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Effects of thermal preparation and *in vitro* digestion on lignan profiles and antioxidant activity in defatted-sesame meal

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ABSTRACT

Defatted-sesame meal (DSM), a byproduct of sesame oil, has attracted considerable interest in the food industry because of its strong antioxidant activity. The aim of this study was to measure the content and distribution of lignans in DSM and evaluate their antioxidant activity after thermal processing and *in vitro* digestion. The results showed that the sesame lignans (SL) content and antioxidant activity were significantly influenced by the temperature and time during thermal preparation, and the maximum antioxidant potency composite index (ACI) was obtained after roasting the samples at 240 °C for 20 min. As sesame seed was processed with longer time and higher temperature, more pinoresinol diglucoside (PD) and sesamol were measured in DSM. According to the correlation matrix under thermal preparation, a significant contribution to the antioxidant potency of DSM was discovered. After *in vitro* digestion, the release amount of lignans increased by 19.6%, and the values of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ACI gradually declined after digestion, with a 40% decrease in both the DPPH radical scavenging activity and the ACI from oral to intestinal phase. These results could be used to help improve the bioavailability of SL and obtaining high quality sesame byproducts.

1. Introduction

Sesame (*Sesamum indicum* L.) is an important oilseed crop, which has been cultivated for over 5000 years. Sesame seed is one of the richest sources of antioxidants, including lignans that are phenyl propane dimers (Anilakumar et al., 2010; Dar and Arumugam, 2013). Sesame lignans (SL) contain sesamin, sesamolin, sesamol, pinoresinol, and other lignan glycosides. Among them, sesamin and sesamolin are two major aglycon lignans in raw sesame seed, which can release sesamol and pinoresinol during the processing of sesame seed (Shi et al., 2017). Clinically, SL exhibit various beneficial effects such as antioxidant activity, anti-hypertensive and anti-cancer activities (Dar et al., 2015; Peñalvo et al., 2006; Visavadiya and Narasimhacharya, 2008). Toorani et al. (2019) reported that sesamol in triacylglycerols from the sesame oil exhibited the highest antioxidant activity. Another study showed that pinoresinol from olive oil displayed a high stability to thermal treatment at 180 °C (Gerstenmeyer et al., 2013).

Sesame seed is a good source of edible oil and is widely used in bakery and confectionery products. Defatted-sesame meal (DSM) can be obtained from the extraction of sesame oils. DSM is mainly used as a feed ingredient for domestic animals or making compost. Generally, the production of sesame oil is typically achieved via several processing steps: removing of impurities, cleaning, roasting, grinding, and oil extraction. Among these steps, roasting is the vital procedure that affects the color, flavor, composition, quality, and digestibility of sesame oil and sesame meal. During the thermal preparation of sesame seed, the water content of sesame seed is gradually reduced, and the colored, fragrant, and tasteful seed is obtained, at the same time, the structure, type, and content of bioactive compounds such as lignans in sesame seed are changed (Wang et al., 2016). Several studies have been reported involving the effect of thermal preparation on sesame seed and its byproducts (Jannat et al., 2013; Makinde and Akinoso, 2013; Tenyang et al., 2017). For example, Kumar et al. (2010) found that infrared roasting of sesame seed at 200 °C for 30 min degraded sesamolin to sesamol. In addition, some antioxidants can be obtained from the byproduct DSM after oil extraction. Kang et al. (1999) found that

Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline) 6-sulphonic acid; ACI, antioxidant potency composite index; ANOVA, analysis of variance; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; DSM, defatted-sesame meal; DW, dry weight; FRAP, ferric reducing antioxidant power; HPLC, high performance liquid chromatography; PD, pinoresinol diglucoside; SD, standard deviation; SL, sesame lignans; TE, trolox equivalent; TL, total lignan; TPTZ, 2,4,6-tripyridyl-s-triazine

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feeding rabbits with DSM showed hypercholesterolemic effects. Therefore, it is important to evaluate how thermal preparation of sesame seed influences the lignan profiles and antioxidant activity of DSM.

On the other hand, it is also of great importance to investigate the behavior of lignans after digestion of DSM. The biological action of these bioactive substances is determined by the bio-accessibility and bioavailability after digestion (Ge et al., 2018; Lucas-González et al., 2018). *In vitro* digestion simulates physiological conditions of the human body and provides information about the changes of target substances and antioxidant potential (Minekus et al., 2014). Recent studies have reported the functional properties of phenolics, flavonoids, and antioxidant activity after gastrointestinal digestion of fruit, vegetable, and seed (Chen et al., 2016; Neto et al., 2017). However, little information is available regarding the antioxidant activity of DSM and its *in vitro* digestion model.

Thus, the objectives of this study were to investigate the effects of thermal preparation and digestion on the SL of DSM and its antioxidant capacity. The results before and after preparation and digestion were compared to generate the scientific information that can be used to improve the bioavailability of active substances and obtain high quality sesame byproducts.

2. Materials and methods

2.1. Chemicals and reagents

Sesame seed (*Sesamum indicum* L.) of cultivars '*Jinhuangma*' was purchased from Yonghui Superstores, Fuzhou, Fujian province, China. Trolox, pinoresinol diglucoside (PD), sesamol, pinoresinol, sesamin and sesamolin standards were obtained from Shanghai Yuanye Biotechnology Co. Ltd. (Shanghai, China). Pepsin, pancreatin, lipase, α-amylase and bile salts were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzthiazoline) 6-sulphonic acid (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), potassium persulfate ($K_2S_2O_8$), calcium chloride ($CaCl_2$), sodium bicarbonate ($NaHCO_3$), hydrochloric acid (HCl) and ferric chloride ($FeCl_3$) were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ, USA). Methanol (HPLC grade) and all other solvents were of analytical grade.

2.2. Thermal processing and DSM preparation

Three hundred grams of sesame seed were washed for 3 min and processed by the following thermal processing steps including frying, roasting, and microwaving. Frying was conducted using a pan and an induction cooker, the sesame seed was fried at different temperatures (160, 200, and 240 °C) for different time (10, 20, and 30 min) after the set temperature was reached. Roasting was conducted in an oven operating at different temperatures (160, 200, and 240 °C) for different length of time (10, 20, and 30 min). Microwaving was completed in a microwave oven operating at different power (80, 400, and 800 W) for different length of time (10, 20, and 30 min).

After thermal preparations, the sesame seed was ground and defatted. The defatting procedures were as follows: anhydrous n-hexane was added (1:10, *w/v*) to 10 g of sesame powders, the mixture was sonicated for 3 min, and then centrifuged at 6 000 × *g* for 5 min at 4 °C. The supernatant was discarded and the process was repeated twice. The residue (DSM) was collected and dried at room temperature, and stored at −20 °C for further analysis.

2.3. Gastrointestinal in vitro digestion

First, the *in vitro* digestion was conducted following the methodology described by Ti et al. (2015) and Maisanaba et al. (2018) with slight modifications. Three phases were simulated: oral, gastric, and intestinal digestion. For oral digestion, DSM (2 g) was blended with water (20 mL) and 500 μL of α-amylase/ $CaCl_2$ solution (32.5 mg of α-amylase dissolved in 25 mL of 1 mM $CaCl_2$, pH = 7.0), and kept at 37 °C for 10 min in a shaking water bath (Pegasus scientific, Rockville, MD, USA). Next, the sample was acidified to pH 2.0 using HCl (6 M) for gastric digestion, then mixed with pepsin (0.1 g) and incubated at 37 °C in a shaking water bath for 1 h. Finally, the pH of sample was adjusted to pH 6.0 using 0.9 M $NaHCO_3$ before mixing with pancreatin-bile salt mixture (5 mL). The mixture was composed of pancreatin (0.1 g), bile salt (0.625 g), lipase (0.25 g) and $NaHCO_3$ solution (0.1 M, 25 mL). Then, the sample was adjusted to pH 7.4 with $NaHCO_3$ (0.9 M) and digested for 2 h in a 37 °C shaking water bath. After each digestion phase, an aliquot of each sample was centrifuged at 8 000 × *g* for 5 min at 45 °C. The supernatant was changed to pH 7.0 and evaporated to dryness at 45 °C. The residue was dissolved in 60% methanol, adjusted to 10 mL and frozen at −40 °C.

All these samples were prepared for the measurement of SL and antioxidant activity. The sesame seed obtained from the optimized thermal preparation were subjected to the three phases of *in vitro* simulated digestion, and the samples were replaced with equal-volume ultrapure water as a blank. The blank was subtracted from the samples.

2.4. Extraction of SL

SL was extracted using methods reported before with some modifications according to the laboratory condition (Lin et al., 2017; Shi et al., 2018). Pre-cooled 80% acetone was added (1:10, *w/v*) to the DSM, ultrasonicated for 5 min, and then centrifuged at 6 000 × *g* for 5 min at 4 °C. The extraction was repeated twice. All the supernatants were collected and evaporated to dryness under vacuum at 45 °C. The residue was dissolved in 60% methanol, diluted to 10 mL. The solution was then dispensed into centrifuge tubes and stored at −20 °C until use.

2.5. Determination of SL

The profile of SL was determined according to Chen et al. (2018). The high performance liquid chromatography (HPLC) system was consisted of a Shimadzu 2030C 3D series Rapid Resolution LC System (Shimadzu, Kyoto, Japan) equipped with an automatic injector and photo-diode array detector. The chromatographic analysis was conducted by Waters XBridge C18 reversed phase column (600 Bar, 250 mm × 4.6 mm, 5 μm). The wavelength of 277 nm was chosen for detection of PD, 295 nm for sesamol, 280 nm for pinoresinol and 287 nm for sesamin and sesamolin. Sample solution was filtered using 0.22 μm syringe filters for analysis.

2.6. Assays of antioxidant activity

The antioxidant activity of the raw, processed, and digested DSM was assessed with four methods.

2.6.1. Assay of DPPH radical scavenging activity

The measurement was performed by the methodology described by Othman et al. (2015). Briefly, 240 μL of methanol, 30 μL of DPPH ethanol solution (1 mM) and 30 μL of extraction solvent, Trolox standard solution or sample extract were mixed. The absorbance was measured at 540 nm using a microplate reader after 30 min of reaction at room temperature. Results were expressed as μmol Trolox equivalent (TE)/100 g sample (dry weight, DW).

2.6.2. Assay of ABTS radical scavenging activity

Referring to the previous method of Almeida et al. (2011) with some modifications, $ABTS^+$ was done by 5 mL of 7 mmol/l ABTS solution and 88 μL of 140 mmol/L $K_2S_2O_8$ aqueous solution in the dark for 12 h. The solution was prepared 1 day in advance and diluted with ethanol to absorbance at 0.70 ± 0.02 at 734 nm before use. $ABTS^+$ solution

(2 mL) was added to sample (25 μL) for 6 min to read the absorbance. Results were expressed as μmol TE/100 g DW.

2.6.3. Assay of ferric reducing antioxidant power (FRAP)

FRAP was determined using the method described by Rocchetti et al. (2017). Briefly, fresh TPTZ working solution was prepared and added to 9 μL of sample and the reaction was kept at 37 °C for 243 s. The absorbance of samples was measured at 793 nm. Results were expressed as μmol TE/100 g DW.

2.6.4. Antioxidant potency composite index (ACI)

ACI was used to comprehensively evaluate the antioxidant activity. It was determined by all assays (DPPH, FRAP, ABTS) and calculated as follows: ACI = (the measured value of the assay/ the maximum value of the assay) * 100. ACI mean = (ACI value of assay 1 + ACI value of assay 2 + … ACI value of assay n)/ n.

2.7. Statistical analysis

Results on SL, DPPH, ABTS, and FRAP after thermal preparation and digestion were expressed as mean value ± standard deviation (SD). All analyses were run in triplicate and data were analyzed using SPSS Statistics software (version 18.0) for analysis of variance (ANOVA, Tukey's multiple range tests, $p < 0.05$). Pearson's correlation coefficients were also calculated using SPSS Statistics software.

3. Results

3.1. Variations of the SL content in the DSM preparation by frying, roasting, and microwaving

The effect of thermal preparation on five kinds of SL (PD, sesamol, pinoresinol, sesamin, sesamolin) is shown in Table 1.

For frying, the lignan contents varied and differed significantly ($p < 0.05$) among samples treated under different conditions. With the extension of frying time at the same temperature, the content of PD, sesamol and total lignan (TL) increased by 122.9%, 162.7%, and 119.1% at 160 °C, increased by 146.4%, 88.5%, and 197.34% at 200 °C, respectively. Frying at 240 °C, PD, sesamol and TL increased first and then decreased (losses of 50.8%, 67.2%, 51.8%, respectively). In addition, the variations of PD, sesamol and TL content at different frying temperatures were similar to those at different frying times (Table 1). The content of sesamin was reduced by 48.2% at 160 °C and was not detected at 200 °C and 240 °C. Sesamolin appeared only in the samples with frying treatment at 160 °C for 10 min, and the content was 0.94 ± 0.01 mg/100 g DW. Pinoresinol was not detected for all the frying conditions.

For roasting, there were significant differences ($p < 0.05$) in the changes of different SL in samples. In addition, the changes of PD, sesamol and TL showed an increasing trend with the deepening of roasting (Table 1). On one hand, with the extension of roasting time, the increases of PD, sesamol and TL content were 35.7%～107.4%, 24.5%～688.5%, 34.3%～97.3%, respectively. On the other hand, with the change of roasting temperature, the increases of PD, sesamol and TL content were 198.4%～293.3%, 182.9%～934.6%, and 112.1%～193.7%, respectively. The results showed that the highest content of PD and TL occurred at 240 °C for 30 min for the roasting treatment. The highest content of sesamol was measured at 240 °C for 20 min and 240 °C for 30 min, and there was no significant difference between these two treatments. Pinoresinol was not detected after roasting at 160 °C for 30 min. The reductions of sesamin and sesamolin were 30.1%～100% and 35.8%～100% at different roasting temperatures (Table 1).

For the microwave treatment, the SL varied and differed significantly ($p < 0.05$). The PD, sesamol and TL content showed an increasing trend with the increase of microwave temperature and time. At different microwave times, the increases of PD, sesamol and TL content were 60.7%～887.5%, 212.8%～1180.6%, 37.4%～801.3%,

Table 1
Effect of different thermal preparations on content (mg/100 g DW) of five kinds of sesame lignans.

Thermal preparation conditions			PD	Sesamol	Pinoresinol	Sesamin	Sesamolin	TL
Frying	160 °C	10 min	80.44 ± 2.15^{h}	2.12 ± 0.08^{l}	nd^{d}	1.12 ± 0.22^{efg}	0.94 ± 0.01^{ef}	84.63 ± 2.30^{i}
		20 min	146.68 ± 3.97^{fg}	4.93 ± 0.14^{g}	nd^{d}	0.61 ± 0.02^{hij}	nd^{g}	152.22 ± 4.10^{fgh}
		30 min	179.31 ± 4.73^{def}	5.57 ± 0.54^{fg}	nd^{d}	0.58 ± 0.00^{ij}	nd^{g}	185.46 ± 4.20^{def}
	200 °C	10 min	131.15 ± 26.25^{g}	6.01 ± 0.34^{ef}	nd^{d}	nd^{k}	nd^{g}	137.15 ± 26.30^{h}
		20 min	165.25 ± 4.26^{efg}	7.02 ± 0.16^{d}	nd^{d}	nd^{k}	nd^{g}	172.27 ± 4.41^{efgh}
		30 min	323.16 ± 32.60^{a}	11.33 ± 0.27^{a}	nd^{d}	nd^{k}	nd^{g}	334.49 ± 32.37^{a}
	240 °C	10 min	159.44 ± 3.92^{efg}	10.40 ± 0.23^{b}	nd^{d}	nd^{k}	nd^{g}	169.84 ± 3.92^{efgh}
		20 min	206.91 ± 7.15^{cd}	11.91 ± 0.06^{a}	nd^{d}	nd^{k}	nd^{g}	218.82 ± 7.12^{cd}
		30 min	78.41 ± 1.28^{h}	3.41 ± 0.21^{hij}	nd^{d}	nd^{k}	nd^{g}	81.83 ± 1.17^{i}
Roasting	160 °C	10 min	38.38 ± 1.35^{ij}	0.26 ± 0.01^{op}	0.59 ± 0.04^{c}	1.67 ± 0.05^{cd}	1.48 ± 0.04^{c}	42.38 ± 1.29^{jk}
		20 min	65.06 ± 1.47^{hi}	0.94 ± 0.02^{nop}	0.28 ± 0.11^{cd}	1.15 ± 0.26^{ef}	1.39 ± 0.06^{cd}	68.81 ± 1.13^{ij}
		30 min	79.59 ± 3.47^{h}	2.05 ± 0.14^{lm}	nd^{d}	1.01 ± 0.12^{efghi}	0.95 ± 0.01^{ef}	83.60 ± 3.31^{i}
	200 °C	10 min	144.06 ± 5.40^{fg}	2.49 ± 0.18^{kl}	nd^{d}	0.93 ± 0.08^{efghij}	0.88 ± 0.00^{f}	148.36 ± 5.54^{fgh}
		20 min	180.97 ± 10.83^{def}	3.02 ± 0.17^{jk}	nd^{d}	0.74 ± 0.02^{fghij}	nd^{g}	184.72 ± 10.91^{defg}
		30 min	195.54 ± 20.57^{de}	3.10 ± 0.27^{ijk}	nd^{d}	0.65 ± 0.00^{ghij}	nd^{g}	199.29 ± 20.75^{de}
	240 °C	10 min	150.94 ± 3.63^{fg}	2.69 ± 0.19^{jkl}	nd^{d}	0.85 ± 0.04^{efghij}	nd^{g}	154.47 ± 3.82^{fgh}
		20 min	194.14 ± 5.47^{de}	6.47 ± 0.96^{de}	nd^{d}	0.53 ± 0.17^{j}	nd^{g}	201.13 ± 4.89^{de}
		30 min	239.74 ± 7.82^{bc}	5.80 ± 0.28^{ef}	nd^{d}	nd^{k}	nd^{g}	245.53 ± 7.74^{bc}
Microwaving	80 W	10 min	20.31 ± 3.31^{j}	0.20 ± 0.00^{p}	3.71 ± 0.20^{a}	2.70 ± 0.41^{a}	2.39 ± 0.04^{a}	29.30 ± 2.86^{k}
		20 min	22.44 ± 1.24^{j}	0.20 ± 0.00^{p}	3.10 ± 0.17^{b}	2.16 ± 0.13^{b}	2.28 ± 0.54^{ab}	30.19 ± 1.90^{k}
		30 min	32.64 ± 1.56^{ij}	1.05 ± 0.03^{no}	2.88 ± 0.55^{b}	1.64 ± 0.19^{cd}	2.04 ± 0.24^{ab}	40.25 ± 2.45^{jk}
	400 W	10 min	28.22 ± 7.29^{ij}	0.31 ± 0.01^{op}	nd^{d}	1.84 ± 0.39^{bc}	1.97 ± 0.13^{b}	32.34 ± 7.02^{jk}
		20 min	57.01 ± 2.15^{hij}	2.28 ± 0.01^{kl}	nd^{d}	1.07 ± 0.12^{efgh}	1.07 ± 0.02^{def}	61.43 ± 2.14^{ijk}
		30 min	255.96 ± 28.36^{b}	3.97 ± 0.13^{h}	nd^{d}	nd^{k}	nd^{g}	259.93 ± 29.33^{b}
	800 W	10 min	36.00 ± 3.14^{ij}	1.25 ± 0.03^{mn}	nd^{d}	1.31 ± 0.23^{de}	1.31 ± 0.02^{cde}	39.88 ± 3.24^{jk}
		20 min	139.72 ± 9.68^{g}	8.27 ± 0.18^{c}	nd^{d}	nd^{k}	nd^{g}	147.99 ± 9.68^{gh}
		30 min	355.51 ± 8.92^{a}	3.91 ± 0.12^{hi}	nd^{d}	nd^{k}	nd^{g}	359.42 ± 9.02^{a}

Values (means ± SD, n = 3) with different letters within a column are significantly different at $p < 0.05$.
PD: Pinoresinol diglucoside; nd: Not detected; TL: Total lignan (the sum of five kinds of lignans).

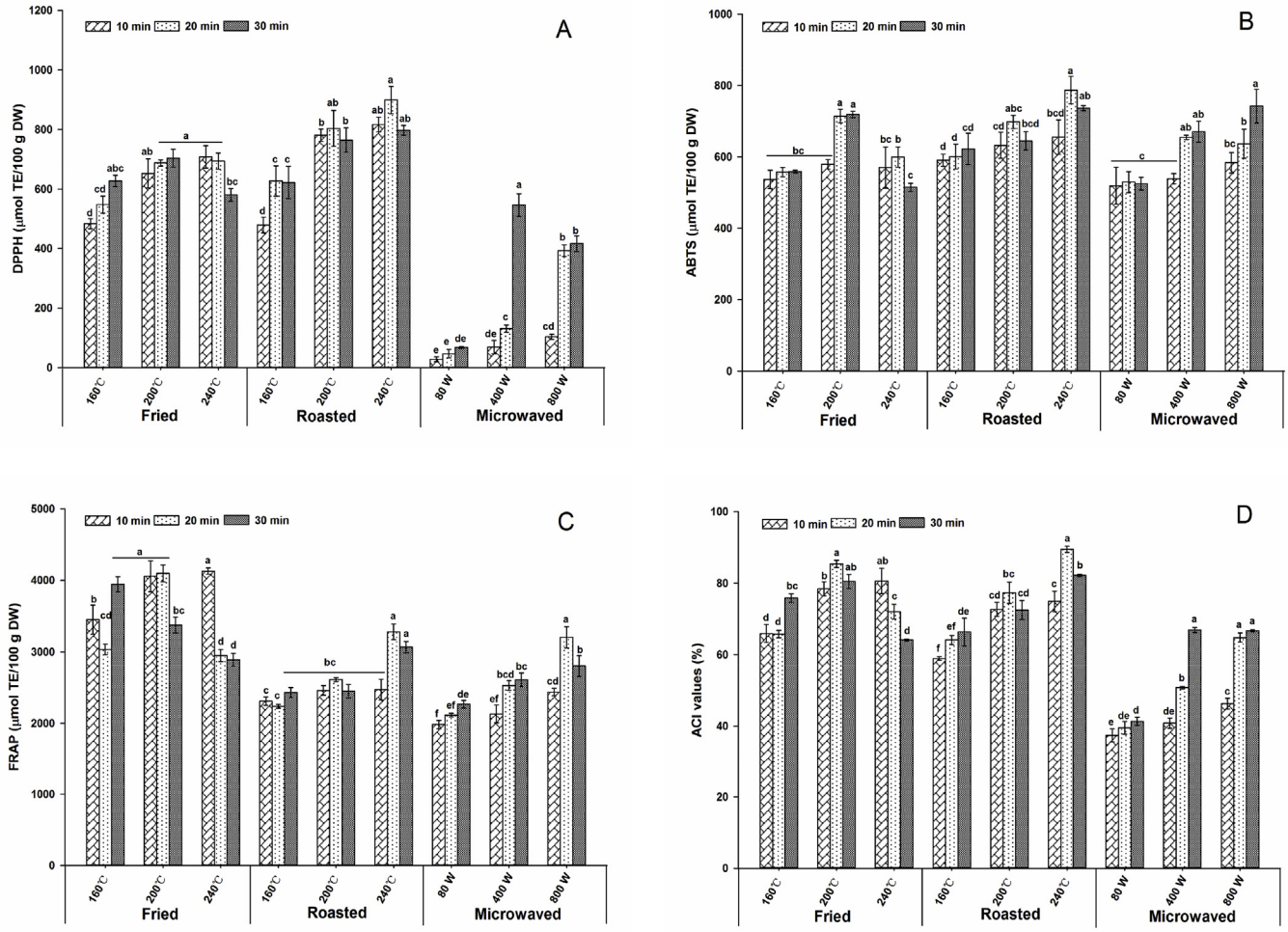

Fig. 1. Effect of different thermal preparations on DPPH (A), ABTS (B), FRAP (C) and ACI (D) of DSM. Mean values with different letters denote significant differences between the same thermal preparation ($p < 0.05$).
ACI: Antioxidant potency composite index.

respectively (Table 1). At different microwave temperatures, the increases of PD, sesamol and TL content were 77.3%～989.2%, 272.4%～4035.0%, and 36.1%～390.2%, respectively. The contents of PD and TL were highest at microwave 800 W for 30 min. The highest content of sesamol was obtained by microwave treatment at 800 W for 20 min. The minimum decreases of pinoresinol, sesamin and sesamolin were 22.4%, 39.3% and 14.6%, respectively (Table 1).

The comparison of three processing methods indicated that the highest content (mg/100 g DW) of PD, sesamol, pinoresinol, sesamin and sesamolin were 355.51 ± 8.92 (microwave 800 W, 30 min), 11.91 ± 0.06 (frying 240 °C, 20 min), 3.71 ± 0.02 (microwave 80 W, 10 min), 2.70 ± 0.41 (microwave 80 W, 10 min), and 2.39 ± 0.04 (microwave 80 W, 10 min), respectively (Table 1).

3.2. Variations of antioxidant activity in the DSM preparation by frying, roasting and microwaving

The variations of antioxidant activity in DSM prepared by frying, roasting, or microwaving are presented in Fig. 1. Different thermal preparations had significant effects on DPPH radical scavenging activity (Fig. 1A), and the values of DPPH radical scavenging activity in frying and roasting samples showed significantly higher than that of microwave treatment ($p < 0.05$). In more detail, it was noted that the levels of DPPH radical scavenging activity increased with the increase of processing time, but decreased to 580.58 ± 21.15 μmol TE/100 g DW at 240 °C for 30 min. For different processing temperatures, similar results were observed. There were significant differences in DPPH radical scavenging activity for roasting at 240 °C for 10 min, 20 min, 30 min, and at 200 °C for 20 min. For the microwave treatments at 400 W for 30 min and 800 W for 20 min, the levels of DPPH radical scavenging activity increased by 691.2% and 278.0%, respectively (Fig. 1A), which were significantly different from the other treatments ($p < 0.01$).

For the assay of ABTS radical scavenging activity (Fig. 1B), the effect of different processing methods on the levels of ABTS radical scavenging activity was not significant ($p > 0.05$), and the values of ABTS radical scavenging activity in frying and roasting samples were slightly higher than that of the microwave treatment. In general, the levels of ABTS radical scavenging activity gradually increased with the increases of temperature and time, but decreased significantly by 14.1%, 7.7% and 6.4% after frying at 240 °C for 30 min and roasting at 200 °C for 30 min, 240 °C for 30 min, respectively. In addition, the results showed that the value of ABTS radical scavenging activity at 240 °C for 20 min was 787.40 ± 38.37 μmol TE/100 g DW, which was significantly higher than other values ($p < 0.05$) (Fig. 1B).

The FRAP analysis reveals that the effect of frying on FRAP was significantly greater than that of roasting and microwaving ($p < 0.05$), and the highest FRAP value was 4129.00 ± 44.19 μmol TE/100 g DW

Table 2
Pearson correlation coefficients between the sesame lignan and antioxidant activity under different thermal preparations.

	PD	Sesamol	Pinoresinol	Sesamin	Sesamolin	TL	DPPH	ABTS	FRAP	ACI
PD	1									
Sesamol	0.653**	1								
Pinoresinol	−0.477*	−0.422*	1							
Sesamin	−0.763**	−0.781**	0.714**	1						
Sesamolin	−0.778**	−0.719**	0.734**	0.928**	1					
TL	1.000**	0.669**	−0.466*	−0.762**	−0.775**	1				
DPPH	0.609**	0.526**	−0.627**	−0.675**	−0.789**	0.605**	1			
ABTS	0.726**	0.374	−0.454*	−0.523**	−0.558**	0.721**	0.553**	1		
FRAP	0.430*	0.727**	−0.451*	−0.697**	−0.666**	0.438*	0.483*	0.202	1	
ACI	0.703**	0.676**	−0.651**	−0.788**	−0.864**	0.702**	0.943**	0.644**	0.703**	1

*$p < 0.05$, **$p < 0.01$.
PD: Pinoresinol diglucoside; TL: Total lignan; ACI: Antioxidant potency composite index.

(frying at 240 °C for 10 min) (Fig. 1C). As the frying time increased, FRAP increased by 14.4% at 160 °C, and decreased by 16.9% and 30.0% at 200 °C and 240 °C, respectively. As the roasting time increased, the value of FRAP increased by 5.3% and 24.1% at 160 °C and 240 °C, respectively, and decreased by 0.4% at 200 °C. Similarly, with the extension of microwave time, the value of FRAP increased by 14.4%, 22.4%, and 15.0% at 160 °C, 200 °C and 240 °C, respectively (Fig. 1C).

As shown in Fig. 1D, the effect of frying and roasting on ACI was significantly greater than that of microwaving ($p < 0.05$). As the frying time increased, ACI increased by 15.1%, 2.6% at 160 °C and 200 °C, respectively, and decreased by 20.5% at 240 °C. With the extension of roasting and microwave time, ACI increased by 12.5%, 0.3%, 9.7% and 10.5%, 64%, 44.2% at 160 °C, 200 °C and 240 °C, respectively. The highest value of ACI (89.51 ± 0.87%) appeared at 240 °C for 20 min (Fig. 1D).

3.3. Correlation analysis

Correlations between the SL and antioxidant activity of DSM prepared by frying, roasting and microwaving were investigated by Pearson's correlation analysis. As shown in Table 2, PD, sesamol, and TL from DSM were significantly positively correlated with antioxidant activity (including DPPH, ABTS, FRAP and ACI) except that sesamol was not significantly associated with the ABTS. Conversely, sesamin, pinoresinol and sesamolin from DSM were significantly inversely correlated with the antioxidant activity (Table 2). It was also found that the correlation coefficients of PD with DPPH, with ABTS, and with ACI were higher than other lignans. And the correlation coefficient of sesamol with FRAP appeared to be higher than other lignans (Table 2).

Considering the correlation between the five kinds of SL, the results suggested that they were significantly correlated for each other (Table 2). In particular, there was a positive correlation between pinoresinol and sesamin (Correlation coefficient $r = 0.714$, $p < 0.01$), as well as between pinoresinol and sesamolin ($r = 0.734$, $p < 0.01$). Whereas, there was a negative correlation between PD and sesamin ($r = -0.763$, $p < 0.01$), as well as between PD and sesamolin ($r = -0.778$, $p < 0.01$) (Table 2).

3.4. Variations of SL content before and after thermal preparation and digestion

Table 3 showed the variations of the SL content during thermal preparation and *in vitro* digestion. The digestion had different repercussions in the stability of the SL. The major and most abundant lignan detected in raw, roasted, and digested sesame was PD, which significantly increased after roasting; whereas a 23.0% loss was observed after the oral digestion ($p < 0.05$). Compared to the undigested sample, 16.3% and 5.2% increases of PD were noticed after the gastric and intestinal phases, respectively, while the two of them didn't differ significantly. Similar results were observed in sesamol and TL after roasting and digestion. Interestingly, the content of sesamol was lost by 98.8% compared to the gastric phase. Pinoresinol appeared only after the digestion and increased by 218.8% after the gastric phase. Regarding sesamin and sesamolin, they were not detected after roasting, but a low content of sesamolin (0.82 ± 0.34 mg/100 g DW) was present after the intestinal phase (Table 3). Overall, the release amount of lignans in the gastric phase was greater than the intestinal phase, except for pinoresinol and sesamin (Table 3).

3.5. Variations in antioxidant activity before and after thermal preparation and digestion

As illustrated in Fig. 2, the antioxidant capacity of roasted DSM was measured by the assays of DPPH radical scavenging activity, ABTS radical scavenging activity, and FRAP, which showed the same increasing trend compared with raw DSM, but differed from each other after digestion. From the oral to intestinal phase, the values of DPPH radical scavenging activity and ACI gradually declined after digestion, with a 94.6% decrease in DPPH radical scavenging activity and a 29.6% decrease in ACI (Fig. 2A and D). ABTS radical scavenging activity showed a 37.4% decrease after the oral digestion, and then significant increased by 130.5% ($p < 0.05$) (Fig. 2B). The measurement of reducing capacity of samples by the FRAP assay yielded higher values for raw, roasted and digested DSM than the assays of DPPH and ABTS radical scavenging activity ($p < 0.05$) (Fig. 2A, B, Fig. 2C). The FRAP assay exhibited a reduced tendency in the oral and gastric phases until a 40.4% loss in gastric digestion, but a significant increase of 40.3% was observed after the intestinal digestion (Fig. 2C).

4. Discussion

4.1. Effect of thermal preparation and in vitro digestion on the SL content

To investigate the effects of temperature and time during thermal preparation on the SL content, sesame seed was heated at different temperatures (160, 200, and 240 °C), times (10, 20, and 30 min) and then analyzed. It is well known that the major lignans in sesame seed are sesamin, sesamolin, and some of their aglycones, as indicated by the composition of raw DSM showed in Table 3. However, the results suggested that PD was the predominant SL after thermal processing, followed by sesamol (Table 1). Further, both PD and sesamol presented an upward trend with the increase of processing temperature and time, but their contents decreased under the condition of 240 °C for 30 min. Therefore, the optimum temperature should be lower than 200 °C when fried in an electric oven. It was also observed that pinoresinol, sesamin and sesamolin were somewhat unstable, and could be affected by frying and roasting at 160 °C, or microwaving at 80 W. Overall, it was found that the effects of thermal preparation on SL under the same conditions were as follows: frying > roasting > microwaving. Previous studies reported that the effects of thermal processing on the concentrations of

Table 3
Effects of roasting and *in vitro* digestion on lignan contents in DSM (mg/100 g DW). Sesame seed was roasted at 240 °C for 20 min and then the polar-soluble part was extracted.

SL	Raw	Roasted	Oral	Gastric	Intestinal
PD	17.69 ± 1.74^{d}	194.14 ± 5.47^{b}	149.54 ± 3.85^{c}	225.76 ± 7.13^{a}	204.15 ± 10.51^{b}
Sesamol	nd^{c}	6.47 ± 0.96^{b}	5.43 ± 0.28^{b}	13.32 ± 1.17^{a}	0.16 ± 0.00^{c}
Pinoresinol	nd^{c}	nd^{c}	16.55 ± 1.24^{b}	11.00 ± 1.98^{b}	35.07 ± 4.72^{a}
Sesamin	3.40 ± 0.27^{a}	0.53 ± 0.17^{b}	nd^{c}	nd^{c}	0.82 ± 0.05^{b}
Sesamolin	3.18 ± 0.34^{a}	nd^{b}	nd^{b}	nd^{b}	nd^{b}
TL	24.27 ± 1.56^{d}	201.13 ± 4.89^{b}	171.52 ± 4.54^{c}	250.08 ± 8.02^{a}	240.61 ± 10.61^{a}

Values (means ± SD) with different letters within a row are significantly different at $p < 0.05$.
PD: Pinoresinol diglucoside; nd: Not detected; TL: Total lignan (the sum of five kinds of lignans).

SL depended on the plant species, cultivation, conditions or processing method (Akhila and Beevy, 2015; Visavadiya et al., 2009). Our results also indicated that high temperature or long processing time would decrease the SL contents. Gerstenmeyer et al. (2013) reported that heating was responsible for the better extractability of the lignans, and moderate heating at 100 °C did not degrade the lignan aglycones and glycosides. Our results are in agreement with those studies.

An *in vitro* digestion model was used in this study to mimic the *in vivo* physiological environment. The results showed that different lignans change differently after digestion. After the oral digestion of roasted DSM, the PD and sesamol contents decreased significantly, while the pinoresinol content increased significantly (Table 3). After the gastric and intestinal digestion, the PD and pinoresinol contents increased significantly, whereas the sesamol content increased at the gastric phase and decreased significantly at the intestinal phase. The simulated digestion of the roasted DSM led to changes in the SL profile compared with the raw and the undigested DSM. The increases or decreases in the SL contents were probably because of the pH and the activity of digestive enzymes. As a result of the enzyme action and pH changes during simulated digestion, pinoresinol and PD can be hydrolyzed and gradually released from the food matrices (Pavan et al., 2014; Thomas-Valdés et al., 2018).

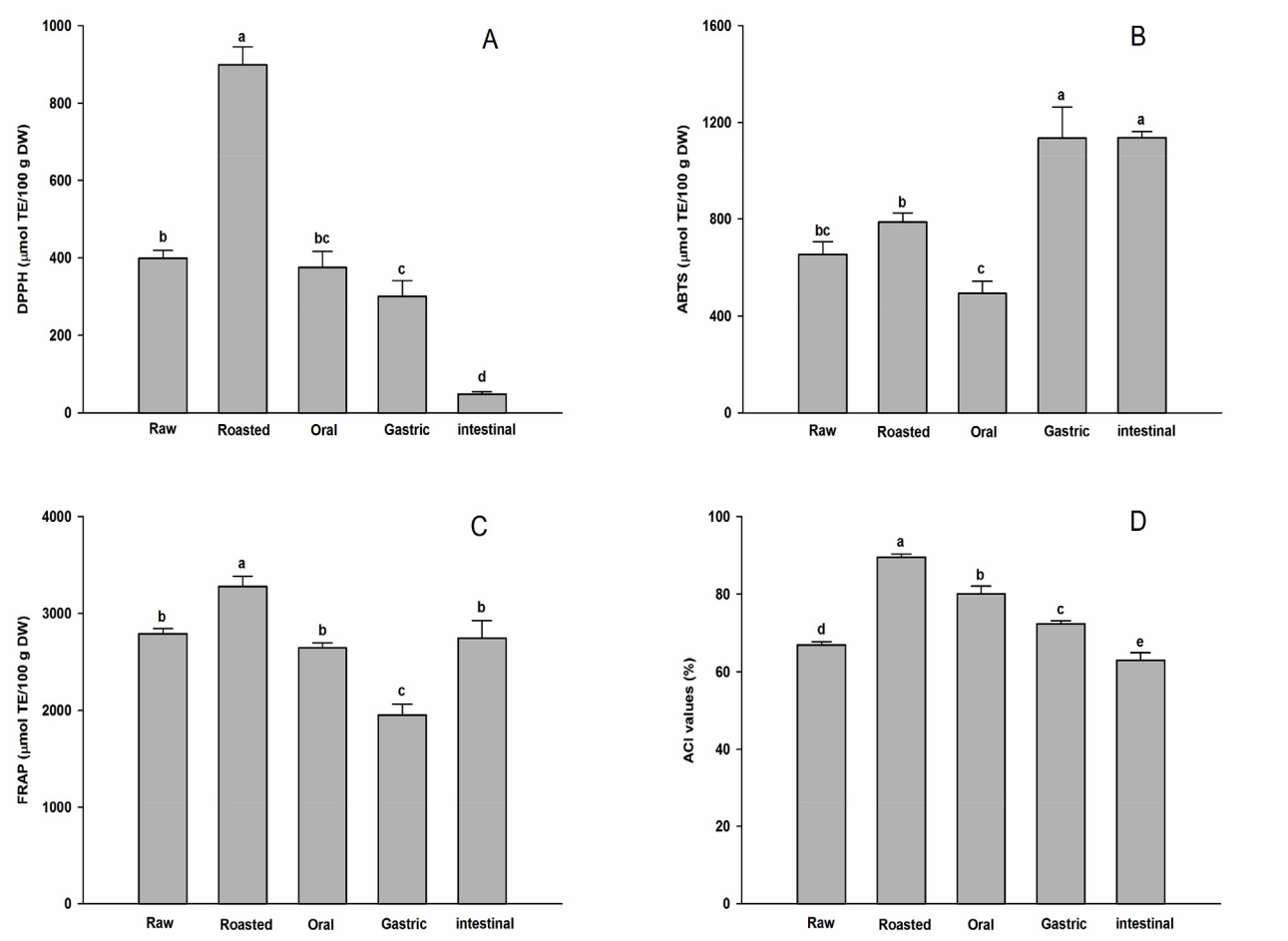

Fig. 2. Changes in DPPH (A), ABTS (B), FRAP (C) and ACI (D) before and after thermal preparation and digestion of DSM. Bars with different superscripts indicate significant difference ($p < 0.05$).
ACI: Antioxidant potency composite index.

4.2. Effects of thermal preparation and in vitro digestion on antioxidant activity

It is well known that lignans contribute to the antioxidant activity, but few studies have investigated the SL antioxidant activity in sesame after processing and *in vitro* digestion. To evaluate the antioxidant activity of the undigested and digested samples induced by the *in vitro* digestion, three different methods of antioxidant assays were conducted. The results indicate that roasted DSM had a higher antioxidant activity than that of raw DSM (Fig. 2), and a product with high antioxidant activity can be obtained by roasting for 20 min at 240 °C under the experimental conditions in this study. The reason for the discrepancy can be attributed to the thermal processing that could lead to the formation of novel compounds with antioxidant activity, and the fact that sesamol has a high antioxidant activity among the lignans. The SL antioxidant activity in sesame measured by the assays of DPPH radical scavenging activity, ABTS radical scavenging activity and FRAP were consistent with the data previously reported (Hung et al., 2016; Shi et al., 2018). Another previous study reported that the antioxidant activity of sweet corn and tomatoes increased after boiling (Dewanto et al., 2002). Antioxidant activity increased in several vegetables such as carrots, mushroom and cabbage after thermal treatment (Halvorsen et al., 2006).

The assay of DPPH radical scavenging activity showed a slight increase after the gastric step, followed by a significant decrease in the intestinal phase ($p < 0.05$) (Fig. 2A). The same effect was observed by Lucas-González et al. (2016) in digested maqui berries. On the contrary, Correa-Betanzo et al. (2014) reported a decrease in the DPPH values of 6% and 51% after gastric and intestinal digestion, respectively. In the FRAP, a sequential loss of the antioxidant activity was observed during the *in vitro* digestion (Fig. 2C). A decrease in the FRAP values of different varieties of apples subjected to *in vitro* digestion was also reported (Bouayed et al., 2011). Regarding the assay of ABTS radical scavenging activity, our results showed a loss of antioxidant capacity after the simulated digestion, with no significant difference between the gastric and intestinal phase (Fig. 2B). In summary, the antioxidant activity of the DSM was partially lost after the *in vitro* digestion. Our results justify the need for various antioxidant assays to evaluate different aspects of the samples.

4.3. Pearson correlation between SL and antioxidant activity

To establish a correlation between the SL and antioxidant activity, the Pearson's coefficient was calculated. As shown in Table 2, a significant correlation was demonstrated between the SL and the antioxidant activity in almost all the cases ($p < 0.01$). The amount of PD and sesamol were positively correlated with the ACI values, but the amount of pinoresinol, sesamin, sesamolin were negatively associated with the ACI values. On the other hand, the results suggest that the five kinds of SL were significantly correlated with each other. In our analysis, the sesamol content showed no statistical correlation with the ABTS values. A similar result was obtained that fat-soluble lignans (sesamin, sesamol, sesamolin) and glycosylated water soluble lignans played a leading role in the antioxidant activities of white sesame seed (Lin et al., 2017). Altogether, our results supported the fact that the changes of antioxidant activity in the DSM were caused by the partial loss or increase of the SL content.

In summary, different thermal preparations and *in vitro* digestion significantly affected the SL content and antioxidant activity in DSM. There was also a strong correlation between the five kinds of SL. These results suggested that the thermal processing affected the antioxidant property of DSM, whereas, interestingly, *in vitro* digestion resulted in beneficial changes to both the content of bioactive compounds and antioxidant activity of the roasted DSM. The roasting method can be used to obtain more physiologically relevant information on the health effects of bioactive compounds in DSM, which can help the food industry to better use byproducts such as DSM.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.fct.2019.03.054.

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