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Changes in the Profile of Phenolic Compounds and in the Antioxidant, Hypoglycemic, and Antidiabetic Activities of a Beverage Based on Sesame By-Product Caused by the Simulated Gastrointestinal Digestion Process

Jonhatan Carrasco-Chávez [1], María Fernanda Quintero-Soto [1,2,*], Rosalio Velarde-Barraza [3], Ignacio Alfredo Rivero-Espejel [3], Ismael Díaz-Peña [4], Martha Elena Vázquez-Ontiveros [1], Ramona Julieta Espinoza-Moreno [1], Luz Adriana Ontiveros-García [1], Jesús Mateo Amillano-Cisneros [1], Janitzio Xiomara K. Perales-Sánchez [5], Oscar Daniel Argüelles-López [5], Fernando Salas-López [6] and Jennifer Vianey Félix-Medina [2,4]

1 Maestría en Biotecnología Agropecuaria, Universidad Politécnica del Mar y la Sierra, Carretera a Potrerillos del Norote Km 3, La Cruz, Elota 82700, Sinaloa, Mexico; 2023030003@upmys.edu.mx (J.C.-C.); mvazquez@upmys.edu.mx (M.E.V.-O.); respinoza@upmys.edu.mx (R.J.E.-M.); lontiveros@upmys.edu.mx (L.A.O.-G.); jamillano@upmys.edu.mx (J.M.A.-C.)
2 Posdoc CONAHCYT, Universidad Politécnica del Mar y la Sierra, Carretera a Potrerillos del Norote Km 3, La Cruz, Elota 82700, Sinaloa, Mexico; jevianey9@gmail.com
3 Centro de Graduados e Investigación en Química, Instituto Tecnológico de Tijuana Unidad Otay-Tecnológico Nacional de México, Blvd. Industrial s/n, Tijuana 22000, Baja California, Mexico; rosaliovb@gmail.com (R.V.-B.); irivero@tectijuana.mx (I.A.R.-E.)
4 Instituto Tecnológico de Culiacán-Tecnológico Nacional de México, Calle Juan de Dios Bátiz 310 pte, Colonia Guadalupe, Culiacán de Rosales 80220, Sinaloa, Mexico; diaz_1986@hotmail.es
5 Facultad de Ciencias Químicas Biológicas, Universidad Autónoma de Sinaloa, Calle Josefa Ortiz de Domínguez, esq. Blvd de las Américas, Ciudad Universitaria, Culiacán 80010, Sinaloa, Mexico; janitzio.perales@uas.edu.mx (J.X.K.P.-S.); oscararguelles@uas.edu.mx (O.D.A.-L.)
6 Facultad de Ciencias Biomédicas, Universidad Autónoma de Occidente, UR Culiacán. Blvd. Lola Beltrán y Blvd. Rolando Arjona, 4 de marzo, Culiacán 80020, Sinaloa, Mexico; fernando.salas@uadeo.mx
* Correspondence: mquintero@upmys.edu.mx

Abstract: The by-product of extracting oil from sesame has good functional properties for use in the development of new food products. In this study, the effect of simulated gastrointestinal digestion on the bioaccessibility of phenolic compounds, as well as the antioxidant, antidiabetic, and hypoglycemic properties of a beverage from sesame by-products was analyzed. Oral digestion significantly decreased the total phenolic content of the beverage, while the total flavonoid content increased. Both phytochemicals increased after gastric and intestinal digestion. Twenty-five phenolic compounds were characterized in the sesame by-product beverage, including feruloylquinic acid, which was present in all the digestive fractions analyzed. The most abundant free phenolic was quercetin, which was also present in all the digested fractions. On the other hand, the most abundant phenolic in the digested fractions was isorhamnetin. During gastrointestinal digestion, the antioxidant activity and inhibitory effects on the α-glucosidase and DPP-IV of the beverage significantly improved, while the ability to inhibit the α-amylase significantly increased during oral digestion, remaining constant throughout digestion. Correlation analysis indicated that flavonoids, including quercetin, may be the compounds with the greatest effect on the evaluated activities. The results of this study not only improve the understanding of the impact of gastrointestinal digestion on the bioaccessibility of phenolic compounds but also suggest potential applications in formulating functional foods with enhanced antioxidant, hypoglycemic, and antidiabetic properties, contributing to the development of health-promoting food products.

Keywords: α-amylase inhibition; α-glucosidase inhibition; dipeptidyl peptidase-IV inhibition; phenolic acids; flavonoids; HPLC-DAD-MS/MS

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Citation: Carrasco-Chávez, J.; Quintero-Soto, M.F.; Velarde-Barraza, R.; Rivero-Espejel, I.A.; Díaz-Peña, I.; Vázquez-Ontiveros, M.E.; Espinoza-Moreno, R.J.; Ontiveros-García, L.A.; Amillano-Cisneros, J.M.; Perales-Sánchez, J.X.K.; et al. Changes in the Profile of Phenolic Compounds and in the Antioxidant, Hypoglycemic, and Antidiabetic Activities of a Beverage Based on Sesame By-Product Caused by the Simulated Gastrointestinal Digestion Process. *Beverages* **2024**, *10*, 115. https://doi.org/10.3390/beverages10040115

Academic Editor: Antonio Cilla

Received: 23 September 2024
Revised: 4 November 2024
Accepted: 22 November 2024
Published: 26 November 2024

1. Introduction

The food industry is one of the main generators of waste or by-products worldwide, which in some cases go directly to landfills, generating a negative environmental impact [1]. The production of food, where conventional raw materials are insufficient to meet global demand, has led to the overexploitation of crops, causing an increase in food costs and poor distribution [2,3]. Therefore, it is important to seek appropriate solutions to establish economically and environmentally sustainable parameters that support the transformation of the food industry towards a circular bioeconomy [3,4]. By-products, with proper processing, can be used as raw materials in the creation of new products or foods. As a result, there has been significant interest in valorizing food waste for the development of new value-added products [1,2]. These by-products represent alternative sources to conventional raw materials, helping to reduce the excessive consumption of the latter, which has been gradually depleting [1].

The by-product of sesame seed oil extraction is often discarded as a secondary raw material despite having a good content of nutritional compounds and phytochemicals [5,6]. When the oil is extracted from the seed (which represents more than 50% of the original components), the remaining compounds increase, mainly protein, fiber, minerals, and phenolic compounds [7,8]. These latter compounds are responsible for the various biological activities of the by-product [9].

Unlike other by-products from the food industry, the by-product of sesame oil extraction retains its techno-functional properties, as well as its nutritional and nutraceutical qualities [10]; this makes it suitable for use as a non-conventional raw material in the production of various food products such as cookies and beverages [5,6]. In this regard, Lucini Mas et al. [5] made wheat cookies enriched with sesame by-product, finding that the addition did not alter the functional parameters of the cookies but significantly increased the antioxidant activity and phenolic compound content by three times the expected values. This was attributed to the generation of Maillard reaction compounds formed during the baking process and the increased bioavailability of phenolic compounds. In a previous study, it was observed that heating combined with the shear force generated by the extrusion process modified the phenolic compound profile of a beverage based on sesame by-product, enhancing its antioxidant and antidiabetic activity [6]. Despite observing an increase in antioxidant and antidiabetic activities due to the extrusion process, it is important to understand the bioavailability of the phenolic compounds present in the beverage during gastrointestinal digestion, as well as the effect that digestion has on biological activity.

Generally, the digestibility of food has a negative impact on the bioavailability of compounds and their biological activities [11]. Most digestibility studies focus on understanding changes in the profile of compounds and antioxidant activity. Chen et al. [8] indicated that the thermal processing applied to six varieties of sesame increased the content of phenolic compounds, flavonoids, and antioxidant activity after roasting. However, they observed a decrease in antioxidant activity when the samples were subjected to the in vitro gastrointestinal digestion process, but depending on the sesame variety, this characteristic either decreased or improved compared to the raw sample. This is due to the type of phenolic compound and the food matrix subjected to the process. On the other hand, Tomé-Sánchez et al. [12] observed that for different wheat-based matrices (germinated wheat, spray-dried wheat bran, and microencapsulated wheat bran), the effects of gastrointestinal digestion were different. In the case of wheat brans, antioxidant activity either decreased or increased depending on the evaluation method (ABTS, DPPH, FRAP, ORAC), while for germinated wheat, an increase in activity was observed during gastric and intestinal digestion. Luo et al. [13] evaluated the effect of gastrointestinal digestion on the antioxidant activity and phenolic compounds of black, brown, and white sesame seeds. It was observed that black and brown sesame seeds presented better stability to antioxidant activity during the digestion process, but the phenolic compounds of beige sesame seeds were the ones that presented the best bioavailability. Among the 12 compounds identified

by these authors, syringic acid was the one that presented the best bioavailability during the different phases of digestion (oral, gastric, and intestinal) of the three types of sesame.

Only one report was found where the hypoglycemic activity of sesame by-product was evaluated after the in vitro gastrointestinal digestion process. Doungwichitrkul et al. [14] isolated and identified 29 phytochemical compounds, mainly furofuran lignans, in the black sesame by-product and evaluated its inhibitory effect against the rat intestinal α-glucosidase. The sesaminol triglucoside compound did not present inhibitory effects against the said enzyme. However, when subjected to the gastrointestinal digestion process, its potential to inhibit the α-glucosidase enzyme increased; this is due to the transformations it underwent during digestion (elimination of a glucose group and two methylene groups).

Other studies focus on examining the importance of phenolic compounds in plant extracts, foods, or beverages and are limited to analyzing their content and their bioactivity. This study is one of the few that focuses on analyzing the effects of in vitro gastrointestinal digestion on the phenolic compound profile and their bioactivities, especially on the phenolic compounds of the sesame by-product-based beverage. No reports were found that evaluate the effect of in vitro gastrointestinal digestion on the antidiabetic activity of sesame or sesame by-products. Therefore, the objective of this research is to determine the changes caused by the gastrointestinal digestion process in the phenolic compound profile, as well as the antioxidant activity, hypoglycemic activity, and antidiabetic activity of a novel beverage made from extruded sesame by-products.

2. Materials and Methods

2.1. Preparation of the Functional Beverage

The methodology reported by Quintero-Soto et al. [6] was followed for the formulation of the beverage, where a 10% concentration of the by-product previously selected as the sensory acceptance was highest. Twenty grams of previously extruded by-product flour [10] was mixed with 5 g of sugar substitute (BC Metco® Sugar) and 200 mL of purified water. The mixture was stirred (500 rpm/20 min) and stored at 8 °C until further use.

2.2. Gastrointestinal Digestion Process

The digestion process was carried out in three phases, as described by Cárdenas-Castro et al. [15] and Lucini Mas et al. [5], with some modifications. For the oral phase, 200 mg of lyophilized beverage was mixed and digested with a human salivary α-amylase solution (A0521, Sigma-Aldrich, St. Louis, MO, USA, 1000–3000 units/mg protein; 100 µL of a 0.049 mg/mL solution in 0.05 M phosphate buffer, pH 6.9, 37 °C, for 2 min). For the gastric phase, a pepsin solution was added to the samples (P-7000, Sigma-Aldrich, ≥250 units/mg solid; 400 µL of a 300 mg/mL solution in 0.2 M HCl-KCl buffer, pH 1.5, 40 °C) and placed in a water bath with agitation for 2 h. For the intestinal digestion, a pancreatin solution (P-1750, Sigma-Aldrich, 4 × USP specifications; 3 mL of a 5 mg/mL solution in 0.1 M $NaHCO_3$, pH = 7.5) and bile salts (1,071,304, US Pharmacopeia, USP, Rockville, Maryland, USA; 2 mL of a 25 mg/mL solution in 0.1 M $NaHCO_3$, pH = 7.5) were added. The final mixture was placed in a cellulose membrane (6000–8000 Da, 25.5 mm × 30 m, Fisherbrand™, Waltham, MA, USA) to simulate the small intestine and dialyzed in 0.1 M $NaHCO_3$ (pH = 7.5) with agitation (150 rpm, 3 h, 37 °C). The fraction that passed through the membrane was considered the fraction of compounds available for passive diffusion into the circulatory system in the small intestine. The fraction remaining inside the membrane was considered the indigestible fraction, which was mixed with 10 mL of methanol (400 rpm/30 min) and centrifuged to recover the supernatant containing the non-digestible phenolic compounds.

During each phase of digestion, aliquots were collected, adjusted to pH 7.0, and washed with ethyl acetate to recover phenolic compounds. The recovered ethyl acetate was evaporated to dryness, and the samples were reconstituted in 2 mL of 80% methanol for analysis by HPLC-DAD-MS/MS. These same aliquots were used to determine biological activities.

2.3. Determination of In Vitro Bioaccessibility of Released Phenolic Compounds

Bioaccessibility expressed as a percentage was calculated as the ratio of phenolic compounds released from the beverage during digestion and the initial content of phenolic compounds in the beverage before digestion [11], using the following formula:

$$\% \text{ bioaccessibility} = \left(\frac{\text{Phenolics after the digestion process}}{\text{Phenolics before the digestion process}} \right) \times 100$$

Values over 100% are indicative of an increase in the bioaccessible amount of phenolic compounds in each digestion phase. The bioaccessibility of phenolic compounds that were not initially found in the undigested beverage (UD) was considered to be 100%.

2.4. Characterization of the Profile of Phenolic Compounds

The phenolic compound profile of the undigested, digested (oral, gastric, and intestinal phases), and indigestible samples was performed using HPLC-DAD (1100 Series, Agilent Technologies, Santa Clara, CA, USA) coupled to a mass detector (1100 Series LC/MSD Trap, Agilent Technologies, USA) as reported by Quintero-Soto et al. [6]. Twenty-five microliters of the sample were separated on a Zorbax SB-C18 column (150 × 3 mm, 5 µm, Agilent Technologies, Santa Clara, CA, USA) using water-formic acid (99:1 v/v) and acetonitrile as mobile phases (A and B, respectively). A linear gradient from 1 to 60% of B over 60 min, with a flow rate of 0.4 mL/min and detection wavelengths of 280 nm (hydroxybenzoic acids and flavan-3-ols), 320 nm (hydroxycinnamic acids), and 350 nm (flavonoids) were used. The identification of phenolic compounds was based on retention time, UV spectra, and mass spectra. The mass spectra were acquired on a mass spectrometer with an ion trap analyzer and an electrospray ionization source. The mass spectrometer parameters for compound identification were as follows: positive and negative ion scanning at 35 V and 300 °C, collision-induced dissociation fragmentation (10–45 V), m/z = 100 to 2000, nitrogen for drying, and helium for collision. Data was analyzed with Agilent ChemStation for LC 3D software (Rev. A. 09. 01 [1206] Agilent Technologies, USA) and LC/MSD Trap software 5.2 (Build 63.8, Bruker Daltonik GmbH). The individual phenolic compound content was calculated using commercial standard curves.

2.5. Quantification of Total Phenolic Compounds and Flavonoids

The total phenolic content (TPC) was determined by mixing 0.2 mL of the sample and 2.2 mL of Folin-Ciocalteu reagent in the dark for 3 min. Then, 0.6 mL of sodium carbonate (7%) was added, and the samples were kept in the dark for 90 min. Finally, the absorbance of the samples was measured at 750 nm and compared to a gallic acid standard curve [16]. Total flavonoid content was determined as described by Xu and Chang [17] by mixing 0.1 mL of the sample, 0.5 mL of water, and 0.25 mL of sodium nitrate (5%) in the dark for 6 min. Subsequently, 0.5 mL of silver chloride (5%) and 0.25 mL of sodium hydroxide (1 M) were added to the mixture. The absorbance of the samples was measured at 510 nm and compared to a quercetin standard curve.

2.6. Determination of Antioxidant Activity

The in vitro antioxidant activity was determined using the ABTS [18] and DPPH [19] methods. For ABTS, three milliliters of the ABTS radical previously activated in potassium persulfate (2.6 mM) and adjusted with phosphate buffer (10 mM, pH 7.4) to an absorbance of 0.70 at 734 nm were mixed with 0.75 mL of the sample and incubated for 6 min at room temperature. The samples were then monitored at 734 nm. For DPPH, 20 µL of the sample was mixed with 180 µL of DPPH radical solution (0.1 mM) and incubated for 30 min at 37 °C. The absorbance of the samples was measured at 510 nm. A trolox standard curve was used in both assays to calculate the results.

2.7. Determination of Hypoglycemic Activity

The hypoglycemic activity was determined by the inhibition of the α-amylase and α-glucosidase enzymes [6]. Ten microliters of the sample and 10 µL of α-amylase solution (2 U/mL) were mixed and incubated for 10 min at 25 °C. Then, 10 µL of soluble starch (1%) was added, and the mixture was incubated again (10 min at 25 °C). Subsequently, 20 µL of a 3,5-dinitrosalicylic acid solution was added and incubated for 5 min at 25 °C. Finally, 200 µL of distilled water was added, and the absorbance was read at 540 nm. The results were expressed as the percentage inhibition of the α-amylase enzyme (AA) and IC_{50}.

For the determination of α-glucosidase enzyme inhibition, 50 µL of the sample and 100 µL of α-glucosidase solution (1 U/mL) were mixed and incubated for 10 min at 37 °C. Subsequently, 50 µL of *p*-nitrophenyl-α-D-glucopyranoside (5 mM) was added, followed by incubation (10 min at 37 °C). Finally, the absorbance was read at 405 nm. The results were expressed as the percentage inhibition of the α-glucosidase enzyme (AG) and IC_{50}.

2.8. Determination of Antidiabetic Activity

The antidiabetic activity was determined by the samples' potential to inhibit the dipeptidyl-peptidase IV (DPP-IV) enzyme using the MAK203 kit (Sigma-Aldrich, St. Louis, MO, USA). The results were expressed as the percentage inhibition of the DPPIV enzyme (%DPP-IV) and IC_{50}.

2.9. Statistical Analysis

The statistical analyses were performed in triplicate (n = 3), and the data were analyzed through a mean comparison using Fisher's least significant difference test ($p < 0.05$) with the STATGRAPHIC plus version 5.1 program (Statistical Graphics Corporation™, Rockville, MD, USA). Pearson correlation analysis and principal component analysis were conducted using the MINITAB 17 program (Minitab, LLC., PA, USA).

3. Results and Discussion

3.1. Changes in the Profile and Content of Phenolic Compounds After the Gastrointestinal Digestion Process

Table 1 shows the profile of phenolic compounds in the sesame seed oil by-product beverage before and during gastrointestinal digestion, as well as the compounds available for the microbiota. In total, 25 phenolic compounds were identified, 13 of which were phenolic acids and 12 flavonoids. Some of these had been previously identified in sesame and its products [6–8].

Table 1. Tentative identification of phenolic compounds from sesame by-product beverage before and after in vitro gastrointestinal digestion.

#	TR (min)	*m/z* $[M-H]^-$	Error (ppm)	MS/MS (Relative Intensity, %)	Proposed Compounds *
1	2.20	169.45	−1.95	125 (100), 97 (8)	Gallic acid [20]
2	6.11	153.35	−1.50	109 (100)	Protocatechuic acid [21]
3	6.53	341.35	−0.56	162 (100), 135 (25)	Caffeic acid hexoside [22]
4	7.01	167.35	−1.26	151 (36), 108 (100)	Vanillic acid [6]
5	8.22	179.59	−2.40	145 (20), 135 (100), 117 (36)	Caffeic acid [6]
6	9.05	353.89	−1.64	191 (100), 179 (10), 173 (37)	Caffeoylquinic acid [23]
7	9.32	325.11	−0.22	163 (100), 119 (41)	*p*-Coumaric acid hexoside [24]
8	10.56	289.89	−2.18	245 (100), 221 (7), 203 (3)	Catechin [20]
9	18.11	163.55	−3.07	146 (62), 119 (100)	p-Coumaric acid [24]
10	19.23	353.68	−1.05	191 (100), 177 (25), 145 (59)	Chlorogenic acid [6]
11	23.06	355.71	−1.38	193 (28), 117 (69)	Ferulic acid hexoside [20]
12	25.63	147.66	−3.47	103 (100), 122 (23), 102 (13)	Cinnamic acid [21]
13	25.98	193.22	−0.21	175 (100), 115 (42), 143 (49)	Ferulic acid [6]
14	26.15	771.89	−0.47	609 (56), 301 (100)	Rutin hexoside [25]

Table 1. *Cont.*

#	TR (min)	*m/z* [M-H]$^-$	Error (ppm)	MS/MS (Relative Intensity, %)	Proposed Compounds *
15	27.46	447.96	−1.63	285 (100), 229 (23), 216 (12)	Kaempferol hexoside [24]
16	27.68	623.85	−0.35	463 (34), 301 (100)	Quercetin dihexoside [26]
17	27.99	609.96	−0.74	301 (100), 300 (88), 151 (10)	Rutin [20]
18	30.12	463.37	−0.30	301 (100), 179 (12)	Quercetin hexoside [27]
19	30.56	367.87	−2.07	134 (30), 193 (100), 278 (10)	Feruloilquinic acid [12]
20	31.62	301.87	−2.12	179 (100), 151 (7)	Quercetin [20]
21	32.13	285.67	−1.51	193 (16), 151 (100)	Luteolin [6]
22	34.19	431.78	−1.55	269 (100), 117 (56)	Apigenin hexoside [28]
23	35.01	285.35	−0.46	257 (100), 229 (13), 216 (19)	Kaempferol [20]
24	35.26	268.98	0.26	161 (13), 152 (100), 117 (52)	Apigenin [6]
25	36.97	315.43	−0.54	301 (10), 300 (100), 285 (49)	Isorhamnetin [6]

TR: retention time. * The numbers in brackets correspond to the reference number.

Of the 25 phenolic compounds identified, only 12 were observed in the undigested sample (UD), while the other 13 were generated by the degradation and transformation of the initial phenolic compounds. Quercetin was present in the highest concentration in UD, while isorhamnetin was found in the three digestive phases and in the fraction available for the intestinal microbiota (Table 2).

The phenolic compounds identified in this study have shown various health effects. Gallic acid, in addition to its antioxidant activity, has been reported to attenuate ulcerative colitis in vivo [29]. Protocatechuic acid promotes the lysosomal autophagy pathway and alleviates neuronal damage in in vivo and in vitro models of Alzheimer's disease [30]. Caffeic acid mitigates myocardial fibrosis and improves cardiac function after myocardial infarction by inhibiting the signaling pathways of the transforming growth factor-β receptor 1 [31]; additionally, caffeic acid and its derivatives, such as chlorogenic acid (3-caffeoylquinic acid) and 5-caffeoylquinic acid, have been observed to have anti-inflammatory activity by inhibiting proinflammatory cytokines and increasing anti-inflammatory cytokine secretions [32].

On the other hand, flavonoids like catechin demonstrated antioxidant, antidiabetic, anti-infectious, and anticancer effects, specifically against gastric cancer cells [33]. Quercetin and rutin have also demonstrated anti-inflammatory activity [34,35]. Kaempferol has shown positive effects on metabolic diseases like diabetes mellitus, obesity, non-alcoholic fatty liver disease, steatohepatitis, and atherosclerosis [36]. Apigenin and its derivatives have demonstrated antimicrobial, anticancer, anti-inflammatory, and antioxidant effects [37]. Isorhamnetin has shown various pharmacological properties associated with the prevention of neurodegenerative disorders like Alzheimer's disease [38], bladder cancer [39], breast cancer [40], colon cancer [41], and ulcerative colitis [42].

Changes in the profile of phenolic compounds during gastrointestinal digestion have been reported by various authors and are dependent on the type of matrix, the concentration of the compound, and whether the compound is in its free, bound, or conjugated form [5,8,11,12,15,43,44]. The changes observed in the phenolic profile of the beverage are described below.

Gallic acid first appeared at the beginning of digestion in the oral phase (OP) (Table 2), with a significant increase observed during the gastric phase (GP) ($p < 0.05$). Subsequently, there was a marked decrease caused by the dehydroxylation of gallic acid, leading to the formation of protocatechuic acid in the intestinal phase (IP).

Table 2. Quantification of phenolic compounds from sesame by-product beverage at different stages of gastrointestinal digestion.

Phytochemical	UD mg/100 g (mg/100 mL)	OP mg/100 g (mg/100 mL)	GP mg/100 g (mg/100 mL)	IP mg/100 g (mg/100 mL)	ID mg/100 g (mg/100 mL)	LOD mg/mL
Gallic acid	ND	7.28 ± 0.06 ^{b}	9.46 ± 0.37 ^{a}	4.78 ± 0.21 ^{c}	0.25 ± 0.02 ^{d}	0.0012
	ND	(0.91 ± 0.01 ^{b})	(1.18 ± 0.05 ^{a})	(0.60 ± 0.03 ^{c})	(0.03 ± 0.00 ^{d})	
Protocatechuic acid	16.73 ± 0.60 ^{a}	ND	ND	3.06 ± 0.05 ^{c}	10.06 ± 0.66 ^{b}	0.0025
	(2.09 ± 0.07 ^{a})	ND	ND	(0.38 ± 0.01 ^{c})	(1.26 ± 0.08 ^{b})	
Caffeic acid hexoside	0.76 ± 0.11 ^{b}	ND	1.91 ± 0.07 ^{a}	1.88 ± 0.07 ^{a}	ND	0.0006
	(0.09 ± 0.01 ^{b})	ND	(0.24 ± 0.01 ^{a})	(0.24 ± 0.01 ^{a})	ND	
Vanillic acid	ND	ND	ND	64.35 ± 4.50	ND	0.0750
	ND	ND	ND	(8.04 ± 0.56)	ND	
Caffeic acid	ND	0.71 ± 0.03 ^{b}	4.58 ± 0.33 ^{a}	ND	ND	0.0006
	ND	(0.09 ± 0.00 ^{b})	(0.57 ± 0.04 ^{a})	ND	ND	
Caffeoylquinic acid	4.44 ± 0.14 ^{b}	4.31 ± 0.35 ^{b}	ND	14.09 ± 0.97 ^{a}	2.17 ± 0.03 ^{c}	0.0004
	(0.55 ± 0.02 ^{b})	(0.54 ± 0.04 ^{b})	ND	(1.76 ± 0.12 ^{a})	(0.27 ± 0.00 ^{c})	
p-Coumaric acid hexoside	ND	ND	ND	ND	11.38 ± 0.81	0.0099
	ND	ND	ND	ND	(1.42 ± 0.10)	
Catechin	19.85 ± 0.08 ^{b}	4.77 ± 0.11 ^{d}	9.86 ± 0.67 ^{c}	10.49 ± 0.14 ^{c}	39.42 ± 3.42 ^{a}	0.0029
	(2.48 ± 0.01 ^{b})	(0.60 ± 0.01 ^{d})	(1.23 ± 0.08 ^{c})	(1.31 ± 0.02 ^{c})	(4.93 ± 0.43 ^{a})	
p-Coumaric acid	ND	ND	ND	3.18 ± 0.04 ^{a}	1.86 ± 0.03 ^{b}	0.0099
	ND	ND	ND	(0.40 ± 0.01 ^{a})	(0.23 ± 0.00 ^{b})	
Chlorogenic acid	30.18 ± 1.33 ^{a}	22.30 ± 0.33 ^{b}	ND	ND	15.65 ± 1.78 ^{c}	0.0241
	(3.77 ± 0.17 ^{a})	(2.79 ± 0.04 ^{b})	ND	ND	(1.96 ± 0.22 ^{c})	
Ferulic acid hexoside	34.59 ± 0.58 ^{a}	21.10 ± 1.04 ^{b}	19.05 ± 0.40 ^{c}	19.09 ± 0.10 ^{c}	11.00 ± 0.47 ^{d}	0.0150
	(4.32 ± 0.11 ^{a})	(2.64 ± 0.13 ^{b})	(2.38 ± 0.05 ^{c})	(2.39 ± 0.01 ^{c})	(1.37 ± 0.06 ^{d})	
Cinnamic acid	14.25 ± 0.13 ^{b}	6.96 ± 0.43 ^{e}	12.27 ± 0.50 ^{c}	24.82 ± 0.62 ^{a}	8.86 ± 0.13 ^{d}	0.0099
	(1.78 ± 0.02 ^{b})	(0.87 ± 0.05 ^{e})	(1.53 ± 0.06 ^{c})	(3.10 ± 0.08 ^{a})	(1.11 ± 0.02 ^{d})	
Ferulic acid	ND	12.54 ± 0.038 ^{c}	26.42 ± 1.61 ^{b}	ND	49.55 ± 1.62 ^{a}	0.0151
	ND	(1.57 ± 0.05 ^{c})	(3.30 ± 0.20 ^{b})	ND	(6.19 ± 3.27 ^{a})	
Rutin hexoside	12.02 ± 0.14 ^{a}	ND	ND	ND	11.79 ± 0.49 ^{a}	0.0019
	(1.50 ± 0.02 ^{a})	ND	ND	ND	(1.47 ± 0.06 ^{a})	
Kaempferol hexoside	ND	ND	ND	ND	72.97 ± 0.49	0.0094
	ND	ND	ND	ND	(9.12 ± 0.06)	
Quercetin dihexoside	41.39 ± 0.62	ND	ND	ND	ND	0.0016
	(5.17 ± 0.08)	ND	ND	ND	ND	
Rutin	ND	9.71 ± 0.16 ^{b}	10.10 ± 0.23 ^{a}	ND	4.97 ± 0.09 ^{c}	0.0005
	ND	(1.21 ± 0.02 ^{b})	(1.26 ± 0.03 ^{a})	ND	(0.62 ± 0.01 ^{c})	
Quercetin hexoside	25.07 ± 0.84	ND	ND	ND	ND	0.0016
	(3.13 ± 0.10)	ND	ND	ND	ND	
Feruloilquinic acid	22.30 ± 2.03 ^{b}	21.38 ± 1.09 ^{b}	14.53 ± 0.64 ^{c}	14.50 ± 0.13 ^{c}	32.40 ± 0.54 ^{a}	0.0150
	(2.79 ± 0.25 ^{b})	(2.67 ± 0.14 ^{b})	(1.82 ± 0.08 ^{c})	(1.81 ± 0.02 ^{c})	(4.05 ± 0.07 ^{a})	

Table 2. *Cont.*

Phytochemical	UD mg/100 g (mg/100 mL)	OP mg/100 g (mg/100 mL)	GP mg/100 g (mg/100 mL)	IP mg/100 g (mg/100 mL)	ID mg/100 g (mg/100 mL)	LOD mg/mL
Quercetin	295.98 ± 5.69 ^{c}	404.79 ± 12.35 ^{b}	414.13 ± 5.28 ^{b}	448.96 ± 1.49 ^{a}	ND	0.0015
	(37.00 ± 0.71 ^{c})	(50.60 ± 1.54 ^{b})	(51.77 ± 0.66 ^{b})	(56.12 ± 0.19 ^{a})	ND	
Luteolin	ND	11.93 ± 0.42 ^{a}	ND	8.19 ± 0.18 ^{b}	ND	0.0023
	ND	(1.49 ± 0.05 ^{a})	ND	(1.02 ± 0.02 ^{b})	ND	
Apigenin hexoside	25.80 ± 1.60 ^{a}	ND	ND	16.80 ± 1.32 ^{b}	ND	0.0057
	(3.23 ± 0.20 ^{a})	ND	ND	(2.10 ± 0.16 ^{b})	ND	
Kaempferol	ND	ND	99.02 ± 5.52 ^{b}	110.88 ± 0.51 ^{a}	ND	0.0094
	ND	ND	(12.38 ± 0.69 ^{b})	(13.86 ± 0.06 ^{a})	ND	
Apigenin	ND	16.10 ± 0.57 ^{a}	15.91 ± 0.59 ^{a}	16.81 ± 0.22 ^{a}	ND	0.0057
	ND	(2.01 ± 0.07 ^{a})	(1.99 ± 0.07 ^{a})	(2.11 ± 0.03 ^{a})	ND	
Isorhamnetin	ND	710.48 ± 15.39 ^{a}	696.68 ± 1.63 ^{a}	496.02 ± 7.97 ^{b}	708.92 ± 14.58 ^{a}	0.0709
	ND	(88.81 ± 0.20 ^{a})	(87.08 ± 0.20 ^{a})	(62.00 ± 1.00 ^{b})	(88.61 ± 1.82 ^{a})	

UD: Undigested (free extract), OP: Oral phase, GP: Gastric phase, IP: Intestinal phase, ID: Indigestible fraction (available to the microbiota), LOD: Limit of detection (mg/mL), ND: Not detected. The results are expressed in mg/100 g dw and mg/100 mL of beverage (values in parentheses). Values (means ± SD) with different letters within a row are significantly different at $p < 0.05$ among the means according to Fisher's test.

Caffeic acid hexoside was one of the phenolics observed for the first time in UD and then disappeared in OP as a result of the hydrolysis of the hexose molecule, generating caffeic acid. This acid appeared for the first time in OP. During GP, a significant increase in caffeic acid ($p < 0.05$) was observed, resulting from the hydrolysis of the quinic group of caffeoylquinic acid and chlorogenic acid. During IP, caffeic acid is transformed into p-coumaric acid by the dehydroxylation of the hydroxyl group. Both caffeic acid hexoside and caffeoylquinic acid reappeared in IP, likely as a result of hydrolysis caused by the mixture of enzymes. Phenolic compounds bound to the food matrix can be released under the acidic conditions of the stomach, where proteins, lipids, and other macromolecules begin to denature, releasing phenolic compounds bound to the food matrix such as caffeic acid hexoside and caffeoylquinic acid or other precursors involved in their synthesis. Ali et al. [45] demonstrated the binding of caffeoylquinic acid to coffee bean storage protein.

Ferulic acid hexoside and feruloylquinic acid were present both in UD and in all phases of digestion. A significant decrease in their concentration was observed as digestion progressed (Table 2). This decrease is reflected in the generation of ferulic acid in OP due to the hydrolysis of the hexose from some ferulic acid hexoside molecules. Ferulic acid more than doubled during GP as a result of the release of the quinic group from feruloylquinic acid.

As for flavonoids, rutin hexoside was observed in UD, which, during OP, lost the hexoside group and was transformed into rutin. Rutin appeared for the first time in this phase and remained stable in GP. During IP, the rutinoside group was hydrolyzed, leading to the formation of quercetin. On the other hand, quercetin dihexoside and quercetin hexoside were present in UD, and both flavonoids were transformed into quercetin during OP through the hydrolysis of their hexose molecules. These transformations were reflected in the significant increase ($p < 0.05$) in quercetin concentration during OP. With regard to apigenin, it appeared for the first time in OP due to the hydrolysis of the hexose group from apigenin hexoside, which was initially identified in UP. The concentration of apigenin remained stable throughout the gastrointestinal digestion process. Throughout the three phases of digestion, the presence of isorhamnetin was observed. This flavonoid was not among those identified in UD. It first appeared in OP, suggesting that it was bound to some component of the beverage and was likely released as a result of hydrolysis caused by the enzyme α-amylase. Isorhamnetin remained stable during OP and GP, but a significant decrease ($p > 0.05$) of around 30% was observed in IP.

In the fraction available to the intestinal microbiota, that is, the indigestible (ID) fraction, 15 compounds were identified. Among them were p-coumaric acid hexoside and kaempferol hexoside, which were only observed in this fraction. The presence of isorhamnetin also stands out. This is relevant since this phenolic compound has been shown to have positive effects on colon cancer [41]. Unabsorbed flavonoids play an important role in the intestinal environment by modulating the diversity and microbial composition of the gut microbiota [46]. Aglycones, such as isorhamnetin, are transformed by intestinal microbial enzymes to produce a variety of ring-fission products, including 3,4-dihydroxyphenylacetic acid, 3-hydroxyphenylacetic acid, protocatechuic acid, and hippuric acid [47,48]. Isorhamnetin has been reported to enhance the abundance and diversity of gut microbiota in mice, with dominant genera mainly including Bacteroidales S24-7 group_norank, Erysipelotrichaceae_uncultured, Lactobacillus, Lachnospiraceae_uncultured, Allobaculum, Lachnospiraceae NK4A136 group, Turicibacter, and Lachnoclostridium [49]. It also improves lipid metabolism in patients with type 2 diabetes mellitus due to the reduction in Firmicutes and Bacteroidetes ratios [49].

The total phytochemical content and its behavior during gastrointestinal digestion are shown in Table 3. In UD, a total phenolic compound (TPC) content of 178.19 mg GAE/100 g was observed, similar to the values reported by Lucini Mas et al. [5] for a cookie made from sesame by-products. During OP, a 50% decrease in TPC was observed, followed by an increase of approximately 65% and 50% in GP and IP, respectively. Regarding the total flavonoid content (TFC), a significant increase ($p < 0.05$) of 100%, 130%, and 150% was

observed in OP, GP, and IP, respectively, compared to the initial concentration (61.64 mg QE/100 g). The behavior observed for TPC and TFC is consistent with the findings reported by Chen et al. [8] for six varieties of sesame subjected to an in vitro gastrointestinal digestion process. Luo et al. [13] reported a gradual increase in the TPC content of beige, brown, and black sesame seeds during all phases of digestion.

Table 3. Total phenolic and flavonoid content from sesame by-product beverage at different stages of gastrointestinal digestion.

Sample	Total Phenolic		Total Flavonoid	
	(mg GAE/100 g)	(mg GAE/100 mL)	(mg QE/100 g)	(mg QE/100 mL)
UD	178.19 ± 2.31 ^{b}	22.27 ± 2.31 ^{b}	61.64 ± 2.58 ^{e}	7.71 ± 0.32 ^{e}
OP	88.19 ± 6.96 ^{e}	11.02 ± 0.87 ^{e}	124.03 ± 8.57 ^{c}	15.50 ± 1.07 ^{c}
GP	153.35 ± 3.43 ^{c}	19.17 ± 0.43 ^{c}	159.15 ± 1.50 ^{b}	19.89 ± 0.19 ^{b}
IP	230.10 ± 4.76 ^{a}	28.76 ± 0.63 ^{a}	187.12 ± 3.33 ^{a}	23.39 ± 0.42 ^{a}
ID	107.86 ± 4.76 ^{d}	13.48 ± 0.60 ^{d}	86.91 ± 4.93 ^{d}	10.86 ± 0.62 ^{d}

UD: Undigested (free extract, OP: Oral phase, GP: Gastric phase, IP: Intestinal phase, ID: Indigestible fraction (available to the microbiota). The results are expressed in mg/100 g freeze-dried beverage dw and mg/100 mL of beverage. Values (means ± SD) with different letters within a column are significantly different at $p < 0.05$ among the means according to Fisher's test.

Regarding bioaccessibility, TPC values of 50%, 86%, and 129% were observed for OP, GP, and IP, respectively (Table 4). These results are contrary to those reported by Jara-Palacios et al. [50], who found higher TPC bioaccessibility in GP (128%) than in IP (75%) for white grape vinification by-products (pulp). For TFC, values of 201%, 258%, and 304% were observed for OP, GP, and IP, respectively. This behavior aligns with the findings reported by Jara-Palacios et al. [50], (30% in GP and 47% in IP) and Liu et al. [51] in Prinsepia utilis Royle fruits (30% in GP and 47% in IP). Among individual phenolics, quercetin showed the highest bioaccessibility (137%) in OP, while caffeic acid hexoside was the most bioaccessible in GP (251%) and IP (247%). These values of bioaccessibility are higher than those reported by Sęczyk et al. [11] for phenolic compounds in different types of wheat (21% to 95%) and similar to those reported by Tomé-Sánchez et al. [12] for germinated whole wheat (11% to 300%), spray-dried wheat bran (51% to 583%), and microencapsulated wheat bran (6% to 120%) subjected to gastrointestinal digestion.

Table 4. Bioaccessibility percentage of the phenolic compounds from sesame by-product beverage at different stages of gastrointestinal digestion.

Compounds	Bioaccessibility *		
	OP	GP	IP
Gallic acid	100	100	100
Protocatechuic acid	ND	ND	18
Caffeic acid hexoside	ND	251	247
Vanillic acid	ND	ND	100
Caffeic acid	100	100	ND
Caffeoylquinic acid	97	ND	317
p-Coumaric acid hexoside	100	100	100
Catechin	24	50	53
p-Coumaric acid	ND	ND	100
Chlorogenic acid	74	ND	ND
Ferulic acid hexoside	61	56	55
Cinnamic acid	49	86	174
Ferulic acid	100	100	ND
Rutin hexoside	ND	ND	ND
Kaempferol hexoside	ND	ND	ND

Table 4. *Cont.*

Compounds	Bioaccessibility *		
	OP	GP	IP
Quercetin dihexoside	ND	ND	ND
Rutin	100	100	ND
Quercetin hexoside	ND	ND	ND
Feruloilquinic acid	96	65	65
Quercetin	137	140	152
Luteolin	100	ND	100
Apigenin hexoside	ND	ND	65
Kaempferol	ND	100	100
Apigenin	100	100	100
Isorhamnetin	100	100	100
Total phenolic	50	86	129
Total flavonoid	201	258	304

OP: Oral phase, GP: Gastric phase, IP: Intestinal phase, ND: Not detected. * Bioaccessibility expressed as a percentage was calculated as the ratio of phenolic compounds released from the beverage during digestion and the initial content of phenolic compounds in the beverage before digestion, values over 100% are indicative of an increase in the bioaccessible amount of phenolic compounds in each digestion phase.

3.2. Effect of the Gastrointestinal Digestion Process on Antioxidant, Hypoglycemic, and Antidiabetic Properties

In Figure 1, the antioxidant activity (AAOX) values measured by ABTS (Figure 1A,B) and DPPH (Figure 1C,D) for the beverage before and after gastrointestinal digestion are shown. The AAOX by ABTS for UD was 1913 µmol TE/100 g, higher than the value reported by Lucini Mas et al. [5] for a sesame-based cookie (1080 µmol TE/100 g). Five hundred milliliters of the beverage provide 1195 µmol TE of AAOX, equivalent to consuming a nixtamalized blue corn tortilla (1066 µmol TE/30 g) [52]. During gastrointestinal digestion, the AAOX by ABTS experienced a significant decrease ($p < 0.05$) in OP, followed by an increase in GP and IP. This behavior is similar to that of TPC. For DPPH, the AAOX was 1388 µmol TE/100 g, values higher than those observed by Chen et al. [8] for six varieties of sesame (650 to 800 µmol TE/100 g). On the other hand, 500 mL of the beverage contains 865 µmol TE for AAOX, values within the range reported by Mainente et al. [53] for 24 types of cider (390 to 973.5 µmol TE/500 mL). During the gastrointestinal digestion process, a gradual increase was observed, reaching an AAOX of 2191.70 µmol TE/100 g in IP, increasing by around 40%.

In Figure 2, the IC_{50} values (µg/mL) for the inhibition of α-amylase (Figure 2A) and α-glucosidase (Figure 2B) are shown. The ability of phenolic compounds to inhibit the enzyme α-amylase (AA) increased at the beginning of gastrointestinal digestion, remaining statistically unchanged throughout the three phases of digestion (IC_{50}: 836.13 to 859.15 µg/mL). These values are higher than those reported by Kartelias et al. [54] for kombucha tea supplemented with different herbs, spices, and fruits (158 to 243 µg/mL).

The same authors also evaluated the inhibition of α-glucosidase (AG), reporting better IC_{50} values (0.85 to 22.24 µg/mL) than those observed in this study, where an IC_{50} of 219.78 µg/mL was obtained in UD. These values improved during gastrointestinal digestion, with IC_{50} values of 202.60, 172.29, and 125.69 µg/mL for OP, GP, and IP, respectively. This behavior is similar to that observed in fruits of Rhus chinensis by Fu et al. [55], who reported an increase in AG enzyme inhibition as digestion progressed.

The antidiabetic activity of the beverage was determined by the inhibition of the DPP-IV enzyme (Figure 3). In this study, a similar behavior was observed among the inhibition of DPP-IV, α-glucosidase, and TFC. In UD, an IC_{50} value of 93.36 µg/mL was obtained, a value similar to that reported for the phytochemical extract of a beverage made from extruded sesame by-products (110 µg/mL) [6] and for fruits of *Prinsepia utilis* Royle (150 µg/mL) [51]. During gastrointestinal digestion, antidiabetic activity increased (82.02,

70.24, and 52.97 µg/mL for OG, GP, and IP, respectively). This is similar to what was reported by Liu et al. [51] for gastrointestinally digested fruits of *Prinsepia utilis* Royle.

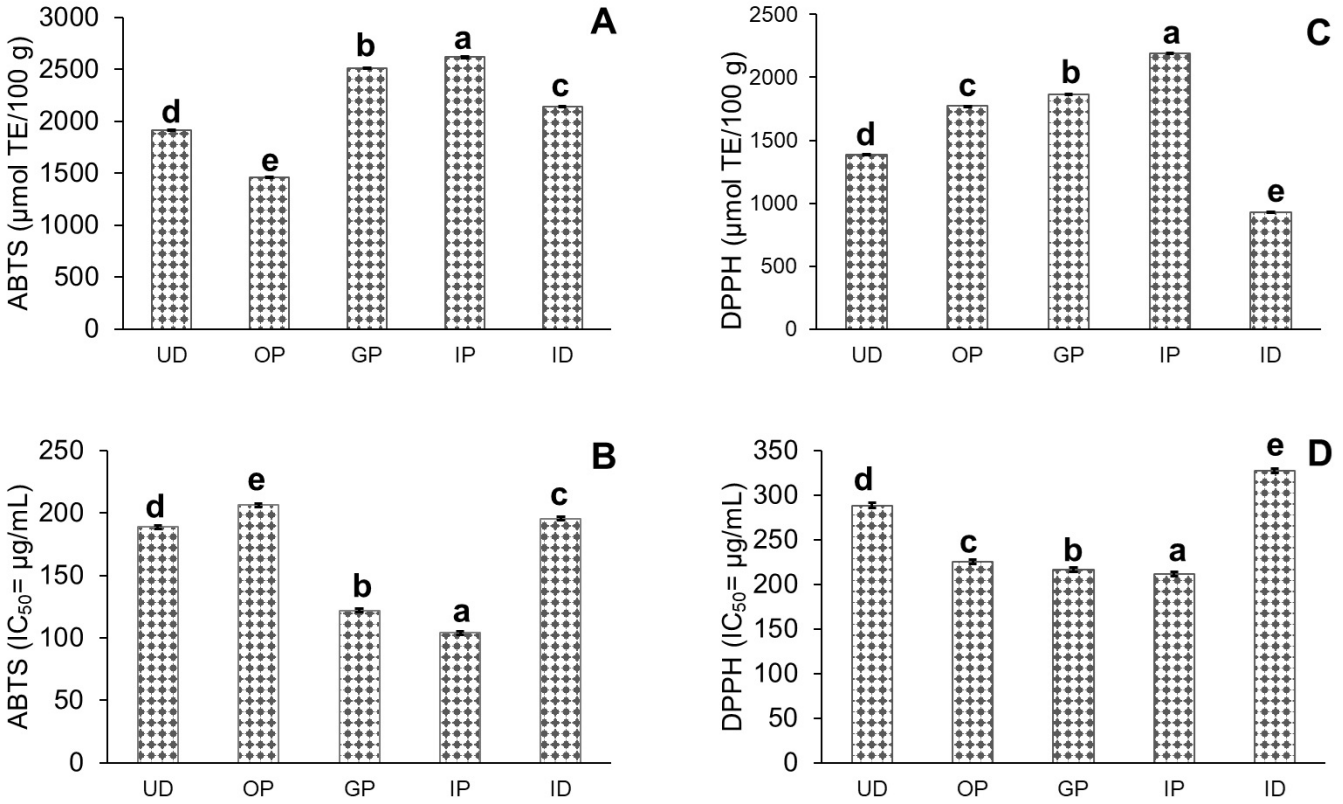

Figure 1. Effect of in vitro digestion on the antioxidant activity of the beverage of by-product sesame seeds. The results of ABTS are expressed in µmol TE/100 g (**A**) and IC_{50} (**B**). The results of DPPH are expressed in µmol TE/100 g (**C**) and IC_{50} (**D**). UD: Undigested (free extract), OP: Oral phase, GP: Gastric phase, IP: Intestinal phase, ID: Indigestible fraction (available to the microbiota). Different letters in the same graph indicate differences ($p < 0.05$) among the means according to Fisher's test.

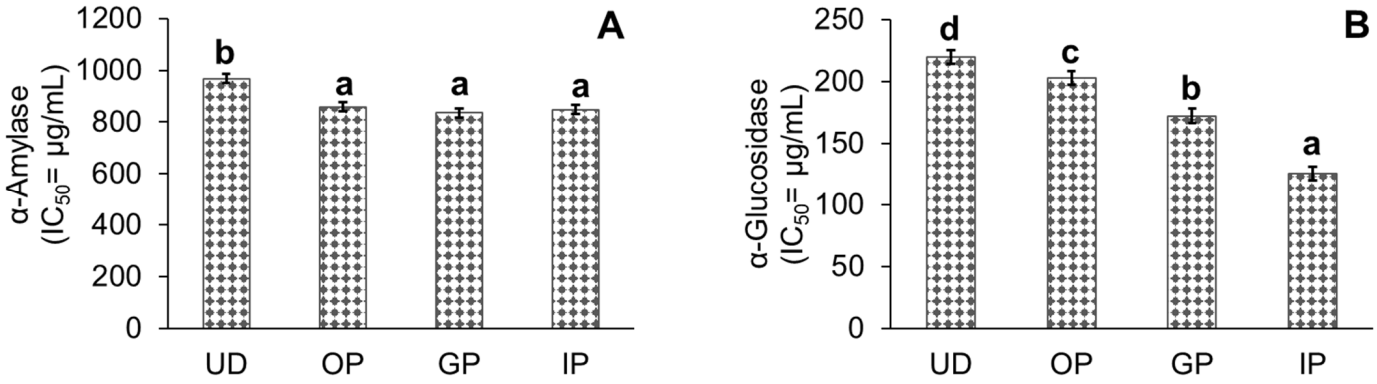

Figure 2. Effect of in vitro digestion on the hypoglycemic activity of the beverage of by-product sesame seeds. Inhibition of α-amylase (**A**) and Inhibition of α-glucosidase (**B**). UD: Undigested (free extract), OP: Oral phase, GP: Gastric phase, IP: Intestinal phase. Different letters in the same graph indicate differences ($p < 0.05$) among the means according to Fisher's test.

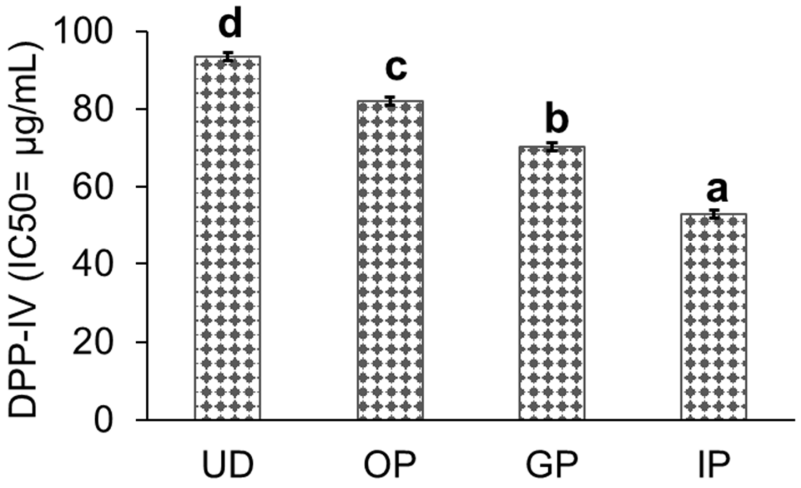

Figure 3. Effect of in vitro digestion on the inhibition of dipeptidyl-Peptidase IV (DPP-IV) of the beverage of by-product sesame seeds. UD: Undigested (free extract), OP: Oral phase, GP: Gastric phase, IP: Intestinal phase. Different letters in the graph indicate differences ($p < 0.05$) among the means according to Fisher's test.

3.3. Relationship Between the Phenolic Compound Profile and the Evaluated Biological Activities

To establish the relationships and similarities between the identified phytochemicals and the evaluated activities, a Pearson correlation analysis (Figure 4) and a principal component analysis (PCA) (Figure 5) were performed.

The positive and highly linear correlations between quercetin and the inhibition of DPPH (r = 0.94; $p > 0.01$), AA (r = 0.98; $p > 0.05$), AG (r = 0.95; $p > 0.05$), and DPP-IV (r = 0.96; $p > 0.01$) suggest that this flavonoid is responsible for these activities. This is also reflected in the PCA, where the vectors are oriented in the same direction. In the case of AAOX, a positive correlation with ABTS vs. TPC (r = 0.79; $p > 0.05$) was observed, while DPPH showed positive correlations with TFC (r = 0.082; $p > 0.05$) and apigenin (r = 0.89; $p > 0.05$). The TPC also showed a positive correlation with DPP-IV (r = 0.72; $p > 0.05$) and α-glucosidase inhibition (r = 0.71; $p > 0.05$). These correlations have been previously reported by various authors [6,20,24].

In addition to positive correlations, negative correlations were observed between bioactive activities and the evaluated compounds. Inhibition of the DPP-IV enzyme showed a negative correlation with feruloylquinic acid (r = −0.096; $p > 0.05$), kaempferol (r = −0.89; $p > 0.05$), and catechin (r = −0.87; $p > 0.05$). Inhibition of α-glucosidases showed a negative correlation with feruloylquinic acid (r = −0.95; $p > 0.05$), kaempferol hexoside (r = −0.89; $p > 0.05$), and ferulic acid (r = −0.80; $p > 0.01$).

The inhibition of α-amylase showed a negative correlation with feruloylquinic acid (r = −0.90; $p > 0.05$), kaempferol hexoside (r = −0.99; $p > 0.001$), catechin (r = −0.92; $p > 0.05$), and p-coumaric acid (r = −0.99; $p > 0.05$). AAOX by DPPH correlated negatively with feruloylquinic acid (r = −0.94; $p > 0.05$), rutin hexoside (r = −0.88; $p > 0.05$), and catechin (r = −0.88; $p > 0.05$).

The PCA showed the differences and similarities between UD, OP, GP, IP, and ID (Figure 5). Together, the first two components explain approximately 73% of the observed variation in the data. The first component (PC1) accounted for 46.6%, while the second component (PC2) accounted for 26.14%. Regarding the distribution of parameters, 22 of the 32 analyzed characteristics were located in the positive quadrant of PC1, including ABTS, DPPH, AA, AG, and DPP-IV. The PCA revealed a separation of the samples into three main clusters: one for the digested samples (purple oval), one for the sample before digestion (green oval), and another for the sample available for the intestinal microbiota (blue oval). IP was positioned in the positive quadrant of the two principal components, indicating that the in vitro gastrointestinal digestion process had a significant positive impact on the samples.

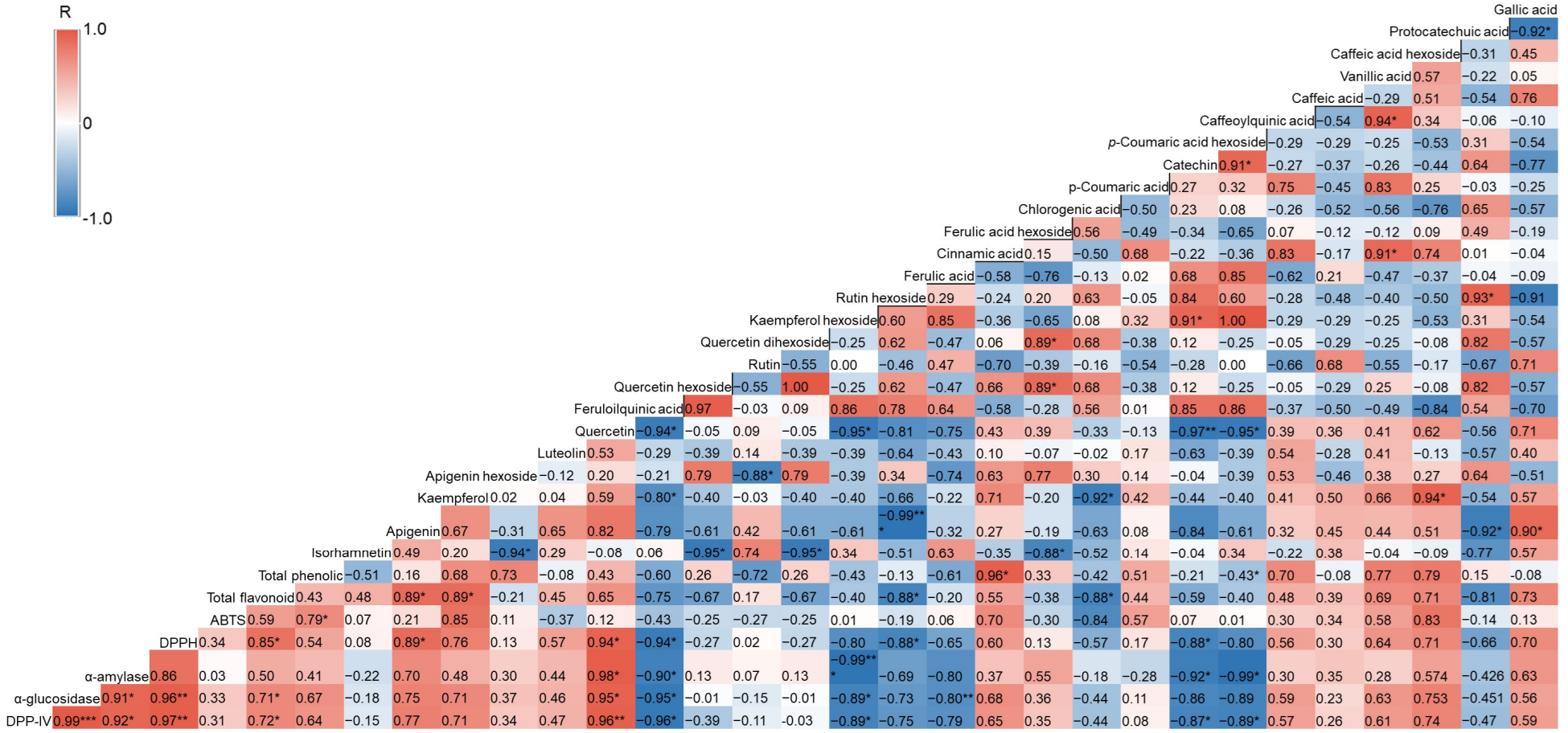

Figure 4. Correlation analysis between identified phytochemicals and evaluated biological activities. *** Correlation is significant at the 0.001 level, ** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level.

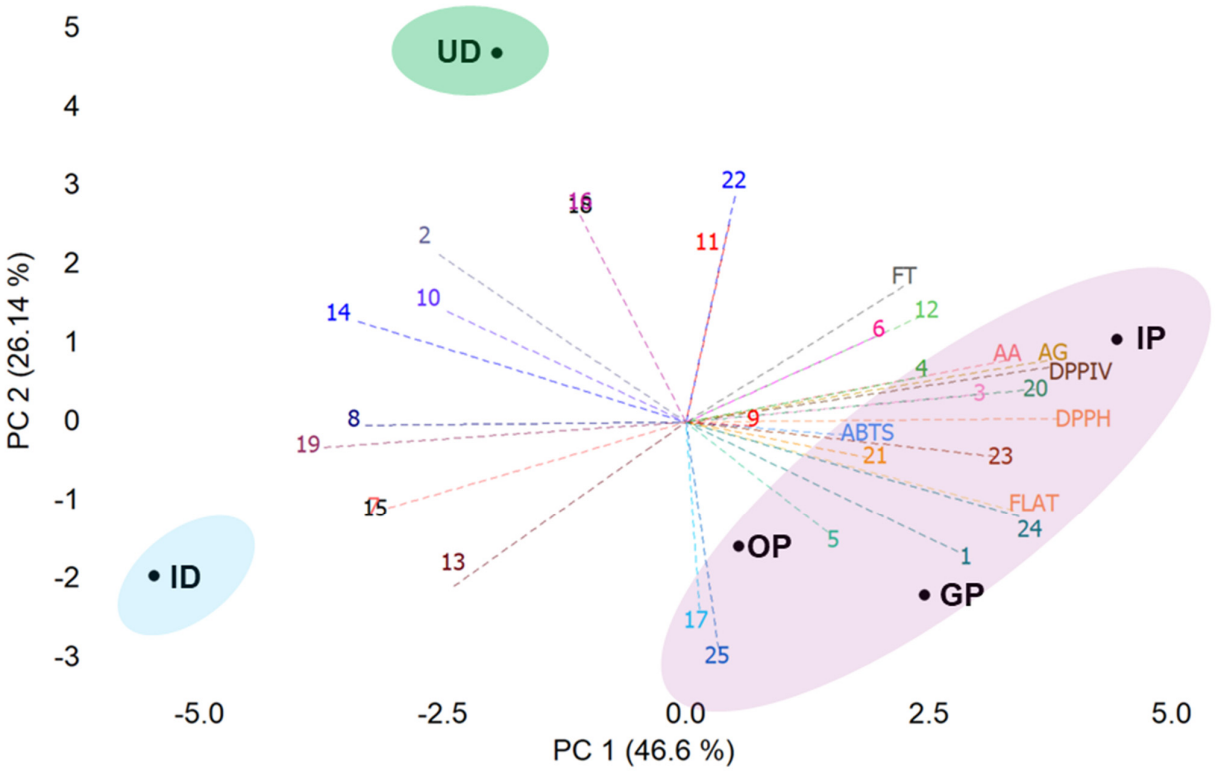

Figure 5. Principal component analysis describing the relationship among different phytochemicals and evaluated biological activities. 1: Gallic acid, 2: Protocatechuic acid, 3: Caffeic acid hexoside, 4: Vanillic acid, 5: Caffeic acid, 6: Caffeoylquinic acid, 7: p-Coumaric acid hexoside, 8: Catechin, 9: p-Coumaric acid, 10: Chlorogenic acid, 11: Ferulic acid hexoside, 12: Cinnamic acid, 13: Ferulic acid, 14: Rutin hexoside, 15: Kaempferol hexoside, 16: Quercetin dihexoside, 17: Rutin, 18: Quercetin hexoside, 19: Feruloilquinic acid, 20: Quercetin, 21: Luteolin, 22: Apigenin hexoside, 23: Kaempferol, 24: Apigenin, 25: Isorhamnetin, FT: Total phenolic, FLAT: Total flavonoid, AA: α-Amylase, α-glucosidase, DPPIV: Dipeptidyl peptidase IV, UD: Undigested (free extract), OP: Oral phase, GP: Gastric phase, IP: Intestinal phase.

4. Conclusions

This study demonstrates the significant impact of gastrointestinal digestion on the compound profile and biological activities of a beverage based on sesame by-products. Notably, compounds like quercetin, isorhamnetin, and caffeic acid hexoside showed strong bioaccessibility after digestion and are linked to enhanced antioxidant, hypoglycemic, and antidiabetic activities. These findings suggest that the digestion process not only improves the bioaccessibility of these bioactive compounds but also potentially enhances their health benefits. Compounds like quercetin showed strong correlations with these activities, suggesting their critical role in mediating health benefits. The principal component analysis highlighted distinct clusters among the samples, reflecting the changes brought about by digestion. Overall, the findings support the hypothesis that gastrointestinal digestion transforms and optimizes phenolic compounds, potentially leading to enhanced bioactivity. Although various studies have evaluated the antioxidant activity and bioaccessibility of phenolic compounds in sesame-based products, this research is the first report reporting the antidiabetic activity of the beverage based on sesame by-products after the in vitro gastrointestinal digestion process. This research contributes valuable insights into the bioaccessibility and bioactivity of phytochemicals, providing a foundation for further exploration of their health benefits and applications in functional foods. Future research, including in vivo studies and clinical trials, is recommended to validate these findings and further explore how this enhanced bioactivity can be harnessed in functional food formulations, contributing to the development of innovative products for health improvement.

Author Contributions: Conceptualization, M.F.Q.-S. and J.V.F.-M.; methodology, J.C.-C., M.F.Q.-S., L.A.O.-G., R.J.E.-M. and J.M.A.-C.; software, I.D.-P., R.V.-B. and O.D.A.-L.; formal analysis, J.C.-C. and M.F.Q.-S.; investigation, J.C.-C., M.F.Q.-S. and J.V.F.-M.; resources, R.V.-B., I.A.R.-E. and J.X.K.P.-S.; data curation, J.C-C., M.E.V.-O. and F.S.-L.; writing—original draft preparation, J.C.-C.; writing—review and editing, M.F.Q.-S.; visualization, M.F.Q.-S. and J.V.F.-M.; supervision, M.F.Q.-S. and J.V.F.-M.; funding acquisition, M.F.Q.-S., M.E.V.-O., J.V.F.-M. and J.X.K.P.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The datasets generated and/or analyzed during this study are available from the corresponding authors upon reasonable request.

Acknowledgments: The authors are grateful to the Consejo Nacional de Humanidades, Ciencia y Tecnología for the postdoctoral fellowship awarded to M.F.Q.-S.

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

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