Food Chemistry 141 (2013) 4166–4170

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

The effect of feed moisture and temperature on tannin content, antioxidant and antimicrobial activities of extruded chestnuts

Brice Wilfried Obiang-Obounou, Gi Hyung Ryu *

Department of Food Science and Technology, Kongju National University, Chungnam 340-802, South Korea

ARTICLE INFO

Article history:
Received 18 February 2013
Received in revised form 26 June 2013
Accepted 27 June 2013
Available online 4 July 2013

Keywords:
Extrusion
Chestnut
Antioxidant
Antibacterial
Tannin
Phenolic compounds

ABSTRACT

This study focuses on the effect of extrusion processing on tannin reduction, phenolic content, flavonoid content, antioxidant and anitimicrobial activity. Extrusion temperature (120 and 140 °C) and feed moisture (25% and 28%) were used on the tannin content, antioxidant and antimicrobial activities. Extrusion cooking reduced tannin content up to 78%, and improved antioxidant activity from 12.89% to 21.17% in a concentration dependant manner without affecting its antimicrobial activity that varied from 250 to 500 mg. The time–kill assay confirmed the ability of extruded chestnut to reduce *Pseudomonas aeruginosa* count below detectable limit that reduced the original inoculum by 3log10 CFU/mL. Overall, the results showed that extrusion cooking might serve as a tool for tannin reduction and could improve the antioxidant and antimicrobial properties of chestnut, which might be helpful for chestnut related products in the food industry.

1. Introduction

Chestnuts are becoming popular nuts for their nutritive nature. These nuts contain a high amount of tannins (Noh et al., 2010) especially the inner shell (Hwang, Hwang, & Park, 2001). Owing to their high affinity for proteins, tannins bind themselves to a variety of digestive enzymes, rendering them ineffective. As a consequence, tannins negatively affect the digestion process, reduce the efficiency of feed utilization and ultimately reduce the growth rate of animals. Additionally, they have an astringent, bitter taste that reduces palatability (Mensah, Adamafio, Amaning-Kwarteng, & Rodrigues, 2012). Chestnuts contain phenolic compounds (de Vasconcelos et al., 2010; Neri, Dimitri, & Sacchetti, 2010) which are bioactive compounds believed to be involved in the defense process against oxidative damage (Barreira, Ferreira, Oliveira, & Pereira, 2008) due to the flavonoids and other polyphenolic compounds they contain (Dragsted, 2003). The phenolic compounds in herbs act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators (Javanmardi, Stushnoff, Locke, & Vivanco, 2003). Whilest oxidation is essential to many organisms for the production of energy to fuel biological processes, oxygen-centered free radicals have been implicated in several ailments, including cancer, diabetes and cardiovascular diseases (Srinivasa, Chaudhury, & Pradhan, 2010).

Extrusion is a mechanical process exposing material to high temperature, shear and pressure over a short period of time (Zhang et al., 2012). One advantage of extrusion cooking lies in the destruction of antinutritional factors, especially trypsin inhibitors, haemagglutinins, tannins and phytates, all of which inhibit protein digestibility (Bargali, Singh, & Singh, 1993), sterilize the finished product and retain natural colours and flavours of foods (Bhandari, D'Arcy & Young, 2001). Extrusion cooking is reported to increase phenolic and flavonoid content (Norajit, Gu, & Ryu, 2011). Phenol and flavonoid are good sources of antimicrobials (Proestos, Boziaris, Nychas, & Komaitis, 2006; Rauha et al., 2000). From this basis we have found that extruded chestnuts could be used as antimicrobials against selected bacteria. The quality of extruded products depends on the conditions under which the extruder is operating (barrel temperature, screw speed, feed rate, feed moisture content, etc.) (Ryu & Ng, 2001). In this study, we investigated the effects of extrusion conditions, process temperature and feed moisture on tannin content reduction, antioxidant and antimicrobial activities of extruded chestnuts.

2. Materials and methods

Chestnut flour was obtained by grinding dried chestnuts (fruit and endocarp) at a local market in Kongju, South Korea. Chemicals were all purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). The microorganisms used in this study were obtained from the Korean Collection for Type Cultures (Daejon, Korea). The bacterial strains used for the study are *Bacillus substilis* (B.S), *Staphylococcus*

* Corresponding author. Tel.: +82 41 330 1484; fax: +82 41 335 5944.
E-mail address: ghryu@kongju.ac.kr (G.H. Ryu).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.06.129

epidermis (S.E) and *Pseudomonas aeruginosa* (P.A). The microbial isolates were subcultured and prepared for assessment of raw and extruded chestnut activity. Gentamycin and streptomycin were used as positive control for antibacterial activities. The negative control used was dimethyl sulfoxide (DMSO).

2.1. Extrusion process

A co-rotating intermeshing twin-screw extruder manufactured by Incheon Machinery Co., Ltd. (Incheon, South Korea) with screw of 32 mm diameter (D) and 768 mm length (L; L/D ratio = 24) was used. The experiments were conducted as follows: screw speed 200 rpm, feed rate 120 g/min and die diameter 3 mm. The moisture content of feed material was adjusted to 15% and stored for 12 h at 4 °C before extrusion processing. The moisture content was adjusted at 25% and 28% by injecting distilled water into the barrel. The melt temperature in the die exit was set at 120 and 140 °C by controlling barrel temperature. The extrudate was dried directly in an oven at 50 °C for 8 h. The dried extrudate was ground to powder using a stainless steel mixer (FM-909T, Hanil Electrical Co., Seoul, South Korea) and then passed through 0.5 mm sieve (No. 35 mesh US Standard Sieve Series) and stored at 4 °C until analysis.

2.2. Antioxidant properties and antimicrobial assay

The ground extrudate samples (0.4 g) were extracted by stirring them at room temperature in a 5 mL methanol: water (80:20 v/v) solution for 5 min, and subsequently placed in a shaking constant-temperature water bath at 80 °C and 150 rpm for 2 h. The resulting slurries were centrifuged at 3000×*g* for 10 min to obtain the supernatant. The residues were subjected to repeat extraction twice under the same conditions and the supernatant collected, combined, filtered using Whatman No. 1 filter paper, and then concentrated to dryness under reduced pressure using a rotary evaporator (Eyela, N–N series, Tokyo, Japan) at 50 °C. After concentration, the extracts were transferred into sample vials for tannin content, total phenolic content, total flavonoid content, DPPH-scavenging activity, and antimicrobial assay.

2.2.1. Tannin contents assay

The quantification of tannins was determined as proposed by the laboratory manual for the FAO division of nuclear techniques in food and agriculture (FAO., 2000). Tannic acid (TA) was used as the standard, and the results expressed in μg of TA equivalent at 0.1 mg/mL of dry based sample.

2.2.2. Total phenolic contents

The total phenolic content of sample solutions was determined according to the Folin–Ciocalteu colorimetric method (Norajit et al., 2011). The reaction mixture was composed of 0.1 mL of sample solution and 1.5 mL of the 10-fold diluted Folin–Ciocalteu's reagent. After 5 min, 1.5 mL of 6% sodium carbonate were added. The reactions were placed in the dark for 90 min, and the absorbance recorded with a UV/Vis-spectrophotometer at 725 nm (Libra S35, Biochrom Ltd., Cambridge, UK). Acidified methanol was used as a blank. Gallic acid was used as the standard, and the results were expressed as mg of gallic acid equivalent per 100 g of dry sample. Samples were assayed in triplicate.

2.2.3. Total flavonoid contents

The sample solutions (0.5 mL) were mixed with 0.1 mL of 10% aluminium nitrate, 0.1 mL of 1 M potassium acetate and 4.3 mL of 95% ethanol. The absorbance was read at 415 nm after 40 min of incubation at room temperature (Woisky & Salatino, 1998). Quercetin was used as the standard, and the total flavonoid content expressed in mg of quercetin equivalents per 100 g of dry sample.

2.2.4. DPPH radical-scavenging activity

The free radical-scavenging activity extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Norajit et al., 2011) . 0.1 mM solution of DPPH in ethanol was prepared and 1.0 mL of this solution was added to 3.0 mL of extract solution in methanol at a concentration of 0.1 mg/mL. Thirty minutes later, the absorbance was measured at 517 nm. The capability of scavenging the DPPH radical was calculated according to the following equation:

$$\text{DPPH radical scavenging activity}(\%) = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance of the sample.

On the other hand, ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. Radical-scavenging activity was expressed as Ascorbic acid equivalent antioxidant capacity (AEAC) at 0.1 mg/mL.

2.2.5. Determination of the minimum inhibitory concentrations

We used 12 h-broth cultures as microbial inocula and adjusted the turbidity of bacterial suspensions to 0.5 McFarland standard solutions. Susceptibility tests were performed using standard broth microdilution method (Obiang-Obounou et al., 2011). Each well was briefly loaded with 100 μL of MHB, 10 μL of antibiotics, and 10 μL of the inoculum (approximately 10^6 colony forming units CFU/mL) to achieve a final inoculum concentration of 5×10^5 CFU/mL in all wells. The minimum inhibitory concentration (MIC) value was the lowest concentration of commercially purchased antibiotics that inhibited visible growth after 24 h of incubation at 37 °C. At the end of the incubation period, the well plates were visually examined for turbidity. Cloudiness indicates that bacterial growth has not been inhibited by the concentration of antimicrobial agent contained in the medium. A colorimetric assay for rapid detection of the presence of bacteria was also performed (see below, Colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide [MTT] test).

2.2.6. Colorimetric assay using MTT test

A colorimetric assay based on MTT for rapid detection of the presence of bacteria was performed as previously described (Obiang-Obounou et al., 2011). Briefly, a stock solution of 5 mg/mL MTT (Sigma) was prepared in phosphate-buffered saline and kept at −70 °C. A final concentration of 1 mg/mL of MTT was used in the assay. After 24 h of incubation at 37 °C, 20 μL of the yellow MTT was added to the 96-well microtiter plate (0.3 mL volume) and incubated for an additional 20 min. The presence of a blue color indicates the presence of bacteria.

2.2.7. Time–kill assay

Viable counts for the determination of living-viable were performed as previously described (Obiang-Obounou et al., 2011) using 100 μL of culture medium. Inocula of 5×10^5 CFU/mL were exposed to the antibacterial either singly or in combination with extruded chestnut and incubated at 37 °C; 0.1 mL aliquots of the culture were removed at 0, 4, 12, and 24 h of incubation; and serial 10-fold dilutions were prepared in saline as needed. The numbers of viable cells were determined on a drug-free Müller–Hinton agar plate after 24 h of incubation. Colony counts were performed on plates, and 30–300 colonies were obtained. The lower limit of sensitivity of colony counts was 100 CFU/mL. Antimicrobials were considered as bactericidal at the lowest concentration that reduced the original inoculum by 3log10 CFU/mL (99.9%) at each time