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Valorization of legume by-products in functional formulations: phytochemicals and their simulated gastrointestinal fate

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The high bioactive content of legume by-products enables their utilization in the development of value-added food products. Thus, this study explores the valorization of legume by-products (chickpea hulls (CH), faba bean hulls (FH), and lentil hulls (LH)) through the formulation of novel functional infusions. The effects of *in vitro* digestion on the phenolic content and antioxidant potential of these infusions were assessed. Aqueous-methanolic (75%) and aqueous extracts of these samples were also evaluated in terms of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity. According to the results, FH and LH showed higher TPC (1002 and 1320 mg GAE/100 g, respectively) and TFC (961 and 986 mg CE/100 g, respectively) values ($p < 0.05$). Similarly, among the infusions (CHI, FHI, and LHI), which were all prepared from their respective samples, the FHI and LHI exhibited higher levels of TPC, TFC, and antioxidant capacity before and after *in vitro* digestion ($p < 0.05$). Additionally, comprehensive LC-ESI-MS/MS phenolic profiling showed the great potential for the retention of individual phenolics in newly formulated infusions. These results suggest that legume by-products have great potential for value-added applications as functional ingredients in infusion formulations, contributing to their sustainable utilization and offering health-promoting properties.

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Sustainability spotlight

Studies on malnutrition, waste utilization, and sustainability, which have attracted global as well as national attention, are gaining increasing importance day by day. Therefore, in this study, the infusions of legume by-products which have been sporadically used for food applications were investigated to determine their value-added application potential aiming to provide valuable information in terms of complete plant utilization and increased economic values. Our work emphasizes the importance of the following UN Sustainable Development Goals: zero hunger (SDG 2) and good health and well-being (SDG 3).

1. Introduction

Phenolic compounds represent a broad category of plant secondary metabolites. They are widely accumulated in several higher plant tissues such as vegetables, fruits, condiments, cereals, pulses, and nuts, and involved in several physiological functions including plant characteristics, color, aroma, and stress tolerance.[1] Phenolic compounds have recently emerged as promising components due to their antioxidant, antibacterial, anticarcinogenic, and anti-inflammatory properties. In addition it has been shown that phenolic compounds can prevent cardiovascular diseases, diabetes, and oxidative stress-related diseases.[1] Legumes, such as chickpea, faba bean, and lentil, which were investigated within this study, contain a variety of phenolic compounds that contribute to their distinct flavor and potential health benefits. These plants can be used as new sources for the production of infusions, which are described as a liquid formulation prepared by pouring boiling water over the plant materials to extract biologically active compounds.[2] Infusions have gained popularity due to their ease of preparation and natural origin; however, one key challenge is ensuring sufficient extraction and stability of bioactive compounds, particularly phenolics, during preparation and gastrointestinal digestion. Most commercial herbal infusions are derived from flowers, leaves, or roots, whereas the use of agri-food by-products such as legume hulls remains underexplored. The valorization of legume hulls for infusion production not only addresses sustainability concerns but also offers a low-

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cost, fiber-rich, and phenolic-rich alternative to conventional herbal materials.

Due to the high amount of proteins, dietary fibers, and bioactive compounds in legume by-products, they can be utilized as valuable resources in the formulation of functional food ingredients. Thus, prior studies have investigated the use of legumes and their fractions in functional beverages and extracts. For instance, chickpea-derived phenolic extracts have been shown to exhibit considerable antioxidant activity and proposed for beverage enrichment.[3,4] Similarly, faba bean hulls and lentil fractions demonstrated high antioxidant activity and phenolic content, particularly in the hull portion.[5,6] Apart from these, by-products of black bean,[7] black soybean cooking water powder[8] and soybean husk[9] were used in plant-based meat, whereas soybean cooking water powder,[10] lupine and chickpea mill residue,[11] and faba bean husk were utilized in bakery products[12] and chickpea husk was added to dairy products.[13] However, limited studies have formulated ready-to-drink infusions using these by-products, and even fewer have assessed their phenolic stability and bioaccessibility under simulated digestion. This gap presents a novel opportunity to develop functional infusion formulations from legume by-products. Such infusion formulations offer practical applicability in daily life while aligning with current consumer demand for sustainable, plant-based functional beverages.[14] On the other hand, legume by-products are also good sources of dietary fibers, phytochemicals, vitamins, minerals, and residual levels of proteins, making them suitable candidates for reutilization in human nutrition and functional product design.[15] According to Johnson and Walsh,[16] the chickpea hull by-product typically showed a higher total phenolic content (56–150 mg gallic acid equivalents (GAE)/100 g) and ferric reducing antioxidant power (38–174 mg Trolox equivalents (TE)/100 g) compared to the kernel part (TPC of 65–105 mg GAE/100 g and FRAP of 44–62 mg TE/100 g), depending on the variety of the chickpea. In another study, faba bean hulls and lentil fractions were investigated, and the results indicated that the hulls exhibited high antioxidant activity, measured by the reducing power (RP), antiradical activity (DPPH), or oxygen radical absorbance capacity (ORAC) assays,[17] indicating the potential of these by-products.

In order to maximize the health benefits of plants and their infusions, it is essential to understand the bioaccessibility of their phenolic compounds. The first step towards understanding the stability of phenolic compounds in the gastrointestinal tract is to estimate their bioaccessibility, which is defined as the number of bioactive compounds released after gastrointestinal digestion. It is evident that polyphenols may interact with other food constituents, may be metabolized by the body, or degraded during the digestion process.[18] Therefore, for improved health outcomes, researchers should develop strategies by understanding which compounds are present in a specific plant and how they are released during digestion. Accordingly, various simulation models for gastrointestinal digestion have been developed to determine the bioaccessibility of bioactive compounds.[14,19] Taken together, these points highlight the value of legume by-products in developing sustainable functional infusion formulations while improving the phenolic content of the products. Therefore, this study aimed to explore the potential of chickpea, lentil, and faba bean hulls in the formulation of functional infusions by investigating the effects of different solvent systems on their phenolic content, phenolic profile, and antioxidant activity; evaluating the retention of phenolic compounds during simulated gastrointestinal digestion, thus characterizing the bioactive composition of the infusions using LC-ESI-MS/MS analysis.

2. Materials and methods

2.1. Chemicals

Pepsin (EC 3.4.23.1, from porcine gastric mucosa), pancreatin (EC 232.468.9, from porcine pancreas, contains trypsin, amylase and lipase), bile, Folin–Ciocalteu reagent, gallic acid, catechin, neocuproine, DPPH (1,1-diphenyl-2-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8 tetramethylchromane-2-carboxylic acid), ABTS (2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid di-ammonium salt), TPTZ (triphenyltetrazolium), TFA (trifluoroacetic acid), and the other chemicals used to prepare simulated salivary, gastric and intestinal fluids were purchased from Merck Life Science. All other reagents were of HPLC or LC-MS grade and provided by Merck Life Science.

2.2. Preparation of plant samples

Legume by-products, including commercial desi chickpea hull (CH), faba bean hull (FH), and lentil hull (LH), were collected after a dehulling and splitting process at AGT Foods R&D Centre (Saskatoon, SK, Canada). Desi chickpea, faba, and lentil hulls were sifted between 14 and 30-mesh screens, and this particular cut/particle size was utilized in the process. Hull samples with 14–30 mesh particle size were washed and rinsed in water. After the washing and rinsing processes, hull samples were roasted and dried at 150 °C for 4 h. Final products were sifted with 30-mesh, and collected as the final product.

2.3. Solvent extraction

In order to extract the polyphenols from the legume by-products, they were subjected to aqueous-methanol (75% of methanol) or water treatments. In this respect, the phenolic extraction procedure was adapted from the method used by Ozkan *et al.*[14] with slight modifications. One gram (1 ± 0.01 g) of ground sample was extracted with 10 mL of either 75% aqueous-methanol (75% MeOH + 25% water) or water (W) at ambient temperature. The mixture was vortexed for 10 s and subsequently sonicated for 15 min in an ultrasonic bath (USC900TH; VWR, Radnor, PA, USA). Then, the treated samples were centrifuged at 4500 rpm for 15 min at 4 °C (Universal 32R; Hettich Zentrifugen, Tuttlingen, Germany), and the supernatants were collected. This extraction procedure was repeated twice for the pellet and the supernatants were pooled and made up to a final volume of 20 mL. Prepared extracts were stored at −20 °C until further analysis.

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2.4. Preparation of the infusions

All infusions, including desi chickpea hull infusion (CHI), faba bean hull infusion (FHI), and lentil hull infusion (LHI), were prepared by grinding the legume by-products followed by hot water treatment, as explained below. First, each sample was separately ground with a common kitchen coffee grinder (Sinbo, Türkiye) and stored at ambient temperature until use. Infusion preparation was adapted from the procedure reported previously.[18] This method was developed based on the conclusions of an internal screening test. For each sample, 10 grams of samples were weighed into a beaker, 80 mL of water at 85 °C was added, and then, the mixture was left for 10 min without heating. Thereafter, the mixture was cooled and filtered through Whatman No. 4 paper. All infusions were stored at −20 °C until further analysis.

2.5. *In vitro* simulated gastrointestinal digestion

The stability of phenolic compounds in the infusions during gastrointestinal digestion was determined based on a protocol reported by Minekus *et al.*[20] with minor modifications. This method consists of three sequential simulations of the oral, gastric, and intestinal phases. Simulated salivary fluid contains potassium chloride (KCl), monopotassium phosphate (KH_2PO_4), sodium bicarbonate ($NaHCO_3$), magnesium chloride hexahydrate ($MgCl_2(H_2O)_6$), ammonium carbonate ($(NH_4)_2CO_3$), hydrochloric acid (HCl), and calcium chloride dihydrate ($CaCl_2(H_2O)_2$). Simulated gastric fluid includes KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2(H_2O)_6$, $(NH_4)_2CO_3$, HCl, and $CaCl_2(H_2O)_2$. Simulated intestinal fluid is composed of KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2(H_2O)_6$, HCl, and $CaCl_2(H_2O)_2$. In order to simulate oral digestion, 5 mL of each infusion was mixed with 4 mL of simulated salivary fluid, 25 μL of 0.3 M $CaCl_2$, and 0.975 mL of distilled water to obtain 10 mL of the oral bolus. The mixture was incubated at 37 °C for 2 min in a shaking water bath (Memmert SV1422; Nürnberg, Germany). For the simulation of the gastric digestion stage, 10 mL of oral bolus was mixed with 7.5 mL of simulated gastric fluid, 1.6 mL of pepsin solution (25 000 U mL^{-1}), and 5 μL of 0.3 M $CaCl_2$, and the pH was adjusted to 3 with 1 M HCl. Thereafter, the volume of the mixture was made up to 20 mL with the addition of distilled water. The mixture was then incubated at 37 °C for 2 h in a shaking water bath, and 5 mL aliquots were collected from the gastric phase for further analyses. Finally, to simulate the intestinal digestion stage, 15 mL aliquot from the gastric phase, 8.25 mL of simulated intestinal fluid, 3.75 mL of pancreatin (800 U mL^{-1}), 1.875 mL of bile solution (160 mM), and 30 μL of 0.3 M $CaCl_2$ were mixed, and the pH was adjusted to 7 with 1 M NaOH. Then, the volume of gastric chyme was made up to 30 mL with the addition of distilled water. The mixture was incubated at 37 °C for 2 h in a shaking water bath.

A blank (without the added sample) was incubated under the same conditions to eliminate interferences due to the digestive enzymes and buffers used in the digestion process. All experiments were performed in triplicate. The samples collected from simulated gastric and intestinal phases were centrifuged at 10 000 rpm for 30 min at 4 °C, and the supernatants were stored at −20 °C until further analysis. The infusions before, during, and after *in vitro* digestion were grouped as undigested infusions (UD), gastric digested (GD), and intestinal digested (ID) infusions, respectively.

In order to calculate the bioaccessibility, the following equation was used, and the calculated values were expressed as percentage:

$$\text{Bioaccessibility (\%)} = (BC_{digested}/BC_{undigested}) \times 100$$

where, $BC_{digested}$ is the quantity of bioactive compounds (TPC, TFC, DPPH, CUPRAC, ABTS, or individual polyphenols) recovered in the supernatants of centrifuged final digesta (BF: bioaccessible fraction) and $BC_{undigested}$ is the undigested infusion.

2.6. Spectrophotometric analyses

Aqueous-methanolic and aqueous extracts of the legume by-products, as well as undigested, gastric digested, and intestinal digested infusion samples, were subjected to spectrophotometric analyses through a UV-visible spectrophotometer (Synergy HT; BioTek Instruments, Winooski, VT, USA). Each measurement was performed at least in triplicate.

2.6.1. Determination of total phenolic content. Total phenolic content (TPC) assay was carried out according to the method of Singleton and Rossi.[21] The absorbances of the samples were measured at 765 nm, and the calibration curve was plotted by using gallic acid in the range of 0.02–0.6 mg mL^{-1} ($y = 3.6388x + 0.0181$; $R^2 = 0.9981$). TPC results were reported as mg gallic acid equivalents (GAE) per 100 g sample.

2.6.2. Determination of total flavonoid content. Determination of total flavonoid content (TFC) was carried out according to Dewanto and Wu.[22] The measurements were conducted at 510 nm. The calibration curve was plotted by using catechin in the range of 0.04–0.4 mg mL^{-1} ($y = 1.1334x + 0.0284$; $R^2 = 0.9926$) and results were reported as mg catechin equivalents (RE) per 100 g sample.

2.6.3. Determination of antioxidant capacity. The antioxidant capacities of the infusions were determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH),[23] cupric ion reducing antioxidant capacity (CUPRAC),[24] and 2,2-azinobis(3-ethylbenzothiazo-line)-6-sulfonic acid (ABTS)[25] assays. Measurements were performed at 450 nm, 517 nm and 734 nm, respectively. Calibration curves were plotted by using Trolox in the range of 0.01–0.1 mg mL^{-1} ($y = 4.4793x - 0.0337$ and $R^2 = 0.9907$ for DPPH; $y = 241\,17x - 0.0164$ and $R^2 = 0.9983$ for CUPRAC; $y = 5.9402x + 0.0307$ and $R^2 = 0.9960$ for ABTS). All results were reported as Trolox equivalents (TE) per 100 g of sample.

2.7. Identification and quantification of polyphenols by HPLC-PDA

The method of Ozkan *et al.*[14] was used to quantify polyphenols in the samples. Concisely, the samples were passed through 0.45 μm membrane filters before being injected into a Waters 2695 HPLC system with a PDA detector (Waters, USA). The stationary phase was a Supelcosil LC-18 (25 cm × 4.60 mm, 5 m

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column, Sigma-Aldrich, Steinheim, Germany). Solvent A: 0.1% TFA in MQ water and Solvent B: 0.1% TFA in acetonitrile were the solvents used for the analysis. Spectral measurements were performed at $\lambda = 280$, 312, and 360 nm and had a flow rate of 1 mL min^{-1} and an injection volume of 10 μL, respectively. A linear gradient was used as follows: at 0 min, 95% solvent A and 5% solvent B; at 45 min, 65% solvent A and 35% solvent B; at 47 min, 25% solvent A and 75% solvent B; and at 54 min, returning to initial conditions. Phenolic compounds were quantified by using their authentic standards. The calibration curves of polyphenol standards showed good linearity (R^2 > 0.99) within the established range (0.01–20 mg/100 mL). LOD and LOQ values ranged from 0.01 to 0.03 mg/100 mL and 0.03 to 0.09 mg/100 mL, respectively. All analyses were carried out in triplicate, and the results were expressed as mg/100 g sample.

2.8. Identification of phenolic compounds by liquid chromatography-mass spectrometry (LC-MS/MS)

In this study, specific components in infusions subjected to gastrointestinal digestion treatment were targeted. In this respect, the samples were chromatographically separated and spectrally identified according to the conditions described previously.[26] Briefly, a C18 Gemini® column (3 μm i.d., with TMS end capping, 110 Å, 100 × 2 mm) connected to a guard column (Phenomenex Inc, Torrance, CA, USA) was used to separate compounds in the following gradient solvent system: 0–60% B for 45 min., next 60–95% B for 1 min, and 95% B for 4 min, at a flow rate of 0.2 mL min^{-1}. Solvent A was water with 0.1% (v/v) formic acid, while solvent B was acetonitrile with 0.1% (v/v) formic acid. Ten μL of the sample was injected into the chromatographic column at 20 °C. The conditions were controlled by using an Agilent 1200 Infinity HPLC coupled to an Agilent 6530B QTOF system (Agilent Technologies, Santa Clara, CA, USA). Mass spectrometry detection was performed in negative ion mode setting with 10 and 30 eV collision energies for every compound. Spectra were acquired in the *m*/*z* range from 100 to 1000. Drying gas temperature and flow were 275 °C and 10 L min^{-1}, respectively, while sheath gas temperature and flow were 325 °C and 12 L min^{-1}, respectively. Nebulizer pressure was set at 35 psig. The voltage of the capillary, skimmer, and fragmentor was 4000, 65, and 140, respectively. Compounds were tentatively identified based on their accurate masses and fragmentation patterns, supported by the available databases (PubChem) and literature sources. The volume/concentration changes during the digestion steps were taken into consideration. The observation of changes in the amounts of monitored compounds after digestion was done by comparison of % peak intensities of detected ions from phenolic compounds in plant infusions before, during, and after *in vitro* digestion. In this respect, the peak intensity values of undigested infusions (UD) were set as 100%, and proportionally compared with the values of gastric digested (GD) and intestinal digested (ID) infusions.

2.9. Statistical analysis

All samples were prepared twice and analyzed at least in three replicates. Error bars on the figures show standard deviations. The results were expressed as mean ± standard deviation. Statistical analysis was performed using SPSS software (version 20.0, SPSS Inc., Chicago, IL, USA). Differences were evaluated using one-way analysis of variance (ANOVA), followed by a Tukey post hoc test ($p < 0.05$). A paired *t*-test was used to reveal differences between solvent types.

3. Results and discussion

3.1. Effect of solvent type on the content of phenolic compounds and antioxidant potential of the plant samples

Fig. 1 shows the effect of solvent type on the phenolic content and antioxidant capacity of the extracts obtained from legume by-products by aqueous-methanolic or aqueous extraction. It is clear from the results that, in general, the TPC, TFC, ABTS, DPPH, and CUPRAC values for the aqueous extracts of the plants were found to be statistically higher ($p < 0.05$) than those of aqueous-methanolic extracts. It has become evident that a significant number of bioactive compounds can be recovered from legume-based by-products (CH, FH, and LH) simply by extracting with water. In detail, TPC, TFC, ABTS, DPPH, and CUPRAC values of legume-based by-products were in the range of 164–1320 mg GAE/100 g, 156–986 mg RE/100 g, 407–3337 mg TE/100 g, 108–1200 mg TE/100 g, and 265–2486 mg TE/100 g, respectively. For aqueous extracts, faba bean hull and lentil hull possessed the highest TPC, TFC, ABTS, DPPH, and CUPRAC values ($p < 0.05$). On the other hand, regarding the aqueous-methanolic extracts, TPC, TFC, ABTS, DPPH, and CUPRAC values of lentil hull were statistically higher than those of others ($p < 0.05$). This trend can be attributed to the inherent differences in the phenolic profiles of the legume species. Faba bean and lentil hulls are known to contain higher levels of condensed tannins, flavonols (such as quercetin and kaempferol derivatives), and hydroxycinnamic acids, which are potent contributors to antioxidant activity.[27] Moreover, the denser cellular structure and pigmentation of these hulls may contribute to a higher accumulation of phenolic compounds compared to chickpea hulls. These compositional differences likely underlie their superior radical scavenging and reducing capacity.[28]

Regarding the efficiency of the solvents used for extraction, contradictory results have been reported in the literature. Similar to our results, higher antioxidant activity values or total phenolic contents in water extracts of different plant materials compared to methanolic or ethanolic extracts have been reported for *Annona muricata* L. (Graviola) leaves[29] and *Carica papaya* L. leaves.[30] On the other hand, ethanolic/methanolic extracts were reported to be superior compared to water extracts with respect to antioxidant activities in some plant materials. In the study of Butsat and Siriamornpun,[31] the maximum antioxidant activity for *Amomum chinense* C. leaves was observed with the use of 80% methanol, followed by 80% ethanol, 80% acetone, and distilled water. Some other examples presenting lower values of total phenolics or antioxidant activity in the water extracts include *Pinus densiflora* S. et Z. bark compared to ethanolic extract,[32] and ginger and *Convolvulus* species compared to their ethanolic and methanolic counterparts.[33]

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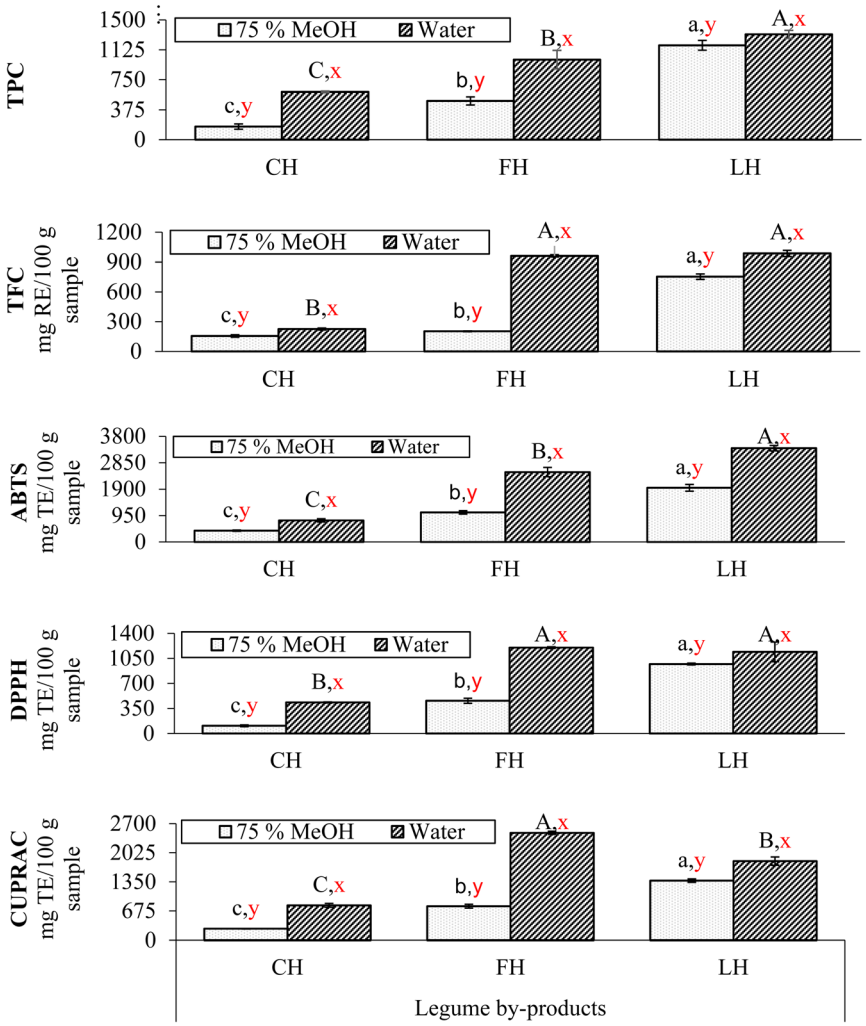

Fig. 1 Effect of solvent type on the content of phenolic compounds and antioxidant properties of legume by-products. CH, chickpea hull; FH, faba bean hull; LH, lentil hull. ^{x,y}Different lowercase letters in the adjacent bars represent significant differences ($p < 0.05$) between the solvent types. $^{a-f}$Different lowercase letters in the light bars represent significant differences ($p < 0.05$) between the samples extracted with 75% methanol. $^{A-E}$Different uppercase letters in the bold bars represent significant differences ($p < 0.05$) between the samples extracted with water.

The differences between the results may arise from (1) variations in the plant's cellular structure, (2) changes in the compositions and antioxidant activities of the extracts caused by different solvents used in extracting the compounds,[34] (3) greater antioxidant capacity of the extract containing phenolic compounds with more hydroxyl groups,[35] and (4) difference in antioxidant activities influenced by the extraction method, characteristics of the extraction solvent (*i.e.* polarity), and extraction parameters including temperature and time.[36,37]

Apart from spectrophotometric determinations, HPLC analysis was also conducted to identify and quantify the effects of solvent type on the concentration of individual polyphenols in the extracts (Table 1). In aqueous-methanolic and aqueous extracts, the maximum amount ($p < 0.05$) of epicatechin was detected in FH and LH, rutin in CH, quercetin in LH, and epigallocatechin gallate in LH. The higher epicatechin and quercetin levels in aqueous-methanolic extracts reflect both the solvent's ability to extract mid-polar phenolics and intrinsic compositional differences.[38] Faba bean hulls are particularly rich in flavan-3-ols,[39] whereas lentil hulls contain flavonols such as quercetin, aligning with their dark pigmented seed coats and reported phenolic profiles.[40] From the results obtained, it is evident that some polyphenols can be detected at their highest levels when extracted with aqueous-methanol, while others can be detected in aqueous extracts. These findings highlight the importance of solvent type in the level of polyphenols and provide valuable insights for further research and applications in various industries.[41] On the other hand, it is noteworthy to mention that the optimum extraction method and solvent should be determined considering the targeted phenolics for the best results[42] due to the fact that not all the phenolics may be extracted with the highest efficiency by using a single method/solvent.

3.2. Retention of infusion polyphenols and their antioxidant capacities during *in vitro* gastrointestinal digestion

In order to evaluate how *in vitro* simulated digestion conditions affected the polyphenol content and antioxidant capacity of

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Table 1 Effect of solvent type on the content of individual polyphenols in the legume by-products

		Type of solvent	
Phenolics	Sample codes*	75% aqueous methanol	Water
Epicatechin (mg/100 g)	CH	ND	ND
	FH	30.11 ± 1.28^{aB}	65.69 ± 0.74^{bA}
	LH	0.88 ± 0.17^{bB}	128.8 ± 1.0^{aA}
Chlorogenic acid (mg/100 g)	CH	ND	ND
	FH	0.28 ± 0.03^{B}	1.10 ± 0.01^{A}
	LH	ND	ND
Rutin (mg/100 g)	CH	12.40 ± 1.79^{A}	14.95 ± 3.96^{A}
	FH	ND	ND
	LH	ND	ND
Quercetin (mg/100 g)	CH	1.33 ± 0.53^{bA}	1.89 ± 1.10^{bA}
	FH	0.18 ± 0.04^{cB}	0.63 ± 0.01^{cA}
	LH	12.68 ± 1.53^{aB}	24.18 ± 0.41^{aA}
Syringic acid (mg/100 g)	CH	ND	ND
	FH	ND	ND
	LH	0.38 ± 0.20^{B}	0.76 ± 0.07^{A}

[a–c] Within each column, different lowercase superscript letters show differences ($p < 0.05$) between samples. [A,B] Within each row, different uppercase superscript letters show differences ($p < 0.05$) between solvent types. *CH, chickpea hull; FH, faba bean hull; LH, lentil hull.

infusions, TPC, TFC, ABTS, CUPRAC, and DPPH assays were performed to analyze their bioaccessible fractions. Table 2 illustrates the effects of *in vitro* gastrointestinal digestion on the phenolic content and antioxidant capacity of infusions prepared from legume by-products (chickpea hull infusion-CHI, faba bean hull infusion-FHI and lentil hull infusion-LHI).

The results show that *in vitro* digestion significantly ($p < 0.05$) lowered the phenolic content and antioxidant capacity of some infusions. Accordingly, TPC, TFC, and antioxidant capacity measured by ABTS, DPPH, and CUPRAC methods decreased in the following order: undigested > gastric digestion > intestinal digestion. As a result of gastric digestion, 12.1% to 50.4% of the phenolic content and antioxidant capacity were lost, whereas this loss after intestinal digestion ranged from 43.4% to 83.6% (Table 2). Accordingly, previous studies have provided evidence indicating dramatic decreases in the levels of polyphenols after undergoing *in vitro* digestion, and this phenomenon has been well-documented and consistently reported in the scientific literature. The loss of phenolics during gastrointestinal digestion can be attributed to various factors.[18,43] Polymerization, epimerization, and auto-oxidation are among the key mechanisms identified under intestinal digestion conditions.[44,45] Understanding these mechanisms is crucial for elucidating the digestion and absorption of nutrients, as well as their potential

Table 2 Changes in the phenolic contents and antioxidant capacities of legume by-product-based infusions during *in vitro* gastrointestinal digestion

		In vitro digestion**			% loss in activities		
	Sample codes*	UD	GD	ID	After GD	After ID	% bioaccessibility
TPC (mg GAE/100 g)	CHI	586 ± 34^{cA}	405 ± 37^{cB}	222 ± 30^{cC}	30.73 ± 4.03^{a}	62.03 ± 2.21^{a}	37.97 ± 2.21^{b}
	FHI	973 ± 95^{bA}	770 ± 64^{bB}	422 ± 38^{bC}	20.36 ± 7.81^{ab}	56.35 ± 4.28^{a}	43.65 ± 4.28^{b}
	LHI	1318 ± 34^{aA}	1174 ± 145^{aA}	706 ± 60^{aB}	10.89 ± 2.30^{b}	46.41 ± 1.38^{b}	53.59 ± 1.38^{a}
TFC (mg RE/100 g)	CHI	125 ± 3^{cA}	62 ± 4^{cB}	44 ± 1^{bC}	49.99 ± 0.70^{a}	64.51 ± 0.50^{a}	35.49 ± 0.50^{b}
	FHI	387 ± 15^{bA}	274 ± 22^{bB}	166 ± 4^{aC}	29.13 ± 2.75^{b}	57.06 ± 1.67^{b}	42.94 ± 1.67^{a}
	LHI	496 ± 14^{aA}	436 ± 11^{aB}	168 ± 13^{aC}	12.05 ± 2.48^{c}	66.11 ± 0.96^{a}	33.89 ± 0.96^{b}
ABTS (mg TE/100 g)	CHI	742 ± 2^{cA}	410 ± 44^{bB}	187 ± 7^{cC}	44.74 ± 0.15^{a}	74.80 ± 0.07^{a}	25.20 ± 0.07^{c}
	FHI	985 ± 9^{bA}	672 ± 5^{aB}	284 ± 5^{bC}	31.77 ± 0.62^{b}	71.17 ± 0.26^{b}	28.83 ± 0.26^{b}
	LHI	1242 ± 5^{aA}	690 ± 2^{aB}	425 ± 1^{aC}	44.44 ± 0.22^{a}	65.78 ± 0.14^{c}	34.22 ± 0.14^{a}
DPPH (mg TE/100 g)	CHI	183 ± 8^{bA}	122 ± 13^{cB}	30 ± 5^{cC}	33.36 ± 3.09^{a}	83.61 ± 0.76^{a}	16.39 ± 0.76^{c}
	FHI	294 ± 16^{aA}	196 ± 11^{bB}	87 ± 9^{bC}	33.20 ± 3.64^{a}	70.35 ± 1.62^{b}	29.65 ± 1.62^{b}
	LHI	304 ± 4^{aA}	227 ± 17^{aB}	143 ± 18^{aC}	25.32 ± 0.98^{b}	52.96 ± 0.62^{c}	47.04 ± 0.62^{a}
CUPRAC (mg TE/100 g)	CHI	415 ± 41^{cA}	255 ± 19^{cB}	225 ± 30^{cB}	38.15 ± 6.14^{a}	45.43 ± 5.42^{a}	54.57 ± 5.42^{a}
	FHI	698 ± 56^{bA}	544 ± 41^{bB}	376 ± 34^{bC}	21.73 ± 6.30^{b}	45.90 ± 4.35^{a}	54.10 ± 4.35^{a}
	LHI	1029 ± 56^{aA}	883 ± 50^{aB}	582 ± 46^{aC}	14.20 ± 4.69^{b}	43.33 ± 3.09^{a}	56.67 ± 3.09^{a}

[a–c] Within each column, different lowercase superscript letters show differences ($p < 0.05$) between infusions. [A–C] Within each row, different uppercase superscript letters show differences ($p < 0.05$) during digestion. *CHI, chickpea hull infusion; FHI, faba bean hull infusion; LHI, lentil hull infusion. **UD, undigested; GD, gastric digestion; ID, intestinal digestion.

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Table 3 Retention of individual phenolics in infusions during *in vitro* gastrointestinal digestion

		In vitro digestion**		
Phenolics	Sample code*	UD	GD	ID
Epicatechin (mg/100 g)	CHI	ND	ND	ND
	FHI	137.0 ± 19.9^{aA}	132.7 ± 11.9^{aA}	54.10 ± 0.22^{B}
	LHI	165.0 ± 22.8^{aA}	16.13 ± 2.90^{bB}	ND
Chlorogenic acid (mg/100 g)	CHI	ND	ND	ND
	FHI	0.31 ± 0.06^{A}	ND	ND
	LHI	ND	ND	ND
Rutin (mg/100 g)	CHI	79.19 ± 2.10^{A}	4.10 ± 0.18^{B}	2.29 ± 0.00^{C}
	FHI	ND	ND	ND
	LHI	ND	ND	ND
Quercetin (mg/100 g)	CHI	6.52 ± 0.18^{bA}	0.65 ± 0.00^{bB}	ND
	FHI	0.69 ± 0.10^{cB}	0.61 ± 0.00^{cB}	0.84 ± 0.00^{bA}
	LHI	17.52 ± 1.32^{aB}	26.79 ± 0.46^{aA}	8.37 ± 2.43^{aC}
Syringic acid (mg/100 g)	CHI	ND	ND	ND
	FHI	ND	ND	ND
	LHI	1.39 ± 0.24^{B}	0.45 ± 0.10^{C}	20.70 ± 3.96^{A}

[a–c] Within each column, different lowercase superscript letters show differences ($p < 0.05$) between infusions. [A–C] Within each row, different uppercase superscript letters show differences ($p < 0.05$) during digestion. *CHI, chickpea hull infusion; FHI, faba bean hull infusion; LHI, lentil hull infusion; ND, not detected. **UD, undigested; GD, gastric digestion; ID, intestinal digestion.

impact on health and disease, given that many phenolic compounds in foods can bind to proteins, carbohydrates, and dietary fibers through chemical bonds, thereby modifying their bioavailability for absorption during gastrointestinal digestion. In the context of intestinal digestion, polymerization refers to the joining of monomers to form larger molecules, such as polysaccharides (complex carbohydrates), proteins, or nucleic acids. This process is critical for the breakdown of complex dietary components into simpler forms that can be absorbed by the body. Epimerization may involve the conversion of one form of a molecule, such as a sugar or an amino acid, into another form with a slightly different configuration. This process can impact the bioavailability and metabolism of nutrients. Besides, during the process of gastrointestinal digestion, auto-oxidation may occur when certain dietary components, such as unsaturated fats or antioxidants, come into contact with oxygen in the gut. This process can lead to the generation of oxidative stress and the production of potentially harmful reactive oxygen species (ROS) within the gastrointestinal tract. During *in vitro* digestion, the oxygen levels are higher compared to those under natural physiological conditions, potentially promoting the epimerization and auto-oxidation of phenolic compounds.[46] In addition, increased pH levels, residual dissolved oxygen, and the probable occurrence of reactive oxygen species due to regular digestive processes might trigger several reactions within the intestinal tract, including epimerization and auto-oxidation.[47] As a consequence, these processes contribute to the degradation and transformation of phenolic compounds, ultimately leading to their reduced concentration in the digestive system.

Determining the bioaccessibility of bioactive compounds in plant infusions is essential to understanding their digestive fate in order to fully exploit their health benefits. The bioaccessibility values according to TPC and TFC changed from 37.97 to 53.59% and 33.89 to 42.94% (Table 2), respectively. On the other hand, LHI and FHI showed the highest bioaccessibility values by both methods, respectively ($p < 0.05$). Based on the antioxidant activity methods, ABTS, DPPH, and CUPRAC assays, the bioaccessibility values changed from 25.20 to 34.22%, 16.39 to 47.04%, and 54.10 to 56.67%, respectively (Table 2). Among the methods used to measure the bioaccessibility of the antioxidant potential during digestion, LHI (ABTS and DPPH) exhibited the highest ($p < 0.05$) antioxidant bioaccessibility value. The decrease in the bioaccessibility values of the infusions can be attributed to several reasons. In general, lentil hull infusion (LHI) and faba bean infusion (FHI) displayed the highest bioaccessibility values because their rich and diverse phenolic profiles, indicating that flavonols and flavan-3-ols are effectively released during gastric digestion and maintain stability under intestinal conditions. These mid-polar compounds show resilience against pH shifts and are less likely to be sequestered by fibers or enzymes.[48] In particular, the DPPH assay favors hydrogen-donating flavonols from LHI, while the ABTS assay better captures electron-transfer capabilities of flavan-3-ols from FHI.[49] The overall decrease in bioaccessibility observed across all infusions aligns with known influences of pH changes, enzymatic degradation, and phenolic–matrix interactions during gastrointestinal digestion. Despite their relative stability, flavanols are prone to structural transformations, especially epimerization and oxidation at near-neutral gastrointestinal pH (6–7.5), which, alongside enzymatic degradation and matrix interactions, critically shape their evolving bioaccessibility during digestion. Flavanols, a subclass of flavonoids, are known for their high stability. However, they can undergo partial degradation under gastrointestinal (GI) conditions. These changes can result in the epimerization of flavanols when the pH exceeds 6. There are also other factors that influence the bioaccessibility and

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Table 4 Phenolic compounds identified by UPLC-ESI-MS/MS analysis in infusions

Phenolic compounds	Molecular formula	$t_{R\ MS}$ (min)	Negative ion mode $[M-H]^-$ (m/z), (Δ, ppm)	(m/z) predicted	Fragment ions (m/z)	References
Chickpea hull infusion (CHI)						
Dihydroxybenzoic acid hexoside	$C_{13}H_{16}O_9$	7.118	315.0734 (−3.94)	315.0722	153.0183, 152.0129, 109.0293, 108.0229	60
Gallocatechin	$C_{15}H_{14}O_7$	8.708	305.0670 (−1.06)	305.0667	174.9546, 125.0240, 179.0359, 137.0214, 165.0179, 219.0668, 261.0779	61
Epigallocatechin	$C_{15}H_{14}O_7$	12.387	305.0657 (3.19)	305.0667	125.0240, 174.9560, 179.0370, 137.0239, 219.0673, 165.0175, 261.0747	Fragmentation
Myricetin rhamnoglucoside	$C_{27}H_{30}O_{17}$	21.587	625.1406 (0.68)	625.1410	316.0232, 178.9991, 271.0247, 151.0036	61
Quercetin-3-*O*-β-D-xylopyranosyl-(1→2)-rutinoside	$C_{32}H_{38}O_{20}$	22.089	741.1882 (0.23)	741.1884	300.0285, 301.0329, 178.9986, 271.0260, 609.1505	60
Tetrahydroxymethoxyflavone *O*-dihexoside *O* – pentoside	$C_{33}H_{40}O_{21}$	22.590	771.1987 (0.30)	771.1989	315.0149, 331.0464, 756.1772, 287.0192, 639.1585	Fragmentation
Rutin	$C_{27}H_{30}O_{16}$	23.343	609.1468 (−1.13)	609.1461	300.0286, 301.0362, 271.0244, 151.0043	60–62
Kaempferol 3-*O*-lathyroside-7-*O*-α-L-rhamnopyranoside	$C_{32}H_{38}O_{19}$	23.427	725.1943 (−1.17)	725.1935	284.0327, 255.0281, 575.1360, 593.1615, 227.0297	60 and 61
Isorhamnetin 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside	$C_{33}H_{40}O_{20}$	23.761	755.2065 (−3.28)	755.2040	315.0526, 299.0227, 300.0288, 271.0333, 357.0563, 623.1698	60
Kaempferol 3-*o*-rutinoside	$C_{27}H_{30}O_{15}$	24.932	593.1500 (2.01)	593.1512	285.0395, 255.0288, 327.0482	60 and 61
Pseudobaptigenin	$C_{16}H_{10}O_5$	35.805	281.0448 (2.65)	281.0455	253.0498, 223.0418, 135.0094, 91.0185, 208.0525, 195.0443	61 and 63
Biochanin A	$C_{16}H_{12}O_5$	41.241	283.0621 (−3.18)	283.0612	268.0399, 148.9547, 240.0427, 117.0347, 239.0339, 151.0015	61–63
Faba bean hull infusion (FHI)						
Gallocatechin	$C_{15}H_{14}O_7$	8.620	305.0684 (−5.63)	305.0667	125.0244, 174.9551, 165.0204, 179.0342, 167.0367, 219.0667	61 and 64
Procyanidin dimer type A isomer 1	$C_{30}H_{26}O_{13}$	9.707	593.1306 (−0.9)	593.1301	407.0732, 177.0170, 289.0688, 125.0232, 245.0791, 290.0709, 305.0659	39 and 61
Chlorogenic acid isomer	$C_{16}H_{18}O_9$	10.049	353.0881 (−0.83)	353.0878	191.0559; 179.0340; 135.0442; 173.0417	Fragmentation, 61
Procyanidin dimer type A isomer 2	$C_{30}H_{26}O_{13}$	12.383	593.1301 (−0.06)	593.1301	125.0242, 289.0721, 407.0781, 177.0189, 245.0839, 423.0711, 305.0673	39 and 61
Epigallocatechin	$C_{15}H_{14}O_7$	12.387	305.0674 (−2.36)	305.0667	125.0247, 179.0333, 219.0660, 137.0262, 174.9558, 167.0344	61, 64 and 65
Procyanidin dimer type B	$C_{30}H_{26}O_{12}$	12.470	577.1355 (−0.61)	577.1351	289.0706, 407.0765, 125.0231, 245.0797, 161.0251, 290.0747	39, 64 and 66
Catechin	$C_{15}H_{14}O_6$	13.136	289.0717 (0.21)	289.0718	245.0829, 205.0502, 125.0242, 179.0343, 109.0299, 137.0244, 151.0381, 165.0206	64–66
Epicatechin	$C_{15}H_{14}O_6$	17.318	289.0717 (0.21)	289.0718	245.0827, 205.0509, 125.0245, 179.0357, 109.0290, 137.0232, 165.0190, 151.0393	64 and 66
Tetrahydroxyflavone *C*-di-hexoside	$C_{27}H_{30}O_{15}$	18.990	593.1506 (1.00)	593.1512	593.1495, 353.0668, 473.1109, 383.0759, 503.1202, 413.0872	Fragmentation
Kaempferol 3-*O*-rhamnoglucoside-7- *O*-rhamnoside	$C_{33}H_{40}O_{19}$	22.503	739.2099 (−1.08)	739.2091	284.0322, 285.0366, 255.0306, 575.1430, 593.1481, 227.0358	67 and 68
Quercetin	$C_{15}H_{10}O_7$	31.284	301.0353 (0.25)	301.0354	151.0045, 178.9995, 174.9578, 107.0149, 121.0294, 65.0052	61, fragmentation

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Table 4 (*Contd.*)

Phenolic compounds	Molecular formula	$t_{R\ MS}$ (min)	Negative ion mode: $[M–H]^-$ (*m*/*z*), (Δ, ppm)	(*m*/*z*) predicted	Fragment ions (*m*/*z*)	References
Lentil hull infusion (LHI)						
Procyanidin trimer	$C_{45}H_{38}O_{20}$	9.123	897.1900 (−1.82)	897.1884	711.1278, 303.0487, 289.0692, 177.0169, 125.0223, 407.0762	69 and 70
Procyanidin dimer type A	$C_{30}H_{26}O_{13}$	9.708	593.1290 (1.79)	593.1301	407.0753, 289.0705, 177.0184, 125.0229, 245.0795, 137.0229	70 and 71
(Epi)catechin glucoside	$C_{21}H_{24}O_{11}$	11.632	451.1244 (0.41)	451.1246	137.0246, 125.0251, 271.0622, 289.0717, 151.0412, 313.0935	71–73
Procyanidin dimer type B	$C_{30}H_{26}O_{12}$	12.468	577.1341 (1.82)	577.1351	289.0718, 407.0776, 125.0245, 245.0812, 161.0249, 137.0246	69, 70 and 72
Epicatechin	$C_{15}H_{14}O_6$	13.054	289.0717 (0.21)	289.0718	245.0793, 203.0688, 205.0490, 125.0233, 179.0339, 109.0293	69, 71, 72 and 74
Procyanidin trimer	$C_{45}H_{38}O_{18}$	13.221	865.1974 (1.31)	865.1985	287.0548, 125.0238, 289.0695, 407.0743, 577.1308, 695.1343	69, 70 and 72
Kaempferol tetraglycoside (kaempferol *O*-dirutinoside)	$C_{39}H_{50}O_{24}$	18.908	901.2617 (0.25)	901.2619	755.1982, 756.2009, 284.0302, 285.0374, 430.0871, 575.1342	72 and 73
Quercetin *O*-rhamnoside	$C_{21}H_{20}O_{11}$	25.850	447.0933 (−0.03)	447.0933	300.0260, 301.0332, 255.0273, 284.0304, 271.0236, 151.0026	72
Kaempferol *O*-glucoside	$C_{21}H_{20}O_{11}$	26.352	447.0931 (0.41)	447.0933	285.0370, 286.0415, 165.0146, 150.9986, 119.0493, 133.0301	69 and 72
Quercetin	$C_{15}H_{10}O_7$	31.202	301.0353 (0.25)	301.0354	151.0023; 174.9565; 178.9964; 121.0324; 130.9661; 179.9942	61, fragmentation
Kaempferol	$C_{15}H_{10}O_6$	31.286	285.0400 (1.61)	285.0405	133.0286, 151.0025, 175.0378, 107.0124, 199.0374, 149.0217	72
Soyasaponin I	$C_{48}H_{78}O_{18}$	39.314	941.5111 (0.47)	941.5115	—	75
Soyasaponin βg	$C_{54}H_{84}O_{21}$	42.493	1067.5440 (−0.72)	1067.5432	—	75

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bioavailability of phenolic compounds, including their chemical structure, interactions with other active compounds in a food matrix, hydrophobicity, absorption in the gastrointestinal tract, digestion of foods, and glucuronic acid level.[50]

Table 3 shows the effects of *in vitro* digestion on the individual polyphenols in legume by-product-based infusions. The polyphenols epicatechin, chlorogenic acid, rutin, quercetin, and syringic acid detected in the extracts of the legume by-products were also detected in their infusions. The analysis revealed that the polyphenols could be easily extracted into the liquid phase during the infusion process.

From Table 3, it is obvious that some polyphenols could be retained in gastric and intestinal digestion stages; in other words, the polyphenols detected in undigested (UD) infusions were also generally detected in gastric digested (GD) and intestinal digested (ID) infusions. However, some polyphenols could not be detected in intestinal digested infusions. For example, chlorogenic acid, which was identified in undigested FHI, was not detected in gastric or intestinal digestion phases. The results of the study demonstrated that these polyphenols were significantly eliminated during the intestinal digestion stage. As a matter of fact, phenolic substances may degrade during digestion because phenolic substances have low stability under alkaline conditions.[51] However, it is also important to note that some compounds were able to pass through the gastric digestion stage without much impact. This suggests that while these compounds may pass the initial stages of digestion, they were not able to withstand the harsh conditions of intestinal digestion.

In addition to these, Table 3 shows that a number of polyphenols increased while the others decreased during gastrointestinal digestion. Quercetin in LHI could be given as an example whose amounts increased ($p < 0.05$) after gastric digestion and decreased after intestinal digestion. In agreement with our results, other investigations on various food samples reported that polyphenol concentrations increased after *in vitro* gastric digestion and that total phenolic concentrations declined significantly following postdigestion.[52,53] The amount of total flavonoids was significantly higher after simulated gastric digestion in raspberry species, as reported by Qin and Wang.[54] In summary, our results indicated that the *in vitro* simulation process had different effects depending on the type, nature, and concentration of the bioactive compounds in infusions, which was consistent with a previous report.[14]

A significant increase in the contents of quercetin in FHI, and syringic acid in LHI was observed after the intestinal digestion stage, which was in parallel with a previous work.[55] Accordingly, epicatechin, quercetin, and syringic acid were assumed to be released during *in vitro* intestinal digestion and showed an increase in the infusions. This result was consistent with the report of Qin and Wang[54] who reported that raspberry samples after intestinal digestion contained the highest levels of total flavonoids, and that intestinal digestion also led to a release of some phenolic compounds. A significant increase in

Table 5 Effects of *in vitro* digestion on % peak intensities of the phenolic compounds quantified by UPLC-ESI-MS/MS analysis

Chickpea hull infusion	UD	GD	ID	Faba bean hull infusion	UD	GD	ID	Lentil hull infusion	UD	GD	ID
Dihydroxybenzoic acid hexoside	100,00	0,00	165.5	Gallocatechin	100,00	72.07	0.00	Procyanidin trimer	100,00	57.63	0.00
Gallocatechin	100,00	69.11	0,00	Procyanidin dimer type A isomer 1	100,00	39.77	0.00	Procyanidin dimer type A	100,00	75.11	0.00
Epigallocatechin	100,00	36.32	0,00	Chlorogenic acid isomer	100,00	44.84	0.00	(Epi)Catechin glucoside	100,00	51.95	0.00
Myricetin rhamnoglucoside	100,00	417.1	0.23	Procyanidin dimer type A isomer 2	100,00	47.96	0.00	Procyanidin dimer type B	100,00	125.3	0.00
Quercetin-3-O-β-D-xylopyranosyl-(1→2)-rutinoside	100,00	194.9	145.8	Epigallocatechin	100,00	64.95	0.00	Epicatechin	100,00	339.7	2,93
Tetrahydroxymethoxyflavone O-dihexoside O - pentoside	100,00	244.2	161.6	Procyanidin dimer type B	100,00	141.2	0.00	Procyanidin trimer	100,00	101.6	0.00
Rutin	100,00	305.4	77.64	Catechin	100,00	104.6	0.00	Kaempferol tetraglycoside (Kaempferol O-dirutinoside)	100,00	290.0	151.4
Kaempferol 3-O-lathyroside-7-O-α-L-rhamnopyranoside	100,00	176.2	222.0	Epicatechin	100,00	69.35	0.00	Quercetin *O*-rhamnoside	100,00	69.24	0.00
Isorhamnetin 3-O-β-D-xylopyranosyl- (1→2)-β- D-glucopyranoside-7-O- α-L-rhamnopyranoside	100,00	183.9	172.6	Tetrahydroxyflavone C-di-hexoside	100,00	517.5	124.3	Kaempferol *O*-glucoside	100,00	0.00	0.00
Kaempferol 3-o-rutinoside	100,00	233.8	117.7	Kaempferol 3-O-rhamnoglucoside-7- O-rhamnoside	100,00	186.9	75.58	Quercetin	100,00	0.00	0.00
Pseudobaptigenin	100,00	0,00	15,09	Quercetin	100,00	0.00	0.00	Kaempferol	100,00	19.18	6,82
Biochanin A	100,00	0,00	15.67					Soyasaponin I	100,00	5,38	8,78
								Soyasaponin βg	100,00	0.00	0.00

*UD, undigested; GD, gastric digested; ID, intestinal digested.

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the bioaccessibility of compounds was observed after moving from the acidic stomach environment to a slightly alkaline intestinal environment, which indicates that intestinal conditions enabled the compounds to be released from the plant matrix and remain stable.[56]

3.3. Liquid chromatography-mass spectrometry (LC-MS/MS) supported identification of individual phenolic compounds in infusions during gastrointestinal digestion

As a part of this study, specific components in infusions after digestion were targeted. Tables 4 and 5 present the MS data of the compounds detected, along with a list of molecular formulae, retention times ($t_{R\ MS}$), mass $[M-H]^-$, predicted m/z values, and main fragments (MS^2 m/z ion fragments) derived from MS/MS analysis. A total of 12 phenolic compounds were present in CHI, 11 in FHI, and 13 in LHI. Percentage peak intensity values of myricetin rhamnoglucoside, quercetin-3-*O*-β-D-xylopyranosyl-(1→2)-rutinoside, tetrahydroxymethoxyflavone *O*-dihexoside *O*–pentoside, rutin, kaempferol 3-*O*-lathyroside-7-*O*-α-L-rhamnopyranoside, isorhamnetin 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside, and kaempferol 3-*O*-rutinoside were observed to increase in CHI during the gastric and intestinal phases. Catechin, tetrahydroxyflavone *C*-di-hexoside, and kaempferol 3-*O*-rhamnoglucoside-7-*O*-rhamnoside in FHI; procyanidin dimer type B, kaempferol tetraglycoside, and Soyasaponin I in LHI were the other phenolic compounds whose % peak intensity value increased during the gastric and intestinal phases. The reason why gastrointestinal digestion increased the amount of these phenolic compounds can be explained by the acidic medium in the gastric phase, which may facilitate the release of polyphenolic compounds in the food matrix by breaking the bonds between bioactive compounds and nutrients, such as fibers, proteins, and carbohydrates,[57] allowing them to be easily measured.[58] A further explanation could be the improvement in the solubility of certain phenolic compounds, before they are linked or present in a reduced form.[59]

4. Conclusions

The present study focused on the polyphenols of legume by-products and their infusions as well as the effect of *in vitro* digestion on antioxidant properties and phenolic compounds of these infusions. It was observed that water extracts of legume by-products had higher TPC, TFC, and antioxidant activity values compared to their methanolic counterparts. FH and LH possessed the highest phenolic contents and the strongest antioxidant capacity. Phenolic content and antioxidant capacity of some infusions were remarkably lowered during *in vitro* digestion, suggesting the impact of the conditions in the small intestine on the antioxidant activities of the infusions, depending on the type, nature, and concentration of the phenolic compounds present.

On the other hand, FHI and LHI were observed to be less affected during the digestion process, and thus their stability and resilience make them valuable components in various industries. By retaining their properties and functionality, FHI and LHI can fulfill their intended purposes and provide the desired benefits to consumers. Based on the results of this study, valuable insights are provided for the potential use of these by-products in infusions and further valorization in different novel applications, including incorporation into functional food formulations.

Conflicts of 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

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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