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

Victor D. Quintero-Castaño [1,2,*], M. Liceth Cuellar-Nuñez [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 Quindio, Armenia, Colombia
2 Programa de Doctorado en Ingeniería, Universidad de Caldas, Manizales, Colombia
3 Facultad de Medicina, Universidad Autónoma de Querétaro, Queretaro, Qro., Mexico; liceth.cuellar@uaq.mx
4 Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Queretaro, Qro., Mexico; ivan.8907@gmail.com
5 Posgrado en Gestión Tecnológica e Innovación, Universidad Autónoma de Querétaro, Queretaro, Qro., Mexico; jose.vasco@uaq.mx
6 Departamento de Ingeniería, Universidad de Caldas, Manizales, 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, Queretaro, Qro., Mexico; chio_cve@yahoo.com.mx
* Correspondence: victordumar@uniquindio.edu.co (V.D.Q.-C.); chio_cve@yahoo.com.mx (R.C.-V.)
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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 (5-12 × 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 a 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 valuables components are essential, and encapsulation might provide advantages not only protecting them but also delivering at 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, being polysaccharides such as starch some of the most commonly explored since they display desirable technological properties such as low cost acquisition, protects the core encapsulating material during the encapsulation process, and can partially degrade at the small intestine

after resisting the oral and gastric conditions during the digestion [4]. Starches from banana (*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, and 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]. As the bioaccessibility of the encapsulated phenolic compounds has not been yet assessed, considering that this is critical to further research on their biological potential [6], 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

Commercial Andean blackberry (*Rubus glaucus* Benth) concentrate was provided by NUTRIUM® company (Valle del Cauca, Colombia). Bananas (cv. Gros Michel) were harvested at Quimbaya (Quindio, Colombia) at 1270 meters above sea level and 25 °C of average temperature. Starch from Bananas was extracted, modified using OSA, and served as encapsulating matrix of the blackberry concentrate following the reported procedure and optimization of Quintero-Castaño et al. [3].

2.2. In Vitro Gastrointestinal Digestion

A simulated gastrointestinal digestion was conducted from the mouth to the small intestine [7]. Informed consent was obtained from people (4 person) participating at the oral stage, chewing the sample (1 g) 15 times for 15 s. The expectorated product was pH-adjusted to 2.0, pepsin (≥2500 U/mg, Sigma Aldrich, St. Louis, MO, US) 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 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. Rats 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 added with the intestinal enzymes (2.6 mg/sample pancreatin: 8 × 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 "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 Compounds Quantification and Identification of Individual Phenolic Compounds

A methanolic extract was prepared from the samples [6], and the total phenolic compounds were measured using the Folin-Ciocalteu procedure [8]. 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 to diode array detection (HPLC-

DAD) was conducted [9]. 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 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

Bioaccessibility of individual phenolic compounds was measured was reported [10]: B (%): $(C_f/C_0) \times 100$ %, where C_f is the final concentration of the compound at a specific time or stage, while 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. [11] 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 $\times 10^{-4}$ cm/s.

2.5. Statistical Analysis

Results from two independent experiments in triplicates were expressed as the means ± SD. Analysis of variance (ANOVA) followed by post-hoc Tukey-Kramer's test was conducted, establishing significance at $p < 0.05$. Analyses were conducted using JMP v. 16 (SAS Institute, US) 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 DF or NDF fractions. However, NDF showed the highest amount at the intestinal stage, agreeing with previous reports from phenolics of other fruits [12]. Authors have reported low bioaccessibility values for total phenolic compounds of digested wild and commercial blackberries (up to ~60%), explained by the overall low stability of these compounds to the gastric and intestinal conditions [13].

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

Sample/Stage	TPC	Individual Phenolic Compounds (HPLC-DAD) (% B)			
	(mg GAE/g Sample)	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 triplicates. Different letters express significant differences by 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 gallic acid for the DF. For the flavonoids, epigallocatechin gallate showed higher bioaccessibility than (+)-catechin (Table 1). Similar to TPC, NDF retained the highest amount of individual phenolics. The absence of flavonoids at 15 and 30 min of the small intestine stage have also been found for *Moringa oleifera* powder phenolics [14] and passion fruit (*Passiflora edulis*) fruits and leaves extracts [15].

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 10^{-4} cm/s range, permeability can be considered high [16] and results from this research are similar to those reported for Andean berry (*Vaccinium meridionale* Swartz) [12] and higher for red globe grape, raspberry, and commercial blackberry (0.98–1.55 × 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 [17]

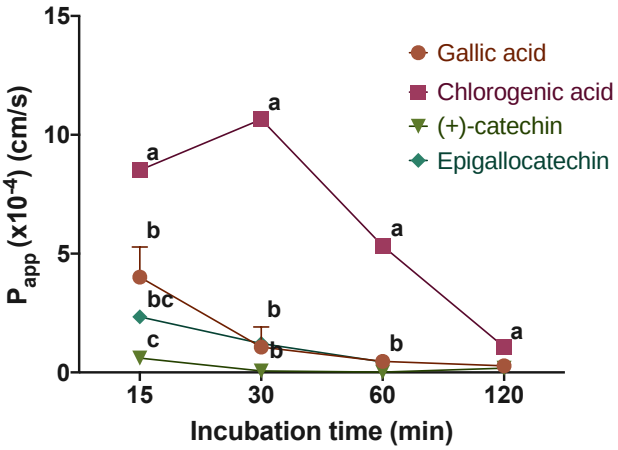

Figure 1. Apparent permeability coefficients (P_{app}) for selected identified phenolics from Andean blackberry (*Rubus glaucus* Benth) encapsulated in OSA-modified Gros Michel banana starch. The results are the means ± SD of two independent experiments in triplicates. Different letters express significant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

4. Conclusions

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

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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 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 available upon reasonable request.

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