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Physicochemical characterization and antioxidant capacity of popcorn nejayote

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Abstract

The pursuit of reusing agribusiness residues towards decreasing their environmental impact has led to the study of its components to know their possible applications. The nejayote is the alkaline wastewater of different food products of nixtamalized corn. This study characterized the physicochemical properties of popcorn nejayote (NP) and determined its total phenolics content (TP) and antioxidant capacity by standard synthetic assays (ABTS and DPPH) and physiologically relevant ones (reducing power -RP-, hydroxyl radical scavenging activity -OH-, and iron chelation IRON). NP antioxidant capacity determined by ABTS, and DPPH methods were 387.9±0.7 and 419.3±2.9 mM Trolox equivalents (mM TE)/mL, respectively. The NP showed 296% RP compared to a control solution of coumaric acid (0.1 M). Also, the NP was obtained at 143% IRON compared to a control solution of caffeic acid (0.1 M). In the case of OH, values ranged from 27% to 45% compared with control solutions. TP was 0.26±0.006 µg gallic acid equivalents/mL (mg GAE/mL). Pearson's correlation analysis found that TP is correlated to the OH, IRON, and DPPH, while IRON activity correlates with OH and ABTS. And finally, OH correlated with DPPH. To the best of our knowledge, this study is NP's first antioxidant capacity report.

1. Introduction

Popcorn is a variety of corn kernels widely used as a healthy snack due to its high fibre content; its annual production represents around £785.7 million (Thakur *et al*., 2021). Popcorn kernels have thicker endosperm that pops up when heated (Sweley *et al*., 2012) changing the size, shape, diameter (Karababa *et al*., 2006) and molecular mapping of popping volume (Thakur *et al*., 2021). Nejayote is the residual water obtained from maize's nixtamalization process to obtain masa and derived products. This process consists of alkaline cooking by soaking and washing the corn kernels in calcium hydroxide with water. This residue water contains a large load of solids and dissolved organic matter, which causes clogging of sewers and drainage, a high calcium salts content, and a pH above 11 that making nejayote an environmental pollutant (Niño-Medina *et al*., 2009).

However, nejayote has been explored as a potential agglutinant and as a chelating agent for water treatment due to its electrochemical properties (González *et al*., 2003; López-Maldonado *et al*., 2017). Research has shown that nejayote can improve the nutritional characteristics of foods through its dietary fibre, calcium, and phenolic compounds content without affecting taste (Niño-Medina *et al*., 2010; Rojas-García *et al*., 2012; Acosta-Estrada *et al*., 2014). Other potential uses of nejayote are related to its anti-inflammatory activity and its use as a bacterial culture medium (Baqueiro-Peña *et al*., 2019; Buitimea-Cantúa *et al*., 2020). Biological properties reported in nejayote include antioxidant capacity, determined by ABTS and DPPH assays (Castañeda-Ruelas *et al*., 2021; Herrera-Balandrano *et al*., 2018; Mora-Rochin *et al*., 2010). The ABTS and

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DPPH assays determine the capacity to reduce redox-active compounds. These assays are widespread and used in fruits, cereals, and vegetables, but they use non-physiological radicals (Floegel *et al*., 2011). Other antioxidants assays, such as the Reducing Power Assay (RP), iron chelation assay (IRON), and the hydroxyl radical test (OH), measure physiologically relevant molecules that can cause damage in the cell (Phaniendra *et al*., 2015). This study aimed to characterize NP, including its antioxidant capacity, physicochemical properties, composition, and FTIR spectral data.

2. Materials and methods

2.1 Materials

The popcorn kernel was obtained in a local store (Great Value, Walmart, USA). All chemicals and reagents used were of analytical grade.

2.2 Extraction method

The nejayote was obtained by cooking 200 g of popcorn kernels in a solution with 1 g of calcium hydroxide dissolved in 400 mL of water for 40 mins at 91°C. The remaining water was filtered to obtain the nejayote (NMX-FF-034/1-SCFI-2002,Norma Mexicana, 2002). The NP pH was adjusted to 5 with HCl 1 N (Salmerón-Alcocer *et al*., 2003) and dried in a convection oven (40°C) (Memmert, UN 110, Germany).

2.3 Chemical proximate composition

The NP moisture was determined with a thermobalance (OHAUS, MB45, USA) by method 925.09 (Association of the Analytical Chemists (AOAC), 1998). Ash content was determined following the AOAC 942.05 method (AOAC, 2005). Lipids were analysed following AOAC 920.75 (AOAC, 1920), and total protein was measured using an automated Kjeltec 8200 with a Nx factor of 6.25 (FOSS, USA). The soluble protein was determined using the Bradford method modified (Bonjoch *et al.,* 2001), in a microplate 10 µL of sample and 100 µL of Bradford's reagent reacted for 10 min and the absorbance was measured at 595 nm in a microplate reader with bovine serum albumin calibration curve (0.062 to 1mg/mL). Total soluble solids were measured by a digital refractometer (ATAGO, Japan) in °Brix. The measurements were expressed as the average of three replicates values ± standard deviation (SD).

2.4 Total and reducing sugars

Total sugar content was determined by the methodology of Dubois *et al*. (1956), 0.6 mL of 5% phenol was mixed with 1 mL of sample (0.05% w/v), then, 3.6 mL of concentrated H_2SO_4 was added to the tubes, shaken, and allowed to stand for 30 mins. Subsequently, they were placed in a cold-water bath to stop the reaction. Finally, the sample was read at a wavelength of 490 nm by a spectrophotometer (Thermo Fisher Scientific, Waltham, USA). A calibration curve of glucose was used to determine the concentration of total sugars (5-60 µg/mL).

The quantification of reducing sugars was determined by the methodology of Miller (1959) with the 3.5 dinitro salicylic acid (DNS). In a test tube, 0.02 mL of sample (0.5%) was mixed with 1 mL DNS and placed in a water bath for 10 mins. Then kept at room temperature for 15 mins. Finally, the sample was read at 540 nm in a spectrophotometer (Thermo Fisher Scientific, Waltham, USA). A glucose calibration curve was used to determine the concentration of reducing sugars (50-500 µg/mL).

2.5 Fourier-transform infrared spectroscopy

The study of NP structure was determined with a Fourier-transform infrared spectroscopy (FT-IR) spectrometer (Agilent Cary model 630, USA) with an integration time of 6 seconds and at room temperature (25°C). Initially, OriginPro 8 (v8.0724, USA) software performed the baseline and spectrums were smoothed. The FT-IR also allowed knowing the secondary structure of proteins in the nejayote using the amide I, amide II, and amide III corresponding bands. The amide I band was observed at 1610–1694 cm^{-1} (Carbonaro and Nucara, 2010; Rojas-Candelas *et al*., 2022; Rojas-Candelas *et al*., 2023), and the assigned structures were β -sheet: 1610-1639 cm^{-1} and 1685-1669 cm^{-1}, turns 1662-1684 cm^{-1}, α-helix: 1650-1658 cm^{-1}, random coil: 1640-1650cm^{-1}. The amide II band was observed at 1500-1560 cm^{-1}. The assigned structures were β-Antiparallel: 1510–1530 cm^{-1}, β-Parallel: 1530-1550 cm^{-1} (Pelton and McLean, 2000). The deconvolution method was used to evaluate the areas of the regions mentioned.

2.6 Total phenols content

Total phenols content was determined according to the methodology reported by Singleton and Rossi (1965), with modifications for the measurement in the microplate reader applied by Bobo-García *et al*. (2015). At the microplate 100 µL Folin-Ciocalteau reactive diluted 1:4 with distilled water and 20 µL NP solution (10% with methanol (80%)) were mixed during 60 seg and was incubated for 4 mins. After incubation 75 µL solution sodium carbonate (100 g/L) was added. It was agitated and incubated at room temperature in the dark for 2 hrs. Finally, the absorbance was measured at 750 nm. TP values are expressed as µg gallic acid equivalents/ml of sample (mg GAE/mL) according to a gallic acid standard curve (10 mg/25 mL). It used a

https://doi.org/10.26656/fr.2017.7(5).174

microplate reader (Multiskan Go, Thermo Fisher Scientific, Waltham, USA).

2.7 Antioxidant capacity

2.7.1 ABTS and DPPH

DPPH radical scavenging activity of NP was determined by the methodology of Bobo-García *et al*. (2015). The radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained by reaction of 150 µMol/L DPPH solution with methanolic solution (methanol 80: water 20), mixed and left at dark for 40 mins. At a microplate, 20 µL of NP solution (10% with methanol (80%)) was added with 180 µL of DPPH and left for 40 min. Then absorbance was read at 515 nm. ABTS radical scavenging activity was determined according to Leite *et al*. (2011). The ABTS radical was obtained with the reaction of 7 mmol/L ABTS stock solution with 2.45 mmol/L of potassium persulfate at room temperature in the dark for 12-16 h. The ABTS radical was diluted up to an absorbance of 0.700 at 754 nm. 250 µL of ABTS radical solution and 50 µL of NP solution (10% with methanol (80%)) were mixed and measured the absorbance after 6 mins at 754 nm. The concentration range of the Trolox calibration curve was 0 to 400 µMol/L. The results are expressed in mM Trolox equivalents (TE)/mL.

2.7.2 Reducing power

The reducing power (RP) of the nejayote was determined according to the methodology of Oyaizu (1986). 20 µL nejayote solution (10% with methanol (80%)) was added with 50 µL phosphate regulator (0.2 M, at pH 6.6) and 50 µL potassium ferricyanide (1%) in the microplate. It was incubated at 40°C for 20 mins. Then it was added 50 µL trichloroacetic acid (10%) and 10 µL $FeCl_3$ (0.1%). Finally, it was incubated for 10 mins at 40°C, and the absorbance was read at 700 nm. The results are presented as a percentage reduction in absorbance compared to four control solutions: ferulic acid, caffeic acid, Trolox, and coumaric acid (0.1 M). It was used a microplate reader (Multiskan Go, Thermo Fisher Scientific, Waltham, USA).

2.7.3 Hydroxyl radical scavenging activity

The methodology of the hydroxyl radical scavenging activity (OH) reported by Li *et al*. (2008) was used in this research. In the microplate reader 50 µL NP solution (10% with ethanol, w/v), 50 µL of 1, 10-phenanthroline, and $FeSO_4$ (3 mM). Then it was aggregated 50 µL H_2O_2 and mixed at 37°C for 60 mins. The absorbance was read at 536 nm. The results are presented as the percentage reduction in absorbance compared to four control solutions: ferulic acid, caffeic acid, Trolox, and coumaric acid (0.1 M).

2.7.4 Iron chelation

The chelating activity of Fe^{2+} was determined by the methodology of Carter (1971) with a few modifications. In the microplate reader, 20 µL of nejayote solution (10% with methanol (80%)) was added with 230 µL sodium acetate buffer (100 mM, at pH 4.9) and 30 µL $FeCl_2$ solution (0.1 mg/mL). The mix was incubated for 30 mins at room temperature and then was added 12.5 µL ferrozine (40 mM). The results are presented as a percentage reduction in absorbance compared to four control solutions: ferulic acid, caffeic acid, Trolox, and coumaric acid (0.1 M).

2.8 Statistical analysis

The measurements are expressed as average values ± standard deviation (SD), n = 3. Data were compared using the ANOVA-Tukey test, and non-significant differences were considered when $p > 0.05$. Pearson analysis was used to obtain the correlations between assays of antioxidants and the total phenols of nejayote. XLSTAT software (v. 2020.1.3, Addinsoft, USA) was used for these analyses.

3. Results and discussion

3.1 Physicochemical parameters

The nejayote is an agro-industrial residue of nixtamalization, so several parts of the kernel, such as endosperm, pericarp, and germ contribute to the nejayote chemical composition. Table 1 shows the composition of dried nejayote. Its moisture value was like the value reported by Castañeda-Ruelas *et al*. (2021). Protein content (6.30±0.30%) and total soluble solids (1.8 °Brix) for nejayote are in accordance with those described by Acosta-Estrada *et al*. (2014), which reported 5.61%, and Díaz-Montes *et al*. (2016) reported a value of 1.53 °Brix respectively. At the same time, Castañeda-Ruelas *et al*. (2021) reported a protein value of 10.34% in nejayote extracted from the white corn kernel. The insoluble protein content is higher than soluble protein; this protein comes from the alkaline hydrolysed pericarp, while the soluble protein comes from the germ and endosperm. These last components are found more internally in the grain, they are present in less quantity in the nejayote (Ayala-Soto *et al*., 2014). Fat and total sugars were different from Castañeda-Ruelas *et al*. (2021), which reported fat values around 6.47% in nejayote of white corn. This is because they studied nejayote collected from a factory such as Castañeda-Ruelas *et al*. (2021), which extracted the nejayote with some modifications in the methodology and used different types of kernel (Acosta-Estrada *et al*., 2014). In our case, the nejayote studied was extracted in the laboratory according to the regulatory Mexican norm. The chemical composition of

https://doi.org/10.26656/fr.2017.7(5).174

nejayote showed that it is a reliable source of protein and sugars for food applications.

Table 1. Physicochemical parameters of dried nejayote.

Parameter	Values
Moisture (%)	7.34±0.14
Total soluble solids (°Bx)	1.8
Ash (%)	51.39±0.43
Fat (%)	0.69±0.14
Total sugars (%)	15.0±0.45
Reducing sugars (%)	5.38±0.13
Non-reducing sugars (%)	9.59±0.35
Nitrogen (%)	1.00±0.04
Crude protein total (%)	6.30±0.30
Crude protein soluble (%)	0.11±0.003
Crude protein insoluble (%)	6.23±0.30

Results expressed as mean value±SD.

3.2 FTIR spectra of nejayote

In addition to sugars, proteins, and lipids, nejayote contains starch, polysaccharides, and phenolic compounds such as p-coumaric, ferulic acid (Baqueiro-Peña *et al*., 2019). These components were observed in the FT-IR spectrum (Figure 1) with their specific absorption areas and the formation of their secondary structure of the proteins of nejayote. The typical absorption areas of the functional groups of carbohydrates in NP's spectra were found at 900-1200 cm^{-1}. The characteristic signals of carbohydrates are present at 1150 and 1080 cm^{-1}. The band at around 1160 cm^{-1} is related to cellulosic material (Wellner, 2013), while the bands typical of the arabinose were observed in 1079 and 1100 cm^{-1}. The signal at 835 cm^{-1} is the characteristic band related to the p-coumaric acid even though it presented a low intensity, so it is concluded that the nejayote has a low level of p-coumaric (Chateigner-Boutin *et al*., 2016). Additionally, Figure 1 shows phenolic groups associated with ferulic acid around 1519 cm^{-1} (Buitimea-Cantúa *et al*., 2020). The signals at 1076 and 1029 cm^{-1} are the fingerprint of characteristic bands related to glucose (Petibois *et al*., 1999). The second part of the spectrum showed lipids (3050cm^{-1}) (Ogbaga *et al*., 2017). The band at around 3350 cm^{-1} is related to the OH and NH stretching, while the CH stretching is 2929 cm^{-1}. Finally, the protein zone (1200-1800 cm^{-1}) and aromatic molecules (1609, 1608, 1516, and 1517 cm^{-1}) (Buitimea-Cantúa *et al*., 2020). The aromatic molecules or intermolecular zone gives foods emulsification properties (de la Rosa-Millán *et al*., 2018), while the protein zone is divided into two parts with the amide I band at 1610–1694 cm^{-1} and amide II at 1500-1560 cm^{-1}. The deconvolution method was applied to obtain the secondary structures of the protein present in NP. The NP obtained 22.73% of the β-sheet structure (Figure 2), and it is divided into two parts β-antiparallel (12.73%) and β-parallel (10.60%). Compared with a 55.73% α-helix structure, these secondary structures give emulsification and gelation properties in food (de la Rosa -Millán *et al*., 2018). The highest value of the secondary structures in nejayote was random coil with 18.15% (Gómez *et al*., 2013), and the 3.37% remaining is from the turn structures. The importance of the knowledge of FTIR spectra of nejayote is to understand the protein's internal structure and if it can have major active sites free to capture oxidative species.

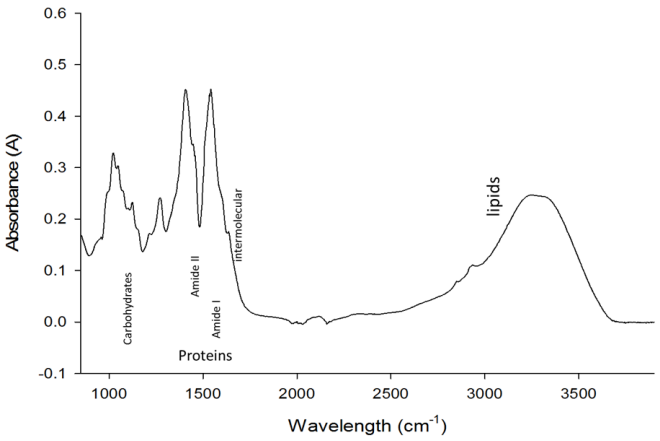

Figure 1. FT-IR spectra of the nejayote.

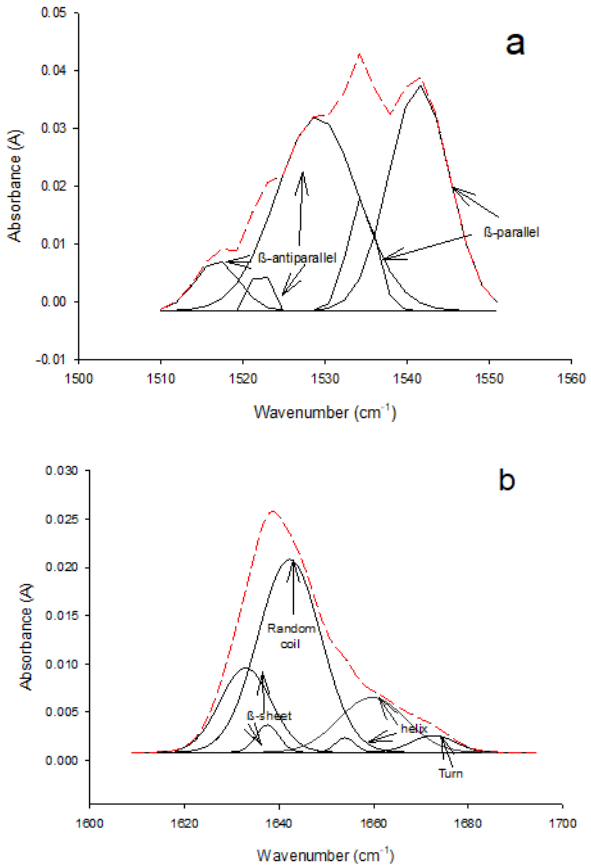

Figure 2. FT-IR spectra of nejayote for the amide II band (a) and amide band I (b) regions. The peaks-fitting corresponding for each secondary structure identified (β-antiparallel, β-parallel, turn, random coil, and α-helix) in the nejayote.

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3.3 Total phenols content

Phenolic compounds found in maize are anthocyanins, p-coumaric acid, vanillic acid, protocatechuic acid, ferulic acid, hydroxybenzoic acid, caffeic acid, syringic acid, gallic acid, and quercetin. Ferulic acid is found in greater quantity in maize than in other ones (Hernández-Martínez *et al*., 2016). The ferulic acid stabilizes reactive radicals by hydrogen atoms and then converts a phenoxy radical. This radical cannot react or generate chain reactions and can only be condensed with another radical and collision (Graf, 1992). The TP values found in NP are lower (0.26±0.006 mg GAE/mL) from the literature because they used other methodologies or control solutions, Castañeda-Ruelas *et al*. (2021) reported 68.62±1.53 mg GAE/100 g in white corn nejayote. Zhang *et al*. (2017) found TP values of 38.00 to 57.04 mg GAE/100 g in eight sweet corn varieties. Another research reported values in different milling cereals and found that the barley and spelt flour fractions showed higher values of TP of approximately 232.9±3.6 mg GAE/g (Ivanišová *et al*., 2012).

3.4 Antioxidant capacity

3.4.1 ABTS and DPPH

Table 2 shows the values of ABTS and DPPH radical scavenging activity of NP. Herrera-Balandrano *et al*. (2018) reported antioxidant capacity values of white corn nejayote of 16.60 to 21.27 µmol TE/g by ABTS and 29.49 to 31.69 µmol TE/g by DPPH. Another report of with corn nejayote antioxidant capacity measured by ABTS was 1670.02 µmol TE/100g (Castañeda-Ruelas *et al*., 2021). In described mechanisms, it is known that the DPPH radical reacts with polyphenols (catechins, proanthocyanins) but not with phenolic acids and sugars. Therefore, the antioxidant values of DPPH and ABTS are partially different. Herrera-Balandrano *et al*. (2018) reported values of DPPH from nixtamalized maize bran of 29.49 to 31.69 µmol TE/g; these values are lower than the values reported in this research. The DPPH of different agro-industrial solid wastes has already been evaluated in other works, and the data show that the antioxidative activity varies widely. Makris *et al*. (2007) conducted a study on this variability and compiled a wide range of DPPH data of other agro-industrial solid waste such as grape seeds, olive tree leaves, apple peels, onion peels, potato peels, and carobs. The grape seeds had the highest value, around 5.94±0.11 mm TE/g expressed as Trolox equivalents. Compared to the values reported in this research, NP antioxidant capacity is higher.

Table 2. Antioxidant activity (ABTS and DPPH) of nejayote and total phenols (TP).

Sample	TP	ABTS	DPPH
Nejayote	0.26±0.006	387.9±0.7	419.3±2.9

Values reported as mM Trolox equivalents (TE)/mL to ABTS and DPPH while total phenols (TP) reported mg GAE/mL.

3.4.2 Reducing power

The results of RP are shown in Figure 3. The activity of the nejayote is compared with the activity shown by Trolox, caffeic acid, ferulic acid, and coumaric acid. The NP exerts an RP of 99% concerning a Trolox, 103% of caffeic acid, 112% of ferulic acid, and 296% coumaric acid. All values present significantly different among them. López-Martínez *et al*. (2011) observed that corn (around 85% compared with Trolox) has significant RP than the masa (41%) and tortilla (45%), data comparable with the values in this research (Figure 3), where NP has more RP than the elements mentioned before because when the raw material is subjected to different transformation processes, it loses certain elements and changes its properties. Ivanišová *et al*. (2012) studied RP variability in the milling of cereals. They compiled a wide range of data on barley, spelt, oat, wheat, rye, and triticale, being barley the ones that obtained higher values (32-38%) in the RP. Compared to the values reported in this research, the nejayote has higher values, around 99%.

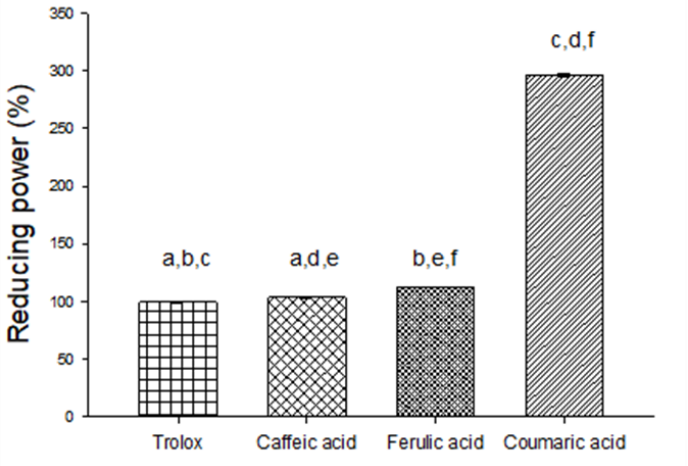

Figure 3. Percentage of reducing power of Nejayote in comparison with Trolox, caffeic acid, ferulic acid, coumaric acid (0.1 M). Values are reported as percentages (%) mean±SD of triplicates. Bars with different notations are statistically significantly different ($p < 0.05$).

3.4.3 Hydroxyl radical

The results of OH are shown in Figure 4. The NP exerts OH scavenging activity at 39% concerning Trolox, caffeic acid at 28%, coumaric acid at 41%, and ferulic acid at 43%. All values present significantly different among them. The bonito fish from Japan had values in OH radical compared with NP around 54±1 compared with ascorbic acid (Sri Kantha *et al*., 1996). Li, Han and Chen (2008) reported OH radicals in protein hydrolysates prepared from corn gluten meal around 20

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to 100% compared with ascorbic acid. Other research obtained results of OH in chickpea protein hydrolysate that was around 36.42 to 81.3%. The two studies occupied protein, but this study's initial values were similar. Wang *et al*. (2008) studied OH radicals in pigments extracted from molasses alcohol wastewater and obtained values from 20 to 98% compared with ascorbic acid.

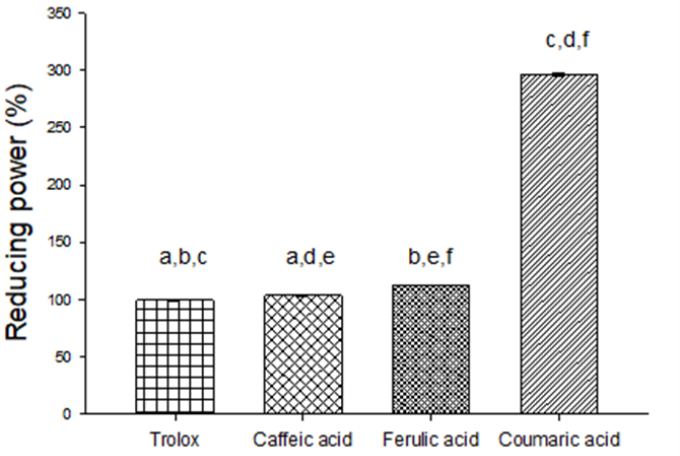

Figure 4. Percentage of hydroxyl radical of nejayote comparison with Trolox, caffeic acid, ferulic acid, coumaric acid (0.1 M). Values are reported as percentages (%) mean±SD of triplicates. Bars with different notations are statistically significantly different ($p < 0.05$).

3.4.4 Iron chelation

The results of IRON are shown in Figure 5. The NP exerts IRON at 135% concerning Trolox, coumaric acid at 117%, ferulic acid at 115% and caffeic acid at 143%. The values that present significant differences are caffeic acid with Trolox, coumaric acid and ferulic acid. Hinneburg *et al.* (2006) carried out a study of antioxidant capacity in herbs and spices from samples low in IRON such as bay leaves (15 mg Na_2EDTA/g extract), huniper (20mg Na_2EDTA/g extract), ginger (30 mg Na_2EDTA/g extract) to high values in IRON such as parsley (178 mg Na_2EDTA/g extract) and fennel (110 mg Na_2EDTA/g extract). At the same time, Wong *et al*. (2014) found values of metal chelation in six medicinal plant extracts where the highest value was 88.2±0.4% of *C. nutans*.

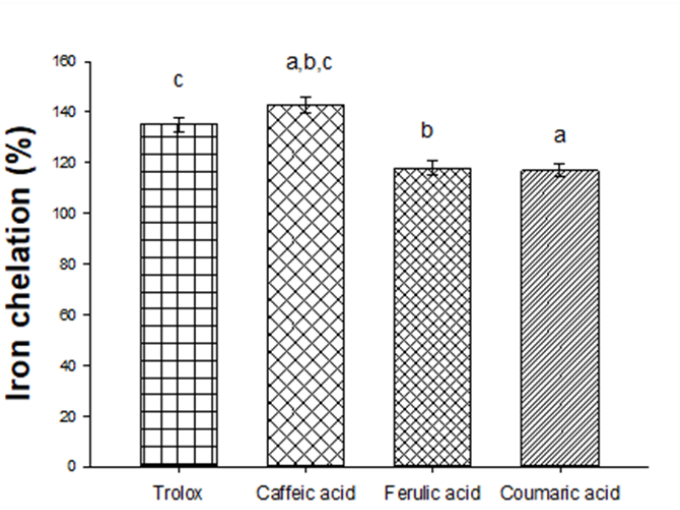

Figure 5. Percentage of iron chelation of nejayote comparison with trolox, caffeic acid, ferulic acid, coumaric acid (0.1 M). Values are reported as percentages (%) mean±SD of triplicates. Bars with different notations are statistically significantly different ($p < 0.05$).

3.5 Pearson correlation in assays of antioxidant capacity

Pearson's correlation analysis (Figure 6) showed the assays of antioxidant capacity used have correlations among them. The intensity of the colorations indicates which assays have the highest positive (green) or negative (red) correlations. The variables with correlation coefficients > 0.70 Pearson criterion can be considered very high correlation (Asuero *et al*., 2006). DPPH had a similarly positive correlation between TP and OH (0.89). Especially this correlation was widely studied in different types of foodstuffs as extracts and seed oil samples (Kozłowska *et al*., 2016), olive oil (Samaniego Sánchez *et al*., 2007), and grapes (Kedage *et al*., 2007). This behaviour by phenols is attributed to the aromatic rings bearing one or more hydroxyl groups. They contribute to scavenging free radicals. The highest values of correlations positive (from 0.96 to 0.99) were OH with TP and IRON. The correlation between OH with TP was observed in a perennial herbaceous plant, *Pouzolzia zeylanica* (L.) Benn, concluding that phenolic compounds are an important contributor to antioxidant capacity (Li *et al*., 2011). But DPPH and ABTS (0.25) do not correlate, and other studies such as Floegel *et al*. (2011) that worked with fruits and vegetables found a correlation positive among these assays. At the same

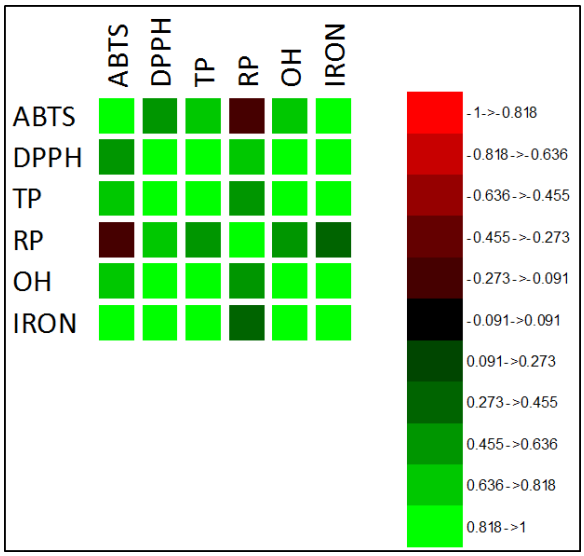

Figure 6. Pearson correlation matrix image using five assays of antioxidant activity and total phenols. ABTS, DPPH, Total phenols (TP), Reducing power (RP), Hydroxyl radical (OH) and Iron chelation (IRON).

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time, a positive correlation exists between DPPH and ABTS with IRON (0.75).

4. Conclusion

Different antioxidant capacity assays were used to measure the antioxidant capacity of NP. It was found that NP has a significant antioxidant capacity compared with other antioxidants such as ferulic acid, caffeic acid, Trolox, and coumaric acid. Pearson correlation analysis showed a positive correlation between physiological antioxidant capacity (RP, OH, and IRON) and the synthetic and standard assays (ABTS and DPPH). The FT-IR spectrum allowed us to know how NP is found because it can have a higher antioxidant capacity with major active sites free and capture reactive species. The study provided some scientific information to reutilize the NP in food benefits and applied it to other industries.

Conflict of interest

The authors declare no conflict of interest.

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