

Camelina (*Camelina sativa* L. Crantz) under low-input management systems in northern Italy: Yields, chemical characterization and environmental sustainability

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Abstract

Camelina can be considered a valuable crop for bio-based products and biofuels, but, to date, there are still many uninvestigated aspects concerning the optimization of its agricultural management and its environmental impact. Consequently, a low-input camelina cultivation has been realized, in northern Italy environment, through a 4-year camelina-wheat rotation in open field. In these conditions, camelina was grown as winter crop. Camelina reached, over the years, a variable (CV=28%) mean seed yield of 0.82 Mg ha⁻¹. This notwithstanding, the oil content - 39.17%

(CV=3%) - and its related quality were rather stable, reaching an oil yield of 320 kg ha⁻¹ particularly rich in omega-3 fatty acids.

The low input cultivation system here adopted implied an energy ratio (output energy/input energy) of 4 and a 30% decrease in Global Warming Potential per hectare, compared to the standard value reported by the European Renewable Energy Directive for sunflower, reducing, at the same time, other relevant environmental burdens. However, due to its relatively low oil production, the full use of all camelina co-products should be considered in order to fulfil the sustainability requirements for European jet fuel production. In fact, stability of yields and quality of oil, oilcake and straws makes low-input camelina eligible for many other novel green chemistry applications.

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Introduction

Camelina [*Camelina sativa* (L.) Crantz] is a minor annual oilseed crop that has been cultivated in Europe since the Bronze Age (Zubr, 1997). Over recent years, it has received increasing attention as a dedicated oilseed feedstock for bio-based products and biofuels. Indeed, commercial ventures and airlines find the features of its oil of great interest, and suitable for many applications, particularly for jet fuel (Corporan *et al.*, 2011). Camelina shows several beneficial agronomic qualities, such as a short growing season, ranging from 70 to 250 days, from sowing to maturity, as spring or winter crop respectively. Furthermore, this crop showed significant compatibility with existing farming practices and high adaptability to a wide range of environmental conditions (Angelini *et al.*, 1997; Berti *et al.*, 2011; Angelini, 2012; Guy *et al.*, 2014; Masella *et al.*, 2014). Camelina can resist at temperatures lower than -15°C and, for this reason, it is well adapted to the northern regions of the Boreal Hemisphere (Schillinger *et al.*, 2012). At this regard, Gesch and Cermak (2011) observed that waterlogged soil could be more harmful to winter camelina survival, than cold stress. Camelina is also resistant to drought conditions, and this characteristic makes it an ideal crop for areas with insufficient rainfall to support other crops (Murphy, 2016). In addition, due to its resistance to pests and diseases and reduced nutritional requirements, camelina requires lower pesticide (Li *et al.*, 2005) and fertilizer amounts, compared to other traditional oilseed crops, such as rapeseed/canola, soybean, and sunflower

(Končius, and Karčauskiene, 2010). For all these reasons, camelina is classified as a well-adapted crop to northern cold areas, southern arid regions, and, generally, suitable for less-favoured areas (LFA) for agriculture. Indeed, in Italian climate conditions, it can be cultivated both as a winter and spring crop (Angelini, 2012; Zanetti *et al.*, 2017).

Camelina siliques contain small, slightly oval seeds with a thousand seed weight (TSW) around 1.0-1.25 g (Schillinger *et al.*, 2012; Zanetti *et al.*, 2017). Seed yield and oil content are highly variable depending on environmental conditions, genotypes and sowing time (Angelini *et al.*, 1997; Zubr, 1997; Vollmann *et al.*, 2007). For instance, in Masella *et al.* (2014), the overall mean seed yield, obtained in a plot trial carried out in northern Italy, was 1340 kg ha⁻¹, with a large fluctuation (130-3900 kg ha⁻¹) according to year, sowing time and genotype. Furthermore, in a 3-year trial, carried out in Canada under different pedo-climatic conditions, Malhi *et al.* (2014) reported seed yields ranging from 261 to 1603 kg ha⁻¹ with no nitrogen fertilization. In central Italy, Angelini (2012) found a seed yield of 610 and 1315 kg ha⁻¹ in winter and spring crops, respectively, in a field experiment with a plant density of 500.000 plants ha⁻¹.

At the same time, according to Righini *et al.* (2016), camelina oil content varies from 26% to 43% in southern and northern Europe, respectively. Similarly, seed quality is particularly affected by environmental factors, such as temperature, precipitation, solar radiation, evapotranspiration, air circulation, and by genotype (Zubr, 2003; Righini *et al.*, 2016; Zanetti *et al.*, 2017). Camelina oil is characterized by a very high content of unsaturated fatty acids (FAs) such as oleic (18:1, 14-16%), linoleic (LA), (18:2, 15-23%), eicosenoic (20:1, 12-15%) and, in particular, α -linolenic acid (ALA) (18:3 n-3, 31-40%) (Berti *et al.*, 2016), which is an omega-3 FA with interesting potential benefits to human and animal diets (Ibrahim and El Habbasha, 2015). Similarly, the acidic profile of camelina oil, as a whole, opens several potential applications for innovative bio-based products, bioenergy, cosmetic, and in many other sectors (Shonnard *et al.*, 2010; Kirkhus *et al.*, 2013; Hixson *et al.*, 2014; Iskandarov *et al.*, 2014; Kim *et al.*, 2015). Furthermore, camelina defatted seed cake, in addition to its interesting protein content, also contains several secondary bioactive compounds such as glucosinolates, polyphenols, carotenoids (Matthäus and Zubr, 2000; Matthäus and Angelini, 2005; Pagnotta, 2019). Promising studies have shown that seed yield and FA profile can be improved through engineered genotypes (Dalal *et al.*, 2015; Liu *et al.*, 2015), but defining optimized and sustainable management is equally important. In Europe, camelina has been observed to have low nutritional requirements and is generally regarded as a low-input crop; however, it responds to high N fertilization rates when grown in environmental conditions that maximize seed yield potential (Solis *et al.*, 2013).

Furthermore, camelina can be introduced in cropping systems of less-favoured areas during fallow period, after wheat harvest. In this case, camelina could increase net profits only if low-cost production practices were adopted. In addition, the crop residues incorporated into the soil are much greater in camelina-wheat than in fallow-wheat rotation, which is likely to improve soil quality and ecological sustainability in the long-term (Chen *et al.*, 2015).

Currently, camelina is considered a valuable oilseed crop able to provide a range of renewable products both for food and non-food uses, but there are still several aspects that should be examined to assess its yield level and environmental impact. This is especially true when external inputs were reduced with a simultaneous reduction of their impact on the environment, but often some of benefits of such cropping systems are offset by lower yields.

Few studies have been performed on camelina adaptability to low-input techniques, in order to follow a proper biorefinery perspective, as required by the EU (Luguel, 2011), where a cascade use of the entire biomass, consisting in a hierarchical utilization of plant components, is applied.

Over recent years, the life cycle assessment (LCA, ISO 14044:2006) has been increasingly applied to support sustainable agricultural cropping systems, and new methodology challenges have been defined (Goglio *et al.*, 2017; Notarnicola *et al.*, 2017). Bioenergy crops are assessed mainly by carbon footprint (BSI, 2011) - following the Intergovernmental Panel on Climate Change (IPCC, 2006) account methods - and by energy depletion (Cherubini *et al.*, 2009).

The main objective of this work was to increase the knowledge associated to environmental impacts, related to camelina cultivation in the Mediterranean area. Accordingly, a 4-year camelina-wheat rotation system was studied with the aim of: i) assessing the agronomic potential of camelina under low-input management (seed and biomass yields, seed oil content and composition) and its stability over time; ii) assessing the global warming potential (GWP) and energy depletion by LCA applied to camelina; and iii) identifying its eco-efficiency in terms of environmental impacts per hectare and per seed yield.

Materials and methods

Experimental design and pedo-climatic conditions

The field trials were carried out at CREA experimental farm located in Budrio (Bologna) in the Po Valley area (Emilia Romagna region, 44°32'00"N; 11°29'33" E, altitude 28 m a.s.l.) over four growing seasons (2012-2016), hereinafter numbered from I to IV, applying a biennial rotation of camelina and wheat. The experimental field was split in two contiguous fields, 500 m² each, in order to rotate the crops both in space and time: i) *field A*: camelina-wheat-camelina-wheat; ii) *field B*: wheat-camelina-wheat-camelina. The physical and chemical soil characteristics, reported in Table 1, were measured on samples collected at the beginning of the experiment, and two years later, when camelina

Table 1. Physical and chemical characteristics of the soils where the experimental trials were carried out. Values are means \pm standard deviation.

Soil parameters*		Field A Silty-clay loam	Field B Silty-clay loam
Sand	(%)	8.3 \pm 1.0	17.7 \pm 3.1
Silt	(%)	56.7 \pm 2.5	50.0 \pm 2.0
Clay	(%)	35.0 \pm 1.6	32.3 \pm 1.2
pH (H ₂ O)	-	8.1 \pm 0.1	8.2 \pm 0.1
Total CaCO ₃	(%)	10.3 \pm 0.3	9.2 \pm 0.1
Active CaCO ₃	(%)	4.8 \pm 0.5	3.2 \pm 0.3
Organic carbon	(g/kg ⁻¹ DM)	12.0 \pm 0.4	10.3 \pm 0.9
Organic matter	(% DM)	2.1 \pm 0.1	1.8 \pm 0.2
Total nitrogen	(g kg ⁻¹)	1.4 \pm 0.1	1.3 \pm 0.2
Available phosphorus	(mg kg ⁻¹)	33.3 \pm 24.5	23.7 \pm 4.9
Exchangeable potassium	(mg kg ⁻¹)	233.5 \pm 4.7	210.0 \pm 15.5
C/N ratio	-	8.8 \pm 0.9	8.0 \pm 0.4

*Soils were sample to the 0.3-m depth. DM, dry matter.

and wheat had been cultivated at least once in the same field.

Soil was silty clay loam with a medium-high content of total nitrogen, available phosphorus and exchangeable potassium, relatively low content of organic matter and C/N, with a moderately alkaline reaction (Table 1). Despite its good fertility, the soil physical characteristics make it prone to weak water infiltration capacity and waterlogging, especially when abundant rainfall occurs.

Meteorological data were collected daily from a weather station located in the farm where the trials were carried out. For each camelina growing season, the GDD (Growing Degree Days) were calculated as: $GDD = \sum T_{\text{mean}} - T_{\text{base}}$, where T_{mean} was the daily mean air temperature, and T_{base} was 5°C, as suggested by Blackshaw *et al.* (2011) and Gesch (2014).

Cultivation techniques

A 2-year cropping system based on a wheat-camelina rotation was planned. Wheat (*Triticum aestivum* L.) var. Bologna was cultivated adopting integrated practices, according to Italian National Integrated Farm Management Guidelines (2019). Camelina was managed with the lowest possible inputs if compared with those applied by other authors (Gesch *et al.*, 2014; Bacenetti *et al.*, 2017) following the scale of importance proposed by SIMAPRO ver. 8.4.0.0 (pesticide>diesel>N-fertilizer>P₂O₅-fertilizer>K₂O-fertilizer>seeds>organic fertilizer). Wheat was sown at the end of October and was harvested every year at the end of June/beginning of July. One treatment of pinoxaden 3%, clodinafop-propargyl 3%, florasulam 0.76%, cloquintocet-mexyl 0.76%, 0.25 dm³ ha⁻¹ for weed control was yearly applied in March/April. One treatment with prothioconazole 12.7% and tebuconazole 12.7%, 1 dm³ ha⁻¹ was yearly applied in order to prevent fusarium and other diseases. One treatment with Tau-Fluvalinate 0.25 dm³ ha⁻¹ against aphids was yearly applied in May. Wheat was fertilized twice, in February and in April, with ammonium nitrate (27% N), 70 kg N ha⁻¹ each.

Camelina sativa var. Italia was provided by the *Brassicaceae* seed collection of CREA-CI (Bologna) (Lazzeri *et al.*, 2013). It was sown in autumn on 5th October 2012, 16th October 2013, 20th October 2014, and 30th September 2015; harvests were accomplished on 7th June 2013, 29th May 2014, 4th June 2015 and 8th June 2016. Cropping techniques and mechanization methods were defined according to the pedoclimatic conditions and the specific characteristics of the area (Table 2), with the aim of performing the experiments under low input management. No products for pest, pathogens nor weed control were applied. A high seeding rate of 12.5 kg ha⁻¹ was used for a better crop competition against weeds. Fertilization was provided through pelletized cattle and horse manure-based amendment (organic C 30%, organic N 2%, and moisture 18%) before sowing, at the rate of 583 kg ha⁻¹ y⁻¹, integrated with a low amount of ammonium nitrate (53 kg ha⁻¹ y⁻¹) in April with standing crop (Table 2). At seed maturity, three sample

areas of one square meter were randomly collected within each experimental field to assess crop yield and yield components - including thousand seed weight (TSW), grain yields, above- and below-ground biomass- which were then used for the subsequent LCA analysis. The plants were harvested manually and threshed by a fixed machine, using sieves suitable for small seeds in order to evaluate the expected grain yield. Thousand seed weight was assessed according to ISTA (2005). Field crop residues were removed.

Seed and oil characterization

After harvesting, seeds were cleaned, partially dried to reach a moisture content around 4%, ground to 0.5 mm size and analysed for their main components by the following procedures:

- *Dry matter* was evaluated by weighing the seeds after oven-drying at 40°C until constant weight.
- *Oil content* was measured by NMR (Nuclear Magnetic Resonance) technique by a MQC benchtop NMR analyser (Oxford Instruments) (ISO 10565:1998) calibrated for camelina seeds following Soxhlet official method (ISO 659:2009).
- *Lower heating value (LHV)* was determined by bomb calorimeter and CHN analyser (LECO corp.) following ASTM E711-87 (2004).
- *Fatty acid composition*. The oil was extracted from ground seeds by hexane and trans-methylated in 2N KOH methanol solution (Conte *et al.*, 1989). FA methyl ester composition was evaluated by a gas chromatography equipped with a flame ionization detector (Carlo Erba HRGC 5300 MEGA SERIES) and a capillary column Restek RT x 2330 (30 m × 0.25 mm × 0.2 µm), following the internal normalization method (ISO 12966-4:2015).

Environmental and energy evaluation

An environmental impact analysis was carried out in order to assess the sustainability of the implemented cultivation techniques. The adopted methodology was the LCA compliant with ISO 14044 (2006) and the guidelines reported in the Renewable Energy Directive (RED) 2009/28/EC (European Parliament, 2009). Methodological framework essentially confirmed the current EU Directive 2018/2001 (European Parliament, 2018), which will be transposed by Member States by 30 June 2021. The impact coefficients of the materials involved in cultivation were those reported in the Global Warming Report (IPCC, 2006) and the Annual European Union Greenhouse Gas Inventory 1990-2011 and Inventory Report 2013 (EEA, 2013). For further considerations regarding a possible production chain based on camelina, the Ecoinvent database ver.3.3 was also used. According to the RED methodology, energy incorporated into machinery was not considered.

Table 2. Camelina crop management protocol adopted in the study area (Budrio, Bologna, Italy).

Farming operations	Field set-up management
Primary tillage	Two-furrow plough following by chisel plough
Seedbed preparation	Spring-tine harrow
Sowing method	12.5 kg ha ⁻¹ y ⁻¹ on 15 cm spaced rows using a plot drill for wheat
Fertilization	Pelletized manure-based amendment (583 kg ha ⁻¹ y ⁻¹ , corresponding to 12 kg N ha ⁻¹ y ⁻¹) Ammonium nitrate (53 kg ha ⁻¹ y ⁻¹ on average, corresponding to 14 kg N ha ⁻¹ y ⁻¹)
Weed and Pest Control	No chemical applications or manual weeding
Residue removal	Tractor with cart

As regards the environmental impact assessment, the GWP with 100-year time horizon, expressed in terms of the amount of CO₂ equivalents (CO₂eq) was considered, as set out in the RED. The LCA includes the choice and definition of a functional unit that characterizes the production purpose of the analyzed system. In this study, the impacts referred to: i) one cultivated hectare; ii) one kg of grain; and iii) one MJ contained therein. As per RED recommendations, straw was not considered as a by-product. In fact, in this analysis only oil and oilcake were allocated on the basis of energy. The allocation factor was defined following D'Avino *et al.* (2015a). Residual oil content in the cake was considered equal to 8.3%, as mean value obtained from other comparable minor *Brassicaceae* defatted seed meals (D'Avino *et al.*, 2015b; Matteo *et al.*, 2018). Meal LHV was calculated from measured seed LHV and LHV of camelina oil reported by Masella *et al.* (2012). Energy analysis was carried out using the Energy ratio (ER) between output energy (OE) and input energy (IE), Energy cost (EC=IE/OE), Energy balance (EB=OE-IE) and Net Energy balance (NEB=EB/IE) following parameters recommended in the literature (Menichetti and Otto, 2009; Basset *et al.*, 2010). Amongst a variety of possibilities, use of the vegetable oil extracted from camelina in Jet-fuel systems was considered. Indeed, the Jet-fuel supply chain was the most quoted example from which impact data were available. The standard values and procedures adopted in software BioGrace (2014) ver. 4d and SIMAPRO ver. 8.4.0.0, in line with the RED sustainability criteria, were used to determine primary energy resource depletion and greenhouse gas (GHG) emissions of the agricultural phase. The specific input values used in this study are reported in Table 3.

In addition, for the calculation of N₂O emissions from fertilizers and crop residues, a specific spreadsheet was defined following the IPCC (2006) guidelines and used to calculate the measured biomass nitrogen content following Spugnoli *et al.* (2012). Moreover, an allocation factor for the extraction phase (only one with useful by-product) was obtained as follows:

$$\text{Allocation factor} = \frac{\text{Oil (mass)} * \text{LHV}_{\text{oil}}}{\text{Oil (mass)} * \text{LHV}_{\text{oil}} + \text{Meal (mass)} * \text{LHV}_{\text{meal}}} \quad (\text{Eq. 1})$$

Statistical analysis

All the analyses were carried out at least in triplicate where not otherwise specified, and statistical analysis of data, expressed as mean ± standard deviation and/or coefficient of variation (CV). To understand the behaviour of the camelina productivity, five Linear Mixed Effect (LME) models (Galecki and Burzykowski, 2013) were fitted to data to determine the relationship between grain yield and oil content (Y) and the explanatory variables (X) Eq. 2-6. Equations were based on R syntaxes in "nlme" package. These models differed in terms of their explanatory variables (with/without year, with/without plot effect and interactions), considering the pseudoreplicates as nested random factor as shown in Eq. 2-6, and the probability distribution of the residual error of the model and the estimation method used (frequentist) based on Maximum Likelihood (ML). All statistical analysis was performed with the R Statistical Software 3.5.1 (Foundation for Statistical Computing, Vienna, Austria). Particularly, LME were evaluated using "nlme" package. Furthermore, ANOVA was applied as post hoc test to models fitted. The criteria applied to select the best model were: i) simplicity, the model choice is always the simplest model that has the lower Akaike information criterion (AIC), and Bayesian information criterion (BIC); ii) Likelihood ratio and *p* value, considering the probability and ratio that indicates the similarity with-

in models:

$$Y \sim 1, \text{ random} = \sim 1 | \text{PLOT}, \text{ method} = \text{"ML"} \quad (\text{Eq. 2})$$

$$Y \sim 1, \text{ random} = \sim \text{REP} | \text{PLOT}, \text{ method} = \text{"ML"} \quad (\text{Eq. 3})$$

$$Y \sim X, \text{ random} = \sim 1 | \text{PLOT}, \text{ method} = \text{"ML"} \quad (\text{Eq. 4})$$

$$Y \sim X, \text{ random} = \sim \text{REP} | \text{PLOT}, \text{ method} = \text{"ML"} \quad (\text{Eq. 5})$$

$$Y \sim X_1 + X_2, \text{ random} = \sim 1 | \text{PLOT}, \text{ method} = \text{"ML"} \quad (\text{Eq. 6})$$

Results

Meteorological data

Precipitation throughout the growing season was generally higher than historical data recorded at the site from October to June (mean annual precipitation = 547 mm as 30-year long-term data). In particular, in the III growing season two intense rainy events occurred in the 3rd decade of January and in the 3rd decade of May (Figure 1). Temperature patterns were consistent with a long-term trend (mean annual temperature = 13.0°C as 30-year long-term data). Generally, the GDD accumulated during the entire growing cycle for camelina (GDD) were more stable across the I, II and III growing seasons, with a mean of 1250 GDD needed to reach maturity (231 days after sowing) (Table 4). In the last growing season, with an early sowing, the thermal time from seeding to harvest was 1513 GDD. Despite sowing in autumn, GDD remained similar to that of most other surveys where spring sowing was adopted. For example, Zanetti *et al.* (2017) recorded an average of 1209 GDD, across years and different locations, for spring-sown camelina crop, and Gesch (2014) reported a GDD range between 1101 and 1216°C for full maturity of this crop. Additionally, Hunsaker *et al.* (2013) reported values ranging from 1259 to 1274°C for camelina sowed in January and harvested in May.

Overall, the weather conditions at the Po valley location were appropriate for adequate camelina development when sown in autumn in a wheat-based cropping system. The earlier harvest time compared to other fall-sown oilseed crops, such as rapeseed, helps to avoid summer drought conditions, typical of the area.

Table 3. Standard values for cultivation input and mechanical oil extraction in terms of GHG emissions and primary energy depletion. Source: BioGrace (2014) and SIMAPRO ver. 8.4.0.0.

Inputs	GHG emissions gCO ₂ eq kg ⁻¹	Input energy MJ kg ⁻¹
<i>Cultivation Input</i>		
Seeds*	729	7.9
Organic N (Manure)	0	10.0
Inorganic N (Ammonium nitrate)	3451	49.0
Diesel	3777	50.0
<i>Mechanical oil extraction</i> ^o		
Electricity consumption for seed crushing and defatting	41.86	0.3

*Value for rapeseed/sunflower; ^oby mean of an industrial pressing plant.

Crop yields and characteristics

Through the four years of wheat-camelina rotation, wheat reached a commercial yield around $7.2 \pm 0.7 \text{ t ha}^{-1}$ (humidity content $10.4 \pm 1.5\%$) with an average crude protein content of 12%. Camelina yield, yield components and seed oil content values are reported in Table 5. TSW was rather stable through the years with an average value of 1.03 g and a notably low variation ($CV=8\%$). Grain yield ranged from 0.60 to 0.94 Mg ha^{-1} , recorded on the III and I growing seasons, respectively, with a relatively high fluctuation ($CV=28\%$, as shown in Table 5), but no significant differences were highlighted, since the effect of the year is close to zero as reported in Table 6. However, in three of the 4 years the grain yield was quite stable (0.94, 0.91 and 0.84 Mg ha^{-1} in the I, II and the IV growing seasons, respectively), and the LME model supports this affirmation. In fact, the overall effect is negligible in both cases (yield and oil content). As reported in Table 6, the simpler model Eq. 2 was chosen for the analysis. Models (Eq. 2-6) are statistically similar and there were no significant effects on the response result-

ed by plot (site) and year. The overall mean value for seed oil content (39.2%) remained stable from year to year ($CV 3\%$). As observed for grain yield, even the aboveground biomass (3.60 Mg ha^{-1} , $CV 32\%$) showed a strong reduction in the III year of trial. The harvest index ranged from 20 to 25%.

Lower heating values (LHV) and biomass nitrogen contents were implemented for LCA to increase reliability of energy allocation and N_2O estimation, respectively. LHVs of above-ground and below-ground residues resulted stable over the years (except for the not available belowground value in 2016). On the contrary, nitrogen content in the crop residues differed between years. It is worth highlighting that, despite their relatively low amount, if all the residues were simply incorporated into the soil, they would be able to supply 28 kg ha^{-1} of organic N on average, in addition to their organic matter, improving, therefore, soil fertility. On the other hand, probably due to the intensive rotation adopted, an N reduction in the whole residual biomass emerged, as showed in Table 5.

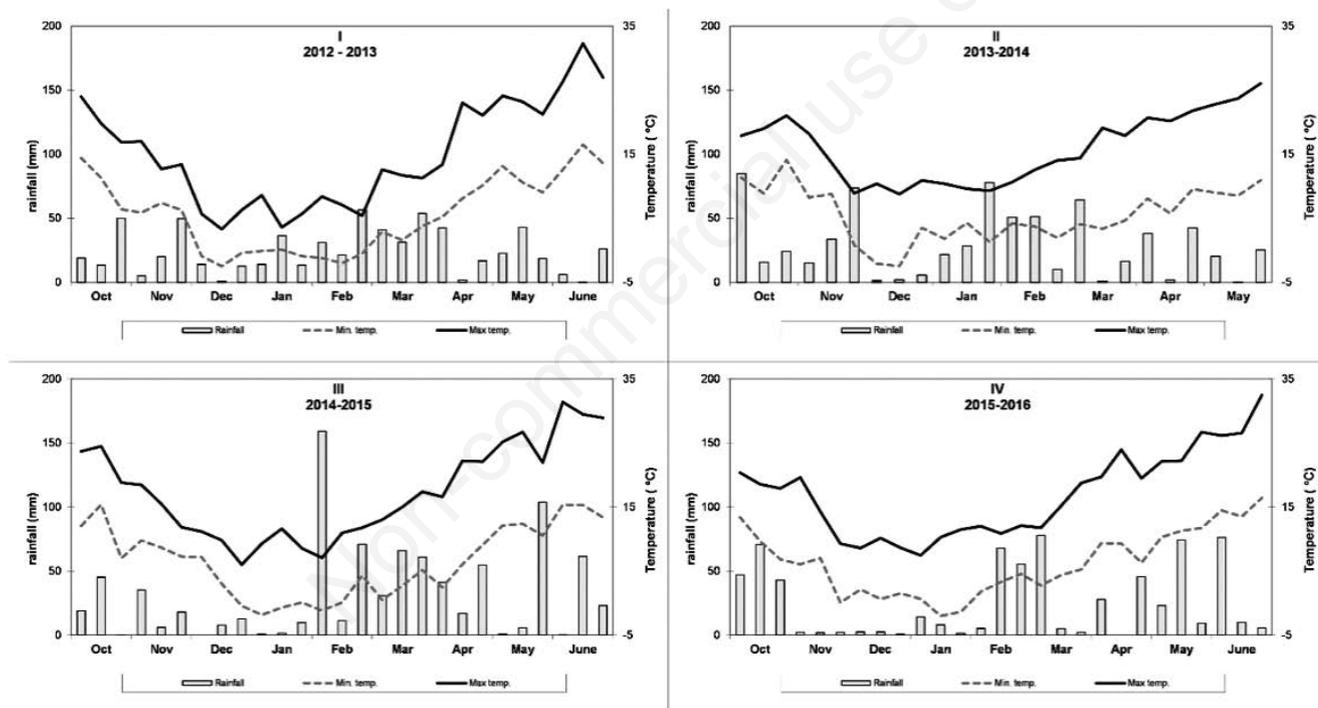


Figure 1. Meteorological data (average monthly maximum and minimum temperatures and total monthly rainfall) of the area (Budrio, Bologna, Italy) recorded in each growing season.

Table 4. Total rainfall, crop cycle length (days), and growing degree days (GDD), registered from sowing to harvest, for each growing season.

Growing season and field	Sowing date	Harvest date	Rainfall (mm)*	Crop cycle	
				Days ^o	GDD (°C) [#]
I - A	10/05	06/07	662.2	244	1190
II - B	10/16	05/29	752.1	224	1296
III - A	10/20	06/04	863.6	226	1264
IV - B	09/30	06/08	683.2	251	1513

*Cumulate rainfall from sowing to harvest. Long-term rainfall = 547 mm; ^oCycle length from sowing to harvest; [#]Base temperature for calculation 5°C (Blackshaw *et al.*, 2011; Gesch, 2014).

Camelina oil was naturally poor in erucic acid, and was, at the same time, characterized by 58% of PUFAs, 32% of MUFAs and 10% of saturated FAs, as mean value over the 4 years (Table 7). The rate between Ω 6 and Ω 3 was around 0.5.

Environmental and energy depletion impact

Table 8 shows the farming inputs applied for camelina (kg ha^{-1}), and the consequent consumption of fossil energy (MJ ha^{-1}). As a result of low rates of fertilizers applied, the main energy consumption was attributable to diesel, ranging around 75% of the total, a value that is quite representative of agricultural mechanization.

As regards cultivation output materials and their corresponding energy, in this particular trial conditions, camelina seed yield was rather low. For this reason, the energy produced (applying LHV reported in Table 5) was 24.3 MJ kg^{-1} (approximately 20 GJ ha^{-1}) and 15.5 MJ kg^{-1} (approximately 55.8 GJ ha^{-1}), as mean values for seeds and above-ground biomass, respectively.

The impact of cultivation expressed as GWP is reported in Table 9, where the CO_2eq emissions per hectare, per kg of grain and per MJ of incorporated energy, are indicated. Regardless of

functional unit, diesel and N_2O emissions caused the main GHG sources.

Average oil and oil-defatted meal yield resulted equal to 281 and 541 kg ha^{-1} , respectively, with corresponding energy values of 10,567 and 12,939 MJ. The required fossil energy - calculated with a presumed energy cost of 0.97 MJ per kg of extracted oil, considering a cold press extraction system (Miller and Kumar, 2013) - was 798 MJ. The energy values of the co-products allowed the allocation of impacts based on energy content, using the percentage of energy of each co-product on the total as a breakdown factor. Thus, a factor of 48% was considered for the oil, and consequently a residual percentage of 52% was applied to the meal.

The emissions of GHG to produce one kg and one MJ of camelina oil was $2428 \text{ gCO}_2\text{eq kg}^{-1}$ oil during cultivation and 136 during the extraction process, which correspond to 62.3 and $3.4 \text{ gCO}_2\text{eq per MJ}$, respectively. Therefore, the total value obtained was $65.7 \text{ gCO}_2\text{eq MJ}^{-1}$.

In addition, when adopting mechanical oil extraction, several uses for the oil-defatted meal could be considered, such as fish-feed feedstock or high valued chemicals (Das *et al.*, 2014). When

Table 5. Productive characteristics of *Camelina sativa* cv. Italia grown under low-input cultivation systems in Bologna, Italy over four growing seasons (I, II, III, IV). Mean values \pm standard deviation are shown ($n = 3$). Variability among growing seasons was measured by coefficient of variation (CV). Due to their stability, Lower Heating Values (LHV) were measured with one single replication per year.

Productive characteristics	I	II	III	IV	Mean	CV (%)
Field	A	B	A	B		
TSW (g)	0.92 ± 0.01	1.14 ± 0.03	1.02 ± 0.03	1.06 ± 0.00	1.03	8
Grain Yield (Mg ha^{-1} DW)	0.94 ± 0.32	0.91 ± 0.20	0.60 ± 0.13	0.84 ± 0.14	0.82	28
Oil content (%)	40.25 ± 0.07	39.65 ± 0.35	38.43 ± 1.61	38.89 ± 0.78	39.17	3
Total above-ground biomass* (Mg ha^{-1} DW)	3.72 ± 1.30	4.59 ± 0.38	2.38 ± 0.33	3.73 ± 0.79	3.60	32
Below-ground biomass (Mg ha^{-1} DW)	0.64 ± 0.17	0.54 ± 0.01	0.29 ± 0.04	0.47 ± 0.10	0.49	34
Seed LHV (MJ kg^{-1})	24.13	23.72	23.37	25.77	24.25	4
Above-ground residues LHV (MJ kg^{-1})	15.77	15.98	14.70	15.56	15.50	4
Below-ground residues LHV (MJ kg^{-1})	13.83	14.29	14.35	n/a	14.16	2
Above-ground residues Nitrogen (kg ha^{-1} DW)	27.5 ± 10.0	32.2 ± 2.0	17.2 ± 5.6	12.8 ± 3.9	22.4	43
Below-ground residues Nitrogen (kg ha^{-1} DW)	8.7 ± 2.4	5.5 ± 0.3	3.0 ± 0.8	4.3 ± 1.2	5.4	46

TSW, thousand seed weight; DW, dry weight. *Total above-ground biomass represents the sum of seed yield and above-ground residues production.

Table 6. ANOVA analysis of the linear mixed effect (LME) model fit by maximum likelihood. Akaike information criterion (AIC), and Bayesian information criterion (BIC) for different LME models.

Model	df	AIC	BIC	Log likelihood	Likelihood Ratio	P-value
Y = Grain yield; X= (Year, Reps, Plots) The best Model (Eq. 2)						
Eq. 2	3	3.66	5.12	1.16	-	-
Eq. 3	6	4.04	6.95	3.97	5.61	0.13
Eq. 4	7	6.03	9.43	3.98	0.01	0.91
Eq. 5	16	24.03	31.79	3.98	0.00	1.00
Eq. 6	6	3.92	6.83	4.03	0.10	1.00
Y = Oil Content; X= (Year, Reps, Plots) The best Model (Eq. 1)						
Eq. 2	3	40.34	41.80	-17.17		
Eq. 3	6	39.13	42.04	-13.56	7.21	0.06
Eq. 4	7	41.02	44.41	-13.51	0.10	0.74
Eq. 5	16	59.02	66.78	-13.51	0.00	1.00
Eq. 6	6	39.13	42.04	-13.56	0.10	1.00

emissions were energy allocated (oil and meal), the impact to produce one MJ of oil resulted 31.4 gCO₂eq.

However, the energy supply chain involves the use of camelina oil for jet biofuel production. An impact of 24 gCO₂eq MJ⁻¹ was attributed to the refinery phase. As described by Li and Mupondwa (2014), this value was estimated following BioGrace (2014) stan-

dards to which a further 1 gCO₂eq MJ⁻¹ was added for transport, as recommended by RED. Adding these values to the totals, without oilcake exploitation, the results returned an impact of 90.7 gCO₂eq MJ⁻¹. Similarly, the total impact resulting from the allocation was 56.4 gCO₂eq MJ⁻¹.

Table 7. Fatty acid (FA) profile (percentage ± standard deviation, n=4) and coefficient of variation (CV) among growing seasons (I, II, III, IV).

FA Field	Common name	I A	II B	III A	IV B	Mean	CV (%)
C 16:0	Palmitic	6.0±0.0	5.7±0.0	6.3±0.1	6.4±0.3	6.2	5
C 18:0	Stearic	2.6±0.0	2.7±0.0	2.5±0.1	2.8±0.1	2.6	6
C 18:1	Oleic	14.9±0.1	15.0±0.1	17.3±0.4	16.0±0.4	16.2	6
C 18:2	Linoleic	18.0±0.1	16.7±0.0	17.7±0.2	17.5±0.3	17.5	2
C 18:3	Linolenic	37.0±0.1	36.6±0.1	36.3±1.2	38.6±0.6	37.3	4
C 20:0	Arachidic	0.0	1.2±0.0	1.3±0.2	1.1±0.1	1.2	12
C 20:1	Eicosenoic	14.0±0.0	14.5±0.1	12.8±0.1	12.7±0.3	13.1	5
C 20:2	Cis-11,14-eicosadienoic	2.0±0.0	2.0±0.1	1.6±0.1	1.5±0.3	1.7	17
C 20:4	Arachidonic	1.5±0.0	1.6±0.0	1.3±0.1	1.4±0.0	1.4	8
C 22:0	Behenic	0.3±0.0	0.3±0.0	0.0	0.0	0.3	0
C 22:1	Erucic	2.7±0.1	2.6±0.0	2.4±0.1	1.9±0.1	2.3	15
C 24:0	Lignoceric	0.0	0.0	0.4±0.0	0.0	0.4	11
C24:1	Nervonic	0.7±0.1	0.7±0.1	0.5±0.1	0.4±0.0	0.5	24
SFA		8.9±0.0	9.8±0.2	9.4±0.7	10.2±0.3	9.7	7
MUFA		31.5±0.0	32.1±0.3	32.9±0.2	30.5±0.6	31.7	4
PUFA		58.4±0.1	56.8±0.14	56.6±1.0	59.9±0.6	57.7	2
Ω-6/Ω-3		0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.5	5
		0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.5	5

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 8. Camelina cultivation inputs and corresponding energy resource consumption per hectare.

Farming inputs	Amount kg ha ⁻¹	Input energy MJ ha ⁻¹
Seeds	12.5	98.4
Organic N (Manure)	12	120
Inorganic N (Ammonium nitrate)	14	685.9
Diesel	80.6	4030
Total		4934.2

Table 9. GWP specific impacts in camelina cultivation, expressed per hectare, per one kg of grain, and as MJ of incorporated energy. Sources included emissions for production and use (i.e. diesel combustion and N₂O emissions from fertilizers and residues).

Sources	GWP specific impacts			
	kgCO ₂ eq ha ⁻¹	%	gCO ₂ eq kg ⁻¹ of grain	gCO ₂ eq MJ ⁻¹ of incorporated energy
Diesel	304.4	45%	369.9	15.3
Seeds (sowing)	5.0	1%	6.1	0.3
Fertilizers (production)	48.3	7%	58.7	2.4
Fertilizers (N ₂ O emissions)	165.8	24%	201.5	8.3
Residues (N ₂ O emissions)	158.4	23%	192.5	7.9
Total	682.0	100%	828.6	34.2

Discussion

Camelina seed yield and quality

As regards agronomic observations carried out during the four growing seasons, the proposed camelina-wheat rotation system provided, for camelina, a rather stable TSW, similar to that reported by other authors (Berti *et al.*, 2011; Angelini, 2012; Masella *et al.*, 2014). On the contrary, grain yields fluctuated across the years. The intense rainy events that occurred during the III growing season, from February onward, caused prolonged waterlogging to camelina from the rosette stage until seed ripening, with a consequent yield reduction of about 33%.

In the other seasons, winter rainfalls were efficiently stored in the soil, thus allowing camelina to meet seasonal water requirements (French *et al.*, 2009). Furthermore, considering the intensive rotation and, above all, the low input strategies adopted (Table 2), the 4-year-average yield ($0.82 \pm 0.15 \text{ Mg ha}^{-1}$) was comparable to those obtained in other studies in which reduced amounts of N fertilizers were adopted (Malhi *et al.*, 2014). In addition, in our study, wheat was able to uptake 140 kg N ha^{-1} by grain and 50 kg N ha^{-1} by straw. Considering that wheat straw was removed from the field, it is possible to state that camelina did not exploited residual N deriving from wheat fertilization.

The overall mean value of seed oil content (39.2%) was similar to, or higher than that reported by the literature (Blackshaw *et al.*, 2011; Guy *et al.*, 2014; Pecchia *et al.*, 2014; Berti *et al.*, 2016; Zanetti *et al.*, 2017).

Similarly to seed yield and oil content, also fatty acid synthesis is deeply influenced by environmental conditions, genotype, and sowing date (Berti *et al.*, 2016; Zanetti *et al.*, 2017; Righini *et al.*, 2019). In the tested Mediterranean environment, the autumn sowing implied relatively low temperatures during the seed filling phase, thus promoting polyunsaturated FA (PUFAs) production and a rather high α -linolenic acid (ALA) content. At this regard, a significant reduction in PUFAs with temperatures above 25°C , during the seed filling stage, was observed by Obour *et al.* (2017) for camelina oil. On the other hand, as expected, the content of monounsaturated FAs (MUFAs) was relatively low. These findings were in line with previous studies carried out in the same environment (Po valley), where different camelina accessions and autumn and spring sowing were evaluated (Zanetti *et al.*, 2017; Righini *et al.*, 2019).

It should be emphasized that the stable content of ALA makes camelina oil an excellent source of this essential fatty acid. If consumed in the human diet, camelina oil could account for, at least partially, the recommended daily intake of 2 g day^{-1} of ALA according to the Regulation CE n° 432/2012 (Official Journal of the European Union, 2012). The rate between $\Omega 6$ and $\Omega 3$ ranged around 0.5, according to modern recommendations for human health. In fact, it has been established that a reduction in the dietary intake of $\Omega 6/\Omega 3$ ratios and $\Omega 6$ -derived metabolites could increase $\Omega 3$ circulating long chain-PUFAs in most individuals (Chilton *et al.*, 2017). This result also confirms the interesting properties of camelina for several applications in the food sectors. Although the $\Omega 3$ content is lower than that of other vegetable oils, such as linseed oil, the high content of natural antioxidants such as tocopherol, phenols and terpenes make it an appreciable oil, with a long shelf life (Berti *et al.*, 2011; Terpinic *et al.*, 2012; Rahman *et al.*, 2018). Moreover, the environmental conditions and the choice of sowing date, especially temperatures during seed filling, are able to influence the FA composition. The higher the temperatures

during seed development, the lower is the ALA content, and, more in general, PUFAs (Zubr and Matthäus, 2002), thus reducing the quality of oil and residual defatted meal for food and feed applications. Under the tested Mediterranean conditions, this problem could be partially overcome by adopting a winter cycle, which allows to anticipate the camelina critical period of seed filling. On the other hand, a lower PUFA/MUFA index and a lower C20-24/C16-18 ratio enhance oil characteristics for industrial uses, especially in terms of oxidation stability (Rodríguez-Rodríguez *et al.*, 2013).

Environmental sustainability

Following SIMAPRO ver. 8.4.0.0, the input referred to camelina with higher GHG impact (as $\text{kgCO}_2\text{eq kg}^{-1}$) were: pesticide>diesel>N-fertilizer> P_2O_5 -fertilizer> K_2O -fertilizer>seeds>organic fertilizer (assumed as 0).

Regarding chemical crop protection, comparing other studies, weed control by chemical products such as Ethfluralin, Trifluralin, Sethoxydim, or Quisalofop were reported, despite pest and disease control was not described (Malhi *et al.* 2014). Gesh (2014) reported an additional hand weeding, whilst Zanetti *et al.* (2017) reported hand weeding in 10 m^2 plots. In this study, 500 m^2 plots were managed avoiding pesticides and weed control interventions, in order to evaluate the adopted strategy in a low-input scaled-up system. The plot dimension helped in having more representative data to base LCA on, in a system where competitive interactions, soil fertility depletion, and other large-scale phenomena were involved.

In this study, diesel consumption was 35% lower than that reported for sunflower cultivated in a similar area (Spugnoli *et al.*, 2012) and it was similar to the reference value reported in the RED for sunflower. In a study conducted by Bacenetti *et al.* (2017), camelina cultivation under Mediterranean conditions required a lower amount of diesel, roughly 7%, but, in our trials, the contribution in terms of N-fertilizer were from 40 to 60% lower than average values reported in other experimental trials performed in Italy (Zanetti *et al.*, 2017), United States of America (Krohn and Fripp, 2012) and Canada (Miller and Kumar, 2013).

The cultivation output materials and their corresponding energy were notably lower than those reported by other authors (Masella *et al.*, 2012; Krohn and Fripp, 2012; Miller and Kumar, 2013) or in the RED for sunflower.

Again, considering sunflower as a term of comparison, the ER (4.04) for camelina was slightly lower, whilst EC, EB and NEB indices, which represent the energy performance in camelina grain yield, were equal to 0.25, 15.02 and 3.33, respectively. This poor energy performance of camelina in comparison to sunflower is not only due to the lower grain yield, but also to the lower oil content (39% in camelina compared to 45% in sunflower). However, as already discussed, this aspect could be improved through different approaches that do not affect the IE flows, such as different sowing dates or new improved genotypes. On the other hand, camelina cultivation implies several agronomical advantages that sunflower cultivation does not: from brief cycle, if a spring sowing is adopted, to the cover crop effect considering a winter sowing. Even the agroecological side effects already discussed need to be considered in the whole balance, due to the very low pesticide and fertilizing input that camelina requires.

The GWP impact (Table 8) resulted significantly lower than that reported by Bacenetti *et al.* (2017) for camelina cultivated in the Mediterranean area ($1701 \text{ gCO}_2\text{eq kg}^{-1}$). It is worth highlighting that GWP per hectare resulted around 30% lower than sunflower (RED value), thus confirming camelina adaptability to very low-

input management, attaining minimum impact. The obtained results were, in any case, in line with those obtained by other authors in different cultivation areas such as Italy (Colombini *et al.*, 2014), United States of America (Moser, 2010; Agusdinata *et al.*, 2011) and Canada (Miller and Kumar, 2013; Li and Mupondwa, 2014).

The GHG emissions in the production of one MJ from camelina oil ($65.7 \text{ gCO}_2\text{eq MJ}^{-1}$) compared to the RED threshold for diesel ($83.8 \text{ gCO}_2\text{eq MJ}^{-1}$), imply an interesting performance by camelina. However, camelina biofuel will not meet the RED sustainability criterion - a minimum savings rate of 35% in terms of GHG emissions compared to diesel oil - when refinery and transport impact are considered, and the oilcake unexploited. Even if, exploiting the oilcake, impact resulting from allocation ($56.4 \text{ gCO}_2\text{eq MJ}^{-1}$ of biofuel) was lower (33% compared to diesel oil), narrowly missing the sustainability criteria required by the RED. The GHG emissions for jet fuel from camelina obtained in these field trials were compared to the values reported by the literature (Agusdinata *et al.*, 2011; Li and Mupondwa, 2014; Lokesh *et al.*, 2015) and to the reference standard fuel values as shown in Figure 2. The importance of improving grain yields and exploiting co-products to reduce the impact of the main product is highlighted. For instance, camelina straws present interesting characteristics as feedstock for pyrolysis due to its low protein content, for further biofuel production after chemical processing (Hernando *et al.*, 2017) or for green building or the automotive sector. In fact, the exploitation of removed aboveground residues (Table 4) would allow an energy allocation (by LHV reported in Table 5) that would reduce the impact of camelina oil. Thereby, in this experiment the exploitation of straws (as part of total aboveground biomass) became crucial to reach the RED sustainability requirement in the use of camelina oil as biofuel.

Conclusions

This study confirmed the interesting adaptability of camelina var. Italia, as winter crop, to the pedo-climatic conditions of northern Italy, reaching, through the four growing seasons, satisfactory and relatively stable seed yield, by adopting a low input cultivation system. Besides this, the seed characteristics confirmed the very high amount of $\Omega 3$ and $\Omega 6$ fractions in the oil, which could provide interesting opportunities, not only for the food and feed sectors, but also for industry, particularly in high value sectors such as cosmetics.

The environmental performance of camelina for biofuel assessed in this study was worse if compared to the main non-food oilseed crops such as rapeseed and sunflower. Furthermore, camelina fuels would be not sustainable according to the RED parameters and other cited research, unless the removed straws are also exploited, reallocating GHG emissions. Nevertheless, the low cropping inputs required for camelina-wheat rotation highlighted interesting perspectives for temperate climates. Consequently, reducing emissions to air (linked to increasing yield) and emissions to water (eutrophication) due to limited use of N-fertilizers, would reduce the burden on the environment.

Considering its by-products, camelina meal has shown interesting applications in: i) animal feed, replacing soy meal; ii) biogas, as feedstock, and; iii) soil management, as fertilizers. However, these uses should be assessed in order to make a feasible environmental balance of its potential reduction or increasing GHG emissions into the system. Nowadays, green chemistry has rekindled interest in a comprehensive promotion of all biorefinery by-products. When these products are raw materials, they can provide an opportunity to replace highly polluting chemicals, *i.e.* chemical origin N-fertilizer, pesticides or coal.

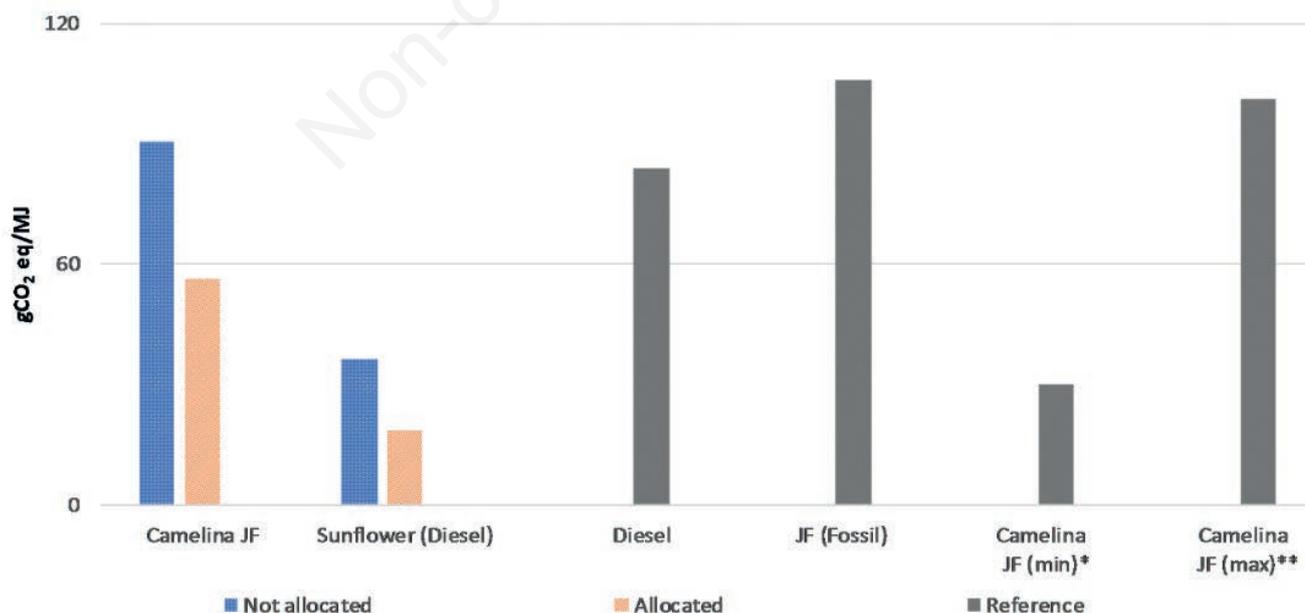


Figure 2. Comparison of camelina Jetfuel (JF) GHG with different propellants (*Li and Mupondwa, 2014; **Lokesh *et al.*, 2015).

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