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Article

# Valorization of agricultural biomass of chili peppers to obtain nutraceuticals

Luis Alfonso Jiménez-Ortega[1]
Pedro de Jesús Bastidas-Bastidas[1]
José Basilio Heredia[1§]
Nayely Leyva-López[1]
Erick Paul Gutiérrez-Grijalva[1]

1 Centro de Investigación en Alimentación y Desarrollo, AC. Carretera a Eldorado km 5.5, Col. Campo El Diez, Culiacán, Sinaloa, México. CP. 80110. (ljimenez222@estudiantes.ciad.mx; pbastidas@ciad.mx; nayely.leyva@ciad.mx; egutierrez@ciad.mx).

Autor para correspondencia: jbheredia@ciad.mx.

## Abstract

Mexico is the largest exporter of chili peppers and the second largest producer worldwide, due to which large quantities of biomass are produced, which are not always treated in a sustainable way, which can have a negative impact on the environment. One of the utilization trends is their use to obtain nutraceuticals. This research aimed to determine the phytochemicals present in poblano, jalapeno, and bell pepper biomasses produced in Culiacán, Sinaloa, Mexico, as well as to evaluate their antioxidant activity. The study was conducted in 2022. Phytochemical screening was performed and total free and bound phenolic compounds, flavonoids, antioxidant activity, capsaicinoids, and volatile compounds were quantified. Chili pepper biomasses are an important source of free phenols (1 010.14 ±41.81 mg GAE 100 $g^{-1}$), bound phenols (158.66 ±8.87 mg GAE 100 $g^{-1}$), flavonoids (158 ±8.87 mg QE 100 $g^{-1}$), dihydrocapsaicin (1.762 µg $kg^{-1}$), phytosterols, terpenes, tannins, saponins, and alkaloids (atropine), in addition to presenting antioxidant activity (35 744.04 ±618.6 µmol TE 100 $g^{-1}$). It was concluded that biomasses contain biofunctional nutraceuticals, so their valorization for this purpose can promote the generation of circular economies in Mexico.

## Palabras clave:

*Capsicum annuum* L., antioxidants, phytochemicals, sustainability.

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## Introduction

Mexico is the second largest producer and main exporter of chili peppers (*Capsicum annuum* L.) worldwide (FAOSTAT, 2022). During fiscal year 2022, the chili peppers with the highest production in the country were jalapeño, bell pepper, and poblano, with 703 420.86, 562 075.1, and 414 656.54 t, respectively (SIAP, 2023).

The fruit represents a small part of the total weight of the plant, almost 50% of it is made up of stems, roots, and leaves, which are discarded at the end of production (Zabot and Cárdenas-Toro, 2017). Some of the disposals of these biomasses are open-air incineration and landfilling, which leads to the generation of pests, foul odors, and greenhouse gases, negatively impacting the environment and public health (Cerda *et al*., 2018).

According to the biomass valorization pyramid, the extraction of nutraceuticals is the activity that provides the greatest added value (Baenas *et al*., 2019); in addition, when exposed to biotic and abiotic stresses, it can biosynthesize a greater amount of secondary metabolites, in contrast to fruits (Khare *et al*., 2020).

In relation to the above, it has been observed that the leaves of *C. annuum* have a content of apigenin, luteolin, and quercetin glycosides higher than the fruits (Cho *et al*., 2020). Also, the stems contain a content of total phenolic compounds higher than the pericarp and placenta (Chen and Kang, 2013), so the inedible parts are a good source of nutraceuticals.

The objective of the research was to determine the groups of phytochemicals, quantify the content of total free and bound phenols, total flavonoids, and capsaicinoids, identify volatile organic compounds and evaluate the antioxidant activity (AOX) of the extracts of agricultural biomasses of chili pepper.

## Materials and methods

### Sample collection and conditioning

The biomasses of bell pepper, Thames variety, poblano pepper, Allende variety, and jalapeño pepper, Orizaba variety, were collected in senescent states (mixture of leaves and stems, 25% and 75%, respectively), in the agricultural valley of Culiacán, Sinaloa, Mexico, in 2022. They were dehydrated in a semi-industrial oven for 12 h at 60 °C, ground in a Pulvex pulverizer, sifted in a 0.45 mm sieve and kept at -15 °C until use.

### Phytochemical screening

The phytochemicals were extracted by maceration, for 24 h at 30 °C, at 700 rpm. Hexane (Hx), methylene chloride (MC), and methanol (MeOH) were used. The extracts were filtered with Whatman No. 1 paper and stored at -20 °C until use. The identification of phytochemicals was carried out using colorimetric techniques (Harborne, 1984).

### Determination of total phenolic compounds (TPCs), bound phenolic compounds (BPCs), and total flavonoids (TFs)

TPCs and TFs were extracted with 80% EtOH by means of an ultrasonic bath at 45 °C for 45 min. The extracts were centrifuged at 10 000 rpm for 15 min at 4 °C. The supernatant was recovered and stored at -15 °C until use. To obtain the BPCs, 10 ml of NaOH 2N was added to the pellet resulting from the previous extraction and it was heated for 30 min at 95 °C. It was then left to stir for 1 h at 25 °C. At the end of the time, 2 ml of concentrated HCl and 10 ml of Hx were added, and it was centrifuged at 10 000 rpm for 10 min at 4 °C.

The supernatant was discarded and 10 ml of ethyl acetate was added to the resulting pellet. The sample was concentrated in a rotary evaporator and the BPCs were recovered with 2 ml of 80%

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EtOH. To determine TPCs and BPCs, the Folin-Ciocalteu (FC) method described by Swain and Hillis (1959) was followed. The results were expressed as mg gallic acid equivalents (GAE) per 100 g of dry sample. The determination of TFs was performed by the aluminum chloride method, described by Ebrahimzadeh *et al*. (2015). Results were expressed as mg quercetin equivalents (QE) per 100 g of dry sample. Both determinations were read on a Synergy HT spectrophotometer (Biotek, USA) at 725 and 415 nm, respectively.

### Antioxidant activity (AOX)

Oxygen radical absorbance capacity (ORAC) was evaluated by using the methodology described by Huang *et al*. (2002), results were expressed as µmoles Trolox equivalents (TE) per 100 g of dry sample. The $ABTS^{\bullet+}$ cationic radical reducing capacity was carried out using the method proposed by Thaipong *et al*. (2006). The reducing capacity of the DPPH radical was carried out following the method of Karadag *et al*. (2009). For both determinations, the results were expressed as mmoles TE 100 $g^{-1}$ of dry sample. Quantification was performed on a Synergy HT microplate reader (Biotek, USA) at 734 and 540 nm, respectively.

### HPLC quantification of capsaicinoids

Capsaicinoids were extracted with acetonitrile (ACN) assisted by sonication for 1 h at 65 °C. The extract was filtered using a 0.45 µm nylon acrodisc. A Varian 9012 HPLC chromatograph was used, which was coupled to a Varian ProStar 363 fluorescence detector. An Agilent C18 Eclipse XBD column was used. The mobile phase consisted of a mixture of 49:50% MiliQ water and ACN, acidifying with 1% v/v acetic acid.

The detection conditions were excitation wavelength 280 nm and emission 325 nm (Daood *et al*., 2015). Analytical grade standards of 99% capsaicin (CAP) (8-Methyl-N-vaillyl-trans-6-nonenamide) and 90% UPS capsaicinoid mixture (dihydrocapsaicin DHC and nordihydrocapsaicin NDHC) were used.

### Determination of volatile organic compounds via GC-MS/MS

MeOH and ACN extracts were analyzed. An Agilent 7890B gas chromatograph with ion trap mass spectrometry detector (CG-MS/MS Agilent 240) was used. A VF-5 MS column, 30 m x 0.25 mm x 0.25 µm, was used. Helium was used as carrier gas at a flow rate of 1 µL/min. The compounds were identified using the mass spectra library of the National Institute of Standards and Technology (NIST).

### Statistical analysis

The results of the content of phenolic compounds, flavonoids and AOX were contrasted with an analysis of variance (Anova) and a general linear model of one factor (type of biomass) and three levels (jalapeno, poblano, and bell pepper). Tukey's test at 95% was used to determine statistical differences between biomasses.

## Results and discussion

### Phytochemical screening

The genetic diversity of chili peppers is wide, resulting in different varieties and types, which differ in their nutritional and phytochemical composition (Hernández-Pérez *et al*., 2020). According to phytochemical screening (Table 1), an abundance and variety of secondary metabolite groups were observed in the methanolic extracts of the three biomasses, with flavonoids, saponins, terpenoids, sterols, alkaloids, coumarins, and quinones standing out.

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**Table 1. Phytochemical screening of chili pepper biomasses.**

| Phytochemical | Test | Bell pepper | | | Poblano pepper | | | Jalapeño pepper | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Hx | CM | MeOH | Hx | CM | MeOH | Hx | CM | MeOH |
| Tannins | $FeCl_3$ | - | - | +++ | - | - | +++ | - | - | +++ |
| | Gelatin-NaCl | - | - | +++ | - | - | +++ | - | - | +++ |
| Flavonoids | Shinoda | - | - | +++ | - | - | +++ | - | - | +++ |
| | $FeCl_3$ | - | - | +++ | - | - | +++ | - | - | +++ |
| | $H_2SO_4$ | - | - | +++ | - | - | +++ | - | - | +++ |
| | NaOH | - | - | +++ | - | - | +++ | - | - | +++ |
| Saponins | Stirring | - | - | +++ | - | - | +++ | - | - | - |
| Terpenes and | Libermann-Buchard | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| Sterols | Salkowski | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| Sterols | $H_2SO_4$ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| >Glycosides | Borntrager's | - | - | - | - | - | - | - | - | - |
| (cardiac, anthraquinones) | Keller killiani | + | + | +++ | + | + | +++ | +++ | +++ | +++ |
| | $H_2SO_4$ | + | + | +++ | + | + | +++ | - | - | +++ |
| Alkaloids | Wagner | - | - | + | - | - | +++ | - | + | +++ |
| | Mayer | - | - | + | - | - | +++ | - | + | +++ |
| | Dragendorff | - | - | + | - | - | +++ | - | + | +++ |
| Cumarinas | NaOH | - | - | +++ | - | - | +++ | - | - | +++ |
| Quinonas | NaOH | - | - | +++ | - | - | +++ | - | - | +++ |
| $H_2SO_4$ | + | +++ | +++ | - | - | +++ | - | - | +++ | |

-= negative; += positive; +++= abundant; Hx= hexane; MC= methylene chloride; MeOH= methanol.

These results are consistent with Swamy *et al*. (2018), where they identified tannins, flavonoids, and terpenoids from stems of *C. annuum longum*. Bhat and Rajanna (2017) reported the presence of polyphenols, tannins, flavonoids, alkaloids, glycosides, terpenes, and saponins in stems of *C. annuum* var. *glabriusculum*. On the leaves of *C. chinense*, tannins, flavonoids, polyphenols, alkaloids, and terpenoids have also been identified (Gayathri *et al*., 2016). The profile of secondary metabolites present in chili pepper biomasses is similar between species (Table 1).

## Quantification of TPCs, BPCs, and TFs

The three biomasses presented significant amounts of TPCs, BPCs, and TFs (Table 2), with the poblano pepper biomass standing out. A greater accumulation of metabolites has been observed in the leaves and stems than in the fruits of different species of *Capsicum*. Barrajón-Catalán *et al*. (2020) analyzed the metabolome of the bell pepper var. Palermo, showing a content of flavonoids higher than in the fruits. These results are relevant for the extraction of flavonoids and phenolic acids from chili pepper biomasses.

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**Table 2. Total phenolic compounds (TFCs), bound phenolic compounds (BPCs), total flavonoids (TFs), and antioxidant activity of chili pepper biomass.**

| Type of biomass | Total phenols (mg GAE 100 $g^{-1}$) | Total bound phenols (mg GAE 100 $g^{-1}$) | Total flavonoids (mg QE 100 $g^{-1}$) | ABTS (mM TE 100 $g^{-1}$) | DPPH (mM TE 100 $g^{-1}$) | ORAC (µM TE 100 $g^{-1}$) |
|---|---|---|---|---|---|---|
| Jalapeño pepper | 930.51 ±25.11a | 79.46 ±4.04b | 351.11 ±16.77b | 2.47 ±0.83a | 0.71 ±0.24b | 35 744.04 ±618.6a |
| Poblano pepper | 1 010.14 ±41.81a | 66.66 ±5.22c | 488.14 ±37.6a | 1.9 ±0.51a | 1.14 ±0.06a | 31 237 ±7 872. 82a |
| Bell pepper | 926.22 ±36.79a | 158.66 ±8.87a | 408.88 ±10.18b | 3.21 ±0.27a | 1.2 ±0.006a | 33 039 ±7 213.33a |

Means and standard deviations. GAE= gallic acid equivalents; QE= quercetin equivalents; TE= Trolox equivalents. Different letters indicate a statistically significant difference according to Tukey's test at 95%.

Chen and Kang (2014) analyzed the TPC content of stems of the PR star and Chengyang varieties, highlighting that the concentrations (9 190 mg GAE $g^{-1}$) were higher than those shown by the pericarp and placenta. Leaves of chili pepper var. Special showed a high TPC content (1 714.20 ±47.72 mg catechin equivalents CATE 100 $g^{-1}$), being higher than that reported in fruits (731.75 ±19.64 mg CATE 100 $g^{-1}$) (Kim *et al*., 2011).

These results are similar to what was reported in the present research, as it could be observed, the leaves and stems of different species of *Capsicum* are rich in phenolic compounds. Variations in TPCs, BPCs, and TFs between species may have been due to cultivation conditions, biotic and abiotic stress, nutrition, exposure to sunlight, genotype, cultural management, among other extrinsic factors (Lozada *et al*., 2023).

## Antioxidant activity

The AOX exerted by chili pepper biomasses on radicals and its ability to donate protons and electrons was demonstrated, with bell pepper biomass standing out (Table 2). The authors Kim *et al*. (2014) analyzed the leaves of three varieties of Korean chili peppers, with the Blackcuban variety standing out for showing an $IC_{50}$ of inhibition of DPPH and ABTS radicals with 49.2 and 26.2 µg $ml^{-1}$, respectively. Leaves of chili pepper var. Sweet Italian have also been shown to inhibit the DPPH and $O_{2-}$ radicals with inhibition percentages of up to 80 and 90%, respectively, with concentrations of 1 mg $ml^{-1}$ (Silva *et al*., 2014).

It was possible to demonstrate the AOX of the extracts of the three chili pepper biomasses, with the ORAC test standing out for obtaining greater inhibition, this test is characterized by simulating the oxidation process at the cellular level caused by oxygen (Prior, 2015). This effect is related to the molecular structure of flavonoids, in particular the presence of OH groups and double bonds, as well as their aromatic nature (Chen *et al*., 2020).

## Capsaicinoid quantification

In this research, despite the fact that phytochemical screening showed an abundance of alkaloids, in HPLC quantification (Figure 1), the presence of DHC was only detected in concentrations of 1.762 and 0.618 µg $kg^{-1}$ in the biomasses of jalapeño and poblano peppers, respectively. No CAP or NDHC were detected.

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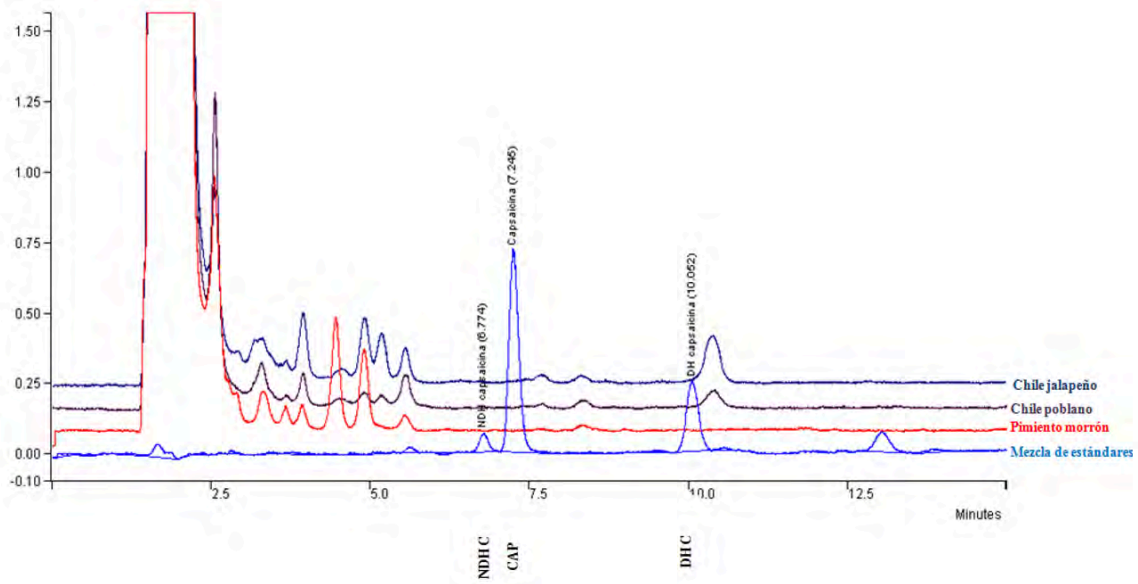
**Figure 1. Chromatogram of the quantification of capsaicinoids in chili pepper biomasses. NDHC= nordihydrocapsaicin; CAP= capsaicin); DHC= (dihydrocapsaicin).**




Simonovska *et al*. (2016) quantified CAP (28.75 mg $kg^{-1}$) in stems of *C. annuum* var. longum conoids. Silva *et al*. (2014) quantified CAP in leaves of chili pepper var. Sweet Italian, reporting 78.3 mg $kg^{-1}$. On the other hand, Estrada *et al*. (2002) quantified CAP (19.99 ±7 µg $g^{-1}$) and DHC (27.28 ±0.3 µg $g^{-1}$), the latter being found in greater quantity in the leaves than in the stems.

The biosynthesis and metabolism of capsaicinoids differ between the types of chili pepper and cultivars, the growth temperature and stage of ripening (Rahman *et al*., 2012), these compounds are normally biosynthesized in the vacuoles of the placenta, in the seeds, and in the septum of the fruits (Aza-González *et al*., 2011).

## Identification of volatile organic compounds via GC-MS/MS

Triterpenes and phytosterols, such as campesterol, stigmasterol, β and γ sitosterol, and β-amyrin, were mainly identified. Some alkaloids such as atropine ($C_{17}H_{23}NO$ ) and its derivatives were also identified, this may explain the abundance of alkaloids in methanol extracts. Phytol, a diterpene alcohol, was identified, which has anti-inflammatory and antioxidant activity.

The phytosterols identified in chili pepper biomasses could be valorized by the pharmaceutical industry to obtain nutraceuticals since these compounds have a direct relationship with lipid metabolism, helping to lower blood cholesterol levels, and help treat pathologies related to hypercholesterolemia (Li *et al*., 2022).

# Conclusions

Biomasses were rich in hydrophilic and hydrophobic compounds with antioxidant activity. Capsaicinoids were identified in the biomass of jalapeño pepper and poblano pepper, so it is suggested to continue researching pungent varieties since these metabolites are appreciated in the food and pharmaceutical industry.

The main findings of this work elucidate the bioactive potential of chili pepper agricultural biomasses, and according to the pyramid of biomass valorization, the extraction of nutraceuticals is the one that provides the greatest added value. This could help generate circular economies in the Mexican countryside due to the high volumes of chili pepper production.

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