



Bioaccessibility and Intestinal Permeability from Andean Blackberry (*Rubus glaucus* Benth) Powders Encapsulated with OSA-Modified FHIA-21 Banana Starch [†]

Victor D. Quintero-Castaño ^{1,2,*}, Mardey Liceth Cuellar-Núñez ³, Ivan Luzardo-Ocampo ⁴, Jose F. Vasco-Leal ⁵, Francisco J. Castellanos-Galeano ⁶, Cristina I. Álvarez-Barreto ⁶ and Rocio Campos-Vega ^{7,*}

¹ Programa de Ingeniería de Alimentos, University of Quindío, Armenia 630004, Colombia

² Programa de Doctorado en Ingeniería, Universidad de Caldas, Manizales 170007, Colombia

³ Facultad de Medicina, Universidad Autónoma de Querétaro, Querétaro 76176, Mexico; liceth.cuellar@uaq.mx

⁴ Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro 76230, Mexico; ivan.8907@gmail.com

⁵ Posgrado en Gestión Tecnológica e Innovación, Universidad Autónoma de Querétaro, Querétaro 76010, Mexico; jose.vasco@uaq.mx

⁶ Departamento de Ingeniería, Universidad de Caldas, Manizales 170007, Colombia; francisco.castellanos@ucaldas.edu.co (F.J.C.-G.); cristina.alvarez@ucaldas.edu.co (C.I.Á.-B.)

⁷ Research and Graduate Program in Food Science, Universidad Autónoma de Querétaro, Querétaro 76010, Mexico

* Correspondence: victordumar@uniquindio.edu.co (V.D.Q.-C.); chio_cve@yahoo.com.mx (R.C.-V.)

[†] Presented at the 2nd International Electronic Conference on Foods—Future Foods and Food Technologies for a Sustainable World, 15–30 October 2021; Available online: <https://foods2021.sciforum.net/>.



Citation: Quintero-Castaño, V.D.; Cuellar-Núñez, M.L.; Luzardo-Ocampo, I.; Vasco-Leal, J.F.; Castellanos-Galeano, F.J.; Álvarez-Barreto, C.I.; Campos-Vega, R. Bioaccessibility and Intestinal Permeability from Andean Blackberry (*Rubus glaucus* Benth) Powders Encapsulated with OSA-Modified FHIA-21 Banana Starch. *Biol. Life Sci. Forum* **2021**, *6*, 111. <https://doi.org/10.3390/Foods2021-10981>

Academic Editor: Antonello Santini

Published: 14 October 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Modified starches for bananas can be used to encapsulate underutilized fruits such as Andean blackberry due to its content of phenolic compounds. This research aimed to assess the bioaccessibility and intestinal permeability of phenolic compounds from Andean blackberry powders encapsulated in octenyl succinic anhydride (OSA)-modified Gros Michel banana starch. Although low bioaccessibilities were found for total phenolics (up to 6%) during the in vitro digestion, most of them were chlorogenic acid and quercetin, released at high apparent permeability values ($5\text{--}12 \times 10^{-4}$ cm/s). OSA-banana starches are suitable encapsulating matrices for blackberry polyphenols, ensuring their targeted release at the small intestine.

Keywords: Andean blackberry (*Rubus glaucus* Benth); apparent permeability coefficient; bioaccessibility; encapsulation; Gros Michel banana; in vitro digestion; modified starches; octenyl succinic anhydride (OSA); phenolic compounds

1. Introduction

Andean blackberry (*Rubus glaucus* Benth) is an underutilized South American blackberry cultivar characterized by its low cost, versatility, and nutritional profile dominated by organic acids and phenolic compounds in a low-sugar and low-lipid food matrix [1]. Although phenolic compounds are bioactive compounds exhibiting a wide range of health effects, they are highly susceptible to environmental conditions such as pH, oxygen, light, and temperature [2]. Therefore, proper techniques protecting these valuable components are essential, and encapsulation might provide advantages that not only protect them, but also be delivered in targeted stages from the gastrointestinal tract at which they could be absorbed or exert their primary health benefit [3].

Several raw materials have been used to encapsulate polyphenols with polysaccharides such as starch being some of the most explored since they display desirable technological properties such as low-cost acquisition, protect the core encapsulating material during the encapsulation process, and can partially degrade at the small intestine after resisting

the oral and gastric conditions during digestion [4]. Starches from bananas (*Musaceae*) meet all of these properties, but chemical modifications are needed since native starches exhibit technological limitations [5]. Octenyl succinic anhydride (OSA) can induce chemical modifications in *Musaceae* starches as OSA provides higher stability of the starch emulsion as the provided half ester substituents from OSA interrupts the linearity of the amylose and the branched sections of amylopectin, stabilizing starch dispersions against phenomena such as gelling or pasting. As a result, increased viscosity and slightly lower gelatinization temperatures are presented, making OSA-modified starches suitable for oil-in-water emulsions in several food and pharmaceutical applications [6]. OSA-modified starches are generally considered as safe for consumption as in vivo studies have shown no adverse effects, and international authorities such as the European Food Safety Authority (EFSA) have approved their consumption [7]. OSA-modified starches have been successfully used to encapsulate Andean blackberry concentrate, resulting in low particle size and low hygroscopic powders, yielding a high amount of total phenolic compounds and encapsulation efficiencies (up to 53.01%) [3]. However, as significant reductions of total phenolics (−46.90%) and DPPH antioxidant capacity (−55.12%) were obtained in the experimental powders vs. the optimal powders in the OSA-modified Gros Michel banana, novel OSA-modifications of additional *Musaceae* starches are needed. In this sense, FHIA-21 bananas are a hybrid banana developed by the Federación Hondureña de Investigación (FHIA) in 1987 that has been commercially introduced in Cuba, Honduras, Nicaragua, Guatemala, Venezuela, Ecuador, Colombia, Perú, and the Dominican Republic due to their tolerance to sub-optimal agronomic conditions, high production yield, and resistance to the Black Sigatoka disease, which is still a highly threat risk to banana production [8]. Moreover, as the bioaccessibility of the encapsulated phenolic compounds has not been assessed yet, considering that this is critical to further research on their biological potential [9], this research aimed to assess the bioaccessibility and intestinal permeability of phenolic compounds from Andean blackberry encapsulated in OSA-modified Gros Michel banana starches.

2. Materials and Methods

2.1. Blackberry Powder Encapsulation

The concentrate of commercial Andean blackberry (*Rubus glaucus* Benth) was provided by the NUTRIUM® company (Valle del Cauca, Colombia). Bananas (cv. Gros Michel) were harvested at Quimbaya (Quindío, Colombia) at 1270 m above sea level and an average temperature of 25 °C. Starch from the FHIA 21 bananas was extracted, modified using OSA, and served as the encapsulating matrix of the blackberry concentrate following the reported procedure and optimization by Quintero-Castaño et al. [3]. Briefly, the bananas were peeled, homogenized in an industrial blender, screened through a 100 µm mesh, and left to stand at 4 °C for 24 h to allow for the supernatant release. Once eliminated, the precipitate was dried at 40 °C for 24 h, and the resulting powder was mixed with water under constant stirring. After pH adjustment (pH: 8.5–9.0), the OSA was added (40 °C, 3.5 h), the pH was adjusted (pH: 7.0), and the solution was left to stand for 24 h at 10 °C. The procedure was repeated three times to eliminate the residual OSA, using acetone to wash the starch. Once washed and dried (50 °C, 24 h), the dry product was stored at 10 °C.

A spray-drying process was followed to produce the powders. For this, a PSALAB spray-dryer (Vibrasec S.A.S., Medellín, Colombia) operating at an air inlet temperature: 130.17 °C, air outlet temperature: 85.21 °C, atomizer disc speed: 26,320.60 rpm, and a feed solution containing the blackberry concentrate (5.59% starch, 1.59% maltodextrin, 47.5% blackberry concentrate, and 45.30% water) was used. The resulting powders were collected in light and oxygen-protected polyethylene bags.

2.2. In Vitro Gastrointestinal Digestion

Simulated gastrointestinal digestion was conducted from the mouth to the small intestine [10]. Informed consent was obtained from the four people participating at the

oral stage, chewing the sample (1 g) 15 times for 15 s. The number of people was selected considering previous assays digesting a wide range of food matrices, indicating that four people is sufficient to obtain replicable results [11,12]. The expectorated product was pH-adjusted to 2.0, pepsin (≥ 2500 U/mg, Sigma Aldrich, St. Louis, MO, USA) was added, and samples were incubated for 2 h at 37 °C in an oscillating water bath (37 °C, 80 cycles/min). For the intestinal stage, everted gut sacs from male Wistar rats (250–300 g) previously fasted for 16 h after housing under appropriate conditions (12 h/12 h light/dark cycle, 22 ± 1 °C, water access ad libitum, and maintenance in individual cages) were obtained. The animals were acquired from the Instituto de Neurobiología (UNAM-Campus Juriquilla, Mexico), and the experimental procedure was previously approved by the Bioethics Committee of the School of Chemistry from Universidad Autónoma de Querétaro (approval ID: CBQ19_1119-2). Rats ($n = 4$ /experiment; total rats: 8) were anesthetized (CO_2 , pentobarbital sodium: 60 mg/kg body weight), opened through a midline abdominal incision, and the jejunum was excised, cut (6 cm segments), and washed with Krebs–Ringer buffer. Sacs were carefully everted using a glass rod, filled with Krebs–Ringer buffer (37 °C, CO_2 -gasified to ensure anaerobiosis, pH: 6.8), and placed in the gastric sample with the added intestinal enzymes (2.6 mg/sample pancreatin: $8 \times \text{USP}$, Sigma-Aldrich; 3 mg/sample bile bovine: Sigma Aldrich) and pH adjustment (7.2–7.4). Samples were incubated for 15, 30, 60, and 90 min at 37 °C in an oscillating water bath (80 cycles/min). Samples quantified outside the small intestinal sac were considered as the non-digestible fraction (NDF), while those at the inner side of the everted sac were referred to as the “digestible fraction” (DF). Aliquots were taken at all stages and immediately stored at -70 °C, protected from light for further analysis. Saliva from the participants was also digested and used as a blank.

2.3. Total Phenolic Compound Quantification and Identification of Individual Phenolic Compounds

A methanolic extract was prepared from the samples [9], and the total phenolic compounds were measured using the Folin–Ciocalteu procedure [13]. Results were expressed in mg. of gallic acid equivalents (GAE)/g dry sample.

For the individual identification and quantification of phenolic compounds, a high-performance liquid chromatography analysis coupled with diode array detection (HPLC-DAD) was conducted [14]. Phenolic compounds were separated in a Zorbax Eclipse XDB column (Agilent Technologies, Palo Alto, CA, USA) in an Agilent 1100 HPLC equipment (Agilent Technologies) at 35 ± 0.6 °C. The sample (20 L) was injected at 1 mL/min, and individual HPLC-grade standards of gallic and chlorogenic acids; (+)-catechin, quercetin, and epigallocatechin were used to quantify the phenolics. Results were expressed in μg equivalents of each phenolic compound/g dry sample.

2.4. Bioaccessibility (% B) and Apparent Permeability Coefficient (P_{app}) Determination

The bioaccessibility of individual phenolic compounds was measured as reported [15]: $B (\%) = (C_f/C_0) \times 100\%$, where C_f is the final concentration of the compound at a specific time or stage and C_0 is the initial concentration of the compound at the undigested sample.

The P_{app} values were calculated using the equation of Lassoued et al. [16] in the chamber model: $(dQ/dt)(1/AC_0)$, where dQ/dt (mg/s) is the amount of phenolic compound transported across the everted gut sac (membrane) per time unit, A is the available surface area for permeation, and C_0 (mg/mL) is the initial concentration of the compounds before the intestinal incubation. Results were expressed as $\times 10^{-4}$ cm/s.

2.5. Statistical Analysis

Results from two independent experiments (two independent digestion procedures) in triplicate were expressed as the means $\pm$ SD. The quantification of phenolics was conducted twice for each replicate. An analysis of variance (ANOVA) followed by the post hoc Tukey–Kramer’s test was conducted, establishing significance at $p < 0.05$. Analyses were conducted using JMP v. 16 (SAS Institute, Cary, NC, USA) software.

3. Results and Discussion

3.1. Total Phenolics and Bioaccessibility of Individual Phenolic Compounds from Capsules

Compared to the methanolic extract (ME), all samples displayed a significantly lower ($p < 0.05$) amount of released (bioaccessible) total phenolic compounds (TPC) (Table 1). The oral and gastric samples exhibited the highest values among the digestive stages, while no differences were found between the DF or NDF fractions. However, NDF showed the highest amount at the intestinal stage, agreeing with previous reports from the phenolics of other fruits [17]. The authors reported low bioaccessibility values for the total phenolic compounds of the digested wild and commercial blackberries (up to ~60%), explained by the overall low stability of these compounds to the gastric and intestinal conditions [18].

Table 1. The total phenolic compounds from the encapsulated Andean blackberry powders along with the digestion.

Sample/Stage	TPC (mg GAE/g Sample)	Individual Phenolic Compounds (HPLC-DAD) (% B)			
		Gallic Acid	Chlorogenic Acid	(+)-Catechin	Epigallocatechin Gallate
ME	909.64 ± 3.67 ^a	-	-	-	-
Oral	49.20 ± 4.23 ^c	4.20 ± 1.18	6.17 ± 0.72	2.91 ± 0.14	0.97 ± 0.19
Gastric	57.58 ± 0.50 ^b	1.59 ± 0.18	51.02 ± 4.04	5.70 ± 0.54	15.59 ± 0.75
Small intestine (DF)					
15 min	2.83 ± 0.33 ^e	4.19 ± 0.37	0.62 ± 0.08	n. d.	n. d.
30 min	3.54 ± 0.11 ^e	11.43 ± 1.23	1.24 ± 0.53	n. d.	n. d.
60 min	2.49 ± 0.94 ^e	10.47 ± 1.18	1.71 ± 0.47	0.69 ± 0.01	1.15 ± 0.17
120 min	2.91 ± 0.36 ^e	17.52 ± 0.69	0.57 ± 0.08	0.11 ± 0.00	1.76 ± 0.04
Small intestine (NDF)					
15 min	24.08 ± 1.18 ^d	20.47 ± 3.34	3.07 ± 0.02	4.01 ± 0.33	4.48 ± 0.16
30 min	21.20 ± 2.35 ^d	17.39 ± 2.82	19.15 ± 0.74	8.05 ± 0.71	4.66 ± 0.05
60 min	21.33 ± 1.46 ^d	13.80 ± 0.99	19.47 ± 0.64	1.08 ± 0.16	4.53 ± 0.07
120 min	21.72 ± 2.17 ^d	24.09 ± 3.81	8.70 ± 0.37	10.06 ± 0.28	4.29 ± 0.10

Results are the means ± SD of two independent experiments in triplicate. Different letters express significant differences by the Tukey–Kramer’s test among samples/stages ($p < 0.05$). % B—bioaccessibility; GAE—gallic acid equivalents; DF—digestible fraction; ME—methanolic extract; NDF—non-digestible fraction; TPC—total phenolic compounds.

Regarding the individual phenolics, chlorogenic acid exhibited the highest bioaccessibilities at the oral, gastric, and NDF stages, while it was gallic acid was for the DF. For the flavonoids, epigallocatechin gallate showed a higher bioaccessibility than (+)-catechin (Table 1). Like TPC, NDF retained the highest amount of individual phenolics. The absence of flavonoids at 15 and 30 min of the small intestine stage were also found for the *Moringa oleifera* powder phenolics [12] and the passion fruit (*Passiflora edulis*) fruits and leaf extracts [11].

3.2. Apparent Permeability Coefficients

Figure 1 shows the P_{app} values of the identified phenolics from the samples. Chlorogenic acid showed the highest values ($p < 0.05$) during the small intestine incubation, followed by gallic acid, while no differences ($p > 0.05$) were found for the flavonoids. As all values were within the 10^{-4} cm/s range, the permeability can be considered as high [19], and results from this research were similar to those reported for Andean berry (*Vaccinium meridionale* Swartz) [17] and higher for red globe grape, raspberry, and commercial blackberry ($0.98\text{--}1.55 \times 10^{-5}$ cm/s), suggesting a potential ability of the encapsulation process to ensure proper release and bioaccessibility, as observed for encapsulated piperine in β -cyclodextrin complexes [20].

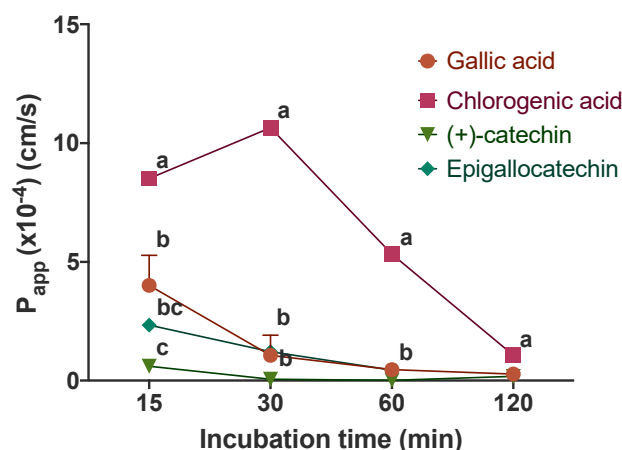


Figure 1. The apparent permeability coefficients (P_{app}) for the selected identified phenolics from Andean blackberry (*Rubus glaucus* Benth) encapsulated in OSA-modified Gros Michel banana starch. The results are the means $\pm$ SD of two independent experiments in triplicate. Different letters express significant differences for each incubation time by the Tukey–Kramer test ($p < 0.05$). P_{app} —apparent permeability coefficient.

4. Conclusions

In conclusion, OSA-Gros Michel banana encapsulation of the phenolic compounds of Andean blackberry displayed a low amount of total phenolic compounds along the digestion, but ensured high bioaccessibility of gallic and chlorogenic acids, allowing for high apparent permeability rates of these compounds during intestinal digestion. Further research is required to validate these properties in vivo.

Author Contributions: Conceptualization, V.D.Q.-C., I.L.-O. and R.C.-V.; Methodology, V.D.Q.-C., I.L.-O., M.L.C.-N. and J.F.V.-L.; Software, I.L.-O. and M.L.C.-N.; Validation, V.D.Q.-C., I.L.-O., M.L.C.-N. and R.C.-V.; Formal analysis, V.D.Q.-C., I.L.-O. and M.L.C.-N.; Investigation, V.D.Q.-C., M.L.C.-N. and I.L.-O.; Resources, V.D.Q.-C., M.L.C.-N., I.L.-O., J.F.V.-L., F.J.C.-G., C.I.Á.-B. and R.C.-V.; Data curation, M.L.C.-N., I.L.-O. and J.F.V.-L.; Writing—original draft preparation, V.D.Q.-C., I.L.-O. and M.L.C.-N.; Writing—review and editing, I.L.-O.; Visualization, I.L.-O.; Supervision, V.D.Q.-C., F.J.C.-G., C.I.Á.-B. and R.C.-V.; Project administration, V.D.Q.-C., J.F.V.-L., F.J.C.-G., C.I.Á.-B. and R.C.-V.; Funding acquisition, V.D.Q.-C., M.L.C.-N., J.F.V.-L., F.J.C.-G., C.I.Á.-B. and R.C.-V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Fondos de Vinculación (FOVIN-UAQ) (grant number: SUV-DVT-2018-08).

Institutional Review Board Statement: The study was conducted according to the guidelines of the National Institute of Health (NIH) and approved by the Ethics Committee of the School of Chemistry from the Universidad Autónoma de Querétaro (approval ID: CBQ16-1116-6).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data will be made available upon reasonable request.

Acknowledgments: V.D.Q.-C. acknowledges the University of Quindío, University of Caldas, and Universidad Autónoma de Querétaro for their support in the development of this project. I.L.-O. acknowledges the postdoctoral fellowship from the Coordinación de Investigación Científica (CIC)-DGAPA UNAM (grant number: 5267). The authors greatly appreciate the technical support provided by M.V.Z., Martín García-Servín, and Alejandra Castilla-León (Instituto de Neurobiología, UNAM-Campus Juriquilla).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Moraes, D.P.; Lozano-Sánchez, J.; Machado, M.L.; Vizzotto, M.; Lazzaretti, M.; Leyva-Jimenez, F.J.J.; da Silveira, T.L.; Ries, E.F.; Barcia, M.T. Characterization of a New Blackberry Cultivar BRS Xingu: Chemical Composition, Phenolic Compounds, and Antioxidant Capacity In Vitro and In Vivo. *Food Chem.* **2020**, *322*, 126783. [CrossRef] [PubMed]
2. Xiong, J.; Chan, Y.H.; Rathinasabapathy, T.; Grace, M.H.; Komarnytsky, S.; Lila, M.A. Enhanced Stability of Berry Pomace Polyphenols Delivered in Protein-Polyphenol Aggregate Particles to an In Vitro Gastrointestinal Digestion Model. *Food Chem.* **2020**, *331*, 127279. [CrossRef] [PubMed]
3. Quintero-Castaño, V.D.; Vasco-Leal, J.F.; Cuellar-Nuñez, M.; Luzardo-Ocampo, I.; Castellanos-Galeano, F.; Álvarez-Barreto, C.; Bello-Pérez, L.A.; Cortés-Rodríguez, M.; Quintero-Castaño, V.D.; Vasco-Leal, J.F.; et al. Novel OSA-Modified Starch from Gros Michel Banana for Encapsulation of Andean Blackberry Concentrate: Production and Storage Stability. *Starch-Stärke* **2021**, *73*, 2000180. [CrossRef]
4. Ramakrishnan, Y.; Adzahan, N.M.; Yusof, Y.A.; Muhammad, K. Effect of Wall Materials on the Spray Drying Efficiency, Powder Properties and Stability of Bioactive Compounds in Tamarillo Juice Microencapsulation. *Powder Technol.* **2018**, *328*, 406–414. [CrossRef]
5. Ogunsona, E.; Ojogbo, E.; Mekonnen, T. Advanced Material Applications of Starch and Its Derivatives. *Eur. Polym. J.* **2018**, *108*, 570–581. [CrossRef]
6. Wurzburg, O.B. Modified Starches. In *Food Polysaccharides and Their Applications*; Stephen, A.M., Phillips, G.O., Williams, P.A., Eds.; Taylor & Francis: Boca Raton, FL, USA, 2006; pp. 88–113. ISBN 978-0-8247-5922-3.
7. Sweedman, M.C.; Tizzotti, M.J.; Schäfer, C.; Gilbert, R.G. Structure and Physicochemical Properties of Octenyl Succinic Anhydride Modified Starches: A Review. *Carbohydr. Polym.* **2013**, *92*, 905–920. [CrossRef] [PubMed]
8. FHIA. Plátano FHIA-21. 2012. Available online: http://www.fhia.org.hn/descargas/Programa_de_Banano_y_Platano/hibridos-FHIA/fhia-21.pdf (accessed on 10 October 2021).
9. Luzardo-Ocampo, I.; Campos-Vega, R.; Gaytán-Martínez, M.; Preciado-Ortiz, R.; Mendoza, S.; Loarca-Piña, G.; Preciado-Ortiz, R.; Mendoza, S.; Loarca-Piña, G. Bioaccessibility and Antioxidant Activity of Free Phenolic Compounds and Oligosaccharides from Corn (*Zea mays* L.) and Common Bean (*Phaseolus vulgaris* L.) Chips during In Vitro Gastrointestinal Digestion and Simulated Colonic Fermentation. *Food Res. Int.* **2017**, *100*, 304–311. [CrossRef] [PubMed]
10. Campos-Vega, R.; Vázquez-Sánchez, K.; López-Barrera, D.; Loarca-Piña, G.; Mendoza-Díaz, S.; Oomah, B.D. Simulated Gastrointestinal Digestion and In Vitro Colonic Fermentation of Spent Coffee (*Coffea arabica* L.): Bioaccessibility and Intestinal Permeability. *Food Res. Int.* **2015**, *77*, 156–161. [CrossRef]
11. Aguillón-Osma, J.; Luzardo-Ocampo, I.; Cuellar-Nuñez, M.L.; Maldonado-Celis, M.E.; Loango-Chamorro, N.; Campos-Vega, R. Impact of In Vitro Gastrointestinal Digestion on the Bioaccessibility and Antioxidant Capacity of Bioactive Compounds from Passion Fruit (*Passiflora edulis*) Leaves and Juice Extracts. *J. Food Biochem.* **2019**, *43*, e12879. [CrossRef] [PubMed]
12. Caicedo-Lopez, L.H.L.H.; Luzardo-Ocampo, I.; Cuellar-Nuñez, M.L.L.; Campos-Vega, R.; Mendoza, S.; Loarca-Piña, G. Effect of the In Vitro Gastrointestinal Digestion on Free-Phenolic Compounds and Mono/Oligosaccharides from *Moringa oleifera* Leaves: Bioaccessibility, Intestinal Permeability and Antioxidant Capacity. *Food Res. Int.* **2019**, *120*, 631–642. [CrossRef] [PubMed]
13. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods Enzymol.* **1999**, *299*, 152–178. [CrossRef]
14. Ramírez-Jiménez, A.K.; Reynoso-Camacho, R.; Mendoza-Díaz, S.; Loarca-Piña, G. Functional and Technological Potential of Dehydrated *Phaseolus vulgaris* L. Flours. *Food Chem.* **2014**, *161*, 254–260. [CrossRef] [PubMed]
15. D’Antuono, I.; Garbetta, A.; Linsalata, V.; Minervini, F.; Cardinali, A. Polyphenols from Artichoke Heads (*Cynara cardunculus* (L.) Subsp. *Scolymus* Hayek): In Vitro Bio-Accessibility, Intestinal Uptake and Bioavailability. *Food Funct.* **2015**, *6*, 1268–1277. [CrossRef] [PubMed]
16. Lassoued, M.A.; Khemiss, F.; Sfar, S. Comparative Study of Two In Vitro Methods for Assessing Drug Absorption: Sartorius SM 16750 Apparatus versus Everted Gut Sac. *J. Pharm. Pharm. Sci.* **2011**, *14*, 117–127. [CrossRef] [PubMed]
17. Agudelo, C.D.; Luzardo-Ocampo, I.; Campos-Vega, R.; Loarca-Piña, G.; Maldonado-Celis, M.E. Bioaccessibility during In Vitro Digestion and Antiproliferative Effect of Bioactive Compounds from Andean Berry (*Vaccinium meridionale* Swartz) Juice. *J. Agric. Food Chem.* **2018**, *66*, 7358–7366. [CrossRef] [PubMed]
18. Sánchez-Velázquez, O.A.; Mulero, M.; Cuevas-Rodríguez, E.O.; Mondor, M.; Arcand, Y.; Hernández-Álvarez, A.J. In Vitro Gastrointestinal Digestion Impact on Stability, Bioaccessibility and Antioxidant Activity of Polyphenols from Wild and Commercial Blackberries (*Rubus* Spp.). *Food Funct.* **2021**, *12*, 7358–7378. [CrossRef] [PubMed]
19. Hubatsch, I.; Ragnarsson, E.G.E.; Artursson, P. Determination of Drug Permeability and Prediction of Drug Absorption in Caco-2 Monolayers. *Nat. Protoc.* **2007**, *2*, 2111–2119. [CrossRef] [PubMed]
20. Quilaqueo, M.; Millao, S.; Luzardo-Ocampo, I.; Campos-Vega, R.; Acevedo, F.; Shene, C.; Rubilar, M. Inclusion of Piperine in β -Cyclodextrin Complexes Improves Their Bioaccessibility and In Vitro Antioxidant Capacity. *Food Hydrocoll.* **2019**, *91*, 143–152. [CrossRef]