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REVIEW

Biodiesel production from camelina oil: Present status and future perspectives

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Abstract

Camelina sativa (L.) Crantz is an oilseed crop with favorable potentials for biodiesel production, such as the high plant yield, high oil content in the seed, high net energy ratio, and low oil production cost. This review paper deals with the present state and perspectives of biodiesel production from camelina oil. First, important issues of camelina seed pretreatment and biodiesel production are reviewed. Emphasis is given to different biodiesel technologies that have been used so far worldwide, the economic assessment of the camelina oil biodiesel (COB) production, the camelina-based biorefineries for the integrated biodiesel production, the COB life cycle analysis, and impact human health and ecosystem. Finally, the perspectives of COB production from the techno-economic and especially genetic engineering points of view are discussed.

KEYWORDS

alcoholysis, biodiesel, camelina, *Camelina sativa*, genetic resources, genome editing

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Department of Biotechnology, New Delhi, India in the form of Indo-US Advanced Biofuel Centre (IUABC) through IUSSTF, New Delhi, India

1 | INTRODUCTION

Camelina or false flax [*Camelina sativa* (L.) Crantz] is gaining attention from scientists world over for its ability to produce oil suitable for many applications such as lubricants, fuel additives, jet fuel, and biodiesel (Moser, 2010). Camelina is considered a better oilseed crop than many others because of its short life cycle of 85–100 days and its ability to grow under stress conditions, in low fertility soils and temperate climate with low water and fertilizer inputs (Moser, 2010; Yuan & Li, 2020). Camelina has high seed yield of 1500–3000 kg ha⁻¹ year⁻¹ and contains 30%–43% of oil having about 45% linoleic and linolenic acid (polyunsaturated acids) and 17% oleic acid (monounsaturated acid) (Gugel & Falk, 2006; Krohn & Fripp, 2012). Furthermore, the availability of various genetic resources is resulting in generation of newer and better engineered *Camelina* lines with improved oil yield (to up to 26%) and composition where the oleic acid contents are significantly enhanced to >60% (Morineau et al. 2017) and amount of di- and tri-unsaturations are reduced dramatically (Abdullah et al., 2018; Ciubota-Rosie et al., 2013; Jiang et al., 2017; Kagale et al., 2014; Zhu et al., 2018). Based on the favorable crop potential of camelina like the large plant yield, large oil content in the seed, high net energy ratio, and low production cost of the oil, camelina oil is highly convenient for biodiesel production (Patil et al., 2009), which is also supported by its high amounts of glucosinolates and erucic acid that limit its use as food (Waraich et al., 2013). Therefore, camelina oil has already been used as an industrial feedstock for road and/or aviation fuel production (Moser 2010; MPPU, 2013).

This paper provides an overview of the present state and perspectives of biodiesel production from camelina oil (Figure 1). First, important issues of camelina seed pretreatment and COB production are reviewed followed by a critical evaluation of the technological, economic, environmental, and social impacts of COB. Finally, the perspectives of COB production from the techno-economic and especially genetic engineering points of view are also discussed.

2 | PRESENT STATE OF COB PRODUCTION

The overall process of biodiesel production from camelina oil involves camelina cultivation and harvesting, seed processing to recover the oil and the meal, the

Highlights

- Camelina is an oilseed crop with favorable potential for biodiesel production.
- Camelina lines with improved oil yield and composition are being generated.
- Biodiesel from camelina will lead to energy security and stronger economy.
- Further improvement through biorefineries producing biodiesel are on the way.
- Genomic resources and genetic engineering platforms for sustainable oil production.

transesterification of camelina oil in the presence of a catalyst to get biodiesel and glycerol, separation of crude biodiesel from glycerol, alcohol recuperation by distillation, and crude biodiesel purification to obtain the final product satisfying the quality standard specification. A schematic presentation of this process is given in Figure 2.

2.1 | Camelina seed pretreatment and oil extraction

The steps of camelina seed processing are the same as for canola seeds (Miller, 2012a). Two main methods that are commonly used to extract oil from camelina seeds are mechanical pressing (Dangol et al., 2017; Fröhlich & Rice, 2005; Restuccia, 2014; Zhao et al., 2014) and solvent extraction (Avram et al., 2015; Belayneh et al., 2015; Pathak et al., 2018; Stroescu et al., 2015), while novel techniques have been rarely employed (Belayneh et al., 2015; Moslavac et al., 2014). Due to a high oil content of camelina seeds, the two-step process is suggested (Ciubota-Rosie et al., 2013; Moslavac et al., 2014), where whole seeds are pressed to recover about 60% of the available oil whereas the oil remained in the cake is separated by solvent extraction. Crude degummed camelina oil is normally required as a raw material to produce biodiesel satisfying the requirements of the standards for biodiesel (Miller, 2012a).

2.2 | The main routes of COB production

Camelina oil is used as a transport fuel as it is (Baernardo et al., 2003) or after conversion into biodiesel by alcoholysis

FIGURE 1 Overview figure summarizing the content of the article

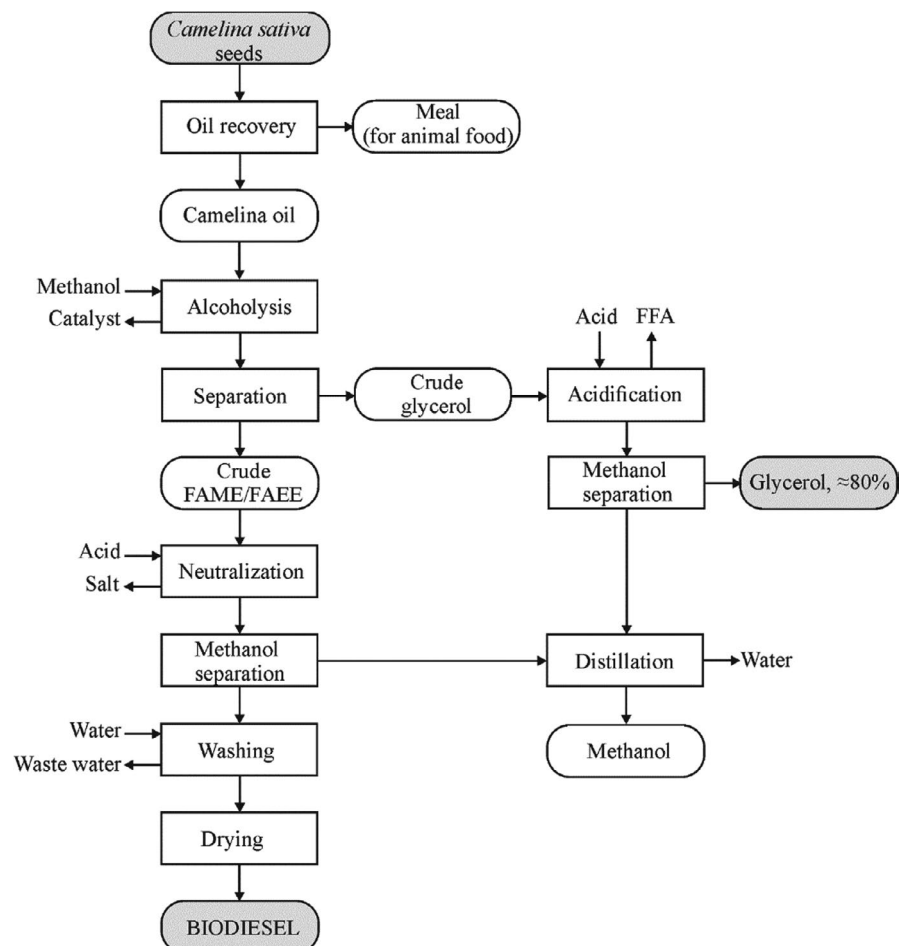
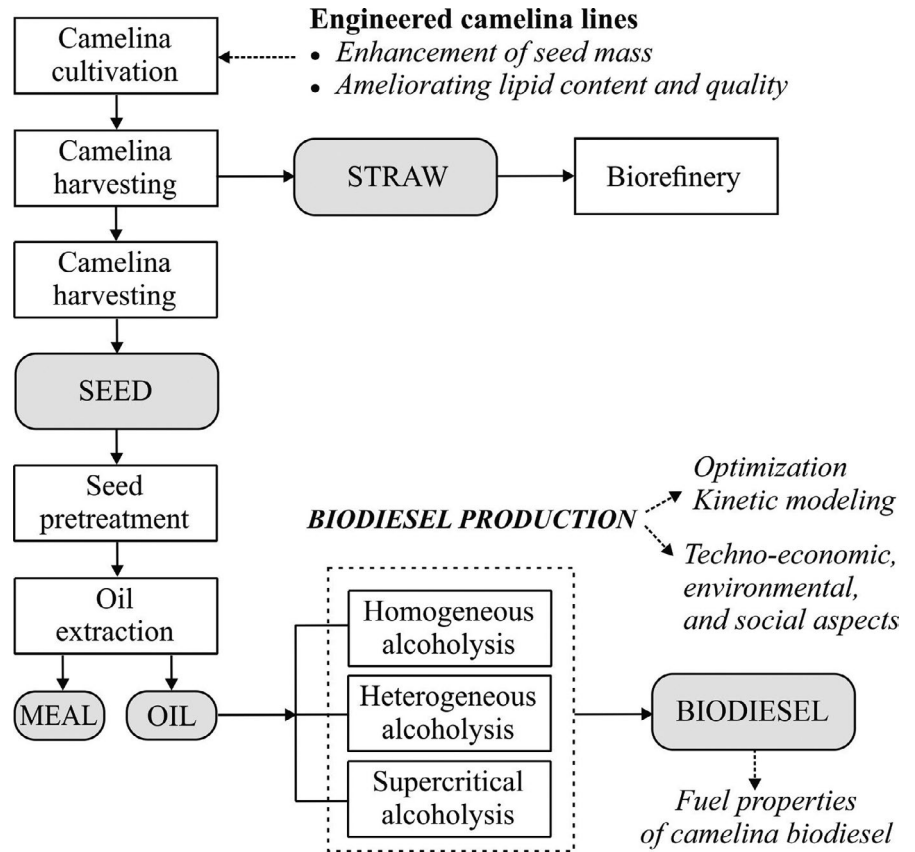


FIGURE 2 Schematic presentation of the process of biodiesel production from camelina seed

reaction with/without the presence of catalysts (Patil et al., 2009). In the alcoholysis reactions, TAGs from camelina oil are most frequently converted into fatty acid methyl, FAME, or ethyl, FAEE, esters using homogeneous base, heterogeneous base, and non-catalytic supercritical catalysis (Table 1). Enzymatic alcoholysis and in situ processes have been rarely used.

2.2.1 | Homogeneous alcoholysis of camelina oil

The base-catalyzed camelina oil alcoholysis reactions were mostly investigated in the presence of methanol at the molar ratio to the oil in the range from 4:1 to 15:1. Generally, the highest ester yields are obtained at the 6:1 mol/mol methanol:oil ratio although some authors suggested higher methanol amounts (up to 9:1 mol/mol) (Patil et al., 2010b; Wu & Leung, 2011; Yang et al., 2015, 2016a, 2016b). KOH and NaOH are commonly used, while methoxides are rarely used (Ciubota-Rosie et al., 2013; Moser & Vaughn, 2010; Yildizhan & Serin, 2015). No significant difference in the activity of these catalysts under the similar reaction conditions has been reported (Patil et al., 2010b). The suggested catalyst KOH amount for obtaining the highest FAME content is in the range from 1% to 1.66%. A decrease in FAME yield is observed at both low and excess catalyst amounts because of the uncompleted alcoholysis reaction and TAG saponification, respectively (Yang et al., 2016b). The optimal reaction temperature is up to 50°C (Bacchetti et al., 2017; Fröhlich & Rice, 2005; Sáez-Bastante et al., 2015; Wu & Leung, 2011; Yang et al., 2015, 2016a, 2016b). Because of its higher polyunsaturation degree, camelina oil has a lower optimal reaction temperature than canola (Yang et al., 2016b). Additionally, high content of C18:3 fatty acid, which has significantly lower activation energy for methanolysis than C18:2 and C18:1 fatty acid (Sharma et al., 2014), enables the conversion of camelina oil at lower temperatures (Yang et al., 2016b). The highest FAME yield is commonly achieved for 40 min (Yang et al., 2016a, 2016b) to 70 min (Wu & Leung, 2011). FAME yields under the optimal reaction conditions are generally higher than 96.5%. The oily feedstocks having higher FFA content are commonly processed in two acid/base-step processes and an intermediate separation of the oil/ester and glycerol/alcohol phases. The FFA esterification using an acid catalyst (sulfuric acid) enables the conversion of FFAs to FAMES, thus reducing the oil acidity from 6.8% to 0.3% within 2 h, whereas in the next step, the alcoholysis of TAGs in the presence of NaOH provides the FAME content above 96.5% (Karčauskienė et al., 2014).

Since recently, novel methods based on the microwave and ultrasonic irradiation have been applied in COB

production (Sáez-Bastante et al., 2015; Patil et al., 2010b). The comparable FAME yields were achieved within 30–60 s and 30–60 min using microwave irradiation and conventional heating, respectively, whereas the energy consumption of the former method was 18–23 times lower (Patil et al., 2010b). The ultrasonic KOH-catalyzed methanolysis of camelina oil provides the highest FAME yield within significantly shorter reaction time than the conventional method (Sáez-Bastante et al., 2015; Patil et al., 2010b).

2.2.2 | Heterogeneous base-catalyzed alcoholysis of camelina oil

The heterogeneous base-catalyzed methanolysis of camelina oil was conducted at higher molar ratios of methanol:oil in a wide range from 9:1 (Patil et al., 2009, 2010b) to 36:1 (Man et al., 2012) and higher temperatures for longer reaction time, compared to the homogeneous base-catalyzed process. Alkaline earth metal oxides, perovskite $\text{Na}_{0.1}\text{Ca}_{0.9}\text{TiO}_3$ nanorods, activated alumina loaded by BaO and SrO, as well as waste materials (eggshells, lobster, mussel, clam, and oyster shells) as a source of CaO, have been tested in the COB production. Among the neat alkali earth oxides, BaO and SrO show higher catalytic activities, due to their higher basic strength (Patil et al., 2009). The reaction time decreased to only 4 min using the activated alumina supported SrO in the microwave-assisted camelina oil methanolysis, whereas the FAME yield was 95% (Patil et al., 2010b). Because of high basic strength and a high specific surface area, $\text{Na}_{0.1}\text{Ca}_{0.9}\text{TiO}_3$ nanorods provided a high ester yield of 93% in 8 h (Man et al., 2012). Also, a MgO/ Fe_2O_3 - SiO_2 core-shell magnetic nanocatalyst ensured almost complete methanolysis of camelina oil under the optimized conditions, could be removed from the reaction mixture by a magnet, and reused for 4 cycles (Rahimi et al., 2021).

The camelina oil methanolysis in the presence of the CaO catalyst obtained from eggshells, lobster (Hangun-Balkir, 2016), mussel, clam, and oyster (Perea et al., 2016) shells has been also studied. All tested catalysts are highly active in methanolysis reaction with an ester yield of over 90%, but they are less active than commercial CaO (Hangun-Balkir, 2016). The CaO catalyst obtained from waste seashells was successfully used in 10 consecutive cycles after washing with methanol and recalcination (Perea et al., 2016).

2.2.3 | Non-catalytic supercritical alcoholysis of camelina oil

The COB production can be performed by non-catalytic processes using supercritical alcohols. In these supercritical processes, FFA esterification and TAG alcoholysis

TABLE 1 Review of the COB production using homogeneous base, heterogeneous base, and non-catalytic supercritical catalysis

Type of catalysis	Catalyst/amount of oil	Type of alcohol	Alcohol:oil, mol/mol	Temperature, °C	Type, volume of reactor/agitation speed, rpm	Yield (purity), %/time (optimal conditions)	References
Homogeneous base	KOH/7.5–12.5 ^a	Methanol	150:1–250:1 ^a	50 and 60	-	96.5/(7.5 g/l KOH; 250 g/l methanol; 50°C)	Bacenetti et al. (2017)
	KOH/1.5 and 2	Methanol	6:1 and 4.5:1	Room	Conical flask, 250 ml/magnetic	97.9/60 min (6:1)	Fröhlich & Rice (2005)
	KOH/1	Methanol	6:1	70	/600	-/1 h	Pathak et al. (2018)
	KOH/1.5	Methanol	6:1	50	Heater-stirrer oven (500 W)/900	(94.53)/40 min	Sáez-Bastante et al. (2015)
	KOH/0.5–2	Methanol	4:1–10:1	40–70	Reactor, 1 l/magnetic	98.4/70 min (8:1; 1% KOH; 50°C) ^d	Wu & Leung (2011)
	KOH/0.5–2	Methanol	6:1–10:1	30–50	Flask, 300 ml/300	98.9/40 min (7.7:1; 1.5% KOH; 38.7°C) ^e	Yang et al. (2015)
	KOH/0.75–1.75	Methanol	6:1–10:1	30–50	Flask, 300 ml/300	(98.5)/40 min (8:1; 1.3% KOH; 40°C)	Yang et al. (2016a)
	KOH/0.75–1.75	Methanol	6:1–10:1	30–50	Flask, 300 ml/300	98.5/40 min (6.9:1; 1.66% KOH; 33.6°C) ^f	Yang et al. (2016b)
	KOH/up to 0.4	Methanol ^c	6:1–40:1	100–220	Microreactor (90 bar)	≈93/20 min (30:1; 0.3% KOH; 180°C)	Patil et al. (2010a)
	KOH/0.8–1.2	Methanol	6:1	-, room, 50	Flask with ultrasonic horn (20 kHz, 70% duty cycle, 50% amplitude, 450 W; 1–3 sonication stage)/with or without stirring	(99.37)/17.6 min (1.2% KOH; 3 sonication stages; stirring, room temperature)	Sáez-Bastante et al. (2015)
	KOH/1.0–2.0	Methanol	2:1–12:1	50	Reactor, ultrasonic horn (24 kHz, 70% amplitude, 70 pulse, 400 W)/	98.91/7.33 min (10.18:1; 1.15% KOH)	Hoseini et al. (2018)
	KOH, NaOH/0.5–2	Methanol	6:1–15:1	-	Reactor, microwave irradiation (800 W)/	97/1 min (9:1; 1% KOH)	Patil et al. (2010b)
	NaOH/0.5	Methanol	6:1	60–65	Round bottom flask/	-/2 h	Soriano & Narani (2012)
	NaOH/0.5	Methanol	6:1	60	-	≈97%	Yildizhan & Serin (2015)
	CH ₃ ONa/2.4	Methanol	6:1	-	Stainless steel drum reactor/	88.6/	Dangol et al. (2017)

(Continues)

TABLE 1 (Continued)

Type of catalysis	Catalyst/amount, % of oil	Type of alcohol	Alcohol:oil, mol/mol	Temperature, °C	Type, volume of reactor/agitation speed, rpm	Yield (purity), %/time (optimal conditions)	References
	CH ₃ ONa/0.5	Methanol	6:1	60	Three-neck flask, 100 ml/mechanical	95/1 h	Moser (2016)
	CH ₃ ONa/1	Methanol	6:1	60	Three-neck round bottom flask, 500 ml/magnetic, 1200	89/1.5 h	Moser & Vaughn (2010)
	CH ₃ OK/0.1:1 ^b	Methanol	6:1	60	Four-neck flask, 200 ml/magnetic	(97.5)/60 min	Ciubota-Rosie et al. (2013)
	KOH/1	Ethanol	9:1	70	Three-neck round bottom flask, 500 ml/magnetic, 1200	84/1.5 h	Moser & Vaughn (2010)
	I stage: H ₂ SO ₄ /0.05–1.5	Methanol	2%–8% ^d	60	250 rpm	0.3% acidity/1 h (6% methanol; 1% H ₂ SO ₄)	Karčauskienė et al. (2014)
	II stage: NaOH/0.3–0.8 and 0.1–0.4		II 10–18% and 4%–7% ^d		Microreactor	97.5/1 h (6% methanol; 0.3% NaOH)	
Heterogeneous base	BaO/0.25–2	Methanol	3:1–15:1	40–130	Glass reactor, 250 ml/	84/3 h (9:1; 1% BaO; 100°C)	Patil et al. (2009)
	SrO					82/2 h (12:1; 0.5% SrO; 60°C)	
	CaO					33/1.5 h (15:1, 0.5%, 100°C)	
	MgO					30/1.5 h (15:1, 1%, 80°C)	
	BaO, SrO, sol-gel activated alumina using BaO and SrO (1–10 mmol/g alumina)/0.5–2	Methanol	6:1–15:1	-	Reactor, microwave irradiation (800 W)	95/4 min (9:1; 2% SrO)	Patil et al. (2010b)
	Commercial CaO, calcined eggshell and lobster shell/0.25–2	Methanol	6:1–18:1	25–75	Round bottom flask, 500 ml/magnetic	(99.1)/3 h (12:1; 1% CaO; 650°C)	Hangun-Balkir (2016)
	Calcined waste seashells/1	Methanol	12:1	65	-/vigorously	95/2 h (mussel shell-based catalyst)	Man et al. (2012)
	Na _{0.1} Ca _{0.9} TiO ₃ nanorods/1–10	Methanol	12:1–48:1	60–120	Batch reactor/mechanical 1000	93/8 h (36:1; 6% Na _{0.1} Ca _{0.9} TiO ₃ ; 60°C)	Man et al., 2012)

(Continues)

TABLE 1 (Continued)

Type of catalysis	Catalyst/amount, % of oil	Type of alcohol	Alcohol:oil, mol/mol	Temperature, °C	Type, volume of reactor/ agitation speed, rpm	Yield (purity), %/time (optimal conditions)	References
Non-catalytic supercritical	MgO/Fe ₂ O ₃ -SiO ₂ core-shell magnetic nanocatalyst/3%–9%	Methanol	6:1–18:1	55–70	Glass reactor 250 mL/mechanical	99/4.1 h (12:1, 4.9%, 700C)	Rahimi et al. (2021)
	-	Methanol	25:1–55:1	240–320	Microreactor (114 bar)	≈88/40 min (45:1; 0.3% hexane; 290°C)	Yang et al. (2010)
	-	Methanol Ethanol	40:1	245–310	Microreactor	90/25 min (300°C) 86/30 min (310°C)	Sun et al. (2014a)
	-	1-butanol	10:1–60:1	290–310	Microreactor	85/45 min (310°C)	Sun et al. (2014b)
	-	Methanol/1-butanol ^f	40:1	290	Microreactor	87.6/80 min (40:1; 305°C) 86.14/30 min (methanol:1-butanol molar ratio 0.5–0.9)	Sun et al. (2015)
	-	Ethanol/1-butanol ^f	6:1–40:1	100–220	Microreactor (90 bar)	85.16/30 min (ethanol:1-butanol molar ratio 0.5–0.7)	Yang et al. (2010a)
	-	Methanol ^g	25:1–55:1	245–320	Microreactor (100 bar)	≈93/20 min (30:1; 0.3% KOH; 180°C) 85/20 min (45:1; 295°C; 0.2% hexane)	Muppaneni et al. (2012)

^ag/L.^bMolar ratio of catalyst to oil.^cSubcritical methanol.^dwt% to the oil mass.^eOrthogonal array design.^fCentral composite design.^gFace-centered central composite design.^hMolar ratio of alcohol mixtures 1:0, 3:1, 2:1, 1:1, 1:2, to 0:1.ⁱUnder subcritical methanol conditions.

occur concurrently, and the yield of the esters does not depend on the presence of FFAs and water in the oily materials. The supercritical processes are conducted with methanol (Patil et al., 2010a; Sun et al., 2014a), ethanol (Muppaneni et al., 2012; Sun et al., 2014a), 1-butanol (Sun et al., 2014b), and the blends of methanol and ethanol with 1-butanol (Sun et al., 2015). The optimal methanol:oil ratio in the supercritical camelina oil methanolysis was 40:1 (Sun et al., 2014a, 2014b, 2015) or 45:1 (Muppaneni et al., 2012; Patil et al., 2010a). Generally, the ester yield depends on the used alcohol and decreases from 90% to 80% with increasing the alcohol carbon chain from methanol to 1-butanol (Sun et al., 2014a). However, the biodiesel heating capacity, cold properties, and cetane number are improved when alcohol with longer alkyl chain is used. Sun et al. (2015) suggested the use of the alcohol mixtures in the production of camelina esters to combine the reactivity of low carbon chain alcohols and the better fuel properties of butyl esters. The optimal reaction temperature is 290–310°C, depending on the other reaction conditions (Patil et al., 2010a; Sun et al., 2014a). With increasing the temperature, the biodiesel yield increases because of the larger equilibrium constant of the endothermic alcoholysis reaction. However, at temperatures above the optimal one, the biodiesel yield decreases because of the decomposition of esters and the favored reverse reaction (Patil et al., 2010a; Sun et al., 2014b). Generally, the optimal reaction time is 20–80 min (Muppaneni et al., 2012; Sun et al., 2014b), depending on the applied reaction conditions and the used alcohol.

The intensive reaction conditions of the supercritical biodiesel production can be facilitated if a cosolvent is added to reduce the critical parameters of the used alcohol and to allow an increase in the alcohol-oil mutual solubility at lower temperature and pressure (Pak & Kay, 1972). The positive influence of various cosolvents, such as hexane, tetrahydrofuran, and triethanolamine, has been already demonstrated for the homogeneously and heterogeneously catalyzed alcoholysis. The addition of hexane at the amount of only 0.05% improved the FAME yield from 46.6% (without hexane) to 73.33% (Yang et al., 2010a) and the FAEE yield from 44.6% to 65.33% (Muppaneni et al., 2012). The optimal hexane amounts for supercritical ethanolysis (Muppaneni et al., 2012) and methanolysis (Yang et al., 2010a) are 0.2% and 0.3%, respectively. The addition of a small KOH amount as a cosolvent/catalyst can also reduce the reaction temperature and pressure, enabling the reaction under the subcritical conditions for alcohol (Yang et al., 2010a). The optimal KOH amount (0.3% of the oil mass) is significantly lower than the amount used in the conventional KOH-catalyzed

alcoholysis whereby the obtained FAME yield is higher for shorter reaction time.

2.2.4 | Other methods of COB production

Enzymatic alcoholysis and in situ transesterification of camelina oil have been rarely studied. Villalba et al. (2016) used commercial Novo-435 (Novozym® 435) lipase and the chemically modified preparations with improved operational stability to catalyze the camelina oil methanolysis and ethanolysis with/without the presence of *t*-butanol as a solvent. In the case of ethanolysis without *t*-butanol, the non-treated Novo-435 lipase is catalytically more active. The highest FAEE yield (92.36 ± 2.03%) was obtained in the presence of Novo-435 in the solvent-free system. When methanol was used as a nucleophile, a significantly smaller FAME yield was obtained (28% with Novo-435) due to the enzyme inactivation. The Novo-435/TNBS derivative is the most stable and active catalyst that provides the highest and almost the same FAME and FAEE yields in 14 cycles. Jung et al. (2017) performed the pyrolysis-assisted in situ transesterification of camelina seeds with dimethyl carbonate in the presence of highly porous silica that improved the contact between the reactants. The optimal temperature of 365°C provided the FAME amount higher than 95 µg/mg seed.

2.3 | Optimization and kinetic modeling of COB production

The statistical method has been most often employed to optimize the KOH-catalyzed COB production using a central composite (Yang et al., 2015), orthogonal array (Wu & Leung, 2011), and face-centered central composite (Yang et al., 2016b) design. In these studies, wide ranges of methanol:oil ratio, KOH concentration, temperature, and time are included. All process factors have a significant influence on ester yield, but the catalyst concentration is most influential. The optimal reaction conditions determined by different research groups using homogeneous and heterogeneous can be found in Table 1.

At present, there are only a couple of the kinetic studies of the heterogeneous base-catalyzed camelina oil alcoholysis using the overall stoichiometric equation:



where A, B, C, and D represent triacylglycerols, methanol, fatty acid methyl esters, and glycerol, respectively, and the general reaction rate equation:

$$-\frac{dC_A}{dt} = kC_A^a C_B^b \quad (2)$$

where C_A and C_B are the concentrations of A and B, respectively, t is time, k is a reaction rate constant, and a and b are the orders of A and B, respectively. By introducing the conversion degree of triacylglycerols, x_A , Eq. (2) can be transformed into Eq. (3):

$$-\frac{dx_A}{dt} = kC_{A0}^{a+b-1} (1-x_A)^a (M-3x_A)^b \quad (3)$$

where C_{A0} and C_{B0} are the initial concentrations of A and B, $C_A = C_{A0}(1-x_A)$, $C_B = C_{B0}(M-x_A)$, and $M = C_{B0}/C_{A0}$.

For the camelina oil methanolysis catalyzed over lobster waste shells, the pseudo-first-order kinetics regarding TAGs was proposed as methanol was in high excess and did not affect the reaction rate order (Hangun-Balkir, 2016):

$$-\ln(1-x_A) = kt \quad (4)$$

The activation energy of this reaction (62.5 kJ/mol) differs from those for different combinations of feedstock/catalyst (Table 2), which can be ascribed to the influence

of different catalyst performances (Ma et al., 2017) and different unsaturation degree and fatty acid profiles of various feedstocks (Pinzi et al., 2011; Sáez-Bastante et al., 2014).

The kinetics of the camelina oil alcoholysis over alkaline earth metal oxides under conventional and microwave heating was modeled using different reaction rate-orders regarding TAGs and methanol (Table 3) (Ma et al., 2017). The type of catalyst significantly affects the kinetics of the camelina oil methanolysis over alkaline earth metal oxides, which could be ascribed to their acid/base-site strengths and surface areas. BaO is a superior catalyst over SrO, CaO, and MgO. In addition, microwave heating improves the reaction rate constant compared to conventional heating (Ma et al., 2017).

2.4 | Techno-economic, environmental, and social aspects of COB production

Besides technological aspects, economic, environmental, and social impacts are important factors for evaluating the sustainability of COB production. They are created at all phases of camelina cultivation, seed processing, and COB

TABLE 2 The activation energy of the alcoholysis of various oils over base solid catalysts assuming the pseudo-first-order kinetics regarding TAGs, Eq. (4)

Feedstock	Catalyst	Activation energy, kJ/mol	References
Canola oil	Alumina-supported potassium	20.9–23.4	Wu et al. (2017)
Sunflower oil	Ba(OH) ₂	32.2	Stamenković et al. (2014)
Waste frying oil	FeCl ₃ -modified resin	35.51	Ma et al. (2017)
Waste lard	Quicklime (CaO)	59.1	Stojković et al. (2016)
Camelina oil	Lobster waste shells	62.5	Hangun-Balkir (2016)
Palm oil	DMC over solid KOH	79.1	Zhang et al. (2010)
Waste frying oil	Calcined snail shells	79.0	Birla et al. (2012)
Corn oil	DMC over solid KOH	83.3	Sun et al. (2014)

TABLE 3 Reaction rate-orders regarding TAGs and methanol for the camelina oil methanolysis over alkaline earth metal oxides (conventional heating)

Catalyst	Model	Reaction order		Reaction rate constant
		TAGs	Methanol	
BaO ^f	$\frac{1}{M-3} \left[\frac{x_A}{1-x_A} - \frac{3}{M-3} \ln \left(\frac{M-3x_A}{1-x_A} \right) \right] = kC_{A0}^2 t$	2	1	0.0526 g ² /(mol ² min)
SrO ^g		2	1	0.0493 g ² /(mol ² min)
MgO	$\frac{x_A}{1-x_A} = kC_{A0} t$	2	0	0.0463 g/(mol min)
CaO	$-\frac{1}{3} \ln \left(\frac{M-3x_A}{M} \right) = kt$	0	1	0.0006 min ⁻¹

^aThe same orders was found for the BaO-catalyzed methanolysis under microwave irradiation.

^bFor the microwave-assisted SrO-catalyzed methanolysis, the overall second-order model (zero-order for methanol) was determined.

production and use. Since there is almost no study of these issues of COB production, the results of assessing the biodiesel produced from other feedstocks may be used for the estimation of their implications.

2.4.1 | Techno-economic issues

Keske et al. (2013) have shown that the growth of camelina to produce biodiesel and protein-containing livestock is economically feasible. A stochastic break-even analysis points out a 0.51 probability of profitable camelina cultivation when petrodiesel price reaches 1.15 \$/l. Moreover, the sale of cake has the highest influence on profitability. If the diesel fuel price goes beyond 0.90 \$/l, the farmer will get more income from avoiding diesel fuel purchase than from the camelina meal sale. Risk analysis shows that a risk-opposed farmer would decide to cultivate camelina if the diesel price is at least equal to 1.31 \$/l. COB can replace petrodiesel on the farm, increasing farm income and diversifying rural economic development, through camelina cultivation.

Miller et al. (2012b) reported the optimum plant size for extracting the oil from camelina seed, which could be used to produce renewable fuels. For the average oil yield and the optimum annual capacity of 90 and 120 million liters of the plants using pressed and solvent-extracted oil, respectively, the minimum production cost is 0.28 \$/l. If meal cake can be sold, the camelina oil production cost is much lower than that of canola oil. If meal cake cannot be sold, the solvent- and pressed-extracted oil production costs at the optimum plant capacities are 0.82 and 1.04 \$/l, respectively. Overall oil cost is affected mainly by field cost (75%–85%), transportation cost (9%–19%), operating and maintenance cost (3%–5%), and capital recovery (2%–4%). Moreover, the production cost is most sensitive to meal cake price and field cost whereas the optimum plant capacity is mostly affected by transportation cost, capital cost, and operating and maintenance costs, as indicated by a sensitivity analysis.

2.4.2 | Camelina-based biorefineries

Camelina is considered a promising energy crop because of high oil content of its seed and high harvested residue (straw) yield. Camelina seeds contain oil (30%–48%), protein (33%–47%), and many valuable bioactive compounds such as phenolic compounds, tocopherols, glucosinolates, polyunsaturated fatty acids, polysaccharides, and lignins. The biorefinery concept requires the valorization of whole camelina biomass by the conversion of seeds, pods, leaves,

and straw, to worthy products (oil and meal), as well as food, feed, biofuels, and other industrial high-added-value products. Therefore, its application in a biorefinery is of deep interest, giving the high-added values to its oil, meal, and straw (above-ground biomass).

Pagnotta et al. (2019) have recently pointed out the perspectives for valorization of camelina in a green chemistry approach based on the integral use of whole biomass. Fatty acid profile of the oil and the polyphenol, flavanol, glucosinolate, and protein contents and antioxidant activities of the press cake (meal) point out the potential uses of camelina-based products in the food and pharmaceutical industries. The residual straw, except as natural soil fertilizer, can be converted into bioethanol, biogas, biochemicals, and nanofibrillated cellulose. Furthermore, the use of camelina biomass in a biorefinery is an environmentally friendly approach with a great energetic value that significantly reduces greenhouse gases (GHG) emissions compared to fossil diesel.

Righini et al. (2016) analyzed the advantages and disadvantages of growing camelina in Europe considering the biorefinery concept. From the agronomy point of view, the positive traits are high and quick emergence, short season, low water use, low input practice, and adaptability to marginal lands while the negative traits are small seed size, little knowledge on cultivation practice, uneven plant maturity, and seed shattering. The selection of the improved camelina varieties and the use of tailored harvesters will greatly decrease the loss of seed in the short cut. Regarding seed and its byproducts, the advantages are high contents of polyunsaturated (especially ω -3) fatty acids, eicosenoic acid, tocopherols, and proteins whereas the drawbacks are the presence of sinapine and glucosinolates and low oxidative stability. The negative traits of camelina like high iodine value, high content of erucic acid, and the negative correlation among oleic, erucic, and behenic acid can be overcome through breeding using the varieties with the desired properties (Li & Mupondwa, 2013).

A case study and sensitivity analysis confirm viability to grow camelina as a rotation crop and exploit the oil and byproducts based on a competitive market in the Canadian Prairies region (Li & Mupondwa, 2013). The camelina oil production cost depends on camelina cost, seed oil content, plant capacity, and meal cake price. Especially, the fluctuation of camelina cost significantly influences the oil production cost and the profitability of the crushing plant. The increase in camelina seed yield and oil content are critical factors in reducing the camelina cost and improving the economy of the camelina-based biorefinery. Maximizing the camelina meal price is critical for making the camelina oil competitive as a raw material for COB production. For a camelina-based biorefinery, Mohammad et al. (2018) consider the utilization of camelina seed to

get oil for biodiesel, meal cake for livestock, and straw for ethanol. Regarding the overall process, the energy input and output are 25.1 and 54.3 MJ/l ethanol, respectively. The net energy ratio of 2.16 MJ/l ethanol is competitive with other energy crops. The biorefinery process reduces GHG emissions by 40% if the produced biodiesel would be used instead of petrodiesel. The meal cake and glycerol provide a supplementary income of 1 \$/kg of produced oil. Therefore, the biorefinery for biodiesel and ethanol production from whole camelina crops is feasible, profitable, and sustainable due to the production of meal and glycerol.

2.4.3 | Environmental assessment of COB production

There are several evaluations of the environmental influence of camelina seed oil used for biodiesel production (Bacenetti et al., 2017; Dangol et al., 2015, 2017; Krohn & Fripp, 2012; Li & Mupondwa, 2013). The environmental impact of COB production is mainly related to seed production (85%–90%), whereas the part of the seed pressing and transesterification is usually limited (2% and 2%–10%, respectively) (Bacenetti et al., 2017). The main environmental impacts have the fertilizer and herbicide production, the diesel fuel, and exhaust gas emissions from the mechanization used in the field-based activities (especially, soil tillage, and sowing) and the emissions of nitrogen and phosphorus compounds associated with the fertilizers utilization.

Camelina avoids many of the possible traps of well-established biodiesel crops like food versus fuel controversy and land-use change. The LCA methodology with the help of a spreadsheet model shows that, when grown as a double crop or in rotation with wheat, camelina decreases GHG emissions by 40%–60% compared to petrodiesel (Krohn & Fripp, 2012). Besides that, by avoiding land-use-change emissions, COB generates less GHGs than canola and soybean biodiesels. Moreover, acceptable yields (1000–2000 kg/ha) should be achieved whereas lowering N fertilizer inputs to maintain and enhance the environmental viability of the camelina crop.

Dangol et al. (2017) compared the life cycle energy, impact on the environment, and economic achievement of the camelina oil-based production of biodiesel and biojet fuel. When fuel additives and transportation are not used, the ratios of the energy of the produced biodiesel and biojet fuel to the corresponding life cycle energy are 3.6 and 1.8, respectively. Biodiesel and biojet fuel reduce life cycle GHG emissions by 69% and 56.6% compared to petrodiesel and Jet-A fuel, respectively. Taking no credit for byproducts, the costs of components and utilities to produce

biodiesel and biojet fuel are US 75 ¢/l and 2.19 \$/l, respectively. Considering the potential demand for biodiesel, as well as the prices of petrodiesel and jet fuels, biojet fuel will not probably be competitive economically with biodiesel soon (Dangol et al., 2020). However, a negative trait of COB is bad oxidative stability (Dangol et al., 2015).

Li and Mupondwa (2013) assessed the influence of biodiesel and biojet fuel produced from camelina oil on the environment concerning global warming, energy resource consumption, ecosystem qualities, and human health. This analysis shows that GHG emissions for COB and biojet in the ranges of 7.61–24.72 and 3.06–31.01 kg CO₂ equivalent/MJ, respectively, are smaller than GHG emissions of other oilseed-based and fossil fuels. Non-renewable energy consumptions for COB and biojet fuel are 0.40–0.67 and –0.13 to 0.52 MJ/MJ. Camelina oil accounts for the largest contribution to the environmental impact, thus requiring the reduction in environmental loads during the seed production. Urea used as nitrogen fertilizer in camelina cultivation has significantly lower environmental impact than a urea/ammonium nitrate combination concerning climate change, ecosystem quality, and human health. On the other hand, in contrast to urea, the combination of urea and ammonium nitrate saves the consumption of energy resources. Higher seed yield will significantly decrease environmental impacts related to the production of camelina seed, oil, and fuel. The main three contributors to global warming, besides camelina oil, are sodium methoxide, and methanol for biodiesel and natural gas and electricity for biojet fuel.

Krzyżaniak and Stolarski (2019) evaluated the influence of camelina cultivation using conventional and reduced tillage by an LCA. The GHG emission is 1.732 or 1.152 kg CO₂ eq./t of dry seeds while converted to the oil and energy content, it is 3.368 and 23.74 kg CO₂ eq./GJ, respectively. Reduced tillage decreases the GHG emission minimally (1.29%). The highest normalized score for cultivation methods was related to fossil depletion, climate change, and particulate matter formation, leading to serious harm to the human health category. Reduced tillage causes a lower environmental impact than conventional one because of the lower utilization of production means despite the soil carbon change is not accounted for. Also, camelina cultivation in reduced tillage on large plantations could reduce GHG emissions. Besides that, the productions of heat or electricity from straw, meal cake, or transporting biofuels can affect additionally the faster accomplishment of national indices as demanded by the EU directives.

From an environmental standpoint and compared to fossil diesel, biodiesel has many advantages: faster biodegradability, low-toxicity, higher oxygen content, no sulfur content, lower GHG emission. The reduction in GHG

emission in the case of Colombian palm biodiesel was estimated to be in the range from 83% to 108% compared to fossil diesel (Acosta, 2017). Additionally, COB emitted fewer GHGs than the biodiesel obtained from soybean and canola oils (Krohn & Fripp, 2012). The exhaust gas emission of biodiesel is related to its oxygen content (10%–12%) although it is also affected by its physicochemical properties, engine performances, and operating conditions (Altun, 2014).

2.4.4 | Human health and ecosystem qualities

Li and Mupondwa assessed the impacts of COB and bio-jet fuel on human health and ecosystem quality (Li & Mupondwa, 2013). Many factors affect human health or ecosystem quality directly or indirectly, thus complicating their relationship with midpoint categories. These two damage categories are aggravated more by land occupation, terrestrial ecotoxicity, respiratory inorganics, and human toxicity. The main factors damaging human health are natural gas, fertilizer, and combine harvesting. The major factor of human toxicity is natural gas that contains carcinogens. Fertilizers contribute to human health damage, while they influence the ecosystem qualities to a smaller degree, except acidification. The agricultural inputs are the major factors damaging ecosystem quality, but their contributions are fairly distributed. The production of electricity, chemicals, and especially natural gas is amenable for eutrophication, acidification, photochemical oxidant formation, and abiotic depletion.

2.5 | Fuel properties of COB in comparison with biodiesel standards

To be commercially used as fuel in diesel engines, the COB must satisfy the biodiesel standards like ASTM D6751 or EN 14214. Generally, many properties of COBs meet the biodiesel quality standards, with exceptions of iodine value, linolenic acid and polyunsaturated methyl ester, viscosity, cetane number, and CFPP.

Iodine value measures the unsaturation degree of biodiesel and influences its stability during storage and use. As a rule, the iodine value of all COBs exceeds the limited value of the EN14214 standard (120 g I₂/g) due to a large content of polyunsaturated fatty acid esters and ranges from 142 (Moser, 2016) to 166.2 (Yang et al., 2010a). Exceptionally, Oni and Oluwatosin (2020) reported a significantly lower iodine value (81.7) with

no explanation. Iodine value can be reduced below the standard limit by blending the camelina methyl esters with the pork lard (Zaleckas et al., 2012), beef tallow (Sendžikienė et al., 2012), or waste frying oil (Zaleckas et al., 2012) esters.

The biodiesel properties like the contents of linolenic acid and polyunsaturated methyl esters are closely related to the camelina oil fatty acid profile. The former property of COB exceeds the EN14214 standard limit of 12% for almost three times and ranges between 32.7% and 38.09% (Ciubota-Rosie et al., 2013; Sáez-Bastante et al., 2015; Yang et al. 2016a; Yang et al., 2016b). However, the mixtures of COB with lard (Zaleckas et al., 2012) and used cooking oil (Zaleckas et al., 2012) methyl esters satisfy the standard specification, which results from their lower linolenic acid content. The polyunsaturated methyl ester content is also above the EN14214 standard limit (Ciubota-Rosie et al., 2013; Yang et al. 2016a).

Cetane number points out the ignition delay of a fuel after its injection into the combustion cylinder. Significantly lower cetane numbers than the limit of both standards have been reported for camelina biodiesels (Ciubota-Rosie et al., 2013; Sun et al., 2015). In most of the studies, the cetane number of COB satisfies or is close (up to 6% less) to the standard limit due to the fatty acid composition and high unsaturation degree of the used camelina oil (Ciubota-Rosie et al., 2013). Sun et al. (2015) observed a lower cetane number for the mixtures of methyl or ethyl esters with butyl esters (42.16–47.63) and its decrease with increasing the butyl ester proportion. Oni and Oluwatosin (2020) reported the cetane number of a camelina oil biodiesel of 55, while the B10 and B5 blends had the cetane number of 45 and 44, respectively.

The kinematic viscosity is of importance for the fuel atomization and combustion in the diesel engine. COBs, generally, fulfill the standard requirements regarding the kinematic viscosity. A higher viscosity is observed for the camelina methyl-butyl and ethyl-butyl ester mixtures, which increases with increasing the butanol proportion in the alcoholic mixture during the esters synthesis (Sun et al., 2015). The increase in the butyl esters amount leads to a higher content of the esters with 20 or more carbons that contributes to a higher kinematic viscosity (Moser, 2016).

The most important parameter for evaluating the fuel flow operability at low temperatures is the CFPP, the lowest temperature at which fuel begins to gush through a standard filter. COB does not satisfy the EN14214 standard specification. Its cold flow operability can be improved by blending it with fossil diesel. A decrease in the biodiesel proportion in the biodiesel/diesel blend from 80% to 20% reduces the CFPP from -7°C to -13°C (Fröhlich and

Rice, 2005). The CFPP of COB decreases more than twice in the B5 and B10 blends (Oni and Oluwatosin, 2020). Another method for reducing the CFPP of COB is to use the suitable additives like CP7134 and Lubrizol (Fröhlich and Rice, 2005). The CFPP of a camelina and used frying oil esters mixture was reduced below the standard limit by the addition of Wintron XC-30 or Infineum R-442 depressant (Zaleckas et al., 2012).

Oxidation stability or oil stability index (OSI) of a biodiesel is measure of its degradation by atmospheric oxygen. Oxidation causes the formation of corrosive acids and polymers that block jets and impede the normal engine operation. The oxidation stability of “pure” COB (0.6–2.9 h) is below the standard limit (Ciubota-Rosie et al., 2013; Moser and Vaughn, 2010; Yang et al. 2016a) because of its high unsaturation degree and high linoleic acid ester content (Knothe, 2005), with an exception (Oni and Oluwatosin, 2020) where the oxidation stability is 12.36 h. The COB stability can be improved by its blending with the methyl esters obtained from other feedstocks (Sendžikienė et al., 2012) or by adding antioxidants (Zaleckas et al., 2012).

3 | PERSPECTIVES OF COB PRODUCTION FROM GENETIC ENGINEERING POINT OF VIEW

Camelina has been tested mainly as a potential biofuel crop, but because of its specific oil composition, it likely deserves higher deliberation as a feedstock to produce biodiesel, aviation fuel, nutritional uses, and animal and fish feeding. The overviews of the prospective uses of camelina crop in food, feed, and chemical industry can be found elsewhere (Aslam et al., 2019; Berti et al., 2016; Faure & Tepfer, 2016). In the following section, the state of the art as far as the genomic and genetic resources available in this plant for manipulation of gene and metabolic pathways to enhance the production and quality of the oil is presented. For brevity sake, only representative examples have been cited.

3.1 | Genetic resources in *Camelina sativa*

3.1.1 | Genomics

Camelina is known to consist of 6–11 species as reported in the literature (Brock et al., 2018). However, there are only 6–7 species, which match the ploidy and chromosome number. These are *C. sativa*, *C. microcarpa* Andr.

ex DC., *C. hispida* (Boiss.) Hedge, *C. rumelica* Velen., *C. neglecta*, and *C. laxa* C.A. Mey (Choudhury et al., 2014). The genome size of *Camelina* ranges from 750 to 785 Mb (Hutcheon et al., 2010; Kagale et al., 2014). The genome analysis, mapping, and sequencing of the camelina genome has been performed using different next-gen approaches such as Hybrid Illumina and Roche 454, which contributed toward the annotation of 89,418 protein-coding genes (Hutcheon et al., 2010; Kagale et al., 2014). The function of genes present in three copies in *C. sativa* genome showed significant divergence, which implicates the complex regulatory pathway for lipid synthesis as regulated by multiple loci (Kagale et al., 2014).

3.1.2 | Transcriptomics

The transcriptome of camelina seeds was used to identify gene regulatory components in the lipid synthesis pathway for oil quality improvement (Abdullah et al., 2018; Nguyen et al., 2013). Illumina GAIIx platform was used for the transcriptomic studies, which could cover most of the genes, especially the lipid synthesis genes that help in the identification of the regulatory networks at different developmental stages and in diverse plant organs (Mudalkar et al., 2014). Similarly, many fatty acid biosynthesis pathway genes were identified by analyzing the transcript abundance for 32,759 genes at different developmental stages of the plant aiding in the identification of key enzymes and molecular mechanisms crucial for the seed development and rendering the oil more suitable for biodiesel production (Wang et al., 2015). A total of 2518 and 3136 transcripts showing differential expression in diacylglycerol acyltransferase (DGAT1) and glycerol-3-phosphate dehydrogenase (GPD1) in camelina transgenic lines, respectively, were identified by Abdullah et al. (2018). This correlation between the transcriptome and metabolome helped in identification of key metabolic switches for lipid production in *Camelina* seeds at different developmental stages.

3.1.3 | Molecular markers

Various techniques such as Random Amplification of Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) were used to investigate the genetic diversity predicated on oil content, seed weight, protein content, etc. in 130 accessions of *Camelina*. The genetic variation in the accessions identified using this technique was used for marker-assisted breeding of *Camelina* (Gehring et al., 2006). This

led to the identification of eight more QTLs for fatty acids (C18:1, C18:2, C18:3, C20:1 ω 7/ ω 9/ ω 11, C22:1 ω 9), plant height, 1000-seed mass, seed yield, and oil content. Also, 15 *Camelina* specific simple sequence repeat (SSR) markers were used to analyze across 40 accessions of *Camelina* that showed high allelic abundance with 57.4% genetic variation (Manca et al., 2013).

3.1.4 | Micro-RNA

In *Camelina*, many known (207) and novel (5) miRNAs have been identified in developing leaves, seeds, and flower buds by deep sequencing technique. In this study, the targets were predicted using In-silico analysis. These miRNAs showed varied function in lipid metabolism in different tissues at different developmental stages (Poudel et al., 2015). Artificial miRNAs (amiRNAs) have also been used for the silencing of the lipid biosynthesis pathway genes as shown in Table 4. Thus, miRNAs can be used as a tool to modify the expression of lipid and fatty acid biosynthesis genes in the seed to improve the quantity and quality of oil/biodiesel.

3.2 | Metabolic engineering in *Camelina* for lipid enhancement

Over the years, genetic engineering techniques such as gene editing and synthetic biology have been developed in various model plants such as *Arabidopsis*. The gene networks in lipid and fatty acid biosynthesis in plants have been well studied, and a plethora of information is now available. This has paved the way for gene/pathway manipulation studies, which have resulted in improving the quality of oil produced by various plants. *C. sativa* is established as a novel model plant and a vital platform for metabolic engineering studies for improvement in oil yield and quality (Yuan & Li, 2020). *C. sativa* is now being exploited as a platform for biodiesel production (Bansal and Durrett, 2016; Collins-Silva et al., 2011; Haslam et al., 2016). More than 90% of the lipid metabolism genes in *Camelina* are similar to that in *Arabidopsis*, which helped in further reliability of its pathways after the reference genome (Kagale et al., 2014). Presently, camelina is receiving significant interest from the scientific community because of its constantly expanding cultivation in the world and the genetic resources generated in recent years. Oil enhancement in *Camelina* seeds can occur either by increasing the biomass yield or by improving the lipid content. In these kinds of genetic improvements, the photosynthetic activity is ameliorated to divert the carbon flux toward the improvement of seed characteristics.

3.2.1 | Enhancement of seed biomass

Biomass yield can be enhanced by either increasing the seed size or seed yield. This has been achieved by genetic manipulation of various enzymes from or outside the lipid biosynthesis pathway. In one of the earlier studies, *Arabidopsis* purple acid phosphatase (AtPAP2) was expressed in *C. sativa* (Zhang et al., 2012). PAP2 has an important role in phosphate absorption and utilization. The majority of phosphate is stored in the form of phytic acid/phytate in the seeds, which is used up during seed germination. Hence, the amount of phytate is directly related to the seed size. Overexpression of AtPAP2 resulted in 50% more seeds per plant and higher seed size as compared to the wild type under specific environmental conditions (Zhang et al., 2012). In addition, the flowering time was reduced and growth was enhanced as compared to the wild type. Further, Choudhary et al. (2014) expressed the γ -subunit of G-protein from *Arabidopsis* in camelina. G-protein signaling is responsible for cell proliferation in the seeds. The gene was expressed using a constitutive *CaMV* 35S promoter and soybean *glycinin* promoter. This resulted in an enhancement in the seed size and seed number with higher oil content (Choudhary et al., 2014). The efficiency of carbon concentration and assimilation was ameliorated in *Camelina* by the expression of CO₂/HCO₃ transporters. These transporters carry the HCO₃ to the cytoplasm for conversion to CO₂ by carbonic anhydrase present in carboxysomes. This aids in concentrating CO₂ near the rubisco, which helps in enhancing photosynthetic processes by inhibiting oxygenase activity. As a result, the CO₂ uptake and assimilation in the leaves were enhanced by 20%–30% and the seed yield also increased by 20%–44% (Paulose et al., 2014). More recently, a transcription factor, WRINKLED 1 (AtWRI1), was expressed resulting in 14% enhancement in seed mass and oil content (An and Suh, 2014). A patatin-related phospholipase (pPLAIII δ) was expressed in *Camelina* such that the phosphatidylcholine (PC) is hydrolyzed into free fatty acids and lysophosphatidylcholine (Li et al., 2015). As a result, the seed oil content was enhanced without affecting the seed yield; however, the plant growth was compromised. In addition, the cellulose content of the seed was affected and the amount of fatty acid with >20 carbons was increased (Li et al., 2015).

Genes from *E. coli* involved in the catabolic process of the glycolate pathway were also expressed in *Camelina*. These genes are responsible for the diversion of carbon flux toward photosynthesis by preventing photorespiratory carbon loss. Expression of partial glycolate dehydrogenase gene or co-expression of full-length bacterial genes namely glycolate dehydrogenase, tartaric semialdehyde reductase, and glyoxylate carboxylase ensued in ameliorating seed yield by 50%–72% and drop in the crop

TABLE 4 Genetic modification studies performed in *Camelina sativa* for improving the quantity and quality of oil

Gene	Promoter	Transformation technique	Phenotypic improvements	References
<i>Arabidopsis</i> purple acid phosphate (ATPAP2)	-	<i>Agrobacterium</i> -mediated	<ul style="list-style-type: none"> 50% increase in number of seeds per plant compared to wild type Enhanced plant growth 	Zhang et al. (2012)
<ul style="list-style-type: none"> RNAi of single gene FAD2 RNAi of double FAD2- FAE1 	Seed-specific soybean glycinin-1	<i>Agrobacterium</i> -mediated using floral dip technique	<ul style="list-style-type: none"> Oil with 50% oleic acid C18:2 and C18:3 amounts decreased to 4.5 wt% and 13 wt%, respectively High oleic acid lines (70%) 18:2 reduced to 4 wt% and 18:3 reduced to 8 wt% C20 and C22 reduced to 4% from 17% in wild type 	Nguyen et al. (2013)
G-protein γ subunit 3 (<i>Arabidopsis</i>)	<i>CaMV35S</i> and seed-specific soybean Glycinin	<i>Agrobacterium</i> -mediated	<ul style="list-style-type: none"> Enhancement in size, mass and number per plant by 15%–40% More oil content per plant 	Choudhury et al. (2014)
Enhancement of carbon fixation and fixed carbon transport	<i>CaMV35S</i>	-	<ul style="list-style-type: none"> Enhancement of CO₂ assimilation and seed yield by 20%–30% and 20%–44%, respectively 	Paulose et al. (2014)
Transcription factor, WRINKLED1	Seed-specific SiW6	<i>Agrobacterium</i> -mediated	<ul style="list-style-type: none"> Increased seed mass Enhancement in seed oil by 14% 	An and Suh (2014)
Phospholipase overexpression (pPLAIII δ)	<i>CaMV35S</i> promoter/seed-specific	<i>Agrobacterium</i> -mediated using floral dip technique	<ul style="list-style-type: none"> Increased seed oil content by 14% No negative effects on plant growth Decreased seed yield Cellulose content decreased by 17% 	Li et al. (2015)
<i>E. coli</i> genes	<i>CaMV35S</i>	<i>Agrobacterium</i> -mediated using floral dip technique	<ul style="list-style-type: none"> Partial bypass- Seed yield increased by 50–57% Full bypass- Seed yield increased by 57%–73% Transgenic lines showed increased biomass and faster development. 	Dalal et al. (2015)
<ul style="list-style-type: none"> Partial photorespiration bypass- Glycolate dehydrogenase Full photorespiration bypass- Glycolate dehydrogenase, tartronic semialdehyde reductase, glyoxylate carboxyligase 	Seed-specific soybean glycinin-1	<i>Agrobacterium</i> -mediated using floral dip technique	<ul style="list-style-type: none"> Increased amounts of C10:0, C14:0, C16:0 fatty acids in seed oil Accumulation of C12:0 and C14:0 Reduced C12:0 in seeds 	Kim et al. (2015)

(Continues)

TABLE 4 (Continued)

Gene	Promoter	Transformation technique	Phenotypic improvements	References
<ul style="list-style-type: none"> Co-expression of $\Delta 9$-acyl-ACP and $\Delta 9$-16:0-ACP desaturase Co-expression with RNAi of KAS II and FatB genes 	Seed-specific	-	<ul style="list-style-type: none"> Amount of omega-7 monounsaturated fatty acids increased to 17% Omega-7 monounsaturated fatty acids further increased to 60–65% of the total fatty acids in <i>Camelina</i> seeds Saturated fatty acids reduced to 5% as compared to 12% in wild type 	Nguyen et al. (2015)
<ul style="list-style-type: none"> Diacylacyl-transferase (EaDAcT) Expression of EaDAcT+ RNAi of diacylglycerol acyl transferase 	Seed-specific soybean glycinin	Agrobacterium-mediated	<ul style="list-style-type: none"> 70 mol% acetyl-TAG in seeds RNAi suppression of DGATI resulted in 85 mol% acetyl-TAG in seeds with uncompromised seed yield and seed germination Total TAG in the seeds increased by 20% Twofold reduction in very long-chain fatty acids 	Liu et al. (2015a, 2015b)
Fatty acid desaturase 2 (FAD2) Knockout of all three CsFAD2s by CRISPR/Cas9	Cas9-Cauliflower mosaic virus35S sgRNA- <i>Arabidopsis</i> U6	Floral dip technique	<ul style="list-style-type: none"> Increase in oleic acid content from 16% to >50% Increase in MUFA^a, 18:1, 20:1, 22:1, from 32% to >70% Decrease in linoleic (16% to 4%) and linolenic acids (35% to 10%) 	Jiang et al. (2017)
Knockout of three, two, and an homologous CsFAD2 (Fatty acid desaturase2) by CRISPR/Cas9	sgRNA1- <i>Camelina</i> U3, sgRNA2- <i>Camelina</i> U6	Agrobacterium-mediated floral dip technique	<ul style="list-style-type: none"> Increase in oleic acid content from 10% to 62% of total FA^a content 	Morineau et al. (2017)
Two <i>Arabidopsis</i> phospholipase D ζ genes (AtPLD ζ 1 and AtPLD ζ 2) Co-expression of AtPLD ζ 1 and AtPLD ζ 2	AtPLD ζ 1- seed-specific glycinin, AtPLD ζ 2- seed-specific β -conglycinin	Agrobacterium-mediated floral dip technique	<ul style="list-style-type: none"> TAG was increased by 2% to 3% compared to wild type. Increase in linolenic acid (18:3) and eicosenoic acid (20:1) FAs was concurrent with decrease in other FAs 	Yang et al. (2017)
Zea mays Leafy cotyledon 1 (LEC1)	Serine carboxypeptidase-like (SCPL17) and acyl carrier protein (ACP5)	Agrobacterium-mediated	<ul style="list-style-type: none"> Increased oil content by 26% in mature <i>Camelina</i> seeds 	Zhu et al. (2018)
<ul style="list-style-type: none"> Co-expression of EaDAcT with single or combination of ChFatB2, cpFatB2, UcfFatB1, CnLPAAT Each co-expression coupled with CsDGATI-RNAi and CsPDATI-RNAi 	Nopaline synthase	Agrobacterium-mediated	<ul style="list-style-type: none"> 50 mol% of MCFA-AcTAGs accumulated With RNAi, MCFA-AcTAGs content was up to 77% 	Bansal et al. (2018)

(Continues)

TABLE 4 (Continued)

Gene	Promoter	Transformation technique	Phenotypic improvements	References
Artificial microRNA mediated suppression of Fatty acyl-ACP thioesterases (CsFATB)	Seed-specific glycinin	Agrobacterium-mediated	<ul style="list-style-type: none"> Palmitic acid (16:0) amount reduced by 45% Stearic acid (18:0) amount reduced by 38% 35% reduction in total saturated FAs and ~65%–70% oleic acid accumulation in seed oil 	Ozseyhan et al. (2018)
Knocking out three CsFAE1 (Fatty acid elongase1) alleles by CRISPR/Cas9	Cas9-Egg cell-specific enhancer and sgRNA- <i>Arabidopsis</i> U6-26	Agrobacterium-mediated vacuum infiltration method	<ul style="list-style-type: none"> Reduction in VLCFAs^b from over 22% to <2% of total fatty acids Concomitant increase in C18 unsaturated fatty acids 	Morineau et al. (2017)
Co-expression <i>AtDGAT1</i> and glycerol-3-phosphate dehydrogenase (yeast)	Seed-specific glycinin	Agrobacterium-mediated floral dip method	<ul style="list-style-type: none"> 13% and up to 52% increase in seed oil and seed mass, respectively 19%, 9% and 8% decrease in C18:1, C18:3 and C20:1 fractions, respectively 	Chhikara et al. (2018)

^aMUFA, Monounsaturated fatty acid.^bVLCFA, Very long-chain fatty acid.^cFA, Fatty acid.

duration by 1–2 weeks. The expression of these genes also increased the nitrogen use efficiency by 15% without compromising the seed quality at ambient or low CO₂ levels but not at higher CO₂ levels (Dalal et al., 2015).

3.2.2 | Ameliorating lipid content and quality

Lipid formation in plants involves three pathways—fatty acid biosynthesis pathway in the chloroplast, fatty acid acylation and elongation in the cytoplasm, and final TAG formation in the endoplasmic reticulum. During fatty acid biosynthesis, acetyl CoA, derived from the glycolytic pathway, with NADPH, is converted to malonyl CoA. Transacylases aid in binding of acetyl CoA and malonyl CoA with acyl carrier protein (ACP) and form acetoacetyl ACP by the action of enzyme β -ketoacyl synthase. Fatty acid synthase catalyzes the elongation of fatty acid chain in multiple cycles. This is followed by reduction, dehydration, and reduction to form saturated fatty acid. The four-carbon fatty acid is now ready to accept another acetyl CoA moiety, which results in further elongation of the fatty acid chain. The thioesterases further catalyze the hydrolysis of acyl chains to form free fatty acids (Cagliari et al., 2011). The fatty acids are transported to the cytosol in the acyl CoA pools. The TAG biosynthesis pathway in the endoplasmic reticulum is also referred to as the Kennedy pathway. It starts with acylation of glycerol-3-phosphate to form lysophosphatidic acid by the action of glycerol-3-phosphate acyltransferase. The successive acylation by lysophosphatidic acid acyl transferase (LPAT) leads to the formation of phosphatidic acid. Phosphatidic acid so produced releases its phosphate to form diacylglycerol (DAG). The ultimate acylation of DAG by diacylglycerol acyltransferase results in the generation of TAGs (Cagliari et al., 2011).

Lipid enhancement using fatty acid biosynthesis pathway genes

The fatty acids present in seed oil can be having small chain (4:0–9:0), medium chain (9:0–14:0), or long chain (14:0–24:0). These fatty acids determine the functional characteristics of the oil such as viscosity, oxidative stability, and combustion properties. Hence, genetic improvements in this pathway yield seed oil with improved qualities (Marmon et al., 2017; Usher et al., 2017).

The FatB genes that synthesize medium-chain fatty acids were earlier isolated and expressed from *Cuphea* in other oil crops to produce jet fuel (Kim et al., 2015). Thus, the overexpression of *CpuFATB4* resulted in the enhancement of C16:0 and C14:0 fatty acid in *Camelina* (Kallio et al., 2014). FAD is responsible for introducing unsaturation in the fatty acids. A higher number of unsaturation

deteriorate the quality of oil as the physical properties of the oil get altered, especially, cold flow properties and oxidative stability (Kang et al., 2011). In a study by Nguyen et al. (2013), the *Camelina* FAD2 expression is diminished using an antisense suppression which resulted in 50% by weight of oleic acid (C18:1) content; however, C18:2 and C18:3 were reduced in amount by 6% and 11%. In another study, simultaneous RNAi suppression of FAD2 and FAE1 in *Camelina* seeds resulted in a rise in C18:1 FA fraction to 70% and reduced C18:2 and 18:3 by 4% and 8% as compared to 17% and 36%, respectively, in the wild type (Nguyen et al., 2013).

Omega-7 monounsaturated fatty acids are known for the improvement of cold flow characteristics, ignition property, and oxidation stability and the reduction in NO_x emissions (Kang et al., 2011). This was achieved in *Camelina* by co-expression of Δ 9-acyl-ACP and Δ 9-16:0-ACP desaturase transgene (Nguyen et al., 2015). The co-expression was coupled with RNAi-mediated silencing of 3-ketoacyl-ACP synthase II and FatB 16:0-ACP thioesterase. 3-ketoacyl-ACP synthase is involved in elongation of ACP-bound species, and FatB is responsible for an essential role of the synthesis of saturated FAs. This resulted in increased generation of ω -7 fatty acid, which was 60%–65% of the total FA content and a concomitant two- to threefold decrease in total saturated FA content (Nguyen et al., 2015). FatB genes were co-expressed with LPAT, an enzyme, which produces the phosphatidic acid precursor required for the synthesis of TAG in camelina seeds (Durrett et al., 2008). This resulted in enhanced content of the medium-chain FAs, particularly C12:0 and C14:0. RNAi-mediated silencing of β -ketoacyl-ACP synthase II enhanced the palmitic acid content with a concomitant decrease in lauric acid (12:0) rendering it suitable for a jet fuel application (Durrett et al., 2008). Apart from genes directly involved in lipid synthesis, the expression of their regulators can also drive the pathway toward higher oil accumulation. Recently, Zhu et al. (2018) investigated the effect of the expression of a master regulator (transcription factor, LEC1) of the FA biosynthesis pathway. It resulted in the enhancement of oil content by 26% in mature *Camelina* seeds, without any detrimental effect on plant growth.

Lipid enhancement using triacylglycerol biosynthesis pathway genes

Triacylglycerols are the stored energy molecules in crop seeds used as feedstock for synthesis of biodiesel. However, the FAs linked to the TAG molecules determine their functionalities and properties. Recently, studies have been carried out to produce designer lipids, which are medium-chain acetyl-TAGs (Bansal et al., 2018; Yuan & Li, 2020). These lipids have less viscosity and better

cold flow properties as compared to long-chain TAGs. Engineered production of these lipids in *Camelina* was performed using the seed-specific promoter driven expression of acyl transferase (EaDacT) gene from *Euonymus alatus*, which resulted in the production of 50% acetyl-TAGs in the seeds (Liu et al., 2015a). In another study by the same group, the expression of this gene was coupled with RNAi suppression of endogenous DGAT1 gene, which also led to enhanced production of acetyl-TAGs (85%) without compromising seed yield or seed germination process (Liu et al., 2015b). Bansal et al. (2018), also conducted similar studies as shown in Table 4 resulting in 77% more production of acetyl-TAG. Phospholipase increases the diacylglycerol flux into the generation of TAG. Phospholipase D ζ 1 and phospholipase D ζ 2 genes from *Arabidopsis* were co-expressed in *Camelina* resulting in a minor enhancement in the TAG content. The rise in C18:3 and C20:1 FA was corresponding to the drop in the content of other FAs (Yang et al., 2017). In another study by Li et al. (2015), *Arabidopsis* phospholipase (phospholipase AIIIId) was overexpressed under a constitutive and a seed-specific promoter to investigate its effect on the seed oil content. It was found that under constitutive expression, the seed oil content increased significantly; however, the cellulose content in the seed was compromised. The seed-specific expression of this gene, however, did not display any negative effects. Seed-specific co-expression of *Arabidopsis* DGAT1 and a yeast cytosolic GPD1 displayed a 13% rise in the seed oil and a 52% rise in the seed mass (Chhikara et al., 2018). In two separate studies, Marmon et al. (2017) and Na et al. (2018) used miRNA-based suppression of CsPDAT (phosphatidylcholine: diacylglycerol acyltransferase) and CsFAD3 (FA desaturases) resulting in the increased formation of linolenic acid and linoleic acid.

CRISPR/Cas9 genome editing technique for lipid enhancements and modifications

The recently developed technique of the CRISPR/cas9 system has been used in *Camelina* for improving lipid content and quality. CRISPR/Cas9 is an advanced technique that allows the insertion/deletion of a gene of interest at a specific locus in the host organism. In a study by Jiang et al. (2017), all three homologs of fatty acid desaturases (CsFAD2) genes were knocked out using CRISPR/cas9. This ensued in a 16%–50% rise in FA content and a reduction in the polyunsaturated FA content. Targeted mutagenesis of CsFAD2 was also performed using CRISPR/cas9 resulting in enhancement of oleic acid content in oil and concomitant reduction in the polyunsaturated FAs (Morineau et al., 2017). The FAE1 gene responsible for elongation of the FA chain required to produce long-chain FAs was inactivated using the CRISPR/cas9 technique

having an egg cell-specific cas9 expression. It resulted in a massive reduction in the content of long-chain FAs (C20-C24) from 22% in wild-type to 2% in the mutant strains. This also resulted in the enhancement of oleic and linolenic acid in *Camelina* oil. Therefore, CRISPR/Cas9 genome editing technique can be successfully used to manipulate various pathways to achieve a profound understanding of the lipid biosynthesis and utilize the information for improvement in the oil content and quality (Ozseyhan et al., 2018).

4 | FUTURE PERSPECTIVE OF COB PRODUCTION

The economic, environmental, and social importance of the bioeconomy is rapidly increasing on the global level. In the actual European scenario, petrol-based products should be substituted by renewable plant-derived products but the food-oriented crops must be avoided for this (Chaturvedi et al., 2019). This scenario recognizes the great role of oilseed crops. Production of vegetable oils has been permanently enlarged during the last two decades achieving presently 207.5 million metric tons to go on in the future (Statista, 2019). About 75% of the world's production of vegetable oils originates from edible oils (soybean, rapeseed, and palm), whereas the remaining oils are from other oilseeds containing peculiar fatty acid profile (Righini et al., 2016). Among the second group of oilseed crops, camelina is identified as one of the main plants for the supervenient EU bioeconomy (Righini et al., 2016).

Throughout the process of biodiesel production from camelina oil, care is needed to enhance the opportunity for its commercialization. Better cultivation practices are needed to increase camelina production worldwide as an energy crop (Berti et al., 2016). With the development of the new high-yielding varieties, with improved seed quality and resistance against disease and insect, camelina may be a promising crop as a future renewable feedstock for the biodiesel industry (Chaturvedi et al., 2019; Berti et al., 2016). Progress of genetics, genomics, and breeding have accelerated developing engineered camelina (Berti et al., 2016) and have led to the production and phenotyping of new lines (Faure & Tepfer, 2016). Hence, the introduction of many metabolic pathways into camelina to produce new lipids has pointed out its potential and versatility that it is going to become an ideal plant for biological lipid synthesis.

Camelina possesses more biodiesel-producing potential per unit area of land than many other crops with the minimum inputs, which is particularly useful for effective spring moisture utilization (Aslam et al., 2019). It

is also important that camelina can be grown into arid regions as a rotation crop (Dangol et al., 2015). From 846,500 ha of land in the Pacific Northwest region of the United States, 443.0 million liters of biodiesel and 1.2 million tons of meal cake could be potentially produced annually. Therefore, COB is considered an advanced biofuel whereas byproduct meal cake could be locally used for livestock (Dangol et al., 2015). These two major products of camelina seed processing are of great commercial value and potential to contribute to the benefits of farmers, companies, and national economies (Mupondwa et al., 2016). Besides that, camelina cultivation offers the advantage of savings in GHG emissions (Aslam et al., 2019). For instance, camelina jet fuel provides about 84% savings in GHG emissions, compared with petrol jet fuel.

Among the unit operations used in the camelina seed processing to get the oil, extraction is probably most critical as it affects the camelina oil quality and quantity. Therefore, a permanent request to the researchers is to increase the camelina oil extraction yield by selecting a better extraction technique and optimizing its operating conditions. Most of the studies on camelina seed oil extraction have been conducted on a laboratory level using conventional techniques (mechanical and solvent extraction), whereas novel, improved techniques have not been used except supercritical-CO₂ extraction. These batch processes have been rarely optimized statistically regarding the major operating variables. Moreover, the kinetics of the camelina seed oil extraction processes are underexplored while their thermodynamics is not analyzed at all. Besides that, the novel extraction methods like aqueous enzyme and ultrasound- or microwave-assisted extractions have the potential to improve the oil extraction rate, shorten the operating time, and minimize the deterioration of oil quality and hence can reduce some drawbacks of the conventional oil extraction techniques. In line with this, the research should be extended to continuous novel extraction techniques in the laboratory and then scaled-up to pilot and industrial levels. In addition, the techno-economic evaluation of various novel extraction techniques should be carried out to assess their feasibility and profitability.

So far, COB production is mainly carried out using homogeneous catalysts like alkali hydroxides. Despite they are inexpensive and speed up the alcoholysis reaction, they should be neutralized and separated from the products by washing that makes the overall process more complex. These drawbacks can be avoided using heterogeneous catalysts that are separated from the products without difficulty and be reused, but the reaction rate is slower than the rate of homogeneous catalysis. A few heterogeneous catalysts have been tested in COB production, and

further investigations are needed to select a suitable solid catalyst regarding its stability, activity, and cost. Also, the reaction conditions should be optimized to select those ensuring the highest biodiesel yield. Moreover, the reaction kinetics should be defined using an adequate kinetic model that can be trustworthy for the process design and scale-up. Besides that, prominent process intensification technologies should be tested, aiming at increasing the product yield, shortening the reaction time, and reducing the amount of alcohol and catalyst, which all will contribute a further reduction in operating cost and energy consumption. These technologies use new reactor types that improve mass/heat transfer or remove the products from the reaction mixture.

5 | CONCLUSION

This review presents camelina as an important, underutilized oilseed crop with great potential as a feedstock in biodiesel production and other industrial applications. However, innovative research is needed to help the improvement of COB production. The kinetics of the camelina oil transesterification should be reflected, whereas this reaction should be optimized regarding the type of reactor, reaction temperature, type and concentration of alcohol, as well as type and concentration of catalyst. It is important to test the cheap, active, and stable solid catalysts in advanced reactors with continuous operation. Further improvement can be achieved through biorefineries able to produce sustainable COB and other worthy chemicals. The impact of COB production on the environment may be reduced by improving the seed yield, minimizing the use of fertilizers, or optimizing tillage.

Camelina is an important oil crop with a fast-developing genetic platform for utilization in the production of designer lipids. The recent advances in genome editing techniques such as RNAi and CRISPR/Cas9 have helped in strategizing better ways for the genetic manipulation of the lipid synthesis pathway in camelina. These techniques have also helped in establishing a more profound understanding of the lipid metabolism in oil-producing plants. However, there is still a critical need to identify rate-limiting enzymes which govern the different pathways of lipid synthesis to gain a stringent control over the manipulation of genetic pathways. Better productivities can also be achieved by the utilization of designer enzymes in the FA triacylglycerol synthesis pathways. Hence, the genetic platform for camelina can be further developed for the accomplishment of more sustainable oil production for varied applications.

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CONFLICT OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

Olivera S. Stamenković involved in conceptualization, methodology, investigation, and writing—original draft. **Kshipra Gautam** involved in data curation, writing—original draft, and writing—review and editing. **Sneh L Singla-Pareek** and **Om P Dhankher** involved in writing—review and editing. **Ivica G. Djalović** involved in conceptualization, investigation, writing—original draft, and project. **Milan Kostić** involved in investigation and writing—original draft. **Petar M. Mitrović** involved in writing—original draft. **Ashwani Pareek** involved in conceptualization, methodology, supervision, and project. **Vlada Veljković** involved in conceptualization, methodology, writing—review and editing, supervision, and project.

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