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Synthetic methods for obtaining conjugated linoleic acids (CLA) by catalysis

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Abstract. Vegetable oils with high CLA content possess high nutritional value. The amount of the bioactive CLA isomers (cis-9,trans-11 and trans-10,cis-12) is very important from that respect. Lots of methods were developed for CLA synthesis. The most environmental friendly methods are based on microbial biosynthesis, but the isomerization yield is very low. Due to this reason, the microbial CLA synthesis is not competitive with the classical (chemical) alkaline isomerization process. With homogeneous catalysis using organometallic catalysts (Ru and Rh complexes), much higher (approximately 80%) yields were obtained with high selectivity related to bioactive isomers.

Key words and phrases: conjugated linoleic acid, CLA, homogeneous catalysis, heterogeneous catalysis, photocatalytic process, hydrotalcite.

The heterogeneous catalysis has the advantage that there is no need for a supplementary separation operation or the recycling of the catalyst at the end of the reaction. In a 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. Yield and selectivity depend on the strength and type of acidic sites as well as on the size distribution of the particles. Based on theoretical considerations, we propose a new hydrotalcite-type alkaline heterogeneous catalyst that may combine the high activity and/or stereoselectivity of the homogeneous alkaline catalyst and the well-known advantages of heterogeneous reaction systems. Besides the existing catalytic methods, a photocatalytic process with UV and visible light irradiation with iodine promoter can be applied, but in this case only highly purified oil substrates are suitable to obtain high CLAs yields.

1 Introduction

Traditionally conjugated linoleic acids (CLAs) were used as additives for oil-based dyes due to the increased reactivity of conjugated double bonds compared to non-conjugated ones, shortening this way the hardening time. Also, the CLAs are precursors (as monomers) for obtaining different biopolymers by radicalic or cationic copolymerization. Recently (*Ha et al.*, 1987, 1989), many new health-benefit effects of the CLAs were discovered. They decrease the body fat quantity and increase muscle mass, possess anti-inflammatory and cancer-preventive effects, exert beneficial effects on the skeletal system, act as immunostimulants and decrease the probability of asthma occurrence.

Theoretically, there are 56 isomers; however, few of them (cis-9,trans-11; trans-10,cis-12 and trans-9,trans-11-CLA) have shown significant biological activity. The bioactivity of the trans-9,trans-11-CLA isomer was discovered two decades ago (*Ha et al.*, 1987,1989) and in some cases its effect is stronger than that of the other isomers.

The predominant isomer (approx. 90%) in the natural products used as foods is the cis-9,trans-11-CLA. The main foods containing this isomer 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 purposes from vegetable oils that are processed at high temperature. The basic method in the industrial approach is the isomerization of linoleic acid (LA) with basic catalysis, using ethylene glycol as solvent and potassium hydroxide or potassium alkoxide as catalyst. The separation of the catalyst at the end of the process is made by neutralization by orto-phosphoric acid. Through basic catalysis, the amount of the two formed isomers (cis-9,trans-11 and trans-10,cis-12-CLA) is almost equal (selectivity $\approx$ 50%) and the yield is quite high (over 80%). This synthetic method cannot be applied to the direct transformation of vegetable oils into CLA-rich triacylglycerols because ester bonds of triacylglycerols hydrolyze resulting free fatty acids (or salts) and glycerine.

The homogeneous catalytic methods using transition metallic complexes were intensively studied and almost quantitative transformation of linoleic acid into CLAs was reported. Despite the great selectivity and conversion, the main problem in this case is the separation of the catalyst from the products.

By using heterogeneous catalytic method to obtain CLAs, the separation problem is avoided, the catalyst can be separated by sedimentation or filtration (at laboratory scale), or even the separation is not necessary (at industrial scale) when fixed bed catalysts are used. The heterogenic catalysis may be a very attractive and efficient way for industrial processing of CLAs. The isomerization of linoleic acid or methyl linoleate by heterogenic catalysis was described, but the conversions were extremely low. The conclusion was that to achieve an acceptable level of conversion, the catalytic active metallic centres need to be partially saturated by absorption of high amount of hydrogen; however, this way, the selectivity was seriously affected by the appearance of a large amount of saturated by-products such as stearic and oleic acids. To avoid the formation of unwanted by-products, a two-step catalytic method was developed. In the first step, a ruthenium-based catalyst was saturated by hydrogen and then, in the second step, the linoleic acid was isomerized in nitrogen atmosphere. In this way, selectivity rose considerably, but still a great amount of hydrogenated product was formed. Using Ru/C catalyst and preactivation of the catalyst by hydrogen absorption (reaction conditions: temperature 120 °C; duration 6 hours), the amount of conjugated products was 53% and the amount of the hydrogenated products was 24%. Without preactivation, catalytic activity decreased significantly. When the carbon support was changed to zeolite or aluminate, the productivity of CLAs formation decreased comparatively to Ru/C, and the formation of the by-product acetylacetonate and $RuCl_3$ was observed.

As heterogeneous catalysts, silica-supported gold and silver catalyst was used in the presence of hydrogen. These metallic active centres possess low hydrogen-binding energy, and therefore the selectivity for hydrogenation (the secondary reaction with unwanted by-product formation) is quite low. In the light of the above, there may be a great opportunity for the development of selective and active heterogeneous catalysts for the production of the main physiological active CLAs': cis-9,trans-11, trans-10,cis-12 and trans-9,trans-11-CLA isomers (*Philippaerts et al.*, 2011a). Simultaneously, it can be concluded that the homogeneous alkaline catalysis at high temperature has many drawbacks. Firstly, the catalyst is in solution and must be removed by neutralization at the end of the process, which is disadvantageous from environmental and economic point of view. The second drawback is that the product is a mixture of many different CLA isomers, not only of the health-benefit ones.

As long as the CLAs are used only for industrial purposes (in paints and inks to enhance their hardening properties), the multitude of isomers does not create any problem, but the use of the CLA isomer mixture is unacceptable as a component in functional foods. Finally, the application of CLA-enriched triacylglycerols would be more advantageous in food than free CLA fatty acids, but they cannot be obtained directly from vegetable oils using homogeneous base catalysis because of the concomitant saponification and transesterification reactions. Therefore, any new methods and procedures increasing the yield of production and able to enhance the purity of CLA mixtures are very welcome.

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

Microbiologic methods for obtaining CLAs

The best methods for the obtaining of CLAs from environmental point of view are the microbial biosyntheses. Some microorganisms contain specific isomerase enzymes that are able to transform linoleic acid into CLAs. This isomerization occurs in the rumen of the ruminant mammals, where the main CLA isomer formed is the cis-9,trans-11 CLA (*Kim et al.*, 2002). Unfortunately, these bacterial species cannot be utilized for the industrial production of the CLAs because at the end of the biotransformation the intermediate product, that is CLA, is transformed into saturated products such as stearic acid. Moreover, bacteria do not synthetize the isomer cis-9,trans-11 until the linoleic acid concentration is high, producing the inhibition of biohydrogenation. Some microorganisms are known for possessing both linoleic acid isomerase and CLA reductase activity, and these are tolerant for linoleic acid. Other microorganisms produce the cis-9,trans-11 isomer only in small quantity; the main product in this case is the trans-10,cis-12 CLA (*Kemp*, 1984;

Kim et al., 2000; *Fukuda et al.*, 2005).

Some of the microorganisms living in the human intestinal tract are also able to produce the cis-9,trans-11 isomer. There are great variations among the tested microflora, and some of the species are able to produce even vaccenic acid. Also important differences exist between the cell lines, producing many different final products (*Coakley et al.*, 2003).

The majority of the rumen microorganisms are obligate or facultative anaerobes, that is, in a linoleic acid-rich medium, they are able to produce CLA isomers. The composition of the CLA mixture is influenced by the properties of the media and the concentration of the linoleic acid. Given the high isomeric specificity, the bacteria have the ability to produce only a minimal quantity of CLAs as the free linoleic acid is a growth-inhibitor for this type of bacterial strains. The majority of the studied species produce mainly the cis-9,trans-11 CLA, but a small amount of trans-9,trans-11 and cis-10,trans-12 isomers is also formed. Some researchers attempted to produce CLAs from LA in different cell cultures. In these cases, the initial LA concentration was set higher than in usual bacterial media to obtain the acceptable CLA concentration. In general, cell cultures could be used only once; however, some lactobacillus cultures were able to produce CLA in five consecutive cycles. Reusability was achieved by the immobilization of lactobacillus cells, but their activity was quite low, the reaction rate was 0.003 g/(L·min). The final concentration of 175 mg/L for CLA was obtained in an hour with 500 mg/L initial concentration of LA (*Ogawa et al.*, 2001). The mechanism of the bacterial biosynthesis of CLA from LA is described below. The first step of the consecutive reactions is water addition to the linoleic acid, and 10-hydroxi-12-octadecenoic acid is formed. In the second step, CLA is formed by the water loss of the intermediate product. This mechanism of CLA formation is absolutely identical with those of the acid-catalysed synthesis.

Beneath the isomerization of LA, the bacteria have the ability to transform the ricinoleic acid into CLA, the product containing preponderantly cis-9,trans-11 and trans-9,trans-11 isomers; selectivity depends on the reaction conditions. In the presence of the reaction mixture of different substances (serine, glucose, sodium chloride, or silver nitrate), the amount of trans-9,trans-11 isomer decreased, and thus the proportion of cis-9,trans-11 isomer may reach even the proportion of 75%. If the initial LA concentration is very low, the proportion of trans-9,trans-11 CLA may reach the value of 97% after a long reaction time due to the thermodynamic control of the isomerization (*Kishino et al.*, 2002). *Salamon et al.* (2006, 2009a) studied the seasonal variation of the fatty acid composition, the CLA content of the cows' milk and the effect of milk processing on the CLA content of the different dairy products (*Salamon et al.*, 2007a). Particular attention was paid to the effect of microwave processing on isomerization of unsaturated fatty acids (*Salamon et al.*, 2007b, 2009b). The experiment was focused on cis-9,trans-11 CLA since this is the most abundant isomer present in milk and in dairy products in an amount of approx. 80%. 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 (*Salamon et al.*, 2009c, 2009d, 2009e). 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 (*Salamon et al.*, 2009d, 2012).

As a conclusion, the CLA production using microbial culture is stereoselective and environmentally friendly in comparison with alkaline methods. Unfortunately, to obtain CLAs by microbiological method is possible only from free LA. For the production of CLA-rich triglycerides, a supplementary reaction step is needed, as triglycerides possess higher bioavailability than free fatty acids. The main problem with the microbiological methods is the low conversion of the reactant, and from that reason the microbiological methods are not competitive with the classical alkaline izomerization method. The best yield obtained microbiologically is 6 mg CLA per minute in a litre of reaction media (*Salamon et al.*, 2009c, 2012), about 500 times lower than in the case of the homogeneous alkaline process. Otherwise, it is obvious that microorganisms have had an important role in obtaining CLA-rich food products instead of the production of pure CLA at large scale. For example, the CLA content of the milk and that of the dairy products could be influenced by changing the fodder composition of the ruminants. The CLA composition of the fermented dairy products or cheeses could be raised by using microbial strains with high CLA-producing potential. Despite of a lot of accumulated knowledge, more studies are needed to elucidate whether the CLA amount produced by lactobacillus or propionobacteria strains during the fermentation process for the obtaining of yoghurt and the ripening of cheeses is sufficient to produce physiological benefit effects in the human organism (*Sieber & Collomb*, 2004; *Philippaert et al.*, 2011a; Salamon et al., 2009c, 2012).

Synthesis of CLAs with metal catalysts

In the metal-catalysed synthesis of CLA, both homogeneous and heterogeneous catalytic methods are developed, but these methods have not been implemented in practice yet. Many researches were focused on the isomerization of linoleic acid or linoleic acid-rich oil using organometallic complexes or supported metal particles as catalyst (*Philippaerts et al.*, 2011a).

Synthesis of CLAs with homogeneous organometallic catalysts

For the obtaining of CLAs and CLA-containing oils, chromium, platinium, ruthenium and rhodium complexes were tested. The highest productivity with such catalysts was 97%. In this case, the substrate for obtaining CLA was methyl linolate (reaction conditions: reaction time 24 hours; temperature 60 °C, ethanol solvent) with ruthenium complex (0.1 mol% $[RhCl(C_8H_{14})_2]$) in the presence of 0.8 mol% $SnCl_2.2H_2O$ and 0.4 mol% $(p\text{-}CH_3C_6H_4)_3P$. At this mild reaction temperature, the substrate is not limited to fatty acids or their alkyl esters like in the base-catalysed or microbial isomerization processes, but triglycerides such as soybean or safflower oils have also been transformed in a single step into their conjugated derivatives. An amount of 100 g soybean oil in 300 mL ethanol solvent was almost completely converted (with selectivity for 95%) into conjugated products within one day, corresponding to a productivity of 0.11 g CLA/(L·min) (*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 the case of the classic alkaline process. The catalytic turnover is about 20 substrate molecules transformed per metal atom in one hour. Depending on the combination of metal complexes and different solvents, the product is a mixture of CLA isomers with different composition. For example, when three types of rhodium-complex catalysts ($[RhCl(C_8H_{14})_2]_2$, $RhCl(PPh_3)_3$ and $RhCl_3\ 3H_2O$) are used under same reaction conditions in ethanolic solution, in the first two cases the cis-trans and trans-cis isomers are dominant, while in the second case the trans-trans isomer is the dominant one. Thus, in methanol solvent, a higher selectivity towards the desirable cis-9,trans-11 and trans-10,cis-12 CLA isomers is obtained. From methyl linoleate substrate, after 24 hrs of reaction time, the obtained CLA yield was 83% and the cumulated selectivity toward cis-9,trans-11 and trans-10,cis-12 isomers was 79%. Using soybean oil as substrate (under the same reaction conditions), the conversion was almost quantitative, with the selectivity toward the desired isomers being 75%. Similar to the isomerization process performed with strong alkaline catalysts, it is generally observed that with the raising of the temperature, the amount of the trans,trans isomers increases due to their higher thermodynamic stability (*Andjelkovic et al.*, 2006; *Larock et al.*, 2001). Despite their obvious advantages, homogeneous metal complexes are not used at industrial scale due to their very high production costs. The price of rhodium is approximately ten times the price of ruthenium, while platinum is two to five times more expensive than ruthenium.

When CLAs are destined to use as food additives, the choice of the solvent is very important since there may remain solvent traces in the product. Practically, only GRAS (Generally Regarded as Safe) solvents are accepted since the total separation of the solvent from the product is very complicated. Another difficulty is the removal of the toxic and soluble metal complexes from the product mixture and the reuse of these very expensive catalysts. To bypass the presented drawbacks for the isomerization of soybean oil and methyl linoleate, a biphasic catalytic system with ionic liquids has been suggested recently. At the end of the reaction, the ionic liquid phase containing the metal complex can be easily separated from the product, preventing the CLA from metal contamination (*Consorti el al.*, 2009).

The mechanism of isomerization in presence of organometallic complexes is somewhat different from the alkaline-catalysed reaction. The key step is the complexation of the fatty acid olefinic double bond by transition-metal catalytic centre. The next step is hydrogen atom abstraction nearby a double bond, followed by a hydrogen addition reaction. The mechanism, in brief, is a consecutive hydrogen addition-elimination, formally similar with the mechanism of heterogeneous isomerization, 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 & Klaus*, 2005a, 2005b). This mechanism consists of three steps. The first step is the chemisorption of hydrogen on the catalyst surface and its dissociation into two hydrogen atoms; the second step is the chemisorption of the double bond of the substrate (linoleic acid or methyl linoleate) on the catalyst surface, concomitant with the opening of the double bond and the formation of two new bonds with the catalyst, which leads to the formation of a stronger adsorbed complex. 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, forming a semi-hydrogenated intermediate. If the hydrogen load on the catalytic surface is rather low, a hydrogen adjacent to the surface-bonded carbon atom is eliminated and a double bonded adsorbed species is formed. This complex at the surface of the catalyst, however, may adopt another configuration, which leads, after desorption, to the formation of different geometrical and positional isomers of linoleic acid.

When the hydrogen load of the catalytic surface is high, a second hydrogen atom is added to the half-hydrogenated intermediate, leading to double-bond saturation, and this way mono-unsaturated fatty acids are formed. This mechanism can be repeated with the primary intermediates (CLA isomers, non-conjugated linoleic acid isomers and mono-unsaturated fatty acids), and hence other CLA isomers, non-conjugated linoleic acid isomers and stearic acid may be formed. *Kreich* and *Claus* (2005a, 2005b) performed the isomerization of the LA with silver catalyst in presence of hydrogen. The Ag/SiO_2 catalyst was synthesized by incipient wetness impregnation of silver lactate on SiO_2, followed by drying at 80 °C and reduced at 325 °C under hydrogen flow – it resulted in an average Ag particle size of 14 nm (Ag content of catalyst 7.7 wt%). At first, they achieved good results with ruthenium catalysts (Ru/C, Ru/Al_2O_3) – in agreement with literature reports. The use of a typical hydrogenation metal catalyst as nickel promised no improvement (*Bernas et al.*, 2003). Afterwards, they developed the silver-mediated catalyst in presence of hydrogen for the obtaining of CLA. In agreement with literature data, silver is the metal that possesses the lowest hydrogen binding energy, silver crystals bond the hydrogen very weak. In the silver catalysed hydrogenation of the polyunsaturated carbon-carbon double bonds, the efficiency is very low in comparison with other metallic catalysts. Because of this behaviour of the silver, the formation of the oleic and stearic acids is hindered. The isomerization with silver ion catalysis was performed under similar conditions as in the case of ruthenium-doped catalysts, namely, in nitrogen atmosphere after catalyst preactivation with hydrogen. Under these conditions, as conversion was not observed as expected, surprisingly good results were achieved in direct synthesis of CLAs over heterogeneous silver catalysts and in the constant presence of hydrogen: the 90% conversion was achieved after 90 minutes over Ag/SiO_2, the selectivity was approx. 60-67% towards CLAs. It was found that the particle size did not exhibit a noticeable influence on the catalytic process. High amount of the trans-9,trans-11 isomer was obtained at high conversions. The linoleic acid conversion increased with the reaction temperature, while the physiologically important cis-9,trans-11- and trans-10,cis-12 isomers remained always the main components of the products. Furthermore, it was observed that the selectivity towards the undesired saturated hydrogenation products (oleic acid and stearic acid) decreased with decreasing substrate/catalyst mass ratio. The transformation of linoleic acid was also carried out without the preactivation of the Ag catalyst with hydrogen and the same degrees of conversion and selectivity were obtained as those with preactivation. Similar results were found using supported gold catalysts (Au on Al_2O_3, Fe_2O_3, CeO_2, MnO_2, TiO_2, ZrO_2, activated carbon, silicalite TS-1) in the presence of hydrogen at 165 °C in a batch reactor. The best results were obtained using a catalyst with 2 wt% Au on TS-1, which exhibits high selectivity (78%) towards CLA (*Bauer et al.*, 2009).

The isomerization of linoleic acid or methyl linoleate toward CLAs by heterogeneous catalysis is made through hydrogenated intermediates. The proposed reaction mechanism has six different steps (*Bernas et al.*, 2004): migration of one double bond of linoleic acid, which results in the formation of CLA; positional and geometric isomerization of CLA; hydrogenation of one double bond of linoleic to mono-unsaturated fatty acid; positional and geometric isomerization of mono-unsaturated fatty acids; hydrogenation of the double bond of a mono-unsaturated fatty acid to stearic acid (*Philippaerts et al.*, 2011b). During the heterogeneous catalytic process, the isomerization and hydrogenation reactions are competitive. Since the isomerization is reversible, the process eventually leads to the formation of the thermodynamically most stable trans,trans CLA isomers. The hydrogenation can be considered as an irreversible consecutive reaction that lowers the CLA yield. In the majority of the heterogeneous catalytic isomerization systems, hydrogen is needed for the forming of the half-hydrogenated intermediates, which will finally transform into final products: the CLAs. On the other hand, the elevated level of hydrogen will lead to the formation of unwanted hydrogenated by-products. Hence, the direct production of CLAs by heterogeneous catalysts is a difficult and complicated process. The activity and selectivity for the isomerization are dependent on the surface structure and the hydrogen adsorption capacity of the metal-dopped catalyst. Metals with a high hydrogen binding capacity (such as palladium) show a high activity and selectivity for the double-bond hydrogenation of LA, while other metals (such as silver and gold) with low hydrogen binding activity show a low activity and selectivity, hence the migration process of the carbon-carbon double bonds. The high activity of ruthenium for double bond isomerization is explained by the fact that the ruthenium contains free d-orbitals, with ability to interact with the π-bonds of linoleic acid and being capable of activating the adjacent C-H bond. These steps are necessary for catalytically migrating double bonds (*Bernas et al.*, 2004; *Bernas & Murzin*, 2003, 2005).

The acid and base active sites on the surface of the catalysts also interact with the carbon-carbon double bonds, and facilitate the isomerization. The Brønsted acids may form carbenium ions. Then, the subsequent stabilization of the ionic intermediate by a proton expulsion occurs, resulting the migration of the double bond, yielding to an initial cis/trans product ratio of around 1:1. Conversion of linoleic acid was strongly affected by the amount and strength of the acidity. Higher amounts of weak-moderate acidic sites (both, Brønsted and Lewis type) favour the conversion of LA over conjugated CLAs and increase the selectivity toward the main isomer of interest, cis9-trans11 CLA. There may exist a synergic effect between Brønsted and Lewis centres. When the acid centres are stronger, the catalytic activity and selectivity decrease faster in time (*Cardó et al.*, 2012). On active basic sites, carbanions are formed, yielding mainly to cis,cis CLA isomers by an allylic mechanism (*Pines & Stalick*, 1977).

Obtaining 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 catalysts can be used in a continuous process in a fixed-bed reactor or a continuous stirred tank reactor (CSTR), enabling the simple and cheap synthesis of functional food ingredients. The continuous operation has a major technological advantage with higher productivity. As the active metal particle or site is well-dispersed on a solid material, heterogeneous catalysts are much easier to be taken apart and recycled, as they overcome the separation difficulties of the soluble metal (*Bauer et al.*, 2009; *Philippaerts et al.*, 2011a). For the isomerization of alkyl linoleates to obtain CLAs, several metals (Ni, Ru, Rh) on various supports were used. At high concentration, nickel was the most active, but ruthenium was found the best from an economic point of view. Ruthenium and rhodium were used in a concentration of 5%, immobilized on active carbon surface, and were activated with hydrogen adsorption. The reaction was carried out at the temperature of 180–240°C, in nitrogen atmosphere. If the reaction was carried out in methanol or isopropanol as a solvent, a high activity and selectivity toward saturated by-products were obtained, whereas using hexane or cyclohexane, the selectivity increased toward the desired CLA isomers. Parallel with isomerization, the polymerization reaction occurs, since this process is catalysed also by metallic centres. The addition of a minimal quantity of nickel to the Ru/C catalyst increased the selectivity toward cis-trans CLA isomers (*Deshpande et al.*, 1985; *Narasimhan 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.*, 2002, 2003, 2004, 2005) for the isomerization of linoleic acid to CLAs. The reactions were carried out in n-decane in the temperature range of 80–120°C. They showed 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. The main disadvantage of such treatment is the emergence of the competitive hydrogenation due to the presence of activated (chemisorbed) hydrogen on the metal surface. However, quite high CLA concentrations were found in the product mixture and the degree of saturation was low compared with the pressurized hydrogenation. Pd possesses great selectivity for hydrogenation, while Ni, Ru and Pt are more selective for isomerization. In the case of Ru, the activity and selectivity is highly dependent on the distribution of Ru particles and on the nature of ruthenium compounds. Larger Ru-clusters increase the selectivity towards CLA formation. The presence of chlorine together with Ru affects the distribution of the CLA isomers. For example, impregnation of γ-aluminate with ruthenium acetyl acetonate results in a catalyst highly selective towards cis-9,trans-11 and trans-10,cis-12 CLA isomer formation (i.e., 68–75% selectivity), whereas impregnation with $RuCl_3.3H_2O$ shows preference for t9, t11 CLA (40% selectivity) (*Bernas et al.*, 2004). Probably, this selectivity difference is caused by the acidity of chlorine, which – similar to acidic zeolites – increases the selectivity toward to all-trans CLA isomers. The temperature has a major influence on reaction rate and selectivity. Contrary to expectations, reaction rate is decreased with temperature increase; in contrast, selectivity increases drastically toward CLAs. The explanation is that higher temperature promotes the hydrogen desorption from the catalyst surface, favouring isomerization.

Philippaerts and co-workers (2011a) developed a novel catalytic method for obtaining CLAs, using highly dispersed ruthenium-dioxide on zeolite support in hydrogen-free condition. As the hydrogenation step was totally missing, the highest yield and selectivity toward the regarded CLA isomers were obtained. The optimal catalyst was identified using several types of zeolites with different Si/Al ratio in their composition (causing structural modification) and changing the amount of countercations (H^+, Na^+, Cs^+) of the aluminosilicate lattice. The most active and selective isomerization catalyst was the Ru/Cs-doped USY zeolite with a Si/Al ratio of 1:40, at the reaction temperature of 165°C. In this condition, from methyl linoleate substrates, mainly cis-9,trans-

11 and trans-10,cis-12 CLA isomers were formed with an overall yield of 67% and a selectivity of 82% at low catalyst concentrations. The main advantage of this catalyst is that no hydrogen pretreatment or addition of hydrogen donors are required, and the obtained productivity was the industrially relevant 0.7 g/(l·min) of CLAs.

Proposal for a new type of heterogeneous catalyst for CLA formation

Despite that different types of hydrotalcites are known as isomerization catalysts, hydrotalcite catalysts were not utilized until now for obtaining CLAs from LA substrate. Their advantage is – as in general with heterogeneous catalysts – that the separation from the reaction mixture is easy at the end of the reaction. In the case of using a fixed bed reactor, even this step may be omitted, as the reaction mixtures flow through the void space of the catalyst bed. Another advantage of the hydrotalcites is that they are cheap, nontoxic and their structure possesses stereoselectivity. Hydrotalcites (HTs) are mixed hydroxides with natural origins, possessing excellent anion exchange capacity and high specific surface, stable at high temperatures, with metal-oxide active centres. Their industrial applications increased in the second half of the last century in the production of different organic substances as catalysts or catalyst supports. The chemical formula of the natural HTs is $Mg_6Al_2CO_3(OH)_{16}\cdot 4H_2O$, layered double hydroxides, magnesium aluminium hydroxyl-carbonate tetrahydrate. The structure of the polymorphic variants depends on the nature of cations included into the layers. The exchange of Mg^{2+} ions with Al^{3+} ions results a positive charge excess in the layers, which will be compensated by hydroxyl or other anions. The structure of HTs strongly depends on many factors such as the synthesis method, the pH of reaction media, the temperature as well as on the applied washing and drying method. Generally, it can be concluded that the nanolayers are elastic, the crystalline water contained by the interlayers is mobile and reversibly removable. The HTs are soluble in acids, and by thermal treatment macro-, meso- and micropores can be formed in them.

The methods for obtaining layered double hydroxides are based on coprecipitation, sol-gel calcinations/rehydration techniques. In most of the cases, Mg/Al or Mg/Zn containing HTs are obtained, but in this way it is possible to synthetise HTs with various metal ion content in their crystalline structure. The morphology, crystallinity degree and the structure of crystalline phases depend on the technique of coprecipitation, reaction temperature and maturation time. By the addition of different anions and organic molecules during the rehydration process, a large variety of structures were obtained (*Mitsudome et al.*, 2008).

Kishore & *Kannan* (2002, 2006) used MgAl-HTs for the basic isomerization of eugenol and safrole, and for obtaining anetole from estragole. While no data were found in literature for the utilization of HT-catalysts for obtaining CLAs, we consider this proposal as a novelty.

Obtaining CLAs by photochemical methods

An alternative way besides microbial methods and metal catalysis is photoisomerization. Japanese scientists (*Seki et al.*, 1998) obtained CLA methyl esters with high yield (80%) from a diluted methyl linoleate solution (5-10%) in petrol ether, using iodine as photocatalyst and with intense visible light irradiation, without reporting the isomer composition of the products. Another research group extended the method to the production of CLA-rich vegetable oil by direct isomerization of soybean oil by UV-light irradiation of solventless substrate, with iodine sensitizer. Under irradiation, the I_2 possesses radical forming potential. In the first experiment (*Gangidi* & *Proctor*, 2004), 6 mg CLA (cis-9,trans-11 CLA isomer) was obtained from 1g soybean oil (0.25 w/w% I_2, irradiation time 85 hrs, irradiation source: mercury lamp, power 100 W). By the optimization of the process and performing the reaction in stirred batch photo-reactor (*Jain* & *Proctor*, 2006), a production of 240 mg CLA/g substrate was obtained (0.15 w/w% I_2, irradiation time 144 hrs), but the isomeric composition was unfavourable, as the rate of trans,trans and that of cis,cis isomers was about 5:1. The main influencing factors are the irradiation flux, the stirring intensity and the concentration of the catalyst.

Recently, the isomerization of soybean oil was performed in pilot scale (1 L volume) laminar flow photo-reactor, irradiated with UV-Vis lamp (reaction conditions: lamp power: 450 W; I_2 concentration: 0.35% w/w; temperature: 48 °C; reaction time: 12 hrs), with a yield of 220 mg CLA/g soy oil. The main isomer (approx. 80%) was the trans,trans CLA (*Jain et al.*, 2008a). Soybean oil should be refined prior to photoisomerization (*Jain et al.*, 2008b); otherwise, the productivity of CLAs decreases drastically (with approx. 98%). Minor components, particularly peroxides and phospholipids, should be removed. Tocopherols enhanced CLA yield at low level, but decreased it at high level. Lutein and free fatty acids seem to have little effect on CLA production (*Tokle et al.*, 2009). The iodine-catalysed photoisomerization of LA has a radical mechanism: the photolytically formed iodine radicals formed in the initiation step can react with the unsaturated substrate in two ways. When the iodine radical directly adds the double bond, an unstable iodinated radical intermediate is formed, which allows bond rotation (stereoisomerization) and the formation of the more stable (trans) isomer. Alternatively, when the iodine abstracts a hydrogen atom, forming hydrogen iodide and allylic radical, then positional isomerization occurs, the trans,trans conjugated product (being the most stable isomer) is formed. It is important to emphasize that – due to the radical nature of the photocatalytic reaction – the isomerization needs to be carried out in inert atmosphere to ensure high chemoselectivity; otherwise, the iodine radicals may be inactivated by other compounds (*Philippaerts et al.*, 2011a).

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