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Fermentation of Corn By-Products: From Agrifood Waste to Higher Value Antioxidant Products

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Abstract: To improve the nutri-functional quality and, in particular, the antioxidant capacity of corn by-products, fermentation with selected lactic acid bacteria was carried out. To this purpose, **white-1** and **2** and **yellow** corn by-products were fermented and then extracted. In all the samples, the fermentation process shows an improvement in antioxidant activity in comparison to non-fermented by-products. It was observed that the **yellow** corn by-product extracts have a higher content of total phenols, especially after fermentation with *P. pentosaceus*, while for **white-1** corn by-product extracts, an increment of antioxidant capability was noticed when fermented with *L. plantarum*. The antioxidant capacity was measured with DPPH and $ABTS^+$ assays, showing that **yellow** corn extracts are more active in comparison with **white-1** and **white-2** ones. Moreover, *L. plantarum* and *P. pentosaceus* provided the best results in increasing the antioxidant activity in all the samples. Analyzing lipid peroxidation in the presence of fermented **white-2** corn by-product extracts, we observed an inhibition of the process after treatment with *L. citreum* compared to the non-fermented control. In all the analyzed samples, through LC-DAD-ESI/MS analysis, the antioxidant dicoumaroyl spermidine (DCS) was detected. The abundance of antioxidant molecules was higher in samples fermented with *P. pentosaceus*, confirming previous observations.

Keywords: fermentation; food by-products; antioxidant

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1. Introduction

In the last few years, it has been recognized that reducing food loss and waste (FLW) represents a key aspect in ensuring a sustainable and healthy diet for the global population [1]. In fact, Target 12.3 of the 2030 Agenda for Sustainable Development of the United Nations (UN) is to shrink by 2030 the global food waste generated along the food supply chain by up to 50% [2]. A reduction such as this would help complete the Sustainable Development Goals (SDGs) of "zero hunger" and significantly improve food production's environmental footprint. This has been a topic of concern due to the high food losses and waste and the consequent decrease in micro and macronutrients [1]. Extracting different compounds from food waste and by-products gives us new possibilities to utilize these molecules in the food industry to make better foods. Some of the waste-derived molecules can confer better nutritional characteristics to existing foods enhancing their antioxidant properties, protein or fiber content, along with the amount of minerals [3,4]. There is a great

opportunity for much food waste and by-products to be used for the production of diverse types of food with better nutritional characteristics [4]. Although not all agro-industrial by-products can be used as ingredients in a newly developed food, proper utilization of these biomaterials can exert beneficial effects on human health. Moreover, the correct use of by-products can solve many environmental problems related to the discharge of this waste [4]. By-products can be reutilized in various ways; for example, in the case of cereals, wet and dry milling and fermentation are carried out [5,6]. Among the fermentable matrices, corn and its processing waste can be included since they are rich in nutrients and have a broad chemical composition [5,6].

Corn, also referred to as maize, is a crop from Central America cultivated for the past 7000 years. This cereal is the highest produced worldwide and is used for human consumption, livestock feed, and fuel [7]. The carbohydrate content in corn is close to 75%, and the water content is about 10%. It provides a wide variety of vitamins (carotenoids, thiamine, riboflavin, niacin, pyridoxine, folate, ascorbic acid, vitamin E, and vitamin K), minerals (calcium, magnesium, phosphorus, potassium, sodium, and zinc) and resistant starches. Lipids in corn are mostly presented in mono- (30%) and polyunsaturated (50%) forms, with a small portion of lipids in the saturated form (20%). Previously, the phytochemicals of corn have received less attention than those of fruits and vegetables. Corn contains significant amounts of bioactive compounds providing desirable health benefits beyond its role as a major source of food. The health benefits of corn are not only from basic nutrients such as the ones cited above but also from their content of phytochemicals such as phenolic acids. However, different varieties of corn contain significantly different phytochemical profiles in terms of flavonoids and carotenoids. Blue, red, and purple corn possess a higher concentration of anthocyanidins (up to 325 mg/100 g dry weight corn), including cyanidin derivatives (75–90%), peonidin derivatives (15–20%) and pelargonidin derivatives (5–10%). Yellow corn is rich in carotenoids (up to 823 μg/100 g dry weight corn), including lutein (50%), zeaxanthin (40%), β-cryptoxanthin (3%), β-carotene (4%), and α-carotene (2%) [8]. Fermentation is convenient since it favors the extension of the shelf life of less durable products. Nevertheless, it is important for the effects on the organoleptic profile as the increase in flavor, consistency, aroma, and nutritional values. This is possible because of the capability of fermenting microorganisms to secrete metabolites [9]. Thanks to the growth of lactic acid bacteria in plant and vegetable fermentations, phenolic compounds such as flavonoids are converted to a greater extent of biologically active metabolites. The expression of enzymes such as glycosyl hydrolase, esterase, decarboxylase, and phenolic acid reductase occurs. Some of these metabolites may undergo reactions cascades that can result in the activation of Nrf2, which is the major regulator of oxidant stress response in mammals since it can induce the expression of antioxidant enzymes exerting a protective role against oxidative damage [10].

Fermentation with selected lactic acid bacteria of other flours for improving its nutritional and functional properties has been demonstrated [6,11].

In addition, the fermentation process is relevant in preventing the development of contaminants during the storage of flours, and this aspect is crucial for food safety [12,13]. In this work, corn processing by-products were fermented in order to improve the antioxidant capacity and nutritional value. To this purpose, several selected lactic acid bacteria were employed, and the entire study was carried out by comparing the non-fermented and fermented products.

2. Materials and Methods

2.1. Corn By-Products Properties

Samples of **white-1** and **-2** and **yellow** corn by-products were provided from Molino Favero (Favero Antonio SRL, Padova, Italy). The by-products were derived from the milling process of commercial hybrids of maize (*Zea mays* L.), in particular from a mix of dent and flint corn.

2.2. Corn By-Products Fermentation

Fermentation of corn by-products was carried out by Veneto Region's Agency for Innovation (Agenzia Veneta per l'Innovazione nel Settore Primario -Biotechnology Laboratory, Thiene, VI, Italy) in 200 g of dough obtained by mixing 100 g of corn by-products with 100 g of deionized sterile water. Each dough was inoculated with a fresh lactic acid bacteria culture to obtain an initial concentration of about 7.0 log CFU/g and was then incubated at 30 °C for 20 h. At the end of the incubation time, the fermented doughs were maintained at −20 °C until use.

The strains used in the fermentation tests are reported in Table 1 and belong to the Culture Collection of Veneto Region's Agency for Innovation. The pH of the doughs was monitored continuously for 20 h by a pH electrode (Micros, Siap + Micros, Treviso, Italy) immersed in the dough and connected to a software system for data acquisition. After fermentation, the samples were analyzed for the presence of lactic acid bacteria; enumeration was performed in de Man, Rogosa, and Sharpe Medium (MRS) added with pimaricine (25 mg/L) to inhibit the growth of contaminating yeasts that may be naturally present in corn by-products. The MRS agar plates were incubated anaerobically at 30 °C for 72 h and then counted considering the different colony morphologies. Since the corn by-products may be contaminated by native lactic acid bacteria, in order to verify the presence and the colonization of the doughs by the inoculated strains, a colony representative of each colony morphology grown on the MRS plates was isolated and submitted to RAPD-PCR with primer D11344 using the amplification conditions described by Andrighetto et al., 2002 [14].

Table 1. Lactic acid bacteria strains used in the fermentation tests.

Name	Fermenting Strains
a	*Lactiplantibacillus plantarum* TH925
b	*Lacticaseibacillus rhamnosus* TH414
c	*Lactobacillus amylovorus* TH875
d	*Lactococcus lactis* TH928
e	*Pediococcus pentosaceus* TH967/968 *
f	*Leuconostoc citreum* TH931

* a mixed culture consisting of two different strains of *Pediococcus pentosaceus* (1:1) was used.

The RAPD-PCR profiles of the colonies isolated from the fermented corn by-products were then compared with the profiles of the inoculated strains. The colonization, expressed as a percentage, was calculated as the ratio between the number of colonies displaying the RAPD-PCR profile of the inoculated strain and the total number of colonies.

2.3. Corn By-Products Hydroalcoholic Extract

A total of 100 mg of the fermented supplied flours were weighed and aliquoted in three replicates. For each type of corn by-product, a non-inoculated and not fermented control, consisting of 100 g of corn by-product added to 100 g of deionized sterile water, was also considered (NC).

They were diluted with 1.5 mL of ethanol 80%, vortexed for 1 min, and placed in the ultrasound bath for 45 min. After that, centrifugation at 16,000× *g* for 20 min was performed. Then, the supernatants were used in further analysis.

2.4. Determination of Antioxidant Activity

To evaluate the antioxidant activity of the extracts, total phenol content determination, ABTS, and DPPH assays were carried out.

2.4.1. Determination of Total Phenol Content

The total phenol content of the extracts was determined using the Folin–Ciocalteau assay [15]. Briefly, 1 mL of Folin–Ciocalteau reagent diluted 1:2 with H_2O was added to both the extracts and the standards and vortexed. After 3 min at room temperature (RT), 2 mL of 10% Na_2CO_3 was added. These samples will react with the gallic acid, used as standard, or the hydroalcoholic extracts of the studied samples for 15 min at RT. The absorbance of 1 mL of the sample was recorded at 750 nm with a spectrophotometer. The obtained values are referred to the calibration curve and are expressed as Gallic Acid Equivalent (mg/100 mL GAE).

2.4.2. 2-2 Azinobis(3-Ethylbenzothiazoline-6-sulfonic acid) (ABTS) Scavenging Assay

This assay evaluates the capacity of the antioxidant species to reduce the cationic radical of the 2-2′azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). A 7 mM ABTS, along with a 2.46 mM KPS (potassium persulfate) solution, was prepared and set aside in the dark for 18 h to form the ABTS radical. Then, according to Tonolo F. et al., 2020, with some modifications, 20 µL of the extract was added to 1 mL of the diluted ABTS solution. After 10 min at RT and in the dark, the absorbance at 734 nm was estimated using a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, CA, USA) [15]. A calibration curve with increasing concentrations of Trolox C (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxilic acid) was set up. Results are expressed as Trolox equivalent antioxidant capacity (TEAC, µM Trolox equivalent/mL product).

2.4.3. 2,2-difenil-1-picrylidrazyl (DPPH) Scavenging Assay

According to Tonolo F. et al., 2020, 800 µL of 100 µM DPPH was added to the samples and then put in the dark for 30 min [15]. Afterward, they were centrifuged for 5 min at 13,600× *g*, and then the absorbance was detected spectrophotometrically at 517 nm (Cary 60 UV-Vis, Agilent Technologies). The DPPH scavenging percentage was obtained using the following formula:

$$\% \text{ DPPH scavenging} = [(\text{Abs control-Abs sample})/(\text{Abs control})] \times 100$$

2.4.4. Lipid Peroxidation Assay

Lipid peroxidation was assessed by determination of the thiobarbituric acid reactive substances (TBARS), according to Buege J.D. and Aust A.S., 1978, with some modifications [16]. Soy beverage was used as a peroxidizable matrix. The production of pink adduct results after the reaction of thiobarbituric acid (TBA) with its reactive products (TBARS). Briefly, 10 mL of soy beverage was diluted in 20 mL of H_2O. Then, 10 µL of the corn by-product extracts were added to 0.5 mL of soy beverage and mixed at RT for 5 min. After, 5 mM cumene hydroperoxide (CHP) and 10 µM hemin were added to induce lipid peroxidation. The samples were shaken for 10 min at 250 r/min at RT and then treated with 1 mL of 35% trichloroacetic acid, 30 µL 1% BHT, and 1 mL of 1% TBA. The samples were placed at 95 °C for 15 min, let to cool down at RT, and subsequently, they were centrifuged at 4000× *g* for 15 min. The spectrum from 500 nm to 580 nm of the supernatant was collected (Cary 60 UV-Vis, Agilent Technologies). The results are expressed as TBARS (nmoles/mL of extract).

2.5. LC-DAD-ESI/MS Measurements

LC-DAD-ESI/MS experiments were performed with an LCQFleet ion trap instrument (ThermoFisher Scientific, Waltham, MA, USA), operating in positive ion mode, coupled with a Surveyor LC Pump Plus (ThermoFisher Scientific, Waltham, MA, USA) and with a UV-Vis detector Accela PDA Detector (ThermoFisher Scientific, Waltham, MA, USA). The used entrance capillary temperature and voltage were at 275 °C and 4 kV, respectively. The ion source temperature was kept at 300 °C. MS^n experiments were performed by applying a supplementary radio frequency (RF) voltage to the end caps of the ion trap (5 V

peak-to-peak). Extracts were injected (25 µL) into a Luna C18(2) (150 × 4.60 mm, 5 µm) (Phenomenex, Torrance, CA, USA) column. The mobile phase consists of solvent A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). From 0–48 min, the eluent composition was changed from 10% to 40% B. The flow rate was 1 mL/min. The UV-VIS detector wavelength ranges from 200 to 600 nm.

2.6. Statistical Analysis

All values were expressed as the mean ± standard deviation of at least three different experiments. The means of 3 experiments were compared with the specific control values (3 replicates for each experiment). The analysis of variance was conducted with the use of the Tukey–Kramer multiple comparison test with a significance level of $p < 0.05$. Origin software was used for the various statistical analyses.

3. Results and Discussion

Corn by-product matrices were fermented with different homofermentative and heterofermentative lactic acid bacteria strains in order to improve their nutritional value and antioxidant properties. The in vitro antioxidant activity of the matrices of interest was analyzed using different assays, such as ABTS and DPPH scavenging assays, and the determination of the total phenol content. The latter was carried out to understand if there are any differences in the phenols content of our samples' extracts. The ABTS and DPPH scavenging tests elicit the extract capability to neutralize the cationic radical ABTS and the DPPH radical, showing their antioxidant activity. In addition, lipid peroxidation in the presence and in the absence of fermented corn by-product extracts was performed, and lastly, LC-DAD-ESI/MS analysis of antioxidant molecules present in the fermented extract was carried out.

3.1. Fermentation

The results of fermentation obtained after incubation with the different lactic acid bacteria (LAB) strains were reported in Table 2. The studied LABs were chosen after testing 18 LABs on corn by-products due to their colonization capacity and specific fermentative metabolism: homolactic species (*L. lactis*, *P. pentosaceus*, *L. amylovorus*); facultatively heterofermentative species (*L. plantarum*, *L. rhamnosus*); obligate heterofermentative (*L. citreum*). Considering that the pH of the doughs before fermentation was about 5.20 in **white-1**, 5.60 in **yellow**, and 5.70 in **white-2**, at the end of fermentation, the pHs of all the inoculated samples were significantly lower, although with differences among strains. In particular, we observed that *L. plantarum* and *P. pentosaceus* determined the lowest pH value for each corn by-product, indicating their capability to produce a higher production of organic acids [13].

Differences among strains were also found in the time required to reach pH 4.6. In all three corn by-product samples, *L. amylovorus* was the fastest strain, while the slowest one was *L. rhamnosus.* The capability of strains to grow and ferment corn by-products is also confirmed by the counts of the lactic acid bacteria and the percentage of colonization at the end of fermentation. In all the corn by-products, the highest counts of lactic acid bacteria were reached in the samples inoculated with *L. plantarum*. These samples not only showed growth during incubation higher than 2 $\log_{10}$ cycles but they were also fully colonized by the inoculated strain confirming the high fermentation capabilities of this species involved in the production of many different types of fermented foods and beverages. *L. plantarum* can be isolated from a wide variety of environments; it is one of the lactic acid bacteria species most commonly isolated from fermented plant materials such as sourdough, sauerkraut, and olives [17–19].

Table 2. Evaluation of corn by-product parameters after fermentation with different bacteria strains: pH values, colony count, and % of colonization were reported.

Corn By-Product	Name	Fermenting Strains	pH at the End	Min at pH 4.60	Colony Count (Log CFU/g)	% Colonization
White-1	**a**	*Lactiplantibacillus plantarum*	3.47	304	9.59	100%
	b	*Lacticaseibacillus rhamnosus*	3.91	753	9.23	78%
	c	*Lactobacillus amylovorus*	4.05	48	8.74	45%
	d	*Lactococcus lactis*	3.86	165	8.82	100%
	e	*Pediococcus pentosaceus*	3.66	480	8.57	87%
	f	*Leuconostoc citreum*	3.87	236	8.93	86%
Yellow	**a**	*Lactiplantibacillus plantarum*	3.79	462	9.57	100%
	b	*Lacticaseibacillus rhamnosus*	4.05	787	9.41	100%
	c	*Lactobacillus amylovorus*	4.04	229	8.97	29%
	d	*Lactococcus lactis*	4.12	274	9.11	100%
	e	*Pediococcus pentosaceus*	3.96	481	9.20	100%
	f	*Leuconostoc citreum*	4.12	370	9.15	94%
White-2	**a**	*Lactiplantibacillus plantarum*	3.75	555	10.23	100%
	b	*Lacticaseibacillus rhamnosus*	3.98	771	9.62	100%
	c	*Lactobacillus amylovorus*	3.84	216	9.08	33%
	d	*Lactococcus lactis*	4.09	242	9.23	100%
	e	*Pediococcus pentosaceus*	3.84	463	9.41	100%
	f	*Leuconostoc citreum*	4.05	344	9.60	100%

On the other hand, *L. amylovorus*, a species known to convert starch to glucose, despite its ability to reach pH 4.6 very quickly, showed a percentage of colonization very low, less than 50% in all the corn by-product samples. The greatest percentages of colonization and the highest counts of lactic acid bacteria were obtained in **white-2** samples; on the other hand, the **white-1** sample provided the worst results in terms of the number of lactic acid bacteria and percentages of colonization.

3.2. Total Phenol Content Increases after Fermentation

The total phenol content of each fermented corn by-product has been estimated. For each extract, 100 µL was added to the Folin–Ciocalteau reagent to determine the total phenols present in our samples, and the absorbance was detected at 740 nm (Section 2.4.3). As reported in Figure 1, the hydroalcoholic extracts of **white** and **yellow** corn by-products fermented with (**a**), (**e**), and (**f**) exhibited an increase in the total phenol content, and generally, **yellow** corn extracts were the richest in phenols. More in detail, in corn by-products fermented with *L. plantarum* (**a**), GAE values of 22.07 ± 2.26, 28.47 ± 3.64, in **white-1** and **yellow**, respectively, were measured. Corn by-product extracts fermented with *P. pentosaceus* (**e**) showed, in all the analyzed corn varieties, a significant increase in total phenol content when compared to the negative control NC (not fermented flours). In particular, in **yellow** and **white-2** corn by-product extracts, 30.30 ± 1.53 and 19.77 ± 0.07 mg GAE/100 mg of product, respectively.

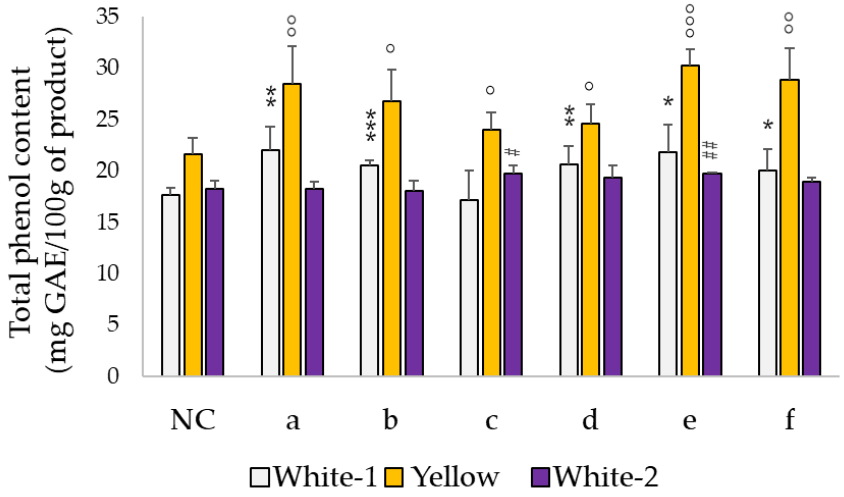

Figure 1. Total phenol content determination of the three corn by-products. Absorbance at 740 nm was measured, and values were expressed as GAE (mg GAE/100 mg of product). The means of 3 experiments were compared with the specific control values (3 replicates for each experiment). Statistic symbol legend: *, °, and # are for **white-1**, **yellow**, and **white-2** corn by-products, respectively. (***, °°° $p < 0.001$, **, °° ,## $p < 0.01$, *, °, # $p < 0.05$).

3.3. ABTS and DPPH

Antioxidant activity was evaluated using the DPPH and ABTS scavenging assay.

Comparing the DPPH scavenging value of the non-fermented white corn by-product (48.68%) with (**a**) and (**e**) (55.65% and 55.07%, respectively), a considerable improvement in antioxidant capacity in fermented samples was apparent (Figure 2A). In **yellow** corn by-products, (**a**) and (**f**) (70.27% and 71.64%, respectively) exhibited a noticeable increase in antioxidant activity when compared to the control, which presented a DPPH scavenging value of 57.58% (Figure 2A). In addition, comparing the antioxidant capacity of the extracts of fermented **white-2** corn by-product, the most remarkable increase in antioxidant activity has been found in (**a**) and (**e**), with DPPH scavenging of 66.52% and 66.06%, respectively (Figure 2A).

These results confirmed what was observed in the total phenol content evaluation where the (**e**) by-product, fermented with *P. pentosaceus*, exhibited the highest antioxidant capacity.

As shown in Figure 2B, a slight increase in ABTS scavenging potential in fermented **white-1** corn by-products was found, as only sample (**d**) presents a statistically significant difference with respect to NC. On the contrary, for all fermented **yellow** corn by-products, a great increase in ABTS scavenging potential was evident. In particular, we obtained a remarkable increase in antioxidant activity for (**e**) (788.60 ± 35.23 TEAC) and (**f**) (790.60 ± 85.41 TEAC) samples, compared to the control (580.90 ± 66.68 TEAC). Then, (**e**) fermented **white-2** corn by-product exhibited only a slight but significant increase in a TEAC value (531.06 ± 13.20) with respect to the negative control (474.97 ± 42.18 TEAC).

Therefore, we can conclude that *P. pentosaceus* (**e**) fermented by-products exert the best antioxidant capacity, as observed for the total phenol groups.

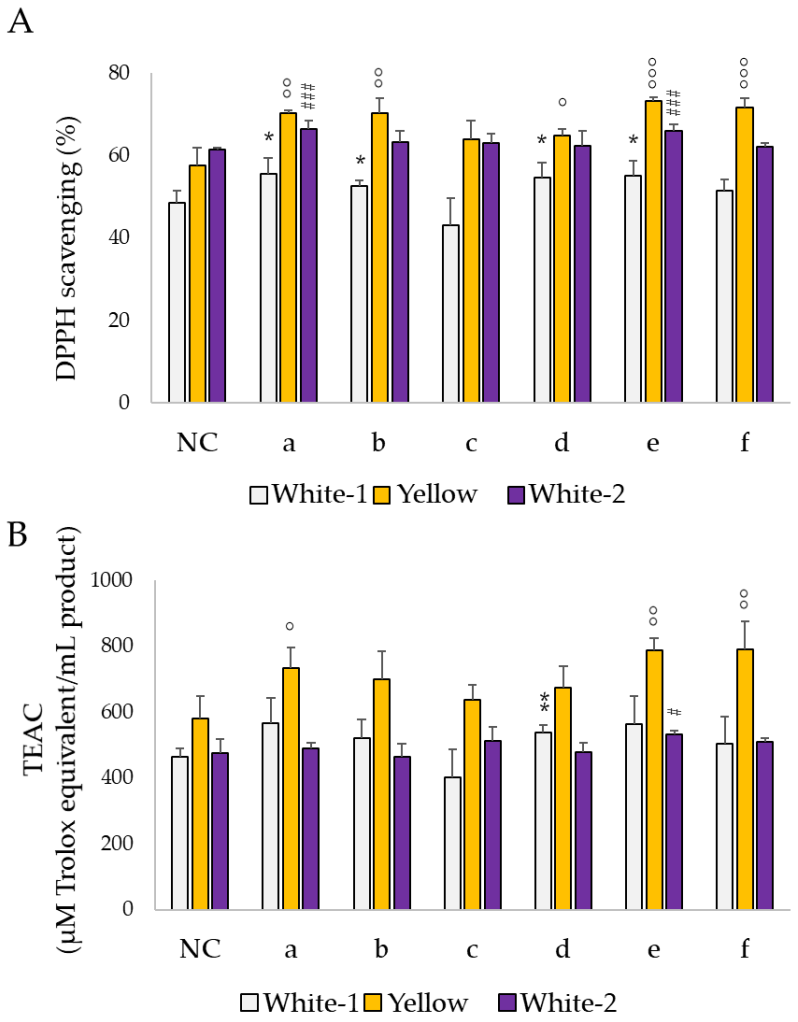

Figure 2. Antioxidant activity of different corn by-product extracts estimated with the DPPH (**A**) and ABTS (**B**) scavenging assays. These tests were carried out by spectrophotometric analysis: DPPH at 517 nm and the cation radical ABTS at 734 nm. In the latter test, the results were reported as Trolox equivalent antioxidant capacity (TEAC, µM Trolox equivalent/mL product). The means of 3 experiments were compared with the specific control values (3 replicates for each experiment). Statistic symbol legend: *, °, and # are reported for **white-1**, **yellow**, and **white-2** corn by-products, respectively. ($^{\circ\circ\circ, \#\#\#}$ $p < 0.001$, $^{**, \circ\circ}$ $p < 0.01$, $^{*, \circ, \#}$ $p < 0.05$).

3.4. TBARS Determination in Soy Beverage in the Presence of Different Corn by-Product Extracts

The capacity of the fermented extracts of the three corn by-products to inhibit lipid peroxidation induced in a food matrix was studied. To this purpose, we used soy beverage as a peroxidizable matrix since soy beverage has a rich content of unsaturated fatty acids that can undergo the peroxidation process. The amount of TBARS products of lipid peroxidation was analyzed in the presence or in the absence of the fermented extracts. Spectrophotometric analysis was carried out, and the absorbance spectra were collected between 500 and 580 nm. The results are expressed as nmoles of TBARS, calculated on the basis of their absorbance at 532 nm. To 0.5 mL of soy matrix, 10 µL of the **white-1, yellow**, and **white-2** fermented corn by-product extracts were added, and lipid peroxidation was induced by cumene hydroperoxide and hemin as reported in Section 2.4.4

By comparing the TBARS production in fermented samples of **white-1** and **yellow** corn by-products to the control sample (**NC**), it is evident that all the values were similar to the control (Table 3). Instead, the TBARS amount in the presence of fermented **white-2** corn by-product extracts was significantly reduced, indicating that the fermentation with specific strains was successful for the improvement of the antioxidant activity. In particular, compared with the non-fermented control (79.66 ± 2.50 nmoles/mL), sample (**e**) (63.36 ± 5.56 nmoles/mL) and especially (**f**) (59.77 ± 9.63 nmoles/mL) significantly decreased the TBARS production.

Table 3. Lipid peroxidation in soy beverage in the presence of corn by-product extracts. Values are expressed in TBARS (nmoles/mL of extract) utilizing the molar extinction coefficient.

Sample	White-1	Yellow	White-2
NC	79.66 ± 2.94	62.61 ± 6.59	79.66 ± 2.90
a	80.04 ± 3.32	59.99 ± 8.49	80.70 ± 6.54
b	80.31 ± 1.28	64.62 ± 5.89	64.46 ± 7.84
c	80.42 ± 1.88	69.09 ± 3.80	86.25 ± 2.78
d	80.10 ± 3.06	77.86 ± 7.36	73.18 ± 6.56
e	81.13 ± 3.74	63.80 ± 4.36	63.37 ± 5.56
f	80.53 ± 1.16	69.53 ± 6.64	59.77 ± 6.63

3.5. LC-DAD-ESI/MS Analysis

Hydroalcoholic extracts of **white-1**, **yellow**, and **white-2** corn by-products non-fermented (NC) and fermented with (**a**) and (**e**) were analyzed by LC-DAD-ESI/MS in order to investigate the polyphenolic derivatives present. In all the analyzed samples, dicoumaroyl spermidine (DCS), diferuloylputrescine (DFP), and *p*-coumaroyl feruloyl putrescine (CFP) were identified by MS/MS spectra of their $[M + H]^+$ ions (Figure S1) and compared with those reported in the literature [20]. These polyphenolic compounds originate from the conjugation of ferulic and *p*-coumaric acids with polyamine and are called hydrocinnamic acid amides (HCAAs) [20]. These hydroxycinnamic acid amides (HCAAs) have been described to be present in maize grain fibers, even if their role is not well known [21]. However, some biological activities of HCAAs have been reported, such as antioxidant, anti-melanogenic, chemopreventive, and antileukemic [22–24]. Due to the presence in their structures of double bonds, different stereoisomers of DCS, DFP, and CFP are detected, which can assume *cis* or *trans*-configuration and can give rise to different combinations, i.e., *trans-trans*, *trans-cis,* and *cis-cis* [25]. These stereoisomers have the same MS/MS spectra but different retention times, and their abundances depend on the varieties of corn. As an example, the chromatograms of ions at *m/z* 441, corresponding to $[M+H]^+$ of DFP, of not fermented (NC), **white-1**, **yellow**, and **white-2** corn by-products are reported in Figure 3. As can be seen, at least two peaks are well defined with r.t. 20.8 and r.t. 17.8. The second one is more abundant for the non-fermented **white-1** variety than for the other two extracts. Literature data indicate that the most abundant peak, with a higher retention time (20.81 ± 0.05), is referred to *trans-trans* isomer, while the other one to *trans-cis* configuration [25]. The same behavior has been observed for DCS and CFP [20,21].

All these stereoisomers slightly increase under the fermentation conditions, and general peaks broadening and shifting were observed for all the samples, probably due to matrix and fermentation effects. For all the extracts, the most abundant polyphenolic derivatives were DCS, as shown in Figure S2.

The obtained data are summarized in Figure 4, where the sum of chromatographic peak areas of all the identified HCAAs is reported vs. the different samples. As a result, all the fermented samples **a** and **e** exhibited an increase in HCAAs. The amount of HCAAs depends on the varieties of corn by-products, and the **yellow** one showed the highest content of polyphenolic derivatives.

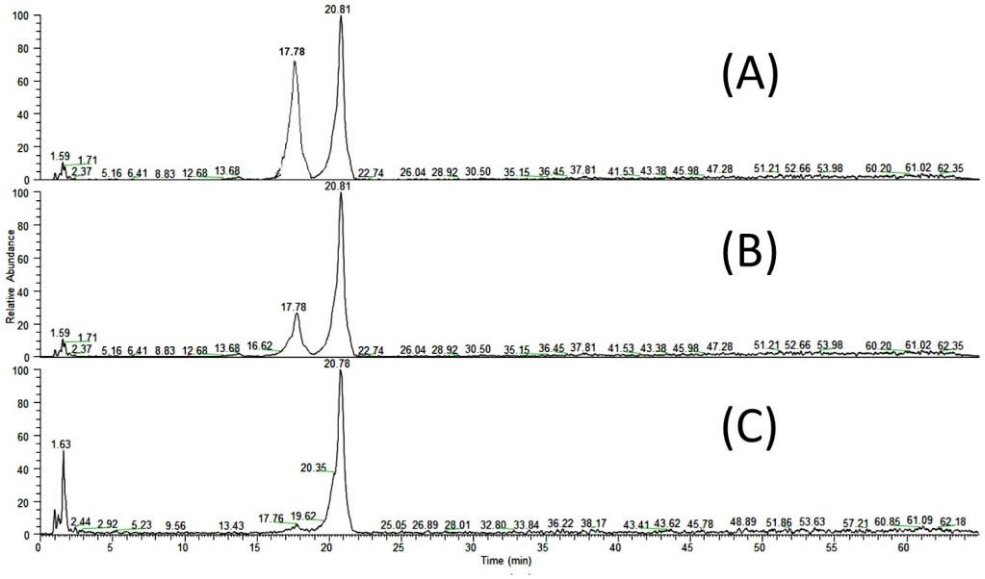

Figure 3. Reconstructed ion chromatograms (RIC) of ions at *m/z* 441, corresponding to $[M + H]^+$ DFP in samples: (**A**) NC **white-1**, (**B**) NC **yellow**, and (**C**) NC **white-2**.

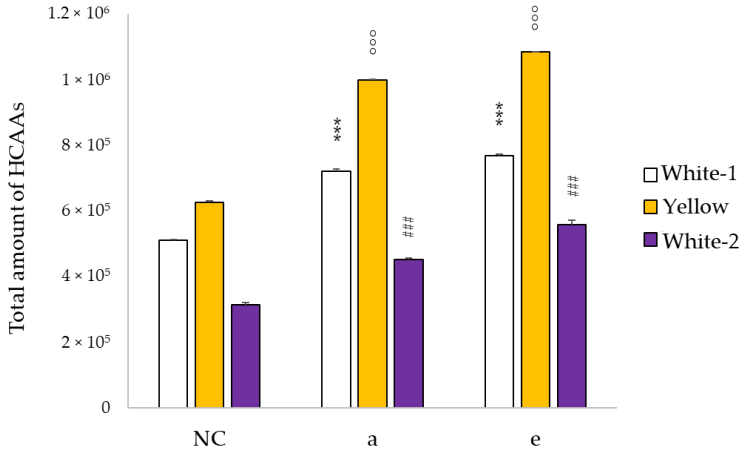

Figure 4. Chromatographic analysis of the fermented extracts for the three analyzed corn by-products. The sum of the chromatographic peak areas of $[M+H]^+$ ions of DCS, DFP, and CFP is reported. The means of 3 experiments were compared with the specific control values (3 replicates for each experiment). Statistic symbol legend: *, °, and # are reported for **white-1**, **yellow**, and **white-2** corn by-products, respectively. (***, °°°, ### $p < 0.001$).

4. Conclusions

In all the corn by-products, the highest counts of lactic acid bacteria were reached in the samples inoculated with *L. plantarum*.

Concerning the antioxidant analysis, it was observed that the **yellow** corn by-product extracts have a higher content of total phenols and antioxidant capacity. Moreover, all the types of corn by-products fermented with *L. plantarum* and *P. pentosaceus* give the best results observed as the increase in antioxidant activity and TPC of the studied extracts of all the corn varieties. In addition, the abundance of antioxidant molecules, in particular DCS, was higher in samples fermented with *P. pentosaceus*, confirming the previous observations. The incremented amount of polyphenols and their derivatives products or their conjugates with other molecules, such as polyamines, are of great interest. Even though they are not considered nutrients, there is a general consensus that these molecules are beneficial for health and potentially able to act in the prevention of pathologies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9040373/s1, Figure S1: MS/MS spectra of $[M + H]^+$ ions of: (a) dicoumaroyl spermidine (DCS, *m/z* 438), (b) diferuloylputrescine (DFP, *m/z* 441) and coumaroyl feruloyl putrescine (CFP, *m/z* 411); Figure S2: Detailed variation of the sum of the chromatographic peak areas of $[M + H]^+$ ions of DCS, DFP, and CFP for the three analyzed corn by-products.

Author Contributions: F.T., A.F. and S.F. obtained the hydroalcoholic extracts, performed antioxidant properties analysis and data analysis, A.L. and C.A. obtained fermented samples, R.S. performed LC-DAD-ESI/MS analysis, A.G. provided samples, conceptualization, M.P.R., O.M. and F.T.; writing—original draft preparation M.P.R. and F.T.; writing—review and editing, M.P.R., F.T., A.L., C.A., R.S., A.G., A.F., S.F. and O.M. All authors have read and agreed to the published version of the manuscript.

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