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Effect of cooking and canning on digestibility and antioxidant potential in chickpea (*Cicer arietinum*) and pigeon pea (*Cajanus cajan*)

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ABSTRACT

Pulses because of their high protein content, have the potential for improving nutritional status and combating malnutrition. A study was carried out at ICAR-Indian Agricultural Research Institute, New Delhi during 2019-20 to see the effects of cooking (boiling) and canning on protein digestibility and antioxidant potential on two contrasting genotypes of chickpea (*Cicer arietinum* L.) and pigeon pea [*Cajanus cajan* (L.) Millsp.] for total protein content. The protein quality was assessed on the basis of essential amino acid score and protein digestibility in terms of Protein Digestibility-Corrected Amino Acid Score (PDCAAS). A pepsin-trypsin-chymotrypsin digestion followed by ninhydrin assay was performed to determine the digestibility. The seeds were boiled and canned in a canning solution of brine solution containing 1.3% (wt/vol) NaCl and 1.6% (wt/vol) sugar. PDCAAS (%) was higher in 'high' protein containing lines than 'low' protein containing lines in case of chickpea. However, no significant variation in PDCAAS % was found between 'low' and 'high' protein pigeon pea genotypes. The antioxidant activity (AOA) was measured by DPPH and FRAP assays and was found to increase in chickpea and pigeon pea genotypes after cooking and canning. Increased AOA in DPPH assay ranged from 62.80–94.69% and from 60.55–95.13% for the cooked and canned seeds respectively. The AOA measured by FRAP assay has shown similar results in the seeds after cooking and canning treatment which ranged from 0.82–13.42 µmol/g and from 2.63–15.71 µmol/g for cooked and canned seeds respectively. The AOA was increased in all the varieties, except in the cooked seeds of Kabuli genotypes.

Keywords: Amino acid score, Antioxidant potential, Canning, Chickpea, Cooking , PDCAAS, Pigeon pea

Pulses constitute an important source of dietary protein particularly in regions where consumption of animal protein is limited. Nutritionally, pulses are among the richest sources of proteins having a content of 20-40% (Duranti 2006). Pulses are also rich in vitamins and minerals specially folate and B-group vitamins (Venkidasamy *et al.* 2019). However, pulses also contain a number of anti-nutritional factors (ANFs) due to which the nutritional value of the proteins and other macronutrients is often compromised (Boye *et al.* 2012). A variety of processing methods are applied to achieve the desirable characteristics and to inactivate reduce, or eliminate the various ANFs (L.X. Lopez-Martínez *et al.* 2017). Canning includes the hydrating of seeds by soaking followed by blanching in canning media and finally sterilizing (Aguilera *et al.* 2009 and Gathu *et al.* 2012). Proteins are the vital components of the human diet having structural and functional roles in growth and development. The protein quality depends on the content essential amino acids, the physiological utilization of them after digestion as well as on the bioavailability of the amino acids (Tavano *et al.* 2016). The Protein Digestibility Corrected Amino Acid Score (PDCAAS) method approved and recommended by FAO in 1991 for use in estimating protein quality is the most widely used method. Initially, amino acid profile of a food protein is compared to a reference value and an amino acid score is determined which is then corrected by multiplying with digestibility of the protein to generate a PDCAAS value (Schaafsma 2012). chickpea (*Cicer arietinum* L.) and pigeon pea [*Cajanus cajan* (L.) Millsp.] also contain a large number of bioactive compounds (Kanatt *et al.* 2011 and Marathe *et al.* 2011) which are beneficial for the health as they have been reported to protect the body against the oxidative stresses and degenerative diseases (Amarowicz and Pegg 2008). There is increasing demand for canned pulses as they provide high consumer value, are convenient to use and have an ease of preparation (Uebersax 2006). The increased consumption of canned pulses necessitates

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improving the knowledge of the changes produced by canning in the nutritional composition and content of bioactive compounds.

MATERIALS AND METHODS

Plant materials: Six genotypes of pulses which consisted of two contrasting genotypes each of *desi* chickpea, *kabuli* chickpea and pigeon pea were selected based on total crude protein content (1019-20). Damaged and broken seeds as well as the foreign materials were handpicked from the sample before the analytical studies. The seeds were crushed to fine powder using grinder and the contents were passed through 80 mm sieve to have uniform powder which was stored for extraction and further assays. Selected seeds were cooked and canned (Parmer *et al.* 2016).

Household cooking (boiling) of selected chickpea and pigeonpea seeds: The 250 mL of distilled water was taken in a 500 mL beaker and was brought to boiling point (100ºC). The 15 g seed was then added and boiling was continued. The boiled grains were drawn at intervals of 2 min and were pressed between the forefinger and thumb to test their softness or tenderness. The time taken to get the desirable softness was recorded as the cooking time of the sample.

Canning of selected chickpea and pigeonpea seeds: The 15 g of selected chickpea and pigeon pea seeds were weighed and soaked in water for 12 h at 25ºC in a ratio of 1:5. The seeds were then poured into cans and brine solution containing 1.3% (wt/vol) NaCl and 1.6% (wt/vol) sugar was added till a 5 mm headspace was obtained. The seeds were then blanched at 85ºC for 30 min in the brine solution. The cans were then sealed, sterilized and processed at 121°C for 14 min. The processed cans were then stored at room temperature for 2 weeks prior to evaluation. After 2 weeks, the seeds were transferred to a screen, rinsed with distilled water and then allowed to drain for 5 min.

Determination of in vitro protein digestibility (gastro intestinal mimic model) in terms of PDCAAS: Briefly, 0.5 g of ground sample flour was mixed with 2 mL of distilled water and kept in boiling water bath for 20-25 min. Then 35 mL of 0.06 HCl 0.06 N was added and incubated overnight at 37°C in a hot air, shaking incubator set at 300 rpm. Further *p*H was adjusted to 2 and 1 mL of pepsin solution was added to each sample and again incubated for 5 hr at 37°C at 300 rpm. After the pepsin incubation was complete, *p*H was adjusted to 7.4 by the addition of 1.0 M Tris buffer and vortexed. The 1 mL of trypsin/chymotrypsin was added and then was incubated overnight at 37°C at 300 rpm. At the end of the trypsin/chymotrypsin incubation, the samples were placed in boiling water bath for 10 min and then were cooled down to room temperature for at least 20 min. Approx. 1.75 mL of the sample was transferred (avoiding the precipitate) to a 2 mL centrifuge tube and centrifuged for 10 min at 15000 x g. All supernatants of the sample solutions, including the sample blanks, calibration samples and the casein control samples, were then further proceeded for Ninhydrin assay for the colourimetric determination of amines. A solution of L-Glycine was used as standard.

Colorimetric determination of amines by Ninhydrin assay: The 0.333 mL of digested sample was taken in 2 mL Eppendorf tube and 0.166 mL of Ninhydrin reagent was added into it. The tubes were kept in dry bath for 5-10 min at 80ºC till the blue color was developed. Then, the tubes were cooled and 0.5 mL of reagent alcohol was added. The absorbance was read at 570 nm.

Antioxidant activity: The total antioxidant capacity of extracts from chickpea and pigeonpea flour was estimated by DPPH assay as given by Shimada *et al.* (1992) for DPPH antioxidant activity and ferric reducing antioxidant power (FRAP) assay method as given by Benzie and Strain (1996). The 1.0 g of the finely ground sample flours were extracted separately with 20 mL methanol by keeping on a shaker overnight and then was centrifuged at 10000 rpm for 15 min.

DPPH radical scavenging activity: The 0.5 mL of methanolic extract of the sample was taken in a test tube. The 4 mL of 0.1 mM DPPH solution was added. The test tube was gently shaken by hand and incubated in dark for 30 min at room temperature and the absorbance was read at 517 nm. A control of DPPH solution without sample was recorded as control value. Results were expressed as percentage of DPPH scavenging relative to control.

$$\text{DPPH antioxidant activity (\%)} = \frac{\text{(Absorbance of control - Absorbance of sample)}}{\text{Absorbance of control}}$$

Ferric reducing antioxidant power: FRAP reagent was prepared freshly and consisted of acetate buffer, TPTZ and $FeCl_3.6H_2O$ mixed in the ratio of 10:1:1, respectively. The 0.1 mL of methanolic extracts was taken in a test tube wherein 2 mL pre-warmed FRAP reagent was added and the solution was incubated at 37°C for 10 min. The absorbance was measured at 593 nm.

Statistical interpretation: Data were represented as mean ± SE (n=3). P value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Effect of cooking (boiling) and canning on protein digestibility and antioxidant potential was carried out on two contrasting genotypes of chickpea (*Desi* and *Kabuli*) and pigeonpea for total protein content. In *desi* chickpea genotypes Pusa 362 (27.49 g/100g) and Pusa 1103 (17.10 g/100 g) were selected as high and low protein types, similarly for *kabuli* chickpea and pigeon pea genotypes BG 3028 (21.86 g/100 g)/Pusa 5023 (14.19 g/100 g) and MAL 13 (21.13 g/100 g)/ Bahar (14.48 g/100 g) were selected as high and low protein types respectively.

PDCAAS (%) of selected chickpea and pigeon pea varieties after cooking (boiling) and canning: The *in vitro* digestibility in terms of PDCAAS (%) of selected chickpea and pigeon pea varieties is given in Table 1.The PDCAAS reflects an attempt to measure the overall quality of a protein as the product of the digestibility of the protein and its amino acid score. In general, the PDCAAS (%) of the analysed genotypes after cooking and canning was found

Table 1 PDCAAS (%) of selected chickpea and pigeon pea varieties after cooking (boiling) and canning

Varieties for low and high protein content	PDCAAS (%)of cooked seeds	PDCAAS (%) of canned seeds	PDCAAS (%) of untreated seeds
Pusa 1103 (low)	53.649 ± 0.055	53.612 ± 0.096	53.577
Pusa 362 (high)	85.543 ± 0.243	85.346 ± 0.130	85.337
Pusa 5023 (low)	66.890 ± 0.029	66.924 ± 0.086	74.553
BG 3028 (high)	74.677 ± 0.115	74.656 ± 0.175	66.753
Bahar (low)	61.625 ± 0.219	61.385 ± 0.139	61.265
MAL 13 (high)	59.957 ± 0.371	59.641 ± 0.126	59.367

The values are mean of two replicates ± SD.

to be higher than the values of the raw (untreated) seeds. Further we found that, the PDCAAS (%) is slightly higher for the cooked seeds than for the canned ones. IVPD through PDCAAS (%) was higher in high protein containing lines than low protein containing lines in case of chickpea. In case of *desi* contrasting genotypes, PDCAAS % was found to be higher in Pusa 362 (high) of 85% as compared to low protein containing variety – Pusa 1103 (53%). In case of *kabuli* contrasting genotypes of chickpea, PDCAAS % was found to be higher in BG 3028 ('high') of 74% as compared to low protein containing variety – Pusa 5023 (66 %). However, no significant variation in PDCAAS % was found between low and high protein pigeonpea genotypes. Increased digestibility of chickpea genotypes after cooking and canning might be due to lower levels of antinutritional factors. It is evident from several reports that, antinutritional factors hinders protein digestibility (Nosworthy *et al.* 2018 and Margier *et al.* 2018). Our results of increased protein digestibility in chickpea genotypes are in agreement with the findings of Margier *et al.* (2018), where they have shown increased protein digestibility of chickpea after cooking and canning.

Effect of cooking (boiling) and canning on antioxidant potential: The antioxidant potential in terms of DPPH antioxidant activity (%) and FRAP (µmol/g) in selected untreated chickpea and pigeon pea varieties and the corresponding change after cooking and canning is given in Table 2. The 2, 2'-diphenyl-1-picrylhydrazyl radical (DPPH•) which has a deep purple colour, is one of the few stable as well as commercially available organic nitrogen radicals, often used in the evaluation of antioxidant potential of natural and synthetic compounds. In case of DPPH antioxidant activity, there is an increase in the activity both after the cooking and canning treatments of the seeds which ranged from 62.80–94.69% and from 60.55–95.13% for the cooked and canned seeds respectively. Among the selected genotypes, *kabuli* chickpea variety Pusa 5023 showed the lowest DPPH antioxidant activity of 62.80%, 60.55% (cooked, canned) while the pigeon pea variety MAL 13 showed the highest DPPH antioxidant activity of 94.69%, 95.13% (cooked, canned). The cooked chickpeas have shown highest antioxidant activity when compared with canned chickpeas. But in case of pigeon pea the antioxidant activity of canned peas is slightly higher than that of cooked pigeon pea.

Originally, the FRAP assay, by Benzie and Strain (1996), was used for the analysis of reducing power in plasma, but later the assay has been adapted for the measurement of antioxidants in plant samples. The assay measures the reduction of ferric 2,4,6-tripyridyl-s-triazine (TPTZ) to a blue-coloured product. The antioxidant activity measured by FRAP assay has shown similar results in the seeds after cooking and canning treatment which ranged from 0.82 µmol/g (Pusa 5023) to 13.42 (MAL 13) µmol/g and from 2.63 (Pusa 372) µmol/g to 15.71 µmol/g (Bahar) for cooked and canned seeds respectively. The antioxidant activity was increased in all the varieties except in the cooked seeds of *kabuli* genotypes, where the activity was shown

Table 2 Antioxidant potential in terms of DPPH antioxidant activity (%) and FRAP (µmol/g) in selected untreated chickpea and pigeon pea varieties and changes in them after cooking (boiling) and canning

Varieties for low and high protein content	DPPH antioxidant activity (%)	FRAP (µmol/g)	DPPH antioxidant activity (%)	FRAP (µmol/g)	DPPH antioxidant activity (%)	FRAP (µmol/g)
	Untreated seeds	Untreated seeds	Cooked (boiled) seeds		Canned seeds	
Desi chickpea						
Pusa 1103 (low)	23.44 ± 0.791	2.37 ± 0.127	77.02 ± 1.38	2.39 ± 0.05	69.84 ± 1.59	2.86 ± 0.53
Pusa 362 (high)	21.73 ± 0.895	3.49 ± 0.304	75.22 ± 2.01	2.31 ± 0.67	71.93 ±1.80	2.63 ± 0.53
Kabuli chickpea						
Pusa 5023 (low)	20.75 ± 1.094	1.77 ± 0.014	65.64 ± 0.32	2.29 ± 1.54	65.19 ± 2.01	3.79 ± 0.80
BG 3028 (high)	18.26 ± 0.724	2.26 ± 0.353	62.80 ± 0.11	0.82 ± 0.09	60.55 ± 3.92	3.26 ± 0.18
Pigeon pea						
Bahar (low)	89.85 ± 0.603	13.77 ± 0.084	94.46 ± 0.21	9.14 ± 0.55	94.61 ± 0.21	15.71 ± 0.18
MAL 13 (high)	68.17 ± 1.086	10.29 ± 0.162	94.69 ± 0.74	13.42 ± 0.09	95.13 ± 0.53	13.66 ± 0.49

The values are mean of two replicates ± SD to be reduced as compared with that of control untreated seeds. Increased antioxidant activity in chickpea and pigeon pea genotypes after cooking and canning might be due to increase in release of bound phytochemicals into food matrices after thermal processing or canning processing, contributing to higher antioxidant potential. Similar kinds of results were shown by Padhi *et al.* (2017) for Canadian pulses and Dewanto *et al.* (2002a, 2002b) in processed tomato and sweet corn.

A major challenge in today's world is to bring a shift in the current diet pattern to healthier and more sustainable diets. In this regard, pulses have an unfathomable role to play and this study demonstrated that processing methods such as cooking (boiling) and canning affects the physico-chemical and nutritional properties of the two pulses. These key findings of this study, suggested the potential of chickpeas and pigeon peas in imparting quality proteins and other nutritional quality and thus can serve as alternative plant-derived proteins which are of good quality to meet the nutritional demand of the human body. Generation of nutritional information in terms of protein digestibility and PDCAAS score can help the industries to formulate various plant based nutritionally rich foods. Additional research is required to study as well to limit the concomitant losses in nutrients, if any, observed during the cooking and the canning process.

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