Orbital
The electronic journal of chemistry

the electronic journal of chemistry

Full Paper | http://dx.doi.org/10.17807/orbital.v15i2.18168

Comparative Study of Fatty Acid Composition in Crude Vegetable Oils from Species Cultivated in Mato Grosso do Sul, Brazil

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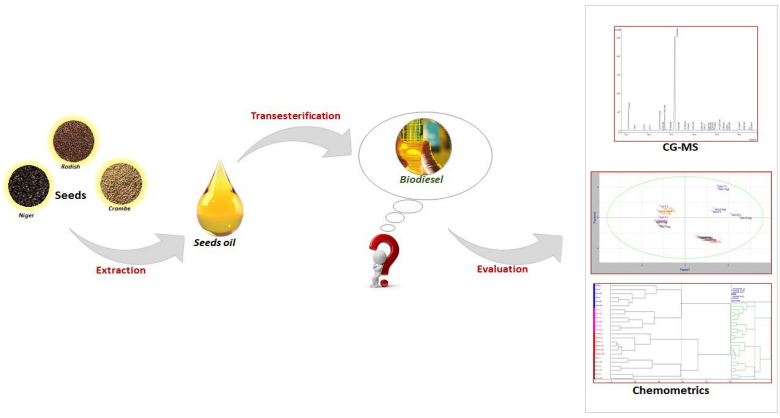
Soybean is the main oilseed cultivated in the Middle-west region of Brazil, once geoclimatic conditions favor its cultivation and underexplored species such as crambe, oilseed radish, and niger too. These species can be alternatives to eliminate the conflict between food and energy production, so the study aimed to evaluate and compare the fatty acids content of soybean, crambe, oilseed radish and niger oils by gas chromatography with flame ionization detector using two quantification methods, followed by multivariate statistical analysis to determine the similarity between the selected species and to provide safe information for the best selection and application of these oilseeds. The oil content in crambe, oilseed radish and niger seeds were 44.7% (m/m), 42.45% (m/m) and 28.19% (m/m), respectively. The major fatty acid in niger and soybean oils was linoleic acid (66.9% and 52.07% m/m, respectively), in crambe and oilseed radish was erucic acid (65.29% and 33.54% m/m, respectively). In addition, for saturated fatty acids, the presence is minor (<17%), except for niger seeds with 19.5% (m/m). Niger oil has greater similarity with soybean oil, whereas crambe and oilseed radish oils are more similar to each other. In this way, it was possible to discriminate the different fatty acids present in the oil samples by the proposed method contributing to the objective of the study and enabling better decision-making in the context like biofuels production.

Graphical abstract



Keywords

Chemometrics
fatty acids
GC-FID
vegetable oil

Article history

Received 10 Mar 2023
Revised 19 Jun 2023
Accepted 30 Jun 2023
Available online 14 Jul 2023

Handling Editor: Adilson Beatriz

1. Introduction

Contemporary Brazilian agriculture is a modern, prosperous and highly competitive division, having been, from

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the beginning of colonization to the 21st century, a driving force of the national economy. Among its various departments, soy and its derivatives are some of the most important, being one of the pillars of the Brazilian economy. Exports originating from the soy agro-industry complex reached an approximate value of US$ 61 billion in 2022, which represented approximately 38.3% of national agribusiness exports. For the year of 2023, the data counted until April revealed that exports reached the approximate value of US$ 9 billion and represented 60.3% of agribusiness exports carried out in the year [1].

The states of the Middle-west region play a fundamental role in the production of cereals, legumes and oilseeds, contributing 12.2% of the national production in 2022 and with a positive contribution of 12.6% until April of 2023. Soy stands out as the main Brazilian crop due to its economic return to the rural producer. The estimated production for the 2023 harvest was approximately 149.1 millions tons of soy [2] in April, which is the main raw material used to produce vegetable oil and biodiesel.

The soybean crop can cause appreciable fluctuations in the Brazilian economy, since this oilseed is an important agricultural commodity and its price is fixed by the Chicago Board of Trade (CBOT), and daily changes in price may happen due to the influence of several factors, from variations in supply and demand to climate and political-economic issues. This makes its use as the main matrix in the production of vegetable oil and/or biodiesel quite vulnerable [3].

In view of possible financial fluctuation faced by the Brazilian economy about the market price of soybeans, the search for alternative oleaginous species to produce vegetable oil and biodiesel has become of crucial for the national economy, especially for those crops that are tolerant to the autumn-winter period (off-season), since during this season period the large arable areas have their production rhythm reduced. The implantation and/or increase of the production of alternative oilseed species in these seasons can improve the farmer's income and contribute to the strengthening of the crop rotation system. The agricultural succession process is deeply important to possibilitate an interruption in the multiplication process of pests, diseases and weeds [4].

Vegetable oil is preferably consumed in human food, being used in the preparation of numerous types of foods, with its composition consisting mostly of long chain unsaturated fatty acids. Recently, there is a growing interest in using vegetable oil in industrial applications due to its potential to replace petroleum-derived sources with renewable products. Vegetable oils can be converted to biofuels and thus generate energy from their burning. This fact helps to minimize the environmental effects produced by burning fossil fuels [5].

Brazil is the second largest producer of biodiesel in the world with a total production in 2021 of 6.8 billion liters, where 72.1% of this production was obtained from soybean oil [6]. However, other oilseed crops have become attractive because their high content of inedible oils mitigate the conflict between energy and food production in their use [7-8].

The distribution of fatty acids in vegetable oils varies considerably due to the type of raw material as well as in the same type of vegetable oil due to the characteristics of each crop [9]. So, is vital in the study of these oils and biodiesel the determination of the composition for the characterization of the products. The fatty acids act directly in several functions of human metabolism, and it is because of these variations of compounds that it is possible to promote the esterification and use of these as a source of energy [10]. Techniques like gas chromatography (GC) and high performance liquid chromatography (HPLC) with different detection systems (MS and FID) can be applied, once they are effective tools for the analysis of fatty acids and their methyl esters presents in these kind of samples [7,11]. The identification of the peaks obtained in the analyses can be made by comparing the retention times with the fatty acid methyl ester standards peaks (FAME) [12] providing the desired information for characterization.

In the state of Mato Grosso do Sul, in addition to soybeans, underexplored oilseed species such as crambe (*Crambe abyssinica*), oilseed radish (*Raphanus sativus L.*) and niger (*Guizotia abyssinica*) are found.

Crambe is a biomass with low production cost. It does not require new machinery or equipment for its cultivation, being possible to grow it in large areas. It has a short production cycle, is a resistant crop (adaptable to different climates) [13] and has a high potential for generating biofuels, since the seeds contain 35.6 to 42.8% of oil [14]. These factors make crambe attractive, resulting in an increase in its cultivation in Brazil, being an interesting source of renewable energy, even more that it is not conflicting with the food industry [13].

Oilseed radish is another example of an oleaginous crop used to produce biodiesel. Worldwide it is the fourth largest behind soy, rapeseed and cotton. In the country, it is mainly concentrated in the South, Southeast and Middle-West regions, as it has a high tolerance for drought, frost and diseases or pests in soils with high acidity, with cultivation being carried out from April to May [8]. The content of oil in the seeds of this species ranges from 26 to 42% [11], being higher when compared to soybean oil (18 to 20%), standing out for the aforementioned purpose [8].

Niger is a native plant of Africa that grows in temperate and tropical zones and can be successfully cultivated in rotation with wheat or corn. Niger seeds contain 30 to 45% of oil, which is used in food, paint and soap manufacturing. Choosing the sowing time is a practice that allows for better water conditions during the development of the crop. The ideal sowing time is the period that offers suitable climatic conditions for the crop and adverse to the incidence of diseases and pests. Based on the climate historic of the region, sowing periods can be defined in which phase there the greater possibility of adequate temperatures and sufficient water supply during the entire growing season of the crop.

In this paper, the authors present the levels of fatty acids found in vegetable oils obtained from the seeds of three different oilseeds cultivated in the state of Mato Grosso do Sul: crambe (*Crambe abyssinica*), oilseed radish (*Raphanus sativus L.*) and niger (*Guizotia abyssinica*), aiming to contribute with information to list one or more promising species for the production of vegetable oil and biodiesel.

2. Results and Discussion

Extraction yield

The highest oil yield, after the chemical extraction process, was observed for crambe seeds, as indicated in Tab. 1, with a percentage in mass corresponding to 44.77%. This value, when compared to others, demonstrates the efficiency of the extraction process. Studies carried out at the Mato Grosso do Sul Foundation Research Station (2007) in the city of Maracajú-MS created the first Brazilian variety of crambe, the

"FMS Brilhante" which presented oil content ranging from 34 to 38 % (m/m) [15]. Seeds belonging to the same variety cultivated in an experimental field in northern Portugal at 2016, presented an oil content in the ranges of 26 to 29.9% (m/m) obtained by chemical extraction [16]. In crambe seeds, cultivated by the Experimental Institute of Industrial Cultures of Bologna, Italy, the oil content in mass was 35 % [17]. In Australia, samples cultivated in 1996 had an oil content ranging from 35.57 to 42.81 % (m/m) [18], while in Austria, the oil content obtained ranged from 22.6 to 38.4 % by mass [19].

The oilseed radish showed oil yield corresponding to 42.45 % by mass. In previous studies, IPR 116 from Paraná's Agronomic Institute, this specie cultivated in Dourados city, showed an oil content equal to 36.85 % (m/m) [20]. Similar values were obtained in which the lipid content in oilseed radish stayed at 30.7-35.6 % interval, with an average of 33.0% in mass [21].

The oil content obtained from niger seeds was 28.19 % (m/m), it was lower than those described in another studies, like in seeds collected in Indian forests, that the oil content was 30 % by mass [22]. FATIMA *et al*. (2015) [23] compared niger seeds grown in the state of South Dakota (USA), during the 2011 season, with two commercially available seed varieties grown in Ethiopia and India. This study showed that seeds cultivated in the USA had oil content equivalent to 36.66% by mass, while imported seeds had oil content corresponding to the range of 36.52 to 38.20 % (m/m). In another study, 153 niger populations, sampled directly from Ethiopian fields during November and December 2003, had oil content at intervals of 27 to 56% (m/m), with 7% of the populations having more than 50% oil by mass [24]. Seeds obtained from Alfred Galke GmbH (Gittelde, Germany), had an oil content equal to 49.9% by weight of seeds [25].

Table 1. Yield of oil obtained from crambe, oilseed radish and niger seeds.

Seeds	Seeds mass* (g)	Oil mass (g)	Yield (%)
Crambe	133.14	59.61	44.77
Oilseed radish	189.19	80.33	42.45
Niger	131.17	36.97	28.19

* Mass of dried and grounded seeds

Method validation

The limit of detection (LOD) varied over a range that extends from 0.03 to 0.6 mg L^{-1}, while the limit of quantification (LOQ) varied over a range that extends from 0.1 to 1.85 mg L^{-1}. For this step, the injections of analytical standards of fatty acid methyl esters (FAME C4-C24) were performed.

Analytical curves were constructed in a linear range with concentrations from 0.1 to 60 mg L^{-1}, with a minimum number of 5 points, resulting in a correlation coefficient (R) ranging from 0.9965 to 0.9999.

The evaluation of the method precision was obtained through coefficients of variation, CV (%), which presented values in the range of 7.0 to 17.6 %. Accuracy was evaluated by percentage of recovery values ranging from 71.43 to 104.4%. This data is shown in Tab. 2.

Table 2. Recovery Values, Coefficients of Variation (CV) and Relative Areas considered for the calculations.

Analytes	C8:0	C10:0	C12:0	C14:0	C16:0
Average area of Standard Ester	28250	32740	36745	39669	44521
Average Area of Recovered Ester	20180	23594	26592	28644	46502
CV (%)	15.9	17.6	10.8	11.7	7.0
Recovery (%)	71.43	72.05	72.36	72.20	104.4

Where: C8:0 is caprylic acid; C10:0 is capric acid; C12:0 is lauric acid; C14:0 is myristic acid and C16:0 is palmitic acid *Chemical composition of fatty acids*

For niger oil, linoleic (C18:2n6c), palmitic (C16:0), oleic (C18:1n9c), and stearic (C18:0) were the major fatty acids observed. Linoleic acid presented the highest percentage of mass, 66.9 % of the total quantified fatty acid, and an average of 55.2 mg of fatty acid per gram of oil (Fig. 1).

In the analysis of fatty acids present in crambe oil, the major fatty acid found was erucic acid (C22:1n9), corresponding to 65.29 % of the total quantified fatty acids and an average of 33.62 mg of this fatty acid per gram of oil, followed by oleic, linoleic and α-linolenic (C18:3n63) acids, as seen in Fig. 2.

For soybean oil, the major fatty acid found was linoleic (52.07 % and 94.39 mg g^{-1}), followed by oleic (21.95 % and 33.90 mg g^{-1}).

Polyunsaturated fatty acids represent the majority fatty acids in soybean and niger oils (58.03 and 67.52, respectively), with linoleic acid from the ω-6 series being the majority in both oils. For soybean oil, there is also α-linolenic acid from the ω-3 series, which had a significant concentration of 5.71 %.

About to monounsaturated fatty acids, soybean oil has a higher concentration than niger oil (21.95 % and 9.0 %, respectively), with oleic acid being the major fatty acid for this class in both oils. The concentration of this acid was 21.95 % for soybean oil and 7.44 % for niger oil. The chromatogram for this oil sample is shown in Fig. 4.

Saturated fatty acids appear as minorities for most oils studied, except for niger oil, where the concentration of this type of acid reaches 19.5 %. For all the other oils, the concentration of saturated fatty acids did not exceed 17 %.

The fatty acid concentrations, expressed in $mg.g^{-1}$, obtained from oil samples, for all determined substances are shown in Tab. 3.

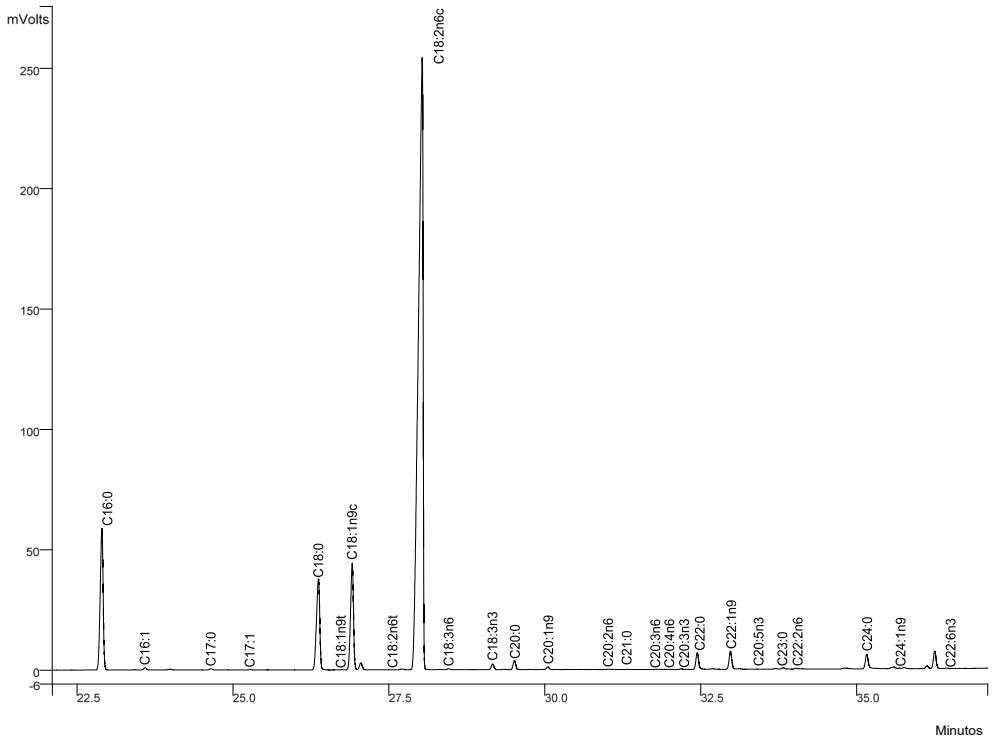

Fig. 1. Chromatogram obtained for the Niger oil samples.

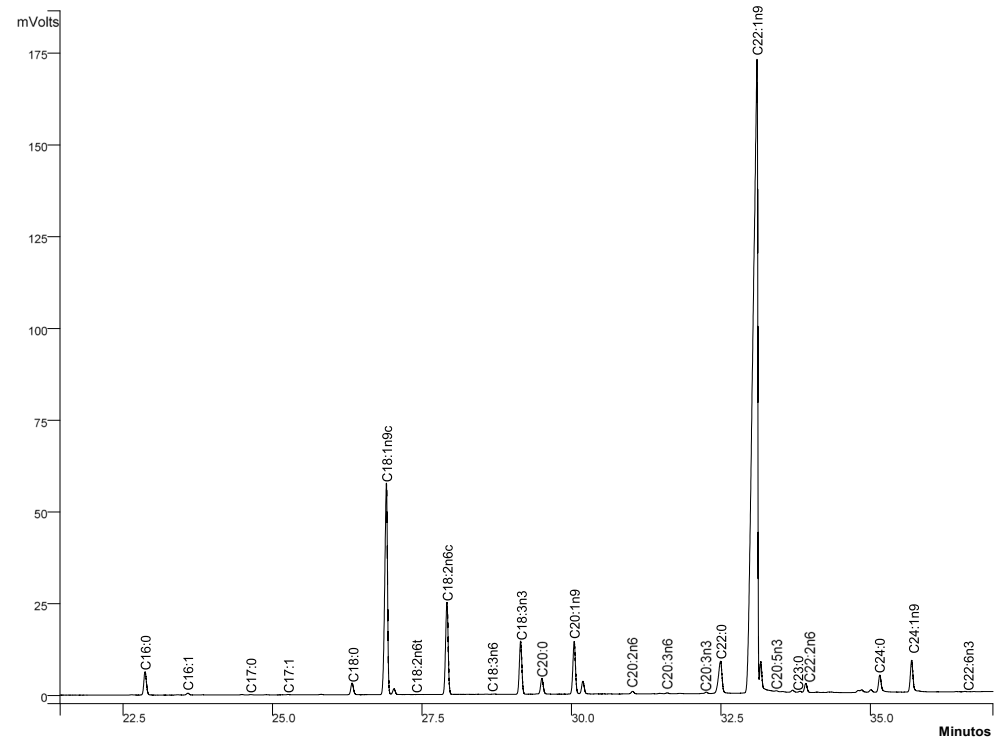

Fig. 2. Chromatogram obtained for crambe oil samples.

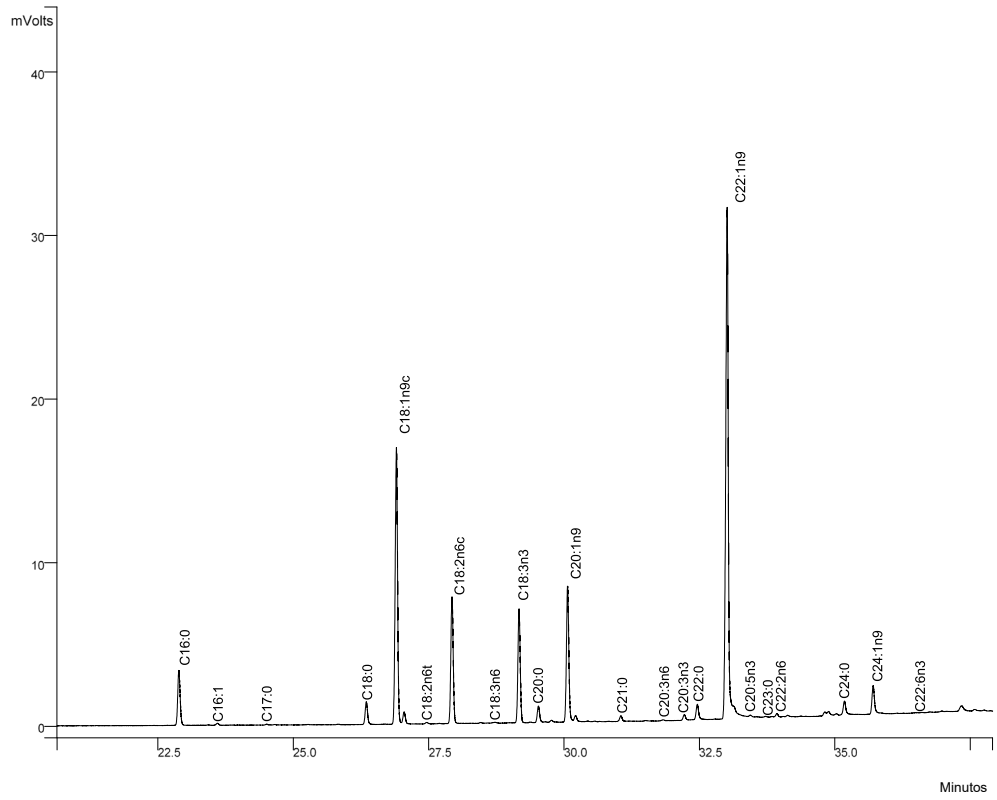

Fig. 3. Chromatogram obtained for the sample of oilseed radish.

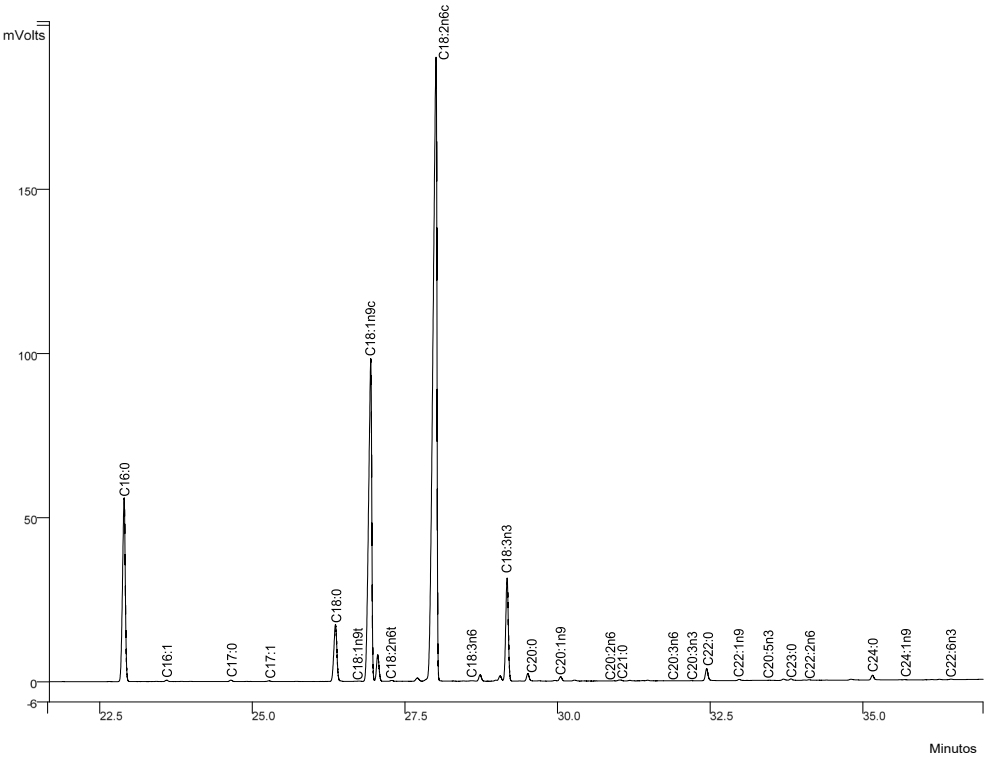

Fig. 4. Chromatogram obtained for the sample of oilseed radish.

Table 3. Concentration of fatty acids expressed in $mg.g^{-1}$ obtained from oil samples.

Compound	Soybean		Niger		Oilseed radish		Crambe	
	Average	% CV	Average	% CV	Average	% CV	Average	% CV
C6:0	nd*	-	$1.407x10^{-2}$	10,73	nd*	-	nd*	-
C8:0	$6.715x10^{-3}$	4.25	$8.656x10^{-2}$	10,67	nd*	-	nd*	-
C12:0	nd*	-	nd*	-	nd*	-	nd*	-
C14:0	$1.479x10^{-1}$	1.16	$4.315x10^{-2}$	15,23	$1.694x10^{-2}$	11.31	$9.389x10^{-2}$	0.79
C15:0	$2.543x10^{-2}$	1.80	$1.894x10^{-2}$	9,63	$4.251x10^{-3}$	2.89	$2.106x10^{-2}$	5.98
C16:0	$23.69x10^{0}$	3.65	$8.86x10^{0}$	12,92	$5.434x10^{0}$	3.54	$8.534x10^{-1}$	11.61
C16:1	$1.552x10^{-1}$	1.03	$1.271x10^{-1}$	11,13	$6.873x10^{-2}$	4.40	$5.892x10^{-2}$	10.75
C17:0	$1.522x10^{-1}$	4.08	$6.528x10^{-2}$	11,63	$8.367x10^{-1}$	4.04	$3.216x10^{-2}$	3.18
C17:1	$7.662x10^{-2}$	1.35	$1.906x10^{-2}$	9,27	$1.940x10^{-2}$	2.41	$1.148x10^{-2}$	13.94
C18:0	$6.681x10^{0}$	1.93	$5.659x10^{0}$	12,47	$1.758x10^{0}$	2.85	$3.703x10^{-1}$	13.59
C18:1n9t	$2.240x10^{-2}$	18.31	$8.810x10^{-3}$	7,69	$6.048x10^{-3}$	13.25	nd*	-
C18:1n9c	$33.90x10^{0}$	1.72	$5.223x10^{0}$	12,91	$19.07x10^{0}$	4.19	$6.099x10^{0}$	13.17
C18:2n6t	$5.064x10^{-3}$	17.31	$5.519x10^{-3}$	7,62	$1.249x10^{-2}$	8.75	$2.607x10^{-3}$	1.72
C18:2n6c	$94.39x10^{0}$	1.70	$55.21x10^{0}$	12,94	$10.71x10^{0}$	2.18	$2.920x10^{0}$	12.82
C18:3n6	$4.416x10^{-2}$	3.33	$4.595x10^{-2}$	5,82	$1.502x10^{-2}$	14.00	$1.052x10^{-2}$	6.83
C18:3n3	$11.77x10^{0}$	11.90	$3.017x10^{-1}$	15,88	$9.684x10^{0}$	0.64	$1.662x10^{0}$	12.39
C20:0	$6.344x10^{-1}$	6.99	$4.074x10^{-1}$	14,55	$1.207x10^{0}$	11.82	$4.632x10^{-1}$	14.56
C20:1n9	$4.066x10^{-1}$	11.07	$1.289x10^{-1}$	12,95	$9.383x10^{0}$	2.68	$1.556x10^{0}$	14.21
C21:0	$7.450x10^{-2}$	3.53	$1.272x10^{-3}$	11,76	$6.496x10^{-2}$	6.56	$4.832x10^{-2}$	14.17
C20:3n6	$2.407x10^{-2}$	18.57	$1.509x10^{-2}$	0,26	$1.144x10^{-2}$	19.47	$3.163x10^{-2}$	13.28
C20:4n6	$7.252x10^{-3}$	1.38	$5.473x10^{-3}$	12,54	nd*	-	nd*	-
C20:3n3	$2.906x10^{-3}$	3.61	$2.628x10^{-2}$	3,67	$4.865x10^{-2}$	14.67	$2.373x10^{-2}$	17.51
C22:0	$1.431x10^{0}$	8.69	$8.882x10^{-1}$	16,63	$3.098x10^{-1}$	0.86	$1.422x10^{0}$	13.96
C22:1n9	$2.245x10^{-2}$	12.08	$8.493x10^{-1}$	14,49	$36.80x10^{0}$	0.40	$33.62x10^{0}$	14.57
C20:5n3	$1.352x10^{-2}$	11.10	nd*	-	$1.987x10^{-2}$	1.46	$2.145x10^{-2}$	5.52
C23:0	$1.566x10^{-1}$	2.51	$7.161x10^{-2}$	10,58	$4.659x10^{-2}$	9.88	$3.021x10^{-2}$	7.21
C22:2n6	$3.642x10^{-2}$	5.75	$1.070x10^{-2}$	18,69	$5.959x10^{-2}$	8.16	$3.018x10^{-1}$	13.93
C24:0	$4.155x10^{-1}$	4.12	$6.859x10^{-1}$	11,19	$9.050x10^{-2}$	9.36	$4.796x10^{-1}$	10.50
C24:1n9	$1.346x10^{-3}$	15.84	$2.751x10^{-2}$	12,83	$4.484x10^{-1}$	6.59	$9.247x10^{-1}$	13.10
C22:6n3	$8.378x10^{-2}$	1.72	$2.799x10^{-2}$	10,53	$8.296x10^{-3}$	3.32	$2.560x10^{-2}$	5.25
Σ Saturated	33.415		16.801		9.768		3.046	
Σ Monounsaturated	34.582		6.383		65.795		42.269	
Σ Polyunsaturated	106.377		55.649		20.569		4.999	

Where: nd* is Not Detected; C6:0 is caproic acid; C8:0 is caprylic acid; C12:0 is lauric acid; C14:0 is myristic acid; C15:0 is pentdecylic acid; C16:0 is palmitic acid; C16:1 is palmitoleic acid; C17:0 is heptadecanoic acid; C17:1 is heptadecenoic acid; C18:0 is stearic acid; C18:1n9t is elaidic acid; C18:1n9c is oleic acid; C18:2n6t is linolelaidic acid; C18:2n6c is linoleic acid; C18:3n6 is γ –linolenic acid; C18:3n3 is α-linolenic acid; C20:0 is arachidic acid; C20:1n9 is gondoic acid; C21:0 is heneicosanoic acid; C20:3n6 is homo-γ-linolenic acid; C20:4n6 is arachidonic acid; C20:3n3 is eicosatrienoic acid; C22:0 is behenic acid; C22:1n9 is erucic acid; C20:5n3 is EPA acid; C23:0 is tricosanoic acid; C22:2n6 is docosadienoic acid; C24:0 is lignoceric acid; C24:1n9 is nervonic acid and C22:6n3 is DHA acid.

Multivariate statistical analysis

Hierarchical cluster analysis (HCA) and principal component analysis (PCA) allow graphical visualization of the entire data set, even when the number of samples and variables is high [25].

Data is previously self-scaled (scaled by variance and centering on the mean) before being subjected to PCA and HCA, once there is a large variation in the concentrations of fatty acids present in oils, in other words, they differ in order of magnitude, attributing by this the same weight to all variables.

On the positive axis PC1 are arranged the groups formed by the oils samples of oilseed radish and crambe, while on the negative axis PC1 are the groups formed by the samples of soybean and niger oils. However, only on the PC2 axis there was a distinction between the sample groups of oilseed radish and crambe oil, which indicates that the niger and soybean oil are more similar to each other (Fig. 5a).

From the analysis of the weight graph (Fig. 5b), it was possible to observe that the most important variable for the samples of soybean and niger oil to assemble them in positive PC1 is the high content of linoleic (C18:2n6c) acid found in these, followed by content of stearic (C18:0), tricosanoic (C23:0) and palmitic (C16:0) acids, which are not the majority, but which are in greater concentration in these oils than those obtained from crambe and oilseed radish. Another variable that contributes to grouping the niger and soybean oil samples in positive PC1 is the caprylic acid (C8:0) content, a fatty acid found only in these samples.

The samples of crambe and oilseed radish oils are similar due to the high content of erucic acid, followed by the content of nervonic acid (C24:1n9), gondoic acid (C20:1n9), arachidic (C20:0) and heneicosanoic acid (C21:0), which have a higher concentration than those found in niger and soybean oils.

It is also observed that the high concentrations of oleic acid in soy samples contribute to assemble the samples of these oils in positive PC2, and this is the variable that has the greatest impact on the distinguish of soybeans from niger oils, as seen in Fig. 5b.

The dendrogram obtained by HCA (Fig. 6) shows the formation of 4 clusters. The oil samples from oilseed radish, crambe, niger, and soybean form a group each. The groups formed by the samples of soybean and niger oils are the most similar, corroborating the PCA data.

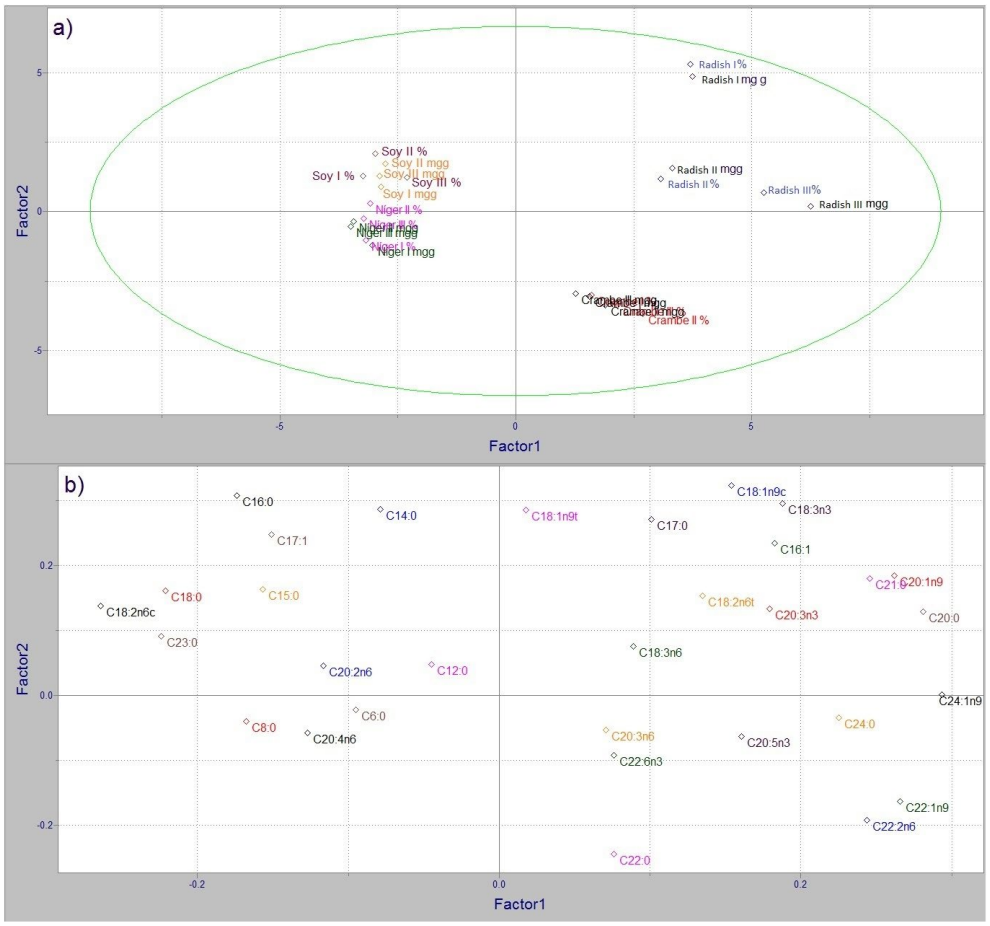

Fig. 5. Graphs of PCA (a) scores and (b) weights obtained from fatty acid concentrations (expressed in mg.g-1 and percentage of mass. PC1xPC2, 34.39 % and 22.16 % of explained variance, respectively).

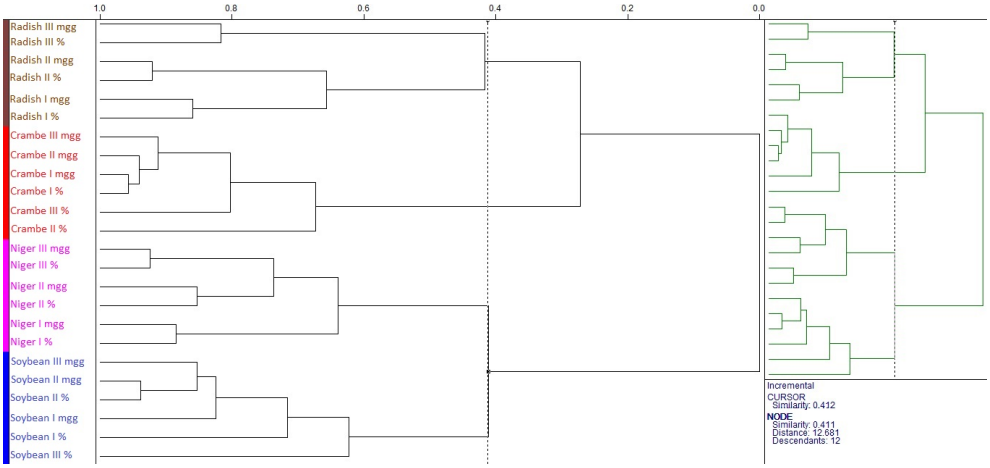

Fig. 6. HCA dendrogram obtained for the studied vegetable oil samples (n=24). Data is auto-scaled, normalized, the incremental connection method was used to measure the Euclidean distance, and the similarity index presented was 0.41.

As observed in PCA, the method of quantification of fatty acids using the official method [28] and the method using calibration curves did not influence the result of the analysis. The samples were assembled regardless of the quantification method used, as shown in Fig. 6.

3. Material and Methods

Reagents and instrumentation

Standard mixture of fatty acid methyl esters, FAME Mix C4-C24, certified reference material, from Supelco. Triglyceride Standard Blend composed of Tricaprin (19.99 mg), Tricaprylin (20.02 mg), Trilaurin (20.00 mg), Trimyristine (19.99 mg) and Tripalmitin (20.00 mg) from Sigma-Aldrich. Magnesium carbonate, sodium hydroxide and n-heptane (99.5%) all ACS grade from Vetec. Hexane and methanol, both HPLC grade, from Spectro-Tedia. Buchi rotary evaporator, model R-3 at reduced pressure, with a vacuum produced by a water tube. Hettich Zentrifugen Rotanta 460 R centrifuge and Sigma centrifuge, model D-37520.

GC-FID and GC-MS conditions

Analyses for quantification were performed using a Varian CP-3800 gas chromatograph with a flame ionization detector (FID), equipped with a injector model CP-8410 (split-splitless). The injected sample volume was 1.0 µL and the sample split was 1:50. For the chromatographic separation, a fused silica capillary column BPX70 with a stationary phase of 70% cyanopropyl polysylphenylene-siloxane, 30 m length, 0.25 mm (i.d.) and 0.25 µm film thickness from SGE was used. Helium (99.9992% purity) was used as carrier gas at a constant flow of 1.0 mL min^{-1}.

Initial oven temperature was 80 ºC (2 min isotherm), raised to 220 ºC with heating at 4 ºC min^{-1} and kept at this temperature for 15 min, with a total time of 52 min. Injector and detector were kept at 200 ºC and 250 ºC, respectively. Qualitative analyses were performed in a gas chromatograph coupled to a mass spectrometer (GC-MS), Varian 3900, equipped with a injector model CP-8410 (split-splitless) and electron impact fragmentation at 70 eV, under the same analyses conditions used in the GC-FID. A fused silica capillary column SLB™-5ms with a stationary phase of 5% phenyl 95% polydimethyl-siloxane, 30 m length, 0.25 mm (i.d.) and 0.25 µm film thickness from Supelco was used.

Samples and sample preparation

An amount of 1 kg of crambe, oilseed radish, and niger seeds were collected from Dourados city (Mato Grosso do Sul, Brazil, Latitude: -22.2235, Longitude: -54.8125). After harvesting, they were stored in paper bags in a dry place, protected from light. Soybean oil samples were purchased from stores in Campo Grande city (Mato Grosso do Sul, Brazil, Latitude: -20.4649, Longitude: -54.6235), and stored in their respective original bottles.

For the extractions, the oilseed radish, niger and crambe seeds were cleaned, removing branches and leaves, then dried in an oven at a temperature of 60 ˚C to remove moisture until reaching a constant mass. Then, they were grounded and inserted in Soxhlet cartridges, prepared with qualitative filter paper, previously leached with hexane, HPLC grade. The extraction of oils was carried out in a Soxhlet apparatus using 500 mL of hexane as the extracting solvent. The extraction time was 9 hours in cycles of 45 min, at a temperature of 65 ˚C.

To remove the extracting solvent, a rotaevaporation step was performed, where the bath was initially kept at room temperature, being increased to 40 ˚C at the end of the process to ensure the complete elimination of the hexane (BP 68.73 °C).

Equation 1 (adapted) [26] was used to calculate the extraction yield, considering grounded and moisture-free seeds.

$$\mathbf{OC} = \frac{\mathbf{M_o}}{\mathbf{BMd}} \times \mathbf{100} \qquad \textbf{(Equation 1)}$$

where: OC is oil content, Mo is oil mass obtained and BMd is Dry biomass.

Subsequently, the oils were degummed and heated at a constant temperature of 70 °C, followed by the addition of 3% by mass of deionized water. The system was kept under agitation and constant temperature for a period of 30 min [17]. To separate the gum, the oil-gum-water mixture was centrifuged at 2000 rpm, for 60 min, at a constant temperature of 70 °C, using the Hettich® ROTANTA 460 R centrifuge. To eliminate water residues from the samples, the oils were filtered with qualitative filter paper 15 cm in diameter and 0.2 mm thick filled with magnesium sulfate ACS grade.

Esterification method and oil extraction

The esterification of the oils was adapted from "ISO 5509 Method- Animal and Vegetable Fats and Oils - Preparation of Methyl Esters of Fatty Acids" [12], which involves basic catalysis. For this, an amount of 0.1 g of oil was weighed and transferred to a screwed tube with 1 ml of n-heptane. Then, 0.05 ml of a 2.0 mol L^{-1} sodium hydroxide solution in methanol media was added to the mixture and stirred for 20 s. A Sigma centrifuge, model 4K 15, at 3500 rpm and constant temperature of 25°C was used for a period of 5 min for phase separation. The supernatant was collected with a Pasteur pipette and inserted into a vial for analysis by gas chromatography.

Chromatographic analysis of fatty acid methyl esters

After analysis by gas chromatography coupled with a flame ionization detector (GC-FID), the identification of fatty acid methyl esters (FAME) in the samples was made by comparing the peak retention time in the sample chromatogram with the retention time obtained by FAME standards.

To determine the elution order and consequently the retention time of each substance in the FAME standard mixture (Sigma), a gas chromatograph coupled with a mass detector (CG-MS) was used.

Method validation

For the determination of parameters such as detection limits, quantification limits and linearity, working solutions prepared by diluting the stock solution of the mixture of FAME standards were used. To prepare the stock solution, the mixture of FAME standards (C4-C24 Sigma) was diluted in 10 mL of n-heptane.

Limits of detection (LOD) and quantification (LOQ)

To determine the smallest amount of analyte present in the sample that could be detected (LOD) and quantified (LOQ) with precision and accuracy in the chromatographic system, the signal-to-noise ratio of the baseline was used. This method can only be applied in analytical procedures that show baseline noise. Working solutions ranging in concentrations from 0.03 to 1.85 mg L^{-1} were used until obtaining a signal for the analytes with an average area equivalent to three (LOD) and ten (LOQ) times the average height in relation to the noise signal [27].

Linearity

To determine the linear regression equation and the linearity of the method, which is the ability to demonstrate the direct proportionality of the results obtained with the analyte concentration in the sample in each range [27], analytical curves were built up with working standard solutions from 0.2 to 60.0 mg L^{-1} F.A.M.E, and were analyzed in triplicate.

Accuracy and precision of the method

In this study, the accuracy of the method was evaluated by recovery tests and the precision by the relative standard deviation, the latter being calculated for the recovery values obtained. A stock solution was prepared by diluting 100.0 mg of a standard mixture of triglycerides (tricaprin, tricaprylin,

trilaurin, trimyristin and tripalmitin) in 10 mL of n-heptane, then an aliquot of this containing 0.1 mg of the standards of triglycerides was added to 0.1 g of a sample of oilseed radish oil.

The fortified sample was submitted to the derivation and extraction process. After analysis of the extract by GC-FID, the areas for methyl esters from triglycerides added to the sample were obtained (the peak area in the fortified sample was subtracted from the peak area in the oil sample). The tests were carried out in 5 repetitions. The recovery was expressed as a percentage of area, calculated in relation to the area of the FAME standard, which was analyzed directly from the n-heptane solution, where the FAME concentration was equal to that of the triglyceride standards added to the oil sample.

Quantification of fatty acid content in oils

Quantification using the Official Method Ce-1e-91, AOCS

The quantification of FAME in crude vegetable oils was initially performed using the Official Method Ce-1e-91: Determination of Fatty Acids in Fats and Edible Oils by Capillary Gas-Liquid Chromatography, described by AOCS (The American Oil Chemist's Society) [28].

By the official method [28], the areas obtained from the chromatogram must be multiplied by the correction factors, to compensate the detector's response by flame ionization. Therefore, the area of each peak shown in the chromatogram was multiplied by a correction factor. Correction factors are calculated from the FAME molecular weight, according to following Equation 2:

$$DIC_x = \frac{MM_x}{(nx-1)(MAc)(DIC16{:}0)} \quad \textbf{(Equation 2)}$$

where: $DICx$ is the DIC factor for component x, *MMx* is molecular mass of component x, *nx* is the number of carbon atoms in the FAME of component x, *MAc* is atomic mass of carbon (12.01 u), *DIC* 16:0 is the DIC correction factor to 16:0 (1.407)

In addition, using Official method [28], the quantification of a particular component is expressed as a percentage of fatty acid and is determined by the corrected area of the analyte peak that is intended to be quantified in relation to the sum of the corrected area of all other peaks presented in the chromatogram. The area corresponding to the solvent peak must be discarded. The calculation is performed according to Equation 3 [28].

$$\frac{A_i}{\sum A_i} \times 100 \quad \textbf{(Equation 3)}$$

where: A_i is analyte peak area and ΣA_i is sum of area of all peaks, except solvent

Quantification using analytical curves

The concentration of each FAME in the oil sample was obtained using the linear equation of the calibration curve for the respective ester.

The FAME of a certain fatty acid presents the methyl group (CH3) in place of the hydrogen atom (H). Thus, the methyl ester presented a greater response in the FID in relation to the corresponding fatty acid. Therefore, the concentrations of FAME obtained in an analysis must be applied to the conversion factor of methyl ester to fatty acid known by the acronym MACF [29].

The MACF is determined by dividing the molecular mass of FAME by the molecular mass of the corresponding fatty acid, as shown in the Equation 4.

$$MACF = \frac{Molecular\ Mass\ of\ FAME}{Fatty\ acid\ molecular\ mass} \quad \textbf{(Equation 4)}$$

After making the appropriate correspondences, the fatty acids found in the samples were listed, along with their average concentrations, in Tab.3.

Multivariate statistical analysis

To verify possible similarities and discrepancies between the samples of the analyzed oils, considering the concentration of fatty acids, Principal Component Analysis (PCA) and Hierarchical Clusters Analysis (HCA) were applied to the data matrix. These are unsupervised pattern recognition techniques, which aim to verify the natural formation of sets of similar samples or clusters [30].

It was decided to assemble the oil samples in the same data matrix with the different methods of quantification of fatty acids. Each oil had 3 samples with fatty acid concentrations expressed in mg g^{-1} and 3 samples with fatty acid concentrations expressed in percentage by mass. Thus, the data matrix was built with a total of 24 samples and 31 variables.

Data was treated with the Pirouette statistical program, version 4.5 (Infometrix, Bothell, WA, USA). For the analysis of the principal components, the pre-processing used on the samples was normalization, while the pre-processing on the variables was self-scaling (scaled by variance and centering on the mean). For the analysis of hierarchical clusters, the Euclidean distance measure was used with the incremental connection method

4. Conclusions

The method used is effective for the extraction and quantification of fatty acids in oil samples. The interpretation of data by chemometric tools proved to be useful to highlight the differences and similarities between the oilseed samples. Among the oils studied, niger was the only that show the greatest similarity to commercial soybean oils. This similarity is due to the high content of linoleic acid found in these species. Crude oils extracted from crambe and oilseed radish presented high content of erucic acid, and cannot be used as food. In addition, both cannot compete with soybean and niger oils for industrial applications, making them a potential raw materials to produce biodiesel.

Acknowledgments

The authors did not preregister the research in an independent, institutional registry. The authors would like to thank to Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Author Contributions

EVRS, SRS were responsable for the conceptualization, rewiening and writing. EVRS made the formal analysis;

investigation; methodology and validation. GVF was responsable for rewiening writing original draft preparation and editing. GBA made the data curation and software. JBGS made the formal analysis; data curation; was responsable for the resources; project administration and supervision.

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How to cite this article

Sanches, E. V. R.; Freire, G. V.; Santos, S. R.; Alcantara, G. B.; de Souza, J. B. G. *Orbital: Electron. J. Chem.* **2023**, *15*, 101. DOI: **http://dx.doi.org/10.17807/orbital.v15i2.18168**