INTERNATIONAL
Agrophysics
www.international-agrophysics.org

Int. Agrophys., 2025, 39, 457-474
doi: 10.31545/intagr/207209

Comprehensive evaluation of *Lagenaria siceraria* and *Cucurbita* species seeds: proximate composition, antioxidant potential, lipid profiling, and oil properties

Abdelghani Ait Nouisse[1,2], *Rachid Belmallam*[1], *Hasna Ait Bouzid*[1], *Mohamed Ibourki*[1], *Angelo Maria Giuffrè*[3]*, *Krishna Devkota*[4], *Khalid Majourhat*[1,2], *El Hassan Sakar*[5], *Said Gharby*[1]*

[1]Biotechnology, Analytical Sciences and Quality Control Team, Polydisciplinary Faculty of Taroudant, Ibn Zohr University, Agadir 83000, Morocco
[2]Geo-Bio-Environmental Engineering and Innovation Laboratory, Molecular Engineering, Biotechnology and Innovation Team, Polydisciplinary Faculty of Taroudant, Ibn Zohr University, Agadir 83000, Morocco
[3]University of Studies "Mediterranea" of Reggio Calabria, Department AGRARIA, 89124 Reggio Calabria, Italy
[4]International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat-institute, Rabat 10090, Morocco
[5]Laboratory of Biology, Ecology, and Health, Faculty of Sciences, Abdelmalek Essaadi University, Tetouan 93002, Morocco

Received March 11, 2025; accepted June 16, 2025

Abstract. Seeds from the *Cucurbitaceae* family are generally considered waste. This study focuses on the seeds of four species: *Lagenaria siceraria* and three species of *Cucurbita* spp., evaluating their nutritional composition, mineral content, bioactive properties, and oil characteristics. *C. moschata* var. "Slaouiya" exhibited the highest protein content (37.12 ± 0.01 g 100 g^{-1}), while *C. moschata* var. "Hamra" had the highest oil content (43.60 ± 0.37 g 100 g^{-1}). Mineral analysis revealed elevated potassium and magnesium levels in *C. moschata* var. "Slaouiya" (875.59 ± 28.87 mg g 100 g^{-1} K and 435.89 ± 3.36 mg g 100 g^{-1} Mg). *L. siceraria* recorded the highest TPC (5.71 ± 0.12 mg GAE g^{-1}) and TFC (16.06 ± 0.27 mg QE g^{-1}). Linoleic acid (C18:2) was the most abundant fatty acid, particularly in *L. siceraria* (70.73 ± 0.10%), which also had the highest content of PUFAs (70.89 ± 0.11%). In terms of oxidative stability and nutritional indices, *L. siceraria* exhibited the highest COX value (7.40 ± 0.01) and OS (3206.81 ± 5.50). *C. moschata* var. "Slaouiya" had the lowest atherogenic indices (AI) (0.23) and thrombogenic indices (TI) (0.09), suggesting potential cardiovascular benefits. These findings highlight the exceptional potential of these seeds, which could serve as a basis for innovations in the food, cosmetics, and pharmaceutical industries.

Keywords: antioxidant activity, *Cucurbita* species, fatty acid, *Lagenaria siceraria*, nutritional indices, proximate composition

1. INTRODUCTION

Fruit and vegetable seeds were long viewed as waste products, but the discovery of their healthy and bioactive components has lately attracted notable interest and attention (Lau *et al.*, 2021). The *Cucurbitaceae* family is one of the largest in the plant kingdom, comprising around 118 genera and 825 species. These plants are among the ten most important vegetable crops in the world (Singh and Kumar, 2024). Turkey, China, India, and the United States are the primary producers of *Cucurbitaceae*. The cultivation of cucurbits for aliment purposes started about 3000 years ago in Western Asia (Rolnik and Olas, 2020).

The important species of *Cucurbitaceae* family include watermelon, melon, gourds, pumpkins/squash, and bitter apples (Cheng *et al*., 2023). Pumpkins (*Cucurbita* spp.), cultivated for thousands of years, are a rich source of carotenoids and boast a wide range of physical variations. The species *Cucurbita moschata*, *Cucurbita maxima*, and *Cucurbita pepo* adapt to a range of temperatures and are commercially significant around the globe (Hosen *et al*., 2021; Priori *et al*., 2016). The pumpkin pulp constitutes 78.69% of its weight, the skin 17.95%, and the seeds 3.63%. The seeds are consumable, with a nutty flavour and

*Corresponding author e-mail: A.M. Giuffrè: amgiuffre@unirc.it
S. Gharby: s.gharby@yahoo.fr, s.gharby@uiz.ac.ma

a tender texture (Singh and Kumar, 2020). Currently, these seeds are known as super-seeds. They are consumed toasted and salted in several countries or used as ingredients in food products (Abou-Zeid *et al*., 2018).

The climbing perennial bottle gourd, scientifically known as *Lagenaria siceraria*, is an important vegetable crop in many tropical countries, including Japan, Egypt, India, and Thailand. Bottle gourds may grow more than a meter long and take on a wide range of forms, from large and spherical to tiny and from bottle-shaped to slender and sinuous (Saeed *et al*., 2022). *L. siceraria* is a low-calorie vegetable characterized by its high-water content, making it a hydrating and healthy food option. The edible part is rich in vitamins, minerals, choline, flavonoids, proteins, terpenoids, and several other phytochemicals. Moreover, this vegetable is recognized for its diversity of bioactive components, such as flavones, sterols, C-glycosides, triterpenoids, and β-glycosides, which enhance its health-promoting properties (Zahoor *et al.*, 2021). *L. siceraria* is the only cultivated species of the genus *Lagenari*a with significant economic value, grown worldwide for a variety of purposes. It is cultivated not only for its edible fruits, which are used in food and medicine, but also for its versatility in making household utensils, decorative items, and musical instruments (Mashilo *et al*., 2017; Roopan *et al*., 2016).

Many fruits and fruit components, such as seeds, defective fruit, and peels, are wasted in the cucurbit food production process. However, these by-products continue to be an excellent resource for compounds (Piccolella *et al*., 2019). Cucurbit seeds offer remarkable nutritional value due to their high protein content and vital elements, such as copper, phosphorus, zinc, iron, and magnesium. They have a slightly sweet flavour with a slightly nutty flavour and are simple to chew (Rolnik and Olas, 2020).

A variety of new vegetable oils, such as maize oil or rice bran oil, have entered the market recently. Classified as minor vegetable oils, their production and usage remain quite limited compared to major oils like soybean, canola, and peanut oil (Yang *et al*., 2018). Despite their richness in oils, most cucurbit seeds are not widely exploited. In some parts of Africa, however, where the climate favours their cultivation, they are used to produce cooking oil (Karrar *et al*., 2019). In recent decades, oils derived from the seeds of the *Cucurbitaceae* family have gained considerable attention due to their fatty acid composition, especially polyunsaturated fatty acids, and their high content of bioactive compounds (Yoshime *et al*., 2018).

Despite a few isolated studies, the current literature offers limited insights into a comprehensive comparative evaluation of multiple species within the *Cucurbitaceae* family. Most existing research focuses on specific aspects, such as nutritional composition or antioxidant properties, without providing an integrated analysis that encompasses lipid profiles, bioactive characteristics, and oil quality. Additionally, species like *L. siceraria* and certain *Cucurbita* species remain underexplored in a multidimensional context, leaving significant gaps in the understanding and utilization of these seeds as both food and industrial resources.

In response to these gaps, this study conducts an exhaustive comparative analysis of the seeds of four specific *Cucurbitaceae* species (*L. siceraria* and three *Cucurbita* species). It examines their nutritional composition, mineral content, and lipid profiles, along with the bioactive properties and quality of their oils. By integrating these diverse parameters, the research aims to elucidate the nutritional and industrial potential of these underutilized seeds, emphasizing their value as promising resources for various applications.

2. MATERIAL AND METHODS

2.1. Seed sampling and processing

In this study, four distinct seeds from specific plant origins were selected for various analyses, as outlined in Table 1. This diversity enables broadly applicable findings, enhancing the study's overall contribution. The seeds were harvested from the fruits of their respective plants, air-dried, and ground into powder. The resulting powder underwent a series of tests to assess ash content, moisture content, protein levels, mineral composition, oil yield, fatty acid profile, and antioxidant activity.

2.2. Proximate composition of seeds

2.2.1. Moisture content and ash content

The moisture content (*MC*, %) in the sample was measured by heating it to a constant weight in an oven at 103°C. The MC was calculated using the following formula:

Table 1. Description of the plants with the studied seeds

Common name	Scientific name	Abbreviation	Genus	Growth status
Bottle gourd	*Lagenaria siceraria*	*L. siceraria*	*Lagenaria*	Cultivated
Winter squash	*Cucurbita maxima* var. "Rtiliya"	*C. maxima* var. "Rtiliya"	*Cucurbita*	
Butternut squash	*Cucurbita moschata* var. "Hamra"	*C. moschata* var. "Hamra"	*Cucurbita*	
Butternut squash	*Cucurbita moschata* var. "Slaouiya"	*C. moschata* var. "Slaouiya"	*Cucurbita*	

$$MC = \frac{P2 - P3}{P2 - P1}100, \quad (1)$$

where: *P*1 represents the initial weight, *P*2 corresponds to the weight of the whole before drying, and *P*3 is the weight after drying. We used the resulting weight differential to find *MC*.

The ash content (*AC*, %) was determined by incinerating the powder in a muffle furnace at 500°C for four hours. The ash content is expressed by the following formula (Ait Bouzid *et al*., 2024):

$$AC = \frac{M1 - M2}{M0}100, \quad (2)$$

*M*0 denotes the mass of the sample before incineration, expressed in grams. *M*1 corresponds to the mass of the empty crucible in grams. Finally, *M*2 refers to the mass of the crucible with the ashes, also measured in grams.

2.2.2. Protein content

The crude protein content (*PC*) was determined from the nitrogen (N) content, measured via combustion using a LECO FP628 elemental analyzer (LECO Corp., MI, USA) following the Dumas method. The nitrogen content obtained was then converted to crude protein (*PC*), expressed in g 100 g^{-1} dry weight (DW), by applying a conversion factor of 6.25 (Jahan *et al*., 2023):

$$PC = 6.25N. \quad (3)$$

2.2.3. Carbohydrate content and energy value

The carbohydrate content (*CC*) of each seed was calculated by subtracting the combined amounts of ash, moisture, oil, and protein from the total content, following the formula established by Oubannin *et al.* (2022):

$$CC = 100\% - (ash + moisture + oil + proteins). \quad (4)$$

Energy value (*EV,* expressed in kcal 100 g^{-1} dry matter) was determined from oil content, protein, and carbohydrate values using the equation (Ibourki *et al*., 2021):

$$EV = (2.62 \times proteins) + (8.37 \times oil) + (4.2 \times carbohydrate). \quad (5)$$

2.2.4. Oil extraction

Oil extraction from the seed powder was performed using the Soxhlet method, with n-hexane as the extraction solvent at a ratio of 20 g of sample weight to 200 mL of solvent, yielding a solid-to-solvent ratio of 1:10. The extraction process lasted 6 h, after which the n-hexane was evaporated under reduced pressure using a rotary evaporator, as described by Gharby *et al.* (2020). The yield of oil (%) was then computed as follows:

$$Oil\ yield = \frac{mass\ of\ extracted\ oil}{mass\ of\ powder}, \quad (6)$$

where: oil yield (g 100 g^{-1}), mass of extracted oil and mass of powder (g).

2.3. Mineral profiling

Mineral content was assessed by incinerating the sample powder in a muffle furnace at 525 °C for 4 h. The resulting ash was treated with 4 mL of 65% nitric acid (NHO_3) and 10 mL of hydrochloric acid (HCl), followed by analysis using a Perkin Elmer Model Optima 8000 DV spectrometer, as outlined by Bijla *et al.* (2021).

2.4. Extraction of bioactive compounds

For each plant, 1 g of seed powder was extracted with 10 mL of 80% aqueous methanol under continuous agitation for 24 h. The mixture was then filtered using Whatman filter paper, and the resulting crude extracts were stored at +4°C until further analysis. These extracts were subsequently tested for total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity.

2.5. Determination of TPC

The total phenolic content (TPC) was measured using the Folin-Ciocalteu method. A mixture of 0.5 mL of a diluted extract solution, 2.5 mL of 1/10 diluted Folin-Ciocalteu reagent, and 4 mL of 7.5% sodium carbonate was prepared and incubated at 45°C for 30 min. Absorbance was subsequently recorded at 765 nm, following the procedure described by Ismaili *et al.* (2016). The results were expressed as mg gallic acid equivalents per gram of dry matter (mg GAE g^{-1} dry matter), using a calibration curve with gallic acid as the standard reference.

2.6. Determination of TFC

The total flavonoid content (TFC) was determined using a colorimetric method. A 1 mL aliquot of the diluted sample (dilution factor = 10) was placed in a 10 mL volumetric flask, and 0.3 mL of 5% sodium nitrite ($NaNO_2$) was added. After 5 min, 0.3 mL of 10% aluminium chloride ($AlCl_3$) was introduced, and the mixture was left to stand for 6 min. Next, 2 mL of 1 M sodium hydroxide (NaOH) was added, and the volume was adjusted to 10 mL with distilled water. After standing for 30 min, the absorbance was recorded at 415 nm. The flavonoid concentration was quantified in terms of quercetin equivalent (mg QE g^{-1}) using a quercetin calibration curve, as described by Ait Bouzid *et al.* (2024).

2.7. Antioxidant activity

2.7.1. Free radical scavenging activity (DPPH)

The antioxidant activity was evaluated by measuring the ability of the extracts to scavenge the DPPH radical (2,2-diphenyl-1-picrylhydrazyl hydrate). For this assay, 2.5 mL of the extract solution, diluted in ethanol, was mixed with 0.5 mL of a 0.2 mM DPPH solution prepared in ethanol. The mixture was stirred quickly and left in the dark for

30 min, following the protocol outlined by Nounah *et al.* (2017). Absorbance was then recorded at 517 nm, and the percentage of DPPH radical inhibition, or radical scavenging activity (RSA, %), was calculated using the following formula:

$$RSA = \frac{(AC - AS)}{AC}100, \quad (7)$$

AC indicates the absorbance of the control (0.5 mL DPPH and 2.5 mL ethanol) and *AS* refers to the absorbance of the sample.

2.7.2. Ferric reducing antioxidant power (FRAP)

The reducing capacity of the seed extracts was determined using the ferric reducing antioxidant power (FRAP) assay, following the procedure described by Aryal *et al.* (2019). A 0.5 mL aliquot of diluted extract (concentration range: 10-200 µg mL^{-1}) was combined with 1.25 mL of a 0.2 M potassium phosphate buffer solution (pH 6.6) and 1.25 mL of a 1% (w/v) potassium ferricyanide solution. The mixture was incubated at 50°C for 20 min. Subsequently, 1.25 mL of a 10% (w/v) trichloroacetic acid solution was added, and the mixture was centrifuged at 3000 rpm for 10 min. From the resulting supernatant, 1.25 mL was mixed with an equal volume of distilled water (1.25 mL) and 0.25 mL of a 0.1% (w/v) ferric chloride solution. The absorbance was measured at 700 nm, and antioxidant capacity was quantified using a Trolox calibration curve. The results were expressed as µmol Trolox equivalents per gram of dry matter (µmol TE g^{-1} DM).

2.7.3. Trolox equivalent antioxidant capacity (ABTS)

The determination of the antioxidant activity of the samples, measured by the ABTS assay, followed the method described by Ismaili *et al.* (2016). The ABTS radical cation (ABTS+•) was prepared by reacting a 2 mM ABTS solution, diluted in deionized water, with 100 µL of 70 mM potassium persulfate ($K_2S_2O_8$). This mixture was kept in the dark at room temperature for 16 h before use. The $ABTS^{+}$• solution was subsequently diluted with ethanol to reach an absorbance of 0.70 at 734 nm. For each sample, 200 µL of extract was added to 2 mL of the diluted ABTS solution, allowed to react for 1 min, and then absorbance was measured at 734 nm. Antioxidant activity in terms of ABTS was reported as mg Trolox equivalent per g of dry matter (mg TE g^{-1} DM).

2.8. Physicochemical analyses of seed oil

2.8.1. Free fatty acid (FFA)

The free fatty acid (FFA) content in the studied seeds was determined following the standard method outlined in ISO 660 (2020). The seed oil was first dissolved in 95% ethyl alcohol, which had been neutralized, and then titrated with a 0.1 M NaOH solution, using phenolphthalein as an indicator. The FFA content was expressed as g 100 g^{-1} of oleic acid.

2.8.2. Peroxide value (PV)

The peroxide value, expressed in milliequivalents of active oxygen per kilogram of oil (mEq O_2 kg^{-1} oil), was determined by iodine titration of an oil solution in isooctane (Analytical grade) and acetic acid (2:3), following the method specified in ISO 3960 (2017).

2.8.3. Iodine value (IV)

The iodine value (IV), an indicator of the unsaturation level, was determined in this study by calculating the proportion of unsaturated fatty acids using the theoretical approach outlined by Gharby *et al.* (2020):

$$IV = (\%C16{:}1 \times 1.001) + (\%C18{:}1 \times 0.899) + (\%C18{:}2 \times 1.814) + (\%C18{:}3 \times 2.737). \quad (8)$$

2.8.4. Saponification value (SV)

The saponification value (SV, mg KOH g^{-1} of oil) is calculated based on the fatty acid composition using Eqs (9) and (10) described below. First, the fractional molecular weight of each fatty acid in the sample is determined by multiplying the fatty acid's proportion (expressed as a percentage divided by 100) by its molecular weight. The mean molecular weight is then obtained by summing the fractional weights of all fatty acids present in the sample, following the approach outlined by Gagour *et al.* (2024):

$$SV = 3 \times n \times 56 \times 1000, \quad (9)$$

where:

$n = \frac{1}{M_{TG}}$ and M_{TG} – mean molecular weight ×3+92.09-(3×18). (10)

2.9. Fatty acid analysis

Fatty acid composition analysis was conducted according to the ISO 12966-2 method (2017). The fatty acids in each sample underwent transmethylation to form their corresponding methyl esters. For each oil sample, 0.1 g was placed into a screw-top test tube with 2 mL of isooctane (2,2,4-trimethylpentane, chromatographic quality), mixed thoroughly, followed by the addition of 0.1 mL of a 2 N potassium hydroxide solution in methanol. The tube was sealed, stirred for 1 min, and allowed to settle for 2 min. Subsequently, 2 mL of a sodium chloride solution was added, mixed, and the isooctane layer was separated and transferred to a sample vial containing approximately 1 g of sodium hydrogen sulphate. The methyl esters were analyzed using a gas chromatograph (Agilent 6890, Santa Clara, CA, USA) equipped with a CP-WAX 52CB column (30 m length × 0.25 mm i.d., 0.25 µm film thickness) and a flame ionization detector (GC-FID).

Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}, with a 1 µL injection in split mode (split ratio 1:50). The temperature settings were 180°C for the oven (held for 5 min, then ramped to 220°C at 15°C min^{-1}), 220°C for the injector, and 230°C for the detector. The results were reported as the relative proportions of each identified fatty acid peak (Gagour *et al*., 2024).

2.10. Oil seed oxidative stability and nutritional quality indices

Evaluating the nutritional value of seed oils involves analyzing various essential indicators, each offering unique insights into the properties of the oils. Indices like the Oxidizability Value (*COX*), Oxidative Susceptibility (*OS*), Desirable Fatty Acids (*DFAs*), Hypercholesterolemic Fatty Acids (*OFAs*), Atherogenic Index (*AI*), Thrombotic Index (*TI*), and the Hypocholesterolemic/Hypercholesterolemic Ratio (*H*/*H*) collectively provide a comprehensive assessment of oil quality. These metrics assess stability, nutritional value, and potential health impacts, offering a nuanced evaluation of oil quality across multiple dimensions.

The formulas for various indices are as follows:

1. Oxidizability Value (*COX*) (Abril *et al*., 2019) is calculated using the formula:

$$\mathrm{COX} = \frac{1\times(\mathrm{C16:1+C17:1+C18:1+C20:1}) + 10.3\times(\mathrm{C18:2})\times 21.6\times(\mathrm{C18:3+C20:3})}{100} \quad (11)$$

2. Oxidative Susceptibility (*OS*) is determined using the formula (Cecchi *et al*., 2011):

$$OS = MUFA + (45 \times C18:2) + (100 \times C18:3). \quad (12)$$

3. Desirable Fatty Acids (*DFAs*), as defined by Paszczyk and Czarnowska-Kujawska (2022), is given by the formula:

$$DFA = UFA + C18:0. \quad (13)$$

4. Hypercholesterolemic Fatty Acids (*OFAs*) (Cais-Sokolińska *et al*., 2023), is calculated using the formula:

$$OFA = C12:0 + C14:0 + C16:0. \quad (14)$$

5. Index of Atherogenicity (*AI*), according to Medeiros *et al.* (2014), is computed using the following formula:

$$AI = (C12:0 + (4 \times C14:0) + C16:0)/ ((n-3)PUFAs + (n-6)PUFAs + MUFAs). \quad (15)$$

6. Index of Thrombogenicity (*TI*) is calculated as follows (Dal Bosco *et al*., 2022):

$$TI=(C14{:}0+C16{:}0+C18{:}0)/((0.5\times \textit{sum og others MUFAs}) + 0.5\times(n\text{-}6)\mathrm{PUFAs}+(3\times(n\text{-}3)PUFAs/(n\text{-}6)PUFAs. \quad (16)$$

7. Hypocholesterolemic/Hypercholesterolemic Index (*H/H*), as described by Winiarska-Mieczan *et al.* (2024), is given by:

$$H/H=((C18{:}1\ (n\text{-}9)+C18{:}2\ (n\text{-}6)+C18{:}3\ (n\text{-}3)/ (C14{:}0+C16{:}0+C16{:}0)n. \quad (17)$$

2.11. Rancimat test

The Rancimat test was employed to determine the induction period (IP) of seed oils by monitoring changes in conductivity in an aqueous solution, as described in ISO 6886 (2016). During this test, oil samples were heated to 110°C (383 K), causing the formation of volatile polar compounds, which were then transferred into distilled water, leading to a gradual increase in conductivity. Each sample, consisting of 3 g of seed oil, was subjected to these conditions with an airflow rate of 20 L h^{-1} (Asbbane *et al*., 2024).

2.12. Statistical analysis

The results presented are the average values obtained from two or three replicates and are expressed as mean ± standard deviation (SD). To identify significant differences among the seeds, a one-way analysis of variance (ANOVA) with Tukey's test was conducted at a 95% confidence level ($p < 0.05$). Additionally, principal component analysis (PCA) was performed using the mean parameter values from the four seeds analyzed. All statistical analyses were carried out using OriginPro, Version 2022 (OriginLab Corporation, Northampton, Massachusetts, USA).

3. RESULTS AND DISCUSSION

3.1. Proximate composition of seeds

3.1.1. Moisture content and ash content

The seeds of four species of the *Cucurbitaceae* family show significant variations in terms of moisture and ash content, as shown in Table 2. *C. moschata* var. "Slaouiya" has the greatest moisture content (*MC*) at 7.81 ± 0.12 g 100 g^{-1}, indicating enhanced water retention, which may affect its nutritional composition and preservation. Conversely, *C. maxima* var. "Rtiliya" has the lowest value at 5.82 ± 0.04 g 100 g^{-1}. The other varieties, *L. siceraria* and *C. moschata* var. "Hamra", have moisture values of 6.24 ± 0.09 g 100 g^{-1} and 6.40 ± 0.11 g 100 g^{-1}, respectively. Ash content (*AC*) also varies across the species, ranging from 3.93 ± 0.03 g 100 g^{-1} for *L. siceraria* to 4.22 ± 0.01 g 100 g^{-1} for *C. moschata* var. "Hamra" *C. maxima* var. "Rtiliya" has an *AC* of 4.12 ± 0.02 g 100 g^{-1}, while *C. moschata* var. "Slaouiya" has an *AC* of 3.97 ± 0.03 g 100 g^{-1}. These differences in moisture and ash content highlight the diversity in nutrient retention and mineral composition among these *Cucurbitaceae* seeds.

Compared to moisture content (*MC*) values reported in the literature, *L. siceraria* (6.24 ± 0.09 g 100 g^{-1}) and *C. maxima* var. "Rtiliya" (5.82 ± 0.04 g 100 g^{-1}) exhibit higher *MC* than those reported by Abdel-Razek *et al.* (2021) at 4.92 ± 0.93 g 100 g^{-1} and Rezig *et al.* (2019) at 8.46 ± 0.62 g 100 g^{-1}, respectively. The MC of *C. moschata* varieties,

ranging from 6.40 to 7.81 g 100 g^{-1}, aligns with the value of 7.67 ± 0.05 g 100 g^{-1} g reported by Indrianingsih *et al.* (2019).

The ash content (*AC*) comparisons show that *L. siceraria* (3.93 ± 0.03 g 100 g^{-1}), *C. maxima* var. "Rtiliya" (4.12 ± 0.02 g 100 g^{-1}), and *C. moschata* varieties (3.97-4.22 g 100 g^{-1}) exceed the values reported in the literature by Abdel-Razek *et al.* (2021) at 2.51 ± 0.73 g 100 g^{-1}, Salehi *et al.* (2021) at 2.42 g 100 g^{-1} and Gade *et al.* (2022) at 3.83 ± 0.01 g 100 g^{-1}, respectively. These findings suggest that the studied seeds contain greater levels of moisture and ash compared to previously documented values, indicating potential variability in nutrient retention based on origin and environmental factors.

3.1.2. Protein content

Proteins are fundamental for human health, being present in all organs and tissues. Plant-derived proteins are increasingly favored due to ethical and environmental benefits, and they also reduce the risk of metabolic disorders (Kumar *et al*., 2022). The protein content (*PC*) in the seeds analyzed is presented in Table 2. *C. moschata* var. "Slaouiya" has the highest PC at 37.12 ± 0.01 g 100 g^{-1}, followed by *C. moschata* var. "Hamra" at 34.14 ± 0.01 g 100 g^{-1}. On the lower end, *L. siceraria* shows a *PC* of 18.21 ± 0.03 g 100 g^{-1}. The *PC* of *L. siceraria* in this study (18.21 ± 0.03 g 100 g^{-1}) substantially surpasses the value reported by Abdel-Razek *et al.* (2021) from Egypt (11.54 ± 1.05 g 100 g^{-1}) and is comparable to flaxseed (*Linum usitatissimum*), with a *PC* of 20.30 ± 0.07 g 100 g^{-1} reported by Gebremeskal *et al.* (2024). Similarly, *C. maxima* shows a *PC* of 30.32 ± 0.03 g 100 g^{-1}, which is slightly below the Tunisian variety reported by Rezig *et al.* (2019) at 33.92 ± 3.16 g 100 g^{-1}, yet exceeds the *PC* reported from Korea (27.48 ± 1.00 g 100 g^{-1}) by Kim *et al.* (2012). The *C. moschata* varieties "Hamra" and "Slaouiya" (34.14 ± 0.01 to 37.12 ± 0.01 g 100 g^{-1}) show considerably higher protein content compared to values from Indonesia (19.23 ± 0.06 g 100 g^{-1} shown by Indrianingsih *et al.* (2019) and India (23.55 ± 0.03 g 100 g^{-1} reported by Gade *et al.* (2022). Their protein levels also surpass that of chia seeds (*Salvia hispanica*), reported as 23.50 ± 0.01 g 100 g^{-1} by Gebremeskal *et al.* (2024), highlighting the strong potential of these varieties as high-protein plant sources.

3.1.3. Carbohydrate content and energy value

The carbohydrate content (*CC*) and energy value (EV) of the seeds demonstrate considerable variation. *L. siceraria* has the highest *CC* at 40.32 ± 0.81 g 100 g^{-1}, while *Cucurbita moschata* var. "Hamra" has the lowest value at 11.64 ± 0.19 g 100 g^{-1}. For *EV*, *C. maxima* var. "Rtiliya" shows the highest value at 504.31 ± 4.39 kcal 100 g^{-1}, whereas *C. moschata* var. "Slaouiya" has the lowest level at 466.61 ± 3.84 kcal 100 g^{-1}. The *CC* of *L. siceraria* (40.32 ± 0.81 g 100 g^{-1}) is significantly higher than the value of 7.55 ± 0.05 g 100 g^{-1} reported in Nigeria by Ogunbusola (2018). *C. maxima* exhibits a *CC* of 18.02 ± 0.84 g 100 g^{-1}, aligning closely with the amount of 17.08 g 100 g^{-1} reported in Brazil by Veronezi and Jorge (2012), but notably higher than the value of 12.75 ± 1.87 g 100 g^{-1} reported in Bangladesh by Jahan *et al.* (2023). The *CC* of *C. moschata* varieties "Hamra" and "Slaouiya" (ranging from 11.64 ± 0.19 to 13.99 ± 1.36 g 100 g^{-1}) aligns with values reported in India (9.83-17.45 g 100 g^{-1}) by Singh and Kumar (2022) and from Korea (14.02 ± 0.76 g 100 g^{-1}) by Kim *et al.* (2012). However, these values are lower than the level of 22.22 ± 0.026 g 100 g^{-1} documented by Gade *et al.* (2022) in India, indicating regional differences in carbohydrate content likely influenced by environmental or genetic factors.

3.1.4. Oil extraction

The oil content (OC) in the four seeds ranges from 31.30 ± 0.62 to 43.60 ± 0.37 g 100 g^{-1}, with *C. moschata* var. "Hamra" recording the highest value, while *L. siceraria* shows the lowest value. *L. siceraria* has an OC of 31.30 ± 0.62 g 100 g^{-1}, which is lower than the value reported by Abd El-Rahman *et al.* (2022) in Egypt (47.22 ± 0.01 g

Table 2. Mean values of physicochemical parameters for the four seeds studied

Seed	MC	AC	PC	OC	CC	EV
	(g 100 g^{-1})					(kcal 100 g^{-1})
L. siceraria	6.24 ± 0.09^{bc}	3.93 ± 0.03^{d}	18.21 ± 0.03^{d}	31.30 ± 0.62^{d}	40.32 ± 0.81^{a}	479.03 ± 1.81^{b}
C. maxima var. "Rtiliya"	5.82 ± 0.04^{d}	4.12 ± 0.02^{ab}	30.32 ± 0.03^{c}	41.71 ± 0.96^{ab}	18.02 ± 0.84^{b}	504.31 ± 4.39^{a}
C. moschata var. "Hamra"	6.40 ± 0.11^{b}	4.22 ± 0.01^{a}	34.14 ± 0.00^{b}	43.60 ± 0.37^{a}	11.64 ± 0.19^{d}	503.27 ± 2.24^{a}
C. moschata var. "Slaouiya"	7.81 ± 0.12^{a}	3.97 ± 0.03^{bc}	37.12 ± 0.01^{a}	37.10 ± 1.14^{bc}	13.99 ± 1.36^{cd}	466.61 ± 3.84^{bc}

Mean ± standard deviation (n = 2). In each line, mean values marked with different letters (a-d) indicate significant differences (Tukey test, $p < 0.05$). MC – moisture content, AC – ash content, PC – protein content, OC – oil content, CC – carbohydrates content, EV – energy value.

100 g^{-1}). The *OC* of *C. maxima* (41.71 ± 0.96 g 100 g^{-1}) is also lower than the range of 47.32-53.59 g 100 g^{-1} in Moroccan regions reported by Boujemaa *et al.* (2024), but exceeds the value reported by Jahan *et al.* (2023) in Bangladesh (35.23 ± 1.08 g 100 g^{-1}) and Montesano *et al.* (2018) in Italy (29.0 ± 0.9 g 100 g^{-1}). The *OC* in the *C. moschata* varieties (43.60 ± 0.37 g 100 g^{-1} to 37.10 ± 1.14 g 100 g^{-1}) surpasses values reported in Brazil (ranging from 42.29 ± 0.09 g 100 g^{-1}) by Veronezi and Jorge (2012), Korea (45.68 ± 1.17 g 100 g^{-1}) by Kim *et al.* (2012), Algeria (37.8 ± 1.70 g 100 g^{-1}) by Bouazzaoui and Mulengi (2018), and India (26.4 ± 0.020 g 100 g^{-1} by Gade *et al.* (2022).

The oil yield from *Cucurbitaceae* seeds is affected by several factors, such as the plant species, environmental conditions, stage of ripeness, timing of seed harvest, and method of extraction (Nyam *et al.*, 2009; Rezig *et al.,* 2022).

3.2. Mineral profiling

Minerals and trace elements, though present in small quantities, are essential micronutrients for human health (Dubey *et al.*, 2020). They form vital components of the skeletal system, influence muscular and nervous function, and play a crucial role in maintaining the body's water balance (Stefanache *et al.*, 2023). Additionally, they are integral to the structure of hormones, enzymes, and other biologically active compounds (Weyh *et al.*, 2022).

In this study, ten minerals, comprising five microelements (Fe, Mn, Cu, Zn, and B) and five macroelements (K, P, Ca, Mg, and Na), were analyzed in four different seeds. Table 3 presents the results, which show substantial variations in macroelement concentrations (K, P, Mg, Ca, and Na) across the seeds. Among the macro-elements, potassium (K) was found to be the most abundant. *L. siceraria* seeds had the highest potassium concentration at 967.06 ± 12.25 mg 100 g^{-1}, whereas *C. maxima* var. "Rtiliya" seeds exhibited the lowest K concentration at 796.01 ± 11.07 mg 100 g^{-1}.

In terms of phosphorus (P), *C. moschata* var. "Slaouiya" seeds had the highest content at 898.85 ± 7.42 mg 100 g^{-1}, followed by *C. moschata* var. "Hamra" seeds at 842.92 ± 4.96 mg 100 g^{-1}. *L. siceraria* seeds have the lowest phosphorus concentration, at 573.43 ± 0.13 mg 100 g^{-1}.

C. *moschata* var. "Slaouiya" seeds showed the highest concentration of magnesium (Mg) at 435.89 ± 3.36 mg 100 g^{-1}, followed by C. *moschata* var. "Hamra" seeds with 415.40 ± 1.46 mg 100 g^{-1}. The lowest Mg concentration was observed in *L. siceraria* seeds with 305.64 ± 0.06 mg 100 g^{-1}. In comparison with the data reported in the literature, these values are higher than those found by Bouazzaoui and Mulengi (2018) for C. *moschata* var. "Slaouiya" seeds (246.12 mg 100 g^{-1}) and by Singh and Kumar (2022) for C. *moschata* var. "Hamra" seeds (283.28 mg 100 g^{-1}).

Calcium (Ca) is most abundant in *C. moschata* var. "Slaouiya" seeds, with a concentration of 82.28 ± 0.75 mg 100 g^{-1}, followed by *L. siceraria* seeds with 68.51 ± 0.05 mg 100 g^{-1}. *C. maxima* var. "Rtiliya" seeds had the lowest Ca content at 42.14 ± 0.41 mg 100 g^{-1}. The results obtained for *L. siceraria* seeds (68.51 ± 0.05 mg 100 g^{-1}), C. *maxima* var. "Rtiliya" seeds (42.14 ± 0.41 mg 100 g^{-1}), and C. *moschata* var. "Hamra" seeds (67.31 ± 1.19 mg 100 g^{-1}) are in contrast with those reported by Ogunbusola (2018), Hussain *et al.* (2021), and Singh and Kumar (2022), respectively. For sodium (Na), *L. siceraria* seeds contain the highest concentration at 21.71 ± 0.45 mg 100 g^{-1}, followed by *C. moschata* var. "Hamra" seeds at 11.55 ± 0.30 mg 100 g^{-1}, while *C. maxima* var. "Rtiliya" seeds have the lowest Na concentration at 5.05 ± 0.05 mg 100 g^{-1}.

Considering the analyses of the micro-elements, the results obtained show significant variations in their concentrations (Fe, Zn, Mn, Cu, and B) among the different seeds studied. *C. moschata* var. "Hamra" has the highest iron (Fe) concentration (10.03 ± 0.24 mg 100 g^{-1}), while *C. maxima* var. "Rtiliya" had the lowest level (5.26 ± 0.00 mg 100 g^{-1}). *C. moschata* var. "Hamra" has the highest zinc (Zn) content (6.91 ± 0.09 mg/100 g), while *L. siceraria* has the lowest Zn amounts (4.47 ± 0.01 mg 100 g^{-1}). For manganese (Mn), *C. moschata* var. "Slaouiya" stands out with the highest concentration (3.61 ± 0.01 mg 100 g^{-1}), and *L. siceraria* has its lowest level (2.23 ± 0.01 mg 100 g^{-1}). *L. siceraria* seeds showed the highest copper (Cu) concentration (1.89 ± 0.02 mg 100 g^{-1}), whereas C. *moschata* var. "Slaouiya" seeds recorded the lowest Cu content (0.33 ± 0.01 mg 100 g^{-1}). Finally, regarding boron (B), *C. moschata* var. "Hamra" displayed the highest level (1.35 ± 0.01 mg 100 g^{-1}), while *C. maxima* var. "Rtiliya" has the lowest content of B (0.37 ± 0.01 mg 100 g^{-1}).

The comparison of our results with the values reported in the literature reveals interesting discrepancies for the microelements in the different seeds studied. In *C. moschata* var. "Hamra" and *C. moschata* var. "Slaouiya", the Fe concentrations (10.03 ± 0.24 mg 100 g^{-1} and 7.33 ± 0.09 mg 100 g^{-1}, respectively) are slightly higher than those reported by Singh and Kumar (2022). On the other hand, our Cu concentrations (0.63 ± 0.01 mg 100 g^{-1} and 0.33 ± 0.01 mg 100 g^{-1}) are slightly lower than those reported by Singh and Kumar (2022) and Bouazzaoui and Mulengi (2018). Nevertheless, the results found for Mn in *L. siceraria* are slightly higher than those reported by Ogunbusola (2018). Similarly, our results for Zn in *L. siceraria* (4.47 ± 0.01 mg 100 g^{-1}) are higher than those reported by the same author, *i.e.* 0.42 ± 0.01 mg 100 g^{-1}.

The variation observed in the mineral composition of the seeds could be attributed to various factors, such as geographical origin, plant variety, harvesting season, and environmental conditions. These significant variations highlight the diversity of the mineral composition of the

seeds of the different species studied, underlining their varied nutritional potential and possible applications in various fields.

3.3. Total polyphenol content (TPC)

One of the most abundant secondary metabolites in plants is phenols, often called phenolics. They have a wide range of biological functions, including antioxidant activity, and are present in many edible and non-edible species (Lin *et al.*, 2016). The total polyphenol content (TPC) in the four seeds investigated is presented in Table 3. These seeds revealed varied quantities of polyphenols. *L. siceraria* exhibited the highest polyphenol content (5.71±0.12 mg GAE g^{-1}), while *C. moschata* var. "Slaouiya" had their lowest level (1.79 ± 0.04 mg GAE g^{-1}).

The TPC in *C. maxima* (1.58 ± 0.02 mg GAE g^{-1}) is significantly lower than the value reported by Hussain *et al*. (2021) at 224.61 ± 1.60 mg GAE 100 g^{-1} from Pakistan and 8.27 ± 0.34 mg GAE g^{-1} obtained in Bangladesh by Jahan *et al.* (2023). Similarly, the TPC in *C. moschata* varieties "Hamra" and "Slaouiya", ranging from 1.03 ± 0.14 mg GAE g^{-1} to 2.44 ± 0.02 mg GAE g^{-1}, is also lower than the value of 175.11 ± 0.020 mg GAE 100 g^{-1} reported by Gade *et al.* (2022) from India.

3.4. Total flavonoid content (TFC)

Flavonoids are the most abundant group of phenolic chemicals found in plants, accounting for more than 50% of the total 8 000 natural phenolic components (Sun and Shahrajabian (2023). The findings, presented in Table 3, reveal that the TFC levels in the investigated seeds varied from 13.45 ± 0.49 mg QE g^{-1} (*C. moschata* var. "Hamra") to 16.06 ± 0.27 mg QE g^{-1} (*L. siceraria*). The TFC in *C. maxima* var. "Rtiliya" (13.83 ± 0.60 mg QE g^{-1}, equivalent to 1383.0 mg QE 100 g^{-1}) and *C. moschata* varieties "Hamra" and "Slaouiya" (13.45 ± 0.49 to 14.60 ± 1.14 mg QE g^{-1}) is significantly higher than the values reported by Hussain *et al.* (2021) in Pakistan (139.37 ± 1.07 mg QE 100 g^{-1}) and by Gade *et al.* (2022) in India (142.82 ± 0.030 mg QE 100 g^{-1}), respectively.

3.5. Antioxidant activity

The antioxidant activity of the seeds was tested using the DPPH, FRAP, and ABTS methods providing a more in-depth evaluation of their antioxidant potential. The results are shown in Table 3. The DPPH assay is frequently used in research to measure antioxidant activity because it effectively accommodates various sample types and detects active compounds at even low concentrations (Oucif *et al.*, 2017). The DPPH of the four seed samples varies significantly, with *C. moschata* var. "Hamra" recording the highest activity (14.34 ± 0.01 mg AAE g^{-1}), while *C. maxima* var. "Rtiliya" shows the lowest value (7.57 ± 0.70 mg AAE g^{-1}). When compared to values reported in the literature for rapeseed (4.61 ± 0.76 mg AAE g^{-1}) and sunflower (17.29 ± 1.23 mg AAE g^{-1}) by Gagour *et al.* (2022), *C. moschata* var. "Hamra" shows a notably higher antioxidant activity than rapeseed but lower than that of sunflower.

The FRAP test has been applied to examine the iron-reducing ability in a range of biological materials. This approach is significant since it gives an early indication of antioxidant presence and potential (Rezanejad *et al.*,

Table 3. Mean values of mineral composition (mg 100 g^{-1}), total phenolic content, total flavonoid content, and antioxidant activities of the investigated seeds

Analysis name	*L. siceraria*	*C. maxima* var. "Rtiliya"	*C. moschata* var. "Hamra"	*C. moschata* var. "Slaouiya"
Ca	68.51 ± 0.05^{b}	42.14 ± 0.41^{c}	67.31 ± 1.19^{b}	82.28 ± 0.75^{a}
Na	20.71 ± 0.45^{a}	5.05 ± 0.05^{d}	11.55 ± 0.30^{b}	6.96 ± 0.10^{c}
K	967.06 ± 12.25^{a}	796.01 ± 11. 07^{d}	855.66 ± 0.08^{bc}	875.59 ± 28.87^{b}
Mg	305.64 ± 0.06^{d}	373.61 ± 3.91^{c}	415.40 ± 1.46^{b}	435.89 ± 3.36^{a}
Fe	6.99 ± 0.04^{c}	5.26 ± 0.00^{d}	10.03 ± 0.24^{a}	7.33 ± 0.09^{b}
Mn	2.23 ± 0.01^{d}	2.34 ± 0.02^{c}	3.44 ± 0.03^{b}	3.61 ± 0.01^{a}
Cu	1.89 ± 0.02^{a}	0.39 ± 0.01^{c}	0.63 ± 0.01^{b}	0.33 ± 0.01^{d}
Zn	4.47 ± 0.01^{d}	4.94 ± 0.04^{c}	6.91 ± 0.09^{a}	5.58 ± 0.01^{b}
B	0.81 ± 0.01^{b}	0.37 ± 0.00^{c}	0.93 ± 0.01^{a}	0.84 ± 0.1^{b}
P	573.43 ± 0.13^{d}	751.37 ± 18.56^{c}	842.92 ± 4.96^{b}	898.85 ± 7.42^{a}
TPC (mg GAE g^{-1})	5.71 ± 0.12^{a}	1.58 ± 0.02^{c}	1.03 ± 0.14^{c}	2.44 ± 0.02^{b}
TFC (mg QE g^{-1})	16.06 ± 0.27^{a}	13.83 ± 0.60^{ab}	13.45 ± 0.49^{b}	14.60 ± 1.14^{ab}
DPPH (mg AAE g^{-1})	11.29 ± 1.44^{b}	7.57 ± 0.70^{ab}	14.34 ± 0.01^{a}	8.59 ± 1.44^{ab}
FRAP (mg AAE g^{-1})	11.11 ± 1.28^{a}	8.55 ± 0.57^{b}	8.36 ± 0.13^{b}	7.77 ± 4.68^{b}
ABTS (mg TE g^{-1})	0.67 ± 0.01^{a}	0.13 ± 0.01^{c}	0.15 ± 0.01^{c}	0.43 ± 0.01^{b}

Mean ± standard deviation (n = 2). In each line, mean values marked with different letters (a-d) indicate significant differences (Tukey test, $p < 0.05$). TPC – total phenolic content, TFC – total flavonoid content, DPPH – 2-2-diphenyl-1-picrylhydrazyl, FRAP – Ferric reducing antioxidant power, ABTS – 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid).

2020). The FRAP results reveal significant differences in iron reduction. *L. siceraria* has the highest ferric-reducing antioxidant power (FRAP) with 11.11 ± 1.28 mg AAE g^{-1}, while *C. moschata* var. "Slaouiya" records the lowest value at 7.77 ± 4.68 mg AAE g^{-1}. In comparison with other plant species, the FRAP values from this research are lower than those reported for *Ziziphus lotus* by Ait Bouzid *et al.* (2022), which range from 9.11 ± 0.04 to 43.67 ± 2.43 mg TE g^{-1} DW. However, they are comparable to or relatively higher than the results for rapeseed (7.73 ± 0.61 mg TE g^{-1}) and sunflower (11.98 ± 0.36 mg TE g^{-1}) published by Gagour *et al.* (2022).

The ABTS test has greater sensitivity in detecting antioxidants with intense pigmentation and water solubility in comparison to the DPPH assay (Lin *et al.*, 2022). The ABTS varies significantly among the four seed samples. *L. siceraria* exhibits the highest ABTS activity (0.67 ± 0.01 mg TE g^{-1}), while *C. maxima* var. "Rtiliya" and *C. moschata* var. "Hamra" show much lower values (0.07 ± 0.09 mg TE g^{-1} and 0.08 ± 0.09 mg TE g^{-1}, respectively). The results of ABTS are inferior to those reported by Ait Bouzid *et al.* (2022) for *Ziziphus lotus*, whose ABTS values range from 5.73 to 5.92 mg TE g^{-1}.

3.6. Physicochemical analyses of seed oil

The mean physicochemical parameters of seed oils from four *Cucurbitaceae* species, presented in Table 4, include free fatty acids (FFAs), peroxide value (PV), iodine value (IV), and saponification value (SV).

3.6.1. Free fatty acids (FFAs)

The concentration of Free Fatty Acids (FFAs) is one of the most important measures for determining the degree of edible oil deterioration during storage (Wang *et al.*, 2024). The FFA results are presented in Table 4. *C. maxima* var. "Rtiliya" recorded the highest value with 24.9 ± 0.43 g 100 g^{-1}, followed by *C. moschata* var. "Hamra") with 9.59 ± 0.34 g 100 g^{-1}. In contrast, *C. moschata* var. "Slaouiya" exhibited the lowest value at 1.28 ± 0.15 g 100 g^{-1}. The FFA values are generally higher than those reported in the literature. *L. siceraria* (3.89 ± 0.32 g 100 g^{-1}) surpasses the value of 2.23 ± 0.04 g 100 g^{-1} reported by Abd El-Rahman *et al.* (2022) for Egyptian samples. *C. moschata* var. "Hamra" (9.59 ± 0.34 g 100 g^{-1}) has considerably higher FFA content than the value of 3.66 ± 0.01 g 100 g^{-1} reported by Ajibe *et al.* (2022) A high level of FFAs observed in *C. maxima* var. "Rtiliya" demands a physical or chemical refining step to improve its quality and stability by reducing the concentration of FFAs.

3.6.2. Peroxide value (PV)

The peroxide value is often used to assess the significance of primary oxidation products in oils. A higher peroxide value indicates a higher degree of lipid oxidation, which can affect the quality, flavour, and shelf life (Zhang *et al.*, 2021). The results presented in Table 4 show that PV in the investigated seeds varied widely, ranging from 8.93 ± 2.39 mEq O_2 kg^{-1} oil (*C. moschata* var. "Hamra") to 21.58 ± 2.34 mEq O_2 kg^{-1} oil (*C. maxima* var. "Rtiliya"). The PV of the examined seed oils shows differences compared to the literature. *L. siceraria* has a much higher PV (14.89 ± 1.73 mEq O_2 kg^{-1} oil) compared to 0.55 ± 0.04 mEq O_2 kg^{-1} oil reported by Abd El-Rahman *et al.* (2022) for Egyptian bottle gourd. The PV of *C. maxima* var. "Rtiliya" (21.58 ± 2.34 mEq O_2 kg^{-1} oil) exceeds the level of 7.39 ± 2.05 mEq O_2 kg^{-1} oil reported by Fokou *et al.* (2009) in Cameroon. Conversely, *C. moschata* var. "Hamra" has a lower PV (8.9393 ± 2.39 mEq O_2 kg^{-1} oil) than the value of 20.47 ± 11.83 mEq O_2 kg^{-1} oil reported by Fokou *et al.* (2009).

These variations indicate that the peroxide value of oil is influenced by such factors as heating during extraction, fatty acid composition, storage conditions, and exposure to oxygen during drying.

3.6.3. Iodine value (IV)

Iodine value (IV) can give an indication on the level of unsaturation in oil and provides its stability to oxidation (Badmus *et al*., 2021). IV indicates double bonds in the molecular structure (Ujm and Sn, 2020). It also reflects the unsaturation degree of FAs (Nwachoko *et al*., 2023). According to Table 4, *L. siceraria* (135.90 ± 0.30 g I_2 100 g^{-1} oil) shows the highest value, followed by *C. maxima* var. "Rtiliya" (120.03 ± 0.31 g I_2 100 g^{-1} oil). *C. moschata* var. "Slaouiya" had the lowest values (102.04 ± 0.31 g I_2 100 g^{-1} oil). The IV of *C. maxima* var. "Rtiliya" (120.03 ± 0.31 g I_2 100 g^{-1} oil) is notably higher than the value of 105.12 g I_2 100 g^{-1} oil reported by Konadu *et al*. (2021) from Ghana. Additionally, the IV of *C. moschata* var. "Hamra" (106.01 ± 0.30 g I_2 100 g^{-1} oil) is comparable to the IV of 105.12 g I_2 100 g^{-1} oil reported by Singh and Kumar (2023). Seed oils are highly recommended medically and industrially due to their low iodine value and high saponification value (Ujm and Sn, 2020).

3.6.4. Saponification value (SV)

The saponification value of oil determines whether it is suitable for making soap (Badmus *et al.*, 2021). All the oils studied exhibit very similar SV values ranging between 191.29 ± 0.81 mg KOH g^{-1} oil (*C. moschata*) to 191.54 mg KOH g^{-1} oil (*C. maxima*) (Table 4). The SV of the seed oils studied are higher than those reported in the literature. *L. siceraria* (191.50 ± 0.81) surpasses the level of 160.42 ± 2.96 mg KOH g^{-1} oil shown by Hachimi *et al.* (2015). *C. maxima* var. "Rtiliya" (191.54 ± 0.81) also exceeds the value of 185.20 mg KOH g^{-1} reported by Konadu *et al.* (2021), while this parameter in *C. moschata* var. "Hamra" (191.29 ± 0.81) is higher than the SV of 176.13 ± 2.46 mg KOH g^{-1} calculated by Singh and Kumar (2023). The high saponification value has highlighted the considerable potential of

Table 4. Mean values of physicochemical parameters, fatty acids composition, fatty acid ratios, oxidative stability and nutritional quality indices

Analysis name	*L. siceraria*	*C. maxima* var. "Rtiliya"	*C. moschata* var. "Hamra"	*C. moschata* var. "Slawiya"
Physicochemical parameters				
FFA (g 100 g^{-1} oil)	3.89 ± 0.32^{c}	24.9 ± 0.43^{a}	9.59 ± 0.34^{b}	1.28 ± 0.15^{d}
PV (mEq O_2 kg^{-1} oil)	14.89 ± 1.73^{b}	21.58 ± 2.34^{a}	8.93 ± 2.39^{bcd}	12.65 ± 1.32^{bc}
IV (g I_2 100 g^{-1} oil)	135.90 ± 0.30^{a}	120.03 ± 0.31^{b}	106.01 ± 0.30^{c}	102.04 ± 0.31^{d}
SV (mg KOH g^{-1} oil)	191.50 ± 0.81^{a}	191.54 ± 0.81^{a}	191.29 ± 0.81^{a}	191.29 ± 0.81^{a}
Fatty acids				
C14:0	ND	0.13 ± 0.01^{a}	0.12 ± 0.01^{a}	0.1 ± 0.01^{ab}
C16:0	12.82 ± 0.10^{c}	12.41 ± 0.10^{c}	16.45 ± 0.10^{a}	15.62 ± 0.10^{b}
C16:1	ND	0.12 ± 0.01^{a}	ND	0.09 ± 0.01^{a}
C18:0	7.6 ± 0.10^{c}	7.63 ± 0.10^{c}	7.84 ± 0.10^{bc}	9.65 ± 0.10^{a}
C18:1	7.96 ± 0.10^{d}	25.87 ± 0.10^{c}	32.57 ± 0.10^{b}	34.59 ± 0.10^{a}
C18:2	70.73 ± 0.10^{a}	53.04 ± 0.10^{b}	42.07 ± 0.10^{c}	38.82 ± 0.10^{d}
C18:3	0.16 ± 0.01^{a}	0.16 ± 0.01^{a}	0.15 ± 0.01^{a}	0.16 ± 0.01^{a}
C20:0	0.47 ± 0.01^{d}	0.53 ± 0.01^{c}	0.61 ± 0.01^{b}	0.79 ± 0.01^{a}
C21:0	0.17 ± 0.01^{a}	0.09 ± 0.01^{b}	0.17 ± 0.01^{a}	0.16 ± 0.01^{a}
Fatty acid types				
SFA	21.06 ± 0.22^{b}	20.79 ± 0.23^{b}	25.19 ± 0.23^{a}	26.32 ± 0.23^{a}
MUFA	7.96 ± 0.11^{d}	25.99 ± 0.11^{c}	32.57 ± 0.11^{b}	34.68 ± 0.11^{a}
PUFA	70.89 ± 0.11^{a}	53.20 ± 0.11^{b}	42.22 ± 0.11^{c}	38.98 ± 0.11^{d}
UFA	78.85 ± 0.21^{a}	79.19 ± 0.22^{a}	74.79 ± 0.21^{b}	73.66 ± 0.22^{b}
Ratio				
MUFA/PUFA	0.11 ± 0.002^{d}	0.49 ± 0.001^{c}	0.77 ± 0.001^{b}	0.89 ± 0.00^{a}
UFA/SFA	3.74 ± 0.04^{a}	3.81 ± 0.04^{a}	2.97 ± 0.03^{b}	2.8 ± 0.02^{b}
PUFA/SFA	3.37 ± 0.04^{a}	2.56 ± 0.03^{b}	1.68 ± 0.02^{c}	1.48 ± 0.01^{d}
Oxidative stability and nutritional quality indices				
COX	7.40 ± 0.01^{a}	5.76 ± 0.01^{b}	4.69 ± 0.01^{c}	4.38 ± 0.01^{d}
OS	3206.81 ± 5.50^{a}	2428.79 ± 5.50^{b}	1940.72 ± 5.50^{c}	1797.58 ± 5.50^{d}
DFA	86.45 ± 0.44^{a}	86.82 ± 0.45^{a}	82.63 ± 0.44^{b}	83.31 ± 0.45^{b}
OFA	12.82 ± 0.14^{c}	12.54 ± 0.16^{c}	16.57 ± 0.16^{a}	15.72 ± 0.16^{b}
H/H	6.15 ± 0.04^{a}	6.31 ± 0.05^{a}	4.51 ± 0.02^{b}	4.68 ± 0.03^{b}
AI	0.17 ± 0.01^{b}	0.17 ± 0.01^{b}	0.23 ± 0.01^{a}	0.23 ± 0.01^{a}
TI	0.04 ± 0.01^{c}	0.05 ± 0.01^{c}	0.08 ± 0.01^{bc}	0.09 ± 0.01^{a}

Mean ± standard deviation (n = 2). In each column, mean values marked with different letters (a-d) indicate significant differences (Tukey test, $p < 0.05$). FFA – fatty acids, PV – peroxide value, IV – iodine value, SV – saponification value, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty, UFA – unsaturated fatty acids, COX – calculated oxidizability value, OS –oxidative susceptibility, DFA – index of desirable fatty acids, OFA – sum of hypercholesterolemic fatty acids, HH – ratio of hypocholesterolemic to hypercholesterolemic, AI – index of atherogenicity, TI – index of thrombogenicity.

the oils in various industrial applications, such as soap, cosmetics, medicine, and pharmaceutical products (Ujm and Sn, 2020).

In conclusion, *C. maxima* var. "Rtiliya" exhibits high levels of FFAs (24.9 g 100 g^{-1} and PV (21.58 mEq O_2 kg^{-1} oil), necessitating physical or chemical refining to improve its oil quality and stability. Although it shows a relatively high iodine value (IV) of 120.03 g I_2 100 g^{-1}, indicating a significant level of unsaturation, this also makes it more vulnerable to oxidation. However, *L. siceraria* and *C. moschata* var. "Slaouiya" display lower FFA content (3.89 g 100 g^{-1} and 1.28 g 100 g^{-1}, respectively) and moderate PV levels (14.89 mEq O_2 kg^{-1} oil and 12.65 mEq O_2 kg^{-1} oil), indicating better oil quality and stability. Both varieties also show similar SV around 191.29-191.54 mg KOH g^{-1} oil, suggesting comparable triglyceride structures.

3.7. Fatty acid composition

Fatty acids, the major abundant components of vegetable oils, have gained significant attention in recent years in the food, cosmetics, and pharmaceutical industries for their natural antioxidant properties and recognized health benefits (Badmus *et al*., 2021; Orsavova *et al*., 2015). The fatty acid composition is summarized in Table 4. Linoleic acid (C18:2) was found to be the dominant fatty acid in the oil samples, ranging from 38.82 ± 0.10 g 100 g^{-1} in *C. moschata* var. "Slaouiya" to 70.73 ± 0.10 g 100 g^{-1} in *L. siceraria*. The high content of omega-6 fatty acids in the seed oils suggests that they are nutrient-rich sources, contributing to a healthy diet (Hagos *et al*., 2023). Oleic acid (C18:1), a monounsaturated fatty acid (MUFA), ranged between 7.96 g 100 g^{-1} in *L. siceraria* and 34.59 g 100 g^{-1} in *C. moschata* var. "Slaouiya", indicating a high MUFA content in the latter. Regarding saturated fatty acids (SFAs), the oils contained two primary SFAs: palmitic acid (C16:0) and stearic acid (C18:0). The palmitic acid content ranged from 12.41 g 100 g^{-1} to 16.45 g 100 g^{-1}, with the *C. moschata* varieties showing notably higher concentrations. Stearic acid levels were found to be between 7.6 g/100 g and 9.65 g 100 g^{-1}, with *C. moschata* var. "Slaouiya" proving to be a particularly good source of stearic acid at 9.65 ± 0.10 g 100 g^{-1}. Stearic acid, a long-chain SFA, is known for its health-promoting properties (Badmus *et al*., 2021).

L. siceraria was particularly rich in PUFAs, representing 70.89% of its total fatty acid content, followed by *C. maxima* var. "Rtiliya" at 53.20%. In contrast, the *C. moschata* varieties, "Hamra" and "Slaouiya", exhibited lower PUFA levels of 42.22% and 38.98%, respectively. MUFAs were most abundant in *C. moschata* var. "Slaouiya" (34.68%) and "Hamra" (32.57%), while *L. siceraria* contained only 7.96%. SFAs were most abundant in *C. moschata* var. "Slaouiya" (26.32%) and "Hamra" (25.19%), while *L. siceraria* had the lowest SFA content (21.06%). On the other hand, the quantities of unsaturated fatty acids (UFAs) varied between 73.66 ± 0.22 g 100 g^{-1} in *C. moschata* var. "Slaouiya" and 79.19 ± 0.22 g 100 g^{-1} in *C. maxima* var. "Rtiliya". The presence of higher levels of PUFAs indicates the significant nutritional value of seed oils. PUFAs are known for their ability to lower plasma concentrations of low-density lipoprotein (LDL) cholesterol, contributing to heart health (Bireche *et al*., 2021). It is now well established that a diet rich in PUFAs and low in SFAs is better for human health. However, high levels of PUFAs can lead to rapid oxidation (Boujemaa *et al*., 2024). The composition of SFAs and UFAs was reported between 13.71-21.40 and 77.50-83.81 g 100 g^{-1}, respectively, by Al-Hwaiti *et al.* (2021). The level of linoleic acid in pumpkin (*C. maxima*) was similar to the percentage found by Hagos *et al.* (2023).

The results of the fatty acid ratios for the four seeds were analyzed across three ratios: MUFA/PUFA, UFA/SFA, and PUFA/SFA. The MUFA/PUFA ratio ranged from 0.11 to 0.89, with *C. moschata* var. "Slaouiya" displaying the largest percentage of MUFAs, while *L. siceraria* exhibited a preponderance of PUFAs. For the UFA/SFA ratio, *C. maxima* var. "Rtiliya" had the highest UFA value (3.81), whereas *C. moschata* var. "Slaouiya" had the lowest level (2.8), indicating a larger prevalence of SFAs. Finally, for the PUFA/SFA ratio, *L. siceraria* showed the greatest ratio (3.37), indicating its richness in PUFAs, while *C. moschata* var. "Slaouiya" had the lowest ratio (1.48).

3.8. Seed oxidative stability and nutritional quality indices

The oxidative stability and nutritional quality indices of oils from four *Cucurbitaceae* seeds, as detailed in Table 4, offer significant insights into their potential applications across food, health, and industrial sectors.

3.8.1. Oxidizability value (COX) and oxidative susceptibility (OS)

The oxidation indices were evaluated based on COX and OS. Lower COX and OS values indicate better oxidative stability and, therefore, a prolonged shelf life (Athanasiadis *et al*., 2024; Belhoussaine *et al*., 2024). *C. moschata* var. "Hamra" and "Slaouiya" exhibited the lowest COX values (4.69 and 4.38, respectively), indicating better oxidative stability and lower susceptibility to oxidation. In contrast, *L. siceraria* had the highest COX value (7.40), suggesting a greater tendency toward oxidation. The COX values of *C. maxima* var. "Rtiliya" (5.76) are comparable to those found in pumpkin oil (*Cucurbita pepo* L.) (5.56) as reported by Banaś *et al.* (2020).

Oxidative susceptibility (OS) also assesses oil's resistance to oxidation. *L. siceraria* has the highest value (3206.81), indicating low oxidative stability. In contrast, the *C. moschata* varieties ("Hamra" and "Slaouiya") have the lowest values (1940.72 and 1797.58, respectively), suggesting higher oxidative stability. *C. maxima* var. "Rtiliya" oil had an OS value similar to that of sunflower oil (2475 ± 14), as reported by M'Rabet *et al.* (2023). The values of OS

for all oils are lower than that of *Linum usitatissmium* L. oil and higher than in *Argania spinosa* L. oil (Belhoussaine *et al*., 2024).

3.8.2. Desirable fatty acids (DFAs), hypercholesterolemic fatty acids (OFAs), and hypocholesterolemic/hypercholesterolemic index (H/H)

The DFA value offers valuable information on the hypocholesterolemic characteristics of the oil under study, showing their capacity to reduce total cholesterol levels (Szpunar-Krok *et al*., 2022). The DFA values of the examined seed oils (Table 4) demonstrate varying degrees of hypocholesterolemic characteristics, with significant differences observed among the species. *C. maxima* var. "Rtiliya" has the highest DFA value of 86.82 ± 0.45. In contrast, *C. moschata* varieties "Hamra" and "Slaouiya" showed lower DFA values of 82.63 ± 0.44 and 83.31 ± 0.45, respectively. These findings indicate that *C. maxima* and *L. siceraria* oils have a stronger potential for lowering total cholesterol levels compared to the *C. moschata* varieties. The DFA values recorded for the four seed oils analyzed are lower than those of walnut oil (92.59 ± 0.03) reported by Lakhlifi El Idrissi *et al.* (2024) but remain close to the values of peanut oil (84.95 ± 0.08) reported by the same authors.

The OFA values for the four seed oils analyzed show significant variation, reflecting different degrees of hypercholesterolemic properties. *C. moschata* var. "Hamra" exhibited the highest OFA value (16.57 ± 0.16), followed by *C. moschata* var. "Slaouiya" (15.72 ± 0.16), while *C. maxima* var. "Rtiliya" has the lowest among these four (12.54 ± 0.16). In comparison, the OFA values of the seed oils are higher than those reported for walnut oil (7.08 ± 0.02) and peanut oil (9.03 ± 0.00) by Lakhlifi El Idrissi *et al*. (2024), indicating a higher hypercholesterolemic potential for the studied *Cucurbitaceae* seed oils.

The H/H ratio findings from the seed oils reveal notable differences in their potential effects on cholesterol metabolism. *C. moschata* var. "Hamra" exhibited the lowest H/H ratio (4.51 ± 0.02). In contrast, *C. maxima* var. "Rtiliya" displayed a higher H/H ratio (6.31 ± 0.05), which, while indicative of a stronger hypocholesterolemic effect, remains below the ratios reported for walnut oil (12.62 ± 0.04) and Shia oil (13.5) by Rokosik *et al*. (2020) and for peanut oil (8.94 ± 0.01) by Lakhlifi El Idrissi *et al*. (2024) Interestingly, the H/H ratios of the studied seed oils are comparable to that of Nigella oil (6.9) reported by Rokosik *et al.* (2020).

3.8.3. Atherogenic and thrombogenic indices (AI, TI)

The analysis of the atherogenic (AI) and thrombogenic (TI) indices for the four seed oils revealed that *C. moschata* varieties "Hamra" and "Slaouiya" had the highest AI values (0.23 ± 0.01), indicating a greater potential to contribute to atherogenic processes. These same varieties also recorded relatively high TI values (0.08 ± 0.01 and 0.09 ± 0.01), suggesting an increased likelihood of promoting thrombosis. In contrast, *L. siceraria* (AI: 0.17 ± 0.01, TI: 0.04 ± 0.01) and *C. maxima* var. "Rtiliya" (AI: 0.17 ± 0.01, TI: 0.05±0.01) showed lower AI and TI values, indicating a reduced potential for cardiovascular risk. The *C. moschata* varieties "Hamra" and "Slaouiya" exhibited the highest AI values (0.23 ± 0.01), surpassing peanut oil (0.11 ± 0.00) as reported by Lakhlifi El Idrissi *et al.* (2024). Additionally, their TI values (0.08 ± 0.01 and 0.09 ± 0.01) were lower than that of peanut oil (0.29 ± 0.00). In contrast, *L. siceraria* and *C. maxima* var. "Rtiliya" had lower AI values (0.17 ± 0.01), falling between the AI values of Nigella oil (0.145) and Shia oil (0.074), as reported by Rokosik *et al.* (2020). Their TI values (0.04 ± 0.01 and 0.05 ± 0.01) were also significantly lower than that of Nigella oil (0.349) and closer to that of Shia oil (0.074).

3.9. Principal component analysis (PCA)

PCA was utilized to decompose the data from the seeds into key dimensions, emphasizing the relationships between their nutritional and bioactive properties. The selected principal components captured 91% of the total variance, offering a comprehensive overview of the dataset. As illustrated in Fig. 1a, the seeds are distinctly distributed along the axes of PC1 (66.11%) and PC2 (24.89%), uncovering significant groupings and differences in their characteristics. The PCA biplot reveals that the four seeds may be categorized into three separate groups according to their nutritional and bioactive characteristics. Cluster 1, which includes *C. moschata* var. "Hamra" and *C. maxima* var. "Rtiliya", is positioned closely along the axes that represent high oil content and protein levels, indicating their potential for oil extraction and nutritional applications. Cluster 2, comprising *C. moschata* var. "Slaouiya", occupies a separate region due to its high protein content and moderate oil levels, reflecting a balance between these two traits. Cluster 3, represented by *L. siceraria*, stands out on the biplot due to its strong association with bioactive properties like total flavonoid content and antioxidant activities (DPPH, FRAP), positioning it as a valuable source for nutraceuticals and functional foods.

The PCA in Fig. 1b captures 97.37% of the total variance (82.44% on PC1 and 14.93% on PC2). Three distinct clusters emerge in this biplot, each representing different seed oils and their unique characteristics. Cluster 1 encompasses *C. moschata* var. "Slaouiya" and *C. moschata* var. "Hamra", indicating a strong similarity in their fatty acid profiles and oxidative stability, which suggests their potential for similar applications in health-promoting products. Cluster 2 is represented by *C. maxima* var. "Rtiliya", which, despite being separated from the first cluster, displays favourable nutritional quality indices, particularly in terms of desirable fatty acids, but also reveals a higher peroxide value, pointing to increased susceptibility to oxidation. Finally, *L. siceraria* forms its own distinct cluster,

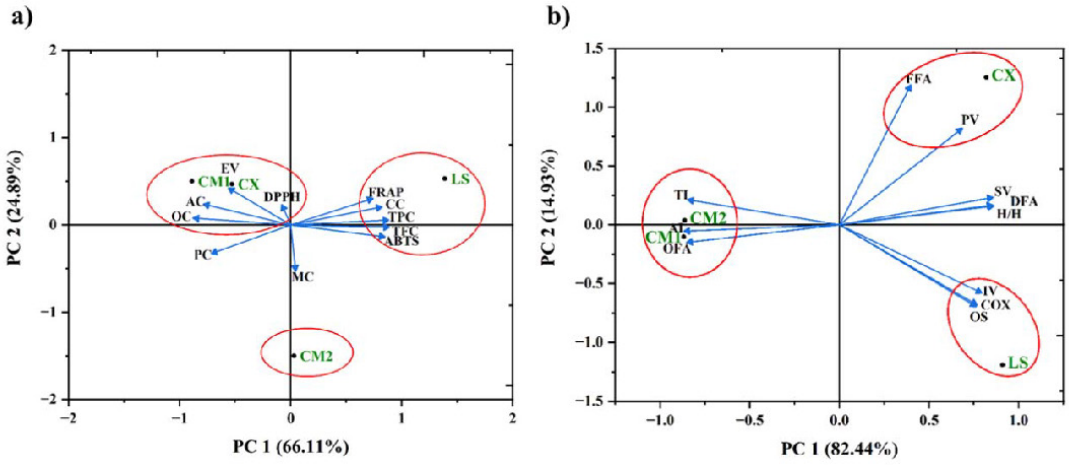

Fig. 1. Principal component analysis of *Cucurbitaceae* seeds. MC – moisture content, AC – ash content, PC – protein content, *OC* – oil content, *CC* – carbohydrates content, *EV* – energy value. LS – *L. siceraria*, CX – *C. maxima* var. "Rtiliya", CM1 – *C. moschata* var. "Hamra", CM2 – *C. moschata* var. "Slaouiya".

characterized by low atherogenicity and thrombogenicity indices, underscoring its potential as a heart-healthy oil. The biplot effectively illustrates these clusters and highlights the diverse functional properties of all these seed oils, paving the way for targeted applications in the food and cosmetic industries.

3.10. Correlation study

The correlation analysis of the examined parameters indicates multiple significant correlations (Fig. 2a). Strong positive correlations exist between *OC* and PC (0.83) and *EV* (0.88). A strong negative correlation between *MC* and *OC* (-0.92) indicates that higher moisture levels reduce the lipid concentration. Conversely, *AC* strongly correlates negatively with PC (-0.85), perhaps indicating distinct compositional attributes between mineral and protein content.

The analysis of the correlations between the parameters of the oil seed studied reveals several significant relationships linked to oil quality and health indices (Fig. 2b). Strong positive correlations are observed between the COX and the IV (0.99) and OS (0.99), indicating that higher unsaturation in the oils is associated with greater susceptibility to oxidation. Similarly, the SV showed a strong positive correlation with the DFA (0.99), suggesting that oils with higher SV tend to contain a higher proportion of beneficial fatty acids. On the other hand, the OFA showed strong negative correlations with the DFA (-0.99) and the SV (-0.98), indicating that as the amount of hypercholesterolemic fatty acids increased, the desirable fatty acid content and the saponification index decreased. In addition, the *AI* and *TI* show strong negative correlations with IV (-0.90 and

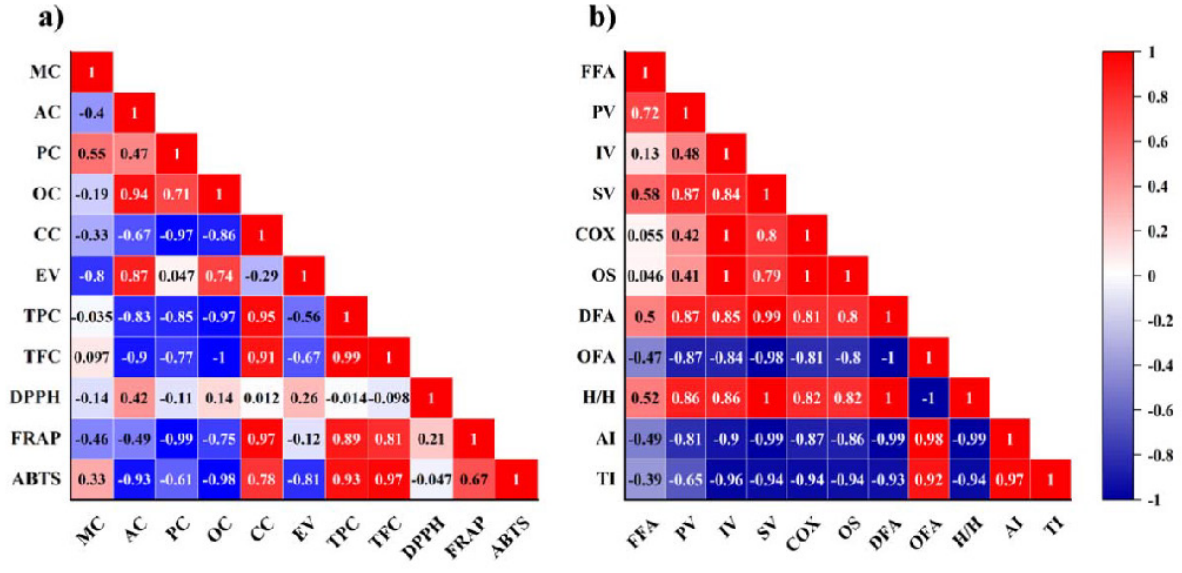

Fig. 2. Correlation coefficients among studied parameters: a) seed analysis, b) oil seed analysis.

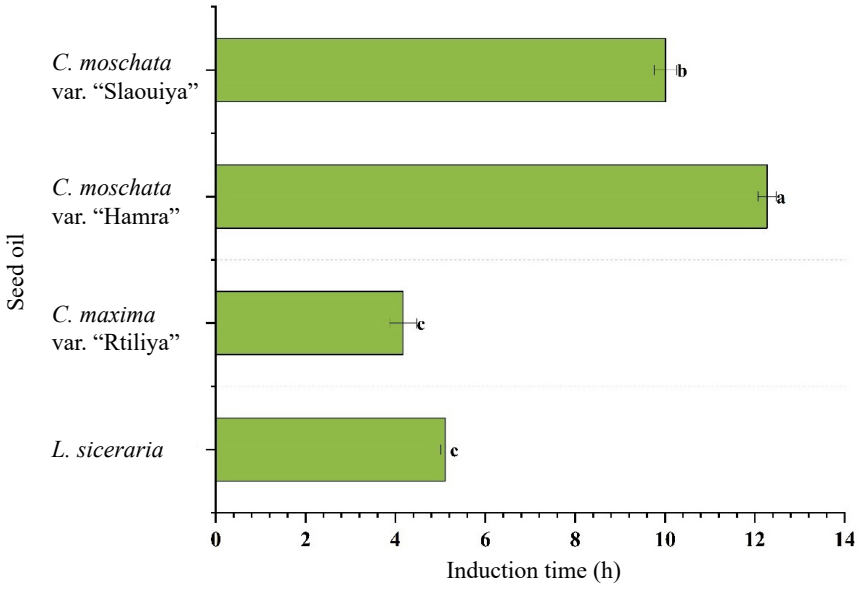

Fig. 3. Induction time (h) of oil seeds. Bars with different letters represent significant differences ($p<0.05$).

-0.96, respectively), indicating that oils with higher unsaturation levels are associated with lower risks of atherogenic and thrombogenic effects, making them healthier options.

The correlation analyses of the two datasets highlight significant relationships between oil composition, nutritional quality, and health indices. Strong positive correlations demonstrate how parameters like oil content, protein content, and energy value are interconnected, confirming that lipid-rich samples contribute significantly to energy intake. Conversely, the second dataset reveals that while higher unsaturation enhances health benefits, indicated by lower atherogenic and thrombogenic indices, it also increases oxidative susceptibility.

3.11. Rancimat test

Oxidative stability is a key indicator of oil quality, reflecting its resistance to oxidation, which can degrade fats and reduce shelf life. High stability ensures better durability and preserves the nutritional and sensory properties of oils (Symoniuk *et al*., 2022). In this research, seed oils were exposed to a temperature of 110°C to assess their induction period (IP). The analysis of oxidative stability, expressed as induction period (IP) (Fig. 3), of the four seed oils reveals significant variability. *C. moschata* varieties "Hamra" and "Slaouiya" exhibited the highest IP values (12.27 ± 0.01 h and 10.01 ± 0.01 h, respectively), indicating superior resistance to oxidation and extended shelf life. These values are comparable to those reported for pumpkin oil (13.01 ± 0.16 h) by Symoniuk *et al.* (2022), highlighting their competitive quality. In contrast, *L. siceraria* (5.11 ± 0.01 h) and *C. maxima* var. "Rtiliya" (4.17 ± 0.01 h) displayed lower IP values, suggesting moderate oxidative stability, similar to cactus oil (*Opuntia ficus-indica*, 6.5 ± 1.0 h) as reported by Asbbane *et al.* (2024). Notably, the oxidative stability of *L. siceraria* and *C. maxima* var. "Rtiliya" exceeds that of pomegranate oil (3.6 ± 0.93 h) reported by Hajib *et al.* (2021), underscoring their potential for certain industrial and nutritional applications.

4. CONCLUSIONS

This study highlights the significant nutritional, mineral, and bioactive potential of the seeds of four species of *Cucurbitaceae*, highlighting their potential applications in the food, pharmaceutical, and industrial sectors. *C. moschata* var. "Hamra" and *C. maxima* var. "Rtiliya" stand out for their high oil content, making them promising candidates for oil extraction, while *L. siceraria* excels in its mineral profile, particularly for potassium and magnesium. The favourable fatty acid composition, particularly in *C. moschata* var. "Hamra", with a high content of monounsaturated fatty acids (MUFA), reinforces the seed's value as a health-promoting product. These results demonstrate the remarkable potential of these seeds for sustainable use in nutraceuticals and functional foods, contributing to innovation in various industries and waste valorization.

Authors' contributions

Abdelghani Ait Nouisse: Resources, Formal analysis, Writing – Original Draft

Rachid Belmallam: Resources, Formal analysis, Data curation

Hasna Ait Bouzid: Resources, Formal analysis, Writing – Original Draft

Mohamed Ibourki: Conceptualization, Methodology, Software

Krishna Devkota: Validation, Supervision, Writing-Original draft preparation

Khalid Majourhat: Visualization, Investigation, Writing – review and editing.
El Hassan Sakar: Supervision, Visualization, Validation, Writing – review & editing
Angelo Maria Giuffrè: Software, Resources, Writing – review & editing, Visualization, Project administration.
Said Gharby: Writing – review & editing, Validation, Supervision, Conceptualization and final approval.

Declaration of Competing Interests: The authors affirm that they have no conflicts of interest.

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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