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SYNTHETIC METHODS TO OBTAIN CONJUGATED LINOLEIC ACIDS (CLAS) BY CATALYSIS – A REVIEW

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The addition of synthetic CLA is a possible way in order to compose foods enriched with conjugated linoleic acids (CLAs). The most environmental friendly methods for CLA synthesis are based on microbial biosynthesis. With homogeneous catalysis using organometallic catalysts (Ru and Rh complexes) high (approximately 80%) yields were obtained with high selectivity related to bioactive isomers. The heterogeneous catalysis has the advantage that at the end of the reaction there is no need for a supplementary separation operation or recycling of the catalyst. In heterogeneous process, the maximum yield may even be higher than 90% and the selectivity remains quite high as the reaction conditions are optimized. The substrates for obtaining CLAs are, in general, linoleic acid or alkyl linoleates and the catalysis is acidic. The yield and the selectivity depend on the strength and the type of acidic sites, as well as on the size distribution of the particles. Beside the existing catalytic methods, a photocatalytic process with UV and visible light irradiation with iodine promoter can be applied.

Keywords: conjugated linoleic acid, CLA, homogeneous catalysis, heterogeneous catalysis, photocatalytic process

Recently many new health-benefit effects of the conjugated linoleic acids (CLAs) were discovered. They decrease the body fat quantity and increase muscle mass, possess antiinflammatory and cancer preventive effects, exert beneficial effects on the skeletal system, act as immunostimulants, and decrease the probability of asthma occurrence (Ha et al., 1989; Pariza et al., 2001).

Theoretically 56 isomers exist, however, few of them (*cis*(*c*)-*9*,*trans*(*t*)-*11*; *t-10*,*c-12*- and *t-9*,*t-11* CLA) have been shown to have significant biologic activity. The bioactivity of the *c*-9,*t-11* CLA isomer (Fig. 1) was discovered two decades ago (Ha et al., 1989) and this isomer is predominant in the natural products used as foods. The main foods rich in CLA are milk, dairy products, meat and fat of the ruminants, as this isomer is produced by the bacteria of the rumen microflora.

Nowadays, CLAs are obtained for industrial purpose from vegetable oils that are processed at high temperature (Philippaerts et al., 2011). The basic method in the industrial approach is the isomerisation of linoleic acid (LA) with basic catalysis by which the amounts of the two formed isomers (*c-9,t-11* and *t-10,c-12* CLA) are almost equal, and the yield is quite high. This synthetic method cannot be applied for the direct transformation of vegetable oils into CLA-rich triacylglycerols, because ester bonds of triacylglycerols hydrolyze to free fatty acids (or salts) and glycerol.

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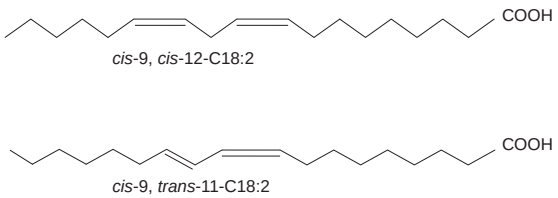

Fig. 1. The structure of linoleic acid (*c-9,c-12* C18:2) and *c-9,t-11* conjugated linoleic acid isomer

The synthetic methods for obtaining CLA, in general, can be categorized in three groups: microbiologic, catalytic (both homogeneous and heterogeneous), and photocatalytic methods (Philippaerts et al., 2011). In this review article we wish to present the different synthetic methods developed for the obtaining of CLAs.

Microbiologic methods for obtaining CLAs

Some microorganisms contain specific isomerase enzymes that are able to transform linoleic acid in CLAs. This isomerisation occurs in the rumen of the ruminant mammals, where the main CLA isomer formed is the *c-9,t-11* CLA (Kim et al., 2000). The bacteria do not synthesize the isomer *c-9,t-11* when the linoleic acid concentration is high, due to substrate inhibition. Some microorganisms are known to possess both linoleic acid isomerase and CLA reductase activity, and these are tolerant for linoleic acid. Other microorganisms produce *c-9,t-11* isomer only in small quantity, the main product in this case is *t-10,c-12* CLA (Kim et al., 2000; Fukuda et al., 2005).

Some of the microorganisms living in the human intestinal tract are also able to produce the *c-9,t-11* isomer. The majority of the studied species produce mainly *c-9,t-11* CLA, but small amounts of *t-9,t-11* and *c-10,t-12* isomers are also formed. Some researchers attempted to produce CLAs from LA in different cell cultures (Coakley et al., 2003). The cell cultures could be used only once, however, some *Lactobacillus* cultures were able to produce CLA in five consecutive cycles. The reusability was achieved by the immobilization of *Lactobacillus* cells, but their activity was quite low (Ogawa et al., 2001).

Beside the isomerisation of LA, some bacteria have the ability to transform ricinoleic acid to CLA (Coakley et al., 2003). When the initial LA concentration is very low, the proportion of *t-9,t-11* CLA may reach the value of 97% after a long reaction time due to the thermodynamic control of the isomerisation (Kishino et al., 2002). Salamon and co-workers (2009) studied the seasonal variation of the fatty acid composition and the CLA content of cows' milk, and the effect of milk processing on the CLA content of the different dairy products. During the experiments, with the aim to increase the CLA content of fermented dairy products, sunflower oil (with high LA content) was added to raw milk. The optimal concentration of the added LA was determined, and the CLA productivity of strains and mixed cultures commercially utilized for industrial production of fermented dairy products was studied.

CLA production using cell cultures or enzyme extracts is highly selective and environmentally friendly in comparison with alkaline methods (Salamon et al., 2012).

The *t-10,c-12* CLA isomer was produced by oleaginous yeast *Yarrowia lipolytica*, in which the linoleic acid isomerase gene from propionibacterium acnes was expressed (ZHANG et al., 2012). Cell-free forms of bacterial linoleic acid isomerases from *Lactobacillus acidophilus* (CCRC14079) and *Propionibacterium freudenreichii* ssp. *shermanii* (CCRC11076) proved to be capable of the production of CLA from linoleic acid (LIN et al., 2002). The multi-component enzyme system of linoleic acid isomerase in *Lactobacillus plantarum* was thoroughly investigated by KISHINO and co-workers (2011) and the immobilization of this enzyme from *Lactobacillus delbrueckii* subsp. *bulgaricus* 1.1480 was accomplished by YOU and co-workers (2011). The main problem with the biological transformation of CLA used to be the low yields, but nowadays, high yields (between 80 and 100%) were achieved with *Lactobacillus reuteri* (MILLIS et al., 2011). Beside their utilization for production of pure CLA at large scale, microorganisms also play an important role in increasing the CLA-content of foods. The CLA content of fermented dairy products or cheeses could be raised by using microbial strains with high CLA-producing potential (PHILIPPAERTS et al., 2011). The enhancement of CLA content in food with strains that are capable of the formation of CLA from linoleic acid was achieved recently by ANDRADE and co-workers (2012).

Synthesis of CLAs with metal catalysts

In metal catalysed synthesis of CLA both homogeneous and heterogeneous catalytic methods are developed, but these methods have not been implemented in practice yet (PHILIPPAERTS et al., 2011).

Synthesis of CLAs with homogeneous organometallic catalysts

For the obtaining of CLAs and CLA-containing oils chromium, platinum, ruthenium, and rhodium complexes were tested. The highest productivity with such catalysts was 97%. At mild reaction temperature triglycerides, such as soybean or safflower oils, have been transformed in a single step to their conjugated derivatives (LAROCK et al., 2001).

Homogeneous Ru and Rh complexes used as catalysts are quite active, but their productivity is one order of magnitude lower than in case of the classic alkaline process. When three types of rhodium-complex catalysts ($[RhCl(C_8H_{14})_2]_2$, $RhCl(PPh_3)_3$ and $RhCl_3$ $3H_2O$) were used under the same reaction conditions in ethanolic solution, in the first two cases the *c-t* and *t-c* isomers, in the third case the *t-t* isomer were dominant. Similar to the isomerisation process performed with strong alkaline catalysts, it is generally observed that with the raising of the temperature, the ratio of the *t-t* isomers increases, owing to their higher thermodynamic stability (LAROCK et al., 2001). High yield can be achieved from vegetable oil with high linoleic acid content by alkali isomerisation in apolar solvents (DIANÓCZKI et al., 2010).

When CLAs are destined to be used as food additives, the choice of the solvent is very important, since solvent traces may remain in the product. Another difficulty is the removal of the toxic and soluble metal complexes from the product mixture, and the reuse of these very expensive catalysts (CONSORTI el al., 2009).

The mechanism of isomerisation in presence of organometallic complexes is somewhat different from the alkali-catalyzed reaction. The mechanism, in brief, is a consecutive hydrogen addition-elimination, formally similar with the mechanism of heterogeneous isomerisation, opening the possibility towards a competitive hydrogenation with the formation of different isomers (FRANKEL, 1970).

Synthesis of CLAs with heterogeneous catalysis

Most heterogeneous catalysts contain transition metals for the reaction with conjugating double bonds in polyunsaturated fatty acids. The bonding on the substrate on metal surfaces includes the addition-elimination steps (KREICH & CLAUS, 2005). This mechanism consists of three steps. The first step is the chemisorption of molecular hydrogen; the second step is the chemisorption of the double bond of the substrate; the third step is the migration of a hydrogen atom on the catalyst surface toward one of the carbon atoms of the adsorbed substrate complex.

When the hydrogen load of the catalytic surface is high, a second hydrogen atom is added to the half-hydrogenated intermediate, leading to the double bond saturation and this way mono-unsaturated fatty acids are formed. KREICH and CLAUS (2005) performed the isomerisation of the LA with silver catalyst in presence of hydrogen. At first, they achieved good results with ruthenium catalysts (Ru/C, Ru/Al_2O_3), afterward, they developed a silver mediated catalyst in presence of hydrogen for the obtaining of CLA.

Good results were achieved in direct synthesis of CLAs with heterogeneous silver catalysts in the constant presence of hydrogen: 90% conversion was achieved after 90 minutes over Ag/SiO_2, the selectivity was approx. 60–67% towards CLAs. The linoleic acid conversion increased with the reaction temperature, while the physiologically important *c-9,t-11* and *t-10,c-12* isomers always remained the main components of the products. Similar results were found using supported gold catalysts in the presence of hydrogen at 165 °C in a batch reactor (BAUER et al., 2009).

The isomerisation of linoleic acid or methyl linoleate toward CLAs by heterogeneous catalysis is made through hydrogenated intermediates. The proposed reaction mechanism has six different steps: migration of one double bond of linoleic acid; positional and geometric isomerisation of CLA; hydrogenation of one double bond of linoleic acid; positional and geometric isomerisation and hydrogenation of the double bond of a mono-unsaturated fatty acid (PHILIPPAERTS et al., 2011).

CLA synthesis with biphasic isomerisation with transition metals

Biphasic catalytic systems have the advantage of high surface area for the reaction as the two immiscible phases of substrate and catalyst dissolved in a suitable solvent are agitated and of an easy recovery of catalyst when the two phases are separated. This method has also been used for the conjugation of different plant oils, among them sunflower oil (QUIRINO & LAROCK, 2012).

Obtaining of CLAs from linoleic acid, alkyl linoleates and triglycerides

By utilizing the main advantage of heterogeneous catalysts, i.e., the easy catalyst/product separation, this type of catalyst can be used in a continuous process in a fixed-bed reactor or a continuous stirred tank reactor (Bauer et al., 2009; Philippaerts et al., 2011). For the isomerisation of alkyl linoleates to obtain CLAs, several metals (Ni, Ru, Rh) on various supports were used (Deshpande et al., 1985).

Different metal catalysts (Ru, Ni, Pd, Pt, Rh, Ir, Os, and bimetallic Pt-Rh) on various supports (carbon, γ-Al_2O_3, SiO_2/Al_2O_3, and on zeolites) have been screened more systematically (Bernas et al., 2004) for the isomerisation of linoleic acid to CLAs. It was shown that pretreatment of the metal catalysts with hydrogen is not required for conjugation, but the activity of the catalyst is drastically increased when the catalyst is brought into contact with H_2 at elevated temperature prior to reaction.

Philippaerts and co-workers (2011) developed a novel catalytic method for obtaining the CLAs, using highly dispersed ruthenium-dioxide on zeolite support under hydrogen-free conditions. The main advantage of this catalyst is that no hydrogen pre-treatment or addition of hydrogen donors are required, and the obtained productivity is the industrially relevant 0.7 g min^{-1} of CLAs.

Obtaining CLAs by photochemical methods

An alternative way besides microbial methods and metal catalysis is photoisomerisation. Japanese scientists (Seki et al., 1998) obtained CLA methyl esters with high yield (80%) using iodine as photocatalyst and with intense visible light irradiation. Another research group extended the method to the production of CLA-rich vegetable oil by direct isomerisation of soybean oil by UV-light irradiation of solventless substrate, with iodine sensitizer. Under irradiation, the I_2 possesses radical forming potential (Gangidi & Proctor, 2004). By optimization of the process and performing the reaction in stirred batch photoreactor (Jain & Proctor, 2006), a production of 240 mg CLA/g substrate was obtained.

Recently the isomerisation of soybean oil was performed in a pilot scale laminar flow photoreactor, irradiated with a UV-Vis lamp yielding 220 mg of CLA/g soy oil. The main isomer (approx. 80%) was the *t-t* CLA (Jain et al., 2008). It is important to emphasize that due to the radical nature of photocatalytic reaction, the isomerisation needs to be carried out in inert atmosphere to ensure high chemoselectivity, other ways the iodine radicals may be inactivated by other compounds (Philippaerts et al., 2011).

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