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Original research paper

IMPACT OF DOMESTIC COOKING (BOILING) ON THE CONTENT OF PHENOLICS AND ANTIOXIDANT ACTIVITY IN VARIOUS LENTIL CULTIVARS

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Abstract: Lentils (*Lens culinaris* Medik) are among the most widely consumed foods in the world; however, they are primarily cooked by boiling, which results in the loss of antioxidant compounds. This study aimed to evaluate the effects of boiling on the phenolic content—specifically total phenolic content (TPC), total flavonoid content (TFC), and total proanthocyanidin content (TPAC) as well as antioxidant activity, including total antioxidant activity (TAA), DPPH-radical scavenging activity (DPPH-RSA), and ferric reducing power (FRP) in various lentil cultivars. Six commercially available lentil samples (black, brown, green, dehulled red, white, and dehulled yellow) were analyzed. TPC, TFC, and TPAC were measured using the Folin-Ciocalteu, $AlCl_3 \cdot 6H_2O$, and butanol–HCl spectrophotometric methods, respectively. These measurements were conducted in conjunction with antioxidant activity assessments (TAA, DPPH assay, and FRP) before and after cooking. The results revealed that whole lentils contained higher levels of TPC, TFC, and TPAC, as well as greater antioxidant activities (TAA, DPPH-RSA, and FRP) compared to dehulled lentils. Furthermore, whole lentils with dark seed coats (green and black) exhibited superior values for TPC, TFC, TPAC, TAA, DPPH-RSA, and FRP compared to varieties with lighter-colored skins (brown and white). These findings indicate that the presence and color of the seed coat significantly influence the phenolic content of lentil seeds. Boiling lentils for 30 minutes resulted in a substantial decrease in TPC, TFC, TPAC, and antioxidant activity (TAA, DPPH-RSA, and FRP). In addition to their nutritional benefits, lentils can serve as excellent sources of antioxidants and may be utilized as functional ingredients in the production of nutraceuticals and health foods within the food industry.

Key words: lentil, cultivar, phenolic compounds, antioxidant activity, raw, boiled

INTRODUCTION

Grain legumes, commonly known as pulses, have attracted growing interest from both consumers and food industry professionals in recent years. This rising trend in consumption is primarily fueled by a growing preference for environmentally sustainable plant-based pro-

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teins, which is essential in addressing global population growth. Additionally, the increasing popularity of legumes can be linked to heightened consumer awareness and education regarding food quality, nutritional profiles, and health benefits (Kaale, Siddiq, & Hooper, 2023).

Lentil (*Lens culinaris* Medik) is an essential legume crop and a staple food in many Asian and African nations. The seeds of lentils are known by various names, including Adas in Arabic, Mercimek in Turkey, Messer in Ethiopia, Heramame in Japan, and Masser or Masoor in India and Pakistan. Over the past two decades, lentils have gained prominence as a major legume crop, with global production increasing by 93% since 2000, rising from 3.39 to 6.54 million metric tons. Canada leads the world in lentil production, accounting for 44% of the total, followed by India at 18% (Dhull, Kinabo, & Uebersax, 2023).

Lentils are nutritionally rich, providing an excellent source of protein (approximately 25%) along with essential micronutrients such as minerals (calcium, iron, potassium, zinc) and vitamins (vitamins A, C, and niacin). Their protein profile is notably superior to that of some other legumes, like peas and chickpeas, which has led to increased interest in their use as value-added ingredients in various food applications, particularly as plant-based meat alternatives or extenders. Additionally, lentils are high in dietary fiber, slowly digestible starch, and numerous bioactive phytochemicals that offer health benefits. Although they contain about 60% carbohydrates, these carbohydrates are digested slowly in the gut, resulting in a low glycemic index of around 30, compared to a reference value of 100 for white wheat bread (Oduro-Yeboah, Sulaiman, Uebersax, & Dolan, 2023).

In addition to being rich in macronutrients and micronutrients, lentils also contain various bioactive phytochemicals, such as phenolics, flavonoids, saponins, carotenoids, phytic acid, tocopherols, and phytosterols. Lentils exhibit strong antioxidant properties due to their high levels of phenolic compounds. Interestingly, lentils have been recognized in traditional medicinal practices. Recent classifications regard these seeds as therapeutic foods due to their valuable components, including bioactive phytochemicals (Dewan, Shams, & Haque, 2024). Studies indicate that pulses, including lentils, offer potential health benefits that extend beyond simply fulfilling basic nutritional needs. Consuming lentils has been associated with reduced rates of various chronic diseases, such as cardiovascular disease (CVD), diabetes, certain cancers, coronary heart disease, degenerative disorders, and the effects of aging. The health benefits attributed to lentils are largely due to their high content of phytochemicals, including polyphenols, saponins, and phytosterols, which are prominent in pulse-based diets (Mustafa et al., 2022).

Boiling is a domestic cooking method that significantly impacts the phytochemicals in pulses. Typically, pulses are boiled in water at 100 °C for a short duration. This cooking method enhances the sensory qualities of the seeds, tenderizes them, and increases consumer acceptability. Additionally, boiling helps eliminate heat-sensitive antinutritional compounds. However, it can also lead to a loss of antioxidant compounds (Zhou et al., 2024). Accordingly, the aim of this study was to examine how domestic cooking (boiling) affects the phenolic compound profiles (TPC, TFC, and TAPC) and antioxidant activity (TAA, DPPH-RSA, and FRP) of six lentil varieties commonly consumed in Algeria.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu reagent and aluminum chloride were provided from Biochem, Chemopharma (Montreal, Quebec). Sodium carbonate, sodium hydrogen phosphate, sodium dihydrogen phosphate, ascorbic acid, acetone, and ethanol were obtained from VWR, Prolabo (CE-EMB). Potassium ferricyanide, ferric chloride, trichloroacetic acid, gallic acid, and quercetin were from Biochem-chemopharma (UK), 1,1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma–Aldrich (Sigma-Aldrich GmbH, Germany).

Plant material

Six samples of lentil seed cultivars (1 kg each) were purchased from local markets. These included four varieties of whole lentils with colored seed coats (black, brown, green, and white) and two cultivars with colored cotyledons (red and yellow). The red and yellow lentils were sold dehulled, without their seed coats. All seeds were hand-sorted to remove

foreign materials and impurities, such as damaged or infested grains. The lentil seeds were then thoroughly washed with a generous volume of tap water to eliminate any adhering materials and subsequently dried. Finally, the seeds were ground into a fine powder using an IKA A 11 basic grinder (Germany) and stored until analysis (Xu & Chang, 2007).

To evaluate the effect of cooking (boiling) on phenolic contents and antioxidant activities, lentil seeds (50 g) were boiled in 1 liter of tap water for 30 minutes in a standard pot (Acito, Fatigoni, Villarini, & Moretti, 2023). After cooking, the samples were cooled, drained, and then ground using an IKA A 11 basic grinder (Germany). The cooked lentil mass was freeze-dried (Alpha1-4 LDplus, Christ, Osterode, Germany) and powdered before extraction, following the same procedure used for the raw lentils.

Quantification of antioxidants

Phenolic analysis

a) Phenolic extraction

The extraction of phenolic compounds from six cultivars of lentil seeds was performed using the method described by Duenas, Hernandez and Estrella (2002), with some modifications. Two grams of powdered lentil seeds were extracted with 30 mL of a solvent mixture composed of ethanol, water, and acetic acid (80/18.5/1.5) at room temperature, using a magnetic stirrer for 40 minutes. The extracts were then centrifuged at 5000 rpm for 10 minutes. The supernatants were filtered through a paper filter and stored at 4 °C until analysis. These extracts were utilized for the determination of total phenolic content (TPC), total flavonoid content (TFC), total proanthocyanidin content (TPAC), and the measurement of antioxidant capacity, including total antioxidant activity (TAA), DPPH-radical scavenging activity (DPPH-RSA), and ferric reducing power (FRP).

b) Total phenolic content (TPC) determination

The total phenolic content (TPC) in lentil seeds was determined using the Folin-Ciocalteu method (Singleton & Rossi, 1965). A volume of 250 µL of the extract was placed into test tubes, followed by the addition of 750 µL of 10-fold diluted Folin-Ciocalteu reagent and 750 µL of sodium carbonate (7.5%, w/v). After vortexing, the tubes were allowed to stand for 30 minutes at room temperature. The absorbance was then measured at 765 nm using a UV/VIS spectrophotometer (Shimadzu, Japan). A standard calibration curve was created using various concentrations of gallic acid. The results were expressed as milligrams of gallic acid equivalent per 100 grams of dry weight (mg GAE/100 g DW).

Total flavonoid content (TFC) determination

The total flavonoid content (TFC) in lentil extracts was determined spectrophotometri-cally using the method described by Quettier-Deleu et al. (2000), based on the formation of a flavonoid-aluminum complex, which has an absorbance maximum at 430 nm. A volume of lentil seed extract was mixed with an equal volume of 2% $AlCl_3$ solution in methanol. After incubating the mixture at room temperature for 10 minutes, the absorbance was measured at 430 nm against a blank. Quercetin was used to create the calibration curve, and the results were expressed as milligrams of quercetin equivalent per 100 grams of dry weight (mg QE/100 g DW).

Total proanthocyanidin content (TPAC) determination

The total proanthocyanidin content (TPAC) was determined using the butanol–HCl assay (Hagerman, 2000). Briefly, 0.5 mL of lentil extract was transferred into test tubes. Following this, 3 mL of butanol–HCl solution (butanol/HCl, 95:5; v/v) and 0.1 mL of a 2% ferric reagent (2% ferric ammonium sulfate in 2 M HCl) were added. The test tubes were vortexed and placed in a boiling water bath for 60 minutes. After cooling, the absorbance was measured at 530 nm against a blank that contained 0.5 mL of solvent instead of the extract. Proanthocyanidins were calculated using the molar extinction coefficient of cyanidin (ε = 34,700 L/mol·cm), and the results were expressed as milligrams of cyanidin equivalent per 100 grams of dry weight (mg CE/100 g DW).

Determination of antioxidant activities

Total antioxidant activity (TAA)

The total antioxidant activity (TAA) of lentil seeds was evaluated using the phosphor-molybdenum method described by Prieto, Pineda and Aguilar (1999). Briefly, an aliquot of 0.1 mL of lentil extract was combined with

1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). For the blank, 0.1 mL of solvent was used in place of the sample. The tubes were capped and incubated in a boiling water bath at 95 °C for 90 minutes. After cooling to room temperature, the absorbance was measured at 695 nm against the blank using a Shimadzu UV/Vis spectrophotometer. A standard calibration curve was constructed using different concentrations of ascorbic acid. TAA was expressed as milligrams of ascorbic acid equivalent per 100 grams of dry weight (mg AAE/100 g DW).

DPPH-radical scavenging activity (DPPH-RSA)

The DPPH-radical scavenging activity (DPPH-RSA) of lentil seeds was estimated using the method described by Blois (1958). Lentil extracts (100 µL) were mixed with 900 µL of a 0.04 mg/mL methanolic solution of DPPH. The absorbance was measured at 517 nm after a 20-minute incubation at room temperature.

The percentage of inhibition was calculated using the following equation:

$$\% \text{ DPPH-RSA} = [(A_c - A_s) / A_c] \times 100$$

where A_c is the absorbance of the negative control (containing 100 µL of solvent instead of the sample) and A_s is the absorbance of the sample.

Ferric reducing power (FRP) activity

The capacity of lentil cultivars to reduce Fe^{3+} was evaluated using the potassium ferricyanide-ferric chloride method(Oyaizu, 1986). Lentil extracts (1 mL) were added to 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide, and the mixture was incubated at 50 °C for 20 minutes.

Following this, 2.5 mL of 10% trichloroacetic acid was added. A fraction (2.5 mL) of the mixture was taken and mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance was then measured at 700 nm. A calibration curve was constructed using ascorbic acid, and the results were expressed as milligrams of ascorbic acid equivalent per 100 grams of dry weight (mg AAE/100 g DW).

Statistical analysis

All assays were conducted in triplicate (n = 3), and the results were expressed as the mean ± standard deviation (SD). Statistical comparisons of the data were performed using one-way analysis of variance (ANOVA) at $p < 0.05$. Tukey's test was applied to determine significant differences for each parameter among the studied lentil cultivars. All analyses were conducted using STATISTICA 5.5 (StatSoft Inc., Oklahoma, USA).

RESULTS AND DISCUSSION

Total phenolic content (TPC)

Table 1 presents the total phenolic content (TPC), total flavonoid content (TFC), and total proanthocyanidin content (TPAC) of both raw and cooked lentils. The raw lentils exhibited higher phenolic content compared to the cooked seeds. The results indicated that the cultivar significantly affected the TPC ($p < 0.05$), which ranged from 12.04 mg GAE/100 g DW for yellow lentils to 186.32 mg GAE/100 g DW for black lentils in the raw samples. For the cooked lentils, TPC values ranged from 3.13 mg GAE/100 g DW for yellow lentils to 10.27 mg GAE/100 g DW for brown lentils.

Additionally, the TPC of dehulled lentils (12.04 mg GAE/100 g DW for yellow and 28.46 mg GAE/100 g DW for red cultivars) was significantly lower ($p < 0.05$) than that of whole lentil varieties (white, brown, green, and black), which had an average TPC of 98.05 mg GAE/100 g DW. Whole lentils with dark seed coats (green and black) contained higher levels of phenolic compounds (107.40) and 186.32 mg GAE/100 g DW, respectively) compared to those with lighter-colored skins (white and brown), which had TPC values of 35.57 and 62.93 mg GAE/100 g DW, respectively.

These findings suggest that the presence and color of the seed coat significantly influence the TPC of lentil seeds. This is consistent with the results of Bubelova, Sumczynski and Salek (2018), who reported higher levels of polyphenolic compounds in whole and darker lentils compared to dehulled seeds. Similarly Xu et al. (2007) found that legumes with higher phenolic content were predominantly the dark-coated varieties. The results indicated that cooking significantly decreased the total phenolic content (TPC) of lentils, with reductions ranging from 73.72% for yellow lentils to 96.77% for black lentils.

Table 1.
Phenolic content (TPC, TFC, and TPAC) of the six studied lentil varieties

Lentil varieties	TPC (mg GAE/100g DW)			TFC (mg QE/100g DW)			TPAC (mg CE/100g DW)		
Raw lentils	Raw lentils	Cooked lentils	Loss (%)	Raw lentils	Cooked lentils	Loss (%)	Raw lentils	Cooked lentils	Loss (%)
Black	186.32 ± 4.93^{a}	6.02 ± 0.6^{bc}	96.77± 0.37^{a}	39.07 ± 4.21a	4.08 ± 0.16^{ab}	89.50 ± 0.72^{abc}	83.78 ± 4.94^{a}	1.25 ± 0.10^{b}	98.50 ± 0.21^{a}
Brown	62.93 ± 0.33^{c}	10.27 ± 0.78^{a}	83.68± 1.24^{d}	33.65 ± 3.06ab	2.87 ± 0.48^{bcd}	91.39%± 1.85^{ab}	21.78 ± 0.53^{c}	2.27 ± 0.07^{a}	89.55± 0.51^{bc}
Green	107.40 ± 2.35^{b}	7.08 ± 0.27^{b}	93.40± 0.38^{b}	36.37 ± 0.80ab	5.30 ± 0.62^{a}	85.41 ± 2.00^{c}	39.88 ± 3.96^{b}	1.24 ± 0.15^{b}	96.90 ± 0.20^{a}
Red (dehulled)	28.46 ± 1.00^{e}	4.38 ± 0.17^{cd}	84.60± 0.67^{d}	26.42 ± 0.89^{c}	3.46 ± 0.44^{bc}	86.86%± 1.90^{bc}	3.54 ± 0.18^{d}	0.32 ± 0.12^{c}	90.93 ± 3.87^{bc}
White	35.57 ± 2.56^{d}	3.32 ± 0.12^{d}	90.65 ± 0.43^{c}	31.63 ± 1.21bc	2.37 ± 0.41^{cd}	92.48± 1.50^{a}	8.86 ± 1.38^{d}	0.57 ± 0.22^{c}	93.58 ± 2.35^{ab}
Yellow (dehulled)	12.04 ± 1.32^{f}	3.13 ± 0.11^{d}	73.72± 3.69^{e}	9.94 ± 0.70d	2.09 ± 0.45^{d}	78.75% ± 5.95^{d}	2.14 ± 0.65^{d}	0.32 ± 0.21^{c}	86.09 ± 6.31^{c}

Different letters indicate significant differences atp < 0.05.
TPC: total phenolic content, TFC: total flavonoid content, and TPAC: total proanthocyanidin content.

This decrease in TPC may be attributed to the chemical degradation or oxidation of phenolic compounds during boiling. The oxidative degradation of phenolic acids, the release of free acids from conjugate forms, and the formation of complex structures involving phenolic substances with related compounds, such as proteins, tannins, and anthocyanins, are the primary reactions occurring during the cooking process (Xu & Chang, 2009).

Many researchers have noted a reduction in the levels of phenolic compounds after applying various heat treatments. Acito et al. (2023) found that the decrease in phenolic compounds following the cooking of lentils varies depending on the cooking method; specifically, pressure cooking resulted in a smaller decline in TPC of 41.74% compared to boiling, which caused a reduction of 45.60%. Djabali, Makhlouf, Ertas, and Barkat (2020) reported that boiling lentils for 45 minutes led to significant losses of 41.98% in TPC. They attributed these reductions to the breakdown of polyphenols during cooking and the solubilization and diffusion of phenolic compounds as heat disrupts cell structures. The presence of higher phenolic levels in cooking water suggests that extraction occurs during this process. Amarowicz and Pegg (2023) highlighted that cooking can have varying effects on phenolic content; while some lentil varieties may experience decreased levels, others may show increases due to the release of bound phenolics from the plant cell walls. Barroga, Laurena, and Mendoza (1985) observed that boiling for 30 minutes resulted in a 73% reduction of polyphenols in ten cultivars of mung bean (*Vigna radiata* L.). Similarly, Yu, Ahmedna, Goktepe, and Dai (2006) reported an 88.9% loss of TPC due to blanching peanut skins. Conversely, Duenas et al. (2016) suggested that processing legumes, including cooking and germination, can lead to positive changes in the composition of phenolic compounds, enhancing phytochemical and nutriational quality while maximizing the health-promoting properties of legumes. Notably, this includes the release of vanillin resulting from the depolymerization of lignin in insoluble dietary fiber in lentils. Garrido, Monagas, Gómez-Cordovés, and Bartolomé (2008) found that heat treatments applied during almond skin processing favored degradation reactions of more polymerized proanthocyanidins and the hydrolysis of glycosylated flavonoids, among other effects. Furthermore, blanching led to the solubilization of the more polar rather than the less polar phenolic structures: flavonoid glycolsides against aglycones; flavan-3-ol monomers against oligomers, quercetin against kaempferol, naringenin against eriodictyol, and so on.

In general, the total polyphenol content in plants is influenced by various factors, including the specific variety, agronomic practices, environmental conditions, growth stage or maturity, and the pigmentation of the tissue (such as red, green, or white) (Cheynier, 2005; Amin, Norazaidah,& Hainida, 2006).

Total flavonoid content (TFC)

The total flavonoid content (TFC) of the lentil seeds was analyzed, and the results are presented in Table 1. The findings indicated that the cultivar significantly affected the TFC ($p < 0.05$), which ranged from 9.94 mg QE/100 g DW for yellow lentils to 39.07 mg QE/100 g DW for black lentils in the raw samples. For cooked lentils, TFC values ranged from 2.09 mg QE/100 g DW for yellow lentils to 5.30 mg QE/100 g DW for green lentils.

The TFC of dehulled lentils (9.94 mg QE/100 g DW for yellow and 26.42 mg QE/100 g DW for red cultivar) was significantly lower ($p < 0.05$) than that of whole lentil varieties (white, brown, green, and black), which had an average TFC of 35.17 mg QE/100 g DW. These results suggest that flavonoids are primarily concentrated in the seed coats of lentils, consistent with previous studies (Duenas et al., 2002; Dueñas, Sun, Hernández, Estrella, & Spranger, 2003; Ganesan & Xu, 2017).

Furthermore, whole lentils with dark seed coats (black and green) exhibited higher TFC levels (39.07 and 36.37 mg QE/100 g DW, respectively) compared to varieties with lighter-colored skins (brown and white lentils, which had TFC values of 33.65 and 31.63 mg QE/100 g DW, respectively).

Our results align with previous studies that demonstrate the impact of cultivar on the TFC of lentils. Xu et al. (2007) reported TFC values ranging from 304 to 454 mg CE/100 g across different lentil cultivars. Similarly, Xu and Chang (2010) found TFC levels between 284 and 687 mg/100 g in 11 lentil cultivars. Additionally, Zhang et al. (2015) showed that green lentils are richer in TFC (66 to 198 mg CE/100 g) compared to red lentils (60 to 162 mg CE/100 g), which is consistent with our findings.

Similar to the total phenolic content (TPC), the total flavonoid content (TFC) significantly decreased ($p < 0.05$) in cooked lentils compared to raw samples. The percentage losses of flavonoid content in cooked lentils ranged from 78.75% for yellow lentils to 92.48% for white lentils. Our findings are consistent with those of Djabali et al. (2020), who reported a significant decrease in TFC of 41.19% in cooked lentils compared to fresh ones. The authors suggested that this reduction may be due to the disruption of cell walls, which facilitates the release of flavonoids into the cooking water. Additionally, flavonoids often exist as glycosides, where one or more hydroxyl groups are bound to sugars. This glycosidic form enhances their solubility in water, explaining their transfer into the cooking water. Aguilera et al. (2010) found that thermal processing of lentils can release some flavonols from bonded forms, resulting in higher levels compared to soaked samples. Specifically, the levels of kaempferoldirutinoside, kaempferolacetylglycoside, and kaempferol 3-glucoside increased after cooking soaked samples. In contrast, the levels of flavones and flavanones decreased due to leaching and thermal or oxidative degradation. Attou, Bouderoua, and Cheriguene (2020) noted that both temperature and cooking time significantly affected the TFC of Algerian lentil varieties, with approximately half of the TFC (46.96% to 48.93%) lost during this process. Similarly, Deng, Padilla-Zakour, Zhao, and Tao (2015) reported a 38.2% reduction in the TFC of boiled buckwheat grains.

Total proanthocyanidin content (TPAC) determination

The effects of cultivar and cooking on the total proanthocyanidin content (TPAC) of the selected lentil seeds are presented in Table 1. The lentil extracts showed significant differences ($p < 0.05$) in their TPAC based on the cultivar, with values ranging from 2.14 mg CE/100 g DW for yellow lentils to 83.78 mg CE/100 g DW for black lentils in their raw form. For cooked lentils, TPAC values ranged from 0.32 mg CE/100 g DW for yellow lentils to 2.27 mg CE/100 g DW for brown lentils. The TPAC of dehulled lentils (2.14 mg CE/100 g DW for yellow and 3.54 mg CE/100 g DW for red cultivars) was significantly lower ($p < 0.05$) than that of whole lentil varieties (white, brown, green, and black), which had an average TPAC of 38.6 mg CE/100 g DW. These results indicate that proanthocyanidins are predominantly concentrated in the seed coats of lentils. Additionally, whole lentils

with dark seed coats (black and green) contained higher TPAC levels (83.78 and 39.83 mg CE/100 g DW, respectively) compared to varieties with lighter-colored skins, such as brown and white lentils, which had TPAC values of 21.78 and 8.86 mg CE/100 g DW, respectively.

Zhang et al. (2015) demonstrated that red lentils contain lower levels of condensed tannins (300 to 582 mg CE/100 g) compared to green lentils (336 to 780 mg CE/100 g), which is consistent with our findings. Jin, Ozga, Lopes-Lutz, Schieber, and Reinecke (2012) reported proanthocyanidin content ranging from 269 to 378 mg/100 g across three lentil cultivars. Additionally, Xu et al. (2007) measured the condensed tannin content (CTC) in 33 cool-season legumes and found that the average CTC value for lentil varieties (approximately 600 mg CAE/100 g) was higher than that of common beans (approximately 400 mg), black soybeans (approximately 200 mg), yellow soybeans (approximately 50 mg), and both green and yellow peas (each less than 50 mg).

Cooking significantly decreased TPAC of lentils, with losses ranging from 86.09% for yellow lentils to 98.50% for black lentils. This reduction in TPAC after cooking has been reported in several studies. Hefnawy (2011) noted that cooking treatments led to a decrease in tannins in lentils. Similarly, Khattab and Arntfield (2009) found that the reduction in tannin content in cowpeas, peas, and kidney beans varied based on the type of physical treatment applied, with boiling resulting in the most significant decrease, followed by autoclaving and microwave cooking. Aguilera, Estrella, Benitez, Esteban, and Martín-Cabrejas (2011) observed a significant reduction in catechins and procyanidins in Pinta bean after heat treatment. Wang, Hatcher, Tyler, Toews, and Gawalko (2010) also reported a significant decline in tannin content after cooking common beans and chickpeas. Zhang et al. (2014) found that while cooking increased the concentrations of certain flavonols, such as kaempferol and quercetin glycosides, it also led to substantial losses in flavanols, including catechin/epicatechin glucoside and oligomeric procyanidins.

The mechanisms behind the differential effects of cooking on various classes of polyphenols remain unclear. Randhir, Kwon, and Shetty (2008) suggested that the increase in phenolic content after thermal processing could result from the release of bound phenolic acids due to the breakdown of cell walls and other cellular components. They proposed that the dissociation of conjugated phenolic forms during cooking, followed by polymerization or oxidation, might contribute to this increase.

Additionally, some phenolic compounds not originally present in the grains could form as by-products of thermal degradation, possibly resulting from the breakdown of tannins into simpler phenolics. Thermal processing may also release phenolics that accumulate in cellular vacuoles, making previously unavailable compounds accessible. Other factors that could influence phenolic content include the Maillard reaction (non-enzymatic browning), caramelization, and chemical oxidation. However, the overall decrease in total phenolic content during cooking likely stems from the degradation of these compounds due to heat.

Antioxidant activities

Total antioxidant activity (TAA)

The total antioxidant activity (TAA) of the selected lentil seeds is presented in Table 2. Lentil extracts from different cultivars exhibited significant differences ($p < 0.05$) in TAA, ranging from 173.77 mg AAE/100 g DW for yellow lentils to 901.53 mg AAE/100 g DW for black lentils in their raw form. For cooked lentils, TAA values ranged from 23.23 mg AAE/100 g DW for white lentils to 51.74 mg AAE/100 g DW for brown lentils. Furthermore, whole lentil seeds with colored coats (black, green, and brown) demonstrated higher antioxidant activity compared to dehulled lentils (red and yellow) or whole lentils with lighter-colored coats (white). This finding aligns with the results of Lin and Lai (2006) who found that dark-coat seeds contained high amounts of phenolic compounds and contributed to high antioxidative ability. Oomah, Caspar, Malcolmson, and Bellido (2011) confirmed that most bioactive phenolics and antioxidants in lentils are concentrated in the hulls, noting strong antioxidant activity in the hulls of green lentils (95.56 mmol TE/100 g) and red lentils (104.05 mmol TE/100 g), compared to their whole seeds (13.61 mmol TE/100 g for green lentils and 13.48 mmol TE/100 g for red lentils) compared to their whole seeds (13.61 mmol TE/100 g for green lentils and 13.48 mmol TE/100 g for red lentils).

They also indicated that the cotyledons primarily contain non-flavonoid compounds, which have lower antioxidant activity than the flavornoids abundant in the seed coats. Xu and Chang (2010) demonstrated that cultivar significantly influenced the antioxidant activity of lentils, with values ranging from 3.89 to 8.20 mmol TE/100 g. Similarly, Xu et al. (2007) reported that antioxidant activity varied from 5.955 to 9.519 mmol TE/100 g across different lentil varieties.

Cooking significantly reduced the TAA of lentils, with reductions ranging from 83.60% for yellow lentils to 94.55% for black lentils. Pal et al. (2017) also observed substantial losses in TAA during the boiling of lentils. In raw lentils, TAA values ranged from 0.712 to 0.819 mM TE/100 g DW, while cooking resulted in a significant decrease in TAA, with reductions between 7.58% and 46.40%. This decline aligns with changes in the concentrations of antioxidants in lentils, suggesting that the reduction in antioxidant activity may be associated with lower phenolic content due to cooking. Aguilera et al. (2010) reported that the changes in antioxidant values could be attributed to the leaching of phenolic compounds into the cooking water. Additionally, other factors may contribute to these changes, including the increased solubility of non-phenolic antioxidant compounds after thermal treatment and the formation of Maillard reaction products, which may exhibit enhanced free radical scavenging properties.

DPPH-radical scavenging activity (DPPH-RSA)

The DPPH-radical scavenging activity (DPPH-RSA) values for the lentil varieties are presented in Table 2. Lentil extracts from different cultivars showed significant differences ($p < 0.05$) in their DPPH-RSA, ranging from 13.29% for yellow lentils to 77.02% for black lentils in their raw form, and from 3.42% for white lentils to 12.76% for green lentils when cooked. The highest DPPH scavenging activity was observed in raw lentils compared to their cooked counterparts.

Additionally, whole lentils with colored seed coats (black, green, and brown) exhibited greater DPPH-RSA compared to dehulled len-tils (red and yellow) or whole lentils with lighter-colored coats (white). This finding aligns with the results of Duenas, Hernandez and Estrella (2006), who reported that lentil seed coats have stronger antioxidant activity (EC_{50} values of 0.05–0.07 mg) compared to lentil cotyledons (EC_{50} values of 21–29 mg). Xu et al. (2007) reported DPPH-RSA values ranging from 1907 to 1987 µmol TE/100 g across 11 lentil cultivars. Fratianni et al. (2014) examined the free radical scavenging activity of grass pea, lentil, and chickpea cultivars originating from the Campania region of Southern Italy, and reported that lentil cultivars demonstrated the highest DPPH-RSA, with EC_{50} values ranging from 170 to 222 mg/100 g.

Cooking markedly diminished the DPPH radical scavenging activity (DPPH-RSA) of lentils, with reductions ranging from 70.09% in yellow cultivars to 90.70% in white cultivars. Acito et al. (2023) demonstrated that both boiling and pressure cooking led to a decline in the DPPH-RSA of Italian lentil seeds. Similarly, Chuwech, Rakariyatham, Ti-noi, Suwitchayanon and Chandet (2023) found that heat treatments of purple rice flour resulted in a reduction in DPPH activity ran-ging from 67.51% to 82.75%. Rocha-Guzmán, González-Laredo, Ibarra-Pérez, Nava-Be-rumen, and Gallegos-Infante (2007) reported that the radical scavenging activity (%RSA) of bean seed coats was affected by cooking time, with longer cooking durations leading to a diminished capacity for trapping free radicals.

This reduction can be attributed in part to the diffusion of phenolic compounds into the cooking water and the cotyledon, as well as the formation of phenolic-protein complexes, which do not contribute to antioxidant effects. Yeo and Shahidi (2017) noted that soluble phenolics (SPs) tend to increase during boiling due to the release of bound phenolics, a process facilitated by the disintegration of the cell wall matrix through hydrothermal energy.

Conversely, insoluble-bound phenolics (IBPs) decreased in both quantity and antioxidant capacity during hydrothermal processing, likely due to the breakdown of cell wall matrices containing these compounds, allowing phenolics to escape. Nevertheless, the rise in soluble phenolics did not compensate for the decline in insoluble-bound phenolics, resulting in an overall decrease in phenolic and flavonoid content following boiling.This reduc-

Table 2.
Antioxidant activities **(**TAA. DPPH-RSA andFRP) of the six studied lentil varieties

Lentil varieties	TAA (mg AAE/100g DW)			DPPH-RSA (%)			FRP (mg AAE/100g DW)		
	Raw lentils	Cooked lentils	Loss (%)	Raw lentils	Cooked lentils	Loss (%)	Raw lentils	Cooked lentils	Loss (%)
Black	901.53 ± 78.34^{a}	48.80 ± 2.44a	94.55± 0.66^{a}	77.02 ± 0.56a	10.78 ± 1.44^{ab}	86.00± 1.97^{ab}	234.54 ± 5.99^{a}	71.70 ± 1.08^{a}	69.42± 0.40^{a}
Brown	521.90 ± 12.83^{b}	51.74 ± 3.16^{a}	90.09± 0.47^{bc}	47.99 ± 4.97c	7.56 ± 0.30^{bc}	84.19± 1.02^{ab}	78.16 ± 7.97^{d}	53.61 ± 1.46^{c}	30.90± 7.69^{e}
Green	565.94 ± 39.63^{b}	45.58 ± 0.54a	91.92± 0.58^{b}	61.04 ± 2.22b	12.76 ± 1.13^{a}	79.05± 2.62^{bc}	126.15 ± 2.4^{b}	57.79 ± 1.42^{b}	54.18 ± 1.44^{c}
Red (dehulled)	390.30 ± 24.99^{c}	40.19 ± 1.90b	89.66± 1.10^{c}	19.55 ± 1.12e	5.43 ± 1.54^{c}	71.94± 9.50^{c}	81.76 ± 8.13^{cd}	45.90 ± 1.12^{d}	43.38± 7.26^{d}
White	283.27 ± 27.16^{c}	23.23 ± 4.45c	91.75± 1.83^{b}	36.68 ± 0.10d	3.42 ± 1.48^{c}	90.70 ± 4.00^{a}	97.89 ± 11.33^{c}	37.59 ± 0.90^{e}	61.17 ± 5.53^{b}
Yellow (dehulled)	173.77 ± 2.06^{d}	28.52 ± 2.62c	83.60± 1.31^{d}	13.29 ± 1.31e	3.99 ± 0.74^{c}	70.09 ± 2.59^{c}	27.77 ± 1.78^{e}	20.69 ± 2.12^{f}	25.57 ± 3.69^{e}

Different letters indicate significant differences at p< 0.05.
TAA: total antioxidant activity, DPPH-RSA: DPPH radical scavenging activity, and FRP: ferric reducing power.

tion may be further compounded by chemical reactions between soluble phenolics and other molecules in the food matrix under high-energy conditions, with the most likely mechanism being the interaction between phenolics and proteins during hydrothermal treatment.

Ferric reducing power (FRP)

The values of the Ferric Reducing Power (FRP) of lentil seeds are presented in Table 2. Lentil extracts from different cultivars demonstrated significant differences ($p < 0.05$) in their FRP, with values ranging from 27.77 mg AAE/100 g DW for yellow lentils to 234.54 mg AAE/100 g DW for black lentils in their raw form, and from 20.69 mg AAE/100 g DW for yellow lentils to 71.70 mg AAE/100 g DW for black lentils for cooked seeds.

Our findings are in agreement with previous studies, which have shown that cultivar significantly affects the FRP of lentils. Xu et al. (2007) reported FRAP values ranging from 8.75 to 13.92 mmol Fe^{2+}equivalents/100 g across 11 lentil cultivars.

FRP in lentils significantly declined after cooking with reductions spanning from 25.57% for yellow lentils to 69.42% for white lentils. This trend aligns with the results of Pal et al. (2017), who noted a substantial decrease in reducing power following lentil cooking. In raw seeds, the reducing power ranged from 0.14 to 0.23 Abs units at 700 nm, while in cooked samples, it varied from 0.05 to 0.11 Abs units at 700 nm, reflecting a significant reduction of 42.11% to 64.29% compared to raw lentils. Yeo and Shahidi (2017) also observed a reduction in reducing power ranging from 8% to 49.1% after cooking four lentil cultivars.

According to these authors, the loss of reducing power may be attributed to the decrease in phenolic content during the boiling process.

CONCLUSIONS

Lentils are typically boiled, a process that reduces both antioxidant compounds and activity. This study aimed to characterize the phenolic compounds and antioxidant activities of six commercial lentil cultivars (black, brown, green, dehulled red, white, and de-hulled yellow) and to investigate the effects of boiling for 30 minutes on total phenolic content (TPC), total flavonoid content (TFC), total proanthocyanidin content (TPAC), and antioxidant activity, including total antioxidant activity (TAA), DPPH-radical scavenging acti-

vity (DPPH-RSA), and ferric reducing power (FRP).

The results indicated that cultivar significantly ($p < 0.05$) affected TPC, TFC, TPAC, and antioxidant activity values of lentil seeds. Among the cultivars, whole lentils (black, green, brown, and white) exhibited the highest concentrations of phenolic compounds and antioxidant activities compared to dehulled lentils (red and yellow). Moreover, whole lentils with dark seed coats (green and black) demonstrated higher values for TPC, TFC, TPAC, TAA, DPPH-RSA, and FRP than varieties with lighter-colored skins (brown and white). These findings suggest that the presence and color of the seed coat have a significant influence on the phenolic content of lentil seeds.

Boiling lentils for 30 minutes resulted in a significant decrease in TPC, TFC, TPAC, and antioxidant activity (TAA, DPPH-RSA, and FRP). Further investigations are necessary to characterize the individual antioxidant components in lentil cultivars. Beyond their nutritional value, lentils can serve as excellent sources of antioxidants and may be utilized as functional ingredients in the development of nutraceuticals and health foods within the food industry.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, validation, formal analysis, writing-original draft preparation, writing-review and editing, supervision, A.M.; Investigation, S.B. and Y.T.; Writing-review and editing, L. A-D., A. M. and S.M-A.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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UTICAJ TERMIČKE OBRADE U KUĆNIM USLOVIMA (KUVANJA) NA SADRŽAJ FENOLNIH JEDINJENJA I ANTIOKSIDATIVNU AKTIVNOST KOD RAZLIČITIH SORTI SOČIVA

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Sažetak: Sočivo *(Lens culinaris* Medik*)* spada među najčešće konzumirane namirnice u svetu; međutim, ono se pretežno priprema kuvanjem, što dovodi do gubitka antioksidativnih jedinjenja. Cilj ove studije bio je da se procene efekti kuvanja na sadržaj fenolnih jedinjenja—konkretno ukupni sadržaj fenola (TPC), ukupni sadržaj flavonoida (TFC) i ukupni sadržaj proantocijanidina (TPAC), kao i na antioksidativnu aktivnost, uključujući ukupnu antioksidativnu aktivnost (TAA), sposobnost uklanjanja DPPH-radikala (DPPH-RSA) i redukcionu moć gvožđa (FRP) u različitim sortama sočiva. Analizirano je šest komercijalno dostupnih uzoraka sočiva (crno, braon, zeleno, crveno- oljušteno, belo i žuto-oljušteno). TPC, TFC i TPAC su određeni korišćenjem spektrofotometrijskih metoda sa Folin-Ciocalteu, $AlCl_3 \cdot 6H_2O$ i butanol–HCl, respektivno. Ova merenja su sprovedena u kombinaciji sa procenom antioksidativne aktivnosti (TAA, DPPH test i FRP) pre i posle kuvanja. Rezultati su pokazali da celo zrno sočiva sadrži viši sadržaj TPC, TFC i TPAC, kao i veću antioksidativnu aktivnost (TAA, DPPH-RSA i FRP) u poređenju sa oljuštenim sočivom. Nadalje, celo sočivo sa tamnim semenskim omotačem (zeleno i crno zrno) pokazalo je superiorne vrednosti za TPC, TFC, TPAC, TAA, DPPH-RSA i FRP u odnosu na sorte sa svetlijom bojom semenskog omotača (braon i belo). Ovi nalazi ukazuju da prisustvo i boja semenskog omotača značajno utiču na sadržaj fenolnih jedinjenja u semenu sočiva. Kuvanje sočiva u trajanju od 30 minuta dovelo je do značajnog smanjenja TPC, TFC, TPAC i antioksidativne aktivnosti (TAA, DPPH-RSA i FRP). Pored svojih nutritivnih vrednosti, sočivo može služiti kao izvanredan izvor antioksidanasa i može se koristiti kao funkcionalni sastojak u proizvodnji nutraceutika i zdravstveno korisnih prehrambenih proizvoda u prehrambenoj industriji.

Ključne reči: *sočivo, sorta, fenolna jedinjenja, antioksidaciona aktivnost, sirovo zrno, kuvano zrno*

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