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Biochemical and Antioxidative Properties of Unprocessed and Sterilized White and Black Sesame By-product from Northern Thailand

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Abstract

The objective of this research was to determine the effects of sterilization on storage stability of white and black sesame by-products. Results showed that sterilization at 120 ^{0}C for 10 min had no effect on proximate compositions and mineral contents of both sesame seed cakes, but the significant reductions of thiamine, riboflavin, sesamin, sesamolin, total phenolic compounds and antioxidant capacity (DPPH and FRAP assays) were observed. During the storage at 37 ^{0}C, all bioactive components and antioxidant properties apparently tended to decrease when the storage time rose. At the end of storage, PV (peroxide value) and TBARS (thiobarbituric acid-reactive substances) values of stored black sesame seed cakes were shown to be significantly lower than that in white sesame seed cakes. This study may suggest the application of black and white sesame seeds cakes as functional food ingredients in the future production.

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Keywords

White sesame;
Black sesame;
By-product;
Sterilization.

Introduction

Sesame (*Sesamum indicum* L.) belongs to the family Pedaliaceae and is popularly cultivated in Thailand, China, India and Burma. Sesame seed cake is a by-product of traditional oil processing. It contains high amounts of nutrients, including protein fragments, free fatty acids, fiber, B vitamins, minerals[1-2], and a number of lignans, for example, sesamin, sesamolin and sesamol, which are phenolic compounds with high antioxidant capacity[3-4]. Ramachandran *et al.,*[5] reported that the components of sesame seed cake were roughly 35.6% protein, 7.6% crude fiber and 11.8% ash. The levels of total phenolics, sesamin, sesaminol triglucoside and antioxidant activity

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(measured by ABTS assay) in sesame seed cakes were found to range between 129.7 and 355.3 mg GAE/100 g, 3.2 and 25.7 mg/100 g, 208.1 and 537.5 mg/100 g, and 8,460 and 24,311 µM TE, respectively[6]. Moreover, the extracts derived from sesame seed cake were shown to have bioactivities, such as effect on polyunsaturated fatty acid metabolism, hypocholesterolemic, anti-cancer and anti-inflammatory properties[1,7].These bioactivities were suggested to associate with risk reduction of cardiovascular, atherosclerosis and oxidative stress diseases[8]. Bigoniya *et al.,*[9] found that intake of sesame seed cakes as supplementation could be adopted as a therapeutic strategy for preventing obesity-induced Type 2 hyperglycemia in rats. In addition, Konsoula and Liakopoulou-Kyriakides[10] and Suja *et al.,*[4,11]showed that the extracts of sesame seed cakes were very effective for lowering the lipid peroxidation of various vegetable oils, such as olive, soybean, sunflower and corn oils.

Sterilization is one of the most effective food preservation methods and has been widely employed in food industry since it requires low investment cost. It is used to eliminate pathogenic and spoilage bacteria as well as their spores for extending the foods' shelf-life and ensuring the consumer safety[12]. In this method, high temperature (> 100°C) is applied for an extended period of time, which, however, may reduce the qualities of foods and result in losses of heat-sensitive nutrients and/ or bioactive components[13-14]. Nonetheless, there were limited studies that investigated the effect of this conventional technique on the quality attributes of sesame seed cakes.

In the present study, the biochemical and antioxidative properties of Thai white and black sesame seed cakes before and after retort sterilization (120 °C/10 min) were evaluated. The purpose was to determine the feasibility of conventional sterilization to extend the shelf-life of sesame seed cakes during storage at 37 °C for 6 months.

Materials and Methods

Sterilization of Sesame Seed Cakes

Various cultivars of white and black sesame seed cakes were obtained from six sesame oil production factories in Chiang Mai, Chiang Rai and Mae Hong Son provinces, Thailand. The samples were air-dried at 60 °C for 3 h using a convective hot-air oven. For sterilization, 100 g of dried seed cakes were filled into the retort pouch bags before vacuum sealing and heating at 120 ^{0}C for 10 min. The sterilized samples were kept at 37 ^{0}C, and, after that, all of the stored samples were subjected to quality assessments every 2 months for up to 6 months.

Determination of Proximate Compositions and Mineral Contents

All samples were determined for the proximate compositions (%, w/w) viz. moisture content, crude protein, crude fiber, fat, ash and carbohydrate according to AOAC methods[14]. To assess the mineral contents, ash residue was dissolved in nitric acid containing 5% (w/v) lanthanum(III) chloride and then analyzed for the mineral constituents (i.e. Ca, P, K, Mg, Fe, Zn, Se and Mn) using an atomic absorption spectrophotometer (Hitachi Z6100, Tokyo, Japan)[14].

Determination of B vitamins and lignans

Thiamine (vitamin B_1) and riboflavin (vitamin B_2) were determined using a spectrophotometric method[14]. To quantify the lignan contents, sesame cakes were ground and 200 mg of their powders were well-mixed with 5 ml of 80% ethanol and then centrifuged at 4,500 rpm for 10 min. The supernatants were filtered through a 0.45 µm nylon membrane before injection (10 µL) into a HPLC system (Agilent 1100, Agilent Technologies, Waldbronn, Germany) that using a reversed phase column (Hypersil BDS C18 5 µm, 150 × 4 mm i.d.; Thermo Electron Co., Southend-on-Sea, UK). An absorbance was set at 290 nm for monitoring both compounds. The mobile phase was a mixture of methanol and deionized water at a ratio of 4:1 (v/v) with a flow rate of 0.8 mL/min[15].

Determination of Total Phenolic Compounds

Total phenolic contents were determined following the modified method of Chaikham and Apichartsrangkoon[16]. Accordingly, 1 g of sesame cake powder was extracted with 19 mL of acidified methanol [1% (v/v) HCl in methanol] for 1 hr and then centrifuged at 4,500 rpm for 10 min. The supernatant (0.5 mL) was mixed with 2.5 mL of 10% Folin-Cicalteau reagent and allowed to react for 10 min. Consequently, 7 mL of 20% sodium bicarbonate solution were added to the mixture and incubated at room temperature for 2 hr. The absorbance was measured at 765 nm using a UV-Visible spectrophotometer (Perkin Elmer UVWINLAB, Perkin Elmer, Waltham, MA). The results were reported as mg gallic acid equivalents per gram of sample (mg GAE/g).

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

In brief, 2 mL of supernatant (as extracted from above section) or methanol (control) were thoroughly mixed with 0.5 mL of 1.5 μM DPPH radicals in methanol, and allowed to react for 30 min at room temperature. The absorbance was measured at 517 nm using a Perkin Elmer UVWINLAB UV-Visible spectrophotometer. The percentage of DPPH radical inhibition was calculated according to the below formula:

DPPH radical scavenging activity = $[1 - (A_1/A_0)] \times 100$,

where, A_0 = absorbance of the control and A_1 = absorbance of the extracted sample[16].

Assessment of Ferric Ion Reducing Antioxidant Power (FRAP) Value

FRAP values of all samples were determined following the method of Zhao *et al.,*[17] with some modifications. Briefly, 7 mL of supernatant (as derived from above section) were mixed with 3 mL of FRAP reagent (10:1:1 of 300 mM sodium acetate buffer at pH 3.6, 10 mM 2,4,6-tripyridyls-triazine solution and 20 mM $FeCl_3.6H_2O$ solution) and incubated at 37 ^{0}C for 30 min. The absorbance was measured at 593 nm. FRAP values were expressed as μmol $FeSO_4$ per gram sample (μmol $FeSO_4$/g).

Determination of Rancidity

Levels of peroxide value (PV) and thiobarbituric acid-reactive substances (TBARS) of samples were determined according to the methods of Sakanaka *et al.,*[18] and Buege and Aust[19], respectively. PV was expressed as mg lipid peroxide/kg sample, while TBARS value was expressed as mg MDA/kg sample.

Microbiological Analysis

Total plate counts, yeast and mold contaminations in unprocessed and sterilized sesame seed cakes were determined following the Bacteriological Analytical Manual (BAM)[20].

Statistical Analysis

The experiments were performed in triplicates and the results were presented as mean ± standard deviation. The statistical differences were determined at $P \leq 0.05$. Data were subjected to Duncan's post hoc test and the differences were detected for homogenous subsets. All statistical analyses were performed using IBM SPSS software version[23].

Results and Discussion

Proximate Compositions and Mineral Contents of Unprocessed and Sterilized Sesame Seed Cakes

In this study, the data showed that sterilization process had no effect on proximate compositions viz. moisture content, carbohydrate, protein, fat, crude fiber and ash of white and black sesame seed cakes (Table 1). Carbohydrate was the major composition of sesame seed cakes, whereby the white one contained higher level of carbohydrate than the black one. In contrast, protein and ash were found in black sesame seed cake at higher levels than that of white sesame seed cake. In addition, fat was the most minute composition in both cakes. These results were comparable to the reports of Kanu[21] and Makinde and Akinoso[1] that showed the high amount of protein in oilseed cakes after extraction. Similarly, Ogunronbi *et al.,*[22] found that flaxseed oil seed cake contained between 38% and 47.3% protein. Rawdkuen *et al.,*[23] reported that sacha inchi seed cakes contained very high amount of protein at approximately 56.61%. According to these findings, oilseed cakes could be good sources for protein and they would be subjected to value-added production. However, future protein configuration is needed to estimate by DSC and CD spectroscopy beside the convectional analytics

Similarly, no effect of sterilization was found on mineral contents, including calcium, phosphorus, potassium, magnesium, zinc, iron, manganese and selenium, of both seed cakes (Table 2). In this case, the most predominant mineral found in both seed cakes, particularly the black one, was calcium, followed by phosphorus, potassium and magnesium. The similar results were found by Makinde and Akinoso[1] that reported the contents of calcium, phosphorus and potassium in white and black sesame seeds at 473.6–521.9, 466.0–482.8 and 465.7–468.8 mg/100 g, respectively. Additionally, Anilakumar *et al.,*[24] revealed that sesame seeds were excellent sources of calcium and they were also rich in phosphorous, iron, magnesium, manganese, zinc and copper.

Table 1: Proximate composition (%, FW) of untreated and sterilized white and black sesame seed cakes

Compositions (%, FW)	White sesame seed cake Untreated cake	Sterilized cake	Black sesame seed cake Untreated cake	Sterilized cake
Moisture	5.38±0.27[a]	5.64±0.33[a]	5.16±0.30[a]	5.47±0.42[a]
Carbohydrate	25.43±2.19[a]	24.90±1.48[a]	16.95±1.19[b]	16.15±0.87[b]
Protein	17.64±0.78[b]	16.68±1.02[b]	20.03±1.05[a]	20.52±2.14[a]
Fat	12.15±2.05[a]	11.64±1.65[a]	12.74±1.82[a]	13.16±1.02[a]
Crude fiber	18.97±2.13[a]	19.07±2.83[a]	19.07±1.93[a]	18.85±2.41[a]
Ash	20.45±2.59[b]	22.05±2.15[b]	26.04±2.18[a]	25.89±2.60[a]

Means in the same rows with the same letters indicate no significant difference(p>0.05). The experiments were performed in triplicates.

Table 2: Mineral contents (mg/100 g, DW) of untreated and sterilized white and black sesame seed cakes

Mineral contents (mg/100 g, DW)	White sesame seed cake Untreated cake	Sterilized cake	Black sesame seed cake Untreated cake	Sterilized cake
Calcium	584.74±2.05[b]	580.13±4.74[b]	600.61±3.91[a]	595.92±5.93[a]
Phosphorus	440.12±3.14[a]	437.64±4.02[a]	538.70±2.47[a]	540.17±4.19[a]
Potassium	419.76±4.08[a]	418.45±2.19[a]	420.86±5.85[a]	422.31±3.96[a]
Magnesium	420.59±2.00[a]	421.05±3.50[a]	418.35±4.91[a]	420.32±2.77[a]
Zinc	6.90±0.84[a]	6.93±1.04[a]	7.01±1.54[a]	7.13±0.19[a]
Iron	7.19±0.62[a]	7.22±0.15[a]	5.98±0.36[b]	6.04±0.30[b]
Manganese	6.05±0.11[a]	6.03±0.35[a]	5.90±0.23[a]	5.88±0.42[a]
Selenium	1.06±0.32[a]	1.11±0.20[a]	1.36±0.27[a]	1.40±0.41[a]

Means in the same rows with the same letters indicate no significant difference (p>0.05). The experiments were performed in triplicates.

In overall, this may indicate that both sesame seed cakes still contained high amounts of nutrients, such as protein, carbohydrate and minerals, after oil extraction and sterilization, and hence may be a good source for functional food ingredients.

Changes of Bioactive Components in Sterilized Sesame Seed Cakes During Storage

As shown in Table 3, the levels of bioactive components (i.e. thiamine, riboflavin, sesamin, sesamolin and total phenolic compounds) and antioxidant capacities (i.e. DPPH and FRAP values) of both sesame seed cakes were found to greatly decrease after sterilization and storage at 37 ^{0}C for 6 months ($p \leq 0.05$). For untreated seed cakes, the black sesame seed cake was found to contain thiamine, sesamin, total phenolic compounds and FRAP value at the levels higher than white sesame seed cake. At the final stage of storage, the contents of all bioactive compounds and the levels of antioxidant capacities in both sesame seed cakes were shown to reduce by roughly 0.5-1 time when compared to the samples at day 0.

Accordingly, Makinde and Akinoso[1] reported the levels of thiamine and riboflavin in whole white and black sesame seeds at the ranges of 0.71-0.83 and 0.36-0.38 mg/100 g, respectively. Suja *et al.,*[4] found that the whole white and black sesame seeds contained sesamin and sesamolin at 203.7-399.3 and 205.4-356.3 mg/100 g, respectively. Lieu and Dang25also reported that the amount of total phenols in white and black sesame seed cakes was about 13.18-13.86 mg GAE/g. In addition, Shahidi *et al.,*[26] determined the levels of total phenolic compounds and free radical scavenging capacity in whole black and white sesame seed extracts and the results showed the considerable antioxidant activity of tested sesame products, especially the black sesame seed. There was a report that suggested the benefits of consuming sesame seeds to increase plasma γ-tocopherol and vitamin E activity and perhaps to prevent cancer and heart diseases[27].

Considering the B vitamins, thiamin (vitamin B_1) is one of the most unstable B vitamins. Therefore, thermal processes viz. sterilization, pasteurization, baking and boiling can reduce the level of thiamin by up to 50%. Also, the stability of thiamin during storage can greatly depend on the moisture content of the food. The foods which having high moisture content would retain thiamin lesser than that having low moisture content[28-29]. On the contrary, riboflavin (vitamin B_2) is very stable during thermal processing, storage and food preparation. However, riboflavin is susceptible to degradation upon exposure to the light[30-31], and light-proof packaging material is therefore necessary to prevent its deterioration.

Sesames generally comprised of two predominant lignans, including sesamin and sesamolin. The health-promoting effects of these lignans viz. decreasing blood lipids, increasing antioxidative ability, and providing anti-inflammatory function were reported by Hsu *et al.,*[32] and Wu[33]. Gerstenmeyer *et al.,*[34] found that lignan aglycones and glycosides in dry foods did not degrade by heating at 100 ^{0}C, but high roasting temperature at 250 ^{0}C could degrade them severely. Also, the lignans in sesame seeds with high moisture content were found to apparently degrade. Wu[33] reported that heating at 200 ^{0}C for 20 min caused a significant loss of lignans in commercial sesame oils, particularly sesamolin. In addition, sesamolin in sesame seeds was also found to be damaged by infrared roasting at the same temperature for 30 min[35].

On the other hand, Abe et al.36 and Anilakumar *et al.,*[24] revealed that sesamin and sesamolin were thermostable compounds and remained at 80-90% of the original levels after roasting. Nonetheless, little has been known regarding the effects of storage on both lignans in sesame seeds. Lee *et al.,*[37] found that the levels of sesamin and sesamolin in sesame oil decreased when the storage time increased (25 ^{0}C for 18 months). The storage temperature and processing condition were suggested to be responsible for degradation of sesamin and sesamolin by perhaps converting them into sesamol[38].

Sterilization of sesame seed cakes was found to cause the reduction of total phenolic compounds and antioxidant activities to the contents/levels lower than that in untreated seed cakes. Heating processes, such as roasting, could also degrade the polyphenols and antioxidant capacity (DPPH assay) in sesame seeds and its oil[10]. Similar findings were reported by Abou-Gharbia *et al.,*[39] and Rababah *et al.,*[38]. In fact, sesamin and sesamolin were shown to have very low antioxidative properties[40]. The reduction of antioxidant capacity in sesame cakes might be due to temperature degradation of phenolic compounds. Besides, significant decreases of total phenolic compounds and antioxidant activities in both sesame cakes throughout the storage period were also observed. These antioxidant compounds and properties were also found to decrease significantly in parboiled germinated brown rice[41], milled rice[42] and other fruits/vegetable products[43-45] during their storage. This may indicate that the conditions for processing and storage are the critical factors for maintaining the levels of phenolic compounds and antioxidant properties.

Table 3: Changes of bioactive compounds and antioxidant capacity in untreated and sterilized white and black sesame seed cakes during storage at 37 °C for 6 months

Bioactive compounds	Storage duration of white sesame seed cake (months)					Storage duration of black sesame seed cake (months)				
	Untreated cake	Sterilized cake				Untreated cake	Sterilized cake			
		0	2	4	6		0	2	4	6
Thiamine (ppm)	22.76±1.15^{b}	17.96±1.01^{c}	16.40±2.53^{cd}	12.69±1.90^{e}	10.39±1.18^{f}	28.64±2.02^{a}	20.94±2.05^{b}	16.75±1.10^{c}	14.07±1.53^{d}	11.69±0.92^{ef}
Riboflavin (ppm)	15.87±0.65^{a}	12.97±0.82^{b}	10.17±0.64^{c}	9.07±1.42^{cd}	7.38±0.58^{e}	16.03±1.34^{a}	12.59±1.00^{b}	9.97±2.60^{cd}	8.75±0.41^{d}	7.09±1.25^{e}
Sesamin (mg/100 g)	37.94±1.28^{c}	30.87±2.73^{d}	25.76±1.83^{e}	20.77±1.80^{f}	17.92±0.16^{g}	45.68±0.95^{a}	40.01±1.24^{b}	38.00±1.19^{c}	30.62±1.04^{d}	25.83±2.19^{e}
Sesamolin (mg/100 g)	22.10±2.58^{a}	17.64±1.90^{c}	15.64±2.07^{ef}	13.03±2.65^{f}	10.03±1.82^{g}	24.97±1.88^{a}	20.89±0.50^{b}	18.61±2.34^{d}	16.79±0.97^{e}	15.45±2.01^{ef}
TPC (mg GAE/g)	30.65±2.19^{c}	25.07±1.85^{e}	22.90±2.06^{f}	17.64±0.54^{h}	15.43±1.02^{i}	40.26±2.97^{a}	34.63±2.70^{b}	27.04±1.75^{d}	24.06±0.28^{ef}	20.80±0.80^{g}
DPPH inhibition (%)	69.42±2.76^{a}	58.05±1.11^{b}	50.48±2.06^{cd}	46.14±2.90^{e}	42.00±2.17^{f}	53.02±1.13^{c}	48.63±2.01^{d}	42.10±3.95^{f}	40.17±2.51^{f}	41.19±1.38^{f}
FRAP value (µmol/g)	26.18±1.06^{b}	20.52±0.75^{c}	18.59±0.61^{d}	16.03±1.00^{e}	14.82±0.49^{f}	32.95±1.26^{a}	27.19±2.55^{b}	22.08±1.86^{c}	19.44±0.48^{d}	16.95±0.71^{e}

Means in the same rows with the same letters indicate no significant difference($p>0.05$). The experiments were performed in triplicates.

Table 4: Changes of bioactive compounds and antioxidant capacity in untreated and sterilized white and black sesame seed cakes during storage at 37 °C for 6 months

Bioactive compounds	Storage duration of white sesame seed cake (months)					Storage duration of black sesame seed cake (months)				
	Untreated cake	Sterilized cake				Untreated cake	Sterilized cake			
		0	2	4	6		0	2	4	6
PV (mg lipid peroxide /kg sample)	12.17 ±0.82^{f}	11.93±0.62^{f}	17.64±0.14^{d}	20.19±0.11^{c}	28.20±0.42^{a}	12.68±1.94^{f}	12.80±1.13^{f}	15.62±0.50^{e}	17.85±0.15^{d}	25.61±1.27^{b}
TBARS (mg MDA /kg sample)	15.04±1.13^{g}	14.80±0.17^{g}	18.90±0.28^{e}	19.65±0.20^{d}	30.52±0.58^{a}	14.95±1.60^{g}	15.17±1.49^{g}	17.38±0.29^{f}	20.07±0.18^{c}	24.99±0.73^{b}

Means in the same rows with the same letters indicate no significant difference($p>0.05$). The experiments were performed in triplicates. PV is Peroxide Value and TBARS is Thio Barbituric Acid Reactive Substance.

Oxidative Stability of Sterilized Sesame Seed Cakes During Storage

The oxidation reaction is a major cause of food deterioration. It is well known that temperature, light, moisture, metals and oxygen are the key factors which can affect the rate of oxidation. In this study, PV and TBARS values of stored white and black sesame seed cakes were investigated. The results in Table 4 showed the insignificant differences of PV and TBARS values between untreated and sterilized sesame cakes (p>p0.05). During storage, both sterilized sesame seed cakes, especially the white one, were shown to have significant increase of the levels of PV and TBARS values ($p \leq 0.05$). In fact, oxidative stability of sesame seeds could be attributed to endogenous antioxidants, including lignans and phenolic compounds[38]. Akinoso *et al.,*[46] reported that PV and oxidative stability of crude sesame oil were considerably depended on moisture content of the seeds, roasting duration and temperature, and also storage conditions. In this study, the results were comparable to the reports of Elleuch *et al.,*[47] and Lee *et al.,*[48] that investigating the sesame seeds and their by-products. The natural antioxidants found in sesame seeds have been shown to influence the oxidative stability. In this regard, the black sesame seed cake was shown to contain sesamin, sesamolin and total phenolic compounds at the levels higher than that of white seed cake, and therefore it may possess high oxidative stability due to their oxygen-scavenging properties49. In contrast, Lee *et al.,*[37] reported that the roasted sesame oil was oxidized slowly during storage at 25 ^{0}C in the dark, and there was no change of PV up to 9 months of storage.

Microbiological Qualities of Sterilized Sesame Seed Cakes During Storage

The colonies of total plate counts (TPC) and yeasts-molds (YM) in untreated and sterilized white and black sesame seed cakes were enumerated using the standard plating methods. The TPC and YM counts in untreated white seed cakes were 6.18±0.62 and 2.50±0.27 log CFU/g, respectively; while those in untreated black seed cakes were 5.97±1.01 and 2.61±0.49 log CFU/g, respectively. After sterilization and storage, no detectable levels of both TPC and YM groups were observed in all samples (data not shown). Based on these results, all the indicator microbes in sterilized and stored seed cakes were well complied with the limits of the Thai Community Product Standard (TCPS No. 686/2004) for roasted sesame[50].

Conclusion

White and black sesame by-products from Northern Thailand can be a good source for functional food ingredients because they contained high amounts of protein, carbohydrate, minerals and various bioactive components. Moreover, the sterilization and storage condition were found to be the key degradation factors of thiamine, riboflavin, sesamin, sesamolin, total phenolic compounds and antioxidant capacity (DPPH and FRAP assays) in these seed cakes. Oxidative stability of both seed cakes during storage was found to depend on the seed types and relate to the amounts of antioxidant compounds.

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