Food Chemistry 524 (2026) 150279

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Comparative analysis of appearance, digestive behaviour, and nutritional quality of grilled alternative meat analogues and beef patty☆

Ruixian Han [a,d,e], Maryam Binti Zulkifli [a], Oscar Abel Sánchez Velázquez [a], Yan Wang [a], Alexander Lim [a], María Lilibeth Manzanilla Valdez [a,d], Martin Mondor [b,c], Evi Paximada [a], Alan Javier Hernández-Álvarez [a,d,*]

[a] School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK
[b] Department of Chemical Engineering and Biotechnological Engineering, Université de Sherbrooke, Sherbrooke, QC J1K 2R1, Canada
[c] Institute of Nutrition and Functional Foods (INAF), Université Laval, Quebec, QC G1V 0A6, Canada
[d] National Alternative Protein Innovate Centre (NAPIC), UK
[e] The James Hutton Institute, Dundee DD2 5DA, UK

ARTICLE INFO

Keywords:
Meat analogues
Textural properties
Digestion
Alternative proteins
Protein quality
DIAAS
DIAAR

ABSTRACT

A wide range of commercial meat analogues have emerged as alternatives to animal protein. This study evaluated six commercial alternative meat analogues (AMAs) against a conventional beef patty (BP), focusing on cooking behaviour, appearance, texture, digestion, and protein quality. AMAs showed distinct structural differences from BP, directly resulting in variations in cooking loss, hardness, and chewiness. Following simulated gastrointestinal digestion, AMAs released more free amino acids, whereas BP demonstrated higher overall protein digestibility. Protein quality assessment revealed that four AMAs (66.1–98.5) had higher *in vitro* protein digestibility-corrected amino acid score (IVPDCAAS) than BP (54.8), while only one (AMA6, 72.3) showed higher *in vitro* digestible indispensable amino acid score (DIAAS) (BP, 64.2). This discrepancy was attributed to the complete digestibility of limiting amino acids (Cys + Met) in BP, highlighting the importance of assessing individual amino acid digestibility. Overall, AMAs show promise but require further improvement in texture and post-cooking nutritional quality.

1. Introduction

The global population has been increasing rapidly over the centuries and reached approximately 8.2 billion in mid-2025 (Worldometer, 2025). Meat is a major source of energy, providing protein and essential micronutrients, including vitamin B_{12}, zinc, and iron, which are necessary to meet the nutritional requirements of the human body (Godfray et al., 2018). Consequently, meat consumption has tripled over the past 50 years, reaching approximately 350 million tonnes annually (Ritchie et al., 2017). However, high levels of meat consumption have been associated with an increased risk of obesity and chronic diseases (Fogelholm et al., 2015). In addition, meat production has significantly negative impacts on the environment, including greenhouse gas emissions, freshwater usage, and agricultural land requirements (Clark & Tilman, 2017). These concerns have prompted scientists to explore and investigate alternative protein sources that could partly or fully replace animal protein, thereby contributing to more sustainable food supply systems and improving public health.

Meat analogues produced from alternative proteins are designed to mimic the appearance, texture, flavour, and cooking experience of conventional meat (El Sadig & Wu, 2024), which makes them appealing not only to vegans and vegetarians but also to meat consumers. Consequently, the development of meat analogues has been regarded as an emerging strategy to promote the integration of alternative proteins into the human diet. Soy and pea proteins are the most used ingredients in commercial meat analogues, such as those produced by Moving Mountains, Beyond Meat, THIS™, and Impossible Foods. In addition, other protein sources, including fava bean, chickpea, and mung bean, have been applied in the formulation of meat analogues due to their functional and nutritional properties (Benković et al., 2023; Siddiqui et al., 2024). Moreover, mycoprotein, a fungal-derived protein source with a featured fibrous structure, has been utilised as an alternative to meat

☆ This article is part of a Special issue entitled: 'NAPIC Conference 2025' published in Food Chemistry.

* Corresponding author at: School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK.
E-mail address: a.j.hernandezalvarez@leeds.ac.uk (A.J. Hernández-Álvarez).

https://doi.org/10.1016/j.foodchem.2026.150279
Received 3 April 2026; Received in revised form 16 June 2026; Accepted 30 June 2026
Available online 2 July 2026

protein in the production of meat analogues since the last century (Khan et al., 2024). Quorn is the commercially available brand.

Compared with animal proteins, plant proteins often lack of one or more indispensable amino acids (Gorissen et al., 2018), and the presence of antinutritional factors can further reduce protein digestibility and nutrient absorption (Manzanilla-Valdez et al., 2024). Consequently, following gastrointestinal digestion, plant proteins are generally considered to exhibit lower nutritional value and amino acid bioavailability than animal proteins. Although the complete amino acid profile of mycoprotein has been reported (Finnigan et al., 2019), studies investigating its gastrointestinal digestibility remain scarce. Therefore, evaluating the nutritional quality of alternative protein-based meat analogues is essential to determine whether they can meet consumer dietary requirements and to address concerns regarding the potential risk of nutrient deficiencies associated with their long-term consumption.

In vitro protein digestibility (IVPD), measured by the pH-drop method, has been widely applied to evaluate the digestibility of individual protein ingredients (Goldberg et al., 2026). To achieve a more comprehensive nutritional evaluation, IVPD is often combined with the amino acid score (AAS) to generate the IVPD-corrected amino acid score (IVPDCAAS), which accounts for limiting amino acids in protein sources (Tavano et al., 2016). Previous studies have consistently demonstrated that animal-derived and soy proteins present high protein quality, with milk, whey, and soy often reporting IVPDCAAS values close to or equal to 1.00 (Qin et al., 2022). In contrast, alternative plant proteins generally exhibit lower and more variable scores, reflecting limitations in both amino acid composition and digestibility. For instance, legumes such as fava bean, chickpea, and red lentil typically present moderate IVPDCAAS values (0.44–0.59) (Han et al., 2025), whereas pseudocereals and seeds, including quinoa and green ramon, tend to show lower scores (0.24–0.35) (Manzanilla-Valdez et al., 2024; Nazir et al., 2025). Chia protein, however, has been reported to exhibit relatively higher values among plant sources (0.68–0.71) (Wang et al., 2023). Despite the widespread use of IVPD and IVPDCAAS for evaluating individual protein ingredients, their application to complex multi-ingredient food formulations remains limited. Moreover, concerns have been raised regarding their suitability for accurately predicting protein quality in formulated foods. Consequently, more advanced evaluation methods are needed to provide reliable insights into the nutritional protein quality in multi-ingredients formulations.

To better mimic gastrointestinal digestion in the human body, the INFOGEST protocol was developed to provide a standardized, cost-effective, and reproducible method for stimulating food digestion (Brodkorb et al., 2019). Following digestion, the digestible fraction may be separated from the undigested digesta by precipitation with 80% (*v*/v) methanol. Protein digestibility can subsequently be quantified by measuring and comparing total nitrogen, total amine groups, and total amino acids in both digestible and undigestible fractions (Sousa et al., 2023). Furthermore, advanced nutritional parameters, including the digestible indispensable amino acid ratio (DIAAR) and the digestible indispensable amino acid score (DIAAS), have been introduced to provide a more accurate assessment of protein digestion and amino acid bioavailability (Sousa et al., 2023). This approach provides more convincing insights into the nutritional value of alternative protein products, particularly in complex formulations (*e.g.*, meat analogues), when compared with conventional meat.

Previous studies on meat analogues have primarily focused on comparing their textural properties, cooking loss, appearance, and sensory characteristics with those of conventional meat. However, little attention has been devoted to differences in digestibility and protein nutritional quality. Therefore, this study aimed to provide a comprehensive evaluation of these aspects. To achieve this, six commercial alternative meat analogues (AMAs) and beef patty (BP) after grilling were considered. The comparison began with assessments of appearance, cooking loss, and textural properties. Given that starch is an important ingredient in AMAs, its digestibility was subsequently evaluated. Regarding protein quality, the nutritional value indices were initially determined using the pH-drop based *in vitro* protein digestibility (IVPD) method. Furthermore, considering the complex formulation of AMAs, which includes starch, fibre, methylcellulose, and other ingredients (**Table S1**), the standardized INFOGEST digestion model was applied to simulate gastrointestinal protein digestion. Protein nutritional values were subsequently quantified, enabling a more physiological relevant evaluation of protein quality. A comparative analysis of the two digestion methods was also discussed. Therefore, the present study should be interpreted as a comparison of commercially available formulated products as consumed, rather than as a direct comparison of isolated plant, fungal and animal protein sources.

2. Materials and methods

2.1. Materials

α-Amylase from porcine pancreas (Type VI-B, ≥ 5 units/mg solid, A3176), trypsin from porcine pancreas (13,000–20,000 BAEE units/mg protein, T0303), chymotrypsin from bovine pancreas (≥ 40 units/mg protein, C4129), protease from *Streptomyces griseus* (≥ 3.5 units/mg solid, P5147), pepsin from porcine gastric mucosa (lyophilized powder, ≥ 3200 units/mg protein, P6887) were purchased from Sigma-Aldrich (Gillingham, UK). The Digestible and Resistant Starch Assay Kit (K-DSTRS) were obtained from Megazyme (Co. Wicklow, Ireland). Pierce™ BCA Protein Assay Kits (23225) and Pierce™ Quantitative peptide Assays & Standards (23295) were purchased from Thermo Fisher Scientific (Gillingham, UK). All other chemical reagents were research grade and were obtained from Sigma-Aldrich (Gillingham, UK), VWR chemicals (Lutterworth, United Kingdom), or Fisher Chemical (Loughborough, UK).

Six AMAs (AMA1 - AMA6) were selected based on their availability and popularity in the UK market and purchased online *via* Ocado between September and October 2024. Notably, AMA5 was produced from mycoprotein, whereas the remaining AMAs were plant-based meat analogues. BP was purchased from a local Morrisons supermarket in Leeds, UK, in September 2024. The ingredient composition of AMAs and BP is presented in **Appendix Table S1**, together with their protein content following grilling and defatting.

2.2. Sample preparation, cooking loss, appearance and textural profiles analysis

Grilling procedure followed the method described by Sousa et al. (2023) with minor modifications. Six AMAs were grilled for 3 min on each side using a commercial electric cooker (MDOC60FW, Montpellier), operated at 70% of its maximum power output, without the addition of spices, fat, or other ingredients. Due to the thicker layer of BP, the grilling time for each side was extended to 4.5 min, while all other conditions remained the same. The internal temperature of BP reached 74.5 ± 5.9 °C, which falls within the internal temperature range of the AMAs (72.4 °C to 81.2 °C) after grilling. The percentage (%) of cooking loss was determined by measuring the change in weight of patties before and after grilling, using the following equation (Zhou et al., 2022):

$$\text{Cooking loss } (\%) = \left(1 - \frac{\textit{weight of patty after grilling } (g)}{\textit{weight of patty before grilling } (g)}\right) \times 100\%$$

The appearance of grilled AMAs and BP was measured using a high-precision colorimeter (DR-10, MERHOVO). The L* value ranged from 0 (black) to 100 (white), where a* represents the redness (positive values) or greenness (negative values), and b* represents the yellowness (positive values) or blueness (negative values). The total colour difference (ΔE) and browning index (BI) were calculated according to the

following equations (Kasim & Kasim, 2015):

$$\Delta E = \sqrt{\left(L^*_{reference} - L^*_{Sample}\right)^2 + \left(a^*_{reference} - a^*_{Sample}\right)^2 + \left(a^*_{reference} - a^*_{Sample}\right)^2}$$

Where the appearance of grilled BP was used as reference.

$$\mathrm{BI} = \frac{100\,(x - 0.31)}{0.17}$$

$$\text{where } x = \frac{a^* + 1.75L^*}{(5.645L^* + a^* - 0.012b^*)}$$

The textural characteristics of grilled AMAs and BP were measured using textural profiles analysis (TPA), performed with a texture analyser (STABLE MICRO SYSTEMS TA.XTplusC, OLIS Ltd.). This method recorded the force *versus* time profiles when the patty was compressed and decompressed twice (Zhou et al., 2022). As the present study evaluated whole patty and accounted for variations in patty size and shape, the grilled samples were tested in their native state (Mabrouki et al., 2024). Textural profiles parameters, including hardness, springiness, cohesiveness, chewiness, and resilience, were calculated according to the procedure described by Bourne et al. (1978).

Subsequently, the grilled AMAs and BP were ground into flour, and hexane was added at a ratio of 4:1 (*v/w*). The mixture was stirred continuously for 1 h at room temperature and then centrifuged at 3000 ×*g* for 15 min at 4 °C. The supernatant was discarded, and the defatting process was repeated three times using fresh hexane. This process reduced the lipid content of patties to 0.63% - 2.54%, which had negligible impact on starch and protein digestibility (Lin et al., 2020). The defatted samples were subsequently used in place of the whole grilled products for all digestion analyses due to improved sample homogenisation.

2.3. Scanning electron microscopy (SEM)

The microstructure of grilled AMAs and BP was examined using a Hitachi High-Tech SU3900 scanning electron microscope (SEM) equipped with a backscattered electron detector. Following the sample preparation method described by Okeudo-Cogan et al. (2023), the patties were coated with gold to a thickness of ~15 nm to ensure adequate thermal and electrical conductivity. Subsequently, a high vacuum condition (> 10 e^{-7} mbar) and low temperature (− 207 °C, achieved using slushed liquid nitrogen) were then applied. The working distance was set to 15.5–16.2 nm, and imaging was conducted at 2 kV and 0.1 nA at magnifications of 500 ×, 1000 ×, and 2000 × to capture detailed and overall microstructural features.

2.4. Digestible starch and resistant starch

Rapidly digestible starch (RDS; digested within 20 min), slow digestible starch (SDS; digested between 20 and 120 min), total digestible starch (TDS; digested within 4 h), and resistant starch (RS, not digested within 4 h) of AMAs and BP were determined using Digestible and Resistant Starch Assay Kit (K-DSTRS). The assay was performed following the instructions provided by Megazyme, available at https://www.megazyme.com/digestible-and-resistant-starch-assay-kit. An overview of the RDS, SDS, TDS, RS, and TS of AMAs and BP is presented in **Appendix Table 2S**.

2.5. In vitro protein digestibility and nutritional quality

The measurement of *in vitro* protein digestibility (IVPD) of AMAs and BP, together with the calculated amino acid score (AAS), indispensable amino acid index (IAAI), predicted biological values (BV), protein efficiency ratios (PER_{1-5}), and *in vitro* protein digestibility-corrected amino acid score (IVPDCAAS), were determined following the same procedures as reported by Han et al. (2025).

2.6. Gastrointestinal digestion of patties simulated using the INFOGEST static model

Defatted and grilled AMAs and BP were digested following the INFOGEST static model described by Brodkorb et al. (2019), with some modifications. Initially, two grams of AMAs were dispersed in 4 mL of simulated salivary fluid (SSF) containing α-amylase (75 U/mL) and were incubated at 37 °C for 2 min at pH 7. Four millilitres of simulated gastric fluid (SGF) was then added to the oral digesta, and the pH was adjusted to 3 with 6 M HCl. Pepsin was added to a final concentration of 2000 U/mL, and the mixture was incubated at 37 °C for 2 h. Gastric digestion was terminated by adjusting pH to 7 with 6 M NaOH. Intestinal digestion was subsequently started by adding 8 mL of simulated intestinal fluid (SIF) containing trypsin (100 U/mL) and chymotrypsin (25 U/mL), followed by incubation at 37 °C for 2 h. The digestion was stopped by heating the mixture at 95 °C for 10 min (Guzmán-Ortiz et al., 2024). Supernatants from both gastric and intestinal phase were collected by centrifugation at 4, 000 ×*g* for 15 min at 4 °C. Due to the high water-holding capacity of BP, the standard fluid volumes were insufficient to form a suspension. To address this issue, the volumes of SSF, SGF, and SIF were each increased by a factor of 1.5, resulting in final volumes of 6 mL, 6 mL, and 12 mL, respectively.

According to Sousa, Portmann, et al. (2023), the digestible fraction was obtained from the supernatant of intestinal digesta through methanol precipitation. The supernatant was mixed with four volumes of absolute methanol and stored at −20 °C for 1 h, followed by centrifugation at 2000 ×*g* for 15 min at 4 °C. The resulting supernatant was considered as the 'bioavailable' fraction. Overall, supernatants collected from gastric phase, intestinal phase, and post-methanol precipitation were freeze-dried and converted into powders for subsequent analyses

2.7. Sodium dodecyl sulfate-polycrylamide gel electrophoresis (SDS-PAGE)

The molecular weight distribution of proteins and peptides in AMAs and BP, as well as digestates collected at gastric and intestinal phases, was analysed using SDS-PAGE according to Laemmli (1970), with minor modifications. Undigested patties containing 20 μg of protein were dissolved in 1× Laemmli buffer containing dithiothreitol (DTT, 15.4 mg/mL). Digested patties containing 60 μg of protein were dissolved in 1× Laemmli buffer without the addition of DTT. All samples were heated at 95 °C for 5 min and subsequently centrifuged at 10,000 ×*g* for 10 min at 4 °C. The resulting supernatants were loaded onto Criterion™ TGX™ precast gels (Bio-Rad). Electrophoresis was performed at 200 V for 30 min. The gels were washed three times with Milli-Q water, stained with Bio-Safe™ Coomassie stain and analysed using a gel imaging system (Gel Doc XR+ system, Bio-Rad). Precision Plus Protein™ standard (2–250 kDa, Bio-Rad) was used as molecular weight markers.

2.8. Degree of hydrolysis, total soluble protein, total peptides, free amino acids and total amino acids analysis

The degree of hydrolysis (DH) of patties in the undigested state (UD), following gastric digestion (G), and after gastro-intestinal digestion (GI) was determined using trinitro-benzene sulfonic acid (TNBS) method according to Adler-Nissen (1979), with L-leucine used as the standard. Briefly, 100 μL of each sample was mixed with 50 μL of TNBS (1% *v*/v) solution and incubated at 37 °C for 2 h. The reaction was terminated by adding 50 μL of 1 M HCl, and the absorbance was measured at 340 nm. The concentration of amine groups in samples was quantified using L-leucine standard curve.

In addition, acid hydrolysis was applied to patties and to the digestible fraction (extracted with methanol) for calculating *in vitro* total protein digestibility. Briefly, 5 mL of 6 M HCl were added to 50 mg of freeze-dried powder, followed by hydrolysis using microwave digestion system (MARS 6, 130 °C, for 1 h). The samples were then centrifuged at

3000 ×*g* for 10 min at 4 °C, and total amine content was quantified following the same procedure described above.

The DH was calculated using the following equation:

$$\text{Degree of hydrolysis (DH)} = \frac{\textit{Amine group in G or GI} - \textit{amine group in UD}}{\textit{Total amine group of patties}} \times 100$$

Soluble protein in the supernatant of patties from UD, G, and GI was quantified using the Pierce™ BCA protein assay kit. Peptides were isolated from the supernatant using 10 kDa molecular weight cut-off membranes (Amicon® Ultra-15, UFC9010, Millipore Sigma, regenerated cellulose) and subsequently quantified using the Pierce™ Quantitative Fluorometric Peptide Assay.

Following the method described by Wang et al. (2024), free amino acids were extracted from 200 mg of UD, G, and GI samples using 2 mL of 0.01 M HCl containing 10 μM / mL norvaline as internal standard. The samples were centrifuged at 2500*g* for 15 min, and the resulting supernatant was analysed by reversed-phase high-performance liquid chromatography (RP-HPLC) and diode array detection (DAD) using an Agilent Zorbax Eclipse Plus C18 column (4.6 × 250 mm. particle size of 5 μm).

Total amino acids from UD, G, GI and the digestible fractions were measured following the protocol described by Manzanilla-Valdez et al. (2024). Briefly, 2 mg of sample was hydrolysed with 4 mL of 6 M HCl at 110 °C for 24 h. Tryptophan was determined separately following basic hydrolysis. Amino acids were then analysed in triplicate after derivation with diethyl ethoxymethylenemalonate, using reverse-phase high-performance liquid chromatography (RP-HPLC) with D,L-aminobutyric acid as an internal standard. A 20 μL aliquot of each sample was injected in a reversed-phase column (Novapack C18, 300 mm × 3.9 mm id., 4 μm; Waters, Milford, MA, USA). Separation was carried out at a flow rate of 0.9 mM/min using a binary gradient system consisting of (A) 25 mM sodium acetate buffer with 0.2% (*w/v*) sodium azide (pH 6) and (B) acetonitrile. The elution program was as follows: 0–3 min, linear gradient from 91:9 (A: B) to 86:14; 3 - 13 min, gradient from 86:14 to 69:31; and 30–35 min, elusion at 69:31. The column temperature was maintained at 18 °C.

2.9. *In vitro* total digestibility, digestible indispensable amino acid ratio (DIAAR), digestible indispensable amino acid score (DIAAS) calculations

In vitro total digestibility of AMAs and BP was calculated according to Sousa, Portmann, et al. (2023), with some modifications. Total nitrogen content was measured using the Dumas combustion method (AOAC, 1990). Meanwhile, total primary amines and total/individual amino acids were quantified in both digestible fraction and the undigested patties. *In vitro* digestibility (%) was calculated separately for each parameter (total nitrogen, total primary amines, and total/individual amino acid) by comparing their amounts in the digestible fraction with those present in the undigested patties, according to the following equation:

$$\textit{In vitro}\ \text{digestibility (\%)} = \frac{\textit{parameter content in digestible fraction}}{\textit{parameterparameter content in patties before digestion}} \times 100 \geq$$

Where the parameter refers to total nitrogen, total primary amines, or total/individual amino acids.

Digestions performed without the addition of patties served as background control. The corresponding background values were subtracted from those of the digestible fraction prior to calculation.

The *in vitro* digestible indispensable amino acid ratio (DIAAR) was calculated for each indispensable amino acid (IAA) using the following equation:

$$\textit{In vitro}\ \text{DIAAR (\%)} = \frac{\textit{mg of IAA per g protein in digestible fraction}}{\textit{mg of the IAA per g protein in reference pattern}^{1}} \times 100$$

[1]Reference pattern: older children, adolescents, and adults (FAO, 2013).

The lowest *in vitro* DIAAR value among all IAAs was used as the *in vitro* digestible indispensable amino acid score (DIAAS) of the patties.

2.10. Residual amino acids after intestinal digestion and methanol precipitation

Residual amino acids following intestinal digestion were calculated by subtracting the amount of each amino acid in the post-intestinal supernatant from that in the undigested patties. Residual amino acids in the pellet after methanol precipitation were determined as the difference between the amounts of each amino acids in the post-intestinal supernatant and the digestible fraction.

2.11. Statistical analysis

Results were expressed as mean ± standard deviation and analysed by GraphPad Prism 10 (GraphPad Software, Boston, MA, USA) and OriginPro 2021 software (OriginLab Crop., MA, USA). All experiments were conducted with three biological replicates, except for the amino acid profiles, which were performed in duplicate. Differences among samples were analysed using one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) test. Statistical significance was set at a confidence level of 95% ($p < 0.05$). Linear correlation analysis was also performed using GraphPad Prism 10.

3. Results and discussion

3.1. Cooking loss, appearance, textural properties, and microstructure

Grilling is a traditional and common method for preparing beef patties. As mentioned by Zhou et al. (2022), this technique is rapid and straightforward, generating similar cooking outcomes when standardized cooking times and temperatures are applied. Consequently, grilling is widely used in burger preparation by both consumers and fast-food restaurants. Table 1 presents the cooking loss of AMAs and BP. BP exhibited a cooking loss of 22.51%. The reduction in BP weight can be attributed to structural shrinkage and moisture loss, associated with the helix-to-coil transition of collagen and the denaturation of actin and myosin during heating (Vu et al., 2022). The cooking loss of BP observed in this study was relatively lower than values previously reported (~ 30% - ~ 40%) (Scheeder et al., 2001; Shen et al., 2022; Zhou et al., 2022). Surface tissue shrinkage during grilling limits the evaporation of internal water (Portanguen et al., 2014; Rohlik et al., 2017), while greater thickness of BP, associated with a lower surface-area-to-volume ratio and reduced exposed surface tissue, promotes rapid surface shrinkage and consequently improves moisture retention (Gardner et al., 2020). Compared with BP, four AMAs exhibited lower cooking losses, ranging from 9.62% to 15.18%. The lower cooking losses of these AMAs may result from the formation of porous networks by denatured protein, fibres, methylcellulose, and modified starch, which enhanced moisture binding and holding capacity (Rasul et al., 2024; Vu et al., 2022). In contrast, higher cooking losses were observed in AMA3 (25.98%) and AMA4 (34.71%). This was attributed to their relatively loose structure, as evidenced by visual observation. Such structural characteristics likely arise from differences in ingredient composition (*e.g.*, the presence of binding agents) and the extent of protein network formation during processing (Beniwal et al., 2021; Dekkers et al., 2018). The formation of a protein–polysaccharide network enhances the matrix's ability to retain water and melted fat, thereby reducing fluid release and resulting in lower cooking loss values. In contrast, a weaker and less cohesive

Table 1
Appearance, cooking loss, and textural properties of grilled commercial alternative meat analogues (AMAs) and conventional beef patty (BP).

	AMA1	AMA2	AMA3	AMA4	AMA5	AMA6	BP
Cooking loss (%)	13.84 ± 1.04^{d}	13.26 ± 2.99^{d}	25.89 ± 0.92^{b}	34.71 ± 2.79^{a}	9.62 ± 0.22^{e}	15.18 ± 1.48^{d}	22.51 ± 0.36^{c}
L*	16.80 ± 1.03^{c}	18.74 ± 1.31^{bc}	25.53 ± 2.16^{a}	13.42 ± 1.24^{d}	18.30 ± 0.34^{b}	16.58 ± 1.05^{c}	19.84 ± 0.11^{b}
a*	20.73 ± 0.43^{bc}	23.17 ± 0.24^{b}	22.01 ± 2.92^{b}	18.71 ± 1.45^{c}	31.85 ± 2.33^{a}	15.84 ± 0.57^{c}	33.44 ± 2.73^{a}
b*	26.45 ± 0.15^{a}	18.23 ± 0.87^{d}	25.45 ± 0.66^{ab}	21.79 ± 2.60^{cd}	20.93 ± 1.27^{cd}	21.12 ± 1.59^{cd}	23.93 ± 1.57^{abc}
ΔE	13.30	11.79	12.86	16.21	3.72	18.12	0.00
BI[1]	73.52	73.35	54.20	81.10	96.18	59.31	93.87
Hardness (N)	25276 ± 1937^{b}	23896 ± 963^{b}	12207 ± 706^{c}	10820 ± 1874^{c}	20622 ± 1138^{b}	22843 ± 740^{b}	33784 ± 3732^{a}
Springiness	0.575 ± 0.008^{b}	0.593 ± 0.006^{b}	0.801 ± 0.038^{a}	0.823 ± 0.047^{a}	0.818 ± 0.011^{a}	0.866 ± 0.015^{a}	0.845 ± 0.016^{a}
Cohesiveness	0.335 ± 0.006^{d}	0.545 ± 0.002^{b}	0.654 ± 0.024^{a}	0.658 ± 0.060^{a}	0.442 ± 0.017^{c}	0.475 ± 0.025^{bc}	0.530 ± 0.020^{b}
Chewiness	5829 ± 577^{e}	7704 ± 261^{c}	6316 ± 279^{d}	6163 ± 1616^{cde}	7485 ± 639^{cd}	11363 ± 1008^{b}	14931 ± 1648^{a}
Resilience	0.191 ± 0.004^{b}	0.198 ± 0.001^{b}	0.232 ± 0.015^{a}	0.243 ± 0.034^{a}	0.153 ± 0.012^{d}	0.182 ± 0.007^{c}	0.208 ± 0.019^{ab}

Data expressed as mean ± SD, $n = 3$. Different lowercase letters within each row indicate statistically significant differences (p-value <0.05).
[1] BI browning index.

network exhibits reduced water- and fat-holding capacity, leading to higher fluid losses. This structural difference facilitated the rapid release of a significant amount of fluid particularly within the first 30s of grilling, thereby resulting in elevated cooking losses in both AMAs.

Food appearance is a critical quality parameter affecting product acceptability and consumer desirability prior to consumption (Maina, 2018). Accordingly, the colour characteristics of AMAs should be compared with those of BP, using lightness (L*), redness (a*), and yellowness (b*) as evaluation parameters. As shown in Table 1, AMA3 exhibited the highest L* value (25.53), followed by BP (19.84), AMA2 (18.74), and AMA5 (18.30). In contrast, BP presented markedly high a* (33.44) and b* (23.93) values. When evaluating differences in overall appearance between AMAs and BP, ΔE values were calculated. Among the samples, only AMA5 showed a slight difference compared with BP, with a value of 3.72, indicating a high level of visual similarity that shows no obvious visual difference to consumers (Mokrzycki & Tatol, 2011). In contrast, AMA6 showed the greatest deviation, with a ΔE value of 18.12, attributed to its lower redness (15.84) compared with BP (33.44). Additional insight was provided by the browning index (BI), which reflects the extent of brown colour formation by Maillard reactions during grilling (Hu et al., 2023). BP recorded a BI of 93.87, which was slightly lower than that of AMA5 (96.18), again indicating the visual similarity between the two samples. The use of plain caramel in AMA5 directly contributed to the observed similarity in BI with BP. The remaining AMAs exhibited lower BI values, with AMA3 showing the lowest BI (54.20). The presence of myoglobin in beef enhances the redness and darkness of meat during grilling (Samard & Ryu, 2019). Although AMAs contain colourants, such as beetroot, to impart red-*ish* colour, they cannot fully mimic the visual characteristics of myoglobin. The absence of myoglobin may therefore explain the lower BI values observed in AMAs.

The textural properties of AMAs are also critical, as they reflect how closely these meat analogues can replicate the eating experience of real meat. Muscle protein denaturation in beef has been reported to result in increased hardness and chewiness after thermal processing (Bakhsh et al., 2021), which is consistent with the findings of the present study. BP exhibited the highest hardness value (33784) and chewiness value (14931). Although AMA5 exhibited the closest visual appearance to BP, it only showed similar springiness (0.818) to BP (0.845). Among the other AMAs, AMA3 and AMA4 showed similar textural properties; however both showed a much lower hardness (12207 and 10820), higher cohesiveness (0.654 and 0.658), and lower chewiness (6316 and 6163) compared with BP. AMA6 exhibited the closest but still distinguishable textural profiles to BP, showing no significant difference values in springiness (0.866 *versus* 0.845), cohesiveness (0.475 *versus* 0.530), and resilience (0.182 *versus* 0.208). Chewiness was slightly lower in AMA6 (11363) than in BP (14931).

To elucidate the overall structural characteristics, SEM images of AMAs and BP were acquired at different magnifications (Fig. 1). BP exhibited a denser and more ordered fibrous structure, which accounts

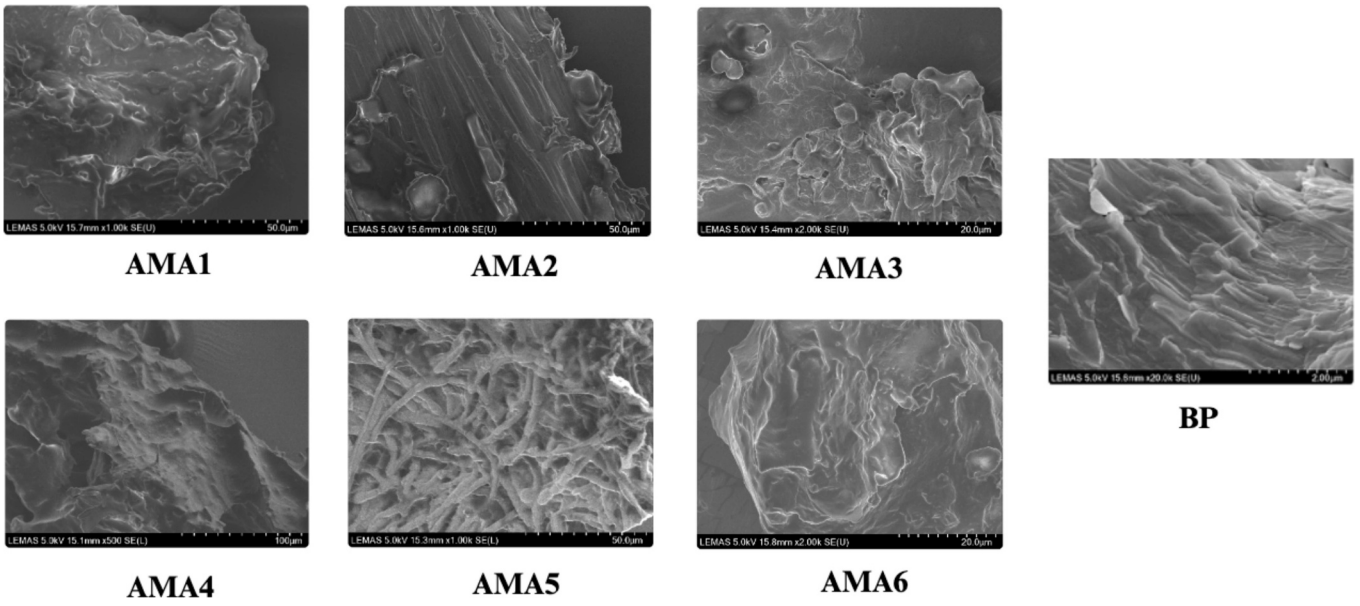


Fig. 1. Scanning electron microscopy (SEM) images of grilled commercial alternative meat analogues (AMA) and conventional beef patty (BP).

for its higher hardness and chewiness. AMA5 displayed a network of interconnected hyphae with variable thickness, reflecting its characteristic structure and contributing to a stronger and more cohesive matrix (Maseko et al., 2025). In contrast, AMA4 did not exhibit a fibrous structure, indicating a marked difference from BP and explaining its distinct textural properties. AMA1 showed a disordered fibrous structure, whereas AMA2 presented a more organized but less dense fibrous arrangement, which contributed to differences in textural properties compared with BP. AMA3 and AMA6 displayed similar internal structures, both characterized by comparable sizes and disordered fibrous patterns. However, AMA6 showed textural properties similar to those of BP, in contrast to AMA3. The presence of air bubbles in AMA3 may have resulted in a looser structure after grilling, which contributed to its markedly different texture compared with BP (Samard & Ryu, 2019).

3.2. *In vitro* protein digestibility and nutritional values

The amino acid profiles of AMAs and BP used for the *in vitro* digestion experiments were analysed, and the results are presented in Fig. 2a. The figure shows the amino acid content per 100 g AMA/BP on a dry matter basis. BP contained the highest total amino acid content (84.95 g/100 g), whereas AMA1 exhibited the lowest (12.63 g/100 g). The huge difference between AMAs and BP can be attributed to the addition of non-protein ingredients in the formulation of AMAs (see **Appendix Table S1** for a detailed list of ingredients).

Protein quality refers to the ability of a food protein to provide amino acids and nitrogen to meet human metabolic requirements (Boye et al., 2012). Therefore, expressing amino acid content per 100 g of protein, rather than per 100 g of AMAs/BP, enables a more accurate and meaningful evaluation. As shown in Fig. 2b, the amino acid composition per 100 g of protein of AMAs and BP were presented, and compared with the amino acid reference pattern for older children, adolescents, and adults (FAO, 2013). AMA2 (3.77) contained lower levels of Lys than the recommend value (4.80). In addition, AMA3 (0.38), AMA4 (1.19), AMA6 (2.10), and BP (1.48) were limited in Met + Cys (2.30).

Legume proteins are typically deficient in sulphur-containing amino acids (Met + Cys) (Ahmed et al., 2023). This characteristic is reflected in the present study, where the lowest AAS for AMA3 (16.66), AMA4 (51.81) and AMA6 (91.30) were associated with Met + Cys (shown in Table 2). In contrast, Lys was identified as the limiting amino acid in AMA2, with an AAS value of 78.44. Notably, AMA1 and AMA5 fulfilled the indispensable amino acid requirements established for older children, adolescents, and adults, indicating a more balanced amino acid composition in these formulations.

Legumes are generally recognized as good sources of Lys, for example, lysine constitutes 6.54% in black bean (Yang et al., 2025), 6.2% in pea (Rodríguez et al., 2008), and 6.0% in soy (Kalman, 2014), when expressed as a percentage of the total amino acid content. The relatively low AAS values observed in this study may be attributed to lysine degradation resulting from Maillard reaction and oxidative process occurring during grilling (Supreetha et al., 2009). Consequently, the lysine content of AMAs did not meet the recommended requirement of 4.8% (FAO, 2013).

On the other hand, mycoprotein has been reported to be rich in indispensable amino acids, meanwhile offering superior branched-amino acid bioavailability and greater stimulation of muscle protein synthesis compared with most plant-derived protein (Majumder et al., 2024). The findings of the present study support these observations, demonstrating an AAS value of 116.56% for Lys in AMA5. BP was expected to show a complete and well-balanced amino acid profile, however, the Met + Cys content did not meet the recommended requirement for young children, with an AAS of only 64.18%, which was lower than that of most AMAs. The low Cys (0.16 g/100 g protein) in BP was the primary factor contributing to its unexpectedly low AAS. Swing et al. (2021) also reported low Cys level in cooked ground beef (2.7 mg/g sample), which is generally lower than those found in cooked meat analogues (2.7–7.1 mg/g sample). Meanwhile, their study demonstrated that Met concentration is higher in cooked ground beef (6.60 mg/g sample), compared to meat analogues (1.80–3.39 mg/g sample). Similar trends were observed in the present study (shown in Fig. 2a). Therefore, the proportion of Met + Cys relative to the total amino acid content was comparatively low, falling below the requirements of the reference pattern. Nevertheless, apart from Cys, the concentrations of the other indispensable amino acids in BP were generally higher than those observed in the AMAs. This was reflected in the protein efficiency ratio (PER) values, which are theoretical indices of protein quality based on animal growth studies and are commonly used to evaluate the capacity of protein to support growth based on weight gain per unit of protein consumed. Among the samples evaluated, BP exhibited the highest PER4 (3.06 *vs.* 2.63–3.03) and PER5 (3.16 *vs.* 2.68–3.09) values.

Using egg white as the refence protein for calculating the indispensable amino acid index (IAAI) provides an effective approach to evaluating the overall indispensable amino acid composition. As described by Chang et al. (2023), protein with an IAAI value greater

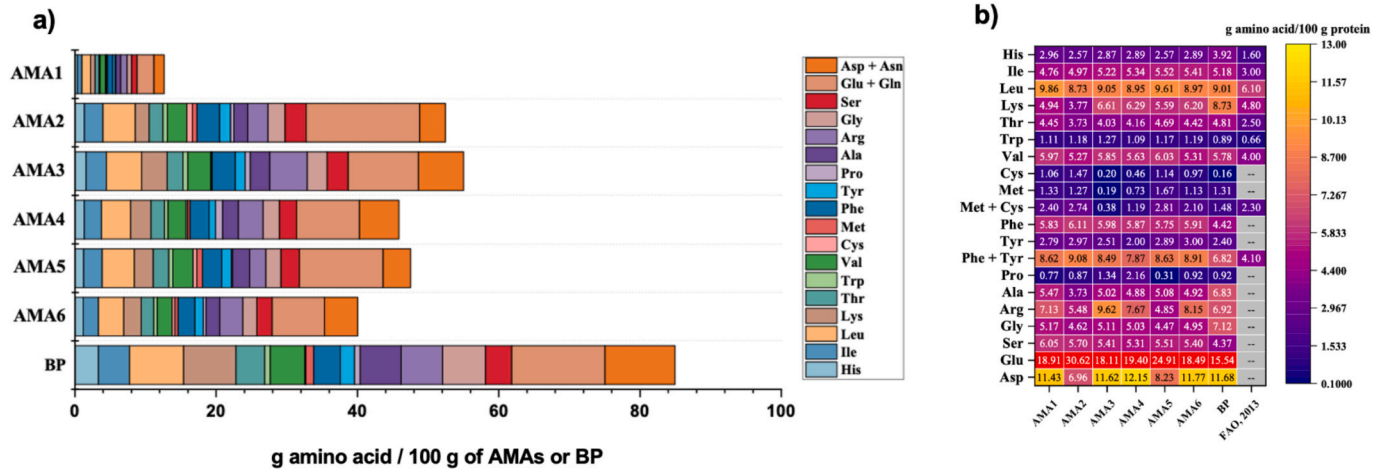


Fig. 2. Amino acid profiles: a) g amino acid per 100 g of AMAs or BP, and b) g amino acid of protein in grilled commercial alternative meat analogues (AMAs) and conventional beef patty (BP). The amino acid requirements of older children, adolescents, and adults were used as the reference pattern (FAO, 2013). The colour scale ranges from dark purple to light yellow, indicating increasing amino acid content from low to high. Red colour is used to present amino acid levels over 13 g /per 100 g of protein. Asp, aspartic acid; Glu, glutamine; Ser, serine; Gly, glycine; Arg, arginine; Ala, alanine; Pro, proline; Tyr, tyrosine; Phe, phenylalanine; Met, methionine; Cys, cysteine; Val, valine; Trp, tryptophan; Thr, threonine; Lys, lysine; Leu, leucine; Ile, isoleucine; His, Histidine.

Table 2
Protein quality parameters of grilled commercial alternative meat analogues (AMAs) and conventional beef patty (BP).

	AAS[1] (%)	IAAI[2] (%)	BV[3]	PER_1[4]	PER_2[4]	PER_3[4]	PER_4[4]	PER_5[4]	IVPD[5] (%)	IVPDCAAS[6] (%)
AMA1	102.85 (Lys)	80.35	75.88	3.77	3.71	4.44	2.89	3.01	75.07 ± 0.31^{c}	77.21
AMA2	78.44 (Lys)	75.55	70.65	3.26	3.19	3.37	2.63	2.68	84.30 ± 1.51^{b}	66.13
AMA3	16.66 (Met + Cys)	67.48	61.85	3.38	3.38	3.58	2.88	3.13	87.38 ± 1.58^{a}	14.55
AMA4	51.81 (Met + Cys)	74.30	69.29	3.30	3.39	4.22	2.88	2.98	84.85 ± 0.36^{b}	43.96
AMA5	116.56 (Lys)	83.79	79.23	3.68	3.59	4.30	3.03	2.95	84.12 ± 0.58^{b}	98.05
AMA6	91.30 (Met + Cys)	81.05	76.64	3.36	3.29	3.47	2.91	3.09	84.67 ± 0.48^{b}	77.31
BP	64.18 (Met + Cys)	79.89	75.38	3.38	3.37	4.14	3.06	3.16	85.39 ± 0.28^{ab}	54.80

Different lowercase letters within the IVPD (%) column indicate significant differences (p-value <0.05), data expressed as mean ± SD, n = 3.
Note: AAS (%), IAAI (%), BV, PER_{1-5}, and IVPDCAAS (%) are calculated values, no standard deviation is available.
[1] Amino acid score.
[2] Indispensabile amino acid index.
[3] Biological value.
[4] Protein efficiency ratio.
[5] *In vitro* protein digestibility.
[6] *In vitro* protein digestibility corrected amino acid score.

than 70 are considered nutritionally adequate. AMA3 appears to be at risk of nutritional inadequacy as indicated by its low IAAI value of 67.48, resulting from an extremely low Met + Cys (AAS: 16.66%). Apart from this, all other AMAs and BP can be considered good sources of indispensable amino acids.

In vitro protein digestibility (IVPD) is an important indicator of amino acid bioavailability and reflects the biological value of dietary protein (Orlien et al., 2023). A previous study has reported IVPD values for beef muscles of ~80% (Barrón-Hoyos et al., 2013). In contrast, BP exhibited a higher IVPD value (85.39%), which may be attributed to the enhanced protein digestibility induced by grilling (Ferreira et al., 2018). The IVPD of AMAs was largely determined by the protein ingredients used in their formulations. For instance, IVPD of black bean has been reported to 65.4% (Guajardo-Flores et al., 2017), which increased to 79.42% (extruded), 75.34% (cooked), and 74.26% (baked) after processing (Nosworthy et al., 2018). These values of processed black bean are comparable to that of AMA1 (75.09%), which also used black bean as its main protein source. Soy, pea, and mycoprotein have been reported to exhibit high protein digestibility (Auer et al., 2024; Wang et al., 2023), which explains why the IVPD of BP (85.39%) showed no significant difference compared with those of AMA2 – AMA6 (from 84.12% to 87.38%).

In vitro protein digestibility-corrected amino acid score (IVPDCAAS), calculated as the product of AAS of most limiting indispensable amino acid and IVPD, provides a more accurate assessment of protein quality in foods (Schaafsma, 2000). The IVPDCAAS of BP was 54.80, exceeding only the values observed for AMA3 (14.55) and AMA4 (43.96). All other AMAs exhibited higher IVPDCAAS values than BP, with AMA5 showing the highest value at 98.05. PDCAAS values of soy protein, beef, and pea protein concentrate were reported as 100, 92, and 73, respectively (Hughes et al., 2011). Meanwhile, Multescu et al. (2024) reported that the PDCAAS of black bean was 70. Although protein quality of ingredients from the same source can be influenced by growing conditions, genotypes, and production processes (Williams et al., 2008), the relatively low IVPDCAAS values observed for AMAs and BP suggest that grilling negatively affect protein quality, through the degradation of certain amino acids (Wang et al., 2021). Therefore, the impact of processing should be carefully considered when optimizing the nutritional value of food formulations (Nosworthy et al., 2023; Osuna-Gallardo et al., 2023; Sánchez-Velázquez et al., 2021), rather than relying solely on nutritional parameters measured and calculated from raw protein ingredients.

3.3. Protein digestion and protein quality parameters assessed by INFOGEST protocol

To better understand the differential digestible behaviours of AMAs and BP during simulated *in vitro* digestion, samples from the oral (undigested), gastric, and intestinal phases were collected. These samples were analysed to determine molecular weight (M_w) distribution, degree of hydrolysis, soluble protein content, peptide content, and the total amount of free amino acids.

3.3.1. Effect of digestion on protein profiles

Fig. 3 presents the molecular weight (M_w) distribution of proteins and peptides released during *in vitro* digestion from AMAs and BP at three different stages of digestion. Based on previous studies, the protein bands corresponding to black bean (Wang et al., 2025), pea (Schmidt et al., 2022), soy (Dumitrascu et al., 2019), rice (Hu et al., 2019), mycoprotein (Lonchamp et al., 2022) and beef (Suwandy et al., 2015) were identified and labelled.

Although AMA2 and AMA4 were both formulated using soy and pea protein, AMA4 exhibited noticeably fewer bands with lower intensity. The protein profiles of AMA4 were similar to those of AMA6, which consists only of soy protein. This indicates that soy protein is the dominant protein ingredient in AMA4. The proportion of pea protein in AMA4 is minimal, resulting in a limited number of detectable bands with sufficient intensity in SDS-PAGE gel. In addition, similarity was also observed among AMA2, AMA3, and AMA4, which can be attributed to the presence of pea proteins in these formulations. AMA1, AMA5, and BP exhibited distinctly different M_w distributions due to their unique protein sources, black bean protein, mycoprotein, and beef protein, respectively. These differences were expected due to variations in protein structure and amino acid sequence length among the different protein sources.

After gastric digestion, proteins and peptides with M_w below 20 kDa become predominant in AMAs. However, in AMA1, bands were still observed in the 37–50 kDa range. Similar findings for black bean proteins was also reported by Sousa et al. (2020), suggesting that certain protein fractions can partially resist pepsin digestion. Similarly, do Evangelho et al. (2017) reported that phaseolin in black beans exhibits resistance to pepsin digestion, which was attributed to its stable, globular tertiary structure, characterized by a high β-sheet content (Mali & Kumar, 2025). Furthermore, phaseolin may interact with other compounds, such as phenolic compounds, which can hinder pepsin access to cleavage sites (Venkatachalam & Sathe, 2003). In contrast, beef protein still displayed a wide range of M_w, indicating the presence of larger, less-digested protein fragments. Baugreet et al. (2019) claimed that the major protein bands in beef after gastric digestion were below 50 kDa. However, in the present study, bands with higher M_w were still observed. This discrepancy may be attributed to the formation of advanced glycation end-products, which can reduce the accessibility of digestive proteases to protein (Bieck et al., 2025). Consequently, the presence of high M_w fractions (*e.g.*, ~82 kDa and ~ 155 kDa) was

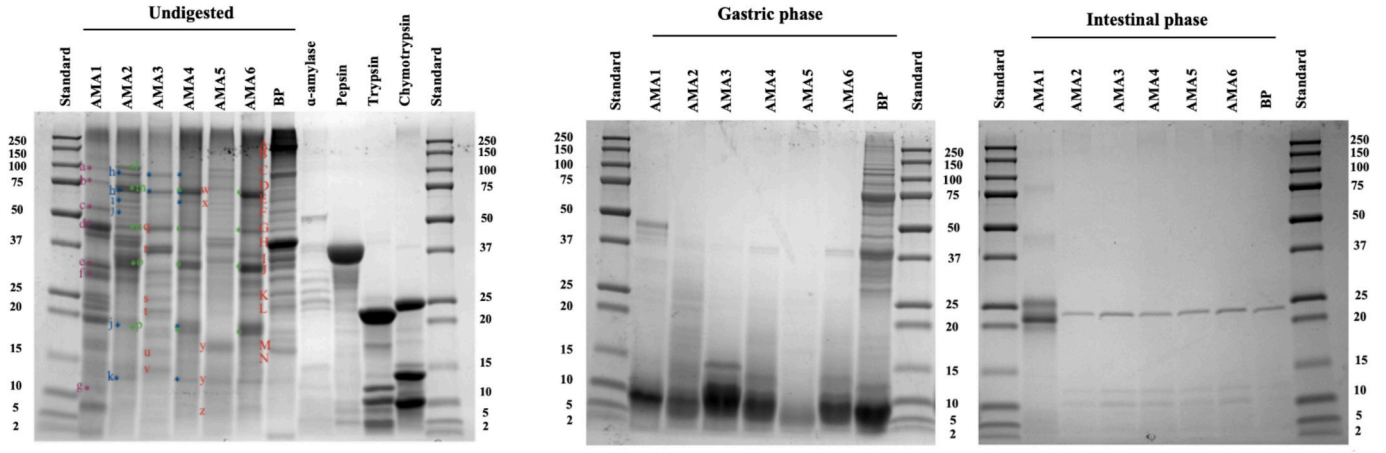


Fig. 3. SDS-PAGE profiles of extractable proteins from a) grilled AMAs and BP, along with the molecular weight marker (2–250 kDa) and the digestive enzymes (α-amylase, pepsin, trypsin, and chymotrypsin) used in the INFOGEST digestion; and b) grilled AMAs and BP after gastric and intestinal digestion phases. Protein bands identifications: **Black bean:** a) lipoxygenase; b) α' subunit β-conglycinin; c) α subunit β-conglycinin; d) α-Phaseolin; e) A_3-glycinin; f) $A_{1,2,4}$-glycinin; g) B-glycinin. **Pea:** h) convicilin; i) legumin monomer; j) vicilin; k) albumins. **Soy:** l) α' subunit β-conglycinin; m) α subunit β-conglycinin; n) β subunit β-conglycinin; o) acidic - glycinin; p) basic- Glycinin. **Rice:** q) proglutelin; r) glutelin-AS; s) globulin; t) globulin-BS; u) prolamin-16 kDa; v) prolamin-13 kDa. **Mycoprotein:** w) nucleoporin; x) RNA-binding protein; y) heat stock protein; z) U6 snRBA-associated Lsm protein. **Beef**: A) Titin and nebulin; B) Filamin; C) Myosin heavy chain-1; D) Myosin heavy chain-7; E) C protein; F) α-actinin-2,3 ATPase-1; G) Desmin; H) Calsequestrin −1; I) tropomyosin β chain; J) tropomyosin α chain; K) Troponin - T; L) myosin light chain; M) Troponin I; N) Troponin C.

observed on the gel after gastric digestion. Nevertheless, glycation does not appear to markedly affect intestinal digestibility (Tang et al., 2023), as evidenced by the complete disappearance of beef protein bands at the end of the intestinal phase. Following the complete INFOGEST digestion, AMA1 still exhibited a protein band around 20 kDa, indicating incomplete protein digestion, which is in agreement with the observation reported by Yang et al. (2025). No visible bands belonging to patties were detected in the other AMAs or in beef protein after further hydrolysis by trypsin and chymotrypsin, indicating extensive hydrolysis and relativity good digestibility.

3.3.2. Effect of digestion on the degree of hydrolysis (DH), soluble protein, peptides, and free amino acids (FAA)

The degree of hydrolysis (DH) directly reflects the extent to which proteins are cleaved into peptides and free amino acids (Rutherfurd, 2010). Therefore, it is a convenient parameter to monitor and compare protein digestion during both gastric and intestinal phases among AMAs and BP. A previous study has demonstrated that plant-based meat analogues exhibited equal or slightly higher protein digestibility than real meat after gastric digestion (Xie et al., 2022). This trend was consistently observed in the present study. As shown in Table 3, apart from AMA1, all AMAs presented comparable or slightly higher DH than BP. Regarding AMA1, the low digestibility of black bean protein may account for its low DH at the end of gastric phase. In addition, AMA1 exhibited the lowest protein content among the samples, and the presence of other components, such as polysaccharides, which may be more likely to interfere with protein digestion. This protein-polysaccharide interaction could hinder enzyme accessibility, thereby reducing pepsin hydrolysis in AMA1 (Fontes-Candia et al., 2025).

After the intestinal phase, the DH continued to increase in all samples. BP ultimately exhibited the highest DH (37.49%), which can be directly attributed to the superior digestibility of animal protein compared with alternative protein sources. Qin et al. (2022) reported a slightly lower DH (32.75%) for beef following gastrointestinal digestion. In the present study, grilling induced protein denaturation, which increases the accessibility of proteins to digestive proteases (Bhat et al., 2021) and may explain the slightly higher DH observed in BP. In contrast, plant proteins and mycoprotein showed relatively lower DH values, primarily due to differences in amino acid sequence composition, structural characteristics (*e.g.*, globular structure and fibrous structure), and the presence of other compounds (*e.g.*, anti-nutritional factors) (Ajomiwe et al., 2024; Day et al., 2022; Manzanilla-Valdez, Ma, Mondor, & Hernández-Álvarez, 2024). AMA6, formulated from soy protein, exhibited a moderate DH (31.70%), whereas the two soy-pea blends displayed markedly different DH values, namely 34.57% for AMA4 and 18.67% for AMA2. Although the protein sources of soy and pea can influence DH, the huge variation observed should largely be attributed to the presence and interactions of non-protein components (Fontes-Candia et al., 2025). Variations in compositional factors, including dietary fibre, starch, phenolic compounds, and other anti-nutritional factors, can promote the formation of protein complexes, thereby creating structural barriers that limit protease accessibility (Cirkovic Velickovic & Stanic-Vucinic, 2018). Furthermore, differences in production conditions and structural characteristics of the AMAs may further modulate the digestibility of protein, contributing to observed variations in the DH values (Beniwal et al., 2021). In addition, rice protein tended to be less digestible than soy and pea proteins (Rutherfurd et al., 2015), thereby contributing to its lower DH values (18.67%) following gastrointestinal digestion. Among all samples, AMA1 exhibited the lowest DH (6.05%), likely due to the lowest digestibly of black beans together with relatively more content of non-protein ingredients and the presence of antinutritional factors (Yang et al., 2025).

Soluble protein, peptide content, and free amino acid levels of all AMAs and BP increase significantly after gastric digestion. Intestinal digestion led to a further increase in these parameters, except for free amino acid levels in AMA3 (from 5.13 g/ 100 g protein dw to 6.62 g/ 100 g protein dw) and AMA6 (from 4.99 g/ 100 g protein dw to 5.98 g/ 100 g protein dw). During digestion, insoluble proteins are cleaved into soluble protein fragments, and in some cases, are further hydrolysed into free amino acids. At the end of the gastric phase, BP (40.24 g/100 g protein dw) exhibited soluble protein levels comparable to AMA1 (42.84 g/ 100 g protein dw) and AMA2 (41.89 g/ 100 g protein dw), and higher than the other AMAs. Its peptide content (18.82 g/ 100 g protein dw) was lower than AMA4 (23.32 g/ 100 g protein dw) and AMA6 (22.17 g/ 100 g protein dw), and its free amino acid content (3.15 g/ 100 g protein dw) did not exceed any AMA. After intestinal digestion, BP (72.12 g/ 100 g protein dw) showed the highest peptide content among all samples. Following complete gastrointestinal digestion, only AMA1

Table 3
Degree of hydrolysis (%), soluble protein (g/100 g protein dw), peptides (g/100 g protein dw), and free amino acids (g/100 g protein dw) during gastrointestinal transit of grilled commercial alternative meat analogues (AMAs) and conventional beef patty (BP).

	Digestion phase	Degree of hydrolysis (%)	Soluble protein (g/100 g protein dw)	Peptides (g/100 g protein dw)	Free amino acids (g/100 g protein dw)
AMA1	U[1]	–	27.87 ± 0.91^{Ac}	8.34 ± 0.28^{Ac}	3.09 ± 0.07^{Ac}
	G[2]	6.05 ± 0.37^{Cb}	42.84 ± 1.68^{Ab}	20.19 ± 0.90^{BCb}	10.59 ± 0.81^{Ab}
	GI[3]	12.99 ± 0.84^{Ea}	77.66 ± 2.01^{Aa}	35.78 ± 2.33^{Ea}	13.43 ± 0.63^{Ba}
AMA2	U[1]	–	14.78 ± 0.70^{Cc}	2.50 ± 0.10^{Ec}	1.14 ± 0.00^{Ec}
	G[2]	9.83 ± 0.29^{Bb}	41.89 ± 1.18^{Ab}	12.99 ± 0.81^{Eb}	2.55 ± 0.36^{Db}
	GI[3]	18.67 ± 0.88^{Da}	46.57 ± 1.87^{Ca}	54.25 ± 3.16^{Da}	7.04 ± 0.34^{Da}
AMA3	U[1]	–	7.97 ± 0.25^{Ec}	3.30 ± 0.12^{Dc}	2.68 ± 0.05^{Bb}
	G[2]	10.51 ± 0.34^{Bb}	26.43 ± 1.19^{Bb}	17.25 ± 0.83^{Db}	5.13 ± 0.99^{Ba}
	GI[3]	19.32 ± 0.72^{Da}	36.66 ± 1.73^{Da}	56.36 ± 2.05^{CDa}	6.62 ± 1.52^{Da}
AMA4	U[1]	–	10.15 ± 0.49^{Dc}	3.75 ± 0.07^{Cc}	1.62 ± 0.00^{Cc}
	G[2]	12.97 ± 0.54^{Ab}	28.67 ± 1.26^{Bb}	23.32 ± 0.44^{Ab}	5.60 ± 0.26^{Bb}
	GI[3]	34.57 ± 1.19^{Ba}	45.28 ± 2.02^{Ca}	66.81 ± 1.33^{ABa}	16.27 ± 1.55^{Aa}
AMA5	U[1]	–	16.15 ± 0.69^{Cc}	4.36 ± 0.08^{Bc}	1.13 ± 0.00^{Ec}
	G[2]	9.36 ± 0.32^{Bb}	27.79 ± 0.62^{Bb}	14.15 ± 0.37^{Eb}	4.41 ± 0.41B^{Cb}
	GI[3]	21.01 ± 0.91^{Da}	57.05 + 2.28^{Ba}	50.68 ± 3.21^{Da}	9.85 ± 0.90^{Ca}
AMA6	U[1]	–	11.67 ± 0.57^{Dc}	3.72 ± 0.13^{Cc}	1.49 ± 0.05^{Db}
	G[2]	10.36 ± 0.62^{Bb}	21.38 ± 0.95^{Cb}	22.17 ± 0.89^{ABb}	4.99 ± 0.40^{Ba}
	GI[3]	31.70 ± 1.07^{Ca}	42.86 ± 1.11^{Ca}	62.22 ± 1.47^{BCa}	5.98 ± 0.62^{Da}
BP	U[1]	–	18.39 ± 0.73^{Bc}	3.43 ± 0.13^{CDc}	0.76 ± 0.01^{Fc}
	G[2]	10.05 ± 1.16^{Bb}	40.24 ± 1.80^{Ab}	18.82 ± 0.51^{CDb}	3.15 ± 0.43^{CDb}
	GI[3]	37.49 ± 0.84^{Aa}	78.80 ± 3.50^{Aa}	72.12 ± 0.79^{Aa}	5.48 ± 0.56^{Da}

Data are expressed as mean ± SD, n = 3, except for free amino acids ($n = 2$). Different uppercase letters within each column indicate statistically significant differences among AMAs and BP at the same digestion phases (p-value <0.05). Different lowercase letters within each column indicate statistically significant differences among digestion phases within the same patty (p-value <0.05).

[1] Undigested.
[2] Ends of gastric digestion.
[3] Ends of gastrointestinal digestion.

(77.66 g/ 100 g protein dw) exhibited soluble protein levels comparable to BP (78.80 g/ 100 g protein dw), whereas all AMAs still contained equivalent or higher levels of free amino acids compared to BP. Notably, substantial differences were observed among the AMAs, even between AMA2 and AMA4, both of which were formulated with soy-pea blends. Consistent with their DH values, AMA4 (34.57%) exhibited greater digestibility than AMA2 (18.47%), as evidenced by its higher peptide content and free amino acid levels. These findings further highlight the complex interactions between proteins and non-protein ingredients and their influence on protein digestibility (Fontes-Candia et al., 2025; Semenova et al., 2014).

In AMA6, peptide content (22.17 g/ 100 g protein dw) was comparable to its soluble protein content (21.38 g/100 g protein dw). In contrast, in the other samples, peptide content was lower than soluble protein content, indicating the presence of larger protein fragments in the supernatant after gastric digestion. Except for AMA1 and BP, peptide content exceeded soluble protein content after intestinal digestion, suggesting that larger proteins or protein fragments were further hydrolysed into small peptides/free amino acid residues. For AMA1, SDS-PAGE analysis demonstrated the presence of several protein bands within the 20–25 kDa M_w range (Fig. 3b **– Intestinal phase**), indicating that large protein molecules remained after digestion. In the case of BP, the relatively small difference observed may be attributed to variations among the analytical methods employed.

Overall, these finding demonstrate that BP exhibited superior protein digestibility when assessed using the INFOGEST digestion model, whereas AMAs, despite tending to generate higher levels of free amino acids, showed greater variability in digestibility depending on formulation and matrix composition.

3.3.3. *In vitro protein digestibility*

This study largely followed the procedures described by Sousa, Portmann, et al. (2023) to compare the *in vitro* protein digestibility of AMAs and BP. In their work, the initial protein content of all samples was standardized to 40 mg. In this study, to better reflect the consumption of grilled patties, the sample weight was normalized to 2 g prior to digestion. Protein digestibility was subsequently evaluated using three analytical approaches: 1) total nitrogen content determined by Dumas method; 2) total primary amines quantified by TNBS assay; and 3) total amino acid composition analysed by HPLC. For TNBS assay, undigested patties and supernatant from methanol-precipitated intestinal digesta were subjected to microwave-assisted hydrolysis with 6 M HCl at 130 °C for 1 h (Afiuni-Zadeh et al., 2011). Regarding total amino acid analysis, samples were hydrolysed with 6 M HCl at 110 °C for 24 h.

Total digestibility of AMAs and BP, evaluated using three different methods, is shown in Fig. 4a. Previous studies reported no significant differences among the three analytical methods when evaluating dietary proteins (Sousa et al., 2023) and burgers (Sousa, Portmann, et al., 2023). Similarly, in this study, no significant difference was observed between total digestibility (%) calculated based on total nitrogen and primary amines. However, when compared with values calculated based on total amino acid, significant differences were detected for AMA3 and for BP. Digestibility values based on total nitrogen (60.27%) in AMA3 was significantly higher than that based on total amino acids (48.06%). Regarding BP, digestibility values calculated using total nitrogen (88.05%) and primary amines (94.85%) were significantly higher than that from total amino acids (74.55%). This difference may be primary attributed to amino acids containing two nitrogen atoms, such as histidine and lysine, which can lead to differences among the values calculated based on total nitrogen, compared with those from primary amines and total amino acids.

The average digestibility values are presented in Fig. 4b. BP exhibited a relatively high average value of total digestibility (85.81%), AMA4 showed a similar digestibility to BP, with an average value of 80.36%. In contrast, AMA1 (62.39%), AMA3 (55.63%), and AMA5 (59.32) exhibited lower digestibility values.

The total digestibility of grilled BP reported by Sousa, Portmann, et al. (2023) was nearly 100%, which is higher than the value observed in the present study (88.05%). This difference may be attributed to variations in BP size and processing conditions, as the thickness of BP and the grilling step could not be fully replicated in this study. Consequently, the Maillard reaction occurring in BP may become more pronounced, leading to a negative impact on protein digestibility (ALjahdali & Carbonero, 2019). On the other hand, their study reported an approximately 8% higher digestibility in alternative meat analogues, which may result from the difference in raw materials and ingredients compositions. A moderate correlation ($R^2 = 0.5522$) between total digestibility (%) and DH was observed, as shown in **Appendix Fig. S1**.

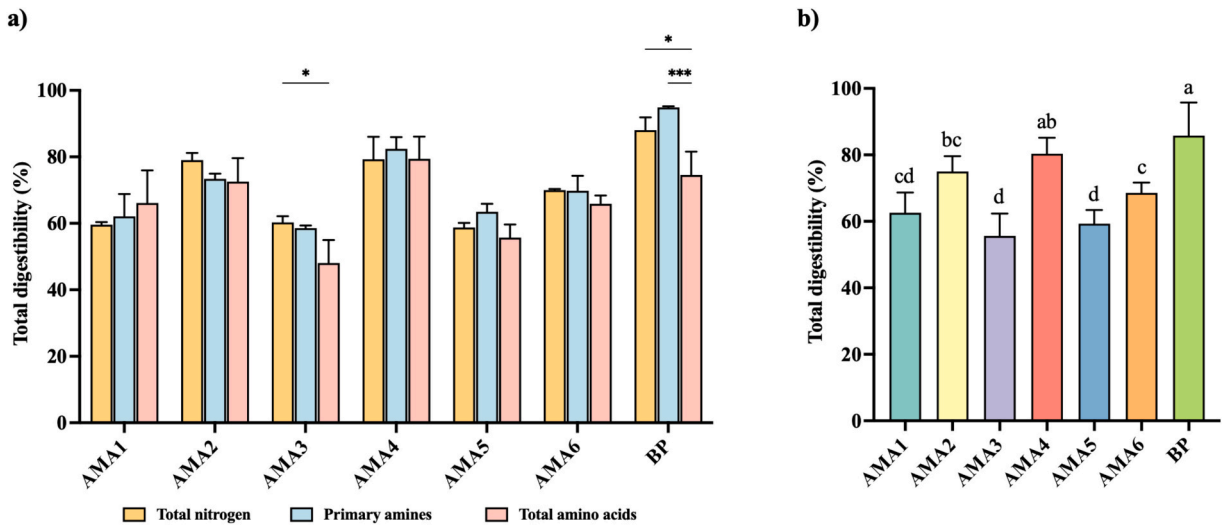
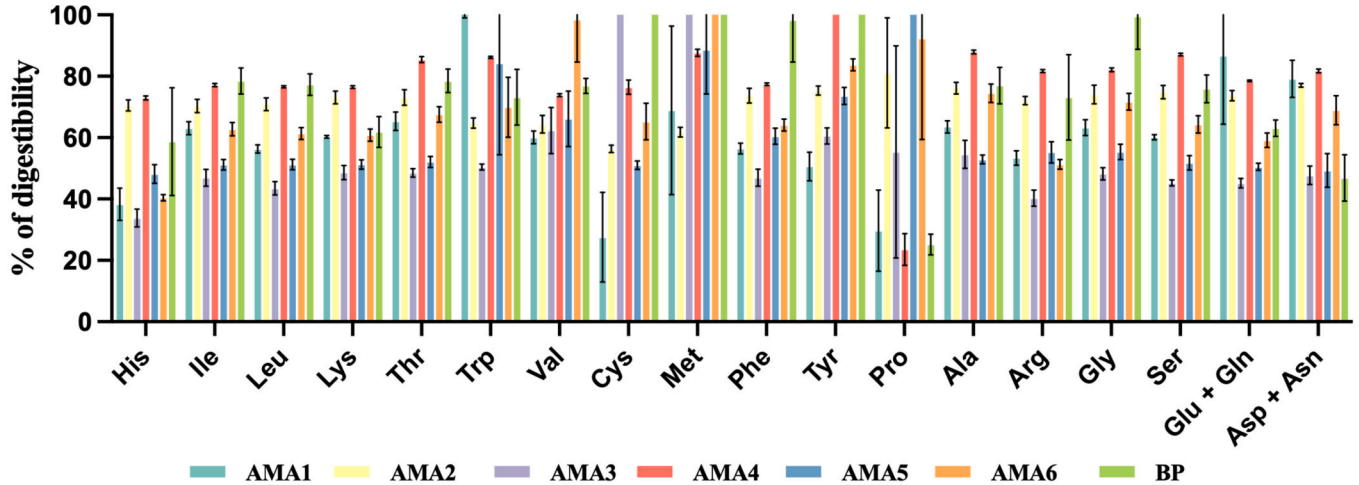


Fig. 4. Total digestibility of grilled commercial alternative meat analogues (AMAs) and conventional beef patty (BP). All substrates were analysed using a) three analytical parameters: total nitrogen ($n = 3$), primary amines (n = 3), and total amino acids (n = 3). Asterisks (* and ***) indicate statistically significant differences at the 0.05 and 0.001 levels, respectively. b) Average total digestibility of AMAs and BP calculated across the three analytical methods. Different lowercase letters indicate statistically significant differences among samples ($p < 0.05$).

This suggests that the supernatant obtained after INFOGEST digestion still contained protein fragments longer than 10 amino acids, which will be further investigated in subsequent analyses (Fig. 7).

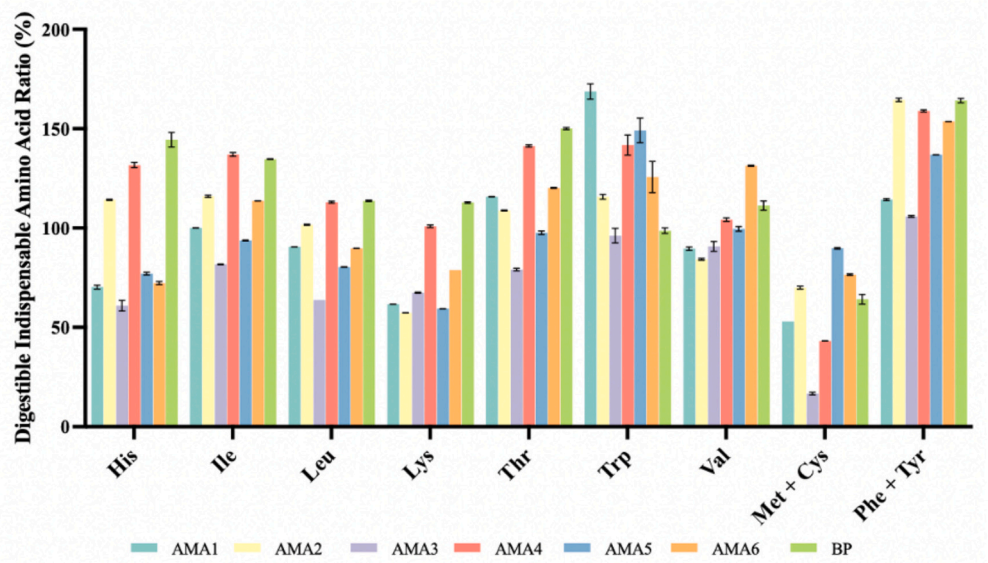
Fig. 5 presents the individual digestibility of amino acid residues. Although BP exhibited the highest overall digestibility among all samples, low digestibility was found in His (59%), Pro (23%), and Asp + Asn (47%). Regarding AMA4, apart from Pro (25%), the digestibility of the remaining amino acids was satisfactory, with overall values over 70%. Pro in AMA2 showed higher digestibility (81%), but the digestibility of all other amino acids was lower than that of AMA4. The remaining AMAs, particularly AMA3 and AMA5, showed low amino acid digestibility, with most values ranging between 45% and 55%.

The *in vitro* DIAAR was calculated according to Sousa, Portmann, et al. (2023). The content of each indispensable amino acids per unit of protein in AMAs and BP was multiplied by its corresponding individual amino acid digestibility, and the resulting values were then divided by the reference amino acid pattern for older children, adolescents, and adults. The *in vitro* DIAAR values were subsequently obtained and presented in Fig. 6. In this study, BP did not meet the reference requirement for Trp (98.7), or Met + Cys (64.2). Regarding AMAs, AMA3 showed particularly low nutritional quality, with all DIAAR values below 100%, apart from Phe + Tyr (105.8). A number of amino acids met the reference requirements in AMA1 (Ile, Thr, Trp, Phe + Tyr), AMA2 (His, Ile, Leu, Thr, Trp, Phe + Tyr), AMA5 (Trp, Phe + Tyr), and AMA6 (Ile, Thr, Trp, Val, and Phe + Tyr). AMA4 exhibited the most favourable nutritional profiles among AMAs, most indispensable amino



	His	Ile	Leu	Lys	Thr	Trp	Val	Cys	Met	Phe	Tyr	Pro	Ala	Arg	Gly	Ser	Glu + Gln	Asp + Asn
AMA1	38	63	56	60	65	100	60	28	69	56	51	30	64	53	63	60	87	79
AMA2	71	70	71	73	73	65	64	56	62	74	75	81	76	72	74	75	74	77
AMA3	34	47	43	49	49	50	62	100	100	47	61	55	55	40	48	45	45	48
AMA4	73	77	77	77	85	86	74	76	88	77	100	24	88	82	82	87	79	82
AMA5	48	51	51	51	52	84	66	51	89	60	74	100	53	55	55	52	51	49
AMA6	40	63	61	61	68	70	99	65	100	64	84	92	74	51	72	64	59	69
BP	59	78	77	62	78	73	77	100	100	98	100	23	77	73	99	76	63	47

Fig. 5. Individual amino acid digestibility of grilled commercial alternative meat analogues (AMAs) and conventional beef patty (BP). Data expressed as mean ± SD, $n = 3$.



	His	Ile	Leu	Lys	Thr	Trp	Val	Met + Cys	Phe + Tyr
AMA1	70.3	100.1	90.5	61.7	115.8	168.7	89.6	52.9	114.3
AMA2	114.2	115.9	101.7	57.3	108.8	115.7	84.3	70.0	164.6
AMA3	60.9	81.8	63.8	67.5	79.1	96.1	90.7	16.7	105.8
AMA4	131.7	137.0	113.0	100.9	141.3	141.8	104.2	43.1	158.9
AMA5	77.1	93.8	80.3	59.4	97.6	149.1	99.5	89.8	136.9
AMA6	72.3	113.7	89.7	78.9	120.2	125.7	131.4	76.5	153.7
BP	144.4	134.7	113.7	112.8	150.0	98.7	111.3	64.2	164.2

Fig. 6. *In vitro* DIAAR values were calculated for grilled commercial alternative meat analogues (AMAs) and conventional beef patty (BP). The values were determined based on the g amino acid/ per 100 g protein, suing the reference patterns for preschool children (6 months – 3 years). Data expressed as mean ± SD, $n = 3$.

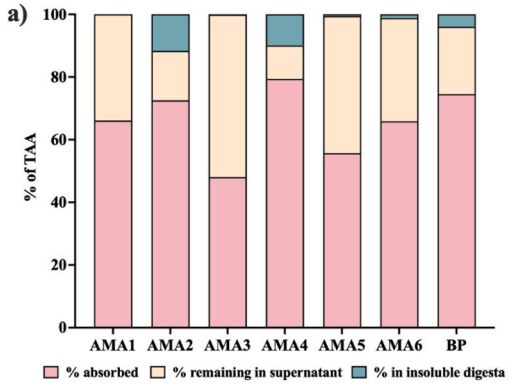
acids (His: 131.7; Ile: 137.0; Leu: 113.0; Lys: 100.9; Thr 141.3; Trp: 141.8; Val: 104.2, and Phe + Tyr: 158.9) meet the reference requirements. However, Met + Cys remained limiting, with an *in vitro* DIAAR value of only 43.1.

Regarding the *in vitro* DIAAS, which refers to the lowest *in vitro* DIAAR value in food protein, AMA6 showed a higher *in vitro* DIAAS (72.3) than BP (64.2). All the other AMAs exhibited lower *in vitro* DIAAS values than BP, with AMA3 presenting the lowest score (16.7), indicating its relatively poor protein quality. In the present study, *in vitro* DIAAS values for all samples were below 75%, indicating that none can be classified as good-quality protein sources and that they may pose a risk of inadequate indispensable amino acid intake (Marinangeli & House, 2017). These findings are consistent with those reported in a recent study on plant-based chicken analogues, which, after recalculating *in vitro* DIAAS values using the reference amino acid pattern for older children, adolescents, and adults, reported scores ranging from 61 to 76 (Alotaibi et al., 2026). Lys or Met + Cys were also identified as the primary limiting amino acids, highlighting that insufficient availability of specific IAA remains a common nutritional challenge in meat analogue products. As previous studies have demonstrated that beef protein (Sousa, Portmann, et al., 2023) and soy protein (Kudełka et al., 2021) are good sources of indispensable amino acids, the relatively low *in vitro* DIAAS values observed in this study (*e.g.*, AMA6: 72.3 and BP: 64.2) are likely attributed to the grilling process, which may reduce the bioavailability of certain indispensable amino acids (Bailey et al., 2020). For instance, Lys is particularly susceptible to heat treatment, in which its ε-amino group reacts with reducing sugars to form nutritionally unavailable 'block' lysine (Mehta & Deeth, 2016). Met + Cys are prone to oxidative reactions leading to disulfide bond rearrangements, formation of dehydroalanine and the generation of cross-linked compounds such as lanthionine and lysinoalanine (Friedman, 1999). His can also be oxidised to form 2-oxo-histidine and may participate in heat- and alkaline- induced cross-linking reactions, including the formation of histidinoalanine (Uchida, 2003). Therefore, supplementing these limiting amino acids in the formulation could represent an effective strategy for improving protein quality.

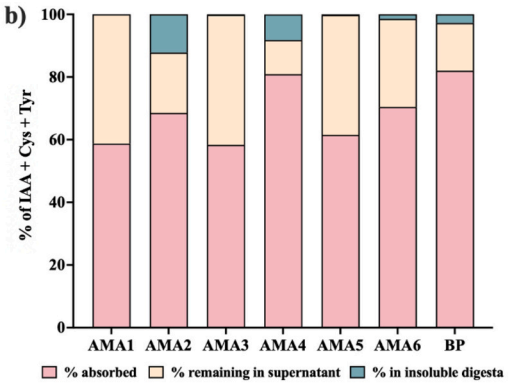
A correlation between IVPDCAAS and *in vitro* DIAAS values for AMAs and BP is shown in **Appendix Fig. S2**. The R^2 value of 0.6420 indicates a moderate relationship between the two parameters, suggesting that IVPDCAAS can provide a general estimate of protein quality. However, it does not consistently correspond to INFOGEST-DIAAS values across all samples. Furthermore, given the limited number of products analysed, this correlation should be considered exploratory rather than predictive. Comparable IVPDCCAS and *in vitro* DIAAS values were observed for AMA3 (14.6 *vs.* 16.7) and AMA4 (44.0 *vs.* 43.1). In contrast, BP exhibited a lower IVPDCAAS (54.8) than *in vitro* DIAAS (64.1), which can be attributed to the complete digestion of its limiting amino acids (Cys: 100% and Met: 100%), despite a lower overall IVPD (85.39%). In contrast, several AMAs, particularly AMA1 (77.2 *vs.* 52.9) and AMA5 (98.1 *vs.* 59.4), showed substantially lower *in vitro* DIAAS values compared to IVPDCAAS. These discrepancies highlight that the digestibility of individual indispensable amino acids may differ from overall protein digestibility and can therefore significantly influence protein quality assessment, potentially limiting the accuracy of IVPDCAAS as indicator of *in vitro* DIAAS.

Martineau-Côté et al. (2024) estimated maximum amino acid absorption based on the amino acid content detected in the digesta supernatant. Following a similar approach, the present study quantified the absorbed (bioavailable) fraction extracted with 80% methanol, the fraction remaining in the digesta supernatant (determined as the total amino acid content in the supernatant minus the absorbed fraction), and the insoluble digesta fraction, as shown in Fig. 7.

Considering total amino acids, AMA4 (79.4%) showed a higher absorbed fraction than BP (74.5%). By contrast, AMA3 exhibited an



	% absorbed	% remaining in supernatant	% in insoluble digesta
AMA1	66.1	33.9	0.0
AMA2	72.6	15.8	11.7
AMA3	48.1	51.9	0.1
AMA4	79.4	10.7	9.9
AMA5	55.7	43.8	0.5
AMA6	65.9	33.0	1.1
BP	74.5	21.6	3.9



	% absorbed	% remaining in supernatant	% in insoluble digesta
AMA1	58.7	41.3	0.0
AMA2	68.6	19.3	12.2
AMA3	58.4	41.5	0.1
AMA4	80.9	10.9	8.2
AMA5	61.6	38.3	0.2
AMA6	70.5	28.1	1.4
BP	82.0	15.3	2.7

Fig. 7. Distribution (%) of a) total amino acids (TAA) and b) indispensable amino acid (IAA) + Cys + Tyr among absorbed fractions, remaining in supernatant, and in insoluble digesta in commercial alternative meat analogues (AMAs) and conventional beef patty (BP).

extremely low absorbed fraction (48.1%). Nevertheless, a substantial proportion of amino acids in AMA3 (51.9%) remained in the digesta supernatant and may still be available for absorption. For the remaining AMAs and BP, the proportion of amino acids remaining in the supernatant showed considerable variation, with values ranging from 10.7% to 33.9%. Particular attention should be given to AMA2 and AMA4, in which 11.7% and 9.9% of amino acids, respectively, were found in the insoluble digesta fraction. As these fractions are less likely to be absorbed, they should not be considered nutritionally available when evaluating the protein quality of food formulations. When the amino acid residues were limited to indispensable amino acids (IAAs) plus Cys + Met, the proportion of the bioavailable fraction decreased in AMA1 (from 66.1% to 58.7%) and AMA2 (from 72.6% to 68.6%). Only minor changes were observed in AMA4 (from 79.4% to 80.9%). Apart from these samples, an increase in the bioavailable fraction were observed among AMA3 (from 48.1% to 58.4%), AMA5 (from 55.7% to 61.6%), AMA6 (from 65.9% to 70.5%) and BP (74.5% to 82.0%). The observed changes in AMAs and BP were associated with shifts between the absorbed fraction and the fraction remaining in the supernatant, because the proportion of amino acids in the insoluble digesta showed no appreciable variation across total amino acids and EAA + Cys + Tyr (< 2%). High M_w peptides present in the digesta supernatant have also been reported in previous studies. For example, Guzmán-Ortiz et al. (2024) observed 42% of peptides generated by lupin after digestion had $M_w > 1$ kDa. Similarly, Wang et al. (2024) reported that 40% of peptides in digested supernatant had $M_w > 1$ kDa. To the best of our knowledge, this is the first study to report the presence of protein fragments in the insoluble pellet obtained after centrifugation of the digesta. These insoluble protein fractions are more likely associated with the formation of insoluble complexes with other macromolecules, resulting from Maillard reactions during grilling (Tamanna & Mahmood, 2015). Further research is needed to determine whether these insoluble fractions can undergo additional fermentation in the colon and subsequently release amino acids with potential beneficial effects, such as Trp (Friedman, 2018).

However, several limitations should be addressed. First, grilling lacks standardized processing protocol, and the selected temperature and processing time may not be suitable for preparing AMAs and BP. In addition, to minimize lipid interference, hexane was used for fat removal. This may lead to an overestimation of protein digestibility, as lipids can reduce enzyme accessibility to protein substrate. Moreover, protein contents were adjusted to equivalent levels across the AMAs and BP, which may lead to an underestimation of the true BP, as it originally contained the highest protein content. Furthermore, the lack of pre-oxidation prior to acid hydrolysis may have led to underestimation of Cys and, to a lesser extent, Met (Rutherfurd et al., 2007). Therefore, AAS, IVPDCAAS, *in vitro* DIAAS values for samples in which Met + Cys were limiting amino acids should be interpret with caution. Reactive lysine was not directly measured in this study, representing a limitation that should be addressed. This is particularly relevant for high-carbohydrate meat analogues, where Maillard reactions may be more extensive and could substantially affect lysine availability (Brestensk et al., 2014). Although the INFOGEST digestion model is considered physiologically relevant and has shown good agreement with previous *in vivo* observations, validation remains necessary. For example, comparable digestibility values have been reported for pea (97 *vs.* 95), bean (96 *vs.* 88), bran (80 *vs.* 82), and zein (63 *vs.* 63) (Sousa et al., 2023). Similar findings were also reported by Fanelli et al. (2022), where DIAAS values for lean beef burgers differed by only 0.18 between predicted and measured values in an *in vivo* pig study, whereas those for Impossible Burgers differed by 1.62. These studies suggested that the INFOGEST protocol could provide promising physiological relevant estimates of protein digestibility and DIAAS. However, the values reported in the present study should be still interpreted as *in vitro* DIAAS, confirmation using *in vivo* digestibility models remains necessary.

4. Conclusion

This study systematically compared 6 AMAs with BP in terms of cooking loss, appearance, textural, digestion characteristics, and protein quality. BP exhibited significant different cooking loss compared to all AMAs. Meanwhile, most AMAs, except AMA5, exhibited reduced browning after cooking, highlighting a remaining gap in reproducing the characteristic appearance of grilled BP. Textural differences were primarily observed in hardness and chewiness, indicating that further improvements in AMA texture are required. When protein quality was assessed using IVPDCAAS values, several AMAs (AMA1, AMA2, AMA5, and AMA6) exhibited higher scores than BP. However, these results should be interpreted with caution, as the pH-drop method appears to underestimate the protein quality of BP, given that its limiting amino

acids (Cys + Met) were fully digested following static INFOGEST digestion. After accounting for the individual digestibility of amino acids, only AMA6 achieved a higher *in vitro* DIAAS value than BP. Although a moderate correlation was observed between IVPDCAAS and *in vitro* DIAAS, the discrepancy between two evaluation methods remains notable. These findings suggest that relying solely on IVPDCAAS may overestimate the nutritional equivalence and substitution potential of AMAs. This highlights the importance of considering the digestibility of individual indispensable amino acids in protein quality assessment. Therefore, *in vitro* DIAAS should be considered as a more physiologically relevant protein quality parameter, particularly for processed protein products. Notably, based on *in vitro* DIAAS values, neither AMAs nor BP fully met the nutritional requirements for older children, adolescents, and adult, highlighting limitations in current formulations. Given that the complete amino acid profile of beef protein is well established and widely recognized, the adverse effects of grilling on amino acid availability and the proportion of amino acids in absorbable forms should be considered in nutritional assessment when evaluating meat and plant-based meat analogues. Overall, current AMAs demonstrate some meaningful progress (particularly in nutritional value), none of them achieved comprehensive equivalence to BP across all evaluated parameters, emphasising the need for continued optimisation of appearance, textural properties, and nutritional quality enhancement in both academic research and industrial development.

CRediT authorship contribution statement

Ruixian Han: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maryam Binti Zulkifli:** Methodology, Investigation. **Oscar Abel Sánchez Velázquez:** Methodology, Investigation. **Yan Wang:** Methodology, Investigation. **Alexander Lim:** Methodology, Investigation. **María Lilibeth Manzanilla Valdez:** Methodology, Investigation. **Martin Mondor:** Writing – review & editing, Validation, Supervision, Methodology. **Evi Paximada:** Supervision, Resources, Methodology. **Alan Javier Hernández-Álvarez:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Funding

This work was funded by Innovate UK, Project number: 10075778, Project title: STARS® x University of Leeds Better Food for All, Competition: Better Food for All: Innovation for improved nutrition, mid-stage. This work was funded by the UK National Alternative Proteins Innovation Centre (NAPIC), which is an Innovation and Knowledge Centre funded by the Biotechnology and Biological Sciences Research Council and Innovate UK (Grant Ref: BB/Z516119/1).

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2026.150279.

Data availability

No data was used for the research described in the article.

References

Adler-Nissen, J. (1979). Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *Journal of Agricultural and Food Chemistry, 27*(6), 1256–1262.
Afiuni-Zadeh, S., Guo, X., Azimi, G., & Lankmayr, E. (2011). Optimization and application of microwave-assisted acid hydrolysis for rapid quantification of protein oxidation markers using LC–MS. *Talanta, 85*(4), 1835–1841.
Ahmed, H. G. M.-D., Naeem, M., Faisal, A., Fatima, N., Tariq, S., & Owais, M. (2023). Enriching the content of proteins and essential amino acids in legumes. In *Legumes biofortification* (pp. 417–447). Springer.
Ajomiwe, N., Boland, M., Phongthai, S., Bagiyal, M., Singh, J., & Kaur, L. (2024). Protein nutrition: Understanding structure, digestibility, and bioavailability for optimal health. *Foods, 13*(11), 1771.
ALjahdali, N., & Carbonero, F. (2019). Impact of Maillard reaction products on nutrition and health: Current knowledge and need to understand their fate in the human digestive system. *Critical Reviews in Food Science and Nutrition, 59*(3), 474–487.
AOAC. (1990). Official methods of analysis. *Journal of the Association of Official Analytical Chemists, 62*, 2742–2744.
Auer, J., Alminger, M., Marinea, M., Johansson, M., Zamaratskaia, G., Högberg, A., & Langton, M. (2024). Assessing the digestibility and estimated bioavailability/bioaccessibility of plant-based proteins and minerals from soy, pea, and faba bean ingredients. *LWT, 197*, Article 115893.
Bailey, H. M., Mathai, J. K., Berg, E. P., & Stein, H. H. (2020). Most meat products have digestible indispensable amino acid scores that are greater than 100, but processing may increase or reduce protein quality. *British Journal of Nutrition, 124*(1), 14–22.
Bakhsh, A., Lee, S. J., Lee, E. Y., Hwang, Y.-H., & Joo, S.-T. (2021). Evaluation of rheological and sensory characteristics of plant-based meat analog with comparison to beef and pork. *Food Science of Animal Resources, 41*(6), 983.
Barrón-Hoyos, J. M., Archuleta, A. R., del Refugio Falcón-Villa, M., Canett-Romero, R., Cinco-Moroyoqui, F. J., Romero-Barancini, A. L., & Rueda-Puente, E. O. (2013). Protein quality evaluation of animal food proteins by in-vitro methodologies. *Food and Nutrition Sciences, 4*(4), 376–384.
Baugreet, S., Gomez, C., Auty, M. A., Kerry, J. P., Hamill, R. M., & Brodkorb, A. (2019). In vitro digestion of protein-enriched restructured beef steaks with pea protein isolate, rice protein and lentil flour following sous vide processing. *Innovative Food Science & Emerging Technologies, 54*, 152–161.
Beniwal, A. S., Singh, J., Kaur, L., Hardacre, A., & Singh, H. (2021). Meat analogs: Protein restructuring during thermomechanical processing. *Comprehensive Reviews in Food Science and Food Safety, 20*(2), 1221–1249.
Benković, M., Jurinjak Tušek, A., Sokač Cvetnić, T., Jurina, T., Valinger, D., & Gajdoš Kljusurić, J. (2023). An overview of ingredients used for plant-based meat analogue production and their influence on structural and textural properties of the final product. *Gels, 9*(12), 921.
Bhat, Z. F., Morton, J. D., Bekhit, A. E. D. A., Kumar, S., & Bhat, H. F. (2021). Thermal processing implications on the digestibility of meat, fish and seafood proteins. *Comprehensive Reviews in Food Science and Food Safety, 20*(5), 4511–4548.
Bieck, K., Ebert, F., Grune, T., & Raupbach, J. (2025). Maillard reaction products in plant-based dairy alternatives and their release during simulated gastrointestinal digestion. *Current Research in Food Science, 10*, Article 100994.
Bourne, M., Kenny, J., & Barnard, J. (1978). Computer-assisted readout of data from texture profile analysis curves 1. *Journal of Texture Studies, 9*(4), 481–494.
Boye, J., Wijesinha-Bettoni, R., & Burlingame, B. (2012). Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. *British Journal of Nutrition, 108*(S2), S183–S211.
Brestensk, M., Nitrayová, S., Heger, J., Patráš, P., Rafay, J., & Sirotkin, A. (2014). Methods for determination reactive lysine in heat-treated foods and feeds. *Journal of Microbiology, Biotechnology and Food Sciences, 4*(1), 13–15.
Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., … Carrière, F. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols, 14*(4), 991–1014.
Chang, L., Gu, Z., Bandillo, N., Chen, B., & Rao, J. (2023). Fractionation, structural characteristics, functionality, aromatic profile, and in vitro digestibility of lentil (Lens culinaris) proteins. *ACS Food Science & Technology, 3*(3), 478–488.
Cirkovic Velickovic, T. D., & Stanic-Vucinic, D. J. (2018). The role of dietary phenolic compounds in protein digestion and processing technologies to improve their antinutritive properties. *Comprehensive Reviews in Food Science and Food Safety, 17*(1), 82–103.
Clark, M., & Tilman, D. (2017). Comparative analysis of environmental impacts of agricultural production systems, agricultural input efficiency, and food choice. *Environmental Research Letters, 12*(6), Article 064016.
Day, L., Cakebread, J. A., & Loveday, S. M. (2022). Food proteins from animals and plants: Differences in the nutritional and functional properties. *Trends in Food Science & Technology, 119*, 428–442.
Dekkers, B. L., Boom, R. M., & van der Goot, A. J. (2018). Structuring processes for meat analogues. *Trends in Food Science & Technology, 81*, 25–36.
Dumitrascu, L., Stanciuc, N., Grigore-Gurgu, L., & Aprodu, L. (2019). Spectroscopic and molecular modeling investigations on heat induced behaviour of soy proteins. *Emirates Journal of Food and Agriculture, 31*(8), 569–580.
El Sadig, R., & Wu, J. (2024). Are novel plant-based meat alternatives the healthier choice? *Food Research International, 183*, Article 114184.
do Evangelho, J. A., Vanier, N. L., Pinto, V. Z., De Berrios, J. J., Dias, A. R. G., & da Rosa Zavareze, E. (2017). Black bean (*Phaseolus vulgaris* L.) protein hydrolysates: Physicochemical and functional properties. *Food Chemistry, 214*, 460–467.
Fanelli, N. S., Bailey, H. M., Thompson, T. W., Delmore, R., Nair, M. N., & Stein, H. H. (2022). Digestible indispensable amino acid score (DIAAS) is greater in animal-based

burgers than in plant-based burgers if determined in pigs. *European Journal of Nutrition, 61*(1), 461–475.
FAO. (2013). *Dietary protein quality evaluation in human nutrition: Report of an FAO expert consultation (food and nutrition paper 92)*.
Ferreira, V. C., Morcuende, D., Madruga, M. S., Silva, F. A., & Estévez, M. (2018). Role of protein oxidation in the nutritional loss and texture changes in ready-to-eat chicken patties. *International Journal of Food Science & Technology, 53*(6), 1518–1526.
Finnigan, T. J., Wall, B. T., Wilde, P. J., Stephens, F. B., Taylor, S. L., & Freedman, M. R. (2019). Mycoprotein: The future of nutritious nonmeat protein, a symposium review. *Current Developments in Nutrition, 3*(6), Article nzz021.
Fogelholm, M., Kanerva, N., & Männistö, S. (2015). Association between red and processed meat consumption and chronic diseases: The confounding role of other dietary factors. *European Journal of Clinical Nutrition, 69*(9), 1060–1065.
Fontes-Candia, C., Díaz-Piñero, L., Vega-Gómez, L. M., Molina-Gilarranz, I., & Martínez-Sanz, M. (2025). Relevance of protein–polysaccharide interactions on nutritional quality and gastrointestinal digestion of protein-based foods. *Journal of Agricultural and Food Chemistry, 73*(9), 4998–5004.
Friedman, M. (1999). Chemistry, biochemistry, nutrition, and microbiology of lysinoalanine, lanthionine, and histidinoalanine in food and other proteins. *Journal of Agricultural and Food Chemistry, 47*(4), 1295–1319.
Friedman, M. (2018). Analysis, nutrition, and health benefits of tryptophan. *International Journal of Tryptophan Research, 11*, 1178646918802282.
Gardner, T., Vierck, K. R., Martini, S., Allen, K., Ban, H., Miller, R. K., … Vierck, K. (2020). Thermophysical properties of beef steaks of varying thicknesses cooked with low and high grill surface temperatures. *Meat and Muscle Biology, 4*(1).
Godfray, H. C. J., Aveyard, P., Garnett, T., Hall, J. W., Key, T. J., Lorimer, J., … Jebb, S. A. (2018). Meat consumption, health, and the environment. *Science, 361*(6399), Article eaam5324.
Alotaibi, M., Muleya, M., Salter, A., Hoad, C., & Eldeghaidy, S. (2026). Comparison of the protein quality of chicken and plant-based chicken analogues using the INFOGEST in vitro digestion system. *Food Chemistry, 520*, 149734.
Goldberg, E. M., Sá, A. G., Franczyk, A. J., Krul, E. S., Lyle, B., Jing, M., … Brisson, G. (2026). A collaborative study to validate in vitro assays for protein digestibility assessment using pH-drop and pH-stat methods. *Journal of Food Composition and Analysis, 151*, 108918.
Gorissen, S. H., Crombag, J. J., Senden, J. M., Waterval, W. H., Bierau, J., Verdijk, L. B., & Van Loon, L. J. (2018). Protein content and amino acid composition of commercially available plant-based protein isolates. *Amino Acids, 50*(12), 1685–1695.
Guajardo-Flores, D., Pérez-Carrillo, E., Romo-López, I., Ramírez-Valdez, L. E., Moreno-García, B. E., & Gutiérrez-Uribe, J. A. (2017). Effect of dehulling and germination on physicochemical and pasting properties of black beans (Phaseolus vulgaris L.). *Cereal Chemistry, 94*(1), 98–103.
Guzmán-Ortiz, F. A., Peñas, E., Frias, J., Castro-Rosas, J., & Martínez-Villaluenga, C. (2024). How germination time affects protein hydrolysis of lupins during gastroduodenal digestion and generation of resistant bioactive peptides. *Food Chemistry, 433*, Article 137343.
Han, R., Wang, Y., Yang, Z., Micklethwaite, S., Mondor, M., Paximada, E., & Hernández-Álvarez, A. J. (2025). Industrial-scale fractionation of fava bean, chickpea, and red lentil: A comparative analysis of composition, antinutrients, nutrition, structure, and functionality. *Current Research in Food Science, 11*, 101152.
Hu, J., Li, X., Yu, Q., Wang, W., & Bi, J. (2023). Understanding the impact of pectin physicochemical variation on browning of simulated Maillard reaction system in thermal and storage processing. *International Journal of Biological Macromolecules, 240*, Article 124347.
Hu, Y., Sun-Waterhouse, D., Liu, P.-z., Cui, C., & Wang, W. (2019). Modification of rice protein with glutaminase for improved structural and sensory properties. International Journal of Food Science and Technology, 54(7), 2458–2467.
Hughes, G. J., Ryan, D. J., Mukherjea, R., & Schasteen, C. S. (2011). Protein digestibility-corrected amino acid scores (PDCAAS) for soy protein isolates and concentrate: Criteria for evaluation. *Journal of Agricultural and Food Chemistry, 59*(23), 12707–12712.
Kalman, D. S. (2014). Amino acid composition of an organic brown rice protein concentrate and isolate compared to soy and whey concentrates and isolates. *Foods, 3*(3), 394–402.
Kasim, R., & Kasim, M. U. (2015). Biochemical changes and color properties of fresh-cut green bean (Phaseolus vulgaris L. cv. Gina) treated with calcium chloride during storage. *Food Science and Technology (Campinas), 35*(2), 266–272.
Khan, R., Brishti, F. H., Arulrajah, B., Goh, Y. M., Abd Rahim, M. H., Karim, R., … Saari, N. (2024). Mycoprotein as a meat substitute: Production, functional properties, and current challenges-a review. *International Journal of Food Science and Technology, 59*(1), 522–544.
Kudełka, W., Kowalska, M., & Popis, M. (2021). Quality of soybean products in terms of essential amino acids composition. *Molecules, 26*(16), 5071.
Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*(5259), 680–685.
Lin, L., Yang, H., Chi, C., & Ma, X. (2020). Effect of protein types on structure and digestibility of starch-protein-lipids complexes. *LWT, 134*, Article 110175.
Lonchamp, J., Stewart, K., Munialo, C. D., Evans, L., Akintoye, M., Gordon, S., … Euston, S. R. (2022). Mycoprotein as novel functional ingredient: Mapping of functionality, composition and structure throughout the Quorn fermentation process. *Food Chemistry, 396*, Article 133736.
Mabrouki, S., Abid, K., Kaihara, H., Patrucco, S. G., Tassone, S., & Barbera, S. (2024). Assessing texture profile analysis in natural state versus texture profile analysis with back extrusion post-homogenization of cooked pea protein-based and meat patties: A comparative study. *Future Foods, 9*, Article 100345.
Maina, J. W. (2018). Analysis of the factors that determine food acceptability. *The Pharma Innovation Journal, 7*(5), 253–257.
Majumder, R., Miatur, S., Saha, A., & Hossain, S. (2024). Mycoprotein: Production and nutritional aspects: A review. *Sustainable Food Technology, 2*(1), 81–91.
Mali, P. S., & Kumar, P. (2025). Effect of microwave irradiation on the functional properties, digestion behavior, and structural characteristics of protein isolate from black beans (Phaseolus vulgaris L.). *Innovative Food Science & Emerging Technologies, 101*, Article 103951.
Manzanilla-Valdez, M. L., Boesch, C., Martinez-Villaluenga, C., Montaño, S., & Hernández-Álvarez, A. J. (2024). Enhancing quinoa (Chenopodium quinoa Willd) protein extraction: Alkaline solubilization coupled to isoelectric precipitation effects on structure, digestibility and antinutrients. *Food Hydrocolloids for Health, 6*, Article 100191.
Manzanilla-Valdez, M. L., Boesch, C., Orfila, C., Montaño, S., & Hernández-Álvarez, A.-J. (2024). Unveiling the nutritional spectrum: A comprehensive analysis of protein quality and antinutritional factors in three varieties of quinoa (Chenopodium quinoa wild). *Food Chemistry: X, 24*, Article 101814.
Manzanilla-Valdez, M. L., Ma, Z., Mondor, M., & Hernández-Álvarez, A. J. (2024). Decoding the duality of Antinutrients: Assessing the impact of protein extraction methods on plant-based protein sources. *Journal of Agricultural and Food Chemistry, 72*(22), 12319–12339.
Marinangeli, C. P., & House, J. D. (2017). Potential impact of the digestible indispensable amino acid score as a measure of protein quality on dietary regulations and health. *Nutrition Reviews, 75*(8), 658–667.
Martineau-Côté, D., Achouri, A., Pitre, M., Karboune, S., & L'Hocine, L. (2024). Improved in vitro gastrointestinal digestion protocol mimicking brush border digestion for the determination of the digestible indispensable amino acid score (DIAAS) of different food matrices. *Food Research International, 178*, Article 113932.
Maseko, K. H., Regnier, T., Bartels, P., & Meiring, B. (2025). Mushroom mycelia as sustainable alternative proteins for the production of hybrid cell-cultured meat: A review. *Journal of Food Science, 90*(2), Article e70060.
Mehta, B. M., & Deeth, H. C. (2016). Blocked lysine in dairy products: Formation, occurrence, analysis, and nutritional implications. *Comprehensive Reviews in Food Science and Food Safety, 15*(1), 206–218.
Mokrzycki, W., & Tatol, M. (2011). Colour difference∆ EA survey. *Machine Graphics and Vision, 20*(4), 383–411.
Multescu, M., Culetu, A., & Susman, I. E. (2024). Screening of the nutritional properties, bioactive components, and antioxidant properties in legumes. *Foods, 13*(22), 3528.
Nazir, H. F., Tapia-Tussell, R., Nosworthy, M. G., Sánchez-Velázquez, O. A., Dowle, A., Ifie, I., & Hernández-Álvarez, A. J. (2025). Nutritional Value, Antinutritional Factors, and Protein Quality of Brosimum alicastrum Seeds: A Sustainable Protein Source. *Journal of Agricultural and Food Chemistry, 73*(37), 23580–23594.
Nosworthy, M. G., Hernandez-Alvarez, A. J., Franczyk, A. J., Medina, G., Neufeld, J., Arcand, Y., … House, J. D. (2023). Effect of cooking on the in vitro and in vivo protein quality of soy, oat and wheat varieties. *Cereal Chemistry, 100*(2), 460–472.
Nosworthy, M. G., Medina, G., Franczyk, A. J., Neufeld, J., Appah, P., Utioh, A., … House, J. D. (2018). Effect of processing on the in vitro and in vivo protein quality of beans (Phaseolus vulgaris and Vicia Faba). *Nutrients, 10*(6), 671.
Okeudo-Cogan, M. C., Murray, B. S., Ettelaie, R., Connell, S. D., Radford, S. J., Micklethwaite, S., & Sarkar, A. (2023). Understanding the microstructure of a functional meat analogue: Demystifying interactions between fungal hyphae and egg white protein. *Food Hydrocolloids, 140*, Article 108606.
Orlien, V., Aalaei, K., Poojary, M. M., Nielsen, D. S., Ahrné, L., & Carrascal, J. R. (2023). Effect of processing on in vitro digestibility (IVPD) of food proteins. *Critical Reviews in Food Science and Nutrition, 63*(16), 2790–2839.
Osuna-Gallardo, E. I., Cuevas-Rodríguez, E. O., Sepúlveda-García, C. I., Reyes-Moreno, C., León-López, L., Han, R., & Hernández-Álvarez, A. J. (2023). Impact of cooking and extrusion processing on nutritional, antinutritional, and techno-functional characteristics of indigenous bean (Phaseolus coccineus). *ACS Food Science & Technology, 3*(11), 1835–1853.
Portanguen, S., Ikonic, P., Clerjon, S., & Kondjoyan, A. (2014). Mechanisms of crust development at the surface of beef meat subjected to hot air: An experimental study. *Food and Bioprocess Technology, 7*(11), 3308–3318.
Qin, D., Wang, L., Fang, R., Yu, Z., Mo, L., & Liu, M. (2022). Peptidomics analysis of enzymatic hydrolysis beef. *Food Science and Biotechnology, 31*(10), 1267–1275.
Qin, P., Wang, T., & Luo, Y. (2022). A review on plant-based proteins from soybean: Health benefits and soy product development. *Journal of Agriculture and Food Research, 7*, Article 100265.
Rasul, S., Tarique, M., Alkaabi, A. O. H., Kamal-Eldin, A., Chiang, J. H., & Yuliarti, O. (2024). Evaluating the effects of insoluble date fruit (Phoenix dactylifera L.) fibers on meat analogue patties composed of pea and wheat protein isolates. *Food Research International, 197*, Article 115158.
Ritchie, H., Rosado, P., & Roser, M. (2017). Meat and Dairy Production. Retrieved from: https://ourworldindata.org/meat-production (Accessed Aug 31 2025)..
Rodríguez, C., Frias, J., Vidal-Valverde, C., & Hernández, A. (2008). Correlations between some nitrogen fractions, lysine, histidine, tyrosine, and ornithine contents during the germination of peas, beans, and lentils. *Food Chemistry, 108*(1), 245–252.
Rohlik, B.-A., Øiseth, S., Garcia, T. B., Sabarez, H., Watkins, P., Oytam, Y., & Buckow, R. (2017). *Muscle structure and water retention in fresh and cooked meat products-FINAL REPORT*.
Rutherfurd, S. M. (2010). Methodology for determining degree of hydrolysis of proteins in hydrolysates: A review. *Journal of AOAC International, 93*(5), 1515–1522.
Rutherfurd, S. M., Fanning, A. C., Miller, B. J., & Moughan, P. J. (2015). Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats. *The Journal of Nutrition, 145*(2), 372–379.

Rutherfurd, S. M., Schneuwly, A., & Moughan, P. J. (2007). Analyzing sulfur amino acids in selected feedstuffs using least-squares nonlinear regression. *Journal of Agricultural and Food Chemistry, 55*(20), 8019–8024.
Samard, S., & Ryu, G. H. (2019). A comparison of physicochemical characteristics, texture, and structure of meat analogue and meats. *Journal of the Science of Food and Agriculture, 99*(6), 2708–2715.
Sánchez-Velázquez, O. A., Ribéreau, S., Mondor, M., Cuevas-Rodríguez, E. O., Arcand, Y., & Hernández-Álvarez, A. J. (2021). Impact of processing on the in vitro protein quality, bioactive compounds, and antioxidant potential of 10 selected pulses. *Legume Science, 3*(2), Article e88.
Schaafsma, G. (2000). The protein digestibility–corrected amino acid score. *The Journal of Nutrition, 130*(7), 1865S–1867S.
Scheeder, M., Casutt, M. M., Roulin, M., Escher, F., Dufey, P.-A., & Kreuzer, M. (2001). Fatty acid composition, cooking loss and texture of beef patties from meat of bulls fed different fats. *Meat Science, 58*(3), 321–328.
Schmidt, F., Blankart, M., Wanger, J., Scharfe, M., Scheuerer, T., & Hinrichs, J. (2022). Upscaling of alkaline pea protein extraction from dry milled and pre-treated peas from laboratory to pilot scale: Optimization of process parameters for higher protein yields. *Journal of Food Measurement and Characterization, 16*(6), 4904–4913.
Semenova, M., Moiseenko, D., Grigorovich, N., Anokhina, M., Antipova, A., Belyakova, L., … Tsapkina, E. (2014). Protein–polysaccharide interactions and digestion of the complex particles. In *Food structures, digestion and health* (pp. 169–192). Elsevier.
Shen, Y., Hong, S., Du, Z., Chao, M., O'Quinn, T., & Li, Y. (2022). Effect of adding modified pea protein as functional extender on the physical and sensory properties of beef patties. *LWT, 154*, Article 112774.
Siddiqui, S. A., Khalifa, I., Yin, T., Morsy, M. K., Khoder, R. M., Salauddin, M., … Khalid, N. (2024). Valorization of plant proteins for meat analogues design—A comprehensive review. *European Food Research and Technology, 250*(10), 2479–2513.
Sousa, R., Portmann, R., Dubois, S., Recio, I., & Egger, L. (2020). Protein digestion of different protein sources using the INFOGEST static digestion model. *Food Research International, 130*, Article 108996.
Sousa, R., Portmann, R., Recio, I., Dubois, S., & Egger, L. (2023). Comparison of in vitro digestibility and DIAAR between vegan and meat burgers before and after grilling. *Food Research International, 166*, Article 112569.
Sousa, R., Recio, I., Heimo, D., Dubois, S., Moughan, P. J., Hodgkinson, S. M., … Egger, L. (2023). In vitro digestibility of dietary proteins and in vitro DIAAS analytical workflow based on the INFOGEST static protocol and its validation with in vivo data. *Food Chemistry, 404*, Article 134720.
Supreetha, S., Tiku, P. K., & Prakash, J. (2009). Retention of lysine in foods processed with microwave and conventional heating. *International Journal of Food Engineering, 5*(3), 1–9.
Suwandy, V., Carne, A., van de Ven, R., Bekhit, A. E.-D. A., & Hopkins, D. L. (2015). Effect of pulsed electric field treatment on the eating and keeping qualities of cold-boned beef loins: Impact of initial pH and fibre orientation. *Food and Bioprocess Technology, 8*(6), 1355–1365.
Swing, C. J., Thompson, T. W., Guimaraes, O., Geornaras, I., Engle, T. E., Belk, K. E., … Utama, I. (2021). Nutritional composition of novel plant-based meat alternatives and traditional animal-based meats. *Journal of Food Science and Nutrition, 7*(109), 1–11.
Tamanna, N., & Mahmood, N. (2015). Food processing and maillard reaction products: Effect on human health and nutrition. *International Journal of Food Science, 2015*(1), Article 526762.
Tang, J., Wichers, H. J., & Hettinga, K. A. (2023). Glycation of soy and pea proteins influences infant gastric digestibility more than intestinal digestibility. *Food Hydrocolloids, 136*, Article 108251.
Tavano, O. L., Neves, V. A., & da Silva Júnior, S. I. (2016). In vitro versus in vivo protein digestibility techniques for calculating PDCAAS (protein digestibility-corrected amino acid score) applied to chickpea fractions. *Food Research International, 89*, 756–763.
Uchida, K. (2003). Histidine and lysine as targets of oxidative modification. *Amino Acids, 25*(3), 249–257.
Venkatachalam, M., & Sathe, S. K. (2003). Phaseolin in vitro pepsin digestibility: Role of acids and phenolic compounds. *Journal of Agricultural and Food Chemistry, 51*(11), 3466–3472.
Vu, G., Zhou, H., & McClements, D. J. (2022). Impact of cooking method on properties of beef and plant-based burgers: Appearance, texture, thermal properties, and shrinkage. *Journal of Agriculture and Food Research, 9*, Article 100355.
Wang, R., Sar, T., Mahboubi, A., Fristedt, R., Taherzadeh, M. J., & Undeland, I. (2023). In vitro protein digestibility of edible filamentous fungi compared to common food protein sources. *Food Bioscience, 54*, Article 102862.
Wang, W., Dong, L., Zhang, Y., Yu, H., & Wang, S. (2021). Reduction of the heterocyclic amines in grilled beef patties through the combination of thermal food processing techniques without destroying the grilling quality characteristics. *Foods, 10*(7), 1490.
Wang, X., Yin, Z., Wang, S., & Zhai, A. (2025). Ultrasonic and enzymatic processing for optimizing gelation and functional properties of black bean protein isolate. *Journal of Food Science, 90*(7), Article e70207.
Wang, Y., Hernández-Alvarez, A. J., Goycoolea, F. M., & Martínez-Villaluenga, C. (2024). A comparative study of the digestion behavior and functionality of protein from chia (*Salvia hispanica* L.) ingredients and protein fractions. *Current Research in Food Science, 8*, Article 100684.
Wang, Y., Sánchez-Velázquez, O. A., Martínez-Villaluenga, C., Goycoolea, F. M., & Hernández-Álvarez, A. J. (2023). Effect of protein extraction and fractionation of chia seeds grown in different locations: Nutritional, antinutritional and protein quality assessment. *Food Bioscience, 56*, Article 103238.
Williams, R., O'Brien, L., Eagles, H. A., Solah, V. A., & Jayasena, V. (2008). The influences of genotype, environment, and genotype× environment interaction on wheat quality. *Australian Journal of Agricultural Research, 59*(2), 95–111.
Worldometer. (2025). Current World Population. Retrieved from: https://www.worldometers.info/world-population/ (Accessed Aug 31 2025)..
Xie, Y., Cai, L., Zhao, D., Liu, H., Xu, X., Zhou, G., & Li, C. (2022). Real meat and plant-based meat analogues have different in vitro protein digestibility properties. *Food Chemistry, 387*, Article 132917.
Yang, J. S., Dias, F. F., & de Moura Bell, J. M. (2025). Optimizing alkaline and enzymatic extraction of black bean proteins: A comparative study of kinetics, functionality, and nutritional properties. *Sustainable Food Technology, 3*(1), 188–203.
Zhou, H., Vu, G., Gong, X., & McClements, D. J. (2022). Comparison of the cooking behaviors of meat and plant-based meat analogues: Appearance, texture, and fluid holding properties. *ACS Food Science & Technology, 2*(5), 844–851.