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Total Phenolic Contents and Antioxidant Activity of Pomegranate (*Punica granatum* L.) Peel Extracts

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ABSTRACT

The phenolic compounds of pomegranate (*Punica granatum* L.) peel extracted by two methods (solvent and ultrasound-assisted) with five solvents (acetone, methanol, ethanol, water and ethyl acetate) were compared with supercritical fluid extraction (SFE). The total phenolic compounds were determined according to the Folin-Ciocalteu reagent using tannic acid as standard. The overall results showed that acetone with sonication produced the maximum amount of phenolic compounds from pomegranate peel extracts (PPE). Furthermore, the effect of the acetone extract of pomegranate peel (0.010-0.050 %) on the stability of soybean oil during heating at 60°C (oven test method) was determined by measuring peroxide and thiobarbituric acid values. At a 0.050 % level of pomegranate peel extract, its antioxidant activity was greater than 0.02 % of the two synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The pomegranate peel extract possessed a relatively high antioxidant activity and might be considered as a rich source of natural antioxidant.

Keywords: Phenolic compounds, Pomegranate peel, SFE, Solvent extraction, Sonication.

INTRODUCTION

The search for cheap and abundant sources of natural antioxidants is attracting worldwide interest. Much research is needed in order to select raw materials; those of residual origin are especially promising due to their lower costs.

The pomegranate (*Punica granatum* L.) is one of the oldest edible fruits and is widely grown in many tropical and subtropical countries [1]. It is an important commercial fruit in Iran with a total production of 665,000 tons in 2003 [2]. Pomegranate juice and peel contain substantial amounts of polyphenols such as ellagic tannins, ellagic acid and gallic acid [3]. It has been used in the preparation of tinctures, cosmetic, therapeutic formula and food recipes [4] and in this regard pomegranate peel is a good source of antioxidants [5].

Antioxidants are the compounds that, when added to food products, especially lipids and lipid-containing systems, can increase the shelf life of the product by retarding the process of lipid peroxidation. Lipid peroxidation in fats and fatty foods not only brings about chemical spoilage in foods but also produces free radicals such as peroxyl and hydroxyl radicals, which are purportedly associated with carcinogenesis, mutagenesis, and aging [6, 7]. On the other hand, the most widely used synthetic antioxidants, butylated hydroxyanisole and butylated hydroxytoluene, which have been used as antioxidants since the beginning of this century, and have been restricted recently, mainly because of their possible carcinogenicity [8] causing liver swelling and changing liver enzyme

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activities [9]. However, in recent years, many attemps have been made to study natural antioxidants, particularly those of plant origin [10].

Great interest has recently been focused on the addition of polyphenols to foods and biological systems, due to their well-known abilities to scavenge free radicals, i. e. antioxidant power. The generation of free radicals plays an important role in the progression of numerous pathological disturbances, such as atherosclerosis [11], brain disfunction [12] and cancer [13]. Extraction is a key step for obtaining antioxidants with an acceptable yield. Solvent extraction is more frequently used for the isolation of antioxidants and the extraction yield and economic viability is dependent on the type of solvent and method of extraction, mostly due to the differing polarity of these compounds. Several extraction techniques have been reported for the extraction of phenolic compounds from different matrices using solvents with different polarities, such as methanol, water, ethyl acetate and petroleum ether [14, 15]. Furthermore, supercritical CO_2 [16,17] and solvent extraction along sonication have been applied for this purpose [18].

The aim of this research was to compare solvent extraction (acetone, methanol, ethanol, water and ethyl acetate) with and without Sonication and with the SFE process. Furthermore, in this study, the effect of concentrated pomegranate peel extracts (PPEs) on the stability of soybean oil during heating has been compared with that of two synthetic antioxidants BHA and BHT.

MATERIALS AND METHODS

Pomegranates (Poost Syah variety) were obtained from the Agricultural Research Centre of Yazd (Iran). The skins were manually removed, sun-dried (ambient temperature= 30 °C and %RH= 32), powdered in a grinder to reach 40-mesh and then were packed and stored at -20 °C until extraction. All chemicals were of analytical grade and of the highest purity available (>99.5 %) and obtained from Merck (Darmstadt, Germany).

A Suprex MPS/225 system (Pittsburg, USA) in the SFE mode was used for the extraction of phenolic compounds. In this study, extractions of 3.0 g of dried powder from the peel were accomplished with a 10 mL volume extraction vessel. Nine extractions were carried out at constant static time of 20 minutes, temperatures of 35, 40 and 45 °C, pressures of 150, 250, and 350 bar, and dynamic times 10, 25 and 35 minutes. Two different concentrations of methanol (10 and 15 %) were used, as a modifier. The extracted phenolics were collected in 5 mL methanol in 10 mL volumetric flasks through a Duraflow manual variable restrictor (Suprex, USA) that avoided plugging and provided a constant flow rate during the extraction process. The compressed supercritical fluid CO_2 at a flow rate of approximately 0.35 ± 0.05 mL was passed through the variable restrictor. During the dynamic time, the volumetric flask was placed in an ice bath for efficiency of collection. Methanol was spiked directly into the extraction vessel with a charged sample prior to extraction to investigate the effect of the modifier. Table 1 shows the SFE experimental conditions for phenolic extraction.

Dried powders of peels (2.5 g) were extracted with 40 mL of each solvent at room temperature for 6 hours. The extract was filtered through Whatman No.42 filter paper to remove fine particles. The residue was re-extracted with the same solvent and the extracts were added to each other [19]. After extraction, the solvent was evaporated using a rotary evaporator (under vacuum and at 30 °C) and the concentrated extracts were stored in a freezer. The same procedure was followed for other solvents and methods.

An Elma Transsonic model 690/H ultrasonic bath (Germany) was used for sonication and extraction of phenolic compounds from mixture of solvents and powdered peel. Forty mL of solvent was

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Table 1. Taguchi experimental design for extraction of phenolic compounds from pomegranate peel using SFE.

Run No.	P (bar)	T (°C)	t (min)	Modifier (MeOH, %)	Phenolic content (%)[a]
1	150	35	10	0	0.73
2	150	40	25	10	0.53
3	150	45	35	15	0.38
4	250	35	25	15	0.75
5	250	40	35	0	0.66
6	250	45	10	10	0.32
7	350	35	35	10	0.77
8	350	40	10	15	0.84
9	350	45	25	0	0.75

[a] Based on dry weight of extracted peel.

added to 2.5 g powdered peel; the mixture was sonicated in this ultrasonic bath for 30 minutes. The extract was filtered through Whatman No.42 filter paper.

The concentrations of phenolic compounds in the extracts were determined according to the Folin-Ciocalteu method [15], and the results were expressed as tannic acid equivalents per gram dry weight of sample (TAE/gdw). The pomegranate peel extracts were dissolved in a mixture of methanol and water (2:1 V/V). Samples (0.2 mL) were mixed with 1.0 mL of 10-fold-diluted Folin-Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate solution, after the mixture had been allowed to stand for 30 minutes at room temperature, the absorbance was measured at 765 nm using Scinco 2120 UV-Vis spectrophotometer (Seoul, South Korea). The estimation of phenolic compounds in the extracts was carried out in triplicate. Antioxidant-free soybean oil was obtained from Parsghoo Co. (Tehran, Iran).

Antioxidant Activity Assay

The pomegranate peel extracted by acetone as solvent along with Sonication had the highest phenolic content. Therefore, this extract was added to soybean oil (refining oil with approximately 40 ppm naturally-occurring tocopherols, measured in the quality control laboratory of Parsghoo Co.) at levels of 0.010, 0.025, 0.035 and 0.050 %. Synthetic antioxidants (BHA and BHT) at 0.010 and 0.020 % levels were added to soybean oil for comparison, and the stability of the oil at 60 °C was monitored, according to the oven test method [20]. Oxidation was periodically assessed by the measurement of peroxide value (PV) [21], and thiobarbituric acid (TBA) value [22]. A control sample was prepared under the same conditions, without adding any antioxidant. All the experiments were carried out in triplicate.

Statistical Analysis

Experimental data was analysed using analysis of variance (ANOVA) and significant differences among means from a triplicate analysis at ($P<0.05$) were determined by Duncan's multiple range test (DMRT) using the SPSS software.

RESULTS AND DISCUSSION

Optimization of SFE Variables

The first step in the SFE of phenolic compounds is to optimize the operating conditions (especially the pressure and the percentage of the modifier) to obtain an efficient extraction of phenolics. In fact, the fluid pressure and temperature, the percentage of modifier and the extraction

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time are generally considered as the most important factors. The optimization of the method can be carried out step-by-step or by using an experimental design. Table 1 shows different conditions of experiments in the extraction of phenolic compounds according to the Taguchi experimental design [23]. All the selected factors were examined using a three-level orthogonal array design with a L_9 (3^4) matrix.

Total Phenolic Content

The concentration of phenolics in the extracts, expressed as tannic acid was dependent on the polarity of solvent and method used in the extraction as shown in Figures 1A and 1B. The amount of phenolic compounds in the acetone extracts (in either solvent or ultrasound-assisted solvent extraction methods) were the highest (40.0 and 35.0 % for sonication and solvent extraction, respectively ($P< 0.05$)), followed by methanol (34.5 and 31.0 %), ethanol (25.3 and 23.0 %), and water (10.0 and 12.0 %), and ethyl acetate extracts (0.2 and 0.2 %). There was a significant difference ($P< 0.05$) in the extraction yields between the extracts of the five solvents used (Figures 1A and 1B). Extraction in acetone by sonication was found to be more efficient than other solvents studied in extracting the antioxidant present in the pomegranate peel. These antioxidative activity results were comparable to the values previously reported by Negi, *et al.* [24]. Ethyl acetate extract and extract of modified SF CO_2 had similar (at $P< 0.05$), but comparatively small extraction yield (as shown in Figures 1A and 1B). As shown in Table 1, in run No. 8 (T= 40 °C, P= 350 bar, dynamic time= 10 min and 15% of modifier), the maximum extraction yield of phenolic acids was obtained (0.84 g/100gdw) that in comparison with solvent extraction, its yield is very low.

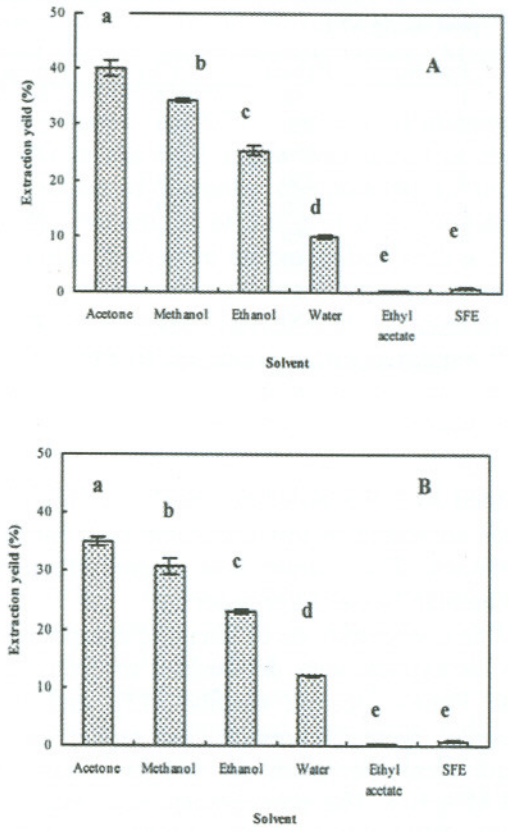

Figure 1. Total phenolic contents of pomegranate peels extracted by (A) sonication and (B) solvent extraction methods.

Effect of Addition of PPEs on the Stability of Soybean Oil

As a general trend, antioxidant activity increased with increasing extract concentration, as indicated by lower PV and TBA values in Figures 2 and 3, but the concentration leading to maximum antioxidant activity is closely dependent on the extracts. Often, natural antioxidants show antioxidant powers lower than those of synthetic ones, but they are not law-limited in quantity. Also, this observation is limited to a certain level, which depends on both the antioxidant and the test [25]. For most natural antioxidant and tests, maximum antioxidant activity was achieved using a 0.05 % concentration.

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The PPEs at 0.010, 0.025, 0.035 and 0.050 % levels and synthetic antioxidants (BHA and BHT) were added at 0.010 and 0.020 %, because the latter were pure compounds whereas the former were complex mixtures, with active components being present at lower levels.

The addition of natural and synthetic antioxidants to soybean oil affected, to differing degrees, the peroxide and TBA values during accelerated oxidation at 60 °C for 12 and 13 days, respectively. The peroxide value (PV) measures primary products of lipid oxidation and the TBA value measures the formation of secondary oxidation products, mainly malonaldehyde, antioxidant (BHA and BHT) at the 0.010 and 0.020 % levels.

CONCLUSION

These results suggest that the PPE may be used as a natural antioxidant to improve the quality, stability and safety of foods such as edible oils. Phenolic compounds are widely distributed in nature and, according to the findings of this study, pomegranate peel is a natural source of phenolic compounds. Acetone extracts were found to contain high phenolic contents (35.0-40.0 %), so it is suggested that the best method for extraction

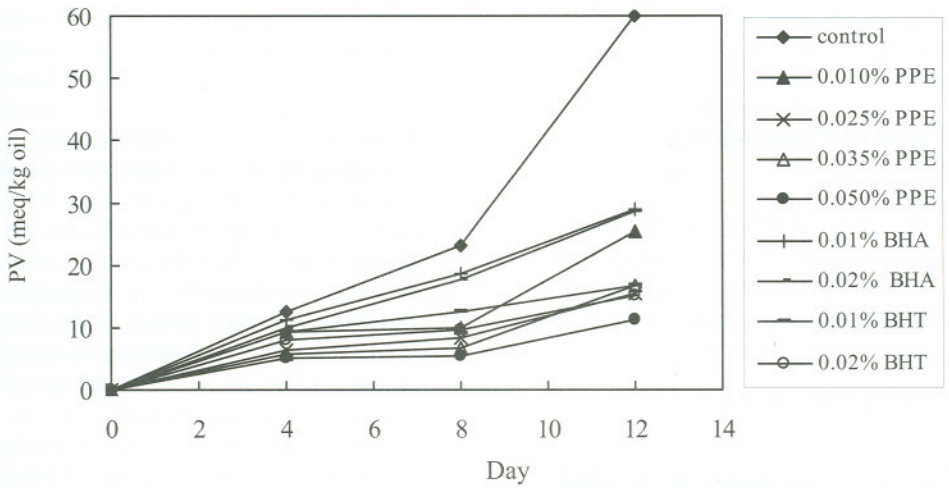

Figure 2. Changes in the peroxide values (PVs) of soybean oil treated with different concentrations of PPEs during storage at 60 °C.

which may contribute to an off-flavour in oxidized oil [26]. All those samples with an added PPEs level at 0.010-0.050 % were more stable on heating at 60 °C than the control, when assessed by the change in peroxide (Figure 2) and TBA (Figure 3) values. The antioxidant effect of PPE increased with concentration and, at a concentration of 0.050 %, its antioxidant activity was higher and significantly different ($P<0.05$) from that of the synthetic

of antioxidant-containing phenolic compounds is by sonication with acetone or methanol. These results suggest that the PPE possesses antioxidant properties and, after purification, could be used as an alternative natural antioxidant. However, extensive research is required on potential sources, optimisation of the extraction process, knowledge about the *in vivo* mechanisms and assimilation. No single compound alone can be considered responsible for this stability.

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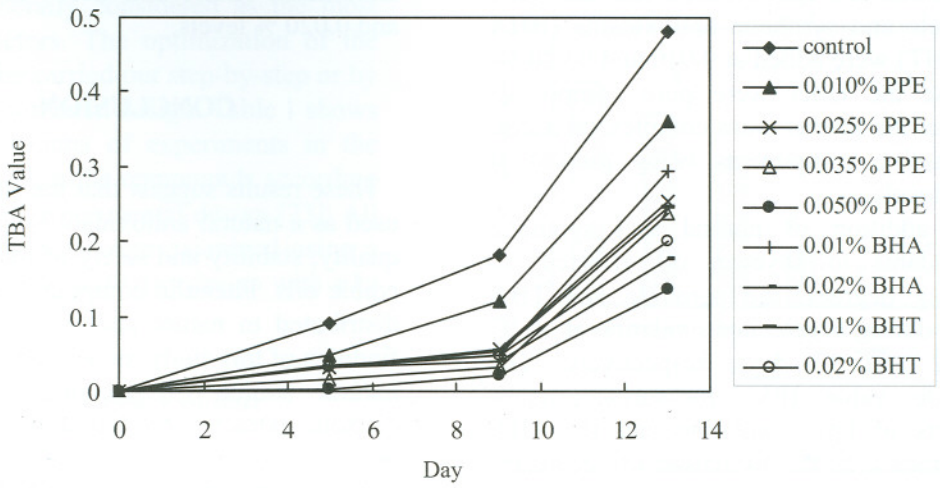

Figure 3. Changes in the TBA values of soybean oil treated with different concentrations of PPEs during storage at 60 °C.

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با امواج فرا صوت (Sonication) دارای بیشترین راندمان است. بعلاوه، تاثیر عصارهٔ استنی پوست انار (۰/۰۵-۰/۰۱ ٪) بر پایداری روغن سویا در دمای ۶۰ درجهٔسانتی‌گراد (آزمون اجاق) تعیین شد (با اندازه‌گیری مقادیر پراکسید و تیوباربیتوریک اسید). در ۰/۰۵ ٪ از عصارهٔ پوست انار، فعالیت آنتی اکسیدانی آن بیشتر از ۰/۰۲٪ دو آنتی اکسیدان سنتزی BHA (Butylated hydroxyanisole) و BHT (Butylated hydroxyltoluene) بود. نتایج نشان دادند که عصارهٔ پوست انار (Pomegranate peel extract, PPE) فعالیت آنتی اکسیدانی بالایی دارد و منبع غنی از آنتی اکسیدان طبیعی است.

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