Universidad Autónoma de Querétaro

Facultad de Contaduría y Administración

Doctorado en Gestión Tecnológica e Innovación

Evaluación estratégica de dos cadenas productivas para el desarrollo agroindustrial en el Estado de Querétaro

Artículos de investigación

Que como parte de los requisitos para obtener el Grado de:

Doctor en Gestión Tecnológica e Innovación

Presenta:
M en T. José Fernando Vasco Leal
Dirigido por:
Dr. Juan José Méndez Palacios
Co-Director:
Dr. Eusebio Ventura Ramos

Querétaro, Qro a Agosto de 2020

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Centro Universitario, Querétaro, Qro.
Agosto, 2020
México

DEDICATORIA

A mis padres, Stella Leal Jiménez y Antonio José Vasco Leyes, por su esfuerzo, apoyo, incondicional amor y por creer siempre en mis sueños, además de inspirarme para seguir en la ardua tarea de lograr mis metas. Por enseñarme el valor del trabajo, que la perseverancia y la educación son fundamentales para el éxito en la vida personal y laboral.

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ÍNDICE GENERAL

RESUMEN

En el Estado de Querétaro, algunos de los problemas que enfrenta la agricultura es la falta de adopciones de innovaciones tecnológicas, la planeación estratégica y la transferencia de tecnologías y conocimientos por parte de los productores agrícolas. Aunado a ello, la falta de desarrollo de capacidades entre los productores en temas empresariales, planeación y manejo eficiente de los cultivos se ve reflejado en la baja productividad de sus unidades productivas; así mismo el fuerte rezago en el uso de tecnologías y por ende los escasos márgenes de utilidad por el excesivo intermediarismo y la falta de integración con otros eslabones de la cadena productiva como transformación y comercialización hacen que el sector agroindustrial no genere una alta derrama económica. Para la presente investigación se realizó el análisis de la gestión tecnológica para la cadena productiva de la higuerilla que se encuentra por establecerse en el Estado de Querétaro y para la cadena productiva establecida de la flor de corte se realizó una metodología mixta, a partir de estudios de políticas de desarrollo agroindustriales, temas agronómicos y gestión tecnológica e innovación. Teniendo en cuenta la investigación realizada, es preciso implementar estrategias para el desarrollo agroindustrial de las cadenas “higuerilla y flor de corte”, que conlleve a los productores del Estado de Querétaro a un incremento en la productividad a través del manejo tecnológico de la producción agrícola, adopción de innovaciones tecnológicas en sus diferentes etapas, transferencia de conocimientos y tecnología, generación de capacidades en temas administrativos y de negocios que ayuden a generar aumento en la productividad en estos sectores productivos de interés nacional.

Palabras claves: cadenas productivas, competitividad, factibilidad, políticas públicas y productividad.

I. INTRODUCCIÓN

Actualmente el campo mexicano posee bajos índices de productividad los cuales repercuten en pérdidas económicas para los productores agrícolas debido a la no adopción de tecnologías y conocimientos, en cierta manera por desconocimiento, así como por la falta de transferencia por parte de los centros de investigación e instituciones afines, los cuales son los encargados de entregar a los agricultores la información adecuada para sus cultivos. Estos bajos rendimientos presentados, ocasionan un desequilibrio en la balanza comercial debido principalmente al aumento de las importaciones de productos básicos. Por lo tanto, el objetivo de este proyecto es desarrollar y transferir información técnica a los agricultores del Estado de Querétaro y generar rentabilidad en el sector agroalimentario. Además de la baja rentabilidad, existen factores sociales como el aumento de la deserción de las personas por los trabajos del campo, quienes encuentran mejores ofertas salariales en los parques industriales como prestadores de servicios o en la migración a países cercanos, lo cual impacta de manera directa en la producción agrícola nacional ya que la mayoría de los núcleos productivos en el Estado son atendidos por personas mayores de edad. Así mismo, la nula transferencia de tecnología y de capacitación a los pequeños agricultores hace que los rendimientos obtenidos no sean suficientes en sus cultivos de autoconsumo y/o comerciales, lo cual podría desencadenar en el creciente riesgo de la seguridad y la soberanía alimentaria, los cuales no serían suficientes para satisfacer las necesidades básicas de la población para una vida activa y saludable.

El desarrollo agroindustrial de la higuerilla y la flor de corte ayudará a mejorar la economía de los productores del Estado de Querétaro y fomentará la creación de nuevas fuentes de trabajo dentro de la zona de influencia, mejorando el entorno social y económico del Estado, además permitirá el desarrollo de nuevos productores capacitados con el fin de obtener rentabilidad en sus agronegocios.

Ventajas estratégicas y competitivas del sector agroindustrial en el Estado de Querétaro.

A continuación se nombran las principales características que posee el sector agroindustrial en el Estado de Querétaro:

1. Conexión de carreteras con mercados especializados como la Ciudad de México, Guadalajara, Monterrey, entre otros más.

2. Aeropuerto Intercontinental de Querétaro con facilidad de movilización de carga.

3. Empresas establecidas como proveedores de insumos, maquinaria, equipos, implementos y servicios especializados para la modernización y desarrollo de proyectos agroindustriales.

4. Facilidad de articular clúster agroindustriales con empresas de la región.

5. Productores agrícolas e inversionistas interesados en generar productos de alto valor y desarrollar agronegocios.

6. Parques industriales agrícolas especializados:
    a. Agropark (Municipio de Colón)
    b. Florapark (Municipio de Amealco de Bonfil)

7. Transporte de carga y logística con unidades móviles adecuadas para las necesidades de espacios, temperatura y atmosferas controladas.

8. Universidades, Instituciones y Asociaciones Civiles que brindan enseñanza, investigación y vinculación entre el sector público y privado:

   a. Universidad Autónoma de Querétaro (UAQ)
   b. Colegio de Ingenieros Agrónomos Queretanos A.C (CIAQ)
   c. Centro Universitario CEICKOR
   d. Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT)
   e. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP)
   f. Comité Estatal de Sanidad Vegetal de Querétaro A.C. (CESAVEQ)

9. Disponibilidad de mano de obra especializada en áreas como química agrícola, horticultura, manejo de invernaderos y biosistemas, agroindustria, agronegocios, ciencia y tecnología de alimentos, entre otros.

10. Apoyos estatales y federales a la capacitación, asistencia técnica, desarrollo de proyectos agroindustriales en temas de agricultura protegida, tecnificación y modernización de sistemas de riego y estímulos a la comercialización, entre otros.

11. Acceso a la financiación por entidades federales como Financiera Nacional Rural de desarrollo agropecuario, rural, forestal y pesquero (FND) y Fideicomisos Instituidos en Relación con la Agricultura (FIRA).

12. Bajos índices de inseguridad que propician la inversión nacional y extranjera.

Consideraciones para el desarrollo agroindustrial

La producción agroalimentaria, el aumento de la producción por área sembrada y el uso sustentable de los recursos naturales abre grandes oportunidades para realizar investigación que genere innovaciones tecnológicas, a fin de ofrecer resultados confiables y soluciones reales a los productores agrícolas en sus necesidades productivas. México posee una brecha que ha dejado vacíos importantes en esta materia, debido a que muchas de las investigaciones generadas no logran depositarse en los productores del campo, pues el proceso de transferencia de los conocimientos, la tecnología e innovación es deficiente en espacio y tiempo y no logra consolidar el uso pleno de la adopción. De esta manera, la evaluación de las cadenas productivas es una necesidad apremiante, por lo que es necesario desarrollar nuevas metodologías, adopción de tecnologías y servicios productivos para los productores agrícolas del Estado de Querétaro, y convertir sus parcelas en zonas potenciales de producción y valor agregado de los diversos cultivos que se poseen en el área de estudio a través de la vinculación entre los productores agrícolas, agroindustriales con especialistas, investigadores y al sector público – privado con el objetivo de desarrollar capacidades productivas que coadyuven en el bienestar social y económico de los productores.

La superficie sembrada, el volumen de la producción y el valor económico que representa la producción agroalimentaria, abre grandes oportunidades para realizar proyectos agroindustriales en el estado de Querétaro que generen impactos económicos, tecnológicos, ambientales y sociales a partir de la adopción de innovaciones tecnológicas, inteligencia de mercados y la planeación estratégica.

Aspectos a considerar:

1. El Estado de Querétaro posee diversas ventajas estratégicas y competitivas para el establecimiento de proyectos agroindustriales de alto impacto.

2. El desarrollo agroindustrial del Estado ayudará a impulsar la economía y fomentará la creación de nuevas fuentes de empleo en la zona de influencia de los proyectos establecidos mejorando el entorno social de los habitantes rurales.

3. Se deberá estrechar los lazos de vinculación estratégica entre los diversos actores de la cadena agroalimentaria con instituciones del sector como la SEDEA, SADER, CIAQ, UAQ, INIFAP, CIMMYT, entre otros, para la generación de investigación, enseñanza y capacitación constante de los agricultores.

4. Implementar el uso de semillas y material vegetativo certificado, buenas prácticas agrícolas, manejo de la agricultura de precisión y conservación, producción orgánica y control biológico.

5. Promover la implementación de sellos de certificación de calidad como trazabilidad, buenas prácticas agrícolas, productos libres de contaminantes para de esta manera acceder a nichos de mercados especializados.

6. Fomentar el interés de los jóvenes a la agricultura de tal manera que el cambio generacional se realice de manera escalonada.

7. Implementar agricultura por contrato en las actividades agrícolas para asegurar la venta y el precio de las cosechas.

8. Implementar el uso de los seguros agrícolas para disminuir los riesgos provenientes de contingencias ambientales como sequía, granizo, incendio, etc.

II. PUBLICACIONES EN REVISTAS INDIZADAS COMO PRIMER AUTOR

II.1 Valorization of Mexican *Ricinus communis* L. Leaves as a Source of Minerals and Antioxidant Compounds

Waste and Biomass Valorization
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ORIGINAL PAPER

Valorization of Mexican *Ricinus communis* L. Leaves as a Source of Minerals and Antioxidant Compounds

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Abstract

Purpose This research aimed to characterize the physicochemical and nutraceutical composition from two Mexican *R. communis* L. leaves accessions (R1 and R2) to valorize their use as a source of macromolecules, minerals, and bioactive compounds.

Methods The physicochemical (proximal composition, X-ray fluorescence and diffraction, FT-IR, and SEM) and nutraceutical composition (phenolic compounds and mono/oligosaccharides, GC–MS, untargeted metabolomics, and in silico interactions) were conducted for the analysis of the leaves.

Results Both accessions exhibited a high amount of protein (41.70–39.58%) and ash (11.81–12.51%). The untargeted metabolomic profiled a major impact on antioxidative pathways. Compared to R1, R2 showed a higher ($p < 0.05$) content of ellagic and *p*-coumaric acids and catechin. Correlations with the in vitro antioxidant capacity and in silico analysis suggested ellagic acid, (+)-catechin, and ricin as candidates for the antioxidant potential. The mineral characterization highlighted calcium and potassium as the most abundant minerals, both confirmed by the SEM analysis. The FTIR spectra of the leaves partially identified the presence of ricin and ricinine, major protein and alkaloid, respectively, of the leaves.

Conclusion These results indicate that *R. communis* L. leaves are an attractive by-product that can serve as an alternative source for the obtention of protein, minerals, and antioxidant compounds.

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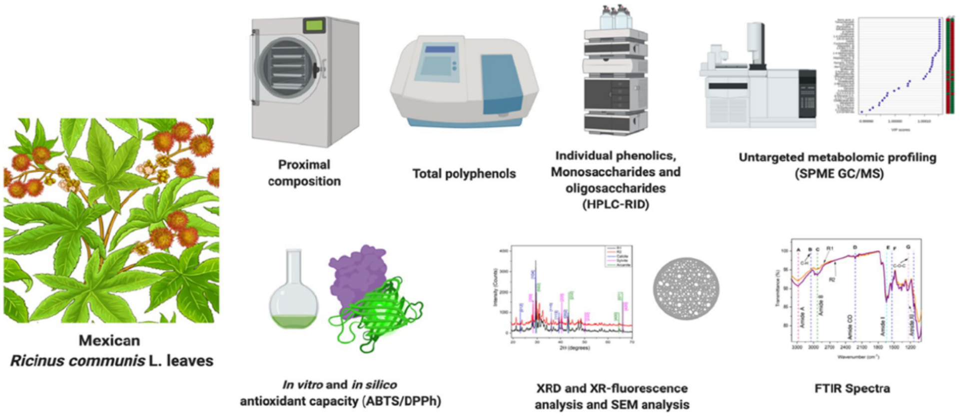
Graphic Abstract



Keywords *Ricinus communis* L. leaves · Residues · Added-value products · Bioactive compounds · Ricin · Ricinine

Statement of Novelty

This study reports the first complete characterization of Mexican R. communis L. leaves accessions integrating in vitro, in silico, and physicochemical characterization.

Introduction

Castor bean (*Ricinus communis* L.) is an African origin bush that usually grows in clay and sandy soils, and highly adaptable to several climatic conditions (tropical, subtropical, or dry climates), rainfalls (700–1500 mm) and temperature (15–38 °C). The main agricultural resource of this plant is the seeds, mainly used in the extraction of castor bean oil. While this product is appreciated for its emollient and lubricant properties associated with wetting and dispersion of dyes, other applications include its use for the manufacturing of synthetic flavors and fragrances [1]. Our research group previously characterized the oil content of seven Mexican *Ricinus communis* L. seeds, confirming its potential for their use as lubricant or biodiesel production [2]. The obtained castor bean oil presented an effective extraction yield (0.43–0.61 L/kg oil), and low acid value (0.54–1.11 mg KOH/g) [3, 4]. As the oil yield depends on the seed variety and the environmental growing conditions, we also proposed a novel classification system at industrial levels using a precise image analysis technique and data mining algorithms [5].

Due to the complexity of the castor bean oil harvesting, the high variation of ripeness stages of seeds (requiring up to five separate harvestings), and the need for plant defoliants to expose the seeds, the oil production requires experience and involves several costs. However, high production yield (up to 2000 kg oil/ha) [6] have subsequently stimulated the selection and development of cultivars [7] and the improvement of the oil extraction methods [8]. This process has led to the crop-rising of this plant [9], bringing a significant generation of several by-products, primarily leaves [10]. *R. communis* L. leaves could be considered another value-added *Ricinus communis* L. good that might economically compensate some of the oil disadvantages, i.e., high viscosity and water content [6]. Castor bean leaves can be used in phytoremediation due to its ability to prevent the incorporation of heavy metals into the food chain [11] and acaricidal properties as well [12]. Besides, the leaves have exhibited wide investigated medicinal properties such as antinociceptive activity [13], antioxidant, and anti-inflammatory properties [14]. Nonetheless, the chemical characterization of leaves is generally restricted to antioxidant phenolics with no identification of additional metabolites [11], minerals, or metabolomics profiling. Moreover, few reports indicate specific compounds in *R. communis* L. leaves that are responsible for their antioxidant capacity nor in silico interactions between the major *R. communis* L. leaves protein and other bioactive components.

Since the Mexican government has proposed the castor bean production as a significant industrial crop [15], putting even more pressure on the generation of leaves as by-products, it is essential to find innovative uses for their incorporation as a value-added product. Although it has been reported that *Ricinus communis* L. extracts have a mild to moderate toxicity [16], the potential of the leaves for industrial manufacturing of medicinal extracts should be considered, especially for their content of bioactive compounds. Among them, proteins from the leaves could be a target macronutrient since the existing industrial processes for their extraction have not reached an industrial production for their incorporation into food applications [17]. Plant carbohydrates such as monosaccharides and oligosaccharides are important components of the human diet, exerting beneficial effects as prebiotics [18]. As minerals from *R. communis* L. leaves has been used for soil phytoremediation, their inclusion into the human diet might provide benefits as a renewable and low-cost source of them [19, 20].

Thus, this research aimed to conduct a physicochemical and nutraceutical characterization of the leaves from two Mexican *R. communis* L. accessions to identify macromolecules, minerals, and bioactive compounds with antioxidant potential. An untargeted metabolomics analysis, in vitro, and in silico approaches were used to deepen into the characterization of value-added compounds.

Experimental Section

Reagents

All reagents were HPLC or analytical grade. Total dietary fiber assay kit (TDF100A-1KT), HCl, H_2SO_4, CuSO4, Na_2SO_4, H_3BO_3, methanol, petroleum ether, Na_2CO_3, 2-aminoethyl diphenylborate, ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picrylhydrazil), gallic acid, (+)-catechin, rutin and HPLC standards were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, US) and J.T. Baker (Mexico City, Mexico).

Plant Material

Two different *Ricinus communis* L. leaves accessions (R1 and R2) were manually harvested twice in May 2017 and were grown near Amazcala (Queretaro Mexico, Lat. 20° 43′ 39.5″ N Lon 100° 15′ 17″ W) at an altitude of 1920 MASL. The average annual estimated rainfalls were 520 mm in mild semi-arid weather. These accessions were selected based on those with the highest oil extraction yield among Mexican varieties (VQ-4 and VQ7 as R1 and R2, respectively) [2]. The cultivation started in December 2016, and the collected leaves were the first buds after winter (131 days of growing). Healthy leaves, characterized by uniform color, upright appearance, and vigorous growth, were selected. A voucher specimen of the plants was deposited at the Herbarium of Universidad Autónoma de Querétaro "Dr. Jerzy Rzedowski" (QMEX) located at the School of Natural Sciences (RCA20191 and RCA20192). The leaves were washed with distilled water, packaged under vacuum, sun-dried (35–60 °C), ground, and passed through a mesh (250 µm). The resulting powder was stored at 4 °C in sealed containers, protected from light.

Chemical and Nutraceutical Characterization of *Ricinus communis* L. Leaves

Proximal Composition

The total content of moisture (method 925.10), lipids (method 920.39), protein (method 920.87), ash (method 923.03), and crude fiber (method 991.43) of the samples were determined following the AOAC procedures [21]. Carbohydrates were determined by difference.

Extracts Preparation

For the methanolic extracts, 100 mg of dried *Ricinus communis* L. leaves (R1 and R2) were mixed with 10 mL of methanol in a 50 mL flask, protected from light, under constant agitation at room temperature (25 ± 1 °C) for 24 h. For solids removal, methanolic extracts were collected and centrifuged at 5000 rpm for 15 min (Hermle Z 326K, Wehingen, Germany) at 4 °C. Depending on the accession (R1 and R2), the methanolic extracts were coded as R1M and R2M.

For the preparation of the aqueous extracts, 100 mg of dried *Ricinus communis* L. leaves (R1A and R2A) were mixed with 10 mL of distilled water in a 50 mL flask and were maintained in a water bath at 40 °C at constant agitation for 1 h. Consequently, samples were cooled to room temperature (25 ± 1 °C) and kept under constant agitation for 23 h. For solids removal, samples were centrifuged at 5000 rpm for 15 min (Hermle Z 326 K, Wehingen, Germany) at 4 °C. All samples were protected from light and stored at − 20 °C for further analysis.

Total Polyphenolic Composition of the Leaves

The total phenolic content of the extracts was determined accordingly to the Folin-Ciocalteu colorimetric method [22], and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of the sample. The total flavonoids content was determined using the method described by Sarker and Oba [23], and the results were expressed as micrograms equivalents of rutin (RE) per gram of extract. The total condensed tannins content was estimated using the procedure reported by Deshpande and Cheryam [24], and results were calculated as milligrams of (+)-catechin equivalents (CE) per gram of the extract.

Free-Phenolics Compounds Quantification by HPLC

The free-phenolic compounds quantification of both methanolic (R1M and R2M) and aqueous extracts (R1A and R2A) were analyzed accordingly to Sarker and Oba [25] with some modifications. A high-performance liquid chromatography-diode array detection (HPLC–DAD) analysis was conducted using an Agilent 1100 Series HPLC System (Agilent Technologies, Palo Alto, CA, US). A Zorbax Eclipse XDB-C18 column (Part 7,995,118–585, Agilent Technologies, 4.6 × 250 mm, 5 µm), thermostatically controlled (35 ± 0.6 °C) with a flow rate of 1 mL/min. The mobile phase consisted of the mixture of two solvents: Solvent A (water adjusted with 0.1% acetic acid) and Solvent B (100% acetonitrile). Solvents were used in a linear gradient as follows: 80–83% A for 7 min, 83–60% for 5 min, 60–50% for 1 min, and 50–85% for 2 min. The detection was performed at 280 nm for phenolic compounds and 320 nm for flavonoids, with an acquisition speed of 1 s. The injected volume of samples was 20 µL and was performed in triplicate. The quantification was carried out using commercial standards curves of gallic, chlorogenic, caffeic, ρ-coumaric, and ellagic acids; and (+)-catechin, rutin, and quercetin (Sigma-Aldrich, St. Louis, MO, US).

Oligosaccharides and Monosaccharides Extraction and Quantification by HPLC

The oligosaccharides from the leaves' powder were extracted using a thermal procedure [26]. For the quantification, a high-performance liquid chromatography-refractive index detector analysis (HPLC-RID) was conducted using an Agilent 1100 Series HPLC System (Agilent Technologies). A C18 Zorbax carbohydrates column (Part 840300-908, Agilent Technologies, 4.6 × 250 mm, 5 µm), thermostatically controlled (35 ± 0.6 °C), and a flow rate maintained at 1 mL/min [27]. The mobile phase consisted in water with 50% acetonitrile, and the quantification was carried out with standard curves of xylose, mannose, raffinose, stachyose, and verbascose (Sigma-Aldrich).

Metabolite Profiling by Solid-Phase Microextraction (SPME) Coupled to Gas-Chromatography–Mass Spectrometry (GC–MS) Analysis

The metabolite profiling from the two *R. communis* leaves methanolic extracts was assessed using the reported procedure of Hussein et al. [28] with some modifications. Before the GC analysis, extracts were separated by SPME, a cost-effective sample preparation technique used to efficiently separate organic compounds using chemical binding affinity with SPME unit components [29]. Briefly, the methanolic extracts were subjected to SPME with a 2-cm polymethylsiloxane-divinylbenzene-carboxen fibre. The sample was extracted at 45 °C, incubated for 5 min with agitation (250 rpm), and then extracted during 120 min. After the extraction, samples were analyzed by GC–MS on an Agilent 7890 GC System (Agilent Technologies, Palo Alto, CA, US.) equipped with an Agilent 5975C VL Mass selective detector with MPS2 XL multi-purpose autosampler (Gerstel). A DB-5MS capillary column (60 mm × 250 mm × 0.25 mm, Agilent Technologies), using helium as carrier gas (1 mL/min), was used to separate the samples. GC injector was held at 250 °C, and both MS source and quadrupole were maintained at 230 °C and 150 °C, respectively. The oven temperature was initially set at 40 °C, raised to 200 °C (5 °C/min), held at 200 °C for 2 min, raised to 230 °C (20 °C/min) and held at 230 °C for 15 min. The metabolite profiling was done comparing mass spectra with NIST/EPA/NIH Mass Spectra Library, v. 1.7.

Untargeted Metabolomic Analysis

An untargeted metabolomic analysis profile was conducted using the MetaboAnalyst software v. 3.0 [30]. The partial least squares-discriminant analysis (PLS-DA) was used to rank each metabolite according to the number of components and variables in each model [31]. Heatmaps were generated using normalized components by sum and Ward sample clustering. The chemical classification of metabolites was done following the database classification of ClassyFire [32].

In Vitro Antioxidant Capacity

The in vitro antioxidant capacity of both methanolic and aqueous extracts of R1 and R2 leaves was estimated by two different antioxidant systems: the 2,2-diphenil-1-picrylhydrazil (DPPH) and the 2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) method. The DPPH assay was conducted following the procedure of Sarker et al. [33] and the ABTS assay accordingly to Sarker et al. [34]. For both assays, the antiradical activity (ARA, %) was calculated using a Trolox calibration curve expressed in terms of ARA.

In Silico Assessment of Antioxidant Capacity and Interactions Between Ricin and Selected Compounds

The in silico evaluation was conducted to assess potential interactions between ABTS and DPPH radicals and selected compounds from *R. communis* L. leaves. Ricin, the main protein of the leaves (Protein Data Bank crystallographic structure: 2AAI); ellagic acid (PubChem CID: 5281855) and (+)-catechin (PubChem CID: 9064) as the main hydroxybenzoic acid and flavonoid, respectively; and ricinine (PubChem CID: 10666) the major alkaloid of the leaves, were used as targets. The potential binding positions for all the evaluated proteins were found using MetaPocket 2.0 web server. The docking procedure selecting flexible torsions, hydrogen bonds, and docking simulations were calculated using the reported procedure of Luna-Vital et al. [35] and AutoDock Tools [36].

Physical Characterization of Leaves and Ashes from *Ricinus communis* L.

X-ray Fluorescence Characterization

The elemental composition of the ashes from *Ricinus communis* L. leaves (R1, R2) was conducted using a NEX QC, NEX QC + Rigaku System (Rigaku Co., Tokyo, Japan). Ashes were combined with a spectroblend binder and mixed to form a homogenized analytical pellet. The operating conditions were 4 W, 50 kV x-ray tube coupled to SDD detector, and the total measurement time was 20 min.

X-ray Diffraction Characterization

The X-ray diffraction pattern of the ash powders from samples R1 and R2 were studied to identify organic compounds from the leaves. For this, a diffractometer (Miniflex®, Rigaku) was used, operating at 35 kV and 15 mA, with a CuK_α radiation wavelength of $\lambda = 0.15406$ nm, from 5 to 50° on a 2θ scale, with a step size of 0.02°. The measurement was conducted at room temperature (25 ± 1 °C).

FT-IR Spectroscopy

Considering that ricinine is the major alkaloid of *Ricinus communis* L. leaves and there has been little research on their potential as a bioactive compound with therapeutic properties [37], FT-IR was used to identify the spectra of this compound. The samples were measured by an FTIR spectrophotometer (Spectrum Two, Perkin Elmer, MA, USA) with an ATR (Attenuated Total Reflectance) accessory in the 600–4000 cm^{-1} range and spectral resolution of 2 cm^{-1}.

Scanning Electron Microscopy (SEM)

A Scanning Electronic Microscope (SEM) Model JSM-6060LV at a high vacuum (JEOL, Tokyo, Japan) was used to study the morphology of ashes from R1 and R2 samples. The samples were fixed on an aluminum specimen holder with double graphite tape and gold-coated by sputtering. The analysis was done employing a 20-kV electron acceleration voltage.

Statistical Analysis

The data were analyzed using the JMP v. 14.0 software, GraphPad Prism v. 8.0, Origin Pro v. 9.0, and the statistical analysis was performed using a one-way ANOVA and the Tukey–Kramer's Test. The differences with a $p < 0.05$ were considered statistically significant. At least three replicates were used for each experiment.

Results and Discussion

Chemical and Nutraceutical Characterization of *Ricinus communis* L. Leaves

Proximal Composition

Table 1 shows the chemical composition of the two *Ricinus communis* L. leaves accessions. R1 presented the highest protein, ash, and crude fiber content, whereas R2 showed the highest lipids and carbohydrates content. Mexican-origin *Ricinus communis* L. leaves presented a higher protein but lower lipids and fiber content than the reported for African-origin castor leaves (10.3%, 5.4%, and 24.8%, respectively) [38], similar ash and crude fiber contents than Indian *Ricinus communis* L. leaves (11.8 and 11.3%, respectively) [39] and higher protein than the values reported for Mexican leaves (27.6%) [40]. Although these variations could be

Table 1 Content of protein, lipids, ash, moisture, crude fiber, and carbohydrates of the evaluated Mexican *Ricinus communis* L. leaves accessions

Sample	Protein	Lipids	Ash	Moisture	Crude fiber	Carbohydrates[a]
R1	41.70 ± 0.52a	3.19 ± 0.25b	12.51 ± 0.20a	11.13 ± 0.05a	10.14 ± 0.77a	31.47 ± 1.02b
R2	39.58 ± 0.48b	3.51 ± 0.24a	11.81 ± 0.03b	10.62 ± 0.15b	8.87 ± 0.49b	34.48 ± 0.90a

The results represent the average of three replicates ± SD and were expressed in percentage. All results were quantified in DW, except for moisture. Means within the same column with different letters differ significantly by Tukey–Kramer's Test ($p < 0.05$)

[a] Calculated by difference

a consequence of the age and genetic background of the plant, or the climatic conditions, the results obtained here suggested that R1 has the potential to be a supplementary source of proteins and R2 an additional source of lipids and carbohydrates.

Total Polyphenolic Composition

The total polyphenolic composition of both methanolic and aqueous extracts from the leaves is depicted in Fig. 1. R1M exhibited the highest total phenolic content (Fig. 1a), while there were no differences among the other extracts ($p > 0.05$). Both methanolic extracts yielded a higher total flavonoid (201.75 ± 12.09—234.92 ± 11.57 mg RE/g DW) (Fig. 1b) and total condensed tannins (Fig. 1c) content than the aqueous extracts.

The total phenolic content for *R. communis* L. Mexican accessions fitted into the reported ranges for several *Ricinus communis* L. leaves populations (174.25–623.70 mg GAE/g DW) [11]. Additionally, this content was higher than those found in other plants such as *Jatropha* (408.34 ± 34.00 mg GAE/g DW) [41] or *Moringa oleifera* leaves (32.75 ± 0.07 mg GAE/g DW) [42]. Regarding flavonoids, *R. communis* L. contents were higher than those reported for Tunisian castor bean leaves [11]. It has been reported that flavonoids are a group of plant metabolites that provide health benefits through via cell signaling pathways with antioxidant and antimicrobial effects [43]. Overall, it has been suggested efficient antibacterial and antifungal activities derived from methanolic extracts of *R. communis* L. leaves [12, 41]. Tannins, saponins, terpenoids, and flavonoids have been linked to such activities by providing a source of stable free radicals forming complexes with nucleophilic amino acids in proteins, contributing to their inactivation and loss of function [44].

Free-Phenolic Compounds Identification and Quantification by HPLC–DAD

Table 2 shows the content of free-phenolic compounds, quantified by HPLC–DAD, of both aqueous and methanolic *Ricinus communis* L. extracts. Overall, R1 leaves showed a higher richness and variety of polyphenols than R2, whereas R1M yielded the highest quantity. Among the hydroxycinnamic and hydroxybenzoic acids, *p*-coumaric and ellagic acids, respectively, were significantly higher ($p < 0.05$) than

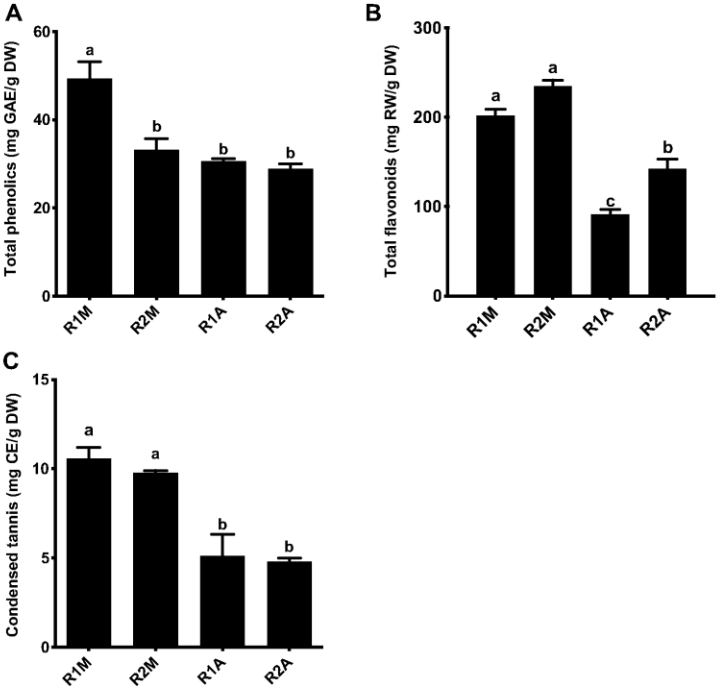


Fig. 1 Total polyphenolic composition of *R. communis* L. leaves. **a** Total phenolic compounds; **b** total flavonoids content; and **c** condensed tannins of both methanolic and aqueous extracts of Mexican *Ricinus communis* L. leaves accessions. The means with different superscript letters significantly differs by Tukey–Kramer's Test ($p < 0.05$). *GAE* gallic acid equivalents, *RE* rutin equivalents, *CE* (+)-catechin equivalents, *DW* dry weight, *R1M Ricinus communis* L. leaves accession 1 methanolic extract, *R2M Ricinus communis* L. leaves accession 2 methanolic extract, *R1A Ricinus communis* L. leaves accession 1 aqueous extract, *R2A Ricinus communis* L. leaves accession 2 aqueous extract

Table 2 Free-phenolic compounds content quantified by HPLC–DAD of the methanolic and aqueous extracts of Mexican *Ricinus communis* L. leaves accessions

Compound	RT (min)	R1M	R2M	R1A	R2A
Hydroxybenzoic acids					
Gallic acid	1.8	35.81 ± 0.14^{a}	35.83 ± 0.12^{a}	17.09 ± 0.09^{b}	10.25 ± 0.13^{c}
Ellagic acid	3.8	728.69 ± 5.10^{a}	nd	18.62 ± 1.16^{c}	28.76 ± 0.04^{b}
Hydroxycinnamic acids					
Chlorogenic acid	2.0	101.88 ± 0.73^{b}	122.08 ± 1.38^{a}	16.56 ± 0.17^{c}	20.47 ± 0.04^{d}
Caffeic acid	2.8	ND	1.99 ± 0.04^{a}	0.11 ± 0.01^{b}	0.15 ± 0.01^{c}
p-Coumaric acid	4.4	517.12 ± 37.44^{a}	nd	46.99 ± 5.29^{b}	0.63 ± 0.04^{c}
Sinapic acid	5.1	nd	nd	0.14 ± 0.01^{a}	0.07 ± 0.01^{b}
Flavonoids					
(+)-Catechin	2.2	147.65 ± 1.07^{a}	115.53 ± 1.90^{b}	6.00 ± 0.06^{d}	10.34 ± 0.09^{c}
Rutin	3.3	nd	nd	1.04 ± 0.02^{b}	5.91 ± 0.01^{a}
Quercetin	13.3	nd	nd	0.82 ± 0.01^{a}	0.98 ± 0.01^{a}

The results represent the average of three replicates ± SD and were expressed in µg equivalents of each phenolic compound/g sample. All results were quantified in DW. The means within the same row with different superscript letters differ significantly by Tukey–Kramer's test ($p < 0.05$)

R1M Ricinus communis L. leaves accession 1 methanolic extract, *R2M Ricinus communis* L. leaves accession 2 methanolic extract, *R1A Ricinus communis* L. leaves accession 1 aqueous extract, *R2A Ricinus communis* L. leaves accession 2 aqueous extract, *RT* retention time, *ND* no detected

the other free phenolic compounds, while (+)-catechin was the main identified flavonoid. These levels of polyphenolics are different to those reported by Wafa et al. [11] for five Tunisian populations of castor bean leaves (Rhiad Andalous, Khanguet Hajej, Aouled Amer, Hamamet and Nefza). However, the authors similarly identified gallic, chlorogenic and *p*-coumaric acids between the evaluated cultivars.

Additionally, Singh and Chauhan [45] and Ghosh et al. [46] also reported gallic and ellagic acids as major phenolic compounds, together with gentisic and ascorbic acids, quercetin, rutin, epicatechin, flavone, and kaempferol in *R. communis* L. Differences in the levels of phytochemicals coincide with the growing conditions of the plant as the phenolic compounds are secondary plant metabolites in response to hydric and temperature stresses [47]. Since the leaves can exhibit variations in the content of these phytochemicals, these disparities should be considered for their application in several study fields, guiding local producers to harvest *R. communis* L. leaves at a certain period. For instance, Wafa et al. [11] reported an effective anti-larvicidal activity (100% of larvae mortality after 24 h of exposure), depending on the assayed *R. communis* L. leaves population. The authors also observed an increment in larvicidal activity with the augmentation of phenolic content. These insecticidal properties (ovicidal and oviposition deterrent activity) has been attributed to their flavonoids content. Quercetin has been reported as the major flavonoids in the *R. communis* L. leaves, but its content varied in *R. communis* L. plants depending on the genetic background and detoxification mechanisms [48]. Hence, a methanolic extract from *Ricinus communis* L. leaves might be more beneficial than an aqueous extract to take advantage of biological control properties. The wide-ranging outcomes of the *R. communis* L. leaves should indicate the need for establishing safe doses for human or animal consumption according to the desired effect.

Oligosaccharides and Monosaccharides Content

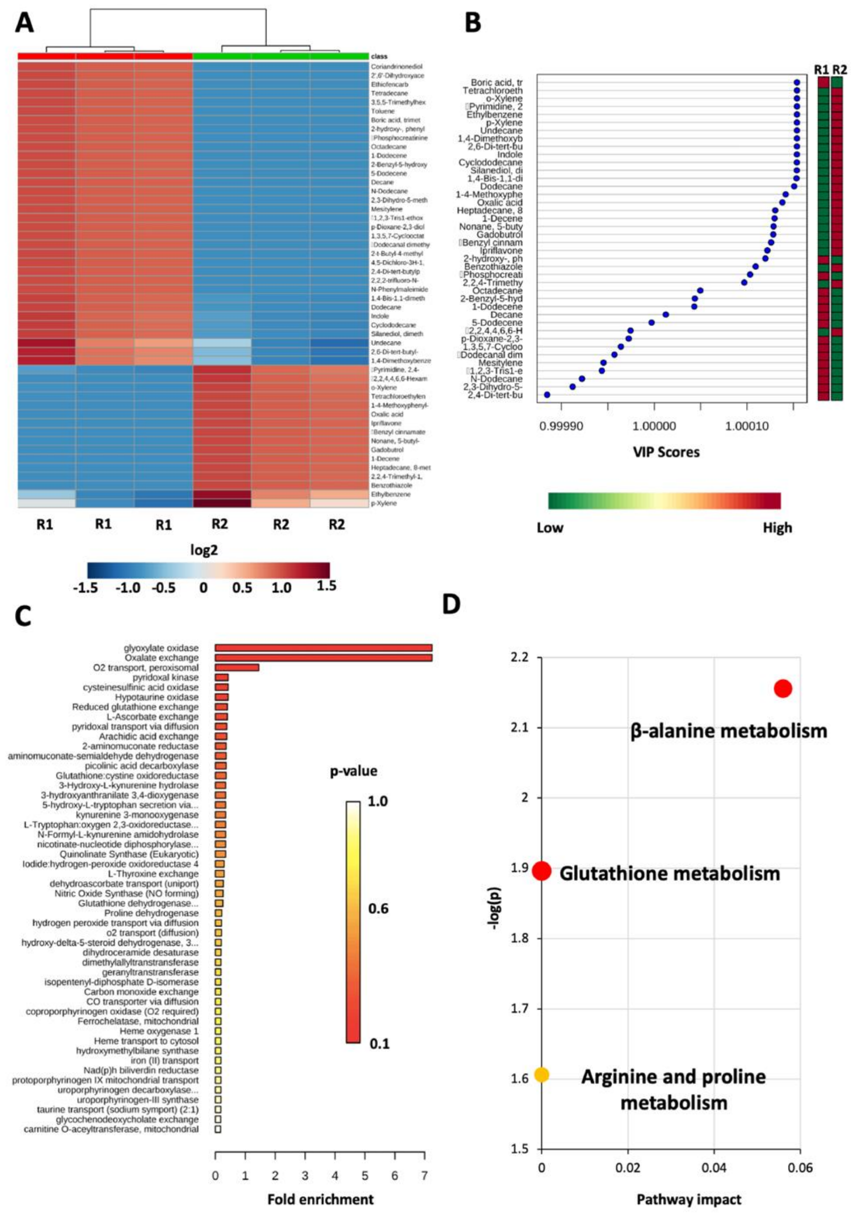
Table 3 shows the oligosaccharide and monosaccharide contents of the two *R. communis* L. leaves accessions. Xylose was the main monosaccharide, while verbascose was the primary oligosaccharide found in the leaves. Mannose and raffinose were significantly different ($p < 0.05$) among the

Table 3 Oligosaccharides and monosaccharides content, quantified by HPLC-RID, of the methanolic and aqueous extracts of Mexican *Ricinus communis* L. leaves accessions

Compound	RT	R1	R2
Monosaccharides			
Xylose	2.9	944.67 ± 20.50^{a}	918.33 ± 32.22^{a}
Mannose	3.1	534.32 ± 23.52^{b}	612.45 ± 35.64^{a}
Oligosaccharides			
Raffinose	3.2	5.98 ± 0.07^{b}	8.93 ± 0.59^{a}
Stachyose	6.2	25.43 ± 1.07^{a}	23.38 ± 0.01^{a}
Verbascose	8.5	803.27 ± 0.49^{a}	946.54 ± 18.09^{a}

The data are the means ± SD. Different letters express significant differences amongst samples (R1 and R2) for each mono/oligosaccharide ($p < 0.05$) by Tukey–Kramer's Test. Mono/oligosaccharides values were calculated in mg equivalents of each monosaccharide or oligosaccharide/g sample, and were extracted directly from the leaves' powder (R1, R2) after a hydro-thermal process

R1 Ricinus communis L. leaves accession 1, *R2 Ricinus communis* L. leaves accession 2, *RT* retention Time (min)


A
class
Coriandrinonediol
2',6'-Dihydroxyace
Ethiofencarb
Tetradecane
3,5,5-Trimethylhex
Toluene
Boric acid, trimet
2-hydroxy-, phenyl
Phosphocreatinine
Octadecane
1-Dodecene
2-Benzyl-5-hydroxy
5-Dodecene
Decane
N-Dodecane
2,3-Dihydro-5-meth
Mesitylene
1,2,3-Tris1-ethox
p-Dioxane-2,3-diol
1,3,5,7-Cyclooctat
Dodecanal dimethy
2-t-Butyl-4-methyl
4,5-Dichloro-3H-1,
2,4-Di-tert-butylp
2,2,2-trifluoro-N-
N-Phenylmaleimide
1,4-Bis-1,1-dimeth
Dodecane
Indole
Cyclododecane
Silanediol, dimeth
Undecane
2,6-Di-tert-butyl-
1,4-Dimethoxybenze
Pyrimidine, 2,4-
2,2,4,4,6,6-Hexam
o-Xylene
Tetrachloroethylen
1-4-Methoxyphenyl-
Oxalic acid
Ipriflavone
Benzyl cinnamate
Nonane, 5-butyl-
Gadobutrol
1-Decene
Heptadecane, 8-met
2,2,4-Trimethyl-1,
Benzothiazole
Ethylbenzene
p-Xylene
R1
R1
R1
R2
R2
R2
log2
-1.5
-1.0
-0.5
0
0.5
1.0
1.5
B
R1
R2
Boric acid, tr
Tetrachloroeth
o-Xylene
Pyrimidine, 2
Ethylbenzene
p-Xylene
Undecane
1,4-Dimethoxyb
2,6-Di-tert-bu
Indole
Cyclododecane
Silanediol, di
1,4-Bis-1,1-di
Dodecane
1-4-Methoxyphe
Oxalic acid
Heptadecane, 8
1-Decene
Nonane, 5-buty
Gadobutrol
Benzyl cinnam
Ipriflavone
2-hydroxy-, ph
Benzothiazole
Phosphocreati
2,2,4-Trimethy
Octadecane
2-Benzyl-5-hyd
1-Dodecene
Decane
5-Dodecene
2,2,4,4,6,6-H
p-Dioxane-2,3-
1,3,5,7-Cycloo
Dodecanal dim
Mesitylene
1,2,3-Tris1-e
N-Dodecane
2,3-Dihydro-5-
2,4-Di-tert-bu
0.99990
1.00000
1.00010
VIP Scores
Low
High
C
glyoxylate oxidase
Oxalate exchange
O2 transport, peroxisomal
pyridoxal kinase
cysteinesulfinic acid oxidase
Hypotaurine oxidase
Reduced glutathione exchange
L-Ascorbate exchange
pyridoxal transport via diffusion
Arachidic acid exchange
2-aminomuconate reductase
aminomuconate-semialdehyde dehydrogenase
picolinic acid decarboxylase
Glutathione:cystine oxidoreductase
3-Hydroxy-L-kynurenine hydrolase
3-hydroxyanthranilate 3,4-dioxygenase
5-hydroxy-L-tryptophan secretion via...
kynurenine 3-monooxygenase
L-Tryptophan:oxygen 2,3-oxidoreductase...
N-Formyl-L-kynurenine amidohydrolase
nicotinate-nucleotide diphosphorylase...
Quinolinate Synthase (Eukaryotic)
Iodide:hydrogen-peroxide oxidoreductase 4
L-Thyroxine exchange
dehydroascorbate transport (uniport)
Nitric Oxide Synthase (NO forming)
Glutathione dehydrogenase...
Proline dehydrogenase
hydrogen peroxide transport via diffusion
o2 transport (diffusion)
hydroxy-delta-5-steroid dehydrogenase, 3...
dihydroceramide desaturase
dimethylallyltranstransferase
geranyltranstransferase
isopentenyl-diphosphate D-isomerase
Carbon monoxide exchange
CO transporter via diffusion
coproporphyrinogen oxidase (O2 required)
Ferrochelatase, mitochondrial
Heme oxygenase 1
Heme transport to cytosol
hydroxymethylbilane synthase
iron (II) transport
Nad(p)h biliverdin reductase
protoporphyrinogen IX mitochondrial transport
uroporphyrinogen decarboxylase...
uroporphyrinogen-III synthase
taurine transport (sodium symport) (2:1)
glycochenodeoxycholate exchange
carnitine O-acyltransferase, mitochondrial
p-value
1.0
0.6
0.1
0
1
2
3
4
5
6
7
Fold enrichment
D
2.2
2.1
2
1.9
1.8
1.7
1.6
1.5
-log(p)
β-alanine metabolism
Glutathione metabolism
Arginine and proline metabolism
0
0.02
0.04
0.06
Pathway impact

◂**Fig. 2** Untargeted metabolomics of *R. communis* L. leaves. **a** Sample clustering (R1, R2) accordingly to their metabolite's composition; **b** variable Importance Projection (VIP) score metabolite ranking; **c** Metabolic pathways association with the metabolite's composition from samples; **d** Predicted major impacted pathways from the metabolomic arrangement. Samples from the VIP scores (**b**) were classified using their relative abundance classified as "low" or "high". The metabolic pathways association (**c**) and predicted major impacted pathways (**d**) are expressed using their *p* value of their reported FDR (false discovery rate)

evaluated accessions. There are no reports about the mono and oligosaccharide content from *R. communis* L. leaves, but the outstanding content of xylose and verbascose might indicate that the leaves can be a good source of them. Mono and oligosaccharides have gained interest due to their several properties in human health as a fiber source and the health benefits derived from their consumption, primarily associated with gut health and the production of specific metabolites (short-chain fatty acids and chemical inductors, among others) [49]. Oligosaccharides also have a wide range of technological and functional applications in the food industry (confectionery, desserts, meat products), drug delivery, stabilizers in the cosmetic industry, pharmaceutical products, packaging, textiles, solubilization of organic components, and removal of organic pollutants and heavy metals from soil and atmosphere [49]. Extraction of oligosaccharides from waste sources such as leaves has been proposed as a feasible alternative for obtaining these compounds of commercial interest [50].

Untargeted Metabolomic Profile

The untargeted metabolomic profile of the accessions (Fig. 2) revealed significant differences among R1 and R2, clustered in two different groups (Fig. 2a). R1 exhibited a higher abundance of trialkylamines, carboxylic acid derivatives, steroid derivatives, and pyridines derivatives. R2 was mainly abundant in aromatic compounds (benzene derivatives) and phenyl derivatives. The variable in importance (VIP) scores of compounds ranked trimethyl ester boric acid, tetrachloroethylene, *o*-xylene, *p*-xylene, undecane, and ethylbenzene as the most significant contributors to the composition of samples (Fig. 2b). This profile of metabolites predicted their involvement in key enzymatic functions (Fig. 2c) linked to glyoxylate and oxalate metabolism, several antioxidant mechanisms (glutathione exchange, glutathione:cystine oxidoreductase, nitric oxide synthase, and hydrogen peroxide transport), and Vitamin B6 metabolism (pyridoxal kinase activity and pyridoxal transport), among others. Likewise, the composition of metabolites mainly impacts pathways such as b-alanine, glutathione, and arginine and proline metabolism (Fig. 2d).

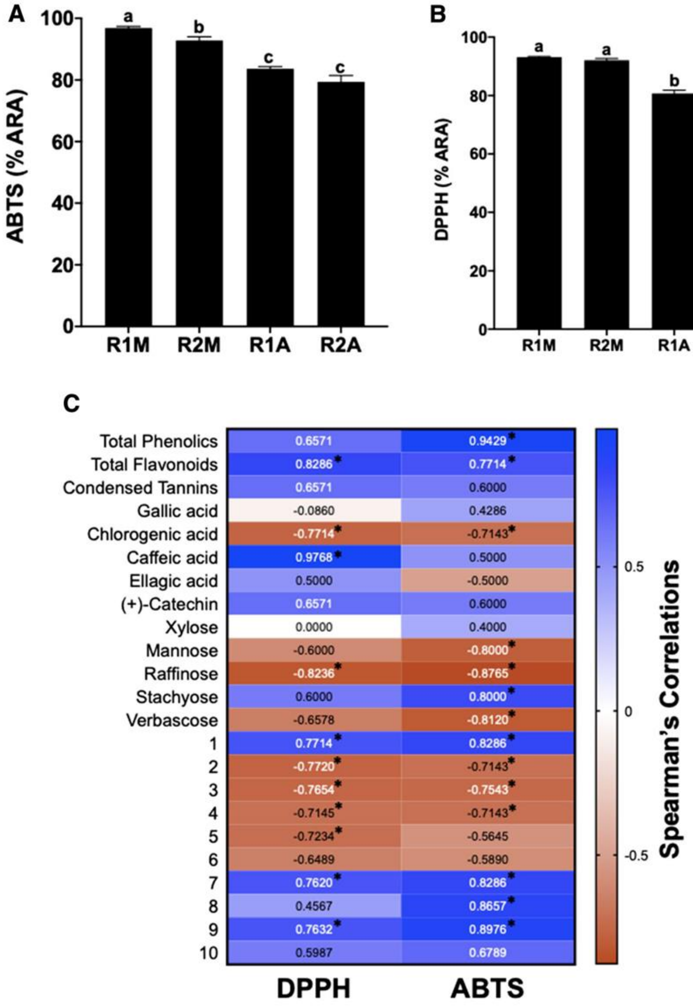
Gramh et al. [51] conducted a GC–MS analysis of *R. communis* L. leaves extracts, identifying alkaloids, phenolics, and flavonoids, which were classified as those components with the major contribution to the biological activities of these extracts such as antioxidant or inhibitory effects on selected human cancer cells (HeLa and HepG2). Compounds such as hexadecanoic and octadecanoic acids were in line with some of the chemical components reported in this article. Untargeted metabolomics has been successfully used in *R. communis* L. seeds as a classification parameter [52] or indirect measurement of the impact of abiotic conditions [53]. This is the first metabolomic characterization of *R. communis* L. leaves from Mexican accessions, indicating the richness of aromatic compounds involved in antioxidant mechanisms that have a significant impact on detoxification of xenobiotics and their metabolites [54].

In Vitro and In Silico Antioxidant Capacity

Figure 3 shows the antioxidant capacity of the two extracts (aqueous/methanolic) from Mexican *Ricinus communis* L. leaves, measured by the ABTS (Fig. 3a) and DPPH (Fig. 3b) methods, and their Spearman's correlations with the chemical composition of the *R. communis* L. leaves accessions as well (Fig. 3c). Both R1 and R2 methanolic extracts displayed the highest antioxidant capacity by ABTS and DPPH methods. R2M showed an increase of more than 1.21-fold regarding R2A in the antiradical activity by ABTS. Whereas, R1M presented an increase of 1.15 regarding its aqueous extract (R1A). Changes in the antioxidant capacity of leaves can be attributed to the solvent used as wells as synergistic combinations or counteractions of several types of chemical reactions, leaching of water-soluble antioxidant composition, and formation or breakdown of antioxidant species [55].

The antioxidant capacity of samples might be correlated with their phytochemical contents. In this regard, correlation coefficients among these parameters were calculated by Spearman's correlation (Fig. 3c). The total flavonoids, chlorogenic acid, caffeic acid, raffinose, boric acid trimethyl ester (1), tetrachloroethylene (2), *o*-Xylene (3); 2,4-bis-trimethylsilyl-oxy-pyrimidine (4), ethylbenzene (5), undecane (7), and 2,6-di-tertbutyl-1,4-benzenediol (9) showed the highest correlations with DPPH. On the other hand, the total flavonoids, chlorogenic acid, caffeic acid, raffinose, boric acid trimethyl ester (1), tetrachloroethylene (2), *o*-Xylene (3); 2,4-bis-trimethylsilyl-oxy-pyrimidine (4), undecane (7); 1,4-dimethoxybenzene (8), and 2,6-di-tertbutyl-1,4-benzenediol (9) exhibited the highest correlations with ABTS. It has been reported that isolated gallic acid, quercetin, and ellagic acid from *R. communis* L. leaves exhibit strong IC_{50} DPPH inhibition [45].

Despite concerns regarding the use of ABTS or DPPH as antioxidant capacity methods, these oxidizing agents can



	DPPH	ABTS
Total Phenolics	0.6571	0.9429*
Total Flavonoids	0.8286*	0.7714*
Condensed Tannins	0.6571	0.6000
Gallic acid	-0.0860	0.4286
Chlorogenic acid	-0.7714*	-0.7143*
Caffeic acid	0.9768*	0.5000
Ellagic acid	0.5000	-0.5000
(+)-Catechin	0.6571	0.6000
Xylose	0.0000	0.4000
Mannose	-0.6000	-0.8000*
Raffinose	-0.8236*	-0.8765*
Stachyose	0.6000	0.8000*
Verbascose	-0.6578	-0.8120*
1	0.7714*	0.8286*
2	-0.7720*	-0.7143*
3	-0.7654*	-0.7543*
4	-0.7145*	-0.7143*
5	-0.7234*	-0.5645
6	-0.6489	-0.5890
7	0.7620*	0.8286*
8	0.4567	0.8657*
9	0.7632*	0.8976*
10	0.5987	0.6789

Fig. 3 In vitro antioxidant capacity assessment of samples. Antiradical activity (ARA, %) by the **a** ABTS and **b** DPPH methods; **c** Spearman's correlations between the phytochemical composition and the antioxidant capacity (ABTS, DPPH). The means with different superscript letters significantly differs by Tukey–Kramer's Test ($p < 0.05$). For the Spearman's correlations, the asterisks indicate the significant correlations ($p < 0.05$). *R1M Ricinus communis* L. leaves accession 1 methanolic extract, *R2M Ricinus communis* L. leaves accession 2 methanolic extract, *R1A Ricinus communis* L. leaves accession 1 aqueous extract, *R2A Ricinus communis* L. leaves accession 2 aqueous extract. **1** Trimethyl ester boric acid; **2** tetrachloroethylene; **3** *o*-xylene; **4** 2,4-bis[(trimethylsilyl)oxy]-pyrimidine; **5** ethylbenzene; **6** *p*-xylene; **7** undecane; **8** 1,4-dimethoxybenzene; **9** 2,6,-ditertbutyl-1,4-benzenediol; **10** Indole

serve as a general screening of the antioxidant potential of phytochemicals and could be a clear strategy for the estimation of their antioxidant properties [56]. Additional experiments evaluating the complex dynamics of antioxidants on in vivo systems provide valuable information that enriches the antioxidant capacity methods [57]. The higher antiradical activity of the methanolic extracts coincides with reported scavenging activity for *R. communis* L. leaves extracts (0.63, 1.25, and 2.50 mg/mL) closer to 100% [58], suggesting that polar solvents are those with the highest efficiency of extraction of antioxidant compounds from *R. communis* L. leaves.

Deepening into the antioxidant potential of selected compounds from *R. communis* L. leaves, several candidates were selected for conducting in silico molecular docking with the radicals, as shown in Fig. 4. A representative protein constituent, ricin with ABTS (Fig. 4a) and DPPH (Fig. 4b), (+)-catechin as a representative flavonoid (Fig. 4C), ellagic acid as a representative phenolic acid (Fig. 4d), and ricinine as a representative alkaloid (Fig. 4e). The selected conformations were chosen as those that exhibited the highest binding energy (Supplementary Table 1). Surprisingly, ricin showed the highest binding affinity with both radicals (-7.70 and -7.00 kcal/mol, respectively for ABTS and DPPH), followed by (+)-catechin, ellagic acid, and ricinine. Particularly for ricin, their affinity is mainly explained by van der Walls, carbon-hydrogen bonding, Pi-anion, and pi-alkyl interactions with several amino acids (Supplementary Table 2). The higher number of hydrogen bonds in the interaction with ABTS, compared with that from DPPH, might explain the higher affinity. It is important to note that this affinity is not an indication of inhibition or enhancement of the radical, considering the reported pro-oxidant properties of ricin, characterized by an increased ribotoxic stress response by MAP3K activation, derived in a pro-inflammatory pathway in organisms [59]. In the case of (+)-catechin, ellagic acid, and ricin, their interaction was mainly governed by pi-stacked (1), conventional hydrogen-carbon bond, sulfur, and pi-alkyl. The lack of interactions between ricinine and both radicals (Fig. 4e) explains the low binding energies and low affinity (Supplementary Table 1).

Physical Characterization of *Ricinus communis* L. Leaves

X-ray Diffraction, X-ray Fluorescence, and SEM Analysis

Figure 5 shows the mineral composition for the ashes for samples R1 and R2, indicating their X-ray diffraction patterns as a function of the 2θ scale (Fig. 5a). The quantification of minerals by X-ray fluorescence is displayed in Fig. 5b, and their confirmation by SEM images are depicted in Fig. 5c. Both *R. communis* L. leaves ashes exhibited a similar pattern (Fig. 5a). The following elements were found in these ashes: calcite [$Ca(CO)_3$] identified with the powder diffraction file PDF# 86–0174, sylvite (KCl) identified with the PDF #41–1476, and arcanite (K_2SO_4) with PDF# 05–0613. Other crystalline phases formed by these minerals are also present in the leaves, but in this work, the compounds mentioned above are mainly forming the ashes. The dotted lines in these patterns were used to identify the main diffracted peaks for each crystalline compound.

The quantification of minerals (Fig. 5b) showed significant differences ($p < 0.05$) in the content of K, S, Cl, Si, Sr, Br, Zn, and Sc. Calcium proved to be the dominant element, followed by K, Mg, and S. The crystalline compounds identified in Fig. 5c coincide with those reported in Fig. 5a for $CaCO_3$ and KCl. Although K_2SO_4 was not identified using SEM, the X-ray diffraction pattern confirmed its presence. Considering that both leaves were cultivated under the same agronomical conditions and harvested at the same time, the differences in the chemical elemental composition of the leaves can be attributed to the genetic origin of the plants [60]. Tadayyon et al. [20] reported Fe, Cu, and Na as other minerals that can be found on *R. communis* plants, and attributed their accumulation to the composition of the soil and the drought stress. Potassium levels increase at higher drought stress, while magnesium exhibits an opposite behavior, being associate with the moisture content of the leaves [61].

FT-IR Spectroscopy and In Silico Interactions of Ricinine and Selected Compounds

Figure 6 shows the FT-IR spectroscopy analysis of *R. communis* L. leaves (R1 and R2). The FT-IR spectra are shown in Fig. 6a, while the correspondent absorption peaks, and their associated functional groups are displayed in Fig. 6b. As indicated, the absorption peak located at 3045 cm^{-1} was due to the C-H stretching frequency of the unsaturated ring. The band at 2224 cm^{-1} was the typical stretching vibration of C-N. Presence of CO groups identified by the absorption band located at 1636 cm^{-1}. Zhu et al. [62] identified a peak located at 1537 cm^{-1} for the C = C stretching vibration of the unsaturated ring, but in the case of R1 and R2 samples, it was found at 1539 and 1542 cm^{-1} respectively. The stretching vibration of C–O–C groups was identified at the band of 1257 cm^{-1}. The band at 777 cm^{-1} was considered to be the characteristic of C-H deformation vibration. Overall, the FTIR spectra coincide with the reported for castor bean leaf extracts [63].

Since these peaks correspond to several functional groups associated with the vibrational state of ricinine [62], the presence of this alkaloid can be inferred. Additionally, typical protein bands in the amide B, amide I, and amide III might be indicating the presence of ricin. For instance, amide I peak (1634 cm^{-1}) represents the anti-parallel b-sheet of protein oligomer of ricin, which is also specific for the recombinant ricin A chain; amide A peak is representing N–H vibration in resonance with amide II overtone band from 3300 to 3250 cm^{-1} [64].

In silico interactions of ricin and selected compounds are represented in Fig. 7. These interactions were considered using ricinine (Fig. 7a), (+)-catechin (Fig. 7b), and ellagic acid (Fig. 7c). The presence of hydrogen bonds explained the

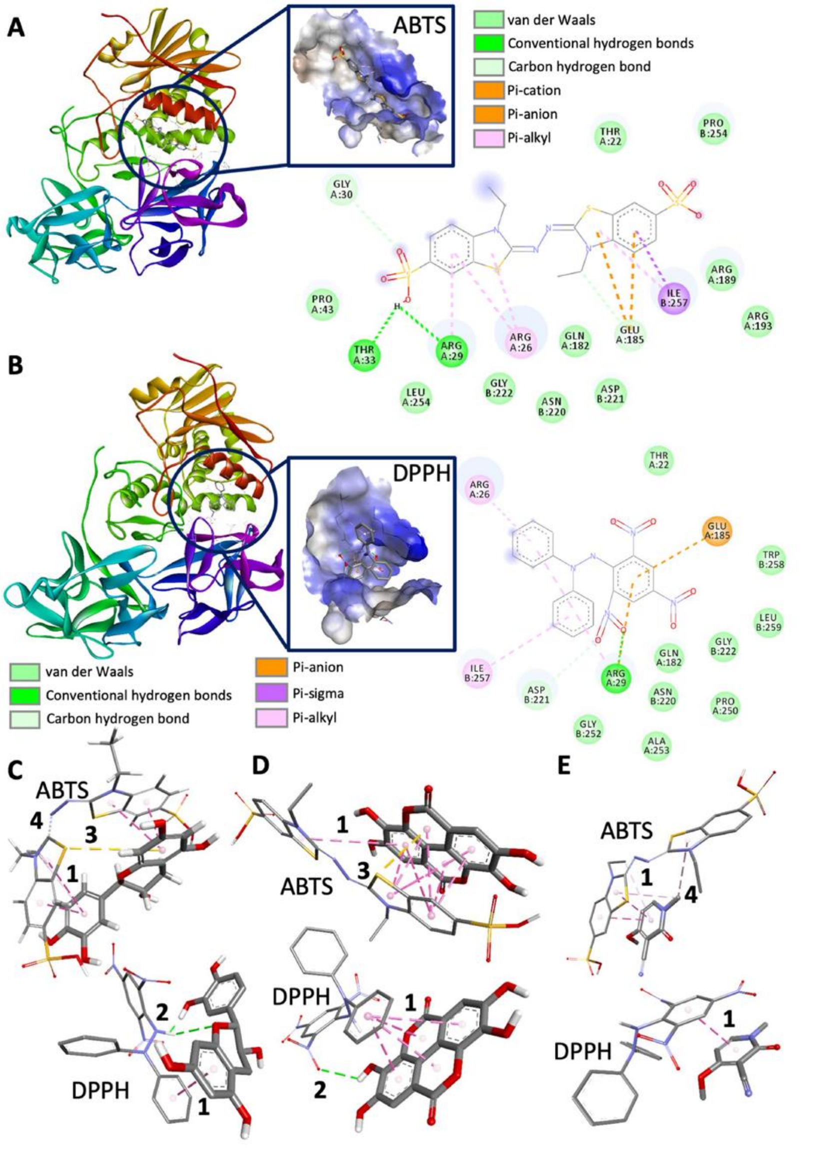
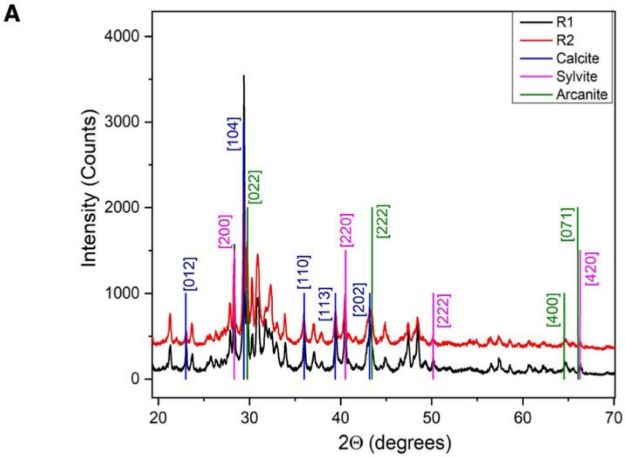


Fig. 4 In silico antioxidant capacity assessment of selected compounds from samples. Best potential interaction between the catalytic site of ricin and **a** ABTS radical, **b** DPPH radical. Potential interactions between ABTS and DPPH for **c** (+)-catechin, **d** ellagic acid, and **e** ricinine. **1** Pi-stacked; **2** conventional hydrogen carbon bond, **3** sulfur, **4** Pi-alkyl



B

Compound	R1	R2
Calcium (Ca)	54.99 ± 4.09^{a}	51.97 ± 0.35^{a}
Potassium (K)	19.34 ± 1.81^{b}	30.30 ± 1.77^{a}
Magnesium (Mg)	8.99 ± 0.68	nd
Sulfur (S)	6.75 ± 0.01^{b}	8.89 ± 0.52^{a}
Chlorine (Cl)	4.66 ± 0.14^{a}	3.55 ± 0.26^{b}
Phosphorus (P)	2.36 ± 0.03^{a}	2.61 ± 0.05^{a}
Silicon (Si)	1.76 ± 0.01^{a}	1.45 ± 0.05^{b}
Strontium (Sr)	0.67 ± 0.01^{b}	0.75 ± 0.01^{a}
Bromine (Br)	0.23 ± 0.01^{a}	0.17 ± 0.01^{b}
Zinc (Zn)	0.14 ± 0.01	nd
Scandium (Sc)	0.10 ± 0.01^{a}	0.07 ± 0.01^{b}

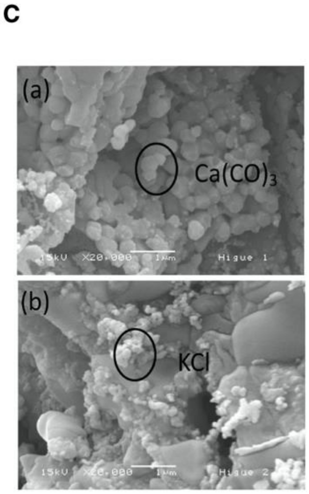


Fig. 5 Elemental composition of *R. communis* L. leaves accessions. **a** X-ray diffraction patterns and **b** mineral composition by X-ray fluorescence, **c** SEM images of ashes from the two *Ricinus communis* L. leaves accessions. For the mineral composition (**b**), the results represent the average of three replicates ± SD, expressed in mg/kg. Means within the same row with different superscript letters differ significantly by Tukey–Kramer's test ($p < 0.05$). Data are expressed in %. The SEM images (**c**) were taken at 20,000 × and represents Ca(CO)$_3$ (subsection "a") and KCl (subsection "b")

higher affinity of ricin with (+)-catechin (− 8.80 kcal/mol) and ellagic acid (-8.60 kcal/mol), while showed low binding energy with ricinine (− 5.40 kcal/mol) (Supplementary Table 3), which lack of this kind of interaction. Notably, ricinine interacts with a higher number of amino acids from ricin than (+)-catechin (Supplementary Table 4), but most of these interactions are weak, represented by van der Waals type bonds.

Conclusions

The results obtained in this work indicated that *R. communis* L. leaves accessions are a source of protein and fiber with low lipid content. The polyphenol and untargeted metabolomic profiling revealed the abundance of selected phytochemicals such as ellagic acid and (+)-catechin, both linked to antioxidant mechanisms. The screening of the

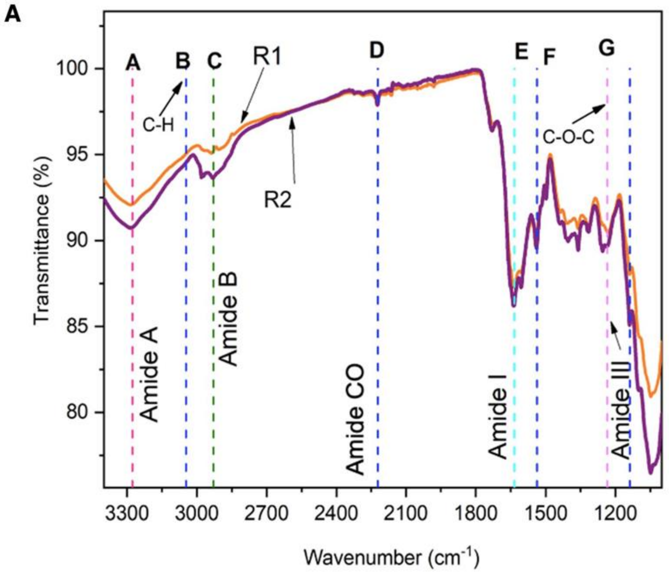


Fig. 6 **a** IR spectra of ricinine and selected identified peptide bonds, **b** FTIR identified peaks by absorption location (cm^{-1}) compared with those found in the literature

B

Functional Groups	Absorption location found (cm^{-1})	Reported absorption Location (cm^{-1})	Reference
Amide A	(A) 3278	3278	Tongnuanchan et al., (2013)
C-H stretching	(B) 3047	3045	Zhu et al., (2018)
Amide B	(C) 2926	2929	Tongnuanchan et al., (2013)
Amide CO	(D) 2224	2224	Mekheimer et al. (2018)
Amide I	(E) 1636	1634	Tongnuanchan et al. (2013)
C=C stretching	(F) 1537	1537 -1538	Quilaqueo et al. (2019); Zhu et al. (2018)
Amide III	(G) 1236	1236	Tongnuanchan et al. (2013)

in vitro and in silico antioxidant capacity correlated polyphenols and oligosaccharides with the inhibition of radicals, molecular bonding, and highlighted ricin and ricinine as target candidates for the antioxidant capacity. Both ricin and ricinine were partially identified in the FTIR spectra of the leaves, and their in silico interactions were characterized, mainly represented by carbon-hydrogen and pi-type bonding. The leaves also displayed an abundant content of minerals identified through several methods, confirming the substantial presence of calcium and potassium in the form of the crystalline phases of calcite and sylvite, respectively. This research contributes to the valorization of unreported

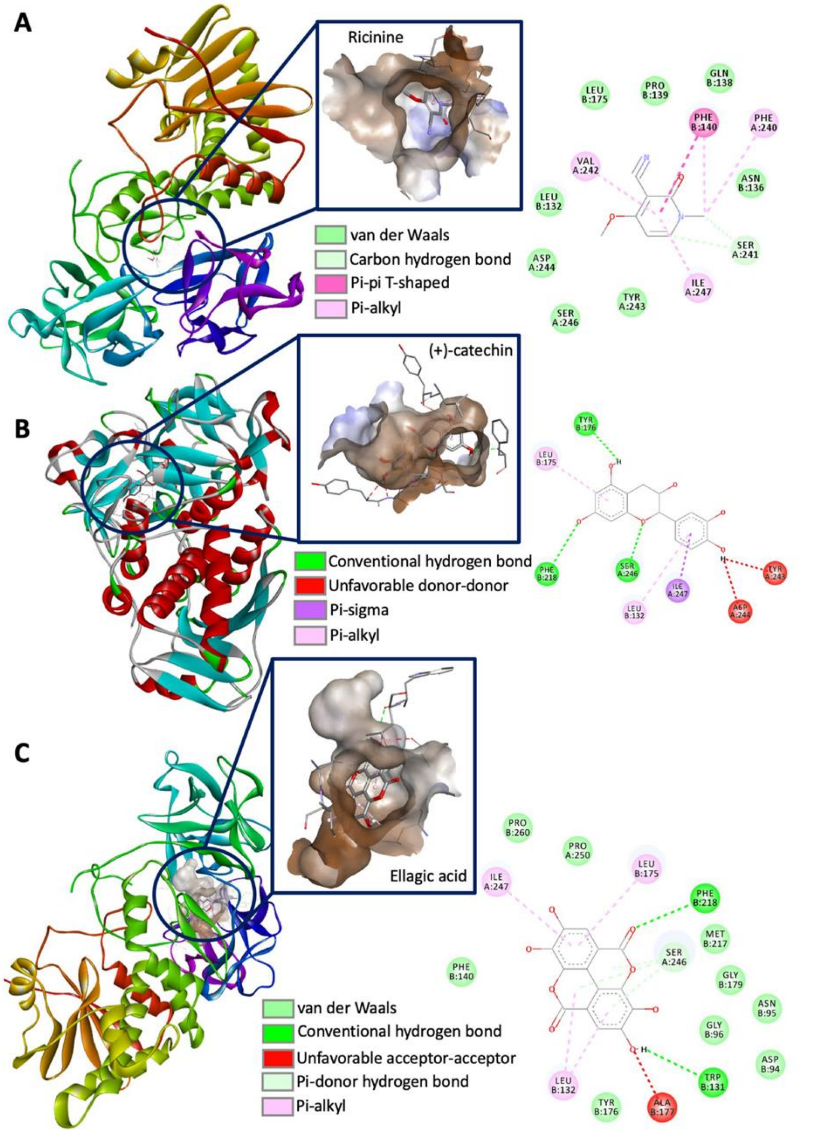


Fig. 7 In silico interactions between ricin and selected compounds from *R. communis* L. leaves. Interactions between ricin and **a** ricinine, **b** (+)-catechin, **c** ellagic acid

Mexican *R. communis* L. leaves accessions, potentially serving as a source of macromolecules, minerals, and antioxidant compounds.

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Data Availability Additional data and material are available upon request of reviewers and editors.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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II.2 Physicochemical characteristics of seeds form wild and cultivated castor bean plants (*Ricinus communis* L.)

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Physicochemical characteristics of seeds from wild and cultivated castor bean plants (*Ricinus communis L.*)

Características fisicoquímicas de semillas de plantas de higuerilla (*Ricinus communis L.*) silvestres y cultivadas

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ABSTRACT

The castor (*Ricinus communis* L.) is an oilseed plant whose main features are its drought resistance, and its adaptation to eroded, polluted, and low fertility soils. Its oil has a great demand in the industrial sector and it has recently attracted considerable interest for its use in the production of biodiesel and jet fuel. In this study, morphological, physical and chemical characterizations were performed to ascertain the quality of wild (VQ-1) and under cultivation (VQ-7) oil castor seeds. The results showed that there are differences in the morphological and physicochemical characteristics regarding oil content (44,95 vs 33,84 %), ash (3,20 vs 2,42 %), and 100-seed-weight (45,87 vs 54,23 g); similar behavior was recorded when characterizing the oil: kinematic viscosity (269,67 vs 266,44 mm^2/s), density (0,9389 vs 0,9465 g/cm^3), and acidity index (0,9918 vs 0,5440 mg KOH/g) for VQ-1 and VQ-7, respectively. Growing conditions to which castor plants were subjected may influence both the final quality of seeds and chemical properties of the oil.

Keywords: characterization of seed, oil quality, castor seed chemistry.

RESUMEN

La higuerilla (*Ricinus communis* L.) es una planta oleaginosa cuyas principales características son su resistencia a la sequía y su adaptación a suelos erosionados, contaminados y de baja fertilidad. Su aceite tiene una gran demanda en el sector industrial y recientemente ha despertado un gran interés para ser utilizado en la producción de biodiesel y bioturbosina. En esta investigación, se realizaron caracterizaciones morfológicas, físicas y químicas de la semilla, así como una evaluación de la calidad del aceite de semillas silvestres (VQ-1) y bajo cultivo (VQ-7). Los resultados demostraron diferencias en las características morfológicas y físico-químicas con respecto al contenido de aceite (44,95 vs 33,84 %), cenizas (3,20 vs 2,42 %) y el peso de 100 semillas (45,87 vs 54,23 g); se observó un comportamiento similar en la caracterización del aceite: viscosidad cinemática (269,67 vs 266,44 mm^2/s), densidad (0,9389 vs 0,9465 g/cm^3) e índice de acidez (0,9918 vs 0,5440 mg KOH g), respectivamente para VQ-1 y VQ-7. Las condiciones de crecimiento a las cuales fueron sometidas las plantas de ricino pueden influir en la calidad final de las semillas y propiedades químicas del aceite.

Palabras clave: calidad de aceite, caracterización de semilla, química del ricino.

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Introduction

Castor bean (*Ricinus communis* L.) is a shrub-like plant whose origin is still discussed; some authors consider it from Asia, but for others it probably originated from Africa (Anjani, 2012). Whatever its origin, the plant has adapted to a number of countries, where it receives different names. This species is widely distributed, especially in tropical and subtropical regions of the world; however, it shows resistance to drought and adaptation to degraded soils (eroded, polluted, and low fertility), with a positive response in growth and performance in different regions, without competing with other oleaginous edible crops. The seed is composed of 25 to 35 % of seed coat, and 65-75 % endosperm; its average chemical composition includes water (5,5 %), crude protein (17,9 %), crude fiber (12,5 %), ash (2,5 %), and carbohydrates (13 %) (Freire, 2001); it also contains oil (up to 55 %), which is the main product (Ogunniyi, 2006) for human purposes. Some characteristics of castor seed oil include a great density, unchanged viscosity at a wide range of temperature, and a freezing point of -10 °C (Lascarro, 2005). This oil is used as raw material in the cosmetic, pharmaceutical, automotive, and aerospace industries, and could be modified for other end usages (Akpan *et al.*, 2006). Also, the increased interest in renewable energy production, such as biodiesel and aircraft biofuels, presents another opportunity for the cultivation of this plant. On the other hand, residues of oil extraction are considered an agro-industrial by-product that can be used as an organic fertilizer or as an ingredient for animal diets, after removal of toxic compounds. Due to the lack of information on native genotypes of México, and the great interest of the government and the chemical industries in promoting this crop, basic information, such as how environmental conditions and agronomic management may affect seed quality and oil content is required. Therefore, the aim of this research was to compare some physicochemical properties, morphological characteristics, and oil quality of seeds collected in the wild and seeds harvested from cultivated plants grown from seeds with the same wild origin.

Materials and methods

Collecting Samples

Wild castor bean seed used for this study was called VQ-1, and it was collected in a place with the coordinates 20°48’55.0”N 100°26’54.2”W, in the State of Querétaro, México. Part of the collected wild seed was conserved, and another part was planted to generate cultivated seed, which was then identified as VQ-7.

Crop establishment

To generate VQ-7 seed, collected wild seed was planted within the facilities of the Center of Applied Physics and Advanced Technology of the National Autonomous University of México, in Juriquilla, Querétaro, México, with the coordinates 20°42’02.80”N 100°26’51.16”W. This place is located at an altitude of 1 946 m, with prevailing agroecological conditions corresponding to a semiarid climate. The region presents annual averages of 22 °C temperature, and 456 mm precipitation. Cropping cultural practices consisted of soil tillage, required irrigation, and control of weeds, pests and diseases. Additionally, measures were taken to avoid pollen contamination from other genotypes or related species, to preserve as much as possible the original genotype and phenotype characteristics of collected wild seed.

Physical characterization of seeds

Weight

The weight (g) of 100 castor bean seeds randomly selected was recorded on an Ohaus analytical balance, with an accuracy of ± 0,0001 g.

Morphometric characterization by image analysis

Thirty seeds were used for morphometric analysis, and images were taken with two Webcams (Microsoft LifeCam HD-3000, USA), and processed using the Matlab 7.1 software. Image size was adjusted to 640 x 480 pixels in red, green and blue (RGB), following the protocol de-scribed by Medina *et al.*, (2010). Seed parameters obtained were length (L), width (W), area, perimeter, and thickness; while mathematical equations below were used to calculate, Feret diameter (DF), elongation index (EI), roundness index (RI) and compaction index (CI) (Wilcox *et al.*, 2002; Isaza *et al.*, 2017):

$$DF = \frac{\sqrt{4\ area}}{\pi} \tag{1}$$

$$EI = \frac{L}{W} \tag{2}$$

$$RI = \frac{4\ \pi\ \text{area}}{perimeter^2} \tag{3}$$

$$CI = \frac{FD}{L} \tag{4}$$

Seed microstructure

Seed coats were removed, and seeds were then frozen in liquid nitrogen to be divided later into longitudinal and transversal sections. Resulting cuts were placed in aluminium sample holders with carbon tape, and observed through an Environmental Scanning Microscope (MEBA, XL-30, Philips, USA), with an acceleration voltage of 25 kV. At the same time, the spectrum of electrons was obtained by Energy Dispersive X-ray Spectrum (EDS) (EDAX, New XL-30, Phillips, USA) and an active area of 10 mm^2, which allowed a qualitative chemical analysis (Stokes, 2008; Perea *et al.*, 2011) of minerals in the endosperm of the two castor seed lots studied.

Chemical characterization of seeds

Oil content was determined with a Soxhlet extraction system, using the method 920.39 (AOAC, 2002). Two grams of ground seeds were deposited in a cellulose thimble, placing it in a volumetric flask and adding 100 ml of petroleum ether, allowing the mixture to repose for six hours. After that time, flask content was dried at 60 °C for 2 hours; oil content (%) was determined by weight difference. Ash content was quantified by the method 923.03 (AOAC, 2002); three grams of ground seeds were placed in a porcelain crucible, which was the placed over the flame of a Bunsen burner for sample carbonization. Then, crucibles and their content were transferred to a muffle furnace (Novatech, México), where samples were exposed to a temperature of 550 °C for eight hours. After that, samples were cooled in a desiccator, and ash content was determined by weight difference. Seed moisture content was performed according to the gravimetric method 925.09B (AOAC, 2002). Two grams of ground seeds were deposited in an aluminum pan, which was placed for 24 h in a forced air drying oven (Felisa, México) set at 105 °C; moisture content (%) was determined by weight difference.

Physicochemical oil properties

Kinematic viscosity and density

This determination was done with a viscometer Stabinger VM3000 (Anton Paar, Austria), using 5 mL of oil at 40 °C, according to the ASTM D 445 standard procedure.

Acidity index

The acidity index was obtained following the methodology proposed by Firestone (1996). It is expressed as the amount of potassium hydroxide required to neutralize the acid components or the free fatty acids contained in one gram of castor bean oil (mg KOH/g).

Statistical analysis

Analyses of variance (ANOVA) and mean tests (t-test, 0,05) were performed for variables measured by triplicate on chemical composition, morphological characteristics, and oil quality of *R. communis* L. seeds. These procedures were performed with Minitab 17®.

Results and discussion

Physical characterization through image analysis

Average values of variables measured in seeds obtained from both wild and cultivated plants are shown in Table 1. There were differences between VQ-1 (wild) and VQ-7 (cultivated) seeds, with the second one presenting higher values in all the cases. These differences could be related to factors such as: soil type and fertility, climate conditions, water availability for plants, harvest time, and the adaptation process, among others. Similar results were observed for calculated parameters: except for elongation rate, a constant tendency to higher values is observed in the cultivated seed, VQ-7. Also, a similar behaviour was observed for the value of Feret diameter, in which VQ-1 gave 9,97, while in VQ-7 it was 11,43. This parameter explains the distance between two parallel lines that are tangential to the contour of the seed projection. Regarding the elongation rate, VQ-1 presented a calculated value of 1.69, while in VQ-7 it was 1.54; this is attributed to a disproportionate internal seed growth, which results in a seed that differs from the ovoid form. The roundness index explains that the closer the value is to 1, the rounder the seed; which, in this case, corresponds to VQ-7 (0,83), compared to VQ-1 (0,8). Finally, the compaction index is higher for VQ-7 (0,80) compared to VQ-1 (0,77). Determination of these physical seed parameters is useful for the design of equipment and implements, such as those used for: husking, seeding, sorting, storage and processing of seeds, among many others.

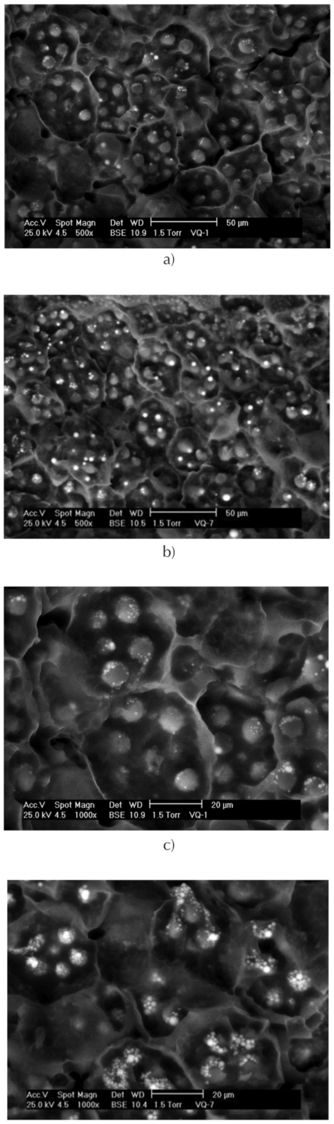
Table 1. Analysis of images of castor seeds

Seed	L (mm)	W (mm)	AR (mm^2)	P (mm)	G (mm)	DF (mm)	EI (%)	RI (%)	CI (%)
VQ-1	13,00	7,71	78,10	35,08	6,07	9,97	1,69	0,80	0,77
	± 0,48^{a}	± 0,22^{a}	± 4,12^{a}	± 0,11^{a}	± 0,28^{a}	± 0,26^{a}	± 0,06^{a}	± 0,02^{a}	± 0,00^{a}
VQ-7	14,23	9,26	102,71	39,30	6,84	11,43	1,54	0,83	0,80
	± 0,63^{b}	± 0,43^{b}	± 7,94^{b}	± 0,58^{b}	± 0,40^{b}	± 0,44^{b}	± 0,07^{b}	± 0,02^{b}	± 0,02^{b}

L = Long, W = Width, AR = Area, P = Perimeter, G = Thickness, DF = Diameter Feret, EI = Elongation index, RI = Roundness index, CI = Compaction index. VQ-1 = Wild seed; VQ-7 = Cultivated in experimental field. The results are expressed as the mean value ± standard deviation. Similar letters refer to the fact that the seeds belong to the same group with a confidence of 95 %.

Castor Seed Microstructure

Figure 1 shows the microstructure images of the studied seeds, obtained with a microscope type MEBA. Lipid microbodies are shown as part of the characteristic cells forming the endosperm (Figure 1), and highlighted as bright spots appear the reserve minerals, which are associated with the structural proteins that cover the lipids. Such structures have been observed in other oilseeds (Ellis *et al.*, 2004, Amonsou *et al.*, 2011, Perea *et al.*, 2011). The results of the EDX spectra are presented in Figure 2, which shows that wild seeds (VQ1) contain carbon, oxygen, phosphorus, sulphur and potassium, while seeds grown in experimental field (VQ7) contain magnesium, in addition to those previously enlisted. Mineral reserves (Mg, P, S and K) found in the seeds are associated with the protein bodies, usually included in an electronic density called globoid crystals (Lott *et al.*, 1982; Prego *et al.*, 1998).



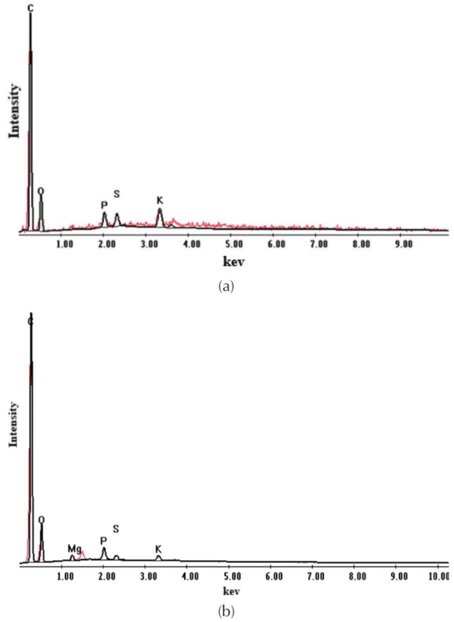
a)

b)

c)

d)

Figure 1. Environmental scanning electron microscopy micrographs of castor seed section of seeds where an stands for endosperm. Figure a,c) VQ-1=wild seed, figure b,d) VQ-7=cultivated in experimental field.
Source: Authors



(a)

(b)

Figure 2. Energy dispersive x-ray spectrum of endosperm cell of castor seed, (a) wild seed (b) cultivated in experimental field
Source: Authors

Weight of seeds

Table 2 shows the weight of 100 seeds. VQ-1 (wild seed) registered an average value of 45,8 ± 1,08 g whereas for VQ-7 (cultivated) it was 54,2 ± 1,28 g. According to these results, the seeds of wild castor bean responded to the application of cultivation practices, producing heavier seeds, compared to seeds from wild plants. This can be related to more developed plants, greater availability of water and nutrients, rapid adaptation to the environment, and conditions that stimulate a greater growing period and accumulation of foliar tissue, which can favor the production of larger seeds. Similar results have been observed in wild and domesticated genotypes of bean, with the latter ones being heavier (Vasco et al., 2018; Morales-Santos *et al.*, 2017); unlike plants growing under limiting conditions, which regularly present a poor vegetative growth as they tend to rapidly reach the reproductive stage to produce seeds, no matter that they are few and of poor quality.

VQ-1 = Wild seed; VQ-7 = Cultivated in experimental field. The results are expressed as the mean value ± standard deviation. Similar letters refer to the fact that the seeds belong to the same group with a confidence of 95 %.

Table 2. Weight and chemical composition of wild and cultivated *R. communis* L. seeds

Seed	Weight (g)	Oil (%)	Humidity (%)	Ashes (%)
VQ-1	45,87± 1,08^{a}	44,95± 0,69^{a}	3,65 ± 0,22^{a}	3,20 ± 0,11^{a}
VQ-7	54,23± 1,28^{b}	33,84± 2,87^{b}	4,79 ± 0,27^{b}	2,42 ± 0,13^{bs}

Source: Authors

Chemical characterization

The oil content in the seeds of VQ-1 (44,95 %) and VQ-7 (33,84 %) is in both cases below those obtained by Acosta-Navarrete *et al.*, (2017), who studied fourteen accessions of seeds from a local collection made in México in 2010; these accessions were later planted and subjected to agronomic management in the field, reporting an oil content between 45,37 and 55,53 % in the harvested grains. These differences could be due to the agroecological conditions in which wild plants are adapted, showing responses to environmental stress, commonly resulting in a greater synthesis of secondary compounds (Akula & Ravishankar, 2011; Selmar & Kleinwächter, 2011). This has been exemplified in *Calendula officinalis*, where water stress induces a greater production of essential oils; while under agronomic management, plants have a better availability of water and nutrients for vegetative growth and biomass accumulation (Anderson *et al.*, 2016), in addition to the effect it has on the production of secondary metabolites. According to these results, the hillock could become an alternative raw material with a high potential for the production of oil for industrial or bioenergetic use. (Vasco et al., 2018; Mosquera-Artamonov *et al.*, 2017; Perdomo *et al.*, 2013). The moisture content in VQ-7 seeds (4,79 %) was higher than that for VQ-1 (3,65 %). In relation to this, Coimbra *et al.* (2007) pointed out the importance of knowing the moisture content present in the seeds as an essential factor in quality evaluation tests. The ash content of 3,20 % was obtained for VQ-1, and 2,42 % for VQ-7. These variations in the chemical composition of the seeds studied here could be related to those compounds and elements in the soil and their availability to the plants during the growth cycle (Table 2).

Oil quality

The oil quality is determined by its physical and chemical proper-ties. Oil characterization from castor seeds of VQ-1 and VQ-7 are presented in Table 3.

Table 3. Physicochemical properties of *R. communis* L. oil

Oil	Viscosity (mm^2/s)	Density (g/cm^3)	Acidity Index (mg KOH/g)	Free Fatty Acids (%)
VQ-1	269,67 ± 1,72^{a}	0,9389 ± 0,0003^{a}	0,9918 ± 0,0062^{a}	0,0620 ± 0,0003^{a}
VQ-7	266,44 ± 2,98^{b}	0,9465 ± 0,0004^{b}	0,5440 ± 0,0075^{b}	0,0290 ± 0,0004^{b}

Source: Authors

VQ-1 = Wild seed; VQ-7 = Cultivated in experimental field. The results are expressed as the mean value ± standard deviation. Similar letters in each variable indicate that there is no statistical difference between seed types (95 % confidence).

Kinematic viscosity

Although there were no differences between wild and cultivated seeds, the observed values (close to 270 mm^2/s) show the high viscosity of castor oil, which is mainly due to the presence of ricinoleic acid, whose structure includes a hydroxyl group, which confers this high viscosity (Benavides *et al.*, 2007). The kinematic viscosity values of castor oil are much higher than those of other vegetable oils, such as sunflower, rapeseed, soybean and jatropha, whose values are only around 30 mm^2/s (Rodríguez-Martínez *et al.*, 2012). Its high viscosity confers to castor oil advantages for the industrial production of coatings, plastics and cosmetics (da Costa Barbosa *et al.*, 2010); however, it represents a limitation for the production of biodiesel, being necessary to mix it with other sub-stances to reduce this index and ensure an efficient engine operation (Benavides *et al.*, 2007), in addition to the need of complying with the quality standards for this use (Berman *et al.*, 2011).

Density

The density of the oils from wild seeds and seeds cultivated in an experimental field did not present differences. The value observed for VQ-1 was 0,9389, while in VQ-7 it was 0,9465 g/cm^3 (Table 3). These values are interesting due to their use in the optimization of the transesterification process. On the other hand, Perdomo *et al.* (2013) reported that castor bean oil has the ability to be miscible in alcohol due to its high density.

Acidity index

The value obtained for the wild seed was 0,9918 mg KOH/g, whereas in the cultivar it only reached a value of 0,5440, representing a reduction of approximately 45 % in this index. Moretto & Fett (1998) argue that free acidity is not a seed constant value; instead, this variable is not much influenced by the genetic characteristics of the plant, but rather by the post-harvest seed management, including storage conditions, particularly high temperature and humidity, known as important causes of and increased oil acidity. On the other hand, it has been observed in olive that a low yield of fruits is associated with a high acidity index of its oil, with a tendency to present low oil acidity in trees with a high fruit yield (Bustan *et al.*, 2014).

Conclusions

There were differences in physicochemical characteristics of seeds from wild and cultivated castor bean plants. Therefore, we can conclude that physical characteristics, chemical composition, and oil content of *R. communis* seeds depend on environmental growing conditions and crop management. This information can be useful for the study of castor bean plants for agro-industrial purposes.

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II.3 Relación entre la composición química de la semilla y la calidad de aceite de doce accesiones de *Ricinus communis* L.

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Relación entre la composición química de la semilla y la calidad de aceite de doce accesiones de *Ricinus communis* L.*

Relation between the chemical composition of the seed and oil quality of twelve accessions of *Ricinus communis* L.

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Resumen

La higuerilla (*Ricinus communis* L.) es una planta que ha generado gran interés a nivel mundial debido al contenido de aceite extraído de la semilla, éste puede ser utilizado en la producción de biocombustibles, productos farmacéuticos y cosmetológicos, entre otros. No obstante, en México pocos estudios describen las características químicas de la semilla y su aceite. El objetivo de este trabajo, fue determinar la composición químico proximal y la calidad del aceite de doce accesiones de *R. communis* provenientes de los estados de Aguascalientes, Jalisco, San Luis Potosí y Zacatecas, colectados en el año 2013. Se realizó análisis químico proximal siguiendo las técnicas recomendadas por la AOAC (2002) y se determinó la calidad del aceite mediante parámetros como: viscosidad, densidad, índice de acidez (IA) y porcentaje de ácidos grasos libres (%AGL). Los datos obtenidos evidenciaron que existe diferencia significativa ($p< 0.05$), en la composición química y calidad de aceite, excepto densidad para las accesiones evaluadas. SLPS11C1 presentó mayor contenido de aceite (51.04 ±0.44%) y proteína (16.02 ±0.36%), mientras JAL3C1

Abstract

Castorbean (*Ricinus communis* L.) is a plant that has generated great interest worldwide due to the oil content extracted from the seed, it can be used in biofuel production, pharmaceutical and cosmetic products, among others. However, in México few studies describe the chemical characteristics of the seed and its oil. The aim of this paper was to determine the proximal chemical composition and oil quality of twelve accessions of *R. communis* from the states of Aguascalientes, Jalisco, San Luis Potosí and Zacatecas, collected in 2013. A proximal chemical analysis was performed following the techniques recommended by the AOAC (2002) and the quality of the oil was determined by parameters such as viscosity, density, acidity index (IA) and percentage of free fatty acids (%AGL). The data obtained showed that there is significant difference ($p<$ 0.05), in the chemical composition and oil quality, except for the accessions density. SLPS11C1 showed higher oil content (51.04 ±0.44%) and protein (16.02 ±0.36%), while JAL3C1 had a higher crude fiber average (21.15 ±0.16%). AGSS2C1 showed higher viscosity (265.84 ±2.54 $mm^2 s^{-1}$)

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posee promedio mayor de fibra cruda (21.15 ±0.16%). AGSS2C1 reportó mayor viscosidad (265.84 ±2.54 mm^2 s^{-1}) y SLPS11C1 un menor IA (0.5415 ±0.0168 mgKOH g^{-1}) y (%) AGL (0.0272 ±0.0008%). Los resultados sugieren que SLPS11C1 y AGSS2C1 son accesiones útiles para la producción agroindustrial. Sin embargo, se debe tener en cuenta que factores fenológicos pueden afectar la composición química de la semilla y la calidad del aceite de manera independiente al lugar de procedencia.

Palabras clave: *Ricinus communis* L., accesiones, análisis químico proximal, calidad del aceite, higuerilla.

Introducción

La 'higuerilla' (*Ricinus communis* L.), pertenece al orden de las *Euphorbiales* y a la familia de la *Euphorbiaceae* (Cronquist, 1981), esta especie oleaginosa se encuentra ampliamente distribuida en México, y posee alto potencial de producción de semilla para la obtención de aceite (Martínez *et al*., 2012; Solís-Bonilla *et al*., 2016). A nivel mundial se conoce comúnmente como 'higuera del infierno', 'tártago', 'higuereta', 'ricino', 'palma de cristo', 'mamoneira', 'mamona', 'castor bean' y 'castor oil plant', entre otros (Falasca *et al*., 2012), es un arbusto cuyo centro de origen es controvertido, aunque se especula que puede ser nativo tanto de Asia como de América, oficialmente se reconoce que proviene de África. Su amplia diversidad genética se traduce en distintas características como altura de planta, color del fruto, tallo y hojas, ausencia o presencia de espinas y dehiscencia en los frutos, así también en tamaño y composición química de la semilla, entre otras características que pueden variar en función del cultivar y de las condiciones agroecológicas donde se encuentren las plantas.

La importancia económica de esta oleaginosa radica en el aceite que contiene su semilla, el cual es empleado como materia prima en diversidad de productos, tales como: pinturas, tintas, lubricantes, poliuretanos, nylon y fluidos funcionales, entre otros (Mutlu y Meier, 2010). Los principales países productores son: India (1.7 millones t), China (40 mil t), Mozambique (69 mil t), Etiopía (11 mil t) y Brasil (37 mil t), aproximadamente (FAO, 2014). Es así y considerando las actuales condiciones de presión sobre la explotación y uso de los hidrocarburos, los aceites vegetales son considerados una fuente alternativa renovable, para

and SLPS11C1 a lower IA (0.5415 ±0.0168 mgKOH g^{-1}) and (%) AGL (0.0272 ±0.0008%). The results suggest that SLPS11C1 and AGSS2C1 are useful accessions for agroindustrial production. However, it must be taken into account that phenological factors may affect the chemical composition of the seed and the quality of the oil independently to the place of origin.

Keywords: *Ricinus communis* L., accessions, castorbean, proximal chemical analysis, oil quality.

Introduction

The 'castorbean' (*Ricinus communis* L.) belongs to the order of *Euphorbiales* and to the *Euphorbiaceae* family (Cronquist, 1981) this oleaginous species is widely distributed in México, and has high potential for seeds production for obtaining oil (Martínez *et al*., 2012; Solís-Bonilla *et al*., 2016). Globally it is commonly known as higuera del infierno, 'tártago', 'higuereta', 'ricino', 'palma de cristo', 'mamoneira', 'mamona', 'castor bean' and 'castor oil plant' among others (Falasca *et al*., 2012), it is a shrub whose origin is controversial, although it is speculated that it may be native to both Asia and America, it is officially recognized that it comes from Africa. Its wide genetic diversity translates into different characteristics such as plant height, fruit color, stem and leaves, absence or presence of spines and dehiscence in the fruits, as well as in chemical size and composition of the seed, among other characteristics that may vary depending on the cultivar and the agroecological conditions where the plants are found.

The economic importance of this oilseed lies in the oil contained in its seed, which is used as raw material in diverse products, such as: paints, inks, lubricants, polyurethanes, nylon and functional fluids, among others (Mutlu and Meier, 2010). The main producing countries are India (1.7 million t), China (40 thousand t), Mozambique (69 thousand t), Ethiopia (11 thousand t) and Brazil (37 thousand t), approximately (FAO, 2014). Thus, and considering the actual pressure on the operation and use of hydrocarbons, vegetable oils are considered a renewable alternative source, for the obtaining of compounds able to replace those from fossil origin (Conceição *et al*., 2007). This is tge reason why castor oil has recently aroused great interest as raw material in the production of biodiesel (Berman *et al*., 2011).

obtener compuestos capaces de sustituir a los provenientes de origen fósil (Conceição *et al*., 2007). Es por ello, que el aceite de ricino recientemente ha despertado gran interés como materia prima en la producción de biodiesel (Berman *et al.,* 2011).

Además del contenido de aceite, la semilla de higuerilla contiene compuestos nutricionales como proteínas, carbohidratos y minerales diversos; así como compuestos tóxicos y alergénicos, los cuales limitan el consumo tanto humano como animal (Audi *et al*., 2005), estos fitoquímicos presentes en el tejido de la planta y las semillas de higuerilla tienen posibles usos medicinales (Morris, 2004), es así como esta actividad citotóxica se utiliza en terapias experimentales y ensayos clínicos capaces de dirigir estos anticuerpos a células cancerígenas sin dañar las células normales (Olsnes *et al*., 1981; Lam *et al*., 2004). A pesar de los múltiples usos industriales que se le ha conferido a la higuerilla, en México se localiza distribuida de manera silvestre, es común considerla como una maleza en áreas urbanas y agrícolas, entre tanto que la producción comercial de esta especie se encuentra en fase inicial en estados como Chiapas, Guanajuato, Querétaro, Sonora y Yucatán, entre otros; mientras en el estado de Oaxaca se siembra desde hace varias décadas.

Particularmente, esta investigación se desarrolla en el marco de un proyecto integral de mejoramiento genético y agronómico de higuerilla, requiriéndose generar información de los materiales genéticos silvestres existentes en el país, para seleccionar y propagar accesiones que tengan potencial como materia prima de uso agroindustrial. Considerando lo anterior, el objetivo de este trabajo fue evaluar doce accesiones de *R. communis*, provenientes de colectas realizadas en los estados de Aguascalientes, Jalisco, San Luis Potosí y Zacatecas, México con base en su composición química de semilla y calidad de aceite.

Materiales y métodos

Material biológico

Las muestras utilizadas pertenecen al banco de germoplasma del Colegio de Postgraduados, *Campus* San Luis Potosí, México (Cuadro 1). Durante el año 2013, se colectaron semillas completamente al azar de doce accesiones de

In addition to the oil content, the castorbean seed contains nutritional compounds like proteins, carbohydrates and diverse minerals; as well as toxic and allergenic compounds, which restrict both human and animal consumption (Audi *et al*., 2005), these phytochemicals present in the plant tissue and the seeds of castorbean have possible medicinal uses (Morris, 2004), this is how this cytotoxic activity is used in experimental therapies and clinical trials capable of directing these antibodies to cancerous cells without harming normal cells (Olsnes *et al*., 1981; Lam *et al*., 2004). In spite of the multiple industrial uses that have been conferred to the castorbean, in México it is distributed wildly, it is common to consider it as a weed in urban and agricultural areas, while the commercial production of this species is in the initial phase in states like Chiapas, Guanajuato, Querétaro, Sonora and Yucatán, among others; while in the state of Oaxaca it has been planted for several decades now.

In particular, this research was carried out within the framework of a comprehensive genetic and agronomic project of castorbean, requiring the generation of information on the wild plants genetic material in the country, in order to select and propagate accessions that have potential as a raw material for agroindustrial use. Considering the above, the aim of this study was to evaluate twelve accessions of *R. communis*, from collections in the states of Aguascalientes, Jalisco, San Luis Potosí and Zacatecas, México based on the seed chemical composition and oil quality.

Materials and methods

Biological material

The samples used belong to the genebank of the Colegio de Postgraduados, San Luis Potosí *Campus*, México (Table 1). During the 2013 year, seeds of twelve castorbean accessions were completely random collected in the states of San Luis Potosí, Aguascalientes, Zacatecas and Jalisco, México; the castorbean plants were in zones between 1 400 and 2 400 masl. The twelve accessions were collected following transects in the different regions of influence, from plants without presence of pests and diseases, vigorous, with seed supply and agronomic production characteristics. Geo positioning data, characteristics of the plant and agri-environmental aspects as well as the description and identification of the collected materials were recorded (Isaza *et al*., 2017).

higuerilla en los estados de San Luis Potosí, Aguascalientes, Zacatecas y Jalisco, México; las plantas de higuerilla se encontraban en zonas entre 1 400 y 2 400 msnm. Las doce accesiones fueron colectadas siguiendo transectos en las diferentes regiones de influencia, a partir de plantas sin presencia de plagas y enfermedades, vigorosas, con dotación de semilla y características de producción agronómica. Se registraron datos de geo posicionamiento, características de la planta y aspectos agroambientales, así como la descripción e identificación de los materiales colectados (Isaza *et al*., 2017*)*.

Análisis químico proximal de las accesiones de *R. communis* L.

Para el análisis químico proximal se utilizaron semillas de higuerilla con cáscara. Las determinaciones fueron realizadas mediante técnicas recomendadas por la AOAC (2002). El contenido de proteína (954.01) se determinó en un sistema Kjeldahl (Labconco, USA) utilizando un factor de conversión a proteína de 6.25. El contenido de fibra cruda (962.09) fue obtenido por digestión con ácido sulfúrico e hidróxido de sodio en un equipo digestor (Ankom, USA). La humedad se determinó por método gravimétrico (7.003), utilizando horno de secado (Felisa, México). Para el contenido de cenizas se empleó la calcinación (923.03) utilizando una mufla de terrígeno (Novatech, México). El contenido de aceite se determinó de acuerdo a la metodología descrita por Loredo *et al*. (2012), en un sistema de extracción de aceite (Soxtec, USA). Finalmente, los carbohidratos fueron calculados por diferencia de los demás componentes ya determinados (Bello-Pérez *et al.*, 2001).

Extracción y evaluación de calidad del aceite de *R. communis* L.

El aceite fue extraído por método mecánico a través de un prototipo en acero inoxidable con capacidad máxima de 400 g de semilla, provisto de un cilindro para contener la semilla y un émbolo para ejercer presión acoplados a una prensa hidráulica (Mikel, México) con capacidad máxima de presión de 700 kg F cm^{-2}. Para evaluar la calidad del aceite obtenido, se determinaron parámetros como: viscosidad, densidad, índice acidez (IA) y porcentaje de ácidos grasos libres (% AGL). Para la determinación de viscosidad y densidad se utilizó un equipo Stabinger VM 3000 (Anton Paar, Austria), con la metodología reportada en la norma ASTM D 445. La densidad fue calculada como la relación entre la masa y el volumen (g

Cuadro 1. Procedencia de las accesiones de *R. communis* L.
Table 1. Origin of the *R. communis* L. accessions.

Núm.	Accesión	Origen	Altitud (m)
1	SLPS11C1	Salinas, San Luis Potosí	2 083
2	SLPS6C1	Capulines, San Luis Potosí	1 955
3	SLPS3C1	Moras Mexquitic, San Luis Potosí	2 003
4	AGSS3C1	Tepezalá, Aguascalientes	1 936
5	AGSS2C1	Calvillo, Aguascalientes	2 095
6	AGSS4C1	San J. Gracia, Aguascalientes	2 004
7	ZACS3C1	N. San Marcos, Zacatecas	2 040
8	ZACS2C1	El Orito, Zacatecas	2 399
9	ZACS1C1	Luis Moya, Zacatecas	1 971
10	JALS2C1	Encarnación Díaz, Jalisco	1 811
11	JALS1C1	Villa Hidalgo, Jalisco	1 920
12	JAL3C1	Tecuán, Jalisco	1 450

Proximal chemical analysis of *R. communis* L. accessions

For the proximal chemical analysis, castorbean seeds with shell were used. The determinations were performed using techniques recommended by AOAC (2002). The protein content (954.01) was determined in a Kjeldahl system (Labconco, USA) using a protein conversion factor of 6.25. The crude fiber content (962.09) was obtained by digestion with sulfuric acid and sodium hydroxide in a digester equipment (Ankom, USA). Moisture was determined by gravimetric method (7.003), using a drying oven (Felisa, México). For the ash content, calcination (923.03) was used using a terrgen muffle (Novatech, Mexico). The oil content was determined according to the methodology described by Loredo *et al.* (2012) in an oil extraction system (Soxtec, USA). Finally, carbohydrates were calculated by difference of other components already determined (Bello-Pérez *et al*., 2001).

Extraction and quality assessment of *R. communis* L. oil

The oil was extracted by mechanical method through a prototype of stainless steel with a maximum capacity of 400 g of seed, equipped with a cylinder to contain the seed and a pressure piston coupled to a hydraulic press (Mikel, México) with a maximum pressure capacity of 700 kg F cm^{-2}. For the evaluation of the oil quality, parameters such as viscosity, density, acidity index (IA) and percentage of free fatty acids (%AGL) were determined. For the determination of viscosity and density, a Stabinger VM 3000 equipment (Anton Paar, cm^{-3}) a las mismas condiciones de presión y temperatura. El porcentaje de ácidos grasos libres (AGL) e índice de acidez (IA) fueron determinados de acuerdo a la ISO660:1983 y a la metodología descrita por Firestone (1996), utilizando titulación con hidróxido de potasio 0.1N y fenolftaleína como indicador. El IA fue expresado como la cantidad en miligramos de KOH, que se requiere para neutralizar los ácidos grasos libres en un gramo de aceite (mg KOH g^{-1}).

Análisis estadístico

Los resultados fueron expresados como la media de tres experimentos independientes ± desviación estándar (DE). Diferencias entre medias se analizaron mediante la prueba de Tukey ($p < 0.05$). Se realizó un análisis de componentes principales (ACP) por el método de Ward y se graficó en un dendrograma. Los análisis estadísticos se efectuaron con los programas Minitab 17® y R®.

Resultados y discusión

Análisis químico proximal

Los resultados obtenidos para la composición químico proximal de las accesiones son presentados en el Cuadro 2. El contenido para cada uno de los parámetros evaluados en las semillas de higuerilla presentaron diferencia estadísticamente significativa ($p < 0.05$). Dichas diferencias se pueden atribuir a distintos factores entre los que se destacan la variabilidad genética, las condiciones del sitio de colecta, la estacionalidad y otros factores ecológicos y de crecimiento (Hidalgo *et al*., 2009). Debido a estas diferencias en todas las variables de respuesta, es aliciente la implementación de estrategias multivariadas.

Austria) was used, following the methodology reported in the ASTM D445 standard. The density was calculated as the ratio between the mass and volume (g cm^{-3}) at the same pressure and temperature conditions. The percentage of free fatty acids (AGL) and acidity index (IA) were determined according to ISO660: 1983 and to the methodology described by Firestone (1996), using titration with 0.1N potassium hydroxide and phenolphthalein as indicator. IA was expressed as the amount of KOH in milligrams, which is required to neutralize the free fatty acids in one gram of oil (mg KOH g^{-1}).

Statistic analysis

The results were expressed as the mean of three independent experiments ±standard deviation (SD). Differences between means were analyzed using the Tukey test ($p < 0.05$). A principal component analysis (ACP) was performed by the Ward method and plotted on a dendrogram. Statistical analyzes were performed using the Minitab 17® and R® programs.

Results and discussion

Proximal chemical analysis

The results obtained for the proximal chemical composition of the accessions are shown in Table 2. The content for each of the parameters evaluated in the castorbean seeds showed statistically significant difference ($p < 0.05$). These differences can be attributed to several factors among which the genetic variability, the conditions of the collection site, seasonality and other environmental and growth factors stands out (Hidalgo *et al*., 2009). Due to these differences in all response variables, it is encouraging to implement multivariate strategies.

Cuadro 2. Análisis químico proximal de las accesiones de *R. communis* L.
Table 2. Proximal chemical analysis of *R. communis* L accessions.

Accesión	Aceite (%)	Proteína[*] (%)	Fibra cruda (%)	Cenizas (%)	Humedad (%)	CHO (%)
SLPS11C1	51.04 ±0.44 ^{a}	16.02 ±0.36 ^{a}	17.88 ±0.03 ^{a}	2.96 ±0.05 ^{c}	5.13 ±0.23 ^{bcde}	12.1 ±0.55 ^{c}
SLPS6C1	43.66 ±2.15 ^{bc}	13.11 ±0.15 ^{e}	12.68 ±0.01 ^{h}	3.41 ±0.09 ^{a}	5.38 ±0.1 ^{abcde}	27.14 ±1.99 ^{a}
SLPS3C1	41.52 ±2.06 ^{c}	12.8 ±0.13 ^{ef}	12.92 ±0.18 ^{h}	3.13 ±0.1 ^{abc}	4.87 ±0.16 ^{ef}	29.63 ±1.97 ^{a}
AGSS3C1	42.1 ± .56 ^{c}	12.61 ±0.43 ^{f}	14.02 ±0.3 ^{g}	3.08 ±0.1 ^{bc}	5.55 ±0.15 ^{abc}	28.18 ±2.17 ^{a}

Los resultados son expresados como la media de tres réplicas ± desviación estándar (expresados en base seca). Diferentes letras en la misma columna expresan diferencias significativas ($p < 0.05$) en la prueba de Tukey. [*]= factor de conversión: 6.25; CHO= carbohidratos obtenido por diferencia.

Cuadro 2. Análisis químico proximal de las accesiones de *R. communis* L. (continuación).
Table 2. Proximal chemical analysis of *R. communis* L. accessions (continuation).

Accesión	Aceite (%)	Proteína* (%)	Fibra cruda (%)	Cenizas (%)	Humedad (%)	CHO (%)
AGSS2C1	48.67 ±2.44 ^{ab}	14.81 ±0.09 ^{cd}	16.95 ±0.24 ^{cd}	2.24 ±0.11 ^{e}	5.75 ±0.27 ^{a}	17.33 ±2.46 ^{bc}
AGSS4C1	42.5 ±0.89 ^{c}	14.75 ±0.12 ^{cd}	14.62 ±0.29 ^{f}	2.89 ±0.04 ^{cd}	5.64 ±0.26 ^{ab}	25.24 ±0.64 ^{a}
ZACS3C1	50.52 ±1.43 ^{a}	15.05 ±0.26 ^{bc}	17.08 ±0.32 ^{c}	3.36 ±0.16 ^{ab}	5.74 ±0.25 ^{a}	13.99 ±1.53 ^{bc}
ZACS2C1	48.84 ±2.62 ^{ab}	15.39 ±0.26 ^{b}	16.52 ±0.21^{de}	2.98 ±0.09 ^{c}	4.51 ±0.17 ^{f}	16.27 ±2.53 ^{bc}
ZACS1C1	46.8 ±2.31 ^{abc}	14.4 ±0.2 ^{d}	16.9 ±0.07 ^{cd}	3.11 ±0.02 ^{bc}	5.01 ±0.15 ^{cdef}	18.79 ±2.51 ^{b}
JALS2C1	50.14 ±1.01 ^{a}	15.16 ±0.02 ^{bc}	16.67 ±0.16 ^{cde}	2.6 ±0.13 ^{d}	4.49 ±0.19 ^{f}	15.43 ±1.15 ^{bc}
JALS1C1	48.31 ±2.37 ^{ab}	15.16 ±0.07 ^{bc}	16.35 ±0.03 ^{e}	3.32 ±0.13 ^{ab}	4.96 ±0.12 ^{def}	16.87 ±2.28 ^{bc}
JAL3C1	46.78 ±1.18 ^{abc}	14.79 ±0.35 ^{cd}	21.15 ±0.16 ^{a}	3 ±0.11 ^{c}	5.54 ± 0.24 ^{abcd}	14.29 ±1.53 ^{bc}

Los resultados son expresados como la media de tres réplicas ± desviación estándar (expresados en base seca). Diferentes letras en la misma columna expresan diferencias significativas ($p < 0.05$) en la prueba de Tukey. *= factor de conversión: 6.25; CHO= carbohidratos obtenido por diferencia.

Aceite

La mayoría de las accesiones de higuerilla presentaron un contenido de aceite promedio de 50%; sin embargo, accesiones como SLPS3C1, AGSS3C1 y AGSS4C1 evidenciaron una disminución hasta de 8%. Contenidos similares, fueron reportados por Armendáriz *et al.* (2015), quienes registraron valores entre 42% y 50.5% para semillas de higuerilla colectadas en varios estados de México. Por su parte, Goytia-Jiménez *et al.* (2011) registraron valores entre 12.2% y 64.84% en 151 accesiones colectadas en el estado de Chiapas. Autores como Bello-Pérez *et al.* (2001), obtuvieron valores similares a los registrados en este trabajo en semillas oleaginosas como cacahuate (*Arachis hypogaeae*) y girasol (*Helianthus annuus*) (47% y 51%, respectivamente). Asimismo, Martín *et al.* (2010) evidenciaron contenidos semejantes en semillas con potencial bioenergético como piñón mexicano (*Jatropha curcas*) (49.1%), nim (*Azadirachta indica*) (39.7%), moringa (*Moringa oleifera*) (38.1%), trisperma (*Aleurites trisperma*) (62%) y nuez de la India (*Aleurites moluccana*) (56.3%). Por consiguiente, se confirma que la semilla de higuerilla constituye una materia prima para la producción de aceite, que puede ser utilizado en la industria farmacéutica, cosmetológica, de lubricantes y para la producción de bioenergía (Mosquera *et al*., 2016)

Proteína

La accesión SLPS11C1 presentó el mayor contenido de proteína (16.02 ±0.36%), éste resultado se encuentra acorde con Reveles *et al.* (2010), quienes reportaron valores de 16.7% y 18.6% en semillas silvestres de higuerilla provenientes

Oil

Most of the accessions of castorbean had an average oil content of 50%, however accessions such as SLPS3C1, AGSS3C1 and AGSS4C1 showed a decrease up to 8%. Similar contents were reported by Armendáriz *et al.* (2015), who registered values between 42% and 50.5% for castorbean seeds collected in several Mexican states. Meanwhile, Goytia-Jiménez *et al.* (2011) recorded values between 12.2% and 64.84% in 151 accessions collected in the state of Chiapas. Authors such as Bello-Pérez *et al.* (2001), obtained similar values to those reported in this paper in oilseeds such as peanut (*Arachis hypogaeae*) and sunflower (*Helianthus annuus*) (47% and 51%, respectively) values. Also, Martín *et al.* (2010) showed similar content in seeds with bioenergy potential such as Mexican pinion (*Jatropha curcas*) (49.1%), neem (*Azadirachta indica*) (39.7%), moringa (*Moringa oleifera*) (38.1%), trisperma (*Aleurites trisperma*) (62%) and cashews (*Aleurites moluccana*) (56.3%). Accordingly, it is confirmed that castorbean seed is a raw material for the production of oil which can be used in the pharmaceutical, cosmetological, lubricants industries and bioenergy production (Mosquera *et al*., 2016)

Protein

The SLPS11C1 accession had the highest protein content (16.02 ±0.36%), this result is consistent with Reveles *et al.* (2010), who reported values of 16.7% and 18.6% in wild castorbean seeds Durango-México. Onyeike and Acheru (2002), who describe average contents of 14.4%

del estado de Durango-México. Onyeike y Acheru (2002), quienes describen contenidos promedio de 14.4% en semillas de higuerilla de origen Nigeriano. Por su parte, contenidos mayores fueron reportados por Perea *et al.* (2011) quienes encontraron hasta 28.48% en semillas de higuerilla variedad Tiripiteo. No obstante, diferencias en el contenido de proteína pueden depender de la composición genética y de las condiciones ambientales de la planta (Ortega y Rodríguez, 1979). Por otra parte, el contenido de proteína obtenido en las accesiones evaluadas podría ser potencialmente utilizado como un complemento en la dieta de ganado vacuno, ovino, caprino y peces, entre otros. Sin embargo, se debe tener en cuenta que la presencia de sustancias tóxicas (alcaloides, alérgenos, entre otros) es una limitante para el uso directo de estas semillas en la alimentación animal o humana, debido a lo cual se requiere de una detoxificación previa.

Fibra cruda

Los valores promedio de fibra cruda, oscilaron entre 12.62% y 21.15%, siendo menor para la accesión SLPS6C1 y mayor para JAL3C1. Makkar, (1998) reportó valores semejantes de fibra cruda en semillas de piñón mexicano (14.6% y 16.4%). De igual forma, datos similares son registrados en algunos tipos de forrajes para animales, tales como harina de soya (12%) y harina de girasol (19%), aunque menores al de heno de alfalfa (45%) (Van-Soest *et al*., 1991). Con base en los datos obtenidos, se recomienda la utilización de la harina detoxificada de higuerilla, en animales rumiantes, pues la ingesta de alimentos con alto contenido de fibra en animales mono gástricos puede ser causante de problemas de digestión.

Cenizas

El contenido de cenizas obtenido para las doce accesiones de higuerilla se encuentra en un intervalo entre 2.24% y 3.41%, siendo mayor para la accesion SLPS6C1 y menor para AGSS2C1. Lucena *et al.* (2010) reportaron valores entre 3.01% y 6.95% en una variedad de *R. communis* brasileña, a diferentes estados de madurez. La cantidad de cenizas presente en las accesiones de higuerilla evidencia el contenido total de minerales, permitiendo conocer de esta forma la presencia de elementos inorgánicos para generar coproductos que generen valor agregado a esta materia prima. Por su parte, Severino *et al.* (2004) destacan el contenido de minerales presentes en las semillas de higuerilla como un excelente acondicionador de suelo.

in Nigerian castorbean seeds. Meanwhile, higher contents were reported by Perea *et al.* (2011) who found up to 28.48% in castorbean seeds of the Tiripiteo variety. However, differences in protein content may depend on the genetic makeup and environmental conditions of the plant (Ortega and Rodríguez, 1979). On the other hand, the protein content obtained in the evaluated accessions could potentially be used as a complement in the diet of cattle, sheep, goats and fish, among others. However, it must be taken into account that the presence of toxic substances (alkaloids, allergens, among others) is a limitation for the direct use of these seeds in animal or human food, which would require a prior detoxification.

Raw fiber

The average crude fiber values ranged from 12.62% to 21.15%, being lower for the SLPS6C1 accession and higher for JAL3C1. Makkar, (1998) reported similar values of crude fiber in Mexican pinion seeds (14.6% and 16.4%). Likewise, similar data are recorded in some fodder types for animals, such as soybean meal (12%) and sunflower meal (19%), although lower to alfalfa hay (45%) (Van Soest *et al*., 1991). Based on the data obtained, it is recommended the use of detoxified castorbean meal, in ruminant animals, since the intake of food with high fiber content in mono-gastric animals can be a cause of digestion problems.

Ashes

The ash content obtained for the twelve accessions of castorbean ranged between 2.24% and 3.41%, being higher for the SLPS6C1 accession and lower for AGSS2C1. Lucena *et al.* (2010) reported values between 3.01% and 6.95% in a variety of *R. communis* brazilian at different maturity stages. The amount of ash in the castorbean accessions shows the total content of minerals, allowing to know in this way the presence of inorganic elements to generate co-products that could generate added value to this raw material. Meanwhile, Severino *et al.* (2004) emphasize the mineral content in the seeds of castorbean as an excellent conditioner for the soil.

Humidity

Moisture contents between 4.49% and 5.75% were obtained in the different accessions of castorbean. Perdomo *et al.* (2013); Perea *et al.* (2011) reported values of 3.89%

Humedad

Contenidos de humedad entre 4.49% y 5.75% fueron obtenidos en las diferentes accesiones de higuerilla. Perdomo *et al*. (2013); Perea *et al.* (2011) reportaron valores de 3.89% y 5.64% respectivamente, en colectas de origen mexicano. Variaciones en el contenido de humedad podrían obedecer a condiciones climáticas, desarrollo del cultivo y el tiempo de cosecha de frutos. Sin embargo, los resultados obtenidos para las semillas de higuerilla muestran bajo contenido de humedad (< 6%), característica que las hace menos susceptibles a procesos de deterioro por acciones de microorganismos, pudiendo ser almacenadas y conservadas durante un tiempo determinado sin afectar su viabilidad (Souza *et al*., 2016).

Carbohidratos

Cuatro accesiones (SLPS6C1, SLPS3C1, AGSS3C1, AGSS4C1) de doce evaluadas presentaron valores superiores al 20%. Annongu y Joseph (2008) encontraron contenidos del 24.88% para carbohidratos en semillas de higuerilla previamente desgrasadas. De acuerdo con los resultados, las semillas de higuerilla pueden ser una buena fuente de energía y complemento para la nutrición de animales, considerando, su previa detoxificación, como ya ha sido mencionado anteriormente. De comprobarse estos contenidos de carbohidratos en las semillas estudiadas, se podría utilizar este subproducto (pasta resultante de la extracción del aceite) en la producción de etanol (Melo *et al*., 2008).

Calidad de aceite

Los resultados obtenidos para la evaluación de calidad de aceite de doce accesiones de higuerilla se presentan en el Cuadro 3.

Viscosidad

Se determinaron valores de viscosidad en el aceite de *R. communis* desde 250.04 a 265.84 mm² s^{-1}. Estos resultados se encuentran dentro de lo reportado por autores como Costa y Rossi (2000); Scholz y Silva (2008) quienes reportan valores de 248.8 mm² s^{-1} y 296.87 mm² s^{-1} para aceites obtenidos de semilla de higuerilla, respectivamente. Se ha reportado que la presencia de un grupo hidroxilo (OH) en el carbono 12, sería el responsable de la alta viscosidad del aceite de ricino (Scholz y Silva, 2008), éstas and 5.64%, respectively, in collections of Mexican origin. Variations in the moisture content could be due to the climatic conditions and development of the crop, as well as the harvest time of the fruits. Nevertheless, the results obtained for the castorbean seeds show a low content of humidity (<6%), characteristic that makes them less susceptible to deterioration processes by microorganisms actions, being able to be stored and conserved during a determined time without affecting its viability (Souza *et al*., 2016).

Carbohydrates

Four accessions (SLPS6C1, SLPS3C1, AGSS3C1, AGSS4C1) of the twelve evaluated showed values higher than 20%. Annongu and Joseph (2008) found contents of 24.88% for carbohydrates in previously defatted castorbean seeds. According to the results, the castorbean seeds can be a good source of energy and complement to animals nutrition, considering, of course, its previous detoxification, as already mentioned above. If these carbohydrate contents are checked in studied seeds, this by-product could be used (paste resulting from the oil extractio) in ethanol production (Melo *et al.,* 2008).

Oil quality

The results obtained for the evaluation of oil quality of twelve accessions of castorbean are shown in Table 3.

Viscosity

Viscosity values were determined in *R. communis* oil from 250.04 to 265.84 mm² s^{-1}. These results are within the reported by authors like Costa and Rossi (2000); Scholz and Silva (2008), who report values of 248.8 mm² s^{-1} and 296.87 mm² s^{-1} for oils obtained from castorbean seed, respectively. It has been reported that the presence of a hydroxyl (OH) group in carbon 12 would be responsible for the high viscosity of castor oil (Scholz and Silva, 2008), these characteristics confer extra stability to oil and its derivatives to prevent the formation of hydroperoxides (Ogunniyi, 2006). A high viscosity and lubricity over a wide range of temperatures and insolubility in fuels and aliphatic petrochemicals make it directly applicable as a lubricant for equipment operating under extreme conditions, as well as in the use in paints and varnishes, among others (Mutlu and Meier, 2010).

características le confieren estabilidad extra al aceite y sus derivados para prevenir la formación de hidroperóxidos (Ogunniyi, 2006). Una alta viscosidad y lubricidad en un amplio rango de temperaturas y la insolubilidad en combustibles y solventes petroquímicos alifáticos, lo hacen directamente aplicable como lubricante para equipos que operan en condiciones extremas, así como en la utilización en pinturas, barnices, entre otras (Mutlu y Meier, 2010).

Density

The average density obtained for oils the extracted of the twelve accessions was 0.9463 g cm^{-3} (Table 3). Similar results are reported by Perdomo *et al.* (2013) who found average values of 0.9418 g cm^{-3} in oils from seeds of Mexican origin. While, Conceição *et al.* (2007) reported values of 0.9573 g cm^{-3} in seeds from Brazil. Differences in the determination of this parameter can be due to small traces of water or impurities in the oils, thus affecting the density of the substance.

Cuadro 3. Calidad de Aceite de las doce accesiones colectadas de *R. communis* L.
Table 3. Oil quality of the twelve accessions collected from R. *communis* L.

Accesión	Viscosidad (mm² s^{-1})	Densidad (g cm^{-3})	Índice de acidez (mg KOH g^{-1})	Ácidos grasos libres* (%)
SLPS11C1	264.97 ±0.87 ^{a}	0.9459 ±0.0012 ^{a}	0.5415 ±0.0168 ^{d}	0.272 ±0.0008 ^{e}
SLPS6C1	262.42 ±0.95 ^{b}	0.9463 ±0.0006 ^{a}	1.1049 ±0.0185 ^{b}	0.555 ±0.0009 ^{c}
SLPS3C1	258.58 ±3.84 ^{d}	0.9449 ±0.0014 ^{a}	2.2178 ±0.0125 ^{b}	1.115 ±0.0006 ^{a}
AGSS3C1	263.37 ±2.84 ^{b}	0.9469 ±0.0003 ^{a}	1.1023 ±0.0208 ^{b}	0.554 ±0.001 ^{c}
AGSS2C1	265.84 ±2.54 ^{a}	0.9471 ±0.0005 ^{a}	0.8159 ±0.0033 ^{c}	0.41 ±0.0002 ^{d}
AGSS4C1	260.53 ±1.95 ^{c}	0.9466 ±0.0017 ^{a}	0.8175 ±0.0106 ^{c}	0.411 ±0.0005 ^{d}
ZACS3C1	263.3 ±1.67 ^{b}	0.9466 ±0.0007 ^{a}	1.8725 ±0.0537 ^{a}	0.941 ±0.0027 ^{b}
ZACS2C1	258.07 ±0.48 ^{d}	0.9466 ±0.0003^{a}	0.5451 ±0.0155 ^{d}	0.274 ±0.0008 ^{e}
ZACS1C1	254.96 ±1.58 ^{e}	0.946 ±0.0003 ^{a}	0.8063 ±0.0191 ^{c}	0.405 ±0.001 ^{d}
JALS2C1	250.04 ±3.34 ^{g}	0.946 ±0.0003 ^{a}	0.5513 ±0.0034 ^{d}	0.277 ±0.0002 ^{e}
JALS1C1	258.55 ±3.59 ^{d}	0.9467 ±0.0007 ^{a}	0.8228 ±0.0076^{c}	0.414 ±0.0004 ^{d}
JAL3C1	253.38 ±1.58 ^{f}	0.9463 ±0.0003 ^{a}	0.5435 ±0.0055 ^{d}	0.273 ±0.0003 ^{e}

Los resultados son expresados como la media de tres réplicas ± desviación estándar. Diferentes letras en la misma columna expresan diferencias significativas (p<0.05) en la prueba de Tukey. Viscosidad y densidad fueron evaluados a 40 °C.

Densidad

La densidad promedio obtenida para los aceites extraídos de las doce accesiones fue de 0.9463 g cm^{-3} (Cuadro 3). Resultados similares son reportados por Perdomo *et al.* (2013) quienes encontraron valores promedio de 0.9418 g cm^{-3} en aceites provenientes de semillas de origen mexicano. Mientras Conceição *et al.* (2007) reportaron valores de 0.9573 g cm^{-3} en semillas provenientes de Brasil. Diferencias en la determinación de este parámetro pueden obedecer a pequeñas trazas de agua o impurezas presentes en los aceites, afectando de esta forma la densidad de la sustancia.

Índice de acidez

Los valores para este índice de calidad oscilaron desde 0.5415 hasta 2.2178 mg KOH g^{-1}, Pradhan *et al.* (2012), caracterizaron aceite de higuerilla para producción

Acidity index

The values for this quality index ranged from 0.5415 to 2.2178 mg KOH g^{-1}, Pradhan *et al.* (2012) characterized castorbean oil for the production of biodiesel with a value of 0.91 mg KOH g^{-1}. On the other hand, Ogunniyi (2006) indicates that castor oil is a polyhydroxylated compound of natural origin, which has as limitation a slight reduction of the hydroxyl number and the acid value during storage. The reduction of these values can be caused by the reaction between the hydroxyl and carboxyl groups of the oil molecule leading to the formation of stolidity. The acidity index in crude oil is influenced by post-harvest and storage treatments such as temperature, humidity and others.

Percentage of free fatty acids

The results obtained for the percentage of free fatty acids showed significant difference (p< 0.05) with a range from 0.272% to 1.1156% for the twelve accessions tested. Lower de biodiesel con un valor de 0.91 mg KOH g^{-1}. Por su parte, Ogunniyi (2006) señala que el aceite de ricino es un compuesto polihidroxilado de origen natural, el cual presenta como limitante una ligera reducción del índice de hidroxilo y del índice de acidez durante el almacenamiento. La reducción de estos valores puede ser causada por la reacción entre los grupos hidroxilo y carboxilo de la molécula de aceite dando lugar a la formación de estolidez. El índice de acidez en el aceite crudo se ve influenciado por los tratamientos poscosecha y de almacenamiento como temperatura, humedad entre otras.

Porcentaje de ácidos grasos libres

Los resultados obtenidos para el porcentaje de ácidos grasos libres presentaron diferencia significativa ($p< 0.05$) con variaciones desde 0.272% a 1.1156% para las doce accesiones evaluadas. Valores menores de (%) AGL fueron encontrados en las accesiones SLPS11C1< ZACS2C1< JALS2C1. De acuerdo con los requerimientos del sector industrial, un porcentaje de ácidos grasos libres óptimo debe ser menor al 0.5%; sin embargo, para la obtención de biodiesel es permitido hasta 3%, utilizando catalizadores básicos homogéneos como hidróxido de sodio o potasio (Moser, 2009).

Análisis multivariado

El análisis de correlación de Pearson al 99% de confianza para las variables estudiadas (Figura 1), expresan valores puntuales de las diferentes relaciones entre las variables. El rango de variación de la correlación es de -1 a 1. Los resultados encontrados permitieron determinar que a mayor contenido de aceite, proteína y fibra cruda (67 - 86%) se tendrá menor contenido de cenizas, humedad y carbohidratos en semilla y viscosidad en aceite. Mientras que el índice de acidez y los ácidos grasos libres tienen una correlación de 100%.

Análisis de componentes principales

Por su parte, los resultados de los agrupamientos y relaciones entre las doce accesiones de higuerilla estudiadas se presentan con base al análisis de componentes principales de seis variables del análisis químico proximal y cuatro variables para la calidad del aceite. En el Cuadro 4, se concluye que con al menos cuatro componentes principales se explica 88.1% de la varianza total acumulada de los datos.

values of (%) AGL were found in the accessions SLPS11C1< ZACS2C1< JALS2C1. According to the requirements of the industrial sector, the percentage of free fatty acids should be less than 0.5%; however, for the obtaining of biodiesel, up to 3% is allowed, using homogeneous basic catalysts such as sodium or potassium hydroxide (Moser, 2009).

Multivariate analysis

The Pearson correlation analysis at 99% confidence for the variables studied (Figure 1), express point values of the different relations between variables. The variation range of the correlation goes from -1 to 1. The results found allowed to determine that a higher content of crude oil, protein and fiber (67-86%) will have lower content of ash, moisture and carbohydrates in the seed and viscosity in the oil. While the acidity index and free fatty acids have a correlation of 100%.

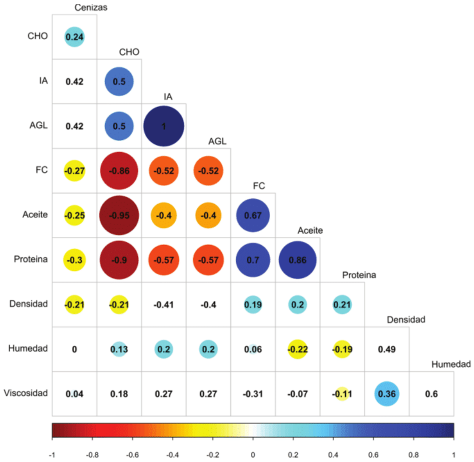

Figura 1. Coeficiente de correlación entre la composición química y la calidad de aceite de *R. communis.* Aceite (%); proteína (%); FC= fibra cruda (%); cenizas (%), humedad (%); CHO= carbohidratos (%); viscosidad ($mm^2 s^{-1}$); densidad ($g cm^{-3}$); IA= índice de acidez (mg KOH g^{-1}); AGL= ácidos grasos libres (%).

Figure 1. Correlation coefficient between the chemical composition and quality of oil *R. communis.* Oil (%); protein (%); FC= crude fiber (%); ash (%), moisture (%); CHO= carbohydrate (%); viscosity ($mm^2 s^{-1}$); density ($g cm^{-3}$); IA= acidity index (mg KOH g^{-1}); AGL= free fatty acids (%).

El componente principal 1 (CP1), permite explicar el 46.9% de la varianza global; con valor propio de 4.69, este componente principal presenta una mayor asociación con las siguientes cuatro variables: carbohidratos (41.8%), proteína (41.1%), lípidos (38.2%) y fibra cruda (38.2%), los cuales tienen alta participación en la definición del CP1, por lo que se puede interpretar que altos valores presentes en este componente principal tienen tendencia a valores promedios altos en estos cuatro nutrientes mencionados anteriormente.

Principal component analysis

On the other hand, the results of the groupings and relations between the twelve accessions of castorbean studied are shown based on the analysis of main components of six variables of the proximal chemical analysis and four variables for the oil quality. In Table 4, it is concluded that with at least four main components, 88.1% of the total cumulative variance of the data is explained.

Cuadro 4. Vectores y valores propios del análisis de componentes principales (CP).
Table 4. Vectors and eigenvalues of main component analysis (CP).

Valor propio	4.6939	1.9825	1.3831	0.7552	0.6483	0.3346	0.1336	0.0687
Proporción	0.469	0.198	0.138	0.076	0.065	0.033	0.013	0.007
Acumulada	0.469	0.668	0.806	0.881	0.946	0.98	0.993	1
Variable	CP1	CP2	CP3	CP4	CP5	CP6	CP7	CP8
Aceite	-0.382	-0.015	-0.393	0.106	0.262	-0.221	-0.321	0.508
Proteína	-0.411	-0.002	-0.24	0.047	0.211	0.148	0.81	-0.166
Fibra cruda	-0.382	0.012	-0.201	-0.087	-0.581	0.136	-0.284	-0.523
Cenizas	0.207	-0.122	-0.328	-0.895	0.134	0.113	0.001	0.027
Humedad	0.093	0.607	-0.113	-0.055	-0.512	0.208	0.217	0.504
Carbohidratos	0.418	0.011	0.355	0.013	0.029	0.04	0.144	-0.058
Viscosidad	0.127	0.555	-0.202	0.149	0.486	0.46	-0.274	-0.299
Densidad	-0.156	0.553	0.19	-0.26	0.114	-0.704	0.044	-0.236
Índice de acidez	0.369	-0.03	-0.462	0.206	-0.095	-0.266	0.084	-0.136
Ácidos grasos libres	0.369	-0.03	-0.46	0.205	-0.098	-0.275	0.086	-0.137

Por su parte, el componente principal 2 (CP2), participa con un valor propio (1.98) y el 19.8% de la varianza explicada, en la cual se encuentra la variable humedad (60.7%), viscosidad (55.5%) y densidad (55.3%), dos variables que hacen referencia a la calidad del aceite y una a la semilla. En el componente principal (CP3), se encuentra índice de acidez (46.2%) y ácidos grasos libres (46%), este componente está conformado solo por atributos de calidad del aceite. Finalmente el último en el CP4 se encuentra cenizas la cual no se asocia con ninguna otra variable, el considerar está componente permite adicionar 7.6% de la variación del proceso explicado.

Análisis por conglomerados

De acuerdo con el análisis por conglomerados (Figura 2), las doce accesiones de higuerilla se clasificaron en grupos relativamente homogéneos, usando una similitud de 66.6%, por

The main component 1 (CP1), allows to explain the 46.9% of the global variance; with a value of 4.69, this main component shows a greater association with the following four variables: carbohydrates (41.8%), protein (41.1%), lipids (38.2%) and crude fiber (38.2%), which have a high participation in the definition of CP1, so it can be interpreted that high values present in this main component tend to have high average values in these four nutrients mentioned above. The main component 2 (CP2), participates with its own value (1.98) and 19.8% of the explained variance, in which the variable humidity is found (60.7%), viscosity (55.5%) and density (55.3%), two variables that refer to the oil quality and one to the seed. In the main component (CP3), there is an acidity index (46.2%) and free fatty acids (46%), this component consists only of attributes of oil quality. Finally the last one in the CP4 is ashes which is not associated with any other variable, considering this component allows to add 7.6% of the variation of the explained process.

medio de la distancia euclidiana como método de agrupación. Se identificaron tres conglomerados predominantes para las doce accesiones. El primero conformado por: SLPS11C1, ZACS3C1 y AGSS2C1, el segundo integrado por: ZACS2C1, JALS1C1, ZACS1C1, JAL3C1 y JALS2C1; y el tercero por: SLPS6C1, AGSS3C1, AGSS4C1 y SLPS3C1.

Conclusiones

Los resultados obtenidos sugieren que la relación entre la composición química y la calidad del aceite de la semilla puede estar afectada por características ambientales y genéticas asociadas a su origen silvestre de manera independiente al sitio de colecta. SLPS11C1 presentó mayor contenido de aceite y proteína, a diferencia de JAL3C1 quien presentó mayor contenido de fibra cruda. Por su parte, AGSS2C1 reportó una mayor viscosidad, mientras SLPS11C1 un menor índice de acidez y porcentaje de ácidos grasos libres. Aunque los resultados sugieren que SLPS11C1 y AGSS2C1 pueden ser accesiones útiles para la explotación integral del cultivo, estas semillas de higuerilla pueden ser consideradas como materia prima estratégica industrial o bioenergética. Para lo cual, se recomienda realizar trabajos de determinación de carbohidratos, contenido de azúcares, almidón y celulosa. Finalmente, los datos obtenidos en el presente trabajo pueden ser considerados para futuros trabajos de mejoramiento genético y agronómico.

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Analysis by clusters

According to the cluster analysis (Figure 2), the twelve accessions were classified into relatively homogeneous groups, using a similarity of 66.6%, by means of the Euclidean distance as a grouping method. Three predominant clusters were identified for the twelve accessions. The first one consists of: SLPS11C1, ZACS3C1 and AGSS2C1, the second consisting of: ZACS2C1, JALS1C1, ZACS1C1, JAL3C1 and JALS2C1; and the third by: SLPS6C1, AGSS3C1, AGSS4C1 and SLPS3C1.

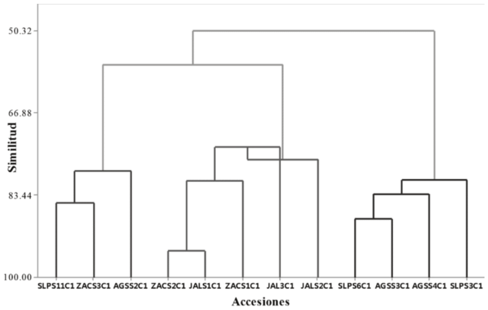

Figura 2. Dendrograma de agrupamiento jerárquico de acuerdo a la composición química y calidad de aceite de doce accesiones de higuerilla.

Figure 2. Dendrogram of hierarchical grouping according to the chemical composition and oil quality of twelve castorbean accessions.

Conclusions

The results suggest that the relationship between chemical composition and seed oil quality may be affected by environmental and genetic characteristics associated with its wild origin regardless of the collection site. SLPS11C1 showed higher content of oil and protein, unlike JAL3C1 that showed higher crude fiber content. On the other hand, AGSS2C1 reported a higher viscosity, while SLPS11C1 had a lower acid number and percentage of free fatty acids. Although the results suggest that SLPS11C1 and AGSS2C1 can be useful accessions for the integral exploitation of the crop, these castorbean seeds can be considered as strategic industrial raw material or bioenergetic. For this, it is recommended to carry out

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researches on the determination of carbohydrates, sugar content, starch and cellulose. Finally, the data obtained in this paper can be considered for future researches of genetic and agronomic improvement.

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III. PUBLICACIÓN EN REVISTAS INDIZADAS COMO CO-AUTOR

III.1 Proyección financiera de cultivo de fresa en biofábrica: inversión, costos y producción

Proyección financiera de cultivo de fresa en biofábrica: inversión, costos y producción

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RESUMEN

El sector agroalimentario de la fresa es en la actualidad uno de los de más alto crecimiento y demanda a nivel nacional e internacional, debido a su versatilidad de usos y la calidad nutricional que aporta a la dieta del ser humano, es usada en postres, ensaladas, conservas, entre otros. El objetivo fue evaluar el rendimiento del fruto, determinar los costos de producción y establecer la inversión inicial para el establecimiento de una unidad productiva tipo biofábrica en invernadero propio o rentado. El presente estudio se realizó en cultivo protegido dentro de un invernadero, con esquema vertical de 5 niveles, para determinar la productividad de este sistema. Se realizó la evaluación del rendimiento del cultivo de fresa por metro cuadro, estimación de costos de inversión, y el cálculo de la Tasa Interna de Retorno (TIR) con datos de las ventas anuales del ciclo agrícola 2018. Los resultados obtenidos indican que la producción en un sistema de biofábrica durante un ciclo de 12 meses fue de 17.37 Kg/m^2, con ventas obtenidas durante el ciclo agrícola 2018 de $ 814,528. La TIR obtenida para la modalidad de compra y renta de invernadero fue de 24 y 37%, respectivamente. De acuerdo con los resultados obtenidos, se propone la implementación del cultivo de fresa tipo biofábrica en cualquier modalidad para generar rendimientos superiores de entre 2.5 y 3 veces más, en comparación con la producción en suelo.

ABSTRACT

The strawberry industry has had a high growth and demand at national and international level because of the versatile nature of the strawberry and the nutritional quality for the human diet. It is used as a dessert, in salads, fruit preserves among others. Objective: To evaluate the crop performance, to determine production costs and to define the initial investment to start production.

Methods: This study was made in a protected cropping system in a Bio Fabric type production greenhouse in a 5 level vertical scheme to determine the system productivity. Crop performance per square meter was evaluated, investment cost estimated and the internal rate of return (IRR) using annual sales of the 2018 agrocycle. Results: In the studied Bio Fabric system during a 12 month period the production performance was 17,37 kg/m2, and the sales in the agrocycle were $814,528. The IRR for greenhouse purchase or rent was 24% and 37% respectively. According to the results its proposed to use the Bio Fabric system instead of the open field production because of the 2.5 to 3 times higher performance.

PALABRAS CLAVE:

Biofábrica, fresas, producción en vertical, rentabilidad.

KEYWORDS:

Plant factory, strawberries, vertical production, profitability.

Introducción

En la actualidad, una alternativa para producir hortalizas, frutas y flores en un ambiente controlado bajo invernadero, ha permitido alta productividad en espacios reducidos con productos de primera calidad y que reúnen los requisitos para exportación a países como Estados Unidos y Canadá generando divisas para el país (Kozai et al., 2019). La importancia de generar tecnología nacional que permita su uso a bajo costo y de manera competitiva a nivel internacional propicia que los pequeños agricultores tengan más posibilidades de ofertar sus productos a mejores precios y la posibilidad de incursionar en el mercado internacional (Felipa, 2019).

La fresa es un producto muy demandado en el mercado nacional e internacional (Simpson, 2018), su producción en ambiente controlado bajo invernadero y en producción vertical permite obtener frutos más confiables y con características de calidad muy elevadas, como color, sabor y firmeza. De igual forma, se pueden obtener frutos con características microbiológicas aceptables, ya que estos sistemas de producción permiten la obtención de frutos libre de patógenos, los cuales se encuentran frecuentemente en los cultivos de campo (Taghavi et al., 2019). Debido a que la gestión de la tecnología y la innovación en el campo mexicano se vuelven cada día más necesarios para acercar a los productores a formas de cultivo más eficientes y seguras (Magallón et al. ,2017); el cultivo de fresa es un modelo apropiado para este análisis, debido a su gran demanda y a la diferencia de producir en sistema vertical, lo que le da un alto potencial productivo.

Los principales países productores de fresa son: China (3.7 millones de toneladas), Estados Unidos (1.4 millones de toneladas), México (658 mil toneladas), Egipto (407 mil toneladas) y Turquía (400 mil toneladas), aproximadamente (FAOSTAT, 2019). Por su parte, según datos del SIAP (2019) la producción de fresa en México para el 2018 fue de 653,639.24 toneladas, siendo el Estado de Michoacán con 454,958.6 toneladas, el primer productor nacional seguido por Baja California con 116,451 toneladas y Guanajuato con 67,178.72 toneladas, mientras que en el estado de Querétaro el cultivo aún es incipiente pero con amplio futuro, teniendo en cuenta las condiciones agroclimáticas, facilidades de vías terrestres y conexiones no mayores a 700 km de distancia de las principales áreas metropolitanas del país: Guadalajara (4,4 millones de habitantes), Monterrey (4.1 millones de habitantes), Ciudad de México (20.1 millones de habitantes), con un mercado potencial de alrededor de 28.6 millones de habitantes (INEGI, 2010) y posibilidad de distribución en menos de 24 horas. La producción de fresa en el país durante los últimos cinco años (Tabla 1) ha crecido en un 42%, y el precio medio rural por hectárea ha incrementado un 71%, por ende, en el año 2018 el valor de la producción ha alcanzado valores de 150% superiores a los registrados en 2014, según datos del SIAP (2019). En México, a pesar de que la producción de fresa en las últimas décadas ha tenido un nivel bajo de adopciones tecnológicas, ha permitido al agricultor cultivar un producto competitivo a nivel nacional e internacional; sin embargo, en los últimos años la exigencia de calidad y sobre todo de inocuidad alimentaria, están conduciendo a los productores agrícolas a optar por sistemas altamente productivos a partir de la integración de producciones verticales en ambientes controlados bajo invernadero, sistemas de riego automatizados o semi automatizados, soluciones nutritivas específicas para cada etapa fisiológica del cultivo, entre otros (Barba Quiles, 2016; Negrete, 2018). Por lo anterior, el objetivo de este proyecto fue evaluar el rendimiento del fruto, determinar los costos de producción y calcularla inversión inicial para el establecimiento de una unidad productiva tipo biofábrica en invernadero propio o rentado.

Tabla 1. Información de la producción de fresa en México 2014 - 2018

Sup. Sembrada (ha)	Sup. Cosechada (ha)	Producción (t)	Rendimiento (t/ha)	Precio medio rural ($/t)	Valor Producción (miles de presos)	Año
13,709.66	13,652.16	653,639.24	47.88	20,503.13	13,401,649.13	2018
13,850.78	13,849.78	658,435.89	47.54	19,200.62	12,642,379.87	2017
11,091.93	11,090.93	468,248.48	42.22	16,716.46	7,827,458.41	2016
10,163.46	10,073.46	392,625.19	38.98	14,716.46	5,779,003.00	2015
9,966.85	9,965.85	458,971.63	46.05	11,923.30	5,472,457.88	2014

Fuente: SIAP, 2019.

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Materiales y métodos

Zona de estudio

El presente estudio se realizó en las instalaciones de la Universidad Autónoma de Querétaro (UAQ), Campus Amealco, ubicado en las coordenadas 20.175284 Norte, -100.136834 Oeste, durante los meses de octubre de 2017 a diciembre de 2018.

Material biológico

Se cultivaron plántulas de fresa variedad Albión, esta variedad fue seleccionada por ser de día neutro, la cual se caracteriza por tener un amplio periodo de floración, ya que tiene menos sensibilidad a factores como la temperatura y el fotoperiodo (Strassburguer et al., 2010).

Descripción de la unidad de producción en biofábrica

La unidad de producción utilizada fue un invernadero tipo dos aguas, con ventanas frontales y laterales, de tres metros de altura y 666 m^2 de superficie. El invernadero se dividió en 10 surcos dobles con cinco niveles verticales y separación de 50 cm entre nivel alcanzando una altura de 2.5 m y una longitud de 46 m en estructura tubular de PVC.

La parte posterior y frontal se ubicó a 0.5 y 1.5 metros de la pared, respectivamente. La distancia entre pasillos fue de 80 cm y se acopló un sistema de riego por goteo con espacios de 30 cm entre cada planta (Figura 1). La densidad de siembra fue de 19 plantas por metro cuadrado en sistema hidropónico cerrado con recirculación (Tabla 2).

Tabla 2. Descripción del modelo de producción de fresas en sistema biofábrica

Descripción	Cantidad
Superficie de módulo (m^2)	666
Líneas de producción	10
Ciclo productivo (meses)	12
Número de plantas por módulo	12,645
Número de plantas por m^2	19

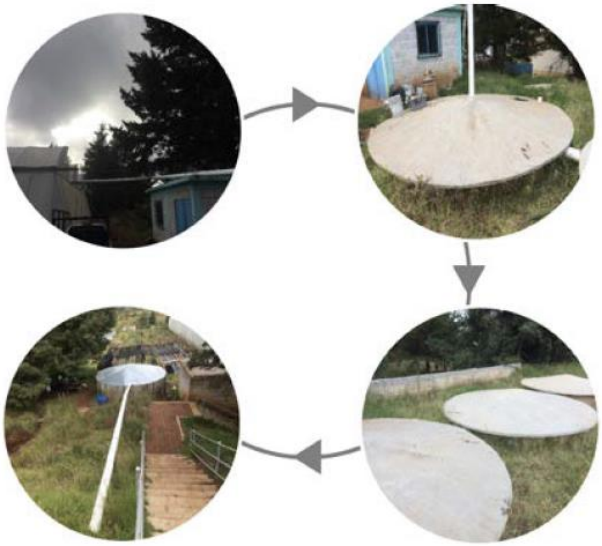
Figura 1. Distribución de planta y sistemas de riego.

Descripción de las condiciones de cultivo

Las plantas se ubicaron en contenedores de 800 mL de capacidad dentro de la tubería de PVC (sanitario) de 4", donde circula la solución nutritiva excedente que la planta no necesita (drenaje). El sustrato utilizado estuvo compuesto por una mezcla de fibra de coco (80%) y perlita (20%). Para el suministro de agua se utilizó un sistema de captación de agua de lluvia (Figura 2), el cual permite el almacenamiento del agua en 3 cisternas de 30 m^3; posteriormente, se suministró mediante bombeo a dos tanques de 2,500 L en riego por goteo, que contenían todos los nutrientes necesarios para el cultivo.

A los 65 días del trasplante, se realizó la primera cosecha y se programaron dos cosechas por semana, derivado de la variedad que es de día neutro. Por otra parte, productos biológicos fueron utilizados en el manejo de plagas y enfermedades.

Figura 2. Sistema de captación de agua de lluvia



Análisis financiero para la produción de fresa mediante el sistema de biofábrica.

Estimación de los costos de inversión y producción

Para la realización de la estimación de los costos de producción se consideró el precio de los recursos actuales en el mercado, así tambien fueron solicitadas cotizaciones a diferentes proveedores y consultas con expertos en el ramo.

Cálculos para el retorno de la inversión

Se determinó la tasa interna de retorno (TIR). La TIR es la tasa de descuento que iguala, en el momento inicial, la corriente futura de cobros con la de pagos, generando un VAN igual a cero. La TIR fue calculada en Excel con la función financiera del mismo nombre.

$$\boldsymbol{VAN} = -I_0 + \sum_{t=1}^{n} \frac{F_t}{(1+TIR)^t} = -I_0 + \frac{F_1}{(1+TIR)} + \frac{F_2}{(1+TIR)^2} + \cdots + \frac{F_n}{(1+TIR)^n} = 0 \quad (1)$$

Ft: Flujo de dinero en cada periodo t
I_0: La inversión inicial (t=0)
n: Número de periodos de tiempo

De acuerdo con el sistema de producción tecnológico de la fresa en biofábrica (Figura 3), éstas fueron guardadas en empaques plásticos con un peso aproximado de 450 g y comercializados en el mercado universitario de la UAQ- Cerro de las Campanas.

Determinación del rendimiento de cultivo

Para calcular el rendimiento total se tomó en cuenta un ciclo de producción compredido de enero a diciembre de 2018. Este ciclo fue utilizado debido a que la variedad de fresa cultivada en el presente estudio (Albión) es de producción constante durante todo el año. Se evaluó la producción promedio de la unidad, sumando la producción total cosechada entre la superficie sembrada (kg/m^2).

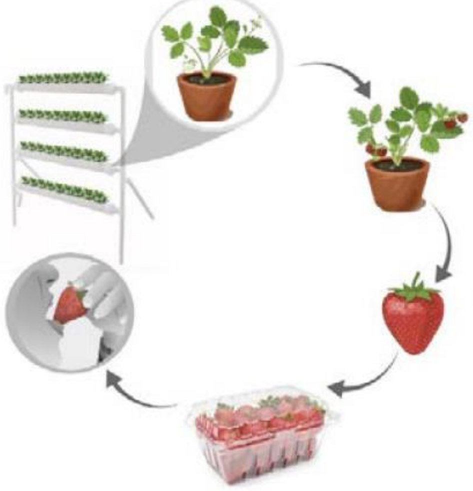


Figura 3. Sistema de producción tecnológico de la fresa en biofábrica

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Resultados y discusión

Rendimiento periódico de la producción de fresas en sistema biofábrica

En la Tabla 3 se encuentran los resultados obtenidos para la producción de fresa en un sistema de biofábrica. Se obtuvo un rendimiento anual de 11,570 kg en una superficie de 666 m^2, es decir un rendimiento de 17.37 kg/m^2. Este rendimiento es mayor a lo reportado para la producción de fresa en suelo, el cual se encuentra en un intervalo de 4 a 6 kg/m^2.

De acuerdo con Pimentel (2010), en México existen tres niveles tecnológicos para la producción de fresa, en los que el espacio no es bien aprovechado. Por tal motivo, la producción de fresa en sistemas verticales genera la posibilidad de tener altas productividades debido a la densidad de las plantas que pueden estar dentro de un invernadero. (Furlani y Fernández, 2007). Los cultivos en biofábrica tienen varias ventajas sobre la agricultura tradicional porque son más seguros y en el caso de la fresa con variedades de día neutro, como la usada en este estudio, producen durante todo el año, la limpieza dentro del invernadero y la no exposición del fruto en la tierra, el aprovechamiento de los espacios, la calidad del fruto entre otras (Kim, 2010). De acuerdo a los resultados obtenidos en el presente trabajo, se propone la implementación del cultivo tipo biofábrica para generar rendimientos superiores de entre 2.5 y 3 veces más, en comparación con la producción en suelo, generando mejores utilidades y alta productividad en espacios reducidos.

Inversión para la producción de fresas en sistema biofábrica

Se consideraron dos tipos de modalidades para la inversión inicial del proyecto: compra y renta de invernadero. La inversión inicial necesaria para la instalación del sistema biofábrica en invernadero rentado fue de $274,948.00. Para el caso de compra del invernadero la inversión es de $ 574,648 en total. Para este proyecto se incluyó la renta del invernadero debido a que el presente trabajo fue realizado en las instlaciones de la Universidad Autónoma de Querétaro. La proyección financiera contemplando la renta ó compra del invernadero para todo el ciclo de 2018 respecto a la inversión fija se presenta en la Tabla 4.

La estimación para el capital de trabajo en las dos modalidades se presenta en la Tabla 5. Para el caso de la renta de invernadero el costo en capital de trabajo fue de $468,539.72 y en el caso de compra de invernadero de $375,299.72. Cabe resaltar que el capital de trabajo considera todos aquellos recursos que requiere el proyecto para poder operar de forma continua; por lo tanto es mayor el gasto en capital de trabajo en la modalidad de renta respecto a la compra de invernadero.

En la Tabla 6 se presentan los valores de inversión total para el sistema de producción de fresa en biofábrica.

Rentabilidad de producción de fresas en sistema de biofábrica

Se tomaron en cuenta todas las ventas del año 2018 con un total de 11,570 kg vendidos de fresa de los cuales se vendieron el 70% (8,099 Kg) a un precio de $77.00 el kg y el 30% (3,471 kg) a un precio de $55.00, generando ingresos totales por $ 814,528.00.

Considerando los datos de la inversión inicial y los flujos de efectivo, se calculó la Tasa Interna de Retorno (TIR), con una proyección a 5 años en pesos constantes. La TIR de retorno considerando que el invernadero fuera rentado fue del 37% y del 24% si se hubiera comprado (Tabla 7).

Tabla 3. Rendimiento periódico de la producción de fresa en sistema biofábrica

Descripción	Unidades	Cantidad
Rendimiento semanal	Kg	241.04
Rendimiento mensula	Kg	964.16
Rendimiento anual	Kg	11,570
Número total de empaques por ciclo anual	Empaques plásticos	25,711

Tabla 4. Inversión fija para el establecimiento del sistema biofábrica

Inversiones	Unidades	Precio UnitarioC	Cantidad	ompra	Renta
Módulo de agricultura protegida (compra)	m^2	$450.00	666	$299,700.00	-
Sistema de riego	Pieza	$77,200.00	1	$77,200.00	$77,200.00
Estructura de PVC	Pieza	$109.91	767	$84,300.97	$84,300.97
Estructura metálica por nave	Pieza	$10,399	3	$31,197.00	$31,197.00
Plántula de fresa	Pieza	$4.50	12,654	$56,943.00	$56,943.00
Macetas	Pieza	$2.00	12,654	$25,308.00	$25,308.00
			Valor total	**$574,648.97**	**$274,498.97**

Tabla 5. Inversión en capital de trabajo de un ciclo anual para la producción de fresa en sistema biofábrica

				Valor	
Capital de TrabajoU	**nidades**	**Precio Unitario**	**Cantidad**	**Compra**	**Renta**
Renta invernadero	m^2	140	666	-	$93,240.00
Fertilización	Dosis	-	3	$7,500.00	$7,500.00
Productos agroquímicos	Dosis	-	-	$15,000.00	$15,000.00
Mano de obra	Jornales por año	211.11	468	$98,799.48	$98,799.48
Asesoría especializada	Servicio por año	692.31	104	$72,00.24	$72,000.24
Empaque plástico	Pieza	3.50	25,714	$90,000.00	$90,000.00
Otros	-	-	-	$40,000.00	$40,000.00
Flete	Traslado	-	-	$52,000.00	$52,000.00
			Valor total	**$375, 299.72**	**$468,539.72**

Tabla 6. Inversión total de un ciclo anual para la producción de fresa en sistema biofábrica

Inversiones	Inversión fija	Capital de trabajo	Inversión total
Compra	$574,648.97	$375,299.72	$949,948.69
Renta	$274,948.97	$468,539.72	$743,488.69

Tabla 7. Rentabilidad de producción de fresas en sistema de biofábrica

Rentabilidad	Valor
Venta de fresas	$814,528.00
Costos IC	$375,299.72
Costos IR	$468,539.72
Utilidad IC	$439,228.28
Utilidad IR	$345,988,28
TIR invernadero rentado (IR)	37%
TIR invernadero comprado (IC)	24%

Conclusiones

De acuerdo a los resultados, se propone la implementación del cultivo tipo biofábrica para generar rendimientos superiores de entre 2.5 y 3 veces más, en comparación con la producción en suelo, generando mejores utilidades y alta productividad en espacios reducidos. Los resultados obtenidos indican que la producción en un sistema de biofábrica evaluado en un ciclo de 12 meses fue de 11,570 kg en una superficie de 666 m², un rendimiento de 17 kg/m², a diferencia de la producción en suelo que está en un rango de 4 a 6 kg/m². La producción en biofábrica es una alternativa para lograr altos rendimientos en espacios reducidos y frutos de alta calidad, con la aplicación de la tecnología en diferentes niveles para proporcionar a los cultivos las necesidades nutritivas y el ambiente que estos necesitan.

Con el crecimiento de la población mundial y la gran demanda de alimentos que en los próximos años tendrá el planeta, esta alternativa es viable y genera confianza, además de poder cultivar productos de forma orgánica y con biofertilizantes amigables con el medio ambiente, así como rendimientos financieros atractivos para los inversionistas.

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III.2 Image analysis and data mining techniques for classification of morphological and color features for seeds of the wild castor oil plant (*Ricinus communis* L.)

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Image analysis and data mining techniques for classification of morphological and color features for seeds of the wild castor oil plant *(Ricinus communis L.)*

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Abstract In this study, a castor seed *(Ricinus communis L.)* classification process was developed using a precise image analysis technique, and several data mining algorithms. Castor seed oil has an excellent demand in the pharmaceutical sector, and it has recently aroused the interest of the biodiesel production companies. However, there are few studies describing the physical characteristics of *Ricinus communis*; thus, any advance in this field contributes to the design of technology that may increase the production of this oil, up to

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industrial levels. In fact, this work aims to contribute not only to understand the physical features of castor seed varieties, but also to unveil key information to develop better castor seed oil extraction machines. Additionally, a novel methodology to study accessions of castor seed gathered from several geographical locations is proposed. Particularly, an automatically accurate image analysis technique was implemented in order to extract color and morphological features from seeds. The data set of seeds was built considering fifty samples per accession. After that, several classification experiments were done using well known data mining algorithms in order to cluster all samples. Experimental results showed that it is possible to cluster studied seeds into ten similar classes with high accuracy (larger than 95 %). Moreover, image analysis and data mining techniques were efficient tools for the classification of seeds, and the color and morphological data gathered are really useful for the design of oil extraction equipment. In fact, the effectiveness in the correct classification instances was 100 %, with a computation time of 0.01 seconds.

Keywords *Ricinus communis* · Seed characterization · Image analysis · Classification

1 Introduction

The seed classification problem has been studied extensively by researchers to distinguish and classify features in several fields that include both botanical and agronomic [22, 27]. Sometimes, seeds must be certify using several tests, such as germination, microbial control and purity [10]. The problem has been shown difficult as the nature of the plants is influenced by factors such as their maturity, weather conditions, location, type of growth substrate, and the accession process that sometimes may include seeds from multiple points [29]. In addition, the feature extraction of seeds is another complex problem which demands the use of the most advance technology and knowledge from areas of study that may include chemistry, physics, pattern recognition, digital image processing, and data mining [25, 42].

Globally, the consumption of seeds and the use of castor oil is rising. Sehgal et al. [46] presented that castor seeds have a good percentage of oil which is extracted and used in different fields such as: pharmaceutical [30], biodiesel production [3], and lubricants [35] among others. Due to the fact that castor bean seeds are an important raw material for the industry, a lot of technology has been developed to extract its oil. However, in the vast majority of the cases the mechanisms have been adapted from other processes without considering the physical properties of *Ricinus communis*. Thus, specific knowledge about these characteristics is missing in the state of the art. Mohsenin et al. [33] argued that seed physical properties constitutes an essential information in the design of castor seed oil extraction machines. Moreover, the study of morphological characteristics has been considered in different works including oil seeds, as an important feature [1, 48]. Thus, in this work a rigorous and detail study over seeds of castor oil is presented.

In general, the methodology applied considers the following stages: the data collection from locations where plants grow wild, the preparation of samples, the acquisition of several silhouette images by using a electromechanical system to rotate and located the sample, the implementation of a camera calibration method, the implementation of several image processing techniques, the feature extraction, the preparation of the data set, and the implementation of cluster algorithms to classify all seeds. Actually, experimental results demonstrates that it is possible to classify castor oil seeds with high accuracy, using the extracted morphological and color features.

The rest of the paper develops as follows: in Section 2, some of the most relevant works of digital image processing techniques used to classify seeds, and current advances in the study of castor oil seeds are presented; then, in Section 3 presents the material and methods used, that include: the data collection stage, and the electromechanical acquisition system. After that, in Section 3.2 the digital image processing techniques implemented are described. In Section 4, experimental results of the feature extraction, selection, and classification algorithms are presented. Finally, in Section 5, the conclusion, contributions, and future works of this paper are listed.

2 Related works

In economic terms, the costs and potential benefits of the use of specific machines to preprocess and extract castor oil, avoid the adaptation of technology that was developed for other purposes. Under similar considerations, some authors had analyzed this issue in other related fields [2, 6]. In this research, the interest is focused on studying morphological and color features of castor seeds gathered from several accessions at different locations in Mexico, by using image analysis. Since the type of seed is an important characteristic for oil extraction process, a previous classification step is required in order to feed machines with the same seed variety [37, 38].

In other fields, Grillo et al. [16] implemented a seed morphometric and colorimetric database to develop statistical classifiers for ten families of the Mediterranean vascular flora. The authors analyzed accessions from 274 taxa belonging to 161 genera, and 52 families. Images were obtained with a flatbed scanner, and processed by a macro software program developed for the morpho-colorimetric measurements. The mean seed weight was also calculated for each accession. Following a similar strategy, Cervantes et al. [8] proposed a model for the description of Arabidopsis seed shape, based on the comparison of the outline of its longitudinal section with a transformed cardioid. The transformation consists of scaling horizontal axis by a factor equal to the Golden Ratio [9]. Moreover, the authors used adjustments of seed shape measurements with simple geometrical forms for the statistical analysis of variations in seed shape under different conditions. Experimental results showed the efficiency of the adjustment to a cardioid in the model plants suggesting that seed morphology may be related to genome complexity.

To solve the problem of seed identification, several machine vision systems have been proposed. As an example, for lentil type identification Shahin and Symons [45] used size measurements by using morphology operations, and color attributes. In castor bean seeds [7] used x-ray analysis to evaluate the quality of seed lots. In that study, seeds were classified according to internal morphology visualized in the radiography image. However, this method can be used to assess the seedling performance, but color and texture information cannot be gathered. Another framework done in castor seed, has considered some mechanical properties. In particular, these characteristics were measured in order to design an oil extraction machine [28, 43]. However, this work was carried out to determine the effect of seed growing regions, and loading speed on some mechanical properties of the seed. Particularly, they did not consider any color, texture or morphological features; thus, the problem of physical characterization of castor seed based on this information is still missing in the literature, and belongs to the main goal of the present research.

Several studies concerning castor been seeds have been conducted in Mexico. Perea-Flores et al. [39], studied a seed variety from the state of Michoacan. The results of its characterization was quite similar to the variety VQ-7 reported by Perdomo et al. [38]. On the other hand, Pecina-Quintero et al. [37], studied 82 accessions from the state of Chiapas (southern Mexico), finding two statistically different families. Similarly, Perdomo et al. [38] studied seven varieties of castor seed from a state of Mexico. In their work, experimental results expressed a deviation in the yield of oil extraction by using the Soxhlet method, with an oil extraction yield between 40.175 % to - 56.218 %. In addition, by a mechanical extraction the values achieved were between 26,334 % to 36.59 %, while for fatty acid composition (FFA %) a variation between 0.0291 % to 0.0547 % was found. Moreover, the proportion of ricinoleic acid measured in the study was between 95.49 % and 74.68 %.

It has been established that castor seed morphological features determine the requirements of the seed oil extraction machinery [12, 43]. Thus, depending on the accession lot, the characterization procedure may represent a challenge that could be solve by pattern recognition, and data mining methods [34]. Considering the previous context, a very important task to either compare or develop an efficient characterization method is to evaluate several classification algorithms. However, to characterize seeds, several features have to be obtained, including length, width, thickness, perimeter, area, thickness ratio, aspect ratio, eccentricity, spread, square root of the seed area, red, green, and blue color components, among others [31, 41, 47]. Even with small samples, building a model to characterize and classify automatically castor bean seeds is a complex problem that demands major efforts. Some of the aspects that make this task difficult, includes the large similarity among parameters such as color, and length. Actually, there are several studies on seed characterization [11, 24, 40], but none of them used morphological and color properties. In this paper, a study about the characterization of castor seeds accessions is presented, and the main goal was to cluster all castor been seeds into several classes that hold similar features, such as size, and color. To achieve this objective, a precise image acquisition system was used. The data set was built with five hundred seeds, collected from the states of Aguascalientes, Jalisco, San Luis Potosi, and Zacatecas Mexico.

3 Material and methods

This research was performed at the *Laboratories of Applied Technological Systems, Department of Telematics Engineering from the Polytechnic University of Queretaro*, Mexico. In particular, this section describes the process for data collection, image acquisition and correction, the digital image processing stage, and the classification algorithms used to cluster the samples.

3.1 Data collection

In this study, castor seeds were collected from wild plants at the states of Aguascalientes, Jalisco, San Luis Potosi, and Zacatecas, Mexico. Prevalent weather conditions in these areas belong to a semi-arid climate. In addition, soils at the collecting sites are all different from each other, and with no agronomic management at all. Collecting points were located along roadways, on soil slopes, next to crops, and close to water channels. The seed bunches collected in the field were stored in paper bags, and properly identified for further management. Before being used in this study, seeds were shelled, and any strange particle removed. Clean seeds were stored under room temperature, and low relative humidity conditions. The collected seeds were gathered from healthy plants with dry bunches. Table 1

Table 1 Geographic location of collected accessions

Accession	Key	Latitude (North)	Longitude (West)	Altitude (m)	Collection date
I	SLPS11C1	2237’28.9”	10142’52.3”	2083	29/03/2013
II	SLPS1C1	2219’32.6”	10112’11.7”	1830	27/03/2013
III	SLPS16C1	2231’40.0”	9934’33.8”	1349	28/03/2013
IV	SLPS6C1	2210’32.08”	10103’7.86”	1955	28/03/2013
V	SLP99C1	2147’54.5”	10100’43.6”	1822	28/03/2013
VI	AGSS3C1	2213’33.4”	10214’13.9”	1936	17/07/2013
VII	AGSS2C1	2154’49.7”	10234’45.7”	2095	13/04/2013
VIII	AGSS4C1	2208’37.2”	10221’26.1”	2004	17/07/2013
IX	ZACS2C1	2245’07.7”	10235’35.9”	2399	20/04/2013
X	JALS1C1	2140’23.6”	10235’34.0”	1920	18/07/2013

shows the geographic location (latitude, longitude, and altitude), and collection date for each accession.

3.2 Image acquisition and correction

A data set containing one thousand images coming from fifty seeds per accession was constructed. All samples were placed manually on the pole of the rotating electromechanical system. Image acquisitions was carried out using a GigE color industrial camera (Imaging Source DFK 23G274) with a format lens 2/3, mount C, and a focal length of 16 millimeters (Imaging Source C1614A). Illumination was provided by a 150 W light source (Fostec Inc, USA) through a double fiber optic bundle. It is important to mention that images are affected by the radial distortion introduced by lens. Thus, images were rectified following a procedure proposed by Bouguet et al. [5]. Images (8-bit RGB, 1600x1200 pixel resolution) of castor seeds were captured at a distance of about 70 mm, illumination field and exposure were manually controlled. Images were saved for later processing. In parallel to the image acquisition process, reference measurements such as length, width, thickness, among others parameters, were measured with a digital precision electronic vernier caliper 500-159-30 (Mitutoyo Inc,) with a resolution of 0.01 mm. Additionally, system calibration was done based on digital image processing techniques, using a 20 mm rectangular steel block checker 516-537 (Mitutoyo Inc,). Thus, a linear relationship between the length of the pattern (in mm), and its corresponding number of pixels was obtained. Figure 1, illustrates the acquisition system. Moreover, the image acquisition process for all samples demanded two images from each seed: the first image was obtained with the elaiosome of the seed pointing to the right side (Fig. 2a), and the second one from the seed rotated clockwise (Fig. 2d).

3.3 Feature extraction

Based on the image properties, a two-step framework was implemented using Matlab (Mathworks Inc.) in order to extract physical features from samples. In general, these steps include various procedures such as image segmentation, and feature extraction methods. Thus, a challenge of this task is the characterization of castor seed *(Ricinus communis L.)* accessions, in order to get a general methodology to ensure the classification process. The first step involves an image segmentation procedure Gonzalez et al. [15], where a

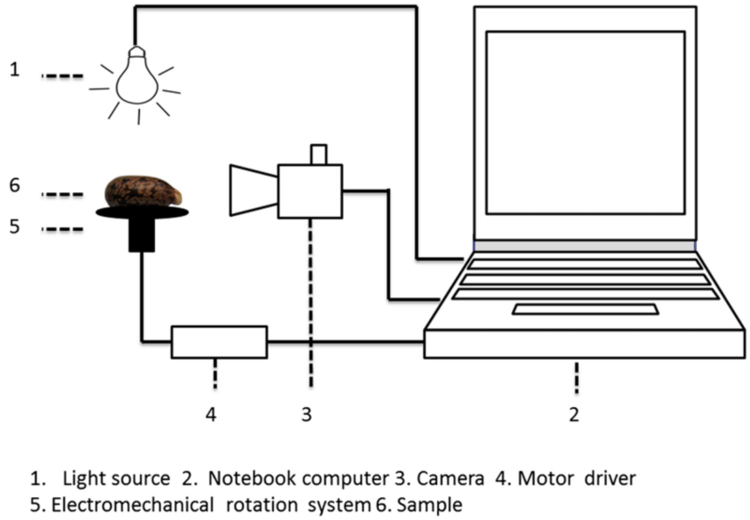

Fig. 1 Image acquisition system

subtraction operation was applied between an input image holding the seed, and the background without it. After that, a binarization operation was applied in order to get an image splitted into two regions: pixels belonging to the seed, and those from the background. A similar binarization strategy was performed by Silva et al. [51]. Then, a mask of the seed pixel information was obtained, and it was used to extract the color and morphological information from the input image. Figure 2, illustrates an example of two acquired images (A and B) from the same seed, the corresponding obtained mask (B and E), and the segmented seed (C and F). The second step considered the measurements applied to the segmented images of each seed. In particular, morphological and color features were measured in both images acquired from every single sample. The morphological features measured were: length, width, thickness, and perimeter, area of the transversal section, geometric mean diameter, surface area, sphericity, length index, sphericity index, Ferret diameter, compaction index, thinness ratio, and aspect ratio [33, 52].

All morphological features where extracted from mask images, where pixel values belongs to background (black pixel without seed information), or foreground (white pixels). Particularly, the features extracted from the image illustrated in Fig. 2b were: length,

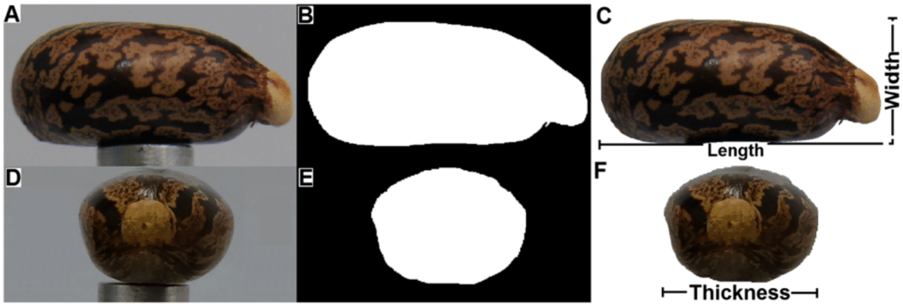

Fig. 2 Example of segmented seeds

perimeter, and area; while width, and thickness were calculated from Fig. 2e. However, the rest of morphological features were obtained by computing relationships between those described above (Perea-Flores et al., [39]; Hernández-Martínez et al., 2011). Table 2, presents a description of each morphological feature analyzed.

The color features extracted were: color components red (R), green (G), blue (B), and the corresponding transformations to the color spaces hue, saturation and value (HSV), and the luma, blue-difference, and red-difference chroma components (YCbCr). Particularly, ten color features were measured with the mean component value of each sample. Since the color component is a matrix, the mean value was estimated in order to be considered as the measured value from each seed. The classification algorithms used in this work are available by using the Weka data mining workbench [19]. This tool, contains an extensive collection of data mining algorithms; in particular, morphological and color features described above, were organized into a single table reference file that was loaded from Weka. All classification experiments were done considering a cross-correlation strategy to train (70 % of samples) and test (30 % of samples) the models gathered. The selected algorithms used in this study are available in Weka tool. All of them, were specially developed to perform data mining task such as classification. In deep, there in the literature several works that described the performance of each strategy to cluster data [50].

4 Results and discussion

Results are presented as follow: first normalized morphological and color features are presented in a graphical representation (Figs. 3 and 4). The normalization process considered the highest value of each feature. Then, results of performance and computational costs of several classification algorithms are presented in Table 3. To realize the computational costs of these algorithms, a personal computer (PC) with an Intel Core 2 Duo E7300 2.6GHz CPU, 2 GB RAM was used. After that, all features measured are presented based on the cluster obtained with the most efficient classification algorithm. Finally, some comparisons with previous results obtained in castor seeds by other authors are done.

Figure 3, illustrates that some clusters such as II, VII, IX, and X are individually isolated. In contrast, clusters I, III, IV, V, VI, and VIII are mixed. Table 3, shows the experimental results of the training phase for all classification algorithms, where three methods presented the same efficiency: naive bayes simple, multilayer perceptron, and decision table naive bayes. In particular, the mean absolute error (MAE), shows that the high dispersion of data is presented by the multilayer perceptron, and the lowest is naive bayes simple. In terms of the computational cost, the fastest method was the naive bayes simple, while the slowest one was the decision table naive bayes.

Some methods have similar computational costs (Bayes Network, Naive Bayes, and Random Forest), even though those methods have different efficiency. The lowest mean absolute error (MAE) was found in the Naive Bayes method, and the highest efficiency in Random Forest. Moreover, some methods have similar computational costs such as Bayes Network, Naive Bayes, and Random Forest, even though those methods expressed different efficiency. The lowest MAE was found in Naive Bayes, and the highest efficiency in Random Tree. A significant fact was found in the training phase, where a good classification performance was obtained by using only some color and morphological features. Thus, other typical techniques like linear discriminant analysis (LDA), or principal component analysis (PCA), were not necessary in order to get a success in the classification process over the clusters. However, the use of machine learning strategies allowed to classify all accessions

Table 2 Morphological features

Morphological Feature		Accession									
		I	II	III	IV	V	VI	VII	VIII	IX	X
Length (mm)	μ	16.32	8.65	11.84	8.71	9.72	12.46	9.66	8.69	11.78	9.41
	σ^2	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02
Width (mm)	μ	9.87	5.46	7.82	6.39	6.51	8.42	6.48	5.70	7.22	5.83
	σ^2	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.03	0.01
Thickness (mm)	μ	6.94	4.11	5.50	4.48	4.63	5.99	4.64	4.24	5.20	4.32
	σ^2	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01
Perimeter (mm)	μ	47.22	24.10	33.20	24.88	26.96	35.16	27.09	24.80	32.59	26.40
	σ^2	0.42	0.03	0.02	0.04	0.03	0.04	0.05	0.05	0.04	0.05
Area (mm^2)	μ	92.67	27.56	50.04	30.56	34.27	58.49	34.63	28.75	47.88	31.51
	σ^2	0.06	0.03	0.54	0.16	0.05	0.20	0.12	0.07	0.26	0.19
Geometric Diameter (mm)	μ	10.35	5.78	7.97	6.29	6.64	8.57	6.61	5.92	7.61	6.20
	σ^2	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01
Superficial Area (mm^2)	μ	326.46	99.07	192.07	118.37	131.04	221.21	130.14	104.91	173.48	113.95
	σ^2	0.51	0.18	5.17	1.86	0.86	0.97	0.89	1.59	8.08	2.37
Sphericity (%)	μ	81.89	84.45	82.16	88.99	80.87	85.08	84.40	83.98	78.87	84.92
	σ^2	120.54	147.15	102.67	164.65	105.59	132.50	120.86	129.39	119.38	123.05
Elongation Index	μ	1.84	1.78	1.67	1.54	1.65	1.68	1.66	1.72	1.84	1.82
	σ^2	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01
Roundness Index (%)	μ	6.91	39.88	16.67	33.50	28.84	12.92	28.40	37.72	17.87	33.41
	σ^2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Feret Diameter (mm)	μ	4.37	3.13	3.67	3.18	3.31	3.77	3.31	3.17	3.63	3.27
	σ^2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Compaction Index (%)	μ	26.80	36.15	31.00	36.45	34.01	30.29	34.31	36.50	30.86	34.78
	σ^2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Thickness Ratio (%)	μ	93.09	60.12	83.33	66.50	71.16	87.08	71.60	62.28	82.13	66.59
	σ^2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aspect Ratio (%)	μ	60.45	63.13	66.04	73.31	67.01	67.57	67.12	65.62	61.29	60.00
	σ^2	0.65	2.99	2.29	2.53	1.66	1.24	2.43	2.42	2.62	1.77

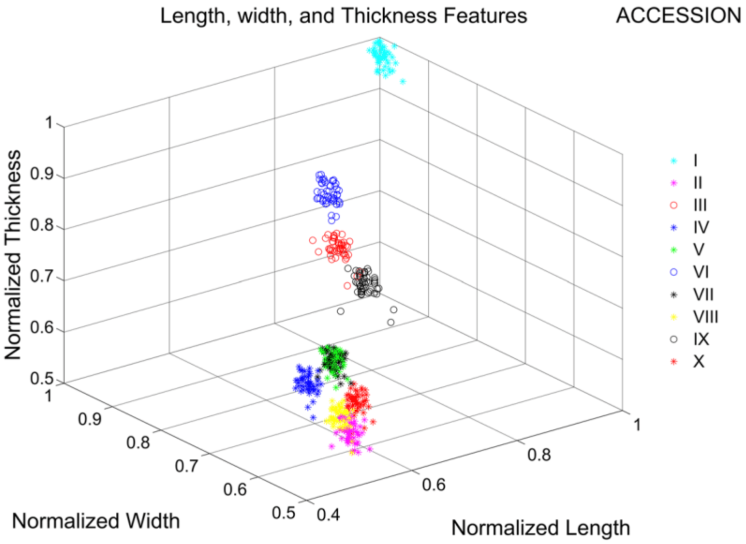

Fig. 3 Normalized length, width, and thickness parameters

with an average of 98 %. The best classification algorithm found at the experimental phase was Random Tree. This algorithm was selected because it has the highest (100 %) Correctly Classify Instances (CCI). Additionally, it is important to mention that any statistical tests were not be applied because the output results are always the same in any iteration. An important characteristics of the Random Tree algorithm is that the built model is not correlated. In fact, the tree has an excellent generalization performance because it was obtained by using a cross correlation strategy. Particularly, 70 % of samples are used to train, and 30 % to test the classification method. In order to represent each cluster as a single class,

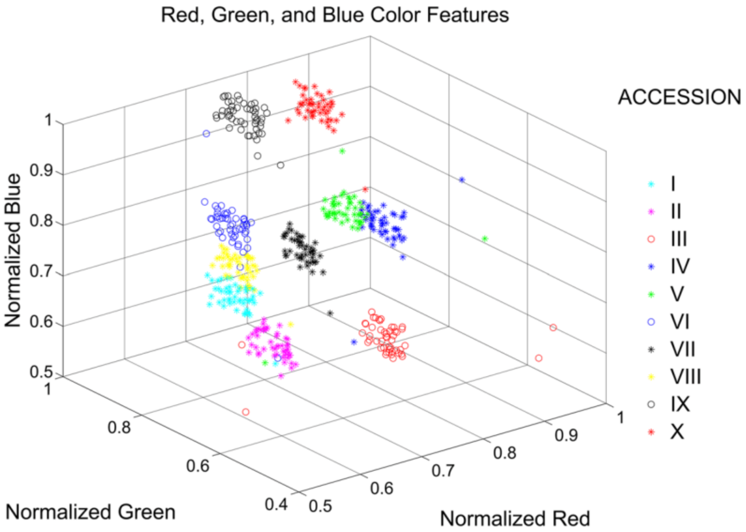

Fig. 4 Normalized red, green, and blue scattered plot

Table 3 Color features

Color Feature		Accession									
		I	II	III	IV	V	VI	VII	VIII	IX	X
Red (%)	μ	9.54	9.33	11.06	12.67	12.13	9.61	11.06	9.88	10.58	12.31
	σ^2	0.01	0.01	0.66	0.12	0.26	0.05	0.01	0.01	0.01	0.02
Green (%)	μ	2.97	2.54	2.18	2.84	2.98	3.04	2.96	3.07	3.31	3.32
	σ^2	0.02	0.01	0.02	0.02	0.02	0.03	0.01	0.01	0.02	0.01
Blue (%)	μ	3.91	3.65	3.69	4.38	4.51	4.68	4.24	4.14	5.64	5.49
	σ^2	0.01	0.01	0.02	0.03	0.04	0.04	0.02	0.01	0.01	0.03
Hue (%)	μ	0.59	0.60	0.58	0.60	0.55	0.62	0.54	0.54	0.62	0.59
	σ^2	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Saturation (%)	μ	0.49	0.48	0.50	0.54	0.52	0.48	0.49	0.51	0.52	0.53
	σ^2	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Value (%)	μ	0.41	0.41	0.41	0.45	0.44	0.43	0.48	0.44	0.41	0.41
	σ^2	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01
Luma (%)	μ	58.12	57.67	56.78	60.77	63.60	62.37	70.25	68.05	59.28	62.72
	σ^2	0.01	0.07	0.34	0.94	0.37	1.06	0.72	0.13	1.15	0.76
Blue-Difference (%)	μ	124.62	124.56	124.48	123.45	123.55	124.85	122.68	123.10	125.18	124.25
	σ^2	0.02	0.02	0.06	0.06	0.04	0.03	0.02	0.02	0.04	0.03
Red-Difference (%)	μ	133.34	133.40	134.42	135.36	134.86	133.04	134.13	133.30	133.26	134.68
	σ^2	0.01	0.01	0.32	0.05	0.11	0.01	0.01	0.02	0.01	0.02
Intensity (%)	μ	49.49	48.97	47.95	52.57	55.85	54.54	63.73	61.16	50.92	54.83
	σ^2	0.01	0.08	0.44	1.26	0.48	1.43	0.99	0.17	1.56	1.04

normalized values of these features were computed. Figure 2 illustrates the results, where one axis represents the normalized length, another the normalized width, and the other one the normalized thickness. In deep, this figure shows that some clusters are completely isolated (clusters I and VI). However, the other clusters are mixed, which is due to a high correlation between samples. In general, these results indicate that a classification process applied to this set of values will not hold a good performance. Figure 5, illustrates a seed sample of each cluster obtained after the classification procedure. In general, some seeds (I, III, IV, VI, VII, VIII, and IX) share many texture features.

Table 4 presents the results of the oil extraction, where the general variation for the ten groups studied were 31.4 to 51.48 %. These results are similar to those reported by Severino *et al*. (2015) (34.6 to 56.6 %). Furthermore, these authors showed that the relationship between certified seed and oil content is directly proportional, which is similar to the results obtained in this research.

The image analysis method implemented in this study to extract morphological features is better than that proposed by Cervantes et al. [8, 9]. The main reason is that these authors approached the silhouette of a seed to an ellipsoid by only using the length and thickness values. In contrast, our measurements have a pixel-based accuracy. It is important to mention that all accessions studied have not been characterized genetically. However, and considering the observations of the physical structure of plants, color of sheets, and characteristics of plant branches, it is possible to assume that there are several varieties of castor seed plants. Based on the experimental results presented in the classification tree (Fig. 5), it is possible to notice that several morphological features, such as: length, width, and thickness parameters, are quite similar between all studied samples, since some of morphological features present a linear combination between them [33, 52].

One of the most significant results of this study is the way the features are clustered, where ten classes appear with samples collected from different geographical locations. Additionally, it was obtained from the ranking of algorithms, a practical method for grading

Fig. 5 Seeds of each cluster obtained by using the random tree classification algorithm

Table 4 Performance and computational cost of classification algorithms

Classfier	CCI(%)	ICI(%)	MAE	RMSE	RAE(%)	RRSE (%)	Time (S)
Bayes Network [14]	98.2000	1.8000	0.0035	0.0582	0.0196	0.1940	0.040
Naive Bayes [44]	99.0000	1.0000	0.0020	0.0447	0.0111	0.1491	0.040
Naive Bayes Multinomial [23]	99.4000	0.6000	0.0480	0.1105	0.2667	0.3683	0.010
Naive Bayes Simple [23]	99.4118	0.5882	0.0012	0.0343	0.0065	0.1142	0.020
Logistic Regression [21]	98.8235	1.1765	0.0025	0.0486	0.0133	0.1616	0.490
Multilayer Perceptron [36]	99.4118	0.5882	0.0066	0.0398	0.0364	0.1324	4.890
Decision Table [18]	98.8235	1.1765	0.0470	0.0865	0.2607	0.2878	0.120
Decision Table Naive Bayes [18]	99.4118	0.5882	0.0021	0.0326	0.0114	0.1086	7.570
Repeated Incremental Pruning	92.3529	7.6471	0.0181	0.1189	0.1005	0.3957	0.080
Nearest-neighbor-like using n-nested-ge	98.8235	1.1765	0.0024	0.0485	0.0130	0.1614	0.070
Best-First Decision Tree Classifier [53]	97.6471	2.3529	0.0051	0.0687	0.0284	0.2286	0.220
Functional Tree [13, 53]	98.2353	1.7647	0.0031	0.0508	0.0169	0.1690	0.500
C4.5 [32]	96.4706	3.5294	0.0071	0.0840	0.0392	0.2796	0.030
Random Forest [4]	98.2353	1.7647	0.0044	0.0399	0.0241	0.1326	0.040
Random Tree [53]	100.000	0.0000	0.0000	0.0000	0.0000	0.0000	0.010

CCI: Correctly Classified Instances, ICC: Incorrectly Classified Instances, MAE: Mean Absolute Error

RMSE: Root Mean Squared error, RAE: Relative Absoulte Error, RRSE: Root Relative Squared Error the seeds. The above stated will contribute to the design of new equipment and industrial machinery intended for extracting castor seed oil. Finally, it was possible to categorize different groups in terms of oil content. Evidently, the estimation of the percentage of extracted oil based on the type of seed and morphological relationship is an important advance to the state of the art [49].

5 Conclusion

An important task in the development of new technologies to optimize the process of castor seed oil extraction is the automatized classification of seed samples. In particular, this paper proposes a strategy to classify castor seeds based on the measurement of several morphological and color features. In fact, it is well known that other features such as image representation may be an alternative method to classify seeds, but we did not consider it because the performance of the classification phase was almost 100 %. Experiments were done in castor seed accessions gathered from different locations. The classification process was achieved by using machine learning algorithms that include: bayes network, naive-bayes multinomial, logistic regression, multilayer perceptron, and random tree, among others.

Considering quantitative results, the highest classification accuracy was found with the Random Tree method. In addition, this research reports simple rules to classify any castor seed, by implementing one of the possible Random Trees. The effectiveness in the correct classification instances was 100 %, with a computation time of 0.01 seconds. Experiments also demonstrated that not all morphological, and color features are required to classify the accessions studied. The classification tree obtained, generates an excellent knowledge to build an embedded system that may classify automatically seeds at industrial levels. Future work will focus on the use of morphological and color features to develop an approach to correlate the amount of castor seed oil content in a given sample. Finally, the classification process is important because it generates the requirements to build a peeling machine, that will extract oil at a higher performance.

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III.3 Plantas silvestres del centro-norte de México con potencial para la producción de aceite.

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Artículo

Plantas silvestres del centro-norte de México con potencial para la producción de aceite

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Resumen

México posee una gran variedad en especies de plantas silvestres que podrían aprovecharse de manera sustentable en la obtención de aceite y productos de alto valor agregado o emplearse como materia prima en el sector industrial, cosmetológico, farmacéutico y/o en la producción de bioenergéticos. El objetivo de este estudio fue determinar el potencial productivo, realizar la caracterización física, química y morfológica de semillas provenientes de plantas silvestres de las regiones de Aguascalientes, San Luis Potosí y Zacatecas con el fin de determinar su potencial en la producción de aceite. Índice de refracción, saponificación, acidez, yodo y peróxido fueron determinados según lo establecido en la Norma Mexicana (NMX). De acuerdo con los resultados obtenidos, *Agave* sp., presentó el mayor rendimiento potencial de semilla de 24305 kg ha^{-1}, sin embargo, presenta un largo periodo de fructificación. Y para el rendimiento de aceite, *A. undulata* mostró el mayor potencial con 1315 kg ha^{-1}. En cuanto a los parámetros morfológicos *C. ficifolia* obtuvo los valores más altos (mm) en dimensiones de largo y ancho y *J. dioica* el mayor valor en espesor (mm) y peso de 100 semillas. Las semillas con altos contenidos de aceite correspondieron a las especies *C. foetidissima* (33.9%) y *P. louisianica* (33.6%) y *J. diodica* (32.86%). La variación entre las características estudiadas permitió la identificación de especies de interés para la producción de aceite de uso industrial y/o bioenergético.

Palabras clave: Aceite, bioenergéticos, plantas silvestres, calidad de aceite, morfología.

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Introducción

La detección de especies vegetales con potencial agroindustrial y bioenergético se han convertido en un tema de gran interés socioeconómico a nivel mundial. Países con gran diversidad ecológica desempeñan un rol importante en la búsqueda de dichas especies. En este sentido, México posee una gran variabilidad ecológica: en el norte y parte del centro del país se encuentran las zonas áridas, semiáridas y subhúmedas secas caracterizadas por los matorrales xerófilos, pastizales y bosques espinosos; en zonas del Pacífico y del centro del golfo de México están los bosques tropicales secos y semisecos; en zonas más húmedas (> 900 msnm) se ubican los bosques tropicales perennifolios, y a mayores altitudes los bosques de niebla; finalmente, en las sierras habitan los bosques de coníferas y de encinos (CONABIO, 2006).

Las zonas secas ocupan más de la mitad del territorio mexicano con 101.5 millones de hectáreas, de esta superficie, las zonas áridas representan 15.7%, las semiáridas 58% y el restante corresponde a las zonas subhúmedas secas (UACH, 2011). De acuerdo a lo anterior, en las zonas áridas y semiáridas existe una gran variedad de vegetación con características adaptadas a este tipo de hábitat, recursos naturales que podrían aprovecharse de manera sustentable en la generación de productos de alto valor agregado o como materia prima en el sector industrial, medicinal, cosmetológico y/o producción de bioenergéticos. Ante el creciente interés de la producción de la bioenergía, es necesario considerar nuevas estrategias tales como centrar la atención en los cultivos, que incluyan árboles forestales de ciclo corto, hierbas perennes y herbáceas, que sean fuentes alternativas de energía como gas natural, biodiesel o bioetanol (Díaz-Ramírez *et al*., 2013).

Aunque, México cuenta con una gran variedad de recursos naturales, su incorporación en el campo bioenergético es incipiente y está siendo impulsada por la implementación de programas y proyectos productivos de mediano plazo. De esta manera, la búsqueda e identificación de especies vegetales endémicas e introducidas con altos contenidos de aceite podrían emplearse en la fabricación de biodiesel. Entre las plantas silvestres con potencial para la producción de aceite que se ha estudiado en el país destacan: higuerilla (Perdomo *et al*., 2013; Mosquera-Artamonov *et al.,* 2016; Vasco *et al.,* 2018), piñón (García *et al.,* 2017), chicalote y cuernitos (Reveles *et al.,* 2010), gatuña (Ortega-Nieblas y Vázquez-Moreno, 1995), entre otras; sin embargo, aún falta por explorar otras especies, con la finalidad de identificar plantas silvestres adaptadas a las condiciones agroambientales de la zona centro-norte de México que pudiesen producir aceite de uso bioenergético.

Tomando en cuenta que tanto especies nativas como introducidas ya adaptadas a cada región, podrían ser aprovechadas con fines energéticos en aquellos suelos no aptos para la agricultura, sin desplazar la producción de alimentos y además requerir pocos insumos, esta investigación pretende colectar especies silvestres del centro-norte de México, determinar su potencial productivo, analizar su contenido de aceite y sus características físico-químicas, e identificar especies vegetales de interés para la producción de aceite.

Materiales y métodos

Selección y colecta de semilla

La colecta de semillas se realizó mediante recorridos de campo entre los meses de junio de 2014 a mayo de 2015, considerando la disponibilidad anual de las plantas silvestres, en 18 sitios pertenecientes a los estados de Aguascalientes (AGS), San Luis Potosí (SLP) y Zacatecas (ZAC), México. Se utilizaron los siguientes criterios para la selección de las especies en campo: 1) abundancia, 2) disponibilidad de semilla, 3) accesibilidad a los sitios, y 4) antecedentes de uso. Las 19 especies colectadas en el área de estudio fueron: chicalote *Argemone mexicana*, calabacilla loca *Cucurbita foetidissima*, chilacayota *Cucurbita ficifolia*, melón hediondo *Apodanthera undulata*, aceitilla *Bidens odorata*, jara *Leonotis nepetifolia*, toloache *Datura inoxia*, cardo *Datura ferox*, cadillo *Xanthium strumarium*, toritos *Proboscidea louisianica,* mostacilla *Brassica* sp., saramago *Eruca sativa*, sangre de grado *Jatropha dioica*, huizache *Acacia farnesiana,* mezquite *Prosopis glandulosa*, gobernadora *Larrea tridentata*, maguey *Agave* sp., pirúl *Schinus molle* y girasol *Helianthus annuus*. Para cada sitio de colecta se registraron las coordenadas de geoposicionamiento, características de las plantas y condiciones agroambientales; éstas se describen en el Cuadro 1.

Cuadro 1. Características y ubicación de las especies colectadas en el estudio.

Estado	Municipio	Latitud (Norte)	Longitud (Oeste)	Altitud (m)	Planta silvestre
AGS	Cosío	22º23´39.8"	102º18´23.4"	2008	*Datura inoxia*
AGS	San José de Gracia	22º08´36.9"	102º21´26.4"	1980	*Helianthus annuus,* *Apodanthera undulata*
AGS	Tepezalá	22º13´33.2"	102º14´14.2"	1887	*Xanthium strumarium,* *Datura ferox,* *Bidens odorata*
SLP	Ahualulco	22º19´38.8"	101º12´15.3"	1862	*Proboscidea louisianica*
SLP	Salinas	22º43´56.0"	101º42´40.0"	2096	*Bidens odorata,* *Eruca sativa*
SLP	Salinas	22º37´32.6"	101º41´15.0"	2110	*Proboscidea louisianica,* *Helianthus annuus,* *Eruca sativa,* *Bidens odorata,* *Xanthium strumarium*
SLP	Salinas	22º37´10.3"	101º43´58.9"	2079	*Acacia farnesiana*
SLP	Salinas	22º35´59.9"	101º42´07.0"	2115	*Larrea tridentata,* *Jatropha dioica*
SLP	Mexquitic	22º19´38.8"	101º12´15.0"	1870	*Bidens odorata,* *Brassica sp,* *Proboscidea louisianica*
SLP	Mexquitic	22º16´23.4"	101º05´35.0"	1980	*Leonotis nepetifolia*
SLP	Venado	22º55´54.3"	101º04´25.6"	1755	*Bidens odorata,* *Xanthium strumarium*

Cuadro 1. Características y ubicación de las especies colectadas en el estudio. (Continuación).

Estado	Municipio	Latitud (Norte)	Longitud (Oeste)	Altitud (m)	Planta silvestre
ZAC	Zacatecas	22º45´26.9"	102º33´51.1	2418	*Bidens odorata,* *Helianthus annuus,* *Datura inoxia*
ZAC	Zacatecas	22º45´51.4"	102º38´21.2"	2325	*Jatropha dioica*
ZAC	Zacatecas	22º46´00.3"	102º35´03.8"	2444	*Argemone mexicana*
ZAC	Guadalupe	22º45´04.2"	102º27´10.4"	2193	*Cucurbita foetidissima*
ZAC	Loreto	22º16´19.3"	101º57´30.4"	2047	*Schinus molle*
ZAC	Guadalupe	22º40´53.5"	102º33´27.1"	2376	*Prosopis glandulosa* *Agave sp.*
ZAC	Genaro Codina	22°29´13.65"	102°27´48.52"	2176	*Cucurbita ficifolia*

Estimación de la productividad en campo

Para determinar su productividad en campo, una vez localizada la planta, se siguió la metodología propuesta por Mostacedo y Fredericksen (2000), quienes recomiendan el método de muestreo por parcela cuadrada de 1 m x 1 m para plantas herbáceas, de 5 m x 5 m para rastreras y de 10 m x 10 m para arbustivas y arbóreas. En cada una de las parcelas se estimó la cobertura, densidad y frecuencia de plantas, así como el número de semillas por planta, de la totalidad de las plantas contenidas en la parcela respectiva. Se emplearon las variables densidad y número de semillas por planta para estimar el rendimiento potencial de semilla extrapolado a una hectárea en condiciones naturales.

Caracterización morfológica de las semillas

Las semillas limpias se secaron a 60°C por un periodo de 18 h. Posteriormente, se registró el peso (g) de 100 semillas tomadas al azar, de cada una de las especies consideradas en el estudio, mediante una balanza analítica (Vlab V300®). A 100 semillas de cada una las especies colectadas se les midió largo, ancho y espesor (cm) utilizando un vernier, de acuerdo a la metodología propuesta por Pérez *et al.* (2006).

Extracción y caracterizacion fisicoquimica de los aceites

La extracción química de aceite se realizó utilizando un determinador de grasa y aceite (Soxtec System HT 1043), de acuerdo a la técnica propuesta por Loredo *et al.,* (2012). Una vez extraidos los aceites se procedió a llevar a cabo la determinación de los siguientes parámetros, tomando en consideración las Normas Mexicanas (NMX): índice de refracción (NMX-F-074-S 1981), índice de saponificación (NMX-F-174-S-1981), índice de acidez (NMX-F-101-1987), índice de yodo (NMX-F-408-S-1981) e índice de peróxidos (NMX-F-154-1987).

Análisis estadístico

Los resultados obtenidos fueron expresados como la media de tres experimentos independientes ± desviación estándar. Para rendimiento potencial de semilla y aceite se realizaron análisis de varianza, el diseño experimental fue completamente al azar y se aplicó la prueba de Tukey ($p \leq 0.05$) utilizando el programa SAS (Statiscal Analysis Software, V9.1).

Resultados y discusión

Colecta de semilla

Se realizaron recorridos de campo en diferentes sitios del Altiplano centro-norte del país, en busca de especies de plantas, que tuvieran potencial para la producción de aceite. Las especies colectadas se distribuyeron en altitudes de 1755 a 2444 msnm. Se observó que la mayoría de las semillas de las plantas colectadas presentan características ruderales de ciclo anual, las cuales se encontraron a la orilla de la carretera, terrenos baldíos o como invasoras de campos de cultivo.

Rendimiento potencial de semilla y de aceite

El análisis estadístico realizado muestra que existe diferencia estadística para el rendimiento potencial de semilla y rendimiento de aceite, en función de las especies vegetales en estudio ($p \leq 0.05$). *Agave* sp., resultó superior y estadísticamente diferente (DSH=2457) al resto de las especies, al registrar un rendimiento potencial de semilla de 24305 kg ha^{-1}. No obstante, ésta representa un caso especial dado que en las especies de porte alto de este tipo de plantas la maduración puede ocurrir entre los 10 y 25 años, mientras que en las de porte bajo de esta especie se puede presentar entre los cuatro y cinco años (García-Mendoza, 2002). En la Figura 1 se destaca que cuatro especies registraron rendimientos potenciales de semilla superiores a 1000 kg ha^{-1}, pero sobresalen, además de *Agave* sp., *A. mexicana, A. undulata y P. louisianica.*

Por el contrario, siete especies presentaron valores de rendimiento de semilla menores a 100 kg ha^{-1}, destacando entre ellas *L. tridentata* con sólo 0.8 kg ha^{-1}, a pesar de haber registrado una de las densidades más altas. Un importante grupo de especies se ubicaron con rendimientos potenciales de semilla intermedios, con excepción de *P. glandulosa,* todas ellas son de porte medio-bajo, herbáceas y rastreras.

Respecto a *B. odorata,* resalta que a pesar de registrar una densidad alta por unidad de superficie y de contar con un alto número de semillas, no se reflejó en su rendimiento potencial debido al bajo peso de éstas. Similar comportamiento se observó con *L. tridentata* dado que sus semillas son muy livianas. En *X. strumarium* su reducido rendimiento pudo estar asociado a la presencia de un bajo número de semillas (dos semillas por fruto); sin embargo, es una especie a considerar ya que su semilla contiene 20.4% de aceite. En el caso particular de *Brassica* sp., su bajo rendimiento pudo ser resultado de su baja densidad y reducido tamaño de semillas; no obstante, posee un alto porcentaje de aceite (30.3%) lo cual resulta interesante, sobre todo sí se considera pueda cultivarse de manera intensiva a fin de incrementar su rendimiento en semilla. En otras especies oleaginosas cultivadas se han observado resultados muy contrastantes en cuanto a rendimientos, sobre todo si se considera que muchos de ellos han sido obtenidos en condiciones experimentales.

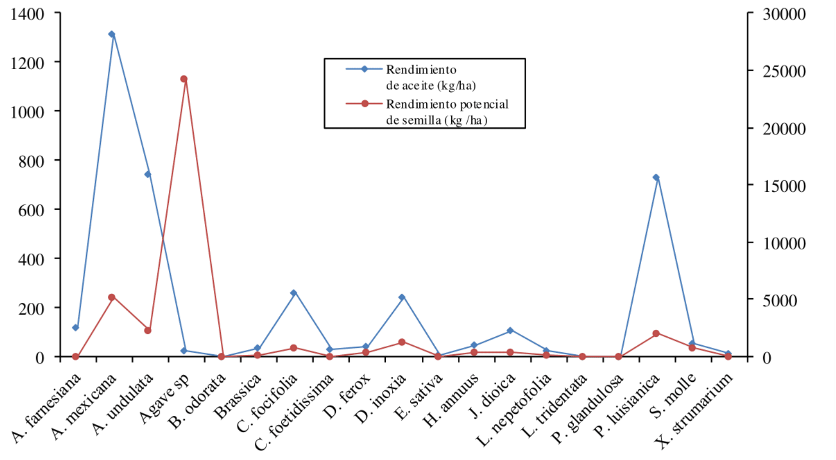

Figura 1. Relación entre rendimiento potencial de semilla y contenido de aceite.

Los resultados obtenidos para rendimiento de aceite de las especies estudiadas se muestran en la Figura 1. *A. mexicana* resultó la especie con el rendimiento más alto de aceite con 1315 kg ha^{-1}, estadísticamente diferente al resto de las especies (DSH=773.2), esta especie resultó tan competitiva o mejor que algunos cultivos comerciales empleados actualmente en la producción de biodiesel, tales como soya *Glycine max* (335 kg ha^{-1}), girasol *Helianthus annuus* (568 kg ha^{-1}), cacahuate *Arachis hypogaea* (712 kg ha^{-1}), colza *Brassica napus* (832 kg ha^{-1}), piñón *Jatropha curcas* (950 kg ha^{-1}), higuerilla *Ricinus communis* (1133 kg ha^{-1}), tung *Aleurites fordii* (1204 kg ha^{-1}) según lo registran Martínez-Valencia *et al*., (2011). *A. undulata* y *P. lousianica* mostraron rendimientos interesantes de aceite de 743 y 730 kg ha^{-1}, estos son derivados de su gran número de frutos, tamaño y cantidad semillas y sobre todo de sus contenidos de aceite superiores a 30%.

Caracterización física y peso de las semillas

Los resultados obtenidos para la caracterización física y peso de las semillas en estudio son presentados en el Cuadro 2.

Cuadro 2. Características físico-químicas de los aceites vegetales.

Semillas	Largo (mm)	Ancho (mm)	Espesor (mm)	Peso 100 semillas (g)
A. mexicana	1.85 ± 0.11	1.73 ± 0.01	1.67 ± 0.10	0.26 ± 0.01
A. undulata	10.72 ± 0.75	8.34 ± 0.59	3.69 ± 0.54	10.60 ± 0.21
A. farnesiana	6.35 ± 0.18	5.86 ± 0.56	4.12 ± 0.23	12.64 ± 1.26
Agave sp.	10.34 ± 0.27	7.30 ± 0.43	0.01 ± 0.03	0.83 ± 0.05
B. odorata	10.22 ± 2.27	0.84 ± 0.15	0.59 ± 0.12	0.14 ± 0.01
Brassica sp.	1.51 ± 0.18	1.36 ± 0.70	1.20 ± 0.30	0.08 ± 0.08
C. ficifolia	17.32 ± 0.30	10.73 ± 0.25	2.65 ± 0.26	3.70 ± 0.04
C. foetidissima	9.62 ± 0.68	2.28 ± 0.36	1.58 ± 0.44	17.31 ± 0.04
D. ferox	3.74 ± 0.31	2.91 ± 0.26	1.49 ± 0.15	0.80 ± 0.03
D. inoxia	4.52 ± 0.63	3.31 ± 0.42	1.31 ± 0.01	0.94 ± 0.02
E. sativa	1.56 ± 0.11	1.24 ± 0.11	0.77 ± 0.14	0.07 ± 0.01
H. annuus	5.64 ± 0.68	2.28 ± 0.36	1.58 ± 0.44	0.63 ± 0.06
J. dioica	11.61 ± 0.92	10.17 ± 0.85	9.66 ± 0.53	154.60 ± 1.95
L. nepetifolia	3.91 ± 0.31	1.48 ± 0.14	1.01 ± 0.18	0.18 ± 0.01
L. tridentata	5.41 ± 0.71	1.59 ± 0.29	2.26 ± 0.33	0.51 ± 0.03
P. louisianica	8.75 ± 0.58	5.10 ± 0.36	2.90 ± 0.37	3.20 ± 0.08
P. glandulosa	5.56 ± 0.30	4.28 ± 0.40	2.40 ± 0.18	3.53 ± 0.06
S. molle	4.51 ± 0.48	4.27 ± 0.45	4.01 ± 0.63	2.65 ± 0.02
X. strumarium	13.75 ± 2.45	4.11 ± 0.61	2.21 ± 0.43	5.61 ± 0.04

Los resultados son expresados como la media de tres réplicas ± desviación estándar.

En cuanto al parámetro “largo” de las semillas evaluadas, los valores (mm) promedio oscilan desde 1.51 ± 0.18 para *Brassica* sp., hasta 17.32 ± 0.30 para *C. ficifolia*. En relación, al “ancho” de las semillas, se determinó en *B. odorata* el promedio menor (mm) con 0.84 ± 0.15 y *C. ficifolia* el valor mayor (mm) con 10.73 ± 0.25. En cuanto a la característica “espesor”, *J. dioica* presentó el promedio mayor (9.66 ± 0.53 mm) con respecto a las semillas de las otras especies en estudio tales como *Agave* spp (0.01±0.03), *B. odorata* (0.59 ± 0.12 mm) y E. sativa (0.77 ± 0.14). Finalmente, el “peso de 100 semillas” presentó una gran variabilidad entre las especies en estudio, las semillas de *J. dioica* resultaron las más pesadas, al registrar un valor promedio de 154.60 ± 1.95 g, mientras que el resto mostraron valores que oscilaron entre 0.26 y 17.31 g por cada 100 semillas. Para todos los casos, los valores de desviación estándar registrados, resultaron bajos, lo que confirma que en los tres parámetros evaluados (largo, ancho y espesor) no existe variabilidad alta en las dimensiones de las semillas de una misma especie. Sin embargo, sí se aprecia ésta en las dimensiones de las semillas de las diferentes especies.

Contenido de aceite de las semillas en estudio

En la Figura 2, se presentan los porcentajes de aceite obtenidos de las semillas colectadas. Se destaca que seis especies registraron contenidos mayores a 30%, entre las que destacan: *C. foetidissima* (33.9%), *P. louisanica* (33.6%), *J. diodica* (32.86%), *C. ficifolia* (31.8%), *A.*

undulata (31.40%) y *Brassica* sp. (30.33%). Asimismo, se aprecia que seis de las especies colectadas obtuvieron un porcentaje de aceite entre 10 y 30% y siete resultaron con valores de aceite menores al 10%. Estos contenidos, en general, son menores a los registrados por Martínez-Valencia *et al.* (2011) para oleaginosas cultivadas comercialmente tales como: girasol *Helianthus annuus* (45-55%), canola *Brassica napa* (40-44%), palma *Elaeis guineensis* (44-57%), coco *Cocus nucifera* (65-75%), cacahuate *Arachis hypogaea* (48-50%) y cártamo *Carthamus tinctorius* (35-40%); o al contenido de aceite de entre, 41.52 a 51.04% obtenidos por Vasco-Leal *et al.* (2017) en doce accesiones de higuerilla procedentes de los estados de Aguascalientes, Jalisco, San Luis Potosí y Zacatecas, México.

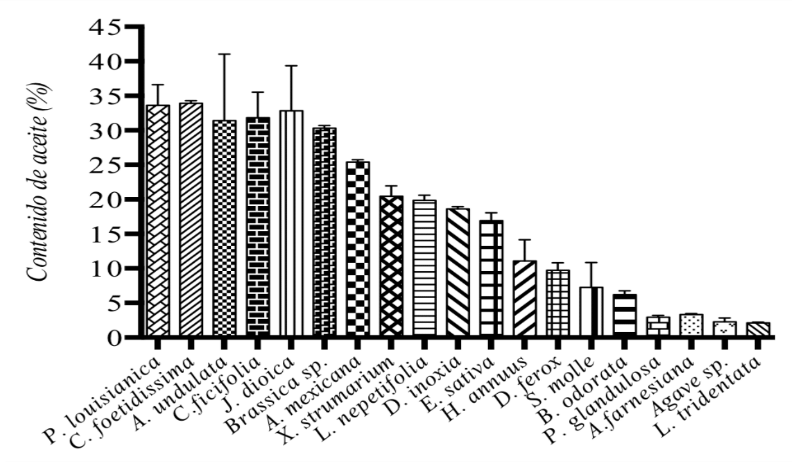

Figura 2. Porcentaje de aceite obtenido de las especies de plantas colectadas.

Caracterización fisicoquímica de aceites

La calidad y la eficiencia del biodiesel dependen del proceso y la calidad del aceite generada por la materia prima; es decir, aceites con baja concentración de ácidos grasos libres, altos en ácidos grasos monoinsaturados y sin gomas e impurezas, entre otras propiedades fisicoquímicas (Martínez-Valencia *et al.*, 2011). Por su parte, Martínez-Sánchez *et al.* (2015) señalan que las características químicas más usadas para la clasificación y determinación de la calidad comercial de los aceites son: índice de yodo, saponificación, peróxidos y acidez; dentro de las características físicas destacan la gravedad específica, el índice de refracción, densidad y el punto de fusión. En el Cuadro 3 se presentan resultados de las características fisicoquímicas de los aceites estudiados.

Cuadro 3. Principales ácidos grasos presentes en los aceites de las especies estudiadas.

Especie	IR	IS (mg KOH g^{-1})	IY ($g\ I_2\ 100\ g^{-1}$)	IP (meq kg^{-1})	IA (% ácido oleico)
A. farnesiana	1.4768	ND	60.68 ± 2.78	ND	ND
A. mexicana	1.4730	119.31 ± 1.42	117.94 ± 1.75	0.39 ± 0.10	11.71 ± 0.02
A. undulata	1.4870	141.48 ± 0.24	123.80 ± 4.54	0.75 ± 0.10	11.25 ± 0.17
Agave sp.	1.4762	ND	ND	0.74 ± 0.05	ND
B. odorata	1.4725	149.81 ± 3.43	96.57 ± 0.86	1.62 ± 1.41	ND
Brassica sp.	1.4715	174.17 ± 0.37	93.82 ± 0.32	0.79 ± 0.04	3.13 ± 0.01
C. ficifolia	1.4748	ND	73.12 ± 1.34	1.08 ± 0.01	0.68 ± 0.22
C. foetidissima	1.4750	180.02 ± 0.05	141.26 ± 22.79	0.47 ± 0.05	11.51 ± 0.03
D. ferox	1.4700	142.31 ± 2.42	ND	ND	29.66 ± 0.19
D. inoxia	1.4720	146.22 ± 0.34	114.95 ± 1.11	1.06 ± 0.01	8.70 ± 0.43
E. sativa	1.4780	129.64 ± 3.37	97.59 ± 0.16	ND	4.24 ± 0.01
H. annuus	1.4723	152.27 ± 3.20	122.29 ± 2.95	0.77 ± 0.05	7.69 ± 0.01
J. dioica	1.4725	ND	113.49 ± 2.58	ND	ND
L. nepetifolia	1.4658	149.93 ± 2.08	88.47 ± 1.20	0.47 ± 0.05	36.17 ± 0.72
P. glandulosa	1.4757	ND	ND	ND	ND
P. louisianica	1.4735	182.83 ± 4.43	116.65 ± 0.32	0.72 ± 0.05	3.50 ± 0.01
S. molle	1.4850	ND	99.40 ± 0.85	0.79 ± 0.05	26.80 ± 0.55
X. strumarium	1.4750	ND	126.63 ± 2.23	0.67 ± 0.05	1.79 ± 0.37

Los resultados son expresados como la media de tres réplicas ± desviación estándar.
IR = Índice de refracción, IS = Índice de saponificación, IY = Índice de yodo,
IA = Índice de acidez, IP = Índice de peróxidos, ND= No determinado.

Índice de refracción (IR)

En el Cuadro 3, se presenta el índice de refracción determinado para los aceites de las especies en estudio, los cuales muestran valores entre 1.4658 y 1.4870; éstos se encuentran dentro del rango de los siguientes resultados obtenidos en aceites provenientes de otras semillas oleaginosas: higuerilla (1,4764 – 1,4778), soya (1,466 – 1,47) y valores promedio mayores comparados con las siguientes materias primas de aceite: piñón (1,4680), algodón y cacahuate (1,460 – 1,465), girasol (1,467 – 1,469), sésamo (1,465 – 1,469), babasú (1,448 – 1,451) de acuerdo a lo señalado en la literatura (Anvisa, 1999; Pinhão manso, 2005; Proquinor, 2003).

Índice de saponificación (IS)

De acuerdo a los resultados obtenidos, las especies con mayor índice de saponificación presentaron valores de 182.83, 180.02 y 174.17 mg KOH g^{-1} (*P. louisiana* > *C. foetidissima* > *Brassica sp.*), mientras los valores de las especies con menor índice fueron de 119.31 y 129.64 (*A. mexicana* < *E. Sativa*). Danlami *et al.,* (2015) observaron valores promedio similares para aceite de *R. communis* (174.6 mg KOH g^{-1}). De igual forma, Yong y Salimon (2006) obtuvieron valores para *Elateriospermum tapos* de 150.90 mg KOH g^{-1}. Resultados similares se obtuvieron en este estudio para las especies de *B. odorata, H. annuus y L. nepetifolia.* Por otra parte, en la industria de los cosméticos, Ruiz y Huesa (1991) observaron valores de IS para el aceite de Karité (*Butyruspermum parki*) de 180-190 mg KOH g^{-1}; dichos valores son similares con los aceites de *C. foetidissima* y *P. louisianica*, lo que posibilita su potencial uso en la industria de los cosméticos. Asimismo, Cruz *et al.* (2015) obtuvieron en *J. curcas*, uno de los aceites más empleados en la obtención de biodiesel con promedios que oscilaron entre 192 y 196 mg KOH g^{-1}, sólo *P. louisianica* registró estos valores en nuestro estudio.

Índice de Yodo

El índice de yodo (IY) evaluado en los aceites de las especies colectadas oscilaron entre 60.68 a 141.26 g I_2 100 g^{-1} (Cuadro 3). Al comparar estos promedios con aquellos aceites vegetales provenientes de otras semillas oleaginosas, Freire (2001) determinó promedios en aceite de *R. communis,* entre 81 a 91 g I_2 100 g^{-1} y Cecchi (2003) en aceite de *Glycine max* registraron valores entre 120 a 141 g I_2 100 g^{-1}; de esta manera se evidencia que cada especie posee un valor característico, el cual podría depender de la variedad y del método empleado en su determinación. De acuerdo, a Saraf y Thomas (2007), este parámetro influye directamente en la calidad del biodiesel, debido a que altos valores de este índice en el aceite, pueden traducirse en una mayor tendencia a la oxidación, contribuir a la formación de gomas en el motor y a la disminución de la lubricidad.

Índice de Peróxidos

Los peróxidos son conocidos como compuestos de la descomposición primaria de la oxidación de las grasas y aceites (Gómez, 2010), por lo que aquellos valores de índice de peróxido (IP) cercanos a 0 meq kg^{-1}, se relacionan con un nivel de rancidez bajo; es decir, se oxidan lentamente permitiendo que el aceite conserve por más tiempo su calidad, dándoles una ventaja para su posible utilización posterior. Un valor alto de IP indica la presencia de oxidación; en este estudio, vale la pena resaltar que los aceites de la mayoría de las especies vegetales estudiadas presentaron un IP menor a 1. De acuerdo con Luna-Guevara y Guerrero-Beltrán (2012) el IP y el IA son considerados indicadores de calidad y frescura de los aceites.

Índice de Acidez

En el Cuadro 3, se aprecia que el aceite de las especies con IA menor a 5% de ácido oleico son *C. ficifolia* (0.68%), *X. strumarium* (1.79%), *Brassica* sp. (3.13%), *P. louisianica* (3.50%) y *E. sativa* (4.24%), mientras *S. molle, D. ferox, L. nepetifolia,* presentan valores de 26.80, 29.66 y 36.17% respectivamente. Esta determinación es indispensable para la elaboración de biodiesel y aceites de uso alimentario o industrial.

Conclusiones

Las especies silvestres estudiadas presentaron características agroproductivas distintivas interesantes en cuanto a productividad de semilla y contenido de aceite. Seis especies (*C. ficifolia, C. foetidissima, A. undulata, J. dioica*, *P. louisianica y Brasica* sp) obtuvieron porcentajes superiores al 30%, por lo que se les considera de interés en la producción de aceite. Considerando las características físicas y químicas de los aceites se sugiere su potencial utilización no solo en la industria de biocombustibles, sino su posible empleo en la industria alimenticia, farmacéutica y cosmetológica, así como en la obtención de productos de mayor valor agregado.

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III.4 Optimization of castor seed oil extraction process using response Surface methodology

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Optimization of castor seed oil extraction process using response surface methodology

Optimización del proceso de extracción de aceite de semillas de ricino utilizando la metodología de superficie de respuesta

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ABSTRACT

This work focuses on the study of the oil extraction yield from castor seed using three different seed conditions: whole, minced and bare endosperm. Taguchi design was used to determine the contribution of the following parameters: seed condition, seed load in the extractor, temperature, and pressure. It was proved that it is necessary to introduce the whole seed and that the presence of the pericarp increases the extraction yield. The contribution of the control factors has an extraction yield limit. After determining which factors contributed to the process, these were left at their optimum levels aiming to reduce the control factors to only two. The complete analysis was done using a surface response methodology giving the best parameter for temperature and pressure that allows a better yielding mechanical extraction. The oil extraction yield can be kept up to 35% of the seed.

Keywords: Experiment design, mechanical extraction, *Ricinus communis L.*

RESUMEN

Este trabajo se centra en el estudio de la extracción y la producción de aceite de semillas de ricino usando tres diferentes estados de semillas: entera, partida y sin testa. Se utilizó un diseño Taguchi para determinar la contribución de los siguientes parámetros: estado de las semillas, cantidad de semilla en el extractor, temperatura y presión. Se demostró que es necesario introducir la semilla entera para aumentar el rendimiento de la extracción. La contribución de los factores de control tiene un límite de extracción en la variable respuesta (rendimiento). Después de determinar cuáles factores contribuían altamente al proceso de extracción y cuáles no, se procedió a dejarlos en los niveles de mayor contribución con la finalidad de reducir el número de factores de control a dos. Posteriormente se utilizó la metodología de superficie de respuesta para la optimización del proceso, dando como resultado los niveles óptimos para los factores de control temperatura y presión. De acuerdo con las pruebas, el rendimiento de la extracción del aceite se puede mantener en un 35% de la semilla.

Palabras clave: Diseño de experimentos, extracción mecánica, *Ricinus communis L.*

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Introduction

Castor bean (*Ricinus communis* L.) is a shrub that originates from Africa. Its seed is commonly known as 'higuerilla', 'ricine' or 'mamona' (Ali *et al.*, 2008;Scholz & Da Silva 2008). The optimal growth conditions are loamy to sandy loam soils and temperatures ranging from 20 to 30 °C. In addition, the annual rainfall should be between 700 and 1500 mm (Valderrama *et al.*, 1994; Ogunniyi, 2006) for the optimal development of the plant. Nevertheless, it adapts to tropical, subtropical, and semiarid conditions, tolerating extreme environmental stresses, including high temperatures and low water availability. At present, the oil extracted from its seeds has many different applications, which include but are not limited to: hydraulic oil, paint thinner, emulsifier, varnishes, pharmaceutical applications, organic fertilizers, biological pest control, manufacture of polymers, and dyes (Ogunniyi, 2006; Lorestani *et al*. 2012), with biodiesel production being another well-known use (Kılıç *et al.,* 2013).

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Oil from castor bean seeds may undergo changes according to the extraction procedure (Ortiz *et al*., 2003). A particularly noticeable change is the modification in the free fatty acid content when heat is used during extraction. Heating around 100 °C generates triricinoleine decomposition of ricinoleic acid and diricinoleine, resulting in increased oil acidity (Salimon *et al*. 2010).

The transesterified biodiesel obtained from *Ricinus communis* L. oil is a potential alternative for solving problems associated with biofuels produced from other sources (first-generation oil, used cooking oils, waste animal fats, etc.), which have shown difficulties in their cold flow properties and oxidative stability, generating storage problems (Perdomo *et al*., 2013).

Hincapié *et al.* (2011) used castor oil to produce biodiesel by transesterification, obtaining a conversion rate of 74,9 % when potassium carbonate was used as a catalyst, and 66,2 % when the catalyst was hemimorphite. On the other hand, Montoya *et al.* (2010) used the response surface method to optimize the ethanolysis process, achieving a transesterification rate of 93,63 %. These authors have focused their research mainly on the transesterification process, but have not considered the oil extraction procedure from seeds.

Rios *et al.* (2007) compared three procedures for oil extraction from cardamom seeds. Fontal (2007) worked on oil extraction by extrusion from coffee seeds, carrying out sensorial tests and the analysis of the volatile compounds in the oil, without considering the oil extraction process. Perdomo *et al.,* (2013) characterized physically and chemically seeds of seven castor bean accessions from central Mexico; also, seed oil was extracted by three different methods (chemical solvent, cold pressing, and warm pressing).

Methods for vegetable oil extraction from seeds

Several processes can be used to obtain oil. The most common oil extraction procedures are classified as: expeller pressing, hydraulic pressing, and solvent-dependent extraction.

The expeller-pressing procedure is widely used in the food industry; raw materials are squeezed under high pressure, either in one step or in batches (Pradhan *et al*., 2011). The extraction is based on a screw that presses the raw materials against the walls of a metallic cylinder; oil is recovered through a mesh that do not allow the passage of solids (Evangelista 2009). The system is commonly equipped with a temperature control device to avoid any damage to the oil that may affect its properties.

Hydraulic pressing is somehow similar to the expeller pressing method, but it yields higher quality oil, and it is more economical at an industry level (Sriti *et al.,* 2011); however, it works only for seed batch extraction. In this case, a hydraulic piston is used to press raw materials against a rigid surface with small openings to separate oil and solid residues. Extraction may be done at room temperature or under increased seed temperature; in the second case, oil yield is enhanced, but oil chemical properties may be altered (Perdomo *et al*. 2013).

Solvent-extraction methods are based on the addition of organic liquid chemicals to ground seeds, and the mixture is then filtered and subsequently heated to around 150 °C to remove solvents by evaporation (Qian *et al.,* 2010). Due to the use of solvents and high temperature, this is a high-cost method, and it is used mostly at a laboratory level.

The aim of this research was to optimize the extraction process of oil from *Ricinus communis* L. using a hydraulic press, and the techniques of experimental designs.

Experimental procedure Material

The seed accession used in this study was collected in the State of Queretaro, Mexico, and named VQ-4 (Perdomo *et al.,* 2013). In terms of experiment design, Figure 1a shows a complete seed ('whole') that defines one of the conditions used for the oil extraction; Figure 1b presents the second seed condition, defined as 'minced'; and Figure 1c defines the third condition, designated as 'bare endosperm'. These three conditions of the seed were used to feed the oil extractor.


(a) (b) (c)

Figure 1. a) Whole seed, b) broken, and c) bare endosperm *Ricinus communis* L.

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Oil extraction equipment

Oil extraction was carried out with a temperature-controlled stainless steel prototype, with a maximum load capacity of 400 g. The extraction device has a cylinder to enclose the seeds and a plunger for pressure application. The prototype is assembled with a hydraulic press (Mikel, México), with a maximum pressure capacity of 68,65 MPa and a 150-mm piston. The system has a thermal casing with a commercial strength of 1000 W at 127 V, and a data logger connected to a k-type thermocouple for temperature-controlled oil extraction.

Oil extraction performance

Immediately after extractions, oil was centrifuged twice at 1300 rpm for 16 min to remove debris.

Kinetic Viscosity

Determination of the oil rheological behavior was done with a Rheomat viscometer (Mettler Toledo, USA). Samples were heated to 40 °C, and measurements were run in triplicate using the standard procedure D445 of the American Society of Testing and Materials (ASTM), designed to determine kinematic viscosity of transparent and opaque liquids.

Color

Oil color was measured with a Minolta colorimeter Model CR300 (Konica Minolta, Japan), according to ASTM D1500-12.

Turbidity

Turbidity was determined with a Hanna Instruments turbidimeter (Hanna, Italia) set with an infrared mode (Lau *et al.*, 2000).

Statistical Analysis

Statistical analysis was performed from a Taguchi experimental design, with an orthogonal array of L9, with two replications (Taguchi, 1987; Miranda, 2009). Temperature values were: 20 °C, 40 °C, and 60 °C; pressure levels were 22,85 MPa, 34,32 MPa, and 45,80 MPa; seed materials used were 100 g, 200 g, and 300 g, for the three seed conditions: whole, minced, and bare endosperm (Figure 1). Analysis of variance ($\alpha=0{,}05$) was used to determine statistical significance of response variables. With the information collected through the Taguchi design, correlation and principal components analyses were performed (Esbense & Geladi, 2009).

The response surface methodology proposed for the analysis, is a central composite design (CCD) consisting of two factors with two blocks, three points central, three points axial cube, with two replications, run randomly (Montgomery, 2013). The central point is replicated several times to provide an independent estimate of the experimental error; α used for this design was 1,414. Temperature factor ranged from 40 °C to 60 °C, and pressure from 22,85 MPa to 45,80 MPa.

Results and discussion

Taguchi design

Table 1 shows the results for Taguchi design for turbidity, viscosity, color, and extraction yield. Regardless of the combination between pressure and temperature, no oil is extracted at all when bare endosperm is used, suggesting that seed coat is required for oil percolation to the collector within the extraction prototype.

Table 1. Results obtained from Taguchi design.

Run	Temp (°C)	Pressure (MPa)	Sample weight (g)	Seed condition	Turbidity (NTU)	Viscosity (Pa.s)	Color (c)	Extraction yield (%)
8	20	22,85	100	1	188,250	0,330	9,55	20,44
4	20	34,32	200	2	212,500	0,360	10,37	13,62
1	20	45,8	300	3	0,000	0,000	0,00	0,00
9	40	22,85	200	3	0,000	0,000	0,00	0,00
3	40	34,32	300	1	194,400	0,340	12,26	30,69
5	40	45,8	100	2	204,500	0,350	11,70	30,65
7	60	22,85	300	2	228,000	0,370	16,25	31,95
2	60	34,32	100	3	0,000	0,000	0,00	0,00
6	60	45,8	200	1	191,600	0,330	14,52	35,55

Seed condition (1) Whole, (2) Minced, (3) Bare endosperm.

The effects of temperature (a), pressure (b), sample weight (c), and seed conditions (d) on extraction yield are presented in Figure 2. There is a slight increase in oil extraction (Figure 2a) when the temperature increases from 20 °C to 60 °C because temperature facilitates the breaking of fatty acids in the seed, allowing the exudation of oil. Figure 2b presents the extraction yield as a function of the pressure, showing an increase in the extraction yield (from 34,32 MPa to 45,80 MPa). Figure 2c shows that increasing the sample weight had just a little contribution to the extraction yield (6 % -100g to 300g). It can also be noticed a significant decrease in the extraction performance affected by the condition of the seed, with no oil extraction at all from bare endosperms Figure 2d; this leads to the inference that the seed coat is required for oil percolation from the top of the collector cylinder to the filter. As it happened with bare endosperm, minced seed (broken) also had a decrease in oil extraction yield, compared to the whole seed, which presented the higher extraction values. In terms of the industry, these results show that it is necessary to use the whole seed in order to increase the extraction yield, suggesting that the paths formed by the pericarp facilitates oil extraction.

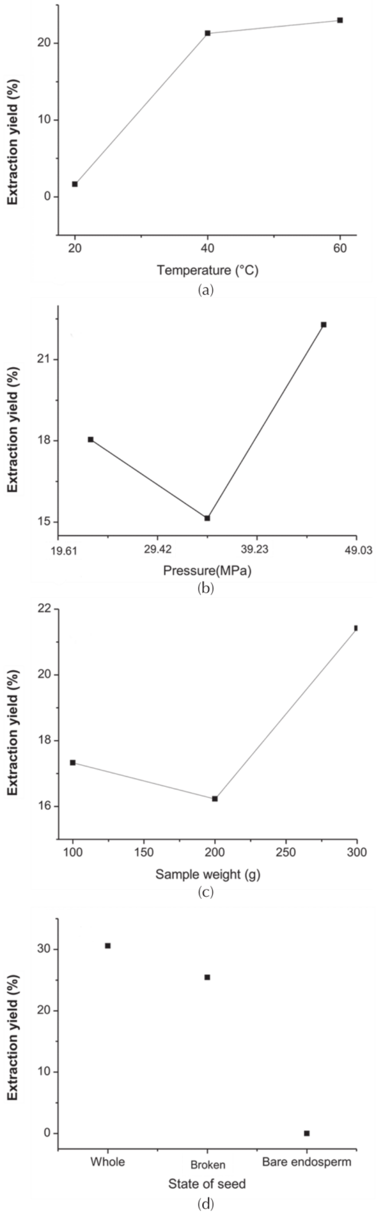

Figure 2. Effects of extraction factors.

The contribution of each factor to oil extraction yield was as follows: seed condition (82 %), temperature (12 %), pressure (4 %) and seed amount in the container (2 %). The extraction of oil increases when the process involves the whole seed and also when the container temperature is increased.

Correlation and principal component analysis

The percentages of correlation between the response variables were: very high, with four iterations over 95 % (viscosity-turbidity, color, turbidity, color-performance, and color-viscosity), and two iterations being less than 90 % (oil extraction performance-turbidity, viscosity-yield) although over 85 %. Thus, raising the level of either variable will have a positive effect on the remaining three variables.

Table 2 shows the principal components for analysis; for the first component (PC1), variation is explained by 95,7 %, and the correlations of the variables are positive, although these are low. In the second component, variation is explained by 99,4 %, and the correlations changed significantly. Then, the experiment contains two major components.

Table 2. Principal component analysis.

Eigenvalue	3,827	0,1500	0,022	0,000
Proportion	0,957	0,0380	0,006	0,000
Accumulated	0,957	0,9940	1,000	1,000
Variable	PC1	PC2	PC3	PC4
Oil extraction	0,487	0,765	-0,410	-0,093
Turbidity	0,503	-0,456	-0,088	-0,729
Viscosity	0,503	-0,430	-0,355	0,660
Color	0,506	0,145	0,835	0,158

When considering two principal components, the correlation matrix shows that increasing the system performance could decrease oil turbidity and viscosity. This type of relationship has also been observed using a supercritical fluid process (Sheibani & Ghaziaskar 2008). Those decreased oil properties could affect the use of castor bean oil for biodiesel production, since low viscosity and acidity are recommended for that purpose (ASTM D6751).

Response surface methodology

For the implementation of the central composite design with two controllable factors, the 'whole' seed condition is used for the high contribution (82 %), and a load of 100 g of seeds, to increase oil extraction performance (Table 2).

The controllable factors are: temperature between 40 °C and 60 °C, and pressure from 22,85 MPa to 45,70 MPa. Table 3 presents the design used with data obtained for each performed run.

Using the average values for extraction showed in Table 3, and the Kolmogorov-Smirnov statistic test with 95 % confidence, it was shown that data collected for performance follows a normal distribution with a p-value of 0,090 (Izraelevitz *et al.*, 2011). For standard deviation, Bonferroni confidence intervals of 95 % were performed for each level. Using the statistics proposed by Bartlett, values are greater than $\alpha = 0{,}05$, stating that it does not have problems of heteroskedasticity.

Table 3. Experimental design runs for central composite design.

Experiment (rum)	Temperature		Pressure		Average extraction yield (%)	Average viscosity (Pa.s)
	Encrypted units	(°C)	Encrypted units	(MPa)		
14	−1	40	−1	22,9	20,166	0,391
11	1	60	−1	22,9	27,849	0,481
9	−1	40	1	45,8	26408	0,473
10	1	60	1	45,8	33,611	0,511
12	0	50	0	34,3	31,210	0,479
13	0	50	0	34,3	31,210	0,462
8	0	50	0	34,3	31,219	0,466
7	−1,41	36	0	34,3	24,008	0,484
2	1,41	64	0	34,3	33,611	0,452
6	0	50	−1,41	18,1	20,166	0,46
5	0	50	1,41	50,6	32,65	0,456
4	0	50	0	34,3	29,769	0,468
3	0	50	0	34,3	31,210	0,529
1	0	50	0	34,3	28,809	0,466

Table 4 presents the iteration pressure*temperature, which had no significant effect on the response variable studied; therefore, it was excluded from the analysis. The response variable had a quadratic response (p-value 0,001), in which both pressure and temperature showed an effect, and had a quadratic relationship.

Table 4. ANOVA for central composite design.

Source of Variations	Degree of freedom	Adjusted sum of squares	Adjusted mean square	F ratio	P value
Regression	5	253,64	20,067	39,78	0,000
Linear	2	40,13	34,997	15,74	0,002
Pressure	1	34,99	7,28	27,44	0,001
Temperature	1	7,28	21,14	5,71	0,044
Square	2	42,29	36,75	16,58	0,001
Pressure*Pressure	1	36,76	7,81	28,82	0,001
Temperature*-Temp	1	7,81	1,27	6,12	0,038
Residual Error	8	10,20	1,82	–	–
Lack-of-fit	4	7,28	0,73	2,49	0,199
Pure error	4	2,92	20,07	–	–
Total	13	263,84	–	–	–

S=1,12929 R-sq. = 96,13 % R-sq.(adjusted) = 93,72%

Equation (1) represents the variation present in the bearing performance, as control variables are temperature and pressure:

$$\text{OEY}\,(\%) = 30{,}571 + 3{,}712P + 3{,}564T - 2{,}241P^2 - 1{,}033T^2 + \varepsilon \quad (1)$$

Where: OEY is oil extraction yield, P is pressure, T is temperature, and ε is the absolute error $\varepsilon \in N\left(\mu,\sigma^2\right)$; regression equation in units unencrypted.

According to the Equation (1) and Table 4, all the terms proposed are significant (p-value <0,05), opposed to the results obtained in a study conducted by Goswami *et al.*, (2009). This research seeks to optimize bioconversion of castor bean oil into ricinoleic acid, finding an equation that characterizes that process. However, due to the lack of significances (p-value > 0,05) in several of the studied variables, these results cannot be conclusive on this pursuit.

The normality of the residuals (p-value = 0,109 Anderson Darling test) is supported by the results and the model presented.

Figure 3 shows the contours produced for increasing the yield in oil extraction. Oil yield increases when levels of both controllable factors are raised.

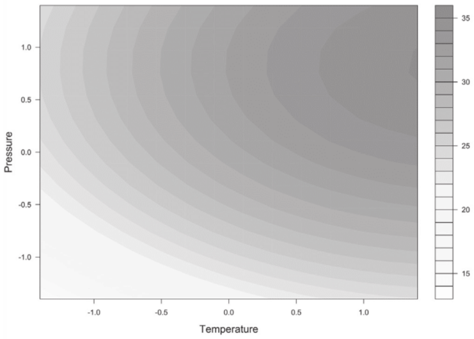

Figure 3. Contour plots for pressure and temperature.

Looking at the response surface for oil extraction yield (Figure 4), the optimization point to get the greatest oil yield performance (35,07 %) was observed at a pressure of 50,31 MPa (0,8403 units unencrypted), and a temperature of 64 °C (1,41 units unencrypted). On the other hand, the control factors had a significant increase in performance (Figure 3), but this significance tends to have a limit, where the control factors did not increase oil yield (Figure 4). This outcome could be due to a high compression of the seed within the extraction device, resulting in a lack of paths for oil flowing to the collector component.

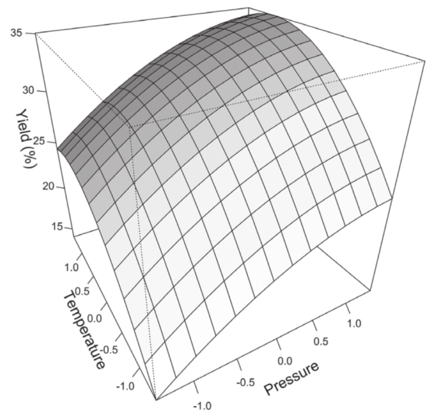

Figure 4.

Scenario analysis

Scenario analysis was developed using optimization techniques (Derringer & Suich, 1980). Three scenarios are detected (Table 5) to determine the optimal levels for the extraction process, taking into account oil yield and viscosity. Table 5 shows the variation related to the possibility of changing pressure and temperature for the extraction process, depending on the importance given to either oil yield or viscosity.

Table 5. Analysis of scenarios to determine the best combination, between variables extraction yield and viscosity.

Scenario	Control factor	Response variable	Target	Value	Desirability
1	Pressure		Max	49,380	0,668
	Temperature		Min	40,830	
		Extraction yield	Max	28,161	
		Viscosity	Min	0,471	
2	Pressure		Min	30,870	0,651
	Temperature		Max	64,000	
		Extraction yield	Max	30,180	
		Viscosity	Min	0,477	
3	Pressure		Min	50,310	0,894
	Temperature		Max	63,030	
		Extraction yield	Max	34,546	
		Viscosity	Min	0,470	

Confirmation runs following the recommended settings

In order to test the results of the analysis developed, two seed oil extraction runs were performed using the optimal setting values obtained (Table 6). According to these tests, oil yield can be increased by 35 % or even more just by controlling those factors that generate higher variability during the extraction process.

Table 6. Confirmation runs.

Experiment (run)	Seed condition	Pressure (MPa)	Temperature (°C)	Extraction yield (%)
1	Whole	50,31	64	36,01
2		50,31	64	34,81

Figure 5 illustrates the extraction yield percent of both process, that one carried out in this work (new), and that performed by Perdomo *et al.* (2013) in four castor seeds varieties. The new extraction process showed an oil yield enhancement in all the seed varieties compared to the extraction yield obtained by Perdomo *et al. (*2013). In addition, the new process decreases the deviation by 12 % ± 5% to 7 % ±2 %, obtaining an improvement of 54 % in comparison with Perdomo *et al,(*2013).

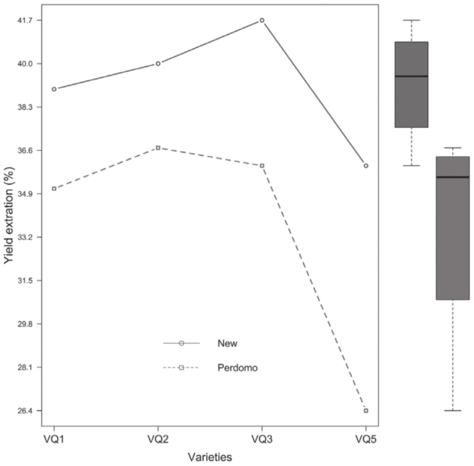

Figure 5.

Conclusions

The condition of the seed has a high contribution to the performance of the oil extraction process (82 %), while the amount of the material load has no effect on the process. Extraction yield, turbidity, viscosity, and color are highly correlated (> 85 %). These variables presented two main components: extraction yield and viscosity, coupled with turbidity. The contribution of the control factors has a yield extraction limit. Increasing pressure (> 50,31 MPa) has no effect on extracting performance, which is due to a high compaction of the seed within the device.

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