Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 92 (2005) 521–525
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts

Amir Hossein Goli, Mohsen Barzegar *, Mohammad Ali Sahari

Department of Food Science and Technology, Faculty of Agriculture, Tarbiat Modarres University, P.O. Box 14115-336 Tehran, Iran

Received 2 February 2004; received in revised form 17 August 2004; accepted 17 August 2004

Abstract

In this study, the phenolic antioxidants of pistachio (*Pistachia vera*) hull were extracted by two different solvent extraction methods (solvent and ultrasound-assisted methods) with three different solvents (water, methanol and ethyl acetate) and the results were compared with supercritical fluid extraction (SFE). The total phenolic compounds were determined according to the Folin–Ciocalteu method. Furthermore, the effects of water and methanolic extracts of pistachio hull on the stability of soybean oil during heating at 60 °C (oven test method) were determined. The pistachio hull extract (PHE) was effective in retarding oil deterioration at 60 °C, with activity increasing with concentration in the range 0.02–0.06%. At a concentration of 0.06%, the PHE was similar in activity to BHA and BHT added at 0.02%. Hence, it is clear that pistachio hulls, which at present are often considered as agricultural waste, contain antioxidant that may usefully be extracted and added to foods.

Keywords: Pistachio hull; Phenolic compounds; Antioxidant activity; Soybean oil; Sonication; SFE

1. Introduction

Antioxidants are the compounds that, when added to food products, especially to lipids and lipid-containing foods, can increase shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants since the beginning of this century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity (Mahdavi & Salunkhe, 1995). Thus, a need for identifying alternative natural and safe sources of food antioxidant is created (Wanasundara & Shahidi, 1998) and the search for natural antioxidants, especially of plant origin, has notably increased in recent years (Zainol, Abd-Hamid, Yusof, & Muse, 2003).

Iran is the leading country that produces pistachio nuts. The total production of Iran was about 249,000 tons in 2003 (Anonymous, 2003) and Iran is the largest exporter (about 86%) in the world.

Great interest has recently been focussed 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 a great number of pathological disturbances, such as atherosclerosis (Steinberg, 1992), brain disfunction (Gordon, 1996) and cancer (Ames, 1983). Due to these facts, it would be interesting to optimize an extraction process to obtain maximum yield of these substances; in addition, undesirable components can be removed. Solvent extraction is frequently used for isolation of antioxidant and extraction yield is dependent on the solvent and method of extraction, due to the different antioxidant

* Corresponding author. Tel.: + 98 21 4196522; fax: + 98 21 4196524.
E-mail address: mbb@modares.ac.ir (M. Barzegar).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.08.020

potentials of compounds with different polarity. Several extraction techniques have been reported for extraction of phenolic compounds from different matrices using solvents with different polarities, such as methanol, water, ethyl acetate and petroleum ether (Cheung, Cheung, & Ooi, 2003; Singh, Murthy, & Jayaprakasha, 2002). Furthermore, supercritical CO_2 (Palma, Taylor, Varela, Cutler, & Cutler, 1999; Persson, Barisic, Cohen, Thorneby, & Gorton, 2002) and solvent extraction by sonication have been applied for this purpose (Bicchi, Binello, & Rubiolo, 2000).

The objective of this work was to compare solvent extraction (with three solvents: water, methanol and ethyl acetate) and solvent extraction by sonication (with similar solvents) with the SFE process by determining the total phenolic content according to the Folin–Ciocalteu method. Furthermore, in this study, the effect of concentration of PHEs on the stability of soybean oil during heating has been compared with that of two synthetic antioxidants BHA and BHT.

2. Materials and methods

2.1. Plant material and chemicals

Pistachio hulls (Fandoghi variety) were obtained from the Yazd province of Iran. Hulls were dried and ground to give 40-mesh size powder. All chemicals and solvents were of analytical grade and obtained from Merck (Darmstadt, Germany).

2.2. SFE apparatus

A suprex MPS/225 system (Pittsburg, PA) in the SFE mode was used for extraction of phenolics. In this study, extraction was accomplished with 10 ml volume extraction vessel. Nine extractions were carried out at constant static time of 5 min, temperatures of 35, 45 and 55 °C, pressures of 100, 200 and 350 atm, and dynamic times 15, 25 and 40 min. Two different concentrations of methanol (0.5 and 1.5 ml) were used, as modifier.

2.3. Solvent extraction

The different solvents (ethyl acetate, methanol, and water) were used to extract phenolic compounds from the pistachio hull. Dried powders of hulls (2.5 g) were extracted by mixing, using a magnetic stirrer, with 10 ml of water at room temperature for 6 h. The extract was filtered through Whatman No. 42 filter paper for removal of hull particles. The residue was re-extracted with the same solvent and the extracts were stored in a refrigerator. The same procedure was followed for other solvents, such as methanol and ethyl acetate, for the extraction of phenolic compounds.

2.4. Ultrasonic extraction

The ultrasound-assisted extraction procedure was used for the extraction of pistachio hull with different solvents (ethyl acetate, methanol, and water). Thus, 20 ml of solvent were added to 2.5 g of powdered hulls; the mixture was sonicated in an ultrasonic bath for 45 min. The extract was filtered and stored in a freezer.

2.5. Supercritical fluid extraction

The extraction vessel (10 ml) was loaded with 2.5 g of dried powder of hull and was placed inside the extraction chamber, which was maintained at 35–55 °C throughout the experiment. Samples were subjected to static supercritical fluid extraction (SFE) for 5 min, followed by dynamic extraction 15–40 min; the extracted analytes were collected in 5 ml methanol in 10 ml volumetric flasks through a variable restrictor that avoided plugging to a great extent and provided a constant flow rate during the extraction process. The supercritical fluid CO_2 flow rate through the variable restrictor was approximately 0.35 ± 0.05 ml (liquid). In order to achieve better collection efficiency, during the dynamic time, the volumetric flask was placed in an ice bath. For studying the effect of modifier, methanol was spiked directly into the extraction vessel with charged sample prior to extraction. The extraction was accomplished with supercritical carbon dioxide under the nine conditions mentioned in Table 1.

2.6. Determination of total phenolics

The concentration of phenolic compounds in the extracts were determined according to the Folin–Ciocalteu method (Singh et al., 2002), and results were expressed as tannic acid equivalents per gramme dry weight of sample (TAE/gdw). The pistachio hull 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-di-

Table 1
SFE experimental conditions for pistachio hull phenolic compounds extraction

Run no.	*P* (atm)	*T* (°C)	*t* (min)	Modifier (%)	Phenolic content (mg TAE/gdw)[a]
1	100	35	15	0	–
2	100	45	25	5	–
3	100	55	40	15	0.2
4	200	35	25	15	2.1
5	200	45	40	0	–
6	200	55	15	5	0.6
7	350	35	40	5	2.4
8	350	45	15	15	7.8
9	350	55	25	0	1.1

[a] mg Tannic acid equivalents per gramme dry weight of sample.

luted Folin–Ciocalteu reagent and 0.8 ml of 7.5% sodium carbonate solution; after the mixture had been allowed to stand for 30 min at room temperature, the absorbance was measured at 765 nm using UNICAM 8620 UV–Vis spectrophotometer. The estimation of phenolic compounds in the extracts was carried out in triplicate, and the results were averaged.

2.7. Antioxidant activity assay

Pistachio hull extracts (except ethyl acetate and SFE extracts due to their low total phenolic contents) were added individually to soybean oil (refining oil with approximately 40 ppm naturally tocopherols) at levels of 0.02%, 0.04% and 0.06%. Synthetic antioxidants (BHA and BHT), at levels of 0.01% and 0.02%, were used for comparison. The oven test method at 60 °C was used to check stability. Oxidation was periodically assessed by the measurement of peroxide value (PV) according to the AOCS method (1989), and thiobarbituric acid (TBA) value, as described by Sidewell, Salwin, Benca, and Mitchel (1954). A control sample was prepared under the same conditions, without adding any

Table 2
Total phenolic content extracted from pistachio hull by different extraction methods[a]

Extraction methods-solvent	Phenolic content (mgTAE[b]/gdw)
Water	34.7 ± 1.16c
Ultrasonic-water	34.2 ± 2.64c
Ultrasonic-methanol	32.8 ± 0.05c
Methanol	32.8 ± 0.96c
SFE-CO_2	6.55 ± 0.69d
Ethyl acetate	5.67 ± 0.24d
Ultrasonic-ethyl acetate	5.02 ± 0.27d

Values with different letters (c, d) were significantly different ($P < 0.05$, Duncan's multiple range test).
[a] Values expressed are means ± SD of triplicate measurements.
[b] TAE, tannic acid equivalent.

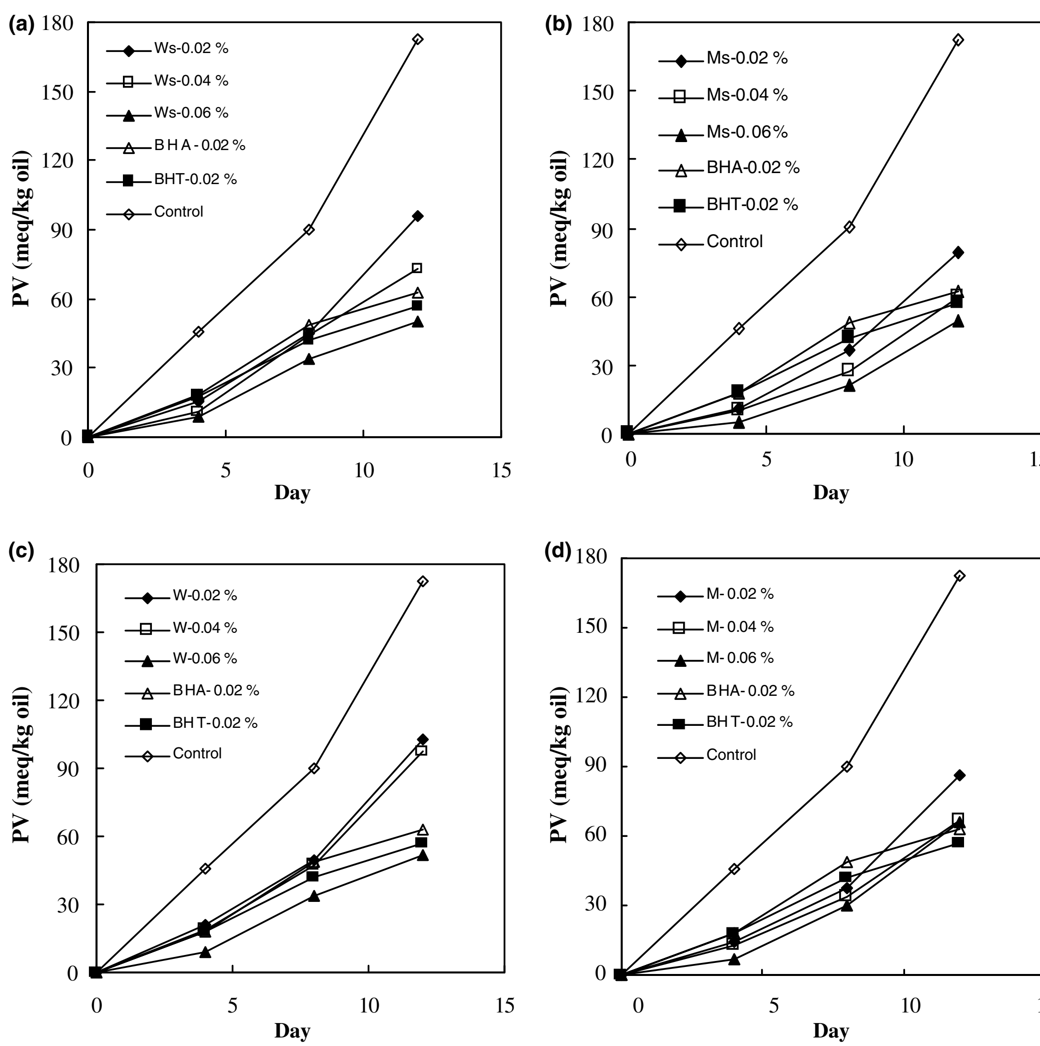

Fig. 1. Effect of pistachio hull extract (PHE) on soybean oil oxidation expressed as peroxide value formation at 60 °C. (a) and (b) Water (Ws) and methanolic (Ms) extracts of hull by sonication method. (c) and (d) Solvent extracts of hull by water (W) and methanol (M), respectively.

antioxidant. All the experiments were carried out in triplicate and results were averaged.

2.8. Statistical analysis

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

3. Results and discussion

3.1. Optimization of SFE variables

The first step in the SFE of phenolic compounds is to optimize the operating conditions (especially pressure and the percentage of modifier) to obtain an efficient extraction of phenolics. In fact, the fluid pressure and temperature, the percentage of modifier and the extraction 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 extraction of phenolic compounds according to the Taguchi experimental design (Roy, 1990). All the selected factors were examined using a three-level orthogonal array design with a L_9 (3^4) matrix.

As shown in Table 1, in run No. 8 (T = 45 °C, P = 350 atm, dynamic time = 15 min and modifier volume = 1.5 ml), the maximum extraction yield of phenolic acids was obtained (7.8 mg/gdw). In comparison with solvent extraction, this yield is low.

3.2. Total phenolic content

The concentration of phenolics in the extracts, expressed as mg TAE/g sample, was dependent on the solvent and method used in the extraction, as shown in Table 2. The amounts of phenolic compounds in the water extracts (in either solvent or ultrasound-assisted

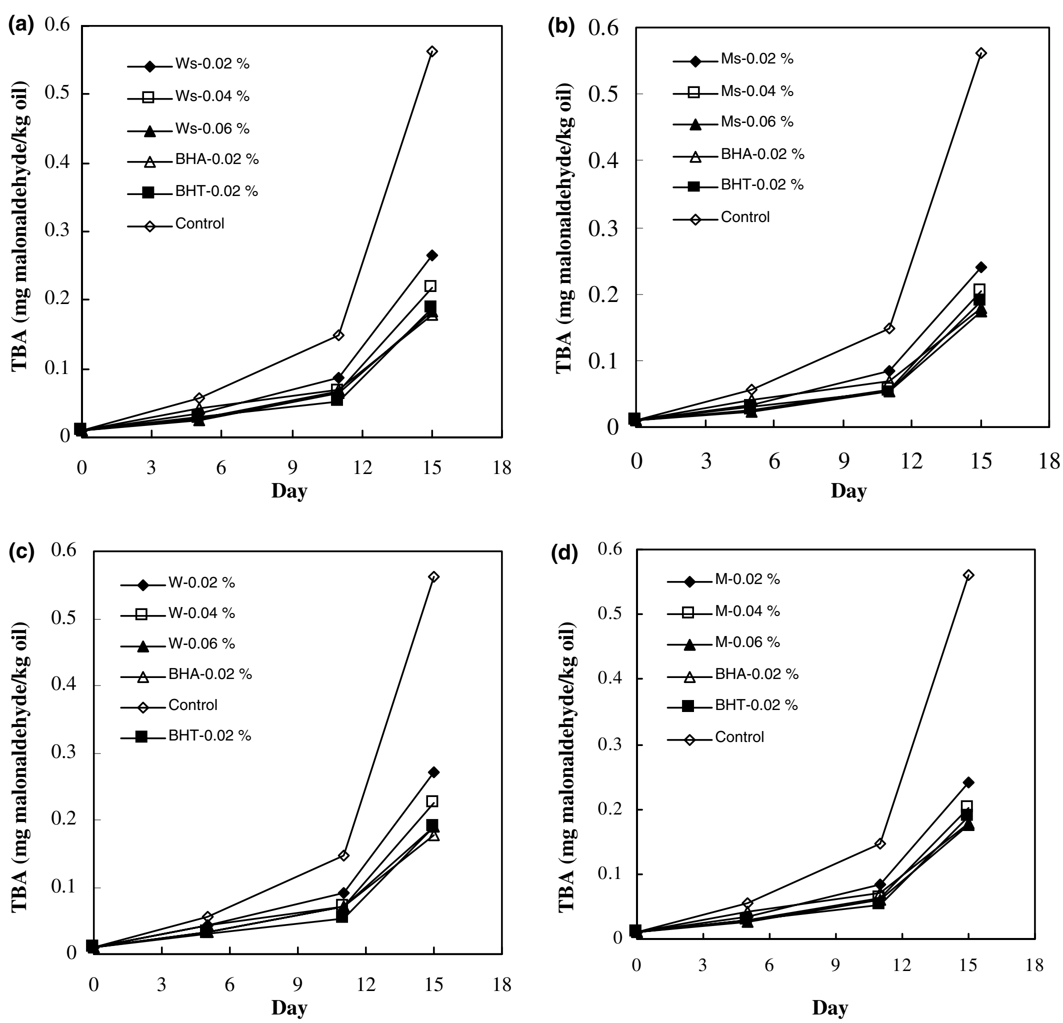

Fig. 2. Changes in the TBA values of soybean oil treated with PHEs during storage at 60 °C. (a) and (b) Water (Ws) and methanolic (Ms) extracts of hull by sonication method. (c) and (d) Solvent extracts of hull by water (W) and methanol (M), respectively.

solvent extraction method) were highest. There was no significant difference ($P < 0.05$) in the extraction yields between the extracts of the two mentioned methods (Table 2). Ethyl acetate extract and extract of modified SF CO_2 had similar (at $P < 0.05$), but comparatively small total phenolic contents (as shown in Table 2). Higher extraction yields of phenolic compounds were obtained with an increase in polarity of the solvent.

3.3. Effect of addition of PHEs on the stability of soybean oil

The PHEs were used at levels of 0.02%, 0.04% and 0.06% and synthetic antioxidants (BHA and BHT) were added at 0.01% and 0.02%, because the latter were pure compounds where as the former were complex mixtures with active components being present at lower levels.

The addition of natural and synthetic antioxidant to soybean oil affected, to different degrees, the peroxide and TBA values during accelerated oxidation at 60 °C for 15 days (Figs. 1 and 2). Peroxide value (PV) measures primary products of lipid oxidation and TBA value measures the formation of secondary oxidation products, mainly malonaldehyde, which may contribute off-flavour to oxidized oil (Rossel, 1994). All samples with PHEs level added at 0.02–0.06% were more stable on heating at 60 °C than the control, when assessed by the change in peroxide (Fig. 1) and TBA (Fig. 2) values. The antioxidant effect of PHE increased with concentration and, at a concentration of 0.06%, the antioxidant activity was not significantly different ($P < 0.05$) from that of the synthetic antioxidant (BHA and BHT) at levels of 0.02%.

4. Conclusion

Phenolic compounds are widely distributed in nature and, according to this paper, pistachio hull is a natural source of phenolic compounds. Water extracts were found to have high phenolic contents (32.0–34.0 mg/gdw of sample), so the best method for extraction of phenolic compounds was solvent extraction with water or methanol. These results suggest that the PHEs possess antioxidant properties and could be used as alternative natural antioxidants (but after toxicological examination). No single compound can be considered responsible for this stability. This study will provide bases for future studies in this area. It is recommended that other common pistachio varieties be examined for antioxidant properties.

Acknowledgements

We gratefully acknowledge financial support from the Tarbiat Modarres University Research Council. We are grateful to Ahmad Rajai for his assistance.

References

Ames, B. M. (1983). Dietry carcinogens and anticarcinogens. Oxygen radicals and degenerative disease. *Science, 221*, 1256–1264.

Anonymous. (2003). *Iran statistical year book 2003, area under cultivation and production of selected perennial crops*. Statistical Center of Iran.

AOCS. (1989). In: D. Firestone, (Ed.), *Official methods and recommended practices of the american oil chemists society* (4th ed.). Champaign: AOCS.

Bicchi, C., Binello, A., & Rubiolo, P. (2000). Determination of phenolic diterpene antioxidant in rosemary (*Rosmarinus officinalis* L.) with different methods of extraction and analysis. *Phytochemical Analysis, 11*, 236–242.

Cheung, L. M., Cheung, P. C. K., & Ooi, V. E. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry, 81*, 249–255.

Gordon, M. H. (1996). Dietry antioxidants in disease prevention. *Natural Product Report*, 265–273.

Mahdavi, D. L., & Salunkhe, D. K. (1995). Toxicological aspects of food antioxidant. In D. L. Mahdavi, S. S. Deshpande, & D. K. Salunkhe (Eds.), *Food antioxidants*. New York: Marcel Dekker.

Palma, M., Taylor, L. T., Varela, R. M., Cutler, S. J., & Cutler, H. G. (1999). Fractional extraction of compounds from grape seeds by supercritical fluid extraction and analysis for antimicrobial and agrochemical activities. *Journal of Agricultural and Food Chemistry, 47*, 5044–5048.

Persson, P., Barisic, Z., Cohen, A., Thorneby, L., & Gorton, L. (2002). Countercurrent supercritical fluid extraction of phenolic compounds from aqueous matrics. *Analytica Chimica Acta, 460*, 1–12.

Rossel, J. B. (1994). Measurements of rancidity. In J. C. Allen & R. J. Hamilton (Eds.), *Rancidity in foods* (third ed., pp. 22–53). UK: Blackie.

Roy, R. K. (1990). *A primer on the Taguchi method*. New York: Van Nostrand Reinhold.

Sidewell, G. G., Salwin, H., Benca, M., & Mitchel, J. A. (1954). The use of thiobarbituric acid as a measure of fat oxidation. *Journal of American Oil Chemists Society, 31*, 603–606.

Singh, R. P., Murthy, K. N. C., & Jayaprakasha, G. K. (2002). Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *Journal of Agricaltural and Food Chemistry, 50*, 81–86.

Steinberg, D. (1992). Metabolism of lipoprotein and their role in the pathogenesis of atherosclerosis. *Atherosclerosis Review, 18*, 1–6.

Wanasundara, U. N., & Shahidi, F. (1998). Antioxidant and pro-oxidant activity of green tea extract in marine oils. *Food Chemistry, 63*, 335–342.

Zainol, M. K., Abd-Hamid, A., Yusof, S., & Muse, R. (2003). Antioxidant activity and total phenolic compounds of leaf, root and petiole of four accessions of *Centella asiatica* (L.) urban. *Food Chemistry, 81*, 575–581.