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Intensification of extraction of bioactive substances from artichoke wastes

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Artichoke (Cynara scolymus L.) is a traditionally cultivated and highly appreciated food in the Mediterranean region. The edible parts are only the heart and inner leaves (bracts). The external bracts, leaves and stems are non-edible by-products considered as wastes. They represent about 60% of the plant, and could be used as raw material for production of useful substances. This article examines potential intensification of the process of extraction of bioactive substances from artichoke wastes through the application of a widely used production process – conventional batch extraction. The target components to be extracted are compounds with high antioxidant activity - polyphenols and flavonoids. The study results in selection of optimal operational parameters that intensify the mass transfer and produce maximum yield of bioactive substances while minimizing the processing costs.

Keywords: artichoke, batch extraction, process intensification, polyphenols, antioxidants

INTRODUCTION

Artichoke *(Cynara cardunculus L. var. Scolymus (L.) Fiori)* is an ancient herbaceous perennial plant, originating from the Mediterranean area, which today is widely cultivated all over the world. Artichoke has been known since 4th century B.C. as a food and remedy, and has been recognized for its healing effects on hepato-biliary diseases and as a digestive aid [1]. In South European countries, the globe artichoke plays an important economic role where its production represents 45% of world production, of which 550 kt are produced in Italy, 200 kt in Spain, and 55 kt in France in 2013 [2]. Only the inner leaves (bracts) and heart of this vegetable are considered edible, whereas external bracts, leaves and stems are non-edible by-products which represent about 60% of the plant. Numerous studies have focused on its beneficial health and antioxidant properties. It appears that they could be related to the phenolic compounds, also known as polyphenols, mainly composed of mono- and dicaffeoylquinic acids and flavonoids. These properties are consistent with a well-known dual role of phenolic compounds as antioxidants and as substrates for oxidative browning reactions, mainly in the presence of high iron concentrations [3-6].

Polyphenols are a class of plant secondary metabolites characterized by the presence of a hydroxyl group attached to a benzene ring or to more complex aromatic structures. Humans cannot synthesize polyphenols and therefore have to rely on their intake to meet dietary needs. This has led to campaigns for promoting the consumption of fruit and vegetables as well as to the idea of enriching common foods with phenolic compounds [7].

Dietary plant polyphenols have received attention for their significant biological functions as antioxidants, anticarcinogens or antimutagens, which have led to their recognition as potential nutraceuticals [8]. In the gastrointestinal tract the polyphenol antioxidant compounds can protect nutrients, such as proteins, lipids and vitamins, from oxidation. In addition, they can also interact with proteins, protecting them from precipitation and loss of nutritional value, enzymatic activity, and other biological effects. It is confirmed that polyphenols have many potential bioactivities in the human body due to interactions with other macromolecules [9].

The extraction of substances from solids using a solvent is applied in many chemical, biotechnological, and pharmaceutical processes for extraction of valuable ingredients. It is the first and important step in isolating and purifying bioactive components from plant materials. The extraction efficiency is influenced by several factors, such as the type and concentration of solvent, solid–solvent ratio, time, temperature, pH, etc. However, in most studies the influence of a single factor has been explained while the interactions among factors have not been examined thoroughly [10]. Prospective and future trends in production processes are indicating new strategies for reducing expenses (energy, time, reprocessing, etc.) without affecting product quality. One such strategy is process intensification: a systematic organization to boost and enhance the mass transfer, optimizing in parallel the use of chemicals and energy, expenses or other benefits through development of efficient techno-

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economical systems [11].

The aim of this article is to determine optimal operational conditions for conventional batch extraction of artichoke wastes (stems and leaves) that intensify the mass transfer and produce maximum yield at reduced solvent consumption and shorter processing time. The target components to be extracted are compounds with high antioxidant activity - polyphenols and flavonoids.

EXPERIMENTAL

Plant material

Stems and leaves of artichoke cultivated in the region of Troyan, Bulgaria (vintage 2014) were used as a raw material. Dry stems and leaves were ground in a household electric grinder, sieved (fraction less than 1 mm was used) and stored in a cold and dark place.

Extraction protocol

A sample of ground raw material (10 g) was mixed in a flask with solvent (ethanol-water mixtures containing 0, 20, 35, 50, 70, 96% ethanol). The extractions were carried out in a thermostatic water bath shaker (Gyrotory Water Bath Shaker, model G76, New Brunswick Scientrific, USA) at 160 rpm. The influence of the main process parameters on the yield was determined by performing extractions with different solvents at different temperatures and different solvent-to-solid ratio (hydromodule). Long contact time was applied in order to await the process completion. The development of the process over time (extraction kinetics) was observed by making parallel extractions of identical samples, each one analyzed after different contact time. 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 big variances between two analyses. Mean values were used. The range of deviation of parallel experiments was 2.5 -7.5 %. Generally, the deviation was greater for smaller measured values.

Single factor ANOVA statistical test was applied to the kinetic results in order to determine whether they are statistically equal (null hypothesis) or significantly different one from another.

Chemicals and reagents

Analyses for total content of polyphenols, flavonoids and antioxidant capacity were made using Folin-Ciocalteu reagent (2N), quercetin, gallic acid, anhydrous Na_2CO_3, DPPH*, methanol and $AlCl_3$ (Sigma). Ethanol-water solvents were prepared by using 96% ethanol (Valerus) in different proportions.

Analyses

1. Determination of total phenolic content

Total polyphenolic content (TPC) of the extracts was determined by the Folin–Ciocalteu method [12] 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 raw material (mg GAE/g rm).

2. Determination of total flavonoids content

The total flavonoids content (TFC) was determined using aluminum chloride colorimetric assay [13]. Absorbance was measured at 420 nm and results were expressed as mg of quercetin equivalents (QE) per one gram of raw material (mg QE/g rm).

3. Antioxidant capacity (AOC)

AOC is determined as described by Brand-Williams [14]. The method is based on a color reaction between the nitrogen atom (from DPPH) and the hydrogen atom of 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 60 min in the dark at room temperature, 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 (one gram) of dry extract.

4. Dry extract yield

It was determined by drying and weighting of liquid extracts at mild temperature until stagnant weight is achieved. Analytical balance Sartorius with 0.1 mg precision was used.

RESULTS

Solid-liquid batch extraction

The mass transfer in solid-solvent systems is affected by several parameters, the main among them being solvent type and concentration, solid/liquid ratio, and temperature. In order to define appropriate conditions the influence of the main parameters has to be studied to select the optimal combination resulting in highest process efficiency. In this study, the process efficiency is quantified by the mass of extracted substance from unit of raw material and by the concentration of target substance in the extract. Higher values indicate higher process efficiency.

The selection of optimal operational parameters is made by applying a four-step experimental approach. Initially, an appropriate solvent has to be used. As known, the extraction of bioactive substances from plants is highly influenced by the solvent nature. Polar solvents such as water and ethanol are widely recognized as adequate for extraction of antioxidant phenolic compounds, which are also of polar type [15]. Consequently, ethanol and water in pure state as well as their mixtures were used as solvents in this study.

The first step was to determine the best solvent concentration. It was done by making extractions with a number of ethanol/water mixtures (0, 20, 35, 50, 70, 96% ethanol) at 70°C near to the ethanol boiling point, which is supposed to ensure better solubility. Abundant solvent was applied (liquid-solid ratio at least 20:1) in order to ensure sufficient amount of liquid phase for good wetting of the solid and for dissolving all extractable matter. Agitation (160 rpm) and long contact time (2 hours) were applied to provide good phase mixing and enough time for attaining pseudo-equilibrium state, at which the mass transfer process should practically complete.

Table 1. Yields of substances extracted with different solvent concentrations

% ethanol	TPC		TFC		Yield of dry extract
	(mg GAE/g rm)	(mg GAE/g de)	(mg QE/g rm)	(mg QE/g de)	(mg de/g rm)
0	12.4	36.6	2.4	7.2	320.0
20	13.3	41.0	2.9	9.1	323.6
35	14.8	45.7	3.2	9.8	324.5
50	16.3	56.00	4.0	11.5	336.9
70	15.6	56.5	4.1	14.9	275.5
96	8.5	46.1	2.8	15.1	183.9

TPC – total polyphenolic content; TFC – total flavonoids content; GA– acid equivalent; QE – quercetin equivalent; rm– raw material; de – dry extract

According to Table 1, maximum yield of total extract and polyphenols is obtained with 50% ethanol. Regarding extracted flavonoids, they are better extracted with more concentrated ethanol. However, they are in minor quantity compared with total polyphenols and do not contribute significantly to the total concentration of the antioxidant substances. Also, yield of polyphenols is the same at 50% and 70% ethanol. Thus, a cheaper 50% ethanol solvent was chosen for further experiments.

Concerning the total extracted mass (yield of dry extract), it is the same up to 50% ethanol and decreases afterwards i.e. water is a better solvent than an ethanolic solvent with concentration more than 50% ethanol.

Table 2. Extraction yield at different temperature

Temp (°C)	TPC		TFC		Yield of dry extract
	(mg GAE/g rm)	(mg GAE/g de)	(mg QE/g rm)	(mg QE/g de)	(mg de/g rm)
20	12.7	46.0	2.6	9.3	276.1
50	14.8	50.2	3.2	11.0	295.9
70	16.3	56.0	4.0	11.5	336.9

The second step was to identify the appropriate temperature. In general, higher temperature will increase the solubility. However, some substances might be thermally unstable, which will be manifested by lower yield at higher temperature indicating that substance degradation exists. Three temperatures have been tested: 20, 50 and 70°C, and the results are depicted in Table 2.

As it is seen, the extraction yield becomes better with higher temperature. Consequently, temperature of 70°C is selected as it results in more intensive mass transfer and highest yield.

The third step is to identify the appropriate liquid-to-solid ratio. In the previous experiments, high ratio was used to ensure sufficient amount of unsaturated solvent to dissolve all extractable matter. However, operation with excessive amount of solvent generates additional expenses for heating, evaporation, recovery and recycling and thus optimization is appropriate.

Extractions at different hydromodules were performed (Fig.1). The other process parameters were tuned at their optimal values (50% ethanolic solvent, temperature 70°C). Again, long contact time (2 hours) was applied in order to ensure equilibrium state.

The results in Fig.1 show that the polyphenols and flavonoids concentration in the dry extracts

rises up to hydromodule 10, while addition of more solvent beyond that does not bring improvement. Therefore, to maximize the active components content with minimum costs, the optimal choice is solvent-to-solid ratio equal to 10.

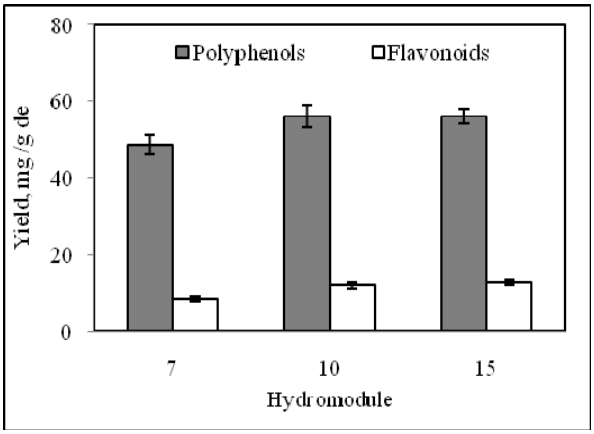

Fig.1. Influence of hydromodule

The fourth step is to determine the minimum time necessary to complete the process. As noted, in all previous experimental runs the phase contact was prolonged to ensure equilibrium state. A long contact time might have some disadvantages, such as: longer and more expensive technological process; increased probability for thermal destruction of unstable active components because of longer exposition at high temperature; unnecessary occupation of reactors, which could be used for production of other goods, etc. Optimization can be done by examination of the process development over time. Extractions were carried out for different contact time at the established optimal process parameters, the obtained yields being plotted against extraction time. The resulting kinetic curves are shown in Fig.2.

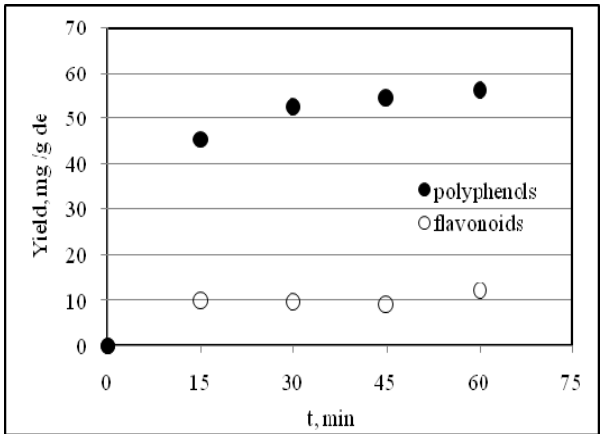

Fig.2. Extraction kinetics

Important increase of the amount of extracted major substances (polyphenols) is observed during the initial period from 0 to about 30 minutes contact time. If the process is run for longer time, minor and insignificant additional amount is extracted (about 3.5%). Thus, further processing might be useless as it does not materially improve the yield, and the processing cost might be higher than the cost of the additional product.

The choice of contact time for polyphenols extraction is justified also by the results of single factor ANOVA tests with significance level 0.05. Statistically, similar (not significantly different) yields of polyphenols are obtained in the interval 30-60 min, i.e. no need to prolong the contact time for more than 30 min.

Antioxidant capacity (AOC)

Fig.3 illustrates the antioxidant capacity of extracts obtained for different contact time at optimal conditions.

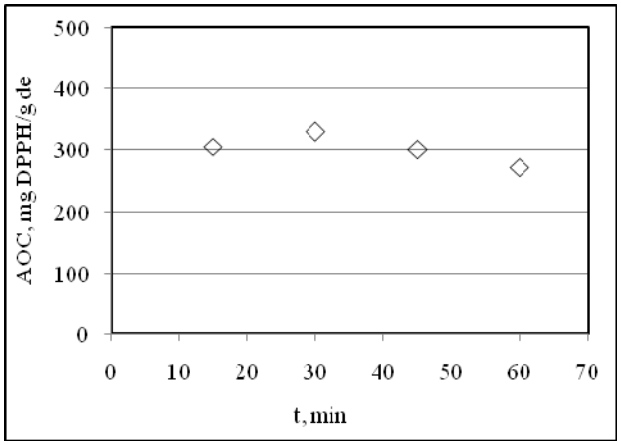

Fig.3. Antioxidant capacity

It is seen that 15-30 min processing results in extracts with maximum AOC. The ANOVA test shows statistically similar values of AOC in the range 15-60 min, which determines 15 min contact as the optimal choice for maximizing AOC. However, in view of the whole extraction process, it seems better to recommend 30 min process duration, because the extract will contain more polyphenols (see Fig.2), although the AOC does not increase proportionally to polyphenol content.

Some decrease of AOC is observed at prolonged contact (more than 30 min). As the yield of polyphenols and flavonoids is not reduced (see Fig.2), the lower AOC can probably be assigned to thermal destruction of non-phenolic antioxidant compounds.

CONCLUSIONS

The extraction of some bioactive compounds (polyphenols and flavonoids) from artichoke wastes was studied with the aim to intensify the process. Operational conditions were determined, at which the mass transfer was improved and yield maximized while minimizing processing costs. In

summary, the optimal operational conditions for the particular case studied here are:

- Solvent composition – 50% ethanol;
- Processing temperature 70°C;
- Solvent-to-solid ratio 10;
- Processing time 30 minutes.

They can be used as basic process parameters necessary for development of technological schemes for production of extracts from artichoke wastes.

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