Endogenous Antioxidants and Stability of Sesame Oil As Affected by Processing and Storage

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ABSTRACT: The effect of processing of coated and dehulled sesame seeds on the content of endogenous antioxidants, namely sesamin, sesamolin, and γ-tocopherol in hexane-extracted oils, was studied over 35 d of storage under Schaal oven test conditions at 65°C. Seeds examined were Egyptian coated (EC) and dehulled (ED) and Sudanese coated (SC) varieties. Processing conditions of raw (RW) seeds included roasting at 200°C for 20 min (R), steaming at 100°C for 20 min (S), roasting at 200°C for 15 min plus steaming for 7 min (RS) and microwaving at 2450 MHz for 15 min (M). The sesamin content in fresh oils from EC, ED, and SC raw seeds was 649, 610, and 580 mg/100 g oil, respectively. Corresponding values for the content of sesamolin in oils tested were 183, 168 and 349 mg/100 g oil, respectively. Meanwhile, the content of γ-tocopherol, the only tocopherol present in the oils, ranged from 330 to 387 mg/kg sample. The effect of processing on changes in the sesamin content in oils from coated seeds was low and generally did not exceed 20% of the original values. On the other hand, oils from dehulled seeds underwent a more pronounced decrease in their sesamin content than the oil from coated seeds after 35 d of storage at 65°C. The corresponding changes in sesamolin and γ-tocopherol contents were more drastic. The RS treatment, which would be the optimal to prepare sesame oil with better quality, was found to retain 86, 80 and 60% of the sesamin, sesamolin and γ-tocopherol, respectively, originally present in the seeds after the storage period. The loss in the content of endogenous antioxidants present in the oils paralleled an increase in their hexanal content.

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KEY WORDS: Antioxidants, hexanal, oxidative stability, sesame oil, sesamin, sesamolin, γ-tocopherol.

Roasted and unroasted sesame seed oils are widely used in the East Asian and Middle Eastern countries. Many of the special properties of sesame oil are due to the presence of 0.4–1.1% sesamin, 0.3–0.6% sesamolin, and traces of sesamol in the oil (1). Both sesamin and sesamolin have the same 2,7-dioxabicyclo-(3,3,0)-octane backbone with two 3,4-methylene dioxyphenyl substituents. These components along with γ-tocopherol confer superior oxidative stability to sesame oil as compared to other sources of vegetable oils. Sesamol, which is usually present in trace amounts in the oil, may be released from sesamolin by hydrogenation, by bleaching earth, or by other conditions of processing and storage (2). However, Fukuda *et al*. (3) have suggested that the main active antioxidative constituent in fresh sesame oil extracted from roasted seeds is γ-tocopherol. After heating at frying temperatures for 1–2 h, sesamol, produced by hydrolysis of sesamolin, was present in the oil extracted from roasted seeds. According to Fukuda *et al.* (3), the content of tocopherol in sesame oil was not higher than that present in other oils (86, 56, and 80 mg/100 g of soybean, cottonseed, and corn oils, respectively). Possible synergistic action of sesame antioxidants in the oil from roasted seeds might be responsible for the superior oxidative stability of sesame oil.

Kamal-Eldin and Appelqvist (4) have reported that the contents of sesamin and sesamolin in oils from *Sesamum indicum* were 0.55 and 0.50%, respectively. These authors also determined the contents of sesamin and sesamolin in three wild species *S. alatum, S. radiatum,* and *S. angustifolium*. They found that *S. radiatum* was rich in sesamin (2.40%) but contained only minor amounts of sesamolin (0.02%); whereas *S. alatum* contained minor amounts of sesamin and sesamolin (0.01% each). The species *S. angustifolium* possessed reasonable amounts of both sesamin (0.32%) and sesamolin (0.16%). The content of γ-tocopherol in *S. alatum, S. radiatum, S. angustifolium,* and *S. indicum* was 210–320, 750, 800, and 490–680 mg/kg oil, respectively. According to Yoshida and Kajimoto (5), the contents of sesamin, sesamolin, and γ-tocopherol in sesame oils were 6824, 5642, and 576 mg/kg oil. The oxidative stability of sesame oil was further dependent on the roasting temperature (6). At roasting temperatures of 250–260°C, a strong flavored oil with a lower quality was obtained (7).

Although there are many assays available for assessing the oxidative stability of oils and fats, hexanal content may serve as an excellent marker for monitoring flavor deterioration of foods (8). Hexanal is a major breakdown product of linoleic acid, and its presence in oils rich in ω6 fatty acids is well documented (9). Moreover, the content of some aldehydes has been shown to correlate with food flavor deterioration (10).

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The main objectives of this work were to study the effect of processing on the endogenous antioxidants of sesame oil after extraction from seeds and storage for 35 d under Schaal oven test conditions. Flavor deterioration of oils was monitored by determining their hexanal contents during storage.

MATERIALS AND METHODS

Seeds from *S. indicum* L. (Giza 24 Egyptian variety and Gabaly Abiad Sudanese variety) were obtained from Sacs Company (Alexandria, Egypt). All chemicals used were obtained from Sigma (St. Louis, MO) or Aldrich Chemical Company (Milwaukee, WI) and were of ACS-grade or better quality.

Sample preparation. Sesame oils were prepared by extraction of 100 g of coated or dehulled seeds with 1000 mL of hexane in a Waring blender at 4°C. The extraction was repeated a second time, and combined extracts were desolventized using a rotary evaporator at 35°C. Seeds used were raw (RW) or subjected to roasting at 200°C for 20 min (R), steaming at 100°C for 20 min (S), roasting at 200°C for 15 min plus steaming at 100°C for 7 min (RS) or microwaving at 2450 MHz for 15 min (M).

Storage conditions. Oil (25 mL) samples were kept in open containers (50 mL conical flasks) in the dark in a Precision (Model 2) oven at 65°C for 35 d. Samples were removed for analyses on days 10, 20, and 35. Separate sample containers were used for each day of analysis. Analyses included determination of hexanal, sesamin, sesamolin, and γ-tocopherol contents.

Oil saponification. Oils were saponified by reflux in 50 mL of alcoholic potassium hydroxide (1 N) for 1 h. Unsaponifiables were extracted twice with 50 mL diethyl ether. The ether extracts were washed with 100 mL water and then dried over anhydrous sodium sulfate according to IUPAC (11). Ether was removed using a rotary evaporator at room temperature (22°C), and the residues were dissolved and kept in chloroform–diethyl ether (5 mL; 4:1, vol/vol) at −20°C for further analysis.

Thin-layer chromatography (TLC). Portions of the unsaponifiables (1.5 mg) were applied to TLC plates as 1.5 cm bands. The following mobile phase was used: chloroform/hexane/methanol (60:30:2, vol/vol/vol). The plates were visualized by spraying with 10% phosphomolybdic acid in ethanol/diethyl ether (1:1, vol/vol) and heating at 110°C for 5 min. Spots were identified against authentic standards (sitosterol, sesamin, sesamolin, sesamol, and γ-tocopherol) applied as reference spots on the two sides of the plate.

Purification of sesamin and sesamolin. One-hundred mL of sesame oil were passed through a packed alumina column (30 cm × 3.5 cm i.d., filled with 125 g alumina) and eluted with petroleum ether. Fractions were collected and examined through Badouins' test which involves heating 2 mL of the oil with 1 mL of concentrated hydrochloric acid containing 1% (wt/vol) sucrose. The portion being eluted immediately below the strong yellow band in the column was removed and continuously extracted with petroleum ether in a Soxhlet apparatus for 3 h (12). A yellow oil was obtained after removal of the solvent; it was saponified with 5% (wt/vol) alcoholic potassium hydroxide for 1 h. Water (100 mL) was added to the mixture, and the resultant solution was extracted three times with 30 mL portions of diethyl ether. Removal of the solvent afforded approximately 2 g of a yellow resin which was subsequently dissolved in 10 mL of diethyl ether and left overnight in a refrigerator whereupon 0.5 g crystalline sesamin was precipitated. Rod-like needles (m.p. 122°C) were obtained upon recrystallization of sesamin from ethanol. The residue, after removal of the ether, was dissolved in 1 mL chloroform, and petroleum ether was added until the onset of cloudiness. Sesamolin, separated as a white solid, was subsequently recrystallized from ethanol as white plates (m.p. 93°C)-yield 0.15 g.

High-performance liquid chromatography (HPLC) analyses. Sesamin and sesamolin were analyzed using a Shimadzu SCL-6B HPLC equipped with a Shim-pack CLC-ODS (M) column (250 cm × 4.6 mm i.d., 5 µm film; Merck, Darmstadt, Germany); a Shimadzu UV-VIS detector; and a Shimadzu Chromatopac CR 501 recording data processor. Samples were filtered through 0.45 µ nylon (Fisher Scientific, Nepeon, ON, Canada) prior to injection onto the column. The mobile phase was a mixture of methanol–deionized water (70:30, vol/vol) at 0.8 mL/min. Peaks were detected at 290 nm. Oils (1.1 g) were dissolved in 10 mL of the mobile phase, 10 µL of which were used for analysis. Quantitation of sesamin and sesamolin was carried out using standard samples. Standards were separated by crystallization and identified by mass spectrometric means.

Tocopherols in the oil were analyzed using normal-phase HPLC using an LC-6A pump; an SPD-6AV ultraviolet (UV)-visible spectrophotometric detector; an SCL-6B system; and an LiChosorb Si 60 (4 cm × 25 mm i.d., 5 µm film; Merck) column. The mobile phase was a mixture of diethyl ether/hexane (5:95, vol/vol) at a flow rate of 1.5 mL/min. Peaks were detected at 290 nm. Oils (1.0 g) were dissolved in 10 mL of mobile phase, 10 µL of which were used for analysis. The amount of each compound was calculated from responses obtained for each standard.

Headspace hexanal analysis. A Perkin-Elmer 8500 gas chromatograph equipped with an HS-6 headspace sampler (Perkin-Elmer Corp., Montréal, PQ, Canada) was used for hexanal analysis of fresh and stored oils. A Supelcowax 10 fused-silica capillary column (30 m × 0.32 mm i.d., 0.10 µm film, Supelco Canada Ltd., Mississauga, ON) was used. The injector and flame-ionization detector (FID) temperatures were 280°C. Helium was the carrier gas employed at an inlet column pressure of 17.5 psig with a split ratio of 7:1. The oven temperature was maintained at 40°C for 5 min and then increased to 115°C at 10°C/min, held there for 1 min, and then ramped to 200°C at 30°C/min and held for 30 min (13).

For headspace analysis, 0.2 g of sesame oil were transferred to 5 mL glass vials which were capped with PTFE butyl septa, crimped, and then subjected to analysis. Vials were preheated in the HS-6 magazine assembly at 90°C for a

45-min equilibration period. Pressurization time was 6 s, and the volume of the vapor phase drawn was approximately 1.5 mL. Individual volatile compounds were tentatively identified by comparing relative retention times of GLC peaks with those of commercially available standards. Quantitative determination of hexanal, the dominant aldehyde present, was accomplished using 2-heptanone as an internal standard (13).

Statistical analyses. All measurements were replicated three times; mean values ± SD were reported for each case. Analysis of variance and Tukey's studentized range test were performed on Statistical Analysis System (14) at a level of $P < 0.05$ to evaluate the significance of differences between mean values. *T*-tests were used to compare means of fresh and stored oils after respective treatments.

RESULTS AND DISCUSSION

One-dimensional TLC for the separation of unsaponifiables obtained from sesame oil of Egyptian coated seeds is shown in Figure 1 using chloroform/hexane/methanol (CHM) (60:30:2, vol/vol/vol). This system (CHM) gave TLC patterns slightly different from those obtained by Kamal-Eldin *et al.* (15). In the CHM system, sesamin had a lower R_f value than those of sesamolin and γ-tocopherol. Similar results were obtained for oils from Sudanese coated and Egyptian dehulled seeds.

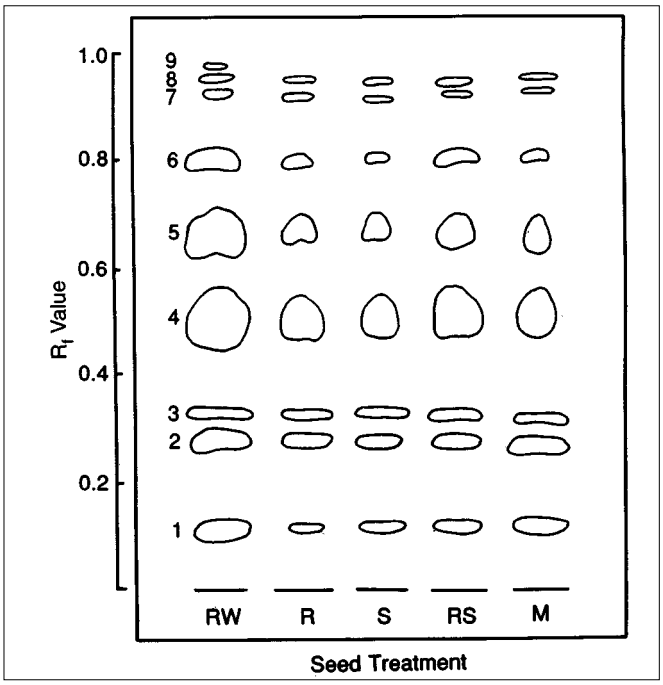

FIG. 1. One-dimensional thin-layer chromatography of unsaponifiables of sesame oil extracted from Egyptian coated seeds after different treatments roasting at 200°C for 20 min (R), steaming at 100°C for 20 min (S), roasting at 200°C for 15 min + steaming for 7 min (RS), microwaving at 2450 MHz for 15 min (M), and in the raw (RW) state, on silicagel 60 plates with chloroform/hexane/methanol (60:30:2, vol/vol/vol). Spots are: 2, desmethylsterols; 3, monomethylsterols; 4, sesamin; 5, seamolin; 6, γ-tocopherol, and (1, 7–9) unknowns.

The content of sesamin and sesamolin in oils from RW, roasted and steamed (RS), and microwaved (M) seeds was moderate as reflected in the size of the spots; corresponding spots for oils after R and S treatments were smaller, especially for sesamolin. This indicates larger degradation of sesamolin than sesamin, which is in agreement with the results reported by Fukuda *et al.* (16) and HPLC data in the present study (see below). On the other hand, no sesamol was detected on TLC plates in this study.

The HPLC separation of sesamin and sesamolin was successfully achieved as these appeared 16 and 21 min after the injection of sample, respectively. Identification of sesamin and sesamolin was done by comparing their retention times and mass spectrometry (MS) fragmentation patterns with those of the standards. Effects of different treatments on sesamin content in extracted oils are shown in Table 1. Sesamin was the dominant lignan present, representing 649 and 580 mg/100 g fresh oil from RW seeds of EC and SC varieties, respectively; after 35 d of storage at 65°C, its content decreased significantly ($P < 0.05$) to 584 and 471 mg/100 g, respectively. There was also a significant ($P < 0.05$) decrease in the content of sesamin in oils from 649 in the RW oil of EC seeds to 576, 601, 583, and 590 for R, S, RS, and M oils, respectively. The sesamin content of all oils decreased significantly ($P < 0.05$) following storage, but this decrease generally did not exceed 20% of the original quantity. The present results are in agreement with those reported by Yoshida and Kajimoto (5).

The sesamin content in oils from ED seeds was less than those of oils from its coated counterpart, 610 mg/100 g in the raw oil. This level decreased significantly ($P < 0.05$) to 489, 531, 555, and 520 mg/100 g oil after R, S, RS, and M treatments, respectively (Table 1). Moreover, the loss of sesamin was higher after storage of oils for 35 d at 65°C as it exceeded 30% in most cases.

There was a significant ($P < 0.05$) difference in the content of sesamolin in the oil from EC and SC seeds for most treatments (Table 2). Approximately twice the amount of sesamolin (349 mg/100 g) was present in the oil from SC seeds than that in the oil from EC seeds (183 mg/100 g). Moreover, the decrease in sesamolin in EC oils following storage was significantly ($P < 0.05$) greater than that from SC oils. In R treatment, EC oils during storage lost 50% of their sesamolin; the effect was less for other oils (32, 42, and 22% for S, M, and RS oils, respectively). However, for oils extracted from SC seeds, the loss of sesamolin after storage generally did not exceed 10%. The significant ($P < 0.05$) decrease in sesamolin of oils from ED seeds exceeded 50% for R and S treatments. The sesamolin content after M treatment of oils from EC and SC seeds was lower than any other treatment of fresh oil (123 and 267 mg/100 g, respectively) or after storage for 35 d (71 and 233 mg/100 g, respectively). This corresponds with results for the hexanal content of oils (see below). The decrease in the sesamolin content was greater than that of sesamin under the same conditions and after storage for both EC and ED oils. Fukuda *et al.* (16) re-

TABLE 1
Effect of Storage for 35 Days on the Content of Sesamin (mg/100 g) in Sesame Oil[a]

	SC		EC		ED	
Treatment	Fresh	Stored	Fresh	Stored	Fresh	Stored
RW	$580 \pm 18^{a,x}$	$471 \pm 12^{a,y}$	$649 \pm 20^{a,x}$	$584 \pm 15^{a,y}$	$610 \pm 21^{a,x}$	$461 \pm 16^{a,y}$
R	$537 \pm 20^{a,x}$	$327 \pm 11^{c\ y}$	$576 \pm 14^{b,x}$	$436 \pm 10^{d,y}$	$489 \pm 15^{c,xc}$	$315 \pm 14^{c,y}$
S	$541 \pm 14^{a,x}$	$390 \pm 13^{b,y}$	$601 \pm 18^{b,x}$	$514 \pm 14^{b,y}$	$531 \pm 16^{b,c,x}$	$325 \pm 13^{c,y}$
RS	$565 \pm 21^{a,x}$	$442 \pm 16^{a,y}$	$583 \pm 15^{b,x}$	$506 \pm 13^{b,c,y}$	$555 \pm 18^{b,x}$	$411 \pm 15^{b,y}$
M	$544 \pm 13^{a,x}$	$377 \pm 10^{b,y}$	$590 \pm 17^{b,x}$	$475 \pm 12^{c,y}$	$520 \pm 12^{b,c,x}$	$422 \pm 16^{b,y}$

[a]Under Schaal oven test conditions at 65°C, in hexane-extracted oil from Sudanese coated (SC), Egyptian coated (EC), and Egyptian dehulled (ED) seeds in the raw (RW) state and after roasting (R), steaming (S), roasting plus steaming (RS), and microwaving (M). Results are mean values of three determinations ± SD. Values in each column with different superscripts (a–c) are significantly ($P < 0.05$) different from one another. Values of fresh and stored oil with different superscripts (x and y) are significantly ($P < 0.05$) different from one another.

TABLE 2
Effect of Storage for 35 Days on the Content of Sesamolin (mg/100 g) in Sesame Oil[a]

	SC		EC		ED	
Treatment	Fresh	Stored	Fresh	Stored	Fresh	Stored
RW	$349 \pm 12^{a,x}$	$315 \pm 9^{a,y}$	$183 \pm 7^{a,x}$	$123 \pm 6^{a,y}$	$168 \pm 5^{a,x}$	$117 \pm 5^{a,y}$
R	$301 \pm 10^{b,x}$	$273 \pm 5^{c,y}$	$146 \pm 5^{b,x}$	$73 \pm 2^{c,y}$	$119 \pm 3^{c,x}$	$55 \pm 1^{d,y}$
S	$331 \pm 9^{a,x}$	$296 \pm 8^{b,y}$	$129 \pm 5^{c,x}$	$88 \pm 4^{b,y}$	$108 \pm 4^{d,x}$	$52 \pm 1^{d,y}$
RS	$327 \pm 8^{a,x}$	$305 \pm 7^{a,b,y}$	$146 \pm 6^{b,x}$	$115 \pm 7^{a,y}$	$139 \pm 4^{b,x}$	$106 \pm 3^{b,y}$
M	$267 \pm 6^{c,x}$	$233 \pm 5^{d,y}$	$123 \pm 3^{c,x}$	$71 \pm 3^{c,y}$	$129 \pm 2^{b,c,x}$	$75 \pm 2^{c,y}$

[a]Under Schaal oven test conditions at 65°C, in hexane-extracted oil from Sudanese coated (SC), Egyptian coated (EC), and Egyptian dehulled (ED) seeds in the raw (RW) state and after roasting (R), steaming (S), roasting plus steaming (RS), and microwaving (M). Results are mean values of three determinations ± SD. Values in each column with different superscripts (a–d) are significantly ($P < 0.05$) different from one another. Values of fresh and stored oil with different superscripts (x and y) are significantly ($P < 0.05$) different from one another.

TABLE 3
Effect of Storage for 35 Days on the Content of γ-Tocopherol (mg/100 g) in Sesame Oil[a]

	SC		EC		ED	
Treatment	Fresh	Stored	Fresh	Stored	Fresh	Stored
RW	$358 \pm 14^{a,x}$	$198 \pm 6^{a,y}$	$387 \pm 7^{a,x}$	$239 \pm 5^{a,y}$	$343 \pm 8^{a,x}$	$210 \pm 7^{a,y}$
R	$285 \pm 9^{c,x}$	$148 \pm 4^{d,y}$	$261 \pm 4^{e,x}$	$140 \pm 2^{e,y}$	$243 \pm 4^{d,x}$	$99 \pm 2^{d,y}$
S	$309 \pm 8^{b,c,x}$	$164 \pm 5^{c,y}$	$285 \pm 5^{d,x}$	$153 \pm 3^{d,y}$	$275 \pm 5^{c,x}$	$106 \pm 2^{d,y}$
RS	$322 \pm 10^{b,x}$	$185 \pm 6^{a,b,y}$	$366 \pm 3^{b,x}$	$215 \pm 4^{b,y}$	$331 \pm 6^{a,b,x}$	$190 \pm 5^{b,y}$
M	$320 \pm 9^{b,x}$	$179 \pm 6^{b,c,y}$	$352 \pm 4^{c,x}$	$200 \pm 4^{c,y}$	$323 \pm 5^{b,x}$	$177 \pm 3^{c,y}$

[a]Under Schaal oven test conditions at 65°C, in hexane-extracted oil from Sudanese coated (SC), Egyptian coated (EC), and Egyptian dehulled (ED) seeds in the raw (RW) state and after roasting (R), steaming (S), roasting plus steaming (RS), and microwaving (M). Results are mean values of three determinations ± SD. Values in each column with different superscripts (a–e) are significantly ($P < 0.05$) different from one another. Values of fresh and stored oil with different superscripts (x and y) are significantly ($P < 0.05$) different from one another.

ported that degradation of sesamolin was more pronounced than that of sesamin, perhaps due to the decomposition of sesamolin to sesamol during thermal processing.

The identification of γ-tocopherol, which we found to be the only tocopherol present in sesame oils, was made by comparison of its retention time with that of a standard, appearing 7 min post-injection, ahead of both sesamin and sesamolin. The content of γ-tocopherol in oils extracted from coated (EC and SC) and dehulled seeds (ED) is shown in Table 3. There was a significant ($P < 0.05$) decrease of about 40% in the amount of tocopherol in oils extracted from seeds following all treatment and after the entire storage period for both EC and SC seeds; this decrease was more pronounced in oils from ED seeds, especially those of R and S. The content of γ-tocopherol in sesame oil did not exceed the amounts in other vegetable oils despite strong resistance of the oil to oxidation (17). Fukuda *et al.* (3) have indicated that γ-tocopherol and other endogenous antioxidants present in sesame oil act synergistically in prevention of oxidation.

The effect of seed treatment and storage of oils on off-

flavor development of products was monitored by determining their hexanal contents. Sesame oil contains a high proportion (38–42%) of linoleic acid, and hexanal is expected to be one of its major secondary oxidation products. Hexanal may be formed directly from the 13-hydroperoxide of linoleic acid through homolytic cleavage or from rearrangement of the 9-hydroperoxide of linoleic acid to the 13-hydroperoxide prior to bond cleavage, or from oxidative decomposition of 2,4-decadienal (18). Dupuy *et al.* (19) have reported that the production of volatiles in peanut samples was related to the extent of oil deterioration.

As shown in Figure 2, hexanal formation was affected by different treatments, the R and S treatments produced more hexanal in oils extracted from ED seeds (420 and 385 mg/kg oil, respectively); whereas, M treatment produced more hexanal in oils extracted from both EC and SC seeds (410 and 430 mg/kg oil, respectively). Figure 2 (A, B, C) also shows that there was a clear increase in the hexanal content after 20 d of storage for all treated oils whereas this increase was very low after 10 d, especially for oils extracted from coated seeds.

The production of hexanal in oils extracted from coated seeds (EC and SC) was in the order of RW < RS < R, S < M, but was in the order of RW < RS < M < S < R for the oils from ED seeds. According to Frankel and Gardner (18), various tocopherols can affect the stability of linoleic acid hydroperoxides. Results for volatile compounds in the oil in this study do not correspond with tocopherol contents after different treatments for oils extracted from both EC and SC seeds following storage. On the other hand, for ED oils, the content of hexanal corresponded with tocopherol contents during the entire storage period. Moreover, hexanal and sesamolin contents during storage corresponded well for oils from both coated (EC or SC) and dehulled (ED) seeds.

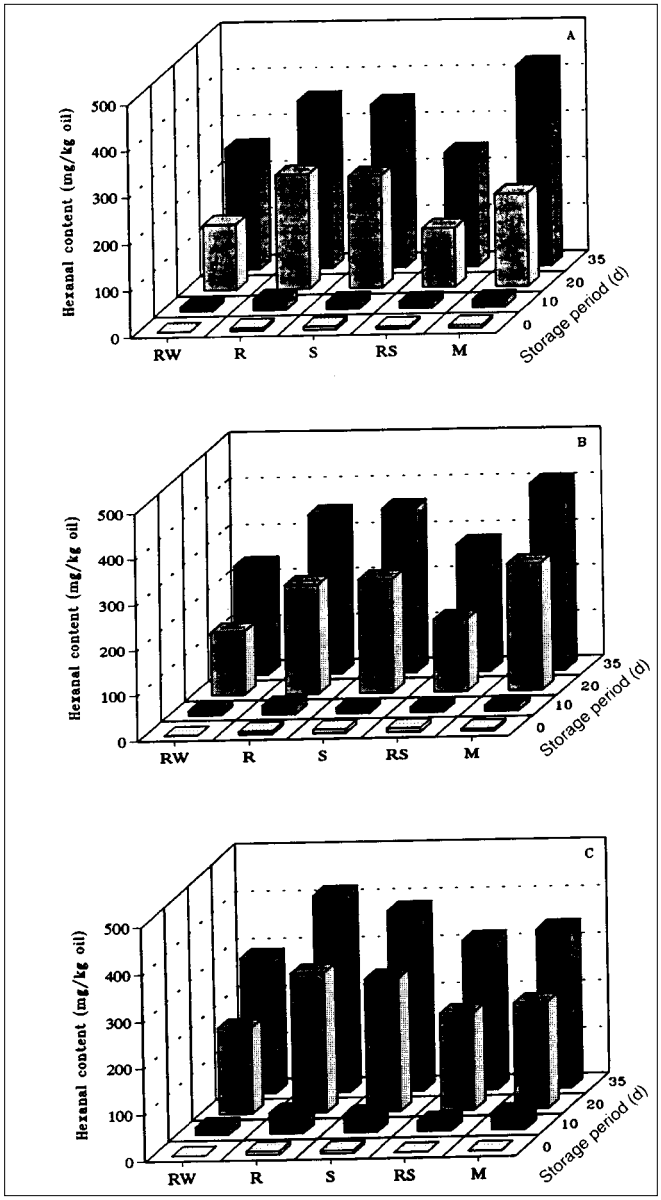

FIG. 2. Effect of different treatments (R, S, RS, M, and RW) on the content of hexanal for; (A): Sudanese coated, (B): Egyptian coated, and (C): Egyptian dehulled seeds after storage for 10, 20, and 35 d. Refer to Figure 1 caption for abbreviations.

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