Potential benefits of *Moringa peregrina* defatted seed: Effect of processing on nutritional and anti-nutritional properties, antioxidant capacity, *in vitro* digestibility of protein and starch, and inhibition of *α*-glucosidase and *α*-amylase enzymes

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A B S T R A C T

This study aimed to eliminate the bitter taste of *Moringa peregrina* press cake (MPC) as a byproduct of oil extraction (by employing safe and conventional methods) and evaluating its potential for formulating value-added food products. The characteristics (nutritional and anti-nutritional properties, monosaccharide composition, *in vitro* starch and protein digestibility, antioxidant capacity, and *in vitro* *α*-glucosidase, and *α*-amylase inhibitory activity) of raw, debittered (soaked in distilled water and boiled), and roasted (after debitterization) MPC flours were determined. Debitterization significantly increased total protein, fiber, arabinose, xylose, antioxidant activity, *in vitro* protein digestibility, and *α*-amylase inhibitory activity, whereas it decreased total starch, resistant starch, starch digestibility, ash, glucose, phytic acid, tannin, and oxalate contents. Fiber content, protein digestibility, *α*-amylase inhibitory activity, and antioxidant activity were further increased as the result of roasting. MPC and its products could inhibit *α*-amylase activity, with the highest inhibition belonging to roasted debittered samples. The current study is the first to report on the comprehensive nutritional and bio- and physicochemical aspects of *Moringa peregrina* press cake and the effect of treatments on improving its sensorial, nutritional, and health-promoting properties. Therefore, these results indicate the potential of treated MPC as a novel natural functional ingredient for various food formulations.

1. Introduction

Moringa peregrina (*M. peregrina*) is a tropical tree belonging to the family of *Moringaceae*, originating in Asia and Africa (Al-Owaisi, Al-Hadiwi & Khan, 2014; El-Hak, Moustafa & Mansour, 2018). *M. oleifera* is the most widely cultivated and the most important commercial species in the Moringaceae family. *M. peregrina*, is the second most economically important species in this family (Salama, Owon, Osman, Ibrahim & Matthäus, 2020). *Moringa* species are well known for medicinal, food, and water purifying applications (Koheil, Hussein, Othman & El-Haddad, 2011; Magaji, Sacan & Yanardag, 2020; Ogunsina, Radha & Indrani, 2011). Among various possible usages of interest for *M. peregrina*, its potential as a functional bioactive-rich ingredient to make fortified food products would stand out (Hernandez-Aguilar et al., 2021). Although there are some studies about the application of *M. oleifera* seed in food products such as cookies (Ogunsina et al., 2011) and wheat bread (Bolarinwa, Aruna & Raji, 2019; Hernandez-Aguilar et al., 2021), there

appears to be no scientific report about the use of *M.peregrina* seed as a food ingredient. Yet, the assessment of biochemical parameters, besides the histological observations, has proved the safety of this seed for human consumption (El-Hak et al., 2018). The seed of *M. peregrina* is a rich source of oil (42–54%) (El-Hak et al., 2018), protein (24%), and minerals with high essential amino acids contents (Al-Dabbas et al., 2010).

The previous studies on the *M. Peregrina* seed are commonly confined to the kernel composition, fatty acid component, and pharmacological efficacy (Al Juhaimi, Ghafoor, Babiker, Matthäus & Özcan, 2017; Al-Dabbas et al., 2010). The previous investigations on the total phenolic contents and antioxidant potential of *M. peregrina* have generally been conducted on leaf extract only (Al-Owaisi et al., 2014; El-Awady, Hassan, El-Sayed & Gaber, 2016). However, Koheil et al. (2011) studied the DPPH• free radical scavenging activity of the ethanolic and aqueous extracts of *M. peregrina* unhulled seed. Earlier studies have shown that *M. peregrina* seeds and leaves possess antidiabetic properties and are used

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in the management of diabetes in folk medicine (El-Hak et al., 2018). Ullah, Bhat and Abuduhier (2015) showed the inhibitory effects of *M. peregrina* 80% methanol leaf extract (IC50= 13,350 μg/mL) on porcine α-amylase. Nonetheless, there are no previous reports about the inhibition of the α-amylase and α-glucosidase enzymes of *M. peregrina* seed kernel/press cake.

The major product obtained from *M. peregrina* seed is its oil (El-Awady et al., 2016). The by-product (seed cake) that is generated after oil extraction of seed has great nutritional value, and due to its high protein content (57%), it is a promising source of various nutrients (Al-Kahtani, 1995). The bitter taste of seed kernel and seed cake limit its industrial usage for human consumption (Al-Dabbas et al., 2010), and the by-product that is generated after oil extraction of *M. peregrina* seed has shown anti-nutritional factors such as phytic acid, trypsin inhibitor, tannin and chlorogenic acid (AL-Kahtani, 1995).

A few attempts have been conducted on the debitterization of *M. oleifera* seed; *e.g.*, boiling, fermentation, and germination. Thermal treatments usually cause a decrease or inactivation of antinutritional factors or increase protein digestibility (Aalim, Wang & Luo, 2021). Nevertheless, there is no detailed report on the seed kernel/defatted seed efficiency for the inhibition of key digestive enzymes related to Type 2 diabetes (*i.e.*, α-glucosidase, α-amylase), monosaccharide composition, total starch, resistant starch, starch digestibility, total phenolic, and oxalate content.

The roasting process has been shown as a method to improve the nutritional and quality attributes such as color, taste, texture, antioxidant properties, and shelf-life of seeds (Ahmed et al., 2021). Most of the works carried out on the characterization of heat treatment on the nutritional composition of *Moringa* species so far, have focused either on *M. oleifera* or limited nutrient or antinutritional factors (Ijarotimi, Adeoti & Ariyo, 2013; Mbah, Eme & Ogbusu, 2012; Ogunsina et al., 2011). Mbah et al. (2012) reported that roasting of *M. oleifera* seed increased fiber, vitamin A, protein, iron, and zinc contents. The level of antinutrients like phytate, saponin, and oxalate was also increased, while tannin was decreased. The only report about roasting of *M. peregrina* seeds was presented by Salama et al. (2020) who studied the effect of roasting on the oil profile, tocopherols, and triacylglycerols. But, no reference is yet available on the nutritional value and bio- and physicochemical properties of *M. Peregrina* seed/press cake after roasting. Nevertheless, the antioxidant capacity of treated *Moringa* species has not been reported, nor have the α-glucosidase and α-amylase inhibitory effects or the protein and starch digestibility of heated/processed seeds been studied. Also, monosaccharide composition of *Moringa* species has not been reported before. Yet, no reference is available on the nutritional value and bio- and physicochemical properties of *M. Peregrina* seed/press cake after roasting.

Additionally, despite *M. Peregrina* seed possessing an enormous potential in the food industry, limited information regarding its bioactive compounds and possible health-promoting effects is available. The knowledge about the bioactive potentials of *M. Peregrina* press cake, as a waste product of oil processing, could stimulate its application in the food industry and improve public nutrition and health. Moreover, using simple and safe treatments to improve sensorial characteristics and nutritional value of this waste seed may lead to increasing demand as an ingredient for the innovation of functional food products and a novel source of feasible business and income. Accordingly, to our knowledge, this is the first work to eliminate the bitter taste and improve the health properties of *M. peregrina* press cake as a by-product of oil extraction by employing safe and domestic methods and evaluating its potential for formulating value-added food products. Hence, the present study aimed to comprehensively investigate the effect of debitterization (soaking and boiling) and roasting on nutritional value of *M. peregrina* press cake including nutrients (ash, carbohydrate, protein, total fiber, total starch, and resistant starch), antinutrients (phytates, oxalate, and tannin), antioxidant capacity, and starch and protein digestibility. Besides, the impact of processings on the inhibition of Type 2 diabetes-related digestive enzymes (*e.g.*, α-glucosidase and α-amylase) and monosaccharide contents were studied.

2. Materials and methods

2.1. Materials

Mature *M. peregrina* seeds were purchased from the GolGoharDaro Company (Sistan, Iran). Pepsin (P7000 from gastric porcine mucosa), pancreatin (P-1750 from the porcine pancreas), amyloglucosidase (P300 units/mL), intestinal acetone powders from rat (I1630), Folin-Ciocâlteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox (6-hydroxy-2,5,7,8 tetramethyl chromane-2-carboxylic acid), gallic acid, *p*ara- hydroxybenzoic acid hydrazide (PAHBAH), D- catechin, and neutral monosaccharide standards (L-fucose, L-rhamnose, D-arabinose, D-galactose, D-glucose, D-mannose, D-xylose, and D-galacturonic acid) were obtained from Sigma-Aldrich (Vallensbaek Strand, Denmark). Trifluoroacetic acid (99%) was from Merck (Darmstadt, Germany). NaOH standard solution (HPLC grade) was purchased from Fluka/Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Oil extraction and treatment of M. peregrina press cake

M. peregrina press cake (MPC) was prepared by dehulling and deoiling seed kernels using a hydraulic press. Debittered press cake was prepared by combining the methods reported by Mbah et al. (2012) and Salama et al. (2020), after carrying out several preliminary/pilot trials and modifications. Briefly, MPC was soaked in distilled water (1:3 w/v) for 24 h at 4 °C. Then, the soaked MPC was added to the boiling distilled water (1:5 w/v) for 20 min (∼ 98 °C). The boiled MPC was soaked in distilled water at 4 °C until the bitter taste was not recognizable by 6 trained panelists. The soaking water was replaced twice a day with fresh distilled water. To investigate the possible effects of roasting on the enhancement of antioxidant properties, the nutritional value and other bio- and physicochemical properties of the debittered sample, a single layer of the sample was heated in a drying oven for 30 min at 170 °C (Salama et al., 2020).

All samples were ground and passed through a 40-mesh sieve. The residual oil of different flours was removed with hexane in a sealed container at the flour to solvent ratio of 1:5 (w/v), using continuous shaking by a magnetic stirrer at room temperature for 5 h. The mixture was filtered through a Whatman filter paper No. 42 (Whatman, Maidstone, UK) and the extraction was repeated two more times. The remaining solvents in the defatted flours were removed by drying in an oven at 40 °C for 8 h, then were ground and passed through a 40-mesh. The defatted flours (*i.e.*, raw, debittered and roasted debittered) were stored at −20 °C until further analysis.

2.3. Determination of bio- and physicochemical properties of defatted flours

2.3.1. Proximate analysis of defatted flours

Protein ($N \times 6.25$), ash, and total fiber contents of defatted flours were determined by 976.05, 923.03, and 978.10 AOAC methods (AOAC, 2005), respectively. The carbohydrate content was calculated by difference.

2.3.2. In vitro *protein digestibility*

The *in vitro* protein digestibility of defatted flours was determined following the method described by Sreerama, Sasikala and Pratape (2008) with some modifications. Briefly, 1 g of different defatted flours was suspended in 75 mL of 0.1 M HCl solution containing 10 mg pepsin. After 3 h of incubation at 37 °C, the mixture was neutralized with 0.2 M NaOH (pH 7.5). A 37.5 mL of phosphate buffer (pH 7.5) containing 20 mg of pancreatin, 1 mM CaCl$_2$, and 0.01% sodium azide was added to the suspension. After 24 h of digestion at 37 °C, the undigested

protein was precipitated using 30% trichloroacetic acid (TCA) solution, separated, and filtered (Whatman No. 42). The nitrogen content of the samples before digestion and of the undigested part was determined by the micro-Kjeldahl method (AOAC, 2005). Protein digestibility was calculated according to the following equation:

$$\% \text{Protein digestibility} = \frac{\text{Total protein in the sample} - \text{undigested protein}}{\text{Total protein}} \times 100$$

$$(1)$$

2.3.3. Measurement of total starch

Total starch was determined according to the method reported by Goñi, Garcia-Alonso and Saura-Calixto (1997). Starch hydrolysis was conducted by suspending 50 mg of defatted flours in 6 mL of KOH (2 M) and shaking vigorously for 30 min at room temperature, followed by the addition of 60 mL amyloglucosidase in a shaking water bath for 45 min (60 °C, pH 4.75). After centrifugation of suspension (3000 × g, 10 min), glucose concentration in the supernatant was measured using an Azura HPLC system with a P60.1 L gradient pump (Knauer, Berlin, Germany). The pump was fitted with a DECADE Elite electrochemical detector with a gold working electrode and an injection valve (Rheodyne 9725i), equipped with a 20 μL injection loop (CA, USA). The separation was conducted by a Dionex CarboPac PA1 anion-exchange column (250 × 4 mm ID, 5 μm particle size) (Dionex Corp., Sunnyvale, CA), and the total starch values were calculated as mg of glucose × 0.9.

2.3.4. Measurement of resistant starch

Resistant starch of the samples was measured by incubation of the defatted flours with pepsin solution for 1 h to remove protein (40 °C, pH 1.5), followed by incubation with amylase for 16 h (37 °C, pH 7) to hydrolyze digestible starch. After centrifugation, the residue was hydrolyzed with KOH (2 M) followed by amyl glucosidase, as explained above for remaining starch (Goñi, Garcia-Diz, Mañas & Saura-Calixto, 1996).

2.3.5. In vitro *starch digestibility*

Starch digestibility was calculated based on total starch and resistant starch, and according to the following equation (Sęczyk, Świeca, Dziki, Anders & Gawlik-Dziki, 2017):

$$\% \text{ Starch digestibility} = 100 - \frac{\text{RS}}{\text{TS}} \times 100 \qquad (2)$$

where, RS and TS present resistant starch and total starch, respectively. *In vitro* digestion methods for RS and TS determination were described in Sections 2.3.3 and 2.3.4.

2.3.6. Monosaccharide composition

The hydrolysis of the samples was conducted by 2 h incubation of 20 mg of defatted flours with 2 M trifluoroacetic acid (600 μL) and 2600 μL Milli-Q water at 121 °C. After cooling to room temperature, hydrolysates were freeze-dried (Labconco Corporation, Kansas City, MO, USA) and kept at −20 °C under N$_2$ until analysis. Monosaccharide content in hydrolysates and standards were defined by high-performance anion-exchange chromatography with a pulsed amperometric detector (Knauer, Berlin, Germany) using a CarboPac PA1 (4 × 250 mm) analytical column (Dionex Corp., Sunnyvale, CA), following the method described by Balaghi, Mohammadifar, Zargaraan, Gavlighi and Mohammadi (2011).

2.3.7. Determination of total phenolic content (TPC)

The phenolic compounds of the raw defatted flour were extracted using five different solvents (acetone, deionized water, ethanol, hexane, methanol) at a flour to solvent ratio of 1:10 (w/v) at 25 °C for 24 h at 150 rpm. The mixture was separated by centrifuging at 6000 × g for 15 min. The extracts were then filtered through a Whatman filter paper No. 42 (Whatman, Maidstone, UK) (Singh, Negi & Radha, 2013),

and the TPC was measured by the Folin-Ciocâlteu method (Slinkard & Singleton, 1977). An aliquot of 20 μL of extract solution was mixed with 1.60 mL of distilled water and 100 μL of 0.2 M Folin-Ciocâlteu phenol reagent. After 8 min of incubation at room temperature, 300 μL of 20% sodium carbonate (Na$_2$CO$_3$) solution was added and stored at 40 °C in a shaking water bath. After 30 min, the absorbance of the sample solutions was monitored at 765 nm with a spectrophotometer (Agilent-Carry 60, USA). The polyphenol content of the extract was expressed as mg gallic acid equivalents (GAE) per g of dry sample.

2.3.8. Production of phenolic extracts

The phenolic compounds in defatted flours were extracted using deionized water according to the method described by Singh et al. (2013). Defatted flours (5 g) were extracted two times with 50 mL of deionized water at 25 °C for 1 h at 150 rpm in dark conditions. After centrifugation of the mixture (6000 × g, 10 min), the supernatant was filtered through a Whatman filter paper No. 42. The filtrate was transferred to −80 °C for 24 h and then freeze-dried (Labconco Corporation, Kansas City, USA). The manufactured dry extract was stored at −20 °C for further analysis.

2.3.9. Antioxidant properties of different phenolic extracts

2.3.9.1. DPPH• radical scavenging assay. An aliquot of 500 μL of different concentrations of extract solution was added to 500 μL of DPPH• methanolic solution (0.1 mM) and stored in dark at room temperature. After 30 min, the reduction in the absorbance was measured at 517 nm with a spectrophotometer (Agilent-Carry 60, USA). The DPPH• radical scavenging activity was expressed as micromoles of Trolox equivalents (μM TE) per g of dry sample (Karimi, Azizi & Ahmadi Gavlighi, 2020).

2.3.9.2. ABTS•+ scavenging activity. The ABTS•+ scavenging activity was measured according to the method from Sarkis et al. (2014). 20 μL of the different concentrations of extract solution was added to 980 μL of diluted ABTS•+solution. The absorbance was read against a blank at 734 nm following the incubation of the well-mixed samples at 30 °C for 10 min in the dark condition.

2.3.10. Inhibitory properties against α-glucosidase and α-amylase enzymes

2.3.10.1. Porcine α-amylase enzyme inhibition test. The α -amylase inhibitory activity of freeze-dried extracts was determined following the method of Karimi et al. (2020). Briefly, 100 μL of different concentrations of extracts in 0.1 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) and 100 μL of α-amylase solution (0.5 U/mL) was pre-incubated at 37 °C for 5 min. Then, a 100 μL of 0.5% (w/v) starch solution was added. After the incubation of the mixture for 20 min at 37 °C, the undigested starch was separated following the inactivation of the enzyme at 100 °C for 10 min and centrifugation of the cooled mixture for 2 min at 13,000 × g. The supernatant (20 μL) was added to 1 mL of PAHBAH solution and placed at 70 °C for 10 min. After cooling the mixture to room temperature, the absorbance was read at 410 nm. The IC$_{50}$ value of acarbose was applied as a control.

2.3.10.2. Rat intestinal α-glucosidase inhibition test. The α-glucosidase inhibitory activity of different extracts was conducted following the method described by Connolly, Piggott and FitzGerald (2014) with some modifications. A 300 mg of rat intestinal acetone powders as an enzyme source were treated by 3 mL of 0.1 M phosphate buffer (pH 6.9) using an ultrasonic bath at 4 °C for 30 min. After centrifugation at 12,000 × g for 30 min at 4 °C, the supernatant was diluted to 36 mU/mL. Aliquots of 100 μL of extract solution (30 mg/mL) were added to 200 μL of α-glucosidase and preincubation was conducted for 10 min at 37 °C. A 100 μL substrate solution of PNPG (5 mM) was added and placed at 37 °C for 30 min. The absorbance of the solution was scanned every 2 min at 405 nm. The method was repeated with the phosphate buffer as the control and the IC$_{50}$ value of acarbose was applied as the positive control.

Table 1
The effect of treatment on the chemical composition of defatted *M. Peregrina* press cake.

Parameters	Samples		
Chemical composition (g/100 g DW)	Raw	Debittered	Roasted debittered
Protein	51.63±0.32[b]	65.78±0.10[a]	65.95±0.05[a]
Crude fiber	10.39±0.27[c]	17.62±0.14[b]	20.14±0.09[a]
Ash	4.62±0.02[a]	2.35±0.01[b]	2.03±0.03[c]
Carbohydrate	33.36±0.07[a]	14.25±0.23[b]	11.88±0.11[c]
Total starch	10.59±0.14[a]	8.31±0.11[b]	8.38±0.06[b]
Resistant starch	7.30±0.16[a]	6.45±0.12[b]	6.35±0.10[b]
Antioxidant activities			
TPC (mg GAE/g DW)	1.1 ± 0.01[c]	1.82±0.02[b]	2.18±0.07[a]
DPPH (μM TE/g DW)	7.54±0.16[c]	8.59±0.36[b]	29.43±0.32[a]
ABTS (μM TE/g DW)	22.44±0.47[c]	65.78±0.82[b]	89.80±0.71[a]
Antinutritional factors			
Tannin (mg catechin/g)	0.17±0.04[a]	ND [b]	ND [b]
Phytic acid (mg/100 g DW)	4211.01±1.05[a]	1530.00±2.04[b]	1516.07±2.53[b]
Oxalate (mg sodium oxalate /100 g sample)	880.03±0.43[a]	220.57±0.35[b]	220.19±0.13[b]
Sugar contents (g/100 g DW)			
Arabinose	2.12±0.01[c]	2.90±0.01[b]	3.49±0.01[a]
Fucose	0.11±0.01[a]	0.05±0.00[b]	3.49±0.01[b]
Galactose	0.71±0.01[a]	0.03±0.00[c]	0.61±0.00[b]
Galacturonic acid	1.59±0.01[b]	1.21±0.01[c]	1.12±0.00[d]
Glucose	6.22±0.01[a]	0.28±0.00[c]	0.24±0.01[c]
Rhamnose	1.25±0.01[a]	0.32±0.02[d]	0.36±0.01[c]
Xylose	0.33±0.01[c]	0.74±0.01[b]	0.90±0.02[a]
Total sugars	**12.33±0.01[a]**	**5.54±0.02[d]**	**6.77±0.02[c]**

Results are displayed as mean ± standard deviation. Values with different superscripted letters within the same row are significantly different ($n = 3$, $p < 0.05$).
DW: dry weight, Raw: defatted *M. Peregrina* press cake (control), Debittered: defatted debittered (soaked in distilled water and boiled) *M. Peregrina* press cake, Roasted debittered: defatted roasted debittered *M. Peregrina* press cake, TPC: total phenolic contents, DPPH: ability to quench DPPH radicals, ABTS: ability to quench ABTS radicals, GAE: gallic acid equivalent, TE: Trolox equivalent, ND: not detected.

2.3.11. Assessment of phytates, oxalate, and tannin in defatted flours

Estimation of phytates was conducted by the spectroscopic method using a standard curve of KH_2PO_4 solution, where a known concentration was reacted with ferrous sulfate-ammonium molybdate reagent as described by Vinay and Kanya (2008). Oxalate was measured by titration of sulfuric acid-treated samples against $KMnO_4$ (0.05 M) and calculated as the equivalent of sodium oxalate (Olawoye & Gbadamosi, 2017). Tannin content was determined with the vanillin-HCL method using D-catechin as a reference (Al-Kahtani, 1995).

2.4. Statistical analyses

All tests were performed in triplicate and the data were subjected to one-way Analysis of Variance (ANOVA) using SPSS 19 software (SPSS Inc., Chicago, IL, USA). Significant mean values were determined at a 95 percent confidence level ($p \leq 0.05$) using Tukey's test.

3. Results and discussion

3.1. Proximate chemical composition of defatted flours

The contents of protein, ash, total fiber, total starch, and resistant starch of defatted flours are presented in Table 1. The main constituents of raw defatted flour were protein (51.63%), carbohydrate (33.36%), and fiber (10.39%). The previous reports on the chemical composition of *M. Peregrina* press cake/seed meal are very limited. Al Juhaimi et al. (2017) reported a much lower protein content (32.09%) in *M. peregrina* seed meal; whereas, AL-Kahtani (1995) reported 57% protein in the seed meal, which agrees with our findings in the present study. Debitterization resulted in a significant increase ($p<0.05$) in total protein content (27.41%). Mbah et al. (2012) showed that 30 min boiling

of seed increased the protein content by up to 19.95% (from 26.71 to 32.04 g/100 g) relative to raw *M. oleifera* seed. The enhancement in protein content could be due to the loss of soluble solids during soaking and boiling which could increase the protein concentration (Avola, Patanè & Barbagallo, 2012). The protein content of roasted debittered flour remained similar to that of the debittered sample.

Debitterization caused a significant ($p<0.05$) increase in fiber content (to the level of 17.62±0.14%), which could be attributed to the formation of the protein–fiber complex. After boiling, a feasible chemical amendment may change the structure of the cell wall, while the storage polysaccharides can result in interrupting protein-carbohydrate integration that leads to the reduction of the soluble fiber (Wang, Hatcher, Tyler, Toews & Gawalko, 2010). Fiber content was more increased after roasting (20.14±0.09). A similar increase has been reported in the case of *M. oleifera* seed post boiling and roasting treatments (Mbah et al., 2012). The debitterization significantly ($p<0.05$) decreased total starch and resistant starch contents in comparison to the raw flour (by about 21.53% and 11.64%, respectively). Debittered and roasted debittered flours showed 8.31±0.11 and 8.38±0.06% total starch, and 6.45±0.12 and 6.35±0.10%, resistant starch contents, with a nonsignificant difference ($p>0.05$) between them. Reduction in resistant starch after debitterization may be attributed to boiling under conditions of high moisture and temperature, which can result in disruption of crystalline structure (Sajilata, Singhal & Kulkarni, 2006). We could not find any reports about the total starch and resistant starch of the *Moringa* seed species, or the effect of heat treatment on these factors.

The carbohydrate content of raw, debittered, and roasted debittered flours were observed to be 33.36±0.07%, 14.25±0.23%, and 11.88±0.11%, suggesting a pronounced effect of treatments. The leaching of soluble sugars into the soaking and boiling medium could be the reason for this reduction. Our findings in the present study are contrary

to Tanwar, Modgil & Goyal, 2018, who reported a significant increase in carbohydrate content of wild apricot kernel using 25% sodium chloride (NaCl) solution as a soaking medium for 12 h. Like the carbohydrate content, a significant decrease ($p < 0.05$) was noticed in the ash content of debittered (2.35±0.01%) and roasted debittered (2.03±0.03%) flours compared to raw flour (4.62±0.02%). Avola et al. (2012) observed a significant decrease in ash content of chickpeas post-cooking, probably due to the ash leakage into the cooking medium. Ahmed et al. (2021) reported a significant loss of ash content in sesame seeds obtained from India and Nigeria after heat treatment (180 °C and 200 °C for 20 and 30 min, respectively). There are no previous reports about the effect of heat treatment on the proximate composition of *M. Peregrina* seed kernel/press cake; thus, this can be the first report on such nutritional factors in this press cake.

3.2. Monosaccharide content

As illustrated in Table 1, seven monosaccharides including arabinose, fucose, galactose, galacturonic acid, glucose, rhamnose, and xylose were determined in raw defatted flour. This cake was high in glucose (50.45%), galacturonic acid (12.90%), and arabinose (17.19%). There is little information in the literature about monosaccharides in the seed kernel of *Moringa* species. Nevertheless, Al Juhaimi et al. (2017) reported that the main monosaccharide in the seed and leaves of *M. oleifera* and *M. peregrina* was glucose. In the present study, the total monosaccharides content decreased significantly ($p < 0.05$) after debitterization, which could be attributed to the leaching out into the soaking and boiling medium during debitterization treatment. In that, the amount of glucose decreased from about 50% in raw defatted flour to approximately 5% in debittered flour; instead, arabinose and xylose showed a significant ($p < 0.05$) increase of 36.79% and 124.24%, respectively. Glucose reduction during debitterization treatment is interesting, suggesting that debittered flour may be appropriate for incorporation into diabetic foods. A significant ($p < 0.05$) increase was observed in the total monosaccharides content after roasting (22.20%). As shown in Table 1, the predominant monosaccharides in roasted debittered flour were found to be arabinose, galactose, rhamnose, and xylose, respectively. The present study is the first report that shows most of the seed kernel monosaccharides in *M. Peregrina* and describes the effect of treatment (soaking, boiling, and roasting) on their contents.

3.3. Effect of solvent on the extraction of phenolic compounds

The amount of total phenolic content from defatted raw flour by various solvents ranged from 0.91±0.07 mg gallic acid equivalence (GAE)/g in the case of ethanol extraction to 3.14±0.06 mg GAE/g for the aqueous extraction. Interestingly, the extraction with deionized water resulted in statistically ($p < 0.05$) higher total phenolic content compared to other solvents.

The total phenolic content results were 1.04±0.05 for acetone, 1.05±0.09 for hexane, and 1.67±0.07 mg GAE/g for methanol extracts. The variation in phenolic compounds is described for different solvents in other *Moringa* species, as well as other plants (Prabakaran, Kim, Sasireka, Chandrasekaran & Chung, 2018; Singh et al., 2013).

3.4. Determination of total phenolic content in defatted flours

As the extraction with deionized water displayed a higher amount of total phenolic content, it was used to produce freeze-dried phenolic extract from different defatted flour. The total phenolic content values in the debittered and roasted debittered flours were found to be significantly ($p < 0.05$) more than that in raw flour. As depicted in Table 1, the total phenolic content values in defatted raw, debittered and, roasted debittered flours were 1.1 ± 0.01, 1.82±0.02, and 2.18±0.07 mg GAE/g DW, respectively.

The previous reports on the total phenolic content of *M. peregrina* are commonly limited to the leaf extract (Al-Owaisi et al., 2014; El-Awady et al., 2016). There are limited reports on the seed kernel total phenolic content of the *Moringa* species. Although the phenolic content of defatted *M. oleifera* seed flour has also been studied. Singh et al. (2013), for example, reported a value of 7.8 mg GAE/g of the sample, using methanol as the extraction solvent. Sarkis et al. (2014) reported phenolic compounds in different seed cakes and nut cakes, among which hazelnut and sunflower (1.51 and 2.47 mg GAE/g, respectively) showed higher amounts while sesame and flaxseed (0.71 and 0.56 mg GAE/g, respectively) showed lower amounts of total phenolic content. These are comparable to the defatted *M. peregrina* seed (1.1 mg GAE/g) in the current study.

In the present study, debitterization resulted in a marked increase of 65.45% in the total phenolic content compared to the raw defatted flour, denoting that boiling water could have increased the extraction of bound phenolic compounds from the food matrix. Similar increases have been observed in boiled breadfruit (Chinedum, Sanni, Theressa & Ebere, 2018). Total phenolic contents substantially increased ($p < 0.05$) to 19.78% in the roasted kernel compared to debittered sample, which could be attributed to the denaturation of the cell wall components, decomposition of linkage between phenolic compounds and fibers, and/or disruption of the bonds between phenols and sugars that may increase the extractability of these compounds (Ahmed et al., 2021; Chinedum et al., 2018). Similarly, Ahmed et al. (2021) detected significant enhancement in total phenol contents post-roasting treatment of sesame seeds. The effect of soaking, boiling, and roasting on the total phenol contents of the seed kernel in *Moringa* species is rarely described in the previous litrature. However, Ijarotimi et al. (2013) reported a slightly lower value of total phenolic contents (0.40±0.32 mg GAE/100 g DW) in soaked *M. oleifera* seed using distilled water for 9 h.

3.5. Antioxidant properties of different phenolic extracts

As illustrated in Table 1, DPPH$^{\bullet}$ and ABTS$^{\bullet +}$ scavenging activities of defatted raw flour were found to be 7.54±0.16 and 22.44±0.47 μmol Trolox equivalent (TE)/g DW, respectively. The earlier investigations on the antioxidant potential of *M. peregrina* have generally been conducted on leaf extract only (Al-Owaisi et al., 2014; El-Awady et al., 2016). However, Koheil et al. (2011) studied the DPPH$^{\bullet}$ free radical scavenging activity of the ethanolic and aqueous extracts of *M. peregrina* whole (kernel and hull) seed. The highest radical scavenging activity for ethanolic extract was exhibited at a 6 mg/mL concentration, while the aqueous extract of 6 mg/mL showed nearly equal free radical scavenging to Trolox at 0.06 mg/mL. Sarkis et al. (2014) reported lower values of ABTS$^{\bullet +}$ scavenging activity for different seed cakes of sunflower, pumpkin, flaxseed, and sesame, and nut cakes of almond and hazelnut (6.24, 0.54, 3.09, 1.15, 0.60, and 1.89 μmol TE/g, respectively), which are comparable to the values observed in the current study for raw defatted flour.

In the present study, DPPH$^{\bullet}$ and ABTS$^{\bullet +}$ scavenging activities of debittered flour were observed to be 8.59±0.36 and 65.78±0.82 μmol TE/g DW, respectively. There was a significant increase ($p < 0.05$) in both DPPH$^{\bullet}$ and ABTS$^{\bullet +}$ scavenging activities of debittered flour compared to raw flour, suggesting that both soaking and boiling treatments could raise the extractability of antioxidants from the plant tissue (Chinedum et al., 2018).

Roasting caused a dramatic increase of 242.61% (from 8.59±0.36 to 29.43±0.32 μmol TE/g DW) and 36.52% (from 65.78±0.82 to 89.80±0.71 μmol TE/g DW) in DPPH$^{\bullet}$ and ABTS$^{\bullet +}$ scavenging activity, respectively, compared to debittered flour. This could be attributed to the heat-induced release of phenolic compounds and the generation of Maillard reaction substances with excellent antioxidant activity (Ahmed et al., 2021). We were not able to find any previous report about the effect of heat treatment on the seed kernel/press cake of *Moringa* species.
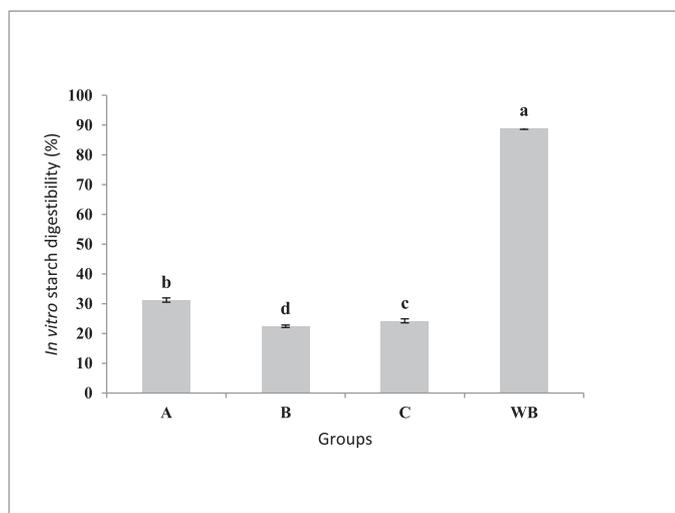
Fig. 1. *In vitro* starch digestibility (%) of defatted *Moringa peregrina* press cake and its different treatments in comparison to white bread. A: defatted *M. peregrina* press cake, B: debittered *M. peregrina* press cake, C: roasted debittered *M. peregrina* press cake. WB: white wheat bread (control). The columns with different letters are significantly different at $p<0.05$. Values and error bars correspond to the mean ± standard deviation of triplicate samples.

Fig. 2. *In vitro* protein digestibility (%) of defatted *Moringa peregrina* press cake and its different treatments. A: defatted *M. peregrina* press cake, B: debittered *M. peregrina* press cake, C: roasted debittered *M. peregrina* press cake. The columns with different letters are significantly different at $p<0.05$. Values and error bars correspond to the mean ± standard deviation of triplicate samples.

3.6. In vitro *starch digestibility*

The i*n vitro s*tarch digestibility of raw, debittered, and roasted debittered flours were observed to be 31.20±0.71%, 22.41±0.40%, and 24.23±0.65%, respectively (Fig. 1). A significant decrease ($p<0.05$) in starch digestibility occurred after the debitterization process (28.17%). The starch digestibility of roasted debittered flour increased significantly to that of debittered flour, but it was 22.34% lower than raw flour. Compared to control (white wheat bread; 88.99%), the digestibility of starch in this experiment was much lower in all samples. To date, no reports are accessible on the starch digestibility of any *Moringa* species seed/ press cake. The high contents of fiber besides the relatively high concentration of polyphenols may affect starch digestibility (Aalim et al., 2021). As a result of such a limited starch digestibility, debittered and roasted debittered flours may promote slow and moderate postprandial glucose and insulin responses and could have advantageous effects in the management of Type 2 diabetes.

3.7. In vitro *protein digestibility*

Fig. 2 shows that the percentage of *in vitro* protein digestibility of defatted raw flour was the lowest value (56.07%) among all samples. It has been reported that *M. peregrina* defatted seed flour had a 69.72% *in vitro* protein digestibility (Al-Kahtani, 1995), which is higher than that we found in the present study. We could not find any other relevant data in the literature regarding the *in vitro* protein digestibility of *M. peregrina* seed/press cake. As a result of debitterization and roasting, a significant increase (14.67% and 32.10%) was observed with a value of 64.30 and 74.07%, which could be attributed to leaching out of antinutritional factors into soaking and boiling media, denaturation, and unfolding of proteins, and higher accessibility of the peptide chain for hydrolytic enzymes during heating (Joye, 2019).

3.8. Inhibition of the *α-amylase activity*

The porcine pancreatic α-amylase inhibitory activity of different extracts is illustrated in Fig. 3. These results represent that α-amylase inhibitory activity enhances in a dose-dependent way as the extract concentration raises. The percentage inhibition of different extracts of raw, debittered, and roasted debittered flours differed from 32.75 to
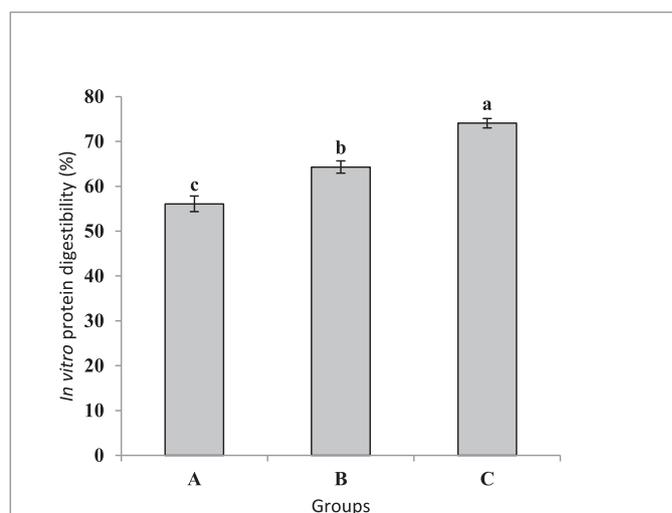
65.32, from 43.73 to 69.90, and from 48.45 to 74.60%, respectively. The highest concentration of 5000 μg/mL displayed the maximum enzyme inhibitory activity in all extracts. In comparison with acarbose (IC_{50} ~29 μg/mL), IC_{50} of different extracts was higher (~861.11, 472.75, and 290.0 μg /mL, respectively), but the data showed the potential of all extracts as the inhibitor of α-amylase.

Previous studies have represented the inhibitory effects of different extracts of *Moringa* species such as *M. oleifera* aqueous leaf and methanol seed extract (IC_{50}= 16,290 and 11,867 μg /mL) (Magaji et al., 2020) and *M. peregrina* 80% methanol leaf extract (IC_{50}= 13,350 μg/mL) on porcine α-amylase (Ullah et al., 2015). These indicate lower inhibition effect of α-amylase than that observed in the present study. The present study is the first report on the inhibition of the amylase enzyme of *M. peregrina* seed kernel. Different IC_{50} values of α-amylase inhibition activity after debitterization and roasting could be explained by the increase of total phenolic content, fiber content, monosaccharide contents, and lower starch digestibility of those samples. The phenolics have been reported as moderate inhibitors of α-amylase activity (Chinedum et al., 2018).

3.9. Inhibition of the *α-glucosidase activity*

Fig. 4 illustrates the α -glucosidase inhibitory activity of different extracts obtained in this experiment. All extracts had a significant difference inhibitory on the α -glucosidase enzyme. The highest (50.63%) and lowest (33.15%) inhibition were related to raw to roasted debittered flour extracts. As expected, the α-glucosidase inhibitory activity of acarbose (IC_{50} = 110 μg/mL) was markedly higher than different *M. peregrina* extracts (30 mg/mL). These results revealed that defatted *Moringa* seed before and after treatment had a low α-glucosidase inhibitory activity. To date, there are no reports on the Inhibition of the α-glucosidase activity of *M. peregrina* seed kernel. However, the findings of the present study agree with those reported by Magaji et al. (2020), who could not detect any α-glucosidase inhibitory activity for aqueous, methanol, or ethyl acetate extract of *M. oleifera* seed.

3.10. *Phytic acid content*

Even though phytic acid has both positive and negative effects on health, it is well known that a high amount of phytic acid interferes
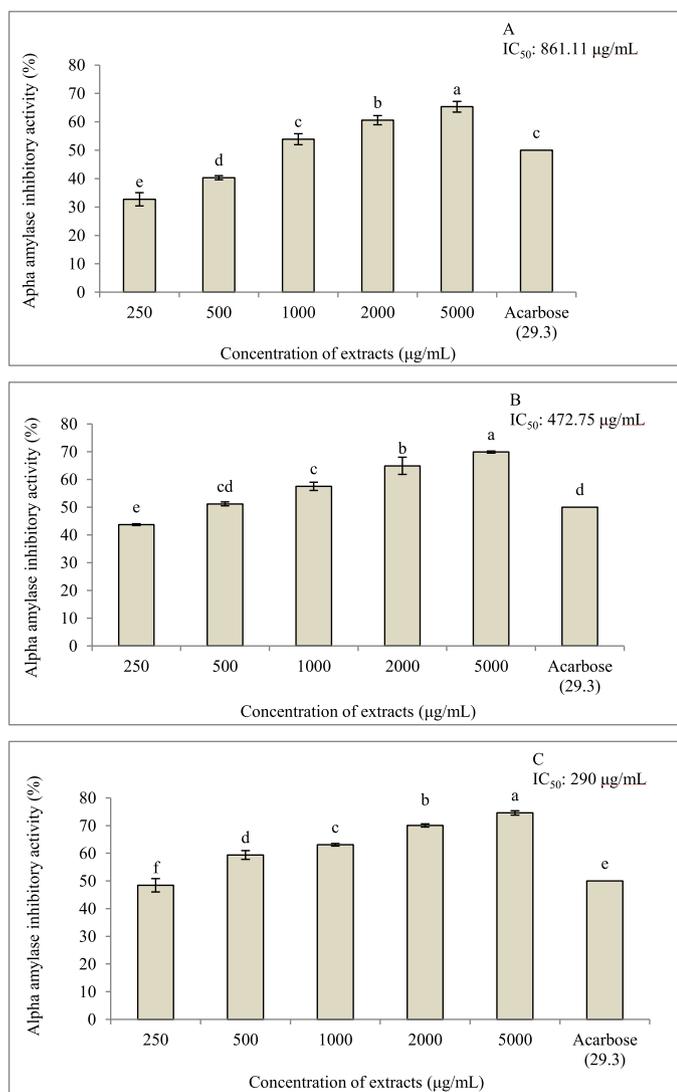
Fig. 3. Percentage of porcine pancreas α-amylase inhibitory activity of defatted *Moringa peregrina* press cake and its different treatments extracts (ppm). A: defatted *M. peregrina* press cake, B: debittered *M. peregrina* press cake, C: roasted debittered *M. peregrina* press cake. The columns with different letters are significantly different at $p<0.05$. Acarbose was used as the positive control (IC50: 29.3 ppm). Values and error bars correspond to the mean ± standard deviation of triplicate samples.

Fig. 4. Percentage of porcine intestine α-glucosidase inhibitory activity of defatted *Moringa peregrina* press cake and its different treatments extracts (test concentration of 30 mg/mL). A: defatted *M. peregrina* press cake, B: debittered *M. peregrina* press cake, C: roasted debittered *M. peregrina* press cake. The columns with different letters are significantly different at $p<0.05$. Acarbose was used as the positive control (IC50: 110 ppm). Values and error bars correspond to the mean ± standard deviation of triplicate samples.

with the digestibility and bioavailability of starch, proteins, and some minerals such as Ca, Fe, and Zn. However, a lower amount of phytic acid is found to have beneficial effects on health. For example, it can act as an antioxidant, anticancer, and antidiabetic compound, while it can also prevent renal stone formation and decrease the risk of cardiovascular diseases (Tanwar et al., 2018). In the current study, the content of phytic acid was 4211.01±1.05 mg/100 g in the raw defatted flour, which decreased to 1530±2.04 mg/100 g after debitterization, denoting a significant ($p<0.05$) decrease of 63.55% (Table 1). This reduction could be attributed to the fact that phytic acid commonly stays int he complexation with salts and minerals, which could have leached out into the soaking and boiling medium.

Hydrolyzation of phytates by activated endogenous phytase, which could lead to leaching out of phytate ions into the soaking medium, might be another reason for this strong effect of boiling (Liang, Han, Nout & Hamer, 2008). Al-Kahtani (1995) reported 2600 mg/100 g phytic acid in *M. peregrina* seed meal, which was lower than the present study and is the only previous report for this seed. No sta-
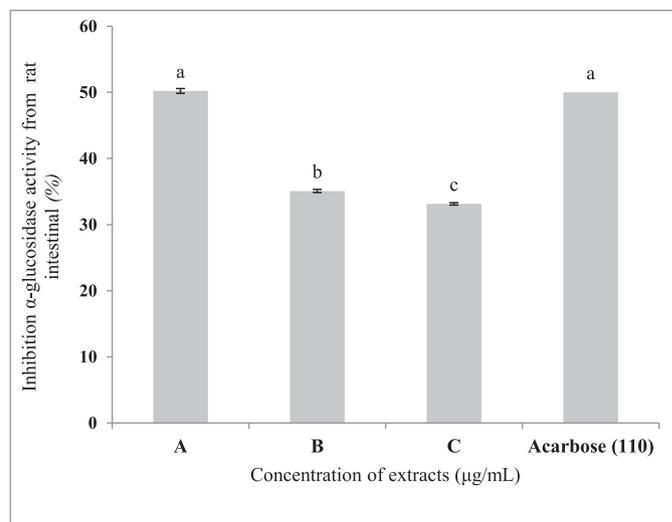
tistically significant differences ($p>0.05$) in phytic acid concentration were observed between debittered and roasted debittered flours in the present study. The results of the present study are in line with those reported by Ijarotimi et al. (2013), who discovered a significant reduction (48.93%) in phytic acid (from 78.33 to 40.00 mg/100 g) due to the pregermination treatment of *M. oleifera* seed kernel using distilled water. Similar decreases have been found after soaking wild apricot kernels (71.83%) (Tanwar et al., 2018). Generally, oilseeds such as soybeans, sesame seeds, and sunflower, and nuts such as walnuts, almond, and cashew nuts have been reported to comprise a very high content of phytic acid (in the range of 1000–5400 and 100–9400 mg/100 g, respectively) (Gupta, Gangoliya & Singh, 2015), which is similar to the values found in raw and treated defatted flours in the present study.

3.11. Oxalate content

As indicated in Table 1, oxalates content in defatted raw flour was 880.03±0.43, which declined to 220.57±0.35 mg/100 g post-debitterization. This counts for about 74.93% reduction, which could be due to the leaching out of soluble oxalate salts into the soaking and boiling medium. Nevertheless, interestingly, roasting did not show any significant effect ($p>0.05$) on oxalates content in the roasted debittered flour compared to debittered flour ($p>0.05$). To date, there are no reports on the oxalate content of *M. peregrina* seed kernel. However, Olagbemide and Philip (2014) reported a much lower oxalates content (38 mg/100 g) in the case of *M. oleifera* defatted seed. The same decrease has been presented by Tanwar et al. (2018), who reported a 38.0% (from 19.58 to 12.14 mg/100 g) reduction in oxalates content after detoxification of wild apricot kernel using 25% sodium chloride (NaCl) solution as a soaking medium for 12 h.

3.12. Tannin content

Raw defatted flour in the current study contained 0.17±0.04 (mg catechin/g) tannins (Table 1). It has been reported that *M. peregrina* defatted seed flour contained a very low concentration of tannins (0.15±0.01 mg catechin/g), with no nutritional concerns (Al-Kahtani, 1995), which agrees with the findings of the present study.

We observed a significant (*p*<0.05) decrease in the tannin content after heat treatment, resulting in non-detectable tannin in debittered and roasted debittered flours. This could be probably due to leaching out into the soaking and boiling medium and/or chemical changes in the structure (Avola et al., 2012). These results agree with those reported by Ijarotimi et al. (2013) who observed a significant decrease in tannin content of *M. oleifera* seed after 9 h of soaking in water. Similar reductions have been found in cooked chickpeas (Avola et al., 2012) and detoxified Karanja seed meal (Vinay & Kanya, 2008).

4. Conclusions

This study showed that defatted *M. peregrina* press cake, a by-product of oil extraction, is a rich source of protein and other functional ingredients. However, its applications in the food industry are confined, owing to bitter taste and antinutrients. Our findings, for the first time, revealed that soaking and boiling, while improving the nutritional properties of *M. peregrina* press cake, markedly decreased its bitter taste, and antinutritional factors. This demonstrates that the combination of these two traditional processes (*i.e.*, soaking and boiling) is a simple and safe approach for the debitterization of *M. peregrina* press cake. Compared to its raw product, the debittered press cake showed a significant enhancement in protein and fiber contents. Meanwhile, the *in vitro* starch digestibility and the contents of carbohydrates, ash, total starch, and resistant starch were decreased. The roasted sample was the best in terms of fiber content, antioxidant properties, and *in vitro* protein digestibility. Overall, the phenolic extracts of all treatments proved activity against the α-amylase enzyme but roasted debittered sample provided the most promising anti-diabetic effect. Moreover, the bio- and physicochemical properties determined in the current study will help the development of novel products such as low glycemic food, alternative proteins, dietary supplements, and novel food ingredients for the application in the available products such as bread, cookies, and snacks. Nevertheless, a vast area for work yet to be carried out in isolating and quantifying the bioactive compounds, as well as the effect of different heat treatments/roasting on the quality, functional properties, and sensory attributes.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2022.100034.

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