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# Effect of roasting in electric oven on oil quality and residue from *Cucurbita maxima* (*Marina di Chioggia*) and *Cucurbita pepo* (*Calabaza Mercado Verde*) seeds from Morocco

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ABSTRACT

This study aimed to assess the impact of the roasting process, conducted in an electric oven, on the oil quality and residue derived from two pumpkin seed species, namely *Cucurbita maxima* (*C. maxima*) and *Cucurbita pepo* (*C. pepo*), cultivated in Morocco. The seeds underwent roasting at temperatures of 60, 90, 120, and 150 °C for 45 min. The cold press extracted oils were characterize in terms of fatty acids, phytosterols, tocopherols and pigment content meanwhile phenolic content and antioxidant activity were evaluated in the residues. The heat treatment did not significantly affect fatty acid content; however, it affected sterols, particularly β-sitosterol, which experienced an 8 % decrease in *C. maxima*. Instead, total sterols significantly increased in *C. pepo* from 153.84 to 181.71 mg/100g. Moreover, the heat treatment influenced the tocopherol contents, revealing a substantial increase in both species. Phenolic content was significantly affected in *C. pepo* whereas the variation in *C. maxima* was statistically nonsignificant. Antioxidant activity exhibited fluctuations during the heat treatment, resulting in an overall increase in the oils. The roasting process influences the composition of bioactive compounds and antioxidant activity in pumpkin seed oil. These findings contribute to a deeper exploration of the functional properties of pumpkin seed products.

## 1. Introduction

Pumpkin seeds are abundant in bioactive compounds known for their physiological benefits to the human body, making them highly valuable in the healthcare industry (Peng et al., 2021). These seeds are rich in various nutritional and healing properties, including unsaturated fatty acids, phytosterols, tocopherols, carotenoids, phenolic compounds, and flavonoids (Broznić et al., 2016). Moreover, pumpkin seeds serve as a plentiful source of essential minerals such as magnesium, potassium, phosphorus, zinc, manganese, iron, calcium, sodium, and copper (Amin et al., 2019).

The bioactive compounds present in these seeds exert simultaneous effects on target sites, thereby reducing the risk of various diseases, including tumors (Chari et al., 2018; Jayaprakasam et al., 2003), microbial infections (Brogan & Mossialos, 2016; Kabbashi et al., 2014), hyperglycemia and diabetes (Adams et al., 2011; Bharti et al., 2013), and complications associated with oxidative stress (Dotto & Chacha, 2020). Moreover, their high content of $\Delta^7$ sterols contributes to urological benefits by countering benign prostatic hypertrophy (Perez Gutierrez, 2016) and exhibits anti-inflammatory properties to combat prostatitis and urinary tract infections (Makni et al., 2008).

Belonging to the Cucurbitaceae family, pumpkins originate from tropical regions, with 90 % of their species primarily found in Africa and Madagascar, Central and South America, and Southeast Asia (Mukherjee

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et al., 2022). The genus *Cucurbita*, which includes pumpkins, consists of annual plants thriving in temperate and subtropical climates worldwide (Boujemaa et al., 2020). In Morocco, strategically positioned with a temperate climate and agriculturally favorable zones, the country plays a significant role in the production of certain *Cucurbita* species. It ranks as the 6th largest global exporter of pumpkins, with a total of 51,000 tons, and the 23rd global producer, mainly cultivating *Cucurbita pepo*, *Cucurbita maxima*, and *Cucurbita moschata*, in the regions of Doukkala, Saïs, and Gharb (Atlasbig, 2021; Cabinet d'études américain, 2019; Khelfaoui, 2002).

Pumpkin seeds, consumed primarily in roasted form, are incorporated into human diets. The heat treatment induces changes in the physical and chemical properties, as well as the cellular structure of seeds. Commonly used heat treatments for oilseeds include roasting, microwaving, infrared radiation, boiling, and steaming (Cai et al., 2021; Waszkowiak et al., 2018). Pumpkin seed oil, traditionally produced by pressing roasted seeds, undergoes significant alterations in organoleptic and physicochemical properties, as well as oxidative stability during roasting. This process reduces the moisture content of oilseeds, increasing oil yield and improving color, flavor, and texture (Hu et al., 2019; Mohamed Ahmed et al., 2020). Heat treatment also leads to an increase in bioactive compounds such as polyphenols and tocopherols, and it promotes the formation of new products through the Maillard reaction, contributing to enhanced antioxidant activity (Ren et al., 2019; Yang et al., 2018).

In contrast to unroasted pumpkin oil, which is green in color and has a typical pumpkin flavor (Raczyk et al., 2018), roasted pumpkin oil, obtained above 100 °C, exhibits a nutty or roasted aroma with a slightly spicy undertone and tends toward a greenish-brown color (Nakić et al., 2006; Siegmund & Murkovic, 2004). However, inappropriate heat treatment can have negative effects, including nutrient degradation, the formation of toxic compounds, and oil oxidation (Oracz et al., 2014).

In the literature, various studies have explored the impact of roasting on pumpkin seeds using different methods such as electric ovens (Goudarzi et al., 2017) and microwaves (Suri et al., 2022) across different roasting temperatures. However, none of these studies have compared the physicochemical parameters, chemical compositions, phenolic compound contents, and antioxidant activity of oil from two Moroccan pumpkin species, *C. maxima* and *C. pepo*.

Therefore, this study aims to investigate the effects of roasting at different temperatures: 60, 90, 120, and 150 °C for 45 min, on the chemical composition (fatty acids, sterols, tocopherols), pigment content (chlorophylls and carotenoids), oil characteristics (acid value, peroxide value, iodine value, specific extinction), total phenolic content, and antioxidant activity (DPPH and ABTS) of the two species *C. maxima* and *C. pepo*. The results of this study can provide insights into how roasting affects the nutritional value of pumpkin seeds and the evolution of their antioxidant activity throughout heating, enabling more effective utilization of these seeds in plant-based diets.

## 2. Material and methods

### 2.1. Sampling

The species studied belong to the *Cucurbita* genus, which is a part of the Cucurbitaceae family. Specifically, *C. maxima* originates from *Marina di Chioggia* variety, while *C. pepo* came from *Calabaza Mercado Verde* variety. The harvesting of these specimens took place in June 2022 in the Khemissat region of Morocco. Subsequently, the seeds underwent meticulous washing with clear water and were air-dried for 48 h before storage, with a maximum storage duration of 2 days. As a control, a sample of unroasted seeds was left untreated for comparative analysis.

### 2.2. Roasting process

100 g of dried seeds were used for each temperature, and a sodium chloride solution (1 %) was added to the mixture as part of the production process. The seeds were transferred on a baking sheet and roasted at 150, 120, 90 and 60 °C for 45 min, using a convection oven (A6-S3 MT 2000 W, LUXELL, TURKEY) to homogenize the oven temperature. For each temperature, three independent experiments were carried out under the same conditions to obtain homogeneous samples. Lastly, the seeds were cooled, initially in a desiccator, then brought back to room temperature in a glass container and stored for a maximum of 5 days.

### 2.3. Oil extraction

The roasted and unroasted seeds were deshelled and placed in a cold press oil machine (1500 W, CGOLDENWALL, CHINA) that had been preheated to 115 °C. The oil obtained from both roasted and unroasted seeds was meticulously separated from the paste through multiple filtrations. This extraction procedure was carried out twice. Finally, the extracted oils were transferred to brown glass bottles and stored at 4 °C for a maximum of 2 days.

### 2.4. Fatty acid composition

Fatty acids are transesterified with KOH/methanol to form fatty acid methyl esters, which were analyzed by gas chromatography with flame ionization and capillary column (Varian CP-3800, Varian Inc., Middelburg, The Netherlands). The column used was a CP-Wax 52CB with the following dimensions (l = 30 m, Ø = 0.25 mm). The carrier gas used was helium for chromatography, with a 1 mL/min flow rate. The initial and final temperatures were 170 °C and 230 °C, respectively, with an increase of 4 °C/min. Data were processed using a Varian Star Workstation v 6.30 and, the results were reported as percentages of each individual fatty acid present in the sample (El Bernoussi et al., 2020).

### 2.5. Sterols composition

The quantification of sterol composition involves saponifying each oil sample, extracting the unsaponifiable fraction, and separating the crude sterols. Subsequently, the crude sterol fraction undergoes trimethylsilylation following the ISO method (ISO12228-1, 2014). To determine the sterol composition, gas chromatography with a flame ionization detector was employed, using a VF-1 ms capillary column (30 m; 0.25 mm; 0.25 μm) thermostated at 270 °C, while both the injector and detector were maintained at 300 °C. Helium was used as the carrier gas for chromatography, with a flow rate of 1 mL/min. Finally, Varian Star Workstation v 6.30 was used for data processing.

### 2.6. Tocopherols composition

Tocopherol contents were determined using high-performance liquid chromatography (Waters e2695, USA) equipped with a fluorometric detector (excitation wavelength 290 nm emission wavelength 330 nm). The separation conducted on a on a C18-Varian silica column (25 cm and 4 mm). Elution was performed using a mixture of isooctane/isopropanol (99:1) at a flow rate of 1.2 mL/min for 20 min. Quantification was performed using external standards (δ-, γ-, β- and α-tocopherols). The data were represented as milligrams of tocopherol per kilogram of oil (Elouafy et al., 2022).

### 2.7. Physicochemical parameters of pumpkin seed oil

Acid value, iodine value, peroxide value and specific extinction coefficients were determined according to the recommended practices of the AOCS method: Ca 5a-40, Cd 1c-85, Cd 8b-90 and Ch 5e91, respectively (AOCS Official Method 7th Edition, n.d.).

AV was determined by titrating a pumpkin oil solution in a 1:1 (v/v) ethanol/ether mixture with an ethanolic solution of KOH, and the results

were expressed as milligrams of KOH per gram of oil (mg KOH/g oil). IV was calculated directly from fatty acid compositions and expressed in milligrams of $I_2$ per 100 g of oil (mg $I_2$/100g oil). PV was measured through iodine titration of a pumpkin oil solution in a 2:1 (v/v) iso-octane/acetic acid mixture with a sodium thiosulfate solution, and the values were represented as milliequivalents of active oxygen per kilogram of oil (mEq $O_2$/Kg oil). $E_{232}$ and $E_{270}$ values were determined using a LLG-uniSPEC 2 spectrometer (LLG Labware, Meckenheim, Germany) for a 1 % (w/v) pumpkin oil solution in cyclohexane.

Chlorophyll (Tch) and carotenoid (Tcar) content were determined by a spectrophotometric method. Samples were dissolved in hexane (1 %), and absorbance was measured at 670 nm for Tch (1) and 470 nm for Tcar (2) (El Moudden et al., 2019), then calculated according to the following formulas:

$$\text{Tch (mg / kg)} = \frac{As \times 10^6}{613 \times 100 \times d} \quad (1)$$

$$\text{Tcar (mg / kg)} = \frac{As \times 10^6}{2000 \times 100 \times d} \quad (2)$$

Where As = absorbance of the sample and d = diameter of the cell.

### 2.8. Total phenolic content

The content of total phenols was extracted from the residue of cold-pressed seeds. The extraction was performed by Twisselmann for 3 h using ethanol as solvent and then removed at 65 °C under reduced pressure using a rotary evaporator. Samples were recovered and stored in a refrigerator at 4 °C for a maximum of 2 days. Total phenols were quantified following the Folin-Ciocalteu spectrophotometric method described by (El-Guezzane et al., 2021) with some slight modifications.

Solutions were prepared by dissolving the extract in ethanol to achieve a concentration of 1 mg/mL. The absorbance was measured using the spectrophotometer (LLG-uniSPEC2) at 765 nm. TPC content was calculated using gallic acid as a standard of 1–200 mg GA/100 mL in ethanol. Results were expressed as mg gallic acid (GAE)/g extract.

### 2.9. Antioxidant activity

#### 2.9.1. DPPH assay

0.5 mL of an ethanolic solution (0.2 mM) of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was added to 2.5 mL of the samples prepared at different concentrations (0.1–1 mg/mL), mixed vigorously, and allowed to stand for 30 min in the dark. The measurement was then performed by spectrophotometer (LLG-uniSPEC 2) at 517 nm. The concentration of DPPH was deduced from a calibration range established with ascorbic acid (0.5–20 μg/mL) (Idrissi et al., 2021).

#### 2.9.2. ABTS assay

The ABTS assay was performed to complement the results obtained in the DPPH assay. A solution of ABTS (2 mM) mixed with $K_2S_2O_8$ in equal amounts was prepared 16 h before use. The solution was then diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. The solution was added to 200 μL at different extract concentrations and then measured at 734 nm after 30 min of incubation. The same was done for the Trolox standard of various concentrations (5–60 μg/mL) (El Moudden et al., 2019).

The results of the two-antioxidant tests were calculated from the following formula (3):

$$\textit{Inhibition}\ (\%) = \frac{A - As}{A} \times 100 \quad (3)$$

Where A = absorbance of the negative control and As = Absorbance of the sample.

### 2.10. Statistical analyses

The levels of Tcar, Tch and total phenolic content along with the physicochemical parameters (AV, IV, PV, $E_{270}$, and $E_{232}$), chemical compositions and antioxidant activity were performed in triplicate, and the data were presented as means ± standard error of the mean using IBM SPSS Statistics 21 software (SPSS Inc., Chicago, USA), followed by Tukey's post hoc test at $p < 0.05$.

## 3. Results and discussion

### 3.1. Physico-chemical parameters of pumpkin seed oil

#### 3.1.1. Acid value

The determination of the acid value (AV) serves the purpose of evaluating the hydrolysis of triglycerides and the production of free fatty acids in oils. It allows for the measurement of the degree of decomposition of oil triglycerides into free fatty acids when exposed to high temperatures. This assessment proves valuable in categorizing certain virgin oils, such as argan and olive oils (Nounah et al., 2021; Suri et al., 2022). The AV values are presented in Table 1.

The *C. maxima* oil samples exhibited no significant difference as the roasting temperature increased ($p > 0.05$), However, a slight increase was observed in the case of *C. pepo*, starting at 90 °C ($p < 0.05$). The AV ranged from 0.56 to 0.79 mg KOH/g of oil during this increase, indicating a notable impact on AV without compromising the oil quality. Importantly, these values remained below 4 mg KOH/g of oil, in compliance with the maximum limit set for crude oils by the FAO (Food and Agriculture Organization of the United Nations, 2016).

The Free Fatty Acid (FFA) content, an indicator of oil shelf life (Boujemaa et al., 2020), is directly related to half of the AV, and thus, these values evolve in parallel. The FFA values in *C. maxima* oil samples ranged from 0.54 to 0.62 % oleic acid ($p > 0.05$), indicating no significant difference. In contrast, the FFA content in *C. pepo* oil samples increased from 0.28 to 0.40 % oleic acid, showing a significant variation. This variation can be attributed to the thermal hydrolysis of triglycerides in the oil samples, as demonstrated by (Tenyang et al., 2017) for the roasting of two Cameron sesame seed varieties. However, (Nounah et al., 2021) showed that cactus seeds underwent no modification during roasting and maintained their oil quality.

#### 3.1.2. Iodine value

The iodine value is commonly used as an approximate indicator of oil stability during roasting (Arab et al., 2022). Indeed, during heating, the double bonds of fatty acids are susceptible to attack by free radicals, leading to a reduction in the number of unsaturation (Kinge et al., 2019). Consequently, IV allows for the assessment of the degree of unsaturation in the oils under investigation. The changes in IV are presented in Table 1.

The oil samples exhibited no significant change in Iodine Value (IV) ($p > 0.05$). In *C. maxima* oil samples, the results ranged between 114.86 and 116.47 g $I_2$/100 g of oil, and between 114.93 and 115.41 g $I_2$/100 g of oil in *C. pepo* oil samples. This is in contrast to the findings reported by (Djikeng et al., 2018), where the IV of cocoa beans increased from 30.0 to 35.0 g $I_2$/100 g of oil during the oven-roasting process. Additionally, (Kinge et al., 2019) observed a decrease in the IV of Djansang seeds, ranging from 104.83 to 98.11 g $I_2$/100 g of oil for traditional roasting and from 105.87 to 102.96 g $I_2$/100 g of oil for the boiling process. The differences in results could be attributed to variations in the thermal processing methods employed, but also to the varying roasting temperatures chosen. Therefore, we can say that the temperatures selected in the current study, in combination with the chosen method, did not exert a significant impact on the IV.

#### 3.1.3. Peroxide value

The Peroxide Value (PV) determines the primary oxidation state of

**Table 1**
Effect of roasting on the changes in the physicochemical properties, chlorophyll, and carotenoid content of pumpkin seed oils.

| Parameters | *C. maxima* | | | | | *C. pepo* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unroasted | 60 °C | 90 °C | 120 °C | 150 °C | Unroasted | 60 °C | 90 °C | 120 °C | 150 °C |
| AV (mg KOH/g oil) | 1.07 ± 0.08$^{a}$ | 1.07 ± 0.08$^{a}$ | 1.12 ± 0.00$^{a}$ | 1.18 ± 0.08$^{a}$ | 1.23 ± 0.00$^{a}$ | 0.56 ± 0.00$^{a}$ | 0.56 ± 0.00$^{a}$ | 0.62 ± 0.08$^{ab}$ | 0.73 ± 0.08$^{ab}$ | 0.79 ± 0.00$^{b}$ |
| FFA (% oleic acid) | 0.54 ± 0.04$^{a}$ | 0.54 ± 0.04$^{a}$ | 0.56 ± 0.00$^{a}$ | 0.59 ± 0.04$^{a}$ | 0.62 ± 0.00$^{a}$ | 0.28 ± 0.00$^{a}$ | 0.28 ± 0.00$^{a}$ | 0.31 ± 0.04$^{ab}$ | 0.37 ± 0.04$^{ab}$ | 0.40 ± 0.00$^{b}$ |
| IV (g $I_2$/100 g oil) | 116.47 ± 0,86$^{a}$ | 115.33 ± 1,87$^{a}$ | 115.86 ± 1,62$^{a}$ | 114.86 ± 2,68$^{a}$ | 115.49 ± 2,30$^{a}$ | 114.93 ± 0,13$^{a}$ | 115.15 ± 0,13$^{a}$ | 115.14 ± 0,82$^{a}$ | 115.41 ± 0,72$^{a}$ | 114.96 ± 0,58$^{a}$ |
| PV (mEq $O_2$/Kg oil) | 4.95 ± 0.07$^{a}$ | 6.05 ± 0.07$^{ab}$ | 7.20 ± 0.14$^{bc}$ | 8.50 ± 0.71$^{cd}$ | 9.50 ± 0.71$^{d}$ | 3.50 ± 0.71$^{a}$ | 4.05 ± 0.78$^{ab}$ | 4.70 ± 0.28$^{ab}$ | 7.00 ± 1.41$^{ab}$ | 8.00 ± 1.41$^{b}$ |
| $E_{232}$ | 2.25 ± 0.01$^{a}$ | 2.55 ± 0.04$^{b}$ | 2.62 ± 0.06$^{bc}$ | 2.75 ± 0.04$^{cd}$ | 2.83 ± 0.04$^{d}$ | 2.23 ± 0.04$^{a}$ | 2.31 ± 0.03$^{ab}$ | 2.47 ± 0.08$^{abc}$ | 2.55 ± 0.07$^{bc}$ | 2.63 ± 0.10$^{c}$ |
| $E_{270}$ | 1.14 ± 0.00$^{a}$ | 1.53 ± 0.00$^{b}$ | 2.06 ± 0.03$^{c}$ | 2.11 ± 0.01$^{c}$ | 2.64 ± 0.01$^{d}$ | 0.98 ± 0.00$^{a}$ | 1.11 ± 0.00$^{b}$ | 1.41 ± 0.00$^{c}$ | 1.51 ± 0.00$^{d}$ | 2.02 ± 0.00$^{e}$ |
| Tcar (mg/kg) | 0.23 ± 0.00$^{a}$ | 0.26 ± 0.03$^{ab}$ | 0.32 ± 0.01$^{bc}$ | 0.37 ± 0.01$^{cd}$ | 0.40 ± 0.01$^{d}$ | 0.29 ± 0.01$^{a}$ | 0.31 ± 0.01$^{ab}$ | 0.38 ± 0.01$^{bc}$ | 0.42 ± 0.01$^{cd}$ | 0.48 ± 0.03$^{d}$ |
| Tch (mg/kg) | 0.35 ± 0.01$^{a}$ | 0.38 ± 0.00$^{a}$ | 0.46 ± 0.01$^{b}$ | 0.58 ± 0.00$^{c}$ | 0.71 ± 0.00$^{d}$ | 0.65 ± 0.03$^{a}$ | 0.69 ± 0.06$^{ab}$ | 0.75 ± 0.04$^{abc}$ | 0.83 ± 0.01$^{bc}$ | 0.88 ± 0.04$^{c}$ |

Results are expressed as the mean values ± standard deviation of the three replicates (mean ± SD, n = 3); (a–d) different letters within a row indicate significant statistical differences ($p < 0.05$). Acid value (AV); iodine value (IV); peroxide value (PV); conjugated dienes ($E_{232}$) and conjugated trienes ($E_{270}$).

oils and fats, primarily by quantifying hydroperoxides, which constitute the principal primary oxidation products of lipids (Tonfack Djikeng et al., 2018). Variations in PV are presented in Table 1.

According to Table 1, the roasting process had an impact on the oils obtained from roasted seeds of both studied species. Indeed, the peroxide value exhibited a significant increase ($p < 0.05$), ranging from 4.95 to 9.50 mEq $O_2$/Kg of oil in *C. maxima* oils and from 3.50 to 8.00 mEq $O_2$/Kg of oil in *C. pepo* oils. The highest PV was registered at 150 °C for both species.

This increase can be attributed to the formation of hydroperoxides during heat treatment. Variances in treatment temperature may account for the observed results. It has been demonstrated that elevated temperatures facilitate the initiation of lipid oxidation, leading to the generation of free radicals, which subsequently react with molecular oxygen to produce hydroperoxides during the propagation stage (Kinge et al., 2019). However, it is worth noting that the values obtained remained below 10 mEq $O_2$/Kg of oil, which is the highest limit recommended by the FAO (Food and Agriculture Organization of the United Nations, 2016).

These results are in line with those reported by (Cai et al., 2021), which showed that PV increased during the heat treatment of sesame, peanut, rapeseed, Camellia seed, sunflower seed, and walnut using various roasting methods, including microwave treatment and oven roasting, at different temperatures.

### 3.1.4. Specific extinction coefficients ($E_{232}$ and $E_{270}$)

The oxidative deterioration and purity of pumpkin seed oils are measured by the specific extinction parameter at 232 nm for the detection of peroxides and 270 nm for the detection of secondary oxidation products (Gharby et al., 2011). The values are presented in Table 1.

The oxidation rate significantly increased during thermal treatment ($p < 0.05$). The absorbance at 232 nm in *C. maxima* oil samples ranged from 2.25 to 2.83, while in *C. pepo*, it ranged from 2.23 to 2.63. However, the results suggest that these oxidation products rapidly increase at 90 °C, promoting oxidation and, consequently, the formation of conjugated dienes. These findings align with those reported by (Nounah et al., 2021), who demonstrated a significant increase in specific extinctions after 20 min of roasting prickly pear seeds at 110 °C, thereby favoring the development of undesirable flavors. The absorbance at 270 nm significantly increased, ranging from 1.14 to 2.64 in *C. maxima* oils and from 0.98 to 2.02 in *C. pepo* oils ($p < 0.05$). This illustrates the formation of conjugated trienes in the oils. The obtained results were in agreement with those reported by (Petkova & Antova, 2019), who established that the absorption at 268 nm increased after microwave and conventional heating of pumpkin seed oil.

### 3.1.5. Chlorophyll (Tch) and carotenoid (Tcar) content

Carotenoids are powerful antioxidants with yellow, orange, or red colors and chlorophylls are responsible for the green color of some oils. However, they can cause rapid degradation of oil stability due to their ability to transfer energy to fatty acid molecules (El Bernoussi et al., 2020). The pigment values are presented in Table 1.

The levels of Tcar increased significantly with temperature ($p < 0.05$), ranging from 0.23 to 0.40 in *C. maxima* oil and from 0.29 to 0.48 in *C. pepo* oil. Meanwhile, Tch content varied from 0.35 to 0.71 for *C. maxima* and from 0.65 to 0.88 for *C. pepo*. This can be explained by the fact that chlorophyll and carotenoid pigments are primarily present in a complex form with proteins, and during thermal treatment, this complex disintegrates, leading to the release of pigments into the oils.

These results align with those reported by (Suri et al., 2022), who showed that microwave treatment and roasting time significantly influenced Tcar and Tch in Nigella sativa L oil, ranging from 3.04 to 9.01 for Tch and from 2.48 to 6.61 for Tcar. (Rekas et al., 2015) suggests that the increase in carotenoid content can be partially explained by the fact that carotenoids bind to proteins inside the seed during thermal treatment, forming stable carotenoid-protein complexes. Consequently, the heat-induced protein denaturation process and the disruption of the seed's internal structure enhance the oil's accessibility to liposoluble carotenoids.

## 3.2. Fatty acids composition

Table 2 presents the changes in fatty acid composition during heat treatment. The results indicate a high abundance of linoleic acid, constituting nearly 50 % of the total fatty acids, followed by oleic acid at 28 %, palmitic acid at 15 %, and stearic acid at 7 %. In addition to these mentioned fatty acids, trace amounts of myristic, palmitoleic, linolenic, and arachidic acids were also identified. These fatty acids can be categorized as saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA). However, understanding the ratio between unsaturated and saturated fatty acids in our oils is essential as it reflects their nutritional qualities. All samples exhibit a range of 22–24 % for total SFA (ΣSFA), 27–29 % for total MUFA (ΣMUFA), and 48–50 % for total PUFA (ΣPUFA). Furthermore, excessive consumption of SFA is associated with elevated plasma cholesterol levels and obesity, in contrast to MUFA and PUFA, which are known to enhance oil stability (Ristić & Ristić, 2003). However, a high quantity of polyunsaturated

**Table 2**
Effect of roasting on the changes in the fatty acid composition of pumpkin seed oils, expressed as a percentage (%).

| Fatty acids (%) | *C. maxima* | | | | | *C. pepo* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unroasted | 60 °C | 90 °C | 120 °C | 150 °C | Unroasted | 60 °C | 90 °C | 120 °C | 150 °C |
| Palmitic acid | 14.89 ± 1.48$^{a}$ | 14.42 ± 0.82$^{a}$ | 14.10 ± 0.42$^{a}$ | 15.05 ± 1.51$^{a}$ | 15.43 ± 2.13$^{a}$ | 14.84 ± 0.78$^{a}$ | 14.91 ± 0.09$^{a}$ | 14.54 ± 0.35$^{a}$ | 14.52 ± 0.33$^{a}$ | 14.73 ± 0.08$^{a}$ |
| Stearic acid | 7.14 ± 0.13$^{a}$ | 7.31 ± 0.45$^{a}$ | 7.85 ± 0.22$^{a}$ | 7.69 ± 1.04$^{a}$ | 7.55 ± 0.81$^{a}$ | 7.03 ± 0.54$^{a}$ | 7.20 ± 0.79$^{a}$ | 6.86 ± 0.52$^{a}$ | 7.16 ± 0.71$^{a}$ | 6.99 ± 0.81$^{a}$ |
| Oleic acid | 27.78 ± 1.15$^{a}$ | 27.72 ± 0.37$^{a}$ | 27.75 ± 1.31$^{a}$ | 27.97 ± 0.79$^{a}$ | 27.22 ± 1.52$^{a}$ | 29.09 ± 0.55$^{a}$ | 28.93 ± 0.61$^{a}$ | 28.77 ± 0.57$^{a}$ | 28.69 ± 0.81$^{a}$ | 29.13 ± 0.69$^{a}$ |
| Linoleic acid | 49.71 ± 0.81$^{a}$ | 48.52 ± 0.99$^{a}$ | 48.38 ± 1.42$^{a}$ | 47.81 ± 2.34$^{a}$ | 49.66 ± 0.54$^{a}$ | 48.30 ± 1.70$^{a}$ | 48.48 ± 1.85$^{a}$ | 48.94 ± 1.32$^{a}$ | 48.51 ± 1.77$^{a}$ | 48.57 ± 1.85$^{a}$ |
| Others | 0.49 ± 0.01$^{b}$ | 1.99 ± 0.05$^{d}$ | 1.94 ± 0.03$^{d}$ | 1.49 ± 0.01$^{c}$ | 0.17 ± 0.04$^{a}$ | 0.75 ± 0.01$^{b}$ | 0.49 ± 0.01$^{a}$ | 0.87 ± 0.03$^{c}$ | 1.11 ± 0.01$^{d}$ | 0.56 ± 0.02$^{a}$ |
| ΣSFA | 22.27 ± 1.64$^{a}$ | 23.52 ± 1.3$^{a}$ | 23.73 ± 0.67$^{a}$ | 23.98 ± 2.55$^{a}$ | 22.88 ± 2.97$^{a}$ | 22.47 ± 1.32$^{a}$ | 22.36 ± 0.91$^{a}$ | 22.01 ± 0.89$^{a}$ | 22.56 ± 1.07$^{a}$ | 22.06 ± 0.91$^{a}$ |
| ΣMUFA | 27.89 ± 1.16$^{a}$ | 27.83 ± 0.38$^{a}$ | 27.86 ± 1.42$^{a}$ | 28.08 ± 0.80$^{a}$ | 27.33 ± 1.53$^{a}$ | 29.11 ± 0.56$^{a}$ | 29.04 ± 0.62$^{a}$ | 28.89 ± 0.58$^{a}$ | 28.80 ± 0.82$^{a}$ | 29.24 ± 0.70$^{a}$ |
| ΣPUFA | 49.84 ± 0.82$^{a}$ | 48.65 ± 1.01$^{a}$ | 48.41 ± 1.42$^{a}$ | 47.94 ± 2.35$^{a}$ | 49.79 ± 0.55$^{a}$ | 48.42 ± 1.72$^{a}$ | 48.60 ± 1.86$^{a}$ | 49.10 ± 1.34$^{a}$ | 48.64 ± 1.79$^{a}$ | 48.70 ± 1.87$^{a}$ |

Results are expressed as the mean values ± standard deviation of the three replicates (mean ± SD, n = 3); (a–d) different letters within a row indicate significant statistical differences ($p < 0.05$).

fatty acids (PUFA) in oils can lead to rapid oxidations (Arab et al., 2022).

Based on the obtained results, it is evident that no significant changes were observed in the studied oils ($p > 0.05$). Furthermore, the method employed in conjunction with the temperatures studied had no effect on the chemical composition of the fatty acids. Notably, mono- and poly-unsaturated fatty acids remained unoxidized.

Similar findings were reported by (Nederal et al., 2012), who observed that roasting pumpkin seed paste did not alter the fatty acid composition. (Petkova & Antova, 2019) reported that conventional heating had no significant impact on the fatty acid composition, whereas microwave roasting to an increase in palmitic acid and a decrease in stearic and linoleic acid. Considering these results, it can be concluded that the oils maintained a certain level of stability during the thermal treatment, despite their high content of polyunsaturated fatty acids.

### 3.3. Sterols composition

Fig. 1 depicts the variation in sterols during the thermal treatment. The predominant sterol in the studied oils, β-sitosterol, exhibited a significant decrease of 4–8 % ($p < 0.05$) in *C. maxima* oils, ranging from 75.11 to 66.80 mg/100g. These values notably increased at 150 °C, reaching 69.25 mg/100g. The oils of *C. pepo* did not show a significant impact during the thermal treatment ($p > 0.05$).

The $\Delta^{5,24}$ stigmastadienol, which is the second major sterol, was minimally affected in *C. maxima* oils, ranging from 43.15 to 44.13 mg/100g, unlike the oils of *C. pepo*, which remained unaffected. The $\Delta^{7}$ campesterol was impacted in the oils of both species. A slight variation was observed in *C. maxima* samples, ranging from 31.19 to 31.64 mg/100g, with minor fluctuations during the thermal treatment. In contrast, a more considerable variation was noted in *C. pepo* oils, ranging from 23.07 to 31.64 mg/100g. The $\Delta^{7}$ avenasterol also underwent a significant change, increasing from 17.17 to 21.09 mg/100g in *C. maxima* oils and from 11.91 to 20.58 mg/100g in *C. pepo* oils. The $\Delta^{7}$ stigmastenol experienced a slight change during the thermal treatment, ranging from 12.36 to 13.87 mg/100g in *C. maxima* oils. Meanwhile, the samples of *C. pepo* remained unchanged.

Similar results were demonstrated by (Arab et al., 2022), who reported that the β-sitosterol content in roasted white and brown sesame seeds decreased from 50.6 to 48.4 mg/100 g between 0 and 180 °C, and then increased at 210 °C. Meanwhile, campesterol increased from 31.4 to 34.2 between 0 and 180 °C but decreased at 210 °C.

The total sterol content of *C. maxima* exhibited a significant decrease,

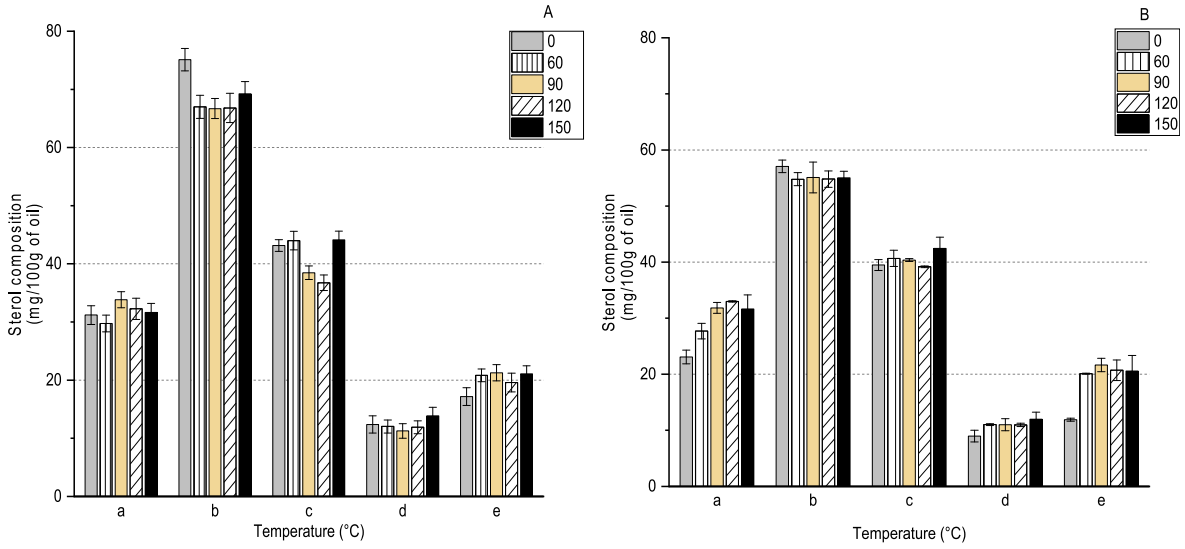


**Fig. 1.** Effect of roasting on the changes in the sterol composition of pumpkin seed oils expressed in mg/100g of oil. (A) *C. maxima*; (B) *C. pepo*; (a) $\Delta^{7}$campesterol; (b) β-sitosterol; (c) $\Delta^{5,24}$stigmastadienol; (d) $\Delta^{7}$stigmastenol; (e) $\Delta^{7}$avenasterol.

ranging from 196.38 to 172.13 mg/100g, after which it increased to reach a value of 194.57 mg/100g at 150 °C. Regarding the species *C. pepo*, the total sterol content increased during thermal treatment, ranging from 153.84 to 181.71 mg/100g (Table 3).

The reductions observed in the levels of individual sterols during heating may arise from degradation due to hydrolysis or dysfunction associated with elevated temperatures. As for the increase in total sterol content, it can be attributed to the rise in specific individual sterols, which implies an anti-polymerization effect, thereby contributing in the protection of the oil compounds against oxidation during heat treatment (Arab et al., 2022).

### 3.4. Tocopherol composition

Vitamin E holds significant importance as a major bioactive compound in the human diet. It belongs to the group of fat-soluble vitamins, comprising eight organic molecules, including four tocopherols and four tocotrienols. These compounds are crucial in determining the nutritional quality of vegetable oils and contribute to their resistance against oxidation during thermal treatments (Yoshida et al., 2006). The various forms of vitamin E homologs (δ-, γ-, β-, α-) are classified based on the number and position of methyl groups within the chromanol ring (Trela & Szymańska, 2019).

The γ-tocopherol content significantly increased during the roasting process ($p < 0.05$), ranging from 362.46 to 476.82 mg/kg in *C. maxima* oils, with a significant decrease at 90 °C. A similar trend was observed in *C. pepo* samples, varying from 360.06 to 473.99 mg/kg during the thermal treatment.

As for the other homologs, they also underwent an impact by increasing during the thermal treatment. β-tocopherol ranged from 96.29 to 241.57 mg/kg and from 117.10 to 210.93 mg/kg in *C. maxima* and *C. pepo* oils, respectively. The third major tocopherol, δ-tocopherol, varied from 40.72 to 75.19 and from 38.73 to 57.78 mg/kg in *C. maxima* and *C. pepo* samples, respectively. The last homolog, α-tocopherol, ranged from 13.80 to 36.25 mg/kg and from 5.18 to 22.01 mg/kg in *C. maxima* and *C. pepo* samples, respectively.

Fig. 2 presents the quantities of tocopherol homologs in the studied pumpkin seed oils. It illustrates four types of tocopherols: δ-, γ-, β-, and α-tocopherol. The content of γ-tocopherol was the highest among the other homologs, representing over 60 % of the total tocopherol content. However, the thermal treatment affected the quantities of each homolog in both varieties.

According to Table 4, the analysis also demonstrated that thermal treatment significantly affected the total tocopherol contents of the studied oils ($p < 0.05$). These contents ranged from 513.27 to 829.83 mg/kg for *C. maxima* and from 521.07 to 764.71 mg/kg for *C. pepo*.

In theory, the increase in total tocopherol content can be attributed to the cellular damage caused by thermal treatment, which has the effect of breaking the bonds connecting tocopherols to proteins or phospholipids, thereby leading to an increase in tocopherol extractability (Vujasinovic et al., 2012).

Comparing our results with those obtained by (Nederal et al., 2012), It is apparent that the total tocopherol contents of roasted and cold-pressed pumpkin seed oils are found within a similar range (639.0–642.4) with an abundance of γ-tocopherol. Also, (Vujasinovic et al., 2012) studied the influence of roasting time on the tocopherol content of pumpkin seed and found that total tocopherol content increased with both duration and roasting temperature (265.79–350.89 mg/kg). However, these values are approximately half of those obtained in the present study. This variation could be attributed to the different temperatures chosen, as well as the moisture content of the seeds and other factors.

**Table 3**
Effect of roasting on the changes in the total sterol content of pumpkin seed oils expressed in mg/100g of oil.

| Total sterols (mg/100g of oil) | *C. maxima* | *C. pepo* |
|---|---|---|
| Unroasted | $196.38 \pm 1.41^{b}$ | $153.84 \pm 2.15^{a}$ |
| 60 °C | $173.53 \pm 2.12^{a}$ | $162.73 \pm 0.73^{b}$ |
| 90 °C | $172.13 \pm 2.12^{a}$ | $170.78 \pm 1.20^{b}$ |
| 120 °C | $174.94 \pm 3.54^{a}$ | $172.94 \pm 0.69^{b}$ |
| 150 °C | $194.57 \pm 2.12^{b}$ | $181.71 \pm 0.79^{ab}$ |

Results are expressed as the mean values ± standard deviation of the three replicates (mean ± SD, n = 3); (a−b) different letters within a column indicate significant statistical differences ($p < 0.05$).

### 3.5. Total phenolic contents

The variation in total phenolic contents (TPC) is presented in Fig. 3. The analysis reveals that the TPC of *C. maxima* remained unaffected during thermal treatment ($p > 0.05$). In contrast, a significant increase in *C. pepo*'s TPC was observed, ranging from 5.37 to 9.45 mg GAE/g, with a significant decrease noted at 90 °C. This aligns with the observed trend in γ-tocopherol content.

Similar results were reported by (Taha & Matthäus, 2018). A slight decrease in TPC was observed at 140 °C, followed by a significant increase at 160 °C. This phenomenon can be attributed to the migration of TPC into the oil phase during the roasting process of safflower oleaginous seeds. This migration may be a result of the release of phenolic contents from their bound form or chemical changes in TPC during thermal treatment. (Durmaz & Gökmen, 2011) reported a 2.8-fold increase after 20 min compared to the unroasted sample of *P. terebinthus* oil, ranging from 7.01 to 19.61 μgGAE/mL. However, no further increase was observed after 40 min of roasting in an electric oven. According to (Durmaz & Gökmen, 2011), the increase in TPC may be linked to phenolic compounds that, during the roasting process, migrate more effectively into the oil phase due to the release of TPC from bound structures or chemical alterations at higher temperatures.

Certainly, the increase at higher temperatures is primarily linked to the accumulation of relatively polar compounds in the oil during the roasting process. Reports indicate that phenolic compounds demonstrate enhanced transfer into the oil phase when derived from roasted seeds. This phenomenon is likely a result of the release of phenolic compounds from bound structures or the chemical alteration of phenolics at elevated temperatures. Moreover, Maillard reaction products formed during the roasting process may have contributed to the augmentation of antioxidant capacity. It can be concluded that the roasting process can influence the distribution of tocopherols in various ways, depending on the seed species or the type and intensity of the heat pretreatment.

### 3.6. Antioxidant activity

The $EC_{50}$ represents the concentration of antioxidants needed to decrease the initial concentrations of free radicals by 50 %. A lower $EC_{50}$ value indicates a more potent antioxidant effect (Eddahhaoui et al., 2023). Table 5 presents the $EC_{50}$ values for the alcoholic extracts obtained from seed residues.

The Antioxidant activity of *C. maxima* showed some fluctuations during heat treatment. The $EC_{50}$ initially started at 0.25 mg/mL and increased between 60 and 90 °C, then began to decrease, reaching 0.29 at 150 °C. A similar trend was observed in the ABTS assay, where the $EC_{50}$ began at 0.68 mg/mL, increased between the two temperatures, and then started decreasing, reaching 1.16 mg/mL.

The $EC_{50}$ values from *C. pepo* exhibited a pattern similar to *C. maxima*. Increasing temperature enhanced antioxidant activity, ranging from 2.37 to 0.76 mg/mL for DPPH and from 5.72 to 2.51 mg/mL for ABTS. A significant increase was observed at 90 °C in the DPPH assay and at 120 °C in the ABTS.

This irregularity in antioxidant activity under heat treatment was also noted by (Taha & Matthäus, 2018). They reported a significant increase in the $EC_{50}$ of safflower seeds at 160 °C, followed by a

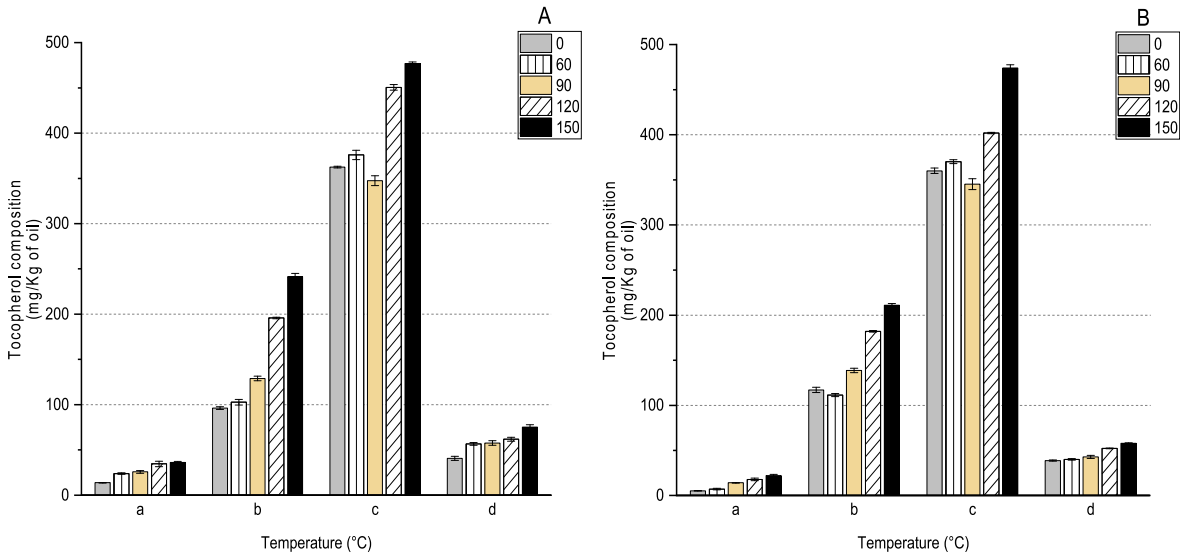


**Fig. 2.** Effect of roasting on the changes in the tocopherol composition of pumpkin seed oils expressed in mg/Kg of oil. (A) *C. maxima*; (B) *C. pepo;* (a) α-tocopherol; (b) β-tocopherol; (c) γ-tocopherol; (d) δ-tocopherol.

**Table 4**
Effect of roasting on the changes in the total tocopherol content in both species of pumpkin seed oil, expressed in mg/Kg of oil.

| Total tocopherols (mg/Kg of oil) | *C. maxima* | *C. pepo* |
|---|---|---|
| Unroasted | 513.27 ± 5.07$^{a}$ | 521.07 ± 7.54$^{a}$ |
| 60 °C | 559.35 ± 6.51$^{a}$ | 528.55 ± 5.46$^{a}$ |
| 90 °C | 559.91 ± 6.28$^{a}$ | 540.91 ± 5.85$^{b}$ |
| 120 °C | 742.76 ± 8.22$^{b}$ | 654.09 ± 5.44$^{c}$ |
| 150 °C | 829.83 ± 9.89$^{c}$ | 764.71 ± 5.64$^{d}$ |

Results are expressed as the mean values ± standard deviation of the three replicates (mean ± SD, n = 3); (a–d) different letters within a column indicate significant statistical differences ($p < 0.05$).

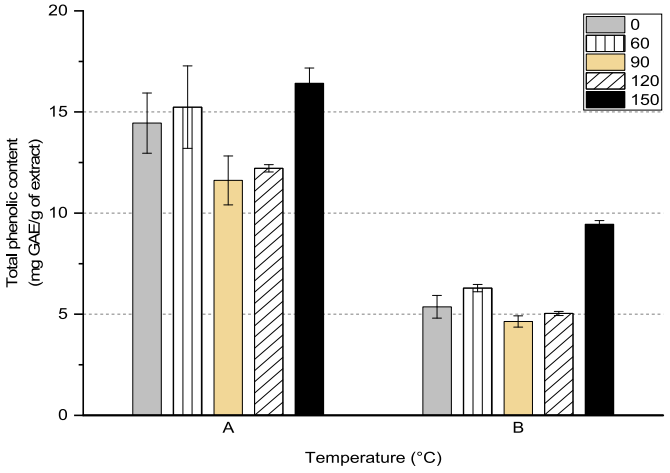


**Fig. 3.** Effect of roasting on the changes in total phenolic contents of extracts from seed residues. (A) C. *maxima*; (B) *C. pepo*.

subsequent decrease. Additionally, (Samaras et al., 2005) found that antioxidant activity increased with higher heating intensity, enhancing the antioxidant capacity of steamed and pressure-cooked soybeans.

This phenomenon can be explicated by the formation of new products from the Maillard reaction (Açar et al., 2009; Jannat et al., 2010). Higher temperatures may alter phenolic components, modifying their activity during the roasting process, as observed earlier. The balance between the formation of new Maillard reaction products with antioxidant capacity and the thermal degradation of naturally occurring antioxidant compounds during the heat treatment of oilseeds is the main factor influencing the overall antioxidant capacity of oilseeds (Taha & Matthäus, 2018).

**Table 5**
Effect of roasting on the changes in $EC_{50}$ of extracts from delipidated seed residues expressed as mg/mL.

| $EC_{50}$ (mg/mL) | *C. maxima* | | *C. pepo* | |
|---|---|---|---|---|
| | DPPH | ABTS | DPPH | ABTS |
| Unroasted | 0.25 ± 0.14$^{a}$ | 0.68 ± 0.10$^{a}$ | 2.37 ± 0.21$^{c}$ | 5.72 ± 0.08$^{e}$ |
| 60 °C | 0.48 ± 0.28$^{b}$ | 1.22 ± 0.03$^{b}$ | 1.48 ± 0.13$^{b}$ | 3.82 ± 0.06$^{d}$ |
| 90 °C | 0.86 ± 0.42$^{d}$ | 2.57 ± 0.12$^{d}$ | 1.94 ± 0.06$^{bc}$ | 2.78 ± 0.14$^{b}$ |
| 120 °C | 0.66 ± 0.42$^{c}$ | 1.91 ± 0.07$^{c}$ | 1.78 ± 0.10$^{b}$ | 3.61 ± 0.04$^{c}$ |
| 150 °C | 0.29 ± 0.42$^{a}$ | 1.16 ± 0.06$^{b}$ | 0.76 ± 0.04$^{a}$ | 2.51 ± 0.03$^{a}$ |

Results are expressed as the mean values ± standard deviation of the three replicates (mean ± SD, n = 3); (a–e) different letters within a column indicate significant statistical differences ($p < 0.05$).

## 4. Conclusion

Our study focused on assessing the variations in nutritional quality, chemical composition, and antioxidant activity of pumpkin seed oils extracted from two species, *C. maxima*, and *C. pepo*, following heat treatment of the seeds using an electric oven at different temperatures: 60, 90, 120, and 150 °C. The roasting process did not have a significant impact on the acidity and iodine value of *C. maxima*. However, the acidity of *C. pepo* samples showed a significant increase starting from 90 °C. As for peroxide value, $E_{232}$, and $E_{270}$, they increased significantly during the heat treatment. Nevertheless, the samples did not exceed the limit required by the FAO. Pigments also underwent a significant increase, consistent with findings from other researchers.

The major fatty acids in the studied oils showed no significant changes. The composition remained unchanged, with a high content of polyunsaturated fatty acids, ranging from 47.94 to 47.94 % for *C. maxima* and 48.42–49.10 % for *C. pepo*. In contrast, the major sterols were affected by roasting. β-Sitosterol exhibited a slight decrease, ranging from 75.11 to 69.25 mg/100g in *C. maxima* samples, while the content of $\Delta^{7}$avenasterol increased significantly, ranging from 17.17 to 21.09 mg/100g for *C. maxima* and 11.91–20.58 mg/100g for *C. pepo*.

The total sterol content fluctuated during the heat treatment. It initially decreased in *C. maxima* samples to a value of 194.57 mg/100g, but increased in the case of *C. pepo*.

Roasting also influenced each homolog by increasing their contents, as well as the total tocopherol, ranging from 513.27 to 829.83 mg/kg for *C. maxima* and 521.07–764.71 mg/kg for *C. pepo*, thereby contributing to maintaining the stability of the oil during thermal treatment.

The phenolic contents showed a significant increase in the case of *C. pepo*, rising from 5.37 to 9.45 mg GAE/g. It remained unchanged in the case of *C. maxima*. As for antioxidant activity, it experienced fluctuations during the heat treatment. Nevertheless, the $EC_{50}$ demonstrated a clear improvement in the anti-radical activity, decreasing from 2.37 to 0.76 mg/mL in the DPPH test and from 5.72 to 2.51 mg/mL in the ABTS test for *C. pepo* extracts. Regarding *C. maxima*, the DPPH test showed stabilization of the extract during the heat treatment, allowing it to maintain its concentration at 0.29 mg/mL.

Based on these findings, it can be stated that roasting pumpkin seeds in an electric oven significantly modified the composition of the extracted oils. The heat treatment indeed enhanced the oil's free radical scavenging capacity by increasing the total content of polyphenols, tocopherols, and carotenoids. Furthermore, prolonged exposure to high temperatures led to a change in oil quality, resulting in a minor oxidation of the seed oils and the formation of primary and secondary oxidation products. It is crucial to assess the pumpkin seed oil's resistance to oxidation by subjecting it to oxidative stress for a specific duration. This will involve comparing the various parameters examined in this study with future results and analyzing oils from both roasted and unroasted seeds.

We believe that these results can significantly contribute to optimizing time and temperature parameters, thereby influencing the duration of heat treatment. Moreover, conducting additional studies on different varieties can further expand the database. Additionally, this study emphasizes the importance of exploring alternative roasting techniques to comprehensively compare their impact on the nutritional quality and composition of pumpkin seed oil.

## CRediT authorship contribution statement

**Ihssan Boujemaa:** Writing – original draft, Methodology, Conceptualization. **Sara El Bernoussi:** Validation, Methodology. **Chakir El Guezzane:** Supervision, Data curation, Conceptualization. **Filippo Maggi:** Supervision, Data curation. **Giovanni Caprioli:** Writing – review & editing, Supervision, Data curation. **Abdelhakim Bouyahya:** Writing – review & editing, Supervision, Data curation. **Hicham Harhar:** Validation, Software. **Mohamed Tabyaoui:** Supervision, Resources.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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