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Effect of Lactic Acid Bacteria Fermentation on the Polar Compounds Content with Antioxidant and Antidiabetic Activity of Avocado Seed Extracts

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Abstract: Avocado seeds, a common waste in the avocado processing industry, have been found to have several bioactivities, such as anticancer, antimicrobial, hypocholesterolemic, antioxidant, and antidiabetic activities, among others. Nowadays, this wastage is causing an environmental problem, so the use of new technologies to take advantage of it is a novel field of research. In this study, the submerged fermentation by lactic acid bacteria was used as a novel tool for improving the bioactive compound extraction from avocado seeds. Avocado seeds were fermented by different strains, their polar compounds were identified and quantified by HPLC-ESI-TOF-MS, the antioxidant activity was measured by DPPH and FRAP assays, and the antidiabetic activity was analyzed by the alpha-amylase assay. A total of 32 polar compounds were identified, with 13 of them being described in avocado seeds for the first time. Avocado seeds fermented by *Pentosaceus acidilactici* showed the highest sum of polar compounds (6279.63 ± 67.74 μg/g d.w.), and by extension, of hydroxytyrosol glucoside (2989.76 ± 3.64 μg/g d.w.). *Lactiplantibacillus plantarum* CECT 9567 showed the highest antioxidant activity measured by both DPPH and FRAP assays (6294.67 ± 19.44 and 6846.91 ± 2.13 μg TE/g d.w., respectively). Furthermore, *Lactiplantibacillus plantarum* CECT 748T had the highest antidiabetic activity (52.15% ± 0.67% inhibition of alpha-amylase activity), attributable to the polyphenols. According to the results, submerged fermentation by lactic acid bacteria led to an interesting increase of the polar compounds' extractability of avocado seeds, consequently improving the bioactivities of the extracts, which could then be used for food nutraceutical or cosmetic purposes.

Keywords: avocado seed waste; HPLC-ESI-TOF-MS; alpha-amylase; submerged fermentation; phenolic compounds

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1. Introduction

Avocado (*Persea americana* Mill., *Lauraceae*) is a fruit dispersed worldwide in tropical and subtropical regions. There are numerous varieties of avocado according to the growth climate, with Hass being the most well-known and marketed [1]. The avocado processing industry, in the production of guacamole or essential oils, generates a solid waste residue that represents 21–30% of the fruit, consisting of seeds, peels, and exhausted pulp [2]. Usually, those residues are discarded as waste, representing an environmental problem, but they are also a prospective source of bioactive compounds [1]. The avocado seed is composed of the endocarp and the kernel. It has been previously reported to have anticancer activity in oral, breast, and liver cells, attributed to its triterpenoid compounds [3,4].

Additionally, antimicrobial activity of the avocado seed extract against Gram-positive and Gram-negative bacteria has been demonstrated for *Salmonella enteritidis*, *Citrobacter freudii*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Escherichia coli*, *P. aeruginosa*, *Mycobacterium intracellulare,* and *Listeria monocytogenes* [5–7]. Furthermore, antifungal activity against *Candida* spp., *Cryptococcus neoformans*, and *Malassezia pachydermatis,* and larvicidal activity against *Artemia salina* and *Aedes aegypti,* were reported [8]. Its hypocholesterolemic activity was also demonstrated in mice, reducing cholesterol and low-density lipoprotein. In addition, the antioxidant [9] and antidiabetic activity [10] of avocado seed has been previously confirmed.

Lactic acid bacteria (LAB) are a heterogeneous group of Gram-positive food-grade microorganisms, historically used in food preservation. Usually, they are mostly reported to ferment carbohydrates as their main source of carbon to produce lactic acid at the industrial level. They can produce a variety of products, including bacteriocins, vitamins, amines, short-chain fatty acids, and exopolysaccharides, during metabolism [11]. Moreover, there are previous data that report increases in antioxidant and phenolic compounds in food submitted to fermentation by LAB, such as apple juice, mulberry juice, soy milk, and wheat dough. LAB enzymes have the capability to break the cell walls, improving the realization of phenolic acids and flavonoids or converting them into simple forms, in both cases improving their bioavailability [12]. Fermentation with acid lactic bacteria is a low-cost and sustainable technology for maintaining or improving the nutritional quality of food and its sensory properties. In fruits and their byproducts, fermentation can occur spontaneously via the lactic acid microbiota naturally present under adequate conditions. Otherwise, it can also be controlled using lactic acid bacteria starter cultures, aiming at different specific objectives, such as improving the digestibility. The fermentation of avocado seeds by LAB could lead to several benefits, such as improving their bioactivities, a field of research that has not been deeply studied. In avocado leaves, the fermentation by LAB has led to satisfactory results [13]. Recently, Rozan et al. reported a reduction in the anti-nutrients tannins, oxalates, and alkaloids contents of 80.76%, 89.95%, and 70%, respectively, by fermenting avocado seed with LAB [14].

Based on the above and the results obtained in previous research [13], this study aimed to evaluate the effect of submerged fermentation with different LAB on the polar profile of avocado seeds, evaluating the antioxidant and the antidiabetic activities, in comparison to an unfermented avocado seed extract.

2. Materials and Methods

2.1. Chemicals and Samples

Most of the reagents, analytical standards, and the pancreatic alpha-amylase were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were previously reported [13]. Avocado seed byproduct samples of the variety Hass were provided by a local company after the guacamole production, in April 2022. The seeds were freeze-dried, sieved to a 100 µm particle size, and frozen at −18 °C until the analyses.

2.2. Fermentation of Avocado Seeds by Lactic Acid Bacteria

The lactic acid bacteria (LAB) strains used were: *Pediococcus acidilactici* CECT 5765T, *Pediococcus pentosaceus* CECT 4695T, *Leuconostoc mesenteroides* subsp. *mesenteroides* CECT 219T, *Levilactobacillus brevis* CECT 5354, *Lactiplantibacillus plantarum* subsp. *plantarum* CECT 748T, and *Lactiplantibacillus plantarum* CECT 9567 (formerly strain C4), and they were obtained and grown as previously reported [13]. Fermentations were carried out according to the methodology described by De Montijo-Prieto et al. [13]. Avocado seeds were submerged in sterile water, previously sterilized at 90 °C, to eliminate the microorganisms previously present. After heat treatment, aliquots were taken to determine viable microorganisms on MacConkey agar (selective medium for enterobacteria, 37 °C), Tryptic soy agar (TSA, enriched medium for bacteria, 37 °C), and Sabouraud agar (medium for fungi, 25 °C). After incubations of the media, the number of viable microorganisms was confirmed to be below

the detection limit of the tests. The quantification of the phenolic compounds was carried out at selected incubation times. Thus, *P. acidilactici* CECT 5765T, *P. pentosaceus* CECT 923, *Le. brevis* CECT 5354, and *La. plantarum* CECT 9567 were analyzed at 24 h of incubation, and *Leu. mesenteroides* CECT 219T and *La. plantarum* CECT 748T at 48 h of incubation.

2.3. Determination of Polar Compounds by HPLC-ESI-TOF-MS

The extraction and analysis of polar compounds in the fermented and non-fermented avocado seeds were carried out following the procedure previously reported by De Montijo-Prieto et al. [13]. Analyses were performed in triplicate. The identification of the phenolic compounds was performed according to the literature. For ensuring the mass accuracy, the tolerances chosen had a score higher than 90% and an error lower than 5 ppm. To quantify the phenolic compounds identified in the avocado seed extracts, calibration curves were used for vanillic acid ($y = 4.1347x + 393.22$; $R^2 = 0.982$), chlorogenic acid ($y = 101.17x - 683.25$; $R^2 = 0.9933$), ferulic acid ($y = 10.502x + 353.77$; $R^2 = 0.9964$), quercetin ($y = 288.76x + 2462.1$; $R^2 = 0.9673$), catechin ($y = 315.87x - 415.59$; $R^2 = 0.9950$), and rutin ($y = 860.4x - 256.21$; $R^2 = 0.9986$). The results are expressed as μg/g d.w.

2.4. Determination of Antioxidant Activity: DPPH and FRAP Assays

Avocado seeds fermented by different LAB were analyzed with DPPH and FRAP assays to determine the antioxidant activity via the procedures described in previous research [15]. The analyses were performed in triplicate and the results are expressed in mg of Trolox equivalents (TE)/g of dry weight (d.w.).

2.5. Determination of Antidiabetic Activity: Alpha-Amylase Assay

The antidiabetic activity of the extracts was performed in vitro by the alpha-amylase assay according to previous research [16,17]. Briefly, 100 μL of fermented or non-fermented methanolic extract of avocado seed (20 mg/mL) was added to 100 μL of porcine pancreatic alpha-amylase (≥2 μd/mL) and pre-incubated during 5 min at 37 °C. Then, 200 μL of 0.2% starch solution in 20 mM of sodium phosphate buffer (pH 6.7) was added and incubated again for 5 min at 37 °C. The reaction was stopped with 1 mL of dinitrosalicylic acid (DNS) color reagent and kept in a boiling bath for 5 min. Once the tubes had cooled on ice until reaching room temperature, they were added to 6 mL of distilled water. The absorbance was measured at 540 nm in a UV-visible spectrophotometer (Spectrophotometer 300 Array, UV-Vis, single-beam, Shimadzu, Duisburg, Germany). The pharmacological inhibitor, acarbose, was included as a positive control at a concentration of 20 mg/mL. Analyses were performed in triplicate. The percentage of inhibition of the alpha-amylase activity was calculated as follows (Equation (1)):

$$\%\ Inhibition = \left(100 - \frac{Absorbance\ control - Absorbance\ sample}{Absorbance\ control}\right) \times 100 \quad (1)$$

2.6. Data Processing

MassLynx 4.1 software (Waters Corporation, Milford, MA, USA) was used for elaborating the HPLC-ESI-TOF-MS results. The Statistica 7.0 package (StatSoft, Tulsa, OK, USA) was used for obtaining statistical differences (Tukey's test) by one-way ANOVA, and Pearson' correlations were performed. The rest of the statistical analyses were performed using MetaboAnalyst 5.0 (Xia Lab, McGill, Montréal, QC, Canada).

3. Results and Discussion

3.1. Identification of Polar Compounds in Fermented Avocado Seeds by HPLC-ESI-TOF-MS

The avocado seeds fermented by the selected strains were analyzed by HPLC-ESI-TOF-MS, and a total of 32 polar compounds were identified. Among them were six phenolic acids, eighteen flavonoids, five terpenoid derivatives, one tyrosol derivative, and other organic compounds. They are presented in Table 1, along with their experimental and

calculated m/z, time (min), error (ppm), score (%), molecular formula, m/z main in source fragments, and the tentative name for each compound. The peaks presented in Table 1 correspond to the numbers shown in Figure 1, which is a representative chromatogram of a fermented avocado seed. To the best of our knowledge, 13 polar compounds were identified here for the first time in avocado seeds.

Table 1. Polar compounds identified by HPLC-ESI-TOF-MS in fermented and non-fermented avocado seeds.

No.	Time (min)	*m/z* Experimental	*m/z* Calculated	Error (ppm)	Score (%)	Molecular Formula	*m/z* in Source Fragments	Compound
1	0.26	343.0304	343.0301	0.9	94.75	$C_{13}H_{12}O_{11}$	201.0226	5-*O*-Galloyl-1,4-galactarolactone
2	0.33	211.0813	211.0823	−4.7	91.29	$C_7H_{16}O_7$	101.0223 89.0238; 59.0111	Perseitol
3	0.37	191.0552	191.0556	−2.1	91.72	$C_7H_{12}O_6$	-	Quinic acid isomer a
4	0.44	191.0551	191.0556	−2.6	98.94	$C_7H_{12}O_6$	-	Quinic acid isomer b
5	1.48	315.1071	315.108	−2.9	99.93	$C_{14}H_{20}O_8$	153.0197; 135.0442	Hydroxytyrosol glucoside
6	2.57	353.0858	353.0873	−4.2	99.99	$C_{16}H_{18}O_9$	179.0302; 191.0532	Caffeoylquinic acid isomer a
7	3.49	337.0914	337.0923	−2.7	99.67	$C_{16}H_{18}O_8$	163.0368; 191.0536	Coumaroylquinic acid isomer a
8	3.89	337.0909	337.0923	−4.2	99.99	$C_{16}H_{18}O_8$	163.0363; 191.0515	Coumaroylquinic acid isomer b
9	4.12	443.1906	443.1917	−2.5	99.76	$C_{21}H_{32}O_{10}$	113.0245; 101.0224	Penstemide
10	4.58	353.0868	353.0873	−1.4	99.99	$C_{16}H_{18}O_9$	179.0334; 191.0531	Caffeoylquinic acid isomer b
11	4.90	337.0914	337.0923	−2.7	91.94	$C_{16}H_{17}O_8$	163.0382; 191.0539	Coumaroylquinic acid isomer c
12	5.02	387.1641	387.1655	−3.6	99.95	$C_{18}H_{28}O_9$	279.0305; 207.0988; 163.1096	Tuberonic acid glucoside
13	5.22	289.0698	289.0712	−4.8	99.63	$C_{15}H_{14}O_6$	137.0219	Catechin
14	5.34	449.1082	449.1084	−0.4	100	$C_{21}H_{22}O_{11}$	287.0541	Eriodictyol 7-*O*-glucoside
15	5.57	337.0915	337.0923	−2.4	99.85	$C_{16}H_{18}O_8$	163.0378; 191.0517	Coumaroylquinic acid isomer d
16	5.62	577.1329	577.1252	13.3	90.86	$C_{19}H_{30}O_{20}$	289.0682	Procyanidin dimer
17	5.77	415.1589	415.1604	−3.6	99.83	$C_{19}H_{28}O_{10}$	339 121	2-Phenylethyl beta-primeveroside
18	5.93	463.2179	463.2179	0.0	100	$C_{21}H_{36}O_{11}$	285.0396; 125.0228	Luteolin/kaempferol derivative isomer a
19	6.85	463.2164	463.2179	−3.2	99.71	$C_{21}H_{36}O_{11}$	285.0390; 125.0202	Luteolin/kaempferol derivative isomer b
20	6.98	1151.2507	1151.2575	−5.9	94.31	$C_{46}H_{56}O_{34}$	863.1739; 289.0672	Procyanidin tretamer
21	7.10	441.1741	441.1761	−4.5	99.97	$C_{21}H_{30}O_{10}$	330.1303	Hydroxyabscisic acid beta-D-glucoside
22	7.31	863.183	863.1823	0.8	90.3	$C_{45}H_{36}O_{18}$	711.1354; 573.1021; 411.068; 289.0684	Procyanidin trimer
23	7.79	625.1409	625.1405	0.6	97.84	$C_{27}H_{30}O_{17}$	300.0247	Quercetin-diglucoside isomer a
24	7.92	625.1389	625.1405	−2.6	96.64	$C_{27}H_{30}O_{17}$	300.0242	Quercetin-diglucoside isomer b
25	8.30	463.2182	463.2179	0.6	100	$C_{21}H_{36}O_{11}$	285.0363; 125.0241	Luteolin/kaempferol derivative isomer c
26	9.13	439.1009	439.1029	−4.6	98.94	$C_{23}H_{20}O_9$	287.0497	Triacetoxy-methoxy-methylflavone isomer a
27	9.25	439.1011	439.1029	−4.1	98.07	$C_{23}H_{20}O_9$	287.0468	Triacetoxy-methoxy-methylflavone isomer b
28	10.07	439.1037	439.1029	1.8	100	$C_{23}H_{20}O_9$	287.0533	Triacetoxy-methoxy-methylflavone isomer c
29	10.66	461.2382	461.2387	−1.1	100	$C_{22}H_{38}O_{10}$	285.0348	Luteolin/kaempferol derivative II isomer a

Table 1. *Cont.*

No.	Time (min)	*m/z* Experimental	*m/z* Calculated	Error (ppm)	Score (%)	Molecular Formula	*m/z* in Source Fragments	Compound
30	10.70	461.2405	461.2387	3.9	100	$C_{22}H_{38}O_{10}$	285.0439	Luteolin/kaempferol derivative II isomer b
31	11.19	461.2397	461.2387	2.2	100	$C_{22}H_{38}O_{10}$	285.0234	Luteolin/kaempferol derivative II isomer c
32	11.32	461.2387	461.2387	0.0	100	$C_{22}H_{38}O_{10}$	285.0355	Luteolin/kaempferol derivative II isomer d

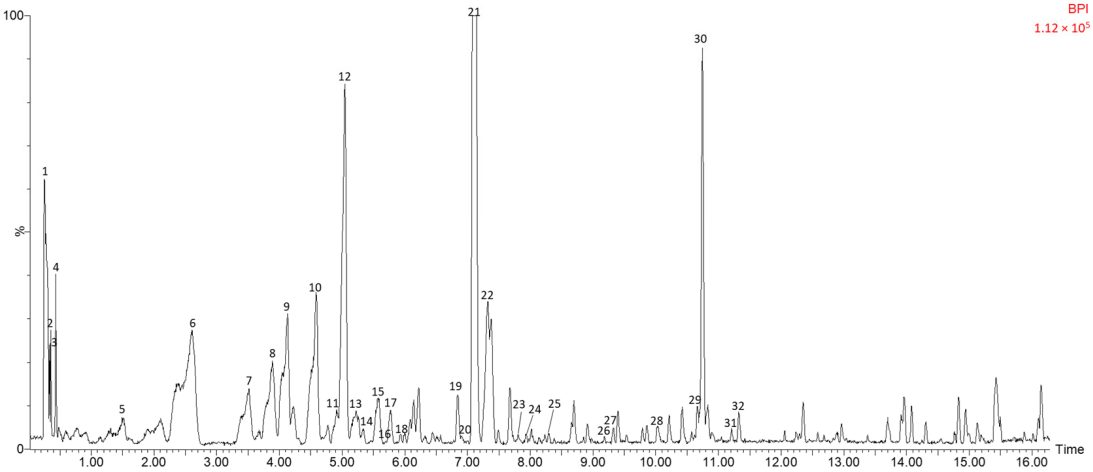

Figure 1. Base peak chromatogram of the polar compounds identified in the fermented avocado seed. Numbers 1–36 correspond to the peaks in Table 2.

Table 2. Log CFU/mL and pH of lactic acid bacteria in avocado seed expressed as average ± standard deviation.

LAB	0 h	24 h		48 h	
	Log_{10} CFU/mL	Log_{10} CFU/mL	pH	Log_{10} CFU/mL	pH
P. acidilactici CECT 5765 T	7.96 ± 0.02	7.14 ± 0.02	4.9	4.17 ± 0.03	4.9
P. pentosaceus CECT 4695 T	7.57 ± 0.01	7.37 ± 0.06	4.9	4.10 ± 0.09	4.8
Leu. mesenteroides CECT 219 T	7.14 ± 0.01	7.04 ± 0.04	5.2	4.03 ± 0.05	5.2
Le. brevis CECT 5354	7.05 ± 0.03	7.01 ± 0.06	5.0	4.04 ± 0.04	4.9
La. plantarum CECT 748 T	7.95 ± 0.02	6.72 ± 0.02	4.9	3.88 ± 0.09	4.9
La. plantarum CECT 9567	7.93 ± 0.06	7.31 ± 0.02	4.6	6.71 ± 0.03	4.5

Corresponding to peaks 3 and 4, two isomers of quinic acid were detected, named as isomers a and b, respectively. Some quinic acid derivatives were detected conjugated with other phenolic acids. Caffeoylquinic acid isomers a and b were detected at 2.57 and 4.58 min (peaks 6 and 10) with the *m/z* in source fragments 179 $(C_9H_7O_4)^-$ of caffeic acid and 191 $(C_7H_{11}O_6)^-$ of quinic acid, respectively. Besides, four isomers of coumaroylquinic acid, named as a, b, c, and d (peaks 7, 8, 11, and 15, respectively), were identified according to its *m/z* in source fragments 163 $(C_9H_7O_3)^-$ and 191 $(C_7H_{11}O_6)^-$, belonging to coumaric and quinic acids, respectively [18].

With the *m/z* 625, two quercetin-diglucoside isomers named a and b were detected according to its *m/z* in source fragment 300 $(C_{15}H_8O_7)^{2-}$ (peaks 23 and 24), that corresponds to quercetin-7-olate, a conjugate base of quercetin arising from selective deprotonation of the 7-hydroxy group [18].

The organic compound perseitol, also known as D-glycero-D-galacto-heptitol, a sugar alcohol, was identified in avocado seeds at 0.33 min (peak 2) according to its m/z in source fragments 59 $(C_2H_3O_2)^-$, 89 $(C_3H_5O_3)^-$, and 101 $(C_4H_5O_3)^-$ [18].

The iridoid penstemide was identified corresponding to peak 9 with the m/z of 443 and the fragmentation according to López-Cobo et al. [18].

A phenethyl alcohol derivative, hydroxytyrosol glucoside (peak 5), was also found in fermented avocado seeds with the molecular formula $C_{14}H_{20}O_8$, the m/z 315, and the m/z in source fragment 153 that corresponds to hydroxytyrosol [18].

At 7.20 min, the compound found with the m/z 441 was named as hydroxyabscisic acid beta-D-glucoside, a derivative of abscisic acid, an isoprenoid plant hormone [18].

Catechin, procyanidin dimer, trimer, and tetramer were detected at 5.22, 5.62, 7.31, and 6.98, respectively, in accordance with other authors [18].

In addition, other compounds were identified here for the first time in avocado seed. With the m/z 343, peak 1 was named as the galloyl ester 5-*O*-galloyl-1,4-galactarolactone according to the PUBCHEM database (PubChem CID: 59235421). The same molecule was also identified, but it was not quantified in *Phyllanthus emblica* L. fruit [19].

Tuberonic acid beta-D-glucoside (peak 12 at 5.02 min) was previously described by some authors in rice [20] and leaves of tobacco [21].

Corresponding to peak 14, a new flavonoid was detected with the m/z 449 and the predicted formula $C_{21}H_{22}O_{11}$. It was named as eriodictyol 7-*O*-glucoside, in concordance with its main m/z in source fragment 287 $(C_{15}H_{11}O_6)^-$, that is eriodyctiol and the phenol explorer database [22]. This compound has been previously identified and quantified in other food, such as almonds [23].

With the m/z 439, three flavonoid isomers were identified and called triacetoxy-methoxy-methylflavone isomer a, b, and c, according to the PUBCHEM database (PubChem CID: 129815511). Its main m/z in source fragment 287 $(C_{15}H_{11}O_6)^-$ suggests that it could also be an eriodictyol derivative, but it has not been previously described.

In addition, other flavonoid derivatives were identified. Three isomers (a, b, and c) of a luteolin/kaempferol derivative were identified according to m/z 463 (peaks 18, 19, and 25) and the m/z in source fragments 285 $(C_{15}H_9O_6)^-$ and 125 $(C_6H_9O_5)^-$, that correspond to luteolin and galloyl glucose with the loss of the carboxyl group, compounds that were previously identified and quantified in avocado seed [24]. Another luteolin derivative (II) was detected at 10.66, 10.70, 11.19, and 11.32 min, named as isomers a, b, c, and d, respectively. Their m/z in source fragment 285 $(C_{15}H_9O_6)^-$ is in agreement as they correspond to luteolin or kaempferol [24].

3.2. Lactic Acid Bacteria Growth in Avocado Seed

Avocado seed mixtures were supplemented with glucose and yeast extract to encourage the growth of LAB strains. As shown in Table 2, the number of viable bacteria in the avocado seed gradually dropped during incubations, until concentrations between 3.88 ± 0.09 and 4.17 ± 0.03 log CFU/mL at 48 h of incubation. Nevertheless, *La. plantarum* CECT 9567 slightly decreased from 7.31 ± 0.02 to 6.71 ± 0.03 log CFU/mL at 24 and 48 h of incubation, respectively. As can be seen in Table 2, all LAB had a negative significant ($p < 0.05$) correlation between the CFU/mL and the time (r = −0.9964). Therefore, when increasing the time, a lower CFU/mL was found in all cases. Despite being part of the LAB metabolism, phenolic compounds are sometimes toxic for them, causing decreases in the growth. The specific pathway of polyphenols that produces bacterial inhibition is still unclear, but changes in the membrane fatty acid composition in *La. plantarum* were previously reported [25]. Therefore, plant-associated LAB have developed metabolic routes for degradation of those polyphenols by enzymes into less toxic compounds. Among the enzymes described in LAB strains capable of degrading or bio-transforming phenolic compounds are tannase, amylase, esterase, β-glucosidase, phenolic acid decarboxylase (PAD), reductase, and benzyl alcohol dehydrogenase enzymes [26]. Tannase enzymes can hydrolyze tannins and gallic acid esters. Hydroxycinnamic acids such as caffeic, p-coumaric, and ferulic acids

can be bio-transformed into dihydrocaffeic, phloretic, or dihydroferulic acids, respectively, by reductase enzymes, or they can be decarboxylated into vinyl derivatives, such as vinyl phenol and vinyl guaiacol, by PAD enzymes. Subsequently, those vinyl derivatives can be reduced into ethyl derivatives [27]. Glucosidase enzymes release phenolic compounds by hydrolysis of glycosylated precursors, including flavonoid glucosides [28]. The tolerance to phenolic compounds depends on the LAB specie. A positive significant correlation was found between the content of quinic acid isomers a and b and perseitol, with the Log_{10} CFU/mL of all lactic acid bacteria (r = 0.6313, 0.6895, and 0.9481, respectively). In these cases, those compounds could have inhibited the growth of LAB during the incubation time or were highly available during fermentation of the avocado seed and were accessible for the bacteria to be used or bio-transformed into other compounds as part of their metabolism. In contrast, quercetin-diglucoside isomer a and luteolin derivative II isomer b showed a negative significant correlation with Log_{10} CFU/mL (r = −0.4490 and −0.5098, respectively). This means that those compounds were formed because of bio-transformations from precursor compounds and that were not toxic for LAB. Regarding *P. acidilactici* CECT 5765 T, it showed a significant ($p < 0.05$) positive correlation of caffeoylquinic acid isomers a and b and the sum of procyanidins, with Log_{10} CFU/mL (r = 0.9999, 0.9999, and 0.9624, respectively). Additionally, hydroxytyrosol glucoside and perseitol had the same tendency ($p < 0.05$, r = 0.9999, in both cases). *P. pentosaceus* CECT 4695 T, *Le. brevis* CECT 5354, and *La. plantarum* CECT 9567 had similar tendencies. In the case of *Leu. mesenteroides* CECT 219 T, the phenolic acid precursor quinic acid isomers a and b showed a significant positive correlation with Log_{10} CFU/mL (r = 0.9278 and 0.8662, respectively). Furthermore, perseitol, catechin, and hydrotytyrosol glucoside showed a significant positive correlation with the Log_{10} CFU/mL (r = 0.9902, 0.8845, and 0.9954, respectively). In contrast, caffeoylquinic acid isomers and coumaroylquinic acid isomers had significant negative correlations with Log_{10} CFU/mL (r from −0.7225 to −0.9995). For procyanidin dimer, trimer, and tetramer, the opposite correlation was found with Log_{10} CFU/mL compared to catechin. In the case of *La. plantarum* CECT 748 T, only quinic acid isomers a and b, perseitol, and hydroxytyrosol glucoside showed significant positive correlations with Log_{10} CFU/mL (r > 0.9622), and negative correlations were found for the rest of the compounds. Previously, the inhibition of the growth of *La. plantarum* with the presence of caffeic acid and its derivatives has been reported by other authors [25]. Others reported inhibition of *La. plantarum* by *p*-coumaric and ferulic acids present in wines [29]. Additionally, the inhibition of other LAB by hydroxycinnamic acids has been reported [30], or inhibition of other Gram-positive bacteria such as *Bacillus cereus* and *Enterococcus faecalis* by caffeoylquinic acid [31]. Furthermore, phenolic compounds found in olive products, such as oleuropein, tyrosol, synapic acid, and syringic acid, showed inhibitory activity against *La. plantarum* strains [32]. Otherwise, there are no previous references of catechin inhibiting the growth of LAB [33]. The use of an avocado seed hydrolysate-based medium to support biomass growth and lactic acid production by *Lactobacillus* sp. has been previously reported [34]. As previously reported [35], avocado seed is a well-known source of carbohydrates (65%), with perseitol and D-mannuheptulose being the sugar compounds that stand out the most. Therefore, according to the results, the content of perseitol was reduced with the increase of Log_{10} CFU/mL, which indicates that its content directly affects the growth of the LAB tested.

Taking everything into account, in order to compare between the LAB strains, the extracts fermented by *Leu. mesenteroides* CECT 219 T and *La. plantarum* CECT 748 T at 48 h and the rest at 24 h were selected. This is also supported by previous research [13].

The pH throughout the fermentations decreased from 5.96 ± 0.02 (initial) to values between 4.5 and 5.2 at 48 h of incubation.

3.3. Quantification of Phenolic Compounds by HPLC-ESI-TOF-MS and Its Bio-Transformations during Fermentation in Avocado Seeds

The phenolic and other polar compounds identified in fermented and non-fermented avocado seeds are summarized in Table 3.

Table 3. Phenolic and other polar compounds quantified by HPLC-ESI-TOF-MS in the fermented avocado seeds and a control. Results are expressed as average ± standard deviation.

	μg/g d.w.						
Compound	***P. acidilactici* CECT 5765T**	***P. pentosaceus* CECT 923**	***Leu. mesenteroides* CECT 219T**	***Le. brevis* CECT 5354**	***La. plantarum* CECT 748T**	***La. plantarum* CECT 9567**	**Control**
5-*O*-Galloyl-1,4-galactarolactone	92.23 ± 1.26 a–c	86.57 ± 1.55 b–d	93.26 ± 1.46 a, b	83.46 ± 5.96 d	85.35 ± 0.93 c–d	88.19 ± 0.38 a–d	94.43 ± 1.02 a
Quinic acid isomer a	107.68 ± 3.90 b, c	108.03 ± 3.99 b, c	98.56 ± 1.79 b, c	112.11 ± 12.08 a, b	102.87 ± 0.17 b, c	123.77 ± 3.04 a	96.89 ± 0.99 c
Quinic acid isomer b	146.31 ± 1.93 a–c	145.11 ± 1.31 a–c	139.39 ± 3.36 c, d	151.87 ± 3.85 a	137.38 ± 0.52 d	148.12 ± 4.09 a, b	140.71 ± 1.35 b–d
Perseitol	12.42 ± 0.02 b	12.18 ± 0.14 b	10.36 ± 0.20 c	12.00 ± 0.35 b	10.90 ± 0.22 c	12.20 ± 0.22 b	15.46 ± 0.05 a
Hydroxytyrosol glucoside	2989.76 ± 3.64 a	1547.58 ± 11.64 c	1740.31 ± 13.67 b	1574.06 ± 10.30 c	886.69 ± 8.32 e	128.44 ± 9.63 f	1255.66 ± 18.34 d
Caffeoylquinic acid isomer a	888.91 ± 1.04 e	787.01 ± 6.32 f	922.80 ± 5.00 d	802.99 ± 2.25 f	1109.82 ± 4.88 c	1168.20 ± 12.57 a	1133.69 ± 4.70 b
Caffeoylquinic acid isomer b	507.57 ± 2.48 e	449.37 ± 3.71 f	520.10 ± 0.57 d	421.71 ± 1.11 g	569.72 ± 1.57 c	652.06 ± 6.83 a	605.25 ± 7.33 b
Coumaroylquinic acid isomer a	302.09 ± 4.99 a	318.50 ± 1.73 a	273.33 ± 11.65 b	238.75 ± 10.77 c	195.23 ± 9.63 d	194.92 ± 0.16 d	210.88 ± 4.20 d
Coumaroylquinic acid isomer b	374.82 ± 13.54 c	341.59 ± 7.27 d	399.01 ± 4.21 c	301.14 ± 16.01 e	468.46 ± 7.37 b	514.91 ± 13.53 a	490.79 ± 3.34 a, b
Coumaroylquinic acid isomer c	148.94 ± 3.80 a	147.85 ± 7.42 a	141.21 ± 1.22 a	119.56 ± 4.14 b	109.00 ± 5.16 b	111.02 ± 2.63 b	116.19 ± 2.57 b
Coumaroylquinic acid isomer d	170.50 ± 6.77 d	153.54 ± 5.15 e	188.37 ± 0.35 c	141.33 ± 5.54 e	207.35 ± 0.94 b	227.54 ± 7.46 a	213.18 ± 5.62 a, b
Penstemide	50.45 ± 1.54 a	52.79 ± 3.15 a	55.24 ± 2.28 a	46.60 ± 11.55 a	56.91 ± 0.77 a	58.34 ± 1.06 a	58.77 ± 0.10 a
Catechin	48.00 ± 0.17 a	39.23 ± 2.03 b	38.46 ± 2.47 b	33.04 ± 4.68 b	33.37 ± 0.79 b	37.48 ± 1.78 b	45.75 ± 1.22 a
Eriodictyol 7-*O*-glucoside	7.85 ± 0.08 b, c	7.99 ± 0.21 a–c	8.47 ± 0.28 a–c	7.33 ± 1.24 c	9.31 ± 0.07 a	8.81 ± 0.01 a, b	8.63 ± 0.04 a–c
2-Phenylethyl beta-primeveroside	10.86 ± 0.26 a	11.24 ± 0.00 a	11.25 ± 0.08 a	10.68 ± 1.37 a	11.22 ± 0.01 a	12.00 ± 0.47 a	11.55 ± 0.13 a
Procyanidin dimer	20.94 ± 0.31 b	18.58 ± 0.32 c, d	21.83 ± 0.24 a, b	18.00 ± 1.21 d	19.96 ± 0.12 b, c	23.04 ± 0.93 a	23.39 ± 0.91 a
Procyanidin tetramer	18.79 ± 1.34 c	16.31 ± 0.24 c	21.97 ± 0.99 b	17.68 ± 0.19 c	23.74 ± 0.15 a, b	25.70 ± 1.53 a	22.82 ± 1.33 b
Procyanidin trimer	166.92 ± 13.88 b, c	133.81 ± 14.92 c	204.51 ± 19.56 a, b	128.79 ± 8.68 c	216.82 ± 1.29 a	240.41 ± 17.46 a	203.83 ± 11.81 a, b
Luteolin/kaempferol derivative isomer a	6.55 ± 0.12 a	6.64 ± 0.09 a	6.77 ± 0.12 a	6.30 ± 0.73 a	6.52 ± 0.06 a	6.90 ± 0.12 a	6.93 ± 0.09 a
Luteolin/kaempferol derivative isomer b	12.28 ± 0.73 a	12.73 ± 0.34 a	12.84 ± 0.33 a	11.74 ± 1.56 a	12.21 ± 0.26 a	13.10 ± 0.42 a	13.01 ± 0.02 a

Table 3. *Cont.*

	μg/g d.w.						
Compound	***P. acidilactici* CECT 5765T**	***P. pentosaceus* CECT 923**	***Leu. mesenteroides* CECT 219T**	***Le. brevis* CECT 5354**	***La. plantarum* CECT 748T**	***La. plantarum* CECT 9567**	**Control**
Luteolin/kaempferol derivative isomer c	5.53 ± 0.08 a	5.72 ± 0.37 a	5.79 ± 0.09 a	5.55 ± 0.64 a	5.63 ± 0.41 a	5.82 ± 0.13 a	6.05 ± 0.08 a
Quercetin-diglucoside isomer a	4.95 ± 0.08 b, c	5.00 ± 0.06 b, c	5.38 ± 0.14 a–c	4.72 ± 0.48 c	6.05 ± 0.48 a	5.52 ± 0.16 a, b	5.42 ± 0.04 a–c
Quercetin-diglucoside isomer b	5.00 ± 0.14 b, c	5.13 ± 0.11 a–c	5.41 ± 0.01 a–c	4.87 ± 0.47 c	5.54 ± 0.14 a, b	5.58 ± 0.01 a	5.51 ± 0.02 a, b
Triacetoxy-methoxy-methylflavone isomer a	3.81 ± 0.07 c, d	4.17 ± 0.02 b	4.84 ± 0.24 a	3.72 ± 0.19 d	4.14 ± 0.00 b, c	5.05 ± 0.04 a	4.84 ± 0.03 a
Triacetoxy-methoxy-methylflavone isomer b	3.88 ± 0.04 d	3.91 ± 0.03 c, d	4.24 ± 0.09 b	3.84 ± 0.23 d	4.19 ± 0.03 b, c	4.54 ± 0.02 a	4.39 ± 0.11 a, b
Triacetoxy-methoxy-methylflavone isomer c	4.66 ± 0.13 d	4.77 ± 0.14 c, d	5.42 ± 0.29 a, b	4.67 ± 0.36 c, d	5.23 ± 0.13 b, c	5.80 ± 0.06 a	5.49 ± 0.12 a, b
Luteolin/kaempferol derivative II isomer a	8.57 ± 0.43 b	9.00 ± 0.67 a, b	9.51 ± 0.18 a, b	8.62 ± 1.20 b	10.39 ± 0.07 a	9.06 ± 0.13 a, b	9.34 ± 0.20 a, b
Luteolin/kaempferol derivative II isomer b	12.43 ± 0.67 b, c	12.29 ± 0.14 b, c	12.79 ± 0.05 b	11.22 ± 1.02 c	25.67 ± 0.10 a	13.31 ± 0.03 b	13.18 ± 0.15 b
Luteolin/kaempferol derivative II isomer c	5.42 ± 0.03 b, c	5.65 ± 0.02 b, c	5.79 ± 0.02 a–c	5.21 ± 0.58 c	6.41 ± 0.04 a	5.82 ± 0.14 a–c	5.90 ± 0.01 a, b
Luteolin/kaempferol derivative II isomer d	8.31 ± 0.54 a, b	8.88 ± 0.55 a, b	8.78 ± 0.02 a, b	7.91 ± 0.89 b	9.54 ± 0.20 a	8.91 ± 0.55 a, b	8.85 ± 0.00 a, b
Sum of phenolic acids and precursors	5636.58 ± 42.08 a	3998.58 ± 48.55 d	4423.06 ± 41.82 b	3863.52 ± 66.05 d, e	3786.53 ± 38.55 e	3268.97 ± 59.95 f	4263.23 ± 48.44 c
Sum of procyanidins	254.65 ± 15.70 b, c	207.93 ± 17.52 c, d	286.77 ± 23.26 a, b	197.51 ± 14.75 d	293.89 ± 2.34 a, b	326.63 ± 21.70 a	295.80 ± 15.27 a, b
Sum of others	388.41 ± 9.96 a	391.68 ± 14.97 a	409.69 ± 8.12 a	373.12 ± 47.29 a	423.50 ± 8.16 a	417.40 ± 6.17 a	428.47 ± 6.19 a
Sum of polar compounds	6279.63 ± 67.74 a	4598.19 ± 81.04 c	5119.52 ± 73.20 b	4434.15 ± 128.09 c	4503.92 ± 49.04 c	4013.01 ± 87.81 d	4987.50 ± 69.90 b

Different letters (a–g) in the same line indicate significant differences.

As can be seen in Table 3, the sum of polar compounds ranged between 4013 and 6280 µg/g d.w. in the avocado seed fermented by LAB. Increments of 26% in avocado seed fermented by *P. acidilactici* and 4.3% by *Leu. mesenteroides* were observed compared to the control, but decreases were found in avocado seeds fermented by other LAB.

The sum of procyanidins decreased in the case of fermentation with *P. acidilactici*, *P. pentosaceus*, and *Le. brevis*, and did not show significant differences by *Leu. mesenteroides* or both strains of *La. plantarum* compared to the control. However, no significant increases were seen. There were no significant differences detected for the flavonoids eriodictyol 7-*O*-glucoside, 2-phenylethyl beta-primeveroside, and quercetin-diglucoside isomer a. Regarding luteolin derivatives, avocado seed fermented by *La. plantarum* CECT 748T stood out, with the highest content. Otherwise, some decreases were detected for quercetin diglucoside isomer b and the three isomers of triacetoxy-methoxy-methylflavone in the avocado seeds fermented by *Le. brevis*, *P. pentosaceus*, and *P. acidilactici*. Furthermore, perseitol significantly decreased in all cases; meanwhile, penstemide did not change compared to the control avocado seed. Most likely, perseitol was converted into D-mannoheptulose [36] by some of the LAB that have aldolase enzymes and consumed as a nutrient for their metabolism [37].

As shown in Table 3, the sum of phenolic acids and precursors increased by 32.21% and 3.74% after fermentations with *P. acidilactici* CECT 5765T and *Leu. mesenteroides* CECT 219T, respectively, but decreased with the other bacteria. Nevertheless, lactic acid bacteria metabolism led to interesting bio-transformations in the avocado seed compounds. Hydroxytyrosol glucoside, an important derivative of hydroxytyrosol in olive fruits [38,39], was significantly increased in fermentations with *P. acidilactici* CECT 5765T, *P. pentosaceus* CECT 923, *Leu. mesenteroides* CECT 219T, and *Le. brevis* CECT 5354. These increases can be explained by the fact that lactic acid bacteria can release phenolic compounds from plant matrices through their hydrolytic enzymes, such as amylase, xylanase, glucosidase, cellulase, tannase, esterase, or chitinase. These enzymes can disrupt the cell wall, where phenolic acids are found linked to lignin, cellulose, hemicellulose, or structural proteins through ether bonds via the hydroxyl group in aromatic rings, or they form esters via the carboxylic group with proteins and carbohydrates [40]. Thus, these enzymes play a key role in disintegrating plant cell wall matrices and increasing the bioavailability of phenolic compounds, which can be used as a new substrate for other bacterial enzymes or can be free for their extraction and recovery as added-value bioactive compounds. On the other hand, the fermentation of avocado seed with the two *La. plantarum* strains significantly decreased the hydroxytyrosol glucoside content. This decrease could be a result of the hydrolysis via the glucosidase activity of both strains to release hydroxytyrosol and glucose. Glycosidase activities are widespread in lactic acid bacteria; among them, *La. plantarum* glycosidases have been especially studied [41]. These enzymes have been reported to release a large number of secondary metabolites from their glycosylated precursors, increasing their antioxidant activity and improving the flavor and aroma of fermented foods [28]. In addition to olive fruit, hydroxytyrosol and hydroxytyrosol glucoside have previously been found in avocado seed [18]. They have shown antioxidant, antibacterial, antifungal, anti-inflammatory, and antitumoral activities in both in vitro and in vivo assays [42–44]. In our previous research with avocado leaves [13], fermentation with both *La. plantarum* strains decreased the concentration of flavonoid glucosides and other glycosylated phenolic compounds, and in the case of *La. plantarum* CECT 748T, it led to a high antioxidant activity.

Regarding chlorogenic acids found in avocado seeds, a significant increase was found in caffeoylquinic acid in fermentation with *La. plantarum* CECT 9567 and in coumaroylquinic acid isomers a and c with *P. acidilactici* CECT 5765T, *P. pentosaceus* CECT 923, *Leu. mesenteroides* CECT 219T, and *Le. brevis* CECT 5354 fermentations. This group of phenolic compounds are produced in plants by esterification of the quinic acid and the hydroxycinnamic acids, including caffeic acid, *p*-coumaric acid, and ferulic acid [45]. They are widely distributed in plant-based foods, such as coffee, tea, and wine, as well as in avocado seed [46]. The most abundant compound is 5-caffeoylquinic acid. Their occurrence in

plants has been associated with lignin biosynthesis, as an indirect defense agent, making tissues more resistant to pathogen attacks and inhibiting fungal toxins, and as a protective agent against oxidative damage caused by exposition to salinity and heat [45]. They have shown antioxidant, antimicrobial, antiviral, and anti-inflammatory activity, as well as risk reduction of developing type 2 diabetes and cardiovascular diseases [47]. Despite the antimicrobial activity, some microorganisms are resistant to chlorogenic acids because of their ability to degrade such compounds [47]. In this study, caffeoylquinic acid isomers a and b and coumaroylquinic acid isomers b and d decreased in fermentations with *P. acidilactici* CECT 5765T, *P. pentosaceus* CECT 923, *Leu. mesenteroides* CECT 219T, and *Le. brevis* CECT 5354, which could be due to the esterase activity of the strains. In human digestion, when chlorogenic acids reach the colon, the microbiota hydrolyses them, releasing hydroxycinnamic acid and quinic acid [48]. A significant increase in the concentration of quinic acid isomers a and b was found when avocado seeds were fermented with *Le. brevis* CECT 5354 and *La. plantarum* CECT 9567, indicating possible hydrolysis of hydroxycinnamic acids. The hydrolysis of these acyl-quinic acids is carried out by cinnamoyl esterases, which have been studied in *Lactobacillus* species, including *La. plantarum* [49] and *L. johnsonii* [50], as well as in *Escherichia coli*, *Bifidobacterium lactis*, and *Lactobacillus gasseri* [51]. Although hydroxycinnamic acids are efficiently absorbed after hydrolysis, intact chlorogenic acids are less well-absorbed only in the small intestine [52]. The hydrolysis of dietary phenolics by fermentation before intake can increase the bioavailability of bioactive compounds in case of dysbiosis of the fermentative microbiota [53].

3.4. Antioxidant Activity in Fermented Avocado Seeds

The antioxidant activity of the LAB-fermented avocado seed extracts was analyzed by DPPH and FRAP assays compared to non-fermented extract of avocado seeds (control), and the results are shown in Figure 2. A significant ($p < 0.05$) positive correlation (r = 0.8884) was found between the antioxidant activity measured by both methods, DPPH and FRAP.

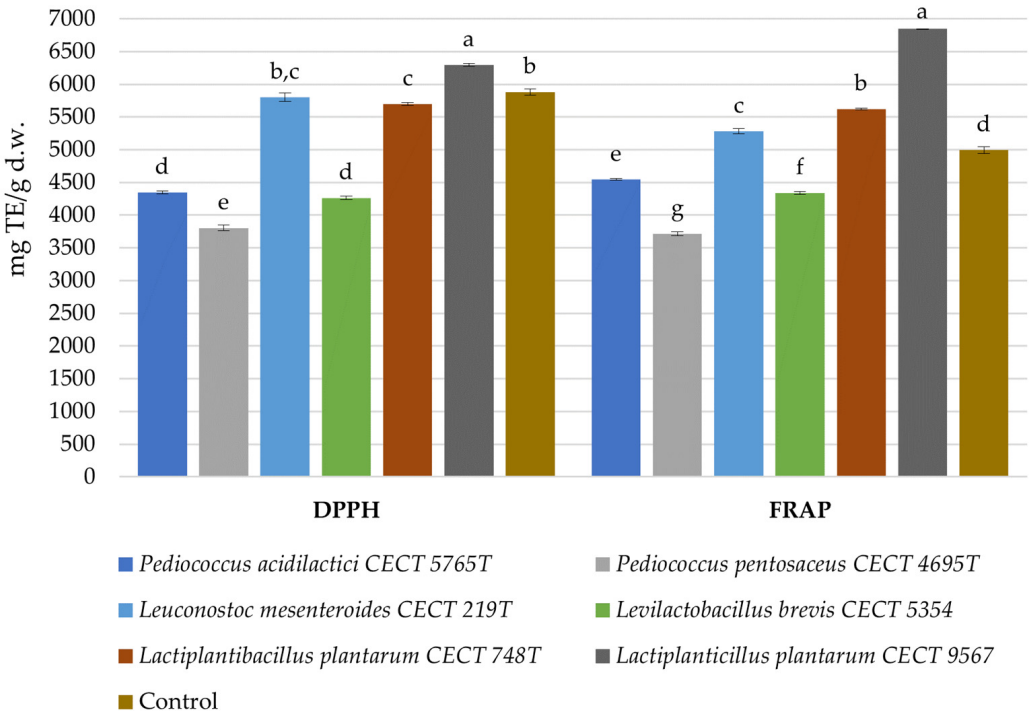

Figure 2. Antioxidant activity of avocado seed fermented by LAB strains and a control measured by DPPH and FRAP assays. Different letters (a–g) for each method indicate significant differences.

As can be seen in Figure 2, the antioxidant activity obtained for the non-fermented and fermented avocado seed extracts ranged 3803–6295 and 3714–6847 μg TE/g d.w. for

DPPH and FRAP assays, respectively. In both cases, the lowest result corresponded to *P. pentosaceus* CECT 4695T, and the highest to *La. plantarum* CECT 9567, with significant differences with respect to the control. For the FRAP assay, *La. plantarum* CECT 748T and *Leu. mesenteroides* CECT 219T also showed significant increases compared to the control. In the DPPH results, no significant reduction in the antioxidant activity was found for *Leu. mesenteroides* CECT 219T. Although avocado seed fermented by *P. acidilactici* CECT 5765T showed the highest content in the sum of polar compounds and, by extension, in the sum of phenolic acids and precursors, it showed a significant decrease in its antioxidant activity compared to the control. In contrast, those that had increases in their antioxidant activity did not show significant increases in the sum of polar compounds. This phenomenon was previously noticed by Li et al. [54] in apple juice fermented by *La. plantarum* ATCC14917. They reported a significant decrease in total phenolic and total flavonoid compounds during fermentation, while the antioxidant activity increased over time. They attributed this to the increase of 5-*O*-caffeoylquinic acid, quercetin, and phloretin [54]. Results found here agree that *La. plantarum* CECT 9567, compared to the control, had significant increments of quinic acid isomer a, and caffeoylquinic acid isomers a and b. This was also statistically confirmed. According to the Pearson's correlation test, the antioxidant activity in the fermented avocado seed can be attributed to the polyphenol content. Furthermore, the sum of procyanidins showed a significant ($p < 0.05$) strong correlation for both methods, DPPH (r = 0.9406) and FRAP (r = 0.8865). Besides, caffeoylquinic acid isomers a and b and coumaroylquinic acid isomers b and d, penstemide, eriodyctiol 7-*O*-glucoside, quercetin-diglucoside isomers a and b, and triacetoxy-methoxy-methylflavone isomers a, b, and c also had significant ($p < 0.05$) strong correlations with the antioxidant activity, as measured by both methods. Previously, Rozan et al. also reported an increment in the DPPH antioxidant activity in Hass avocado kernel flour fermented by *La. plantarum* [14].

3.5. Antidiabetic Activity in Fermented Avocado Seeds

The capacity of inhibiting alpha-amylase was analyzed in the LAB-fermented avocado seed compared to the non-fermented extract of the avocado seed (control). All were tested at 20 mg of avocado/mL, and the results are shown in Figure 3.

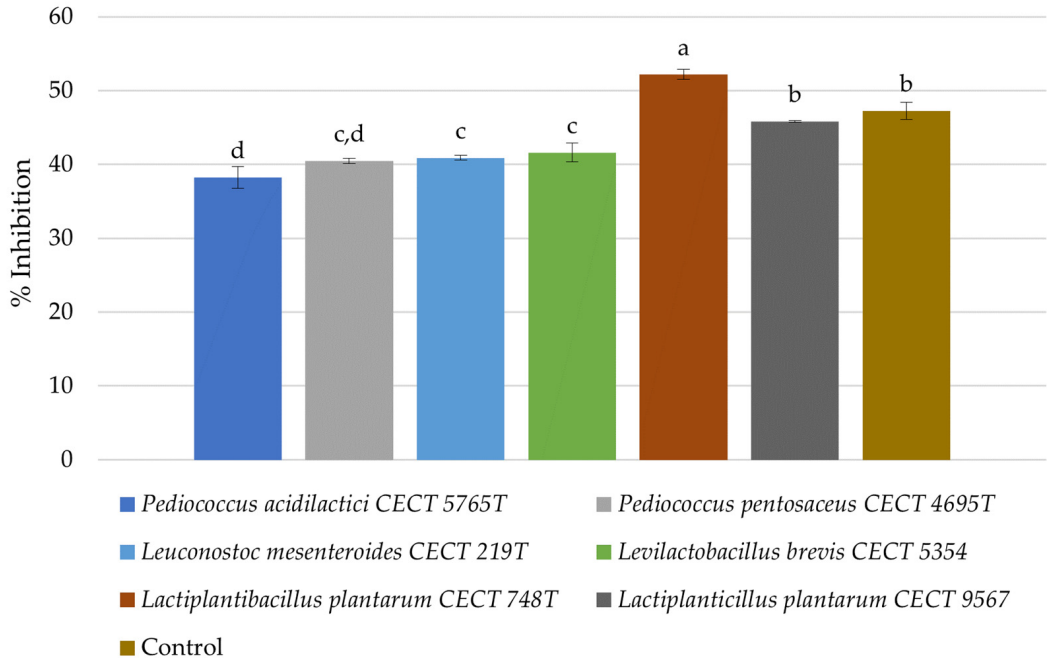

Figure 3. Inhibition of alpha-amylase activity of fermented avocado seed by different strains and non-fermented avocado seed. Different letters (a–d) indicate significant differences.

As can be seen in Figure 3, all the LAB tested presented a % of inhibition higher than 25%. *La. plantarum* CECT 748T had the highest % of inhibition; meanwhile, *P. acidilactici* CECT 5765T had the lowest. *La. plantarum* CECT 9567 did not show significant differences with the unfermented avocado seed, but the rest had significant decreases in the capacity of inhibiting alpha-amylase activity. Ojo et al. reported values similar to those obtained here, with a % of inhibition ≈20% of alpha-amylase at ≈20 mg/mL of avocado seed extract [10]. Moreover, Abd Elkader et al. reported values of the same range of magnitude with avocado leaves and fruits [55]. The Pearson correlation test revealed a significant positive correlation between the antidiabetic activity and the compounds caffeoylquinic acid (isomers a and b, r = 0.7786 and 0.6163, respectively), penstemide (r = 0.5154), procyanidin trimer (r = 0.6112) and tetramer (r = 0.6881), quercetin-diglucoside isomers a (r = 0.7749) and b (r = 0.6540), eriodyctiol 7-*O*-glucoside (r = 0.7022), triacetoxy-methoxy-methylflavone isomers b (r = 0.5741) and c (r = 0.5421), and luteolin/kaempferol derivative isomers a–d (r = 0.5929–0.7970). Moreover, a positive correlation (r = 0.7568) with total flavan-3-ols content was also found. Younis et al. [56] studied the antidiabetic activity of seven varieties of avocado seed by the alpha-amylase method and reported that it could be attributed to its content of polyunsaturated fatty acids, as well as several polyphenolic compounds, such as eriodictyol-7-oneohesperidoside, 4-caffeoylquinic acid, and linoleic acid ethyl ester [56]. Furthermore, the inhibition of alpha-amylase activity showed a significant positive correlation with the antioxidant activity measured by DPPH (r = 0.6376) and FRAP assays (r = 0.5503). This makes sense as most of the compounds correlated with the antioxidant activity also showed correlation with the antidiabetic activity, and by extension, flavonoids. The differentiation compounds between both activities were luteolin derivative isomers. In fact, flavonoids are reported by other authors to have antidiabetic activity [57].

3.6. Clustering Heatmap

A hierarchical clustering heatmap (Figure 4) was performed to provide an intuitive visualization of all the polar compounds quantified by HPLC-ESI-TOF-MS and the antioxidant (DPPH and FRAP) and antidiabetic (alpha-amylase) assays carried out in the fermented avocado seeds by the selected strains, and the non-fermented control.

As can be seen from Figure 4, avocado seed fermented by *La. plantarum* CECT 9567 was clustered with the control samples, showing minor differences. Close to them were the avocado seeds fermented by *La. plantarum* CECT 748T and *Leu. mesenteroides* CECT 219T. Although fermentation of the avocado seed with *La. plantarum* CECT 9567 showed a phenolic profile similar to that of the unfermented seed, key bio-transformations carried out by this strain can be visualized in the figure by color changes. These include increased concentrations of quinic acid and chlorogenic acids, such as some isomers of caffeoylquinic acid, as well as decreased concentrations of hydroxytyrosol-glucoside, by enzymatic hydrolysis for the release of free hydroxytyrosol and glucose. Furthermore, Figure 4 highlights the antioxidant activity by the FRAP assay found in the fermentation with this strain. Another clear group was formed by the rest of the LAB-fermented avocado seeds. In a nutshell, this clustering confirms all the results discussed previously, and shows the differences in the metabolism of the tested LAB on the polar compounds present in avocado seeds.

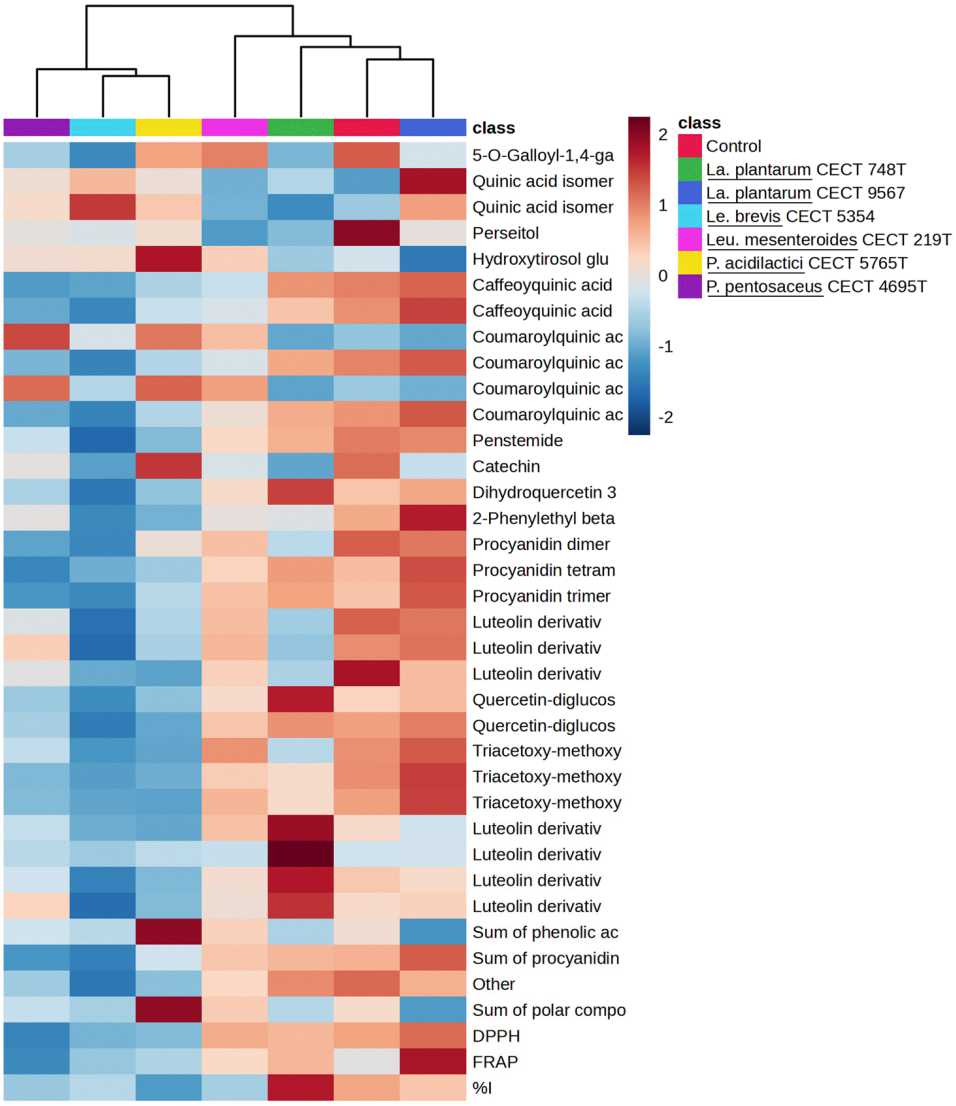

Figure 4. Clustering heatmap of the avocado seeds fermented by LAB and the control according to the analyses performed.

4. Conclusions

In this research, the chemical bio-transformations and changes in antioxidant and antidiabetic activity induced by LAB strains were studied in avocado seeds using submerged fermentations. A total of 32 polar compounds were identified by HPLC-ESI-TOF-MS and, to our knowledge, 13 of them were tentatively identified for the first time. It was found that among all the compounds, caffeoylquinic and coumaroylquinic acids' isomers could have inhibited the growth of *L. mesenteroides*. No effect was found for procyanidins in LAB growth. Quinic acid and perseitol seemed to be the most available compounds to be bio-transformed by LAB or consumed as part of their metabolism. Avocado seeds fermented by *P. acidilactici* showed the highest sum of polar compounds, and by extension, of hydroxytyrosol glucoside, quantified by HPCL-ESI-TOF-MS. Among all the tested LAB, *La. plantarum* CECT 9567 showed the highest significant increase in the antioxidant activity, measured by both DPPH and FRAP, mainly attributable to its content in hydroxycinnamic acids, procyanidins, and flavonoids. Furthermore, *La. plantarum* CECT 748T had the highest alpha-amylase inhibitory activity, attributable to the polyphenols. In conclusion, submerged fermentation by lactic acid bacteria can be used in the exploitation and valorization of avocado seed waste for its use in food formulations or the production of enriched extracts.

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