Advances in biology through agronomy, aquaculture, coastal and environmental sciences

Leandris Argentel Martínez
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Editors

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Prologue

Advances in biology through agronomy, aquaculture, coastal and environmental sciences is an electronic book, edited by Pantanal Editora, based on the compilation of research papers where the authors of the different chapters have used highly current scientific methodologies and research equipment.

The biological sciences as the main object of research in agriculture, aquaculture, coastal and environmental sciences generate every day an understanding of knowledge that allows raising the scientific level of society as part of universal access to knowledge.

This book mainly addresses issues related to the use of plants extracts as sustainable alternatives for biocontrol of pests and bacterial diseases. It also brings together information on viruses and other diseases in aquatic organisms. In addition, studies of mangroves structure and their contribution to carbon sinks in experimental sites in northwestern Mexico are presented. Finally, an analysis on educational strategies for environmental education based on plant biology is carried out.

Editors appreciate the participation of the authors who have come from higher education institutions and research centers of great scientific prestige in Mexico. The majority of them are members of the National Research System of CONACyT, Mexico.

The authors

Summary

Chapter 4

Extraction of carotenoids present in the byproducts of bell pepper (*Capsicum annuum L.*) using the solvent method assisted with ultrasonic pulses

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ABSTRACT

In this project, the bell pepper byproduct was proposed to obtain carotenoids using the solvent-assisted method with ultrasonic pulses to evaluate the best extraction method and the effect of the pulses on the antioxidant capacity of the carotenoids. The extraction of carotenoids from the byproducts of bell pepper (*Capsicum annuum* L.) was carried by solvent extraction. The extractions consisted of using different solvents (hexane, ethanol, and acetone) assisted by ultrasonic pulses for 20 and 40 minutes with an amplitude of 40 kHz. After obtaining the oleoresin extracts, the antioxidant activity was determined using spectrophotometry techniques and physical-chemical analysis, determining pH, humidity, ashes, lipids, and proteins. The results of the extraction of carotenoids show that a better performance of the antioxidant activity was obtained for TEAC (trolox equivalent antioxidant capacity) as for DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) when the assistance of ultrasonic pulses was applied, with the solvents ethanol and acetone. In both methods, the treatments with hexane did not have the expected performance, taking into account the results of the other two solvents that were used during the extractions. Regarding the determination of flavonoids and phenols, a similar behavior was presented in both studies, presenting a minimum yield in the methods where the hexane solvent was used.

Keywords: ultrasound, antioxidants, phenols, flavonoids

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INTRODUCTION

The chili (*Capsicum annuum* L.) is among the main species of domesticated plants in Mexico; within its varieties, the bell pepper is one of the best, and approximately 5,800 hectares are planted throughout the country. Field yield can reach up to 50 tons per hectare per year (Reséndiz-Melgar et al., 2010). Of this enormous production, approximately 3 tons per hectare are discarded, given the demands made during chili packing.

The main component of chili peppers is water, followed by proteins and carbohydrates, as explained by the Spanish Nutrition Foundation (FEN); it is a good source of fiber, minerals, and vitamins as well as a good source of carotenes, among which capsaicin and pigments with antioxidant properties are found (Castillo-Olivera, 2021).

Carotenoids are compounds responsible for coloring a large number of plant and animal foods; it is known that some of these compounds, such as α and β carotene, are provitamin A (Meléndez-Martínez et al., 2004). Recent studies have revealed the antioxidant properties of these pigments, as well as their effectiveness in certain human diseases, increasing interest in these pigments (Acacio et al., 2013).

The traditional method for extracting these pigments is solid–liquid extraction, where high residence times and large amounts of solvents are needed, using heat and agitation. The use of these solvents always generates residues in the oil, and their toxicity causes scientific interest.

Many advanced techniques for the extraction of bioactive compounds have been investigated to improve extraction efficiency and overcome the disadvantages of conventional extractions. Ultrasound is a crucial technology to achieve the goal of sustainable "green" extraction. Recent studies show that ultrasound significantly affects the speed of various processes in the chemical and food industry (Chemat et al., 2017). Ultrasonic-assisted extraction improves the mass transfer of the extraction process by generating cavitation within the material. When cavitation bubbles are produced and collapse, the cell walls of the material are destroyed, and solute release is promoted.

Different studies report a significant increase in the yield of extracts with carotenoids with antioxidant capacity when using ultrasound-assisted extractions, even more than with microwave-assisted extractions (Chutia et al., 2021; Chuyen et al., 2018).

Therefore, this project aims to extract the carotenoids present in bell pepper coproducts through a solvent-assisted method with ultrasonic pulses and to evaluate their antioxidant capacity. The results will allow for determining the best method for extracting carotenoids and establishing the most appropriate temperature conditions, pepper pulp/solvent volume ratio, extraction time, and number of stages to obtain pepper oleoresin.

MATERIALS AND METHODS

Raw material

The peppers (*Capsicum annuum*) used in this study come from "Agrícola Badilla Flores S. A de C. V," in the Yaqui Valley in southern Sonora, grown in shade mesh. The samples were obtained in their highest state of maturation (red) after having been discarded for failing the quality of product packaging. The solvents used to extract carotenoids from bell pepper (*Capsicum annuum*) were hexane, acetone, and commercial-grade ethanol.

Sample pretreatment

The fresh peppers were subjected to a pretreatment with heat to eliminate the excess moisture, the peppers were cut between 1 to 2 cm thick (using only the skin and pulp of the fruit), and the seeds were discarded. They were dried in a food dehydrator for 24 h at below 60 °C to avoid carotenoid denaturation. The dry material was ground in a blade mill to obtain a particle size between 0.5 and 1.7 mm, following the methodology of (AOAC, 2005). This adaptation facilitated the extraction process of the carotenoids in the fruits. Likewise, it allowed better handling of the sample and a better application of the extraction method. A general diagram of the methodology followed is shown in Figure 1.

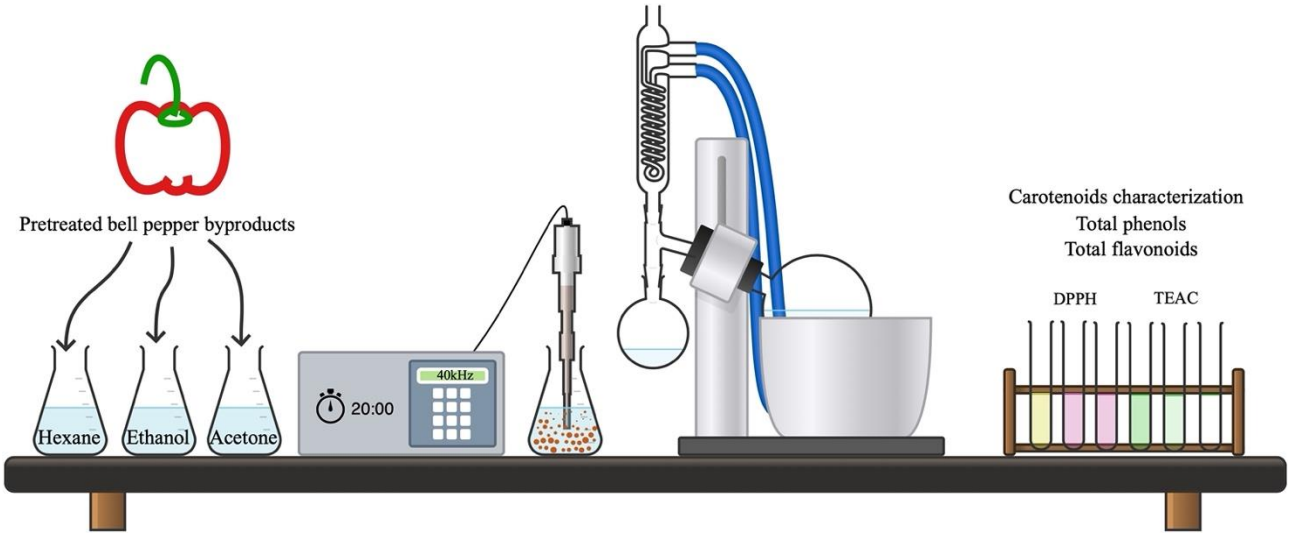

Figure 1. Diagram of bell pepper by-products processing.

Obtaining extracts

Solvent extraction

Fifty grams of processed bell pepper was used for every 100 mL of solvent in a 1:2 W/V ratio. The extraction was carried out in one stage for six h at 50 °C on a hot plate with constant stirring. The extract was separated from the solid material by settling/filtration and then stored in amber glass bottles in a dark place following the methodology proposed by Cardona et al. (2006).

Solvent extraction assisted by ultrasonic pulses

The same methodology was followed, but pulse assistance was given during the warm-up time: pulses with an amplitude of 40 kHz at two different times of 20 and 40 min. The extracts were then placed on a hot plate with constant stirring. The extract was separated from the solid material by settling/filtration and then stored in amber glass bottles in a dark place following the methodology proposed by Cardona et al. (2006) with some modifications.

Obtaining oleoresin

The extracts obtained by the methods above were subjected to a solvent reduction process using a Büchi brand R-215 rotary evaporator coupled with a vacuum pump at a temperature below 40 °C to avoid the loss of carotenoids due to oxidation. The oleoresin obtained was stored in a cool place and in an amber container to protect it from light and other factors that may cause denaturation and loss of antioxidant capacity.

Physical-chemical analysis of the raw material

The physicochemical characteristics were determined following the methodology of the AOAC, 2005, to analyze the pH, moisture, ashes, lipids, and proteins.

pH

The bell pepper (*Capsicum annuum*) was liquefied with distilled water 1:1 P/V to obtain an aqueous solution. The HANNA Instruments brand potentiometer model HI 2211 pH/ORP Meter was calibrated to carry out the analysis (AOAC, 2005).

Moisture content

A Memmert atmospheric pressure oven was used. The crucibles were washed and weighed and then placed in the oven at a temperature of 120°C for two h; the crucibles were weighed and recorded. With the help of an analytical balance, 3-4 g of sample was weighed and placed in the crucibles. Subsequently, they were placed in the oven at 110°C for one h. After this time, the crucibles were removed to room temperature. The dried sample was left to reach a constant weight, and the final weight was registered (925.10 AOAC, 2005).

Ash content

*Dried samples (*1-2 g) were placed in the crucibles and into the muffle. The temperature in the muffle was set to 550 °C for two hours. The weight of the samples was registered, and the inorganic matter was reported as the ash content (940.26 AOAC, 2005).

Lipid content

For the determination of lipids, Soxhlet extraction equipment was used. This method is based on continuously extracting all solvent-soluble substances from the dried bell pepper sample. Approximately 3 to 4 g of dry sample was accurately weighed and transferred to an extraction cartridge, covered with cotton, and placed in the extractor of the apparatus whose flask had already been brought to constant weight. The solvent was added over the cartridge until it siphoned two times. The extraction time was between 4 and 8 hours. After the extraction, it is removed from the heat to recover the solvent; the solvent is taken to the rotary evaporator to eliminate as much of the solvent as possible. While the sample is cooled down at room temperature for 15 minutes, it is placed in an oven with an air current at 80 °C for 30 min, cooled in the desiccator, and weighed to determine the percentage of lipids (963.15 AOAC, 2005).

Protein content

The protein determination of bell pepper (*Capsicum annuum*) was obtained by the Microkjeldahl (1883) method. The method consists of 3 stages, digestion, distillation, and titration; this methodology determines the amount of nitrogen related to the protein content (955.04D AOAC, 2005).

Determination of antioxidant activity

The oleoresins were evaporated in a vacuum rotary evaporator and resuspended in 80% methanol. The new methanolic extracts were diluted 1:20 and used to carry out the reactions of total phenols and flavonoids.

Determination of antioxidant capacity by TEAC

The ABTS radical scavenging capacity of the extracts was assessed by the method of Re [20]. A stock solution of the ABTS radical was prepared and kept in the dark at room temperature for 18 h. The stock solution was diluted with phosphate buffer to prepare the working solution of the ABTS radical with an absorbance of 0.70 ± 0.02 at 734 nm. For this analysis, an aliquot of the sample was mixed with 280 µL of the ABTS radical working solution, which was incubated for 30 min at room temperature in the dark, and then the absorbance was measured at 734 nm by using a microplate reader (Thermo Scientific Multiskan Sky; USA). A standard curve of Trolox was used to express the results as Trolox equivalents/mg sample (TE/g). Six replicates were carried out for each sample.

Determination of antioxidant capacity by DPPH

A 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was performed as reported by Dewanto et al. (2002). The vials of each of the samples were weighed and diluted in 1 mL of DMSO to obtain stock solutions, from which 100 µL was taken and diluted in 900 µL to obtain the first dilution. From this

dilution, 100 µL was taken and diluted in 900 µL to obtain the second dilution. To prepare the DPPH solution, 1.23 mg of reagent was weighed in a previously tared volumetric flask and dissolved in 25 mL of DMSO to obtain a 125 µM solution, which was stirred in a sonicator.

Samples and standards were prepared in triplicate following the methodology (Dewanto et al., 2002). The antioxidant capacity was expressed as µmol equivalents of Trolox/g of lyophilized extract (µmol of TE/g of LE).

Determination of Total Phenols

All extracts' total phenolic content (TPC) was determined spectrophotometrically using the Folin-Ciocalteu method (Singleton et al., 1999). The phenolic content was expressed as mg of gallic acid equivalents per 100 g of lyophilized extract (mg GAE/100 g LE).

Determination of total flavonoids

The total flavonoid content (TFC) was determined using a colorimetric method (Zhishen et al., 1999); (Dewanto et al., 2002). The results were expressed as mg of (+) – quercetin equivalents per 100 g of lyophilized extract (mg EC/100 gLE).

RESULTS AND DISCUSSION

Physicochemical analysis

The results of the characterization of red bell pepper (*Caspsicum annuum*) pulp are shown in Table 1. The pH values are similar to those Carranza (2009) described, with a pH of 5.3. There was no significant difference in the values of the proximal analyses (moisture, ash, lipids, and proteins) carried out in the present study compared to previous studies carried out by Rincón (2017) and Carranza (2009).

Table 1. Proximal composition of the bell pepper pulp.

Proximal composition	Present study	Bell pepper (Red, yellow, orange)*	Bell pepper (Red)**
pH	5.3 ± 0.34	5.2	5.3
Humidity	92.42 ± 0.61	92.1	92.3
Ash	8.19 ± 1.33	9.01	8.1
Lipids	0.74 ± 0.61	0.19	1.3
Proteins	1.04 ± 0.04	0.89	1.2

The results are presented in %,
Results are mean of 3 replicates ± standard deviation
* (Rincón-Alvarez, 2017)
** (Carranza-Rodriguez, 2009)

Antioxidant activity

Table 2 shows the results obtained from the determination of antioxidant activity equivalent to Trolox (TEAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), total phenols (TPC), and total flavonoids (TFC). The values are presented as the average of 3 replicates ± standard deviations and have been expressed in μM equivalents to Trolox for TEAC and DPPH and in Mg/ml for TPC and TFC.

The highest values for the TEAC test were obtained by the treatments where the solvents acetone and ethanol were used without the assistance of ultrasonic pulses, and the lowest value was obtained by hexane.

Table 2. Antioxidant activity.

Treatment	TEAC*	DPPH*	TPC**	TFC***
Acetone	6864.1 ± 11.6	3340.56 ± 10.48	1.29 ± 0.11	2.36 ± 0.51
Acetone/Pulses 20 min	6223.7 ± 3.16	2812.39 ± 6.64	1.73 ± 0.19	2.57 ± 0.07
Acetone/Pulses 40 min	6711.2 ± 1.59	3810.96 ± 19.29	1.45 ± 0.02	2.8 ± 0.05
Ethanol	14169.3 ± 7.45	5131.39 ± 7.50	2.34 ± 0.18	3.26 ± 0.34
Ethanol/Pulses 20 min	18875 ± 3.87	7475.15 ± 3.27	3.59 ± 0.18	4.31 ± 0.03
Ethanol/Pulses 40 min	17343.2 ± 1.98	6171.23 ± 13.80	2.84 ± 0.14	3.27 ± 0.03
Hexane	1093.2 ± 0.98	83.17 ± 64.54	0.69 ± 0.04	1.43 ± 0.08
Hexane/Pulses 20 min	903.3 ± 1.36	-	0.65 ± 0.09	1.26 ± 0.04
Hexane/Pulses 40 min	938.9 ± 6.54	-	0.44 ± 0.04	1.56 ± 0.02

Results are presented in μM TROLOX equivalents/g for TEAC and DPPH; for Total Phenols (TPC) and Total Flavonoids (TFC) were expressed in Mg/ml. * Abs 734 nm, ** Abs 765 nm, *** Abs 415 nm. Results are mean of 3 replicates ± Standard Deviation

When subjected to the assistance of ultrasonic pulses, the lowest times are those obtained by the solvents acetone and hexane at 20 min of treatment, compared to treatments without pulse assistance. Likewise, the highest values obtained when the treatments are submitted to the assistance with ultrasonic pulses are those obtained by acetone and hexane at 40 min of treatment, the lowest being the sample with ethanol at 40 min.

The results corresponding to the DPPH test showed that the highest value was obtained by treatment with the ethanol solvent with 20 min of pulse assistance, followed by acetone with 40 min of pulse assistance.

When not subjected to the assistance of ultrasonic pulses, the values obtained below the treatments with assistance were obtained by acetone followed by ethanol and hexane.

The results obtained for total phenols show a similar behavior in the treatments with solvents assisted by ultrasonic pulses in the DPPH test. The ethanol solvent with 20-min pulses had the highest value, followed by acetone with 20-min pulse assistance. under hexane with pulse assistance.

The values obtained by the solvents without the assistance of pulses were lower in the solvents acetone and ethanol, and only the hexane without treatment did not show a significant difference from the hexane assisted with pulses for 20 min.

Regarding the total flavonoids, they show the highest values for the treatments assisted with ultrasonic pulses, being the solvents with the highest values, ethanol with 20 min of assistance followed by acetone with 40 min of assistance and the lowest value obtained by hexane. with 40 min of assistance.

On the other hand, the lowest values are obtained by the treatments without the assistance of ultrasonic pulses, the lowest being the one obtained by the treatment with acetone as a solvent, the others showed us a significant difference with the minimum values for the assistance with pulses.

Under the conditions of the present study, it was possible to obtain carotenoids from red bell pepper (*Capsicum annuum L.*) byproducts by assisting with ultrasonic pulses.

It is possible to affirm that the use of this emerging technology, such as ultrasonic pulses as an assistant for the extraction of bioactive compounds such as carotenoids, has particular effectiveness depending on the solvent in enhancing their antioxidant properties since by generating membrane rupture, it helps to release almost in its entirety the compound of interest, thus generating a greater extraction.

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Index

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