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Comparison of *in vitro* digestibility and DIAAR between vegan and meat burgers before and after grilling

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ABSTRACT

Plant-based meat alternatives of high quality and digestibility could be a way to reduce meat consumption and, consequently, the environmental impact. However, little is known about their nutritional characteristics and digestion behaviour. Therefore, in the present study, the protein quality of beef burgers, known as excellent source of protein, was compared with the protein quality of two highly transformed veggie burgers, based on soy or pea-faba proteins, respectively. The different burgers were digested according to the INFOGEST in vitro digestion protocol. After digestion, total protein digestibility was determined, either based on total nitrogen (Kjeldahl) analysis, or after acid hydrolysis based on total amino groups (o-phthalaldehyde method) or total amino acids (TAA; by HPLC). The digestibility of individual amino acids was also determined, and the digestible indispensable amino acid score (DIAAS) was calculated based on in vitro digestibility. The impact of texturising and grilling on in vitro protein digestibility and the digestible indispensable amino acid ratio (DIAAR) was evaluated at the level of the ingredients and the finished products. As expected, the grilled beef burger had the highest in vitro DIAAS values (Leu 124 %), and grilled soy protein-based burger reached in vitro DIAAS values that could be rated as good (soy burger, *SAA 94 %)* protein source, according to the Food and Agriculture Organization. The texturing process did not significantly affect the total protein digestibility of the ingredients. However, grilling led to a decrease in digestibility and DIAAR of the pea-faba burger ($P < 0.05$), which was not observed in the soy burger, but led to an increase in DIAAR in the beef burger ($P < 0.005$).

1. Introduction

The current livestock sector occupies about 70 % of global agricultural land (FAO, 2009), is responsible for approximately 14.5 % of global greenhouse gas emissions, and has negative impacts on the environment, global health, water, and land resources (FAO, 2013b; McMichael et al., 2007). Animal-based proteins provide a significant portion of the human diet, and meat consumption has increased significantly over the past century. In addition to the negative environmental impacts, high consumption of meat, especially red and processed meat, is highly associated with health problems (increased risk of cardiovascular diseases, cancer and diabetes type 2) (Zhang et al., 2021). By contrast, vegetarian and meat-reduced diets can help to overcome critical environmental, animal welfare, and health challenges in the food system (Dinu et al., 2017). Therefore, a shift towards a higher consumption of plant proteins is needed. New protein sources for human consumption have emerged in recent years to support the transition towards more sustainable food production. Due to their similar appearance, texture, and taste to that of animal products, plant-based meat analogues have gained acceptance, and the market is rapidly expanding to meet growing consumer demands (Beardsworth & Keil, 1991). Soy protein is historically the most commonly used raw

Abbreviations: AA, amino acid; BCA, bicinchoninic acid; DIAA, digestible indispensable amino acid; DIAAR, digestible indispensable amino acid ratio; DIAAS, digestible indispensable amino acid score; DTT, dithiothreitol; EAA, essential amino acids; FAO, Food and Agriculture Organization of the United Nations; GHG, greenhouse gas; HPLC, high-performance liquid chromatography; IVD, *in vitro* digestion; LC-MS, liquid chromatography-mass spectrometry; MS, mass spectrometry; NEAA, nonessential amino acids; OPA, *o*-phthalaldehyde; RT, room temperature; SDS, sodium dodecyl sulphate; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; TAA, total amino acids; TN, total nitrogen; UHPLC, ultra-high-performance liquid chromatography; UV, Ultraviolet; UV/VIS, ultraviolet/visible.

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ingredient in the preparation of meat analogues, making it the best-known alternative to animal protein (Zhang et al., 2021). However, sources such as chickpeas, faba beans, rice, and green peas are also gaining popularity (Bohrer, 2019).

Unlike animal proteins, plant proteins may lack some indispensable (essential) amino acids in the required proportions to meet human nutritional needs, and a strict vegan diet might lead to nutritional deficiencies (Elorinne et al., 2016). In addition, plant proteins are associated with lower protein digestibility (Bohrer, 2019). Taken together, this can constitute a challenge for replacing animal proteins with plant proteins. Food manufacturers try to overcome these hurdles by partially or fully replacing traditional animal food proteins with alternative plant-based foods and ingredients to provide optimal nutrition, taste, and functionality. However, little is currently known about the gastrointestinal behaviour of newly developed meat analogues compared to real meat products (Lee et al., 2020). This knowledge is important because the transformation affects the nutritional profile and, consequently, the digestion and absorption of these products, ultimately affecting human health (Ogawa et al., 2018). Therefore, better knowledge of the nutritional quality of new and alternative protein sources is important for providing nutritional recommendations.

The aim of the present work was to analyse how food processing (drying, texturisation, and extrusion of proteins) and food preparation (e.g. grilling) affect protein digestibility. Therefore, *in vitro* digestibility was determined according to the INFOGEST protocol (Brodkorb et al., 2019; Sousa et al., 2022) in two different finished plant-based products, soy burgers and pea-faba burgers, and their corresponding ingredients (soy concentrate, texturised soy, pea isolate, faba bean concentrate, and extruded pea-faba). In addition, the digestibility and digestible indispensable amino acid score (DIAAS) of the finished plant burgers were compared with beef burgers before and after grilling.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and enzymes used in the present study were purchased from MERCK.

2.2. Sample preparation for in vitro digestion (IVD)

All protein sources were digested according to the INFOGEST protocol (Brodkorb et al., 2019), including adjustments for protein digestibility (Sousa et al., 2022). Briefly, minced beef was shaped into a burger without the addition of spices or other ingredients. The two plant-based burgers, pea-faba burgers and soy-based burgers, as well as beef meat burgers, were grilled for 3 min on each side at 70 % of the power of the stovetop without the addition of fat. Both the grilled and raw burgers were cut into pieces of 2–3 mm to mimic the chewing process. The protein sources were normalised according to their protein content, and 0.04 g of total protein per mL of food was used for *in vitro* digestion. As a blank digestion, a protein-free cookie (Moughan et al., 2005), containing only fat and carbohydrates was digested in parallel with the test foods, as previously described. The cookie was made from 40.8 g purified corn starch, 15.7 g sucrose, 4.9 g cellulose, 0.7 g baking powder, 0.5 g ground ginger, and 36.9 g margarine and baked at 175 °C in portions of ~ 35 g for 30 min. The effect of other nutrients on *in vitro* digestion was tested by mixing the individual ingredients consisting of isolated proteins (soy concentrate, texturised soy, pea isolate, faba bean concentrate, and extruded pea-faba) (normalised to 0.04 g protein) with 0.25 g of the ground cookie to better simulate meal composition, as previously described (Moughan et al., 2005).

2.3. Pancreatin solubilisation and activity determination

Pancreatin solubilisation was performed as previously described (Sousa et al., 2022) in order to avoid the formation of undissolved particles, which leads to non-reproducible measurements. Trypsin activity in this suspension was measured according to a previous protocol (Brodkorb et al., 2019). Briefly, for the activity assay or the digestion experiment, pancreatin was dissolved in simulated intestinal fluid, right before the experiment, at a concentration of 100 U trypsin activity/mL of digest, then vortexed for 10 s, followed by sonication (45 Hz, 130 W) at room temperature (RT) for 5 min. The suspension was then centrifuged (2000 × *g*, at RT, for 5 min), and the supernatant was transferred into a new tube, placed on ice, and immediately used for the experiment (enzyme activity or digestion).

2.4. In vitro digestion with the INFOGEST static model

Enzyme activities and bile concentrations were measured according to the assays described in the harmonised protocol (Brodkorb et al., 2019). All substrates were digested *in vitro using* the INFOGEST protocol (Brodkorb et al., 2019) with the adjustment for pancreatin solubilisation described above and the addition of the previously described workflow for digestibility assessment (Sousa et al., 2022). Briefly, the substrates were normalised to a protein content of 0.04 g, diluted to 1 mL with water, and then mixed with 1 mL of simulated salivary fluid (pH 7, 37 °C) containing amylase (300 U/mL of digesta) for 2 min. Then, 2 mL of simulated gastric juice (pH 3, 37 °C) containing pepsin (2000 U/mL of digesta) was added to the reaction tube and incubated at 37 °C for 120 min. Next, 4 mL of simulated intestinal juice (pH 7, 37 °C) containing pancreatin (100 U trypsin activity/mL of total digesta) and bile (10 mmol/L of total digesta) was added and incubated at 37 °C for 120 min. The entire digestion experiment was performed under continuous gentle mixing on a rotating wheel (16 rpm). Gastric digestion was stopped after 120 min by increasing the pH to pH 7 with NaOH (1 mol/L) and the intestinal phase was stopped by adding the protease inhibitor 4-(2 aminoethyl) benzenesulfonylfluoride (AEBSF, trademark Pefabloc®, 500 mmol/L, Roche, Basel, Switzerland). All samples were immediately snap frozen in liquid nitrogen. For each set of samples digested, 1 g of a protein-free enzyme blank (cookie) was digested in parallel.

2.5. Sample separation into digestible and indigestible fractions

After thawing, the digested samples were separated into digestible and indigestible fractions by precipitation with MeOH (80 %, v/v, final concentration) at −20 °C for 1 h, followed by centrifugation (2000 × *g* at 4 °C for 15 min) as previously described (Sousa et al., 2022). For each digested sample and enzyme blank (protein-free cookie), a representative aliquot of the supernatant was collected into new tubes. The pellets (P) were washed twice with MeOH (100 %), centrifuged between the washing steps (2000 × *g* at 4 °C for 5 min), and then dried in a CentriVap (Labconco, Kansas City, Missouri, USA). The weights of the dry pellet and total supernatant (S) (supernatant of digesta plus MeOH) in mg were recorded as previously described (Sousa et al., 2022) and used to calculate digestibility.

2.6. Analysis of total nitrogen (TN) by Kjeldahl

The TN present in the pellet, and in the supernatant after precipitation with MeOH 80 % was quantified using the Kjeldahl method, according to ISO 8968–3:2004/IDF 20–3: 2004 (ISO-8968-3, 2004).

2.7. Acid hydrolysis

The samples were subjected to acid hydrolysis with 6 mol/L HCl to measure the total amino acids (TAA) and total amino group (OPA) content. Briefly, 220 μL of the supernatant were pipetted and dried directly in the glass vials using a CentriVap (Labconco, Kansas City, Missouri USA) and resuspended in 220 μL H_2O, 120 μL 3,3′-dithiodipropionic acid (DDP)/0.1 % NaOH (0.2 mol/L), 120 μL HCl (0.2 mol/L),

40 μL norvaline (NVa; 10 mmol/L), and 500 μL HCl (37 %). The entire digesta pellet was directly weighed into a vial and resuspended with 880 μL H_2O, 480 μL DDP 0.1 %/NaOH (0.2 mol/L), 480 μL HCl (0.2 mol/L), 160 μL NVa (10 mmol/L), and 2 mL HCl (37 %). All samples were incubated for 15 h at 110 °C.

2.8. Quantification of total amino groups (R-NH_2, OPA method)

After acid hydrolysis (Section 2.7), the total amino groups (R-NH_2) in the supernatant and pellet of the precipitated samples were measured using the *o*-phthalaldehyde (OPA) method (Kopf-Bolanz et al., 2012). Briefly, the clear supernatant (if needed after centrifugation at 13,000 × *g*, 5 min) of the hydrolysed samples was diluted 10 times with perchloric acid (0.5 mol/L). After derivatisation with OPA and in the presence of 2-mercapto-ethansulfonic acid, the produced 1-alkylthio-2-alcylisonindol compounds were measured by UV/VIS photometry at 340 nm. The results were calculated based on a glutamic acid standard curve. A blank digestion (supernatant and pellet of a protein-free cookie) was used as the background.

2.9. Determination of total amino acids

The total amino acids (TAA) of each undigested substrate were determined as described in ISO 13903:2005 (ISO 13903, 2005). The TAA in the digests was analysed using the adapted AOAC method 2018.06 for infant formula (Jaudzems et al., 2019). After hydrolysis (Section 2.7), each sample was neutralised and derivatised with AccQ-Tag Ultra reagent (Waters, 2007). The amino acid pattern was determined by ultra-high-performance liquid chromatography (UHPLC) (Acquity UPLC BEH C18 2.1 × 150 mm, 1.7 μm, Waters) coupled with a UV detector (Vanquish, Thermo Scientific, Reinach, Switzerland). The UHPLC conditions were as follows: 2 μL injection volume, column temperature of 50 °C, UV detection at 260 nm, and a flow rate of 0.4 mL/min.

2.10. In vitro total digestibility, DIAAR, and proxy-DIAAR calculations

The total digestibilities of *in vitro* digested substrates were determined with three different analytical endpoints by calculating the total amounts of N, total primary amines (R-NH_2), or amino acids in the supernatants and pellets in mg (N) or mmol (R-NH_2, amino acids), taking into account all dilution steps performed during the analytical process, according to formula (1) (Sousa et al., 2022).

$$\textit{invitro}\,\text{digestibility}\,[\%] = (\text{Fs} - \text{Cs}) / ((\text{Fs} - \text{Cs}) + \max(0; \text{Fp} - \text{Cp}))\,\text{x}\,100 \quad (1)$$

where Fs = Food supernatant, Cs = Cookie supernatant, Fp = Food pellet, Cp = Cookie pellet.

The amino acids in the supernatant and pellet of the protein-free cookie digest, representing the enzyme background, were subtracted from the fractions of the food digests to account for the autolysis of the digestive enzymes. In addition, the term (max(0;Fp-Cp)) indicates that the amount of amino acids from the protein-free cookie digest was set as a minimum, and values below the enzyme background (resulting from analytical bias for highly digestible substrates) were set to zero.

The digestible indispensable amino acid ratio (DIAAR) was calculated for each indispensable amino acid (IAA) according to formula (2) by calculating the DIAA for each IAA.

In vitro DIAA = mg of IAA per g of food protein × *in vitro* digestibility of IAA

$$\textit{invitroDIAAR}(\%) = 100x\frac{\textit{mgofinvitrodigestibledietaryIAAin1gofdietaryprotein}}{\textit{mgofthesamedietaryIAAin1gofthereferenceprotein}} \quad (2)$$

As reference protein, preschool children (6 months to 3 years) was used as recommended by the FAO (FAO, 2013a).

The lowest DIAA ratio (DIAAR) corresponded to the DIAAS of the tested dietary protein.

Proxy *in vitro* DIAAR was calculated by multiplying each IAA with the total *in vitro* digestibility rather than by the digestibility of each individual IAA.

2.11. Statistical evaluation

Statistical differences between digestibilities with regard to the values of TN, R-NH_2, and TAA obtained with the three methods were not significant, as calculated with ANOVA for repeated measures and total digestibilities (Analysis ToolPak in Excel) for all investigated substrates, including the substrates of this project and those previously analysed (Sousa et al., 2022). Therefore, the results of all three methods were combined for the assessment of differences in digestibility between food sources using paired t-tests (Analysis ToolPak in Excel). Unless otherwise indicated, P values indicate two-tailed significance.

3. Results

3.1. Substrates

The raw samples were analysed for their composition in protein, fat, and moisture (Table 1), and for the amino acid distribution per g of kg of protein source (Fig. 1). The substrates had a variable composition, with protein contents ranging from 12.9 g to 78.6 g. Carbohydrate values in the finished vegan burgers reached 1.6 g (soy) and 15.4 g (pea-faba). The content of individual amino acids for each sample under raw conditions was analysed using UHPLC. As expected, the isolated/concentrated protein powders used as ingredients for the vegan burgers had higher protein and amino acid contents than the finished products (Table 1 and Fig. 1). Despite the difference in amino acid content between the ingredients and the final products, all had a very similar and high essential/nonessential amino acid ratio (soy: 0.8, pea-faba: 0.9, beef: 1) (Supplemental Table 1).

3.2. In vitro protein digestibility

To allow the comparability of protein hydrolysis between the samples, all the digestions were normalised to a protein content of 0.04 g, which was based on a conversion factor of 6.25 for all sources, as recommended by the FAO (FAO, 2013a). Digestibilities were calculated using three different analytical approaches: TN by Kjeldahl, total

Table 1
Composition of substrates in protein, fat, carbohydrates, and moisture.

(g/100 g)	Protein (TN × 6.25)	Fat (OICC)	Carbohydrates (by difference)	Moisture (Oven)
Faba bean concentrate	54.6	3.3	15.43	7.5
Pea isolate	78.6	9.1	0	5.8
Extruded pea & faba	28.7	3.1	2.2	61.1
Pea & faba burger (raw)	18.5	16.8	4.0	55.9
Pea & faba burger (grilled)	20.3	n.d	n.d	n.d
Soy concentrate	64.4	0.26	0	6.0
Texturised soy	27.3	0.31	1.3	65.6
Soy burger (raw)	12.9	13.3	1.6	65.2
Soy burger (grilled)	13.9	n.d	n.d	n.d
Beef meat (raw)	20.7	n.d	n.d	n.d
Beef burger (grilled)	24.1	n.d	n.d	n.d

n.d. = not determined.

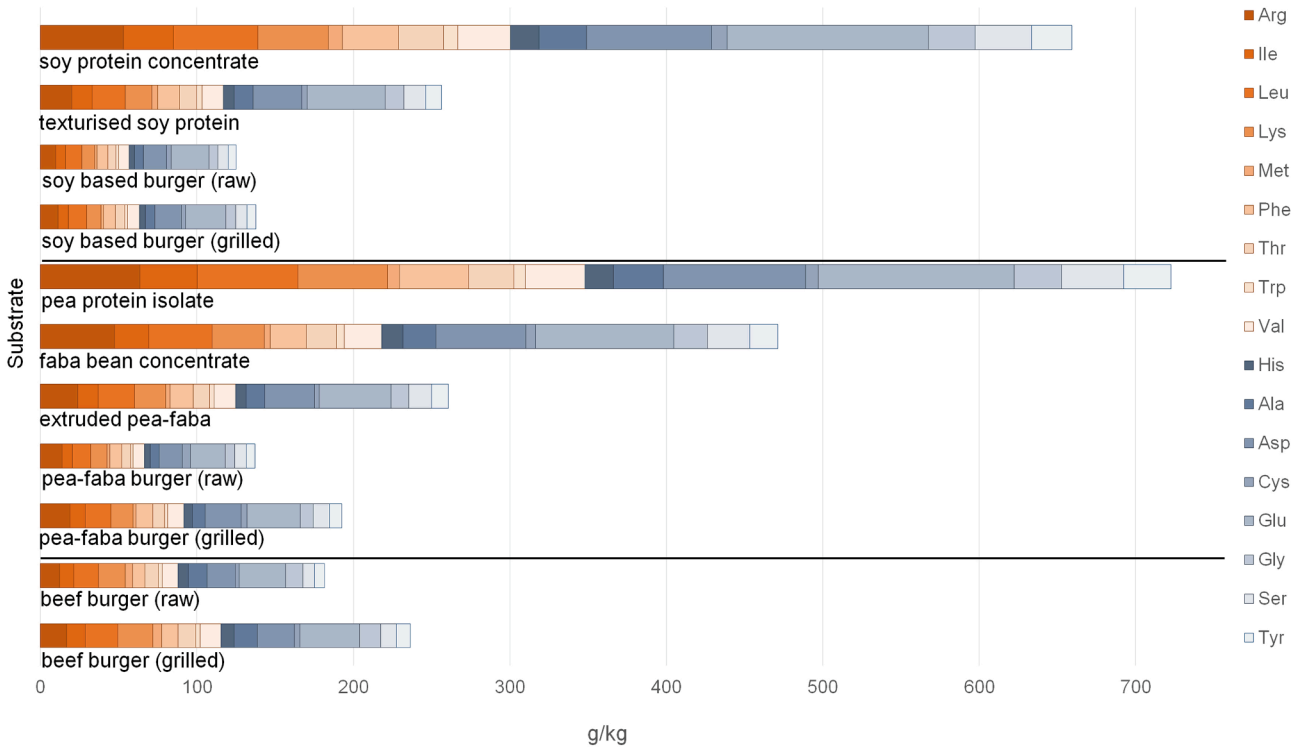


Fig. 1. Amino acid composition (g/kg of protein source) of the ingredients and the finished products (raw, and grilled samples). Orange: essential amino acids; blue: nonessential amino acids.

primary amines (R-NH_2) by OPA, and TAA by HPLC. For OPA and HPLC measurements, the supernatants and pellets from MeOH precipitated intestinal digests were first hydrolysed with 6 mol/L HCl at 110 °C for 15 h.

For all three methods, total digestibility was calculated using the formula (1) in section 2.10 of material and methods and as described in detail in Sousa et al. (Sousa et al., 2022). All the ingredients (faba bean concentrate, pea isolate, extruded pea-faba, soy concentrate, and texturised soy) were digested together with 0.25 g of cookie to simulate a complete meal. The finished products (soy burger, and pea-faba burger) and beef were digested alone (Sousa et al., 2022). In the raw state, the digestibility of the tested plant-based protein sources (ingredients and final products) was around 85 % or higher for all the methodologies (Fig. 2). Nevertheless, the digestibility of beef meat of nearly 100 % was higher ($P < 0.005$) compared to the plant-based protein sources. Grilling had no effect on the digestibility of meat protein, but it had a slightly negative effect on the digestibility of the pea-faba burger ($P_{one\ tailed} < 0.05$). A comparison of the three different methods revealed that the OPA (R-NH_2, black bars) generally gave lower values for protein digestibility, while Kjeldahl (TN, light grey bars) gave the highest values, and HPLC (TAA, dark grey bars) was in between, but no statistical difference was found between the methods.

3.3. Individual amino acid digestibility and the effect of grilling

The results of the total amino acid analysis by HPLC were used to calculate the digestibilities of each individual amino acid for each substrate, performed as described in Section 2.10. The digestibilities of

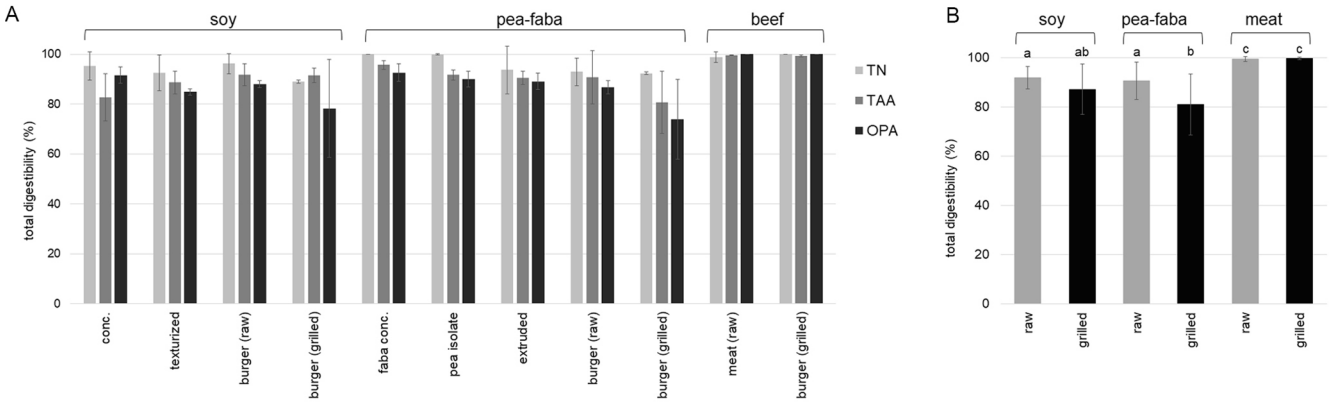


Fig. 2. Total digestibility of the ingredients and finished products. All substrates were analysed using three different methods. Release of total nitrogen (light grey), primary amines (black), and total amino acids (dark grey). All the ingredients (faba bean concentrate, pea isolate, pea-faba extruded, soy concentrate, and texturised soy) were digested together with 0.25 g of cookie to mimic a real meal in terms of macronutrient content. At least three independent experiments were performed, and error bars represent the standard deviation (SD) (A); average total digestibility across all three analytical methods (TN, NH_2, and TAA, $N \geq 9$) for raw and grilled burgers; significant different digestibilities are indicated with different letters (B).

individual amino acids were calculated for all ingredients (digested together with cookie), for the finished burgers, and for the beef burger, serving as a control for high digestibility. Moreover, all the burgers were grilled to assess whether digestibility was affected by grilling (Fig. 3). Amino acid digestibility is close to 100 % for raw and grilled beef burgers. The lowest values were found in grilled pea-faba burger with significant decrease in digestibility for some amino acids was observed (Fig. 3) and also a high variability between duplicates was found. However, this high variability cannot be attributed to the grilling process since it was not observed in grilled soy burger. Graphics showing the digestibilities of the ingredients in comparison with the corresponding finished products were plotted to determine the effect of texturisation on digestibility (Soy burger: Supplemental Fig. 1, pea-faba burger: Supplemental Fig. 2).

3.4. *In vitro* DIAAR values

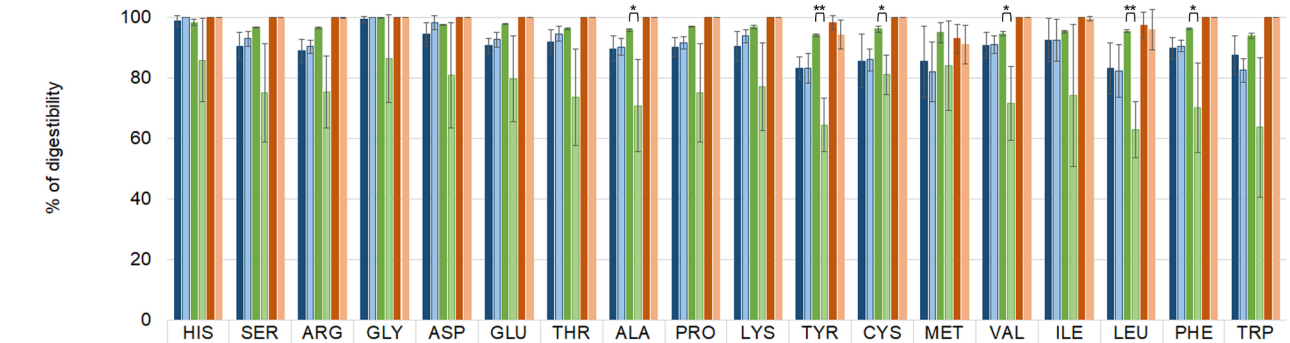
The *in vitro* DIAAR values were calculated based on the amount of the corresponding amino acids present in one gram of food protein (based on the total nitrogen × nitrogen conversion factor of 6.25), divided by the reference requirement values for that amino acids for preschool children (6 months to 3 years; (FAO, 2013a)), and multiplied by the digestibility of each individual indispensable amino acid assessed by HPLC (Formula 2 in Section 2.10). In accordance with the digestibility results (Fig. 3), the DIAAR values were calculated by comparing plant burgers with beef burgers under raw and grilled conditions, respectively. The DIAAR values for each ingredient and the corresponding finished product under raw and grilled conditions were also calculated and compared to test whether technological treatment and texturisation affected protein quality (soy burger: Supplemental Fig. 3, pea-faba burger: Supplemental Fig. 4). For the soy products, the DIAAR values did not differ between concentrate and finished burgers before and after grilling. Interestingly, the texturised soy had lower values (Fig. 3 and Supplemental Fig. 3). By contrast, no clear effect of the texturising process on amino acid digestibility was observed when we compared the amino acid digestibility of the faba bean concentrate and pea isolate with the extruded pea-faba product. However, a higher DIAAR value was observed for the raw pea-faba burger compared to all its ingredients (Fig. 4), which was decreased by grilling (Fig. 3 Supplemental Fig. 4). The DIAAR values of the beef burger were higher compared to both plant-based products, and grilling had a clear positive effect, resulting in a higher DIAAR compared to the raw burger.

4. Discussion

This study focused on three main research objectives: (i) assessing the protein quality of novel protein sources, such as plant-based meat analogues, and comparing them with a traditional source of known high quality, such as a beef burger; ii) understanding whether the protein quality of the ingredients is related to or affects the quality of the finished products; and iii) determining the impact of the grilling process on digestibility and protein quality. Therefore, two different plant-based burgers were studied: one made from soy only and the second from two different sources, faba and pea. The digestibility of the finished products and their corresponding ingredients before or after texturisation was analysed according to the recently published digestibility workflow (Sousa et al., 2022), based on the INFOGEST protocol (Brodkorb et al., 2019). As previously described, all the ingredients of the plant-based burgers were digested together with 0.25 g of protein-free cookie to better simulate the macronutrient composition of a real meal (Sousa et al., 2022), whereas the three burgers were digested alone before and after grilling.

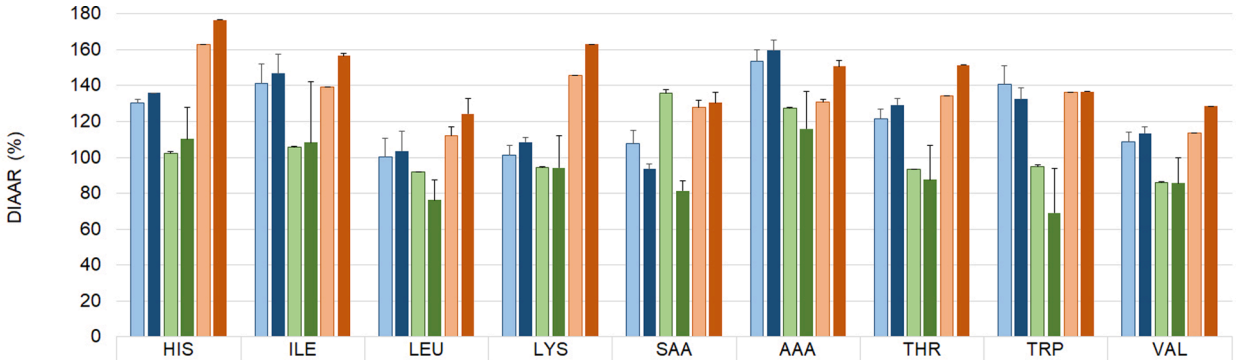
Total protein digestibility, assessed by three different analytical methods, gave comparable results, although the *in vitro* digestibilities based on TN were slightly but not significantly higher than the values based on primary amines (OPA, R-NH_2), and TAA (Fig. 1). Therefore, as expected, the average digestibility encompassing all analytical methods showed that the digestibility of the meat burgers was higher (>99 %) than that of the vegan variants ($P < 0.005$). However, the digestibilities of both plant-based burgers were over 85 %, which can also be considered highly digestible. Interestingly, grilling did not reduce the protein digestibility of beef and soy burgers, but it slightly negatively affected the digestibility of pea-faba burgers ($P_{\text{one sided}} < 0.05$) (Fig. 2B) and, consequently, the DIAAR ($P < 0.05$) of this product (Fig. 4). By contrast, an increase in DIAAR ($P < 0.001$) was observed for the grilled beef burger (Fig. 4), which was not due to changes in digestibility (Fig. 2) but can be explained by differences in protein content between raw and grilled products (Fig. 1). Further, at the level of digestibility of specific individual amino acids of the pea-faba burger, a significant reduction was observed after grilling. The amino acids tyrosine and leucine were the most affected ($P < 0.05$), while alanine, cysteine, valine, and phenylalanine were also affected, but with lower significance ($P \leq 0.1$). By contrast, the effect of grilling on the digestibility of individual amino acids was not statistically significant for beef and soy burgers (Fig. 4).

The occurrence of Maillard reactions and consequent generation of Maillard reaction products are undesirable effects of the



	HIS	SER	ARG	GLY	ASP	GLU	THR	ALA	PRO	LYS	TYR	CYS	MET	VAL	ILE	LEU	PHE	TRP
soy (raw)	99	91	89	99	94	91	92	90	90	91	83	86	85	91	93	83	90	87
soy (grilled)	100	93	90	100	98	93	95	90	92	94	83	86	82	91	93	82	91	83
pea-faba (raw)	98	97	97	100	98	98	96	96	97	97	94	96	95	95	95	96	96	94
pea-faba (grilled)	86	75	75	86	81	80	74	71	75	77	65	81	84	72	74	63	70	64
beef (raw)	100	100	100	100	100	100	100	100	100	100	98	100	93	100	100	98	100	100
beef (grilled)	100	100	100	100	100	100	100	100	100	100	94	100	91	100	100	96	100	100

Fig. 3. Effect of grilling on individual amino acid digestibility. Comparison of digestibility of individual amino acids of plant-based burgers from soy (blue bars) and pea-faba (green bars) with beef meat burgers (orange bars) under raw and grilled conditions, respectively. Digestibilities were calculated as described earlier (Sousa et al., 2022). The error bars represent the SD of the triplicate analysis. Significant differences are indicated (*: $P \leq 0.1$ and **: $P < 0.05$).



	HIS	ILE	LEU	LYS	SAA	AAA	THR	TRP	VAL
soy (raw)	130	141	100	101	108	153	121	141	109
soy (grilled)	136	147	104	108	94	160	129	133	113
pea-faba (raw)	102	106	92	94	136	127	93	95	86
pea-fabe (grilled)	110	108	76	94	81	116	88	69	86
beef (raw)	163	139	112	146	128	131	134	136	114
beef (grilled)	177	157	124	163	130	151	151	137	129

Fig. 4. The DIAAR values were calculated for the pea-faba burger (green), soy-based burger (blue), and beef meat burger (orange) under raw (darker colour) and grilled (lighter colour) conditions, respectively. DIAAR values were based on total protein (TN*6.25) content and the reference requirement values for preschool children (6 months to 3 years) given by the FAO (FAO, 2013). The error bars are the SD of at least three analyses.

protein–carbohydrate complexes present in processed foods exposed to heat (Jaeger et al., 2010). Maillard reactions can cause nutritional losses of amino acids and decreased amino acid digestibilities (Almeida et al., 2014; González-Vega et al., 2011). As indicated in Table 1, the carbohydrate content in the pea-faba burger was twice as high as in the soy burger; and the probability of Maillard reactions during grilling of the pea-faba burger could be higher than for the soy burger. Therefore, the analysis of Maillard products in these products would be of interest in the future, as it could explain the decrease in digestibility observed for the grilled pea-faba burger. However, the marked increase in the DIAAR of sulphur-containing amino acids in the faba-pea burgers compared to the ingredients (Supplemental Fig. 4) might have additional reasons. Sulphur-containing amino acids can be added to meat analogues to enhance meat flavours (Zhang et al., 2018). Moreover, differences in the susceptibility of individual amino acids to heat treatment during meat preparation might also apply to meat analogues (Hodgkinson et al., 2018).

Animal proteins are considered high-quality/complete proteins due to their amino acid profile and high protein digestibility. By contrast, most plant proteins (except soy protein) are incomplete (lacking one or more indispensable amino acids) and have lower digestibilities due to the presence of antinutritional factors (Mariotti & Gardner, 2019). In agreement with this, our digestibility and DIAAR values for beef were higher than those of the tested meat analogues. However, the amino acid profiles of plant proteins with deficiencies in certain amino acids can be improved by combining different sources (Jiménez-Munoz et al., 2021). The difference in DIAAR values was greater between beef and pea-faba burgers than between soy and beef, which is in alignment with the concept that soy is a complete protein. However, grilled soy-based burger can be considered a good-quality protein source (DIAAS $\geq$ 75 % $<$ 99 %) according to FAO, while the grilled beef meat burger is, as expected, an excellent protein source (DIAAS $\geq$ 100 %) (FAO, 2013a).

Compared to the *in vivo* DIAAR values determined in growing pigs (Herreman et al., 2020), our results for faba bean, pea, and soy concentrate showed good agreement (Supplemental Fig. 6), indicating that the recently developed *in vitro* workflow (Sousa et al., 2022) could also be applicable for digestibility predictions in highly transformed protein sources. However, this needs to be further confirmed with *in vivo* data collected with the exact same products, if possible.

5. Conclusion

In recent years, plant-derived proteins have been widely used as ingredients in the food industry due to their relatively low cost, higher sustainability, reduced environmental impact, and reduced ethical concerns when compared with animal-derived proteins. Soy protein is the best-known and most widely used plant protein source, and has been used for many years in a variety of food products (e.g. tofu, soy-milk, yogurts, snacks, and meat analogues). However, other legumes, such as green peas, chickpeas, lentils, and faba beans, are gaining more and more attention from the food industry. Therefore, it is important to better understand these new protein sources, especially regarding their digestibility, since it is well known that plant proteins generally have a lower protein quality and digestibility than animal proteins. The alternative protein sources tested in the present work proved to be good alternatives to meat due to their high digestibility values and good-quality amino acid profiles. Soy proteins appeared to be more temperature stable than pea and faba proteins; thus, improving the recipe and characteristics of the pea-faba burger is recommended. It can be concluded that it is difficult to predict the amino acid digestibility and DIAAR values of a final product based on its ingredients. More *in vivo* data are needed to validate the application of the protocol in highly transformed foods.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.112569.

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