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Effects of Technological Processes on Functional Bioactive Compounds, Antioxidants, and Nutrients Levels of Summer Squash (*Cucurbita pepo*) Seeds

O. E. Tadros, M. K. Khalil, M. A. Attia, M. S. Tawfik*

Department of Food Sciences and Technology, Faculty of Agriculture, Al-Shatby, PO Box 21454, Alexandria University, Alexandria, Egypt
*Corresponding author: Manal Said Tawfik, mana92t@yahoo.com

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Abstract Summer squash seeds are highly nutritious functional food, rich in tocopherols, carotenoids, total phenolic compounds, flavonoids, and antioxidants. Egypt is a major producer of pumpkins, squash, and gourds, with pumpkin seeds being the main part consumed. The present study investigated the effect of all local manufacturing parameters, including soaking, roasting, and soaking followed by roasting, on the concentration of these compounds. The findings showed that the total concentration of tocopherols was highest in raw hulled seeds, with alpha-tocopherol being the most abundant. However, all processed seeds had lower tocopherol levels than raw seeds, with soaked roasted seeds having the lowest levels. The total carotenoid content varied significantly in soaked seeds, while the rind had lower carotenoid levels compared to raw hulled seeds. The rind also had higher levels of total phenolic content (TF) and flavonoids, showing greater antioxidant activity compared to raw hulled seeds. The combination of soaking and roasting led to the highest TF content, while it resulted in the lowest level of flavonoids. All the treated seeds exhibited higher antioxidant activity compared to raw hulled seeds, with the greatest antioxidant activity found in soaked seeds. Overall, the study highlights the importance of processing methods in determining the nutritional value of summer squash.

Keywords: *Summer Squash; Tocopherols; Carotenoids; Total Phenolic Compounds; Flavonoids; Functional Foods; Technological Treatments*

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1. Introduction

Summer squash (*Cucurbita pepo* L.), a member of the Cucurbitaceae family and a common species within the Cucurbita genus, is a highly valued plant known for its significant therapeutic abilities. Summer squash is one of the most widely consumed vegetables globally, and all its edible parts, including fruit and seeds, contain numerous active compounds with high nutritive value. These plants are rich in fatty acids, proteins, amino acids, carbohydrates (including pectin and non-pectin polysaccharides), vitamins (C, E, K, B1, and B2), beta-carotene, flavonoids, tocopherols, dietary fiber, and minerals (such as potassium, phosphorus, magnesium, iron, and selenium), as reported in various studies [1-5].

The biological functions of *C. pepo* include anti-inflammation, antioxidation, glucose-lowering activity, anticarcinogenic, antiangiogenesis, and antilipogenic effects, which help prevent various chronic diseases. These health benefits are attributed to the presence of phytochemicals, such as tocopherols (α- and ɣ-tocopherol), carotenoids (β-carotene, β-cryptoxanthin, lutein, and zeaxanthin), phenolics, triterpenes, and secondary metabolites like β-sitosterol, dehydrodiconiferyl alcohol, and tetrasaccharide glycerol glycolipid, as reported in previous studies [6-8]. Additionally, the seeds of *Cucurbita pepo* have an exceptional concentration of α, γ, and δ-tocopherols and tocotrienols in their oil, as reported by Nakić et al. [9].

Furthermore, *C. pepo* contains compounds like β-carotene (which gives an orange color) and lutein (which gives a bright yellow color), and its seed oil has a high concentration of carotenoids. β-carotene is converted to vitamin A in the body, which is responsible for vision, growth, and fetal development. Vitamin A deficiency can lead to vision loss and infant mortality, as reported in studies [10,11]. Carotenoids are highly beneficial for athletes, individuals engaged in heavy physical activity, and those experiencing prolonged stress. These carotenoids act as antioxidants, reducing the risk of certain diseases, such as cancer and cardiovascular diseases by eliminating free radicals. Carotenoids can also neutralize

singlet oxygen and react with free radicals. Foods rich in carotenoids can reduce the risk of lung tumors, coronary disease, skin damage, and urinary diseases, as reported in previous studies [11-13]. *Cucurbita pepo* seed oil contains certain phenolics such as tyrosol, vanillic acid, caffeic acid, and p-coumaric acid in significant concentrations besides trans-cinnamic acid in small amounts. Also, the flowers of *Cucurbita pepo* may encompass significant concentrations of phenolic compounds [14,15].

Seeds are exposed to different process operations such as soaking, roasting, drying, and salting, which affect the level of bioactive compounds. Egypt is a major producer of summer squash [16] and this study aims to evaluate the levels of various bioactive compounds in Egyptian varieties and their changes during processing techniques such as soaking, roasting, and soaking followed by roasting. The study will also examine the potential use of squash rind, which is often discarded and considered waste.

2. Materials and Methods

2.1. Chemicals

Dichloromethan, methanol, acetonitrile, acetone, petroleum ether, anhydrous sodium sulfate, aluminuim nitrate, sodium carbonate, sodium nitrite, folin-ciocalteu reagent, sodium carbonate, gallic acid, sodium nitrite, aluminum nitrate, sodium hydroxide, rutin, and 2, 2-Diphenyl -1-picrylhdrazyl (DPPH) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA).

2.2. Samples Collection

In this study, the *C. pepo* fruit samples had an average weight of 3±0.5 kg and were collected from the El-Sewed field in Kum El-Farag "Kum Abu Hanash" village, located in Abu El-Matamir city, Beheira Governorate, Egypt. The samples were collected between May and June 2021 and were obtained from a specific location with the coordinates of latitudes 30° 54' 25.78"N, longitude 30° 10' 28.21"E, and an elevation of 1.99 M.

2.3. Methods

2.3.1. Samples Preparation and Processing

In this study, we followed a simulated local manufacturing and preparation process for summer squash seeds, as presented in Figure 1. First, mature summer squash fruits were washed with tap water and deionized water to remove dirt. The fruits were then cut open and the seeds were separated. The rind (the part of the fruit without seeds) was sliced lengthwise and cut into small pieces. The small pieces were dried in a laboratory oven at 60°C with air circulation, until they reached a constant weight (about 7% moisture content) [11,17,18]. The dried rind was then crushed with a mortar, pestle, and ground into a fine powder. The powder was dried again at 60°C for 3 hours in the laboratory oven to ensure that it was completely dry [19]. The powder was then stored in a dark, sealed glass bottle in a freezer to protect it from light until the analyses were carried out [20].

The seeds were also washed with tap water and deionized water. The washed seeds were then divided into two groups. The first group of seeds was dried at 60°C in a laboratory oven with air circulation until they reached a constant weight (about 7% moisture content). They were then screened to remove empty and deformed seeds, later on they were divided into two groups: one group was left untreated (raw seeds) and the other group was roasted at 160°C for 10 minutes with hot air circulation (roasted seeds).

The second group of seeds was soaked in deionized water (at a ratio of 1 seed: 5 water) at 25°C for 24 hours (12 hours of daylight and 12 hours of darkness). This process simulated the traditional soaking process used in Egypt. The soaked seeds were then dried at 60°C in a laboratory oven with air circulation until they reached a constant weight (about 7% moisture content). The dried soaked seeds were also screened to remove empty and deformed seeds. They were then divided into two groups: one group was left untreated (soaked seeds) and the other group was roasted at 160°C for 10 minutes with hot air circulation (soaked roasted seeds) [20].

Finally, the treated seeds in each group were dehulled (the outer layer was removed) and ground into a fine powder. The powder of the dried seeds in each group was stored in a dark, sealed glass bottle in a freezer to protect it from light until the analyses were carried out.

2.3.2. Determination of Tocopherols

Tocopherols were determined in raw, soaked, roasted, and soaked roasted seeds of mature summer squash (*Cucurbita pepo*) fruits using high-performance liquid chromatography (HPLC).

The samples were prepared by weighing 1 gram of each sample into a 250-mL volumetric flask. Then, 10 mL of dichloromethane was added and the sample was mixed for 10 minutes. Next, 30 mL of HPLC-grade methanol was added to each sample to make the final volume 40 mL. The mixture was then mixed for another 10 minutes. The mixture was centrifuged at 3000 rpm for 30 minutes and filtered through Whatman filter paper number 1. The supernatant was then filtered through a 0.45-µm syringe filter and set aside in a flask to be injected into the HPLC.

The HPLC analysis was performed using a Shimadzu SCL-10Avp HPLC equipped with a fluorescence detector (Shimadzu, Japan). A portion of 25 µL of sample was injected and the oven temperature was maintained at 40°C. The chromatographic separation was carried out using a C18 column (150 × 4.6 mm). The mobile phase consisted of acetonitrile HPLC grade (eluent A) and methanol HPLC grade (eluent B).The separation of tocopherols was achieved by isocratic elution (50% acetonitrile and 50% methanol) with a flow rate of 1.0 mL/minute for 20 minutes. The fluorescence detection was set at wavelengths of 295 and 330 nm for excitation and emission, respectively.

The identity of the tocopherols in the samples was determined by comparing their retention times to those of standard tocopherols. Fifty milligrams of each standard tocopherol was dissolved in 50 mL of HPLC-grade methanol [19,21].

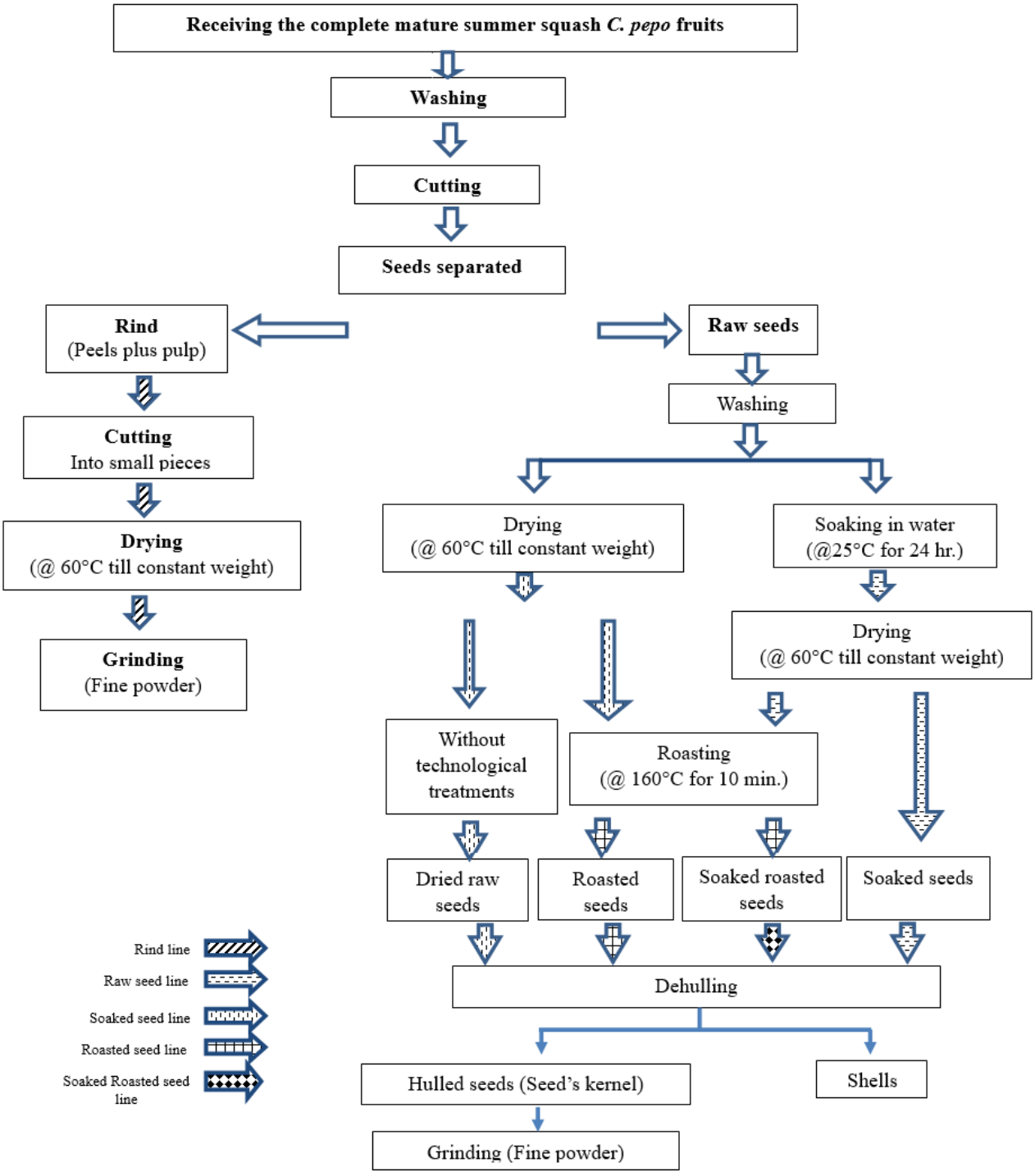

Figure 1. Flow chart illustrates the manufacturing process line of rind and hulled seeds tested throughout the study.

2.3.3. Determination of Total Carotenoids Content

To determine the carotenoid content of the rind and hulled seeds [18,22] of summer squash (*Cucurbita pepo*), 2 grams of each sample powder was mixed with 30 mL of acetone using a magnetic stirrer. The mixture was stirred for 10 minutes, then transferred to centrifuge tubes. The residue was washed with 10 mL of acetone until it became colorless. The mixture was centrifuged at 3000 rpm (Sigma laborzentrifugen, Germanay) for 30 minutes, then the material was filtered through Whatman filter paper number 1.

The filtrate was transferred to a separating funnel, and 30 mL of petroleum ether and 100 mL of distilled water were added. The two phases were allowed to separate, and the petroleum ether phase was transferred to a volumetric flask. The absorbance of the petroleum ether extract was measured at 450 nm using a UV-Vis spectrophotometer (Laxco-Alpha 1506, WA, USA).

The total carotenoid content of the samples was determined using the following equation:

Total carotenoids content (µg/g) = A × volume (mL) × 104 / A1%1cm × sample weight (g)

where:

- A = the absorbance of the sample
- Volume = 30 (the total volume in milliliters of petroleum ether extract)
- A1%1cm = 2592 (the absorption coefficient of β-carotene in petroleum ether)

2.3.4. Determination of Total Phenolic Content

Five grams of each powder sample was soaked in 50 mL of methanol for 24 hours in an orbital shaker. The samples were then centrifuged at 3000 rpm for 30 minutes and filtered through Whatman filter paper number 1. The filtered extracts were stored frozen at -20°C until the analysis is carried out. The total phenolic content of the extracts was determined using the Folin–Ciocalteu reagent. 0.5 mL of each extract was transferred to a suitable volumetric flask, then 0.5 mL of 0.5 N Folin–Ciocalteu reagent was added. 1 mL of sodium carbonate (75 g/L) was added to make the medium alkaline, which is necessary for the oxidation-reduction reaction between the phenolic compounds and the reagent. Then, 8 mL of distilled water was added to make the final volume 10 mL.

In the case of rind extracts, 0.25 mL of the extract was diluted with 0.25 mL of methanol before going through the same procedures to obtain an adequate absorbance. The mixtures were kept at 25°C for 2 hours before measuring their absorbance at 760 nm using a UV-Vis spectrophotometer against a blank. A calibration curve was prepared using a gallic acid solution, and the total phenolic content of the samples was calculated as gallic acid equivalents (GAE) in mg/100 g of sample. Three replicates of absorbance values were recorded, and the results were expressed as mean ± standard deviation [11,23].

2.3.5. Determination of Flavonoid Content

The total flavonoid content of the methanolic extracts of rind and hulled seeds was determined using the $Al(NO_3)_3$-$NaNO_2$ method. A 2 mL aliquot of each extract was transferred to a volumetric flask. Then, 0.6 mL of 5% $NaNO_2$ was added and mixed for 6 minutes. After that, 0.5 mL of 10% $Al(NO_3)_3$ was added and mixed well for 6 minutes. Next, 3 mL of 4.3% NaOH was added and mixed as well. Finally, 3.9 mL of distilled water was added to make the final volume 10 mL.

All samples were kept at 25°C for 15 minutes before measuring the absorbance at 500 nm using a UV-Vis spectrophotometer. A blank was used to subtract the absorbance of the reagent blank. A calibration curve was prepared using rutin solution, and the total flavonoid content was calculated as rutin equivalents in mg/100 g of sample. Three replicates of absorbance values were recorded, and the mean was considered. Flavonoids were calculated as rutin equivalent (RE) in mg/100 g sample [24].

2.3.6. Determination of Antioxidant Activity

The DPPH radical scavenging activity of the methanolic extracts of rind and hulled seeds was examined. Several concentrations of the extracts were reacted with 2,2-diphenyl-1-picrylhydrazyl (DPPH) at room temperature. Different volumes of the prepared extracts (0.2, 0.4, 0.6, 0.8, and 1 mL) were transferred to 10 mL volumetric flasks. Then, 1 mL of DPPH (0.002% in methanol) was added to each volumetric flask, and the final volume was made to 5 mL with methanol. In the case of raw and treated hulled seeds, the volume of extracts was increased to 0.4, 0.8, 1.2, 1.6, and 2 mL to get adequate absorbance. All samples were left to stand in the dark for 30 minutes [25].

The absorbance of the samples was measured at 517 nm using a UV-Vis spectrophotometer. The scavenging activity was calculated using the following equation:

DPPH scavenging activity (%) = (A0 - A1) × 100 / A0

where:

- A0 = absorbance of blank
- A1 = absorbance of the sample

The higher the DPPH scavenging activity, the greater the antioxidant capacity of the extract.

2.3.7. Statistical analysis

Analytical determinations for the samples were performed in triplicate, and the data were represented as mean values ± standard deviations (SD). The data were subjected to one way ANOVA with Tukey's test ($p< 0.05$) using SPSS program version 21.0 (SPSS Inc., Chicago, IL, USA)) to determine the significant difference between the mean values.

3. Result and Discussion

3.1. Tocopherol(s) in Raw Hulled Seeds of Mature Summer Squash

Total tocopherols (vitamin E) were examined in raw hulled seeds, which contain high level of lipids (46.65 %) compared to rind, which has a less fat content (2.69%). The concentration of α-, β- and γ-tocopherol was detected by HPLC (Figure 2-**a**). It is amounted to 45.08, 23.95, and 32.60 mg/100g raw hulled seeds weight (SW) respectively, with total concentration 101.63 mg/100g (Figure 3). The α-tocopherol was the highest (44%) class of the total tocopherols ($p<0.05$), followed by γ-tocopherol (32%), and β-tocopherol (24%).

The concentration of tocopherols in the raw hulled seeds was significantly higher compared to the Dietary Reference Value (DRV) of 13 mg/day [26], which means that 12.8 g of raw hulled seeds would be enough to cover the recommended daily intake of vitamin E. Hence, summer squach seeds and their oil can be outstanding sources of vitamin E [9,27]. In parallel to present results, Eltohamy and Ibrahim [28] reported that two types of an old Egyptian summer squash (Shamamy and Cop) had oil contents in range of 40.4-43 %. The high concentration of tocopherols in summer squash seeds is attributed to their abundant oil content. Oil serves as an effective solvent for tocopherols, and the high oil content in summer squash seeds aids in perserving the tocopherols [9,27].

The results of this study are in line with previous studies that have shown that the tocopherol content of summer squash seeds is high. A study by Akin et al. [29] found that the total tocopherol content of *Cucurbita pepo* seeds harvested from Anatolia regions, Turkey was 94.89 mg/100g. Lower concentrations were reported in studies carried in Slovenia (45.4 to 70.9 mg/100g oil), Serbia

(38.02 to 64.10 mg/100g), and Tunisia (29.49 to 39.73 mg/100g) [9,22,30]. This difference of tocopherol concentration between studies reflects the effects of plant varieties, soil type, weather, and other environment conditions.

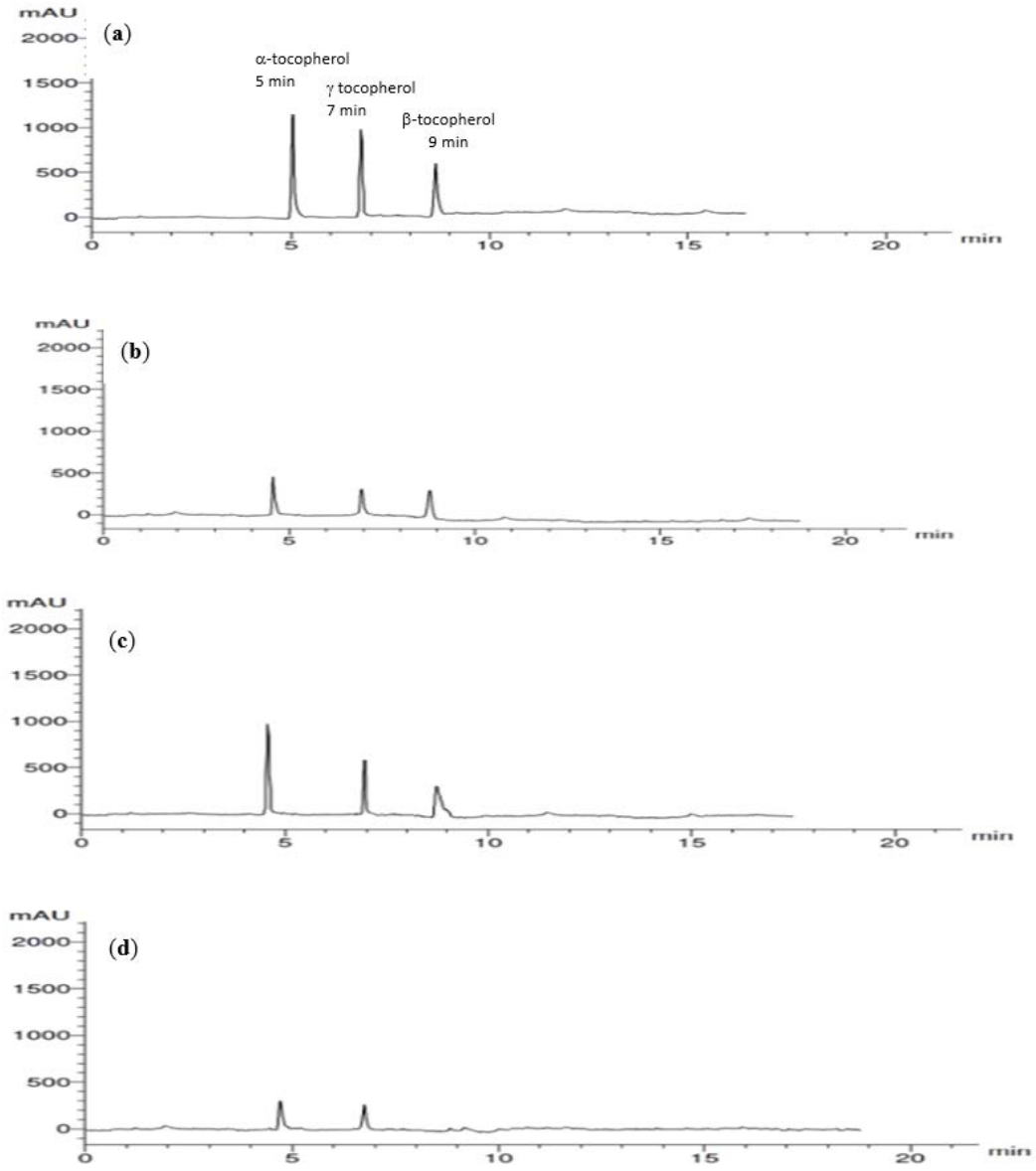

Figure 2. Presence of α-, β- and γ-tocopherol in raw hulled seeds of mature summer squash (a). And in seeds after soaking (b), roasting (c), and soaking followed by roasting (d).

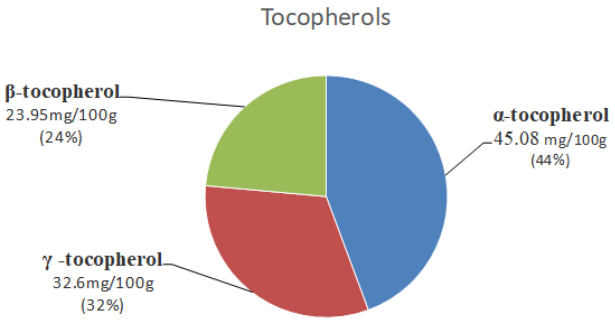

Figure 3. Tocopherol(s) composition of raw hulled seeds of mature summer squash (*Cucurbita pepo).* Means followed by superscript different letters are significant at p>0.05 levels according to LSD analysis

The tocopherols in summer squash seeds have several health benefits. They act as antioxidants, which mean that they help protecting the body from damage caused by free radicals. Free radicals are unstable molecules that can damage cells and tissues. Tocopherols can also help to lower cholesterol levels and protect against heart diseases [31]. Overall, the results of this study suggest that summer squash seeds are good source of tocopherols and have several health benefits.

3.2. Total Carotenoids in Rind and Raw Hulled Seeds of Mature Summer Squash

The rind contained 2.46 mg/100g fruit weight (FW), while the raw hulled seeds contained 4.15 mg/100g seed weight (SW) (Table 1). One molecule of β-carotene is

converted into two molecules of vitamin A in the human body. Therefore, consuming 100g of the rind would provide an adult male with 702.85% of the Dietary Reference Value (DRV) of vitamin A (700µg/day). This goes up to 1185.71% and 2542.86% with daily consumption of 100g of raw hulled seeds and their oils, respectively. These results suggest that mature summer squash, whether the rind, raw hulled seeds, or their oil can be an excellent source of vitamin A. The results of this study are in line with previous studies that have shown that the carotenoid content of summer squash is high. For example, a study by Akin et al. [29] found that the total carotenoid content of four samples of cold-pressed oil obtained from *Cucurbita pepo* seeds cultivated in Anatolia regions, Turkey ranged from 6.95 to 7.60 mg/100g oil. Low content of carotenoids (0.29 mg/100g oil) was found in warehouse cultivated plant in Poland [32]. However, it is important to note that the carotenoid content of summer squash can vary depending on the variety of squash, the growing conditions, and the ripeness of the fruit. Overall, the results of this study suggest that mature summer squash is a good source of vitamin A and can be a healthy addition to the diet.

Table 1. Total phenolic content, flavonoids and antioxidant activity of rind and raw hulled seeds of mature summer squash *(Cucurbita pepo)*.

Characteristics	Rind	Raw hulled seeds
Total carotenoids	2.46±0.56[b]	4.15±0.74[a]
Total phenolic content (mg GAE /100g sample powder)	1622.95±2.35[a]	124.59±1.12 [b]
Flavonoid content (mg RE/100g sample powder)	9.81±0.55 [a]	5±0.30 [b]
Antioxidant activity IC_{50} (mg)	18± 0.75[b]	247±1.60 [a]

*Means in the same row followed by superscript different letters are significant at p>0.05 levels according to LSD analysis.

3.3. Total Phenolic Content

Table 1 illustrates the concentration of total phenolic compounds in the rind of mature summer squash (1622.95 mg GAE/100g) was significantly higher ($p<0.05$) compared to the raw hulled seeds (124.59 mg/100g). Nawirska-Olszanska et al. [33] found that the concentration of total phenolics in *Cucurbita pepo* seeds varied depending on the variety. For example, the Junona variety had a total phenolic content of 82.4 mg GAE/100g, while the Miranda variety had a total phenolic content of 113 mg GAE/100g.

The level of total phenolics in the peel of pumpkin also varies depending on the variety, species, and extraction solvent. Gaweł-Beben et al. [34] found that the total phenolic content of pumpkin peel ranged from 462.3 to 1759.9 mg GAE/100g.

The high concentration of total phenolic compounds in mature summer squash is likely due to the presence of various phenolic compounds, including flavonoids, stilbenes, and lignans. These compounds have antioxidant and anti-inflammatory properties, which may contribute to the health benefits of summer squash. In conclusion, the rind of mature summer squash has a significantly higher concentration of total phenolic compounds compared to the raw hulled seeds. This suggests that the rind may be a more concentrated source of these beneficial compounds.

3.4. Total Flavonoids Content

The rind of mature summer squash contains significantly more total flavonoids (9.81 mg RE/100g) than the raw hulled seeds (5.0 mg/100g) as presented in Table 1. This difference was statistically significant ($p<0.05$). The high concentration of total flavonoids in the rind of mature summer squash might be due to the presence of various flavonoids, such as quercetin, kaempferol, and rutin. These compounds have antioxidant and anti-inflammatory properties, which may contribute to the health benefits of summer squash.

The high concentration of total flavonoids in the rind of mature summer squash suggests that it may be a good source of these beneficial compounds. However, more research is needed to confirm these findings and identify the major compounds. In addition to the high concentration of total flavonoids, the rind of mature summer squash also contains a variety of other beneficial compounds, including carotenoids, vitamin C, and vitamin A. These compounds may contribute to the health benefits of summer squash, including reducing the risk of cancer, heart disease, and other chronic diseases. While there is limited information available about the flavonoid content and types in *Cucurbita pepo* flesh and seeds, the results of this study suggest that the rind may be a more concentrated source of these beneficial compounds.

A study by Peng et al. [20] found that the Chinese seed flower contains even higher levels of total flavonoids at 70 mg RE/100g. This suggests that the flower may be an even more concentrated source of these beneficial compounds compared to the rind.

Overall, the results of this study suggest that the rind and flower of mature summer squash may be good sources of flavonoids and other beneficial compounds. More research is needed to confirm these findings and to determine the specific health benefits of these compounds.

3.5. Antioxidant Activity

Table 2. The percentage of DPPH radical scavenging activity for the methanolic extract of rind and raw hulled seeds of mature summer squash (Cucurbita pepo).

Concentration (mg)	DPPH radical scavenging activity %	
	Rind	Raw hulled seeds
20	59.49±3.34 [a]	11.20±1.77 [b]
40	61.39±2.65 [a]	14.58±2.23 [b]
60	62.66±1.88 [a]	14.60±2.78 [b]
80	80.38±3.45 [a]	19.89±1.88 [b]
100	82.28±2.90 [a]	20.50±2.34 [b]

*Means in the same row followed by superscript different letters are significant at *p*>0.05 levels according to LSD analysis.

Table 3. The effect of different technological treatments on tocopherol fractions in hulled seeds (SW) of mature summer squash (*Cucurbita pepo)* fruit.

Tocopherol (s)	Technological treatments (mg/100 g SW)			
	Raw	Soaking (25°C/24 hr)	Roasting (160°C/10 min)	Soaking + Roasting
α-tocopherol	45.08±0.34[a]	19.58±0.22[c]	33.35±0.19[b]	12.68±o.42[d]
β-tocopherol	23.95±0.12[a]	8.35±0.56[c]	20.28±0.25[b]	0.0 [d]
γ-tocopherol	32.60±0.45[a]	7.72±0.37[c]	28.43±0.32[b]	5.66±0.22[d]
Total tocopherols	101.63±0.57[a]	35.65±0.75[c]	82.06±0.90[b]	18.34±0.85[d]

*Means in the same row followed by superscript different letters are significant at $p>0.05$ levels according to LSD analysis.

The antioxidant activity of the methanolic extract of rind powder was significantly higher ($p < 0.05$) than the methanolic extract of raw hulled seeds. The inhibitory concentrations for half of the free radicals (IC_{50}) of the methanolic extracts were 18 and 247 mg for rind and raw hulled seeds, respectively (Table 1). The DPPH radical scavenging activity of rind methanolic extract was 59.49%, 61.39%, 62.66%, 80.38%, and 82.28% for 20, 40, 60, 80, and 100 mg of rind, respectively as presented in Table 2. The DPPH radical scavenging activity of raw hulled seeds methanolic extract was 11.20%, 14.58%, 14.60%, 19.89%, and 20.50% for 20, 40, 60, 80, and 100 mg of raw hulled seeds, respectively (Table 2).

The results of the antioxidant activity in rind and raw hulled seeds were consistent with the determined amounts of phenolic content and flavonoids. The high antioxidant activity of rind can be explained by the dual action of phenolic compounds and flavonoids.

The results of this study suggest that the rind of mature summer squash is a good source of antioxidants. The high antioxidant activity of rind is likely due to the presence of various phenolic compounds and flavonoids, which have been shown to have several health benefits, including reducing inflammation, lowering blood pressure, and protecting against cancer.

The results of this study are consistent with the findings of previous studies. For example, a study by Nyam et al. [35] found that the rind of Cucurbita pepo has a higher level of total phenolic compounds and antioxidant activity than the seeds.

3.6. Effect of Different Technological Processes on Bioactive Components

3.6.1. Tocopherol Content

In the present study, the soaking for 24 hrs at 25C° had a significante effect ($p<0.05$) on the tocopherol level in raw hulled seeds to reach 19.58, 8.35, and 7.72 mg/100g of α-, β- and γ-tocopherol, respectively in soaked seeds (Table 3 and Figure 2-b, c and d). The obtained results illustrate that the highest effect on tocopherol content was due to the combining effect of soaking and roasting processes (81.95%). It is worth notably that the soaking process destructs more vitamin E (64.92%) compared to the roasting process (19.25%).

This is proportional to destruction rate reached to 56.56%, 65.14%, and 76.32 % in the three tocopherol classes, respectively. It is noticed γ-tocopherol was the highest affected fraction followed by β- and α- tocopherol, which was the least affected. After roasting (160C°/10 min), the level of α-, β- and γ-tocopherol in roasted hulled seeds was diminished significantly ($p<0.05$) to 33.35, 20.28, and 28.43 mg/100g, respectively. The amount of α-, β- and γ-tocopherol determined after roasting was reduced by 26.02%, 15.32%, and 12.79% respectively, as compared to the original contractions in raw seeds (Figure 2). The roasting had affected the tocopherol fractions in the same trend as in soaking treatment, e.g. γ- > β- > α-tocopherol.

Moreover, the successive application of roasting after soaking led to the lowest level of tocopherol. The concentration of α- and γ-tocopherol decreased to 12.68 and 5.66 mg/100g, respectively; while β-tocopherol was completely eliminated in the soaked roasted seeds. The quantity of α-, β- and γ-tocopherol destructed by soaking followed by roasting were 71.85, 100.0, and 82.64%, respectively of the original levels in seeds without treatments (Figure 2).

These results showed that the soaking treatment had the greatest effect on the tocopherol content. The same effect of soaking was reported in sorghum by Afify et al. [36]. In sorghum, the soaking for 20 h at room temperature dropped the tocopherols by more than 20%. It's well known that pre-soaking of raw pumpkin seeds neutralizes anti-nutrients, e.g. phytate and degraded the membrane enzymes. This vital process will please the stomach and improve nutrients absorption. It is recorded that during soaking the respiration increased led to drop the nutrients level, which had been reserved in seeds [37]. The reduction of vitamin E also might be attributed to its protective role against lipid oxidation.

Similar effect of roasting was indicated in various reports [32,38,39]. The diminished total tocopherols was more pronounced in gourd pepo oil seeds, leveled to 36% @140°C/5 min [39]. Loss of different tocopherol fractions during roasting was showed to be varied between studies. It seems to be that heat stability of tocopherol fractions during roasting process is depending on temperature, exposure time, and matrix content.

3.6.2. Carotenoids

The total carotenoids content in hulled seeds powder is altered throughout the application of technological treatments, and it changed significantly ($p<0.05$) from 4.15 mg/100g of raw hulled seeds to 2.66, 3.97 and 2.89 mg/100g of hulled soaked, roasted, and soaked roasted seeds, respectively as shown in Table 4.

Table 4. The effect of different technological treatments on the level of carotenoids, total phenolic content, flavonoids, and antioxidant activity of hulled seeds of mature summer squash (*Cucurbita pepo*).

Characteristics	Technological treatments			
	Raw	Soaking	Roasting	Soaking + roasting
Total carotenoids	4.15 ±0.20[a]	2.66 ±0.25[b]	3.97±0.27 [a]	2.89±0.30 [b]
Total phenolic content (mg GAE/100g powder sample)	124.59±2.40[c]	95.08±3.44[d]	150.82±2.66 [b]	175.41±2.54 [a]
Flavonoids content (mg RE/100g powder sample)	5±0.59 [a]	1.15±0.11 [c]	3.08±0.23 [b]	0.77±0.16 [d]
Antioxidant activity (IC_{50} mg)	247±3.56 [a]	102±3.12 [d]	140± 2.11[b]	116±3.07 [c]

*Means in the same row followed by superscript different letters are significant at $p>0.05$ levels according to LSD analysis.

On the other hand, the total carotenoid content in mature summer squash seeds is subsequently at its lowest concentration. The concentration of total carotenoids reduced significantly ($p<0.05$) by soaking and roasting process, consequently all the treated seeds have lower concentration of carotenoids compared to raw seeds. The level of total carotenoids decreased by 35.90, 4.04, and 30.36% for soaked, roasted, and soaked roasted seeds, respectively, compared to the main contractions in raw seeds. The highest effect on total carotenoid content was found in the soaked seeds (2.66 mg/100g). On the contrary, roasting had no impact on total carotenoids content compared to raw hulled seeds ($p<0.05$).

There is no information in the literature about the effect of pre-soaking and roasting on carotenoids in pepo seeds. In pearl millet, β-carotene content was stable after 9 hr. of soaking, however after 12 hr. the carotene was reduced [40]. In agreement with the present results, Vadya and Choe [41] found that heating of mustard seeds at 160°C was not significantly affected carotenoid content.

3.6.3. Total Phenolic Content and Flavonoids

The total phenolic content in *Cucurbita pepo* seeds varied with significant differences ($p<0.05$) after applying technological treatments from 124.59 mg Gallic acid /100g in raw hulled seeds to 95.08, 150.82 and 175.41 mg Gallic acid /100g of soaked, roasted, and soaked roasted hulled seeds, respectively, as shown in Table 4. Further, total flavonoids content decreased with significant difference ($p<0.05$) after applying technological treatments on seeds. It reduced from 5 mg Rutin/100g of raw hulled seeds to 1.15, 3.08, and 0.77 mg Rutin/100g of soaked, roasted and soaked roasted hulled seeds correspondingly (Table 4). The soaking process decrease phenolics by 23.68% and flavonoids by 77%. Numerous studies documented the effect of soaking on the loss of phenolic content in different grains and legumes [36,40,42,43]. The reduction of phenolics was explained through the leaching into the soak water [44,45]. Another mechanism attributed to binding of some polyphenols with organic matters e.g. carbohydrates and proteins [46,47]. Moreover, the oxidation process during soaking could lead to the activation of polyphenoloxidase, which will result in degradation of phenolic compounds [48,49].

Exposing raw seeds to the heat of the roasting process increased ($p<0.05$) the phenolic content in the roasted seeds. As well, exposing the soaked seeds to the roasting process elevated the total phenolic content in the soaked roasted seeds. The obtained results showed that the roasting process increases the level of phenolics by 21%, while it decreases the level of flavonoids by 38.4%. Several investigators showed that roasting was enhancing polyphenolic content in pumpkin seeds [20,50,51]. Two mechanisms have been proposed. The first is assuming that roasting caused cell membrane destruction and led to release bound phenols [20]. The second is attributed to Millard reaction products which could be interacted with FC reagents ended to enhance polyphenol content. Total phenolic content was amplified more in soaked roasted seeds compared to roasted seeds. These results could be clarified through the increase in proteolytic activity during soaking, leading to the increase in hydrolyzed products of protein, which liberated amino acids [52,53] accordingly elevated more Millard reaction products than only roasted seeds. The increase in overall phenolic content is higher in soaked roasted seeds compared to roasted seeds.

3.6.4. Antioxidants

The effect of soaking and roasting alone or in combination on the total antioxidants are presented in Table 4 and Figure 4. The results showed increase, with significant differences ($p<0.05$) in the antioxidant activity of the processed seeds vs. raw ones. The methanolic extract of soaked seeds had the highest DPPH radical scavenging activity by 58.7%. The lowest IC50 belonged to the methanolic extract of soaked seeds (102 mg) compared to the IC50 of the methanolic extract of raw hulled seeds (247 mg), roasted seeds (140 mg), and soaked roasted seeds (116 mg/ml) as shown in Table 4.

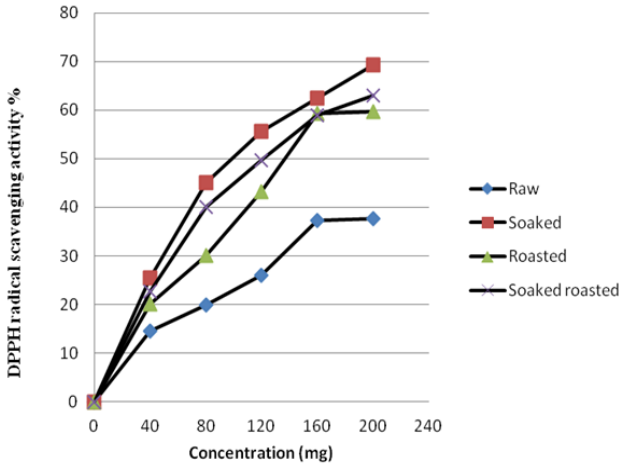

Figure 4. Effect of different technological treatments on the antioxidant activity of methanolic extracts of hulled seeds of mature summer squash (Cucurbita pepo) fruits.

An increase was found in the antioxidant activity of soaked seeds (Figure 4), besides a change in the sensory characteristics. The increase in the antioxidant activity of soaked seeds can be explained through the action of

hydrolytic enzymes, which made a partial hydrolysis in the protein of soaked seeds as well as the activity of antioxidant enzymes [54]. Nkosi et al. [55] found that the protein isolated from *Cucurbita* seeds showed about 80% radical scavenging activity and chelating activity. In *Cucurbita pepo* oil cake protein isolate, which was hydrolyzed using two food grade enzymes (alcalase and trypsin), could be working as anti-oxidative agent from nature with strong pH and thermal stability [56]. Ming-Tai et al. [57] stated that D-methionine, such as sulfur-containing amino acid, can act as a powerful antioxidant. Thus, it can be a chemo-protectant in patients getting cisplatin as part of a chemotherapy regimen.

Peng et al. [20] reported that the roasting process over range from 120 to 200˚C for 10 min enhanced the phenolics content and antioxidant property of *Cucurbita pepo* seeds. The outcomes showed that overall phenolic compounds and total antioxidant capacity amplified as the roasting temperature increased. Consequently, the increasing in overall phenolics content is one of the most important causes for the enhancement of antioxidant activity of the roasted seeds. On the other hand, Millard reaction products significantly promoted the antioxidant capacity of roasted seeds. In the study of Minh [58] on *Cucurbita* seeds, the results showed that there were noteworthy changes between unroasted and roasted samples. Total phenolic content and scavenging activity were less in unroasted seeds compared to roasted ones.

4. Conclusion

Raw mature summer squash seeds can be outstanding source of tocopherols (vitamin E) and carotenoids (vitamin A), while rind can be an excellent spring of phenolic compounds which have a great antioxidant capacity comparing to seeds. The level of total tocopherols, total carotenoids, total phenolics, and total flavonoids in mature summer squash seeds was affected by the applied technological treatments. Roasting of seeds had the lowest impact on the level of total tocopherols, total carotenoids, and total flavonoids compared to soaking. Soaking of mature summer squash seeds in water decreased the level of total carotenoids and total phenolic compounds to the lowest level. On the other hand, combination of soaking and roasting led to a decrease in the levels of total tocopherols and total flavonoids, reaching to the lowest value. In contrast, an increase with significant differences in antioxidant activity was found in all the processed seeds. The highest antioxidant activity was found in the soaked seeds followed by soaked roasted seeds. Roasted seeds had antioxidant activity higher than raw seeds but lower than soaked and soaked roasted seeds. The great antioxidant activity of soaked seeds might be linked to the hydrolytic products of protein, as the local technological process of mature summer squash seeds enhances the amount of total phenolics, while essential nutrients like vitamin E, carotenoids and flavonoids are affected. The analysis of rind indicated the importance of using it as nutrional supplement, especially for its ability as powerful antioxidants. More investigations are needed in order to maintain and maximize these important compounds, while maintaining the best organoleptic qualities.

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