# Study on preparation of conjugated linoleic acid

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**Abstract**

Conjugated linoleic acid has good physiological functions. The reactive mechanism of conjugation catalyzed by alkali and the factors affecting the conjugation such as reaction time and alkali dosage were discussed. The optimal conditions were obtained by orthogenesis test, which resulted in a conjugation yields above 90%.

**Key words:** linoleic acid; alkali catalizatioin; conjugation

## Introduction

Conjugated Linoleic Acids (CLA) were first reported in 1987 (Ha et al., 1987). It is a term used to describe a group of positional and geometric isomers of linoleic acid in which the two double bonds are conjugated (Ha, et al.,1989). It has many kinds of physiological activities such as anticarcinogenic and anti-atheromatous, participating on the disassemble and metabolism of fat, increasing body immunity (Zhang et al., 2000).

Natural CLA is found in meat and milk of ruminant such as cattle and sheep. It is seldom in other animal and vegetable oils. So artificial synthesis is of importance. There were many methods for the synthesis, such as dehydration of allylic hydroxy oleate, alkali catalyzation and enzyme catalyzation (Chen et al., 2000). Among these methods, alkali catalyzation is the most efficient one. In this study, the reactive mechanism and optimal conditions of alkali catalyzing method were studied.

## Material and Methods

Materials: Sunflower oil was purchased from Inner Mongolia. NaOH and $H_2SO_4$ were obtained from Luoyang Chemical Co. Inc. Glycol and hexane were purchased from Tianjin Chemical Co. Inc.

Alkali-catalyzed conjugation (Nie et al.,1997) : Sunflower oil, hexane and alkali were transferred to three joint flasks with a condenser, an agitator, a temperature sensor and air intaker. Temperature sensor should be protected from the solution by vitreous duct with bottom closed. The reaction was performed under nitrogen. The product was cooled to about 40°C, neutralized by oil of vitriol and water fluid. The topper was separated and dried by sodium sulfate and conserved in refrigerator.

Fatty acid analysis: Fatty acids of alkali-catalyzed product were converted to the corresponding fatty acid methyl esters with a mixture of 14% $BF_3$ in methanol and toluene (1:1,v/v) under nitrogen at 90°C for 45min. Fatty acid methyl esters were analyzed on a flexible silica capillary column with an Angelent 5980N gas chromatograph, equipped with a flame-ionization detector and an automated injector. Column temperature was programmed from 180 to 220°C at a rate of 1°C/min and then held for 10 min. Injector and detector temperatures were set at 250 and 300°C, respectively. Hydrogen was used as the carrier gas at a head pressure of 15 psi.

## Results

*Composition of sunflower oil*

**Table 1 The fatty acid composition of sunflower oil**

| Fatty acid | Palmic acid | Stearic acid | Oleic acid | Linoleic acid | Linoleinic acid |
|---|---|---|---|---|---|
| Content | 6.87 | 5.38 | 22.94 | 63.73 | 0.56 |

Linoleic acid is the main fatty acid in sunflower oil (Table 1). Five carbons of the double bonds in linoleic acid are in a plane, so the trans-construct has low energy. The product of alkali-catalyed conjugation is mostly the formation of cis-trans. 9c,11t-18:2 and 10t,12c-18:2 acids account for above 70%. And 9c,11t-18:2 has be proved to have physiological activities.

There were many other reactions along with conjugation. So it is difficult to reflect the rule of the reaction. In this test, palmic acid that is steadiness in the reaction was used as contrast. Relative concentration of other fatty acid is the ratio between the content of a particular acid and the content of palmic acid.

*Effect of time on the reaction*

The test was performed at 190°C with a solvent dosage of 3 times of sunflower oil volume and alkali dosage of 1.4 times of the saponification equivalent of sunflower oil. As shown in Fig 1, with the time prolonged, oleic acid content did not change. The content of CLA increased rapidly within 3 hours and reached the peak at 5 hour. After 5 hour, little change could be detected. When the reaction time was extended, the amount of byproduct increased too, thus the physiological activities should be decreased. In sunflower oil, the content of linoleinic acid is below 1%. So the influence of linoleinic acid on the reaction is neglectable. Hence, 4-5 hours is suitable for the reaction.

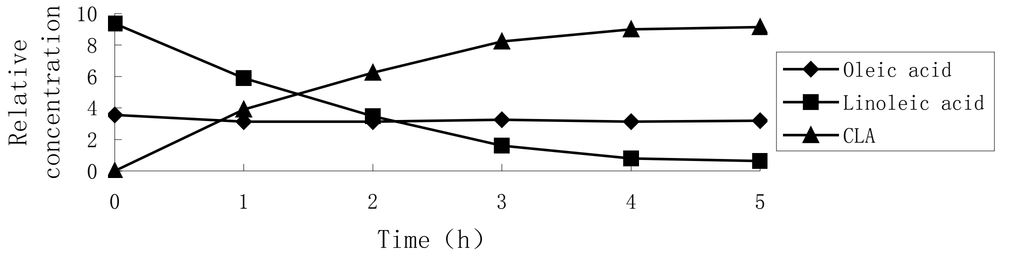


Fig 1 The effect of time on reaction

*Effect of alkali amount on the reaction*

With the increase of alkali amount, the content of oleic acid did not change (Fig 2). When the alkali dosage was 1.1 times of saponification equalivent, the conversion rate of linoleic acid was very low. When it ws 1.7 times, the conversion rate reached the peak. Continuous increase of the alkali dosage did not result in any change of the conversion rate. The reaction turned slower if the alkali dosage was only equal to saponification equalivent, because saponification is easier than conjugation. As a catalyzer, when alkali concentration reached a certain value, the effective collision among molecules reached the peak. After that, increase of the catalyzer will not change the effective collision.

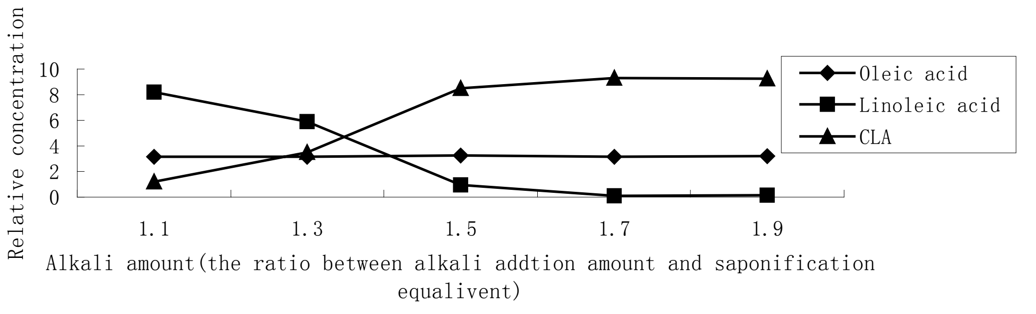


Fig 2 The effect of alkali amount on the reaction

*Effect of solvent dosage on the reaction*

It was observed that superfluous foam occurred when solvent dosage is very small, for example, at 1:2 (v/v). It would be difficult to separate after conjugation and cause the lose of CLA significantly. If the solvent dosage is very high, it will decrease the effective collision,results in the decrease of the conjugation.

*Selection of optimal condition*

According to the single factor experiment, four factors and three levels was designed as Table 2, 3,4.

**Table 2 Factors and levels of orthogonal experiment**

| | A:Temperature (°C) | B:Time (h) | C:Ratio between alkali amount and saponification equalivent | D:Ratio between volumes of sunflower oil and solvent |
|---|---|---|---|---|
| 1 | 190°C | 1 | 1.7 | 1:7 |
| 2 | 170°C | 7 | 1.5 | 1:3 |
| 3 | boiling | 4 | 1.3 | 1:5 |

Alkali amount and time has observably effect on the reaction. The optimal condition was A (boiling),

B (7h), C (1.7), D (1:5) in Table 3. It can be calculated that the optimal condition is A (190°C), B (4h), C (1.7), D (1:3). Its conjugation rate was 99.5% under the condition.

## Conclusions

Alkali-catalyed conjugation of oil is a complex reaction. Under proper conditions, conjugation is taking place mainly. The content of linoleic acid in sunflower oil is high. The optimal condition of alkali-catalyed conjugation is that alkali dosage of 1.7 times of saponification equalivent, with solvent volume 3 times of sunflower oil, and the reaction for 4 hours at 190°C.

**Table 3 The condition of orthogonal experiment**

| | A | B | C | D | Relative concentration | | Average value | Convertion rate |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 6.14 | 5.68 | 5.91 | 63.8% |
| 2 | 1 | 2 | 2 | 2 | 9.21 | 9.15 | 9.18 | 99.1% |
| 3 | 1 | 3 | 3 | 3 | 6.06 | 6.38 | 6.22 | 67.1% |
| 4 | 2 | 1 | 2 | 3 | 4.85 | 4.77 | 4.81 | 51.9% |
| 5 | 2 | 2 | 3 | 1 | 2.44 | 2.1 | 2.27 | 24.5% |
| 6 | 2 | 3 | 1 | 2 | 8.46 | 8.32 | 8.39 | 90.6% |
| 7 | 3 | 1 | 3 | 2 | 3.64 | 3.42 | 3.53 | 38.1% |
| 8 | 3 | 2 | 1 | 3 | 9.3 | 9.22 | 9.26 | 99.9% |
| 9 | 3 | 3 | 2 | 1 | 8.49 | 8.41 | 8.45 | 91.2% |
| I | 42.62 | 28.5 | 47.12 | 33.26 | | | | |
| II | 30.94 | 41.42 | 44.88 | 42.2 | | | | |
| III | 42.48 | 46.12 | 24.04 | 40.58 | | | | |
| R | 11.68 | 17.62 | 23.08 | 8.94 | | | | |

**Table 4 Analysis of variance on date of orthogonal experiment**

| Soruces of variation | A | B | C | D | $S_{e1}^2$ | $S_{e2}^2$ | $S_e^2$ |
|---|---|---|---|---|---|---|---|
| Sum of square | 14.9785 | 27.7489 | 54.0005 | 7.5628 | 22.5413 | 0.1634 | 22.7047 |
| df. | 2 | 2 | 2 | 2 | 4 | 9 | 13 |
| Mean squared deviation | 7.4893 | 13.8745 | 27.0003 | 3.7814 | 5.6762 | / | / |
| F value | 4.2881 | 7.9441 | 15.4595 | 2.1651 | $F_{0.95}$ (2, 13) =3.80 | | |
| Significance | * | * * | * * | / | $F_{0.99}$ (2, 13) =6.70 | | |

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