Industrial Crops and Products 49 (2013) 668–674

Contents lists available at SciVerse ScienceDirect

Industrial Crops and Products

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

Effect of solvents extraction on phenolic content and antioxidant activity of the byproduct of eggplant

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ARTICLE INFO

Article history:
Received 20 February 2013
Received in revised form 30 May 2013
Accepted 11 June 2013

Keywords:
Solvents
Extraction
Antioxidants
Antioxidant activity
Eggplant Peel
Solanum melongena

ABSTRACT

Eggplant is one of most common vegetables consumed all around the world. This study has assayed antioxidants from the byproduct (peel) of eggplant (*Solanum melongena*), using three extraction solvents: 70% methanol, 70% ethanol and 70% acetone. For each solvent, content of total phenolics, flavonoids, tannins, and total anthocyanins were quantified. Antioxidant activity of different extracts were screened using the ferric reducing power, 1,1-diphenyl-2-picryl hydrazyl (DPPH•) radical scavenging, hydrogen peroxide (H_2O_2) scavenging and metal chelating activities. The results showed that 70% methanol is the best solvent for the extraction of anthocyanins (82.83 ± 1.07 mg DGE/100 g DP), whereas, 70% acetone is the best solvent for the extraction of total phenolics, flavonoids and tannins (29.3 ± 1.23 mg GAE/100 g DE; 18.5 ± 0.07 mg QE/100 g DE and 5.37 ± 0.22 mg TAE/100 g DE, respectively). Anthocyanic extracts have exhibited the higher reducing power (39 ± 2.5 mg QE/100 g DE) and scavenging activity (IC_{50} = 2.88 ± 0.02 mg/mL), whereas the phenolic extracts have shown the highest metal chelating activity (18.53 ± 0.4%).

1. Introduction

Antioxidant components are microconstituents present in the diet that can delay or inhibit lipid oxidation, by inhibiting the initiation or propagation of oxidizing chain reactions, and are also involved in scavenging free radicals (Othman et al., 2007). Epidemiological studies have shown that high fruit and vegetable consumption has health benefits in the prevention of chronic diseases (Cheel et al., 2007). These foods are reported to contain a wide variety of antioxidant components, including phenolic compound (Arancibia-Avila et al., 2008). Phenolics are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. They have also metal chelation properties (Proestos et al., 2006). The oxygen consumption inherent in cell growth leads to generation of a series of reactive oxygen species (ROS), these ROS are molecules such as superoxide anion radicals ($O^{\bullet}_2$) and hydroxyl radicals ($OH^{\bullet}$). However, non free radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are formed *in vivo* also. Both oxygen species play a positive role in energy production, phagocytosis, regulation of cell growth intercellular signaling, and synthesis of biologically important compounds (Gülçin et al., 2005). However during oxidative stress, large amounts of these ROS can be products and may be dangerous because of their ability to attack numerous molecules, including proteins, lipids (Halliwell et al., 1992) and DNA (Gülçin et al., 2005).

Eggplant, *Solanum melongena*, is a common and popular vegetable crop grown in the subtropics and tropics (Sarker et al., 2006). Eggplant is native to southeastern Asia and great proportion of world production is produced in Asia and Mediterranean basin. The most cultivated variety in Algeria is the elongated ovoid in a dark purple skin. Its fruit is primarily used as a cooking vegetable for the various dishes all over the world (Demir et al., 2002; Hanson et al., 2006). It contains ascorbic acid and phenolics, both of which are powerful antioxidants (Vinson et al., 1998). Studies have shown that eggplant extracts suppress the development of blood vessels required for tumor growth and metastasis (Matsubara et al., 2005), and inhibit inflammation that can lead to atherosclerosis (Han et al., 2003).

Different solvent systems have been used for the extraction of polyphenols from plant material. The yield and antioxidant activity of natural extracts is dependent on the solvent used for extraction. Several procedures have been proposed (Pokorny and Korczak, 2001): extraction using fats and oils, organic solvents, aqueous alkaline solutions and supercritical carbon dioxide.

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http://dx.doi.org/10.1016/j.indcrop.2013.06.009

Aqueous mixtures of ethanol, methanol and acetone, are commonly used (Hayouni et al., 2007). Wang and Helliwell (2001) reported that aqueous ethanol was superior to methanol and acetone for extracting flavonoids from tea. However, in another work, water was found to be better solvent, for extracting tea catechins, than were 80% methanol or 70% ethanol (Hayouni et al., 2007). For this reason, the extraction method of phenolics differs from plant substrate to another and an ideal extraction method for particular phenolic classes has to be individually designed and optimized.

The choice of our investigation is based on two criteria: first, to enhance the peel (byproduct) of eggplant, which is a good source of bioactive substances (anthocyanins) as long as the Algerian people do not consume it. Therefore the first objective of this study is to evaluate several types of phytochemicals that are present in the dried powder of peel eggplant. The second criterion is to select the solvent that led to the extracts with the highest antioxidant capacity.

In this study extracts were obtained from dried powder of peels eggplant using different organic solvents: 70% methanol, 70% ethanol and 70% acetone. Efficiency of extraction was determined by measuring the total phenols, flavonoids, tannins, total anthocyanin and antioxidant activity (ferric reducing power, scavenging effect of 1,1-diphenyl-2-picryl hydrazyl (DPPH•) radical, scavenging capacity of hydrogen peroxide and metal chelating activity).

2. Materials and methods

2.1. Chemicals and sample preparation

All chemicals were purchased from Sigma (represented by Algerian Chemical Society, Setif, Algeria). Fresh eggplant (*S. melongena*) was purchased from local market, Bejaia city, Algeria. A previously developed method described and suggested peel separation from pulp tissue by immersion in ethylene glycol at 35 °C. But this practice did not preserve anthocyanin from polyphenol oxidase oxidative activity (Spagna et al., 2003), for this reason peels were removed using a sharp knife (Todaro et al., 2009), dried in the drying oven at 40 °C during 4 days, and ground to granulometry lower than 250 μm.

2.2. Quantification of antioxidants

2.2.1. Carotenoids

Carotenoids are pigments insoluble in water and soluble in apolar solvents like hexane. The carotenoids content was evaluated by Soto-Zamora et al. (2005) method. 10 mL of solvent mixture (hexane, acetone and ethanol, 1:5/2:5/2, v/v/v) were added to 0.5 g of dried powder. After 3 min of stirring, 1 mL of potassium hydroxide (KOH, 1 M) was added and the mixture was incubated during 40 min, then the absorbance of upper phase was determined at 450 nm. β-Carotene was used as the standard and the results were expressed as mg β-carotene equivalent per 100 g of dry powder (mg βCE/100 g DP).

2.2.2. Anthocyanins

10 mL of solvents (70% methanol, 70% ethanol and 70% acetone) containing 0.2% of formic acid were added to 1 g of dried powder. After stirring for 40 min, the homogenized samples were then centrifuged at 5000 rpm for 20 min at 4 °C. 5 mL of different solvents were added to the pellet, the same operation was repeated, then the supernatants were collected (Wang et al., 2008).

Total anthocyanins were determined by a pH differential method (Prior et al., 1998). Absorbance was measured at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5. The concentration of anthocyanins was obtained using the following equation:

$$C\ \ (\mathrm{mg/l}) = \frac{A \cdot MW \cdot DF \cdot 1000}{\Sigma L}$$

where $A = [(A_{510} - A_{700})\ \mathrm{pH}_{1.0} - (A_{510} - A_{700})\ \mathrm{pH}_{4.5}]$, MW (molecular weight) of delphinidin-3-glucoside (465 g/mol), Σ is the molar extinction coefficient of delphinidin-3-glucoside (29,000 L/mol/cm), DF is the dilution factor and L is the length of vessel (1 cm).

Results were expressed as delphinidin-3-glucoside equivalent per 100 g of dry powder (mg DGE/100 g DP).

2.3. Phenolic compounds

2.3.1. Preparation of the extracts

Dried powder (0.5 g) was extracted with 50 mL of 70% methanol, 70% ethanol and 70% acetone. The extraction was carried out at room temperature, using magnetic blender. After 40 min, the solution was centrifuged for 25 min at 4000 × *g* (10 °C), the supernatant was filtered (Whatman paper no. 4) and stored under refrigerated conditions until used.

2.3.2. Quantification

The amount of total phenolics in the extract was determined using the Folin–Ciocalteu reagent and gallic acid as standard as described by Singleton and Rossi (1965).

The total flavonoids content was determined by the methodology of Quettier-Deleu et al. (2000) and quercetin was used as standard.

Tannins were estimated spectrophotometrically according to the protocol developed by Hagerman and Butler (1978) and tannic acid was used as standard. All analyses were performed in triplicate and the mean value was calculated.

2.4. Antioxidant activity

Several methods have been developed to assay free radical scavenging capacity and total antioxidant activity of plant extracts. The most common and reliable method involves the determination of the disappearance of free radicals using a spectrophotometer. In our study, we used four methods: reducing power, metal chelating activity, scavenging of the radical DPPH and H_2O_2 activities.

2.4.1. Free radical-scavenging activity

The ability of the extracts to scavenge DPPH free radicals was determined by the method of Suja et al. (2005). 100 μL of various concentrations of the samples were mixed with 3 mL of DPPH in methanol (0.1 mM). After 30 min of incubation in the dark and ambient temperature, absorbance was measured at 515 nm. The percentage scavenging was calculated according to the following equation:

$$\%\mathrm{Scavenging} = \left[\frac{\mathrm{Abs}_{\mathrm{contr}} - A_{\mathrm{extr}}}{A_{\mathrm{contr}}}\right] \times 100.$$

where $\mathrm{Abs}_{\mathrm{contr}}$ is the absorbance of the control (without extract) after 30 min and A_{extr} is the absorbance of extract after 30 min. IC_{50} was calculated as the concentration of extracts causing a 50% inhibition of DPPH radical.

2.4.2. Reducing power

The reducing power of the extracts was evaluated according to the protocol of Hseu et al. (2008). 1 mL of different concentrations of the samples was mixed with phosphate buffer (1 mL, 0.2 M, pH = 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1 mL, 1 g/100 mL). The mixture was incubated at 50 °C for 20 min.