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Effect of Solvent Type and Extraction Conditions on the Recovery of Phenolic Compounds from Artichoke Waste

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The recovery of phenolic compounds from the two main components of artichoke waste, the outer bracts and the stems, was investigated. The total polyphenol content, expressed as gallic acid equivalents (GAE) per g of dry weight, was 24.14 ± 0.73 mg GAE/g for bracts and 35.71 ± 1.65 mg GAE/g for stems. Different solvents, including acetone, ethanol, hexane, ethyl lactate, water and 50:50 ethanol–water mixture, were preliminarily screened for their extraction efficiency. Aqueous ethanol was the most effective solvent and was used to assess the effects of temperature (T = 40–60 °C), extraction time (E = 30 – 90 min) and liquid-to-solid ratio (R = 10 –30 mL g^{-1}) on the extraction yields. Under the best conditions (T = 60 °C, E = 90 min, R = 30 mL g^{-1}), the amount of phenolics recovered from bracts and stems were 19.44 and 28.02 mg GAE/g, respectively. For both materials, E was the most influential factor, followed by T and R. Overall, the results obtained support the use of artichoke waste as a source of phenolic antioxidants and give useful directions on how to improve recovery by proper selection of extraction conditions.

1. Introduction

Globe artichoke (*Cynara scolymus* L.) is a perennial plant originating from the Mediterranean region and grown for its edible flower buds. Italy is the world's largest producer of artichokes, with an annual production of about 400,000 t (FAOSTAT, 2012). During the industrial processing of artichokes, about 80 – 85 % of the total plant biomass is discarded and turned into a solid waste. This material consists mainly of the stems and the external parts of the flowers, commonly known as bracts.

In recent years, following the general trend towards the reuse of agro-industrial wastes, attempts have been made to find a use for this waste. The production of biofuels (Fabbri et al., 2014), the extraction of high-molecular-weight inulin (López-Molina et al., 2005) and the recovery of peroxidase to remove phenolic pollutants from wastewater (Sergio et al., 2010) are some of the proposed applications.

Examination of the composition of artichoke waste reveals that it is a rich source of polyphenols, a class of plant secondary metabolites with a wide range of bioactivities, including antioxidant, anticancer and anti-inflammatory properties (Padney and Rizvi, 2009). The major polyphenols in artichoke are caffeoylquinic acids, a group of esters of quinic and caffeic acids, but many other bioactives, such as luteolin and apigenin derivatives, are also present (Pandino et al., 2011). According to Negro et al. (2012), chlorogenic acid, cynarin (1,5-dicaffeoylquinic acid), luteolin 7-O-rutinoside and luteolin 7-O-glucoside are the predominant polyphenols in bracts and stems. Studies have also shown that the amount of polyphenols in artichokes may vary significantly with developmental stage and genotype, and that they tend to accumulate preferentially in different plant parts (Pandino et al., 2013). However, despite the presence of important health-promoting compounds, little attention has so far been devoted to their recovery from artichoke waste. In particular, studies on the effects of solvent type and process conditions on the extraction yields are currently lacking.

In this contribution we have investigated the feasibility of recovering phenolic compounds from artichoke waste by a simple and environmentally friendly solvent-extraction procedure. Different solvents were preliminarily screened for their ability to extract polyphenols. The solvent with the most suitable properties was then used to perform a systematic study aimed at assessing the effects of the main process parameters on the recovery of polyphenols. To highlight matrix effects and/or possible differences in

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extraction efficiency, the main components of artichoke waste, the bracts and the stems, were individually tested and characterized.

2. Experimental

2.1 Chemicals and plant material

Acetone, ethanol, hexane, hydrochloric acid and sodium carbonate were obtained from Carlo Erba (Milano, Italy). Gallic acid (3,4,5-trihydroxybenzoic acid), ethyl lactate and the Folin-Ciocalteu reagent were from Sigma-Aldrich Co. (St. Louis, Mo, USA). All chemicals were reagent grade and used without further purification. Aqueous solutions were prepared with deionized water.

Fresh artichokes were purchased from a local market. External bracts and stems were removed by a knife and chopped into small pieces. Then they were blanched in water (85 °C for 15 min) in order to inactivate polyphenol oxidase, the enzyme responsible for oxidation of polyphenols. The thermally treated plant material was dried to a final moisture content of about 6 % in a forced-air dehydrator (Stöckli, Switzerland) operated at 50 °C, milled with an electric grinder (Moulinex, Italy) and stored at room temperature.

2.2 Analytical methods

Moisture content was measured by an electronic moisture analyzer (model MAC 50/1, Radwag, Poland). A three-stage extraction procedure was used to evaluate the initial phenolic content of the waste material (Panusa et al., 2013). Total phenolics were determined by the Folin-Ciocalteu method. The results were expressed as gallic acid equivalents (GAE) per unit weight of dry solid using a calibration curve obtained with gallic acid standards.

2.3 Solvent screening

Solvent screening was performed by contacting 1 g of artichoke bracts or stems with 100 mL of solvent in magnetically stirred and thermostated (25 ± 0.1 °C) screw-cap flasks. After 1-h extraction, a sample of the liquid was withdrawn, passed through a 45 μm nylon filter and assayed for total phenolics. The solvents investigated were: acetone, ethanol, water, hexane, ethyl lactate and the 50:50 ethanol–water mixture.

2.4 Study of the recovery process

The recovery of phenolic compounds from artichoke waste was investigated using aqueous ethanol (50 % v/v) as extraction solvent. Extraction experiments were carried out in batch mode following the procedure described elsewhere (Zuorro and Lavecchia, 2011).

A full-factorial design was used to evaluate the effects of temperature (T), extraction time (E) and liquid-to-solid ratio (R), on the recovery of polyphenols from the plant material. The levels of each factor were chosen to cover a range of values of practical interest and are reported, in natural and coded values, in Table 1. Coded values were obtained using the following equations:

$$x_1 = \frac{T - 50}{10} \tag{1}$$

$$x_2 = \frac{E - 60}{30} \tag{2}$$

$$x_3 = \frac{R - 20}{10} \tag{3}$$

Four center-point replicates were added to estimate the experimental error, for a total of $2^3 + 4 = 12$ runs (Table 2). They were conducted randomly to minimize the effects of uncontrolled factors. The extraction yield of phenolic compounds (y), expressed as the amount of extracted phenolics per unit weight of dry solid, was used as the response variable. The design of experiments was performed using the statistical software Minitab®, version 16.2.3 (Minitab Inc., PA, USA).

Table 1: Natural and coded levels of the factors included in the experimental design

Factor	Level			Unit
	–1	0	+1	
Temperature (T)	40	50	60	°C
Extraction time (E)	30	60	90	min
Liquid-to-solid ratio (R)	10	20	30	mL g^{-1}

3. Results and discussion

3.1 Characterization of artichoke waste

The total phenolic content of bracts was 24.14 ± 0.73 mg GAE/g and that of stems was 35.71 ± 1.65 mg GAE/g. These results are in line with those reported in previous studies (Gaafar and Salama, 2013) and confirm that stems are a richer source of polyphenols than bracts. Of course, when considering the industrial processing waste, the amount of phenolics could vary greatly depending on the proportion of the two main waste components and the presence of leaves, which may also be found in the industrial waste (Pandino et al., 2013). It should also be considered that the amount of polyphenols in the industrial waste is dependent on the applied blanching conditions. Blanching is a thermal treatment used to minimize the deterioration of vegetables products during storage (Nayak et al., 2013). In the case of artichokes, the primary purpose of blanching is to inactivate polyphenol oxidase, the enzyme catalysing the oxidation of caffeoylquinic esters and other ortho-dihydroxyphenolic substrates, in order to avoid the development of flavour and browning (Lattanzio et al., 2009). During this treatment, which is generally accomplished by immersing the plant material in hot water for a specified time, some polyphenols are lost and others produced (Lutz et al., 2011), the net effect depending on their initial amount and the severity of the treatment.

Table 2: Experimental design layout. q is the amount of extracted phenolics per unit weight of dry plant material; SO is the standard order and RO the run order of experiments

SO	RO	x_1	x_2	x_3	q (mg GAE/g)	
					Bracts	Stems
1	7	–1	–1	–1	11.23	12.66
2	8	+1	–1	–1	11.48	16.58
3	6	–1	+1	–1	17.45	25.06
4	1	+1	+1	–1	19.26	26.89
5	2	–1	–1	+1	12.97	16.86
6	4	+1	–1	+1	13.25	20.67
7	9	–1	+1	+1	18.86	24.45
8	5	+1	+1	+1	19.44	28.02
9	12	0	0	0	16.84	22.03
10	3	0	0	0	16.61	21.84
11	11	0	0	0	16.44	21.92
12	10	0	0	0	16.71	22.43

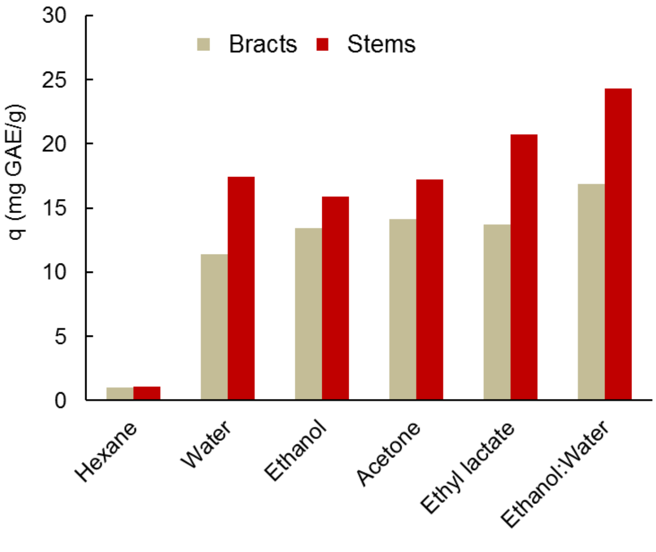


Figure 1: Effect of solvent type on the recovery of polyphenols from artichoke waste

3.2 Solvent screening

Solvent screening experiments gave the results presented in Figure 1. It can be seen that solvent type affected significantly polyphenol recovery and that there were some differences between the two waste components. The six solvents can be ranked, according to their average extraction efficiency, in the following order: 50:50 ethanol–water mixture > ethyl lactate > acetone > ethanol > water > hexane.

Differences in efficiency can be primarily ascribed to the higher affinity of phenolic compounds for polar solvents, but other factors such as weakening of the solute–matrix interactions and swelling of the plant material could also be involved. As is known, swelling is caused by the adsorption of solvent molecules on specific functional groups of biomass components (Obataya and Gril, 2005). This phenomenon results in an expansion of the plant material, which facilitates solvent penetration and the extraction of matrix-bound phenolics. Low-molecular-weight polar compounds like water, ethanol and acetone are effective swelling promoters (El Seoud, 2009). Therefore, it can be hypothesized that the observed solvent effects are, at least in part, due to the swelling of artichoke plant tissue.

3.3 Phenolic extraction and influential factor analysis

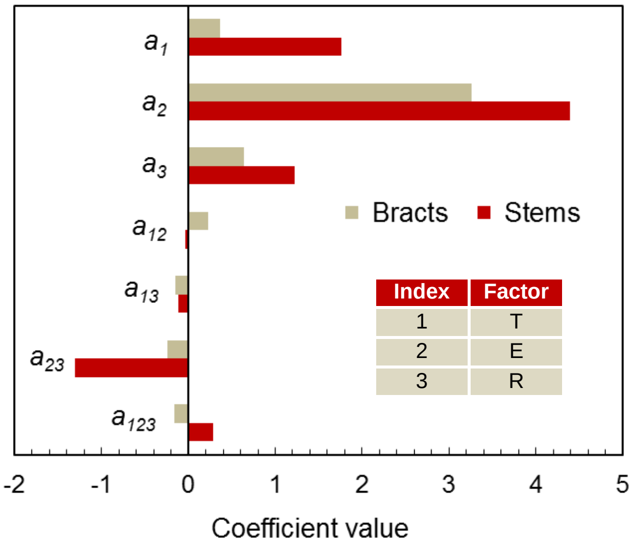
The results of the experimental design are shown in Table 2. The amount of extracted phenolics ranged from 11.23 to 19.44 mg GAE/g for bracts and from 12.66 to 28.02 mg GAE/g for stems. For both materials, the maximum extraction yield was obtained at T = 60 °C, E = 90 min and R = 30 mL g^{-1}. Under these conditions, the resulting percentage yield were 80.5 % for bracts and 78.5 % for stems.

The experimental data were correlated by the following equation:

$$y = a_0 + \sum_{i=1}^{3} a_i x_i + \sum_{i=1}^{3} \sum_{j=i+1}^{3} a_{ij} x_i x_j + a_{123} x_1 x_2 x_3 \tag{4}$$

where y is the response variable, x's are the coded independent variables, a_i are the coefficients associated with the main effects, a_{ij} are those related to the binary interactions and a_{123} is the ternary interaction coefficient.

The eight unknown coefficients in Eq(4) were determined using the data of runs 1–8 in Table 2. Their values are shown in the Pareto chart in Figure 2. To assess the statistical significance of these coefficients, we followed the procedure described in a previous paper (Zuorro et al., 2013). In particular, the standard deviation of the experimental response was first estimated from the central points of the factorial design (runs 9–12 in Table 2). Then, the 95 % confidence interval of each coefficient was determined by the Student's *t*-test and its statistical significance assessed. The coefficients associated with the main effects (T, E, R) and those related to the interactions T×E and E×R (bracts) or E×R (stems) were statistically significant.



Index	Factor
1	T
2	E
3	R

Figure 2: Pareto chart showing the effects of factors on the recovery of polyphenols from artichoke waste

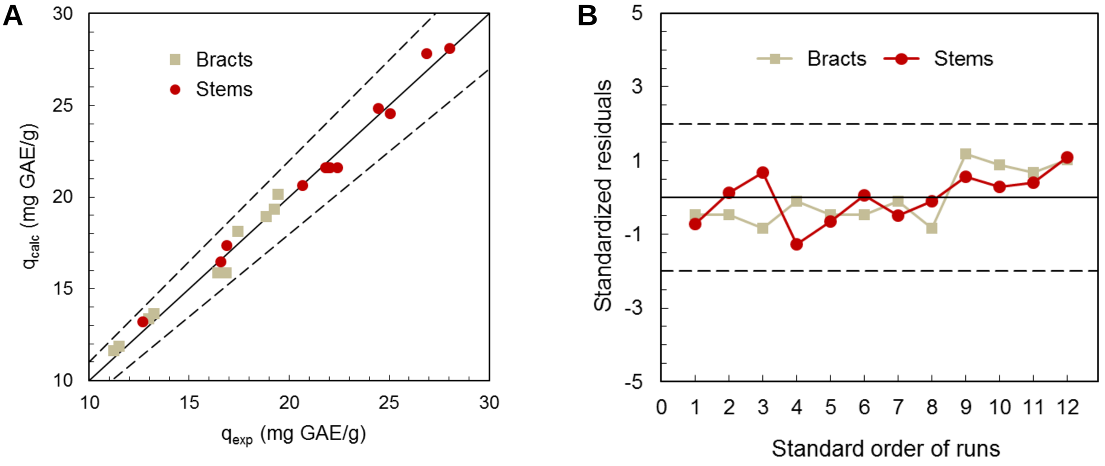


Figure 3: Statistical analysis of the reduced models (Eq. 5 and 6): (a) Comparison between experimental and calculated yields. The dashed lines delimit the ±10 % deviation band; (b) Standardized model residuals

As can be seen from the values of a_1, a_2 and a_3, T, E and R exerted a beneficial effect on polyphenol recovery. The higher values of these coefficients for stems, compared to bracts, are probably the results of differences in the structural properties of the two plant tissues and/or in the strength of the phenolic compound–matrix interactions. For both materials, E was the most influential, followed by T and R. Furthermore, there was a significant negative interaction between E and R, which means that, at a given temperature, the influence of extraction time on polyphenol recovery is more pronounced at lower liquid-to-solid ratios. The reason may be that at low liquid-to-solid ratios the mass-transfer of tissue-bound phenolics into the solvent is reduced (Cussler, 2009). Accordingly, under these conditions the positive effect of extraction time becomes more prominent.

Removal of non-significant terms from the full model Eq(4) provided the following simplified expressions for polyphenol recovery from bracts (q_B) and stems (q_S):

$$q_B = 7.623 - 0.01T + 0.086E + 0.112R + 7.75\times10^{-4}\,T\cdot E - 8.01\times10^{-4}\,E\cdot R \tag{5}$$

$$q_S = -2.089 + 0.164T + 0.222E + 0.304R - 3.24\times10^{-3}\,E\cdot R \tag{6}$$

A good agreement was found between experimental and calculated yields (Figure 3a), the mean percentage error being 3.29 % for bracts and 1.92 % for stems. Analysis of standardized residuals (SR) was also performed. They were calculated as:

$$SR = \frac{q_{\exp} - q_{,calc}}{\left(\frac{1}{n-p-1}\sum_n \left(q_{\exp} - q_{calc}\right)^2\right)^{0.5}} \tag{7}$$

where n is the number of data points and p is the number of model parameters (p = 6 for bracts and 5 for stems). If the errors were normally distributed, 95 % of the points would fall within the ±2 band (Montgomery, 2012). Figure 3B shows that all data points were randomly scattered around the zero line and no outlier (i.e., SR < –2 or SR > +2) was detected. This suggests that the simplified models described by Eq(5 and 6) can be considered statistically significant and used to describe the influence of process conditions on the recovery of phenolic compounds from artichoke waste.

4. Conclusions

The recovery of natural antioxidants or other health-promoting compounds from agro-industrial wastes is a topic of growing interest in the field of applied biotechnology. This study demonstrates that the phenolic compounds found in artichoke bracts and stems, the two main components of artichoke processing waste, can be easily recovered by a solvent-extraction procedure using aqueous ethanol as solvent. The results obtained indicate that the extraction efficiency is affected by both the solvent type and the process conditions. We have also shown that an analysis of influential factors can provide useful indications on the effects of process variables on polyphenol recovery.

Future research should focus on the real industrial waste as well as the minimization of polyphenol loss in the plant material during blanching. The utilization of the solid residue remaining after recovery of polyphenols should also be investigated. An interesting possibility would be the use as a low-cost adsorbent. Recently, we have demonstrated the feasibility of this approach for other lignocellulosic wastes including spent tea leaves (Lavecchia et al., 2010), coffee silverskin (Zuorro et al., 2013), defective green coffee and spent coffee grounds (Zuorro et al., 2014). Application of this strategy to artichoke processing waste would allow its integral exploitation and also contribute to reducing its environmental impact.

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