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Effect of Extrusion Parameters on Conjugated Linoleic Acids of Corn Extrudates

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The effects of extrusion temperature, 150−190 °C, and torque, 50−70%, on the content and configuration of conjugated linoleic acids (CLA) in corn extrudates were analyzed by GC and HPLC. At a temperature of 150 °C, CLA content increased from 1.2 mg/g of oil in feed to 7.8 mg/g of oil in corn extrudates. A decrease in total CLA ($P < 0.05$) was obtained when the product temperature was further increased to 190 °C. Alteration of CLA geometrical configuration was observed at higher extrusion temperatures. *trans*,*trans*-CLA significantly increased ($P < 0.05$) from 10.2% in feed to 11.9% of CLA at the extrusion condition of 190 °C and 70% torque. The highest expansion of extrudates was found at the product temperature of 150 °C and 70% torque. This extrusion condition also gave the maximum total CLA content and minimum *trans*,*trans*-CLA formation.

KEYWORDS: Conjugated linoleic acid; CLA; extrusion

INTRODUCTION

Conjugated linoleic acid (CLA) was first identified in grilled ground beef (*1*). Isomerization and desaturation were found to be involved with the CLA synthesis in ruminants (*2*), whereas commercial CLA is mainly produced by alkaline isomerization. A number of studies have reported its anticarcinogenic property in several animal organs, such as the forestomach, lung, large intestine, and colon (*3*−*6*). Moreover, CLA also has been shown to contain antioxidant and antiatherosclerotic properties as well as body fat reduction activity (*6*−*9*). CLA has gained considerable attention after being found to have these physiological benefits, and, as a result, the enhancement of CLA in natural food has been widely studied. In food processing, Ha et al. (*1*) reported that CLA formation involved the oxidation of linoleic acid. It was explained that radicals from the oxidation reacted with protons from hydrogen donors, such as proteins, and then rearranged to form conjugated diene structures. Therefore, lipid and protein contents are important for CLA formation. Shantha et al. (*10*) and Lin et al. (*11*) also reported this finding in dairy products.

Besides food components, the influences of food processing such as temperature, air, and starter cultures on CLA contents were also reported in Cheddar-type cheese processing (*12*). However, there are no published reports in terms of thermomechanical processing. Mechanical and thermal treatment in extrusion probably modulates the conversion of linoleic acid into CLA. The objective of this study was to investigate the effect of extrusion conditions on CLA content and configurations in corn extrudates. Total CLA contents and fatty acid profiles were analyzed by using a gas chromatographic (GC) method (*13*), whereas silver high-performance liquid chromatography (Ag^+-HPLC) (*14*) was used to determine the proportion of *cis*,*cis*, *cis*,*trans*/*trans*,*cis*, and *trans*,*trans* isomers of CLA.

MATERIALS AND METHODS

Materials and Chemicals. Corn meal was obtained from Thai Maize Industrial Co., Ltd. (Bangkok, Thailand). Sunflower oil was purchased from Tanakorn Oil Product Ltd. (Samutprakran, Thailand). Three standard CLA methyl ester isomers, *cis*9,*cis*11, *cis*9,*trans*11, and *trans*9,*trans*11, 98% purity, were purchased from Matreya, Inc. (Pleasant Gap, PA) and stored at −20 °C. Standard fatty acid methyl ester mixtures and the C17 internal standard were purchased from Sigma (St. Louis, MO) and stored at −20 °C. All chemicals and solvents were of reagent grade and purchased from Sigma and Fisher Scientific, Inc. (Pittsburgh, PA).

CLA Synthesis by Alkaline Isomerization Method. Two reaction steps were used to synthesize a mixture of CLA methyl ester isomers from sunflower oil. Sunflower oil was methylated to fatty acid methyl ester (FAME) by sodium methoxide and extracted by hexane. After the evaporation of hexane at 40 °C using a rotary evaporator, linoleic acid methyl ester was changed to CLA by isomerization and acidification according to the method of Berdeaux et al. (*15*), but ethylene glycol was used instead of dichloromethane and methanol. This CLA oil was used in preparation for corn extrusion.

Viscosity Measurement. The viscosity of sunflower oil and isomerized oil was measured at 25 °C with a Brookfield model RVTD rotational viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA) using a no. 2 spindle.

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Extrusion and Sample Preparation. The extrusion was carried out using a MPF19:25 twin-screw extruder (APV Baker, Peterborough, U.K.) with an L/D (barrel length/barrel diameter) ratio of 15:1 and equipped with a 3 mm diameter die. Screw configuration was set for direct-expanded product, which was a combination of mixing and conveying elements. The barrel temperatures were set to accommodate the product temperatures of 150, 170, and 190 °C. The water feed rate was controlled to acquire the torque of 50, 60, and 70%. The extrudates were collected and dried at ambient temperatures.

Corn meal mixed with 2% sunflower oil was used as starting feed for assessing total CLA content affected by extrusion conditions, whereas corn meal mixed with 2% CLA oil was used to estimate the alteration of CLA configuration during extrusion. All experiments were operated at constant screw speed (232 rpm) and a feed rate of 9.08 kg/h for the treatment with 2% sunflower oil or 9.24 kg/h for the treatment of corn meal mixed with 2% CLA oil.

Specific Mechanical Energy (SME). The SME was calculated according to the method of Choudhury and Gautam (*16*) as

$$\text{SME (kJ/kg)} = \frac{n(\text{actual})}{n(\text{rated})} \times \frac{\%T}{100} \times \frac{P(\text{rated})}{m}$$

where n = screw speed (rpm), T = net torque, P = motor power (kJ/s), and m = feed rate (kg/s).

Expansion Ratio. The diameter of five corn extrudates was measured with a vernier caliper. The average diameter was used for determining expansion ratio, which is the ratio of extrudate diameter to the die.

Oil Extraction. Thirty grams of corn extrudates from each treatment was mixed with 100 mL of solvent (chloroform/methanol, 2:1) and homogenized by Ace homogenizer (Nihonseiki Kaisha Ltd., Japan) at 7000 rpm for 3 min and, then, the vessel was rinsed with 50 mL of the solvent. The mixture was centrifuged at 2000*g* for 20 min and filtered through filter paper. Fifty milliliters of distilled water was added to a 150 mL aliquot of the filtrate, and the solvent layer was separated in a separatory funnel. Finally, solvent was evaporated by a rotary evaporator at 40−45 °C.

FAME Analysis. *Preparation of FAME.* Approximately 30 mg of the extracted oil was placed into a 15-mL reaction tube fitted with a Teflon-lined screw cap. Sodium hydroxide (1.5 mL, 0.5 M) was added. The tube was flushed with nitrogen, capped, heated at 100 °C for 5 min with occasional shaking, and then cooled to room temperature. One milliliter of C17 (fatty acid form) internal standard (2.00 mg/mL in hexane) and 2 mL of 4% HCl in methanol were added and heated at 60 °C for 15 min with occasional shaking (*17*). After methylation was completed, 10 mL of deionized water was aded. The solution was transferred to a centrifuge tube, and 6 mL of hexane was aded for FAME/CLAME extraction. The solution was centrifuged at 2000*g* and 10 °C for 20 min, and then the hexane layer was dried over sodium sulfate and analyzed by gas chromatography (GC) and high-performance liquid chromatography (HPLC).

FAME and CLA Methyl Ester (CLAME) Analysis by GC. FAME and CLAME were analyzed using a GC (HP 6890, Hewlett-Packard Inc., Palo Alto, CA) equipped with a 100 m × 0.25 mm fused silica capillary column SP2560 (Supelco Inc., Bellefonte, PA). Injector and detector temperatures were set at 240 °C. The column temperature was kept at 75 °C for 1 min, then increased at 20 °C/min to 185 °C, held at 185 °C for 15 min, then increased at a rate of 4 °C/min to 220 °C, and held at 220 °C for 35 min (*13*).

CLAME Analysis by HPLC. CLAME were analyzed using an HPLC (HP 1100, Hewlett-Packard) equipped with a 20-μL Rheodyne injection loop and a UV detector set at 233 nm. Two ChromSpher 5 lipid analytical silver-impregnated columns (4.6 mm i.d. × 250 mm stainless steel, 5 ,m particle size) with a guard column were used in series. HPLC separation was peformed isocratically with the mobile phase of 0.1% acetonitrile in hexane freshly prepared and at the flow rate of 1.0 mL/min (*14*).

4,4-Dimethyloxazoline (DMOX) Derivatives. CLAME samples were collected from HPLC fractions and hydrolyzed to free fatty acid by 10 mL of 0.5 N KOH/MeOH at 80 °C for 40 min (*13*). After hydrolysis, 10 mL of water was added. The mixture was adjusted to pH 3 with 1 N HCl and salting out with NaCl. The mixture was transferred to a 40-mL centrifugal tube, and then 10 mL of petroleum ether was added and centrifuged at 4000 rpm and 10 °C for 20 min. The petroleum ether layer containing free fatty acids was dried over sodium sulfate and concentrated under nitrogen gas to 1 mL. The aliquot was placed into a screw-cap reaction tube, and a 3-fold amount of 2-amino-2-methyl-1-propanol was added. The tube was purged with nitrogen and then heated at 170 °C for 5 h. After reaction completion, 10 mL of HPLC water was added. The mixture was transferred to a 40-mL centrifugal tube, and then 10 mL of petroleum ether was added. Two milliliters of saturated NaCl was added to break the emulsion. The mxiture was centrifuged at 4000 rpm and 10 °C for 20 min. The petroleum ether layer was dried over sodium sulfate and concentrated under nitrogen gas. DMOX derivatives were analyzed by GC-MS.

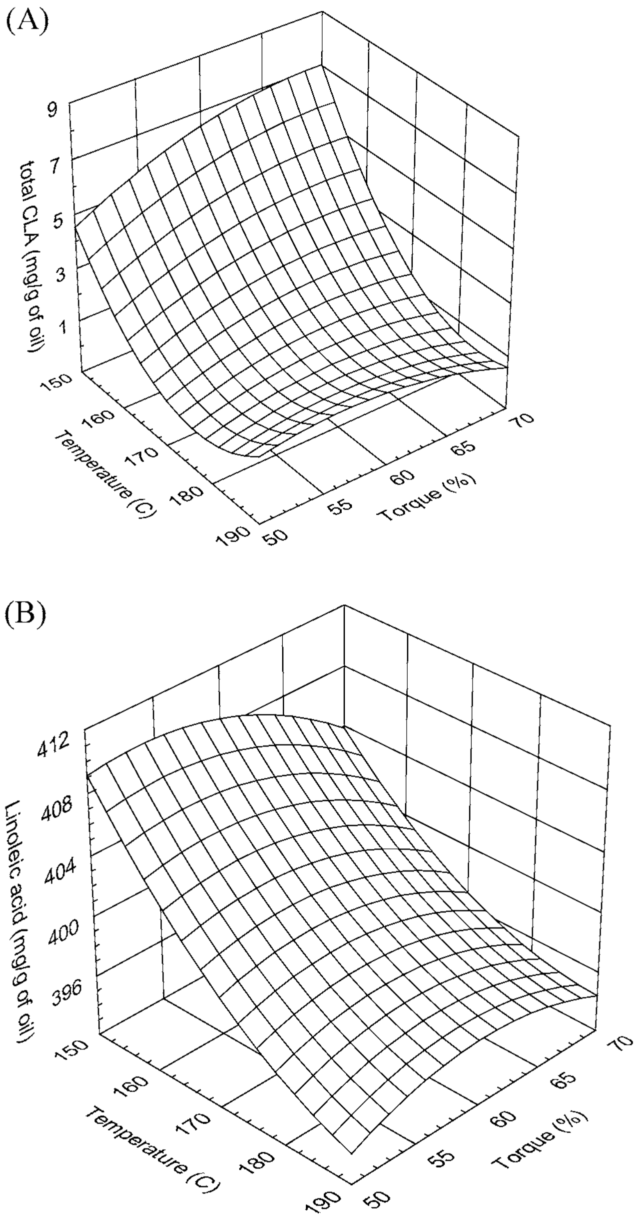

Figure 1. Response surface plot of (**A**) total CLA of corn extrudates containing sunflower oil affected by extrusion conditions and (**B**) changing of linoleic acid affected by extrusion conditions.

DMOX Derivative Analysis by GC-MS. DMOX derivatives were analyzed by GC-MS (GC, Varian, Star 3400 dx; MS, Varian, Saturn 2000) equipped with a 100 m × 0.25 mm fused silica capillary column (SP2560, Supelco Inc.). Injector and transfer line temperatures were 220 °C. The column temperature was kept at 75 °C for 1 min, then increased at 20 °C/min to 185 °C, held at 185 °C for 15 min, then increased at 4 °C/min to 220 °C, and held at 220 °C for 45 min (*13*).

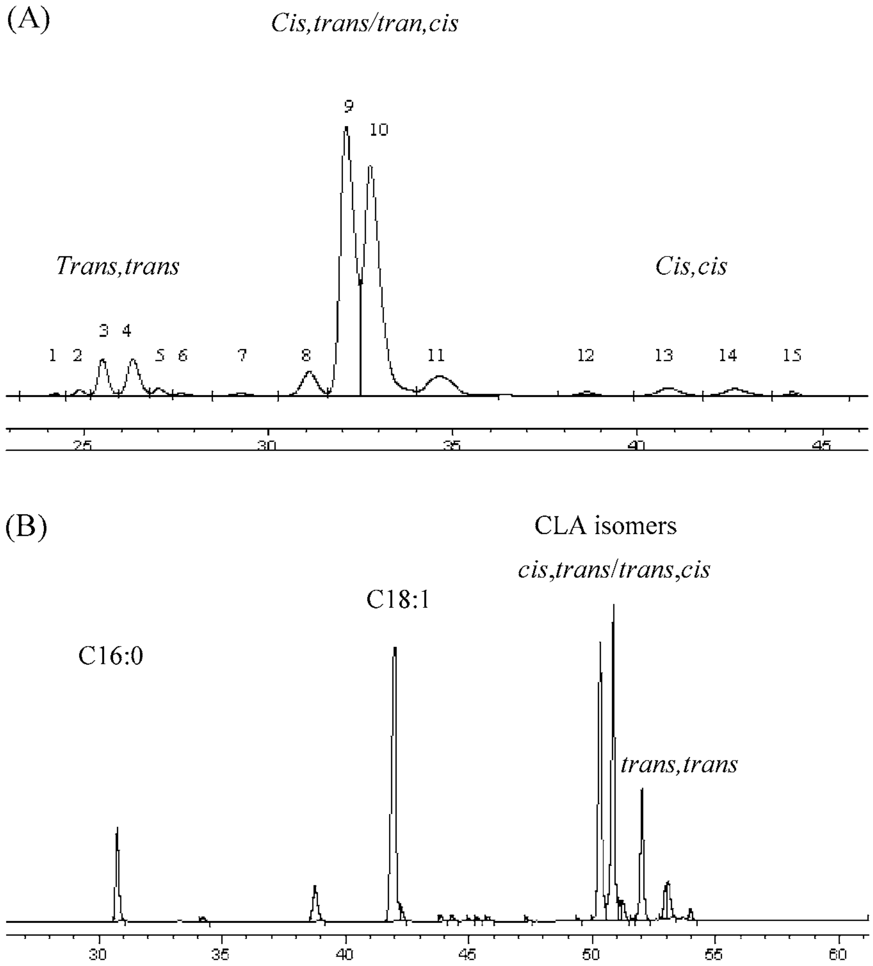

Figure 2. (**A**) Partial HPLC chromatogram of CLA oil used as starting feed. Peaks: (1) *t*12, *t*14; (2) *t*11, *t*13; (3) *t*10, *t*12; (4) *t*9, *t*11; (5) *t*8, *t*10; (6) *t*7, *t*9; (7) *c*12, *t*14/*t*12, *c*14; (8) *c*11, *t*13/*t*11, *c*13; (9) *c*10, *t*12/*t*10, *c*12; (10) *c*9, *t*11/*t*9, *c*11; (11) *c*8, *t*10/*t*8, *c*10; (12) *c*11, *c*13; (13) *c*10, *c*12; (14) *c*9, *c*11; (15) *c*8, *c*10. (**B**) Partial GC chromatogram of CLA oil.

Statistical Analysis. A 3 × 3 factorial design with three replications was employed. The data were analyzed by ANOVA, and a *t* test was conducted to identify differences between means ($P < 0.05$).

RESULTS AND DISCUSSION

Effect of Extrusion Parameters on Total CLA Using Sunflower Oil. Sunflower oil in the experiment contained CLA at 3.6 ± 0.2 mg/g of oil. Corn meal was mixed with 2% sunflower oil and used as starting feed. The oil extracted from the starting feed contained 9.3, 3.4, 30.5, 55.3, 1.0, and 0.6% of oleic acid, stearic acid, palmitic acid, linoleic acid, linolenic acid, and total CLA, respectively. **Figure 1** illustrates that the formation of CLA during extrusion was dependent on product temperature and torque ($P < 0.05$) due to thermal and mechanical energy as a result of extrusion processing. Increasing product temperature from 150 to 170 and 190 °C resulted in a decrease in total CLA content ($P < 0.05$). At the lowest percent torque of 50, total CLA decreased from 4.3 to 0.2 mg/g of oil, whereas at 70% torque, a decrease in total CLA from 7.8 to 1.5 mg/g of oil was obtained when product temperature was increased to 190 °C. A similar effect of temperature on the formation of CLA by chemical reaction using homogeneous RhCl $(PPh_3)_3$ catalyst and Rh/C heterogeneous catalyst was also reported (*18*, *19*).

Alteration of percent torque directly affected the mechanical energy; therefore, an increase in percent torque from 50 to 70% led to an increase in SME from 184 to 258 kJ/kg. Besides frictional heat from mechanical energy, changing of product temperature affected CLA alteration as well. At the product temperature of 150 °C, s significantly greater yield ($P < 0.05$) of total CLA content was obtained at a higher percent torque. However, a slight increase ($P > 0.05$) in total CLA was noticed as percent torque increased even at the product temperature of 190 °C.

The interaction between product temperature and percent torque had an influence on total CLA content ($P < 0.05$). The highest yield of CLA (7.8 mg/g of oil) was obtained by extrusion at a product temperature of 150 °C and a torque of 70%. With respect to the Ag^+-HPLC results, CLA configurations of corn extrudates extruded at this condition were 79.0, 12.1, and 8.8% of *cis*,*trans*/*trans*,*cis*-, *trans*,*trans*-, and *cis*,*cis*-CLA, respectively.

It was assumed that the combination of mechanical and thermal energies affected the double bonds of linoleic acid and formation of conjugated diene. In general, the double bonds at *cis*9 and *cis*12 of linoleic acid are shifted to *cis*9,*trans*11 and *trans*10,*cis*12 in the first step of bond shifting, resulting in the predominant *cis*,*trans*/*trans*,*cis*-CLA isomers (*20*). Because only *cis*,*trans*/*trans*,*cis*-CLA was reported to have health-benefit properties such as anticancer, antiatherosclerosis, and body fat reduction activities (*2*, *6*), further studies to enhance these isomers and total CLA content under other extrusion conditions or with other food-processing methods should be investigated.

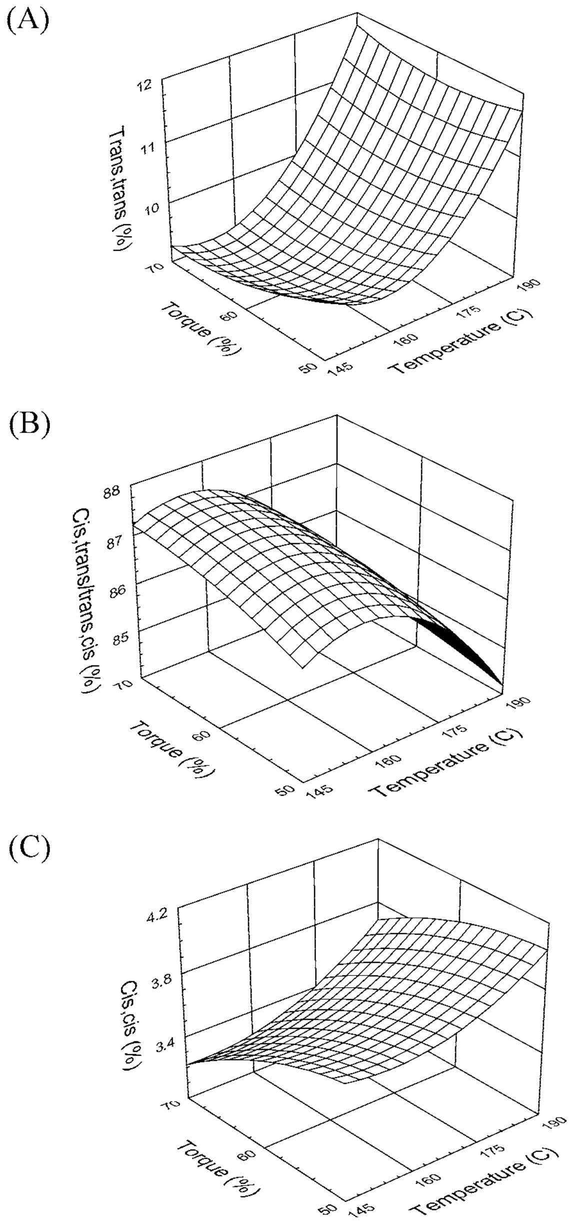

Figure 3. Response surface plots of *trans,trans*-CLA (**A**), *cis,trans*/*trans,cis*-CLA (**B**), and *cis,cis*-CLA (**C**) of corn extrudate containing CLA oil as a result of extrusion conditions.

In this experiment, an advantage of the extrusion process was that both heat and shear could enhance CLA content of the starting feed (1.2 mg/g of oil) up to 6.6-fold (7.8 mg/g of oil) in the extrudates by selecting optimum extrusion conditions. The combination of heat and shear effects has not previously been reported, but there are several papers reporting an increase of CLA content due to processing of dairy products. An increase in CLA content was presented up to 11.2−32.1% from raw materials that contained 3.4−6.1 mg of CLA/g fat in cheese (*11*) and butter (*21*). However, the papers also reported that those processes did not alter the ratio of *cis*9,*trans*11 to total amounts of CLA.

High energy could cause bond shifting from *cis,trans*/*trans,cis* isomers to *trans,trans* isomers, which was observed at a product temperature of 190 °C (**Figure 1**). Furthermore, very high extrusion temperature may cause the transformation of conjugated diene to nonconjugated dienes and a breaking down of the C18 structure to some short-chain fatty acids (data not shown).

Table 1. Die Pressure and Expansion Ratio of Corn Extrudates

treatment [temperature (°C)/ torque (%)]	experiment A[a] die pressure (psi)	experiment A[a] expansion ratio[b]	experiment B[a] die pressure (psi)	experiment B[a] expansion ratio[b]
190/70	180	2.22 ± 0.06b	215	1.51 ± 0.06e
190/60	150	1.81 ± 0.02c	185	1.49 ± 0.06e
190/50	130	1.41 ± 0.05d	140	1.36 ± 0.03f
170/70	280	2.79 ± 0.05a	190	1.76 ± 0.05c
170/60	210	2.51 ± 0.03a	170	1.61 ± 0.06d
170/50	180	1.96 ± 0.06c	145	1.32 ± 0.04f
150/70	290	2.89 ± 0.08a	255	2.21 ± 0.06a
150/60	230	2.67 ± 0.03a	210	1.97 ± 0.05b
150/50	185	2.25 ± 0.10b	150	1.77 ± 0.02c

[a] Experiment A, containing 2% sunflower oil; experiment B, containing 2% CLA oil. [b] Means with different letters in a column are significantly different ($P < 0.05$).

Effect of Extrusion Conditions on Alteration of CLA Configurations Using CLA Oil. As analyzed by the Ag^+-HPLC, the CLA used in corn extrusion contained 15 peaks in three groups of isomers: *trans,trans*-, *cis,trans*/*trans,cis*-, and *cis,cis*-CLA (**Figure 2A**). Individual peaks were identified by comparison with the standard CLA, *cis*9,*cis*11−18:2, *cis*9,*trans*11−18:2, and *trans*9,*trans*11−18:2. Further identification of other isomers was done by the analysis of DMOX derivatives by GC-MS and using references *16* and *22* from molecular ions of *m*/*z* 333 and a series of even mass ions (*16*, *22*). Elution orders and identification of isomers were as follows: (1) *t*12, *t*14; (2) *t*11, *t*13; (3) *t*10, *t*12; (4) *t*9, *t*11; (5) *t*8, *t*10; (6) *t*7, *t*9; (7) *c*12, *t*14/*t*12, *c*14; (8) *c*11, *t*13/*t*11, *c*13; (9) *c*10, *t*12/*t*10, *c*12; (10) *c*9, *t*11/*t*9, *c*11; (11) *c*8, *t*10/*t*8, *c*10; (12) *c*11, *c*13; (13) *c*10, *c*12; (14) *c*9, *c*11; and (15) *c*8, *c*10. However, only eight peaks were observed on the GC chromatogram (**Figure 2B**), due to coelution of isomers within positional and geometrical groups (*23*). The GC elution order of CLA isomer groups was *cis,trans*/*trans,cis* followed by *cis,cis* and finally *trans,trans*. Quantification by GC using C17 fatty acid as the internal standard showed that the CLA oil contained a total of 509.5 mg of CLA/g of starting oil, of which 81.8% were *cis,trans*/*trans,cis* isomers. In addition, 73.92% of health-beneficial isomers obtained were *cis*9,*trans*11 and *trans10,cis12*.

The starting feed contained 85.8 and 10.2% of *cis,trans*/*trans,cis* and *trans,trans* isomers, respectively. Extrusion at the lowest product temperature of 150 °C and highest torque of 70% could increase *cis,trans*/*trans,cis*-CLA to 87.4%, whereas *trans,trans* decreased to 9.1%. This could be due to the conversion of linoleic acid in corn meal into CLAs as mentioned previously. Thus, at proper extrusion conditions, the proportion of *cis,trans*/*trans,cis*-CLA could be manipulated to be higher with minute amounts of the *trans,trans* isomers.

Variation of extrusion conditions could cause alteration of CLA geometrical configuration as shown in **Figure 3**. Higher product temperature was an important factor inducing greater *trans,trans*-CLAs. At 70% torque, an increase in product temperature from 150 to 190 °C led to a significant increase ($P < 0.05$) in *trans,trans* isomers from 9.1 to 11.9%, whereas *cis,trans*/*trans,cis*-CLAs significantly decreased ($P < 0.05$) from 87.4 to 84.6%. This seems to confirm the above experiment using sunflower oil in which *cis,trans*/*tran,cis* conjugated bonds were formed at lower product temperatures. When the energy increased, the *cis*,*trans*/*trans*,*cis* isomers could shift their configurations to the *trans*,*trans* ones as shown in **Figure 3A**.

The proportion of CLAs in *cis*,*cis* configurations slightly changed during extrusion (**Figure 3C**). However, *cis*,*cis*-CLAs have been shown to have neither harmful nor beneficial properties.

Changing torques also affected CLA configurations. The mechanical energy in this study was slightly lower than in the above study because of a higher dry feed rate. Obviously, using 2% sunflower oil and 2% CLA oil gave similar results. Increasing SME from 181 to 253 kJ/kg and torque from 50 to 70% could increase *cis*,*trans*/*trans*,*cis* isomers, whereas most *trans*,*tran* isomers did not significantly decrease ($P < 0.05$). These suggested that the combination of mechanical and thermal energy influenced the transformation of *cis*,*trans*/*trans*,*cis* and *trans*,*trans* configurations. However, product temperature was obviously the most effective factor inducing lipid transformation.

Different die pressures and expansion ratios were observed in all processing conditions as shown in **Table 1**. At the same extrusion conditions, treatments with sunflower oil had lower dry feed rates and lower water feed rates (data not shown), higher SMEs, higher die pressures, and consequently higher expansion ratios. This was because the viscosity of sunflower oil (86 mPa·s) was greater than that of CLA oil (64 mPa·s). In addition, oil viscosity contributed differences in lubrication of starting feed and in dough viscosity, resulting in higher expansion ratios.

The results in **Table 1** showed that the higher the product temperature, the lower the expansion ratio due to a lower melt viscosity. This is similar to the finding of Valley et al. (*24*). In contrast, higher percent torque would increase die pressure, resulting in higher expansion ratios. Therefore, the extrusion condition at the product temperature of 150 °C and 70% torque provided the highest expansion ratio in this study. In addition, this extrusion condition also fabricated bright yellow, homogeneously aerated, high-puffing extrudates with very good appearance.

In summary, extrusion conditions affected CLA contents and also altered CLA configurations of corn extrudates. Thermal and mechanical energy carried out at a product temperature of 150 °C and 70% torque provided the maximum total CLA content with minimum *trans*,*trans* isomers (9.1% of total CLA) and maximum *cis*,*trans*/*trans*,*cis* isomers (87.4% of total CLA). In addition, corn extrudates obtained from this condition had a good appearance with the highest expansion.

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