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Extraction and Quantification of Capsaicin in Chili Varieties

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Abstract

Capsaicin is an ingredient that contains chili varieties. The objective of the study was to determine the concentration of capsaicin in 4 varieties of chili and 5 extraction methods. The varieties were: piquín, habanero, tree and apple, the methods were: water at room temperature, water at 50 ° C, vinegar at 10%, methanol at 10% and ethanol at 10%. Capsaicin was analyzed with HPLC and a 4x5 factorial with 4 repetitions was completely randomized for concentration analysis. There was a highly significant difference in varieties and methods, but the interaction was only significant. Piquin chili presented the highest concentration of capsaicin with 84.3 µg g^{-1}, followed by habanero with 42.8 µg g^{-1}, within the methods ethanol was the best with 84.3 µg g^{-1}, followed by methanol with 74.3 µg g^{-1}, these being the best interactions. Piquin chili with alcohols is the best choice for a sustainable product.

Keywords: Varieties, Spiciness, Organic, Pests

Introduction

Capsaicin (CAP, N-[(4-hydroxy-3-methoxyphenyl) methyl]-8-methyl(-6-nonenamide) and dihydrocapsaicin (DH,N-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methylnonanamide) are responsible for more than 90% of the spiciness in chili peppers (López, 2003; Cázares *et al*., 2005) [15, 9].

Capsicum annuum L., is the most important crop among the species of cultivated chiles, with origin, domestication and diversification in Mexico, you can find a great variety of chiles: ancho, mulato, miahuateco, de chorro or cristalino, huacle, gordo, pasilla de Oaxaca, dulce, de agua, jalapeño, rayado, serrano, loco, criollos de Tecomatlán (red, yellow and black), coxle, criollo de Morelos, ya'ax ik, xcat'ik, gallo-gallina, guajillo, de árbol, puya, copi, soledad, pasilla, chawa, achilito, cora, costeño, tabaquero, taviche, mirador, apaxtleco, bolita, tusta, piquín, güiña shirunduu, güiña shuladi, pico de paloma, ojo de cangrejo, manzano, habanero and chilpaya, cascabel, chocolate, de onza, escuchito, lajoyero, parado, de monte, mirasol, nanche, soltero, zacapaleño and tecpin; however, it is known that in Mexico there are around 1,300 collections of chili peppers (Aguilar *et al*., 2010) [2].

Capsaicin is synthesized and accumulated in the tissue adjacent to seeds (Ben-Chaim *et al*., 2006) [5], and its content depends on genotype, fruit maturity, and growing conditions (Zewdie and Bosland, 2000) [33]. Capsaicin has antimicrobial and antioxidant (Riquelme and Matiacevich, 2017) [23] and antifungal (Moreno *et al*., 2012) [19] properties.

The habanero pepper (*Capsicum chinense*) has a concentration of 679.4 mg kg^{-1} of capsaicin and 514.1 mg kg^{-1} of dihydrocapsaicin, for a total of capsaicinoids of 1,193.6 mg kg^{-1}. In studies from the Sierra de Norte de Puebla, chili peppers were found with 21.54 to 556.78 µg g^{-1} capsaicin and 19.54 to 348.26 µg g^{-1} dihydrocapsaicin (Morán *et al*., 2008) [18].

Repellency studies for *Bemisia tabac*i with habanero pepper extracts (*Capsicum chinense*) showed a repellency of 95% at a capsaicin concentration of 40% over a period of 8 h, indicating that mortality is less than an hour and repellency lasts up to eight hours, and from there, the repellency effect is lost (Castillo *et al*., 2012) [8].

The objective is to evaluate four species of chili peppers from Mexico, for their concentration of capsaicin with five forms of extractants to obtain the highest capsaicin content.

Materials and Methods

Two factors were worked on: A) extraction methods and B) Chili pepper varieties. The extraction methods were 1) Water at room temperature, 2) Water at 50° C, 3) 10% vinegar in water, 4) 10% methanol in water and 5) 10% ethanol in water. The chili varieties were a) Piquín pepper (*Capsicum annuum* var. *Glabriusculum*), b) habanero pepper (*Capsicum chinense*), c) tree

chili (*Capsicum annuum* L) and d) apple pepper (*Capsicum pubescens*).

To have 5 extraction methods and 4 varieties of chili with 4 repeats for a completely randomized design with two factors.

The extraction was performed by weighing 20 g of each chile with its repeats, and it was placed in a 250 mL Erlenmeyer flask, where the 100 mL extraction solutions were applied; it was stirred for one minute and ground in a blender, the ground mixture was placed in a Büchner funnel with a filter No. 615 CMSLLC 3856 of 8 µm and with a chemker 300 vacuum pump towards a 500 mL Kitasato flask, where about 50 mL were collected, in this solution (Brown *et al*, 2015) [7], in each sample it was diluted in 1:10 in a volumetric flask of 10 mL and from the dilution it is passed to a beaker of 10 mL and 2 mL are taken, with a 5 mL syringe then the water is changed and a 0.45 µm agilent filter is placed in the syringe and the sample is passed to a verex vial of 9 mm and 2 mL, for analysis.

The analysis was performed with an Agilent 1200 HPLC, 60% acetonitrile was used as a solvent, with a flow of 1 mL/min and a maximum pressure of 400 bar, the injection was 10 µL and the analysis time was 12 minutes. The method in the HPLC was CAPSAICIN-2021. M and the sequences were Cuant-Habanero.S, Cuant-Piquín.S, Cuant-Manzano.S, Cuant-Arbol.S; to quantify the capsaicin of the chili extracts with the Agilent Sorbax SB C18 column, 5 microns inside and 250 x 4.6 mm in dimensions. This process was carried out in one day for each chili pepper (Martins *et al*., 2011) [17].

The calibration curve for capsaicin was performed with 10, 20, 30, 50, 100 and 200 ppm using a Sigma Aldrich Capsaicin M2028 standard, with the HPLC with the same method and the CALIB-CAPSAICIN sequence. S, to obtain chromatography in milli-Absorbance Units per second (mAU*s) and thus fit it with the ppm value to a linear regression model (Abdalla *et al*., 2017) [1].

The analysis of variance of the factors and their interaction was performed with the SAS 9.4 program (SAS, 2022) [28].

Results and Discussion

Capsaicin calibration was obtained at a retention time of 8.3 minutes so that the absorbance was presented in the chromatography, the values that were obtained and their adjustment are presented in Fig 1.

The model has an almost exact adjustment of 0.99, but since the response variable is area and the values must be in ppm, so the conversion is made to:

$$ppm = \frac{(area - \beta_0)}{\beta_1}$$

Where β_0 is the intersection on the axis of the area and β_1 is the slope of the line of fit. So ppm will be:

$$ppm = \frac{(area - 66.039)}{2.917}$$

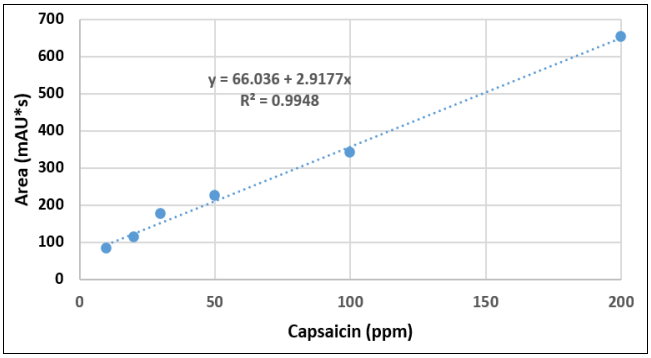


Fig 1: Capsaicin calibration curve

The analysis of the factors indicates that the effect of the extraction methods is highly significant and like the effect of the chili varieties, the interaction of the extraction method with chili varieties was only significant. The concentration of capsaicin by the extraction method (Table 1) indicates that ethanol is the best extractant (Arteaga, 2019) [3] and is statistically similar to water at 50° C, but it is superior to vinegar by 92% and hot water by 42%, another method that can be considered due to its extraction value is methanol, it is higher than vinegar by 30%, but lower than ethanol by 61%.

Table 1: Extraction method of Capsaicin in chili

Extraction	Capsaicin	
	(µg g^{-1})	
Water	19.81	b
Water 50° C	26.25	ab
Ethanol	35.44	a
Methanol	24.06	b
Vinegar	18.44	b

a, b, = Values with different literals in a column, are different Tukey ($p \leq 0.05$).

Using an extractant as a chemical compound, such as ethanol, has a greater effect on the separation of capsaicin from chili peppers (Restrepo *et al*., 2007) [22], but if it is a biorational product, water at 50° C would be the best option to obtain capsaicin from chili peppers. It should be noted that ethanol comes at 10%, that is, a low concentration, which, if it meets the requirements of COFEPRIS, can be considered a sustainable product for pest control (Lugo *et al*., 2017) [16].

Of the chili varieties, the piquin pepper has the highest concentration of capsaicin and is significantly higher than the other varieties with 64.85 µg g^{-1} (Table 2). It is more than 1,000% higher than the apple and tree varieties, and only 182% higher than the habanero pepper (Vera *et al*., 2011) [30].

Table 2: Chili varieties in the concentration of capsaicin

Chili varieties	Capsaicin ($\mu g\ g^{-1}$)	
Piquín	64.85	a
Habanero	22.95	b
Tree	9.2	c
Apple	2.2	c

a, b, c = Values with different literals in a column, are different Tukey ($p \leq 0.05$).

The superiority of the piquín pepper may be due to its growing environment, because the habanero pepper was obtained from the center of Veracruz, although studies indicate that in the last stages of cutting this chili has a higher concentration of capsaicin (Borges *et al*., 2010) [6]; the Puebla tree chili and the Morelos apple pepper developed in environments with adequate temperature and rainfall for their growth (Sánchez *et al*., 2010) [25], the piquín pepper was obtained from the forest in the central area of Tamaulipas, which is why the piquín pepper is confused with the mountain chili (Rodríguez *et al*., 2004) [24], this chili is a semi-perennial plant subject to an average rainfall of 400 mm per year in the region, which differs from the other regions (Orellana *et al*., 2013) [20].

In the concentration of capsaicin, only the piquín pepper and the habanero pepper are within the values described by Morán *et al*. (2008) [18], the apple pepper and the tree pepper are not suitable for obtaining a large amount of capsaicin, because they have a lower concentration. The effect of genotype - species - environment influenced the capsaicin content, because the piquín pepper being in a more adverse environment produced a greater amount (Pardey *et al*., 2009; Yánez *et al*., 2015) [21, 32].

The value of capsaicin obtained in the study is lower than that reported (Contreras *et al*., 1998) [11] and may be due to the method of analysis, because the higher values obtained use acetonitrile as an extractant with a temperature of 70° C and agitation for 4 hours, and after filtrations a 1:10 dilution of the sample is performed with acetonitrile, in a vial of 1 mL (Collins *et al*., 1995) [10], but in the methodology described above it is not done in this way because 60% acetonitrile is used as a solvent in the HPLC, not in the preparation of the sample.

The best interaction of extraction and varieties was found in the piquín pepper with 84.3 $\mu g\ g^{-1}$, because it responds better with ethanol, in the individual effect it is denoted that hot water with 26.25 $\mu g\ g^{-1}$, is an option after ethanol, but in the interaction, methanol is more representative with 74.3 $\mu g\ g^{-1}$ (Table 3); these two types of alcohols are suitable for extraction and with requirements to make a sustainable product (Shen *et al*., 2012; FAO 2016) [26, 14].

The habanero pepper only has an effect with ethanol, which can be managed as an option for obtaining capsaicin (De la Cruz *et al*., 2020) [12]. The apple pepper and the tree pepper have no effect, they only interact as extraction methods between the chili pepper varieties, as can be seen in Table 3.

Table 3: Interaction of extraction methods and varieties of chili in the concentration of capsaicin

Extraction	Capsaicin ($\mu g\ g^{-1}$)				
	Apple	Tree	Habanero	Piquin	$\bar{X}$
Water	1.75 x	3.3 cx	17.8 by	56.5 cz	19.81b
Water 50° C	2.0 z	16.3 ay	24.8 by	62.0 bcz	26.25ab
Ethanol	2.5 w	12.3 abx	42.8 ay	84.3 az	35.44a
Methanol	1.75 z	9.5 abcy	10.8 cy	74.3 abz	24.06b
Vinager	3.0 x	4.8 cx	18.8 by	47.3 cz	18.44b
$\bar{X}$	2.2z	9.2z	22.95y	64.85x	

a, b, c = Values with different literals in a column, they are different Ficher ($p \leq 0.05$).

x, y, z = Values with different literals in a row, they are different Ficher ($p \leq 0.05$)

Multiple studies of capsaicin have been conducted for pest control on diverse families of phytophagous insect and mites of order coleoptera with 24%, lepidotera with 20%, hemiptera with 13%, homoptera with 11%, acari with 10%, the other taxonomic orders with less than 10%. Myzus persicae at doses of 12.2 mg/mL (12,200 $\mu g\ g^{-1}$) de capsaicin cause mortality of 97% (Barreto *et al*., 2025) [4]. The maximum concentration in the studies was 84.3 $\mu g\ g^{-1}$ and the maximum solubility in aqueous solutions is 100 $\mu g\ g^{-1}$ and in this study is less (Su-Fen *et al*., 2023) [27], suitable for a biopesticide.

Applications at 6.8 $\mu g\ g^{-1}$ of capsaicin in Diamond back butterfly (*Plutella xylostella*) a lepidoptera has a repellency effect of more than 60% (Falsini *et al*., 2025) [13], in our study we have 84.3 $\mu g\ g^{-1}$so its effect will be greater. Applications of 800 $\mu g\ g^{-1}$, to control cross-stripped cabbage worm in 61% at 10 weeks after transplanting (WAT), snails and cabbage web worms was 43% at 10 WAT, this is the major pests of cabbages under rain feed condition (Vimbai *et al*., 2023) [31].

Capsaicinoids exhibit insecticidal, repellent, and antifeedant activities through multiple mechanisms, including membrane disruption, oxidative stress induction, and interference with ion transport and neuronal signaling. The capsaicin has in its structure aromatic ring, amide linkage, and hydrophobic side chain. That induces as a consequence of capsaicin in pests the modulation of ionic channels provokes the neurophysiological impairment, reduce locomotor activity and paralysis, to observe in pests the effect of antifeedant, repellent, reduced growth and development, reduced reproduction, induces neuromuscular dysfunction and insecticidal mortality (Toledo *et al*., 2026) [29]. All these are the benefits that capsaicin has as a sustainable biopesticide to reduce environmental deterioration.

Conclusion

To obtain the highest concentration of capsaicin, piquín pepper and ethanol should be selected as an extractor if a sustainable product with this ingredient is desired.

The habanero pepper can be the second option, only in case

you do not have piquín pepper, but its effect is less.
The extraction methodologies and analysis differ from other authors, but one thing is basic science and another is applied science, and this study is focused on applied science in a biopesticide.

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