

Effect of different heat treatments on physicochemical properties and antioxidant characteristics of black beans

XIAOLONG JI^{1,2}, SHULI ZHANG¹, XIN DU¹, YUNING ZHANG¹, YANG YAO^{3*},
YINGYING ZHU^{1,2*}

¹College of Food and Bioengineering, Zhengzhou R&D Center for High-quality Innovation of Green Food (Green Premium Agricultural Products), Zhengzhou University of Light Industry, Zhengzhou, P.R. China

²National & Local Joint Engineering Research Center of Cereal-Based Foods (Henan), Zhengzhou, P.R. China

³Key Laboratory of Grain Crop Genetic Resources Evaluation and Utilization, Ministry of Agriculture and Rural Affairs, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, P.R. China

*Corresponding authors: yaoyang@caas.cn; 67171720@qq.com

Citation: Ji X.L., Zhang S.L., Du X., Zhang Y.N., Yao Y., Zhu Y.Y. (2025): Effect of different heat treatments on physicochemical properties and antioxidant characteristics of black beans. Czech J. Food Sci., 43: 300–310.

Abstract: This study systematically evaluated the effects of four thermal processing methods-boiling, steaming, extrusion, and roasting-on the physicochemical properties and *in vitro* antioxidant activity of black beans. Notably, ash content decreased following boiling and steaming, reaching 45.5 mg·g⁻¹ and 43.5 mg·g⁻¹, respectively, corresponding to reductions of 8.5% and 13.3%. In contrast, extrusion and roasting led to moisture loss, resulting in ash content increases of 3.2% and 6.8%. Among the treatments, boiling significantly increased powder clumping (5.1%), primarily due to elevated moisture content, a value markedly higher than that observed for other methods. Both boiling and steaming diminished brightness, while extrusion deepened colour intensity and enhanced redness. Regarding chemical composition, polyphenol content declined after boiling and steaming (3.8 mg·g⁻¹ and 2.9 mg·g⁻¹, respectively) relative to untreated black bean powder (4.3 mg·g⁻¹). Extrusion, however, elevated polyphenol levels, whereas all heat treatments reduced flavonoid content, with boiling exerting the greatest impact. Antioxidant activity also declined post-processing, with boiling having the most pronounced effect on 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH) radical scavenging and steaming most affecting hydroxyl radical elimination. Extrusion emerged as the optimal processing method for black beans, and superior retention of bioactive compounds, enhanced antioxidant capacity, improved physicochemical properties (lower clumping, stable colour parameters). These findings could provide actionable insights for food industries to select processing methods that maximize nutritional value and functional properties of black bean products.

Keywords: Glycine max (L.) Merr.; heat processing method; extrusion

Black beans (*Glycine max* (L.) Merr.) are the dried and mature seed of a leguminous plant belonging to the family *Fabaceae*, subfamily *Faboideae*, genus *Glycine* and

subgenus *Soja*, form a significant component of diets in Mexico, Indonesia, and India, and they are also widely popular in China (Rosa-Millán et al. 2019; Li et al. 2024).

Supported by National Key Research and Development Program of China (2021YFD1600100) and Earmarked Fund for China Agriculture Research System (CARS-08-G21).

© The authors. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0).

<https://doi.org/10.17221/144/2024-CJFS>

According to data from the Food and Agriculture Organization of the United Nations in 2021, the top five countries in terms of global soybean production were Brazil (138 million tons), the United States (115 million tons), Argentina (46.2 million tons), China (18.6 million tons), and India (10.45 million tons). Black soybeans accounted for approximately 1–2% of the total soybean consumption, indicating its relatively limited roles in the overall soybean market. However, black beans are abundant in diverse nutrients, notably containing a higher level of protein. This makes them easily digestible while effectively meeting the body's protein needs (Mitharwal et al. 2024). Simultaneously, black bean is abundant in various functional active compounds, including anthocyanins, polysaccharides, isoflavones, and dietary fibre etc., which making it a dual-purpose legume with both medicinal and culinary applications (Felix et al. 2020; Teixeira-Guedes et al. 2020). Black beans are good sources of proteins (41.4–44.3%), carbohydrates (30.4–32.8%), dietary fibres (27.6–30.5%), lipids (10.4–18.6%), vitamins (E, K, and B), and minerals (Na, K, P, and Fe), based on dry matter (Li et al. 2024).

The quality of black beans is markedly influenced by the choice of processing methods. Traditional approaches such as boiling and steaming are widely employed to enhance palatability and digestibility, while simultaneously reducing anti-nutritional compounds including phytic acid and tannins. However, prolonged steaming might result in the degradation of thermolabile nutrients. Roasting is primarily utilised to intensify flavour, aroma, and texture, imparting a desirable crispness; yet exposure to elevated temperatures could induce partial lipid oxidation and diminish amino acid content. Extrusion processing offers notable advantages in improving both nutritional value and sensory attributes, although it may also contribute to the loss of certain heat-sensitive nutrients. Each technique presents a distinct balance between nutritional preservation and enhancement of organoleptic qualities (Li et al. 2024). The changes in functional active ingredients in processed black beans are increasingly attracting the attention of researchers. According to the Compendium of Materia Medica (Li et al. 2024), black beans are believed to be beneficial for regulating spleen function, reducing edema, clearing the lungs, detoxifying the body, nourishing the kidneys, preventing night sweats, and promoting longevity (Ismail et al. 2021; Sun et al. 2022; Li et al. 2024). In terms of trace elements, black beans exhibit higher levels of vitamins B and E, which could contribute to reducing cholesterol

levels in the bloodstream. Yamamoto et al. (2022) reported that black beans possessed strong antioxidant activity, and extracts from black beans hold potential anti-aging properties. Heat processing could remove certain antinutritional compounds from black beans, improve their aroma, and eliminate unwanted beany flavours (Carter et al. 2019). Thermal processes such as boiling and steaming have important effects on the synthesis and conversion of amino acids within black beans, and high temperatures cause notable changes in the nutrient content of black beans (Li et al. 2021; Borges-Martínez et al. 2022). Various conditions of heat-induced extrusion processes have been applied to improve certain nutritional characteristics of legumes, including increasing the bioavailability of carbohydrates and proteins, while reducing the content of antinutritional factors concurrently (Abera et al. 2023).

In recent years, different published research papers have highlighted variations in nutritional composition among different legumes, as well as differences in the composition of various types of black beans in the same region. However, there has been limited comparative analysis of black beans subjected to different processing methods. Despite the widespread use of thermal processing in food preparation, the specific effects of common heat treatment methods on the nutritional composition of black beans remain inadequately characterised. In this study, black beans were subjected to four distinct processing techniques – boiling, steaming, extrusion, and roasting – with the objective of systematically analysing and comparing their impacts on fundamental nutritional constituents and *in vitro* antioxidant activity. The findings aim to offer a scientific basis for the comprehensive utilization and value-added development of black beans in functional food applications.

MATERIAL AND METHODS

Materials and chemicals. The black beans were bought from local distributors in Fukang city, Xinjiang, China. The black beans particles used in the experiment were plump and there was no marked difference in particle weight, which met the experimental requirements. Hermo Scientific™ Process 11 were bought from Leistritz AG (Germany). K42FK619 Oven was supplied by Zhejiang Supor Co., Ltd. (Zhejiang, China). The rutin standard, gallic acid standard, forinol, 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH) reagents were obtained from Yuanye Biotechnology Co.,

Ltd. (Shanghai, China). All other reagents were of analytical grade and were supplied by the Kemiou Chemical Reagent Co., Ltd. (Tianjin, China).

Experimental procedure. The process for preparing raw black bean powder involved the following steps with minor adjustments (Wang et al. 2022). We selected intact and plump black beans, rinsed them thoroughly with deionised water, and dried them in an oven set to 55 °C, used a grinder to process the beans, and then passed the ground material through a 100-mesh sieve to produce raw black bean powder. The prepared raw bean powder was stored in glassine bags at 4 °C for later use. The methods for various treatments were outlined as follows. Steaming: soak the cleaned black beans in deionised water for 2–3 h, then utilise high-temperature steam to treat them (black beans to deionised water ratio was 1 : 10) for 60 min (Wu et al. 2023). Boiling: place the washed black beans in a pot, with a material to liquid ratio of 1 : 5 for 4 h, and finally boil them in water for 30 min (Qubbaj et al. 2022). Roasting: soak the cleaned black beans in deionised water, then place them in an oven preheated to 180 °C (both upper and lower temperatures) for 40 min (Alves et al. 2020). Extrusion puffing: put the prepared raw bean powder into a pre-set twin-screw extruder (the pre-set temperature range was 40, 50, 60, 70, 80, 110, 120, 149 °C for each section, with a speed of $7.25 \times g$ and a torque of 15%) for extrusion and dry them (Jeong et al. 2021). All the above treated black bean samples were dried, grinded, sieved and stored in the same procedure of raw bean flour.

Analytical methods. The contents of protein, fat, and ash in all samples were detected by Kjeldahl method, Soxhlet extraction method, and gravimetric method *via* muffle furnace, respectively, and the total carbohydrate was calculated by subtraction (Saldívar et al. 2011). The physical properties (angle of repose, rate of agglomeration, bulk density, moisture content) were measured according to the method referred to Guo et al. (2014).

Colour parameters. The colour of black bean samples was measured using a colorimeter (CR-400, Konica Minolta, Japan). The results of L^* , a^* and b^* were recorded, and followed by calculation of the mean \pm standard deviation.

Total phenolic content. The total phenolic content (TPC) was measured using the Folin-Ciocalteu method with slight modifications (Neder-Suarez et al. 2021). Using deionised water as the solvent, a standard curve was prepared by gallic acid standard solution, and the

absorbance was measured at 765 nm using a UV-visible spectrophotometer (UV 752, Shanghai, China). The results were expressed as milligrams of gallic acid equivalent per gram of samples [$\text{mg gallic acid equivalent (GAE)} \cdot \text{g}^{-1}$].

Total flavonoid content. The total flavonoid content (TFC) was determined using a method involving aluminium nitrate after extraction with polyphenol solution (Lee et al. 2023). The absorbance was measured at 510 nm using a UV-visible spectrophotometer, and a standard curve was prepared using rutin standard solution with deionised water as the solvent. The results were expressed as milligrams of rutin equivalent per gram of samples ($\text{mg RE} \cdot \text{g}^{-1}$).

DPPH scavenging activity assay. With a few modest adjustments, the DPPH radical scavenging rate was determined using a prior technique. 2 mL of sample extract was mixed with 2 mL of DPPH-ethanol solution (0.1 mM) thoroughly, and the mixture was incubated in the dark at 37 °C for 30 min. The absorbance of solution was measured at 517 nm wavelength to calculate the scavenging rate of samples against DPPH radicals (Fonseca-Hernandez et al. 2021). The calculation formula was as follows:

$$\text{DPPH scavenging activity}(\%) = \left(1 - \frac{A_1 - A_2}{A_0} \right) \times 100 \quad (1)$$

where: A_1 – absorbance of the sample group; A_2 – absorbance of the blank group (ethanol without DPPH); A_0 – absorbance of the control group (deionised water instead of the sample).

Hydroxyl radical scavenging ability assay. The prior procedure was chosen to ascertain the rate of hydroxyl radical scavenging (Xue et al. 2016). Add 1 mL of 6 mM FeSO_4 solution, and 6 mM salicylic acid-ethanol solution, 6 mM H_2O_2 solution into 1 mL of sample solution with a certain concentration (1.0, 5.0 and 10.0 $\text{mg} \cdot \text{mL}^{-1}$), and react at 37 °C for 30 min without light. The absorbance of the solution was measured at 517 nm. The calculation formula was as follows:

$$\text{Hydroxyl radical scavenging}(\%) = \left(1 - \frac{A_1 - A_2}{A_0} \right) \times 100 \quad (2)$$

The A_1 was the absorbance of sample group, A_2 the blank group (deionised water replaced H_2O_2 solution), and A_0 the control group (deionised water replaced the sample).

<https://doi.org/10.17221/144/2024-CJFS>

Statistical analysis. Each treatment was performed in triplicate on three independently prepared sample batches ($n = 3$), and the results expressed as mean and standard deviation. Data analysis was performed using SPSS 26.0 (2021) and Origin 8.0 (2022) software.

RESULTS AND DISCUSSION

Chemical composition. In terms of plant-based protein, the protein content of black beans could be comparable to that of meat, making it an excellent source of high-quality dietary protein (Choudhary et al. 2024). Additionally, black bean protein contains eight essential amino acids required by human body (Melini et al. 2023). Figure 1 lists the chemical composition of various black bean samples after different processing methods. Extrusion and roasting led to significant protein concentration and denaturation due to elevated temperature, yielding protein levels of $373.5 \text{ mg}\cdot\text{g}^{-1}$ and $401.7 \text{ mg}\cdot\text{g}^{-1}$, respectively (Sanchez-Tapia et al. 2022). Thermal processing-induced changes in protein content were likely attributable to protein denaturation, which disrupted native protein structures, enhanced protease accessibility,

facilitated peptide bond hydrolysis, and ultimately accelerated protein degradation. In the case of extrusion puffing, the high-temperature twin-screw extrusion process promoted the diffusion of interfacial molecules within the bean matrix, leading to partial lipid release. Among the four processing methods investigated, extruded black beans exhibited the lowest fat content ($130.9 \text{ mg}\cdot\text{g}^{-1}$). In contrast, roasting increased the fat content to $168.5 \text{ mg}\cdot\text{g}^{-1}$. This elevation was primarily due to the high-temperature, moisture-free conditions of roasting, which dehydrated the beans, altered cellular architecture, and concentrated lipids. Additionally, roasting suppressed undesirable alcohol-derived flavours, intensified the Maillard reaction, and enhanced the formation of volatile compounds such as pyrazines, thereby obviously improving the characteristic aroma profile of black beans (Mariscal-Moreno et al. 2021; Machado-Velarde et al. 2023).

Steamed black beans exhibited the lowest ash content ($43.1 \text{ mg}\cdot\text{g}^{-1}$), whereas roasted samples demonstrated the highest levels. Moisture-based processing methods, such as steaming and boiling, facilitated the leaching of organic salts, thereby contributing to a reduction in ash content. In contrast, both extrusion

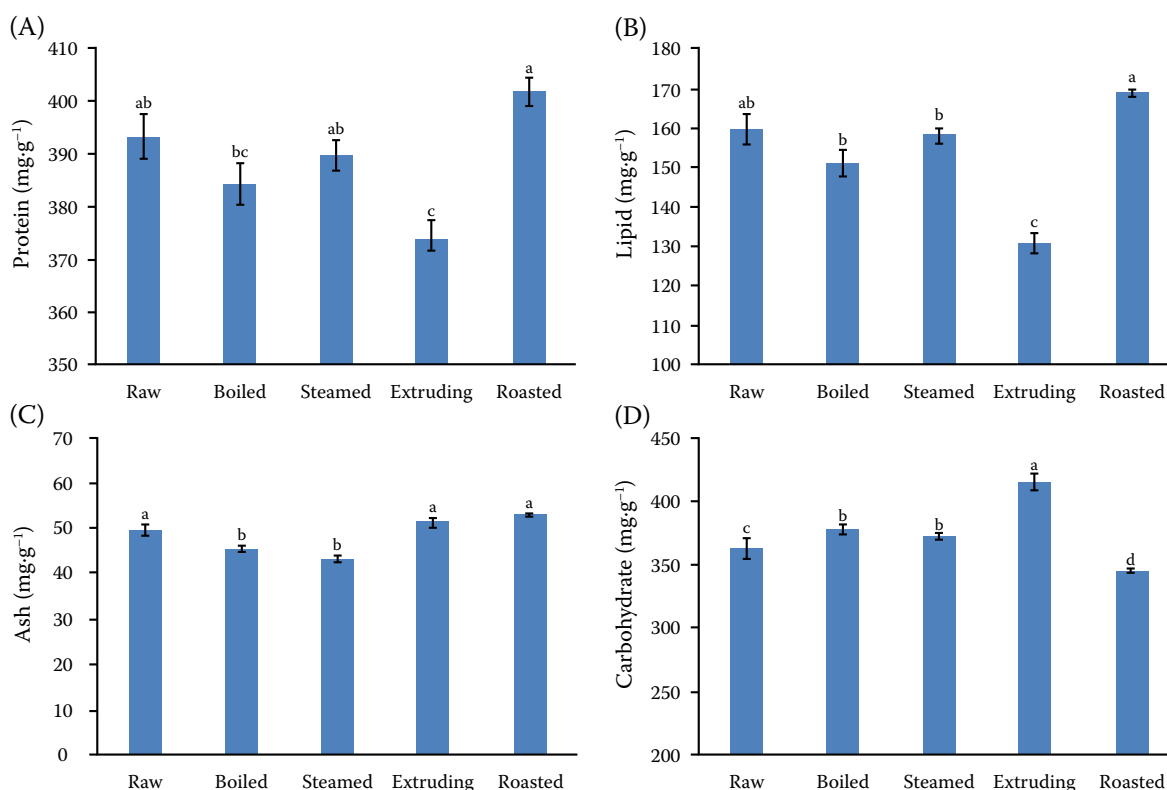


Figure 1. Chemical composition of black bean under different thermal processing methods

Data with different letters are significantly different among different groups ($P < 0.05$)

puffing and roasting induced substantial moisture loss, which likely concentrated residual inorganic matter and resulted in a relative increase in ash content. The ash content decreased after boiling and steaming, reaching $45.5 \text{ mg}\cdot\text{g}^{-1}$ and $43.5 \text{ mg}\cdot\text{g}^{-1}$, respectively, representing reductions of 8.5% and 13.3%. In contrast, extrusion and roasting caused moisture loss, leading to increases in ash content by 3.2% and 6.8%, respectively. Carbohydrate levels varied only slightly across the different treatments, a trend that might be explained by partial hydrolysis of starches and sugars caused by mechanical disruption and thermal effects during processing (Barreto et al. 2021; Damian-Medina et al. 2022). The most noticeable difference was observed in samples subjected to extrusion puffing, where changes in molecular characteristics occurred under the action of thermoplastic extrusion, resulting in changes in carbohydrate content.

Physical properties. Physical properties are critical indicators for determining the processing methods of black beans. Figure 2 lists the physical properties of black beans processed by different methods, revealing variations across these methods. During high-temperature processing, the protein molecular

structure of bean flour changed, black bean protein exhibited multiple properties leading to variations in physical properties of black beans (Hernandez-Velazquez et al. 2020). The angle of repose mainly evaluated the dispersibility of dry powder, which also related to its hygroscopicity; higher hygroscopicity could cause dry powder to clump, resulting in a larger angle of repose. Powders with a lower clumping rate and a smaller angle of repose, with higher bulk density, possessed better dispersibility and flowability (Alpos et al. 2021).

The moisture content of dry powder after steam and boil treatments was obviously higher than other treatments. The energy might be transferred in the form of steam during the seed maturation process, causing seeds to absorb a large amount of steam, which promoted the maturation and softening of black beans (Evangelho et al. 2016). Following extrusion puffing and roasting, both the moisture content and clumping rate of the resulting black bean powders decreased markedly, likely due to the higher processing temperatures associated with these methods compared to boiling and steaming. Boiled samples exhibited the highest clumping rate (5.1%) and retained the greatest moisture content, significantly exceeding values

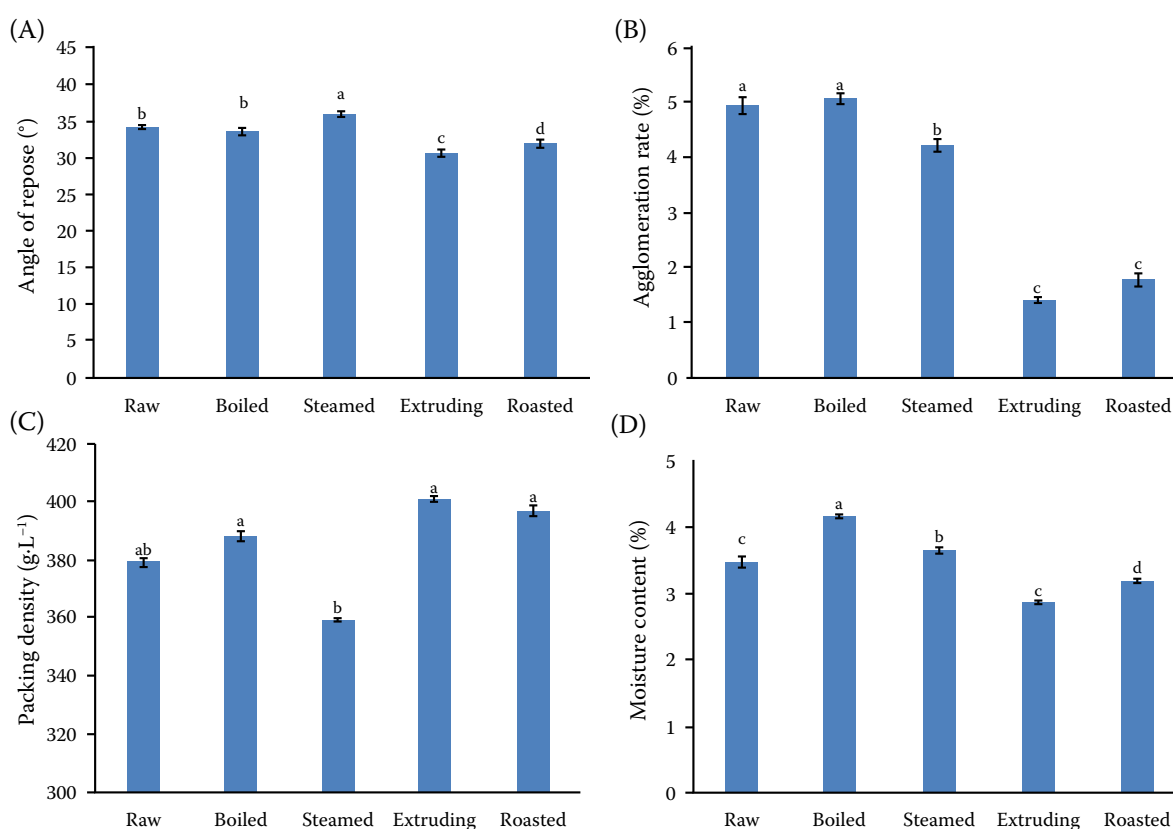


Figure 2. Physical properties of black bean under different thermal processing methods

Data with different letters are significantly different among different groups ($P < 0.05$)

<https://doi.org/10.17221/144/2024-CJFS>

observed in the other treatments. While the bulk densities across all processed samples were relatively similar, the extrusion-puffed powder demonstrated the highest bulk density at 401.3 g·L⁻¹. Collectively, these findings indicate that extrusion puffing yields superior physical properties in black bean powders, offering valuable insights for optimising processing strategies to enhance product quality.

Table 1 reveals the colour characteristics of black bean powder, with untreated raw black beans exhibiting a high brightness ($L^* = 75.3$), slightly leaning towards red ($a^* = -4.7$) and yellow ($b^* = 22.7$). The brightness of black beans showed a downward trend after steaming, boiling, extrusion, and roasting treatments, indicating that heat processing led to a darker appearance of black beans. During the roasting process, the colour turned to a dark yellow-brown due to the transformation of rutin (yellow-green) into quercetin (a dark green substance), and the thermal decomposition of anthocyanins (black-purple) into darker colours (Evangelho et al. 2017; Feitosa et al. 2018). In black soybean flour, the Maillard reaction facilitated the reaction between reducing sugars and free amino acids, leading to the generation of dark-coloured compounds and a notable decrease in lightness values. Meanwhile, the steaming, boiling, extrusion, and roasting treatments also caused changes in redness, where steaming and extrusion increased redness, with roasting resulting in a greater increase. Because the black bean contained a lot of anthocyanins, this pigment appeared red, usually deposited in the epidermis of the protective film, after high-temperature processing of the protective film was destroyed by high temperature, anthocyanins were released leading to redness (Neder-Suarez et al. 2021). The yellowness overall showed a decreasing trend, with boiling, steaming, and extrusion treatments reducing yellowness, while roasting slightly increasing it. The different heat treatment methods impacted the colour characteristics of black beans significantly. The data indicated that boiling and steaming could lead to a de-

crease in brightness of black beans, while redness and yellowness either increase or decrease. Extrusion made the black beans overall darker, while also increased redness (Ruiz-Armenta et al. 2019). Roasting enhanced the redness of black beans, obviously, and increased yellowness slightly.

These findings offered critical insights into the influence of thermal processing on the colour characteristics of black beans, underscoring their relevance in food manufacturing and quality assurance. The selection of an appropriate heat treatment method could be pivotal for aligning product attributes with specific functional requirements or consumer preferences, as thermal processing directly modulates visual quality and, consequently, market acceptance of the final product.

The TPC and TFC content. The polyphenols (TPC) and flavonoids (TFC) are compounds naturally present in plants, with various health benefits, including improving digestion, brain function, blood sugar levels, and prevention of thrombosis, heart disease, etc. Black soybeans are a good source of phenolic compounds (Li et al. 2024). As shown in Figure 3A, different processing methods impacted the polyphenol content of black beans. Polyphenols are unstable compounds at high temperatures, the heat processing could cause degradation, decarboxylation, and polymerisation, led to reduce their content, extraction rate, and antioxidant activity (Kim 2016; Jin et al. 2019).

Following thermal processing, the polyphenol content of black beans decreased in the order: extrusion > roasting > steaming > boiling. Among these methods, extrusion was most effective in preserving polyphenolic compounds, likely due to its streamlined processing and its ability to enhance the physical structure of the grains. Nonetheless, further investigation was warranted to optimise extrusion parameters in order to maximize the retention of polyphenols and antioxidant capacity. Roasting, a widely used thermal treatment, not only imparted distinctive sensory attributes but also altered the nutritional profile of grains. Interestingly, the impact of roasting on polyphenol content was relatively modest, potentially due to thermal degradation of complexes formed between carbohydrates, organic acids, and phenolic compounds-resulting in the partial release of bound polyphenols (Aregueta-Robles et al. 2018). The boiling and steaming processing methods reduced the polyphenol content of black beans to varying degrees, and the polyphenol content of steam-treated beans was 131.8% of that in boiled beans. Steaming, which did not involve direct contact with water, could better preserve water-

Table 1. Colour attributes of black bean under different thermal processing methods

Sample	L^*	a^*	b^*
Raw	75.3 ± 0.2 ^a	-4.7 ± 0.3 ^e	22.7 ± 0.1 ^b
Boiled	70.4 ± 0.1 ^b	1.5 ± 0.1 ^c	20.3 ± 0.3 ^c
Steamed	69.3 ± 0.2 ^c	3.3 ± 0.1 ^b	16.3 ± 0.1 ^e
Extruding	63.3 ± 0.4 ^e	1.1 ± 0.3 ^d	17.3 ± 0.2 ^d
Roasted	64.6 ± 0.5 ^d	9.9 ± 0.1 ^a	28.4 ± 0.1 ^a

L^* – brightness; a^* – redness; b^* – yellowness

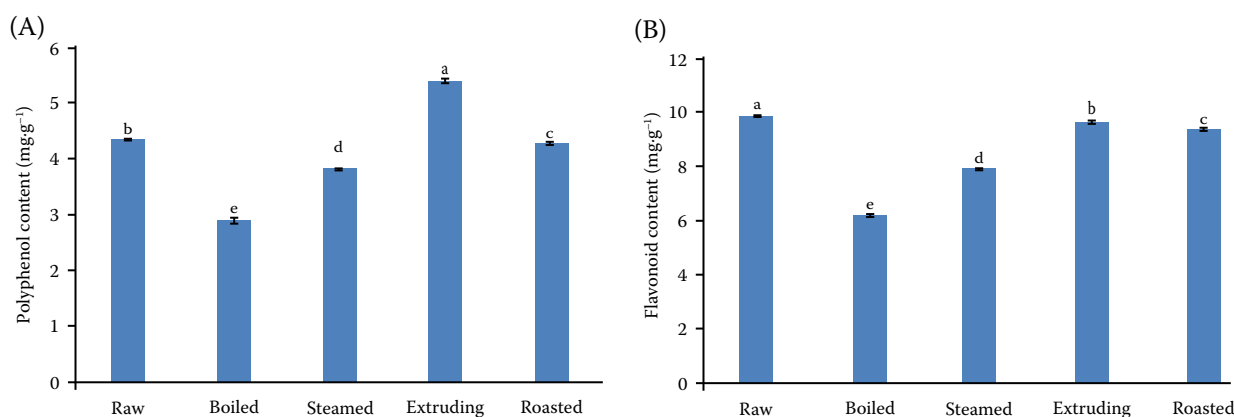


Figure 3. Polyphenol content (A) and flavonoid content (B) of black bean flour under different heat treatments
Data with different letters are significantly different among different groups ($P < 0.05$)

soluble substances. In contrast, boiling involved direct contact with water, affecting polyphenols differently, and resulting in more phenolic compounds dissolving in water during the process.

The flavonoids are a class of natural compounds synthesized by plants to adapt to ecological environments, garnering considerable attention due to their antioxidative, antibacterial, anti-aging, and lipid-lowering effects (Kumar et al. 2017). As indicated in Figure 3B, the content of flavonoids showed varying degrees of decline under different heat treatments. This was attributed to the thermal processing methods wherein flavonoids undergo degradation and breakdown under higher temperature conditions, resulting in decreasing content (Hostetler et al. 2013). Among the thermal processing methods evaluated, boiling exerted the most pronounced effect on flavonoid content, resulting in a substantial reduction of 37.3%. This loss was primarily attributed to the direct contact with water during boiling, which facilitated the leaching of water-soluble flavonoids. In addition, the large surface area and intense heat exposure likely accelerated the degradation and subsequent release of flavonoid compounds. Roasting led to a comparatively modest decrease of 5.0% in flavonoid content, accompanied by a noticeable darkening in colour. The relatively limited impact of roasting might be explained by the absence of direct water interaction, which reduced solubilization losses. Among all processing methods, extrusion puffing exhibited the least influence on flavonoid levels. This could be ascribed to its extremely short processing duration, despite the application of high temperature and pressure, thereby minimizing thermal and oxidative degradation of flavonoid compounds (Rockenbach et al. 2018).

In summary, high-temperature thermal processing adversely affected the retention of polyphenols and flavonoids in black beans, with water-mediated treatments exhibiting the most pronounced losses. Both processing temperature and duration were identified as critical factors influencing the stability of these bioactive compounds. To enhance the preservation of nutritional and functional components, careful optimisation of thermal processing conditions was essential. Among the methods evaluated, extrusion puffing emerged as the most effective and rational approach, offering a favourable balance between processing efficiency and the retention of key phytochemicals.

Antioxidant properties. The antioxidant properties of black beans are critically important in reducing the damage to cells and molecules caused by reactive oxygen species (Xue et al. 2016; Chen et al. 2022). These properties were also changed to varying degrees during the processing stages.

Figure 4 displays the antioxidant capacity *in vitro* of black bean powder extracts at different concentrations. This study assessed the antioxidant capacity through analysis of phenolic compound content with utilizing DPPH radical scavenging ability and hydroxyl radical scavenging ability. As shown in Figure 4, the black bean powder post-extrusion processing exhibited superior DPPH and hydroxyl radical scavenging abilities compared to other levels, consistence with the earlier discussed findings on polyphenol and flavonoid contents.

The extrusion process could not only facilitate the formation of melanoidins via Maillard reaction, enhancing the free radical scavenging ability of bean powder, but also break down plant cell wall to release bound phenolic substances, and increasing the polyphenol

<https://doi.org/10.17221/144/2024-CJFS>

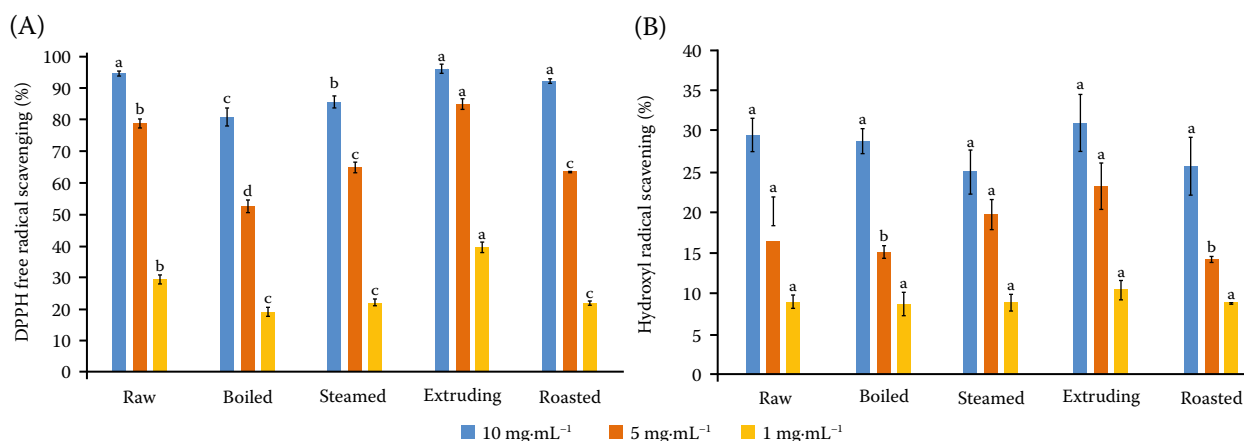


Figure 4. Antioxidant capacity of black bean flour under different heat treatments

DPPH – 1,1-diphenyl-2-trinitrophenylhydrazine; data with different letters are significantly different among different groups ($P < 0.05$)

content and subsequently improving the antioxidant capability of bean powder (Shin et al. 2014). Steaming, boiling, and roasting reduced the antioxidant capacity of black bean powder to varying degrees, with boiling exerting the most pronounced effect on DPPH radical scavenging—showing a 13.6% decrease at a concentration of 10 mg·mL⁻¹, while steaming led to the greatest reduction in hydroxyl radical scavenging with a 4.6% decreasing at the same concentration. These results suggested that, relative to other processing methods, extrusion processing more effectively preserved phenolic compounds and maintained antioxidant activity. Black bean extracts exhibited stronger DPPH radical scavenging activity and moderate activity against hydroxyl radicals, likely attributable to the distinct profiles of antioxidant constituents. In particular, the levels of total phenolics and flavonoids might play a critical role in neutralising different types of reactive oxygen species (Park et al. 2013).

CONCLUSION

Black beans are a highly nutritious legume, recognized for their stronger antioxidant properties. In this study, the effects of four thermal processing techniques – steaming, boiling, extrusion, and roasting – on the physicochemical characteristics and *in vitro* antioxidant activity of black beans were systematically investigated. All processing methods induced compositional changes to varying degrees. Moisture-based treatments, such as steaming and boiling, resulted in the leaching of organic salts and a consequent reduction in ash content. In contrast, extrusion and roast-

ing led to significant moisture loss, likely contributing to the concentration of dry matter constituents. From a physical standpoint, the clumping rate of black bean powder was markedly higher following boiling, possibly due to elevated moisture content, whereas extrusion yielded the highest bulk density among all treatments. In terms of colour, both boiling and steaming reduced brightness and altered chromatic properties, while extrusion deepened bean coloration and intensified redness. Boiling exerted the most pronounced impact on nutritional composition, as direct water contact facilitated the dissolution and degradation of key bioactive compounds. Conversely, extrusion processing was most effective in preserving nutritional integrity, leading to an increase in phenolic compound content while also enhancing the black beans' physical properties and antioxidant potential. Collectively, these findings demonstrated that thermal treatments not only modulated the phenolic profile of black beans but also influenced their associated bio functional effects. Extrusion processing, in particular, appeared to offer a promising strategy for maximising nutritional retention and functional quality in black bean-based food products.

In conclusion, each thermal processing technique possesses distinct advantages and limitations, and the strategic selection of processing conditions could effectively mitigate nutrient loss. This has important implications for the preservation of nutritional quality in value-added black bean products and the development of functional foods with enhanced health benefits.

Acknowledgment. All authors are grateful for the help of other colleagues during the course of the study.

REFERENCES

- Abera S., Yohannes W., Chandravanshi B.S. (2023): Effect of processing methods on antinutritional factors (oxalate, phytate, and tannin) and their interaction with minerals (calcium, iron, and zinc) in red, white, and black kidney beans. *International Journal of Analytical Chemistry*, 2023: 6762027.
- Alves P.L.S., Berrios J.D.J., Pan J., Yokoyama W.H. (2020): Black, pinto and white beans lower hepatic lipids in hamsters fed high fat diets by excretion of bile acids. *Food Production, Processing and Nutrition*, 2: 25.
- Alpos M., Leong S.Y., Oey I. (2021): Combined effects of calcium addition and thermal processing on the texture and *in vitro* digestibility of starch and protein of black beans (*Phaseolus vulgaris*). *Foods*, 10: 1368.
- Aregueta-Robles U., Fajardo-Ramirez O.R., Villela L., Gutiérrez-Urbe J.A., Hernández-Hernández J., López-Sánchez R.D.C., Scott S.P., Serna-Saldívar S. (2018): Cytotoxic activity of a black bean (*Phaseolus vulgaris* L.) extract and its flavonoid fraction in both *in vitro* and *in vivo* models of lymphoma. *Revista De Investigacion Clinica (Clinical and Translational Investigation)*, 70: 32–39.
- Barreto N.M.B., Pimenta N.G., Braz B.F., Freire A.S., Santelli R.E., Oliveira A.C., Bastos L.H.P., Cardoso M.H.W.M., Monteiro M., Diogenes M.E.L., Perrone D. (2021): Organic black beans (*Phaseolus vulgaris* L.) from Rio de Janeiro State, Brazil, present more phenolic compounds and better nutritional profile than nonorganic. *Foods*, 10: 900.
- Borges-Martínez E., Gallardo-Velázquez T., Cardador-Martínez A., Moguel-Concha D., Osorio-Revilla, G., Ruiz-Ruiz J.C., Martínez C.J. (2022): Phenolic compounds profile and antioxidant activity of pea (*Pisum sativum* L.) and black bean (*Phaseolus vulgaris* L.) sprouts. *Food Science and Technology*, 42: e45920.
- Carter C.E., Manthey F.A. (2019): Seed treatments affect milling properties and flour quality of black beans (*Phaseolus vulgaris* L.). *Cereal Chemistry*, 96: 689–697.
- Chen Y., Zheng Z., Ai Z., Zhang Y., Tan C.P., Liu Y. (2022): Exploring the antioxidant and structural properties of black bean protein hydrolysate and its peptide fractions. *Frontiers in Nutrition*, 9: 884537.
- Choudhary D., Andreani G.A., Mahmood S., Wen X., Patel M.S., Rideout T.C. (2024): Postnatal consumption of black bean powder protects against obesity and dyslipidemia in male adult rat offspring from obese pregnancies. *Nutrients*, 16: 1029.
- Damian-Medina K., Milenkovic D., Salinas-Moreno Y., Corral-Jara K.F., Figueroa-Yáñez L., Marino-Marmolejo E., Lugo-Cervantes E. (2022): Anthocyanin-rich extract from black beans exerts anti-diabetic effects in rats through a multi-genomic mode of action in adipose tissue. *Frontiers in Nutrition*, 9: 1019259.
- Evangelho J.A., Berrios J.J., Pinto V.Z., Antunes M.D., Vanier N.L., Zavareze, E.R. (2016): Antioxidant activity of black bean (*Phaseolus vulgaris* L.) protein hydrolysates. *Food Science and Technology*, 36: 23–27.
- Evangelho J.A., Vanier N.L., Pinto V.Z., Berrios J.J., Dias A.R.G., Zavareze E.R. (2017): Black bean (*Phaseolus vulgaris* L.) protein hydrolysates: Physicochemical and functional properties. *Food Chemistry*, 214: 460–467.
- Felix J.W., Sánchez-Chávez E., De-la-Cruz-Lázaro E., Márquez-Quiroz C. (2020): Edaphic and foliar biofortification of common black bean (*Phaseolus vulgaris* L.) with iron. *Legume Research*, 44: 192–196.
- Feitosa S., Greiner R., Meinhardt A.K., Müller A., Almeida D.T., Posten C. (2018): Effect of traditional household processes on iron, zinc and copper bioaccessibility in black bean (*Phaseolus vulgaris* L.). *Foods*, 7: 123.
- Fonseca-Hernandez D., Lugo-Cervantes E.D., Escobedo-Reyes A., Mojica L. (2021): Black bean (*Phaseolus vulgaris* L.) polyphenolic extract exerts antioxidant and antiaging potential. *Molecules*, 26: 6716.
- Guo Y., Wassgren C., Ketterhagen W., Hancock B., Curtis J. (2018): Discrete element simulation studies of angles of repose and shear flow of wet, flexible fibers. *Soft Matter*, 14: 2923–2937.
- Hernandez-Velazquez I., Sanchez-Tapia M., Ordaz-Nava G., Torres N., Tovar A.R., Galvez A. (2020): Black bean protein concentrate ameliorates hepatic steatosis by decreasing lipogenesis and increasing fatty acid oxidation in rats fed a high fat-sucrose diet. *Food & Function*, 11: 10341–10350.
- Hong J.Y., Shin S.R., Kong H.J., Choi E.M., Woo S.C., Lee M.H., Yang K.M. (2014): Antioxidant activity of extracts from soybean and small black bean. *Food Science and Preservation*, 21: 404–411.
- Hostetler G.L., Riedl K.M., Schwartz S.J. (2013): Effects of food formulation and thermal processing on flavones in celery and chamomile. *Food Chemistry*, 141: 1406–1411.
- Ismaiel S.A., Salama H.M. (2021): Allelopathic effects of black nightshade (*Solanum Nigrum* L.) on germination, growth and yield of broad bean (*Vicia Faba* L.) and common bean (*Phaseolus Vulgaris* L.). *Applied Ecology and Environmental Research*, 19: 3431–3441.
- Jeong E.W., Park S.Y., Yang Y.S., Baek Y.J., Yun D.M., Kim H.J., Go G.W., Lee H.G. (2021): Black soybean and Adzuki bean extracts lower blood pressure by modulating the renin-angiotensin system in spontaneously hypertensive rats. *Foods*, 10: 1571.
- Jin H., Zhao Q., Feng H., Wang Y., Wang J., Liu Y., Han D., Xu J. (2019): Changes on the structural and physicochemi-

<https://doi.org/10.17221/144/2024-CJFS>

- cal properties of conjugates prepared by the Maillard reaction of black bean protein isolates and glucose with ultrasound pretreatment. *Polymers*, 11: 848.
- Kim A.J. (2016): The inhibitory effects of roasted black bean (*Rhynchosia nulubilis*) extracts on RANKL-mediated RAW264.7 cells differentiation. *Food Science and Biotechnology*, 25: 839–846.
- Kumar S., Sharma V.K., Yadav S., Dey S. (2017): Antiproliferative and apoptotic effects of black turtle bean extracts on human breast cancer cell line through extrinsic and intrinsic pathway. *Chemistry Central Journal*, 11: 56.
- Lee M., Lee K.G. (2023): Effect of ultrasound and microwave treatment on the level of volatile compounds, total polyphenols, total flavonoids, and isoflavones in soymilk processed with black soybean (*Glycine max* (L.) Merr.). *Ultrasonics Sonochemistry*, 99: 106579.
- Li L., Luo C., Zheng X. (2021): Purification of anthocyanins derived from black kidney bean (*Phaseolus vulgaris* L.) by a simulated moving bed. *Journal of Chemistry*, 2021: 580756.
- Li S., Chen J., Hao X., Ji X., Zhu Y., Chen X., Yao Y. (2024): A systematic review of black soybean (*Glycine max* (L.) Merr.): Nutritional composition, bioactive compounds, health benefits, and processing to application. *Food Frontiers*, 5: 1188–1211.
- Mariscal-Moreno R.M., Chuck-Hernández C., Figueroa-Cárdenas J.D., Serna-Saldívar S.O. (2021): Physicochemical and nutritional evaluation of bread incorporated with Ayocote bean (*Phaseolus coccineus*) and black bean (*Phaseolus vulgaris*). *Processes*, 9: 1782.
- Machado-Velarde L.X., Davila-Vega J.P., Gutierrez-Urbe J., Espinosa-Ramírez J., Martínez-Avila M., Guajardo-Flores D., Chuck-Hernández C. (2023): Black bean hulls as a byproduct of an extraction process to enhance nutraceutical and glycemic-related properties of nixtamalized maize tostadas. *Foods*, 12: 1915.
- Melini F., Lisciani S., Camilli E., Marconi S., Melini V. (2023): Effect of cooking on phenolic compound content and *in vitro* bioaccessibility in sustainable foods: a case study on black beans. *Sustainability*, 16: 279.
- Mitharwal S., Saini A., Chauhan K., Taneja N.K., Oberoi H.S. (2024): Unveiling the nutrient-wealth of black soybean: A holistic review of its bioactive compounds and health implications. *Comprehensive Reviews in Food Science and Food Safety*, 23: e70001.
- Neder-Suarez D., Lardizabal-Gutierrez D., Zazueta-Morales J.J., Meléndez-Pizarro C.O., Delgado-Nieblas C.I., Wong B.R., Gutiérrez-Méndez N., Hernández-Ochoa L.R., Quintero-Ramos A. (2021): Anthocyanins and functional compounds change in a third-generation snacks prepared using extruded blue maize, black bean, and chard: An optimization. *Antioxidants*, 10: 1368.
- Park H.S., Shin S.R., Hong J.Y., Yang K.M. (2013): Comparison of the antioxidant activities of small-black-bean-Chungkukjang-added black food and soybean Chungkukjang extracts. *Journal of Food Preservation*, 20: 735–743.
- Qubbaj T., Samara R. (2022): Efficacy of three entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* isolates against black bean aphid, *Aphis fabae* (Scop.) (Hemiptera: Aphididae) on faba bean (*Vicia faba* L.). *Legume Research*, 45: 1572–1579.
- Rockenbach R., Ávila B., Bragança G., Monks J., Peres W., Gualarte M., Elias M. (2018): Effect of different hydration temperatures on the sensory, nutritional, and instrumental profile of black beans. *Revista Chilena de Nutrición*, 45: 144–152.
- Rosa-Millán J., Heredia-Olea E., Perez-Carrillo E., Guajardo-Flores D., Serna-Saldívar S. (2019): Effect of decortication, germination and extrusion on physicochemical and *in vitro* protein and starch digestion characteristics of black beans (*Phaseolus vulgaris* L.). *LWT - Food Science and Technology*, 102: 330–337.
- Ruiz-Armenta X.A., Zazueta-Morales J.J., Delgado-Nieblas C.I., Carrillo-López A., Aguilar-Palazuelos E., Camacho-Hernández I.L. (2019): Effect of the extrusion process and expansion by microwave heating on physicochemical, phytochemical, and antioxidant properties during the production of indirectly expanded snack foods. *Journal of Food Processing and Preservation*, 43: e14261.
- Sanchez-Tapia M., Hernandez-Velazquez I., Pichardo-Ontiveros E., Granados-Portillo O., Gálvez A., Tovar A.R., Torres N. (2020): Consumption of cooked black beans stimulates a cluster of some clostridia class bacteria decreasing inflammatory response and improving insulin sensitivity. *Nutrients*, 12: 1182.
- Saldívar X., Wang Y.J., Chen P., Hou A. (2011): Changes in chemical composition during soybean seed development. *Food Chemistry*, 124: 1369–1375.
- Sun M., Li D., Hua M., Miao X., Su Y., Chi Y., Li Y., Sun R., Niu H., Wang J. (2022): Black bean husk and black rice anthocyanin extracts modulated gut microbiota and serum metabolites for improvement in type 2 diabetic rats. *Food and Function*, 13: 7377–7391.
- Teixeira-Guedes C., Sanchez-Moya T., Pereira-Wilson C., Ros-Berruazo G., López-Nicolás R. (2020): *In vitro* modulation of gut microbiota and metabolism by cooked cowpea and black bean. *Foods*, 9: 861.
- Wang K., Gao Y., Zhao J., Wu Y., Sun J., Niu G., Zuo F., Zheng X. (2022): Effects of *in vitro* digestion on protein degradation, phenolic compound release, and bioactivity of black bean tempeh. *Frontiers in Nutrition*, 9: 1017765.
- Wu T., Sheng Y.N., Tian Y., Yu M., Bai L., Wang C.Y. (2023): Exploring the effect of boiling processing on the metabolic

<https://doi.org/10.17221/144/2024-CJFS>

- components of black beans through *in vitro* simulated digestion. LWT–Food Science and Technology, 184: 114987.
- Xue Z., Wang C., Zhai L., Yu W., Chang H., Kou X., Zhou F. (2016): Bioactive compounds and antioxidant activity of mung bean (*Vigna radiata* L.), soybean (*Glycine max* L.) and black bean (*Phaseolus vulgaris* L.) during the germination process. Czech Journal of Food Sciences, 34: 68–78.
- Yamamoto M., Yoshioka Y., Kitakaze T., Yamashita Y., Ashida H. (2022): Preventive effects of black soybean polyphenols on non-alcoholic fatty liver disease in three different mouse models. Food & Function, 13: 1000–1014.
- Received: July 15, 2024
Accepted: May 30, 2025
Published Online: August 1, 2025