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Posted Date: 26 February 2024

doi: 10.20944/preprints202402.1467.v1

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Re-Valorization of Red Habanero Chili Pepper (*Capsicum chinense*) Waste by Recovery of Bioactive Compounds: Effect of Different Extraction Process

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Abstract: Inadequately managed agricultural waste significantly impacts the environment, health, and economy. This form of pollution arises from the underutilization, poor awareness, and insufficient treatment of agricultural waste. Fruit and vegetable wastes are valuable sources of bioactive compounds. This study aims to revalorize discarded waste from red habanero chili peppers (*Capsicum chinense*) by extracting bioactive compounds through different extraction processes: maceration (ME), maceration assisted by ultrasound (US), Soxhlet extraction (SE), supercritical fluid extraction (SFE), and supercritical fluid extraction with a co-solvent (SFEC). Extraction processes have significant effects on extraction efficiency and phytochemical profile (capsaicinoids and carotenoids recovery). The results indicate that the highest efficiency process is obtained with SFEC, also a high phytochemicals recovery (14.9 mg of total capsaicinoids and total carotenoids 292.09 µg per gram of sample). Concerning to phytochemical profile in the extract, the maceration process gets the highest concentration of compound followed by US and SFEC. These data reveal that the use of SFE and SFEC extraction is recommended for extraction of phytochemicals with biologically activity from red habanero chili pepper waste for diverse industries application.

Keywords: agrifood waste; bioactive compounds; capsaicinoids; carotenoids; extraction process; habanero pepper; re-valorization

1. Introduction

Red habanero chili pepper (*Capsicum chinense*) is a high aromatic chili pepper and is an important crop in Mexico. The main edible part of the red habanero chili peppers is the pericarp which contains high amounts of ascorbic acid, vitamins A and E, carotenoids, and phenolic compounds, therefore it has significant potential health benefits [1–3]. In fact, around 70% of the production is consumed fresh and the rest is processed and largely dedicated to export. The quality of peppers is defined by consumer perception, mainly for sensorial characteristics, such as, size, color or texture. In the case of habanero peppers, a significant factor impacting their quality is the variation in fruit size and weight[4]. Smaller habanero peppers (classified as second or third quality) tend to command lower

 doi:10.20944/preprints202402.1467.v1

prices in the market, thereby leading to financial losses for farmers. Furthermore, approximately 20-30% of the yearly production incurs postharvest losses due to physiological damages resulting from improper postharvest handling practices [5–7].

Habanero peppers graded as second or third quality, along with defective fruits, present an opportunity as potential sources for deriving nutraceuticals and other biomolecules. This is due to their inherent content of compounds exhibiting antioxidant activity, thereby facilitating the valorization of these byproducts (Figure 1). The implementation of various unitary operations (such as drying, extraction, or milling) stands out as a pivotal stage in the process of obtaining bioactive compounds from this residual material [8–11].

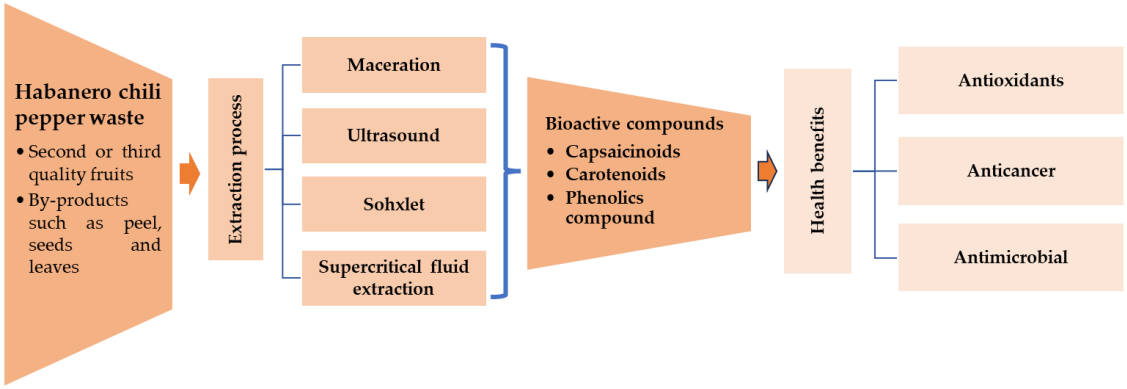

Figure 1. Schematic representation of different extraction process for bioactive compounds recovery from habanero chili pepper waste and their health benefits adapted to Kumar *et al*. [12].

Different authors have shown that *Capsicum* extract present high biological effects such as antioxidant, anti-inflammatory, antifungal and anti-carcinogenic properties, therefore it is possible their use in many applications in pharmaceutical, chemical and food industries [13–16], due to its bioactive compounds content (mainly capsaicinoids and carotenoids). Among capsaicinoids, capsaicin (C) and dihydrocapsaicin (DHC) are the major compounds, representing 89 – 98% in this variety [17]. Capsanthin is the major carotenoid present in pepper oleoresin, although it does not possess provitamin A activity, it has shown to be effective as a free radical scavenger and it also has an HDL cholesterol raising effect in plasma [18]. However, the bioactivity of pepper oleoresin depends on the maturity stage [19], the specific variety of *Capsicum spp*., and the extraction method [20]. In order to successfully recover bioactive compounds and downstream applications in the industry, it is recommended to apply selective extraction techniques for the target compound, thus providing final extracts with greater added value.

Among the industrial products obtained from habanero chili peppers, *Capsicum* oleoresin, an oily nature extract of intense color and fragrance, characteristic to chili peppers, has a diverse industrial application. Traditional *Capsicum* extraction process consists in three stages: dehydration of fresh red peppers, milling and extraction by organic solvents (n-hexane, ethanol and others), using techniques as maceration or Soxhlet [21]. However, the process time, the need for large quantities of solvent and the requirement to treat the waste generated due to the dangerous effects on human health and the environment are some disadvantages associated with the conventional process. The affinity between solvent and solute, the mass transfer, the use of co-solvent, and the properties of the solvents (environmental safety, non-human toxicity, and financial feasibility), should also be considered in the selection of the suitable solvent for an extraction process [22]. Carbon dioxide (CO_2) and water supercritical, organic salt-based solvents such as ionic liquids and deep eutectic solvents, are recognized as environmentally friendly solvents. On the other hand, ethanol, terpenes, glycerol, and methyl esters of fatty acids from vegetable oils are identified as agricultural solvents[23]. Ethanol is widely used as an organic solvent in the extractions of *Capsicum*, due to its low toxicity and good extraction capacity [24–26]. The authors have also observed that, sometimes, the addition of water to

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the extraction solvent helps to increase the effectiveness of the extraction, in terms of extractable solids and capsaicinoids extraction [24,26].

Recently different technologies were developed for *Capsicum* extractions, such as, microwave-assisted extraction, ultrasound-assisted extraction, and supercritical fluid extraction [20,27–30]. Supercritical fluids extraction has been investigated extensively as an alternative to traditional methods that use organic solvent for extraction and fractionation of bioactive compounds from natural matrices, due to its low cost, non-toxicity, non-flammability, inertness, and relatively low supercritical conditions (31.35 °C and 7.39 MPa) when CO_2, the most common supercritical fluid, is used [28,31]. Supercritical CO_2 is limited to dissolve compounds with medium-high molecular weight and low polarity such as carotenoids. An alternative for the extraction of organic compounds with a greater polarity is the addition of CO_2 modifiers such as co-solvents (ethanol, methanol, water, etc.), increasing the extraction yield [28]. In addition, this alternatives extraction process, has focused the attention of bioactive compounds recovery from habanero chili pepper by-products (Table 1).

Table 1. Bioactive compounds recovery from different habanero chili pepper waste.

Habanero chili pepper waste	Extraction process	Bioactive compounds recovery	Reference
Leaves, peduncles, and stems	Ultrasound extraction	Phenolic compounds, carotenoids, and capsaicinoids	[32]
Wasted pulp and seeds	Ultrasound extraction	Capsaicinoids	[33]
Leaves	Ultrasound extraction	Phenolic compounds	[34]
Leaves and stems	Ultrasound extraction using NADES	Phenolic compounds and vitamin C	[35]
Leaves and stems	Maceration, Soxhlet, and SFE	Phenolic compounds	[36]
Seeds	Enzyme-assisted extraction	Phenolic compounds and capsaicinoids	[37]

NADES: natural deep eutectic solvents.

Therefore, in this study different extraction processes, including conventional methods such as Soxhlet (SE) and maceration (ME), as well as non-conventional methods such as ultrasound-assisted maceration (US) and high-pressure processes using supercritical CO_2 (SFE) and co-solvent (SFEC), were employed to determine their influence on biochemical compounds (capsaicinoids and carotenoids) in red habanero chili pepper waste. Some aspects of the process efficiency are discussed in terms of the chemical profile of the extract and the extraction efficiency.

2. Materials and Methods

2.1. Chemicals

The reference standards of capsaicinoids, capsaicin (97%) and dihydrocapsaicin (90%), and carotenoids, capsanthin (95%), zeaxanthin (97%) and β-carotene (93%), were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). The water was obtained from a Milli-Q water deionization system (Millipore, Bedford, MA, USA). The methanol, glacial acetic acid, and methyl tert-butyl ether (MTBE) for chromatographic separation were HPLC grade and were purchased from Merck (Darmstadt, Germany). Carbon dioxide (99.995%) was provided by Abello-Linde S.A. (Barcelona, Spain). Ethanol used for extractions process, was provided by Golden Bell Reagents (Zapopan, Jalisco, México).

2.2. Sample Preparation

Second or third quality fruits of red habanero chili pepper (*C. chinense*) were collected from a local market (Veracruz, México). The fruits were pretreatment for extraction process as reported by

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Olguín-Rojas *et al*. [38]. Slices of 5 ± 1 mm were dried at 70 °C and 1.5 m s^{-1} of temperature and air velocity respectively, until reach 10% of moisture in wet base. This process was performed using a tray drying oven model A39854-14 (Apex Engineering, Calvert City, KY, USA). The dried product was stored in laminated bags that were perfectly sealed under vacuum and stored at −20 °C prior to extraction process.

2.3. Extractions Experiments

2.3.1. Maceration Extraction (ME)

The maceration process was carried out using a sample:solvent ratio of 1:5 (w:w), and a mix of ethanol:water (70:30, w:w) was used as solvent. The process was performed in a Benchtop Orbital Shaker model MaxQ 4450 (Thermo Fisher Scientific, Waltham, MA, USA). The operating conditions were as follows: temperature 50 °C, 150 rpm (orbital radius: 9.5 cm), 150 min of extraction time.

2.3.2. Soxhlet Extraction (SE)

Soxhlet extraction was conducted with 15.0 ± 0.5 g of dried habanero sliced and 250 g of solvent (ethanol:water 70:30, w:w). The samples were packed in a filter paper and inserted in the Soxhlet extractor. The heating power was set to reach 2 cycles per hour, in such a way that 5 extraction cycles were achieved within 150 min.

2.3.3. Maceration Extraction Assisted by Ultrasound (US)

The maceration assisted by ultrasound was carried out using a sample:solvent ratio of 1:5 (w:w), a mix of ethanol:water (70:30, w:w) was used as solvent. The process was performed in an ultrasonic bath (Westprime Systems, Cat. No. B90-055H; Chino, CA, USA) that was operated at 45 kHz and 100 W for 30 min. A cooling bath with recirculation was coupled to maintain the extraction temperature of 50 °C.

2.3.4. Supercritical Fluid Extraction (SFE and SFEC)

Supercritical fluid extractions (SFE) and supercritical fluid extraction with co-solvent (SFEC) were carried out in a high-pressure apparatus supplied by Thar Technology, model SF100 (Pittsburgh, PA, USA). The set-up included an extraction vessel (capacity of 100 mL) with a thermostatic jacket to control the extraction temperature, two pumps with a maximum flow rate of 50 g min^{-1} (one for CO_2 and the other for the co-solvent), a back pressure valve regulator to control the system pressure, and a cyclonic separator. For SFE and SFEC the extraction vessel was loaded with 15.0 ± 0.5 g of sample. Supercritical extraction was carried out at 15 MPa, 40 °C, 90 min and a CO_2 flow of 1.2 kg h^{-1} [39]. For SFEC ethanol was added at a flow of 0.3 kg h^{-1}. Extracts were recovered in a cyclonic separator.

2.4. Extraction Efficiency

The extraction efficiency was determined based on the calculated yield (η), which is the ratio of the amount of solids in the extract (Ex_{E2}) and the extractable solids in the sample (Fx_{F2}), using the Equation 1 [40].

$$\eta\ (\%) = \frac{Ex_{E2}}{Fx_{F2}} * 100 \qquad \text{Eq. (1)}$$

where E is the mass of the extract obtained, x_{E2} is the extractable solids mass fraction in the extract, F is the mass of habanero chili pepper in the extraction process, and x_{F2} is the extractable solids mass fraction.

2.5. Analysis

2.5.1. Determination of Extractable Solids

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Extractable solids in dried red habanero waste (x_{F2}) of habanero chili pepper waste was determined for sequential extractions with an ethanolic solution of 70% (w/w) at a ratio of 1 g of sample with 100 g solvent as reported Vázquez-León et al. [40]. x_{F2} was determined in 0.440 ± 0.011 g per g de raw material.

Extractable solids in extracts (x_{E_2}) were determined as follows: an aliquot (5 mL) of each extract was concentrated and dried with a vacuum rotary evaporator model R-205 (Büchi, Flawil, Switzerland) at 60 °C and 7.2×10³ Pa. After that, the samples were put in a vacuum oven (Lab Line Instrument, Mod. 3818-1, Tripunithura Kochi, India) at 60 °C and 6×10⁴ Pa until reaching constant weight. The extractable solids mass fraction in the extract (x_{E2}) was calculated by the weight difference according to the equation 2 [40].

$$x_{E_2} = \frac{w_1}{w_0} \quad \text{Eq. (2)}$$

where w_0 is the initial weight and w_1 is the final weight. The analyses were done in triplicate.

2.5.2. Determination of Total Capsaicinoids

Total capsaicinoids were obtained as the sum of capsaicin and dihydrocapsaicin. Total capsaicinoids were evaluated in the different extracts obtained as follows: 3 mL of each extract (except for SFEC process) were filtered by a syringe filter and analyzed by UHPLC-Fl. The extracts obtained by SFEC process were dissolved in a knowledge volume of ethanol, and 3 mL was filter by a syringe filter and analyzed by UHPLC-Fl. The separation and quantification of individuals capsaicinoids were carried out on a UHPLC (ACQUITY UPLC H-Class, Waters, Milford, MA, USA) system equipped with an ACQUITY UPLC Quaternary Pump System, an ACQUITY UPLC Auto Sampler with temperature control adjusted at 15 °C, a column oven set at 50 °C for the chromatographic separation, an ACQUITY UPLC® Photodiode Array (PDA) Detector and an ACQUITY UPLC® Fluorescence (FLR) Detector. Empower 3 software (Waters) was used to control the equipment and for data acquisition. Capsaicinoids were analyzed on a Waters ACQUITY UPLC BEH C18 column (50 mm x 2.1 mm I.D., particle size 1.7 µm). A gradient method was used for the chromatographic separation with acidified water (0.1% acetic acid, solvent A) and acidified acetonitrile (0.1% acetic acid, solvent B) working at a flow rate of 0.8 mL min^{-1}. The gradient used was as follows: 0 min, 0%B; 0.50 min, 45%B; 1.60 min, 45%B; 1.95 min, 50% B; 2.45 min, 55% B; 2.80 min, 63% B; 3.00 min, 63% B; 4.00 min, 100% B; 6.00 min, 100% B. The temperature of the column was kept constant at 50 °C. The wavelengths used for fluorescence detection were 278 nm (excitation) and 305 nm (emission) [41]. For the capsaicinoids quantification, calibration curves for capsaicin (C) and dihydrocapsaicin (DHC) were used (y = 2046731.37x + 55924.05 for capsaicin and y = 2194087.16x + 36229.73 for dihydrocapsaicin), which are the two capsaicinoids standards that are commercially available. Correlation coefficients (r^2) (0.9997 for C and 0.9999 for DHC) were calculated. Limits of detection (0.074 mg L^{-1} and 0.062 mg L^{-1} for C and DHC respectively) and limits of quantification (0.247 mg L^{-1} and 0.207 mg L^{-1} for C and DHC respectively), were determined as the analyte concentration corresponding to the standard deviation of the signal of the blank values ($n = 10$) plus 3 or 10 times, respectively, divided by the slope of the linear regression.

2.5.3. Determination of Total Carotenoids

Total carotenoids were obtained as the sum of principal carotenoids (capsanthin, zeaxanthin and β-carotene). The determination of individual carotenoids was performed according to Minguez-Mosquera and Hornero-Mendez [15], which is based on the quantification of insaponificable carotenoids. The sample in contact with a nonpolar solvent mixture (20 mL of petroleum ether:ethyl ether, 1:1) is placed in a separating funnel. The organic and inorganic phases were separated using distilled water. The organic phase was incubated with a solution of 20% KOH in methanol for 12 h, and then concentrated in vacuum to dryness and suspended in methanol. Total carotenoids were evaluated in the different extracts obtained as follows: 3 mL of each extract (exception for SFEC process) were filtered by a syringe filter and analyzed by HPLC following the analysis method

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reported by Giuffrida *et al*. [17] with modifications. The analysis was carried out in a Dionex chromatographic system (Sunnyvale, CA, USA), consisting of an automated sample injector (ASI-100), a pump (P680), a thermostatic column compartment (TCC-100), a photodiode array detector (PDA-100) and a universal chromatography interface (UCI-50). The software used for data analysis was Chromeleon 6.60. The carotenoids were separated using a C30 reverse phase column Develosil® (Phenomenex Inc., Torrance, CA, USA, 5 µm, 150 x 4.6 mm). The chromatographic method utilized a gradient of two solvents ratios MeOH:MTBE:H_2O (82:16:02, solvent A) and (10:88:02, solvent B), working at a flowrate of 0.5 mL min^{-1}. The gradient method used was as follows: 0 min, 0% B; 8 min, 0% B; 26 min, 100% B; 36 min, 100% B. The temperature of the column was held constant at 30 °C.

Carotenoids quantification was performed by comparing retention times and absorption spectra with principal carotenoids (capsanthin, zeaxanthin and *β*-carotene). Standard calibration curves for these three carotenoids were performed at a concentration range between 0.5 and 10 mg/L. The correlation coefficients obtained (R^2) were 0.9997, 0.9996 and 0.9997 for capsanthin, zeaxanthin and *β*-carotene respectively.

2.6. Statistical Analysis

Experimental results were given as mean ± standard deviation and statistical analyses were performed by one-way ANOVA followed by Tukey's pairwise test using Minitab 16 statistical software (State College, PA, USA). Differences were considered statistically significant at the value of probability less than 5% ($p < 0.05$).

3. Results and Discussion

3.1. Physical Characteristics of Red Habanero Chili Pepper Extract

In recent years, the valorization of agrifood by-products through extraction processes has gained significant attention in the agrifood industry. In this context, selective extraction processes are a critical stage, not only in the recovery of bioactive compounds but also in downstream industrial applications. The extensive use of solvents and post-extraction processes such as evaporation or concentration must be considered in workflow design and cost process. According to our results, as expected, the extraction process had an influence on the physical attributes of red habanero chili pepper waste extracts. Typical liquid-phase extracts were obtained using maceration (ME), Soxhlet extraction (SE), maceration assisted by ultrasound (US) and supercritical fluid extraction with co-solvent (SFEC). Conventional extraction process offers easily handling, low operating pressures (ambient conditions), and relatively low temperatures. In alignment with the valorization of agrifood by-products, there is a shift away from traditional, time-consuming methods towards cleaner, more efficient, and environmentally friendly alternatives [42]. In this sense, US is an alternative for reduce the time process. With supercritical fluid extraction (SFE) solid as a powder was obtained as extract, due the absence of any liquid solvent in the SFE process, where the diffusion of solutes is facilitated by supercritical CO_2 in its gaseous phase under atmospheric conditions.

The commercial viability of capsicum extracts relies significantly on their red coloring capacity, regardless of whether they possess a pungent flavor or not. The coloring potential of the extracted oleoresins has an important role in determining their quality and, consequently, their ultimate market price. All the obtained extracts exhibited the high pungency characteristic of habanero chili pepper. However, the extraction process employed had a substantial impact on the color of the extracts. ME, US, and SFEC process produced extracts with red hues, whereas SE and SFE processes yielded extracts with yellow tones. These color variations can be attributed to differences in the composition and profile of bioactive compounds present in the extracts. Furthermore, the coloring capacity of an oleoresin serves as an indicator of its carotenoid content, a topic that will be explored in the subsequent sections of this discussion.

3.2. Extraction Yield from Different Extraction Processes

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To design the process, not only is the effect of the process on extraction yield and the quality of the obtained extract important, but the extraction time and operability will also determine the selection of the most appropriate process. Table 2 presents the principal composition of extracts obtained by different extraction processes from red habanero chili pepper wastes. The conventional ME process yielded the highest concentration of extractable solids, exceeding the SE and SFEC processes by an order of magnitude. In comparison with the US process, no significant differences were reported; however, the total extraction time is shorter. These results are similar to those reported by Fernández-Ronco et al.[43], for paprika extraction. The ultrasonic-assisted maceration process encompasses cavitation, thermal, and mechanical impacts. The improved procedure could be linked to the liberation of extra bioactive compounds resulting from the breakdown of cell walls and the disintegration of chromoplasts induced by cavitation, which is influenced by both thermal and mechanical forces [44].

Table 2. Principal composition, extraction yield and recovery of the capsaicinoids and carotenoids from red habanero chili pepper by different extraction process.

Extraction process	Extractable solids concentration*	η (%)	Recovery from raw material*		Concentration in extract**	
			Total capsaicinoids	Total carotenoids	Total capsaicinoids	Total carotenoids
ME	40.22 ± 2.53^{a}	30.82 ± 0.24^{a}	9.26 ± 0.16^{b}	77.52 ± 1.19^{bc}	3.1 ± 0.07^{a}	25.99 ± 0.55^{a}
SE	1.55 ± 0.07^{b}	4.6 ± 0.01^{c}	10.44 ± 1.15^{b}	111.36 ± 7.85^{b}	0.79 ± 0.13^{b}	8.38 ± 0.08^{c}
US	42.5 ± 1.67^{a}	31.49 ± 3.63^{a}	10.64 ± 0.2^{b}	97.24 ± 0.51b^{c}	3.29 ± 0.17^{a}	30.27 ± 2.6^{a}
SFE	ND	18.68 ± 0.03^{b}	8.56 ± 1.27^{b}	56.53 ± 2.08^{c}	3.34 ± 0.37^{a}	17.43 ± 0.14^{c}
SFEC	3.95 ± 0.01^{b}	29.24 ± 0.07^{a}	14.91 ± 0.38^{a}	292.09 ± 10.59^{a}	0.48 ± 0.01^{b}	9.35 ± 0.82^{b}

ND: not determined. Different letters in the same column indicate that the means differ significantly by Tukey's test ($p < 0.05$). The values are the means of triplicates ± SD. * g/kg d.w. **g/kg of extract.

In the SE process, a sample:solvent ratio of approximately 1:16 (w:w) was employed This ratio resulted in a lower concentration of extractable solids due to the excess solvent used. However, a notable drawback of the SE process lies in its high solvent consumption, which is a consequence of the system configuration requirements. These requirements include maintaining sufficient solvent for reflux while ensuring an adequate amount for the recovery vessel. In this sense, the high quantity of liquid solvent used in SFEC reduce significantly the concentration of extractable solids.

In terms of extraction efficiency, the highest values were achieved by ME, US, and SFEC, followed by SFE. These results are similar to reported by Aguiar et al. [45] for Malagueta chili pepper (*C. frutescens*) using supercritical CO_2 with yields ranging between 11.8 and 13.6% using temperatures and pressures of 40 °C and 150 bar.

The recovery of compounds from habanero chili peppers is dependent on the process and the solvent employed. The SCFE process achieved the highest recovery of bioactive compounds (capsaicinoids and carotenoids). The total yield of capsaicinoids (C + DHC) varied from 8.56 ± 1.27 g kg^{-1} of sample (SFE) to 14.91 ± 0.38 g kg^{-1} of sample (SFEC) in the different extraction processes studied. These high concentration values of capsaicinoids were expected in Habanero pepper since it is popularly known in Mexico as a very pungent pepper [17].

The difference in the extraction yield of total capsaicinoids in ME (9.3 g kg^{-1} of sample) and SE (10.4 g kg^{-1} of sample) processes could be explained by the presence of water in the extraction solvent and the contact between the solvent and the sample. Barbero et al. [24] found that water, being a highly polar solvent, reduced the extraction of less polar capsaicinoids such as dihydrocapsaicin compared to capsaicin. SE showed the lowest extraction yield; however, this process provided extracts with high concentrations of capsaicinoids, similar to SFE. Therefore, it was possible to obtain extracts with higher purity. As Aguiar et al. [28] reported, the solubility of capsaicinoids in CO_2 at 15 MPa and 50 °C is probably higher than the solubility of other compounds present in Second quality red habanero chili pepper , such as triacylglycerols and carotenoids.

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Comparable findings were noted by Chel-Guerrero et al. [32], for bioactive compounds from stems of habanero pepper plants. The SFE process showed exhibited promising results for obtaining compounds with anti-inflammatory activity. Conversely, the ME and SE processes demonstrated superior performance in extracting antioxidant compounds and polyphenols.

As is known, the advantage of supercritical CO_2 processes is that the fluid exhibits both gas-like properties, including diffusion, viscosity, and surface tension, and liquid-like properties, demonstrating density and solvation characteristics typical of liquids. Moreover, the incorporation of a co-solvent such as ethanol enhances the specificity and speed of the target molecule, attributed to the physicochemical properties of the solvent [28]. The primary solvent in SFE is predominantly the supercritical CO_2 due to its high diffusivity, low mass transfer limitations and high surface tension. These properties could break cell walls of the raw material and so allow deeper penetration into the small pores of the matrix and, therefore the extraction efficiency of the co-solvents used can be increases [46]. The solvent employed in the extraction processes (EtOH) exhibited the capability to extract both polar and non-polar substances. Consequently, the selectivity of solute recovery was diminished, resulting in an increase of the solids content in the final product. This differs from SFE, where only non-polar components are extracted due to the distinct properties, as previously mentioned with supercritical CO_2.

These results demonstrate that the selection of the extraction process significantly influences the composition, yield, and recovery of capsaicinoids and carotenoids from red habanero chili peppers, with ME and SFEC generally yielding higher concentrations and recoveries compared to SE and SFE. In the following section, the profile of extract obtained from red habanero chili pepper waste with respect to the main bioactive compounds (capsaicinoids and carotenoids) is discussed.

3.3. Bioactive Compound of Red Habanero Chili Pepper Extract from Different Extraction Processes

When examining the extraction process, it is essential to consider various factors, including their impact on yield, extraction time, and the quality of the extract. In this instance, the quality of chili extracts is assessed based on attributes such as color and pungent capacity. Due to their bright color and spicy taste, chilies are frequently used in the preparation of condiments and food items. In addition, the chilies extracts are recognized for the health beneficial aspects, medicinal and phytopharmacy potential, in addition for health-promoting functional attributes [47]. Most commercially valued oleoresins are those that exhibit a high red coloring capacity, primarily attributed to carotenoid pigments, either with or without a pungent flavor. Among these carotenoids, capsorubin, capsanthin, zeaxanthin, and β-cryptoxanthin are the primary compounds responsible for the red coloration, while β-carotene primarily serves as an antioxidant.

Table 2 present the total capsaicinoids and carotenoids content in the extract obtained by different extraction process. It is observed that when using SFE, the highest concentration of capsaicinoids (3.34 ± 0.37g kg^{-1} of extract) is obtained, which is statistically similar to the result obtained with US and ME. However, when EtOH is used as a co-solvent, the concentration of capsaicinoids (0.48 ± 0.01 g kg^{-1} of extract) is one order of magnitude lower. Although the yield of total capsaicinoids was improved by using a co-solvent (SFEC), the use of more solvent reduces the final concentration of capsaicinoids.

Regarding the bioactive compound profile in the extract (Table 3), as expected, in all cases the extract obtained showed a higher concentration of capsaicin than dihydrocapsaicin; this corresponds to what was reported for the genus *C. chinense* [17]. Aviles-Betanzos [48] report the highest concentration of capsaicin (37.9 ± 0.84 g kg^{-1} extract) and dihydrocapsaicin (10.17 ± 0.18 g kg^{-1} extract) using SFEC at 45 °C, ≈100 bar, and 60 min. The results of capsaicinoids and carotenoids for the evaluated materials were similar or higher than those found in the literature, which can be explained by the difference of capsicum varieties, maturate stage or cultivation regions [19].

Table 3. Principal bioactive compound composition of red habanero chili pepper extract by different extraction process.

Extraction	Capsaicin*	Dihidrocapsaicin*	Capsanthin**	Zeaxanthin**	β-carotene**

 doi:10.20944/preprints202402.1467.v1

process					
ME	2.70 ± 0.03[a]	0.40 ± 0.03[a]	15.50 ± 0.67[a]	9.15 ± 0.13[a]	1.34 ± 0.32[c]
SE	0.60 ± 0.09[b]	0.19 ± 0.03[c]	4.79 ± 0.03[c]	0.91 ± 0.06[b]	2.68 ± 0.08[b]
US	2.84 ± 0.14[a]	0.45 ± 0.02[a]	18.05 ± 1.56[a]	10.33 ± 0.93[a]	1.85 ± 0.16[bc]
SFE	3.05 ± 0.21[a]	0.29 ± 0.05[b]	10.59 ± 0.07[b]	0.97 ± 0.01[b]	5.87 ± 0.04[a]
SFEC	0.42 ± 0.01[b]	0.06 ± 0.03[d]	5.31 ± 0.28[c]	0.60 ± 0.03[c]	3.44 ± 0.27[b]

Different letters in the same column indicate that the means differ significantly by Tukey's test ($p < 0.05$). The values are the means of triplicates ± SD. * mg/g of extract, ** µg/g of extract.

On the other hand, the highest concentration of total carotenoids was founded in ME and US extracts (25.99 - 30.27 g kg^{-1} of extract). With respect to carotenoids profile, the dominant carotenoid found in the extracts obtained is capsanthin (57.2% - 77.4%). The red color of chili fruits is due capsanthin, capsorubin, and cryptocapsin. In *C. chinense* fruits, the capsanthin and capsorubin constitute > 60% of the total carotenoids. Yellow pigmentation in fruits is due to α-, β-carotene, zeaxanthin, lutein and β-cryptoxanthin.

The CO_2 is a non-polar solvent, which preferentially extracts non-polar components. β-carotene, being a relatively nonvolatile compound with no functional groups, represents a hydrophobic natural product [46]. The results indicate a significant extraction of β-carotene when CO_2 is used as the solvent (33.7% - 36.8%), in contrast to ME process (4.0% - 5.1%). Moreover, any modifications in the SFE conditions influence the polarity of the extraction fluid, resulting in the extraction of pigment fractions within specific polarity ranges. Additionally, the inclusion of EtOH in the SFE process enhances the extraction of red pigments [49,50]. When employing SFEC, a higher concentration of carotenoids per gram of sample is extracted (Table 2). The properties of high diffusivity of pressurized CO_2 fluid, coupled with low limitations in mass transfer and surface tension, enable SFE to disrupt cell structures, facilitating the penetration of the organic solvent into the small pores of the matrix. As carotenoids are typically found within chromoplasts, these effects enhance extraction efficiency [46]. However, the concentration in the extract is reduced due to the presence of other soluble compounds, such as free fatty acids [51].

The data underscores the importance of extraction process selection in obtaining habanero chili pepper second quality extracts with specific bioactive compound profiles for various applications, including food and pharmaceutical industries.

5. Conclusions

Different extraction process for bioactive compounds recovery from second or third quality red habanero chili peppers, were employed. The results revealed varying profiles of bioactive compounds in the extracts, depending on the extraction process used. The supercritical fluid extraction process using CO_2 resulted in lower extraction yields when compared to low-pressure methods. However, the quality of the SFE extract was significantly superior in terms of total capsaicinoids compared to other extraction methods. In this context, the revalorization of food waste emerges as a technological and innovative research area with beneficial effects for the population, the economy, and the environment. The bioactive compounds present in these extracts could find further applications in the food and pharmaceutical industries. The results obtained from this study will be used for evaluating and applying different extraction techniques in industrial processes, offering the best alternatives for obtaining high-quality commercial chili pepper extracts.

Author Contributions: Conceptualization, J.A.O.-R., L.A.V.-L. and G.d.C.R.-J.; methodology, J.A.O.-R., L.A.V.-L., G.F.B. and M.T.F.-P.; software, J.A.O.-R. and L.A.V.-L; validation, G.F.B. and G.d.C.R.-J.; formal analysis, J.A.O.-R., L.A.V.-L., G.F.B. and G.d.C.R.-J.; investigation, J.A.O.-R. and L.A.V.-L.; resources, G.F.B., M.P., L.C. and G.d.C.R.-J.; data curation, G.F.B. and G.d.C.R.-J.; writing—original draft preparation, J.A.O.-R. and L.A.V.-L; writing—review and editing, J.A.O.-R., L.A.V.-L., G.F.B. and G.d.C.R.-J.; visualization, G.F.B. and G.d.C.R.-J.;

supervision, G.F.B. and G.d.C.R.-J.; project administration, G.F.B. and G.d.C.R.-J.; funding acquisition, G.F.B., M.P., L.C. and G.d.C.R.-J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Acknowledgments: The authors express their acknowledgments to Consejo Nacional de Humanidades Ciencia y Tecnología (CONAHCyT). Special acknowledgment is extended to the Mass Spectrometry Division of the Central Research Services for Science and Technology (SC-ICYT) of the University of Cadiz for the collaboration throughout the analysis of the samples.

Conflicts of Interest: The authors declare no conflict of interest.

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