

Probiotic plant-based yogurts from common beans (*phaseolus vulgaris*): physicochemical properties, bioactive peptides, and health-promoting activities before and after in vitro digestion

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Research Article

Keywords: Antihypertensive, Bioactive peptides, Gastrointestinal simulation, Antioxidant activity, Total phenolic content

Posted Date: December 22nd, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-8339775/v1>

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Additional Declarations: No competing interests reported.

Abstract

Soybean and common beans (navy, great northern, pinto, and red kidney) were used to prepare plant-based milks and their corresponding probiotic yogurts. Fermentation significantly enhanced textural properties of all bean milks, particularly in great northern yogurt, which also presented the highest whiteness along with the lowest syneresis (3.20%). Pinto yogurt had the greatest free radical scavenging activity (60.61 g/mL) and total phenolic content (2,736.92 mg/L). Total peptide content considerably increased in all fermented samples, highest in red kidney yogurt, as confirmed by SDS–PAGE. After gastrointestinal simulation, yogurt digestates showed improved bioactivity over bean milks, including higher phenolics (up to 6,869.84 mg/L in pinto), radical quenching (greatest in pinto with 112.76 g/mL), and antihypertensive activity (29.75% in red kidney). Viable probiotics remained above 10⁶ CFU/g after 28 days. These findings suggest that bioactive-rich, bean-based functional yogurts can have potential health-promoting effects on the digestive system, targeting hypertension while serving as carriers of probiotics.

1. Introduction

Nowadays, with increasing public awareness about "healthy living," a nutritious diet for maintaining the body's well-functioning and -being has increasingly become an influential trend. Foods rich in probiotics and bioactive compounds have been shown to improve health and reduce the risk of chronic diseases such as hypertension and gastrointestinal (GI) tract illnesses [1]. Common beans (*Phaseolus vulgaris*), often referred to as "the meat of the poor" due to their affordability and high protein content, ranging from 18.5% to 32%, are a valuable source of plant protein [2]. The various bioactive components in common beans, including polyphenols and proteins, play a crucial role in therapeutic effects on colonic mucosal damage and inflammatory responses. They also influence the bacterial populations in the lower bowel, alter short-chain fatty acid profiles in fecal fermentations, and affect lipid profiles in the blood of both animals and humans [3]. Despite their annual global yield exceeding 28 million tons, the industrial utilization of common beans, especially for value-added food products, remains limited [4]. Various processing technologies, such as fermentation and enzymatic treatment, have been employed to enhance the bioactivity and bioavailability of phenolic compounds and the release of bioactive peptides from proteins. This results in functional foods and beverages with improved health benefits [3, 5, 6].

Plant-based yogurt is gaining popularity due to its low cholesterol and lactose content, making it an excellent alternative for those who are lactose intolerant or allergic to milk proteins. Lactic acid bacteria (LAB), used as probiotic strains in yogurt fermentation, are key starters due to their developed proteolytic systems and ability to metabolize nutrients in beans to produce bioactive compounds, aroma, flavor, and preservative compounds such as organic acids and bacteriocins [7]. Additionally, enzymatic hydrolysis during the physiological processes of GI digestion can modulate biological activities, increase bioactivity, and naturally break down proteins into amino acids and peptides [8]. Previous research has shown that peptides derived from common bean proteins exhibit antioxidant properties [3] and antihypertensive effects [9]. The antihypertensive activity is mainly due to the inhibition of Angiotensin I-Converting Enzyme (ACE) in the Renin-Angiotensin-Aldosterone System (RAAS) [2], which helps prevent high blood pressure. In clinical practice, synthetic ACE inhibitors like captopril, enalapril, and ramipril are used to treat cardiovascular diseases but can cause serious side effects such as gastrointestinal issues, skin rashes, and taste disturbances [9]. Therefore, discovering new, safe, and natural ACE inhibitors from food sources is of great importance. Wu et al [10] demonstrated that fermentation of mung bean milk can generate peptides with enhanced ACE-inhibitory activity, highlighting its potential as a functional food. Similarly, Montemurro et al [11] reported that fermenting a hemp–rice blend improved both antioxidant capacity and sensory attributes. Despite these promising findings, such studies have typically focused on a single legume type and a narrow range of parameters, with limited comparative evaluations across multiple common bean cultivars.

Although plant-based milk and yogurt alternatives have attracted considerable attention, relatively few studies have focused on formulations based on common beans. Most existing work has either concentrated on a single bean type, typically soybean, [12–14] or restricted its scope to basic compositional and functional properties [7, 15, 16]. Research to date has also been largely confined to yogurt products, with little examination of the corresponding bean milks or of how

fermentation affects peptide profiles and post-digestion bioactivity [7, 17–19]. Furthermore, previous studies have either not included an appropriate control such as soy or have instead used animal milk as the reference [6, 9, 19, 20].

This gap in comparative, in-depth analysis limits our ability to identify the most suitable bean cultivars for probiotic yogurt development and functional food applications. Therefore, the present study aimed to develop and evaluate yogurt alternatives derived from four commonly consumed bean cultivars (pinto, navy, great northern, and red kidney beans), using soybean as a reference. This work integrates fermentation performance, physicochemical analysis, peptide profiling, and in vitro digestion to assess antioxidant and ACE-inhibitory activity. Furthermore, the structural and microbiological stability of bean-based yogurts was assessed after 28 days of refrigerated storage. By addressing limitations in prior studies, this research provides a more complete understanding of the suitability of different bean types for plant-based functional yogurt development.

2. Materials and Methods

2.1. Materials

Dry beans, including great northern (GN), pinto (P), navy (N), red kidney (RK), and soy (S), along with mixed yogurt culture (Yogourmet, Lyo-San Inc., Lachute, QC, Canada), sugar, and coconut milk (Thai Kitchen, Simply Asia Foods, Thailand), were sourced from commercial suppliers (Lincoln, NE, USA). Unless otherwise specified, all chemicals used were of analytical reagent grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of bean milks and yogurts

The biotechnological process for making plant-based yogurts is shown in Supplementary Fig. S1. To prepare the bean milks, dry beans were mixed with distilled water (dH₂O) at a ratio of 1:4 (w/w) and then pressure-cooked and sterilized in an electric pressure cooker (Instant Pot®, China) for 30 min. The cooked beans were then mixed with other ingredients, including sugar (25:1 w/w) and coconut milk (12.5:1 w/v), and homogenized using a handheld immersion blender (Oster, 2609, USA). To make the bean yogurts, the prepared bean milks were cooled to 42°C and fermented with a mixed yogurt culture containing *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus* at a ratio of 40:1 (w/w) for yogurt beans to starter culture at 42°C for 10 h until the pH reached 4.5 [21]. After fermentation, the mixture was cooled to 4°C within 5 min. The bean samples were then lyophilized before analysis. To ensure repeatability, three separate samples were prepared for each run, and each experiment was conducted in triplicate.

2.3. Analysis of physiochemical properties

The physicochemical properties of the bean milks and yogurts including pH, fat, and protein content were analyzed using the AOAC method from 1995. Moisture content was evaluated with a moisture analyzer (Mettler Toledo HB43-S Halogen). Soluble solids (Brix) were measured with a refractometer (Leica Inc., Buffalo, NY, USA). Titratable acidity was determined by titrating the samples with 0.1 N NaOH to a pH of 8.2. The volume of sodium hydroxide solution consumed during titration was recorded, and titratable acidity was calculated according to the following equation [5]:

$$\text{Titratable acidity (\%)} = \frac{10 \times V_{\text{NaOH}} \times 0.009 \times 0.1}{W} \times 100\% \text{ (Eq. 1)}$$

where 10 is the dilution factor, V_{NaOH} represents the volume of NaOH, 0.009 is the conversion factor (1 mL of 0.1 N NaOH neutralizes 0.009 g of lactic acid), 0.1 denotes the normality of NaOH, and W is weight of the sample.

The mineral composition of plant-based yogurt samples (K, P, S, Mg, Ca, Na, Fe, B, Zn, Mn, Cu, Mo, and Ni) was determined using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500cx, Santa Clara, CA, USA) at the University of Nebraska-Lincoln. Samples were pretreated with trace-metal grade nitric acid, digested overnight at 65°C, and diluted 20-fold with ultrapure water before analysis. The ICP-MS was operated in mixed-gas collision/reaction mode (H₂ and He) to

minimize interferences, and quantification was performed using external calibration standards with gallium (^{71}Ga , 50 $\mu\text{g/L}$) as the internal standard.

2.4. Syneresis determination

The centrifugal acceleration test, with minor modifications based on Nehaa et al [16], was used to assay the syneresis rate of the bean yogurts. In this test, 5 g of samples was placed in a test tube and centrifuged at 1,200 $\times$ g for 15 min at 4°C. The whey was then separated, and its mass was measured. Syneresis was calculated as follows:

$$\text{Syneresis (\%)} = \frac{\text{Weight of whey (g)}}{\text{Initial weight of sample (g)}} \times 100\% \text{ (Eq. 2)}$$

2.5. Color measurement

The color of the samples was evaluated according to Karbasi et al [5]. Color values were determined using a Minolta Colorimeter CR-300 (Minolta Camera Co., Osaka, Japan) and expressed as L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness) parameters of the CIELab color system. Whiteness (W) was calculated using the following equation:

$$W = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \text{ (Eq. 3)}$$

Color difference (ΔE) represents the distance in three dimensions of the CIE Lab color space and was determined using the following equation:

$$\Delta E = [(L_t^* - L_0^*)^2 + (a_t^* - a_0^*)^2 + (b_t^* - b_0^*)^2]^{1/2} \text{ (Eq. 4)}$$

where, L_0^* , a_0^* , and b_0^* represent the initial color parameters ($t = 0$) and L_t^* , a_t^* , and b_t^* denote the values at time t .

2.6. Texture analysis

The texture profile was determined using a TA-XT2i texture analyzer (Stable Microsystems, Surrey, UK) with a 25 kg load cell and the “Texture Expert” software at room temperature according to Zhao et al [22] with slight modifications. A 25 mm diameter cylindrical probe was used in the “double-bite” compression test to penetrate to 35% of the sample height, with a pre-test speed and test speeds of 1 mm/s, and a post-test speed of 10 mm/s. The data acquisition rate was 400 pps. The three most important texture parameters, including firmness (g), cohesiveness (g), and consistency (g.s), were calculated from the texture profile analysis plot.

2.7. Total phenolic content (TPC) analysis

The total phenolic content of samples was measured according to the Folin–Ciocalteu method as described by Karbasi et al [23]. Briefly, 200 μL of each sample was mixed with 1000 μL 10% (v/v) Folin–Ciocalteu reagent and left to react for 5 min at room temperature. Then, 800 μL of 7.42% (w/v) aqueous sodium carbonate was added, and the mixture was vortexed vigorously. The samples were then allowed to stand in the dark at room temperature for 1 h. The absorbance was read at 760 nm. The TPC values were determined from a standard curve of known concentrations of gallic acid solutions and expressed as milligram gallic acid equivalents (GAE) per liter of sample.

2.8. Antioxidant activity analysis

Antioxidant activity was determined using the method described by Karbasi et al [23]. To prepare the stock solution, 25 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl) was dissolved in 1000 mL methanol (0.0625 mM). 100 μL of each sample was mixed with 3.9 mL of stock solution and allowed to rest in the dark at room temperature for 1 hour. A control sample was prepared using the same volume of methanol. Absorbance was read at 515 nm. The DPPH concentration was calculated using the calibration equation, and the percentage of remaining DPPH at steady-state was calculated as follows:

$$\% \text{DPPH}_{\text{Rem}} = \frac{[\text{DPPH}\cdot]_t}{[\text{DPPH}\cdot]_{\text{control}}} \text{ (Eq. 5)}$$

where $[DPPH\cdot]_t$ represents the concentration at steady-state conditions and $[DPPH\cdot]_{control}$ denotes the initial concentration of DPPH. The amount of remaining DPPH at steady state was plotted against the sample concentration to determine the half-maximal efficient concentration (EC_{50}) value. EC_{50} is defined as the amount of sample required to reduce the initial concentration of DPPH $\cdot$ by 50% and is expressed as the volume of sample (mL) per gram of DPPH. The antiradical efficiency (AE) was calculated as follows:

$$AE = \frac{1}{EC_{50}} \text{ (Eq. 6)}$$

2.9. Protein extraction and determination of total soluble protein content

Soluble protein was extracted and used to measure the total protein content [24]. Briefly, 30 mg of lyophilized samples and 1.5 mL of a 2% NaCl (w/v) solution were added to 2 mL conical tubes containing 2.8 mm stainless steel beads (D1033-28; Benchmark Scientific, Sayerville, NJ, USA). The samples were homogenized using a benchtop homogenizer (BS-BEBU-3; Benchmark Scientific, Sayerville, NJ, USA) in 40-seconds intervals at 4000 rpm. After homogenization, the samples were centrifuged at 10,000×g for 10 min. To ensure method repeatability, three separate samples were prepared for each run, and each experiment was conducted in triplicate. The supernatant was collected and diluted for total soluble protein content and further analyses. Total soluble protein content of the extracted supernatant was measured using the Bradford method [25], adjusting for sample weight, and expressed as grams of protein per 100 g of sample.

2.10. Total peptide content analysis

The Pierce™ Quantitative Fluorometric Peptide Assay (23290; Thermo Scientific, Waltham, MA, USA) was employed to quantify the total peptide content in extracted supernatant and filtered digest (FD). The samples were adjusted by the amount of soluble protein and expressed as mg peptide per 100 g of sample.

2.11. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The effect of fermentation on the extracted supernatant was assessed by SDS-PAGE. The Supernatant was mixed in a 1:1 ratio with 2x Laemmli sample buffer (1610737; Bio-Rad, Hercules, CA, USA) to obtain a 2 mg/mL protein solution, which was then heated at 95°C for 5 min. 15 µL of the sample was loaded into wells of a polyacrylamide gel (4568096; Bio-Rad, Hercules, CA, USA) to achieve a protein concentration of 30 µg per well. Precision Plus Protein Dual Color Standard (1610374; Bio-Rad, Hercules, CA, USA) was used as a molecular weight marker. The gel was electrophoresed at 80 V and 0.3 A for 90 min using 10X Tris/Glycine/SDS running buffer (1610732; Bio-Rad, Hercules, CA, USA). Subsequently, the gel was stained with Coomassie Brilliant Blue R-250 for 20 min and then de-stained with a solution of methanol: acetic acid: distilled water (20:10:70, v/v/v).

2.12. Simulated *in vitro* gastrointestinal digestion

The simulated *in vitro* gastrointestinal digestion followed the INFOGEST protocol, using simulated salivary, gastric, and intestinal fluids, according to [26]. The composition and content of various chemicals in the stock solutions of simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) are detailed in Supplementary Table S1. The intestinal phase concluded with the addition of 8 mL of trypsin-chymotrypsin inhibitor. The entire solution was then centrifuged at 10,000×g for 20 min to separate digested and undigested fractions. A portion of the digested fraction underwent ultracentrifugation filtration using Amicon™ Ultra-15 Centrifugal Filter Units with a < 3 kiloDalton molecular weight cutoff (UFC903024; Fisher Scientific, Waltham, MA, USA) to obtain a filtrate (< 3 kDa MW). All fractions were subsequently frozen at -80°C and lyophilized. For further analysis, the < 3 kDa filtered digest, referred to as FD, was utilized. To ensure method repeatability, three separate samples were prepared for each run, and each experiment was conducted in triplicate.

2.13. Measurement of ACE inhibitory activity

In vitro ACE inhibitory activity was assayed according to Cushman et al [27] with slight adjustments. 100 µL of HHL solution (5 mM in 0.1 M borate buffer pH 8.3, containing 0.3 M NaCl) were incubated with 40 µL of FD samples at 37°C for 5 min. Then, 80 µL of ACE solution (0.025 U/mL) was added and further incubated at 37°C for 30 min using a Temp-Block module heater. The reaction was stopped by adding 22 µL of 1 M HCl. The hippuric acid released by ACE was quantified using HPLC system equipped with a Zorbax Eclipse XDB C18 column (4.6 mm i.d. × 150 mm, Agilent, Palo Alto, CA, USA). The control was prepared using the buffer instead of the inhibitor, while the negative control was prepared by adding HCl before the addition of the enzyme and inhibitor. ACE inhibition (%) was calculated as follows:

$$\text{ACE inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{inhibitor}}}{A_{\text{control}}} \times 100\% \text{ (Eq. 7)}$$

where A_{control} denotes the hippuric acid content of the control (without the inhibitor) and $A_{\text{inhibitor}}$ represents the hippuric acid content of the reaction with the inhibitor.

2.14. Peptide separation and identification using untargeted HILIC LC-MS/MS

The small molecular weight peptide profiles of FD samples were analyzed by the Proteomics and Metabolomics Facility at the Nebraska Center for Biotechnology, and protocols adapted from Nolasco, et al [1, 8], with slight adjustments. Lyophilized FD was resuspended in LC-MS/MS grade water to a concentration of 20 µg/µL. Samples were further diluted two times with 100% acetonitrile for a 50 µg injection. The injection was loaded onto an XBridge Amide 3.5 µm column (186004868; Waters Corp., Milford, MA, USA) using a Vanquish HPLC (Thermo Fisher, Waltham, MA, USA) at 45°C and a flow rate of 400 µL/min. The gradient profile was as follows: starting at 90% B, reducing to 30% B over 13 min, then returning to 90% B in 0.5 min. The data was performed on a QE-HF mass spectrometer (Thermo Fisher, Waltham, MA, USA) in positive ion mode, scanning a mass range of 60 to 900 m/z for single charge ions at 60,000 resolution with an AGC target of 3×10^6 and a maximum ion time of 50 ms. Ions were further fragmented by HCD with an isolation window of 1.6 m/z at 15,000 resolution. Data obtained from the hydrophilic interaction chromatography (HILIC) separation was analyzed for peptide quantification and identification using Progenesis QI (v. 2.4; Waters Corp., Milford, MA, USA). Peak abundance was normalized for differences in sample loading using a total ion chromatogram. NIST MS/MS v. 1.0 was employed for MS/MS library searches, and compounds were identified with a mass accuracy of < 5 ppm and an isotopic similarity of at least 90%. MS2 spectra were manually reviewed to match against the database MS2 spectra.

2.15. Enumeration of viable cells

Viable counts of lactic acid bacteria, aerobic mesophilic bacteria (aerobic plate count), yeasts, and molds in bean yogurts were determined during 28-day storage at 4°C. To assess the number of surviving microbial cells, 25 g of samples were diluted (1:10) using 0.1% sterile peptone solution. For lactic acid bacteria, dilutions were spread plated in duplicate on deMan, Rogosa, and Sharpe agar (MRS; Acumedia) and incubated at 37°C for 48 hours. For aerobic plate count, dilutions were spread plated in duplicate on Standard Methods Agar (SMA; Acumedia) and incubated at 35°C for 48 hours. Yeast and mold counts were determined by spreading dilutions in duplicate on Dichloran Rose Bengal Chloramphenicol agar (DRBC; Acumedia) and incubated at 25°C in the dark for 5 days. The limit of detection for all microbial enumeration methods was 10 CFU/g.

2.16. Statistical examination

The measurements were conducted in triplicate to ensure method repeatability, and the results are presented as means with corresponding standard deviations. Statistical analysis was performed using one-way analysis of variance (ANOVA) in SPSS software (version 16, IBM, NY, USA) to identify significant differences between mean values. Duncan's test was applied at a significance level of 0.05.

3. Results and Discussion

3.1. Biochemical, nutritional, and physiochemical characterization of bean milks and yogurts

The proximate composition of bean milks (before fermentation) and bean yogurts (after fermentation) was analyzed and presented in Table 1. The pH of the bean milks, initially around 6.5, dropped sharply to approximately 4.5 after 10 hours of fermentation by LAB. This pH reduction is attributed to the growth of LAB and the metabolic breakdown of high-molecular-weight compounds, such as carbohydrates, which serve as a carbon source, leading to the accumulation of organic acids like lactic acid and short-chain fatty acids [28]. This decrease in pH can act as a preservative, inhibiting the growth of undesirable spoilage and pathogenic microorganisms [6, 7]. Additionally, titratable acidity significantly increased from about 0.26% mL in the bean milks to roughly 0.55% mL after fermentation in all samples (Table 1). The reduction in pH and increase in titratable acidity may be associated with specific active bean-derived ingredients that play a prebiotic role, promoting the growth of lactic acid bacteria [7]. Similar findings were reported by Zahir et al [20] for the fermentation of black, white, kidney, and cranberry bean whey, and by Xu et al [7] for soybean and hemp protein-fermented yogurt.

Table 1
Proximate composition of bean milks and yogurts.

	Bean milks					Bean yogurts				
	Soy	Navy	Great Northern	Pinto	Red Kidney	Soy	Navy	Great Northern	Pinto	Red Kidney
pH	6.56 ± 0.01 ^a	6.54 ± 0.06 ^a	6.55 ± 0.01 ^a	6.55 ± 0.04 ^a	6.54 ± 0.11 ^a	4.56 ± 0.04 ^b	4.47 ± 0.05 ^b	4.57 ± 0.08 ^b	4.46 ± 0.06 ^b	4.56 ± 0.01 ^b
Titrateable acidity (%)	0.26 ± 0.01 ^b	0.25 ± 0.00 ^b	0.25 ± 0.00 ^b	0.26 ± 0.01 ^b	0.25 ± 0.01 ^b	0.55 ± 0.01 ^a	0.52 ± 0.01 ^a	0.56 ± 0.01 ^a	0.53 ± 0.01 ^a	0.57 ± 0.01 ^a
Soluble solids (°BX)	11.30 ± 0.00 ^b	10.60 ± 0.00 ^e	12.20 ± 0.00 ^a	10.50 ± 0.00 ^f	10.80 ± 0.00 ^d	10.00 ± 0.00 ^g	9.40 ± 0.00 ⁱ	10.90 ± 0.00 ^c	9.10 ± 0.00 ^j	9.60 ± 0.00 ^h
Syneresis (%)	-	-	-	-	-	4.12 ± 0.54 ^d	5.14 ± 0.14 ^b	3.20 ± 0.08 ^e	5.49 ± 0.26 ^a	4.83 ± 0.11 ^c
Moisture (%)	78.34 ± 0.42 ^b	80.21 ± 0.49 ^a	80.83 ± 2.07 ^a	80.72 ± 2.34 ^a	79.47 ± 0.25 ^{ab}	78.29 ± 0.82 ^b	82.08 ± 2.71 ^a	80.90 ± 0.34 ^a	81.29 ± 1.80 ^a	81.11 ± 1.98 ^a
Protein content (g/100g)	3.39 ± 0.18 ^a	1.91 ± 0.03 ^c	2.11 ± 0.07 ^b	2.01 ± 0.05 ^{bc}	2.13 ± 0.03 ^b	3.35 ± 0.08 ^a	1.84 ± 0.06 ^c	2.03 ± 0.09 ^{bc}	2.09 ± 0.15 ^{bc}	2.12 ± 0.12 ^b
Fat content (g/100g)	4.04 ± 0.09 ^a	0.92 ± 0.03 ^f	2.61 ± 0.25 ^c	2.98 ± 0.17 ^b	1.55 ± 0.05 ^d	4.33 ± 0.21 ^a	1.04 ± 0.16 ^e	2.93 ± 0.09 ^{bc}	3.16 ± 0.10 ^b	1.72 ± 0.12 ^d
Macroelements (mg per 100 g)										
K	2531.7 ± 78 ^c	2210.3 ± 229 ^d	2913.0 ± 287 ^b	2403.0 ± 178 ^d	2709.0 ± 135 ^b	1740.2 ± 185 ^e	2308.6 ± 189 ^d	2529.7 ± 173 ^c	3660.3 ± 184 ^a	2501.3 ± 96 ^c
P	1120.3 ± 39 ^b	746.6 ± 81 ^d	1035.8 ± 105 ^b	808.1 ± 64 ^c	976.1 ± 170 ^b	764.3 ± 99 ^d	852.4 ± 68 ^c	903.5 ± 64 ^b	1474.2 ± 70 ^a	930.5 ± 40 ^b
S	513.2 ± 12 ^b	317.4 ± 33 ^c	378.9 ± 33 ^c	342.0 ± 25 ^c	344.9 ± 54 ^c	345.5 ± 17 ^c	344.8 ± 27 ^c	334.93 ± 21 ^c	606.2 ± 29 ^a	340.6 ± 12 ^c
Mg	398.9 ± 13 ^b	303.6 ± 30 ^c	317.8 ± 33 ^c	313.6 ± 22 ^c	258.6 ± 40 ^d	262.4 ± 13 ^d	318.8 ± 24 ^c	289.9 ± 20 ^b	651.4 ± 31 ^a	235.0 ± 8 ^d
Ca	299.4 ± 9 ^a	246.1 ± 22 ^b	303.1 ± 28 ^a	146.0 ± 11 ^d	146.3 ± 23 ^d	208.0 ± 10 ^c	269.3 ± 18 ^a	253.1 ± 17 ^b	292.3 ± 14 ^a	144.9 ± 7 ^d
Na	17.7 ± 0.6 ^{cd}	19.4 ± 2 ^c	18.8 ± 1 ^c	18.8 ± 1 ^c	18.6 ± 4 ^c	19.6 ± 8 ^{bc}	26.0 ± 2 ^b	23.9 ± 2 ^b	62.8 ± 2 ^a	24.7 ± 0.5 ^b
Microelements (mg per 100 g)										
Fe	10.2 ± 0.4 ^a	7.3 ± 0.7 ^{bc}	7.5 ± 0.7 ^{bc}	7.5 ± 0.6 ^{bc}	6.4 ± 1 ^c	7.2 ± 0.4 ^b	8.4 ± 0.6 ^b	6.7 ± 0.5 ^c	8.3 ± 0.3 ^b	6.3 ± 0.4 ^c
B	5.8 ± 0.2 ^a	2.2 ± 0.2 ^c	2.4 ± 0.3 ^c	2.0 ± 0.2 ^c	2.3 ± 0.3 ^c	3.9 ± 0.2 ^b	2.3 ± 0.2 ^c	2.1 ± 0.2 ^c	4.3 ± 0.3 ^b	2.0 ± 0.1 ^c
Data are presented as the means ± standard deviation of at least three separate experiments; values with different superscripts within a row differ significantly ($p < 0.05$).										

	Bean milks					Bean yogurts				
	Soy	Navy	Great Northern	Pinto	Red Kidney	Soy	Navy	Great Northern	Pinto	Red Kidney
Zn	3.9 ± 0.2 ^a	3.2 ± 0.3 ^b	4.2 ± 0.4 ^a	3.6 ± 0.3 ^a	3.9 ± 0.7 ^a	2.9 ± 0.1 ^{cb}	3.5 ± 0.2 ^b	3.5 ± 0.2 ^b	4.0 ± 0.3 ^a	3.7 ± 0.2 ^a
Mn	3.3 ± 0.1 ^a	2.4 ± 0.2 ^{bc}	2.6 ± 0.2 ^b	1.9 ± 0.1 ^c	2.1 ± 0.3 ^c	2.5 ± 0.1 ^b	2.8 ± 0.2 ^b	2.4 ± 0.2 ^{bc}	2.2 ± 0.2 ^c	2.1 ± 0.1 ^c
Cu	1.5 ± 0.0 ^c	1.5 ± 0.1 ^c	1.3 ± 0.1 ^c	1.4 ± 0.1 ^c	1.6 ± 0.3 ^{bc}	1.1 ± 0.0 ^d	1.8 ± 0.1 ^b	1.1 ± 0.1 ^d	2.1 ± 0.1 ^a	1.4 ± 0.1 ^c
Mo	0.3 ± 0.0 ^e	0.3 ± 0.0 ^e	0.8 ± 0.1 ^b	0.5 ± 0.0 ^d	0.5 ± 0.1 ^{cd}	0.2 ± 0.0 ^f	0.3 ± 0.0 ^e	0.6 ± 0.0 ^c	1.0 ± 0.0 ^a	0.5 ± 0.0 ^d
Ni	0.4 ± 0.0 ^c	0.6 ± 0.0 ^b	0.1 ± 0.0 ^f	0.4 ± 0.0 ^c	0.2 ± 0.0 ^e	0.3 ± 0.0 ^d	0.6 ± 0.0 ^b	0.1 ± 0.0 ^f	1.0 ± 0.0 ^a	0.2 ± 0.0 ^e
Data are presented as the means ± standard deviation of at least three separate experiments; values with different superscripts within a row differ significantly ($p < 0.05$).										

Soybean yogurt had the highest protein and fat content, with 3.35 g/100g and 4.33 g/100g, respectively, followed by red kidney bean yogurt containing 2.12 g/100g of protein and 1.72 g/100g of fat. This suggests that common beans like red kidney beans are suitable as supplementary components for developing novel low-calorie products due to their lower fat level. Although the moisture, protein, and fat content of bean milks showed minimal changes after fermentation, the level of soluble solids decreased significantly in bean yogurts due to the consumption of sugars by lactic acid bacteria. The decrease in pH and soluble solids, along with an increment in titratable acidity, indicates the growth performance of the starter cultures during fermentation.

Syneresis, also known as "wheying off," refers to the separation of the liquid phase from the gel, which is considered a quality defect in gel structures like yogurts and negatively affects consumer acceptance. Syneresis is strongly linked to the microstructure, indicating the colloidal properties of yogurts. It is a sign of nonhomogeneity in the yogurt's gel network and can occur due to factors such as excessive heating pretreatments, low lactic acid level/ high pH, moving when the gel is still weak, low total solids, and high incubation temperatures [20]. Therefore, managing syneresis is crucial for improving the shelf life of yogurt products. In our study, yogurts made from great northern beans showed the lowest syneresis level at 3.20%, indicating maximum homogeneity. This might be due to the stable and robust three-dimensional gel network structure in this fermented yogurt, which can trap water molecules and prevent whey separation/precipitation. This contrasts with the findings of Xu et al [7], who reported an increase in syneresis rate and whey precipitation in a plant-based yogurt made from soy and 5% hemp protein. As well, the syneresis rate was influenced by the yogurt's flow behavior [16]. In our study, great northern bean yogurt demonstrated the highest consistency and textural properties (Fig. 2A), likely due to the strong molecular bindings and interactions that help retain water during syneresis.

Minerals are essential for organic functions, existing in ionic form and as components of compounds like hormones, enzymes, and tissue proteins. They constitute approximately 4% of total body weight and play a vital role in human well-being [29]. The mineral composition of bean yogurt samples is detailed in Table 1. Among these inorganic elements, K had the highest concentration across all bean yogurts, followed by P, S, Mg, Ca, Na, Fe, B, Zn, Mn, Cu, Mo, and Ni. Yogurt made from pinto beans exhibited the highest levels of all minerals, significantly surpassing those found in African Yam (AY) bean yogurt, which recorded concentrations of 270.9 mg/100g for K, 99.6 mg/100g for P, 38.7 mg/100g for Mg, 0.58 mg/100g for Fe, and 0.01 mg/100g for Cu [30]. Similarly, potassium and phosphorus were the most prevalent minerals in AY bean-based yogurt. In a study by Gawalko et al [31], various trace elements were identified in peas, with ranges of 48.2–155.0 mg/100g for Ca, 0.3–1.5 mg/100g for Cu, 2.9–8.0 mg/100g for Fe, 580.4–1331.0 mg/100g for K, 101.1–151.5 mg/100g for Mg, 0.4–2.1 mg/100g for Mn, 127.8–601.8 mg/100g for P, < 0.005–0.2 mg/100g for Se, and 1.8–8.0 mg/100g for Zn. Da Cunha et al

[32] analyzed the inorganic element profile of soybean extract, revealing concentrations of 935.6, 1110.1, 299.2, 510.8, 160.1, 49.3, 3.5, 2.9, 0.9 mg/100g for K, P, Mg, Ca, Na, Fe, Zn, Mn, Cu, respectively.

Color is a crucial quality criterion that significantly influences the acceptability, popularity, and sensory appeal of food products among consumers [19]. Plant-based yogurts made from soy, navy, great northern, pinto, and red kidney beans are depicted in Fig. 1. Soy, navy, and great northern bean yogurts exhibited a creamy hue, while pinto and red kidney bean yogurts displayed a reddish color attributed to the presence of polyphenols in these beans. Following LAB fermentation, there was minimal color difference observed between bean milks (Fig. 2A) and yogurts (Fig. 2B), except for those made from pinto and red kidney beans, which showed increased lightness (L^*), whiteness (W), and total color difference (ΔE) after fermentation (Fig. 2B). The ΔE values exceeded 2, making the color difference noticeable to the average observer [33]. Yogurts prepared from pinto and red kidney beans exhibited the highest ΔE values, around 5, indicating distinct color changes compared to their respective milks post-fermentation. In plant-based products, alterations in the fermented matrix can significantly impact color. Flavonoids present in beans, such as flavonol glycosides, anthocyanins, and proanthocyanidins, contribute to their color by forming complexes with proteins, metals, or polysaccharides, or undergoing hydrolysis [34] and lightening the color of the aforementioned yogurts. Among bean-based yogurts, great northern bean exhibited higher L^* and W values compared to others ($p < 0.05$), indicating increased lightness and whiteness. Conversely, its a^* and b^* values were lower, suggesting a less reddish and yellowish hue. The CIELab color parameters (Fig. 2B) derived in this study correlated well with the visual appearance of the samples (Fig. 1), suggesting that chromaticity variations could aid the human eye in distinguishing color differences effectively.

Texture analysis mimics the breakdown of food as experienced when taking a spoonful of yogurt, during oral processing, or throughout manufacturing, and its outcomes are frequently correlated with the sensory textural attributes [35]. Figure 2C and D illustrate texture properties such as firmness, consistency, and cohesiveness of bean milks and yogurts. Firmness refers to the force required to achieve a specific deformation in the food product, represented by the peak force on the initial compression cycle [7]. Consistency describes the uniformity and stability of a product's texture over time and under varying conditions, while cohesiveness measures the strength of internal structural bonds. It is evident that fermentation significantly influenced the textural parameters of all bean milks ($p < 0.05$). Firmness increased by 11.15% and 67.76% in pinto and great northern bean yogurts, respectively, consistency rose by 15.26% and 64.01% in pinto and great northern bean yogurts, respectively, and cohesiveness increased from 29.51% and 208.77% in red kidney and great northern bean yogurts, respectively (Fig. 2D). Accordingly, yogurt made from great northern beans exhibited superior gel qualities and the highest textural parameters. Previous studies by Xu et al [7] about soybean-hemp protein yogurt, Qin et al [19] regarding pea, chickpea, and fava bean yogurts, Zhang et al [14] about soybean sprout yogurt, Yang et al [17] about pea protein and mung bean protein yogurts, and Mishra et al [15] regarding soybean yogurt all reported lower values for textural properties. The variation in bean composition may explain differences in gel strength under identical processing conditions. Fat content can inhibit yogurt gel network formation, whereas protein content promotes its development [36]. Therefore, the low-fat, high-protein profile of great northern bean yogurt might contribute to its enhanced texture characteristics. Interestingly, pinto and red kidney bean yogurts with similar low-fat and high-protein profiles exhibited reduced hardness, cohesion, and consistency. This disparity may be attributed to the superior gelation capability of great northern bean protein under acidic conditions induced by lactic acid bacteria [19], which stabilizes yogurt gel structures by creating small and uniform micropores. The gel formation process involves acid-induced gelation through the accumulation of organic acids during LAB growth. As pH decreases, protein net charges are neutralized, facilitating protein gel formation [14]. Montemurro et al [11] noted a significant viscosity decrease of up to 48% in yogurts made from rice and hemp flour after 16-hour fermentation, likely due to acetic and lactic acid formation, negatively impacting physicochemical characteristics.

3.2. Antiradical efficiency and total phenolic content of bean milks and yogurts

The production of foods with radical quenching ability holds special significance in functional food research as natural antioxidants are preferred over synthetic ones, which is attributed to their lower risk of carcinogenic effects. There is a good

association between antioxidant capacity and both the total phenolic content and the total peptide content generated during fermentation [3]. Polyphenols and antioxidant peptides found in food prevent the formation of free radicals or scavenging them and active oxygen species, which can cause oxidative damage to biomolecules and potentially lead to various diseases, thereby being important in maintaining antioxidant defense systems [6, 37]. A modest yet significant increase in the antioxidant activity of bean samples was observed after fermentation (Fig. 3A). Similar trends were noted in previous studies [6, 11, 38, 39]. Fermentation increases antioxidant activity by liberating bound phenolic acids, converting glycosylated flavonoids and isoflavones into aglycones, transforming complex phenolics into more bioactive derivatives, depolymerizing tannins, improving bioavailability, and promoting synergistic interactions with other fermentation metabolites [6, 12, 40]. In addition, the hydrolysis of proteins by proteinases during fermentation produces numerous active peptides and amino acids, enhancing the antioxidative activity of beans. These peptides act as electron donors, intercepting free radicals to halt radical chain reactions and converting them into less harmful products [37]. The bioactivity of protein hydrolysates depends on factors such as amino acid sequence, size, and structure of the peptides [3]. Li et al [38] reported that peptides generated from chickpea fermentation exhibited DPPH radical scavenging activity. As it can be seen in Fig. 3B, the total phenolic content of bean yogurts slightly decreased compared to bean milks. Torino et al [6] found that the total phenolic content of lentil seeds decreased after natural and 96-hour induced fermentation by *L. plantarum*. This may be attributed to interactions between antioxidant components, which can have antagonistic or synergistic effects and influence the final antiradical efficiency. Among the yogurt samples, pinto beans exhibited the highest scavenging activity against free DPPH radicals (60.61 g/mL; Fig. 3A) as well as the highest total phenolic content (2,736.92 mg/L; Fig. 3B). It has been reported that colored beans, like pinto beans, possess superior antioxidant and antiradical properties compared to less colored beans [2], such as navy and great northern beans, indicating the lowest levels of total phenolic content and antioxidative capacity in our study. Therefore, pinto beans fermented with LAB show promise in reducing oxidative stress and may be beneficial for therapeutic purposes.

3.3. Total soluble protein and peptide content of bean milks and yogurts

Figure 4 shows the electropherogram of bean milks and bean yogurts, highlighting that the intensity of the dye decreased due to fermentation. This indicates significant changes in protein profiles, underscoring the strong proteolytic activity of LAB, which degraded the bean proteins [35]. Large and medium-to-large proteins in beans, especially those within the 30–250 kDa range, were hydrolyzed into low-molecular-weight polypeptides below 25 kDa or individual amino acids. These findings align with Wu et al [10], who noted the disappearance of most mung protein bands after fermentation. Similarly, Li et al [38] found that large chickpea proteins degraded into small peptides after 24 hours of fermentation by *B. subtilis* lwo due to the secretion of proteolytic enzymes like metalloproteinases, aminopeptidases, and serine endopeptidases. Another study by Shi et al [41] reported a significant increase in small peptides under 25 kDa after corn-soybean meal fermentation. Protein hydrolysis by LAB enhances the nutritional value and reduces the toxicity of beans by degrading antinutritional proteins such as phytohemagglutinin and α -amylase inhibitors while also increasing protein digestibility [9]. Thus, electrophoretic analyses confirm the suitability of common beans as a substrate for proteolytic enzymes from LAB.

Figure 3C shows that the total soluble protein in bean milks either decreased or remained statistically unchanged after fermentation. Similar results were observed by Rui et al [9] with navy bean fermentation by *L. bulgaricus*, *L. plantarum*, and *L. helveticus*, and by Jakubczyk et al [42] with pea seeds fermented by *L. plantarum* 299v. Additionally, Aguirre et al [43] found that about one-third of soybean proteins hydrolyzed during a 6-hour LAB fermentation. LAB have a complex proteolytic system involving various cell-envelope proteinases and peptidases, along with efficient transport systems that facilitate secondary protein hydrolysis into small peptides and amino acids [24]. Each proteinase or peptidase type, with its unique substrate specificity, domain composition, and anchoring mechanism, can influence the production of protein hydrolysate [18]. Additionally, proteolysis can be initiated by beans' endogenous proteases activated at low pH, leading to protein degradation and reduction [24]. Figure 3D shows a significant increase ($p < 0.05$) in total peptide content in all bean yogurts, with the lowest and highest concentrations in soybean yogurt (34.15 mg/100 g) and red kidney bean yogurt (64.37 mg/100 g), respectively. Compared to the unfermented matrix, total peptide content rose by 18.25% to 25.32% in great northern bean

yogurt and red kidney bean yogurt, respectively, due to intense proteolytic activity. This aligns with SDS–PAGE results (Fig. 4). The varying levels of water-soluble proteins and peptides in the yogurts can be attributed to the different solubility of bean proteins and the activity of endogenous bean proteases [24]. Therefore, the proteolytic activity of LAB and the spontaneous protein/peptide breakdown in beans raised peptide levels during fermentation, potentially offering benefits such as antioxidant, anticancer, ACE inhibition, antihypertensive, antithrombotic, and antimicrobial properties [18, 32, 37].

3.4. Antiradical efficiency, total phenolic content, ACE inhibitory activity, and total peptide content of bean milks and yogurts after *in vitro* gastrointestinal simulation

Bean milks and yogurts were subjected to simulated gastrointestinal digestion and ultrafiltration, following the physiologically relevant model of human digestion, i.e., INFOGEST protocol. Total phenolic content and oxidative inhibition potential increased significantly ($p < 0.05$) for both bean milks and yogurts after digestion (Fig. 5A and B), following the same patterns observed before digestion (Fig. 3A and B). While the antioxidative capacity and total phenolic content of bean yogurts did not show a noteworthy increase compared to bean milks before digestion (Fig. 3A and B), all yogurt samples demonstrated a significant increase in both parameters after digestion ($p < 0.05$). Gastrointestinal digestion produces fractions with molecular weights below 10 kDa, including diverse soluble constituents like amino acids, small peptides, and phenolic compounds, especially those from the seed coat of beans [44]. These antioxidative components in digested beans can hinder radical formation or react with radicals to convert them into less harmful or inert substances. During LAB fermentation, complex high-molecular-weight polyphenolic compounds break down into simpler, more biologically active ingredients [45, 46]. Simulated gastrointestinal digestion of fermented cooked soy meal releases microbial enzymes, enhancing phenolic and flavonoid content, including genistin, daidzin, glycitin, and malonylgenistin [46]. Within the yogurt samples, pinto and red kidney beans showed the highest radical quenching ability (112.76 and 109.74 g/mL, respectively) (Fig. 5A) and total phenolics (6869.84 and 6482.74 mg/L, respectively) (Fig. 5B). Zahir et al [2] reported that digested yogurt formulated with kidney bean whey exhibited significantly higher scavenging activity, which was attributed to its superior phenolic content, followed by yogurts made with black, white, and cranberry beans.

ACE regulates blood pressure by converting the inactive decapeptide angiotensin I into the potent vasoconstrictor angiotensin II, an octapeptide, as well as by degrading bradykinin, a vasodilator, into inactive peptides. Inhibiting ACE with natural or synthetic inhibitors has been shown to lower arterial blood pressure in both animals and humans by preventing this conversion. In our study, all digested bean milks exhibited ACE-inhibitory activity, which significantly increased after fermentation (Fig. 5C). Fermentation not only promotes the generation of ACE-inhibitory peptides from precursor proteins but also enhances the release and transformation of phenolic compounds, both of which may contribute to ACE-inhibitory activity [3, 9, 42]. The highest ACE inhibitory activity was observed in the fermented red kidney bean digestate (29.75%). Changes in ACE inhibitory activity among various bean-based yogurts are usually attributed to differences in their parent protein structures, which impact the release of peptides during fermentation [6]. The inhibitory potential of these peptides depends on their sequence, hydrophobicity, and size. In addition, variations in the amount and types of phenolic compounds may also contribute, either through direct ACE-inhibitory effects or through interactions with proteins and peptides that modulate peptide activity and the bioavailability of polyphenols [47]. Torino et al [6] observed that fermentation of lentils with *L. plantarum* CECT 748^T increased ACE inhibitory activity by approximately 25%, which is related to intensive proteolysis. Similarly, the ACE inhibitory activity of digested pea seeds increased markedly after a 7-day fermentation at 22°C [42]. Phenolic compounds, particularly flavonoids and tannins, have been reported to contribute to ACE inhibition. Their activity is thought to result from the ability to chelate the zinc atom in the catalytic site of ACE, thereby interfering with enzyme function [48]. Paiva et al [49] also reported strong correlations between ACE inhibition and total phenolic content, suggesting that polyphenols contribute to ACE-inhibitory activity. They further concluded that the combined effects of peptides and released polyphenols likely underlie the dual antihypertensive and antioxidant properties observed in protein hydrolysate fractions. Therefore, the fermentation process reveals the promising potential of antihypertensive peptides generated during *in vitro* gastrointestinal digestion, while also enhancing the release and activity of phenolic compounds that can contribute synergistically to ACE inhibition.

The total peptide content of gastrointestinal digestates with MW < 3 kDa, measured using the Pierce™ Quantitative Fluorometric Peptide Assay, was significantly higher in bean yogurt samples compared to their bean milk counterparts ($p < 0.05$), as shown in Fig. 5D. This finding aligns with the total peptide content observed before gastrointestinal digestion (Fig. 3D), where the amount significantly increased after fermentation. Among the fermented hydrolysates, soybean yogurt demonstrated the highest peptide concentration (203.76 mg/100 g), followed by red kidney bean yogurt (180.50 mg/100 g) (Fig. 5D). Similarly, Zahir et al [2] showed that yogurt fortified with red kidney bean whey had the highest peptide level compared to yogurts made with black, white, and cranberry beans at the end of duodenal digestion.

3.5. Pearson correlation heatmaps of bioactivity parameters in bean-based samples before and after *in vitro* digestion

The Pearson correlation matrices (Fig. 6) demonstrate that antiradical efficiency is most strongly associated with total phenolic content both before and after digestion, confirming phenolics as the primary contributors to antioxidant potential in bean-based samples. By contrast, the correlations involving peptides were very weak after digestion, suggesting that peptides in their digested forms contribute minimally to antioxidant activity compared with phenolics. The strong correlation between total phenolic content and antioxidant activity can be explained by the chemical properties of phenolics. They possess multiple hydroxyl groups capable of donating electrons or hydrogen atoms, stabilize radicals via resonance, chelate pro-oxidant metals, and often act synergistically with other bioactive molecules [50]. Mechanistically, gastrointestinal hydrolysis further enhances the release and transformation of bound phenolic acids (e.g., ferulic, caffeic, gallic, and p-coumaric acids) and flavonoid aglycones, thereby increasing their solubility, accessibility, and radical-scavenging efficiency [51]. Similarly, Wongsu et al [46] reported a significant correlation between total phenolic content and antioxidant activity in soy- and Job's tears-based yogurt-like products supplemented with rice protein hydrolysates, further supporting the dominant role of phenolics in determining antioxidant potential. In contrast, interactions between peptides and phenolics, including hydrogen bonding or hydrophobic stacking, can reduce the free availability of peptides to act as antioxidants, which may explain their weak correlation with antiradical efficiency after digestion [52]. Another contributing factor may be assaying specificity. While phenolics typically show direct and dose-dependent responses in single radical-scavenging assays such as DPPH, peptides often exert subtler or mechanism-specific effects (e.g., metal chelation, singlet oxygen quenching), which are not fully captured by such assays [53]. Overall, these findings indicate that although both phenolics and peptides can be released or modified during fermentation and digestion, phenolics remain the dominant determinants of antioxidant capacity, while peptides play a more secondary and context-dependent role.

3.6. Determination of possible antihypertensive peptides in bean milks and yogurts after *in vitro* gastrointestinal simulation

Peptide profiling of the digested bean milks and yogurts revealed generally similar peptide compositions (Supplementary Table S2); though the relative intensities varied (Supplementary Fig. S2). Quantification of free amino acids, dipeptides, and tripeptides showed that total peptide intensity significantly increased after fermentation ($p < 0.05$), supporting the enhanced protein breakdown observed earlier (Fig. 5D).

To interpret the potential functionality of these peptides, their sequences were compared to previously reported ACE-inhibitory peptides (Supplementary Table S3). Many of the di- and tripeptides identified contained amino acid residues, such as Tyr, Trp, Pro, Met, Leu, Ile, Phe, Thr, and His, that are commonly associated with antihypertensive activity. These structural motifs, particularly at the N- or C-terminal positions, have been shown to influence biological functionality.

For antihypertensive potential, multiple peptides identified in the samples shared structural features with known ACE-inhibitory peptides, such as the presence of Pro, Tyr, Phe, and Trp at the C-terminal end, which facilitates binding to ACE [41, 54–56]. Additionally, Arg and branched-chain amino acids like Val and Ile at the N-terminal region are also known to strengthen the interaction between peptides and ACE [54]. The cumulative presence of these motifs suggests that the fermented bean yogurts can possess enhanced ACE-inhibitory activity. The importance of peptide length and hydrophobicity is further supported by literature, indicating that low molecular weight peptides, particularly di- and tripeptides, are more

readily absorbed and more effective as ACE inhibitors than larger peptides [9, 42, 57, 58]. While tetrapeptides or longer chains may not be absorbed intact, enzymatic digestion during gastrointestinal transit or fermentation may break them down into shorter, bioactive units [59]. Siow et al [60] observed that peptides with repetitive amino acid sequences conferred increased antihypertensive activity, attributed to their superior biological effectiveness and quicker absorption compared to their constituent amino acids.

Taken together, our findings suggest that fermentation enhances the release of peptides with structural features linked to antihypertensive activity. However, it remains to be determined whether the observed effects result from individual peptides or synergistic interactions among peptide mixtures and other components like phenolics in the matrix. Further studies involving purified peptide fractions and functional assays will be necessary to confirm these bioactivities.

3.6. Storage stability

Over 28 days of refrigerated storage, there were no significant changes observed in moisture percentage, soluble solid level, colorimetric components (L^* , a^* , b^* , W , and ΔE), and textural profile parameters (firmness, consistency, and cohesiveness) of plant-based yogurts (data are not shown). Table 2 presents the pH values and lactic acid bacteria counts for bean yogurts stored at 4°C over the same period. The pH of yogurt samples averaged around 4.5 and exhibited a slight decrease (mean = 0.12 units) throughout the storage duration. The initial lactic acid bacteria count for soy, navy, great northern, pinto, and red kidney beans was log 6.32, 6.54, 6.41, 6.38, and 6.50 CFU/g, respectively, at the end of fermentation, with increases observed during the first three weeks of storage (Table 2). This underscores beans as rich sources of carbon, nitrogen, essential amino acids, and vitamins critical for supporting LAB growth and metabolism [18, 20]. It is noteworthy that bacterial growth and protein breakdown are intricately linked [18], facilitating the accumulation of bioactive peptides during fermentation and subsequent microbial population increase [9]. Recent studies on lupin oat yogurt [18] and cashew yogurt [61] have shown that probiotic cell viability increased only after 7 days of cold storage. However, our study documented that bean yogurts maintained high LAB viability throughout 28 days of refrigeration. The slight decline in microbial growth after 21 days may be attributed to reaching the stationary phase, marking a stabilization period where carbon and nitrogen sources gradually diminish.

Table 2
Cell counts and acidification of bean yogurts during 28-day storage at 4 °C.

Bean Yogurt	Time (day)	pH	Microbial population (CFU/g)			
			Lactic Acid Bacteria	Aerobic Plate Count	Yeast Count	Mold Count
Soy	0	4.56 ± 0.04 ^a	2.10E + 06 ^d	1.50E + 02 ^e	< 10	< 10
	7	4.54 ± 0.01 ^a	1.90E + 0e	6.50E + 07 ^c	< 10	< 10
	14	4.54 ± 0.03 ^a	1.40E + 07 ^a	4.00E + 08 ^a	< 10	< 10
	21	4.51 ± 0.01 ^a	1.20E + 07 ^b	1.80E + 08 ^b	< 10	< 10
	28	4.52 ± 0.01 ^a	4.40E + 06 ^c	1.60E + 06 ^d	< 10	< 10
Navy	0	4.47 ± 0.05 ^a	3.50E + 06 ^e	1.20E + 03 ^e	< 10	< 10
	7	4.43 ± 0.01 ^a	6.40E + 07 ^b	5.30E + 07 ^c	< 10	< 10
	14	4.44 ± 0.05 ^a	2.90E + 07 ^c	1.20E + 08 ^b	< 10	< 10
	21	4.35 ± 0.03 ^b	1.20E + 08 ^a	1.80E + 08 ^a	< 10	< 10
	28	4.34 ± 0.02 ^b	8.10E + 06 ^d	7.10E + 05 ^d	< 10	< 10
Great Northern	0	4.57 ± 0.08 ^a	2.60E + 0 ^e	6.90E + 02 ^e	< 10	< 10
	7	4.49 ± 0.01 ^a	1.40E + 07 ^c	6.10E + 07 ^c	< 10	< 10
	14	4.47 ± 0.01 ^b	4.80E + 07 ^b	1.50E + 08 ^a	< 10	< 10
	21	4.41 ± 0.01 ^c	6.50E + 07 ^a	1.40E + 08 ^b	< 10	< 10
	28	4.37 ± 0.03 ^c	7.80E + 06 ^d	2.70E + 05 ^d	< 10	< 10
Pinto	0	4.46 ± 0.06 ^a	2.40E + 06 ^e	4.10E + 02 ^e	< 10	< 10
	7	4.46 ± 0.02 ^a	6.60E + 07 ^b	5.20E + 07 ^c	< 10	< 10
	14	4.40 ± 0.03 ^a	2.00E + 07 ^c	1.90E + 08 ^a	< 10	< 10
	21	4.40 ± 0.04 ^a	7.40E + 07 ^a	1.40E + 08 ^b	< 10	< 10
	28	4.36 ± 0.06 ^{ab}	6.70E + 06 ^d	5.00E + 05 ^d	< 10	< 10
Red Kidney	0	4.56 ± 0.01 ^a	3.20E + 06 ^e	3.30E + 02 ^e	< 10	< 10
	7	4.46 ± 0.04 ^b	4.20E + 07 ^b	6.10E + 07 ^c	< 10	< 10
	14	4.46 ± 0.05 ^b	1.00E + 07 ^c	1.50E + 08 ^b	< 10	< 10
	21	4.42 ± 0.01 ^b	6.80E + 07 ^a	2.40E + 08 ^a	< 10	< 10
Data are presented as the means ± standard deviation of at least three separate experiments; values with different superscripts within a column differ significantly ($p < 0.05$).						

Bean Yogurt	Time (day)	pH	Microbial population (CFU/g)			
	28	4.42 ± 0.04 ^b	3.70E + 06 ^d	1.10E + 05 ^d	< 10	< 10
Data are presented as the means ± standard deviation of at least three separate experiments; values with different superscripts within a column differ significantly ($p < 0.05$).						

LAB starters are recognized for their therapeutic effects at concentrations of at least 10^6 CFU of viable bacteria per gram of yogurt at the time of consumption [62]. Moreover, the high LAB density (6 log CFU/g) and low pH are among the factors that ensure a long microbial shelf-life in fermented products. Monitoring the yogurts during the storage demonstrated that all produced bean-based yogurts met the necessity of having 10^6 CFU of viable bacteria per gram and low pH even after 28 days. This contrasts with findings from Kanda et al [63], who stated that storing soy yogurt at 5°C for more than 19 days could lead to a decline in viable cell counts below desirable levels for consumers. Differences in maximum cell concentrations may stem from variations in nutrient compositions of raw materials and fermentation conditions affecting LAB strain growth in yogurt [19]. Throughout the four-week storage period, yeast and mold counts remained below 10 CFU/g. Thus, soy, navy, great northern, pinto, and red kidney beans demonstrate excellent potential as substrates for LAB growth and metabolic activity in producing probiotic yogurts.

4. Conclusion

The observed reduction in pH and soluble solids, along with the increase in titratable acidity of bean milks after fermentation, reflects the strong growth performance of the LAB starter culture. Syneresis, a common quality defect in fermented products, was lowest in great northern bean yogurt, likely due to its robust three-dimensional gel network, as indicated by its superior textural properties and consistency. This yogurt also exhibited lower reddish and yellowish hues, coupled with higher lightness and whiteness values. Among inorganic elements, potassium was the most abundant across all bean yogurts, with pinto bean yogurt showing the highest concentrations of all measured minerals. Pinto bean yogurt also displayed the greatest oxidative inhibition potential and total phenolic content. SDS-PAGE electropherograms revealed a significant increase in total peptide content in all bean milks after fermentation, with soy and red kidney beans having the lowest and highest concentrations, respectively. Simulated gastrointestinal digestion of both bean milks and yogurts resulted in marked increases in antioxidative capacity and total phenolic content. While antiradical efficiency and total phenolic content did not show significant changes in bean yogurts compared to bean milks before digestion, they increased significantly in all yogurt samples after digestion.

All digested bean milks exhibited ACE-inhibitory activity, which was further enhanced after fermentation, with the fermented red kidney bean digestate showing the highest level. The presence of Pro, Tyr, Phe, and Trp at the C-terminus, and Arg together with branched-chain aliphatic amino acids such as Val and Ile at the N-terminus, were strongly associated with ACE inhibition. These findings highlight the excellent bioaccessibility of bean-based yogurts after simulated GI digestion and provide valuable insights into how LAB fermentation promotes the breakdown of bean proteins and the release of bioactive peptides. Importantly, the results underscore that the optimal common bean choice should depend on the targeted functionality or feature, for example, selecting red kidney beans for maximizing ACE-inhibitory activity or great northern beans for superior texture and color.

Declarations

Conflicts of interest

The authors declare no conflict of interest.

Author Contribution

MKB: Conceptualization, Methodology, Data curation, Formal analysis, Validation, Investigation, Writing -original draft, Visualization, Software; XX: Conceptualization, Methodology, Investigation, Visualization, Writing - review & editing; CX: Conceptualization, Investigation, Supervision, Resources, Project administration, Funding acquisition, Writing - review & editing.

Acknowledgement

The authors acknowledge support from the U.S. Department of Agriculture, Agricultural Marketing Service, Specialty Crop Block Grant Program, through the Nebraska Department of Agriculture (Award Number: 18-13-407). The authors also acknowledge Hatch funding from the USDA via the College of Agricultural, Consumer and Environmental Sciences at the University of Illinois Urbana-Champaign (ILLU Number 698-302), which supported the publication of this work.

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Figures

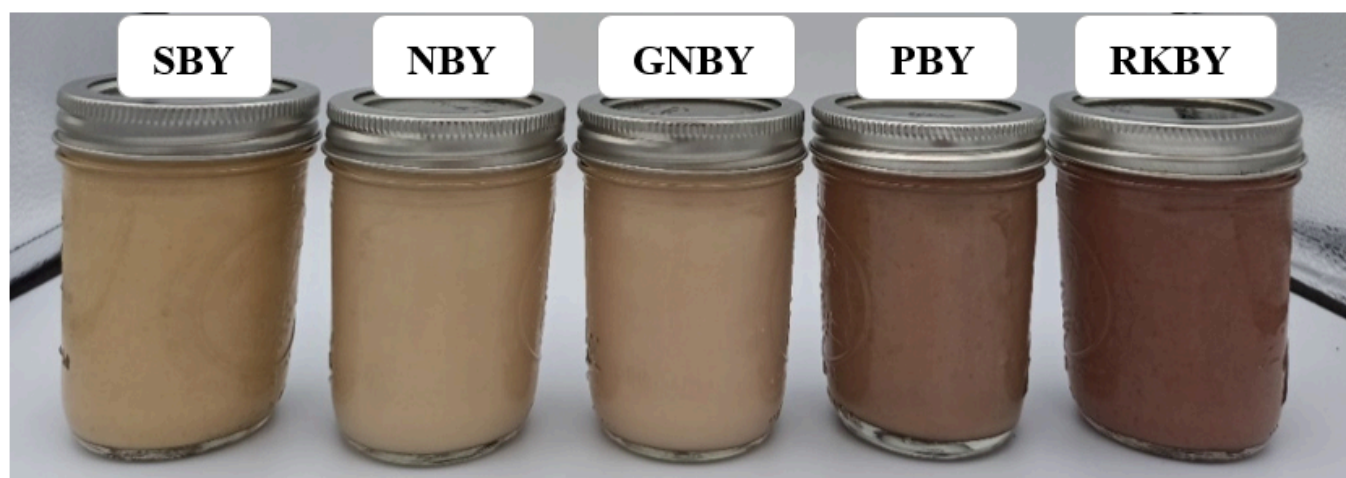


Figure 1

Visual appearance of bean yogurts made from soybean, navy bean, great northern bean, pinto bean, and red kidney bean.

SBY, soybean yogurt; NBY, navy bean yogurt; GNB, great northern bean yogurt; PBY, pinto bean yogurt; RKB, red kidney bean yogurt.

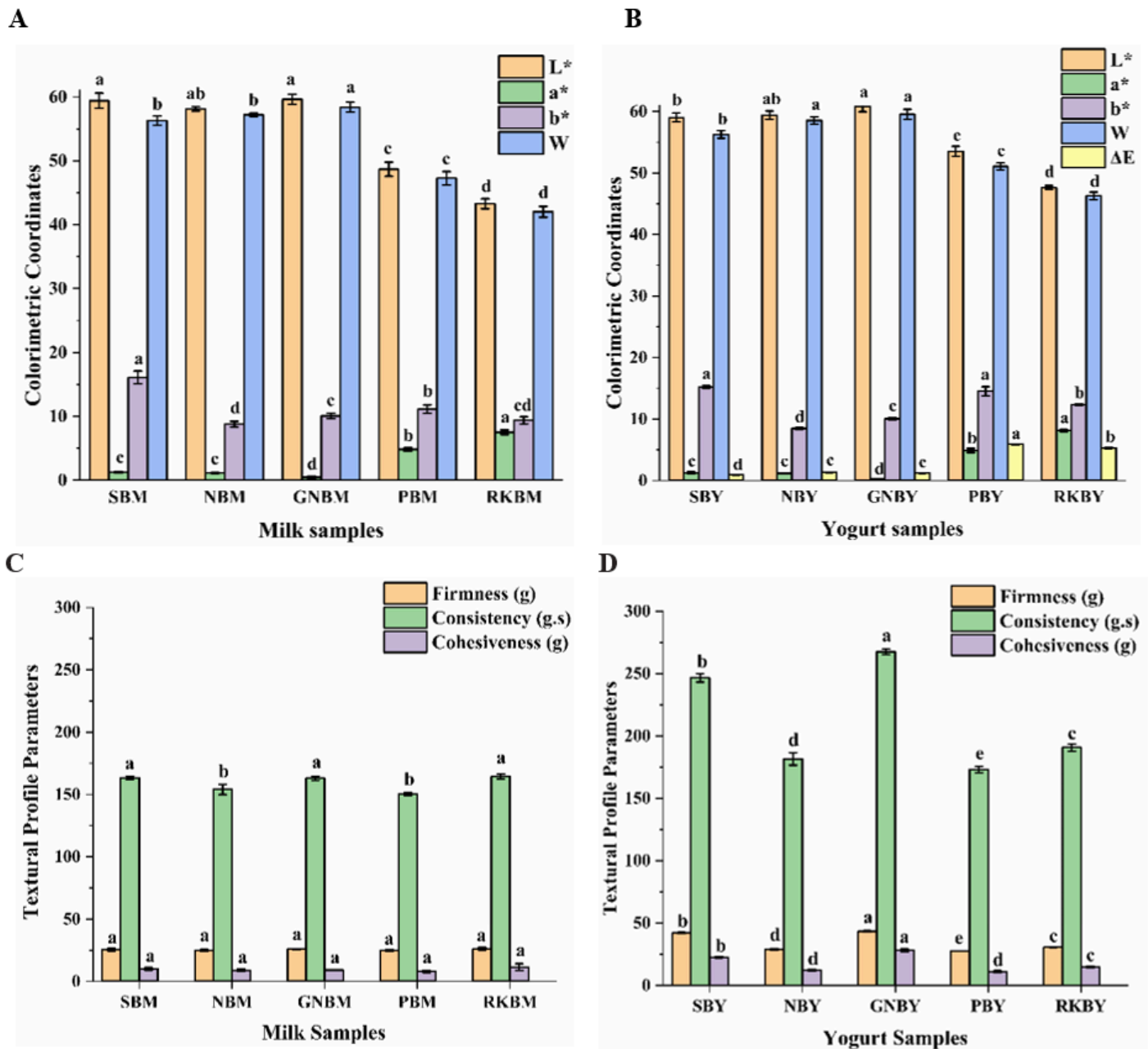


Figure 2

Colorimetric coordinates of bean milks (A) bean yogurts (B); and textural profile parameters of bean milks (C) and bean yogurts (D).

SBY, soybean yogurt; NBY, navy bean yogurt; GNB, great northern bean yogurt; PBY, pinto bean yogurt; RKBY, red kidney bean yogurt; SBM, soybean milk; NBM, navy bean milk; GNBM, great northern bean milk; PBM, pinto bean milk; RKBM, red kidney bean milk. L*, lightness; a*, redness to greenness; b*, yellowness to blueness; W, whiteness; ΔE, color difference. Values with different superscripts within a sample differ significantly ($p < 0.05$).

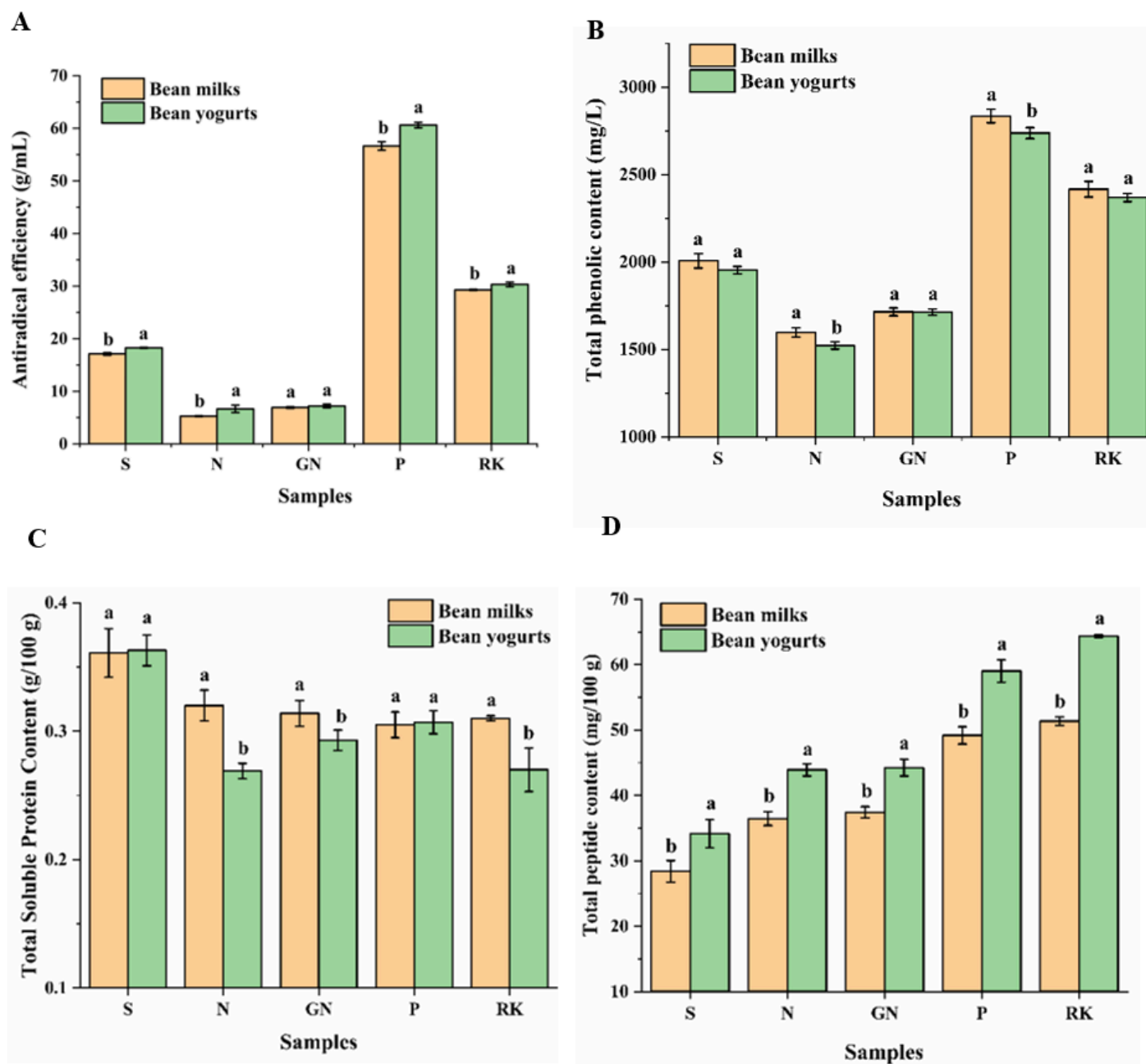


Figure 3

Antiradical efficiency (A); total phenolic content (B); total soluble protein (C); and total peptide content (D) of bean milks and yogurts.

S, soybean; N, navy bean; GN, great northern bean; P, pinto bean; RK, red kidney bean. Values with different superscripts within a sample differ significantly ($p < 0.05$).

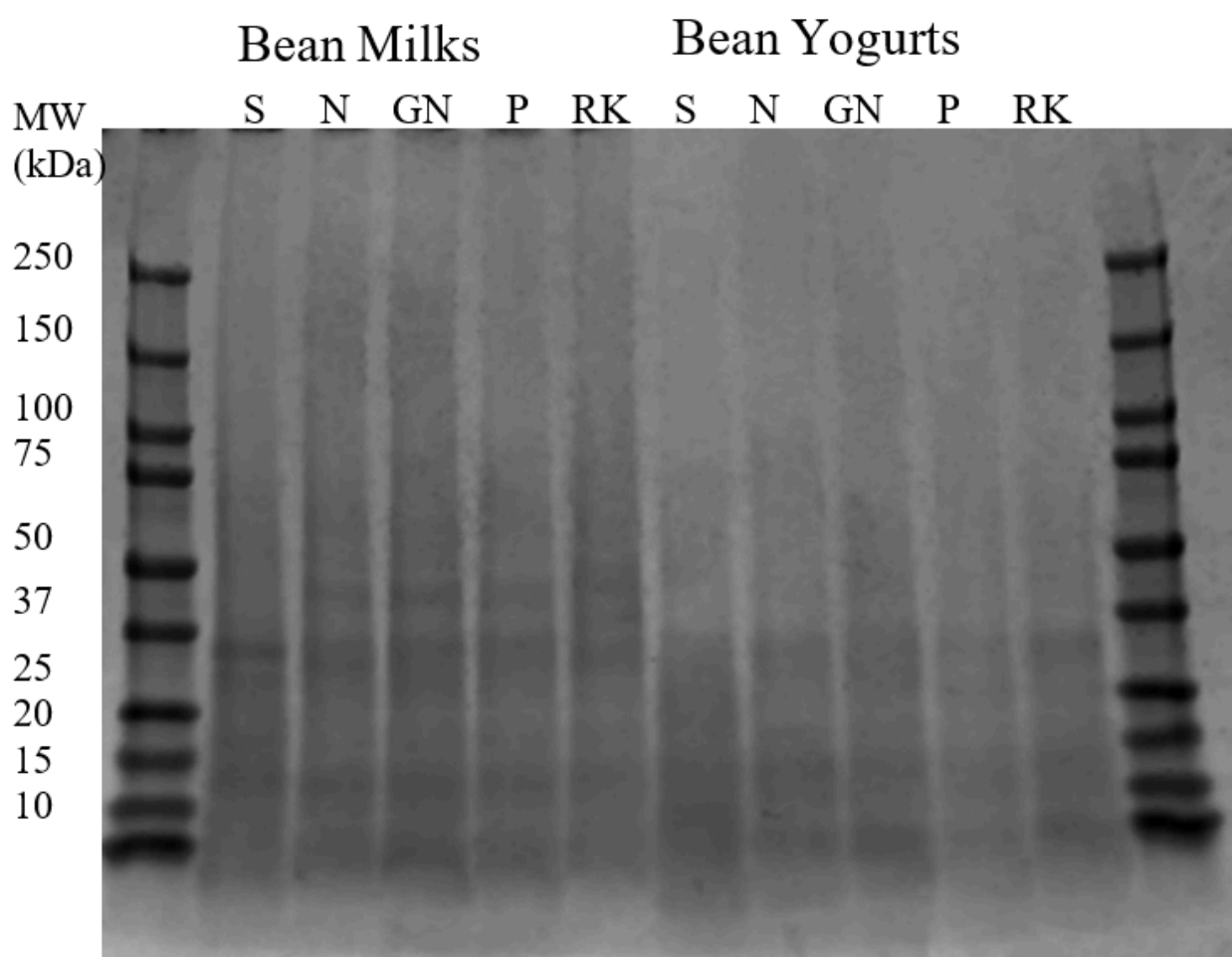


Figure 4

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profile of bean milks and yogurt.

S, soybean; N, navy bean; GN, great northern bean; P, pinto bean; RK, red kidney bean.

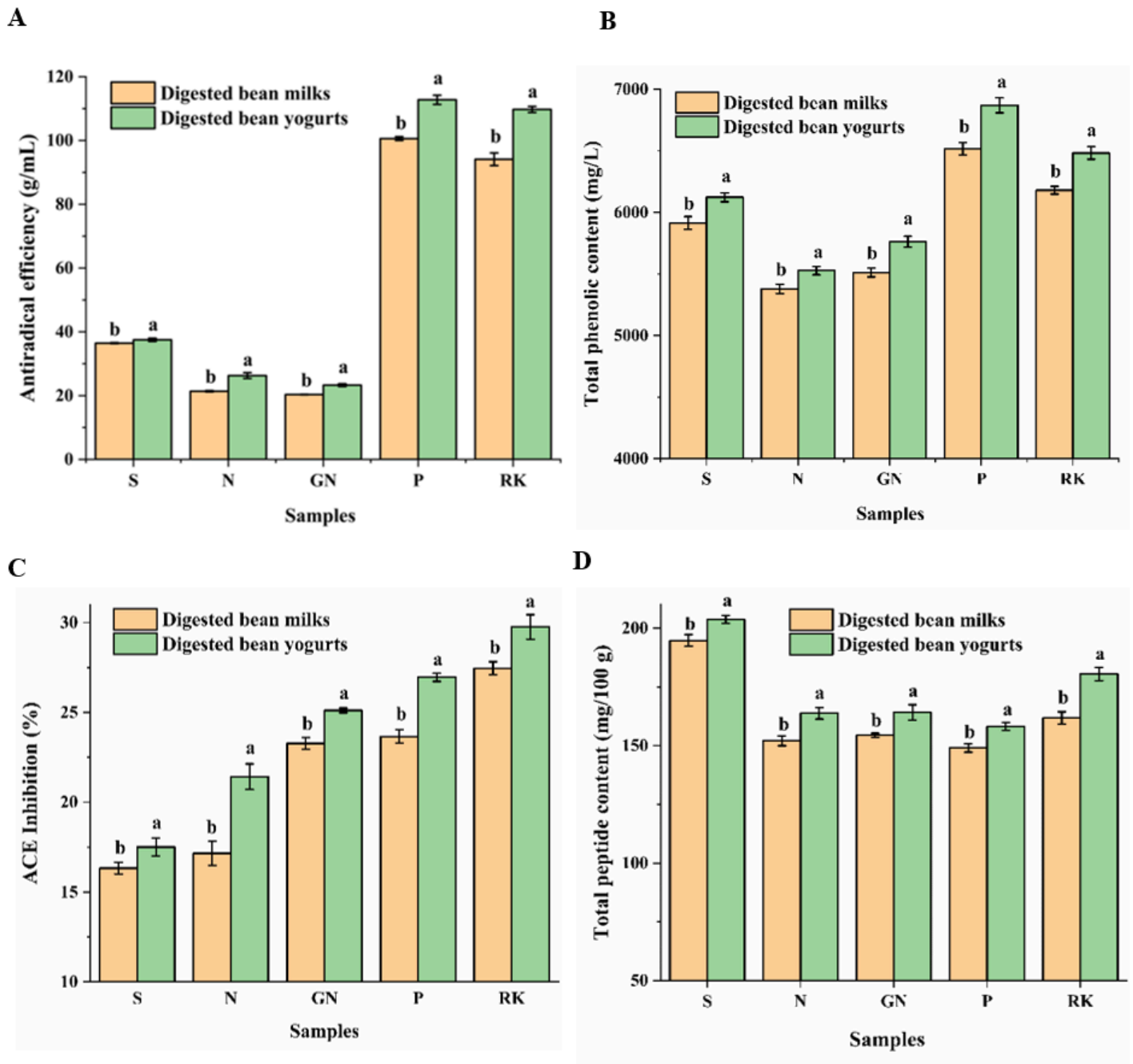


Figure 5

Antiradical efficiency (A); total phenolic content (B); ACE inhibitory activity (C); and total peptide content (D) of bean milks and yogurts after *in vitro* gastrointestinal simulation.

S, soybean; N, navy bean; GN, great northern bean; P, pinto bean; RK, red kidney bean. Values with different superscripts within a sample differ significantly ($p < 0.05$).

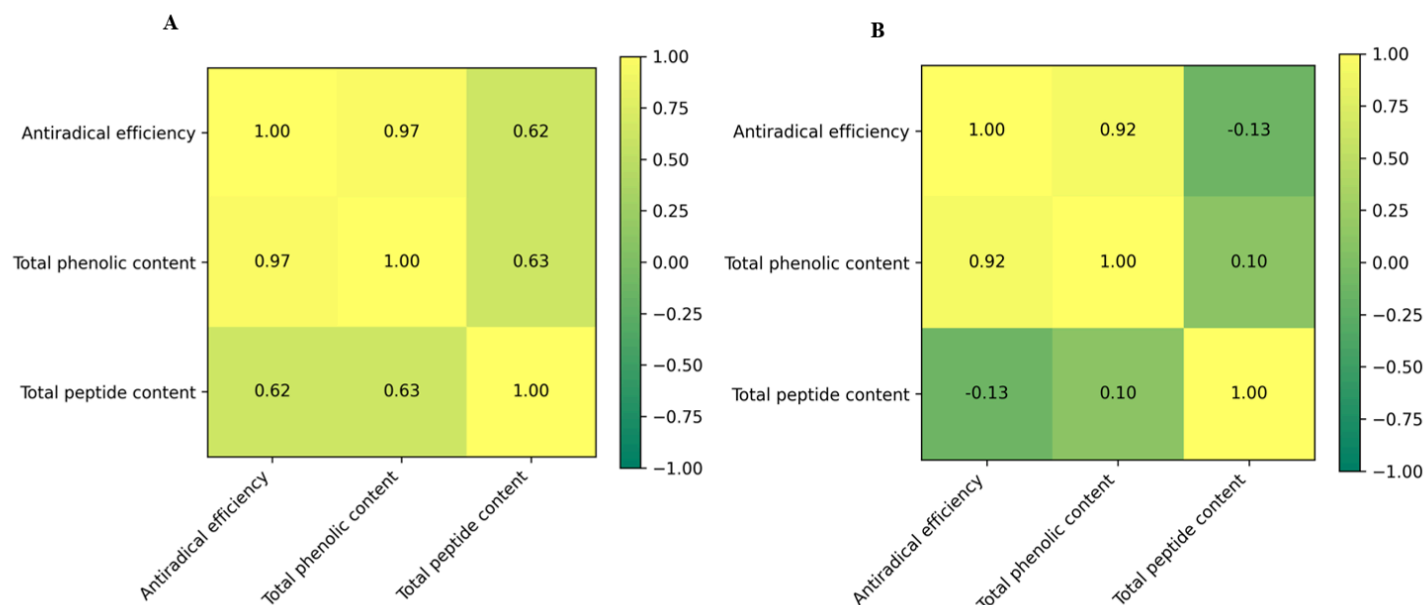


Figure 6

Pearson correlation heatmaps of bioactivity parameters in bean-based samples before and after in vitro digestion. The parameters include antiradical efficiency (g/mL), total phenolic content (mg/L), and total peptide content (mg/100 g). (A) Correlation matrix prior to digestion; and (B) Correlation matrix after digestion.

Supplementary Files

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