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Nutritional composition of green asparagus (*Asparagus officinalis L.*), edible part and by-products, and assessment of their effect on the growth of human gut-associated bacteria

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ABSTRACT

Asparagus is considered a healthy food with a high content of bioactive compounds. In this study, the proximate and mineral composition, non-digestible carbohydrates and bioactive compounds of edible spear, spear by-product and root have been evaluated. Their activity on the growth of human gut-associated bacteria has been studied. The results support the high nutritional and functional value of the asparagus, including its by-products, highlighting the potential of the non-edible parts to be used as prebiotics. A remarkable content in xylose, inulin, flavonoids and saponins has been found. It has been shown that the spear by-product can be selectively used to promote the growth of commensal or probiotic lactobacilli and bifidobacteria strains. It has been confirmed that any part of the asparagus has a potential future as a healthy food or as health-promoting ingredients, however more work is required to identify the compounds able to modulate the human gut microbiota.

1. Introduction.

Asparagus (*Asparagus officinalis L.)* spears are considered a healthy food because of their low calories, high fiber content, and the presence of several phytochemicals including, among others, fructans, flavonoids, vitamins, saponins or cinnamic acids (Chin & Garrison, 2008; Ku et al., 2018). However, asparagus production and processing also generate a significant amount of agri-food by-products since a big proportion of both the aerial parts (~6 Tm/ha), including stems, leaves and fruits, and the underground parts (>30 Tm/ha), including roots, are discarded (Zhao et al., 2011; Fuentes-Alventosa et al., 2013; Viera-Alcaide, Hamdi, Rodríguez-Arcos, Guillén-Bejarano, & Jiménez-Araujo, 2020). In fact, the removal of asparagus-related waste has become a significant agronomic, economic and environmental challenge for asparagus producers (Viera-Alcaide et al., 2020).

In the last decades, it has been observed that asparagus roots and other by-products may be good sources of the same nutrients and phytochemicals that are present in the spears and, therefore, they could have a high potential to be used as food ingredients with a remarkable nutritional value and health-promoting properties (Fuentes-Alventosa et al., 2009; Viera-Alcaide et al., 2022). Thus, such non-edible parts should no longer be considered as mere cultivation waste but, actually, as food by-products with interest for several industrial sectors and contributing to a sustainable agriculture. In Spanish provinces such as Granada and Cadiz, the production of green asparagus is showing great interest in the by-products generated and their revaluation.

The asparagus cell walls are rich in phenolic–polysaccharides complexes, especially after the post-harvest process of hardening (Rodriguez et al, 2005). As a result, asparagus spears and asparagus by-products are a good source of dietary fiber (Agudelo Cadavid, Restrepo Molina, & Cartagena Valenzuela, 2015). Roots are particularly rich in fructans (Witzel & Matros, 2020), which are often associated with immunomodulatory and antioxidant activities due to, among other characteristics, their association to saponins and phenolic compounds (Fuentes-Alventosa et al., 2013). In addition, this dietary fiber, as well as most of the other fiber-associated compounds, remain undigested during their

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transit through the mouth, stomach and small intestine (Saura-Calixto, García-Alonso, Goni, & Bravo, 2000). Once in the large intestine, the fiber complex could act as a prebiotic since it may be used by selected members of the colonic microbiota and, particularly, by commensal or mutualistic anaerobic bacteria, which generate active metabolites (e.g., short chain fatty acids) that play key roles for the host's health (Gibson et al., 2017).

In this context, the objectives of this work were, first, to know the proximate and mineral composition, non-digestible carbohydrates and bioactive compounds content of edible (spear) and non-edible (spear and root by-products) parts of green asparagus, and second, to study their possible prebiotic effect on the stimulation of the proliferation of some relevant cultivable members of the human gut microbiota.

2. Materials and methods

2.1. Materials

Asparagus (*A. officinalis, L.)* variety *Herkolim* were obtained from Centro IFAPA Rancho de la Merced de Jerez (Chipiona, Cadiz, Spain). Freshly harvested, green asparagus spears were provided along with their roots. Each asparagus was hygienically cut to obtain three fractions, one edible and two by-products: (a) the ~ 15 cm length upper portion (edible part); (b) the rest of the spear (15–18 cm) (non-edible part or by-product); and (c) the root (by-product). Roots were cleaned to remove the sand and the three fractions were kept in a freezer at −18 °C. Subsequently, they were freeze-dried using a TELSTAR LyoQuest freeze dryer at −53 °C and a pressure of 0.101 mbar for 5 days. Prior to the analyses, freeze-dried samples were shredded to a fine powder and passed through a sieve of<1 mm.

2.2. Proximate composition

The chemical composition, including moisture, protein, fat, ash, total carbohydrates, reducing sugars, and total dietary fiber (TDF) was determined in the three asparagus portions. Moisture was determined by oven-drying at 105 ± 1 °C. Proteins were analysed as total nitrogen content by the Kjeldahl procedure and the conversion factor used to transform nitrogen into protein was 6.25 (920.87 Method, AOAC 2012). Fat content was measured by extraction with diethyl ether in a Soxhlet system (920.39 Method, AOAC 2012). Ash content was determined by incineration of samples at 550 °C in a muffle furnace (930.22 Method, AOAC 2012). Anthrone (James, 1995) and 3,5-dinitrosalicylic acid (DNS) (Miller, 1959) methods were used to quantify total carbohydrates and reducing sugars, respectively. Both colorimetric methods were adapted to a Synergy HTX Absorbance Microplate Reader (BioTek Co., USA). TDF and insoluble dietary fiber (IDF) were isolated enzymatically by sequential hydrolysis with amylase (pH 6, 100 °C, 30 min), protease (pH 7.5, 60 °C, 30 min) and, finally, amyloglucosidase (pH 4.5, 60 °C, 30 min), following the AOAC Method 992.16 and 991.42 (AOAC, 1995). All the enzymes were purchased from Sigma–Aldrich (St Louis, MO, USA). Soluble dietary fiber (SDF) was calculated by subtracting the IDF proportion from the TDF.

2.3. Mineral composition

Samples were incinerated at a temperature that increased linearly to 550 °C for 1 h and then at 500 °C for 20 h in a microwave muffle furnace (Milestone MLS-1200 Pyro, Shelton, CT, USA). The resulting ashes were dissolved in 2 mL of 12 M HCl: 14.5 M HNO_3 (1:1, v:v) and then diluted to 25 mL with distilled water. An additional dilution was performed for Ca and Mg determination with La_2O_3 (expecting a final concentration of 10 g/ L) and with CsCl (final concentration of 2 g/ L) for Na and K analysis. Mineral element concentrations were measured using a Perkin Elmer Analyst 200 Atomic Absorption Spectrophotometer (MA, USA). The wavelength (nm) of the minerals analysed was: K = 766.5, Na = 589.1, Ca = 422.7, Mg = 285.3, Fe = 248.4, Zn = 213.9, Cu = 324.8, and Mn = 279.5). This method showed good accuracy (93 – 99 % recoveries), repeatability (below 2 %) and reproducibility (<4.7 %) (Mateos-Aparicio, Redondo-Cuenca, Villanueva-Suárez, Zapata-Revilla, & Tenorio-Sanz, 2010).

2.4. Dietary fiber monosaccharides and uronic acids

Hydrolysis of the gravimetric TDF residues was carried out and neutral sugars quantification was performed following the Englyst protocol (Englyst, Quigley, & Hudson, 1994) and analysed by gas liquid chromatography (GLC) in a Perkin-Elmer Autosystem Chromatograph equipped with a hydrogen flame ionization detector, using β-d-allose (Fluka) as internal standard.

The uronic acids were determined spectrophotometrically according to the colorimetric method of 3,5-dimethylphenol (Scott, 1979) adapted to microplate with a Synergy HTX Multi-Mode (Bio-Tek Instruments, Winooski, USA).

2.5. Inulin and low molecular weight carbohydrates by HPLC

Sample extracts were obtained under optimized conditions. In brief, a distilled water extraction was carried out using a solution of 1 g of lyophilized sample in 40 mL of hot distilled water. The solution was kept in a water bath at 70 °C and in constant agitation for 1 h. Then, it was filtered before performing the HPLC assay.

Identification and quantification of inulin and low molecular weight carbohydrates (LMWC) in the above obtained extracts was conducted by liquid-chromatography analysis using an Agilent 1100 Series HPLC, equipped with a refractive index detector (RID) on a RezexTM ROA-Organic Acid H+ (8 %), 300 mm × 7.8 mm column, protected with a Carbo-H 4 × 3.0 mm ID security guard cartridge (Phenomenex España, Madrid, Spain). Ultrapure Milli-Q water (Milli-Q Integral 5 Water Purification System from Millipore) acidified with 2.5 mM H_2SO_4 was used as mobile phase, at a flow rate of 0.4 mL/ min. The column was maintained in a thermostatic oven at a constant temperature of 25 °C. Both standards and samples were filtered through 0.45 μm syringe filters for aqueous solutions and 5 μL volume injected into the HPLC. Inulin and different LMWC standards (DP4 = stachyose, DP3 = 1-Kestose, DP3 = raffinose, DP2 = cellobiose, DP2 = sucrose, DP1 = fructose, and glucose) were injected in triplicate at various concentrations (1.0, 0.5 and 0.25 mg/mL) and used for calibration. Sample extracts were diluted in ultrapure water (5 mg/mL), filtered and then injected. Regression standard curves were obtained for concentration *versus* area (mV × s). Peaks in chromatograms of samples were identified by coincidence of their retention times with available LMWC standards and they were quantified by comparison of their areas with the corresponding calibration curves.

2.6. Phenolic compounds and saponins

2.6.1. Low molecular polyphenols (LMP) and macromolecular polyphenols (MP)

Samples were previously extracted following the methodology described by Saura-Calixto & Goñi (2006). Briefly, samples were sequentially extracted at room temperature with a solution of methanol/ water (50:50, v/v) in acid medium and acetone/water (70:30, v/v). Each solid sample (0.5 g) was placed in a capped centrifuge tube, 20 mL of methanol/water (50:50, v/v) and 2 N HCl, to obtain a pH 2.0 was added and the tube was thoroughly shaken at room temperature for 1 h. The tube was centrifuged at 2500g in a Thermo Heraeus Megafuge 11 (Thermo Fisher, Waltham, MA, USA) for 10 min and the supernatant was recovered. Twenty mL of acetone/water (70:30, v/v) was added to the residue, and shaking and centrifugation were repeated. The methanol and acetone extracts were combined and used to determine LP by Folin–Ciocalteu colorimetric method (Singleton et al., 1999) using a

Synergy HTX Absorbance Microplate Reader (BioTek Co., USA). Results were expressed in terms of mg of gallic-acid equivalents (GAE) per g of dry matter.

Residues from the double extraction were subjected to two different procedures previously reported (Saura-Calixto, Serrano, & Goñi, 2007) in order to determine the main MP, proanthocyanidins (MPP) and hydrolysable polyphenols (MHP). All the extraction and analytical procedures were performed in triplicate. Results were expressed as mean values ± standard deviation, on a dry matter basis.

MHP comprise hydrolysable tannins, phenolic acids, and hydroxycinnamic acids that are released from the food matrix by strong acidic hydrolysis. They were determined by a methanol/H_2SO_4 90:10 (v/v) hydrolysis of 200 mg of residues from methanol/acetone/water extraction at 85 °C for 20 h (Hartzfeld, Forkner, Hunter & Hagerman, 2002). The hydrolysate was collected for polyphenols analysis with Folin-Ciocalteau reagent (Singleton, Orthofer & Lamuela-Raventós, 1999). The results were expressed as mg of GAE.

Residues from the methanol/acetone water extraction were treated with 5 mL/L HCl-butanol (3 h, 100 °C) for MPP determination (Reed, McDowell, Van Soest, & Horvarth, 1982). MPP were calculated from the absorbance at 550 nm of the anthocyanidin solutions. MPP from Mediterranean carob pod (*Ceratonia siliqua* L) supplied by Nestlé S.A. (Vevey, Switzerland) were treated under the same conditions to obtain standard curves.

2.6.2. Flavonoids and phenolic acids

2.6.2.1. Preparation of ethanolic extract. Phenols and saponins were identified and quantified in the ethanolic extracts from the different samples. Ethanolic extraction was performed using ethanol:water (80:20, v/v) in a ratio 1:40 (dw/v). The mixture was homogenized in an Ultraturrax (Ultra-Turrax T25, Janke & Kunkel/IKA Labortechnik, Munich, Germany) for 1 min at maximum speed and filtered through filter paper. The residue was extracted again in the same conditions. Ethanolic extracts were pooled together and stored at −20° C until analysis. All extractions were made in duplicate.

2.6.2.2. Analysis of phenolic compounds. Flavonoids and phenolic acids were quantified in a Jasco-LC-Net II ADC (Jasco, Madrid, Spain) liquid chromatograph system equipped with a DAD (Hamdi et al, 2017). Separation was carried out in a Mediterranea Sea C18 reverse-phase analytical column (25 cm lengthx4.6 mm i.d., 5 μm particle size; Teknokroma, Barcelona, Spain). An elution gradient was used with solvents A (water with 1 % formic acid) and B (acetonitrile with 1 % formic acid): the proportion of solvent B was increased from 0 % to 15 % in 10 min, then maintained at 15 % for 5 min, then raised to 20 % over the next 10 min, maintained at 20 % for 5 min, and raised to 100 % over the next 5 min, maintained at 100 % B for 5 min and finally returned to the initial conditions over the following 5 min. The column end was connected directly to a diode array detector (DAD) (Waters 996, Millipore, Manchester, UK). Spectra from all peaks were recorded in the 200–600 nm range and the chromatograms were acquired and quantified at 280 nm.

The quantification of individual flavonoids was directly performed using an eight-point regression curve in the range of 0–250 μg on the basis of standards. The results were calculated from the mean of two replicates.

2.6.3. Quantification of saponins by LC-MS

The evaluation of the saponin content was carried out as described by Vazquez-Castilla et al. (2013a). An HPLC Waters Alliance (Manchester, UK) system fitted to a Mediterranean Sea C18 reverse phase analytical column (25 cm length × 4.6 mm i.d., 5 μm particle size; Teknokroma, Barcelona) was used. An elution gradient was used with solvents A (water with 1 % formic acid) and B (acetonitrile with 1 % formic acid): 0–30 min, 20 % B; 30–60 min, linear gradient to 30 % B; 60 to 70 min, linear gradient to 100 % B; and 70–80 min, linear gradient to 20 % B. Saponins were detected using an online connected quadrupole mass analyzer (Waters Acquity QDa Detector, Waters Inc., Manchester, UK) the flow in the MS was regulated using a split (flow 0.3 mL/min). ESI mass spectra were obtained at ionization energies of 50 and 100 eV (negative mode) and 50 eV (positive mode) with scans from m/z 200 to 1200. The capillary voltage was 3 kV; the desolvation temperature was 200° C; the source temperature was 100° C and the extractor voltage was 12 V.

Two different external standards were used: protodioscin and shatavarin IV. For each standard, 10 dilutions from 0 to 500 μg/mL were prepared and injected into the LC-MS system. For each standard, the selected ion chromatogram corresponding to its molecular ion in negative mode at 100 eV was integrated and the peak area was plotted against the concentration and subjected to regression analysis.

2.7. Assessment of the prebiotic or/and antimicrobial activity of different asparagus fractions or/and extracts

A total of 20 bacterial strains belonging to 14 bacterial species that may inhabit or transit through the human gastrointestinal tract were used to test the effect of the three asparagus fractions (edible spear, non-edible spear and root) on their growth. On the one hand, they included some species and strains that usually play commensal or beneficial roles for the host: *Ligilactobacillus salivarius* MP98, *L. salivarius* MP100, *Limosilactobacillus reuteri* MP301, *Lacticaseibacillus rhamnosus* GG, *Lactiplantibacillus plantarum* MP303, *Bifidobacterium breve* MP297, *B. breve* MP307, *Bifidobacterium longum* MP324, *B. breve* MP347, and *Bifidobacterium animalis* MP320. On the other hand, they also included some species and strains isolated from cases of food poisoning: *Staphylococcus aureus* MP364, *S. aureus* MP369, *Listeria monocytogenes* MP377, *L. monocytogenes* MP380, *Bacillus cereus* MP383, *Escherichia coli* MP386, *E. coli* MP388, *Klebsiella pneumoniae* MP390, *Salmonella enterica* serovar *enteritidis* MP395 and *S. enterica* serovar *typhimurium* MP399. All the strains belonged to the collection of the research group UCM920080 (Complutense University of Madrid, Spain).

To carry out the assays, 20 mL cultures of each strain were performed. The lactic acid bacteria and bifidobacteria strains were inoculated in tubes of MRS broth (Oxoid, Basingstoke, UK) supplemented with L-cysteine (2.5 g/L) (MRS-C), which were incubated anaerobically (85 % nitrogen, 10 % hydrogen, 5 % carbon dioxide) in an anaerobic workstation (DW Scientific, Shipley, UK), at 37 °C for 72 h. The rest of the strains were grown in tubes of BHI broth (Oxoid), which were aerobically incubated at 37 °C for 48 h. All the tubes had the same initial concentration (~5 $\log_{10}$ colony-forming units [cfu]/mL) of the respective strain. Four tubes were prepared for each strain. The first was inoculated with 0.1 g/L of the freeze-dried edible fraction of the asparagus while the second and the third ones were supplemented with the same concentration of the non-edible and root fraction, respectively. The fourth tube was not supplemented and served as a control. Tubes containing one of the asparagus fractions but without inoculating the strains were also included to assure that there was no bacterial growth due to contaminations of the asparagus-derived material. After the incubations, serial decimal dilutions of the cultures were performed using sterile peptone water and bacterial enumeration was carried on MRS-C plates (for lactic acid bacteria and bifidobacteria) or on BHI plates (for the other strains). The incubation conditions of the plates were identical to those described for the respective broth media.

These assays were repeated five times per each strain and each asparagus fraction.

2.8. Statistical analysis

Analyses were performed in triplicate and data were presented as mean ± standard deviation (SD). To establish the statistical significance

of differences ($p < 0.05$), ANOVA unifactorial was applied, Duncan's Multiple Range Test. SAS version 9 software was used for this purpose.

Microbiological data were recorded as CFU/mL and transformed to logarithmic values before statistical analysis. The normality of data distribution was analyzed using the Shapiro–Wilks test. Then, the quantitative variables were expressed as means and standard deviations (SD). Two-way ANOVA tests were used to compare the experimental groups and pairwise *t*-test with Bonferroni correction was used to identify which pairs of means were statistically different.

Principal Component Analysis (PCA) was performed using the method of singular value decomposition in order to identify key microorganisms and spot outliers with the R software (×64) 4.1.0 desktop version.

3. Results and discussion

3.1. Proximate composition of the samples

The results for the proximate composition of the three asparagus fractions analysed are shown in Table 1 (g/100 g dry matter). Regarding the moisture content, the two areas of the spear showed similar values (86.25 g/100 g by-product and 88.03 g/100 g edible portion) while the root presented a much lower value (61.25 g/ 100 g). A previous work showed higher values of moisture (91.60 g/100 g) in the edible part of white asparagus (Redondo-Cuenca, Villanueva-Suarez, Rodriguez-Sevilla, & Heredia-Moreno, 1997). Such differences are not strange since this parameter may be affected by different factors, including the different agricultural practices that are used for green or white asparagus. Previous values of moisture content in roots from *Asparagus stipularis* (57.4 g/100 g) are similar to the values for roots obtained in this study (Adouni et al., 2022).

The composition of the three samples revealed a high nutritional value, being remarkable the high content of proteins, carbohydrates, and dietary fiber. Nonetheless, some differences were observed among them. Protein content was significantly higher in the edible sample (46.23 g/100 g) in relation to by-products (17.91 and 11.92 g/100 g for root and spear, respectively). By-products contained less fat (0.87 and 1.38 g/100 g in root and spear, respectively) than the edible portion (4.66 g/100 g), while total carbohydrates and dietary fiber were higher in the root and spear by-products (Table 1). In contrast, the mineral content was almost double in the edible part (8.90 g/100 g) than in the by-products (5.79 and 4.89 g/100 g in root and spear, respectively). Amaro-López, Zurera-Cosano, & Moreno-Rojas (1999) indicated that there is a spatial distribution of minerals in asparagus spears so that the highest concentration of minerals occurs at the apical portion, probably due to higher cellular growth and development in this portion. In general, the results of the present study regarding the composition of the edible part of asparagus were similar to those shown by Chitrakar, Zhang, & Adhikari (2019), with slight differences with respect to proteins and total carbohydrates.

Table 1
Proximal composition of green *Asparagus officinalis* L., var. *Herkolim* (root by-product, spear by-product and spear edible portion). Results are expressed as g/100 g dry matter[1].

	Root by-product	Spear by-product	Spear edible portion
Protein	17.91 ± 0.14^{b}	11.92 ± 0.08^{a}	46.23 ± 0.66^{c}
Fat	0.87 ± 0.08^{a}	1.38 ± 0.28^{a}	4.66 ± 0.34^{b}
Total Carbohydrates	40.56 ± 0.42^{c}	28.88 ± 0.08^{b}	21.40 ± 0.12 ^{a}
Sucrose	1.66 ± 0.02^{c}	0.24 ± 0.05 ^{a}	0.82 ± 0.04^{b}
Glucose	1.25 ± 0.03 ^{a}	6.92 ± 0.01^{c}	5.70 ± 0.06^{b}
Fructose	1.94 ± 0.02 ^{a}	9.79 ± 0.17^{c}	7.91 ± 0.01^{b}
Total dietary fiber	38.28 ± 1.62^{b}	58.10 ± 0.35^{c}	23.80 ± 0.85 ^{a}
Insoluble dietary fiber	26.45 ± 0.82^{b}	45.72 ± 0.28^{c}	8.69 ± 1.38 ^{a}
Soluble dietary fiber[2]	11.84 ± 1.09 ^{a}	12.38 ± 0.56 ^{a}	15.11 ± 0.86^{b}
Ash	5.79 ± 0.18^{b}	4.89 ± 0.07 ^{a}	8.90 ± 0.17^{c}

[1] Data are the mean of ≥ 3 determinations ± SD. Values followed by different superscript letter significantly differ ($p < 0.05$).
[2] Calculated by difference between total and insoluble dietary fibre values.

In relation to the total carbohydrates, both spear fractions shared similar values (28.88 and 21.40 g/100 g in by-product and edible portion, respectively) while that observed in the root was much higher (40.56 g/100 g). Similarly, the content of sucrose was higher in the root with respect to the other two areas of the asparagus. Another difference among them was the ratio of reducing and non-reducing sugars. In the case of the root, the reducing sugars (glucose plus fructose) only represented 8 % of the total carbohydrates but their proportion was much higher in both spear portions (58 % and 64 % in by-product and edible portion, respectively). This indicates a lower proportion of free sugars (glucose and fructose) in the root where they are forming oligosaccharides or polysaccharides, probably as a reserve source. The major carbohydrates in the edible part of asparagus are fructose and glucose, with a minor amount of sucrose (Bhowmik, Matsui, & Kawada, 2000). Similar results of sucrose, glucose and fructose to those obtained in this work in the edible part of green asparagus have been recently provided by Soteriou, Antoniou, Rouphael, Kyratzis, & Kyriacou (2021). In the same way, as indicated above with respect to mineral content, the total sugar content of the asparagus also showed a spatial distribution, with the highest content at the bottom and the lowest content at the tip portion (Slatnar et al., 2018).

In general, the dietary fiber values were remarkable in all the samples, although they reached higher amounts in the two by-products, where TDF content was particularly noticeable (58.10 and 38.38 g/100 g in spear by-product and root, respectively). In the case of the edible part, the amount of dietary fiber was lower (23.80 g/100 g) but it is also worth noting the large proportion of SDF (63 %) compared to IDF (37 %); this is in contrast with the by-products, where IDF predominated. The ration IDF/SDF was 2.2, 3.7 and 0.6 in root, spear by-product and edible spear, respectively. IDF/SDF ratios between 4.6 and 7.2 in dietary fiber fractions of different fiber-rich powder from asparagus spear by-products have been reported previously (Fuentes-Alventosa et al., 2009).

Asparagus spears and spear by-products are rich sources of nutritional and phytochemical compounds, but their composition and concentration are affected by a variety of factors, including asparagus variety, spear part, harvesting season or cultivation method (Chitrakar et al., 2019). Anyway, the results of this work confirm that a large quantity of the by-products generated during production and processing of asparagus could be converted into high value products, including bioactive dietary fiber powders. The Regulation (EC) No 1924/2006 on nutrition and health claims made on foods indicates that by-products could be considered as high in fiber when they contain>6 g per 100 g and the values obtained by the root and spear by-product in this work were 14.83 and 7.99 g/100 g of fresh matter, respectively. Although the value obtained by the edible portion was lower (2.85 g/100 g fresh matter), it also approached the threshold to be considered as a source of fiber (3 g/100 g). Since the recommended European consumption of fiber is estimated to be 20 g/person/day (Adouni et al., 2022), a supplementation of the diet with the by-products analysed in this study may contribute to increase fiber consumption.

Asparagus and its by-products could be also considered products of interest from a functional point of view due to their contents of inulin, dietary fiber and other phytochemicals, such as phenolic compounds (Rodríguez et al., 2005). Many of these bioactive compounds are mainly located in the lower portions of the spears and in the root. The first are discarded during the industrial processing, while the second one is an agricultural by-product. Such bioactive compounds are included in the non-digestible fraction of asparagus and will be discussed in section 3.3.

3.2. Minerals

Table 2 shows the results for the mineral elements in the asparagus

Table 2
Contents of macro- and microelements of green *Asparagus officinalis* L., var. *Herkolim* (root by-product, spear by-product and spear edible portion). Results are expressed as g/100 g or mg/100 g dry matter.[1]

Element	Root by-product	Spear by-product	Spear edible portion
K (g/100 g)	1.82 ± 0.23^{a}	2.20 ± 0.16^{b}	2.76 ± 0.156^{c}
Na (g/100 g)	0.07 ± 0.02^{a}	0.02 ± 0.01^{b}	0.04 ± 0.01^{b}
Ca (g/100 g)	0.26 ± 0.01^{c}	0.12 ± 0.00 ^{a}	0.24 ± 0.00^{b}
Mg (g/100 g)	0.10 ± 0.00^{b}	0.05 ± 0.01 ^{a}	0.21 ± 0.01^{c}
Fe (mg/100 g)	33.85 ± 1.13^{b}	8.10 ± 1.16 ^{a}	10.38 ± 1.53 ^{a}
Zn (mg/100 g)	6.69 ± 0.81^{b}	3.77 ± 0.02 ^{a}	11.12 ± 0.44^{c}
Cu (mg/100 g)	1.30 ± 0.29 ^{a}	1.18 ± 0.12 ^{a}	2.81 ± 0.75^{b}
Mn (mg/100 g)	2.18 ± 0.38^{b}	0.85 ± 0.20 ^{a}	1.21 ± 0.10 ^{a}
Ash (g/100 g)	5.79 ± 0.18^{b}	4.89 ± 0.07 ^{a}	8.90 ± 0.17^{c}

[1] Data are the mean of ≥ 3 determinations ± SD. Values followed by different superscript letter significantly differ ($p < 0.05$).

fractions. The essential minerals or macroelements (K, Na, Ca and Mg) are expressed as g/100 g dry matter and the trace minerals or microelements (Fe, Zn, Cu and Mn) as mg/100 g dry matter. Regarding the first ones, K was high in the three parts of the asparagus studied, the differences between them being significant. The edible portion provides a higher amount (2.76 g/ 100 g) and a progressive decrease was observed in the other fractions (2.20 g/100 g, spear by-product and 1.82 g/100 g root). Potassium concentration increased from root to apical portion, being the predominant mineral detected. In fact, the asparagus potassium content is considered quite high, compared to other vegetables, hence its consumption is recommended to ameliorate human hypertension (Pegiou, Mumm, Acharya, de Vos, & Hall, 2020).

The rest of the macroelements, Na, Ca and Mg, were found in much lower quantities, and there was not the progressive decrease from the top to the root as occurred with potassium. Na was the macroelement found in lower proportions, with the root having the highest content (0.07 g/100 g). In the root and in the edible part, a higher content of Ca was observed (0.26 g/100 g and 0.24 g/100 g, respectively), being the second in quantity after K. Mg, along with Na, was the macroelement found in the lowest proportion. In all the asparagus fractions analysed, the order from the highest to the lowest concentration was always the same: K, Ca, Mg and Na.

Comparing our data with the literature, the data presented by Moreiras, Carbajal, Cabrera, & Cuadrado (2013) for the edible part of asparagus are slightly higher in K 3.91, Na 0.08 and Ca 0.41, expressed in g/100 g dry matter, and similar for Mg where there is coincidence in the two studies (Mg 0.21 g/100 g dry matter). Zhang, Xia, & Zhu (2002) reported Ca amounts in the edible part of green asparagus from 0.30 to 0.37 g in 100 g dry matter, slightly higher than those presented in this study. Amaro-López et al (1999) carried out a study with green *A. officinalis*. The spears were cut into lengths of 20 cm and divided into ten portions of 2 cm, corresponding to the first apical portion to the tenth portion, the most basal portion of the asparagus. In the edible part, the K data range from 4.47 to 3.43 g/100 g dry matter, from the upper to the lowest zone. In the by-product or basal zone, the value is 3.22 g/100 g dry matter. These values were slightly higher than those obtained in the present work but have the same tendency to decrease from the youngest to the oldest zone of the spear. However, this decrease in mineral content along the green asparagus spear did not occur in the same way for the rest of the macroelements analysed (Na, Ca and Mg). Ca and Mg are also higher in the work of Amaro-López et al. (1999) than in the present study. In the case of Na the concentration is similar in both studies and asparagus areas. Soteriou et al. (2021) study the edible part of five green asparagus cultivars (Ercole, Eros, Giove, Italo and Vittorio) and present K, Ca and Mg values similar to those presented in this work although slightly higher. The Na concentration is very low and agrees with that reported in this work.

For the microelements Fe, Zn, Cu and Mn, results show that Fe had the highest concentration in the samples, with the root (33.85 mg/100 g dry matter) standing out above the rest (8.10 spear by-product and 10.38 spear edible portion, mg/100 g dry matter). In the case of the others microelements, Zn (11.12 mg/100 g dry matter) and Cu (2.81 mg/100 g dry matter) in edible portion and Mn (2.18 mg/100 g dry matter) in root stood out. Spear by-product, the basal part of the spear, always had intermediate contents of the trace elements. From the data presented by Pegiou et al. (2020) for the edible portion of asparagus, the higher Fe content (17.83 mg/100 g dry matter) and lower Zn content (4.50 mg/100 g dry matter) can be observed in comparisson to the data provided in this study. Similarly, in the data provided by Moreiras et al. (2013), higher values of Fe (20.79 mg/100 g dry matter) and lower values of Zn (5.67 mg/100 g dry matter) are given. Zhang et al. (2002) reported Fe amounts in the edible part of green asparagus of 12.86–18.57 mg/100 g dry matter. Accordingly, the results obtained by Amaro-López et al. (1999) on different portions of asparagus are similar for Fe, Zn and Cu and lower for Mn. Adouni et al. (2022) studied roots of wild *Asparagus stipularis Forssk* from Monastir (Tunisia) and found similar values for Fe 37.20 mg/100 g dry matter but lower values for Zn (2.23 mg/100 g dry matter) and Mn (0.67 mg/100 g dry matter), and almost double for Cu (2.35 mg/100 g dry matter).

Of the fractions analysed (edible part, basal part or by-product and root), the presence of K among the macroelements, and of Fe in the case of the microelements, is noteworthy, being K mainly found in the edible part and Fe in the root.

3.3. Non-digestible fraction

A large number of the components of the non-digestible fraction of vegetables behaves as a prebiotic, which is defined as a substrate that is selectively utilized by host microorganisms, conferring a health benefit (Gibson et al., 2017). This definition includes carbohydrate and non-carbohydrate substances, from dietary fiber and fructans to polyphenols.

In addition to the classic fiber components, non-starch polysaccharides (NSP), the samples contained other non-digestible compounds of proven resistance to the action of digestive enzymes, such as fructans and polyphenols, and other possible associated compounds (Garcia-Alonso et al., 2022; Viera-Alcaide et al., 2022). The non-digestible components of the samples studied (expressed as g/100 g dry matter) are shown in Tables 3 and 4.

3.3.1. Non digestible carbohydrates

In relation to TDF components (Table 3), the amounts of neutral sugars (cellulose or hemicelluloses) were the main difference among the three samples (Fig. 1). Glucose, xylose and arabinose were majority in the root although the content in galactose was very close to that of arabinose. In the spear by-product, glucose and xylose represented 89 % of the NSP while the rest of the sugars were present in much smaller proportions. In the case of the edible portion of the spear, the difference in the contribution of each monosaccharide was smaller than in the other two samples, although glucose, xylose galactose and arabinose were the predominant ones.

The high values of glucose indicate the presence of a high percentage of cellulose in the three fractions, a fact that agrees with the results obtained in other studies focused on spear by-products of green asparagus (Fuentes-Alventosa, et al., 2009; Jaramillo-Carmona et al., 2019). These authors showed that glucose was the major sugar (nearly 70 %) in green asparagus spear by-products. Anotherstudy also reported that glucose was the main neutral sugar of the fiber in the edible part of white asparagus (Redondo-Cuenca et al., 1997).

Xylose was the next major sugar, indicating that xylans and xyloglucans were also among the major components, while arabinans, mannans, galactans may be the origin of the high percentage of arabinose, mannose, and galactose. Studies *in vitro* and *in vivo* both in animals and humans have shown the bifidogenic effect of xylans (Corzo et al., 2015). In white and green asparagus, xylose and arabinose account for

Table 3
Non digestible components of green *Asparagus officinalis* L., var. *Herkolim* (root by-product, spear by-product and spear edible portion). Results are expressed as g/100 g dry matter.[1]

	Root by-product	Spear by-product	Spear edible portion
TDF neutral sugars			
Arabinose	1.54 ± 0.05^{c}	0.56 ± 0.01^{a}	1.05 ± 0.01^{b}
Xylose	5.24 ± 0.11^{b}	16.36 ± 0.19^{c}	1.13 ± 0.05 ^{a}
Mannose	0.62 ± 0.01 ^{ab}	0.52 ± 0.01 ^{a}	0.64 ± 0.09^{b}
Galactose	1.06 ± 0.02^{b}	0.89 ± 0.01 ^{a}	1.11 ± 0.01^{c}
Glucose	16.26 ± 0.08^{b}	29.10 ± 0.66^{c}	7.26 ± 0.02 ^{a}
TDF uronic acids	3.37 ± 0.03 ^{a}	3.84 ± 0.06^{b}	3.96 ± 0.04^{c}
Total NSP	28.08 ± 0.09^{b}	51.26 ± 0.83^{c}	15.15 ± 0.18 ^{a}
Klason Lignin[2]	10.20 ± 1.68^{b}	6.84 ± 0.69 ^{a}	8.65 ± 0.81^{b}
Fructans			
Inulin	16.18 ± 0.24^{b}	1.46 ± 0.01 ^{a}	1.30 ± 0.01 ^{a}
DP4	0	0	0
DP3	0.97 ± 0.01	0	0
	1.01 ± 0.02		
Phenolic compounds [3]LP			
(Extractable Polyphenols)	1.11 ± 0.03^{b}	0.64 ± 0.01 ^{a}	1.11 ± 0.01^{b}
MP			
(No extractable	0.59 ± 0.01^{b}	0.50 ± 0.02 ^{a}	0.71 ± 0.03^{c}
Polyphenols)MPP	1.19 ± 0.01^{c}	0.98 ± 0.01^{b}	0.80 ± 0.04 ^{a}
(Proanthocyanidins)MHP			
(Hydrol. Polyphenols)			

[1] Data are the mean of 3 determinations ± SD. Values followed by different superscript letter significantly differ ($p < 0.05$).
[2] Calculated by difference between total dietary fiber (TDF) value and non starch polysaccharides.
[3] LP, low-molecular polyphenols; MP, macromolecular polyphenols; MPP, macromolecular proanthocyanidins polyphenols; MHP, macromolecular hydrolysable polyphenols.

Table 4
Flavonoids, phenolic acids and saponins of green *Asparagus officinalis* L., var. *Herkolim* (root by-product, spear by-product and spear edible portion). Results are expressed as mg/100 g dry matter.[1]

		Root by-product	Spear by-product	Spear edible portion
Flavonoids		n.d.	**1.38 ± 0.05**	**8.87 ± 0.08**
Quercetin-triglycoside			0.10 ± 0.01	0.36 ± 0.01
Isorhamnetin-triglycoside			0.12 ± 0.01	0.270 ± 0.00
Quercetin-3-O-rhamnoglucoside (Rutin)			1.16 ± 0.03	8.10 ± 0.08
Kaempferol-3-O-rhamnoglucoside (Nicotiflorin)			n.d.	0.07 ± 0.01
Isorhamnetin-3-O-rhamnoglucoside (Narcissin)			n.d.	0.06 ± 0.01
Phenolic acids				
Caffeic acid		**1.92 ± 0.06**	n.d.	n.d.
Saponins		**13.07 ± 0.63**	**1.03 ± 0.04**	n.d.
[M−H]$^{-}$	Previously described as			
1051.5	HTSAP1	5.60 ± 0.19		
919.5	HTSAP2	1.03 ± 0.19		
1047.7	Protodioscin		1.03 ± 0.04	
1033.6	HTSAP11	0.99 ± 0.19		
1035.5	HTSAP6	0.75 ± 0.14		
1177.8		0.33 ± 0.00		
1033.6		0.49 ± 0.01		
1043.6		0.11 ± 0.01		
887.6	ACSAP2	0.17 ± 0.03		
755.6		1.06 ± 0.03		
871.5		0.22 ± 0.00		
739.5		0.33 ± 0.02		
871.5		1.55 ± 0.14		
739.5		0.44 ± 0.12		

[1] Data are the mean of 2 determinations ± SD.

higher than 20 %, and arabinoxylans are postulated as the main hemicellulose in cell walls, together with xyloglucans (Rodríguez et al., 1999; Jaramillo-Carmona, Guillén-Bejarano, Rodríguez-Arcos, & Jiménez-Araujo, 2017). In asparagus by-product samples, xylose, arabinose and arabino-xylooligosaccharides are the most abundant ones (Jaramillo-Carmona, Rodríguez-Arcos, Guillén-Bejarano, & Jiménez-Araujo, 2019). Considering the percentage of individual sugars in the total content of neutral sugars of the spear by-product, its high content in xylose seems particularly remarkable (Fuentes-Alventosa et al., 2009). In relation to the presence of uronic acids, which are the main components of the pectic substances, the absolute values were very similar in the three samples analysed although the percentage that they represented with respect to total NSP were different as explained below.

TDF expressed in terms of NSP (Table 3) was 28.08 g/ 100 g, 51.26 g/ 100 g and 15.15 g/ 100 g in root, spear by-product and spear edible portion, respectively. The percentage of uronic acids and neutral sugars was similar in the two by-products (uronic acids: 7–12 %; neutral sugars: 88–93 %) but different in the edible part (uronic acids: 26 %%; neutral sugars: 74 %). Redondo-Cuenca et al. (1997) studied the modifications of white asparagus under different storage conditions and, within the TDF, the proportions of NSP were similar to those obtained for the edible part of green asparagus in this work.

Fructans were other non-digestible components present in the asparagus fractions (Table 3). Inulin was the most abundant fructan, and both inulin and the determined oligosaccharides (DP4 and DP3) are potential fermentation substrates for the colonic microbiota (Sun et al., 2020; Valcheva et al., 2019). It is worth mentioning the concentration of inulin in the root (16.18 g/100 g), which was much higher than those achieved in the spear by-product (1.46 g/100 g) and the edible spear part (1.30 g/100 g). These results indicate that the root of asparagus, which is a residue in agricultural practices, causing environmental problems, could be very usable as a raw material to obtain this prebiotic component. A novel inulin-type fructan from *Asparagus cochinchinensis* has been reported recently and the authors demonstrated its beneficial impact on human intestinal microbiota (Sun et al., 2020). The oligosaccharides DP4 and DP3, which are also part of the fructans, were only present in the root and in quantities much lower (0.97 and 1.01 g/100 g, respectively) than those obtained for inulin. Fructans are important storage carbohydrates in plants. The storage carbohydrates in asparagus roots are inulin and inulin neoseries-type fructans (DP3 and DP4). Fructan is degraded through hydrolysis by the fructanby fructan exohydrolase (FEH), which does not act on sucrose. FEH exhibits fructan 1-exohydrolase activity via the hydrolysis of inulin-type fructan and this hydrolysis is considered to be an energy source for the emerging asparagus spear (Ueno, Sonoda, Yoshida, Shiomi, & Onodera, 2018).

3.3.2. *Phenolic compounds and saponins*

The three samples studied showed a high content of polyphenolic compounds, both extractable polyphenols with a low degree of polymerization (LP), and non-extractable polyphenols with a high degree of polymerization (MP) (Table 3). It should be noted that both the LP and

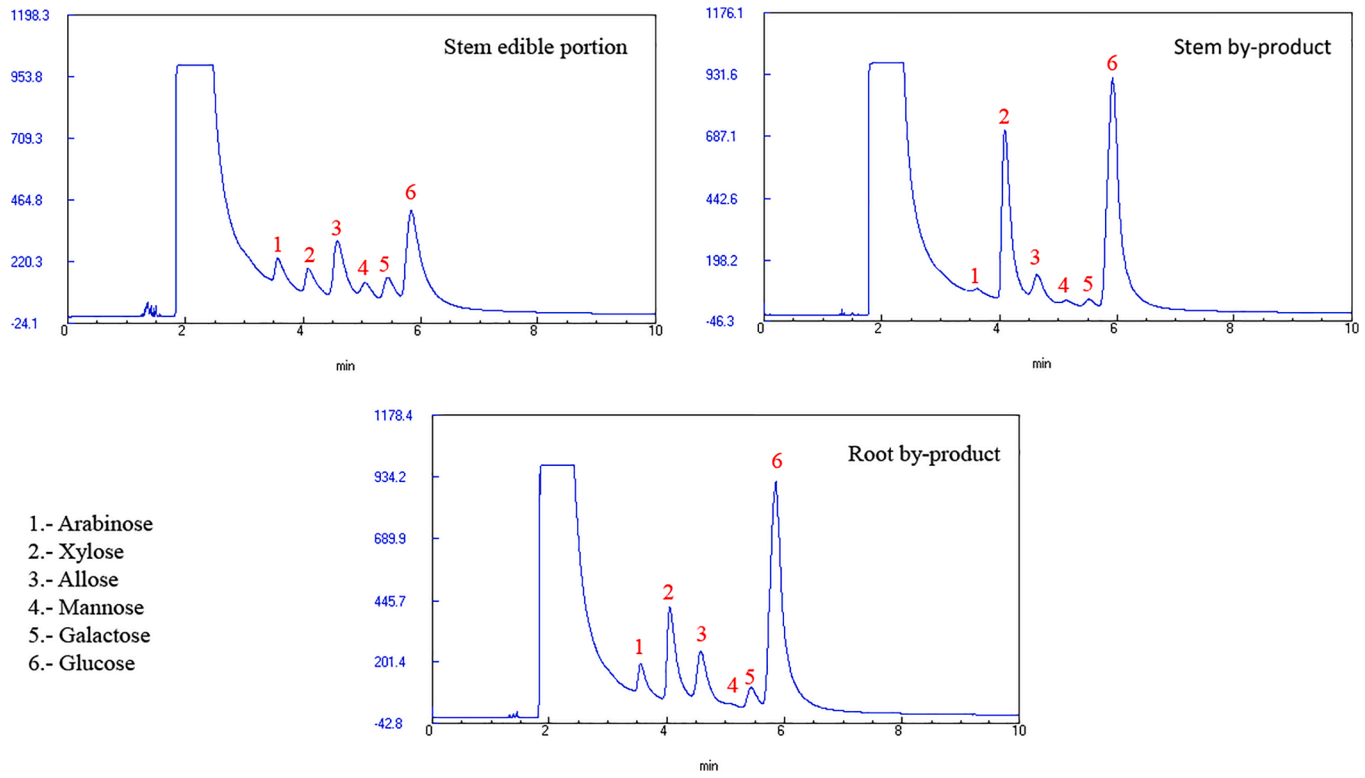

Fig. 1. - Monosaccharides profile of Total Dietary fiber by CG Chromatography (1.- Arabinose, 2.- Xylose, 3.- Allose, 4.- Mannose, 5.- Galactose, 6.- Glucose).

the MP contents were higher in the asparagus samples than in any other plant food from a Western diet analysed using the same methodology (Saura-Calixto et al., 2007). It is also noteworthy that the content in MP (MPP and MHP) was much higher than in LP in all the samples. MHP were a quantitatively important fraction of polyphenols in all groups (Table 3).

The amounts and type of polyphenolic compounds contained in any food have nutritional interest because of the physiological effects derived from their consumption. In this context, phenolic compounds can be classified according to their solubility in the intestinal medium (Goñi & Hernández-Galiot, 2019). Most of them are associated with other constituents of the fiber matrix (Saura-Calixto et al., 2007). An important part of LP is usually soluble in the intestinal medium and would be absorbed in the small intestine. On the other hand, MP reach the colon where they interact with the microbiota and produce some active metabolites. Despite their relevance, MP have rarely been considered when quantifying polyphenol intake although they account for more than half of the daily dietary polyphenols (Saura-Calixto et al., 2007).

To elucidate the significance of polyphenols in human health, it is essential to know the amount of polyphenols consumed in the diet and their bioavailability. Polyphenols have to be available to some extent in the target tissue in order to exert their biological properties. Therefore, physiological effects are closely associated with their degree of bioaccessibility in the different intestinal niches.

Phenolic compounds may become bioactive in the human gut once they are released from the food matrix by the action of digestive enzymes in the small intestine and/or bacterial enzymatic activity in the large intestine (Jenner, Rafter, & Halliwell, 2005). It has been estimated that about 48 % of dietary polyphenols are bioaccessible in the small intestine while 42 % become bioaccessible in the large intestine. However, only a small part of small intestine bioaccessible polyphenols can be absorbed through the intestinal mucosa and, therefore, metabolized. In fact, bioavailability of bioaccessible polyphenols in the small intestine has been reported be very low, with values ranging between 5 % and 10 % (Clifford, 2004).

The samples studied contained LP and MP in appreciable amounts, so the inclusion of these samples in the diet could provide the expected nutritional effects for these polyphenolic components, which, as the literature indicates (Mithul Aravind, Wichienchot, Tsao, Ramakrishnan, & Chakkaravarthi, 2021) can be very varied and interesting effects from the health point of view. However, it would be necessary to carry out intestinal bioavailability and bioaccessibility studies to be able to conclude on the effects derived from its consumption.

On the other hand, it is necessary to know the phenols and saponins composition as they are the main phytochemicals present in asparagus and they were quantified in significant amounts not only in the edible portion, but also in the different by-products. In fact, while phenols were mostly concentrated in the tip of the spears, saponins were accumulated in the spear by-product. Asparagus roots can be a good source of phenolic acids, mainly caffeic acid, and saponins.

The detailed composition of phenols and saponins of asparagus samples investigated in this study are summarized in Table 4. The phenolic profile of the spears consists of different types of flavonoid glycosides, derived from three aglycones: quercetin, kaempferol and isorhamnetin, rutin (quercetin-3-O-rhamnoglucoside). In the edible portion, five individual compounds were identified and quantified, being rutin the most abundant (>90 % total flavonoid complement). This quercetin triglycoside was also the main flavonoid present in the spear by-product, where it was only accompanied by minor quantities of two other compounds. It is remarkable that, even when the spear by-product contained over 6 times less flavonoids than the edible part, they represent a magnificent source of these bioactive compounds, especially rutin, which has high added value as a bioactive ingredient and nutraceutical. Our data are in consonance with those from Fuentes-Alventosa et al (2008). They studied the phytochemical composition of most cultivated commercial hybrids of green asparagus and reported that their flavonoid contents varied between 259 and 763 mg/kg of fresh weight, being rutin the main flavonoid glycoside in all samples. Assuming that the average humidity of green asparagus spears was 92

%, their contents referred to dry weight ranged between 3.237 and 9.537 mg/g. Therefore, we can consider that the *Herkolim* cultivar used in this study has a high potential as a functional food, as it is among those with the highest content of antioxidant flavonoids (8.856 mg/g dry weight).

The phytochemical characterization of the spear by-product revealed that this asparagus-coproduct contained significant amounts of flavonoids and saponins. Flavonoid content was similar to that previously described by other authors (Fuentes-Alventosa et al, 2013; Santiago, Feijoo, Moreira, González-García, 2021). The latter have developed different processes to achieve an optimized rutin extraction that increases their yields from asparagus waste, and at the same time that reduces the eco-environmentally loads derived from the use of solvents and energy requirements. The amounts of rutin that were extracted with these optimized techniques ranged between 0.94 and 2.34 mg/g of dry sample, which is comparable to our results. It is noteworthy that rutin (1.38 mg/g) was accompanied by similar amount of saponins (1.033 mg/g), which may confer this interesting asparagus by-product several bioactive properties, such as hypocholesterolemic and antitumoral activity, in addition to the antioxidant capacity mainly derived from phenols (Hamdi et al., 2018).

Roots by-product from *A officinalis* has also turned out to be a very good source of bioactive compounds, especially due to their great content of saponins (13.068 mg/g), significantly higher than the contents previously reported by Motoki et al. (2019), who found that buds, rhizomes and roots from *A. officinalis* contained between 0.94 and 7.51 mg/g dry weight of protodioscin. Saponin composition in the samples investigated in this study is in consonance with our previous results, which revealed that while protodioscin was the only saponin present in the spear by-product, the roots present a more complex profile. This consists in a combination of several saponins, which are the same kind as those present in different *Asparagus* wild species (Hamdi et al., 2017; 2021). Among the 13 distinct saponins quantified in this study, 8 of them are new, while the remaining 5 had already been previously identified in several genotypes of *triguero* asparagus from Huétor-Tájar (Vázquez-Castilla et al., 2013b) and other wild asparagus species (Hamdi et al., 2021). In addition to saponins, the root samples contained significant amounts of caffeic acid (1.915 mg/g), which is in consonance with other studies in which was stated that caffeic acid contents ranged from 0.151 mg/g to 1.8 mg/g depending on the extraction solvent. The interest of caffeic acid and its derivatives lies in the fact that they exhibit significant biological activities such as antioxidants to control lipid peroxidation and have a potential therapeutic effect in treating neurodegenerative diseases (Symes et al., 2018).

3.4. Effect of the asparagus fractions on bacterial growth

The results describing the effect of the different asparagus parts on the growth of the indicator organisms are shown in Tables 5 and 6. In the case of the commensal or probiotic strains (Table 5), the three fractions were able to promote their growth although, overall, the growth-promoting effect of the non-edible spear was significantly higher and broader in comparison to both the edible spear and the root. In contrast, the growth of the pathobionts strains was not promoted by any fraction while the non-edible spear showed an inhibitory effect against all of them (Table 6).

The PCA analysis showed that each fraction grouped separately from the other fractions and, also, from the control group (Fig. 2). In comparison to the control group, the profiles of the three asparagus fractions moved towards the right of the graph and, among them, that of the non-edible fraction had a particularly strong positive association with the lactic acid bacteria and bifidobacteria strains while it was negatively associated to the pathobionts (Fig. 2).

The fact that the three fractions somehow stimulated the growth of the *Lactobacilli* and *Bifidobacteria* strains may be explained on the basis of their richness in some compounds that can be used by these either in a non-selective (e.g., free sugars) or in a selective (e.g., fructans) manner. These kinds of bacteria display a wide spectrum of enzymes enabling the use of these substrates through different highly specialized metabolic pathways. The different proportions of some compounds in the three parts of the asparagus may explain why the effect of the non-edible spear was more pronounced. As it has been stated above, there is a spatial distribution of some compounds throughout the asparagus (Amaro-López et al., 1999.; Slatnar et al., 2018). The concentrations of some of them decrease from the edible spear to the root (e.g., minerals, SDF, uronic acids) while the opposite happens for other compounds (e.g., total sugars, fructans, IDF). The non-edible spear is located in the middle of the asparagus and, therefore, may provide a well-balanced mix of

Table 5
Effect of the different green *Asparagus officinalis* L., var. *Herkolim* fractions (root by-product, spear by-product and spear edible portion) on the growth of the probiotic strains included in this study. Bacterial counts are expressed as $\log_{10}$ CFU/mL ± SD. Control group means the culture without supplementation with any asparagus fraction. Each assay was performed in quintuplicate.

Strain	Control	Root by-product	Spear by-product	Spear edible portion	P-value*
L. salivarius MP98	9.40 ± 0.16	10.32 ± 0.08	10.56 ± 0.05	10.2 ± 0.10	<0.001
L. salivarius MP100	9.18 ± 0.08	10.16 ± 0.09	10.48± 0.08	9.96 ± 0.21	<0.001
L. reuteri MP301	9.22 ± 0.08	9.76 ± 0.11	10.16± 0.11	9.66 ± 0.17	<0.001
L. rhamnosus GG	9.20 ± 0.10	9.90 ± 0.16	9.96 ± 0.21	9.62 ± 0.08	<0.001
L. plantarum MP303	9.24 ± 0.11	9.44 ± 0.11	10.02 ± 0.13	9.52 ± 0.13	<0.001
B. breve MP297	8.82 ± 0.08	9.14 ± 0.09	9.42 ± 0.08	9.14 ± 0.11	<0.001
B. breve MP307	8.74 ± 0.13	9.14 ± 0.15	9.44 ± 0.11	9.16 ± 0.11	<0.001
B. longum MP324	8.56 ± 0.11	8.98 ± 0.08	9.36 ± 0.11	9.04 ± 0.09	<0.001
B. longum MP347	8.58 ± 0.13	8.96 ± 0.11	9.36 ± 0.15	9.04 ± 0.11	<0.001
B. animalis MP320	9.00 ± 0.12	9.32 ± 0.08	9.6 ± 0.07	9.10 ± 0.07	<0.001

*Two-way ANOVA tests were used to evaluate differences in mean values of CFUs/mL of samples Clear grey color indicates values having differences that are statistically significant with respect to the control group. Dark grey color means statistically significant differences among the three asparagus fractions (pairwise *t*-test comparisons between group levels with Bonferroni correction for multiple testing).

Table 6
Effect of the different green *Asparagus officinalis* L., var. *Herkolim* fractions (root by-product, spear by-product and spear edible portion) on the growth of the pathogenic strains included in this study. Bacterial counts are expressed as log10 CFU/mL ± SD Control group means the culture without supplementation with any asparagus fraction. Each assay was performed in quintuplicate.

Strain	Control	Root by-product	Spear by-product	Spear edible portion	P-value*
S. aureus MP364	10.24 ± 0.11	10.40 ± 0.12	9.76 ± 0.24	10.10 ±0.10	<0.001
S. aureus MP369	10.24 ± 0.11	10.26 ± 0.15	9.42 ± 0.15	10.12 ±0.08	<0.001
L. monocytogenes MP377	9.20 ± 0.16	9.20 ± 0.10	8.78 ± 0.08	9.18 ± 0.13	<0.001
L. monocytogenes MP380	9.20 ± 0.10	9.26 ± 0.13	8.64 ± 0.17	9.18 ± 0.15	<0.001
B. cereus MP383	9.38 ± 0.08	9.28 ± 0.19	8.52 ± 0.13	9.14 ± 0.11	<0.001
E. coli MP386	9.36 ± 0.11	9.26 ± 0.11	8.60 ± 0.12	8.78 ± 0.13	<0.001
E. coli MP388	9.32 ± 0.16	9.30 ± 0.14	8.54 ± 0.11	8.76 ± 0.05	<0.001
K. pneumoniae MP390	9.08 ± 0.08	8.98 ± 0.15	8.44 ± 0.18	8.70 ± 0.16	<0.001
Salmonella enterica serovar *enteritidis* MP395	9.20 ± 0.12	9.14 ± 0.13	8.64 ± 0.15	8.94 ± 0.23	<0.001
Salmonella S. enterica serovar *typhimurium* MP399	9.24 ± 0.15	9.26 ± 0.09	8.58 ± 0.15	9.20 ± 0.07	<0.001

*Two-way ANOVA tests were used to evaluate differences in mean values of CFUs/mL of samples Clear grey color indicates values having differences that are statistically significant with respect to the control group. Dark grey color means statistically significant differences among the three asparagus fractions (pairwise *t*-test comparisons between group levels with Bonferroni correction for multiple testing).

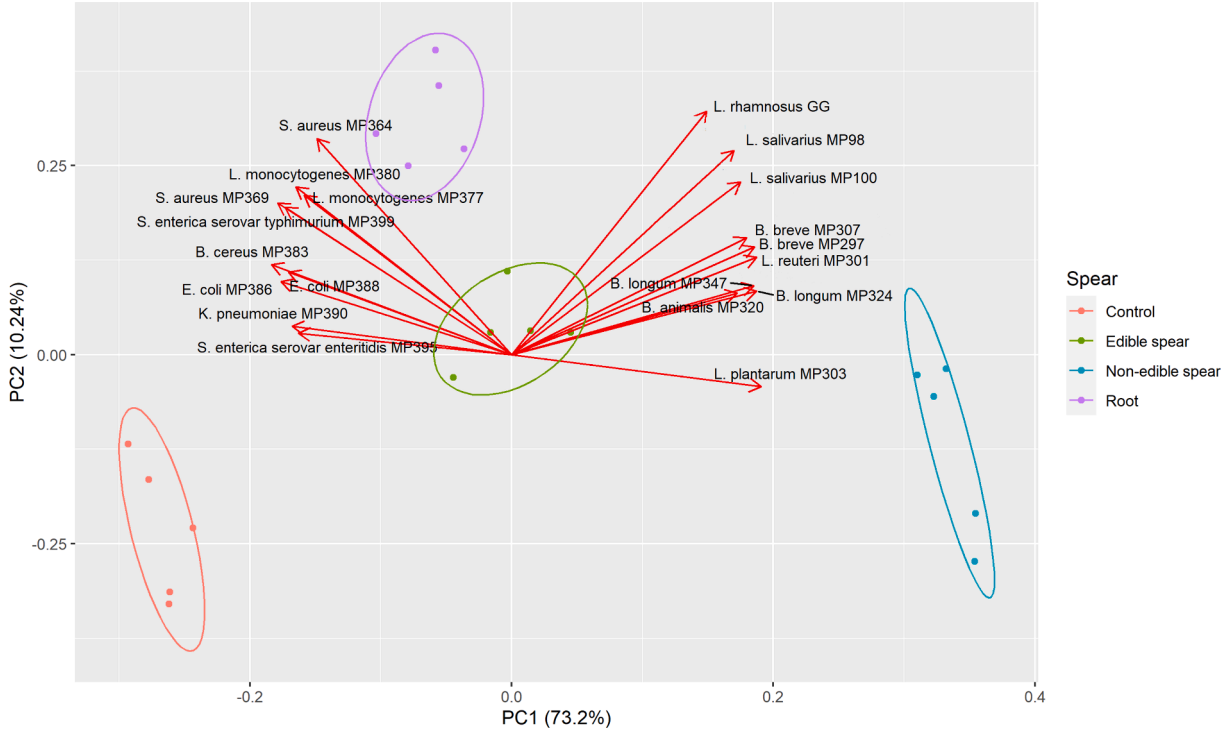

Fig. 2. Principal Components Analysis (PCA) plot of the 4 groups of samples from the edible, non-edible, root asparagus fractions and the control group. This plot is showing the relationships between the 20 bacterial strains and the samples, and the stimulating effect of the edible and non-edible spear on the growth of the lactobacilli and bifidobacteria strains assayed in this work.

nutrients and bioactive factors with regards to the growth of these beneficial bacteria.

The fact that the three fractions showed a high content of both LP and MP polyphenolic compounds may also help to explain why they did promote the growth of the *Lactobacilli* and *Bifidobacteria* but not that of the pathogen strains. On the one hand, different studies have revealed that polyphenols may act as prebiotics for commensal and mutualistic bacteria (Sanders et al., 2019; Shortt et al., 2018; Singh et al., 2019). Up

to 95 % of the consumed dietary polyphenols cannot be absorbed in the small intestine and are passed on to the colon, where they are metabolized by the residing bacteria (Marín, Miguélez, Villar, & Lombó, 2015). The ability of such compounds to produce beneficial effects are mainly due to a bi-directional relationship with the gut microbiota: polyphenols can beneficially impact the composition of the gut microbiota while beneficial gut bacteria metabolize polyphenols into bioactive compounds that produce health benefits (Mithul Aravind et al., 2021).

On the other hand, several works have demonstrated the inhibitory effect of different plant-derived polyphenols against *S. aureus*, *B. cereus*, *L. monocytogenes*, *E. coli* or *Salmonella* spp., including antibiotic-resistant strains and those forming biofilms in food and medical surfaces (Zhang et al., 2014;Kumar et al., 2021; Zhang et al., 2016; Zhao et al., 2019). There are many mechanisms explaining the inhibitory activity of polyphenols, including their ability to chelate iron, a mineral that is essential for the survival of pathogenic bacteria (Kumar et al., 2021).

4. Conclusion

As a conclusion, the results presented in this study further support the high nutritional and functional value of the asparagus, including its by-products, highlighting the potential of the non-edible parts to be used as prebiotic compounds. The composition of the three portions revealed an interesting content of proteins, carbohydrates, and dietary fiber. Nonetheless, some differences were observed among them. A remarkable content in xylose was found, mainly in by-products, indicating the presence of xylans and xyloglucans, that have shown bifidogenic effect. It is worth mentioning the concentration of inulin in the root, confirming that this residue of agricultural practices could be very usable as a raw material to obtain this prebiotic component. The phenolic profile of the spears consists of different types of flavonoid glycosides, derived from three aglycones: quercetin, kaempferol and isorhamnetin. Spear by-product and spear edible portion represent a magnificent source of these bioactive compounds, especially rutin, which has high added value as bioactive ingredient. Beside, roots by-product has also turned out to be a very good source of bioactive compounds, especially due to their great content of saponins. In this work, it has been shown that the non-edible spear is able to be selectively used to promote the growth of commensal or probiotic lactobacilli and bifidobacteria strains. While it seems clear that any part of the asparagus has a potential future either as a healthy food or as health-promoting ingredients or supplements, more work is required to elucidate those specific compounds with the highest ability to beneficially modulate the composition of the human gut microbiota. Subsequently, and in order to fulfil the definition of prebiotic, it will be necessary to demonstrate their beneficial roles on the host's health.

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CRediT authorship contribution statement

Araceli Redondo-Cuenca: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft. **Alejandra García-Alonso:** Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Rocio Rodríguez-Arcos:** Data curation, Methodology, Investigation, Software, Validation, Visualization, Writing – original draft. **Irma Castro:** Data curation, Methodology, Investigation. **Claudio Alba:** Data curation, Methodology, Investigation. **Juan Miguel Rodríguez:** Data curation, Methodology, Investigation, Software, Validation, Visualization, Writing – original draft. **Isabel Goñi:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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