



Synthesis of Linoleic Acid of Conjugated Isomers from Sesame (*Sesamum indicum*) Seed Oil: its use and Effect in a Microstructured Product Type Oil-In-Water Emulsion

DAVID RAMÍREZ-BREWER, LUIS MADRID-PALENCIA, LUIS MONROY-RODRÍGUEZ, SOMARIS QUINTANA- MARTÍNEZ and LUIS A. GARCÍA-ZAPATEIRO*

Research Group of Complex Fluid Engineering and Food Rheology, University of Cartagena, Colombia.

Abstract

The development of functional foods is an area of great interest and innovation in the food industry. The use of conjugated linoleic acid (CLA) in food formulations has been growing in recent years due to its multiple health benefits. In this study, conjugated linoleic acid was obtained from sesame oil, and its use in the formulation of oil-in-water food emulsions was evaluated. Conjugated linoleic acid (CLA) was synthesized from the linoleic acid present in sesame oil using the alkaline isomerization method using propyleneglycol as a solvent. The effect of alkali concentration (NaOH) and reaction time on the conversion of linoleic acid to CLA was evaluated. A 96.6% conversion of CLA was obtained with a NaOH concentration of 7% and a reaction time of 2 h. Emulsions were prepared using CLA as oil phase and soy lecithin, tween 80, carboxymethylcellulose as emulsifying agents. Emulsions with mixtures of carboxymethylcellulose and tween 80 were stable, presenting a non-Newtonian fluid behavior of pseudoplastic type ($n < 1$). The Ostwald-de-Waele model shows an optimal fit to the experimental data of apparent viscosity ($R^2 > 0.99$), and its microstructural characterization shows a homogeneous particle distribution. These results show that the alkaline isomerization process using propylene glycol as a solvent is an excellent alternative for the synthesis of CLA from vegetable oils such as sesame oil and its application in the development of microstructured products such as functional emulsions, and their subsequent application in the development of new food products with beneficial health characteristics.



Article History

Received: 14 February 2024

Accepted: 25 August 2024


Keywords

Conjugated Linoleic Acid; Emulsions; Ostwald-De-Waele Model; Rheology; Sesame Oil.

CONTACT Luis A. García-Zapateiro ✉ lgarciaz@unicartagena.edu.co 📍 Research Group of Complex Fluid Engineering and Food Rheology, University of Cartagena, Colombia.



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Doi: <https://dx.doi.org/10.12944/CRNFSJ.12.2.26>

Introduction

Sesame (*Sesamum indicum*) is an important oil seed crop, belonging to the *Padaliaceae* family, cultivated throughout the world.^{1,2} Sesame seeds have a high oil content of 50-55% and contain proteins, carbohydrates, fiber, vitamin E, minerals, tocopherols, and lignans such as sesamol and sesamin.²

Sesame oil is produced from sesame seeds by roasting, pressing, and refining and is widely used for cooking, medicinal, and cosmetic purposes.³ It is mainly produced in Africa and Asia, especially in Tanzania, Myanmar, India and China.⁴ Sesame oil is rich in polyunsaturated fatty acids such as linoleic acid, oleic acid palmitic acid and stearic acid, is also rich in various bioactive substances, such as tocopherols, phytosterols and lignans, and exhibits greater oxidation stability than that of other vegetable oils,^{5,6} which have shown strong antioxidant, anti-inflammatory, and antimutagenic functions.^{7,8}

Conjugated linoleic acid (CLA) is a general term for a mixture of positional and geometric isomers of linoleic acid (18:2), in which two double bonds are conjugated.⁹ *Cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA¹⁰ are the most abundant CLA isomers, representing approximately 95% of all isomers and have been a topic of increasing interest due to their therapeutic and nutritional properties.¹¹ In recent years, numerous investigations have been carried out on CLA due to its biological activities potentially beneficial to health, including prevention of cardiovascular diseases, anti-inflammatory properties, reduction of body fat, anticancer effects and improvements immunerelated responses^{12,13} approved by the U.S. Food and Drug Administration, CLA is labeled "Generally Regarded as Safe" and can be applied to a wider range of functional foods or food additives.¹⁴ Also, The European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition, and Allergies has recognized CLA combination (c9,t11 and t10,c12) as a safe new food ingredient in amounts up to 3.5 g/day.¹⁵

However, CLA, like most highly non-polar bioactive lipids, cannot be used in water-based foods and beverages due to its insolubility in water, and CLA is not stable during thermal processing and significant loss occurs. of biologically active CLA through

oxidation. For these reasons, CLA is generally converted into an emulsion, allowing its application in various food products and, in turn, allowing it to act as an effective protection system for the delivery of CLA in food products.^{11,12}

Emulsions are thermodynamically unstable systems from a physicochemical point of view, rapidly or slowly separating into two immiscible phases over a period of time.¹⁶ and are stabilized by improving their kinetic stability,¹⁷ where stability can be defined as the resistance to physical changes.

Oil-in-water (o/w) emulsions consist of small oil droplets dispersed in an aqueous medium, with each droplet being coated by a thin layer of emulsifier molecules,¹⁸ including protein, polymers, ionic and nonionic surfactants.^{19,20} Thermodynamically, the emulsion is an unstable system that undergoes physicochemical mechanisms such as coalescence, creaming, and Ostwald ripening, leading to its separation into initial phases.²¹⁻²³ The presence of an emulsifier or surfactant during emulsification minimizes the interfacial energy between immiscible phases through various mechanisms such as adsorption, encapsulation, or chemical links,²³ therefore the selection of an appropriate emulsifier or surfactant is important to avoid emulsion instability, such as gravitational separation, flocculation coalescence and phase separation, during processing, storage and use.¹² The use of stabilizers is an effective method to improve the stability of emulsion.²⁴ Biopolymers (especially polysaccharides) have been used as stabilizers in emulsions.²⁵ These polymers are intended to reduce the Brownian motion of the system and thus maintain stable emulsified components for longer periods.²⁶ Then the emulsion is considered an excellent system for delivering nutraceuticals, and are considered superior rather than a typical oil-in-water emulsion, since they are highly concentrated in terms of incorporated ingredients such as bioactive lipids or flavour oils.²⁷ Concentrated emulsions are stable to cream because a higher concentration of oil in a continuous phase retards the movement of the molecules and, in turn, prevents gravitational separation. Beverage emulsions are specially designed so that the specific component is released under controlled conditions or highly stable than in its natural form.^{27,28} Therefore, the objective of this study was i.) synthesize conjugated

linoleic acid (CLA) from the linoleic acid present in sesame oil (Sesamum indicum) and, ii) prepare oil-in-water emulsions using CLA as the oil phase and evaluate its rheological properties and stability.

Material and Methods

Material

The sesame seeds were purchased at the central market of the city of Cartagena. Soy lecithin, tween 80, and carboxymethylcellulose were purchased from Tecnas (Colombia). Propylen glycol, sodium hydroxide, acid hydrochloric and sodium sulfate were obtained from Sigma–Aldrich (St. Louis, MO, USA). Palmitic acid ($\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$), linoleic acid ($\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}_2\text{H}$), oleic acid ($\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$), stearic acid ($\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$), the ethyl ester of eicosapentaenoic acid of ethyl ester ($\text{C}_{22}\text{H}_{34}\text{O}_2$) and docosahexaenoic acid ($\text{C}_{24}\text{H}_{36}\text{O}_2$) from Merck. All other reagents were analytical grade.

Synthesis of Conjugated Isomer Linoleic Acid from Sesame Oil

Sesame oil was obtained by extraction using the Soxhlet method following the methodology proposed by Abdiani *et al.*² The extraction yield was calculated using Equation 1:

$$\text{Yield (\%)} = \frac{g \text{ sesame oil}}{g \text{ sesame seed}} \times 100 \quad \dots(1)$$

The extracted sesame oil was subjected to an alkaline isomerization process following the method previously described by Rocha *et al* and Shayanmehr *et al*^{29,30} with some modifications, evaluating the influence of NaOH concentration (6 and 7%) on propylene glycol and reaction time (1.5 and 2 h), according to 2² factorial design, as can be seen in Table 1. Sesame oil (~ 80 mL) was placed in a 300 mL three-neck flask equipped with magnetic stirrers, thermometer, reflux condenser, and nitrogen supplied by a bubble using a heating plate; after that, NaOH in propyleneglycol was added, and the mixture was heated to 180 °C in an inert atmosphere. Afterward the reaction time, the blends were cooled at room temperature. Then, 25 mL of hydrochloric acid (HCl) was added to the mixture constantly stirred for 15 min. Subsequently, the pH of the mixture was adjusted to pH 3 with the addition of HCl (1 N); then, the mixtures were

transferred to a separate funnel. The lipid fraction (fatty acids) was extracted with two 25 ml portions of hexane and washed in a separation funnel and 12.5 ml aliquots of NaCl aliquots (5% w/v). After that, Na_2SO_4 (5% v/v) and allowed to dry. Finally, the solution of fatty acids in hexane was subjected to heating under vacuum to remove the solvent. The conjugated linoleic acid (CLA) isomers were cooled and stored at -20 °C. The percentage of conversion of linoleic acid to conjugated linoleic acid was determined using Equation 2:

$$\text{Conversion CLA (\%)} = \frac{\% \text{ Linoleic acid conjugated}}{\% \text{ Linoleic acid}} \times 100 \quad \dots(2)$$

Table 1: The Isomerization Process Conditions to obtain the conjugated linoleic acid sesame oil.

No. Reaction	NaOH %	Time Reaction h
1.	6	1.5
2.	6	2.0
3.	7	1.5
4.	7	2.0

Fatty Acid Profile

The fatty acid profile was determined to include sesame oils, and the conjugated linoleic acid isomers were analyzed by Gas Chromatography with Mass Spectrometric Detection (CG-MS), according to the method reported by Mielles-Gomez *et al*³¹ with modifications. Methyl esters were prepared by *trans*methylation according to ISO 5509 using NaOH in 2 M methanol and petroleum ether medium. Fatty acid methyl esters were analyzed using an Agilent 4890D gas chromatograph equipped with a split injection system set at 250 °C and a flame ionization detector (FID) set at 300 °C using helium as carrier gas. A capillary column Stabilwax 30 m long by 0.25 mm in diameter and a stationary phase film of 0.25 μm, was used. Compounds were identified against the NIST 2014 mass spectral library. Standards-based calibration curves were used to determine the content of these fatty acids in the different samples.

Formulation and Standardization of the Emulsion

Oil-in-water (o/w) emulsions were prepared using blends of conjugated isomers of linoleic acid (20% w/w) and distilled water (80% w/w) evaluating lecithin

(0.3 and 0.4% w/w) and tween 80 (0.3 and 0.4% w/w) as emulsifiers and carboxymethylcellulose (CMC) (0.5 % w/w) as stabilizer (Table 2). Initially, the aqueous phase was prepared by solubilizing the emulsifier in distilled water until constant stirring (100 rpm) for 15 min; after that, CMC was added and mixed for 15

min at 60 °C in distilled water. The oil phase (CLA) was added and homogenized using an ultraturrax (IKA T25 basic, Deutschland, Germany) with an S25 N-10ST dispersing tool at 11400 rpm for 15 min at room temperature. Finally, they were stored at 4 °C until analysis.

Table 2: Formulation of oil in water (o/w) emulsion of conjugated linoleic acid.

Sample code	Oil phase (CLA) %	Aqueous phase %	Lecithin %	Tween 80 %	CMC %
Lec-0.3	20	80	0.3	--	--
Lec-0.4	20	80	0.4	--	--
Twe-0.3	20	80	--	0.3	--
Twe-0.4	20	80	--	0.4	--
CMC-0.5	20	80	--	--	0.5
CMC-0.5-Twe-0.4	20	80	--	0.4	0.5

Rheological and Microstructural Characterization

The viscous flow behavior of stable emulsions was performed by an employee at Brookfield DV-E viscometer strain (Brookfield Engineering Laboratories, Massachusetts, USA) following the methodology proposed by Brewer *et al.*³² Readings between 0 and 100 scale units (spindle no. 3) were taken at rotational speeds between 5 to 100 rpm at 25 °C. The scale values were read after 90 seconds under shear. The rheological behavior was analyzed using the Ostwald-de-Waele rheological model (Equation 3):

$$\eta = K\dot{\gamma}^{n-1} \quad \dots(3)$$

where η is the apparent viscosity (Pa·s), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency index (Pa·sⁿ) and n is the flow behaviour index (dimensionless). The model parameters were adjusted using the OriginPro software. The particle size distribution of the stabilized emulsions was evaluated by optical microscopy according to the methodology proposed by Brewer *et al.*³² The microstructure was measured using a standard Leica D500 Germany light microscope with 100X.

Statistical Analysis

Data were analyzed using Statgraphics 19 software. Measurements were performed in duplicates and were expressed as mean \pm standard deviation.

The coefficient of variation (C.V) was used to analyze the relationship between the standard deviation and the means. The multivariate ANOVA was used to analyze the effect of the variables under study on the response variable. Fisher's LSD test was used to identify significant differences between the means with $p < 0.05$

Results and Discussion

Conjugated Isomers of linoleic Acid from Sesame oil

Sesame oil was obtained using the Soxhlet method, recovering 46.6 ± 1.05 % of the total oil present in the seed. These results were similar to those reported by Abdiani *et al* and Zhang *et al*^{62,33} who reported extraction yields with the Soxhlet method and screw pressing of 50-60% and 50% respectively, and slightly lower than those reported by Brewer *et al* and Zhang *et al*^{62,33} who obtained extraction yields of 63.75% with the mechanical pressing method and 96.7% with the subcritical propane extraction. Table 3 shows the acid profile of sesame oil. These results are consistent with what was reported by Brewer *et al.* and Huang *et al*^{6,32} for the sesame oil obtained by the pressing method, which indicates that the Soxhlet and pressing method used do not significantly modify the fatty acid composition of the sesame oil. The percentage of CLAs and the conversion of linoleic acid to conjugated linoleic acid by the isomerization process can be shown in Table 4.

The results obtained show a maximum conversion of conjugated linoleic acid to conjugated linoleic acid of 96.60% under reaction conditions of 7% NaOH and reaction time of 2 h using. This result was similar to that reported by Rocha *et al* and Silva-Ramirez *et al.*^{30,34} who synthesized conjugated linoleic acid by alkaline isomerization and microwave-assisted alkaline isomerization, with 96% and 98.96% conversion respectively. The results of the ANOVA show that the concentration of NaOH and the reaction time have a significant effect ($p < 0.05$) on the CLA content. The results indicate that an increase in the concentration of NaOH (6 to 7%) and the reaction time (1.5 to 2 h) results in a higher percentage of conversion of linoleic acid to CLA. The above can be explained due to the interaction of the solvent with the alkali, which according to what was reported by Shayanmehr *et al.*,²⁹ who studied the effect of the interaction of different types of solvents (water, ethanol and propyleneglycol) with alkalis (NaOH, KOH, CH₃ONa and CH₃OK), and the reaction times (0.25, 0.5, 1, 2, 3.5, 5 and 6.5) concluding that there is a significant interaction effect between the solvent and the alkali and that when using propylene glycol with NaOH, obtained a greater amount of CLA isomers, and that the isomerization time when increased, up to 2 hours, has a significant effect on the amount of isomers obtained, while at longer times (3.5 - 6 h) no significant change is observed in the amount of isomers produced, which is consistent with the results of our study, where a reaction time of 2 h produces the highest percentage of CLA. Similar results were obtained by Rocha *et al.*³⁰ for the conversion of linoleic acid to CLA from safflower oil with 7% NaOH and 2.15 h of reaction time.

Table 3: Fatty acid profile of sesame (Sesamum indicum) oil.

Fatty Acid	Composition (%)*
Palmitic acid (C16:0)	9.23
Stearic acid (18:0)	5.95
Oleic acid (C18:1)	34.58
Linoleic acid (C18:2)	49.38
Eicosapentaenoic acid (EPA) (C20:5)	0.23
Docosahexaenoic acid (DHA) (C22:6)	0.63

*Data with C.V.< 0.05

Table 4: Conversion of linoleic acid to total conjugated linoleic acid-CLA.

No. Reaction	CLAs (%) *	Conversion (%)
1.	25.34 ^a	51.32 ^a
2.	31.57 ^b	63.93 ^b
3.	39.44 ^c	79.87 ^c
4.	47.70 ^d	96.60 ^d

*Total conjugated linoleic acid. Data with C.V < 0.05. Different letters in the same column express statistically significant differences ($p < 0.05$).

Stability and Microstructural Characterization of Emulsions with CLA

Figure 1 shows the macroscopic images of fresh emulsions prepared using CLA obtained with 7% NaOH and 2 h and different percentages and types of emulsifiers as shown in Table 2. The stability of the emulsions was evaluated in storage for 48 h at 25°C (Fig 1b). As seen in Figure 1a, fresh emulsions prepared with Tween 80 at 0.3% and 0.4% (Twe-0.3 and Twe-0.4) showed phase separation. This can be attributed to the thin interfacial membrane produced by Tween 80, which in the presence of the high revolutions used in the preparation of the emulsions, destroyed the internal structure of the emulsions, allowing phase separation.³⁵ After 48 h of storage at 25°C, the emulsions prepared with lecithin (Lec-0.3 and Lec-0.4) showed phase separation phenomenon, which may be due to the use of natural lecithin when used alone, they are not usually effective agents to stabilize O/W emulsions or W/O, otherwise they must be in combination with other factors such as the addition of proteins, polysaccharides or inorganic salts.^{36,37} The emulsions formulated with the CMC mixture (CMC-0.5) and CMC-Tween 80 (CMC 0.5- Twe-0.4) were stable after 48 h of storage at 25°C; these samples did not present the phenomenon of coalescence. This can be explained because polysaccharides such as CMC, being thickening agents, modify the rheological behavior of the continuous phase, reducing the movement of oil droplets, thus avoiding the phenomenon of coalescence that contributes to the stability of the emulsion.^{26,38,39} The microstructure of the stabilized emulsions was evaluated freshly prepared (at 0 h) at 25 °C. Figure 2 shows the distribution of particle-

stabilized emulsions with CMC and CMC-Tween 80 mixtures. A reduction in drop diameter is observed in the emulsion with CMC and Tween 80, which have a small drop diameter. Vicente *et al.*,²⁶ observed similar results in which the stabilized emulsions with xanthan gum and Tween 80 resulted in drop diameters smaller than those of the stabilized pectin emulsions. This phenomenon can be explained by

the presence of CMC in the emulsions, increasing the viscosity of the emulsion and reducing the Brownian movement and therefore reducing the coalescence of the droplets and in the presence of Tween 80 contributing to the reduction of the droplet diameter. This reduction in droplet size is important in emulsions to minimize the effect of gravity cream.²⁶

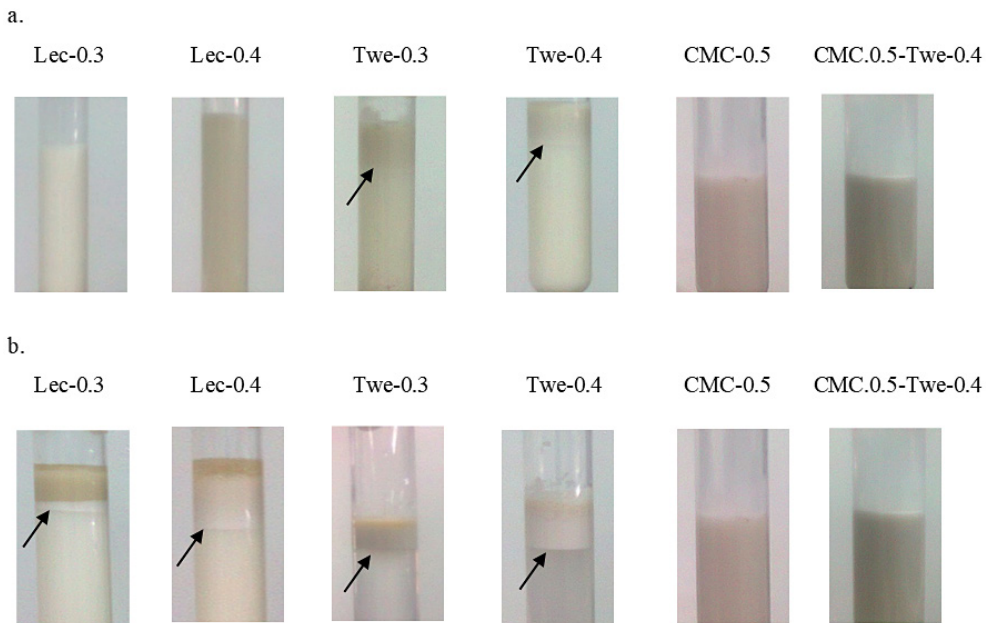


Fig. 1: Stability of oil-in-water (o/w) emulsions of conjugated linoleic acid. a.) at 0 h (freshly prepared). b.) after 48 h at 25°C. Black arrows indicate phase separation.

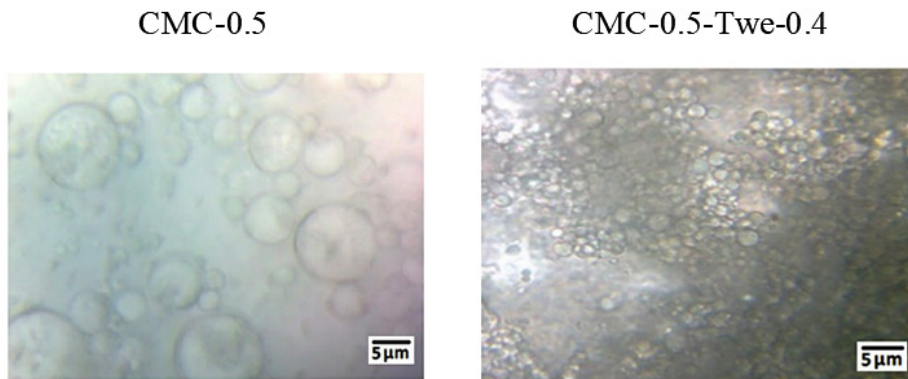


Fig. 2: Micrograph of stabilized oil-in-water (o/w) conjugated linoleic acid emulsions at 25 °C.

Rheological Characterization of Emulsion with CLA

The rheological were performed on stable oil-in-water (o/w) emulsions of conjugated linoleic acid with 0.4% Tween 80 and 0.5% CMC (CMC-0.5- Twe-0.4) after 48 h of preparation, which presented a better distribution and droplet size. Table 5 shows the results of the adjustment of the parameters of the Ostwald-de Waele model and the viscosity at 100 rpm. The viscosity values of the CMC-0.5-Twe-0.4 emulsion for a value of 100 rpm were 632 cP (0.632 Pa s). This result was superior to that reported by Nasrabadi *et al.*,⁴⁰ who evaluated the viscosity of CLA emulsions stabilized with acacia gum (10% w/w) and xanthan gum (0.3% w/w) at a speed of 100 rpm with a value of 47.7– 53.9 cP, indicating that the CMC-Tween 80 mixture confers higher viscosity values to the emulsion, which is related to the improved stability of the emulsions.³⁵ The viscous flow behavior of the emulsions is shown in Figure 3. The apparent viscosity presents a potential decrease with the increase of shear rate, showing a typical non-Newtonian fluid shear thinning. Similar results were obtained by Xiang *et al* and Yu *et al*^{11, 41} who prepared emulsions with CLA

stabilized with gum arabic and olegel emulsions with CLA safflower seed oil stabilized with pea protein respectively, and when evaluating their rheological behavior, they observed a decrease of viscosity by increasing the shear rate.^{11,41} The results show that the Ostwald-de-Wale model shows a good fit to the experimental data. ($R^2>0.999$). The values of the adjusted parameters of the Ostwald de Waele model indicate that the emulsions stabilized with CMC-Tween 80 show a pseudoplastic behavior ($n<1$). This result is similar to that reported by Yang *et al*⁴² who reported n values between 0.9921 and 0.4170 for emulsions with CLA nanoparticles stabilized with OSA starch and xanthan gum. The consistency index obtained in this study for emulsions with CLA stabilized with CMC-Tween 80 mixtures was 3.26 Pa sⁿ. This value is lower than that reported by Yang *et al* for emulsions with a CLA nanoparticle content of 50g/100g in the emulsion (13.21 - 11.42 Pa sⁿ) and higher for emulsions with a CLA nanoparticle content of 5.20 g/100g of the emulsion (0.0349 - 1.039 Pa sⁿ). These differences may be due to the CLA content and the stabilizer used in the emulsion.^{35,42}

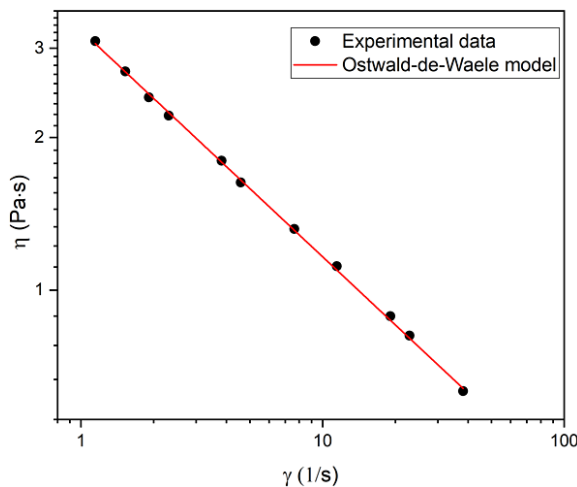


Fig. 3: Viscous flow behavior of oil in water (o/w) conjugated linoleic acid emulsions with 0.4% Tween 80 and 0.5% CMC at 25 °C.

Table 5: Adjusted parameter emulsions with CLA

Sample	K (Pa s ⁿ)	n	R ²	η (Pa s)
CMC-0.5-Twe-0.4	3.26±0.018	0.553 ±0.005	0.99	0.632

Conclusions

The alkaline isomerization process led to the obtaining of conjugated linoleic acid (CLA) from linoleic acid present in sesame oil with a conversion of 96% under reaction conditions of 7% NaOH and 2 h. The emulsions prepared with CLA and carboxymethylcellulose (CMC-0.5) and CLA and mixture of Carboxymethylcellulose with Tween 80 (CMC-0.5-Twe-0.4) were stable after 48 h. The addition of Tween 80 contributed to a reduction in the droplet size of the emulsions, indicating better stability in the emulsion due to which minimizes the effects of cream due to gravity. The emulsions prepared using conjugated linoleic acid as the oil-phase and a CMC-Tween 80 mixture presented non-Newtonian behavior of pseudoplastic type ($n < 1$), adjusted to Ostwald-de-Waele model ($R^2 > 0.99$). These results show that an alkaline isomerization process is an excellent option for obtaining conjugated linoleic acid from oils such as sesame, in addition to its application in the formulation of microstructured products such as emulsions, and its subsequent application in the development of new food products with functional and beneficial health characteristics, due to the presence of conjugated linoleic acid.

Acknowledgement

The authors thank the University of Cartagena for its support in the development of this research.

Funding Source

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Statement

Not applicable in this section.

Authors' Contribution

Conceptualization, D.R-B., L.M-P., L.M-R., S.E.Q., and L.A.G.Z.; methodology, D.R-B., L.M-P., L.M-R., S.E.Q., and L.A.G.Z.; software, D.R-B., validation, D.R-B., L.M-P., L.M-R., S.E.Q., and L.A.G.Z.; formal analysis, D.R-B., L.M-P., L.M-R., S.E.Q., and L.A.G.Z.; investigation, D.R-B., L.M-P., L.M-R., S.E.Q., and L.A.G.Z.; writing—original draft preparation, D.R-B.; writing—review and editing, D.R-B., S.E.Q. and L.A.G.Z.; All authors have read and agreed to the published version of the manuscript.

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