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Articulo Original / Original Article Potential antioxidant effect of fruit peels for human use from northern Peru, compared by 5 different methods

[Potencial efecto antioxidante de las cáscaras de frutas para uso humano del norte de Perú, comparado por 5 métodos diferentes]

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Ganoza-Yupanqui ML, Muñoz-Acevedo A, Ybañez-Julca RO, Mantilla-Rodríguez E, Zavala E, Gajardo S, Ríos M, Benites J, Martínez JL. Potential antioxidant effect of fruit peels for human use by 5 different methods **Bol Latinoam Caribe Plant Med Aromat** 20 (6): 611 - 637 (2021). https://doi.org/10.37360/blacpma.21.20.6.44 **Abstract:** The objective of the work was to determine the antioxidant potential *in vitro* of freeze-dried peel extracts of 20 fruits from the northern region of Peru through five tests (Folin-Ciocalteu, DPPH; ABTS⁺, FRAP and CUPRAC). According to multivariate statistical analyzes, five groups were found: (i.) peel extracts with the highest values of antioxidant capacity (AC) from custard apple, and star fruit; (ii.) rind extracts with high AC values from quince, sweet granadilla, guava, and black grape; (iii.) husk extracts with middle values of AC from passion fruit, and red mombin; (iv.) shell extracts with low AC values from tangerine, mandarine, and bitter orange; and, (v.) coating extracts with the lowest AC values from pawpaw, red pawpaw, muskmelon, dragon fruit, yellow and red indian figs, pear, apple, and green grape. To conclude, the fruit lyophilized-husk extracts of custard apple, star fruit, quince, sweet granadilla, guava, and black grape obtained the best AC.

Keywords: Antioxidant assays; Peruvian fruit rinds; CUPRAC; FRAP; DPPH

Resumen: El objetivo del trabajo fue determinar el potencial antioxidante *in vitro* de extractos de cáscara liofilizada de 20 frutos de la región norte del Perú mediante cinco pruebas (Folin-Ciocalteu, DPPH, ABTS⁺, FRAP y CUPRAC). Según análisis estadísticos multivariados, se encontraron cinco grupos: (i.) Extractos de piel con los valores más altos de capacidad antioxidante (CA) de chirimoya y carambola; (ii.) extractos de cáscara con altos valores de CA de membrillo, granadilla dulce, guayaba y uva negra; (iii.) extractos de cáscara con valores medios de CA de maracuyá y mombina roja; (iv.) extractos de cáscara con valores medios de CA de maracuyá y mombina roja; (iv.) extractos de cáscara con valores bajos de Papaya, papaya roja, melón, fruta del dragón, higos indios amarillos y rojos, pera, manzana y uva verde. Para concluir, los extractos de cáscara liofilizada de chirimoya, carambola, membrillo, granadilla dulce, guayaba y uva negra obtuvieron el mejor CA.

Palabras clave: Ensayos de antioxidantes; Cortezas de frutas peruanas; CUPRAC; FRAP; DPPH

INTRODUCTION

exposed multiple Human being is to endogenous/exogenous factors that affect its health condition which would cause to the body some decompensation leaving it unprotected to any harmful agent, which could trigger damages at the cellular and systemic scales. Nonetheless, the same body will seek self-regulation via the homeostasis of multiple systems, but if this is not achieved, a series of pathologies will arise at different levels; this is why today the health care through the proper/healthy management of food, exercise and lifestyles are of vital importance to obtain and maintain the homeostasis in the body (Krishnaiah et al., 2007; Thatoi et al., 2014).

It is widely known that the consumption of fruits, vegetables and cereals contributes beneficially to human health, helping to maintain homeostasis. Particularly, the intake of fruits and vegetables (recommended by WHO) with bright colors (produced by the bioactive constituents type phenolics and/or carotenoids) has been related to a positive impact for the prevention of: (i) chronic diseases such as atherosclerosis, arthritis/rheumatism, cardiac disorders, Alzheimer disease, or cancer, etc.; and (ii.) decrease in the age-related functions. It is worth noting that the general health benefits are not only due to the contribution of phytochemical components type phenolics/carotenoids but also by other phytochemical constituents (e.g., phytosterols, organo-nitrogenous/sulfur compounds) that together with vitamins and minerals help to the nutrition (Kaur & Kapoor, 2001; Collins & Harrington, 2002; Arouma, 2003; Naczk & Shahidi, 2006; Willcox et al., 2007; Yahia, 2010; Liu, 2013; Lima et al., 2014; Williamson, 2017: McDougall, 2017: Ybañez-Julca et al., 2020).

Likewise, it has been suggested that phenolic compounds (e.g., monophenols, phenolic acids, flavonoids and polyphenols) would be involved for a favorable biological response through different mechanisms, such as the imitation of sex hormones, and the inhibition of enzymes and inflammatory events (radical scavenging or antioxidant capability) (Akiyama et al., 1987; Middleton et al., 2000; Pietta, 2000; Barnes et al., 2005). For instance, the inflammatory cascade (related to chronic sicknesses) could be prompted by oxidative stress/damage, which favors the formation of radical and non-radical oxidant species - ROS (e.g., oxygen (triplet/singlet), superoxide anion-radical, hydrogen peroxide. hypochlorous acid, peroxynitrite, etc.) that are responsible for the process of cell deterioration; thus,

the whole body will stimulate defense mechanisms (e.g., antioxidant defense) for protection against these ROS.

Then, those substances (endogenous or exogenous) that could "trap" oxidizing species (delaying or preventing the oxidation of a substrate), through several mechanisms, e.g., by donating a hydrogen atom or an electron/hydrogen ion, are antioxidants (or scavengers) (Halliwell, 1995; Halliwell *et al.*, 1995; Halliwell, 1999; Davies, 2000; Kohen & Nyska, 2002; Benites *et al.*, 2019a; Benites *et al.*, 2019b), just like the phenolic compounds are (at low concentrations, they are one of the most powerful agents for protection against oxidation) (Halliwell, 1995; Karakaya, 2004; Collins, 2005; Soobrattee *et al.*, 2005).

Particularly, the tropical/sub-tropical fruits are rich in carotenoid and phenolic compounds, in addition to containing vitamins and minerals (Yahia, 2010; Dominguez-Avila et al., 2018). Some of these fruits were the subject of this research such as the native fruits from Annona cherimola (custard apple), Averrhoa carambola (star fruit), Carica papaya (yellow/red pawpaw), Hylocereus megalanthus (dragon fruit), Opuntia ficus-indica (Red/yellow indian fig), Passiflora edulis (passion fruit), Pa. ligularis (sweet granadilla), Psidium guajava (common guava), Spondias purpurea (red mombin) along with other fruits introduced in America, e.g., Citrus x aurantium (bitter orange), Ci. reticulata (mandarine), Ci. x tangelo (tangerine), Cucumis melo (muskmelon), Cydonia oblonga (quince), Malus domestica (common apple), Pyrus communis (common pear), and Vitis vinifera (black/white grapes), all of them widely distributed and consumed in Latin America.

According to the reviewed scientific literature, most of these fruits (pulp extracts) have reports on antioxidant capacities and/or phenolic compound contents, f.i., An. Cherimola (Vasco et al., 2008; Isabelle et al., 2010; Gupta et al., 2011; Loizzo et al., 2012; Murillo et al., 2012; Barreca et al., 2013; Spinola et al., 2015; Sanchez-Gonzales et al., 2019), Av. Carambola (Lim et al., 2007; Hassimotto et al., 2009; Barreca et al., 2013; Saghir et al., 2013; Yan et al., 2013; Noor Asna & Noriham, 2014; Panteleón-Velasco et al., 2014; Khanam et al., 2015; Silva & Sirasa, 2018; Stafussa et al., 2018; Aladaileh et al., 2019; Silva et al., 2020), Ca. papaya (Lim et al., 2007; Isabelle et al., 2010; Rivera-Pastrana et al., 2010; Almeida et al., 2011; Addai et al., 2013a; Addai et al., 1213b; Maisarah et al., 2013; Zunjar et al., 2015; Calvache et al., 2016; Iamjud et al., 2016;

Seow et al., 2016; Jarisarapurin et al., 2019), Ci. x aurantium (Peterson et al., 2006; Ramful et al., 2011; Jabri-Karoui & Marzouk, 2013; Divya et al., 2016; Zeghad et al., 2019), Ci. Reticulate (Ghasemi et al., 2009; Tumbas et al., 2010; Isabelle et al., 2010; Ramful et al., 2011; Barros et al., 2012; Barreca et al., 2013; Kelebek & Selli, 2014; Zhang et al., 2014; Wang et al., 2017; Stafussa et al., 2018; Zhang et al., 2018), Cu. Melo (Ismail et al., 2009; Isabelle et al., 2010; Barreca et al., 2013; Morais et al., 2015; Ibrahim & El-Masry, 2016; Stafusa et al., 2018; Muzykiewicz et al., 2018), Cy. Oblonga (Silva et al., 2002; Magalhaes et al., 2009; Legua et al., 2013; Wojdylo et al., 2013; Szychowski et al., 2014; Kabir et al., 2015; Teleszko & Wojdylo, 2015; Umar et al., 2015; Stojanovic et al., 2017; Baroni et al., 2018; Torres et al., 2018; Sut et al., 2019), H. megalanthus (Torres-Grisales et al., 2017), M. domestica (Sun et al., 2002; Valavanidis et al., 2009; Vieira et al., 2009; Hassimotto et al., 2009; Isabelle et al., 2010; Karaman et al., 2013; Teleszko & Wojdylo, 2015; Lutz et al., 2015; Wang et al., 2015a; Raudone et al., 2017; Inal et al., 2017; Navarro et al., 2018), O. ficus-indica (Chirinos et al., 2013; Andreu et al., 2018; Zeghad et al., 2019; Aruwa et al., 2019; García - Cayuela et al., 2019), Pa. edulis (Vasco et al., 2008; Ismail et al., 2009; Stangeland et al., 2009; Zeraik & Yariwake, 2010; Isabelle et al., 2010; Barreca et al., 2013; Chirinos et al., 2013; da Silva et al., 2014; Spinola et al., 2015; Bravo et al., 2016; Stafussa et al., 2018; Rotta et al., 2019; Guimaraes et al., 2020), Pa. ligularis (Vasco et al., 2008; Saravanan & Parimelazhagan, 2014; Rotta et al., 2019), Ps. guajava (Luximon-Ramma et al., 2003; Nilsson et al., 2005; Thaipong et al., 2006; Lim et al., 2007; Pattamakanokporn et al., 2008; Hassimotto et al., 2009; Stangeland et al., 2009; Isabelle et al., 2010; Barreca et al., 2013; da Silva et al., 2014; Flores et al., 2015; Paz et al., 2015; dos Santos et al., 2017; Rojas-Garbanzo et al., 2017; de Almeida et al., 2017; Stafussa et al., 2018; Liu et al., 2018a; Hartati et al., 2020), S. purpurea (Lim et al., 2007; Vasco et al., 2008; Almeida et al., 2011; Omena et al., 2012; de Almeida et al., 2017; Stafussa et al., 2018; Vasconcelos et al., 2020), P. communis (Sun et al., 2002; Isabelle et al., 2010; Li et al., 2014; Kolniak-Ostek & Oszmianski, 2015; Wang et al., 2015b; Kolniak-Ostek, 2016a; Kolniak-Ostek, 2016b; Dutra et al., 2017; Liu et al., 2018b), and V. vinifera (Karakaya et al., 2001; Sun et al., 2002; Balasundram et al., 2006; Isabelle et al., 2010; Breksa et al., 2010; Zeghad et al., 2019; Li et al., 2019). In like manner and based on the same manuscripts referred to above,

the husks of most of these fruits have been studied (except for Ci. x tangelo, H. megalanthus and Pa. ligularis) in terms of the content of their phenolic compounds and antioxidant capacities; however, there is not scientific report on the study of antioxidant capacity from the peels of these fruits found in the northern Peruvian region. Usually, common people prefer to ingest only the fruit pulps, removing and discarding peels even though some of them contain fiber and bioactive constituents (Banerjee et al., 2017; Can-Cauich et al., 2017; Perez-Jimenez & Saura-Calixto, 2018; de Albuquerque et al., 2019); this action is due to the consumers do not know on the phytochemical richness/fiber found in the fruit rinds.

In this work, the *in vitro* antioxidant potential of lyophilized-peel extracts from 20 fruits of the northern Peruvian region was assessed by using the Folin-Ciocalteu reagent and DPPH, ABTS⁺, FRAP and CUPRAC methods, with the aim of establishing which husks would have a new high- added value (possibility of dietary intake or exploitation when some of them were discarded as an agroindustrial waste).

MATERIALS AND METHODS

Reagents

The reagents used were Folin & Ciocalteu's phenol 2,2'reagent (Sigma-Aldrich), Azino-bis(3ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Sigma-Aldrich), 2,2-diphenyl- 1-picrylhydrazyl (Sigma-Aldrich), 2,4,6-tris(2-pyridyl)-s-triazine (Sigma-Aldrich), (Sigma-Aldrich), neocuproine Gallic acid (±)-6-hydroxy-2,5,7,8-(Merck), tetramethylchromane-2-carboxylic acid (Trolox, Sigma-Aldrich). quercetin dihvdrate (Sigma-Aldrich), butylhydroxytoluene (Merck), Iron(III) chloride hexahydrate (Sigma-Aldrich), sodium carbonate (J.T.Baker), hydrochloric acid fuming 37% (Merck), copper(II) chloride dihydrate (Merck), sodium acetate anhydrous (J.T.Baker), ammonium acetate (J.T.Baker), ethanol 96% (CKF).

Preparation of fruit husk samples and obtaining the extracts

Samples of each fruit were chosen according to their sizes (intermediate), discarding those of greater/ smaller sizes, as well as some that showed certain damages/deterioration. Then, the selected samples were washed and peeled manually. Starting from this, 50-100 g of each fresh fruit husks were cut into pieces ca. 1 cm², which were frozen (at -80°C for 48 h) and lyophilized. The extract of each lyophilized

fruit rinds (20-50 g) was obtained using a Soxhlet equipment and ethanol (96%), at 80°C for 24 h (Zavala-Urtecho *et al.*, 2018). Later, each extract was concentrated (to dryness under reduced pressure), lyophilized, and stored hermetically (4°C in Falcon® tubes) until the respective test.

Qualitative colorimetric tests for phenolic compounds

The lyophilized peel extracts were consequently subjected to a qualitative screening to identify the presence of compounds potentially responsible for the antioxidant power. The phytochemical tests were based on the reactions with FeCl₃, AlCl₃/NaOH, vanillin/H₂SO₄, HCl/NaOH, and Mg/HCl for determination of flavonoids, phenols, anthocyanins, and/or betalains (Ganoza, 2001; Rao *et al.*, 2016).

Measurement of the total content of phenols

The assessment of the total content of phenols on each fruit peel extract was carried out pursuant to the procedure described by Singleton et al. (1999) and Suárez-Rebaza et al. (2019), using the Folin-Ciocalteu reagent and visible spectrophotometry. Like so, lyophilized peel extracts were first dissolved (1.25-5 mg/mL) according to the type of fruit, and then each of them (25 µL) was placed and shaken with the Folin-Ciocalteu reagent (10%, 125 µL), at 45°C during 20 min. After this time, Na₂CO₃ (7%, 100 µL) was added and the mixture was allowed to stand for 10 min. Subsequently, each solution was read at 760 nm. The total content of phenols for each sample was expressed as mg of total phenols equivalent to gallic acid per gram of lyophilized extract (mg TPEGA/g LE) quantified by means of calibration solutions (0.02-0.16 mg/mL) from gallic acid (1 mg/mL), which were processed in the same manner as the samples. All the tests were performed in triplicate. Quercetin was used as a control flavonoid.

Assay of the radical scavenging capacity

The estimation of the radical scavenging capacity of each husk extract was performed using the 2,2diphenyl-1-picrylhydrazyl radical (DPPH) according to the method by Brand-Williams *et al.* (1995), Alam *et al.* (2013), and Suárez-Rebaza *et al.* (2019). Thus, the lyophilized extracts were first dissolved (2.5-5 mg/mL), and each of them (10 μ L) was placed and shaken with the DPPH reagent (0.2 mM, 300 μ L) during 15 min. Successively, each solution was measured at 517 nm. For quantitation, from a stock solution of Trolox® (1 mg/mL) were prepared different solutions of calibration (0.025-0.25 mg/mL), which were processed as described above. The radical-scavenging capacity (related to antioxidant ability) for each sample was expressed as mg of Trolox® equivalent per gram of lyophilized extract (mg TE/g LE). All the experiments were done by triplicate. Butylhydroxytoluene was used as a control antioxidant.

Test of the cation-radical scavenging capacity

The appraisal of the cation-radical scavenging capacity of each shell extract of fruit was effected by means of the 2,2'-azinobis(3-ethyl benzothiazolline-6-sulfonic acid) cation-radical (ABTS⁺) based on the process by Re et al. (1999) and Suárez-Rebaza et al. (2019). The fruit rind extracts were first dissolved (2.5-5 mg/mL), and each of them (10 μ L) was placed and shaken with the ABTS+. reagent ($A_{\lambda-750}$: 0.7, 300 µL) during 5 min. In order, each solution was read at 750 nm. For quantitation, from a standard solution of Trolox® (1 mg/mL) were prepared nine different solutions of calibration (0.013-0.2 mg/mL), which were treated as described formerly. The cationradical scavenging capability (related to antioxidant effect) for each sample was expressed as mg of Trolox® equivalent per gram of lyophilized extract (mg TEAC/g LE). All experiments were carried out in triplicate. Butylhydroxytoluene was used as a control antioxidant.

Assay of the ferric reducing/antioxidant power

The FRAP test was carried out based on the adapted method by Benzie and Strain (1996) using 2.4.6tris(2-pyrydyl)-s-triazine (TPTZ) and ferric chloride (FeCl₃.6H₂O). The FRAP reagent was prepared freshly mixing and incubating (37°C) the 300 mM acetate buffer (25 mL), 10 mM TPTZ/10 mM HCl (2.5 mL), and 20 mM FeCl₃.6H₂O (2.5 mL). Promptly, the lyophilized extracts were first dissolved (2.5-5 mg/mL), and each (8 µL) was placed to react with the FRAP reagent (200 µL), at 37°C for 30 min. Successively, each solution was measured at 593 nm. The antioxidant power for each sample was expressed as mg of Trolox® equivalent per gram of lyophilized extract (mg TE/g LE), Ramirez et al. (2014), calculated through a calibration curve (0.013-0.13 mg/mL) prepared from a standard solution of Trolox® (1 mg/mL). All experiments were carried out in triplicate.

Test of the cupric ion reducing antioxidant capacity The CUPRAC assay was performed according to process by Celik *et al.* (2010) and Özyürek *et al.*

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(2011), using neocuproine (Nc) and copper chloride (CuCl₂). Initially, the husk extracts were dissolved (5 mg/mL), and then each of them (10 μ L) was mixed with CuCl₂ (250 μ L), neocuproine (250 μ L), ammonium acetate buffer (250 μ L), and distillated water (265 μ L). After 30 min of reaction (at room temperature), each was read at 450 nm. The antioxidant capacity for each sample was expressed as mg of Trolox® equivalent per gram of lyophilized extract (mg TE/g LE) (Celik *et al.*, 2010) based on the calibration curve (0.063-1.5 mg/mL) obtained from a stock solution of Trolox® (1 mg/mL). All experiments were carried out in triplicate.

Statistical treatment of data

The results of each trial were expressed as media \pm standard deviation, and the data were treated using one-way ANOVA, the Tukey post hoc test, and the Pearson index (to establish correlation between trials). The *p* value < 0.05 was considered statistically

significant. All acquired data from antioxidant methods and yield of extracts were statistically treated and subjected to the principal component analysis (PCA), cluster (CA) and K-means analysis as tools of multivariate statistical analysis (MVA) by IBM SPSS Statistic 25 (2017) and Statgraphics (2016) software.

RESULTS AND DISCUSSION

From the lyophilized ethanol extracts obtained of the husks of the 20 fruits (Table No. 1, Figure No. 1), the yields for each of them were determined, which are presented in Table No. 2. According to the Table, the percentage yields were ranked among ~ 3-62 % with the highest yields [> 19.8 ± 0.2 % (median)] found in the rind extracts of custard apple, green and black grapes, guava, red mombin, star fruit, pear, apple, and quince. It is worth noting that 15 of the 20 fruit shell extracts (75%) exhibited a yield higher than 11%.



Figure No. 1 Fruits grown in northern region of Peru

a: Carica papaya L. var. pauna, b: Carica papaya L. var. maradol, c: Cucumis melo L., d: Annona cherimola Mill., e: Hylocereus megalanthus (K. Schum. ex Vaupel) Ralf Bauer, f: Cydonia oblonga Mill., g: Citrus × aurantium L., h: Passiflora ligularis Juss., i: Averrhoa carambola L., j: Opuntia ficus-indica (L.) Mill. var. roja, k: Passiflora edulis Sims, l: Pyrus communis L., m: Citrus × tangelo J.W. Ingram & H.E. Moore, n: Malus domestica (Suckow) Borkh., o: Opuntia ficus-indica (L.) Mill. var. amarilla, p: Citrus reticulata Blanco var. clementine, q: Psidium guajava L., r: Spondias purpurea L., s: Vitis vinifera L. var. italia, t: Vitis vinifera L. var. quebranta

On the other hand, Table No. 3 shows the data of qualitative colorimetric screening for phenolic compounds of lyophilized shell extracts from 20 fruits. Based on the table, the most peel extracts were

positive for flavonoids using the $AlCl_3$ test; nonetheless, two extracts resulted negative (pawpaw and red pawpaw) and one of them (Dragon fruit) was the one with least color. In addition, five extracts of

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fruit peels (bitter orange, tangerine, mandarine, star fruit and black grape) colored pink on the Shinoda test.

In contrast, all extracts were positive for simple phenols through the $FeCl_3$ test, showing the strongest colors those extracts from bitter orange, tangerine, mandarin, and guava fruit shells. Also, nine husk extracts (pawpaw, muskmelon, custard

apple, sweet granadilla, red and yellow indian figs, passion fruit, mandarine, and guava) were not susceptible (negative results) to vanillin/H₂SO₄ reagent. In particular, the husk extracts of the black grape/red mombin and red indian fig fruits contained anthocyanins (AC)/pro-AC and betalains, respectively.

Fruits grown in northern region of Peru							
Code	Scientific name	Common name (English/Spanish)					
а	Carica papaya L. var. Pauna	Pawpaw/Papaya					
b	Carica papaya L. var. Maradol	Red pawpaw/Papaya roja					
с	Cucumis melo L.	Muskmelon/Melón coquito					
d	Annona cherimola Mill.	Custard apple/Chirimoya					
e	Hylocereus megalanthus (K. Schum. ex Vaupel) Ralf Bauer	Dragon fruit/Pitahaya					
f	Cydonia oblonga Mill.	Quince/Membrillo					
g	Citrus \times aurantium L.	Bitter orange/Naranja de jugo					
h	Passiflora ligularis Juss.	Sweet granadilla/Granadilla					
i	Averrhoa carambola L.	Star fruit/Carambola					
j	Opuntia ficus-indica (L.) Mill. var. roja	Red indian fig/Tuna roja					
k	Passiflora edulis Sims	Passion fruit/Maracuyá					
1	Pyrus communis L.	Common pear/Pera de agua					
m	Citrus × tangelo J.W. Ingram & H.E. Moore	Tangerine/Naranja-tangelo					
n	Malus domestica (Suckow) Borkh.	Common apple/Manzana Santa Rosa					
0	Opuntia ficus-indica (L.) Mill. var. Amarilla	Yellow indian fig/Tuna amarilla					
р	Citrus reticulata Blanco var. Clementine	Mandarine/Mandarina					
q	Psidium guajava L.	Common guava/Guayaba					
r	Spondias purpurea L.	Red mombin/Ciruela					
S	Vitis vinifera L. var. Italia	Green grape/Uva verde					
t	Vitis vinifera L. var. Quebranta	Black grape /Uva negra					

Table No. 1

Yields of the extracts from lyophilized fruit husks							
Fruit name	Yield (%)	Fruit name	Yield (%)				
Pawpaw	8.6	Passion fruit	8.9				
Red pawpaw	9.1	Common pear	38.2				
Muskmelon	17.4	Tangerine	11.0				
Cherimoya	25.9	Common apple	50.2				
Dragon fruit	4.2	Yellow indian fig	19.9				
Quince	61.6	Mandarine	19.6				
Bitter orange	19.2	Common guava	31.4				
Granadia	2.7	Red mombin	35.2				
Star fruit	36.2	Green grape	26.4				
Red indian fig	15.3	Black grape	45.3				

Yields of the extracts from lyophilized fruit husk

F	Phenols		Flavonoids		AC/PAC/BL
Fruits	FeCl ₃	V/H ₂ SO ₄	Mg/HCl	NaNO ₂ /AlCl ₃ /NaOH	HCl/NaOH
Pawpaw	+				N/A
Red pawpaw	+	+			
Muskmelon	+			++	N/A
Custard apple	++			+++	N/A
Dragon fruit	+	++		+	N/A
Quince	+	+		+++	N/A
Bitter orange	+++	++	+	++	N/A
Sweet granadilla	++			++	N/A
Star fruit	+	+	+	+++	N/A
Red indian fig	+			++	+++ (red/green - BL)
Passion fruit	+			++	N/A
Common pear	+	+		+++	N/A
Tangerine	+++	+	+	+++	N/A
Common apple	+	+		++	N/A
Yellow indian fig	+			++	N/A
Mandarine	+++		+	++	N/A
Common guava	+++			+++	N/A
Red mombin	++	++		+++	+ (red/green - PAC)?
Green grape	+	++		++	N/A
Black grape	+	+	++	++	+++ (red/green - AC)

 Table No. 3

 Presumptive identification of phenolic compounds from lyophilized extracts of the Peruvian fruits

V-Vanillin, BL-Betalains, PAC-Proanthocyanin, AC-Anthocyanins, N/A-Not applicable, +: positive result, -: negative result

By last, considering all the colored tests, eight of the 20 fruit peel extracts were presumptively found with a lower content of phenolic compounds (pawpaw, red pawpaw, muskmelon, passion fruit, yellow indian fig, apple, dragon fruit, and sweet granadilla). These results would be in accordance with literature (Bocco et al., 1998; Jimenez-Escrig et al., 2001; Yilmaz & Toledo, 2004; Rehman, 2006; Nurliyana et al., 2010; Celik et al., 2010; Tumbas et al., 2010; Ozyurek et al., 2011; Ghafoor et al., 2013; Nile et al., 2013; Ramirez et al., 2014; Liu et al., 2018b; Ordoñez-Gomez et al., 2018; Karasawa & Mohan, 2018; Li et al., 2019), who also reported that some of the fruits (e.g., tangerine, bitter orange, mandarine, guava, black grape, red indian fig) under study presented the same families of phenolic compounds identified.

The results related to the antioxidant potential of the shell extracts from 20 fruits, measured through five methods are shown in Figure No. 2 to Figure No. 6. Thereby, the measurement of the reducing power for each fruit peel extract by Folin-Ciocalteu reagent, expressed as the total phenolic content (TPC) equivalent to gallic acid, was found between 14.4 \pm 0.3 mg GAE/g and 92 \pm 1 mg GAE/g (Figure No. 2).

Due to the heterogeneity presented by the values of TPC, the median of the data (26.8 \pm 0.4 mg GAE/g) was used as a selection criterion. In consequence, nine peel extracts (from passion fruit, guava, mandarine, bitter orange, tangerine, sweet granadilla, quince, custard apple, star fruit) presented values greater than the median (28.2 \pm 0.6 - 92 \pm 1 mg GAE/g) and they were considered promising. From this group, the mandarine and bitter orange extracts had similar values of TPC $(37.1 \pm 0.8 - 38 \pm 1 \text{ mg})$ GAE/g), as well as the tangerine, sweet granadilla, quince extracts whose TPC values were ~ 47 ± 1 mg GAE/g (for each), and custard apple and star fruit extracts showed the highest values of TPC (65.2 \pm 0.7 mg GAE/g and 92 \pm 1 mg GAE/g, respectively). The comparison of the reducing power between these extracts revealed that the star fruit peel extract was ~ 2.5-2.0 and \sim 1.5 times higher than the citrus fruits and custard apple husk extracts, correspondingly.

In the same way, the antiradical capacity (expressed in mg equivalent to Trolox®) as a measure of the antioxidant potential of the 20 fruit peel extracts by DPPH assay was established between 7.2 ± 0.2 mg TE/g and 59.2 ± 0.3 mg TE/g (results are presented in Figure No. 3). The median of

the data was 13.7 ± 0.1 mg TE/g and ten extracts were chosen from values higher than the median (14 ± 1 - 59.2 ± 0.3 mg TE/g). The rind extracts were pawpaw, tangerine, red mombin, passion fruit, black grape, sweet granadilla, guava, star fruit, quince and custard apple. Then, red mombin, passion fruit and black grape husk extracts obtained similar values of mg TE/g (23.3 $\pm 0.8 - 26.2 \pm 0.2$); other group of extracts (sweet granadilla, guava and star fruit) showed values of mg TE/g $(32.2 \pm 0.4 - 38.7 \pm 0.4)$ close to each other; and to close, the highest values of mg TE/g were for the quince and custard apple extracts $(46 \pm 2 \text{ and } 59.2 \pm 0.3, \text{ individually})$. The free-radical-scavenging capacity related to antioxidant power from custard apple peel extract was ~ 2.6-1.3 times greater than the other nine extracts.

Figure No. 2 Graph of the comparison of TPC values determined for lyophilized peel extracts from 20 fruits of the northern Peruvian region



Likewise, the metering of the antioxidant power of peel extracts from 20 fruits, according to the free-radical-scavenging capacity and expressed in mg equivalent to Trolox® (TEAC), was determined by using ABTS⁺ test [similar method to DPPH assay, based on both single electron and/or H atom transfers (Prior & Cao, 1999; Prior & Cao, 2000; Prior *et al.*, 2005; Karasawa & Mohan, 2018). Figure No. 4 exhibits the comparison of the TEAC values calculated for 20 fruit shell extracts; these values were between $14.2 \pm 0.7 - 72 \pm 1$ mg TE/g, and in agreement with the median value (25.1 ± 0.7 mg TE/g) were selected nine extracts of fruit rinds (red mombin, passion fruit, tangerine, quince, sweet granadilla, star fruit, black grape, guava and custard apple) whose TEAC values were greater than the median $(32.3 \pm 0.6 - 72 \pm 1 \text{ mg TE/g})$. In descending order of TEAC value, the highest values were for the custard apple and guava extracts $(72 \pm 1 \text{ mg TE/g})$ and $62 \pm 2 \text{ mg TE/g}$, independently), followed by black grape, star fruit, sweet granadilla and quince extracts with values ranked among $56 \pm 1 - 48.4 \pm 0.9 \text{ mg}$ TE/g, and ending with the tangerine, passion fruit and red mombin extracts, whose values were $34.0 \pm 0.1 - 32.3 \pm 0.6 \text{ mg TE/g}$. Comparing the antiradical potential of the nine extracts was observed that the relationship between custard apple rind extract was ~ 2.2-1.2 times greater than for the remaining eight extracts.

Figure No. 3

Graph of the comparison of antiradical capacity values determined by DPPH· assay for lyophilized peel extracts from 20 fruits of the northern Peruvian region



Figure No. 4

Graph of the comparison of free-radical scavenging capacity values determined by ABTS⁺ assay for lyophilized peel extracts from 20 fruits of the northern Peruvian region



Other interesting test that measure the reducing power, involving a possible single-electron transfer mechanism, is the FRAP assay. In this sense, the antioxidant capacity, measure as ferric reducing potential, of the shell extracts from 20 fruits was expressed as mg equivalent to Trolox® and was determined among $17.8 \pm 0.5 - 60 \pm 2$ mg TE/g (Figure No. 5). From the median value (23.2 \pm 0.6 mg TE/g) of the data, nine extracts (tangerine, red mombin, star fruit, quince, black grape, sweet granadilla, passion fruit, custard apple and guava) with higher values $(25.4 \pm 0.2 - 60 \pm 2 \text{ mg TE/g})$ were chosen. In ascending order of mg TE/g, two extracts from tangerine and red mombin had comparable values of mg TE/g (25.4 \pm 0.2 - 29.6 \pm 0.8); followed by the star fruit, quince, black grape, sweet granadilla and passion fruit extracts whose values of mg TE/g (41 \pm 1 - 50.5 \pm 0.9) were close to each other; and, the highest values of mg TE/g were

for the custard apple and guava extracts (54 ± 2 and 60 ± 2 , separately). The antioxidant capacity related to reducing power from guava peel extract was ~ 1.1-2.4 times better than for the other eight extracts.

As a final point on results, the last applied assay that measured the reducing power of the rind extracts was the CUPRAC test (mechanism of singleelectron transfer, similar to FRAP). Figure 6 presents the cupric reducing potential, as a measure of the antioxidant capacity, of the husk extracts from 20 fruits expressed as mg equivalent to Trolox® and was found between 31 ± 2 mg TE/g and 111 ± 2 mg TE/g. Consistent with the value of median (51 ± 4 mg TE/g), ten extracts of fruit peels (tangerine, red pawpaw, red mombin, passion fruit, sweet granadilla, star fruit, black grape, guava, quince and custard apple) whose Trolox®-equivalent values were greater than the median ($54 \pm 2 - 111 \pm 2$ mg TE/g) were selected.

Figure No. 5 Graph of the comparison of reducing capacity values determined by FRAP assay for lyophilized peel extracts from 20 fruits of the northern Peruvian region



Figure No. 6 Graph of the comparison of reducing capacity values determined by CUPRAC assay for lyophilized peel extracts from 20 fruits of the northern Peruvian region



In descending order of mg TE/g value for these extracts, the greatest values were for the custard apple and quince/guava extracts (111 \pm 2 mg TE/g and 96 \pm 2/95 \pm 1 mg TE/g, particularly); subsequently the other extracts from black grape, star fruit, sweet granadilla and passion fruit ranked among 85 \pm 2 - 71 \pm 2 mg TE/g; and the red pawpaw and mombin, and tangerine extracts showed values amongst 67 \pm 2 - 54 \pm 2 mg TE/g. Comparing the reducing power of the ten extracts was found that the relationship between the shell extract of custard apple was ~ 2.1-1.2 times bigger than for the remaining nine extracts.

The collection and distribution of all results related to the five antioxidant tests and their medians are presented in Figure No. 7 (diagram of box and whisker). As can be seen in it, due to the median was not found in the center of the rectangle, the distribution of the data on antioxidant potential for trials was not symmetrical. However, for each assay was found that 50-75% de data was dispersed. In general, the most scattered data, in descending order, were for F-C, CUPRAC, DPPH, ABTS⁺, and FRAP tests. The variability in the data (dispersions) could be considered usual due to the differences in the chemical compositions of the extracts, particularly in

the content of phenolic/flavonoid compounds. This group of compound would be responsible of the antioxidant effect/capacity for each extract.

Once the data were obtained from the five antioxidant tests applied on the peel extract samples, they were statistically treated and subjected to the multivariate analyses with the purpose to found any relationship (similarity/difference) between them. The MVA exploration was initiated by applying the principal component analysis, in which the Factors 1 (~ 85%) and 2 (~ 11%) were able to explain together ca. 96% of the variability of original data. These factors presented eigenvalues greater or equal than to 0.5 (F1 - ~ 4.2; F2 - ~ 0.6). Figure No. 8 shows the resulting graph of PCA and according to it, the variables of greatest contribution (based on correlation) to Factor 1 were the values of antioxidant capacity by ABTS+ (0.22453), DPPH (0.22430), CUPRAC (0.22195) and FRAP (0.20402) tests; whereas the value by Folin-Ciocalteu (0.81975)contributed mainly to Factor 2 (e.g., the adjusted equation to the second principal component was 0.337042*FRAP 0.228139*CUPRAC +0.120617*ABTS^{+.} 0.007391*DPPH⁻ - 0.905402*FC, which is clearly showing the highest contribution by Folin-Ciocalteu value).

Figure No. 7 Box and whisker plot comparing the distribution of the data obtained (20 extracts of fruit peels) related to the five antioxidant tests (in a general way)



Figure No. 8 PCA plot involving to the antioxidant capacity values of the peel extracts from 20 fruits, measured through Folin-Ciocalteu, DPPH⁻, ABTS⁺⁻, FRAP and CUPRAC methods





If the cases are considered, the highest contribution to Factor 1 were the effects (related to antioxidant) of peel extracts of custard apple (26.498), star fruit (10.835) and guava (10.644); while the values for star fruit (42.710), guava (14.677) and black grape (9.711) were main to the Factor 2. As a final observation, in agreement to PCA plot, five main groups were found based on the similarities between the results of the antioxidant potential: A) composed by star fruit extract; B) including to custard apple extract; C) constituted by tangerine, bitter orange and mandarine extracts; D) conformed by guava, quince, sweet granadilla, black grape and passion fruit extracts; and, E) characterized by red pawpaw and mombin, pawpaw, dragon fruit, red and yellow indian figs, muskmelon, pear, apple and green grape extracts.

In addition to the above, two 3D plot were made to compare the extracts with the antioxidant tests. In the first plot, the results of the FRAP, CUPRAC and Folin-Ciocalteu methods were compared, and it was observed that seven extracts stood out over the others; nevertheless, only four of them (star fruit, custard apple, quince and sweet granadilla) showed the highest values (Figure No. 9). In the second graph, the values obtained by the FRAP, ABTS^{+.} and DPPH[.] assays were contrasted, and again, seven extracts surpassed to the others. Nonetheless, star fruit, custard apple, guava and sweet granadilla extracts were the most active.

For its part, from the CA by using the complete linkage as a joining rule and Euclidean distances (non-standardized) as a linkage measure, the vertical hierarchical tree plot including to the 20 extracts related to the antioxidant capacity values determined by five tests was built. Pursuant to similar/difference characteristics from the 20 extracts, five conglomerates were established (Figure No. 10): I - passion fruit, sweet granadilla, quince, guava and black grape extracts; II - custard apple and star fruit extracts; III - tangerine, bitter orange, red pawpaw and mombin extracts; IV - yellow and red indian figs and mandarine extracts; V - apple, pear, muskmelon, green grape, dragon fruit and pawpaw extracts.

Figure No. 9 3D plot resulting from the comparison between antioxidant effects measured by: i.) FRAP, CUPRAC and Folin-Ciocalteu assays; ii.) DPPH·, ABTS⁺·and FRAP test







The clusters with the highest media values were II and I, constituted by custard apple (72.205), star fruit (61.528), guava (57.353), quince (56.553), sweet granadilla (49.709), black grape (48.048) and passion fruit (41.770) extracts. As a complementary statistical analysis to CA, K-means clustering analysis was carried out. For the K-means analysis, the same cluster number (five) was established such as for CA. The analysis of variance applied to K-means cluster, as differentiation criteria, showed that all variables (five) related to antioxidant assays [Folin-Ciocalteu (0.000002), DPPH (0.000001), ABTS^{+.} (0.000000), FRAP (0.000001) and CUPRAC (0.000003)] were significant (p<0.05).

Figure No. 11 displays the plot of means for the five clusters based on the 20 extracts and the values of antioxidant power measured by five tests. Thus, cluster 1 (circle bullet) was constituted by citrus fruit extracts (tangerine, mandarine and bitter orange); cluster 2 (square bullet) contained to the extracts from quince, sweet granadilla, guava and black grape; while cluster 3 (diamond bullet) was composed by passion fruit and red mombin extracts; cluster 4 (triangle bullet) included to extracts from pawpaw, red pawpaw, muskmelon, dragon fruit, yellow and red indian figs, pear, apple, and green grape peels; and, cluster 5 (solid circle bullet) was formed by custard apple and star fruit extracts. For closing the interpretation of K-means, the fruit peel extracts that showed the expected behavior (the best antioxidant potential) based on the antioxidant capacity values of five tests were located in cluster 5 followed by cluster 2.

Figure No. 11 Plot of means for five cluster based on the antioxidant capacity values of the peel extracts from 20 fruits, measured through Folin-Ciocalteu, DPPH⁻, ABTS⁺⁻, FRAP, CUPRAC methods



Thus, the most active extracts were obtained from the peels of custard apple, star fruit, sweet granadilla, quince, guava and black grape fruit. It is important to clarify that the extracts of custard apple and star fruit showed the highest values of antioxidant capacity in the majority of the experiments performed, when compared with the other four extracts; however, for the FRAP method, the values between the six extracts were close.

At present, the trend of consumption of "nutraceutical" or functional foods has been extended due to existing reports (Wildman & Kelley, 2007; Yadav *et al.*, 2012; Nasri *et al.*, 2014; Gul *et al.*, 2016; Jampilek *et al.*, 2019; Gupta *et al.*, 2020) on the capability to prevent both degenerative/chronic diseases as well as helping to maintain good the consumer health (de Ancos *et al.*, 2009). It has been assumed that the phytochemicals responsible for these benefits are compounds of phenolic nature (e.g., simple phenols, phenolic acids and flavonoids), which possess a high radical-scavenger capacity related to the decrease/inhibition/control of different oxidation processes (Soobrattee *et al.*, 2005; Balasundram *et al.*, 2006). This would be one of the

reasons why there is a great interest in establishing the antioxidant potential of the horticultural products and analyzing in many cases, the variations in the maturation and senescence processes during the postharvest (Hurtado-Vidarte & Ortiz-Robles, 2018).

According to the reviewed scientific literature, various reports on antioxidant capacity (related to the five test used) from some fruit peels under study were found, which permitted to compare between results. The first data considered were reported by Hurtado-Vidarte and Ortíz-Robles (2018), which studied the antioxidant capacity (by Folin-Ciocalteu reagent, DPPH and CUPRAC assays) and chemical constituents (total polyphenol, flavonoids, etc.) from peels of the most consumed fruits in Peru; among these fruits were mandarine, apple, orange, pawpaw, and grape. The TPC (0.37-1.13 mg GAE/g), DPPH (0.49-2.86 mg TE/g), and CUPRAC (2.9-3.3 mg TE/g), values reported by these authors differed significantly; videlicet, such values were lower than those of this work, possibly, due to the manner they prepared the husks for obtaining extracts which was different (thermal dehydration: 103°C/3 h) (Correa & Bernal, 1989). Furthermore, Ordoñez-Gómez *et al.* (2018), found that total polyphenol content and antioxidant capability of peels from orange and mandarine (included among twelve varieties of citric fruits) collected at Departamento de Huánuco (Peru) were also lower, with TPC and IC₅₀ (DPPH and ABTS⁺) values of 14.0-32.2 mg GAE/g, 1.9-3.4 mg TE/mL and 0.17-0.24 mg TE/mL, respectively.

As regards to the shell extract of An. cherimola, this showed the best antioxidant effect (less by the test with Folin-Ciocalteu reagent, where the fruit peel extract obtained the second best value for TPC). When comparing our result with the available literature, it was found that the report by Loizzo et al. (2012), showed some coincidence related to that the Italian fruit shell had a high TPC value $(15 \pm 1 \text{ mg chlorogenic acid equivalents}/100 \text{ g})$ fresh weigh); however, the TPC value for the Peruvian fruit crust was much higher (65.2 \pm 0.7 mg EAG/g). Besides, the antioxidant potential based on the TAC values by ABTS+, DPPH and FRAP between Italian (I) and Peruvian (P) fruits were: TEAC-P (72 \pm 2 mg TE/g) > TEAC-I (3.6 \pm 0.2); DPPH-I (IC₅₀ 58 \pm 2 µg/mL) < DPPH-P (59.2 \pm 0.3 mg TE/g); and FRAP-I (53 \pm 3 μ M Fe(II)/g) < FRAP-P (54 \pm 2 mg TE/g). As a historical fact, it would be interesting to mention, that custard apple played an important role in the life of the Incas [it is native to the inter-Andean valleys of Peru and Ecuador (Calzada, 1993; Gardiazabal & Rosenberg, 1993)], as a food resource based on suggested by Gardiazabal & Rosenberg (1993).

In this same order, the second greatest antioxidant effect was presented by the husk extract from A. carambola fruit; however, it achieved the highest value for TPC, compared with the values of the other fruit rind extracts under study. This high reducing capacity would be in agreement as described by Asna & Noriham (2014) and Ruvini et al., 2007), who established that the fruit had a good antioxidant power. Nonetheless, Muñoz-Jáuregui et al. (2019), reported the antioxidant potential (by Folin-Ciocalteu, DPPH. and ABTS+.) of carambola fruit peel extract from Lima (Peru), with values of 10.0 ± 0.4 mg GAE/g, 43.8 ± 0.2 mg/mL (IC₅₀) and 9 mg TE/g, individually, which were lower than those found in this manuscript. Star fruit also called "kamrakh" (India) o "babingbing" (Philippines) is a tropical fruit native to southwestern Asia (Janick & Paull, 2008; Hii & Ogugo, 2014), besides distributed in subtropical areas of America, eg, Peru (González et al., 2001); a complete study of culture and adaptation can be seen in Mattheus-Cagua et al. (2015).

The peel extract with the highest anti-radical potential determined by FRAP method, in this research, was for *Psidium guajava* L. This result is interesting when was compared with the report by Liu *et al.* (2018a), who found that peel extract of Chinese guava presented a similar value of 66 ± 1 mg TE/g (FRAP – 60 ± 2 mg TE/g, Peru). Nevertheless, values of mg TE/g by ABTS⁺ and DPPH⁻ were different; ie, ABTS⁺-Ch (91 ± 1) > ABTS⁺-P (62 ± 2); and DPPH-Ch (66 ± 1) > DPPH⁻P (38.6 ± 0.4). Previously, Martínez *et al.* (2012), mentioned that guava fruit waste had a higher antioxidant power than pineapple and passion fruit.

Although all lyophilized extracts of husks from fruits grown in northern Peru generated TAC values according to the methods used, certain results were not correlated with studies published on peels of some of the fruits, such as *Cucumis melo* L. (Vouldoukis *et al.*, 2004; Alagar Raja *et al.*, 2015; Zeb, 2016; Rolim *et al.*, 2018). This fruit is of high consumption, with an abundant production and commercialization due to its great demand; Brazil is one of the main consumers (Rolim *et al.*, 2018). The intake of muskmelon has shown to be useful for the treatment and prevention of cancer due to the content of resveratrol, lycopene, astaxanthin and phenolic acids (Li *et al.*, 2012; Deng *et al.*, 2012).

When the review of the scientific literature was carried out for the antioxidant capacity of the fruit peels included in this work (low potential), some reports were found on them: *Citrus reticulate* (Ghasemi *et al.*, 2009; Tumbas *et al.*, 2010; Omer *et al.*, 2015; Hamdan *et al.*, 2016; Safdar *et al.*, 2017), *Malus domestica* (Navarro *et al.*, 2018), *Carica papaya* (Ang *et al.*, 2012; Calvache *et al.*, 2016; Jamal *et al.*, 2017); although, some works related to the antioxidant potential on their pulps were reported, e.g., *Passiflora ligularis* (Cabrera-Navarro *et al.*, 2014), *Citrus reticulate* (Zou *et al.*, 2016), *Spondias purpurea* (Elufioye & Berida, 2018), *Hylocereus megalanthus* (Choo & Yong, 2011).

In the case the indian fig peels, the values of antioxidant capacities and TPC were different from those reported by Andreu *et al.* (2018); ie, the two Peruvian fruit peels (red – 21 ± 1 mg EAG/g, yellow - 26.6 ± 0.3 mg EAG/g) showed greater TPC values than Spain fruit husk (17.4 ± 0.5 – 19 ± 1 mg EAG/g); however, the values on antioxidant capacities by ABTS⁺, DPPH and FRAP from Spain fruit were better [mmol Trolox®/kg (dw) - ABTS⁺: 33.3 ± 0.2 - 37.3 ± 0.9; DPPH: 54.8 ± 0.7 – 60 ± 2; FRAP: 40 ± 2 – 114 ± 5] than two Peruvian fruits.

It should be noted that the results obtained in this research differed to those of the consulted science literature, possibly due to the peel extracts were not prepared as dry/fluid extract (concentration by thermal/pressure evaporation) or tinctures, but they were lyophilized, which could improve the conservation and greater concentration of the components responsible for bioactivity.

CONCLUSIONS

The extracts with the highest values of antioxidant power were obtained from the peels of custard apple and star fruit, and they were followed by the peel extracts of sweet granadilla, quince, guava and black grape fruits. From them, it can be suggested that during the consumption of the fruit pulps from star fruit, quince, guava and black grape should also be ingest their rinds and not remove/discard them due to the high antioxidant potential they presented; while, the husks of the cherimoya could be used for the isolation of the antioxidant phytochemicals before their final disposal.

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