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Protein Digestibility and Iron Bioaccessibility of Plant-Based Meat Analogues

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Abstract

Understanding the nutritional quality of plant-based meat alternatives (PBMAs) is important for consumers, manufacturers, and health professionals. This study examined nine commercial PBMAs, focusing on protein digestibility and iron dialyzability. *In vitro* protein digestibility ranged from 81–96%, comparable to meat (86–90%), and was highest in protein concentrates and isolates (97–99%). Extrusion conditions (150–750 rpm, 100–160 °C) had minimal impact on digestibility but significantly affected texture and sensory properties. Dialysable iron in PBMAs was lower (2–5%) than in fungi-based products (15–32%) and meat (2–40%), though PBMAs had higher total iron content. Iron fortification using ferrous citrate, fumarate, sulphate and ferric pyrophosphate yielded dialysable iron values of 1.9–3.4%. These findings provide valuable insights into the nutritional composition of PBMAs and highlight opportunities to optimize processing for improved iron bioavailability.

Keywords:

Plant-based meat alternative; Digestibility; Protein; Iron; Extrusion

1.0 Introduction

Plant-based meat alternatives (PBMAs) are defined as plant-derived products that mimic conventional meat in appearance, texture, taste, and colour, without containing animal-derived ingredients (C. Sun, Fu, Chang, Li, & Fang, 2022). The growing interest in PBMAs is reflected in their rapid market expansion, with projections estimating the sector's value at USD 21 billion by 2030, representing approximately 9% of the global meat market share (Andreani, Sogari, Marti, Froldi, Dagevos, & Martini, 2023; OECD/FAO, 2021; Zhao, Wang, Hu, & Zheng, 2023). This interest is driven by multiple factors, including the increasing awareness of the ethical and environmental impacts of meat production, as well as the perceived nutritional benefits of a plant-based diet (Ferreira, Sharma, & Zannad, 2021; González, Marquès, Nadal, & Domingo, 2020; Papier et al., 2021; Willett et al., 2019). Ideally PBMAs would be able to mimic the taste of animal-derived products while offering similar or improved nutrition. Ensuring PBMAs deliver key nutrients at levels comparable to meat is essential to avoid nutrient deficiencies; in this context, the availability of nutrients like protein and essential micronutrients like iron, zinc and B vitamins is particularly important as meat is known to be a rich source of these compounds (N. W. Smith, Fletcher, & McNabb, 2022).

The quality of protein found in a food depends on both its amino acid profile and its digestibility; this can be quantified using the Protein Digestibility Corrected Amino Acid Score (PDCAAS) (WHO/FAO/UNU, 2007). Here, the amino acid score is determined by comparing the quantity of essential amino acids to a reference pattern of essential amino acids to determine the amino acid score; this number is then multiplied by the digestibility of the protein to give the final PDCAAS. Animal-derived products typically have high amino acid scores, while cereals are typically limited by the availability of lysine and legume proteins are limited by the availability of cysteine and methionine. Processing plant-based products can change the amino acid score; for example, the calculated score for raw soybeans is 0.96, while it is 1.12 for soy protein isolate. For comparison, calculated values for chicken breast and bacon are of the order 1.3 (values are from the USDA database (USDA, 2025)).

The digestibility of a protein depends on the structure of the protein and the matrix in which it is found. It is generally thought that the digestibility of proteins in plant-based sources is lower than that for animal products, this has been attributed to both the difference in protein structures (Diao et al., 2025) as well as the presence of compounds such as phytate and polyphenols that complex proteins and inhibit digestive enzymes (Amin, Petersen, Malmberg, & Orlien, 2022; Gilani, Xiao, & Cockell, 2012; Joye, 2019). Legumes are known to contain protease inhibitors; these can interact with digestive enzymes and thereby reduce the rate of protein hydrolysis and hence uptake. Polyphenols (e.g. tannins) reduce protein digestibility through a different mechanism; in this case they bind to the protein and reduce its solubility, this can lead to a reduction in in the rate of enzymatic hydrolysis. The role of phytate is more complex, it can reduce the activity of digestive enzymes by chelating necessary cofactors, or through interactions with either the enzyme or protein substrate (Gilani et al., 2012). Processes involved in the production of plant protein isolates, such as air classification and alkaline extraction of proteins from plant sources, can increase protein digestibility by reducing the content of antinutrients in the isolates (Amin et al., 2022). Processes such as alkaline extraction also reduce the content of insoluble proteins, making proteins more digestible (Kumagai et al., 2006; Z. Zhang et al., 2019).

The majority of PBMA products are produced by extrusion cooking, using protein isolates or concentrates as the initial ingredients. The extrusion cooking process employs a combination of temperature, pressure and shear to convert ingredients into a meat-like structure. Various processes occur inside the extruder, including denaturation of the proteins, aggregation, and the formation and breaking of chemical bonds (Boukid, 2021; McClements & Grossmann, 2021; M. Singh et al., 2021; Zahari, Östbring, Purhagen, & Rayner, 2022). The main parameters that influence the final product are the temperature (a maximum of 140-180 °C), screw speeds (ranging from 300-1200 RPM), the feed rate, and the moisture content (55-75%) (Dinali et al., 2024). During this process, the digestibility of the proteins can be affected by changes to the structure of the protein, breakdown of antinutritional factors, oxidation of the proteins and reactions between amino acids and other compounds present (e.g. the Maillard reaction) (Mosibo,

Ferrentino, Alam, Morozova, & Scampicchio, 2022). As yet, there is little information in the literature about the digestibility of PBMAs and how this is affected by processing conditions; such information is essential in evaluating the nutritional characteristics of PBMAs.

In addition to being a source of protein, meat is a good source of essential minerals, with iron being one of the most important. Published data indicates that iron deficiency anaemia affects approximately 3% of the UK population, with pregnant women being disproportionately impacted, accounting for 10–20% of cases (M. Smith et al., 2024; Snook et al., 2021). It is worth noting that the prevalence of iron deficiency is 4–29% higher among vegetarians and vegans, highlighting the need for careful monitoring of iron intake in these populations (Pawlak, Berger, & Hines, 2018; Slywitch, Savalli, Duarte, & Escrivão, 2021; Waldmann, Koschizke, Leitzmann, & Hahn, 2004).

The nutritional quality of food depends on both the concentration of nutrients and how well they are absorbed during the digestion process. In meat, iron is found in the haem form, which is present in myoglobin and haemoglobin (Hooda, Shah, & Zhang, 2014; Lynch & Cook, 1980); in this form, iron is absorbed relatively efficiently (Perera et al., 2023; Piskin, Cianciosi, Gulec, Tomas, & Capanoglu, 2022). Conversely, PBMAs contain non-haem iron, which is often bound to other components in the plant matrix (e.g. phytate and phenolic compounds) (Hooda et al., 2014; Lynch et al., 1980). These compounds hinder iron absorption by forming insoluble complexes (Hooda et al., 2014; Lynch et al., 1980).

While *in vivo* methods exist to quantify the absorption of iron in humans, these are generally expensive, time-consuming and challenging from an experimental perspective (Dias, Costa, Nutti, Tako, & Martino, 2018). Animal studies share many of the same challenges, with the additional consideration that they may not reliably represent human metabolism. It is for this reason that *in vitro* approaches are widely used; these offer a way of assessing the iron availability in a quicker and more cost-effective fashion. *In vitro* approaches can be broadly divided into two categories; those which evaluate the solubilisation and diffusion of iron (i.e. dialysis-based methods), and those which include

a cell culture step which includes uptake of the iron by enterocytes. A key step in the uptake of iron is the enzyme catalysed reduction of iron(III) to iron(II) which is then transported across the cell membrane (Sawai, 2025), cell culture based approaches have the advantage of accounting for this. Both dialysis based and cell culture models do not fully model the physiology of iron uptake (Dias et al., 2018; Glahn, 2009), which means their predictions of bioavailability do not necessarily align with *in vivo* studies. However, it has been shown (Bohn et al., 2018) there is a good correlation between the *in vitro* and *in vivo* methods, meaning the *in vitro* methods can be used as screening tools to compare different iron sources and processing conditions.

There is relatively little published data about the bioavailability of iron in PBMAs and how the processing affects this; again, such information is key in evaluating their nutritional quality. Recent work in this area using cell culture based methods has reported varied results, with one study (Kose, de Bie, Wang, Eilander, Wanders, & Sharp, 2025) finding that the iron bioavailability was higher in animal based mince compared to the plant-based alternative, while another study (Li, Luo, Wong, Zhang, Xiao, & McClements, 2025) found that plant-based burgers had a higher iron bioavailability than the meat-based alternative, while others Latunde-Dada et al. (2023) reported that the iron bioavailability in a range of PBMAs was generally comparable to animal-based burgers. Based on these results, there is a clear need to further examine the nutritional properties of PBMAs available on the market.

Therefore, this study aimed to quantify the nutritional quality of commercially available PBMAs, particularly concerning the protein digestibility and iron content and availability. The effect of the processing conditions on these parameters was also examined, in order to understand to what extent they are affected by the processing conditions. Findings from this research are expected to contribute to a deeper understanding of the nutritional properties of PBMAs and provide valuable insights for both consumers and manufacturers.

2.0 Materials and Methods

Nine commercially available plant-based meat alternatives (PBMAs), along with wholegrain soybean, pea, and fava bean flours, were obtained from Tesco, a major UK supermarket chain. For comparative analysis, two meat-based bacon products and one chicken product were also procured from the same retailer. Soy protein concentrate, pea protein, fava bean protein, soy textured vegetable proteins (TVP) and pea TVP, ferric pyrophosphate, ferrous citrate, ferrous fumarate and ferrous sulphate were supplied by THIS. The composition of the procured samples is summarised in Table 1 based on the nutritional information provided on the respective product labels.

All enzymes and analytical reagents used in this study were purchased from Merck Great Britain (Gillingham, UK), except items provided in the Megazyme *in vitro* protein digestion pack, which was purchased from Megazyme Global (Wicklow, Ireland).

Dialysable iron was determined using dialysis tubing Spectra/Por 7 (20.4 mm diameter) with a molecular weight cut-off of 10 kDa (Scientific Laboratory Supplies, Nottingham, UK).

All the samples were frozen and stored at -20 °C before being blended in a Model BB340S blender (Waring Commercial, Connecticut, USA) to achieve a particle size of approximately 500-1000 µm. Two separate packs for each sample were combined and blended; these samples were stored at -20 °C before analysis.

2.1 Determination of Moisture Content

A 5 g sample was placed in a pre-weighed moisture tin, and its mass was determined with precision using a single-pan digital balance (ABT 220-5DNM, Kern and Sohn, Balingen, Germany) with a sensitivity of 0.0001 g. The sample was then dried in an electric oven (HAS 75F, Genlab, Widnes UK) set to a temperature of 105 ± 1 °C overnight. After drying, the sample was removed from the oven, allowed to cool in a desiccator to prevent moisture absorption, and subsequently reweighed to determine its moisture content.

Table 1: Nutritional information declared on the food label of commercially available PBMA products

Product	Protein Ingredients	Protein (g/100 g)	Carbohydrate (g/100 g)	Fat (g/100 g)	Iron (mg/100 g)	Iron Fortificant Added on Ingredient List	Fibre (g/100 g)	Salt (g/100 g)
Grilled chicken breast	Animal-Chicken	21.40	2.40	1.30	Not reported	Not Applicable	0.00	0.54
Unsmoked bacon rashers	Animal-Pig	16.20	0.30	14.80	Not reported	Not Applicable	0.50	2.80
Smoked bacon rashers	Animal-Pig	17.90	0.30	14.40	Not reported	Not Applicable	0.50	2.80
Plant-based chicken #1	Soy Concentrate, Fava Isolate	22.00	1.80	3.50	3.20	Yes	6.40	0.75
Plant-based chicken #2	Soy Concentrate, Gluten	16.00	3.30	7.20	2.30	No	4.80	0.90
Plant-based bacon	Soy Concentrate, Soy Isolate, Pea Isolate	13.50	8.10	9.60	1.90	Yes	3.40	1.50
Plant-based streaky bacon #1	Soy Concentrate, Gluten	15.00	3.90	10.00	Not reported	Yes	5.20	2.80
Plant-based streaky bacon #2	Soy concentrate	14.00	7.90	15.00	Not reported	No	3.30	2.00
Plant-based smoked bacon	Soy Concentrate, Soy Isolate, Pea Isolate	25.00	14.00	1.20	3.80	No	5.20	2.70
Plant-based sausages	Soy Concentrate	8.70	12.00	6.00	Not reported	No	6.40	1.50
Fungi-based meat-free pieces	Mycoprotein	14.00	1.70	2.60	Not reported	No	7.10	0.79
Fungi-based vegan pieces	Mycoprotein	15.00	2.10	1.40	Not reported	No	6.90	0.40

2.2 Determination of Protein Digestibility

The protein quality of the samples was evaluated using the *in vitro* Protein Digestibility-Corrected Amino Acid Score (PDCAAS) methodology (Megazyme K-PDCAAS 12/19), incorporating modifications to the manufacturer's protocol (Megazyme, Wicklow, Ireland). Samples were analysed in duplicate on two separate days, resulting in four replicates per measurement.

Samples (1 g) were combined with 19 mL of 0.06 N HCl, followed by vortex mixing and incubation at 37 °C for 30 minutes at 300 rpm. Subsequently, 1 mL of pepsin solution (1 mg/mL) was added, and the mixture was vortexed and incubated for 1 hour at 37 °C and 300 rpm. The pH was then adjusted to 7.4 using 2 mL of 1.0 M Tris buffer. To this 200 µL of a trypsin/chymotrypsin solution (5 mg/mL) was added, and the samples were incubated at 37 °C and 300 rpm for a further 4 hours. Enzymatic activity was terminated by heating the samples in a boiling water bath for 10 minutes. Following this, the samples were vortexed and allowed to cool to room temperature and the mixture was incubated overnight at 4°C. The samples were centrifuged at 22,000 g for 10 minutes, and the resulting supernatants were diluted 10-20 fold in acetate buffer (50 mM, pH 5.5) for subsequent colourimetric analysis.

The concentration of α-amino acids was determined via a ninhydrin assay, employing *L*-glycine as the calibration standard. A 12-point calibration curve was prepared in the range 0-2 mM. Absorbance was measured at 570 nm using a Shimadzu UV 1800 spectrophotometer.

The concentration of the primary amino acids per mass of sample (*C*) was determined from the calibration curve, and corrected for the dilution factor (*D*) and the sample weight (*W*):

$$C = \frac{D}{W} \times \frac{(A_{570} - b)}{m} \qquad (1)$$

Where A_{570} is the measured absorbance at 570 nm, and *m* and *b* are the gradient and intercept of the calibration curve, respectively. The corrected amine concentration (*CN*) was then determined:

$$CN = C + \frac{20[Proline]}{5[Lysine]} + 2[Histidine] + 2[Arginine] \qquad (2)$$

The corrected amine concentrations (*CN*) were then used to determine the *in vitro* digestibility using a correlation provided by the supplier. Amino acid concentrations provided by the supplier were used in Equation (2).

2.3 Sample extrusion and analysis

A co-rotating twin-screw extruder was used to produce high moisture PBMAs. The heating barrels were set to a temperature between 30 °C and 160 °C, to achieve the maximum temperature employed in the production of PBMAs. Water was fed into the system at a dosing rate of 4.2-4.6 L h^{-1} (to obtain a final barrel moisture content of between 62-67%), and the powder feed rate was 2.6 kg h^{-1}. A cooling die was attached at the end of the heating barrels and set to a temperature between 50 °C and 80 °C. The cooling die had an opening of 8 mm × 40 mm, and the screw speed was varied between 150 and 750 rpm. The extrudates were sealed in moisture-proof bags to prevent moisture loss and stored at -20 °C before analysis.

To quantify the effect of the extrusion conditions on the texture, protein digestibility and iron dialysability, experiments were performed by varying the screw speeds and maximum temperature of the barrel using the recipe of the commercial product “THIS isn’t chicken pieces” (supplied by THIS™, UK). It contains predominantly soy protein (28%) and 2% or less of natural flavouring, fava protein, rapeseed oil, potato starch, pea fibre, and calcium sulphate. The extrusion was performed at screw speeds of 150, 550 and 750 rpm and maximum temperatures of 100, 130 and 160 °C while maintaining a constant powder feed rate and rate of water addition.

2.4 Texture Analysis

A TA.XTPlusC Texture Analyser from Stable Micro Systems (Godalming, UK) with a load cell of 50 kg was used to measure the penetration force of the extruded material. The testing consisted of a 5 mm cylindrical probe compressing at the centre of the 40 mm wide piece of extruded material. The probe moved at a speed of 2 mm s^{-1} and compressed to 90% of the sample's original height. The peak force was calculated as the largest force during the test, which occurred at 90% strain for all of the samples. Materials were defrosted and brought to room temperature (approximately 20 °C) prior to analysis. Each measurement was repeated six times; the reported results are the average and error bars show one standard deviation about the mean.

Bending analysis of the samples was performed within one minute after exit from the extruder, while the sample was still hot. The extruded material was folded over itself, crossways, to create a double layer. Photographs of the sample were then taken to allow visualisation of the internal structure.

2.5 Iron analysis

Acid digestion of the samples was performed according to the EPA method 3051A (U.S.EPA, 2007) using yttrium as an internal standard and elemental iron as an external calibration standard. Solid samples (~1.5 g) with 50–60% moisture content were weighed into crucibles, dried overnight at 105 °C, and cooled. Dried samples were finely ground using a mortar and pestle, with equipment cleaned (Milli-Q water and HNO_3) between samples to prevent contamination. Approximately 0.5 g of each dried sample was digested using a CEM MARS 6^{TM} (North Carolina, United States of America) microwave digestion system. Each sample was mixed with 4 mL Milli-Q water, 1 mL 30% H_2O_2, 4 mL 68% HNO_3, and yttrium internal standard to achieve 1 mg/L yttrium post-dilution. Samples were pre-digested for 15 minutes under a fume hood before sealing the vessels. The samples were heated to a temperature of 200 °C using a controlled multistage ramp composed of a 15-minute increase in temperature to 200 °C, held at 200 °C for 15 minutes and then cooled for 10 minutes. Digests were transferred to 15 mL centrifuge

tubes, centrifuged at 2000 × *g* for 10 minutes, and diluted by transferring 5 mL of supernatant into 10 mL Milli-Q water.

The iron content in the samples was quantified using inductively coupled plasma-optical emission spectrometry (ICP-OES, PerkinElmer Optima 7000DV). The Method Detection Limit (MDL), defined as the minimum concentration of an analyte that could be identified, measured, and reported with 99% confidence at an analyte concentration that is greater than zero, was determined to be 3 µg/L. A standard iron solution (purchased from Thermo Fisher Scientific) was prepared at concentrations of 0.0075-5 mg/L (10 standard points) to obtain a standard calibration curve with an R^2 of at least 0.9995. The yttrium standard and iron analyte were detected at wavelengths of 371.03 nm and 238.20 nm, respectively.

The measured values were converted to an as-is basis using the previously determined moisture content.

2.6 Iron dialysability analysis

An *in vitro* assay was performed to quantify the dialysability of iron according to the methodology of Miller, Schricker, Rasmussen, and Van Campen (1981). Here, a two-stage process is used that mimics digestion in the stomach and iron uptake in the intestine. A macerated sample (12 g) was combined with 30 g of deionised water in a 250 mL Erlenmeyer flask. The pH of the mixture was adjusted to 2.0 using 6 M HCl, and pepsin was added to achieve a final activity of 2000 U/mL in the digestion mixture. The sample was then diluted to a final weight of 60 g with deionised water and incubated at 37°C for 120 minutes in a shaking water bath set at 200 rpm. Following incubation, 12 g aliquots of the gastric digest were withdrawn for subsequent intestinal digestion and titratable acidity analysis. For titratable acidity determination, a 12 g aliquot of the gastric digest was mixed with a pancreatin (10 mM) bile extract solution formulated to achieve a final pancreatin activity of 2000 U/mL in the digestion mixture. The pH was incrementally raised to 7.5 by titration with 0.5 M NaOH, and the volume of NaOH required was

recorded. This value was subsequently used to calculate the molar equivalent of sodium bicarbonate ($NaHCO_3$) necessary for the intestinal phase.

A 12 g aliquot of the gastric digest was combined with the pancreatin-bile extract solution prepared as described above, ensuring a final pancreatin activity of 2000 U mL^{-1}. Dialysis tubing (20 cm long) containing 15 g of a $NaHCO_3$ solution, formulated according to the previously calculated NaOH molar equivalent, was added to the mixture. The tubing was securely sealed with clamps, and the system was incubated at 37°C in a water bath for 2 hours. After incubation, the contents of the dialysis tubing were carefully transferred into 15 mL centrifuge tubes. The dialysates were acidified with 0.5 mL of concentrated nitric acid (68% HNO_3) to stabilise the mineral content and were subsequently stored at -20°C until further analysis.

The iron content in the dialysates was measured using ICP-OES using the method described previously. This was used to determine the percentage of dialyzable iron:

$$Mass\ of\ dialyzable\ iron = \frac{V_{dialysate}[Fe]}{0.2m_{sample}} \qquad (3)$$

where the volume of the dialysate ($V_{dialysate}$) was calculated using the measured mass of the dialysate and a density of 1 g/mL; [Fe] is the iron concentration measured using ICP and m_{sample} is the sample mass. The percentage dialyzable iron was found by dividing the mass of dialyzable iron by the total iron content:

$$\%D\text{ialyzability} = \frac{Mass\ of\ dialyzable\ iron}{Total\ iron\ content} \times 100 \qquad (4)$$

The contribution to the Total Absolute Requirement (TAR) for iron was calculated by multiplying the iron content (on an 'as is' basis) by the dialyzability (as a proxy for absorption):

$$\%\,TAR = \frac{Iron\ content\ \times \%D\text{ialyzability}}{1.68} \qquad (5)$$

The value of 1.68 mg per day is the TAR for young women (FAO/WHO, 2001).

To investigate the effect of changing the iron source four compounds (ferrous sulphate, ferrous fumarate, ferric pyrophosphate and ferrous citrate) were examined). These were added to either starch (as a blank) or the mixture used to prepare plant-based chicken to

a final concentration of 0.1% (w/w). The formulation was then mixed with water at a rate of 30:70 w/v (formulation: water), transferred into a heat-stable Ziplock bag and heated in a shaking water bath at 90 °C for 1 hour.

2.7 Statistical Analysis

Unless stated otherwise each experiment was performed three times, and one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test was used to compare samples. GraphPad Prism version 10.4.1 (GraphPad Software, Boston, Massachusetts, USA) was used to perform the statistical analysis.

3.0 Results and Discussion

3.1 In vitro Protein Digestibility

Figure 1 shows the *in vitro* protein digestibility values for the products examined. The digestibility of wholegrain soy, pea and fava flours was 88%, 84% and 87%, respectively. These findings are consistent with previous studies, as summarised in Table 2. The protein concentrates demonstrated significantly ($p < 0.01$) higher digestibility (approximately 97%) than their wholegrain flour counterparts, as shown in Figure 1. As previously discussed, the processes used to produce the isolates and concentrates can reduce the content of antinutrients (like phenolics and phytate which interfere with protein availability and absorption) as well as solubilise insoluble proteins, thereby increasing the digestibility of the resulting concentrate or isolate (Amin et al., 2022; Kumagai et al., 2006; Z. Zhang et al., 2019). This is consistent with the results shown in Figure 1 where the plant-based concentrates and isolates were observed to have higher *in vitro* protein digestibility than the wholegrain flours.

Commercially available PBMAs examined displayed relatively high *in vitro* digestibility, with values ranging from 81-96% (Figure 1), in comparison, the values for the animal-based meats examined were 86-90% (Figure 1 and Table 2). The relatively high digestibility of the PBMAs examined is most likely attributable to the high digestibility of the protein concentrates and isolates used in their formulation. As shown in Figure 1, the

soy, pea and fava protein concentrates examined had digestibility values of 97-99%. It appears that the processing (extraction of the proteins into high protein ingredients and extrusion into meat analogues) of these ingredients to produce PBMAs leads to a small loss in digestibility (Figure 1). The reduction could be attributed to the addition of other ingredients and changes in the protein structure (e.g. denaturation, Maillard reactions) which occur during processing (Huelsebusch, Heyn, Amft, & Schwarz, 2025; S. K. Singh, Singha, & Muthukumarappan, 2019). It is noteworthy that this reduction is more pronounced in TVPs than in the high moisture extruded PBMAs. This could be attributed to the difference in processing employed; high moisture extrusion is employed in the production of PBMAs, while TVPs are produced using low moisture extrusion, where both processes result in the denaturation and unfolding of the protein structures (Schmid, Farahnaky, Adhikari, & Torley, 2022; Wang, Zheng, Zhang, Wu, Tan, & Liu, 2024). While both processes involve high temperature, shear and pressure, the high moisture used in the production of PBMAs results in the aggregation of the protein structures at a micro and macroscopic level, a phenomenon absent in the production of TVPs, which may explain the differences in the digestibility.

It is important to note that the method employed to assess protein digestibility in this study was conducted *in vitro* and does not fully reflect *in vivo* physiological processes. With this in mind it was found that that the protein digestibility of PBMAs is comparable to that of conventional meat.

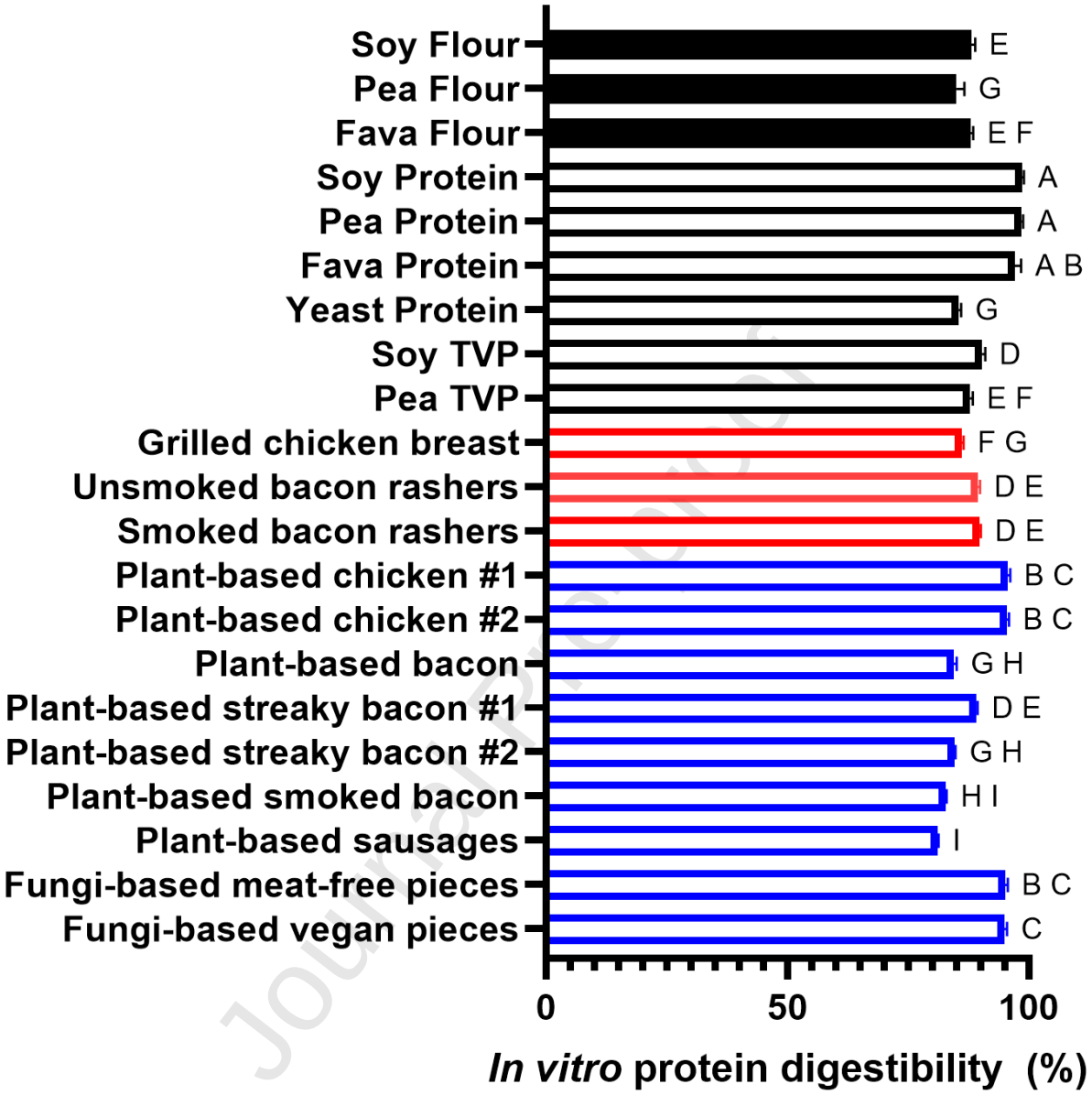

Figure 1 – Results of the *in vitro* protein digestibility experiments. Reported values are the average of four replicates, with the error bars denoting one standard deviation about the mean. Results with different letters are significantly ($p < 0.01$) different. Statistical analysis was performed using one-way ANOVA ($F(20,63) = 257.9$, $p < 0.0001$); Tukey's test was used for multiple comparisons; results with different letters are significantly ($p < 0.01$) different.

Table 2 - *In vitro* protein digestibility and total iron content of whole grains used as protein sources in plant-based meat alternatives (PBMAs) in this study, compared to values reported in the literature

Sample	Analysed In Vitro Protein Digestibility (%)	Reported In Vitro Protein Digestibility (%)	Reference	Analysed Iron Content (mg/100 g)	Reported Iron Content mg/100 g	Reference
Soy Flour	88	89	(Berno, Guimaraes-Lopes, & Canniatti-Brazaca, 2007)	7.38	9-16.6	(Ibáñez, De Blas, Cámara, & Mateos, 2020)
		83	(Ojokoh & Yimin, 2011)		6.16-7.31	(Lestienne, BESANCon, Caporiccio, Lullien-Péllerin, & Tréche, 2005)
		85	(Ogodo, Ugbogu, Onyeagba, & Okereke, 2018)			
Fava Flour	88	78	(Alonso, Aguirre, & Marzo, 2000)	1.99	1.5-6.7	(Dhull, Kidwai, Noor, Chawla, & Rose, 2022)
		80	(Elsheikh, El Tinay, & Fadul, 1999)		1.8-21.3	(Labba, Frøkiær, & Sandberg, 2021)
		94	(Ayala-Rodríguez, López-Hernández, Lomelí, González-Martínez, & Vázquez-Rodríguez, 2022)		4.18	(Millar, Gallagher, Burke, McCarthy, & Barry-Ryan, 2019)
Pea Flour	85	82	(Linsberger-Martin, Weiglhofer, Phuong, & Berghofer, 2013; G. Sun et al., 2023)	3.08	3.92	(Millar et al., 2019)
		84	(Ma, Boye, & Hu, 2017)		2.6-3.9	(Khan et al., 2015)

Soy Protein	98	91-95	(Scottá, Albino, Rostagno, Gomide, Campos, Vieira, & Demuner, 2013)	11.4	10.4-13.9	(Auer, Alminger, Marinea, Johansson, Zamaratskaia, Högberg, & Langton, 2024)
Fava Protein	97	88-92	(Njeri, Nu, Schulze, & Kiarie, 2024)	34.4	8.5-38.9	(Auer, Alminger, Marinea, Johansson, Zamaratskaia, Högberg, & Langton, 2024)
Pea Protein	98	76-89	(Njeri, Nu, Schulze, & Kiarie, 2024)	17.5	11.7-20.0	(Auer, Alminger, Marinea, Johansson, Zamaratskaia, Högberg, & Langton, 2024)

Sample	Analysed In Vitro Protein Digestibility (%)	Reported In Vitro Protein Digestibility (%)	Reference	Analysed Iron Content (mg/100 g)	Reported Iron Content mg/100 g	Reference
Grilled chicken breast	86	75-90	(Faber et al., 2010)	0.45	0.3-1.4	(Cross, Harnly, Ferrucci, Risch, Mayne, & Sinha, 2012)
					1.04	(USDA, 2025)
Unsmoked bacon rashers	89	70-90	(Bailey, Mathai, Berg, & Stein, 2020)	0.36	1.4-2.5	(Cross et al., 2012)
					1.4	(USDA, 2025)
Smoked bacon rashers	90	70-90	(Bailey et al., 2020)	1.33	1.4-2.5	(Cross et al., 2012)
					1.4	(USDA, 2025)

Building on the insights from commercial samples, we conducted experiments to evaluate how extrusion parameters influence the *in vitro* digestibility; results are shown in Figure 2. It was found that for the range of parameters examined, changing the extruder settings had a small but significant effect on the *in vitro* protein digestibility, with slightly higher values being observed when the temperature was above 130 °C and a screw speed greater than 550 rpm.

Changing the extruder settings had a much more pronounced effect on the mechanical properties of the samples, as shown in Figure 2 (b) and (c). It was found that at a temperature of 100 °C no fibres formed for all screw speeds examined (Figure 2 (c)) indicating that these settings cannot be used to produce a viable product. Unsurprisingly, increasing the temperature led to the samples becoming harder (Figure 2(b)), a conclusion in agreement with previous work (de Boer, Capuano, Kers, & van der Goot, 2025; Fu, Li, Zhang, Li, Zang, & Liu, 2024; Mateen, Mathpati, & Singh, 2023; Zhang, Chen, Kaplan, & Wang, 2022; Zhang, Liu, Liu, Yoon, Rizvi, & Wang, 2019).

It was found that the protein isolates and concentrates used as ingredients in the production of PBMAs have relatively high *in vitro* protein digestibility, and that the processing to form the products leads to a relatively small change in the digestibility (Figure 1). Changing the extruder settings leads to large changes in the product texture, while having a relatively small effect on the protein digestibility (Figure 2). Such results are of obvious interest as they demonstrate that manufacturers can vary the sensory properties of their products across a wide range without affecting the protein digestibility.

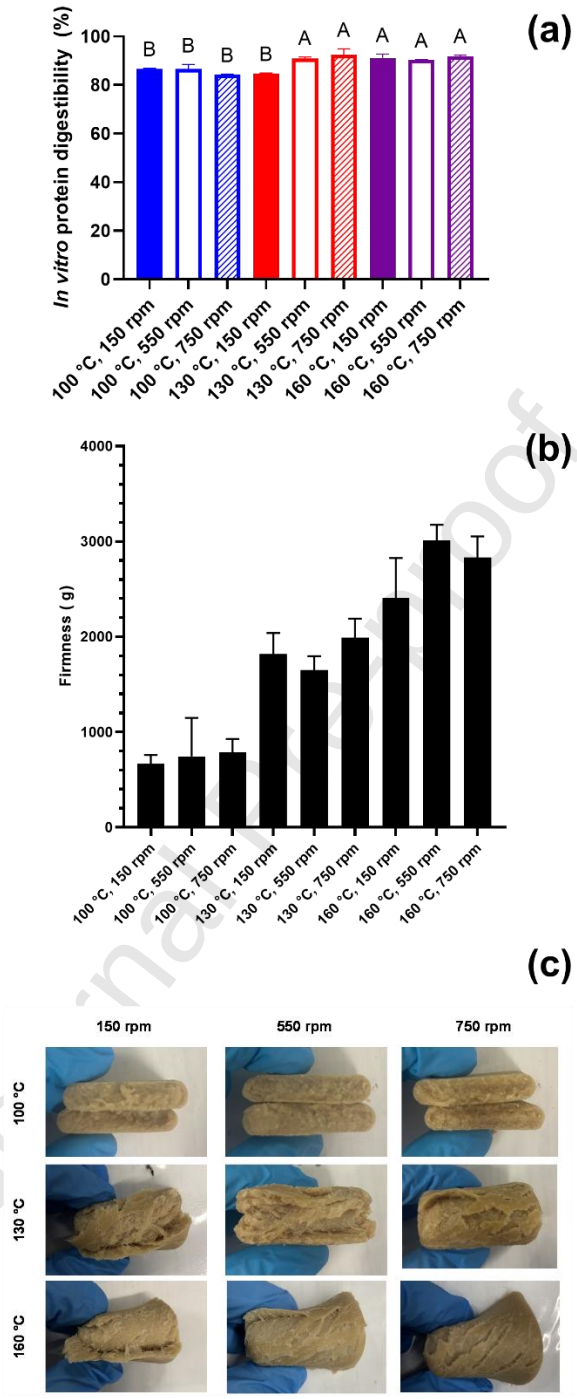

Figure 2 – Plot showing the effect of the extruder settings on (a) the *in vitro* protein digestibility (b) the firmness of the sample and (c) a cross-section of the extrudate. Statistical analysis was performed using one-way ANOVA for the in vitro digestibility ($F(8,27) = 29.37$, $p < 0.0001$). Tukey's test was used for multiple comparisons, results with different letters are significantly ($p < 0.01$) different.

3.2 Iron content and availability

Figure 3 shows the results from the analysis of the iron content of plant-based ingredients, commercial PBMA products, and meat products. A relatively wide range of iron contents (0.37 – 34 mg/100 g sample) was observed; values have been reported on an 'as is' basis (data about the moisture content for the samples is available in the Supplementary Material). A comparison with literature values for iron content has been provided in Table 2; it was found that values determined in this work agree with those previously published, with the exception of the unsmoked bacon, where the measured value was less than the published data.

It was found that the soy, pea and fava protein isolates had significantly ($p < 0.0001$) higher iron concentrations (11.4, 17.5 and 34.4 mg/100 g respectively) than the wholegrain products examined, suggesting that the processing steps involved enriched the protein isolates in iron. This result is consistent with the published literature (Table 2).

Interestingly, the plant-based meat products typically had higher iron contents (0.44 – 7.99 mg/100 g) than the animal-derived products examined (0.37 – 1.33 mg/100 g). The higher iron content in PBMAs in this study is consistent with previous findings that reported a higher iron content in soy-based burgers when compared to traditional beef burgers (Latunde-Dada et al., 2023). In some instances this is likely due to the products being fortified with iron (a full list of ingredients for the products tested is supplied in the Supplementary Material).

Beyond the total iron content, the physiologically relevant value is the amount of iron which is bioavailable and can be effectively absorbed. The percentage of dialysable iron was used as an *in vitro* proxy for iron bioavailability, with results being shown in Figure 3.

Interestingly, smoked bacon had the lowest value (2%). This may be attributed to the nitrites used in curing that can bind iron and significantly reduce its bioaccessibility (Parthasarathy & Bryan, 2012; Shakil et al., 2022). The observed differences in dialyzability between the plant-based and traditional meat products are most likely due to the different forms of iron present. The haem-iron found in animal sources is understood to be more bioaccessible than the non-haem iron found in plants (Bastide, Pierre, &

Corpet, 2011; Cross et al., 2012; Zielińska-Dawidziak, Białas, Piasecka-Kwiatkowska, Staniek, & Niedzielski, 2023). Additionally, plants are known to contain compounds such as tannins, flavonoids and phytic acid, which are known to impede mineral absorption (Sá, Moreno, & Carciofi, 2020).

Generally speaking, the percentage of dialysable iron was lower (2-5 %) for the plant-based products; the products produced using mycoprotein had comparable (15-32 %) values to the traditional meats (2-40 %). Higher bioaccessibilities (70-90 %) have been reported for PBMAs by previous authors (Latunde-Dada et al., 2023; Li et al., 2025). The disparity between results is most likely due to differences in the methodology used; here we have used dialysis tubing to model iron uptake, whereas other studies have used the solubilization of iron as a proxy for its availability during digestion.

Figure 3(c) shows the contribution of a 100 g serving of each of the products to the Total Absolute Requirement (TAR) of iron; here, a value of 1.68 mg per day was used (FAO/WHO, 2001). With the exception of the unsmoked bacon, all of the products examined contributed between 8-15% of the TAR. Interestingly, the PBMAs examined tended to have a relatively high iron content but low dialysability, with the opposite being found for the meat; it was found these factors compensated for one another, leading to comparable contributions to the TAR.

Here, it must be noted that the *in vitro* method used does not fully replicate the complex processes which happen *in vivo.* Specifically, the method used here accounts for solubilisation and diffusion of the iron, but it does not account for uptake by enterocytes and subsequent transport steps. As previously discussed, iron dialysability correlates well with *in vivo* uptake, however care must be taken to use the values as indicators of relative, and not absolute iron availability (Bohn et al., 2018). *In vitro* methods do have the advantage of being cheaper, faster and having less ethical challenges than *in vivo* work; having a reliable and standardised method for quantifying the availability of iron (and other metals) would undoubtedly be beneficial in analysing the nutritional properties of PBMAs (Etcheverry, Grusak, & Fleige, 2012; Sulaiman, Givens, & Anitha, 2021).

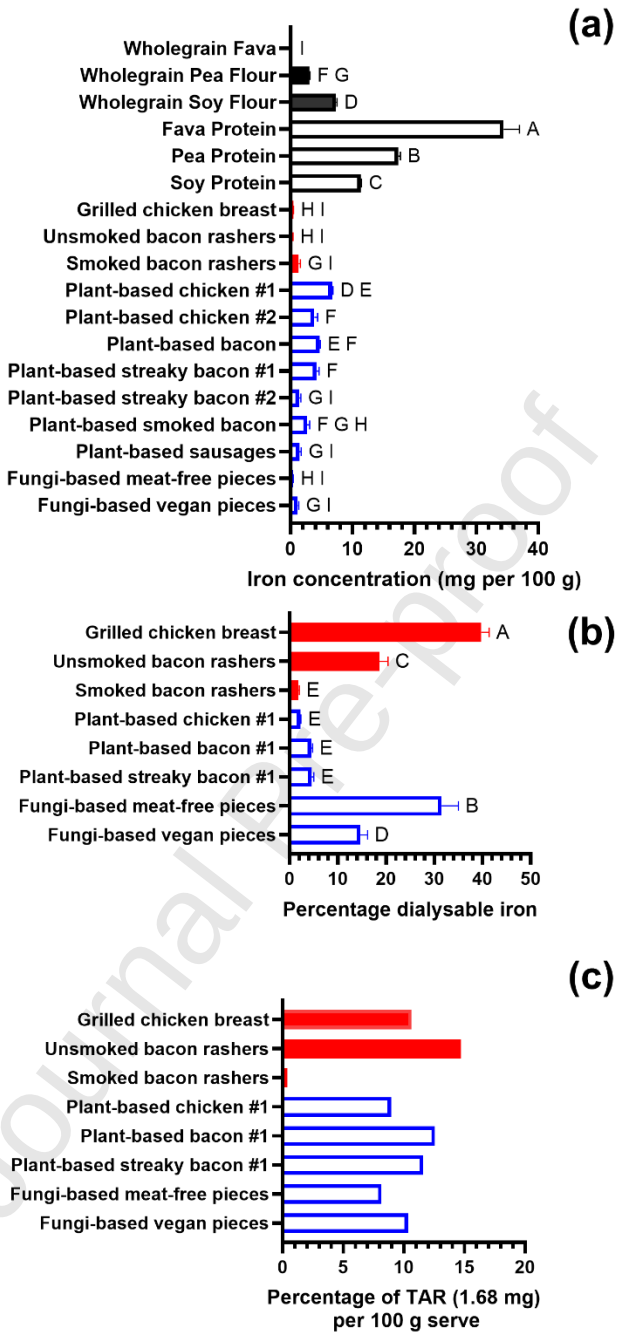

Figure 3 – Plot showing (a) the iron concentration per 100 g of sample (on an as is basis); (b) the percentage dialysable iron and (c) the contribution of a 100 g serving to the TAR. Results in (a) and (b) are the average of three replicate measurements with error bars denoting one standard deviation about the mean. Average values are shown in (c). Values with different letters are significantly different ($p < 0.01$). Statistical analysis was performed using one-way ANOVA ($F(18,38) = 449.1$, $p < 0.0001$ for the iron concentrations and $F(7,64 = 724$, $p < 0.0001$ for the percentage dialysable iron), Tukey's test was used for multiple comparisons.

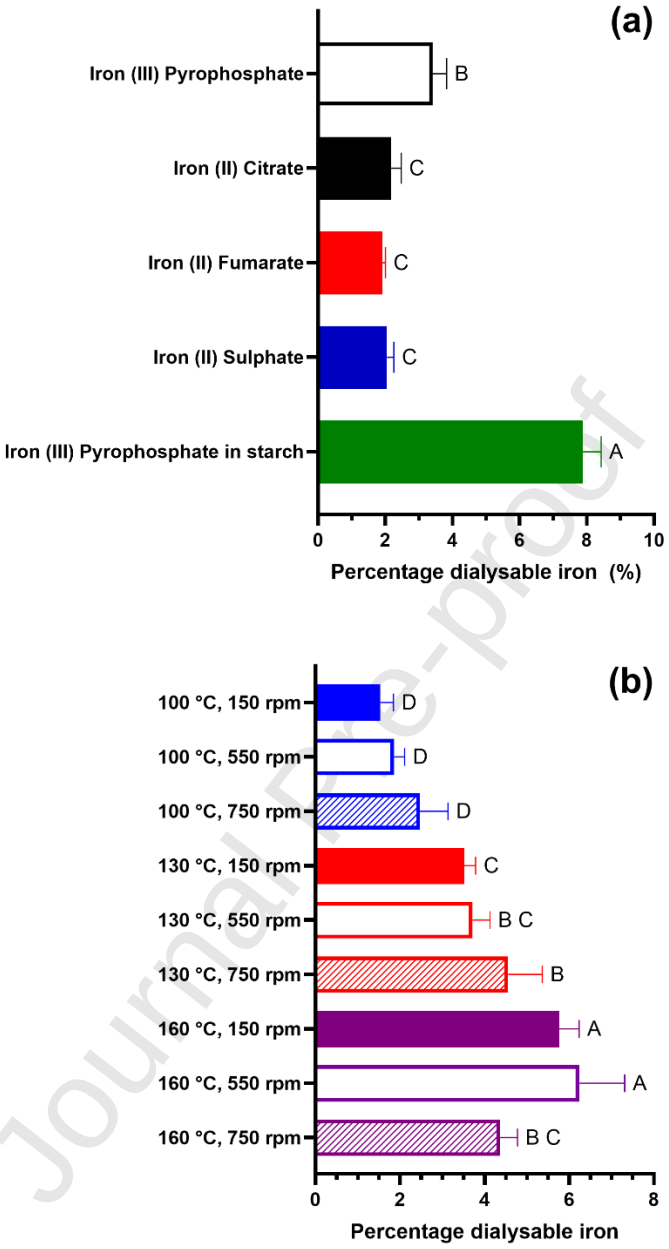

Figure 4 – Plot showing (a) the percentage of dialysable iron for the different fortificants evaluated and (b) the effect of the extrusion conditions on the percentage of dialysable iron. The fortificants have been evaluated in a PBMA matrix, aside from the final experiment where starch was used (Iron (III) Pyrophosphate in starch). Reported results are the average of nine data points, with the error bars denoting one standard deviation about the mean. Results have been compared using one-way ANOVA ($F(4, 40) = 472.5$, $p < 0.0001$ for the fortificants and $F(8, 72) = 72.07$, $p < 0.0001$) for the different extrusion conditions). Tukey's test was used for multiple comparisons; results with different letters are significantly ($p < 0.01$) different.

The effect of the processing parameters (temperature and screw speed) on the dialysability of the iron was also examined, with the results being shown in Figure 4. It was observed that increasing extrusion temperature and screw speed significantly ($p < 0.01$) increased the dialyzable iron with the exception of extrudates at 160 °C at a screw speed of 750 rpm. Compounds like phytate and polyphenols which are present in plant-based products are known to interfere with iron uptake through the formation of stable complexes with iron that reduce its uptake (Lubaale, Taylor, Emmambux, & Duodu, 2022; Ortiz-Cruz et al., 2020; Osuna-Gallardo et al., 2023), we speculate that the breakdown of these compounds may be the cause of the increase in dialyzable iron. An interesting avenue for further work would be to quantify the concentration of these compounds before and after processing in order to test this hypothesis.

PBMAs can be fortified with iron, which is typically added in the form of iron salts. Figure 4 shows the percentage of dialysable iron for the fortificants examined; these were evaluated using either starch or the PBMA premix as the matrix. It was observed that the matrix had a significant ($p < 0.01$) effect on the dialysibility, with starch matrix having a higher dialysability compared to PBMA matrix; this can most likely be attributed to the absence of compounds which bind to the iron and reduce its ability to diffuse across the membrane. It was found that there was no significant difference in iron dialysability between ferrous citrate, ferrous fumarate and ferrous sulphate, while ferric pyrophosphate was observed to have significantly ($p < 0.01$) higher dialysability. This contrasts with the literature, for example the WHO gives the relative bioavailability of ferric pyrophosphate as 21-74% of ferrous sulphate (World Health Organization, 2006). However, it is important to note that processing can have a significant effect on the bioavailability of ferric pyrophosphate. For example, processing increased the bioavailability of ferric pyrophosphate by approximately 2.4 fold in a soy-based infant formula and 1.7 fold in a dairy milk based infant formula (Theuer, Kemmerer, Martin, Zoumas, & Sarett, 1971; Theuer, Martin, Wallander, & Sarett, 1973). Contrastingly, processing was found to decrease the bioavailability of ferric pyrophosphate in chocolate drink powder by approximately 3.8 fold (Hurrell, Reddy, Dassenko, Cook, & Shepherd, 1991). It has been reported that heat treatment has a minimal effect on the bioavailability of ferrous sulphate

(Theuer et al., 1971). As the samples here were processed (via heating at 90 °C) this may explain why the ferric pyrophosphate was observed to have a higher dialysibility than the ferrous sulphate.

These results highlight the importance of evaluating the iron availability in foods after the normal processing has occurred. Having a more detailed mechanistic explanation for the effect of the processing conditions and matrix components on the percentage of dialysable iron would be valuable in the formulation of food products. Similarly, it would be it would be interesting to understand whether the addition of haem-iron sources to a plant-based matrix would lead to an increase in the percentage of dialysable iron.

4.0 Conclusions

Given the increasing uptake of PBMAs, it is important to understand their nutritional properties and how these can be affected by processing. In this work we have measured the protein digestibility and the iron concentration and dialysability of nine commercially available PBMAs along with three traditional meat products and the ingredients used to produce PBMAs.

It was found that the protein digestibility for the PBMAs was generally high (83-96%) and comparable to traditional animal-based meat (86-90%). This study found that for commercial PBMAs the protein digestibility is primarily determined by the digestibility of the ingredients used in the product formulation, while the extrusion parameters had a relatively minor effect for the conditions examined (see Figure 2). Contrastingly, it was found that varying the processing conditions had a significant effect on the firmness and texture of the product. The practical consequence of this is that manufacturers can vary the processing conditions (within the range examined) to obtain the desired product quality without causing a large change in the *in vitro* protein digestibility.

It was found the iron content in the PBMAs (0.44 – 7.99 mg/100 g) was generally higher than the animal-derived products examined (0.37 – 1.33 mg/100 g). Contrastingly, the iron dialysability (a proxy for uptake during the digestion process) was low (2-5%) for the PBMAs, with the exception of the mycoprotein based products (15-32%); values for the animal-derived products were 2-40%. The combination of these factors meant the contribution to the total absolute iron requirement was similar for both the PBMAs and animal-derived products. The non-haem sources of iron tested (ferrous sulphate, ferrous fumarate, ferrous citrate and ferric pyrophosphate) showed low dialysability and of those, the ferric pyrophosphate had the highest dialysability (3.4%). While the percentage of dialyzable iron from PBMAs is low, the iron content of PBMAs can contribute significantly to daily total absolute requirements for iron without fortification, provided the protein ingredients used are high in iron.

Results from this work suggest several areas for further investigation, for example *in vivo* studies to determine whether the bioavailability of iron in PBMAs can be increased by changes to the processing conditions, or by the development of novel, vegan iron sources. Similarly, it would be of interest to understand the content and availability of other essential nutrients known to be deficient in vegan diets (e.g. zinc, B vitamins, omega-3 fatty acids) in PBMAs.

In summary, this work evaluated a range of commercially available PBMAs, finding they had comparable protein digestibility to similar animal-derived products. The PBMAs examined tended to have higher iron contents but a lower iron dialysability than the meat products examined; the consequence of this was that they both made comparable contributions to the total absolute iron requirement. It was observed that the processing conditions had a relatively small effect on the *in vitro* protein digestibility while having more of an impact on the iron dialysiability. As PBMA products are gaining wider adoption and increasing governmental support, documented evidence of their nutrition becomes critical. Therefore, these findings hold significant relevance for consumers, companies, and policymakers seeking to make informed dietary and regulatory decisions about PBMAs.

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Declaration of interests

☐ 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.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Kelsey Kanyuck reports financial support was provided by THIS. Nur Liyana Sulaiman reports financial support was provided by THIS. Wanrui Zhang reports financial support was provided by THIS. Wanrui Zhang reports a relationship with THIS that includes: employment. Kelsey Kanyuck reports a relationship with THIS that includes: employment. Nur Liyana Sulaiman reports a relationship with THIS that includes: employment. Kelsey Kanyuck, Nur Liyana Sulaiman and Wanrui Zhang were employed by THIS, a manufacturer of plant-based meat analogues when the work was conducted. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

- PBMAs show high protein digestibility, ranging from 81–96%.
- Protein isolates used in PBMAs reach digestibility levels of 97–99%.
- Extrusion speed and temperature affect texture more than digestibility.
- PBMAs have lower iron dialysability but higher total iron content.
- Iron bioavailability improves with higher screw speeds and temperatures.