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Physico-chemical characteristics and oxidative stability of oils from different Peruvian castor bean ecotypes

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SUMMARY: The aim of this research was to assess the physico-chemical properties and shelf-life of oils press-extracted at two temperatures (60 °C and 80 °C) from five Peruvian castor bean ecotypes. A wide variation for all traits was observed. Low acidity index, low peroxide index and absence of *p*-anisidine were recorded. The total tocopherol contents ranged from 798 to 1040 mg/kg. A higher antioxidant capacity was detected in methanolic extracts than in hexane extract. From the Rancimat performed at 150-170 °C, the predicted shelf-life at 25 °C ranged from 0.15 to 8.93 years; the higher extraction temperature led to a longer shelf-life, probably because of enzyme inactivation.

KEYWORDS: *Antioxidant capacity; Fatty acids; Rancimat;* Ricinus communis; *Tocols.*

RESUMEN: ***Características físico-químicas y estabilidad oxidativa de aceites de diferentes ecotipos de ricino peruano.*** El objetivo de esta investigación fue estudiar las propiedades fisicoquímicas y la vida útil de aceite de ricino extraido a presión a dos temperaturas (60 y 80 °C) de cinco ecotipos peruanos. Se notó una amplia variación para todas las características. Se observaron bajos índice de acidez, bajo índice de peróxido y ausencia de *p*-anisidina. El contenido total de tocoferoles osciló entre 798 y 1040 mg/kg. Se detectó una mayor capacidad antioxidante en los extractos en metanol que en los extractos en hexano. A partir del Rancimat realizado a 150-170 °C, la vida útil prevista a 25 °C osciló entre 0.15 y 8.93 años; la mayor temperatura de extracción condujo a una vida útil más larga, probablemente debido a inactivación de las enzimas.

PALABRAS CLAVE: *Ácidos grasos; Capacidad antioxidante; Rancimat;* Ricinus communis; *Tocoles.*

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1. INTRODUCTION

The castor bean (*Ricinus communis* L.), a perennial flowering plant from the Euphorbiaceae family of probable African origin, is currently firmly rooted in all the warm areas of the world. Castor beans present a wide variation in many characteristics, including size and precocity of the plant, color of stems, leaves and petioles, dehiscent or indehiscent fruit, as well as color, shape, size and chemical composition of the seeds (Wang *et al*., 2010). The seeds, highly toxic for the presence of an easily-extractable protein, ricin, which is a deadly natural poison (Patel *et al*., 2016), are rich in a prized viscous oil of relevant interest for the industry, utilized in the manufacturing of paints, plastics, lubricants, aeronautical fluids, biodiesel, cosmetics and pharmaceutical products (Mutlu and Meier, 2010). The unique characteristics of castor bean oil are a consequence of its high content in mono-unsaturated ricinoleic acid (12-hydroxy-9-octadecenoic acid), whose molecule has three functional reactive groups: a carboxyl group (COOH), an unsaturation point in carbon nine and a hydroxyl group in carbon 12 (Mutlu and Meier, 2010). The carboxyl group consents different esterification reactions; the single unsaturation point may be altered by epoxylation, hydrogenation or vulcanization while the hydroxyl group can be acetylated, alcoxylated or removed by dehydration, increasing the unsaturation and generating a semi-dry acid. The presence of the hydroxyl group in carbon 12 promotes a high and stable viscosity index, and great lubricity (Scholz and da Silva, 2008).

In recent years, concerns over global climate change and knowledge of the limited fossil oil reserves has stimulated the search for alternative and renewable sources which are apt for the industrial production of bio-based polymers, such as castor oil. Because of its great economic value and ever-increasing demand, the castor bean is widely cultivated in tropical, sub-tropical and temperate countries; its diffusion is favored by its short generation time, good drought and salt tolerance, and adaptation to marginal soils (Timko *et al*., 2014). India (1,198,000 Mg) was by far the biggest producer in 2018, followed by Mozambique (85,436 Mg), China (27,000 Mg), Brazil (14,224 Mg), Myanmar (12,068 Mg) and Ethiopia (10,930 Mg) (www.fao.org/faostat, last accessed 26/03/2020). An evaluation of the worldwide castor bean germplasm has evidenced a relatively low genetic variation (Allan *et al*., 2008), but only scattered information is available about differences among accessions for morpho-agronomic traits and oil composition (e.g. Akande *et al.*, 2012; Armendáriz *et al*., 2015; da Silva Ramos *et al*., 1984; Lavanya *et al*., 2012;).

Ricinus communis is not cropped in Peru, nor has the recent importation of varieties been recorded, except for some adaptation trials in the hot and humid lowlands of the Amazon (Villalobos *et al*., 2008). Castor bean plants, regarded as unwanted weeds, are often eradicated by farmers. Hence, local ecotypes are likely the survivors of old, perhaps unintentional, introduction episodes and have adapted to specific growing environments.

Castor oil is obtained from castor beans by mechanical pressing, solvent extraction or their combination (Mutlu and Meier, 2010; Perdomo *et al*., 2013). A solvent extraction under thermo-sonication at 70 and 60 °C was proposed by López-Ordaz *et al*. (2019) and Palconite *et al*. (2018), respectively. The mechanical process has lower costs and is more environmentally-friendly but gives inferior yields to the solvent extraction method (Mutlu and Meyer, 2010). Cold pressing is sometimes performed (Ananth *et al*., 2019), but heating to around 60 °C is often applied (Perdomo *et al*., 2013) to harden the interior of the beans and to improve the extraction efficiency (Patel *et al*., 2016). Nevertheless, specific studies investigating the best thermal conditions are still lacking.

The aim of the research was to study the differences among ecotypes in physico-chemical characteristics and oxidative stability of press-extracted oil from five Peruvian castor beans.

2. MATERIALS AND METHODS

2.1. Castor bean seeds

Five castor bean seed samples (5 kg each) were collected from four different eco-geographical regions in the Central-North part of Peru, i.e. Huarmey (coast, 14 m a.s.l.), Carhuaz (highlands, 2688 m a.s.l.), Casma (coast, 207 m a.s.l.) and La Carbonera (coast, 140 m a.s.l.). The seeds were dried to 3-5% humidity. Their dimensions (length,

width and thickness) were measured with a caliper on 200 seeds per accession, while their weight was assessed with a 220R lab balance (Precisa Gravimetrics AG, Dietikon, Switzerland) on three 1000-seed batches per accession. Moisture, ash, lipids and protein contents were determined according to methods 934.06, 942.05, 922.06 and 953.01 (AOAC International, 2019), respectively; total carbohydrate content was computed by difference, by subtracting the measured protein, fat, ash, fiber and water from the total weight. The analyses were repeated twice.

2.2. Oil extraction

Two different subsamples of each ecotype were heated at 60 or 80 °C for 20 and 30 min, respectively in an UN55 oven (Memmert, Schwabach, Germany). The oil was extracted using a PH1020 hydraulic press (Neo & Neo Next, China) under a 20-t pressure, centrifugated at 5000 rpm for 30 min with a Sigma 2-16P centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) to remove any impurities, and stored at 4 °C in dark glass containers until analysis.

2.3. Oil characteristics

Oil density was measured in accordance to the Codex Alimentarius (1999) and oil viscosity was assessed following method D2983 (ASTM International; www.astm.org). The free fatty acids (FFA, % oleic acid) were determined according to the official method Ca 5a-40 (AOCS, 1998). The refractive index was measured according to method 921.08 (AOAC International, 2019) working at 25 °C and using a digital A 24051 refractometer (Rudolph Research Analytical, NJ, USA) kept at 20 °C. The acidity index was determined by direct titration as described in method Cd 3d-63 (AOCS, 1998); the peroxide index was measured according to method 965.33 (AOAC International, 2019); the iodine index was determined by the Wijs method, in accordance with method 993.20 (AOAC International, 2019); the *p*-anisidine value was monitored by method Cd 18-90 (AOCS, 1998).

Oil color was determined by the CIELAB method using a Chroma Meter II tristimulus colorimeter (Minolta Italia SpA, Milan, Italy), using a standard white reflector plate and the illuminating C; three parameters were assessed: *L** (luminosity), *a** (red-green), *b** (yellow-blue).

The fatty acid composition was assessed only on the 60 °C-extracted samples by gas-chromatography following the method described by Simonetti *et al*. (2002). Tocopherol content and composition was evaluated by normal phase HPLC as outlined by Rodríguez *et al.* (2021). To measure the antioxidant capacity, the 2-2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical cation scavenging capacity tests were performed on hydrophilic (HF) and lipophilic (LF) fractions (Varas Condori *et al*., 2020) as follows: exactly 2 g of oil and 2 mL of n-hexane were vortexed together until complete dissolution. Subsequently, 2 mL of 80:20 methanol-water were added and vortexed. The mixture was centrifuged at 9870 *g* for 10 min. Finally, the methanolic phase (located in the lower part of the centrifuge tube) and the n-hexane phase (found in the upper part of the tube) were separated, and their volumes measured. ABTS and DPPH tests were performed as described by Yilmaz *et al*. (2015). The results are reported as mmol Trolox equivalent (TE)/kg oil.

The oxidation stability index (OSI; h) was evaluated using a Rancimat equipment (743 Rancimat Metrohm Co., Switzerland) following Official Method Cd 12b-92 (AOCS, 1998) at 150, 160 and 170 °C with an air flow of 20 L/h; the shelf-life (years) at 20, 25 and 20 °C was extrapolated from the OSI results.

All the chemical analyses were performed twice, while color and oxidative stability thrice, on independent samples.

2.4. Statistical analysis

The analysis of variance (ANOVA) was performed considering castor oil ecotypes (E) and extraction temperature (T) as main factors. Within each extraction temperature, a one-way ANOVA was also carried out and when significant differences were observed the Fisher`s Least Significant Difference test (LSD) at $p < 0.05$ was performed. Means and standard errors were computed using Excel 2013 (Microsoft, Redmond, WA, USA), while the ANOVAs and LSD analyses were computed with the software Statgraphics® Centurion XVI (Statpoint Technologies Inc., Warrenton VA, USA).

3. RESULTS AND DISCUSSION

3.1. Seed characteristics

Figure 1 shows the beans of the five ecotypes (Huarmey grande, Huarmey chico, Carhuaz, Casma and La Carbonera); the dimension parameters length, width, thickness and 1000-kernel weight are summarized in Table 1. The ANOVA (not shown) evidenced significant differences ($p < 0.05$) among the samples for all four traits. The Huarmey grande ecotype had the biggest seeds, followed by La Carbonera, and Casma; Huarmey chico and Carhuaz had the smallest seeds, with comparable size and weight. With the exception of Huarmey grande, the dimensions were within the ranges (length: 0.865-1.710 cm, width: 0.546-1.000 cm, thickness: 0.300-709 cm, 1000 seeds weight: 101.0-735.7 g) reported by Perdomo *et al*. (2013), Velasco *et al*. (2015) and Wang *et al*. (2010). Additionally, the two Huarmey ecotypes differed from the others in the color of the teguments, i.e. black vs. brown-grey with black lines.

The chemical composition of the seeds is reported in Table 1. The ANOVA (not shown) highlighted the existence of significant differences ($p < 0.05$) for

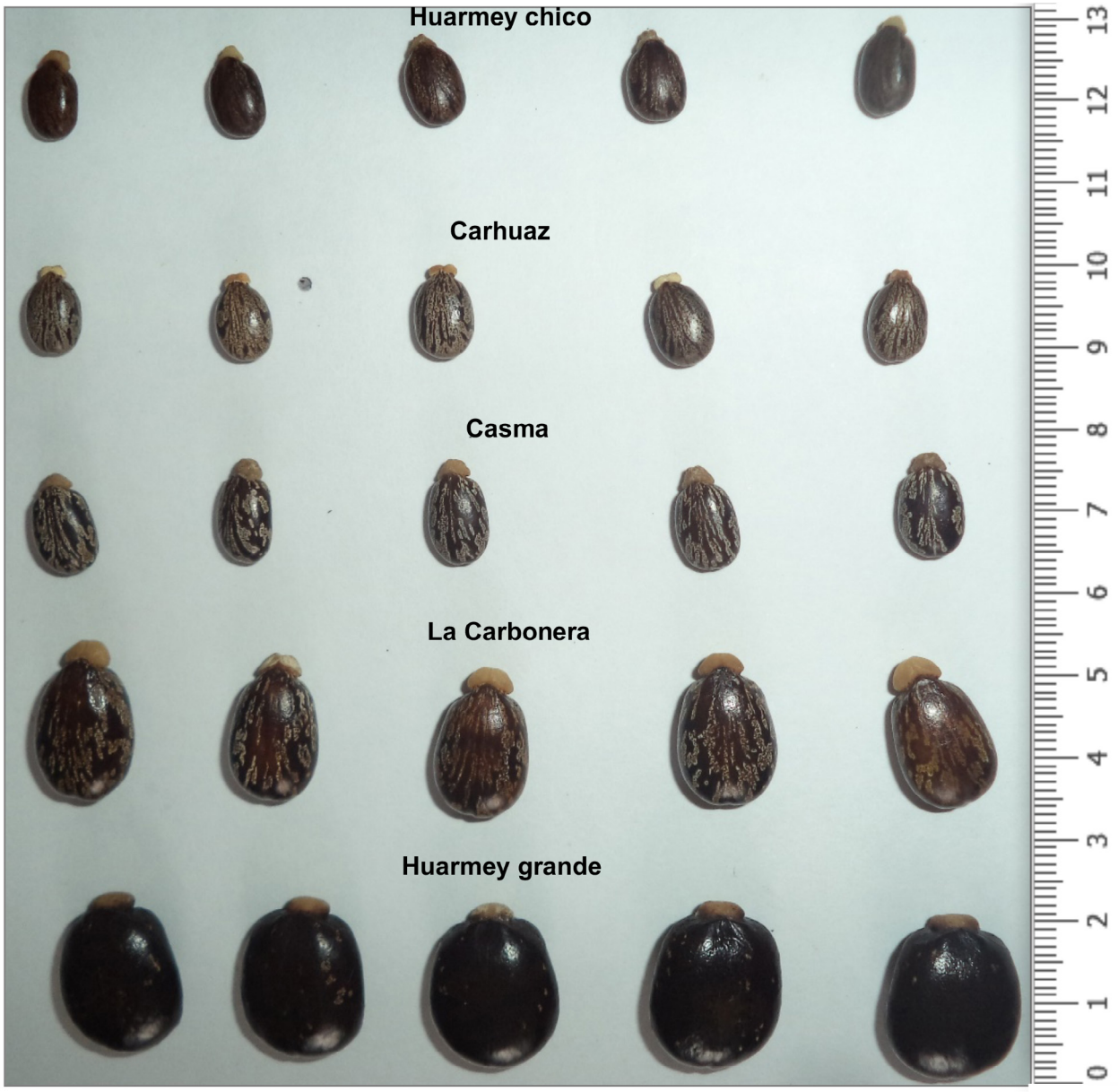

Figure 1. Castor beans of the five Peruvian ecotypes analyzed.

Table 1. Morphological characteristics and chemical composition (mean±standard error) of the seeds of five Peruvian *Ricinus communis* ecotypes. Length, width and thickness were measured on 200 seeds, 100-kernel weight on three 1000-kernel samples, moisture, ash, lipids, proteins and fiber on two independent samples for each ecotype. Carbohydrate content was computed by difference, subtracting the measured protein, fat, ash, fiber and water from the total weight.

	Huarmey grande	Huarmey chico	Casma	Carhuaz	La Carbonera
Length (cm)	1.77±0.05^{a}	0.95±0.03^{d}	1.17±0.04^{c}	0.94±0.03^{d}	1.61±0.05^{b}
Width (cm)	1.43±0.05^{a}	0.59±0.02^{d}	0.66±0.03^{c}	0.60±0.02^{d}	0.99±0.04^{b}
Thickness (cm)	0.78±0.02^{a}	0.44±0.01^{d}	0.50±0.02^{c}	0.44±0.02^{d}	0.67±0.02^{b}
1000-kernel weight (g)	877.91±3.11^{a}	122.58±0.14^{d}	184.06±1.21^{c}	122.62±0.84^{d}	464.42±3.24^{b}
Moisture (g/100 g)	3.26±0.03^{c}	3.82±0.06^{a}	3.33±0.07^{c}	3.14±0.07^{d}	3.55±0.04^{b}
Ash (g/100 g DM)	3.52±0.11^{c}	4.09±0.10^{a}	2.69±0.07^{e}	2.92±0.05^{d}	3.87±0.09^{b}
Lipids (g/100 g DM)	60.54±0.36^{a}	53.99±0.06^{d}	57.79±0.07^{c}	58.85±0.60^{b}	57.66±0.47^{c}
Proteins (g/100 g DM)	15.22±0.56^{d}	19.95±0.14^{a}	19.42±0.27^{a}	18.62±0.41^{b}	16.83±0.14^{c}
Fiber (g/100 g DM)	10.53±0.04^{c}	14.68±0.08^{a}	14.38±0.03^{b}	14.73±0.07^{a}	10.33±0.06^{d}
Carbohydrates (g/100 g DM)	10.19±0.24^{b}	7.29±0.07^{c}	5.72±0.33^{d}	4.89±0.21^{e}	11.31±0.25^{a}

Different letters in a row indicate significant differences ($p \leq 0.05$) among ecotypes according to the LSD test.

moisture, ash, lipids, protein, fiber and total carbohydrate contents. The seed moisture, always low, ranged from 3.1 to 3.8 g/100 g. The ash content was highest in Huarmey chico and lowest in Casma. Similar values (2.24 to 3.41 g/100 g DM) were reported by Vasco Leal *et al*. (2017) for 12 castor bean accessions from Mexico, which are largely inferior to the value (6.44 g/100 g) described for one Pakistani variety (Panhwar *et al*., 2016).

Huarmey chico showed the lowest lipid content, while Huarmey grande had the highest; the other three ecotypes were similar (on average, 58.1 g/100 g DM). The values are within the ranges (39.6-59.5, 34.6-56.6, 12.2-64.8, 37.2-60.6 g/100 g DM) reported, respectively, by da Silva Ramos *et al.* (1984) for 36 varieties, by Severino *et al.* (2015) for 40 breeding lines and commercial genotypes, by Goytia Jiménez *et al*. (2011) for 151 accessions collected in the state of Chiapas, Mexico, and by Wang *et al*. (2010) for the USDA world collection. However, these results exceed those reported by Vasco Leal *et al*. (2017) (41.5-51.0 g/100 g DM), Armendáris *et al*. (2015; 42.0-48.5 DM), and are partially higher than the values (44.6-54.8 g/100 g) reported by Velasco *et al*. (2015) in 121 accessions from southern Spain. Different extraction methods influence lipid recovery; for example, values ranging from 57.0 (Soxhlet) to 48.0-61.1% (thermosonication) were observed by López-Ordaz *et al*. (2019).

Protein concentration was maximum in Huarmey chico and Casma and minimum in Huarmey grande, all results comparable to those (12.61-16.02 g/100 g DM) described by Vasco Leal *et al*. (2017). Nevertheless, Perea-Flores *et al*. (2011) found a higher protein content (28.48 g/100 g DM) in the Mexican variety Tiripiteo.

The fiber content was low (10.3 and 10.5 g/100 g DM) in the big-seeded ecotypes La Carbonera and Huarmey grande, and significantly higher (14.4-14.7 g/100 g DM) in the other three castor oil accessions. Conversely, the carbohydrate concentration was superior in the former two ecotypes (11.3 and 10.2 g/100 g DM, respectively), and lower in the latter three (4.9-7.3 g/100 g DM).

The composition of castor oil seeds may be influenced not only by genetic causes (e.g. seed size, Severino *et al*., 2015; Velasco *et al*., 2015), but also by environmental factors (Ramanjaneyulu *et al*., 2013). The climatic conditions in Huarmey, Casma and La Carbonera are generally warm, sunny, with little rain; while Carhuaz has a cold climate with seasonal rains.

3.2. Oil characteristics

3.2.1 Color

The ANOVA (not presented) carried out on the color parameters *L**, *a**, *b** evidenced significant effects for ecotype and for ecotype x temperature

Table 2. Color coordinates *L**: luminosity, *a**: red-green and *b**: yellow-blue (mean±standard error; n=3) of castor oil extracted at two temperatures (60 °C and 80 °C) from five Peruvian ecotypes.

	°C	Huarmey grande	Huarmey chico	Casma	Carhuaz	La Carbonera
*L**	60	32.4±0.8^{a}	33.7±0.1^{a}	32.9±0.1^{a}	32.8±0.9^{a}	28.3±0.1^{b}
	80	35.8±0.4^{a}	32.3±0.1^{c}	33.0±0.2^{b}	28.9±0.1^{e}	30.5±0.2^{d}
*a**	60	1.4±0.2^{b}	1.1±0.1^{b}	1.5±0.1^{b}	1.1±0.3^{b}	2.5±0.1^{a}
	80	0.4±0.0^{c}	1.4±0.0^{b}	1.3±0.2^{b}	2.2±0.1^{a}	2.2±0.1^{a}
*b**	60	5.1±0.6^{b}	6.6±0.2^{a}	6.8±0.3^{a}	5.9±0.7^{ab}	2.4±0.3^{c}
	80	7.7±0.4^{a}	6.0±0.2^{c}	6.6±0.3^{b}	2.9±0.1^{e}	4.6±0.3^{d}

Different letters in a row indicate significant differences ($p \leq 0.05$) among ecotypes according to the LSD test.

interaction, but not for oil extraction temperature *per se*. Because the interaction was relevant, Table 2 reports the results separately for 60 and 80 °C. At 60 °C the luminosity was similar except for La Carbonera, which was slightly darker; while at 80 °C Huarmey grande was more luminous than the others, and La Carbonera was still the darkest. The other two parameters showed that *a** was slightly higher (i.e. redder) for La Carbonera oil, while *b** was greater (i.e. yellower) for Huarmey chico, Casma and Carhuaz (at 60 °C) and for Huarmey grande (at 80 °C). The low luminosity of the samples is partially a consequence of the extraction method utilized. Mechanical pressing tends to drag different components present in the seeds, such as gums: these impurities hinder the penetration of the light, thus reducing the luminosity. In fact, solvent-extracted oil is markedly clearer, as noticed by Conceição *et al*. (2007) and by Palconite *et al*. (2018).

3.2.2. Physicochemical parameters

Figure 2 presents the physicochemical characteristics of the castor oil extracted at two different temperatures (60 and 80 °C). The ANOVAs (not presented) showed significant effects due to the ecotype as well as the extraction temperature; the ecotype always had the largest effect, except for oil density when the temperature was preeminent. The interaction between the two factors was also significant but generally of minor relevance except for the peroxide and iodine indices.

Compared to 60 °C, the 80 °C treatment led to better oil yield, superior oil density and kinematic viscosity, slightly higher refraction index, and inferior acidity index and free fatty acid content. For the peroxide and iodine indices, the interaction between temperature and ecotype was more relevant than the temperature *per se*, as each genotype behaved differently. The *p*-anisidine was always undetectable.

Heating influences the process of rupture of lipid bodies, thus favoring oil extraction; the better yields at the superior temperature were probably linked to more drastic shattering of lipid bodies and superior oil fluidity. Interestingly, the yield was significantly correlated to the weight of the seeds ($r = 0.78$ and $r = 0.83$ at 60 and 80 °C, respectively). At 60 °C the oil yield varied from 33.4 (Huarmey chico) to 39.0% (Huarmey grande), and at 80 °C from 33.5 to 42.0% (same ecotypes). These values match those reported by Perdomo *et al*. (2013), who obtained a yield of 36.6% using a mechanical press at 60 °C on a Mexican variety. Similarly, our results are within the variation (31.99-48.39%) summarized in their review by Ahmad *et al*. (2020). In general, yield seems to be influenced by the size of the seed (Huarmey grande seeds were exceptionally large).

At 60 °C, the oil density of Carhuaz and La Carbonera was the highest (0.949 and 0.949 g/cm^3), while Casma had the lowest (0.947 g/cm^3); at 80 °C Carhuaz and Huarmey grande showed superior results (0.949 and 0.949 g/cm^3). The differences between the results of the two extraction temperatures may be related to the fact that higher temperatures favor the migration of compounds such as gums, proteins, fiber, carbohydrates, etc. into the oil, thus increasing its density (Vasco Leal *et al*., 2017). Our results are within the variation (0.946-0.958 g/cm^3) described by Conceição *et al*. (2007), Panhwar *et al*. (2016) and Vasco Leal *et al*. (2017) for Brazilian, Pakistani and Mexican accessions.

At 60 °C the kinematic viscosity of Huarmey grande was the highest (227.1 mm^2/s), followed by

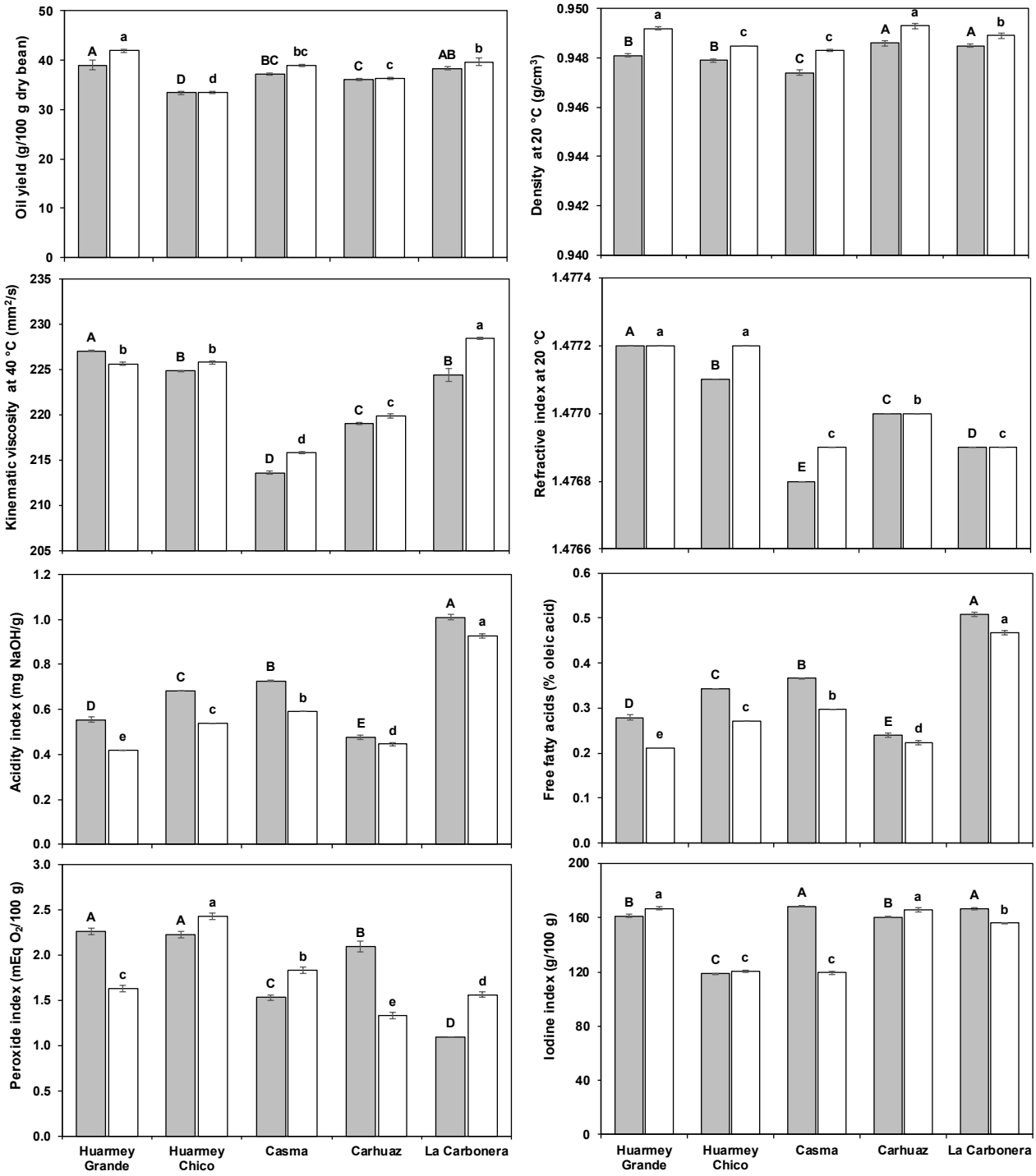

Figure 2. Physico-chemical characteristics of the oil obtained by pressing extraction at 60 °C (grey bars) and 80 °C (white bars). Error bars represent the standard error (n=2). Different letters indicate significant differences ($p \leq 0.05$) among ecotypes for each extraction temperature (capital letters: 60 °C; small letters: 80 °C) according to the LSD test.

those of Huarmey chico and La Carbonera, while Casma presented a relatively low value (213.6 mm^2/s, respectively). At 80 °C La Carbonera showed the maximum value (228.4 mm^2/s) and Casma, again, the minimum (215.8 mm^2/s). These results are marginally lower than those (239.39 mm^2/s) reported by Conceição *et al*. (2007) but are far inferior to those (250.04 to 265.84 mm^2/s) by Vasco Leal *et al*. (2017), although these last researchers used different analytical equipment.

The refraction index at 60 °C was greatest in La Carbonera (1.477), and at 80 °C in Huarmey grande and Huarmey chico (1.477 in both samples) but the differences among ecotypes were generally minimal. A similar value (1.479) was reported by Canoira *et al*. (2010); while an inferior result (1.431) was observed by Panhwar *et al*. (2016).

Concerning the acidity index and the free fatty acid content, only La Carbonera evidenced high values, while all the other ecotypes showed 0.420-0.727 mg NaOH/g and 0.211-0.366 g/100 g, respectively; the minimum values were those of Carhuaz at 60 °C and of Huarmey grande at 80 °C. In general, lower values were observed at 80 °C extraction. Perdomo *et al*. (2013) stated that the temperature affects the free fatty acid content and thus the acidity index. In fact, all seed oils have lipolytic enzymes which release free fatty acids. However, if the enzymes are inactivated by high temperatures, hydrolysis is limited, free fatty acids are scarce and acidity is low. The results of this research are comparable to those (0.542-1.218 mg KOH/g) reported by Vasco Leal *et al*. (2017) and to those (0.823 to 1.390 mg KOH/g) determined by Perdomo *et al*. (2013). Therefore, the acidity indices of the five Peruvian ecotypes are ideal and in general the oil has a low content in free fatty acids. Poor post-harvest handling of the seeds and poor storage of the oil often affect this quality indicator, hence low acidity values are achieved when the raw material are damaged or exposed to extreme conditions (high temperatures, humid environments, etc.).

The lowest peroxide index scores were reached by La Carbonera and Casma at 60 °C, and by Carhuaz and La Carbonera at 80 °C. A similar situation was evident also for the iodine index: Casma and La Carbonera had the lowest values at 80 °C while the two Huarmey and Carhuaz had the smallest at 60 °C. The peroxide values were lower than those (4.63 to 4.90 meq O_2/kg oil) reported by López-Ordaz *et al*. (2019) but similar to the one (2.25 meq O_2/kg) described by Panhwar *et al*. (2016), while the iodine values (Figure 2) were higher than those scored by López-Ordaz *et al*. (2019), Torrentes-Espinoza *et al*. (2017), and Panhwar *et al*. (2016), i.e. 84.9-85.9, 91.0 and 83.6 g I_2/100 g lipid, respectively, probably because of different ecotypes and origins of the seeds as well as different extraction methods

3.2.3. Fatty acids

The castor oil contained mainly ricinoleic acid (on average, 86.8%), followed by linoleic acid (4.2%), oleic acid (3.1%) and other minor fatty acids (Table 3). The one-way ANOVA (not presented) did not show significant differences for ricinoleic or linoleic acids among samples; the variation of the other fatty acids, even if significant, was very limited.

Table 3. Fatty acids composition (%; mean±standard error; n=2) of castor oil extracted at 60 °C from five Peruvian ecotypes.

Fatty acid	Huarmey grande	Huarmey chico	Casma	Carhuaz	La Carbonera
C16:0	0.83 ± 0.02^{c}	1.22 ± 0.04^{b}	1.58 ± 0.01^{a}	0.89 ± 0.02^{c}	1.17 ± 0.02^{b}
C18:0	0.69 ± 0.05^{b}	0.97 ± 0.03^{a}	1.07 ± 0.03^{a}	1.07 ± 0.05^{a}	1.03 ± 0.11^{a}
C18:1n9	$3.04 \pm 0.19b^{c}$	2.54 ± 0.05^{c}	3.54 ± 0.13^{a}	3.19 ± 0.20^{ab}	3.07 ± 0.03^{ab}
C18:1n7	0.43 ± 0.03^{c}	0.46 ± 0.01^{bc}	0.55 ± 0.03^{ab}	0.42 ± 0.02^{c}	0.60 ± 0.04^{a}
C18:2n6	4.21 ± 0.19	4.29 ± 0.18	4.72 ± 0.18	3.84 ± 0.09	4.17 ± 0.23
C18:3n3	0.54 ± 0.02^{a}	0.52 ± 0.01^{ab}	0.48 ± 0.02^{bc}	0.50 ± 0.01^{ab}	0.44 ± 0.01^{c}
C20:1n9	0.29 ± 0.01^{a}	0.23 ± 0.01^{b}	0.30 ± 0.00^{a}	0.32 ± 0.02^{a}	0.18 ± 0.02^{b}
C18:1OH	87.09 ± 0.54	87.31 ± 0.52	85.68 ± 0.49	86.92 ± 0.49	86.89 ± 0.52
Others	2.88 ± 0.08^{a}	2.47 ± 0.19^{ab}	2.06 ± 0.09^{b}	2.85 ± 0.09^{a}	2.45 ± 0.12^{ab}

Different letters in a row indicate significant differences ($p \leq 0.05$) among ecotypes according to the LSD test.

Overall, the oil of our ecotypes was constituted by about 91% mono-unsaturated fatty acids (MUFA), 7% poly-unsaturated fatty acids and 2% saturated fatty acids. Similar percentages for ricinoleic acid (from 75.0 to 90.0%), linoleic acid (4.1-9.7%) and oleic acid (3.0%-7.7%) are reported in the literature (Ahmad *et al*., 2020; da Silva Ramos *et al.,* 1984; Harhar *et al*., 2016; Torrentes-Espinoza *et al*., 2017; Velasco *et al*., 2015; Wang *et al*., 2011).

3.2.4. Tocopherols

The ANOVA (not shown) demonstrated the existence of significant differences merely among ecotypes. No differences were observed between the two extraction temperatures, suggesting good thermal stability of the compounds. Only tocopherols were detected (Table 4); the most abundant homologue was δ-tocopherol (on average, 520.2 mg/kg), followed by γ-tocopherol (366.8 mg/kg), α-tocopherol (9.5 mg/kg) and β-tocopherol (7.5 mg/kg), for an average total tocopherol content of 904.1 mg/kg. Interestingly, and unlike the other ecotypes, Huarmey grande had more γ-tocopherol than δ-tocopherol (506.8 vs. 448.7 mg/kg). The Huarmey chico samples showed the highest total tocopherol content (1038.4 mg/kg) and Carhuaz the lowest (780.1 mg/kg). Very little information is available in the literature on the presence of tocopherols in castor oil. Total tocopherol content of all ecotypes was higher than the values reported by Ananth *et al*. (2019), i.e. 461.3 mg/kg, and by Harhar *et al*. (2016), i.e. 183 mg/kg but lower than the levels recorded by Velasco *et al*. (2005) in a natural high-oleic acid mutant (2617 mg/kg) and in a standard castor oil line (1345 mg/kg). Velasco *et al*. (2015) observed a very large variation (99.6-282.2 mg/kg) for total tocopherol content in the seeds of 121 accessions from southern Spain.

On average, α- and β-tocopherol showed a minimal percentage (1.1 and 0.8%, respectively) of total tocopherols, while γ-tocopherol represented 40.2%

Table 4. Mean (±standard error; n=2) tocopherol content (mg/kg DM) and antioxidant capacity (ABTS and DPPH methods; mmol TE/kg oil) of the hydrophilic (methanol 80%) and lipophilic (hexane) fractions recovered from castor oil extracted at two temperatures (60 °C and 80 °C) from five Peruvian ecotypes.

	°C	Huarmey grande	Huarmey chico	Casma	Carhuaz	La Carbonera
α-tocopherol	60	13.1±0.3^{a}	9.8±0.5^{b}	9.8±0.5^{b}	8.2 ±0.8^{bc}	6.2±0.7^{c}
	80	13.0±1.2^{a}	9.6±0.1^{b}	9.5±0.7^{b}	8.5±0.5^{b}	7.1±1.0^{b}
β-tocopherol	60	10.9±0.2^{a}	7.9±0.5^{b}	6.7±0.3^{b}	5.7±0.3^{b}	7.6±1.1^{b}
	80	11.6±1.0^{a}	6.5±0.3^{bc}	6.6±0.4^{b}	5.3±0.3^{c}	7.4±0.4^{b}
γ-tocopherol	60	506.8±4.7^{a}	390.0±0.3^{b}	382.8±11.6^{b}	251.5±6.2^{d}	308.7±0.1^{c}
	80	500.3±4.7^{a}	389.9±2.4^{b}	379.4±9.5^{b}	253.1±3.6^{d}	305.6±23.0^{c}
δ-tocopherol	60	448.7±2.5^{c}	632.6±6.8^{a}	523.1±1.3^{b}	513.0±1.2^{b}	475.5±18.7^{c}
	80	457.7±6.4^{d}	631.5±12.9^{a}	527.8±1.7^{b}	514.8±1.8^{bc}	477.8±23.0^{c}
Total tocopherols	60	979.5±7.3^{b}	1040.3±8.1^{a}	922.5±12.1^{c}	778.5±8.5^{d}	797.9±19.0^{d}
	80	982.6±13.3^{b}	1037.5±15.1^{a}	923.4±10.9^{c}	781.7±6.1^{d}	797.8±8.6^{d}
$ABTS_{MeOH\ 80\%}$	60	34.0±1.9^{c}	39.6±1.2^{ab}	33.3±1.4^{c}	41.1±0.7^{a}	35.4±1.1^{bc}
	80	38.9±1.4^{a}	32.7±0.9^{c}	33.9±0.6^{bc}	31.4±0.6^{c}	36.9±0.7^{ab}
$ABTS_{Hexane}$	60	9.3±0.7^{a}	9.8±0.2^{a}	8.6±0.1^{ab}	6.9±0.4^{b}	7.6±0.6^{b}
	80	10.3±0.4^{a}	9.4±0.1^{b}	9.1±0.1^{b}	7.5±0.1^{c}	7.8±0.1^{c}
$DPPH_{MeOH\ 80\%}$	60	30.1±0.1^{a}	26.2±0.3^{b}	26.7±0.3^{b}	21.7±0.4^{d}	24.1±0.1^{c}
	80	26.4±1.1^{a}	27.1±0.0^{a}	26.6±0.2^{a}	22.7±0.4^{b}	23.3±0.1^{b}
$DPPH_{Hexane}$	60	5.4±0.4^{a}	5.1±0.2^{a}	3.8±0.1^{b}	1.7±0.2^{c}	5.9±0.3^{a}
	80	5.3±0.2^{c}	7.1±0.4^{a}	5.8±0.2^{bc}	2.8±0.3^{d}	6.3±0.0^{ab}

Different letters in a row indicate significant differences ($p \leq 0.05$) among ecotypes according to the LSD test.
ABTS: 2-2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation; DPPH: 2,2-diphenyl-1-picrylhydrazyl radical cation: MeOH 80%: 80:20 methanol-water extraction solvent; Hexane: n-hexane extraction solvent.

and δ-tocopherol 59.7%. Similarly, Velasco *et al.* (2005) and Velasco *et al.* (2015) identified γ-tocopherol and δ-tocopherol as the two most relevant homologues. Nevertheless, Ananth *et al*. (2019) and Harhar *et al.* (2016) identified γ-tocopherol as the most abundant homologue.

3.2.5. Antioxidant capacity

The antioxidant capacities of the hydrophilic and the lipophilic extracts are presented in Table 4. The ANOVA (not presented) highlighted a relevant ecotype influence and a significant temperature effect only for the DPPH results of hexane extracts. The interaction between ecotype and extraction temperature was predominant in the 80% methanol extracts for the ABTS test, although it was less important for DPPH. At first glance, it is easy to see that most of the antioxidant capacity was due to the hydrophilic compounds, extracted by the 80% methanol solution. We tested the total polyphenol content by the Folin-Ciocalteu method as described by Varas Condori *et al*. (2020), but we did not observe detectable values (not reported). Therefore, the high antioxidant capacity of this fraction is probably attributable to the ricinoleic acid. The antioxidant capacity in lipophilic extract is likely due to the tocopherols.

In general, Huarmey grande showed the highest antioxidant capacities in the methanolic phase at both extraction temperatures. For the ABTS test, Huarmey chico and Carhuaz also had top results at 60 °C and La Carbonera at 80 °C. For DPPH, instead, the other best performers (along with Huarmey Grande) were Huarmey chico and Casma, at 80 °C. For the lipophilic extracts, Huarmey grande, Huarmey chico and Casma had the best ABTS antioxidant capacity, in accordance with their tocopherol contents. The situation was somewhat different for DPPH because of La Carbonera's outstanding values.

It is difficult to compare our results with the scarce data in literature because of the different variables involved. Ananth *et al*. (2019) observed that the ABTS radical scavenging capacity of hydrophilic castor oil extract was superior to that of the lipophilic extract (0.63±0.02 vs. 0.13±0.04 μM TEAC/g oil DM), but the opposite was true for the DPPH assay (0.18±0.02 vs. 0.61 ± 0.03 μM TEAC/g oil DM). Santos *et al*. (2018), working with methanolic extracts, observed a free radical inhibitory capacity of 15.31±0.13 mg ascorbic acid equivalent/100 mL for ABTS, while for DPPH a 1.87±0.18% inhibition was found.

3.2.6. Oxidative stability index (OSI) and shelf-life

The OSI values of the oils analyzed by Rancimat at 150, 160 and 170 °C, with a constant 20 L/h airflow, are presented in Table 5 while the extrapolated shelf life at 20, 25 and 30 °C is reported in Table 6. The ANOVA (not presented) recorded a significant effect of the ecotype and, to a lesser degree, of the extraction temperature; the interaction was significant only for OSI and of minor relevance. The effect of the extraction temperature was higher for shelf-life than for OSI.

Huarmey grande generally showed the highest oxidative stability, and La Carbonera the lowest (Table 5). The huge difference (with a ratio around 2.0) between these two ecotypes at 150 °C decreased with increasing temperatures, reaching the lowest ratio (1.38) at 170 °C. To allow comparison with the values reported in the literature, the extrapolations at 110 and at 120 °C were performed. The OSI of the oils extracted at 80 °C ranged from 66.2 h to 145.6 h at 110 °C, and from 32.4 h to 69.2 h at 120 °C. Over-

Table 5. Oxidative stability index (h; mean±standard error; n=3) of castor oil extracted at two temperatures (60 °C and 80 °C) from five Peruvian ecotypes, and tested by Rancimat at 150, 160 and 170 °C.

	°C	Huarmey grande	Huarmey chico	Casma	Carhuaz	La Carbonera
150 °C	60	6.88±0.06^{a}	6.34±0.16^{b}	5.86±0.02^{c}	6.70±0.10^{a}	3.72±0.02^{d}
	80	7.41±0.07^{a}	6.24±0.05^{c}	5.79±0.08^{d}	6.99±0.14^{b}	3.69±0.09^{e}
160 °C	60	3.42±0.01^{a}	3.22±0.05^{b}	2.93±0.07^{c}	3.26±0.02^{b}	2.38±0.02^{d}
	80	3.55±0.02^{a}	3.37±0.03^{b}	2.87±0.07^{d}	3.18±0.06^{c}	1.95±0.05^{e}
170 °C	60	1.57±0.06^{ab}	1.63±0.02^{a}	1.44±0.02^{c}	1.52±0.02^{bc}	1.46±0.02b^{c}
	80	1.68±0.05^{a}	1.61±0.02^{a}	1.34±0.04^{b}	1.57±0.04^{a}	0.88±0.02^{c}

Different letters in a row indicate significant differences ($p \leq 0.05$) among ecotypes according to the LSD test.

Table 6. Shelf-life (years; mean±standard error; n=3) at 20, 25 and 30 °C of castor oil extracted at two temperatures (60 °C and 80 °C) from five Peruvian ecotypes.

	°C	Huarmey grande	Huarmey chico	Casma	Carhuaz	La Carbonera
20 °C	60	9.58±0.21^{b}	4.91±0.53^{c}	6.18±0.41^{c}	11.67±0.64^{a}	0.19±0.01^{d}
	80	10.67±0.62^{b}	4.93±0.20^{d}	9.00±0.70^{c}	12.98±0.18^{a}	4.77±0.52^{d}
25 °C	60	6.62±0.18^{b}	3.50±0.36^{c}	4.35±0.28^{c}	8.06±0.43^{ab}	0.15±0.01^{d}
	80	7.37±0.44^{b}	3.51±0.13^{d}	6.24±0.46^{c}	8.93±0.12^{a}	3.34±0.35^{d}
30 °C	60	4.58±0.16^{b}	2.49±0.25^{c}	3.06±0.19^{c}	5.56±0.28^{a}	0.12±0.01^{d}
	80	5.09±0.32^{b}	2.50±0.09^{d}	4.33±0.31^{c}	6.15±0.08^{a}	2.33±0.24^{d}

Different letters in a row indicate significant differences ($p \leq 0.05$) among ecotypes according to the LSD test.

all, the oxidative stability of the Peruvian accessions was high, in line with or often better than those reported by other authors. For example, Harhar *et al*. (2016) found an OSI of 35.5±4.0 h at 110 °C in Moroccan castor oil, while Adam Ali *et al*. (2016) obtained a value of 48.0 h at 120 °C in solvent-extracted castor oil. The oxidative stability of castor oil is therefore superior to that of other oils, such as chia (1.49 h at 110 °C; Villanueva *et al*., 2017), sacha inchi (0.493 h at 110 °C; Rodríguez *et al*., 2015), argan, olive, sesame and nigella (31.0, 27.0, 28.5 and 17.0 h at 110 °C, respectively (Harhar *et al*., 2016). Furthermore, all the samples exceeded the minimum 8 h oxidation stability at 110 °C requirement prescribed in the EN 14214 specification for biodiesel.

The shelf-life of all castor oils was enhanced when the seeds were extracted at 80 °C, indicating that the temperature played an important role in stability, probably through enzyme inactivation. Carhuaz and Huarmey grande consistently had the longest shelf-life, whereas La Carbonera had the shortest, particularly when extracted at 60 °C. The oxidation of polyunsaturated fatty acids generates volatile compounds that impart undesirable flavors and aromas, endangering the nutritional quality of the oil and limiting its shelf-life (Ixtaina *et al*., 2012). However, the hydroxyl behavior of the unsaturated ricinoleic acid makes castor oil a natural polyol, boosting oxidative stability and shelf-life compared to other vegetable oils. For example, the shelf-life of sacha inchi oil is 3.29, 1.79 and 0.79 years at 20, 25 and 30 °C, respectively (Rodríguez *et al*., 2015), and that of cottonseed oil is 46 days at 25 °C (Kurtulbaş *et al*., 2018). At 25 °C the shelf-life as determined by the Rancimat extrapolation of the biodiesel oil obtained from the transesterification of cooking oil waste was lower (3.1 months), and even after the addition of different strong antioxidants (BHA, BHT, PY, PG, and TBHQ), it only reached a maximum of 37.9 months (3.2 years; Zhou *et al*., 2016). Therefore, the excellent shelf-life of castor oil guarantees a protracted storage without appreciable deterioration.

4. CONCLUSIONS

A wide variation was observed in all the traits analyzed. The abundant ricinoleic acid, coupled with high total tocopherol contents and antioxidant capacity (mainly due to the hydrophilic extracts) promoted good oxidative stability and long shelf-life.

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