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Optimization of the extraction of natural antioxidants from avocado seeds

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Food supplements of plant origin are widespread for dietary use. Most often they are in the form of extracts rich in natural antioxidants with high radical-scavenging action. Avocado (*Persea americana*) is a fruit widely cultivated throughout the world. There are many studies that attest to its health benefits. Avocado peels and seeds are considered as waste. However, there are indications that avocado seeds, which represent about 23 % of fruit weight, have even higher antioxidant activity than that of its flesh. So, the aim of this work is to study the influence of process parameters on the extraction of avocado seeds in order to determine the optimal conditions for obtaining extracts containing maximum amount of bioactive substances and having high antioxidant activity. An experimental approach is developed, which allows the optimal process parameters to be found by a reduced number of experiments. As a result, the following important process parameters are determined: selection of appropriate "green" solvent, which dissolves more antioxidant compounds; minimum solvent-to-solid ratio and minimum process duration necessary for complete extraction, which minimizes process costs; process temperature and ensures an acceptable compromise between higher solubility and thermal stability of the antioxidant compounds. The results obtained are useful for development of technological schemes for production of antioxidant extracts for use as functional supplements obtained from bio-wastes.

Keywords: Avocado seeds, Extraction, Polyphenols, Optimization, Antioxidant activity

INTRODUCTION

Avocado fruit is widely cultivated throughout the world. In 2014, world production of avocados was 5 million tons, with Mexico alone accounting for 30% (1.52 million tons) of the total [1].

The avocado fruit is valued not only for its flavor and nutritional profile, but also for its pharmacological properties. One important medicinal property of avocado fruit is its cancer-preventive effect due to combination of nutrients and phytochemicals (alkanols, terpenoid glycosides, various furan ring-containing derivatives, flavonoids and coumarins) [2]. Many polyphenol compounds can be found in avocado, but in the greatest quantity are lutein and zeaxanthin. Avocado fruit is also rich in several B vitamins and vitamin K, with good content of vitamin C, vitamin E and potassium [3]. The pulp of the avocados is rich of monounsaturated lipids (as oleic acid (67% of total lipids), polyunsaturated (linoleic acid), and saturated lipids (palmitic acid). Nutrient-rich avocado oil has diverse uses for salads or cooking and in cosmetics and soap products [4].

Polyphenols are known for their antioxidant properties. They are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens [5]. In general, they are attracting the attention, due to their role in the plant's immune response, their influence in the oxidative stability and organoleptic characteristics of foods, and the wide variety of health-promoting effects attributed to them [6]. In food, polyphenols may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability. Towards the end of 20th century, epidemiological studies and associated meta-analyses strongly suggested that long-term consumption of diets rich in plant polyphenols offered some protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases [7-9].

The growing interest in the replacement of synthetic food antioxidants has led to multiple investigations in the field of naturally-sourced antioxidants. The search for cost-efficient natural antioxidants has led to the exploration of raw materials of residual origin [10]. Recently, fruit waste has become one of the main sources of municipal solid wastes, which have been an increasingly tough environmental issue. One of the solutions to this problem is to use fruit wastes as a source of valuable compounds - the bioactive constituents, especially phenolic compounds, and use them in the food, pharmaceutical, as well as cosmetics industry. Thus, utilization may be of considerable economic benefits and has become increasingly attractive [11]. Vegetables and some

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fruits yield between 25% and 30% of nonedible products [4, 5]. The by-products of fruits and vegetables are made up of skins and seeds of different shapes and sizes that normally have no further usage and are commonly wasted or discarded [12]. Avocado seeds represent about 20% from its mass and are considered as waste at a volume more than one million tons per year. This waste has a significant environmental impact due to the organic charge. It also requires additional costs for handling and storage [13]. There are a number of studies that find use of seeds as activated carbon [14], natural food dye (it produces orange color) [15], and as protection of oils and fats from oxidation [13].

Extraction is the first step in the studies of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization [16]. Many factors, such as solvent composition, time of extraction, temperature, pH, solid-to-liquid ratio and particle size, may significantly influence the solid–liquid extraction. The big difference in the polarity of polyphenols requires the use of solvents with different polarity (water, acetone, methanol, ethanol, or their mixtures with water). Water/ethanol mixtures are possibly the most suitable solvent systems for the extraction of polyphenols due to the different polarities of the bioactive constituents, and the acceptability of this solvent system for human consumption. There is a growing interest in efficient and environmentally acceptable extraction methods. The desirable features of 'green' extraction methods are low solvent consumption, short extraction time and high extraction yield. Attention is now being directed to the extraction techniques that rely on solvents that are not hazardous to human health [17-19].

The aim of this work is to study the influence of process parameters on the extraction of avocado seeds in order to determine the optimal conditions for obtaining extracts containing maximum amount of bioactive (polyphenol) substances and having high antioxidant activity. An experimental approach is developed, which allows the optimal process parameters to be found by a reduced number of experiments.

EXPERIMENTAL

Raw material

Avocado fruits were purchased from the local market. The seeds were manually separated from the fruits. Before air drying they were cut and ground to particle size of 1 mm by using a chopper. Finally, the ground and dried seeds were stored in dark until use.

Chemicals and reagents

Analyses for total content of polyphenols were made using Folin-Ciocalteu phenol reagent (2N), gallic acid and anhydrous Na_2CO_3, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and methanol were obtained from Sigma. Ethanol-water solvents were prepared by using 96% ethanol obtained from Valerus.

Extraction procedure

Extraction from the plants is an empirical exercise in which different solvents are utilized under a variety of conditions such as time, liquid/solid ratio and temperature of extraction. The success of the extraction process depends on the most appropriate assay [20].

In order to simplify the extraction process and save time and costly reagents, we have developed a four-step experimental procedure for finding the conditions for obtaining maximally enriched extracts. In general, a sample of ground raw material (10 g) was mixed in a flask with corresponding amount of solvent (depending on solvent-to-solid ratio). The extractions were carried out in a thermostated water bath shaker (Gyrotory Water Bath Shaker, model G76, New Brunswick Scientific, USA) at 160 rpm. After extraction, the mixture was filtered, and the filtrate was collected and stored at 4^0C for analyses. Each test was repeated in duplicate or in triplicate in case of bigger difference between two analyses. Mean values were used.

Analyses

Analyses for total polyphenols and antioxidant activity of the extracts were made.

Determination of total phenolic content. Total polyphenolic content (TPC) of the extracts was determined by the Folin–Ciocalteu method [21,22] using UV-VIS spectrophotometer (UNICAM®-Helios β). The absorbance of samples was measured at 765 nm. TPC was expressed as mg of gallic acid equivalent (GAE) per 1 gram of dry extract (mg GAE/g de).

Determination of in vitro antioxidant capacity (AOC). AOC was determined by DPPH method [23,24]. The method is based on a color reaction between the nitrogen atom (from DPPH) and the hydrogen atom of a hydroxyl group of the antioxidant compound. 1 ml extract was mixed with 4 ml solution of DPPH in methanol (0.004%). After keeping the sample in dark at room temperature for 60 min, the absorbance was measured at 517 nm. AOC was expressed as IC50 (quantity of extract neutralizing 50% of DPPH amount).

Graphically antioxidant capacity is expressed as mg DPPH which is neutralized by the corresponding amount (grams) of dry extract.

Statistical treatment. One-way Analysis-of-Variances software (ANOVA, Microsoft) with significance level 0.05 was applied to the treatment of experimental data in order to distinguish statistically equal mean results as opposed to statistically different ones.

RESULTS AND DISCUSSION

As a result of the four-step experimental procedure, the following important process parameters were determined:

Step 1: Selection of appropriate concentration of a “green” solvent, which dissolves maximum amount of antioxidant compounds (polyphenols).

Usually, solvents as methanol, ethanol, acetone, ethyl acetate, etc., at different volume fractions in water have commonly been used for extraction of polyphenols from different plants [25-27]. In this research water, ethanol, and aqueous solutions of ethanol (30, 48, 70%) were used for the extraction of avocado polyphenols. In order to achieve complete extraction, high solvent-to-solid ratio (20:1), as well as high temperature and long contact time (70^{o}C and 120 min respectively), were applied.

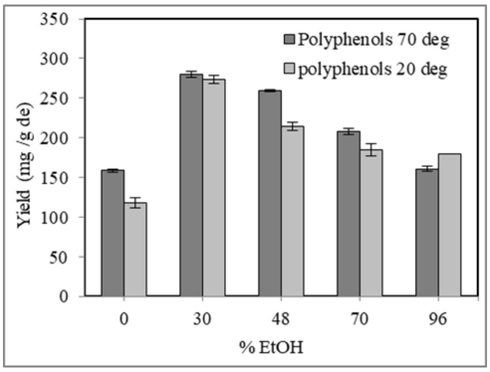

Fig. 1. Extraction yield at different solvent concentrations

From the results shown in Fig. 1, it is evident that an increase in the volume fraction of ethanol in the aqueous solutions does not have positive influence on the extraction efficiency. The yield of TPC has a maximum at 30 % ethanol and decreases afterwards with increasing percentage of ethanol. The results of ANOVA test confirm this conclusion.

Step 2: Determination of minimum solvent-to-solid material (hydromodule).

In determining the impact of the basic parameters on the extraction of target components at atmospheric pressure, the variation of hydromodule was investigated [28]. The aim was to minimize the amount of solvent, at which maximum amount of target components is derived. Experiments were performed at different ratios of solvent/solid phase: from 5/1 to 20/1. Our experience based on results of previous studies on plant materials extraction has shown that two hours of phase contact usually are more than sufficient to achieve equilibrium and to complete the mass-exchange process. For this reason, the extraction was conducted for two hours. The results are shown in Figure 2. According to them, at a ratio of 5/1 the amount of solvent is not sufficient for dissolving of all active components, while at a ratio of 8, 10, 12.5 and 20:1 the yield of extracted polyphenols is approximately equal – about 290 mg/g de. ANOVA test has also shown that hydromodules in the range 8 – 20 produce statistically equal yield. Consequently, from economic point of view, the most advantageous liquid-to-solid ratio is 8:1, which ensures high extraction at a lesser solvent quantity. Therefore, the following studies were conducted with this hydromodule.

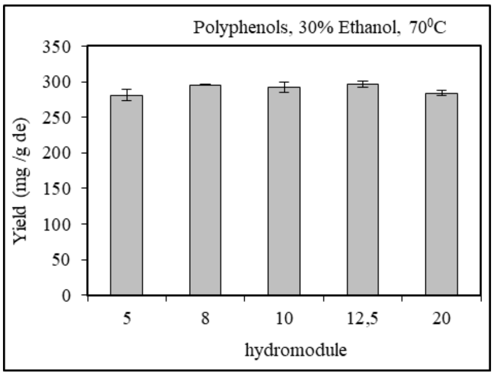

Fig. 2. Extraction yield at different hydromodules

Step 3: Determination of the process temperature:

Knowing in advance that higher temperature will probably lead to better solubility of the solid matter, our aim here was to check how the temperature affects the thermal stability of extracted antioxidant compounds [29,30]. The influence of temperature on the yield of polyphenols is shown in Figures 1 and 3. Three temperatures below the boiling temperature of the solvent were examined (20, 45 and 70^{O}C). From Fig. 1 it is seen that the yield at 70^{o}C is always higher than at 20^{o}C except for 96% ethanol. However, regarding the details in Fig. 3, which represents the temperature dependence of the yield for the optimal solvent concentration, it can be seen that at 70^{o}C the yield of the extracted polyphenols is significantly higher than that at 45^{o}C (14.5 %).

It is a sign for no temperature degradation of polyphenols. For this reason 70^{o}C has to be chosen as an appropriate process temperature. ANOVA analysis has shown that the results for 20 and 40ºC are statistically equal, while the yield at 70ºC is different from that obtained at other tested temperatures.

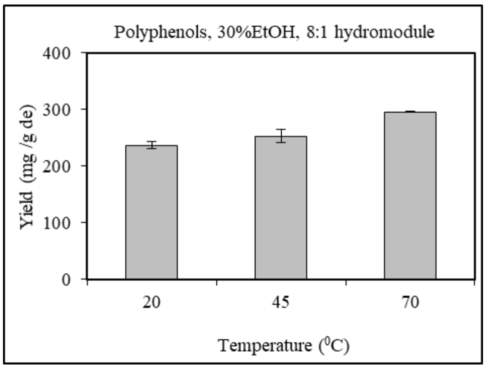

Fig. 3. Extraction yield under different temperature conditions

Step 4: Determination of minimum process duration necessary for extraction completion:

A) Kinetics of polyphenols extraction

The duration of the process necessary for maximizing the polyphenols yield was determined by a kinetic study tracking the evolution of the process over time. The results are presented in Figure 4. It is observed that after about 60 minutes, the extraction curve seems to reach a plateau, and no significant additional quantities are extracted afterwards (281.8 mg/g de for 60 min against 295.4 mg/g de for 120 min or 4.6 % more). This observation was confirmed by ANOVA test, which determined the group of results in the interval 60 – 120 min as statistically equal. Consequently, 60 minutes process duration can be selected as sufficient and optimal extraction time.

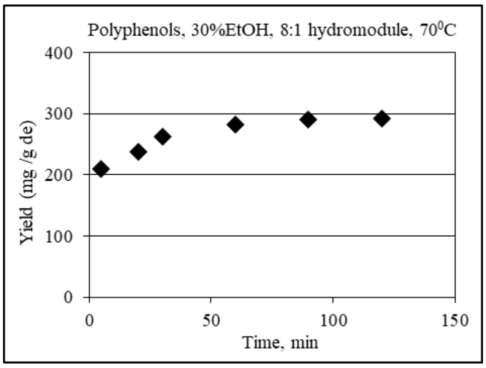

Fig. 4. Kinetics of polyphenols extraction

B) *In vitro* antioxidant capacity (AOC)

The phenolic compounds have an antioxidant effect due to the delivery of a hydrogen atom or an electron that stabilizes and neutralizes the free radicals, thus preventing their damaging oxidative action [31].

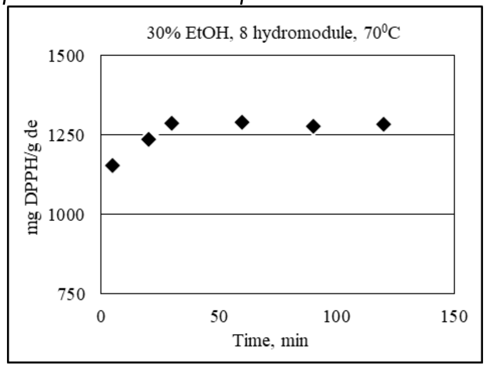

Fig. 5. AOC of the extracts obtained at optimized conditions

Figure 5 shows the time evolution of the antioxidant capacity (AOC) of the extracts obtained at optimal process parameters. According to ANOVA test, AOC values keep statistical equality after 30 minutes. However, in view of the fact that more polyphenols are extracted for 60 min than for 30 min, it is recommended to apply 60 minutes of extraction.

CONCLUSION

Four-step experimental procedure is developed for optimizing the extraction of bioactive substances from vegetables. It gives results with a low number of experiments (about 15). The method is applied to the extraction of antioxidant polyphenolic compounds from avocado waste (seeds), and the optimal processing conditions are determined as follows: 30% ethanol, 70^{0}C, solvent-to-solid material ratio 8, process duration 60 min. Generally, there is correlation between AOC values and the amount of extracted polyphenols. Extracts with higher polyphenols concentration possess higher antioxidant capacity.

The results are useful for development of technological schemes for production of antioxidant extracts for use as functional supplements obtained from bio-wastes.

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ОПТИМИЗИРАНЕ НА ЕКСТРАКЦИЯТА НА ПРИРОДНИ АНТИОКСИДАНТИ ОТ КОСТИЛКИ НА АВОКАДО

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(Резюме)

Хранителни добавки от растителен произход са широко разпространени за диетична употреба. Най-често те са под формата на екстракти, богати на природни антиоксиданти със силно радикал-прихващащо действие. Авокадото (*Persea americana*) е широко култивиран плод в целия свят. Има много изследвания, доказващи неговите ползи за здравето. Люспите и костилките на авокадото са отпадък. Има обаче индикации, че костилките на авокадото, които представляват около 23 % от теглото на плода, имат дори по-силно антиоксидантно действие от месестата му част. Целта на настоящата работа е да се изследва влиянието на параметрите на процеса върху екстракцията на костилки от авокадо с оглед да се определят оптималните условия за получаване на екстракти, съдържащи максимално количество биоактивни вещества и имащи висока антиоксидантна активност. Разработен е експериментален подход, даващ възможност за намиране на оптималните параметри на процеса посредством намален брой експерименти. В резултат са определени следните важни параметри на процеса: избор на подходящ „зелен“ разтворител, който да разтваря по-голям брой антиоксидантни съединения с минимално съотношение разтворител:твърдо вещество и минимална продължителност на процеса, необходима за пълна екстракция при минимална температура, с което минимизира разходите за процеса и осигурява приемлив компромис между по-високата разтворимост и термичната стабилност на антиоксидантните съединения. Получените резултати могат да се използват за разработване на технологични схеми за производство на антиоксидантни екстракти, които да се използват като функционални добавки, получени от био-отпадъци.