Antioxidant and Antimutagenic Activities of Optimized Extruded Desi Chickpea (*Cicer arietinum* L) Flours

José Antonio Garzón Tiznado[1,2], Mar de Jesús Heiras Palazuelos[2], Ramona Julieta Espinoza Moreno[2], Mirta Cano Campos[2], Francisco Delgado Vargas[1,2], Roberto Gutiérrez Dorado[1,2], Jorge Milán Carrillo[1,2] and Cuauhtémoc Reyes Moreno[1,2,*]

[1]*Maestría en Ciencia y Tecnología de Alimentos, Facultad de Ciencias Químico Biológicas (FCQB), Universidad Autónoma de Sinaloa (UAS), Ciudad Universitaria, A.P. 1354, CP 80000, Culiacán, Sinaloa, México*

[2]*Doctorado en Biotecnología (Programa Regional del Noroeste para el Doctorado en Biotecnología), FCQB-UAS, Ciudad Universitaria, A.P. 1354, CP 80000, Culiacán, Sinaloa, México*

Abstract: The objective of this study was to evaluate the effect of optimized extrusion cooking process on antioxidant and antimutagenic properties of desi chickpea cultivars. Three desi chickpea cultivars (Brown-ICC3512, Red-ICC13124, Black-ICC3761) were studied. Extrusion was carried out in a single-screw extruder; the operation conditions were previously optimized to obtain maximum antioxidant activity in extruded chickpea flour: Extrusion temperature (ET) = 155°C, and screw speed (SS) = 240 rpm. The antioxidant activity (*AOA)* was evaluated using the oxygen radical absorbance capacity (*ORAC*) assay. Antimutagenic activity (*AMA*) of ground samples extracts was tested against 1-Nitropyrene (1-NP) with the Kado microsuspension assay using *Salmonella typhimurium* strain TA98. The total phenolic (*TPC*) and flavonoid (*TFC*) contents, *AOA, and AMA* of raw desi chickpea cultivars ranged from 1.31 to 1.35 mg GAE g^{-1} sample, dw, from 0.464 to 1.006 mg CAE g^{-1} sample, dw, from 54.9 to 57.3 µmol TE g^{-1} sample, dw, and from 57.8-62.3% inhibition, respectively. Brown-ICC3512 showed the highest *TFC* and *AOA*, while Red-ICC13124 had the highest *AMA*. The extrusion cooking process increased the *TPC, AOA and AMA* of whole desi chickpea grains in 5.3-9.2%, 9.9-12.2%, and 17.5-21.9%, respectively. The optimized extrusion cooking process is a recommended technology for increasing *AOA* and *AMA* in desi chickpea grains, which could be used as functional foods.

Keywords: Antioxidant activity, antimutagenic activity, phenolic content, desi chickpeas, extrusion cooking.

INTRODUCTION

Flavonoids are the most important group of the phenolics family and they represent two thirds of dietary phenolics; the rest are mostly represented by the phenolic acids [1]. There is increasing awareness and interest in the antioxidant behavior and potential health benefits associated with phenolics because these compounds have been related to the prevention of chronic diseases, such as cancer, cardiovascular problems and diabetes [2]. Dietary sources of phenolics include fruits, grains, teas and spices. Phenolics exhibit antimutagenic effects against aflatoxin B_1 [3], benzo[α]pyrene and 1-nitropyrene [4]; they also exhibited an antioxidant effect, which has been linked to inhibition of oxidative damage related conditions such as coronary heart diseases, stroke, and cancers [5-7]. Antioxidant activity of phenolics is of interest for the food industry given the demand for natural antioxidants [8].

Cancer is a health concern and it has been suggested that several environmental factors (e.g. diet, lifestyle and smoking) are correlated with the development of this condition; in fact, around 35% of the cancer cases may be associated with diet [9, 10]. DNA mutation is considered a key event in cancer development [11] and dietary antimutagenic substances have been suggested as important factors in the prevention or treatment of cancer; moreover, *in vitro* evaluation of antimutagenicity is commonly used as a first stage to identify new anticarcinogenic substances. Plants are the main source of bioactive metabolites with antimutagenic and anticarcinogenic activities (e.g. phenolics, quinones, glucosinolates, allyl sulfides, terpenoids and alkaloids), and several studies have showed a relationship of these activities with antioxidant capacity [12-14].

On the other hand, nitroarenes are present in diesel and gasoline emissions, ash particles, cigarette smoke condensates, home heater emissions and in the urban atmosphere. Nitroarenes, such as 2-nitrofluorene (2-NF), 1-nitropyrene (1-NP) and 1,8-dinitropyrene (1,8-DNP), typically act as potent mutagens for *Salmonella typhimurium* strains. 1-NP is a direct-acting mutagen, but it requires metabolic activation toward arylhydroxilamines by acetyl-CoA: *N*-hydroxyarylamine *o*-acetyltranferase (OAT) which is present in bacterial cells [15-17]. Benzo[α]pyrene is a polycyclic aromatic

*Address corresponding to this author at the Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa, México. Postal address: Lichis # 1986, La Campiña, CP 80060, Culiacán, Sinaloa, México; Tel/Fax: +52 (667) 7157641; E-mail: creyes@uas.uasnet.mx

ISSN: 2223-3806 / E-ISSN: 1927-5951/13

hydrocarbon (PAH) carcinogen that undergoes metabolic activation to reactive benzo[α]pyrene dihydrodiol epoxide, which is an electrophilic species capable of binding to DNA, RNA and some other macromolecules [18].

Chickpea (*Cicer arietinum* L) is one of the most important grain-legume crops in the world, with a world production of 10.4 Mt [19]. Chickpeas contain high levels of proteins (17 - 25%, dw), and carbohydrates; they also supply some minerals (Ca, Mg, Zn, K, Fe, P) and vitamins like thiamine and niacin as well as unsaturated fatty acids (oleic, linolenic). Chickpea contain a wide range of phenolic compounds, which could be considered as bioactive compounds due to their antioxidant capacity [20]. Chickpeas contain isoflavones such as Biochanin in free forms (Biochanin A and B) and those linked to other compounds (Biochanin glucoside); the content of isoflavones represents the highest percentage of identified phenolics [21, 22]; several biological activities have been associated with isoflavones, including a reduction in osteoporosis, cardiovascular disease, prevention of cancer and the treatment of menopause symptoms [23-25].

Chickpea, like other legume seeds, must be processed before consumption; it is consumed parched, fried, roasted, boiled, as snack food, sweet and condiments; seeds are ground and the flour can be used as soup, dhal, and to make bread [26]. Cooking procedures improve the flavor and palatability of the food product and increase its protein digestibility and nutritional bioavailability [27, 28]. However, they also decrease the levels of bioactive compounds and antioxidant activity of the grains, as it has been shown for several seed legumes, including kabuli-type chickpea lines [20, 29, 30].

Extrusion is a high temperature/short time technology that offer numerous advantages including versatility, high productivity, low operating costs, energy efficiency, high quality of resulting products and an improvement in digestibility and biological value of proteins [31]. The versatility of the extrusion process has allowed its use to elaborate several food products, including breakfast cereals, snacks, and precooked flours. The use of extruded flours to elaborate some food products has several advantages, since the extrusion process is accompanied by pre-gelatinization of starch granules, resulting in loss of the molecular order and the complete degradation of polymers with the formation of highly soluble fragments. Therefore, suspensions of flours precooked by extrusion are able to increase their viscosity rapidly, with a low tendency to form lumps, since starch granules have been modified showing high swelling capacity under both cold and hot conditions, which makes extruded flours highly recommended for preparation of instant food products [32].

The objective of this study was to evaluate the effect of optimized extrusion cooking process on the levels of phytochemicals (phenolics and flavonoids) and antioxidant and antimutagenic properties of whole desi chickpeas grains. This was done to determine their potential use as functional foods.

MATERIALS AND METHODS

Chemicals

The reagents 2,2´azobis(2-amidinpropane), (+)-catechin, flourescein (FL) and Folin-Ciocalteu were obtained from Sigma Chemical Co (St Louis, MO, USA). Sodium hydroxide, hexane, methanol, ethanol and ethyl acetate used were analytical grade.

Legumes

Three desi chickpea cultivars (Red-ICC5383, Red-ICC13124, Black-ICC3761) from the Core Collection/World Germplasm Bank of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) were grown on irrigated land at the Culiacan Valley Experimental Station of the National Research Institute for Forestry, Agriculture and Livestock (INIFAP), Sinaloa, México. Chickpeas were harvested, shelled, cleaned and stored in tightly sealed containers at 4ºC until use.

Proximate Composition

The following AOAC [33] methods were used to determine the proximate composition: Drying at 130ºC for moisture (method 925.09B); incineration at 550ºC, for ashes (method 923.03); defatting in a Soxhlet apparatus with petroleum ether, for lipids (method 920.39C); microKjeldahl for protein (Nx6.25) (method 960.52). Carbohydrate content was estimated by difference. All determinations were made by triplicate.

Production of Extruded Desi Chickpea Flours

The procedure recommended by Espinoza-Moreno *et al.* [34] optimized for producing extruded chickpea flour with maximum values of antioxidant activity and acceptability was used: Whole chickpea kernels were

placed in a domestic blender at low velocity to obtain grits that passed through a 40-US mesh (0.425 mm) screen. Each 100 g of chickpea grits was mixed with 15 mL distilled water + 5 mL vegetable oil + 1 g NaCl + 0.5 mL soybean lecithin. Each lot was packed in a polyethylene bag and stored for 12 h at 4ºC. Prior to extrusion, grits were tempered at 25ºC for 1 h. Extrusion cooking was done using a single – screw laboratory extruder Brabender model 20 DN (CW Brabender Instruments, Inc, NJ, USA) equipped with a 19 mm screw diameter, length–to–diameter 20:1, nominal compression ratio 1:1, and die opening of 3 mm. The inner barrel was grooved to ensure zero slip at the wall. The barrel was divided into two independent electrically heated feed ends and central zones cooled by air. A third zone, at the die barrel, was also electrically heated but not cooled by air. The three zones were set at the same temperature. A screw-operated hopper fed the feedstock into the extruder at 30 rpm. The extruder optimized operation conditions were: Extrusion temperature (ET)= 155°C, and screw speed (SS)= 240 rpm. Extrudates were cooled, equilibrated at environmental conditions (25ºC, 65% RH), milled to pass through an 80-US mesh (0.180 mm) screen, and packed in plastic bags. Extruded whole desi chickpea flours were stored at 4ºC until use.

Extraction of Free Phytochemicals

Free phytochemicals in ground samples were extracted as previously reported by Dewato *et al*. [35] with some modifications. Briefly, 1 g of ground sample was blended with 10 mL of 80% chilled ethanol for 10 min and then centrifuged at 2,500 x g for 10 min; the supernatant was evaporated to 2 mL under vacuum at 45ºC. The resulting extracts were frozen at -20ºC and stored until evaluation. For each sample, quadruplicate extractions were performed and used for analyses.

Extraction of Bound Phytochemicals

Bound phytochemicals in ground samples were extracted using the method reported by Mora-Rochín *et al*. [36]. After extraction of free phytochemicals, the residue was digested with 10 mL of 2 M sodium hydroxide in a water bath at 95ºC for 30 min with previous removal of O_2 using nitrogen gas. Finally, the sample was agitated for 1 additional hour at room temperature. The mixture was acidified (pH<2.0) with 2 mL of 2M hydrochloric acid and extracted with hexane to remove lipids. The final solution was extracted five times with 10 mL of ethyl acetate for each extraction. The ethyl acetate fraction was pooled and evaporated to dryness under vacuum at 35ºC. Bound phytochemicals were reconstituted in 2 mL of methanol - water (50:50, v/v) to improve the solubility of the compounds and to obtain a clear and homogeneous solution. The extracts were frozen and stored at -20ºC until evaluation. For each sample, quadruplicate extractions were performed and used for analyses.

Total Phenolic Content (TPC)

The phenolic content of free and bound extracts from ground samples was determined using the colorimetric method described by Singleton *et al*. [37]. Briefly, 20 µL of appropriate dilutions of extracts were oxidized with 180 mL of Folin - Ciocalteu reagent. After 20 min, absorbance of the resulting blue color was measured at 750 nm using a Microplate Reader (Synergy™ HT Multi-Detection, BioTek Inc, Winooski, VT, USA). A calibration curve was prepared using gallic acid as standard and total phenolics were expressed as micrograms of gallic acid equivalents (µg GAE) g^{-1} sample (dw).

Total Flavonoid Content (TFC)

The flavonoid content of free and bound phytochemical extracts from ground samples was determined according to Heimler *et al*. [38] and Zia-Ul-Haq *et al*. [39]; the results were expressed as micrograms of catechin equivalents (µg CAE) g^{-1} sample, dw, using a calibration curve of (+)-catechin. Linearity range of the calibration curve was 10-1,000 mg mL^{-1} (r=0.99). The extraction was conducted in triplicate and the extracts were diluted to the linear range for determination.

Antioxidant Activity (AOA)

Free and bound hydrophilic antioxidant capacities were determined using the oxygen radical absorbance capacity (ORAC) assay [40]. This assay is based on the degree of inhibition of fluorescein (FL) oxidation by antioxidants that scavenge peroxyl radicals generated from the thermal degradation of 2,2'-azobis (2-methyl-propionamide) dihydrochloride (AAPH). A stock solution of FL (1.2 mM) was prepared dissolving 39.87 mg of FL in 100 mL of phosphate buffer (PBS) (75 mM, pH 7.4), and stored (darkness, 4ºC). A diluted solution (10 µM) was prepared from the FL stock solution, and a diluted solution (0.1 µM) was prepared daily taking 0.25 mL of the second solution and adjusting to 25 mL with PBS. The AAPH radical (200 mM) was prepared daily by taking 207 mg of AAPH and making it up to 5 mL with PBS. The reference standard used was a 1 mM trolox solution that was prepared in PBS from a 5

mM stock standard solution kept in the freezer at -20ºC. Aliquots of 25 µL of free and bound phytochemical extracts of ground samples diluted in PBS were transferred into 96-plate wells, loaded into the plate holder of a Microplate Reader (Synergy™ HT Multi-Detection, BioTek Inc, Winooski, VT, USA), where 150 µL of FL (0.1 µM) were dispensed, mixed and incubated at 37ºC during 30 min before adding 25 µL of AAPH. The reaction was performed at 37ºC and the fluorescence (485 nm for excitation and 538 nm for emission) was measured at 2 min intervals during 60 min. Calculation of protective effects of samples and control is from the net integrated areas under the fluorescence decay curves (AUC) [AUC_{sample} - $AUC_{control}$]. Results were expressed as micromoles of Trolox equivalent (µmol TE) g^{-1} sample, dw.

Mutagenicity and Antimutagenicity Testing

The microsuspension assay was used, which is a simple and sensitive modification of the Ames test and it is based on absolute amounts of the compound added per tube [41, 42]. Tester strain YG1024 was kindly provided by Dr. Takehiko Nohmi, Division of Genetics and Mutagenesis, Biological Safety Research Center, National Institute of Hygienic Sciences, Japan. The strain was maintained, propagated, routinely tested for genetic markers and re-isolated whenever necessary. For the assay 1-NP was dissolved in DMSO and the phenolic extracts from raw (RWCE) and extruded whole chickpea (EWCE) grains in water; bacteria were grown overnight in Oxoid Nutrient Broth No. 2 (Oxoid Ltd., Hants, UK) to approximately 1–2×10^9 cells mL^{-1} and harvested by centrifugation (4,500×g, 4°C, 10 min). Bacteria cells (1×10^{10} cells mL^{-1}) were resuspended in ice-cold PBS (0.15 M PBS, pH 7.4) after which ingredients were added in the following order to 12×75 mm sterile glass culture tubes kept on ice: 0.1 mL of cocktail, 0.1 mL of bacteria (1×10^{10} cells mL^{-1} PBS), 0.01 mL of RWCE or EWCE (100, 200, 300 and 500 µg/tube) or 0.005 mL of 1-NP (50 and 100 ng/tube) + 0.005 mL of RWCE or EWCE (100, 200, 300 and 500 µg/tube). The mixture was incubated in the dark at 37°C with vigorous shaking. After 90 min, the tubes were placed into an ice bath. Tubes were removed one at the time, and 2 mL of molten top agar containing 90 nmol of histidine and biotin were added. The combined solutions were vortex-mixed and poured onto minimal glucose plates. Plates were incubated at 37°C in the dark for 48 h and the colonies were counted. The plates were microscopically observed for thinning or absence of a background lawn and/ or presence of micro-colonies, which are considered indicators of toxicity induced by the test material. Samples were tested in triplicate for each independent experiment performed. The mixture was incubated for 90 min, mixed with top agar, and poured onto minimal glucose plates as previously described. Antimutagenicity was expressed as percentage of mutagenicity inhibition following the formula:

$$\% \text{ Inhibition} = 100 - [X_1/X_2(100)]$$

where X_1=number of revertants per plate in the presence of extract, expressed as equivalents of catechin; X_2=number of revertants per plate in the absence of extract. The slope values were used to calculate the mutagenic potency.

Statistical Analysis

The results were analyzed using one-way analysis of variance followed by Duncan´s multiple range test comparisons among means with significance level of 5%. Pearson correlation coefficient was used to determine correlations among means with a significance level of 10%.

RESULTS AND DISCUSSION

Total Phenolic Content (TPC)

The total phenolic content (*TPC*, calculated as the sum of free and bound phenolic) of raw and extruded desi chickpea cultivars are shown in Table **1**. In the case of raw chickpea grains, the highest *TPC* was obtained for Black ICC3761 [1.35 mg GAE g^{-1} sample (dw)], followed by Red ICC13124 [1.33 g GAE g^{-1} sample (dw)] and Brown ICC3512 [1.31 mg GAE g^{-1} sample (dw)]. These values were higher than those reported by Zia-Ul-Haq *et al*. [43]; they observed *TPC* in the range of 0.92 -1.12 mg GAE g^{-1} sample (dw) for four desi chickpea varieties indigenous to Pakistan. Other researchers reported that the *TPC* in whole seeds of desi chickpea cultivars varied from 1.5 to 6.8 mg GAE g^{-1} sample (dw) [44]. The observed differences in *TPC* could be attributed to the genetic background, grain physical properties and particularly to the seed coat color since the seed coat is the structure containing more phenolic compounds [45]. Our results show that most of the phenolics (70.92-82.44%) in raw desi chickpea seeds occurred in the bound or attached to cell wall form (Table **1**). Bioactive phytochemicals exist in free, soluble-conjugated, and bound forms; bound phytochemicals, mostly in cell wall materials, are difficult to digest in the upper gastrointestine and may be digested by bacteria in the

Table 1: Effect of Extrusion Cooking Process on the Phenolics Content of Desi Chickpea Cultivars

Processing / Cultivar	Phenolic compounds* (mg GAE g^{-1} sample, dw)		
	Free	Bound	Total
Raw			
Brown-ICC3512	0.29 ± 0.007^{F}	1.08 ± 0.017^{A}	1.31 ± 0.004^{F}
Red-ICC13124	0.31 ± 0.006^{E}	1.02 ± 0.013^{B}	1.33 ± 0.003^{E}
Black-ICC3761	0.34 ± 0.007^{D}	1.07 ± 0.014^{A}	1.35 ± 0.007^{D}
Extruded			
Brown-ICC3512	0.48 ± 0.009^{C}	0.95 ± 0.007^{D}	1.43 ± 0.008^{B}
Red-ICC13124	0.51 ± 0.008^{B}	0.89 ± 0.010^{E}	1.40 ± 0.007^{C}
Black-ICC3761	0.54 ± 0.011^{A}	0.98 ± 0.010^{C}	1.44 ± 0.005^{A}

*Data are expressed as means ± standard deviations.
$^{A-F}$Means with different superscripts in the same colum are significantly different (Duncan, $p \leq 0.05$).

colon to provide health benefits and reduce the risk of colon cancer [45, 46].

Extrusion cooking resulted in a significant increase (5.3-9.2%) of *TPC* in the three desi chickpea cultivars (Table **1**); this increase could be related with the extrusion temperature (155ºC) and the release of bound polyphenols, which decreased 8.4-12.8% during processing (Table **1**), or Maillard reaction products formed during extrusion that have been reported possess scavenging activity on reactive oxygen species [47,48]. Maillard reaction involves condensation reactions between sugars and amino acids and it has been found to be linked to polyphenols *via* inhibition of polyphenol oxidase [49]. Similar observations of increased *TPC* and antioxidant activity during processing under high temperatures (baking, frying, roasting) have been reported by Segev *et al.* [50].

Total Flavonoid Content (TFC)

Total flavonoid content (*TFC*, calculated as the sum of free and bound flavonoids) was analyzed in order to examine their potential role on the antioxidant activity of the selected desi chickpea cultivars, and how they are affected by the extrusion process. The *TFC* of raw and extruded desi chickpea cultivars are showed in Table **2**. Significant differences ($p<0.05$) were found among the cultivars studied. The highest *TFC* for raw desi chickpea grains was for Brown ICC3512 (1.006 mg CAE g^{-1} sample, dw), whereas the lowest value was observed for Black ICC3761 (0.464 mg CAE g^{-1} sample, dw). Other researchers [43] reported *TFC* in the range of 0.79 - 0.99 mg CAE g^{-1} sample, dw, for four desi chickpea varieties indigenous to Pakistan, which are close to those found in this study.

Extrusion cooking resulted in an increase (4.1-8.2%) of *TFC* in the three desi chickpea cultivars

Table 2: Effect of Extrusion Cooking Process on the Flavonoids Content of Desi Chickpea Cultivars

Processing / Cultivar	Flavonoids* (mg CAE g^{-1} sample, dw)		
	Free	Bound	Total
Raw			
Brown-ICC3512	0.255 ± 0.003^{B}	0.751 ± 0.012^{A}	1.006 ± 0.012^{B}
Red-ICC13124	0.162 ± 0.003^{E}	0.530 ± 0.006^{C}	0.692 ± 0.008^{D}
Black-ICC3761	0.122 ± 0.004^{F}	0.342 ± 0.007^{E}	0.464 ± 0.007^{F}
Extruded			
Brown-ICC3512	0.356 ± 0.004^{A}	0.691 ± 0.013^{B}	1.047 ± 0.011^{A}
Red-ICC13124	0.249 ± 0.005^{C}	0.493 ± 0.006^{D}	0.742 ± 0.010^{C}
Black-ICC3761	0.187 ± 0.003^{D}	0.315 ± 0.006^{F}	0.502 ± 0.00^{E}

*Data are expressed as means ± standard deviations.
$^{A-F}$Means with different superscripts in the same colum are significantly different (Duncan, $p \leq 0.05$).

(Table **2**); this increase could be related to the extrusion temperature (155ºC) and the release of bound flavonoids, which decreased 7.0-7.9% during processing (Table **2**), or Maillard reaction products formed during extrusion. Other researchers [50] studied the effect of three thermal treatments on *TFC* in colored chickpea seeds and reported that baking, frying and roasting colored seeds had higher *TFC* than raw grains.

Flavonoids are common constituents of legumes, and they can provide health-promoting functions. Epidemiological research suggested that flavonoid intake is positively associated with a reduction in the risks of coronary heart disease and certain types of cancer [51] induced by free radicals. The antioxidative properties of flavonoids are considered to be due to radical scavenging by donating hydrogen. Metal-chelating is another feature of certain flavonoids, and those with the catechol structure in the B-ring or probably with both 5-hydroxyl and 4-oxo groups can suppress the iron - or copper- catalyzed Fenton reaction [52,53]. The importance of the antioxidant constituents of legumes in the maintenance of health and nutritive value of food is also increasingly of interest among food manufacturers and consumers as the future trend toward developing functional food [54].

Antioxidant Activity (AOA)

Table **3** shows the total hydrophilic antioxidant activity (*AOA*) (sum of antioxidant capacities of free and bound phenolic) or ORAC values of raw and extruded desi chickpea cultivars. The ORAC values of raw desi chickpea ranged from 54.9 µmol TE g^{-1} sample, dw (Black ICC3761) to 57.3 µmol TE g^{-1} sample, dw (Brown ICC3512). ORAC values ranging from 8.58 to 11.40 µmol TE g^{-1} sample, dw, have been reported by other researchers for desi chickpea varieties from Pakistan [53] which were approximately five times lower than those found in this study. These differences may be attributed partly to the chickpea and the quantification methods used.

Processing of the whole raw desi chickpea grains using extrusion cooking increased ($p<0.05$) the total ORAC value of the extruded kernels when compared with the unprocessed materials [61.6-63.3 vs 54.9-57.3 µmol Trolox equivalent (TE) g^{-1} sample (dw)] (Table **3**). It was also observed that the ORAC value of free phenolic compounds significantly increased ($p<0.05$) and ORAC value of bound phenolic decreased ($p<0.05$) in extruded grains (Table **3**). This behavior could be attributed to (i) breaking of conjugated phytochemicals and release of free phytochemicals [35], (ii) prevention of enzymatic oxidation and, (iii) darker colors of the extruded grains indicating formation of Maillard reaction products having antioxidant properties [55]. Our results show that free phenolics were the primary contributors to ORAC value in both unprocessed (62.13-66.37%) and extruded (68.56-72.49%) desi chickpea grains (Table **3**).

The ORAC method is usually employed to estimate the *AOA* of foods and to evaluate *in vivo* responses to dietary antioxidant manipulations; it is the only method so far that combines both inhibition time and degree of inhibition into a single quantity [56]. The US Department of Agriculture, and the food and nutraceutical industries have accepted this method to the point that some manufacturers now include ORAC values on the product labels [57-59].

Other researchers [60] studied the antioxidant activity in extruded products prepared from purple potato and dry pea flours and they observed that extruded products had significant higher ($p<0.05$) ORAC antioxidant activities compared to raw

Table 3: Effect of Extrusion Cooking Process on the Hydrophilic Antioxidant Activity of Desi Chickpea Cultivars

Processing / Cultivar	Hydrophilic antioxidant activity* (µmol TE g^{-1} sample, dw)		
	Free	Bound	Total
Raw			
Brown-ICC3512	35.6 ± 0.5^{E}	21.7 ± 0.4^{A}	57.3 ± 1.1^{C}
Red-ICC13124	37.3 ± 0.7^{D}	18.9 ± 0.4^{E}	56.2 ± 1.3^{C}
Black-ICC3761	34.2 ± 0.6^{F}	20.7 ± 0.4^{B}	54.9 ± 1.1^{D}
Extruded			
Brown-ICC3512	43.4 ± 0.6^{B}	19.9 ± 0.3^{C}	63.3 ± 1.0^{A}
Red-ICC13124	44.8 ± 0.7^{A}	17.0 ± 0.2^{F}	61.8 ± 1.2^{B}
Black-ICC3761	42.4 ± 0.7^{C}	19.2 ± 0.5^{D}	61.6 ± 0.9^{B}

*Data are expressed as means ± standard deviations.
$^{A-F}$Means with different superscripts in the same colum are significantly different (Duncan, $p \leq 0.05$).

formulations. Other researchers [50] studied the effect of three thermal treatments on antioxidant activity in colored chickpea seeds; their results indicated that baking, frying and roasting colored seeds result in a significant increase of antioxidant activity when compared with unprocessed samples.

A high correlation (Pearson´s correlation coefficient r=0.942; p=0.005) was found between *TPC* of raw and extruded desi chickpea flours and their total hydrophilic *AOA*. The positive linear correlation obtained is considered extremely significant due to the high coefficient of determination (r^2=0.887). A similar effect has been found in raw grains by other researchers [20, 44, 56, 61-64]. Phenolic compounds are considered the major compounds that contribute to the total antioxidant activities of grains [63-65]. These compounds have been associated with a reduction in the risk of cancer, heart disease and diabetes, but they also have antibacterial, antiviral, anti-inflammatory and anti-allergenic activities; most of these benefits result from their *AOA* [66].

Antimutagenic Activity (AMA)

The phenolic extracts from raw (RWCE) and extruded whole chickpea (EWCE) grains were not toxic and not mutagenic to the bacteria at the concentrations tested; RWCE and EWCE produced a number of revertant colonies of 190.7-259.3 and 152.5-220.5 per plate, respectively (Table **4**). The *AMA* of RWCE, evaluated using 500 mg/tube against 1-NP in tester strain TA98 of *Salmonella typhimurium* and expressed as % inhibition, ranged from 57.8 % (Brown-ICC3512) to 62.3 % (Red-ICC13124). Others [67] evaluated *AMA* in acetonic extracts from three common bean varieties, unprocessed and processed, ranging from 50.0 to 78.6%.

Processing of the whole raw desi chickpea grains using extrusion cooking increased ($p<0.05$) in 17.5-21.98% the *AMA* values of the extruded kernels when compared with the unprocessed materials (68.1-73.2 vs 57.8-62.3% inhibition) (Table **4**).

AMA in raw and extruded desi chickpea cultivars were highly correlated with their *TPC and AOA* (*AMA-TPC*: Pearson's correlation coefficient r=0.826; p=0.043; *AMA-AOA*: Pearson´s correlation coefficient r=0.955; p=0.003). The positive linear correlations obtained are considered extremely significant due to the high coefficients of determination (*AMA-TPC*: r^2=0.6823; *AMA-AOA*-: r^2=0.912). Rocha-Guzman *et al*. [67] reported a high correlation between total phenol content and antioxidant and antimutagenic activities for acetone extracts from common bean in agreement with our results. Others [68] also reported that newly harvested beans (highest phenolic compounds content) showed higher antimutagenic activity against aflatoxin B_1 mutagenicity than stored beans (lower phenolic content).

The correlations obtained indicate that phenolic compounds were responsible for the antioxidant and antimutagenic activity exhibited in this study. Thus the total phenolic content can be used to predict the ability of the phenolic extracts to scavenge DPPH radical and to decrease the mutagenicity induced by 1-NP. Therefore, raw and extruded desi chickpea cultivars can be considered an important source of phenolic compounds, which exhibit antioxidant and antimutagenic activity. Thus they may be helpful in the

Table 4: Effect of Extrusion Cooking Process on Antimutagenic Activity of Desi Chickpea Cultivars Evaluated in the Lyophilized Methanolic Extract Using 500 mg/tube Against 1-NP in Tester Strain TA98 of *Salmonella typhimurium*

Processing / Cultivar	Antimutagenic activity*	
	Revertants/plate	% inhibition
Raw		
Brown-ICC3512	247.9 ± 13.1^{A}	57.8 ± 1.71^{E}
Red-ICC13124	190.7 ± 10.2^{C}	62.3 ± 0.98^{D}
Black-ICC3761	259.3 ± 12.4^{A}	57.9 ± 1.03^{E}
Extruded		
Brown-ICC3512	203.3 ± 8.5^{BC}	70.5 ± 0.94^{B}
Red-ICC13124	152.5 ± 11.5^{D}	73.2 ± 1.14^{A}
Black-ICC3761	220.5 ± 12.6^{B}	68.1 ± 1.37^{C}

*Data are expressed as means ± standard deviations.
$^{A-E}$Means with different superscripts in the same colum are significantly different (Duncan, $p \leq 0.05$).

prevention of degenerative diseases such as cancer. However, further work is required to determine mechanisms involved in the antioxidant and antimutagenic effects. In addition, more *in vivo* evidence and identification of active phenolics involved are needed [67].

CONCLUSIONS

Our results indicate that optimized extrusion cooking process might be applied to produce precooked instant extruded flours with significantly higher levels of total phenolics and flavonoids content, and antioxidant and antimutagenic activities than unprocessed desi chickpea flours. Thus, extruded desi chickpea flours might be considered as a functional food in addition to its traditional role of providing dietary proteins. Due to their high antioxidant and antimutagenic activities, extruded pigmented desi chickpea flours might also contribute significantly to the management and/or prevention of degenerative diseases associated with free radical damage.

ACKNOWLEDGEMENTS

This research was supported by FOMIX-CONACyT (Fondos Mixtos del Gobierno del Estado de Sinaloa, México y Consejo Nacional de Ciencia y Tecnología) and PROFAPI-UAS (Programa de Fortalecimiento y Apoyo a Proyectos de Investigación, Universidad Autónoma de Sinaloa, México) 2011, 2012.

REFERENCES

[1] Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. J Nutr 2000; 130: 2073S-85S.

[2] Robbins RJ. Phenolic acids in foods: an overview of analytical methodology. J Agr Food Chem 2003; 51: 2866-87. http://dx.doi.org/10.1021/jf026182t

[3] Loarca-Piña G, Kuzmicky PA, González de Mejía E, Kado NY, Hsich DPH. Antimutagenicity of ellagic acid against aflatoxin B1 in the *Salmonella* microsuspension assay. Mutat Res 1986; 360: 15-21.

[4] Gonzalez de Mejia E, Castaño-Tostado E, Loarca-Piña G. Antimutagenic effects of natural phenolic compounds in beans. Mutat Res 1999; 441: 1-9. http://dx.doi.org/10.1016/S1383-5718(99)00040-6

[5] Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr Cancer 1992; 18: 1-29. http://dx.doi.org/10.1080/01635589209514201

[6] Powles JW, Ness AR. Fruit and vegetables, and cardiovascular disease: a review. Int J Epidem 1996; 26: 1-13.

[7] Singh RP, Chidambara-Murthy KN, Jayaprakasha GK. Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. J Agri Food Chem 2002; 50: 81-6. http://dx.doi.org/10.1021/jf010865b

[8] Ibáñez E, Kubatova A, Señorans FJ, Casvero S, Reglero G, Hawthorne SB. Subcritical water extraction of antioxidant compounds from rosemary plants. J Agri Food Chem 2003; 51: 375-82. http://dx.doi.org/10.1021/jf025878j

[9] Doll R. The lessons of life: keynote address to the nutrition and cancer conference. Cancer Res 1992; 52: 2024s-9s.

[10] Benigni R. Structure-activity relationship studies of chemical mutagens and carcinogens: mechanistic investigations and prediction approaches. Chemical Rev 2005; 105: 1767-800. http://dx.doi.org/10.1021/cr030049y

[11] Ames BN, Durston WE, Yamasaki E, Lee FD. Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proceedings of the National Academy of Sciences *USA* 1973; 70: 2281-5. http://dx.doi.org/10.1073/pnas.70.8.2281

[12] Shankel DM, Kuo S, Haines C, Mitscher LA. Extracellular interception of mutagens. In: Bronzetti G, Hayatsu H, De Flora S, Waters MD, Shankel DM (eds), Antimutagenesis and anticarcinogenesis mechanisms III. Plenum, New York, USA, 1993; p. 65-74. http://dx.doi.org/10.1007/978-1-4615-2984-2_5

[13] Johnson IT. Antioxidants and antitumor properties. In: Pokormy J, Yanishlieva N, Gordon M (eds), Antioxidants in food. CRC, Boca Raton FL, USA, 2003; p. 100-23.

[14] Knasmüller S, Majer BJ, Buchmann C. Identifying antimutagenic constituents of food. In: Remacle C, Reusens B (eds) Functional foods, ageing and degenerative disease. CRC, Boca Raton FL, USA, 2004; p. 581-14. http://dx.doi.org/10.1533/9781855739017.4.581

[15] Mermelstein R, Kiriazides DK, Butler M, McCoy EC, Rosenkranz HS. The extraordinary mutagenicity of nitropyrenes in bacteria. Mutat Res 1981; 89: 187-96. http://dx.doi.org/10.1016/0165-1218(81)90236-6

[16] Rosenkranz HS, Mermelstein R. Mutagenecity and genotoxicity of nitroarenes. All nitro-containing chemicals were not created equal. Mutat Res 1983; 114: 217-67. http://dx.doi.org/10.1016/0165-1110(83)90034-9

[17] Watanabe M, Ishidate M Jr, Nohmi T. Sensitive method for detection of mutagenic nitroarenes and aromatic amines: new derivatives of *Salmonella typhimurium* tester strains possessing elevated o-acetyl transferase levels. Mutat Res 1990; 234: 337-48. http://dx.doi.org/10.1016/0165-1161(90)90044-O

[18] Josephy PD, Lord HL, Snieckus VA. Inhibition of benzowaxpyrene dihydrodiol epoxide mutagenicity by synthetic analogues of ellagic acid. Mutat Res 1992; 242: 143-9.

[19] FAOSTAT, "Statistical database" 2011 [homepage on the Internet] [cited 2011 October 28]: Available from: http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

[20] Han H, Baik B-K. Antioxidant activity and phenolic content of lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.), peas (*Pisum sativum* L.) and soybeans (*Glycine max*) and their quantitative changes during processing. Int J Food Sci and Technol 2008; 43: 1971-8. http://dx.doi.org/10.1111/j.1365-2621.2008.01800.x

[21] Campos-Vega R, Loarca-Piña G, Oomah BD. Minor components of pulses and their potential impact on human health. Food Res Int 2012; 43: 461-82. http://dx.doi.org/10.1016/j.foodres.2009.09.004

[22] Aguilera Y, Dueñas M, Estrella I, Hernández T, Benítez V, Esteban RM *et al*. Phenolic profile and antioxidant capacity of chickpeas (*Cicer arietinum* L) as affected by a dehydration process. Plant Foods Hum Nutr 66: 187-95.

[23] Ricketts ML, Moore DD, Banz WJ, Mezeiand O, Shay NF. Molecular mechanisms of action of the soy isoflavones

includes activation of promiscuous nuclear receptors. A review. J Nutr Biochem 2005; 16: 321-30. http://dx.doi.org/10.1016/j.jnutbio.2004.11.008

[24] Messina M, McCaskill-Stevens W, Lampe JW. Addressing the soy and breast cancer relationship: Review, commentary, and workshop proceedings. J Nat Cancer Inst 2006; 98: 1275-84. http://dx.doi.org/10.1093/jnci/djj356

[25] Trock BJ, Hilakivi-Clarke L, Clarke R. Meta-analysis of soy intake and breast cancer risk. J Nat Cancer Inst 2006; 98: 459-71. http://dx.doi.org/10.1093/jnci/djj102

[26] Nizakat B, Khattak AB, KhattakGSS, Mehmood Z, Ihsanullah I. Quality and consumers acceptability studies and their inter-relationship of newly evolved desi type chickpea genotypes (*Cicer arietinum* L.). Quality evolution of new chickpea genotypes. Int J Food Sci Technol 2007; 42: 528-34. http://dx.doi.org/10.1111/j.1365-2621.2006.01246.x

[27] Chau CF, Cheung PC, Wong YS. Effect of cooking on content of amino acids and antinutrients in three chinese indigenous legume seeds. J Sci Food Agri 1997; 75: 447-52. http://dx.doi.org/10.1002/(SICI)1097-0010(199712)75:4<447::AID-JSFA896>3.0.CO;2-5

[28] Milán-Carrillo J, Reyes-Moreno C, Camacho-Hernández IL, Rouzaud-Sandez O. Optimization of extrusion process to transform hardened chickpeas (*Cicer arietinum* L) into a useful product. J Sci Food Agri 2002; 82: 1718-28. http://dx.doi.org/10.1002/jsfa.1242

[29] Xu BJ, Chang SKC. Effect of soaking, boiling and steaming on total phenolic content and antioxidant activities of cool season food legumes. Food Chem 2008; 110: 1-13. http://dx.doi.org/10.1016/j.foodchem.2008.01.045

[30] Segev A, Badani H, Galili L, Hovav R, Kapulnik Y, Shomer I *et al*. Total phenolic content and antioxidant activity of chickpea (*Cicer arietinum* L) as affected by soaking and cooking conditions. Food Nut Sci 2011; 2: 724-30. http://dx.doi.org/10.4236/fns.2011.27099

[31] Gutiérrez-Dorado R, Ayala-Rodríguez AE, Milán-Carrillo J, López-Cervantes JA, Garzón-Tiznado JA, *et al*. Technological and nutritional properties of flours and tortillas from nixtamalized and extruded quality protein maize (*Zea mays* L). Cereal Chem 2008; 85: 808-16. http://dx.doi.org/10.1094/CCHEM-85-6-0808

[32] Vasanthan T, Yeung J, Hoover R. Dextrinization of starch in barley flours with termostable alpha-amylase by extrusion cooking. Starch/Stârke 2001; 53: 616-22. http://dx.doi.org/10.1002/1521-379X(200112)53:12<616::AID-STAR616>3.0.CO;2-M

[33] AOAC. Official Methods of Analysis 16th edn, Association of Official Analytical Chemists. Washington, DC, USA; 1999.

[34] Espinoza-Moreno. Desarrollo de nuevos productos de valor nutricional / nutracéutico alto a partir de maíz, garbanzo y frijol. M.Sc. Thesis 2011, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa, México.

[35] Dewato V, Wu X, Liu RH. Processed sweet corn has higher antioxidant activity. J Agri Food Chem 2002; 50: 4959-64. http://dx.doi.org/10.1021/jf0255937

[36] Mora-Rochín S, Gutiérrez-Uribe JA, Serna-Saldívar SO, Sánchez-Peña P, Reyes-Moreno C, Milán-Carrillo J. Phenolic content and antioxidant activity of tortillas produced from pigmented maize processed by conventional nixtamalization or extrusion cooking. J Cereal Sci 2010; 52: 502-8. http://dx.doi.org/10.1016/j.jcs.2010.08.010

[37] Singleton VL, Orthoferand R, and Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 1999; 299: 152-78. http://dx.doi.org/10.1016/S0076-6879(99)99017-1

[38] Heimler D, Vignolini P, Diniand MG, Romani A. Rapid tests to assess the antioxidant activity of *Phaseolus vulgaris* L. dry beans. J Agri Food Chem 2005; 53: 3053-6. http://dx.doi.org/10.1021/jf049001r

[39] Zia-Ul-Haq M, Iqbal S, Ahmad S, Imran M, Niaz A, Bhanger MI. Nutritional and compositional study of desi chickpea (*Cicer arietinum* L.) cultivars grown in Punjab, Pakistan. J Food Chem 2007; 105: 1357-63. http://dx.doi.org/10.1016/j.foodchem.2007.05.004

[40] Ou B, Hampseh-Woodill M, Prior RL. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. J Agric Food Chem 2001; 49: 4619-26. http://dx.doi.org/10.1021/jf010586o

[41] Kado NY, Langley D, Eisenstadt E. A simple modification of the Salmonella liquid incubation assay. Increased sensitivity for detecting mutagens in human urine. Mutat Res 1983; 121: 25-32. http://dx.doi.org/10.1016/0165-7992(83)90082-9

[42] Kado NY, Guiguis GN, Flessel CP, Chan RC, Chang K, Wselowski JJ. Mutagenicity of fine (<2.5 mm) airborne particles: diurnal variation in community air determined by *Salmonella* micro preincubation procedure. Environ Mol Mutagen 1986; 8: 53-66.

[43] Zia-Ul-Haq M, Iqbal S, Ahmad S, Bhanger MI, Wiczkowskiand W, Amarowicz R. Antioxidant potential of desi chickpea varieties commonly consumed in Pakistan. J Food Lipids 2008; 15: 326-42. http://dx.doi.org/10.1111/j.1745-4522.2008.00122.x

[44] Adom KK, Liu RH. Antioxidant activity of grains. J Agric Food Chem 2002; 50: 6182-7. http://dx.doi.org/10.1021/jf0205099

[45] Segev A, Badani H, Kapulnik Y, Shomer I, Oren-Shamir M, Galili S. Determination of polyphenols, flavonoids, and antioxidant capacity in colored chickpea (*Cicer arietinum* L.). J Food Sci 2010; 75: S115-9. http://dx.doi.org/10.1111/j.1750-3841.2009.01477.x

[46] Liu RH. Whole grain phytochemicals and health. J Cereal Sci 2007; 46: 207-219. http://dx.doi.org/10.1016/j.jcs.2007.06.010

[47] Hayase F, Hirashima S, Okamoto G, Kato H. Scavenging of active oxygens by melanoidins. Agric Biol Chem 1990; 54: 855-62.

[48] Yen GC, Hsieh PP. Antioxidative activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction products. J Sci Food Agric 1995; 67*:* 415-20. http://dx.doi.org/10.1002/jsfa.2740670320

[49] Lee MK, Park I. Inhibition of potato polyphenol oxidase by Maillard reaction products. Food Chem 2005; 91: 57-61. http://dx.doi.org/10.1016/j.foodchem.2004.05.046

[50] Segev a, Badani H, Galili K, Hovav R, Kapulnik Y, Shomer I *et al*. Effects of baking, roasting and frying on total polyphenols and antioxidant activity in colored chickpea seeds. Food Nutr Sci 2012; 3: 369-76. http://dx.doi.org/10.4236/fns.2012.33053

[51] Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, *et al*. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. American J Med 2002; 113 (Suppl 9B): 71S-88S. http://dx.doi.org/10.1016/S0002-9343(01)00995-0

[52] Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine 1996; 20: 933-56. http://dx.doi.org/10.1016/0891-5849(95)02227-9

[53] Mira L, Fernández TM, Santos M, Rocha R, Florencio HM, Jennings KR. Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity. Free Radic Res 2002; 36: 1199-8. http://dx.doi.org/10.1080/1071576021000016463

[54] Ren SC, Liu ZL, Wang P. Proximate composition and flavonoids content and *in vitro* antioxidant activity of 10 varieties of legume seed grown in China. J Med Plants Res 2012; 6: 301-8.

[55] Fares C, Menga V. Effects of toasting on the carbohydrate profile and antioxidant properties of chickpea (*Cicer arietinum* L) flour added to durum wheat. Food Chem 2012; 131: 1140-8. http://dx.doi.org/10.1016/j.foodchem.2011.09.080

[56] Cao GH, Prior RL. Measurement of oxygen radical absorbance capacity in biological samples. Methods Enzymol 1999; 299: 50-62. http://dx.doi.org/10.1016/S0076-6879(99)99008-0

[57] Bank G, Schauss A. Antioxidant testing: An ORAC update. Nutraceuticals World 2004 [article on the Internet]: [cited 2012 Oct 2]: Available from: http://www.nutraceuticalsworld.com/march042.htm

[58] USDA. Antioxidants and Health, ACES publications, 2010; p. 4.

[59] USDA. Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods. United States Department of Agriculture, USA, 2007; p. 1-34.

[60] Nayak B, Liu RH, Berrios J de J, Tang J, Derito C. Bioactivity of antioxidants in extruded products prepared from purple potato and dry pea flours. J Agric Food Chem 2011; 59: 8233-43. http://dx.doi.org/10.1021/jf200732p

[61] Xu BJ, Yuan SH, Chang SKC. Comparative analyses of phenolic composition, antioxidant capacity and color of cool season legumes and other selected food legumes. J Food Sci 2007a; 72: 167-77. http://dx.doi.org/10.1111/j.1750-3841.2006.00261.x

[62] Xu BJ, Yuan SH, Chang SKC. Comparative studies on the antioxidant activities of nine common food legumes against copper - induced human low - density lipoprotein oxidation *in vitro.* J Food Sci 2007b; 72: 522-7. http://dx.doi.org/10.1111/j.1750-3841.2007.00464.x

[63] Yao Y, Sang W, Zhou M, Ren G. Antioxidant and α-glucosidase inhibition of colored grains in China. J Agric Food Chem 2010; 58: 770-4. http://dx.doi.org/10.1021/jf903234c

[64] Yao Y, Cheng X, Wang L, Wang S, Ren G. Biological potential of sixteen legumes in China. Int J Mol Sci 2011; 12: 7048-58. http://dx.doi.org/10.3390/ijms12107048

[65] Garzón-Tiznado JA, Ochoa-Lugo MI, Heiras-Palazuelos MJ, Domínguez-Arispuro DM, Cuevas-Rodríguez EO, Gutiérrez-Dorado R, *et al*. Acceptability properties and antioxidant potential of desi chickpea (*Cicer arietinum* L) cultivars. Food Nutr Sci 2012; 3: 1281-9. http://dx.doi.org/10.4236/fns.2012.39169

[66] Randhir R, Shetty K. Mung beans processed by solid-state bioconversion improves phenolic content and functionality relevant for diabetes and ulcera management. Innovation Food Sci Eng Technol 2007; 8: 97-04.

[67] Rocha-Guzmán NE, Herzog A, González-Laredo RF, Ibarra-Pérez FJ, Zambrano-Galván G, Gallegos-Infante JA. Antioxidant and antimutagenic activity of phenolic compounds in three different colour groups of common bean cultivars (*Phaseolus vulgaris*) Food Chem 2007; 521-7. http://dx.doi.org/10.1016/j.foodchem.2006.08.021

[68] Aparicio-Fernández X, Manzo-Bonilla L, Loarca-Piña GF. Comparison of antimutagenic activity of phenolic compounds in newly harvested and stored common beans *Phaseolus vulgaris* against aflatoxin B1. J Food Sci 2005; 70: 73-8. http://dx.doi.org/10.1111/j.1365-2621.2005.tb09068.x

Received on 23-10-2012 Accepted on 20-12-2012 Published on 23-01-2013

DOI: http://dx.doi.org/10.6000/1927-5951.2013.03.01.5