

Effect of Roasting and Dehulling on Antioxidant activity, Oil quality and Protein functionality of Sesame Seeds used in Tahina and Halawa

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Abstract

The aims of this study were to evaluate the effect of roasting and dehulling on the antioxidant activity and some of protein functionalities of sesame seeds used in tahina and halawa production. Samples were taken throughout tahina and halawa production steps. Seed dehulling and roasting resulted in a 30% reduction in total phenol content (TPC). The production steps of tahini and halawa increased TPC by about 61% and 108%, respectively. Although Tahina and Halawa had higher TPC than dehulled and roasted seeds they showed lower DPPH scavenging activity. The IC₅₀ for tahina and halawa was about 40-50% greater than those of dehulled and roasted. Proteins isolated from dehulled seeds showed greater emulsion capacity (EC) and stability (ES) than protein from roasted seeds except at pH 2 where roasted protein showed greater EC and ES. Roasted seeds' protein showed greater water and oil absorption capacities than that of dehulled protein.

Keywords: Antioxidant activity; Tahina and halawa; Roasted seeds; Emulsion capacity (EC).

Introduction

Sesame seed (*Sesamum indicum* .L) has been considered as one of the most important and healthful crops in the world for many years. It is renowned as a nutritious food for human health and is used as an ingredient in sweets and confectionaries [1]. Sesame spots, cakes, and seeds exhibit high reducing power and free radical scavenging activity as well as protection against oxidative deterioration. Sesame seeds provide highly stable oil, nutritious protein and meals [2] and have various beneficial health properties, including hypocholesterolaemic, hepatoprotective, and antimutagenic effects [3-5]. In the Middle East, dehulled sesame seeds are used primarily in the production of tahini which is made from a paste of dehulled roasted sesame seeds and halawa and is similar to peanut butter. The dehulling and roasting procedure may change the antioxidant activity, oil quality and protein functional properties of the dehulled sesame. These changes may affect positively or negatively the food products prepared from dehulled and roasted sesame such as tahina and halawa.

Sesame seed is an important source of oil (44-58%), protein (18-25%), carbohydrate (~13.5%) and ash (~5%). The oil fraction is remarkably stable to oxidation which can be attributed to endogenous antioxidants, namely lignins and tocopherols [6,7]. Recent studies indicate sesame protein is an excellent quality protein (nearly 80% α -globulin and 20% β -globulin) with high nutritional and biological (high netprotein utilization and digestibility) [8]. Sesame proteins have been classified in four classes of protein based on Osborne sequential extraction and different solubility: water soluble albumins, salt

soluble globulins, prolamins soluble in alcohol/water mixtures and glutelins soluble in dilute acid or alkali. Rivas [9] reported that the proteins in sesame flour consisted of 8.6% albumin, 67.3% globulin, 1.4% prolamin and 6.9% glutelin. Furthermore, they reported that the alkali protein isolate (extracted in water at pH 10 and precipitated at pH 4.0) were comprised of 41.3% albumin, 14.8% globulin, 0.8% prolamin, 41.0% glutamine. Onsaard et al. [10]; Zhao [5] who found that water- and oil-holding capacities of roasted sesame seeds meal protein ranged from 1.29 to 3.30 gm water/ gm protein and from 1.19 to 3.08 g oil/ g. Maximum emulsifying activity of sesame seed meal protein (83%) was obtained at a pH of 10. Emulsifying activity decreased with increasing pH reaching the minimum value (37%) at pH 6.0; increasing pH to more than 6.0 increased emulsifying activity [5]. Minimum emulsion stability of native sesame seed meal protein was recorded at pH 4 (42.86%), followed by subsequent increase in emulsion stability as the pH increased. The maximum emulsion stability of sesame seed meal protein (86.75%) was obtained at pH10 [5]. To the best of our knowledge, there is little or no data on the effect of roasting and dehulling of sesame seed on antioxidant activity and protein functionality, therefore, the objectives of this study were to evaluate the effects of processing of tahina and halawa on the polyphenol content and its antioxidant activity, the effects of roasting on functional properties of protein, and the effects of roasting on oil quality.

Materials and Methods

Sample collection

Ethiopians sesame seeds *Sesamum indicum. L* (about 3 kg) were purchased from two (Jordanian local producer).

Steps of Sesame paste (Tahina) and Halawa processing

The production of Tahina and Halawa include the following steps (Figure 1):

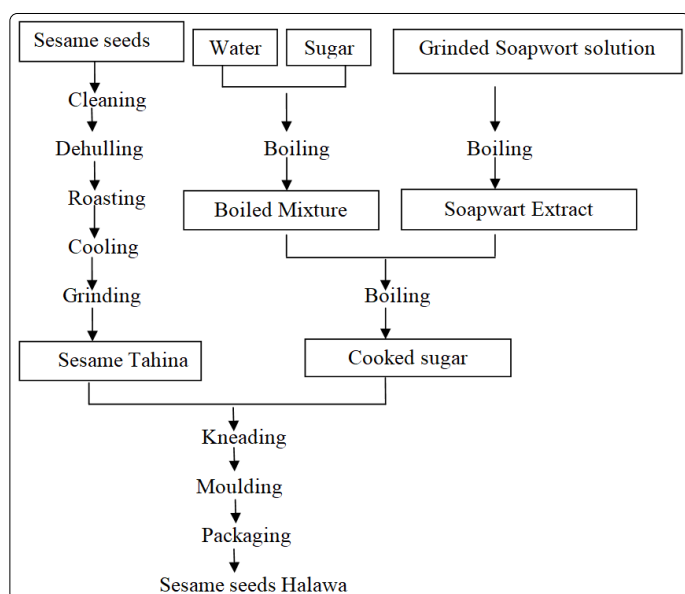


Figure1. Flow chart for Tahina and Halawa processing steps

1-Raw sesame seeds were sieved to remove stones and soil.

2-Seeds dehulling: The first producer dehulled seeds by

soaking seeds in a concentrated salt solution (14-18%) for 2 hours. Hulls were floated to the surface then removed. The second producer dehulled seeds using a peeling machine.

3- Seeds roasting: The first producer roasted seeds at 130°C in a steam heated oven for two hours with shaking. The second producer roasted seeds under pressure (5 bar) at an oven temperature of 130°C using six rotating cylindrical tanks that are heated electrically.

4-Tahina production: Tahina production (by both producers) was carried out by grinding and mixing the roasted seeds using two stones wheels, one of them fixed, the other with continuous rotation until to obtain sesame paste (2 rpm for 1-2 min).

5- Production of Halawa: Halawa production (by both producers) included mixing of Soapwarte (*Saponaria officinalis*) water extract (1%) with sucrose solution containing citric acids then cooking at 130°C for 45-60 min. The cooked mixture (natef) was added to tahina past (1:1) in a kneading tank and mixed until a homogenous mixture was obtained (15 min).

Preparation of raw, dehulled, roasted sesames seeds, tahina and halawa extracts

Two grams each of fresh, dehulled and roasted sesame seeds, and 10 g of tahina and halawa were suspended in 100 ml of 90% ethanol with continuous shaking for two hours at room temperature. The extract was then filtered and stored at 4°C until analysis [11].

Determination of total phenolic content

The total phenolic contents of the extracts were determined according to the method Kaur and Kapoor [12]. Extract (300 µl) was transferred into a 10 ml volumetric flask, 3 ml of Na₂CO₃ (20% w/v) were added and shaken by hand for 3 min. Folin Caleuclitea reagent (FCR; 0.5 ml) was added and the volume was made up to 10 ml with distilled water. The solution was allowed to stand at room temperature in the dark for one hour before absorbance was measured at 750 nm using UV/ visible spectrophotometer (Model UVD-2950, Labomed, Inc). Total phenolic content was expressed as mg gallic acid equivalent/kg seeds.

Scavenging ability of the extracts on the 2,2-diphenyl-1-picrylhydrazyl radical

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability of the extracts of sesame seeds and its products was measured according to method of Hatano et al. [13]. An aliquot (1 ml) of each extract at different concentrations was added to 0.25 mL of 0.2 mmol/L DPPH methanolic solution in 5 ml test tubes. Absorbance was measured at 517 nm after 30 minutes of storage in the dark using a UV/visible spectrophotometer (Model UVD-2950, Labomed, Inc). The antiradical activity was expressed as IC₅₀ (mg/mL) which is the concentration required to cause a 50% inhibition. The scavenging activity of the extracts was calculated as follows:

$$\text{Inhibition\%} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100\%$$

Oil extraction from sesame seeds

Ground seeds were soaked in petroleum ether for 24 hours then solvent was decanted and defatted ground seeds were air dried at room temperature for about 24 hour to remove residual solvent. The process was repeated twice. The oil obtained was kept at -18°C until analysis.

Preparation of protein isolate from sesame seeds

Protein isolate from fresh raw, and roasted dehulled sesame seeds was prepared according to the method of Sosulski and Mccurdy [14]. The defatted (raw, dehulled and roasted seeds) were dispersed in water then pH was adjusted to 10 using 0.1N NaOH for 1 hour with continuous stirring followed by centrifugation (centrifuge 5810 R; EPPENDORF, Germany) at 3800 rpm for 30 minutes at 4°C. The process was repeated twice. The pH of the combined supernatant was adjusted to the isoelectric point PI (pH4-5) with 0.2 N HCL. The mixture was centrifuged at 3800 rpm for 30 minutes at 4°C. Precipitated protein was then washed with distilled water and pH was adjusted to 7 using 0.2 N NaOH. Protein isolate was then freeze dried using Freeze dryer (GPERGN, model FDB-5502, Gmopo-city, Korea).

Emulsion capacity and stability of sesame seeds protein isolates

Emulsification capacity of proteins (raw and roasted sesame) was evaluated according to the method of Marshal [15]. One gram of sample was whipped with 100 ml of distilled water at pH 2, 4, 6, 8, and 10 adjusted using 0.1 M HCL or 0.1 M NaOH then titrated with corn oil to the break point (separation of emulsion into two phases) of the emulsion using a blender at low speed. Emulsion capacity (EC) was expressed as grams of oil emulsified per gram of sample before phase inversion. Emulsions were transferred into 250 ml graduated cylinders and emulsion stability was recorded after 1, 3, 24 and 48 hr at room temperature by measuring the amount of water separated from the oil.

Oil and water absorption capacity

For oil and water absorption capacity determinations (raw and roasted sesame protein), the method of Beuchat [16] was followed. One gram of sample was mixed with 10 ml of corn oil (purchased from local market) or distilled water for 30 seconds in a 25-ml centrifuge tube. Samples were allowed to stand at room temperature for 30 minutes then centrifuged at 3000 rpm for 30 min (Hettich Zentrifugen., model Universal 32, UK). The volume of the supernatant was measured in a 10 ml graduated cylinder. Results were expressed as milliliters of corn oil or water absorbed per gram of sample.

Quality evaluation of the dehulled and roasted sesame seeds oils

The quality of the dehulled and roasted sesame seeds oils was evaluated by free fatty acids and peroxide value test according the AOAC standard methods [17-19].

Statistical Analysis

For phenol content and free radical scavenging activity, all assessments were carried out in triplicate while for those of functional properties proteins of sesame seed were carried out in duplicate [20-22]. The design followed in experiment is complete randomized (CRD). Analysis of variance (ANOVA) was performed using Statistical Analysis Software [23]. Differences among the mean values were tested using Least Significant Difference (LSD).

Results and Discussions

Total Polyphenol Contents (TPC)

The difference in total polyphenols content (TPC) of the products of the various production steps followed by the two producers lies in the dehulling and roasting steps. Dehulling was carried out by the first producer by soaking seeds in salt solution, while the second producer used a peeler machine which is based on friction. Roasting was done by first producer using a mechanical oven at 130°C for 2 hours, while second producer roasted under pressure (5bar) at 130°C using six rotating cylindrical tanks. Raw seed samples from both producers had similar TPC, which may be due to the fact that the source of these seeds was Ethiopia. The TPC of these seeds were slightly lower than those of Rizki et al. [24] who reported that the content of these compounds extracted with 80% ethanol was 370 mg/kg. It is evident from data in table 1 that the TPC of the two producers showed a significant decrease after dehulling by about 18% for the first producer and 10% for the second. This indicates that the hulls may contain significant amounts of polyphenols. Roasting dehulled seeds negatively affected polyphenol content; TPC content decreased significantly ($p \leq 0.05$) after 2 hrs of roasting at 130°C by about 12.5% compared to dehulled seeds. This finding agrees with that of Rizki et al. [24] who reported that TPC decreased in sesame seeds roasted by microwave. Riziki [25] reported that roasting sesame seeds at 150°C for up to 90 min significantly increased the TPC but then decreased it toward the end of roasting period (360 min). They attributed the increase in TPC to production of Maillard reaction products generated during roasting which can be measured by Folin-Ciocalteu reagent. The amount of TPC in tahina of the two producers was about twice that of the dehulled sesame seeds. The increase in TPC in tahina could be due to the severe mixing during processing which ruptures seed cells releasing more polyphenols extractable by the solvent used in this study. The amount of TPC in halawa was about 2.5 times that in the dehulled seeds. This increase could also be due to the severe mixing of the seeds and to polyphenols from the wart root extracts (soap root) which was added as an emulsifier. This increase could also be due to Maillard reactions. The results shown in table 1 indicate that there were significant ($p \leq 0.05$) effect of the processors on TPC. The TPCs of the dehulled, roasted, tahina and halawa produced by the second processor were significantly higher than those produced by the first processor.

Table 1. Influence of Production Steps of Tahina and Halawa on Polyphenols

Treatment	Polyphenols (mg/kg)	
	First Producers	Second producers
Raw	A440 ± 13 ^c	A412 ± 7 ^c
Dehulled	A359 ± 4 ^c	A372 ± 8 ^c
Roasted	A314 ± 8 ^c	A330 ± 6 ^c
Tahina	B680 ± 20 ^b	A735 ± 18 ^b
Halawa	B827 ± 11 ^a	A1012 ± 23 ^a

-Values within the same row with different capital letters are significantly different ($p \leq 0.05$).

-Values within the same column with different lower case letters are significantly different ($p \leq 0.05$).

Effect of processing steps on the antioxidant activity

The antioxidant activities of the extracts of sesame seeds products in this study were evaluated using DPPH radical. This radical is commonly used for the assessment of antioxidant activity *in vitro*. DPPH is a very stable organic free radical with a deep violet color, with an absorption maximum in the 515-528 nm range. Upon receiving a proton from a hydrogen donor, mainly from phenolics, it loses its chromophore and becomes yellow. As the concentration of phenolic compounds or degree of hydroxylation of the phenolic compounds increases, the DPPH radical scavenging activity (antioxidant activity) also increases [26]. Because these radicals are very sensitive to the presence of hydrogen donors, the system operates at very low concentrations. Therefore, a large number of samples can be tested in a short time. Data in table 2 shows that processing steps had significant effect on the antioxidant activity of their product extracts. Although, tahina and halawa extracts had the highest TPC, they showed the lowest antioxidant. For example, the phenolics required to scavenge 50% of DPPH radicals from dehulled seeds extracts were 9.8 and 18 µg, and 18.8 and 33.3 µg for tahina extracts from the first and second producers, respectively. This could be due to the change in phenolic structure or to the chelating effect of their component of minerals. In both cases, the compound activity might be decreased.

Table 2. IC₅₀ Phenolic content (µg) causing 50% inhibition of DPPH

Sample	First Producer	Second Producer
Dehulled	B9.80 ± 1.4 ^c	A18.0 ^c
Roasted	B11.8 ± 1.3 ^c	A15.7 ^d
Halawa	B15.8 ± 1.5 ^b	A24.0 ^b
Tahina	B18.0 ± 1.6 ^a	A33.3 ^a

-Values within the same row with different capital letters are significantly different ($p \leq 0.05$).

-Values within the same column with different small letters are significantly different ($p \leq 0.05$).

Water and Oil Absorption Capacity

Data in table 3 shows that no significant effect of producers on the water absorption capacity or oil absorption capacity. Roasting had a slight but significant ($P \leq 0.05$) effect on water holding capacity. The increase in water absorption by the roasted seeds was about 32.55% when compared to that of raw dehulled seeds. These results may be due to protein unfolding during roasting which exposed hydrophilic amino acids which bind water.

Oil absorption capacity depends on several factors such as type and source of protein, protein particle size and whether or not it is subjected to heat treatment. As shown in

table 3, oil absorption capacity for the raw dehulled sesame seeds was about 1.2 g/g protein, similar to that reported by Kanu [27]. However, these results disagree with those of Zhao [5] who reported higher oil holding capacity of sesame protein isolate (2.7 g/g protein), which might be due to the type and source of sesame seeds and to the method of protein extraction. However, roasting at 130°C for 2 hours increased the oil holding capacity by about 2 fold. These results could be due to the partial denaturation of proteins with exposition of hydrophobic amino acid groups during roasting. The presence of several non-polar side chains may bind the hydrocarbon chains of fats, thereby increasing absorption of oil. Results in table 3 indicate that the raw dehulled sesame seed exhibited lower oil holding capacity than water holding capacity.

Table 3. Water and oil holding capacity* of the isolated proteins of dehulled and roasted sesame seeds at 130°C for 2 hrs.

Parameter	First Processor		Second Processor	
	Dehulled	Roasted	Dehulled	Roasted
Water absorption capacity (g water/g protein)	2.0 ± 0.1 ^b	2.6 ± 0.2 ^a	1.9 ± 0.2 ^b	2.8 ± 0.1 ^a
Oil absorption capacity (g oil/g protein)	1.1 ± 0.1 ^b	2.8 ± 0.1 ^a	1.3 ± 0.2 ^b	2.9 ± 0.2 ^a

-*Measured as volume (ml) of oil or water absorbed per one gram of protein.

-Values within the same row with different letters are significantly different ($p \leq 0.05$).

Emulsion capacity (EC)

The effects of pH on the emulsifying capacity (EC) of dehulled and roasted sesame seeds from the two producers are shown in table 4. As shown in table 5, no significant effect occurred due to the producers on the emulsion capacity (EC) of the dehulled and roasted seeds. The minimum emulsion capacities of dehulled and roasted sesame seeds from the two producers at pH 4 (near isoelectric point) were 20 and 10 ml oil/g protein, respectively. This is due to the fact that at the pI of protein, the net charge is zero which enhances protein-protein interaction. Therefore, protein precipitate and will not be able to migrate to protein oil interface leading to minimum EC at this pH [28]. These results agree with those of Kanu et al. [27] and Khalid et al. [29]. These results partially agree with those of Zhao et al. [5] who reported that EC for roasted sesame seed protein was minimal at pH 2-7 range and these EC were not significantly different.

Table 4. Emulsion capacity* at different pHs for the isolated proteins of dehulled and roasted sesame seeds at 130°C for 2 hrs.

pH	First Producers		Second producers	
	Dehulled	Roasted	Dehulled	Roasted
2	150 ^b	200 ^a	150 ^b	200 ^a
4	20 ^d	10 ^d	20 ^d	10 ^d
6	50 ^c	40 ^c	50 ^c	40 ^c
8	150 ^b	100 ^b	150 ^b	100 ^b
10	170 ^a	120 ^b	170 ^a	120 ^b

-*Emulsion capacity expressed as amount of oil (ml) absorbed per gram of protein.

-Values are the means of two replicates ±SD.

-Values within the same column with different letters are significantly different ($p \leq 0.05$).

A higher EC was observed on both sides of the isoelectric point for the raw dehulled and roasted sesame seeds proteins. At pH 2, ECs of 150 ml oil/g protein for raw and 200 ml oil/g protein for roasted proteins was found. This indicates that roasting had a positive effect on EC of sesame seed protein. The EC for roasted protein at pH 2 was 1.33 times greater than that of raw protein. The emulsion produced by roasted protein at this pH was very thick and similar to that of mayonnaise. Further studies are needed to explain this behavior. The highest EC for the raw dehulled seed protein occurred at pH 10 (170 ml/1 g protein). These results agree with those of Kanu et al. [27] and Khalid et al. [29]. The roasted sesame seed protein showed similar behavior to that of raw dehulled protein. As shown in table 4, roasting significantly decreased the EC at all pH values tested except at pH 2 where it increased. For example, at pH 10, EC decreased about 30% compared to that found for dehulled protein. These results indicate that emulsion capacity was pH-dependent and that alkaline pH was found to improve the emulsion capacity more than acidic pH.

Emulsion stability (ES)

The effects of pH on emulsion stability (ES) of the dehulled sesame protein isolate were similar to the effects of pH on protein emulsion capacity (Table 5). The minimum ES was in the pH range of 4-6 increasing on both sides of this range reaching maximum values at pH 2, 8 and 10. At these pH values, no separation of liquid was observed after storage at room temperature for 48 hrs.

On the other hand, roasting of sesame seeds had a negative effect on the ES at all pH values except at pH 2. The stability was minimal at pH 10 and then at pH 8. This behavior could be due to the decrease in the forces responsible for the formation of a strong viscoelastic film at the interface which results in lower stability of the formed emulsion.

Table 5. Emulsion stability* of raw and roasted sesame seeds protein isolate at different pH values for the two producers.

PH	First Producer								Second producers								
	Dehulled				Roasted				Dehulled				Roasted				
	Storage time (hours)		Storage time (hours)		Storage time (hours)		Storage time (hours)		Storage time (hours)		Storage time (hours)		Storage time (hours)				
	1	2	24	48	1	2	24	48	1	2	24	48	1	2	24	48	
2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	24	48
4	8.0	8.2	8.3	8.3	8.0	8.2	8.3	8.3	8.0	8.2	8.3	8.3	0	0	0	0	
6	30	32	33	33	30	32	33	33	33	34	34	35	8.0	8.2	8.3	8.3	
8	0	0	0	0	41	50	51	52	0	0	0	0	30	32	33	33	
10	0	0	0	0	41	52	53	53	0	0	0	0	41	50	51	52	

*Emulsion stability expressed as volume (ml) of water separated from the emulsion at room temperature.

** 1 and 2 refers to the first and second producers.

Sesame seeds oil quality

The results reported in table 6 show that the roasting process did not affect PV and the acidity of the extracted oil. These results disagree with those of Manal and Hassan [26] who reported that the roasting process for sesame seeds (200°C for 15 min) resulted in an increase in PV and in the acidity of their oils.

Table 6. Effect of roasting of sesame seeds roasting on PV and Acidity of its oil

Parameter	Dehulled	Roasted
PV (Meq O2/kg oil)	ND*	ND
Acidity% (as oleic acid)	1.9 ± 0.05	1.8 ± 0.1

*ND: not detected

Conclusion

Based on the results obtained in this study, it can be concluded that hulls of sesame seeds contain polyphenols. Roasting or dehulling seeds at 130°C for 2 hours decreased poly phenol content. Although, polyphenol decreased due to roasting, they exhibited good antioxidant activity. Production of tahina and halawa increased polyphenols content, but decreased antioxidant activity. Roasting had no significant effect on the oxidation of fat of sesame seeds based on fatty acid composition and peroxide value. Roasting negatively affected both emulsion capacity and emulsion stability except at pH2 (where emulsion capacity and stability increased). Roasting positively affected both water and oil absorption capacity.

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