CAMELINA (*Camelina sativa* L. CRANTZ) VARIETIES FOR BIODIESEL PRODUCTION. CRITERIA FOR CONVENTIONAL PLANT BREEDING

Estudio de diferentes variedades de camelina (*Camelina sativa* L. Crantz) para la producción de biodiésel. Criterios para la mejora vegetal

Maria del Mar DELGADO ARROYO¹*, Sergio ÁLVAREZ GALLEGO², Aníbal CAPUANO ASINARI³ and Sara MARTÍNEZ DELGADO⁴

¹ Departamento de Medio Ambiente y Agronomía, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, km 7.5 carretera de La Coruña, 28040 Madrid, España
² Departamento de Sistemas y Recursos Naturales, Universidad Politécnica de Madrid, Ciudad Universitaria s/n, 28040 Madrid, España
³ Camelina Company España, Camino de la Carrera 11, Fuente el Saz del Jarama, 28140 Madrid, España
⁴ Departamento de Ingeniería y Morfología del Terreno, Universidad Politécnica de Madrid, Ciudad Universitaria s/n, 28040 Madrid, España
*Author for correspondence; delgado@inia.es

(Received May 2019; accepted October 2019)

Key words: fatty acid profile, viscosity, iodine value, cold filter plugging point, cetane number

ABSTRACT

Biodiesel and its diesel blends have been accepted worldwide as potential renewable alternative fuels to substitute petroleum diesel. Perspectives for biodiesel commercialization using *Camelina sativa* L. Crantz as raw material are presented in this paper. Fatty acid profiles and the morphological characterization, including production, number of seeds, thousand seed weight and fatty acid percentage were analyzed for different varieties. A further assessment, considering four parameters, viscosity, iodine value, cold filter plugging point, and cetane number, was carried out to evaluate the biodiesel quality. It was concluded that camelina varieties have a similar percentage of fatty oil content. This work shows large differences in productivity and in the selected physical and chemical properties of derived biodiesel from camelina. Results show that varieties 1072, 117 and N5 (1.9) from Camelina Company Spain could be suitable due to their high productivity (over 4.2 g per plant). Variety 1109 could also be used due to its low iodine value (131). Varieties 1008, 1085 and 1109 could be used due to their high cetane number (over 51). This research has identified suitable varieties for biodiesel production. Further improvement of camelina’s characteristics is needed by means of plant breeding and manipulation of culture conditions.

Palabras clave: perfil de ácidos grasos, viscosidad, valor de yodo, punto de obturación de filtro frío, número cetónico

RESUMEN

El biodiésel y sus mezclas de diésel han sido aceptados en todo el mundo como posibles combustibles renovables alternativos al diésel procedente del petróleo. En este trabajo...
se presentan las perspectivas para la comercialización de biodiésel utilizando Camelina sativa L. Crantz como materia prima. En primer lugar, se analizaron los perfiles de ácidos grasos y la caracterización morfológica, incluida la producción, el número de semillas, el peso de mil semillas y el porcentaje de ácidos grasos para diferentes variedades. En segundo lugar, con el fin de evaluar la calidad del biodiésel, se llevó a cabo una evaluación adicional, teniendo en cuenta cuatro parámetros: la viscosidad, el valor de yodo, el punto de obturación del filtro frío y el índice de cetano. Se concluyó que las variedades de camelina tienen un porcentaje similar de contenido de aceite graso. Además, en este trabajo se observan diferencias significativas en la productividad y en las propiedades físicas y químicas del biodiésel derivado de camelina. Los resultados muestran que las variedades 1072, 117 y N5 (1.9) de Camelina Company Spain podrían ser aceptables, ya que presentan una alta productividad (más de 4.2 g por planta). La variedad 1109 se podría utilizar debido a su bajo valor de yodo (131). Las variedades 1008, 1085 y 1109 presentan un alto valor cetónico (más de 51), por lo que también se recomendaría su empleo. Este trabajo ha destacado variedades que son aptas para su empleo como biodiésel. No obstante, se necesita una mejora adicional de las características de la camelina mediante la optimización y manipulación de las condiciones del cultivo.

INTRODUCTION

Plant breeding is conceived as the art and science by which the traits of plants are exchanged aiming the creation of new varieties with desired characteristics. Plant breeding can be developed by different techniques which range from the simple selection of plants with selected characteristics to more complex genomic techniques (Acquaah 2015). Inside plant breeding, there is room for conventional plant breeding when the improvement is developed using traditional tools within the natural boundaries of the species. This consists of the selection among natural variability and is practiced worldwide by individuals such as gardeners or by professional plant breeders such as government institutions, universities, or crop-specific industry associations. Traditionally, plant breeding has been used for the selection of features such as faster growth, higher yields, pest and disease resistance, larger seeds, or sweeter fruits. Breeders now seek to produce crop varieties that appeal to satisfy new objectives such as climate change mitigation. Plant’s viability to substitute fossil fuels has been a solid strategy to decrease transport greenhouse gas emissions.

Global transport emissions reached 7.3 Gt CO2e in 2014. This figure represents 23 % of global emissions and it has increased by 28 % since 2000. Getting transportation on track to meet the two-degree scenario targets requires implementing a broad set of policies. Biodiesel and its diesel blends have been accepted worldwide as potential renewable alternative fuels to substitute petroleum diesel. In addition to being renewable, biodiesel has other advantages such as reducing regulated air pollutants emissions (Devan and Mahalakshmi 2009, Huang et al. 2010), reducing greenhouse emissions (Dorado 2003, Szybist et al. 2007), and being non-toxic, environmentally safe and biodegradable (Mondala et al. 2009). Biodiesel can be produced by several techniques, among them, transesterification is the most common one (Kakati et al. 2017). In transesterification, a fatty acid component reacts with an alcohol (methanol/ethanol) in the presence of a catalyst to form a mixture of fatty acid ester and alcohol (Barnwal and Sharma 2005). Methanol has become the most used alcohol and the reason is twofold. On the one hand, it has good physical and chemical properties; on the other hand, the commercial costs are very low compared to other possible glycerol (Berrios et al. 2009). The catalyst varies from homogeneous acids or bases to heterogeneous with enzymes (Da Conceiçao et al. 2016).

Fatty acids for biodiesel production come from oilseed crops. Many authors have studied biodiesel production from non-edible oil crops. For example, jatropha oil (Jatropha curcas L.) (Rabiliah et al. 2014), karanja oil (Pongamia pinnata L.) (Thiruvengadaravi et al. 2012), okra oil (Hibiscus esculentus L.) (Anwar et al. 2010), rubber seed oil (Hevea brasiliensis Muell. Arg) (Reshad et al. 2015), moringa oil (Moringa oleifera Lam.) (Fernandes et al. 2015), kusum oil (Schleichera oleosa L.) (Silintona et al. 2015), andiroba oil (Carapa guianensis Aubl.), castanhola oil (Terminalia catappa L.) (Iha et al. 2014), mahua oil (Madhuca indica J.F. Gmel.) and simarouba oil (Simarouba glauca D.C.) (Jena et
Materials and Methods

Plant description

Camelina (Camelina sativa L. Crantz) is an annual oilseed crop in the Brassicaceae family coming from regions of southeast Europe and southwest Asia (Larsson 2013). It has a short growing season and is tolerant to droughts, cool weather, and insect pests. Moreover, camelina, unlike other traditional oilseed crops (rapeseed/canola, soybeans, and sunflowers, for example), thrives in cool, arid climates and is nicely adapted to the more northerly regions of North America, Europe and Asia (Iskandarov et al. 2014, Vollmann and Eynck 2001). These properties allow camelina to be used as a rotational crop with winter wheat, helping in the control of undesirable weed and pest cycles (Moser and Vaughn 2010). Its seeds have between 35 and 43 % of oil content on a dry matter basis with a high percentage of unsaturated fatty acids (about 90 %) (Kirkhus et al. 2013, Jiang et al. 2014). This fatty acid profile may vary from endogenous to exogenous causes such as genotype, geographic location, climate, and fertilizer inputs. For more information about camelina see the review on uses, genetics, genomics, production, and management made by Berti et al. (2016).

Experimental procedure

The experiment was conducted in a greenhouse at the National Institute for Agricultural Research (INIA) in Madrid, Spain, from December 2015 to June 2016. One hundred and thirty-nine varieties from several countries (Russia, Ukraine, Byelorussia, Germany, Poland, Denmark, USA and some unknown) were selected for the investigation. These varieties came from the Germplasm Bank owned by Camelina Company Spain, which offers technical solutions for the industry through the study of varieties for application (as in the production of oil). This varieties identification is codified due to confidentiality with CEE codes for different plants species: CCE 1061, 1076, 1077, 1090, 1092, 1100, 1104, 1169, 1200, 1226, 1234, 1236, 1246, 1259 and 1260 are pilosa and the rest of the codified plants of camelina shown in Appendix 1 are sativa.

A randomized complete block design with three replicates per each variety was used in the experiment. The plants were seeded in pots of 1 L (11 × 11 × 12 cm). The material used was a special blend containing 40 % of blonde peat (0.20 mm), 15 % of perlite, 30 % of coconut fiber and 15 % of substratum with a pH around 6. To obtain a fast absorption of nutrients in the first stages of the plant, during seeding, each pot received an application of 1g/L of nitrogen, phosphorus, and potassium (NPK, 8-24-8). A week later, 1g/L of osmocote bloom was applied containing NPK with tellurium (12-17-18 + Te), which is released slowly during all cycle, magnesium (1.5 %) and all necessary micro-elements (0.02 % boron, 0.045 % copper, 0.35 % iron, 0.05 % manganese, 0.020 % molybdenum, and 0.013 % zinc). Before seeding, the substrate was taken to field capacity and Previcur (active component: propamocarb fosetilate, 840 g/L) was applied in doses of 0.2 ml/L in order to prevent diseases. In December, camelina varieties were seeded (1 seed/pot) and grown in the greenhouse at a temperature of approximately 20 ºC. Camelina seeds were collected from each variety in plastic bags. Seeds were cleaned and dried in sunlight for reducing its moisture content. Fatty acid methyl esters (FAMES) of the camelina seeds were performed by duplicate, following the method described by Sukhija and Palmquist (1988). All samples were analyzed for FAMES using a Perkin-Elmer Autosystem-1 (Massachusetts, USA) gas chromatographer with flame ionization detection (Muñño et al. 2014). The process to obtain FAME biodiesel is known as transesterification, which is a reversible reaction that consists of the reaction of vegetable oil with methanol in the presence of a catalyst to give methyl ester, biodiesel and an amount of glycerin (Harreh et al. 2018). The transesterification process produces raw biodiesel, which after a refining process meets the standards of the European Biofuel Technology Platform (EBTP 2011). The transesterification process for obtaining camelina biodiesel is not included in the scope of this paper. For more information regarding transesterification process of camelina seed see Bacenetti et al. (2017).
Technical standards and reference properties

Technical standards, such as those provided by the European EN 14214 and the American ASTM 6751, must be met when biodiesel is commercialized. These standards provide boundaries for physical and chemical properties to guarantee a safe use. To do so, there are indicators that provide information on relevant properties such as efficiency in the transesterification process (e.g., the amount of free glycerol or water). Other indicators depend on the fatty acid profile, standing out among them, viscosity, cetane number, cold properties, and iodine value. Lubricity was not assessed because it is only addressed in petrodiesel standards (Knothe 2009).

Following Viola et al. (2011) and Mahmudul et al. (2017), in this work iodine value, viscosity, cetane number, and cold filter plugging point were selected as the most important physical and chemical properties to accomplish biodiesel standards (Table I). Iodine value is an index of the number of double bonds in biodiesel that control the extent of unsaturation; it is an important property of biodiesel suitability for long-term engine uses. Viscosity represents a liquid’s resistance to flow. Cetane number measures the ignition quality of diesel fuel, the higher this number, the easier it is to start. Finally, cold filter plugging point is the lowest temperature at which a given volume still passes through a standardized filtration device. It gives an estimate of the lowest temperature that fuel will give trouble-free flow. In this study, the limit of 0 ºC has been chosen as a reference, since it is the common limit for European countries such as Spain and Germany, among others. These four properties have been estimated from the fatty acid profile following the recommendations of the American Oil Chemists’ Society for the calculation of the iodine value (Petursson 2002); the Grunberg-Nissan equation for predicting biodiesel viscosity, as described in the paper of Allen et al. (1999); a linear model to predict the cetane number of biodiesel, and the equations developed by Ramos et al. (2009) to predict cold filter plugging point. Due to the lack of data for the prediction of viscosity, hypothetical values calculated from a linear extrapolation (R² > 0.99) were assumed.

RESULTS

Tables II, III and IV show the statistical results of the fatty acid profile, the morphology characterization, and the selected physical and chemical properties of the derived biodiesel. The complete data of all varieties under study are given in Appendix I. From the fatty acid profile, it can be observed that the unsaturated fatty linolenic acid (C₁₈H₃₆O₂) [18:3] has the highest mean value, where 55 % of the varieties achieved a value above the mean content for this specific fatty acid. On the other hand, lignoceric acid (C₂₄H₄₈O₂) [24:0] was the saturated fatty acid with the lowest values, where 55 % of the varieties did not achieve content above the mean of these fatty acids. It is also important to distinguish between saturated and unsaturated fatty acids. Within the saturated fatty acids, the palmitic acid (C₁₆H₃₃O₂) [16:0] is found to be the most influential, being the variety CEE 1118 33 % higher than the average value. It has noticeably higher unsaturated fatty acid content compared to saturated acids (Table II). As mentioned before,

**TABLE I. MAIN PHYSICAL AND CHEMICAL PROPERTIES REGULATED UNDER BIODIESEL STANDARDS**

| Property                        | EN 14214 | ASTM 6751 |
|---------------------------------|----------|-----------|
| Iodine value (mg I₂/g)          | < 120    | Not specified |
| Viscosity at 40 ºC (mm²/s)      | 3.5–5.0  | 1.9–6.0   |
| Cetane number                   | > 51     | > 47      |
| Cold filter plugging point (ºC) | Country specific | Country specific |

EN: European, ASTM: American Society for Testing and Materials

**TABLE II. STATISTICAL RESULTS OF THE FATTY ACID PROFILE**

| Statistical | [16:0] | [18:0] | [18:1] | [18:2] | [18:3] | [20:0] | [20:1] | [20:2] | [20:3] | [22:0] | [22:1] | [22:2] | [22:3] | [24:0] | [24:1] |
|-------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| μ           | 7.0   | 2.9   | 12.5  | 17.4  | 37.9  | 1.9   | 12.6  | 1.7   | 1.5   | 0.4   | 2.8   | 0.2   | 0.5   | 0.2   | 0.6   |
| σ           | 0.6   | 0.4   | 2.0   | 2.0   | 3.1   | 0.3   | 0.9   | 0.3   | 0.3   | 0.1   | 0.4   | 0.0   | 0.1   | 0.0   | 0.1   |
| C<sub>v</sub> | 9%    | 14%   | 16%   | 12%   | 8%    | 18%   | 7%    | 15%   | 17%   | 18%   | 16%   | 21%   | 25%   | 23%   | 14%   |
| Max         | 9.3   | 5.2   | 24.3  | 25.4  | 43.0  | 3.9   | 17.4  | 2.2   | 2.0   | 0.8   | 4.0   | 0.3   | 0.8   | 0.4   | 0.8   |
| Min         | 4.8   | 2.1   | 8.8   | 10.8  | 19.0  | 1.2   | 9.6   | 0.9   | 0.4   | 0.3   | 1.6   | 0.1   | 0.1   | 0.1   | 0.4   |

μ: mean, σ: standard deviation, C<sub>v</sub>: coefficient of variation, Max: maximum, Min: minimum
TABLE III. STATISTICAL RESULTS OF THE MORPHOLOGY CHARACTERIZATION

| Statistical | Production (g) | Seeds | TSW (g) | FA % |
|-------------|----------------|-------|---------|------|
| μ           | 3.0            | 2613  | 1.11    | 33.2 |
| σ           | 1.0            | 821   | 0.23    | 2.2  |
| C_v         | 33%            | 31%   | 21%     | 7%   |
| Max         | 4.4            | 4651  | 1.56    | 37.1 |
| Min         | 0.1            | 93    | 0.3     | 24.2 |

Seeds: number of seeds, TSW: thousand seed weight, FA: fatty acid content, μ: mean, σ: standard deviation, C_v: coefficient of variation, Max: maximum, Min: minimum

TABLE IV. STATISTICAL RESULTS OF THE SELECTED PHYSICAL AND CHEMICAL PROPERTIES OF DERIVED BIODIESEL

| Statistical | Viscosity (μ) | Iodine value (IV) | Cold filter plugging point | Cetane number |
|-------------|--------------|-------------------|----------------------------|---------------|
| μ           | 4.1          | 167               | –2.0                       | 45            |
| σ           | 0.0          | 6                 | 1.9                        | 1             |
| C_v         | 1%           | 3%                | 94%                        | 3%            |
| Max         | 4.4          | 177               | 7.8                        | 53            |
| Min         | 4.0          | 131               | –5.3                       | 43            |

μ: mean, σ: standard deviation, C_v: coefficient of variation, Max: maximum, Min: minimum

The fatty acid [18:3] is the predominant acid. It is in the fatty acid [22:3] where a remarkable difference between the maximum and minimum values is observed.

Morphological characterization of camelina seeds is also presented in this work. In Appendix 1, a small variation in the fatty acid percentage (FA %) can be seen among the different varieties. It can be pointed out that significant differences can only be noticed in varieties with very low production, such as 1164 and 1167. The maximum value for fatty acid content is 37.1 % corresponding to the variety N11 (5.10) with an average height of 115.0 cm, biomass of 10.87 g, number of branches 15, total silicles 54 and shattering 7. The minimum value is found in variety 1164 with an average height of 100.0 cm, biomass of 7.8 g, number of branches 6, total silicles 35 and shattering 5, which is also the one with the lowest production. However, no direct relation can be confirmed between low production and low FA %. In fact, variety 1385 has a production of 2 g, which is lower than the production of 1036 (3.2 g), and 1085 has a fatty acid percentage 2.5 % higher. It is worth mentioning that in varieties where production is very low, such as 102, 1109, 1164 and 1167, FA % is also very low (27.7, 27.1, 24.2 and 25.5 %, respectively).

The mean value for the number of seeds is 2613 (table III). More than 50 % of the varieties investigated achieved a number of seeds above the mean. Variety 1118 is the one with the highest number, 4651. On the other hand, the lowest number of seeds, 93, was produced by the variety 1164. This variety, together with 1167, present the lowest productions, 0.1 g.

It should be noted that, in relation to the thousand seed weight (TSW), 47 % of the tested varieties did not achieve the mean value for this parameter. Another noteworthy aspect is that 27 % of the varieties, such as 1205 and 1226 (with a number of seeds of 3424 and 3654, respectively), have a very low TSW value (0.93 and 0.98 g, respectively). This means that, even though the production is high, the size of these seeds is unsatisfactory. The ideal option would be to achieve equilibrium between the production of seeds and their size. This compromise can be observed in N5 (1.9), where the number of seeds is very high (3010), as well as the TSW value (1.41).

In order to commercialize biodiesel, four parameters, included in table IV, have been selected to benchmark the varieties investigated in this paper. According to viscosity, Camelina-derived biodiesel will have an average value of 4.1 mm²/s. Variety 1109 is the one with the maximum value, whilst the minimum value of 4.0 mm²/s is observed in several varieties. Despite the fact that variety 1109 presents the highest fluidity due to its high viscosity, its value is within the range suggested by the European EN 14214 and the American ASTM 6751.

The high results for iodine values reveal that these varieties of camelina present a large number of double bonds. Oils tend to have more double bonds than other substances, which explains their liquid state at room temperature. The iodine value of these varieties is very high, with an average value of 167 because camelina seeds are characterized for being unsaturated oily seeds.

In relation to the cold filter plugging point (CFPP), the mean value is –0.2 ºC. Just by considering this average value, it seems that biodiesel produced from these varieties will not be suitable for countries with low temperatures, as it will easily clog the vehicles’ engines. However, some of the varieties could be used in biodiesel production (Appendix 1). Sixteen varieties have been identified to have a CFPP above the established limit (0 ºC), for instance, 1109 has a CFPP of 7.8 ºC.
The last parameter taken into consideration to evaluate biodiesel quality is the cetane number. The lowest value for this parameter is shown in table IV. From the total amount of varieties studied, only 13 achieved a suitable cetane number for biodiesel according to the American ASTM 6751. It can also be highlighted from Appendix 1, that in most cases with favorable cetane numbers (CN), the CFPP is also above the limit. Taking these two parameters in consideration, the most appropriate variety is 1109 because of its high CFPP (7.8 °C) and high ignition capacity (53).

DISCUSSION

The results obtained confirm the relevance of the fatty acid content in all camelina varieties being studied (minimum 24 %, maximum 37 %, average 33 %). The comparison between average fatty acid contents and compositions from this work and other studies is presented in table V. Our figures are within the expected results. However, this study shows large differences between camelina varieties in terms of net productivity, the number of seeds and thousand seed weight. These properties clearly differ between varieties, presenting a coefficient of variation over 20 %, maximum 33 %. The wide range of studied varieties could explain these differences. Some of them come from regions with extreme climate conditions, such as Russia. In these areas, competition between species is reduced and plants do not need large numbers of seeds to assure its effective reproduction.

The comparison of the physical and chemical properties of biodiesel with reference numbers shows important insights. Viscosity figures widely accomplish the standard references; however, all varieties failed to accomplish the reference values for iodine and most of them fell short of the reference values for cold filter plugging point and cetane number. This clearly prevents the widely use of camelina for biodiesel production and stresses the need of plant breeding.

The fatty acid profile differs from the chain length and number of unsaturations. Generally, as the chain length increases, there is an increase in the cetane number and other properties such as heating value, viscosity, and melting range-E, which influence the cold filter plugging point. Likewise, a high unsaturation degree of the methyl esters leads to high percentages in the previously mentioned properties (Viola et al. 2011). This is the case of camelina varieties, which have a high degree of unsaturation in the fatty acid profile. This will lead to poor oxidative stability. Berti et al. 2016 previously detected this weakness of camelina for biodiesel production. The improvement in biodiesel use could be developed by different strategies. Firstly, the extraction of fatty acids could be segmented, leaving unsaturated fatty acids for other uses. Secondly, camelina varieties with better profiles, such as variety 138, which has a higher percentage of saturated acids (which will enhance the oxidative stability), could be studied and improved by means of plant breeding and manipulation of culture conditions. Finally, it should be considered that in oilseeds, the linolenic acid [18:3] content varies with temperature during seed development. At high temperatures, the synthesis of linolenic acid decreases causing an increase in the two other major constituents, oleic and linoleic acid ([18:1] and [18:2], respectively).

Future plant breeding must consider fatty acid productivity and estimated properties for biodiesel. On the one hand, varieties 1072, 117 and N5 (1.9) have large fatty acid productivity but the estimated properties for biodiesel production are far from the reference figures. On the other hand, the estimated properties for biodiesel production from varieties 1008, 1085 and 1109 are close to accomplishing the standards.

It has been reported that camelina is a flexible crop and that different climatic and soil conditions can affect the fatty acid profile (Zubr and Matthäus 2002). The application of effective tools, such as image

| Location | Content [16:0] | [18:0] | [18:1] | [18:2] | [18:3] | [20:1] | [22:1] | Others | Reference |
|----------|---------------|-------|-------|-------|-------|-------|-------|-------|-----------|
| Spain    | 33.2          | 7.0   | 2.9   | 12.5  | 17.4  | 37.9  | 12.6  | 2.8   | 13.9      | This study |
| Canada   | 40.5          | 6.6   | —     | 15.0  | 17.1  | 37.0  | 14.5  | 3.2   | 13.2      | (Gugel and Falk 2006) |
| Canada   | 37.5          | —     | 2.2   | 14.5  | 18.2  | 34.1  | 15.0  | 3.7   | 12.3      | (Jiang et al. 2014) |
| Germany  | 39.0          | 6.5   | 2.3   | 16.2  | 18.1  | 39.0  | 13.2  | 2.6   | 8.6       | (Zubr and Matthäus 2002) |
| Slovenia | 33.0          | 6.0   | 3.0   | 16.3  | 18.7  | 33.0  | 16.0  | 3.0   | 10.0      | (Rode 2002) |
descriptors can improve selective breeding and plant phenotype, which will ultimately produce enhanced sowing seeds (Wiwart et al. 2019). In addition, conventional breeding techniques have been studied to improve camelina’s properties (Vollmann and Eynck 2015). Hybridization breeding between Camelina sativa and C. alyssum, C. microcarpa and C. rumelica resulted in successful breeding, while breeding was unsuccessful with other more distant related crop species like Brassica napus, B. rapa, and B. juncea (Séguin-Swartz et al. 2013). Although conventional breeding has proven to improve to some extent the fatty acids profile of camelina, this process is slow. Recent investigations have been carried out to improve fatty acid content through bioengineering. Mutation breeding has resulted in an improved seed lipid composition aimed at the production of high-value industrial products (Sainger et al. 2017). As a complement to conventional breeding, bioengineering has opened a new window to obtain new accessions with desirable characteristics that may be of interest for future industrial applications.

CONCLUSIONS

The fatty acid content and profile of camelina varieties are the cornerstone to assess its potential for quality biodiesel production and establish criteria for plant breeding. All camelina varieties have a similar percentage of fatty oil content. There are large differences in productivity and in the estimation of selected physical and chemical properties of the derived biodiesel from the varieties of camelina studied. The key to turn camelina into a good component for production of biofuels is to reduce the high degree of unsaturation and promote varieties with high productivity. These improvements could be done by conventional plant breeding. The cultivation of camelina and the products derived from camelina’s production, such as oil and flour, will provide a sustainable and viable short-term solution for both the fuel industry and the food sector.

ACKNOWLEDGMENTS

The authors are grateful to the Ministry of Economy, Industry and Competitiveness for funding this study as part of the RTC2015-3265-5 project. The authors extend their sincere thanks to Jesús García for his technical assistance.

REFERENCES

Acquaah G. (2015). Conventional plant breeding principles and techniques. In: Advances in plant breeding strategies: breeding, biotechnology and molecular tools (Jameel M., Mohan J.S. and Dennis J., Eds.) Springer, Cham, Switzerland, 115-58. https://doi.org/10.1007/978-3-319-22521-0_5
Allen C.A.W., Watts K.C., Ackman R.G. and Pegg M.J. (1999). Predicting the viscosity of biodiesel fuels from their fatty acid ester composition. Fuel 78 (11), 1319-1326. https://doi.org/10.1016/S0016-2361(99)00059-9
Anwar F., Rashid U., Ashraf M. and Nadeem M. (2010). Okra (Hibiscus esculentus) seed oil for biodiesel production. Appl. Energy 87 (3), 779-785. https://doi.org/10.1016/j.apenergy.2009.09.020
Bacenetti J., Restuccia A., Schillaci G. and Failla S. (2017). Biodiesel production from unconventional oilseed crops (Linum usitatissimum L. and Camelina sativa L.) in Mediterranean conditions: Environmental sustainability assessment. Renew. Energ. 112, 444-456. https://doi.org/10.1016/j.renene.2017.05.044
Barnwal B.K. and Sharma M.P. (2005). Prospects of biodiesel production from vegetable oils in India, Renew. Sustain. Energ. Rev. 9 (4), 363-378. https://doi.org/10.1016/j.rser.2004.05.007
Berrios M., Gutiérrez M.C., Martín M.A. and Martín A. (2009). Application of the factorial design of experiments to biodiesel production from lard. Fuel Process. Technol. 90 (12), 1447-1451. https://doi.org/10.1016/j.fuproc.2009.06.026
Berti M., Gesch R., Eynck C., Anderson J. and Cermak S. (2016). Camelina uses, genetics, genomics, production, and management. Ind. Crops Prod. 94, 690-710. https://doi.org/10.1016/j.indcrop.2016.09.034
Da Conceição L.R.V ., Carneiro L.M., Rivaldi J.D. and de Castro H.F. (2016). Solid acid as catalyst for biodiesel production via simultaneous esterification and transesterification of macaw palm oil. Ind. Crops Prod. 89, 416-424. https://doi.org/10.1016/j.indcrop.2016.05.044
Devan P.K. and Mahalakshmi N.V . (2009). A study of the performance, emission and combustion characteristics of a compression ignition engine using methyl ester of paradise oil-eucalyptus oil blends. Appl. Energ. 86 (5), 675-680. https://doi.org/10.1016/j.apenergy.2008.07.008
Dorado M. (2003). Exhaust emissions from a Diesel engine fueled with transesterified waste olive oil. Fuel 82 (11), 1311-1315. https://doi.org/10.1016/S0016-2361(03)00034-6
EBTP (2011). Fatty acid methyl esters (FAME). European Biofuel Technology Platform [online]. http://www.etipbioenergy.eu/images/fame-fact-sheet.pdf

Fernandes D.M., Sousa R.M.F., de Oliveira A., Morais S.A.L., Richter E.M. and Muñoz R.A.A. (2015). Morinda oleifera: A potential source for production of biodiesel and antioxidant additives. Fuel 146, 75-80. https://doi.org/10.1016/j.fuel.2014.12.081

Harre D., Saleh A. and Hamdan S. (2018). An experimental investigation of Karanja biodiesel production in Sarawak, Malaysia. Journal of Engineering 2018, 4174205. https://doi.org/10.1016/2018/18474205

Huang G., Chen F., Wei D., Zhang X. and Chen G. (2010). Biodiesel production by microalgal biotechnology. Appl. Energ. 87 (1), 38-46. https://doi.org/10.1016/j.apenergy.2009.06.016

Iha O.K., Alves F.C.S.C., Suarez P.A.Z., Silva C.R.P., Meneghetti M.R. and Meneghetti S.M.P. (2014). Potential application of Terminalia catappa L. and Carapa guianensis oils for biofuel production: Physical-chemical properties of neat vegetable oils, their methyl-esters and bio-ols (hydrocarbons). Ind. Crops Prod. 52, 95-98. https://doi.org/10.1016/j.indcrop.2013.10.001

Iskandarov U., Kim H.J. and Cahoon E.B. (2014). Camelina: An emerging oilseed platform for advanced biofuels and bio-based materials. In: Plants and bioenergy (Maureen C., Marcos A. and Nicholas C., Eds.). Springer, New York, USA, 131-140. https://doi.org/10.1007/978-1-4614-9329-7_8

Jena P.C., Rahaman H., Prasanna G.V. and Machavaram R. (2010). Biodiesel production from mixture of mahua and simarouba oils with high free fatty acids. Biomass Bioenergy. 34 (8), 1108-1116. https://doi.org/10.1016/j.biombioe.2010.02.019

Jiang Y., Caldwell C.D. and Falk K.C. (2014). Camelina seed quality in response to applied nitrogen, genotype and environment. Can. J. Plant Sci. 94 (5), 971-980. https://doi.org/10.4141/cjps2013-396

Kakati J., Gogoi T.K. and Pakshirajan K. (2017). Production of biodiesel from amari (Amoora wallichii King) tree seeds using optimum process parameters and its characterization. Energy Convers. Manag. 135, 281-290. https://doi.org/10.1016/j.enconman.2016.12.087

Kirkhus B., Lundon A.R., Haugen J.E., Vogt G., Borge G.I.A. and Henriksen B.I.F. (2013). Effects of environmental factors on edible oil quality of organically grown Camelina sativa. J. Agric. Food Chem. 61 (13), 3179-3185. https://doi.org/10.1021/jf304532u

Knothe G. (2009) Improving biodiesel fuel properties by modifying fatty ester composition. Energ. Environ. Sci. 2 (7), 759-766. https://doi.org/10.1039/B903941D

Larsson M. (2013). Cultivation and processing of Linum usitatissimum and Camelina sativa in southern Scandinavia during the Roman Iron Age. Veg. Hist. Archaeobot. 22 (6), 509-520. https://doi.org/10.1007/s00334-013-0413-3

Mahmudul H.M., Hagos F.Y., Mamat R., Adam A.A., Ishak W.F.W. and Alenezi R. (2017). Production, characterization, and performance of biodiesel as an alternative fuel in diesel engines – A review. Renew. Sustain. Energ. Rev. 72, (C) 497-509. https://doi.org/10.1016/j.rser.2017.01.001

Mondala A., Liang K., Toghihani H., Hernandez R. and French T. (2009). Biodiesel production by in situ transesterification of municipal primary and secondary sludges. Bioresour. Technol. 100 (3), 1203-1210. https://doi.org/10.1016/j.biortech.2008.08.020

Moser B.R. and Vaughn S.F. (2010). Evaluation of alkyl esters from Camelina sativa oil as biodiesel and as blend components in ultra-low-sulfur diesel fuel. Bioresour. Technol. 101 (2), 646-653. https://doi.org/10.1016/j.biortech.2009.08.054

Muñoz I., Apeleo E., de la Fuente J., Pérez-Santaescalástica C., Rivas-Cañedo A., Pérez C., Diaz M.T., Cañete V. and Lauzurica S. (2014). Effect of dietary supplementation with red wine extract or vitamin E, in combination with linseed and fish oil, on lamb meat quality. Meat Sci. 98 (2), 116-123. https://doi.org/10.1016/j.meatsci.2014.05.009

Patil P.D., Gude V.G., Camacho L.M. and Deng S. (2010). Microwave-assisted catalytic transesterification of Camelina sativa oil. Energ. Fuel. 24 (2), 1298-1304. https://doi.org/10.1021/ef9010065

Petursson S. (2002). Clarification and expansion of formulas in AOCs recommended practice 1C-85 for the calculation of iodine value from FA composition. J. Am. Oil Chem. Soc. 79, 737-738.

Rahbigh M.F., Taufiq Y.H., Rashid U., Teo S.H., Shajarutun Z.A. and Islam A. (2014). Production of biodiesel from non-edible Jatropha curcas oil via transesterification using Bi2O3-La2O3 catalyst. Energ. Convers. Manag. 88, 1257-1262. https://doi.org/10.1016/j.enconman.2014.02.072

Ramos M.J., Fernández C.M., Casas A., Rodríguez L. and Simarouba L. (2014). Production of biodiesel and antioxidant additives in Sarawak, Malaysia. Journal of Engineering 2018, 4174205. https://doi.org/10.1016/2018/18474205

Reshad A.S., Tiwari P. and Goud V.V. (2015). Extraction of oil from rubber seeds for biodiesel application: Optimization of parameters. Fuel 150, 636-644. https://doi.org/10.1016/j.fuel.2015.02.058

Saiyer M., Jaiwal A., Saiyer P.A., Chaudhary D. and Jaiwald R. (2017). Advances in genetic improvement
of *Camelina sativa* for biofuel and industrial bio-products. Renew. Sust. Energ. Rev. 68 (1), 623-637. https://doi.org/10.1016/j.rser.2016.10.023

Séguin-Swartz G., Nettleton J. A., Sauder C., Warwick S. I. and Gugel R. K. (2013). Hybridization between *Camelina sativa* (L.) Crantz (false flax) and North American camelina species. Plant Breed. 132, 390-396. https://doi.org/10.1111/pbr.12067

Silitonga A.S., Masjuki H.H., Mahlia T.M.I., Ong H.C., Kusumo F., Aditiya H.B. and Ghazali N.N.N. (2015). *Schleichera oleosa* L. oil as feedstock for biodiesel production. Fuel 156, 63-70. https://doi.org/10.1016/j.fuel.2015.04.046

Sukhija P.S. and Palmquist D.L. (1988). Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36 (6), 1202-1206. https://doi.org/10.1021/jf00084a019

Szybist J.P., Song J., Alam M. and Boehman A.L. (2007). Biodiesel combustion, emissions, and emission control. Fuel Process. Technol. 88 (7), 679-691. https://doi.org/10.1016/j.fuproc.2006.12.008

Thiruvengadaravi K.V., Nandagopal J., Baskaralingam P., Sathya V. and Sivanesan S. (2012). Acid-catalyzed esterification of karanja (*Pongamia pinnata*) oil with high free fatty acids for biodiesel production. Fuel 98, 1-4. https://doi.org/10.1016/j.fuel.2012.02.047

Viola E., Zimbardi F. and Valerio V. (2011). Graphical method to select vegetable oils as potential feedstock for biodiesel production. Eur. J. Lipid Sci. Technol. 113 (12), 1541-1549. https://doi.org/10.1002/ejlt.201000559

Vollmann J. and Eynck C. (2015). Camelina as a sustainable oilseed crop: Contributions of plant breeding and genetic engineering. Biotechnol. J. 10 (4), 525-535. https://doi.org/10.1002/biot.201400200

Wiwart M, Kurasiak-Popowska D., Suchowilska E., Wachowska U. and Stuper K. (2019). Variation in the morphometric parameters of seeds of spring and winter genotypes of *Camelina sativa* (L.) Crantz. Ind. Crop. Prod. 139, 111571. https://doi.org/10.1016/j.indcrop.2019.111571

Yang J., Caldwell C., Corscadden K., He Q.S. and Li J. (2016). An evaluation of biodiesel production from *Camelina sativa* grown in Nova Scotia. Ind. Crop. Prod. 81, 162-168. https://doi.org/10.1016/j.indcrop.2015.11.073

Zaleckas E., Makarevičienė V. and Sendžikienė E. (2012). Possibilities of using *Camelina sativa* oil for producing biodiesel. Fuel Transport. 27 (1), 60-66. https://doi.org/10.3846/16484142.2012.664827

Zubr J. and Matthäus B. (2002). Effects of growth conditions on fatty acids and tocopherols in *Camelina sativa* oil. Ind. Crop. Prod. 15 (2), 155-162. https://doi.org/10.1016/S0926-6690(01)00106-6
### Appendix 1: Fatty Acid Profile and Derived Parameters from All Varieties Under Study

| Variety | P(g) | CN | IV | CFPP | FA (%) |
|---------|------|----|----|------|--------|
| 1       | 8.8  | 1.8| 1.3| 1.9  | 41     |
| 2       | 3.5  | 2.0| 1.9| 2.0  | 37.2   |
| 3       | 8.8  | 1.8| 1.3| 1.9  | 41     |
| 4       | 3.5  | 2.0| 1.9| 2.0  | 37.2   |
| 5       | 8.8  | 1.8| 1.3| 1.9  | 41     |
| 6       | 3.5  | 2.0| 1.9| 2.0  | 37.2   |
| 7       | 8.8  | 1.8| 1.3| 1.9  | 41     |
| 8       | 3.5  | 2.0| 1.9| 2.0  | 37.2   |
| 9       | 8.8  | 1.8| 1.3| 1.9  | 41     |
| 10      | 3.5  | 2.0| 1.9| 2.0  | 37.2   |
| 11      | 8.8  | 1.8| 1.3| 1.9  | 41     |
| 12      | 3.5  | 2.0| 1.9| 2.0  | 37.2   |
| 13      | 8.8  | 1.8| 1.3| 1.9  | 41     |
| 14      | 3.5  | 2.0| 1.9| 2.0  | 37.2   |
| 15      | 8.8  | 1.8| 1.3| 1.9  | 41     |
| 16      | 3.5  | 2.0| 1.9| 2.0  | 37.2   |
| 17      | 8.8  | 1.8| 1.3| 1.9  | 41     |
| 18      | 3.5  | 2.0| 1.9| 2.0  | 37.2   |
| 19      | 8.8  | 1.8| 1.3| 1.9  | 41     |
| 20      | 3.5  | 2.0| 1.9| 2.0  | 37.2   |

**Fatty Acid Profile and Derived Parameters:**
- **CFPP:** Cold filter plugging point
- **CN:** Cetane number
- **Prod:** Productivity
- **TSW:** Thousand seed weight
- **FA:** Fatty acid
## APPENDIX 1. FATTY ACID PROFILE AND DERIVED PARAMETERS FROM ALL VARIETIES UNDER STUDY

| Variety | 1076 | 1077 | 1079 | 1081 | 1083 | 1085 | 1089 | 1092 | 1093 | 1096 | 1098 | 1093 | 1096 | 1098 | 1099 | 1100 | 1102 | 1104 | 1108 | 1112 | 1115 | 1119 | 1123 | 1126 | 1129 | 1132 | 1135 | 1138 | 1141 | 1144 | 1147 | 1150 | 1153 | 1156 |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| CFPP    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| CN      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Prod    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| TSW     |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| FA      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Seeds   |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| P(g)    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| IV      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| μ       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |

CFPP: cold filter plugging point, CN: cetane number, Prod: productivity, TSW: thousand seed weight, FA: fatty acid
| Variety | [160] | [161] | [162] | [163] | [164] | [165] | [166] | [167] | [168] | [169] | [170] | [171] | [172] | [173] | [174] | [175] | [176] | [177] |
|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| TSW (g) | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    |
| FA (%)  | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    |
| IV      | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    |
| CFPP:   | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    |
| CN:     | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    |
| Prod:   | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    |
| TSW:    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    |
| FA:     | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    | 4.1    |

APPENDIX 1. FATTY ACID PROFILE AND DERIVED PARAMETERS FROM ALL VARIETIES UNDER STUDY

CFPP: cold filter plugging point, CN: cetane number, Prod: productivity, TSW: thousand seed weight, FA: fatty acid
## APPENDIX 1. FATTY ACID PROFILE AND DERIVED PARAMETERS FROM ALL VARIETIES UNDER STUDY

| Variety | 16:0 | 18:0 | 18:1 | 18:2 | 20:0 | 20:1 | 20:2 | 20:3 | 22:0 | 22:1 | 22:2 | 22:3 | 24:0 | 24:1 | μ  | IV | CFPP | CN | P (g) | Seeds | TSW (g) | FA (%) |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|----|-----|------|----|------|-------|---------|------|
| N1(5.5) | 6.8  | 2.8  | 10.7 | 18.0 | 38.5 | 1.9  | 12.4 | 2.0  | 1.7  | 0.4  | 3.0  | 0.2  | 0.7  | 0.2  | 0.7 | 4.1 | 170 | -2.3 | 45   | 3.3  | 2386 | 1.38 | 35.3 |
| N1(2.2) | 6.6  | 3.0  | 13.1 | 15.5 | 39.5 | 1.8  | 13.1 | 1.4  | 1.5  | 0.3  | 2.8  | 0.1  | 0.5  | 0.1  | 0.6 | 4.1 | 169 | -2.4 | 45   | 3.5  | 3693 | 0.95 | 34.1 |
| N5(1.9) | 7.1  | 2.9  | 10.3 | 19.3 | 37.4 | 1.9  | 12.3 | 2.0  | 1.5  | 0.4  | 3.0  | 0.2  | 0.6  | 0.2  | 0.7 | 4.1 | 169 | -1.9 | 45   | 4.2  | 3010 | 1.41 | 35.2 |
| N5(4.14)| 6.7  | 2.5  | 11.8 | 17.5 | 39.1 | 1.6  | 12.3 | 1.8  | 1.6  | 0.3  | 3.0  | 0.2  | 0.6  | 0.2  | 0.7 | 4.1 | 171 | -4.0 | 45   | 3.7  | 2984 | 1.25 | 34.6 |
| N8(2.8) | 6.8  | 3.0  | 12.0 | 16.4 | 38.0 | 2.1  | 13.4 | 1.5  | 1.5  | 0.4  | 3.3  | 0.2  | 0.6  | 0.2  | 0.6 | 4.1 | 166 | -1.0 | 46   | 3.8  | 3411 | 1.11 | 34.8 |
| N8     | 6.9  | 2.7  | 10.4 | 17.9 | 38.8 | 1.8  | 12.8 | 1.9  | 1.6  | 0.4  | 3.1  | 0.2  | 0.7  | 0.2  | 0.7 | 4.1 | 170 | -2.6 | 45   | 3.0  | 2635 | 1.16 | 34.4 |
| N11(1.5)| 7.0  | 2.5  | 10.1 | 17.6 | 38.7 | 1.9  | 12.8 | 2.0  | 1.6  | 0.4  | 3.6  | 0.3  | 0.7  | 0.2  | 0.5 | 4.1 | 170 | -2.6 | 45   | 4.0  | 3030 | 1.32 | 34.8 |
| N11(3.7)| 6.9  | 2.5  | 10.1 | 17.1 | 39.0 | 2.0  | 13.0 | 2.0  | 1.7  | 0.4  | 3.7  | 0.3  | 0.7  | 0.2  | 0.5 | 4.1 | 170 | -2.4 | 45   | 3.6  | 2549 | 1.43 | 34.6 |
| N11(5.10)| 6.9 | 2.5  | 11.0 | 16.4 | 39.1 | 1.9  | 13.0 | 2.0  | 1.8  | 0.4  | 3.6  | 0.2  | 0.7  | 0.2  | 0.5 | 4.1 | 170 | -2.8 | 45   | 4.1  | 3104 | 1.32 | 37.1 |
| 6.1    | 2.6  | 12.7 | 17.0 | 38.1 | 1.6  | 13.8 | 1.9  | 1.7  | 0.3  | 2.7  | 0.2  | 0.5  | 0.2  | 0.6 | 4.1 | 169 | -3.9 | 45   | 3.2  | 3004 | 1.07 | 36.1 |

CFPP: cold filter plugging point, CN: cetane number, Prod: productivity, TSW: thousand seed weight, FA: fatty acid