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Antioxidant effect and chemical composition of *Ananas comosus* [L.] Merr. peels from Peruvian Northern

[Efecto antioxidante y composición química de las cascaras de *Ananas comosus* [L.] Merr. del Norte Peruano]

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Abstract: Pineapple peels has several beneficial properties including antioxidant activity. We investigated the antioxidant effect of five different peels of pineapple lyophilized extracts, not adsorbed and adsorbed onto Amberlite. They were examined using total phenolic contents (TPC), antioxidant effect by 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP). In addition, we analyzed the chemical composition by HPLC-ESI-QTOF-MS/MS. The main constituents of pineapple peels were tentatively identified as quercetin glycosides and N,N'-diferuloylspermidine. We conclude that the antioxidant activity in pineapple peels from District of Poroto, Province of Trujillo, Region of La Libertad, can be associated with the presence of flavonoid and spermidines.

Keywords: *Ananas comosus* [L.] Merr.; Antioxidant effect; TPC; DPPH; FRAP; HPLC-ESI-QTOF-MS/MS.

Resumen: Las cáscaras de piña tienen varias propiedades beneficiosas, incluida la actividad antioxidante. Investigamos el efecto antioxidante de cinco exfoliaciones diferentes de extracto liofilizado de piña, no adsorbidas y adsorbidas en Amberlita. Se examinaron utilizando los contenidos fenólicos totales (TPC), el efecto antioxidante mediante la eliminación del radical 1,1-difenil-2-picril-hidrazilo (DPPH) y el poder férrico antioxidante reductor (FRAP). Además, analizamos la composición química por HPLC-ESI-QTOF-MS/MS. Los principales constituyentes de las cáscaras de piña se identificaron tentativamente como glucósidos de quercetina y N,N'-diferuloylspermidina. Concluimos que la actividad antioxidante en las cáscaras de piña del Distrito de Poroto, Provincia de Trujillo, Región de La Libertad, puede estar asociada con la presencia de flavonoides y espermidinas.

Palabras clave: *Ananas comosus* [L.] Merr.; Efecto antioxidante; TPC; DPPH; FRAP; HPLC-ESI-QTOF-MS/MS..

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INTRODUCTION

Fruit and vegetable processing generates around one third of byproducts or waste (O'shea *et al*., 2012), constituting a form of environmental pollution, even representing a risk to human health (Uchoa *et al*., 2008; Galindo-Estrella *et al*., 2009). It is known that many of these byproducts contain vitamins, minerals, fiber, and antioxidants that are important for physiological functions (Matias *et al*., 2005; Felipe *et al*., 2006; Sousa *et al*., 2011; Ribeiro da Silva *et al*., 2014), thus, the use of these byproducts in the production of new food products appears to be a nutritious alternative source of low cost.

The pineapple (*Ananas comosus* (L.) Merr.), botanically is a member of the Bromeliaceae family, comprises about 2000 species and is originated in tropical South America but is now widely grown in all tropical and subtropical areas of the world (Morton, 1987; Mhatre *et al*., 2009; Li *et al*., 2014; Steingass *et al*., 2015).

More than 25 million metric tons of pineapple are produced each year, (FAO, 2017), approximately 30% of its weight is wasted as the peel of the fruit. (Hepton & Hogson, 2003). This waste, can be used to extract and isolate potential bioactive compounds that might be beneficial in the food, pharmaceutical, cosmetics, and textile industries (Sagar *et al*., 2018). As waste products of the pineapple cannery, pineapple peel waste could be a potential source for the extraction of beneficial bioactive compounds as bromelain (Ketnawa *et al*., 2009)

Polyphenolic compounds are secondary plant metabolites that exist ubiquitously in the plant kingdom where they have a wide range of different structures (Kammerer *et al*., 2007) and physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective, and vasodilatory effects (Benavente-Garcia *et al*., 1997; Samman *et al*., 1998; Middleton *et al*., 2000; Manach *et al*., 2005)

In this study, we investigated five different lyophilized extracts of pineapple peels, not adsorbed and adsorbed onto Amberlite, the free radical scavenging capacities were followed via their total phenolic contents, their reaction with the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical, their ferric reducing antioxidant power (FRAP). In addition, we analyzed the chemical composition by HPLC-ESI-QTOF-MS/MS. This is the first report of antioxidant effect and chemical composition analysis of Poroto pineapple peels.

MATERIAL AND METHODS

Ananas comosus Samples: Geographic origin and extraction

Five *Ananas comosus* samples were provided in March 2017 by farmers from the district of Poroto, Province of Trujillo, in the Region of La Libertad, Perú. Therefore, they represent the composition of pineapple from farmer from the same area. The farmers were selected on the basis of the main providers of fruit products in the region. The collection places are shown in Figure No. 1.

The samples were identified the "Herbarium Truxillense de la Facultad de Ciencias Biológicas de la Universidad Nacional de Trujillo". A voucher sample under accession HUT were deposited in this herbarium (see Table No. 1).

The lyophilized ethanol extracts of pineapple peels were prepared as follows: A) The fruits were peeled, to separate the peels from fruits followed by washing them with tap water to remove all dirt particles. Then, 20 g of dried peels were weighted and added to 200 mL of ethanol (EtOH), preheated at 50ºC. The mixture was subjected to soxhlet extraction for 2 hours. The extraction process was twice repeated with another 200 mL of EtOH. Then the extracts were joined, filtered using a filter to remove insoluble particles and evaporated under reduced pressure. The extract was frozen an -80ºC (Arctiko) and then lyophilized with a freeze-dryer (Labconco). The lyophilized was stored at +4 ºC until tested and analyzed. B) The lyophilized extracts of peels were redissolved in water, filtered and adsorbed onto Amberlite XAD-7, pre-treated as described in Jiménez-Aspee *et al*. (2014). Phenolic compounds were desorbed from the resin using MeOH and MeOH:H2O 7:3 (v/v) and the combined extracts of each sample were taken to dryness and lyophilized. The phenolic-enriched peels extracts (PEPE) were concentrated under reduced pressure and lyophilized for its analysis.

Chemicals

Folin-Ciocalteu phenol reagent (2 N), reagent grade Na_2CO_3, $AlCl_3$, HCl, $FeCl_3$, $NaNO_2$, NaOH, quercetin, trichloroacetic acid, sodium acetate, HPLC-grade water, HPLC-grade acetonitrile, reagent grade EtOH and formic acid were obtained from

Merck (Darmstadt, Germany). Gallic acid, TPTZ (2,4,6-Tris-(2-pyridyl)-s-triazine), Trolox, and DPPH (1,1-diphenyl-2-picrylhydrazyl radical) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

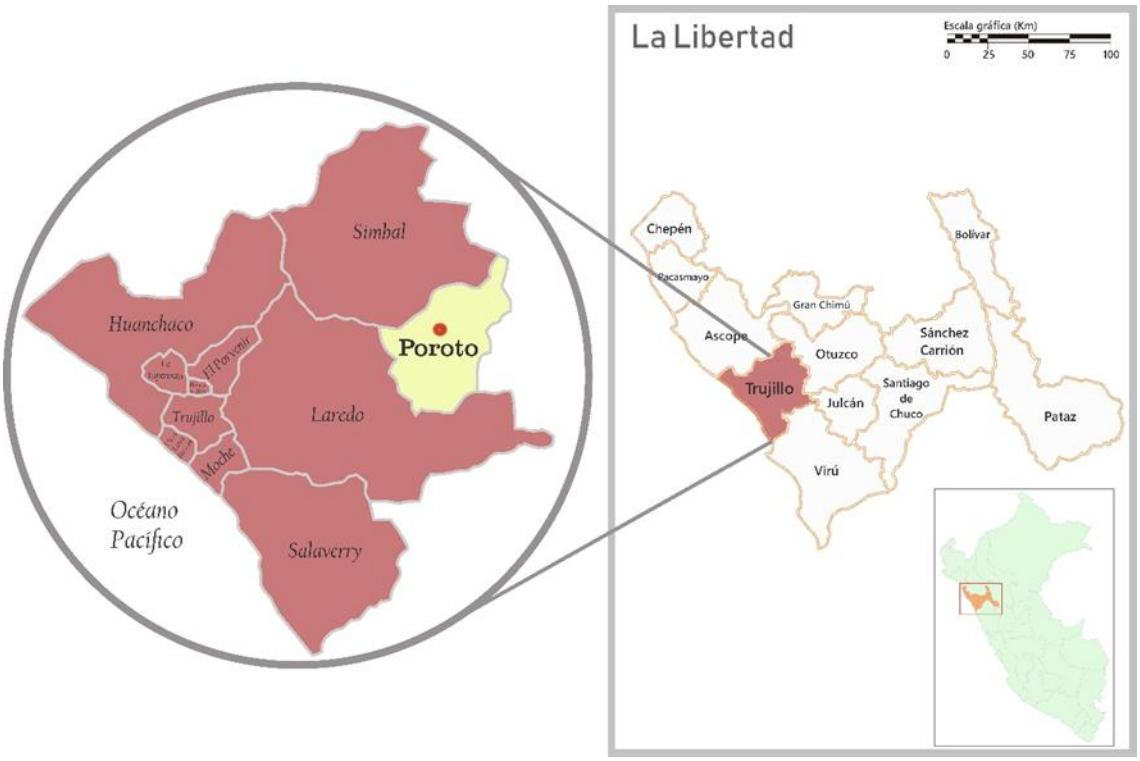


Figure No. 1
Map of Perú showing the location of the District of Poroto, Province of Trujillo, Region of La Libertad, and the pineapple collection place.

Table No. 1
Pineapple fruits varieties, popular name and voucher sample HUT of *Ananas comosus* [L.] Merr. var. from the district of Poroto, Province of Trujillo, Region of La Libertad, Perú

***Ananas comosus* [L.] Merr. var.**	**Popular name**	**HUT**
Roja trujillana	Piña roja	59633
Cayenne lisa	Piña blanca	59634
MD2	Piña golden	59635
Vainilla	Piña vainilla	59636
Tortuga	Piña con pepa	59637

Total Phenolic Contents (TPC)

The total phenolic contents (TPC) of five varieties of pineapple peels of lyophilized ethanol extracts were determined by the Folin-Ciocalteu method with some modifications (Yildirim *et al*., 2001). Stock solutions (1 mg/mL) were prepared in EtOH. TPC, the Folin-Ciocalteu method was followed. The results are expressed as mg gallic acid equivalents (GAE)/g extracted from the peel of pineapple (EPP). All determinations were carried out in triplicate and are reported as mean values ± SD.

Antioxidant Activity Assays

DPPH Assay

The free radical scavenging capacity of the extracts was determined by DPPH assay as previously described with some modifications (Rojo *et al*., 2009). Liophylizated extracts were dissolved in EtOH to a final concentration of 1 mg/mL, filtered and kept in the dark. The stock solutions were serially diluted in 96-well microplates to final concentrations of 1, 0.8, 0.6, 0.4, 0.2 and 0.1 mM. The DPPH solution was freshly prepared in ethanol 0.2 mM mixed with 10 µL of the extract at different concentrations. Ethanol was used as the negative control, and trolox was used as the positive control. The reaction mixture was incubated for 10 min at room temperature and absorbance was measured at 517 nm in Fisherbrand accuSkan GO UV/Vis Microplate Spectrophotometer (Hampton, USA). Afterwards, a curve of % DPPH bleaching activity versus concentration was plotted and IC_{50} values were calculated. IC_{50} denotes the concentration of sample required to scavenge 50% of DPPH free radicals. The determinations were performed in triplicate and are reported as mean values ± SD.

FRAP (Ferric Reducing Antioxidant Power) Assay

The determination of ferric reducing antioxidant power or ferric reducing ability was performed as previously described by Benzie & Strain, (1996). The FRAP working solution was prepared mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM of HCl and 20 mM $FeCl_3$ solution in a 10:1:1 v/v/v ratio. A 8 µL of extract was mixed with 2 mL of FRAP solution pre-warmed at 37ºC and left to stand in the dark for 30 min. Absorbance was measured at 593 nm using a Fisherbrand™ accuSkan™ GO UV/Vis Microplate Spectrophotometer (Hampton, USA). Quantification was performed using a standard curve of the antioxidant Trolox. Samples were performed in triplicate and results were expressed as mmol Trolox equivalents per gram of lyophilized extract (mg TE/g). The determinations were performed in triplicate and are reported as mean values ± SD.

HPLC-ESI-QTOF-MS/MS Analysis

The solutions were prepared at 5 mg/mL in methanol. The chromatographic separation was done in HPLC Rapid Resolution (Agilent, 1200 series) composed by a binary pump, degreaser and automatic injector and using Agilent Zorbax C-18 column (4.6 x 150 mm, 1.8 µm) with a flow rate of 0.5 mL/min and 10 µL injection. The elution gradient was acetonitrile (B) and water (A) in the following ratio: 0.0 -15 min 12-20% B; 15-20 min 20% B; 20-35 min 20-12% B. Column effluent was divided by T-valve and a fraction equivalent to 20 µL/min was introduced into the mass spectrometer. The chemical identification was performed using a Q-TOF orthogonal mass spectrometer (micrOTOF-QTM, Bruker Daltonics) equipped with electrospray ionization source (ESI). The analysis parameters were provided for the positive mode, with a mass range of 100-1000 m/z: 4500 V capillary voltage; set end plate offset -500 V; set charging voltage 2000 V; drying gas temperature 200ºC; drying gas flow 10.0 mL/min; gas pressure 4 bar; collision energy (MS/MS) 35 eV; collision gas N_2. The mass data obtained were processed in Bruker Compass Data Analysis 4.2 software (Bruker Daltonics).

Statistical Analysis

GraphPad Prism software (San Diego, CA 92037, USA) was used. The determination was repeated at least three times for each sample solution. Analysis of variance was performed using ANOVA. Significant differences between means were determined by Tukey comparison test (p values <0.05 and 0.001 were regarded as significant).

RESULTS AND DISCUSSION

Few researches have concentrated on the antioxidant capacity of extracts of pineapple peels. In this studied, we investigated the *in vitro* total phenolic contents (TPC) and antioxidant activity (DPPH and FRAP) of five different lyophilized extracts of pineapple peels, not adsorbed and adsorbed onto Amberlite. The samples are summarized in Table No.

2 and Table No. 3, respectively.

The variation of the pineapple fruits samples presented different hues according to the colour of the peels are shown in Figure No. 2.

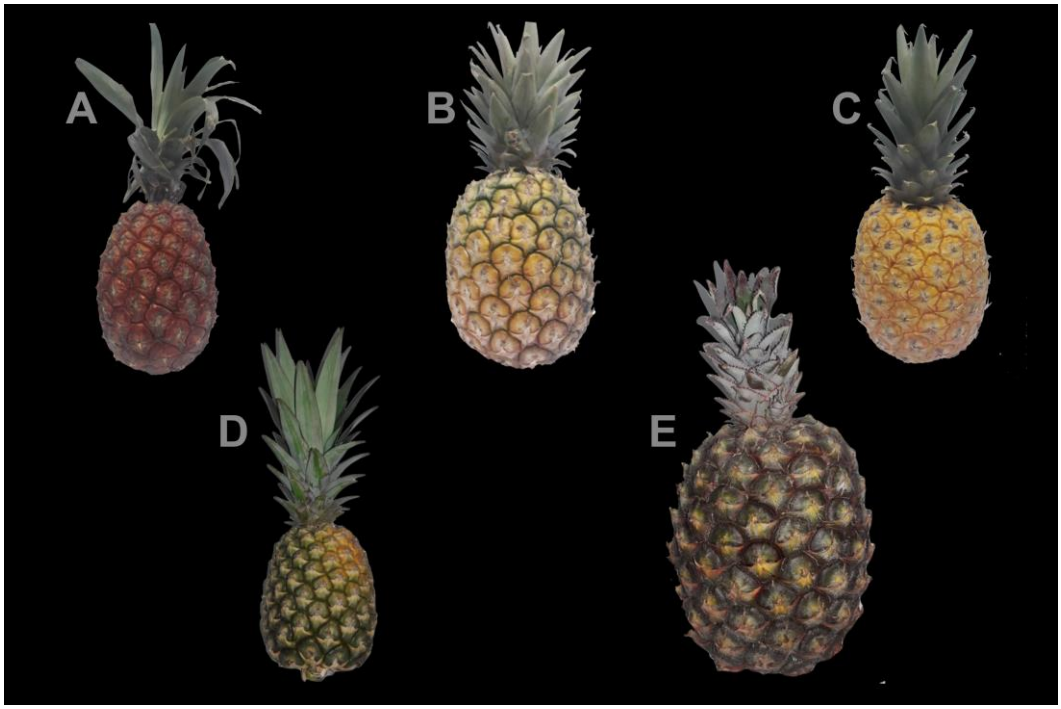


Figure No. 2
Photograph of A: Piña roja; B: Piña blanca; C: Piña golden; D: Piña vainilla; E: Piña con pepa

The range of total phenolic content (TPC) of lyophilized extracts of pineapple peels not adsorbed onto Amberlite was 22.79-41.46 mg GAE per g EPP (Table No. 2). The higher values were from "piña vainilla" (41.46 mg GAE/g EPP), "piña golden" (34.39 mg GAE/g EPP) and "piña roja" (33.27 mg GAE/g EPP).

Table No. 2
Total phenolic contents, DPPH scavenging activity and Ferric reducing antioxidant power (FRAP) of lyophilized extracts of pineapple peels (not adsorbed onto Amberlite).

Pineapples Sample	Total Phenolic (mg GAE/g EPP)	Antioxidant Activity	
		DPPH IC_{50} (mg/mL)	FRAP (mg TE/g EPP)
Piña roja	33.27 ± 0.55	4.17 ± 0.03	20.15 ± 0.36
Piña blanca	24.70 ± 0.29	5.79 ± 0.17*	14.40 ± 0.43
Piña golden	34.39 ± 0.89	4.07 ± 0.03	22.79 ± 1.64*
Piña vainilla	41.46 ± 1.61**	4.88 ± 0.39*	18.13 ± 0.17
Piña con pepa	22.79 ± 0.40	6.40 ± 0.18*	14.67 ± 0.51

GAE: Gallic acid Equivalent; EPP: extracted from the peel of pineapple; TE: trolox equivalents. Values are averages ± standard deviation of triplicates. *p<0.05; **p<0.001.

The most active samples in the DPPH assay, with IC_{50} values ranging from 4.07 to 6.40 mg/mL were those of piña golden (4.07 mg/mL), piña roja (4.17 mg/mL), piña vainilla (4.88 mg/mL), piña blanca (5.79 mg/mL) and piña con pepa (6.40 mg/mL). The activity is not related with the TP content of the samples. In the FRAP assay there was no clear relation between TP content and DPPH but the samples with lower TP content and DPPH were the least active in the FRAP assay (Table No. 2). As TPC lyophilized extracts of pineapple was low, the samples were enriched in phenolics for antioxidant activity studies and phenolic profiling. The lyophilized samples of pinapple peels were extracted with EtOH and phenolics were retained onto Amberlite XAD-7 to obtain the phenolic-enriched extract of pineapple peels (PEPE). The same samples adsorbed onto Amberlite was 56.78-220.64 mg GAE per g EPP (Table No. 3). The highest PEPE was of "piña roja" sample (220.64 mg GAE per g EPP). Lower PEPE values for the different samples of *Ananas comosus* ranged from 56.78 to 112.15 mg GAE per g EPP for piña blanca, piña con pepa, piña vainilla and piña golden as shown in Table No. 3.

Table No. 3
Total phenolic contents, DPPH scavenging activity and Ferric reducing antioxidant power (FRAP) of extracts of pineapple peels (adsorbed onto Amberlite).

Pineapple Samples	Total Phenolic (mg GAE/g PEPE)	Antioxidant Activity	
		DPPH IC_{50} (mg/mL)	FRAP (mg TE/g PEPE)
Piña roja	220.64 ± 1.05**	0.60 ± 0.02**	161.78 ± 7.09**
Piña blanca	56.78 ± 0.61	1.83 ± 0.27	25.97 ± 1.44
Piña golden	112.15 ± 0.58**	1.24 ± 0.13	59.61 ± 4.33
Piña vainilla	100.06 ± 1.95**	1.47 ± 0.10	60.53 ± 1.21
Piña con pepa	65.36 ± 1.17	2.26 ± 0.18	31.39 ± 1.70

GAE: Gallic acid Equivalent; PEPE: phenolic-enriched extract of pineapple peels; TE: trolox equivalents. Values are averages ± standard deviation of triplicates. ** $p<0.001$;

The most active samples in the DPPH assay, with IC_{50} values ranging from 0.60 to 2.26 mg/mL. The activity is related with the TP content of the samples ($p<0.001$). In the FRAP assay there was clear relation between TP content and DPPH but the samples with lower TP content and DPPH were the least active in the FRAP assay (Table No. 3).

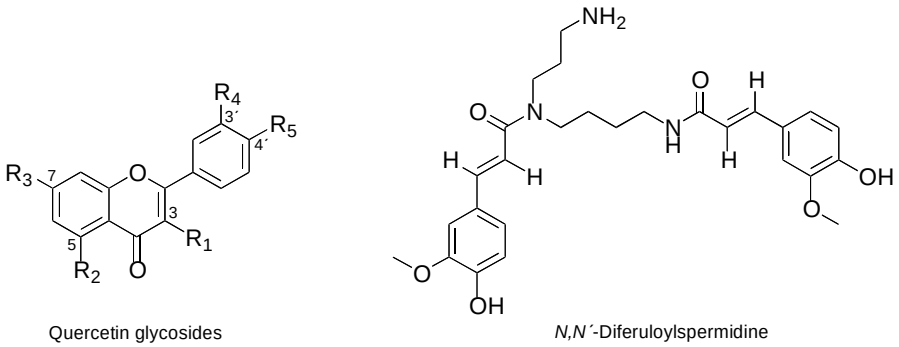
The tentative identification of compounds in the Amberlite-retained phenolic enriched extracts of pineapple peels from the district of Poroto was investigated by HPLC-ESI-QTOF-MS/MS techniques. The data was analysed and compared with previously published reports. The main constituents of pineapple peels were tentatively identified as quercetin glycosides and *N,N'*-diferuloylspermidine by their molecular weight and mass spectrometric, data are given in Table No. 4.

Table No. 4
Tentative identification of phenolic constituents in the EtOH lyophilizated extracts from pineapple peels by HPLC-ESI-QTOF-MS/MS.

Tentative identification	Rt (min)	$[M + H]^+$ (m/z)	Main Fragment MS^1 (m/z)	Main Fragment MS^2 (m/z)
Quercetin-hexoside-rhamnose-hexoside	**2.5**	**773**	**303**	**[303]: 195, 285**
Quercetin-hexoside-hexoside	**4.6**	**627**	**627**	**[627]: 303, 465**
***N,N'*-diferuloylspermidine**	**5.7**	**496**	**498**	**[498]: 177, 234, 322**

Rt: Retention time; m/z = mass/charge ratio; MS = mass spectrometry.

All samples were analysed in the EtOH extracts made from pineapple peels revealed that among chromatographic peaks giving $[M + H]^+$ ion at m/z 627 and 303 agrees with that quercetin-hexoside-rhamnose-hexoside and quercetin-hexoside-hexoside, respectively. They were tentatively identified as *O*- conjugates of sugars, according to the MS data and literature. The MS of the flavonoid glycosides clearly indicates two groups of flavonol glycosides based on quercetin (quercetin-hexoside-rhamnose-hexoside and quercetin-hexoside-hexoside). The $[M + H]^+$ ion at m/z 498 is compatible with that *N,N'*-diferuloylspermidine (Figure No. 2).



Quercetin glycosides *N,N´*-Diferuloylspermidine

Figure No. 2
Quercetin glycosides and *N,N'*-diferuloylspermidine of pineapple peels from the district of Poroto, Perú.

The ion trap MS^2 spectra of the protonated molecules, showed abundant product ions at m/z 322, 234, and 177, in agreement with the main product ions reported in the collision cell spectra of protonated *N,N'*-diferuloylspermidine (Kite *et al*., 2013). Accurate mass measurements of these product ions (m/z 322.212 = $C_{17}H_{28}N_3O_3^+$; m/z 234.112 = $C_{13}H_{16}NO_3^+$; m/z 177.055 = $C_{10}H_9O_3^+$) were also in agreement with the fragments postulated by Youhnovski *et al*. (1998).

The chemical composition of pineapple peels from the district of Poroto is similar to pineapple peels samples from Europe (Steingass *et al*., 2015; Difonzo *et al*., 2019).

CONCLUSIONS

In this study, several *in vitro* assays were applied to evaluate the antioxidant potential of lyophilized extracts of five different pineapple peels, not adsorbed and adsorbed onto Amberlite and the

tentative identification of phenolic constituents by HPLC-ESI-QTOF-MS/MS. The results of the present study would certainly help to ascertain the potency of the lyophilized extract of peels of *Ananas comosus* as a potential source of natural antioxidants. Therefore, the results on the antioxidant activity of pineapple peels from Poroto, can be associated with the presence of quercetin glycosides and *N,N'*-diferuloylspermidine.

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