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Production of a Safe and Efficient Antioxidant-Rich Extract from Sweet Orange *(Citrus sinensis* cv Shahsavari) Peel

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ABSTRACT: The aim of this study is to develop an extraction method for the producing of a safe and efficient source of antioxidant compounds from fresh and pre-treated sweet orange (*Citrus sinensis cv shahsavari*) peel. Three types of solvents (100% ethanol, 100% water and ethanol-water mixtures at the ratios of 35:65 and 65:35, v/v), three duration times (60, 120 and 240 min) were chosen as the parameters affecting the phenolic compounds and flavonoid contents, extraction yield and antioxidant activities. These properties were evaluated by using the Folin-Ciocalteu method, DPPH radical scavenging activity assay and the aluminum chloride colorimetric method, respectively. The results showed that the extraction times within the current range of 60-240 min have no significant effect on the investigated properties. The binary water-ethanol mixture at the ratio of 65:35 showed the best extraction yield for total phenolic compounds of fresh orange peel. The lowest IC_{50} and the highest antioxidant activity observed in this extract sample indicated a possible synergistic effect among the phenolic compounds with different polarities. Pretreatment of the orange peel by liquid nitrogen resulted in maximum antioxidant activity that may be related to the releasing the bounded phenolic compounds.

Keywords: *Antioxidant Source, Freeze Drying, Liquid Nitrogen, Phenolic Compound, Sweet Orange Peel.*

Introduction

In recent years, functional foods have become much more popular because of increasing concern about the diet-related diseases such as cancer, heart disease, obesity and diabetes. Several epidemiological studies have reported on the protective effects of the phytochemicals especially phenolic compounds against numerous diseases (Pokorny *et al.*, 2001). Waste and byproducts of fruits are good sources of sugars, minerals, dietary fibers and phenolic compounds and using these waste materials may be considered as an efficient and economic and environmentally-friendly way of producing natural antioxidant rich extracts (Singh & Immanuel, 2014).

Citrus fruits are one of the world's most consumed fruits with significant antioxidant activities eaten fresh and used for juice production (Mokbel *et al.*, 2006). During the juice production, a large amount of by-products including peel and seeds are remained as the waste materials that constitutes 45-50% of the fruit mass (Omoba *et al.*, 2015). Sweet orange (*Citrus sinensis*) is the most important class of commercial citrus products worldwide (Kimball, 1991; Berger, 2007), which is usually used for the

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juice production. Therefore, a huge quantity of peel is generated in these industries that are treated as waste materials without proper intended applications. Several studies reported that orange peel contains high concentration of total phenolic compounds and presented antioxidant activities in the food products (Yalcin *et al.,* 2011; Arora & Kaur, 2013; Asha *et al*., 2015). Thus, orange peel might be considered as a rich source of antioxidants and could be added in the formulations of different food products. Such an application has been reported by Rasouli *et al*. (2017) for producing a functional drink.

Among different solvents used for the extraction of the antioxidant compounds from the plant sources, the polar types including ethanol, methanol and water are known to be more suitable for recovering polyphenolic compounds from the plant matrices (Do *et al*., 2014). The type of solvent selected for such applications are mainly based on the following aspects (Ghasemzadeh *et al*., 2015; Waszkowiak and Gliszczyn´ska‑S´wigło, 2016; Złotek *et al*., 2016).

a) Extraction yield and antioxidant activity
b) Toxicity of the residual solvent and its risk to human health
c) Solvent waste disposal problems

According to directive 2009/32/EC, ethanol is classified in the group of solvents which may be used in compliance with good manufacturing practice for all uses and have been found acceptable from the point of view of safety to the consumers. But for methanol, the maximum residue limit of 10mg/Kg in the extracted foodstuff has been established (Anon, 2009). Furthermore, in 26 August 2010, the European commission declared that the above-mentioned directive might not be applied to the extraction solvents used in the production of food additives and this limit should be lowered to 1.5mg/Kg in order to be considered as safe for food additives (Anon, 2010). Therefore, it might be concluded that ethanol and water are preferred to methanol from the safety point of view.

Up to now, different solvents have been used to extract phenolic compounds from orange peel. But, only a few studieshave considered the toxicity of the solvents that might remainin the final extract. Therefore, the current study is focused on introducing a method to produce a safe extract of phenolic compounds from sweet orange peelusing water, ethanol or a mixture of them as the solvents.

Materials and Methods

- Materials

Fresh sweet orange *(Citrus sinensis*cv Shasavari) was purchased from north of Iran. Gallic acid, quercetin and 2, 2 diphenyl-1-picrylhydrazyl (DPPH) were supplied from Sigma-Aldrich (St. Louis, MO). Folin-Ciocalteu reagent and α-tocopherolwere purchased from Merck Chemical Company (Darmstadt, Germany). All of the other reagents were of analytical grade and obtained from Merck Chemical Company.

- Methods

- Preparation of orange peel extract from fresh and treated samples

The orange fruits were first peeled and the albedo was removed manually. Ten grams of the fresh shredded peel was extracted with 150 ml of four different solvents including 100% ethanol, 100% water and two ethanol:water mixtures (at the ratios of 35:65 and 65:35, v/v) (Rezaei *et al.*, 2013). The extraction process was accomplished at 40°C in a thermo-shaker (Gerhardt Co., Konigswinter, Germany) at 150 rpm for 1,2 and 4 hours. The mixture was then centrifuged at 12,000 rpm for 15 min at room temperature and the supernatant was refrigerated for further analysis. In the

second group of analysis, pretreated orange peel samples were first dried by a freeze-dryer (Christ, Osterode, Germany) at -56°C, 50 KPa pressure for 24 hoursor frozen under liquid nitrogen. Then, the pretreated samples were extracted under the optimum extraction conditions (extraction time and solvent) determined from the first group of tests (Rasouli *et al.*, 2017).

- Determination of total phenolic content in the extracted samples

Total phenolic contents of the extracts were determined using Folin-Ciocalteu colorimetric method (Chen *et al.*, 2008). Forty µl of the extract was mixed with 200 µl of the Folin–Ciocalteu reagent and 3.2 ml of distilled water and kept at room temperature. After 5.0 min, 600 µl of a saturated sodium carbonate solution was added and the mixture was placed at room temperature for 2 hours. The absorbance was measured at 760 nm via a UV–visible spectrophotometer (Ultrospec 2000, Manassas, Virginia, USA). Total phenolic content was calculated from the calibration curve ($y=0.0005x+0.0082$, $r^2=0.993$) and the results were expressed as mg of gallic acid equivalent to 100g of dry orange peel.

- DPPH radical scavenging activity

The antioxidant activity of the extractswere measured with the DPPH method (Brand-Williams *et al.*, 1995) with slight modifications (Duarte-Almeida *et al.*, 2006). Two mlof (0.01, 0.02, 0.05 and 0.1) v/v of the orange peel extract in ethanol and 4.0 ml of DPPH solution (2×10^{-4} M) were mixed in the test tube. The test tube was then incubated in the dark for 60 min at room temperature.

The decrease in the absorbance was measured at 517 nm using a UV-visible spectrophotometer. The inhibition of radicals was calculated using the following formula:

$$\%\ \text{inhibition} = \frac{A0 - As}{As} \times 100$$

Where A_0 is the absorbance of the control (i.e., no extract added) and A_s is the absorbance of the sample with DPPH solution. The half-maximal inhibitory concentration (IC_{50}) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%.

- Total flavonoid content

Total flavonoids contents of orange peel extracts were determined by the aluminum chloride colorimetric method (Zhishen *et al.*, 1999). According to this procedure, 0.5ml of orange peel extract was mixed with 1.5 ml ethanol, 0.1 ml of a-10%ethanolic solution of aluminum chloride (10 g $AlCl_3$ in 100 ml ethanol), 0.1ml of a 1 mol/l potassium acetate solution and 2.8 ml distilled water. The mixture was allowed to stand for 30 min and absorbance was measured at 415 nm. Total flavonoid content was calculated from a calibration curve ($y=0.0064x+0.0239$, $r^2=0.985$) and the results were expressed as mg quercetin equivalent per g dry weight.

- Statistical analysis

All of the tests were conducted in triplicate orderand means and standard deviations were calculated. Analysis of variance (ANOVA) followed by Duncan's test was performed using the version 22 statistical package for the social science (SPSS, IBM Co., Armonk, NY,USA).

Results and Discussion

- Total phenolic contents in the extract samples

The effect of the extraction time on total phenolic contents in the prepared extracts is shown in Table 1. The results indicated that the intended extraction times (i.e., 60,120 and 240 min) did not have significant ($p<0.05\%$) effect on the total phenolic contents of the extracts. Therefore, the shortest extraction time (60 min) was selected for further tests. According to the data in Table 2, total phenolic content in the

ethanol extract was significantly lower than that in the water extract.But, both binary ethanol/water solvents showed better performance for extracting the phenolic compounds. Generally, ethanol–water mixtures seem to be the most suitable solvents for the extraction because of different polarity of both solvents and possibility of mixing them in any proportion to provide a range of polarities (Li *et al.*, 2016) and their acceptability for human consumption. Therefore, the prepared extract might be safely added into the food product without the risk of hazardous solvents (Waszkowiak and Gliszczyn´ska-S´wigło, 2016).

Table 1. The effect of the extraction time on the total phenolic contents in the orange peel extracts.

Extraction time (min)	Total phenolic content (mg/100 g of orange peel)[1]
60	882±139[a]
120	850±224[a]
240	887±154[a]

[1] The results are expressed as mean ± standard deviation.
[a] The superscript shows that there is no significant differences (*P*>0.05) among them.

Table 2. The effect of solvent type on the total phenolic contents in the orange peel extracts (extraction time= 60 min)

Type of solvent[1]	Total phenolic content (mg/100 g of orange peel)[2]
W	933±148[a,b]
65% W+35% E	820±101[b,c]
35%W+65%E	996±128[a]
E	744±194[c]

[1] W=Water, E=Ethanol
[2] The results are expressed as mean ± standard deviation and values with different superscript letters show significant differences (*P*<0.05) among the means.

The results of the current study demonstrated that ethanol-to-water ratio is an important factor affecting the total phenolic compound extraction andincrease in ethanol volume in the tested solvents impacted negatively on the extraction. The highest content was observed for water extraction. When 35 and 65% ethanol were mixed with water, significant changes did not occurred in the extraction of the phenolic compounds. Total phenolic content was decreased significantly when 100% ethanol was used as the extraction solvent.

It has been previously proven that ethanol and water may extract different types of phenolic compounds (Do *et al.*, 2014). The content of phenolic acids and phenolic acid glycosides are usually decreased by increasing the ethanol concentration in the extraction solvent. On the other hand, phenolic acid esters are more successfully extracted by ethanol-rich solvents (Waszkowiak and Gliszczyn´ska-S´wigło, 2016). Sun *et al.* (2015), reported that water extracts may contain higher concentrations of polar phenolic acids but ethanolic extracts included a higher content of weakly polar phenolic compounds such as phenolic acid esters and flavonoids (Coulter, 2007; Sun *et al.*, 2015). Phenolic compounds of sweet orange peel mainly consisted of phenolic acids (sinapic acid, ferulic acid, coumaric acid and caffeic acid) (Zefang *et al.*, 2016) and glycosylated flavonones (narirutin and hesperidin), flavones and polymethoxylated flavones (diosmin, luteoin and sinensetin) as well asflavonols (rutin, quercetin, kaempferol) (Anagnostopoulou *et al.*, 2005; Tokusgolu & Hall, 2011). It seems that the first two groups of compounds that have higher polarities are present in higher concentrations in the peel part of our selected cultivar (i.e., *Shahsavari*) of sweet orange. Thus, the total phenolic contents were higher in the high-polar extracts (water and mixed extracts) as compared to the low-polar extracts (in the ethanolic extract).

- Antioxidant activities of the extracts

The effect of extraction time on the antioxidant activity of the extracts are given in Table 3 based on their 50% inhibition capacities (IC_{50}). The results did not show significant differences ($p<0.05$) and therefore the lowest extraction time of 60

min was selected again. Table 4, presents the IC_{50}values of the extracts obtained by different solvents. Significant differences were not observed between the IC_{50} values of the extracts when water and ethanol were used as solvents. It means that these two extracts of phenolic compounds of sweet orange peel have similar antioxidant activities. But, by using solvent mixtures (water+ ethanol), the results differed significantly ($p<0.05$). Lagourli *et al.* (2010) reported that extracts obtained by solvent with different polarity levels might contain different antioxidant constituents that demonstrated a varying DPPH reactivity (Lagourli *et al.*, 2010). Since sweet orange peel contains both polar and non-polar phenolic compounds (Anagnostopoulou *et al.*, 2005; Tokusgolu & Hall, 2011; Zefang *et al.*, 2016), it could be extract d more efficiently by binary water-ethanol solvent thatresulted in an extract with higher antioxidant activity as compared to mono-solvent systems (Waszkowiak and Gliszczyn´ska-S´wigło, 2016). Also a synergistic effect might be found between the phenolic compounds with different polarities and in our research, this effect is higher for a mixture of 35% water and 65% ethanol as solvent. Such effect has also been reported by Sun *et al.* (2015), who investigated on the effect of binary ethanol-water solvents on the antioxidant activity of propolis extracts and concluded that the solvent containing 75% ethanol showed the lowest IC_{50} and highest antioxidant activity.

Table 3. The effect of extraction time on the inhibition concentration and IC_{50}values of orange peel extracts

Extraction time (min)	Inhibition concentration (%)[1]	IC_{50} (mg/g)[1]
60	65±6[a]	48±19[a]
120	58±21[a]	95±63[a]
240	52±12[a]	106±77[a]

[1] The results are expressed as mean ± standard deviation.
[a] The superscript shows that there is no significant differences (*P*>0.05) among the means in each column.

Table 4. The effect of solvent type on the inhibition concentration and IC_{50}values of orange peel extracts (extraction time= 60 min)

Solvent[1]	Inhibition concentration (%)[2]	IC_{50} (mg/g)[2]
Water	62±17[a,b]	75±50[a,b]
65% W+35% E	48±17[b]	141±84[a]
35% W+65% E	68±7[a]	45±20[b]
Ethanol	55±10[a,b]	70±39[a,b]

[1] W=Water, E=Ethanol
[2] The results are expressed as mean ± standard deviation and values in each column with different superscript letters represent significant differences (*P*<0.05) among the means.

- Flavonoid contents in the extract samples

The effect of extraction time on the flavonoid contents of the extracts is presented in Table 5. Similar to the results obtained for the previous tests significant difference ($p<0.05$) was not observed for the extraction of flavonoids within 60-240 min of extraction. But, type of solvent shows a significant difference on the amounts of flavonoids extracted ($p<0.05$). Ability of ethanol for extracting the low- and non-polar flavonoids (Sun *et al.*, 2015) is obviously distinguishable from the results given in Table 6. The flavonoid concentration in the extract is continuously increased as the ethanol content in the solvent increased from zero up to 65% and then it is slightly decreased for pure ethanol solvent. It was previously reported that for the extraction of the phenolic constituents with different polarities, a mixed solvent (water+ethanol) with a balanced polarity will act better (Sun *et al.*, 2015; Waszkowiak & Gliszczyn´ska-S´wigło, 2016).

Table 5. The effect of extraction time on the flavonoid contents in the orange peel extracts.

Extraction time (min)	Flavonoid content (mg/100 g orange peel)[1]
60	62±24[a]
120	68±37[a]
240	65±32[a]

[1] The results are expressed as mean ± standard deviation.
[a] The superscript shows that there is no significant differences (P>0.05) among them.

Table 6. The effect of solvent type on the total phenolic contents of the orange peel extracts.

Solvent[1]	Total phenolic contents (mg/100g orange peel)[2]
Water	33±11[d]
65% W+35% E	53±6[c]
35%W+65%E	110±11[a]
Ethanol	71±4[b]

[1] W=Water, E=Ethanol
[2] The results are expressed as mean ± standard deviation and values with different superscript letters represent significant differences (P<0.05) among the means.

- Total phenolic contents, antioxidant activity and flavonoid contents in the pretreated orange peel extract samples

The effect of pretreatment of orange peel by freeze drying and liquid nitrogen on the total phenolic contents, antioxidant activity and flavonoid contents of the extracts are presented in Figure 1. The results indicated that pre-treating by freeze drying resulted in the lowest total phenolic and flavonoid contents as well as highest IC_{50}. On the other hand, using fresh orange peel is preferred for producing an extract rich in phenolic compounds as well as flavonoids from orange peel but pretreatment by liquid nitrogen might result in preparing an extract containing lower concentration of phenolic compounds with the highest antioxidant activity than fresh orange peel extract. It has been previously reported that in some cases of plant extracts, low or no correlation might be observed between the phenolic content and antioxidant activity (Parejo *et al.*, 2002; Hinneburg *et al.*, 2006). It has been proven that the insoluble/bound polar phenolics located in the cell wall matrix of the plant cells are released more successfully under liquid nitrogen during grinding by mortar and pestle (Torti *et al.*, 1995).

Conclusion

In this study, it was demonstrated that the type of solvent and the pretreatment might clearly influence the extraction yield of total phenolic compounds, flavonoids and the antioxidant activity of the extracts obtained from sweet orange peel. Binary water-ethanol mixture at the ratio of 65:35 was found to be the best solvent for extraction of orange peel. The orange peel extract is a rich source of phytochemicals such as phenolic

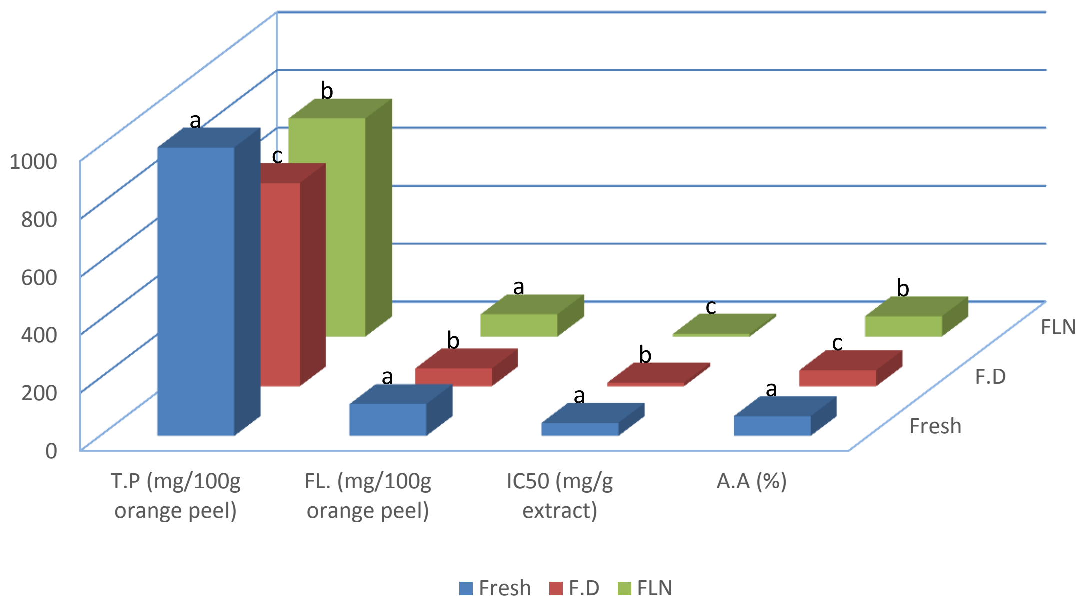

Fig. 1. Comparison of total phenolic contents (TP), flavonoid contents (FL), IC_{50} and antioxidant activities (AA) in the extracts samples produced from fresh, freeze dried (FD) and frozen by liquid nitrogen (FLN) of orange peel.

compounds and flavonoids and can be used for preparation of functional drinks. Since the types and polarities of the phenolic compounds are the main key parameters in their extraction process, it will be necessary to accomplish precise analysis of the peel of *shahsavari* sweet orange in order to determine these chemical specifications for the phenolic compounds existed in this known and abundant Iranian cultivar.

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References

Anon. (2009). European Parliament, Council of the European Union. Extraction solvents used in the production of foodstuffs and food ingredients. Directive 2009/32/EC. Official Journal of European Union/2009L0032.

Anon. (2010). European Parliament, Council of the European Union. Extraction solvents used in the production of foodstuffs and food ingredients. Directive 2010/59/EU. Official Journal of the European Union/ L 225/10

Anagnostopoulou M. A., Kefalas P., Kokkalou E., Andreana N. Assimopoulou A. N. & Papageorgiou V. P. (2005). Analysis of antioxidant compounds in sweet orange peel by HPLC–diode array detection–electrospray ionization mass spectrometry. Biomedical Chromatogrphy, 19, 138–148.

Arora, M. & Kaur, P. (2013). Antimicrobial & antioxidant activity of orange pulp and peel. International Journal of Science and Research, 2 (11), 2319-7064.

Asha, A., Manjunatha, M., Rekha, R. M., Surendranath, B., Heartwin, P., Rao, J., Magdaline, E. & Sinha, Ch. (2015). Antioxidant activities of orange peel extract in ghee (butter oil) stored at different storage temperatures. Journal of Food Science and Technology, 52(12), 8220–8227.

Brand-Williams, W., Cuvelier, M. E. & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology, 28, 25-30.

Berger, R. G. (2007). Flavours and Fragrances Chemistry, Bioprocessing and Sustainability. Springer. New York, NY, pp. 648.

Chen, Q., Zhao, J., Liu, M., Cai, J. & Liu, J. (2008). Determination of total polyphenols content in green tea using FT-NIR spectroscopy and different PLS algorithms. Journal of Pharmaceutical and Biomedical Analysis, 46, 568–573.

Coulter, I. D. (2007). Evidence based complementary and alternative medicine: Promises and problems. Research in Complementary and Natural Classical Medicine, 14, 102-108.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, Ph. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S. & Ju, Y. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatic. Journal of Food and Drug Analysis, 22, 296 -302.

Duarte-Almeida, J. M., Salatino, A., Genovese M. I. & Lajolo, F. M. (2006). Phenolic composition and antioxidant activity of culms and sugarcane (SaccharumofficinarumL.) products. Food Chemistry, 125, 660-664.

Ghasemzadeh, A., Jaafar, H. Z. E., Juraimi, A. SH. & Tayebi-Meigooni, A. (2015). Comparative evaluation of different extraction techniques and solvents for the assay of phytochemicals and antioxidant activity of hashemi rice bran. Molecules, 20, 10822-10838.

Hinneburg, I., Damien Dorman, H. J. & Hiltunen, R. (2006). Antioxidant activities of extracts from selected culinary herbs and spices. Food Chemistry 97, 122–129.

Kimball, D. A. (1991). Citrus Processing, Quality Control and Technology. Springer, NewYork. NY. pp.182.

LagourIi, V., Bantouna, A. & Stathopoulos, P. (2010). A comparison of the Antioxidant activity and phenolic content of nonpolar and

polar extracts obtained from four endemic Lamiaceaespecies grown in Greece. Journal of Food Processing and Preservation 34, 872–886.

Li, H., Zhang, Zh., Xue, J., Cui, Li., Hou, T., Li, X. & Chen, T. (2016). Optimization of ultrasound-assisted extraction of phenolic compounds, antioxidants and rosmarinic acid from perilla leaves using response surface methodology. Journal of Food Science and Technolology, 36(4), 686-693.

Mokbel, M.S., Watanabe, Y., Hashinaga, F. & Suganuma, T. (2006). Purification of the antioxidant and antimicrobial substance of ethyl acetate extract from Buntan (Citrus grandisOsbeck) fruit peel. Journal of Biological Science, 9 (1), 145-150.

Omoba, O. S., Obafaye, R. O., Salawu, S. O, Boligon, A. A. &Athayde, M. L. (2015). HPLC-DAD phenolic characterization and antioxidant activities of ripe and unripe sweet orange peels. Antioxidants, 4, 498-512.

Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Flerlage, N., Burillo, J. & Codina, C. (2002). Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilledmediterranean herbs and aromatic plants. Journal of Agricultural and Food Chemistry, 50, 6882-6890.

Pokorny, J., Yanishlieva, N. & Gordon, M. (2001). Antioxidant in food, practical application. CRC Press. New York, Washington, DC. pp. 210-246.

RasouliGhahroudi, F. (2017). Novel functional packaging for tea bag by using microencapsulated mixture of green tea and orange peel extracts. Ph.D. Dissertation. Islamic Azad University, Tehran, Iran.

Rasouli Ghahroudi, F., Mizani, M., Rezaei, K. & BameniMoghadam, M. (2017). Mixed extracts of green tea and orange peel encapsulated and impregnated on black tea bag paper to be used as a functional drink. International Journal of Food Science and Technology (in press).

Rezaei, S., Rezaei, K., Haghighi, M. & Labbafi, M. (2013). Solvent and solvent to sample ratio as main parameters in the microwave-assisted extraction of polyphenolic compounds from apple pomace. Food Science and Biotechnology, 22(5), pp.1269-1274.

Singh, S. & Immanuel, G. (2014). Extraction of antioxidants from fruit peels and its utilization in Paneer. Food Processing Technology, 5 (7), 348-349.

Sun, Ch., Wu, Zh., Wang, Z. & Zhang, H. (2015). Effect of ethanol/water solvents on phenolic profiles and antioxidant properties of Beijing propolis extracts. Evidence-Based Complementary and Alternative Medicine, 1-9.

Tokusglu, O. & Hall, C. (2011). Fruit and Cereal Bioactives, Soucrose, Chemistry & Application. CRC Press. Taylor & Francis. New York. pp.124-128.

Torti, S. D., Dearing, M. D. & Kursar, Th. A. (1995). Extraction of phenolic compounds from fresh leaves: a comparison of methods. Journal of Chemical Ecology, 21(2), 117-125.

Waszkowiak K. &Gliszczyn´ska-S´wigło A. (2016). Binary ethanol–water solvents affect phenolic profiland antioxidant capacity of flaxseed extracts. European Food Research and Technology, 242, 777–786.

Yalcin, H. S., Karaman, S. & Ozturk, I. (2011). Evaluation of antioxidant efficiency of potato and orange peel and apple pomace extracts in sunflower oil. Italian Journal of Food Science, 23 (23), 55-61.

Zefang, L., Zhao, Zh., Hongmei, W., Zhiqin, Z. & Jie, Y. (2016). Phenolic composition and antioxidant capacities of chinese local pummelo cultivars' peel. Horticultural Plant Journal, 2 (3), 133–140.

Zhishen, J., Mengcheng, T. & Jianming, W. (1999). The determination of flavonoids content in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64, 555-559.

Zlotek, U., Mikulska, S., Małgorzata Nagajek, M. & S´wieca, M. (2016). The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (Ocimumbasilicum L.) extracts. Saudi Journal of Biological Sciences, 23, 628–633.