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# *Physicochemical, Nutritional, and Antioxidant Properties of Two Iranian Lentil Cultivars: A Comparative Study of Cooking and Germination Effects*

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***ABSTRACT***

**Background**: Lentils are one of the most important rainfed legumes in the world. They are an excellent source of minerals, vitamins, and amino acids. **Methods:** Proximate composition (moisture, total ash, total fat, protein, fiber, and carbohydrate), mineral content, antioxidant activity (DPPH $IC_{50}$), and total phenolic compounds (TPC) were determined in the raw, germinated, and cooked samples of two cultivars of Iranian lentils. **Results:** Cooking and germination showed a significant effect on TPC, antioxidant activity, and minerals. The highest amount of phenolic compounds was obtained in raw black lentil, followed by raw green and germinated lentils. The black cultivar showed a higher quantity of K, Cu, Ca, and Zn and treatments decreased the concentrations of mineral elements in the investigated samples. Moreover, losses of the minerals in the cooked samples were higher than the germinated ones. **Conclusions:** Cooking and germination brought about a significant reduction in the phenolic compounds and antioxidant activity. Meaningful reductions were also recognized in the mineral content during cooking and germination.

**Keywords**: *Antioxidants; Polyphenols; Fabaceae; Nutritive value*

## Introduction

Food security is restricted by severe climate changes, global warming, and water shortage crisis around the world. Legumes are sources of carbohydrates, protein, dietary fiber, micronutrients, vitamins, and phenolic compounds. However, the principal consumption of proteins and carbohydrates is supplied by animal products and cereals with much higher water requirements and lower nutritional value than legumes. People are prone to obesity, heart disease, cancer, and diabetes by consuming a great amount of meat and cereal-based food. In addition, rice fields and livestock farms are known as two primary sources to increase methane greenhouse gas around the world (Rebello *et al.*, 2014).

For many countries, the low amount and quality of proteins are the main nutritional issues. But, the adverse effects of low protein and particular

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vitamins can be supplied by legumes since they are the second dominant source of food after cereals. Epidemiological studies also showed that the consumption of legumes is inversely associated with the risk of coronary heart disease, type II diabetes, obesity, lower LDL, and higher HDL cholesterol (Siva *et al.*, 2018).

Legumes can be a good substitute for animal proteins but their cost is equal to one-fifth of milk production. Compared with cereals, the crop yields of legumes are two or three times higher and create job opportunities in rural areas (Bhavya and Prakash, 2021, Xu *et al.*, 2007). As a result, 2016 was declared the international year of legumes by Food and Agriculture Organization (FAO).

Lentils (*Lens culinaris Medik*) are one of the most important rainfed legumes around the world. They contain approximately 18-32% protein, and 60% carbohydrate, with soluble and insoluble dietary fiber which decrease cholesterol, fat, and blood sugar. They are an excellent source of molybdenum, copper, manganese, iron, zinc, potassium, phosphorus, folate, vitamin B1, and B6 (Dueñas *et al.*, 2009). Lentil is composed of several varietal groups based on testa and cotyledon color. Brown lentils are the most common variety, their color range from khaki brown to dark black, and they have a mild, earthy flavor. Green lentils are incredibly similar to brown lentils, have a more robust and slightly peppery flavor, and come in a variety of sizes. Pardina or Spanish brown types have brown speckled testa with yellow cotyledons. Red lentils have traditionally been produced and consumed in India and the Middle East. More recently, the USA, Canada, and Australia have become significant producers of red lentils. Black beluga lentils are shiny, tiny, and black and look like caviar (Stefaniak and McPhee, 2015).

The consumption of lentils with wheat or rice improves the composition of essential amino acids in human diet because lentils contain a high quantity of lysine and tryptophan (Portman *et al.*, 2019). Green lentils (also known as brown, yellow, or macrosperma) are characterized by a green to brown seed coated with yellow cotyledons. Red lentils (known as microsperma or Persian) also have a pale to dark grey seed coated with red cotyledons (Kaur *et al.*, 2010).

In addition to valuable nutritional composition, lentils have anti-nutritional agents such as trypsin inhibitors and oligosaccharides. The preparation processes can significantly decrease or eliminate anti-nutritional components but damage their dietary properties. For instance, soaking, germinating, boiling, and cooking improve the availability of proteins, starch, some minerals, and vitamins (Fabbri and Crosby, 2016). Therefore, the basic objective of the present study is to survey physicochemical, nutritional, and antioxidant properties of the two cultivars of Iranian lentils, including green (with green hull and yellow cotyledon) and black (with black hull and red cotyledon) lentils. Furthermore, changes in physicochemical characteristics are compared in raw, germinated, and cooked samples.

## Materials and Methods

*Chemicals and reagents:* Folin Ciocalteau phenol reagent, gallic acid monohydrate, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), and 1,1-diphenyl-2-picrylhydrazyl-hydrate (DPPH) were received from Sigma-Aldrich (Sternheim, Germany). Sodium carbonate, potassium acetate, ferrous sulfate, sodium acetate, and sodium sulfate were also acquired from Sigma. Organic solvents, including methanol and ethanol, were purchased from Merck (Darmstadt, Germany). All the chemicals were used as received without any further purification.

*Preparation of germinated and cooked lentils:* The green lentil sample was purchased in March 2019 from a local supermarket in Mashhad, Khorasan Razavi province, Iran. The black lentil was also obtained from a local market in Bahabad, Yazd, Iran. Foreign materials and broken and damaged seeds were removed, and the cleaned samples were stored in polyethylene bags at 4˚C.

The raw samples were prepared as follows. A minimum sample size of 100 g of each cultivar was mixed and grounded using an electric mill

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(IKA, A11 basic) and passed through a No.35 (500-µm) sieve set. The resulting powders were packed in properly labeled polyethylene bags and stored at 4˚C for further analysis.

The seeds must be exposed to water and oxygen for germination. Generally, the cleaned seeds were soaked in water for one to several days to be completely hydrated. The following procedure was obeyed to prepare the germinated samples. Lentil (10 g) of each cultivar was transferred to a Petri dish and soaked in water for one overnight. Then, the excess water was discarded and the seeds were covered with filter paper. The dishes were kept at room temperature (23-25 ˚C) over the next few days for germination, and sufficient moisture was maintained by occasionally adding drops of water to wet the filter paper. The seeds were germinated in 3 to 4 days.

The cooked green and black lentils were prepared by transferring 10 g of each sample to 500 mL of boiling water. The process was continued for the following 15 min, and then, the samples were filtered.

*Chemical composition analysis:* Standard test methods were used to determine ash content, total fat, and crude fiber (de Almeida Costa *et al.*, 2006). Moisture was determined gravimetrically by drying the samples at 110 ˚C until constant weight is reached. Kjeldahl method was used to determine nitrogen content, and a factor of 6.25 was employed to estimate protein content (Association of Official Analytical Chemists (AOAC), 1990). A GBC SensAA atomic absorption spectrometer (GBC Scientific Equipment, Australia) with a continuum deuterium source as the background correction system was used to specify metal elements. The instrument was equipped with flame and graphite furnace atomizers and single-element hollow cathode lamps. The operating conditions adjusted in the atomic absorption spectrometer were carried out according to the standard guidelines of the manufacturer.

*Preparation of sample extracts:* An ethanolic extract was prepared from lentil samples. The samples were grounded with an electric mill, and 25 ml ethanol solution (70 %v/v) was added to 0.5 g of the resulting powder and stirred for 30 min. Then, the mixture was filtered, and the filtrate was kept in a closed polyethylene tube at 4 ˚C until analysis. The extracts were used to determine antioxidant activities and total phenolic compounds (TPC).

*Determination of total content of phenolic compounds:* PC was quantified following the standard spectrophotometric method with Folin-Ciocalteu reagent diluted in distilled water at a 1:10 ratio (Esmaeelian *et al.*, 2020). Then, the diluted reagent (1.5 ml) was added to 200 µl of each sample extract. The mixtures were shaken for 5 min and followed by the addition of 2 mL of the saturated sodium carbonate solution (about 75 g/l). The samples were kept in the dark at room temperature (23-25 ˚C) for the following 30 min, and the absorbance was read at 765 nm (DR 5000™ UV-Vis, Hach Company, USA). After that, a calibration curve was prepared with gallic acid as the standard, and TPC was expressed as milligram gallic acid equivalents (mg GAE) in 100 g dry weight of the sample.

*Determination of antioxidant activity:* The antioxidant activity of lentil extracts was determined using the standard DPPH method (Zhou *et al.*, 2017). The sample extracts were diluted at different concentrations, and 2 ml of each solution was mixed with 2 ml of the daily prepared DPPH solution (40 mg/l in ethanol). The samples remained at room temperature (23-25 ˚C) for 30 min before spectrophotometric measurements at 517 nm. Subsequently, free radical-scavenging activity was calculated according to Equation (1) and was expressed as the percentage inhibition of DPPH.

**Equation (1)**

$$\text{Inhibition (\%)} = \left[\frac{A_{blank} - A_{sample}}{A_{blank}}\right] \times 100$$

Where, $A_{blank}$ and $A_{sample}$ are the absorbance of blank and test solutions, respectively. The sample concentration providing 50% inhibition ($IC_{50}$) was calculated by exponential regression analysis from the curve of inhibition% versus the extract

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concentration (1000-50000 mg/ml) with a regression equation of Y=25.59 Ln(X)-256.4, R2=0.996.

*Data analysis:* All measurements were performed in three replications, and T-test statistical analysis was carried out to compare mean values (Duncan's test at a P-value of 0.05). Finally, ANOVA was conducted to determine significant differences at P-value<0.05 (using Tukey HSD multiple range test).

## Results

*Proximate composition:* As shown in **Table 1**, the content of moisture and volatiles was increased during processing for both cultivars. The green lentil sample had higher moisture content in all the three states and showed a superior capability to absorb water. .

**Table 1.** Chemical composition (g/100 g) of black and green lentils*.

| Sample | Moisture | Total ash | Total fat | Protein | Fiber | Carbohydrate |
|---|---|---|---|---|---|---|
| Green lentil | | | | | | |
| Raw | 7.95±0.02 $^{c, D}$ | 3.28±0.25 $^{a, B}$ | 1.72±0.11 $^{a, B}$ | 26.57±0.41 $^{a, D}$ | 4.59±0.37 $^{a, B}$ | 55.89±0.49 $^{a, A}$ |
| Germinated | 15.29±0.54 $^{a, A}$ | 2.04±0.06 $^{b, DE}$ | 1.31±0.15 $^{b, CD}$ | 27.34±0.20 $^{a, CD}$ | 1.87±0.23 $^{c, DE}$ | 52.14±0.12 $^{c, BC}$ |
| Cooked | 14.28±0.29 $^{b, B}$ | 1.60±0.15 $^{c, E}$ | 1.02±0.16 $^{b, D}$ | 26.64±0.70 $^{a, D}$ | 2.63±0.31 $^{b, CD}$ | 53.83±1.07 $^{b, ABC}$ |
| Black lentil | | | | | | |
| Raw | 6.81±0.30 $^{c, E}$ | 3.83±0.21 $^{a, A}$ | 2.12±0.17 $^{a, A}$ | 28.43±0.58 $^{b, BC}$ | 7.41±0.41 $^{a, A}$ | 51.39±1.04 $^{b, C}$ |
| Germinated | 8.96±0.20 $^{a, C}$ | 2.86±0.11 $^{b, BC}$ | 1.60±0.16 $^{b, BC}$ | 30.37±0.91 $^{a, A}$ | 1.28±0.40 $^{c, E}$ | 54.93±1.35 $^{a, AB}$ |
| Cooked | 8.15±0.36 $^{b, CD}$ | 2.42±0.20 $^{b, CD}$ | 1.30±0.10 $^{b, CD}$ | 29.85±0.66 $^{ab, AB}$ | 3.32±0.38 $^{b, C}$ | 54.96±1.65 $^{a, AB}$ |

*: Results are in mean ± standard deviation (n=3). Different superscripts show statistically significant differences (P < 0.05). Means with at least one identical superscript (uppercase letters) do not differ significantly (effect of lentil sample). Treated samples were also evaluated separately for each cultivar (lower case letters).

The total ash content of 1.60-3.28 and 2.42-3.83 g/100 g was determined for different states of green and black lentils, respectively. Black lentil had higher total ash, and raw lentils had higher ash content in both cultivars. As presented in **Table 1**, cooking and germination resulted in a meaningful reduction in fat content. The results showed that the black lentil had a higher amount of protein than the green cultivar. No significant changes were observed for the germinated and cooked green lentil, but germinated black lentil had higher protein compared with the raw sample.

The results suggested that raw samples contained the highest amount of fiber, and 4.59 and 7.41 g/100 g of fiber were measured in green and black lentils, respectively. Carbohydrate was also determined in all the samples. As shown in **Table 1**, green lentils in the raw state had higher carbohydrate (55.89 g/100 g) than the germinated (52.14 g/100 g) and cooked samples (53.83 g/100 g). The carbohydrate content of raw black lentil (51.39 g/100g) was increased and reached 54.93 and 54.95 g/100g in the germinated and cooked samples.

*Changes in phenolic compounds and antioxidant activity:* Phenolic content was determined in the raw and treated samples. As shown in **Table 2**, the raw black lentil had a higher TPC value than the green cultivar. Also, germination and cooking resulted in a significant decrease in phenolic compounds in both cultivars.

Antioxidant activity was also decreased in germinated and cooked samples (**Table 3**). The results showed lower IC50 values or higher antioxidant activities for black lentils in all the three states.

The results confirmed a higher amount of phenolic compounds and antioxidant activity for the raw black lentil than the green cultivar. The findings followed the previous works which revealed higher antioxidant activity of black lentils among the other seven different studied cultivars. Also, higher antioxidant activity of legumes with a darker skin color was reported.

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**Table 2.** Total phenolic content (TPC) in black and green lentils.*

| Sample | TPC (mg GAE/100 g) |
|---|---|
| Green lentil | |
| Raw | 52.59±4.45 $^{a, B}$ |
| Germinated | 30.37±1.97 $^{b, CD}$ |
| Cooked | 14.44±2.60 $^{c, E}$ |
| Black lentil | |
| Raw | 69.84±5.48 $^{a, A}$ |
| Germinated | 31.50±2.94 $^{b, C}$ |
| Cooked | 21.20±2.13 $^{c, DE}$ |

*: Results are in mean ± standard deviation (n=3). Different superscripts show statistically significant differences ($P < 0.05$). Means with at least one identical superscript (uppercase letters) do not differ significantly (effect of lentil sample). Treated samples were also evaluated separately for each cultivar (lower case letters).

*Change in mineral contents:* The mineral contents of raw, germinated, and cooked lentil samples are presented in **Table 4**. The black cultivar showed a higher quantity of mineral elements, particularly K, Ca, Mg, Fe, Cu, and Zn

**Table 3.** Antioxidant activity ($IC_{50}$) in black and green lentils.*

| Sample | $IC_{50}$ (mg/Kg) |
|---|---|
| Green lentil | |
| Raw | 4528.11±2.96 $^{c, E}$ |
| Germinated | 12094.57±1.96 $^{b, D}$ |
| Cooked | 31819.80±3.80 $^{a, A}$ |
| Black lentil | |
| Raw | 2410.59±2.46 $^{c, F}$ |
| Germinated | 15269.83±1.27 $^{b, C}$ |
| Cooked | 28223.03±2.62 $^{a, B}$ |

* Results are in mean ± standard deviation (n=3). Different superscripts show statistically significant differences ($P < 0.05$). Means with at least one identical superscript (uppercase letters) do not differ significantly (effect of lentil sample). Treated samples were also evaluated separately for each cultivar lower case letters).

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**Table 4.** Effect of different treatments on selected mineral contents (mg/kg) of lentils.*

| Sample | K | Cu | Fe | Mg | Ca | Zn | Mn | Na |
|---|---|---|---|---|---|---|---|---|
| Green lentil | | | | | | | | |
| Raw | 6297.8 $^{a, B}$ | 17.53 $^{a, BC}$ | 53.43 $^{a, BC}$ | 901.09 $^{a, C}$ | 626.32 $^{a, C}$ | 63.99 $^{a, D}$ | 30.18 $^{a, B}$ | 3908.4 $^{a, A}$ |
| Germinated | 6164.2 $^{a, B}$ | 12.83 $^{b, D}$ | 59.73 $^{a, ABC}$ | 725.06 $^{b, D}$ | 465.14 $^{b, D}$ | 45.61 $^{b, E}$ | 29.38 $^{a, B}$ | 152.4 $^{b, C}$ |
| Cooked | 3648.3 $^{b, C}$ | 12.75 $^{b, D}$ | 36.75 $^{b, D}$ | 684.70 $^{b, D}$ | 463.22 $^{b, D}$ | 42.12 $^{b, E}$ | 27.85 $^{a, B}$ | 97.1 $^{b, C}$ |
| Black lentil | | | | | | | | |
| Raw | 7525.9 $^{a, A}$ | 28.65 $^{a, A}$ | 67.44 $^{a, A}$ | 1164.59 $^{a, A}$ | 1276.51 $^{a, A}$ | 156.67 $^{a, A}$ | 38.84 $^{a, A}$ | 3251.3 $^{a, A}$ |
| Germinated | 7691.3 $^{a, A}$ | 19.56 $^{b, B}$ | 61.50 $^{a, AB}$ | 1008.41 $^{b, B}$ | 902.58 $^{b, B}$ | 139.75 $^{b, B}$ | 37.07 $^{a, A}$ | 869.3 $^{b, BC}$ |
| Cooked | 3772.9 $^{b, C}$ | 15.75 $^{c, C}$ | 50.68 $^{b, C}$ | 965.64 $^{b, BC}$ | 812.41 $^{c, B}$ | 120.25 $^{c, C}$ | 28.79 $^{b, B}$ | 1192.4 $^{b, B}$ |

*: Results are in mean ± standard deviation (n=3). Different superscripts show statistically significant differences ($P < 0.05$). Means with at least one identical superscript (uppercase letters) do not differ significantly (effect of lentil sample). Treated samples were also evaluated separately for each cultivar (lower case letters).

The results showed great losses of K (48.9%), Cu (45.1%), Ca (36%), Mn (25.9%), Fe (24.9%), and Zn (23.1%) in the black lentil during cooking. Similarly, cooking caused a significant reduction of K (42.1%), Zn (34.2%), Fe (31.3%), Cu (27.3%), Ca (26.1%), and Mg (24.1%) in the green lentil cultivar.

## Discussion

In general, lentil seeds had a higher moisture content compared with other legumes (Aryee and Boye, 2017). However, storage conditions could affect the seeds' characteristics. The major storage conditions that affect any grain are temperature and moisture content (Sravanthi *et al.*, 2013). An increase in moisture content in cooked and germinated samples was due to water absorption. Also, a significant decrease of ash in the germinated and cooked samples could be due to the loss of some grain husk (Bubelová *et al.*, 2018) or the dissolution of water-soluble minerals during these processes (Duenas *et al.*, 2016). Other studies also noticed an improvement in some nutritional characteristics with an ash reduction during cooking and germination of

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lentils (Guo *et al.*, 2012). Compared with other legumes, lentils had less ash content than beans and chickpeas and more ash content compared with peas (Hoover and Ratnayake, 2002).

Lentils had a low content of fats approximately equal to chickpea and pea and most of the grains such as rice and wheat (Iqbal *et al.*, 2006). During germination, carbon is used as the primary source for growth, and fatty acids are oxidized to carbon dioxide and water to produce energy (Megat Rusydi *et al.*, 2011). Other similar investigations also reported a decrease in fat content after cooking (Aryee and Boye, 2017).

The increase of protein content in germination can be a result of the synthesis of enzymatic proteins, hormonal changes, increase in amino acids, peptides, nitrogenous compounds, and release of free amino acids after hydrolysis (Nonogaki *et al.*, 2010). Moreover, increasing the amount of protein in the cooked samples can be due to the rise in solubility, and consequently, increase the concentration. Also, similar changes were reported in protein content of dark beans and lentils after germination and cooking (Aryee and Boye, 2017, Duenas *et al.*, 2016).

The amount of fiber is reduced in thermal processes like cooking, due to the volume reduction of pectic polysaccharides and dissolution/destruction of hemicellulose polymer into simple carbohydrates (Rehinan *et al.*, 2004). Due to the increase of alpha-galactosidase activity during legumes germination, a reduction in oligosaccharides content occurs and leads to a decrease in the crude fiber (Ghavidel and Prakash, 2007).

Carbohydrates are the main component in the lentil seeds, ranging from 43.4 to 74.9 g/100 g of dry matter (Iqbal *et al.*, 2006). Starch is the dominant polysaccharide in lentils and serves as the main source of energy. Lentil seeds contain starch within the range of 34.7 to 65 % in the matter (Hoover and Ratnayake, 2002).

A reduction in phenolic content due to germination process was also reported in red lentils (Moslem *et al.*, 2016) and germinated legumes and rice (Megat Rusydi *et al.*, 2011), and androgen enzymes influenced phenolic content changes in the germination process (Gharachorloo *et al.*, 2013). The anti-radical activity of phenolic compounds depends on their molecular structure and phenolic hydrogen availability (Singh *et al.*, 2014). Other studies also showed a significant decrease in phenolic compounds after cooking different legumes, including lentils (López *et al.*, 2017). The reduction of phenolic content in the cooking process resulted from dissolution of phenols during hydrothermal events, breaking of phenolic compounds, chemical transfer, and decomposition into phenol-protein complexes (Sasipriya and Siddhuraju, 2012, Siddhuraju and Becker, 2007).

Decrease in antioxidant activity after cooking was due to the loss of antioxidant compounds and would lead to increase in solubility (López *et al.*, 2017). The increase observed in antioxidant activity after germination may result from the rise in the number of antioxidant compounds except for polyphenols. Furthermore, legumes had other bioactive compounds beside phenolics, such as vitamins and carotenoids, that could apply synergistic reactions among themselves or with phenolic compounds. It could be the main reason for the difference in the number of antioxidant compounds (Singh *et al.*, 2014). Zou reported higher antioxidant capacity of lentils than common vegetables and fruits like apples, cherries, plums, broccoli, cabbages, grapes, dried beans, onions, and potatoes (Zou *et al.*, 2011).

The results showed mineral losses for green and black lentils. Such losses were attributed to the leaching of minerals from lentil seeds into cooking water. Therefore, cooking would not reduce mineral levels if the cooking water is not discarded. Other studies also showed similar results for lentils during processing due to water diffusion of certain minerals (Aryee and Boye, 2017). The lowering of ash content, as presented in **Table 1** was also observed in the treated samples. The low ash content in both cooked and germinated samples was due to the removal of husks, which were abundant in minerals (Bubelová *et al.*, 2018).

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## Conclusion

This study demonstrates the highest amount of ash, fat, protein, crude fiber, total phenol, and antioxidant activity in black lentils. But, the green lentil shows higher carbohydrates only in the raw state. Therefore, it can be concluded that the black lentil has a higher nutritional quality and antioxidant capacity than the green lentil. Germination and cooking process decreases all the measured properties except for protein content. The cooked lentils (compared to raw ones) possess fewer polyphenols, ash, fat, and fiber and, additionally, lower (though still relevant) antioxidant activity. On the contrary, the germinated samples (compared to the cooked ones) exhibited higher contents of ash and polyphenols, better antioxidant activity, and reduced fiber content. The minerals in cooked samples were significantly reduced (compared to the raw ones). However, cooking would not reduce mineral levels if the cooking water is not discarded.

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## Authors' contributions

All the authors contributed to the study's conception and design, commented on previous versions of the manuscript, and approved the final manuscript.

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## Conflict of interest

The authors declared no conflict of interest

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