horticultura brasileira

Research

RAMIREZ-ARAGÓN, MG; TROYO-DIÉGUEZ, E; PRECIADO-RANGEL, P; BORROEL-GARCIA,VJ; GARCIA-CARRILLO, EM; GARCIA-HERNÁNDEZ, JL. 2022. Antioxidant profile of hot and sweet pepper cultivars by two extraction methods. *Horticultura Brasileira* 40: 411-417. DOI: http://dx.doi.org/10.1590/s0102-0536-20220409

Antioxidant profile of hot and sweet pepper cultivars by two extraction methods

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ABSTRACT

Chili peppers are among the most important vegetables in the world. The demand of this fruit reveals a noticeable rapid increasing, which importance is mainly due to its nutraceutical composition. These fruits are rich in capsaicinoids, phenolic compounds, carotenoids, and others, including vitamins. In this study, a comparative evaluation between two extraction methods of bioactive compounds of fourteen chili pepper cultivars was performed. Two extraction methods for antioxidants, the time-solvent and the ultrasound were evaluated. The design of the experiment was completely randomized with three repetitions where variables evaluated were total phenolic compounds, flavonoids content, antioxidant capacity and capsaicin. Results showed that the phenolic compounds oscillated between 48.7 - 634.1 mg GAE/100 g dry weight (DW), the flavonoids content varied from 1 - 97 mg QE/100 g DW, the antioxidant activity from 65 – 348 µmol Trolox/g DW and the capsaicin content oscillated from 0.3 - 922 mg/100 g DW. The extraction method with higher values of bioactive compounds for each of the chili pepper types was the ultrasound for all the measured variables.

Keywords: *Capsicum annuum*, phenolics, flavonoids, capsaicin, ultrasound, antioxidant capacity.

RESUMO

Perfil antioxidante de cultivares de pimenta e pimentão por dois métodos de extração

A pimenta malagueta está entre os vegetais mais importantes do mundo. Sua demanda revela notável e rápido aumento de sua importância, principalmente devido à sua composição nutracêutica. Essas frutas são ricas em capsaicinóides, compostos fenólicos, carotenóides e outros, incluindo vitaminas. Neste estudo, foi realizada uma avaliação comparativa entre dois métodos de extração de compostos bioativos de quatorze cultivares de pimenta malagueta. Foram avaliados dois métodos de extração de antioxidantes, o tempo-solvente e o ultrassom. O delineamento do experimento foi inteiramente casualizado com três repetições onde as variáveis avaliadas foram compostos fenólicos totais, teor de flavonoides, capacidade antioxidante e capsaicina. Os resultados mostraram que os compostos fenólicos oscilaram entre 48,7 - 634,1 mg GAE/100 g de peso seco (PS), o teor de flavonóides variou de 1 - 97 mg QE/100 g de PS, a atividade antioxidante variou de 65 - 348 µmol trolox/g de PS e o teor de capsaicina oscilou de 0,3 - 922 mg/100 g de PS. O método de extração com maiores valores de compostos bioativos para cada um dos tipos de pimenta foi o ultrassom para todas as variáveis medidas.

Palavras-chave: *Capsicum annuum*, compostos fenólicos, flavonóides, capsaicina, ultrassom, capacidade antioxidante.

Received on July 8, 2022; accepted on October 31, 2022

Chili pepper (*Capsicum annuum*) fruits are among the most consumed vegetables worldwide (Palma *et al.*, 2020). World production exceeds 19 million tons of fresh capsicum fruits, with 1.5 million ha across Nigeria, which is the largest producer in Africa (Sarwar *et al.*, 2020). The diversity of pepper varieties is massive and they are differentiated by shape, size, pulp (pericarp) thickness and final color at the ripe stages. Pepper contains important levels of secondary metabolites with antioxidant capacity, including carotenoids, flavonoids, different polyphenols, and others (Fratianni *et al.*, 2020). From these compounds, capsaicinoids are the most stounding, mainly capsaicin (Sá Mendes *et al.*, 2020); however, their concentration can vary according to the amount of sunlight, soil, season, crop region, temperature changes, variety of fruit and maturity level (Dias *et al.*, 2016).

It is worth mentioning that the chemical composition and content of bioactive compounds may be due not only to the difference between species but also to the type of extraction used (Gurnani *et al.*, 2016). Conventional extraction (solvent use in long periods of time) is the most common method for extracting secondary metabolites from plants (Kumar *et al*., 2021). However, it has several drawbacks, such as wasting a large number of solvents and energy, consuming a long time, degrading thermally unstable compounds and the use of toxic solvents (Yang *et al*., 2019). Therefore, several green and novel techniques have been developed for extracting bioactive compounds from plants, some examples are microwave-assisted extraction, enzyme-assisted extraction, deep eutectic solvent extraction, supercritical fluid extraction, and ultrasound-assisted extraction (Yusnawan, 2018).

Ultrasonic method increases the bioactive compounds extraction efficiency because it can accelerate the release and dispersion of these intracellular compounds into the solvents and tear plant cells (Mai *et al*., 2022). High-frequency ultrasonic waves induce contraction and expansion cycles that subsequently cause cavitation, breakage of plant cell walls, resulting in the infiltration of solvents into cells. The ultrasonic extraction rate and yield are influenced by different factors, including amplitude and ultrasonication time (Tang *et al*., 2021). Literature stated that ultrasonic extraction method offers efficiency and reproducibility advantages compared to conventional techniques due to their time-saving, ease of procedure, and environment-friendly properties, as well as yielding a cost-effective output of high-quality antioxidants extracts (Leksawasdi *et al*., 2022). With recent advances in materials science, and the development of robust and energy efficient transducers, the application of ultrasonic extraction is increasingly used on an industrial scale. This type of environmentally friendly extraction has grown and in turn has increased the interest of the food industry since production costs are reduced, processes are accelerated or extraction yields are increased. To encourage greater acceptance by the industry and develop these types of large-scale extraction techniques, it is necessary to demonstrate the safety, sustainability, cost-effectiveness, process efficiency, and environmental friendliness of ultrasonic extraction (Ojha *et al*., 2020). The importance of pepper's bioactive compounds is due to their analgesic, cardioprotective, pharmacological, and neuroprotective properties, among others (Gurnani *et al*., 2016; Lu *et al*., 2017). In addition, all peppers contain a wide range of bioactive compounds with functional and technological attributes with relevant industrial interest (Sá Mendes *et al*., 2020). These substances determine the qualitative, organoleptic and nutritional properties of chili for different uses other than food (Tripodi *et al.*, 2020). The aim of this work was to evaluate the antioxidant profile and quantity of capsaicin of 14 different chili pepper cultivars comparing two extraction methods.

MATERIAL AND METHODS

Sample preparation

Fresh fruits from fourteen chili pepper cultivars (Habanero, Piquin, De arbol, Serrano, Jalapeño, Puya, Mirasol, Yidu, Chilaca, Hungaro, Caribe, Poblano, Paprika and Bell pepper) were used in this work. The peppers were purchased at a local supermarket at City of Gómez Palacio, Durango, Mexico. Fruits were all taken to the Biotechnology Laboratory Polytechnic University. Fruits were cut and pulp was separated from seeds. Afterward, samples (only pulp) were placed over blotting paper to be dehydrated at room temperature (25°C). After 20 days the samples already dry were pulverized in a blender (Hamilton Beach) and stored at 5°C. Each sample was replicated three times. Each replicate included 10 fruits obtained in supermarket.

Extracts by time-solvent method

Ethanol was used as extraction solvent. For each sample, 1000 mg of chili material was mixed with 10 mL solvent. The mixture was kept at room temperature for 24 h in constant movement in a tube shaker (Benchmark M2100). The resulting mixture was centrifuged at 9200 rpm for 3 min. The supernatant was then recovered and filtered with a syringe filter of 45 μm of pore. After that the extracts were stored at -20°C for posterior analysis.

Extracts by ultrasound method

Ethanol was the solvent used for all samples. The quantity of chili pepper was the same employed by time-solvent method. Each preparation was sonicated (Ultrasonics®) for 20 min. The sound waves had an intensity of 20 KHz for 20 min. Following this procedure mixture was centrifuged at 9200 rpm for 3 min, the supernatant was removed from the tubes and stored in Eppendorf tubes at -20°C until analyzed (Mai *et al*., 2022).

Determination of total phenolic compounds

The content of total phenolic compounds (TPC) was quantified using a modification of the Folin-Ciocalteu method (Ainsworth & Gillespie, 2007). First, 50 μL of extract was mixed with 3 mL distilled water in a tube test. After that, 250 μL of Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis MO, USA) were added. The mix was vortexed for 10 sec and allowed to react for three minutes. After, 750 μL of sodium carbonate (20% w/v) were added and stirred for 10 s, followed by 950 μL of distilled water and stirred again in vortex. Samples were left to react for 2 hours at room temperature and in a dark place. Over time the solution was subjected to a spectrophotometer 10 UV (Genesys, USA) to obtain the absorbance at 765 nm. TPC was calculated in base of a calibration curve with gallic acid as standard. Results were reported in mg GAE/100 g dry weight (DW). Analyzes were carried out in triplicate.

Quantification of flavonoids content

Total content of flavonoids was determined using the technique of Baba & Malik (2015). Initially 50 μL of extract were placed into an assay tube, then methanol was added to obtain 1.0 mL of solution. Afterward, it was mixed with 4 mL of distilled water and 0.3 mL of $NaNO_3$ solution at 5%. Then 0.3 mL of $AlCl_3$ solution at 10% was added. Samples were incubated for 5 min. Subsequently, 2 mL of NaOH solution at 1 M were added and the final volume of the mixture was completed to 10 mL with double-distilled water. The mixture was left in reaction for 15 min. Obtained solutions were observed at a spectrophotometer to measure the absorbance at 510 nm. The total flavonoid content was expressed in mg of quercetin equivalent per 100 g of dry weight sample (mg QE/100 g DW). Analyzes were carried out in triplicate.

Determination of total antioxidant capacity (ABTS)

Antioxidant capacity analysis was performed following the technique of Domínguez and Ordoñez (2013) using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) as radical (ABTS Sigma-Aldrich®, USA). Thus, to carry the tests, there were used 10 µL of sample and 990 µL of the adjusted radical. After 30 min of reaction, the absorbance (734 nm) was read by spectrophotometry. The calibration curve was performed using Trolox (6-hydroxy-2, 5, 7,8-tetramethylchroman-2-carboxylic acid) as antioxidant agent; result was expressed as equivalent micromoles in Trolox per gram of sample in dry weight (µmol Trolox/g DW).

Capsaicin quantification

Determination of capsaicin was achieved with the technique described by Moreno-Ramírez *et al*. (2018). This methodology was adapted to the conditions of the equipment (HPLC, Agilent Technologies, USA) used. The injection volume was 10 µL, the column was a C-18 Agilent Technologies, USA (5 lm, 4.6 mm x 50 mm). The mobile phase consisted of a gradient of acetonitrile: water (65:35), the flow rate was 1.0 mL/min, the runtime was 12 min. The capsaicin was identified by a comparison of their retention times to those of the capsaicin standard (SIGMA-ALDRICH, USA).

Statistical analysis

A completely random design was used, with three repetitions. The variables evaluated were: total phenolic compounds, flavonoids content and antioxidant capacity and capsaicin. The data were statistically analyzed using ANOVA to compare the means. LSD test was used to verify the statistically significant differences at a 95% confidence level ($p<0.05$). Data were analyzed using the Statistica 6.0 ® software.

RESULTS AND DISCUSSION

Total phenolic compounds (TPC)

Polyphenolic compounds gained the attention of health-related scientists and specialists because they act as antioxidants and protect the human body from the oxidative stress (Gulcin, 2020). Due to their positive human health benefits, consumers have increased the demand of these secondary metabolites, particularly in vegetables like peppers, that are consumed in large amounts worldwide (Sagar *et al.*, 2018).

TPC showed a very wide rank of values from 48.7 to 364.8 and from 104.1 to 634.1 for the two assessed extraction methods: the time-solvent and the ultrasound method, respectively (Figure 1). Our results express that habanero chili pepper was the cultivar with the highest value of TPC, while the type of chili with the lowest amount of such compounds was the Bell pepper in both extraction methods. It is an outstanding result, regarding that habanero type of pepper generally has a very high concentration of capsaicinoids (Soares *et al.*, 2020). This suggests that the concentration of capsaicinoids and TPC are correlated, or even that they have a kind of synergic association. All the samples evaluated showed significant differences ($p<0.05$), regarding the extraction method. According to our results, the differences between the extraction methods reflects a superior efficiency, with an increase of 46% in phenolics extraction when the ultrasound method was used. The cultivars with the highest values of TPC were (in descending order): habanero, paprika and serrano, while the samples with the lowest concentration were (in ascending order) mirasol, yidu and bell pepper. TPC results steadily showed a remarkable variability that can be found in chili peppers (Ribes-Moya *et al.*, 2020). Our results showed similar trend to those published recently by Alam *et al.* (2020), who pointed out that peppers will be more pungent according to higher TPC concentrations, than sweeter peppers with lower TPC content.

On the other hand, the results obtained in this reaserch were consistently higher in comparison with a previous study performed with 63 Balkan peppers, where values varied from 42 to 267 mg/100 g in phenolics content where the obtention of chili extracts were by traditional method (time- solvent) and all samples were dry at room temperature (Denev *et al.*, 2019) in the same way that our samples were managed. Furthermore, these experiments have noticed the complex characterization of TPC from the high diversity of the evaluated pepper cultivars. This variability in phenolics is developed in the biogenetically arise from the shikimatephenyl propanoids flavonoids pathways due to plants need phenolic compounds for pigmentation, growth, resistance to pathogens and for many other functions. Therefore, they represent adaptive characters that have been subjected to natural selection during evolution of the fruit (Sonaniya *et al.*, 2022).

Total flavonoid contents (FC)

In chili fruit, the primary flavonoids are quercetin, luteolin, kaempferol, catechin, epicatechin, rutin, luteolin, apigenin and myricetin (Alvarez-Parrilla *et al.*, 2011; Vera-Guzmán *et al.*, 2011). Flavonoid concentrations may change according to the diversity of genotypes, landraces, cultivars and the ripening

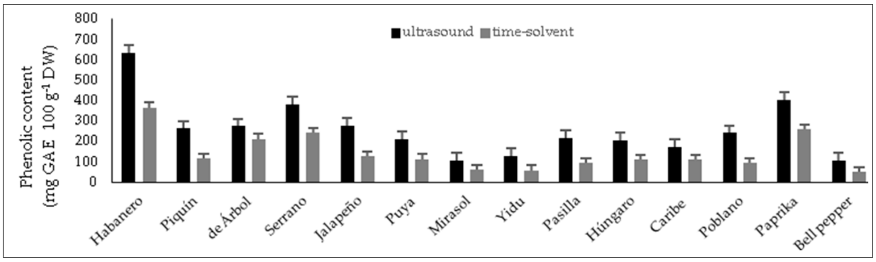

Figure 1. Total phenolics compounds of fourteen chili pepper cultivars obtained by time-solvent and ultrasound extraction method. México, Universidad Politécnica de Gómez Palacio, 2021.

phase of the fruit, among other factors, including the main analytical laboratory parameters such as sample preparation, extraction method and quantification methods (Vera-Guzmán *et al*., 2017).

The analysis of FC demonstrated that the selection of the extraction method is relevant when the principal objective of the study is to increase the detection of molecules of interest. Our results showed high variation among samples due to its own nature. Same effect was obtained in the results reported for TPC (Figure 1). The evaluation between methods for FC extraction revealed a higher efficiency with the ultrasound method (Figure 2), accordingly, it is observed that FC values were 83% higher in FC extracted by this method. The sample of habanero pepper showed the highest value in FC with 97 and 41 mg QE/100 g, with the ultrasound and time-solvent extraction method, respectively. However, samples of yidu, húngaro, caribe, and bell pepper types did not reflect any amount of FC in the time-solvent extraction method.

Evidences suggest that concentration of flavonoids is correlated to maturity degree of the fruit, morphotype, landrace and varietal group or cultivar (Meckelmann *et al*., 2015).

A lab analysis of 63 Balkan pepper (*Capsicum annuum*) accessions reported values from 1.0 to 64 mg/g of FC, showing a wide range of variation in concentration of FC (Denev *et al*., 2019); in this sense, the variability of results in this study is inherent to the pepper types and genotypes.

Some experiments reveal a high variability of FC in different types of chili peppers, but also at the same type of pepper the variation could reflect a huge value in the concentration of such compound. For example, jalapeño has ranged from 10 to 332 µg/g of total flavonoids, piquín has oscillated from 97 to 544 µg/g, among others (Alvarez-Parrilla *et al*., 2011; Blanco-Ríos *et al*., 2017; Vera-Guzmán *et al*., 2011).

Total Antioxidant capacity (TAC)

Antioxidant capacity is an important parameter as an indicator of health functionality of a food product (Vera-Guzmán *et al*., 2017). Literature has reported that FRAP (Benzie & Strain, 1996) and ABTS (Re *et al*., 1999) methods are effective to determine antioxidant activity. These methods are convenient and easy to use, giving reliable results (Huei *et al*., 2020; Sora *et al*., 2015).

The differences in the total antioxidant activity observed among the different peppers in this study possibly obey to the diversity and complexity of antioxidant compounds present in fruits, these differences may be attributed to different factors, including fertilization, fruit ripening, genotype and others (Rodríguez-Maturino, 2012; Alvarez-Parrilla *et al*., 2011).

The average TAC from chili peppers samples as a result of ultrasound extraction method ranged from 97 to 348 micromoles in trolox base per gram of dry weight-sample (µmol Trolox/g DW). The time-solvent method extraction obtained antioxidant components in concentrations that oscillated from 65 to 222 µmol Trolox/g DW (Figure 3). The analyzed samples with the highest concentration were from the type of chili pepper tree by ultrasound method, while poblano pepper samples showed the major concentration of antioxidants compounds when the time-solvent extraction was employed. The percentage of extraction efficiency by ultrasound was on average 32% higher

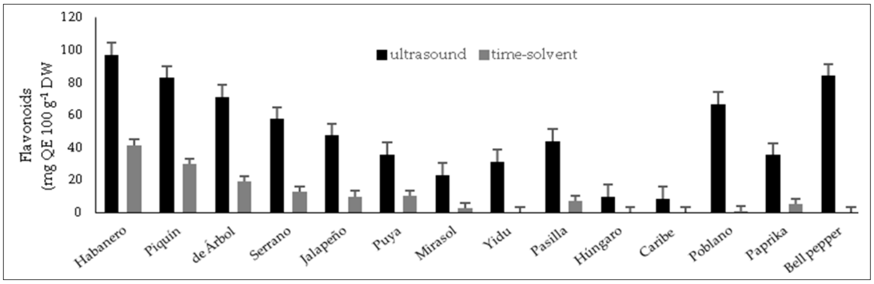

Figure 2. Total flavonoid content of fourteen chili pepper cultivars obtained by the time-solvent and ultrasound extraction method. México, Universidad Politécnica de Gómez Palacio, 2021.

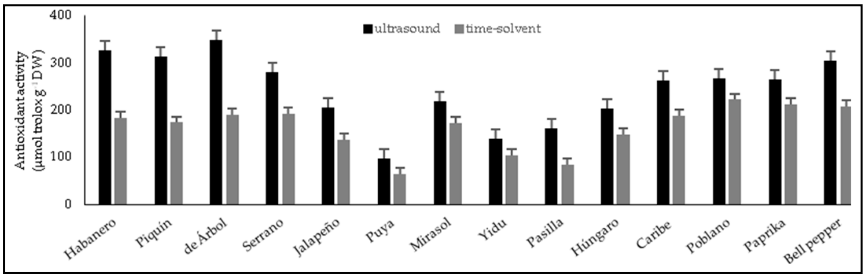

Figure 3. Antioxidant activity of fourteen chili pepper cultivars obtained by time-solvent and ultrasound extraction method. México, Universidad Politécnica de Gómez Palacio, 2021.

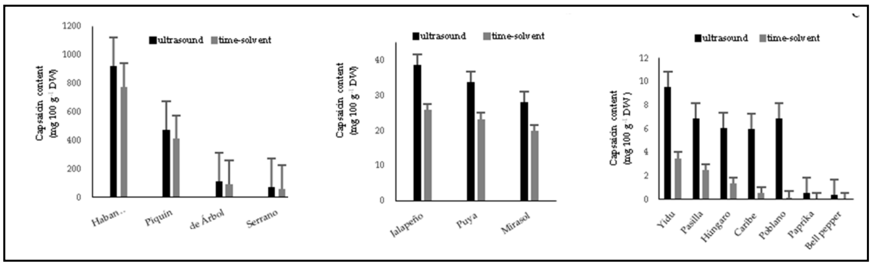

Figure 4. Chili peppers samples with major quantity of capsaicin in two extraction methods (a), chili pepper samples which capsaicin content was below 50 mg comparing two extraction methods (b) and samples of chili peppers with less than 10 mg of capsaicin content in two extraction methods (c). México, Universidad Politécnica de Gómez Palacio, 2021.

Table 1. Capsaicin, flavonoids (FC), total phenolic compounds (TPC) content and total antioxidant capacity (TAC) of fourteen chili pepper extracts by ultrasound method. México, Universidad Politécnica de Gómez Palacio, 2021.

Cultivars	capsaicin (mg/100 g)	FC (mg/g)	TPC (mg/g)	TAC (μmol Trolox/g)
Habanero	922.3 ± 5.49[a]	0.97 ± 0.011[a]	6.3 ± 0.055[a]	326.5 ± 1.06[b]
Piquín	474.1 ± 4.01[b]	0.83 ± 0.011[b]	2.6 ± 0.019[e]	314.3 ± 0.83[c]
De árbol	115.5 ± 3.30[c]	0.71 ± 0.021[c]	2.7 ± 0.041[d]	348.8 ± 1.0[a]
Serrano	74.2 ± 2.01[d]	0.57 ± 0.021[d]	3.7 ± 0.033[c]	279.6 ± 1.48[e]
Jalapeño	38.5 ± 1.38[e]	0.47 ± 0.014[e]	2.7 ± 0.053[d]	206.5 ± 1.48[h]
Puya	33.6 ± 1.44[ef]	0.35 ± 0.015[f]	2.1 ± 0.031[g]	97.0 ± 1.18[k]
Mirasol	27.9 ± 1.69[fg]	0.23 ± 0.018[g]	1.0 ± 0.027[j]	217.9 ± 1.98[g]
Yidu	9.5 ± 0.24[h]	0.31 ± 0.021[f]	1.2 ± 0.02[i]	139.8 ± 2.28[j]
Chilaca	6.8 ± 0.18[hi]	0.43 ± 0.014[e]	2.1 ± 0.023[g]	161.7 ± 1.98[i]
Húngaro	6.0 ± 0.37[hi]	0.1 ± 0.014[h]	2.0 ± 0.022[g]	204.6 ± 0.50[h]
Caribe	5.9 ± 0.25[hi]	0.08 ± 0.014[h]	1.7 ± 0.005[h]	263.6 ± 1.15[f]
Poblano	6.8 ± 0.11[hi]	0.66 ± 0.018[c]	2.4 ± 0.035[f]	266.9 ± 1.15[f]
Paprika	0.5 ± 0.04[ij]	0.35 ± 0.018[f]	4.0 ± 0.055[b]	265.0 ± 0.98[f]
Bell Pepper	0.3 ± 0.03[jk]	0.84 ± 0.029[b]	1.0 ± 0.006[j]	305.2 ± 3.3[d]

[a – k] Different superscripts in the same column indicate statistical differences at the p<0.05 for each sample.

than the time-solvent method.

Other study reported TAC for 18 samples of chili peppers at the mature stage, where habanero evidenced the highest ABTS scavenging capacity, while Fresno reflected the lowest antioxidant potential (Hamed *et al.*, 2019). Those results coincide with our results, where habanero showed the highest score using ABTS assay from all chili peppers used in this study. A previous experiment reported that the ABTS in pepper varied from 89.25 to 141.25 μmol TE/g when extracts were made from seeds, and from 17.17 to 97.40 μmol TE/g for pulp extracts (Sora *et al.*, 2015). On the other hand, the samples evaluated in a different study showed elevated TAC when the extracts were made with pulp (Huei *et al.*, 2020), reporting values of TAC from 91.95 mg/mL to 0.4 mg/mL for the highest and the lowest concentration in samples, respectively. Such results have similarity with the samples analyzed in the current experiment, but in our case the essay was carried out with pulp samples. Other authors (Carvalho *et al.*, 2015) found variability for TAC of eight *Capsicum* peppers genotypes with values from 46.7 to 113 μmol TE/g; in this study, their analysis showed similarity with our results for the chili cultivars Puya, Yidu and Pasilla.

Capsaicin content

Capsaicinoids are compounds synthesized by the condensation of vanillylamine, which is a phenolic portion with a branched short chain of fatty acid (Arce-Rodríguez & Ochoa-Alejo, 2019). Those molecules confer pungency or spice to the chili fruits. Chemical structure of this components comprises a phenolic nucleus connected to a fatty acid by an amide union (Lu *et al.*, 2017).

In this study, capsaicin content from the analized samples showed a high variability that could be influenced by several factors, including the climatic and edafic conditions, wet regime, fruits' stage of maturity, and others. Results were divided into three groups according to the content of capsaicin obtained in the analyses (Figure 4). The significance of differences (p<0.05) for mean values was determined by an analysis of variance, which indicated that habanero chili pepper had the highest capsaicin content in both, the ultrasound and time-solvent extraction methods, with 922 and 772 milligrams of capsaicin (mg/100 g DW), respectively. Group one was conformed by samples with average capsaicin content above 60 mg/100 g DW (Figure 4a). Group two (Figure 4b) includes the chili pepper samples below 50 mg/100 g DW of capsaicin, where the jalapeño pepper showed the highest concentration of the group with 38 and 25 mg/100 g DW for ultrasound and time-solvent methods, respectively. The third group represents samples with the lowest amounts of capsaicin from the total chili peppers evaluated; such group expressed less than 10 mg/100 g DW (Figure 4c). One of the most noticeable results of this study was observed with this biochemical variable. In this context, the third group strongly reflected the efficiency of the extraction method in two types of chili pepper (paprika and bell pepper) for capsaicin content (0.58 and 0.38 mg/100 g DW respectively) with the ultrasound method, certainly low values, but such detection was not possible with the time-solvent method.

In a study with 14 chili pepper fruits, Ridzuan *et al.* (2019) reported a variation from 23 to 207 μg/mL in capsaicin content, which are lower than the values reached in our study. Other study reported capsaicin data ranging from 0.02 to 0.05 mg/g, which are similar to group three (Figure 4c) in our experiment. Those results represent the pungency in no-spicy peppers (Alam *et al.*, 2020). The capsaicin values recorded in our study reflected a wide variation, similar to the results reported by Bajer *et al.* (2015), varying from 46.451 to 285 µg/g, where two different extraction methods were applied. All results published by different authors confirm diversity of capsaicinoids profile in *Capsicum* species. According to our results, the best method of extraction used was the ultrasound where the data reported that all variables (TPC, FC, TAC, CC) presented a mayor concentration in each sample measurement. It is noticeable that higher values of bioactive compounds in each of chili pepper types were observed. All cultivars evidenced statistical differences regarding their antioxidant profile (Table 1.)

Ultrasound is a key-technology in achieving the objective of sustainable "green" chemistry and extraction, exerting a significant effect on the rate of various processes in the chemical and food industry (Chemat *et al.*, 2017). A previous study reported a list of different extraction methods for capsaicinoids (Vázquez-Espinosa *et al.*, 2019), where it was remarked that ultrasound is adequate, quick, and effective for the extraction of capsinoids. As conclusions, chili peppers present different bioactive compounds which are recognized with functional properties, such as capsaicinoids, antioxidant compounds, between others; also, peppers are currently main vegetable component in the food processing industries due to their antimicrobial or antioxidant activities, contributed in part by capsinoids. For these reasons, the use of techniques that may increase the possibility to extract a larger amount of secondary metabolites should be considered. The efficiency of the ultrasound method for the extraction of active compounds was demonstrated in comparison with the time-solvent method. All the results obtained showed that the extraction of active compounds is greater by the ultrasound method for all the variables measured. It is important to continue with the study of these fruits due to there being a great diversity of cultivars and therefore the composition of compounds varies in a wide range between species. It is of great interest to continue exploring the most suitable techniques for extraction of beneficial molecules that contribute to health and also represent an open field for different areas.

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