INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 7, No. 4, p. 22-30, 2015

RESEARCH PAPER OPEN ACCESS

The effect of packaging and storage of vitamin-C in fresh and commercial orange juices

Afshan Kaleem[1*], Asma Tanveer[2], Irfana Iqbal[3], Moneeza Abbas[4], Roheena Abdullah[1], Mehwish Iqtedar[1], Mahwish Aftab[1], Tehreema Iftikhar[5], Shagufta Naz[1]

[1] *Department of Biotechnology, Lahore College Women University, Lahore, Pakistan*

[2] *Department of Zoology, Lahore College Women University, Lahore, Pakistan*

[3] *Institute of Industrial Biotechnology, Government College University, Lahore, Pakistan*

[4] *Department of Environmental Sciences, Lahore College Women University, Lahore, Pakistan*

[5] *Department of Botany, Lahore College Women University, Lahore, Pakistan*

Key words: Vitamin-C, Fresh, Commercial orange juices, Storage, Packaging.

http://dx.doi.org/10.12692/ijb/7.4.22-30 Article published on October 10, 2015

Abstract

Vitamin-C in commercial and fresh orange juice samples was investigated. The commercially available juices included packed and powdered orange juices. Different brands of commercial liquid and powdered orange juices were included in this study. The seasonal variation of vitamin-C in fresh oranges was also explored. Fresh cultivar of oranges including Ambersweet, Tangarin and Mandrin were selected for vitamin-C content. Furthermore the effect of storage, packing material lined and unlined with different fabrics and preservatives on the stability of vitamin-C in orange juice samples were investigated. Redox titration was used for measurement of vitamin-C content in all samples. Maximum vitamin-C content was observed in end of the season fresh juice samples. Some of the commercially available orange juices (within expiry date) showed highest vitamin-C content, while mostly showed loss of vitamin-C during preservation process.

* **Corresponding Author:** Afshan Kaleem ✉ afshankaleem@yahoo.dk

Introduction

Vitamin-C, also called L-ascorbic acid (AA) is an important vitamin present in almost all fruits and vegetables. It is an important antioxidant, water soluble vitamin, sensitive to oxygen, heat, alkali and many other factors (Bolling *et al.,* 2013; Lavelli *et al.,* 2011; Licciardello & Muratore, 2011). It has been used to prevent and control scurvy, but is also vital for many other important biological functions such as modulation of the immune system and control of inflammatory diseases (Sigusch, 2013; Sorice *et al.,* 2014).

The amount of vitamin-C depends on the cultivar and ripeness of the citrus fruit and storage conditions (Andrews & Driscoll, 1977; Djordjevic *et al.,* 2013; Ramaiya *et al.,* 2013). Moreover the packaging material has a direct effect on the availability of vitamin-C in orange juice. Vitamin-C containing juices stored in metal or glass containers is stable, but when stored in plastic bottles its shelf-life decreases. Bacigalupi and colleagues (2013) showed that orange juices packed in PET bottles had a loss of 53% vitamin-C. During processing of juices, temperature and oxygen also play a major role in the loss of vitamin-C. Non-thermally processing of juices give higher levels of vitamin-C, but financial factors has minimized the use of such methods in the citrus industry. Another emerging problem in the fruit juice industry is the adulteration of orange juice with less expensive juices and sugars, artificial sweeteners, colors and other materials such as organic phosphate, calcium, added sugar, sodium chloride, magnesium sulphate, which also reduces the stability of vitamin-C (Ribeiro *et al.,* 2011; Stander *et al.,* 2013).

The presence of vitamin-C in fruits, juices, fruit pulp etc has been evaluated by many different methods. HPLC is the method of choice for this purpose (Valente *et al.,* 2014). Recently there has been an explosion of new methodologies including automated methodologies for the determination of pharmaceutical formulations and fruit juices for vitamin-C content (Llorent-Martinez *et al.,* 2013). Thus multi-commutated flow injection analysis has been used for the evaluation of vitamin-C in pharmaceuticals (Llorent-Martinez *et al.,* 2013). A molecularly imprinted copolymer has been described as a sensor for vitamin-C with a concentration sensitivity of 0.1-10 mM (Kong *et al.,* 2014). Similarly a redox method involving polyanalin film has been developed that is sensitive to vitamin-C (Bossi *et al.,* 2000). Spectroscopic methods for the determination of vitamin-C are commonly used. This involves oxidation of vitamin-C with manganese III following reduction of the unreacted manganese with diphenyl amine, detected at λ570 nm (Shyla & Nagendrappa, 2013). Voltammetric iodometric titration of vitamin-C in pharmaceutical preparations has also been reported (Verdini & Lagier, 2000). Redox methods for an evaluation of vitamin-C are also commonly used where sophisticated methods may not be easily available (Justi *et al.,* 2000; Muszalska *et al.,* 2000). There are some certified reference materials available for the estimation of vitamin-C in food matrices, fruits and juices (Valente *et al.,* 2014).

Prolonged use of preservatives such as sodium nitrite is not without consequences. oxidative properties of sodium nitrite are known for hepatic damage and several other organs (Salama *et al.,* 2013; Shinn *et al.,* 2013). No regulatory control has been imposed by the Government of Pakistan for use of these preservatives in orange juices. Manufacturers of the packed orange juice use any preservative any amount at their free will causing public health hazard. Stability of vitamin-C can be maintained in an acidic environment or in the presence of other additives such as sodium nitrite or rosemary extract (Doolaege *et al.,* 2012). Other preservatives such as perchloric and metaphosphoric acids have been used to increase the shelf life of vitamin-C (Valente *et al.,* 2014). The stability of vitamin-C incorporated in whey protein and thermal decomposition kinetics and shelf life of vitamin-C in air and nitrogen have been reported (Janjarasskul *et al.,* 2011; Juhasz *et al.,* 2012). These studies have indicated that the atmosphere influences the stability of vitamin-C. The industrial processing and long-term storage of food material also has strong impact on the stability of vitamin-C. The long-term storage at ambient temperatures results in the loss of vitamin-C and only 13% of the total vitamin-C remains after 12 months of its storage (Koh *et al.,* 2012).

With innumerable orange juice brands on Pakistan market with little control on quantity and quality of preservatives, storage conditions and packing materials, public health is severely at risk. Therefore, this study was designed to investigate the loss of vitamin-C in different orange juices and different citrus fruits produced locally.

Material and methods

The determination of vitamin-C content in fresh oranges of different cultivar and commercial orange juices was conducted. All samples were collected to meet the requirement for analyzing the vitamin-C concentration from fresh oranges and commercial juice samples. Commercial juices samples were collected from the local market of Lahore, Pakistan. Samples were stored at 4ºC, for further analysis. All the packed samples were ensured to get analyzed at least 4-5 weeks before the marked expiry date on the pack. vitamin-C tablets were used as a control.

Evaluation of vitamin-C content in standard solution

Vitamin-C tablets (Cawood-C or CaC-1000) were obtained from a local pharmacy. The vitamin-C tablets were ground to a fine powder using a mortar and pestle. 250 mg of the powdered material was poured into a 250 mL Erlenmeyer flask and dissolved in 100 mL of deionized water. 20 mL of the solution was taken into a 250 mL conical flask and 1mL 0.1M starch solution and 150 mL of water were added and mixed well. For titration potassium Iodide 5 mM solution was dropwise added from the burette, until a dark blue color appeared. The titration was repeated three times and means reading was taken. Titration was repeated for different concentrations of vitamin-C (2-100 mg/mL) and a standard curve was drawn and the linear equation was generated and used for determining the vitamin-C concentration in the unknown samples. The concentration of vitamin-C (mg/mL) in the unknown sample was calculated from the linear equation generated for the standard curve.

Sample preparation and procedure for analysis of vitamin-C content in fresh orange juice

A piece of fresh orange (Mandarin, Ambersweet or Tangrin) was taken and juice was squeezed out and collected in three plastic bottles. The juice was passed through double layer of cheese cloth to remove any suspended material. To prevent oxidation of vitamin-C, oxalic acid (1mg/mL, final concentration) was added to samples. After addition of starch solution (50 µl), samples were titrated with 5mM solution of potassium iodide solution.

The endpoint of the titration was identified as the first permanent dark blue-black colour formed by the starch-iodine complex. Titration was repeated three times and means volume was taken. As KI is added to the sample, the ascorbic acid is oxidised to dehydroascorbic acid, while the iodine is reduced to iodide ions. The iodine formed is immediately reduced to iodide, as long as there is any ascorbic acid present. Once all the ascorbic acid has been oxidised, the remaining iodine is free to react with the starch indicator, which forms the blue-black starch-iodine complex.

Analysis of vitamin-C content in liquid and powdered orange juices

150 mL of the different commercial juices (Fresher, Pulpy orange, Mitchell's orange juice, Sammi orange juice, Honest orange juice, Nestle orange juice, Rani orange juice, Vivo orange juice, Tang powder orange juice, RASNA powder orange juice, Aroset powder orange juice, Hi-Nutri orange juice National powder orange juice) were obtained and labelled (the powdered juices were first dissolved in a known volume of water as given on the sachet). 50 mL of filtered juice through cheese cloth was taken into a 250 mL Erlenmeyer flask and 50 µl of starch indicator was added. Iodine solution was drop-wise added to the titration flask. To determine the precise color-change in the solution a splitting drop technique was used. This procedure was repeated in triplicates for the entire orange juice sample and results were calculated using the linear equation generated from the standard solution of vitamin-C.

Statistical analysis

All experiments were performed in triplicates. The results have been analyzed by using ANOVA (SPSS version 16) with a significance level of $p \leq 0.05$.

Results and discussion

Vitamin-C is an important anti-oxidant, which functions as a co-substrate for many enzymes and also helps to protect against several diseases such as cancer, heart disease, stress and in building of cartilage (Yokoyama *et al.,* 2000; Riemersma *et al.,* 2000; Sargeant *et al.,* 2000).

In this study vitamin-C content in freshly squeezed oranges (Mandarin, Ambersweet and Tangrin), commercial liquid and powdered orange juices, collected from the local market, was determined. Furthermore the seasonal vitamin-C variation in fresh oranges, and the effect of orange storage and preservatives was investigated. The pharmaceutical preparations, Calwood-C and tablets, were used as standard vitamin-C. The vitamin-C content in the commercial vitamin-C tablets was found to be 500.06±0.03 mg/tablet in CaC-1000 and 497.13±0.09 mg/tablet in Calwood-C (Figure 1, Table 1).

Table 1. Vitamin-C content in commercial orange juices (liquid and powder).

Brand	Nature of sample	Source of sample	No. of sample	Initial amount	Manufactured	Expiry date	Range of values	Mean value	Experiment performed	Packing material
1	Commercial Orange drinks	Fresher	3	100%/250ml	4/10/09	4/10/10	0.065-0.07	0.068	12/3/2010	Plastic bottle
2	-	Pulpy orange	3	30mg/100ml	---	5days after opening	0.064-0.068	0.067	As opened	Plastic bottle
3	-	Mitchell's orange	3	60mg/100ml	---	2/5/10	0.085-0.09	0.089	16/3/2010	Plastic bottle
4	-	Sammi	3	29mg/100ml	1/10/08	3/9/10	0.056-0.064	0.062	20/3/2010	Tin pack
5	-	Honest orange	3	Enrich	14/1/09	13/1/11	0.021-0.025	0.026	20/3/2010	Tin pack
6	-	Nestle orange	3	17mg/100ml	4/12/09	12/6/10	0.21	0.21	1/4/2010	Cardboard carton
7	-	Rani float	3	Enrich	2/5/09	2/5/10	0.054	0.054	12/4/2010	Tin pack
8	-	Vivo orange	3	26.6mg/100ml	20/1/10	30/1/11	0.49-0.5	0.52	12/4/2010	Plastic bottle
9	Instant powder juice	Tang orange	3	33mg/25g	1/9/09	1/9/11	0.1157-0.1187	0.12	12/4/2010	Plastic enamel
10	-	Mitchell's orange	3	41mg/25g	---	27/6/11	0.101-0.102	0.101	13/4/2010	Plastic enamel
11	-	Rasna orange	3	240mg/25g	29/7/09	27/7/10	0.154-0.155	0.162	15/4/2010	Plastic enamel
12	-	Aroset	3	10mg/9g	1/8/08	1/8/10	0.058-0.068	0.079	16/4/2010	Plastic enamel
13	-	Hi-Nutrition	3	100%/25g	1/12/09	2/2/11	0.1705-0.175	0.2	17/4/2010	Plastic enamel
14	-	National orange	3	13mg/20g	1/11/09	1/1/11	0.157	0.222	17/4/2010	Plastic enamel
15	Vitamin-C tablets	CaC-1000	3	500mg/6.6g	11/12/09	10/11/10	497.1-497.5	497.13	1/1/2010	Aluminum foil
16	-	Calwood-C	3	500mg/6.65g	9/12/09	9/11/10	500-500.09	500.06	31/12/2009	Aluminum foil

These results were in close agreement to the information provided on the package (500 mg/6.6g). The vitamin-C content in freshly squeezed oranges (natural) and commercially available orange juice samples are shown in Figure 2 and 3 and tabulated in Table 1 and 2. The amount of vitamin-C did not vary significantly in different brands of vitamin-C available from the market. However, of all orange juice samples included in this study were found to be the best in terms of vitamin-C content. Pasteurization and packaging processes easily destroy vitamin-C. When a container of any type was opened, the vitamin-C content decreased by about 2%.

Different orange cultivars showed different vitamin-C content. Ambersweet had highest vitamin-C followed by Mandarin and Tangrin respectively (Figure 2, Table 2). The vitamin-C contents in Mandarin, Ambersweet and Tangrin orange in the beginning of the season were 66.2± 2.58mg/100 ml, 77.13±9.06 mg/100 mL and 37.4±4.97mg/100 mL respectively, while the vitamin-C content of the same cultivar at the end of the season was 105.52±14 mg/100 mL, 117.90±5.43 mg/100 mL and 56.54±8.32 mg/100 mL respectively (Figure 2, Table 2). In commercial fruit juice samples Vivo orange (0.52mg±0.004) and Nestle orange (0.21mg) and in orange instant powder Hi-nutrition (0.2mg±0.017) and National orange

(0.22mg±0.004) showed highest level of vitamin-C (Figure 3, Table 1). Unripe fruit had the highest level of vitamin-C content. Vitamin-C losses during ripening process may be due to higher rate of photosynthesis (Brenes *et al.,* 2005). The position of fruit on the tree also affects vitamin-C content and sunlight exposure enhances vitamin-C content. Fruit positioned on the outside of the tree and on the south side are reported to have higher level of vitamin-C (Aruoma, 1998). The experimental results show that in Ambersweet, Mandarin and Tangrin get more ascorbic acid at certain stages of maturity. The ending season had the highest vitamin-C, while starting season showed lowest vitamin-C content (Figure 3, Table 2).

Table 2. Vitamin-C content in fresh oranges (mandarin, ambersweet and tangrin) with the seasonal variation.

Brand	Nature of sample	Source of sample	No. of sample	Range of values	Mean value	Oxalic acid	Experiment preformed
1	Mandarin	Starting season	3	63-71.3	66.2	Added	As plucked
2	-	Mid season	3	54-66	61	Not added	preserved for 4-5 days at room temp
3	-	Ending season	3	122-165	150	Not added	As plucked
4	Ambersweet	Starting season	3	65.6-95	77.1	Added	As plucked
5	-	Mid season	3	55.5-95	72.57	Not added	preserved for 4-5 days at room temp
6	-	Ending season	3	109-128	117	Not added	As plucked
7	Tangrin	Starting season	3	28.8-46	37.4	Added	As plucked
8	-	Mid season	3	21.8-32	27	Not added	preserved for 4-5 days at room temp
9	-	Ending season	3	44.8-72.6	56	Not added	As plucked

Moisture contents of the fruit vary from 82.8 to 90%, when just ripe, and it decreases, when the fruit is over-ripe. The ripe oranges contain 4.0 to 11-5% fibre and the value increases as fruit matures (Davalos *et al.,* 2005). When different varieties of citrus are stored in pyramidal structure under ambient conditions, the shelf-life extension is observed upto 28 days (La Vecchia *et al.,* 2001). This is because of the reduction in the rate of evaporation of water and respiratory gases (CO_2 and O_2), which improve the appearance of fruit shelf-life of the citrus fruit. It can be further extended by wax coating of the fruit or by using lining material like polyethylene, butter paper and cellophane as packing material (Ramadan *et al.,* 2003).

Citrus fruit shows an increase in reducing sugars during storage. The conversion of non-reducing sugar into reducing sugar in different variety of citrus fruit, stored at ambient temperature is known to be more than those stored in pyramidal structure. When fresh orange juice is preserved in containers, vitamin-C loss is due to oxidation by a residual air layer trapped within the container during processing. The loss is faster in the first 2 weeks and is more evident at higher storage temperatures (Proteggente *et al.,* 2002). Therefore, it is recommended that orange juice must be kept cool to prevent vitamin-C degradation (Ramadan & Moersel, 2007).

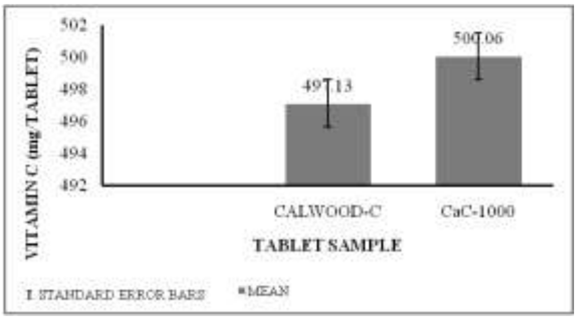

Fig. 1. Vitamin-C in Calwood and CaC-100 tablets.

Studies have shown that orange peel has the highest level of vitamin-C, followed by the pulp, and juice. Only 26% of vitamin-C of a citrus fruit can be found in the juice. The peel has 53% and the pulp and rag has 21%. Vitamin-C content of commercial orange juices ranges from 2.4 to 43 mg/100 mL (Ramadan &

Moersel, 2007). Storage of commercial orange juices in closed containers at room temperature for 4 months results in vitamin-C losses ranging from 29 to 41%. Commercial orange juice, when stored in open containers in the refrigerator for 31 days, lost 60 to 67% of its ascorbic acid, while fresh orange juice lost ascorbic acid at much slower rate of 7 to 13% (Terry *et al.,* 2001). Open containers of commercial orange juice, when stored outside the refrigerator for 10 days, may lose 12.5% of their ascorbic acid content, while refrigerated juice for the same period, the ascorbic acid loss reached - to 9%. Canned orange juices have lower vitamin-C levels due to heating during the canning process (Willett, 1991).Vitamin-C content is less in pasteurized juices than in fresh orange juices. This loss is due to the addition of sugar, which dilute liquid juices. A similar reduction in vitamin-C content of citrus juices and concentration of mineral content increases the final concentration of vitamin-C content. This is probably due to the addition of the sugar which invariably contains certain minerals that complex with vitamin-C (Rodríguez-Bernaldo & Costa, 2006).

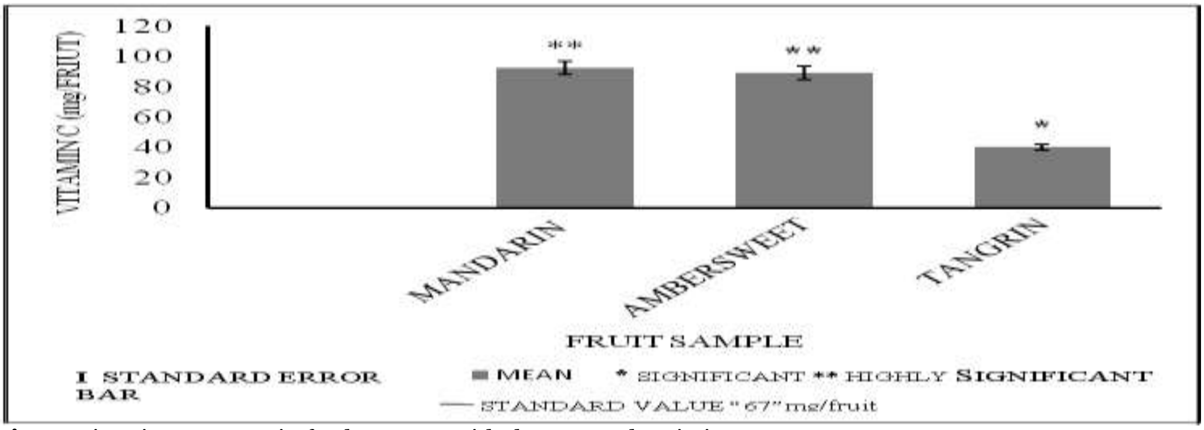

Fig. 2. Vitamin-C content in fresh oranges with the seasonal variations.

In caned orange juice there is residual oxygen that oxidises ascorbic acid to dehydroascorbic acid and also vitamin-C reacts with the tin. Glass packed orange juice provides poor retention of vitamin-C, losing 10% after 4 months of storage, oxidation starts due to photo oxidation through transparent glass. Cardboard carton have specially designed multi-layered oxygen and light barriers to protect both loss of vitamin-C, flavour, and to enhance shelf-life (Richelle *et al.,* 2001). Orange juice packed in foil-lined cardboard cans retain greater than 90% of their vitamin-C after 12 months at -20°C. Mostly sugar found in orange juices is fructose that can cause breakdown of vitamin-C. The higher the fructose content the greater the loss of vitamin-C has been reported (Kahkonen *et al.,* 1999).

The Pharmaceutical preparations e.g. tablets containing vitamin-C have also been analyzed for the determination of vitamin-C content. The determination of vitamin-C content in tablets was 500.06±0.03 mg/tablet in $CaCl_2$ 1000 in Calwood-C 497.13±0.09 mg/tablet. The same method was used to analyze vitamin-C content in instant powdered juices. However, the results were not reliable. In samples with early manufacturing date the loss of vitamin-C was greater, Tang orange juice had only 0.0283±0.004 mg/100 mL vitamin-C content (Figure 3). For the production of powdered orange juice (Tang) the fresh orange juice is evaporated at high temperature that results in the total loss of vitamin-C (Gillman *et al.,* 1995). The samples having early manufacturing date had low vitamin-C content, while the samples having late manufacturing date had a comparatively high vitamin-C content e.g. Vivo 100% orange inside have 0.512±0.002 mg/100ml vitamin-C content and Sammi orange has least 0.054mg/100mL vitamin-C content. For the purpose of dilution of orange drinks sugar is added, which causes the breakdown of ascorbic acid (Daglia *et al.,* 2000).

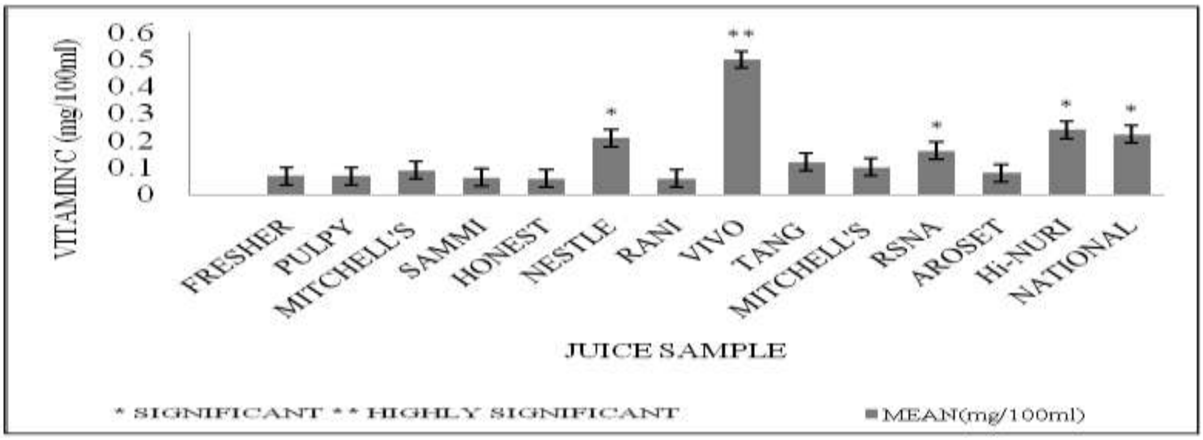

Fig. 3. Vitamin-C content in commercial orange juices.

Packed juices (liquid and powdered) purchased from the local market of Lahore contain very low content of vitamin-C suggesting the loss of vitamin-C is considerably high due to storage and the process of preservation. The conclusion of this study is that the vitamin-C content of the commercial juices are very low and do not reach the threshold value i.e 2.4mg/serving. When fresh juices are treated by oxalic acid the oxidation process is reduced. Ending season fresh orange has highest amount of vitamin-C in mandarin.

References

Andrews FE, Driscoll PJ. 1977. Stability of ascorbic acid in orange juice exposed to light and air during storage. Journal of the American Dietetic Association **71,** 140-142.

Aruoma OI. 1998. Free radicals, oxidative stress and antioxidants in human health and disease. Journal of the American Oil Chemists' Society **75,** 199-212.

Bacigalupi C, Lemaistre MH, Boutroy N, Bunel C, Peyron S, Guillard V, Chalier P. 2013. Changes in nutritional and sensory properties of orange juice packed in PET bottles: an experimental and modelling approach. Food Chemistry **141,** 3827-3836.

Bolling BW, Chen YY, Chen CY. 2013. Contributions of phenolics and added vitamin-C to the antioxidant capacity of pomegranate and grape juices: synergism and antagonism among constituents. International Journal of Food Science & Technology **48,** 2650–2658.

Bossi A, Piletsky SA, Piletska EV, Righetti PG, Turner AP. 2000. An assay for ascorbic acid based on polyaniline-coated microplates. Analytical Chemistry **72,** 4296-4300.

Brenes CH, DelPozo-Insfran D, Talcott ST. 2005. Stability of copigmented anthocyanins and ascorbic acid in a grape juice model system. Journal of Agricultural and Food Chemistry **53,** 49–56.

Daglia M, Papetti A, Gregotti C, Berte F, Gazzani G. (2000). In vitro antioxidant and ex vivo protective activities of green and roasted coffee. Journal of Agricultural and Food Chemistry **48,** 1449-1454.

Davalos A, Bartolome B, Gomez-Cordoves C. 2005. Antioxidant properties of commercial grape juices and vinegars. Food Chemistry **93,** 325-330.

Djordjevic B, Savikin K, Zdunic G, Jankovic T, Vulic T, Pljevljakusic D, Oparnica C. 2013. Biochemical properties of the fresh and frozen black currants and juices. Journal of Medicinal Food **16,** 73-81.

Doolaege EH, Vossen E, Raes K, De Meulenaer B, Verhe R, Paelinck H, De Smet S. 2012. Effect of rosemary extract dose on lipid oxidation, colour stability and antioxidant concentrations, in reduced nitrite liver pates. Meat Science **90,** 925–931.

Gillman MW, Cupples LA, Gagnon D, Posner BM, Ellison RC, Castelli WP, Wolf PA. 1995. Protective effect of fruits and vegetables on development of stroke in men. The Journal of the American Medical Association **273,** 1113-1117.

Janjarasskul T, Min SC, Krochta JM. 2011. Storage stability of ascorbic acid incorporated in edible whey protein films. Journal of the Science of Food and Agriculture **59,** 12428–12432.

Juhasz M, Kitahara Y, Takahashi S, Fujii T. 2012. Thermal stability of vitamin C: thermogravimetric analysis and use of total ion monitoring chromatograms. Journal of Pharmaceutical and Biomedical Analysis **59,** 190-193.

Justi KC, Visentainer JV, Evelazio de Souza N, Matsushita M. 2000. Nutritional composition and vitamin-C stability in stored camu-camu (Myrciaria dubia) pulp. Archivos Latinoamericanos de Nutrición **50,** 405-408.

Kahkonen MP, Hoia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. Journal of the Science of Food and Agriculture **47,** 3954–3962.

Koh E, Charoenprasert S, Mitchell AE. 2012. Effects of industrial tomato paste processing on ascorbic acid, flavonoids and carotenoids and their stability over one-year storage. Journal of the Science of Food and Agriculture **92,** 23-28.

Kong Y, Shan X, Ma J, Che M, Chen Z. 2014. A novel voltammetric sensor for ascorbic acid based on molecularly imprinted poly(o-phenylenediamine-co-o-aminophenol). Analytica Chimica Acta **809,** 54-60.

La Vecchia C, Altieri A, Tavani A. 2001. Vegetables, fruit, antioxidants and cancer: a review of Italian studies. European Journal of Nutrition **40,** 261-267.

Lavelli V, Corey M, Kerr W, Vantaggi C. 2011. Stability and anti-glycation properties of intermediate moisture apple products fortified with green tea. Food Chemistry **127,** 589-595.

Licciardello F, Muratore G. 2011. Effect of temperature and some added compounds on the stability of blood orange marmalade. Journal of Food Science **76,** C1094–1100.

Llorent-Martinez EJ, Molina-Garcia L, Kwiatkowski R, Ruiz-Medina A. 2013. Application of quantum dots in clinical and alimentary fields using multicommutated flow injection analysis. Talanta **109,** 203-208.

Muszalska I, Zajac M, Wrobel G, Nogowska M. 2000. Redox methods validation of paracetamol and ascorbic acid in pharmaceutical preparations. Acta Poloniae Pharmaceutica **57,** 27–32.

Proteggente AR, Pannala AS, Paganga G, Va BL, Wagner E, Wisema S, Vande PF, Dacombe C, Rice-Evans CA. 2002. The antioxidant activity of regular consumed fruit and vegetables reflects their phenolic and vitamin-C composition. Free Radical Research **36,** 217-233.

Ramadan MF, Kroh LW, Moersel JT. 2003. Radical scavenging activity of black cumin (Nigella sativa L.),(Guizotia abyssinica Cass.) crude seed oils and oil fractions. Journal of the Science of Food and Agriculture **51,** 6961–6969.

Ramadan MF, Moersel JT. 2007. Impact of enzymatic treatment on chemical composition, physicochemical properties and radical scavenging activity of goldenberry (Physalis peruviana L.) juice. Journal of the Science of Food and Agriculture **87,** 452-460.

Ramaiya SD, Bujang JS, Zakaria MJ, King WS,

Shaffiq Sahrir MA. 2013. Sugars, ascorbic acid, total phenolic content and total antioxidant activity in passion fruit (Passiflora) cultivars. Journal of the Science of Food and Agriculture **93,** 11981205.

Ribeiro DO, Pinto DC, Lima LM, Volpato NM, Cabral LM, de Sousa VP. 2011. Chemical stability study of vitamins thiamine, riboflavin, pyridoxine and ascorbic acid in parenteral nutrition for neonatal use. Nutrition Journal **10,** 47.

Richelle M, Travazzi I, Offord E. 2001. Comparison of the antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa and tea) prepared per cup serving. Journal of Agricultural and Food Chemistry **49,** 3438-3442.

Riemersma RA, Carruthers KF, Elton RA, Fox KA. 2000. Vitamin-C and the risk of acute myocardial infarction. The American Journal of Clinical Nutrition **71,** 1181-1186.

Rodríguez-Bernaldo de Quirós A, Costa HS. 2006 Analysis of carotenoids in vegetable and plasma samples: A review. Journal of Food Composition and Analysis **19,** 97-111.

Salama MF, Abbas A, Darweish MM, El-Hawwary AA, Al-Gayyar MM. 2013. Hepatoprotective effects of cod liver oil against sodium nitrite toxicity in rats. Pharmaceutical Biology **51,** 1435-1443.

Sargeant LA, Jaeckel A, Wareham NJ. 2000. Interaction of vitamin-C with the relation between smoking and obstructive airways disease in EPIC Norfolk. European Prospective Investigation into Cancer and Nutrition. European Respiratory Journal **16,** 397-403.

Shinn C, Marco A, Serrano L. 2013. Influence of low levels of water salinity on toxicity of nitrite to anuran larvae. Chemosphere **92,** 1154-1160.

Shyla B, Nagendrappa G. 2013. Redox spectrophotometric method involving electrolytically generated manganese(III) sulphate with diphenylamine for the determination of ascorbic acid present in the samples of various fruits, commercial juices and sprouted food grains. Food Chemistry **138,** 2036-2042.

Sigusch BW. 2013. The role of vitamin-C (ascorbic acid) in the prevention and therapy of oral diseases. Archives of Oral Biology **58,** 905-906.

Sorice A, Guerriero E, Capon, F, Colonna G, Castello G, Costantini S. 2014. Ascorbic acid: its role in immune system and chronic inflammation diseases. Mini Reviews in Medicinal Chemistry **14,** 444-452.

Stander MA, Kuhn W, Hiten NF. 2013. Survey of South African fruit juices using a fast screening HILIC-MS method. Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment **30,** 1473-1484.

Terry P, Terry JB, Wolk A. 2001. Fruit and vegetable consumption in the prevention of cancer: an update. Journal of General Internal Medicine **250,** 280-290.

Valente A, Sanches-Silva A, AlbuquerqueTG, Costa HS. 2014. Development of an orange juice in-house reference material and its application to guarantee the quality of vitamin-C determination in fruits, juices and fruit pulps. Food Chemistry **154,** 71-77.

Verdini RA, Lagier CM. 2000. Voltammetric iodometric titration of ascorbic acid with dead-stop end-point detection in fresh vegetables and fruit samples. Journal of the Science of Food and Agriculture **48,** 2812-2817.

Willett WC. 1991. Micronutrients and cancer risk. The Journal of the American Medical Association **53,** 265S-269S.

Yokoyama T, Date C, Kokubo Y, Yoshiike N, Matsumura Y, Tanaka H. 2000. Serum vitamin-C concentration was inversely associated with subsequent 20–year incidence of stroke in a Japanese rural community. The Shibata study. Stroke **31,** 2287-2294.