

Review article

## *Camelina sativa* (L.) Crantz A mercantile crop with speckled pharmacological activities

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### Abstract

*Camelina sativa* (L.) Crantz (Brassicaceae) is an emerging short season, climate proof, biofuel crop. Popularly hailed as 'gold of pleasure' or 'false flax', *Camelina* plant requires low agronomic inputs. The seeds are commercially important as it includes 35-40% of oil (rich in  $\omega$ -3 fatty acid, tocopherols), 27-32% protein and glucosinolates (GSLs). Evidences suggest that Europe and Central Asia are the landmarks of *Camelina* instigation owing to its use since Bronze Age. Traditionally, the oil was used for culinary purpose, massage and as a lamp fuel. The oil has been found to possess antihypercholesterolemic, antioxidant properties and increases insulin sensitivity. Recently, the seed cake (meal, a by-product) has developed as a source of polyunsaturated fatty acids (PUFA) and proteins in animal feed to improve the  $\omega$ -3 fatty acid content of animal based food products such as eggs, meat, milk and fish oil. Additionally, the components have been found to modulate the expression of inflammatory proteins and GSLs are reported to possess anticancer activity. In this review, we aim to highlight the commercial application and medicinal properties of *C. sativa* along with newly discovered use and its utilization as feedstock for biofuel and biolubricant production.

**Key Words:** *Camelina sativa* (L.) Crantz, biofuel, biolubricant, glucosinolates, anticancer, insulin sensitizer

### Abbreviations used

ALA	: Alpha-Linolenic Acid
CP	: Cloud Point
CFPP	: Cold Filter Plunging Point
CM	: Camelina Meal
CO	: Camelina Oil
COX-2	: Cyclooxygenase-2
GSLs	: Glucosinolates
IENICA	: Interactive European Network for Industrial Crops and their Applications
IL-6	: Interleukin-6
MAPK	: Mitogen Activated Protein Kinase
MUFA	: Monounsaturated Fatty Acid
mm <sup>2</sup> /s	: Millimeter <sup>2</sup> /second
NF $\kappa$ B	: Nuclear Factor kappa-light-chain-enhancer of activated B cells
OSI	: Oil Stability Index
PUFA	: Polyunsaturated Fatty Acid
ppm	: Parts Per Million
RFS	: Renewable Fuels Standards

TIA	: Trypsin Inhibitor Activity
TNF- $\alpha$	: Tumour Necrosis Factor- $\alpha$
USAF	: The United States Air Force
UV	: Ultraviolet

### 1. Introduction and historical perspective

The use of *Camelina sativa* (L.) Crantz dates back to ages of 1500-400 BC (Bronze Age) and between 400 BC to 500 AD. Seeds and capsules of false flax, *C. microcarpa* and *C. linicola* have been uncovered in human corpses and in archaeological excavations in Scandinavia and Western Europe. This evidence shows that during the period of the Iron Age, the seeds of *C. linicola* and *C. sativa* have been used as a part of human diet along with contemporary feed sources such as flax (*Linum usitatissimum*) and cereals (Zubr, 1997). *Camelina* was grown intermittently during the Middle Ages. In the beginning of the 20<sup>th</sup> century and up to 1930's, the crop was cultivated in France, Belgium, Holland, the Balkan region and Russia in the region from Caucasus to Siberia and during the mid of 20<sup>th</sup> century (1945-1955), *Camelina* was cultivated in European countries like Poland and Sweden after which the cultivation of flax was decreased by many folds (Zubr, 1997). The lately reintroduced attention on *Camelina* was encouraged by its high amounts of  $\alpha$ -linolenic acid (18:3, n-3) in seeds, which made *Camelina* as a new vegetable source of  $\omega$ -3 fatty acids, due to which *Camelina* oil may be adopted as an alternative to fish oil. *C. sativa*, a member of cruciferous family, synonymously known as *Camelina parodii* Ibara & La Porte, *Myagrums sativum* L. (basionym), *Camelina caucasica* (sinskaya) Vassilcz and *Camelina glabrata* (DC.) Fritsch ex N.W. Zinger (Francis and Warwick, 2009) (Table 1 and Table 2).

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**Table 1:** Common or popular names of *Camelina* plant

Language (s)	Name (s)
English	<i>Camelina</i> , False flax, Wild flax, Gold of pleasure, Linseed dodder, Siberian oilseed, German sesame, Largeseed false flax, Dutch flax
French	Caméline, camélineciliée, linbâtard, sesame d'Allemagne
German	Leindotter
Italian	Camellina, dorella, drodetta
Japanese	Nagami-no-ama-nazuna
Portuguese	<i>Camelina</i>
Russian	Ryžik
Spanish	<i>Camelinapilosa</i>
Swedish	Oljedådra

**Table 2:** Botanical classification of *Camelina* species

Kingdom	Plantae	Plants
Sub-Kingdom	Tracheobionta	Vascular plants
Super-Division	Spermatophyta	Seed plants
Division	Magnoliophyta	Flowering plants
Class	Magnoliopsida	Dicotyledons
Sub-Class	Dilleniidae	Polyphyletic, centrifugal stamens and binucleate pollen
Order	Capparales	Eurosids II group of dicotyledons, production of glucosinolate
Family	Brassicaceae	Mustard family
Tribe	Camelineae	Distinct chromosome characteristic
Genus	<i>Camelina</i> Crantz	Ground and flax
Species	<i>C. alyssum</i> , <i>C. linicola</i> , <i>C. microcarpa</i> , <i>C. rumelica</i> , <i>C. Sativa</i>	

## 2. Sources of information retrieval

A comprehensive bibliographic search on *C. sativa* was carried out during September 2014 to March 2016 by referring the abstracts, articles, notes, textbooks, and peer reviewed publications catalogued in globally acknowledged scientific databases of INFLIBNET, NISCAIR, PUBMED, SCIELO, SCOPUS, Google Scholar, Science Direct and Sci-Finder. The key words applied for retrieving the information from published literature included 'Camelina sativa', 'false flax', 'flax gold', 'Camelina meal', 'Camelina oil', 'Glucosinolates', 'Camelina as biofuel' and 'Camelina as biolubricant'

## 3. Geographical distribution and habitat

Even though the exact region remains uncertain, *Camelina* species were likely to be originated in South-Eastern Europe and South-Western Asia. Ghamkhar *et al.* (2010) carried out a molecular analysis of 53 accessions of *C. sativa*, collected from Russia, China (1) and Australia (1) of which accessions from Russia-Ukrainian regions disclosed that this region is the main land for genetic assortment in *C. sativa* and proposing it could be the starting point of this species (Ghamkhar *et al.*, 2010) and few report advocates

that *Camelina* is native to Europe (Frame *et al.*, 2007). Archaeological evidences suggests that the farming of *Camelina* initiated in Neolithic times in South-East Europe and by the time of Iron Age. *Camelina* has become as an essential crop all over the Europe. Before the emergence of *Camelina* as a potential crop, it was introduced as a contaminant weed in seed of flax and other crops in North America. False flax is now being cultivated on a large scale in Canada, USA, European countries, China and Australia as a feedstock for biofuel production (www.inspection.ga.ca).

*Camelina* can be seen growing in natural grasslands, in the fields of grain, flax and alfalfa, open woods, lakeshore, roadside, beside the railway tracks and waste places. *Camelina* has the ability to develop in most of the soil types of cold and semi-arid climate zones, steppes and prairies (Gugel and Falk, 2006). *Camelina* is generally reported to grow well on marginal lands and it can withstand the drought conditions to an extent, but severe drought conditions and unexpected climate changes like sudden rainfall and heavy wind flow, especially during delicate phases such as maturing and harvesting can negatively affect the crop yield (Vollmann *et al.*, 2001). It has been also observed that false flax can germinate at low temperatures (2-8°C) and seedlings are tolerable to frost (Ehrensing and Guy, 2008). In a comparative study, Steppuhn *et al.* (2010) examined the growth and performance parameters of canola and *Camelina* under wavering salinity, it showed that *Camelina*'s performance was not as good as canola under moderate to high salinity (Steppuhn *et al.*, 2010).

## 4. Plant morphology

*C. sativa* is a cruciferous, annual or winter annual herb with a tap root system. *Camelina* generally grows to touch height from 30 to 90 cm. Stems are single, usually branched in the air; turn out to be woody when matured. Shoots may be somewhat hairy or smooth. Rosette with no lobes and are withered at flowering. Leaves are lance shaped and alternate on the stem, deficient in petiole and generally gripped along with the shoot. Leaves are 2-8 cm long and 2-10 cm wide, may be smooth or have few forks or hairs on the surfaces. Flowers are tiny, greenish-yellow or pale-yellow in colour with 4-5 mm long four spatulas like petals and are surrounded by four erected sepals. Six stamens of uneven length are grouped into three pairs. The silicles are smooth, leathery and pear shaped, 7-9 mm long and casually looks like the balls of flax. Seeds are minute and pale yellow-brown in colour, generally 2-3 mm long and are rough with an intensely grooved surface. Each plant weighs (capable of producing 100 -1000 seeds) just 1g on an average 1000 seeds (Schuster and Friedt, 1998; Francis and Warwick, 2009) and they do not exhibit dormancy (issg.database; Robinson, 1987a; Putnam *et al.*, 1993).

### 4.1 Camelina cultivation and use of fertilizers

Although, the origin of *Camelina* is somewhere in Europe, it is now being cultivated in higher altitudes, especially along the US-Canada border, Northern China, India, Australia and Northern Europe for research and other industrial applications. *Camelina* is generally sown in the spring and winter. *Camelina* crop can also be propagated with the help of existing farm practices. It is highly tolerant to cold weather, drought, semi-arid conditions, and low-fertile or saline soils; this indicates *Camelina* has minimal requirements of water, pesticide, and fertilizer than other commercial oilseed crops (Moser,

2010). Proper contact of seed with soil should be taken care while sowing. Seeds sowing can be done manually or by using similar equipment, used for canola farming, such as seed drill and forage seeder, but forage seeders are more preferably recommended over seed drill for better crop establishment (Gugel and Falk, 2006; Urbaniak *et al.*, 2008). The acclaimed and ideal seed sowing rate is 3-7 kg/ha (approximately 250-600 seeds/m<sup>2</sup>) to achieve an optimal plant density of 125-200 plants/m<sup>2</sup> (Zubr, 1997; Johnson, 2011). Higher seeding rate than recommended can develop rivalry among the plants of the crop and decrease the maturity time, which may adversely affect the crop yield (Johnson, 2011). The rate of seed germination and development of *Camelina* has been observed for an average of 40% (Johnson *et al.*, 2007; Urbaniak *et al.*, 2008; Johnson, 2011). Since *Camelina* has shorter crop duration (85-100 days), it can also be grown as an intercrop or a mix crop with other crops. *Camelina* yields on an average from 900 to 2240 kg of seeds per hectare area of land at maturity (Zubr, 2003b; Ciubota-Rosie *et al.*, 2013).

Although nutrient pre-requisite is moderate to low for the growth and it has been reported that *Camelina* can grow devoid of fertilizer use. Depending on the type of soil, studies have shown that seed yield of *Camelina* was significantly raised by the use of nitrogen fertilizers; yield also depends on sowing time and weather conditions. There was a huge (1.1-2.2 fold) increase in seed yield in fields with nitrogen fertilizer (Konèius and Karèauskienè, 2010; Zubr, 1997), phosphorous and sulphur (Jackson, 2008), compared to unfertilised fields. *Camelina* crop grown in nitrogen deficient soil looks poor with small and greenish yellow leaves. Moreover, crop matures early; it doesn't form many pods and yields small seeds (Zubr, 1997). *Camelina* crop yield was observed to be influenced by many parameters like location, climate, and season of seed sowing, irrigation and use of fertilizers (Jackson, 2008). In an experiment by Zubr (2003a) at different locations of Europe like Finland, Sweden, Denmark, England, Ireland, Germany and Scotland, a significant variation has been observed in the oil contents, crude fibre and crude protein of *Camelina* seeds. The seeds of summer sown varieties comprise around 42% oil on a dry matter basis, which is a bit low compared to winter varieties (45%). On the other hand, a trial conducted at three different locations of Canada in different seasons by Gugel and Falk (2006) found variations in various crop traits like days-to-flowering, days-to-maturity, crop height, seed yield, oil content, protein content and levels of unsaturated fatty acids. Rode (2002) reported variations in the *Camelina* seed oil content of fatty acids and *Camelina* oil characteristics grown by various farmers in Slovenia (Hrastar *et al.*, 2012) and France. Studies have shown that the yield of *Camelina* seeds in winter varieties is very high compared to summer varieties, so as the oil content of the seed; this indicates winter season is the best suitable season for *Camelina* cultivation (Hrastar *et al.*, 2012). The results of preliminary studies conducted in India, revealed that the crop has shown better adaptability to the cooler climate of hilly areas. The low yields of *Camelina* may be attributed to the fact that the crop was grown during summer-rainy season inside the polyhouse without any fertilizer, leading to quick maturity due to high temperature compared to published reports where the crop was grown in winter-spring season allowing better growth and yield with a dry spell during harvesting (Sadhuram *et al.*, 2010).

## 4.2 Weed control and diseases of *Camelina*

*Camelina* is very subtle to many herbicides, so manual weed control is much preferred over the use of herbicides. Though, *Camelina* is tolerant to few herbicides such as ethalfluralin, pendimethalin, trifluralin, Assure® in Canada and Poast® in USA are the acclaimed herbicides to control the weed in *Camelina* crop (<http://www.inspection.gc.ca>). *Camelina* has gained attention because of its potential industrial application such as biofuel feedstock, animal feed and human consumption. Short crop duration, drought resistance, high seed yield capacity and resistance to most of the conjoint pests and diseases of canola and other brassica crops, hence, *Camelina* may be cultivated adjacent to the crucifer crops (Scholze and Hammer, 1997). Decreased susceptibility to diseases may be due to resistance built by the species against unfavourable environmental conditions or small area cultivation. Large scale cultivation may increase the risk of pathogens (Table 3). A review of literature shows *Camelina* is highly resistant some common pathogens of crucifer plants like *Alternaria* black spot (Föller *et al.*, 2002) and blackleg (Li *et al.*, 2005) which cause massive crop damage (Séguin-Swartz *et al.*, 2009). A greater resistance of *Camelina* to pests was associated with the production of antimicrobial compounds in the roots upon infected with pathogens. These include two types of phytoanticipins and two camelexins called phytoalexins, namely; camelexin and methoxycamelexin (Conn *et al.*, 1988; Kenneth *et al.*, 1994). Moreover, stem rot caused by *Sclerotinia sclerotiorum* and gray mold caused by *Botrytis cinerea* were found to diminish the seed yield in wet weather conditions of spring season (Scholze and Hammer, 1997).

## 4.3 *Camelina* seed oil

*Camelina* oil is golden yellow in colour with a mild, almond-like essence, and has a characteristic odour like mustard with mild nutty taste (Abramovic and Abram, 2005). The reported yield of *Camelina* seed oil (mechanical pressing and pellet solvent extraction) is nearly 43.9 % weight of dry seeds (Rosie *et al.*, 2013). *Camelina* oil is depicted by its vast amounts of unsaturated fatty acids (~90 %) of which about 35% of  $\alpha$ -linolenic acid (18:3; n-3), 15% of linoleic acid (18:2; n-6) and 15% of gondoic acid (20:1; n-9) are predominated, besides the oil contains 6-12% of saturated fatty acids and about 3% of erucic acid (Domil *et al.*, 2015). In addition, this oil is uniquely rich in powerful antioxidants such as tocopherols (vitamin E) and *Camelina* oil is one among the highest of all natural tocopherol (Sizova *et al.*, 2003; Ramotar-John, 2014). Natural antioxidants (tocopherols) present in *Camelina* oil may contribute to a shelf life of 12 to 24 months without any change in flavour and aroma of the oil under ambient storage conditions (Crowley and Fröhlich, 1998). Plant pigments like carotenoid, the precursor substance of vitamin A and chlorophyll synthesis, have been found using UV spectroscopy in *Camelina* oil (Sizova *et al.*, 2003). The unrefined *Camelina* oil is highly vulnerable to degradation by direct light than by temperature (Abramovic and Abram, 2005). *Camelina* oil is extremely rich in cholesterol, which is uncommon and relatively very high even compared to other vegetable oils with high-cholesterol. The major sterols identified in the *Camelina* oil included sitosterol (1,884 ppm), campesterol (893 ppm),  $\Delta$ 5-avenasterol (393 ppm), cholesterol (188 ppm), brassicasterol (133 ppm) and stigmasterol (103 ppm, (Shukla *et al.*, 2002).

**Table 3:** Common diseases of Brassicaceae crops and susceptibility of Camelina

Disease	Pathogen	Characteristics/Symptoms	Susceptible / Resistant	Reference
Damping off	<i>Rhizoctonia solani</i> and <i>Pythium debaryanum</i> .	Brown discolouration of root and rotten root crown	Resistant	Conn <i>et al.</i> , 1988
Brown girdling root rot	<i>Rhizoctonia solani</i> and <i>Fusarium spp.</i>	Lesions, Root girdling, Root stub (Advanced stage), Root cut off/decay (Advanced stage)	Resistant	Gugel <i>et al.</i> , 1987
Club root	<i>Plasmidophora brassicae</i>	Development of large galls/clubs on root, Compromised root function, Drooping and stunting of aerial parts	Susceptible	Scholze and Hammer, 1997
Alternaria black spot	<i>Alternaria brassicae</i>	Grey to black spots on stem and leaves, Shrunken and diseased seed	Resistant	Roessel, 1995
Blackleg	<i>Leptosphaeria maculans</i>	Greyish-white lesions on stem, leaves and pods	Resistant	Li <i>et al.</i> , 2005
Downy mildew	<i>Albugo candida</i> , <i>Peronospora parasitica</i>	Greyish-white mycelial growth on lower surfaces of leaf, stems and siliques	Susceptible	Föller <i>et al.</i> , 2002; Roessel, 1995; Vollmann <i>et al.</i> , 2001
Sclerotinia stem rot	<i>Sclerotinia sclerotiorum</i>	Lesions on stem, leaves, girdling of the stem	Susceptible	Crowley, 1999; Roessel <i>et al.</i> , 1996
White rust	<i>Albugo candida</i>	White powdery spots on lower leaf surfaces, hypertrophied siliques	Susceptible	Föller <i>et al.</i> , 2002
Aster yellows	<i>Candidatus phytoplasmaasteris</i>	Distorted, sterile inflorescence, Phyllody pods and abnormal green pigmentation of floral organs	Susceptible	Robinson, 1987b
Grey mold	<i>Botrytis cinerea</i>	Grey-black coloured fungal development on stems and pods	Susceptible	Crowley, 1999; Roessel, 1995
Smuts	<i>Ustilago spp.</i>	Black powdery spots stems and branches	Susceptible	Akk and Ilumäe, 2005
Rusts	<i>Puccinia aristidae</i> <i>P. trabutii</i> <i>P. isiacae</i>	Rust like spore spots on leaves	Susceptible	Akk and Ilumäe, 2005
Verticillium wilt	<i>Verticillium longisporum</i>	Early yellowing and drying out of leaves and stem	Susceptible	Pikul <i>et al.</i> , 2014
Powdery mildew	<i>Erysiphe polygoni</i> <i>E. cruciferarum</i>	Bleaching of chlorophyll in leaves	Susceptible	Roessel <i>et al.</i> , 1996
Light leaf spot	<i>Pyrenopeziza brassicae</i>	Very small white spots on green leaves which turn into lesions with pinkish centre spots	Susceptible	Akk and Ilumäe, 2005
Ring spot	<i>Mycosphaerella brassicicola</i> and <i>Septoria camilinae</i>	Light brown-black concentric necrotic spots on leaves	Susceptible	Séguin-Swartz <i>et al.</i> , 2009
Grey stem and White leaf spot	<i>Pseudocercospora capsallae</i>	Stem turns into grey and white spot lesions on leaves throughout all the growing stages	Susceptible	Séguin-Swartz <i>et al.</i> , 2009
Bacterial blight	<i>Pseudomonas syringae</i>	Small to elongated black oval spots on stems, leaves and pods	Susceptible	Pikul <i>et al.</i> , 2014
Mottling	<i>Turnip crinkle virus</i> <i>Turnip rosette virus</i>	Discrete mottling, leaf crinkling and stunting	Susceptible	Broadbent and Heathcote, 1958; Hein, 1984

#### 4.3.1 Uses of *Camelina* oil

Historical evidences show that the oil was used for human consumption and also for culinary purpose in combination with the rapeseed oil. In 2010, the aptness of *Camelina* oil for human purposes was certified by Health Canada. The oil is suitable for cooking, baking, frying food materials and to use in salads, but not suitable for deep-frying. Pilot studies were conducted to ascertain and confirm the applicability of oil in certain food products made of mixed fats, egg yolk, ice cream and dressing of others (Zubr, 2009). The false flax seed oil should be deodorized prior to use. Since *Camelina* oil has exceptional amounts of omega-3 and 6 fatty acids, it can be considered as an alternative and vegetable source of these essential fatty acids over fish originated omega-3 fatty acids which are less aesthetic for human consumption due to its fish like odour (Hixson *et al.*, 2014). The unexceptional content of useful fatty acids and vitamin E helps *Camelina* oil as an alternate to the commercially available food oils such as mustard, sunflower, canola and others (Tables 4 and 5). The oil is also very rich in natural antioxidants, such as tocopherols, making this highly stable oil

very resistant to oxidation and rancidity. Earlier this oil was used in lights, in recent years the oil has attracted various manufacturers of soaps and varnishes, dermatological goods, cosmetic products such as cosmetic oils, lotions and skin creams (Conn *et al.*, 1988). In order to lower the negative influence of biodiesel production on food sector, it is obligatory to discover substitute feedstock for the production of biodiesel, including commercial oilseed crops and other fatty waste (Zaleckas *et al.*, 2012). *Camelina* oil could be one of its kind of feedstock well-suited for biofuel production. In spite of an excessive iodine number (>160), pilot studies successfully converted the oil into methyl ester (Rice, 1995) and experiments upkeep the oil for its use as biofuel in a mixture with diesel oil. *Camelina* oil is categorised into fast drying oil due to large content of polyunsaturated fatty acid because of which it is widely preferred as a raw material for production of eco-friendly polymers, paints and varnishes. Oil with this kind of properties is appropriate for making some medicines (Zaleckas *et al.*, 2012). Previously, the seed oil was utilised in folk medicine to treat burns, eye inflammation, wounds, stomach ulcers, and now-a-days, it is being used in veterinary medicine (Rode, 2002).

**Table 4:** Properties of *C. sativa* oil

R triense	R C20+d	R polyunsaturated	CP (°C) PP (°C) OSI, 110°C (h)	t, 40°C (mm <sup>2</sup> /s)	Lubricity 60°C (lm)	Acidity Index AV (mgKOH/g)	Iodine Value IV (gI <sub>2</sub> /100g)	C, 24°C (mN/m)	C, 40°C (mN/m)	R saturated	R unsaturated
20.4	16.5	54.3	10 (1) f, 17 (1), 2.2 (0.2)	28.28 31.49 (0.05)	128 (2)	2.06 (0.04)	141	32.3 (0.1)	30.7 (0.1)	10.3	88.3

Adapted and modified from Domil *et al.*, 2015

**Table 5:** Nutritional data of *Camelina* oil and its comparison with Canola, Sunflower, Soybean, Flax seed and Olive oils

Parameter	<i>Camelina</i> oil	Canola oil	Sunflower oil	Soy oil	Flax seed oil	Olive Oil
Oil content (%) weight of dry seed	43.3	> 43	40-50	20.6	42.2	45
Fatty acid content						
Saturated fatty acids (%)	9.7	9.6	9.5	15.6	10.0	14.3
Palmitic acid (16:0)	5.4	5.2	4.3	10.0	5.5	11.5
Stearic acid (18:0)	2.5	4.4	2.8	4.5	3.5	1.8
Arachidic acid (20:0)	1.5	0.7	2.3	NL	0.65	0.22
Monounsaturated fatty acids (%)	32.8	59.3	31.5	28.5	22.1	78.4
Oleic acid (18:1)	12.6	17.0	20.0	28.0	16.0	75.0
11-Eicosenoic acid (20:1)	1.7	14.5	0.96	0.2	0.1	0.31
Erucic acid (22:1)	2.9	41.0	NL	NL	NL	NL
Polyunsaturated fatty acids (%)	55	30.7	59.5	57.5	68.3	7.0
$\alpha$ -Linolenic acid (18:3, n-3)	38.1	9.1	0	6.8	55.0	0.7
Linoleic acid (18:2, n-6)	16.0	18.6	68.8	50.4	16.8	9.8
Tocopherols (ppm)	810	468	609	829	367	177

Various countries like Australia, Canada, European union and the United States had set a limit of erucic acid (C22:1, n-9) content in food ingredients which should be less than 2% as per Food Standards Australia, New Zealand (FSANZ) for canola (Food Standards Australia, New Zealand 2003) and <5% according to the European Industry (European Commission, 1976). For human consumption purposes,

seed oils should accommodate a well-adjusted ratio of PUFAs,  $\alpha$ -linolenic acid (18:3, n-3) to linoleic acid (18:2, n-6) (Sizova *et al.*, 2003). Considering the health benefits of *Camelina* oil, Health Canada has approved the use of *Camelina* oil as a food ingredient and is approved and registered as commercial food oil in Canada and many European countries (Ghamkhar *et al.*, 2010; Campbell *et al.*, 2013).

#### 4.3.2 *Camelina* oil as a biofuel

American Society for Testing and Materials (ASTM) defined biodiesel as a fuel comprised of mono-alkyl esters of long-chain fatty acids and has to meet the ideal requirements of ASTM standard D6751 to be considered as a biodiesel. A wide range of lipids of different origin serves as a feedstock for biodiesel production. Biodiesel and biofuel are meritorious over fossil fuel in terms of positive energy balance, biodegradability, domestic and renewable origin, high flash point, tremendous lubrication, low or no sulphur content, lower exhaust emission and can be miscible with petrodiesel fuels in all proportions (Hixson *et al.*, 2014). However, at this stage, biodiesel cannot completely replace fossil fuel based diesel due to several limitations of biodiesel, such as lower oxidative stability, higher production costs, storage stability and low temperature operability. However, canola is not well adapted to cropping environments such as low rainfall regions, least irrigated, or those with water logging, sandy soils, or saline conditions, whereas *Camelina* has been shown to withstand the early water deficits, dry conditions and can grow in lower rainfall regions (Bramm *et al.*, 1990; Putnam *et al.*, 1993; Campbell *et al.*, 2013).

Rapid depletion of conventional energy resources and increased utilization and demand for fossil fuel energy sources and has provoked the researchers to concentrate on alternative, economic and sustainable resources for energy production. Plant derived biofuels are top of the priority list against fossil fuel (Table 6). Canola (*Brassica napus*) has emerged as a major source of feedstock for the oilseed industry around the world. The yield of canola is potentially restricted to temperate and semi-arid areas (Campbell *et al.*, 2013). An extensive research is being carried out throughout the globe in search of potential biofuel feedstock to fulfil the requirements to an extent. Compared to other biofuel feedstocks, *Camelina* has been shown potential to endure the early water

deficits, arid conditions and can grow up in a lesser rainfall area (Moser, 2010), can grow in less fertile land and can be propagated as a mix crop (Bramm *et al.*, 1990; Putnam *et al.*, 1993; Zaleckas *et al.*, 2012). *Camelina* oil has become the choice of natural resource for biodiesel and satisfies most of the standards to be a biofuel feedstock except its higher iodine value (Crowley, 1999). Most of the conventional biodiesel feedstocks around the world come under food crops for human consumption category, adaptation of these crops as feedstock for biodiesel production may interfere with their use in food industry and affect it very adversely. As *Camelina* oil can't be approved as food ingredient for human consumption due to high cholesterol and eicosenoic acid (15%) content, so it can be considered as biodiesel feedstock over traditional feedstocks (Soriano and Narani, 2012). It is very easy to extract, refine and trans-esterify the oil from *Camelina* seeds. Two major limitations of *Camelina* oil are its unsaturation and high iodine number (>160). An ideal biodiesel feedstock must have low amounts of unsaturated (2 or 3 double bonds) and polyunsaturated ( $\geq 4$  double bonds) fatty acids and a great amounts of monounsaturated fatty acids, whereas *Camelina* oil encompasses 8.63% of saturated fatty acids; 33% of monounsaturated fatty acids, 54% of unsaturated fatty acids and 2.48% of polyunsaturated fatty acids (Domil *et al.*, 2015).

Trans-esterification is the method involves conversion of lipids, fats and oils into fatty acid methyl esters (FAMEs) in the presence of an alkali catalyst and excessive methanol at 60°C, is a classical method and green diesel *via* catalytic hydro-treating is the choice of methods for production of biofuel from seed oils and is considered as best suited method for extraction of biodiesel from *Camelina* oil. The *Camelina* derived biofuel properties such as cetane number, cold flow properties, kinematic viscosity, oxidative stability, *etc.*, are similar to soybean oil derived biodiesel and acceptable for the use as biodiesel (Moser, 2010).

**Table 6:** Critical parameters associated with *Camelina* derived biodiesel and biodiesels produced from other vegetable oils

Parameters	Camelina	Canola	Coconut	Mustard	Palm	Soybean	Sunflower
Kinematic viscosity, 40 °C (mm <sup>2</sup> /s)	4.32	4.52	2.85	5.07	5.38	4.07	4.26
Flash point (°C)	172	170	ND	169	178	ND	175
Sulphur (ppm)	5.46	2.22	0.48	2.45	0.75	0.27	0.42
Cloud point (°C)	2.7	-1.1	4.1	4.4	11.6	0.0	3.3
Cold Flow properties (°C)	1.1	-8.8	1.6	3.3	11.6	-5.5	0.0
Distillation temp (°C) T90	369	355	343	ND	360	354	ND
Oil Stability Index (h)	0.6	2.8	11.8	4.2	21.4	1.1	0.9

\*ND = Not Described

#### 4.3.3 *Camelina* oil: A potential renewable jet fuel feedstock

Besides, the *Camelina* oil and seed cake are being considered as the feedstock for biodiesel production, seed oil also serves as raw material for production of renewable jet fuel. The seed oil should be hydro-deoxygenated similar to biodiesel for preparation of jet fuel from *Camelina* seed oil and isomerization of linear paraffins to tributary paraffins to enhance cold flow properties to avoid freezing of linear paraffins at lower temperatures encountered at the operational altitudes. Several commercial biofuel industries have already produced and Accelerger Corp, Altair Corp, Biojet Corp and Sustainable Oils are currently working towards *Camelina* derived jet fuel and biodiesel (Boateng *et al.*, 2010).

#### 4.3.4 Limitations of *Camelina* seed oil as feedstock for biofuel production

The physicochemical characteristics of biodiesel hinge on the carbon chain length and unsaturated fatty acid content of vegetable oil or animal oil serves as a feedstock. *Camelina* biodiesel contains 10-12% saturated, 37-40% monounsaturated and 48-50% of polyunsaturated fatty acid methyl esters. The data from various study reports showed that *Camelina* oil derived biodiesel doesn't meet ASTM D6751-14 and EN 14214 standards which are believed to regulate the safe use of biodiesel. The US and EU biofuel regulatory bodies had restricted the content of  $\alpha$ -linolenic acid (ALA) to 12% in biodiesel feedstock, excessive ALA dictates many biodiesel

properties negatively, like cetane number, the iodine value, linolenic acid methyl ester content and the oxidation stability. Hence, the *Camelina* oil derived biodiesel fails to meet the standards set up by these agencies and this infers that *Camelina* oil won't be suitable for production of biodiesel, which meets the standard criteria (Ciubota-Rosie *et al.*, 2013; Rosie *et al.*, 2013). But *Camelina* oil is still one of the top contending biofuel feedstocks, if the above mentioned properties could be amended and becomes a top priority of many corporations involved in biofuel generation. Apparently, genetic modification, production of transgenic plants and new breeds of *Camelina* may promise the desired fatty acid composition of *Camelina* oil, hence, a biodiesel with improved characteristics can be attained (Ciubota-Rosie *et al.*, 2013). Even though the iodine value of *Camelina* seed oil in winter variety (164.6g I<sub>2</sub> /100g) is

lower than spring variety (169.6g I<sub>2</sub> /100g), it won't help the oil to reach the optimum requirements to be ideal feedstock. But it can be used within mixtures with other lipids having lower iodine value like pork lard or frying oil. If *Camelina* oil be used in large quantity for biodiesel production, at least 20% of other oils, lipids or fats are needed in a mixture containing a major portion of *Camelina* oil (Zaleckas *et al.*, 2012).

None of the biodiesel derived from traditional feedstock are supposed to be used alone and they can only be used in blends with commercial petrodiesel. Moreover, *Camelina* seem to be an alternative to above stated traditional feedstock due to its agronomical advantages, but the characteristics of *Camelina* oil that sternly discourage its utility as biofuel feedstock is presented in Table 7.

**Table 7:** Physicochemical properties of *Camelina* derived biofuel

Parameter	ASTM D6751-14	UNE-EN-14214; 2014	Camelina derived biofuel
Acid value (mg KOH/g)	≤ 0.5	≤ 0.5	0.15
Carbon residue (%)	≤ 0.05 (on 100% sample)	≤ 0.3 (on 10% distillation residue)	0.019 (on 100 % sample)
Cetane number	≥ 47	≥ 51	42.76
Cloud point (°C)	According climate zone	-	0 – 3
Cold filter plunging point (°C)	-	According climate zone	- 4
Copper strip corrosion (3h, 50°C), Classification	≤ 3	1	1A
Cold soak filterability test (S)	≤ 360	-	246
Density (at 15°C, Kg/m <sup>3</sup> )	-	860 – 900	888
Distillation temperature atmospheric equivalent temperature (90% recovered, °C)	≤ 360	-	369
Flash point (°C)	≥ 93	≥ 101	152
Iodine value (g I <sub>2</sub> /g)	-	≤ 120	> 160
Kinematic viscosity (at 40°C, mm <sup>2</sup> /s)	1.9 – 6	3.5 – 5	4.3
Methanol content (%)	≤ 0.2	≤ 0.2	0.0121
Methyl ester content (%)	-	≥ 96.5	97.5
Oxidation stability (110 °C, H)	≥ 3	≥ 6	1.3
Phosphorus content (mg/kg)	≤ 10	≤ 4.0	≤ 0.1
Sulphur content	≤ 15	≤ 10	N1
Sulphated ash content	≤ 0.02	≤ 0.02	0.0013
Linolenic acid methyl ester (%)	-	≤ 12.0	34.2 – 38.4
Polyunsaturated methyl esters (%)	-	≤ 1	2.08
Monoglyceride content (%)	-	≤ 0.8	0.579
Diglyceride content (%)	-	≤ 0.2	0.171
Triglyceride content (%)	-	≤ 0.2	0.107
Free glycerol (%)	≤ 0.02	≤ 0.02	0.006
Total glycerol (%)	≤ 0.240	≤ 0.25	0.189
Group I metals (mg/kg)	≤ 5.0	≤ 5.0	0.11
Group II metals (mg/kg)	≤ 5.0	≤ 5.0	0.16
Water content (mg/kg)	-	≤ 500	120
Water sediment (%)	≤ 0.2	-	0
Total contamination (mg/kg)	-	≤ 24	7.3

### **i. High amounts of unsaturated fatty acids**

A biofuel feedstock must have high monounsaturated fatty acid esters (MUFE) to polyunsaturated fatty acid esters (PUFE) ratio to be acknowledged as feedstock for biodiesel generation (Monyem and Van Gerpen, 2001; Moser and Vaughn, 2010; Pinzi *et al.*, 2011). A greater fraction of monounsaturated fatty esters ensures high kinematic viscosity, which helps in clog free flow of biodiesel through the engine (Knothe and Steidley, 2005). A kinematic viscosity of a mixture of 5% *Camelina* alkyl monoester and 95% diesel was found satisfactory according to the petrodiesel (ASTM D975) and petrodiesel-biodiesel blend (ASTM D7467) standards (Moser and Vaughn, 2010). Lower emissions of CO, CO<sub>2</sub>, and smoke than mineral fuels were recorded with trials on *Camelina* biodiesel (Bernardo *et al.*, 2003; Campbell *et al.*, 2013; Rosie *et al.*, 2013).

### **ii. High content of $\alpha$ -linolenic acid**

According to the requirements of the Lithuanian standards (LST EN14214:2003), the content of  $\alpha$ -linolenic should not be more than 12%. The content of methyl esters of  $\alpha$ -linolenic acid, negatively affects other biodiesel properties such as the cetane number, distillation temperature, iodine value and oxidative stability (Ciubota-Rosie *et al.*, 2013).

### **iii. Higher distillation temperature**

The distillation temperature of biodiesel relies on the length of the polyunsaturated fatty acid (PUFA) ester carbon chains. High amounts of linolenic and eicosanoic acids lead to a very high distillation temperature of *Camelina* biodiesel, which can cause gradual dilution of lubricating oil and result in serious damage to the engine (Ciubota-Rosie *et al.*, 2013). The distillation temperatures of *Camelina* biodiesel do not meet any of the USA and EU standards.

### **iv. High iodine value**

*Camelina* seed oil has an iodine value around 165 g of I<sub>2</sub> per 100 g of oil which is believed to be very high that leads to polymerization of biodiesel and upon heating, biodiesel deposits as a film on engine parts like injector nozzles, piston ring grooves and piston rings, hence, *Camelina* seed oil can't be used alone and directly for biofuel production.

### **v. Lower cetane number**

Cetane number is the quantification of combustion quality of biodiesel during ignition. The cetane number also relies on two factors of fatty acids present in feedstock oil, one is the length of fatty acid chain and the other is the number of double bonds present in fatty acids. For an optimal cetane number the oil should contain saturated fatty acids with long carbon chains. *Camelina* derived biodiesel has a cetane number of 42.76, which has to be more than 47 (ASTM D6751-09) or 51 (UNE-EN 14214:2010). Biodiesel with a lower cetane number tend to cause diesel detonation that leads to incomplete combustion of biodiesel and results in elevated smoky and particulate exhaust emissions (Ciubota-Rosie *et al.*, 2013).

### **vi. Low oxidation stability**

Oxidative stability has become an important concern for biodiesel produced from vegetable oil feed stocks rich in polyunsaturated fatty acids (PUFA). PUFA esters are vulnerable to radical attack due

to the neighbouring methylene groups of the double bonds. The hydroperoxides formed in the primary stages of oxidative collapse of biodiesel are assumed to attack elastomers present in the fuel line system. Polymerization of hydroperoxides with other free radicals may lead to the formation of insoluble deposits and gums, which is associated with plunging of fuel filter and further sedimentation of polymers in the fuel injection system and combustion chamber. External addition of antioxidants to the *Camelina* derived biodiesel or amalgamation with other more oxidatively stable feedstock may help in enhancing the oxidative stability (Ciubota-Rosie *et al.*, 2013).

### **vii. Cold filter plunging point (CFPP) and low temperature behaviour**

Cold filter plunging point is the lowest temperature at which, given volume of biodiesel or diesel passes through a standard filtration unit at a specified time. This parameter has an important relation to the use of petrodiesel or biodiesel fuels at low temperature especially during winter season. The CFPP of fatty acid methyl esters of *Camelina* oil is comparatively high ( $-4^{\circ}\text{C}$ ) which can be lowered using commercial additives (Fröhlich and Rice, 2005).

### **viii. Decreased kinematic viscosity**

Kinematic viscosity of a biofuel is proportionately depends on the PUFA content of feedstock oil. The flow of *Camelina* biodiesel is adversely influenced by large amounts of polyunsaturated fatty acid methyl esters (Knothe and Steidley, 2005).

### **4.3.5 Trial runs using *Camelina* derived biofuel**

The United States Air Force (USAF) has conducted undoubted trials on A-10 Thunderbolt II fighter jet and F/A-18 Super Hornet fighter jet, using a mixture of *Camelina* derived jet fuel and commercial JP-8 jet fuel. The USAF targets to obtain 50% of its domestic flight fuel from unconventional sources. Other airlines such as Japan Airlines (JAL) and Royal Dutch (KLM) successfully accomplished flight trial runs using proportions of *Camelina* derived jet fuel with JP-8 jet fuel ([www.biofuelsdigest.com/blog2/](http://www.biofuelsdigest.com/blog2/)). Energy and Environmental Research Centre of the University of North Dakota entered into an agreement with Great Plains Oil and Exploration, LLC to carry out research and produce combinations using *Camelina* derived biofuels, diesel, gasoline and jet fuels. Altair, Inc. has tied up with 14 airline companies in Seattle-Tacoma International Airport to provide up to 750 million gallons of *Camelina* derived and other renewable jet fuels (Moser, 2010).

Targeted Growth, LLC and Sustainable Oils, Inc. analysed the life cycle of *Camelina* derived jet fuel and biodiesel and concluded that it emits minor levels of greenhouse gases and the demand for cumulative energy and fossil energy are considerably with their use is lower than conventional petroleum derived fuels, these results helped biodiesel and renewable jet fuels derived from *Camelina* to be certified as advanced biofuels according to the Renewable Fuels Standards (RFS2) set by the United States Environmental Protection agency (Moser, 2010). Experiments conducted to examine the use of *Camelina* seed oil in proportions along with methyl esters of pork lard and beef tallow in the mixture with petrodiesel fuel and reported no harmful components found in engine emissions (Zaleckas *et al.* 2012). In 2012, three international airlines piloted

trial runs of biojet flights using a blend ratio of 50:49:1, containing standard Jet A1, *Camelina* oil, and carinata oil, respectively and no technical difficulties were reported (Campbell *et al.*, 2013).

Kang *et al.* (2011) carried out trials on Peugeot Xad saloon and Isuzu Trooper, light transport vehicles using *Camelina* derived biodiesel and found to have 1.5 to 4.6% lower fuel economy compared to conventional diesel fuel and also observed the accumulation (about 16%) of esters of *Camelina* oil in lubricating oil in trial run on Isuzu trooper but not in a Peugeot. No adverse signs of a high iodine number were observed in engine performance over the use of *Camelina* derived biofuel and these findings are similar to the results obtained from trial runs carried out on tractor and car engines (Prankl and Wörgetter, 1996; Fröhlich and Rice, 2005).

Most of the limitations of *Camelina* biodiesel such as CFPP, decreased kinematic viscosity, higher distillation temperature, high iodine value, lower cetane number, low oxidation stability and low temperature behaviour are due to the presence of large quantities (over 90%) of unsaturated fatty acid methyl esters. These limitations can either be minimised by blending the *Camelina* derived biodiesel with other superior biodiesel feedstock or petrodiesel. The other viable options for minimization of the limitations of *Camelina* biodiesel are the production of *Camelina* varieties with more favourable fatty acid composition through genetic engineering of conventional breeding methods. Kang *et al.* (2011) have developed a low  $\alpha$ -linolenic acid (18:3) and low PUFA yielding variety of *Camelina*. Therefore, the fatty acid content of *Camelina* oil may be altered with the help of modern technologies like mutagenesis, genetic engineering and conventional breeding methods. Manipulation of fatty acid content could help *Camelina* oil to evolve as a potential biodiesel feedstock and biodiesel and jet fuel derived from *Camelina* hopefully accomplish the requirements set by USA and EU agencies for its use as biodiesel, alone or in mixtures with petrodiesel (Kang *et al.*, 2011). Findings of pilot studies and experiments conducted over the use of *Camelina* seed oil as biodiesel feedstock speculate that *Camelina* is not a golden opportunity for biodiesel production, but genetic engineering and conventional breeding are the two most viable options available today for production of *Camelina* variety with the desired ratio of saturated and unsaturated fatty acids (Ruiz-Lopez *et al.*, 2014).

#### 4.4 Camelina meal

The second most valuable and useful part of *Camelina* with economic value is seed meal also called as oil cake or *Camelina* meal or expeller. It is the remaining crushed portion of seeds obtained after removal of oil from seeds by cold compression or mechanical or solvent extraction methods. The meal that comprises of crude protein (45%), fibers (13%), phytochemicals (~14%) high in glucosinolates (GSLs), residual oil (10%), soluble carbohydrates (10%), minerals (5%), nucleic acids (0.2%) and minute quantities of vitamins (Das *et al.*, 2014; Zubr, 1993). The nutrient profile of false flax seed is comparable to other oil seeds such as soybean, rapeseed and flax seeds and has potential to hold a good market share. Zubr (2003a) reported that *Camelina* seeds contain 18 amino acids, most of which are essential amino acids including isoleucine, leucine, valine, methionine, phenylalanine, histidine, lysine, tryptophan, threonine. CM is likely to express a similar pattern of

fatty acids content of *Camelina* seed oil. According to their abundance, the fatty acid found in CM are  $\alpha$ -linolenic acid (18:3, n-3) > linoleic acid (18:2, n-6) > Oleic acid (18:1) > eicosenoic acid (20:1) besides the traceable amounts of other saturated, monounsaturated and polyunsaturated fatty acids (Aziza, Quezada, and Cherian, 2010a; Cherian *et al.*, 1996). Zubr and Matthäus (2002) stated that *Camelina* oil on average contains 28.07 ppm of  $\alpha$ -tocopherol ( $\alpha$ -T), 742 ppm of  $\gamma$ -tocopherol. Blades and Zubr (2010) found that *Camelina* seed had high levels of thiamin (B1, 18.8  $\mu$ g/g), niacin (B3, 194  $\mu$ g/g) and pantothenic acid (B5, 11.3  $\mu$ g/g) as it relates to the vitamin content. Among the minerals in *Camelina* meal, potassium occupies the major portion followed by traceable amounts of magnesium, sulphur, calcium and phosphorus. Micronutrients like iron (Fe) content (329  $\mu$ g/g) were high with considerable amount of manganese (Mn, 40  $\mu$ g/g) and zinc (Zn, 40  $\mu$ g/g, (Ramotar-John, 2014). *Camelina* meal cake found to contain three types of glucosinolates (GSL's) including tannins, glucocamelin and sinapine (Matthäus and Zubr, 2000), it may also contain Trypsin Inhibitor Activity (TIA, 12-28 mg/g CM) which along with GSLs may limit the effectiveness of *Camelina* when used as a feed ingredient. However, plant breeding and transgenic plant production techniques are being explored to reduce the TIA and GSL levels in *Camelina* meal (Budín, *et al.*, 1995). Furthermore, heating of soybean meal for 40 min successfully reduced TIA content by 90% (Zubr, 2003a); a similar cooking process like heating, baking, *etc.*, could help to reduce the TIA in *Camelina* seeds and meal (Table 8). In appurtenance, recently reported experimental stipulations have shown ethyl acetate extracts of seeds and meals of *Camelina sativa* showed lower values for antioxidant activity, phenolic compounds, and flavonoids than the methanolic extracts, however, meal showed higher antioxidant activities, phenolics and flavonoid contents than seed (Quezada and Cherian, 2012).

**Table 8:** Chemical composition of Camelina meal and Camelina oil

Nutritional Parameter	Seed cake / meal	Seed oil
Gross energy (kcal/kg)	4,688 – 4,822	NL
Dry matter (g/kg)	934.5	NL
Crude protein (%)	35.2 – 46.9	NL
Crude fat (%)	4.9 – 11.9	NL
Crude fibre (g/kg)	11.00 – 11.27	NL
Ash (%)	5.25 – 6.5	NL
Neutral Detergent fibre (%)	41.8	NL
Total phenolic compounds ( $\mu$ g/g)	773	128
Flavonoids (mg/g)	18.5	NL
$\alpha$ -Tocopherol (ppm)	7.2	14 – 41
$\gamma$ -Tocopherol (ppm)	330	660-990
Glucosinolates (imol/g)	14 – 36	NL
Fatty acids (%)		
Palmitic acid (16:0)	6 – 9.0	5 – 6.5
Palmitoleic acid (16:1)	0.2 – 0.32	NL
Stearic acid (18:0)	2.3 – 2.5	1.5 – 3.5
Oleic acid (18:1)	14 – 21.7	14 – 17.5
Linoleic acid (18:2, $\omega$ -6)	19.04 – 28.8	13.5 – 19
$\alpha$ -Linolenic acid (18:3, $\omega$ -3)	24.2 – 41.3	27 – 40
Eicosanoic acid (20:1)	10.1 – 15.57	12.3 – 16.2
Ecosadienoic acid (20:2)	1.4 – 1.67	1.7 – 2.0
Ecosatrienoic acid (20:3)	0.9 – 1.2	1.3 – 1.7
Erucic acid (22:1)	1.7 – 1.8	1.6 – 3.0

Amino acids (% Protein)		
Aspartic acid	7.7	NL
Threonine	3.9	NL
Serine	4.0	NL
Glutamic acid	16.1	NL
Glycine	4.9	NL
Alanine	4.3	NL
Valine	5.4	NL
Isoleucine	3.9	NL
Leucine	6.6	NL
Tyrosine	2.4	NL
Phenylalanine	4.0	NL
Lysine	4.6	NL
Histidine	2.3	NL
Arginine	7.5	NL
Tryptophan	1.2	NL
Methionine	2.0	NL
Carbohydrates (% fat free dry meal)		
Fructose	0.04	NL
Glucose	0.42	NL
Sucrose	5.5	NL
Raffinose	0.64	NL
Stachyose	0.36	NL
Starch	1.21	NL
Pectin	0.96	NL
Mucilage	6.7	NL
Crude Fibre	12.8-18	NL
Lignin	7.4	NL
Vitamins ( $\mu\text{g/g}$ dry meal)		
Thiamin (B1)	18.8	NL
Riboflavin (B2)	4.4	NL
Niacin (B3)	194	NL
Pantothenic acid (B5)	11.3	NL
Pyridoxin (B6)	1.9	NL
Biotin (B7)	1.0	NL
Folate (B9)	3.2	NL
Minerals (mg/kg dry meal)		
Phosphorus	7,468	NL
Potassium	11,502	NL
Calcium	2,100	NL
Magnesium	3,794	NL
Aluminium	1.78	NL
Sulphur	8,024	NL
Sodium	14.5	NL
Iron	148	NL
Manganese	25.2	NL
Zinc	50.9	NL
Copper	6.55	NL
Nickel	3.3	NL
Cadmium	0.17	NL

NL: Not Listed, **Source:** Adapted from (Guinda *et al.*, 2012; Imbrea *et al.*, 2011)

#### 4.4.1 Uses of *Camelina* meal

*Camelina* meal (CM) was documented for its use in animal feed since ancient times (Neuss, 1978; Ollech, 1884), which is strongly supported by the amino acid content. *Camelina* meal is a potential protein source. In recent years, it is being exploited as a component in animal feed for poultry and dairy animals. Increased price and demand for the conventional animal feeding material suggests bringing an alternative protein source like *Camelina* meal into the market (Colombini *et al.*, 2014). In recent years, CM was successfully incorporated as an element in animal feed for egg-laying hens, broilers, pigs, fishes, rabbits, cows, ewes and other animal species. Several experiments proved that the addition of CM up to 15% fodder mixture had a comparable nutritional value to the extracted soybean meal, corn meal and other commercially available meals (Moriel *et al.*, 2011; Moriel *et al.*, 2016). CM has also been found to interact with the immune systems of the animals and alters the protein and gene expression of proinflammatory cytokines (COX-2, IL-6, IL-8, TNF- $\alpha$ ) and mRNA levels of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and modulates the expression of signalling molecules such as MAPK and NF $\kappa$ B (Taranu *et al.*, 2014).

#### 4.4.2 *Camelina* meal as a component of animal feed

An outstanding nutrient profile of *Camelina* meal strongly supports its use as a component in animal food. However, there are few other reasons behind using CM as a part of food of many animal species; some of them are: (i) increased price and decreased supply of conventional animal fodder, (ii) low agronomical inputs for cultivation and processing of *Camelina*, (iii) availability, cost and minimal adverse effects of *Camelina* meal and (iv) to provide the increased human needs of PUFA and proteins through various by-products of animal such as eggs, fish oil, meat and milk (Table 9). Clinical and biomedical investigations have suggested that heavy intake of *trans* fatty acids and SFA (12:0, 14:0 and 16:0) is linked with increased cardiovascular disease risk in humans (Mensink *et al.*, 2003). Experiments are being conducted with various plant origin oils rich in PUFA obtained from flax seed, canola, *Camelina* and marine fish oils, in order to elevate the PUFA levels in animal by-products which can be utilised by humans for their health purposes.

#### 4.4.3 Feed of broiler birds

The idea of including *Camelina* seed meal into the feed of broiler chicken is to improve the  $\omega$ -3 fatty acid in the meat of broiler chickens. Due to the positive health benefits, significance of  $\omega$ -3 fatty acids increases and it has become a challenge to meet the requirements to obtain these essential fatty acids. To increase the content of  $\omega$ -3 fatty acids in poultry based products, broilers have been fed with flax (Ryer and Givens, 2005) and marine fish oils (Gonzalez-Esquerra and Leeson, 2001). Feeding *Camelina* meal to broilers led to a noteworthy increase in  $\alpha$ -linolenic acid (18:3) and longer chain  $\omega$ -3 fatty acids, such as docosapentaenoic acid (22:5), docosahexaenoic acid (22:6) and eicosapentaenoic acid (20:5) in breast (white), thigh (dark) meat, liver and adipose tissues. The fatty acid content increase in meat and tissues was directly proportional to the percentage of *Camelina* meal incorporated into

the feed (Ryhänen *et al.* 2007). In another study, it was revealed that  $\gamma$ -tocopherol levels in thigh meat of hens upon feeding with CM prevented the lipid peroxidation in the respective tissue. CM feeding to broilers neither affected the physical characteristics such as body weight gain, carcass weight, and weights of major edible organs, nor the sensory qualities (taste, juiciness and tenderness)

of broiler meat (Aziza *et al.*, 2010b). Despite many advantageous effects, feeding hens with more than 10% CM impaired feed intake and reduced the feed conversion rate in broilers and Turkey poults in the starter phases of the study (Ryhänen *et al.*, 2007; Frame *et al.*, 2007), CM also caused deprivation in feed efficiency of broilers (Aziza *et al.*, 2010b).

**Table 9:** Effects of *Camelina* meal based food on performance of various animal species

Animal species	Advantageous effects	Adverse effects	Parameters not being affected	Reference
Broiler chicken	Levels of $\alpha$ -Linolenic acid (18:3, n-3), Eicosapentaenoic acid (EPA) (20:5, n-3), Docosapentaenoic acid (DPA) (22:5, n-3), and Docosahexaenoic acid (DHA) (22:6, n-3) significantly elevated in white (breast) meat, dark (thigh) meat, liver and adipose tissues	Lower feed efficiency (FE) in the initial period in 10% <i>Camelina</i> meal fed broilers, which may be due to presence of anti-nutritive compounds such as GSLs but overall FE not affected	No significant differences were found in body weight gain, carcass weight and no change in weight of abdominal fat, heart, spleen, gastrointestinal tract and lungs (expressed as percentage of carcass weight)	Aziza <i>et al.</i> , 2010a
	Incorporation of <i>Camelina</i> meal at 5 and 10% increased $\gamma$ -tocopherol levels in thigh meat prevents lipid peroxidation in chicken thigh meat than in breast meat and works as natural antioxidant.		No change in total phenolic content of the chicken meat.	Aziza <i>et al.</i> , 2010b
	Increased Omega-3 fatty acid levels in broiler meat (muscle and back fat)	Impaired feed conversion and decreased feed intake in starting phase of the study (1-14 days).  Increased gondoic acid levels by three fold in broiler meat	<i>Camelina</i> meal neither affected the sensory quality of broiler meat nor the size of thyroid gland and liver histology	Ryhänen <i>et al.</i> , 2007
		Decreased feed consumption in 15% and 20% CM fed poultry and decreased body weight gain and poor feed conversion in 20% <i>Camelina</i> meal fed groups		Frame <i>et al.</i> , 2007
Layer hens	Eggs with increased levels of total $\omega$ -3 fatty acids, docosahexanoic acid (8 fold); decreased saturated and monounsaturated fatty acid content; and increased albumin weight were produced by hens fed with <i>Camelina</i> meal	Feed containing 15% <i>Camelina</i> meal negatively affected egg production, yolk weight, yolk size, yolk total lipid content and colour	No difference in egg weight, shell weight and shell thickness	Cherian <i>et al.</i> , 2009
	Increased total egg n-3 fatty acids and docosahexanoic acid (DHA); higher egg shell strength. No detectable GSLs was found in eggs	Higher shell thickness of eggs produced from hens fed with 5% <i>Camelina</i> meal than control and 10% with no change in egg quality	Layer performance, egg production, egg weight, yolk colour, yolk cholesterol content and egg sensory characteristics such as odour and taste	Kakani <i>et al.</i> , 2012
	Increased n-3 fatty acid levels especially $\alpha$ -linolenic acid		No sensory characters of eggs produced by layers fed with <i>Camelina</i> meal were found to be altered	Rokka <i>et al.</i> , 2002
<i>Tilapia</i> fish	Increased n-3 fatty acids in <i>Tilapia</i> fillets		No significant changes in feed utilization and growth were observed in 25% <i>Camelina</i> meal fed fishes	Ramotar-John, 2014

Cattle-Finnish Ayrshire Cow	Decreased levels of total saturated fatty acids (SFA; 12:0, 14:0, 16:0) and increased concentrations of 18:3, n-3 fatty acids in cow milk. Quality of butter produced from low fat containing milk is improved in terms of softness and spreadability	Decreased feed intake, milk fat, protein and lactose content. Increases concentrations of trans C18:1 isomers	No changes were recorded in dry meal intake, milk yield and milk composition	Halmemies-Beauchet-Filleau <i>et al.</i> , 2011; Hurtaud & Peyraud, 2007
Polish Ewe	Decreased total SFA (Lower indices of atherogenicity and thrombogenicity); increased total MUFA and PUFA (especially n-3) content in ewe's milk	Milk with low fat and decreased total solid content obtained from Ewe's fed with 20% CM	No significant changes in milk yield	Szumacher-Strabel <i>et al.</i> , 2011
Broiler rabbit	Increased PUFA in Longissimusdorsi muscle and perirenal fat and decreased levels of atherogenic and thrombogenic saturated fatty acids in rabbit meat		No significant differences in live weight, live weight gain, feed consumption, feed efficiency, carcass yield and weight of edible organs	Masoero <i>et al.</i> , 2007; Peiretti <i>et al.</i> , 2007
Pigs	Decreased plasma glucose and lipid levels in fattening pigs. Increased feed efficiency and hepatic activity. Increased $\alpha$ -linolenic and linoleic acid levels and decreased SFA levels in back fat and intramuscular fat. <i>Camelina</i> oil Increased ALA, eicosapentanoic acid (EPA), docosahexanoic acid (DHA) levels in plasma and 10% oil included pig feed reduced serum cholesterol and triglyceride content	Reduced average daily feed intake during starter phase in animals fed with high CM included diet. Increased liver weight and decreased the weight of thyroid gland. Reduced daily weight gain of pigs fed with 10% CM. Declined consistency of carcass. Decreased vitamin E content in most of the tissues. Decreased plasma levels of arachidonic acid (AA) and docosapentanoic acid (DPA)	Feed intake, average weight gain, feed efficiency. Plasma levels of docosapentanoic acid (DPA) weren't altered	Eidhin <i>et al.</i> , 2003; Flachowsky <i>et al.</i> , 1997; Meadus <i>et al.</i> , 2014; Taranu <i>et al.</i> , 2014

#### 4.4.4 Feed of layer hens

Since the poultry based products (meat and eggs) are very rich in protein and easily available to all groups of people around the world irrespective of their capital income and these products has become the choice of researchers to meet the demand for essential PUFA. Egg yolk components governed by the dietary nutrient supply. Feeding poultry birds with  $\omega$ -3 fatty acid rich food may directly or indirectly promote further elongation of fatty acid acyl chain and desaturation of fatty acids in the liver and which helps to increase the  $\omega$ -3 and 6 fatty acid content of the egg yolk (Watkins *et al.*, 2003; Cherian *et al.*, 1996; Yamamoto *et al.*, 1996). Hen eggs are considered as highly nutritive and consumption of eggs helps in human physical health development. Nutritionists and scientists are more concerned to improve the nutrient profile of hen eggs even further, as an initiation they started feeding hens with plant based food (seed cakes and seed oils) which found to be very rich in protein and lipid content. *Camelina* meal can also serve as a vegetable source of nutrients and can be included in the feed of layer hens. Different studies conducted by Moriel *et al.* (2011; 2016) observed a 2-3 fold and a dose-dependent augmentation of total  $\omega$ -3 fatty acids in eggs of birds fed with CM (5%, 10% and

15%) compared to eggs produced by birds fed without CM. Cherian *et al.* (2009) also noted a substantial decline in weight and colour of the yolk and total fat content of the eggs in CM (10 and 15%) fed bird eggs, whereas albumen protein weight was also increased which lead to a plunge in yolk:albumen ratio. Similar results regarding reduction in yolk weight upon feeding with CM were also documented from studies carried out elsewhere (Bean and Leeson, 2003; Novak and Scheideler, 2001). The reduction in yolk weight may be linked to an elevated level of n-3 fatty acids in egg due to incorporation of CM. Feeding layers with CM led to a decreased SFA level in eggs, this activity of CM may be explained by the diminished operation of stearoyl-coenzyme, a desaturases enzyme responsible for the conversion of SFA into MUFA due to increased levels of PUFA (Ntambi, 1999). A considerable fall in total MUFA which suppresses the synthesis of VLDL and leads to a prominent reduction in total egg lipid content and yolk weight. None of the research groups have reported any adverse effects on bird performance, egg production, egg weight, shell weight, egg sensory qualities such as taste and odour except minimal alterations in egg shell thickness and egg shell strength of eggs collected from CM fed layers (Rokka *et al.*, 2002; Cherian *et al.*, 2009; Kakani *et al.*, 2012).

A noticeable reduction in feed consumption of layers fed with more than 10% of CM and no traces of GSLs or any of their metabolites were found in eggs.

#### 4.4.5 *Camelina meal as fish feed*

The fish meal and soybean meal are the leading food products of aquaculture industries. Due to an increased demand of these two protein sources, researchers have focused on identifying and developing alternative ingredients as a protein source in fish feeds. Trials were conducted in Nile tilapia (*Oreochromis niloticus*) which is a substantial and suitable species for aquaculture in many tropical and sub-tropical regions of the world. Inclusion of *Camelina* meal and oil (0, 5, 10, 15, 20 and 25%) in the fish diet for 60 days indicated similar growth performance indices and feed consumption to fish fed with commercial food. Significant differences for body organ indices and body composition were also observed. Inclusion of *Camelina* meal (15%) in fish diet was found to be cost effective feed formulation and as an alternative ingredient for soymeal. The addition of *Camelina* meal and oil in tilapia diets caused substantial escalation of  $\omega$ -3 fatty acid content of fillets. Tilapia diets with inclusion levels up to 25% of *Camelina* meal and 4.7% *Camelina* oil did not significantly affect feed utilization and growth rates and suggest that the diets were sufficiently palatable for tilapia (Ramotar-John, 2014).

#### 4.4.6 *Camelina meal as cattle feed*

Experiments on the replacement of grass silage with red clover silage indicated that the enhancement of milk fat and PUFA content, particularly 18:3 with minute rise in *trans* oleic acid content and significantly lowered SFA levels in milk (Dewhurst *et al.*, 2006; Vanhatalo *et al.*, 2007; Shingfield *et al.*, 2012). Moderate inclusion of CM in red clover silage-based diets of lactating cows did not affect dry meal intake, milk yield, or milk composition, but decreases medium length carbon chain fatty acids (12:0, 14:0, 16:0) and total SFA concentrations and enrich the content of unsaturated fatty acids (PUFA) inherent to lipid supplements. Milk ALA (18:3) content was lower on CM than CO due to a reduced 18:3 intake and low levels of 18:3 in CM compared to CO. In a separate study by (Hurtaud and Peyraud, 2007) reported that the addition of CM in dairy cow feed led to a reduced feed intake, milk fat content, milk fat yield, milk protein content, lactose content and FCM production but did not affect the milk production and milk fatty acid composition. Reduced milk fat content due to feeding CM resulted in softer and probably more spreadable butter. Feeding CM led to some strong and desirable effects on milk and it is, therefore, advisable to feed CM up to a maximum limit of 2 kg/cow/day, which minimizes the adverse effects on milk fat content and inhibits the rise of potentially unhealthy *trans* isomer concentrations of oleic acid (Hurtaud and Peyraud, 2007; Halmemies-Beauchet-Filleau *et al.*, 2011).

#### 4.4.7 *Camelina meal enhance PUFA in Ewe's (Female sheep) milk*

Milk fats are rich in saturated fatty acid (such as palmitic, oleic, myristic and lauric acids) and are known to play a vital role in the precipitation of coronary heart disease (Williams, 2000). Studies have proved that consumption of medium (C12:0, C14:0) and long-chain saturated fatty acids (C16:0, C18:0 and C20:0) and *trans*

isomers of monounsaturated fatty acids accelerate the thrombus formation or are atherogenic, whereas monounsaturated (C16:1, C18:1 and C20:1) and polyunsaturated (C18:3, C22:5 and C22:6) fatty acids are reported to possess inhibitory effects on thrombogenesis and atherogenesis (Novak and Scheideler, 2001). Supplementation of plant oils and seed cakes or other rich sources of  $\alpha$ -linolenic and linoleic fatty acids through animal diet helps in escalation of MUFA and PUFA content in milk fat. Inclusion of CM in the ewe's diet has increased the content of total monounsaturated and polyunsaturated fatty acid levels and significantly reduced the total saturated fatty acid concentration of milk. CM inclusion in the ewe's diet didn't affect the milk yield and composition but decreased total solid content due to lower fat yield in milk, which may be correlated to suppressed *de novo* milk fat synthesis by increased PUFA and *trans*-10 and *cis*-12 isomers of linoleic acid (Sinclair *et al.*, 2007) of which linoleic acid is a potential blocker of milk fat synthesis compared to linolenic acid (Bauman and Griinari, 2003).

#### 4.4.8 *Effect of Camelina oil on porcine blood lipids*

The effect of fish oil (FO) and *Camelina* oil (CO) on blood lipid levels of pigs was monitored at two different concentrations (5 and 10%). Both the oils containing diets boosted the plasma  $\omega$ -3 fatty acids levels, but  $\omega$ -6 fatty acid concentrations were reduced. Fish oil diets were significantly increased the plasma eicosapentaenoic acid (C20:5) compared to CO diets. CO also reduces the level of serum triglyceride and cholesterol in a dose-dependent manner (Eidhin *et al.*, 2003; Flachowsky *et al.*, 1997). Jaskiewicz and Matyka (2003) observed an elevated levels of linoleic and linolenic acids in the backfat and muscle of pigs fed with 5 or 10% CM, these results were found similar to the preceding experiments conducted on animals elsewhere (Hurtaud and Peyraud, 2007; Kahindi *et al.*, 2014).

#### 4.4.9 *Camelina meal as feed for broiler rabbits*

Broiler rabbits fed with CM (0%, 10%, or 15%) included diets for a period of 50 days observed a substantially amplified magnitude of long chain unsaturated PUFA (especially n-3 fatty acids) in perirenal fat and longissimusdorsi muscle which are considered to be anti-thrombogenic and antiatherogenic, while the CM inclusion in the diet was responsible for the reduction in atherogenic and thrombogenic SFA levels. The experiment revealed that feeding broiler rabbits with CM included diet helps in improvement of nutritional value and fineness of rabbit meat, besides the CM had no harmful effects on rabbit growth characteristics, feed consumption, feed efficiency, live weight, live weight gain, carcass yield and weights of edible organs (Scholze and Hammer, 1997). *Camelina* meal is being exploited for its use in animal diets due to its exceptional nutrient profile and its richness in proteins, fatty acids, amino acids and minerals. Several studies conducted on animal species strongly support the use of CM in animal diets (Peiretti *et al.*, 2007). Though the addition of CM in animal diets has significantly increased PUFA (n-3 and n-6) levels in respective animal by-products (Masoero *et al.*, 2007). Inclusion of CM above 10% in animal diets has minimal but non-negligible adverse effects on animal growth and performance, such as reduced feed intake (Frame *et al.*, 2007), impaired feed conversion (Ryhänen *et al.*, 2007; Frame *et al.*, 2007), low feed efficiency (Aziza *et al.*, 2010a), decreased body weight gain (Frame *et al.*, 2007) in broiler birds. Inclusion of CM (15%) in

the diet of layer hens negatively affected the egg production, yolk weight, yolk size, yolk total lipid content (Cherian *et al.*, 2009), low fat and decreased total solid content in ewe's milk fed with diet containing CM (20%, (Szumacher-Strabel *et al.*, 2011). CM included diet resulted in reduced growth and performance in mice (Korsrud and Bell, 1967; Korsrud *et al.*, 1978). However, no experiment has reported any mortality in animals fed with CM. The detrimental effects in animals upon feeding with CM included diet are linked to the presence of antinutritive compounds in *Camelina* seeds. Like other cruciferous plants, *Camelina* contains high amounts of non-starch polysaccharides, GSLs, phytic acid, sinapine, condensed tannins and erucic acid (Matthäus and Zubr, 2000; Matthäus and Angelini, 2005) that can affect feed consumption and feed conversion rate in animals. Although, GSL's as such do not produce any toxic effects on animals, but their metabolites formed after breakdown can be harmful to the gut microflora, affecting growth and feed efficiency (Schuster and Friedt, 1998), while amino acid profiles justify the use of CM in animal fodder (Miller *et al.*, 1962).

Although, the CM is rich in protein and essential n-3 and n-6 fatty acids (Colombini *et al.*, 2014) and could be incorporated into poultry and cattle feed up to 100 g/kg of dietary dry meal (Schill, 2010) as a source of energy, but its use in animal feed is restricted due to its high GSLs content as it was found to depress the feed intake and growth in different animal species. To overcome this limitation, it is suggested to maintain the CM amount below 10% in animal feed to minimise the negative effects on animal growth and performance (Hurtaud and Peyraud, 2007). In other ways, the researchers are looking forward to produce low GSLs yielding varieties or to adopt the technologies to treat and improve the nutritive value of CM to be useful in animal feed (Ryhänen *et al.*, 2007). The USDA and Health Canada have approved and permitted to use the CM up to 10% in the animal feed especially for broiler and layer hens and up to 2% in diets of growing pigs (<http://www.biodieselmagazine.com>).

## 5. Bioactive components of *C. sativa* and pharmacological activities

Data regarding the practice of *C. sativa* in remedies and therapies is barely acknowledged. Very little information was documented in Hagers Handbook of Pharmaceutical Praxis to support the use of *Camelina* seeds to treat eye inflammations and cancer list (Honermeier and Agegnehu, 1994). *Camelina* seeds encompass a diverse class of bioactive compounds (Table 10) such as phenolics, sinapic acid, ellagic acid, quercetin, rutin, catechin, and salicylic acid, GSLs, phytic acid, sinapine, tocopherols and  $\omega$ -3 fatty acids (Abramovic and Abram, 2005; Abramović *et al.*, 2007; Blades and Zubr, 2010; Terpinc *et al.*, 2012).

**Table 10:** Antinutritive compounds of *Camelina* meal

Glucosinolates	Tannins	Phytic acid	Sinapine	Reference
24 $\mu$ mol/g	2.2 mg/g	19 mg/g	2 mg/g	Matthäus and Zubr, 2000

### 5.1 Antioxidant activity

Different types of extracts of *Camelina* oil (CO) and *Camelina* meal (CM) comprising huge amounts of phenolic compounds have been examined and known for their antioxidant activities. Alcoholic

extracts were also found to have DPPH (free radicals) scavenging and iron chelation properties (Terpinc *et al.*, 2012; Polak, *et al.*, 2012). The tocopherols in CO prevent the oxidation of oil and enhance the shelf life and use of oil for a longer period.

*Camelina* seed oil possesses extraordinary polyunsaturated fatty acid content and is thought to accommodate high amounts of antioxidants such as phenolic and flavonoid compounds. Taranu *et al.* (2014) also reported increased mRNA expression of antioxidant enzymes, catalase (CAT), glutathione peroxidase 1 (GPx1) and superoxide dismutase (SOD) by 3.43, 1.83 and 2.47 folds, respectively. A significant (9.02%) increase in total antioxidant capacity was also observed in pig spleen upon feeding with CM and mRNA expression of enzymes responsible for nitric oxide (NOS) synthesis, especially endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) by 3.23 and 4.60 folds, respectively (Taranu *et al.*, 2014). A similar study was carried out to explore the effects of large doses of *Camelina* seed oil (CO) on the antioxidant enzyme (SOD and CAT) levels in rat plasma and liver of Sprague-Dawley revealed that CO did not alter or improve reduced levels of antioxidant enzymes due to high fat diet. Moreover, CO also did not affect the lipid peroxidation and total antioxidant capacity in plasma and the livers of rats fed with high fat diet (Deng *et al.*, 2011).

### 5.2 Immunomodulatory

Highly distinguished polyunsaturated fatty acid rich sources like fish oil and vegetable oils are widely studied for their notable positive effects on immune mediators, serum parameters, inflammatory responses and reactive oxygen species (ROS). In support of the immune-modulatory effects of CM, experiments conducted on the spleen of fattening pigs demonstrated that CM inclusion up to 12% in animal diets did neither affected feed intake nor average weight gain, but it modulated immune response in spleen cells through diminishing the gene and protein expression of proinflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), cyclooxygenase-2 (COX-2), interleukin-8 (IL-8), interleukin-6 (IL-6) and interleukin-1 $\beta$  (IL-1 $\beta$ ), except elevated levels of IL-4 gene. A comparable effect of CM on cytokine gene expression was observed in pig plasma and CM has also reduced plasma glucose and cholesterol content. Immuno-blotting results suggested that the components of CM also decreased protein expression of mitogen-activated protein kinase (MAPK), nuclear factor of kappa light polypeptide gene enhancer in  $\beta$ -cells (NF $\kappa$ B) besides increasing the peroxisome-proliferator activated receptor gamma (PPAR- $\gamma$ ) mRNA levels in spleen lysates (Taranu *et al.*, 2014).

### 5.3 CYP enzyme induction

The weaned pigs of 28 days were fed with diets (including 3.7% and 7.4% CM) for 28 days revealed a soaring feeding efficiency as a result of which the liver weights get increased. An increased gene expression Phase I and Phase II detoxifying enzymes, aldehyde dehydrogenase 2, Cytochrome 8b1 (CYP8B1) and thiosulfate transferase in liver was also observed which was associated with methyl-sulfinyldecyl isothiocyanate (*Camelina* GSL metabolite) also testified to be liable for amplified liver metabolism and increased liver weight and the altered iodine uptake due to higher GSL content in CM lead to decreased thyroid weight (Meadus *et al.*, 2014).

Previously, a similar effect on hepatic detoxifying enzymes has also been stated in the case of GSLs of other brassica crops (Zhang *et al.*, 1992).

#### 5.4 Lipid lowering property

*Camelina* seed oil (cold-pressed) rich in  $\alpha$ -linolenic acid dose-dependently suppresses the increase in body and liver weight of *Sprague-Dawley* rats served with high fat diet. Besides, the body weight of HFD fed rats treated with high doses of CO (4.4 g/kg body weight) did not show any significant change as compared to control. CO also decreases triglycerides, total cholesterol, alanine amino transferase, aspartate amino transferase and plasma low density lipoprotein levels compared to high fat diet rats in a dose-dependent manner. Consequently, the plasma and liver high density lipoproteins levels were increased in treated (1.1, 2.2 and 4.4 g/kg body weight) groups with CO (Deng *et al.*, 2011). The lipid lowering potential of CO is comparable to  $\omega$ -3 fatty acid rich commercial fish oil. CO has also been found to limit the hepatocellular damage triggered due to high fat diet (Deng *et al.*, 2011)

Hypercholesterolemic human subjects fed with *Camelina* oil (CO) for six weeks showed significant increase in the proportion of fatty acid ( $\alpha$ -linolenic acid) of serum lipids and its metabolites (Eicosapentanoic acid and docosapentanoic acid) compared to rapeseed and olive oil fed group subjects. At the end of the six week study, CO was found to decrease the serum LDL levels by 12.2% which is more and prominent compared to two other oils without affecting the serum HDL and triglyceride levels and blood pressure of the subjects (Karvonen *et al.*, 2002).

#### 5.5 Enhancement of insulin sensitivity

In a normal condition, insulin gets released into the blood after meal intake to facilitate the glucose uptake by muscle, liver and fat cells and simultaneously inhibits lipolysis (Choi *et al.*, 2010). Late pregnancy in ruminants is associated with temperate insulin resistance. During late pregnancy, plasma non-esterified fatty acid levels are squeezed due to insulin sensitivity. Before parturition, ruminants take very less dry matter that leads to enhanced lipid movements and increases plasma non-esterified fatty acids that may further reduce the insulin sensitivity in whole body and the glucose uptake. Treatments consisted of abomasal infusion of *Camelina* oil administered in 10 equal doses daily elevated basal plasma non-esterified fatty acids (0.25mmol/l) concentrations on day 5 as compared to control (0.17 mmol/l) and also increased the plasma C18:3, n-3 content. CO infusion disrupted glucose clearance and, hence, decreased insulin secretion, thereby augmenting the insulin clearance already present in the plasma and, hence, insulin sensitivity was enhanced (Salin *et al.*, 2012).

#### 5.6 Synergistic activity of Camelina meal components

Defatted *Camelina* meal up regulated quinone reductase (NQO1), a phase II detoxification enzyme without affecting cytochrome P450 (CYP) 1A1 activity in mouse hepatoma (Hepa1c1c7) cells. Stimulation of NQO1 and mixed effects on CYP450 enzymes are also the hallmark effects of GSLs of other brassica crops. GSL 10, GSL 9, quercetin, and rutin, the major bioactive components of CM-induces NQO1 activity in a dose-dependent manner when tested alone and in combination. Isobologram graphs reveal the synergistic

effect of bioactive components of CM such as GSL9 and quercetin on NQO1 induction. This study also revealed that neither GSLs extracted from defatted CM nor methanolic extracts of *Camelina* seed showed any cytotoxicity in Hepa1c1c7 cells except at higher doses (5 $\mu$ M) of GSL 10 (Das *et al.* 2014).

#### 5.7 Antinutritive compounds in Camelina seed

Glucosinolates (GSLs; 14-34), sinapine (4 mg/g), phytic acid or inositol phosphate (IP; 13-15mg/g) and condensed tannins (1 mg/g) are the predominant antinutritive compounds found and reported in *Camelina* seeds, all of which can unenviable affect the nutritional value of *Camelina* seed oil and meal (Matthäus and Zubr, 2000; Bertrand *et al.*, 2005).

GSL's are the main secondary metabolites synthesised in the plants of *Capparales* order of the *Brassicaceae* family. They are commonly known as 'Mustard oil glycosides' and 'Thioglucosides'. GSL's are a class of water soluble organic compounds that contain sulphur, nitrogen and a glucose derived group. During adverse or stress conditions, plant cells secrete myrosinase enzyme and in presence of water, myrosinase cleaves off glucose moiety from GSL's (Mithen *et al.*, 2000). The remaining molecule after myrosinase activity quickly converts to active defence molecules such as thiocyanate, isothiocyanate or nitrile that act to prevent the damage due to stress conditions, GSL's also act as natural pesticides (Jeffery and Araya, 2009). Usually, seeds of cruciferous plants contain distinctive amounts of GSL's and considered as the reservoirs of GSL's. The cumulative glucosinolates content of *C. sativa* lies within 14.5-34 mmol/kg (Lange and Stoller, 1995; Matthäus and Zubr, 2000). The season of cultivation can influence the GSL content of *Camelina* seed. The GSL content of seeds obtained from winter varieties (20 pmol/g) is high compared to summer varieties (15 pmol/g, Berhow *et al.*, 2013).

In *Camelina* seeds, three aliphatic sulfinyl-glucosinolates were determined using high performance liquid chromatography. Glucocamelin (10-methyl-sulfinyl-decyl-glucosinolate) which confers about 65% of total GSL's in *Camelina* seed, other GSL's include 9-methyl-sulfinyl-nonyl-glucosinolate and 11-methyl-sulfinyl-undecyl-glucosinolate (Kjaer, 1956; Lange and Stoller, 1995; Schuster and Friedt, 1998; Das *et al.*, 2014). The GSLs and their metabolites from *Camelina*, canola, rapeseed and other crucifer plants faintly vary in the type and quantity (Shahidi and Gabon, 1989).

Since the United States of America and Canada had set a maximum limit for GSL levels of canola meal (30  $\mu$ mol/g dry matter) and must not go beyond 20  $\mu$ mol/g, as per the EU regulations, the GSL content of *Camelina* falls under the limits as compared to canola meal (Moss, 2002). Although, the GSL content of CM is lower than the approved limit, it has produced negative effects on performance and development characteristics of animals fed with >10% CM included diets as a consequence of this use of CM in animal diets is restricted to a level of 10%. Researchers are now more attentive towards developing new techniques to isolate the glucosinolates or abate their quantity in CM, so that it can be utilised for human and animal purposes (Russo and Reggiani, 2012a; Berhow *et al.*, 2013). On the other side of GSLs, the isothiocyanates, active metabolites of GSLs from other brassica crops were explored for their anticancer

properties and, hence, CM can also be examined for other related activities (Talalay and Zhang, 1996). Glucosinolates are considered bitter to humans (Mithen *et al.*, 2000; Russo and Reggiani, 2012b), chickens (Pekel *et al.*, 2009), fish (Morais *et al.*, 2012) and pigs (Eidhin *et al.*, 2003) and has been found to reduce feed intake in a dose-dependent manner. Large doses of thiocyanate, a glucosinolate metabolite affects iodine transport to thyroid (Schone *et al.*, 1990). GSL metabolites are primarily associated with stimulation of Phase I & II biotransformation enzymes in liver (Zhang *et al.*, 1992). The chemical structure of 10-methylsulfinyldecyl is familiar to the sulforaphane, a compound widely distributed in cruciferous vegetables such as broccoli, cabbage and mustard, which has shown a protective effect against cancer and cardiovascular diseases (Anwar-Mohamed and El-Kadi, 2009).

## 6. Translational research and future perspectives in *Camelina* species

Contemporary fatty acid constitution of *Camelina* seed does not fulfil the requirements of any particular application and the seed composition needed to be optimized in order to use the *Camelina* seed oil and meal for various industrial applications. Technological advances helped researchers to discover the polyploid genome structure (Hutcheon *et al.*, 2010; Kagale *et al.*, 2014) and transcriptome (Nguyen *et al.*, 2013) of *C. sativa* to improve the quality and chemical composition of seed oil and meal so that they can be used and exploited for various industrial applications like feedstock for biodiesel and seed oil for human consumption. The unfolded transcriptome data could help to modify *Camelina* oil and meal compositions for fuel and various other industrial applications, respectively (Johann *et al.*, 1996).

Efforts are being made to bring up the transgenic *Camelina* plants though metabolic engineering techniques to meet the requirements of PUFA rich oil with high amounts DHA as a food and health supplement, less PUFA and high amounts of SFA containing *Camelina* seed oil serves as a suitable biodiesel feedstock (Bansal and Durrett, 2016; Faure and Tepfer, 2016; Vollmann and Eynck, 2015). Russo and Reggiani (2012b) established the production of transgenic *C. sativa* with significantly increased amount of  $\omega$ -3 fatty acids, especially DHA (12%) like in fish oil with limited intermediate fatty acid synthesis. The study also revealed an excessive accumulation of new  $\omega$ -3 fatty acids like stearidonic acid, eicosatetraenoic acid besides EPA and DHA content in *Camelina* seeds. *Camelina* seed meal was also found to be rich in DHA like *Camelina* oil, which is favourable for feed applications (Petrie *et al.*, 2014). In another report, biosynthetic pathways involved in EPA and DHA production were manipulated through metabolic engineering and a significant increase in the synthesis of long chain PUFA like EPA (20:5) and DHA (22:6) in *Camelina* seeds with minimal precipitation of intermediate fatty acids was observed. The study revealed the creation of two transgenic varieties of *Camelina*; one with up to 31% EPA and the other is with 12% EPA and 14% DHA content (Bansal and Durrett, 2016; Prankl and Wörgetter, 1996) also published similar results and demonstrated over production of transgenic *Camelina* with significantly high amounts of EPA in seed oil (Anwar-Mohamed and El-Kadi, 2009). Transgenic *Camelina* seeds were produced by *Agrabacterium* interceded inoculation with previously designed plasmid carrying

castor fatty acid hydroxylase (FAH12) into plants during early flowering stages which yielded over 1% of transgenic seed with decreased long chain PUFA compared to non-transgenic cultivar (Nguyen *et al.*, 2013). In January 2010, Health Canada notified Canpressco Products Inc., Midale, Saskatchewan about its no objection to the use of cold-pressed *Camelina* oil as a food ingredient (<http://www.hc-sc.gc.ca>).

## 7. Conclusion

*Camelina* popularly called as 'false flax' is being chosen since ancient times for anthropological and animal uses. Presently, *Camelina* is highly exploited for its use in biofuel and biolubricants production apart from its use in various animal feed industries. Few studies have kick started to find out the medical and biological uses and applications of components of *Camelina* oil and press cake which can promote human health and animal well-being. *Camelina* oil has already been in use for nutraceuticals and cosmeceuticals relevance. Besides a small number of activities, further studies are needed for the production of application specific transgenic *Camelina* plants to discover novel bio active compounds and to standardize and fix the limits of *Camelina* meal in animal feed and to reduce the levels of anti-nutritive compounds, all of which can contribute towards making *Camelina* a money plant with diverse industrial applications.

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## Useful websites and patents on *Camelina*

<http://www.feedipedia.org/node/4254>  
<http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/camelina-cameline-eng.php>  
<https://www.sciencedaily.com/releases/2014/08/140804122909.htm>  
<http://www.nature.com/ncomms/2014/140423/ncomms4706/full/ncomms4706.html>  
<http://www.biofuelnet.ca/2014/12/18/biodiesel-camelina/>  
[http://biofuelstp.eu/oil\\_crops.html](http://biofuelstp.eu/oil_crops.html)  
<http://www.scirp.org/journal/PaperInformation.aspx?PaperID=24118>  
<http://www.tandfonline.com/doi/pdf/10.1080/00071669986657>  
<http://www.greenerpro.com/Camelina.html>  
<http://japr.oxfordjournals.org/content/19/2/157.abstract>  
<http://www.siberiantigernaturals.com/camelinaoil.htm>  
[http://extension.usu.edu/files/publications/publication/AG\\_Poultry\\_2008-03pr.pdf](http://extension.usu.edu/files/publications/publication/AG_Poultry_2008-03pr.pdf)  
<http://articles.extension.org/pages/70230/including-camelina-in-organic-poultry-diets>  
[http://www.wageningenacademic.com/doi/abs/10.3920/978-90-8686-804-9\\_18](http://www.wageningenacademic.com/doi/abs/10.3920/978-90-8686-804-9_18)  
<http://onlinelibrary.wiley.com/doi/10.1002/jsfa.6408/pdf>  
<http://www.lowens.ca/camelina-sativa-seed-oil-for-the-skin/?v=c86ee0d9d7ed>

<https://www.mountainroseherbs.com/products/camelina-oil/profile>  
<https://www.strictlymedicinalseeds.com/product.asp?specific=2500>

<http://www.alive.com/food/cooking-with-camelina-oil/>

<http://www.omegamaidenoils.com/blog/advantages-of-camelina-over-fish-oil-supplements/>

<http://www.currentscience.ac.in/Volumes/107/03/0359.pdf>

<http://www.environmentportal.in/files/Camelina%20sativa.pdf>

A patent on Herbicide resistant *Camelina sativa* by Kaijalaine *et al.* (2010) relates to a method to transform the *Camelina* plant for herbicide resistant especially for those who inhibiting acetolactate synthase and develop improved method for in vitro regeneration of transformed plant. Patent No- WO2010147636 A1. Authors- Seppo Kaijalainen, Kimmo Koivu, Viktor Kuvshinov, Eric Murphy. <https://www.google.co.in/patents/WO2010147636A1?cl=en>

A patent on Transformation in *Camelina sativa* by Kuvshinov *et al.* (2011) provide a method for transforming camelina via using Agro-bacterium mediated transformation system. Patent No- US 7,910,803 B2. Authors-Viktor Kuvshinov; Anne Kanerva; Kimmo Koivu; Svetlana Kuvshinova; Eija Pehu. <http://www.google.com/patents/US7910803>

A patent on *Camelina sativa* variety 'SO-50' by Fernando (2012) proposed method to developed a new variety which is pest or pathogen resistance by using sexual hybridization. Patent No- 20120124693. Author- Fernando Guillen-Portal <http://www.faqs.org/patents/app/20120124693>

A patent on modifying the fatty acid profile of *Camelina sativa* oil by Puttick *et al.* (2014) presents the compositions and methods for modifying fatty acids in *Camelina sativa* oil. Patent No- US 2014/0107361 A1. Authors- D. Puttick; A.Todd; C. Sarvas; H. Damude; Brian McGonigle. <http://www.google.com/patents/US20140107361>

A patent on Transformation system in *Camelina sativa* Kuvshinov *et al.* (2014) reveals the method for screening and assessing the efficacy of homologous and heterologous recombinant products and plant transformation. Patent No- US 2014/0223607 A1. Authors- Viktor KUVSHINOV; Anne Kanerva; Kimmo Koivu; Eija Pehu; Svetlana Kuvshinova. <http://www.google.com/patents/US20140223607>

A patent on floral dip method for transformation of *Camelina* by Brier *et al.* (2014) reported a better and less complex method that offers advantage over pre-existing methods. Patent No - 8779238 (JUSTIA patents). Authors-Thu Nguyen, Xunjia Liu, Jay Derocher. <http://patents.justia.com/patent/8779238#history>

A patent on Isolation and use of FAD2 and FAE1 from *Camelina* by Hutcheon *et al.* (2015) provide gene, protein sequence and mutation for FAD2 and FAE1. Additionally it proposed the method to improve the quality of seed oil /meal. Patent No- US 9,035,131 B2. Authors – Carolyn Hutcheon; Renata F.Ditt; Christine K. Shewmaker. <http://www.google.com/patents/US20110239323>

### Conflict of interest

We declare that we have no conflict of interest.

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