

Nutritional quality, fatty acids content and antioxidant capacity of pecan nut fruits from Criolla and Improved walnut varieties

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Abstract

In the current study were evaluated the nutrimental quality, fatty acid and mineral content, and antioxidant capacity of pecan nut of Criolla and Improved walnut varieties. Nut fruit samples of three Criolla walnut varieties ('Nazas I', 'Nazas II' and 'Norteña'), and two Improved varieties ('Western' and 'Wichita') were collected in La Laguna (Nazas, Durango) in Northern Mexico. The analyses run in nut samples were: bromatological and mineral content, fatty acids profile and antioxidant capacity. The results showed that Criolla and Improved varieties had similar bromatological content except 'Nazas I', which had the lowest protein and fat content. Furthermore, nuts from Criolla varieties had a higher mineral content, including iron (~38 mg/100 g dw), zinc (~51 mg/100 g dw) and manganese (25 mg/100 g dw). Besides, the fatty acid profile indicates a higher rate of oleic acid (71%) in nut oil of Criolla varieties than in Improved varieties (61%). Regarding antioxidant capacity (AC) the obtained values were similar in all varieties, with 'Norteña' variety (850 μ M trolox equivalent/g of oil) in Criolla, and 'Western' (800 μ M trolox equivalent/g of oil) in Improved as the highest values of AC among all varieties. Hence, pecan nut fruits of Criolla varieties are good nutrients and beneficial health compounds source for consumers, and their production and marketing represent interesting opportunity areas for nut producers.

Keywords: health benefits; nut; nutriments; oleic acid

Introduction

Pecan walnut [*Carya illinoensis* (Wangenh.) K. Koch] is a native tree from northern Mexico and southern U.S. (Su *et al.*, 2004; Beuchat and Pegg, 2013), although is also cultivated in other regions like Australia, South Africa, Israel and South America (Wakeling *et al.*, 2001). Walnut tree is considered as one of the most important species among the *Carya* gender. Nut is the fruit obtained from this tree, and one of its

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main attributes is its high oil content that can be up to 75% depending on growth conditions, maturity and variety nut (Wakeling *et al.*, 2001). Nut oil is mostly composed by unsaturated fatty acids such as oleic (60-70%) and linoleic acid (up to 30%), and very low amounts of others fatty acids including palmitic, stearic and linoleic acids (Beuchat and Worthington, 1978; Santerre, 1994). Hence, nut fruit can be an important antioxidant supply to diet due to its unsaturated fatty acids (Senter and Horvat, 1978) and tocopherol content (Domínguez-Avila *et al.*, 2013; Nishi *et al.*, 2014), and its regular consumption can help to prevent chronic degenerative diseases associated to lipids consumption (Dominguez-Avila *et al.*, 2015).

México is the fifth pecan nut world producer, with a walnut cultivated surface of 110,530.82 ha, and an annual production of 147,198 ton of nut (SIAP-SAGARPA, 2018). However, most of the world market is composed by Improved varieties, which result from either grafts or genetic cross of selected varieties of a high quality and yielding features (Thompson and Grauke, 2012). Besides, yielding, and oil and sensorial quality of nut fruits from these modified (or 'Improved') walnut varieties can be affected by pests and diseases as well as by climatic conditions during its production cycle (Aguilar Perez *et al.*, 2015). One of the alternatives to decrease such undesired effects in nut fruit quality and yielding are to search 'criollo' nut genotypes.

In Mexico there are several native genotypes classified as Criolla varieties, which have an early maturation and are resistant to infectious diseases like villus stain (*Mycosphaerella caryigena*) and roña, a micotic disease caused by *Cladosporium carygenum* (Aguilar Perez *et al.*, 2015). Criolla nut genotypes are those varieties resulting from ungrafted trees, and regularly are either medium or small size fruits, with a thick and hard-shell nut, and highly adapted to a delimited region, thereby resulting an interesting alternative for nut producers. Furthermore, there are also Criolla nut varieties with a high nutritional quality fruit mostly due to its unsaturated fatty acids content (Wakeling *et al.*, 2001), in addition to its mineral and vitamin content (Martínez-Peniche, 2001). Hence, such Criolla varieties can be an interesting production alternative for nut fruit production due to its lower production cost and better adaptation to different production conditions (Grauke, 1988).

The aim of this study was to determine bromatological composition, fatty acid profile and content and antioxidant capacity of nut fruit from Criolla and Improved varieties cultivated in La Laguna, Durango (Mexico).

Materials and Methods

Vegetable samples

The pecan nut fruits evaluated in this study were selected genotypes from three Criolla nut varieties ('Nazas I', 'Nazas II' and 'Norteña'), and two Improved varieties ('Western' and 'Wichita'). Five kg of nut fruit of each variety were collected from walnut trees cultivated in La Laguna (Durango), located in northern Mexico. Selected walnut trees were 18-year-old with a similar irrigation and fertilization conditions. Sample collection was performed in Nazas, Durango, located at 25° 23' north latitude and 104° 3' west longitude, with an altitude of 1250 masl, dry climate, and an annual average temperature of 20.8 °C.

Reagents

HPLC grade solvents (chloroform, methanol, hexane, and water); hydrochloric and nitric acid; sodium sulphate and hydroxide, boron trifluoride, fatty acids mixture standard; and elements standard trace grade (calcium, copper, iron, manganese and zinc) were all acquired from Sigma-Aldrich.

Bromatological analyses

Analytical tests were performed according to AOAC methods (Horwitz, 2010) of protein (950.48), fat (948.22), ashes (920.48), crude fiber (985.29), humidity (930.15) content, and total carbohydrates by weight difference.

Oil extraction

Oil nut was extracted using an adaptation of Bligh and Dyer method (1959). Approximately 8 g of grounded nut almond sample were placed in 50 mL plastic tubes, and mixed with 30 mL of a chloroform-methanol-water (1:2:2 v/v) solution. The blend was vortexed for 2 min and left without agitation for 20 more min. Afterwards the blend was centrifuged (Centurión Pro-Research, UK) at 3000 rpm for 5 min at 20 °C, and the lipidic (chloroform-oil) phase was extracted and dehydrated in sodium sulphate. The samples residues were removed from the sodium sulphate by washing it twice with chloroform, and finally the remaining solvent was removed in a rotavapor at 65 mm de Hg and 40 °C. The obtained oil sample was stored at 5 °C until further analyses (≤ 6 days).

Fatty acids methylated esters extraction

Fatty acids methylated esters (FAME) extract was obtained using the AOAC method 969.33 (Horwitz, 2010). Approximately 0.5 g of oil sample and 1 mL of 1 N NaOH in methanol were mixed. The mixture was vigorously agitated and heated under boiling bath for 15 min, and cooled at room temperature. Afterwards 1 mL of 14% boron trifluoride was added and the mixture was vortexed for 2 min and allowed to rest without agitation for 30 more min. Then, 2 mL of hexane and 1 mL of distilled water were added, and the mixture was vortexed for 1 min and centrifuged at 1000 rpm for 5 min. The hexane (FAME) phase was extracted and made up to a 10 mL volume with hexane. The FAME extract was finally stored in amber vials at -40 °C until its analyses (≤ 6 days).

Fatty acids identification and quantification

One μ L of the methylated esters (FAME) extract was injected into an Agilent 6820 gas chromatograph (Agilent Technology, CA, EUA) equipped with an ionization flame detector. FAME were separated in a Supelco SP2560 capillary column (120 m x 0.25 mm x 0.2 μ m) (Supelco Analytical, PA, USA) using helium as a carrier gas at a 50-psi pressure. The chromatograph operation conditions were: Injector in a splitless mode at a temperature of 260 °C, initial column temperature of 110 °C with an increase of 5 °C/min until reaching 225 °C during 6 min. High purity standards of FAME were used not only to identify but also quantify these compounds ($R^2 \geq 0.99$). Fatty acid expressed as percentage of total fatty acid content.

Mineral analyses

The evaluated minerals were calcium, copper, iron, manganese and zinc. One gm of grounded nut almond sample was mixed with 10 mL of concentrated nitric acid and 7 mL of 7% Mg (NO₃)₂ (digestion procedure), and dried in a stove at 90-95 °C. Afterwards the mixture was placed in a Thermolyne 48000 oven (Barnstead International, Iowa, USA) at 80 °C, and oven temperature was gradually increased 3 °C/min up to 350 °C, maintaining such temperature until the sample was fully burned. Then, the oven temperature was increased to 525 °C and maintained for 16 h, and finally the sample was allowed to cool to room temperature. The remaining sample was mixed with 2 mL of 50% HNO₃ and heated at 120 °C until got dried. Later, the sample was placed in the oven at 80 °C, and oven temperature was gradually increased 3 °C/min until reaching 525 °C, maintaining the oven temperature for 30 min. The remaining ashes were dissolved in 4 mL of 50% hydrochloric acid and made up to a 25 mL volume (SSA, 1994) with HPLC grade distilled water. Analyses were run using a blank (no sample), and a fortified sample, which was added with a mineral element spike during the digestion procedure. Samples were analyzed in an atomic absorption spectrophotometer Buck model 210 (Buck Scientific Inc. Norwalk, U.S.) by using the flame method. Mineral element quantification was done using standard curves with mineral element standard of calcium, iron, manganese, copper and zinc ($R^2 \geq 0.99$). Results are presented on mg/100 dry weight.

Antioxidant capacity

The antioxidant capacity was evaluated using the β -carotene-linoleic acid bleaching method following the procedure published by Duarte-Almeida *et al.* (2006). A stock solution was prepared mixing 2 mg of β carotene, 45 mg of linoleic acid and 400 mg of Tween 40 in 10 mL of chloroform. Chloroform was removed using a vacuum oven, the final stock solution was prepared adding to the remaining linoleic acid- β carotene-tween mixture 100 mL of distilled water. A 0.5 mL sample of oil nut diluted in ethanol was mixed with 4.5 mL of final stock solution in an amber vial, and immediately was read the mixture absorbance in a spectrophotometer at a wavelength of 470 nm (zero time), and the mixture sample was incubated at 50 °C, reading its absorbance after 2 hours. A standard curve was prepared using Trolox ($R^2 \geq 0.99$), and the results were reported in μ M Trolox equivalent/g of oil.

Statistical analysis

Results were analyzed using a one-way ANOVA, and mean differences were found by means of the multiple comparison test of Fisher ($P < 0.05$), using the SAS software version 8 (SAS Institute Inc, 2005).

Results and Discussion

Bromatological quality

Nowadays consumers demand high nutritional content food products since it is known that their consumption is related to health benefits. The bromatological analyses results of the Criolla ('Nazas I', 'Nazas II' and 'Norteña') and Improved ('Wichita' and 'Western') pecan nut varieties are shown in Table 1. Regarding fat content all nut varieties had a similar composition except the 'Nazas I', which had the lowest fat content ($P < 0.05$). Besides, all nut varieties had a high protein ($7.35 \pm 0.48\%$) and fiber ($12.05 \pm 2.74\%$) content. These results are similar to those published by other researchers (Singanusong *et al.*, 2003; Ozkan and Koyuncu, 2005), with some slight differences attributable to agricultural practices, season and geographic location (Aguilar Perez *et al.*, 2015). Hence, these evaluated nut varieties are excellent fiber and protein source (Mudgil and Barak, 2013; Ötles and Ozgoz, 2014), and can be comparable to high nutritional quality food products like fresh eggs.

Table 1. Bromatological analyses (%) of pecan nut fruits from Criolla and Improved varieties grown in Laguna region (Durango, Mexico)

Variety	Humidity	Ashes	Fiber	Fat	Protein	CH*
'Nazas I'	3.6 ± 0.1^b	1.7 ± 0.2^a	11.2 ± 2.9^{bc}	53.8 ± 11.6^b	5.6 ± 0.4^c	20.9 ± 13.4^a
'Nazas II'	4.2 ± 0.1^a	1.5 ± 0.3^{abc}	8.3 ± 1.9^c	67.3 ± 1.2^a	8.2 ± 0.6^a	10.9 ± 1.5^b
'Norteña'	3.0 ± 0.2^c	1.3 ± 0.1^c	10.1 ± 1.8^{bc}	65.5 ± 1.8^a	7.9 ± 0.5^{ab}	11.6 ± 3.7^b
'Wichita'	3.4 ± 0.2^b	1.4 ± 0.1^{bc}	16.9 ± 4.2^a	65.4 ± 8.5^a	7.3 ± 0.5^b	4.9 ± 6.7^c
'Western'	2.9 ± 0.2^c	1.6 ± 0.1^{ab}	13.6 ± 2.9^{ab}	58.7 ± 2.4^{ab}	7.7 ± 0.4^{ab}	14.9 ± 3.8^{ab}

Results (%) stand for mean \pm standard deviation ($n = 5$). Different characters in the same column mean significantly different values according to Fisher test ($P < 0.05$).

*Carbohydrates

Mineral content

The results of mineral content showed that iron, zinc and manganese content in nut fruits from Criolla varieties was higher than in Improved genotypes ($P < 0.05$), meanwhile copper content was higher in Improved varieties. Calcium content was similar in all evaluated varieties. These results disagree with those reported by Singanusong *et al.* (2003), who published a higher Cu, Mn and Ca content in other nut varieties, attributing such differences to the geographic location, nut genotype, soil and climate conditions (Aguilar Perez *et al.*, 2015). Zinc is one of the most required nutrients for walnut trees, since its deficiency results in vein chlorosis,

slow leaf growth and shortening of branches length, in addition that this mineral plays an important role as a chlorophyll stabilizer (Ojeda-Barrios *et al.*, 2012). Besides, it is known that zinc consumption contributes to maintain important metabolic functioning in humans, such as protein and energy synthesis (Rose, 2016), and also as an anti-inflammatory agent (Jarosz *et al.*, 2017); and its deficiency can delay growth and neuronal development, as well as immunity alterations and even death (Rubio *et al.*, 2007). Zinc content in Criolla varieties can be considered as high, implying that Laguna region walnut trees requirements of this mineral are fulfilled, and that consumption of nut fruits from these Laguna Criolla varieties would promote health benefits for consumers.

Table 2. Mineral content (mg/100 g dw) of pecan nut fruits from Criolla and Improved varieties grown in Laguna region (Durango, Mexico)

Variety	Fe	Cu	Zn	Mn	Ca
'Nazas I'	58.5 ± 4.2 ^a	12.5 ± 1.4 ^d	59.6 ± 4.4 ^a	27.4 ± 2.3 ^a	131.4 ± 13.2 ^a
'Nazas II'	35.7 ± 3.3 ^{bc}	14.8 ± 2.1 ^c	47.4 ± 5.1 ^b	30.3 ± 3.5 ^a	84.10 ± 7.70 ^b
'Norteña'	23.2 ± 3.7 ^d	16.7 ± 2.0 ^{bc}	47.0 ± 5.1 ^b	16.4 ± 1.5 ^b	140.1 ± 14.2 ^a
'Wichita'	29.9 ± 3.2 ^c	18.1 ± 1.8 ^a	38.1 ± 3.9 ^c	7.11 ± 1.0 ^c	102.5 ± 9.80 ^{ab}
'Western'	36.8 ± 4.1 ^b	17.0 ± 2.9 ^{ab}	25.1 ± 3.2 ^d	14.1 ± 1.2 ^b	97.8 ± 10.1 ^{ab}

Results (mg/100 g dw) stand for mean ± standard deviation (n = 5). Values in the same column followed by different character indicate significant difference according to the Fisher LSD test P <0.05.

Fatty acids profile

One of the most important nutritional attributes of nut fruit is its high unsaturated fatty acids content, which are beneficial to human health (Atanasov *et al.*, 2018). Results of fatty acids content in nut fruits of Criolla and Improved walnut varieties evaluated in this study indicate that oleic acid had the highest fatty acid content in all nut varieties, followed by the linoleic acid, and with low amounts palmitic, stearic and linolenic acid (Table 3). Pecan nut fruits from Criolla walnut varieties had higher oleic acid content (68-73%), meanwhile Improved varieties had higher linoleic (27.7 ± 2.85%) and linolenic acid content (1.51 ± 0.15% in 'Wichita' variety). Regarding the saturated fatty acids, palmitic acid content was higher in Improved varieties, meanwhile stearic acid was similar in all nut varieties evaluated.

These results are similar to those reported in other studies, having Criolla nut varieties (Toro-Vazquez *et al.*, 1999; Villarreal-Lozoya *et al.*, 2007; Polmann *et al.*, 2019) a higher oleic acid content than Improved varieties (Oro *et al.*, 2008; Nishi *et al.*, 2014). It has been proven that diets containing food products with a high oleic acid content are beneficial for health (Menendez *et al.*, 2005; Carrillo *et al.*, 2012), which is mostly due to its anti-lipidemic (Nishi *et al.*, 2014, Dominguez-Avila *et al.*, 2015), anti-inflammatory and anti-carcinogenic properties (Banim *et al.*, 2018). Therefore, ingest of nut fruits from these Criolla walnut varieties grown in La Laguna can promote health benefits for consumers.

Table 3. Fatty acid content (%) of pecan nut oil of Criolla and Improved varieties cultivated in La Laguna (Mexico)

Variety	Palmitic	Stearic	Oleic	Linoleic	Linolenic
'Nazas I'	4.15 ± 0.52 ^b	2.57 ± 0.36 ^{ab}	71.91 ± 5.78 ^a	19.01 ± 1.05 ^{cd}	1.38 ± 0.18 ^b
'Nazas II'	4.57 ± 0.45 ^b	2.87 ± 0.27 ^a	73.01 ± 8.64 ^a	17.37 ± 1.36 ^d	1.25 ± 0.15 ^c
'Norteña'	5.19 ± 0.97 ^b	2.96 ± 0.31 ^a	68.42 ± 6.72 ^b	21.17 ± 1.97 ^c	1.16 ± 0.12 ^c
'Western'	6.19 ± 0.65 ^a	2.48 ± 0.25 ^{ab}	63.13 ± 7.88 ^c	25.82 ± 2.44 ^b	1.22 ± 0.23 ^c
'Wichita'	7.23 ± 0.71 ^a	1.95 ± 0.17 ^b	58.32 ± 5.94 ^d	29.64 ± 3.25 ^a	1.51 ± 0.18 ^a

Results (%) stand for mean ± standard deviation (n = 5). Values in the same column followed by different character indicate significant difference according to the Fisher LSD test P <0.05.

Antioxidant capacity

Nut fruits contain phyto chemical compounds that could exert beneficial health effects due to their antioxidant properties (Villarreal-Lozoya *et al.*, 2007; Laura *et al.*, 2014). Antioxidant capacity of nut fruit oil from evaluated walnut varieties in this study (Figure 1) show that 'Norteña' variety (a Criolla variety) had $873.1 \pm 78.8 \mu\text{M}$ Trolox equivalent/g of oil, meanwhile 'Western' (an Improved variety) had $634.6 \pm 61.1 \mu\text{M}$ Trolox equivalent/g of oil; although there were not differences among all varieties (Table 3, $p > 0.05$).

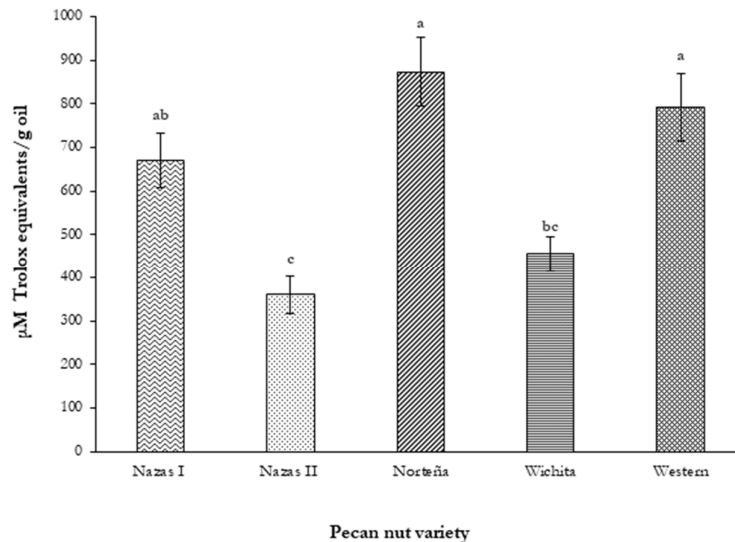


Figure 1. Antioxidant capacity (μM Trolox equivalent/g of oil) in Criolla ('Nazas I', 'Nazas II' and 'Norteña') and Improved ('Wichita' and 'Western')

Column with different character indicate significant difference according to the Fisher LSD test $P < 0.05$.

The antioxidant capacity of nut oil is due to its unsaturated fatty acid composition as well as its vitamin E content, which are proven as excellent antioxidant agents (Jiang, 2014; Yang *et al.*, 2015). There is not available literature regarding antioxidant capacity of oil nut, therefore these results could provide valuable information about antioxidant properties of oil nut from the Laguna walnut varieties evaluated in this study as good nutraceutical food product.

Conclusions

Pecan nut fruits of Criolla varieties cultivated in La Laguna have good nutrimental attributes and antioxidant capacity similar to those of Improved varieties such as 'Western' or 'Wichita'. Besides, Criolla varieties nut had a higher zinc, iron and oleic acid content than in Improved varieties evaluated. Hence, regular consumption of nut fruit of Criolla varieties can promote beneficial health effects to consumers.

Authors' Contributions

Methodology: NFCM; Funding acquisition: JGAA; Writing - review and editing: JRER, JRMF; Validation: ESC; Supervision and Writing - original draft: JAMV

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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