Industrial Crops & Products 112 (2018) 90–97

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Research paper

Bioactive compounds of organic goji berry (*Lycium barbarum* L.) prevents oxidative deterioration of soybean oil

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ARTICLE INFO

Keywords:
Response surface methodology
Natural antioxidant
Superfruit
Atoxic solvent

ABSTRACT

The protecting ability of the organic goji berry extract as an alternative to synthetic antioxidants against oxidation of soybean oil was investigated. For this, the extraction of phenolic compounds with high antioxidant activity was optimized. The main bioactive compounds of the optimized extract were identified and quantified. Different solvents were used in the extraction process. The effects of temperature (25–45 °C), time (60–180 min), and solid:solvent ratio (1:10–1:30, w/v) in the extraction were evaluated by a Box–Behnken experimental design. Analyses of phenolic acids and flavonoids were performed by Ultra Performance Liquid Chromatography (UPLC), in optimal extraction conditions. The effect of the organic goji berry extract on improving the soybean oil oxidative stability was evaluated using the Rancimat test. A solution of ethanol/water (70/30, v/v) was the most efficient in the extraction of phenolics with a high antioxidant activity. The optimum conditions for the extraction is obtained by the experimental design were 45 °C, 162 min and a solid:solvent ratio of 1:30, resulting in an extract with 1338.80 mg/100 g of phenolics, and antioxidant activity of 0.73, 3.66, and 2.81 mmolTE/100 g for FRAP, ABTS, and DPPH, respectively. The phenolic acids identified were as follows: syringic, chlorogenic, gallic, caffeic, *p*-coumaric, 4-hydroxybenzoic, ferulic, and trans-cinammic. The flavonoids were as follows: rutin, naringin, quercetin, catechin, and kaempferol. The protection factor (PF) values of soybean oil with organic goji berry extract (500–3000 mg/kg) were significantly higher than that of oil with butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (100 mg/kg). These results suggest that the organic goji berry extract may be a substitute for synthetic antioxidants in stabilizing oil against oxidative deterioration.

1. Introduction

The fruits of the *Lycium barbarum* L., known as goji berry, wolfberry, or “gou-qi-zi” are grown mainly in Asian countries (Potterat, 2010). In China, goji berry is cultivated under two different systems, organic and conventional. The demand for organic food has increased throughout the world, mainly due to the awareness of the population about possible risks regarding the presence of chemical residues in food (Llorent-Martínez et al., 2010).

Goji berries are known as “superfruit” and have been used for over 2500 years in Asian countries for medicinal purposes (Amagase and Farnsworth, 2011). In scientific literature, several beneficial effects, such as the following, have been reported: treatment for eye problems (Amagase and Farnsworth, 2011; Qian et al., 2004), body weight reduction (Amagase, 2010), immunomodulatory effects (Arroyo-Martinez et al., 2011) as well as antiproliferative activity (Hogan et al., 2010).

The antioxidant properties of the goji berry are used for the development of drugs, cosmetics and special purpose foods (Pan et al., 2011). Accordingly, goji berry is considered to be a raw material for the extraction of phenolic compounds. These biologically active compounds have been added to food products to provide protection against oxidation. Many studies show that natural antioxidant compounds are as potent as synthetic antioxidants in vegetable oils (Yang et al., 2016; Franco et al., 2016; Comunian et al., 2016). These results are extremely important considering that the prolonged consumption of synthetic antioxidants may cause carcinogenic and mutagenic effects on the human body (Cordeiro et al., 2013).

Therefore, in order to obtain the vegetable extract for applying as a natural antioxidant, some factors must be considered, such as the choice of a non-toxic solvent in an adequate volume and polarity for

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https://doi.org/10.1016/j.indcrop.2017.10.052
Received 23 May 2017; Received in revised form 12 September 2017; Accepted 29 October 2017
0926-6690/

extraction, the influence of temperature and time, biological activity and yield upon extracting bioactive compounds (Pedro et al., 2016).

Food industries apply many technological operations to obtain vegetable extracts with a high yield and low cost. The response surface methodology (RSM) is a suitable approach to obtain an extract with such optimized features (Bassani et al., 2014). Therefore, to obtain a natural extract with high antioxidant content, the mathematical model of RSM may be efficiently applied, besides investigating the influences of different factors on extraction (Pedro et al., 2016).

Studies regarding the organic goji berry have not yet been explored in the literature. In addition, this is the first study to indicate the application of organic goji berry extract as a natural antioxidant in edible oils, as there is no optimization study about the extraction process of these compounds with the use of non-toxic solvents for the application in the food industry. Therefore, the main objectives of this study were: (1) to optimize the extraction of total phenolic compounds with antioxidant activity by response surface methodology, (2) to identify and quantify the bioactive compounds in the optimized extract by UPLC–Ultra Performance Liquid Chromatography, and (3) to evaluate the capability of organic goji berry extract in protecting soybean oil against oxidation by the Rancimat method and comparing it with the efficacy of synthetic antioxidants: butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and t-butyl-hydroxyquinone (TBHQ).

2. Materials and methods

2.1. Materials

Folin-Ciocalteau reagent, TPTZ, DPPH, ABTS, chemical UPLC-grade standards (purity ≥ 95%), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and t-butyl-hydroxyquinone (TBHQ) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Soybean oil without added antioxidants were provided by Granol Industry, Trade and Export S/A. Methanol, acetic acid, ethanol and acetone were purchased from Vetec® (Rio de Janeiro, RJ, Brazil). Ultrapure water (Milli-Q) was used in all experiments.

2.2. Preparation of samples

The dehydrated samples of organic goji berry (*Lycium barbarum* L.) were acquired at Municipal Market of Curitiba, PR, Brazil, from the 2015 harvest and certified organic: IMO Control, Manufactured: Qingdao Ri Tai Food Co., Ltd. The fruits were ground in a mill (Marconi MA 630/1, São Paulo, Brazil) to 10 mesh and stored in dark in a vacuum packaging at 4 °C until analysis.

2.3. Selection of solvents

Various solvents, such as ultrapure water, acetone, ethanol and methanol, in different concentrations and mixtures were evaluated for the extraction of bioactive compounds from the goji berry. The selection of the best solvent was achieved in response to the presence of the highest quantity of phytochemicals in the extracts with high antioxidant activity (by FRAP, ABTS and DPPH assays).

Solutions of ethanol/water and methanol/water were tested for the extraction of phytochemicals at the proportions of 20/80, 30/70, 50/50, and 70/30 (v/v). A solution of acetone/water at the proportion of 70/30 (v/v) was also used in the experiments of extraction (Liyana-Pathirana and Shahidi, 2005). Two grams of sample were mixed with 20 mL of each extractive solution and stirred in an incubator shaker (Marconi MA 420, São Paulo, Brazil) at 25 °C, 210 rpm for 60 min. The supernatant was obtained by centrifugation (MPW-350R, Warsaw, Poland) at 4300 rpm for 20 min. For each extract, the volume was completed to 50 mL with the solution used in the extraction (ethanol/water, methanol/water or acetone/water).

Sequential extraction was carried out according to Larrauri et al. (1997). Two grams of sample were mixed with 20 mL of methanol/water (50/50, v/v) and stirred in an incubator shaker at 25 °C, 210 rpm for 60 min. The mixture was centrifuged at 4300 rpm for 20 min and the supernatant recovered. The sediment was added of 20 mL of acetone/water (70/30, v/v), and the steps of stirring and centrifugation were repeated. The extracts were combined and the volume was completed to 50 mL with ultrapure water.

The sequential extraction using acetone/water/acetic acid (70/28/2, v/v/v) was performed according to Kevers et al. (2007), and the same extraction conditions used by Liyana-Pathirana and Shahidi (2005) (cited above) were employed.

All the extracts were stored in amber glass bottles at −20 °C for further analysis.

2.4. Experimental design

A Box–Behnken design (Table 1) was used to evaluate the effect of three independent variables in the extraction of the bioactive compounds and *in vitro* antioxidant activity: temperature (x_1, 25–45 °C), time (x_2, 60–180 min) and solid:liquid ratio (x_3, 1:10–1:30 mL). These values were established previously, data not shown. The response parameters were: total phenolics, flavonoids, anthocyanins, carotenoids and antioxidant activity (FRAP, ABTS and DPPH assays). The complete design consisted of 17 combinations, including 5 replicates of the central point in order to estimate pure error and to assess the lack of fit of the proposed models. The experiments were performed randomly and in triplicate.

2.5. Total phenolic compounds (TPC)

The TPC was determined using the Folin-Ciocalteau method (Singleton and Rossi, 1965). In test tubes of 1 mL were added 600 µL of ultrapure water, 200 µL of diluted sample (1/20, v/v) and 50 µL of Folin-Ciocalteau reactive. Afterwards, 150 µL of Na_2CO_3 (15%, w/v) was added to each tube followed by agitation. After 60 min of reaction, the absorbance was monitored at 760 nm. A calibration curve was prepared using gallic acid as standard and the results of TPC were expressed as mg of gallic acid equivalents per 100 g dry weight sample.

2.6. Total flavonoids content (TFC)

The TFC was quantified using the aluminum chloride colorimetric assay (Jia et al., 1999). In test tubes of 1 mL were added 100 µL of sample, 400 µL of ultrapure water and 30 µL of $NaNO_2$ (5%, w/v). After 5 min, 60 µL of $AlCl_3{\cdot}6H_2O$ (10%, w/v) was added and the solution was allowed to react for 6 min. Then, 200 µL of NaOH 1 mol/L solution and 210 µL of ultrapure water were added and mixed. After 5 min of reaction, the absorbance was measured at 510 nm. Catechin was used as a standard and the results were expressed as mg of catechin equivalents per 100 g of dried fruit.

2.7. Total anthocyanins (TA)

The TA was performed by the pH-differential method (Giusti and Wrolstad, 2001). The extract was diluted in a pH 1.0 solution (0.1 mol/L HCl, 25 mmol/L KCl) and in a pH 4.5 solution (0.4 mol/L CH_3COONa). The absorbance of the mixtures was then measured at 515 and 700 nm. The value of $(Abs_{515}–Abs_{700})_{pH1.0}–(Abs_{515}–Abs_{700})_{pH4.5}$ corresponds to the absorbance of the anthocyanins. Calculation of the TA was based on a cyanidin-3-glucoside, molar extinction coefficient of 26,900 and a molecular mass of 449.20 g/mol. The results were expressed as mg of cyanidin-3-glucoside equivalents per 100 g of dried fruit.