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Recovery of antioxidant phenolic compounds from avocado peels by solvent extraction

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The extraction of a vegetal waste (avocado peels) is studied aimed at quantification of polyphenolic content and antioxidant activity of the extracts. Ethanol-water mixtures are used as solvents for the reason that ethanol is the most common "green" solvent having high solubility power, and being completely biodegradable. The main task is to determine the optimal operational conditions, at which the extraction of antioxidant substances from avocado peels is maximized and correspondingly, extracts with higher antioxidant capacity are obtained. A simplified four-step experimental procedure is applied for optimization of the main process parameters, at which the yield of the target bioactive components (polyphenols) is maximized. The process kinetics is studied with the use of Peleg's equation, and model equilibrium concentration is calculated. The experimental concentration at equilibrium state fits well the model results (2.5 % difference).

Key words: Avocado peels, Extraction, Optimization, Antioxidants, Polyphenols

INTRODUCTION

There are two types of antioxidants used in the food, cosmetic and pharmaceutical industries: natural antioxidants obtained entirely from natural sources, and synthetic antioxidants created by chemical processes. In recent years, there is an increasing interest in natural (bio) antioxidant substances, because synthetic antioxidants are supposed to have toxic and mutagenic effects on the human body [1, 2]. Currently, the food industry is focused on replacing the use of synthetic by natural antioxidants [3].

The main source of natural antioxidants are plants such as fruits, vegetables, cereals, mushrooms, flowers, herbs and spices [4, 5]. Vitamins soluble in lipids and selenium also occur in food derived from animals (milk and fish lipids, eggs), but in smaller amounts, and in dependence on the kind of feed consumed (e. g. carotenoids content in milk lipids, eggs). For this reason, products derived from animals are not significant sources of antioxidants in human diet [5].

Due to considerable consumption and industrial processing of the edible parts of the plants, significant amount of biological waste is generated (such as citrus fruit skins, pineapple residues, avocado peels and seeds, sugarcane bagasse and other plant residues). One of the most beneficial approaches is to recover the bioactive constituents, especially the phenolic compounds, making full use of them in the food, pharmaceutical and cosmetics industry. Thus, utilization of fruit wastes as sources of bioactive compounds may be of considerable economic benefits and has become increasingly attractive [6-8].

With a global production exceeding 5.5 million tons in 2016, avocado has become a major agro-industrial commodity [9, 10]. The principal use of avocado fruit is for human consumption, although other applications related to the production of cosmetics, nutritional supplements and livestock feed have been reported [10, 11]. Only the avocado pulp is employed for commercial applications, while avocado peels and seeds are considered as waste [11, 12]. The antioxidant activity of avocado pulp and wastes has been evaluated in a number of studies [12-18]. It is reported that in view of higher polyphenolic content and antioxidant activity, the downward ranking is: peels, seeds, pulp [12, 15, 16].

A number of solvents have been used for recovery of polyphenols from avocado wastes: petroleum ether, water, methanol, 80% ethanol, acetone, ethyl acetate [12, 15-18]. Typically, the extraction has been made at selected constant conditions (solvent, solid-to solvent ratio, time and temperature) without variation of the process parameters, i.e. no attempts have been made to optimize the extraction process.

In this study, avocado peels, which are the richest part of avocado fruit, are examined as a source of antioxidant substances (polyphenols). The main task is to determine the optimal operational conditions at which their extraction from avocado peels is maximized and correspondingly, extracts with higher antioxidant capacity are obtained. Ethanol-water mixtures are used in our study for the reason that ethanol is the most common "green" bio-solvent

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used on a large scale, because it is easily available, it has high solubility power, and is completely biodegradable [19].

EXPERIMENTAL

Raw material

Avocado fruits were purchased from the local market. The peels were separated from the fruits. After air drying they were ground to particle size not bigger than 1 mm by using a chopper. Finally, the dried and ground peels were stored in a dark place until use.

Chemicals and reagents

Standard Folin-Ciocalteu phenol reagent (2N), gallic acid, 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

In general, a sample of ground raw material (5 g) was mixed in a flask with a corresponding amount of solvent (depending on solvent-to-solid ratio). The extractions were carried out in a thermostatic 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 stored in a refrigerator. Each extraction was repeated in duplicate or in triplicate in case of bigger difference between two analyses. Mean values were used.

Samples of liquid extracts were dried and weighed in order to obtain the quantity of extracted dry matter (de)

Analyses

Determination of total phenolic content. Total polyphenolic content (TPC) of the extracts was determined by the Folin-Ciocalteu method [20, 21] using UV-VIS spectrophotometer (UNICAM®-Helios β). The absorbance of the samples was measured at 765 nm. Calibration curve for gallic acid was made, and TPC was expressed as mg of gallic acid equivalent (GAE) per 1 gram of dry extract (mg GAE/g de).

In vitro antioxidant capacity (AOC). AOC was determined by the DPPH method [22, 23], based on a color reaction between the nitrogen atom (from DPPH) and the hydrogen atom of the hydroxyl group of the antioxidant compound. 1 ml of extract was mixed with 4 ml of DPPH solution 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).

Usually, AOC is expressed as extract concentration (mg/ml), which neutralizes 50% of a standard DPPH solution (IC50 value). In this presentation, a lower IC50 value means a higher AOC. In order to obtain a more logic presentation, we have recalculated and represented the values of IC50 as mg DPPH neutralized by 1 g of dry extract. In this case, a higher value refers to a higher AOC.

Statistical treatment

One-way Analysis-of-Variances software (ANOVA, Microsoft) with a significance level of 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

To simplify the experimental procedure in view of saving reagents and time, we have applied a four-step experimental approach for finding the operational conditions at which maximum bioactive components (polyphenols) are extracted.

Determination of appropriate solvent composition

The target substances might have different polarity, which plays on the selection of an appropriate solvent (polar or non-polar). In our case, as polyphenol compounds are mainly of polar type, they can be successfully extracted by polar solvents, such as water, methanol, ethanol, acetone, etc. [15]. As the extracts are intended for human applications, safe “green” solvents should be used. In this study, water (polarity 1.84 D) and ethanol (1.69 D) were chosen as being safe. The solvent polarity can be additionally tuned by mixing these solvents in different proportions.

At this initial stage, the optimal process parameters are not known, so it is convenient to proceed at conditions, which will ensure better solubility of the target substances resulting in a complete extraction. These are: high temperature, excessive amount of solvent (high solvent-to-solid ratio), long contact time, agitation.

The concentration of polyphenols in the extracts obtained by water, concentrated ethanol and different ethanol-in-water mixtures, is presented in Fig. 1a. Fig. 1b shows analogous results for the antioxidant capacity. The other constant process parameters are: temperature 70^{o}C (close to solvent boiling point), solvent-to-solid ratio 20, contact time 150 min under agitation.

According to Fig.1a, a clear maximum of extracted polyphenols is obtained with 48%

ethanolic solvent. The concentrated ethanol seems to be the worst solvent in our case study, which dissolves even less polyphenols than pure water.

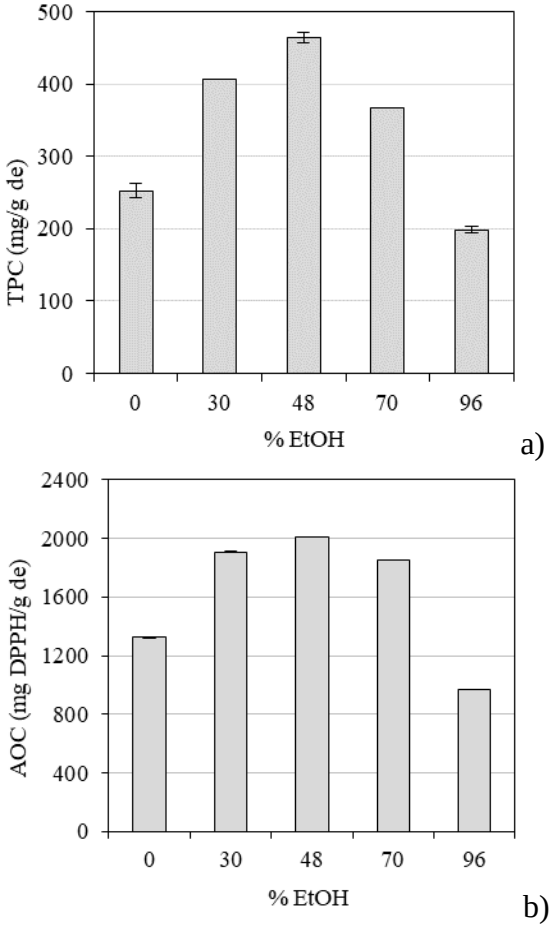

Fig. 1. Influence of ethanol concentration on: a) the concentration of polyphenols TPC); b) the antioxidant capacity (AOC) of the extracts

Similar is the situation with the antioxidant capacity (Fig. 1b) – maximum at 48% ethanol, lowest at 96% ethanol. Evident correlation exists between the amount of polyphenols and antioxidant capacity. A higher polyphenol concentration corresponds to a higher antioxidant capacity of the extracts.

Fig. 2 presents the amount of polyphenols (TPC) extracted from the raw material using different ethanol concentrations.

Again, best results were obtained with 48% ethanol. An interpolation of this graph for 80% ethanol (rectangular point) gives the value 66.77 mg/g rm. It is quite similar to the result 63.5 of Tremocoldi *et al.* [18] obtained with the same ethanolic concentration.

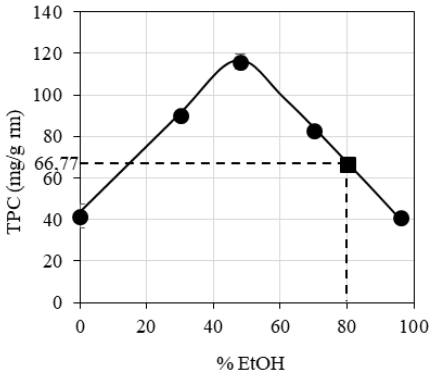

Fig. 2. Influence of ethanol concentration on TPC extracted from the raw material (rm)

With the optimal ethanol concentration 48%, both water-soluble and ethanol-soluble antioxidants were extracted at a maximum grade. Consequently, further experiments have to be carried out with this solvent composition.

Selecting a suitable temperature

Usually, the solubility of solids in a solvent is expected to be better at a higher temperature. From practical point of view, the influence of temperature should be studied in the range from room to solvent boiling point temperature (in our case about 70^0C). However, in case of thermally unstable substances, a high temperature can give rise to their destruction, and this possibility should be inspected. Figs. 3a and 3b illustrate the influence of temperature on the polyphenols content and on the antioxidant capacity of the extracts. The other process parameters keep constant values, namely 48% ethanolic solvent, solvent-to-solid ratio 20, contact time 150 min under agitation.

When the temperature is increased from 20 to 70°C, the polyphenol content shows a proportional rise, too. This fact can be interpreted as an evidence for better dissolution and for thermal stability of the active substances even in the upper temperature range. Similar observation is valid for antioxidant capacity (Fig.2b) – it grows with increasing temperature.

Based on the above results, the temperature selected for further experiments was 70^0C.

Influence of hydromodule on TPC and AOC

If the amount of solvent is insufficient, the target compounds cannot be fully dissolved. To avoid this situation, an excessive amount of solvent is often used in practice. Acting in this empirical way raises the expenses for the larger amount of solvent in use, more energy is spent for its regeneration and elimination from the extract, and reactors with larger volume are needed as well. There is some minimum

amount of solvent necessary for full dissolution of the target. It can be determined by making extractions with gradual increase of solvent-to-solid ratio, registering the minimum ratio at which the yield becomes constant and does not depend on further ratio increase.

Figs. 4a and 4b illustrate the impact of hydromodule on polyphenols concentration and AOC of the extracts. Some process parameters are kept at already optimized values, namely 48% ethanolic solvent and temperature 70°C. The solid-liquid system is agitated for a long time (150 min).

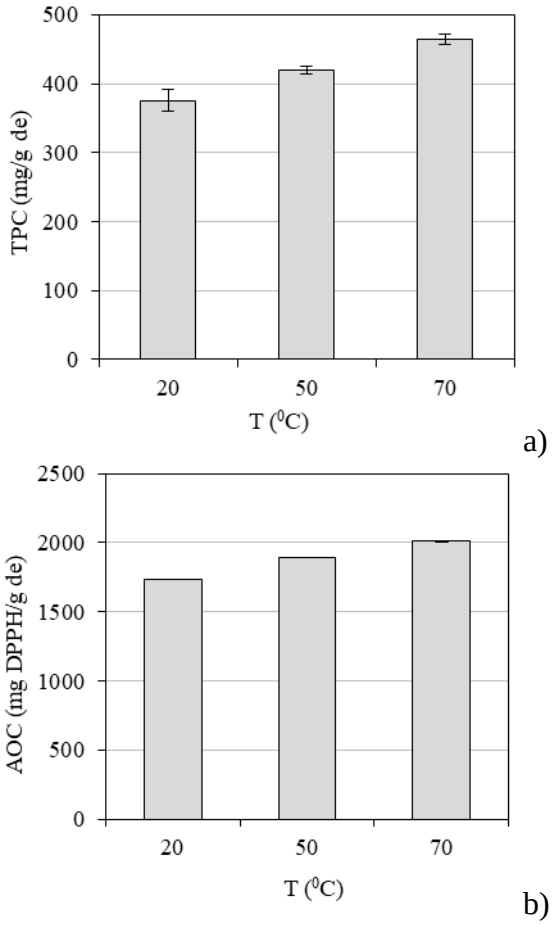

Fig. 3. Influence of temperature on TPC (a) and on AOC (b)

As can be seen from Fig. 4a, increasing the solvent amount from hydromodule 5 to 20 results in increased amount of extracted polyphenols. It is an indication that more solvent is necessary for complete dissolution of the target. Unlikely, at hydromodule 20 and more, the quantity of extracted polyphenols stays almost the same; i. e. further increase of hydromodule does not improve the extraction. So, a hydromodule of 20 can be selected as the minimum value of solvent-to-solid ratio required for complete recovery of the valuable components and for obtaining extracts with maximum AOC (Fig. 4b). ANOVA analysis confirms this observation by indicating statistically different values of TPC in the interval 5 – 20 and statistically similar results for hydromodule 20 and 25. It is worth mentioning that the relevant literature usually reports a solvent-to-solid ratio for extraction of avocado peels of 10, which seems insufficient in view of our results.

Process kinetics and determination of optimal contact time

Establishing the duration of phase contact required for achieving pseudo-equilibrium state is essential for optimizing the extraction process. The necessary contact time can be determined by tracking the process development in the course of time. The resulting kinetic curve has asymptotic shape, the plateau being an indication for no further extraction (pseudo-equilibrium state is attained). So, the optimized (minimum) processing time corresponds to the point where the plateau begins. In our case, pseudo-equilibrium state seems to be reached after 20 min (Fig. 5), as far as ANOVA test has shown statistically equal values of TPC in the interval 20 – 120 min.

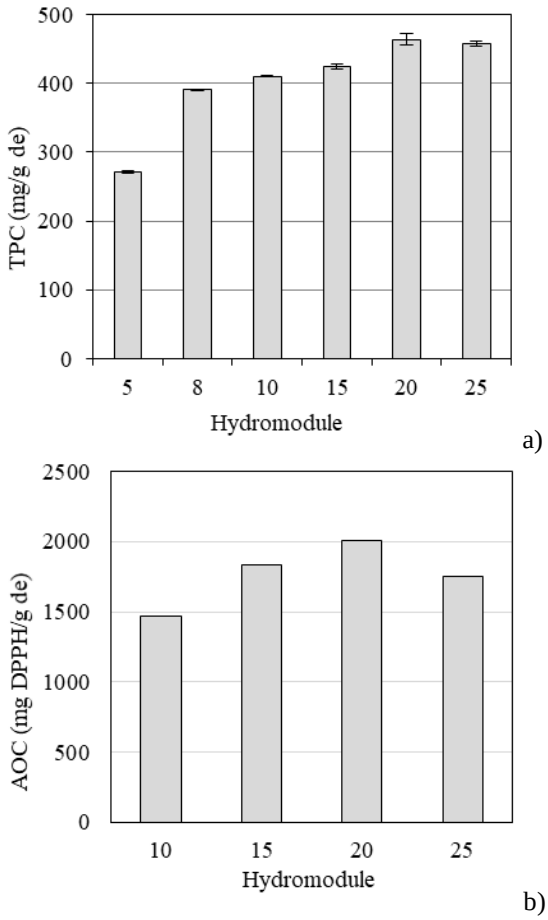

Fig. 4. Influence of hydromodule on TPC (a) and on AOC (b)

Modeling of extraction kinetics

Modeling was performed by using Peleg's equation. Although it is originally used to describe absorption kinetics [24], it can also be applied to the

extraction kinetic curves (extracted matter over time), because both curves have similar asymptotic shape.

The Peleg`s equation has the form:

$$C(t) = C_0 + \frac{t}{K_1 t + K_2} \quad (1)$$

In case of an extraction process, $C(t)$ is the concentration of extracted substance (mg/g de) at time t (min), C_0 is the concentration of extracted substance at the initial time t = 0, K_1 and K_2 are constants.

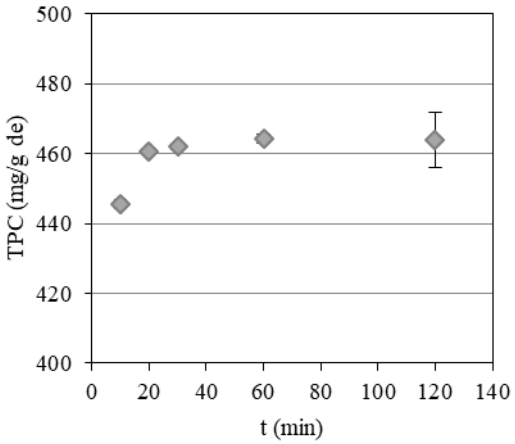

Fig. 5. Extraction kinetics of TPC of extracts obtained with 48 % ethanol, temperature 70°C, hydromodule 20

Since C_0 in all experimental runs is zero, Eq. (1) can be rewritten as:

$$C(t) = \frac{t}{K_1 t + K_2} \quad (2)$$

Eq. (2) can be rearranged in linear form:

$$\frac{t}{C(t)} = K_1 t + K_2 \quad (3)$$

Thus, K_1 and K_2 can be determined from the intercept and the slope of this straight line.

The extraction rate at time t can be obtained by differentiation of (2):

$$\frac{dC(t)}{dt} = \frac{K_2}{(K_1 t + K_2)^2} \quad (4)$$

At time t = 0, Eq. (4) takes the form:

$$\frac{dC(0)}{dt} = \frac{1}{K_2} = R_0 \quad (5)$$

So, the physical meaning of K_2 is related to the initial extraction rate R_0.

When $t \rightarrow \infty$, i.e. at equilibrium state, Eq. (2) becomes:

$$C(t)\,|_{t\rightarrow\infty} = C_e = \frac{1}{K_1} \quad (6)$$

Thus, the constant K_1 is related to the concentration at equilibrium state Ce.

Fig. 6 represents the linear form of Peleg's equation described by the expression:

$$y = 0.0021x + 0.0006 \quad (7)$$

The coefficients K_1=0.0021 and K_2=0.0006 serve to calculate the velocity and equilibrium constants, namely initial extraction rate R_0 = 1666.67 (mg GAE/g de min) and model equilibrium concentration C_e = 476.19 (mg GAE/g de).

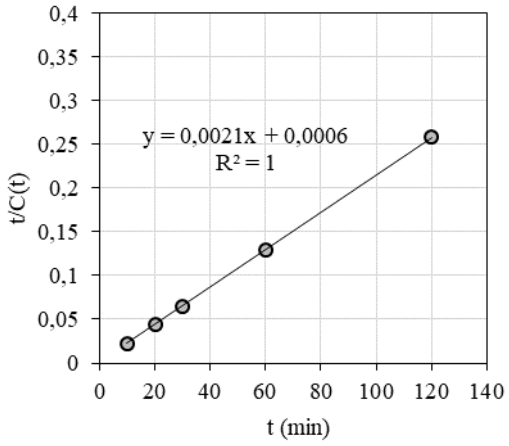

Fig. 6. Linear form of Peleg`s equation for TPC

Fig. 7 represents equation (2) (line) superimposed to real experimental data for the extraction kinetics of polyphenols (points). Good match is observed. Also, the values of calculated equilibrium concentration of polyphenols 476.19 (mg GAE/g de) and the experimentally determined equilibrium concentration from Fig. 5 (mean value 463,37) coincide rather well, the difference being only 2.5 %.

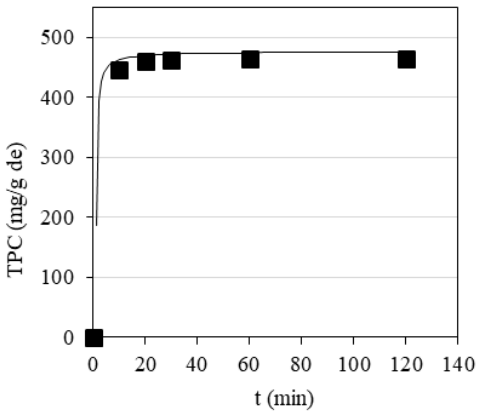

Fig. 7. Equation (7) confronted to real experimental data for extraction kinetics of polyphenols.

CONCLUSION

In view of valorization of food bio-wastes, the extraction of antioxidant phenolic substances from avocado peels with a "green" solvent (aqueous ethanol) was studied. Process optimization was

made using a simplified approach with a reduced number of experiments. It was found that the main process parameters, at which the yield and antioxidant activity of the extracts are maximized, are: extraction under agitation for 20 min with 48 % ethanol at temperature 70°C and solvent-to-solid ratio (v/w) 20.

The experimental data for process kinetics were treated by Peleg's equation, and model equilibrium concentration was determined. The experimental pseudo-equilibrium concentration matches closely the calculated equilibrium concentration (2.5 % difference).

In conclusion, an unused agricultural waste, avocado peels, was found to be rich of phenolic compounds. The results of this study specified the conditions for optimal processing in view of production of enriched extracts with higher antioxidant activity.

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ИЗВЛИЧАНЕ НА АНТИОКСИДАНТНИ ФЕНОЛНИ СЪЕДИНЕНИЯ ОТ ОБЕЛКИ НА АВОКАДО ЧРЕЗ ТЕЧНА ЕКСТРАКЦИЯ

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(Резюме)

Екстракцията на растителен отпадък (обелки от авокадо) е изучена с цел количествено определяне на съдържанието на полифеноли и антиоксидантната активност на екстракта. Водно-етанолни смеси са използвани като разтворител, тъй като етанолът е най-широко разпространеният „зелен" разтворител с висока разтваряща способност и е напълно биоразградим. Основната задача е да се определят оптималните условия на процедурата, при които екстракцията на антиоксидантни вещества от обелките на авокадо е максимална и се получава екстракт с максимален антиоксидантен капацитет. Четиристепенна експериментална процедура е използвана за оптимизиране на основните параметри на процеса, при които добивът на целевите биоактивни компоненти (полифеноли) е максимален. Кинетиката на процеса е изучена с помощта на уравнението на Peleg и е изчислена моделната равновесна концентрация. Експерименталната концентрация в равновесно състояние съвпада много добре с резултатите от модела (2.5 % разлика).