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

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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 B₁ [3], benzo[a]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-acetyltransferase (OAT) which is present in bacterial cells [15-17]. Benzo[a]pyrene is a polycyclic aromatic
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-amidinopropane), (+)-catechin, fluorescein (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 4°C. Prior to extrusion, grits were tempered at 25°C 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 (25°C, 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 4°C 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 45°C. The resulting extracts were frozen at -20°C 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 95°C for 30 min with previous removal of O2 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 35°C. 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 -20°C 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, 4°C). 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 -20°C. 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 37°C during 30 min before adding 25 μL of AAPH. The reaction was performed at 37°C 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 1–2×10⁹ cells mL⁻¹ and harvested by centrifugation (4,500×g, 4°C, 10 min). Bacteria cells (1×10⁸ 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 (1×10¹⁰ 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:

\[
\text{% Inhibition} = 100 - \left[ \frac{X_1}{X_2} \right] \times 100
\]

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 gastrointestinal and may be digested by bacteria in the
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].

<table>
<thead>
<tr>
<th>Processing / Cultivar</th>
<th>Phenolic compounds* (mg GAE g⁻¹ sample, dw)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Free</td>
</tr>
<tr>
<td>Raw</td>
<td></td>
</tr>
<tr>
<td>Brown-ICC3512</td>
<td>0.29 ± 0.007^E</td>
</tr>
<tr>
<td>Red-ICC13124</td>
<td>0.31 ± 0.006^E</td>
</tr>
<tr>
<td>Black-ICC3761</td>
<td>0.34 ± 0.007^D</td>
</tr>
<tr>
<td>Extruded</td>
<td></td>
</tr>
<tr>
<td>Brown-ICC3512</td>
<td>0.48 ± 0.009^C</td>
</tr>
<tr>
<td>Red-ICC13124</td>
<td>0.51 ± 0.008^B</td>
</tr>
<tr>
<td>Black-ICC3761</td>
<td>0.54 ± 0.011^A</td>
</tr>
</tbody>
</table>

*Data are expressed as means ± standard deviations.
^Means with different superscripts in the same column are significantly different (Duncan, p ≤ 0.05).

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>
<thead>
<tr>
<th>Processing / Cultivar</th>
<th>Flavonoids* (mg CAE g⁻¹ sample, dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free</td>
</tr>
<tr>
<td>Raw</td>
<td></td>
</tr>
<tr>
<td>Brown-ICC3512</td>
<td>0.255 ± 0.003^B</td>
</tr>
<tr>
<td>Red-ICC13124</td>
<td>0.162 ± 0.003^E</td>
</tr>
<tr>
<td>Black-ICC3761</td>
<td>0.122 ± 0.004^E</td>
</tr>
<tr>
<td>Extruded</td>
<td></td>
</tr>
<tr>
<td>Brown-ICC3512</td>
<td>0.356 ± 0.004^A</td>
</tr>
<tr>
<td>Red-ICC13124</td>
<td>0.249 ± 0.005^C</td>
</tr>
<tr>
<td>Black-ICC3761</td>
<td>0.187 ± 0.003^D</td>
</tr>
</tbody>
</table>

*Data are expressed as means ± standard deviations.
^Means with different superscripts in the same column are significantly different (Duncan, p ≤ 0.05).
Antioxidant and Antimutagenic Activities of Optimized Extruded

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 antioxidative 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

<table>
<thead>
<tr>
<th>Processing / Cultivar</th>
<th>Hydrophilic antioxidant activity* (μmol TE g⁻¹ sample, dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free</td>
</tr>
<tr>
<td>Raw</td>
<td></td>
</tr>
<tr>
<td>Brown-ICC3512</td>
<td>35.6 ± 0.5⁶</td>
</tr>
<tr>
<td>Red-ICC3124</td>
<td>37.3 ± 0.7₂</td>
</tr>
<tr>
<td>Black-ICC3761</td>
<td>34.2 ± 0.6⁵</td>
</tr>
<tr>
<td>Extruded</td>
<td></td>
</tr>
<tr>
<td>Brown-ICC3512</td>
<td>43.4 ± 0.6⁸</td>
</tr>
<tr>
<td>Red-ICC3124</td>
<td>44.8 ± 0.7⁵</td>
</tr>
<tr>
<td>Black-ICC3761</td>
<td>42.4 ± 0.7⁵</td>
</tr>
</tbody>
</table>

*Data are expressed as means ± standard deviations.

Means with different superscripts in the same column are significantly different (Duncan, p ≤ 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***

<table>
<thead>
<tr>
<th>Processing / Cultivar</th>
<th>Antimutagenic activity*</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Revertants/plate</td>
<td></td>
</tr>
<tr>
<td><strong>Raw</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brown-ICC3512</td>
<td>247.9 ± 13.1$^a$</td>
<td>57.8 ± 1.71$^b$</td>
</tr>
<tr>
<td>Red-ICC13124</td>
<td>190.7 ± 10.2$^c$</td>
<td>62.3 ± 0.98$^b$</td>
</tr>
<tr>
<td>Black-ICC3761</td>
<td>259.3 ± 12.4$^a$</td>
<td>57.9 ± 1.03$^f$</td>
</tr>
<tr>
<td><strong>Extruded</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brown-ICC3512</td>
<td>203.3 ± 8.5$^{cd}$</td>
<td>70.5 ± 0.94$^b$</td>
</tr>
<tr>
<td>Red-ICC13124</td>
<td>152.5 ± 11.5$^c$</td>
<td>73.2 ± 1.14$^d$</td>
</tr>
<tr>
<td>Black-ICC3761</td>
<td>220.5 ± 12.6$^d$</td>
<td>68.1 ± 1.37$^d$</td>
</tr>
</tbody>
</table>

*Data are expressed as means ± standard deviations.

$^a$-$^e$ Means with different superscripts in the same column 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.

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