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Physicochemical Composition of Seed Oil of Wild Jojoba Populations in Northwestern Mexico

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Abstract This research analyzed physicochemical parameters of lipids, as well as the fatty acid profile and diversity of three jojoba ecotypes of northwestern Mexico. Oil content was from 43 to 49% in the three ecotypes; iodine value ranged from 82.08 to 83.11 g / 100 g; acidity value was 0.33 to 0.39 mg KOH/g. The four most abundant fatty acids in the three ecotypes were eicosanoic (52-62.43%), oleic (13.80-27.36%), 13-docosanoic (5.25-9.45%), and palmitic (6.43-9.70%) acids. The Inter Simple Sequence Repeat (ISSR) analysis showed that polymorphic accessions were 83%. The ecotypes analyzed in this study represent an alternative for selection and conservation of wild germplasm; furthermore, they also represent a potential for their use in cosmetics and biodiesel manufacturing industries.

Keywords: *fatty acid, characterization, ecotypes, seeds*

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1. Introduction

Jojoba (*Simmondsia chinensis*) is a dioecious shrub, native to the Sonoran Desert, located in the states of Arizona and California, in the United States of America, and the states of Sonora, Baja California, and Baja California Sur in Mexico [1]. Jojoba seeds contain from 40 to 60% of oil which is chemically classified as liquid wax because unlike most vegetable seed oils that are composed of triglycerides, jojoba oil consists of esters. This unique chemical structure gives jojoba special features [2]. Jojoba oil is a source for developing cosmetics and lubricants and has the potential for biodiesel production [3,4]. Shailesh *et al*. [5] determined that the viscosity and refractive indexes, specifically gravity and fatty acid composition were important properties that indicated stability when used as additives in lubricants or biodiesel production. Jojoba seed oil composition depends on environmental factors such as climate, soil type or specific environment (ecotype), and genetic factors [6]. Some studies have shown that the content and composition of jojoba oil is strongly related to the genetic variability of the plant [7]. At first large-scale jojoba planting attempts failed due to low seed production and low oil content, so the study of genetic variability could be the key to achieve high yields in the near future [7]. The Inter Simple Sequence Repeat (ISSR) is understandable, affordable, and useful to determine genetic variability techniques [8]. The choice of primers used in ISSR is critical for the detection of high levels of polymorphism. This technique has been used successfully in genome mapping in a variety of species such as corn, rice, barley, wheat, and grass [9] . On the other hand, they have been proposed as a new source of genetic markers that can overcome the technical limitations of restriction fragment length polymorphism (RFLP) and random amplification of polymorphic DNA (RAPD) [17]. Because of the importance of jojoba, it is necessary to conduct studies related to the physicochemical characteristics of oil seeds from wild populations and their genetic variability. In this paper, these studies were performed using seeds of three wild ecotypes from northwestern Mexico to evaluate the use of germplasm in the region and promote their conservation and use in breeding and production programs.

Table 1. Climate conditions of the three ecotypes of jojoba *Simmondsia chinensis* in northwestern México, Sonora (Sonoyta); Baja California Sur (BCS.); Baja California (BC). Maximum temperature (T. max); average temperature (T.A); minimum temperature (T. min); relative humidity (RH); precipitation (Prec); wind speed (Vv) and global radiation (Rad. G) (INIFAP, 2011, CONAGUA, 2010)

Ecotypes	T. max (°C)	T. A. (°C)	T. min (°C)	RH (%)	Prec (mm)	Vv (km/hr)	Rad. G. (w/m^2)
Sonoyta	30.6	21.8	13.0	41.04	215	3.18	518.54
Todos Santos	32.8	23.9	15.0	53.07	483.4	4.36	420.37
ndígena la Huerta	24.7	15.0	5.2	43.30	266.3	5.49	498.06

2. Materials and Methods

2.1. Plant Material

Fruits of wild plants of jojoba (*Simmondsia chinensis*) were obtained from three ecotypes of Mexico: Sonoyta in Sonora (31° 86' 13" N and 112° 85' 44" W); Indígena la Huerta in Baja California (31° 51' 16" N and 116° 09' 51" W), and Todos Santos in Baja California Sur (23° 25' 6.12" N and 110° 9' 14.4" W), which are regions of origin of the species. Climate information from different ecotypes (Table 1) in the station network was obtained from Agroclimate Mexico [10,11]. Fruits were transferred to the Centro de Investigaciones Biológicas del Noroeste (CIBNOR). The seeds were separated from the fruit and stored in glass vials at 5°C.

2.2. Oil Extraction

The 960.39 (AOAC International Method MD, U.S.A.) [12] was used to evaluate oil content. The seeds were ground in a blender (Osterizer, model 4108, USA). The 10 g sample was placed in a pre-weighed thimble and set in the Soxhlet extractor to perform extraction with n-hexane for 16 h; subsequently, the solvent was distilled off in vacuum on a rotary evaporator (Buchi, ModelR-205, USA). The thimble was placed at 103 ± 2°C in the oven for 24 h to remove residual solvent, cooled in a desiccator, and weighed on an analytical balance (Sartorius AX124, Goettingen, Germany). The oil was stored in amber bottles for physicochemical analysis.

2.3. Determination of Oil Content and Physicochemical Parameters

The refractive index was determined with a Mettler-Toledo RE40D (Schwerzenbach, Switzerland) refractometer at 25°C; viscosity with a viscometer (Cannon Fenske, Germany) at 40°C-ASTM using D445-06 method; acid by 940.28 method; iodine with the 993.20, method; and peroxide with 965.33 method [12]. Specific gravity was determined by the pycnometer method (Model 43205, Brand, Germany).

2.4. Fatty Acid Composition

Oil extraction and methylation were done following the method 969.33 [12] and analyzed by gas chromatography (Varian, U.S.A.) coupled to a mass spectrometer (Titan 4000, U.S.A.) using a capillary column CP-SIL 43CB (25 mx 0.32 mm x 0.2 um). The column temperature was programmed at 120°C (ramp 0.0 cm/min and held for 1.0 min); 210°C (ramp 10°C/min for 4 min); 215°C (ramp 1.0 maintained for one min); and 220°C (ramp 0.5°C / min for one min). The injector temperature was 205°C. The ramp split was approximately 50:1. The carrier gas (helium) was at a constant flow of 1.0 mL/min. Separation of methyl ester fatty acid was identified by electron impact. The quantitative analysis of fatty acids was determined by using internal standards (F. A. M. E. Mix-C4-C24, Supelco, Cat. 18919-AMP). The percentage of individual fatty acid was calculated by comparing the peak areas with the same internal standards, and it was expressed as the total amount of fatty acids in each lipid fraction.

2.5. DNA Extraction

Leaves of apical meristems in different ecotypes were collected, placed in plastic bags, and moved in a modified container with ice at CIBNOR's laboratory. DNA extraction was performed immediately by CTAB method 2% (1.4 M NaCl, 10 mM EDTA, 10 mMTris-HCl pH 8.0, 0.2% β-mercaptoethanol)[13]. In 0.5mL Eppendorf tubes, 0.3g of fresh tissue buffer were placed with 200 µl of 2% CTAB and incubated at 60°C for 15 min. Subsequently, 400 mL of chloroform were added to isoamyl alcohol (24:1v/v), centrifuged at 13000 rpm for 10 min, and supernatant was recovered; 15µL of RNAse (10µg /µL) were added; enzyme was incubated at 37°C for 30 min; subsequently, 400 µL of chloroform were added to isoamyl alcohol (24:1v/v), centrifuged at 13000 rpm for 10 min, and supernatant was recovered. The DNA was precipitated with 400 µL of 100% isopropanol at -20°C, immediately centrifuged at 13000 rpm for 10 min, and decanted. The precipitate was washed with 100% isopropyl alcohol and centrifuged at 13000 rpm for 5min; the solvent was evaporated and re-suspended in 20 µl of sterile distilled ultrapure water. The DNA obtained was stored at -20°C for analysis in the laboratory.

2.6. Analysis of Jojoba Genetic Variability (ISSR)

DNA samples were analyzed with the primers (ISSR) M2, M10 and M11 (5'AGAGAGAGAGAGAGAGCT-3 ', 3'-5'GTCGTCGTCGTCGTCGT, 5'GAGGAGGAGGAGGC-3'), (Integrated DNA Technologies brand). PCR reactions were performed in a volume of 15 µL containing: 100 ng/mL of DNA, 5 U/mL of Taq polymerase (Promega Corporation, U.S.A.), 5X Buffer, 25 mM MgCl2, 10m MdNTPs, 10 pM of primers, and ultrapure water. Amplification conditions were: initial denaturation of 94°C x 2 min, followed by 35 cycles consisting of 94°C x 2 min, 49-51°C (suitable temperature alignment each first x 1 min), extension 72ºC x 1.3min and a final extension at 72°C for 10 min. After completing the PCR reaction, the products were stored at 4°C. Preliminary verification of amplification was carried out in agarose gels stained with 2% gel network (Promega Corporation, U. S. A.) using a 1

Kb ladder marker (Invitrogen, U.S.A.). Finally, gels were analyzed on a Gel Doc (BioRad, serial number 765/07029, Germany), and system image was recorded with the Quantity One (BioRad, Hercules, CA, U.S.A.) software.

2.7. Cluster Analysis

The binary matrix was based on presence (1) and absence (0) of the polymorphic bands observed among individuals. The relationship between the groups was estimated by the Jaccard similarity coefficient with the FREETREE program, statement based on the Unweigthted Pairwise Method with Arithmetic mean (UPGMA). Trees were visualized by using the TREVIEW (3.2) software.

Polymorphism percentage among the ecotypes under study was obtained by considering the total number of polymorphic bands for each of them divided by the sum of those obtained in all primers, and multiplied by 100.

2.8. Statistical Analysis

All the analyses were done in triplicate. The normality Kolmogorov-Smirnov and homoscedasticity (Levene) tests were performed. Pearson correlation test was performed to compare the physicochemical characteristics of oil with environmental factors. The data were analyzed using one-way ANOVA procedures, mean, and standard deviation. The differences between treatment means were compared with the multiple range Tukey test with 0.05 confidence level α and 95%. Sigma Stat statistical software 3.5 (2007) and SigmaPlot 10.1 (2009) were used.

3. Results

3.1. Oil Content

Oil content of 10 g of jojoba seeds varied significantly from 43 to 49%, (F = 16.65, $p \leq 0.05$) (Table 2). Sonoyta ecotype had higher oil content (49%) and Indígena la Huerta lower oil content (43%).

Physicochemical characteristics of jojoba seed oil

Table 2 shows the results of jojoba oil analysis. The refractive index was 1.46. No significant difference (F = 1.00, $p \leq 0.05$) was found in the refractive index for the three study ecotypes. Specific density was 0.86g/cm3. No significant differences (F = 1.00, $p \leq 0.05$) were shown in the specific gravity of the three ecotypes. The refractive index and specific density of jojoba oil was within IJEC (International Jojoba Export Council, 1998) standards. The viscosity of jojoba oil was 21.60 cSt at 21.12 in the three ecotypes studied. The acid value of the three ecotype-samples was from 0.36 to 0.39 mg KOH/g. In both parameters, no significant differences (F = 0.07, $p \leq 0.05$) (F = 0.82, $p \geq 0.05$) were observed. The iodine value was 82.86 81.08 in g/100 g. No significant differences (F = 0.97, $p \leq 0.05$) were observed in the three ecotypes, and iodine value was found within IJEC quality standards. Peroxide index was 2.00 meq of peroxide/kg for the three ecotypes. No significant differences (F = 1.00, $p \leq 0.05$) were observed, and peroxide index was within IJEC quality standards.

3.2. Fatty Acid Composition

The fatty acid composition of jojoba oil is shown in Table 3. The content of saturated fatty acids (palmitic, myristic, lauric, pentadecanoic) was 7.9% and that of unsaturated fatty acids (eicosanoic, docosenoic, oleic, linoleic and linolenic acid) was 92% of the total fatty acids. The fatty acids eicosenoic C20: 1 (52.36 to 64.56%), docosenoic C22: 1 (6.86 a 9.95%) and palmitic C16:0 (7.38 to 9.70%) were significantly different (F = 29.84, $p \leq 0.05$). The oleic fatty acids C18: 1 (16.31 to 27.36%) (F = 4.91, $p \leq 0.05$), linoleic C18: 2 (0.37 to 0.95%) (F = 55.48, $p \leq 0.05$), and linolenic acid C18: 3 (0.87 2.50%) (F = 37.75, p 0.05) showed differences in the three ecotypes. Lauric fatty acids C12: 0, myristic C14: 0 and pentadecanoic C15: 0 appeared in small quantities (< 0.1%).

Table 2. Physical and chemical characteristics of oils of three *Simmondsia chinensis* ecotypes of northwestern México. The value of the mean ± sd. Different superscripts in the same column indicate significant difference between ecotypes (Tukey Test, $p < 0.05$). Number of samples (n = 3)

Characteristics	Sonoyta	Todos Santos	Indígena la Huerta	Range (IJEC)
Oil content (%)	49 ± 2.80[a]	44 ± 1.35[b]	43 ± 1.00[b]	
Index of refraction (25 °C)	1.46 ± 0.00[a]	1.46 ± 0.00[a]	1.46 ± 0.00[a]	1.45-1.46
Viscosity (cSt) 40 °C	21.60 ± 0.00[a]	21.12 ± 0.00[a]	21.12 ± 0.00[a]	
Specific density (g/cm^3)	0.86 ± 0.00[a]	0.86 ± 0.00[a]	0.86 ± 0.00[a]	0.86-0.87
Iodine index (g/100g)	83.11 ± 0.43 [a]	81.46± 0.01[a]	82.35 ± 0.00[a]	80-85
Acid index (mg KOH/g)	0.39 ± 0.00[a]	0.35 ± 0.01 [a]	0.36 ± 0.02 [a]	1 max.
Peroxide index (meq/kg de sample	2 ± 0.00[a]	2 ± 0.00[a]	2 ± 0.00[a]	2 max.

Table 3. Composition of acid degree of oil in three *Simmondsia chinensis* ecotypes of northwestern México. The value of the mean ± sd. Different superscript on the same line indicates significant difference between ecotypes (Tukey Test, $P < 0.05$). Number of samples (n = 3). BCS (Baja California Sur) B.C. (Baja California)

Fatty acid composition	Sonora (Sonoyta)	BCS. (Todos Santos)	BC Indígena la Huerta
Lauric (C12:0)	0.02 ± 0.00 [a]	0.04 ± 0.01 [a]	0.02 ± 0.00 [a]
Myristic (C14:0)	0.20 ± 0.03 [ab]	0.11 ± 0.03 [a]	0.15 ± 0.02 [a]
Pentadecanoíc (C15:0)	0.08 ± 0.00 [a]	0.09 ± 0.02 [a]	0.11 ± 0.00 [a]
Palmitic (C16:0)	7.67 ± 1.44 [a]	7.38 ± 2.40 [a]	9.70 ± 0.74 [b]
Oleic (C18:1)	18.90 ± 5.40 [a]	16.31 ± 3.65 [a]	27.36 ± 5.90 [b]
Linoleíc (C18:2)	0.37 ± 0.09 [a]	0.47 ± 0.18 [a]	0.95 ± 0.19 [b]
Linolenic (C18:3)	0.87 ± 0.20 [a]	1.09 ± 0.65 [a]	2.50 ± 0.25 [b]
Eicosenoic (C20:1)	62.43 ± 9.38 [b]	64.56 ± 9.04 [b]	52.36 ± 4.15 [a]
13-docosanoic (C22:1)	9.45 ± 7.40 [a]	9.95 ± 2.48 [a]	6.86 ± 2.34 [b]

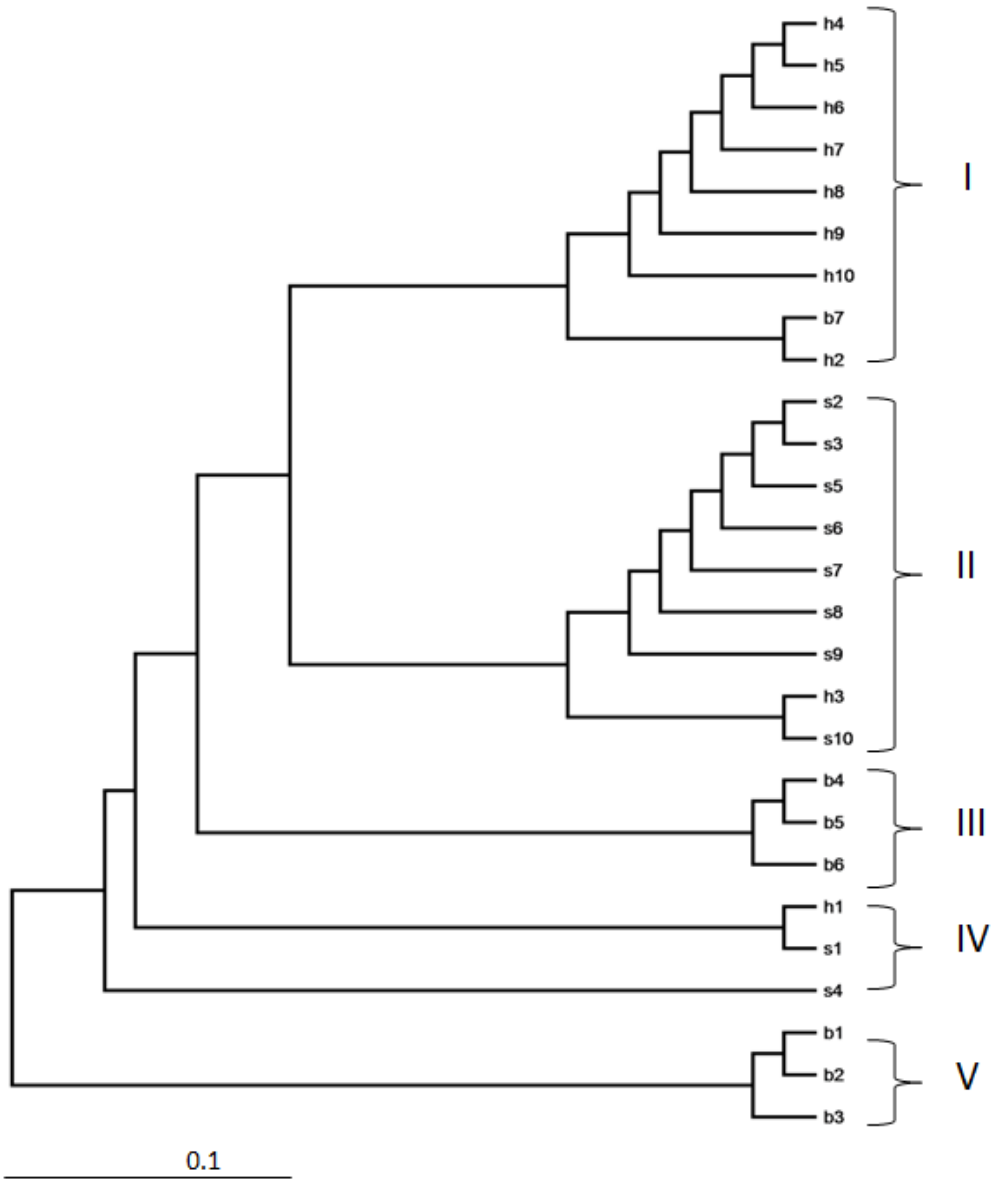

Figure 1. Dendrograma from 27 accessions of *Simmondsia chinensis* based on genetic distances generated with the combination of ISSR oligonucleotides. Based on the Jaccard's values of similarity. s = Sonoyta; b = Todos Santos; h = Indígena la Huerta.

3.3. Genetic Variability

The results obtained by using three ISSR primers to analyze 27 accessions of *S. chinensis*, a total of 20 bands were observed; 11 were polymorphic and 9 monomorphic. The number of bands generated by each one was first from four to 12, observing that the first M2 had the highest number of polymorphic bands while the first non-polymorphic M11 bands were observed. The size of amplified products varied from 200 to 2.000 bp. The first M2 generated the highest percentage of polymorphisms (83%), and the first M10 generated 25%.The average Jaccard similarity coefficient of all ecotypes was 0.85. The similarity index indicated that the maximum distance of genetic similarity was one; accessions of Indígena la Huerta (H3) and Sonoyta (s10), Indígena la Huerta (h2) and Todos Santos (b7) were found to be genetically identical because they showed a Jaccard similarity index of one. In addition, a high degree of similarity was found among individuals of each ecotype. The dendrogram obtained by the UPGMA method (Figure 1) showed five main groups: (I) nine accessions, seven Indígena la Huerta (h2, h4, h5, h6, h7, h8, h9, and h10) and one of Todos Santos (b7); (2) nine accessions, eight Sonoyta (s2, s3, s5, s6, s7, s8, s9, and s10), and one Indígena la Huerta (h3); (3) three accessions of Todos Santos (b4, b5, and b6); (4) three accessions, two Sonoyta (s1 and s4), and Indígena la Huerta (h1); and (5) three accessions of Todos Santos (b1, b2 and b3).

4. Discussion

4.1. Content and Physicochemical Characteristics of Jojoba Oil

Jojoba seeds used in this study showed no physical damage (necrosis or spots). Gayol *et al*. [14] assessed the quality of jojoba seeds in Argentina, and found that oil content was higher in undamaged seeds (50.82%) than in those damaged (39.11%), demonstrating that oil content of jojoba seed varies according to the quality of the seed and its environment. It coincides with what it was observed in the physicochemical characteristics of oil from different

ecotypes analyzed, which were within the IJECT standards. On the other hand, oil content was similar to those proposed by Gayol *et al*. [14], 2009 (for undamaged seeds). Moreover, seeds with high oil content can be used in industrial processes for obtaining biodiesel and cosmetics [15]. Sonoyta, Todos Santos, and Indígena la Huerta are alternatives for the development of plants for this purpose.

On the other hand, temperature and precipitation were highly related to oil content (r =0.996 temperature) and (r = 0.872 precipitation), as observed in Sonoyta and Todos Santos. On the contrary Indigenous seeds ecotype la Huerta developed in lower temperature conditions (24.70°C) and rainfall (63 mm) to obtain lower oil content (43%).Additionally, oil content in the three ecotypes showed a low ratio regarding altitude (r = 0.160, $p > 0.05$). As to ecotypes at an altitude of 146 msnm with 700-oil content, it was lower than in those found in Sonoyta with higher altitude (584 msnm).

Seed moisture of the different ecotypes was less than 10%, which allowed proper storage of seeds [16]. On the other hand, a negative correlation between relative humidity and oil content (r = -0. 912) was observed. As mentioned before jojoba seeds for this study were obtained from wild populations. The variability analyses showed a polymorphism of 83% with the first M2 (Figure 1), which could explain the variations in oil content among individuals from the three ecotypes Sonoyta, Indígena la Huerta, and Todos Santos. When Al Sogeer *et al*. [7] compared the genetic variation of seven jojoba genotypes (from Argentina) in relation to oil content, they reported that the coefficient of genetic similarity was from 0.60 to 0.90. Micro satellite ISSR analysis showed a high polymorphism among the seven genotypes whose oil content of was from 47.17 to 54.95 ± 1.80%, similar to those reported for Sonoyta in this study.

Previous studies have shown that jojoba seed oil from Jordan, Argentina, Egypt, and India have refractive indexes from 1.44 to 1.46 [17,18,19,20,21], similar to the jojoba seed oil ecotypes studied in northwestern Mexico, indicating that there is no significant relationship of this parameter with respect to environmental factors. Studies have been reported on the characteristics of jojoba seed oil from Australia and India with a specific gravity of 0.86 and 0.85 g/cm^3 [21,22], similar to those found in this work.

The physical properties of the jojoba oil samples were compared following the IJEC quality standards (Table 2) by observing that the refractive index and specific gravity of all samples in this study were within the range of their quality standards.

Furthermore, a significant difference was observed in the physical properties of jojoba oil from the three ecotypes, which did not show a significant relationship with environmental factors according to the statistical analysis performed by the Pearson test.

Regarding the chemical properties of oil, viscosity was 21.12 cSt at 21.60 in the three ecotypes located at an altitude from 146 to 700 m, which was similar to that reported by Savita *et al*. [21] on jojoba oil samples (22.40 cSt), located 432 m from Rajasthan, India.

The acid value of the three ecotypes of northwestern Mexico was from 0.35 to 0.39 mg KOH/g, with less than one mg KOH/g within IJEC quality (Table 2) standards. These results were equal to those observed by Tobares *et al*. [19] and Savita *et al*. [21], who reported an acid value (mg KOH/g) of 1.10 and 0.49 of seeds from Argentina and India.

Iodine was observed from 83.11 to 81.46 g/100g. No significant differences were found in the studied populations, and it was observed within IJEC quality standards. Other studies have shown iodine content in 82 jojoba oil samples from 82.98 to 84 g/100g [17,21]. A low iodine index indicates better oxidation stability and polymerization [23]. Jojoba oil has a smaller number of iodine with respect to coconut and palm, indicating that at low temperatures, oil solidifies causing viscosity problems in the production of biodiesel, at room temperature though, and it is more stable [24].

Peroxide index was two meq/kg for the three ecotypes (Table 2). No significant differences were found in the three ecotypes and within IJEC quality standards. A high peroxide index (≥ 5 meq/kg) indicates an oxidation process underway [25]. Based on the above, jojoba oil of the different *S. chinensis* ecotypes studied in northwestern Mexico does not have oxidation. Gayol *et al*. [14] reported a lower peroxide level (0.97 meq/kg), and Savita *et al*. [21] showed a peroxide level of 4.41 meq/kg, higher than IJEC standards in samples from Ratangarh, India, attributing it to the conditions and storage time of the seeds until oil extraction (sunlight, bacteria, fungi, heating).

4.2. Fatty Acid Composition

Fatty acids are shown in Table 3. The content of saturated (palmitic, myristic, lauric, and pentadecanoic) fatty acids constituted approximately 7.9%, while 92.02% of unsaturated (eicosanoic, docosenoic, oleic, linoleic, and linolenic) constituted fatty acids. In some industries, such as biodiesel manufacturing, fatty acid composition is important because it directly determines the properties of this product. Some changes in the chain length of fatty acids influence these properties. As a rule, the seeds with a high proportion of palmitic, oleic, and stearic acids are suitable to produce better quality biodiesel [15].

Some authors have suggested that environmental factors play an important role in fatty acid content, and temperature is one of the most important ones in the composition [26]. A correlation analysis showed that in different ecotypes, the maximum temperature was positively correlated with fatty C14 acids: 0, C20: 1, C22: and negatively with fatty acids 1 (r = 0.861, 0.419 and 0.505) C12: 0, C15: 0, C16: 0, C18: 1, C18: 2, and C18: 3 (r = -0435, -0801, -0463, -0362, -0.69 and -0.66), which agrees with that observed in Indígena la Huerta where a lower temperature was recorded and content of C16, C18: 1, C18: 2, and C15:0 was significantly higher (Table 3). Salisbury and Ross [27] mentioned that the variation in fatty acid composition was related to temperature because it affects primary metabolism and biosynthesis. With increasing temperature, the interaction between fatty acid chains (unsaturated) decreases; the cell membrane is more flexible, and more fluid lipid accumulation impacts the seed.

Precipitation was highly correlated with C14 fatty acids, insignificantly with C12 fatty acid 1 (r = 0.705, 0.811 and 0.819): 0 (0064) and negatively with other fatty acids 0,

C20: 1 and C22. These results demonstrated that what was observed in Sonoyta and Todos Santos was the highest rainfall recorded (81 and 73 mm, respectively). In addition, low rainfall affects primary metabolism of seeds by slowing the rate of fatty acid biosynthesis [28]. Gayol *et al*. [29] evaluated the fatty acid composition of jojoba seeds of samples from Bañados de la Rioja, located 865 m from La Rioja, Argentina and found a higher proportion of C20: 1 (66.35%) and C22: 1 (14.24%) than those found in jojoba seeds of the three studied populations. These results coincide with the correlation analysis where altitude showed to be negatively correlated with C20: 1 and C22: 1 (r = -0776 and -0767), and Todos Santos ecotype located at higher altitude (146 m) than La Rioja showed the lowest content of these fatty acids.

4.3. Genetic Diversity of Wild Ecotypes of *Simmondsia chinensis*

The ISSR analysis of jojoba accessions showed a polymorphic percentage of 54%. The first M2 generated the highest percentage of polymorphic bands. Despite the geographical distance among Sonoyta (Sonora), Indígena la Huerta (Baja California), and Todos Santos (Baja California Sur) ecotypes, a similarity index of 0.85 was observed. Accessions h2 (Indígena la Huerta, Baja California) and b7 (Todos Santos, Baja California Sur), h3 (Indígena la Huerta, Baja California) and s10 (Sonoyta, Sonora) were genetically identical, which may be due to the evolution of the species or the introduction of genetic material to such ecotypes influenced by man.

Currently in Mexico, studies that focus on evaluating genetic diversity of *S. chinensis* are not available. Some studies in India have used molecular markers of agave in accessions of jojoba to identify female and male plants, reporting fragment sizes similar to those in this study: 500 and 2000 bp; however, they do not report polymorphic percentage [8]. On the other hand, Al Sooger *et al*. [7] evaluated the genetic diversity of accessions of jojoba from Saudi Arabia, which were previously phenotypically selected, finding a number of amplified bands from two to 11 similar to the first M2 of this study. They found polymorphism percentage that varied from 0.25 to 100, which is one similarity index from 0.60 to 0.90 as seen in the first M2.

5. Conclusion

Physicochemical parameters of lipids from the seeds of the three *Simmondsia chinensis* ecotypes were within the required quality standards and were not influenced by environmental conditions of the different ecotypes. Ecotypes of Sonora and Baja California Sur had higher oil content, which was important for the implementation of the projects to improve seed production. Moreover, significant differences of unsaturated fatty acids were found in the three ecotypes, which is useful for the production of biofuels. Jojoba seeds from *S. chinensis* in northwestern Mexico are an alternative for biodiesel production because they have the necessary characteristics for their production. It is important to perform further studies on genotypic characterization of the wild population ecotypes studied to help explain the differences in lipid content and composition for gene selection with the best features for the biodiesel industry and lubricants.

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Appendix

Table A1. Jojoba Fatty Acids

Sonora (Sonoyta)							
	Sonora 1		Sonora 2		Sonora 3		
	Area	%	Area	%	Area	%	Average
Lauric (C12:0)	2579	0.02732531	1259	0.02339128	2008	0.02533797	0.02535152
Myristic (C14:00)	22813	0.24171086	11784	0.21893791	14740	0.18599684	0.21554853
Pentadecanoic (C15:0)	8425	0.08926551	4521	0.0839968	6207	0.07832309	0.0838618
Palmitic (C16:0)	871016	9.22868668	427698	7.94630893	553060	6.97879308	8.0512629
Oleic(C18:1)	2296000	24.3268374	878208	16.3164478	1452000	18.3220764	19.6551205
Linoleic (C18:2)	38157	0.40428534	28275	0.52532835	20896	0.26367638	0.39776336
Linolenic (C18:3)	87094	0.92278814	40818	0.75836791	82686	1.04337411	0.90817672
Eicosenoic (C22:1)	5528000	58.5708873	3660000	68.0000624	4366000	55.0924142	60.5544546
13-Docosenoic (C22:1)	584052	6.18821344	329785	6.12715863	1427269	18.010008	10.10846
Total	9438136	100	5382348	100	7924866	100	100

Baja California Sur (Todos Santos)							
	JBC35(1)		JBC35(2)		JBC35(3)		
	Area	%	Area	%	Area	%	Average
Lauric (C12:0)	3554	0.07746323	2178	0.05170056	2527	0.04185606	0.05700662
Myristic (C14:00)	14709	0.3205984	6627	0.15730929	12402	0.20542098	0.22777622
Pentadecanoic (C15:0)	5077	0.11065865	1848	0.04386715	4117	0.06819208	0.07423929
Palmitic (C16:0)	513160	11.1848714	142327	3.37850605	461250	7.63993124	7.40110291
Eladic (C18:1)	995416	21.6961571	498527	11.8338508	898678	14.8852859	16.1384313
Linoleic (C18:2)	42782	0.93247948	18119	0.43010217	28864	0.47808992	0.61355719
Linolenic (C18:3)	66506	1.44956945	45302	1.07536224	79338	1.31411786	1.27968318
Eicosenoic (C22:1)	2540000	55.3620186	997207	23.6713335	4478000	74.1715167	51.0682896
13-Docosenoic (C22:1)	406779	8.86618368	2500585	59.3579682	72182	1.1955892	23.1399137
	4587983	100	4212720	100	6037358	100	100

Baja California (Indígena de la Huerta)							
	JBC1		JBC1		JBC1		
	Area	%	Area	%	Area	%	Average
Lauric (C12:0)	1778	0.02117985	1457	0.02209361	1442	0.02402583	0.0224331
Myristic (C14:00)	13415	0.15980188	11464	0.17383746	7761	0.12930961	0.15431632
Pentadecanoic (C15:0)	9156	0.10906791	8131	0.12329662	6539	0.1089493	0.11377128
Palmitic (C16:0)	838839	9.99240003	674343	10.2255823	530262	8.83494055	9.68430763
Eladic (C18:1)	1846000	21.9898818	2221000	33.6787337	1586000	26.4250799	27.3645651
Linoleic (C18:2)	62720	0.74713185	64290	0.97487879	68106	1.13474558	0.95225207
Linolenic (C18:3)	193783	2.30837772	182902	2.7734839	141836	2.36319523	2.48168562
Eicosenoic (C22:1)	4688000	55.8442935	3150000	47.7658762	3210000	53.4832954	52.3644884
13-Docosenoic (C22:1)	741079	8.82786544	281079	4.26221737	449928	7.49645861	6.86218047
	8394770	100	6594666	100	6001874	100	100

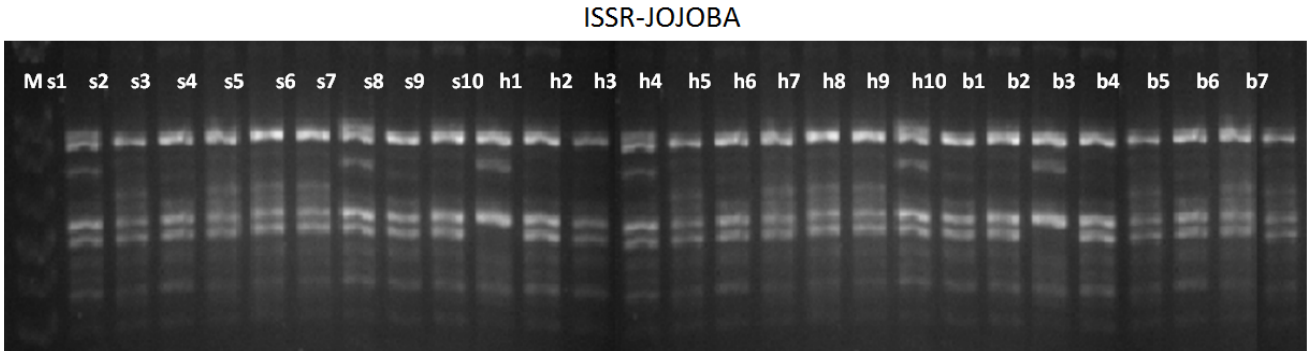

Figure A1. Agarose gel showing the amplified product of jojoba oil of three *Simmondsia chinensis* ecotypes (Indígena la Huerta =h, Todos Santos = b, and Sonoyta = s) using ISSR primers (M2). ISSR-PCR band profiles generated by ISSR primers. M = Molecular weight marker

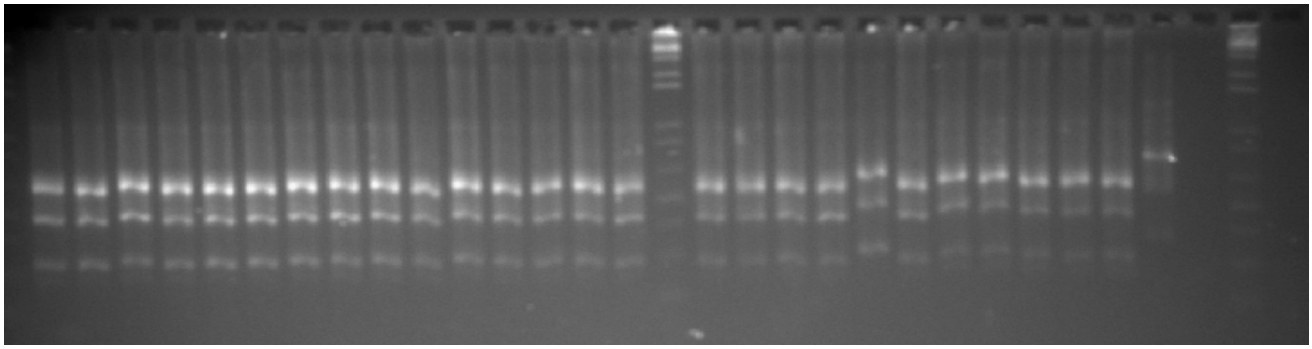

Figure A2. Agarose gel showing the amplified product of jojoba oil of three *Simmondsia chinensis* ecotypes (Indígena la Huerta =h, Todos Santos = b, and Sonoyta = s) using ISSR primers (M11). ISSR-PCR band profiles generated by ISSR primers. M = Molecular weight marker

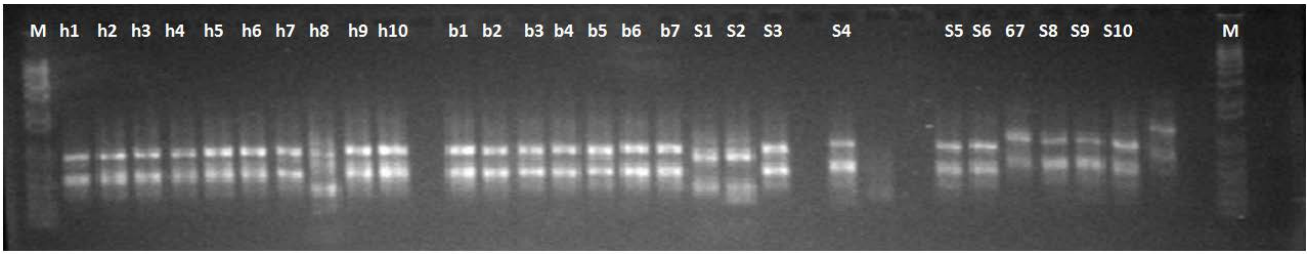

Figure A3. Agarose gel showing the amplified product of jojoba oil of three *Simmondsia chinensis* ecotypes (Indígena la Huerta =h, Todos Santos = b, and Sonoyta = s) using ISSR primers (M10). ISSR-PCR band profiles generated by ISSR primers. M = Molecular weight marker