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Abstract: Capsaicin, vitamin C, carotenoids and phenolic compounds from cumari-do-Pará peppers (*Capsicum chinense* Jacq.) harvested from two different locations from Pará, Brazil, and at different ripening stages were extracted employing ultrasonic-assisted extraction (UAE). Edible vegetable oils from soybean (*Glycine max*), Brazilian nuts (*Bertholettia excelsa* H.B.), and palm olein were used as the extraction solvent, as an alternative to organic solvents combined with UAE. The proximate composition of the pepper extracts and vitamin C were determined through AOAC methods, while total phenolic compounds and carotenoids were assessed by UV/Vis spectrophotometry, and capsaicin, by high-performance liquid chromatography. The antioxidant activities of cumari-do-Pará extracts were evaluated by the ABTS radical scavenging and β-carotene/linoleic acid system assays. Bioactive compounds and antioxidant activity varied with harvesting location and pepper ripening stage. Soybean oil was the most effective in extracting hydrophobic molecules particularly linoleic acid, corresponding to about 60% of the total fatty acid. Carotenoids were extracted whith highest yields by all oil extracts. Oily cumari-do-Pará extracts can be used as spices in foodstuffs and as additives in pharmaceuticals and nutraceuticals. Edible vegetable oils combined with UAE are, thus, efficient for bioactive compound extraction, also comprising an environmentally friendly, safe, low-cost, and fast alternative.

Keywords: green extraction; soybean and Brazilian nut oils; palm olein; vitamin C; capsaicin; carotenoids; phenolic compounds

1. Introduction

Capsicum spp. peppers, commonly known as cumari-do-Pará peppers, are very popular worldwide due to certain sensorial features, such as color, pungency, and aroma, also comprising a source of bioactive compounds that offer several health benefits. Adding pepper extracts to foods or consuming them on their own can boost antioxidant food power, as these extracts are a source of vitamins C, E, provitamin A, carotenoids, phenolic compounds and, mainly, capsaicin [1–5]. Pepper

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consumption has been associated with significantly lower all-cause, cardiovascular, and cancer-related mortalities when consumed regularly, according to a meta-analysis evaluation, although a consumption regime has not yet been established [6]. In addition to interest due to flavor and sensorial aspects, capsaicin and other capsaicinoids found in peppers are also considered pharmacological agents, as they interact with the transient receptor receptor potential cation channel subfamily V member 1 (TRPV1), which increases intracellular calcium levels and activates the sympathetic nervous system, releasing catecholamines [7–10]. These events increase and improve fat metabolism, thermogenesis, and blood glucose control, reducing the risk of obesity and metabolic syndrome, all of which underly the risk of cardiovascular events, strokes and death [6,7,11,12]. Along with capsaicin, other compounds present in pepper extracts, such as vitamin C, E, provitamin A, carotenoids, and phenolic compounds, can also reduce cardiovascular and cancer-related complications, attenuating cellular oxidative status by scavenging reactive oxygen and nitrogen reactive species and reducing inflammation by acting through the nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor-kappaB (NFκB) signaling pathways [13–17]. Because of this, pepper products and extracts have been recognized both as food ingredients and potential pharmacological agents [18–21].

Traditionally, pepper processing aiming at obtaining bioactive compounds includes dehydration, milling, and conventional extraction employing organic solvents, such as methanol, ethanol, or petroleum-derived solvents [22–24]. However, organic solvents can be unsafe due to their residual presence in final products and the emission of potentially harmful volatile derivatives. These emissions can affect the respiratory tract and may be neurotoxic or carcinogenic [25–27]. Furthermore, organic solvents are environmentally harmful pollutants that negatively affect the atmosphere and climate quality [28], and their removal from food and pharmaceutical additives is also time-consuming and costly [29–32].

Current regulations concerning non-environmental friendly processes and restrictions imposed on food processing employing organic solvents have encouraged research on the application of green technologies to recover valuable compounds from edible plants through low-cost and safe processing methods [33–36]. In this sense, edible vegetable oils have been noted as promising green extraction solvents and have become popular for extracting bioactive compounds [37–44]. These oils are also renewable and non-toxic resources, as their methyl esters do not emit volatile organic compounds, and exhibit comparable technical performances to organic solvents used for extracting bio-compounds from vegetable matrices [31,37,38,45]. The high viscosity of vegetable oils, however, is a major constraint concerning bioactive compound extraction, due to low diffusivity and, consequently, low compound yields, even when applying high processing temperatures [40]. Recently ultrasonic-assisted extraction (UAE), has become a widely applied tool used to circumvent these and other limitations inherent to conventional extractions, *i.e.*, extraction time and solvent consumption, while improving bioactive compound yields, particularly regardinh highly hydrophobic compounds found in food matrices [31,33,34,40,46–48]. The UAE method is based on the cavitation generated by ultrasonic energy, which disrupts cell walls, allowing for highly efficient bioactive compounds release and diffusion rates from vegetable matrices [31,33,34,40,46–48]. Finally, the quantity, quality, and yield of bioactive agents in vegetable matrices vary significantly depending not only on the cultivar, farming practices, and management, but mostly on ripening stage [29,49–51].

In this context, the suitability of a green bioactive compound extraction method from cumari-do-Pará peppers (*Capsicum chinense* Jacq) harvested at two different ripiening stages employing UAE combined with vegetable oil extraction was investigated. Oils from soybeans, Brazilian nuts, and palm olein can comprise organic solvent alternatives used to produce oily pepper extracts naturally enriched with bioactive compounds, employing an innovative, efficient, low-cost, safe, fast, and green methodology.

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2. Materials and Methods

2.1. Plant Material and Agroclimatic Study Area Characteristics

Peppers (*Capsicum chinense* Jacq) were harvested from two different locations in the state of Pará, in Northern Brazil, within the Amazon rainforest biome. The first comprises Igarapé-Açu, at 01º07'44" S, 47º37'12" W, at an average altitude of 50.0 m a.s.l., with an annual average maximum temperature of 32.2°C, and relative humidity of 85%. Annual precipitation is high, reaching 2,460 mm, strongly concentrated between January and June, while drier periods occur between August and December [52,53]. Peppers were also harvested at Santo Antônio do Tauá at 01º09'07" S, 48º07'46" W, at an average altitude of 20.0 m a.s.l, with an annual average maximum temperature of 31.7°C, relative humidity of 85%, and precipitation of 2,600 mm, with the rainiest period occurring between December and May, and a low hydric deficiency of 69 mm noted during drier periods between September and November. An annual water surplus of 1,100 mm, refering to the maximum water soil retention, is noted in this area. The soils in the municipality of Santo Antônio do Tauá exhibit low natural fertility levels, due to low essential nutrient and mineral contents [54]. Peppers were harvested in September 2015 at two different maturation stages, immature, comprising fully developed fruit just prior to the onset of maturation, and mature, exhibiting a completely yellow skin. Over-mature and damaged peppers were discarded. For processing, peppers were sanitized in a 200 mg/L sodium hypochlorite solution for 15 min and washed with distilled sterilized waterm and the seeds and pulps were ground, freeze-dried, and immediately stored at -18ºC, until use.

2.2. Physicochemical Analyses

Centesimal composition analyses, namely moisture (method nº. 932.12), protein (method nº. 920.109), lipid (method nº. 963.15) and ash (method nº. 972.15), were carried out according to the Association of Official Analytical Chemists (AOAC) [55]. Total carbohydrates were estimated by subtracting the sum of moisture, protein, ash, and lipid content from 100%. pH values were determined using an microcomputer pH-vision 246072 (EXTECH Instruments, Waltham, MA, USA), while titratable acidity was expressed as a citric acid percent and soluble solids were measured using a refractometer (ABBE, AR 1000S, RPC), determining the refractive indices of each samples, reported in °Brix. Pepper vitamin C contents were determined in mg per 100 g, according to AOAC method 43065 [56], where metaphosphoric acid, used as the solvent, was replaced by oxalic acid. All measurements were conducted in triplicate.

2.3. Fatty Acid Composition

The fatty acid composition of the employed vegetable oils was determined by their conversion into fatty acid methyl esters (FAMEs) according to Rodrigues, et al. [57],, followed by subsequent analyses employing a CP-3380 gas chromatograph (Varian Inc., CA, USA) equipped with a flame ionization detector (FID) and a CP-Sil 88 60 cm capillary column, with an internal diameter of 0.25 mm, and film thickness of 0.25 μm (Varian Inc.). The operating conditions were as follows: helium was employed as the carrier gas at a 0.9 mL/min flow rate, the FID detector was set at 250 ºC, the injector (split ratio 1:100) was set at 250 ºC, and a 1.0 μL injection volume was applied. The column temperature programmed was set at 175 ºC for 8 min, followed by a 2.0 ºC/min increase up to 180 ºC for 28 min, and, finally, a 2.0 ºC/min increase up to 205 ºC, for 10 min. Individual fatty acid peaks were identified by comparing their retention times with those of known Nucheck 74X standard fatty acid blends (Nu-Chek Inc., MN, USA) run under the same operating conditions. The retention time and area of each peak were calculated using the Varian Star 3.4.1 software (Varian Inc.). The results were expressed as relative percentages of total fatty acids.

2.4. Ultrasonic-Assisted Extraction (UAE)

Ultrasonic-assisted extraction (UAE) was carried out using a domestic ultrasonic bath device (Unique, Maxiclean 1450, BRA) set at a constant power of 400W and 40 kHz. The apparatus was

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equipped with a digital control system for sonication time standardization. The freeze-dried pepper samples (1.0 g) were placed in 15 mL capped plastic tubes and mixed individually with 5 mL of oil obtained from from soybean, Brazilian nut, or palm olein. The samples were then placed at the center of the ultrasonic device, 7 cm in depth and sonicated for 60 min. After this procedure, the mixtures were centrifuged at 13,000 x g, for 20 min at 25 ºC, and the supernatants, comprising the oily extracts (OEs), were separated for further analyses.

2.5. Oily Extract Pretreatment

Prior to antioxidant activity, capsaicin and total phenolic content determinations, all OE underwent pretreatment by mixing 2 mL of each sample with 2 mL hexane and 10 mL methanol: water (50:50, v/v). The suspensions were then stirred by magnetic agitation at 1,500 g, for 30 min at room temperature followed by methanol phase removal, filtering through 0.45 µm PVD filters (Merck Millipore, MA, USA), and re-extraction. After two extractions, the methanol extracts were pooled and transferred to dark glass bottles, bubbled with nitrogen (N_2), and stored in the dark at −20 °C until use.

2.6. Total Phenolic Content (TPC) Determinations

The total phenolic contents (TPC) of the pepper extracts obtained by the organic solvent extraction (OSE) or OE methods were estimated using the Folin–Ciocalteu assay, according to the method developed by Singleton and Rossi [58] and modified by [59]. A 70% aqueous acetone solution was used as the organic solvent for the OSE, while extracts acquired in the pre-treatment stage were used for OE. TPC was determined through absorbance measurement at 725 nm using a UV/Vis spectrophotometer (Nova, NI 2000, BRA). TPC were expressed as gallic acid equivalents (GAE) mg.100 g of sample.

2.7. Capsaicin Quantification

The OSEs were obtained by mixing with 50 mL of absolute ethanol followed by magnetic stirring for 12 h, while OE were obtained by mixing with 10 mL of absolute ethanol followed by homogenization at 40 rpm for 20 min, repeated twice. The ethanol extracts were filtered through 0.45 µm syringe filters (Merck Millipore). Capsaicin determinations were performed by high-performance liquid chromatography (HPLC) employing an LC-A10 Prominence (Shimadzu, JPN) apparatus equipped with a UV detector at 280 nm and a C18 column (150 mm x 4.6 mm x 5 µm). The mobile phase comprised acetonitrile/water at a 60:40 ratio (v/v) and 1.0 mL/min flow rate at 40ºC, according to Perucka and Oleszek [60], with modifications. Samples (40 µL) were injected into the HPLC system and capsaicin identification and quantification were performed using capsaicin standards. Data were expressed as mg of dry weight.

2.8. Total Carotenoid (TC) Determinations

Carotenoids were extracted from OSE peppers using acetone and petroleum ether as the solvent mixture, while hexane at a 10:2 (v/v) ratio was used for the OE samples. Total carotenoids (TC) were determined by measuring sample absorbances at 450 nm on an NI 2000 spectrophotometer (Nova, BRA), according to Godoy and Rodriguez-Amaya [61]. The results were expressed as µg of carotenoids per g of dry weight (µg lutein/g DW), using the molar extinction coefficient of lutein in petroleum ether (2589 M^{-1} cm^{-1}).

2.8. Antioxidant Activity Determinations

2.8.1. ABTS Radical Scavenging Assay

The $ABTS^+$ assay was performed according to Rufino, et al. [62]. The ABTS solution was prepared by mixing 5 mL of 7.0 µM ABTS and 88 µL of 145 µM potassium persulfate solution, which was left to react for 12–16 h at room temperature in the dark. Ethanol (99.5%) was added to the

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solution until reaching an absorbance value of 0.700 ± 0.05 at 734 nm, assessed using an NI 2000 UV/Vis spectrophotometer. Trolox (100–2000 μM TE) was used as the reference antioxidant compound. The ABTS solution was added to the samples and the absorbances of the samples and standard solutions were determined after 6 min at 734 nm at room temperature. Results were expressed as μmol Trolox equivalent (TE) g^{-1}.

2.8.2. β-Carotene/Linoleic Acid Determinations

The antioxidant activities of the sample extracts were determined according to Matthäus [63], with modifications. A stock solution of a β-carotene-linoleic acid mixture was prepared by dissolving 3.34 mg β-carotene in 1 mL chloroform, with the addition of 40 mg linoleic acid and 400 mg Tween 20. The chloroform was completely evaporated and 100 mL of water were added with vigorous shaking. Then, 5 mL of the mixture were dispensed in test tubes and a 200 μL aliquot of the extract (pretreatment extract) was added and incubated at 50°C, for 60 min. Absorbances were then determined at 470 nm at time zero (A_0) and after 60 min incubation (A_{60}), on an NI 2000 UV-Vis spectrophotometer. Antioxidant activities were calculated according to the following equation:

$$\textbf{Antioxidant activity}\ (\%) = \left[1 - \frac{(A_0 - A_{60})}{(A_0^c - A_{60}^c)}\right] \textbf{x100} \quad (1)$$

where A_0 and $\mathbf{A_0^c}$ are the initial incubation time sample and control absorbances, respectively, while A_{60} and $\mathbf{A_{60}^c}$ are the sample and control absorbances, respectively, determined at the end of the reaction.

2.9. Statistical Analyses

All determinations were performed in triplicate and expressed as means ± standard deviations (SD). The results were subjected to ANOVA and Pearson's correlation tests, and potential differences between means were evaluated by Tukey´s multiple comparison test. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using the Statistica® 7.0 software (Statsoft Inc., OK, USA).

3. Results and Discussion

3.1. Physicochemical Analyses

The physicochemical characteristics of immature and mature peppers from Taua and Igapare-Açú, are presented in Table 1.

Table 1. Proximate composition of immature and mature cumari-do-Pará peppers from two locations, Tauá and Igaparé-Açu in the state of Pará, Brazil.

Analyses	TAUÁ		IGARAPÉ-AÇU	
	Immature	Mature	Immature	Mature
Moisture (g.100 g^{-1})	91.46±0.17^{a}	90.92±0.17^{a}	87.90±0.24^{c}	87.76±0.45^{c}
Total proteins (g.100 g^{-1})	1.22±0.09^{b}	0.97±0.22^{c}	1.49±0.04^{a}	1.50±0.026^{a}
Total lipids (g.100 g^{-1})	1.41±0.09^{a}	1.49±0.05^{a}	0.94±0.07^{b}	1.64±0.06^{c}
Ashes (g.100 g^{-1})	0.65±0.04^{b}	0.71±0.05^{b}	0.76±0.05^{ab}	0.94±0.04^{a}
Carbohydrate (g.100 g^{-1})	5.26±0.21^{d}	5.91±0.13^{c}	8.91±0.35^{a}	8.16±0.69^{b}
Soluble solids (ºBrix)	8.57±0.26^{d}	9.30±0.06^{b}	9.2±0.00^{c}	10.26±0.06^{a}
Titratable acidity (% citric acid)	0.45±0.01^{a}	0.34±0.01^{c}	0.40±0.01^{b}	0.35±0.00^{c}
pH	5.07±0.00^{c}	5.25±0.02^{b}	5.33±0.02^{a}	5.36±0.00^{a}
Bioactive compounds				
Vitamin C (mg.100 g^{-1})	128.16±0.79^{a}	77.15±0.46^{c}	101.91±0.55^{b}	64.55±0.36^{d}
Total phenolic content (mg GAE.100g^{-1})	697.64±5.36^{d}	757.49±6.39^{b}	724.69±6.42^{c}	825.21±3.32^{a}

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Capsaicin (mg.g^{-1})	1.42±0.01^{d}	1.77±0.00^{c}	3.12±0.01^{b}	3.26±0.01^{a}
Total carotenoids (µg.g^{-1})	11.43±0.07^{d}	22.97±0.08^{b}	17.17±0.01^{c}	29.41±0.06^{a}
Antioxidant analyses				
β-carotene/linoleic acid (%AA)	24.48±0.46^{c}	27.26±0.48^{b}	27.06±0.27^{b}	31.77±0.49^{a}
ABTS (µM trolox.g^{-1})	46.79±1.67^{c}	50.23±0.47^{b}	50.17±1.98^{b}	65.83±2.20^{a}

The results are presented on a dry weight basis following three determinations (mean ± standard deviation). Same letters on the same line indicate no significant difference between maturation stages and/or locations according to the Tukey test ($p \leq 0.05$).

The influences of maturation stage and location on the determined physicochemical parameters are displayed in Table 1. Pepper moisture contents varied from 87.76% to 91.46% in both maturation stages and locations, similar to values reported in peppers from other Capsicum genus members [5,64,65]. Moisture content did not vary with maturation stage, with no differences observed between immature and mature peppers, but varied per geographic location. In this sense, peppers from Tauá exhibited a higher moisture content (from 90.92±0.17 to 91.46±0.17) than those harvested at Igarapé-Açú (from 87.76±0.45 to 87.90±0.24), probably due to the fact that the Tauá hydrographic network is made up of several small rivers, including the Bituba, Caripé, Patauateua, São Francisco and Tauá Rivers, while also receiving the influence of the Sol and Furo da Laura Bays, forming a large water drainage network used in agriculture and potentially favoring water capture by local crops [54]. Although moisture did not differ significantly between maturation stages, a trend of decreased moisture with increasing maturation was observed for the peppers from both locations. Moisture status as a function of maturation corroborates [66], who reported moistures contents from 81.46% to 91.42% for immature peppers, and from 78.19% to 89.39% for mature ones. Moisture losses during the ripening process may be related to respiratory cell functions or to the climatic characteristics inherent to the Amazon region, comprising high evapotranspiration demands combined with water losses to the atmosphere through plant surface evaporation and transpiration [67]. The peppers were harvested in September, during the local dry season and during one of the hottest months of the year, according to the National Institute for Space Research Weather Prevision Center and Climate Studies [68].

The total protein contents of peppers from Tauá varied between immature and mature specimens, with values of 1.22±0.09 and 0.97±0.22 mg.100 g^{-1}, respectively, while no protein content variation was observed between immature and mature peppers from Igarapé-Açú. Lower protein contents during ripening have been reported for other fruits, such as Barbados cherries (Malpighia punicifolia L.) [69]. Reduced proteins contents during maturation can result from the breakdown of proteins into amino acids used todevelop carbon skeletons for the synthesis of volatile compounds that contribute to aroma, which are enhanced in mature fruits [69]. On the other hand, Igarapé-Açú peppers exhibited protein content values of 1.49±0.040 and 1.50±0.026 mg.100 g^{-1} in immature and mature fruits, respectively, higher than values observed in peppers from Tauá (Table 1).

No significant difference in total lipid contents was observed between immature and mature peppers from Tauá (Table 1), in contrast to Igarapé-Açu peppers, where an increase in lipid contents from 0.94±0.07 to 1.64±0.06 g.100 g^{-1} in immature and mature fruits was observed. Similar results were reported by Carvalho, et al. [70] when investigating sweet peppers (Capsicum chinense) at different ripening stages, with average lipid contents of 0.367 mg.g^{-1} in immature peppers and 0.456 mg.g^{-1} in mature ones. Increased phosphatidylcholine and phosphatidic acid contents during fruit ripening are due to increased respiratory rates, a characteristic of climacteric fruits [71].

Differences in ash contents were also noted between mature fruits from both locations (Tauá 0.71±0.05 mg.100 g^{-1} and Igarapé-Açu 0.94±0.04 mg.100 g^{-1}), ranging from 0.65±0.04 to 0.94±0.04%, corroborating previous reports for Brazilian peppers, which indicated values ranging from 0.6 to 1.7% [72].

Regarding carbohydrates, differences were observed concerning maturation stage and geographic locations, as immature and mature Igarapé-Açu peppers already exhibited higher carbohydrate contents (Table 1). However, during ripening, carbohydrate contents varied between locations, with a 35% increase in carbohydrate contents in mature peppers, ranging from 5.26±0.21 to

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5.91±0.13 mg.100 g^{-1}, while peppers from Igarapé-açú presented a 8.5% carbohydrate content reduction from immature to mature fruits, from 8.91±0.35 to 8.16±0.69 mg.100 g^{-1}. Polysaccharides are mobilized during the aerobic metabolism during fruit maturation,, increasing monosaccharide contents, such as glucose and fructose, which in turn improve fruit texture and flavor. This explains the higher sugar contents noted during Tauá pepper maturation [73]. On the other hand, the reduced sugar contents observed in Igarapé-Açú peppers may be due to fruit aging, as these peppers were probably harvested at the end of their life cycle, which may comprise a study limitation. In this sense, the anabolic metabolism was predominant in Igarapé-Açú peppers, resulting in higher consumption of simple sugars.

Soluble solid contents increased throughout pepper ripening at both sampled locations, with 7.8 % and 10.3 % increases observed in Tauá and Igarapé-Açu peppers, respectively. Increased soluble solids commonly occur during fruit ripening following the mobilization of starch and other polysaccharides from the cell wall, resulting in the monosaccharide accumulation alongside alkalination and consequent pH increases [74]. Similar soluble solid increases have been reported for Brazilian chilies (Capsicum annuum L. and 'Zarco HS' Yellow) [75,76].

The titratable acidity of the investigated peppers varied for both maturation stage and geographical location, with 25 % and 12.5 % acidity levels decreases observed in mature Tauá and Igarapé-Açu peppers, respectively compared to immature peppers (Table 1). This is expected for Capsicum spp. during ripening, when the synthesis of organic acids occurs, with decreased organic acid contents noted at the end of maturation period and beginning of senescence, as these compounds are consumed during the pepper respiratory metabolism [70,77].

Vitamin C contents decreased by approximately 40% in peppers harvested at both locations, associated with advanced ripening, from 128.16±0.79 mg.100^{-1} in immature peppers to 77.15±0.46 mg.100^{-1} in mature peppers from Tauá, and from 101.91±0.55 mg.100^{-1} in immature to 64.55±0.36 mg.100^{-1} in mature peppers from Igarapé-Açu. Vitamin C decreases observed during fruit maturation may be due to ascorbic acid oxidase (ascorbinase) and peroxidase activities [77]. Similar results have been reported for sweet peppers and bell peppers (C. annuum L.), with vitamin C values ranging from 58.8 and 361.65 mg.$100g^{-1}$ in immature peppers, and between 36.70 and 220 mg.$100g^{-1}$ in mature peppers [70,78,79]. Vitamin C confers resistance against biotic and abiotic stresses, and although the contents determined herein reduced with pepper ripening, the levels were still enough to meet the recommended dietary allowance of 60 to 90 mg/day for adults [80].

3.2. Total Phenolic Contents

Total phenolic compounds (TPC) following OSE and OE extractions were different considering both maturation stages and geographic locations (Table 2). Immature peppers contained lower phenolic compound concentrations compared to mature ones, and peppers with Igarapé-Açu containing the highest amounts of these compounds (Table 2).

Table 2. Total phenolic contents of OSE peppers and OE from soybean, Brazil nut, or palm olein from Tauá and Igarapé-Açu, Para, Brazil.

Total phenolic contents	**TAUÁ**		**IGARAPÉ-AÇU**	
(mg GAE $100g^{-1}$)	**Immature**	**Mature**	**Immature**	**Mature**
OSE/acetone 70%	1332.25±8.02^{Ca}	1435.23±17.73^{Ba}	1367.27±12.88^{Ca}	1542.21±8.02^{Aa}
OE/Soybean	53.02±0.73^{Db}	77.46±1.69^{Cb}	87.10±0.71^{Cb}	113.58±0.73^{Ab}
OE/Brazilian nut	52.05±0.62^{Db}	70.18±1.04^{Cb}	85.99±1.54^{Bb}	106.98±0.62^{Ab}
OE/Palm olein	51.08±0.43^{Db}	67.73±1.81^{Cb}	80.01±0.89^{Bb}	100.56±0.43^{Ab}

Results are presented on a dry weight basis following triplicate extractions and determinations (mean ± standard deviation). Means followed by the same capital letters in lines indicate no significant difference between maturation stage and geographic location, while the same lowercase letters in columns indicate no significant difference between extraction solvents according to the Tukey test ($p \leq 0.05$). OSE = organic solvent extracts, OE = oily extracts.

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Increased phenolic compound contents were observed, regardless of the extraction solvent, with advancing maturation in pepperes harvested from both geographic locations (Table 2). Several studies have reported this trend, as many of phenolic compounds are synthesized during the last fruit maturation stages [81,82]. The flavor and color of most mature fruits indicate increased phenolic compound contents at maturity, with polyphenols confering several sensory and flavor characteristics to mature fruits [83].

No differences ($p < 0.05$) concerning extracted phenolic compounds were noted among the investigated extraction oils employed for pepper compound extraction. The OE/soybean, however, was the most efficient, extracting the highest yield compared to OE/Brazilian nut and OE/Palm olein considering maturation stages and geographic locations (from 53.02 to 113.58 mg GAE $100g^{-1}$). In another study, 82.0 and 99.0 mg GAE $100g^{-1}$ were obtained, respectively, when employing hexane or ethyl acetate to extract total phenolic compounds from *Capsicum baccatum* L. [84]. Thus, vegetable oils seem to have the potential to be used for biocompound extraction, mainly for food purposes, compared to other lipophilic solvents. Herein, vegetable oils were efficient in extracting phenolic compounds when combined with UAE.

The investigated OSEs led to significant phenolic compound content extractions, higher than the values reported by Carvalho, de Andrade Mattietto, de Oliveira Rios, de Almeida Maciel, Moresco and de Souza Oliveira [66], ranging 215.73 to 1103.20 mg $100g^{-1}$ on a dry basis, in different pepper species. Other studies reported phenolic compound contents ranging from 284.6 to 570.7 mg GAE $100g^{-1}$ in mature peppers, and from 256.5 to 354.8 mg GAE $100g^{-1}$ in immatures ones [1].

In the present study, vegetable oils extracted between 4% and 7% of the total phenolic compounds when compared with organic solvents considering both pepper maturation stage and geographic location (Table 2). It is important to note that significant polarity differences exist between organic solvents and vegetable oils. Phenolic compounds exhibit a higher affinity with polar compounds, such as aqueous acetone, used herein for OSE extraction, compared to non-polar and hydrophobic ones, such as vegetable oils, explaining the poor performance of vegetable oils regarding phenolic compound extraction from the investigated pepper matrices. On the other hand, edible vegetable oils, although resulting in lower phenolic compound extraction efficiency compared to organic solvents, are environmentally friendly and do not not require an additional solvent evaporation step, due to their edible nature.

3.3. Capsaicin

Total capsaicin increased with advancing maturation in both geographic locations, higher in Igarapé-Açu peppers when employing both organic and oil solvents (Table 3). These findings corroborate previous data describing increasing capsaicin contents from the immature to mature stages in *Capsicum chinense* Jacq. *cv Habanero*, *Capsicum annuum* var. *acuminatum* L., and *Capsicum annuum* L. [49,51,85]. Higher capsaicin contents in Igarapé-Açu peppers (Table 5) may be associated to crop management characteristics, such as the applied irrigation regime, as deficient fruit hydration can result in increased capsaicinoid contents as a result of hydric stress, as reported in another study for habanero peppers subjected to hydric stress [86]. Furthermore, the period between September and December at Igarapé-Açu is characterized by rainfall deficiencies, which may comprise a triggering factor for water stress responses, resulting in higher capsaicin synthesis and accumulation in peppers and other fruits raised in Northeastern Pará, Brazil [53].

Table 3. Total capsaicin from peppers extracted employing organic solvents (OSE) or edible vegetable oils (OE) harvested in Tauá and Igarapé-Açu, Pará, Brazil.

Capsaicin ($mg.g^{-1}$)	**TAUÁ**		**IGARAPÉ-AÇU**	
	Immature	**Mature**	**Immature**	**Mature**
OSE/ethanol	2.73 ± 0.02^{Da}	3.38 ± 0.01^{Ca}	5.86 ± 0.03^{Ba}	6.13 ± 0.02^{Aa}
OE /Soybean	0.271 ± 0.03^{Db}	0.394 ± 0.06^{Cb}	0.492 ± 0.05^{Bb}	0.576 ± 0.03^{Ab}
OE /Brazil nut	0.269 ± 0.04^{Db}	0.354 ± 0.04^{Cb}	0.482 ± 0.01^{Bb}	0.500 ± 0.05^{Ab}
OE /Palm olein	0.252 ± 0.03^{Db}	0.273 ± 0.01^{Cc}	0.479 ± 0.08^{Bb}	0.496 ± 0.04^{Ab}

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Capsaicin contents from three distinct extractions are reported on a dry weight basis following triplicate determinations (mean ± standard deviation). Means followed by the same capital letters in lines indicate no significant difference between maturation stage and location, while the same lowercase letters in columns indicate no significant difference between samples concerning each investigated solvent according to the Tukey test ($p \leq 0.05$). OSE = organic solvent extracts, OE = oily extracts.

Vegetable oils used to prepare the OEs investigated herein did not differ from each other concerning capsaicin yields, although the use of OE/soybean tended to extract higher amounts in the immature and mature stages (0.271 to 0.576 $mg.g^{-1}$) compared to OE/Brazil nut (0.269±0.04 to 0.500±0.05 $mg.g^{-1}$) and OE/palm olein (0.252±0.03 to 0.496±0.04 $mg.g^{-1}$), suggesting greater extraction potential.

The capsaicin contents of OE concerning all pepper maturation stages and locations ranged from 0.252±0.03 to 0.576±0.03 $mg.g^{-1}$ corresponding to about 10% of the total capsaicin content from OSE extracted with ethanol, which varied from 2.73±0.02 to 6.13±0.02 $mg.g^{-1}$, probably due to the greater polarity and capsaicin affinity of ethanol compared to vegetable oils (Table 3). Although the capsaicin content in OE samples was lower than in the OSE samples, another study reported 0.442 $mg.g^{-1}$ capsaicin content in immature and 0.530 $mg.g^{-1}$ in mature hot peppers (C. *annuum L.*) following ethanolic extractions [3]. Other reports for *Capsicum chinese* indicated capsaicin values from 0.132 to 0.022 $mg.g^{-1}$, 0.022 to 0.045 $mg.g^{-1}$, 0.065 to 0.177 $mg.g^{-1}$ and 0,020 $mg.g^{-1}$ to 0,025 $mg.g^{-1}$ respectively, when employing hexane, ethanol, acetone, and methanol extraction, combined with different methods such as UAE, soxhlet extraction, and maceration, respectively [87]. This indicates that edible vegetable oils combined with UAE may indeed adequately substitute organic solvents when employed in capsaicin pepper recovery.

3.4. Total Carotenoids

Total carotenoid contents increased with increasing pepper maturation stage and differed by location (Table 4). The peppers from Igarapé-Açu contained the highest concentration of extracted carotenoids compared to those from Tauá. Increases in total carotenoid compound contents in ripening peppers have been reported in other assessments. Reduced chlorophyll content occurs during ripening, with increased carotenoid synthesis, responsible for fruit coloring [50,51,82,88]. Herein, organic solvent pepper extraction resulted in the highest total carotenoid yields, followed by OE/Soybean, OE/Brazil nut, and OE/palm olein.

Table 4. Total carotenoid contents from peppers extracted employing organic solvents (OSE) or edible vegetable oils (OE) harvested in Tauá and Igarapé-Açu, Pará, Brazil.

Total carotenoids (µg.g^{-1})	**TAUÁ**		**IGARAPÉ-AÇU**	
	Immature	**Mature**	**Immature**	**Mature**
OSE/acetone, petroleum ether	21.90±0.14^{Da}	43.87±0.16^{Ba}	32.25±0.30^{Ca}	55.23±0.12^{Aa}
OE/Soybean	18.86±0.17^{Db}	34.63±0.11^{Bb}	24.68±0.19^{Cb}	38.22±0.16^{Ab}
OE/Brazilian nut	13.77±0.12^{Dc}	23.81±0.17^{Bc}	19.58±0.22^{Cc}	30.91±0.06^{Ac}
OE/Palm olein	12.62±0.17^{Dd}	21.65±0.23^{Bd}	18.72±0.36^{Cd}	28.04±0.28^{Ad}

The results are presented on a dry weight basis following triplicate extractions and determinations (mean ± standard deviation). Means followed by the same capital letters in lines indicate no significant difference between maturation stages and location, while the same lowercase letters in columns indicate no significant difference between samples concerning each extraction solvent according to the Tukey test ($p \leq 0.05$). OSE = organic solvent extracts, OE = oily extracts.

The OE peppers presented high total carotenoid contents when compared with OSE peppers (Table 4), at least two-fold higher than those reported for *Capsicum* spp. In one study, values of 7.65 µg g^{-1} for immature and 9.55 µg g^{-1} were reported for mature *Capsicum annuum* peppers, expressed as lutein following ethanol extraction [1]. The higher extraction efficiency of vegetable oils is probably

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due to the lipophilic character of carotenoid compounds combined with UAE, which results in greater carotenoid diffusion from the pepper matrix to the lipid solvent.

3.5. Fatty Acid Vegetable Oil Profiles

The TPC, capsaicin, and total carotenoid compound analyses of cumari-do-Pará peppers (Tables 2–4) indicated that the most effective vegetable oil for bioactive compound extraction was soybean oil. This may be due to higher soybean oil unsaturated fatty acid content, *i.e.*, as oleic acid and linoleic acid (Table 5). Extraction yields were, in fact, directly affected by linoleic content (Table 5).

Table 5. Major fatty acids found in palm olein, Brazilian nut oil, and soybean oil.

Vegetable oil	Fatty acid (g.100g^{-1})		
	Palmitic acid (C16:0)	**Oleic acid (C18:1, ω-9)**	**Linoleic acid (C18:2, ω-6)**
Soybean	12.40 ± 0.05^{c}	23.30 ± 0.05^{c}	58.21 ± 0.07^{a}
Brazilian nut	15.25 ± 0.03^{b}	38.48 ± 0.02^{b}	35.87 ± 0.02^{b}
Palm olein	37.81 ± 0.10^{a}	44.62 ± 0.09^{a}	10.70 ± 0.12^{c}

Different letters in the same column indicate significant differences according to the Tukey test ($p \leq 0.05$).

In addition, oil viscosity also interferes with extraction yield, affecting diffusivity by lowering interactions between solution and solvent molecules [45,89]. Thus, high viscosity oils may result in lower extraction yields. This was verified herein, as different extraction efficiencies were noted due to different viscosities of the investigated oils at 40ºC, with palm olein presenting the highest viscosity (33.79 mPa.s), followed by Brazil nut oil (31.86 mPa.s) and, finallu, soybean oil (29.5 mPa.s) [90–92].

Solvent evaporation can lead to the loss of bioactive compounds due to several factors. The main one comprises the thermal sensitivity of many bioactive compounds. Furthermore, the high temperatures required for solvent evaporation can cause compound degradation. For example, Soxhlet extractions involve prolonged exposure to heat, which can be detrimental to thermolabile bioactive compounds.

3.5. Antioxidant Activity Determined by the ABTS and β-Carotene/Linoleic Acid System Assays

Pepper extract antioxidant activities evaluated by the ABTS and β-carotene/linoleic acid system assays followed the same trend as observed for the extracted bioactive compound contents, revealing differences according to pepper ripening stage, with a tendency towards higher antioxidant activity in mature peppers, corroborating previous reports (Table 6) [1,50]. Furthermore, several biochemical changes occur in response to biotic and abiotic factors throughout the fruit maturation process, culminating in the synthesis and increased content of new phytochemicals and, consequently greater antioxidant capacity.

Table 6. Antioxidant activity evaluated by the ABTS and β-carotene/linoleic acid system assays of OSE peppers and OE peppers in soybean, Brazilian nut, or palm olein harvested at Tauá and Igarapé-Açu, Para, Brazil.

ABTS	**TAUÁ**		**IGARAPÉ-AÇU**	
(μM trolox g^{-1})	**Immature**	**Mature**	**Immature**	**Mature**
OSE/methanol, acetone	89.58±1.73^{Ca}	95.89±0.77^{Ba}	94.27±1.30^{Ba}	123.61±2.61^{Aa}
OE/Soybean	16.08±0.26^{Db}	24.05±0.50^{Cb}	26.44±0.18^{Bb}	42.45±0.68^{Ab}
OE/Brazil nut	13.57±0.14^{Cb}	22.84±0.21^{Bb}	24.16±0.50^{Bb}	40.50±0.35^{Ab}
OE/Palm olein	10.56±0.11^{Cb}	21.17±0.44^{Bb}	22.92±0.30^{Bb}	39.12±0.45^{Ab}
β-carotene/linoleic acid	**TAUÁ**		**IGARAPÉ-AÇU**	
%AA (60 min)	**Immature**	**Mature**	**Immature**	**Mature**
OSE/methanol, acetone	46.88±0.79^{Ca}	52.06±0.80^{Ba}	50.86±0.51^{Ba}	59.66±0.92^{Aa}
OE/Soybean	9.24±0.19^{Db}	16.20±0.40^{Bb}	14.97±0.33^{Cb}	24.09±0.51^{Ab}

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OE/Brazil nut	7.90±0.32^{Dbc}	14.82±0.19^{Bbc}	12.25±0.54^{Cc}	22.18±0.83^{Ab}
OE/Palm olein	6.70±0.38^{Cc}	13.22±0.19^{Bc}	11.34±0.57^{Bc}	19.08±0.24^{Ac}

The results are presented on a dry weight basis following triplicate extractions and determinations (mean ± standard deviation). Means followed by the same capital letters in lines indicate no significant difference between the maturation stage and harvesting location, while the same lowercase letters in columns indicate no significant difference between samples in each extraction solvent according to the Tukey test ($p \leq 0.05$). %AA= Percentage of antioxidant activity, OSE = organic solvent extracts, OE = oily extracts.

As demonstrated by the Pearson correlation coefficient, bioactive compounds obtained by OSE were positively correlated with antioxidant activity determined by both the ABTS method ($r = 1.00$) and the β-carotene/linoleic acid system ($r = 0.97$) assays (Table S1). The same correlation was also established in other studies [3,51,66,82,93]. Concerning OE, several compounds, *i.e.*, capsaicin, phenolic compounds, and carotenoids, increased during the pepper maturipeningration process, positively influencing antioxidant activity inferred by the two methods ($r > 0.78$). This proves that antioxidant activity is not dependent on a single compound but on several, as well as their interactions [70,85].

3.6. A Brief Overview on the Cost-Benefits of Organic Solvent Extraction Compared to Vegetable Oils in Obtaining Bioactive Compounds Yields

The use of organic solvents requires a subsequent evaporation step that can result in the loss of bioactive compounds, as many of these are thermally labile. The high temperatures required for solvent evaporation can also degrade bioactive compounds. As stated previouslt, the Soxhlet extraction emthod, for example, involves prolonged exposure to heat, which can be harmful to heat-labile compounds [94,95].

Furthermore, the volatility of certain bioactive compounds must also be critically considered. During solvent evaporation, especially in processes such as rotary evaporation, volatile compounds can evaporate along with the solvent, resulting in a final dry extract and antioxidant activity losses, which can further reduce compound contents in organic extracts and the apparent higher OSE efficiency compared to vegetable oils [94–96]. In this sense, vegetable oils employed as solvents are ready for consumption, cost-effective, and sustainable.

4. Conclusion

The physicochemical characteristics, bioactive compound profiles, and antioxidant activities of mature and immature *Capsicum chinense* Jacq. were assessed herein. These profiles were influenced by harvesting location, where unfavorable local conditions, such as low soil hydration due to long dry periods, can culminate in low moisture levels and increase bioactive compound contents in response to abiotic stresses. Although this increase may be beneficial for human health, it indicates earlier pepper ripening due to poor farming conditions. Fruit ripening is directly associated to increased bioactive compound contents and antioxidant activities. However, fruits must be harvested under satisfactory farming conditions, such as adequate hydration levels and favorable soil and climate conditions. One study limitation is the fact that no systematic or instrumental controls concerning fruit ripening degree (unripe, medium ripe, ripe, and overripe) were applied, and fruits were categorized only into immature or mature peppers, and not harvested after the same number of days.

The use of vegetable oils for pepper bioactive compound extraction was deemed satisfactory. The combination of UAE and edible vegetable oils do not exhibit the same extraction efficiency as the organic solvents investigated herein, probably due to the low lipophilicity of certain bioactive compounds such as polyphenols, while carotenoids, that display high hydrophobicity, were more prone to diffusion from the pepper matrix to the employed vegetable oils. On the other hand, the bioactive contents in oily extracts were close to or higher than those found in organic solvent extracts in other assessments. The use of vegetable oils associated with UAE allows for the ready production of edible bioactive- and unsaturated fatty acid- rich extracts from pepper and oils. Furthermore, no

additional solvent evaporation step is required, unlike organic solvents, in which bioactive and/or flavor molecule losses and the potential generation of toxic subproducts that may negatively affect the environment and human health may occur.

On the other hand, edible oils, such as soybean oil, which exhibited the greatest extraction efficiency among those tested herein, are adequate, cheap, as they are widely consumed and commercialized in Brazil, and accelerate industrial production, as they result in consumable and environmentally friendly extracts beneficial to human health. Finally, in addition to employing green extraction method, a final product enriched with bioactive compounds can be developed and consumed daily. This not only adds flavor but also enhances the antioxidant status of everyday foods.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S1:**-** Pearson's correlation coefficient (r) between bioactive compounds and the antioxidant capacity of pepper extracts obtained with organic solvents and vegetable oils.

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Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics.

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