

Article

Non-Conventional Oilseeds: Unlocking the Global Potential for Sustainable Biofuel Production

Bushra Ahmed Alhammad ¹, Aftab Jamal ² , Claudia Carlucci ^{3,*} , Muhammad Farhan Saeed ⁴ , Mahmoud F. Seleiman ^{5,6}  and Marcelo F. Pompelli ^{7,*} 

¹ Biology Department, College of Science and Humanity Studies, Prince Sattam Bin Abdulaziz University, Al Kharj Box 292, Riyadh 11942, Saudi Arabia

² Department of Soil and Environmental Sciences, Faculty of Crop Production Sciences, The University of Agriculture, Peshawar 25130, Pakistan; aftabses98@gmail.com

³ CNR-Institute of Nanotechnology (CNR-NANOTEC), c/o Campus Ecotekne, Via Monteroni, 73100 Lecce, Italy

⁴ Department of Environmental Sciences, COMSATS University Islamabad, Vehari Campus, Vehari 61100, Pakistan; farhansaeed@cuivehari.edu.pk

⁵ Plant Production, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia; mseleiman@ksu.edu.sa

⁶ Department of Crop Sciences, Faculty of Agriculture, Menoufia University, Shibin El-Kom 32514, Egypt

⁷ Facultad de Ciencias Agrícolas, Universidad de Córdoba, Montería 230002, Colombia

* Correspondence: claudia.carlucci@nanotec.cnr.it (C.C.); marcelo@fca.edu.co (M.F.P.)

Abstract: Renewable energy sources have become an urgent worldwide concern due to the impacts of global warming. Globally, biofuels can significantly reduce greenhouse gas emissions, which are major contributors to global warming. The use of biofuels has the potential to transform the energy landscape while mitigating the adverse effects of traditional fossil fuels. This study examines the water features, biochemical compositions, and fatty acid profiles among various plant species. The results reveal significant variations in water features as a consequence of the relative water content and water potential of each seed. Also, we note that some non-edible species like *A. blanchetii*, *C. procera*, *E. oleracea*, *P. juliflora*, *M. oleifera*, and *J. curcas* have good attributes that confer a biofuel-like species. These attributes are high in oil content and have a good profile content of long-chain polyunsaturated fatty acids (LC-PUFAs), ranging from 35% to 80% among the different oilseeds. Fatty acid profiling reveals distinct compositions among the plant species. Stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) were the principal oils in *A. blanchetii*, *J. curcas*, *P. juliflora*, *M. oleifera*, and *S. tuberosa* compared to other species. *M. oleifera* stands out with a high linoleic acid (C18:1) content, while *C. maxima*, *J. curcas*, and *P. juliflora* are even higher (C18:2). A principal component analysis (PCA) and Pearson correlations analysis also confirmed that alternative oilseeds exhibited similarities to standard oilseeds and have the potential to replace them for biofuel production. These findings demonstrate the potential of non-conventional oilseeds for sustainable biofuel production. By unlocking their global potential, we can advance towards mitigating environmental impacts and fostering a sustainable biofuel industry.

Keywords: edible oil seeds; non-edible oil seeds; biofuel; biodiesel; *Moringa oleifera*; *Jatropha curcas*; *Prosopis juliflora*



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1. Introduction

1.1. The Oil Seed Species

The increasing global demand for renewable energy sources and the need to reduce greenhouse gas emissions have led to a growing interest in biofuels as a sustainable alternative to fossil fuels [1]. It is essential to design appropriate long-term strategies based on the use of renewable fuels that would gradually replace the declining fossil fuel production, as the world's accessible oil resources are gradually running out. Additionally,

the production of fossil fuels has harmed the environment by leading to an increase in the atmospheric concentration of CO₂ [2]. It has been reported that about 98% of carbon emissions stem from combustion fossil fuels [3].

Biodiesel is a fuel made from used vegetable or waste vegetable oils, animal fats, or both. Chemically, biodiesel is a mono-ester alkali that can serve as an alternative for conventional diesel fuel [4], and due to its complete miscibility, biodiesel can be utilized in its pure form or blended with petroleum diesel [5]. It can also be synthesized from industrial crops, agricultural by-products, and urban wastes [6].

Biodiesel made from vegetable oils and animal fats is being utilized in numerous countries worldwide, including the USA, China, India, Malaysia, Brazil, and several European countries, as a means to reduce air pollution and decrease dependence on fossil fuels [7].

Edible resources (e.g., soy, corn, cotton, sunflower, and canola) are normally used to fuel vehicles by converting edible oils into biodiesel. It is thought that the large-scale manufacturing of biodiesel from edible oils may cause a global imbalance in the market for food [6,8]. Environmentalists have recently begun debating the harmful effects of producing biodiesel from edible oils [1,3,8,9]. They assert that the widespread growth of oil crop plantations for the manufacturing of biodiesel could hasten deforestation in nations like Malaysia, Indonesia, and Brazil [10]. Additionally, this has increased the price of producing biodiesel due to rising feedstock costs, rising market demands, and the competition for food [9]. Even if the production of crop oil is constantly rising, the stockpiles of vegetable oils are constantly falling as a result of the rising production of biodiesel. So, non-conventional oilseeds have gained considerable attention due to their potential for producing biodiesel and other bio-based fuels [8]. Brazil has become a significant player in the biofuel market due to its rich agricultural resources and cutting-edge agro-industrial infrastructure [4]. According to the published literature, Brazil produced 3.35 billion m³ in 2018 [11] and continues to increase its production over the years [2]. Currently, Brazil has 51 power plants to produce biodiesel, totalizing 26,602.26 m³ day⁻¹ [12–14]. Brazil may increase the area planted with biodiesel feedstocks without affecting regions utilized for grazing or for growing feed crops [15]. Therefore, the cultivation is based on soy, needing new species to meet the explanation and use of marginal soils for future biodiesel production in Brazil and other countries.

Non-conventional oilseeds offer numerous advantages as feedstocks for biofuels. When compared to conventional oilseed crops, they often possess a higher oil content and distinct oil profiles [16]. In a recent study, Shaah et al. [8] reviewed non-edible oil crops and highlighted their potential as reliable feedstock for biodiesel production, citing advantages such as low production costs, high oil yields, and no conflicts with food products. Moreover, cultivating non-conventional oilseeds on marginal soils is practical, as it minimizes competition with food crops and helps alleviate the indirect effects of biofuel production on land use [9]. A recent study by Mukhtar, et al. [17] indicated that non-edible oilseed species like castor bean (*Ricinus communis*) can yield stability even on marginal lands due to their tolerance to water stress, low soil fertility, high soil pH, and arid conditions. Similarly, Ramos, et al. [5] observed that non-edible crops can be grown on lands unsuitable for arable crops, with lower water and fertilizer requirements, thus enhancing their economic viability. Such resources present promising and eco-friendly alternatives for enhancing biofuel characteristics through new and advanced technologies [18]. These non-edible oils offer advantages as diesel fuels due to their liquid nature, portability, ready availability, renewability, higher combustion efficiency, lower sulfur and aromatic content, and increased biodegradability [17,18]. Additionally, they can thrive in various climatic conditions [18]. Therefore, the objective of current research is to shift the focus from edible resources towards low-cost potential feedstocks such as non-edible crops for biodiesel synthesis.

1.2. Catalytic Transesterification on Non-Edible Oils

The transesterification reaction is the most used process to produce biodiesel from non-edible oil. Generally, non-edible oils contain high viscosity, free fatty acids, and

moisture that have to be reduced [19]. This reaction occurs by a triglyceride and an alcohol, most commonly methanol and ethanol, with or without a catalyst. The catalytic transesterification process occurs to convert non-edible oil into biodiesel in the presence of a catalyst through homogenous or heterogeneous catalysis. The catalyst used in the biodiesel transesterification process could be either basic or acidic, depending on the fatty acid compositions in the source. Different parameters influence the catalytic process, including the reaction temperature, time, amount of catalyst, as well as the alcohol/oil molar ratio [20]. In homogeneous catalysis, the catalyst and the reactants are in the same gaseous or, above all, liquid phase. The main catalysts used in homogeneous catalysis are sodium and potassium hydroxide, sodium methoxide, hydrogen chloride, and sulfuric and sulfonic acid [21]. The advantages of homogeneous catalysts lie in the contact surface with the reactants and the excellent selectivity. The most important disadvantages, however, are the low thermal stability, relatively high reaction time, and further purification to separate the catalyst, resulting in increased energy consumption and production costs. Furthermore, the homogeneous catalytic process is not suitable for producing biodiesel from the non-edible oils containing a high amount of free fatty acids that causes a saponification side reaction, reducing the biodiesel conversion and catalyst efficacy [22].

Based on the above background, it was hypothesized that non-conventional oilseeds have the potential to considerably increase Brazil's biodiesel production, becoming a leader in the production of these oilseed species. To test this hypothesis, we conducted a comprehensive analysis of the key parameters to comprehend the comparison of the edible and non-edible biofuel species. The main goal of this research is to study the potential of alternative oilseeds for the International Biofuels Program and Renewable Energy Programs. The results of this study will clarify the benefits and feasibility of using unconventional oilseeds for biodiesel production in Brazil and other interested countries. This study will provide information on the potential advantages and difficulties of integrating these alternative feedstocks into the biodiesel value chain to industry stakeholders, researchers, and policymakers, ultimately assisting in the growth of a more efficient and sustainable bioenergy sector in Brazil.

2. Results

2.1. Water Features

In this study, we illustrate the variation in fresh weight, ranging from 0.01 g to 2.82 g, with a weight difference (Δ) of 2.81 g (Table 1). Following a 120 h seed imbibition period, there was a notable increase in the fresh weight, ranging from 0.02 g to 3.98 g. Notably, *C. procera* displayed the lightest seed whereas *L. rigida* exhibited the heaviest. Additionally, the relative water content (RWC) ranged from 6.3% in *A. hypogaea* to 31.4% in *E. oleraceae*. After 120 h of imbibition, the RWC ranged from 22.2% (*P. juliflora*) to 69.2% (*M. oleifera*). The driving force behind the water imbibition was the osmotic potential, exemplified by *A. hypogaea*. This specie, with an initial RWC of 6.3%, experienced a substantial increase in RWC to 39.7%, resulting in a change (Δ) of 33.4%. Simultaneously, its osmotic potential increased from -66.9 MPa to -0.8 MPa after the 120 h imbibition period. The same pattern was described for *P. juliflora* where its Δ RWC was 13.6% but its $\Delta\Psi_w$ was -88.8 MPa. The same pattern was verified for *M. oleifera* where its Δ RWC was 60.8% and its $\Delta\Psi_w$ was -72.4 MPa. In distinct form, *E. oleraceae* increased its RWC by 3.5%, being the species which modulated its Ψ_w ($\Delta-4$ MPa) the least. *M. glabra* showed an inverse pattern because its Δ RWC was 58.9% and its $\Delta\Psi_w$ was -71.6 MPa, with the same pattern for *G. hirsutum* with a Δ RWC of 46% and a $\Delta\Psi_w$ of -78.1 MPa. *M. oleifera* had a Δ RWC of 60.8% and its $\Delta\Psi_w$ was -72.4 MPa. *P. juliflora* was the species with less delta weight (0.03 g), but its imbibition potential was higher because its seed showed a $\Delta\Psi_w$ of -88.2 MPa. *M. glabra* impressively show a Δ RWC of 58.9% and a $\Delta\Psi_w$ of -71.6 MPa (Table 1). These data were corroborated with fast imbibition governed by $\Delta\Psi_w$. *M. glabra* had a $\Delta\Psi_w$ of -71.6 MPa and an imbibition in the first 12 h of 278%, followed by *C. procera* at 112% and *M. oleifera* (95%) (Table 1).

Table 1. Scientific name, common name, family, seed number used in each repetition (N), fresh weight, relative water content, and water potential (Ψ_w) of the 14 plant species studied. The mean differences within the columns are represented by lowercase letters (SNK, $p \leq 0.05$). Each point represents the mean (\pm SD) of 20 repetitions. *Glycine max* (Gm) and *Zea mays* (Zm) were included as an edible and common biodiesel oilseed.

Scientific Name	Common Name	Family	N	Seed Fresh Weigh (g)		Seed Relative Water Content (%)		Ψ_w (MPa)	
				Initial	Final	Initial	Final	Initial	Final
<i>Allamanda blanchetii</i> A.DC. [1]	Purple allamanda	Apocynaceae	8	0.11 \pm 0.01 d	0.17 \pm 0.01 e	13.3 \pm 0.8 d	46.3 \pm 0.1 fg	−60.0 \pm 4.1 c	−0.9 \pm 0.3 bc
<i>Annona squamosa</i> L. [2]	Sugar-apple	Annonaceae	8	0.27 \pm 0.03 d	0.38 \pm 0.02 de	18.2 \pm 0.3 c	40.9 \pm 1.3 g	−19.7 \pm 2.6 a	−1.4 \pm 0.5 bc
<i>Arachis hypogaea</i> L. [3]	Peanut	Fabaceae	5	0.65 \pm 0.04 cd	1.01 \pm 0.07 de	6.3 \pm 0.2 g	39.7 \pm 1.0 g	−66.9 \pm 3.5 c	−0.8 \pm 0.2 bc
<i>Calotropis procera</i> (Aiton) Dryand. [4]	Milkweed	Asclepiadaceae	30	0.01 \pm 0.00 d	0.02 \pm 0.00 e	18.2 \pm 0.6 c	61.6 \pm 0.8 cd	−74.3 \pm 4.0 cd	−0.6 \pm 0.1 bc
<i>Cucurbita maxima</i> subsp. <i>maxima</i> [5]	Winter squash	Cucurbitaceae	8	0.05 \pm 0.01 d	0.09 \pm 0.01 e	9.1 \pm 0.2 f	56.1 \pm 6.0 de	−61.4 \pm 1.5 c	−0.3 \pm 0.1 a
<i>Euterpe oleracea</i> Mart. [6]	Açaí palm	Arecaceae	5	1.18 \pm 0.13 c	1.25 \pm 0.14 d	31.4 \pm 0.7 a	37.9 \pm 0.9 g	−6.6 \pm 1.0 a	−2.6 \pm 0.3 d
<i>Gossypium hirsutum</i> L. [7]	Cotton	Malvaceae	10	0.11 \pm 0.01 e	0.22 \pm 0.02 e	8.8 \pm 0.1 f	54.8 \pm 0.1 def	−78.7 \pm 5.8 cd	−0.6 \pm 0.1 bc
<i>Helianthus annuus</i> L. [8]	Sunflower	Compositae	30	0.02 \pm 0.00 e	0.05 \pm 0.01 e	9.5 \pm 0.4 ef	64.0 \pm 1.1 bc	−64.7 \pm 5.7 c	−0.3 \pm 0.1 ab
<i>Jatropha curcas</i> L. [9]	Purging nut	Euphorbiaceae	5	0.71 \pm 0.05 cd	1.24 \pm 0.06 d	8.8 \pm 0.3 f	53.3 \pm 1.1 ef	−63.1 \pm 0.5 c	−0.7 \pm 0.2 bc
<i>Licania rigida</i> Benth. [10]	Oiticica	Chrysobalanaceae	3	2.82 \pm 0.38 b	3.98 \pm 0.55 b	4.9 \pm 0.4 g	30.7 \pm 0.4 h	−76.2 \pm 8.7 cd	−1.4 \pm 0.3 bc
<i>Malpighia glabra</i> L. [11]	Acerola	Malpighiaceae	5	0.03 \pm 0.00 e	0.13 \pm 0.01 e	18.1 \pm 0.5 c	77.0 \pm 0.8 a	−71.7 \pm 5.0 cd	−0.1 \pm 0.1 a
<i>Moringa oleifera</i> Lam. [12]	Drumstick tree	Moringaceae	5	0.37 \pm 0.03 e	0.83 \pm 0.06 de	8.4 \pm 0.1 f	69.2 \pm 1.9 b	−73.1 \pm 0.3 cd	−0.7 \pm 0.1 bc
<i>Prosopis juliflora</i> (Sw.) DC. [13]	Mesquite	Fabaceae	10	0.04 \pm 0.01 e	0.07 \pm 0.02 e	8.6 \pm 0.1 f	22.2 \pm 0.7 i	−90.0 \pm 4.3 d	−1.8 \pm 0.1 c
<i>Spondias tuberosa</i> Arruda [14]	Umbu plant	Anacardiaceae	2	1.38 \pm 0.18 c	2.44 \pm 0.32 c	11.0 \pm 0.2 e	46.0 \pm 3.5 fg	−71.5 \pm 0.4 cd	−0.7 \pm 0.1 bc
<i>Glycine max</i> (Gm)	Soybean	Fabaceae	10	0.46 \pm 0.08 d	0.97 \pm 0.09 de	5.83 \pm 0.12 g	33.3 \pm 0.8 gh	−85.3 \pm 1.7 e	−0.5 \pm 0.0 ab
<i>Zea mays</i> (Zm)	Maize	Poaceae	10	0.15 \pm 0.04 d	2.36 \pm 0.17 c	12.84 \pm 0.96 d	32.4 \pm 0.2 gh	−89.3 \pm 1.8 f	−1.6 \pm 0.4 c

2.2. Relative Imbibition

Comparing the 12 h imbibition with the 120 h imbibition results, it can be observed that most species displayed an increase in water uptake over time (Table 2). *M. glabra* demonstrated the highest relative imbibition at both 12 h and 120 h with values of 150% and 320%, respectively, showcasing its sustained and efficient water absorption capacity. *C. procera* also exhibited a notable increase in imbibition from 100% at 12 h to 150% at 120 h. Similarly, several other species such as *A. hypogaea*, *C. maxima*, *G. hirsutum*, *H. annuus*, *J. curcas*, *G. max*, and *Z. mays* showed an increased imbibition at 120 h compared to the 12 h imbibition levels (Table 2). *E. oleracea*, *A. squamosa*, *L. rigida*, *A. blanchetii*, *S. tuberosa*, and *P. juliflora* also displayed some increase in imbibition at 120 h, although the values remained relatively low compared to other species. *M. oleifera* showed an increase from 90% to 120% at 120 h, while the imbibition potential of *Z. mays* significantly increased from 30% to 130% at 120 h.

Table 2. Imbibition relative measured at 12 and 120 h after the start of imbibition that was measured in 14 alternative oilseed species, plus the control as soybean and maize. Each value denotes mean \pm SE. Each value, followed by different lowercase letters, denotes a significant difference at 12 h and when followed by uppercase letters, values denote a significant difference at 120 h. Asterisks (*) denote a statistical difference between 12 and 120 h.

Species	Imbibition Relative (12 h)	Imbibition Relative (120 h)	
<i>A. blanchetii</i>	30.43 \pm 2.05 g	56.87 \pm 2.60	G *
<i>A. squamosa</i>	16.32 \pm 1.56 h	37.16 \pm 5.23	H *
<i>A. hypogaea</i>	52.77 \pm 5.02 f	55.92 \pm 2.36	G
<i>C. procera</i>	109.95 \pm 7.85 b	158.75 \pm 13.64	B *
<i>C. maxima</i>	71.36 \pm 8.64 d	89.63 \pm 9.07	E *
<i>E. oleraceae</i>	0.10 \pm 0.20 i	6.66 \pm 6.10	I *
<i>G. hirsutum</i>	72.91 \pm 13.00 d	100.91 \pm 10.41	D *
<i>H. annuus</i>	59.93 \pm 10.54 ef	116.00 \pm 14.57	C *
<i>J. curcas</i>	63.90 \pm 2.49 de	74.96 \pm 2.41	F
<i>L. rigida</i>	16.12 \pm 1.26 h	41.20 \pm 7.71	H *
<i>M. glabra</i>	275.89 \pm 21.80 a	319.79 \pm 17.04	A *
<i>M. oleifera</i>	99.56 \pm 5.73 c	125.14 \pm 8.03	C *
<i>P. juliflora</i>	25.83 \pm 8.40 g	89.25 \pm 23.41	E *
<i>S. lutea</i>	61.93 \pm 3.06 ef	77.15 \pm 7.63	F *
<i>G. max</i>	38.61 \pm 0.00 ef	89.41 \pm 0.00	F *
<i>Z. mays</i>	73.28 \pm 0.00 g	116.37 \pm 0.00	C *

2.3. Integument Hardness

The integument hardness (Table 3) of this study's species exhibits significant variation, with mean values ranging from 21.96 \pm 1.17 N to values exceeding the maximum detection limit of the measurement equipment, which is 200 N. Specifically, the integument hardness of the species *E. oleraceae*, *P. juliflora*, and *S. tuberosa* could not be accurately measured, but it is known to surpass 200 N. To facilitate comprehension, the hardness values were normalized relative to the function of these three species, which were assigned a hardness value of 100%. Subsequently, the integument hardness values of the species *A. hypogaea* (10.98 \pm 0.59 N), *M. oleifera* (19.06 \pm 0.89 N), *M. glabra* (21.53 \pm 0.82 N), *C. maxima* (22.81 \pm 0.98 N), *J. curcas* (24.90 \pm 0.62 N), *G. max* (19.05 \pm 0.87 N), and *H. annuus* (26.84 \pm 0.62 N) were found to fall within a range of up to 30% of the maximum limit of the measurement equipment or the hardness of the integument of the aforementioned three species.

Table 3. Integument hardness, soluble carbohydrates, starch, amino acids, and soluble proteins measured in 14 alternative oilseed species, plus the control as soybean and maize. In each feature, the different lowercase letters denote significance between means. Each value denotes the mean (\pm SE).

	Integument Hardness ¹	Soluble Carbohydrates ²	Starch ²	Amino Acids ²	Soluble Proteins ³
<i>A. blanchetti</i>	149.4 \pm 4.7 b	42.9 \pm 1.7 g	201.7 \pm 37.1 bcd	55.1 \pm 6.9 c	89.7 \pm 0.9 c
<i>A. squamosa</i>	164.0 \pm 4.7 a	84.2 \pm 4.9 d	176.4 \pm 27.6 cde	86.3 \pm 10.1 a	101.5 \pm 1.2 b
<i>A. hypogaea</i>	22.0 \pm 1.2 g	61.0 \pm 3.1 ef	229.8 \pm 48.3 bcd	30.2 \pm 3.0 fg	72.2 \pm 1.6 d
<i>C. procera</i>	74.2 \pm 2.2 c	128.7 \pm 3.1 b	375.1 \pm 22.9 a	70.5 \pm 2.7 b	217.4 \pm 3.6 a
<i>C. maxima</i>	45.6 \pm 2.0 def	68.3 \pm 5.8 de	158.2 \pm 35.5 cde	41.2 \pm 4.7 d	34.6 \pm 1.0 g
<i>E. oleaceae</i>	200.0 \pm 0.0	75.6 \pm 1.2 cd	129.1 \pm 21.0 e	19.2 \pm 0.9 h	1.9 \pm 0.2 i
<i>G. hirsutum</i>	68.5 \pm 2.5 c	102.6 \pm 1.1 c	373.5 \pm 52.3 a	25.0 \pm 2.4 g	66.0 \pm 1.2 e
<i>H. annuus</i>	53.7 \pm 1.2 d	94.8 \pm 6.4 c	236.5 \pm 32.4 bc	43.9 \pm 2.8 d	74.1 \pm 2.8 c
<i>J. curcas</i>	49.8 \pm 2.0 de	218.9 \pm 7.6 a	150.1 \pm 13.5 de	33.4 \pm 1.8 f	101.5 \pm 1.8 b
<i>L. rigida</i>	68.2 \pm 2.8 c	51.5 \pm 1.4 fg	136.0 \pm 24.4 de	36.3 \pm 3.5 e	10.7 \pm 0.1 h
<i>M. glabra</i>	43.1 \pm 1.6 ef	63.9 \pm 1.4 ef	172.6 \pm 19.6 cde	58.1 \pm 4.6 c	53.7 \pm 2.2 f
<i>M. oleifera</i>	38.1 \pm 1.8 f	99.1 \pm 1.2 b	259.5 \pm 32.4 b	60.8 \pm 1.2 c	30.6 \pm 0.2 g
<i>P. juliflora</i>	200.0 \pm 0.0	58.1 \pm 4.7 ef	217.2 \pm 46.0 cde	40.0 \pm 2.3 d	92.9 \pm 1.3 d
<i>S. lutea</i>	200.0 \pm 0.0	62.9 \pm 2.2 ef	209.5 \pm 33.3 bcd	31.1 \pm 21.1 f	10.2 \pm 0.5 h
<i>G. max</i>	45.9 \pm 1.3 ef	69.0 \pm 2.2 e	202.0 \pm 12.7 bcd	27.0 \pm 3.7 g	82.7 \pm 2.1 d
<i>Z. mays</i>	167.6 \pm 6.2 a	59.0 \pm 4.1 ef	159.0 \pm 12.5 cde	36.0 \pm 3.0 f	112.9 \pm 4.1 b

¹ = N m⁻²; ² = mmol kg⁻¹ DW; ³ = g kg⁻¹ DW.

Furthermore, three other species, namely *L. rigida* (34.10 \pm 1.42 N), *G. hirsutum* (34.27 \pm 1.26 N), and *C. procera* (37.08 \pm 1.10 N), exhibited integument hardness values falling within a range of 30 to 60% relative to the previously mentioned species. Lastly, three additional species, *A. blanchetii* (74.69 \pm 2.37 N), *A. squamosa* (81.98 \pm 2.33 N), and *Z. mays* (82.2 \pm 2.44 N), demonstrated integument hardness values falling within the range of 60 to 90% of the maximum value depicted in the data presented in Table 3.

2.4. Biochemical Analysis

The biochemical analysis among the different plant species revealed intriguing characteristics across various parameters. Species including *M. oleifera*, *A. blanchetii*, *C. procera*, and *J. curcas* exhibited notably higher levels of soluble carbohydrates, with respective content values of 85, 41, 120, and 200 mmol kg⁻¹ DW (Table 3). In contrast, the edible species displayed a relatively lower but varied range of carbohydrate contents, spanning from 45 to 90 mmol kg⁻¹ DW. Noteworthy edible species with higher carbohydrate contents include *G. hirsutum* and *H. annuus*, exhibiting values of 90 and 85 mmol kg⁻¹ DW, while *A. squamosa* and *Z. mays* had the lowest values of 80 and 62 mmol kg⁻¹ DW, respectively. Additionally, soybean and *C. maxima* shared the same value of 70 mmol kg⁻¹ DW. (Table 3). Comparing the starch content, we observed that the non-edible species *A. blanchetii* and *C. procera* exhibited starch levels of 198 and 360 mmol kg⁻¹ DW, respectively (Table 3). Among the edible species, the starch content varied from 120 to 360 mmol kg⁻¹ DW. Notably, the edible species *G. hirsutum*, *H. annuus*, and *A. hypogaea* displayed starch values of, in order, 373 \pm 52, 237 \pm 32, and 230 \pm 48 mmol kg⁻¹ DW, while the non-edible species *C. procera*, *M. oleifera*, and *P. juliflora* had starch values of 375 \pm 23 mmol kg⁻¹ DW, 260 \pm 32 mmol kg⁻¹ DW, and 217 \pm 46 mmol kg⁻¹ DW, respectively. It should be noted that soybean and corn, as an edible reference, show 202 \pm 13 mmol kg⁻¹ DW and 159 \pm 13 mmol kg⁻¹ DW of starch (Table 3).

We observed notable differences among all the species in their amino acid contents. Among the non-edible species, *A. blanchetii* and *C. procera* showed amino acid contents of 50 and 70 mmol kg⁻¹ DW, respectively, while *E. oleracea*, *G. hirsutum*, *J. curcas*, *L. rigida*, and *S. tuberosa* displayed relatively lower amino acid contents ranging from 15 to 30 mmol kg⁻¹ DW (Table 3). To compare, the edible species *H. annuus*, *A. hypogaea*, and *G. hirsutum* displayed values of 43 ± 11 mmol kg⁻¹ DW, 30 ± 11 mmol kg⁻¹ DW, and 23 ± 9 mmol kg⁻¹ DW (Table 3), respectively.

The results reveal the soluble protein content for each plant species. These values range from 10 to 210 g kg⁻¹ DW (Table 3). *C. procera* demonstrated the highest soluble protein content at 217 ± 3.6 g kg⁻¹ DW, followed by *J. curcas* (102.5 ± 1.8 g kg⁻¹ DW), *A. squamosa* (101.5 ± 1.2 g kg⁻¹ DW), and *P. juliflora* (92.9 ± 1.3 g kg⁻¹ DW); all of these are non-edible species. On the other hand, *E. oleracea*, *L. rigida*, and *S. tuberosa* exhibited the lowest soluble protein content at 10, 20, and 20 g kg⁻¹ DW, respectively (Table 3). Additionally, *G. max* and *Z. mays* contributed to these findings, with soybean having a soluble protein content of 60 g kg⁻¹ DW and *Z. mays* with a content of 20 g kg⁻¹ DW. The results in (Table 4) show the total protein content of each plant species. The values range from 2% to 40%. *A. hypogaea*, *C. maxima*, and *C. procera* exhibited relatively higher total protein contents at 40%, followed by *P. juliflora* (34.5%), *M. oleifera* (29.6%), and *J. curcas* (25.8%), all non-edible species. Among the edible species, *G. max* (34%) and *G. hirsutum* (23%) gave emphasis. On the other hand, *E. oleracea*, *L. rigida*, *S. tuberosa*, and *Z. mays* had lower protein contents at 5% or less. The remaining species displayed total protein contents ranging from 15% to 27% (Table 4).

Table 4. Total proteins, fibers, ash, oil, and long-chain polyunsaturated fatty acids (LC-PUFAs) measured in 14 alternative oilseed species, plus the control as soybean and maize. In each feature, different lowercase letters denote a significance between the means. Each value denotes the mean (±SE).

	Total Proteins (%)	Fibers (%)	Ash (%)	Oil (%)	LC-PUFAs (%)
<i>A. blanchetii</i>	21.9 ± 0.8 f	20.6 ± 1.0 c	2.8 ± 0.1 g	13.4 ± 0.2 f	78.1 ± 0.8 a
<i>A. squamosa</i>	16.1 ± 0.8 g	28.5 ± 0.5 a	1.8 ± 0.1 i	20.0 ± 0.5 e	74.8 ± 0.0 ab
<i>A. hypogaea</i>	38.4 ± 1.9 a	2.4 ± 0.2 i	2.3 ± 0.2 h	50.5 ± 1.3 a	80.1 ± 0.3 a
<i>C. procera</i>	31.8 ± 3.2 c	16.0 ± 0.6 de	4.3 ± 0.2 c	12.8 ± 0.2 f	70.4 ± 3.1 bc
<i>C. maxima</i>	38.3 ± 1.2 a	15.3 ± 1.2 def	4.3 ± 0.1 c	34.8 ± 1.0 c	74.2 ± 0.1 ab
<i>E. oleraceae</i>	5.7 ± 0.4 i	10.2 ± 0.7 g	1.5 ± 0.1 j	0.7 ± 0.1 h	50.5 ± 1.0 e
<i>G. hirsutum</i>	23.1 ± 1.1 f	16.1 ± 1.5 de	4.6 ± 0.2 b	19.6 ± 1.2 e	76.7 ± 0.5 a
<i>H. annuus</i>	26.1 ± 3.4 e	12.9 ± 0.5 efg	3.0 ± 0.1 f	43.1 ± 0.2 b	80.3 ± 0.4 a
<i>J. curcas</i>	25.8 ± 0.5 e	12.6 ± 0.5 fg	4.7 ± 0.1 a	50.2 ± 1.0 a	61.0 ± 3.1 d
<i>L. rigida</i>	6.2 ± 0.4 i	11.2 ± 0.9 g	1.9 ± 0.1 i	5.5 ± 0.2 g	31.6 ± 0.4 g
<i>M. glabra</i>	14.4 ± 0.6 h	17.1 ± 1.1 d	1.4 ± 0.2 j	2.2 ± 0.2 h	41.3 ± 3.0 f
<i>M. oleifera</i>	29.6 ± 0.8 d	19.8 ± 0.9 c	3.5 ± 0.2 e	30.1 ± 0.8 d	81.0 ± 0.5 a
<i>P. juliflora</i>	34.5 ± 4.4 b	7.2 ± 0.3 h	3.6 ± 0.4 d	1.4 ± 0.1 h	69.1 ± 0.2 bc
<i>S. lutea</i>	1.4 ± 0.4 j	24.2 ± 1.1 b	1.1 ± 0.1 k	0.6 ± 0.1 h	66.5 ± 1.1 c
<i>G. max</i>	34.0 ± 2.8 b	15.4 ± 2.0 de	5.0 ± 0.6 a	21.6 ± 1.0 e	85.2 ± 3.5 a
<i>Z. mays</i>	7.9 ± 0.0 i	9.6 ± 0.3 gh	1.9 ± 0.1 i	3.9 ± 0.1 gh	87.3 ± 3.0 a

The results indicate the fiber content of each plant species as a percentage. The fiber content ranges from 5% to 28%. Notable findings include *A. squamosa* with a high fiber content of 28%, followed by *S. tuberosa* (25%), *A. blanchetii* (21%), *M. oleifera* (19.8%), and *C. procera* (16%). Edible species like *A. hypogaea*, *C. maxima*, *G. max*, and *G. hirsutum* had relatively lower fiber contents at 5–15%. Other species such as *E. oleracea*, *H. annuus*, *J. curcas*, *L. rigida*, *M. glabra*, *Z. mays*, and *P. juliflora* fell within the range of 10–18% (Table 4).

The ash content ranged from 1.2% to 5%, with the following falling order: *J. curcas* (4.7%), *G. max* (5.1%), *G. hirsutum* (4.6%), and *C. procera* (4.3%). *C. maxima* and *H. annuus* also exhibited relatively high ash contents at 4% and 3%, respectively. The remaining species fell within the range of 1.2% to 3.5% (Table 4).

The oil content ranged from 0.6% to 51% (Table 4). Notable findings include the contents of *A. hypogaea* (50.5%), *J. curcas* (50.2%), and *H. annuus* (43.1%). In this category, *J. curcas* should be underlined. Other non-edible species like *C. maxima* (34.8%), *M. oleifera* (30.1%), and *A. squamosa* (20%) also must be underlined. Among the edible species, *G. max* (21.6%), *G. hirsutum* (19.6%), and *Z. mays* (3.9%) should be highlighted. On the other hand, several species such as *E. oleracea*, *L. rigida*, *M. glabra*, *P. juliflora*, and *S. tuberosa* had very low oil contents, ranging from 0.3% to 0.5% (Table 4).

LC-PUFA is the best oil for biofuel, ranging from 35% to 80% (Table 4). Several species, including *Z. mays* (87.3%), *G. max* (85.2%), *H. annuus* (80.3%), *A. hypogaea* (80.1%), and *G. hirsutum* (76.7%), presented higher LC-PUFA contents. However, many other non-edible species like *M. oleifera* (81%), *A. blanchetii* (78.1%), *A. squamosa* (74.8%), *C. maxima* (74.2%), *P. juliflora* (69.1%), *S. tuberosa* (66.5%), and *J. curcas* (61%) must be highlighted.

2.5. Fatty Acid Profiling of Different Oilseed Species

The statistical analysis of the fatty acid profiling revealed that the composition of various fatty acids varied significantly among the plant species (Table 5; Supplementary Figures S1 and S2). *M. glabra* stands out with a higher concentration of palmitic acid C16:0 (27.9%), compared to *L. rigida* (26.1%), *G. hirsutum* (24.5%), *E. oleracea* (19.9%), *S. tuberosa* (19.7%), *J. curcas* (19.1%), and *C. procera* (17.1%). Other species ranged from 10% to 15% (*C. maxima*, 15.6%; *A. blanchetii*, 14.2%; *P. juliflora*, 14.1%; *A. squamosa*, 12.9%; and *A. hypogaea*, 10.5%). Among LC-PUFA (higher C18), we emphasize the oleic acid (C18:1) present in *M. oleifera* with a value of $73.5 \pm 0.6\%$, followed by *A. blanchetii* (61.6%), *A. squamosa* (50.3%), *S. tuberosa* (35.8%), *C. procera* (34.8%), and *P. juliflora* (32.3%); all of these are non-edible species. Among the edible species, we underline *A. hypogaea* (48.9%), *Z. mays* (35.7%), *G. hirsutum* (24.1%), and *H. annuus* (23.6%). The highest concentration of linoleic acid (C18:2) was present in *G. max* (59.1%), *H. annuus* (56.7%), and *G. hirsutum* (53.1%) with respect to the edible species. Among the non-edible species, the highest concentration of C18:2 was *C. maxima* (57.1%), *P. juliflora* (36%), *C. procera* (32.9%), *S. tuberosa* (31.6%), and *J. curcas* (29.1%). The alternative species *M. oleifera*, *C. procera*, *L. rigida*, and *E. oleracea* are highlighted with concentrations of linolenic acid of 3.6%, 3.6%, and 2.2%, respectively. Only two species presented a significant concentration of eicosenoic acid (C20:1), with *A. hypogaea* (1.4%) as a representant of the edible species and *M. oleifera* (2.5%) as a representant of the non-edible species.

Table 5. Fatty acid (FA) measured in fourteen oilseed species listed in Table 1 plus the control as soybean and maize. Each FA was measured as a percentage of the total FA. The mean differences within the columns are represented by the lowercase letters (SNK, $p \leq 0.05$). Each point represents the mean (\pm SD) of 10 repetitions. *Glycine max* (Gm) and *Zea mays* (Zm) were included as an edible and common biodiesel oilseed.

Species Number	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C24: 0	Others
[1]	n.d.	n.d.	14.2 \pm 1.0 ef	n.d.	4.7 \pm 0.1 fg	61.6 \pm 2.0 b	16.6 \pm 1.2 h	<1%	1.1 \pm 0.1 f	<1%	<1%	<1%	n.d.
[2]	n.d.	n.d.	12.9 \pm 0.3 f	n.d.	11.4 \pm 0.1 d	50.3 \pm 0.1 c	24.5 \pm 0.1 h	n.d.	0.9 \pm 0.1 g	n.d.	n.d.	n.d.	n.d.
[3]	n.d.	n.d.	10.5 \pm 0.4 g	n.d.	2.8 \pm 0.1 h	48.9 \pm 0.6 c	30.3 \pm 0.8 f	<1%	1.3 \pm 0.1 e	1.4 \pm 0.1 b	2.9 \pm 0.1 c	1.8 \pm 0.3 a	n.d.
[4]	n.d.	<1%	17.1 \pm 1.2 cd	1.7 \pm 0.9 a	8.8 \pm 0.6 e	34.8 \pm 3.6 d	32.9 \pm 4.5 e	3.6 \pm 1.1 a	<1%	<1%	<1%	<1%	n.d.
[5]	n.d.	n.d.	15.6 \pm 0.1 de	n.d.	9.8 \pm 0.2 e	17.0 \pm 0.2 g	57.1 \pm 0.3 a	<1%	<1%	n.d.	n.d.	n.d.	n.d.
[6]	7.1 \pm 1.4	16.9 \pm 2.3	19.9 \pm 2.3 b	n.d.	2.6 \pm 1.0 hi	23.9 \pm 2.2 f	26.8 \pm 2.4 g	1.9 \pm 0.1 b	n.d.	n.d.	n.d.	n.d.	n.d.
[7]	n.d.	<1%	24.5 \pm 0.3 a	<1%	2.4 \pm 0.1 i	24.1 \pm 0.6 f	53.1 \pm 1.5 c	<1%	n.d.	n.d.	1.1 \pm 0.1 d	n.d.	1.6 \pm 0.1 b
[8]	n.d.	n.d.	7.0 \pm 0.4 h	n.d.	3.5 \pm 0.2 fgh	23.6 \pm 0.6 f	56.7 \pm 0.6 b	<1%	<1%	n.d.	<1%	<1%	n.d.
[9]	n.d.	n.d.	19.1 \pm 4.4 bc	0.6 \pm 0.1 c	9.2 \pm 0.5 e	25.1 \pm 0.7 f	29.1 \pm 5.1 f	<1%	1.9 \pm 0.2 c	n.d.	3.9 \pm 0.1 b	n.d.	n.d.
[10]	n.d.	n.d.	26.1 \pm 0.8 a	n.d.	30.1 \pm 0.9 a	23.5 \pm 0.1 f	5.9 \pm 1.1 j	2.2 \pm 0.1 b	n.d.	n.d.	n.d.	n.d.	1.98 \pm 0.1 a
[11]	n.d.	<1%	27.9 \pm 6.4 a	<1%	15.6 \pm 4.1 c	23.9 \pm 9.2 f	17.8 \pm 4.7 h	<1%	1.5 \pm 0.4 d	<1%	<1%	<1%	n.d.
[12]	n.d.	n.d.	5.5 \pm 0.1 h	1.0 \pm 0.1 b	4.0 \pm 0.5 fg	73.5 \pm 0.6 a	1.1 \pm 0.2 k	3.6 \pm 0.4 a	2.5 \pm 0.2 b	2.5 \pm 0.1 a	5.4 \pm 0.2 a	0.9 \pm 0.1 b	n.d.
[13]	n.d.	n.d.	14.1 \pm 0.2 ef	n.d.	7.7 \pm 0.1 f	32.3 \pm 0.5 e	36.0 \pm 0.6 d	1.0 \pm 0.1 c	4.0 \pm 0.1 a	<1%	2.8 \pm 0.1 c	1.5 \pm 0.1 ab	n.d.
[14]	n.d.	n.d.	19.7 \pm 0.4 b	n.d.	11.5 \pm 0.6 c	35.8 \pm 0.9 d	31.6 \pm 1.9 ef	<1%	<1%	n.d.	n.d.	n.d.	n.d.
G. max	n.d.	n.d.	11.5 \pm 0.8 fg	n.d.	22.4 \pm 0.9 b	n.d.	59.10 \pm 0.3 a	3.7 \pm 0.6 a	<1%	n.d.	n.d.	<1%	n.d.
Z. mays	n.d.	n.d.	11.7 \pm 0.6 fg	<1%	1.8 \pm 0.4 i	35.7 \pm 0.2 d	49.2 \pm 0.6 cd	<1%	<1%	n.d.	n.d.	<1%	n.d.

Fatty acid: 12:0 is lauric acid; 14:0 is myristic acid; 14:1 is tetradecenoic acid; 16:0 is palmitic acid; 16:1 is palmitoleic acid; 18:0 is stearic acid; 18:1 is oleic acid; 18:2 is linoleic acid; 18:3 is linolenic acid; 20:0 is arachidic acid; 20:1 is eicosenoic acid; 22:0 is behenic acid; 24:0 is lignoceric acid. n.d is not detected in the chromatographs. Trace values are shown as <1%.

2.6. Principal Component Analysis

The principal component analysis (Figure 1) resulted in a cartesian plane that explains 84.7% of the variations in the data, where PC1 and PC2 explain, respectively, 47.8% and 36.9% of the variations. In this context, three distinct groups were formed according to their similarity at 66%. In group 1, the species *A. squamosa*, *A. blanchetii*, *E. oleraceae*, *S. tuberosa*, and mainly *P. juliflora* are similar to *Z. mays*, and therefore these species have similar characteristics, mainly in oil concentration and LC-PUFAs. Likewise, the species *A. hypogaea*, *C. maxima*, *H. annuus*, and mainly *M. oleifera* and *J. curcas* resemble *G. max*, one of the main oilseeds currently used for biofuels worldwide. Finally, the species *M. glabra*, and mainly *C. procera* and *L. rigida* resemble *G. hirsutum*, another standard oleaginous species. In these terms, we verified that each group was formed by a standard oilseed and other alternative oilseed species. Comparisons were made with all the features, but the most important and weighted features were the percentage of oil and the composition of the LC-PUFAs. Thus, we believe that this study has shown quite firmly that standard oilseeds, used as food sources, can be replaced by other non-edible oilseeds, which in general are easy to grow, are renewable, and have the possibility of producing them in less fertile soils, which would be inappropriate for the cultivation of food species, ultimately helping, to a great extent, food security, since in groups 1, 2, and 3, respectively, we have the species *E. oleraceae* and *P. juliflora* (group 1), *M. oleifera* and *J. curcas* (group 2), and *C. procera* (group 3), all of these resembling their positive control with a high concentration of oil and/or a good concentration of LC-PUFAs.

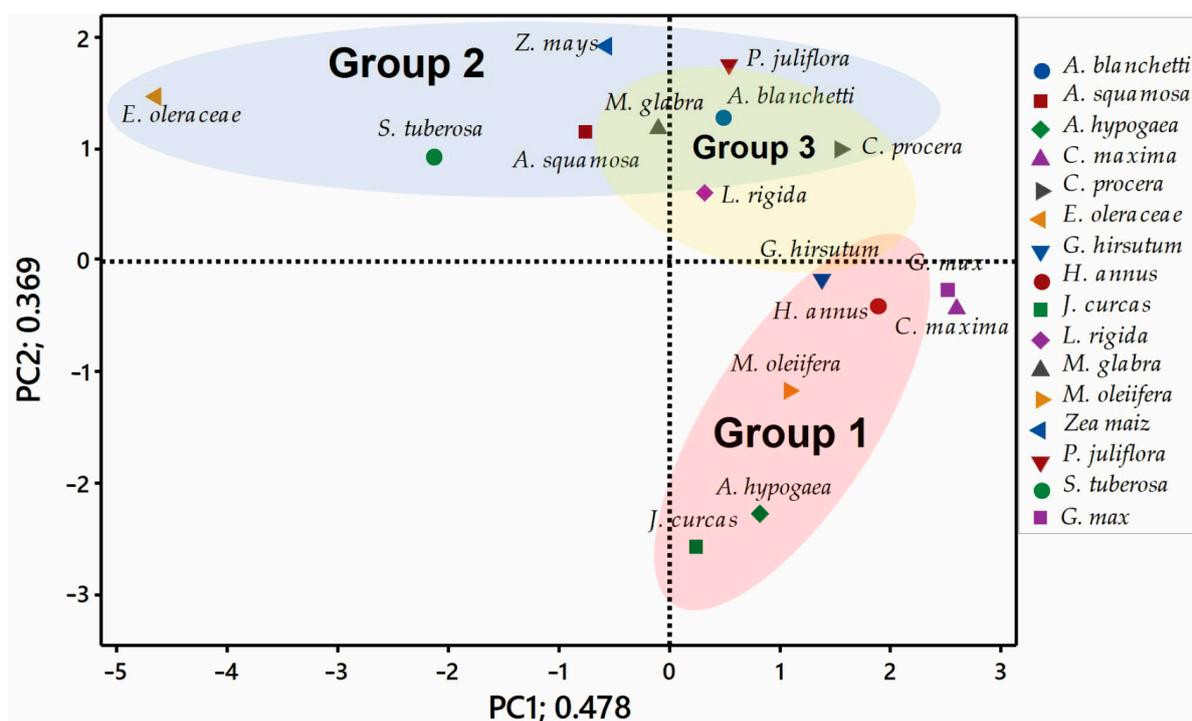


Figure 1. Principal component analysis, a multivariate feature to assess all physiological and biochemical components in each seed species, showing all treatments displayed in PC1 and PC2 to show cluster formations.

The Pearson correlations between all variables measured in this study are shown in Figure 2. It can be seen that the imbibed water is directly proportional to the size of the seed ($r = 0.966$ and 0.993 to $FW_{(i)}$ and $FW_{(f)}$) and that there is a difference in water potential (Ψ_w) between the seed and the external environment ($r = -0.732$ and -0.401 to $\Psi_{w(i)}$ and $\Psi_{w(f)}$). Also, we show that the higher the integument value, the negative its Ψ_w ($r = -0.803$ and -0.675 to $\Psi_{w(i)}$ and $\Psi_{w(f)}$) and the lower its RWC ($r = -0.532$). Integument hardness

was also negatively influenced by the total protein concentration ($r = -0.555$) but not the soluble proteins ($p = 0.595$), and seed hardness is provided by its higher fiber concentration ($r = 0.529$). Regarding the oil content, it was verified that the oil concentration is inversely proportional to the hardness of the tegument ($r = -0.673$) as well as to the water potential, both in the dry seed ($r = 0.612$) and in the imbibed seed ($r = 0.454$). One of the main results of these correlations should be that the oil concentration in the seeds is directly proportional to the LC-PUFA profile ($r = 0.887$) and the LC-PUFAs are inversely proportional to the fresh weight, both in the dry seed ($r = -0.675$) as well in the imbibed seed ($r = -0.605$).

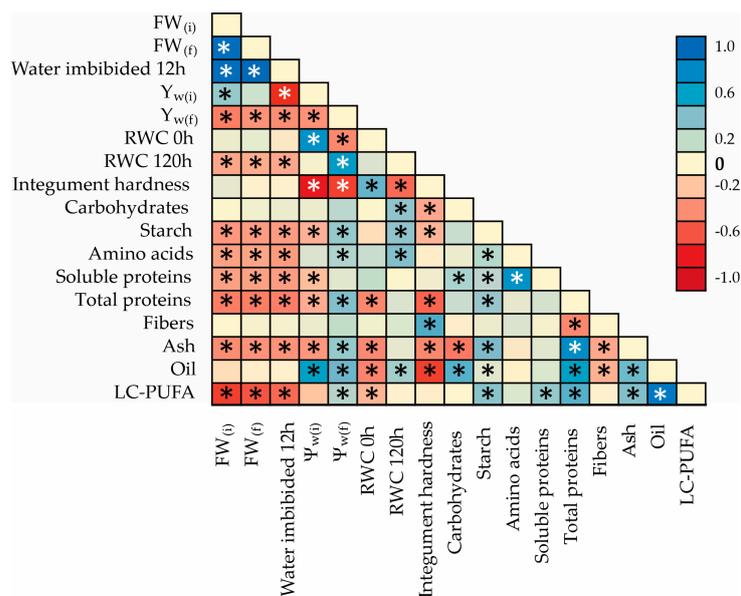


Figure 2. Pearson correlation between all analyzed features. A color degree was performed, and a legend is provided on the right-hand side. Asterisks (*) denote significance at $p \leq 0.05$.

3. Discussion

Water imbibition of seeds plays a crucial role in oil production by triggering germination, activating lipolytic enzymes, mobilizing storage reserves, and facilitating the subsequent extraction of oil from oilseed crops [23]. The triphasic model of water uptake in seeds, as proposed by Baskin and Baskin [24], provides a framework for understanding the different stages of water absorption. Phase I represents the initial rapid uptake of water by the seeds, followed by phase II, where water absorption becomes more established and reaches a plateau. Phase III, also known as the post-germination phase, is characterized by the emergence of the primary root and a significant increase in seed moisture. It should be noted that phase III is only reached by viable seeds.

The relative water content (RWC) measurements provide information on the water retention abilities of the seeds [25]. In this study, species with a higher RWC (69.2%) after the imbibition period, such as *M. oleifera*, demonstrated efficient water absorption and storage capacities. It is important to note that the relative water content of non-conventional oilseeds can vary depending on the specific seed. For example, the RWC of *J. curcas* ranged from 3.3 to 7.7% [26], *Camelina sativa* ranged from 4–6% [27], and 8–10% for *M. oleifera* [28]. During the initial stages of seed imbibition, the movement of water is mainly governed by the matric component, which involves the physical properties of the seed [29]. As water availability increases and seed metabolism becomes more active, the osmotic component's participation in the imbibition process increases [30]. The water potential, a critical measure of a plant's water condition, reflects the balance between water intake, transpiration, and water loss through various pathways [30]. Plants with more negative water potential values, according to the research, are more likely to have greater water absorption [31].

For instance, in our study, species like *A. blanchetii*, *A. squamosa*, *A. hypogaea*, and *C. procera* exhibited lower water potential values (−74.3 to −60 MPa) and a higher RWC (13.3% to 61.6%) after the imbibition period. On the other hand, species like *C. maxima*, *E. oleracea*, and *G. hirsutum* showed relatively less negative water potential values and a moderate seed RWC. Other studies have also reported similar trends. For instance, da Silva, et al. [32] found that *J. curcas* had low water potential values, indicating its ability to absorb and retain water effectively. Babaei and Ajdanian [33] observed similar results in *Cannabis sativa*, where the plant exhibited low water potential values associated with efficient water absorption. Furthermore, Afzal, et al. [28] studied the water potential and water absorption in *M. oleifera* and reported that the species had relatively negative water potential values, suggesting its efficient water absorption capacity. The changes in osmotic potential observed in this study during water imbibition highlight the role of osmotic regulation in facilitating water absorption by oilseeds. The ability of certain species, such as *A. hypogaea*, to significantly increase their RWC and increase their osmotic potential suggests efficient water uptake mechanisms. This characteristic can be advantageous for conventional oilseeds used in biodiesel production, as it indicates their potential to thrive under varying environmental conditions and water availability.

Integument resistance refers to the ability of the seed coat (integument) to withstand mechanical or chemical stress during processing or storage [34]. Bayer and Appel [35] propose that the seeds of *A. squamosa* commonly contain oils.

In contrast, Svoma [36] described the integument of *A. squamosa* as rich in fibers, inhibiting water absorption. Regarding *J. curcas* seeds, these are characterized by a well-defined testa and tegmen. However, it was noted that not all exotesta cells are lignified throughout the seed. The inner portion of the *J. curcas* seed coat contains numerous macrosclereids, which are associated with seed rigidity and waterproofing [37,38]. Interestingly, in *J. curcas*, the macrosclereids found in the exotesta do not act as a physical barrier to water and gas movement [37]. Corte-Real, et al. [37] mention that the *Jatropha* seed's macrosclereids do not restrict the passage of water and gases, a characteristic supported by previous studies [39]. The integument of *J. curcas* seeds is described as having a large concentration of pores, which makes it more fragile and promotes rapid imbibition (Table 2), as also described by Pompelli, et al. [30] and Corte-Real, et al. [37].

In this study, we observed a positive correlation between seed weight and water imbibition, indicating that as seed weight increased, water uptake also increased. In accordance with a previous study [30], various factors are known to influence seed imbibition, and in this context, the hardness of the seed coat, exemplified by species like *E. oleracea*, *P. juliflora*, and *S. tuberosa*, is an important consideration. The integrity and hardness of the seed coat can significantly impact water imbibition. Additionally, the composition of seed reserves plays a crucial role, particularly in terms of water imbibition capacity. Seeds with reserves rich in carbohydrates and proteins tend to imbibe more water compared to oilseed species [40,41].

Furthermore, the osmotic potential, in conjunction with the seed surface in contact with water, the integument hardness, and the biochemical composition of the reserves collectively influence seed imbibition [30]. The osmotic potential of seeds affects the movement of water into the seed. Seeds with a lower osmotic potential exhibit greater water imbibition compared to seeds with a less negative osmotic potential. However, it is important to note that the osmotic potential acts in concert with other factors such as the seed coat's surface properties, integument hardness, and biochemical composition of the reserves. The biochemical composition of the reserves also plays a role in seed imbibition. Starchy seeds tend to absorb more water than protein-rich seeds, and in turn, protein-rich seeds typically exhibit a higher imbibition rate than oilseed species. These findings indicate that the composition of seed reserves, including the presence of carbohydrates, proteins, or oils, has a significant impact on the water imbibition capacity of seeds. Soluble proteins and total proteins in non-conventional oilseeds can impact biodiesel production by influencing enzymatic hydrolysis, oil extraction, and the biodiesel quality [18]. Proteins

can act as catalyst poisons during the transesterification reaction, which is the process of converting the extracted oil or free fatty acids into biodiesel [42]. In our study, species like *C. procera* showcased a significantly higher soluble protein content compared to the edible species. This indicates that non-edible oilseeds have the potential for co-product utilizations, where both biodiesel and protein-rich by-products could be generated simultaneously [18]. However, achieving the simultaneous production of biodiesel and protein-rich products requires specialized processing techniques and optimizations. The biorefinery approach aims to extract oil and valuable components like proteins from oilseeds to maximize their utilization [43]. Recent advancements in synchronous waste mitigation with energy developments focus on generating biofuels and co-products, such as protein-rich residues, from various feedstocks [44]. These oil seeds have specific qualities that make them suitable for animal feeds as well. When we extract oil from the seeds using pressing, we obtain a by-product called oil cake. When we use solvent extractions, we obtain a by-product called oil meal [45]. These by-products contain important components like carbohydrates, proteins, minerals, fiber, and some fats [46]. The cakes produced from edible oil seeds are called edible oil cakes and have a high protein content ranging from 15% to 50% [47]. They are commonly used as animal feed [46]. On the other hand, the oil cakes from non-edible seeds that cannot be used as animal feed due to toxic compounds and impurities are called non-edible oil cakes [45]. Non-edible oil cakes like the *Azadirachta indica*, *Ricinus communis*, *Madhuca longifolia*, and *Millettia pinnata* cakes are often used as organic fertilizers because they contain nitrogen, phosphorus, and potassium [48].

Globally, non-conventional oilseeds have received considerable attention from researchers due to their high oil content and fatty acid composition that can be used as potential feedstocks for biodiesel production [49]. In this study, the highest oil content of 50% was determined in *J. curcas*, which is comparable to the oil content of edible species like *A. hypogaea* (peanut). Additionally, we identified a total protein content of 30–35% in *J. curcas*, *M. oleifera*, and *P. juliflora*, which is similar to the soybean. These characteristics make non-edible species like *J. curcas*, *M. oleifera*, and *P. juliflora* suitable candidates for the production of biodiesel. These results are in line with the work of Pandey, et al. [50] who reported that *J. curcas* seeds have an oil content ranging between 30% and 40%. Similarly, in another study, it was reported that *J. curcas* oil has a crude protein content of approximately 24.60%, a crude fat content of about 47.25%, and a moisture content of around 5.54% [51]. The fatty acid composition of a biodiesel feedstock is an important factor in determining how efficiently it can be converted into biodiesel. The process used to convert the feedstock into biodiesel usually does not change the types of fatty acids present in it. However, the specific types and amounts of fatty acids in non-edible oils can vary depending on the plant species, how the plants were grown, and the method used to extract the oil [8]. The structural parameters of fatty esters, such as the chain length, degree of unsaturation, and branching, influence important fuel properties like the viscosity, cold flow, oxidative stability, lubricity, density, heat of combustion, and ignition quality [52]. Hoekman, et al. [53] noted that medium-chain fatty acids, such as lauric acid, or unsaturated ones, such as oleic or linoleic acid, are adequate to improve the cold properties of biodiesel. Therefore, as the content of unsaturated fatty chains increases, the oil becomes more susceptible to oxidation by air, and, as a consequence, the biodiesel stability decreases [54]. Nevertheless, the use of biodiesel with a high cold filter plugging point in countries that experience very low temperatures can result in the formation of wax crystals, gels, and insoluble compounds that may clog up vehicle engines when the fuel passes through the filtration system [55]. Fatty acids are the primary components of biodiesel. Biodiesel typically contains a range of fatty acids, including palmitic acid (C16:0), linoleic acid (C18:2), oleic acid (C18:1), stearic acid (C18:0), and linolenic acid (C18:3) [8]. These fatty acids contribute to the composition and properties of biodiesel and accentuate their significance in lipid metabolism [56]. The analysis of our results suggests that certain non-edible oil seed species possess fatty acid profiles comparable to or even better than those of edible oil seed species commonly used for biodiesel production. For example, the *J. curcas* seed oil exhibited a higher level of

saturated fatty acids at 28% (19.1% palmitic acid and 9% stearic acid), while *L. rigida* showed 56% saturated fatty acids (26.1% palmitic acid and 30.1% stearic acid), and *M. glabra* had 43% saturated fatty acids (27.9% palmitic acid and 15.6% stearic acid). In contrast, soybean had 10.5% palmitic acid and 2.8% stearic acid, while maize contained 11% palmitic acid and 1.8% stearic acid (Table 2). In terms of the unsaturated fatty acids, *G. max* exhibited high amounts of oleic acid (48.9%) and linoleic acid (59.1%), while *Z. mays* had 35.7% oleic acid and 49.2% linoleic acid. Non-edible species like *J. curcas* and *L. rigida* displayed good levels of oleic acid (25.1% and 23.5%, respectively) and linoleic acid (29.1% and 5.9%, respectively). Linoleic acid (18:2) was found to be the most prevalent among the polyunsaturated fatty acids, with soybean (*G. max*) having the highest content of linoleic acid (59.1%) compared to other species. Previous studies [57] reported that *J. curcas* seed oil contains 22.50% saturated fatty acids (16% palmitic acid and 6.5% stearic acid) and 78.7% unsaturated fatty acids (43.5% oleic acid, 34.4% linoleic acid, and 0.8% linolenic acid). Similarly, another study by Sahoo and Das [58] found that the seeds of non-edible species like *P. pinnata* contain 19.2% saturated fatty acids (11.7% palmitic acid and 7.5% stearic acid) and 70.7% unsaturated fatty acids (51.6% oleic acid, 16.5% linoleic acid, and 2.7% linolenic acid). Additionally, Ramadhas, et al. [59] reported that rubber seed oil contains 18.9% saturated fatty acids (10.2% palmitic acid and 8.7% stearic acid) and 80.5% unsaturated fatty acids (24.6% oleic acid, 39.6% linoleic acid, and 16.3% linolenic acid). A higher concentration of saturated fatty acids results in a higher viscosity due to which the performances are poor at low temperatures [60]. Therefore, a higher unsaturated oil ratio is needed to produce biodiesel. The highest ratio was calculated in the case of *H. annuus* (6.72), but the following species had a better unsaturated:saturated ratio than *G. max*: *A. blanchetii*, *A. squamosa*, *A. hypogaea*, *C. procera*, *C. maxima* subsp. *maxima*, and *G. hirsutum*, with highlights of *M. oleifera* and *P. juliflora*.

Biodiesel is produced by transesterifying triglycerides with short-chain alcohols using a catalyst. Catalysts boost reaction rates without being consumed. Homogeneous catalysts function in the same phase as the reaction mixture, while heterogeneous catalysts operate in different phases [5]. So, the heterogeneous catalysis of non-edible oils is preferred in the presence of free fatty acids [61]. The reactants and the catalyst are in different phases (solid, liquid, gaseous, or immiscible liquids), generally with the catalyst in the solid phase and the reactants in the gaseous or liquid phase. The catalysts have good thermal stability and present more active sites, which can improve the selectivity factor, and the recovery of the catalyst is easy and inexpensive. An alternative to a heterogeneous catalytic transesterification of non-edible oils is a biocatalytic process, where enzymes such as lipases isolated from different microorganisms are used to catalyze the reaction [62]. However, many enzymes can be activated or inactivated at specific pH ranges or in the presence of a signal molecule. A non-catalytic transesterification process for biodiesel production from non-edible oils can be conducted using supercritical fluids and co-solvents such as tetrahydrofuran, acetone, isopropanol, or diethyl ether [63]. Generally, the non-catalytic transesterification of non-edible oils is rapid as it requires a relatively lower reaction time compared to other catalytic transesterification processes.

The transesterification of non-edible oils can be conducted using supercritical methanol, with the advantage that the free fatty acid amount does not affect the process and the water content enhances the biodiesel conversion with low energy consumption [64]. Furthermore, the immiscibility of biodiesel and glycerol at room temperature favors a straightforward separation process. The drawbacks of this process are the high operating costs for the harsh reaction conditions; however, the investment can be rewarded with higher productivity.

In this study, non-edible oil seeds, including species like *J. curcas*, *L. rigida*, *M. oleifera*, and *M. glabra*, have shown promising potential for biodiesel production. While these non-edible species may exhibit higher levels of saturated fatty acids, their unique composition can be optimized to achieve desirable fuel properties. Moreover, these non-edible species offer distinct advantages in terms of oil availability and reduced competition with food production. By focusing on the development of efficient extraction and processing

techniques, we can harness the full potential of these non-edible alternatives for sustainable biodiesel production.

4. Materials and Methods

4.1. Seed Species and Processing

The choice of species was carried out following some criteria: (i) the species should be a species of economic or ecological interest; (ii) it should be easily acquired in the market or in Brazilian nature; (iii) it should represent some of the native or foreign species introduced in Brazil; (iv) there should be a lack of studies with these species with these objectives. Table 1 contains all the studied species, as well as their botanical family, common name, and the number of seeds that compose one experimental unit. All commercial fruits (*A. squamosa*, *A. hypogaea*, *C. maxima*, *G. hirsutum*, *H. annuus*, *M. glabra*, and *S. tuberosa*) were freshly acquired from Pernambuco Center and Logistics (located at 8°04'15" S; 34°56'34" W; 7 m.asl.). The Brazilian native fruit species (*A. blanchetii*, *E. oleracea*, *J. curcas*, and *L. rigida*) were collected from the Federal University of Pernambuco (located at 8°03'01" S, 34°56'33" W; 15 m.asl.) or from the Rocha Negra Waterfall, Santarém, PA, Brazil (*E. oleracea*) (located at 2°29'49" S, 54°45'13" W; 87 m.asl.), and the invasive fruit species (*C. procera*, *M. oleifera*, and *P. juliflora*) were collected from the city of Serra Talhada in a natural Brazilian savanna-like ecosystem (7°58'51" S, 38°19'02" W; 418 m.asl.) between March and September 2022. Immediately after the collection, all fruits were pulped, and seeds were manually removed from fruits. All seeds classified as unviable (dark-colored seeds with irregular developments) were discarded. The proper seeds were spread in an absorbent paper and left under the sun in a ventilated place for natural drying till a moisture content of 7.5% was reached (on the basis of fresh weight), which was measured by the loss of water and in an analytical balance (Sartorius Analytical Balance mod. ENTRIS224-1S, Bradford, MA, USA; accurate to 0.1 mg) after determining their dry weight, which was measured after 72 h in 75 °C. After this, all seeds were stored in air-tight plastic containers at 4 °C, as described in Moncaleano-Escandon [65]. All plant scientific names were checked by WFO [66].

4.2. Water Potential, Seed Imbibition, and Integument Hardness

These features were measured as previously described in Pompelli, et al. [31].

4.3. Biochemical Analysis

A total of 3 g of the seeds were promptly immersed in liquid nitrogen and macerated in a fine powder and were stored in a −40 °C ultrafreezer until use.

4.3.1. Soluble Carbohydrates

A total of 50 mg of the seed poll was homogenized with 600 mL of ethanol 80% (Sigma-Aldrich, Darmstadt, Hesse, Germany, part number 493511) in a polypropylene tube (Sigma-Aldrich, part number Z760951). All tubes were mixed and inserted in a thermo-shaker (Multitherm, Benchmark Scientific, Sayreville, NJ, USA) and incubated for 90 min at 70 °C at 500 rpm. Following this, the tubes were centrifuged at 4 °C for 10 min at 15,000× *g*. Next, the supernatant was transferred to a new polypropylene tube. In the pellet, 600 mL of ethanol 80% was added, mixed, and incubated in thermo-shaker for 90 min at 70 °C at 800 rpm. This last procedure was repeated 1 fold more. The pellet was reserved at −40 °C to measure starch, and all supernatants were combined, and the volume was filled up to 2 mL with ethanol 80%. A standard pattern using 1 mm L^{−1} anhydrous glucose (Sigma-Aldrich, part number D9434) was constructed using phenol, sulfuric acid [67]. Both the standard curve and samples were kept in standby for 10 min. After, all tubes, including the standard curve, were used, and 200 μL was disposed in a glass microplate and read in a microvial reader (ThermoScientific™, Multiskan™ GO, Missouri City, TX, USA). The absorbances were measured at 490 nm.

4.3.2. Starch

A total of 1000 mL of ethanol 80% was added to the resulting pellet of the previous reaction. The tubes were mixed and centrifuged at 4 °C for 10 min at 15,000× g. The supernatant was discarded, and the pellet was washed 4 more times with ethanol 80% to remove any sugar residues. After, the pellet was mixed with 800 mL of a 2 M KOH solution. All tubes were mixed and incubated for 120 min at 95 °C at 500 rpm. After, 200 mL of a 1 M glacial acetic acid (Sigma-Aldrich, part number 1.00063) was added to all tubes. After mixing, all tubes were centrifuged at 4 °C for 10 min at 15,000× g. After that, the supernatants were transferred to a new polypropylene tube, and the pellets were discarded. For the reaction, the samples were mixed with 300 mM of a sodium citrate buffer (Sigma-Aldrich, part number P4922) and incubated with amiloglucosidase (Sigma-Aldrich, part number 10113; 2 U per reaction) at 55 °C for 60 min. After mixing, all tubes were centrifuged at 4 °C for 10 min at 15,000× g. All supernatant and standard curve tubes were kept in standby for 10 min. After, all tubes, including the standard curve, were used, and 200 µL was disposed in a glass microplate and read in a microvial reader at 490 nm.

4.3.3. Amino Acids

A total of 50 mL of the same extract described in Section 4.3.1 was mixed with 2 mL of bi-distilled water plus 1.5 mL of a 2% ninhydrin Reagent (Sigma-Aldrich, part number N7285). After mixing, all tubes were incubated for 20 min at 100 °C. After, all tubes were chilled in an ice bath. More 8 mL of ethanol was added to all tubes. After mixing, all tubes were kept in standby for 10 min. After, all tubes, including the standard curve, were used, and 200 µL was disposed in a glass microplate and read in a microvial reader at 570 nm.

4.3.4. Soluble Proteins

The previously reserved pellet described in Section 4.3.2 was washed as described before and mixed with 800 µL of 0.1 M NaOH. All tubes were mixed and incubated for 60 min at 95 °C at 800 rpm. Subsequently, the tubes were centrifuged for 5 min at 13,000× g at 4 °C. After preparing the BSA standard curve, the samples were prepared according to the manufacturer's instructions (Protein Assay Dye Reagent Concentrate, Bio-Rad, Hercules, CA USA, part number #5000006).

4.3.5. Total Proteins, Ash, Fibers, and Oil

All these components were extracted and measured in accordance with Bradford [68] for the total proteins, and the ash was measured as described in AOAC 923.03/1980 [69]. For the fibers and oils, the procedure described in Barreto and Bezerra Neto [70] was used. To extract the fatty acid and measure its profile, the procedure described in detail in de Araújo Silva, et al. [71] was used, with fatty acids identified by a GC coupled to a mass spectrometer (GC/MS Agilent 6859/5975B) using an Agilent J&W DB-5HT capillary column (30 m × 0.32 mm × 0.10 µm) (Agilent Technologies, Santa Clara, CA, USA) and He as the carrier gas for 1.5 mL min⁻¹. The peaks were identified by comparison with the standard mass spectrum and compounds of the Wiley 229 (Wiley Ford, WV, USA) and NIST 08 [72] databases.

4.4. Experimental Design and Statistical Analysis

The experiments were conducted in a completely randomized design composed of 16 species. All analyzed features were composed of 5 repetitions. All data were processed by a one-way ANOVA in SigmaPlot for Windows v. 14.0 (Systat Software, Inc., San Jose, CA, USA). The principal component analysis was estimated after the multivariate analysis for all analyzed features in Minitab 18.1 (Minitab, Inc., Chicago, IL, USA).

5. Conclusions

In this study, we tried to show the chemical composition of the seeds of edible and non-edible species in order to look for alternative species of biofuels, in addition to soy and corn. We showed that there are many species that can perfectly replace soybean or corn oil in biofuel production. Many non-edible species have very interesting characteristics, such as a high oil and LC-PUFA content. Other edible species have a certain destination for the fruit, but not for the seed, as is the case of *A. squamosa*, *C. maxima*, *E. oleracea*, *G. hirsutum*, *M. glabra*, and *S. tuberosa*, so that the production chain can be expanded with the full use of the fruit and seed and sometimes the residue cake from oil extractions, which can contain high levels of carbohydrates, proteins, and fibers and a low ash content. Among the alternative species studied here, *J. curcas*, *M. oleifera*, *C. procera*, and *P. juliflora* are of great interest as non-edible biofuel species that compete harmoniously with edible fuels. Other studies may result from this research, such as the sustainable use of the cake resulting from oil extractions, as already carried out with *J. curcas* by this team of researchers.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/catal13091263/s1>, Figure S1: Fatty acid profile measured in gas chromatography coupled with mass spectrometer. Each species is written in each chromatograph, Figure S2: Fatty acid profile in *Glycine max* and *Zea mays* as positive pattern in all edible and non-edible oilseed species. The fatty acids were measured in gas chromatography coupled with mass spectrometer.

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