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Impact of food technological processing on biochemicals of plant-based protein-rich products: A ^{1}H NMR metabolomics study

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ABSTRACT

Plant-based foods are essential component in healthy human diets. Recently, plant-based protein-rich (PBPR) foods—made from cereals and legumes using innovative processing technologies that enhance taste, texture, and nutritional value—have been driving market growth. However, processing techniques significantly alter the biochemical composition of these foods, impacting their sensory and nutritional properties. Using ^{1}H NMR metabolomics, this study analyzed PBPR foods across processing techniques, revealing distinct biochemical profiles. Products classified as highly processed (made from protein concentrates or isolates) were characterized by elevated levels of propylene glycol, pyroglutamate, levulinic acid, hydroxymethylfurfural (HMF), and glucose, and lower levels of myo-inositol and asparagine. In particular, fermented products, such as tempe, were associated with high levels of amino acids, 4-aminobutyrate (2-fold higher than in whole beans), and nicotinate (12.6-fold higher). These findings highlight that processing technique is associated with distinct biochemical profiles of PBPR foods, which may have implications for health, warranting precision design and optimization of the processing of PBPR.

1. Introduction

Plant-based foods are important constituents in the human diet worldwide. In the last decades, the consumption of products from plant origin has become increasingly significant due to sustainability reasons and health benefits (Willett et al., 2019). Relative to livestock production, cultivating vegetables, fruits, cereals, and legumes yields lower greenhouse gas emissions, reduces land demand and antibiotic resistance risks, and thus represents a more sustainable food pathway. The health benefit of plant-based diets is attributed to, e.g. the presence of dietary fiber, vitamins, minerals, phytochemicals, and reduced saturated fats. PBPR products can be manufactured with various processing techniques to make them palatable, convenient, and even meat-like in texture (Boukid, 2020). A key driver of market growth has been innovative technologies that enable complex, realistic structures mimicking animal protein. The processing transforms vegetable protein into fibrous layers that match the appearance and texture of meat or fish, resulting in a vast range of products for consumers. Soy, peas, fava beans, etc., are widely used PBPR foods due to their high protein content, nutritional quality, and health benefits (Zhang et al., 2024). Proteins from these sources also have great functional properties, such as emulsification and gelling properties, and are therefore widely used in the production of various PBPR foods (Keivaninahr et al., 2021; Nishinari et al., 2014). These products include a wide spectrum of meat-free options, for example, products resembling burger patties, sausages, and nuggets, which serve as substitutes for animal proteins and simulate the taste and texture of animal meat (Boukid, 2020).

Different technologies may be applied, differing in the physical procedures (for example, extrusion, fermentation), the amount of biochemical constituents removed from the product, and the amount of

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added ingredients, resulting in differences in the biochemical composition of the differentially processed PBPR products (Monteiro et al., 2018; Raita et al., 2025). In recent years, evidence has accumulated demonstrating how highly processed foods are associated with adverse health issues, including impaired cardiometabolic health and obesity, as evidenced by various prospective and intervention studies (González-Gil et al., 2025; Lane et al., 2024; Sharma et al., 2025; Touvier et al., 2023). Typically, these highly processed foods are characterized by refined raw materials, added food additives and often also contain higher levels of saturated fat and salt compared to fresh, minimally processed foods, which are linked to metabolic issues like hypercholesterolemia and hypertension (Da Silva, 2025). Emerging evidence indicates that food processing can modulate gut microbiota and their associated metabolites, which in turn influences host metabolic health (Huang et al., 2024). In the case of PBPR foods, consumption of PBPR processed foods has also faced criticism regarding their overall health effects, high consumption of which has been associated with increased energy intake and weight gain relative to unprocessed PBPR foods in recent studies (Hall et al., 2019).

As the consumption of novel PBPR foods continues to rise, there is a growing demand to characterize their biochemical composition, which is closely linked to their health attributes. Although plant-based foods are generally a beneficial choice based on epidemiological evidence, concerns regarding highly processed PBPR foods deserve to be addressed. Previously, we used liquid chromatography-mass spectrometry (LC-MS)-based metabolomics to investigate phytochemicals of PBPR foods, especially focusing on products made of soy (Raita et al., 2025). Here, we utilized ^{1}H NMR metabolomics to analyze the overall biochemical composition of various PBPR food products across different processing categories, alongside meat products, to shed new light on how different processing methods could influence nutritional quality and hence potential health implications.

It should be noted that this study is exploratory and cross-sectional in nature. The observed biochemical differences across product categories reflect the combined effects of raw material selection, formulation, and processing technology, which are inherently confounded in commercial products. Therefore, the term “processing-related differences” is used throughout to indicate associations rather than causal attributions.

2. Materials and methods

2.1. Samples

Here, we utilized ^{1}H NMR metabolomics—complementing our earlier LC-MS phytochemical analysis of the same samples (Raita et al., 2025). A total of 169 PBPR foods including whole beans, tofu, extruded chunks, tempe, and products resembling meat like nuggets, sausages, cold cuts, and burger patties, along with 8 unseasoned and unprocessed animal-based products (including two samples of minced beef, chicken strips, salmon fillets, and pork strips each), were sourced from local markets in Turku, Finland, between September and December 2021 (Raita et al., 2025). Detailed information on the products analyzed is provided in Supplementary Table 1.

Products were classified according to three systems. First, product type was assigned based on ingredient labels and processing information, resulting in six categories: whole bean/plant, extruded chunks, tofu, tempe, protein concentrate/isolate, and meat. Second, NOVA classification grouped products into unprocessed/minimally processed, processed culinary ingredients, processed, and ultra-processed, where ultra-processed products were defined as those containing at least one substance not commonly used in culinary preparations (e.g., protein isolates, modified starches, or additives). Third, the classification system proposed by Poti et al. was applied, yielding four categories: unprocessed/minimally processed, basic processed, moderately processed, and highly processed, with the latter indicating products derived from purified or extracted ingredients (e.g., protein concentrates/isolates) and containing at least one additive.

For NOVA classification, products were assigned based on the nature, extent, and purpose of processing and on the ingredient list. Unprocessed/minimally processed foods included intact or minimally modified plant or animal foods without added salt, sugar, oil, or cosmetic additives. Processed foods included products made by adding culinary ingredients such as salt, oil, or sugar to minimally processed foods for preservation or palatability, while the original food matrix remained recognizable. Ultra-processed foods included industrial formulations containing protein isolates/concentrates, texturized proteins, refined ingredients, flavorings, colorants, stabilizers, emulsifiers, or other additives, particularly when the original plant matrix was no longer recognizable.

For the Poti et al. classification, products were categorized as unprocessed/minimally processed, basic processed, moderately processed, or highly processed. Unprocessed/minimally processed products were single-ingredient foods with no or only slight modification. Basic processed products were single foods altered by processes such as extraction, pressing, milling, refining, canning, or related processing steps. Moderately processed products were still recognizable as their original plant or animal source but contained added ingredients such as salt, oil, sweeteners, or flavorings. Highly processed products were multi-ingredient industrial formulations, often ready-to-eat or ready-to-heat, containing refined ingredients, protein fractions, flavor systems, stabilizers, emulsifiers, or other additives.

Extruded chunks were defined as products manufactured through extrusion, as indicated on their nutrition labels. All items were prepared following package instructions, mimicking typical cooking methods before consumption, but without the addition of frying oil. Each product was divided into five equal portions and stored at −20 °C until further processing. Before analysis, thawed samples were homogenized using Bamix processors (Model M133) at maximum speed (mode 2) or manually ground with a pestle and mortar for more intricate samples, such as whole beans.

2.2. NMR sample preparation

Approximately 100 mg of pre-homogenized samples were weighed and 4 mL/g of ice-cold methanol, 0.82 mL/g of ice-cold ultrapure water, and 2 mL/g of ice-cold chloroform were added and vortexed prior to homogenization (TissueLyser II, Qiagen, Germany). After homogenization, 2 mL/g ice-cold chloroform and 2 mL/g ice-cold water were added, vortexed, and the homogenization was repeated. Samples were left on ice (15 min) and centrifuged (2000 ×*g*, 15 min, +4 °C, VWR Mega Star 600R). The upper phase containing the polar metabolites was collected, and solvents were removed using a freeze dryer. 530 μL of phosphate buffer (90 mmol/L KH_2PO_4, pH = 7.4) and 70 μL of Chenomx Internal Standard solution (Chenomx Inc., Edmonton, Alberta, Canada) containing 5 mM DSS-d_6 were added to the dried aqueous extract. After vortexing, pH was adjusted to 6.85, and the samples were centrifuged before transferring the supernatant into 5 mm NMR tubes. The solvent of the lower phase was removed with a nitrogen flow. The dried lipid extract was dissolved in 600 μL of chloroform-*d* containing 0.03% trimethylsilane (TMS). The solutions were transferred into 5 mm NMR tubes. Each commercial product was purchased as a single batch. From each product, five equal portions were prepared following package instructions. These five portions were homogenized and pooled to obtain a representative sample per product. NMR measurements were performed on this pooled sample without technical replicates, given the high inherent reproducibility of NMR. Thus, each product represents one independent biological observation ($n = 1$ per product).

2.3. NMR analysis

The NMR experiments were performed at 298 K using a 600 MHz Bruker AVANCE III NMR spectrometer (Bruker BioSpin AG, Fällanden,

Switzerland) equipped with a Prodigy TCI cryoprobe and a precooled SampleJet sample changer. ^{1}H NMR spectra from aqueous and lipid extracts were recorded using the one-dimensional (1D) NOESY (*noesygppr1d*) and *zg30* pulse sequences, respectively. The parameters used for the 1D NOESY were as follows: spectral sweep width, 16.02 ppm; acquisition time, 3.41 s; D1, 5 s; D8, 0.1 s; the number of scans was 128. The parameters used for *zg30* were collected with 32 scans; sweep width of 14 ppm; receiver gain of 32; acquisition time of 4 s; D1, 5 s.

All the spectra were manually phase- and baseline-corrected with Topspin 3.5 software (Bruker BioSpin GmbH, Rheinstetten, Germany). The NMR signal assignment and metabolite quantification for aqueous extract spectra were done with the aid of Chenomx Profiler 7.5 software (Chenomx Inc., Edmonton, Alberta, Canada) and the Human Metabolome Database (HMDB, http://www.hmdb.ca).

Metabolite assignment was performed using the Chenomx Profiler 7.5 software with its proprietary spectral library, supplemented by manual verification against the Human Metabolome Database (HMDB) and published literature (Chen et al., 2020, 2021; Pariyani et al., 2024). Table of chemical shift assignments of the metabolites observed in the ^{1}H NMR spectra using one dimensional (1D) NOESY, two-dimensional HSQC, *J*-resolved spectra was constructed (Supplementary table sheet 2; Fig. S1A—D). The chemical shifts of the peaks in the lipid samples are rather well known and can be identified based on NMR literature (Chen et al., 2021) and lipid libraries, such as https://www.aocs.org/resource/nmr/. The NMR acquisition and processing parameters were adapted from previously published protocols (Chen et al., 2020).

Prior to the batch NMR metabolomics experiment, we performed a reproducibility test using four representative samples: raw butter bean, pork, an oat-based product, and tofu. Each sample was prepared in triplicate and analyzed under identical NMR conditions. The coefficient of variation (CV) for major metabolite signals was below 5% across replicates. Representative overlaid spectra are shown in Fig. S2A—D.

The 1D ^{1}H NMR spectra of the lipid extracts were manually integrated (TOPSPIN V3.0, Bruker Biospin, Germany), and the integrals are normalized to the TMS peak area. The NMR signal assignment and quantification were done with the aid of the literature (Jiang et al., 2013; Satyarthi et al., 2009).

2.4. Statistical analyses

Principal component analyses (PCA) for PBPR products and meats were prepared using SIMCA version 16 (Sartorius Stedim Data Analytics AB, Umeå, Sweden). Prior to PCA, the data were mean-centered and Pareto-scaled. K-means clustering was made with ComplexHeatmap with R Version 4.2.146. The Sankey diagram was made with SankeyMATIC (https://www.sankeymatic.com). The boxplots and stacking plots were performed with ggplot2 using R Version 4.2.1. The Kolmogorov−Smirnov test was used to check the normality of the data distribution in GraphPad Prism V 6.0 (GraphPad Software Inc., San Diego, USA). If data were normally distributed, a parametric one-way analysis of variance (ANOVA) was performed; otherwise, the nonparametric Kruskal−Wallis test was applied, and post hoc Fisher's LSD test or Dunn's

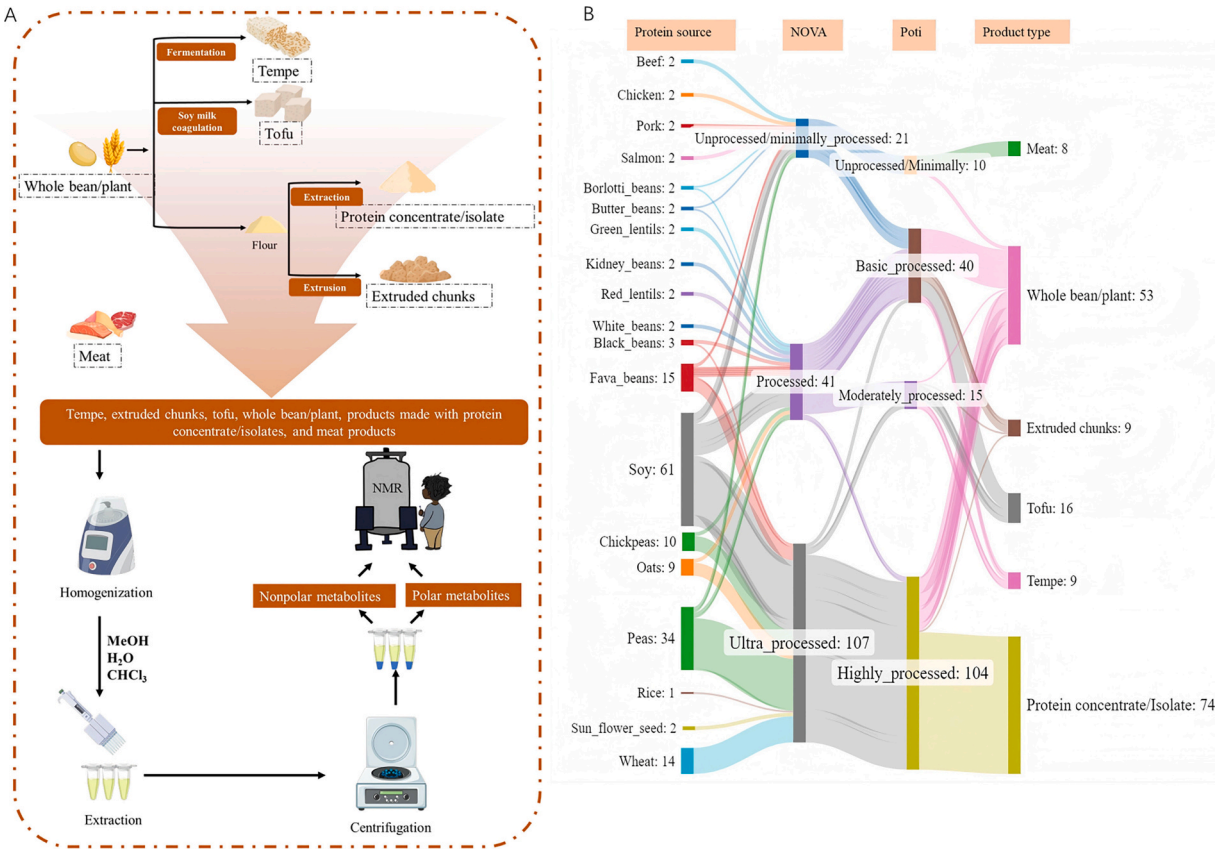

Fig. 1. Common processing technologies used for PBPR foods include tempe, extruded chunks, tofu, whole bean/plant, products made with protein concentrate/isolates, and meat products and the ^{1}H NMR metabolomics approach in this study illustrates the sample preparation pipeline (A). Sankey diagram of the products classified based on the protein source, NOVA and Poti, et al., classification systems, as well as product type according to the ingredient label (B). The Sankey diagram illustrates the classification flow of all analyzed samples across four dimensions: protein source, NOVA classification, Poti et al. classification, and final product type. The width of each flow is proportional to the number of samples in each category. Major protein sources include soy, pea, fava bean, chickpea, oat, wheat, and common beans; product types comprise whole bean/plant, extruded chunks, tofu, tempe, protein concentrate/isolate, and meat. Most protein concentrate/isolate products are classified as highly/ultra-processed, whereas tempe, tofu, extruded chunks, and whole beans are mainly distributed in minimally/basic/moderately processed categories. This diagram clearly shows the inherent linkage between raw material, processing intensity, and product type.

test was applied between groups. Values with different letters are significantly different ($p < 0.05$).

3. Results and discussion

3.1. NMR metabolomics analysis brings out differences in PBPR food samples owing to the protein source and product type

The present study provides a comprehensive analysis of the biochemical profiles by NMR metabolomics of PBPR foods, focusing on the impact of processing technologies and raw materials. This investigation encompassed an array of foods produced by various processing methods, spanning from intact whole beans to food products formulated from plant protein concentrates/isolates. Fig. 1A presents workflows to elucidate the distinctions among the prevalent PBPR food processing methodologies. Extruded chunks and protein concentrates/isolates undergo different processing methods, impacting their nutritional composition and functional properties. For example, in the case of soy, the beans are usually dehulled and defatted into soy flour. Defatted flour goes through an extruder to produce puffed meat-like protein chunks. Protein concentrates/isolates are made from defatted flour through additional extraction, where carbohydrates and fibers are removed using aqueous alcohol extraction or acid leaching, resulting in products that contain 60–90% protein. Thus, protein concentrates/isolates go through more rigorous processing compared to extruded chunks (Ishaq et al., 2022; Li et al., 2023; Webb et al., 2023). Tempe, a fermented product prepared from cooked whole beans (mainly soybeans) by using *Rhizopus* spp., is a popular Indonesian food. Tofu is made from coagulated soy milk. In total, 78 metabolites were identified and quantified in the aqueous phase (Supplementary Table 1 sheet2), and 12 lipid-related signal groups were analyzed in the lipid phase.

The flow diagram (Fig. 1B) illustrates the classification of the PBPR foods analyzed in this study according to the protein source, NOVA, and Poti et al. classification systems, and product type in a different order. Soy and peas are the most widely used PBPR food sources worldwide and also in this study (Arora et al., 2023). Both soy and pea protein concentrates/isolates are notable for their high protein content, good digestibility, and comprehensive amino acid profiles, containing all essential amino acids. These qualities make them comparable to animal-based protein sources such as casein and whey, making them suitable for use in various food applications (Guillin et al., 2022; Z. Huang et al., 2023). Almost all the protein concentrate/isolate products fall into the more processed category, the majority of extruded chunks, tempe, and tofu products fall into the less processed categories, whole bean/plant

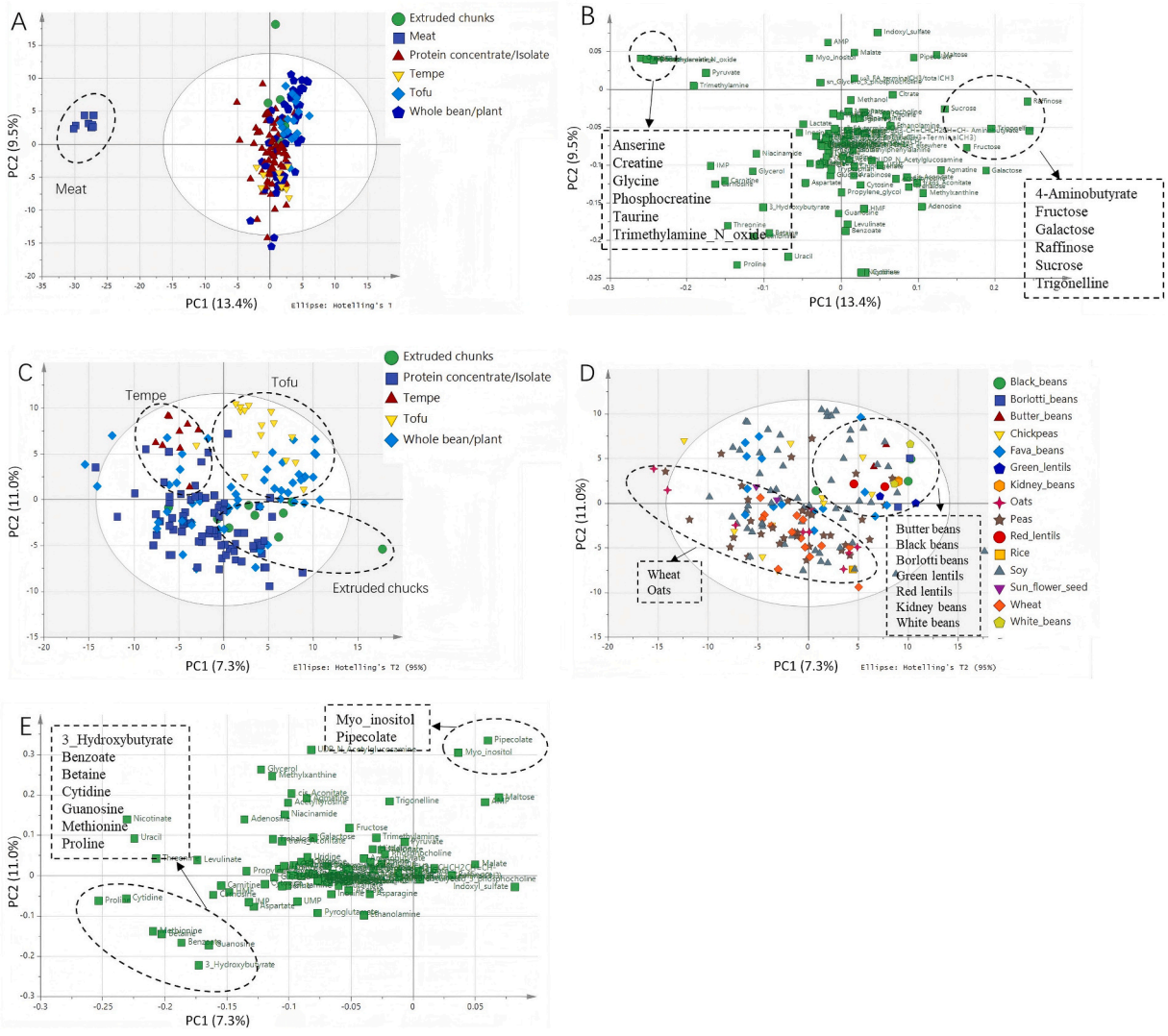

Fig. 2. PCA score plot (A) and corresponding loading plot (B) made using identified compounds from ^{1}H NMR metabolomics in PBPR foods and meat products. PCA score plot performed using identified compounds from ^{1}H NMR metabolomics in PBPR products based on different grouping strategies: product type (C), protein source (D), and corresponding loading plot (E).

products are identified in all four categories due to different processing methods (frying, dying, and the addition of oil and additives, etc.).

PCA of the ^{1}H NMR data revealed separation of food categories (by processing degree, product type, and source) based on their biochemical composition (Fig. 2). These differences have important implications for nutritional quality, functional properties, and potential health effects. These differences have important implications for nutritional quality, functional properties, and potential health effects. PBPR foods and meat controls are separated in the score plot, and the corresponding loading plot shows higher levels of anserine, creatine, glycine, phosphocreatine, taurine, and trimethylamine N-oxide and lower levels of 4-aminobutyrate, fructose, galactose, raffinose, sucrose, and trigonelline in meat compared to the PBPR products, as main contributors to the separation (Fig. 2A and B, Fig. S3). A previous study also showed an increase in 4-aminobutyrate and butyrate-producing gut microbiota in the PBPR foods intervention compared to the control group (Toribio-Mateas et al., 2021).

When the PBPR products are labelled by product type in the PCA analysis, tofu products fall into the first quadrant, tempe products fall into the second quadrant, and the extruded chunks in the fourth quadrant (Fig. 2C), the products made from isolated proteins occupy mostly the third and fourth quadrants, and whole bean/plant products are scattered in all the quadrants due to the origins. When the products are labelled by protein source, butter beans, black beans, borlotti beans, green lentils, red lentils, kidney beans, and white beans are located in the first quadrant, while oats and wheats are separated from them (Fig. 2D). Butter beans, black beans, borlotti beans, green lentils, red lentils, kidney beans, white beans, and tofu products represent PBPR foods with less processing, and these products were characterized by higher levels of myo-inositol and pipecolate and lower levels of 3-hydroxybutyrate, benzoate, betaine, cytidine, guanosine, methionine, and proline (Fig. 2E). The higher levels of anserine, creatine, phosphocreatine, taurine, glycine, and trimethylamine N-oxide in meat are consistent with animal muscle-derived metabolic features, including muscle energy metabolism and nitrogen-containing muscle metabolites (Purchas et al., 2004; Wu, 2020). In contrast, the relatively higher levels of 4-aminobutyrate, fructose, galactose, raffinose, sucrose, and trigonelline in PBPR products are consistent with plant-derived matrices, including plant carbohydrate metabolism, seed/legume oligosaccharides, and plant secondary metabolites (Elango et al., 2022; Nguyen et al., 2024).

Lower levels of myo-inositol were found in products based on protein concentrates/isolates (considered as highly processed foods). Since abnormalities in myo-inositol metabolism are linked to polyneuropathies in diabetes and chronic renal failure, changes in dietary myo-inositol intake in foods may have the potential to affect disease course (DiNicolantonio & O'Keefe, 2022).

3.2. K-means clustering of metabolic profile

When focusing on the individual biochemicals and categorizing the products either according to their protein sources (Fig. 3A) or by food product type (Fig. 3B), we can see different clustering owing to the differential levels of the measured compounds. Meat products showed metabolite profiles different from those of PBPR foods as was evident also in the Fig. 2A and B. Among the investigated PBPR foods, sunflower seeds, wheat, soy, fava beans, oats, peas, and chickpeas exhibited a higher concentration of the metabolites within cluster 3 (nicotinate, glucose, and some amino acids like proline, lysine, phenylalanine, glutamate, tryptophan, leucine, and isoleucine), cluster 4 (lipid-related signals), as well as some in cluster 2 (alanine, valine, and methionine) compared to a group including black beans, borlotti beans, butter beans,

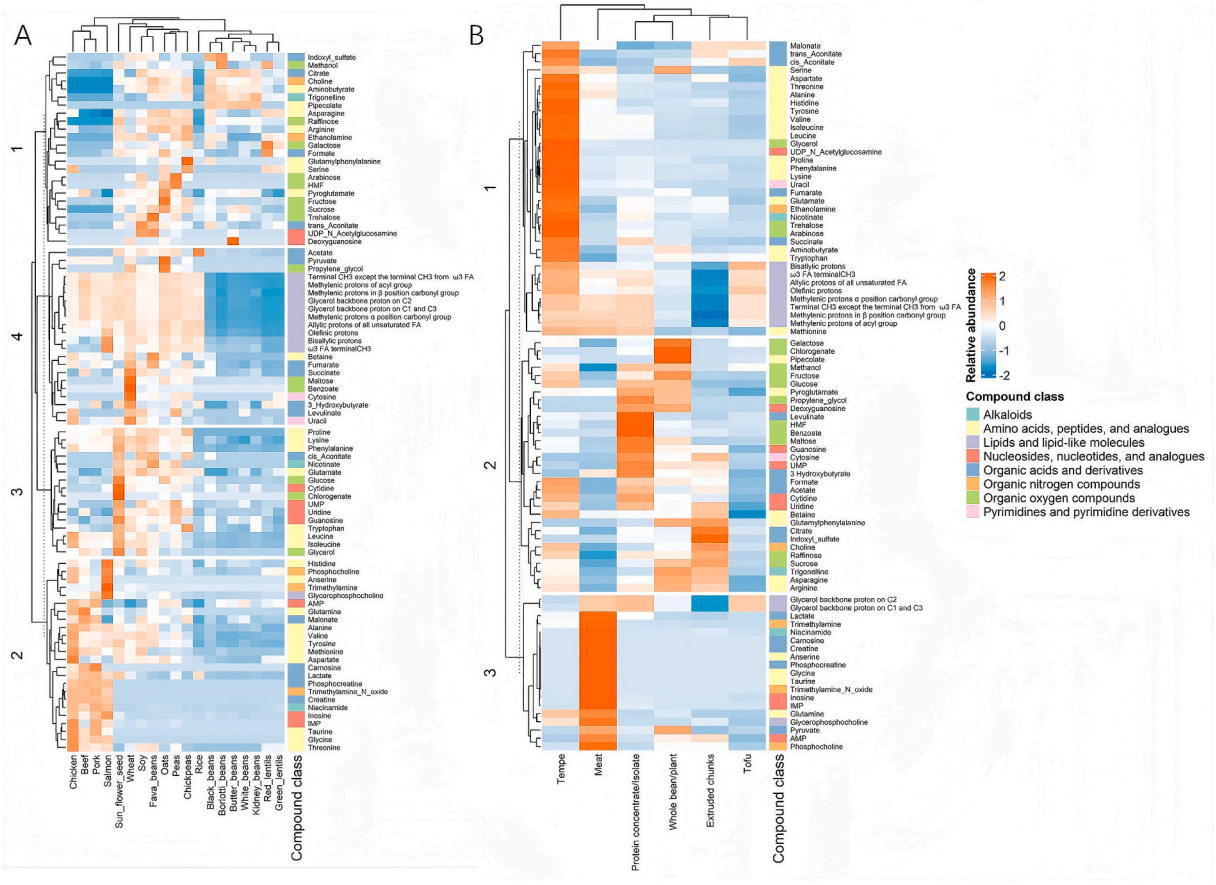

Fig. 3. K-means clustering made from metabolite concentrations and bin integrals. Products are classified based on the protein source (A) and product type (B).

white beans, kidney beans, red lentils, and green lentils (Fig. 3A).

When focusing on the cluster analysis based on the product type, clearly different profiles were observed, especially for tempe (Fig. 3B), which exhibited a higher content of unsaturated lipids and amino acids (especially lysine and threonine) in general, particularly in cluster 1 of Fig. 3B. Tempe also showed elevated levels of 4-aminobutyrate, nicotinate, aconitate, and glycerol compared to other food types. It has been reported that 4-aminobutyrate retarded the elevation of systolic blood pressure and improved discrimination learning in rats (Aoki et al., 2003). In Japan, highly purified 4-aminobutyrate is used as a medication

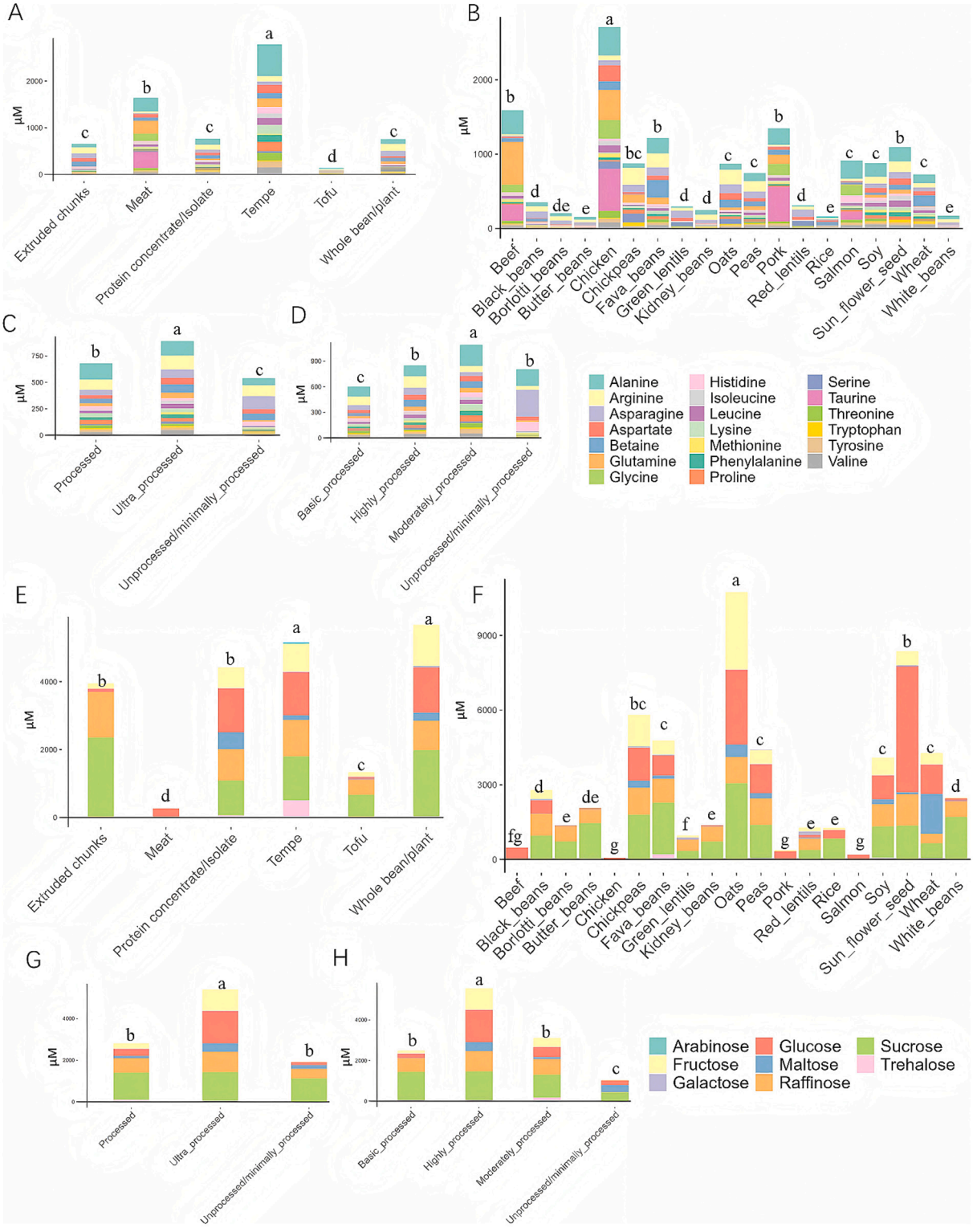

Fig. 4. Amino acids and sugars in foods produced from different sources using various processing methods. Amino acid levels (μM) in PBPR products are shown in stacking plots in different category groups: product type (A), protein source (B), Nova classification (C), and Poti et al. classification (D). Sugar levels (μM) in PBPR products are shown in stacking plots in different category groups: product type (E), protein source (F), Nova classification (G), and Poti et al. classification (H). Different lower-case letters indicate statistical significance according to one-way ANOVA followed by Tukey's post hoc test.

for the improvement of cerebral blood flow. Previously a tempe fermentation study also found that the fermentation process increased 4-aminobutyrate levels by up to 6 times compared to unfermented soys (Aoki et al., 2003). In our study, tempe showed close to 2-fold more 4-aminobutyrate compared to the whole bean/plant. Furthermore, 4-aminobutyrate has been found to increase in parallel with butyrate production in the gut (Toribio-Mateas et al., 2021). Nicotinate is a water-soluble vitamin of the B complex occurring in plant tissues. Studies have shown that tempe contains up to 20 times more nicotinate compared to its raw material, soy, as a result of 72 h of fermentation (Murata, 1967). In our study, tempe showed 12.6 times more nicotinate compared to the product type whole bean/plant.

Products classified as protein concentrates/isolates (considered highly processed) were characterized by higher levels of propylene glycol, pyroglutamate, and HMF. (5-hydroxymethylfurfural) (Fig. 3B). Propylene glycol is commonly used in food processing, including PBPR products, as a humectant, solvent, and stabilizer (Elmore et al., 2014). Pyroglutamate is also formed from the cyclization of glutamine or glutamic acid residues, which can occur naturally or during food processing, especially under heat or enzymatic action (Yoshimune et al., 2006). HMF, higher in protein concentrate/isolate products, is formed in carbohydrate-rich foods during heat processing and the Maillard reaction, when reducing sugars react with amino acids. This can happen during baking, roasting, drying, and other thermal processes (Kowalski et al., 2013). The presence of these compounds raises an important point: while these molecules may contribute to functionality, they also reflect the degree of processing and potential alteration of the nutritional profile.

3.3. *Effect of protein source and product type on amino acids, sugars, and lipids*

Since amino acids are an essential nutritional component, we focused in more detail on their composition across all the differentially processed food products. Tempe is distinguished by its high amino acid concentration, as depicted in Fig. 4A. The proteolytic capacity of *Rhizopus* strains used in tempe fermentation has been shown a significant increase in free amino acids (Baumann & Bisping, 1995). A previous study has confirmed that tempe products contain significantly more amino acids compared to unfermented soy, with individual amino acid levels increased by up to 85 times (Murata et al., 1967). Chickpeas, fava beans, oats, peas, and sunflower seeds exhibit higher levels of arginine, as shown in Fig. 4B. Fava beans and wheat specifically show an increased concentration of betaine (Fig. 4B). After FDR correction, the majority of metabolites showing nominal differences remained significant ($q < 0.05$). Key findings—such as elevated glucose, HMF, propylene glycol, and pyroglutamate in highly processed products, as well as higher amino acids, 4-aminobutyrate, nicotinate, and trehalose in tempe—all had $q < 0.05$. A few metabolites with only nominal significance are discussed as trends where noted.

Sunflower seeds, wheat, oat, and soy exhibited a higher concentration of methionine compared to a group including black beans, borlotti beans, butter beans, white beans, kidney beans, red lentils, and green lentils (Fig. S4 and Fig. 3A). A previous study has also shown that cereals generally contain less lysine, whereas legumes (soy, other common beans, peas, and chickpea) are deficient in sulfur-containing amino acids like cysteine and methionine (Langyan et al., 2022), which is similar to the current study showing that bean products contain less methionine.

Higher levels of asparagine were observed in less processed products (whole bean/plant and extruded chunks), whereas lower levels were observed in protein concentrate/isolate products (Figs. 4D and 3B). While asparagine is known to be metabolized during Maillard reactions under heat processing, alternative explanations include inherent differences in raw material composition or formulation choices. Nevertheless, the consistent association of higher asparagine with less processed categories suggests that it may serve as a potential marker associated with lower processing intensity. Further controlled studies using identical raw materials with varying processing conditions would be needed to confirm causality.

Sucrose, fructose, and glucose are the dominant sugars in PBPR products (Fig. 4E). Glucose was found in the highest concentrations in ultra-processed or highly processed products, as shown in Fig. 4G–H. A previous study has shown that 2–20% of sucrose was lost during extrusion at 170–210 °C in protein-rich products (Singh et al., 2007); however, significant loss of sucrose was not observed in the current study. This discrepancy may be due to differences in raw material composition (legumes vs. cereal-based proteins) and extrusion conditions (e.g., temperature, moisture content, residence time), which influence the extent of thermal degradation of sucrose. Public concern is growing regarding the glucose content in food products, with glucose being found in the highest concentrations in processed products, particularly those derived from protein concentrates/isolates (Monteiro et al., 2018). These high glucose levels, prevalent in protein concentrates/isolates, can serve as a marker distinguishing these products from those of other processing categories. The elevated glucose in protein concentrates/isolates is likely due to both the breakdown of more complex carbohydrates during processing and the addition of glucose or glucose-derived sweeteners to enhance flavor and palatability (Monteiro et al., 2018).

Maltose levels are reduced in extruded chunks and tofu compared to other product types (Fig. 4E), which can be attributed to the effects of heat processing. The thermal hydrolysis that occurs during extrusion and cooking, along with the addition of acids in tofu production, likely facilitates the reduction in maltose (Singh et al., 2007). Furthermore, processed products often have added maltodextrin, a substance used to enhance flavor and texture, which could account for the observed alterations in sugar profiles. Tempe commonly showed a higher level of trehalose, a non-reducing disaccharide consisting of two glucose units linked in an α,α-1,1-glycosidic bond (Fig. 4E). Although no previous literature has reported the trehalose level in tempe products, *R. oryzae*, a species closely related to *R. oligosporus*, accumulates trehalose in response to environmental stresses such as heat, osmotic stress, and nitrogen starvation (Uyar et al., 2010). This suggests a similar mechanism in *R. oligosporus*, where trehalose acts as a stress protectant to enhance fungal survival and functionality during fermentation.

Glycerol backbone protons on C1/C3 and glycerol backbone proton on C2 (Fig. 5A and B) signals represent total glycerides. Fig. 5C and D represent total lipids. Higher levels of glycerides and total lipids were observed in tofu, protein concentrate/isolate (a protein source for ultra-processed or highly processed products in this study) products, and meat. Lower levels of glycerides were observed in extruded chunks. Although, extruded chunks as well as protein concentrates/isolates are defatted, products based on protein concentrates and isolates usually contain high amounts of added oil, such as seed oil in several products, as confirmed by a previous study showing vegetable oil, canola oil, sunflower oil, sunflower kernels, rice bran oil, and coconut oil are commonly added to PBPR foods in Australia (Curtain & Grafenauer, 2019). Recent studies have shown that the addition of hydrocolloids (Bhuiyan et al., 2024) and the order of ingredient addition (Bhuiyan et al., 2025) significantly influence the structural properties and texture of extruded plant-based meat analogs.

One noteworthy difference is that tempe contains the highest total lipids, which was not the case in the glycerides represented by the glycerol backbone signal of glycerides. Therefore, tempe might contain more non-glyceride lipids, such as free fatty acids, compared to other product types. A pronounced shift in glycerides toward fatty acids has also been observed during the tempe fermentation in a previous study (de Reu et al., 1994). Fig. 5 H and I represent omega-3 fatty acyls and their ratio. Total unsaturated lipids (Fig. 5F and G), total polyunsaturated lipids (Fig. 5E), and omega-3 fatty acyls shared similar patterns among the products, with tempe products being the highest one, followed by protein concentrate and isolates. Extruded chunks

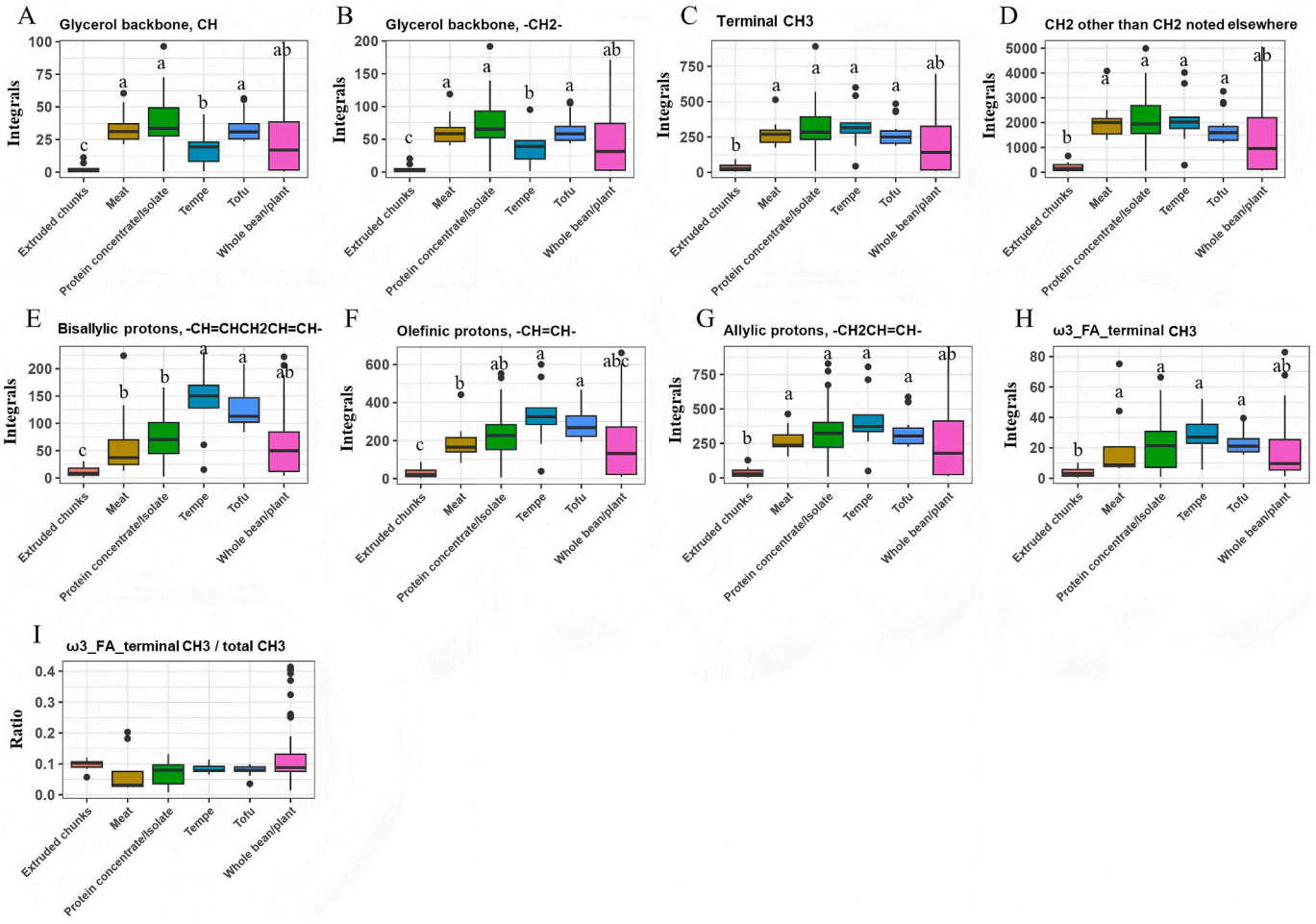

Fig. 5. Box plots of bin integrals of identified lipid signals from ^{1}H NMR. Different lower-case letters indicate statistical significance according to one-way ANOVA followed by Tukey's post hoc test.

contained the lowest level of unsaturated lipids.

The lipid content in wheat, soy, oats, peas, chickpeas, favabeans, and rice (mainly belonging to more processed products) was similar to the meat controls in general (Fig. S5A-B and Fig. 3A). Sunflower seeds, wheat, soy, fava beans, oats, peas, and chickpeas contained more unsaturated fatty acids and omega-3 fatty acids (signals from olefinic protons, bisallylic protons, allylic protons, and ω-3 FA terminal CH_3) than the rest of the plant products.

3.4. Limitations and future perspectives

A key limitation of this study is the inherent confounding between processing degree, raw material, and product formulation in commercially available products. For instance, protein concentrates/isolates are predominantly derived from soy or pea, while tempe is almost exclusively made from whole beans. Future studies using controlled processing of identical raw materials would be necessary to isolate the specific effects of processing technology from those of raw material composition.

Another limitation relates to sample preparation: products were prepared according to package instructions rather than under standardized cooking conditions. While this reflects real-world consumer practices, it introduces additional variability that may affect certain heat-labile metabolites differently across products.

4. Conclusion

This study provides a comprehensive analysis of the biochemical composition of PBPR foods using ^{1}H NMR metabolomics. The findings highlight that the biochemical profiles of PBPR foods reflect the combined effects of raw material, formulation, product type, and processing technology. Soy and peas are the most widely used protein sources, with protein concentrates/isolates and whole beans/plants being the predominant product types. Products classified as highly processed (protein concentrates/isolates) were characterized by higher levels of simple sugars (notably glucose), fatty acids, and Maillard reaction products (e.g., HMF). In contrast, less processed products (whole bean/plant and extruded chunks) were characterized by higher levels of asparagine. Fermentation (as in tempe) was associated with substantially higher levels of free amino acids—particularly lysine, threonine, and 4-aminobutyrate—as well as nicotinate and trehalose. Concurrently, the lipid profile of tempe showed a shift toward putative free fatty acids and phospholipids, underscoring its dual benefits for enhancing nutrient bioavailability and functional properties, and positioning tempe as an outstanding option among PBPR foods. These compositional differences may influence sensory attributes and, more importantly, modulate potential health implications.

CRediT authorship contribution statement

Kang Chen: Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis. **Jasmin Raita:**

Writing – review & editing, Methodology, Investigation, Formal analysis. **Maaria Kortesniemi:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Zhengxuan Jiao:** Writing – review & editing. **Baoru Yang:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Kati Hanhineva:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2026.150648.

Data availability

Data will be made available on request.

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