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Garlic (*Allium sativum* L.) husk waste as a potential source of phenolic compounds: Influence of extracting solvents on its antimicrobial and antioxidant properties

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ABSTRACT

This paper reports the extraction process of polyphenols from the industrially disposed garlic husk (GH) using different studied solvents (water, methanol, ethanol and 50% aqueous solutions of methanol and ethanol). The highest extraction yield was achieved with water (26.5%) and high bioactive potential was shown by the samples extracted with 50/50 methanol/water (25 mg GAEs/g dry GH, 0.617 mg QE/g dry GH, IC_{50} = 0.26 mg/mL for DPPH assay, $RP_{0.5AU}$ = 2.8 mg/mL for reducing power, and IC_{50} = 0.45 mg/mL for hydroxyl radical assay). All the extracts exhibited antimicrobial activity against Gram-positive bacteria, when applied at different concentrations (1–10 mg/mL). Only 50/50 methanol/water and absolute methanol extracts inhibited the growth of *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. These interesting biological properties could be attributed to the major identified phenolic compounds, such as caffeic, *p*-coumaric, ferulic and di-ferulic acids. With this procedure, the GH waste polyphenols could be used as a cheap source of natural compounds, with potential applications in the food and health sectors.

1. Introduction

In last few years, there has been a growing interest in phytochemicals as new sources of natural antioxidants and antimicrobial agents (Robards, 2003). The commonly used synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ascorbyl palmitate (PA) are severely restricted by legistative rules concerning both application and permitted levels (Regulation EC, 2008). Furthermore, there is some safety concerns related to the residual toxicity of chemical preservatives (EFSA, 2012). The increasing consciousness of consumers with regard to food additive safety, created a need for identifying alternative natural and probably safer sources of food antioxidants. The replacement of synthetic antioxidants by natural ones may have benefits due to health implications and functionality such as solubility in both oil and water, of interest for emulsions, in food systems. The demand for the use of such compounds has produced in recent years a clear increase in the number of studies based on natural extracts which come from plants, food processing industry or agricultural waste products (Cruz et al., 2007; Moure et al., 2001).

Garlic (*Allium sativum* L.) has been used throughout its history for both culinary and medicinal purposes (Rivlin, 2001). Garlic is a particularly rich source of organosulfur compounds which are partly responsible for health beneficial effects of garlic (Amagase et al., 2001). Garlic bioactive components are well known to possess antioxidant (Prasad et al., 1995; Ide et al., 1997; Amagase, 2006). During harvesting period garlic bulb yields a considerable amount of husk, stem and leaf which is simply thrown or disposed causing a severe problem in the community. Garlic stem and leaf contains allicin, the major bioactive component of garlic; which is comparatively lower than garlic bulb (Mohsen and Shahab, 2010). However, to date, no information is available on garlic husk chemical composition which is a nonedible material, has a characteristic spicy flavor as a potential source of polyphenols.

Several agricultural and industrial residues have been studied as sources of potentially safe natural additives with antimicrobial

Abbreviations: GH, garlic husk; AQ, aqueous; AM, absolute methanol; AE, absolute ethanol; MW, 50/50 methanol–water (v/v); EW, 50/50 ethanol–water (v/v); GAE, gallic acid equivalent; QE, quercetin equivalent; TDF, total dietary fiber; SDF, soluble dietary fiber; IDF, insoluble dietary fibers.

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and/or antioxidant properties for the food industry; various compounds have been isolated, many of them being polyphenols. In addition, polyphenolic compounds have positive health benefits in the prevention of human diseases associated with oxidative stress (Scalbert et al., 2005). Different solvent systems have been used for the extraction of phenolic compounds from agriculture waste products and since the activity depends on the polyphenol compounds and the antioxidant assay, comparative studies for selecting the optimal solvent providing maximum antioxidant activity are required for each substrate. In fact, several procedures have been proposed (Pokorny and Korczak, 2001): extraction using fats and oils, organic solvents, aqueous alkaline solutions and supercritical carbon dioxide.

The aim of this study was to obtain a crude extract that could be used as a source of antioxidants for use in the food industry. For this purpose, the influence of the solvent on the properties of garlic husk extracts was investigated for polyphenols recovery from the industrially disposed garlic husk. The major phenolic compounds were identified and quantified by liquid chromatography/mass spectrography (LC/MS). In addition, the radical-scavenging activities of the different extracts and their antimicrobial effects were tested against Gram-positive and Gram negative bacteria.

2. Materials and methods

2.1. Reagents and standards

1,1-Diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), potassium ferrycianide, trichloroacetic acid (TCA), ferric chloride, 1,10-phenanthroline, Gallic acid, Quercetin, Folin Ciocalteu reagent and sodium carbonate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Aluminum chloride was obtained from Fluka (Buchs, Switzerland). All other chemicals and solvents were of analytical grade.

2.2. Raw material

The garlic husk (GH) used in this study, was discharged from the manufacturing process of conservation of garlic. The outer dry of skin garlic bulbs (*A. sativum* L.) were collected, air-dried at 40 °C, grounded into fine particles and passed through 2 mm sieve screen. All analyses were performed using triplicate samples and analytical results were expressed on a dry matter basis. The samples were stored at 4 °C prior to analysis.

2.3. Physicochemical analysis of GH

Dry matter was determined according to the Association of Official Analytical Chemists (AOAC, 1997) method. Total protein was determined by the Kjeldahl method. Protein was calculated using the general factor (6.25) (Vandercook et al., 1979). Fat content was determined according to French Association of Standardization using standard NF V03-713 (AFNOR, 1986). Lignin was isolated from GH as residual precipitate after total hydrolysis of cellulose and hemicellulose by sulfuric acid according to the method TAPPI T 222 om-88, 1988). Acid-insoluble lignin estimation test method involves addition of 2 mL 72% H_2SO_4 into 1 g of oven-dry-basis sample in a 100 mL beaker, followed by addition of 13 mL of 72% H_2SO_4. The mixture was kept in the beaker in a water bath at 20 °C with continuous stirring for 2 h. The contents of the beaker were filtered through a tarred G2 crucible that weighed (W1). The residue was dried with the crucible in an oven at 105 °C overnight and weighed (W2). The acid-insoluble lignin was assessed by the following expression: Acid insoluble lignin% = (W2 − W1) × 100/O.D weight of sample.

The filtrate obtained from the G2 crucible was kept for soluble lignin estimation, which was measured spectrophotometrically at 280 nm. Acid-soluble lignin was determined according to TAPPI UM 250 standards. It can be estimated through the following expression (Laboratory manual 2001): Acid soluble lignin% = {(Absorbance at 280 nm × Dilution factor/20) × 100}/1000 × O.D weight of sample.

2.4. Dietary fiber

Insoluble and soluble dietary fibers (DF) were determined according to the AOAC enzymatic-gravimetric method of Prosky et al. (1988). Briefly, the defatted samples were gelatinized with heat-stable alpha amylase (A-3306, Sigma Chemical Co., St. Louis, MO, USA) (100 °C, pH 6, 30 min) and then digested with protease (P-5380, Sigma Chemical Co., St. Louis, MO) (60 °C, pH 7.5, 30 min), followed by incubation with amyloglucosidase (A-9268, Sigma Chemical Co., Poole, Dorset, UK) (60 °C, pH 4.5, 30 min). Then, the samples were filtered, washed (with water, 95% ethanol and acetone), dried and weighed to determine insoluble fiber. Four volumes of 95% ethanol were added to the filtrate and to the water washings. Then, the precipitates were filtered and washed with 78% ethanol, 95% ethanol and acetone. After that, the residues (soluble DF) were dried and weighed. The obtained values were corrected for ash and protein. Total DF was determined by summing insoluble DF and soluble DF.

2.5. Extracts preparation

For the aqueous (AQ) extraction, 5 g of the powdered sample were extracted with 250 mL of boiling water for 45 min and filtered through Whatman no. 4 paper. In the extractions with absolute methanol (AM), absolute ethanol (AE), 50/50 methanol–water (v/v, MW) and 50/50 ethanol–water (v/v, EW), 1.5 g of sample were extracted with 25 mL of the tested solvent for 45 min at room temperature and filtered through Whatman no. 4 paper (Fernández-Agulló et al., 2013). The solvents were evaporated and the phenolic extracts obtained were redissolved in water to a final concentration of 50 mg/mL and stored in the dark at 4 °C for further use. All the extractions were done in triplicate.

2.6. Total phenol contents

Total phenol content of the GH extracts was determined by using Folin–Ciocalteu reagent following a slightly modified method of Singleton and Rossi (1965). Briefly, 1 mL of sample was mixed with 1 mL of Folin–Ciocalteu's reagent for 3 min and 1 mL of saturated sodium carbonate (Na_2CO_3) solution was added to the mixture and 10 mL distilled water was then adjusted. After 1 h of incubation in the dark at room temperature, the absorbance at 750 nm was measured. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds expressed as mg/g gallic acid equivalent (GAE) of dry GH.

2.7. Total flavonoid content

Total flavonoid content was determined using the method described by Zhishen et al. (1999). A 500 μL of sample was mixed with 75 μL of 5% sodium nitrite solution, 150 μL of 10% aluminum chloride ($AlCl_3$) solution, and 500 μL of 1 N sodium hydroxide solution. After incubation at room temperature for 5 min, the reaction mixture absorbance was measured at 510 nm. The content of total flavonoid compounds expressed as mg/g quercetin equivalent (QE) of dry GH.