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Research Article

Hydrothermal Treatment on Reducing Saponins, Phytic Acid, and Oxalates of Sesame (*Sesamum indicum* L.) Seeds

Harold Toros and Romel Guzmán-Alvarez*

Instituto de Ciencia y Tecnología de Alimentos, Facultad de Ciencias, Universidad Central de Venezuela, Los Chaguaramos, Caracas, Venezuela

***Corresponding Author:** Romel Guzmán-Alvarez, Instituto de Ciencia y Tecnología de Alimentos, Facultad de Ciencias, Universidad Central de Venezuela, Los Chaguaramos, Caracas, Venezuela.

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Abstract

We studied the effect on three different varieties of sesame seeds (DV-9; Guesa-4 and Guesa-150 with bark and peeled, the application of a hydrothermal treatment at 55°C for 5; 10 and 15 minutes, on the reduction of antinutritional factors (saponins, phytic acid and oxalates). The most effective treatment in reducing antinutritional factors was the hydrothermal treatment without bark for 15 minutes because it reduced the content for the DV-9 variety, of saponins, phytic acid and oxalates by 22.54; 80.61 and 83.25% respectively, for the Guesa-4 variety by 12.78; 71.71 and 79.59% respectively and for the Guesa-150 variety by 10.43; 66.72 and 90.46% respectively. These results indicate that anti-nutritional factors (saponins, phytic acid and oxalates) can be reduced in sesame seed with simple and effective processes that could be used by growers without any expensive equipment.

Keywords: Sesame, Oxalate, Saponins, Phytic Acid, Nutritional Factors, Antinutritional Factors

Introduction

Anti-nutrients are natural or synthetic compounds that interfere with the release or uptake of nutrients, thereby reducing the bio-accessibility and bioavailability of nutrients in food [1]. A diet rich in whole grains and plant foods, low in total fat but high in soluble fibres and monounsaturated and polyunsaturated fatty acids, reduces the risk of chronic non-communicable diseases. This food group includes sesame seeds, which have various nutritional properties [2]. Sesame, whose scientific name is Sesamum indicum L., belongs to the Pedaliaceae family, which is composed of 16 genera and 60 species [3]. Sesame is grown in tropical and subtropical regions on just over seven million hectares to produce six million tonnes per year [4]. The demand for sesame seed has increased every year due to commercial and industrial interest in the high oil content. Myanmar, India and China are now the world's leading producers, followed by Sudan, Nigeria, Ethiopia and Uganda. In America the largest producers are: Mexico, Guatemala, and Venezuela [4].

Sesame is used for the production of edible oil, margarines (it is appreciated in countries that consume it for its pleasant taste and being easily digestible), as an ingredient in the pharmaceutical industry, in the manufacture of soaps, cosmetics and paints. After the extraction of the oil, the residual part (cake) remains, useful for feeding livestock and poultry. It contains 40 to 50% protein. Sesame seed is used in the preparation of biscuits and confectionery [5,6]. The term antinutrients is used to qualify those compounds that affect the nutritional value of some foods, especially seeds, because they hinder or inhibit the assimilation of nutrients that come from foods generally of plant origin (proteins and minerals), from the biochemical point of view these factors are of varied nature and can become toxic or cause undesirable physiological effects such as flatulence, Stomach distension, pancreatic affectations, agglutination of red blood cells, decrease in the assimilation of nutrients, among others, antinutritional factors are natural non-fibrous substances, generated by the secondary metabolism of plants as a defense mechanism to stressful situations or against the attack of moulds, bacteria, insects and birds [1,7].

Authors [8] studied the effect on three different varieties of sesame seeds (DV-9; Guesa-4 and Guesa-150 whole and dehulled, the application of an alkaline treatment (KOH 0.7%) at 55°C for 5; 10 and 15 minutes, on the reduction of antinutritional factors (saponins, phytic acid and oxalates). The most effective treatment in reducing antinutritional factors was the alkaline treatment dehulled for 15 minutes because it reduced the content for the DV-9 variety, of saponins, phytic acid and oxalates by 27.76; 93.27 and 88.63% respectively, for the Guesa-4 variety by 23.79; 92.22 and 92.06% respectively and for the Guesa-150 variety by 23.79; 86.88 and 91.36% respectively. Others [9] evaluated the effect of various treatments on the anti-nutritional factors of both whole and dehulled varieties of sesame seeds in Nigeria and observed that the use of water soaking, germination, autoclaving, roasting and cooking significantly reduced the levels of phytates and oxalates in whole and dehulled sesame seeds, with a maximum reduction in these levels after germination. Authors [10] determined the content of anti-nutritional factors in sesame seeds during four days of fermentation, and it was observed that there was a 50% reduction in phytic acid content, while the oxalate content was reduced by 69%, after 96 hours of fermentation.In accordance with the above, it was proposed in the present work to evaluate the application of a hydrothermal treatment to reduce anti-nutritional factors in sesame seeds for use in the human diet.

Materials and Methods

Collection/preparation of sesame seed flour

The sesame seeds (*Sesamun indicum* L.) varieties Guesa-150, Guesa-4 and DV-9, were provided by the company Comercializadora Guesa C.A, located in Turen, Portuguesa state, in the Bolivarian Republic of Venezuela. Sesame seeds were washed with potable water, then hydrothermal treatment was applied at a temperature of 55°C for 5, 10 and 15 minutes. Whole and dehulled seeds were obtained. In the case of dehulled seeds, once the treatment in study was applied, the raw material was homogenized in a Metvisa® brand blender for 1 minute. Then, the seeds were placed inside a 14 mesh sieve and successive washings (minimum 3) were performed, under water pressure in order to retain the bark of the seeds. To obtain whole seeds, after applying the treatment in study, successive washings with jet water were realized (minimum 3), trying to manipulate the seeds as less as possible. Once obtained the seeds (whole and dehulled) they were dried in a tray dehydrator (Mitchel Dryers 6451/59) to a temperature of 55 ºC during 5 hours. After drying the seeds, they were cold pressed, using a hydraulic press (Fisher Scientific Co. Carver Laboratory Press model B), under a pressure of approximately 24,000 psi, with which oil and cake (residue) were obtained. Once the oil was extracted, it was centrifuged and filtered and then stored in clean, dry glass containers. The cake was then reduced in size using a Corona® brand disc mill, and the material obtained was passed through a 60 mesh sieve to obtain sesame flour which was then stored in plastic bags for later analysis.

Determination of anti-nutrients

Determination of saponins

The saponin content was determined according to the standard method of [8,11,12]. 1 g of the defatted sample was mixed with 10 mL of a 20% ethanol solution in a test tube. The mixture was heated in a water bath for 90 minutes at 55 ºC. It was then filtered through a filter paper. The residue was extracted with 10 mL of 20% ethanol. The extract was reduced by approximately half in a water bath at 90°C and transferred to a centrifuge tube in which 5 mL of diethyl ether was added and agitated vigorously in vortex and centrifuged at 3000 rpm for 2 minutes. Extraction was performed twice until the aqueous layer became light coloured. The recovered aqueous layer was mixed with 5 mL propanol and 2 mL 5% NaCl solution, agitated vigorously in vortex and centrifuged at 3000 rpm for 2 minutes. The recovered aqueous layer evaporated to dryness on a previously weighed evaporation plate. The residue was dried at 60°C in a stove and reweighed after cooling in a desiccator. The content of saponins, expressed in %, was calculated by applying the equation [12].

Determination of phytic acid

The determinations were made under the methodology of [8,13,14], where 2 g of dry matter were soaked in 50 mL of 2% HCl for three hours and then filtered with Whatman N° 1 paper. 25 mL were recovered from the filtrate and 5 mL of potassium thiocyanate (KSCN) were added at 0.3% and 4 drops of ammonia (NH_3) were added as an indicator. 53.5 mL of distilled water was added to generate the appropriate acidity. The mixture was titrated with a solution of 0.01 M iron chloride ($FeCl_3$). The titration was stopped until a yellow color appeared that persisted for about 3 to 5 minutes. The phytic acid content, expressed in mg%, was calculated by applying the equation [14].

Determination of oxalates

The standard method of [8,15] were used. 1 g of dry matter was weighed in a beaker and mixed with 75 mL of H_2SO_4 3 M. It was agitated intermittently for 1 hour, with magnetic agitator. The mixture was filtered through Whatman Filter Paper No. 1. 25 mL of filtrate were used and 5 mL of H_2SO_4 6N and 50 mL of distilled water were added. It was titrated hot (80-90 ºC) against 0.1 N $KMnO_4$ solution till a faint pink color appeared and persisted for at least 30 seconds. The content of oxalates, expressed as mg%, was calculated by applying the following equation [16].

Statistical analysis

Significant differences between means were calculated using analysis of variance (ANOVA) and a multiple comparison test (Fisher's minimal significant difference) with a significance level of 5%. The statistical program Statgraphics Centurion was used.

Results and Discussion

Saponine

The content of saponins in sesame flour obtained from seed without applying any treatment was 16.34 ± 0.05, 15.42 ± 0.15 and 15.15 ± 0.01% for varieties DV-9, Guesa-4 and Guesa-150 respectively (table 1). These values are higher than those reported by other authors for some varieties of *Sesamun indicum* such as: 4.91-5.03% [16]; 2.45-2.49% [17]; 9.20-10.14% [18]; 2.91-2.95% [19]; 3.14-4.46% [20] and 5.60% [21]. In table 1, it is shown, for the varieties of seeds in study, that the content of saponins was decreasing with the different treatments applied, observing statistically significant differences (p < 0.05) between them, however, no statistically significant differences were observed between the times applied for the same treatment, as well as it could be determined that the best treatment was: hydrothermal dehulled during 15 minutes, with a percentage of reduction of 22.65%; 12.91 and 10.43 for DV-9; Guesa-4 and Guesa-150 respectively. One of the most widely used hydrothermal treatments to reduce anti-nutritional factors has been cooking. Some authors [17] reduced the saponin content in sesame seeds from Nigeria after different treatments (fermentation, roasting and cooking). In the case of cooking (temperature of 100 °C for 10 minutes), a reduction in saponin content of 2.47 to 1.25% was observed, i.e. a 49% reduction. Others [16], used sesame seeds from Nigeria, which were cooked at 100°C for 10, 20 and 30 minutes and observed a reduction in saponin content of 59.5, 71.8 and 67.6% respectively. Others [22], used debarked giant bean (*Canavalia plagiosperma*) seeds, which were cooked at a temperature of 100 °C for 30, 40 and 50 minutes, obtaining a reduction in saponin content of 89.3; 92.2 and 96.3% respectively. Authors [23], worked with peeled yellowtail seeds (*Hura crepitans* Linn), which were cooked for two hours at 100 °C, obtaining a reduction in saponin content of 11%. Hydrothermal treatments reduced the content of saponins due to their high solubility in water because of their amphiphilic nature [24-26]. The reduction of the saponin content was carried out by the leaching process, by which the saponins are removed when they come into contact with water during the first 30 minutes, at temperatures between 20 and 60 °C, the leaching rate being significantly higher at 60 °C, and after 120 minutes at 20 °C, i.e. at higher temperatures, the solubility of saponins in water increases and the softening of tissues is also faster and the driving force of the leaching process also becomes more significant, consequently the saponin diffusion rate increases. The hydrated seed crusts allowed water to penetrate deep into the seed matrix to release more saponins by simple diffusion [27].

This study showed that the saponins are distributed throughout the seed, since the dehulled by hydrothermal treatment eliminated part of the saponin content that was in the hull and pericarp, this generated the exposure of the embryo, whose saponin content was released by simple diffusion when in contact with water. The seeds with hull, when in contact with water, allowed the hydration of the hull and thus caused the passage of water to the interior of the seed, which was put in contact with the embryo, thus releasing the content of saponins [27]. Regarding the thermal stability of saponins, they are resistant to temperatures below 100 °C [27,28], therefore there was no significant difference between the times of 5, 10 and 15 minutes at 55 °C, because from 60 °C the leaching rate increases [27].

Phytic acid

The phytic acid content in sesame flour obtained from seed without applying any treatment was 30.86 ± 0.61, 27.01 ± 0.35 and 25.36 ± 0.40 mg% for varieties DV-9, Guesa-4 and Guesa-150 respectively, (table 2), these values are within the range reported by other authors for some varieties of *Sesamun indicum* such as: 30.64-32.54 mg% [10] and 29.00-31.00 mg% [29], in the case of DV-9 varieties; 27.00-27.86 mg% [18], for the case of varieties Guesa-4; 23.75-25.96 mg% [30], for the case of varieties Guesa-150, and both for varieties Guesa-4 and Guesa-150 with a range of 20.45-29.65 mg% [16]. In addition, they are higher than the value observed in sesame seeds from Nigeria, which was 1.42 mg% [17], as well as for white dehulled sesame seeds, with a range of 0.83-0.85 mg% [31] and lower than observed in NCRI-98-60 (white) seed varieties of 60.15-65.19 mg% and for NCRI-97-28 (black) varieties of 51.07-54.13 mg% [9]. The phytic acid content can vary depending on the variety of crop, climatic conditions, location, irrigation conditions, type of soil, and the year during which they are grown [32]. In table 2, it is shown, for the varieties of seeds in study, that the phytic acid content was decreasing with the different treatments applied, observing statistically significant differences (p < 0.05) between them, and it could be determined that the best treatment was: hydrothermal dehulled during 15 minutes, with a percentage of reduction of 80.61%; 71.71% and 66.72% for DV-9; Guesa-4 and Guesa-150 respectively.

	Seed varieties		
Treatments	DV-9 (%)	Guesa-4 (%)	Guesa-150 (%)
Control	16,34 ± 0,05 a	15,42 ± 0,15 a	15,16 ± 0,01 a
Whole 05'.	15,43 ± 0,01 b	15,36 ± 0,09 a	14,43 ± 0,28 b
Whole 10´	15,36 ± 0,07 b	15,28 ± 0,04 a	14,39 ± 0,12 b
Whole 15´	15,35 ± 0,15 b	15,26 ± 0,02 a	14,35 ± 0,26 b
Dehulled 05´	12,85 ± 0,02 c	13,45 ± 0,30 b	13,61 ± 0,12 c
Dehulled 10´	12,75 ± 0,05 c	13,45 ± 0,01 b	13,61 ± 0,25 c
Dehulled 15´	12,64 ± 0,06 c	13,43 ± 0,15 b	13,57 ± 0,07 c

Table 1: Saponin content of treated seeds.

Values are mean ± SD from triplicate determinations; different letters in the same column are significantly different p ≤ 0.05.

Different treatments such as: dehulled, autoclave, soaking, extrusion, microwave, cooking, germination and fermentation, are the main ones to reduce the amounts of phytic acid and other antinutritional factors in food [33]. Some authors [16] used sesame seeds from Nigeria, which were cooked at 100°C for 10, 20 and 30 minutes and found that the phytic acid content decreased by 28.94, 34.33 and 54.09% respectively. Others [17] determined the reduction of phytic acid content in sesame seeds from Nigeria, after applying different treatments (fermentation, roasting and cooking). In the case of cooking (temperature of 100 °C for 10 minutes), a reduction in phytic acid content of 1.42-0.57 mg% was observed, i.e. a 59.86% reduction.

Hydrothermal treatments contribute to the reduction of the phytic acid content because phytic acid is soluble in water and therefore the process of leaching phytate ions into water is carried out under the influence of the concentration gradient. This reduction can be attributed to the permeability of the seed hull that has been modified when in contact with water [33-35], and leaching is also favoured in the case of low-molecular-weight compounds of an ionic nature [37]. The reduction in phytic acid content observed after hydrothermal treatment is likely to be by partial chemical hydrolysis of inositol hexaphosphate to penta and tetraphosphates [36-38]. Phytic acid is stable at temperatures below 100 °C [39], therefore, the decrease in phytic acid content under applied heat treatments is due to the formation of insoluble binary complexes with proteins or minerals such as calcium and magnesium and these complexes are not detected by the method used [35,38,40,41]. Phytic acid is synthesized during the development of the seed and is deposited in structures called globoids in the form of magnesium and potassium salts [42]. These structures are located inside the protein corpuscles of the cells of the cotyledon, in the hull and pericarp of the oilseeds [43]. In sesame seeds, specifically in the bark, a significant amount of anti-nutritional factors such as phytic acid are found and the removal of this hull implies a reduction in the phytic acid content [9].

	Seed varieties		
Treatments	**DV-9 (mg%)**	**Guesa-4 (mg%)**	**Guesa-150 (mg%)**
Control	30,86 ± 0,61 a	27,01 ± 0,35 a	25,36 ± 0,40 a
Whole 05′.	22,43 ± 0,57 b	19,26 ± 0,48 b	16,97 ± 0,24 b
Whole 10′	17,77 ± 0,34 c	16,84 ± 0,25 c	12,02 ± 0,48 c
Whole 15′	14,40 ± 0,25 d	14,63 ± 0,27 d	11,23 ± 0,19 d
Dehulled 05′	10,75 ± 0,49 e	10,31 ± 0,21 e	10,56 ± 0,30 e
Dehulled 10′	8,12 ± 0,23 f	8,19 ± 0,22 f	10,17 ± 0,27 e
Dehulled 15′	5,98 ± 0,27 g	7,64 ± 0,28 g	8,44 ± 0,19 f

Table 2: Phytic acid content in treated seeds.

Values are mean ± SD from triplicate determinations; different letters in the same column are significantly different p ≤ 0.05.

	Seed varieties		
Treatments	**DV-9 (mg%)**	**Guesa-4 (mg%)**	**Guesa-150 (mg%)**
Control	2,11 ± 0,02 a	2,15 ± 0,03 a	1,85 ± 0,03 a
Whole 05′.	1,37 ± 0,05 b	1,31 ± 0,01 b	1,19 ± 0,03 b
Whole 10′	1,19 ± 0,04 c	1,30 ± 0,03 b	1,10 ± 0,03 c
Whole 15′	1,06 ± 0,01 d	1,15 ± 0,03 c	0,81 ± 0,02 d
Dehulled 05′	0,49 ± 0,02 e	0,61 ± 0,03 f	0,35 ± 0,02 e
Dehulled 10′	0,41 ± 0,01 f	0,52 ± 0,02 g	0,24 ± 0,01 f
Dehulled 15′	0,35 ± 0,02 g	0,44 ± 0,01 h	0,17 ± 0,01 g

Table 3: Oxalate content in treated seeds.

Values are mean ± SD from triplicate determinations; different letters in the same column are significantly different p ≤ 0.05.

Oxalates

The oxalate content of sesame flour obtained from seed without applying any treatment was 2.11 ± 0.02, 2.15 ± 0.03 and 1.85 ± 0.03 mg% for varieties DV-9, Guesa-4 and Guesa-150 respectively, (table 3), these values are similar compared with those reported by other authors for varieties DV-9 such as: 2.10 mg% [17]; while the values of 2.03-2.37 mg% [44], were similar for both DV-9 and Guesa-4 varieties. They are also higher than the values reported by several authors such as: 0.0049-0.0057 mg% [18]; 0.95-1.15 mg% [10]; 0.41-0.44 mg% [19]; 1.28-1.30 mg% [31]; and are lower than the value observed for Nigerian sesame seeds which was 85.44-85.90 mg% [29]; 15.21-16.11 mg% [16], as well as for NCRI-98-60 (white) seed varieties which was 181.74-185.10 mg% and for NCRI-97-28 (black) varieties which was 150.40-157.60 mg% [9]. The amount of these antinutrients in the seeds depends not only on the plant, but also on the season, soil nutrients and local water conditions of the soil where they are grown [45]. In table 3, it is shown, for the seed varieties under study, that the oxalate content was decreasing with the different treatments applied, observing statistically significant differences ($p < 0.05$) among them, and it could be determined that the best treatment was: hydrothermal dehulled for 15 minutes, with a percentage reduction of 83.25%; 79.59% and 90.46% for DV-9; Guesa-4 and Guesa-150 respectively.

Different treatments such as: dehulled, autoclaving, soaking, extrusion, microwave, cooking, germination and fermentation, are the main ones to reduce the amounts of oxalates and other anti-nutritional factors in food [33]. Some authors [16] used sesame seeds from Nigeria, which were cooked at 100°C for 10, 20 and 30 minutes and found that the oxalate content decreased by 46.04, 47.70 and 70.11% respectively. Others [17] determined the reduction of oxalate content in sesame seeds from Nigeria, after applying different treatments (fermentation, roasting and cooking). In the case of cooking (temperature of 100 °C for 10 minutes), a reduction in oxalate content of 2.10 to 1.04% was observed, i.e. a 50.47% reduction. Hydrothermal treatments contribute to the reduction of the oxalate content because the oxalate is water soluble and therefore the leaching process takes place [9]. The diffusion of oxalate occurs very slowly at 30 °C and as the temperature increases, the solubility of oxalate in water increases [46] and the reaction of decomposition of calcium oxalate crystals into calcium oxide also occurs rapidly at high temperatures [47]. In addition, small changes (ruptures) in the hull can be generated, thus facilitating the escape of the oxalate [48]. Oxalate is synthesized in the form of crystals, in structures called idioblasts, located in the cotyledons and hull of oilseeds [49,50]. In sesame seeds, specifically in the hull, a significant amount of anti-nutritional factors such as oxalate are found and the removal of this bark implies a reduction in the oxalate content [9].

Conclusion

The anti-nutritional factors (saponins, phytic acid and oxalates) can be reduced in sesame seed with simple and effective processes that could be used by growers without any expensive equipment.

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