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## Characterization of biochemical compounds and antioxidant activity of “dedo-de-moça” chili pepper accessions

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### ABSTRACT

*Capsicum baccatum* is one of the main chili pepper species grown and consumed in South America. In Brazil, *C. baccatum* var. *pendulum* is widely cultivated, especially “dedo-de-moça” type. For most horticultural crops, including *Capsicum* species their diversity remains under-explored for traits related to fruit quality, since over the past half century breeding efforts have focused mainly on good agronomic performances. The investment in screening traits such as flavor, nutritional compounds and antioxidant content in traditional varieties is very important to support fruit quality breeding efforts. Thus, the objective of this study was accessing the variations into content of soluble solid, acidity, capsaicinoid, vitamin C, total phenolic compounds and antioxidant activity (*in vitro* and *in vivo*) of five “dedo-de-moça” peppers accessions. The results evidenced that UEL111 and UEL112 accessions showed highest TSS and ratio. Accessions UEL110 and UEL112 showed highest concentration of vitamin C and total phenolic content and UEL113 accession showed highest concentration of capsaicinoids. *In vitro* antioxidant assays suggest the UEL114 with highest antioxidant activity. *In vivo* antioxidant assays have not identified differences among the accessions and proved the antioxidant effect of chili pepper extracts on cell protection against stress agent. The results highlighted accessions that can be exploited in chili pepper breeding programs.

**Keywords:** *Capsicum baccatum* var. *pendulum*, *Saccharomyces cerevisiae*, fruit quality, plant breeding.

### RESUMO

#### Caracterização de compostos bioquímicos e atividade antioxidante de genótipos de pimenta “dedo-de-moça”

*Capsicum baccatum* é uma das principais espécies de pimenta cultivadas e consumidas na América do Sul. No Brasil, *C. baccatum* var. *pendulum* é amplamente cultivada, especialmente a pimenta do tipo “dedo-de-moça”. Para a maioria das culturas horticolas, incluindo as espécies de *Capsicum*, a diversidade genética permanece subexplorada para características relacionadas à qualidade dos frutos, uma vez que nos últimos anos os esforços dos programas de melhoramento têm concentrado principalmente no bom desempenho agrônomo. Assim, o investimento na avaliação de características como sabor, compostos nutricionais e características de promoção da saúde, como o teor de antioxidantes em variedades tradicionais, é muito importante para apoiar os esforços de melhoramento para a qualidade dos frutos. Assim, o objetivo deste estudo foi acessar as variações nos teores de sólidos solúveis, acidez, capsaicinoides, vitamina C, compostos fenólicos totais e atividade antioxidante (*in vitro* e *in vivo*) de cinco acessos de pimentas “dedo-de-moça”. Os resultados indicaram que os acessos UEL111 e UEL112 apresentaram os maiores valores de sólidos solúveis totais (TSS) e relação (TSS/acidez total titulável). Os acessos UEL110 e UEL112 apresentaram maior concentração de vitamina C e conteúdo fenólico total e o acesso UEL113 apresentou maior concentração de capsaicinoides. Ensaio antioxidante *in vitro* sugerem UEL114 com maior atividade antioxidante. Ensaio antioxidante *in vivo* não identificaram diferenças entre os acessos e provaram o efeito antioxidante dos extratos de pimenta na proteção celular contra o agente de estresse. Estes resultados permitiram identificar acessos que podem ser explorados em programas de melhoramento de pimenta para qualidade de frutos.

**Palavras-chave:** *Capsicum baccatum* var. *pendulum*, *Saccharomyces cerevisiae*, qualidade dos frutos, melhoramento de plantas.

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The genus *Capsicum* (Solanaceae) is native from tropical zones of Central and South America and comprises five domesticated species: *C. pubescens*, *C. baccatum*, *C. annuum*,

*C. chinense* and *C. frutescens* (Nuez *et al.*, 1996), which spread rapidly since their introduction in Europe replacing the most popular spice, the black pepper (*Piper nigrum*), possibly because of

their ease cultivation and great diversity, including sweet and spicy varieties differing in fruit size, shape and color. Among five domesticated species, *C. baccatum* is one of the main chili

pepper species grown and consumed in South America (Albrecht *et al.*, 2012; Cardoso *et al.*, 2018). This species has a wide genetic variability, being divided in three botanical varieties: the domesticated *C. baccatum* var. *pendulum* and wild species *C. baccatum* var. *praetermissum* and *C. baccatum* var. *baccatum* (Scaldfarfero *et al.*, 2018).

For most horticultural crops, including *Capsicum* species their diversity remains under-explored for traits related to fruit quality, since over the past half century breeding efforts have focused mainly on good agronomic performances in terms of yield and adaptation to biotic and abiotic stress. However, the goals of horticultural plant breeding programs have slowly been expanded to meet the new requirements specifically linked to consumer preferences, such as improved flavor, nutritional compounds and health-promoting traits, such as antioxidant content (Kyriacou & Rouphael, 2018).

Antioxidants are usually present in fruits and vegetables and its consumption has been associated with many health benefits in consequence to its fundamental role in prevent oxidative stress caused by free radicals, which has been identified as a major causative factor in the development and progression of several life-threatening diseases (Finkel & Holbrook, 2000; Kaur & Kapoor, 2001). Chili peppers are a good source of antioxidants including widespread compounds, such as carotenoids, flavonoids, ascorbic acid (vitamin C), tocopherol (Vitamin E) and specific constituents such as capsaicinoids, responsible for the pungency. The capsaicinoids are produced in the placenta glands, wherein capsaicin, dihydrocapsaicin and nordihydrocapsaicin are the most frequent (Bogusz *et al.*, 2018).

The determination of antioxidant activity is paramount in the evaluation of food products for determining antioxidant benefits. Currently there are several methods (*in vitro* and *in vivo*) of assessing antioxidant activity which may differ in reaction mechanisms, application and complexity (Rice-Evans *et al.*, 1996; Pulido *et al.*, 2000; Bogusz *et al.*, 2018). *In vitro* determination

methods are widely used to evaluate the antioxidant activity of plant samples and are based on the free radical scavenging ability (Pisoschi & Negulescu, 2012; Sora *et al.*, 2015). Examples of *in vitro* assays include ferric reducing ability of plasma (FRAP), copper reduction assay (CUPRAC), oxygen radical absorbance capacity (ORAC), total peroxy radical trapping antioxidant parameter (TRAP), 2,2'-azino-bis-3 o 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonate (ABTS) (Alam *et al.*, 2013). Although relatively simple to perform, *in vitro* assays do not always reflect the cell condition (López-Alarcón & Denicola, 2013; Stinco *et al.*, 2015). Thus, *in vivo* assays with cellular models are considered a useful tool in determining the mechanisms of action as well as the protective effect of antioxidants in the presence and absence of stressors. *Saccharomyces cerevisiae* yeast has been widely used as a cellular model to determine the antioxidant activity of foods, indicating a similar effect that could be observed in cells of higher eukaryotes (Odriozola-Serrano *et al.*, 2016).

In addition to the presence of antioxidant compounds, the concentration of sugars and organic acids are important parameters for the quality of the chili pepper fruits, contributing to the flavor intensity (Acunha *et al.*, 2017). High sugar content in fruits also ensures a higher yield in the processing stage, requiring lower sugar addition and energy expense for dehydration. Therefore, the development of chili pepper cultivars rich in biochemical compounds including health and taste-related attributes arouses interest for both fresh market and industry that processes dry pepper products.

The investment in screening these emerging traits in traditional varieties is very important to support fruit quality breeding efforts. In Brazil, *C. baccatum* var. *pendulum* is widely cultivated by family farmers, especially “dedo-de-moça” type. This variety produces elongated fruit with mild to medium pungency and is consumed *in natura*, as dehydrated flakes and as spice in food products because of their typical color,

pungency, taste and distinct aroma. Thus, the objective of this study was accessing the variations into content of soluble solid, acidity, capsaicinoid, vitamin C, total phenolic compounds, and antioxidant activity (*in vitro* and *in vivo*) of five “dedo-de-moça” chili peppers accessions.

## MATERIAL AND METHODS

### Plant material

The experiments were performed from January to November 2015 with five “dedo-de-moça” chili peppers (*C. baccatum* var. *pendulum*) accessions (UEL110, UEL111, UEL112, UEL113, and UEL114) belonging to the germplasm collections from the Universidade Estadual de Londrina (UEL). These accessions come from commercial cultivars and inbred lines from the *Capsicum* breeding program of UEL.

All accessions were grown according to a completely randomized block design with three replicates (7 plants/replicate) in a greenhouse located in the experimental area of Agronomy Department of UEL, Paraná, Brazil (23°22'S, 51°10'W; 585 m elevation) following standard agronomic practices for chili pepper cultivation. Fruits were harvested at the red-ripest age (70 to 80 days after anthesis). Seeds were removed and fresh fruits were evaluated for soluble solids, acidity, pH and vitamin C. The remaining fruits were frozen, lyophilized and stored at -20°C (Christ, Model 500) for analysis of phenolic and flavonoid compounds, capsaicinoids, and antioxidant activity *in vivo* and *in vitro*.

### Soluble solids, acidity and pH

Total soluble solids (TSS) were determined by digital refractometer (Atago®) using juice of fresh pressed fruit pericarp, and the results were expressed as °Brix. Titratable acidity (TA) and pH were determined from the extracted fruit juice. TA was measured by titration with NaOH 0.1M (up to pH 8.2), and results were expressed as percentage of citric acid (m/m) (IAL, 2008). The pH was measured in a

potentiometer (Quimis, Q400A). All the analyses were performed in triplicate for each accession replicate. The ratio was calculated by the relation between TSS and TA.

### Vitamin C

The vitamin C (ascorbic acid) content was obtained by the AOAC's official titrimetric method (1984), modified by Benassi & Antunes (1988). To summarize, 10 g of fresh sample and 50 mL of 2% oxalic acid (m/v) (Synth) were homogenized and titrated with 2,6-dichlorophenol-indophenol (Merck, Germany) until the occurrence of distinct rose-pink color. The analyses were performed in triplicate for each accession replicate and the results expressed as milligrams of ascorbic acid per 100 g fresh weight (mg 100 g<sup>-1</sup>).

### Phenolic and flavonoid compounds

One gram of lyophilized fruits without seeds was homogenized with 10 mL methanol 80% (v/v) during 30 min and centrifuged during 5 min at 2500 rpm (Vázquez *et al.*, 2008). The supernatant was collected for quantification of phenolic compounds and flavonoids. To determine the total phenolic content, a mixture with 1 mL chili peppers fruits extract, 1 mL methanol, 1 mL Folin-Ciocalteu reagent 0.20 N and 1 mL sodium carbonate 10% (m/v) was incubated at room temperature in the dark during 30 min. Subsequently, the absorbance was measured at 765 nm in a Micronal spectrophotometer (AJX1600). A calibration curve of gallic acid as standard phenolic compound was plotted (range from 10 to 100 mg L<sup>-1</sup>). The total phenolic contents from the chili peppers fruits extract were expressed as mg gallic acid equivalents (GAE) per 100 g fresh weight (mg GAE g<sup>-1</sup>) (Swain & Hillis, 1959).

To determine the flavonoids content, a mixture with 1 mL chili peppers fruits extract, 1 mL aluminum chloride 5% (w/v) and 2 mL methanol were incubated at room temperature in the dark for 30 min (Woisky & Salatino, 1998). Subsequently, the absorbance was measured at 425 nm in a Micronal spectrophotometer (AJX 1600). A calibration curve of Quercetin as standard flavonoid was plotted

(range from 1 to 50 mg L<sup>-1</sup>). The total flavonoids contents from the chili pepper fruits extract were reported as Quercetin equivalent (QE) per 100 g fresh weight (mg QE g<sup>-1</sup>) (Swain & Hillis, 1959).

### Antioxidant activity *in vitro*

One gram of lyophilized fruits without seeds was homogenized with 10 mL methanol 80% (v/v) during 30 min and centrifuged during 5 min at 2500 rpm (Vázquez *et al.*, 2008). The supernatant was collected for quantification of antioxidant assays. Antioxidant activity was determined via free radical DPPH• (2,2-diphenyl-1-picryl-hydrazine), according to Brand-Williams *et al.* (1995), modified by Casagrande *et al.* (2007). In short, 50 µL methanolic extract was homogenized with 1 mL acetate buffer solution (100 mM; pH 5.5), 1 mL ethanol, and 0.5 mL ethanolic solution of DPPH (250 µM). Tubes were kept at room temperature in the dark during 15 min and DPPH radical absorbance was measured at 517 nm in spectrophotometer (Thermo-Genesys) in triplicate. The analytical curve for quantification was prepared using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (0.20 to 1.00 mmol L<sup>-1</sup>) and the results were expressed in µmol Trolox equivalent antioxidant capacity (TEAC) per gram of sample. The antioxidant activity was also determined by the FRAP method. In short, 30 µL methanolic extract was added into test tubes with 70 µL distilled H<sub>2</sub>O and 900 µL FRAP reagent. They were kept at 37°C during 30 min and absorbance was measured at 595 nm in spectrophotometer (Thermo-Genesys) in triplicate. The analytical curve for quantification was prepared as described above. Results were expressed in µmol TEAC per gram of sample (Benzie & Strain, 1996).

### Antioxidant activity *in vivo*

The evaluation of the antioxidant activity of the extracts from different chili pepper accessions was performed by survival trials and mitochondrial dysfunction of *Saccharomyces cerevisiae* cells BY4741 (MATa; his3; leu2; metra; Euroscarf). Cells were

cultured in 2% YPD liquid medium using an orbital shaker at 28°C and 160 rpm at the volume/medium ratio 5:1. The cell concentration was determined by measuring the absorbance at 570 nm. Absorbance conversion in dry weight was calculated by filtration of 10 mL of the cell suspension through a Milipore filter (0.45 µm) and dehydrated at 80°C to constant weight.

Yeast cells were collected in the 1<sup>st</sup> exponential growth phase (1.0 mg dry weight/mL), and then incubated with 10 µg mL<sup>-1</sup> of chili pepper extracts during 60 min at 28°C and 160 rpm. Cells were collected by centrifugation and washed twice with 50 mM phosphate buffer, pH 6.0. The pellet was resuspended in the same phosphate buffer containing 1.0 mM H<sub>2</sub>O<sub>2</sub>, and kept during 60 min at 28°C and 160 rpm. Before the cell viability assays and mitochondrial dysfunction, the toxicity of chili pepper extracts at the concentration of 10 µg mL<sup>-1</sup> during 1 and 2 hours was determined by the growth of colonies in 2% YPD solid medium.

Cell viability was determined by plating a volume equivalent to 40 µg of cells in 2% YPD solid medium, before and after the oxidative stress conditions. Plates were incubated at 28°C during 48 hours and the colonies were counted. To detect mitochondrial dysfunction, the cells passed through the same treatment, but the plating was performed in solid medium YPGly (1% yeast extract, 2% glycerol, 2% peptone and 2% agar), used to test the inability of cells to grow under strictly aerobic medium (glycerol). The results were expressed as percent survival of yeast cells.

### Capsaicinoid content

For the analysis of capsaicinoids, 1g lyophilized fruits was homogenized with 25 mL methanol (Merck, Germany) in an ultrasound (EQM Cristófoli-CF, Brazil) at 160 W, 42 kHz frequency, during 20 min (Barbero *et al.*, 2008). The samples were filtered and the extracts were dried in a rotary evaporator. The analysis was carried out by High Performance Liquid Chromatography (CLAE) (Waters LC616 Alliance) coupled with a diode array detector (Waters, 2996), and monitored absorbance at 250 to 300 nm.

The capsaicinoids were separated in C18 column of Betasil Thermo reverse phase (25 cm, 4.6 mm, 5  $\mu$ m), and the volume injected was 20  $\mu$ L, with run time of 15 minutes at a flow rate of 1 mL min<sup>-1</sup>. The mobile phase was constituted of two solutions: ultrapure water acidified with acetic acid at 1% in (A) and acetonitrile at 60% (v/v) (B) (Collins *et al.*, 1995; Estrada *et al.*, 2000). The analytical curves for quantification of capsaicinoids were prepared using the capsaicin, dihydrocapsaicin and nordihydrocapsaicin (0.02 - 0.1 mg mL<sup>-1</sup>) (Cayman Chemical, Michigan, USA). Chromatographic analysis was performed in triplicate, and the results were expressed in milligrams of capsaicin, dihydrocapsaicin or nordihydrocapsaicin per gram of sample (mg g<sup>-1</sup>).

#### Statistical analysis

Data were submitted to analysis of variance (ANOVA) and significant differences in mean values were separated using Tukey's test at  $\alpha=0.05$ . Statistical analyses were performed using R software (<http://www.r-project.org>) and the agricolae package.

## RESULTS AND DISCUSSION

Biochemical compounds content in “dedo-de-moça” chili pepper accessions are presented in Table 1 and Figure 1. Analysis of variance showed a significant effect ( $P<0.05$ ) among the five “dedo-de-moça” chili pepper accessions for most traits, except for titratable acidity (TA) and pH. These results

reveal accessions with distinguished values for the development of chili pepper cultivars with high fruit quality. Several studies have also indicated a wide variability of *C. baccatum* var. *pendulum* for fruit quality traits and bioactive compounds (Rêgo *et al.*, 2009; Rodríguez-Burruezov *et al.*, 2009; Eggink *et al.*, 2014).

Total soluble solids (TSS) ranged from 5.20 to 7.10°Brix and among the five accessions, UEL111 and UEL112 presented the highest values. The same accessions also presented the highest values for the ratio (TSS/AT), reflecting the TSS contents since there was no significant difference for the titratable acidity (TA). The concentration of sugars and organic acids highly contribute to the fruits flavor intensity, an important quality parameter for both fresh consumption and processing. Fruits with high SST contribute for a higher yield in the processing stage, requiring lower sugar addition and reducing the energy cost for water evaporation (Acunha *et al.*, 2017). Similar investigation studies have reported even higher values for TSS in *C. baccatum* genotypes. Rêgo *et al.* (2009) and Ferrão *et al.* (2011) observed a variation of 7.20 to 13.53°Brix and 5.5 to 11.9°Brix, respectively. The same was observed in other species, where in TSS ranged from 8.11 to 11.02°Brix in accessions of *C. chinense* (Moreira *et al.*, 2018). However, unlike our study, these examples were performed in fruits produced in the field. In addition to being an inherent characteristic of the genotype, the TSS also suffers

interference from the environment. Thus, the lower values for TSS may be related to the cultivation in greenhouse, which in some cases may promote less sunlight capture and, as a consequence, lower concentrations of photoassimilates (Lee & Kader, 2000).

The values for vitamin C content ranged from 34,6 to 110,8 mg 100g<sup>-1</sup> fresh weigh and the highest amount was found in the UEL110 and UEL112 accessions. Similar values were found by Rodríguez-Burruezov *et al.* (2009) who evaluated 23 accessions of *C. baccatum*. In greenhouse cultivation the values ranged from 34.5 to 90.8 mg 100 g<sup>-1</sup> fresh weigh and in field cultivation 44.3 to 157.7 mg 100 g<sup>-1</sup> fresh weigh. Besides an important physiological antioxidant, vitamin C is also required for several important functions in the organism and immune cell development, which makes it an essential dietary component (Granger & Eck, 2018). According to the values determined in this study, the ingestion of 50-100 g fresh pepper fruits can provide about 100% of the recommended daily amounts of vitamin C recommended by the Food and Drug Administration (FDA) (60 mg day<sup>-1</sup>) (FDA, 2016).

The analyses found a significant variation in phenolic contents, ranged from 200 to 440 mg GAE 100 g<sup>-1</sup> fresh weight in the five peppers accessions, highlighting the UEL110 and UEL112. In contrast, for flavonoids there was a small variation among the genotypes (170 and 240 QE 100 g<sup>-1</sup> fresh weight) and only the accession UEL112 differed statistically from the others, having

**Table 1.** Biochemical compounds from “dedo-de-moça” chili pepper accessions. Londrina, UEL, 2016.

Accessions	Total soluble solids (TSS, °Brix)	Titratable acid (TA, % citric acid)	Ratio (TSS/TA)	pH	Vitamin C (mg AA* 100 g <sup>-1</sup> )	Total phenolic content (mg GAE** 100 g <sup>-1</sup> )	Total flavonoid content (mg QE*** 100 g <sup>-1</sup> )
UEL110	5.20 c	49.93 a	0.10 b	4.79 a	110.83 a	440 a	210 a
UEL111	7.10 a	48.80 a	0.14 a	4.71 a	49.65 cd	260 bc	230 a
UEL112	7.10 a	49.33 a	0.14 a	4.72 a	99.31 ab	420 a	170 b
UEL113	5.80 b	49.03 a	0.12 b	4.79 a	34.58 d	300 b	230 a
UEL114	5.50 bc	50.03 a	0.11 b	4.72 a	73.59 bc	200 c	240 a
CV (%)	2.62	6.69	8.54	2.20	10.51	5.25	5.24

\*AA= ascorbic acid; \*\*GAE= gallic acid equivalent; \*\*\*QE= quercetin equivalent; Means followed by same letters in the column, do not differ, Tukey test ( $P<0.05$ ).

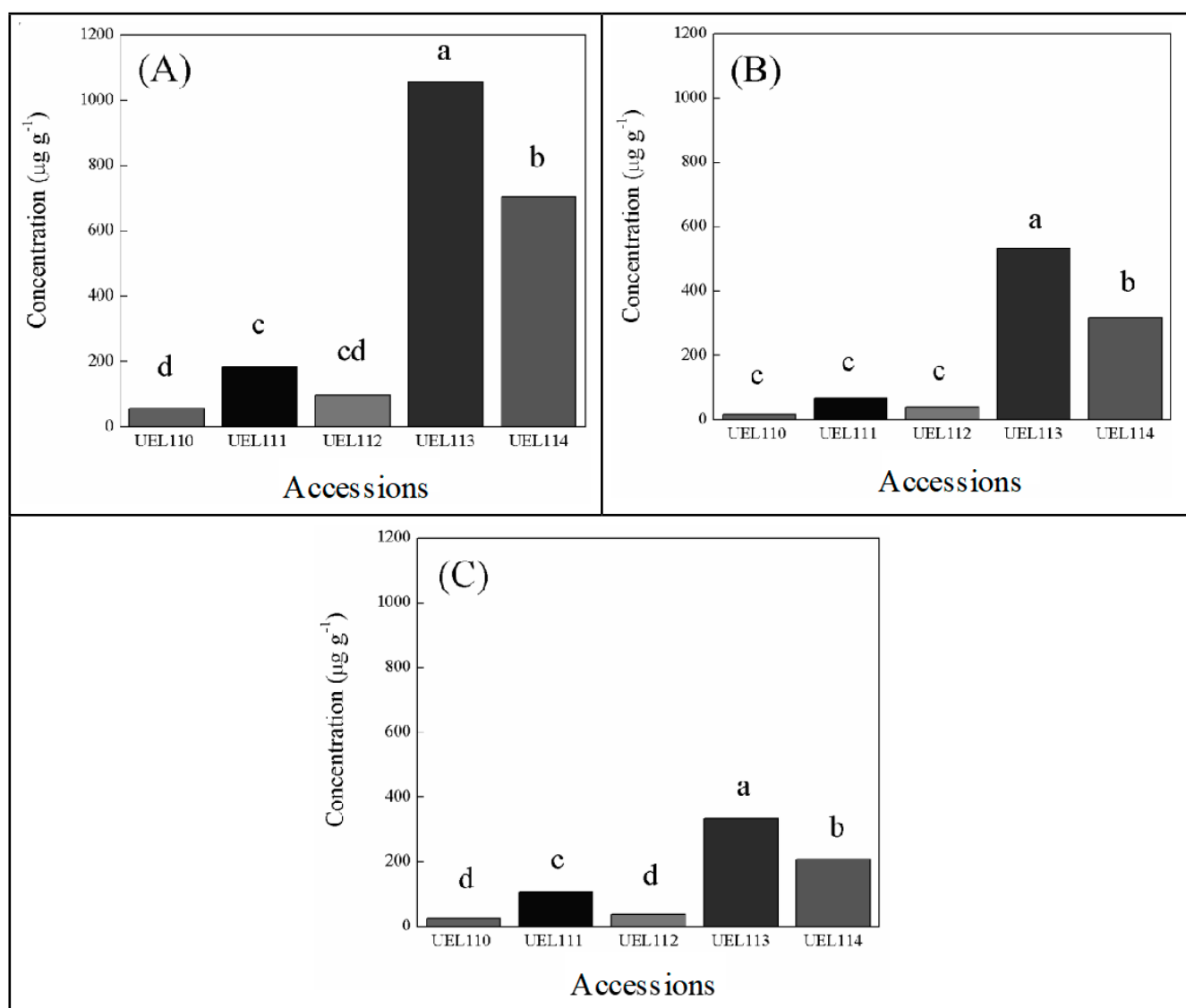
the lowest value. In general, the total phenolic and flavonoid compounds from the five chili pepper accessions were higher than those reported in the literature for another *Capsicum* species (Ghasemnezhad *et al.*, 2011; Zhuang *et al.*, 2012; Carvalho *et al.*, 2015). These differences may be related to the accession, but also to external factors, such as fruit maturity, extraction and analytical method and growing conditions. Phenolic compounds are secondary metabolites widely found in fruits, contributing to the color and sensory characteristics. The great interest in these substances is related to their wide range of biochemical activities, such as antioxidant, antimutagenic

and anticarcinogenic (Haminiuk *et al.*, 2012).

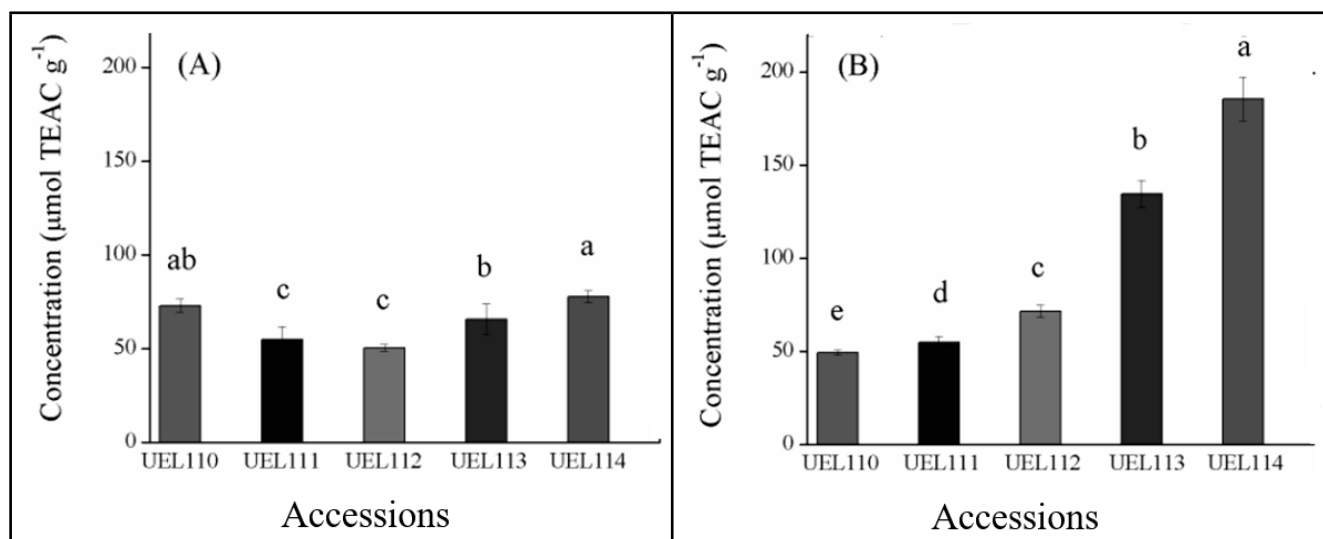
The obtained data showed a diversified content of capsaicinoids among the accessions. The values of capsaicin ranged from 57.27 to 1057.50  $\mu\text{g g}^{-1}$ , while for dihydrocapsaicin and nordihydrocapsaicin ranged from 17.78 to 532.70  $\mu\text{g g}^{-1}$  and 25.7 to 333.60  $\mu\text{g g}^{-1}$ , respectively (Figure 1). The highest values of capsaicinoids were observed in the accession UEL113. The capsaicinoid is the constituent that confers the fruits pungency, an intrinsic characteristic of the *Capsicum* genus and one of the most important quality traits considered by the spice industry. The capsaicinoids content and pungency varies between

the chili pepper species, while *C. baccatum* var. *pendulum* presents fruits with mild to median pungency, and may even be absent in some cases. Acunha *et al.* (2017) measured the levels of capsaicin and dihydrocapsaicin in 72 *Capsicum* spp. accessions and observed values differing from 0.15 to 1935.77  $\text{mg } 100 \text{ g}^{-1}$  and 0.03 to 186  $\text{mg } 100 \text{ g}^{-1}$ , respectively, wherein the accessions of *C. baccatum* showed the lowest concentrations.

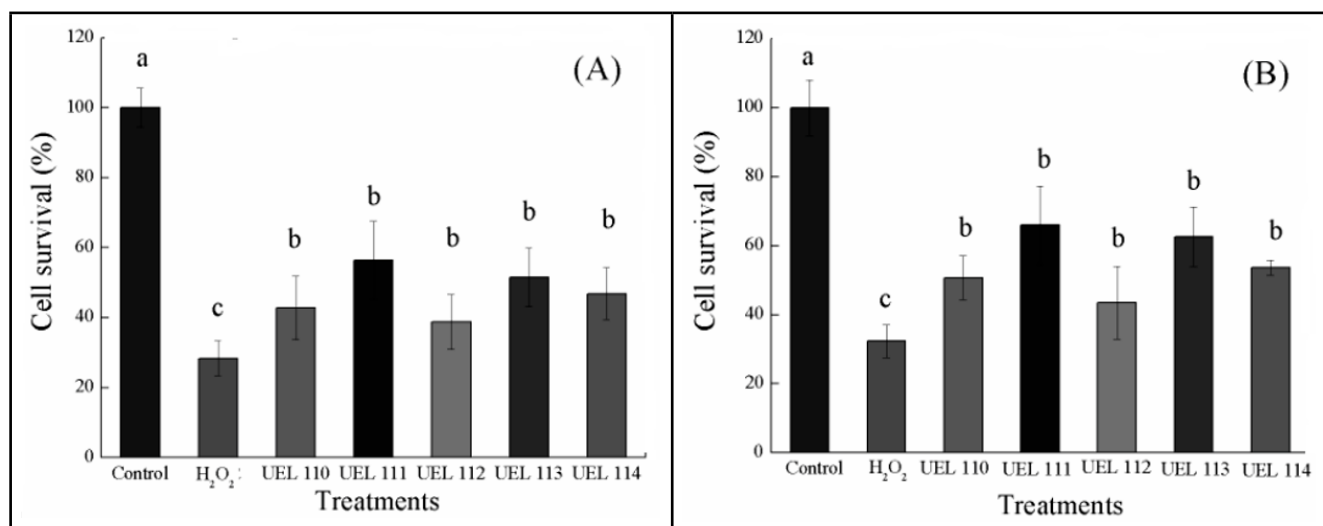
The determination of the antioxidant activity was performed by FRAP and DPPH, both methods widely used in studies of *Capsicum* spp. The FRAP is based on the reduction of metal complexes by sample antioxidants while



**Figure 1.** Content of capsaicinoids in “dedo-de-moça” chili pepper genotypes, (A) capsaicin, (B) dihydrocapsaicin and (C) nordihydrocapsaicin. Means followed by same letters do not differ, Tukey test ( $P < 0.05$ ). Londrina, UEL, 2016.



**Figure 2.** Antioxidant activity *in vitro* of “dedo-de-moça” chili pepper accessions; (A) DPPH assay and (B) FRAP assay. Means followed by same letters do not differ, Tukey test ( $P < 0.05$ ). Londrina, UEL, 2016.



**Figure 3.** Antioxidant activity *in vivo* of “dedo-de-moça” chili pepper accessions by oxidative stress test; (A) cell viability and (B) control of mitochondrial dysfunction. Means followed by same letters do not differ, Tukey test ( $P < 0.05$ ).

DPPH is based on the measurement of the scavenging capacity of antioxidants towards the free radical neutralization (Alam *et al.*, 2013). In this study, antioxidant activity ranged from 50.50 to 72.9 μmol TEAC g<sup>-1</sup> and 40.5 to 185.50 μmol TEAC g<sup>-1</sup> by DPPH and FRAP methods, respectively (Figure 2). According to the results obtained in the first method, the highest antioxidant activity was observed in the UEL110 and UEL114 accessions. The second method also pointed to UEL114 accession with the highest antioxidant activity, although the accession UEL110 gave the lowest activity. The determination

of antioxidant activity by the DPPH method can produce very divergent results when compared to the FRAP method, because it is very sensitive to certain compounds (Müller *et al.*, 2011). Similarly to our study, Sora *et al.* (2015) also found variation in the results of antioxidant capacity by the DPPH assay (2.28 to 15.6 μmol TEAC g<sup>-1</sup>) and by the FRAP assay (3.99 to 84.67 μmol TEAC g<sup>-1</sup>) in chili pepper. Thus, for a more robust evaluation it is indicated the application of both DPPH and FRAP antioxidant method since the FRAP assay has a more selective character in the evaluation of the antioxidant activity.

*In vitro* antioxidant assays give an evidence of free radical scavenging ability of different extracts (Sora *et al.*, 2015). However, they are not able to access the effect of an antioxidant on cell survival (Oliveira *et al.*, 2014). Thus, the antioxidant capacity of the extracts from different pepper accessions was also assessed by measuring cellular protection of *S. cerevisiae* in the presence of a stressor agent. The cells of yeast *S. cerevisiae* showed sensitivity to the application of oxidative stress by H<sub>2</sub>O<sub>2</sub> (1.0 mM) and only 22.16% survived when compared to the control (Figure 3a). However,

the addition of chili pepper extract allowed partial suppression of the damage, increasing the mean survival rate to 36.95%. Similarly, the evaluation of mitochondrial dysfunction of *S. cerevisiae* shows significant differences between stressed cells and treatments with chili pepper extract (Figure 3b). The application of H<sub>2</sub>O<sub>2</sub> alone resulted in a survival rate of 22.56%, whereas the treatment with chili pepper extracts led to a survival rate of 37.45%, an increase of 66%. In both evaluations, unlike *in vitro* methods, there was no difference between the five chili pepper accessions. The rate of increase in survival was higher than that reported by Lingua *et al.* (2016) which evaluated the oxidative stress damage by H<sub>2</sub>O<sub>2</sub> (2.0 mM) in *S. cerevisiae* cells (ATCC36900) treated with grape extracts, a fruit known for its antioxidant properties. The authors obtained an increase between 14 and 20% of the survival rate of the cells exposed to H<sub>2</sub>O<sub>2</sub>. Rodrigues (2004) also evaluated the antioxidant activity of ethanolic extract from *C. cerifera* leaves on *S. cerevisiae* cells damage by H<sub>2</sub>O<sub>2</sub>, which promoted a survival rate of 11.5%. These results suggest that chili pepper extracts may have performed an antioxidant protection capable of partially avoiding the damage in the cells caused by exposure to H<sub>2</sub>O<sub>2</sub>.

Several studies have demonstrated a correlation between antioxidant capacity and phenolic, capsaicinoids and vitamin C content. In the present investigation, the antioxidant activity determined by the FRAP and DPPH methods was higher for UEL114 accession, although this genotype did not show the highest levels of any of the bioactive compounds evaluated. Likewise, the accessions that presented higher contents of bioactive compounds did not present superior *in vivo* antioxidant activity, i.e., there was no difference between the different accessions, indicating that even the genotypes with lower contents of bioactive compounds presented a good antioxidant activity.

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