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Conjugated Linoleic Acid Production and Optimization Via Catalytic Reaction Method Using Safflower Oil

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ABSTRACT

Conjugated linoleic acid (CLA) is one of the most physiological active fatty acids due to its positive effect on prevention of diseases such as obesity, hyperlipidemia, atherosclerosis and cancer. The aim of this study was to optimize the production of conjugated linoleic acid (CLA)% in alkali isomerization of safflower oil including 71% linoleic acid (LA) using response surface methodology (RSM). The effect of three reaction variables such as temperature, reaction time and amount of KOH on CLA production was investigated using design-expert software. The optimal condition determined at this point, 72% total CLA with the yield of 84% temperature reaction time and the amount of KOH were 187.6 ºC, 2.12 h 48.7 g; respectively. Further results indicate that just two reaction parameters including temperature and the amount of catalyst have significant effects on CLA production %; however,the reaction time showed negligible impact on CLA production.

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1. INTRODUCTION[1]

Today, diseases such as obesity, hyperlipidemia, atherosclerosis, diabetes, cancer and high blood pressure are increasing in industrialized countries. Although, the exact mechanism of these disease associated with lifestyle is not so clear. But, it seems that the quality of dietary fat can be an important factor to be considered in this context.

Conjugated fatty acids due to their positive effect on the prevention of these diseases, have attracted much attention. Conjugated linoleic acid (CLA) is one of the most physiological active fatty acids [1]. CLA is a collective term used to describe a mixture of octadecadienoic acids (18:2) that possess a conjugated double-bond system [2]. The *9c,11t*-18:2 and *10t,12c*-18:2 isomers of CLA are believed to be responsible for the beneficial physiological effects of CLA [3]. Natural sources of CLA include beef (0.43% of total beef lipids) and dairy products (0.40 to 0.55% of total dairy lipids) [4]. The *9c,11t*-18:2 CLA, also called rumenic acid, is the main isomer, constituting 90% of the total CLA found in dairy and beef lipids [5]. CLA exhibits many biological effects such as anti-carcinogenic activity [6], immune stimulation [7], body fat reduction [8], cholesterol reduction in blood [9], and lowering atherosclerosis symptoms [10]. About 3 grams of CLA per day are required to provide health needs, although our receive from natural resources is about 10% of this value. Therefore, it is necessary to produce CLA as a dietary supplement or enriching the vegetable oils and dairy products with CLA [11].

Commercial production of CLA includes, alkali isomerization of linoleic acid, dehydration of ricinoleic acid methyl ester [12] and microbial synthesis of *9c,11t* -18:2 from linoleic acid using cultures of different microorganisms [13]. Each of these methods produces a different mixture of CLA isomers. Among these methods, alkali isomerization of linoleic acid is the most common method for production of CLA because it is economically valuable [14].

This study presents the optimization of CLA% (two essential isomers) production in alkali isomerization of safflower oil. It was effected using response surface methodology (RSM) and the effect of three reaction

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variables (amount of KOH, temperature, and reaction time) on CLA production using design-expert software.

2. MATERIALS METHOD

2. 1. Materials Safflower oil was obtained from a local grocery store (Saveh, Iran). Chemicals and solvents were all of analytical grade and purchased from Merck (Darmstadt, Germany). Pure fatty acids as standard, including palmitic, stearic, oleic, linoleic and linolenic acids were purchased from Sigma Chemical Co. (St Louis, MO, USA).

2. 2. Determination of the Composition of Fatty Acids in Safflower Oil In order to realize the fatty acid composition of safflower oil used to produce CLA, the safflower oil was methylated according to AOCS Ce 2-66 methodc [15]. 5 ml of sodium hydroxide solution (0.5 M) were added to 250 mg of safflower oil in a round bottom flask and boiled under total reflux for 10 min, 5 ml of methanolic boron trifluoride were added and boiled for another 2 min, then 3 ml of n-heptane added and boil for 1 min. After addition of saturated sodium chloride solution, heptane phase was removed and 1µl of that was injected to GC MSD [16].

The gas chromatographic analysis of fatty acid methyl esters (FAMEs) was performed on an Agilent 5975c (Agilent Technologies, Santa Clara, CA, USA) gas chromatograph. The column used was a HP-5 MS silica capillary (30 m, 0.32 mm i.d., 0.25 µm film thickness) type. The temperature program consisted of 110ºC for 2min and then increasing the temperature to 230ºC at a rate of 5 ºC/min and held for 5min, then increased to 270ºC at a rate of 10ºC/min and held for 5 min. Temperatures for injector and detector were 270 ºC. Ultra high pure helium was used as the carrier and makeup gas. The flow rate of the makeup gas was 30 ml/min. The pressure was maintained at 10 psig on the column in order to obtain the flow rate of the carrier gas at 1.2 ml/min. The split ratio was set at 1:100 [17].

2. 3. CLA Production The method described by Kim et al. [18] with improvement was utilized to synthesize CLA. 100 g safflower oil 71% linoleic acid (LA) was added to a three-neck round-bottom flask contains a solution of KOH (according to RSM runs) in 150 g propylene glycol, nitrogen was bubbled through the ethylene glycol and flask heating in an oil bath. After a reaction time (according to RSM runs) the mixture was left to cool down to 80 ºC in 2 hours. The solution was transferred to a separation funnel and acidified (pH <2) with hydrochloric acid. After dilution with 50 ml of deionized H_2O (d H_2O), the CLA was extracted with 3×50 ml of hexane. The hexane extract was washed with 3×50 ml solution of 30% methanol in distilled (d) H_2O and then with 3×50 ml dH_2O. Anhydrous sodium sulfate was added to remove water. The hexane was evaporated by a vacuum rotary evaporator at 70 ºC to obtain CLA. The CLA was stored in dark containers which occupied with nitrogen in the refrigerator and CLA content after esterification was determined by gas chromatography.

2. 4. Determination of The Composition of CLA in the Product The methyl esters of CLA were prepared based on the AOCS Ce 2-66 method [15]. 5 ml of methanolic boron trifluoride were added to 100 mgCLA mixture in a 50 ml round-bottom flask. Then the flask was boiled under total reflux condition for 2 min. This followed by addition of 3 ml n-heptane and boiled for another 1 min. After addition of saturated sodium chloride solution, heptane phase was removed and 1µl of that was injected to GC MSD.

The conversion of CLA% production was evaluated with the following equation: Equation (1)

$$\text{Conversion of CLA} = \frac{\text{desired product (GC MSD response\%)}}{\text{desired product + unreactioned LA (GC MSD response\%)}} * 100 \quad (1)$$

According to Equation (1), 100% conversion could be obtained when the whole amount of the raw material turned into a desired product.

2. 5. Experimental Design For RSM Optimization of production amount of two essential isomers (*9c,11t*-18:2 , *10t,12c*-18:2) of CLA were the most important issues in this study. The surface response method was used to optimize the process in order to maximize production of *9c,11t*-18:2 and *10t,12c*-18:2 from a chemical pathway. The experimental plan was designed and the results obtained were analyzed using Design Expert version 7.0.0 (Stat-Ease Inc., Minneapolis, MN) software to build and evaluate models. The planning of tests was proceed based on the central combinational plan of three factors with each factor in 3 levels with 2 iterations in a central point containing 16 runs [19-21]. The amount of catalyst (A: 30,45,60g) reaction temperature (B: 150,170,190 ˚C) and reaction time (C: 1,2,3 h) were used as the variables to maximize the responses of (*9c,11t*-18:2 (%) and *10t,12c*-18:2 (%)).

2. 6. Test Validation Method validation done by repeating injections to GC MSD, These results are the average of response of 3 injections to GC MSD. Also if the results of a run was doubtful, the run was repeated and the results was checked out again.

3. RESULTS AND DISSCOTION

3. 1. Fatty Acids Profile of Safflower Oil GC MSD analyses of the methylated safflower oil revealed

a mixture of 71.11 % linoleic acid (18:2), 16.98 % oleic acid (18:1), 8.75% palmitic acid (16:0) and 3.17% stearic acid (18:0) methyl esters. Figure 1 presents the chromatogram result of methylated safflower oil.

3. 2. Optimization of CLA Production To optimize and maximize the amount of CLA production, the response surface methodology (RSM) was used. This method allows us to investigate the effect of different variables on the result with the lowest number of experiments and it gives a relationship between CLA production and independent variables included the amount of catalyst (KOH), reaction temperature and reaction time as a mathematical equation.

In this study two responses were evaluated *9c,11t*-18:2 and *10t,12c*-18:2 production% (Table 1). The results were similar to each other for both isomers; therefore, it was concluded here that we only consider the results of *9c,11t*-18:2 CLA production. For creating response surfaces, the data obtained based on the above design was fitted to a second-order polynomial equation of the form [22]. The coefficients of independent variables determined for the model for the *9c,11t*-18:2 production% is given by Equation (2)

$$\text{CLA } 9c,11t \% = -115.78937+4.64608*A-0.052690*B+20.55889*C-0.015462*AB-0.052750*AC-0.054062*BC-0.018295*A^2+3.22155E-003*B^2-1.90638*C^2 \quad (2)$$

Analysis of variance (ANOVA table, Table 2) also showed that the regression model for *9c,11t*-18:2 production was statistically good with a significance level of $p < 0.0008$ and the models had no significant ($p > 0.05$) lack of fit. Thus, well-fitting models for *9c,11t*-18:2 production were successfully established. Also, the actual and predicted plot for the responses presented in Figure 2, confirm the mentioned results.

The "Pred R^2" is 0.7027 and the "Adj R^2" is 0.9204, these two values are not close but it is normally expected. This may indicate a block effect.

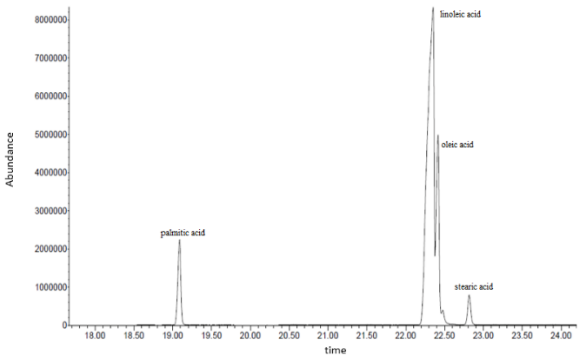

Figure 1. Chromatogram of methylated safflower oil (19.1 min palmitic acid 8.75%, 22.3 min linoleic acid 71.11%, 22.4 oleic acid 16.98% and 22.8 stearic acid 3.17%)

TABLE 1. Experimental conditions and results for CLA% production

Run	A:KOH (g)	B:tempreture (°C)	C:Time (h)	*9c,11t* %	*10t,12c* %
1	60.00	190.00	3.00	32.79	33.31
2	30.00	150.00	1.00	9.93	11.44
3	45.00	170.00	2.00	33.6	34.77
4	45.00	170.00	3.00	31.53	34.89
5	30.00	170.00	2.00	24.55	26.21
6	45.00	150.00	2.00	30.63	31.15
7	45.00	170.00	2.00	33.68	31.7
8	60.00	150.00	1.00	30.34	32.38
9	30.00	190.00	3.00	34.1	35.12
10	30.00	150.00	3.00	17.51	17.92
11	60.00	190.00	1.00	32.7	33.7
12	30.00	190.00	1.00	33.24	36.03
13	60.00	150.00	3.00	32.36	32.82
14	60.00	170.00	2.00	30.95	31.45
15	45.00	190.00	2.00	35.68	35.93
16	45.00	170.00	1.00	28.39	29.79

According to Table 2, p-value of time variable is not significant and Figure 3 (a, b) also states that changes in time effects on *9c,11t*-18:2 production% was very slightly and 2 hours residence time is enough for desired production of CLA. The temperature of the reaction was an important factor in the CLA production% and as the temperature increased, the production increased as well.

TABLE 2. ANOVA results for the model of design expert 7.0.0 for *9c,11t* CLA% production

Response		*9c,11t*CLA% Production
P-value	model	0.0008
	A:KOH	0.0006
	B:temprature	0.0002
	C:time	0.0654
Lack of fit		0.0204
R^2		0.9682
Adj.R^2		0.9204
Pred.R^2		0.7027
Adeq. precision		18.035
S.d.*		1.92
C.V.**		6.52

*standard deviation

**Coefficient of Variation

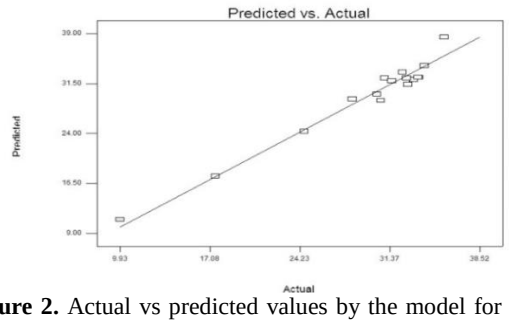

Figure 2. Actual vs predicted values by the model for *9c,11t* CLA

This is more noticeable when the amount of catalyst is lower than 50 g. The amount of KOH used for the reaction was another important factor in CLA production%. With increasing the amount of KOH up to 50 g, CLA production % increased but at higher amounts it is slightly decreased. According to Figure 3(c), at high temperatures, the catalyst amount is not as effective as the low temperatures.

The uncoded coordinate of stationary point for the CLA yield was 184 ˚C, 2.29 h and 50.64 g of KOH. At the optimum point, the maximum predicted value of the CLA yield was 36.06% for *9c,11t*-18:2 and 36.19% for *10t,12c*-18:2. The results of experimental studies under the same conditions for CLA production revealed nearly 35.94% of *9c,11t*-18:2 and 36.21% of *10t,12c*-18:2. Figure 4 shows the optimal point for CLA production by chromatogram.

In Table 3, results of this study was compared with the other methods for CLA production [5, 14, 21] and a similar chemical method [18] to this study. It is clear that photochemical method is not suitable for the production of CLA as a separate product and this method can be used just for oil enrichment with CLA.

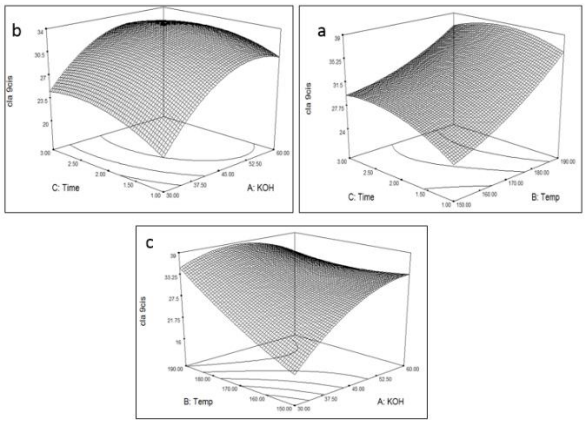

Figure 3. Response surface plot for the effect of temperature and time at center level of catalyst (a), effect of catalyst and time at center level of temperature (b) effect of catalyst and temperature at center level of time (c) on the *9c,11t*18:2%production

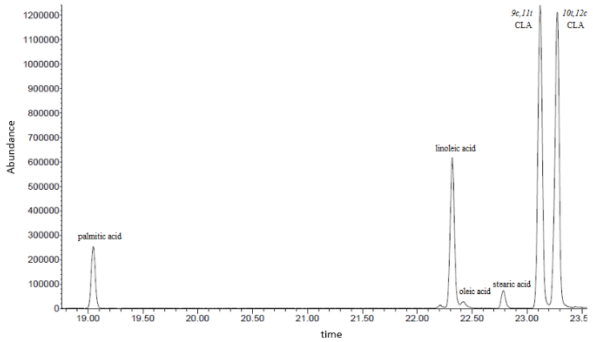

Figure 1. Chromatogram of maximum CLA production (23.11min *9c,11t*-18:2 35.94%, 23.3 min *10t,12c*-18:2 36.21%)

TABLE 3. Comparative results for CLA production via different methods by different starting material

Production method	Starting material	CLA content: *9c,11t +10t,12c*	yield
Microbial synthesis [21]	Ricinoleic acid (89%)	44%	36%
Dehydration of ricinoleic acid methyl ester [14]	Ricinoleic acid (89%)	72%	77%
Alkali isomerization of linoleic acid [18]	Linoleic acid (80%)	70%	-
Photochemical synthesis [5]	Linoleic acid (53%)	0.6%	-
This study	Linoleic acid (71%)	72%	84%

Also, microbial synthesis produces CLA in very low amounts meanwhile, is not recommended for high CLA production and even the content of undesired isomers is high. Dehydration of ricinoleic acid methyl ester can produce CLA with considerable yield and the isomers content are suitable either, but the reaction is complicated, long and due to utilization of different chemical materials in the reaction, isolation of CLA is confusing.

The result for the present investigation was compared with similar work performed by Kim et al. [18]. As, they did not optimize CLA production, they reached 70% total CLA from 80% initial linoleic acid content.

4. CONCLUSION

An applicable method was developed to prepare CLA from safflower oil that can be also applied on a commercial scale. The starting material, safflower oil, is plentifully abundant. In this study, CLA production was optimized and maximized by RSM method. Obtained

results showed that this method has the potential of being scaled up to produce an inexpensive CLA product. The resulting oil contained more than 72% CLA and the conversion efficiency for linoleic acid to CLA was 84%. Optimal reaction conditions were: 184˚C of reaction temperature, 2.29 h of reaction time and 50.64 g of KOH as catalyst and just two reaction parameters including temperature and the amount of catalyst has significant effects on CLA production %.

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Conjugated Linoleic Acid Production and Optimization Via Catalytic Reaction Method Using Safflower Oil

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چکیده

لینولئیک اسید مزدوج به مجموعه ای از ایزومرهای فضایی و موقعیتی لینولئیک اسید گفته می‌شود که فعالیت های بیولوژیکی منحصر به فردی خصوصاً پیشگیری از سرطان و درمان چاقی از خود نشان داده‌اند. هدف از این پژوهش بهینه کردن مقدار تولید CLA در واکنش ایزومریزاسیون قلیایی روغن گلرنگ حاوی 71٪لینولئیک اسید با استفاده از طرح RSM و بررسی تاثیر سه متغیر دمای واکنش، زمان واکنش و مقدار قلیا مورد استفاده است. برای این منظور از نرم افزار design-expert بهره گرفته شد و نقطه بهینه با شرایط دمای187/6 ˚c، زمان واکنش2/12ساعت و مقدار قلیا48/7گرم تعیین شد که در این نقطه مجموعا 72٪ CLA با بهره 84٪تولید شد. نتایج نشان می‌دهند که دو پارامتر تاثیر گذار بر این واکنش دما و مقدار قلیا است و زمان واکنش تاثیر چندانی بر میزان تولید ندارد.

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