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Research Article

Contrast between Brazil and other countries in nutraceutical components of *Camelina sativa* grains

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Abstract

Camelina sativa, also known as false flax, is a species can be cultivated in a variety of climates, seasons and soil types, due to its short development cycle and tolerance to drought and low temperatures. In the composition of its grains, it presents a high amount of oil and rich in unsaturated fatty acids. In view of this, the objective of this work was to compare the composition of the nutraceutical components of *Camelina sativa* L. grains cultivated in Brazil and countries in Europe, Asia and North America. About 20 kg of grains were collected and then a homogeneous sample of 1kg of grains was cleaned to perform the centesimal composition of the grains. Afterwards, a search for information was carried out on the cultivation of *Camelina sativa* L. in other countries and the collection of information on the centesimal composition of the grains of this oleaginous plant. The collection of information was based on obtaining published scientific articles on the nutraceutical composition of *Camelina sativa* in regions of Europe, Asia and North America. Brazil presents a promising environment for the cultivation of *Camelina sativa*, with superior performance for the content of stearic acid, linoleic acid, linolenic acid and palmitic acid. The fatty acids profile decreased with the reduction of the minimum air temperature. The mineral material, palmitic acid and linolenic acid are positively correlated.

Keywords: Nutritional profile; variability; biodiesel; protein; low fertility; lipid metabolism; plant breeding.

Introduction

Camelina sativa, also known as false flax, is an herbaceous plant of the Brassicaceae family, recognized for its good tolerance to drought, low fertility saline soils and cold climates (Neupane, Lohaus, Solomon, & Cushman, 2022). This species presents a relatively short growth cycle, ranging from 85 to 100 days. (Fröhlich & Rice, 2005; Moser & Vaughn, 2010). In addition, it requires low demand for agrochemicals and fertilizers, due to its favorable performance in poor soils and tolerance to pests that affect other oilseed species (Zubr, 1997; Seguin-Swartz et al., 2009). Recently, important research has been carried out with crops aiming to make better use of climate and soil conditions (Furlan et al., 2022; Resende et al., 2023; Hailemariam, 2023; Ducatti & Tironi, 2023; Bester, et al 2024).Due to these characteristics of *Camelina sativa*, the distribution of the species extends from Europe to southwest Asia, and it was introduced in America and Canada (Ghidoli, Ponzoni, Araniti, Miglio, & Pilu, 2023). Studies reinforce that this species grows in a variety of climates, seasons and soil types, due to its short development cycle and tolerance to drought and low temperatures (Moser, 2010; Berti, Gesch, Eynck, Anderson, & Cermak, 2016; Vollmann & Eynck, 2015).

Furthermore, its short cycle and easy genetic transformation, combined with the available genome data, allowed camelina to become a model oilseed plant to study the regulation of lipid metabolism and genetic improvement. Particularly, camelina is capable of rapid metabolic engineering to synthesize and accumulate high levels of unusual fatty acids and modified oils in the seeds, which are more stable and environmentally friendly. Such engineered camelina oils have been increasingly used as the super resource for edible oil, health-promoting food and medicine, biofuel oil and high-valued chemical production (Yuan & Li, 2020). It is a high-quality edible oil (Orczewska-Dudek, Pietras, Puchała, & Nowak, 2020). Still, in folk medicine, the oil is considered a *good remedy for* the treatment of burns and wounds (Ibrahim & Habbasha, 2015).

The weight of 1,000 seeds ranges from 0.8 to 2.0 grams. The seeds contain oil and 27% to 32% protein. Camelina reproduces by seeds and is primarily a self-pollinating species (Francis & Warwick, 2009; Sainger et al, 2017). Thus, its oilseeds (Fröhlich & Rice, 2005; Borges & Torres, 2017) naturally have a high oil content (between 28 and 40% by weight of vegetable oil) (Fröhlich & Rice, 2005; Moser & Vaughn, 2010). The oil is highly unsaturated (~90%), has a desirable n-6:n-3 ratio (1:1.8), and contains high concentrations of tocopherols (Zubr & Matthäus, 2002). Thus, it has a nutritional profile comparable to conventionally used oilseeds (Waraich et al, 2013).

Camelina sativa seed is an underutilized oil source rich in omega-3 fatty acids; however, camelina oil is not fully exploited for food applications. Its high omega-3 content makes it susceptible to oxidation, which may limit food applications. Still on unsaturated fatty acids, the following stand out: 30-40% fraction of α-linolenic acid, 15-25% fraction of linoleic acid, 15% fraction of oleic acid and about 15% of eicosenoic acid (Zubr, 1997).

Many studies have been conducted in the most diverse regions of the globe with different average annual precipitation levels, to investigate grain yield, oil content, protein content and fatty acid composition. Differences in genotypes, water availability, environment, soil physical and chemical characteristics, and management practices clearly affect the overall productivity of this crop (Neupane et al., 2022). Low grain yields are largely associated with variables such as air temperature and precipitation, especially in the stages of full flowering and siliqua filling (Berti et al., 2016).

According to Załuski, Tworkowski, Krzyżaniak, Stolarski and Kwiatkowski (2020), climatic factors were responsible for approximately 73% of the oscillation in

the productivity of this oilseed. These same authors still report only 6% of genetic contribution in the expression of productivity. Through the importance of environmental factors in the development and expression of the fatty acid profile of *Camelina sativa*, this work aimed to compare the composition of the nutraceutical components of *Camelina sativa* L. grains cultivated in Brazil and countries in Europe, Asia and North America.

Materials and Methods

The collection of *Camelina sativa* samples took place in the Brazil. The soil is classified as a typical dystroferric Red Latosol, characterized by a deep, dark red color profile, with high clay content and well-drained (Streck et al., 2018). According to the Köeppen climate classification, the climate of the region fits the description of Cfa (humid subtropical) (Galvani, 2012). About 20 kg of grains were collected and then a homogeneous sample of 1kg of grains was cleaned to perform the centesimal composition of the grains.

The determination of the centesimal composition of *Camelina sativa* (humidity in an oven with air circulation at 105 ºC, ash in a muffle furnace at 550 ºC, proteins by the Kjeldahl method, lipids by the Soxhlet method and carbohydrates by difference) took place according to the AOAC (2000). The results were expressed in $g.100g^{-1}$. The oil obtained in the lipid analysis was used for analysis of acidity, peroxides, determination of specific absorption at 232 and 270 nm and composition of fatty acids.

To evaluate the acidity content, the lipid fraction of the sample was dissolved in a solution of ethyl ether:ethyl alcohol (2:1, v/v) in an oil:solvent ratio of 1:10 (w/v), adding phenolphthalein was added and titrated with a KOH solution (0.1 N), according to the AOCS methodology (1992). The results were expressed in mg of KOH g^{-1}.

To determine the peroxide number, five grams of the lipid fraction were dissolved in 30 mL of acetic acid:chloroform solution (3:2, v/v), The mixture was stirred and 0.5 mL of saturated potassium iodide solution was added and the flask was kept in the dark for one minute. Then, 30 mL of distilled water and 0.5 mL of starch solution (1%) were added and titrated with sodium thiosulphate (0.1 N) until the blue color disappeared, according to the AOCS methodology (1992). The results were expressed in meqO2 kg^{-1} sample.

The specific extinction coefficients (K232 and K270) were determined using a 10 mL volumetric flask; 100 mg of oil (cleaned and filtered and dissolved in isoctane) were weighed. The absorbance of the solution was measured using a spectrophotometer at 232 and 270 nm and used to determine the specific extinction coefficients (K232 and K270), using the method proposed by the American Oil Chemists Society's (Firestone, 2001).

The fatty acid composition of the lipid fraction samples was determined by gas chromatography coupled to mass spectrometry (GCMS), following the method described by COI (2015). For the transesterification reaction, 0.1 grams of sample, 2 mL of hexane and 0.2 mL of methanolic potassium hydroxide (2M) were mixed under vigorous stirring for 30s. The upper layer containing methyl esters was collected and injected into the gas chromatograph. A Shimadzu QP2010 Ultra GC-MS with AOC-20i autoinjector and NIST 2011 mass spectrum library was used. 1µL of sample was injected with injector temperature at 200ºC, in splitless mode. Helium was used as carrier gas 34 with flow rate of 1.78 mL min^{-1} and linear velocity as flow control mode.

The capillary column used was Rxi-1MS (30m x 0.25mm x 0.25µm).

The temperature gradient was maintained at 78 °C for 6.5 min, increased at a rate of 60 °C min -1 to 180 °C for 13.44 min, then increased at a rate of 35 °C min -1 until 280 °C and remained so for 5.5 min. The ion source and interface temperatures were set to 200°C, and the mass scan range was m/z 35 to 500 and 0.3 scans per second. Fatty acid methyl esters were identified by comparison with the retention times of

reference standards, and the results were expressed as a relative percentage of fatty acids.

Subsequently, a search for information on the cultivation of *Camelina sativa* in other countries took place and information was collected on the centesimal composition of the grains of this oleaginous plant. The collection of information was based on obtaining published scientific articles on the nutraceutical composition of *Camelina sativa* in regions of Europe, Asia and North America. With this survey, it was possible to obtain information on crops in Spain 2013 (Ciubota-Rosie, Ruiz, Ramón, & Pérez, 2013), New Jersey-US 2009 (Moser & Vaughn, 2010), Wilmington-US 2015 (Belayneh., Wehling., Cahoon., & Ciftci., 2015; Belayneh, Wehling, Zhang, & Ciftci, 2017), Minnessota-US 2016 (Walia et al., 2018), Poznán-Poland 2018 (Krzyżaniak et al., 2019), Turin-IT 2006 (Pierreti et al., 2007), Romania (Toncea, Ghilvacs, & Prisecaru, 2013), New Scotia-Canada 2016 (Yang, Caldwell, Corscadden, He, & Li, 2016), Quebec-Canada 2020 (Kasiga et al., 2020), Iran 2018 (Raziei, Kahrizi, & Rostami-Ahmadvandi, 2018) and Germany, United Kingdom, Ireland, Finland and Denmark 2009 (Zubr , 2010), New Jersey-US 2012 (Quezada & Cherian, 2012), Poland 2019 (Popowska et al., 2020), Ankara-Turkey 2010 (Katar), and Argentina 2022 (Figure 1).

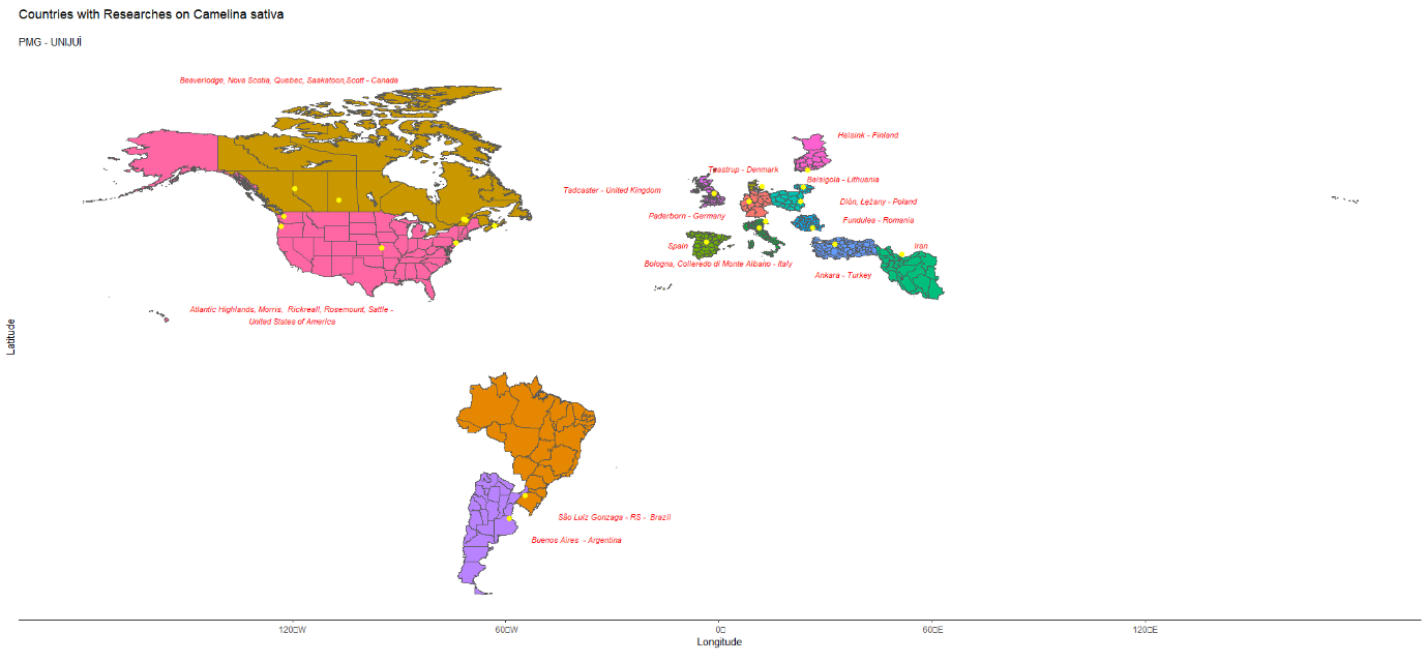

Figure 1. Location of environments studied in Argentina, Brazil, United States of America, Canada, Spain, Italy, Germany, Finland, Iran, Turkey, Poland, Romania, Denmark and Lithuania.

From the Nasa Power platform (National Aeronautics and Space Administration [NASA], 2022), the variables maximum daily air temperature (T max., ºC), daily mean air temperature (T mean, ºC), minimum daily air temperature (T min, ºC) and annual daily precipitation (Prec, mm) in each country of ambient cultivation.

In each environment, the average of all nutraceutical characteristics of *Camelina sativa* and meteorological variables was calculated. Then, the mean and standard deviation of each nutraceutical variable for all environments were calculated.

Pearson's linear correlation coefficients were calculated between pairs of variables, then, between pairs of variables in each environment. For example, correlations were made between the protein obtained in Brazil and the protein obtained in Germany. The significance of correlation coefficients was verified by Student's t

test at 5% error probability.

To verify the dissimilarity between the nutraceutical components of *Camelina sativa*, the dissimilarity matrix was determined using the Euclidean distance and the grouping was done using the UPGMA algorithm. The dissimilarity matrix was also calculated to verify divergence in the expression of nutraceutical components between countries.

Parameters of the multiple linear regression model were estimated for all nutraceutical variables, considering Tmean, Tmin, Tmax, and Prec as explanatory. All analyzes were performed using the R software (R Core Team, 2023) using the packages agricolae (Mendiburu, 2021), metan (Olivoto & Lucio, 2020) and ggplot2 (Wickham, 2016).

Results and Discussion

Based on the descriptive analysis of *Camelina sativa* response in the different cultivation countries, it was observed that Brazil and the United States of America had higher values of stearic acid (5.84% and 3.29%, respectively) (Figure 2). On the other hand, Canada stood out for the higher expression of lipid values (37.59%). In Brazil, higher values than other countries were observed in relation to linoleic, linolenic and palmitic acids (21.16, 44.64 and 14.74%, respectively). In addition, crops in Argentina had the highest magnitudes of mineral material, while crops in Italy stood out for the highest concentrations of oleic acid (16.82%) and crops in Germany had higher protein contents. These results show the variability in the nutritional characteristics of *Camelina sativa* among different growing countries (Figure 2).

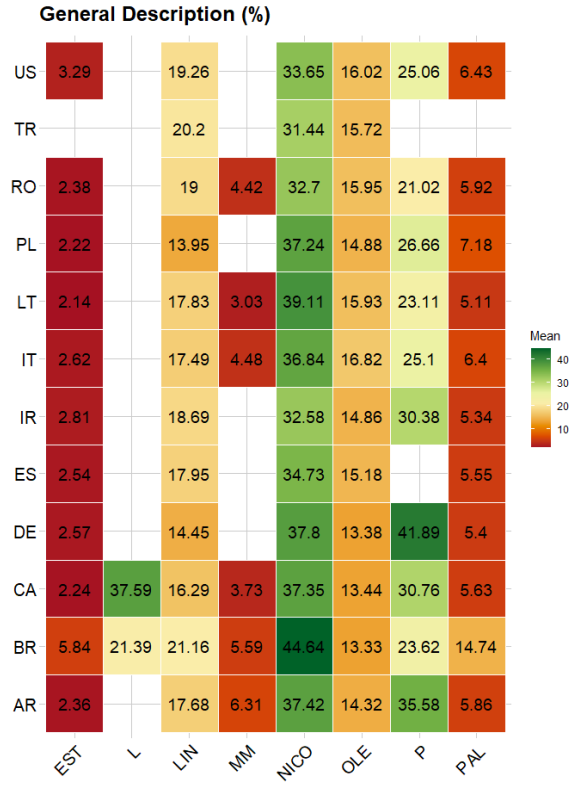

Figure 2. Descriptive analysis of the average centesimal composition of *Camelina sativa* oil in Argentina (AR), Brazil (BR), United States of America (US), Canada (CA), Spain (ES), Italy (IT), Germany (DE), Iran (IR), Turkey (TR), Poland (PL), Romania (RO) and Lithuania (LT).

A resilient crop to different environments, cultivated in the most diverse continents, characterizes *Camelina sativa (*Figure 3). In Brazil, the highest mean and maximum annual air temperatures were recorded. Iran had the highest mean air temperatures, while Canada had the lowest mean and minimum air temperatures. A study carried out by Sanehkoori et al. (2023) demonstrated that *Camelina sativa* germination reached the highest percentage between temperatures of 5 to 20ºC. However, temperatures above 25ºC negatively affect germination, flowering and grain filling, as observed by Berti et al. (2016) and Sanehkoori et al. (2023). Thus, all environments presented favorable temperatures for the development of this oilseed.

Average daily precipitation plays an important role in the performance of *Camelina sativa*. In Brazil, higher values vary between 3.4 and 6.7 mm daily, indicating an adequate supply of water for crop growth. On the other hand, Turkey presented lower values, between 0.8 and 1.3 mm daily, suggesting a lower availability of water. Studies conducted by Hergert et al. (2016) revealed that the water demand of *Camelina sativa* throughout the cycle estimates between 450 mm and 500 mm.

The centesimal composition of ash of *Camelina sativa* cultivated in Brazil was 5.43% (Table 1). Studies conducted by Pieretti et al. (2006) in Italy, Kasiga et al. (2020) in Canada, Rezmaité et al. (2021) in Lithuania reported lower values of crude ash, with contents of 3.2%, 3.7% and 2.98%, respectively. Mean protein values were 22.94%. This protein concentration of 22.94% is lower than other studies took place in different countries in Europe, Asia and North America. In particular, Zubr (2003) conducted a study in the city of Paderborn-Germany and revealed a crude protein content of 42.5%. However, it presented a lower result in Romania, in a study conducted by Toncea et al. (2013), who reported a protein content of 20.42%.

For the lipid content, values of 20.34% were found. Kasiga et al. (2020) revealed a higher value of lipids with a value of 36.6%. The percentage of total carbohydrates observed was 43.18%. According to Krzyżaniak et al. (2019) the causes of differences in the composition of *Camelina sativa* grains, is due to the difference in cultivation environments, such as soil and climate characteristics.

The observed seed acidity content was 0.80 mg KOH g^{-1} (Table 2), a value higher than that observed by Wu and Leung (2011), which was 0.354 mg KOH g^{-1}. Raczyk, Popis, Kruszewski, Ratusz and Rudzińska (2015), in Poland, with oils from four different locations, observed a value of 0.61 mg KOH g^{-1}. These values indicate the quality of this raw material, since the acidity is related to the quality of the raw material, with the processing and, mainly, with the conservation conditions of vegetable oils (Morais, Christiani, Cestari, & Flumignan, 2012). The peroxide index showed a lower performance (0.00%) than studies carried out by Raczyk et al. (2015), in Poland, who found a value of 2.36 meq/kg. Values within Codex Alimentarius standards, being considered safe for human consumption (Codex Alimentarius, 1999).

The specific extinction coefficients of K232 and K270 are important indicators of the degree of degradation of an oil in the initial stage, that is, formation of primary and secondary oxidation products (Fadda et al., 2022). Thus, in Brazil, values of 0.07 of K232 and 0.10 of K270 were observed, values considered low, these two indices are related to the stabilization of the radical via double bond rearrangement (Barriuso, Astiasarán, & Ansorena, 2013), and are used as an efficient parameter to estimate the degree of oxidation of polyunsaturated fatty acids (Tura, Mandrioli, Valli, & Toschi, 2023).

The results of the fatty acid profile revealed palmitic acid levels (C16:0) of 15.15%, a value higher than studies carried out in Canada by Yang et al. (2016) (Table 3). A stearic acid content (C18:0) of 5.71% was observed, which represents a lower performance compared to the value of 2.49% reported by Quezada and Cherian (2012) in the United States of America. Regarding oleic acid (C18:1) and linoleic acid (C18:2), values of 13.31% and 21.07% were recorded, respectively. Katar (2013), in

a study carried out with different genotypes in Turkey, found a higher value for oleic acid (16.07%) and a lower value for linoleic acid (20.03%). The content of linolenic acid (C18:3) in Brazilian cultivation observed was 44.73%. Raziei et al. (2018), in a multi-environment study in Iran, found values between 25.83 and 38.82%. Similar values were found by Gugel and Falk (2006) in Canada, with a content of 37.6%. These authors also mention that the air temperature has an effect on the expression of the polyunsaturated fatty acid profile.

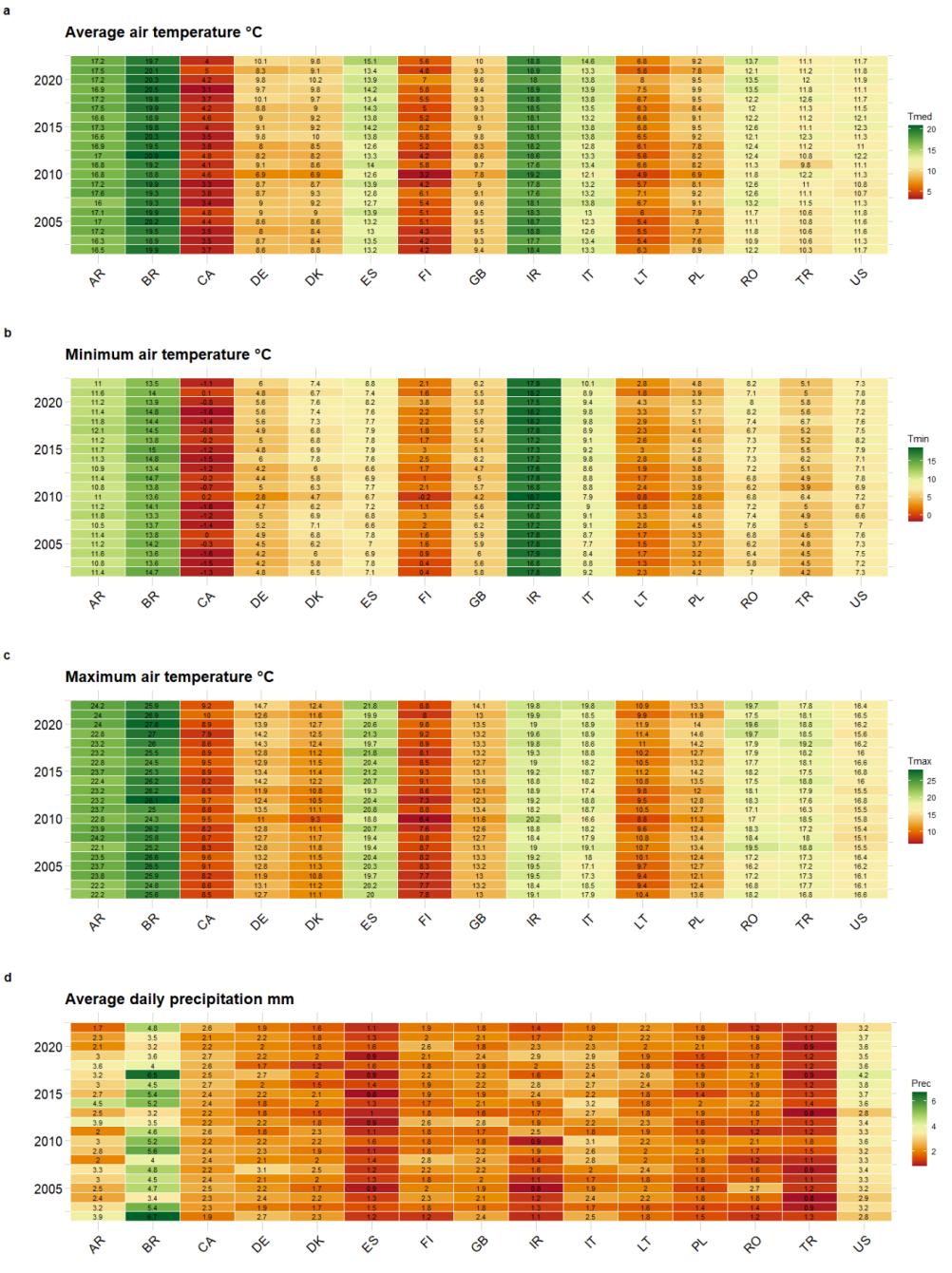

Figure 3. Descriptive analysis of the annual average of climate variables in Argentina (AR), Brazil (BR), United States of America (US), Canada (CA), Spain (ES), Italy (IT), Germany (DE), Finland (FN), Iran (IR), Turkey (TR), Poland (PL), Romania (RO), Finland (FN) Denmark (DN) and Lithuania (LT).

Table 1. Mean values ± standard deviation of all environments for the centesimal composition of *Camelina sativa* cultivated in Brazil.

Cultivar	Humidity (%)	Ashes (%)	Proteins (%)	Lipids (%)	Carbohydrates (%)
Camelina sativa	8.21±0.05	5.43±0.05	22.94±0.57	20.34±030	43.18±0.19

Values presented as mean±standard deviation (n=3).

Table 2 - Acidity (mg KOH g^{-1}), peroxide value (meq/kg^{-1}) and extinction coefficient (K232, K270) of *Camelina sativa.*

	Acidity (mg KOH g^{-1})	Peroxide index (meq/kg)	K232	K270
Camelina sativa	0.80±0.05	0.00±0.00	0.07±0.00	0.10±0.01

Values presented as mean±standard deviation (n=3).

Table 3. Profile of fatty acids (%) of *Camelina sativa*, cultivated in Brazil.

	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linolenic acid (C18:3)
Camelina sativa	15.15	5.71	13.31	21.07	44.73

Estimates of correlation coefficients aimed to reveal the magnitude and direction of associations between the evaluated *Camelina sativa* traits in countries. Linear correlation performed for eight characters in nine countries revealed 396 associations, ten of which were significant (Table 4). Thus, significant correlations of low magnitude were observed for the protein content of camelina cultivation in the United States of America and Iran (r=0.25) with values of 25 and 30% of protein respectively (Quezada et al., 2012; Raziei et al., 2018), these environments presented similar maximum air temperatures and precipitation. For Lithuania and Italy, a correlation of the same magnitude (r=0.21) was observed, with protein values of 23.35% and 24.5% (Raizmaté et al., 2021; Peiretti, Mussa, Prola, & Meineri, 2007), similarity this can be explained by the average precipitation of these environments. Studies by Kon'kova, Shelenga, Gridnev, Dubovskaya, & Malyshev, (2021), report the influence of environmental stimuli on oil and protein content.

Weak positive correlations were given for the percentage of palmitic acid in Italy and Iran (0.27), oleic acid in Iran and Brazil (0.25) these with similar average annual temperatures. For the percentage of oleic acid in Spain and Italy (0.28), mineral material in Canada and Argentina (0.20), linolenic acid in Spain and Poland (0.24) and the United States and Italy (0.21) showed weak positive associations. Weak positive association was still observed for the percentage of linoleic acid between the United States and Italy (0.21), correlation of the same strength, but negative between Turkey and Spain (-0.18).

Multiple linear regression models for predicting stearic acid, lipid, oleic acid and protein were not significant. Tmin showed influence in the prediction of linoleic acid, mineral material and linolenic acid. The linoleic acid content has a negative influence of the minimum temperature, that is, environments with low temperatures promote the reduction of the compound (Table 5). The mineral material, on the other

hand, presents a positive relationship with the mean temperature, that is, environments with low thermal amplitude and precipitation and a negative relationship with the minimum temperature. The linolenic acid content showed a positive response with precipitation and a negative response with minimum temperature. Palmitic acid has a negative relationship with the mean temperature and a positive relationship with the maximum temperature. Bozoo et al. (2021) also found an effect of precipitation on the expression of the fatty acid profile of this crop.

Table 4. Pearson Linear Correlation for the variables total protein (TP), palmitic acid (PAL), oleic acid (OLE), mineral material (MM), linolenic acid (LLEN) and linoleic acid (LLEI) in the United States of America, Iran, Lithuania, Italy, Brazil, Spain, Canada, Argentina, Turkey and Poland.

Pearson's linear correlation	
MM x PAL	0.27**
MM x LLEN	0.20*
TP _US x TP _IR	0.25*
TP _LT x TP _IT	0.21*
PAL_IT x PAL_DE	0.27**
OLE_IR x OLE_BR	0.25*
OLE_ES x OLE_IT	0.28**
MM_CA x MM_AR	0.20*
LLEN _ES x LLEN _PL	0.24*
LLEN _US x LLEN _IT	0.21*
LLEI_IT x LLEI_IR	0.21*
LLEI_TR x LLEI_ES	-0.20*

Only significant linear correlation coefficients ($p \leq 0.05$) were presented.

Table 5. Environmental predictor model for the fatty acid profile of *Camelina sativa*. Tmean: mean air temperature (ºC); Tmax: maximum air temperature (ºC); Tmin: minimum air temperature (ºC); Prec: Precipitation (mm).

Model: Y= a + Tmean+ Prec + Tmax+ Tmin
ST= NS
L=NS
LLEI= 18.02721- 0.02745 (Tmin)
MM= 4.22923 + 0.05788 (Tmean)+ 0.08416 (Prec)- 0.0519 (Tmin)
LLEN = 36.03047+ 0.21867 (Prec)- 0.05019 (Tmin)
OLE= NS
TP = NS
PAL= 0.1760- 0.2245(Tmean)+0.1891(Tmax)

NS= Not significant. ST- Stearic Acid; L- Lipid; LLEI- Linoleic Acid; MM- Mineral Material; LLEN- Linolenic Acid; OLE- Oleic Acid; TP- Total Protein; PAL- Palmitic Acid.

In the dissimilarity dendrogram (Figure 4) the formation of two large groups and four subgroups was observed. For the large group one, the variables protein, linolenic acid and lipid content were grouped, where linolenic acid and protein formed the first subgroup and the percentage of lipid subgroup two. The large group two had the grouping of the variable's oleic acid, linoleic acid, mineral material, stearic acid and palmitic acid, where subgroup one was composed of oleic and linoleic acid. Subgroup

two by palmitic acid, stearic acid and mineral material. Studies by Kurt and Gore (2020) prove that changing the environment, genotype and management can change the fatty acid profile of this crop.

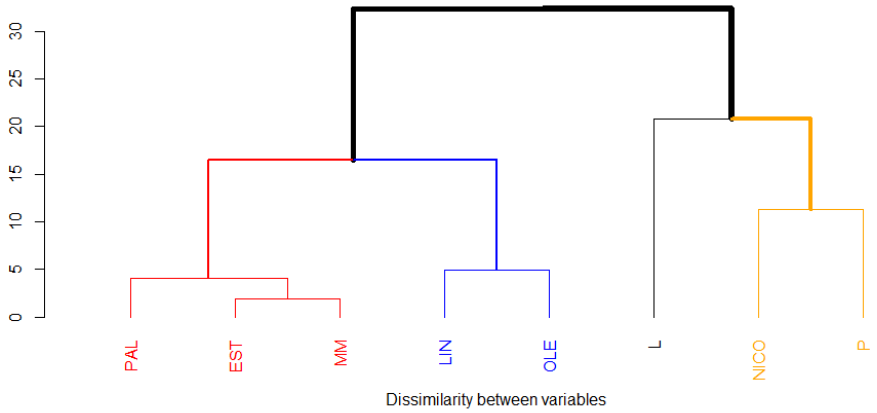

Figure 4. Dendrogram of dissimilarity for the variable's palmitic acid, stearic acid, mineral material, linoleic acid, oleic acid, lipids, linolenic acid and protein.

According to the fatty acid profiles presented in the different countries under study, it was observed in the dissimilarity dendrogram, the formation of two large groups was observed, the first large group is constituted only by Turkey due to the difference in the expression of acid contents linoleic acid and linolenic acid (Figure 5). The large group two, formed by three subgroups, the first covers the countries of Spain, Romania, Poland, Italy, Lithuania, and Argentina these countries present similarity for the content of stearic acid and oleic acid. The second subgroup has only Brazil, which shows a great discrepancy for other countries, in relation to the values of palmitic acid and linoleic acid. The third subgroup is formed by the United States of America, Canada, Germany, and Iran, which have similarity in terms of oleic acid content.

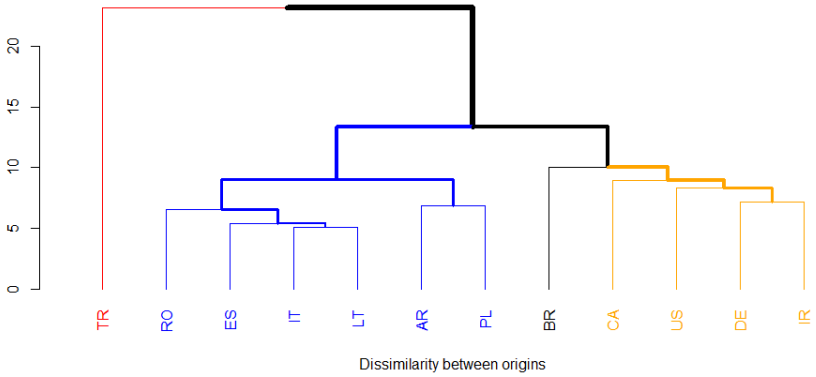

Figure 5. Dendrogram of dissimilarity between Argentina, Brazil, United States of America, Canada, Spain, Italy, Germany, Finland, Iran, Turkey, Poland, Romania, Denmark and Lithuania.

Conclusion

Brazil presents a promising environment for the cultivation of *Camelina sativa*, with superior performance for stearic acid, linoleic, linolenic and palmitic acid content.

The fatty acid profile decreases with the reduction of the minimum air temperature.

The mineral material, palmitic acid and linolenic acid are positively correlated.

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