



***In vitro* Bioaccessibility of Phenolic Compounds from the Halophytes *Suaeda Edulis* and *Suaeda Esteroa*: Opportunity for the Development of Novel Foods**

FRANCYELLI REGINA COSTA-BECHELENI¹, ENRIQUE TROYO-DIÉGUEZ^{2*},
ALAN AMADO RUIZ- HERNÁNDEZ³, FERNANDO AYALA-NIÑO⁴, LUIS ALEJANDRO
BUSTAMANTE-SALAZAR⁵, NORMA JULIETA SALAZAR-LÓPEZ⁶
and ROSARIO MARIBEL ROBLES-SÁNCHEZ³

¹Center for Biological Research of Northwest Mexico S. C. (CIBNOR), Graduate Studies in the Use, Management and Preservation of Natural Resources, Av. Polytechnic Institute, Mexico.

²Center for Biological Research of Northwest Mexico S. C. (CIBNOR), Arid Zone Agriculture Program, Av. Polytechnic Institute, Mexico.

³Functional Foods and Nutraceuticals Laboratory, Research Department and Postgraduate in Food (DIPA), University of Sonora (UNISON), Mexico.

⁴Biotechnology and Prototypes Unit (UBIPRO), Faculty of Superior Studies Iztacala, Autonomous National University of Mexico, Mexico.

⁵Department of Instrumental Analysis, Faculty of Pharmacy, University of Concepcion Chile.

⁶Institute of Agricultural Sciences. Baja California Autonomous University. Mexicali, B.C., México.

Abstract

Halophytic plants grow in high salinity environments and present phytochemicals with antioxidant properties, such as phenolic compounds; due to the uncertain availability of healthy foods, there is a growing interest in their nutritional potential. However, their bioactive compounds with beneficial health effects are limited in their bioaccessibility. The objective of this study was to subject *S. edulis* and *S. esteroa* to an *in vitro* digestion process to evaluate the bioaccessibility and total antioxidant capacity of phenolic compounds during three phases of digestion. We determined phenolic compounds, flavonoids, and antioxidant capacity by colorimetric methods and phenolic composition by UHPLC-DAD. Total phenols, total flavonoids, and total antioxidant capacity by DPPH and TEAC in the three phases of digestion (oral, gastric, and intestinal) of *S. esteroa* were higher than in *S. edulis*, founding 4.84 % higher in total phenol content, and 0.05 % in total flavonoid content; also, and 28.94 and 23.93 % higher in total antioxidant



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CONTACT Enrique Troyo-Diéguez ✉ etroyo04@cibnor.mx 📍 Center for Biological Research of Northwest Mexico S. C. (CIBNOR), Arid Zone Agriculture Program, Av. Polytechnic Institute, Mexico.



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capacity by DPPH and TEAC, respectively in the intestinal digestion phase. The bioaccessibility of *S. edulis* was higher than in *S. esteroa*; the intestinal was the phase reflecting more bioaccessible compounds. The bioaccessibility percentages of total phenols and flavonoids were 590.16 and 1012.93 %, and the percentage recovery of total antioxidant capacity by DPPH and TEAC were 181.37 and 139.74 %. We identified phenolic acids ferulic, p-Coumaric, and synaptic (hydroxycinnamic), gallic and protocatechuic (hydroxybenzoic), the flavonoids catechin (flavan-3-oles), myricetin and ruthin (flavonols), naringenin and naringin (flavonones). *S. esteroa* presented bioactive compounds in higher concentrations than *S. edulis* due to the stress imposed by its habitat; nevertheless, the determined bioactive compounds of *S. edulis* showed a higher bioaccessibility because it was managed under local improvement.

Introduction

Digestion is the process of breaking down mechanically and enzymatically the food for absorption.¹ This process begins in the mouth, where food is crushed into smaller pieces by the teeth, while both enzymes, such as salivary amylase and lingual lipase, begin chemical digestion.² Subsequently, peristaltic contractions of the smooth muscle and mechanical digestion occur in the stomach. Then, further food degradation takes place with the activity of hydrochloric acid and enzymes such as pepsin.³ Finally, through the action of enzymes such as trypsinogen, pancreatic amylase, pancreatic lipase, chyme trypsinogen, and procarboxypeptidase, among others, chemical digestion in the intestine prepares the food components for absorption by the intestinal cells.⁴

Within the process of food digestion, in addition to breaking down nutrients into absorbable elements, during that mechanism, various compounds present in the food matrix of plant-derived products are also released.^{5,6} Phenolic compounds are secondary plant metabolites involved in defense against pathogens, protection against herbivores, and antioxidants, among others.⁷ Their structure is varied, as they can present one or more hydroxyl groups in their composition and an aromatic ring.⁸ Phenolic compounds are classified as simple and polyphenolic, the simple ones have an aromatic ring and an alkyl, aryl, alkenyl, alkoxy, hydroxy or amino group. Polyphenolics, on the other hand have more than one phenolic unit and generally have C15.⁹ These compounds are free, bound to organelles, conjugated to macromolecules, confined to cell

walls, and trapped in matrices.¹⁰ High bioaccessibility depends on the simple structure of some of the compounds with C6, C6-C1 and others.^{11,12} Small phenolic compounds can be absorbed directly in the intestine, whereas complex compounds cannot.¹³ Several factors can influence the bioaccessibility of phenolic compounds, the chemical state of the food matrix, interactions or the presence of cofactors or suppressors. It is estimated that 48% of all phenolic compounds are absorbed in the small intestine, 42% in the large intestine and only 10% are not digested.¹⁴ Therefore, the release of these compounds after digestion is called bioaccessibility. Those compounds released from the food matrix that is subsequently absorbed and reach intact or metabolized target tissues are called bioavailable. Likewise, compounds that produce biological effects in the body represent bioactivity.¹⁵

Among the observed benefits of phenolic compounds are the antioxidant activity by neutralizing free radicals resulting from metabolic processes,¹⁶ anti-inflammatory effects by reducing the synthesis of proinflammatory cytokines,^{17,18} as well as cardiovascular,^{19,20} neurological^{21,22} and anticancer effects.^{23,24}

Due to these health benefits, new foods are developed on whole grains,^{25,26} fruit and vegetable-based beverages,^{27,28} fortified dairy products,^{29,30} and functional foods,^{31,32} have been developed. However, due to the current conditions on food production and climate change, new sources for food development, which also present beneficial health effects, are being sought.³³⁻³⁵

Among the new sources of phenolic compounds considered to obtain food responsibly are the use of cereals (sorghum, millet), with low water requirements and resistance to climate change,³⁶ the use of by-products,^{37,38} as well as the search for new plant resources such as algae and edible halophytes.^{39,40}

Among the plants, halophytes can develop salt glands, have compatible solute accumulation, specialized root systems, specialized metabolism, and high tolerance to osmotic stress, which allows them to grow in soils with high salt concentrations such as mangroves and coastal areas.^{41,42} Due to these stress conditions during their growth, they present considerable concentrations of phenolic compounds,⁴³ exploitable for the development of potentially healthy foods, which in turn would help counteract the effects of climate change (remediation of saline soils) and provide new crops for marginal areas.

Therefore, to know the potential beneficial health effects of halophytes, it is proposed to determine the bioaccessibility of phenolic compounds of *S. edulis* and *S. esteroa*, which would bring us closer to understanding the release processes of these compounds during digestion processes.

Materials and Methods

Study Area and Experiential Installation

We carried out this study at the Functional Food Laboratory of the Research and Postgraduate in Food Department (DIPA) of the University of Sonora (UNISON), Campus Centro, Hermosillo, Sonora, northwest Mexico.

Obtaining Flour Samples

Twenty-five plants of *S. edulis* and the same number of *S. esteroa* were collected in Xochimilco, southwest Mexico City, and La Paz, Baja California Sur, Mexico, in December 2021, respectively. We collected plant samples by apical cutting of stems and leaves using pruning shears, then cleaning them with distilled water and subsequently dried and stored in 26.8 x 27.27 cm Ziploc® plastic bags for freezing. To obtain freeze-dried plants, they were frozen in a Thermo Scientific Revco Value Series freezer at -70 °C for 1-h and freeze-dried in a LABCONCO® FreeZone® Freeze Dry System freeze dryer at -50 °C for three days. Next, through a milling and pulverization process, powder with a particle size < 0.45 mm were obtained (Figure 1).



Fig. 1: Powders obtained from freeze-dried biomass from *S. edulis* (A), and *S. esteroa* (B).

In vitro Simulation Gastrointestinal Digestion Assay

We performed the *in vitro* simulation gastrointestinal digestion assay following the procedure described

Salazar-López *et al.* (2018),⁴⁴ processing the samples of both species, (1) *S. edulis* and (2) *S. esteroa* for digestion. The assay consisted of three digestion phases: 1. Oral, 2. Gastric, and 3.

Intestinal (Figure 2), following the ethical guidelines of the Council for International Organizations of Medical Sciences.⁴⁵ Descriptions of procedures for each phase of digestion are below.

Oral Phase

Three asymptomatic fasting volunteers chewed for 15 s 1 g each of *S. edulis* and *S. esteroa* meal. Subsequently, they placed each chewed sample in 50 mL Falcon tubes and rinsed their mouths twice with 5 mL of distilled water for 30 s each and expelled the liquid into the corresponding Falcon tubes. Next, they were homogenized and stored on ice for approximately 2 to 3 min.

Gastric Phase

For gastric digestion, 5 mL of hydrochloric acid-potassium chloride (HCl-KCl) buffer (2 M, pH 1.5)

was added. The pH was adjusted to 1.5 with hydrochloric acid (HCl) (6 M) and sodium hydroxide (NaOH) (2 M) on a HANNA 211 digital pH meter. 667 μ L of pepsin solution (300 mg/mL) in HCl-KCl buffer was added. This was then incubated in a shaking water bath Precision Scientific, Winchester, VA, USA, 66800, at 37 °C and 100 rpm for 1 h.

Intestinal Phase

For intestinal digestion, 9 mL of saline phosphate buffer (1 M, pH 7.5) was added. The pH was adjusted to 7.5 with hydrochloric acid (HCl) (6 M) and sodium hydroxide (NaOH) (2 M), using the pH meter mentioned above. Then, 1 mL of pancreatin solution (17 mg/mL) in saline phosphate buffer and 80 mg of bile salts were added. Subsequently, it was incubated in the shaking water bath at 37 °C and 100 rpm for 6 h.

In vitro simulation gastrointestinal digestion

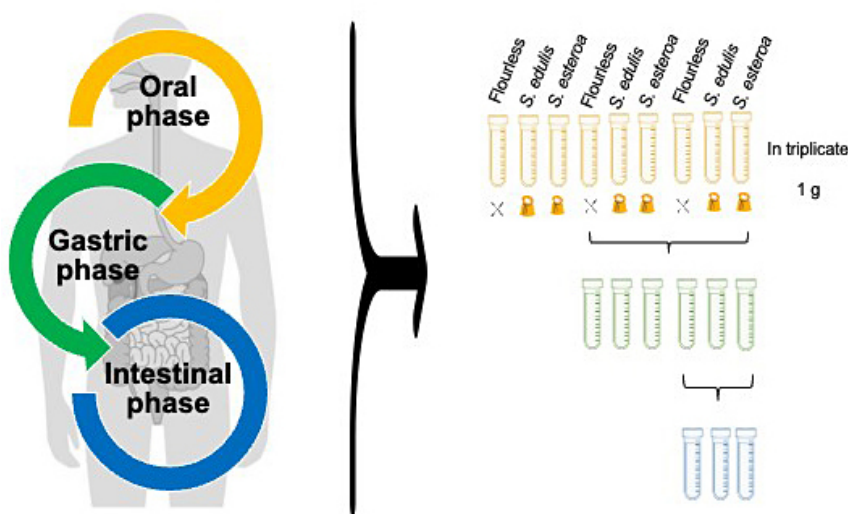


Fig. 2: General diagram of the *in vitro* simulation gastrointestinal digestion of *S. edulis* and *S. esteroa*.

Samples obtained in the three digestion steps were centrifuged in a Sigma 3-16KL centrifuge at 4 °C and 6000 rpm for 10 min. The supernatants recovered by filtration were frozen in an ARCTIKO ULUF 450 ULUF ultrafreezer at -80 °C for 1 h and lyophilized in a LABCONCO lyophilizer at -52 °C for 7 days. The lyophilized digests were dissolved in 3 mL of 50% methanol (MeOH). They were then filtered using cellulose acetate membrane filters VWR-

International North America (0.25 mm, 0.45 μ m) and stored at -20 °C, until further analysis.

Determination of Total Phenols Compounds

The content of total phenols compounds of *S. edulis* and *S. esteroa* digests was quantified spectrophotometrically by the colorimetric method using the Folin-Ciocalteu reagent (FCR), described by Salazar-López *et al.* (2016).⁴⁶ Briefly, using

a Nunc™ Edge multiwell plate, 30 µL of each digest was mixed with 150 µL of Folin's reagent (1:9) and 120 µL of sodium carbonate (Na₂CO₃) (0.075 mg/mL). The mixture was homogenized and incubated in the dark for 1-h, and absorbance was measured at 765 nm using a FluoStar Omega BMG Labtech Inc. microplate reader spectrophotometer, Ortenberg, Germany. The results were expressed as mg gallic acid equivalents per gram of dry weight sample (mg GAE/g, dw), using a curve as a reference standard.

Determination of Total Flavonoids Compounds

The content of total flavonoid compounds of *S. edulis* and *S. esteroa* digests was quantified spectrophotometrically by the colorimetric method using the aluminum trichloride, by Valenzuela-González *et al.* (2022).⁴⁷ We mixed 30 µL of each digest with 9 µL of sodium nitrite (NaNO₂) (0.05 mg/mL) and 120 µL of distilled water, using a Nunc™ Edge multiwell plate, allowing it to stand for 5 min. Next, 9 µL of aluminum trichloride (AlCl₃) (0.1 mg/mL) was added and allowed to stand for another 5 min, adding finally 60 µL of sodium hydroxide (NaOH) (0.04 mg/mL) and 72 µL of distilled water. We stirred the mixture and measured the absorbance at 415 nm using a FluoStar Omega BMG Labtech Inc. microplate reader spectrophotometer, Ortenberg, Germany. The results were expressed as mg quercetin equivalents per gram of dry weight sample (mg QE/g, dw), using a curve as a reference standard.

Determination of Antioxidant Capacity

DPPH '2,2-Diphenyl-1-picrylhydrazyl' Assay

We performed a spectrophotometric assay of antioxidant capacity by DPPH (2,2-Diphenyl-1-picrylhydrazyl) of *S. edulis* and *S. esteroa* digests, described by Ruiz-Hernández *et al.* (2021).²⁵ Subsequently, the working solution of the radical was prepared immediately before use by mixing 2.5 mg of DPPH with 100 mL of methanol (MeOH), the solution obtained in an intense purple color, was adjusted to an absorbance of 0.7 ± 0.02 at 515 nm using a FluoStar Omega BMG Labtech Inc. microplate reader spectrophotometer, Ortenberg, Germany. Using a Nunc™ Edge multiwell plate, we mixed 20 µL of each digest with 280 µL of reagent (DPPH radical) (0.025 mg/mL). Once we homogenized the mixture and incubated it in the dark for 1-h, we

determined the absorbance changes at the same wavelength using the microplate reader mentioned above. The results were expressed as µmol Trolox equivalents per gram of dry weight sample (µmol TE/g, dw), using a curve as a reference standard.

TEAC 'Trolox Equivalent Antioxidant Capacity' Assay

On the other hand, we performed a spectrophotometric assay of antioxidant capacity by TEAC (6-hydroxy-2,5,7,8-tetramethyl-chromium-2-carboxylic acid) of *S. edulis* and *S. esteroa* digests, described by Salazar-López *et al.* (2017).⁴⁸ First, we prepared an ABTS activating stock solution by mixing 88 µL of a potassium persulfate (K₂S₂O₈) solution (37.8 mg/mL) with 5 mL of an aqueous ABTS solution (19.3 mg/mL), identifying the solution presented as a deep blue color incubated in the dark at room temperature for 16-18 h. Subsequently, we prepared the working radical solution before use by mixing 1.5 mL of the stock solution with 100 mL of ethanol (C₂H₆O), adjusting it to an absorbance of 0.7 ± 0.02 at 734 nm with the spectrophotometer mentioned above. Using a Nunc™ Edge multiwell plate, we mixed 20 µL of each digest with 280 µL of reagent (ABTS radical). Then, we homogenized the mixture and incubated it in the dark for 5 min, determining the absorbance changes at the same wavelength using the microplate reader mentioned above. The results were expressed as µmol Trolox equivalents per gram of dry weight sample (µmol TE/g, dw), using a curve as a reference standard.

Estimation of Bioaccessibility

We estimated the bioaccessibility of *S. edulis* and *S. esteroa* by the concentration of bioactive compounds present in each stage of digestion and the concentration of bioactive compounds found in each extract of the undigested samples; these results are presented in Costa-Becheleni, *et al.* (2024).⁴⁹ The results were expressed in terms of percentage bioaccessibility and recovery of antioxidant activity using the following formula:

$$BA (\%) = \frac{TP/TFLA/TAC \text{ phenolic compound after digestion (oral, gastric and intestinal)}}{TP/TFLA/TAC \text{ phenolic compound in undigested samples (free and conjugated)}} \times 100$$

Where: BA: bioaccessibility, TP: is the total phenols, TFLA: is the total flavonoids, TAC: is the total antioxidant capacity.

Quantification of Total Phenols and Flavonoids by Ultra High-Performance Liquid Chromatography equipped with a Diode Array Detector (UHPLC-DAD)

Quantification of the total phenol and flavonoid compounds in digests of three phases followed the procedure described by Lee *et al.* (2012),⁵⁰ with slight modifications. We used an ultra high-performance liquid chromatography (UHPLC) system from Agilent Technologies 1260, Germany, equipped with a diode array detector (DAD), separating five phenol acids and five flavonoids with a Zorbax Eclipse Plus-C18 reversed-phase column (2.0 mm x 50 mm 1.8 micron), at a temperature of 30 °C. A two-phase mobile binary elution system of (A) water: formic acid (99.9 : 0.1) and (B) acetonitrile: formic acid (99.9 : 0.1) was applied. A linear elution gradient was as follows:

(1) 0-3 min (97-93 % phase A, and 3-7 % phase B); (2) 3-5 min (93-90 % phase A, and 7-10 % phase B); (3) 5-8 min (90-88 % phase A, and 10-12 % phase B); (4) 8-10 min (88-85 % phase A, and 12-15 % phase B); (5) 10-15 min (85-85 % phase A, and 15-15 % phase B); (6) 15-18 min (85-45 % phase A, and 15-55 % phase B); (7) and 18-20 min (45-10 % phase A, and 55-90 % phase B).

The flow rate was 0.4 mL/min, and the injection volume was 5- μ L. To identify phenol and flavonoid compounds, we prepared aqueous methanol extracts by comparing retention time and peaks of the UV spectra of the samples with those of pure reference standards. The results were expressed as μ g of phenol acids and flavonoids per gram of dry weight sample (μ g phenol ac and fla/g, dw) (using reference curves with each of the acids at different concentrations).

Data Collection and Statistical Analysis

For the numerical analysis, we tabulated the data of the registered variables and classified them in spreadsheets using Excel® 365 version 2022. The results were analyzed with a parametric statistical method, using the Kolmogorov-Smirnov normality test⁵¹ and Levene's test of similarity of variances test⁵² to verify the normal distribution of the data, except for the quantification of phenolic and flavonoid compounds determined by UHPLC-DAD, since statistical programs require at least five data to

perform statistical tests and, in this case, we only have three data for each variable. We compared the differences between species with the t-Student test at a 95 % significance level ($p < 0.05$), generating the resulting graphs with the free statistical software PAST version 4.10.⁵³

Results and Discussion

Free Total Phenols and Flavonoids During Gastrointestinal Simulation

Phenolic compounds can be found in different conformations in the food matrix, during the digestion process these can be released to be absorbed and realize their bioactivity or interact with the rest of the food components and digestion elements.⁵⁴

Under digestion conditions, the acid pH of the stomach can cause the breaking of ester, glycosidic, and disulfide bonds, causing the release from the food matrix of phenolic compounds.⁵⁵ By the effect of pH, denaturation of proteins and depolymerization of polysaccharides can release those compounds that were trapped or bound to them.⁵⁶ Likewise, by enzymatic action, hydrolysis of the food matrix structure can cause a release of the compounds trapped in it.⁵⁷ On the other hand, the alkaline pH of the intestine can help dissolve the food matrix, releasing phenolic compounds, as well as the presence of enzymes such as pancreatin, consisting of pancreatic amylase and lipase, trypsin, and chymotrypsin, can further degrade the food matrix, predisposing the release of antioxidant compounds.⁵⁸⁻⁶⁰

Although digestion releases phenolic compounds, hydrolysis of food matrix components will also cause interactions among them.⁵⁴ Phenols and proteins can interact, then forming protein-phenol complexes, where these interactions (non-covalent bonds) can affect the structure of proteins and the availability of phenols for absorption.⁶¹ At the same time, phenolic compounds can cause inhibition of digestive enzymes by binding to the active site, inhibiting their bioactivity, which in turn decreases the release of compounds by reducing the number of enzymes that hydrolyze structures where more phenolic compounds are trapped.⁵⁴ On the other hand, free phenolic compounds can interact with those carbohydrates hydrolyzed during digestion, forming hydrogen bonds, where hydroxyl groups

of phenols and carbohydrates interact;⁶² likewise, hydrophobic interactions can occur where non-polar portions can interact with carbohydrates, minimizing the interaction with water.⁶³ Also, inclusion complexes may occur, where phenols are inserted into the carbohydrate structures, as commonly occurs with the glucose rings in starch.⁶⁴ Figure 3 displays the effects of released phenolic compounds from the food matrix, reflecting their interactions. The above reflects an increase of free total phenols and flavonoids during the "in vitro" digestion process, possibly due to enzymatic effects and the pH adjustment, as discussed above.

The results indicate an increase through the different phases; in this sense, the intestinal bioavailable fraction of phenols was higher for *S. esteroa*, being 4.84 % higher in phenol content compared to

S. edulis (18.57 and 17.67 mg GAE/g), showing high significant differences between species ($p < 0.05$). In contrast, total flavonoid content was statistically equal during all phases; however, the bioavailable intestinal fraction of total flavonoids was higher for *S. esteroa*, being 0.05% higher in total flavonoid content compared to *S. edulis* (42.06 and 42.04 mg QE/g), showing non-significant differences ($p > 0.05$) between species compared to the other phases.

On the other hand, when comparing the content of free phenols of the intestinal fraction with that of both undigested halophytes, we found that after the digestion process, the content of free phenols and flavonoids of the intestinal fraction is 4.84 and 8.01 times more in *S. esteroa* and 7.68 and 12.51 times more in *S. edulis* than the content found in the undigested plants.

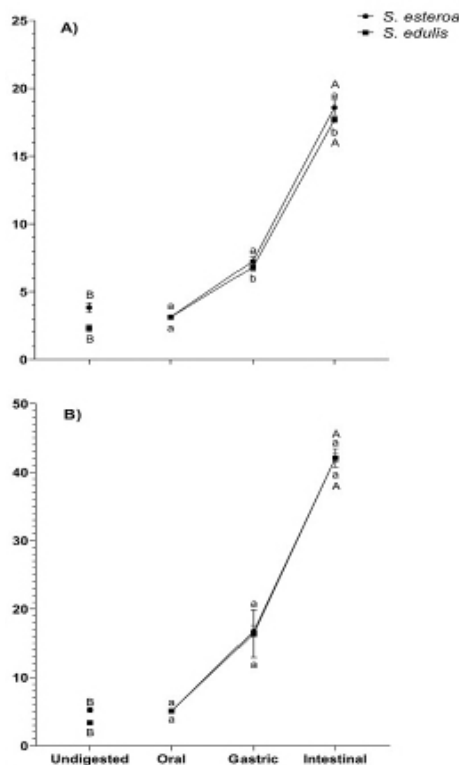


Fig. 3: (A) Total phenols (mg GAE/g, dw: dry weight), and (B) total flavonoids (mg QE/g, dw: dry weight) of oral, gastric, and intestinal phase digests of *S. edulis* and *S. esteroa* during the in vitro gastrointestinal simulation process. Lines correspond to the mean \pm standard deviation of three replicates. Superscripts a and b indicate statistically significant differences between species for each phase (t-Student test, $p < 0.05$). Comparisons between intestinal phases and undigested samples are presented in capital letters.

Bioaccessibility of Free Total Phenols and Flavonoids during the Assessed Gastrointestinal Simulation Process

The results indicate bioaccessibility percentages in *S. esteroa* of 355.71 for total phenols and 653.90 for

total flavonoids, while in *S. edulis*, the values were 590.16 and 1012.93 % (total phenols and flavonoids, respectively) (Table 1).

Table 1: Estimation of bioaccessibility (%) for total phenols and total flavonoids of oral, gastric, and intestinal phase digests of *S. edulis* and *S. esteroa*.

Species	Oral Phase	Gastric Phase	Intestinal Phase
Bioaccessibility (%) of total phenols (mg GAE/g, dw)			
<i>S. edulis</i>	104.08	226.73	590.16
<i>S. esteroa</i>	60.04	138.83	355.71
Bioaccessibility (%) of total flavonoids (mg QE/g, dw)			
<i>S. edulis</i>	122.13	395.22	1012.93
<i>S. esteroa</i>	78.99	259.99	653.90

Notes. mg GAE/g, dw (mg of gallic acid equivalents/g of sample, dry weight); mg QE/g, dw (mg of quercetin equivalents/g of sample, dry weight); each value is the mean of three replicates.

Evidence suggest that phenols and flavonoids trapped in the food matrix were released in the digestion process. Similar results were found in *Hypericum perforatum* L. after *in vitro* digestion, where it significantly increases the content of phenols, phenolic acids, and flavonoids.⁶⁵ In contrast, different results were observed in quinoa sprouts, where some varieties present lower content of phenols and flavonoids in the intestinal fraction.⁶⁶ On the other hand, in *Salicornia ramosissima* and *Sarcocornia fruticosa* the highest content of total phenols was found in the gastric phase, which was significantly different.⁶⁷

Although the Folin-Ciocalteu technique is widely used to determine the content of total phenols, it is important to consider that this can overestimate or give false positives to the presence of polyphenols, since during extraction it is possible to obtain some reducing compounds such as sugars,⁶⁸ and some amino acids⁶⁹ that react in the technique. On the other hand, it is important to know that under the aluminum trichloride technique to determine flavonoids can indicate false positives or false negatives, since, being a colorimetric technique, it must be considered that the absorption spectrum of each flavonoid is different, as well as some flavonoids cannot form a complex with aluminum.⁷⁰ Therefore, identifying the types of compounds present in the extracts is essential.

Estimation of the Total Free Antioxidant Capacity during the Gastrointestinal Simulation Process

The results indicate an increase in antioxidant capacity in all phases; however, the intestinal fractions bioavailable by both colorimetric methods was higher for *S. esteroa*, being 28.94 % higher in antioxidant capacity content by DPPH (35.03 and 24.89 $\mu\text{mol TE/g}$) and 23.93 % by TEAC (32.72 and 24.89 $\mu\text{mol TE/g}$), compared to *S. edulis*, showing highly significant differences between species ($p < 0.05$) (Figure 4).

On the other hand, when comparing the antioxidant capacity of the intestinal fraction with that of both undigested halophytes, we found that after the digestion process, the antioxidant capacity of the intestinal fraction increased 1.61 and 1.83 times by DPPH and 1.22 and 1.41 times by TEAC for *S. esteroa* and *S. edulis* compared to that found in the undigested plants.

Changes in antioxidant activity during digestion correspond to individual changes in the structure of the phenolic compound.^{71,72} In the oral phase, the binding of salivary enzymes to phenols by partial deglycosylation may occur, decreasing the antioxidant activity.⁷³

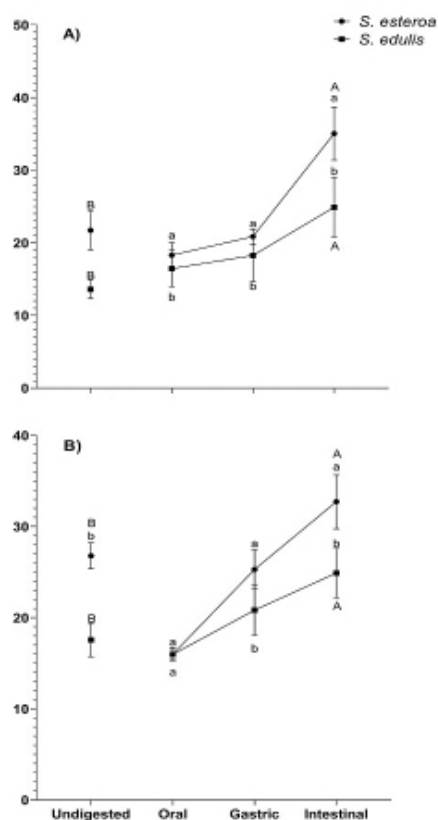


Fig. 4: Total antioxidant capacity by (A) DPPH, and (B) TEAC ($\mu\text{mol TE/g}$, dw: dry weight) of oral, gastric, and intestinal phase digests of *S. edulis* and *S. esteroa* during the *in vitro* gastrointestinal simulation process. Lines correspond to the mean \pm standard deviation of three replicates. Superscripts a and b between lines indicate statistically significant differences between species for each phase (t-Student test, $p < 0.05$). Comparisons between intestinal phases and undigested samples are presented in capital letters.

The fact that there is no correlation between the content of phenolic and flavonoid compounds and antioxidant activity may be because during chewing and because of salivary enzymes, some of these compounds are released; however, their antioxidant capacity may be much lower than those released during other phases because they are more basic.⁷⁴ This may be due to the presence of hydroxyl groups, the substitution of these in the aromatic ring, up to the antioxidant mechanisms that these released compounds may have, such as the of hydrogen atoms transfer (HAT), single electron transfer followed by proton transfer (SET-PT), and sequential protons loss with electron transfer (SPLET).⁷⁵ In addition, the synergistic and antagonistic effect of some of these compounds is

known, which can positively or negatively influence antioxidant activity.⁷⁶

Estimation of Antioxidant Activity Recovery during the Gastrointestinal Simulation Process

Antioxidants such as phenols are molecules involved in free radical scavenging, participating in the inhibition of peroxides and singlet oxygen, and various oxygen reactive species, as they function as chelators of metal ions and scavenge oxidation products.⁷⁷

There are different classifications, including primary and secondary, where primary antioxidants inhibit chain oxidation by acting as free radical acceptors or hydrogen donors, which compete with the

propagation of lipid oxidation, producing stable products.⁷⁸ On the other hand, secondary antioxidants prevent or retard oxidation by suppressing oxidation promoters such as prooxidative enzymes, metal ions, and singlet oxygen.⁷⁹ Therefore, determining the antioxidant recovery during the gastrointestinal simulation process brings us closer to the potential beneficial effect of consuming foods rich in phenolic compounds, which could promote some benefits, such as a decrease in arteriosclerosis, aging, and diabetes, among others.⁸⁰

The results of the antioxidant recovery during the digestion process of *S. edulis* and *S. esteroa* show that during the different phases of digestion, the antioxidant capacity increases, which could

be due to the greater availability of phenolic compounds resulting from the hydrolysis of the food matrix.⁸¹ When comparing the antioxidant recovery of the intestinal phase, we recognized the value of halophytes as potentially functional food; however, *S. edulis* presents significantly higher antioxidant capacity compared to *S. esteroa*, which could be due to the presence of more different types of antioxidant compounds than *S. esteroa*. This difference seems to be caused by the extreme growth and stress conditions prevailing in the habitat of these plants.⁸² When comparing both plants, antioxidant recovery in DPPH of 157.15 and 181.37 % and in TEAC of 118.07 and 139.74 % were observed for *S. esteroa* and *S. edulis*, respectively (Table 2).

Table 2: Estimation of antioxidant capacity recovery total (%) by DPPH and TEAC of oral, gastric, and intestinal phase digests of *S. edulis* and *S. esteroa*.

Species	Oral Phase	Gastric Phase	Intestinal Phase
Antioxidant capacity recovery total (%) by DPPH ($\mu\text{mol TE/g, dw}$)			
<i>S. edulis</i>	117.85	130.58	181.37
<i>S. esteroa</i>	82.44	93.91	157.15
Antioxidant capacity recovery total (%) by TEAC ($\mu\text{mol TE/g, dw}$)			
<i>S. edulis</i>	89.39	116.13	139.74
<i>S. esteroa</i>	57.77	91.36	118.07

Notes. DPPH (2,2-Diphenyl-1-picrylhydrazyl); TEAC (Trolox Equivalent Antioxidant Capacity); $\mu\text{mol TE/g, dw}$ (micromoles of Trolox equivalents/g of sample, dry weight); numbers correspond to the mean of three replicates.

A previous study reported different results for *Salicornia ramosissima* and *Sarcocornia fruticosa*, where the gastric phase presented the highest antioxidant recovery during the gastrointestinal simulation process.⁶⁷ When identifying the antioxidant potential of any extract rich in phenolic compounds, it is necessary to keep in mind that the observed results may be due to different conditions, including antioxidant synergy,⁸³ antagonistic effects⁸⁴ and the chemical structure⁸⁵ of the compounds present. Therefore, by identifying each of the compounds individually, it will be known how these possibly increased or decreased the antioxidant potential of the samples.

Content of Free Phenols and Flavonoids found during the Gastrointestinal Simulation Process

After knowing the content of free phenols and total

free flavonoids, as well as the antioxidant recovery during the gastrointestinal simulation, by using liquid chromatography, phenolic acids such as ferulic, gallic, p-Coumaric, protocatechuic and synaptic, as well as flavonoids such as catechin, myricetin, naringenin, naringin and ruthin were identified according to the retention times and spectrum of each compound compared with different standards. These compounds were also identified in undigested samples (data not shown).

When comparing the phenolic compounds found in *S. edulis* and *S. esteroa* with the content of total phenols and flavonoids, we could attribute that much of its content is given by the identified compounds, however, other compounds that could not be identified could positively influence the antioxidant capacity of these plants, this is

because compounds such as caffeoylquinic acid, hexosyl rhamnetin pentoside and dihydroquercetin among others, have been abundantly identified in different halophytes.⁶⁷ When observing the content of the bioactive compounds identified, it was found that some phenolic acids and flavonoids increase and decrease through the different gastrointestinal phases, which demonstrates the release and interactions that these compounds have with the food matrix. When comparing what happens with each compound in the intestinal phase, an important phase since it is where compounds would be available for absorption by the intestine to carry their bioactivity, only some compounds such as gallic acid, p-Coumaric acid, catechin, myricetin and ruthin in *S. edulis*, p-Coumaric, protocatechuic, synaptic, catechin, myricetin, naringenin and ruthin in *S. esteroa* presented the highest concentration significantly in the intestinal phase compared to the oral and gastric phases.

However, both phenolic acids and flavonoids from the intestinal phase in both plants, the concentration found bioaccessible compared to the concentration of undigested plants is varied according to the different types of compounds. Where most of the compounds except p-Coumaric, catechin and naringin decreased after digestion in *S. edulis*. Compared to *S. esteroa*, p-Coumaric acid and ruthin were the only compounds that did not decrease after simulation. On the other hand, when comparing the content of bioactive compounds of halophytes before and after digestion, as observed, most of the compounds presented low percentages of bioaccessibility, however, p-Coumaric acid, catechin and naringin in *S. edulis* presented very good percentages of bioaccessibility. This reflects the dynamic effect that digestion has on the food matrix and the content of bioactive compounds. The results are shown in Tables 3 and 4.

Table 3: Identification for phenols acids from oral, gastric, and intestinal phase digests of *S. edulis* and *S. esteroa* by UHPLC-DAD.

Species	Compounds (µg/g, dw)	Oral	Gastric	Intestinal	Bioaccessibility (%)
<i>S. edulis</i>	Ferulic	603.10±82.93 ^{bA}	157.57±5.00 ^{nsC}	178.71±6.37 ^{bB} ↓	3.99
	Gallic	1967.97±37.66 ^{aC}	3395.58±273.33 ^{aA}	3099.94±213.03 ^{aB} ↓	91.49
	p-Coumaric	9.70±0.05 ^{bC}	35.98±0.60 ^{nsB}	428.88±33.71 ^{aA} ↑	488.08
	Protocatechuic	1.04±0.11 ^{nsC}	2.02±0.18 ^{aB}	9.65±0.67 ^{nsA} ↓	5.04
	Synaptic	66.93±7.83 ^{aC}	75.77±2.39 ^{nsA}	75.38±4.51 ^{nsB} ↓	73.07
<i>S. esteroa</i>	Ferulic	1114.64±84.00 ^{aA}	118.32±47.97 ^{nsC}	264.74±2.55 ^{aB} ↓	10.40
	Gallic	1.83±0.20 ^{bA}	1.17±0.05 ^{bB}	1.14±0.09 ^{bC} ↓	81.42
	p-Coumaric	30.57±4.66 ^{aC}	37.27±4.45 ^{nsB}	70.63±3.67 ^{bA} ↑	114.47
	Protocatechuic	0.99±0.11 ^{nsC}	1.32±0.06 ^{bB}	8.29±0.74 ^{nsA} ↓	15.46
	Synaptic	43.95±7.70 ^{bC}	60.04±8.99 ^{nsB}	78.07±4.72 ^{nsA} ↓	9.09

Notes. µg/g, dw (micrograms/g of sample, dry weight); numbers correspond to the mean ± standard deviation of three replicates; superscripts a and b between columns indicate statistically significant differences of the same compound between species; superscripts A, B and C between lines indicate statistically differences of the same compound across digestion phases (t-Student test, $p < 0.05$); superscripts ns indicate statistically non-significant differences according to the above comparisons (t-Student test, $p > 0.05$); the arrows ↑↓ indicate increase or decrease in the concentration of bioactive compounds compared to undigested sample.

Table 4: Identification for flavonoids from oral, gastric, and intestinal phase digests of *S. edulis* and *S. esteroa* by UHPLC-DAD.

Species	Compounds ($\mu\text{g/g}$, dw)	Oral	Gastric	Intestinal	Bioaccessibility (%)
<i>S. edulis</i>	Catechin	20.47 \pm 2.74 ^{a C}	39.79 \pm 1.90 ^{a B}	48.91 \pm 3.82 ^{a A} \uparrow	764.21
	Myricetin	20.37 \pm 1.22 ^{a B}	1.93 \pm 0.02 ^{ns C}	25.57 \pm 0.28 ^{a A} \downarrow	15.60
	Naringenin	32.99 \pm 1.59 ^{ns A}	13.24 \pm 1.10 ^{b C}	23.30 \pm 1.01 ^{b B} \downarrow	28.21
	Naringin	173.92 \pm 1.66 ^{b B}	284.47 \pm 39.48 ^{a A}	113.19 \pm 8.25 ^{b C} \uparrow	252.20
	Ruthin	5956.39 \pm 157.77 ^{a B}	4271.25 \pm 641.85 ^{a C}	6911.01 \pm 711.06 ^{a A} \downarrow	44.58
<i>S. esteroa</i>	Catechin	11.08 \pm 1.97 ^{b B}	7.05 \pm 0.13 ^{b C}	13.18 \pm 0.55 ^{b A} \downarrow	10.67
	Myricetin	2.49 \pm 0.04 ^{b B}	1.78 \pm 0.10 ^{ns C}	3.02 \pm 0.29 ^{b A} \downarrow	5.51
	Naringenin	32.92 \pm 3.02 ^{ns C}	64.18 \pm 5.95 ^{a A}	63.21 \pm 7.38 ^{a B} \downarrow	63.24
	Naringin	395.38 \pm 46.40 ^{a A}	142.20 \pm 17.41 ^{b C}	208.54 \pm 31.99 ^{a B} \downarrow	80.52
	Ruthin	0.74 \pm 0.04 ^{b B}	0.29 \pm 0.04 ^{b C}	8.49 \pm 0.34 ^{b A} \uparrow	194.41

Notes. $\mu\text{g/g}$, dw (micrograms/g of sample, dry weight); numbers correspond to the mean \pm standard deviation of three replicates; superscripts a and b between columns indicate statistically significant differences of the same compound between species; superscripts A, B and C between lines indicate statistically differences of the same compound across digestion phases (t-Student test, $p < 0.01$); superscripts ns indicate statistically non-significant differences according to the above comparisons (t-Student test, $p > 0.05$); the arrows $\uparrow\downarrow$ indicate increase or decrease in the concentration of bioactive compounds compared to an undigested sample.

Other authors reported similar results in barley, observing that through digestion, some compounds such as vinylic, ferulic, and p-coumaric acids, among others, vary in their concentration. Therefore, when calculating the release of some compounds, high and low percentages of bioaccessibility of some of them are shown.⁸⁶ Similar results were found in wheat, where after digestion, some compounds vary in concentration; likewise, bioaccessibility percentages above 500 % are presented in some compounds.⁸⁷

In contrast, in halophytes such as *Sarcocornia fruticosa* and *Salicornia ramosissima* compounds, such as caffeoylquinic acids and galocatechin identified before digestion were not detected in the intestinal phase.⁸⁷

The localization of phenolic compounds in different plants can cause a variety of results during the digestion process since some of them are free, can be conjugated or trapped in more complex elements such as proteins, oligosaccharides, polysaccharides, as well as those trapped could be released, but conjugated again.¹⁰ For example, phenolic compounds tend to be conjugated or

trapped between proteins and polysaccharides,^{88,89} whereas those compounds such as ferulic acid tend to be mostly bound to the arabinose chains of plant cell walls,⁹⁰ while gallic acid is abundantly free in plant cells.⁹¹

Evaluating the effect of digestion on the content of phenols and flavonoids in halophytes such as *S. edulis* and *S. esteroa* helps to understand the behavior of these compounds during the gastrointestinal tract, as well as to elucidate the potential beneficial effect that these compounds could have by their consumption. However, even if good bioaccessibility is present, bioactivity in target tissues will depend on bioavailability, as well as the transformations that occur to the bioactive compounds in the liver for being transported in the bloodstream, which could modify the antioxidant potential and beneficial effects.^{92,93} With the above considerations, it is possible to use *S. edulis* and *S. esteroa* halophytes as potential beneficial foods to counteract some chronic degenerative- diseases, as some bioaccessible compounds in these plants have anticancer properties,⁹⁴ antidiabetic⁹⁵ and other effects. In this context, promoting the production of this kind of plant biomass could help to remediate

saline soils, enhancing food production and helping to counteract food insecurity problems.⁹⁶

Conclusions

The content of phenols, flavonoids, and antioxidant recovery of halophytes increased during digestion. However, *S. esteroa* presented significantly higher contents of free total phenols and flavonoids in the intestinal fraction than *S. edulis*. Meanwhile, *S. edulis* has higher antioxidant recovery when compared to *S. esteroa* in the intestinal fraction. Very high percentages of bioaccessibility and antioxidant recovery (> 100%) were present in both halophytes. Gallic acid was the compound with the highest concentration in *S. edulis* in the intestinal fraction; however, p-Coumaric acid presented the highest percentage of bioaccessibility. In *S. esteroa*, ferulic acid presented the highest concentration in the intestinal phase. According to these results, we observed considerable bioaccessibility in this plant. Ruthin in *S. edulis* presented high concentrations in the intestine, while catechin and naringin presented the best bioaccessibility. In *S. esteroa*, naringin is the most abundant compound, while ruthin presented percentages above 100% bioaccessibility.

The halophytes *S. edulis* and *S. esteroa* reflect a high potential as a health food, which could help to counteract chronic degenerative diseases related to obesity and help to solve marginal and saline soils, as well as a potential crop to counteract the problems of food security and climate change.

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Conflict of Interest

All authors declare no conflict of interest. The authors have read and approved the final version of the manuscript.

Data Availability Statement

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement

The document accurately and thoroughly presents the authors original research and analysis.

Authors Contribution

Each author has significantly and directly contributed intellectually to the manuscript. Author Contributions: FRCB, ETD, RMRS proposed and designed the experiments; FRCB, AARH, RMRS, NJSL performed the lab procedures and methods; FRCB, FAN organized and assisted in field sampling; FRCB, FAN executed the biomass processing; LABS, RMRS helped in interpreting the biochemical and nutritional data; FRCB, ETD, AARH wrote the first version of manuscript. FRCB, ETD, LABS, RMRS reviewed the overall quality of the manuscript.

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