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Pumpkin landraces from southern Brazil as functional foods

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Abstract

Squash is a food that meets the requirements of a healthy diet and is highly appreciated by consumers for its mild flavour and high nutritional value. In southern Brazil, many farmers grow several pumpkin landraces that are widely used in food. The consumption of its fruits and derivatives benefits human health due to the bioactive compounds, minerals, and antioxidants in the fruit pulp, making pumpkin a functional food. The objective of this study was to characterize the bioactive compounds, antioxidant activity, and minerals in the pulp of fruits of pumpkin landraces (*Cucurbita maxima*) from southern Brazil. Phenolic compounds, carotenoids, antioxidant activity, and minerals were evaluated in 10 accessions of pumpkin landraces from the Cucurbitaceae Genebank of Embrapa Temperate Agriculture (Pelotas, RS, Brazil). In mature fruits, the seeds and peels were discarded, and opposite longitudinal slices of pulp were manually prepared for analysis. All analyses were performed in triplicate. The data obtained showed genetic variability in total phenolic compounds, carotenoids, antioxidant activity, and minerals. Accessions C49, C307, and C216 had high levels of total phenolic compounds and carotenoids and high antioxidant activity. Of these accessions, accession C49 had the highest antioxidant capacity (504.1 $\mu g \cdot g^{-1}$). C216 and C178 were high in copper and iron, and the latter was highest in potassium, calcium, and phosphorus. Thus, accessions C216 and C178 have great potential for exploitation by genetic improvement programs for the development of biofortified cultivars in efforts to promote consumer health.

Keywords: bioactive compounds; antioxidant activity; genetic resources; Cucurbitaceae; *ex situ* conservation; variability, cultivars of pumpkin.

Practical application: pumpkin landraces as source of nutrients for a healthier diet.

1 Introduction

Food is fundamental to people's lives. A varied diet provides the nutrients (carbohydrates, proteins, lipids, vitamins, and minerals) that we need for growth, maintenance of health, and well-being. In recent years, the majority of the population has become aware of the importance of healthy eating (Ministério da Saúde, 2014; Cañas & Braibante, 2019). In this context, pumpkin is a food that meets the requirements of a healthy diet and is highly appreciated by consumers for its mild flavour and high nutritional value (Zhou et al., 2014). Pumpkin is an annual plant that has been domesticated in the Americas. Belonging to the genus *Cucurbita* and the family Cucurbitaceae. Pumpkin landraces of the five domesticated species *(Cucurbita argyrosperma* Huber. *C. ficifolia* Bouche, *C. maxima* Duchesne, *C. moschata* Duchesne, and *C. pepo* L.) are grown in different regions of the country (Heiden et al., 2007).

C. maxima is one of the main crops of the Guarani Indians of north-eastern Argentina and Paraguay, and there are numerous variations of this species in the Americas (Lira et al., 2009). In the South region of Brazil, farmers grow pumpkin landraces of it, and there is great genetic variability between and within the populations of these species (Ferreira, 2008). The pumpkin landraces are those developed, through the selection of plants, by the farmers themselves over time, whose seeds are passed from generation to generation and exchanged between neighbours and relatives. In this process, knowledge is also exchanged related to planting, management, harvesting, and storage (Barbieri, 2012). Squashes and pumpkins are very popular vegetable in many tropical and subtropical countries and is very versatile in cooking, having an advantage over other vegetables because it can be stored for up to six months at room temperature before being consumed (Ahamed et al., 2011; Silva & Silva, 2012). Several regional and local dishes are prepared with *Cucurbita* fruits (Heiden et al., 2007).The chemical composition of squash pulp is quite diverse and depends on both the species and the variety. It is low in proteins, fats, and carbohydrates, making it a low-calorie and easily digestible vegetable. The energy value of its pulp is approximately 30 kcal/100 g (United States Department of Agriculture, 2015). Squashes with more intense orange pulp are particularly valuable because they are high in carotenoids, especially β-carotene and lutein (Seleim et al., 2015).

The consumption of pumpkin pulp and its derivatives can bring benefits to human health due to its pectin, mineral salts, α- and β-carotene, lutein, fibers and minerals, in addition to bioactive compounds and high content of vitamin A, which

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are antioxidants that can inhibit the action of free radicals in the body. Due to its use (fruits and derivatives, bark, seeds, flowers, leaves) and nutritional properties, pumpkin can be considered a functional food (Ferreira et al., 2017; Zhou et al., 2014; Carvalho et al., 2021).

Functional foods are those that have components that promote and maintain health and prevent diseases. In addition to its nutritional value, biological properties such as antidiabetic, antihypertensive, antibacterial, and antioxidant activity are also attributed to squash (Pająk et al., 2014; Xanthopoulou et al., 2009; Zhou et al., 2014). These foods provide not only essential nutrients but also provide bioactive compounds that help improve the human diet (Saltzman et al., 2013; Vizzotto, 2005). Several studies in Brazil and other countries have shown the levels of carotenoids, minerals and antioxidant activity in different pumpkin species, as well as their functionality, taking into account that leaves, flowers, bark, seeds and stalks can also be used in food human and present interesting values of bioactive compounds (Ramos & Queiroz, 2005; Jiang & Du, 2011; Boschi, 2015; Priori et al., 2017; Kulczyński & Gramza-Michałowska, 2019; Amadeu et al., 2021; Carvalho et al., 2021).

Taking into account the functionality of pumpkins, it is important consider the biofortification, which is a process of development of natural foods that present amounts of nutrients capable of meeting the needs of the human body when inserted in the population's diet. Changes in the content of the pulp do not change the appearance, taste, texture or way of preparing the food. It is also an important strategy to combat malnutrition, made by crossing plants of the same species, generating more nutritious cultivars, excellent sources of one or more essential micronutrients, such as iron, zinc, calcium and vitamin A (Loureiro et al., 2018). Embrapa coordinated the BioFORT network, for the biofortification of foods with a focus on the improvement of pumpkin, rice, beans, cowpea, cassava, sweet potatoes, corn and wheat. Bioactive compounds are an area of interest for nutritionists and food technologists. They endow foods with properties that are important for health promotion. The food industry is increasingly willing to add bioactive compounds to improve the nutritional properties of food products (Kulczyński & Gramza-Michałowska, 2019). The consumption of functional foods with bioactive properties has been associated with protection against various non communicable diseases, delayed ageing, and prevention of certain chronic diseases such as cancer, diabetes, and cardiovascular diseases (Ashour et al., 2011; Alvarez-Parrilla et al., 2012). Currently, research focussing on the human genome and lifestyle habits is being done to find out whether and how the consumption of functional foods can prevent diseases (Bland, 2018). Thus, there is growing interest in quantifying the levels of bioactive compounds in fruits and vegetables to determine their potential functionality in promoting quality of life and preventing disease (Zimmer et al., 2012; Oliveira, 2015).

Given the above, this study aimed to characterize the bioactive compounds, antioxidant activity, and minerals in the pulp of pumpkin landraces (*C. maxima*) cultivated in southern Brazil.

2 Materials and methods

Bioactive compounds (total phenolic compounds and carotenoid compounds), antioxidant activity, and minerals (Ca, Mg, K, Cu, Fe, Mn, Zn, P) were evaluated in the pulp of fruits of 10 accessions of pumpkin landraces *C. maxima* from the Cucurbitaceae Genebank of Embrapa Temperate Agriculture. The 10 accessions (Figure 1) were chosen based on passport data, which in turn were based on fruit characteristics such as shape, size, and colour of the peel and pulp and on the availability of seeds (Table 1). We chose to evaluate the pulp as this is the part widely consumed as food by people.

In addition to the 10 pumpkin landrace, fruits of the Tetsukabuto cultivar, an interspecific hybrid, were used in the analyses to compare the levels of compounds in the pumpkin landraces and this commercial cultivar. The fruits of the Tetsukabuto cultivar were purchased from a supermarket in the municipality of Pelotas, Rio Grande do Sul (RS), Brazil.

In September 2015 in a greenhouse, accessions of pumpkin landraces were sown in black polystyrene bags filled with mixed soil and substrate (1 : 1). In October, when the plants reached the stage of two to three true leaves, 10 seedlings from each accession were transplanted to the experimental field, located at Embrapa Temperate Agriculture, with spacing of 2.5 m between plants and 3 m between rows, in a completely randomized design, considering each plant as a replicate. The soil was prepared by ploughing and harrowing, and fertilization was performed with NPK 04–14–08 incorporated into the planting furrow. The cultivation area was kept clean, with weed control by manual weeding. Drip irrigation was performed three times a week or as needed. No fungicides were applied.

The fruits were harvested when ripe. Opposite longitudinal portions of the fruits were manually prepared by removing the peel and seeds. The pulp fresh weight of 10 fruits from each accession was manually chopped and homogenized. Four 2.5-g samples of pulp from each accession were used for the analysis of total phenolic compounds, carotenoids, and antioxidant activity. All analyses were performed in triplicate. The total phenolic compounds were measured by the method of Swain & Hillis (1959). Carotenoids were quantified by the Talcott and Howard method (Talcott & Howard, 1999). The antioxidant activity was evaluated by the method of Brand-Williams et al. (1995).

The minerals Ca, Mg, K, Cu, Fe, Mn, Zn, and P were quantified from lyophilized pulp samples. After lyophilization, the samples were ground and stored in plastic bags inside desiccators to avoid contact with moisture until the minerals were quantified, which followed the method of Tedesco et al. (1995).

For quantification of macronutrients (Ca, Mg, K, P), 0.2 g of lyophilized sample were mixed with 0.7 g of digestion mixture (CuSO4 + Na2SO4), 1.0 mL of hydrogen peroxide and 2 mL of sulfuric acid. The samples were kept in a digester block for 30 min (160 to 180 °C). Afterwards, the temperature was raised to 350 to 375 °C, until the solution cleared (greenish yellow tone) and kept for 60 min for complete digestion. To determine Ca and Mg, 1 mL of the digested sample added with 4 mL of 0.1% lanthanum oxide and water was evaluated in an atomic absorption equipment. In the same equipment, K readings

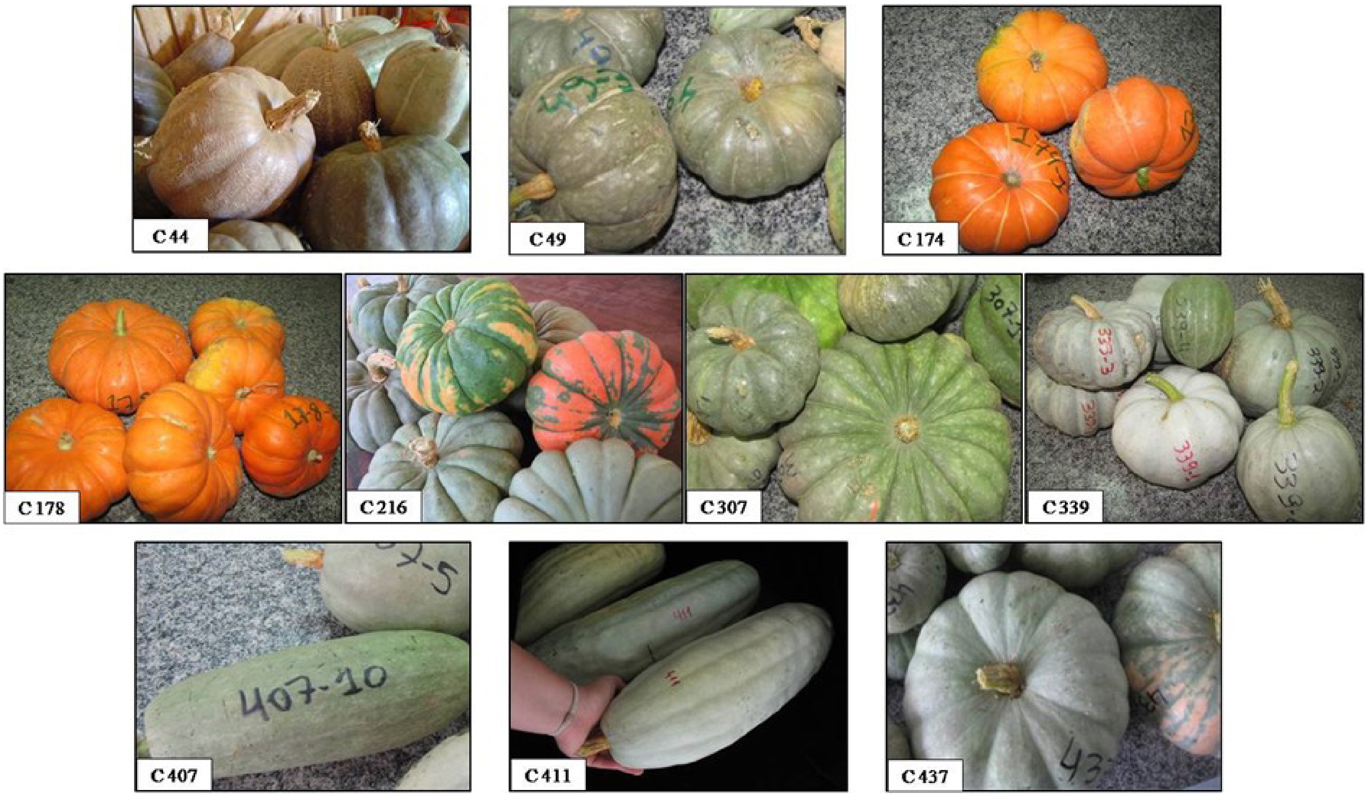

Figure 1. Accessions of pumpkin landraces (*Cucurbita maxima)* from the Cucurbitaceae Genebank of Embrapa Temperate Agriculture used for the analysis of bioactive compounds, antioxidant activity, and minerals. Photos: Rosa Lía Barbieri and Eduardo Valduga.

Table 1. Accessions of pumpkin landraces (*Cucurbita maxima*), from the Cucurbitaceae Genebank from Embrapa Temperate Agriculture, characterized for bioactive compounds, antioxidant activity and minerals.

Access	Common name	Municipality of origin
C44	abóbora-verde-alongada	Garibaldi, RS
C49	moranga-enxuta	Renascença, PR
C174	abóbora	Pelotas, RS
C178	abóbora-moranga-vermelha	São Lourenço do Sul, RS
C216	abobrinha-rajada	Pelotas, RS
C307	abóbora	Casca, RS
C339	moranga-para-tortéi	David Canabarro, RS
C407	abóbora-perna-de-moça	Mostardas, RS
C411	moranga-grande	Caçapava do Sul, RS
C437	abóbora-de-casco	Tavares, RS

were performed in 1 mL of digested sample diluted in water. For determination of P, 1 mL of the digested sample was used, added with 2 mL of distilled water, 3 mL of PB solution (HCl 0.87M and $(NH4)6Mo7O24.4H2O$ 0.38%), 3 drops of PC solution (1-amino-2-naphthol-4-sulfonic acid, sodium sulfite and sodium metabisulfite). After shaking and resting for 30 min, readings were taken in a spectrophotometer at a wavelength of 660 nm (Silva, 2009).

For quantification of micronutrients (Cu, Fe, Mn, Zn), 1.0 g of the sample was added to 6 mL of concentrated nitric acid, in a digestion tube. After 24 h of rest, the samples were placed in the digestion block for 30 min with a temperature between 80 and 90 °C and, subsequently, a temperature between 120 and 130 °C until 0.5 and 1 mL of solution remained. After 10 min of rest, 1 mL of perchloric acid was added, keeping the solution for 2 h between 180 and 190 °C. After digestion, the samples were read in an atomic absorption equipment (Silva, 2009).

The data obtained were subjected to analysis of variance to find any significant differences between the materials. When verifying the existence of a significant difference between treatments, according to the *p* value from the F test, the magnitude of these differences was evaluated by multiple-comparisons testing. Tukey's test was used to compare means with 95% confidence. Statistical analyses were performed using the statistical software SAS 9.2 (SAS Institute, 2011) and the statistical software Genes (Cruz, 2001). The histograms were prepared in Microsoft Office Excel.

3 Results and discussion

The chemical analysis showed variability in the bioactive compounds, antioxidant activity (Table 2), and minerals (Table 3) in the fruit pulp of the accessions of the *C. maxima* pumpkin landraces evaluated.

In addition to being recognized as a bioactive product and a functional food, due to scientific research (Ferreira et al., 2016; Mahmoodpoor et al., 2018; Vale et al., 2019 it is also important to highlight they nutritional composition of pumpkins. Their pulp is rich in complex B vitamins, vitamin C, dietary fiber, phosphorus, potassium, calcium, sodium, magnesium, iron, and carotenoids (beta-carotenes that have excellent performance as provitamin A). Antioxidant properties are also reported, which confer the inhibition of free radicals, thus reducing the risk of developing cardiovascular diseases and cancer (Anjos et al., 2017; Anastácio et al., 2020).

The values for total phenolic compounds found in the different accessions ranged from 16.8 mg/100 g in accession C178 to 65.5 mg/100 g in accession C49. In the commercial cultivar Tetsukabuto, the value was also high, 58.4 mg/100 g, which did not differ statistically from the value in C49. Other authors, who also used the Folin-Ciocalteu reagent to measure total phenolic compounds, obtained higher values for this species (160 mg/100 g) (Tiveron, 2010) and for *C. moschata* accessions (79.9 mg/100 g) (Priori et al., 2017). Sátiro et al. (2020) found lower values of total phenolic compounds (20.35 mg/100 g) in *C. moschata*. The values found by Nobre (2016) in *C. moschata)* were lower: 33 mg/100 g. In *maxima*, contents of total phenolic compounds of 56 mg/100 g were found, lower than those found in this work (Sharma & Rao, 2013).

Table 2. Means for total phenolic compounds, total carotenoids, and total antioxidant activity in accessions of *Cucurbita maxima* from Cucurbitaceae Genebank of Embrapa Temperate Agriculture and in the hybrid cultivar Tetsukabuto.

Accession	Total phenolic compounds[1]	Total carotenoids[2]	Antioxidant activity[3]
C44	24.5 ef	13.3 c	163.2 d
C49	65.5 a	29.3 a	504.1 a
C174	22.2 ef	10.4 c	164.2 d
C178	16.8 f	10.4 c	135.2 d
C216	44.1 bcd	31.2 a	221.4 cd
C307	40.9 cd	29.2 a	325.1 bc
C339	36.6 cde	14.8 bc	275.1 cd
C407	45.2 bc	18.7 b	328.4 bc
C411	32.1 cdef	18.1 b	247.6 cd
C437	28.2 def	17.9 b	200.2 cd
Tetsukabuto	58.4 ab	30.4 a	445.8 ab

[1]Total phenolic compounds, expressed in mg of chlorogenic acid equivalent/100 g of fresh weight; [2]Carotenoids, expressed in mg of β-carotene equivalent/100 g of fresh weight; [3]Antioxidant activity, expressed in µg of Trolox equivalent/g of fresh weight. Means followed by the same letter in each column do not differ significantly by Tukey's test at 5% error probability.

Like antioxidant activity, phenolic compound levels can be affected by several factors in plants, including the environment (seasonality, temperature, water availability, ultraviolet radiation, atmospheric pollution) degree of fruit maturation, solvent used in the extraction of phenolic compounds and genetics. These compounds are widely used in the food industry because of their effectiveness in preventing lipid oxidation and because the consumption of foods rich in phenolic compounds is linked to the prevention of non-communicable chronic diseases (Gobbo-Neto & Lopes, 2007; Llorach et al., 2008; Shimano, 2012; Pereira & Angelis-Pereira, 2014; Guimarães et al., 2019).

The range of total carotenoid concentrations found in this study is similar to those reported by Ramos et al. (2009) in *C. maxima* fruits, who measured values ranging from 10.5 to 35.6 mg/100 g and a mean of 25.3 mg/100 g. In this study, the highest concentrations of carotenoids were in the accessions C216 (31.2 mg/100 g), C49 (29.3 mg/100 g), and C307 (29.2 mg/100 g). It was 30.4 mg/100 g in the Tetsukabuto cultivar (Table 2). Lower values for carotenoids were found in other studies with *Cucurbita* ssp. (Boschi, 2015; Seroczyńska et al., 2006; Molica, 2015; Provesi, 2010). A study carried out with *C. moschata* showed that the levels of total carotenoids ranged from 23.4 mg/100 g to 40.4 mg/100 g (Carvalho et al., 2012).

Carotenoids, as represented in the pulp pigmentation of *C. maxima* fruits (yellow, orange, and red), are known for their benefits to humans. Animals cannot synthesize carotenoids again and, therefore, those found in animals are directly accumulated from food or partially modified through metabolic reactions, presenting a great structural diversity and important biological roles as precursors of vitamin A, photoprotectors, antioxidants, immunity enhancers and contributors to reproduction (Maoka,

Table 3. Content minerals present in the fruit pulp of the accessions of *Cucurbita maxima* from the Cucurbitaceae Genebank of Embrapa Temperate Agriculture and Tetsukabuto hybrid cultivar. Pelotas, RS, Brazil.

Accession	Calcium (Ca)	SD	Magnesium (Mg)	SD	Potassium (K)	SD	Phosphorus (P)	SD	Copper (Cu)	SD	Iron(Fe)	SD	Manganese (Mn)	SD	Zinc (Zn)	SD
C44	13.23 c	0.9	7.42 bcde	0.3	233.55 cd	10.1	6.52 de	1.1	0.03 bcd	0.0	0.08 a	0.0	0.01 e	0.0	0.08 c	0.0
C49	4.55 e	0.2	7.00 bcde	0.2	206.30 de	7.4	7.70 de	2.1	0.03 bc	0.0	0.04 b	0.0	0.01 e	0.0	0.05 d	0.0
C174	20.46 b	0.5	6.35 de	0.4	240.88 c	1.7	2.41 f	0.3	0.03 bcd	0.0	0.03 bc	0.0	0.04 bc	0.0	0.03 ef	0.0
C178	27.16 a	0.8	8.10 bc	0.5	361.11 a	5.3	18.39 a	0.4	0.04 b	0.0	0.03 bc	0.0	0.01 e	0.0	0.03 def	0.0
C216	12.65 cd	3.0	8.01 bcd	1.6	333.29 a	3.3	14.73 b	1.0	0.06 a	0.0	0.10 a	0.0	0.03d	0.0	0.10 b	0.0
C307	12.05 cd	0.2	6.19 e	0.5	246.70 c	12.3	11.76 bc	2.1	0.03 bcd	0.0	0.01 c	0.0	0.03 cd	0.0	0.04 de	0.0
C339	9.64 d	0.8	6.33 de	0.4	240.21 c	1.4	6.12 de	2.0	0.02 d	0.0	0.01 c	0.0	0.03 d	0.0	0.04 def	0.0
C407	11.97 cd	0.9	6.59 cde	0.5	227.78 cd	21.4	8.64 cd	0.5	0.02 d	0.0	0 d	0.0	0.01 e	0.0	0.01 f	0.0
C411	14.08 c	0.1	8.0 bcd	0.1	232.36 cd	11.7	5.78 def	0.6	0.02 d	0.0	0.09 a	0.0	0.07 a	0.0	0.11 b	0.0
C437	18.16 b	1.3	9.92 a	0.3	295.49 b	4.7	12.70 b	0.7	0.04 bc	0.0	0.08 a	0.0	0.05 b	0.0	0.04 def	0.0
Tetsukabuto	14.60 c	0.4	8.71 ab	0.1	193.55 e	10.1	4.89 ef	0.1	0.02 cd	0.0	0.09a	0.0	0.03 cd	0.0	0.19 a	0.0

Means followed by the same letter in each column do not differ significantly by Tukey's test at 5% probability. Calcium in mg/100 g of fresh weight; magnesium in mg/100 g of fresh weight; phosphorus in mg/100 g of fresh weight; potassium in mg/100 g of fresh weight; copper in mg/100 g of fresh weight; iron in mg/100 g of fresh weight; manganese in mg/100 g of fresh weight; zinc in mg/100 g of fresh weight; SD = Standard deviation.

2020), which has contributed to its valuation within the market of bioactive compounds (Ferreira et al., 2021).

The results for the antioxidant capacity among the evaluated accessions showed great variation (Table 2). Accession C49 had the highest antioxidant capacity, 504.1 μg·g^{-1}, while accession C178 had the lowest value, 135.2 μg·g$^{-1.}$

In foods, antioxidant activity may come from nutrients such as vitamins A, C and E, or from non-nutrients such as carotenoids, flavonoids, total phenolic compounds, phenolic compounds and minerals such as copper, zinc, magnesium and iron (Saxena et al., 2007). In particular, total phenolic compounds and carotenoids are the most important antioxidants for the human diet, as they have physical or chemical mechanisms of action to neutralize the effects of reactive oxygen species resulting from cellular activities and oxygen metabolism (Cerqueira et al., 2007; Universidade Federal do Estado do Rio de Janeiro, 2021). These factors help protect against oxidative processes in the body and are important in intercepting free radicals (Duarte-Almeida et al., 2006).

Pumpkin, along with carrots and spinach, are cited by Universidade Federal do Estado do Rio de Janeiro (2021) as examples of plant foods abundant in vitamin A precursor carotenoids. The consumption of these vitamin A-rich foods, such as pumpkin pulp, for example, it is very important for embryonic development, protection of the body against oxidative stress, vision and immune system functioning.

Using the same method that we used, Priori et al. (2017) also found a wide range of values between accessions, with the lowest range of values for *C. moschata*, whose highest-antioxidizing accession had an activity of 357.8 μg·g^{-1}. Tiveron (2010) and Duarte-Almeida et al. (2006) found a wide range of values among *C. maxima* accessions, with a lower mean value than we saw (12.7 μg·g^{-1}). The use of different analytical methods may also yield results that are not comparable with each other (Chun et al., 2005). Attarde et al. (2010) evaluated the antioxidant capacity in *C. maxima* by the DPPH method and found values of 393, 355 and 155 μg•g-1. Another point to consider is the fact that the use of different solvents to obtain the extracts (methanol, water/methanol, water/ethanol) makes it difficult to compare the different studies (Gonçalves et al., 2015). However, differences in results between studies may be related to the genotypes of the species studied and environmental factors such as soil characteristics, temperature, and humidity, as well as the harvest season, which may cause great variations in the contents of bioactive compounds and thus in the antioxidant capacity of the vegetables.

There was a high correlation (r = 0.910) between the content of total phenolic compounds and the antioxidant activity in the *C. maxima* accessions evaluated (Figure 2). The same was also reported in the study by Priori et al. (2017), who found a high correlation between total phenolic compounds and antioxidant activity in *C. moschata* accessions (r = 0.801). Vizzotto et al. (2007) also found a high correlation between total phenolic compounds and antioxidant activity in peaches (r = 0.85) and plums (r = 074). For Jacobo-Velázquez & Cisneros-Zevallos (2009), this positive correlation is common because the antioxidant activity is directly related to the phenolic profile.

The correlation between antioxidant activity and total carotenoid concentration (Figure 3) was lower (r = 0.653), though it was still higher than that reported by Priori et al. (2017) in *C. moschata* accessions (r = 0.217) and Amariz (2011), who did not observe a correlation in *C. moschata* accessions of the Active Germplasm Bank of Embrapa Semi-Arid.

Minerals play an important role in the human body. By weight, the human body is 96.05% water and only 3.95% mineral matter. The main minerals that make up the human body are calcium (2.34%), phosphorus (1.06%), potassium (0.29%), and sodium (0.21%). Other minerals, such as iron, fluorine, zinc, copper, manganese, and iodine, together make up only 0.15% of the body weight (Dhaar & Robbani, 2008).

The levels of minerals in fruits depend highly on the soil, fertility, climate, and variety (Nour et al., 2011). They are divided into micro- (Cu, Fe, Mn, Zn) and macronutrients (Ca, Mg, K, P). Micronutrients play a central role in the metabolism and maintenance of tissue function. Therefore, an adequate intake of these components is necessary. There is a growing interest

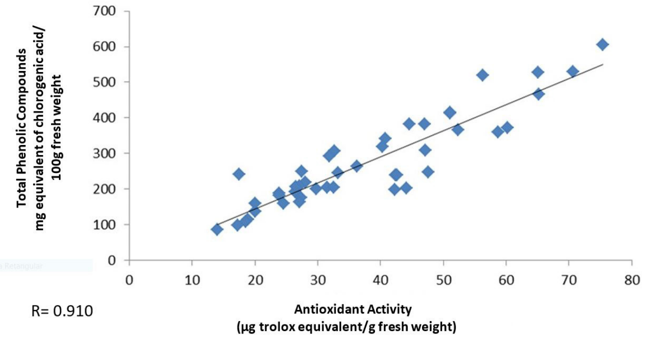

Figure 2. Correlation between total phenolic compounds and total antioxidant activity in 10 accessions of *Cucurbita maxima* from the Cucurbitaceae Genebank of Embrapa Temperate Agriculture and the hybrid cultivar Tetsukabuto.

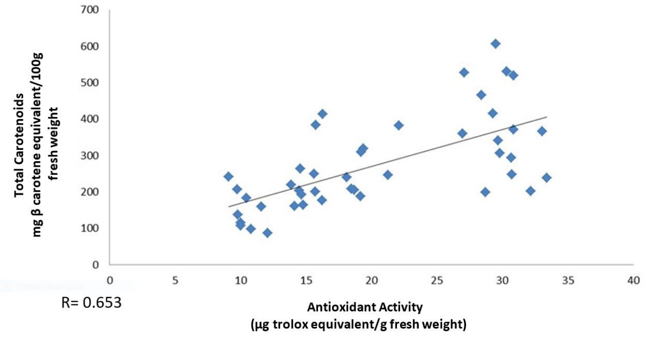

Figure 3. Correlation between total antioxidant activity and total carotenoids in 10 accessions of *Cucurbita maxima* from the Cucurbitaceae Genebank of Embrapa Temperate Agriculture and the cultivar Tetsukabuto hybrid.

in the role of micronutrients in optimizing health and in the prevention or treatment of diseases; this is largely due to increased knowledge and understanding of the biochemical functions of these nutrients (Shenkin, 2006). Wide variation was observed in the contents of all minerals across the accessions (Table 3).

Calcium ranged between 4.55 mg/100 g in accession C49 and 27.16 mg/100 g in accession C178 (Table 3). Blessing et al. (2011) observed a lower calcium concentration (24.4 mg/100 g) for pumpkin (*Cucurbita* spp.) than some of the values found in this study. Calcium is a fundamental mineral for organisms due to its role in blood coagulation, neurological function, muscle contraction, metabolic processes, and bone and tooth formation (Kitumbe et al., 2013). Broccoli is considered a source of calcium; for every 100 g of broccoli, it has 86 mg of calcium. The daily recommendation of calcium for food is 1,000 mg/day for adult men and women (Institute of Medicine, 2001). Based on this information, we can say that, for an adult's nutrition, an average portion of 200 g of pumpkin (accession C178) would contribute 5.4% of their daily calcium needs.

Magnesium ranged from 6.19 to 9.92 mg/100 g fresh weight, the highest value being in accession C437. Accession C437 showed a higher magnesium content in the pulp (9.92 mg/100 g) than the cultivar Tetsukabuto (8.71 mg/100 g). United States legislation recommends an average daily intake of 420 mg/day magnesium for adult men and 320 mg/day magnesium for adult women (Institute of Medicine, 2001). Our findings mean that, for an adult man, an average portion of 200 g of pumpkin (accession C437) would contribute 4.7% of his daily magnesium needs. Thus, the supply of magnesium could come in part from the consumption of the pulp of *C. maxima* variety fruits. Magnesium plays a key role in the body in many reactions, including the metabolism of carbohydrates, lipids, proteins, and nucleic acids. Thus, it is very important that the human body's levels of magnesium be adequate to prevent diseases, including cardiovascular and systemic diseases. Low regular intake of this mineral is associated with a higher chance of developing depression in adults (Tarleton & Littenberg, 2015). The main dietary sources of magnesium are vegetables, legumes, seafood, nuts, cereals, and dairy products (Dutra-de-Oliveira & Machini, 2003). Some 55% to 60% of the magnesium in the body is found in bone tissue, associated with phosphorus and calcium, and the rest is found in amorphous form (Cônsolo, 2015).

Phosphorus ranged from 2.41 to 18.39 mg/100 g, accession C178 having the highest value. Phosphorus has one of its most important functions in the formation and mineralization of the bone organic matrix. It is also one of the components of nucleic acids (DNA and RNA), a constituent of energy reserve molecules (ADP and ATP), functions in the metabolism of fats (formation of lecithin), and participates in the maintenance of osmotic pressure and basic acid balance and in the enzymatic systems involved in glucose metabolism (Litz, 2013). Accession C178 presented higher amounts of phosphorus than those reported in the Brazilian Table of Food Composition – TACO (Table of Food Composition, 2004), which reports an average of 12 mg/100 g in pumpkin (Universidade Estadual de Campinas, 2011). The recommended average daily intake is 700 mg/day of phosphorus for adult men and women (Institute of Medicine, 2001). Thus, to feed an adult, an average portion of 200 g of pumpkin (accession C178) would contribute 5.2% of their daily phosphorus needs.

For mineral potassium, the accessions showed values of 193.5 mg/100 g in the Tetsukabuto cultivar, 333.29 mg/100 g in C216, and 361.11 mg/100 g in C178 (Table 3), indicating the superiority of the accessions over the hybrid cultivar regarding the concentration of this mineral in the fruit pulp. The *C. maxima* accessions analysed in this study showed higher amounts of potassium than the values in TACO (2004), which showed values of 126 and 165 mg/100 g for pumpkin (*Cucurbita* ssp.) (Universidade Estadual de Campinas, 2011). Blessing et al. (2011) observed lower potassium levels in squash (*Cucurbita* spp.) (ranging from 123.89 to 217.669 mg/100 g) than those found in this study. Potassium is a very important mineral for the human body because it is present in most cells, and *C. maxima* accessions have proved to be an important source of this mineral. The recommended daily intake is 4.7 mg/day of potassium for adult men and women (Institute of Medicine, 2001); thus, we can say that, for the feeding of an adult, an average portion of 200 g of pumpkin (accession C178) would contribute 15.3% of their daily potassium needs. This mineral has functions such as regulating the osmotic balance of the cell, acting as an available base to neutralize acids in the acid–base balance, maintaining the water balance in the body, and activating several enzymatic systems (Araújo et al., 2010).

Copper varied from 0.02 to 0.06 mg/100 g (Table 3), accession C216 having the highest value. Copper is a micronutrient necessary for human physiology and is the third most common trace element in the human body. The recommended average daily intake of copper for adult men and women is 0.9 mg/day (Institute of Medicine, 2001). Following this daily recommendation, an adult consuming 200 g of pumpkin/day would supply 13% of their daily copper needs. Accession C216 showed more copper than the values reported in TACO (2004), which has 0.05 mg/100 g for pumpkin (*Cucurbita* ssp.) (Universidade Estadual de Campinas, 2011).

Manganese ranged from 0.01 to 0.07 mg/100 g in our accessions, C411 having the highest value. The main functions of manganese in the human body include participation in the synthesis of mucopolysaccharides and indirect intervention in the processes of cartilage and bone formation. Its deficiency may be associated with impaired carbohydrate and lipid metabolism, inadequate bone development, and reduced fertility (Waitzberg, 2009). In addition to being present in the soil, manganese is also found in large amounts in foods such as nuts (4.7 mg/kg), cereals (4.1 mg/kg), grains (4.1 mg/kg), fruits (0.2-10.4 mg/kg), cattle meat, and fish and eggs (0.1-3.99 mg/kg) (Martins & Lima, 2001; Gropper et al., 2009). The daily manganese recommendation is 2.3 mg/day for men and 1.8 mg/day for women (Institute of Medicine, 2001). From this daily recommendation, an adult man consuming 200 g of pumpkin/day would supply 6.0% of his daily manganese needs. Values for manganese reported in Table of Food Composition (2004) are low (0.01 mg/100 g), equivalent to the lowest value found in this study for pumpkin (*C. maxima*).

The zinc concentration ranged from 0.01 (accession C407) to 0.19 mg/100 g (Tetsukabuto) (Table 3). Zinc is an essential mineral to the human body, is involved in various aspects of cellular and molecular metabolism, and is necessary for the activity of more than 200 enzymes and for the functioning of the immune system, cell division, and protein and DNA synthesis (Bailey et al., 2015; Institute of Medicine, 2001). The daily recommendation for zinc is 11 mg/day for men and 8 mg/day for women (Institute of Medicine, 2001). From this recommendation, we can say that for the feeding of an adult, an average portion of 200 g of pumpkin (cultivar Tetsukabuto) would contribute 3.4% of their daily zinc requirements.

Iron had values between 0.01 and 0.10 mg/100 g (Table 3), C216 having the highest value. The value for iron reported in TACO (0.2 mg/100 g) was below the value found in this study. Iron is a metal involved in the regulation of growth and is an integral part of proteins and enzymes that perform various physiological functions, and most iron is contained in hemoglobin, whose function is oxygen transport and cellular respiration (Jomova & Valko, 2011). Iron is one of the most important minerals in the human diet (Dhaar & Robbani, 2008). The daily recommendation for iron is 8 mg/day for men and 18 mg/day for women (Institute of Medicine, 2001). From this recommendation, we can say that for the feeding of an adult, an average portion of 200 g of pumpkin (accession C216) would contribute 25% of their daily iron needs.

Accession C178 stood out for its calcium, phosphorus, and potassium concentrations, and accession C216 showed excellent values for copper, iron, and potassium. The Tetsukabuto cultivar showed high values for iron (0.09 mg/100 g), magnesium (8.71 mg/100 g), potassium (193.55 mg/100 g), and zinc (0.19 mg/100 g). This means that the accessions of *C. maxima* of the Cucurbitaceae Genebank of Embrapa Temperate Agriculture offer important characteristics as part of a genetic improvement programe for a variety of biofortified pumpkin.

4 Conclusion

The contents of bioactive compounds, the antioxidant activity, and the mineral concentrations vary widely between the fruit pulps of accessions of pumpkins landraces, *Cucurbita maxima,* from the Cucurbitaceae Genebank of Embrapa Temperate Agriculture. Accessions C49, C307, and C216 have high levels of phenolic compounds, carotenoids, and antioxidant activity. Accessions C216 and C178 show high values of copper and iron, and the latter also has the highest values of potassium, calcium, and phosphorus. Accessions of *C. maxima* landraces, especially C216 and C178, can be exploited in breeding programs for the development of biofortified cultivars in an effort to promote consumer health.

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