

THE RELATIONSHIP BETWEEN ANTIOXIDANT ACTIVITIES AND PHENOLIC COMPOUNDS IN SUBCRITICAL WATER EXTRACTS FROM ORANGE PEEL

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Abstract

Subcritical water extraction, also called green technology, is used to extract phytochemicals from different plants and foods. Orange peel, a valuable by-product in the food industry, is a rich source of antioxidant compounds such as phenolics, β -carotene and essential oils. In this study, determination of the relationship between antioxidant activities and phenolic compounds in subcritical water extracts from orange peel was aimed. For this aim, The Ferric Reducing Antioxidant Assay (FRAP), The Cupric Reducing Antioxidant Capacity (CUPRAC) and DPPH radical scavenging activity (IC_{50}) of subcritical water extracts were measured. Total phenolic contents were statistically significant ($P < 0.01$) correlated as $r^2 = 0.98$ and 0.92 with FRAP and CUPRAC values in subcritical water extracts obtained from orange peel. Also, total flavonoid contents were statistically significant ($P < 0.01$) correlated as $r^2 = 0.97$ and 0.93 with FRAP and CUPRAC values in subcritical water extracts from orange peel. Besides, total phenolic and total flavonoid content in subcritical water extracts from orange peel were statistically significant ($P < 0.01$) correlated as $r^2 = -0.68$ and -0.70 with IC_{50} value, respectively.

Keywords: Antioxidant activity, DPPH, orange peel, subcritical water extraction, phenolics, flavonoids.

PORTAKAL KABUĞUNDAN ELDE EDİLEN KRİTİK ALTI SU EKSTRAKTLARINDA ANTİOKSİDAN AKTİVİTE DÜZEYLERİ İLE FENOLİK BİLEŞİKLER ARASINDAKİ İLİŞKİ

Öz

Yeşil teknoloji olarak da adlandırılan kritik altı su ekstraksiyonu işlemi farklı bitkilerden ve gıdalardan fitokimyasal bileşiklerin ekstrakte edilmesi amacı ile kullanılmaktadır. Gıda endüstrisinde değerli bir yan ürün olan portakal kabuğu fenolik bileşikler, β -karoten ve esansiyel yağlar gibi antioksidan bileşikler açısından zengin bir kaynaktır. Bu çalışmada, portakal kabuğundan elde edilen kritik altı su ekstraktlarında antioksidan aktivite değerleri ile fenolik bileşikler arasındaki ilişkinin saptanması amaçlanmıştır. Bu amaçla, kritik altı su ekstraktlarının Demir indirgeme Gücü Aktivitesi (FRAP), Bakır indirgeme Gücü Aktivitesi (CUPRAC) ve DPPH radikal süpürme aktivitesi (IC_{50}) ölçülmüştür. Portakal kabuğundan elde edilen kritik altı su ekstraktların toplam fenolik madde içeriği ile FRAP ve CUPRAC değerleri arasındaki ilişki istatistiksel ($P < 0.01$) açıdan önemli olup sırası ile $r^2 = 0.98$ ve 0.92 düzeylerinde bulunmuştur. Aynı zamanda, portakal kabuğundan elde edilen kritik altı su ekstraktların toplam flavonoid içeriği de FRAP ve CUPRAC değerleri ile istatistiksel ($P < 0.01$) açıdan önemli düzeyde ilişkilidir (sırası ile $r^2 = 0.97$ ve 0.93). Ayrıca, portakal kabuğundan elde edilen kritik altı su ekstraktların toplam fenolik madde ve toplam flavonoid içerikleri ile IC_{50} değeri arasındaki ilişki sırası ile $r^2 = -0.68$ ve -0.70 düzeylerinde olup istatistiksel ($P < 0.01$) açıdan önemlidir.

Anahtar kelimeler: Antioksidan aktivite, DPPH, portakal kabuğu, kritik altı su ekstraksiyonu, fenolikler, flavonoidler.

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INTRODUCTION

Subcritical water extraction has an increasing attention due to short extraction time and high extraction yield as compared with conventional extraction methods. Furtherly, subcritical water is an alternative extraction solvent against to the toxic organic solvents such as especially methanol and acetone (Ko et al., 2011). In the literature, it was reported that especially fruit, vegetable and oilseed waste products were rich sources of different bioactive compounds. Subcritical water was used for phenolic compounds extraction from grape skins and defatted grape seeds (Duba et al., 2015), olive fruit dreg (Yu et al., 2015), flaxseed meal (Özkaynak Kanmaz, 2014), onion peel (Lee et al., 2014), various food by-products (Ko et al., 2014), also antioxidant compounds from grape pomace (Vergara-Salinas et al., 2015), β -glucan from barley (Kodama et al., 2016), resveratrol from Barks of *Shorea Roxburghii* G. Don. (Chainukool et al., 2014), secoisolaricresinol diglucoside (SDG) lignan from flaxseed (Özkaynak Kanmaz and Ova, 2013).

Polyphenols are major antioxidants in human diet. Polyphenols protect cell constituents against oxidative damage and therefore, limit the risk of various degenerative diseases associated with oxidative stress (Jiménez, 2005). Citrus peels contain main flavanone glycosides (hesperidin and naringin), polymethoxylated flavones and numerous hydroxycinnamates (Manthley and Grohman, 2001). The attention on food by-products are notecably increasing because of the recycling processes or environmental purposes for the future. Citrus by-products have been used for animal feeding, pectin production and fuel production. Orange and grapefruit have high amount of peel and these peels are discarded as by-product in the food industry (Lagha-Benamrouchea and Madania, 2013). However, citrus peels have high concentration of flavonoids as compared citrus fruits. Orange (*C. sinensis* L.) peel is also a rich source of phenolic compounds and contains narirutin and hesperidin flavonoids which have antioxidant effect (Sawalha et al., 2009).

In the literature, there is not information about the effect of extraction temperature and static extraction time on antioxidant compounds in subcritical water extracts from orange peel using accelerated solvent extractor. Also, there is a lack

of publication regarding the correlation between antioxidant activities and phenolic compounds in subcritical water extracts from orange peel by accelerated solvent extractor. The aim of the present study was to determine the effect of extraction temperature (from 50 to 180°C) and static extraction time (5 and 15 min) on antioxidant activities, total phenolic content and total flavonoid content of subcritical water extracts from orange peel using accelerated solvent extractor. Also, the correlations between antioxidant activities and phenolic compounds in subcritical water extracts from orange peel were investigated in this study.

MATERIAL AND METHOD

Material

Sweet Washington oranges (*C. sinensis* L.) were used in this study. Oranges were cultivated with natural farming and were harvested in November 2014 from a natural orchard in Köyceğiz, Muğla. The orange peels were not covered with wax and they were peeled with a stainless steel knife and then dried in the vacuum air oven at 50°C until constant weight. Dried orange peels were vacuum packed for prevention of oxidation and then stored at -20°C until the extraction process. Samples were ground with a coffee grinder (Bosch, KM 13) between 600 and 1500 μ m just before the extraction.

All the chemicals and solvents were analytical or HPLC grade. The chemicals and reagents used in this study were Folin-ciocalteau phenol reagent, luteolin, aluminium chloride, sodium hydroxide, methanol, ethanol, DPPH, acetate buffer, hydrochloric acid, 2,4,6-tripyridyl-s-triazine (tptz), iron (III) chloride hexahydrate, iron(II) sulfate heptahydrate, copper(II) chloride, neocuproine, ammonium acetate, trolox (Sigma); sodium carbonate, sodium nitrite (Merck); ferrulic acid (Fluka).

Method

Subcritical water extraction

Subcritical water extracts from orange peel were obtained using accelerated solvent extractor (ASE 350, Dionex Corporation). During subcritical water extraction, temperature is the most important

parameter so wide temperature range (50, 100, 120, 140, 160 and 180°C) was used in this study. Besides, static extraction time is one of the most effective parameters during subcritical water extraction (Çam and Hışıl, 2010; Özkaynak Kanmaz and Ova, 2013). In this study, short extraction time was aimed so only 5 and 15 min were chosen as static extraction time. It is not possible to change the pressure in the Dionex ASE 350 system so 1500 psi pressure was used in this study. Particle size is also an effective parameter and higher extraction efficiency was obtained between 600 and 1500 µm during subcritical water extraction by Çam and Hışıl (2010). In this study, the particle size was used between these values. Subcritical water extraction was carried out in a metal extraction cell (10 mL) and 1 g of dried orange peel was used for each extraction process. Besides, flush volume was used as a fixed factor because it has no effect on the extraction yield during subcritical water extraction (Çam and Hışıl, 2010). In this study, the least flush volume (5%) was used to decrease the volume of water extract because water extracts were adjusted to 25 mL and then, subcritical water extracts were stored at -20°C. The extraction procedure was carried out two times.

Determination of antioxidant activities

The Ferric Reducing Antioxidant Assay (FRAP) was carried according to the method of Benzie and Starin (1999). FRAP reagent was prepared by mixing 25 mL of 300 mM acetate buffer (pH 3.6) with 2.5 mL of 10 mM 2,4,6-tripyridyl-s-triazine (tptz) solution in 0.5 mL of HCl (40 mM) and 2.5 mL of 20 mM iron (III) chloride hexahydrate solution. 3 mL FRAP reagent was mixed with 100 µL of sample extract and incubated at 37°C for 4 min. Absorbance of the solutions was measured by a Shimadzu UV-VIS 1800 spectrophotometer at 595 nm against a reagent blank containing distilled water. Trolox was used a positive control to construct a reference curve (62.5-1000 µM). FRAP values were expressed as µmol iron (II) sulfate heptahydrate equivalent of g.

The scavenging activity of DPPH radical was determined using the method of Molyneux (2004). 1.5 mL of the sample extract was mixed with 1.5 mL of DPPH (0.1 mM in methanol),

vortexed and incubated at room temperature in the dark for 50 min. Absorbance of the solutions was measured by spectrophotometer at 517 nm. Besides, the control solution without sample extract was used. The results were expressed as IC₅₀ (mg/mL), which was calculated from the curves by plotting absorbance values. IC₅₀ values represent the concentration of the extract (mg/mL) required to inhibit 50% of the radicals.

The Cupric Reducing Antioxidant Capacity (CUPRAC) was determined according to the method of Apak et al. (2004). 1 mL of Copper(II) chloride solution (1.0×10^{-2} M), 1 mL of ethanolic neocuproine solution (7.5×10^{-3} M) and 1 mL of ammonium acetate (1M, pH 7.0) were mixed in a test tube. The sample extract with different concentrations was added to the initial mixture. The tubes were stoppered and the absorbance of the solutions was measured by spectrophotometer at 450 nm against a reagent blank after 30 min. The result was calculated using the molar absorption coefficient (ϵ ; 1.7×10^4 L.mol⁻¹.cm⁻¹) against trolox, which was the standard reference compound. The result was expressed as mM Trolox/100 g.

Determination of total phenolic and total flavonoid content

Total phenolic content of subcritical water and solvent extracts was assayed as described by Skerget et al. (2005). 0.5 mL of extract was mixed with 2.5 mL of 0.2 N Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate and then the solution was incubated at 50°C for 5 min in the (Memmert, WNB 14) and cooled immediately. The absorbance was measured by spectrophotometer at 760 nm. The calibration curve was prepared with ferrulic acid solutions at five concentrations of aqueous methanol (80%). The results were expressed as ferrulic acid equivalent (mg of ferrulic acid per L of extract).

Total flavonoid content of subcritical water and solvent extracts was analysed by a spectrophotometric method by Chang et al. (2006). 0.5 mL of extract was mixed with 2.5 mL of distilled water and 150 µL of 5% sodium nitrite solution. The vortexed solution was allowed to stand for 5 min and then 300 µL of 10% aluminium chloride solution was

added to the mixture and allowed to stand for 5 min. Lastly, 1 mL of 1 M sodium hydroxide was added and 450 µL distilled water was added and final solution was vortexed at medium speed. The absorbance was measured by spectrophotometer at 510 nm. The calibration curve was prepared with luteolin solutions at five concentrations aqueous methanol (80%). The results were expressed as luteolin equivalent (mg of luteolin per L of extract).

Statistical analysis

One-way analysis of variance, least significant difference (LSD) for extraction temperature and t-test for static extraction time and also univariate analysis of variance for the temperature effect, time effect and temperature-time interaction was applied at significance level 0.05 using SPSS statistical package whereas, Pearson's correlation coefficients were at significance level 0.01. All the analyses were performed in triplicate.

RESULTS AND DISCUSSION

Antioxidant activities are generally related with phenolic compounds in plants due to their redox properties, which help them to act as hydrogen donors, singlet oxygen quenchers and reducing agents (Chang et al., 2001). For IC_{50} test, there is a negative correlation between IC_{50} value and antioxidant activity so, the lowest IC_{50} value was the highest antioxidant activity. Also, IC_{50} value of subcritical water extracts from orange peel was compared with IC_{50} value of trolox in this study. Besides, ferric reducing antioxidant power (FRAP) and the cupric reducing antioxidant capacity (CUPRAC) were measured to evaluate the antioxidant activity of subcritical water extracts from orange peel.

In the present study, the effect of extraction temperature and static extraction time on antioxidant activities, total phenolic content and total flavonoid content of subcritical water extracts from orange peel were determined statistically significant ($P < 0.05$) and also there was a statistically significant ($P < 0.05$) interaction between extraction temperature and static extraction time in subcritical water extracts obtained from orange peel.

The correlation between antioxidant activities and total phenolic content in subcritical water extracts from orange peel

In this study, total phenolic content of subcritical water extracts from orange peel had statistically significant ($P < 0.01$) positive correlations as $r^2 = 0.98$ and 0.92 with FRAP and CUPRAC values (Table 1). In the literature, it was also reported that there was a strong correlation ($r^2 = 0.81$) between FRAP value and total phenolic content of orange peel (Lagha-Benamrouchea and Madania, 2013). Fu et al. (2011) also determined high correlation ($r^2 = 0.84$) between total phenolic content and FRAP value of 62 fruits. Ozgen et al. (2008) also reported statistically significant ($P < 0.05$) correlation as $r^2 = 0.93$ between total phenolic content and FRAP value of the eight pomegranate cultivars.

Table 1. The Pearson's correlation coefficients* in subcritical water extracts obtained from orange peel.

Trait	IC_{50}	CUPRAC	TPC	TFC
IC_{50}		-0.85*	-0.68*	-0.70*
FRAP	-0.78*	0.96*	0.98*	0.97*
CUPRAC			0.92*	0.93*
TPC				0.99*

* Correlation is significant at the $P < 0.01$ level

Besides, in this study, the negative correlation between total phenolic content and IC_{50} value was determined statistically significant ($P < 0.01$) and the correlation coefficient was found as $r^2 = -0.68$ in subcritical water extracts from orange peel (Table 1). In the literature, Çam et al. (2009) also observed statistically significant ($P < 0.01$) negative correlation as $r^2 = -0.81$ between total phenolic content and EC_{50} value of pomegranate juices. Besides, Tan et al. (2015) determined statistically significant ($P < 0.05$) negative correlation ($r^2 = -0.71$) between total phenolic content and DPPH radical in the different polar solvent extracts of *Magnolia officinalis* leaves. However, Lagha-Benamrouchea and Madania (2013) reported a strong negative correlation ($r^2 = -0.92$) between DPPH radical and total phenolic content of orange (*Citrus sinensis* L. and *Citrus aurantium* L.) peel. These results suggested that phenolic acids played a significant role in the antioxidant mechanism with the DPPH radical. In the literature, Wiboonsirikul et

al. (2007) also reported that the strong radical scavenging activity against the DPPH radical mainly resulted from the phenolic substances.

Besides, in this study, subcritical water extracts from orange peel at 180°C and 15 min had great attention due to its statistically ($P < 0.05$) highest IC_{50} value (Table 2 and Figure 1). Also, the statistically ($P < 0.05$) highest FRAP (76.29 $\mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O/g}$) and CUPRAC (102.39 mM troloks/g) value in subcritical water extracts from orange peel were found at 180°C and 15 min (Table 2, Figure 2 and 3). Subcritical water extracts from orange peel at 180°C and 15 min also had great attention due to its statistically ($P < 0.05$) highest total phenolic content (Table 2 and Figure 4). In the literature, it was reported that high extraction temperature decrease the surface tension of water to penetrate into the sample well and also improve the mass transfer of bioactive compounds from the sample to the water in the extraction cell during subcritical water extraction. Static extraction time was also reported an effective parameter which is related with the exposure between water and the sample during subcritical water extraction but, it is not as effective as extraction temperature (Çam and Hışıl, 2010; Özkaynak Kanmaz, 2014).

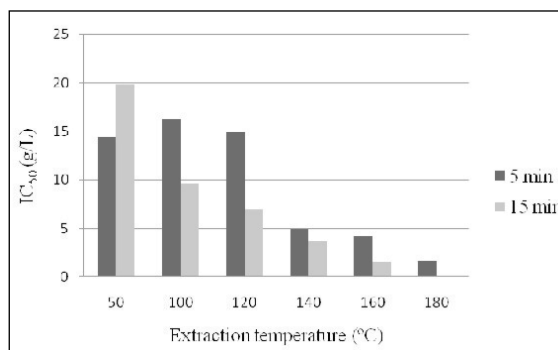


Figure 1. IC_{50} value of subcritical water extracts from orange peel.

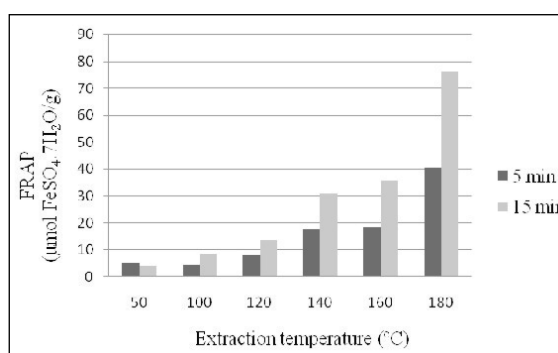


Figure 2. FRAP value of subcritical water extracts from orange peel.

Table 2. Antioxidant activity and phenolic content of subcritical water extracts from orange peel.

Extraction temperature (°C)	Extraction time (min)	IC_{50} (g/L)	FRAP* ($\mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O/g}$)	CUPRAC# (mM troloks/g)	TPC (g/L)	TFC (g/L)
50	5	14.44±0.00 ^b	5.25±0.19 ^d	6.37±0.09 ^d	1.36±0.04 ^a	0.71±0.04 ^a
100		16.25±0.22 ^a	4.58±0.24 ^d	6.16±0.28 ^d	1.28±0.06 ^a	0.64±0.06 ^f
120		14.93±1.28 ^b	8.04±0.12 ^c	14.67±0.88 ^c	1.93±0.08 ^d	0.80±0.06 ^d
140		4.93±0.15 ^c	17.86±0.45 ^b	31.61±0.48 ^b	4.17±0.32 ^b	1.17±0.06 ^b
160		4.22±0.41 ^c	18.48±0.68 ^b	35.07±0.36 ^b	3.59±0.12 ^c	0.92±0.07 ^c
180	15	1.70±0.19 ^d	40.72±1.26 ^a	85.14±4.79 ^a	11.96±1.09 ^a	2.87±0.19 ^a
50		19.82±0.57 ^a	4.10±0.39 ^f	4.51±0.06 ^f	0.99±0.05 ^f	0.62±0.00 ^e
100		9.64±0.36 ^b	8.38±0.49 ^e	9.85±0.28 ^e	2.32±0.07 ^e	0.88±0.06 ^d
120		6.94±0.25 ^c	13.62±0.23 ^d	21.83±1.07 ^d	2.64±0.12 ^d	0.89±0.06 ^d
140		3.72±0.04 ^d	30.87±1.24 ^c	48.68±1.91 ^c	5.57±0.46 ^c	1.37±0.09 ^c
160		1.55±0.05 ^e	35.64±1.30 ^b	68.31±3.85 ^b	10.07±0.58 ^b	2.81±0.22 ^b
180		0.20±0.01 ^f	76.29±0.03 ^a	102.39±1.63 ^a	26.26±2.23 ^a	5.13±0.30 ^a
Trolox		0.06±0.00				

Values are means±standard deviations of three (n=3) measurements

^{a-f} Values with different superscript letters within a column are significantly different at $P < 0.05$

*: $\mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O/g}$

#: mM troloks/g

TPC: Total phenolic content

TFC: Total flavonoid content

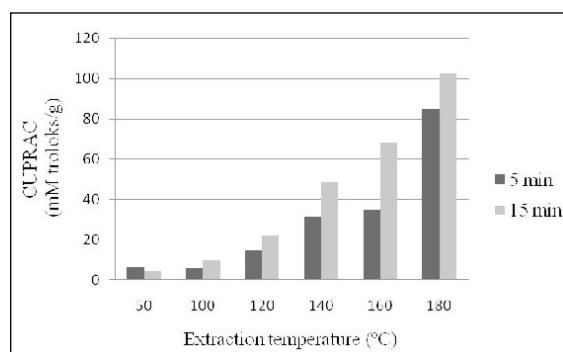


Figure 3. CUPRAC value of subcritical water extracts from orange peel.

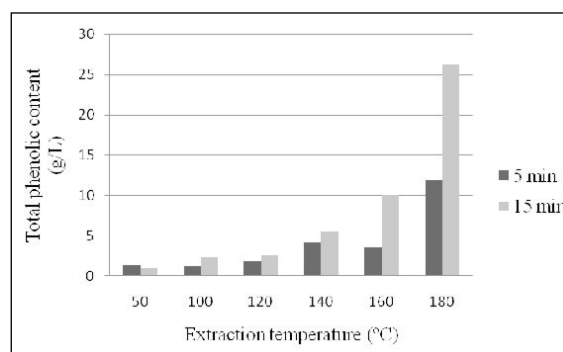


Figure 4. Total phenolic content of subcritical water extracts from orange peel.

Özkaynak Kanmaz and Ova (2013) also reported that SDG lignan content of subcritical water extracts from flaxseed meal increased 15.6 times as extraction temperature increased from 120 to 180°C. Besides, Duba et al. (2015) found that total polyphenols of subcritical water extracts from grape skin (from 44.3 to 77 mg/g) and defatted seeds (44 to 124 mg/g) significantly increased as the extraction temperature increased from 80 to 120°C. Yu et al. (3) also reported that total phenolic content of subcritical water extracts of olive fruit dreg increased as extraction temperature increased remarkably from 100 to 160°C.

The correlation between antioxidant activities and total flavonoid content in subcritical water extracts from orange peel

In this study, total flavonoid content of subcritical water extracts from orange peel had statistically significant ($P < 0.01$) positive correlations as $r^2 = 0.97$ and 0.93 with FRAP and CUPRAC values (Table 1). These results suggested that flavonoids played a major role in the antioxidant mechanism with FRAP and CUPRAC test. Also, the negative correlation between total flavonoid content and IC_{50} value in subcritical water extracts from orange peel was statistically significant ($P < 0.01$) and the correlation coefficient was found as $r^2 = -0.70$ in subcritical water extracts from orange peel (Table 1). In the literature, Tan et al. (2015) also determined a statistically significant ($P < 0.05$) negative correlation as $r^2 = -0.80$ between total flavonoid content and DPPH radical in the different polar solvent extracts of *Magnolia officinalis* leaves. These results suggested that flavonoids also played a significant role in the

antioxidant mechanism with DPPH radical. Also, melanoidins could played a significant role in the antioxidant mechanism with DPPH radical because melanoidins were occurred during Maillard reaction at high extraction temperatures. In this study, the colour of water extracts from orange peel turned to dark brown at high extraction temperatures and long static extraction times during subcritical water extraction (Figure 5). In the literature, it was also reported that melanoidins showed high correlation with DPPH radical (Delgado-Andrade et al., 2005). Manzocco et al. (2001) also reported that antioxidant activity increased with the increasing heating time during the Maillard reaction. However, to better understand the benefits of melanoidins, these compounds need to be chemically characterised and the in vivo biological effects of these compounds should be investigated.

Besides, IC_{50} value of subcritical water extracts from orange peel decreased 2.5 times (from 4.22 to 1.70 g/L) and 7.8 times (from 1.55 to 0.20 g/L) as extraction temperature increased from 160 to 180°C for 5 and 15 min, respectively (Table 2 and Figure 1). Besides, CUPRAC and FRAP value of subcritical water extracts from orange peel increased 2.43 and 2.20 times as extraction temperature increased from 160 to 180°C for 5 min. Also, CUPRAC and FRAP value of subcritical water extracts increased 1.5 and 2.1 times as extraction temperature increased from 160 to 180°C for 15 min (Table 2, Figure 2 and 3). Total flavonoid content of subcritical water extracts from orange peel increased 3.1 times (from 0.92 to 2.87 g luteolin equivalent/L) and 1.8 times (from 2.81 to 5.13 g luteolin equivalent/L) as extraction temperature increased from 160 to 180°C for 5 and 15 min, respectively in this study

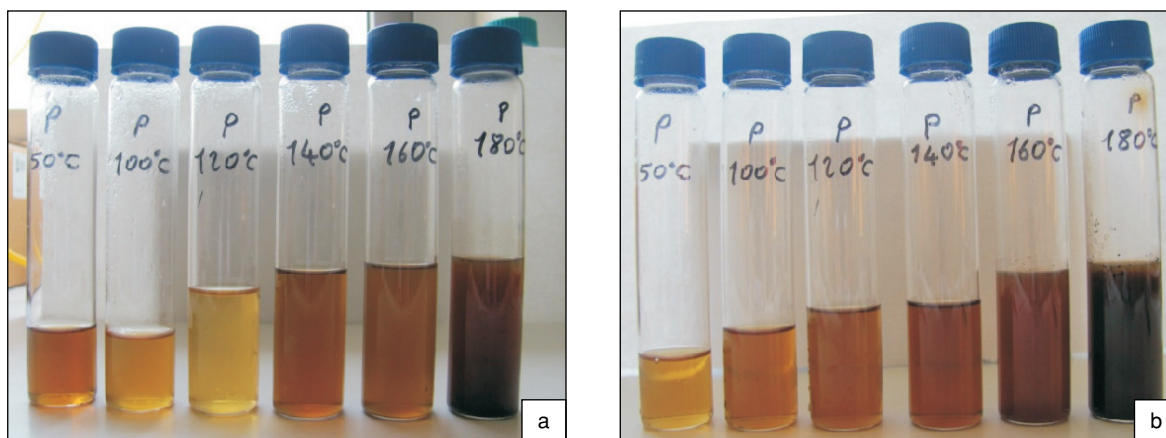


Figure 5. Subcritical water extracts from orange peel at different extraction temperatures for 5 (a) and 15 (b) min by accelerated solvent extractor, respectively.

(Table 2 and Figure 6). Subcritical water extracts from orange peel at 180°C and 15 min also had great attention due to its highest total flavonoid content (5.13 g luteolin equivalent/L). The statistically ($P < 0.05$) second highest total flavonoid content (2.87 g luteolin equivalent/L) was found at 180°C for 5 min in this study. These results might be explained with decreasing of water polarity and dielectric constant as extraction temperature increased up to 180°C during subcritical water extraction. High extraction temperatures decrease the hydrogen bonding strength and also polarity of the water so selection of the extraction parameters is too important for the extraction of polyphenols (Duba et al., 2015). In the literature, it was also reported that the molecule structure of compounds in the material had an important effect on the extraction yield and flavonoids with OH side chain were highly extracted at lower temperatures than O-CH₃ side chains during subcritical water extraction (Ko et al., 2014).

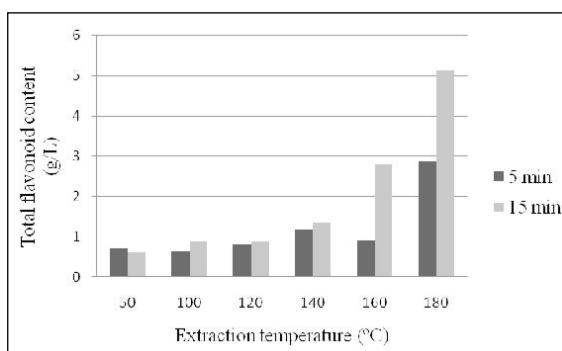


Figure 6. Total flavonoid content of subcritical water extracts from orange peel.

Ko et al. (2014) studied with subcritical water extraction of flavonoids from different materials using accelerated solvent extractor and the highest extracts were obtained for quercetin at 170°C/10 min (from onion skins), kaempferol and luteolin at 190°C/15 min (from carrots), naringin at 170°C/10 min and naringenin at 170-190°C/15 min (from grapefruit peels), hesperidin at 170°C/10 min (from orange peels) and also hesperetin at 190°C/10 min (from lemon peels). Furtherly, in this study, it was reported that the glycoside forms including sugar were highly extracted at lower temperatures than that of less polar aglycones and also, flavonoids with double bonds were highly extracted at higher temperatures during subcritical water extraction (Ko et al., 2014).

CONCLUSION

Phenolics and flavonoids played significantly higher role in the antioxidant mechanism with FRAP and CUPRAC value than IC₅₀ value in subcritical water extracts from orange peel. These results suggested that other antioxidant compounds such as β -caroten, essential oils and also melanoidins could played a major role in the antioxidant mechanism with DPPH radical. Subcritical water extracts obtained from orange peel could be alternative functional ingredients to produce functional water or functional drink in the food industry because of their high phenolic content and antioxidant activity.

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