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Original

Fatty acids and conjugated linoleic acid in organic milk produced in the southeast of Mexico

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ABSTRACT

Objective. To determine the fatty acid (FA) profile, conjugated linoleic acid (CLA) and isomers in cow's milk produced under organic conditions in southeastern Mexico. **Materials and methods.** Forty-eight milk samples were collected during one year from three production units (PU 1, 2 and 3) and a collecting tank (CT) in the municipality of Tecpatán, Chiapas (12 months x 4 = 48), following the guidelines established in Mexican regulations. Fat was extracted with detergent solution; AG analysis was performed by gas chromatography with flame ionization detector and ALC and isomers were obtained by high performance liquid chromatography (HPLC) with UV-Vis detector. **Results.** Chromatographic analyses identified and quantified 23 FA in all milks, from C4 to C20:1. Statistical analysis (ANOVA, Tukey) showed significance ($p<0.05$) at C4-C10, C16, C18, C18:1n9t, C18:2n6 and C20; in all cases the CT was similar in at least two PU. The mean values over time exhibited homogeneous regularity behavior. CLA and 9 of its isomers were also determined; ANOVA did not show significance. The isomer C18:2 9c-11t had the highest percentage of total CLA with a mean value of 90.06%. **Conclusions.** Gas and liquid chromatographic analyses allowed characterization of FA, CLA and isomers profiles in organic milk produced in southeastern Mexico, which had not been studied so far.

Keywords: Gas chromatography; high performance liquid chromatography; milk fat; lipids; cows (*Source: CAB Thesaurus*).

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RESUMEN

Objetivo. Determinar el perfil de ácidos grasos (AG), ácido linoleico conjugado (ALC) e isómeros en leche de vaca producida en condiciones orgánicas en el sureste de México. **Materiales y métodos.** Durante un año se colectaron 48 muestras de leche, proveniente de tres unidades de producción (UP 1, 2 y 3) y tanque colector (TC) del municipio de Tecpatán, Chiapas (12 meses x 4 = 48), siguiendo las pautas establecidas en normatividad mexicana. La grasa se extrajo con solución detergente; el análisis de AG se realizó por cromatografía de gases con detector de ionización de flama y el ALC e isómeros se obtuvieron mediante cromatografía líquida de alta resolución (CLAR) con detector UV-Vis. **Resultados.** Los análisis cromatográficos identificaron y cuantificaron 23 AG en todas las leches, desde el C4 hasta el C20:1. El análisis estadístico (ANOVA, Tukey) arrojó significancia ($p<0.05$) en C4-C10, C16, C18, C18:1n9t, C18:2n6 y C20; en todos los casos el TC fue similar en al menos dos UP. Los valores medios a través del tiempo exhibieron comportamiento con regularidad homogénea. Asimismo, se determinaron el ALC y 9 de sus isómeros; el ANOVA no arrojó significancia. El isómero C18:2 9c-11t tuvo el mayor porcentaje del ALC total con un valor medio de 90.06%. **Conclusiones.** Los análisis de cromatografía de gases y de líquidos permitieron caracterizar los perfiles de AG, ALC e isómeros en leche orgánica que se produce en el sureste mexicano, que hasta el momento no se habían estudiado.

Palabras clave: Cromatografía de gases; cromatografía líquida de alta resolución; grasa de la leche; lípidos; vacas (*Fuente: CAB Thesaurus*).

INTRODUCTION

Milk production in the world is constantly increasing, just look at the production values of 794, 802, 814, 855, 880 and 893 billion liters obtained during the years 2014, 2015, 2016, 2017, 2018 and 2019, respectively (1). According to FAOSTAT data, Mexico, in 2018 ranked eighth in milk production worldwide with 12 billion liters and was estimated to be so also in 2019 with 12.2 billion liters; the European Union obtained the first place (156.2 billion) followed by the United States (99 billion) and India (89 billion liters); fourth place went to Brazil (34.9 billion liters); fifth, sixth and seventh China, Russia and New Zealand with 31.1, 30.3, 21.4 billion liters respectively. The total milk produced is divided into cow's milk (81.7%), buffalo (14.4%), goat (2.4%), sheep (1.2%) and camel (0.3%) and of the total cow's milk, 1.8% is obtained in organic production systems (1). In 2017, world organic milk production was estimated at 7.1 billion liters; the United States was the country with the largest share (16.4%), followed by Germany (13.2%), China (12.4%), France (9.0%), Austria (8.6%), and Denmark (7.8%). However, the participation of each of these countries with respect to the total production of cow's milk is 1.2, 2.9, 2.2, 2.5, 16.5 and 9.7%, respectively (1), which makes it evident that Austria and Denmark are the countries with the highest national production of organic milk.

In 2008, Mexico registered an area of 10728 hectares dedicated to organic livestock production and of these 482 (4.9%) were set aside for bovine milk production (2). In 2019, organic milk production was estimated at 22 million L, which meant approximately 0.2% of national production. The state of Chiapas registered the first place in organic milk production and Tecpatán was the municipality that contributed the largest number of liters (5000000 L per year) (3).

Given the interest in organic milk production in Mexico and the world, this research focuses on identifying fatty acids and conjugated linoleic acid in organic milk produced in southeastern Mexico. This is because it has been documented that cow's milk contains 2 to 5% of lipids with 70% of saturated fatty acids and 30% of unsaturated fatty acids; and within the fatty acids is the conjugated linoleic acid, which has awakened the interest of researchers for its potentially beneficial properties for a good human health, such as the prevention of atherosclerosis and cancer (4).

Likewise, studies of fatty acid content have been published in conventional raw goat milk from the State of Mexico and Guerrero and in raw and pasteurized cow milk marketed in supermarkets in Mexico City (5,6,7,8), however, in the literature search conducted for this work, no publications on lipid content in organic milk in Mexico were identified and in particular no study describing the profile of fatty acids and CLA in organic milk from Chiapas was recorded. Due to the above and the importance of the topic at the national and international level, the objective of this work was to establish, during one year, the profile of fatty acids, conjugated linoleic acid and isomers in organic milk produced in Tecpatán, Chiapas, Mexico.

MATERIALS AND METHODS

Origin of the samples. Milk samples were obtained from Creole breed cattle (Zebu-Swiss Brown) from the municipality of Tecpatán, Chiapas. This municipality has about 3500 cows between three and seven years of age, distributed in 80 ranches that meet the characteristics of organic production units (PU). The cows receive mineral salt as a supplement and are fed in complete grazing, mainly with Insurgente (*B. brizantha*), Mombaza (*P. máximum*), Rajador (*L. divaricatum*), Cabezón (*P. virgatum* L.) and Mulato (*Bracharia hibrido 36087*) grasses. In all production units milking is done by hand, once a day with calf at foot, between 5 and 6 b.m. and the volume of production per animal is on average 4 liters.

Tecpatán is located in the northwest zone of the state of Chiapas, bordered to the north by the municipality of Ostuacán, to the northeast by the municipality of Francisco León, to the east by the municipalities of Ocotepec and Copainalá, to the south by the municipalities of Cintalapa and Ocozocuautla de Espinoza, and to the northwest by the municipality of Las Choapas of the state of Veracruz de Ignacio de la Llave (9). It occupies 1.68% of the state's surface and has 37543 inhabitants (Figure 1). The climate is hot humid with abundant rainfall in summer (50.37%), hot humid with rainfall throughout the year (49.11%) and semi-warm humid with rainfall throughout the year (0.52%) (9).

Sample collection. Organic milk samples were obtained during one year (January to December 2018), at 30-day intervals. One L of milk was collected, immediately after milking, from three PU of 80 that are located in Tecpatán. In addition, one L from the collecting tank (CT) and cooling tank available to all organic milk producers in Tecpatán, totaling four L per month for one year (48 samples in total). The samples were labeled and kept refrigerated until laboratory analysis.

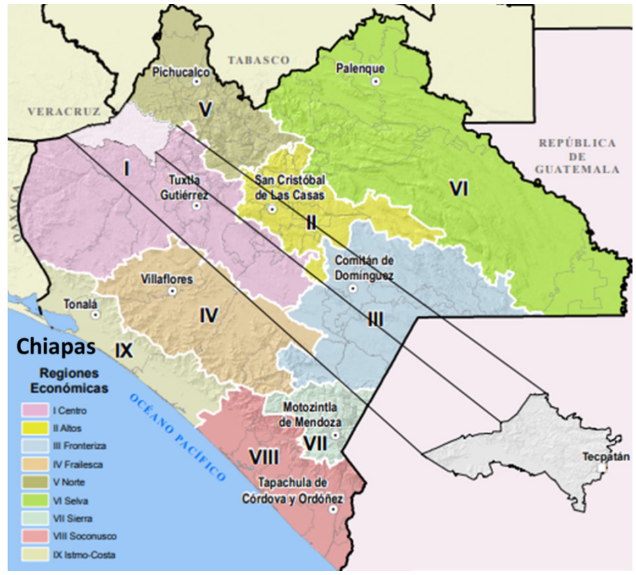

Figure 1. Location map of the Municipality of Tecpatán, Chiapas, Mexico. Source: (10).

Extraction of milk fat. Milk fat was extracted by detergent solution to break the fat emulsion and the fat extract was filtered through anhydrous sodium sulfate (J.T. Baker, USA) to ensure the absence of water. The obtained fatty matter was kept in freezing (-4°C), to avoid peroxidation, until analysis.

Analysis of fatty acids by gas chromatography with flame ionization detector. The determination of FA was carried out by means of its methyl esters, previously derivatized with potassium hydroxide in methanol 2N (5) and injected, in duplicate, to the gas chromatograph with flame ionization detector.

Chromatographic conditions. A Shimadzu GC 2010 Plus gas chromatograph (Japan) with 100 m long fused silica capillary column with 0.25 inner diameter and 0.2 µm layer thickness (SP ™ 2560, Cat. No. 24056, USA) was used. Temperatures: 140, 270 and 250°C of the furnace, detector and injector respectively. Temperature ramp: T1 = 140°C for 5 min, with 5°C x min increase until reaching T2 = 195°C, after 1 min increased 6°C x min until reaching T3=220°C, held for 20 min and then increased 5°C x min until reaching T4 = 24°C, held for 4 min. The total run time was 50.17 min. Nitrogen was used as carrier gas with a pressure of 32.5 psi with a flow rate of 10 mL/min; the injection was split type. The identification and quantification of chromatographic signals (peaks) was carried out by the external standard method and using Shimadzu GC Solution Crhomatography Data System Version 2.4 software (Japan). The standard used was 37 fatty acid mixture (37 component FAME Mix analytical standard, Supelco Cat. No. 47885-U, USA). The injection volume of the sample and standard was 1 µL.

Analysis of conjugated linoleic acid (CLA) and isomers by high performance liquid chromatography (HPLC) with UV-Vis detector. The analysis of CLA and isomers was similar to fatty acids: by derivatization to methyl esters (5).

Chromatographic conditions. A Hitachi Elite La Chrom high performance liquid chromatograph (HPLC) (Hitachi High Technologies Corporation, Japan), with UV-Vis detector at 233 nm wavelength; with stainless steel column of 250 mm length x 4.6 mm internal diameter and 5 µm particle size (ChromoSpher 5 lipid column, USA) was used. The EZCrhom Elite-Enterprise software (Agilent Technologies, Santa Clara, CA, USA) was used to record and integrate the chromatographic signals.

The column was conditioned with hexane-acetonitrile mobile phase (99:1 v/v, J.T. Baker, USA) at a flow rate of 1 mL/min for two hours with constant agitation. Separation of the CLA isomers was carried out with hexane-acetonitrile mobile phase (99.9:0.1 v/v), operated isocratically at a flow rate of 1 mL/min, 30 min were allowed to elapse between injection and injection to stabilize the baseline. The injection volume was 30 µL for each sample, with a run time of 50 min. The identification and quantification of CLA and isomers was done by the external standard method. The standard used was Linoleic acid, conjugated methyl ester (Sigma Cat. No. 05632-250 mg, USA).

Statistical analysis. The statistical program IBM® SPSS ®version 24.0 for Windows (Armonk NY, USA) was used. The parameters measured were fatty acids and conjugated linoleic acid.

The study was descriptive and longitudinal for one year, with monthly observation. With the values obtained, a database was constructed and subjected to exploratory analysis to observe the distribution behavior and, where appropriate, outliers. Grouping and inferential statistical tests were applied to contrast mean values and find similarities in the levels of fatty acids, CLA and isomers in the analyzed milks from the three production units (PU) and collecting tank (CT) (ANOVA, Tukey's test at 95% confidence and Pearson's correlation coefficient). For all analyses the significance was $p<0.05$.

General scheme of the work. Figure 2 shows the general scheme of the work.

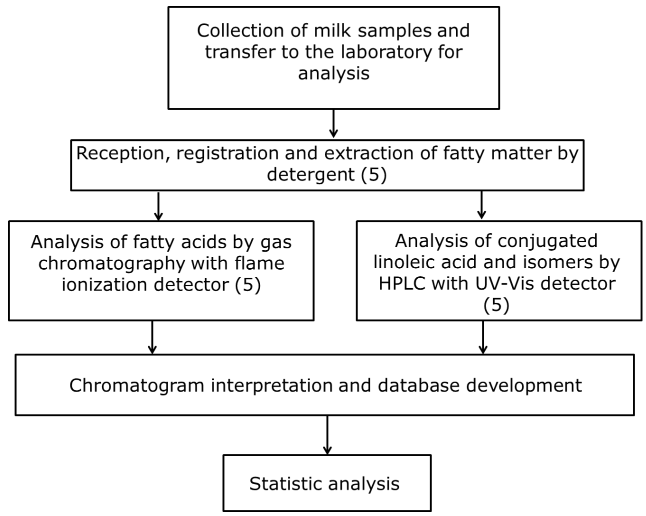

Figure 2. General scheme of the work carried out.

RESULTS

Fatty acids. In the chromatographic analysis, the composition (% w/w) of fatty acids (FA) was observed, the majority FA C14, C16, C18 and C18:1 were identified. Short and long chain, saturated and unsaturated fatty acids were also detected to establish the fatty acid profile in milk fat (Figure 3).

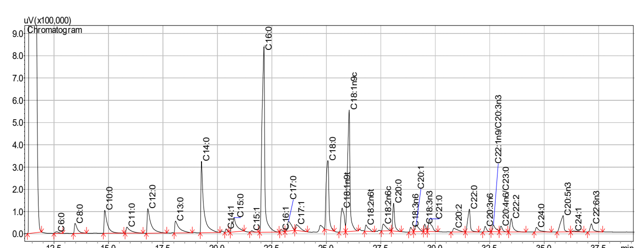

Figure 3. Chromatogram of the fatty acid standard, chromatograph injection volume: 1 µL (FAME Mix analytical standard, Supelco Cat. No. 47885-U, USA).

In the 48 milk samples analyzed, 23 FA were determined, from butyric (C4) to gadoleic (C20:1), including short (C6, C8), medium (C10, C12, C14), long (≥C16), saturated and unsaturated FA (Figure 4). Table 1 shows the means, standard deviations, minimum and maximum FAs found in the organic milks of the three PUs and the CT. ANOVA showed significance ($p<0.05$) for C4, C6, C8, C10, C16, C18, C18:1n9t, C18:2n6 and C20; Tukey's maximum significant difference multiple comparisons ($\alpha=0.05$) indicated that PU2 in C4, C6 and C8 presented significant statistical differences to the other PU and the collecting tank. The C16 content was the same in PU 1, 3 and CT. In C18 similarity was found between PU 1 and 3 and at the same time similarity in PU 2, 3 and CT. For C18:1n9t PU1 and CT were statistically different ($p<0.05$) and in C18:2n6c PU 1 and 3 were equal ($p\geq0.05$), so were PU 2, 3 and CT. For C20, PU1 was different from the other PU and CT.

Figure 5 shows the contents (% w/w) of C4, C16, C18 and C18:1n9t through time. In C4 constant values were visualized in all the studied milks with a decrease in the period from July to November, only PU2 presented a higher value in the month of October. The tendency of C16 was increasing from March to September in all cases, PU2 presented lower values from May to October. The behavior of C18 was higher from January to April with a constant trend from April to December; a decrease in values was noted from June to November in PU1. The C18:1n9t described a decrease in values from March to September, in time the behavior was similar in all PU and CT.

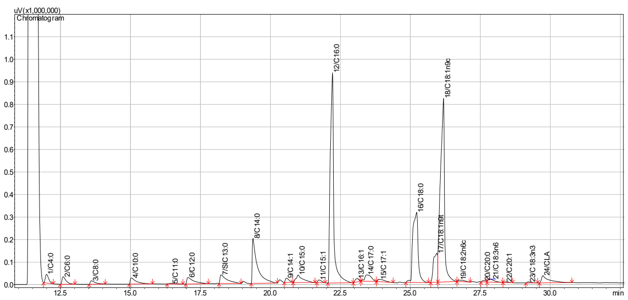

Figure 4. FA profile in organic milk from Tecpatán, Chiapas, Mexico (chromatograph injection volume: 1 μL).

Table 1. Fatty acid composition (% w/w) in milk from the municipality of Tecpatán, Chiapas.

FA	PU 1				PU 2				PU 3				Collecting tank			
	$\bar{x}$	SD	Min	Max	$\bar{x}$	SD	Min	Max	$\bar{x}$	SD	Min	Max	$\bar{x}$	SD	Min	Max
C4	3.3a	0.4	2.4	3.8	3.9b	0.9	2.6	5.9	3.2a	0.6	2.5	4.6	3.2a	0.5	2.6	4.6
C6	1.8a	0.2	1.5	2.1	2.0b	0.2	1.8	2.4	1.9a	0.2	1.6	2.3	1.9a	0.1	1.7	2.1
C8	0.9a	0.1	0.6	1.1	1.0b	0.1	0.8	1.3	0.9a	0.1	0.7	1.1	0.9a	0.1	0.7	1.1
C10	1.8ab	0.3	1.3	2.3	1.9ab	0.3	1.4	2.3	1.7a	0.3	1.2	2.2	2.0b	0.3	1.6	2.4
C11	0.2a	0.0	0.1	0.3	0.2a	0.0	0.1	0.2	0.2a	0.0	0.1	0.2	0.2a	0.0	0.1	0.2
C12	2.0a	0.4	1.4	2.5	2.0a	0.3	1.2	2.4	2.0a	0.4	1.5	2.6	2.1a	0.3	1.6	2.5
C13	0.1a	0.0	0.1	0.1	0.1a	0.0	0.0	0.1	0.1a	0.0	0.1	0.1	0.1a	0.0	0.1	0.1
C14	8.7a	1.3	6.5	10.5	9.0a	0.6	8.0	9.7	9.0a	1.0	7.1	10.5	9.0a	1.0	7.4	10.4
C14:1	1.8a	0.2	1.2	2.1	1.8a	0.4	1.0	2.2	1.7a	0.3	1.2	2.2	1.5a	0.4	0.9	1.9
C15	1.2a	0.2	0.8	1.5	0.9a	0.3	0.3	1.2	0.9a	0.3	0.4	1.2	0.8a	0.3	0.3	1.1
C15:1	0.4a	0.0	0.3	0.5	0.6a	0.5	0.3	2.1	0.8a	0.8	0.3	2.3	0.9a	0.7	0.3	2.2
C16	27.3a	3.0	22.4	31.1	25.6b	1.4	23.3	28.9	27.2a	1.7	24.2	29.6	26.4ab	1.7	23.1	28.8
C16:1	0.3a	0.0	0.3	0.5	0.3a	0.0	0.3	0.4	0.4a	0.0	0.3	0.4	0.4a	0.0	0.3	0.4
C17	0.6a	0.1	0.5	0.8	0.5a	0.1	0.1	0.6	0.6a	0.1	0.5	0.7	0.5a	0.0	0.5	0.6
C17:1	0.5a	0.1	0.4	0.6	0.6a	0.1	0.4	0.8	0.6a	0.0	0.5	0.7	0.5a	0.0	0.5	0.6
C18	11.7a	1.7	8.1	14.2	13.1b	1.2	11.4	15.6	12.4ab	0.9	11.1	14.4	12.5b	0.7	11.4	13.8
C18:1n9t	3.8a	0.9	2.6	6.1	3.9ab	0.6	2.6	4.7	4.1ab	0.6	3.4	5.3	4.3b	0.7	3.6	5.9
C18:1n9c	22.1a	2.8	17.5	27.3	21.3a	1.5	19.4	23.3	21.1a	2.2	17.1	24.6	21.3a	1.6	18.9	23.5
C18:2n6c	0.3a	0.1	0.3	0.5	0.4b	0.0	0.3	0.4	0.3ab	0.0	0.3	0.5	0.4b	0.1	0.3	0.5
C18:3n6	0.1a	0.0	0.0	0.1	0.1a	0.0	0.0	0.2	0.1a	0.0	0.1	0.1	0.1a	0.0	0.1	0.2
C18:3n3	0.3a	0.1	0.2	0.4	0.3a	0.0	0.3	0.4	0.4a	0.0	0.3	0.4	0.4a	0.0	0.3	0.4
C20	0.1a	0.0	0.1	0.2	0.2b	0.0	0.1	0.2	0.2b	0.1	0.1	0.3	0.2b	0.1	0.2	0.4
C20:1	0.1a	0.1	0.0	0.2	0.1a	0.0	0.0	0.1	0.1a	0.0	0.0	0.1	0.1a	0.0	0.0	0.1

FA: fatty acid; $\bar{x}$: arithmetic mean, SD: standard deviation, Min: minimum; Max: maximum.
Different letters in means of the same row indicate difference at 95% confidence.

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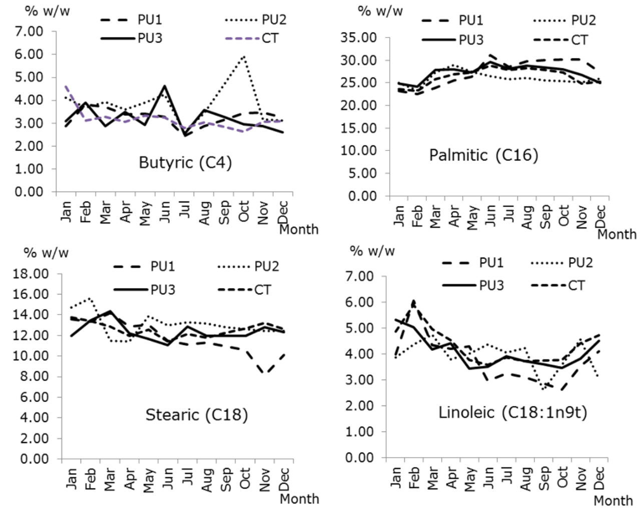

Figure 5. Distribution trend over time of the mean values of C4, C16, C18 and C18:1n9t (% w/w) in organic milk from PU 1, 2, 3 and CT.

The correlation was positive for short and medium chain FA (C4 to C14) and C16. The correlation of palmitoleic (C16:1) with the other FA was negative and, from stearic (C18) onwards, the correlations were negative and positive (Table 2).

Conjugated linoleic acid (CLA). The CLA standard was composed of nine CLA isomers (12t-14t; 11t-13t; 10t-12t; 9t-11t; 8t-10t/7t-9t; 11t-13c; 10t-12c; 9c-11t; 7c-9t). Table 3 describes the means and 95% confidence intervals of CLA (% w/w) and isomers (% CLA) determined in the milks studied. One-way ANOVA showed no significant difference ($p \geq 0.05$) in CLA and isomers in PU and TC milks.

Table 2. Correlations between fatty acids of PU 1, 2, 3 and CT milks produced in Tecpatán, Chiapas.

FA	C4	C6	C8	C10	C12	C14	C14:1	C16	C16:1	C18	C18:1n9t	C18:1n9c	C18:2n6c	C18:3n3
C4	1													
C6	0.6**	1												
C8	0.4**	0.9**	1											
C10	-0.1	0.5**	0.7**	1										
C12	-0.3	0.4**	0.6**	0.9**	1									
C14	-0.2	0.5**	0.6**	0.9**	0.9**	1								
C14:1	-0.1	0.1	0.2	0.3*	0.3*	0.3*	1							
C16	-0.2	0.4*	0.4**	0.6**	0.7**	0.8**	0.2	1						
C16:1	-0.2	-0.6**	-0.6**	-0.6**	-0.5**	-0.6**	-0.1	-0.5**	1					
C18	0.1	-0.1	-1	-0.2	-0.3	-0.4*	-0.1	-0.7**	0.4*	1				
C18:1n9t	0.02	-0.3	-0.3*	-0.2	-0.4**	-0.5**	-0.1	-0.7**	0.3	0.4**	1			
C18:1n9c	0.1	-0.6**	-0.7**	-0.9**	-0.9**	-0.9**	-0.3*	-0.7**	0.6**	0.2	0.4*	1		
C18:2n6c	0.1	-0.1	-0.1	-0.3*	-0.4**	-0.4**	-0.3	-0.7**	0.4*	0.5**	0.7**	0.3*	1	
C18:3n3	-0.2	-0.1	-0.02	-0.1	-0.1	-0.1	-0.1	0.004	-0.1	-0.03	-0.1	0.05	-0.1	1

FA: fatty acid; *: significant correlation with α=0.05 (bilateral); **: significant correlation with α=0.01 (bilateral).

Table 3. Mean values and confidence intervals of CLA (% w/w) and isomers (% CLA) in organic milk from Tecpatán, Chiapas.

CLA	**PU 1**	**PU2**	**PU3**	**CT**
Isomers	$\bar{x}$ **(CI)**	$\bar{x}$ **(CI)**	$\bar{x}$ **(CI)**	$\bar{x}$ **(CI)**
CLA	1.09 (0.83,1.34)	1.15 (1.00,1.31)	1.22 (1.06,1.38)	1.33 (1.11,1.28)
12t-14t	0.66 (0.43,0.89)	0.44 (0.10,0.78)	0.46 (0.09,0.84)	0.61 (0.51,0.72)
11t-13t	0.92 (0.37,1.46)	0.80 (0.67,0.94)	0.88 (0.53,1.24)	0.99 (0.89,1.08)
10t-12t	0.12 (0.07,0.17)	0.36 (-0.37,1.08)	0.37 (-0.29,1.04)	0.27 (-0.02,0.55)
9t-11t	0.28 (0.06,0.46)	0.37 (0.11,0.63)	0.29 (0.0,0.55)	0.32 (0.07,0.57)
8t-10t/7t-9t	0.18 (0.11,0.26)	0.24 (0.07,0.41)	0.35 (0.04,0.66)	0.22 (0.06,0.37)
11t-13c	0.41 (-0.14,0.95)	0.50 (-0.33,1.32)	0.28 (-0.10,0.68)	0.15 (0.13,0.17)
10t-12c	4.46 (1.48,7.44)	4.29 (2.96,5.62)	4.52 (3.92,5.12)	4.87 (3.30,6.44)
9c-11t	90.42 (86.85,93.98)	90.19 (88.86,91.52)	90.51 (88.72,92.30)	89.14 (86.95,91.32)
7c-9t	2.57 (1.99,3.16)	2.80 (2.48,3.12)	2.32 (0.99,3.65)	3.43 (2.38,4.47)

$\bar{x}$: arithmetic mean, CI: confidence interval for the mean at 95% confidence.

DISCUSSION

Fatty acids. Gas and liquid chromatography methods are known for their favorable performance to determine the composition of milk fat. In this work, the contents (% w/w) of FA were determined in milk samples produced under organic production conditions, from three PU and CT of the municipality of Tecpatán, Chiapas. The significant differences ($p<0.05$) found in 9 of the 23 FA between PU and CT (Table 1) refer that at least two PU are similar in certain FA to CT, which is understood because in the collecting tank the total production of organic milk of the municipality is concentrated and the individual effect is diluted, so the distance of the mean values for each one of the PU is the minimum distance. Benbrook et al (11) analyzed organic milk from seven regions of the United States during 18 months, reported 29 FA, most of them with values close to the values found in this research. However, lower values in C4, C18:1 and CLA and higher values in C10, C14, C16 and C18:2 stand out. According to scientific studies (12,13) the benefits of the consumption of certain FA have been recognized, for example butyric acid (C4) has an antitumor effect in prostate, breast and colon; C6, C8 and C10, besides giving the characteristic aroma of the different types of milk (goat, sheep and cow), have been associated in inhibition of microbial and viral growth and dissolution of cholesterol deposits in *in vitro* tests and in test animals. C12, C14 and C16 FA have been linked to the elevation of "bad cholesterol" (LDL) in blood, it is worth mentioning that myristic acid (C14) is more hypercholesterolemic, with a potency 3 to 6 times greater than lauric (C12) or palmitic (C16) acids, however, stearic (C18) acid has been found to be neutral (14). CLA has been attributed anticarcinogenic, antiteratogenic, antiadipogenic, antidiabetogenic and anti-inflammatory properties, however, the information comes from *in vitro* studies (15). In other studies (11,16-19) carried out around the world in organic and conventional milk, the production conditions were observed to be particular for each region, such as climate, time of the year, breed, age and type of feeding, showing a diverse content of FA, however, the FA profile is similar (Table 4).

The FA values obtained in this work are an advance in the characterization of the lipid fraction of organic milk from southeastern Mexico.

Regarding the distribution of fatty acid content over time, it was observed that it was constant in all the milks analyzed (PU 1, 2, 3 and CT), however, some variations were observed during the year; there are times when the trend increases and others when it decreases (Figure 3).

Table 4. Mean values of fatty acids (% w/w) and conjugated linoleic acid found in this study and other countries.

FA	This study	EEUU*	New Zealand**	Poland***	Korea****	Denmark*****
		2013	2015	2017	2020	2020
	(% w/w)	(% w/w)	(% w/w)	(% w/w)	(% w/w)	(% w/w)
C4	3.39	2.42	2.30	1.38	2.23	-
C6	1.90	1.95	1.78	2.08	1.77	2.56
C8	0.93	1.31	1.18	1.54	1.09	1.43
C10	1.84	4.14	2.19	3.09	2.51	3.18
C12	2.03	3.41	3.20	3.78	3.16	3.52
C14	8.93	11.23	11.29	12.1	11.12	10.97
C14:1	1.71	0.93	0.99	1.76	0.87	0.92
C16	26.73	30.06	32.79	36.7	30.18	27.00
C16:1	0.35	1.50	1.64	1.80	1.43	1.29
C18	12.43	11.05	10.47	9.45	14.15	9.04
C18:1	25.46	20.93	20.86	19.88	24.00	19.29
C18:2	0.36	2.05	1.50	1.74	0.36	2.64
C18:3	0.46	0.82	0.90	0.52	0.57	0.81
ALC	1.20	0.73	1.63	-	-	-

*: (11); **: (16); ***: (17); ****: (18); *****: (19).

Popovic-Vranjes et al (20), during one year analyzed the FA in organic milk fat from Serbia, the data showed higher values of polyunsaturated fatty acids in the period from May to July. Benbrook et al (11) when studying organic milk from seven regions of the United States observed seasonal periods during the year and differences in the composition and contents of fatty acids, the C18:2 presented lower values from May to November and the CLA from May to October. Tunick et al (21) analyzed the fatty acids of organic milk during spring-summer and autumn-winter, where they observed statistically significant difference in seven of 16 identified FA (C6, C8, C14:1, C16, C18:1 and C18:2), they did not find statistical significance in CLA. According to Stergiadis et al (22) in organic milk produced in England during one year, they obtained higher values of C16 during October, November and December, this coincides with the values found in this research, based on the grazing conditions of dairy cattle, with warm humid climate and rains during the whole year, it was not possible to observe statistical differences in the content of FA.

Pearson's correlation analysis between FA showed positive correlations from C4 to C16 in the milk studied and negative correlations from C16:1 to C18:3n3. These results coincide with those found in Mexican pasteurized milk commercialized in Mexico City (8), with the difference that the positive and negative correlations were from C18.

Conjugated linoleic acid. CLA is a group of at least 28 isomers of linoleic acid, which have a beneficial effect on human health (13). Table 3 shows that the CLA and isomers of the studied milks did not present statistically significant differences ($p<0.05$), this is probably due to the breed of the cows and the feeding that is similar in all the PU of the studied municipality. The confidence intervals at 95% of CLA and its isomers are similar to the values found for cow milk in Poland (23). The presence of C18:2, 9c-11t with 91.5% is notorious, while in the present study 90.1% was obtained, due to the lower number of isomers (6 *vs.* 9), the higher the number of isomers, the contents tend to decrease and vice versa. Other investigations carried out in milks from the United States and New Zealand report similar isomers profile, however, the contents vary due to geographical zones, climate, handling, breed, time of the year and mainly to feeding (16,22). In fact, the C18:2 9c-11t is an isomer of the CLA with a higher proportion in bovine milk fat; it is the result of the ruminal biohydrogenation of C18:2n6 and the major source is the endogenous conversion of C18:1 11t (vaccenic acid, VA), the scientific evidence proposes that VA is desaturated to C18:2 9c-11t (13). Studies in cell lines and experimental animals have found anticancer effects of CLA isomers, for example, C18:2 10t-12c inhibits the growth of colon cancer cells and induces their death, while C18:2 9c-11t is known to mediate anticancer effects through apoptosis. Also, positive correlations have been observed between CLA supplementation and improvement in body mass index, body weight, body fat mass, abdominal adiposity and lean body mass; consumption of 3 to 4.2 g/day of 1:1 concentrations of C18:2 9c-11t/C18:2 10t-12t in overweight and obese children has been found to decrease body fat. Other globular membrane lipids in milk, such as phospholipids and sphingolipids, offer benefits in dysfunctional lipid metabolism, intestinal dysbiosis and cardiovascular disease (12,13,24,25). Table 6 shows that the average CLA value found in this work is higher than in other countries, which offers an opportunity to promote organic milk production in this area of southeastern Mexico.

In conclusion, it was possible to characterize the fatty acid profiles, conjugated linoleic acid and isomers in organic milk produced in Tecpatán, Chiapas, Mexico, which had not been studied so far. Butyric, myristic, palmitic, stearic and oleic represented approximately 77% of the total FA and the 9c-11t isomer represented 90.1% of the total CLA isomers. Given the conditions of the municipality, production system, breed of cows (zebu-Swiss Brown cross), sufficient pasture all year round and above all the constant climate (warm-humid with rain all year round), the effect of the time of the year on the contents of fatty acids, conjugated linoleic acid and isomers was not observed.

Conflict of interest

The authors of this paper declare that there is no conflict of interest with the publication of this manuscript.

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