Chemistry* 112.1 (2009): 258-266.

24. Fischer UA., *et al*. "Impact of processing and storage on the phenolic profiles and contents of pomegranate (Punica granatum L.) juices". *European Food Research and Technology* 233 (2011): 797-816.

25. Sellappan S and Akoh CC. "Flavonoids and Antioxidant Capacity of Georgia-Grown Vidalia Onions". *Journal of Agricultural Food Chemistry* 50.19 (2002): 5338-5342.

26. Kessler M., *et al*. "Anti- and pro-oxidant activity of rutin and quercetin derivatives". *Journal of Pharmacy and Pharmacology* 55.1 (2003): 131-142.

27. Frankel EN and Finley JW. "How to standardize the multiplicity of methods to Evaluate natural antioxidants". *Journal of Agricultural Food Chemistry* 56.13 (2008): 4901-4908.

28. Du G., *et al*. "Antioxidant capacity and the relationship with polyphenol and vitamin C in actinidia fruits". *Food Chemistry* 113.2 (2009): 557-562.

29. Amarowicz R., *et al*. "Free radical-scavenging capacity, antioxidant activity, and phenoliccomposition of green lentil (Lens culinaris)". *Food Chemistry* 121.3 (2010): 705-711.

30. Nagavani V., *et al*. "Free radical scavenging activity and qualitative analysis of polyphenols by RP-HPLC in the flowers of Couroupita guianensis Abul". *Electronic Journal of Environmental, Agricultural and Food Chemistry* 9.9 (2010): 1471-1484.

31. Touati N., *et al*. "Screening of phytochemical content of commercial apricot- and orange-based beverages and its relationship with antioxidant capacity". *International Food Research Journal* 20.6 (2013): 3177-3184.

32. Barba FJ., *et al*. "A comparative study of the analysis of antioxidant activities of liquid foods employing spectrophotometric, fluorometric, and chemiluminescent methods". *Food Analytical Methods* 6.1 (2013): 317-327.

33. Lee KW., *et al*. "Major phenolics in apple and their contribution to the total antioxidant capacity". *Journal of Agricultural Food Chemistry* 51.22 (2003): 6516-6520.

34. Serrano M., *et al*. "Chemical constituents and antioxidant activity of sweet cherry at different ripening stages". *Journal of Agricultural and Food Chemistry* 53.7 (2005): 2741-2745.

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