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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.

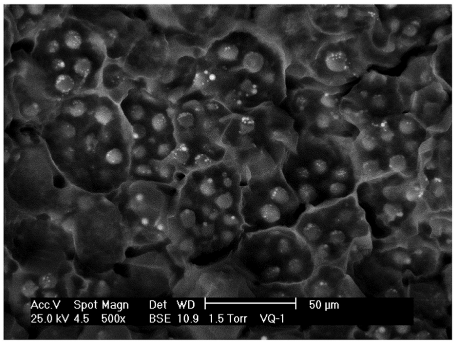
Table 1. Analysis of images of castor seeds

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]

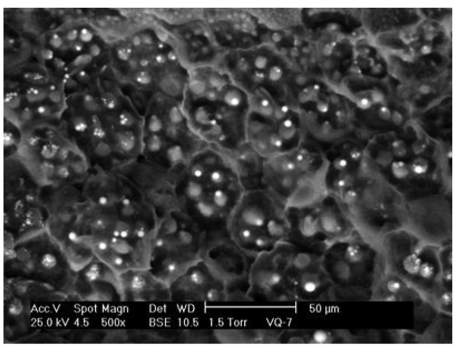
L = Long, W = Width, AR = Area, P = Perimeter, G = Thickness, DF = Diameter Feret, EI = Elongation index, RI = Roundness index, CI = Compaction index. VQ-1 = Wild seed; VQ-7 = Cultivated in experimental field. The results are expressed as the mean value ± standard deviation. Similar letters refer to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

Castor Seed Microstructure

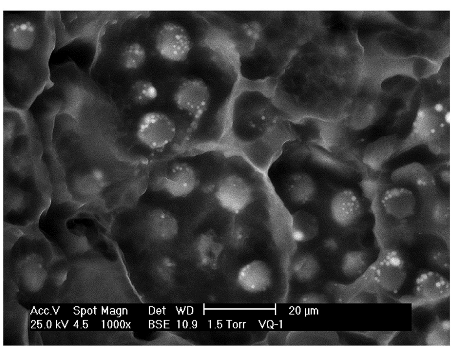
Figure 1 shows the microstructure images of the studied seeds, obtained with a microscope type MEBA. Lipid microbodies are shown as part of the characteristic cells forming the endosperm (Figure 1), and highlighted as bright spots appear the reserve minerals, which are associated with the structural 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).



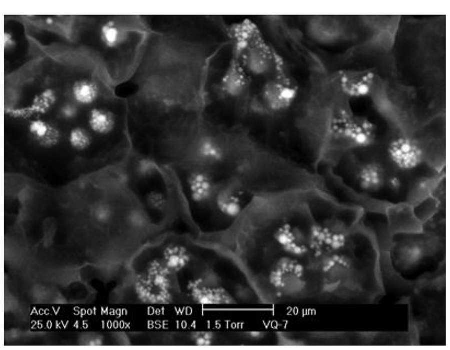
a)



b)

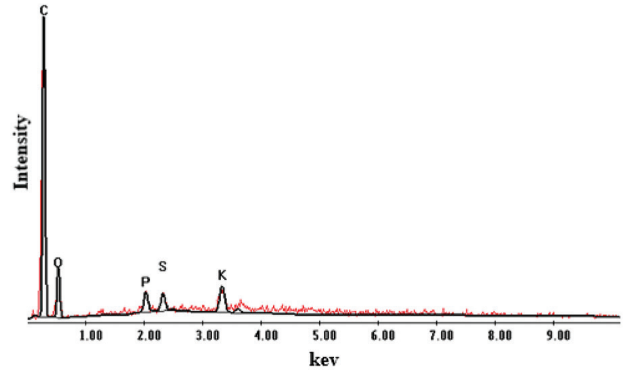


c)

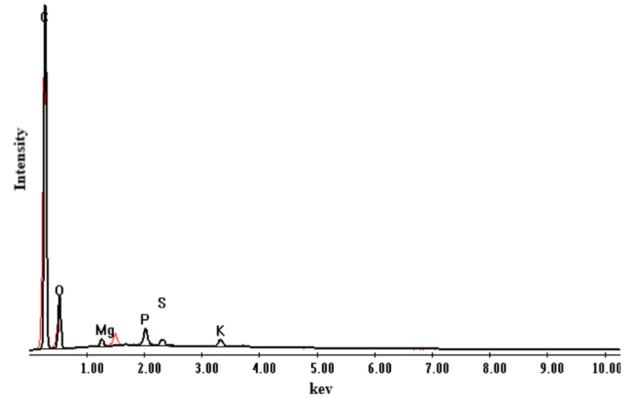


d)

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± 1,08ª	44,95± 0,69ª	3,65 ± 0,22ª	3,20 ± 0,11ª
VQ-7	54,23± 1,28ᵇ	33,84± 2,87ᵇ	4,79 ± 0,27ᵇ	2,42 ± 0,13ᵇˢ

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).

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 ± 1,72ª	0,9389 ± 0,0003ª	0,9918 ± 0,0062ª	0,0620 ± 0,0003ª
VQ-7	266,44 ± 2,98ᵇ	0,9465 ± 0,0004ᵇ	0,5440 ± 0,0075ᵇ	0,0290 ± 0,0004ᵇ

Source: Authors

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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