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Expeller Pressing of Pumpkin Seed Oil: Oil Yield, Quality, Fatty Acid Profile, and Bioactive Compounds

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ABSTRACT

The seeds constitute approximately 3.34% (w/w) of pumpkin (*Cucurbita moschata*), a byproduct rich in bioactive compounds. Cucurbita seeds may contain approximately 33% oil with a unique composition and nutritional value. The present study aimed to obtain pumpkin (*C. moschata*) seed oil by screw pressing (expeller) from four superior lines obtained through self-fertilization cycles, as well as a hybrid resulting from the crossing of two lines developed at the Embrapa Semi-arid plant breeding program. A commercial sample was used as a control. The oil was extracted using an expeller press with a maximum capacity of 3–5 kg/h, and the oil yield, oil recovery, and cake residual oil content were evaluated. The oil quality, fatty acid profile, tocochromanol, and carotenoid composition were assessed. There was a significant difference in seed oil content (27%–37%, dry weight), oil yield after pressing (13.6%–26.2%) and cake residual oil content (14.1%–18%) ($p < 0.05$) that presented a positive and negative correlation with the seed oil content, respectively. Peroxides and free fatty acid content were up to 2.28 meq/kg and 0.22%, respectively, showing the high initial quality of screw pressed oils. There were significant differences ($p < 0.05$) for the fatty acid profile, and the main fatty acids were C18:2 (35.6%–38.9%), C18:1 (32.2%–37.5%), C16:0 (13.5%–15.3%), and C18:0 (9.4%–11.4%). The screw-pressed pumpkin seed oil presented high amounts of *gamma*-tocopherol (442–686 mg/kg). The results showed the feasibility of the process in obtaining an oil rich in bioactive compounds.

1 | Introduction

The consumption of healthy and nutritionally rich foods has been growing due to their great importance in the diet and has become a quality-of-life parameter. Studies have shown that good nutrition plays a crucial role in the prevention and treatment of diseases.

Pumpkin, belonging to the *Cucurbitaceae* family and the *Cucurbita* genus, is a variable-shaped and sized vegetable and can be harvested at different development stages. Due to its ease of cultivation, durability, and low production cost, the genus has been gaining ground among consumers. *Cucurbita moschata* is a *Cucurbita* species originally from the central region of Mexico, which is the main current exporter. It is regarded as one of the richest pumpkin species in terms of essential nutrients, as well as economically feasible and one of the most cultivated pumpkin species in the world (Montesano et al. 2018).

This vegetable is composed of skin, pulp, and seeds. After removing the pulp, the largest fraction, 18%–21%, is discarded. The seeds constitute approximately 3.34% of the pumpkin's

total weight. They are a byproduct rich in bioactive compounds and are usually consumed roasted and added to various foods. Promoting their valuation contributes to the advancement of the circular economy concept (Shajan et al. 2024).

Pumpkin seeds contain approximately 33% oil (w/w), with a unique composition and nutritional value (Jarret et al. 2013). The major fatty acids are the essential n-6 polyunsaturated fatty acid (PUFA) linoleic (C18:2), oleic (C18:1 n-9, monounsaturated), palmitic (C16:0), and stearic (C18:0), whose concentration varies depending on the species and variety and the growing period (Petkova and Antova 2015). Palmitic, stearic, and oleic are fatty acids, providing pumpkin seed oil with some oxidative stability and less need for antioxidants. Tocopherols are bioactive compounds present in pumpkin seed oil with antioxidant properties (Boujemaa et al. 2020).

Continuous pressing is currently the most commonly used method to obtain edible oil from oleaginous materials, particularly on a smaller scale (Antoniassi et al. 2022). As far as we know, there are few reports of screw pressing of pumpkin seeds. Butinar et al. (2011) and Aktaş et al. (2018) analyzed pressed pumpkin seed oil from *C. pepo*, whereas Zhang et al. (2024) compared pumpkin seed oils from pressing, solvent extraction, and enzymatic extraction; however, no information was provided about the efficiency of the expeller pressing process.

Embrapa Semi-Arid, through its breeding program, has been developing pumpkin (Cucurbita spp.) varieties with high yield potential and improved resistance and tolerance to diseases, targeting industrial applications. The present study aimed to promote the full utilization of the fruit by applying mechanical (expeller) extraction and assessing the quality and process parameters, as well as chromatographic profiles of the seed oil obtained from selected breeding lines and a developed hybrid. This approach seeks to add value to the oilseed fraction, which is typically considered an industrial by-product, thereby contributing to the sustainability and economic viability of pumpkin processing.

2 | Materials and Methods

2.1 | Samples

The samples consisted of seeds from genotypes of *Cucurbita moschata* from the Embrapa Semi-arid plant breeding program: an intraspecific hybrid resulting from crossings of two lines (HYB), four superior lines obtained in self-fertilization cycles of high commercial quality (LIN1, LIN2, LIN3, LIN4), and a commercial sample (COM), used as control. The seeds were cultivated in an experiment conducted from January to May 2023, in the Embrapa Semi-arid Experimental Field, in Petrolina, State of Pernambuco (09° 09′ S, 40° 22′ W, 365.5 m altitude), Brazil. Typically, the climatic variables observed during the experiment were characterized by an average temperature of 25.6°C, with a minimum of 19.6°C and a maximum of 34.5°C, an average relative humidity of 55%, and accumulated precipitation of 13.3 mm.

The mucilage was manually separated from the seeds. Then, the seeds were washed in a sieve and placed in net fabric bags to dry in the shade, in the post-harvest warehouse of Embrapa Semi-arid under the same environmental conditions of the hybridization experiment described above. These dried samples were transported to Embrapa Food Technology, packaged in vacuum-sealed bags, and stored in a cold chamber (−14°C ± 1°C).

2.2 | Moisture Content

For each expeller pressing experiment, the moisture content of the seeds was determined by the oven method at 103°C ± 2°C until constant weight. Likewise, this procedure was carried out on the de-oiled cakes (American Oil Chemists' Society (AOCS) 2009).

2.3 | Oil Quality

The acidity (free fatty acid content expressed as % of oleic acid, FFA) and peroxide value (PV) of the seed oil were determined according to American Oil Chemists' Society Official Methods AOCS Ca 5a-40 and Cd 8-53, respectively (American Oil Chemists' Society (AOCS) 2009).

2.4 | Oil Content

The seeds and partially defatted cake oil content were obtained by solvent extraction (American Oil Chemists' Society (AOCS) 2009). The samples were ground using a blender, properly packed in cellulose cartridges, and added to a Soxhlet extractor for solvent extraction using petroleum ether at 30°C–60°C for 16 h. The solvent was removed using a rotary evaporator and a nitrogen stream.

2.5 | Mechanical Extraction

The dried seeds in shell were lightly cracked using a household blender to ease the oil extraction. Two replicate crude oil extractions from a 500 g sample were carried out using a bench-scale expeller press, model CA 59 G (IBG Monforts Oekotec GmbH & Co. KG, Germany) (Figure 1), with a seed input capacity of 3–5 kg/h, without heating, except in the initial minutes, to ease the oil flow.

Preliminary tests demonstrated that the best condition for extracting oil from pumpkin seeds using the expeller press was a 10 mm opening and a screw speed of 8 rpm.

The onset of steady-state conditions was identified by the observation of a consistent material flow and uniform press cake appearance. At this stage, measurements of the feed (dried seeds—Figure 2), extracted oil, and press cake mass were recorded. These same measurements were repeated at the end of processing to quantify the relevant processing parameters. Visual assessment of press cake homogeneity was used as a practical indicator for confirming steady-state operation.

The experiments were performed in duplicate, with outlet temperatures from 52°C to 63°C, indicating that the extraction

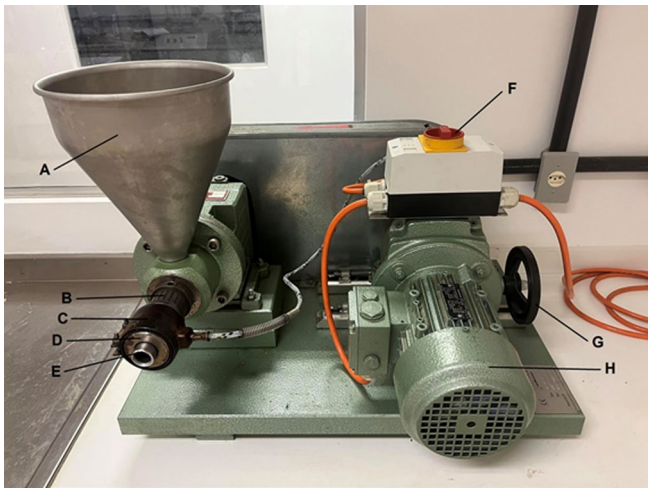

FIGURE 1 | Expeller press used in the experiments, (A) feed hopper, (B) perforated cylinder (oil outlet), (C) strip heater, (D) screw head, (E) outlet nozzle of press cake, (F) start button, (G) gearbox, (H) motor.

FIGURE 2 | Pumpkin dried seeds.

process cannot be classified as cold pressing. The oil obtained from the extraction of each sample was centrifuged (Thermo Scientific, Sorvall Legend XTR) for 10 min at 10,000 rpm to remove sediments and stored for analysis (Figure 3). The oil extraction yield (Y) was calculated by mass balance between the amount of seed used and its oil content and the amount of cake generated by pressing and its residual oil content, as shown in Equation (1). The oil recovery efficiency (R) was determined according to Equation (2) (Antoniassi et al. 2022).

$$Y = \frac{(P_s \times O_s) - (P_c \times O_c)}{P_s} \times 100\,\% \quad (1)$$

where P_s is seed weight (g) (DW), O_s is the seed oil content ($g \times 100\,g^{-1}$) (DW), P_c is the cake weight (g) (DW), O_c is the cake residual oil content ($g \times 100\,g^{-1}$) (DW).

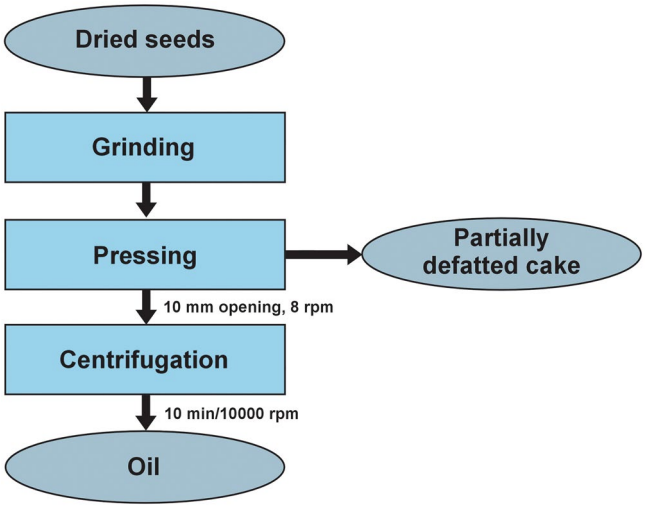

FIGURE 3 | Flow diagram of pumpkin seed oil extraction through screw pressing.

$$R = \frac{Y}{O_s} \times 100\,\% \quad (2)$$

It is worth mentioning that the continuous press extraction method may be carried out at low temperatures. However, due to the friction caused between the extractor and the sample, the process was monitored to avoid overheating by choosing the best opening and screw speed determined in preliminary tests, and by reducing the time of sample preheating.

2.6 | Fatty Acid Composition

The methyl esters were obtained according to Antoniassi et al. (2018) and analyzed by gas chromatography on an Agilent 7890 with a flame ionization detector operated at 280°C and using an HP FFAP capillary column (25 m × 0.2 mm × 0.30 μm). Oven temperature was programmed as follows: initial temperature of 150°C for 1 min; from 150°C to 180°C with a rate of 30°C/min; from 180°C to 200°C (20°C/min); 200°C–230°C (3°C/min) and at the final temperature of 230°C for 10 min. The injector was set to 250°C and operated in a 1:50 split mode, and 1 μL of sample was injected. The standards of NU-CHEK PREP Inc. (Elysian, MN) and Supelco (Bellefonte, PA, USA) were used for identification, and quantification was performed by internal normalization.

The iodine value (IV) was calculated according to the AOCS Official Methods AOCS Cd 1c-85, and the saponification value (SV) was calculated based on the fatty acid composition:

$$SV = \frac{(3 \times 56.1 \times 1000)}{\left[(\text{average fatty acid molecular weight} \times 3) + 92.09 - (3 \times 18)\right]} \quad (3)$$

In which: 3 (in the numerator) is the number of fatty acid residues per triglyceride, 1000 is the conversion factor for milligrams to grams, 56.1 is the molar mass of KOH, 92.09 is the glycerol molecular weight (g/mol), and 18 is the water molecular weight.

2.7 | Simultaneous Analysis of Carotenoids and Tocopherols

Analyzes were performed simultaneously using a Waters Technologies Alliance 2695 liquid chromatograph (HPLC) Milford, Massachusetts, USA connected to Waters 2998 photodiode array (PDA) and Waters 2424 fluorescence detectors, according to Antoniassi et al. (2024). The oil was dissolved in acetone. The compounds were separated on a YMC C30 reverse phase analytical column (0.25 m × 3.0 mm I.D., 5.0 μm particle size) (Kyoto, JAPAN) conditioned to 35°C. Samples were kept at 15°C. The separation was performed using a gradient (flow 0.8 mL/min) with the mobile phase methanol/methyl tert-butyl ether (9/1, v/v) for 20 min, methanol/methyl tert-butyl ether (1/9, v/v) for 5 min, and methanol/methyl tert-butyl ether (9/1, v/v) for 3 min, totaling 28 min. The identification of the compounds was performed by comparing the sample retention time with the peak retention time of the carotenoid and tocochromanol standards and by the absorption spectra of the carotenes (Davies 1976). The fluorescence detector was set at 290 nm (excitation) and 330 nm (emission) for tocopherols. The PDA detector was operated in scan mode between 200 and 800 nm, and quantification at 450 nm. The quantification of tocopherols was done through external standardization. The concentration of each tocochromanol stock solution (*alpha*-, *gamma*-, *delta*-tocopherol, *alpha*-, *gamma*-, *delta*-tocotrienol, Sigma Aldrich, St. Louis, USA) was calculated according to AOCS Ce 8–89 (2009). For total carotenes, the quantification was performed by spectrophotometry, considering the wavelengths, molar absorptivities, and the solvent used in the dilution, according to Davies (1976).

2.8 | Identification of Tocopherols and Tocotrienols by LC/MS

The extraction of tocopherols and tocotrienols from the pumpkin seed oil was performed with acetonitrile and methanol according to Ko et al. (2008). In brief, the oil (1 g) was vortexed with 5 mL of solvent for 5 min and centrifuged at 10,000 RPM for 3 min. The extract was collected, and the re-extraction was repeated twice. The solvent was removed, and the final extract was dissolved in acetonitrile. The separation and identification were carried out using a Waters Aquity ultra high-performance liquid system and Xevo TQ MS quadrupole mass spectrometer (Waters, UK) with an electrospray ionization (ESI) source operating in negative mode. A Waters Acquity UPLC BEH C18 1.7 μm (2.1 × 100 mm) column (Waters, UK) was employed for the analysis at 40°C with a mobile phase comprising 95% methanol and 5% water containing 1% formic acid (0.3 mL/min), and the injector was maintained at 20°C. The mass spectrometer settings were: source temperature 150°C, collision energy 30 V, capillary 3 kV, cone 30 V, desolvation temperature of 500°C, desolvation gas flow of 1000 L/h, and cone gas flow of 30 L/h. The multi-reaction monitoring mode (MRM) was applied to detect tocopherols and tocotrienols at the following transitions: gamma tocotrienol (409 > 149), gamma tocopherol (415 > 149), delta tocotrienol (395 > 135), and delta tocopherol (401 > 135).

2.9 | Statistical Analysis

The Pearson correlation analysis, Analysis of variance, and Tukey test were performed using Statgraphics (Statgraphics Technologies Inc.) at a significance level of 0.05.

3 | Results and Discussion

3.1 | Moisture Content and Oil Content

The moisture content of the dried seeds provided by Embrapa Semi-arid was determined immediately prior to the oil extraction process, and the levels varied slightly, with differences insufficient to impact the process performance. It was 7.17% average, which is considered suitable for expeller pressing (Antoniassi et al. 2022).

Based on the mass measurements, taken before and after the process reached the steady state, the data displayed in Table 1 were obtained.

The seed oil content varied from 26.02% to 36.57% (DW) (Table 1), which is within the variation observed for pumpkin seeds cultivated in other countries, such as the range reported by Jarret et al. (2013) for *C. moschata* germoplasm from the USDA (15.8% to 34.2%). However, Petkova and Antova (2015) found an oil content of 47% for *C. moschata* from Hungary.

TABLE 1 | Quality of pumpkin seed, oil, and cake, and parameters of screw pressing of pumpkin seeds, DW*.

Sample	Ms (%)	FFA (%)	PV (meq/Kg)	O_s (%)	O_c (%)	Mc (%)	*Y* (%)	*R* (%)
HYB	7.61 ± 0.13	0.18 ± 0.016^{c}	nd	36.05 ± 1.63^{a}	14.08 ± 1.37^{c}	9.03 ± 0.3	26.19 ± 1.26^{a}	72.6
COM	7.8 ± 0.10	0.17 ± 0.005^{c}	2.28 ± 0.455	36.56 ± 0.06^{a}	15.67 ± 0.46^{b}	9.22 ± 0.19	25.53 ± 0.36^{b}	69.8
LIN 1	8.91 ± 0.04	0.22 ± 0.003^{a}	nd	27.57 ± 0.66^{c}	17.86 ± 0.64^{a}	9.63 ± 0.06	14.11 ± 0.37^{e}	51.1
LIN 2	7.75 ± 0.09	0.22 ± 0.010^{a}	nd	26.02 ± 1.59^{c}	16.24 ± 0.39^{b}	9.57 ± 0.07	13.56 ± 0.24^{f}	52.1
LIN 3	7.65 ± 0.01	0.20 ± 0.006^{b}	nd	31.24 ± 0.19^{b}	16.30 ± 0.50^{b}	8.79 ± 0.08	18.95 ± 0.09^{c}	60.9
LIN 4	7.42 ± 0.05	0.20 ± 0.004^{b}	nd	27.69 ± 2.69^{c}	15.68 ± 0.41^{b}	8.51 ± 0.29	16.68 ± 0.12^{d}	58.9

Note: Press nozzle: 10 mm; screw speed: 8 rpm.
Abbreviations: COM, commercial; DW, dry weight; FFA, free fatty acids; HYB, hybrid; LIN 1, line 1; LIN 2, line 2; LIN 3, line 3; LINE 4, line 4; Mc, cake moisture; Ms., seed moisture; nd, not detected; O_c, residual oil content in cake; O_s, oil seed content; PV, peroxide value; *R*, oil recovery efficiency; *Y*, oil yield.
*Mean ± standard deviation. Different means with different lowercase letters in the same column are significantly different ($p < 0.05$) by the Tukey test.

3.2 | Oil Yield and Oil Recovery Efficiency

Through mechanical pressing, the oil yield ranged from 13.56% to 26.19% (DW) and the residual oil content in cake of 14%–18% (DW) ($p < 0.05$) was obtained, and then the oil recovery was calculated ranging from 51% to 73%, and there is a trend of higher oil recovery for high seed oil content. The oil yield was higher for the samples with higher oil content, which was confirmed by a positive correlation (Pearson) between seed oil content and oil yield ($p < 0.01$) (Table 4). Additionally, there was a negative correlation between cake oil content and oil yield and seed oil content ($p < 0.01$), pointing out the advantage of the higher seed oil content for the feasibility of the process. In this sort of process, incomplete recovery of the oil is expected. Antoniassi et al. (2022) and Wilhelm et al. (2014) reported cake residual oil content ranging from 4% to 7% for expeller pressing of passion seed, whereas higher figures were observed for other seeds and nuts.

The oil recovery depends on the pressing conditions, which were optimized with a 10 mm opening and screw speed of 8 rpm (data not shown). It is necessary to regard the relationship between the distance between the screw and the extractor wall to avoid overheating the sample and, at the same time, the equipment extracts as much oil as possible from the input material. The increase in temperature is deleterious to the oil quality. The extraction temperature varied from 53°C to 59°C for the COM sample, and from 55°C to 57°C, from 53°C to 64°C, from 57°C to 63.5°C, from 58°C to 63°C for LIN1 to LIN4, and 53°C to 58°C during HYB seed extraction. Despite the slight increase in temperature caused by friction in the press, no burning aroma was detected in the oils. Thus, the screw pressing of the pumpkin seed oil was not a cold pressing as well as was claimed for other seeds and kernels.

Neđeral et al. (2012) compared the oxidative stability of roasted seed oil (110°C for 45 min) that was expeller-pressed up to 120°C, unroasted seed oil (pressed up to 90°C), and cold-pressed pumpkin seed oil (C. pepo). The oil recovery of roasted seeds was 90%, whereas naked seeds and husked seeds reached oil recovery of 73% and 48%, respectively, with initial oil content of 34.9 and 44.6; however, no further information was reported about the process.

3.3 | Oil Quality Parameters

The quality indexes followed international legislation recommendations (FAO 2022), whose limits for cold-pressed and unrefined oils are a maximum acid value of 4.0 mg KOH/g (~2% of FFA) and 15 meq/kg peroxide value. The FFA content varied from 0.17% to 0.22%, thus demonstrating the efficacy of the treatment of seeds (separation and cleaning) and drying to reduce seed moisture to levels suitable for pressing, avoiding enzymatic hydrolysis by lipases and safe storage before processing. FFA showed a negative correlation (Pearson) with seed oil content and oil yield and a positive correlation with cake residual oil content (Table 4). Peroxides were not detected in the screw-pressed oils, despite the high level of unsaturated fatty acids, except in the COM oil, in which the oxidative process had already started (2.28 ± 0.455 meq/kg). Furthermore, the slight heating of the oil flowing through the screw press was not high enough to affect the quality of the crude oils, probably due to the presence of tocochromanols that will be further discussed. Acidity results lower than 0.34% for pressed pumpkin seed oils were cited by Zhang et al. (2024) and Aktaş et al. (2018); however, peroxide value was 14.5 and 11 meq/kg, respectively, pointing out the need for seed pre-treatment good practices for pumpkin seeds.

3.4 | Fatty Acid Compositions

Table 2 presents the fatty acid composition of the oils obtained. The main fatty acids were C18:2 (35.6%–38.9%), C18:1 (32.2%–37.5%), that comprises C18:1 n-9 and C18:1 n-11, C16:0 (13.5%–15.3%), C18:0 (9.4%–11.1%) while small amounts were observed for C20:0 (0.6%) and up to 0.2% for C18:3, C14:0, C16:1, C20:1, C22:0, C22:1 and C24:0. There were significant differences ($p < 0.05$) among oils, possibly indicating genetic differences, however, these differences were very small. Pumpkin seed oil belongs to the group of seed oils rich in oleic (C18:1 n-9) and linoleic (C18:2) acids but the content of C18:0 was higher than that observed for edible and liquid oils (FAO 2022). The C16:0 and C18:2 content were lower, whereas C18:1 was higher than the results reported by Petkova and Antova (2015) and Jarret et al. (2013) for oil from seeds of *C. moschata* grown in Bulgary and United States, respectively and for *C. moschata, C. pepo*, and *C. maxima* cultivated in Morocco (Boujemaa et al. 2020). The high C18:1 content is an advantage for the oxidative stability of the oil and can distinguish Brazilian pumpkin seed oil. The iodine value is consistent with oils with higher contents of C18:1 and C18:2, and SV results are in the range of most edible oils, except lauric oils (FAO 2022).

It is observed that the content of one of the major fatty acids, C18:1, is closest to the ranges established by Brazilian legislation (ANVISA 2021). COM showed the highest oil content, followed by HYB, with no significant difference between them, while HYB was the cultivar with highest C18:1 content. Embrapa cultivars had lower C18:2 content, than marketed cultivars. The COM seed also slightly differed from Brazilian legislation regarding C18:2 and some minor fatty acids. This demonstrates that there may be variability in the fatty acid composition of oils from the same pumpkin species grown in different soils and climate and soil conditions around the world, so regulatory bodies may consider such differences. In certain cases, it is necessary to support these regulatory bodies with reliable data to widen the range of levels of these fatty acids.

3.5 | Tocopherol and Carotenoid Content

Tocopherols and tocotrienols are found in lipid-rich fractions of cells. In vegetable oils, they have a protective effect against oxidative processes, especially if they contain high levels of polyunsaturated fatty acids, such as pumpkin seed oils (Shahidi and De Camargo 2016).

The quantification of tocopherols and tocotrienols in oils and foods is usually performed by HPLC with fluorescence detector (HPLC-FLR) which is selective and sensitive for these compounds. Figure 4 shows the chromatogram of the pumpkin seed

TABLE 2 | Fatty acid composition of expeller-pressed pumpkin seed oil (%)**.

Fatty acid	HYB	COM	LIN 1	LIN 2	LIN 3	LIN 4	Brazilian legislation*
C14:0	0.08 ± 0.001	0.10 ± 0.002	0.10 ± 0.001	0.10 ± 0.002	0.10 ± 0.001	0.10 ± 0.001	0.18–0.5
C16:0	14.13 ± 0.091^{c}	15.27 ± 0.094^{a}	13.89 ± 0.059^{d}	13.48 ± 0.077^{e}	14.61 ± 0.068^{b}	14.11 ± 0.036^{c}	8.0–25.4
C16:1	0.10 ± 0.001	0.09 ± 0.000	0.10 ± 0.001	0.09 ± 0.000	0.11 ± 0.001	0.10 ± 0.000	0.1–0.7
C17:0	0.07 ± 0.000	0.09 ± 0.000	0.06 ± 0.001	0.09 ± 0.001	0.07 ± 0.000	0.07 ± 0.000	n.d.—0.2
C18:0	9.41 ± 0.025^{e}	10.12 ± 0.012^{d}	11.39 ± 0.016^{a}	11.42 ± 0.046^{a}	10.63 ± 0.026^{c}	11.10 ± 0.032^{b}	3.0–11.2
C18:1	37.49 ± 0.049^{a}	32.18 ± 0.036^{f}	34.99 ± 0.029^{c}	33.64 ± 0.017^{e}	35.23 ± 0.172^{b}	34.22 ± 0.025^{d}	17.0–44.1
C18:2	35.58 ± 0.066^{f}	38.93 ± 0.052^{a}	36.32 ± 0.043^{d}	37.83 ± 0.059^{b}	36.13 ± 0.123^{e}	37.16 ± 0.024^{c}	39.7–65.0
C18:3	0.16 ± 0.001^{e}	0.16 ± 0.000^{e}	0.187 ± 0.001^{b}	0.186 ± 0.000^{c}	0.17 ± 0.001^{d}	0.19 ± 0.000^{a}	0.1–0.9
C20:0	0.64 ± 0.007	0.59 ± 0.002	0.61 ± 0.003	0.63 ± 0.004	0.60 ± 0.004	0.60 ± 0.002	0.3–1.0
C20:1	0.12 ± 0.001	0.11 ± 0.001	0.10 ± 0.001	0.10 ± 0.003	0.10 ± 0.001	0.10 ± 0.001	—
C22:0	0.22 ± 0.004	0.20 ± 0.004	0.21 ± 0.004	0.22 ± 0.005	0.20 ± 0.006	0.20 ± 0.004	—
C22:1	0.18 ± 0.004	0.20 ± 0.002	0.18 ± 0.003	0.21 ± 0.005	0.18 ± 0.004	0.20 ± 0.003	n.d.—0.8
C24:0	0.29 ± 0.005	0.30 ± 0.002	0.28 ± 0.002	0.30 ± 0.003	0.29 ± 0.003	0.28 ± 0.001	—
N.I.	1.56 ± 0.050	1.66 ± 0.057	0.13 ± 0.005	1.66 ± 0.032	1.57 ± 0.025	1.56 ± 0.039	—
∑SAT	26.53	26.67	26.53	26.24	26.47	26.44	
∑MUFA	35.37	32.57	35.37	34.04	35.62	34.62	
∑PUFA	36.51	39.09	36.51	38.01	36.31	37.35	
IV	94.43	94.43	93.77	95.22	93.63	94.43	
SV	195.32	195.82	195.39	195.52	195.44	195.42	

Abbreviations: COM, commercial; HYB, hybrid; IV, iodine value; LIN 1, line 1; LIN 2, line 2; LIN 3, line 3; LIN 4, line 4; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SAT, saturated fatty acids; SV, saponification value.
*ANVISA (2021).
**Mean ± standard deviation. Different means with different lowercase letters in the same row are significantly different ($P < 0.05$) by the Tukey test.

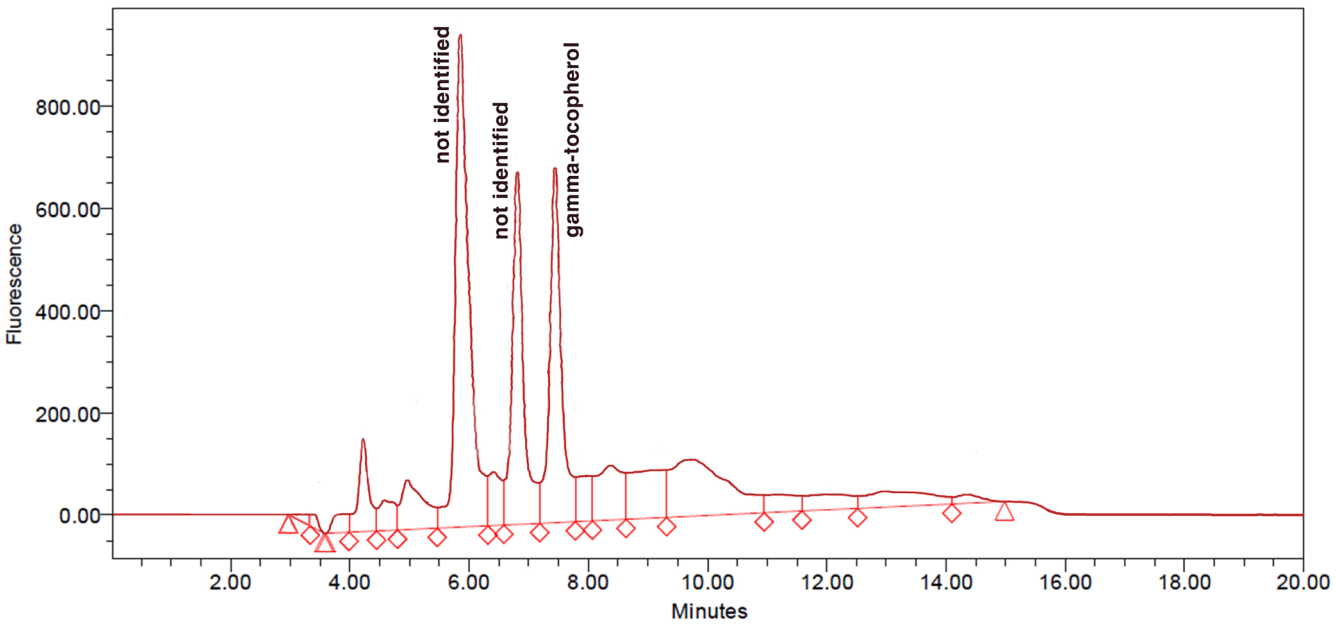

FIGURE 4 | HPLC fluorescence detector chromatogram of tocopherols from pressed pumpkin seed oil.

oils evaluated by HPLC-FLR, and three peaks with retention times consistent with *gamma*-tocotrienol, *delta*-tocopherol, and *gamma*-tocopherol were observed. *Alpha*-tocopherol was not detected. *Gamma*-tocopherol is usually the main tocochromanol of pumpkin seeds among species of the genus Cucurbita, while *gamma*-tocotrienol, *alpha*-, and *delta*-tocopherol were quantified at very lower levels (Boujemaa et al. 2020; Petkova and Antova 2015). Considering the intensity of the peaks present in the HPLC-FLR analysis, the identification was carried out by LC/MS, and the result is shown in Figure 5. *Gamma*-tocopherol was confirmed as the main tocopherol present in the oil. *Gamma*-tocotrienol and *delta*-tocopherol were not detected. Thus, only *gamma*-tocopherol was quantified by HPLC-FLR (Table 3). Although the FLR detector is selective for tocopherols and tocotrienols, Butinar et al. (2011) identified new tocochromanols isomers (*gamma*-tocomonoenol and *alpha*-tocomonoenol) in seeds, roasted seeds, and roasted seed oil of *C. pepo*, and these compounds were also found in the HPLC-FLR analysis. As far as we know, there is no report of these compounds in *C. moschata* seeds, but the identification by LC/MS is useful for confirming the tocopherols of pumpkin seed oils.

There were significant differences for *gamma*-tocopherol content ($p < 0.05$) among samples ranging from 441 to 688 mg/kg of pumpkin seed oils and the lower and higher levels were found for HYB and LIN 4, respectively. This range observed is consistent with the results of 586 and 530 mg/kg of pressed pumpkin seed oils obtained by Butinar et al. (2011) and Zhang et al. (2024), respectively, for roasted seed (*C. pepo*) and for dried seed (*C. maxima*). However, the *gamma*-tocopherol content of pressed pumpkin seed oils (*C. pepo*) varied from 132 to 881 mg/kg depending on the pre-treatment, such as sun drying, roasting, wet and dry salt addition before pressing (Aktaş et al. 2018). The tocopherol content of pumpkin seed oil depended on the varieties, oil extraction method, and pre-treatment of the seeds.

Regarding the correlation analysis, there was a negative correlation of *gamma*-tocopherol and seed oil content and oil yield, and a positive correlation with cake residual oil content, C18:0, and C18:3 fatty acids (Table 4). These results are consistent with the findings of Petkova and Antova (2015) who showed a reduction of content of *gamma*-, *alpha*, and *delta*-tocopherols, C18:0, and C18:3 of seed oils, and an increase of seed oil content during the ripening of fruits of *C. moschata*.

The total carotenoid content varied from 27 to 33 mg/kg, and *beta*-carotene was the main component (13.4–24.6 mg/kg)

TABLE 3 | Tocopherol and carotenoid content of the expeller-pressed pumpkin seed oils (mg/kg).

	Gamma-tocopherol	Lutein	*Beta*-carotene	Total carotenoid
HYB	441.90 ± 55.20^{d}	0.57	13.37	27.08 ± 2.41
COM	537.40 ± 52.72^{c}	0.66	21.19	28.05 ± 1.60
LIN 1	674.30 ± 71.53^{ab}	0.90	21.73	27.44 ± 1.53
LIN 2	555.90 ± 64.10^{c}	0.78	17.58	28.63 ± 1.20
LIN 3	584.56 ± 67.37^{bc}	0.97	22.58	28.56 ± 0.89
LIN 4	686.26 ± 53.09^{a}	1.10	24.59	33.18 ± 4.42

Note: Different means with different lowercase letters in the same column are significantly different ($P < 0.05$) by the Tukey test.
Abbreviations: COM, commercial; HYB, intraspecific hybrid; LIN 1, line 1; LIN 2, line 2; LIN 3, line 3; LIN 4, line 4.

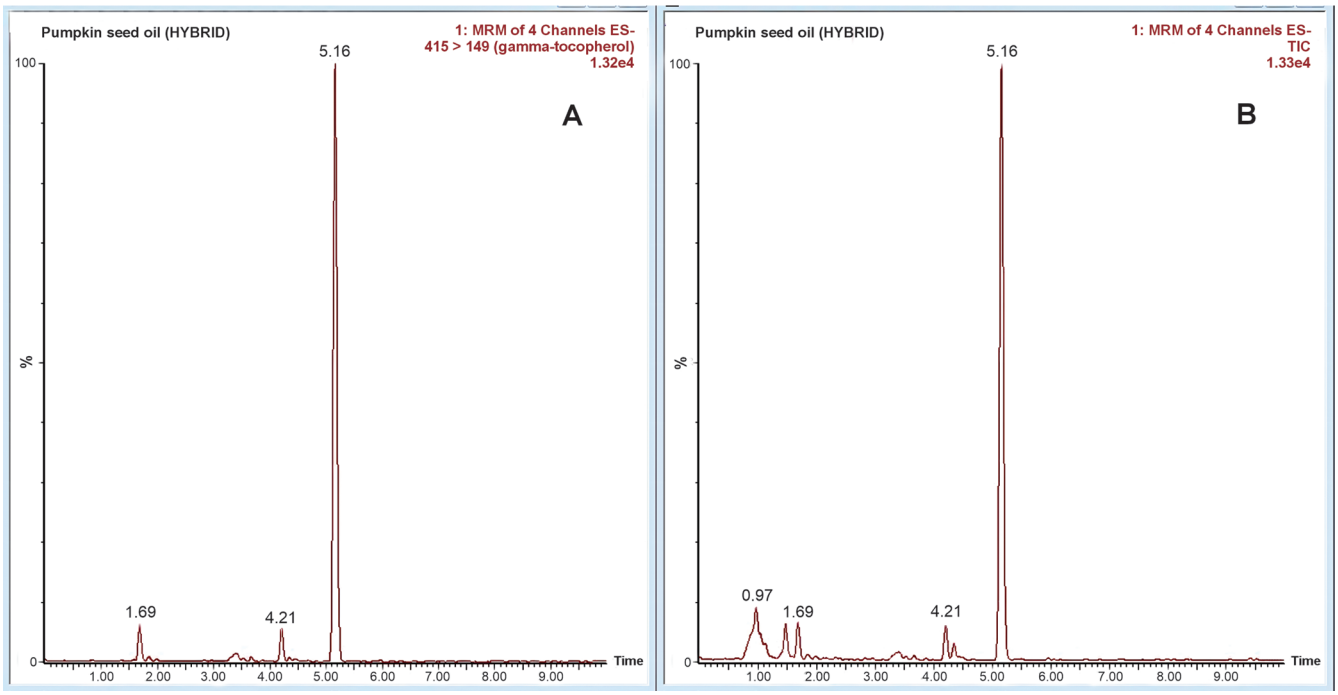

FIGURE 5 | MRM chromatogram of pumpkin seed oil extract (HYB), (A) transition of *gamma*-tocopherol (415 > 149), (B) four monitored transitions: *gamma*-tocotrienol (409 > 149), *gamma*-tocopherol (415 > 149), *delta*-tocotrienol (395 > 135), and *delta*-tocopherol (401 > 135). Peak intensity is shown in the top right-hand corner of the chromatogram.

TABLE 4 | Pearson correlation coefficient among process parameters (seed oil content, oil yield, cake residual oil content), oil quality (Free fatty acids content—FFA), major fatty acids (C16:0, C18:0, C18:1, C18:2, C18:2) and *gamma*-tocopherol content.

Parameter	Oil yield	Cake residual oil content	FFA	*Gamma*-Tocopherol	C16:0	C18:0	C18:1	C18:2	C18:3
Seed oil content	0.955**	−0.486**	−0.758**	−0.576**	0.729**	−0.898**	0.14	0.035	−0.934**
Oil yield		−0.655**	−0.864**	−0.626**	0.678**	−0.972**	0.208	0.006	−0.963**
Cake residual oil content			0.752**	0.552**	−0.201	0.721**	−0.304	0.07	0.577**
FFA				0.458**	−0.718**	0.833**	−0.06	−0.09	0.802**
Gamma-tocopherol					−0.18	0.659**	−0.255	0.05	0.691**
C16:0						−0.534**	−0.35*	0.366*	−0.664**
C18:0							−0.415*	0.196	0.924**
C18:1								−0.947**	−0.134
C18:2									−0.076

*, **: significant at 5% and 1% level, respectively.

while lutein was quantified up to 1.1 mg/kg. These results are very low, and no statistical comparison was performed. The total carotene content of 44.8 mg/kg was obtained by Al-Turky et al. (2024) by hexane extraction of *C. moschata* seeds. The range of 0.25–0.66 mg/kg of total carotenoids was observed for oil extracted by hexane from seeds of *C. moschata, C. pepo*, and *C. maxima* (Boujemaa et al. 2020).

4 | Conclusions

The process of obtaining pumpkin seed oil (*C. moschata*) by pressing presented an oil recovery ranging from 51% to 73%, and there is a trend in the higher oil recovery for high seed oil content among the lines and hybrids studied. The oil content of the partially defatted cake was up to 18%, demonstrating the feasibility of the process, which considers the design of the equipment and the operating parameters.

The oil from *C. moschata* seeds from Embrapa's breeding program (Brazil) showed higher content of C18:1 fatty acid, differing from samples of this species from other countries. In the tocopherol profile, *gamma*-tocopherol, which usually has superior antioxidant activity among tocopherols, stands out at high levels.

The most pronounced differences among the samples were observed in oil content, whereas their composition—both in major and minor compounds—showed only minor variation. However, the observed variations in fatty acid composition exceeded the acceptable ranges established by regulatory agencies. We believe that these results provide valuable insights to regulatory agencies for the evaluation and potential revision of identity and quality standards for pumpkin seed oil in Brazil.

Furthermore, this work contributed to adding value to the pumpkin oilseed fraction, which is typically considered an industrial by-product.

Author Contributions

A.M.M.G. conceived and designed the study, wrote the first draft of the manuscript, and carried out the research on the data. S.P. was responsible for the development of methodology for tocochromanols by HPLC-MS. A.E.W. performed formal analysis and carried out the research. R.M.E.B. is responsible for the production of writing. V.N.R. performed formal analysis and carried out the research. R.A. is responsible for the conceptualization, the development of methodology for tocochromanols by HPLC-MS and carotenoids by HPLC, the production of writing, and statistical analysis. All authors contributed to and approved.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

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