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Quantification of Bioactive Compounds of Pink Pepper (Schinus Terebinthifolius, Raddi)

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Abstract*— Pink pepper (Schinus terebinthifolius, Raddi) is a healthy food due to nutritional and medicinal qualities. Capsaicin is an energetic and analgesic substance which confers the spice taste of pink pepper. The pink pepper is rich in vitamins and promotes reduction of blood clots, acting as vasodilator; stimulates the production of endorphins in the brain, which is a hormone that produces a well-being feeling; has antioxidant, anticancer and anti-inflammatory activities; and reduces appetite, which is beneficial to obesity treatment. This study aimed to analyze the nutritional and physicochemical characteristics of pink pepper. Also, its antioxidant was determined by DPPH (1,1-diphenyl-2-picrilidrazil) assay. Nutritional content of pink pepper presented 27.5 µg/g of carotenoids, 17.3 mg/100g of vitamin C and 12.8 mg / g of capsaicinoids. Pink pepper showed higher antioxidant activity (83.33±2.07%) when compared to other peppers. The content of the phenolic compound vanillic acid (1076.1 mg/100g) was higher compared to other tropical fruits marketed globally. Thus, pink pepper can be used as a functional food due to its bioactive properties, besides to its regular uses in cooking.

KEYWORDS: **Antioxidant activity, bioactive compounds, physical-chemical characteristics, pink pepper.**

I. INTRODUCTION

The Schinus terebinthifolius Raddi is a plant native to Brazil, Argentina and Paraguay, popularly known as pink pepper or red mastic. When ripe, the red peel of the fruit turns into a sort of shell around the seed. The seed is unique; it measures approximately 0.3mm in diameter [7]. This small fruit stands out among the many existing spices and it is usually used to add flavor and refinement to dishes of the universal cuisine. Its smooth and spicy flavor as well as its appearance, allow its use in various culinary preparations and also can be used in the form of whole grain or milled. However, the pink pepper is especially suitable for making sauces that accompany white meats, poultry and fish, for not stifling its subtle taste. In direct survey to a trader, the Brasfoods Importação e Exportação, the export price of a kilogram of the product is around US$ 12 to 14 / kg, depending on the size of the harvest [2]. Its seed has several active compounds such as flavonoids, carotenoids and alkaloids. Phenolic compounds and carotenoids are economically important because they are used in the food industry as flavorings, colorants and antioxidants for food and beverages. The "burnt" characteristic of pink pepper, called pungency, is attributed to an alkaloid capsaicin, which is accumulated in the inner part of the fruit and this is what confers beneficial properties to health [21]. Given the growing trade of pink pepper, the need to identify its functional compounds and their concentrations has been observed. Through research it was found that the carotenoids, vitamin C and capsaicin have no quantification reports on the fruit in question. The knowledge of the content of these components present in the species under study may ultimately foster its trade and, especially, its consumption due to its functional properties. The interest in the study of antioxidants is increasing due mainly to the effect of free radicals continuously produced by the body [3]. These radicals react with proteins, DNA and other oxidizable substances, promoting irreversible damage, such as premature aging and degenerative diseases [17],[20].The main way of getting the body antioxidants is inserting some vitamins (eg: ascorbic acid.), phenolic compounds and carotenoids in the diet, then we support the need for consumption of potentially antioxidant foods [23]. So, by being an alternative in the food condiments industry, this paper aims to perform the physical-chemical characterization of in-natura pink pepper (Schinus terebinthifolius, Raddi), and to determine the antioxidant activity by DPPH essays (1.1 diphenyl-2-picrilidrazil) and quantify fractions of phenolic compounds, in order to inform consumers about the bioactive properties that these fruits can offer.

II. MATERIAL AND METHODS

A. Preparation of Samples

The samples of pink pepper were collected in the Lower São Francisco River (Sergipe) - region of semi-humid tropical climate, sandy soil and annual average temperature of 26°C. In the collection, it was decided to select approximately 100 g of ripe fruits with whole peel and without soiling from three different lots of fruits and mix them. From this sampling, analysis for physicochemical characterization of the fruits (moisture, minerals, aw, pH and acidity) were performed.

B. Physicochemical analysis

The determinations were performed in triplicate and in accordance with the Standards of the Adolfo Lutz Institute [16]. The determination of ascorbic acid was performed by the standard method, 43,065, of A.O.A.C and modified by Benassi Antunes [4] in which the extractor solvent meta phosphoric acid was replaced by oxalic acid. The levels of total carotenoids were determined by the method of Lichtenthaler [18] and expressed in µg/g. For the

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determination of capsaicin content 0.2 g of sample was weighed and mixed with 25 mL of methanol / water (9: 1, v / v), being left under continuous stirring for 30 minutes. After stirring, the solution was filtered with a quantitative filter paper and gauged with methanol in a 50 mL flask. The absorbance readings were performed at Micronal-B582 spectrophotometer at a wavelength of 248 and 296 nm. For quantification of capsaicin, standard curves were constructed using the Sigma pure capsaicin at concentrations of 0:01, 0:02, 0:03, 0:04 and 0:05 g / L, whose equations were: at 248 nm, y = 2.7537, R^2 = 0.9791; at 296 nm, y = 1.5774, R^2 = 0.9952. For the final calculation of the capsaicin content of pink pepper the equation y = 1.5774, R2 = 0.9952 was used for quantifying a better result. The average values was expressed in mg / g.

C. Obtaining of the extract of pink pepper for the extraction of phenolic compounds and antioxidant compounds.

The extracts of pink pepper were obtained according to the methodology proposed by Shivashankara et al. (2004) [28] apud Villa-Rodríguez et al. (2011) [32] with modifications. Approximately 6 g of pepper were homogenized with 20 mL of methanol / water (8: 2, v / v) using a glass rod. The mixture was sonicated for 30 minutes in ultrasound and centrifuged at 4200 rpm for 15 minutes at 4°C. The supernatant was collected and the precipitate was extracted again with 5 mL of methanol / water (8: 2, v / v) under the conditions previously described. The collected supernatants were mixed and filtered through Watman N° 1 filter paper. The filtrates were concentrated on a rotary evaporator, diluted in 3 ml of methanol and stored at -26°C until time of analyses.

D. Determination of antioxidant activity by DPPH essays (1.1-diphenyl-2-picrilidrazil).

The determination of antioxidant activity using the DPPH• free radical reduction was based on the methodology of Brand-Williams et al. (1995) [8] with some adaptations. A methanol solution containing 0.06 mM DPPH• was prepared. The spectrophotometer was calibrated with methanol (white) on a 515 nm absorbance. Then a mixture with 100 µL of pink pepper extract with 3.9 mL of the DPPH• solution was prepared. After mixing, the absorbance was read at an interval of 1 minute to achieve stabilization. Antioxidant activity was expressed as inhibition percentage according to the method of Sanchez-Moreno et al. (1998) [26] using the following equation: % Inhibition = $[(A_a - A_b) / A_a] \times 100$, in which A_a is the absorbance of the DPPH solution and A_b is the absorbance of DPPH solution reduced by the sample.

E. Phenolic compounds (Ultra-Fast Liquid Chromatography -UFLC)

The phenolic compounds present in the extracts were separated and quantified according to the methodology of Gomes et al. (2013) [26] in the column C18 Shimadzu XR-ODS (50 x 3.0 mm, 2,2µm) an ultra-fast liquid chromatograph LC-20AD (Shimadzu) equipped with degasser (DGU - 20A3), binary pump (LC -20 AD), an auto sampler (SIL-20A) and an UV / VIS – DAD diode array detector (SPD-M20A) with monitoring at between 190 and 800 nm were used. The elution was isocratic and the mobile phase had acetonitrile as solvent (8%) and potassium phosphate monobasic (KH_2PO_4) 5 mM, with pH 2.2 adjusted with phosphoric acid at a flow rate of 0.6 ml / per minute for 15 minutes. The volume of sample injected was 2 µl. All analyses were performed in triplicate and monitored at wavelengths of 254 nm and 310 nm, at 40 ± 2°C. For quantification of the phenolic compounds, external calibration curves were constructed with ten points, using the standard phenolic acids (vanillic acid, ρ-coumaric acid and ferulic acid), at concentrations from 0.0005 to 0.28 mg / mL of the extract under the same conditions, whose equations were: $y = 7*10^6x - 3614$, $R^2 = 0.999$; $y = 1*10^7X - 9601$, $R^2 = 0.999$; $y = 8 * 10^6 x - 6659$, $R^2 = 0.999$. Standards of gallic acid, protocatechuic acid, chlorogenic acid were also used to identify their presence by comparing spectra and retention times of the obtained data samples.

III. RESULTS AND DISCUSSION

A. Physicochemical characterization of pink pepper in natura.

The results of water activity and pH (Table I) indicate that the pink pepper is not a matrix prone to the development of microorganisms. A high content of capsaicin (12.8 mg / ml) was observed. A significant carotenoids (27.5 mg / g) and ascorbic acid (17.3 mg / 100g) (Table I) presence was noticed. The RDC Resolution nº. 269 of September 22, 2005 of the ANVISA recommends a daily intake of 45 mg of vitamin C for adults of both sexes, 55 mg for pregnant women, 70 mg for infants and 30-35 mg for children in the 1 to 10 age range, respectively.

Table I. Physico-chemical parameters of fresh pink pepper (Schinus terebinthifolius, Raddi).

Parameters physicochemical	percentage	deviation standard
Moisture (%)	14,70	±0,12
Ash (%)	3,30	± 0,23
Water activity (Aw)	0,50	± 0,02
Ph	5,10	± 0,26
Acidity (Ac. Citrus / 100g)	0,75	± 0,02
Vitamin C (mg / 100g)	17,30	± 0,50
Carotenoids (mg / g)	27,50	± 0,23
Capsaicin (mg / ml)	12,80	± 0,02

B. Antioxidant Activity

The Table II shows the comparison between the antioxidant activities of the extracts of pink pepper obtained from different solvents. It can be seen that the methanol

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extract had a greater inhibition of DPPH • followed by the alcoholic and aqueous extracts. The degree of polarity of the solvents explains inhibition sequence of the extracts, once pink pepper phenolic compounds present in the fractions are soluble in organic solvents. Currently, there are numerous varieties of pepper spread across the world and the concentrations of phenolic compounds that are directly linked to their antioxidant capacity vary according to the species, genus and external factors [29]. Table III shows the comparison of antioxidant activity by DPPH • of different pepper varieties. It was observed that the extract of "cheiro" peppers showed a higher potential to reduce DPPH• radical among varieties analyzed in Table III. The antioxidants act by interacting with free radicals before they can react with biological molecules, preventing chain reactions to occur [6].The pink pepper showed good antioxidant activity results, showing the intermediary percentage of inhibition compared to other peppers mentioned herein and superior to the pulp of *Citrus Sinensis* (orange), which showed 51.88% inhibition of DPPH • in the analyses performed by Bernardes et al. (2011) [5], revealing the pink pepper as a trend-setting functional food.

Table II. Antioxidant activity (% inhibition) by the DPPH • method of different extracts of pink pepper.

Extracts	Antioxidant activity (% Inhibition)	References
Methanolic	**83,33 [a] ± 2,07**	**This work**
Ethanolic	53,00 [b] ± 3,00	DEGÁSPARI *et al.*, 2004[12]
Aqueous	25,00 [c] ± 1,00	DEGÁSPARI *et al.*, 2004[12]

Means followed by the same letter do not differ statistically from each other. The Tukey test at 5% probability level was applied.

Table III. Antioxidant activity (% inhibition) by the DPPH • method of different varieties of pepper.

Varieties of peppers	Antioxidant activity (% Inhibition)	References
Pepper parsley	97,10[a] ± 0,97	MENICHINI *et al.*, 2009 [22]
green peppers	85,00[b] ± 2,00	CONFORTI *et al.*, 2007 [11]
pink pepper	**83,33[c] ± 2,07**	**This work**
red pepper	80,00[d] ± 2,00	CONFORTI *et al.*, 2007[11]
Black pepper	62,00[e] ± 1,00	SUHAJ *et al.*, 2006

As médias seguidas pela mesma letra não diferem estatisticamente entre si. Foi aplicado o Teste de Tukey ao nível de 5% de probabilidade.

C. Phenolic Compounds

Table IV shows the values obtained from the quantification of phenolic compounds of red mastica (*Schinus terebinthifolius*, Raddi), as reported by the UFLC chromatograms in Figures 1 and 2. Among the compounds tested, the most outstanding were: p-coumaric acid, vanillic acid, and ferulic acid. The results were compared to data from other studies whose analyses sought to quantify fractions of phenolic acids in various fruits, among them: Dragovic-Uzelac et al. (2007) [13] quantified 1.30 mg / 100g of p-coumaric acid and 0.80 mg / 100g ferulic acid in plums (*Prunus salicina*), and Podesta (2009) [24] quantified in the same species, in fruits of three different orchards, a quantity of vanillic acid ranged from 3.47 ± 2.04 to 30.69 ± 0.05 mg / 100g, proving that the content of phenolic compounds is directly influenced by conditions of biotic and abiotic stresses to which the fruits are submitted, as these compounds tend to be synthesized as a protection to such conditions, justifying the higher concentration of these in the peels, the outer part of the fruit, which is more exposed to possible damage (Simões et al, 2007.). Rampazzo et al. (2012) [25] quantified in strawberry (*Fragaria vesca*) 7.0 mg / 100 g of ferulic acid, in raspberry (*Rubus idaeus*) 0,20mg / 100g of p-coumaric acid, and none of these compounds were detected in samples of mulberry (*Morus nigra*); Broinizi et al. (2007) [7] quantified 14520.0 mg / 100g p-coumaric acid and 15.0 mg / 100 g of ferulic acid in cashew false fruits. Such bioactive compounds identified in the samples of pink pepper, usually, are not completely utilized by the human body, depending on factors such as chemical nature, size, structure, solubility, degree of glycosylation position, and the conjugation of these with other compounds. At last, all these items can influence directly in the bioavailability, absorption, distribution, metabolism and excretion in humans [1]. This research results it was found that the pink pepper contains a great concentration of phenolic compounds when compared to research that quantified these compounds in plum, strawberry, mulberry, raspberry, and cashew, giving to the pink pepper the condition of bioactive food due to its high antioxidant power.

Table IV. Phenolic Acids (mg / ml) in the hydrophilic fraction of a pink pepper UFLC.

	PHENOLIC ACIDS (mg / 100g)		
Samples	**Acid. p-coumaric (310nm)**	**Acid. vanillic (254nm)**	**Acid. ferulic (310nm)**
pink pepper	**449,0[a]**	**1076,1[a]**	**393,8[a]**
Mulberry	-	-*	-
Raspberry	0,20[b]	-*	-
Strawberry	-	-*	7,0 [b]
Cashew	14520,0[c]	-*	15,0[c]
Plum	1,3[d]	30,69[>e]	0,8 [d]

*: Not analyzed; Different letters indicate different authors. a: this work; b: Rampazzo et al. (2012) [25] c: Broinizi et al.

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(2007) [7]; d: Dragovic-Uzelac et al. (2007) [17]; > e: higher concentrations found by Podesta (2009) [24].

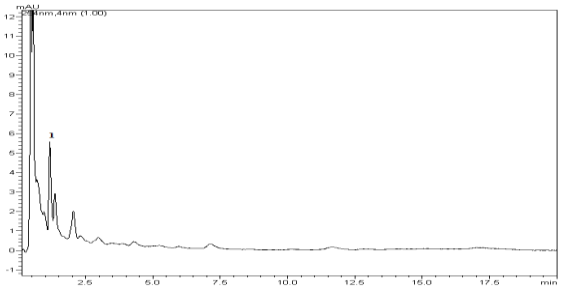

Fig 1. Profile of chromatograms of phenolic acids (254nm) of the methanol extract of pink pepper. Vanillic acid (1).

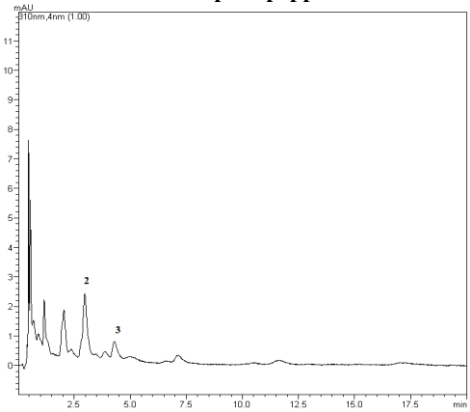

Fig 2. Profile of chromatograms of phenolic acids (310nm) of the methanol extract of pink pepper. P-coumaric acid (2) and ferulic acid (3).

IV. CONCLUSION

The commercial expansion of the pink pepper (*Schinus terebinthifolius*, Raddi) is promising due to its bioactive potential and versatility of dishes in which these fruits can be eaten, meeting the new trends in the food market.

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The preferred spelling of the word “acknowledgment” in American English is without an “e” after the “g.” Use the singular heading even if you have many acknowledgments. Avoid expressions such as “One of us (S.B.A.) would like to thank ....” Instead, write “F. A. Author thanks ....” Sponsor and financial support acknowledgments are placed in the unnumbered footnote on the first page.

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