

Effects of Baking, Roasting and Frying on Total Polyphenols and Antioxidant Activity in Colored Chickpea Seeds

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ABSTRACT

Chickpea lines with colored testa (seed coat) contain high levels of polyphenolic compounds that exhibit high levels of antioxidant activity. In a previous study, we showed that common processing procedures, such as soaking and cooking, decrease the levels of these bioactive compounds and subsequent overall antioxidant activity. The observed reduction in total phenolic content was due to the movement of polyphenols from the seed coat to the soaking or cooking water. Here, the effects of baking, roasting and frying processes were examined in relation to total phenolic content (TPC), total flavonoid content (TFC) and ferric-reducing ability of plasma antioxidant activity (FRAP AA) of colored chickpea seeds. Baked, fried and roasted colored chickpea seeds had significantly higher levels of TPC, TFC and FRAP AA than regular cream- and beige-colored seeds subjected to the same treatments. In contrast to our previous results with soaking and cooking, baking, frying and roasting retained most of the TPC, TFC and FRAP AA in the final products. Thus, colored chickpeas subjected to these three processing methods might be considered a functional food in addition to its traditional role of providing dietary proteins.

Keywords: Colored Chickpea Seed; FRAP; Processing; Total Phenolics; Total Flavonoids

1. Introduction

Chickpea (*Cicer arietinum* L.) is an annual grain legume (pulse crop) that is extensively cultivated for human consumption throughout the world, including the Mediterranean basin, the Near East, Central and South Asia, East Africa, South and North America, and Australia. It is the second-most important pulse crop in the world (after dry bean), covering 15% (10.2 million ha) of the area dedicated to pulse cultivation and accounting for 14% (7.9 million tons) of pulse production worldwide (FAOSTAT, <http://faostat.fao.org/default.aspx>). In Israel, chickpea is the main pulse crop, grown on about 6,000 to 10,000 ha spread throughout the country. Chickpea seeds vary in size, shape and color [1]. Based on these variations, chickpea cultivars are classified into two categories: kabuli and desi [2]. The kabuli-type seeds have a thin seed coat ranging in color from white to cream and a 100-seed weight of 28 to 70 g. Desi-type chickpea seeds have a thicker, irregularly shaped seed coat, which ranges in color from light tan to black, and a 100-seed weight of not more than 28 g.

Dry legumes, including chickpea, are a good source of protein, dietary fibers, minerals and bioactive polyphenols [3,4], and they also contribute to polyphenol intake from other foods [5]. The antioxidant capacity [6], and antimutagenic [7-10], apoptosis-related [11] and antiproliferative effects of legumes are associated with the presence of phenolic compounds in the seeds [11-13]. The abundance of phenolic compounds implies that legumes, such as the common bean (*Phaseolus vulgaris*) [9], faba (broad) bean (*Vicia faba*) [14,15], beach pea (*Lathyrus maritimus*) [16], mung bean (*Vigna radiata*) [17], lentil (*Lens culinaris*) [18], peanuts [19] and chickpea (*Cicer arietinum*) [20-22] may be significant food sources for active antioxidants.

Amounts of polyphenols and flavonoids and antioxidant activity vary greatly among chickpea lines [4]. For example, in a previous study with chickpea, we found that isolated hulls from colored chickpea lines contain higher amounts of polyphenols and flavonoid compounds than those of regular beige-colored seeds, and exhibit high levels of antioxidant activity [4]. This variation in both seed coat color and antioxidant activity makes colored

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chickpea a strong potential subject of study as a functional food.

Chickpea is usually consumed after cooking [23], frying [24], baking [25] or roasting [23,26]. These processing procedures improve the flavor and palatability of the food product and increase its nutritional bioavailability by inactivating antinutritional factors [27,28]. However, they may also affect its bioactive compounds and antioxidant activity [20,21]. The objective of this study was to determine the effects of baking, frying and roasting on levels of bioactive compounds (polyphenols and flavonoids) and antioxidant activity *in vitro*, in different food stuffs made from chickpea accessions with different-colored seed coats to determine their respective potentials for use as functional foods.

2. Materials and Methods

2.1. Plant Materials

Two chickpea lines were used in this study. Line 731, a desi type with black seed coat, originally derived from the National Germplasm Resources Laboratory of the USDA-ARS, Beltsville, MD, USA, and cv. Zehavit, a commercial Israeli kabuli cultivar with cream seed coat. Both lines were produced in our laboratory.

2.2. Baking

Baking was performed in a digital breadmaker (Morphy Richards 48290) utilizing the basic white bread setting for 3 h that included: kneading for 10 min at room temperature (RT), rising for 20 min at RT, second kneading for 15 min at 30°C, rising for 70 min at 32°C and baking for 65 min at 121°C. Chickpea flour was made by grinding the seeds to a fine powder (60 mesh). Chickpea bread was made from dough containing 400 g chickpea flour and 225 ml tap water. Samples were taken from the dough, and from the crust and inside parts of the baked bread. All samples were freeze-dried and kept at 4°C until use.

2.3. Frying

Frying was performed in a deep fryer (Graetz DF-730). Chickpea seeds (70 g) were soaked at RT in 500 ml of tap water overnight. Soaked seeds were then removed from the soaking water and ground with a small amount of tap water (about 10 ml) in a blender. Small balls (falafel balls) of this dough were fried in canola oil preheated to 190°C for 5, 10 and 15 min. Samples were taken from the soaked seeds, dough, and inside and crusted parts of the falafel ball. All samples were freeze-dried, defatted and kept at 4°C until use.

2.4. Roasting

Two types of roasting (wet and dry) were performed. For

wet roasting, three samples of chickpea seeds, 20 g each, were separately soaked in 100 ml tap water, overnight. For dry roasting, dry seeds were used directly without pre-soaking. All samples (wet or dry) were roasted at 125°C, 150°C and 175°C for 30 min in a Philco kitchen oven. Roasted seeds were freeze-dried and kept at 4°C until use.

2.5. Polyphenol Extraction

Samples of each treatment were ground into a fine powder (60 mesh) in a Retsch MM301 grinder. A 0.1 g portion of the powder was extracted in a 2 ml microfuge tube with 1 ml of acetone/water (50:50, v/v) as previously described. Extracts were kept in the dark at 4°C until use for determination of total phenolic content (TPC), total flavonoid content (TFC), and ferric reduction ability of plasma antioxidant activity (FRAP AA). All extractions were conducted in triplicate.

2.6. Determination of TPC, TFC and FRAP AA Levels

TPC was determined by the Folin-Ciocalteu assay [29,30] and TFC was determined using a colorimetric method [6] in 50- μ l extraction samples as previously described [4]. Both TPC and TFC were expressed as mg + catechin equivalent /g sample through the calibration curve. Linearity ranges of the calibration curves were 5 to 200 μ g/ml ($r = 0.997$) and 5 to 800 μ g/ml ($r = 0.99$) for TPC and TFC, respectively. The FRAP AA method was used to evaluate the antioxidant activity of chickpea extracts by measuring the ferric reducing ability of plasma at low pH levels [31]. Standard solutions of 0.1 to 1 mM TROLOX (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Aldrich Germany) in methanol were prepared.

2.7. Statistical Analysis

All experiments were performed in triplicate. Data were analyzed by ANOVA using the statistical software package JMP Version 5.0. Tukey HSD multiple range test was carried out to detect significant differences between lines used, while Pearson correlation test was conducted to determine the correlation among variables. Significant levels were defined using $P \leq 0.05$.

3. Results and Discussion

3.1. Effect of Baking on TPC, TFC and FRAP AA

The effect of baking on TPC, TFC and FRAP AA in black desi and beige kabuli lines is shown in **Figure 1**. The levels of TPC, TFC and FRAP AA in whole dry seeds were lower than in our previous report [4]. As shown in **Figure 1**, flour, dough and bread made from black seeds

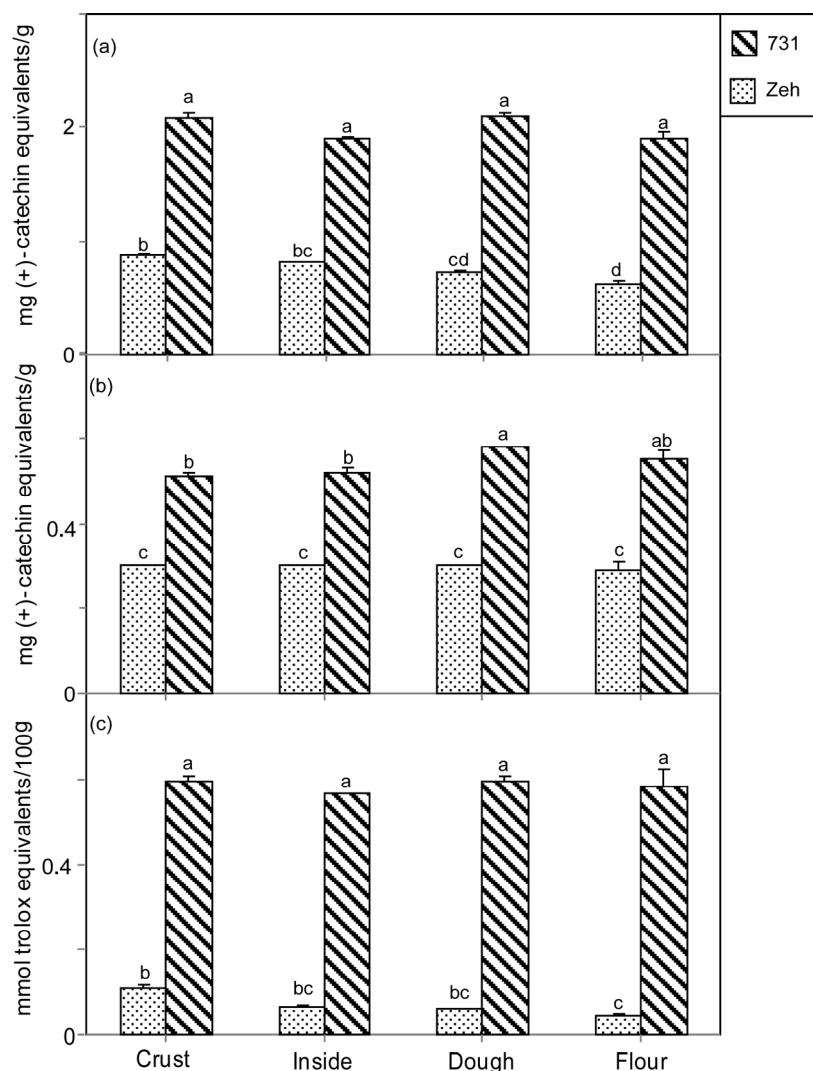


Figure 1. Effect of baking on total polyphenolic content (a), total flavonoid content (b) and antioxidant activity (c) in black desi line (731) and beige kabuli cv. Zehavit (Zeh) flour, dough and bread crust and inside parts. Bar represents mean \pm standard error from at least three replicates. Columns headed by the same small letter are not significantly different ($P < 0.05$).

(line 731) contained significantly higher levels, up to 3-, 2- and 13-fold, of TPC, TFC and FRAP AA, respectively, than those made from regular cream and beige-colored cv. Zehavit seeds (**Figures 1(a-c)**). In line 731, baking did not affect the levels of TPC or FRAP AA relative to the flour and dough, but slightly decreased the levels of TFC. This might be due to omission of a presoaking stage before baking, and thus no discarding of the baking water. These results strengthen our previous observation that polyphenols and flavonoids most likely leak into the surrounding water during the soaking and cooking processes [32]. In cv. Zehavit, however, a slight but significant increase in TPC and FRAP AA was observed in the bread crust. This might be due to the production of Maillard reaction products in the crust during thermal processing [33]. Similar observations have been made when baking rhubarb, whereby both TPC and FRAP AA were higher

during the first 20 min and then decreased to low levels [34], and when baking chocolate cookies and chocolate cakes made with baking powder rather than baking soda [35,36].

3.2. Effect of Frying on TPC, TFC and FRAP AA

The effect of frying on TPC, TFC and FRAP AA in black desi and beige kabuli lines is shown in **Figure 2**. Similar to baking, dry seeds, dough and fried falafel from black seeds (line 731) contained significantly higher levels, up to 3-, 2.6- and 14-fold, of TPC, TFC and FRAP AA, respectively, than those from regular cream-colored cv. Zehavit seeds (**Figures 2(a-c)**). Unfried dough from line 731 contained about 20% - 40% lower levels of TPC, TFC and FRAP AA than dry seeds. This was expected

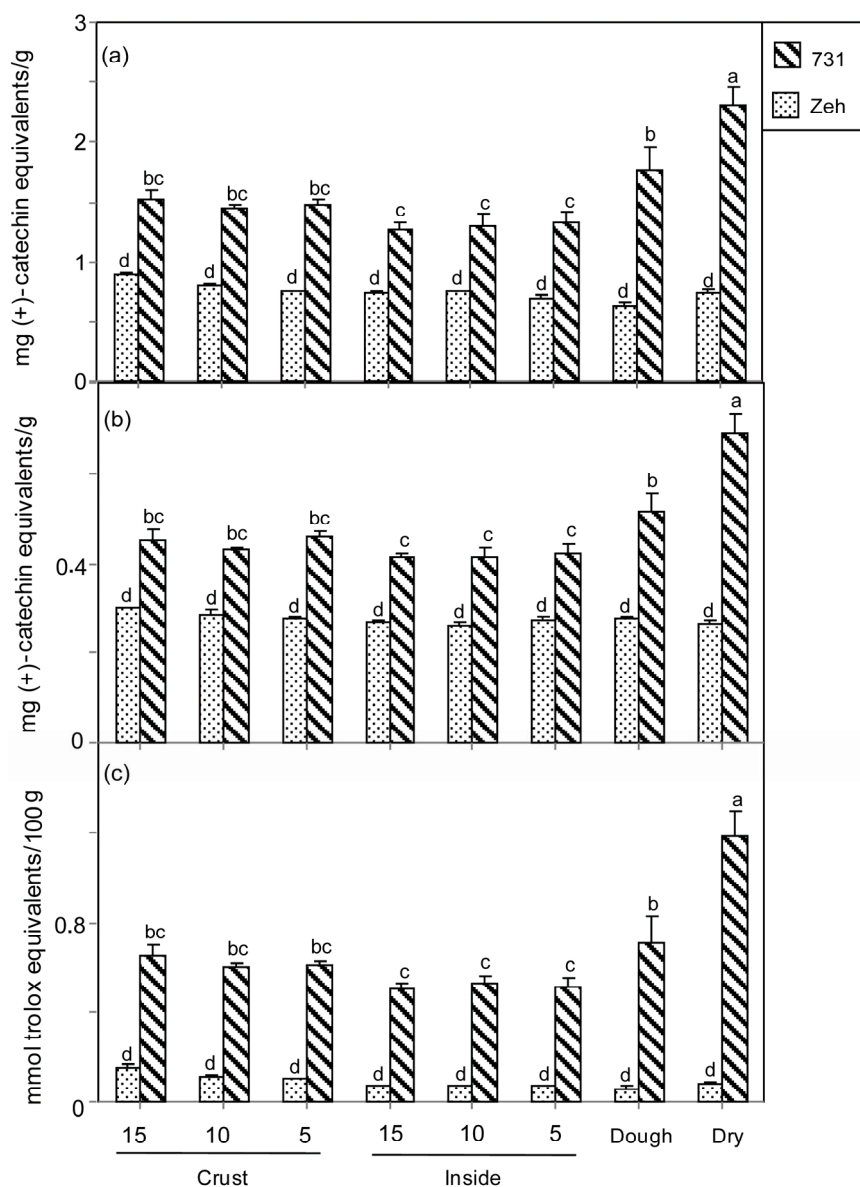


Figure 2. Effect of frying on total phenolic content (a), total flavonoid content (b) and antioxidant activity (c) in black desi line (731) and beige kabuli cv. Zehavit (Zeh) dry seeds, dough and crust and inside parts of the falafel ball. Bar represents means \pm standard error of at least three replicates. Columns headed by the same small letter are not significantly different ($P < 0.05$).

because, in contrast to baking, frying chickpea falafel balls requires a presoaking treatment during which TPC and TFC leak into the soaking water [21,32]. In line 731, frying resulted in a further significant decrease in all three studied parameters inside the fried balls, but not in the crust (**Figures 2(a)-(c)**). These reductions occurred only during the first 5 min of frying: longer frying did not cause changes in any of the three parameters. As solubility of polyphenols and flavonoids in oil is low, the reduction in TPC and TFC in the inside part of the falafel ball during frying might be due to degradation [37,38]. In cv. Zehavit, there were no significant changes in any of the

three parameters in the fried treatments.

3.3. Effect of Roasting on TPC, TFC and FRAP AA

The effects of roasting chickpea seeds on their TPC, TFC and FRAP AA levels are shown in **Figure 3**. In the current study, two roasting methods were tested: wet roasting (**Figures 3(a)-(c)**) and dry roasting (**Figures 3(d)-(f)**). With all roasting treatments, roasted black seeds (line 731) contained significantly ($p < 0.05$) higher levels, up to 2.3-, 1.9- and 10-fold, of TPC, TFC and FRAP AA than the respective levels obtained for the regular cream-

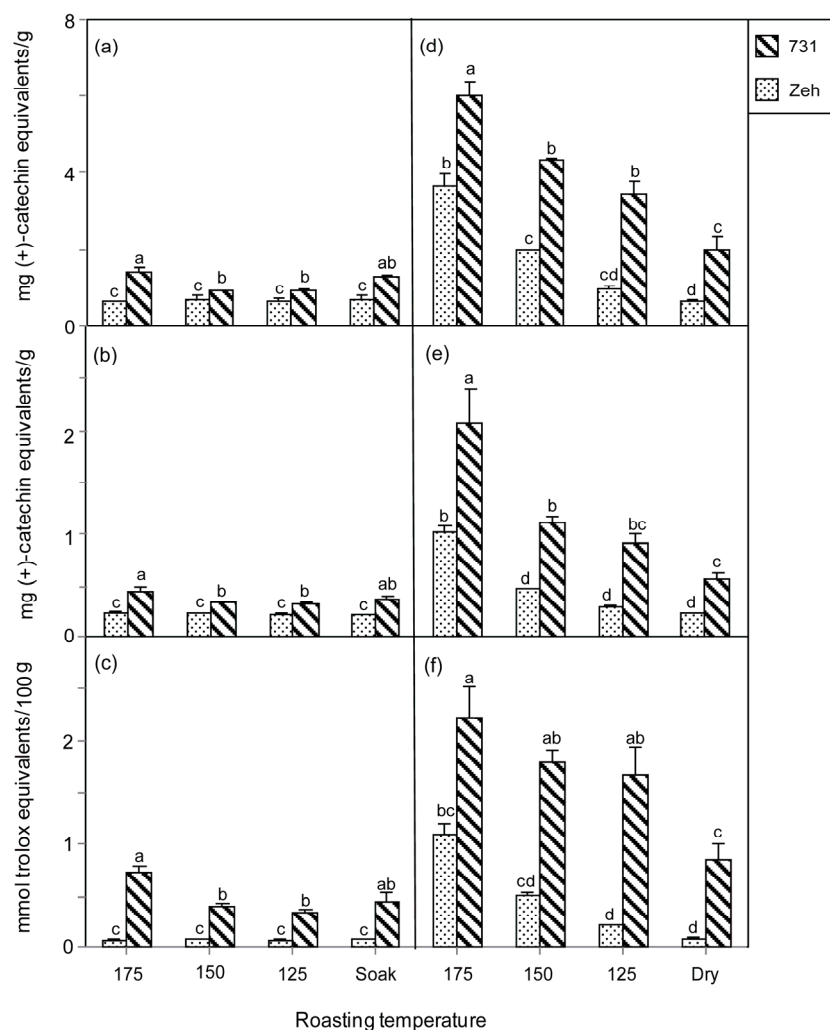


Figure 3. Effect of wet ((a)-(c)) and dry ((d)-(f)) roasting on total phenolic content ((a) and (d)), total flavonoid content ((b) and (e)) and antioxidant activity ((c) and (f)) in black desi line (731) and beige kabuli cv. Zehavit (Zeh) dry and soaked seeds and seeds roasted at different temperatures. Bar represents means \pm standard error of at least three replicates. Columns headed by the same small letter are not significantly different ($P < 0.05$).

colored seeds of cv. Zehavit (**Figure 3**). Results from the wet roasting resembled those of fried chickpeas (**Figure 2**), as both required a presoaking treatment which resulted in TPC and TFC leakage into the soaking water [21,32]. Wet roasting did not produce any significant changes in any of the three parameters in either line (**Figures 3(a)-(c)**). Dry roasting, in contrast to all other treatments performed in this study, resulted in a significant increase in TPC, TFC and FRAP AA in both line 731 and cv. Zehavit. This increase in levels of the three parameters was positively correlated with the increase in roasting temperature from 125°C to 175°C (**Figures 3(d)-(f)**). The increase in all three parameters could result from release of bound polyphenols or from Maillard reaction products formed during roasting that have been reported to possess scavenging activity on reactive oxygen species [39,40]. Maillard reaction involves condensation reac-

tions between sugars and amino acids and has been found to be linked to polyphenols [41] via inhibition of polyphenol oxidase [42]. Similar observations of increased TPC and antioxidant activity with increased roasting temperature and duration have been found for carob powder [43] and apricot kernels [44].

4. Conclusions

Our results indicate that baking, frying and roasting colored chickpea seeds result in significantly higher levels of TPC, TFC and FRAP AA than in similarly treated cream- and beige-colored seeds. In contrast to our previous results with soaking and cooking, in which almost all TPC, TFC and FRAP AA leaked into the surrounding water [32], baking, frying and roasting retained most TPC, TFC and FRAP AA in the final products. Thus, colored chickpea subjected to any of these three processing methods

might be considered a functional food, in addition to its traditional role of providing dietary proteins. Colored chickpea, due to its high antioxidant activity, might also contribute significantly to the management and/or prevention of degenerative diseases associated with free radical damage. Future research should be conducted to determine whether colored chickpeas can be used for management and/or prevention of such degenerative diseases.

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