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EXTRACTION OF NATURAL ANTIOXIDANTS FROM LEMON PEELS. KINETICS AND ANTIOXIDANT CAPACITY

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ABSTRACT

Experimental study of the effect of the particle size and solvent concentration on the extraction kinetics of lemon peels with ethanol-in-water solutions, total polyphenols contents and antioxidant activity was carried out. It was found that the process is sensitive to the particles' sieve fraction. The effect of solvent concentration was almost negligible for the system studied. The total polyphenols contents and the antioxidant activity of the obtained extracts were quantified. The lemon peels are promising cheap residue and may find potential applications in food and cosmetic industries as a source of natural antioxidants.

Keywords: natural antioxidants, polyphenols, extraction, lemon peels.

INTRODUCTION

Nowadays the oxidative stress is one of the serious problems of the modern society. The air pollution, smoking, UV-radiation, mode of life lead to so called oxidative stress of cells, responsible for many diseases as dermatitis, melanomas or photo aging of the skin. One of the basic applications of the natural plant extracts in cosmetic formulations is due to their antioxidant properties. Usually the natural antioxidants do not cause allergic reactions, therefore they are suitable for formulation of hypoallergic cosmetics. Another interesting property of the natural extracts is their antimicrobial activity. Antioxidant and antimicrobial activities of different plant extracts are not fully investigated. In the literature appear new data concerning unstudied plants or other raw materials. The oxidative deterioration of fats and oils in foods is responsible for rancid odours and flavours, with a consequent decrease in nutritional quality and safety caused by the formation of secondary, potentially toxic, compounds. The addition of antioxidants is required to preserve flavour and colour as well as to avoid vitamin destruction [1].

Citrus residues are attractive source of natural antioxidants because of their important crop, with production estimated at 80 million tones per year [1-3]. The main uses of citrus in food industries include fresh juice or citrus-based drinks [4]. Citrus processing by-products potentially represent a rich source of natural flavonoids, owing to the large amount of peel produced, and that citrus peels contain a high concentration of phenolic compounds. Moreover, while flavonoids are abundant elsewhere in the plant kingdom, there are several compounds (e.g., flavanones, flavanone glycosides and polymethoxylated flavones) unique to citrus, which are relatively rare in other plants [5]. Usually valuable natural materials have been extracted with organic solvents. However, some of them are toxic, and the extraction conditions are often severe. For this reason, a food-grade ethanol instead of methanol is widely used for the extraction of phenolic compounds from various citrus peels. The effects of different parameters (conditions of the preparation of peel samples, repeated extraction, organic solvents used and their concentration, temperature, etc.) on the extraction process were investigated by different authors [1-5].

The aim of this research is to study the possibility to use lemon peels extracts as a source of natural antioxidants, to quantify the extracted phenolic contents in lemon peels, to compare the amounts obtained using solvent extraction and to evaluate the antioxidant activities (AOA) of the extracts.

For this purpose the extraction kinetics of lemon peels with ethanol-in-water solutions is followed, the effect of ethanol concentration and the particle size on the total extract quantity, the amount of extracted phenolic compounds and the antioxidant activity of extracts are studied.

EXPERIMENTAL

Plant materials

Lemons were purchased from local fruit market, Sofia, Bulgaria (country of origin – Greece). All the fruits were of eating quality, without blemishes or damage. The fruits were manually pealed. The tissue removed was the pericarp region (peel), which includes the epicarp and mesocarp. The material was dispensed in paper bags and dried at ambient conditions in order to decrease their humidity from 85 % to 10 % of mass. Dried lemon peels were stored at ambient conditions before any further treatment. Afterward the peels were grinded and sieved using vibrating metal sieves with mesh sizes of 2.5; 2 and 1mm.

Chemicals

Folin-Chiocalteu reagent, Sodium carbonate, Gallic acid were purchased from Sigma Chemical Co. The ethanol used was 96 % PA, supplied by Valerus, Bulgaria.

Extraction procedure

The kinetics of extraction was studied in both liquid and solid phases. The quantity of extracted substance in the liquid phase increases and, simultaneously, the mass of the solid diminishes. A series of samples of each fraction were prepared. Each of them contained 1g ($\pm$0.0005 g) dry, ground and packed in cheesecloth lemon peels of the corresponding fraction. The packets were also weighted. The balance used was Sartorius analytic, precision 0.1mg. The solvent was a mixture of ethanol and distilled water in different proportions: 20 %, 50 % and 70 % vol. of ethanol. The operational solid-to-liquid ratio was 0.05 or 1g (lemon peels) / 20 cm^3 (ethanol + H_2O). After preparing the samples, they were placed in a laboratory shaker (THUS 2) and brought in contact with the solvent. This moment was considered as a beginning of the extraction process. The samples were taken between the 3rd and the 80th minute. The packet containing the solid phase was withdrawn and dried. The flasks containing the liquid phase with the extracted substance were also dried at 50°C in thermostated oven until constant weight. The obtained experimental kinetic curves are presented on Fig. 1.

After drying at 50°C the total extract amount was determined gravimetrically.

Total polyphenols contents

It is considered that Total Polyphenols Content (TPPC) is representative for rough determination of antioxidant capacity. As the molecular mass of polyphenols mixture is unknown, TPPC can be estimated as Gallic Acid Equivalent ($TPPC_{GAE}$). The photometric method, based on colour reaction of phenolic compounds with Folin-Ciocalteu reagent is largely used for determination of total polyphenols content because of its simplicity and reliability [6].

Antioxidant activity determination

Other methods for determination of the antioxidant activity are FRAP (Ferric Reducing Antioxidant Power), ORAC (Oxygen Radical Absorbance Capacity), or TEAC (Trolox Equivalent Antioxidant Capacity). As it is underlined in the literature, all these methods give quite different results and the choice of suitable method for antioxidant activity determination is of great importance. A method largely applied is the $DPPH^+$ method because of its simplicity and stable results [7, 8]. It is based on discoloration reaction between nitrogen electron (from $DPPH^+$) and hydrogen atom of hydroxyl group (from antioxidant substance). An inconvenience of this method is its light sensitivity. For this reason, the reaction must be carried out in dark and in non-alkaline medium. In our study we applied this method using a spectrophotometer Helios β (Unicam, USA). To compare the AOA of different materials we used IC_{50} parameter. It represents the amount of antioxidant sample, which inhibits 50 % of the initial concentration of $DPPH^+$.

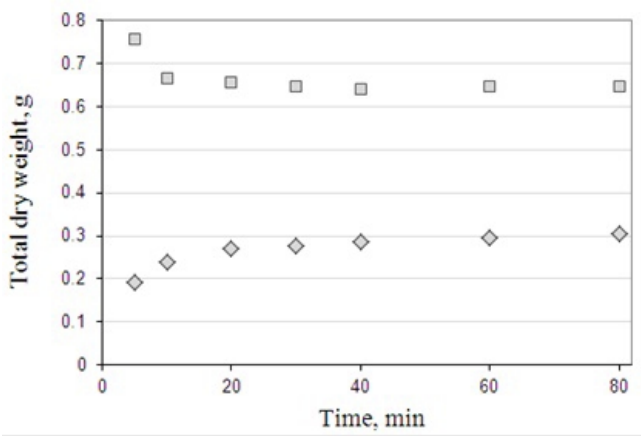

Fig. 1. Experimental kinetic curve at solid-to-liquid ratio 0.05 g cm^{-3}, particle size d < 1 mm and 50 % ethanol concentration. □– solid phase; ◇– liquid extract.

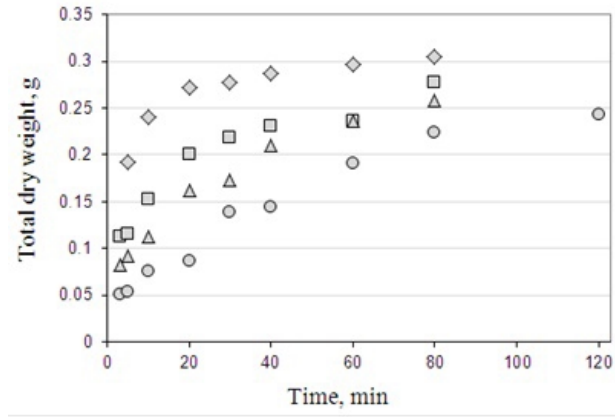

Fig. 2. Influence of the particle size on the extract amount: ◇- $< 1\ mm$; □ - $1\ mm < d_p < 2\ mm$; △- $2\ mm < d_p < 2.5\ mm$; ○- $d_p > 2.5\ mm$.

RESULTS AND DISCUSSION

Extraction kinetics

The kinetics of extraction from dried lemon peels was followed. The total dry weight was determined in the solid and the liquid phase. Fig.1 presents typical kinetic curves for peel fraction less than 1 mm with 50 % ethanol-in-water solution. The solid-to-liquid ratio was 1 g raw material in 20 cm^3 of solvent.

The kinetic curve exhibits three parts: the first steep part corresponds to dissolution of easily available substances at a particle surface. The middle part describes the simultaneous dissolution of the reminded surface substances and the substances in the internal part of the solids (both external and internal diffusion control). Slowly increasing (decreasing in the solid phase) part of the curves depicts the entire exhausting of the surface part of soluble substances – internal diffusion controls.

Effect of particle size

To study the effect of particles' size experiments with 50 % ethanol concentration and different sieve fractions described in the experimental section were carried out. The total dry extract weight was investigated. The results are presented in Fig. 2. It is evident that the best results were obtained for the fraction $< 1\ mm$, which can be explained with increased solid phase surface enhancing the phase contact. For the largest sieve fraction used (> 2.5 mm) the process was carried out until 120 min. It was found that the total extract's quantity slowly tends to the values reached at 60-80 min for fractions between 1 and 2 mm and between 2 and 2.5 mm. Our results confirm other authors' experimental data on the positive effect of particles demolition on the polyphenols extraction [1].

Effect of the solvent concentration

In order to study the influence of the solvent concentration experiments with 20 % and 70 % of ethanol-in-water were also carried out. The solid fraction used was less than 1mm, giving the best results for the total extract. The results are presented in Fig. 3.

In can be seen that the solvent concentration does not affect significantly the extraction rate and the total amount of the dry extract. Therefore, the solvent concentration should be chosen according to economical reasons.

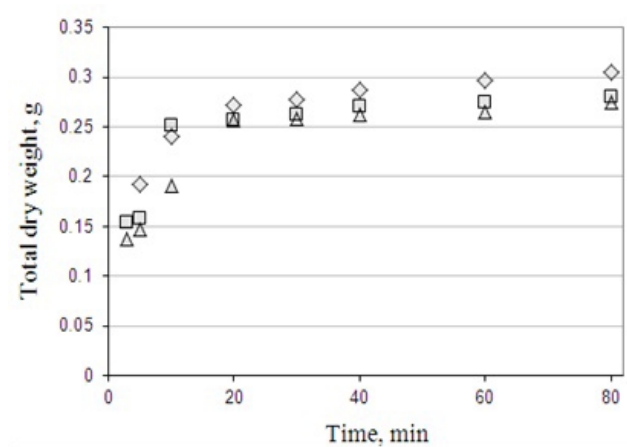

Fig. 3. Effect of the solvent concentration on total dry extract: ◇ - 50 % ethanol, □– 20 % ethanol, △ - 70 % ethanol.

Table 1. Lemon peels extracts: TPPC and AOA.

Ethanol concentration (% vol.)	Particle size (mm)	$TPPC_{GA}$ (mg l^{-1})	IC50% (mg l^{-1}) sample	$IC50\%_{AA}$ (mg ml^{-1})
20	<1	832.73	80.52	0.19
20	1-2	779.09	86.94	0.18
70	<1	224.55	46.87	0.33
70	1-2	202.73	46.18	0.33
50	<1	928.18	40.4	0.38
50	1-2	821.82	40.13	0.382
50	>2.5	454.55	33.35	0.46

Total polyphenols content and antioxidant activity

The results obtained are summarized in Table 1. It can be seen that the extracts obtained with 70 % ethanol contain approximately 4 times less total polyphenols compared to 20 % and 50 %. As for the data concerning total dry extracts the particles of sieve fraction less than 1 mm exhibit the largest TPPC and this content diminishes increasing the particle size for the same ethanol-in-water concentration (50 %).

It can not be seen an obvious relation between phenolic content and antioxidant activity.

This fact coincides with other authors' results for malts, citrus residues and plant extracts [1].

The comparison between the data obtained for the TPPC and AOA for lemon peels studied and other plant extracts is presented in Fig.4. It is evident that the lemon peels exhibit even higher values for total polyphenols' content and reasonable data for AOA. This result makes them a promising and cheap source for natural antioxidants for food and cosmetic industries.

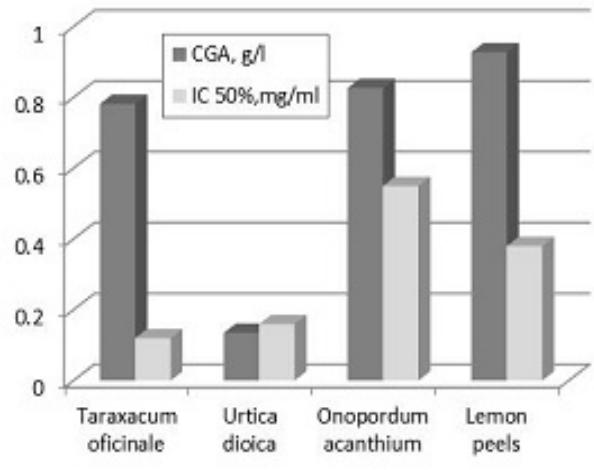

Fig. 4. Comparison between TPPC and AOA for three medicinal plants and the lemon peels [9, 10, this work].

CONCLUSIONS

The possibility of use the lemon peels as a source of natural antioxidants was studied. The extraction kinetics was investigated for the system lemon peels-ethanol-in-water solutions for the solid and the liquid phase. The effect of particle size and the solvent concentration was estimated. It was found dependence on the sieve fraction of solids and almost negligible influence of the ethanol concentration on the total extract amount. The total polyphenols content and the antioxidant activity of the extracts obtained were determined. There was no found any direct dependence between both quantities. Comparing to three medicinal plants characteristics the lemon peels can be characterized as a promising source of natural antioxidants for food and cosmetic industries.

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