Production of Conjugated Linoleic Acid Isomers by Dehydration and Isomerization of Castor Bean Oil

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ABSTRACT: The possibility of combining dehydration and isomerization of castor bean oil as a means to obtain CLA as TAG forms was studied. First, dehydration was carried out using various catalysts and reaction parameters. Best results were obtained using phosphoric acid (0.1% w/w) at 280°C for 5 h. Under such conditions, satisfactory proportions of CLA were obtained (54% of total FA) with a majority of 9-*cis*,11-*trans* isomer (61% of total CLA). Other catalysts such as bisulfate-bisulfite, sulfuric acid, tungstic and phosphotungstic acids, or resins and zeolites were also tested. With the exception of resins and zeolites, these catalysts also led to CLA production but in limited amounts in comparison with phosphoric acid. In a second step, an isomerization reaction was carried out to transform the residual nonconjugated linoleic acid also produced during dehydration into CLA. Using Wilkinson catalyst [$RhCl(PPh_3)_3$] in ethanol solvent, dehydrated castor bean oil was isomerized in high yields (>98%), allowing a complete disappearance of nonconjugated linoleic acids. The resulting dehydrated/isomerized oil contained more than 87% CLA with the 9-*cis*,11-*trans* isomer being predominant (40% of CLA fraction). Finally, urea fractionation was also applied on dehydrated/isomerized castor bean oil FA to obtain FFA products containing about 93% CLA.

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CLA are a group of FA isomers with a chain length of 18 carbons and two nonmethylated interrupted double bonds. Depending on the double-bond positions and their configuration (*cis* or *trans*), different isomers can be found. CLA are naturally present in milk fat in quantities varying from 6 to 16 mg/g and in lesser amounts in various meats (1,2). For a few years now, CLA have been the subject of a growing interest owing to their suspected nutritional and therapeutic properties. Indeed, since the first study by Pariza *et al.* (3) showing an inhibitory effect of CLA on mutagen formation in rat liver cells, many other studies have demonstrated the beneficial effect of CLA isomers on human and animal health (4). In particular, the 9-*cis*,11-*trans* isomer, also known as rumenic acid, seems to be the most active against cancer cells. For example, it was observed that a CLA supplementation in rats' diet led to a reduction of mammalian or skin tumors (5,6). Similarly, Lavillonnière and Bougnoux (7) and Ochoa *et al.* (8) concluded that increased levels of CLA in mammalian tissues resulted in a decrease, respectively, of breast or prostate cancer risk. Moreover, it appears that CLA formation also may have some very advantageous nutritional properties. Several studies tend to show that some isomers can contribute to the reduction of body fat (9–13). This property is largely attributed to the 10-*trans*,12-*cis* isomer. Finally, CLA are said to be involved in immune function preservation (14–16), arthritis and inflammatory diseases (17–21), and atherosclerosis in animals (22–24).

Endogenous production of CLA isomers by humans from vaccenic acid (18:1, 11-*trans*) is very limited (25). Therefore, a very large proportion of CLA found in body tissues is from dietary origin. At present, the interest in CLA as commercial nutritional supplements is great, and different products are now commercially available (26). In most cases, these commercial products contain about 50 to 80% CLA and correspond to a complex mixture of isomers, the 9-*cis*,11-*trans* and 10-*trans*,12-*cis* isomers being the most abundant. Various methods are available to produce synthetic CLA. The alkaline isomerization of linoleic acid is the most common. Generally, this reaction cannot be carried out on natural linoleic vegetable oils such as sunflower, soy, or safflower, but instead must be produced from their corresponding soaps, which, once conjugated through the action of a strong base, are then transformed into FFA by dilute acid. The reaction temperature is around 200 to 250°C, and the FFA obtained are generally purified by distillation at the end of the process. Such isomerization reactions also can be applied to methyl esters. For example, using potassium hydroxide, Chin *et al.* (27) obtained a mixture of methyl esters containing nearly 90% CLA with a majority of 9-*cis*,11-*trans* and 10-*trans*,12-*cis* isomers in equivalent quantities. Nowadays, alkaline isomerization of linoleic acid is often performed in propylene glycol in order to limit reaction temperature (<100°C) and catalyst quantities (2%). Under such conditions, the formation of undesired isomers, such as 8-*trans*,10-*cis* and 11-*cis*,13-*trans* is limited (26,28) More recently, new methods have been described in which transition metal catalysts are used that allow satisfactory CLA production (29–31). For example, Larock *et al.*

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(32), using rhodium or ruthenium transition metals at 60°C, could produce high CLA yields (90%) from the conjugation of soy methyl esters.

Dehydration of castor bean oil acid is another well-known process that was extensively studied about 60 years ago (32–34). Indeed, this oil contains about 85 to 90% ricinoleic acid (12-hydroxy-9-*cis* 18:1), which can be easily dehydrated at high temperatures under acid catalysis to form an additional double bond in the aliphatic chain. Depending on the dehydration conditions, this newly formed double bond can be conjugated or not with the one initially present, leading to various CLA isomers and nonconjugated linoleic acid. It is worth noting that the main objective of these early publications was to obtain CLA-enriched TAG for painting and varnishing applications, not for the nutritional benefits of CLA isomers. Moreover, owing to the limited resolution capacity of chromatographic techniques at that time, data regarding the repartition of the different CLA isomers formed during the process are unavailable. However, dehydration of ricinoleic acid still appears to be a good strategy to obtain CLA in satisfactory yields. For example, Berdeaux *et al*. (36) showed that methyl ricinoleate could be transformed into the corresponding mesylate (12-mesyloxy-octadec-9-enoate), which was then reacted with a base (1,8-diazabicyclo [5.4.0]-undec-7-ene, or DBU) to give a product containing 66% of the desired 9-*cis,*11-*trans* CLA isomer. Recently, a similar process was applied in which DBU was replaced by potassium hydroxide to reduce the economic costs of the process (37). A 77% CLA production was observed at 80°C, with the mixture composition corresponding to 72% 9-*cis*,11-*trans* and 26% 9-*cis*,11-*cis*.

Some other methods have used microbiological processes to produce CLA. For example, *Propionibacterium freudenreichii* ssp. has been employed to transform linoleic acid into CLA with conversion yields ranging from 57 to 87%. The main isomers formed were 9-*cis*,11-*trans* and 9-*trans,*11-*cis* (38). Similarly, all 9,11 isomers were produced using *Lactobacillus acidophilus* with 80% conversion yield (39). Generally, lactic bacteria species are good catalysts for CLA synthesis using linoleic or ricinoleic acid substrates (40–43). However, although these microbiological techniques appear attractive, they are not yet economically competitive with classical chemical processes such as the alkaline isomerization of linoleic acid or the dehydration of castor bean oil.

In the present paper, we have investigated and combined these two strategies to produce CLA as TAG since it was recently shown that the absorption and bioavailability of such CLA products is improved in comparison with CLA FFA or FAME (40).

MATERIALS AND METHODS

Materials. (i) Oils. Castor bean (*Ricinus communis*) and sunflower oils were purchased from Sigma-Aldrich (Saint Quentin, France). The typical FA composition of castor bean oil was the following: C16:0 (1.2%), C18:0 (1.1%), C18:1n-9 *cis* (2.8%), C18:1n-9 *cis*, n-7 OH (ricinoleic, 90.0%), 18:2n-6 *cis* (linoleic, 4.3%), C18:3n-6 (0.4%), and C20:0 (0.2%).

(ii) Dehydration catalysts. Sodium bisulfate, sodium bisulfite, sulfuric acid, phosphoric acid, tungstic and phosphotungstic acids, Amberlyst IR 120 and IR 400 resins, and zinc were all purchased from Sigma-Aldrich (Saint Quentin, France). Zeolite CBV 712 and CBV 600 were obtained from Zeolyst International (Valley Forge, PA). Lewatit K2629 resin was from Bayer-Sybron Chemical (Pittsburgh, PA). Dowex 50 WX8 resin was purchased from Acros Organics (Noisy le Grand, France). All resins and zeolites were dried at 110°C for 48 h before use. For recycling, Zeolite CBV 712 was recovered from reaction medium by simple filtration and washed with 5×50 mL chloroform to eliminate traces of dehydrated castor bean oil.

(iii) Chemicals. Wilkinson catalyst [$RhCl(PPh_3)_3$], $SnCl_2 \cdot 2H_2O$, tri-*O*-tolylphosphine, urea, sodium methylate, and acetyl chloride were from Sigma-Aldrich. Potassium hydroxide, sodium sulfate, HCl, phenolphthalein, methanol, octanol, ethanol, 2-methyl 2-propanol, chloroform and hexane, all analytical grades, were from Carlo Erba (Montpellier, France).

Methods. (i) Castor bean oil dehydration. Castor bean oil (250 mL) was magnetically stirred (250 rpm) and heated in a 500-mL three-necked round-bottomed flask placed in a thermostated mineral oil bath at the selected temperature in the presence of zinc powder (1 g) as antipolymerization agent. After 20 min equilibration at the selected temperature, the studied catalyst was added, and the dehydration reaction was then initiated. The reaction medium was maintained under vacuum to allow the removal of water formed during the process, and gentle bubbling with nitrogen was used to limit the presence of oxygen and possible oxidation reaction. Samples (50 mg) were periodically taken from the reaction medium using a syringe and transformed into FAME for GC analysis and determination of FA composition. In subsequent experiments with the final selected catalysts, a three-order experimental design (temperature, reaction time, amount of catalyst) was carried out to optimize CLA production and especially 9-*cis,*11-*trans* isomer formation.

(ii) Isomerization of dehydrated castor bean oil. In a hermetically sealed 50-mL round-bottomed flask under nitrogen atmosphere, dehydrated castor bean oil (10 g) was mixed in ethanol (or octanol or 2-methyl 2-propanol) in the presence of the selected weight amounts of Wilkinson catalyst, $SnCl_2 \cdot 2H_2O$, and tri-*O*-tolylphosphine. Samples were periodically removed from the reaction mixture and analyzed by GC for FA composition.

(iii) Urea fractionation. Dehydrated/isomerized castor bean oil (50 g) was refluxed for 90 min in a 1 N ethanolic KOH solution (1000 mL). After cooling to ambient temperature, 1 L of 4 N HCl was added and the resulting FFA were extracted by 3×350 mL hexane. FFA extracts were then washed with water to neutrality. The organic phase was dried over anhydrous sodium sulfate and filtered, and the solvent was evaporated under vacuum. A fraction of the recovered FFA (5 g) was then added to an equivalent weight of urea previously dissolved in hot methanol (1 g of urea/3 mL of MeOH). After urea inclusion, the reaction mixture was cooled to ambient

temperature and stored at −25°C overnight. Then the urea adduct fraction was separated from the mother liquor by vacuum filtration. To extract FFA from the mother liquor, 50 mL of water was added to this latter, and medium was acidified to pH < 2 by 6 N HCl (50 mL). FFA were then extracted by 2 × 50 mL hexane. The organic phase was subsequently washed by 25 mL water, 3 × 50 mL aqueous MeOH (30% vol/vol), and then by 3 × 50 mL water. The hexane fraction was then dried over anhydrous sodium sulfate, filtered, and the solvent evaporated under vacuum. FA composition was then determined by transformation into FAME and GC analysis. FFA were extracted from the urea fraction by adding 50 mL of water and heating to complete urea dissolution. FFA then appeared in the upper phase. After cooling to ambient temperature, the medium was acidified to pH < 2 by 6 N HCl (50 mL). FA were then extracted by 2 × 50 mL of hexane, then washed according to the same procedure as for the mother liquor. Samples were analyzed by GC for FA composition.

Transformation into FAME and GC analysis for FA composition. In 25-mL round-bottomed flasks, samples (10 mg) were added to 3 mL sodium methylate solution with phenolphthalein. The reaction medium was refluxed for 10 min. Then 3 mL methanolic HCl was added to phenolphthalein discoloration, and the mixture was refluxed again for 10 min and then cooled to ambient temperature. Next, 8 mL of hexane and 10 mL of water were added and the organic phase was recovered, dried over anhydrous sodium sulfate, and filtered for the subsequent GC analysis: Agilent 6890 series using a SUPELCOWAX 10 capillary column (SGE, Courtaboeuf, France) with the following characteristics: length, 30 m; internal diameter, 0.32 mm; film thickness 0.25 μm. FAME were directly injected into the GC. Carrier gas: helium, flow 2.2 mL/min; split ratio: 1:80. Injector temperature: 250°C; FID detector temperature: 270°C. The temperature settings were as follows: 150 to 225°C at 5°C/min, 225°C for 20 min. CLA isomers were identified in comparison with commercially available CLA standards.

All assays concerning castor bean oil dehydration, isomerization, and urea fractionation or FA compositions were run in triplicate with less than 3% deviation. Data presented in the tables correspond to the average value of three determinations.

RESULTS AND DISCUSSION

Among the different CLA products that are commercially available, a very large majority correspond to FFA or alkyl ester mixtures of various isomers. However, some recent findings showed that TAG forms were the most suitable for incorporation into functional foods and for bioavailability of CLA isomers in comparison with the alkyl ester forms (44). Therefore, we decided to study methods that allow the production of CLA isomers preferably as TAG. Such CLA TAG are usually obtained through the lipase-catalyzed production of restructured TAG (45). However, these technologies generally require particular enzyme selectivities and multistep product synthesis and purification. Moreover, they often lead to TAG containing limited amounts of CLA (<50%). In this context, we estimated that the combination of ricinoleic acid dehydration with new methods of linoleic acid isomerization with transition metal catalysis was attractive for the purpose of producing TAG highly enriched in CLA and especially the 9-*cis*,11-*trans* isomer.

Castor bean oil dehydration. Although several recent publications described the dehydration of ricinoleic acid as FFA or FAME (36,37), we considered that direct dehydration of castor bean oil was more attractive since the recovered final products would be as TAG forms. In the presence of a Lewis or Brönsted acid catalyst and heat, castor bean oil can be easily dehydrated with good conjugation yields. The effects of three factors on the dehydration process were tested: type and quantity of catalyst, temperature, and reaction time. No dehydration reaction occurred in the absence of catalyst.

The first trials were carried out with a mixture of sodium bisulfate–sodium bisulfite following the experimental procedures described in the early literature (35). The catalyst efficiency was evaluated at 230°C with 1.5% w/w sodium bisulfate and 0.5% w/w sodium bisulfite for a 2% catalyst total amount. In this case, sodium bisulfite plays the role of an antipolymerizing agent. CLA formation was followed by GC over the course of the dehydration reaction (Table 1). The dehydration reaction was very fast since residual ricinoleic acid was only 38.4% after the first hour. After 24 h, dehydration

TABLE 1
FA Composition over Reaction Course of Dehydrated Castor Bean Oil at 230°C with 2% Sodium Bisulfate (1.5%)–Sodium Bisulfite (0.5%) Catalyst

Reaction time (h)	Formed CLA isomer repartition (%)[a]				Total CLA (%)	9*c*,11*t* from total CLA[c] (%)	Linoleic acid[d] (%)	Residual ricinoleic acid (%)
	9*c*,11*t*[b]	10*t*,12*c*	10*c*,12*c*	9*t*,11*t*				
1	9.6	0.4	6.7	5.0	21.5	44.7	29.6	39.4
2	11.8	1.3	8.7	7.1	28.9	41.0	37.6	24.2
4	14.5	1.6	10.1	10.3	36.5	39.6	47.0	7.0
6	14.4	2.0	9.5	12.5	38.4	37.6	48.1	4.8
8	14.2	1.8	9.0	13.3	38.4	37.1	49.2	3.7
24	14.7	2.2	6.1	15.1	38.0	38.6	51.3	1.9

[a]% given from total FA composition. This table does not mention other nonreacting FA: C16:0 (1.2%), C18:0 (1.1%), C18:1n-9 *cis* (2.8%), C18:3n-6 (0.4%), C20:0 (0.2%).
[b]9*c*,11*t* is the abbreviated term for 9-*cis*,11-*trans* isomer.
[c]This value corresponds to the ratio (%) of 9*c*,11*t* isomers in the fraction of total CLA.
[d]Data correspond to total methylene-interrupted linoleic acid as 9-*trans*,12-*cis* and 9-*cis*,12-*cis* forms.

was nearly complete, as there was a minimal content (1.9%) of unreacted residual ricinoleic acid. However, the reaction produced not only conjugated isomers but also methylene-interrupted (nonconjugated) linoleic acid in higher proportions (29.6%) than the total CLA. Moreover, we observed that both nonconjugated linoleic acid isomers, namely, 9-*trans*,12-*cis* and 9-*cis*,12-*cis*, were formed. The typical molar ratio between these two isomers was about 7:3 in favor of 9-*cis,*12-*cis*. For prolonged reaction times, nonconjugated linoleic acid remained the FA that was predominantly produced. After 24 h, it accounted for 51.3% of the total FA. Concerning CLA formation, a significant quantity (21.5%) was produced within the first hour, with 9-*cis*,11-*trans* being predominant (44.7% of total CLA). At the completion of the reaction, total CLA was 38%. However, the proportion of 9-*cis*,11-*trans* isomers decreased to 38.6% of total CLA following prolonged reaction times. We observed that prolonged reaction times favored the formation of 9-*trans*,11-*trans,* which increased from 5.0% (60 min) to 15.1% when dehydration was complete. Finally, the 10-*trans*,12-*cis* isomer was produced in very small quantities (<3%).

Metallic oxides have been described in the literature as powerful catalysts for castor bean oil dehydration. These catalysts were initially tested at 200°C and 0.5% quantity using zinc powder as an antipolymerizing agent. From these experiments, we concluded that phosphotungstic acid was not a suitable catalyst since total polymerization of castor bean oil was observed within 15 min. Similar results were obtained with higher catalyst amounts. On the contrary, no polymerization was observed with tungstic acid. However, dehydration was very slow. After 24 h, residual ricinoleic acid was still 75% of total FA, and CLA production did not exceed 7%. Increasing the catalyst amount to 2% did not result in significant improvement in dehydration yields. After 24 h, whereas 55.4% of unreacted ricinoleic acid remained, total CLA formation was only 12.8%, with equivalent production of four isomers: 9-*cis*,11-*trans*, 10-*trans*,12-*cis*, 10-*cis*,12-*cis*, and 9-*trans*,11-*trans*. We estimated that both temperature and catalyst quantities had to be increased to favor CLA production. Therefore, additional reactions were carried out with tungstic acid at 250°C using higher catalyst amounts (Table 2). With 2% tungstic acid catalyst, about 5.7% residual ricinoleic acid was present after 24 h. Total CLA production reached 34.4% whereas nonconjugated linoleic acid accounted for 49.9%. Concerning the repartition of CLA isomers, 9-*cis*,11-*trans* was preferentially produced with a maximum of 59.8% when the reaction was stopped at 24 h. Interestingly, CLA repartition was somewhat different with 5% catalyst. In that case, both 9-*cis*,11-*trans* and 9-*trans*,11-*trans* were synthesized predominantly. In terms of CLA production, although the dehydration was faster with 5% catalyst, final total CLA amounts were very comparable (34.4%). However, with 5% tungstic catalyst, the formation of nonconjugated linoleic acid was favored and reached 55.5% after 24 h. At that time, the dehydration reaction was complete and residual ricinoleic acid was very low (<1%). These results with tungstic acid showed that total CLA production and isomer repartition were clearly dependent on the catalyst amount, operating temperature, and reaction time.

The importance of reaction temperature was confirmed when using strong acidic resins as ion exchangers, such as Dowex 50W X8, Amberlyst IR 120 and IR 400, or Lewatit K2629. Castor bean oil dehydrations were carried out at the maximal stability temperature of these resins (150°C) using a 2% catalyst load. CLA formation was very limited after 24 h with a 5% maximum for Lewatit K2629. As ricinoleic acid amounts remained constant (around 89.3%), we concluded that dehydration did not occur. The reaction was tried at higher temperature (250°C) using Lewatit K2629 catalyst (Table 3). In that case, reasonable amounts of CLA had formed after 24 h (31.6%). However, dehydration was not quantitative since 14.9% residual ricinoleic acid remained. Moreover, nonconjugated linoleic acid formation was slightly higher than CLA formation. Finally, concerning the repartition of CLA isomers, we

TABLE 2
FA Composition over Reaction Course of Dehydrated Castor Bean Oil at 250°C with 2% and 5% Tungstic Acid Catalyst[a]

Reaction time (h)	Formed CLA isomer repartition (%)				Total CLA (%)	9*c*,11*t* from total CLA (%)	Linoleic acid (%)	Residual ricinoleic acid (%)
	9*c*,11*t*	10*t*,12*c*	10*c*,12*c*	9*t*,11*t*				
Catalyst amount = 2% w/w								
1	1.8	0.9	1.5	1.5	5.6	31.6	11.9	74.9
2	8.6	0.0	4.5	4.2	17.4	49.5	27.4	47.1
4	11.5	0.0	5.5	5.2	22.3	51.6	33.0	36.8
6	12.1	0.0	5.0	7.2	24.2	50.0	33.6	32.5
8	14.0	0.0	5.5	6.0	25.6	54.9	40.0	26.2
24	20.6	0.4	4.6	8.8	34.4	59.8	49.9	5.7
Catalyst amount = 5% w/w								
1	2.6	1.3	1.9	2.0	7.7	32.2	14.5	70.4
2	5.1	2.0	4.2	4.3	15.7	32.4	37.2	38.9
4	8.9	3.5	7.5	9.2	29.1	30.5	44.0	16.4
6	9.2	3.9	7.3	10.5	31.0	29.8	49.0	9.3
8	10.0	3.6	7.8	11.0	32.5	30.9	51.0	6.1
24	13.6	2.5	6.1	12.4	34.4	38.6	55.5	0.5

[a]For footnotes see Table 1.

TABLE 3
FA Composition over Reaction Course of Dehydrated Castor Bean Oil at 250°C with Lewatit K2629 or Zeolite CBV 712[a]

Reaction time (h)	Formed CLA isomer repartition (%)				Total CLA (%)	9*c*,11*t* from total CLA (%)	Linoleic acid (%)	Residual ricinoleic acid (%)
	9*c*,11*t*	10*t*,12*c*	10*c*,12*c*	9*t*,11*t*				
Lewatit K2629 (2% w/w)								
1	3.8	1.4	1.5	10.5	17.2	22.1	20.5	50.3
2	6.3	1.3	2.2	12.0	21.9	29.0	22.7	41.4
4	7.6	1.6	2.5	14.0	25.6	29.6	27.0	33.8
24	10.8	1.9	3.4	15.4	31.6	34.3	35.7	14.9
Recycled Lewatit K2629 (2% w/w)								
1	0.0	0.0	0.0	0.0	0.0	0.0	5.1	87.5
2	0.3	0.0	0.1	0.2	0.7	47.7	5.7	85.9
4	0.6	0.0	0.3	0.5	1.4	44.6	5.8	85.7
24	2.0	0.0	0.9	1.8	4.8	41.6	9.5	77.9
Zeolite CBV 712 (2% w/w)								
1	1.6	0.0	0.7	1.8	4.0	38.7	10.2	78.2
2	1.9	0.0	0.8	2.1	4.7	38.5	10.7	77.1
4	2.0	0.0	0.8	2.3	5.2	40.0	11.3	76.2
24	3.7	0.0	0.8	2.8	7.3	51.3	13.9	69.9
Zeolite CBV 712 (5% w/w)								
1	3.4	0.5	1.5	4.5	9.8	34.2	17.7	64.2
2	3.8	0.6	1.3	4.5	10.3	37.3	20.7	60.0
4	5.5	0.7	1.5	5.6	12.5	44.4	20.8	58.6
24	6.9	1.1	1.2	6.0	15.1	45.4	25.0	48.4

[a]For abbreviations see Table 1.

observed that 9-*trans,*11-*trans* formation was favored in comparison with 9-*cis*,11-*trans*, which was only 34.3% of total CLA. However, since Lewatit K2629 was used here at a temperature above its maximal thermal stability, we checked the impact of such thermal treatment on the catalyst recyclability and efficiency. The same Lewatit K2629 was filtered from the reaction medium and washed by organic solvent to remove any traces of dehydrated castor bean oil. Then it was recycled for another dehydration reaction that was carried out at 250°C. A drastic decrease in the catalyst efficiency was observed with very low CLA formation (<5%) and limited ricinoleic acid dehydration (Table 3). These experiments demonstrated that resin catalysts were inefficient owing to their limited thermal stability, which did not allow carrying out reactions at sufficiently high temperatures for castor bean oil dehydration. In further experiments, we selected strongly acidic zeolites with better thermal stability and evaluated their ability to catalyze the dehydration process at 250°C. Zeolite CBV 600 was not a satisfactory catalyst, and no CLA production was possible with this material whatever quantity of it was used in the process (from 2 to 5%). Results were better with Zeolite CBV 712 but still limited. Only 7.3% CLA formation was observed after 24 h with 2% catalyst. Increasing the catalyst quantity (5%) resulted in additional CLA formation (15.1%), but it was still insufficient (Table 3).

Finally, nonoxidizing mineral acids such as sulfuric and phosphoric acid were also tested at 250°C at various catalyst levels. Concerning sulfuric acid, we observed that the dehydration reaction was very fast with only 5.3% residual ricinoleic acid after 60 min and 1.3% after 6 h (Table 4). Total CLA formation was very comparable to that obtained with sodium bisulfate–sodium bisulfite catalysis. However, some disadvantages appeared in the case of sulfuric acid. Indeed, the CLA isomer repartition showed that 9-*cis*,11-*trans* isomers represented only 25–26% whereas 9-*trans*,11-*trans* was predominant. Moreover, after 2 h, it was observed that prolonged reaction times lead to isomer rearrangement, resulting in a slight increase of 10-*trans*,12-*cis* and 10-*cis*,12-*cis* isomers as well as an increase in the nonconjugated linoleic acid ratio. No further experiments were carried out with this catalyst. For phosphoric acid, the initial assays were run using a 2% catalyst load. The reaction was almost complete within 6 h according to the minimal unreacted ricinoleic acid content (3.3%), with 46.7% maximal CLA formation. For the first hours, formation of the 9-*cis*,11-*trans* isomer was favored with a 63.4% maximum at 4 h. For prolonged reaction times, the 9-*trans,*11-*trans* isomer was preferably produced, thus lowering the 9-*cis*,11-*trans* content in the total CLA (54.4%). No 10-*trans,*12-*cis* formation was detected. To accelerate the dehydration reaction and try to favor 9-*cis*,11-*trans* production, experiments were carried out with increased phosphoric acid content (5%) (Table 4). The reaction kinetics then improved: Residual ricinoleic acid was 5.2 and 1.9% for 60 min and 2 h reaction time, respectively. Only traces of ricinoleic acid were noted after 6 h. However, total CLA formation was lower in comparison with the 2% catalyst quantity. Only 31.9% total CLA was obtained. Moreover, formation of the 9-*trans*,11-*trans* isomer was favored throughout the reaction. In parallel, we observed that a strong castor bean oil polymerization occurred (increase in viscosity and discoloration). Finally, as increased phosphoric acid quantities were not advantageous for CLA production, reactions were repeated with 0.5% catalyst amount (Table 4). This resulted in slower dehydration kinetics, but a satisfactory CLA formation was observed after 24 h (47.5%). Moreover, 9-*cis*,11-*trans* formation was greatly favored, reaching around 70% of total CLA

TABLE 4
FA Composition over Reaction Course of Dehydrated Castor Bean Oil with Sulfuric or Phosphoric Acid at 250°C[a]

Reaction time (h)	Formed CLA isomer repartition (%)				Total CLA (%)	9*c*,11*t* from total CLA (%)	Linoleic acid (%)	Residual ricinoleic acid (%)
	9*c*,11*t*	10*t*,12*c*	10*c*,12*c*	9*t*,11*t*				
Sulfuric acid (2% w/w)								
1	9.4	2.6	3.9	20.3	36.2	26.3	47.3	5.3
2	9.7	2.7	4.0	20.6	37.0	26.2	48.8	2.3
4	9.1	2.8	4.0	19.5	35.5	25.8	48.9	1.5
6	9.0	2.9	4.1	19.5	35.6	25.4	49.3	1.3
Phosphoric acid (0.5% w/w)								
1	3.5	0.0	1.6	0.2	5.3	66.0	9.7	76.0
2	7.4	0.0	3.0	0.8	11.3	66.0	13.8	65.1
4	17.0	0.0	6.5	1.1	24.8	69.0	22.4	42.2
6	24.0	0.0	8.9	1.9	34.8	69.0	29.4	25.8
8	29.4	0.0	9.8	2.8	41.9	70.0	34.5	14.0
24	30.8	0.0	8.1	9.6	47.5	64.9	39.2	3.6
Phosphoric acid (2% w/w)								
1	4.6	0.0	2.5	1.2	8.3	55.3	13.1	67.1
2	13.0	0.0	5.9	1.6	20.6	63.3	21.5	48.5
4	26.7	0.0	11.8	3.7	42.2	63.4	37.3	11.2
6	25.4	0.7	11.9	8.8	46.7	54.4	40.7	3.3
Phosphoric acid (5% w/w)								
1	6.2	3.1	4.8	19.1	33.3	18.9	48.3	5.2
2	6.3	2.8	4.2	22.7	35.8	17.1	44.7	1.9
4	8.1	2.5	4.0	21.2	36.5	22.1	43.7	1.9
6	7.1	2.3	2.8	19.1	31.9	23.8	41.0	0.5

[a]For footnotes see Table 1.

after 8 h. The production of the 10-*trans*,12-*cis* isomer was very limited (<1%) as observed with 2% catalyst.

This catalyst screening for castor bean oil dehydration showed that the choice and quantity of catalyst, in association with the reaction temperature and duration, were of crucial importance for CLA production and isomer repartition. From our experiments, phosphoric acid seemed to be the most promising catalyst. Therefore, we selected this catalyst for further experiments and applied experimental design strategy to optimize total CLA production, especially of the 9-*cis*,11-*trans* isomer. The influence of three parameters, namely, catalyst quantity (0.1 to 5%), reaction temperature (180 to 300°C), and reaction time (60 min to 24 h) was studied. Temperature was observed to be the most important parameter. Below 200°C, the dehydration reaction was not possible. At the 200°C minimal value, CLA total production was directly correlated with catalyst amount. For 0.2% catalyst, CLA total production was very limited (<3%) after 24 h. For higher catalyst quantities (2.5 and 5%), we observed a fast disappearance of ricinoleic acid, which represented, respectively, 6.7 and 4.3% after 24 h. However, in such conditions, CLA production only reached a 40% maximum. Moreover, 9-*cis*,11-*trans* was only 20.3% of the total CLA, *trans-trans* isomers being produced preferentially. For reactions carried out at 300°C, dehydration was very fast whatever the catalyst amount. For example, with 5% phosphoric acid, the reaction was complete after 2 h. Interestingly, at such temperature, 9-*cis*,11-*trans* isomer production was inversely correlated with catalyst quantities. Indeed, it was 20% for a 5% catalyst load and increased to 70% of total CLA when using 0.2% phosphoric acid. For prolonged reaction times, a sigmatropic rearrangement (26) was observed between CLA isomers, leading to a decrease in 9-*cis*,11-*trans* isomer and an increase in *trans-trans* CLA. Finally, we estimated that a 300°C reaction temperature was not suitable for a dehydration reaction owing to the elevated risk of polymerization side-reactions. Such reactions were indeed observed at this temperature. In our experimental design, a 280°C reaction temperature was shown to be the most appropriate. At this temperature, 0.1% catalyst amount gave the best results. After 5 h, dehydration was almost complete and the dehydrated castor bean oil obtained contained about 54% CLA, with the 9-*cis*,11-*trans* isomer accounting for 61% of the total CLA. In parallel, we also found that prolonged reaction times had to be avoided to limit sigmatropic rearrangement and drastic modification of CLA repartition. Typically, under optimized conditions (phosphoric acid 0.1%, 280°C, 5 h), the FA composition of the obtained dehydrated castor bean oil was C16:0 (1.2%), C18:0 (1.1%), C18:1n-9 *cis* (2.8%), ricinoleic acid (<2%), nonconjugated linoleic acids (33%), C18:3n-6 (0.4%), C20:0 (0.2%), and total CLA (54%) with 61% of 9-*cis*,11-*trans* isomer/total CLA.

Isomerization of dehydrated castor bean oil. The dehydration of castor bean oil allowed us to obtain TAG containing around 54% CLA and 33% of nonconjugated linoleic acid both *cis,cis* and *cis,trans* isomers. We then evaluated the feasibility of transforming *in situ* the remaining nonconjugated linoleic acids into CLA isomers by isomerization reaction to produce highly CLA-enriched TAG. For such isomerization reactions, classical alkaline catalysis with KOH (26–31) cannot be applied on TAG. However, some recent work demonstrated that rhodium or ruthenium transition metals could be used as catalysts for isomerization of TAG (32). Following the

experimental procedure described in this study, we studied the efficiency of Wilkinson catalyst [$RhCl(PPh_3)_3$] to isomerize dehydrated castor bean oil. After 24 h of reaction, isomerization of nonconjugated linoleic acid was determined by GC (Table 5). The addition of phosphine ligand (methoxy triphenylphosphine) was not advantageous, as could be concluded from trials 6 and 7, whereas reactions conducted in the absence of the Lewis acid $SnCl_2$ were unsuccessful, giving no isomerization (data not shown). In the absence of solvent, isomerization was not possible whatever the temperature and catalyst quantity used (trials 1 to 5). We evaluated the influence of the solvent nature using ethanol, octanol, and 2-methyl 2-propanol (Table 6). Best results were obtained with ethanol. The isomerization reaction was very fast and almost complete within 24 h (yield 98.8%). The final dehydrated/isomerized castor bean oil contained about 87.8% CLA. Concerning the repartition of isomers, reaction favored the formation of full *trans* 9,11 isomer. Consequently, the amount of 9-*cis*,11-*trans* in the CLA fraction decreased throughout the reaction time. Such 9-*trans,*11-*trans*-favored production could be explained by the presence of both *cis,cis* and *cis,trans* nonconjugated linoleic acid in the initial dehydrated castor bean oil. During isomerization, the *cis,trans* would be transformed into fully *trans* CLA. This was verified when the isomerization reaction was carried out under the same conditions with sunflower oil, which contains only *cis,cis* nonconjugated linoleic acid. In this case, we observed a preferred synthesis of 9-*cis*,11-*trans* and 10-*trans*,12-*cis* isomers in comparable amounts, whereas production of all-*trans* isomers was very limited (data not shown). Reaction with octanol as solvent was much slower and only reached 82.4% isomerization yield after 24 h with a CLA isomer repartition in favor of *trans*,*trans* isomer. Finally, reaction with 2-methyl 2-propanol tertiary alcohol was unsatisfactory, with a very low yield (1.5%). Therefore, ethanol was selected

TABLE 5
***In situ* Isomerization of Dehydrated Castor Bean Oil (10 g scale) with Wilkinson Catalyst[a]**

Trial	Wilkinson catalyst[b] (mg)	$SnCl_2 \cdot 2H_2O$ (mg)	$(O\text{-}CH_3C_6H_4)_3P$ (mg)	Solvent (10 mL)	Temp. (°C)	Isomerization yield (%)
1	50	40	20	None	60	0
2	50	None	None	None	60	0
3	50	40	20	None	120	0
4	200	160	80	None	60	0
5	50	None	None	None	120	0
6	50	40	20	EtOH	60	95.4
7	50	40	None	EtOH	60	98.8
8	50	40	None	EtOH	25	0
9	50	40	None	Octanol	60	82.4
10	50	40	None	2-Methyl 2-propanol	60	1.5
11	10	40	None	EtOH	60	92.7

[a]Reactions were run under a nitrogen atmosphere for 24 h in 10 mL of solvent.
[b]$RhCl(Pph_3)_3$.

TABLE 6
Influence of Solvent Nature on Reaction Yields and CLA Repartitions in the Isomerization of Dehydrated Castor Bean Oil with Wilkinson Catalyst

Reaction time (h)	Formed CLA isomer repartition (%)[a]				Total CLA (%)	9*c*,11*t* from total CLA[c] (%)	Residual linoleic acid[d] (%)	Isomerization yield (%)
	9*c*,11*t*	10*t*,12*c*	10*c*,12*c*	9*t*,11*t*				
Ethanol (Table 5, trial 7)								
1	35.0	4.7	7.7	20.1	67.5	51.8	20.3	38.5
2	37.9	6.6	8.9	28.6	82.0	46.2	4.9	85.1
4	38.6	7.1	8.6	33.3	87.6	44.0	0.9	97.3
6	37.3	7.2	8.5	34.0	87.7	43.0	0.7	97.9
24	35.0	6.8	8.8	36.6	87.8	40.2	0.4	98.8
Octanol (Table 5, trial 9)								
1	31.6	2.7	6.1	12.9	54.1	59.2	32.3	2.1
2	31.9	2.8	6.5	13.8	55.0	58.0	31.1	5.8
4	30.9	2.8	6.9	14.7	55.3	55.9	28.7	13.0
6	30.8	3.0	7.5	17.2	58.7	52.7	24.6	25.6
24H	20.7	4.8	9.5	32.3	67.4	30.8	5.8	82.4
2-Methyl 2-propanol (Table 5, trial 10)								
1	31.7	2.8	6.7	14.0	54.1	57.4	33.0	0
2	31.9	2.8	6.3	12.5	54.3	59.8	32.8	0.6
4	30.0	2.6	6.5	12.7	54.5	58.0	32.8	0.6
6	30.6	2.6	6.6	12.6	54.9	58.4	32.7	1.0
24	30.4	2.7	6.3	13.0	55.0	57.9	32.5	1.5

[a]% given from total FA composition. Initial dehydrated castor bean oil FA composition: C16:0 (1.2%), C18:0 (1.1%), C18:1n-9 *cis* (2.8%), C18:1 ricinoleic acid (<2%), nonconjugated linoleic acids (33%), C18:3n-6 (0.4%), C20:0 (0.2%), and total CLA (54%) with 61% of 9-*cis*,11-*trans* isomer/total CLA.
[b]9*c*, 11*t* is the abbreviated term for 9-*cis*, 11-*trans* isomer.
[c,d]See Table 1 footnotes.

for further experiments. The feasibility of the reaction was tested at 250°C and did not show any isomerization (Table 5, trial 8). Reaction temperatures higher than 60°C were not tested due to the risk of hydrogenation side-reactions that may occur with elevated temperatures (32). The reaction was also carried out with lower Wilkinson catalyst quantities (Table 5, trial 11). In this case, a good isomerization yield was still obtained; however, it was not quantitative and was slightly lower than the one previously observed with 50 mg catalyst. In conclusion, using the optimized reactions conditions (Table 5, trial 7), the dehydrated/isomerized castor bean oil that was obtained had the following FA composition: C16:0 (1.2%), C18:0 (1.1%), C18:1n-9 *cis* (2.8%), ricinoleic acid (<2%), nonconjugated linoleic acids (<0.5%), C18:3n-6 (0.4%), C20:0 (0.2%), and total CLA (87.8%) with 40.2% of 9-*cis*,11-*trans* isomer/total CLA.

Urea fractionation. The combined dehydration and isomerization of castor bean oil led to an efficient production of TAG highly enriched in CLA. We were also interested in applying urea fractionation technology to try to increase this CLA content even more. Following a recent process describing CLA purification (46), we evaluated this technology on FFA of dehydrated/isomerized castor bean oil. Indeed, the urea fractionation cannot be applied directly on TAG. Results showed an improvement in CLA content in the mother liquor (Table 7). Indeed, CLA counted for 93.6% of total FA in this liquor with a majority of 9-*cis*,11-*trans* (60.8%). On the contrary, a tendency for 9-*trans*,11-*trans* depletion was observed in the liquor. Concerning the urea fraction, we observed that all residual saturated FA were located here. Its CLA content was about 79%, with a majority of *trans,trans* isomer, whereas rumenic acid was just 32% of the CLA isomers in this fraction.

The combination of dehydration and isomerization of castor bean oil is a promising process for production of TAG highly enriched in CLA isomers. This is a clear advantage in comparison with most existing methods, in which CLA are obtained as FFA or alkyl esters, as it was recently demonstrated that TAG forms are best for CLA bioavailability (40). We also showed that urea fractionation of FFA from dehydrated/isomerized castor bean oil could lead to products containing more than 93% CLA as FFA. This amount is superior to most of the commercially available CLA products. Depending on the reaction conditions, catalyst choice and amount, reaction time, and temperature, the repartition of CLA isomers can be modulated to favor 9-*cis*,11-*trans* production.

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REFERENCES

1. O'Shea, A.M., R. Devery, F. Lawless, K. Koegh, and T. Stanton, Enrichment of the Conjugated Linoleic Acid Content of Bovine Milk by Dry Fractionation, *Int. Dairy J. 10*:289–294 (2000).
2. Parodi, P.W., Conjugated Octadecadienoic Acids of Milk Fat, *J. Dairy Sci. 60*:1550–1553 (1977).
3. Pariza, M.W., S.H. Ashoor, F.S. Chu, and D.B. Lund, Effects of Temperature and Time on Mutagen Formation in Pan-Fried Hamburger, *Cancer Lett. 7*:63–69 (1979).
4. Fernie, C.E., Conjugated Linoleic Acid, in *Lipids for Functional Food and Nutraceuticals,* edited by F.D. Gunstone, The Oily Press, Bridgewater, 2003, pp. 291–318.
5. Ip, C., S.P. Briggs, A.D. Saegele, H.J. Thompson, J. Storkson, and J.A. Scimeca, The Efficacy of Conjugated Linoleic Acid in Mammary Cancer Prevention Is Independent of the Level or Type of Fat in the Diet, *Carcinogenesis 17*:1045–1050 (1996).
6. Belury, M.A., A.P. Nickel, C.E. Bird, and Y. Wu, Dietary Conjugated Linoleic Acid Modulation of Phorbol Ester Skin Tumor Promotion, *Nutr. Cancer 26*:149–157 (1996).
7. Lavillonnière, F., and P. Bougnoux, Conjugated Linoleic Acid (CLA) and the Risk of Breast Cancer, in *Advances in Conjugated Linoleic Acid Research, Volume 1,* edited by M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Parisa, and G.J. Nelson, AOCS Press, Champaign, 1999, pp. 276–282.
8. Ochoa, J.J., A.J. Farquharson, I. Grant, L.E. Moffat, S.D. Heys, and K.W.J. Wahle, Conjugated Linoleic Acids (CLAs) Decrease Prostate Cancer Cell Proliferation: Different Molecular Mechanisms for *cis*-9,*trans*-11 and *trans*-10,*cis*-12 Isomers, *Carcinogenesis 25*:1185–1191 (2004).
9. Zambell K.L., N.L. Keim, M.D. Van Loan, B. Gale, P. Benito, D.S. Kelley, and G.J. Nelson, Conjugated Linoleic Acid Supplementation in Humans: Effects on Body Composition and Energy Expenditure, *Lipids 35*:777–782 (2000).
10. Jahreis, G., J. Kraft, F. Tischendorf, F. Schone, and C. von Loeffelholz, Conjugated Linoleic Acids: Physiological Effects in Animal and Man with Special Regard to Body Composition, *Eur. J. Lipid Sci. Technol. 102*:695–703 (2000).
11. Blankson, H., J.A. Stakkestad, H. Fagertun, E. Thom, J. Wadstein, and O. Gudmundsen, Conjugated Linoleic Acid Reduces Body Fat Mass in Overweight and Obese Humans, *J. Nutr. 130*:2943–2948 (2000).
12. Petridou A., V. Mougios, and A. Sagredos, Supplementation with CLA: Isomer Incorporation into Serum Lipids and Effect on Body Fat of Women, *Lipids 38*:805–811 (2003).
13. Malpuech-Brugere, C., W.P.H.G. Verboeket-van de Venne, R.P. Mensink, M.A. Arnal, B. Morio, M. Brandolini, A. Sæbo, T.S. Lassel, J.M. Chardigny, J.L. Sébédio, and B. Beaufrere, Effects of Two Conjugated Linoleic Acid Isomers on Body Fat Mass in Overweight Humans, *Obesity Res. 12*:591–598 (2004).

TABLE 7
FA Composition of Urea Fraction and Mother Liquor After Urea Fractionation of Dehydrated/Isomerized Castor Bean Oil FFA

	Formed CLA isomer repartition[a] (%)				Total CLA (%)	9*c*,11*t* from total CLA (%)	Linoleic acid (%)	Residual ricinoleic acid (%)
	9*c*,11*t*[b]	10*t*,12*c*	10*c*,12*c*	9*t*,11*t*				
Initial substrate	38.3	7.3	9.0	33.2	87.8	40.2	0.4	1.8
Mother liquor	56.9	10.5	9.4	16.8	93.6	60.8	0.5	1.9
Urea fraction	25.3	4.9	9.2	39.6	79.0	32.0	0.2	0.9

[a,b]For footnotes see Table 2.

14. Field, C.J., and P.D. Schley, Evidence for Potential Mechanisms for the Effect of Conjugated Linoleic Acid on Tumor Metabolism and Immune Function: Lessons from n-3 Fatty Acids, *Am. J. Clin. Nutr. 79*:1190S–1198S (2004).
15. Albers, R., R.P.J. van der Wielen, E.J. Brink, H.F.J. Hendriks, V.N. Dorovska-Taran, and I.C.M. Mohede, Effects of *cis*-9, *trans*-11 and *trans*-10,*cis*-12 Conjugated Linoleic Acid (CLA) Isomers on Immune Function in Healthy Men, *Eur. J. Clin. Nutr. 57*:595–603 (2003).
16. Kelley, D.S., J.M. Warren, V.A. Simon, G. Bartolini, B.E. Mackey, and K.L. Erickson, Similar Effects of *c*9,*t*11-CLA and *t*10,*c*12-CLA on Immune Cell Functions in Mice, *Lipids 37*:725–728 (2002).
17. Shen, C.L., D.M. Dunn, J.H. Henry, Y. Li, and B.A. Watkins, Decreased Production of Inflammatory Mediators in Human Osteoarthritic Chondrocytes by Conjugated Linoleic Acids, *Lipids 39*:161–166 (2004).
18. Watkins, B.A., and M.F. Seifert, Conjugated Linoleic Acid and Bone Biology, *J. Am. Coll. Nutr. 19*:478S–486S (2000).
19. Cheng, W.L., C.K. Lii, H.W. Chen, T.H. Lin, and K.L. Liu, Contribution of Conjugated Linoleic Acid to the Suppression of Inflammatory Responses Through the Regulation of the NF-κB Pathway, *J. Agric. Food Chem. 52*:71–78 (2004).
20. Bassaganya-Riera, J., R. Hontecillas, and D.C. Beitz, Colonic Anti-inflammatory Mechanisms of Conjugated Linoleic Acid, *Clin. Nutr. 21*:451–459 (2002).
21. Yu, Y., P.H. Correll, and J.P. Vanden Heuvel, Conjugated Linoleic Acid Decreases Production of Pro-inflammatory Products in Macrophages: Evidence for a PPARγ-Dependent Mechanism, *Biochim. Biophys. Acta 1581*:89–99 (2002).
22. McLeod, R.S., A.M. Le Blanc, M.A. Langille, P.L. Mitchell, and D.L. Currie, Conjugated Linoleic Acids, Atherosclerosis, and Hepatic Very-Low-Density Lipoprotein Metabolism, *Am. J. Clin Nutr. 79*:1169S–1174S (2004).
23. Nagao, K., N. Inoue, Y.M. Wang, J. Hirata, Y. Shimada, T. Nagao, T. Matsui, and T. Yanagita, The 10 *trans*,12 *cis* Isomer of Conjugated Linoleic Acid Suppresses the Development of Hypertension in Otsuka Long-Evans Tokushima Fatty Rats, *Biochem. Biophys. Res. Commun. 306*:134–138 (2003).
24. Kritchevsky, D., S.A. Tepper, S. Wright, P. Tso, and S.K. Czarnecki, Influence of Conjugated Linoleic Acid (CLA) on Establishment and Progression of Atherosclerosis in Rabbits, *J. Am. Coll. Nutr. 19*:472S–477S (2000).
25. Salminen, I., M. Mutanen, M. Jauhiainen, and A. Aro, Dietary *trans* Fatty Acids Increase Conjugated Linoleic Acids Levels in Human Serum, *J. Nutr. Biochem. 9*:93–98 (1998).
26. Sæbo, A., Commercial Synthesis of Conjugated Linoleic Acid, in *Advances in Conjugated Linoleic Acid Research, Volume 2,* edited by J.-L. Sébédio, W.W. Christie, and R. Adlof, AOCS Press, Champaign, 2003, pp. 71–81.
27. Chin, S.F., W. Liu, J.M. Storkson, Y.L. Ha, and M.W. Pariza, Dietary Sources of Conjugated Dienoic Isomers of Linoleic Acid, a Newly Recognized Class of Anticarcinogens, *J. Food Compos. Anal. 5*:185–197 (1992).
28. Delmonte, P., J.A.G. Roach, M.M. Mossoba, G. Losi, and M.P. Yurawecz, Synthesis, Isolation, and GC Analysis of All the 6,8- to 13,15-*cis/trans* Conjugated Linoleic Acid Isomers, *Lipids 39*:185–191 (2004).
29. Bernas, A., N. Kumar, P. Maki-Arvela, N.V. Kul'kova, B. Holmbom, T. Salmi, and D.Y. Murzin, Isomerization of Linoleic Acid over Supported Metal Catalysts, *Appl. Catal. A–General 245*:257–275 (2003).
30. Bernas, A., P. Maki-Arvela, N. Kumar, B. Holmbom, T. Salmi, and D.Y. Murzin, Heterogeneously Catalytic Isomerization of Linoleic Acid over Supported Ruthenium Catalysts for Production of Anticarcinogenic Food Constituents, *Ind. Eng. Chem. Res. 42*:718–727 (2003).
31. Bernas, A., N. Kumar, P. Maki-Arvela, B. Holmbom, T. Salmi, and D.Y. Murzin, Heterogeneous Catalytic Production of Conjugated Linoleic Acid, *Org. Process Res. Develop. 8*:341–352 (2004).
32. Larock, R.C., X.Y. Dong, S. Chung, C.K. Reddy, and L.E. Ehlers, Preparation of Conjugated Soybean Oil and Other Natural Oils and Fatty Acids by Homogeneous Transition Metal Catalysis, *J. Am. Oil Chem. Soc. 78*:447–453 (2001).
33. Radlove, S.B., W.M. Dejong, and L.B. Falkenburg, A Continuous Process for the Dehydration of Castor Oil, *Ibid. 25*:268–271 (1948).
34. Terrill, R.L., Dehydration of Castor Oil, *Ibid. 27*:477–481 (1950).
35. Waheeduddin, M., M.A. Siva Samba, and J.S. Aggarwal, A Comparative Study of Some Catalysts for the Dehydration of Castor Oil, *Paintindia 16*:24–26 (1966).
36. Berdeaux O., W. Christie, F.D. Gunstone, and J.L. Sébédio, Large-Scale Synthesis of Methyl *cis*-9,*trans*-11-Octadecadienoate from Methyl Ricinoleate, *J. Am. Oil Chem. Soc. 74*:1011–1015 (1997).
37. Yang, L., Y. Huang, H.Q. Wang, and Z.Y. Chen, Production of Conjugated Linoleic Acids Through KOH-Catalyzed Dehydration of Ricinoleic Acid, *Chem. Phys. Lipids 119*:23–31 (2002).
38. Rainio, A., M. Vahvaselka, T. Suomalainen, and S. Laakso, Reduction of Linoleic Acid Inhibition in Production of Conjugated Linoleic Acid by *Propionibacterium freudenreichii* ssp. *shermanii*, *Can. J. Microbiol. 47*:735–740 (2001).
39. Ogawa, J., K. Matsumura, S. Kishino, Y. Omura, and S. Shimizu, Conjugated Linoleic Acid Accumulation *via* 10-Hydroxy-12-octadecaenoic Acid During Microaerobic Transformation of Linoleic Acid by *Lactobacillus acidophilus*, *Appl. Environ. Microbiol. 67*:1246–1252 (2001).
40. Alonso, L., E.P. Cuesta, and S.E. Gilliland, Production of Free Conjugated Linoleic Acid by *Lactobacillus acidophilu*s and *Lactobacillus casei* of Human Intestinal Origin, *J. Dairy Sci. 86*:1941–1946 (2003).
41. Ando, A., J. Ogawa, S. Kishino, and S. Shimizu, CLA Production from Ricinoleic Acid by Lactic Acid Bacteria, *J. Am. Oil Chem. Soc. 80*:889–894 (2003).
42. Lee, S.O., G.W. Hong, and D.K. Oh, Bioconversion of Linoleic Acid into Conjugated Linoleic Acid by Immobilized *Lactobacillus reuteri*, *Biotechnol. Prog. 19*:1081–1084 (2003).
43. Lin, T.Y., C.W. Lin, and Y.J. Wang, Production of Conjugated Linoleic Acid by Enzyme Extract of *Lactobacillus acidophilus* CCRC 14079, *Food Chem. 83*:27–31 (2003).
44. Fernie, C.E., I.E. Dupont, O. Scruel, Y.A. Carpentier, J.L. Sébédio, and C.M. Scrimgeour, Relative Absorption of Conjugated Linoleic Acid as Triacylglycerol, Free Fatty Acid and Ethyl Ester in a Functional Food Matrix, *Eur. J. Lipid Sci. Technol. 106*:347–354 (2004).
45. Diks, R.M.M., and J.A. Bosley, The Exploitation of Lipase Selectivities for the Production of Acylglycerols, in *Enzymes in Lipid Modification,* edited by U.T. Bornscheuer, Wiley-VCH Press, Weinheim, Germany, 2000, pp. 3–22.
46. Ma, D.W.L., A.A. Wierzbicki, C.J. Field, and M.T. Clandinin, Preparation of Conjugated Linoleic Acid from Safflower Oil, *J. Am. Oil Chem. Soc. 76*:729–730 (1999).

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