NUTRITIONAL CONTENT AND CAPSAICIN IN CHILE DE AGUA FRUITS(Capsicum annuum L.)

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ABSTRACT

Chili fruits are a distinctive feature of Mexican diets. Chile de agua (Capsicum annuum L.) in the Central Valleys of Oaxaca, Mexico, are important economically, social, culturally, and especially as food. Chili fruits were collected directly from the field to quantify the content of macro- and micro-elements, protein, fats and capsaicin in the pericarp, seed and placenta and determine their nutritional value and pungency. Nutrient content was determined in an atomic absorption spectrophotometer, and P and S content was determined in a UV spectrophotometer. Protein was quantified in an elemental organic analyzer, and for fat content the Soxhlet method was used. To determine capsaicin in pericarp, seed and placenta of fresh chili fruits, extraction, purification and quantification was performed with the Soxhlet method and in a UV spectrophotometer. The seeds showed a significantly high content of protein (3.49 g 100g-1) and fats (1.19 g 100g-1). Differences in capsaicin concentration were significant: placenta (19256.3Su) >seed (11034.7Su) > pericarp (9525.3Su).

Keywords: Fat, Placenta, Protein, Pungency, Seed.

1. INTRODUCTION

Plants have been basic elements in the development of human societies; they have satisfied different biological and cultural needs (Bañuelos *et al.*, 2008).All organisms need to ingest a series of substances that serve to renew, repair and reproduce their structure or as precursors of substances that control and coordinate their metabolism. These substances essential for life are called nutrients (macro and micro), which are ingested in food (Bourges, 2011; Montaña *et al.*, 2008). Vitamins, minerals, fatty acids and essential amino acids are needed in lower quantities but are fundamental for the organism because they intervene in many processes; that is, each of the nutrients is characterized by the functions it carries out in the organism (Montaña *et al.*, 2008).

Chilis are considered a basic food in nutrition (Ben *et al.*, 2006; Bourges, 2011; Zegbe *et al.*, 2012), and in Mexican diets since its most important use is as an ingredient in the basic diet (Long, 2011; Zegbe *et al.*, 2012).They are consumed fresh or dried, in drinks (Bourges, 2011), or as a condiment (Zegbe *et al.*, 2012).Interest in chili is not centered only onits economic importance. It has been shown that it is an excellent source of natural dyes, vitamins (A, C, E and

B), minerals (N, P, K, Ca Mg, Na, Fe, Cu, Zn and Mn), and energy (Arcos *et al.,* 1998), as well as phytochemical compounds (phenolic acids) such as capsaicinoids.

Capsaicin is a very important secondary metabolite with diverse medicinal uses and biological activity. In the medical literature reports have been published that show the effect of capsaicin in several pathological processes such as allergies. It decreases the concentration of chemical substances that participate in the transmission of pain in postsurgical dietsrich in vitamin C (present in chilis). It participates in synthesis of collagen, a fundamental protein in the process of scarring. It reduces cardiovascular disorders and other chronic degenerative diseases (Waizel and Camacho 2011). Capsaicin and dihydrocapsaicin, located mainly in the placenta tissue adjacent to the seeds, are responsible for more than 90% of the spiciness (Ben *et al.*, 2006). Their contents depend on the genotype, fruit maturity and growing conditions.

The potential of chilis as sources of capsaicinoids in the pharmaceutical industry has promoted its phytochemical study. Research has focused on improved varieties of different types of chilis, but regional varieties have been ignored. This is the case of chile de agua, which is a highly important crop economically, social and culturally. It is endemic in the Central Valleys of Oaxaca where it is used as food, medicine, in rituals and as an ornamental (Montaño *et al.*, 2014).For these reasons, in this study we quantified the contents of macro and microelements, protein, fats and capsaicin in pericarp, seed and placenta of chile de agua fruits (*Capsicum annuum* L.) to determine the nutritional contribution and pungency of this vegetable.

2. MATERIALS AND METHODS

Description of the study area

The study was conducted in the region of the Central Valleys of Oaxaca, Mexico (16º 20' and 17º 40' N; 95º 55' and 97º 30' W) on a high plateau with an averagealtitude of 1 500 m. The region has an extension of 8 762.36 km^2 and makes up 9.2% of the state's area. It comprises seven political-administrative districts: Centro, Ejutla, Etla, Ocotlán, Tlacolula, Zaachila and Zimatlán.It is distinguished from other regions by its dynamic interaction with the capital city and among its rural communities because of its geographic proximity and its commercial activity. The geographic configuration of this territory is varied: there are alluvial plains with hills and some mountains that reach heights of 2 050 m. This heterogeneity conditions climate variation, from warm subhumid on the plains with average temperatures of 22ºC to temperate subhumid in the high parts of the sierra with mean annual temperatures of 19.5ºC (INEGI, 2014). Average annual precipitation if 727.7 mm in the center of the region and the rainy season occurs in summer.

Collection of fruits

Chile de agua (*Capsicum annuum* L.) fruits were collected at commercial maturity in eight locations of six districts of the region: San Sebastián (Etla District), San Baltazar Guelavila, San Jerónimo Tlacochahuaya (Tlacolula District) Cuilapam de Guerrero, San Antonio de la Cal (Central District), Zimatlán de Álvarez (Zimatlán District), Ejutla de Crespo (Ejutla District) and Ocotlán de Morelos (Ocotlán District).

Fruit characteristics

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At commercial maturity, the fruits are firm, lustrous, and yellow green. At physiological maturity, the fruit is soft and red. Fruits are 8 to 12 cm long and 4 to 5 cm in diameter on average.

Determination of nutrient content, protein and fat

Samples were prepared and analyzed in the Laboratory of Environmental Diagnostics of the Instituto Tecnológico del Valle de Oaxaca (ITVO). Fruits were dried at 60ºC in an oven to determine moisture content in pericarp, seed and placenta. Pericarp and seedwere milled separately in an electric mill (Kups GX4100) the placenta in a porcelain mortar. Pulverized samples were stored in individual sealed plastic recipients.

Seed and pericarp samples were assessed for contents of sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), phosphorus (P), sulfur (S) and protein (total nitrogen). Content of the elements was determined following the manual of analytical procedures for analysis of soils and plants (Álvarez and Marín, 2011) in an atomic absorption spectrophotometer (Thermo Scientific ICE 3000 Series AA spectrometer). The data were transformed with the following formula:

$$\text{mg kg}^{-1} = \frac{\text{mg L}^{-1}\ \text{A A} \ \text{x} \ \text{Df} \ \text{x} \ \text{Vt}}{\text{W}}$$

where mg L^{-1} A A= mg L^{-1} obtained in the atomic absorption spectrophotometer; Df = dilution factor; Vt = gauged volume of digested material; W = sample weight.

The content of P was analyzed with the colorimetric method of the vanadatomolybdic reagent with a spectrophotometric (UV-VIS Cintra 10) with absorbance of 470 nm. Data were transformed with the formula:

$$\text{mg kg}^{-1} = \frac{\text{mg L}^{-1}\ \text{UV} \ \text{x} \ \text{Df} \ \text{x} \ \text{Vt}}{\text{W}}$$

where: mg L^{-1} UV= mg L^{-1}obtained in the UV spectrophotometer; Df= dilution factor; Vt= gauged volume of digested material; W= sample weight.

Sulfur content was analyzed with the turbidimetric method using a spectrophotometer (UV-VIS Cintra 10). The data were transformed with the formula:

$$\text{mg kg}^{-1}{}_{S} = \frac{\text{mg L}^{-1}\ \text{U V}_{SO_4} \ \text{x} \ \text{Df} \ \text{x} \ \text{Vt x 32}}{\text{W}}$$

where: mg L^{-1} U VSO_4 = mg L^{-1} SO_4obtained in theUV spectrophotometer; Df= dilution factor; Vt= gauged volume of digested material; W= sample weight; Factor=32.

Protein was quantified by weighing 2 to 3 mg sample per duplicate in an element organic analyzer (Perkin Elmer 2400). The results obtained were transformed with the following formula:

$$\% \text{ Protein} = \%\text{N x } 6.25$$

where: % protein = % dry sample protein; %N = % nitrogen; 6.25 = conversion factor for proteins.

To quantify the fat content, the Soxhlet method was used with 2.0 g of sample. The results obtained were transformed with the formula:

$$\% \text{ fat} = \frac{W_2 - W_1}{W} X100$$

where: W= sample weight; W_1= flask weight alone; W_2= flask weight with fat.

Determination of capsaicin content

To determine capsaicin content, extraction, purification and quantification were performed on pericarp, seed and placenta from fresh chile de agua fruits (Bosland and Votava, 2000).

a) Soxhlet extraction: the fresh fruit was cut into small pieces and 5.0 g were weighed and placed into Whatman cellulose cartridges (22 mm in diameter by 80 mm long). Extraction was performed with 99.7% pure isopropyl alcohol maintaining the reflux for 3 h.

b) Purification: Once the extract cooled, 5.0 mg active carbon was added, and the mixture was placed in a rocking shaker at 180 rpm for 5 min. Extracts were later filtered with Nº 42 Whatman filter paper. The filtrate was transferred to a 200 mL flask and gauged with isopropyl alcohol.

c) Quantification: Absorbance of the extracts were measured at 281 nm in a visible ultraviolet spectrophotometer (GBC CINTRA 10). The calibration curve was prepared with natural capsaicin that contained 65% capsaicin and 35% dihydrocapsaicin at concentrations of 9.3, 18.6, 37.2, 46.5 and 55.8 mg mL^{-1} using isopropyl alcohol. The concentration of the alkaloid was determined with the following formula:

$$\text{Capsicum (mg mL}^{-1}\text{)} = \text{mg mL}^{-1} \text{ CC x Dm}$$

where:mg mL^{-1} CC = calibration curve reading; Dm= extractant volume g^{-1}sample; 1 mg mL^{-1} = 15 Su.

Statistical analysis

The analysis of variance was performed using a completely randomized design for content of macro and micronutrient elements, protein and fats. The treatments were fruit parts (pericarp and seed) with 32 replications (four observations on samples from each of the four locations). With the data on capsaicin, an analysis of variance was performed using a completely randomized design for each fruit part: pericarp, seed and placenta. The treatments were the six locations where fruits were collected. Treatments were replicated four times. Finally, mean values of the capsaicin found in each fruit part (24 observations) were compared with the Tukey test ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

The principal use of chile de agua is culinary; it is an essential ingredient in artisan dishes. Its consumption depends on the food habits that are part of social, cultural and economic value of the population (Montaña, 2008) as well as on its availability. Nutrition is biopsychosocial and has three components: 1) nutritional value (biological), 2) sensorial and emotional value, and 3) social and cultural value (Bourges, 2011). Consumption of chile de agua fruits in the Central Valleys region is essentially based on the two last components since participation of the family is of vital importance among the populations that produce it (Aparicio *et al*., 2013).

Moisture content

Moisture content of fruits on the day they were picked was 91.7% in pericarp. This section had the highest moisture content was found (Figure 1) due to its thickness; that is, the pericarp is fleshy, a good characteristic for its consumption. Mendoza *et al*. (2015) determined 93.7% moisture on picking day in *C. annuum* var. Annuum (serrano chili). In other species low moisture contents have been reported: 7.5 in *C. baccatum*, and 7.9 in *C. chinense* (Yánez *et al*., 2015).

Chile de agua seeds had 79.9% moisture content. Ayala *et al*. (2014) determined moisture content of 7.6% in chile de árbol, chile ancho and chile guajillo. Studies conducted by Reveles *et al*. (2013) on diverse types of chilis mention that seed should have 8 to 10% moisture content to maintain its germination viability.

The chile de agua placenta had 85.3% moisture. Fruits of the genus *Capsicum* characteristically have high moisture content (Caballero, 2017).

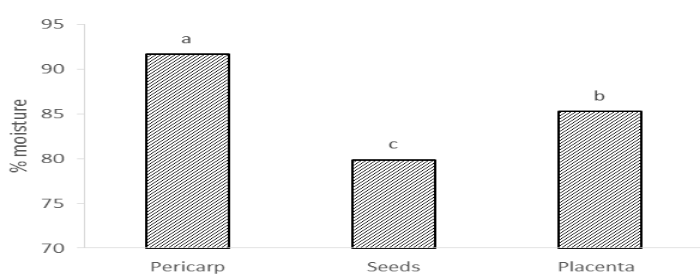

Figure 1.Moisture content in analyzed sections of chile de árbol fruits. Bars with different letters indicate significant differences, Tukey ($p \leq 0.05$) (data obtained from 64 observationson each fruit part).

Nutrient content

The results reflect concentrations of the nutrients in the chile de agua analyzed. The nutrient contribution of a produce depends on nutrient content and of the amount of the food consumed.

The analyses of variance revealed significant differences ($p\leq0.05$) in macro and microelements. The concentrations of the macroelements Na, Ca, Mg, K, P, S and the microelements Fe, Mn and Zn were significantly higher (Tukey, $p\leq0.05$) in seeds than in the pericarp (Table 1), except for Cu for which there was no significant difference. The high or low concentration of minerals in the fruits (Chávez *et al.*, 2016) may be due to crop management, that is, availability and supply of nutrients (Cruz *et al.*, 2014; Martínez *et al.*, 2016), as well as the substrate that favors plant growth (Beltrán *et al*., 2016) and has characteristics of high cationic exchange and other factors.

Of the microelements analyzed, Mn was detected in a lower concentration than Cu, Fe and Zn. Chávez *et al*. (2016), in studies conducted with different morphotypes of *Capsicum* including chile de agua, argue that the different morphotypes are important sources of necessary minerals in the diets of consumers since they form part of their dietary habits.

Table1. Concentrations of macro and microelements in pericarp and seeds of chile de agua fruits (100 g simples, average of 32 replications)

Element	Pericarp	Seed
	---------------------------- mg $100g^{-1}$	-------------------------
Sodium	0.80 b ± 0.024	1.56 a ±0.128
Calcium	7.11 b ± 0.673	16.08 a ± 7.560
Magnesium	5.68 b ± 0.886	14.93 a ± 3.998
Potassium	63.98 b ± 4.819	254.58 a ±35.112
Phosphorus	32.46 b ± 9.347	09.72 a ±1.800
Sulfur	18.36 b ± 9.585	57.47 a ±7.208
Copper	0.10 a ± 0.000	0.15 a ±0.000
Iron	0.23 b ± 0.020	0.65 a ±0.053
Manganese	0.04 b ± 0.000	0.12 a ±0.000
Zinc	0.10 b ± 0.012	0.23 a ±0.032

Values with different letters in each row indicate significant differences (Tukey $P \leq 0.05$).

Protein and fat contents

The analyses of variance revealed significant ($P \leq 0.05$) differences in protein and fat contents. Protein concentration was significantly higher mayor (Tukey, $P \leq 0.05$)in seed (3.49 g $100g^{-1}$) than in pericarp (1.27 g $100g^{-1}$) (Figure 2). This may be because protein is the main nitrogen reserve material of the seed. Accumulation of reserves is one of the three seed development phases that enable it to have high germinating quality and to be resistant in storage conditions (Ayala *et al*., 2014).

Seed fat content (1.19 g $100g^{-1}$) was significantly higher (Tukey $P \leq 0.05$). Fats are a source of energy in foods; they are combinations of saturated and unsaturated fatty acids. According to Martínez *et al*. (2006), in studies on fatty acid profiles in chili pepper varieties, mention that fatty acids are highly important structurally and metabolically for both the plant and the fruit; they are precursor of the biosynthesis of essential volatile compounds in aroma, and their content depends on the degree of fruit maturation. Proteins, fats and carbohydrates are all seed energy reserves, and their function is to sustain the plant during the first life stages such as germination (Doria, 2010).

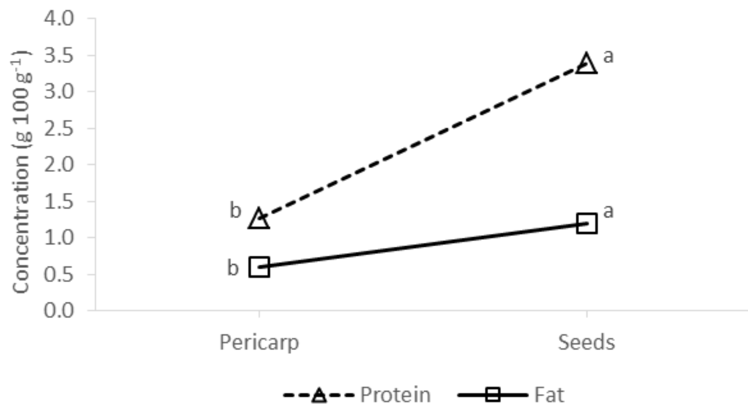

Figure 2. Protein and fat content in pericarp and seeds of chile de agua fruits (100 g fresh sample, average of 32 replications). Different letters in a row indicate significant differences (Tukey $P \leq 0.05$).

Capsaicin content

The analysis of variance showed significant differences ($p \leq 0.05$) in capsaicin content in pericarp (9525.3Su), seed (11034.7Su) and placenta (19256.3Su) among chile de agua fruits (Table 2). In general, the highest concentration of capsaicin (Tukey, $p \leq 0.05$) was detected in the placenta of the analyzed fruits. These results coincide with other authors (Long, 2011; Zegbe *et al.*, 2012), who argue that capsaicinoids are synthesized in fruit placenta tissues and have the function of chemical protection for the seed (García *et al.*,1995).Biosynthesis and accumulation of capsaicinoids occurs in the epidermal cells of the interlocular septum (secretion organs) in the fruit placenta (Ruiz *et al.*, 2011).Capsaicinoids are compounds characteristic to fruits of the genus *Capsicum* and are responsible for their spiciness (Yánez *et al.*, 2015).

The chili fruits from San Antonio de la Cal had the highest concentration of capsaicin in the pericarp, 13291.00Su, while the highest concentration of capsaicin in fruits from Ejutla was found in the seed, 15998.40Su. The lowest concentration, 8620.2Su, was in chilis from Ocotlán. The placenta of fruits from Etla had the highest concentration of capsaicin (28691Su). Chilis, such as poblano chilis, that have a lower level of pungency in general have a low content of capsaicin (0.230 mg g^{-1}fresh weight) (Villa *et al*., 2014), than a spicy chili like chile de agua (9525.33Suinpericarp).

Zegbe *et al.*(2012) argue that there are no criteria that establish how spicy a chili will grow to be since that would depend largely on climate (temperature), solar radiation, amount of water, soil fertility, bioavailability of nutrients (Valadez *et al*., 2016), agronomic management during crop development and phenotypical differences. In this respect, Estrada *et al.*(1999) studied the effect of water stress on *Capsicum annuum* L. var. *annuum*fruits and found a higher quantity of capsaicinoids when the plants were subjected to water deficit. Ruiz *et al.*(2011)mention that levels of spiciness of the genus *Capsicum* is determined by plant genetics, environment and their interaction.

Table 2. Capsaicin concentration in components of chile de agua fruits (values obtained from 24 observations.

Fruit components	Capsaicin (Scoville units)
Placenta	19256.33 a ± 915.732
Seed	11034.70 b ± 635.968
Pericarp	9525.33 c ± 564.436

Values with different letters indicate significant differences, Tukey, ($p \leq 0.05$).

Nutrient content of chili fruits of the genus *Capsicum* has been the object of study by researchers (Borges *et al.*, 2010; Velasco *et al.*,1998; Velasco *et al.*, 2001). However, the relationship between mineral concentration and the content of capsaicin has produced diverse results.

4.CONCLUSIONS

The pericarp had the highest moisture content (91.7%), followed by the placenta (85.3%) and seed (79.9%). The seeds exhibited higher contents of Na, Ca, Mg, K, P, S, Cu, Fe, Mn and Zn than the pericarp. K was the element that was detected in greater quantity in the pericarp than in the seed. Seeds had a higher content of protein (3.49 g $100g^{-1}$)and fat (1.19 g $100g^{-1}$) than pericarp. The concentration of capsaicin was significantly higher in placenta (19256.3Su), followed by seed (11034.7Su) and pericarp (9525.3Su).

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