Valorization of Cereal By-Products from the Milling Industry as a Source of Nutrients and Bioactive Compounds to Boost Resource-Use Efficiency

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Abstract: Cereal by-products (wheat germ, maize bran–germ mixture, rye bran, and wheat bran) from the flour milling industry were characterized for their nutritional value and chemical composition, as well as for antioxidant and antibacterial activities. Carbohydrates (including sucrose) were the major nutritional constituents (56.35–78.12 g/100 g dw), followed by proteins (11.2–30.0 g/100 g dw). The higher energy value (432.3 kcal/100 g dw) was presented by the wheat germ. This by-product also presented the highest citric acid content (0.857 g/100 g dw), the most abundant organic acid detected. Unsaturated fatty acids predominated in all samples given the high content of linoleic (53.9–57.1%) and oleic (13.4–29.0%) acids. Wheat germ had the highest levels of tocopherols (22.8 mg/100 g dw) and phenolic compounds (5.7 mg/g extract, with a high apigenin-C-pentoside-C-hexoside content). In turn, while the wheat bran extract was particularly effective in inhibiting the formation of thiobarbituric acid reactive substances (TBARS), the rye bran extract was the only sample capable of protecting erythrocytes from oxidative hemolysis. Regarding antibacterial properties, in general, the lowest minimum inhibitory concentrations were observed against methicillin-resistant Staphylococcus aureus. These results highlight the characterized by-products as sustainable ingredients for the development of novel bakery and functional food products and contribute to a better bioresource-use efficiency and circularity.

Keywords: cereal bran/germ; nutritional quality; chemical composition; antioxidant activity; antimicrobial activity; bioresource-use efficiency

1 Introduction

Cereal grains are rich in phytochemicals and nutrients, such as phenolic acids, flavonoids, carbohydrates, dietary fibers, proteins, and tocopherols, among other constituents, which have a vital role in preventing cardiovascular and digestive system diseases, overweight and obesity, inflammation, type 2 diabetes, and some types of cancer [1]. Some studies have shown that consumers are increasingly aware of the relationship between diet and disease, and there is a trend towards a gradual decrease in the consumption of animal-derived protein and a demand for plant-based diets, which have well-known physical and environmental health benefits [2,3].

The food industry has focused on the production of functional foods based on different types of cereals, due to the growing consumer’s demand for healthier foods [4,5]. For this purpose, both cereals and their constituents offer unlimited potential and are an excellent raw material for the production of functional foods and functional ingredients, in particular for the design of novel food products based on cereals or their by-products [6]. Cereals...
include rice, wheat, rye, maize, barley, sorghum, millet, and oats, among others, and their
global production is very extensive as they are the basis of many human diets worldwide.
Therefore, the sector is taking into account the sustainability and the efficient use of the
by-products generated by the crops or during cereal processing [7]. These result in valuable
by-products during milling, such as bran, germ, coat, husk, or endosperm, which could
be a good source of potentially marketable ingredients and bioactive compounds [6]. The
milling industries commonly release these by-products in the field or direct them to animal
feed, bioethanol production, cosmetics, meat substitutes, and nutraceutical/pharmaceutical
products, among other applications [8].

During the milling operation, the endosperm is broken down into fine particles
(flour), while the germ and bran are removed. The germ is a good source of vitamins (B
and tocopherol), minerals, proteins, dietary fiber, carbohydrates, fatty acids, flavonoids,
glutathione, and sterols [8–11]. The bran contains fibers and antioxidants, heteroxylans,
cellulose, proteins, starch, phenolic acids, lipids, and minerals [12,13].

With the growing world population and given the limited resources our planet can
provide, it is essential to produce enough food to meet the growing demands and needs of
the human population and also to ensure food security. However, with restricted arable
land, the agri-food industry by-products should become recycled within the food chain
and, thus, be valorized as a sustainable source of food and food ingredients [6,14], also
promoting the circular economy. Therefore, this study was performed to provide a detailed
nutritional and chemical characterization of cereal by-products, namely wheat, maize, and
rye bran and/or germ, currently produced in large quantities by food ingredients indus
trial groups, but which have low or no commercial value. Antioxidant and antibacterial
activities were also evaluated after preparation of hydroethanolic extracts with these cereal
by-products. Overall, it was intended to demonstrate that these milling by-products can
have value-added potential in the food market as a low-cost material for the development
of nutrient-rich ingredients and bioactive compounds.

2. Materials and Methods

2.1. Samples and Samples Preparation

Cereal by-products (wheat germ, maize bran–germ mixture, rye bran, and wheat bran)
were kindly supplied by the “Dacsá Group”, a food ingredients industrial group from
Almàssera-Valencia, Spain, in March 2018. The dry samples were reduced to a fine powder,
packaged in sealed plastic bags, and stored at −20 °C until further analysis.

2.2. Standards and Reagents

High-performance liquid chromatography (HPLC)-grade acetonitrile (99.9%), n-hexane
(95%), and ethyl acetate (99.8%) were purchased from Fisher Scientific (Lisbon, Portugal).
The fatty acid methyl ester standard mixture 47885-U, formic acid, 6-hydroxy-2,5,7,8-
tetramethylchroman-2-carboxylic acid (Trolox), L-ascorbic acid, 2,2′-Azobis(2-aminopropane)
dihydrochloride (AAPH), and the tocopherol (α, β, γ, and δ isoforms), free sugar, and
organic acid standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). Pheno-
lolic compounds standards were acquired from Extrasynthèse (Genay, France). Thiamine,
casamino acids, malt extract, and agar were obtained from Panreac AppliChem (Barcelona,
Spain). PDA and PDB mediums were acquired from Oxoid microbiology products (Ham
shire, UK). p-Iodonitrotetrazolium chloride (INT) was purchased from Panreac AppliChem
(Barcelona, Spain), Tryptic Soy Broth (TSB), and Mueller- Hinton (MH) from Biolab®
(Budapest, Hungary). All other reagents and solvents were of analytical grade and obtained
from common sources. Water was treated in a Milli-Q water purification system (TGI Pure
Water Systems, Greenville, SC, USA).

2.3. Determination of Nutrients and Energy Value

Fat, protein, and ash contents were determined by AOAC procedures [15]. The crude
fat content was determined by Soxhlet extraction of the samples with petroleum ether
The ash (total mineral) content was determined by samples incineration at 550 ± 15 °C (AOAC 923.03). The crude protein content was analyzed using an automatic distillation and titration unit (Pro-Nitro-A model, JP Selecta, Barcelona) by the macro-Kjeldahl method (AOAC 978.04) (N × correction factor, namely 5.70 for wheat germ, 6.25 for maize bran/germ mixture, 5.83 for rye bran, and 6.31 for wheat bran) [16]. The results were expressed in g per 100 g of dry weight (dw).

Total carbohydrates were calculated by difference according to Equation (1).

\[
\text{Carbohydrates (g/100 g dw) = 100} - (\text{g protein + g fat + g ash}) \quad (1)
\]

The energy was calculated according to the Regulation (EU) No 1169/2011 of the European Parliament and of the Council [17], as shown in Equation (2).

\[
\text{Energy (kcal/100 g dw) = 4 \times (g protein + g carbohydrates) + 9 \times (g fat)} \quad (2)
\]

### 2.4. Evaluation of Chemical Constituents

#### 2.4.1. Organic Acids

The organic acids profile was analyzed by ultra-fast liquid chromatography (UFLC; Shimadzu 20A series, Kyoto, Japan) following a procedure previously described and optimized by Pereira et al. [18]. Briefly, the samples (1 g) were stirred with 25 mL of meta-phosphoric acid for 45 min and filtered, first through Whatman No. 4 filter paper and then through 0.2 µm nylon filters. Chromatographic separation was achieved in reverse phase on a C18 column (5 µm particle size, 250 × 4.6 mm; Phenomenex, Torrance, CA, USA). Detection was performed in a photo-diode array detector (PDA), at 215 and 245 nm (for ascorbic acid). The detected compounds were identified and quantified by chromatographic comparison of the peak area with calibration curves obtained from commercial standards of oxalic (\(y = 1 \times 10^7x + 231,891; r^2 = 0.9999\); limit of detection (LOD) = 6.3 µg/mL; limit of quantification (LOQ) = 20.8 µg/mL), malic (\(y = 950,041x + 6255.6; r^2 = 0.9999\); LOD = 15.9 µg/mL; LOQ = 52.9 µg/mL), ascorbic (\(y = 4 \times 10^7x + 1 \times 10^6; r^2 = 0.9909\); LOD = 0.29 µg/mL; LOQ = 0.96 µg/mL), shikimic (\(7 \times 10^7x + 175,156; r^2 = 0.9999\); LOD = 10.2 µg/mL; LOQ = 56.5 µg/mL), citric (\(y = 1 \times 10^7x - 10,277; r^2 = 0.9997\); LOD = 4.4 µg/mL; LOQ = 14.5 µg/mL), and fumaric (\(y = 185,062x + 117,588; r^2 = 1\); LOD = 42.5 µg/mL; LOQ = 141.7 µg/mL) acids. The results were expressed in g per 100 g dw.

#### 2.4.2. Free Sugars

Soluble sugars were analyzed in a HPLC system coupled to a refraction index (RI) detector as previously described by Spréa et al. [19]. Briefly, the samples (1 g) were spiked with melezitose (internal standard, 5 mg/mL) and extracted with 80% ethanol at 80 °C. The mixture was centrifuged and the supernatant was concentrated and defatted with ethyl ether. After concentration at 40 °C, the residues were dissolved in 5 mL of water and filtered through 0.2-µm nylon filters. Identification was achieved by comparing the sample retention times with those of the authentic standards, while quantification was based on the internal standard method, with calibration curves constructed with commercial standards of fructose (\(y = 1.04x; r^2 = 0.9999\); LOD = 0.05 mg/mL; LOQ = 0.18 mg/mL), glucose (\(y = 0.935x; r^2 = 0.9999\); LOD = 0.08 mg/mL; LOQ = 0.25 mg/mL), sucrose (\(y = 0.977x; r^2 = 0.9999\); LOD = 0.06 mg/mL; LOQ = 0.21 mg/mL), trehalose (\(y = 0.991x; r^2 = 0.9999\); LOD = 0.07 mg/mL; LOQ = 0.24 mg/mL), and raffinose (\(y = 0.891x; r^2 = 0.9999\); LOD = 0.09 mg/mL; LOQ = 0.30 mg/mL). The results were expressed in g per 100 g dw.

#### 2.4.3. Fatty Acids

After transesterification of the lipid fraction obtained by Soxhlet extraction [19], the fatty acid methyl ester (FAME) mixture was analyzed by gas-liquid chromatography with flame ionization detection, using a YOUNG IN Chromass 6500 GC System apparatus equipped with a split/splitless injector, a flame ionization detector (FID), and a Zebron-
Fame column. Identification were made by chromatographic comparison of the retention times of the sample FAME peaks with those of the standard 47885-U (Sigma-Aldrich, St. Louis, MO, USA). The results were recorded and processed using Clarity DataApex 4.0 Software (Prague, Czech Republic) and expressed in relative percentage of each fatty acid.

2.4.4. Tocopherols

Tocopherols were analyzed accordingly to a procedure described by Spréa et al. [19], using the HPLC system coupled to a fluorescence detector (FP-2020; Jasco), programmed for excitation at 290 nm and emission at 330 nm. Briefly, the samples (500 mg) were spiked with a BHT solution (10 mg/mL) and tocol (internal standard, 50 µg/mL), and homogenized first with 4 mL of methanol and then with 4 mL of hexane. Then, 2 mL of saturated NaCl aqueous solution were added, the mixture was homogenized, centrifuged, and the upper layer was collected. The extraction was repeated twice with hexane. The extracts were dried under a nitrogen stream, redissolved in 2 mL of n-hexane, dehydrated, and filtered through 0.22-µm syringe filters. Chromatographic separation was performed in normal phase on a Polyamide II column (5 µm particle size, 250 × 4.6 mm; YMC, Kyoto, Japan). Identification was made by chromatographic comparison with authentic standards and quantification was based on the fluorescence signal response of each standard, using the internal standard (tocol) method and calibration curves constructed from commercial standards of α-tocopherol (y = 1.295x; \( r^2 = 0.991 \); LOD = 18.06 ng/mL; LOQ = 60.20 ng/mL), β-tocopherol (y = 0.396x; \( r^2 = 0.992 \); LOD = 25.82 ng/mL; LOQ = 86.07 ng/mL), γ-tocopherol (y = 0.567x; \( r^2 = 0.991 \); LOD = 14.79 ng/mL; LOQ = 49.32 ng/mL), and δ-tocopherol (y = 0.678x; \( r^2 = 0.992 \); LOD = 20.09 ng/mL; LOQ = 66.95 ng/mL). The results were expressed in mg per 100 g dw.

2.5. Evaluation of Phenolic Compounds and Bioactivities

2.5.1. Extracts Preparation

The dry material was used to prepare hydroethanolic extracts as described by Mariotti et al. [16]. The samples (2.5 g) were mixed with 30 mL of ethanol/water (80:20, v/v) and stirred for 1 h at 25 °C. The mixtures were then filtered through Whatman No. 4 filter paper and the residues were re-extracted with 30 mL of the same hydroalcoholic mixture. The extracts were first concentrated under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland) at 40 °C and then lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA).

2.5.2. Phenolic Compounds

Phenolic compounds were analyzed in the hydroethanolic extracts, which were re-dissolved in ethanol/water (80:20, v/v) to a final concentration of 10 mg/mL and filtered through 0.22-µm disposable filter disks. The analysis was performed in an HPLC system (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA) coupled with a diode-array detector (DAD, using 280 and 370 nm as preferred wavelengths) and a Linear Ion Trap (LTQ XL) mass spectrometer (MS, Thermo Finnigan, San Jose, CA, USA) equipped with an electrospray ionization (ESI) source. Chromatographic separation was performed on a Waters Spherisorb S3 ODS-2 column (3 µm, 4.6 mm × 150 mm; Waters, Milford, MA, USA). The operating conditions were previously described by the Bessada et al. [20], as well as the identification and quantification procedures. The results were given as mg per g of extract.

2.5.3. Antioxidant Activity

The thiobarbituric acid reactive substances (TBARS) formation inhibition and oxidative hemolysis inhibition (OxHLIA) assays were performed in vitro. Trolox was used as a positive control in both assays. For TBARS, the lyophilized extracts were re-dissolved in ethanol/water (80:20, v/v) and subjected to successive dilutions from 10 to 0.019 mg/mL. The lipid peroxidation inhibition was evaluated by the decrease in TBARS formation, using
porcine (*Sus scrofa*) brain cell homogenates. The color intensity of the malondialdehyde–thiobarbituric acid adduct in the mixtures was monitored spectrophotometrically at 532 nm. The inhibition ratio (%) was calculated using the following formula: 
\[
\left( \frac{A-B}{A} \right) \times 100
\]
where A and B correspond to the absorbance of control and sample solution, respectively [16]. The results were expressed in IC$_{50}$ values (mg/mL), which translate the extract concentration providing 50% of antioxidant activity. For OxHLIA, the anti-hemolytic activity was assessed as described by Silva de Sá et al. [21]. Briefly, an erythrocyte solution (2.8%, v/v; 200 µL) was mixed with 400 µL of either: (i) extract solution (0.106–3.4 mg/mL in PBS); (ii) PBS (control); (iii) water (for complete hemolysis); or (iv) trolox (7.81–250 µg/mL). After pre-incubation at 37 °C for 10 min with shaking, 200 µL of AAPH (160 mM in PBS) were added and the optical density was measured at 690 nm every ~10 min in a microplate reader (Bio-Tek Instruments, ELX800) until complete hemolysis. The results were expressed as IC$_{50}$ values (mg/mL), translating the extract concentration able to promote a Δt hemolysis delay of 60 and 120 min.

2.5.4. Antibacterial Activity

The antibacterial activity was evaluated by the broth microdilution method coupled to the rapid p-iodonitrotetrazolium chloride (INT) colorimetric assay [22]. The tested microorganisms were clinical isolates from patients hospitalized in various departments of the Local Health Unit of Bragança and Hospital Center of Trás-os-Montes and Alto-Douro Vila Real, Northeast of Portugal, and included three Gram-positive (*Enterococcus faecalis*, *Listeria monocytogenes*, and methicillin resistant *Staphylococcus aureus*) and five Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*) bacteria. The microorganisms’ identification and susceptibility (Table S1) tests were performed as described in Supplementary Material. The minimum inhibitory concentration (MIC) was determined by the colorimetric microbial viability based on the reduction of the INT colorant (0.2 mg/mL). The minimum bactericidal concentration (MBC) was evaluated by plating the wells content without coloration in the MIC assay. Antibiotics were used as positive controls, namely ampicillin (20 mg/mL) and imipenem (1 mg/mL) for Gram-negative bacteria, and vancomycin (1 mg/mL) and ampicillin (20 mg/mL) for Gram-positive bacteria.

2.6. Statistical Analysis

All analyses were performed in triplicate and the results were presented as mean ± standard deviation (SD) (except for antibacterial activity). Since the number of significant figures of the mean value is conditioned by the SD, the SD was rounded to one significant figure, which indicated in which decimal place the uncertain digit of the mean value was. The results were analyzed by a one-way analysis of variance (ANOVA) followed by a Tukey’s HSD test, with α = 0.05. A Student’s *t*-test was applied when only two samples were compared. SPSS v. 23.0 was used for all statistical analysis.

3. Results and Discussion

Cereal by-products represent an unexploited source of nutrients and bioactive compounds and could serve as low-cost materials for the development of novel functional and fortifying ingredients for foods and functional foods, as well as for non-food products. Therefore, the macronutrient and micronutrient composition of wheat, rye, and maize bran and/or germ supplied by the milling industry are herein described, as well as their composition in phenolic compounds and in vitro antioxidant and antibacterial activities.

3.1. Compositional Features of the Cereal By-Products

The results in Table 1 show the proximate composition of the different cereal by-products, including fat, protein, ash, and carbohydrates content and energy value. Carbohydrates were the most prevalent constituents, ranging from 56.35 g/100 g dw in wheat germ to 78.12 g/100 g dw in maize bran–germ mixture. The results are slightly higher
than the 46.07 g/100 g dw previously reported by Mahmoud et al. [23] for wheat germ sample from Egypt. Proteins were the second most abundant nutrients and the contents differed significantly between cereal by-products. Interestingly, the samples with the lowest carbohydrate content had the highest protein level. As shown in Table 1, 30.0 g/100 g dw were quantified in wheat germ, whose 100-g portions can provide 53.6 and 65.2% of the recommended dietary allowances of protein for adult male and female individual, respectively [24]. The two by-products with germ, namely wheat germ and the maize bran–germ mixture, presented higher quantities of crude fat (9.64 and 8.08 g/100 g dw, respectively) than the bran samples. Navarro et al. [25] and Mahmoud et al. [23] reported a higher fat content in maize bran–germ (10.74 g/100 g dw) and wheat germ (10.29 g/100 g dw) samples from Brazil and Egypt, respectively. Fats are important nutritional constituents of cereal by-products, being a source of essential fatty acids and energy, and play an important role in their quality [26]. Overall, in terms of energetic contribution, wheat germ presented the highest value (432.3 kcal/100 g dw) in accordance with the higher fat and protein contents, followed by the maize bran–germ mixture (429.8 kcal/100 g dw) and wheat bran (412.4 kcal/100 g dw). The rye bran samples showed the lowest energy value, reaching only 405.4 kcal/100 g dw. Mahmoud et al. [23] reported similar energy values for wheat germ (430.3 kcal/100 g dw), while the National Food Institute [27] presents lower values for wheat germ (379 kcal/100 g), wheat bran (291 kcal/100 g), and common rye flour (322 kcal/100 g). The INRAE-CIRAD-AFZ [28] composition tables present high energy values for maize germ (690 kcal/100 g) and bran (457 kcal/100 g). However, to the best of the authors’ knowledge, this is the first study describing the energy value of the maize bran–germ mixture and rye bran.

### Table 1. Proximate composition and energy value of the cereal by-products.

|                        | Wheat Germ | Maize Bran–Germ | Rye Bran | Wheat Bran |
|------------------------|------------|-----------------|----------|------------|
| Fat (g/100 g dw)       | 9.64 ± 0.01 a | 8.08 ± 0.01 b | 3.9 ± 0.1 d | 5.05 ± 0.02 c |
| Proteins (g/100 g dw)  | 30.0 ± 0.1 a | 11.2 ± 0.2 d   | 15.3 ± 0.1 c | 16.4 ± 0.1 b |
| Ash (g/100 g dw)       | 3.97 ± 0.05 a | 2.66 ± 0.04 d  | 3.54 ± 0.04 b | 3.12 ± 0.04 c |
| Carbohydrates (g/100 g dw) | 56.35 ± 0.02 d | 78.12 ± 0.04 a | 77.2 ± 0.1 b | 75.42 ± 0.01 c |
| Energy (kcal/100 g dw) | 432.3 ± 0.1 a | 429 ± 0.2 b    | 405.4 ± 0.01 d | 412.8 ± 0.1 c |

In each line, different letters indicate significant differences (p < 0.05) between samples. The number of significant figures of each mean value was conditioned by the standard deviation, which was rounded to one significant figure.

Table 2 shows the free sugars and organic acids detected in the cereal by-products, namely five sugars and six organic acids characterized by HPLC-RI and UFLC-PDA, respectively. Regarding free sugars, sucrose was the most abundant in all cereal by-products, reaching 10.4 g/100 g dw in wheat germ, 3.84 g/100 g dw in the maize bran–germ mixture, and approximately 2.9 g/100 g dw in the bran samples. These results are in agreement with those of Rizzello et al. [29], who also reported sucrose as the main soluble sugar in wheat germ (7.2 g/100 g dw). Table 2 also shows that the free sugar profile of the different samples is different, as some of these water-soluble molecules were not detected in some of the studied cereal by-products. Trehalose and fructose were detected only in wheat germ (0.25 g/100 g dw) and maize bran–germ mixture (0.15 g/100 g dw), respectively, while rye bran did not contain glucose. Overall, the total amount of free sugars ranged from 3.51 g/100 g dw to 15.2 g/100 g dw in wheat germ, this last sample with a considerable difference towards the other cereal by-products. The National Food Institute [27] reported a similar value for a wheat germ sample from Denmark, namely 15.7 g/100 g dw of total free sugars. It is also interesting to note that free sugars correspond to 27% of the carbohydrate fraction of wheat germ and less than 6% in the remaining samples.
Table 2. Composition in sugars and organic acids of the cereal by-products.

|                     | Wheat Germ | Maize Bran–Germ | Rye Bran | Wheat Bran |
|---------------------|------------|-----------------|----------|------------|
| Free sugars (g/100 g dw) |            |                 |          |            |
| Fructose            | nd         | 0.15 ± 0.02     | nd       | nd         |
| Glucose             | 0.18 ± 0.01 c | 0.16 ± 0.01 b | nd       | 0.14 ± 0.01 a |
| Sucrose             | 10.4 ± 0.1 a | 3.84 ± 0.01 b   | 2.92 ± 0.03 c | 2.9 ± 0.1 c |
| Trehalose           | 0.25 ± 0.01 | nd              | nd       | nd         |
| Raffinose           | 4.65 ± 0.03 a | 0.4 ± 0.1 d | 0.59 ± 0.01 c | 1.69 ± 0.01 b |
| Total of free sugars| 15.2 ± 0.1 a | 4.4 ± 0.1 c    | 3.51 ± 0.02 d | 4.7 ± 0.1 b |
| Organic acid (g/100 g dw) |        |                 |          |            |
| Oxalic acid         | 0.090 ± 0.001 b | 0.105 ± 0.001 a | 0.0471 ± 0.0001 d | 0.0593 ± 0.0001 c |
| Malic acid          | nd         | nd              | tr       | tr         |
| Ascorbic acid       | tr         | tr              | nd       | nd         |
| Shikimic acid       | nd         | nd              | tr       | tr         |
| Citric acid         | 0.857 ± 0.002 a | 0.204 ± 0.001 d | 0.424 ± 0.001 c | 0.539 ± 0.001 b |
| Fumaric acid        | tr         | tr              | tr       | tr         |
| Total of organic acids | 0.946 ± 0.002 a | 0.309 ± 0.001 d | 0.471 ± 0.001 c | 0.598 ± 0.001 b |

In each line, different letters indicate significant differences (p < 0.05) between samples. The number of significant figures of each mean value was conditioned by the standard deviation, which was rounded to one significant figure. nd—not detected; tr—traces (below LOQ).

Regarding organic acids, malic, ascorbic, shikimic, and fumaric acids were detected in trace amounts and just in some samples (Table 2). Citric acid was the main compound identified in the cereal by-products, and its levels contribute greatly to the total amounts of organic acids recorded in wheat germ (0.946 g/100 g dw), followed by wheat bran (0.598 g/100 g dw), rye bran (0.471 g/100 g dw), and maize bran–germ mixture with the lowest levels (0.309 g/g 100 g dw). Oxalic acid was also detected in all samples, especially in the maize bran–germ mixture (0.105 g/100 g dw). To the best of the authors knowledge, this is the first report describing the organic acids composition of these by-products. However, wheat is recognized as a complete cereal because it has much higher soluble sugars and organic acids than other cereals, regardless of the cereal part under analysis (bran or germ). Sugars and organic acids are important quality indicators that can be related to preservation and storage conditions [30,31].

Table 3 shows the lipophilic compounds detected in the cereal by-products, namely fatty acids and tocopherols. The GC-FID analysis allowed the detection of 13 fatty acids, whose contents are presented in relative percentage. Linoleic acid (C18:2n6c) was the most abundant fatty acid, with levels up to 53% in all samples. Similar C18:2n6c contents (55.1 and 54.4%) were reported by Mahmoud et al. [23] for a wheat germ oil sample from Egypt and by Abdelghany et al. [32] for soybean seed accessions collected in China, respectively. This essential fatty acid is precursor of eicosanoids, which take part in many biological processes, and required for normal human health. Oleic acid (C18:1n9c) ranked second and prevailed in the maize bran–germ mixture (29%). This omega-9 fatty acid was also previously reported as the second most abundant in soybean in a slightly lower relative percentage (24.6%) [32]. Regarding saturated fatty acids (SFA), palmitic acid (C16:0) was the major contributor and prevailed in wheat bran and germ samples (with approximately 18%), while stearic acid (C18:0) predominated in the maize bran–germ mixture (2.96%). In turn, the higher amounts of monounsaturated fatty acids (MUFA) were quantified in the maize bran–germ mixture (29.2%). In general, polyunsaturated fatty acids (PUFA) predominated in all samples, with levels ranging from 55.9 to 65.4%. The fatty acid profiles observed in the characterized cereal by-products are in agreement with previous reports [33–36]. Fatty acids are good food quality indicators, since lipid peroxidation affects the overall quality and odor of cereals when stored for a long period [30,37].
Table 3. Composition in fatty acids and tocopherols of the cereal by-products.

| Fatty acids (relative %)                      | Wheat Germ | Maize Bran–Germ | Rye Bran | Wheat Bran |
|---------------------------------------------|------------|-----------------|----------|------------|
| C14:0                                       | 0.12 ± 0.01 b | nd              | 0.14 ± 0.01 a | 0.126 ± 0.001 b |
| C15:0                                       | 0.073 ± 0.003 c | nd              | 0.15 ± 0.01 a | 0.104 ± 0.001 b |
| C16:0                                       | 0.12 ± 0.02 a | 10.6 ± 0.3 c    | 15.59 ± 0.04 b | 18.3 ± 0.2 a |
| C16:1                                       | 0.17 ± 0.02 c | 0.101 ± 0.004 d | 0.27 ± 0.02 a | 0.20 ± 0.01 b |
| C18:0                                       | 0.86 ± 0.01 c | 2.955 ± 0.001 a | 1.3 ± 0.1 b  | 1.32 ± 0.01 b |
| C18:1n9c                                    | 13.4 ± 0.1 d | 29.0 ± 0.1 a    | 17.31 ± 0.04 b | 16.07 ± 0.04 c |
| C18:2n6c                                    | 57.1 ± 0.1 a | 54.8 ± 0.2 c    | 53.9 ± 0.1 d | 56.3 ± 0.2 b |
| C18:3n3                                     | 8.0 ± 0.1 b  | 1.04 ± 0.01 d   | 8.6 ± 0.1 a  | 5.17 ± 0.05 c |
| C20:0                                       | 0.205 ± 0.001 c | 0.48 ± 0.01 a | nd        | 0.24 ± 0.01 b |
| C20:1                                       | 1.35 ± 0.03 b | 0.168 ± 0.005 d | 1.456 ± 0.003 a | 1.0 ± 0.1 c |
| C22:0                                       | 0.25 ± 0.02 c | 0.36 ± 0.03 b   | 0.42 ± 0.01 a | 0.41 ± 0.01 a |
| C20:5n3                                     | 0.30 ± 0.02 c | nd              | 0.47 ± 0.01 a | 0.40 ± 0.02 b |
| C24:0                                       | 0.20 ± 0.01 d | 0.46 ± 0.01 b   | 0.49 ± 0.03 a | 0.29 ± 0.01 c |
| SFA                                         | 19.73 ± 0.02 b | 14.9 ± 0.3 d   | 18.1 ± 0.1 c | 20.8 ± 0.2 a |
| MUFA                                        | 14.9 ± 0.1 d  | 29.2 ± 0.1 a    | 19.03 ± 0.02 b | 17.3 ± 0.1 c |
| PUFA                                        | 65.4 ± 0.1 a  | 55.9 ± 0.2 d    | 62.9 ± 0.1 b | 61.9 ± 0.3 c |

Tocopherols (mg/100 g dw)

| Tocopherol                  | Wheat Germ | Maize Bran–Germ | Rye Bran | Wheat Bran |
|-----------------------------|------------|-----------------|----------|------------|
| α-Tocopherol                | 13.46 ± 0.01 a | 3.12 ± 0.02 d  | 4.1 ± 0.1 c | 5.23 ± 0.04 b |
| β-Tocopherol                | 9.27 ± 0.04 a | 0.12 ± 0.03 d   | 1.27 ± 0.01 c | 2.99 ± 0.01 b |
| γ-Tocopherol                | nd         | 1.61 ± 0.03 a   | nd       | 0.140 ± 0.001 b |
| δ-Tocopherol                | 0.046 ± 0.001 | nd     | nd | nd         |
| Total of tocopherols        | 22.8 ± 0.1 a  | 5.10 ± 0.02 d  | 5.4 ± 0.1 c | 8.35 ± 0.04 b |

In each line, different letters indicate significant differences (p < 0.05) between samples. The number of significant figures of each mean value was conditioned by the standard deviation, which was rounded to one significant figure. SFA—saturated fatty acids; MUFA—monounsaturated fatty acids; PUFA—polyunsaturated fatty acids; nd—not detected.

The results of the tocopherols composition are also shown in Table 3. α-Tocopherol was found in all cereal by-products and as the predominant isoform, especially in wheat germ (13.46 mg/100 g dw), where it was possible to detected δ-tocopherol. The study of Görnáš et al. [38] reports lower α-tocopherol values for wheat and rye bran (1.9 and 1.4 mg/100 g dw, respectively) than those achieved in the present study. In turn, higher levels were obtained by Ansolin et al. [39] in wheat germ oil (28.5 mg/100 g) and by Navarro et al. [25] in maize bran–germ oil (13 mg/100 g dw). Wheat bran and germ samples stand out with the highest amounts of β-tocopherol (2.99 and 9.27 mg/100 g dw, respectively). Only two samples contained γ-tocopherol, namely the maize bran–germ mixture and wheat bran (1.61 and 0.14 mg/100 g dw, respectively). The tocopherol profiles herein reported are in agreement with those previously reported [9,25,38]. Overall, wheat by-products showed the highest concentration of tocopherols, with 22.8 mg/100 g dw quantified in the germ and 8.35 ± 0.04 mg/100 g dw in the bran. Based on the α-tocopherol values in Table 3 and the recommended dietary allowances for vitamin E of 15 mg/day for healthy adults [24], it could be concluded that a 100-g portion of wheat germ contributes 61.8% to the intake of this liposoluble vitamin.

3.2. Phenolic Composition of the Cereal By-Products

Cereal by-products are important sources of phytochemicals such as phenolic compounds, which have gained major attention since they exhibit a wide range of biological activities, including antioxidant and antimicrobial effects [40,41]. The chromatographic information (retention time, wavelengths of maximum absorption in the visible region (λmax), and mass spectral data) used in the tentative identification of the phenolic compounds detected in the hydroethanolic extracts prepared with the cereals by-products are shown in Table 4. Eleven phenolic compounds were tentatively identified in the extracts, including 4
phenolic acids (p-coumaroyl, caffeic, and chlorogenic acid derivatives), 6 flavonoids (luteolin and apigenin C-glycosylated derivatives) and one unknown compound that was found in maize bran–germ and rye bran samples. Regarding phenolic acids, peak 1 presented a pseudomolecular ion [M-H]− at m/z 487 and a unique MS² fragment at m/z 163 (loss of two hexosyl moieties), coupled to a characteristic UV spectrum of p-coumaric acid at 301 nm, the peak was tentatively identified as p-coumaric acid dihexoside. Peaks 2 and 3 were tentatively identified as caffeic acid hexoside, presenting a pseudomolecular ion [M-H]− at m/z 341 and MS² fragments at m/z 179, 161, and 135, that correspond to the break of the caffeic acid unit and the loss of one hexosyl moiety (341 – 179 = 162 u). Finally, peak 9, with [M-H]− at m/z 515 and λmax 311 nm (characteristic of chlorogenic acid derivatives), was tentatively identified as 3,5-O-dicaffeoylquinic acid using the hierarchical fragmentation pattern previously reported by Clifford et al. [42,43].

Table 4. Phenolic compounds tentatively identified in the hydroethanolic extracts of the cereal by-products. It is presented the retention time (Rt), wavelengths of maximum absorption in the visible region (λmax), and mass spectral data.

| Peak | Rt (min) | λmax (nm) | [M-H]− (m/z) | MS² (m/z) | Tentative Identification |
|------|----------|-----------|---------------|-----------|-------------------------|
| 1    | 4.7      | 301       | 487           | 162 (100) | p-Coumaric acid dihexoside |
| 2    | 4.8      | 311       | 341           | 179(100), 161(15), 132(5) | Caffeic acid hexoside |
| 3    | 5.43     | 311       | 341           | 179(100), 161(15), 132(5) | Caffeic acid hexoside |
| 4    | 11.45    | 345       | 579           | 459(35), 429(10), 357(5), 327(10), 309(5) | Luteolin-O-pentoside-C-hexoside |
| 5    | 12.11    | 287       | 385           | 267 (100), 249(20) | Unknown compound |
| 6    | 12.86    | 336       | 563           | 545(43), 473(100), 443(7), 383(31), 353(28), 311(5) | Apigenin-C-pentoside-C-hexoside |
| 7    | 13.21    | 326       | 563           | 545(20), 473(92), 443(100), 383(28), 353(25), 311(5) | Apigenin-C-hexoside-C-pentoside |
| 8    | 14.21    | 336       | 563           | 545(32), 473(100), 443(98), 383(38), 353(31), 311(5) | Apigenin-C-pentoside-C-hexoside |
| 9    | 17.11    | 324       | 515           | 353(60), 191(100), 179(30), 173(5), 161(5), 135(5) | 3,5-O-Dicaffeoylquinic acid |
| 10   | 19.65    | 331       | 769           | 563(11), 545(81), 425(100), 335(31) | Sinapic acid ester of apigenin-C-diglycoside |
| 11   | 20.53    | 331       | 769           | 563(10), 545(89), 425(100), 335(12) | Sinapic acid ester of apigenin-C-diglycoside |

The flavonoids group represented the majority of the compounds identified in the cereal by-products, being apigenin derivatives the ones with higher expression in all samples (except in maize bran). Peaks 6, 7, and 8 all presented the same pseudomolecular ion [M-H]− at m/z 563, and characteristics MS² fragment at m/z 473 (90 u), 383 (90 u), 353 (30 u), that correspond to the loss of units in multiples of 30, leading to the identification of C-glycosylated derivatives. The differentiation between the three peaks, take into account the abundance of 100% in MS² fragment at m/z 473 in peaks 6 and 8, and the MS² fragment at m/z 443 in peak 7, which lead to the tentative identification of apigenin-C-pentoside-C-hexoside (peaks 6 and 8) and apigenin-C-hexoside-C-pentoside (peak 7) [44]. Peaks 10 and 11 presented a pseudomolecular ion [M-H]− at m/z 769 and an MS² fragmentation pattern coherent with that previously described by Hirawan and Beta [45] in whole-wheat Spaghetti, being both tentatively identified as sinapic acid esters of apigenin-C-diglycoside. Finally, the last flavonoid found was peak 4 tentatively identified as luteolin-O-pentoside-C-hexoside ([M-H]− at m/z 579), using the chromatographic profile previously described by Roriz et al. [46].

As presented in Table 5, the flavonoids group represents the majority (in content) of the compounds found in all samples (except for maize bran), more specifically, 81% (4.7 mg/g extract) in wheat germ and 77% (3.4 mg/g extract) in wheat bran of the total phenolic compounds. These concentrations are mainly due to the presence of apigenin-C-pentoside-C-hexoside, with 2.95 mg/g of extract in the wheat germ and 2.09 mg/g of extract in wheat bran. In maize bran and rye bran, phenolic acids were detected in higher concentrations, 0.046 mg/g extract in maize bran (peak 2 corresponded to the only phenolic compound found in this sample) and 0.93 mg/g extract in rye bran (mainly due to the
presence of p-coumaric acid dihexoside, in agreement with previous reports found in the literature [47–49].

Table 5. Phenolic compounds content in the hydroethanolic extracts of the cereal by-products.

| Peak | Tentative Identification | Wheat Germ | Maize Bran–Germ | Rye Bran | Wheat Bran |
|------|--------------------------|------------|-----------------|----------|-----------|
| 1    | p-Coumaric acid dihexoside | nd         | 0.046 ± 0.001 c | nd       | 0.115 ± 0.006 a |
| 2    | Caffeic acid hexoside     | 0.088 ± 0.001 b | 0.57 ± 0.02     | nd       | nd        |
| 3    | Caffeic acid hexoside     | nd         | nd              | nd       | nd        |
| 4    | Apigenin-C-pentoside-C-hexoside | 0.121 ± 0.004 * | nd       | 0.084 ± 0.001 |
| 5    | Unknown compound          | nd         | nd              | nd       | nd        |
| 6    | Apigenin-C-pentoside-C-hexoside | 0.602 ± 0.01 a | nd       | 0.39 ± 0.01   |
| 7    | Apigenin-C-hexoside-C-pentoside | 1.004 ± 0.058 * | nd       | 0.80 ± 0.05   |
| 8    | Apigenin-C-pentoside-C-hexoside | 2.9 ± 0.1 a     | 0.067 ± 0.002 c | 2.1 ± 0.1 b |
| 9    | 3,5-Dicaffeoylquinic acid | 0.34 ± 0.01 a | 0.293 ± 0.001 b | 0.35 ± 0.01 a |
| 10   | Sinapic acid ester of     | 0.16 ± 0.01 a | nd              | nd       | 0.140 ± 0.001 |
|      | apigenin-C-diglycoside    |             |                 |          |           |
| 11   | Sinapic acid ester of     | 0.470 ± 0.001 a | nd       | 0.057 ± 0.001 c | 0.413 ± 0.007 b |
|      | apigenin-C-diglycoside    |             |                 |          |           |

Calibration curves used in quantification: apigenin-6-C-glucoside (y = 107025x + 61531, \( r^2 = 0.9989 \), LOD = 0.19 µg/mL; LOQ = 0.63 µg/mL; peak 6, 7, and 8); caffeic acid (y = 388345x + 406369, \( r^2 = 0.9939 \), LOD = 0.78 µg/mL, and LOQ = 1.97 µg/mL; peaks 2 and 3); chlorogenic acid (y = 168823x – 161172, \( r^2 = 0.9999 \), LOD = 0.20 µg/mL, and LOQ = 0.68 µg/mL; peak 9); luteolin-6-C-glucoside (y = 4087.1x + 72589, \( r^2 = 0.9988 \), LOD = 0.20 µg/mL, and LOQ = 0.45 µg/mL; peak 4); p-coumaric acid (y = 301950x + 6966.7, \( r^2 = 0.9999 \), LOD = 0.68 µg/mL, and LOQ = 1.61 µg/mL; peak 1); sinapic acid (y = 197337x + 30036, \( r^2 = 0.9997 \), LOD = 0.17 µg/mL, and LOQ = 1.22 µg/mL; peaks 10 and 11). Statistically significant differences (p < 0.05) between more than two samples were assessed by a one-way ANOVA, using Tukey’s significant difference (HSD), and are indicated by different letters; significant differences (p < 0.001) between two samples were assessed by a Student’s t-test. The number of significant figures of each mean value was conditioned by the standard deviation, which was rounded to one significant figure. nd—not detected; nq—not quantified.

Wheat germ and bran were the samples presenting the highest concentration of phenolic compounds (5.7 and 4.4 mg/g extract, respectively), and also a higher concentration of total phenolic acids (1.066 and 1.017 mg/g extract, respectively). The phenolic compounds of wheat were previously studied by different authors, with different phenolic acids and results obtained. Zou et al. [50] reported ferulic acid as the major compound found in wheat germ (250 µg/g of extract) and 554 µg/g of extract of total phenolic acids. Vaher [51], in bran spring wheat, also reported ferulic acid was the main one (268.9 µg/g dw) and the amount of total phenolic acids was 569 µg/g dw. López-Pereá et al. [52] reported caffeic acid as the major compound (0.86 µg/g dw) and the total of phenolic compound was 100 µg/g dw in wheat bran. Skrajda-Brdak et al. [49] reported ferulic acid (222.1 µg/g dw) as the main compound and the total phenolic acids content was 273.2 µg/g dw for common wheat.

3.3. Bioactivity of the Cereal By-Products

Whole cereal grains have been described as having great bioactive properties due to their fractions, bran and germ, that comprise health-promoting bioactive compounds [41]. Therefore, the antioxidant activity of the cereal by-product hydroethanolic extracts was evaluated in vitro by the thiobarbituric acid reactive substances (TBARS) formation inhibition, using porcine brain tissue homogenates, and the oxidative hemolysis inhibition assay (OxHLIA), using sheep erythrocytes. The results are presented in Table 6 and were expressed in IC50 values, translating the extract concentration providing 50% of antioxidant activity in the TBARS assay or the concentration able to protect 50% of the erythrocyte population in OxHLIA. The cereal by-product with the best TBARS formation inhibition
capacity was the wheat bran (with the lowest IC$_{50}$ value of 0.26 mg/mL), followed by the maize bran–germ mixture and rye bran. The lowest activity was shown by the wheat germ extract, not agreeing, therefore, with the highest content of phenolic compounds quantified in this sample (Table 5), but coinciding with the highest levels of citric acids (Table 2) and tocopherols (Table 3). For OxHLIA, only rye bran presented capacity to protect the erythrocyte membrane from the free radicals generated in the system by the thermal decomposition of AAPH. The other extracts did not delay hemolysis compared to the used negative control (PBS). It should be noted that the lower IC$_{50}$ values of trolox are due to the fact that this positive control is a pure antioxidant compound, while the natural extracts tested are complex mixtures of different constituents, some of which have no activity. The antioxidant activity of the studied cereals has been demonstrated by previous studies, but most of them used chemical methods, such as reducing power, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and Folin–Ciocalteu assays, among others [53–55]. However, cell-based assays have been described as more suitable to measure the antioxidant activity of natural products than chemical methods [56]. It is also worth noting that, according to some studies, cereal phenolic acids display antioxidant properties in vitro due to the presence of an aromatic phenolic ring [41].

Table 6. Antioxidant activity (IC$_{50}$ values, mg/mL) of the cereal by-product hydroethanolic extracts.

|                | Wheat Germ | Maize Bran–Germ | Rye Bran | Wheat Bran | Trolox  |
|----------------|------------|-----------------|----------|------------|---------|
| TBARS          | 4.8 ± 0.1  | 0.62 ± 0.01     | 0.98 ± 0.01 | 0.26 ± 0.01 | 0.023 ± 0.001 |
| OxHLIA, Δt 60 min | na         | na              | 0.58 ± 0.02 | na         | 0.020 ± 0.001 |
| OxHLIA, Δt 120 min | na         | na              | 1.02 ± 0.04 * | na         | 0.041 ± 0.001 |

For TBARS, statistically significant differences ($p < 0.05$) between samples were assessed by a one-way ANOVA, using Tukey’s significant difference (HSD), and are indicated by different letters; for OxHLIA, significant differences ($p < 0.001$) between the two samples * were assessed by a Student’s $t$-test. The number of significant figures of each mean value was conditioned by the standard deviation, which was rounded to one significant figure. na—no activity.

The antibacterial activity of the cereal by-products was also tested against Gram-positive and Gram-negative bacteria and the results are presented in Table 7 as minimum inhibitory and bactericidal concentrations (MIC and MBC, respectively). In general, the extracts were not very effective against the selected foodborne microorganisms. The best results were achieved against methicillin-resistant Staphylococcus aureus with the maize bran–germ moisture extract (MIC of 2.5 mg/mL) and rye bran and wheat bran extracts (MIC of 5 mg/mL). MBC values above 20 mg/mL were obtained in all cases. Among the tested microorganisms, the Gram-positive bacteria appear to be more susceptible to the extracts than the Gram-negative bacteria. The positive controls ampicillin, imipenem, and vancomycin yielded much lower MIC and MBC values, as expected for commercial antibiotics. Despite the low efficacy of the tested extracts, isolated compounds such as phenolic acids can display remarkable antimicrobial effects [41], which highlights their potential for application as preservatives in food and food-packing materials, among others. Regarding previous studies, Călinoiu and Vodnar [57] reported better results for heat processed wheat bran, with an MIC of 1.875 mg/mL against Enterococcus faecalis. In turn, a moderate antibacterial effect toward Pseudomonas aeruginosa was achieved with heat processed wheat bran and fresh wheat bran extracts, with MIC values of 3.75 mg/mL and 7.5 mg/mL, respectively. The same authors attributed moderate antibacterial activity to heat processed wheat bran against Escherichia coli (MIC of 3.75 mg/mL) and Salmonella typhimurium (MIC of 7.5 mg/mL).
Table 7. Antibacterial activity (mg/mL) of the cereal by-product hydroethanolic extracts.

|                        | Wheat Germ |          | Maize Bran–Germ |          | Rye Bran |          | Wheat Bran |          | Ampicillin |          | Imipenem |          | Vancomycin |          |
|------------------------|------------|----------|-----------------|----------|----------|----------|------------|----------|------------|----------|----------|----------|------------|----------|
|                        | MIC MBC    | MIC MBC  | MIC MBC         | MIC MBC  | MIC MBC  | MIC MBC  | MIC MBC    | MIC MBC  | MIC MBC    | MIC MBC  | MIC MBC  | MIC MBC  | MIC MBC    | MIC MBC |
| Gram-negative bacteria  |            |          |                 |          |          |          |            |          |            |          |          |          |            |          |
| *Escherichia coli*      | >20 10 >20 | >20 10   | >20 20 >20      | >20 20 >20 | <0.15   | <0.15   | <0.0078    | <0.0078 | nt         | nt       |          |          |            |          |
| *Klebsiella pneumoniae* | >20 >20 >20| >20 >20  | >20 >20 >20 >20 | >20 >20 >20 | 10 20   | <0.0078 | <0.0078    | <0.0078 | nt         | nt       |          |          |            |          |
| *Morganella morganii*   | 20 >20 20  | >20 20   | >20 20 >20 >20 | >20 >20 >20 | >20 >20 | <0.15   | <0.15   | <0.0078    | <0.0078 | nt         | nt       |          |          |            |          |
| *Proteus mirabilis*     | >20 >20 >20| >20 >20  | >20 >20 >20 >20 | >20 >20 >20 | >20 >20 | <0.015  | <0.15   | <0.0078    | <0.0078 | nt         | nt       |          |          |            |          |
| *Pseudomonas aeruginosa*| >20 >20 >20| >20 >20  | >20 >20 >20 >20 | >20 >20 >20 | >20 >20 | 0.5     | 1       | nt         | nt       |            |          |          |            |          |

| Gram-positive bacteria   |            |          |                 |          |          |          |            |          |            |          |          |          |            |          |
| *Enterococcus faecalis*  | 10 >20 20  | >20 20   | >20 >20 >20 >20 | >20 >20 >20 | <0.15   | <0.15   | nt         | nt       | <0.0078    | <0.0078 |
| *Listeria monocytogenes* | 10 >20 20  | 2.5 >20  | 20 >20 >20 >20 | >20 >20 >20 | >20 >20 | <0.15   | <0.15   | nt         | nt       | 0.25       | 0.5      |          |            |          |

MRSA—Methicillin-resistant *Staphylococcus aureus*; MIC—minimum inhibitory concentration; MBC—minimal bactericidal concentration; nt—not tested.

4. Conclusions

This study contributes to the valorization of cereal by-products generated worldwide by the flour milling industry. The findings herein described are valid arguments to support the use of the industry’s cereal germ and bran by-products as underexploited alternative sources of nutrients and bioactive compounds with potential health benefits for consumers. Among the studied cereal by-products, wheat germ has emerged as an interesting source of protein and vitamin E, given the high contribution to the recommended dietary allowances. Wheat germ, maize bran–germ mixture, rye bran, and wheat bran could be directed to human nutrition as a sustainable way to promote the development of novel and functional foods, the bioresource-use efficiency, and the circular bioeconomy in this so important food sector. The results can also be useful to complete food and/or feed composition tables.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11050972/s1, Table S1: Resistance profile of the tested Gram-positive and Gram-negative bacteria to different antibiotics. Minimum inhibitory concentration (MIC) in µg/mL.

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