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Evaluation of Antioxidant Properties of Barberry Fruits Extracts using Maceration and Subcritical Water Extraction (SWE)

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***Abstract*—**The quality and shelf life of foods of containing lipids (fats and oils) significantly reduces due to rancidity.Applications of natural antioxidants are one of the most effective manners to prevent the oxidation of oils and lipids. The antioxidant properties of juice extracted from barberry fruit (*Berberris vulgaris*.L) using maceration and SWE (10 bars and 120 - 180°C) methods were investigated and compared with conventional method. The amount of phenolic compound and reduction power of all samples were determined and the data were statistically analyzed using multifactor design. The results showed that the total amount of phenolic compound increased with increasing of pressure and temprature from 1861.9 to 2439.1 (mg Gallic acid /100gr Dry matter). The ability of reduction power of SWE obtained antioxidant extract compared with BHA (synthetic antioxidant) and ascorbic acid (natural antioxidant). There were significant differences among reduction power of extracts and there were remarkable difference with BHA and Ascorbic acid ($P<0.01$).

***Keywords*—**Subcritical water, Antioxidant, Barberry, Phenolic compound, Reduction power

I. Introduction

ANTIOXIDANTS have often been used to protect against free radicals by scavenging reactive oxygen or ending radical chain reactions. Recently, health concerns draw much attention to the use of natural antioxidant compounds in medicine and food technology. The quality and shelf life of foods of containing lipids significantly reduced due to rancidity of fats and oils. The application natural antioxidants is one of the most effective way to prevent the oxidation

Many studies have shown that polyphenolic compounds are effective antioxidants against lipid oxidation in phospholipid bilayers [1]. It has been proved that during oxidative stress, reactive oxygen components (ROS) such as superoxide, hydroxyl and peroxyl radicals are generated [2].

One of the promising sources of natural antioxidants is different part of barberry tree such as fruit, bark of stems and roots. This plant is a shrub with 1–3 m height, spiny, with yellow wood and oval shape leaves, bearing pendulous yellow flowers succeeded by oblong red fruits. Many studies on chemical composition of barberry extract shows that the main components of barberry extract are alkaloid constituents with an isoquinolinic nucleus such as berberine, berbamine and palmatine [6]. Conventional extraction techniques based on organic solvents have been applied to the extraction of natural antioxidants from the plant sources but those are very expensive, long time extraction period and baneful effect on product. Subcritical water extraction (SWE) is an emerging extraction technique that using water as the solvent, but with modified physical properties; it is considered a recent alternative for the isolation of antioxidant constituents from plant materials [9].

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In SWE systems, the temperatures between are 100 and 374˚C (the critical point of water is at 374˚C and 22 MPa) generally employed and the pressure must be high enough to keep the water in the liquid state. Under subcritical conditions, the dielectric constant of water, e, (i.e., its polarity) can be lowered easily to that of ethanol or methanol by increasing the temperature, low enough to dissolve many intermediate compounds by low polarity of water [11]. The aims of this study were to investigate antioxidant activity of barberry fruit extract using SWE and maceration and comparing with traditional method by evaluation of phenolic compound, reduction power, radical scavenging properties and rancidity test.

II. Experimental

A. Extraction by maceration

Maceration method carried out under constant stirring1000 rpm, using aqueous distilled water in the ratio of 1:20 solid - solvent (w/v) at room temperature. The extraction processing was carried out in absent the light for 18 h. The extracted suspension then filtered and subsequently solvent evaporated by rotary vacuum evaporator at 40°C.

B. Subcritical water extraction (SWE)

The subcritical water processing was performed in SWE devices made by Sib Food Tech. GMBH. The apparatus of subcritical water extraction system consisted of a HPLC pumps (Comet Type: MTP Ax 2/70 m) used for delivering water and solvent, electrical elements for receive to high temperature and a digital temperature controller (Abtin Mfg Eng CO, Iran) which regulated the vessel temperature. Samples were placed in the extraction cell and extractions were carried out at two different extraction temperatures (120 and 180°C), in 10 bar pressure and two solid-solvent ratio (1:and1:30) whereas the extraction time was 30 min.

C. Determination of total phenolic content (TPC)

Total phenolic content was determined as previously described by Xu. Et al. (2002) with a few modifications . 2.5 mL of Folin–Ciocalteo reagent (diluted 10 times) and 2 mL sodium carbonate (7.5%) reagent were added to 0.5 ml sample, and subsequently the mixture was incubated in the dark for 30 min. After incubation, the absorbance was measured at 765 nm. Gallic acid was used as a standard to obtain the calibration curve. The phenolic content is reported as mg gallic acid per 100 g dry material [12].

D. Reducing power

The reducing power of SWE and maceration extracts was determined according to the method of Shymala et al. (1986) [10].

Different amounts of antioxidant extracts in 2.5 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M/L, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)6$] (2.5 ml, 1%). The mixture was incubated at 50 8C° for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (5 ml) was mixed with distilled water (5 ml) and FeCl3 (1 ml, 0.1%) and the absorbance was measured at 700 nm. The reduction power of extracts measured with EC_{50} (a minimum concentration of antioxidants that can be reduces 50% of Fe^{+3} to Fe^{+2}). The antioxidant properties of treated samples were compared with BHT (Butylated Hydroxy Toluene) and Ascorbic acid as synthetic and natural antioxidant agents respectively [10].

E. Radical scavenging

The antioxidant activity was determined using the procedure that described by Brand-Williams et al (1995). with a few modifies. According to this procedure, 2 mg of DPPH (2, 2-diphenyl-1-picrylhydrazyl) were dissolved in 50 ml of methanol as this solution had an absorbance between was 1-0.9. Different concentrations of barberry extracts were tested and 0.1 ml of these solutions along with 3.9 ml of DPPH solution was placed in test tubes to complete the final reaction media (4 ml). Reaction was completed after 30 min at room temperature and absorbance was measured at 517 nm in a UV/VIS spectrophotometer [8]. Methanol was used to adjust zero and DPPH–methanol solution as reference sample.

$$RSA\% = (Abs_{control} - Abs_{sample} / Abs_{control}) \times 100$$

The percentage of remaining DPPH against the extract concentration was then plotted to obtain the amount of antioxidant necessary to reduce the initial DPPH concentration by 50% or IC_{50}. Generally, lower amount of IC_{50} means the higher antioxidant power.

F. Determination of melanoidins

The degree of browning compounds formation such as melanoidins was measured at absorbance of 420 nm. This wavelength has been previously used to detect browning effects in fruit juices. The extracts were diluted four times before measuring the absorbance while some of the extracts had very high absorbance (>2.50) [4].

G. Rancimat test

The antioxidant activities of soybean oil were studied as control with a Metrohm Rancimat Model 743, at 110˚C. The air flow rate was fixed at 20 l/h. The constant concentration 300 ppm of treated samples were added to Bleached ,Decolorized and clarified soybean oil and the anti oxidant properties were compared with BHT and vitamin E as synthetic and natural antioxidant respectively. Each of oil (3 g) samples was poured in reaction vessel. After start heating, the vapors released during the oxidation process together with the air are passed into the flask containing 60 ml of demineralized water and fitted with an electrode for measuring the conductivity. The electrode is also connected to a recording device.

It indicates the end of induction period (IP) when the conductivity begins to increase rapidly. This accelerated increase is caused by the dissociation of volatile carboxylic acids produced during the oxidation process and absorbed in the water. When the conductivity of this solution is recorded continuously, an oxidation curve is obtained whose point of inflection, known as the induction period (IP) that can be calculated by the point of intersection of two tangents

The effect of samples on retarding the soybean oxidation, interpreted as the protection factor (P_f), was calculated according to the following expression:

$$P_f = IP_{antiox.}/IP_{contrl.}$$

Where $IP_{antiox.}$ and $IP_{contrl.}$ were the induction period (IP) of oil oxidation with and without antioxidant, respectively [3,6].

H. Determination of soluble solids

Soluble solids in the extracts were determined using a digital refractometer (ATAGO, Rx- 5000α) and exhibit as Bx(Total Soluble Solids).

I. Determination pH of extracts

The pH of extracts were determined by pH meter (WTW inolab level2) that calibrated using buffer solution at pH 4 and 7.

III. Results and Discussions

A. Determination of total phenolic content

The significant variations were observed in the amount of total phenol content among the samples. The total phenolic content varied from 1861.9 to 2439.1 (mg G.A). $(100gr\ D.M)^{-1}$. The amount of the total phenolic content was the highest in 1:30 ratio. In fact, the ratio had the higher influence on TPC. Rise ratio and temperature cause to increase TPC.The amounts of total phenolic content of treated samples using SWE are shown in Table 1.All treated SWE samples at 180°C were produced the highest TPC. The TPC extracted juice yield was increased with increasing the temperature. This phenomenon can be described as the polar solvent such as water normally dissolves polar compounds much more readily than nonpolar compounds. At high temperature and pressure the polarity of water reduces and acts as nonpolar solvent. Consequently, the capability of water to dissolve less polar compounds is enhanced at higher temperatures and pressure. Raising the temperature- pressure of water also reduces its surface tension and viscosity, as a result increases the diffusion rate and facilitate of mass transfer during extraction [9]. The effects of these phenomena should all lead to greater TPC at higher SWE temperatures. Ibanez et al. (2003) showed that at 25°C, rosmanol was the major component of an SWE extract, accounting for >50% of total extract composition. When the temperature increased from 25 to 200°C, an increase in the extraction capability of subcritical water toward less polar compounds such as carnosic acid and carnosol was observed [5].

The interaction effects of temperature and solid- liquid ratio on phenol content of SWE extracts were remarkable as shown in Fig 1.

However, no significant differences observed between the TPC of SWE extract at 180°C with 1:10 solid-solvent ratio compared with maceration extract.

TABLE I
"TPC" OF "SWE" EXTRACTS AT DIFFERENT TEMPERATURES AND SOLID-LIQUID RATIOS

	Temperature (°C)		Ratio	
	120	180	1:10	1:30
TPC (mg G.A/100g D.M)	2150.6[b]	2372.02[a]	1990.48[b]	2532.14[a]
EC_{50} (ppm)	323.59[a]	319.69[b]	325.1[a]	318.18[b]
IC_{50} (ppm)	3493.16[a]	2611.45[b]	3138.36[b]	2966.25[a]
Melanoidin (abs at 420 nm)	1.37[a]	0.90[b]	1.66[a]	0.59[b]
P_f	1.16[b]	1.44[a]	1.38[a]	1.23[b]

B. Reducing power

The various methods developed to measure the efficiency of dietary antioxidants were focused on different mechanism of the oxidant defense system, i.e., scavenging active oxygen species and hydroxyl radical, reduction of lipid peroxyl radicals, inhibition of lipid peroxidation, as well as chelation of metal ions [11]. The reducing power assay are based on the reductants (antioxidants) in the test compound or extract reduces the Fe^{3+}/ferricyanide complex [$FeCl_3/K_3Fe(CN)_6$] to the ferrous (Fe^{2+}) form [9].

The reducing power of different extracts of barberry for reducing the ferric ions were determined and compared with BHT and ascorbic acid. The higher amount of reducing power or least of EC_{50} (Effective concentration 50) was observed at120°C with 1:30 solid- liquid ratio. Maceration extract had minimum reduction power with maximum of EC_{50}. In addition, BHT and ascorbic acid showed the minimum of EC_{50} concentration. The ascorbic acid had the best of reduction power as a natural antioxidant. Slope of reduction power standard curves showed that among of SWE extracts, The obtained samples at 180°C with 1:30 solid- solvent ratio had minimum of reduction power. Ascorbic acid standard curve had theatrical slope (fig 2).

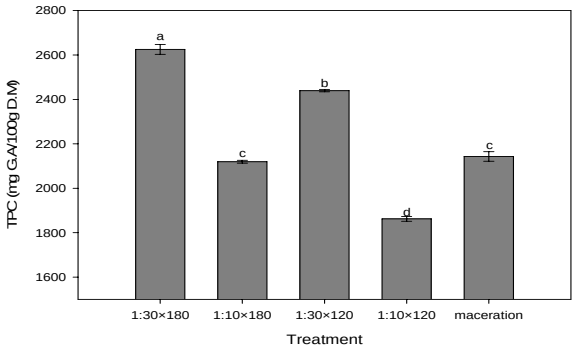

Fig. 1 TPC in SWE and maceration extracts.

C. Radical scavenging

The DPPH radical as stable organic nitrogen radical is commercially available. This compound has a deep purple color [9]. The quenching capacity of DPPH in SWE extracts was different at different conditions (temperature and solid - liquid ratio).

Increasing of temperature and solid - solvent ratio in extraction process, significantly exhibited lower in IC_{50} index or higher radical scavenging ability ($P<0.01$). IC_{50}. The amounts of various extracts are shown in table 1. Lower IC_{50} at 180°C implies better vis-à-vis DPPH solution while the lowest sensitivity was observed at 120°C extract.

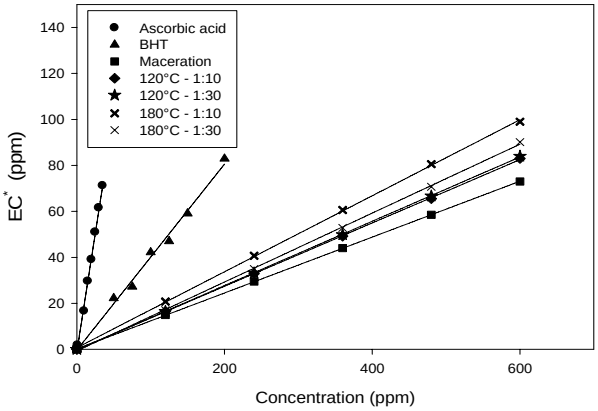

Fig. 2 Reduction power of barberry extract samples with standard

The scavenging percent of DPPH by SWE, maceration, BHT and ascorbic acid are shown in Fig.3. Evaluation of interaction effects between temperature and ratio extraction, indicated that not significant difference ($P>0.01$) between the highest radical scavenging activity at 180°C with solid – liquid ratios of 1:10 and 1:30, The similar radical-scavenging activity was observed in extracts from SWE at 120°C with 1:30 ratio and maceration extract, ($P>0.01$).

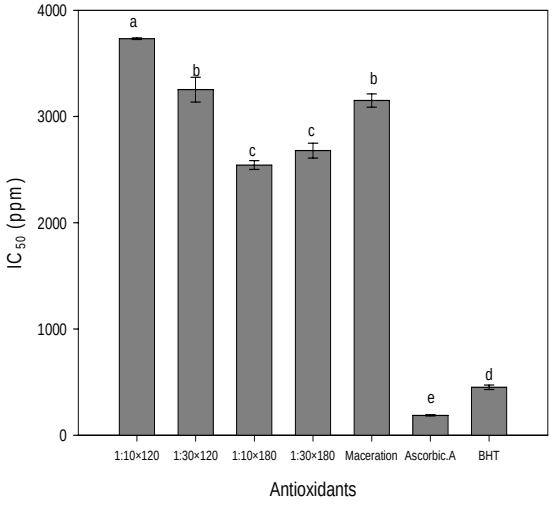

Fig. 3 IC_{50} for SWE, maceration, BHT and Ascorbic acid antioxidants solutions

D. Melanoidins

The melanoidin compounds of barberry fruit extract were evaluated in 420 nm by spectrophotometeric method after treated samples using SWE at 120 and 180°C for 30 min.The formation of Millard products (melanoidins) measured after SWE process and compared with maceration extract.

The results showed that the amount of melanoidin compound of SWE extracts at 120°C were significantly higher than at 180°C (table1). The amount of melanoidins increased with decreasing the temperature and it can be due to production of Millard Reaction Compounds (MRC) at lower temperature. In addition, it seems that the increasing of TPC and antioxidant activity in the extracts obtained at 180°C may be inhibited and change these products (MRC). Hossein et al (2011) reported that the production of Maillard compound potentially can be producing the health harmful substances [4].

The amounts of MRC were Increase with decreasing the solid- liquid ratio elevated from 1:30 to 1:10. Presumably the concentration of MRC at 1:30 was lower than 1:10 due to decrease absorbance in 420 nm.

The interaction effects of temperature and solid-solvent ratio in SWE process on MRC were evaluated and compared with maceration extract. The obtained results indicated that the minimum MRC produced in maceration extract.

TABLE II
Bx, pH and "MRC" IN "SWE" PARAMETERS INTERACTION AND MACERATION EXTRACTS

Temp*Ratio	Bx	pH	Melanoidins
120×1:10	5.37[d]	2.96[e]	1.834[a]
120×1:30	2.16[b]	3.036[b]	0.901[c]
180×1:10	5.54[a]	3.031[c]	1.517[b]
180×1:30	4.46[c]	3.106[a]	0.284[e]
maceration	2.133[e]	2.985[d]	0.33[d]

E. Rancimat test

The Metrohm Rancimat apparatus is frequently used to measure oil stability index (OSI) and the term ''Rancimat'' and ''OSI'' are often used interchangeably in the literature while referring to the test method.

The air coming out of the sample is finally passed through water contained in a tube fitted with a conductivity meter; a sharp rise in conductivity is interpreted as indication of the formation of short chain water soluble carboxylic acids, i.e., secondary oxidation products. Studies have indicated that the primary acidic species formed in the Rancimat OSI test is formic acid [7].

The results indicated that the maximum IP were obtained at 180°C and solid- liquid ratio 1:10. There were remarkable differences between temperature and ratio ($P<0.01$). The most of P_f at this work was 1.44 when extraction temperature was reached to 180°C and after that amount of 1:10 ratio was maximum (table 1).

The application of the Rancimat test to interaction of extraction parameter in SWE, BHT and vitamin E after that these antioxidants added to soybean oil showed that interaction of 180°C temperature and 1:10 ratio had higher amount of P_f higher and like to the BHT. after that interaction between 180° and 1:30 ratio, had a good effect on induction period of oil among SWE extracts (fig 4).

The evaluation of antioxidant properties of vitamin E were evaluates and the results showed that a weak pro-oxidant effect occurred due to induction period. The P_f for vitamin E was 0.95.

These results indicate that the SWE extract are suitable antioxidant for edible industrial oil production. Bx and pH of SWE and maceration extracts showed in table 2. Bx, increase with temperature and decrease with increase ratio from 1:10 to 1:30. The pH increased with increasing the temperature and ratio increase.

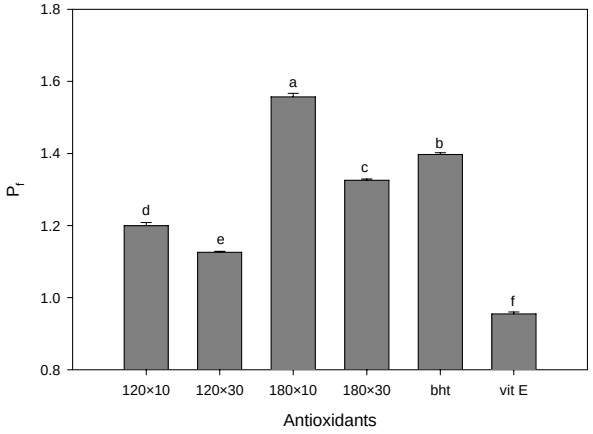

Fig. 4 P_f of antioxidants compound from rancimat test at 300 ppm concentration, 110°C and 20 l/h air flow.

IV. CONCLUSIONS

This study depicted the application of succritical method for extraction of antioxidant components in barberry fruits and compared with traditional method .the results show that SWE is a promising and safe method for extraction of phenolic compound. The phenolic compounds of barberry fruits were showed the high ability of antioxidant properties. Additionally, SWE extracts had crucial effect for preservation of edible food oil under frying process and can be used instead of synthesis BHA and BHT antioxidant.

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