ABSTRACT

Peppers of the genus Capsicum are rich in antioxidants and considered to be an excellent source of compounds, which can bring benefits for human health, such as vitamin C and phenolic compounds. These compounds are influenced by the ripening stage of the fruits. Thus, the aim of this study was to determine the ideal harvest point for ‘Murupi’ pepper fruits, targeting the consumer market, based on physical, chemical, and functional attributes. These attributes were evaluated during postharvest, being the fruits packed in low density polyethylene plastic film (LDPE) and submitted to cold storage (25±1°C and 95±3% U.R.) for 20 days. The treatments consisted of three different fruit maturation stages at harvest time (fruit peel color: green, partially red and totally red). The authors analyzed the vitamin C, total and soluble fibers, ORAC (Oxygen Radical Absorbance Capacity), DPPH (2,2-diphenyl-1-picrylhydrazyl), total and reducing sugars, total phenolics, carotenoids, capsaicin and anthocyanins. The fruits harvested in immature stage (green) showed the highest values of vitamin C, total phenolics and, capsaicin contents, and also the highest averages of fruit antioxidant activity. The use of 0.10 mm-thick LDPE packages showed to be effective in delaying the maturation of pepper fruits in relation to sensory attributes, especially in terms of total fiber contents, being also observed, in this case, better maintenance of vitamin C and capsaicin contents, phenolic compounds and the highest antioxidant activity.

Keywords: Capsicum chinense, antioxidants, postharvest, phenolics, carotenoids, anthocyanins.

RESUMO

Determinação do ponto de colheita de pimentas ‘Murupi’ acondicionadas em embalagens plásticas e armazenamento refrigerado

As pimentas do gênero Capsicum são ricas em antioxidantes sendo consideradas excelentes fontes de compostos que podem trazer benefícios à saúde humana, como vitamina C e compostos fenólicos que, por sua vez, são influenciados pelo estádio de maturação dos frutos. O objetivo deste trabalho foi determinar o ponto ideal de colheita em pimentas ‘Murupi’, direcionando seu mercado consumidor, com base nos atributos físicos, químicos e funcionais. Esses atributos foram avaliados durante a pós-colheita, onde os frutos foram embalados em filmes plásticos de polietileno de baixa densidade (PEBD) ou não, submetidos ao armazenamento refrigerado (25±1°C e 95±3% U.R.) por 20 dias. Os tratamentos utilizados consistiram nos três diferentes pontos de maturação dos frutos no momento da colheita (coloração da epiderme: verde, parcialmente vermelha e totalmente vermelha). Foram realizadas as análises de vitamina C, fibras totais e solúveis, ORAC (Oxygene Radical Absorbance Capacity), DPPH (2,2-difenil-1-picrilhidrazil), açúcares totais e reduutores, fenólicos totais, carotenoides, capsaicina e antocianinas. As pimentas colhidas em estádio imaturo (verde) apresentaram os maiores valores de vitamina C, fenólicos totais e teores de capsaicina, além das maiores médias em relação à atividade antioxidante dos frutos. Da mesma forma, o uso de embalagens de PEBD de 0.10 mm de espessura mostrou-se eficaz em retardar a maturação das pimentas em relação aos atributos sensoriais, em especial quanto ao teor de fibras totais, sendo também observado, neste caso, a melhor manutenção dos teores de vitamina C e capsaicina, de compostos fenólicos e a maior atividade antioxidante dos frutos.

Palavras-chave: Capsicum chinense, antioxidantes, pós-colheita, fenólicos, carotenoides, antocianinas.

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properties, and the antioxidant activity of capsaicinoids would play a role similar to that of tocopherol, which justifies its use as natural antioxidant (Yuan et al., 2015). The fruits have several compounds beneficial to human health even preventing cardiovascular diseases, cancer, weight loss, appetite inhibition, combating premature aging, among others (Yuan et al., 2015). However, the amount of these compounds may vary according to the genotype, degree of maturity, cultivation and processing conditions.

‘Murupi’ pepper is very well known as the most Brazilian, in comparison to the others, being domesticated by the Amazonian people. It is used in several forms, for fresh consumption or even to prepare jellies, condiments for typical dishes or candies. In the indigenous culture, ‘Murupi’ pepper is also widely used due to its therapeutic and prophylactic properties against “pano branco”, rheumatism, toothache, among others (Barbero et al., 2014).

In the food industry, ‘Murupi’ pepper can be used as natural dye, replacing the artificial dyes and, due to its antioxidant properties, can also suppress unpleasant flavor and smell of dishes (Costa et al., 2010). This fact is important, being the ideal harvest time determined by the final product desired by consumers. For the commercial exploitation of functional products, pepper should be harvested earlier, whereas to be commercially explored as food, like spice, it should be harvested later, to provide better development of sensory attributes. Thus, we highlight that maturation of ‘Murupi’ can be considered one of the most precise indicators to determine this specie’s harvest time properly.

According to Sothe et al. (2018), the appropriate control of storage conditions for vegetables is essential to keep the final quality. The cellular metabolism of these species continues even during post-harvest storage, influencing sensory aspects and nutritional composition of the products (Edusei & Ofosu-Anim, 2013). That means that cold storage, as well as the use of flexible plastic packages in post-harvest, can be considered techniques of great importance on a longer useful life of fruits and vegetables, slowing down the biochemical and physiological processes during ripening and senescence.

This study aimed to determine the ideal harvest time for ‘Murupi’ pepper fruits, submitted or not to plastic packages, better targeting its consumer market, based on the development of physical, chemical and functional attributes evaluated during the cold storage of the fruits during post-harvest for 20 days.

**MATERIAL AND METHODS**

The fruits were harvested in a private rural property, in Rural Zone of Boa Vista-RR, in the region of Projeto de Assentamento Nova Amazônia. After harvest, the fruits were taken to the Laboratory of Food Technology at Universidade Federal de Roraima, where the experiment was carried out.

The harvest was performed at three maturation stages, based on the peel color: green, partially red, totally red. After harvesting, the fruits were sanitized using 5% sodium hypochlorite solution, for 10 minutes, rinsed, air dried, and separated into lots according to maturation stages: E1= green fruits (T1= green peel, without packing, T2= green peel, packed); E2= partially red peel (T3= partially red peel, without packing; T4= partially red peel, packed) and E3= totally red peel (T5= totally red peel, without packing; T6= totally red peel, packed). In each container, the treatments were arranged in lots consisting of 250±15 g of pepper fruits.

Pepper fruits were packed with Low Density Polyethylene (LDPE) plastic film, 0.10 mm thick, (TPO2, oxygen permeability rate, 11,234 cm²/m²/day at 25°C and 1 atm; TPCO2, carbon dioxide permeability, 36,705 cm²/m²/day at 25°C and 1 atm; permeability area of 805 cm²). Then, the fruits of all treatments were kept in a refrigerating chamber at 25±1°C and 95±3% U.R. and stored for 20 days.

The experimental design was of randomized blocks, in a 6x5 factorial scheme, being 6 treatments (different maturation stages at harvesting and use or not of plastic packing at postharvest) and five-day analysis (conservation time), using 10 sample units in each one of the three replicates. The analyses were carried out each four days, during 20 days. The experiment was carried out in four harvests in a row (from March to October) and the results were presented using the average values of all the parameters evaluated during these four experimental cycles. The obtained data were evaluated using the variance analysis and, when significant difference was verified, we applied Tukey test at 5% probability, considering that the unfolding of effects was presented using regression analysis. The presented graphs demonstrate the sources of variation where no significance between the evaluated parameters was verified.

**Performed analyses**

**Vitamin C content** - determined by HPLC analysis (ascorbic acid), following AOAC methodology (2010), expressed in mg of ascorbic acid/100 g wet basis (b.u.).

**Total and soluble fibers** - determined by enzymatic-gravimetric method (AOAC, 2010), with results expressed in %.

**Total and reducing sugars** - determined according to Nelson’s methodology (1944), with results expressed in mg of glucose/100 g of pulp.

**Antioxidant capacity (DPPH)** - DPPH radical scavenging activity was measured as described by Brand-Williams et al. (1995) with some modification. The essay was performed in a 96-well reader (Synergy HT Multi-ModeMicroplate Reader, BioTek Industries), and the decrease in absorbance at 517 nm was monitored every 5 minutes until the reaction reached a plateau. The determinations were carried out adding 250 µL DPPH solution and 40 µL methanol to each well of the microplate for control, or the same volume for standard solutions (BHA, BHT, ascorbic acid, chlorogenic acid, quercetin and trolox) and the sample extracts. DPPH remained at the end of the reaction was determined and quantified as the DPPH radical scavenging activity using a standard Trolox curve. The antioxidant activity

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by the DPPH method was expressed in μmol Eq Trolox/100 g of sample.

**Antioxidant capacity (ORAC)** - based on the method of Ou et al. (2001) adapted by Huang et al. (2002), used in microplates, with fluorescein. The essay was carried out in a 96-well reader (Synergy HT Multi-ModeMicroplate Reader, BioTek Industries). A 25 μL volume of the sample was mixed with 150 μL of fluorescein (555 mM) and incubated for 15 minutes at 37°C in the microplate before automatic injection of 25 μL of the AAPH solution (155 mM). Fluorescence was monitored for 50 minutes by readings (λ excitation = 485 nm; λ emission = 520 nm). Trolox solutions were prepared for the calibration curve (8, 16, 24, 32 and 40 μM). All solutions were diluted in phosphate buffer (75 mM, pH 7.4). The samples were evaluated in three dilutions, considering the average as a final ORAC value, as recommended by Huang et al. (2002). The quantification of antioxidant activity was based on the calculation of the area under the fluorescence decay curve proposed by Prior et al. (2005). The results were expressed in μmol Eq Trolox/100 g of sample.

**Total phenolics** - determined by spectrophotometer method using the Folin-Ciocalteau reagent (Merck), following the methodology proposed by Wettasinghe & Shahid (1999), being the results expressed in mg of gallic acid/100 g of sample.

**Total carotenoids** - 0.2 g of the samples (pulp and peel) were weighed for extraction. The samples were crushed and homogenized and put in test tubes covered with aluminum foil, being 10 mL of hexane-acetone extractor solution (6:4) added to these samples. The extracts were shaken for 1 minute. After 9 minutes, the extracts were filtered through cotton, being read in triplicate immediately after that, using a spectrophotometer at 450 nm. We used β-carotene as a standard for making the calibration curve. The results were expressed in mg of β-carotene/100 g of sample, wet basis (b.u.), AOAC (2010).

**Total anthocyanins** - we used the methodology adapted by Francis (1982), correcting the sample masses to 0.2 g of the sample (pulp and peel) in a beaker for the extraction. Then, we added 6 mL ethanol extracting solution 95% + HCl 1.5 N (85:15). The sample extract was transferred to a 10 mL flask, also corrected, completing the volume with the extracting solution. Afterwards, this material was transferred to essay tubes and shaken in tubes wrapped in aluminum foil and stored in the refrigerator. After 24 hours, the sample extracts were filtered and immediately stored in the freezer. For the determination of the absorbance (λ = 520 nm), an UV-VIS spectrophotometer (model Genesys 10) was used. The absorbance was measured in triplicate, being 10 mL of hexane-acetone added to these samples. The extracts were filtered and immediately stored in the freezer. 1 minute. After 9 minutes, the extracts were filtered through cotton, being read in triplicate immediately after that, using a spectrophotometer at 450 nm. We used β-carotene as a standard for making the calibration curve. The results were expressed in mg of β-carotene/100 g of sample, wet basis (b.u.), AOAC (2010).

**Capsaicin** - the fruits were cut and macerated with grail and pistil. Then, 1 g of macerated and homogenized fruits was transferred to a beaker, adding 20 mL methanol. The solution was submitted to an ultrasound bath for 20 minutes. After, the extracted was filtered, using qualitative analytical paper. We filled up the filtrate with methanol up to the 25 mL mark in the volumetric flask. A 1 mL aliquot of this extract was filtered through a membrane filter, storing the obtained extract in a vial for further analysis. The quantification of capsaicin content in the fruits of each accession was performed by HPLC, Shimadzu apparatus, system category LC VP HPLC, a pump (LC-6AD) and a UV-Vis detector (SPD-10AV VP). The column used was Thermo Gold® C18 (octadecyl), 100 mm long. 3.0 mm internal diameter and 1.9 μm particle size. The initial mobile phase was composed of 30% ultra-pure water (A) and 70% methanol:acetone/nitile (95:5) mixture (B) at a flow rate of 0.4 mL/min. The UV detector was set at 280 nm. Quantification was performed by integrating the peak area obtained with the injection of 3 μL of the sample extract. The data obtained from the analysis were interpolated to the calibration curve constructed with seven points of the standard substance (Ha et al., 2010). The results were expressed in mg/100 mL of pulp and peel. Before the beginning of the analyses, we obtained the calibration curve in order to compare with the commercial capsaicin standard (Cayman Chemical, Michigan-USA), with purity over 95%.

**RESULTS AND DISCUSSION**

In relation to vitamin C contents in pepper fruits, we verified significant interactions between maturity stage and packing. ‘Murupi’ pepper fruits showed gradual loss of vitamin C throughout the experiment (Figure 1A). We also observed that the decrease in these contents was constant and that the content decreased since the first cold storage days. For Wathyuni et al. (2011), several factors can be related to vitamin C decrease, besides the post-harvest storage period, maturation time can also be considered an important trait. These factors can explain the results found in this study. Thus, at the end of the experiment, the fruits which were harvested at green stage showed higher averages in relation to the others, 14.34 mg/100 g, followed by the ones harvested at partially red stage, 12.87 mg/100 g and the ones harvested at fully red stage, 9.67 mg/100 g. All of them were packed.

In relation to total phenolic compounds (Figure 1B), we observed triple interaction among variation sources: maturity stages, use or not of packings and post-harvest storage time. Using the results obtained in this experiment, at the end of the 20th day of cold storage, we verified that higher averages were noticed in fruits harvested still green and packed in plastic film, average 5191.03 mg/100 g. Using the same packing, for fruits harvested partially red, the average found was 3825.53 mg/100 g. For the fruits harvested at fully red stage and packed, the average result was 2986.98 mg/100 g. When the fruits are not submitted to packing, the green, partially red and fully red fruits, respectively, showed average values of 4929.14 mg/100 g, 3751.135 mg/100 g and 2928.895 mg/100 g for phenolic compounds. Marin et al. (2014) reported that a decrease in phenolic content was noticed with the advance of the maturity stage (from green to red), just like the observed in this study.
For carotenoid contents in fruits (Figure 1C), we observed significant interactions between maturity stage and post-harvest conservation of the fruits, not being significant in relation to packing, though. Thus, we noticed that the fruits harvested at fully red stage showed highest values for carotenoid contents, 37.87 mg/100 g, at the end of the experiment. However, the fruits which were harvested at partially red stage showed average values of 26.795 mg/100 g and, when the fruits were harvested green, the average values were 16.32 mg/100 g. The authors also observed that fruits harvested at fully red and partially red stages showed increasing values, up to 12th day of evaluation, decreasing right after this evaluation. Nevertheless, we obtained different results for green fruits, which showed increasing values throughout the experiment. Wahyuni et al. (2011) assumed that the carotenoid content increases as fruits mature. Menichini et al. (2009), studying the influence of maturation in total fruit carotenoid content of Capsicum chinense cv. Habareno, observed values of 62.7 mg/100 g and 362 mg/100 g of fresh mass, respectively, for immature and mature fruits. If the increase in carotenoid content and the change in fruit color was due to the advance of the fruit maturation stage, a decrease observed after the 12th day, probably, is due to the beginning of the senescence of the fruits harvested at partial or fully red stages.

In relation to quantification of anthocyanin contents (Figure 1D), we observed significant interactions between maturity stage and post-harvest storage time of the fruits. We have not verified any significant influence in relation to package use. Thus, higher averages observed at the end of the experiment were related to fruits harvested at fully red stage, 19.43 mg/100 g of anthocyanins. These fruits showed, during this same period, average values of 11.90 mg/100 g of anthocyanins, being the lowest concentrations of anthocyanins found in the green fruits, with average values of 4.93 mg/100 g of anthocyanins. We
observed a decrease in anthocyanin contents for all fruits evaluated in this study and throughout all the evaluations. The anthocyanins probably present this behavior due to the fact these are unstable pigments which can be degraded during storage. Thus, the degradation process of anthocyanins leads to loss of color and influences the formation of dark pigments, which may even compromise the quality of the product (Wang & Xu, 2007).

After quantifying capsaicin (Figure 1E), we observed triple interaction among all variation sources, considering that green fruits, packed in LDPE films, showed the highest averages at the end of the experiment, with values of 95.21 mg/100 g. The fruits harvested at partially red stage and packed, showed average values of 63.60 mg/100 g, whereas the fruits harvested at fully red stage, also packed, showed average value of 60.16 mg/100 g capsaicin. The authors observed an increase in capsaicin content in fruits up to the 12th day, being observed right after this day, a decrease in these contents up to the end of the research in the fruits harvested at partial or fully red stages, pointing out, as mentioned before, advanced maturity stage of the fruits. However, the same was not observed for packed fruits which were harvested at green stage, which only presented a decrease after the 16th day of analysis. These results were also observed by Almeida et al. (2015), studying detection of capsaicin in extracts of immature and mature fruits of Capsicum baccatum. These results confirm the influence of maturity stage in degradation of the components responsible for the pungency of pepper. The capsaicin contents in fruits not submitted to packing at the end of the experiment were 88.91, 63.27 and 59.46 mg/100 g, respectively, for fruits harvested at green, partial and fully red stages. Finally, considering capsaicin as a relevant attribute to determine the commercial quality of pepper fruits, in addition to being the main capsaicinoid responsible for the pungency of peppers (Guillen et al., 2018), we strongly recommend that cosmetics industries use pepper at immature stage (green peel), precisely because of the amount of capsaicin presented at that time. Nevertheless, fresh consumption is recommended when ‘Murupi’ pepper is harvested at more advanced maturity stage (partial or fully red stage), due to the sensory traits by the time part of the compounds responsible for the pungency have already been degraded during the maturation process.

For quantifying the total fibers (Figure 2A), no significance was observed in the interaction of variation sources, being necessary individual discussions for each of the sources tested here. Thus, we observed that fruit maturity stage at harvest time resulted in a lower reduction of total fiber contents throughout the experiment; the fruits harvested at green stage showed higher values, followed by the ones harvested at full and partially red stages, respectively. We also observed that the packed fruits showed lower losses concerning total fiber contents when compared with the unpacked ones, regardless of the fruit maturity stage at harvest. And finally, noticing the advance in fruit maturity stage, we observed a decrease in these compounds over the experimental period. This result can be considered as being part of the fiber depolymerization metabolism itself, resulting in a decrease in total fiber contents, due to the respiratory metabolism during maturation and, consequently decrease in soluble fiber content (Figure 2B).

In relation to quantifying the soluble fiber content (Figure 2B), the authors observed interaction between maturity stages and post-harvest storage life, being detected no significance in relation to packing use. The fruits harvested at fully red stage showed higher soluble fiber contents, followed by fruits harvested at partially red and green stages, with average values of 2.29, 1.44 and 1% of soluble fibers, respectively. This behavior is related to an increase of compound solubilization and consequent fiber depolymerization during maturation, making soluble fiber content higher in fruits at more advanced maturity stage. Regarding time, we observed a decrease in soluble fiber contents, for all fruits evaluated in this study, throughout all the evaluations.

For total sugars (Figure 2C), we observed an interaction between maturity stage and post-harvest storage period. In this sense, we could observe over the experimental time a decrease in total sugar concentrations in fruits harvested at all maturity stages. At the end of the 20-day analysis, we verified that fruits harvested at fully red stage showed average values of 3.55 mg/100 g of glucose, followed by fruits harvested at partially red stage, 3.33 mg/100 g of glucose, and the green ones, with average values of 2.95 mg/100 g of glucose.

To quantify reducing sugars in fruits (Figure 2D), no significant interaction between variation sources was observed, being necessary individual discussions for each of the sources tested. First, we highlight that no significant difference among the fruits in relation to packing was verified, regardless of maturity stage and/or post-harvest storage time. In relation to storage time of fruits, we noticed an increasing in reducing sugar contents up to the end of the research, regardless of the other variation sources. In relation to fruit maturity stage at harvest time, the fruits harvested at fully red stage showed the highest values, whereas the ones harvested at partially red and green stages showed the lowest values for soluble sugars.

We may infer that converting more complex sugars to simple sugars is one of the most notable changes during fruit maturation, starch, for example, is mainly transformed into reducing sugars, glucose, fructose and sucrose. We can conclude that when the non-reducing sugar goes through the depolymerization process, an increase in reducing sugar concentration is potentialized, being this process clearly influenced by fruit maturation, as verified in this study.

We noticed triple interaction among all variation sources in this experiment in relation to fruit antioxidant activity, measured by both used methods (DPPH, Figure 3A and ORAC, Figure 3B).

In relation to antioxidant capacity measurements obtained by DPPH...
method (Figure 3A), the authors observed that the fruits harvested at green stage, and submitted to plastic packing, showed, at the end of the research, the highest values, 569.78 umolEq Trolox/100 g. Fruits harvested at partial or fully red stages, showed average values of 328.70 and 276.64 umolEq Trolox/100 g, respectively. Pepper fruits harvested at green stage, not-packed, showed the highest antioxidant activity, with averages of 442.70 umolEq Trolox/100 g at the end of the research, followed by fruits harvested at partial and fully red stages, 271.20 and 228.25 umolEq Trolox/100 g of sample, respectively. We highlight that, according to Costa et al. (2010), the antioxidant potential of pepper fruits would not only be related to the concentration of total phenolics, but also to the capsaicinoid content in pepper, which can donate electrons to the DPPH radical, stabilizing it. However, comparing the studies carried out on fruits grown in the Amazonian region (Carvalho et al., 2014), we can consider that the results in this study are superior.

The ORAC method (Figure 3B) showed fruits harvested at green stage and packed in plastic film at the end of the 20 days of cold storage, showed average values of 555.27 umolEq Trolox/100 g; the ones harvested at partial and fully red stages showed average values of 316.09 and 258.26 umolEq Trolox/100g of sample, respectively. We highlight that both were submitted to the use of packing. When not packed, the green fruits showed higher antioxidant activity in relation to the other maturity stages, with average of 431.18 umolEq Trolox/100 g of sample. The lowest averages, in the same period, were presented by fruits harvested at partial and fully red stages, 260.80 and 213.09 umolEq Trolox/100 g, respectively. These data are in accordance with Neves et al. (2015) who evaluated the quality of murici pepper fruits harvested at different maturity stages, and could also observe that fruits in earlier maturity stage showed higher antioxidant potential compared to more mature fruits. According to Sousa et al. (2011) and Cazarin et al. (2014), the increase in the antioxidant potential of the fruits is mainly related to the presence of high concentrations of phenolic compounds. Thus, according to the results presented in Figure 1B (analysis of quantity of total phenolics), we can infer that the results for antioxidant activity in this study, in both DPPH and ORAC methods, can be related to phenolic compound contents. The same results were found by Wang & Xu (2007): the fruits decreased their antioxidant capacity during maturation, due to a decrease in some metabolite concentration, such as the phenolic compounds. Nevertheless, we point out that, according to Jacobo-Vellazquez & Cisneros-Zevallos (2009), probably, the phenolic compound contents are not the only traits related to the high antioxidant capacity of fruits, being necessary to evaluate other biochemical components.

Considering this fact, we can determine that the ideal harvest point of ‘Murupi’ peppers evaluated in this study is directly related to the final product preferred by the consumers. Thus, immature fruits, the green ones, which presented the highest capsaicin concentrations, are recommended for those who appreciate fruits with greater pungency. Green fruits are also recommended for agro-industrial processing in the cosmetics industry, since higher values of vitamin C, total phenolics and antioxidant activity, and higher capsaicin content are verified, which are extremely valuable for manufacturing anti-aging products.

Fruits harvested at partial or fully red stages are recommended for fresh consumption, for consumers who are less tolerant to spicy taste, typical characteristic of peppers, since they present less pungency. For these fruits, we also observed higher contents of anthocyanins and carotenoids.

The use of 0.10 mm thick LDPE packing, regardless of fruit maturity stage at the time of harvest, proved to be effective in delaying maturation of ‘Murupi’ pepper fruits. In this sense, in relation to sensory attributes, especially for total fiber content, the authors also observed a better maintenance of vitamin C contents, capsaicin, phenolic compounds and higher antioxidant activity of fruits of the genus Capsicum.

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