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# EXTRACTION AND CHARACTERIZATION OF THE FATTY ACID PROFILE OF QUINTONIL (*Amaranthus hybridus*)

# EXTRACCIÓN Y CARACTERIZACIÓN DEL PERFIL DE ÁCIDOS GRASOS DEL QUINTONIL (*Amaranthus hybridus*)

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## Abstract

Quintonil (*Amaranthus hybridus*) grows as shrub and it is commonly used as animal feed. This typical Mexican vegetable is consumed in certain regions after a thermal processing, namely, boiling, frying or steaming. Some reports indicate that quintonil contains nutritive compounds such as, vitamins, proteins, chlorophyll, phenolic compounds and polyunsaturated fatty acids (PUFA). The aim of the present work was to identify and quantify lipid fractions and to obtain the fatty acid profile of quintonil leaves, as well as to assess the effect of thermal processing (boiling and steaming) on such profile. Our data showed that palmitic, palmitoleic, stearic, oleic, linoleic and $\alpha$-linolenic (ALA) acids were present. ALA was the predominant residue in all the treatments: 1417.8 - 1667.5 mg/100g (fresh), 1621.4 - 1667.5 mg/100g (boiled) and 1437.9 - 1912.6 mg/100g (steamed). A similar behaviour was observed for the other fatty acids, indicating that thermal processing did not affect the fatty acid profile of quintonil. Conversely, it seemed to favour their availability. This research promotes quintonil consumption because of its health implications in an attempt to preserve Mexican biodiversity and revaluate this ancient crop.

*Keywords*: Quintonil, fatty acid, extraction, characterization, thermal processing.

## Resumen

El quintonil (*Amaranthus hybridus*) crece como maleza y se emplea como alimento para animales. Esta planta autóctona de México es consumida en ciertas regiones del país de forma cruda o después de haberse sometido a un tratamiento térmico: hervido, frito o cocido al vapor. Algunos estudios refieren que el quintonil contiene compuestos bioactivos como vitaminas, proteínas, clorofila, compuestos fenólicos y ácidos grasos poliinsaturados (PUFA). Por ello, el objetivo de este trabajo era identificar y cuantificar los diferentes grupos de lípidos y perfiles de ácidos grasos, así como el efecto del tratamiento térmico (hervido y cocción al vapor). De los resultados obtenidos se encontró que el perfil de ácidos grasos del quintonil es el siguiente: ácidos palmítico, palmitoleico, esteárico, oleico, linoleico y $\alpha$-linolénico (ALA). El ALA se identificó como el componente mayoritario en todos los tratamientos: 1417.8 - 1667.5 mg/100g (quintonil crudo), 1621.4 - 1667.5 mg/100g (hervido) y 1437.9 - 1912.6 mg/100g (cocido al vapor). Se observó un comportamiento similar para los demás ácidos grasos, indicando que el procesamiento térmico no afecta el contenido de ácidos grasos sino que podría favorecer su disponibilidad. Esta investigación busca promover el consumo del quintonil por sus beneficios a la salud en un intento por preservar la biodiversidad mexicana y revalorizar este cultivo ancestral.

*Palabras clave*: Quintonil, ácidos grasos, extracción, caracterización, procesamiento térmico.

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Fig. 1. *Amaranthus hybridus* (leaf, flowering and seeds).

## 1 Introduction

A remarkable aspect of Mexico is its richness in plant species. Since pre-Hispanic times, ancient civilizations have consumed different crops that are still preserved. However, such indigenous edible plants are undervalued, due to the lack of reliable data related to their nutritional value, and also because consumption of such plants is considered as of low social stature.

*Quelites* is one of those plant species, which are recognized for their large diversity. This is a group of wild and arable plants whose foliage is edible. Currently, more than 250 species are known, belonging to different botanical families. (Vázquez García *et al*., 2004). SAGARPA reported in 2013 some agricultural statistics suggesting the low economic exploitation of *Quelites*, caused by the reduced cultivation surface and their low price. *Quelites* are mainly cultivated in Mexico City and the states of Puebla, Tlaxcala, Querétaro, Hidalgo, Morelos and México. However, *Quelites* could play a very important role in the health of Mexicans for their contents of fiber (8.61%), vitamins (thiamine 2.75 mg, riboflavin 4.24 mg, niacin 1.54 mg and 25.40 mg ascorbic acid in 100 g of sample) and minerals (44.15 mg Ca, 34.91 mg P, 54.20 mg K, 231.22 mg Mg and 13.58 mg Fe in 100 g of sample).

Among *Quelites* varieties, the present study focuses on quintonil (*Amaranthus hybridus*), which grows as a shrub and it is usually employed as animal feed. Quintonil is an herbaceous plant of 0.5-1 m high, branched and often tending to have reddish colour. Leaves are 5-15 cm long and 1-7 cm wide. Tiny and numerous flowers arranged in single or branched, cylindrical, elongated compact assemblies up to 18 cm long and up to 4 cm in diameter, are located at the ends of the branches or in the axilla of the leaves, of greenish, yellowish or reddish, usually formed of 5 elongated pieces up to 2 mm long, straw and spiny at the tip; dry fruit, almost globose, with a single dark and bright seed of about 1 mm in diameter (Figure 1). Quintonil is found in flowers from April to January. It grows among maize, alfalfa, peanut, chickpea and oats, as well as on roadsides. Data reported by SIAP (2015) showed that the annual production of quintoniles in 2013 was 1272.96 ton, which is low compared to huazontle (3189 ton) and pápalo (5855 ton). The surface area for quintonil cultivation was only 8 ha's and the commercial price is $1520/ton (Mexican peso).

As an herbaceous plant, leaves are the edible part. It has been reported that *A. hybridus* leaves are rich in polysaccharides, vitamins and minerals (Grubben, 1993). Akubugwo *et al*. (2007) pointed out the chemical and nutritional value of *A. hybridus* leaves from Nigeria. According to these authors, leaves contain (in dry weight): Na (7.43 mg/100 g), K (54.2 mg/100g), Ca (44.15 mg/100g), Mg (231.22 mg/100g), Fe (13.58 mg/100g), Zn (3.8 mg/100g) and P (34.91 mg/100g); vitamins such as carotene (3.29 mg/100g), thiamine (2.75 mg/100g), riboflavin (4.24 mg/100g), niacin (1.54 mg/100g), pyridoxine (2.33 mg/100g), ascorbic acid (25.40 mg/100g), tocopherol (0.5 mg/100g), as well as amino acids: I, L, K, M, C, F, Y, T, V, H, A, R, D, E, G, P and S.

For the above reasons, *A. hybridus* has a very important nutritional value. The present study deals with the fatty acid content of quintonil and even though this plant cannot be considered as an oilseed crop, some reports have identified interesting fatty acid residues. The fat contents found were: 5.5% (Budin *et al*., 1996), 9.92 ± 1.29% (Bruni *et al*., 2001), 11

and 14% (Dhellot *et al*., 2006), 7.1 and 8.5% (Gamel *et al*., 2007) and 8.3 ± 0.28% (Ogrodowska *et al*., 2014). According to those observations, two important residues were found to be the main components: oleic (C18:1 n-9) and linoleic (C18:2 n-6) acids. Also, squalene has been quantified in significant amounts (Guil-Guerrero *et al*., 2000; He *et al*., 2003).

Typically, quintonil (as other green leafy plants) is consumed fresh (as salad) as well as after being submitted to traditional processing techniques. For instance, in Nigeria it is combined with condiments to make soup (Oke, 1983; Mepha *et al*., 2007). In Congo, leaves are eaten like spinach or green vegetables (Dhellot *et al*., 2006). The leaves are boiled, mixed with a peanut sauce and eaten as a salad in Mozambique and West Africa (Oliveira & DeCarvalho, 1975; Martin & Telek, 1979). With respect to Mexico, quintonil is consumed basically in the same ways but only in certain regions of the country.

As mentioned before, there is no technical data describing neither the fatty acid content of quintonil nor the effect of thermal processing. Because quintonil is important in local diets; the aim of the present research was to identify and quantify lipid fractions and obtain their fatty acid profile from quintonil (*Amaranthus hybridus*) leaves, as well as to investigate the effect of thermal processing (boiling and steaming) on such profile. Our research intends to promote quintonil consumption provided its content of several health protecting nutrients in an attempt to preserve Mexico's biodiversity and revaluate this ancient crop.

## 2 Experimental

### 2.1 Materials

Quintoniles were harvested in a local area in the community of San Lorenzo Tlacotepec, Atlacomulco, state of México, during two different seasons: spring and fall (2015-2016). Leaves are the edible portions and were employed to conduct this study. Leaves were washed and disinfected. Quintonil was identified and authenticated at the National Herbarium of Mexico, by Dra. Hilda Flores Olvera and was registered with the number 1434640. The fatty acid standard Supelco 37 Component FAME Mix was purchased from Supelco (Bellefonte, PA). All the solvents employed were HPLC-grade from SIGMA-ALDRICH (St. Louis, MO).

Table 1. Efficiency of the solvent systems employed to recover lipids from fresh quintonil leaves

| Solvent system | Lipid content* (mg/g) |
|---|---|
| Hexane (100%) | 28 |
| $CHCl_3$:$CH_3OH$ (2:1) | 152.48 |
| $CHCl_3$:$CH_3OH$ (1:2) | 38.5 |

*The entries represent the mean of triplicate determinations (dry matter). 5 g of fresh quintonil leaves were used to assay each solvent system.

### 2.2 Lipid content

In order to recover the maximum amount of lipids, we assayed three solvent extraction systems. System 1 consisted of hexane, system 2 of $CHCl_3$:$CH_3OH$ (2:1) and system 3 of $CHCl_3$:$CH_3OH$ (1:2). 5 g of fresh quintonil leaves were set in Soxhlet equipment set at 60ºC with the different solvent systems and magnetically stirred for 1 h. The oil-solvent mixtures were filtered through filter paper to separate the leaves and the liquid phase was placed in a rotary evaporator to remove the corresponding solvents at 40ºC. The lipid fraction obtained from the extracts was estimated by weight difference. The results obtained are depicted in Table 1 (dry matter).

### 2.3 Lipid fractions

Separation of lipid classes was conducted using thin-layer chromatography (TLC). Samples (30 $\mu$L) of the extracts obtained above were dissolved in chloroform (120 $\mu$L) and loaded on TLC plates from Merck (Silica gel 60 $F_{254}$) to be further developed using different mobile phases: (1) hexane:diethyl ether (96:4, v/v) to separate triacylglycerides (TAG), (2) hexane:diethyl ether:acetic acid (100:2:0.2, v/v/v) to separate free fatty acids (FFA) and (3) hexane:ethyl acetate (85:15, v/v) to separate diacylglycerides (DAG), according to Prieto *et al*. (1992). The plates were further submitted to an ultraviolet light tube at 254 nm to permit the identification of the different bands. Secondly, the bands corresponding to the different lipid fractions were scraped off and dissolved in $CHCl_3$:$CH_3OH$ (2:1, v/v). After that, the resultant solution was filtered and then subjected to selective derivatization for GC analysis following the protocol reported by Miranda *et al*. (2013). Derivatization was conducted as follows: 200 $\mu$L of the filtered solutions were mixed with 1 mL of 0.2N HCl-$CH_3OH$ and then heated at 60°C during 4 h; then, 0.2 mL of distilled water and 2 mL hexane were added.

Table 2. Lipid fractions and fatty acid profiles of lipid extracts from fresh quintonil leaves

| Lipid fractions | Fatty acid profile | Fatty acid (area %)* | Fraction yield (%) |
|---|---|---|---|
| **TAG** | C16:0 | 13.2+0.61 | 83 |
| | C16:1 (n-7) | 2.8±0.29 | |
| | C18:0 | 5.1±0.45 | |
| | C18:1 (n-9) | 16.5±0.92 | |
| | C18:2 (n-6) | 23.6±1.57 | |
| | C18:3 (n-3) | 38.7±0.76 | |
| **1,2-DAG** | C16:0 | 40.7±0.23 | 7.2 |
| | C16:1 (n-7) | 0.9±0.14 | |
| | C18:0 | 19.5±1.59 | |
| | C18:1 (n-9) | 22.8±0.37 | |
| | C18:2 (n-6) | 6.7±0.79 | |
| | C18:3 (n-3) | 8.3±1.01 | |
| **1,3-DAG** | C16:0 | 14.9±0.83 | 4.5 |
| | C16:1 (n-7) | 2.9±0.14 | |
| | C18:0 | 6.7±0.63 | |
| | C18:1 (n-9) | 52.4±1.30 | |
| | C18:2 (n-6) | 1.9±0.05 | |
| | C18:3 (n-3) | 19.3±0.65 | |
| **FFA** | C16:0 | 9.6±1.20 | 3.6 |
| | C16:1 (n-7) | 0.5±0.54 | |
| | C18:0 | 0.2±0.06 | |
| | C18:1 (n-9) | 2.4±0.43 | |
| | C18:2 (n-6) | 62.9±1.45 | |
| | C18:3 (n-3) | 23.9±1.76 | |

TAG: triacylglycerides, 1,2-DAG: 1,2-diacylglycerides, 1,3-DAG: 1,3-diacylglycerides, FFA: free fatty acids

* Entries in the table are the mean of triplicate determinations (dry matter).

After vortexing, the methyl esters were extracted in the hexane layer and then collected for GC analysis. The results obtained are depicted in Table 2 (dry matter).

## 2.4 Fatty acid composition

Two $\mu$L of the latter extract was injected into a Varian 3800 GC (Palo Alto, CA) fitted with an Agilent HP-Innowax polar capillary column (30 m X 0.32 mm X 0.25 $\mu$m). Injector (CP-8410) and FID temperatures were both set at 250ºC. The oven temperature was kept at 50ºC for 2 min, then raised to 220ºC at rate of 30ºC/min and held for 25 min. After that point, temperature was taken to 255ºC and held for 7 min. Fatty acids were identified by comparing their retention times with those of the Supelco 37 FAME Mix standard. The results obtained are depicted in Table 2 (dry matter).

## 2.5 Thermal processing

As it was indicated before, quintoniles are typically consumed either fresh or after a thermal processing. In the present study, we selected boiling and steaming as thermal processes to evaluate their effect on quintonil fatty acid profile.

*Boiling*. 5 g of fresh quintonil leaves were immersed in boiling water at atmospheric pressure for 10 minutes. After that, samples were placed in a water bath at 4ºC during 30 seconds to stop cooking. Excess of water was further removed with desiccant paper.

*Steaming*. 5 g of fresh quintonil leaves were cooked in a stainless steel Ekco steamer by the effect

of saturated steam (produced with 200 mL of boiling water at atmospheric pressure) for 10 minutes. A stove, fitted with temperature control, was used. After that, samples were placed in a water bath at 4ºC during 30 seconds to stop cooking. Excess of water was further removed with desiccant paper.

Both boiled and steamed quintonil samples were submitted to lipid fraction (see section 2.3) and fatty acid composition (see section 2.4) analysis.

### *2.6 Statistical analysis*

All the trials were performed by triplicate (r), using a completely randomized design to evaluate the effects of thermal processing and season of the year on fatty acid content. The treatments (t) were fresh, boiled and steamed quintonil leaves and the seasons (s) were spring and fall (see Table 3). Data were analysed by ANOVA using SAS 9.0, when needed mean treatments were compared using Tukey´s multiple range procedure. A p-value of less than 0.05 was regarded as significantly different.

Table 3. Statistical analysis to evaluate the effects of thermal processing and season of the year on fatty acid content

| **Dependent Variable: C16:0** | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean square** | **F value** | **Pr>F** |
| t | 2 | 3288.19 | 1644.095 | 0.52 | 0.6058 |
| s | 1 | 15330.005 | 15330.005 | 4.87 | 0.0475 |
| r | 2 | 1002.66333 | 501.33167 | 0.16 | 0.8544 |
| **Dependent Variable: C16:1 (n-7)** | | | | | |
| **Source** | **DF** | **Type III SS** | **Mean square** | **F value** | **Pr>F** |
| t | 2 | 710.67 | 355.335 | 2.63 | 0.1128 |
| s | 1 | 3595.52 | 3595.52 | 26.63 | 0.0002 |
| r | 2 | 280.973333 | 140.486667 | 1.04 | 0.3831 |
| **Dependent Variable: C18:0** | | | | | |
| **Source** | **DF** | **Type III SS** | **Mean square** | **F value** | **Pr>F** |
| t | 2 | 803.73 | 401.865 | 5.56 | 0.0195 |
| s | 1 | 62.72 | 62.72 | 0.87 | 0.3698 |
| r | 2 | 188.043333 | 94.0216667 | 1.3 | 0.3079 |
| **Dependent Variable: C18:1 (n-9)** | | | | | |
| **Source** | **DF** | **Type III SS** | **Mean square** | **F value** | **Pr>F** |
| t | 2 | 2072.71 | 1036.355 | 2.78 | 0.1019 |
| s | 1 | 1021.52 | 1021.52 | 2.74 | 0.1238 |
| r | 2 | 234.12 | 117.06 | 0.31 | 0.7364 |
| **Dependent Variable: C18:2 (n-6)** | | | | | |
| **Source** | **DF** | **Type III SS** | **Mean square** | **F value** | **Pr>F** |
| t | 2 | 2103.62111 | 1051.81056 | 0.79 | 0.4775 |
| s | 1 | 72796.5606 | 72796.5606 | 54.45 | <.0001 |
| r | 2 | 1251.86778 | 625.93389 | 0.47 | 0.6371 |
| **Dependent Variable: C18:3 (n-3)** | | | | | |
| **Source** | **DF** | **Type III SS** | **Mean square** | **F value** | **Pr>F** |
| t | 2 | 53817.57 | 26908.785 | 1 | 0.3954 |
| s | 1 | 268058.42 | 268058.42 | 10 | 0.0082 |
| r | 2 | 50587.8533 | 25293.9267 | 0.94 | 0.4165 |

t: treatment, s: season of the year, r: replication

# 3 Results and discussion

Traditional Mexican cuisine includes many vegetables and fruits that were the basis of good nutrition in Mesoamerica, as they offer, in addition to the macro, micronutrients, phytochemicals and molecules whose importance for health has not been fully explored. Many plant species, *Quelites* included, are wasted and undervalued because they are considered as neglected and underutilized species (NUS) in the prevailing supply and marketing systems that do not favour them, with the consequent decrease in demand and supply (Gálvez Mariscal & Peña Montes, 2015).

Basically, the research conducted about NUS has been focused on cultivation, adaptation and some aspects of nutritional value. In an effort to promote its consumption and contribute to generate new technical data, the present study focused on the fatty acid profile of quintonil.

## *3.1 Lipid content*

Fresh quintonil leaves were utilized to extract the highest amount of lipids to be further analysed to identify and quantify the different lipid fractions and fatty acid compositions. Soxhlet equipment was utilized to evaluate the efficacy of three solvent systems. From the entries listed in Table 1 (dry matter), it may be observed that the mixture consisting of $CHCl_3$:$CH_3OH$ (2:1) allowed us to extract the highest lipid content, 152.48 mg/g (762.4 mg), achieving a yield of 15.3%. Conforti *et al*. (2012) attained a similar yield for *A. retroflexus*. Venskutonis & Kraujalis (2013) stated that leafy parts of amaranth contain low amounts of lipids, thus they are rarely analysed. However, we decided to assay quintonil leaves because they represent the traditional edible part.

Soxhlet extraction has been typically employed for recovering lipids in amaranth seeds and the yield has been found to depend on particle size, process time and the repeated extraction cycles (Venskutonis & Kraujalis, 2013). Members of the *Amaranthaceae* family have also been subjected to milling which increased the recovery yield (Lyon & Becker, 1987; Gamel *et al*., 2007; Barba de la Rosa *et al*., 2009). Instantaneous controlled pressure-prop has been applied too; however, it was suggested that after a very fine grinding, no special pre-treatments would be required. Supercritical fluid extraction using $CO_2$ as a solvent is a novel technique for the extraction of amaranth seeds (Venskutonis & Kraujalis, 2013).

## *3.2 Lipid fractions and fatty acid compositions*

Once the most efficient solvent system was selected, we proceeded to characterize the different lipid fractions and their corresponding fatty acid compositions. After being recovered, lipid extracts were analysed by TLC. Three fractions were identified: TAG, DAG (including both 1,2-DAG and 1,3-DAG) and FFA. The yields for each fraction are 83, 7.2, 4.5 and 3.6%, respectively (see Table 2). However, it should be noted that the fatty acid residues present in each fraction were different. In their review, Venskutonis & Kraujalis (2013) suggested that the main components in the lipophilic fraction of *Amaranthus* seeds included TAG, phospholipids, squalene, and tocopherols. These authors also refer various minor components, such as phytosterols, waxes, and terpene alcohols for different *Amaranthus* species. Gamel *et al*. (2007) characterized both *A. caudatus* L. and *A. cruentus* L. seed oils and they observed that lipid fractions consisted of 80.3-82.3% of TAG, 9.1-10.2% of phospholipids and the squalene content ranged 4.8-4.9% in both types of oil.

With respect to the fatty acid composition, we quantified the following residues: palmitic (C16:0), palmitoleic (C16:1 n-7), stearic (C18:0), oleic (C18:1 n-9), linoleic (C18:2 n-6) and $\alpha$-linolenic (ALA, C18:3 n-3) acids. These fatty acids were identified in all the fractions but the major fatty acid residue was different in each fraction (see Table 2). The predominant fatty acids for each fraction were ALA (38.7%) in the case of TAG, palmitic acid (40.7%) for 1,2-DAG, oleic acid (52.4%) in 1,3-DAG and linoleic acid (40.7%) in the FFA. Other studies on leaves referred to the following composition: ALA (56.5-62%), linoleic (15.5-24.7%) and palmitic (13.5-15.5%) (Guil-Guerrero *et al*., 2000; He *et al*., 2003). On the other hand, Table 4 summarizes the fatty acid profiles for different *Amaranthaceae* members. The entries are according to the fatty acids identified in the quintonil leaves assayed in this research and other profiles previously reported in the literature for different *Amaranthus* seeds. We can outline interesting and promising technological parameters from Table 4: (a) plant species and cultivar, (b) the fatty acid profile for leaves differs to the ones obtained for seeds, (c) ALA was the major component for quintonil leaves while linoleic acid was for seeds, (d) the contents

Table 4. Fatty acid profiles for different *Amaranthaceae* species

| **FATTY ACID** | | *A. hybridus* | | | *A. caudatus* | | | *A. cruentus* | | | | | | | *A. dubis* | *A. hypochondriacus* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **(%)** | **This work** | **[1]** | Var. 1 **[2]** | Var. 2 **[2]** | **[1]** | **[4]** | **[6]** | **[1]** | **[3]** | **[5]** | **[6]** | **[7]** | **[8]** | **[9]** | **[1]** | **[1]** | **[3]** |
| **C16:0** | **13.2** | 20.5 | 19.35 | 18.47 | 18.3 | 16.54 | 20.5 | 20.1 | 22.2 | 23.45 | 19.4 | 20.4 | 20.75 | 17.02 | 16.9 | 21.8 | 21.4 |
| **C16:1 (n-7)** | **2.8** | - | - | - | - | - | - | - | 0.11 | - | - | 0.4 | 16.57 | 0.09 | - | - | 0.1 |
| **C18:0** | **5.1** | 2.8 | 2.95 | 3.48 | 3.1 | 4.64 | 2.2 | 3.3 | 3.57 | 4.16 | 4.5 | 3.9 | 3.79 | 2.12 | 3.5 | 3 | 3.98 |
| **C18:1 (n-9)** | **16.5** | 20.8 | 30.64 | 26.44 | 28 | 26.19 | 25.5 | 27.5 | 30.1 | 24.66 | 32.9 | 32.1 | 23.57 | 19.13 | 20.4 | 16.3 | 22.8 |
| **C18:2 (n-6)** | **23.6** | 46.4 | 37.13 | 40.48 | 35.6 | 46.91 | 49.8 | 43 | 42.2 | 47.05 | 40 | 38.2 | 35.31 | 24.84 | 46.9 | 52.5 | 49.1 |
| **C18:3 (n-3)** | **38.7** | - | 0.69 | 0.74 | 0.3 | - | 0.6 | - | 0.69 | 0.69 | 0.5 | 0.7 | - | 1.29 | 0.4 | - | 0.93 |
| **S/U ratio** | **0.22** | **0.35** | **0.33** | **0.32** | **0.33** | **0.29** | **0.3** | **0.33** | **0.35** | **0.38** | **0.32** | **0.34** | **0.32** | **0.42** | **0.3** | **0.36** | **0.35** |

[1] Budin *et al*. (1996), [2] Dhellot *et al*. (2006), [3] Jahaniaval *et al*. (2000), [4] Bruni *et al*. (2001), [5] Ogrodowska *et al*. (2014), [6] Gamel *et al*. (2007), [7] León-Camacho *et al*. (2001), [8] Escudero *et al*. (2004), [9] Sujak & Dziwulska-Hunek (2010).
S/U ratio: saturated fatty acids/unsaturated fatty acids ratio

are listed according to different isolation procedures, applied solvents mixtures or particle size (in the case of grinding), (e) agrotechnological practices and growing location and, (f) Saturated/Unsaturated fatty acids (S/U) ratios.

About the S/U ratios depicted, the mean values were 0.33, 0.31, 0.36, 0.3 and 0.355 for *A. hybridus*, *A. caudatus*, *A. cruentus*, *A. dubis* and *A. hypochondriacus*, respectively, indicating elevated unsaturated fatty acids contents. In comparison to related species, the S/U ratio obtained for quintonil leaves is highly unsaturated (0.22 S/U ratio), suggesting that it is a promissory source of valuable nutritional omega-3 (38.7%) and omega-6 (23.6%) PUFA. In previous detailed reviews, we have described the biochemical and biological functions of PUFA (Baeza-Jiménez *et al*., 2014; Baeza Jiménez & García Galindo, 2014). In agreement to our results, Conforti *et al*. (2012) published that *A. hybridus* contains higher amounts of PUFA (11.0 ± 1.40% C18:2 and 58.80 ± 2.20% C18:3) in comparison to other green leafy vegetables: *Anchusa azurea* (2.57 ± 0.28% C18:2 and 5.02 ± 0.48% C18:3), *Cichorium intybus* (0.36 ± 0.01% C18:2 and 1.58 ± 0.13% C18:3), *Portulaca orelacea* (0.70 ± 0.05% C18:2 and 1.78 ± 0.19% C18:3), *Raphanus raphanistrum* (1.65 ± 0.15% C18:2 and 9.43 ± 0.89% C18:3) and *Sochus oleraceus* (1.26 ± 0.14% C18:2 and 2.6 ± 0.25% C18:3).

On the other hand, it is worth noting that a low S/U ratio also indicates that oils can be easily oxidized. We have already mentioned that these leafy vegetables are consumed fresh, as well as after a thermal processing. Thus, quintonil leaves underwent boiling and steaming treatments in order to evaluate their effect on PUFA content.

## *3.3 Thermal processing*

*Amaranthaceae* leaves or seeds may be processed for consumption in different ways. However, processing may affect the bioactive compounds present. Our results obtained after thermal processing are depicted in Table 5 (dry matter). It can be observed that regarding the form of consumption, as well as the season of the year, ALA is the main component for all the treatments assayed: 1417.8 mg/100g sample (fresh), 1621.4 mg/100g (boiled) and 1437.9 mg/100g (steamed) for spring; and in the case of fall: 1667.5 mg/100g (fresh), 1629.2 mg/100g (boiled) and 1912.6 mg/100g (steamed). For the other fatty acids present in quintonil leaves, variations on their contents were also noted. These observations are in accordance with other reports. In a similar study, Torres Acosta *et al*. (2006) found the following ALA contents: 26.55 mg/100g (fresh), 34.85 mg/100g (boiled) and 34.61 mg/100g (steamed), as well as 99.97, 81.75 and 174.3 mg/100g contents for linoleic acid in fresh, boiled and steamed samples, respectively. Our contents for such residue were higher. The different concentrations can be explained by differences in analytical protocols, processing time and cultivations.

Venskutonis & Kraujalis (2013) reviewed some studies related to processing parameters for *Amaranthaceae* members. They cited that during puffing or popping of *A. cruentus* seeds, the percentage of unsaturation decreased from 75.5% to 62.3%, the content of linoleic acid decreased from 46.8 to 27.0% and for squalene a significant increase was noted. They also mentioned that popping and cooking reduced the lipid contents in *A. caudatus* and *A. cruentus* seeds by 5.6% and 7.7%, and by 1.7% and 3.7%, respectively. Similarly, Bruni *et al*. (2001) explored supercritical fluid extraction using carbon dioxide for lipid extraction. The authors did not have significant effects on the fatty acid profile as compared with traditional methods for both wild *A. caudatus* from Ecuador and Italian *A. caudatus*. Their best results were obtained at 40 MPa in terms of total extract yield; however, fatty acid compositions were similar.

Table 5. Effect of thermal processing on the fatty acid profiles of quintonil at different seasons of the year

| **Fatty Acid** | **Fresh (mg/100g)** | | **Boiled (mg/100g)** | | **Steamed (mg/100g)** | |
|---|---|---|---|---|---|---|
| **Season** | **Spring** | **Fall** | **Spring** | **Fall** | **Spring** | **Fall** |
| **C16:0** | 458.1±9.3 | 483.9+9.6 | 449.6±16.5 | 444.9±48.4 | 402.1±45.1 | 556.1+15.6 |
| **C16:1 (n-7)** | 100.2±29.2 | 135.4±33.0 | 96.5±5.8 | 108.4±12.2 | 90.3±4.2 | 128.0±8.5 |
| **C18:0** | 62.9±29.2 | 61.8±3.0 | 43.6±0.1 | 52.8±5.9 | 46.6±6.5 | 49.7±0.4 |
| **C18:1 (n-9)** | 89.9±37.7 | 116.9±40.9 | 82.1±14.3 | 100.8±22.1 | 77.4±15.9 | 76.9±5.0 |
| **C18:2 (n-6)** | 281.9±25.2 | 424.7±0.1 | 319.5±11.0 | 398.6±24.3 | 296.5±20.2 | 459.5±53.8 |
| **C18:3 (n-3)** | 1417.8±81.1 | 1667.5±95.2 | 1621.4±158.4 | 1629.2±137.8 | 1437.9±104.9 | 1912.6±6.2 |

*The values shown in the table are the mean of triplicate determinations (dry matter).

Some other interesting observations can be noticed from Table 5. As we indicated in section 2.1, quintonil was harvested during two different seasons: spring and fall. With some exceptions, the higher contents for the different residues were measured on fall regarding the treatment assayed it is worth highlighting the content reached for ALA on fall: 1667.5 mg/100g (fresh), 1629.2 mg/100g (boiled) and 1912.6 mg/100g (steamed).

Fatty acid synthesis occurs in the chloroplast, where NADPH is also produced by the light reactions of photosynthesis. Palmitic (C16:0) is the precursor of stearic (C18:0), as well as palmitoleic (C16:1 $\Delta^9$) and oleic (C18:1 $\Delta^9$). Mammals cannot convert oleic to linoleic (C18:2 $\Delta^{9,12}$) or ALA (C18:3 $\Delta^{9,12,15}$), whereas in plants, oleic is produced by stearoyl-ACP desaturase in the chloroplast stroma that uses reduced ferredoxin as the electron donor. Plants can also synthesize the desaturases that introduce double bonds at the $\Delta^{12}$ and $\Delta^{15}$ positions and they are located in the endoplasmic reticulum and the chloroplast. The endoplasmic reticulum enzymes act not on FFA but on a phospholipid, phosphatidylcholine, which contains at least one oleic linked to glycerol. Both plants and bacteria must synthesize PUFA to ensure membrane fluidity at reduced temperature (Nelson & Cox, 2014). These biochemical implications permit us to understand the entries listed in Table 5.

### *3.4 Statistical analysis*

A statistical evaluation was conducted to evaluate the effects of thermal processing and season of the year on the fatty acid profile of quintonil leaves. Table 3 summarizes the ANOVA carried out. According to the results obtained there was not significant difference for the thermal processing except for stearic acid, when a reduced concentration was observed. In the case of season of the year, except for oleic acid, fall is the best time for consuming quintonil leaves. On spring, due to a high photosynthetic activity, lipids become a practical form of stored carbon and energy for the growing shoots and roots of seedlings whereas on fall, lipids are mainly maintained for membrane biogenesis. That is why a larger concentration of PUFA was measured on fall. According to Torres Acosta *et al*. (2006) the degree of unsaturation is higher when vegetables grow in cooler zones.

## Conclusions

Most plants consumed contain beneficial components such as lipids. The composition of fatty acids found in those lipids (or oils) determines their nutritional, technological, and stability properties. In an attempt to preserve the Mexican biodiversity and revaluate this ancient crop, we carried out this research focused on the lipid content of quintonil leaves. The fatty acids identified were palmitic, palmitoleic, stearic, oleic, linoleic and ALA. Compared to other reports, ALA was found as the predominant fatty acid residue in all treatments. The fatty acid contents were not adversely affected by thermal processing but they actually increased. Even though quintonil is not an oilseed, the high PUFA content (38.7% of ALA and 23.6% of linoleic acid) can be potentially exploitable as a foodstuff. On the other hand, quintonil has been reported to contain fiber, vitamins, minerals and various phytochemicals, which can be a coadjutant to improve Mexican health that is strongly affected by obesity and diabetes.

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