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Reduction of antiproliferative capacities, cell-based antioxidant capacities and phytochemical contents of common beans and soybeans upon thermal processing

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ABSTRACT

The effects of boiling and steaming processes on the antiproliferative and cellular antioxidant properties, as well as phytochemicals, of two types of common beans (pinto and black beans) and two types of soybeans (yellow and black) were investigated. All thermal-processing methods caused significant ($p < 0.05$) decreases in total phenolic content (TPC), total saponin content (TSC) and phytic acid content (PAC) values in all bean types (except for TPC values in pressure-steamed yellow soybeans) as compared to those of the raw beans. All types of uncooked raw beans exhibited cellular antioxidant activities (CAA) in dose-dependent manners. Black soybeans exhibited the greatest CAA, followed by black beans, pinto beans and yellow soybeans. The CAA of cooked beans were generally diminished or eliminated by thermal processing. The hydrophilic extracts from raw pinto beans, black beans and black soybeans exhibited antiproliferation capacities against human gastric (AGS) and colorectal (SW480) cancer cells in dose-dependent manners. The raw yellow soybeans exhibited dose-dependent antiproliferation activities against the SW480 cells. Most of the cooked beans lost their antiproliferation capacities as observed in the raw beans. These results indicate that different processing methods may have various effects on phytochemical profiles and bioactivities. Overall, thermal processing caused a significant reduction of the health-promotion effects of beans.

1. Introduction

Soybeans (*Glycine max*) and common edible beans (*Phaseolus vulgaris* L.) are the world's first and second most important food legumes. Soybeans and soy-based foods are extensively consumed in the Asian diet. Common edible beans are one of the basic foods in Africa, India, and Latin America. Pinto beans are preferred in Mexico and the southwestern United States, while central American and south American and African populations eat mostly coloured beans (including black beans). Particularly, Brazil, which has the highest food legume intake per capita, mostly in the form of black beans.

In recent years, food legumes have attracted a great deal of attention due to their functional components and health-promoting effects in relation to the prevention of chronic diseases, including cardiovascular diseases, obesity and diabetes (Geil & Anderson, 1994). Epidemiological studies have found links between the low incidence of cancer and consumption of beans. For example, epidemiological data from 41 countries revealed that countries with the greatest consumption of beans had the lowest death rates due to breast, prostate, and colon cancer (Bawadi, Bansode, Trappy, Truax, & Losso, 2005; Bobe et al., 2008; Correa, 1981; Geil & Anderson, 1994; Hangen & Bennink, 2003; Hughes, Ganthavorn, & Wilson-Sanders, 1997; Shi et al., 2004). Some case-control studies have provided evidence of protection by food legumes against cancer of the colon and rectum (Franceschi, 1999) as well as prostate (Jain, Hislop, Howe, & Ghadirian, 1999). Preclinical studies have also shown that feeding black or navy beans to laboratory animals reduced both the incidence and number of colon tumors by 50% (Hangen & Bennink, 2003). The World Cancer Relief Fund/American Institute for Cancer Research Committee recognised the potential of food legumes consumption to decrease cancer risk and emphasised the need for additional research in this area (World Cancer Research Fund, 1997). Isoflavones, protease inhibitors (such as trypsin inhibitors), saponin, phytosterols, and phytic acids have been found to be anticarcinogens in soybeans (Messina, Persky, Setchell, & Barnes, 1994). In addition, in recent years, most of the so-called antinutrients (such as tannins, saponins, and phytic acids) have been found to actually possess health-enhancing properties if used properly in the context of food for disease prevention (Bawadi et al., 2005).

In vitro antioxidant activities and phenolic compounds in raw (unprocessed) pinto and black beans, yellow and black soybeans

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have been reported in several earlier communications (Madhujith, Naczk, & Shahidi, 2004; Oomah, Cardador-Martinez, & Loarca-Pina, 2005; Xu & Chang, 2007). These studies indicate that common beans and soybeans may serve as excellent dietary sources of natural antioxidants for disease prevention and health promotion. However, the health-promoting capacities of common beans and soybeans strictly depends on their processing history, since beans must be cooked or processed before consumption. Food processing not only improves flavour and palatability of legume foods, but also increases the bioavailability of nutrients. Cooking brings about a number of changes in the physical characteristics and chemical composition of food legumes. Food legumes are usually cooked by a boiling process before use. Pressure boiling and steaming can also be used for this purpose. High pressure processing technology may provide high quality (flavour, colour and biological active components) food products (Knorr, 1999).

The phenolic components and chemical antioxidant activities of thermal processed common beans and soybeans have been investigated in our recent studies (Xu & Chang, 2008a; 2008b; 2009a) and in one report on pressure-cooked common beans (Rocha-Guzmán, González-Laredo, Ibarra-Pérez, Nava-Berúmen, & Gallegos-Infante, 2007). These preliminary studies showed that soaking, boiling, and steaming processing significantly affected the phenolic components and antioxidant activities (determined by *in vitro* chemical assays) of the beans studied. However, how thermal processing affects saponins and phytic acid in these beans and how the major potential health-promoting effects (anticancer activity and cellular antioxidant activity) are influenced by thermal processing have not been studied. As a result of our continued study on thermal processing effects of food legumes, the present study was undertaken to investigate the effects of boiling and steaming processes on the total phenolics, saponins, phytic acids, cellular antioxidant activities, and antiproliferation properties of pinto beans, black beans, yellow and black soybeans.

2. Materials and methods

2.1. Chemicals and reagents

Dimethyl sulfoxide (DMSO), 2′,7′-dichlorofluorescin diacetate (DCFH-DA), gallic acid, soyasaponin (contained minimum 80% saponin), phytic acid, sulphosalicylic acid, Folin–Ciocalteu reagent, sodium carbonate, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and vanillin and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). The 2,2′-azobis (2-amidino-propane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). Analytical grade solvents used for extraction were purchased from VWR international (West Chester, PA). Two human cancer cell lines (AGS and SW480) were purchased from American Type Culture Collection (ATCC, Manassas, VA). Hanks balanced salt solution (HBSS) and 0.4% trypan blue solution were purchased from Cambrex Bio Science Walkersville, Inc. (Walkersville, MD). Phosphate-buffered saline (PBS), trypsin–EDTA solution, penicillin–streptomycin, fetal bovine serum (FBS), and all of the cell culture media (Eagle's MEM, Leibovitz's L-15, F-12K) were purchased from Mediatech, Inc. (Herndon, VA). Sterilised cell culture materials, such as T-25 and T-75 flasks, syringe filters, 24-well and 96-well plates, as well as pipettes, were purchased from Corning Incorporated (Corning, NY).

2.2. Legume materials

The two common beans used in this study were pinto bean (*Phaseolus vulgaris* L. cv. Pinto) and black beans (*P. vulgaris* L. cv. Turtle Eclipse), which were obtained from the University of Idaho Foundation Seed Program Kimberly Research and Extension Center (Kimberly, ID). The two soybeans used were one yellow cultivar (*Glycine max* (L.) Merr. cv. Proto) and one black cultivar (*G. max* (L.) Merr. cv. C-1), which were obtained from Sinner Brothers & Bresnahan (West Fargo, ND). Broken seeds, damaged seeds, and foreign materials were removed from the samples. Moisture content was determined by drying the sample in an air oven at 110 °C until a constant weight was obtained. All calculations for the determination of phenolics and quantification of antioxidants activities are on a dry weight basis.

2.3. Soaking, boiling, steaming, and cooking time

The soaking procedures of beans and determination method of hydration ratio as reported in our earlier communication (Xu & Chang, 2008a) were followed. The soaked yellow soybeans (with a 100% hydration ratio), as well as black soybeans and common beans (with a 50% hydration ratio), were drained and then boiled or steamed by the methods described below. The reason for choosing a 50% hydration ratio for soaking coloured beans (including black soybeans, pinto and black beans) was based on the study on black bean (Xu & Chang, 2008a), where 50% hydration was found to achieve a particular softness and retained more water-soluble phenolic substances than a higher hydration ratio did. All thermal processes were performed according to our previously described procedures (Xu & Chang, 2008a). Briefly, regular (atmospheric) boiling and steaming treatments were conducted using a domestic atmospheric cooker and a domestic atmospheric steam cooker, respectively; pressure boiling and steaming were conducted using an M-0512-H Mirro pressure cooker (Mirro Co., Manitowoc, WI), respectively. The cooking time was determined based on a tactile method (Vindiola, Seib, & Hoseney, 1986). The boiling and steaming time, as well as pressure conditions, were selected for pinto bean based on preliminary experiments, and selected for black bean based on the results from our previous report (Xu & Chang, 2008a). After cooking treatments, the beans were drained and cooled to room temperature in covered plastic containers. Subsequently, the cooked samples were frozen and then freeze-dried. The original raw beans and the freeze-dried cooked beans were ground into flour with an IKA® all basic mill (IKA Works Inc., Wilmington, NC) and passed through a 60-mesh sieve.

2.4. Extraction of hydrophilic phenolics from legumes

Extraction procedures of total phenolics were conducted according to our earlier communication (Xu & Chang, 2007). Briefly, yellow soybean flours (0.5 g in triplicate) were extracted twice with 5 ml of acetone/water (50:50, v/v), whereas the coloured bean flours (including black soybeans) were extracted twice with 5 ml of acetone/water/acetic acid (70:29.5:0.5, v/v/v). A portion of hydrophilic phenolic extracts was directly used for the total phenolic assay, as below. Another portion of the hydrophilic total phenolic extract was freeze-dried after removing the organic solvents; and then the freeze-dried extract (10 mg) was dissolved in a cell culture medium as a stock sample solution. The final concentrations of samples (0.125, 0.25, 0.5, 1, 2, and 5 mg/ml) were obtained by diluting the sample with the cell culture medium. The diluted legume sample solutions were subjected to a cellular antioxidant activity (CAA) assay and an antiproliferation assay.

2.5. Determination of total phenolic content (TPC)

The TPC was determined by a Folin–Ciocalteu assay (Singleton & Rossi, 1965) with slight modifications (Xu & Chang, 2007). The TPC was expressed as milligrams gallic acid equivalents per gram