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Capacidad antioxidante y contenido de polifenoles totales en manzanas de distintas variedades cultivadas en Chile

Antioxidant capacity and total polyphenol content in different apple varieties cultivated in Chile

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Resumen

Se determinó el contenido de polifenoles totales por método de Folin Ciocalteu y capacidad antioxidante por método FRAP, en 3 variedades de manzanas cultivadas en Chile: Granny Smith, Royal Gala y Fuji (enteras y sin piel). El contenido de polifenoles totales en manzanas enteras y sin piel no presenta diferencias significativas. La capacidad antioxidante de la variedad Granny Smith es significativamente mayor que las variedades Royal Gala y Fuji. Se deshidrataron hojuelas de manzana a 60° C por 4 horas y éstas presentaron alto contenido de polifenoles totales. La adición de ácido cítrico y bisulfito de sodio – como agentes antipardeamiento- antes de la deshidratación presentó un efecto protector sobre los polifenoles totales y capacidad antioxidante.
Se encontró una correlación entre el contenido de polifenoles totales y capacidad antioxidante en manzanas sin piel (R^2 = 0.8081) y manzanas enteras (R^2 = 0.7585).

Palabras clave: Polifenoles; Capacidad antioxidante; Deshidratación; Manzana.

Abstract

Three apple varieties cultivated in Chile were studied in total polyphenol content by Folin Ciocalteu method and antioxidant capacity by FRAP method: Granny Smith, Royal Gala and Fuji (whole and peeled apples). The total polyphenol content in whole and peeled apples do not show significant differences. The antioxidant capacity of the Granny Smith variety is significantly higher than Royal Gala and Fuji. Apple dehydration at 60 °C for 4 hours to obtain flakes keeps polyphenol content high. The addition of citric acid and sodium bisulfite - as antibrowning agents- before drying has a protective effect on polyphenols and its antioxidant capacity.
There is a correlation between the polyphenol content and the antioxidant capacity in peeled apples (R^2 = 0.8081) and whole apples (R^2 = 0.7585).

Keywords: Polyphenols; Antioxidant capacity; Dehydration; Apple.

Introduction

Fruit and vegetable consumption has been linked with lower morbidity and mortality rates due to degenerative diseases (1).

Some chemical compounds which occur naturally in plants are the cause of this relationship. Among them, polyphenols stand out. They contain more than two –OH groups attached to a benzene ring and are divided into several classes according to the number of benzene rings that they contain and the structural elements that bind these rings to one other. Because of their nearby -OH groups and conjugated double bonds, polyphenols show antioxidant capacities and their mechanisms are electron donation, metal ion chelation, ascorbic acid sparing and ROS quenching (2).

Apples are grown on a large scale worldwide and the different apple varieties are highly consumed. Apple attributes of texture and flavor are preferred by consumers. Consumption of apples has been linked with the prevention of chronic diseases and with a lower incidence of cancer (3).

Apples are a good source of polyphenols and have a high antioxidant capacity (4). The purposes of this study are to determine which apple varieties grown in Chile – Fuji, Granny Smith and Royal Gala- present the highest polyphenol content and antioxidant activity, to evaluate the contribution of apple skin in the polyphenol content and

antioxidant capacity, and to analyze the effects of dehydration and the addition of citric acid and sodium bisulfite -as antibrowning agents- on apple polyphenol content and antioxidant capacity.

Materials and methods

Chemicals

All chemicals and reagents used were of analytical grade. Folin-Ciocalteau reagent (FCR), Na_2CO_3, gallic acid standart,methanol, acetate buffer, HCl, 2,4,6-tripyridyl-*s*-triazine (TPTZ), $FeCl_3$ andFeSO4 from Merck.

Samples

The samples of the different apple varieties (Granny Smith, Royal Gala and Fuji) at commercial maturity were purchased from a vegetable market in Santiago of Chile in 2011. The selected fruits were of uniform size and absence of defects and placed in cold storage at 4 °C until they were analized.

Treatments included 11 groups: (1) Granny Smith whole (G1); (2) Royal Gala whole (R1); (3) Fuji whole (F1); (4) Granny Smith pulp (G2); (5) Royal Gala pulp (R2); (6) Fuji pulp (F2); (7) Granny Smith snack (G3); (8) Granny Smith snack pretreatment with citric acid (G4); (9) Granny Smith snack pretreatment with citric acid and sodium bisulfite (G5); (10) Royal Gala snack pretreatment with citric acid and sodium bisulfite (R3) and (11) Fuji snack pretreatment with citric acid and sodium bisulfite (F3).

Samples of whole fresh apples and fresh apple pulp were analyzed.On the other hand, apple snacks were elaborated according to the following methodology: whole apples were sliced using an apple slicer in order to obtain ± 5 mm apple flakes. Apple flakes were submerged in both citric acid (15 g/L) and sodium bisulfite (0.7 g/L) solutions for 10 minutes to prevent enzymatic browning. Later, apple flakes were rinsed in water and taken to the dehydration tunnel with forced-air circulation at a temperature of 60 ºC (140 ºF) for 4 h. For the Granny Smith variety, untreated slices were dehydrated, treated with citric acid alone and both citric acid and sodium bisulfite. The snacks were packed in polypropylene bags and stored at room temperature until evaluation.

Extraction procedure

The samples were ground using a grinder to yield fine particles. The sample (1 g) was added 10 ml methanol, stirred for 1 hour, centrifuged for 15 min at 3600 rpm and finally the supernatant was removed to perform the analysis.

Determining total polyphenols

Total phenolic contents from the extracts were quatified using Folin-Ciocalteu´s method as described by Singleton *et al.* (5).

Antioxidant capacity

Ferric reducing antioxidant power (FRAP) was determined in the sample extracts according to Benzie & Strain (6).

Statistical analysis

The data are shown as the mean ± standard deviation of five repeated samples. The results obtained were compared by analysis of variance (ANOVA) using the SPSS 15.0 program (SPSS, Inc.) for Windows. Multiple comparison was performed using the Duncan's test at the 0.05 level.

Results and discussion

Polyphenol content in the samples of Granny Smith, Royal Gala and Fuji whole apple varieties goes from 142.72 to 106.63 mg GAE/100g, presenting no significant differences (p>0.05) among the apple varieties.

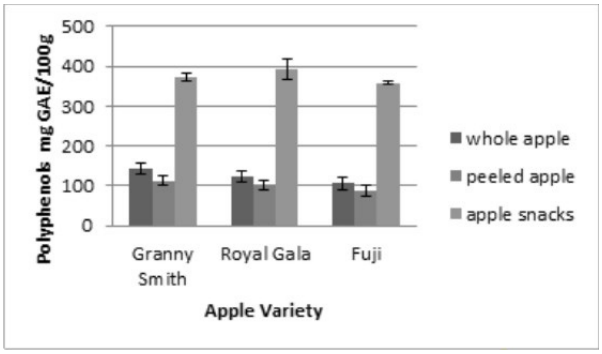

Figure 1: Total polyphenols of fresh whole apples, fresh peeled apple and apple snacks previously immersed in citric acid and bisulfite (dehydrated at 60 °C4 h) in Granny Smith, Royal Gala and Fuji apple varieties.

Henriquez *et al.* (7) found significant differences between different varieties of Chilean whole apples. Other authors point out that Royal Gala apples have the highest polyphenol concentration -very similar to that presented by Granny Smith apples, though- and that Fuji apples have the lowest (8).

The polyphenol content determined in this study is lowest than that established by Imeh & Khokhar (8), since the initial treatment of the sample was different: they cut and mashed the apples under liquid nitrogen conditions and then dried the samples using the lyophilization process, thus preventing the action of the polyphenol oxidase enzyme.

Other studies show different values of polyphenol contents in different apple varieties, which go from 71 mg GAE/100g to 335.9 mg GAE/100g (4, 9,10).

The difference found in several studies is explained by the complexity of these compounds and the extraction and analysis methods. Phenolic compounds which are present in fruits occur free or combined with glycosides in nature. When combined with glycosides, phenolic compounds cannot be completely extracted. For that reason, the total polyphenol content may be underestimated (11). During the sample preparation stage, the way in which apples are cut and the homogenization process may induce a reaction of the polyphenol oxidase enzyme, thus decreasing the concentration of phenolic compounds (12).

Moreover, the concentration of phenolic compounds may be affected by apple variety, cultivar and genus and also by extrinsic factors, such as soil, seasonality, agronomic factors, light exposure, etc. (13).

To compare the polyphenol content among peeled fruits, it was found that there are no significant differences between them(p> 0.05).The content of phenolic compounds varies in the different fruit tissues. Polyphenol content in apple skin is approximately three times higher than that found in the flesh. Carbone *et al.* (14) analyzed phenolic compounds in apple flesh and skin, they determined 1.5 times more phenolic compounds in fruit skin than flesh.In the case of apples - as in many other fruits– phytochemical compounds are present mainly in the skin, since this is the part of the fruit which offers protection against adverse agents from the environment. For that reason, antioxidant accumulationin the skin is a frequent phenomenon (7, 15). This result agrees with those obtained by different authors (10, 16).

Consuming unpeeled fruits is highly recommended than eating the flesh only.

Khanizadeh *et al.,* (17) determined the polyphenol composition of apple flesh and skin in different apple varieties using HPLC, finding that apple skin contained 4.3 times more polyphenols than apple flesh. Furthermore, the polyphenol composition is different, as some polyphenols are exclusive for apple skin and, therefore, they are not present in the flesh. Apple flesh shows a higher concentration of chlorogenic acid, hydroxycinnamic acids, and procyanidins, whereas apple skin has a higher concentration of catechins, epicatechins, flavonols and dehydrochalcones when compared to those contained in the flesh. Other authors agree that apple skin has a higher concentration of flavonols than apple flesh.

In the apple snacks concentration of fruit compounds occurs, due to dehydration, and the increase in the polyphenol content is significantly higher than those of fresh apples (p<0.01).

Polyphenols are accumulated mainly in plant cell vacuoles, which are destroyed by the application of heat. As a result, polyphenols are released to the environment, turning more accessible to extraction and more available during the consumption of the fruit. The increase in the polyphenol content that takes place in dehydrated fruits is due to the formation of molecules with a lower molecular weight at moderately high temperatures, such as 60ºC (140ºF) temperature applied during the apple drying process. By contrast, at extreme temperatures, a destruction of the phenolic structure would occur. Moreover, the polyphenol release would be the result of the hydrolysis reactions of the glycosylated molecules during the dehydration process (18, 19). On the other hand, temperature inhibits pro-oxidant enzymes, as in the case of the polyphenol oxidase enzyme (20).

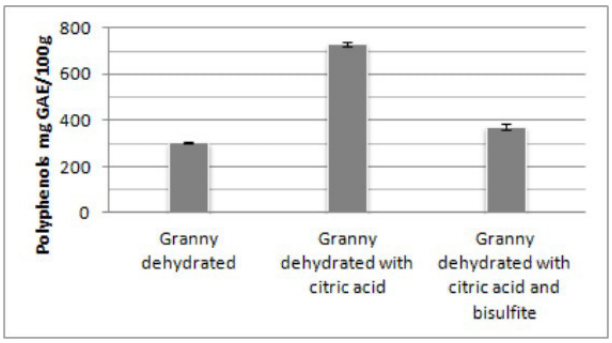

Figure 2: Total polyphenols of apple snacks (dehydrated at 60°C for 4 h), apple snack treated with citric acid,apple snack treated with citric acid and bisulfite. Granny Smith variety.

In several studies, an increase in the total polyphenol levels by effect of fruit drying (plums, figs, peaches and raisins) has been demonstrated, as in dried apples, rises in the total polyphenol levels - which went from 20 to 200 %- were observed when compared to the levels of fresh fruit (21). Studies conducted in sun-dried green leafy vegetables showed a change in the total polyphenol content, when increases in a range of 6,45% to 223,08% were observed (22).

Citric acid and sodium bisulfite are added in order to prevent apples from the enzymatic browning. The degree of browning depends on the phenolic compound content and the polyphenol oxidase activity. Citric acid produces a pH reduction, thus decreasing the enzyme activity, whereas sodium bisulfite is the most potent polyphenol oxidase inhibitor in apples.

Figure 2 shows that there are significant differences among Granny Smith apples which were only dehydrated, those immersed in a citric acid solution before drying, and those which were immersed in both citric acid and bisulfite sodium solutions before being dried. The higher concentration of polyphenols was shown by apples treated with citric acid only.

Rababah *et al.* (21) found no significant differences in the polyphenol content in samples of fresh, mashed, and dehydrated apples when ascorbic acid was added. Also, during the dehydration process, ascorbic acid disappears quickly due to the dehydration temperature or because of

the important role of this acid in the o-quinone regeneration. O-quinones are the result of the phenol oxidation by the polyphenol oxidase enzyme during the enzymatic browning. For that reason, ascorbic acid may be oxidized to dehydroascorbic acid (23).

Enzymatic browning occurs almost immediately after the destruction of the cellular structure and the mixing of enzyme and substrate, which happens immediately after cutting or peeling fruits (24). Sodium bisulfite addition to fruits before drying produces a slight protective effect on the polyphenol content (25). It would be expected to increase both citric acid and bisulfite the polyphenol content, but it is unclear why it exclusively increases with citric acid.

In relation to the antioxidant capacity evaluated by using the FRAP technique, Granny Smith variety shows a value which is significantly higher ($p<0.05$) than those of the Royal Gala and Fuji varieties.

Comparing the antioxidant activity in whole apples, using the FRAP assay, Granny Smith has significantly higher antioxidant capacity than the other two varieties. These results agree with those obtained by Henriquez *et al.* (7), who worked with Chilean apples. Imeh & Khokhar (8) determined the following decreasing order ofantioxidant capacity: Fuji > Granny Smith > Royal Gala.

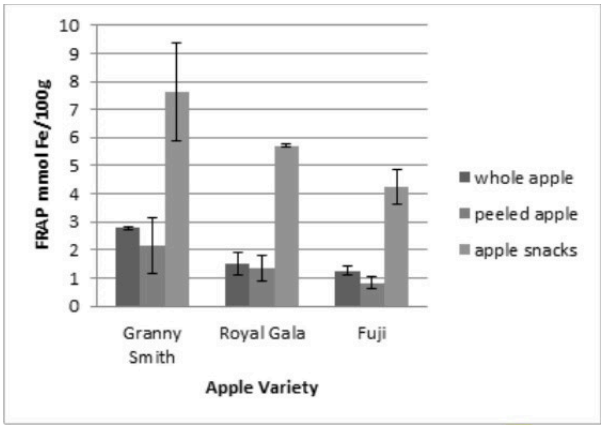

Figure 3: Antioxidant capacity FRAP of fresh whole apples, fresh peeled apple and apple snacks treated with citric acid and bisulfite(dehydrated at 60°C for 4 h) in Granny Smith, Royal Gala and Fuji apple varieties.

Araya *et al.* (26) found that Royal Gala apples had a higher antioxidant activity than Fuji apples, with values of 0.671 and 0.458 mmol Fe/100g respectively. These values are lower than those determined in this study.

The values obtained in the present study are higher than those determined by Lotito & Frei (4) in Granny Smith and Fuji apples, with FRAP values of 0.914 y 0.780 mmol Fe/100g, respectively. The great variability observed in different studies regarding the apple antioxidant activity may be explained by the influence of the fruit tree's location, harvest season, type of soil, agronomic factors, postharvest conditions, etc., all which affect the antioxidant levels present in vegetables (3, 13, 27). However, storage, refrigeration and controlled atmosphere conditions do not affect the antioxidant activity of the apples (28).

Antioxidant activity measured by FRAP in peeled apples compared to whole fruit, has no significant differences.Araya *et al.* (26) worked with Fuji and Royal Gala apples and obtained a bigger diminution in their FRAP value (73,58% for Fuji apples and 62,30% for Royal Gala apples). Wolfe *et al.* (15) determined the antioxidant capacity of 4 apple varieties using methods different from FRAP and concluded that the skin of these 4 varieties contained a higher antioxidant capacity than apple flesh and whole apples (flesh and skin).

The antioxidant capacity measured by FRAP in apple skin is -on average- 6.6 times higher than that found in the flesh in apples from 8 different varieties (17).

In apple snacks, the increase in the antioxidant activity is produced in the order of 175%, 278% and 237% for Granny Smith, Royal Gala and Fuji apples respectively. There is a significant difference between fresh and apple snacks for Granny Smith,Royal Gala and Fuji varieties.

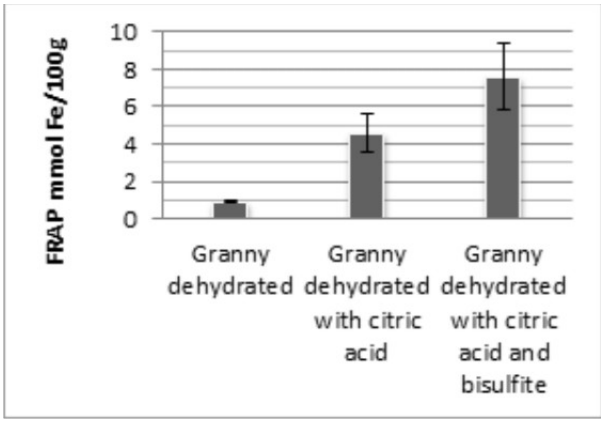

Figure 4: Antioxidant capacity FRAP of apple snacks (dehydrated at 60°C for 4 h), apple snack treated with citric acid, apple snack treated with citric acid and bisulfite. Granny Smith variety.

Apple flakes were submerged in both citric acid (15 g/L) and sodium bisulfite (0.7 g/L) solutions for for 10 minutes.

The antioxidant capacity of apple snacks increased in almost 5 times when citric acid was added, and 8 times in the case of the addition of sodium bisulfite regarding dehydrated apple without treatment.

The correlation between polyphenol concentration and antioxidant capacity measured by FRAP for all the apple samples is low (R^2= 0.428), but it increases when only whole apples or apple flesh is correlated. In this study, the correlation is in the order of R^2 = 0.7585 for whole apples and R^2 = 0.8081 for apple flesh (Figure 5 and Figure 6).

Müller *et al.* (29) obtained a R^2 value of 0.85 when they correlationed the polyphenol content and FRAP values in different fruit whipped creams, concentrates and juices. Rodriguez-Vaquero *et al.* (30) demonstrated that the correlation between polyphenol content and FRAP values in Argentinian herbal infusions is in the order of R^2 = 0.812.

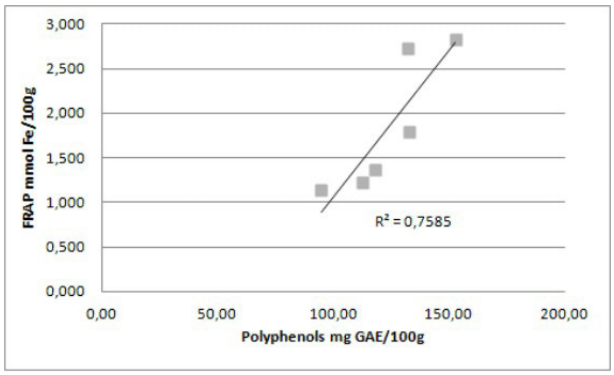

Figure 5: Correlation between polyphenol content and antioxidant capacity (FRAP) in fresh whole apples.

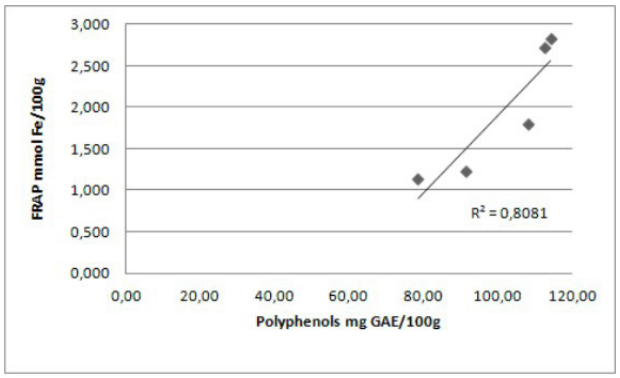

Figure 6: Correlation betwween polyphenol content and antioxidant capacity (FRAP) in peeled fresh apples.

In apples from 67 different varieties, Wojdyło *et al.* (31) determined a R^2 value of 0.804, which may be considered fairly high.

Khanizadeh *et al.* (17) worked with different apple culture and obtained a correlation between the polyphenol content and FRAP values in the order of $R^2 = 0.74$ and $R^2 = 0.51$ in apple flesh and skin respectively, whereas Vieira *et al.* (13) obtained very similar correlations for apple flesh and skin when they correlationed the polyphenol content and the antioxidant capacity measured by ABTS assay ($R^2 = 0.717$ and $R^2 = 0.716$). Henríquez *et al.* (7) worked with different varieties of Chilean apples and found a higher correlation between polyphenol content and FRAP in apple skin, being followed by whole apples. However, in apple flesh, the correlation was not significant. In this study, the correlation is in the order of $R^2 = 0.8081$ for apple flesh and $R^2 = 0.7585$ for whole apples.

Conclusions

From the results obtained in the present study, it can be concluded that the polyphenol content in Chilean apples from three different varieties are similar and that the antioxidant capacity of Granny Smith apple variety is significantly higher. In apple skin, bioactive compounds are accumulated, since whole apples presented a higher antioxidant capacity and concentration of total polyphenols. Apple dehydration under the conditions described in this study (5 mm thick flakes dried at a temperature of 60 ºC for 4 h) does not affect the polyphenol concentration and antioxidant capacity.

The addition of citric acid and sodium bisulfite as anti-browning agents has a positive effect, since they improve the antioxidant capacity of dehydrated apples significantly.

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References

1. **Mirmiran, P.; Noori, N.; Zavareh, M. B. ; Azizi, F.** Fruit and vegetable consumption and risk factors for cardiovascular disease. Metabolism, 58: p 460-468. 2009.
2. **Kalt, W.** Effects of production and processing factors on major fruit and vegetable antioxidants. Journal of Food Science, 70: p 11-19.2005.
3. **Jakopic, J.; Stampar, F.; Veberic, R.** The influence of exposure to light on the phenolic content of 'Fuji' apple. Scientia Horticulturae, 123: p 234-239. 2009.
4. **Lotito, S.; Frei, B.** Relevance of apple plyphenols as antioxidants in human plasma: contrasting in vitro and in vivo effects. Free Radical Biology & Medicine, 36: p 201-211.2004.
5. **Singleton, V. L.; Orthofer, R.; Lamuela-Raventos., R. M.** Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology,299: p 152-178. 1999.
6. **Benzie, I.F.F.; Strain, J.J.** The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Analytical Biochemistry, 239: p 70-76. 1996.
7. **Henriquez , C.; Almonacid, S.; Chiffelle, I.; Valenzuela, T.; Araya, M.; Cabezas, L.; Simposon R., Speisky H.** Determination of antioxidant capacity, total phenolic content and mineral composition of different fruit tissue of five apple cultivars grown in Chile. Chilean Journal of Agricultural Research, 70: p 523-536.2010.
8. **Imeh, U.; Khokhar, S.** Distribution of conjugated and free phenols in fruits: antioxidant activity and cultivar variations. Journal of Agricultural and Food Chemistry, 50: p 6301-6306.2002.
9. **Rop, O.; Jurikova, T.; Sochor, J.; Mlcek, J.; Kramarova, D.** Antioxidant capacity, scavenging radical activity and selected chemical composition of native apple cultivars from central europe. Journal of Food Quality, 34: p 187-194.2011.

10. **Drogoudi, P. D.; Pantelidis, G.** Effects of position on canopy and harvest time on fruit physico-chemical and antioxidant properties in different apple cultivars. Scientia Horticulturae, 129: p 752-760. 2011.
11. **Balasundram, N.; Sundram, K.; Samman, S.** Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chemistry, 99: p 191-203.2006.
12. **Patthamakanokporn, O.; Puwastien, P.; Nitithamyong, A.; Sirichakwal, P.** Changes of antioxidant activity and total phenolic compounds during storage of selected fruits. Journal of Food Composition and Analysis, 21: p 241-248.2008.
13. **Vieira, F.G.K.; Da Silva,G.; Copetti, C.; Faria, P.; Da Costa, E.; Fett R.** Phenolic compounds and antioxidant activity of the apple flesh and peel of eleven cultivars grown in Brazil. Scientia Horticulturae, 128: p 261-266. 2011.
14. **Carbone, K.; Giannini, B.; Picchi, V.; Lo Scalzo, R.; Cecchini, F.** Phenolic composition and free radical scavenging activity of different apple varieties in relation to the cultivar, tissue type and storage. Food Chemistry,127: p 493-500. 2011.
15. **Wolfe, K.; Wu, X.; Liu, R.H.** Antioxidant activity of apple peels. Journal of Agricultural and Food Chemistry, 51: p 609-614.2003.
16. **Le Bourvellec, C.; Bouzerzour, K.; Ginies, C.; Regis, S.; Plé, Y.;Renard, C.M.** Phenolic and polysaccharidic composition of applesauce is close to that of apple flesh. Journal of Food Composition and Analysis, 24: p 537-547.2011.
17. **Khanizadeh, S.; Tsao, R.; Rekika, D.; Yang, R.; Charles, M.T.; Rupasinghe, V.** Polyphenol composition and total antioxidant capacity of selected apple genotypes for processing. Journal of Food Composition and Analysis, 21: p 396-401.2008.
18. **Soong, Y.; Barlow, P.** Antioxidant activity and phenolic content of selected fruit seeds. Food Chemistry, 88: p 411-417.2004.
19. **Turkmen, N.; Sari, F.; Velioglu, S.Y.** The effect of cooking method on total phenolics and antioxidant activity of selected green vegetables. Food Chemistry, 93: p 713-718.2005
20. **Del Caro, A.; Piga, A.; Pinna, I.; Fenu, P.M.; Agabbio, M.** Effect of drying conditions and storage period on polyphenolic content, antioxidant capacity and ascorbic acid prunes. Journal of Agricultural and Food Chemistry, 52: p 4780-4784. 2004.
21. **Rababah, T.M.; Ereifej, K.I.;Howard, L.** Effect of ascorbic acid and dehydration on concentrations of total phenolic, antioxidant capacity, antocyanins and color in fruits. Journal of Agricultural and Food Chemistry, 53:p 4444-4447.2005.
22. **Oboh, G.; Akindahunsi, A.** Changes in the ascorbic acid, total phenol and antioxidant activity of sun-dried commonly consumed green leaf vegetables in Nigeria. Nutrition Health, 18: p 29-36. 2004.
23. **Devic, E.; Guyot, S.; Daudin, J-D. ; Bonazzi, C.** Effect of temperature and cultivar on polyphenol retention and mass transfer during osmotic dehydration of apples. Journal of Agricultural and Food Chemistry, 58: p 606-614.2010.
24. **24. Jang, J-H.; Moon, K-D.** Inhibition of polyphenol oxidase and peroxidase activities on fresh-cut apple by simultaneous treatment of ultrasound and ascorbic acid. Food Chemistry, 124: p 444-449. 2011.
25. **Latapi, G.; Barrett, D.** Influence of pre-drying treatments on quality and safety of sun-dried tomatoes. Part I: Use of steam blanching, boiling brine blanching and dips in salt or sodium metabisulfite. Journal of Food Science, 71: p 24-31. 2006.
26. **Araya, H.; Clavijo, C. ; Herrera, C.** Capacidad antioxidante de frutas y verduras cultivadas en Chile. Archivos Latinoamericanos de Nutrición, 56: p 361-365. 2006.
27. **Felicetti, D.A. y Schrader, L.E.** Postharvest changes in pigment concentrations in "Fuji" apples with "Fuji" stain. Scientia Horticulturae, 125: p 283-288. 2010.
28. **Van der Sluis, A.; Dekker, M.; Van Boekel, M.** Activity and concentration of plyphenolic antioxidants in apple juice.3. Stability during storage. Journal of Agricultural and Food Chemistry, 53: p 1073-1080. 2005.
29. **Müller, L.; Gnoyke, S.; Popken, A.M.; Böhm, V.** Antioxidant capacity and related parameters of different fruit formulations. LWT-Food Science and Technology, 43: p 992-999. 2010.
30. **Rodriguez-Vaquero, M.J.; Tomassini Serravalle, L.R.; Manca de Nadra, M.C.; Strasser de Saad, A.M.** Antioxidant capacity and antibacterial activity of phenolic compounds from argentinean herbs infusions. Food Control, 21: p 779-785. 2010.
31. **Wojdyło, A.; Oszmianski, J.; Laskowski, P.** Polyphenolic compounds and antioxidant activity of new and old Apple varieties. Journal of Agricultural and Food Chemistry, 56:p 6520-6530.2008.

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