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Effect of *in vitro* gastrointestinal digestion on the release of phytochemicals from nanche (*Byrsonima crassifolia* L.) fruit

Efecto de la digestión gastrointestinal *in vitro* sobre la liberación de fitoquímicos del fruto de nanche (*Byrsonima crassifolia* L.)

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Technological innovation: New evidence on stability during gastrointestinal digestion stages and consumption as a source of compounds with health benefits.

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Resumen

El fruto de nanche (*Byrsonima crassifolia* L.) representa una importante fuente nutritiva por su alto contenido de fibra, vitaminas y minerales, así como también de fitoquímicos con actividad biológica. El objetivo de esta investigación fue evaluar la liberación de fitoquímicos durante la digestión gastrointestinal utilizando un modelo de digestión *in vitro* que simula las condiciones químicas y biológicas, además de estudiar los cambios en la actividad antioxidante durante el proceso de digestión. Los resultados de la investigación mostraron que los frutos de la selección UAA1 presentaron el mayor contenido de fenoles solubles totales en la extracción química (35 mg EAG/g), así como en la etapa gástrica (11.02 mg EAG/g) e intestinal (8.9 mg EAG/g). La transición del ambiente ácido a las condiciones alcalinas causó una disminución en la cantidad de polifenoles hidrolizables. En cuanto a los flavonoides totales estos se incrementaron al pasar a la fase intestinal.

La estabilidad de los compuestos fenólicos a los cambios de pH en las diferentes etapas de digestión afectó la biodisponibilidad de los mismos, aun cuando cerca del 35 al 45% del contenido de fenoles totales fue liberado en la etapa gástrica, estos compuestos disminuyeron al pasar a la etapa intestinal. Los cambios en la actividad antioxidante durante la digestión *in vitro* se correlacionaron con los cambios en la concentración de flavonoides, así como con el pH. Los frutos de la selección UAA1 presentaron la mayor capacidad antioxidante tanto a nivel gástrico como intestinal. Para ensayo ABTS los frutos de la selección UAA1 presentaron una mayor capacidad antioxidante en la etapa gástrica (561 mmol ET/g). En cuanto a la etapa intestinal, la selección UAA1 presentó mayor capacidad antioxidante mediante el ensayo DPPH (763 mmol ET/g) y FRAP (1326 mmol ET/g). El extracto químico de la selección UAA1 mostró una actividad antioxidante medida con el ensayo FRAP de 1442 mmol ET/g de nanche en base seca. La actividad antioxidante medida al final del procedimiento de digestión fue de 1326 mmol ET/g de nanche en base seca, correspondiente a 91% de la actividad antioxidante total en el extracto químico. En conclusión, por sus características intrínsecas, los frutos de nanche de la selección UAA1 podrían ser aprovechados para la obtención de fitoquímicos con aplicaciones en el desarrollo de alimentos funcionales.

Palabras clave: *Byrsonima crassifolia*, capacidad antioxidante, compuestos fenólicos, fitoquímicos.

Abstract

The nanche fruit (*Byrsonima crassifolia* L.) represents an important nutritional source due to its high content of fiber, vitamins and minerals, as well as phytochemicals with biological activity. The objective of this investigation was to evaluate the release of phytochemicals during gastrointestinal digestion using *an in vitro* digestion model that simulates chemical and biological conditions, and to study changes in antioxidant activity during the digestion process. The results of the research showed that the fruits of the UAA1 selection showed the highest content of total soluble phenols in the chemical extraction (35 mg EAG/g) as well as in the gastric (11.02 mg EAG/g) and intestinal (8.9 mg GAE/g) stages. The transition from the acid environment to alkaline conditions caused a decrease in the amount of hydrolysable polyphenols. As for total flavonoids, these increased when passing to the intestinal phase. The stability of phenolic compounds to pH changes in the different stages of digestion affected their bioavailability, even though about 35 to 45% of the total phenol content was released in the gastric stage, these compounds decreased when passing to the intestinal stage. The changes in antioxidant activity during *in vitro* digestion correlated with changes in flavonoid concentration as well as pH. The fruits of the UAA1 selection showed the highest antioxidant capacity both gastric and intestinal levels. For the ABTS test, the fruits of the UAA1 selection presented a higher antioxidant capacity in the gastric stage (561 mmol ET/g). Regarding the intestinal stage, the UAA1 selection showed higher antioxidant capacity in the DPPH (763 mmol ET/g) and FRAP (1326 mmol ET/g) assays. The chemical extract of the UAA1 selection showed an antioxidant activity measured with the FRAP assay of 1442 mmol TE/g of nanche on a dry basis. The antioxidant activity measured at the end of the digestion procedure was 1326 mmol TE/g of butter on a dry basis, corresponding to 91% of the total antioxidant activity

in the chemical extract. In conclusion, due to their intrinsic characteristics, the nanche fruits of the UAA1 selection could be used to obtain phytochemicals with applications in the development of functional foods.

Keywords: Antioxidant capacity, *Byrsonima crassifolia*, phenolic compounds, phytochemicals.

1. Introduction

Plant foods are products of great interest since macronutrients and micronutrients contain a series of substances that can have a significant impact on the course of some diseases and may be indispensable in the long term for our health. These bioactive substances are called phytochemicals (Palafox-Carlos *et al.,* 2011). Tropical fruits have been especially noted for being a rich source of a wide variety of biological active phytochemicals, such as phenolic compounds, phytosterols, carotenoids, and some vitamins (De La Rosa *et al*., 2010). The fruit of the nanche (*Byrsonima crassifolia* L.) is a tropical species native to Mexico and Central America (CONABIO, 2019), which is highly valued as a food supplement for its great contribution of dietary fiber, vitamins, and minerals, as well as phytochemicals, especially phenolic compounds. Compounds such as gallic acid and quercetin have been mainly identified as well as catechin, epicatechin, rutin and kaempferol (Pires *et al*., 2019; Rodrigues *et al*., 2016) as well as ferulic acid, resveratrol, and caffeic acid (Mariutti *et al*., 2013; López *et al*., 2014). Several properties have been attributed to phenolic compounds. In this regard, one of the most important is the antioxidant capacity. This activity seems to be related to its chelating capacity, lipoxygenase inhibition, and free radical scavenging (Alamed *et al*., 2009). However, these properties depend on how bioaccessible these compounds are at the time of digestion for absorption (Saura-Calixto *et al*., 2007; Bohn, 2014). The bioavailability of a compound depends on its digestive stability, its release from the food matrix (known as bioaccessibility), and the efficiency of its transepithelial passage. Bioavailability differs greatly from one phenolic compound to another, and for some compounds, it depends on the food source (Manach *et al*., 2005). In addition, most polyphenols exist in foods as esters, glycosides, or polymers that cannot be absorbed in their native form (Crozier *et al*., 2009). Only aglycones and some glycosides can be absorbed in the small (Bouayed *et al*., 2012). The most important factors to determine the possible beneficial effects of phenolic compounds and ensure their bioavailability are stability and bioaccessibility in gastrointestinal conditions, in this sense, the phytochemical compounds present in nanche fruit will be stable to the conditions of gastrointestinal digestion *in vitro,* increasing their bioaccessibility. Therefore, the aim of this research was to evaluate the release of phytochemicals using an *in vitro* gastrointestinal digestion model that simulates chemical (pH, temperature) and biological (gastric and enzyme) conditions as well as to study the changes in antioxidant activity in the digestion process.

2. Materials and methods

2.1 Material

The fruits of selections UAA1, UAA6, UAA15 were collected from trees after natural abscission located in the germplasm bank of the Academic Unit of Agriculture of the Autonomous University of Nayarit (21.42555° LN, 105.89103° LO, 966 masl) during the agricultural cycle August-October, 2019. This germplasm bank has a semi-warm sub-humid climate with an annual rainfall of

1,267.3 mm and an average annual temperature of 20.5 °C.

For this research, 30 fruits per tree were selected according to size, color, maturity for consumption and without apparent mechanical damage. The fruits were transferred in polyethylene bags (18 x 20 cm) to the laboratory of the Food Technology Unit of the Autonomous University of Nayarit on the same day of harvesting. The fruits were washed with water and sodium hypochlorite solution at 1% for 10 min. Then, the seed was removed to grind the pulp and shell. The paste obtained was lyophilized (Labconco® Free zone 2.5) and stored at −20 °C until further analysis.

2.2 In vitro gastrointestinal digestion

The nanche selections were subjected to *in vitro* gastrointestinal digestion using the method described by Saura-Calixto *et al.* (2000) with modifications by Blancas-Benítez *et al.* (2018). Each stage of the digestion was performed separately to ensure a better interpretation of the results. For the simulation of the gastric phase, 150 mg of nanche fruits were incubated on a dry basis with pepsin (300 mg/mL, in HCl-KCl buffer, pH 1.5, 40° C, 1 h, P-7000, Sigma-Aldrich). After the gastric phase, nanche selections were subjected to the simulated intestinal phase with pancreatin solution (5 mg/mL in phosphate buffer, pH 7.5, 6 h, 37 °C, P-1750, Sigma-Aldrich) containing α- amylase (120 mg/mL in Tris-maleate buffer, pH 6.9, 16 h, 37 °C, A-6255, Sigma-Aldrich). Extractable polyphenols (EP, mg GAE/g) from the supernatant of each stage of the *in vitro* gastrointestinal digestion were considered as the phenolic compounds present in the gastric fraction (GasF) and in an intestinal fraction (IntF).

2.3 Chemical extraction (CE)

An aqueous organic extraction was performed with an acidified methanol-water solution (50:50 v/v) (Pérez-Jiménez *et al.*, 2008). 250 mg of nanche fruit were weighed on a dry basis in 50 mL centrifuge tubes to make an organic extraction of the total extractable polyphenols. 10 mL of the acidified methanolic solution were added, and the tubes were kept under constant agitation at 25 °C ± 2 °C for 1 h. Afterwards, the samples were centrifuged at 3000 rpm, at 4 °C for 10 min and the supernatants were separated into 25 mL volumetric flasks. Then, 10 mL of acetone-water solution were added to the residue of the previous extraction and kept under stirring at 25 °C ± 2 °C for 1 h. The supernatant was recovered in the same 25 mL flask to mix the extracts and made up to volume with the methanol/HCl/water, acetone-water solution (50:50 v/v). Non-extractable polyphenols (NEPs) were obtained by hydrolysis from the residues of the aqueous organic extraction and the digested fractions by the method described by Hartzfeld *et al.* (2002). The NEPs were spread with a glass rod and 20 mL of methanol, as well as 2 mL of H_2SO_4, were carefully added. Subsequently, they were incubated in a shaking bath at 85ºC for 20 h. After the incubation time, the tubes were allowed to cool at 25 °C ± 2 °C and then centrifuged at 3000 rpm for 10 min, recovering the supernatant in a 50 mL flask. Subsequently, the residues were washed twice with 10 mL of distilled water, centrifuging under the same conditions between each of the washes. The supernatants were mixed and made up to 50 mL.

2.4 Determination of phenolic compounds, hydrolyzable polyphenols, and flavonoids

The content of extractable polyphenols (EP) in the extracts of the digested and chemically digested fractions obtained were quantified

by the Folin-Ciocalteu method (Montreau, 1972), measuring the absorbance at 765 nm with a microplate reader (Biotek, Synergy HT, USA). The gallic acid was used as standard and the results were expressed as mg GAE/g dw. NEPs were quantified as hydrolyzable polyphenols (HP). The quantification was carried out with the same methodology described above for EPs and the results were expressed in mg GAE/g dw according to a gallic acid curve. Total polyphenols (TP, mg GAE/g dw) were calculated as EP + NEP. Total flavonoids (TF) content was evaluated according to the colorimetric method described by Oomah *et al.* (2005). Briefly, 50 µL of the digested fractions and chemically extracted samples were placed in 180 µL of distilled water. Then, 20 µL of 1% 2-aminoethyldiphenylborate solution was added. The absorbance was read in a microplate reader (Biotek, Synergy HT, USA) at 404 nm, and the total flavonoid content was expressed as mg Rutin equivalents (RE)/g dw.

2.5 Antioxidant activity

Reducing capacity and radical scavenging activity of digested fractions and chemically extracted samples were measured. The determination of the reducing capacity was carried out using the protocol based on the ferric antioxidant/reducing power assay (FRAP) described by Benzi and Strain (1996). Briefly, 900 µL of FRAP reagent, TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), $FeCl_3$, acetate buffer, and 90 µL of distilled water were mixed with 30 µL of the extract and then the absorbance was measured at 595 nm every 20 s for 30 min at 37 °C. Radical scavenging was evaluated by the ABTS (2,2-azinobis-(3-ethylbenzothioazolin-6-sulfonic acid) colorimetric method (Re *et al.*, 1999). Briefly, 7 mmoL/L of ABTS solution and 2.45 mmoL/L of potassium persulfate were mixed in a 1:1 ratio and allowed to stand in the dark for 12-16 h to produce the ABTS radical cation. This solution was diluted with acidified methanol and acetone: water to reach an absorbance of 0.07 ± 0.02 at 734 nm. Subsequently, 30 µL of extract and 250 µL of the radical were added to a microplate and incubated in the dark for 7 min. The decrease in the absorbance of the sample at 734 nm was observed. The radical scavenging activity was determined following the method of (Brand-Williams *et al.*, 1995). A solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) containing 3.9 mg/100 mL of methanol 80% was stirred for 60 min. Subsequently, it was diluted with methanol (80%) until reaching an absorbance of 0.07 ± 0.02 at 515 nm. An aliquot of 30 µL was added to 250 µL of the DPPH solution to incubate in the dark for 30 min. The decrease in the absorbance of the sample at 515 nm was observed. The absorbances were observed in a microplate reader (Biotek, Synergy-HT, USA). Results were expressed as mmol of Trolox equivalents (TE)/g dw.

2.6 Statistical analysis

Data were analyzed by a completely randomized design using analysis of variance (ANOVA). Comparison of means of the Tukey test with 5% significance level was performed when ANOVA showed significant differences. The JMP 11.0 software (SAS, Institute Inc., 2013) was used for all statistical analysis. Results were expressed as the mean values of three biological replicates ± standard deviation (SD).

3. Results

3.1 *Phenolic compounds, hydrolyzable polyphenols, and flavonoids*

The content of TSP released during *in vitro* gastrointestinal digestion and by CE is shown in Figure 1. Regarding CE, the UAA1 selection (35 ± 1.6 mg GAE/ g dw) showed significant differences ($P<0.05$) compared to the other selections. It should be noted that the amount of bioaccessible polyphenols in

food can differ quantitatively and qualitatively from the polyphenols extracted with chemical methods, so not all the polyphenols present in the food matrix, but only those that are released in the gastrointestinal tract are actually bioaccessible in the intestine and, therefore, potentially bioavailable (Tagliazucchi *et al.,* 2010). Regarding the different stages of *in vitro* digestion, the highest quantification of soluble phenols occurred in the gastric phase. These results could be due to the hydrolysis of some phenolic compounds linked to some other component of the food matrix such as proteins and/or carbohydrates, mainly due to the acidic pH and the action of digestive enzymes that hydrolyze the non-covalent bonds between the hydroxyl groups of phenolic compounds and the polar groups of polysaccharide molecules; while in proteins, they hydrolyze the hydrogen bridging bonds between the hydroxyl groups and carboxyl groups of the peptide bonds (Saura-Calixto *et al.,* 2007; Palafox-Carlos *et al.,* 2011; Rodríguez-Roque *et al.,* 2013). High concentrations of phenols have been reported in gastric conditions, in contrast to the intestinal phase (Rodríguez-Roque *et al.,* 2013). Likewise, an absorption of phenolic acids in the gastrointestinal tract has been reported within the first and second hour after ingestion, therefore, they are the first compounds to be released in the gastric phase. Once these compounds are subjected to a drastic pH change in the intestine (pH 7.5), can undergo degradation (Lafay and Gil-Izquierdo, 2008). As for the nanche selections in the GasF, a higher content of soluble phenols was found in the UAA1 selection (11.02 ± 0.01 mg GAE/g dw, presenting significant differences (P<0.05) compared to the UAA6 selection. These results may be related to the pH of the fruits; which indicates the presence of acid groups, including organic acids, phenols, and amino acids (Paull, 1997). In fact, the UAA1 selection is characterized by having an acidic pH (Agredano-De La Garza *et al.,* 2021). In addition, the UAA1 selection (8.9 ± 2.9 mg GAE/g dw) continues to present higher content of soluble phenols in the IntF, although there are no significant differences in comparison with the other selections. The bioavailability of these compounds will vary widely and will depend on various factors such as the food source and chemical interactions with other phytochemicals and biomolecules (Manach *et al.,* 2005).

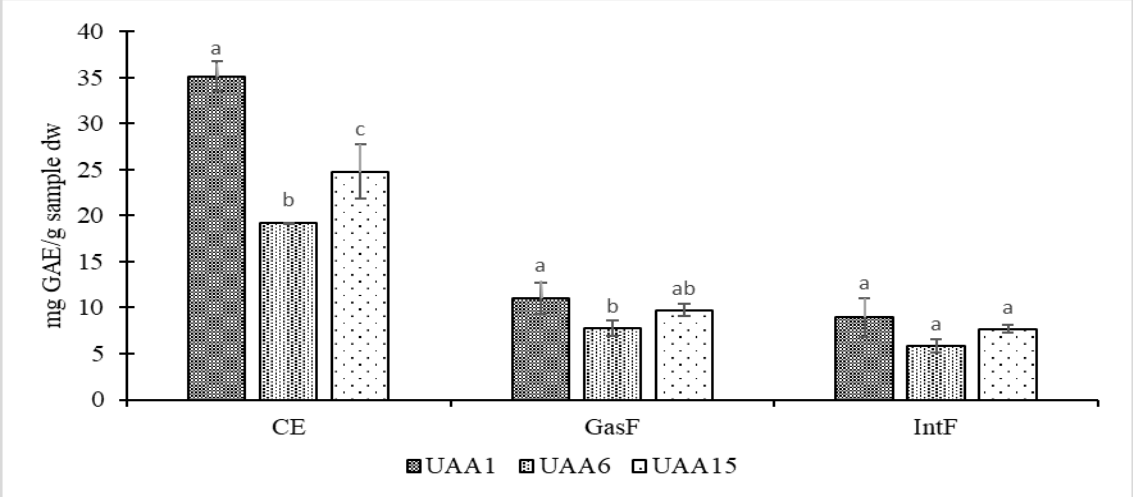

Figure 1. Total soluble phenols of the nanche selections during the chemical extraction, *in vitro* gastric phase and intestinal phase. Different letters per selection indicate a significant difference (P<0.05). Vertical lines mean the SD of three replicates. CE=Chemical extraction, GasF=Gastric fraction, IntF=Intestinal fraction. UAA represents the different nanche selections.

The content of HP obtained in the CE (2.2 mg GAE/g dw) of nanche fruits was lower than those of the GasF (2.9 mg GAE/g dw) (Fig 2). It has been shown that the phenolic compounds that are released by chemical extraction and hydrolysis may differ from those released in the human intestine, possibly because these procedures involve different sample treatments (Arranz *et al.*, 2010). In addition, these compounds are made up of phenolic acid polymers, which form larger complexes with the food matrix, making them difficult to extract them by conventional methods. However, during the *in vitro* digestion process, the food matrix is transformed due to the interaction of the medium and digestive enzymes, which facilitates or increases the release of these compounds (García-Gutiérrez *et al.,* 2017). The content of some HP in the first stage is possibly related to ellagitannins when exposed to acids, ester bonds are hydrolyzed and ellagitannins are rearranged into ellagic acid, which generates an increase release of hydrolyzable tannins in the gastric phase (Alminger *et al.,* 2014). The transition from acidic to alkaline conditions caused a decrease in the amount of HP. This behavior is similar to that reported by Krook and Hagerman (2012) who showed that some hydrolyzable tannins such as penta-galoyl glucose are unstable at pH greater than 7, causing degradation. Therefore, since the intestinal phase takes place at 7.5 pH, all those compounds of this nature were less stable when entering this stage. On the other hand, the UAA6 selection (2.9 ± 0.1 GAE/g dw) presented significant differences (P<0.05) compared to the UAA1 and UAA15 selection in the IntF. This may be due fact that the fruit of the UAA6 selection is characterized by having a high lignin content, which is highly related to the fiber content in the fruit peel. The HP are mainly found in the fruit peel, it should be noted that in this research the peel and pulp of nanche were analyzed.

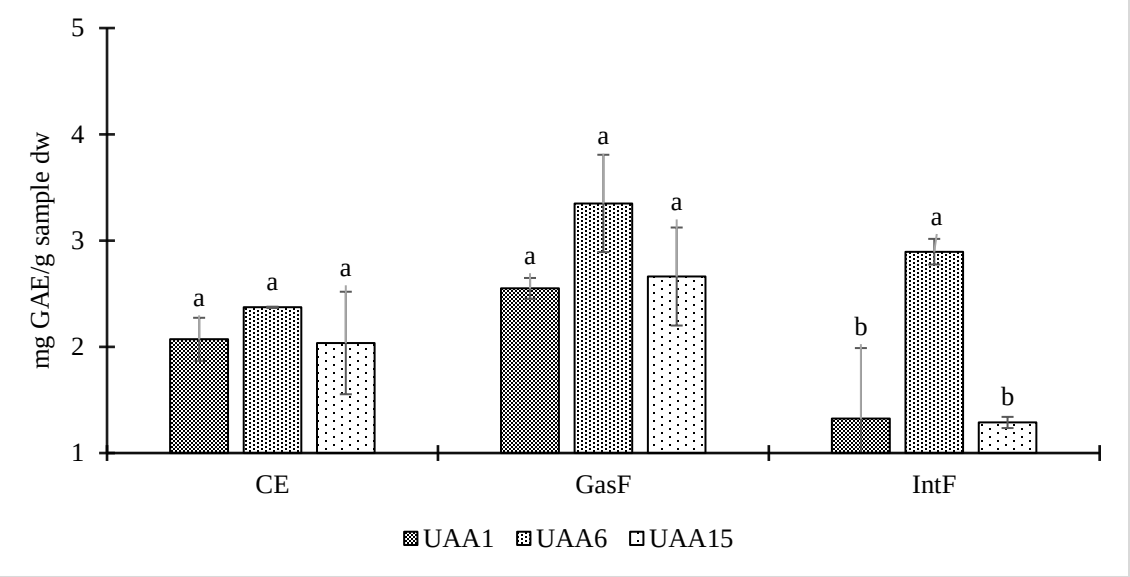

Figure 2. Hydrolyzable polyphenols of the nanche selections during chemical extraction (CE), *in vitro* gastric phase and intestinal phase. Different letters per selection indicate a significant difference (P<0.05). Vertical lines mean the SD of three replicates. CE=Chemical extraction, GasF=Gastric fraction, IntF=Intestinal fraction. UAA represents the different nanche selections.

According to the results of this research, TF increased when passing to the intestinal phase (Fig 3). In fruits, flavonoids can be found in free form (aglycones), as linked to sugars to form heterosides, which is the most frequent (Crozier *et al.,* 2009). Some flavonoids are released more rapidly as aglycones than those that are glycosylated, and these tend to be better absorbed in the small intestine (Bouayed *et al.,* 2012). Glycosides generally resist acid hydrolysis in the stomach and reach the duodenum intact (Gee *et al.,* 1998). On the other hand, gastric absorption of flavonoids has been observed in mice surgically treated so that absorption is restricted to the stomach, showing that absorption at gastric level is possible for some flavonoids, but not for their glycosides (Crespy *et al.,* 2002). Therefore, glycosylation clearly influences its absorption. As for the flavonoids present in the GasF, higher values were observed for the UAA15 selection (3.2 ± 0.6 mg RE/g dw) as well as for the IntF (6.2 ± 1.6 mg RE/g dw), although there are no significant differences in comparison with the other selections (Fig 3). Various flavonoid compounds form bonds between lignin fragments through ether bonds, which limits their absorption and release in the gastric phase (Bily *et al.,* 2003); the selections within their phytochemical characterization showed a high content of lignin (0.30 g/100g dw), which could cause a limited bioavailability at the gastric level. Nanche also has abundant flavonoid compounds, especially rutin (quercetin-3-rutinoside), a glycosylated compound that, when hydrolyzed, releases its aglycone (quercetin) (Pires *et al.,* 2019; Rodrigues *et al.,* 2016). Hence, some of the glycosylated compounds that are present in the fruit could resist gastric digestion to be bioavailable in the intestinal phase.

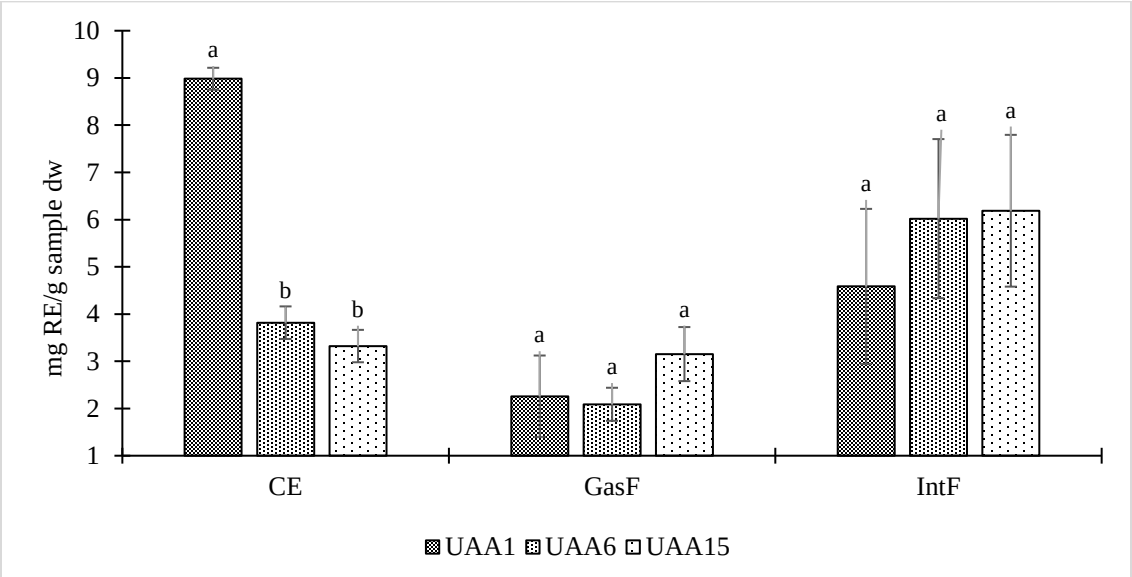

Figure 3. Flavonoids of the nanche selections during chemical extraction, *in vitro* gastric phase and intestinal phase. Different letters per selection indicate a significant difference (P<0.05). Vertical lines mean the SD of three replicates. CE=Chemical extraction, GasF=Gastric fraction, IntF=Intestinal fraction. UAA represents the different nanche selections.

In the intestinal phase, the release of phytochemical compounds is affected by changes in pH and the action of the enzymes pancreatin and α-amylase, responsible for hydrolyzing starch into its simpler constituents (Hur *et al.,* 2011). Tagliazucchi *et al.* (2010) evaluated the bioavailability of phenolic compounds during *in vitro* digestion

of grapes. The authors showed that incubation with pancreatic solution increased the bioaccessibility of total flavonoids after two hours of digestion, increasing by 7.44 mg catechin/100 g grapes (an increase of 20.5%). They also suggest that flavonoids other than anthocyanins are stable at alkaline pH since these were greatly affected, with a loss of 58%. Total polyphenol content was quantified by obtaining all those extractable and non-extractable phenolic compounds (Fig 4). In this sense, the UAA1 selection was significantly (P<0.05) higher in the release of total phenols in both the gastric (13.6 mg GAE/g dw) and intestinal (10.2 mg GAE/g dw) fractions.

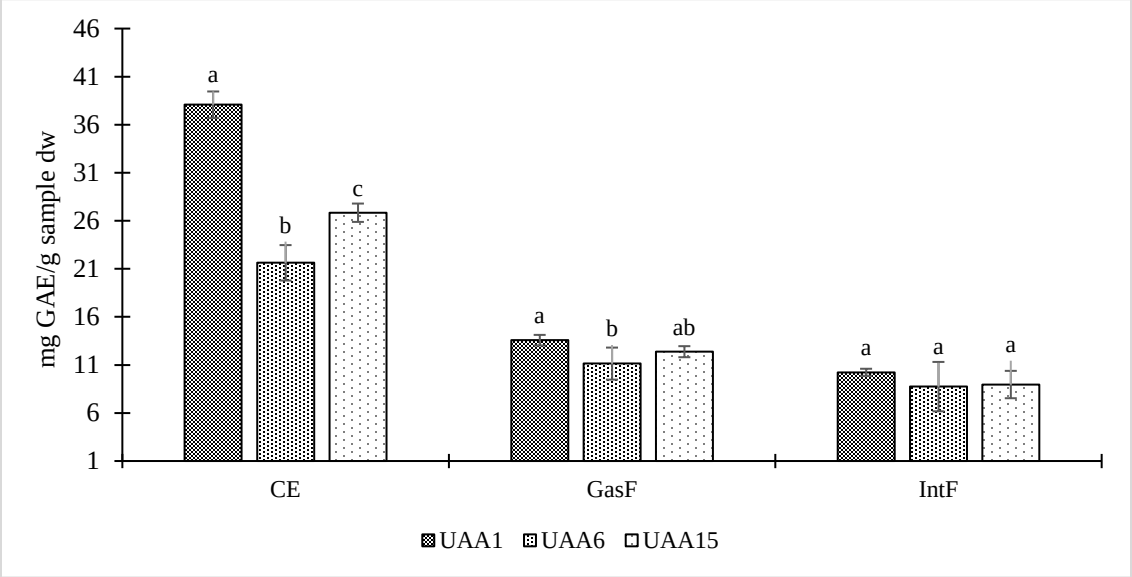

Figure 4. Total polyphenols of the nanche selections during chemical extraction, *in vitro* gastric phase and intestinal phase. Different letters per selection indicate a significant difference (P<0.05). Vertical lines mean the SD of three replicates. CE=Chemical extraction, GasF=Gastric fraction, IntF=Intestinal fraction. UAA represents the different nanche selections.

The stability of phenolic compounds to changes in pH in the different stages of digestion affects their bioavailability, and this is reflected in the results found, since they showed differences between the stages of digestion (gastric and intestinal). Although about 35 to 45% of the total phenol content was released in the gastric phase, these compounds decreased in the intestinal phase. This behavior is similar to the reported by Hernández-Maldonado *et al.,* (2019), who showed that in the *in vitro* digestion of a mango snack, the content of phenolic compounds in the gastric phase was slightly higher than the observed in the intestinal phase. Kosinska-Cagnazzo *et al.* (2015) point out that this behavior could be due to the incomplete release of phenols from the matrix due to possible interactions with other compounds, such as fiber, proteins, and lipids, poor enzymatic hydrolysis, and, especially, instability in alkaline conditions. Considering the amount, the total polyphenols quantified by the CE, only 64, 91, and 80% of the selections UAA1, UAA6, and UAA15, respectively were bio-accessible and therefore potentially bioavailability, while the rest could have been degraded and partly not extracted from the food matrix.

3.2 Antioxidant activity

The bioaccessibility of phytochemical compounds is extremely important to counteract the oxidative damage generated throughout the gastrointestinal tract,

promoting good intestinal health due to the antioxidant capacity of the compounds (Masibo and He, 2008). Table I shows the antioxidant capacity evaluated by the ABTS, DPPH, and FRAP methods in the different stages of *in vitro* digestion. The antioxidant activity of the nanche selections increased significantly (P<0.05) during the transition from acidic gastric environment to the alkaline intestinal environment. This could be attributed to the increased release of TF in the intestinal phase (Fig 3). From the point of view of antioxidant activity, the solubility of flavonoids is important, since the aglycon structure offers better antioxidant properties than the glycosides. The alkaline pH favored the solubility of these compounds, as most of them are poorly soluble in neutral aqueous solutions, but are soluble in alkaline aqueous solutions. In this way, the equilibrium structures (neutral or ionized) that predominate in a solution are totally dependent on pH (Jovanovic *et al.*, 1994).

Table I. Antioxidant capacity of the nanche selections during chemical extraction, *in vitro* gastric phase and intestinal phase.

	ABTS (mmol TE/g dw)	%CV	**DPPH** (mmol TE/g dw)	%CV	**FRAP** (mmol TE/g dw)	%CV
Chemical extraction (CE)						
UAA1	582 ± 8.5^{a}	1.5	608 ± 13.6^{a}	2.2	1442 ± 4.1^{a}	0.3
UAA6	581 ± 7.2^{a}	1.2	605 ± 10.8^{a}	1.8	908 ± 0.7^{b}	0.1
UAA15	584 ± 6.0^{a}	1.0	613 ± 8.3^{a}	1.3	1274 ± 3.6^{a}	0.3
Gastric fraction (GasF)						
UAA1	561 ± 28^{a}	5	556 ± 34^{a}	6.1	465 ± 28^{a}	6.1
UAA6	536 ± 5^{ab}	0.97	540 ± 10^{a}	1.9	301 ± 7^{b}	2.4
UAA15	507 ± 13^{b}	2.58	466 ± 24^{b}	5.1	341 ± 18^{b}	5.3
Intestinal fraction (IntF)						
UAA1	820 ± 38^{a}	4.63	763 ± 25^{a}	3.3	1326 ± 10^{a}	0.7
UAA6	823 ± 49^{a}	5.89	666 ± 48^{b}	7.1	1286 ± 37^{b}	2.9
UAA15	821 ± 28^{a}	3.41	541 ± 9^{c}	1.6	1305 ± 15^{b}	1.1
MSD CE	14.6		22.2		639.6	
MSD GasF	36.22		49.34		42.21	
MSD IntF	53.85		40.95		47.07	

Values are means of three repetitions ± SD, different letters per column indicate a significant difference (P<0.05), MSD; Minimum significant difference.

According to Martínez-Flórez *et al.*, (2002) the acid-base properties of some flavonoids show that phenoxyl radicals are neutral in an acid medium and acquire a negative charge at pH 7. This means that at physiological pH of plant tissues (5-7.5) and human plasma (7.4), the most likely way to find these flavonoids is as a phenoxide anion. Such is the case with quercetin, since it has been shown that its catechol group is completely deprotonated at physiological pH, which implies that it can exert a high antioxidant activity within the human body. However, these properties do not apply to all flavonoids, since these properties vary according to the structure. Also, the transition from gastric environment to the alkaline intestinal environment can induce structural changes in the phenolic

molecules, which is mainly attributed to the ionization of the hydroxyl groups present in the aromatic rings of the phenolic compounds, generating changes in the antioxidant activity in relation with the variation in the pH value (Mukai *et al.*, 1997). This behavior was demonstrated by Tagliazucchi *et al.* (2010) in a grape extract that was subjected to *in vitro* digestion, showing a significant increase in antioxidant activity during the transition from gastric to intestinal environment. These changes can be studied with the ABTS assay since it is carried out in an alkaline medium (pH 7-7.5). In the GasF, the UAA1 selection (561 ± 28 mmol TE/g dw) presented the highest antioxidant capacity by ABTS. This selection has a higher content of phenolic compounds which could influence the total antioxidant capacity. On the other hand, the antioxidant activity of the nanche selections evaluated with FRAP and DPPH assays showed a similar trend with respect to the ABTS assay during gastric digestion. Regarding the IntF, the UAA1 selection showed significant differences (P<0.05) compared to the other selections evaluated using the DPPH (763 ± 25 mmol TE/g dw) and FRAP (1326 ± 10 mmol TE/g dw) assays. The FRAP method is a method that does not evaluate the free radical neutralizing capacity but rather its reducing capacity by electron transfer, specifically capable of reducing the ferric ion to the ferrous state. In this context, the antioxidant activity of flavonoids results from their chelating properties of metal ions, such as Fe^{2+}, Cu^{2+}, Zn^{2+}. Hence, the significant (P<0.05) increase in antioxidant activity in the intestinal phase by FRAP assay ($r^2 = 0.80$) has been attributed to this property. The chemical extract of the UAA1 selection showed antioxidant activity by FRAP assay of 1442 mmol TE/g dw of nanche. The antioxidant activity measured at the end of the digestion procedure was 1326 mmol TE/g dw, corresponding to 91% of the total antioxidant activity in the chemical extract.

4. Conclusions

The stability of the phytochemical compounds present in the nanche fruits was affected by changes in pH at different stages of *in vitro* gastrointestinal digestion, minimizing their bioaccessibility at the intestinal level. The CE of the nanche fruits of the UAA1 selection showed a high content of TSP. This action was also observed in the different stages of *in vitro* gastrointestinal digestion. Indeed, with this same extraction, there is a minimal release of HP due to its chemical composition; increasing the bioaccessibility of TF during the transition from the acidic gastric environment to the alkaline intestinal environment, promoting a significant increase in antioxidant capacity. The UAA1 selection presented the highest antioxidant activity at the gastric and intestinal phase.

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