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Research article

Effect of germination, roasting, and variety on physicochemical, techno-functional, and antioxidant properties of chickpea (*Cicer arietinum* L.) protein isolate powder

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ABSTRACT

Chickpeas are a very important part of the human diet due to their nutritional and bioactive composition. Ethiopia is one of the top chickpea producers and consumers of chickpea-based products daily. However, limited studies were conducted on the effect of common processing methods, roasting and germination, on techno-functional and nutritional properties of chickpea protein isolates. Two varieties of chickpea, Arerti (Kabuli type) and Natoli (Desi type), were selected and treated with different roasting temperature (150 and 180 °C) and germination time (24, 48, and 72 h). The protein was isolated with alkaline-solubilization followed by isoelectric precipitation. Freeze-dried isolates were investigated for proximate composition, techno-functional properties, antioxidant properties, and antinutritional content. Chickpea protein isolates (CPIs) mean protein content was between 79.72 and 87.43%, comparatively lower for those from roasted and higher for those from germinated chickpea. Mean values of CPIs' water holding capacity (WHC), oil holding capacity (OHC), protein solubility (PS), foaming capacity (FC), and Emulsifying capacity (EC) for both varieties were in a range of 1.07–2.47 g/g, 1.40–2.21 g/g, 43.88–69.99%, 14.00–94.00%, and 56.44–84.16%, respectively. Roasting at 150 °C improved most of the techno-functional properties (WHC, OHC, PS, and FC) while roasting at 180 °C negatively affected almost all the techno-functional properties. Both heat treatments significantly increased the antioxidant properties of the isolates. Germination for 72 h was the best treatment in improving all antioxidant properties. CPIs from treated chickpea had lower antinutritional content than those from native chickpea except for phytate on Natoli variety where no statistical difference ($p > 0.05$) was observed. The finding showed that based on the intended use the different techno-functional properties of the isolates can be altered by applying those treatments. Proximate, techno-functional, antioxidant, and antinutritional characters indicated that CPIs can be a good ingredient for the food industry to formulate functional foods.

1. Introduction

Plant-based proteins, other than wheat and soy which have allergen concerns, are gaining popularity as low cost, healthier, and sustainable sources with reduced environmental impacts and acceptable product quality and safety (Singhal et al., 2016). In connection to this, legume protein products are becoming the most appropriate alternative due to their high nutritive quality, good techno-functional properties, and low cost (Barać et al., 2015; Keskin et al., 2021; Semba et al., 2021). Among the many other legumes, chickpeas are a very important part of the human diet due to their nutritional and bioactive composition (Raza et al., 2019; Savic et al., 2018) and their protein is considered better than some pulses (Jukanti et al., 2012).

Ethiopia is one of the largest chickpea producers and consumers of chickpea-based products. According to the Ethiopian Central Statistical Agency report, about 0.5 million tonnes of chickpea was produced in the main cropping season of 2020/2021 (CSA 2021). Traditionally, flour from roasted chickpea is made into stew, locally named *shiro wot*, to be served along with *Injera*, a fermented cereal-based traditional bread, on daily basis by the majority. Other chickpea-based products such as *Kollo* (roasted chickpea) and *Nifro* (boiled chickpea) are common foods in the country.

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Protein isolates are the refined forms of protein containing a substantial amount of protein with better digestibility (Garba and Kaur, 2014). They are used increasingly in food and non-food applications (Ariyarathna and Karunaratne, 2014). Different methods for the extraction of protein from pulses such as alkaline extraction-isoelectric precipitation, salt extraction-dialysis, micellar precipitation, and ultrafiltration have been reported. These extraction methods have different levels of impact on the composition and functional properties of the isolates (Boye et al., 2010; Stone et al., 2015). Moreover, processing methods can affect the physicochemical, functional, thermal, and structural properties of protein isolate to a varying extent (Xu et al., 2017). Roasting and germination can significantly alter flour protein solubility, consequently, impacting the overall efficiency of protein extraction (Skylas et al., 2017). Germination is known for improving functional (Sofi et al., 2020a) and antioxidant properties and reducing antinutrients substantially (Haileslassie et al., 2016).

Despite there is high production and regular consumption of chickpea-based products in Ethiopia, studies that focus on characterization of the changes in the properties of protein isolate due to the common processing methods of Ethiopian chickpea varieties are limited. The objective of this study was, therefore, to isolate protein from roasted and germinated Ethiopian chickpea cultivars and to characterize the proximate composition, techno-functional, antioxidant properties, and antinutritional content to investigate the effect of those treatments and to suggest for future product development efforts.

2. Materials and methods

2.1. Raw materials

Two varieties of chickpea namely Arerti (Kabuli type) and Natoli (Desi type) were collected from Debre Zeit agricultural research center which is located in Bishoftu town, East Shewa Zone of the Oromia Region of Ethiopia, and has an elevation of about 1,920 m. The varieties were selected because of their higher productivity and farmers' preference (Rao et al., 2013).

2.2. Sample preparation

Roasting was done in a hot air oven at two different time-temperature combinations; at 150 °C for 30 min and 180 °C for 15 min. The germination treatment was done as described by Malunga et al. (2012) with some modification. Chickpea seeds were soaked in distilled water (1:5; w/v) for 12 h and then left to germinate at 25 °C inside an environmental test chamber for 24, 48, and, 72 h.

The subsequent milling, defatting, and isolation procedures were performed as described by Mondor et al. (2009) with modifications. The chickpea beans were milled into a fine flour, then sieved through a 250-mesh sieve and defatted with ethanol (97%). The protein was isolated from chickpea flour with an alkaline solubilization-isoelectric precipitation method. Chickpea flour (300 g) was weighed and mixed with distilled water, in a flour to water ratio of 1:5 (w/v). Then the pH of the solution was adjusted to 9.5 with 1 N NaOH and stirred for 1 h. It was placed in a test tube and centrifuged at 2683 g for 30 min. Then the supernatant was collected in a container and the pH was adjusted to 4.5 using 1 N HCl to establish isoelectric precipitation. The mixture was centrifuged at 2683 g for 20 min and the residue protein isolate was washed using distilled water. The pH of the protein isolate was adjusted to 7.0 using 1 N NaOH and dried in a freeze drier.

2.3. Proximate analysis

Protein, fat, ash, and moisture content was determined according to the AOAC method (AOAC 2016) with official method numbers 979.09, 4.5.01, 941.12, and 925.10, respectively. Crude fiber content was quantified using ISO 6865, Official Method (2000). Available carbohydrate content was calculated by difference. The energy value of the protein isolate was determined by the method described by Southgate (1976).

2.4. Techno-functional properties

Water holding capacity (WHC) and oil holding capacity (OHC) were determined using methods described by Rodríguez-Ambriz et al. (2005). To determine the protein solubility the method mentioned by Morr (1985) was used. Bulk density was determined as described by Okaka and Potter (1977). Tapped density was measured as described in Ji et al. (2015). Emulsifying capacity (EC) and emulsion stability (ES) were determined by the method described in Aguilera et al. (2009). The foaming capacity and stability were determined by the method of Lin et al. (1974).

2.5. Determination of antioxidant properties

Sample extraction was done as described by Abdeltaif et al. (2018) with some modifications (using methanol instead of ethanol for extraction and employing sample to solvent ratio of 1:10 instead of 1:25). Total polyphenol content determination was done using the Folin-Ciocalteu method as described by Peschel et al. (2006). Briefly, 1 mL of Folin-Ciocalteu reagent was added to a mixture that consists of 1 mL of extract and 9 mL of distilled. After 5 min, 10 mL sodium carbonate (7% conc.) solution was added to the mixture and the total volume of the mixture was made up to 25 mL by deionized water. A set of standard solutions of gallic acid (20, 40, 60, 80, and 100 μg/mL) were prepared in the same way as described above and incubated for 90 min at room temperature. The absorbance for test and standard solutions were determined against the reagent blank at 550 nm with a UV/Vis spectrophotometer (Lambda 950 UV/Vis, Agilent Technologies German). The total polyphenol (TPC) content was expressed as milligram gallic acid equivalents per 100 g of extract (mg GAE/100 g).

Flavonoid content was determined using the method of Muanda et al. (2011). Briefly, 0.5 mL of extract was mixed with 2 mL of deionized water and 0.15 mL of sodium nitrite (5% w/v). After 5 min, 0.15 mL of 10% aluminum chloride was added followed by the addition of 1 mL of 1 M sodium hydroxide after 6 min. The total volume was adjusted to 5 ml deionized water and absorbance was read at 510 nm using UV/Vis spectrophotometer (Lambda 950 UV/Vis, Agilent Technologies German). Different concentrations of catechin standard solutions (0.002–0.125 μg/mL) were used to establish the calibration curve. Total flavonoid content was expressed in milligram catechin equivalents per 100 g of extract (mg CEQ/100 g).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was done using the method described by Desmarchelier et al. (1997). Briefly, a solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of chickpea protein isolate extract in methanol at different concentrations (20–120 μL/mL). The reaction mixture was measured using UV/Vis spectrophotometer at 517 nm. Percentage DPPH radical scavenging activity was calculated using equation number 1.

$$\%\ \text{DPPH radical scavanging activity} = \frac{(Ao - A1)}{Ao} * 100 \quad (1)$$

Where; A_0 = is the absorbance of the control, and.

A_1 = is the absorbance of the extract/standard.

The H_2O_2 scavenging capacity of the protein isolate was determined using the method described by Ruch et al. (1989) with modification. Briefly, hydrogen peroxide (40 mM) was prepared in a phosphate buffered saline (PH = 7.4). Aliquot of 0.4 mL of protein isolate extract (20–1000 ppm) which was prepared using 95% ethanol was mixed with 0.6 mL of hydrogen peroxide. After 10 min, the absorbance of the mixture was measured at 230 nm against blank solution using UV/Vis

spectrophotometer. The percentage of scavenged hydrogen peroxide was calculated by equation number 2.

$$\%\ \text{of scavenged hydrogen peroxide} = \frac{(A_o - A_1)}{A_1} * 100 \quad (2)$$

Ao = absorbance of control.

A1 = absorbance of CPI (Sample).

2.6. Determination of antinutritional factors

Phytate content was determined using AOAC (2016) method number 986.11. Tannin was determined using the method described by Burns (1971) with slight modification. Briefly, 1 g of chickpea protein isolate was weighed in a test tube. Then 10 mL of 1% HCl in methanol was prepared for extracting the tannin from the sample. The sample was extracted for 24 h at room temperature using a horizontal shaker. After extraction, the sample was centrifuged at 1000 rpm for 5 min. One milliliter of the extract was mixed with 5 mL of vanillin-HCl reagent (prepared by mixing 8% of concentrated HCl and 4% vanillin both prepared using methanol). *D*-catechin was used as a standard. The mixture was left for 20 min and the absorbance was measured at 500 nm.

2.7. Statistical analysis

Replicate measurement was done for all parameters and results were reported as Mean±standard deviations. Data were subjected to analysis of variance using SPSS software, version 26. Statistical differences between means were separated using Duncan's Multiple Range Test and significant differences were declared at $p < 0.05$.

3. Results and discussion

3.1. Proximate composition

The proximate composition of chickpea protein isolates (CPIs) from raw and treated chickpea is presented in Table 1. There was a decrease in protein content by 4.6 and 4.2% for CPIs obtained from roasted Arerti chickpea at 150 and 180 °C, respectively, compared to the native sample. This can be attributed to a decreased protein solubility as a result of thermal denaturation and polymerization of amino acids (Kato et al., 1985). CPIs obtained from germinated chickpea were higher in protein content than those obtained from raw and roasted chickpea. The increase in protein during germination could be attributed to the proteolytic action of endogenous enzymes and a similar finding was reported by Sofi et al. (2020a).

Native CPIs were higher in fat content (2.47 ± 0.00 and 2.25 ± 0.04% for Arerti and Natoli, respectively). CPIs that were obtained from roasted chickpea at 150 °C were relatively lower in fat compared to that roasted at 180 °C. An intense roasting procedure can increase fat content (Santos et al., 2018) which can be attributed to the concentration of fat due to reduction of other components, such as moisture (Adekanmi et al., 2009) and ash. CPIs from germinated chickpea were low in fat content compared with their native counterpart and this was in agreement with Kavitha and Parimalavalli (2014).

The moisture content of the CPIs ranged from 3.3 to 4.8%. The report of Xu et al. (2017) is also within this range. CPIs from roasted chickpea at 180 °C had the lowest moisture content for both varieties and it agreed with Yu et al. (2007).

CPIs from native chickpea (4.58 ± 0.0% for Arerti and 4.76 ± 0.11% for Natoli) were higher and those from 72 h germinated chickpea (3.86 ± 0.04 and 3.66 ± 0.28% for Arerti and Natoli, respectively) were lower in their ash content. Both roasting and germination significantly reduced the ash content, except for Natoli variety germinated for 24 h, and this is in line with Xu et al. (2017) and Kumar et al. (2020). The leaching of minerals from the seeds during soaking could be the possible reason for low ash content in CPIs from germinated samples (Bulbula and Urga, 2018).

The fiber content of CPIs obtained using different treatments falls in the range between 0.31 ± 0.00 and 0.84 ± 0.00% for Arerti, and 0.14 ± 0.02 and 0.84 ± 0.00% for Natoli variety. The results were in agreement with the finding of Oo et al. (2017). CPIs from native chickpea generally had low fiber content. The trend observed on the CPIs from germinated Arerti was the same as the report of Udensi and Okoronkwo (2006) as there was an initial increase in fiber content at 24 h germinations, compared to the native CPI, which was then decreased when germination time was increased. There was an increase of 86.4 and 90.9% fiber for CPIs from Arerti chickpea that was roasted at 150 and 180 °C, respectively. The increase was also observed on CPIs from roasted Natoli chickpea, by a three-fold, which is in line with Kumar et al. (2020) and it could be the result of roasting induced formation of indigestible products (Ee et al., 2013) and in case of germination, it could be due to an increase of cellulose, accompanied by an increase of pectic polysaccharides (Benítez et al., 2013).

The carbohydrate content of the CPIs was between 2.68 and 10.0%. It was lower than that reported by Oo et al. (2017). CPIs from roasted Natoli chickpea at 150 °C had significantly higher carbohydrates content than those from germinated ones while CPIs from roasted Arerti chickpea at 180 °C had significantly higher carbohydrate content than those

Table 1. Mean ± SD value for proximate composition and energy of protein isolates from native and treated chickpea.

Variety	Sample name	Protein content (%)	Fat content (%)	Moisture (%)	Fiber (%)	Ash (%)	Carbohydrate (%)	Energy value (Kcal/100 g)
Arerti	A-raw	84.00 ± 0.38^{b}	2.47 ± 0.0^{d}	3.67 ± 0.10^{c}	0.44 ± 0.02^{b}	4.58 ± 0.00^{e}	4.84 ± 0.31^{b}	377.62 ± 0.39^{d}
	A-150	80.17 ± 0.45^{a}	1.31 ± 0.09^{a}	4.71 ± 0.15^{d}	$0.82 \pm 0.02e$	4.33 ± 0.10^{d}	8.67 ± 0.60^{c}	367.11 ± 0.19^{a}
	A-180	80.49 ± 0.79^{a}	1.80 ± 0.11^{b}	3.23 ± 0.19^{a}	0.84 ± 0.00^{e}	3.63 ± 0.03^{a}	10.0 ± 0.90^{c}	378.16 ± 1.41^{d}
	A-24	87.43 ± 0.33^{c}	1.24 ± 0.06^{a}	3.9 ± 0.12^{c}	0.71 ± 0.00^{d}	4.0 ± 0.05^{c}	2.68 ± 0.36^{a}	371.65 ± 0.59^{b}
	A-48	85.99 ± 0.30^{c}	1.71 ± 0.04^{b}	3.38 ± 0.05^{b}	0.54 ± 0.01^{c}	4.17 ± 0.09^{c}	4.27 ± 0.21^{b}	376.42 ± 0.74^{cd}
	A-72	79.72 ± 1.02^{a}	2.15 ± 0.02^{c}	4.78 ± 0.16^{d}	0.31 ± 0.00^{a}	3.86 ± 0.04^{b}	9.19 ± 0.93^{c}	374.95 ± 0.57^{c}
Natoli	N-raw	80.57 ± 0.47^{a}	2.25 ± 0.04^{d}	3.34 ± 0.06^{a}	0.14 ± 0.02^{a}	4.76 ± 0.11^{b}	8.95 ± 0.35^{c}	378.28 ± 0.81^{c}
	N-150	80.81 ± 1.54^{a}	1.64 ± 0.00^{c}	4.32 ± 0.14^{b}	0.43 ± 0.00^{bc}	3.32 ± 0.74^{a}	9.48 ± 0.94^{c}	375.96 ± 2.40^{bc}
	N-180	82.44 ± 1.39^{ab}	2.55 ± 0.06^{e}	3.3 ± 0.06^{a}	0.41 ± 0.01^{b}	3.79 ± 0.05^{a}	7.50 ± 1.31^{bc}	382.71 ± 0.21^{d}
	N-24	84.09 ± 1.28^{bc}	1.23 ± 0.07^{a}	3.47 ± 0.08^{a}	0.42 ± 0.03^{bc}	4.74 ± 0.27^{b}	6.06 ± 0.97^{ab}	371.66 ± 1.88^{a}
	N-48	84.06 ± 0.53^{bc}	1.54 ± 0.04^{b}	4.82 ± 0.12^{c}	0.46 ± 0.014^{c}	3.32 ± 0.00^{a}	5.80 ± 0.36^{ab}	373.30 ± 0.34^{ab}
	N-72	86.07 ± 0.97^{c}	1.57 ± 0.01^{bc}	4.13 ± 0.10^{b}	0.51 ± 0.01^{d}	3.66 ± 0.28^{a}	4.06 ± 1.14^{a}	374.70 ± 0.59^{ab}

Means sharing the same letter in a column with in variety are not significantly different ($p > 0.05$). A-raw = Arerti raw; A-150 = Arerti roasted at 150 °C; A-180 = Arerti roasted at 180 °C; A-24 = Arerti germinated for 24 h; A-48 = Arerti germinated for 48 h; A-72 = Arerti germinated for 72 h; N-raw = Natoli raw; N-150 = Natoli roasted at 150 °C; N-180 = Natoli roasted at 180 °C; N-24; Natoli germinated at 24 h; N-48 = Natoli germinated for 48 h; N-72 = Natoli germinated for 72 h.

obtained from germinated chickpea for 24 and 48 h. The result is supported by the report of Kumar et al. (2020). The energy value of the CPIs was within a narrow range, between 367.1 and 378.2 kcal/100 g for Arerti, and between 371.7 and 382.7 kcal/100 g for Natoli CPIs. The result is slightly lower than the report of Sánchez-Vioque et al. (1999).

3.2. Functional properties

3.2.1. Water holding capacity (WHC)

Table 2 presents the functional properties of isolates from native and treated chickpea. The highest water-holding capacity was observed for CPIs from both varieties roasted at 150 °C. Improvement of WHC by dry heating was also reported elsewhere (Bühler et al., 2020). This is because, at the beginning of heat denaturation, the protein molecules will unfold. The unfolding might expose more hydrophilic groups than the hydrophobic, thereby increasing the hydrophilic binding sides (El-Adawy, 2000). On the other hand, CPIs from roasted chickpea at 180 °C had significantly lower WHC compared to their native counterparts. This result is supported by Zayas (1997) which states that water retention capacity of proteins usually decrease with increase in temperature. This could be due to the reduction of the availability of hydrophilic amino acids when the polypeptide chain is unfolded and changed to random coil structure at high temperature.

Germination for 24 h significantly improved the WHC as compared to the native CPIs which is in agreement with Akaerue and Onwuka (2010). The increase observed could be as a result of the production of compounds having better WHC, such as soluble sugars (Ocheme et al., 2015).

3.2.2. Oil holding capacity (OHC)

The OHC of CPIs ranged between 1.40-2.21 g/g which is in line with Oo et al. (2017). The only treatment with a positive impact on the oil holding capacity (OHC) of the Arerti CPI was roasting at 150 °C, which increased it by 8.7%. All the rest of the treatments resulted in a decrease of OHC, though it was not significant for the 48 h germinated CPIs. The decrease in fat absorption capacity on germinated CPIs could be due to the negative effect of germination on the hydrophobicity of the proteins (El-Adawy, 2003). Looking at the effect of processing on the Natoli variety, both roasting treatments resulted in a loss in OHC compared to the native CPI. This is in agreement with Umezuruike and Nwabueze (2017). The remaining two germination treatments (24 and 72 h) significantly improved the OHC of the Natoli CPI and it is in agreement with Sharma et al. (2019). This increase might be due to enhanced interaction between non-polar amino acids groups and hydrocarbon moieties of oil exposed to the protein chain during germination (Ocheme et al., 2015).

3.2.3. Protein solubility (PS)

Roasting at 150 °C positively affected the protein solubility. There was a 14.6 and 12.6% increase for Arerti and Natoli CPIs, respectively. On the other hand, roasting at 180 °C decreased quarter and one-fifth of the protein solubility of Arerti and Natoli CPIs, respectively. This is in accordance with the findings of Xu et al. (2017). Skylas et al. (2017) stated that high temperature used during roasting can denature and modify protein structure which consequently reduces the solubility of the protein. Arerti CPI, germinated for 24 h, had a significantly improved PS while there was no significant effect on Natoli CPIs. Protein isolate obtained from 48 h germinated Arerti chickpea and those obtained from 24 and 48 h germinated Natoli chickpea had no significant difference ($p > 0.05$) compared with their native CPIs. On the other hand, 72 h germination significantly decreased the PS of CPIs obtained from both varieties. The finding is in contrast to what is reported by Ferreira et al. (2019).

3.2.4. Bulk density (BD) and tapped density (TD)

Roasting significantly increased the bulk density of the isolates. Iyenagbe et al. (2017) also reported an increase in BD of heat-treated conophor nut protein isolates. Germination (72 h) has caused a significant decrease ($p < 0.05$) in the bulk density for CPIs obtained from the Natoli variety and the finding is in agreement with the report by Sofi et al. (2020b). Other germination treatments didn't bring any significant effect on the BD.

There was an increase in tapped density for CPIs from roasted chickpea at 150 °C by 9.1 and 6.6%, and at 180 °C by 14.3 and 16.0% for Arerti and Natoli, respectively, compared to their native counterparts. The 72 h germination caused a significant decrease, by ~13%, for Arerti CPI. But the Natoli variety was not significantly affected by the treatment. A decrease in TD due to germination was also observed by Ocheme et al. (2015) which could be attributed to the breakdown of complex compounds, such as protein and starch.

3.2.5. Emulsifying capacity (EC)

Both roasting treatments decreased the emulsifying capacity of the CPIs and it is supported by the report of Xu et al. (2017). This could be due to protein dissociation during heat treatment. The protein forms smaller particles that expose more hydrophobic groups and tend to form smaller emulsion droplets (Imtiaz, 2007). The emulsifying capacity of

Table 2. Mean ± SD value for functional properties of isolates from native and treated chickpea.

Variety	Sample	WHC (g/g)	OHC (g/g)	PS (%)	FC (%)	FS (%)	EC (%)	ES (%)	BD (g/cm^3)	TD (g/cm^3)
Arerti	A-raw	1.93 ± 0.01^{b}	2.03 ± 0.05^{c}	61.08 ± 0.88^{b}	50.0 ± 2.83^{c}	76.00 ± 2.83^{b}	70.30 ± 1.40^{b}	87.34 ± 1.74^{b}	0.70 ± 0.00^{a}	0.77 ± 0.0^{b}
	A-150	2.47 ± 0.02^{c}	2.21 ± 0.04^{d}	69.99 ± 2.15^{d}	59.0 ± 1.41^{d}	83.00 ± 1.41^{c}	63.37 ± 2.80^{a}	76.59 ± 1.18^{a}	0.75 ± 0.04^{b}	0.84 ± 0.00^{c}
	A-180	1.07 ± 0.07^{a}	1.83 ± 9.30^{b}	45.24 ± 0.31^{a}	14.0 ± 0.00^{a}	89.00 ± 1.41^{d}	58.42 ± 1.40^{a}	74.54 ± 3.01^{a}	0.77 ± 0.00^{b}	0.88 ± 0.05^{c}
	A-24	2.34 ± 0.14^{c}	1.58 ± 0.02^{a}	65.16 ± 2.13^{c}	40.0 ± 2.83^{b}	69.00 ± 1.41^{a}	72.28 ± 1.40^{b}	93.13 ± 2.07^{c}	0.67 ± 0.00^{a}	0.77 ± 0.00^{b}
	A-48	2.01 ± 0.05^{b}	2.03 ± 0.09^{c}	59.42 ± 0.61^{b}	67.0 ± 1.41^{e}	79.00 ± 1.41^{bc}	80.20 ± 4.20^{c}	87.73 ± 2.85^{bc}	0.65 ± 0.03^{a}	0.72 ± 0.00^{ab}
	A-72	1.83 ± 0.11^{b}	1.8 ± 0.04^{b}	45.47 ± 0.60^{a}	40.0 ± 0.00^{b}	75.00 ± 1.41^{b}	82.18 ± 1.40^{c}	89.17 ± 1.52^{bc}	0.63 ± 0.00^{a}	0.67 ± 0.00^{a}
Natoli	N-raw	1.80 ± 0.07^{bc}	1.83 ± 0.02^{c}	59.34 ± 0.28^{c}	38.0 ± 2.83^{b}	81.00 ± 1.41^{c}	68.32 ± 4.20^{b}	85.61 ± 3.21^{a}	0.65 ± 0.03^{b}	0.75 ± 0.04^{ab}
	N-150	2.21 ± 0.04^{e}	1.66 ± 0.0^{b}	66.80 ± 1.17^{d}	48.0 ± 0.00^{c}	84.00 ± 0.00^{d}	61.39 ± 0.00^{a}	85.48 ± 2.28^{a}	0.72 ± 0.00^{cd}	0.80 ± 0.04^{bc}
	N-180	1.13 ± 0.11^{a}	1.40 ± 0.05^{a}	47.42 ± 0.57^{b}	26.0 ± 0.00^{a}	82.00 ± 0.00^{cd}	56.44 ± 1.40^{a}	85.96 ± 0.35^{a}	0.74 ± 0.04^{d}	0.87 ± 0.05^{c}
	N-24	2.05 ± 0.22^{de}	2.13 ± 0.09^{d}	61.00 ± 1.17^{c}	94.0 ± 2.83^{d}	76.00 ± 0.00^{b}	77.23 ± 0.00^{c}	93.59 ± 1.81^{b}	0.67 ± 0.00^{bc}	0.72 ± 0.02^{ab}
	N-48	1.89 ± 0.01^{cd}	1.72 ± 0.05^{bc}	58.39 ± 1.85^{c}	53.0 ± 1.41^{c}	73.00 ± 1.41^{a}	81.19 ± 2.80^{cd}	89.11 ± 4.80^{ab}	0.63 ± 0.00^{b}	0.69 ± 0.03^{a}
	N-72	1.61 ± 0.10^{b}	2.01 ± 0.08^{d}	43.88 ± 2.00^{a}	22.0 ± 2.83^{a}	91.00 ± 1.41^{d}	84.16 ± 1.40^{d}	88.23 ± 0.20^{ab}	0.57 ± 0.02^{a}	0.69 ± 0.03^{a}

Means sharing the same letter in a column with in variety are not significantly different ($p > 0.05$). A-raw = Arerti raw; A-150 = Arerti roasted at 150 °C; A-180 = Arerti roasted at 180 °C; A-24 = Arerti germinated for 24 h; A-48 = Arerti germinated for 48 h; A-72 = Arerti germinated for 72 h; N-raw = Natoli raw; N-150 = Natoli roasted at 150 °C; N-180 = Natoli roasted at 180 °C; N-24; Natoli germinated at 24 h; N-48 = Natoli germinated for 48 h; N-72 = Natoli germinated for 72 h. WHC-Water holding capacity; OHC-Oil holding capacity, PS-protein solubility; FC-Foaming capacity; FS-Foaming stability; ES-Emulsion stability; BD-Bulk density; TD-Tapped density.

CPIs was improved by germination and this is in line with Sofi et al. (2020b). The increase observed in emulsion capacity could be due to an increase in the area of stabilized oil droplets at the interface which is a function of the food components (Al-Ismail, 2018).

3.2.6. Emulsion stability (ES)

Both roasting treatments significantly decreased the ES in the case of Arerti CPIs. Xu et al. (2017) also reported that emulsion stability is significantly decreased by roasting. This behavior could be due to the decrease in the forces responsible for the formation of a strong visco-elastic film at the interface which results in lower stability of the formed emulsion (Delahaije et al., 2019). CPIs from germinated samples had better ES than those from roasted ones, despite Natoli CPIs germinated for 48 and 72 h which had no significant difference with the roasted CPIs. A previous study also reported that germination improved ES in pigeon pea (Sharma et al., 2019).

3.2.7. Foaming capacity (FC)

There was a significant increase in foaming capacity for chickpea protein isolates of both varieties that were roasted at 150 °C. The finding is supported by Kinsella (1981) as it stated that limited heating, which induces partial unfolding of globular proteins without causing thermal coagulation facilitates foam formation.

The impact of the 24 h germination result was different for the two varieties as it caused a 25% decrease in Arerti and a 147% increase in the Natoli variety. CPIs from germinated chickpea for 48 h showed similar results for both varieties. It improved the foaming capacity by 34 and 39.5% for Arerti and Natoli, respectively, which is in agreement with Sharma et al. (2019).

3.2.8. Foam stability (FS)

While foam volumes were enhanced, the stability of foams was reduced. CPIs with high foaming capacity were weak in their foam stability and it is in line with Delahaije et al. (2019). CPIs with the lowest foaming capacity (Arerti roasted at 180 °C and Natoli germinated for 72 h) were the highest in their foam stability. In the case of CPI from Natoli chickpea germinated for 72 h, it is in agreement with Sharma et al. (2019). Native Arerti and Natoli CPIs lost 24% and 19%, respectively of their foam, after an hour. The least stable foam was developed for CPIs from Arerti chickpea germinated for 24 h which lost 31% of its foam (Table 2). The action of proteolytic enzymes on the protein and peptides and the subsequent structural changes in those compounds during germination could be the possible reason for the existing difference in the foam stability.

3.3. Antioxidant properties

3.3.1. Total phenolic content

Table 3 presents the antioxidant properties of CPIs from raw and treated chickpeas. The total phenolic content of CPIs prepared from native Arerti and Natoli flours was 179.8 and 186.0 mg GAE/100 g, respectively. Roasting significantly increased the total phenol content of the isolate, and this could be attributed to the production of Maillard reaction products during roasting (Al-Ismail, 2018). Germination also improved the total phenolic content by 16.2–51.6% for CPIs obtained from Arerti chickpea and by 39.1–76.6% for CPIs obtained from Natoli chickpea. As germination time increased the TPC was also increased. Solubilization of condensed tannin could be the possible reason for the increase of total phenolics during germination (James et al., 2020). The finding is in agreement with the report of Sofi et al. (2020b).

3.3.2. Total flavonoid content

The total flavonoid content (TFC) of CPI from native Arerti and Natoli flour was 68.2 and 88.3 mg CEQ/100 g, respectively. Roasting at 150 and 180 °C increased TFC by 142.2 and 138.6% for CPI from Arerti and 73.7 and 80.5% for CPI from Natoli chickpea. Germination treatment also increased TFC content for both varieties. As germination time increased flavonoid content also increased, which is the same finding as León-López et al. (2020). This could be attributed to the enzymatic biosynthesis of flavonoids and other secondary plant metabolites due to germination from seed coats and cotyledons due to the enzymatic activation (Lin and Lai, 2006; Duodu 2014).

3.3.3. 1,1-Diphenyl-2-picrylhydrazyl (DPPH˙) inhibition assay

The DPPH radical inhibition value of the native Arerti and Natoli CPIs was 37.3 and 44.5%. Roasting significantly increased the percent inhibition, despite the lack of significant difference between CPIs from chickpea roasted at 150 and 180 °C. The increased availability of phenolic compounds or the formation of new antioxidant compounds during the heating process could justify the increment during roasting (Lemos et al., 2012). Germination also increased the percent inhibition of the DPPH; with the highest percentage increase observed for CPIs from Areti variety germinated for 72 h (47.2%). The longer the germination time the higher is the antioxidant capacity. This could be associated with the increase in total phenolics, the most potent antioxidant compounds in

Table 3. Mean ± SD value for antioxidant properties of isolates from native and treated chickpea.

Variety	Sample name	Total phenolics (mg GAE/100 g)	Total flavonoid (mg CEQ/100 g)	DPPH % inhibition	IC_{50} of DPPH (mg/mL)	Hydrogen peroxide scavenging %
Arerti	A-raw	179.8 ± 1.79^{a}	68.2 ± 1.55^{a}	37.3 ± 3.06^{a}	167.4 ± 14.35^{c}	62.0 ± 0.85^{a}
	A-150	286.5 ± 1.79^{c}	165.2 ± 5.20^{d}	53.8 ± 1.16^{b}	107.7 ± 1.65^{ab}	78.8 ± 2.68^{bc}
	A-180	311.8 ± 16.93^{d}	162.7 ± 3.47^{d}	54.6 ± 1.57^{b}	101.5 ± 2.11^{a}	80.5 ± 2.13^{cd}
	A-24	208.9 ± 5.75^{b}	115.3 ± 3.96^{b}	51.6 ± 0.61^{b}	106.4 ± 2.99^{ab}	75.9 ± 1.16^{b}
	A-48	265.6 ± 16.12^{c}	141.5 ± 12.31^{c}	50.3 ± 0.00^{b}	118.7 ± 2.70^{b}	77.5 ± 0.97^{bc}
	A-72	272.5 ± 0.51^{c}	185.4 ± 7.75^{e}	54.9 ± 2.79^{b}	110.3 ± 3.40^{ab}	83.6 ± 1.46^{d}
Natoli	N-raw	186.0 ± 15.8^{a}	88.3 ± 9.71^{a}	44.5 ± 1.09^{a}	130.2 ± 7.90^{c}	66.7 ± 0.18^{a}
	N-150	233.4 ± 3.92^{b}	153.4 ± 14.35^{c}	53.4 ± 1.77^{bc}	107.2 ± 2.80^{ab}	81.6 ± 1.70^{b}
	N-180	271.2 ± 7.79^{c}	159.4 ± 1.36^{c}	51.9 ± 0.48^{b}	108.7 ± 0.16^{ab}	83.0 ± 5.29^{b}
	N-24	258.8 ± 5.49^{c}	126.8 ± 2.87^{b}	46.9 ± 1.57^{a}	123.8 ± 2.30^{c}	82.7 ± 2.62^{b}
	N-48	298.6 ± 0.30^{d}	185.2 ± 4.86^{d}	54.1 ± 1.43^{bc}	113.7 ± 0.05^{b}	82.9 ± 3.10^{b}
	N-72	328.5 ± 11.82^{e}	197.6 ± 8.28^{d}	55.9 ± 1.50^{c}	97.3 ± 3.00^{a}	80.8 ± 4.56^{b}

Data are expressed as mean ± standard error of replicate determinations. Means sharing the same letter in a column with in variety are not significantly different ($p > 0.05$). A-raw = Arerti raw; A-150 = Arerti roasted at 150 °C; A-180 = Arerti roasted at 180 °C; A-24 = Arerti germinated for 24 h; A-48 = Arerti germinated for 48 h; A-72 = Arerti germinated for 72 h; N-raw = Natoli raw; N-150 = Natoli roasted at 150 °C; N-180 = Natoli roasted at 180 °C; N-24; Natoli germinated at 24 h; N-48 = Natoli germinated for 48 h; N-72 = Natoli germinated for 72 h; GAE = gallic acid equivalent, CEQ = catechin equivalent; IC50 = 50% inhibition capacity.

plants. The finding is in accordance with the reports of James et al. (2020) and Saleh et al. (2019).

The CPIs from germinated and roasted chickpea were better in their IC_{50} value compared to their native counterparts. Both native CPIs required a comparatively higher amount of sample to inhibit 50% of the DPPH. IC_{50} values of the native CPIs were 167.4 and 130.2 mg/mL for Arerti and Natoli, respectively. A lower IC_{50} value means, the antioxidant activity of the sample is higher and the lowest IC_{50}, 97.3 mg/mL, was obtained for 72 h germinated Natoli CPI although it is not statistically significant compared with the roasted Natoli CPIs (Table 3).

3.3.4. Hydrogen peroxide scavenging capacity

The hydrogen peroxide scavenging value of the raw CPIs was 62 and 66.7% for Arerti and Natoli CPIs, respectively. Roasting significantly improved scavenging capacity for both varieties compared to their native counterparts. The highest percentage increase was observed for CPIs obtained from Arerti variety roasted at 180 °C (29.8%). According to Hatamian et al. (2020) roasting at 180 °C significantly increased the antioxidant activity of chia seed. The percentage inhibition of the CPIs prepared from germinated seed increased significantly compared to the native CPIs. In CPIs prepared from germinated Arerti flour the increase is gradual, as the germination time increased the inhibition also increased. The maximum increment was achieved for CPIs obtained from Arerti variety germinated for 72 h (34.8%). The increase in antioxidant capacity during germination and roasting could be attributed to the release of bound phenolics from the seed coats which have significant contributions for antioxidant properties (Duodu 2014).

3.4. Antinutritional content

3.4.1. Phytate content

Table 4 presents the antinutritional content of CPIs from raw and treated chickpeas. The phytate content of the native CPIs was 385.70 mg/100 g and 319.32 g/100 g for Arerti and Natoli, respectively. The lowest phytate content was obtained for CPI prepared from Arerti chickpea flour roasted at 150 °C where the treatment decreased the phytate content by 40.2%. The decrease in phytate content could be attributed to a heat-induced decrease of extractability of phytate (Kumar et al., 1978). The germination process also significantly reduced the phytate content of the Arerti CPI. The 72 h germination was the most effective as it decreased the phytate by 18.6% and this is in line with Sofi et al. (2020a).

Table 4. Mean ± SD value for antinutritional content of isolates from native and treated chickpea.

Variety	Sample name	Phytate content (mg/100 g)	Tannin content (mg/100 g)
Arerti	A-raw	385.70 ± 3.11^{c}	105.18 ± 14.88^{b}
	A-150	230.54 ± 21.67^{a}	84.27 ± 14.9^{ab}
	A-180	311.33 ± 12.20^{b}	63.00 ± 14.85^{ab}
	A-24	325.87 ± 10.56^{b}	62.68 ± 14.77^{ab}
	A-48	341.53 ± 6.18^{b}	52.17 ± 29.51^{a}
	A-72	313.95 ± 28.93^{b}	41.83 ± 14.79^{a}
Natoli	N-raw	319.32 ± 48.40^{a}	125.34 ± 14.77^{c}
	N-150	371.72 ± 6.03^{a}	73.63 ± 0.00^{ab}
	N-180	352.08 ± 24.24^{a}	62.74 ± 14.79^{a}
	N-24	377.31 ± 3.13^{a}	114.93 ± 29.55^{bc}
	N-48	354.10 ± 21.87^{a}	63.10 ± 14.87^{a}
	N-72	374.99 ± 18.36^{a}	41.90 ± 14.81^{a}

Data are expressed as mean ± standard error of replicate determinations. Means sharing the same letter in a column with in variety are not significantly different ($p > 0.05$). A-raw = Arerti raw; A-150 = Arerti roasted at 150 °C; A-180 = Arerti roasted at 180 °C; A-24 = Arerti germinated for 24 h; A-48 = Arerti germinated for 48 h; A-72 = Arerti germinated for 72 h; N-raw = Natoli raw; N-150 = Natoli roasted at 150 °C; N-180 = Natoli roasted at 180 °C; N-24; Natoli germinated at 24 h; N-48 = Natoli germinated for 48 h; N-72 = Natoli germinated for 72 h.

Activation of endogenous phytase during germination could result in the decrease of phytate content (Azeke et al., 2011; Nkhata et al., 2018). On contrary to this, no significant effect was observed due to germination for Natoli variety.

3.4.2. Tannin content

The tannin content of CPIs from the native Natoli and Arerti varieties were 105.18 and 125.34 mg/100 g, respectively. Roasting significantly decreased the tannin content of CPIs for both varieties. In the case of Arerti CPIs, roasting at 150 and 180 °C decreases the tannin content by 19.9 and 40.1%, respectively. The germination treatment highly decreased the tannin content though there was a huge difference in the decreasing trend between the varieties germinated for 24 h. The Arerti CPI germinated for 24 h removed 40.4% of the tannin, and the Natoli CPI tannin content was decreased by only 8.3%. The decrease in tannins may result from leaching into the soaking water (Agume et al., 2017) or hydrophobic association of tannins with seed proteins and enzymes (Megat Rusydi and Azrina, 2012). The results are in accordance with Olika et al. (2019).

4. Conclusion

Chickpea protein isolates prepared from defatted chickpea flour were good in terms of their nutritional, techno-functional, antioxidant, and antinutritional factors. Both roasting and germination treatments modify those parameters to a different extent. Therefore, based on the intended purpose the desired character of the isolates can be enhanced using a specific processing method. Further studies are required on the use of protein isolates from chickpea obtained by applying different treatments for functional foods product development.

Declarations

Author contribution statement

Nobel Mesfin: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Abera Belay, Endale Amare: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data included in article/supplementary material/referenced in article.

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The authors declare no conflict of interest.

Additional information

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