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Food Technology/ Original Article

Phenolic compounds, carotenoids, and antioxidant activity in a super-sweet corn hybrid

Abstract – The objective of this work was to determine the total carotenoids, total phenolics, and antioxidant activity of 'UENF SD 08' (super-sweet corn) and to compare them with those of 'UENF50611' (field corn). The total carotenoid content was determined according to Rodriguez-Amaya, the total phenolic content by the Folin-Ciocalteau method, and the antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging method. For 'UENF SD 08', the average values were: 936.76±16.34 μg 100 g^{-1} for carotenoids, 346.0±3.80 mg 100 g^{-1} for phenolics, and 45.53±0.01% for antioxidant activity. For UENF50611, the average values were: 1,173.38±21.66 μg 100 g^{-1} for carotenoids, 563.33±7.97 mg 100 g^{-1} for phenolics, and 59.82±0.11% for antioxidant activity. UENF50611 showed a higher antioxidant activity observed due to its higher levels of carotenoids and phenolic compounds; however, the values observed for the 'UENF SD 08' super-sweet corn are within the ranges already described in the literature. 'UENF SD 08' contains a lower concentration of carotenoids and phenolic compounds than 'UENF50611', its nonmutant genetic background. However, the super-sweet corn 'UENF SD 08' has agronomic advantages, is considered a source of the evaluated secondary metabolites, and its consumption can contribute to a diet with a higher content of antioxidants.

Index terms: *Zea mays*, bioactive substances, secondary metabolites.

Compostos fenólicos, carotenoides e atividade antioxidante em híbrido de milho superdoce

Resumo – O objetivo deste trabalho foi determinar os carotenoides totais, os fenólicos totais e a atividade antioxidante de 'UENF SD 08' (milho superdoce) e compará-los com os de 'UENF50611' (milho comum). O conteúdo de carotenoides totais foi determinado de acordo com Rodriguez-Amaya, o de fenólicos totais pelo método de Folin-Ciocalteau e a atividade antioxidante pelo método de sequestro de radicais livres de 2,2-difenil-1-picrilhidrazila (DPPH). Para 'UENF SD 08', os valores médios foram: 936,76±16,34 μg 100 g^{-1} para carotenoides, 346,0±3,80 mg 100 g^{-1} para fenólicos e 45,53±0,01% para atividade antioxidante. Para 'UENF50611', os valores médios foram: 1.173,38±21,66 μg 100 g^{-1} para carotenoides, 563,33±7,97 mg 100 g^{-1} para fenólicos e 59,82±0,11 para atividade antioxidante. O milho 'UENF50611' apresentou maior atividade antioxidante devido aos seus maiores teores de carotenoides e compostos fenólicos; porém, os valores observados para o milho superdoce 'UENF SD 08' estão dentro das faixas já descritas na literatura. 'UENF SD 08' contém menor concentração de carotenoides e compostos fenólicos que 'UENF50611', seu background genético não mutante. Contudo, o milho superdoce apresenta vantagens agronômicas, é considerado fonte dos metabólitos secundários avaliados e seu consumo pode contribuir para dieta com teor mais elevado de antioxidantes.

Termos para indexação: *Zea mays*, substâncias bioativas, metabólitos secundários.

Introduction

Brazil is one of the largest grain producers in the world. Among the most prominent crops is the corn crop (*Zea mays* L.), whose production in 2019/2020 was around 101 million metric tons. Despite the high production, about 70% of the corn produced in Brazil is destined for animal consumption (Faustino et al., 2020). Even though the genetic richness of corn is conserved in active germplasm banks, the selection of materials for the generation of new cultivars is mainly focused on agronomic characteristics. Adaptation to climatic conditions, high yield, high resistance to pest and disease attacks, attractive appearance, and ability to support different handling and processing operations and not sensory characteristics are the most desired attributes (Pinto et al., 2021).

In Brazil, most studies of corn crops do not focus on components of nutritional importance and bioactive substances, which could provide value to this food and encourage the use of this crop in natura or for the development and commercialization of food products with claims of health benefits. Furthermore, meeting the characteristics of the field does not guarantee that the nutritional characteristics will be achieved (Teixeira et al., 2013).

The interest of researchers and the population itself for foods that, in addition to providing nutrients, are also sources of biologically active substances that provide additional health benefits, has grown (Küster-Boluda & Vidal-Capilla, 2017). The higher prevalence of chronic non-communicable diseases, which are often triggered by degenerative processes resulting from excess reactive oxygen species, which cause damage at the cellular level and activate some components of the inflammasome, could be prevented or have its complications minimized through the consumption of substances with antioxidant capacity (Abais et al., 2015).

In edible parts of vegetables, bioactive substances can have other functions such as odor, color, and a more astringent taste. In addition, when consumed, they can perform some beneficial biological activities in the human organism, such as antioxidant activity (Gonzalez de Mejia et al., 2020).

Corn crops are good sources of bioactive phytochemicals. It is noteworthy that the grains contain polyphenols, phenolic acids, flavonoids, anthocyanins, carotenoids, and other substances of medicinal importance (Barabási et al., 2020).

The super-sweet corn hybrid, 'UENF SD 08', resulting from genetic improvement research, at Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), was registered in 2018 at Registro Nacional de Cultivares of Ministry of Agriculture, Livestock and Supply (MAPA). Researches that have evaluated its agronomic characteristics have already been carried out (Pereira et al., 2019b). However, to aggregate knowledge to this cultivar, studies on its nutritional characteristics, levels of bioactive substances, and its antioxidant capacity are of great importance, for possible use in industry or even to encourage local production and trade.

The objective of this work was to determine the total carotenoids, total phenolics, and antioxidant activity of 'UENF SD 08' (super-sweet corn) and to compare them with those of 'UENF50611' (field corn).

Materials and Methods

The super-sweet corn cultivar used in this study has the shrunken-2 gene (sh2), a mutant recessive allele that leads to a failure in the sucrose-to-starch conversion mechanism. The hybrid 'UENF SD 08' is the result of genetic improvement research carried out by the UENF, for over 20 years. The common corn cultivar used as a control was UENF50611, also developed by UENF. This genetic background has the same parent populations of common corn, cultivar UENF SD 08, however, before the introduction of the mutant gene and obtaining the lines.

Hybrids were grown at Escola Agrícola Antônio Sarlo of UENF, located in the municipality Campos dos Goytacazes, in the Norte Fluminense region, in the state of Rio de Janeiro, Brazil (21º24'48"S, 41º44'48"W, at 14 m of altitude), in the 2017/2018 harvests. According to Köppen-Geiger (Beck et al., 2018) the climate classification is Aw (tropical climate with summer rainfall). Since it is close to the coast, the climate is controlled by equatorial and tropical air masses with influence from the tropical maritime mass. The soil in this region is predominantly Acrisols and Cambisols (Driessen et al., 2001; Santos et al., 2018). The base fertilization was performed with 800 kg ha^{-1} of the chemical fertilizer formulated N-P_2O_5-K_2O 04-14-0. The first covering fertilization was performed

30 days after sowing with 300 kg ha^{-1} of the fertilizer formulated $N-P_2O_5-K_2O$ 20-05-20 and the second covering fertilization was performed with 200 kg ha^{-1} urea (45% N) 45 days after sowing. Harvesting was performed 22 days after female flowering, considered the optimal period, both for productivity (Guan et al., 2013), and for taste and texture quality (Camilo et al., 2015).

The content of total carotenoids (TC) was determined from samples of super-sweet and field corn in natura were lyophilized using the L202 lyophilizer (Liotop, São Carlos, SP, Brazil), ground in a Willey knife mill**,** and sieved on Tyler series particle size sieves (25 mesh, 0.71 mm opening). After cold extraction, performed with acetone and petroleum ether, the TC was quantified by spectrophotometry at 450 nm (Rodriguez-Amaya, 2001). The results were expressed in µg of carotenoids per 100 g fresh material. This analysis was performed in duplicate. The concentration of total carotenoids was calculated using the following equation: TC (µg carotenoids per gram of sample) = $100(A \times V \times 10^4/ E1\%_{1cm} \times m)$; in which A is the absorbance at 450 nm; V is the final volume of the sample (mL); m is the sample mass (g); $E1\%_{1cm}$ is the extinction coefficient of beta-carotene in petroleum ether = 2592.

To prepare the ethanolic extracts, the corn kernels were ground in the FP05 multiprocessor (Multilaser, São Paulo, SP, Brazil) and were then transferred to glass jars, capped and wrapped in aluminum foil and then left at 25°C, for 72 hours. The extracts were prepared in a 1:2 (solute: solvent) ratio; the used solvent was ethyl alcohol. After this period, the extracts were filtered in a Whatman filter (North Bend, OH, United States) and left in a water bath, at 20ºC (temperature was monitored with a thermometer), under the hood, and protected from light, until complete evaporation of the solvent. Then, they were stored in a refrigerator at 7°C until the analyses were performed. The experiment was carried out in triplicate.

The total phenolic content of the extracts prepared previously, ethanolic extracts was determined according to the method described by Singleton et al. (1999) with modifications. Gallic acid (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) was used for the calibration curve and concentrations ranged from 0 a 500 µg mL^{-1}. A sample aliquot (0.1 mL) diluted in methanol (1.0 mg mL^{-1}) was added to 1.0 mL methanol and 0.1 mL Folin-Ciocalteau reagent (Sigma-Aldrich, Germany). The mixture was homogenized and then 1.0 mL Na_2CO_3 (7%) was added after 5 min. The reaction was carried out for 90 min in the dark at 25°C. The absorbance was read at 760 nm and the results were expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE 100 g^{-1}). The analysis was performed in triplicate.

The in vitro photocolorimetric method of the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) (Blois, 1958) was used to evaluate the extracts (super-sweet corn and field corn) antioxidant activity.

Samples were prepared in methanol at concentrations ranging from 2.0, 0.2**,** and 0.02 mg mL^{-1}. Samples (0.5 mL) were added to 0.5 mL of methanolic solution of DPPH (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) (4%) for final concentrations of 1.0, 0.1, and 0.01 mg mL^{-1}. The reaction was incubated in the dark for 60 min at 25ºC. After incubation, the absorbance was determined in the UVmini-1240 spectrophotometer (Shimadzu, Kyoto, Japan) at 515 nm.

The free radical scavenging activity of each extract was expressed by the ratio of DPPH uptake based on the DPPH solution absent from the extract (negative control) and a solution of a standard aromatic substance (positive control), 2,6-di-(tert-butyl)-4-methyl phenol or butylated hydroxytoluene (BHT). Subsequently, the free radical scavenging percentage (SP) was calculated. The free radical scavenging ability was expressed as a percentage of inhibition of radical oxidation and was calculated using the following equation: % Inhibition = $[(Ac - Aa)/Ac] \times 100$, where Ac is the absorbance of the negative control (DPPH + methanol solution); and Aa is the absorbance of the sample after 60 min.

The normality of the data was initially tested by Shapiro-Wilk's test. The results were expressed as mean ± standard deviation. Student's t-test was used to analyze the total carotenoid and total phenolic data. For antioxidant activity, the analysis of variance was used, and when a significant difference was detected, the means were compared using Tukey's test at 5% probability. To better understand the contribution of total carotenoids and total phenolic contents to the antioxidant activity, the correlation estimated by Pearson's correlation coefficient (r) was performed. For all the analyses performed, the significance level established was $p<0.05$. All data were analyzed by the IBM SPSS Statistics 19 software (Armonk, NY, USA).

Results and Discussion

The mean concentration of total carotenoids (TC) and total phenolics (TP) were higher ($p<0.05$) in the 'UENF50611' corn when compared with those determined for the 'UENF SD 08' hybrid (Table 1).

Antioxidant activity results are in Table 2. There was a statistically significant difference between the samples of ethanolic extracts and among the samples and the control (BHT), regardless of the concentration used. When considering only the ethanolic extracts of the different types of corn, 'UENF50611' presented a higher antioxidant capacity than 'UENF SD 08'.

Among the secondary metabolites (SM) analyzed, a strong correlation ($r>0.70$) was observed between TP content and antioxidant activity, regardless of the concentration used (Figure 1 A). This strong correlation indicates that the contribution of phenolic compounds to the antioxidant activity of the genotypes developed is relevant. However, the correlation between antioxidant activity and carotenoid content was moderate ($r<0.70$) (Figure 1 B).

The concentration of TC observed for 'UENF SD 08' super-sweet corn was lower than observed for 'UENF50611' common corn ($p<0.05$). However, the observed values, regardless of the type of corn, were within the concentration ranges reported by other authors as Cardoso et al. (2009) and Lopez-Martinez & Garcia (2015). In the first study, the researchers evaluated more than 134 corn genotypes for use in biofortification programs. The average concentration range varied from 946 μg 100 g^{-1} to 4,284 μg 100 g^{-1}. Lopez-Martinez & Garcia (2015) evaluated more than 44 types of sweet corn and found values ranging from 400 μg 100 g^{-1} to 3,300 μg 100 g^{-1}.

Similar to those mean carotenoid contents, TP contents were also higher for the 'UENF50611' ($p<0.05$). TP concentrations observed in this study for 'UENF50611' were similar to those observed by Lopez-Martinez et al. (2009), who evaluated 18 different types of corn and found average values of 551 mg 100 g^{-1}. For 'UENF SD 08', the mean values observed were similar to those reported by and higher than those described by Hu et al. (2011), which were of 340 mg 100 g^{-1} and 124 mg 100 g^{-1} respectively.

In general, the SM concentrations of plants do not remain stable as they do with other qualitative or quantitative traits. The main factors that may be responsible for variations in these concentrations can be divided into four main groups: genetic (only 15 to 25% of genes contribute to SM synthesis), ontogenic, morphogenetic, and environmental factors (Verma & Shukla, 2015).

For corn crops, it is well established that different growing conditions and external factors that affect the plants, such as microorganisms, insects, and herbivorous animals, can influence SM concentrations. Phenolic compounds are known to be more responsive to environmental factors than carotenoids (Zaynab et al., 2018). The degree of ripeness of the harvesting season would also be another factor to be considered, since different ripening stages may imply different phytochemical concentrations, including the content of carotenoids would be more affected than that of phenolic compounds (Hu et al., 2021). Nevertheless, there is no consensus on which factors would

Table 1. Total carotenoids and total phenolic contents of 'UENF SD 08' and UENF50611 maize (*Zea mays*) genotypes.

	Maize genotype	
	'UENF SD 08'	UENF50611
Total Carotenoids (μg 100 g^{-1} fresh material)	936.76±16.34*	1,173.38±21.66*
Total Phenolic (mg 100 g^{-1} sample)(1)	345.81±3.80*	563.28±7.97*

Results expressed as mean ± standard deviation: total carotenoids (n=2), total phenolic (n=3). (1)mg in gallic acid equivalent 100 g^{-1}. *Averages differ statistically from each other by Student's t test at 5% probability.

Table 2. Antioxidant activity of ethanolic extraction of 'UENF SD 08' and UENF50611 maize (*Zea mays*) genotypes(1).

Maize genotype	Ethanolic extract concentration		
	1 μg mL^{-1}	0.1 μg mL^{-1}	0.01 μg mL^{-1}
'UENF SD 08'	45.53±0.01a	17.42±0.01a	13.95±0.01a
UENF50611	59.82±0.11b	21.49±0.01b	19.86±0.01b
BHT (control)	79.44±0.01c	78.79±0.19c	67.70±0.01c

(1)Averages followed by same letter in the column do not differ from each other by Tukey''s test at 5% probability. BHT, 2,6-di-(tert-butyl)-4-methylphenol or butylated hydroxytoluene.

specifically affect the content of total carotenoids and total phenolics in different maize crops since there is a wide variety of species (Lopez-Martinez & Garcia, 2015).

A potential reason for the different levels of TC and TP concentrations observed between the two cultivars in this study could be the influence of the genetic factor since the experimental conditions of the growing season and harvest were the same. Although the genetic basis of 'UENF SD 08' and UENF50611 is the same, except for the presence of the gene shrunken-2 (sh2), 'UENF SD 08' is a simple hybrid. It was generated from two lines and by backcrossing (Pereira et al., 2019b), whereas UENF50611 is an interpopulation hybrid, meaning that it was generated by crossing between two populations (Pereira et al., 2019a). Therefore, the presence of the mutant gene could allow different gene interactions to occur, which would modify the metabolism of the cultivar and consequently affect the synthesis of SM (Entringer et al., 2017).

Regarding antioxidant activity, the results observed for both 'UENF50611' and 'UENF SD 08' were similar to those described by Lopez-Martinez et al. (2009). These researchers evaluated 18 types of corn for antioxidant activity and observed that for yellow corn hybrids, regardless of whether sweet or super-sweet, the antioxidant activity ranged from 40 to 60%. The percentage observed in this study is relevant and expressive since inhibition percentages lower than 40% show a weak capacity to sequester the DPPH (Ferreira et al., 2015). Therefore, both cultivars could have their consumption associated with the supply of substances with antioxidant capacity.

The mean values observed for antioxidant activity (Table 2) are consistent with the contents of TC and TP determined in the samples, since the higher the concentration of SM, the higher is the antioxidant

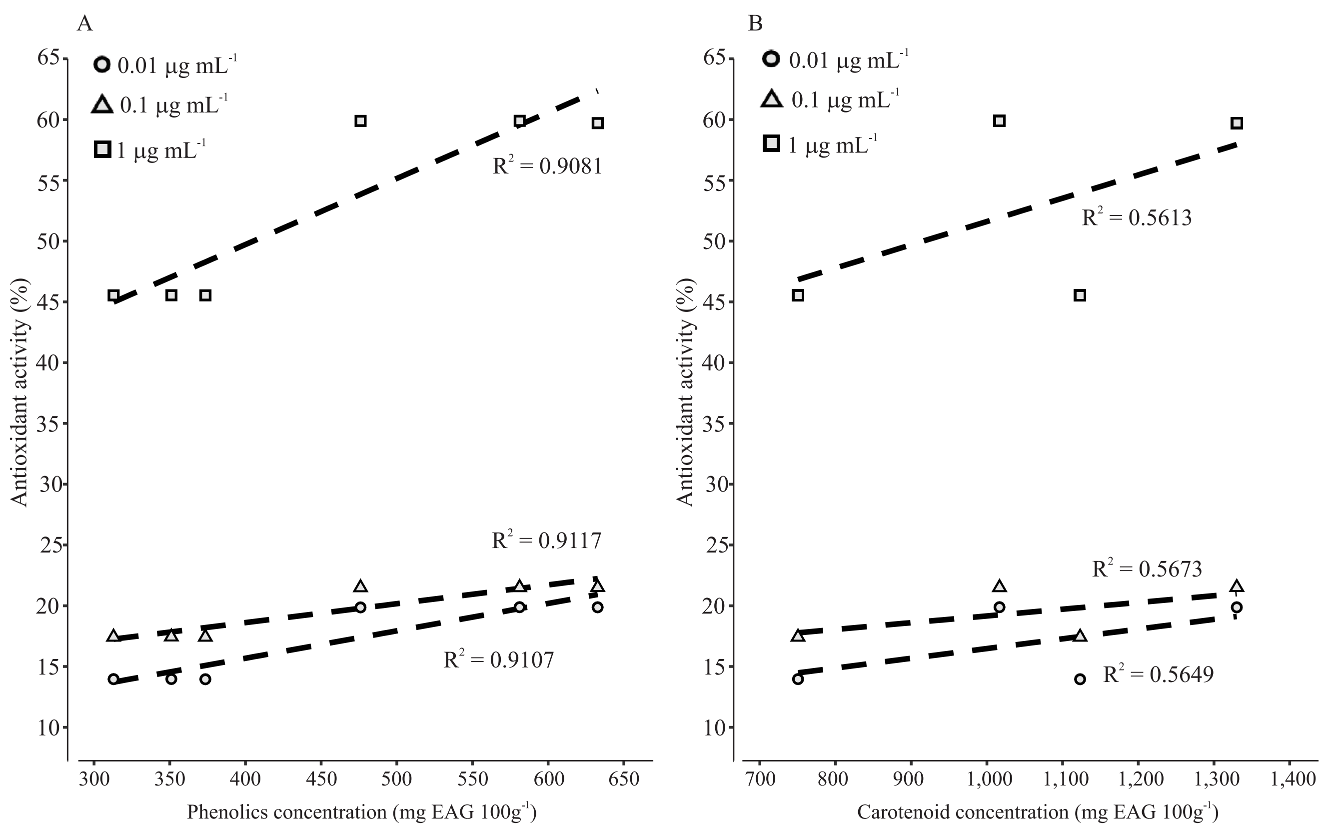

Figure 1. Correlation between total phenolic and antioxidant activity (A) and total carotenoids and antioxidant activity (B) of different maize (*Zea mays*) ethanolic extracts: 'UENF SD 08' and UENF50611. The correlation value between phenolics and carotenoids was 0.7259.

potential of the plant (Isah, 2019). However, the correlation analysis revealed that the phenolic compounds contribute more strongly to the total antioxidant activity than the TC, regardless of the concentration used. Therefore, TP contributes more to the reactive oxygen species (ROS) sequestering action, and as they are in greater proportion in the 'UENF50611' sample, this genotype presented higher antioxidant activity.

The importance of quantifying the SM lies in the fact that they determine important aspects in the quality of human food (Uarrota et al., 2011). In vitro studies have already demonstrated that phenolic substances have antimicrobial, antioxidant, antiviral, anti-inflammatory, and vasodilatory properties (Thirumurugan et al., 2018). For carotenoids, antioxidant properties, protection against oxidative damage to cellular components, prevention of cardiovascular diseases and cancer are described (Oluba & Oredokun-Lache, 2018). Therefore, the consumption of foods that are sources of these substances results in health benefits and contributes to an improved quality of life (Castejón-Veja et al., 2020).

In addition, evaluating the antioxidant activity of the samples is also relevant, since antioxidants are substances that can neutralize ROS and maintain a redox balance. The excess ROS arising from redox imbalance has been investigated as a trigger for activation of the inflammasome, a signaling complex with multiple proteins that would be involved in an inflammatory immune response and the pathophysiology of degenerative diseases (Anand, 2020).

Conclusions

1. 'UENF SD 08' super-sweet corn (*Zea mays*) hybrid has lower contents of total carotenoids, total phenolics, and antioxidant activity when compared with 'UENF50611', its nonmutant genetic background; however, 'UENF SD 08' has agronomic advantages, is considered a source of these secondary metabolites, and its consumption can contribute to a diet with a higher content of antioxidants.

2. 'UENF SD 08' total carotenoids and total phenolic contents, as well as the antioxidant activity, are within the average concentrations described in the literature and expected for maize crops.

3. In both genotypes, total phenolic compounds are more strongly associated with antioxidant activity than total carotenoids.

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