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Sicana odorifera seeds as a new source of punicic acid determined by NMR analyses

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ABSTRACT

The seed oil composition of South American cucurbits, including the Chilean giant pumpkin (*Cucurbita maxima*) and the Paraguayan *Sicana odorifera*, was assessed by high-field nuclear magnetic resonance (NMR). The main objective of the study was to describe the composition of the seed oils using NMR as the main analytical tool and to detect possible conjugated linolenic acid isomers (CLnA). A previous study of *S. odorifera* seed oil identified linolenic and linoleic acids as the main fatty acids (FAs). Reexamination of the *S. odorifera* seed oil by NMR showed that the C18:3 fatty acid previously reported is the CLnA isomer punicic acid (PA). PA occurs in *S. odorifera* cultivars atropurpurea and fragans, with higher content in atropurpurea (37.42 and 31.70%, respectively). The identification of PA as a major constituent in *S. odorifera* oil opens new perspectives on the possible applications and consumption of seeds as a healthy food. The new source of PA is a fast-growing cucurbit, adapted to tropical and subtropical climates. The seed oil composition of the Chilean giant pumpkin is like that of other *C. maxima* cultivated in South America and Europe. NMR spectroscopy was a rapid and reliable method for identifying and quantifying the main constituents in the seed lipids, clearly differentiating CLnA isomers and linolenic acid.

1. Introduction

The pumpkin, *Cucurbita maxima* Duch ("zapallo"), is a crop that originated in South America. Its relative, *Cucurbita pepo* L., is native to Mesoamerica and Mexico (Lira et al., 2016). In Chile, the "zapallo de guarda" is the most widespread and appreciated landrace of *C. maxima* (Ordenes, 2015). The fruits are large (about 15–25 kg) and can be stored for several months. The seeds are de-husked, roasted, and eaten as a snack. The lipid composition of pumpkin seeds was reported (Montesano et al., 2018). However, less is known about the lipids from the Chilean "zapallo de guarda" seeds. Wild or semi-domesticated Cucurbitaceae in South America include *Sicana odorifera* (Vell.) Naudin, known in Paraguay as "kurugua". Two *S. odorifera* cultivars occur in Paraguay, namely cv. *atropurpurea*, with deep purple skin, and cv. *fragans*, with reddish-brown skin (Fig. 1). Previous work on "kurugua" seed oil (Caballero et al., 2021; Mereles et al., 2021) reports linolenic acid (32.80–38.08 %), linoleic acid (28.62–29.51 %), and oleic acid (13.16–19.39 %) as the main fatty acids (FA).

Conjugated linolenic acids (CLnA) can sometimes be reported by mistake as C18:3 by GC/FID and assigned as linolenic acid when other spectroscopic means are not used. The CLnA isomers present the double bonds (DB) in positions Δ9,11,13 and Δ8,10,12, differing also in the geometry of the DB. Eleostearic acid (9E, 11E, 13E isomer, β-eleostearic acid and 9Z, 11E, 13E isomer, α-eleostearic acid) as well as punicic acid (9Z, 11E, 13Z isomer) are among the CLnA isomers occurring in seed oils and are less common than linolenic acid (Venianakis et al., 2021). CLnA isomers show a narrow distribution in plants. The 9Z,11E,13Z isomer (PA) occurs in the seeds of *Punica granatum* (Cairone et al., 2023) and *Trichosanthes kirilowii*, while 9Z,11E,13E (α-eleostearic acid) was reported from the Cucurbit *Momordica charantia* and tung seed oil. The 9E, 11E,13Z isomer was found in *Catalpa* (Bignoniaceae) seeds, while

Abbreviations: FA, fatty acid; CLnA, conjugated linolenic acids; DB, double bond; NMR, Nuclear magnetic resonance; PA, punicic acid.

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β-eleostearic acid (9E,11E,13E isomer) occurs in *Momordica charantia, P. granatum,* and *Catalpa* seeds. The 8Z,10E,12Z jacaric acid was found in *Jacaranda* and pomegranate seed oil (Sassano et al., 2009).

Nuclear Magnetic Resonance (NMR) is an analytical technique that provides information on the main components in oil samples (Castejón et al., 2014; Guillén & Ruiz, 2003;Miyake et al., 1998). NMR has been successfully applied to olive oil (Lia et al., 2020; Sacchi et al., 1997), other vegetable oils (Siudem et al., 2022), native seed oils from Botswana (Yeboah et al., 2017), and Amazonian fruits (Dos Santos et al., 2018). One advantage of NMR is that it does not require derivatization and provides chemical profiles of the entire lipid content from the samples in a short time. Additional information, including unsaturation value, acidity, saponification index, average molecular mass, and olefinic-to-aliphatic ratio, can be obtained from the same sample in a short time (Hidalgo & Zamora, 2003; Knothe & Kenar, 2004). The results are usually comparable with the GC/FID data (Guillén & Ruiz, 2003; Miyake et al., 1998). Characterization of polyunsaturated, conjugated FA requires comparison with geometric isomers and careful literature search (Cao et al., 2007).

Several bioactivities have been reported for CLnA isomers, including an apoptosis-inducing effect for α-eleostearic acid and its dehydroxylated derivative (Kobori et al., 2008).

The pomegranate (*Punica granatum*) seed oil contains triglycerides with high levels of PA (Cairone et al., 2023; Fatope et al., 2002). A comprehensive review on PA was published (Aruna et al., 2016). PA has been investigated for the prevention of neurological disorders and Alzheimer's disease, but lacks animal trials (Sen et al., 2025).

Guerra-Vázquez et al. (2022) review the anti-inflammatory and antioxidant effects of PA and the mechanism involved in the modulation of peroxisome proliferator-activated receptors. While in vivo studies are

Chilean "zapallo de guarda"

Storage of *Cucurbita maxima* in central Chile

Fruits of *Sicana odorifera*

Upper: *S. odorifera* cv. atropurpurea (deep purple peel); Lower: *S. odorifera* cv. fragans (reddish brown peel).

Fig. 1. The Chilean squash "zapallo de guarda" (*Cucurbita maxima*) in storage, and the fruits of Paraguayan *Sicana odorifera* cultivars atropurpurea (deep purple skin) and fragans (reddish brown peel).

needed, the available data suggest health-promoting potential for PA. Other bioactivities of PA relate to glycaemia, as hypolipidemic and anti-obesity agents (Holic et al., 2018). The main natural source of PA is the seeds of *P. granatum*. However, due to low yield, unstable seed production, and restricted cultivation, other sources of PA are needed for industrial production. Biotechnological approaches, genetic engineering of high-producing pomegranate plants or modified microorganisms, are among the alternatives considered (Holic et al., 2018). An intervention of PA to human volunteers showed that PA can be incorporated into red blood cells and human plasma, without toxicity (Yuan et al., 2009). The beneficial role of PA and omega-3 fatty acids in the gut microbiota was reported using an in vitro fecal fermentation model (Salsinha et al., 2024). Pomegranate oil and fish oil, rich in omega-3, increase acetate and butyrate in the high-fat, high-sugar diet as well as GABA and tyrosine.

Seeking new sources of PA in edible seeds of Cucurbitaceae, we examined two South American cucurbits (*C. maxima* and *S. odorifera*) using NMR as an analytical technique. The *S. odorifera* seeds from the *atropurpurea* (deep purple peel) and *fragans* (reddish brown peel) cultivars are both from the ancient agriculture of the Guarani Amerindians in Paraguay. The *C. maxima* seeds from two different harvest years were analyzed to assess possible differences in composition. NMR offers the advantage of rapid detection of CLnA in the samples and provides relevant information on the oil composition.

2. Materials and methods

2.1. Reagents

Hexane (CAS 110–54–3, 98.5 % purity), deuterated chloroform ($CDCl_3$) (CAS 865–49–6, 99.8 % deuteration degree), and heneicosanoic acid (C21:0, analytical standard, CAS 2363–71–5, purity $\geq$ 99 %) were from Merck (Darmstadt, Germany). For the fatty acid identification and quantification by GC/FID, the 37 fatty acid methyl esters standard (37 FAMEs) Supelco CRM47885, and heneicosanoic acid were used.

2.2. Plant material

The seeds from *C. maxima* were from Chile, and the plant was cultivated on the outskirts of Talca, Región del Maule, Chile. The *S. odorifera* plants were cultivated in Paraguay from seeds obtained and stored by native peasants in two geographical locations. They comprised the morphological cultivars atropurpurea and fragans. The cv. atropurpurea fruits were from Juan de Mena, Cordillera Department (24 57' 35.8″ S, 56 44' 20.0″ W), while the cv. fragans was from the district Yasy kañy-Acepar, Canindeyú Department (24 37′ 12.6″ S, 55 48′ 59.2″ W). Samples from *S. odorifera* atropurpurea were collected in October 2024, and samples from *S. odorifera* fragans were harvested in December 2024, respectively. The fruits with no visible signs of damage were processed at the Department of Food Biochemistry, Faculty of Chemical Sciences, National University of Asunción, Paraguay (FCQ-UNA). Seeds from *Punica granatum* L. were used as a source of punicic acid (PA) for comparison.

2.3. Extraction

The *C. maxima* seeds were washed with tap water, dried at room temperature, and lyophilized before processing. Lyophilized seeds were separated into cotyledons (the edible part) and husk. The cotyledons were powdered in a Sindelen LCM-18000GF blender (Santiago de Chile, Chile) and extracted with hexane in a Soxhlet for 6 h (method No 948.22). The seeds and epicarp of *S. odorifera* were manually separated from the endocarp, dried for 8 h at room temperature, and freeze-dried to extract the oil by cold pressing using a manual press (CDr Food press, Florence, Italy). Pomegranate seed oil was obtained after hand separation of the arils from the fruit purchased at the Talca market in Chile. The juice was removed from the aryls by pressure. Seeds were washed with tap water, lyophilized, powdered, and extracted under the same conditions as *C. maxima* cotyledons. The oils were collected in clean, dry glass vials, minimizing headspace and avoiding direct exposure to light, oxygen, and heat. Samples were stored at −20 °C until analysis.

2.4. NMR analyses

The seed oils were analyzed on a Bruker Ascend NMR spectrometer (Bruker, Karlsruhe, Germany), operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Bidimensional NMR, including heteronuclear single-quantum coherence (HSQC), total correlation spectroscopy (TOCSY), and heteronuclear multiple-bond correlation (HMBC). The samples (20–30 mg) were dissolved in 0.5 mL of $CDCl_3$ using 5 mm NMR tubes. Measurements were performed at 24 °C. For the ^{13}C NMR spectra, the parameters were: broadband proton decoupling mode, 65 K data points, spectral width of 236 ppm, acquisition time of 1.10 s, and 1024 scans. The compounds were identified according to the literature (Cao et al., 2007; Guillén & Ruiz, 2003; Hidalgo & Zamora, 2003; Knothe & Kenar, 2004; Miyake et al., 1998).

The relation of the descriptor used for assignation and quantification in the ^{1}H NMR spectra (letters and numbers), the identity of the H signal, and the chemical shift of the different protons in our samples is summarized in Table S1. The content of triglyceride in the samples was estimated by comparing the area of the H from the acyl CH_2 of the fatty acids (H-2 or α-H of the fatty acid chains) at δ 2.30 (F) and the signals for the four glyceryl H-1 and H-3 protons at δ 4.29 and δ 4.13 (H). Six α-methylene H are present in triglycerides, with a typical ratio of 1.5 with H-1 and H-3 of glycerol. The absence of the terminal methyl at δ 1.00 (t) (B) rules out the occurrence of linolenic acid. The methyl signal at δ 0.88–0.90 (A) belongs to saturated, monounsaturated, and di-unsaturated FA, but also agrees with the methyl of C18:3 isomers with conjugated double bonds. The ratio of conjugated C18:3 isomers of linolenic acid vs. di-unsaturated, mono-unsaturated, and saturated FA should be calculated, based on structural features different than the terminal methyl. All allyl methylene protons for the usual unsaturated FA appear as a multiplet at δ 2.04 (E). The divinyl methylene H for both linoleic and linolenic acid appears at δ 2.76 (G). Additional H signals at δ 6.45 (4, Fig. 1), 6.04 (3, Fig. 1), and 5.44–5.46 ppm (2, Fig. 1) indicate conjugated double bonds, pointing to a conjugated linolenic acid (CLnA) isomer.

The formula used to calculate the ratio of di-unsaturated C18:2 (linolenic acid), monounsaturated C18:1 (oleic acid), and saturated fatty acids (FA) is shown in equations (Eq.) (1) and (2). The results are expressed in percentages.

$$\text{Percent linolenic acid(di}-\text{unsaturated)}\,\text{C18}:2 = \left(\frac{G}{F}\right) \times 100 \qquad (1)$$

$$\text{Percent oleic acid (monounsaturated) } 18:1 = \left(\frac{E}{2F}\right) - \left(\frac{G}{G}\right) \times 100 \qquad (2)$$

The total monounsaturated fat (MUFA) is equal to Eq. (2).

Total monounsaturated fat (MUFA) = C18:1

The total polyunsaturated fat (Eq. (3)) must include the di-unsaturated FA (Eq. (1)) and tri-unsaturated compounds, if present.

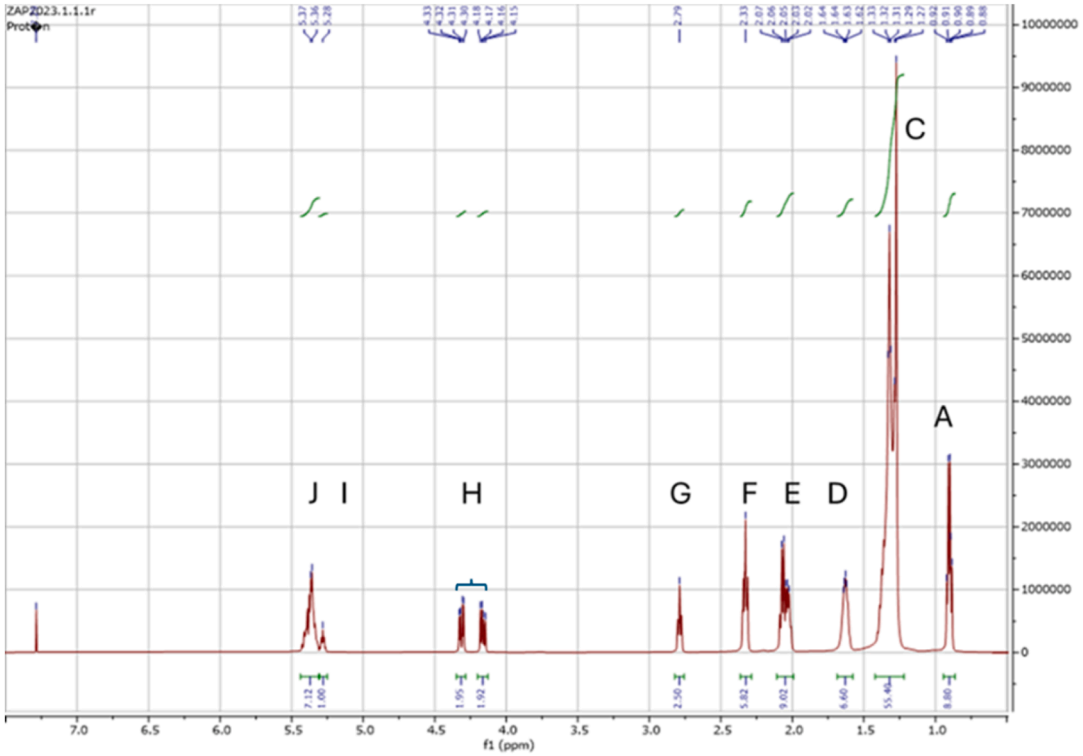
$$\text{Total polyunsaturated fat (PUFA)} = \text{C18}:2 + \text{C18}:3 \qquad (3)$$

The total percent saturated FA is calculated as in Eq. (4):

$$\text{Total saturated fatty acids (SFA)} = 100 - (\text{MUFA} + \text{PUFA}) \qquad (4)$$

Alternative data for the iodine value can be obtained by ^{1}H NMR, as reported by Knothe and Kenar (2004). They comprise the allylic position equivalents (APE) (Eq. (5)) and bis-allylic position equivalents (BAPE) (Eq. (6)), calculated as follows:

A *Cucurbita maxima* seed oil (Chilean “zapallo de guarda”)



B *Sicana odorifera* cv. atropurpurea (deep purple skin)

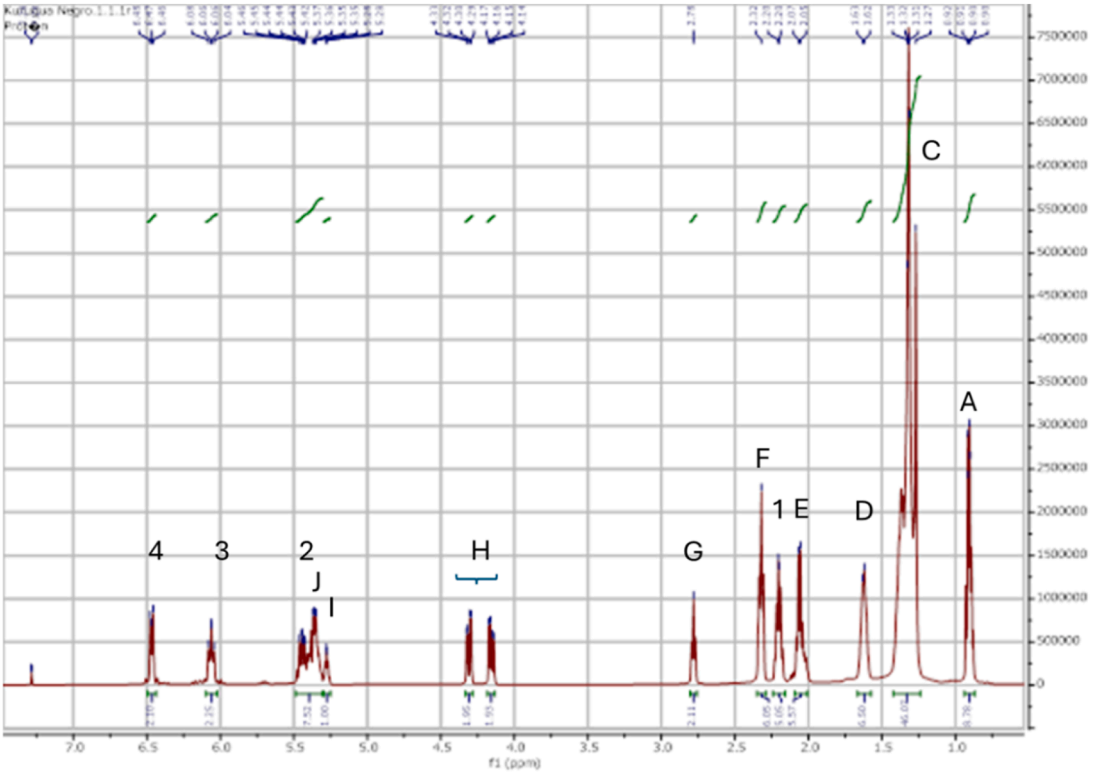
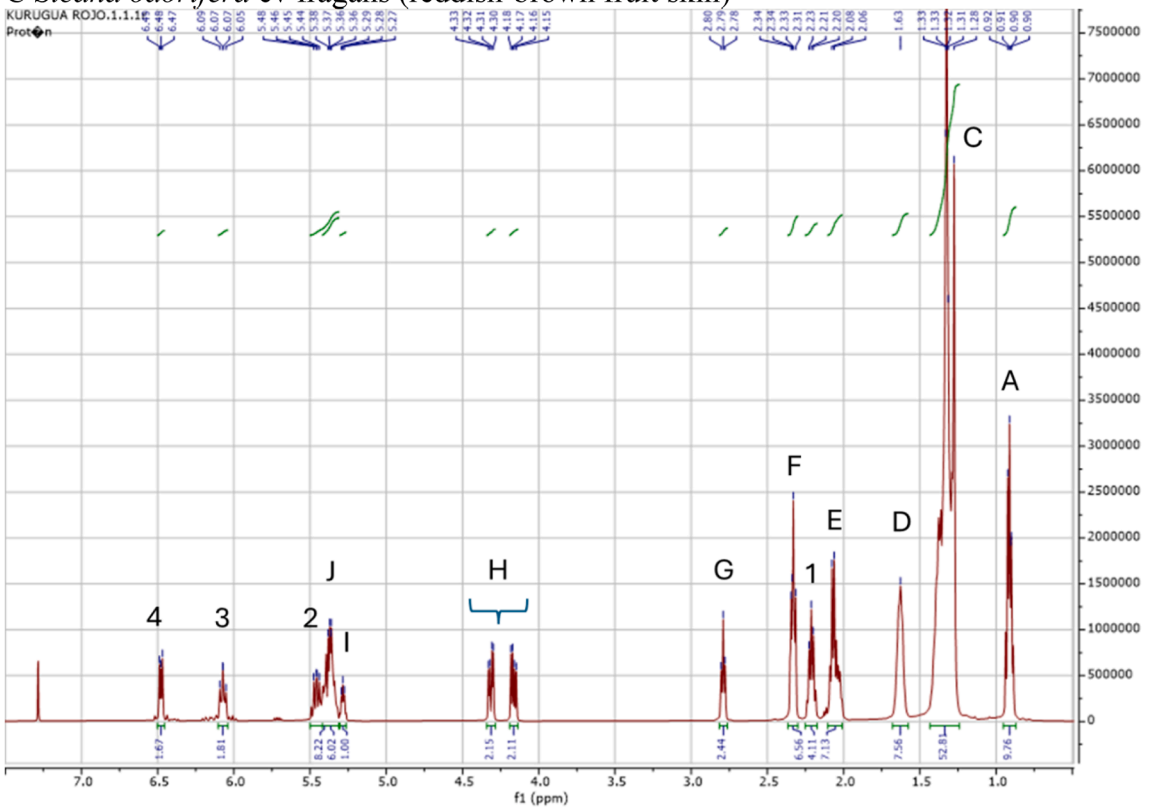


Fig. 2. ^{1}H NMR spectra (500 MHz, $CDCl_3$) of (A) *Cucurbita maxima* seed oil, (B-C) *Sicana odorifera* seed lipids (B: atropurpurea; C: fragans), and (D) pomegranate (*Punica granatum*) seed oil.

C *Sicana odorifera* cv fragans (reddish-brown fruit skin)



D *Punica granatum* seed oil

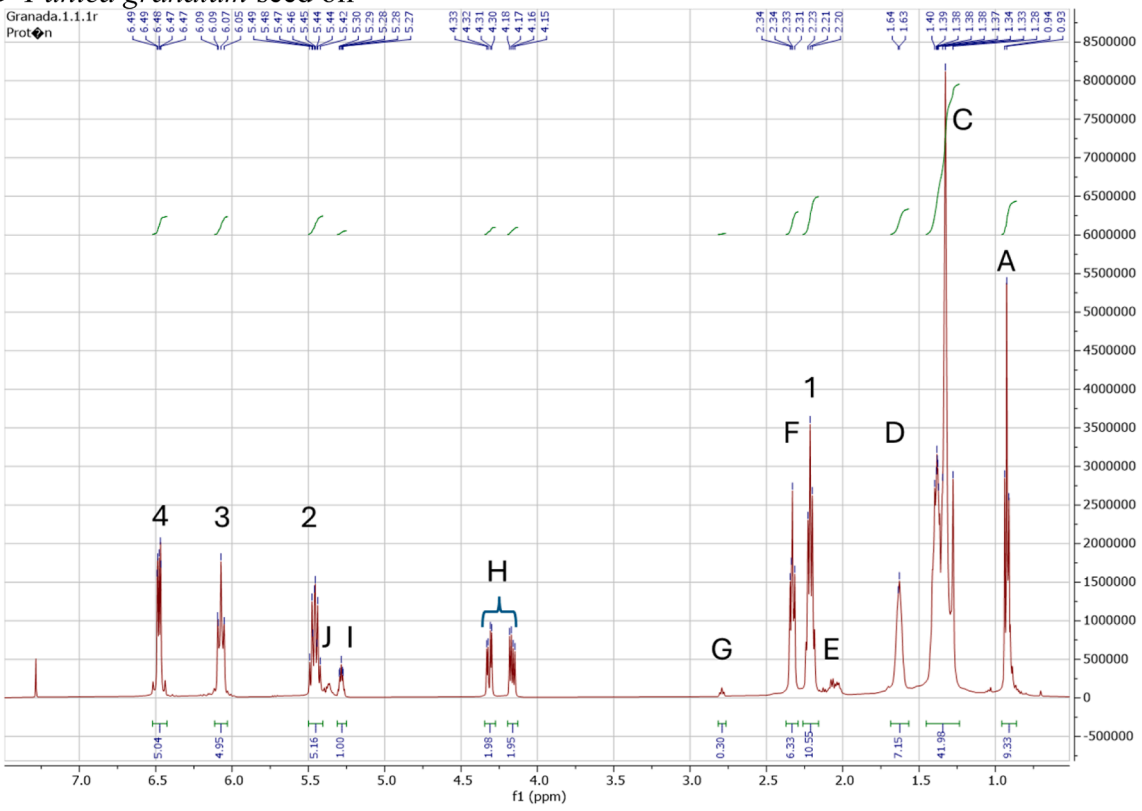


Fig. 2. (*continued*).

$$APE = 2x(C18:1 + C18:2 + C18:3) \quad (5)$$

$$BAPE: C18:2 + 2xC18:3 \quad (6)$$

The alternative iodine value (A_{IV}) can be calculated as follows (Yeboah et al., 2017) (Eq. (7)).

$$A_{IV} = 253.8x[(J + 2* + 3* + 4*) - (H/4)/2xA_{MW}x(H/4)]x100 \quad (7)$$

The first term includes all olefinic H. The traditional Hanus and ^{1}H NMR methods for iodine value have a good correlation (Guillen & Ruiz, 2003).

For alternative saponification value (A_{SV}), the following formula is used (Eq. (8)) (Yeboah et al., 2017):

$$A_{SV} = 343.55 - 0.1911xA_{MW} \quad (8)$$

The average molecular weight of the lipids was determined using Eq. (9) (Yeboah et al., 2017):

$$A_{MW} = 15.034x(A/3) + 14.026(C + D + E + F + G + 1*)/2 + 173.100x(H/4) + 26.016[(I + J + 2* + 3* + 4*) - (H/4)/2] \quad (9)$$

The first term of the equation refers to the terminal methyl, the second to the methylene (CH_2), the third to the glyceryl (CH_2O-R), and the fourth to the olefinic H. The 1*, 2*, 3*, and 4* are from the CLnA (Fig. 2).

2.5. GC-FID analyses

A new analysis of *S. odorifera* oil was performed by GC-FID to verify the identification of polyunsaturated FA and compare the results with NMR. The method used was 40.1.05 AOAC Official Method, 948.22, Fat (crude) in nuts and nut products: Gravimetric Methods (https://doi.org/10.1093/9780197610145.003.3475). After saponification and fatty acid esterification according to the AOCS Ce 2–66 method (Firestone, 2009), the methyl esters were analyzed by GC-FID using an Agilent (Hewlett-Packard) 6890 Plus GC, equipped with a flame ionization detector (FID) from Agilent Technologies model G1530A (Wilmington, USA) and manual injection. The injection was split at a ratio of 20:1, with a volume of 1 μL, using a Zebron™ ZB-FAME (100 $m \times 0.25$ mm ID) Phenomenex Inc. (Torrance, CA, USA) column. Nitrogen was used as the carrier gas, and hydrogen as the auxiliary gas. The inlet and detector temperatures were 240 °C and 400 °C, respectively. The temperature program started at 60 °C, increased to 180 °C at 10 °C/min (with a 5-minute hold), then rose to 215 °C at 5 °C/min and finally reached 240 °C at 5 °C/min, with a 16-minute hold. These parameters were established in the Ce 1j-07 method (Firestone, 2009).

2.6. Statistical analysis

All experiments were conducted in triplicate ($n = 3$), and results are expressed as mean ± standard deviation (SD). Differences among samples were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons ($p < 0.05$).

For NMR analyses, seed oil was extracted in triplicate, and analyses were carried out in triplicate for each sample. Integrals for each signal were measured three times for each NMR measurement.

Statistical comparisons between analytical methods (^{1}H NMR and GC-FID) (Table 3) were performed for the atropurpurea and fragrans cv. of *S. odorifera*. For each fatty acid and lipid class, differences between methods were evaluated using Student's *t*-test for independent samples (two-tailed). All analyses were conducted using triplicate measurements ($n = 3$), and results are expressed as mean ± standard deviation (SD). All analyses were performed using GraphPad Prism 5.0 software (GraphPad Software, Inc, CA, USA). When $p \leq 0.05$, with $n = 3$ differences were considered statistically significant.

3. Results and discussion

The composition of *C. maxima* and *S. odorifera* seed lipids was determined by NMR, and the *S. odorifera* fatty acid composition was reassessed by GC-FID. A sample of *Punica granatum* seed oil was included as a reference for punicic acid (PA).

3.1. NMR analyses

The ^{1}H NMR spectra of the *C. maxima* (Fig. 2A) and *S. odorifera* oils (Fig. 2B and C) showed characteristic signals for triglycerides, differing in the identity of the FA. The FA comprises a CLnA isomer and linoleic acid, identified by the characteristic divinyl CH_2 signal at δ 2.76, coupling with the m at δ 5.32–5.36 ppm. Heteronuclear single-quantum coherence (HSQC) experiments established the identity of the C associated with the olefinic proton signals at δ 5.44–5.46 (132.67 d, 132.43 d), 6.04 (128.86 d, 128.76 d) and 6.45 (127.80 d, 127.89 d), as well as with the m at δ 5.32 (128.08 d, 127.95 d) and 5.36 (130.19 d, 129.96 d), supporting two different unsaturated FA, one of them with three conjugated double bounds and the second one with alternate double bonds. The identification of the CLnA isomer follows from the chemical shift of the olefinic ^{1}H (Fig. 2B, C) and ^{13}C signals (Fig. 3A, C) as well as from the HSQC, HMBC, and TOCSY experiments. While α-eleostearic acid (c9, t11, t13) requires six different olefinic H signals, β-eleostearic (t9, t11, t13) and PA (c9, t11, c13) show three sets of olefinic H but differ in the coupling constants *J* and the chemical shifts of the olefinic C signals. PA is the main fatty acid in pomegranate seed oil. To confirm the identification, we investigated the oil from *P. granatum* seeds, extracted and assessed in the same conditions as the cucurbit lipids. Comparison of the ^{1}HNMR spectrum of *S. odorifera* (Fig. 2B, C) with that of pomegranate (Fig. 2D) shows that both contain the same PA triglyceride, supporting our assignment. Enlarged ^{13}C NMR spectra of the vinyl C of *S. odorifera* and pomegranate oil (Fig. 3) show a clear coincidence of the signals belonging to PA, and, in addition, the C signals for two double bonds from linoleic acid in the *S. odorifera* oil. The NMR data for the CLnA in *S. odorifera* agree with PA, according to the chemical shifts reported by Venianakis et al. (2021) for geometric isomers of CLnA and by Cao et al. (2007) for CLnA isomers. Therefore, we corrected the previous identification of linolenic acid in *S. odorifera* seed oil and confirmed that the compound is PA. For the calculations using the NMR data, new terms are needed to include the H identified by the numbers 1, 2, 3, and 4 in the spectra (Fig. 2B, C, and D). The ^{1}H and ^{13}C NMR data of PA and linolenic acid triglycerides are summarized in Table S1, and the structure is shown in Fig. S1.

Studies on the oil composition of pumpkin seeds (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb.) cultivated in Croatia showed that the main FA in all seed oil samples were linoleic (40.14 % to 55.33 %) and oleic (29.20 % to 41.07 %), followed by palmitic and stearic acid (Nederal et al., 2014). In our pumpkin seeds (Table 1), linoleic acid was higher (42.92 %) in the 2023 sample, and oleic acid was higher (49.71 %) in the 2025 sample.

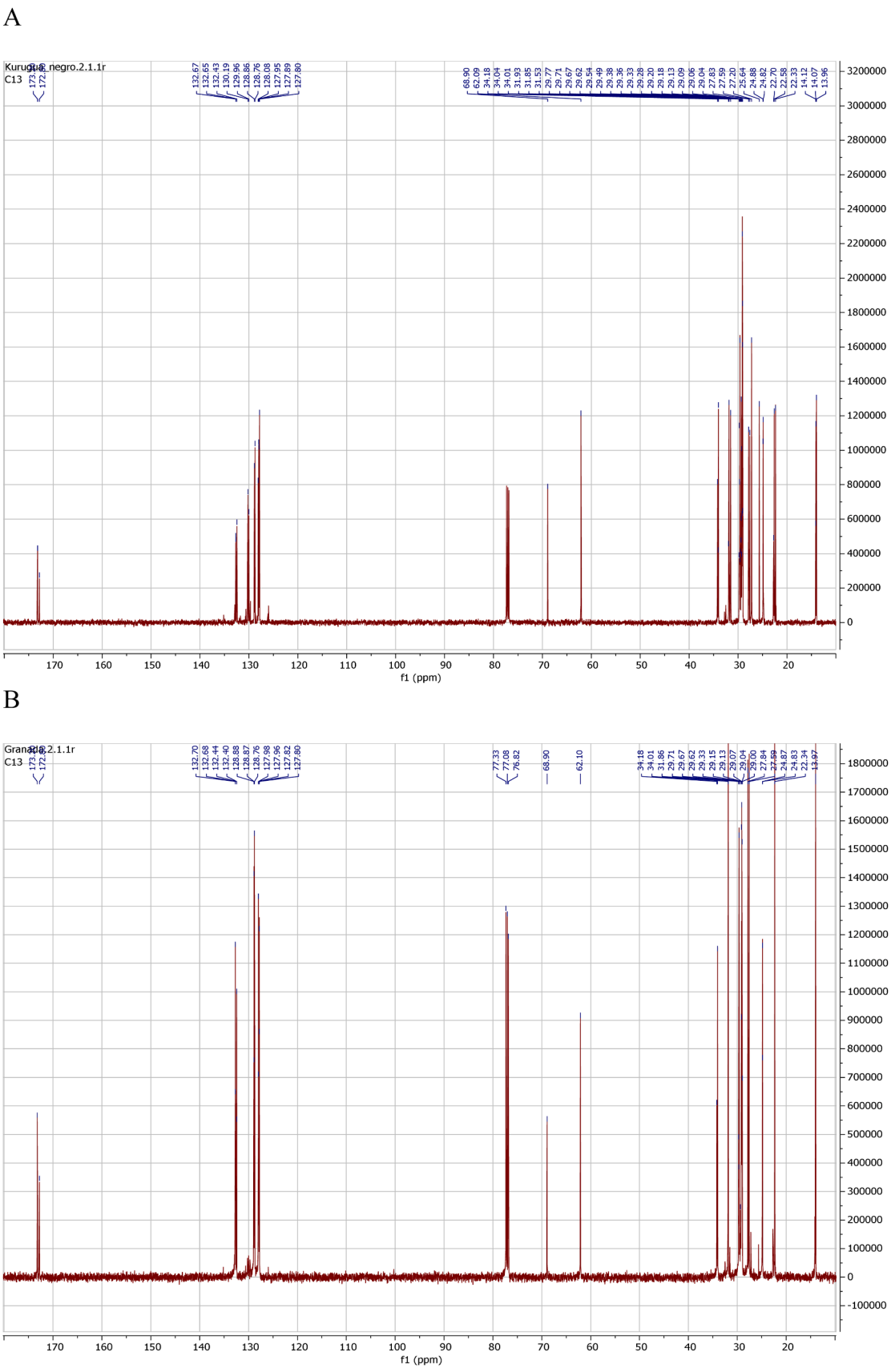
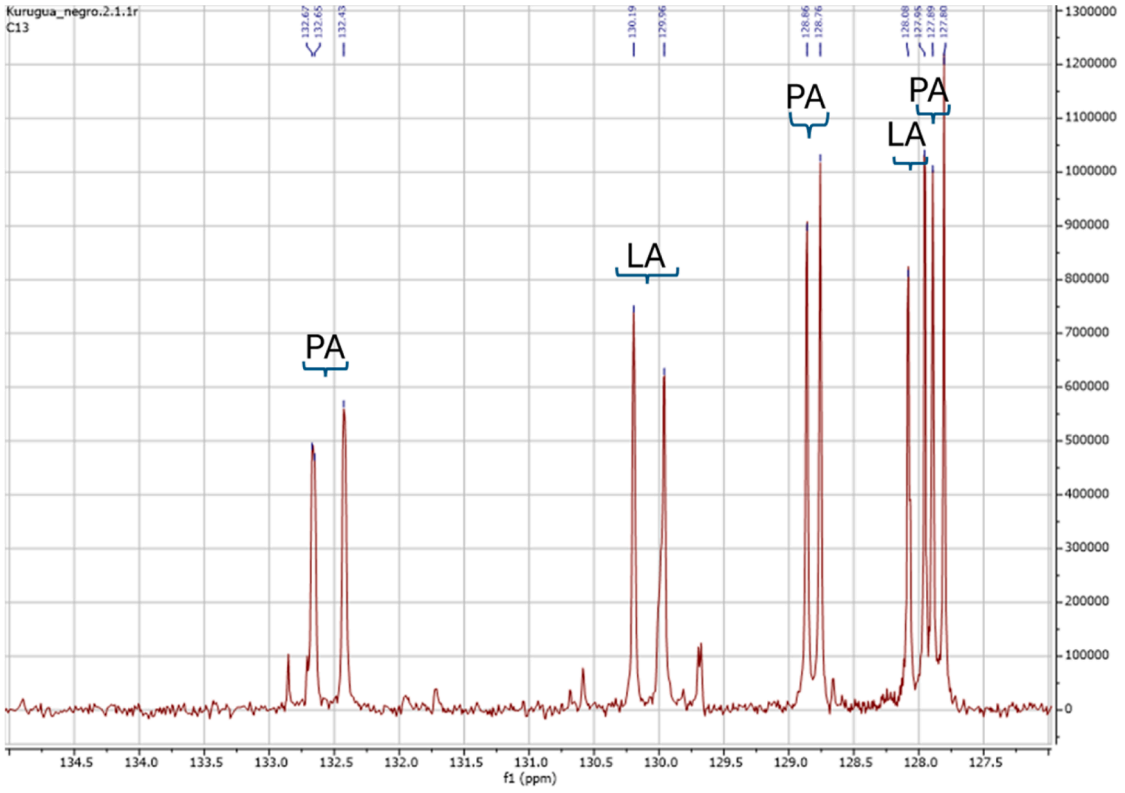


Fig. 3. ^{13}C NMR spectrum (125 MHz) of *Sicana odorifera* (A) and pomegranate (B) seed lipids and the olefinic carbon signal region of *S. odorifera* oil (C) and pomegranate seed oil (punicic acid reference) (D). PA: Punicic acid; LA: Linolenic acid.

C



D

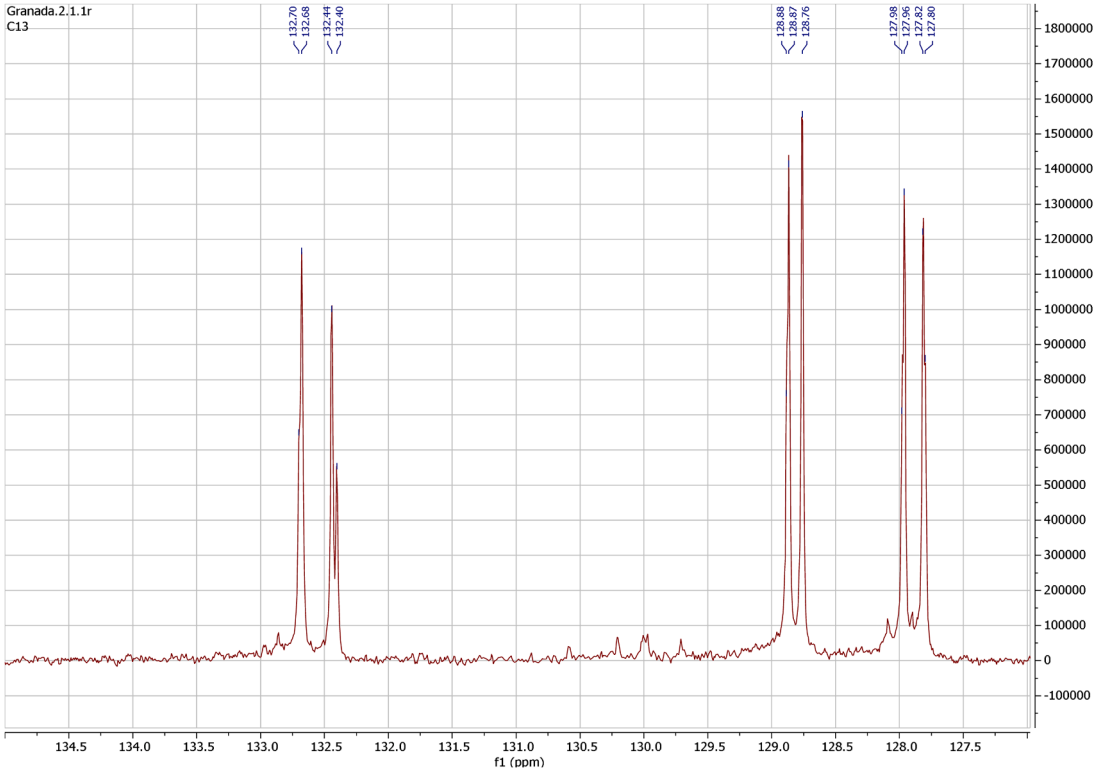


Fig. 3. (*continued*).

Table 1
Fatty acid composition of giant pumpkin (*C. maxima*) and kurugua (*S. odorifera*) seed oil determined by NMR analysis. Pomegranate (*Punica granatum*) seed oil was used as a reference for punicic acid (PA).

	Pumpkin 2023	Pumpkin 2025	*S. odorifera* atropurpurea	*S. odorifera* fragans	Pomegranate
C18:1	34.85±0.31 ^{b}	49.71±0.36^{a}	9.87±1.11 ^{d}	17.75±0.55 ^{c}	0.00±0.00 ^{e}
C18:2	42.92±0.09 ^{a}	28.04±0.08 ^{c}	34.80±0.46 ^{b}	36.43±0.81 ^{b}	5.67±0.12 ^{d}
Punicic acid	0.00±0.00 ^{d}	0.00±0.00 ^{d}	36.11±0.45 ^{b}	29.18±0.21 ^{c}	83.19±2.11 ^{a}
MUFA	34.85±0.31 ^{b}	49.71± 0.36 ^{a}	9.87±1.11 ^{d}	17.75±0.55 ^{c}	0.00±0.00 ^{e}
PUFA	42.92±0.09 ^{d}	28.04± 0.08 ^{e}	70.91±0.23 ^{b}	65.61±0.12 ^{c}	88.86±1.14 ^{a}
SFA	22.24±0.25 ^{a}	22.25± 0.41 ^{a}	19.20±0.31 ^{b}	16.64±0.18 ^{c}	11.14±0.34 ^{d}
TG content	1.50±0.01 ^{c}	1.61±0.00 ^{ab}	1.51±0.06 ^{bc}	1.62±0.04 ^{ab}	1.68±0.04 ^{a}
A_{MW}	840.68±6.90^{c}	980.30±32.13 ^{a}	857.80±75.57 ^{c}	965.41±10.71 ^{ab}	892.47±10.97 ^{bc}
A_{SV}	182.90±1.32 ^{a}	156.21±6.14 ^{c}	179.57±14.44 ^{ab}	159.06±2.05 ^{c}	173.01±2.10 ^{b}
A_{IV}	96.15±1.00 ^{d}	74.06±1.89 ^{e}	160.94±15.60 ^{c}	142.33±1.32 ^{b}	221.44±4.12 ^{a}

A_{MW}: Mean molecular weight; A_{SV}: Alternative saponification index (SV); A_{IV}: Alternative iodine value (IV). Different superscript letters within the same row indicate significant differences between samples (columns) according to one-way ANOVA and Tukey's post-test ($p < 0.05$).

Table 2
Fatty acid profile in *Sicana odorifera* seeds from the atropurpurea (deep purple skin) and fragrans (reddish-brown skin) forms by GC-FID. Results are presented as g/100 g oil.

Fatty acid	Abbreviated formula	Kurugua atropurpurea	Kurugua fragrans
Myristic	C14:0	0.06±0.00 ^{b}	0.08±0.01 ^{a}
Palmitic	C16:0	6.89±0.02 ^{b}	7.84±0.07 ^{a}
Heptadecanoic	C17:0	0.07±0.00 ^{a}	0.05±0.01 ^{b}
Stearic	C18:0	3.72±0.03 ^{b}	3.91±0.06 ^{a}
Arachidic	C20:0	0.28±0.01 ^{a}	0.28±0.01^{a}
Behenic	C22:0	0.04±0.01 ^{a}	0.04±0.01 ^{a}
Tricosanoic	C23:0	1.09±0.16 ^{a}	1.24±0.21 ^{a}
Lignoceric	C24:0	0.04±0.00 ^{a}	0.04±0.01 ^{a}
Total SFA		**11.10±0.03** ^{b}	**12.24±0.14** ^{a}
Palmitoleic	C16:1	0.02±0.00 ^{b}	0.04±0.01 ^{a}
Oleic	C18:1	9.08±0.06 ^{b}	14.30±0.17 ^{a}
Gondoico	C20:1	0.15±0.03 ^{a}	0.09±0.01 ^{b}
Total MUFA		**9.25±0.08** ^{b}	**14.43±0.17** ^{a}
8,11 Octadecadienoic	C18:2 c ω6	0.47±0.05 ^{a}	0.44±0.21 ^{a}
Linoleic	C18:2 c ω6	39.15±0.28 ^{a}	38.96±0.25 ^{a}
Punicic	C18:3 ω5 (9c,11t,13c)	37.42±0.06 ^{a}	31.04±0.91 ^{b}
Docosadienoic	C22:2 ω6	1.45±0.06 ^{a}	1.57±0.17 ^{a}
Total PUFA		**78.49±0.39** ^{a}	**71.57±1.02** ^{a}
Total UFA		**87.74±0.47** ^{a}	**86.00±1.19** ^{a}

The values are expressed as mean ± SD ($n = 3$). SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids. UFA: Unidentified fatty acids. Different superscript letters within the same row indicate significant differences between species by Student's *t*-test ($p < 0.05$).

3.2. GC FID analyses

The NMR analysis of *S. odorifera* oil revealed the presence of the conjugated linoleic acid (CLnA) PA instead of linolenic acid (Table 1). A new GC analysis of the FA was performed to confirm and refine the previous assignment (Table 2). The main difference was the identification and assignment of the major C18:3 fatty acids in the C18 region of the chromatogram, confirming PA as a predominant component. A total of 8 saturated fatty acids (SFAs), 3 monounsaturated fatty acids (MUFAs), and 4 polyunsaturated fatty acids (PUFAs) were identified using FAME standards. PA was one of the predominant FA acids in both *S. odorifera* seed oils (atropurpurea and fragans), along with linoleic and oleic acids. According to GC-FID analysis, linoleic acid ranged from 38.96 ± 0.25 % (cv. fragrans) to 39.15 ± 0.28 % (cv. atropurpurea), while oleic acid ranged from 14.30 ± 0.17 % to 9.08 ± 0.06 %, respectively. PA showed values of 31.04 ± 0.91 % (cv. fragrans) and 37.42 ± 0.06 % (cv. atropurpurea), with statistically significant differences between cultivars ($p < 0.05$). These differences in FA composition are consistent with variations due to environmental and cultivation conditions, as reported in other cucurbit species (Shelenga et al., 2020). Compared to previous GC–MS data (Mereles et al., 2021), where linolenic acid (C18:3) was reported as 32.82 ± 0.01 % and 38.08 ± 0.01 % for *S. odorifera* fragans and *S. odorifera* atropurpurea, respectively, the present study clarifies that these values correspond to PA.

Table 3
Comparison of the main FA content of the seeds of *S. odorifera* by NMR and GC.

Fatty acid	*S. odorifera* atropurpurea		*S. odorifera* fragans	
	NMR	GC-FID	NRM	GC-FID
C18:1	9.87±1.11^{a}	9.08±0.06 ^{a}	17.75±0.55^{a}	14.34±0.17^{a}
C18:2	34.80±0.46^{b}	39.15±0.28^{a}	36.43±0.81^{b}	38.96±0.25^{a}
C18:3/CLnA Punicic acid	36.11±0.45^{b}	37.42±0.06^{a}	29.18±0.21	31.70±0.91
MUFA	9.87±1.11^{a}	9.25±0.08 ^{a}	17.75±0.55^{b}	14.43±0.17^{a}
PUFA	70.91±0.23 ^{b}	78.49±0.39 ^{a}	65.61±0.12^{b}	71.57±1.02^{a}
SFA	19.2 ± 0.31 ^{a}	11.10±0.03 ^{b}	16.64±0.18^{a}	12.24±0.14^{b}

Different superscript letters within the same row indicate significant differences between analytical methods (NMR vs GC-FID) by Student's *t*-test ($p < 0.05$).

3.3. Comparison of NMR and GC FID analyses

NMR analysis showed that the contents of C18:1, C18:2, and PA were 17.75 ± 0.55 %, 36.43 ± 0.81 %, and 29.18 ± 0.21 % for *S. odorifera* fragrans, and 9.87 ± 1.11 %, 34.80 ± 0.46 %, and 36.11 ± 0.45 % for *S. odorifera* atropurpurea, respectively. When comparing the analytical methods, significant differences ($p < 0.05$) were observed between NMR and GC-FID for several variables, particularly for PUFA and SFA contents in both species, as well as for oleic acid (C18:1) in *S. odorifera* fragrans oil. In general, NMR and GC-FID provided comparable trends in fatty acid composition, although significant quantitative differences were observed. NMR offers the advantage of distinguishing the geometric configuration of conjugated double bonds in CLnA, while GC-FID provides greater sensitivity and allows for the detection and quantification of minor fatty acids. Therefore, both techniques should be considered complementary for a comprehensive characterization of lipid profiles.

According to Fatope et al. (2002), pomegranate seed oil is composed mainly (88 %) of the monoacylglycerol of PA. Our NMR analyses of Chilean pomegranate seed lipids, used for comparison with *S. odorifera*, show that most lipids are PA acid triglycerides. Based on the integrals for the divinyl protons (G) and the acyl chain (J) of unsaturated acids other than PA, the only other unsaturated fatty acid is C18:2, accounting for 5.67 % of the total. The mean area of the three double bonds from PA (6H, signals 2 + 3 + 4, Fig. 2) is 15.35, and the mean area for the terminal methyl (3H) of the fatty acids (A) is 9.22. The 6:3 ratio of the PA double bonds to the FA methyl group requires an area of 7.67 for the methyl and supports 83.10 % PA triglycerides. An additional 5.67 % are

linoleic acid (C18:2) triglycerides, accounting for 88.86 % of polyunsaturated FA.

The identity of linoleic acid in *S. odorifera* oil was confirmed by previous work using standards and by comparing NMR data on conjugated linoleic acid isomers (Davis et al., 1999). The ^{1}H and ^{13}C NMR data of the main triglycerides of *S. odorifera* oil are summarized in Table S2.

The average molecular weight (A_{MW}) of the glycerides from our samples, determined by NMR, was 840.68–980.30 for pumpkin, 857.80–965.41 for *S. odorifera*, and 892.47 for pomegranate, respectively. The average saponification value (A_{SV}) obtained by NMR was 156.21–182.90 for pumpkin, 159.06–179.57 for *S. odorifera* and 173.01 for pomegranate, respectively. The mean alternative iodine value by NMR (A_{IV}) was 74.06–96.15 for pumpkin, 142.33–160.94 for *S. odorifera*, and 221.44 for pomegranate oil (Table 1). According to literature, the molecular weight for triolein, trilinolein and trilinolenin ranges between 873–885, with iodine indices of 86, 173, and 261, respectively. The information obtained by NMR is comparable to the data reported by Mereles et al. (2021).

Due to their potential effect on obesity, CLnA has been considered a functional food ingredient. Functional yoghurts containing conjugated FA, the effect of the compounds reducing the accumulation of lipids in hepatocytes, and the release of triglycerides in adipocytes have been reported (Machado et al., 2022).

4. Conclusions

The lipid composition of Chilean giant pumpkin seeds falls within the range reported in previous studies from South America and Europe. Our results show that the identity of the main FA from *S. odorifera* seeds must be corrected, reporting PA instead of linolenic acid (ώ−3). The PA content in the *S. odorifera* samples was 29 to 37 %. Our findings highlight a new source of PA in the seeds of a fast-growing food plant. Additional studies are needed to assess the content of PA in the seeds of different accessions/landraces of *S. odorifera* in subtropical and tropical countries, as well as the possible effects of selection/breeding and agronomic practices to increase the production of this relevant compound. NMR proved to be a suitable and fast method for the identification of CLnA in plant oils.

Ethical statement

Not applicable.

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CRediT authorship contribution statement

Laura Mereles: Writing – review & editing, Writing – original draft, Resources, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Alberto Burgos-Edwards:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Felipe Leyton:** Methodology, Investigation, Formal analysis. **María Beatriz Carvajal:** Methodology, Investigation, Formal analysis. **Eva Coronel:** Methodology, Formal analysis. **Guillermo Schmeda-Hirschmann:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2026.101325.

Data availability

Data will be made available on request.

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