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Phenolic composition changes of processed common beans: their antioxidant and anti-inflammatory effects in intestinal cancer cells

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ABSTRACT

Four varieties of common beans, Negro 8025 (N), Bayo Victoria (BV), Pinto Durango (PD), and Pinto Saltillo (PS) were evaluated and compared for phenolic composition, antioxidant activity and anti-inflammatory effects by *in vitro* human intestinal cell model. Beans were processed by canning and boiling in open pot. Acetone/Water extracts were analyzed for phenolic composition by HPLC-PAD and HPLC-MS, screened for antioxidant activities, as lipid peroxidation inhibition and chelating capacities by inhibition of deoxi-D-ribose degradation. It was investigated their anticarcinogenic effect by inhibiting cell proliferation, decreasing interleukin-8 (IL-8), modulating interleukin-10 (IL-10), inhibiting tumor necrosis factor alpha (TNFα) and regulating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ). Canning induced antioxidant compounds in order N > PD > BV > PS associated with potential for scavenging hydroxyl radicals and metal chelating capacities. Effect of cooking on bioactive compounds was cultivar dependent, being more quantitative than qualitative due to release of bonded phenolics. Inhibition of cyclooxygenase-2 (COX-2), TNFα and NF-κβ was observed, and the induced expression of IL-10. Both effects were also cultivar and process dependent, particularly in PD beans.

1. Introduction

Legumes have become important in the human diet because of their nutritional properties, low cost and the physiological effects associated with its intake. The major legumes consumed in Latin America are common bean (*Phaseolus vulgaris* L.).

Currently, the consumption of this legume has changed due to different factors such as increasing availability of common bean varieties, regional and cultural changes associated with modern life as the lack of domestic time to cook them at home. The latter has led to the developing of convenience foods by the industry based on the four more preferred common bean varieties: Bayo, Pinto, Black and Peruvian. The most commercially offered presentation of common beans and the most requested by the consumers is the canned form (82.3%), and from these 52% are processed as whole seeds, being black beans the most demanded variety (Rodríguez-Licea, García-Salazar, Rebollar-Rebollar, & Cruz-Contreras, 2010).

Commercial acceptance of industrial products such as common beans depends directly on the adequate thermal processing and determination of the optimal cooking time. A well-designed thermal process, improves the palatability, texture and increased bioavailability of nutrients as a result of gelatinization of the starch, as well as to protein denaturation. Hence the importance of the type of bean processing, since it can significantly determine the effectiveness of its natural biological action, due to the release of bioactive compounds that play an important role in the antioxidant system of the organism (Champ, 2002; Rocha-Guzmán, González-Laredo, Ibarra-Pérez, Nava-Berumen, & Gallegos-Infante, 2007; Wolosiak et al., 2010). The antioxidant activity of polyphenols is the functional property of interest, as has been the target of numerous studies. Their chemical structure encloses in key positions a variable number of reactive hydroxyl groups, which allow the antioxidant to react and stabilize free radicals. Consequently, it is extremely important to determine the amount of polyphenols in legume species. The number of natural polyphenols has been estimated at almost half a million, and many of them occur as glycosides and polymers. However, the polyphenols bioactivity is attributed to aglycone fragments of its metabolites and not to sugars (Sakakibara, Honda, Nakagawa, Ashida, & Kanazawa, 2003).

Although there is a major emphasis on the antioxidant properties of phenolics, there are evidences (Williams, Spencer, & Rice-Evans, 2004) that flavonoids, as precursors and their *in vivo* metabolism products,

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do not act only as conventional hydrogen-donating antioxidants, but also may exert modulatory activity in cells through modulation of protein and lipid kinase signaling cascades.

Today the increase of chronic degenerative diseases has been linked to the declined intake of traditional foods such as whole grains and legumes (Ranilla, Genovese, & Lajolo, 2009). Common bean consumption has been associated with beneficial implications in human health such as a treatment and prevention of cancer, cardiovascular diseases and other pathologies of inflammatory origin, due mainly to its antioxidant properties (Martínez Valverde, Periago, & Ros, 2000). Recent investigation has found that the varieties Pinto Durango (PD) and Negro 8025 (N) of common beans have outstanding effects in lowering glucose and cholesterol levels, and preventing colon cancer *in vivo* (Reynoso-Camacho et al., 2007). Several studies have shown how is the beneficial effect of polyphenols present in legumes, on various physiological activities related to its antihypertensive, antibacterial and antioxidant activities. Potential benefits related to health are associated to the protective effect of polyphenols against the cell damage caused by oxidative stress that is closely linked to toxic responses in cells, resulting in the inhibition of carcinogenesis (Xu & Chang, 2011). Focusing on cancer prevention, phenolic compounds have been identified as potent preventive agents, acting as antioxidants and modulators of intracellular signaling processes included in the initiation/promotion of cancer. Inflammation inducing cytokines released by damaged/transformed cells play an important role in colorectal carcinogenesis, wherein the expression of tumor necrosis factor alpha (TNFα) is related to tumor progression of colorectal adenocarcinomas (Grimm et al., 2010). Transcription factors are important in regulating the response to inflammation and oxidative stress. In particular, the increase of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκβ) transcription of genes involved in cell death and survival, and their activation, is the crucial step for interleukin-8 (IL-8) gene transcription in most cells. IL-8 is expressed in many different cell types, including monocytes, keratinocytes, endothelial cells and several human tumor cell lines (Lee, Kim, Choi, & Kim, 2005). Interleukin-10 (IL-10) is a cytokine with potent anti-inflammatory activity, which inhibits the production of inflammatory cytokines such as TNFα that induces production of reactive oxygen species (Narushima et al., 2003).

The epidemiological relationship between common bean consumption and the risk or incidence of chronic degenerative diseases should take into account differences in composition between varieties/phenotypes, in addition to the phenolic groups present and the thermal treatments applied. Recent studies have reported compounds such as catechins and procyanidins in Pinto beans flour, and hydroxycinnamic and hydroxybenzoic acids in cannellini beans (Aguilera, Estrella, Benítez, Esteban, & Martín-Cabrejas, 2011). Another research has shown the existence of flavonols such as quercetin and kaempferol, as well as isoflavones like genistein and daidzein, and phenolic acids in raw and cooked beans in autoclaving. It has been determined that the concentration of phenolic compounds is affected by the thermal treatment; however, in both wild and cultivated varieties, this type of compounds is still present after thermal treatment (Díaz-Batalla, Widholm, Fahey, Castaño-Tostado, & Paredes-López, 2006). Therefore, the purpose of this research was to evaluate the influence of thermal processing (canning and open pot) of common beans (*Phaseolus vulgaris* L) varieties Black 8025 (N), Bayo Victoria (BV), Pinto Durango (PD) and Pinto Saltillo (PS) in their chemical composition, and their antioxidant and anti-inflammatory activities in a human intestinal cell model.

2. Material and methods

2.1. Biological material

HT-29 (colorectal adenocarcinoma) cells were obtained from the American Type Culture Collection (ATCC number HTB-38™). Low-density lipoprotein (LDL) obtained from human plasma was donated by the General Hospital of Durango, Mexico. Four selected dry bean cultivars (Black 8025, Bayo Victoria, Pinto Durango, and Pinto Saltillo, from the 2009 harvesting season) were donated by INIFAP-Bajío (Celaya, Gto., Mexico).

2.2. Chemicals

Methanol, acetonitrile, formic acid, acetic acid (HPLC grade) were purchased from J.T. Baker (Toluca, Mexico). Reagent chemicals, catechin, epicatechin, ellagic acid, rutin, gallic acid, hydrochloric acid, dimethyl sulfoxide (DMSO), deoxy-D-ribose, thiobarbituric acid (TBA), trichloroacetic acid (TCA), Dulbecco's Modified Eagle Medium (DMEM), (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), fetal bovine serum (FBS), penicillin, streptomycin, hydrogen peroxide and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma Chemical (St. Louis, MO, USA).

2.3. Phenolic extract preparation

Four common bean varieties were thermally processed as indicated by Rocha-Guzmán et al. (2013), following a canning processing and a traditional cooking in open pot. For chemical comparisons, processed and unprocessed common bean flours were lyophilized. The freeze-dried flours were subjected to extraction with acetone/water (70:30) for 24 h at room temperature (25 °C) and the extracts lyophilized and stored at −84 °C for later analysis.

2.4. Chemical characterization

2.4.1. Analysis of phenolic profile by liquid chromatography-mass spectrometry

Individual analysis of phenolic compounds was carried out in a HPLC-PAD and HPLC-MS systems (Dueñas, Hernández, & Estrella, 2004). It was used with a high performance liquid chromatograph with photodiode array detection, equipped with an autosampler 717 plus, a quaternary pump (Waters, Milford, MA, USA). It was used with a reversed phase column 300 × 3.9 mm, 4 μm, Nova-Pack packed with C18 (Waters) and isocratic flow biphasic. Phase A was a mixture of water/acetic acid, 98/2 (v/v) and phase B consisted of a mixture of water/acetonitrile/acetic acid, 78/20/2 (v/v/v). The flow rate used was 0.7 mL/min, and the detection of compounds was performed at wavelength of 210 to 410 nm at intervals of 1 s.

The identification and quantification of phenolic compounds was performed using standard chemicals, applying the analysis of spectral parameters and confirmation by HPLC coupled to mass spectrometry, according to methodology by Dueñas et al. (2004). The standards, phenolic acids, caffeic and gallic acids; flavan-3-ols (+)-catechin, (-)-epicatechin, and procyanidin B2; flavonols myricetin 3-*O*-rhamnoside, kaempferol 3-*O*-rutinoside, and kaempferol 3-*O*-glucoside; kaempferol 3-O-robinoside-7-O-rhamnoside; flavanones eridictyol 7-O-rutinoside and eridictyol were obtained from Extrasynthése (Lyon, France); anthocyanins delphinidin-3glucoside, malvidin-3-glucoside, petunidin-3-glucoside, pelargonidin-3-glucoside, and cyanidin-3-glucoside were obtained from Polyphenols Laboratories (Sandnes, Norway). Other compounds, for which no standards were available were identified based on the study of UV spectral parameters and by HPLC-MS data following the methodology of Dueñas et al. (2004). The unknown polyphenol compounds were quantified with the calibration curves of the most similar compounds. MS conditions included an electrospray ionization interface (ESI) with nitrogen gas and a flow of 10 L/min at 350 °C, nebulizer pressure of 55 psi, and the capillary voltage was 4000 V. The ESI interface was operated in negative mode, scanning m/z from 100 to 2000.