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Genetic variability of bioactive compounds in *Capsicum chinense*

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Abstract

Capsicum is one of the most important genera of the *Solanaceae* family. Among the domesticated species, *C. chinense* is considered the most Brazilian pepper, besides being known for having high levels of phytochemical compounds and, consequently, high antioxidant capacity. This work was intended to perform the chemical characterization of bioactive compounds present in *C. chinense* accessions from the *Capsicum* Genebank of Embrapa Temperate Agriculture. Fruits from 19 accessions were evaluated regarding the concentration of total phenolic compounds, total carotenoids, total anthocyanins and antioxidant activity. It was possible to observe a high genetic variability among the evaluated accessions. The highest concentration of phenolic compounds was found in accession number P391; the highest concentrations of total carotenoids were found in accession numbers P240; P348 and P399; the highest concentration of total anthocyanins was in accession number P240; and the highest antioxidant activity was found in accession numbers P386, P391, P366, P350, P346 and P399. There is a correlation between antioxidant activity and the content of phenolic compounds, as well as between antioxidant activity and carotenoids in *Capsicum chinense*. The genetic variability noticed can be exploited in breeding programs for the selection of accessions with desirable characters.

Keywords: genetic resources; germplasm; functional foods.

Practical Application: Characterization of bioactive compounds in Brazilian landraces of *Capsicum chinense*.

1 Introduction

Capsicum is a genus of the *Solanaceae* family, considered as one of the most economically important (Antonio et al., 2018). Five species are domesticated: *Capsicum annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens* (Pickersgill, 1997). Among the domesticated species, *C. chinense* is considered the most Brazilian peppers. This species is largely cultivated in the Amazon region, which is a probable domestication center. Presenting a great genetic variability for size, shape, color and pungency of the fruits, *C. chinense* is broadly used in Brazilian cuisine, especially the types known as *pimenta-de-cheiro*, *pimenta-de-bode*, *cumari-do-Pará*, *murupi*, *habanero* and *pimenta biquinho* (Alvares Bianchi et al., 2020).

The main substance responsible for the pungency in peppers is capsaicin. Besides capsaicin, other compounds can be quantified in cultivated pepper species, such as phenolic compounds, carotenoids, anthocyanins and antioxidant activity (Thuphairo et al., 2019; Hernández-Peréz et al., 2020).

Bioactive compounds from pepper species are known for their pharmacological properties such as analgesic, anti-obesity, cardioprotective, neurological and dietetic activity (Mendes & Gonçalves 2020). Several studies, both *in vitro* and *in vivo*, have linked *C. chinense* to protective effects such as antioxidant and anticancer activity (Sharma et al., 2017; Sarpras et al., 2018; Sherova et al., 2019), useful in reducing or preventing chronic diseases (Antonious 2018; Salehi et al., 2018).

These functional compounds can be used as additives in multifunctional foods, with potential in the food industry (Lu et al., 2017). Is increasing the interest in using natural antioxidants, as those of *Capsicum* for example, since the synthetic antioxidants present carcinogenic potential and other damages to health associated with them (Fratianni et al., 2020; Franco et al., 2012; Radha Krishnan et al., 2014; Cabral et al., 2021). Capsaicin has been used as a flavoring and preservative agent in food formulations and as an active compound in packaging film and functional foods, as its antioxidant and antimicrobial activity (Rezazadeh et al., 2021). For this purpose, the development of new cultivars with characteristics that meet the needs of growers and consumers is necessary. In order to achieve this goal, plant breeders need genetic resources and must have access to the widest genetic diversity available. In addition, the characterization of these genetic resources is essential information for conservation and use in breeding programs (Alvares Bianchi et al., 2020).

Incorporating nutrient-rich pepper genotypes that contain high levels of bioactive compounds into human diets can help combat nutrient deficiencies by meeting daily needs (Antonio et al., 2018). Accordingly, the objective of this work was to perform

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the chemical characterization of bioactive compounds present in *C. chinense* accessions from the *Capsicum* Genebank of Embrapa Temperate Agriculture (one of the 43 Decentralized Units of Brazilian Agricultural Research Corporation).

2 Material and methods

2.1 Germplasm

Ripe fruits of 19 randomly chosen *C. chinense* accessions (Table 1) from the *Capsicum* Genebank of Embrapa Temperate Agriculture (Pelotas, Rio Grande do Sul state, Brazil) were evaluated. Sowing was performed in October 2018, in expanded polystyrene trays filled with sterilized commercial substrate, which were maintained in a greenhouse located in the facilities of Embrapa Temperate Agriculture. When they reached around 10 cm of height, the seedlings were transplanted into 7 L capacity pots containing substrate. Pots with the plants were maintained in the greenhouse, irrigated manually. Experimental design was entirely randomized, considering each pot an experimental unit (one accession) with five repetitions (five pots). Ripe fruits from each plant were stored at -18 °C in a freezer in the Food Science and Technology Laboratory of Embrapa Temperate Agriculture. In order to perform the analyses, the seeds were discarded and opposite longitudinal portions of the fruits were manually prepared. The 19 accessions were analyzed for total phenolic content, total carotenoids, total anthocyanins and antioxidant potential.

Table 1. *Capsicum chinense* accessions from the *Capsicum* Genebank of Embrapa Temperate Agriculture, characterized for bioactive compounds.

Accessions	Common name	Origin	Color of the ripe fruit
P195	Pimenta-de-cheiro amarela	Belém, PA	Yellow
P201	Pimenta	Rio de Janeiro, RJ	Orange
P240	Pimenta	Porto Seguro, BA	Red
P341	Pimenta	Tubarão, SC	Orange
P346	Pimenta	Londrina, PR	Red
P348	Pimenta	Londrina, PR	Red
P350	Pimenta	Londrina, PR	Orange
P356	Pimenta habanero	Pelotas, RS	Orange
P366	Pimenta	Olinda – PE	Yellow
P367	Pimenta	Olinda – PE	Red
P381	Pimenta	Teresina, PI	Red
P382	Pimenta	Teresina, PI	Red
P385	Pimenta	Teresina, PI	Red
P386	Pimenta	Teresina, PI	Orange
P387	Pimenta	Teresina, PI	Red
P391	Pimenta	Pelotas, RS	Orange
P399	Pimenta	Florianópolis, SC	Red
P407	Pimenta	Lagoa dos Três Cantos, RS	Yellow
P420	Pimenta cheirosa do Pará	Pelotas, RS	Red

Information derived from *Capsicum* Genebank passport data.

2.2 Quantification of phenolic compounds

The methodology for determination of total phenolic compounds was adapted from Swain & Hillis (1959). 250 µL of the sample was pipetted into a test tube, soon after then were added 4 mL of ultrapure water and 250 µL of Folin-Ciocalteu reagent (0.25 N). The tubes were shaken for 1 minute and left for 3 minutes to react. Later, 500 µL of sodium carbonate (1 N) was added. Tubes were shaken for 1 minute again lefting for 2 hours to react. Absorbance readings were taken in a spectrophotometer at a wavelength of 725 nm, after it was zeroed with the methanol, using glass cuvettes. When the absorbance was higher than 0.6, samples were diluted and readings repeated. The concentration of total phenolics was estimated from a standard curve developed for chlorogenic acid. Results were expressed as mg chlorogenic acid equivalent/100 g sample.

2.3 Quantification of carotenoids

Carotenoids were quantified by the methodology adapted from Talcott & Howard (1999), with some modifications. In the absence of direct light, two grams of fruit samples were homogenized in Ultra-Turrax® device with 20 mL of acetone/ethanol solution (1:1) containing 200 mg/L of BHT (butylhydroxytoluene). After filtration, 50 mL of hexane was added to the sample. After stirring and visual separation of the phases in the test tube, due to the addition of hexane, only the supernatant was removed for reading, and 25 mL of ultrapure water was added. Absorbance readings were taken in a spectrophotometer at a wavelength of 470 nm, after it was zeroed with hexane solvent blank, using glass cuvettes. Concentration of carotenoids was estimated from a standard curve developed for β-carotene and results were expressed as mg of β-carotene equivalent/100 g sample.

2.4 Quantification of anthocyanins

In order to perform analysis of total anthocyanins, we used the methodology used was proposed by Fuleki & Francis (1968), with adaptations. A 5 g sample and 15 mL of acidified ethanol (85:15 ratio) were added in a Falcon® tube and the sample was homogenized in an in Ultra-Turrax® device at maximum speed until reaching uniform consistency. The extract was centrifuged for 20 minutes at 4000 RPM at 0 °C. After partitioning with hexane to separate and remove carotenoids, the lower part was removed for anthocyanins reading. The extract was stored in an environment protected from light incidence for 30 minutes. After 30 minutes, the spectrophotometer was zeroed with acidified ethanol. The absorbance was read in a quartz cuvette at 535nm. When the absorbance was higher than 0.7, the samples were diluted and readings repeated. The results were expressed in mg cyanidin 3-glucoside/100 g of sample. A standard curve for cyanidin-3-glucoside was generated.

2.5 Quantification of antioxidant activity

The antioxidant potential was determined using the 2,2-diphenyl-1-picrylhydrazyl radical-scavenging (DPPH) method (Brand-Williams et al., 1995). One hundred microliters of the methanolic extract of the fruits (the same extract used in the determination of phenolic compounds) were added to 3.9 mL of DPPH solution in methanol (100 mM). The solution was then shaken and maintained in a closed flask in the dark. The absorbance was measured at 517 nm after 24 h of reaction. The antioxidant potential was expressed as micrograms of Trolox® equivalent/mg $^{-1}$ in fresh weight (µg Trolox®/mg $^{-1}$ in fresh weight).

2.6 Statistical analysis

The data were submitted to analysis of variance (ANOVA $p \leq 0.05$). When significant, the means were compared by Scott-Knott test, in addition, Pearson correlation was performed, all usingthe Genes Computational Package (Cruz, 2016). Histograms were generated using Microsoft Office Excel®.

3 Results and discussion

Variation was observed in the results for total concentrations of phenolic compounds (Figure 1), carotenoids (Figure 2), anthocyanins (Figure 3), and antioxidant activity (Figure 4), in the 19 evaluated *C. chinense* accessions, which highlights the presence of genetic variability among accessions from the *Capsicum* Genebank.

Concentration of phenolic compounds ranged from 113.115 mg of chlorogenic acid equivalent/100g fresh mass (P420) to 233.58 mg of chlorogenic acid equivalent/100 g fresh mass, in accession P391, which stood out with the highest quantity of phenolic compounds (Figure 1). Phenolic compounds entail health benefits due to their ability to scavenge *in vitro* and *in vivo* free radicals in biological systems (Hernández-Pérez et al. 2020). Acunha et al. (2017), when evaluating 51 *C. annuum*, *C. baccatum*, *C. chinense* and *C. frutescens* accessions from the *Capsicum* Genebank of Embrapa Temperate Agriculture for total phenolic content, total carotenoid content and antioxidant potential, found a range for phenolic compounds from 54.4 to 243.47 mg of GAE 100 g^{-1}. The genotypes with the highest and lowest phenolic compound contents among them were *C. baccatum* accessions, and the overall average phenolic compound content among all accessions tested by Acunha et al. (2017) was 122.08 mg GAE 100 g^{-1}. Regarding the indices of phenolic compounds in *C. chinense*, Acunha et al.

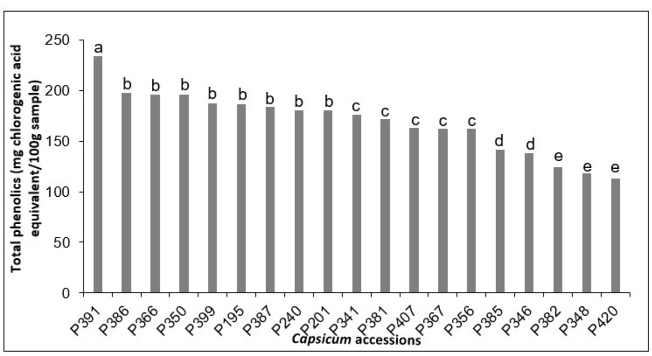

Figure 1. Total phenolic content expressed as mg chlorogenic acid/100g fresh weight of 19 *Capsicum chinense* accessions from the *Capsicum* Genebank of Embrapa Temperate Agriculture. Different lowercase letters in the histogram bars indicate statistical difference by Scott-Knott test ($P < 0.05$).

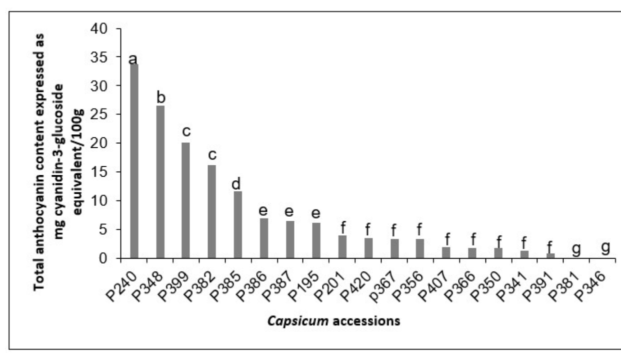

Figure 3. Total anthocyanin content expressed as mg cyanidin-3-glucoside equivalent/100g fresh weight of 19 *Capsicum chinense* accessions from the *Capsicum* Genebank of Embrapa Temperate Agriculture. Different lowercase letters in the histogram bars indicate statistical difference by Scott-Knott test ($P < 0.05$).

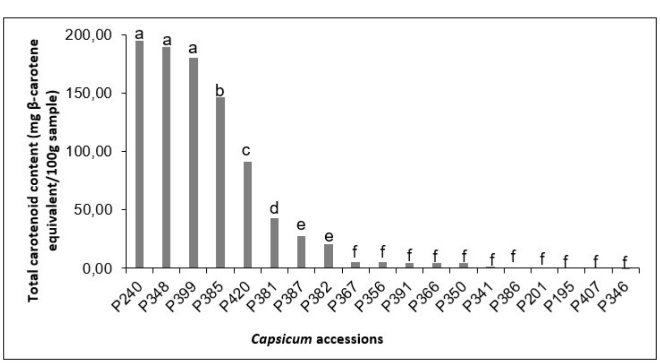

Figure 2. Total carotenoid content expressed in mg β-carotene equivalent/100g fresh weight of 19 *Capsicum chinense* accessions belonging to the *Capsicum* Genebank of Embrapa Temperate Agriculture. Different lowercase letters in the histogram bars indicate statistical difference by Scott-Knott test ($P < 0.05$).

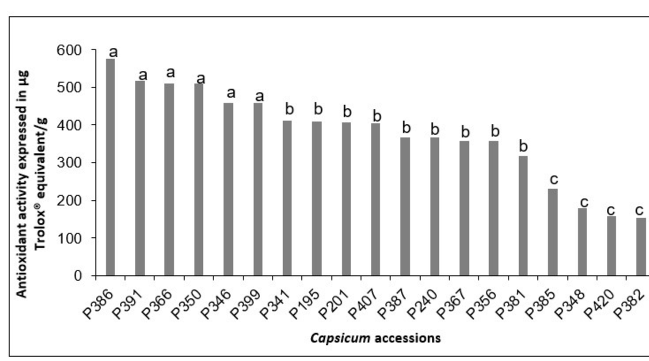

Figure 4. Antioxidant activity expressed in µg Trolox® equivalent/g in fresh weight of 19 *Capsicum chinense* accessions belonging to the Capsicum Genebank of Embrapa Temperate Agriculture. Different lower case letters in the histogram bars indicate statistical difference by Scott-Knott test ($P < 0.05$).

(2017) found 144.39 ± 30.61 mg GAE 100 g^{-1}, higher than the average found among all species studied by them; however, it is still numerically lower than that found in the current work. It is important to consider that there is a wide genetic variability for many characters in the domesticated species of *Capsicum* (Neitzke et al., 2015). Such variability is shown by the data obtained in this work in comparison with those cited above. The knowledge about the variability of the population allows the selection of superior genotypes and, consequently, the increase of allele frequency favorable for selection (Gonçalves et al., 2008).

Carotenoids are bioactive compounds broadly found in plants. They are responsible for the coloring of *Capsicum* spp. (Sá Mendes & Branco de Andrade Gonçalves, 2020). These colors can range in different shades of orange, yellow and red. These compounds play an important role in nutrition and health, because they have antioxidant and anticarcinogenic activities and act as precursors of other essential compounds, such as vitamin A and retinoic acid (Antonio et al., 2018). In this work, the highest values (180.33 mg of β-carotene 100 g^{-1}; 188.97 mg of β-carotene 100 g^{-1} and 195 mg of β-carotene 100 g^{-1}) were found, respectively, in accessions P399, P348 and P240 (Figure 2), all of them producing red fruits. According to Meléndez-Martínez et al. (2004), capsanthin and capsorubin are carotenoids found almost exclusively in fruits of the *Capsicum* genus, being the main pigments that give color to red peppers. Fratianni et al. (2020), when analyzing seven types of yellow and red bell peppers (*Capsicum annuum*), found that the carotenoid content ranged from 0.255 mg/100 g to 0.579 mg/100 g of fresh product in the yellow varieties, while the red varieties always showed a higher β-carotene content than the yellow varieties, with values ranging from 0.396 mg/100g to 0.705 mg/100 g of fresh product. Despite presenting numerically lower values, these data corroborate with those analyzed in this work and with that cited by Meléndez-Martínez et al. (2004). According to Blind et al. (2018), variability is an essential condition for the establishment of any breeding program; however, the efficiency of the selection of superior genotypes will depend on genetic and environmental parameters related to the characteristics of interest.

When evaluating 14 *Capsicum annuum* accessions from the *Capsicum* Genebank of Embrapa Temperate Agriculture, Padilha et al. (2015) found that accessions P39 and P143 presented carotenoid values equal to 134.83 and 147.72 mg/100 g, respectively. In turn, Neitzke et al. (2015), when evaluating 24 *Capsicum baccatum* accessions also from the same *Capsicum* Genebank, highlighted accession P179, a sweet pepper that presented the highest content of total carotenoids (152.06 mg/100 g), indicateding it for breeding programs for *in natura* consumption. Comparing to values reported by Padilha et al. (2015) and by Neitzke et al. (2015), we have found higher content of total carotenoids in the current study. Thus, according to the presented data, *C. chinense* seems to be the most carotenoid-rich pepper species.

Regarding anthocyanins, accession P240 stood out with 33.69 mg of cyanidin 3-glucoside 100 g^{-1} (Figure 3). Neitzke et al. (2015) and Padilha et al. (2015) found low contents of total anthocyanins (12.37 mg/100 g) in *C. baccatum* and (4.92 mg /100 g) in *C. annuum*, respectively. On the other hand, Vasconcelos et al. (2012), when analyzing 18 landraces of *C. baccatum*, found higher values of total anthocyanins in 5 accessions (10.49 mg/100 g; 10.52 mg/100 g; 11.38 mg/100 g; 11.41 mg/100 g and 13.30 mg/100 g), which did not differ statistically among themselves. These accessions had elongated and red fruits when ripe. All the previously mentioned studies differ from the value found for the anthocyanin content in *C. chinense*. Thus, it demonstrates the great intraspecific genetic variability with respect to bioactive compounds and also the potential of this accession for use in breeding programs.

In addition, it should be underlined that carotenoids and anthocyanins are responsible for the red color, a characteristic found in many fruits of *Capsicum* species. Accession P240 has red fruits, as mentioned above, and can therefore be used for the purpose of enhancing food products and, consequently, play an important role in food quality, due to the strong influence on color and flavor properties (Sá Mendes & Branco de Andrade Gonçalves, 2020). Accession P240 can be indicated for obtaining varieties with high content of total carotenoids and anthocyanins in breeding programs.

Considering that pepper is a very popular condiment in Brazilian cuisine, it is important to make a comparison with other condiments, regarding their antioxidant potential. In the current work, the antioxidant activity (Figure 4) the accessions P386, P391, P366, P350, P346 and P399 had the highest reference values, which did not differ statistically and presented values ranging from 458 µg of Trolox® equivalent/g in fresh mass to 577 µg of Trolox® equivalent/g in fresh mass. In turn Yang et al. (2020), reported that ginger (220 ± 10 µg of ascorbic acid equivalent/g) shows higher antioxidant capacity than garlic (130 ± 10 µg ascorbic acid equivalent/g) and onion (140 ± 10 µg ascorbic acid equivalent/g) ($p < 0.05$). This comparison demonstrates that peppers have significantly higher antioxidant potential values than these other spices used in cuisine.

Rosário et al. (2021), when investigating antioxidant activity in *C. annuum* and *C. chinense*, found that *C. annuum* (180 µg of Trolox® equivalent/g) had close antioxidant activity values to *C. chinense* (178.80 µg of Trolox® equivalent /g). In turn, Menichini et al. (2009), investigating *C. chinense*, *habanero* peppers, found a value of 287 µg of Trolox® equivalent /g in ripe peppers. These values were lower than those obtained in this study; however, highlight the *C. chinense* accessions as having high levels of bioactive compounds. Pivovarov et al. (2022) reported the content of total antioxidants varies greatly between varieties and species of *Capsicum*.

This comparison highlights the importance of these peppers, which stand out for their diversified use (Figure 5). They are broadly used in the food industry as raw material for dyes, flavorings, oleoresins, condiments, sauces and spices, being also very valuable in cuisine, pharmacology, dentistry and medicine (Pinto et al., 2013).

The correlations among the independent variables were analyzed with Pearson correlation coefficient (Table 2). High correlations (correlation higher than 0.70) were observed between antioxidant activity and total phenolic compounds, as well as between total anthocyanins and total carotenoids

Figure 5. *Capsicum chinense* fruits evaluated for total phenolic content, total carotenoids, total anthocyanins and antioxidant activity. Accessions P391, P240, P348, P399, P399, P386, P366, P350 and P346. Photos: Daniela Priori.

Table 2. Pearson correlation coefficients between pairs of evaluated variables of *Capsicum chinense* accessions from the *Capsicum* Genebank of Embrapa Temperate Agriculture.

Variables	Total phenolic compounds	Total carotenoids	Total anthocyanins	Antioxidant activity
Total phenolic compounds	1			
Total carotenoids	-0.322	1		
Total anthocyanins	-0.298	0.833**	1	
Antioxidant activity	0.842**	-0.440	-0.332	1

**It differed significantly at 1% Pearson correlation.

(Table 2). Low correlations were not observed (correlations from 0.30 to 0.50) and the other correlations (from 0.00 to 0.30) were considered negligible.

The high correlation between antioxidant activity and phenolic compounds verified here (R = 0.842) is commonly observed in other works (Burin et al., 2014). Accession P391 showed statistically higher phenolic content and higher antioxidant activity. Other compounds can influence the antioxidant activity of this accession, such as vitamin C and capsaicinoids (Chávez-Mendoza et al., 2015). We recommend these additional analyses in a next experiment to characterization of genetic variability of bioactive compounds in *Capsicum chinense*. Camargo et al. (2017) found a high correlation between the content of total phenolic compounds and the antioxidant capacity (0.89) with respect to the content of other phytochemicals, agreeing with the data of this study. It should be underlined that this strong correlation between antioxidant activity and phenolic compounds suggests that these compounds are the main responsible for the antioxidant capacity of peppers. Nonetheless, it is not only an isolated compound, but a synergy of compounds presents in peppers that are responsible for their antioxidant properties (Carvalho et al., 2015). Due their composition of phenolic compounds and capsaicin, habanero pepper (*C. chinense*) is considered a good source of bioactive compounds, which give it antioxidant capacity and antimicrobial activity against foodborne microorganisms (Jattar-Santiago et al., 2022).

The total anthocyanins also presented a strong correlation with the total carotenoids (R = 0.833). This correlation can be observed earlier in this work, being possible to notice due to the high levels of these compounds in the same accession (P240). Carvalho et al. (2015), when evaluating eight genotypes from the *Capsicum* Genebank of Embrapa Eastern Amazon (another Decentralized Unit of Brazilian Agricultural Research Corporation), also found a high content of anthocyanins and carotenoids in the accession, IAN 186305 (*C. baccatum*), which presented 58.09 mg/100 g of carotenoids and 18.30 mg/100 g of anthocyanins. These values are lower than those found in this study with *C. chinense* accessions, with genotypes rich in bioactive compounds and showing the high genetic variability. The same did not happen in a study with other solanaceous plants, 15 varieties of potatoes (*Solanum tuberosum*) presenting different flesh coloration: white, yellow, red and purple; and two varieties of *Solanum phureja*. Hejtmánková et al. (2013) found the correlation of R = 0.33, and report that these varieties have a high content of anthocyanins and also contain a considerable quantity of total carotenoids, which would explain such a correlation that exists, but is nevertheless low.

Accordingly, to the results presented the 19 characterized accessions of *C. chinense* are indicated for use in breeding programs that seek the development of varieties with high levels of bioactive compounds.

This work showed high content in phenolic compounds, carotenoids, anthocyanins and antioxidant activity in some accessions of *C. chinense*. These bioactive compounds could be added to human diet as in nature as through food industry, as a way to reduce synthetic additives (food colorings, stabilizers and antimicrobials). This could be an differential for food industry, exploring the available natural variation in *Capsicum* genetic resources to benefit the human health.

4 Conclusion

Capsicum chinense accessions from the *Capsicum* Genebank of Embrapa Temperate Agriculture present genetic variability for the concentrations of bioactive compounds.

There is a high correlation between the antioxidant activity and the concentration of phenolic compounds, as well as between the concentration of anthocyanins and carotenoids in *Capsicum chinense* access.

Accessions P391 (high concentration of phenolic compounds and antioxidant activity), P240, P348 and P399 (high concentration of total carotenoids), P240 (high concentration of total anthocyanins and carotenoids), P386, P391, P366, P350, P346 and P399 (high antioxidant activity) are good options for use in breeding programs.

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