Modification of Fatty Acid Profiles of Rapeseed (*Brassica napus* L.) Oil for Using as Food, Industrial Feed-Stock and Biodiesel

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ABSTRACT Rapeseed is a member of family Brassicaceae, cultivated as oil crop. Rapeseed oil is being utilized from early civilization, but its popularity being declined from the mid-nineteenth century due to presence of erucic acid (C22:1) and glucosinolates. Thereby, several attempts have been made to develop cultivars free from those toxins. In the past 20 years, breeders got success in developing '00'- quality rapeseed, known as 'Canola'. The target mutagenesis of *fae-1* and *fae-2* of *Brassica napus* ensured such success. Thereafter, 'canola' regains its market as a healthy vegetable oil. Moreover, high oleic acid rapeseed lines, with 86% oleic acid, have been developed by using chemical mutagenesis of *FAD2* alleles responsible for desaturation of oleic acid (C18:1) to linoleic acid (C18:2). Recently, high erucic acid rapeseed oil regained interest for biodegradable plastic, cosmetic, emollient industries and for biodiesel. Therefore, breeding approaches have been pursued; unfortunately, that were failed to reach erucic acid level beyond 50% in seed-oil. Rapeseed genotypes over-expressed with *Ld-LPAAT* separately and *Ld-LPAAT-FAE* chimaric construct together were tried but failed to reach the erucic acid content more than 60%. Thereof, combined effort of conventional breeding and transgenic approaches are brought together to overcome three hypothesized bottlenecks; reviewed in this article, which restricted erucic acid level near to 60%. Finally, rapeseed genotypes with 78% erucic acid were developed successfully. This material is now available in Germany for using in emollient industries and for biodiesel. Therefore, this article is reviewed on the current status and future outlook for modification of fatty acid profiles of rapeseed oil for its end-use as food, industrial feed-stock and biodiesel.

Keywords *Brassica napus*, Fatty acid composition, Food, Biodiesel

INTRODUCTION

Brassica is the genus with more than 51 genera in the family Brassicaceae, it is economically important genus containing 37 different species (Gómez-Campo 1980). *Brassica* genus included many crop species provided edible parts and seeds. *Brassica* are of great importance as vegetables throughout the world. The main species are *Brassica oleracea* and *B. rapa* included cabbages, brussel sprouts, cauliflower, broccoli, Kimchi cabbage, Pakchoi etc. (Rakow 2004). Besides, the vegetable crops, some species of this genus are equally important as source of vegetable oils. The oleiferous *Brassica* represented by rapeseed and mustard play an important role for vegetable oil production worldwide. Oil plays an important role in

our daily diet. From nutritional point of view, fats and oils in our daily life are mostly needed for calories and fat soluble vitamins absorbent. At present, rapeseed is the second most important oilseed in the world, after soybean and palm; and is used for both nutritional and industrial purposes (FAO 2014).

Brassica oilseed production has increased over the last 50 years (FAO 2014). The largest acreage of *Brassica* oilseeds is found in India, followed by China and Canada. Highest seed yields are contributed from Europe (France, Germany and the UK) with average yields of about 3.0 tonnes/ha, more than twice the seed yields obtained in Canada and Australia (Rakow 2004). This is due to the fact that, in Europe, the winter annual forms of *B. napus* are grown under favourable growing conditions, which are

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comparatively more productive than spring *B. napus* forms. Canada and Australia are major rapeseed-exporting countries while Japan and China are major importers (Kimber and McGregor 1995). More than half of the rapeseed oil marketed internationally is refined in the European Union (EU). The production in India, China and other countries is locally consumed.

The past fifty years have seen significant growth in rapeseed production due to the introduction of food rapeseed (canola), low in erucic acid (22:1; *cis*-13 docosenoic acid) and glucosinolates (Lühs and Friedt 1994; Przybylski and Mag 2002). Presently, fatty acids profile of rapeseed oil has been modified according to the market prospects with health benefits. However, there are several arguments about the rapeseed oil-health relationship. The major ones were point-outs originally by Trautwein and Erbersdobler (1998) and supported by the “Reference Values for Nutrient Intake” of the German Nutrition Society (2002). Secondly, there are the considerations of the US Food and Drug Administration; FDA (2006) when discussing a qualified health claim for rapeseed oil as vegetable oil. These arguments refer to the original (erucic acid free) variety of rapeseed oil containing around 10% linolenic acid. This vegetable oil quality is referred to as “canola” in North America (Barth 2009). In the following

some nutritional considerations are pinpointed comparing newly introduced high oleic/low linolenic acid (so-called HOLLi) varieties with the original variety containing around 10% linolenic acid (Table 1). The term “rapeseed oil” is used for the original (erucic acid free) variety with around 10% linolenic acid (Barth 2009; The Paleo Diet 2015). Recently, researches are being conducted to develop rapeseed lines with higher amount of Lauric and myristic acid for direct use of rapeseed oil as margarine following transformation and antisense techniques (Möllers 2016; personal communication). These two short chain fatty acids are absence in naturally occurring rapeseed fatty acid *de novo* biosynthesis pathway (Puyaubert *et al.* 2005).

In the last decade this genera also gain attention to being not only as the source of edible vegetables and oils but also source of healthy oils, bio-fuel and industrial feed-stock (Möllers 2004; Nath 2008). Therefore, the fatty acid compositions of the rapeseed oils have been modified according to specific objectives through conventional and molecular breeding. Very Long Chain Fatty Acids (VLCFAs) is currently used in cosmetic, polymer and emollient industry for lubricant, adhesive and biodegradable plastics products. The rapeseed oil with more than 60% erucic acid could be used directly as industrial

Table 1. Fatty acids composition of different rapeseed oil quality type compare with different other crop plants available for food and industrial use (Source: Möllers 2004; Nath 2008; The Paleo Diet 2015).

Rapeseed oil type and/crop type	Fatty acids < C16	Saturated fatty acids ²⁾	Oleic acid C18:1	Linoleic acid C18:2	Linolenic acid C18:3	Erucic acid C22:1
Rapeseed						
‘00’ Canola	< 1	7	60	20	10	< 2
High Erucic (HEAR)	< 1	2	13	3	3	78 ^{y)}
Low-Linolenic (LLi)	< 1	7	60	30	< 2	< 2
High Oleic (HOAR)	< 1	5	86	4	4	< 2
High Oleic/Low-Linolenic (HOLLi)	< 1	5	85	6	2	< 2
Palm	< 1	48	37	9	< 1	< 2
Soybean	< 1	14	24	52	7	< 2
Cotton	< 1	25	18	52	< 1	< 2
Sunflower	< 1	11	20	66	< 1	< 2
HO sunflower line ^{x)}	< 1	6	90	3	0	< 1

²⁾Mainly Palmitic acid (C16:0) and stearic acid (C18:0).

^{y)}Included ~6% Eicosenoic acid (C20:1).

^{x)}HO-High Oleic.

feedstock without any fractional distillation (Piazza and Foglia 2001). Thus, researches (renewable energy sources, RES) have gained much support in policy level and research grants to increase the share of RES in energy sectors have been adopted.

The EU target is to increase its RES from 5.4% in 1997 to 40.0% by 2020 (Rosillo-Calle 2006). Recently, Biodiesel among other RES is highly being promoted. This is because the resources are abundant, can be generated in a short period of time and obtained from different sources (waste products, forest and crops). Biodiesel production would provide employment opportunities through the cultivation, harvesting, transporting and conversion to bioenergy (Rosillo-Calle 2006). Also, it can be used to generate different forms of energy including heat, electricity and fuel. In 2000, 79.8% out of 13.8% of RES used worldwide was from biomass (IEA 2002) and in Europe, 66.1% of RES used was biomass and biodiesel (EC 2006; Ofori 2008). Therefore, this review concentrated on the potentiality of rapeseed oil as source of healthy vegetable oil and alternate renewable energy or industrial feedstock.

MAIN SUBJECT

Importance of rapeseed

Rapeseed is an important source of vegetable oil and regarding world oilseed production it is now the second largest oilseed crop after soybean (FAO 2014). Rapeseed has gained acceptance worldwide largely because of major improvements in the seed oil and meal quality. World vegetable oil markets are highly competitive requiring a steady improvement in oil quality to increase market prospects. The objective of modifying oil quality is to develop oils with enhanced nutritional and functional properties and which require if possible no further processing for specific end-use markets. The market of rapeseed oil is primarily for human consumption, but also for a range of industrial applications (Craig and Millam 1995). Presently, different types of rapeseeds with a modified fatty acid composition are available for different purposes. With a worldwide production of 65 million tonnes of rapeseed was

produced in 2013, it ranks behind only soybeans in world oilseed crop production. Rapeseed/Canola is grown principally as a source of edible oil; however it is being used more frequently in the production of biodiesel, especially in the EU where almost ten million tonnes of biodiesel were produced in 2013, mainly from canola oil (EBB 2014).

Modification of fatty acid composition of rapeseed oil

Most of the rapeseed oil is produced for human consumption in the form of margarine, fried and backed products and salad oil. Several attempts have been made through conventional breeding and transgenic approaches for modifying the fatty acid compositions to make the rapeseed oil more suitable for specific food and non-food utilization (Möllers 2004). Among the different approaches, development of high oleic acid rapeseed (HOAR) oil is important for both food and non-food purposes. Previously, this oil was used only for human consumption as salad oil or partially hydrogenated for frying and deep-frying purposes. However, studies published by Káb (2001); Piazza and Foglia (2001) summarized impressively the various application of HOAR. Those findings brought hope of the ever increasing market of HOAR at the present state (von Borcke 2014). Modifications of the fatty acids composition in rapeseed have been achieved beyond the natural occurring variation by mutation breeding and genetic engineering. As the EU do not accept the transgenic crop, therefore the utilization of mutation breeding cultivars with high oleic (HO) acid content has currently better chances being realized (Ayton 2014).

Development of high oleic acid oilseed rape (HOAR)

HO acid vegetable oil is being popular as source of stable and healthy fried oil. Therefore, HO-sunflower, HO-soybean and HO-safflower cultivars have taken lead in vegetable market in Europe, Australia and North America. In rapeseed breeding, efforts have made for many years focusing on increasing the oleic acid content. In Germany, mutation experiments were started about 20 years ago and first mutant with HO acid content was selected by Rücker and Röbbelen (1996). Genetic studies confirmed that the selected mutant line was inherited by single gene in an

additive manner (Schierholt *et al.* 2001). In a doubled haploid population segregating for oleic acid revealed that mutation itself increased of 11% oleic acid from 60% to 71% compared to wild type (Schierholt 2001). Molecular mapping of the mutated genes confer that oleic acid desaturase gene (*FAD2*; Δ -12 oleate desaturase) being mutated (Fig. 1). *FAD2* gene catalyses the introduction of the second carbon bond into oleic acid and thereby producing polyunsaturated fatty acids (Slabas *et al.* 2001; von Borcke 2014). Previously, it was assumed that there are two *FAD2* alleles present in *B. napus*; one from *B. oleracea* and other from *B. rapa* brought change the oleic acid content in the oil of *B. napus* (Möllers 2004).

Now Ian Bancroft and Rachel Wells at the John Innes

Centre (JIC), Canada have been able to increase the oleic acid content in rapeseed oil significantly beyond the level of current varieties. Their work has confirmed the precise number of alleles of the *FAD2* gene found in *B. napus*. Through detailed genetic studies and sequencing they have found that there are four alleles of the *FAD2* gene present in oilseed rape. Combined with their insights into the properties of each individual *FAD2* allele they have been able to manipulate the optimal combination of *FAD2* alleles in order to obtain new oil-seed rape lines with oleic acid contents of up to 86% (von Borcke 2014). The JIC team used Ethyl-Methyl Sulphonate mutagenesis to gain oleic acid profile over 86% and poly-unsaturated fatty acid content below 5% compared with HO acid sunflower oil (Table 1).

Development of VLCFA rapeseed lines

The fatty acids of eicosenoic (C20:1) and erucic acid (C22:1) are considered as VLCFA in rapeseed oil. The occurrence of erucic acid is considered as anti-nutritional factor for human consumption. Therefore, it was minimized by breeding and finally developed Canola- or '00'-quality (Lühs and Friedt 1994; Przybylski and Mag 2002). High Erucic Acid Rapeseed (HEAR) cultivars are regained interest for industrial purposes. Erucic acid (*cis*-13-docosenoic acid, 22:1) a VLCFA having 22 carbon atoms with one double bond at the *cis*-13 position of the carbon chain. Genetic studies show that in rapeseed, which arose from a spontaneous cross between *B. rapa* (A-genome) and *B. oleracea* (C-genome); 22:1 content is controlled by the two gene loci, *E1* (*Bn-fae1.1*; A-genome) and *E2* (*Bn-fae1.2*; C-genome), which have additive effects (Harvey and Downey 1964; Stefansson 1983; Lühs *et al.* 1999; Nath *et al.* 2009b). Studies of *Arabidopsis thaliana* mutants deficient in VLCFAs showed that the fatty acid elongase (*fae1*) gene product is required in the seeds for the elongation from 18:1 to 22:1 (Fig. 1) (Kunst *et al.* 1992; James *et al.* 1995; Nath 2008). The development of seed oils bearing a high percentage of erucic acid for industrial applications is a subject of research (Taylor *et al.* 1995; Kott *et al.* 1996) because it is a very excellent renewable raw material used in plastic film manufacture, in the synthesis of nylon and in the lubricant and emollient

Fig. 1. Cellular and molecular mechanism of biosynthesis of fatty acids in *Brassica napus* utilising ACP substrates in the plastid and subsequent reactions on CoA substrates occurring in the cytoplasm (adapted from Slabas *et al.* 2001; Nath 2008). ACCase: acetyl-CoA carboxylase, ACP: acyl carrier protein, FAS: fatty acid synthetase, CoASH: activated CoA.

industries (Leonard 1994; Sonntag 1995; Murphy 1996). A rapeseed line containing high proportions of erucic acid would significantly reduce the processing costs of itself for using as the industrial feed-stock.

Genetic (Kunst *et al.* 1992) and biochemical (Cassagne *et al.* 1994; Domergue *et al.* 1998) studies have suggested the existence of several elongase activities in *Brassica*. In the presence of labeled malonyl-CoA, VLCFAs could be synthesized from acyl-CoAs (acyl-CoA elongation) of the microsomal lipids; there is an absolute requirement for adenosine triphosphate (ATP) (ATP dependent elongation; Domergue *et al.* 1999). In the case of acyl-CoA elongation, Lessire *et al.* (1985) demonstrated that successive additions of C2 units to stearoyl-CoA (18:0-CoA) were responsible for the sequential synthesis of arachidonoyl-CoA (20:0-CoA), behenoyl-CoA (22:0-CoA) and lignoceroyl-CoA (24:0-CoA). Several studies suggested that two biochemical steps are critical for improvement of erucic acid production in rapeseed: membrane bound fatty-acid elongation and lysophosphatidic acid acyltransferase (LPAAT) activity leading to the biosynthesis of trierucin. The gene of an erucoyl-CoA preferring acyltransferase (*Ld-LPAAT*) has been successfully cloned from meadow-

foam (*Limnanthes douglasii* L.) and over expressed in rapeseed (Brown *et al.* 1995; Hanke *et al.* 1995; Lassner *et al.* 1995; Brough *et al.* 1996; Han *et al.* 2001). However, the overall proportions of 22:1 in the seed oil did not increase. However, the *Ld-LPAAT* transgene approach were successful in achieving high trierucin content in HEAR *B. napus* seed oil. Weier *et al.* (1997) suggested that the level of trierucin depends not only on the activity of the introduced *sn*-2-acyltransferase but also on other biosynthesis or incorporation steps. It is possible that the levels of erucoyl-CoA in the seed acyl-CoA pool may be too low to allow high levels of trierucin biosynthesis. If this is the case, then over-expression of genes regulating VLCFAs biosynthesis may be required to boost very long-chain acyl-CoA availability for incorporation into seed triacylglycerols (TAGs).

Attempts are being made by conventional breeding to increase the erucic acid content. This has so far resulted in breeding lines with up to 60% erucic and eicosenoic acid. These fatty acids are found in the *sn*-1 and the *sn*-3 position of the TAGs but are excluded from the *sn*-2 position (Fig. 2B). This *sn*-2 exclusion limits the erucic acid content theoretically to total of 66% (33% in each of the *sn*-1 and

Fig. 2. Schematic diagram of biosynthetic pathway of erucic acid in developing seeds of rapeseed of 2 steps elongation starting from 18:1-CoA pool (A) and probable 3 limiting factors for elongation of fatty acids from 18:1-CoA to 22:1 (B).

sn-2 position) and prevents the synthesis of trierucin (Cao *et al.* 1990; Frentzen 1993; Katavic *et al.* 2001; Nath *et al.* 2009a). The gene of an erucyl-CoA preferring *sn-2* acyltransferase from *Limnanthes douglasii* (*Ld-LPAAT*) has been successfully cloned and over expressed in rapeseed (Brown *et al.* 1995; Hanke *et al.* 1995; Lassner *et al.* 1995; Brough *et al.* 1996; Friedt and Lühs 1998; Han *et al.* 2001). However, the overall proportions of 22:1 in the seed oil did not increase (Han *et al.* 2001). In a next step, interest focused on the fatty acid elongation mechanism from oleic acid to eicosenoic acid and then to erucic acid (Fig. 2A). This elongation is the result of two cycles of a four-step mechanism, in which 18:1-CoA and 20:1-CoA are used as substrates. The first step, the initial condensation reaction of these fatty acids with malonyl-CoA is catalysed by the β -ketoacyl-CoA synthase (KCS; Fig. 1). It is believed that this initial reaction is the rate-limiting step (Cassagne *et al.* 1994). The *fae1* gene encoding the KCS, has been cloned from a range of plant species and has been over expressed under control of a seed specific promoter in HEAR. However, only very minor increases in 22:1 content were reported (Han *et al.* 2001; Katavic *et al.* 2001). Even in combination with the *Ld-LPAAT* no substantial increase in 22:1 content has been found (Han *et al.* 2001). It seems that there are other bottlenecks in the pathway, such as the pool of oleic acid available for elongation to eicosenoic and erucic acid (Fig. 2B). To study the question if availability of 18:1-CoA is limiting for 22:1 synthesis, Sasongko and Möllers (2005) crossed HEAR (cv. Maplus) to HOAR (Schierholt *et al.* 2001) to recombine the genes for high 22:1 with those for high 18:1 (i.e., low content of polyunsaturated fatty acids). However, the recombinant line High Erucic and Low Polyunsaturated fatty acid (HELP) did not show a significant change of 22:1 acid content, indicating that in this material the β -ketoacyl-CoA synthase activity may be limiting (Fig. 2B). To test this hypothesis, two separate approaches were followed involving two transgenic lines over expressing the *fae1* gene in combination with the *Ld-LPAAT* gene. Those lines were crossed to the line 6575-1 HELP. Segregating populations were studied for the inheritance of erucic acid content and other traits (Nath *et al.* 2009a; 2009b). From their study, they have been reported first to develop high erucic acid

rapeseed lines with more than 78% VLCFA including 6% eicosenoic acid in the seed oil. Those lines are now available for the researchers and growers, to whom are interested for HEAR, in the plant breeding department, Georg-August University, Germany.

***B. napus* as medicinal food plant**

The rapeseed oil is usually used for medicinal or food purposes. In Iranian traditional medicine, the root parts of Colza were used for the therapeutic purposes as diuretic, anti-scurvy, anti-inflammatory of bladder and anti-cold (Zargari 2001). The seeds are also used for treatment of hepatic and kidney disease. Rapeseeds are also used in the Eastern folk medicine as bronchial cathartic (Evans 1997; Zargari 2001). It has been reported that rapeseed oil diet contained 326 mg more plant sterols than the olive oil diet. Rapeseed oil decreases cholesterol absorption by 11%, and increased excretion of cholesterol, bile acids, and their sum as sterols by 9, 32, and 51% compared to olive oil. The serum cholesterol level could be decreased by 7% (Ellegård *et al.* 2005).

Saeidnia and Gohari (2012) reported that intake a diet with 10% rapeseed oil for 13 weeks increased systolic blood pressure, plasma levels of Na⁺ and lipids, and decreased the level of K⁺ compared to those in the soybean oil group. They suggested that an increase in body fluid *via* activation of Na⁺ pump or Na⁺, K⁺-ATPase and/or a blunt endothelium-dependent vasodilation by increased superoxide might be related to increase of blood pressure and hence, these would promote the peripheral vascular lesions and atherosclerotic vascular injury (Naito *et al.* 2000). Presently, rapeseed oil has also been used as component of bio-pesticides against fungi. The antifungal activity have been confirmed by linolenic, linoleic and oleic acids against plant pathogenic fungi; *Rhizoctonia solani*, *Pythium ultimum*, *Pyrenophora avenae* and *Crinipellis perniciosus* (Walters *et al.* 2004).

Rapeseed oil as biodiesel

Fossil fuels from coal, natural gas and petroleum (oil) have been the main sources of energy since the mid 1800s. In 2005, fossil energy is contributed about 79% of the total energy consumption in the EU countries and 81%

worldwide (EC 2007). However, there is large global threatened of shortage of fossil fuel due to continuous mining of this resources. Therefore, it is necessary to develop technology and alternative plants for renewable and green energy as well as ensure security in the ever expanding energy sector for our upcoming generations. The mining, processing, and combustion of fossil fuels also produce CO₂ and other gases which are contributing significantly to increase in concentration of atmospheric CO₂ and finally greenhouse effect (Houghton 2001). The first global attempt to search for solutions to these problems was the Kyoto protocol was introduced in 1997 by the United Nations. Thereafter, RES of bio-, water-, wind- and solar-energy is being promoted because they are neutral to the production of CO₂ and replaceable.

Biodiesel is a fuel defined as methyl esters (FAME) of long-chain fatty acids derived from renewable biological sources. Compatibility of biodiesel with mineral oils allows combining them in order to obtain a stable fuel mixture (Dworakowska *et al.* 2011). It can be used in the form of pure methyl esters of fatty acids as well as mixed with diesel fuel containing up to 30% of biological oil (Knothe *et al.* 1997; Fukuda *et al.* 2001; Tyson *et al.* 2004; Leung *et al.* 2010). Biofuels production has been raised rapidly in the last twenty years. Growing tendency can be expected in the future, if national governments of different countries continue in achievement of higher share of 'green energy'. Diversification and self-sufficiency in energy and environmental aspects should underpin national and international policy. Consumption of biofuels in the transport sector has been significantly increasing since 2000. Biofuels produced in the EU are mostly first-generation biofuels, mainly produced from agricultural raw materials. Vegetable oils belong to the renewable materials that are currently used also for the production of biodiesel. Production of biodiesel by trans-esterification of vegetable oils is currently the primary source of biodiesel. The most widely used input material for biodiesel production in the EU is rapeseed oil made from rapeseed, which accounts for the production of biodiesel in the range of 57% to 70% (Zentková and Cvengrošová 2013). Therefore, HEAR oil bring an idea to use as biodiesel for their characteristic properties with very high number of carbon atoms (C22:1)

and potentiality to produce more energy.

Serra *et al.* (2010) reported that rapeseed has become more 'independent' and gradually has been changing into a crop with significant industrial use (biodiesel). This claim is consistent with the results achieved in the paper. Change in the use of rapeseed has been occurred since 2003. In the year 2003, the EU Biofuels Directive (2003/30/EC) implemented and set targets for biofuels. Shares of rapeseed production for industrial purposes on the total rapeseed production until 2003 ranged from 15% to 40%. The rest of produced rapeseed was used for its primary purpose, which is food and feed processing.

In the EU, 77% of biodiesel production is produced from rapeseed oil (Fig. 3). Other plants oil involved in the production of biodiesel are soybean oil and palm oil, which are mostly imported into Europe. The shares of soybean oil and palm oil in the production of biodiesel in the EU are 9% and 12%, respectively. The rest of the production of biodiesel coming from waste materials is estimated to make 2% (USDA 2011). Zentková and Cvengrošová (2013) reported that the ever increasing demand of biodiesel leads to an increase in production and acreage of rapeseed as an input for biodiesel production. Production of biodiesel represents new opportunities for farmers for the placement of their production. The growth of biodiesel production also leads to changes in orientation of rapeseed consumption and utilization.

Fig. 3. Different kinds of plant oil used directly as biodiesel in the year 2010 in the EU. Source: USDA 2011, adopted from Zentková and Cvengrošová (2013).

CONCLUSIONS AND FUTURE OUTLOOK

Rapeseed oil plays a vital role in the world vegetable oil market. Fatty acids composition has been modified as per the end-user demand. However, to offer the best choice of healthy and acceptable food to the consumer a coordination of plant breeding, food processing and nutrition science is required. Here, the nutritional aspects of modified fatty acid rapeseed oil have not reviewed in detail. Although the use of HOLLI oil in consumption by the consumer should have high priority from the standpoint of public health. To pursue this aim confusion of the consumer must be avoided by creating a new name and a new brand for HOLLI varieties. However, there is vast scope in future outlook for modifying the fatty acid composition of rapeseed oil. It is possible to produce rapeseed oil with satisfactory amount of lauric acid and myrestic acid for using as vegetable margarine through over-expression of genes from coconut.

Furthermore, during oilseed development, significant amounts of sugar provided by photosynthesis are converted to storage TAGs by the developing seed in rapeseed. In oilseed plants, at which the pathway commits the fixed carbon to lipid biosynthesis is the conversion of acetyl-CoA. The temporal distribution of ATP-citrate lyase (ACL) activity in developing seeds of rapeseed closely paralleled both that of acetyl-CoA carboxylase (ACCase) in the cytosol and the overall rate of lipid biosynthesis (Fig. 4; Fatland *et al.* 2005). Therefore, over-expression of ACL gene will help to produce more acetyl-CoA in the cytosol from mitochondria, which might have possibility to produce necessary malonyl-CoA for long chain fatty acid biosynthesis. In combination with transgenic and mutation to stop competition of acetyl-CoA pool for synthesis of plant secondary metabolites (Fig. 4) could bring further breakthrough in develop higher amount of long chain fatty acid rapeseed genotypes beyond the current level.

Fig. 4. Scheme of putative fatty acids and different metabolites (isoprenoids, malonate, flavonoids, etc.) biosynthetic pathways in plants (following Fatland *et al.* 2005; Nath 2008). ACL is depicted together with a postulated citrate cycle that would provide citrate from the mitochondria. In the cytosol, acetyl-CoA can be carboxylated by ACCase to form malonyl-CoA and hence converted to long chain fatty acids (22:1).

FAS: fatty acid synthase, TCA: tricarboxylic acid cycle, leu: leucine, ACL: ATP-citrate lyase, ACC: acetyl-CoA carboxylase.

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