LWT - Food Science and Technology 61 (2015) 346–351

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Chemical characterization and use of artichoke parts for protection from oxidative stress in canola oil

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ARTICLE INFO

Article history:
Received 4 August 2014
Received in revised form
13 December 2014
Accepted 21 December 2014
Available online 30 December 2014

Keywords:
Antioxidants
Fat acids
Oxitest
QUENCHER procedure
PCA

ABSTRACT

The residues of artichoke processing are commonly discarded by the food industry owing to lack of detailed knowledge of their chemical composition, which would facilitate research into practical uses for them. We therefore evaluated proximate and fatty acid compositions, total phenolic compound content and total antioxidant activity of artichoke parts (bracts, receptacle and spikes) cultivated in Brazil and performed principal components analysis. Analysis indicated higher concentrations of phenolic compounds and antioxidant activity in the spikes than in the bracts and receptacle. In a follow-up experiment we assessed the lipid protective value of artichoke parts in tests on canola oil using a new instrument, called "Oxitest". The addition of 5 g/100 g of spikes almost doubled the canola oil induction point. These results suggest that artichoke spikes may have industrial applications as natural antioxidant additives to foods such as canola oil.

1. Introduction

The artichoke plant (*Cynara cardunculus* var. *scolymus* (L.) Fiori) originated in southern Europe, in the Mediterranean region, and it belongs to the Asteraceae family, which also includes daises and sunflowers (Lombardo et al., 2010). It is the most important horticultural crop in Italy, followed by potato and tomato (Fratianni, Tucci, De Palma, Pepe, & Nazzaro, 2007). Italy is also the world's biggest producer of artichokes with about 480,000 tons harvested in the year of 2010, followed by Spain (167,000 tons) and France (42,000 tons). Together these three countries are responsible for 85% of the total global cultivation area (Lutz, Henríquez, & Escobar, 2011; Saez, Fasoli, D'Amato, Simó-Alfonso, & Righetti, 2013). Artichoke was introduced to Brazil at the beginning of the 20th century by Italian immigrants, and nowadays it is mainly grown in the states of São Paulo and Rio Grande do Sul. It is important to remember that Brazilian consumption much lower than European consumption and sale and production costs are higher because of this lower demand, which reduces accessibility (Di Giulio, 2004). Costs are also high because only a small part of the artichoke is edible; cultivation produces a large volume of byproducts which are usually discarded.

The edible parts of artichoke and its byproducts contain many chemical compounds known for their beneficial effects in the human body, such as phenolic acids, flavonoids and sesquiterpenes, these compounds help to protect the liver, reduce blood cholesterol levels, have anticarcinogenic activity, and increase the volume of bile secretion from liver as well as the rate of discharge from the digestive system (Jacociunas et al., 2013; Lattanzio, Kroon, Linsalata, & Cardinali, 2009; Noldin et al., 2003; Pandino, Lombardo, Mauromicale, & Williamson, 2011; Shen, Dai, & Lu, 2010; Sonnante, De Paolis, Lattanzio, & Perrino, 2002). As well as these benefits the compounds listed above are also responsible for the antioxidant activity of artichoke parts; they stabilize free radicals in the human body (Biglari, Alkarkhi, & Easa, 2008; Ferracane et al., 2008; Rufino, Alves, Fernandes, & Brito, 2011; Vasco, Ruales, & Kamal-Eldin, 2008).

The ability of antioxidant compounds to delay or inhibit oxidation of substrates has other applications, including protecting edible fats and oils – and even engine lubricants (Focke et al., 2012; Impellizzeri et al., 2012) – against oxidation.

In many cases the additive used is a synthetic antioxidant such as 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT) due to the high activity per unit weight of these compounds, however there is

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http://dx.doi.org/10.1016/j.lwt.2014.12.050
0023-6438/

evidence that they may have harmful effects on human health (Arabshahi-Delouee & Urooj, 2007; Botterweck, Verhagen, Goldbohm, Kleinjans, & Van Den Brandt, 2000; Dolatabadi & Kashanian, 2010; LeClercq, Arcella, & Turrini, 2000). Chemical and pharmacological research is therefore underway to find natural antioxidants which are suitable for use in the food industry (Moure et al., 2001). Byproducts of artichoke represent a potential alternative to synthetic antioxidants, because of their low cost and the reported antioxidant activity of artichoke cultivated in other countries; however there is very limited data on the antioxidant content and activity of artichokes cultivated in Brazil.

Samples of this type can also be tested for ability to protect a substrate against oxidation. Lipid matrices rich in unsaturated fatty acids (UFAs) are usually more prone to oxidative degradation than saturated fatty acids (SFAs). The fatty acid composition of canola oil (7.3 g/100 g saturated fatty acids; SFAs, 66.9 g/100 g monounsaturated fatty acids; MUFAs and 25.8 g/100 g polyunsaturated fatty acids; PUFAs; Huang et al., 2008) makes it an excellent substrate for lipid degradation tests.

The aim of this study was therefore to determine the antioxidant capacity of artichoke using DPPH radical capture assay, alone and in combination with the QUENCHER procedure, and to evaluate the proximate and fatty acid composition of artichoke parts in terms of total phenolic content using high performance liquid chromatography (HPLC). The ability of samples to delay canola oil oxidation was tested using a new instrument, "Oxitest". Data on antioxidant activity were correlated with themselves and with Oxitest data and assays of total phenolic content to extract more information about the chemical composition of sampled artichoke parts.

2. Materials and methods

2.1. Sampling

About 36 kg of artichokes (*Cynara cardunculus* var. *scolymus* (L.) Fiori) were acquired commercially in three periods separated by 30-day intervals, in the city of Maringá, Paraná state, Brazil. Samples were washed with running water, dried with paper towels and manually separated into bracts, spikes and receptacle (heart). Samples from the three different sampling periods were combined and homogenized in order to minimize the effects of edaphoclimatic variation, which might affect the properties of interest in these samples. Artichoke parts were dried in a laboratory oven with forced ventilation at 45 °C until constant weight was attained. Dried samples were packed under vacuum in polypropylene bags and kept in a freezer at −18 °C. Prior to analysis samples were triturated in a knife mill and passed through a 0.177 mm sieve to ensure that particle size did not influence the analytical procedures (Gökmen, Serpen, & Fogliano, 2009).

2.2. Reagents and standards

Reagents and solvents in analytical and HPLC grades were acquired from Fisher Scientific (Fair Lawn, NJ) and Sigma Chemicals Co (St. Louis, MO). Chlorogenic acid, p-coumaric acid, ferulic acid, apigenin and cynarin standards for HPLC were acquired from Sigma Chemicals Co (St. Louis, MO). Ultrapure water (Milli-Q system, Millipore Corp, Bedford, MA) was used in all analyses.

2.3. Chemical analysis

All analyses were carried out in triplicate. Total lipid content (TL) of the samples was determined according to the procedure described by Bligh and Dyer (1959). Moisture content was determined using AOAC Official Method 930.15, ash content using AOAC Official Method 942.05 and crude protein (CP) was measured according to AOAC Official Method 960.52 (1990), using a factor of 6.25 to convert percentage nitrogen to percentage protein (Association of Official Analytical Chemists AOAC, 2000). Crude fiber content was determined according to the IAL method (Instituto Adolfo Lutz, 2008, chap. 4) using digestion with sulfuric acid 0.23 mol/L and sodium hydroxide 0.66 mol/L.

2.4. Fatty acid composition

Fatty acid methyl esters were prepared by saponification of sample lipid contents with sodium hydroxide 0.5 mol/L (in methanol) followed by methylation with triboronfluoride (12.0 ml BF_3 in 100.0 ml methanol) (Joseph & Ackman, 1992) and separated by gas chromatography (GC) using a Thermo 3300 gas chromatograph equipped with a flame ionization detector (FID) and a fused-silica CP-7420 (SELECT FAME) capillary column (100 m × 0.25 mm i.d. × 0.25 mm cyanopropyl). The operation parameters were as follows: detector temperature, 240 °C; injection port temperature, 230 °C. Column temperature was maintained at 165 °C for 7 min, then raised by 4 °C/min to 185 °C and maintained at this temperature for 4.67 min. Following this the temperature was raised to 235 °C at a rate of 6 °C/min and maintained at this level for 5 min, giving a 30-min chromatographic run. Carrier gas flow (hydrogen) was 1.2 ml/min; nitrogen at 30 ml/min was used as the makeup gas. The sample split ratio was 1:80.

Fatty acids were identified by comparing retention times to those of standard methyl esters (Sigma, USA). Quantification of fatty acids (FAs) was performed using tricosanoic acid methyl ester (Sigma, USA) as an internal standard (Joseph & Ackman, 1992). Peak areas were determined using the Chromquest 5.0 software.

2.5. Preparation of extracts

Methanolic, ethanolic and aqueous extracts were obtained from all prepared sample in proportions of 1:10 (m/v). Methanolic and ethanolic extracts were stirred for 4 h at room temperature (25 °C) then subjected to rotary evaporation to remove solvents. Aqueous extracts were stirred for 8 min at 80 °C. After cooling the solvent was sublimated in a lyophilizer (model Alpha 1-2 LD plus, Martin Christ, Germany). All extracts were stored in eppendorfs, protected from light, in a freezer at −18 °C until the analysis.

2.6. Analysis of antioxidant capacity using conventional DPPH assay

Initially, the antioxidant capacity of extracts was measured through a conventional DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical capture method, following the procedure described by Brand-Williams, Cuvelier, and Berset (1995), with some modifications (Ma et al., 2011). Extracts were prepared as 2.0 mg/ml solutions then 25.0 μl aliquots were mixed with 2.0 ml of a methanolic solution of 6.25×10^{-5} mol/L DPPH. The mixture was kept in the dark for 30 min and absorbance at 517 nm was measured in a spectrophotometer (Genesys 10 uv, Thermo Scientific, Madison, USA). Methanolic solutions of Trolox ((±)-6-hydroxi-2,5,7,8-tetramethylcromane-2-carboxylic acid) with different concentrations were used to achieve the calibration curve and the antioxidant capacity was expressed in mmol Trolox equivalent (TE)/100 g dried sample.

2.7. Antioxidant capacity analysis through DPPH assay paired with QUENCHER procedure

The QUENCHER (QUick, Easy, New, CHEap and Reproducible) procedure is a recently developed procedure for measuring the antioxidant capacity of foods directly (Gökmen et al., 2009). It has