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Influence of processing on protein quality and environmental impact assessment of soy-based meat analogues

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ABSTRACT

While meat is an established source of high-quality protein, limited data exists on plant-based meat analogues, particularly regarding how specific production steps and extrusion conditions affect their protein quality and ecological footprint. We used the Protéix soybean cultivar to produce dry soy protein intermediate products with varying degrees of refinement and employed them to obtain meat analogues by high-moisture extrusion. As a reference, a commercial soy protein concentrate was used to produce meat analogues by high- and low-moisture extrusion. *In vitro* amino acid (AA) digestibility and *in vitro* DIAAS of intermediate products and extrudates were assessed and compared to traditional soy-based foods and chicken breast. The meat analogues had high total protein *in vitro* digestibility (>95 %) irrespective of extrusion type, energy input, and soybean variety. The extrusion process substantially enhanced protein digestibility of mildly refined soy protein powders which had low protein digestibility (<60 %). Consequently, meat analogues based on these raw materials showed the lowest environmental footprint per kg quality-corrected protein - with a fourfold lower global warming potential than chicken, compared to only a 17 % reduction observed for meat analogues based on soy protein isolate. *In vitro* DIAAS values for all studied meat analogues ranged from 81 to 102 for children aged 0.5 to 3 years and were only limited in sulfur-containing AA. Soy-based meat analogues were equally digestible as tofu and cooked chicken breast, had similar protein quality as soymilk and tofu, and can be good to excellent protein sources for humans.

1. Introduction

Nature conservation and emission reduction targets require a transition towards environmentally sustainable diets, particularly in high- and middle-income countries (Springmann et al., 2018). An increased consumption of legumes, nuts, fruits, and vegetables has been recommended to replace added sugars and red meat in global diets (Willett et al., 2019). Soybeans (*Glycine* max) are a versatile legume being widely used in food production due to its high protein content, favourable amino acid (AA) profile, and technical characteristics such as gelling, emulsion, water binding and foaming properties (Riaz, 2006). Soy is among the predominant protein sources for meat analogues, which replicate the sensory attributes of meat (Siegrist et al., 2024). These may facilitate the transition to a more plant-based diet by allowing consumers to maintain familiar consumption patterns and cooking habits (Michel et al., 2021). However, limited data exists on the effect on protein digestibility and quality of specific food processing unit operations involved in meat analogue production. Similarly, from an environmental perspective, available life-cycle assessment (LCA) studies on soy-based meat analogues are limited to few case studies, which often lack transparency regarding utility consumption for individual unit operations (Shanmugam et al., 2023) resulting in a limited understanding of the influence of specific processing steps on the environmental impact of soy-based extrudates.

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Meat analogues can be produced *via* high- or low-moisture extrusion (HME or LME) of plant proteins (Kyriakopoulou et al., 2019). In the extruder barrel, ingredients are hydrated, kneaded and conveyed towards the die. The mixture plasticizes due to heat generated by friction, barrel heating and steam injection while pressure builds up due to the flow constriction at the exit die. In LME, a mixture with less than 40 % water content is forced through the die, with a sudden release of pressure at the outlet causing water vaporization and leading to product expansion, resulting in a porous and fibrous structure. Upon hydration, this sponge-like structure rapidly absorbs water and serves as ground meat analogue for burger patties and similar preparations or as meat extender in animal-based meat preparations (Kyriakopoulou et al., 2019). In HME, up to 70 % of the mixture consists of water. A cooling die is attached to the extruder outlet to prevent product expansion due to the evaporation of water caused by a sudden pressure release. The resulting extrudate contains meat-like fibers and may be torn into chunks which are directly used as meat analogues. While the mechanism behind the structure formation is not fully elucidated, it is generally agreed that proteins play a major role and the protein content based on dry matter should comprise at least 50 % (Sägesser, 2024; van der Sman & van der Goot, 2023). Although plant cultivation generally has lower environmental impacts compared to animal farming (Poore & Nemecek, 2018), plant-based meat analogue production *via* HME requires raw materials with a concentrated protein content for extrusion, which increases their environmental footprint. Different protein extraction methods do not only yield intermediate products with varying protein concentration, composition and techno-functional properties, but also have substantially different environmental footprints (Lie-Piang et al., 2021). Additionally, processing affects nutritional quality and LCAs shall be evaluated in relation to nutrition (Green et al., 2020).

Since animal-based foods are a source of high-quality proteins which may play an important role in preventing AA deficiencies in vulnerable population groups, it is important to assess the protein quality of meat analogues (Hu et al., 2019). The digestible indispensable amino acid score (DIAAS) is the recommended protein quality metric which considers the food's AA profile and ileal AA digestibility (FAO, 2013), describing its ability to meet the daily indispensable amino acid (IAA) requirements when the total daily protein intake equals the estimated average requirement (FAO, 2013; Wolfe et al., 2016). Since direct assessment of ileal AA digestibility in animals and humans is not always feasible nor ethically justified (Moughan & Lim, 2024), an *in vitro* approach showing good agreement with *in vivo* assessments (Brodkorb et al., 2019; Sousa et al., 2023) can be used to predict human AA digestibility in a food processing context (Moughan & Lim, 2024).

While processing can enhance food safety and shelf life, it can also improve digestibility of nutrients, *e.g.*, by reducing antinutrients, or, on the contrary, decrease heat-sensitive nutrients and increase glycemic index. Thus, the impact of processing on the nutritional quality of food should be systematically assessed (Ahrné et al., 2025) in combination with ecological considerations. We aimed to assess the effects of soybean processing in form of soy protein extraction and texturization on both nutritional protein quality and environmental impact of finished soy-based meat analogues.

2. Materials and methods

2.1. Study design

Nutritional and protein quality analyses were performed for (i) pilot-scale produced protein powders (soy white flakes, concentrate, and isolate) and meat analogues from soybeans of a defined soybean cultivar (Protéix), which had previously been used for protein quality analysis of traditional soy-based food products (cooked soybeans, soymilk, and tofu) (Hammer et al., 2024); and for (ii) meat analogues from an industrially-produced soy protein concentrate *via* high- and low-moisture extrusion and with low *vs* high energy input. The LCAs were conducted based on primary data for the pilot-scale process and based on literature data for the industry-scale process. By combining nutritional and environmental impact analysis of food processing operations, we aimed to assess whether the effect of considering protein quality in the functional unit (FU) of an LCA can influence the overall LCA results.

2.2. Raw materials

Dry soybeans of the cultivar Protéix (2009, Agroscope) developed for the Swiss climate and optimized for human nutrition (with higher protein content and improved taste) were obtained from Mühle Rytz (lot number 4348; Biberen, Switzerland). A commercial SPC (Alpha 8, Solae LLC, Missouri, USA) was included in the study for comparison of the pilot-scale with an industrial-scale process.

2.3. Processing steps and system boundaries

The dried raw soybeans of the Protéix cultivar were used to produce soy white flakes (SWF), which were further processed to soy protein concentrate (SPC) and soy protein isolate (SPI) at pilot scale. The processing steps were defined to model the industrial process, constrained by certain feasibility aspects. Namely, no conditioning of the soy grits (steam heating to 70 °C and 11 % additional moisture, (Riaz, 2016)) was performed prior to flaking to minimize the formation of fines by softening the grits. Instead, the oil extraction was carried out at elevated temperature to compensate for the deactivation of antinutrients and protein denaturation. Additionally, oil was extracted by supercritical carbon dioxide ($scCO_2$) instead of conventionally used hexane. Since pilot-scale hexane extractors typically operate with batch sizes exceeding 100 kg, no access to such a facility could be obtained and implementing hexane extraction with conventional lab equipment would have resulted in unacceptable safety hazards as well as a lack in verified process control. This solvent substitution was expected to slightly reduce the oil yield, as only fully apolar substances can be extracted with $scCO_2$, excluding phospholipids. However, as apolar triglycerides constitute 95 % of the soy fat (Sheehan et al., 1998), this reduction was anticipated to be minor. SWF, SPC, and SPI were then extruded to produce meat analogues. The pilot- and industrial-scale processes, including the system boundaries for the LCA, are presented in Fig. 1 and a detailed description of the individual pilot-scale processing steps is provided in the following paragraphs. Commercial SPC was included in the extrusion trials as comparator and to enhance experimental flexibility. The commercial SPC may have undergone an additional functionalization step, such as a heat treatment, which was not accounted for in the LCA. Functionalization is widely applied, but companies treat the specific processes as trade secrets to maintain differentiation (Yang et al., 2014).

2.3.1. SWF production process

A hammer mill without sieves (FreDrive-LAB, Frewitt, Switzerland) was used at 6200 rpm with a feed rate of 55 rpm to split 62.5 kg dry soybeans (57.5 kg dry matter) into partially dehulled grits. Subsequently, a malt mill (MattMill Master, MattMill, Germany) was employed at the widest gap to detach residual hulls from the grits. The grits were then separated from hulls and fines using an air classifier (LST 1, Strecker & Schrader, Germany) with a feed rate setting of 8.5 (53 kg/h) and air intake setting of 6 and 5 on the two separate intake control dials. Subsequently, the soy grits were flaked by a service provider using a traditional cereal flaker (assumed gap width 1.5 mm, feed rate ~ 25 kg/h, Brunners Getreide-Produkte, Schwarzenburg, Switzerland) to rupture cell walls, increase surface and reduce diffusion distance for the following oil extraction. Conditioning was not applied in our study due to the lack of appropriate technical equipment for uniform steam treatment and subsequent drying of grits. The soy flakes were then defatted *via* supercritical carbon dioxide ($scCO_2$) extraction at NATEX (Ternitz, Austria). 2180 kg of CO_2 were used to defat 44.5 kg of soy grits,

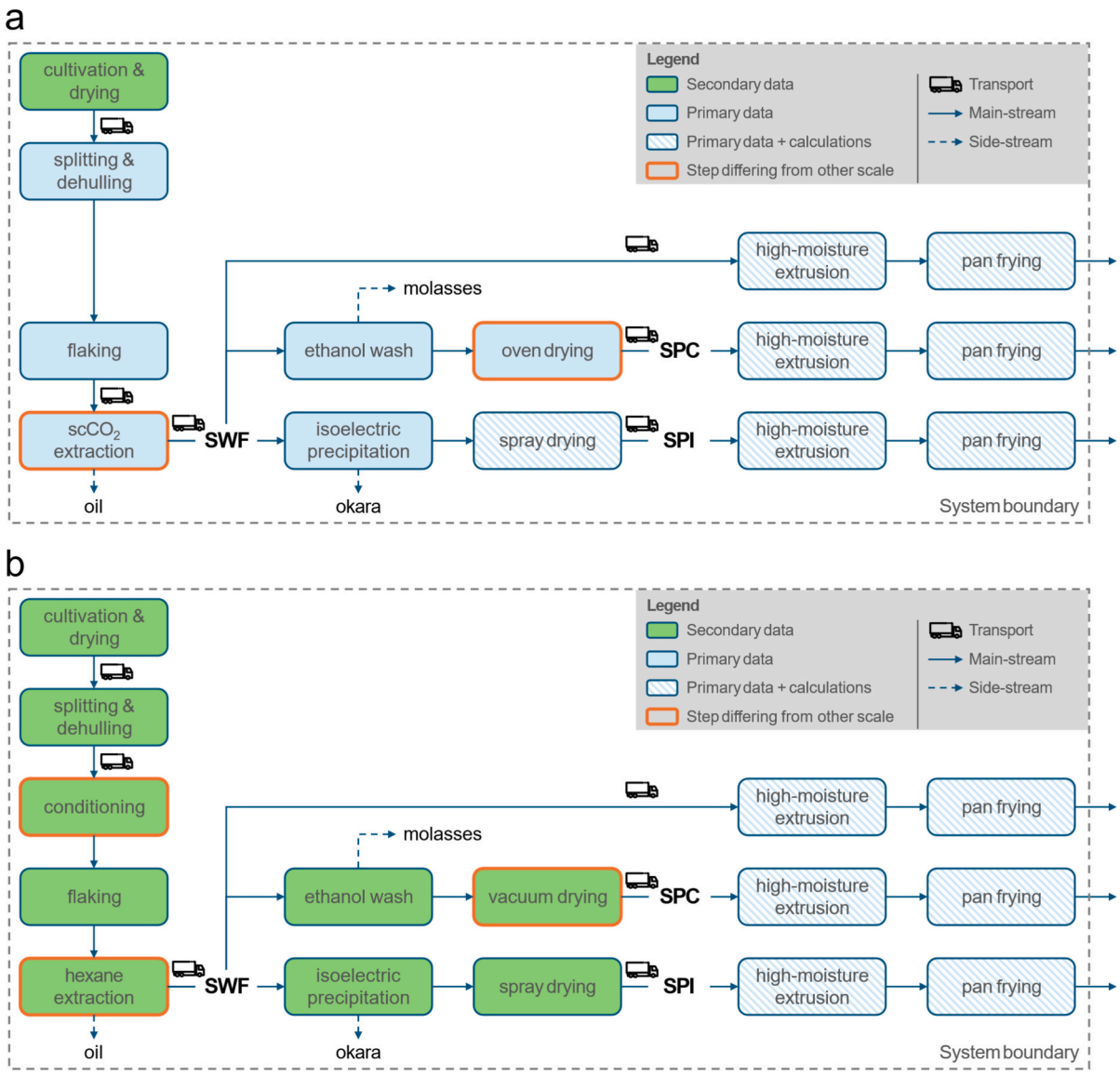

Fig. 1. Processing steps for soy-based meat analogue production from cradle-to-use are shown at a) pilot scale and b) industry scale. High-moisture extrusion was performed with soy white flakes (SWF), soy protein concentrate (SPC) or soy protein isolate (SPI). At pilot scale all steps except the supercritical CO_2 ($scCO_2$) extraction, conducted in Austria, were carried out in Switzerland. The modelled industry process was assumed to take place entirely in Switzerland. Process flow, relevant tranportation and system boundaries, integral to the LCA, are displayed.

41.8 kg dry weight (CO_2-to-material ratio of 48.3:1). The extraction process was conducted at 460 bar and 65 °C for 2 h and 30 min and at 75 °C for the remaining time with a CO_2 flow rate of 363 kg/h over 6 h. The extracted soybean oil was separated from carbon dioxide using 2 separators, where the pressure was reduced to 60 and 48 bar in separators 1 and 2, respectively, at 46 and 36 °C. 5 kg of defatted flakes (SWF) were ground in the hammer mill with a 1 mm sieve in preparation for the extrusion trials. The rest was used for further SPC and SPI production.

2.3.2. SPC production process

10 kg of SWF were suspended in 24 L of aqueous EtOH (60 % *v*/v) and macerated for 20 min at 30 °C. The solvent was drained manually with a spider strainer, cheese cloth, and a cheese press. A washing step with pure tap water was subsequently applied to remove residual ethanol, followed by another draining step. The wet SPC was air dried overnight at 40 °C using a food dehydrator (JerkyMaster 600, Klarstein, Switzerland). The dried product was then ground in the hammer mill with a 1 mm sieve in preparation for the extrusion trials.

2.3.3. SPI production process

22 kg of SWF were suspended in 160 kg of water. After adjusting the pH to 9.5 with 200 g of NaOH pellets, wet milling was applied with a colloid mill (MZ100, FrymaKoruma Mills, Germany) with a gap size of 0.15 mm at 2900 rpm. The milled macerate was fractionated into a soluble and an insoluble fraction using a decanter (MD80, Lemitec GmbH, Germany) with a throughput of 84 L/h at 2600 rcf. Proteins in the soluble fraction were then precipitated with approximately 2 L of 3.6 M HCl to reach pH 4.3. The precipitate was collected and concentrated with a disk separator (Clara 20, Alfa-Laval, Germany) operated at a throughput of 120 L/h at 10600 rcf and 1.5 bar back pressure. The separated precipitate was neutralized to pH 7 with 64.7 g NaOH pellets prior to spray drying. The concentrate was then dried at 12 L/h with a spray dryer Niro (GEA, Germany), at a dry air temperature of 186 °C and an outlet temperature of 80–82 °C.

2.3.4. Meat analogue production

Meat-like structures were produced *via* high- and low-moisture extrusion (HME and LME) on a Process 16 twin-screw extruder

(Thermo Fisher Scientific, Karlsruhe Germany) with 8 barrels and a length-to-diameter ratio (L/D) of 40. The same screw configuration, consisting primarily of forward conveying elements with four kneading blocks of 1.5 L/D and 3 L/D in barrel three, six, eight and seven, respectively, was used for all processes. For HME, a cooling die with a length of 480 mm and a channel cross-section of 28 × 6 mm was attached to the extruder. HME was performed for each intermediate product using two specific process settings chosen to achieve two distinct levels of energy input, specifically high energy input (HEI) and low energy input (LEI). The energy input was increased through elevated temperatures, higher screw speeds and/or increased dry matter content. The throughput was kept at 2 kg/h and the cooling die temperature was set to 40 °C for all trials. The barrel temperature increased towards the end plate where it reached 120–140 °C. The screw speed was varied between 300 and 1000 rpm and the powder feed between 30 and 60 % (Table 1). The pH of the self-produced SPC and SWF was neutralized by extruding with 0.1 M KOH. All other trials were performed with tap water.

LME was performed with commercial SPC only. An end plate with a nozzle of the diameter of 3 mm was attached to the extruder and the throughput was increased to 3 kg/h. The powder-to-liquid feed ratio was increased to above 65 % and the end plate temperature was reduced to 100 °C while the screw speed was varied between 500 and 800 rpm (Table 1).

2.3.5. Food preparation

Meat analogues were prepared consistently with customary cooking procedures for meat and meat analogues in commercial products to yield edible meat like cooked preparations. Brielfy, high-moisture extrudates were cut into 4 cm long pieces and grilled over high heat for 5 min in a frying pan without the use of any cooking oil. Preparation of low-moisture extrudates included rehydration by soaking products in water (1:3 wt/wt) for 15 min and subsequent cooking over medium heat for 10 min. Product weights were monitored during the food preparation steps.

2.4. Product characterisation

2.4.1. Extrudate structure analysis

The fibrous structure of all extrudates was assessed visually and recorded with photographs. For high-moisture extrudates, tensile and cutting test were conducted to characterize their mechanical properties. For low-moisture extrudates, rehydration capacity was determined. The detailed procedures are provided in SI-B.

2.4.2. Nutrient composition

Nutrient analyses were conducted with the intermediate products and selected cooked extrusion products. Dry matter content was determined following the ISO standard 5534:2004 (ISO, 2004b). Fat content was analyzed with the Schmid-Bondzynski-Ratzlaff method (ISO, 2022). Total nitrogen (TN) was measured by Kjeldahl analysis (ISO, 2004a). Protein content was calculated by multiplying TN by the nitrogen-to-protein conversion factor (NPCF) of 6.25 (TN x 6.25), as stipulated by the definition of DIAAS (FAO, 2013). In addition, protein content was assessed with a soy-specific NPCF of 5.7 (TN x 5.7) (Krul, 2019). The AA content (excluding tryptophan) was quantified by ultra-high performance liquid chromatography (UPLC; Vanquish Flex, Thermo Fisher Scientific, Switzerland) after an acidic hydrolysis (HCl 6 mol/L, 110 °C, 24 h), according to the AOAC method 2018.06 for infant formula (Jaudzems et al., 2019), with modifications described in Sousa et al. (2023). Tryptophan content was determined by UPLC (Ultimate3000, Thermo Fisher Scientific, Reinach, Switzerland) after an alkaline hydrolysis (NaOH 6 mol/L, 110 °C, 20 h), as reported in Walther et al. (2022).

2.4.3. Protein extraction and SDS-PAGE

To examine possible changes in protein composition due to protein isolation steps, proteins of the Protéix intermediate products (dehulled soybeans, SWF, SPC, and SPI) were extracted and separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and proteins in the main bands identified based on a recent publication (Hammer et al., 2024) where peptide mass fingerprinting had been performed for raw soybeans (as described in SI-C).

2.5. Protein digestibility and DIAAS analyses

2.5.1. In vitro digestion

The intermediate products (SWF, SPC, and SPI), the cooked LME and selected HME products were *in vitro* digested by applying the harmonized static INFOGEST protocol (Brodkorb et al., 2019), with an adapted pancreatin solubilization procedure required for the subsequent *in vitro* digestibility assessment (Sousa et al., 2023). The selected HME products included all HEI extrudates along with the LEI of commercial SPC to assess the impact of energy input by extrusion on digestibility. In preparation of the *in vitro* digestion, activities of digestive enzymes and concentrations of bile salts were measured, and pH adjustment experiments were performed for all products. To mimic the mastication during the oral phase, extrusion products were cut into 2–3 mm pieces with a knife mill (GRINDOMIX GM200, Retsch GmbH, Germany). The products in the *in vitro* digestion were normalized to an input of 40 mg of total protein (TN x 6.25) per g of food. Products were prepared as they would typically be consumed, as recommended by Brodkorb et al. (2019): finished products (extruded products) were cooked or grilled and then digested alone, whereas food intermediates (SWF, SPC, and SPI) were

Table 1
Extrusion processing setting for each meat analogue, produced *via* high- or low-moisture extrusion (HME or LME) with high or low energy input (HEI or LEI) from self-produced soy white flakes (SWF), soy protein concentrate (SPC) or soy protein isolate (SPI) as well as commercial soy protein concentrate (SPC_{com}). For HME the cooling die was set to 40 °C.

Operation mode	Throughput	Powder content	Water content	Barrel temperatures 2–8*	End plate temperature	Screw speed
	[kg/h]	[%]	[%]	[°C]	[°C]	[rpm]
HME HEI SWF	2	50	50	80, 80, 100, 100, 130, 130, 135	135	1000
HME LEI SWF	2	45	55	80, 80, 100, 100, 120, 120, 120	120	500
HME HEI SPC	2	50	50	80, 80, 100, 100, 130, 130, 135	135	1000
HME LEI SPC	2	45	55	80, 80, 100, 100, 120, 120, 120	120	500
HME HEI SPI	2	60	40	80, 80, 100, 100, 130, 130, 140	140	500
HME LEI SPI	2	60	40	80, 80, 100, 100, 120, 120, 120	120	300
HME HEI SPC_{com}	2	40	60	80, 80, 100, 100, 130, 130, 135	135	1000
HME LEI SPC_{com}	2	30	70	80, 80, 100, 100, 120, 120, 120	120	500
LME HEI SPC_{com}	3	75	25	40, 60, 80, 100, 100, 100, 100	100	500
LME LEI SPC_{com}	3	65	35	40, 60, 80, 100, 100, 100, 100	100	800
LME $_{v}$LEI** SPC_{com}	10	65	35	40, 60, 80, 100, 100, 100, 100	100	800

* Barrel 1 was not heated.
** To further decrease the energy input ($_{v}$LEI) in LME, the throughput was increased to 10 kg/h.

supplemented with 0.25 g of protein-free cookie to reflect real-life consumption, which is consistent with both *in vivo* (Devi et al., 2020; Fanelli et al., 2021; Moughan et al., 2005) and *in vitro* methodologies (Sousa et al., 2023). *In vitro* digestion experiments ($n = 3$) were performed, and 1 g of protein-free cookie was digested in parallel as a blank digestion to account for the enzyme background for total AA and primary amines (R-NH_2) analyses. *In vitro* digestion was conducted as previously described in Hammer et al. (2024).

2.5.2. In vitro digesta analyses

After completion of the *in vitro* digestion, digestibility was assessed by following the analytical workflow by Sousa et al. (2023). Briefly, the intestinal phase was stopped by precipitating undigested proteins and larger peptides (> 8–10 AA) with ice cold MeOH (80 % *v*/v final concentration, −20 °C, 1 h). The supernatants (absorbable fraction) and pellets (undigested fraction) were separated by centrifugation (4000 rcf, 4 °C, 15 min) and pellets washed twice with ice cold 80 % MeOH. Firstly, 220 μL of supernatants and full pellets were dried using a CentriVap (Labconco, Kansas City, USA) and a freeze-drier (Alpha 1–4 with LyoCube, Christ, Germany), respectively. Next, both fractions were hydrolyzed to free AA by acid hydrolysis (HCl 6 mol/L, 110 °C, 15 h). Finally, total AA contents (TAA method) were measured by neutralization of hydrolyzed samples, derivatization with AccQ-Tag Ultra reagent and analysis by UHPLC (Vanquish, Thermo Scientific, Switzerland) according to the AOAC method 2018.06 for infant formula (Jaudzems et al., 2019). As an alternative analysis, total primary amines (R-NH_2) were quantified in both hydrolyzed fractions using the o-phthalaldehyde (OPA) method described by Kopf-Bolanz et al. (2012).

2.5.3. Calculation of in vitro digestibility

The *in vitro* digestibility was calculated according to the equations published in Sousa et al. (2023). The total AA (TAA method) and total primary amines (R-NH_2 method) contents were calculated for the supernatants and pellets of the food digests by taking dilution steps into account and correcting for *in vitro* digestion background (measured in the digests of the protein-free cookie). The corrected amount of AA or R-NH_2 in the supernatant (absorbable fraction) was divided by the corrected amount of AA or R-NH_2 in the whole digesta (supernatant + pellet). With the first analytical approach (TAA method), *in vitro* digestibility of individual AA was calculated, and the mean AA digestibility is referred to as *in vitro* digestibility of total protein. The second analytical approach (R-NH_2 method) allows the calculation of *in vitro* digestibility of total protein only.

2.5.4. In vitro DIAAR and DIAAS

The digestible indispensable amino acid score (DIAAS) was calculated according to the FAO report on protein quality (FAO, 2013) and as previously reported in detail (Hammer et al., 2024). First, the content for each IAA was calculated in mg per 1 g of food protein, based on a conversion factor of 6.25 (TN x 6.25). Secondly, the *in vitro* digestible IAA content was calculated by multiplying the amount of each IAA in the food (mg IAA per g food protein) by the *in vitro* digestibility of the same IAA. Thirdly, the *in vitro* digestible IAA contents were divided by the recommended amino acid scoring patterns to assess protein quality for the following age groups: a) infants (birth to 6 months), b) young children (0.5 to 3 years), and c) older children, adolescents and adults (> 3 years), resulting in *in vitro* digestible IAA (reference) ratio (DIAAR) for each IAA. Finally, the lowest *in vitro* DIAAR (limiting AA) was identified as the DIAAS for each studied food and age group. Based on the DIAAS for young children (0.5 to 3 years), the quality-corrected protein (qc-protein) was determined as the product of protein content based on NPCF 6.25 and DIAAS (Herrmann et al., 2024). The scoring pattern for young children is discussed more prominently for comparison purposes as recommended by FAO (2013).

2.6. Life-cycle assessment (LCA)

An attributional LCA was used to quantify the environmental impacts arising from the production of soy-based meat analogues at pilot scale as described above. An industry-scale process was modelled based on literature data for comparison. The system boundaries were cradle-to-gate whereas pan frying was also modelled for the final extrudates because they were fried before the protein digestibility and DIAAS measurements (subsection “food preparation”). The system boundaries are depicted together with the unit processes considered at pilot and industry scale in Fig. 1a and b, respectively. The main difference between pilot and industry scale was that $scCO_2$ extraction was used for defatting at pilot scale instead of the commonly used hexane-based solvent extraction. The $scCO_2$ extraction was done by NATEX in Austria, whereas the hexane-based defatting at industry scale was assumed to take place at the same location as all other processing steps in Switzerland. Additionally, there was no conditioning step at pilot-scale production.

All material flows at pilot scale were documented during processing (SI-B). Energy inputs were directly measured, except for the spray drier, the extrusion unit, and the pan frying for which the electricity consumption was calculated. It was beyond the scope of this study to measure all emission flows from fertilizer and pesticide application during soybean cultivation. Therefore, a representative process for the Swiss context from the Agrifootprint database v6.3 (Blonk et al., 2022) was used. The industry-scale process was modelled for Switzerland based on datasets from the Agrifootprint database v6.3 (Blonk et al., 2022) for soybean protein concentrates and isolates, which were modified by changing input and emission flows from the Dutch to the Swiss context. Additionally, the combined flaking and defatting process from the Agrifootprint database was split and complemented based on information from scientific literature. After consultation with industry, it was concluded that the estimated energy consumption per kg for the extrusion unit at pilot scale was also representative for industry scale. Energy consumption and water losses from pan frying were considered to be the same as in the pilot-scale process. For all other steps, the environmental impacts were based on the modelled data described above. Transport distances for the pilot- and industry-scale model were obtained from Google Maps. Where applicable, generic waste and wastewater treatment processes were modelled. Capital goods were excluded from the assessment. Detailed inventories for each unit process can be found in SI-B.

Economic allocation was used throughout this study to model multi-output processes. All applied allocation factors can be found in SI-Table B8. The impacts were calculated for each (intermediate) product using four different functional units (FU), namely (i) 1 kg of product, (ii) 1 kg of total protein (N x 5.7), (iii) 1 kg of digestible protein (N x 5.7), and (iv) 1 kg of quality-corrected protein (qc-protein) in the product. The environmental impacts in the categories global warming potential (GWP), land use (LU), water use (WU), and particulate matter (PM) were calculated based on the ReCiPe 2016 (Huijbregts et al., 2017) midpoint indicators using a hierarchist perspective. The LCA model was created in openLCA v2.3 (Hildenbrand et al., 2024). The figures were created using pandas (The pandas development team), NumPy (Harris et al., 2020), and matplotlib (Hunter, 2007) python packages.

3. Results

3.1. Pilot-scale process vs. industry standards

The yields in the soybean preparation for extraction were reduced compared to those achievable at an industrial scale due to non-optimized processing conditions, the use of lab scale and pilot equipment and the lack of a conditioning step (mass balance in SI-Fig. B1). Consequently, the soy flake yield after splitting, dehulling, and flaking reached 73 %, compared to reported industrial yields of approximately

90 % (Sheehan et al., 1998). In the $scCO_2$ extraction, 3.8 kg of soybean oil was extracted from 44.5 kg raw material (9 %). However, with 15 % fat content the resulting SWF still contained a substantial amount of fat (Table 2). Thus, while the protein content of SWF fell within the expected range of 44–54 %, fat content substantially exceeded the target value of below 1 % (Demarco & Gibon, 2020; Sheehan et al., 1998), indicating an incomplete defatting process. The residual fat content was then further enriched in the subsequent processing steps resulting in 17 % and 21 % fat for SPC and SPI, respectively. This was the primary factor explaining the reduced protein content of only 47 % and 64 % for SPC and SPI, respectively, compared to industrial concentrates or isolates (>60 % and > 90 % protein content, respectively). Nonetheless the yields (per dry matter) of the pilot-scale protein extraction from SWF, at 79 % and 37 % for SPC and SPI, respectively, were comparable to industrially reported values of 75 % and 35 % (Berk, 1992; Deak et al., 2008; Verfaillie et al., 2023). For benchmarking, a commercial SPC with lower fat (1.2 %) and higher protein content (56.9 %) than the "Protéix" SPC was included to represent the target composition, as the pilot-scale process did not fully achieve the intended fat reduction (Table 2).

The relative protein composition of dehulled soybeans, SWF, SPC and SPI was visualized by SDS-PAGE showing no observable differences in gel band patterns or intensity (SI-Fig. C1), suggesting that the composition of major soy proteins was largely unaffected by the extraction processes. Consistently, the IAA profile of intermediate products was also comparable (Table 2, SI-Table C1).

The SPI had reduced flowability caused by its small particle size compared to the other products. Additionally, the high fat content in the self-produced intermediate products presumably reduced water-holding capacity and restricted operational flexibility for extrusion. Temperatures above 135 °C could not be reliably reached under consistent extrudate outflow and initial target recipes for SPI resulted in an unstable process. The extrusion energy expenditure was only minimally influenced by the raw material (see detailed results in SI-Table A1). The energy consumption of roughly 2 MJ/kg extrudate was marginal compared to the processing steps prior to extrusion. By extruding at lower temperature, reduced rotational speed, and lower dry matter, extrusion energy expenditure could be reduced by up to 20 %. The increase in LME throughput to 10 kg/h had the biggest effect on energy expenditure by reducing it to 0.9 MJ/kg extrudate.

3.2. Product quality assessment

For HME, extrudates produced at HEI featured the desired fibrous structures while at LEI the fibrousness was less pronounced (SI-Table A3). A prior study showed that commercial SPC forms more pronounced fibrous structures with stronger textures at higher melt temperatures (Sägesser et al., 2025), but this could not be applied for the SWF, SPC and SPI of this study. Except for SPI, the texture of the LEI samples was weaker compared to the corresponding HEI sample (SI-Fig. A1). At LEI, commercial SPC featured nearly no fibers and had a

Table 2
Macronutrients (g/kg product) and indispensable amino acid content (IAA, mg/g protein (TNx6.25)) of intermediate products and cooked meat analogues (means ± SD; $n = 3$) produced either with a commercially available soy protein concentrate (Alpha-8) or by manufacturing intermediate products from Protéix soybeans. IAA composition is shown in comparison to the recommended AA pattern for DIAAS calculation[1].

	AA pattern	Commercial SPC					Protéix soybean					
	Child[2]	SPC_{com}	HME LEI SPC_{com}	HME HEI SPC_{com}	LME LEI SPC_{com}	LME HEI SPC_{com}	SWF	SPC	SPI	HME HEI SWF	HME HEI SPC	HME HEI SPI
Dry matter	–	923.1 ± 0.1	352.1 ± 3.0	467.3 ± 12.4	293.4 ± 4.4	308.3 ± 4.8	973.3 ± 0.1	935.2 ± 0.0	952.3 ± 1.9	527.0 ± 0.5	535.3 ± 1.0	646.4 ± 0.7
Fat	–	12.2 ± 0.4	< 10.0	< 10.0	< 10.0	< 10.0	148.5 ± 3.5	166.5 ± 14.8	213.5 ± 6.4	79.5 ± 0.4	93.2 ± 3.9	120.0 ± 4.2
Protein (TNx5.7)	–	569.2 ± 5.6	212.0 ± 6.4	280.6 ± 13.2	183.2 ± 8.6	191.9 ± 2.4	454.1 ± 0.9	473.9 ± 1.4	636.5 ± 6.6	249.7 ± 12.8	269.4 ± 14.5	436.9 ± 0.4
Protein (TNx6.25)	–	624.2 ± 6.2	232.5 ± 7.0	307.7 ± 14.4	200.8 ± 9.4	210.4 ± 2.6	497.9 ± 1.0	519.6 ± 1.6	697.9 ± 7.2	273.8 ± 14.1	295.4 ± 15.9	479.1 ± 0.4
Histidine	20	26.2 ± 0.1	27.5 ± 0.0	25.5 ± 0.2	25.1 ± 0.0	26.4 ± 0.1	24.8 ± 0.1	25.2 ± 0.2	24.8 ± 0.1	25.2 ± 0.1	25.7 ± 0.1	25.9 ± 0.3
Isoleucine	32	48.5 ± 0.2	50.8 ± 0.0	46.9 ± 0.2	46.9 ± 0.1	49.3 ± 0.1	45.1 ± 0.2	45.7 ± 0.4	47.2 ± 0.2	45.3 ± 0.2	46.4 ± 0.2	50.8 ± 0.7
Leucine	66	75.7 ± 0.3	79.5 ± 0.0	73.8 ± 0.2	73.9 ± 0.2	77.4 ± 0.1	70.5 ± 0.3	71.8 ± 0.7	73.7 ± 0.3	71.5 ± 0.3	73.5 ± 0.2	77.4 ± 0.9
Lysine	57	63.9 ± 0.3	67.4 ± 0.3	62.8 ± 1.5	60.4 ± 0.1	60.7 ± 0.5	58.3 ± 0.2	59.1 ± 0.3	58.4 ± 0.2	58.8 ± 0.3	60.3 ± 0.2	60.9 ± 1.0
SAA	27	28.2 ± 0.1	29.6 ± 0.2	27.0 ± 0.3	**25.2** ± 0.3	**24.4** ± 0.2	27.5 ± 0.2	27.5 ± 0.3	**25.2** ± 0.4	27.3 ± 0.1	**26.7** ± 0.3	**24.1** ± 0.3
AAA	52	85.4 ± 0.2	89.4 ± 0.0	83.9 ± 0.2	83.3 ± 0.1	86.8 ± 0.1	82.3 ± 0.2	83.2 ± 0.5	86.2 ± 0.3	83.3 ± 0.2	84.4 ± 0.1	90.0 ± 0.8
Threonine	31	39.3 ± 0.2	41.4 ± 0.0	38.5 ± 0.1	38.2 ± 0.1	39.8 ± 0.1	35.9 ± 0.1	36.7 ± 0.3	34.8 ± 0.0	36.6 ± 0.1	37.4 ± 0.1	35.1 ± 0.4
Tryptophan	8.5	13.3 ± 0.0	16.2 ± 0.0	15.8 ± 0.0	16.3 ± 0.0	14.6 ± 0.0	15.7 ± 0.1	14.6 ± 0.0	15.7 ± 0.0	14.4 ± 0.0	16.7 ± 0.0	12.6 ± 0.6
Valine	43	50.2 ± 0.2	52.9 ± 0.1	48.7 ± 0.1	48.6 ± 0.1	51.6 ± 0.2	46.6 ± 0.1	47.5 ± 0.2	48.3 ± 0.2	46.2 ± 0.1	48.2 ± 0.2	50.7 ± 0.6
Total AA[3]	–	607.9 ± 1.6	238.7 ± 0.5	293.5 ± 1.0	189.9 ± 0.3	206.8 ± 0.4	464.6 ± 0.9	489.5 ± 1.3	666.4 ± 1.3	258.4 ± 0.7	284.0 ± 0.6	473.6 ± 2.2
Molar ratio (IAA/DAA)[4]	–	0.59	0.60	0.60	0.60	0.59	0.58	0.58	0.58	0.57	0.58	0.58

[1] SAA = sulfur-containing amino acids (cysteine + methionine), AAA = aromatic amino acids (tyrosine + phenylalanine), SWF = soy white flakes, SPC = soy protein concentrate, SPI = soy protein isolate, SPC_{com} = commercial soy protein concentrate, HME = high-moisture extrusion, LME = low-moisture extrusion, LEI = low energy input, and HEI = high energy input.
[2] Amino acid scoring pattern for children aged 6 months to 3 years (FAO, 2013).
[3] Sum of indispensable and dispensable amino acids in g/kg product.
[4] Indispensable to dispensable amino acids molar ratio (IAA/DAA).

weaker texture than all other raw materials, while its texture at HEI was among the strongest even though it was extruded at lower dry matter content. Only SPI extrudates with a particularly high powder feed ratio of 60 % were harder and had similar stiffness compared to the meat analogue from commercial SPC. Overall, the HEI extrudates contained fibers and exhibited texture parameters comparable to the reference. However, given that the reference was not extruded at optimal temperatures, the texture parameters of all extrudates were generally weak. In addition to the high fat content, the lack of a functionalization step may have also contributed to this outcome. The rehydration capacity of the LME extrudates with 2.2 and 2.9 for HEI and LEI, respectively (SI-Table A2), was comparable to the one of commercial products with a reported average at 2.9 for soy-based LME (van Esbroeck et al., 2024). Optically, the HEI product, which was produced with lower water content, appeared darker and slightly overprocessed.

The resulting cooked extruded products from SWF and SPC (commercial and produced) contained 20–30 % of protein with similar AA profiles, characterized by a molar ratio of indispensable to dispensable AA (IAA/DAA) between 0.57 and 0.60 across the extruded products. The SPI-based extrudates had a protein content close to 50 % with an IAA/DAA ratio of 0.58. All cooked extruded products were rich in IAA, sufficient to meet or exceed the IAA requirements for young children (0.5 to 3 years), if digestibility is not considered. Only the sulfur-containing AA (SAA) were slightly below the required level for the LME products and the SPC- and SPI-based products (Table 2, SI-Table C1).

3.3. *In vitro digestibility of AA and total protein*

The total protein *in vitro* digestibility of intermediate products from the Protéix cultivar, assessed by TAA analysis (Fig. 2), was enhanced by the extrusion process (HME, high energy input) if the digestibility of the intermediate was low (self-produced SWF and SPC). For intermediates with a higher digestibility (> 89 %), the extrusion had a slightly positive or no effect on the total protein *in vitro* digestibility (self-produced SPI and commercial SPC). The cooked extruded products were highly digestible with values ranging from 96.6 to 99.3 % based on TAA analysis. No differences were observed in respect to extrusion type (LME or HME) or energy input (low or high energy during the extrusion process). Comparing the digestibility of intermediate products, cooked extruded products, and traditional soy foods derived from Protéix soybeans showed an improvement upon increasing structural disruption due to processing: SWF, SPC < cooked soybeans < soymilk, SPI ≤ cooked extruded products and tofu. Notably, extruded products were equally digestible as cooked chicken breast (Fig. 2). In contrast to self-produced SPC, commercial SPC was already highly digestible, possibly due to additional processing steps, such as conditioning and functionalization.

Similar total protein *in vitro* digestibility values were obtained by OPA (total R-NH_2) analysis compared to the TAA (total AA) method (SI-Fig. C2, SI-Table C2), leading to the same conclusion. The *in vitro* digestibility of individual AA is available in the supplemental materials (SI-Table C2).

3.4. *Protein quality: In vitro DIAAR and DIAAS*

In Fig. 3a, *in vitro* DIAAR values of extruded products of this study and tofu (Hammer et al., 2024) produced from the same soybean cultivar are compared to chicken breast (Hammer et al., 2023). Chicken breast, as an example of a high-quality protein, had *in vitro* DIAAR values above 100 % for all IAA, indicating that it can fully meet the IAA

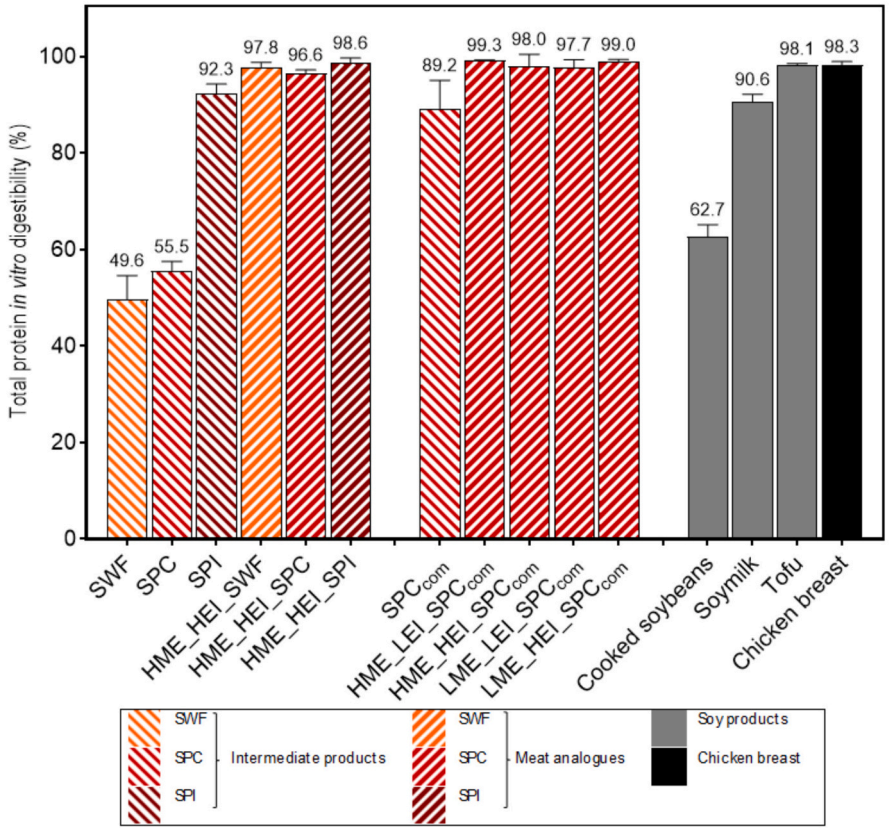

Fig. 2. Total protein *in vitro* digestibility is presented as mean ± SD (n = 3) of self-produced intermediate products (soy white flakes (SWF), concentrate (SPC), isolate (SPI)) and corresponding extruded products; commercial soy protein concentrate (SPC_{com}) and its corresponding extruded products compared to traditional soy foods (cooked beans, soymilk, tofu) produced from Protéix soybeans (Hammer et al., 2024) and to cooked chicken breast (Hammer et al., 2023), based on total amino acid (TAA) analysis. HME = high-moisture extrusion, LME = low-moisture extrusion, LEI = low energy input, HEI = high energy input.

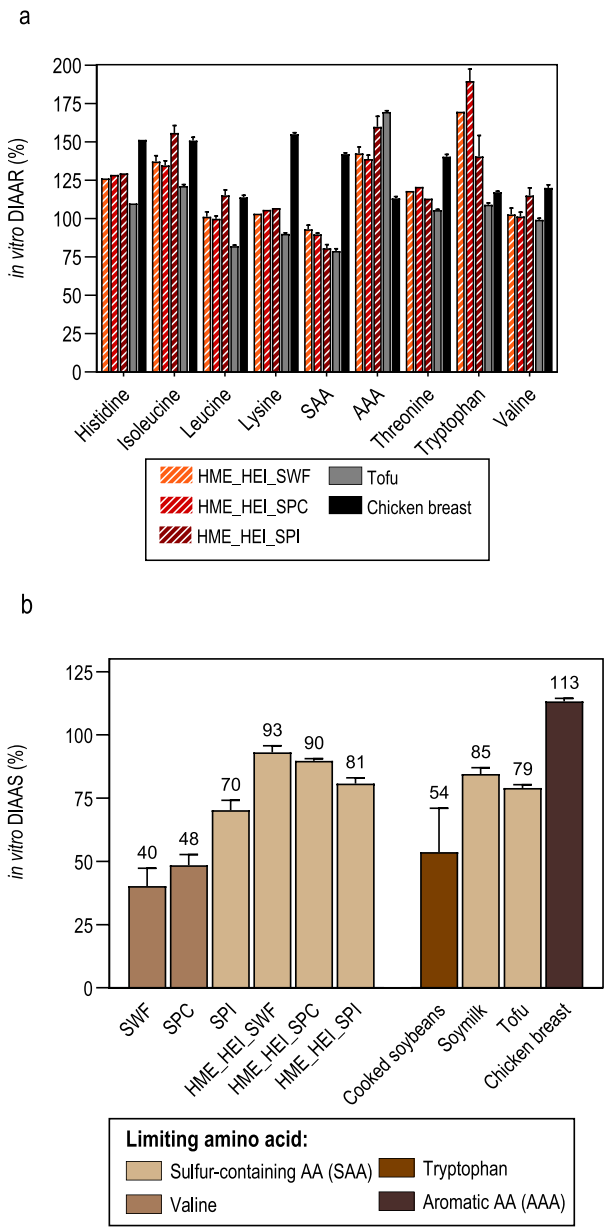

Fig. 3. Protein quality of soy-based meat alternatives compared to chicken breast. a) Comparison of *in vitro* digestible indispensable amino acid (reference) ratio (DIAAR) of cooked meat analogues produced with high-moisture extrusion (HME) using high energy input (HEI) from soy white flakes (SWF), concentrate (SPC), and isolate (SPI) with tofu from Protéix soybeans (Hammer et al., 2024) and cooked chicken breast (Hammer et al., 2023); and b) *In vitro* digestible indispensable amino acid score (DIAAS, and lowest DIAAR) of intermediate products and corresponding cooked meat analogues compared to traditional soy products (cooked soybeans, soymilk and tofu) (Hammer et al., 2024) and to cooked chicken breast (Hammer et al., 2023). The *in vitro* DIAAR and DIAAS calculations were based on *in vitro* digestibility of individual amino acids (TAA analysis) and recommended amino acid pattern for preschool children (0.5 to 3 years) (FAO, 2013). DIAAR values with a SD of zero (histidine, lysine, threonine and tryptophan) have 100 % digestibility (SI-Table C2).

requirements (reference pattern: 0.5–3 years) (FAO, 2013). The three extrudates also reached *in vitro* DIAAR ≥100 % for all IAA except sulfur-containing AA (SAA), while tofu had three *in vitro* DIAAR values ≤90 % (leucine, lysine, and SAA). The SWF- and SPC-based extrudates had similar *in vitro* DIAAR values for all IAA which were lower than those of chicken breast for most IAA, but higher for tryptophan and aromatic AA (AAA). The SPI-based extrudate generally had slightly higher *in vitro* DIAAR values (histidine, isoleucine, leucine, AAA, and valine) than the SWF- and SPC-based, but was lower for SAA, threonine, and tryptophan. In comparison to tofu, all three extrudates had higher *in vitro* DIAAR values for many IAA (histidine, isoleucine, leucine, lysine, threonine, and tryptophan).

The lowest DIAAR value, referred to as the *in vitro* DIAAS is determining the food's protein quality (Fig. 3b). *In vitro* DIAAS of the intermediate products ranged from 40 to 70, whereas cooked extruded products from Protéix soybeans had DIAAS between 81 and 93. The limiting AA for most soy products was SAA, whereas SWF and SPC were limited in valine, and the cooked soybeans in tryptophan. The extrusion process strongly increased the DIAAS of SWF and SPC, while extruding the SPI did not result in this large increase due to a higher DIAAS of 70 prior to extrusion. The cooked extruded products were of similar protein quality (by *in vitro* DIAAS) compared to soymilk and tofu but had lower DIAAS values than chicken breast. The commercial SPC (alpha-8) had a high DIAAS of 83, which remained similar after low-moisture extrusion with both low and high energy inputs but increased to 102 with high-moisture extrusion cooking with low energy input (SI-Table C3). The *in vitro* DIAAR and DIAAS values of all products, calculated for the different AA scoring patterns (birth to 6 months; 0.5 to 3 years; and > 3 years), can be found in the supplementary materials (SI-Table C3).

3.5. Environmental impact assessment

When using a mass-based FU, SPI-based extrudates had the highest impacts in all four impact categories, followed by SPC- and SWF-based extrudates (Fig. 4). While the pilot- and industry-scale models were consistent in this aspect, they diverged substantially in the magnitude of resulting environmental impacts and their main contributors (SI-Fig. B2). The primary contributors in the industrial-scale model were cultivation and drying for GWP, extraction for WU, and cultivation for LU and PM. At pilot scale, the main contributors were cultivation and defatting for GWP, cultivation for LU, defatting, extraction, and drying for WU, and cultivation, defatting, and drying for PM.

Fig. 5 illustrates environmental impacts along the industry-scale production chain when calculated per kg of product, total protein, digestible protein, and qc-protein. Even though the defatting step created some impacts by itself (Fig. 4), the impacts for GWP, LU, and PM of SWF were slightly lower compared to dried soybeans (Fig. 5). This is because a share of the impacts was allocated to the co-product, soybean oil. The production of SPC and SPI requires an increasing degree of processing compared to SWF, which lead to an increase in environmental impacts when using a mass-based FU. At the same time, the additional processing increased the protein content and particularly for SPI also the protein digestibility and quality (Fig. 2). Thus, when using protein-based FUs, the impacts for dried soy and SWF strongly increased relative to SPI. For LU, for example, the impacts of dried soybeans and SWF exceeded the impacts of SPI per kg of digestible protein. Due to the lower amount of digestible protein, SPC showed the highest impacts among all ingredients in GWP, LU, and PM per kg of digestible protein and in LU and PM per kg of qc-protein. The final processing step, extrusion, had a negligible environmental impact, except for WU (Fig. 4). Thus, while the addition of water in the recipes reduced the mass-based impacts of extrudates compared to their respective ingredients, they remained constant per kg of total protein except for WU. At the same time, extrusion did significantly increase the digestibility and DIAAS of SWF- and SPC-based extrudates compared to the raw ingredients (Fig. 2). Consequently, although at different absolute levels, the relative distribution of impacts from the production of SWF-, SPC-, and SPI-based extrudates appears comparable across all four functional units with SPI-based extrudates having the highest impacts followed by SPC- and SWF-based extrudates (Fig. 5).

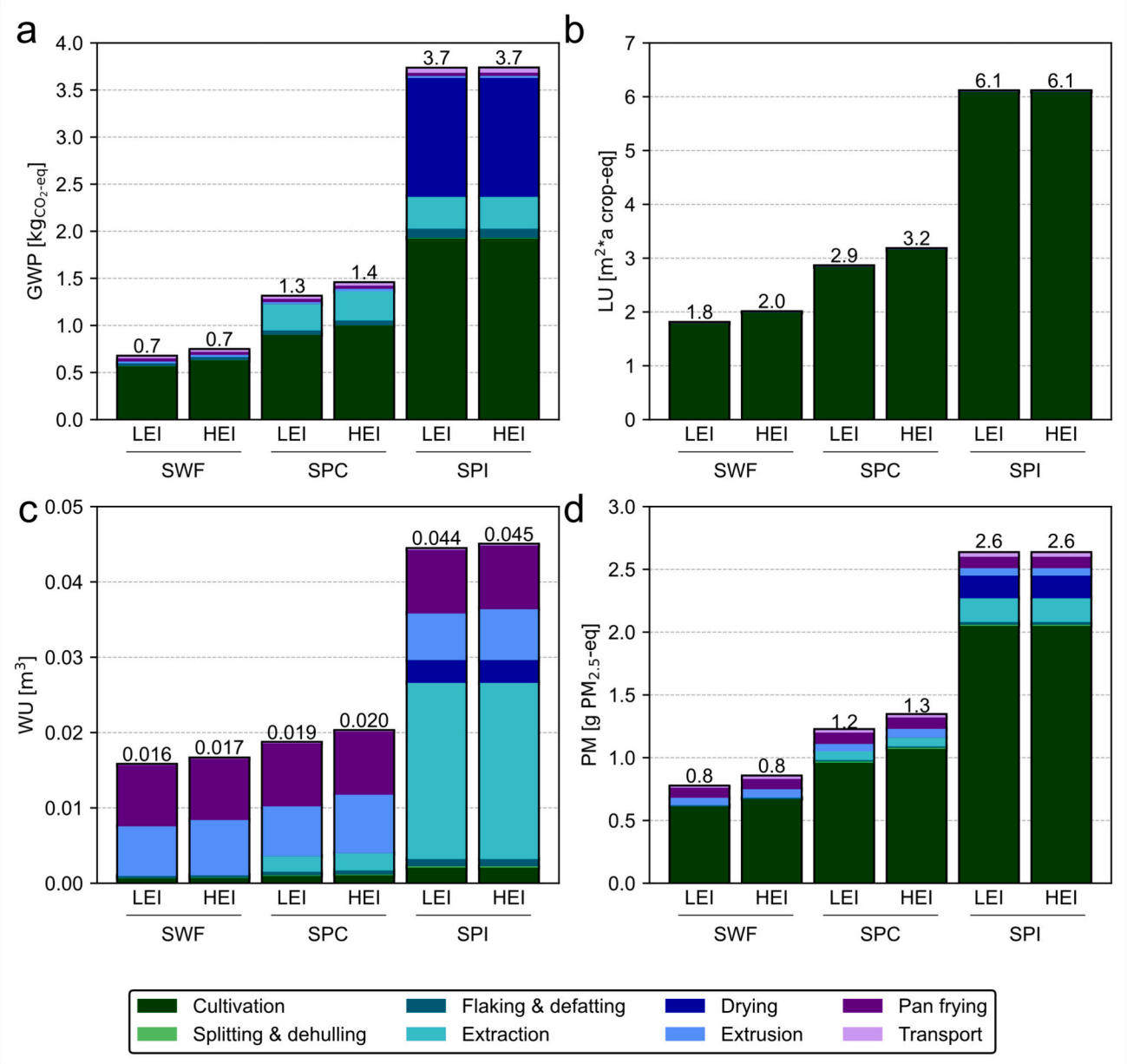

Fig. 4. Environmental impacts arising from the production of 1 kg of soy-based high-moisture extrudate using low (LEI) and high energy input (HEI) extrusion and soy white flakes (SWF), protein concentrate (SPC) and isolate (SPI) as ingredients, respectively. The impacts are based on industry-scale data and allocated to the respective processing steps. Results are shown for a) global warming potential (GWP), b) land use (LU), c) water use (WU), and d) particulate matter emissions (PM).

4. Discussion

The main findings of this study are that: 1) Soy-based meat analogues were highly digestible (> 95 %) regardless of soybean cultivar, extrusion type and energy input; 2) The extrusion process could strongly enhance the total protein *in vitro* digestibility of mildly processed soy protein intermediate products with low protein digestibility; 3) *In vitro* DIAAS values for the produced soy-based meat analogues ranged from 81 to 102 for children aged 0.5–3 years, and were only limited in the sulfur-containing AA; and 4) Cultivation, drying and extraction were identified as main contributors to the environmental impact, highlighting the potential of extruding mildly processed ingredients to optimize protein supply relative to environmental footprint.

Protein digestibility of soybean products generally correlated positively with the degree of processing and the refinement level of the ingredients. Raw soybeans have a low standardized ileal digestibility (SID; pig trial: 48–61 % (Yin et al., 2008)). Food preparation steps improve taste and protein digestibility by inactivating protease inhibitors and lectins (Riaz, 2016) as described for cooking (*in vitro*: 62.7 % (Hammer et al., 2024)), roasting (pig trial: 72.3 % (Kaewtapee et al., 2018)), or milling-associated cell wall disruption, rendering macronutrients more accessible (Holland et al., 2020). The intermediate products (SWF, SPC, and SPI) and extruded soy-based meat analogues in this study were produced from Protéix soybeans, as previously evaluated traditional soy foods (Hammer et al., 2024), allowing the observed effects to be attributed to processing and food preparation steps. Our data suggests that these meat analogues are more digestible than cooked soybeans (63 %) and have similar protein digestibility to soymilk (91 %) and tofu (98 %). The high protein digestibility (> 95 %) of all soy-based extrudates aligns with a recent study where a burger based on SPC (Impossible Burger), was found to be 99.1 % digestible in growing pigs (Fanelli et al., 2022). No detrimental effect on digestibility was observed in our study

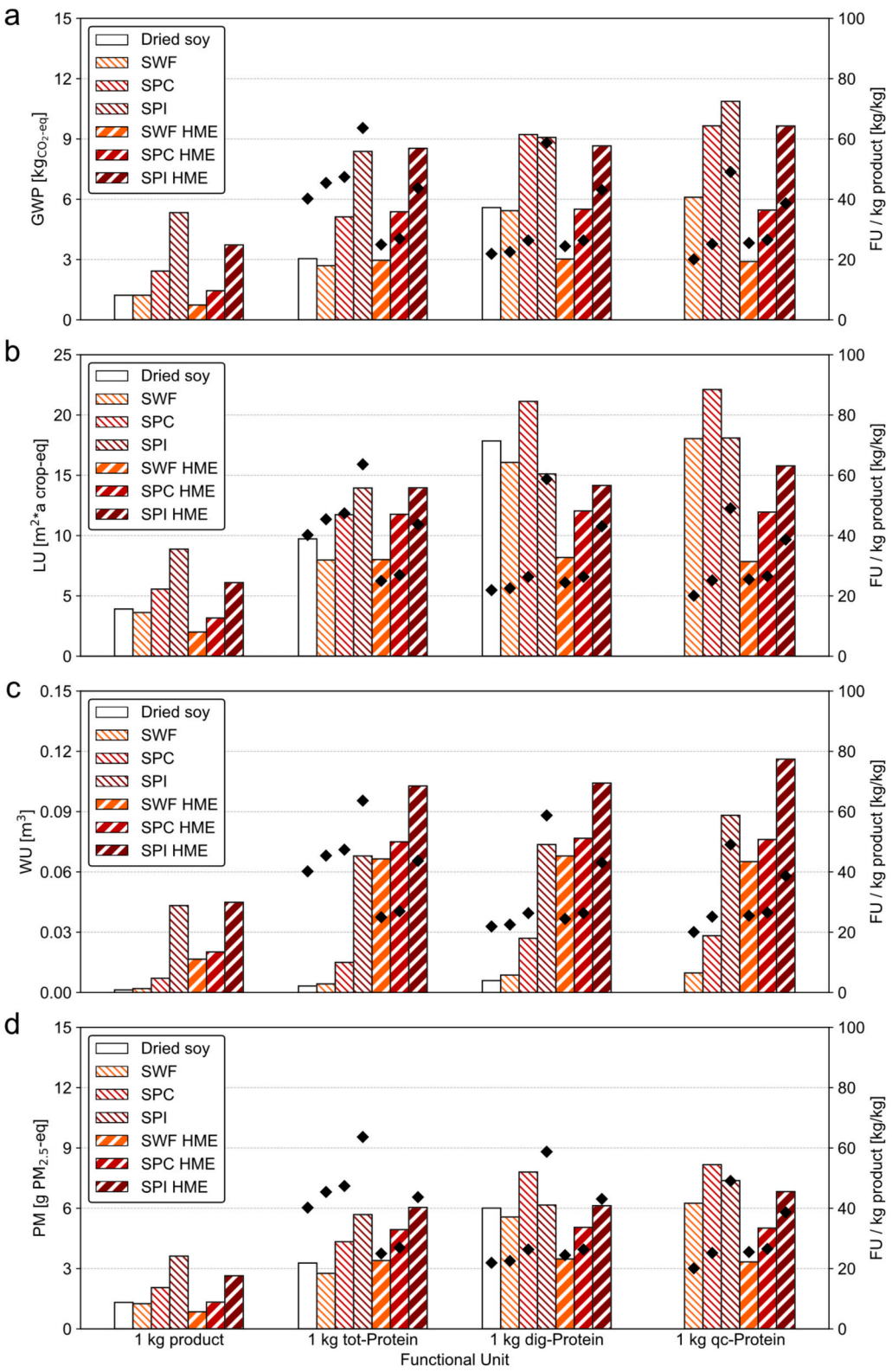

Fig. 5. Environmental impacts arising from the production of raw soybeans, soy white flakes (SWF), soy protein concentrate (SPC), soy protein isolate (SPI), and high-moisture extrudates from SWF, SPC, and SPI, respectively. The impacts are calculated per kg of fresh product, 1 kg of total (tot-) protein (N x 5.7) (FU 2), 1 kg of digestible (dig-) protein (N x 5.7) (FU 3), and 1 kg of DIAAS corrected (qc-) protein (N x 6.25) (FU 4). Impacts were calculated for a) global warming potential (GWP), b) land use (LU), c) water use (WU), and d) particulate matter emissions (PM). The black diamonds refer to the secondary y-axis on the right and indicate the content of total (FU 2), digestible (FU 3), and qc-protein (FU 4) in the respective products (FU 1). Dried soybeans assumed to have a digestibility of raw soybeans, (Yin et al. (2008)). No DIAAS values found for raw soybeans (blank for the qc-protein FU).

(product melt temperature at up to 150 °C). Other studies have reported a decrease in protein digestibility after extrusion, mostly caused by very high temperatures (≥200 °C) and high levels of reducing sugars, which can promote insoluble compound formation and Maillard reaction products (Moughan & Lim, 2024; Singh et al., 2007) or by protein aggregation into insoluble, indigestible conglomerates (Rekola et al., 2023). However, most studies found protein digestibility of raw material to be increased by mild HME (Singh et al., 2007). Likewise, in a recent human study, LME increased IAA digestibility in expanded chickpeas and yellow pea products by 18.7 % and 22.2 %, respectively, compared to pressure-cooked legumes (Devi et al., 2020). A recent study investigating HME specifically, similarly found increased *in vitro* protein digestibility compared to the starting materials (SPC and SPI), with a positive correlation of *in vitro* digestibility with product moisture content, which however our study did not identify (de Boer et al., 2025). The extrusion process in the current study improved the low total protein *in vitro* digestibility of SWF and SPC (<60 %) and did not negatively affect the protein digestibility of the SPI and commercial SPC, which had relatively high initial digestibility values (≥90 %). The produced SWF (dehulled, ground to flakes, defatted) were poorly digestible, similarly to the ileal digestibility of defatted soy flour (pig trial: 37.4 % (Li et al., 1998)). This low digestibility was likely caused by partially intact cell walls and/or the presence of antinutrients, indicating that mechanical and/or thermal treatment were not sufficient. Soybeans are the richest source of dietary trypsin inhibitors, and the trypsin inhibitors may have been concentrated during fat extraction (Gilani et al., 2012) or insufficiently inactivated due to the omitted conditioning step in SWF and SPC. In the latter, aqueous ethanol extraction was performed, resulting in increased protein content, while its total protein *in vitro* digestibility was not enhanced and remained much lower than the commercially obtained SPC. Thus, the applied elevated temperature during extraction was insufficient to compensate for the lack of conditioning, and as a potentially digestibility-improving functionalization step was omitted, the low value is consistent with expectations. In contrast, the high total protein *in vitro* digestibility of the commercial SPC aligns with available *in vivo* data (pig trial: 92 % (Cervantes-Pahm & Stein, 2008)). The commercial SPC had also higher protein and lower fat content than self-produced SPC. The lower fat extraction yield of the self-produced SPC cannot be solely explained by the polarity of the chosen solvent ($scCO_2$) but can also be attributed to other material characteristics such as bulk density and particle size of the SWF. The resulting high residual fat content potentially negatively impacted the protein digestibility by interacting with proteins and digestive enzymes, in addition to the effects of antinutrients. Finally, heat-induced protein denaturation could have enhanced protein digestibility by unfolding polypeptides and making them more accessible to digestive enzymes in the commercial SPC. In contrast, the self-produced SPI reached a high protein *in vitro* digestibility comparable to previously reported true ileal digestibility of commercial SPI assessed in humans of 91 % (Mariotti et al., 1999). Ultimately, extrusion leveled the protein digestibility of SWF and SPC to that of SPI-based extrudates, likely due to protein unfolding and thermal inactivation of antinutritional factors under elevated temperature, pressure, and shear.

Protein quality of meat analogues assessed by *in vitro* DIAAS ranged from 81 to 102, indicating good (DIAAS 75–99) to excellent (DIAAS ≥100) protein quality (reference pattern: 0.5–3 years) (FAO, 2013). Their protein quality is, therefore, similar albeit lower than animal-based proteins (DIAAS ≥100), but generally higher than other plant-based proteins (Adhikari et al., 2022), which can be explained by the soy IAA profile. Consistently, in a pig trial, Fanelli et al. (2022) found DIAAS of a SPC-based burger (91, SAA) to be higher than a pea protein isolate based burger (71, SAA), but both were lower than a beef burger (111). Sousa et al. (2023) found that *in vitro* DIAAS for a grilled soy burger (94, SAA) was lower than for a grilled beef burger (124) but was higher than a pea-faba burger (69, tryptophan). Soy-based meat analogues produced from the Protéix culivar (DIAAS: 81–93) had comparable protein quality by DIAAS as soymilk (DIAAS: 85) and tofu (DIAAS: 79) produced with the same cultivar, and processing steps of all three end products strongly improved protein quality compared to cooked soybeans (Hammer et al., 2024). Self-produced SWF and SPC had relatively low DIAAS values (< 50), because of their low protein digestibility, but the commercial SPC, representative of commercial products had higher protein quality (DIAAS: 83). A recent quantitative review reported a mean ± SD DIAAS of 84.5 ± 11.4 (SAA; 0.5–3 years) for various soy products (van den Berg et al., 2022). The differences in DIAAS were attributed to undefined food preparation steps, soybean cultivar, and study conditions. The compiled DIAAR data for SPC and SPI of this quantitative review are similar to our *in vitro* DIAAR values for the commercial SPC and self-produced SPI, while the self-produced SPC had a lower DIAAR due to the low IAA digestibility. Upon HME, the DIAAS increased the least for SPI (from 70 to 81), resulting in the lowest final value for the extrudate due to a reduction in SAA content. A similar decrease in SAA being reflected in a small increase in DIAAS upon extrusion was observed in LME (for LEI: from 83 to 83; for HEI from 83 to 86), where the highest product melt temperatures were reached, suggesting that SAA degradation is influenced by process harshness (SI-Table C3).

As for macronutrients, the representativeness of our pilot-scale process for LCA was limited (SI-Fig. B2) due to reasons outlined in SI-B. At the same time, the results obtained from the industry-scale data align with previous findings. For example, the GWP impacts per kg of SWF, SPC, and SPI found here (Fig. 5a) are at the lower end of ranges reported in literature, which are 0.34 to 0.90 $kg_{CO2\text{-}eq}$ (Dalgaard et al., 2008), 1.5 to 10.8 $kg_{CO2\text{-}eq}$ (Thrane et al., 2024), and 6.7 to 20.2 $kg_{CO2\text{-}eq}$ per kg product (Berardy et al., 2015; Thrane et al., 2024), respectively. Thus, while nutritional quality can theoretically be represented at pilot scale, industry-scale modelling based on secondary data remains the preferred approach for LCA. Cultivation was the primary driver of all studied environmental impacts except for water use (Fig. 4). Thus, maximising yields during processing is crucial to minimize inputs from the cultivation phase. Besides cultivation, drying and extraction were major LCA contributors, especially for the GWP. Consequently, reducing the degree of refinement holds considerable potential to reduce environmental impacts. Unlike SWF, which requires minimal process water, SPC and SPI rely on wet extraction steps, from which all water must be removed by drying to obtain the powdery intermediate product. Streamlining these wet extraction steps or introducing slurry intermediate products instead of powders are promising levers for the future. Additionally, commercial extrudates are typically not solely based on highly refined ingredients, such as SPI, but incorporate other components (Schmid et al., 2022). Modifying the formulation presents opportunities to mitigate the environmental impact per FU kilogram of product. However, employing only a mass-based FU fails to account for nutritional quality.

By integrating the nutritional product quality assessment, a nutritional LCA (nLCA) was conducted to comprehensively assess the influence of processing on nutritional quality and environmental impacts. The nLCA demonstrated that the choice of the FU strongly influences the interpretation of the results (Fig. 5). While processing generally increases the environmental impacts when evaluated based on 1 kg of product or total protein, it can reduce the impact per kg of qc-corrected protein due to the gained increase in protein digestibility. Namely, GWP, LU and PM per kg of qc-protein increased with protein extraction but decreased with HME with a more pronounced effect for mildly processed intermediates products, resulting in the lowest impacts for extrudates produced from SWF. Hence, although processing has a significant environmental impact, it may enhance digestibility, highlighting the relevance of nLCA in tailoring process designs.

Soymilk and tofu are established protein sources of good quality and our data indicates that soy-based meat analogues are equal in this respect (Figs. 2 & 3). For comparison, chicken meat produced in Switzerland has a protein content of approximately 26.8 g/100 g

(Hammer et al., 2023), a DIAAS of 113 (Fig. 3) and a GWP of around 3.5 kg CO_2-eq per kg total mass (Alig et al., 2012). This results in 13 kg CO_2-eq per kg protein and 11.6 kg CO_2-eq per qc-protein. Hence, the GWP impacts for industrially produced extrudates from SPI, SPC, and SWF reported in Fig. 5 are 17, 53, and 75 % lower per kg of qc-protein compared to Swiss chicken meat. However, the investigated products contain other essential nutrients apart from protein and may be sources of important secondary components. Thus, more comprehensive studies beyond protein quality are required to fully understand the nutritional and environmental consequences of replacing substantial portions of animal-based foods in current diets.

The strengths of this study are that meat analogues were manufactured by self-produced intermediate products with a single defined soybean cultivar allowing direct comparison across the processing value chain and with previous studies that used the same cultivar. Processing operations were transparently reported, including mass balances, allowing to draw conclusions regarding yield, texturization characteristics, protein quality, and environmental footprint and relate it to their production, enabling the use of nutritional-quality based FUs in the nLCA. The *in vitro* digestibility assessment by Sousa et al. (2023) based on the INFOGEST protocol with good agreement with *in vivo* data was used to estimate the ileal AA digestibility and DIAAS. Environmental impacts were estimated for industrial-scale production using industrial data complemented by modelling where primary data were unavailable, and results were compared to actual pilot-scale data to elucidate the influence of production scale and emphasize the relevance of scale-appropriate data in LCA studies. The limitations of the study are that the fat extraction process was not optimal and no functionalization step of the proteins was included, which resulted in meat analogues that were not fully representative of industrially produced ones. Thus, an industrial SPC had to be included as a reference. Further, the *in vitro* model has so far only been validated with a limited number of food sources, not including meat analogues, and more comparative data between *in vitro* and *in vivo* methods including human studies on highly transformed products are needed.

5. Conclusion and outlook

In conclusion, this study is among the first to investigate protein quality from meat analogues across the food production value chain. Despite limitations in replicating the industrial scale process, soy processing increases the content of quality-corrected protein, while increasing environmental impacts. An optimal process would combine mild extraction with extrusion, yielding extrudates with 75 % lower global warming potential per quality-corrected protein compared to chicken meat. In contrast, this impact was only 17 % lower for extrudates based on wet-extracted soy protein isolate. While the DIAAS values for these specific products need further confirmation, they are in good agreement to previously published *in vivo* data. This study further underscores the potential of nLCA and advocates for its broader application in decision-making frameworks integrating nutrition with environmental considerations.

Declaration of generative AI and AI-assisted technologies

In the writing process large language models and AI-assisted technologies were used to improve the readability and language of the manuscript. The authors take full responsibility for the content of the publication.

CRediT authorship contribution statement

Laila Hammer: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Corina Sägesser:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Armin Siegrist:** Writing – original draft, Investigation, Formal analysis, Conceptualization. **Mario Arcari:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Moritz Goessler:** Investigation, Formal analysis. **Pornpimol Scheuchzer:** Investigation, Conceptualization. **Moritz Müller:** Methodology, Investigation. **Christoph Denkel:** Writing – review & editing, Supervision. **Joseph Dumpler:** Writing – review & editing, Supervision. **Alexander Mathys:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Lotti Egger:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Reto Portmann:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Diego Moretti:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Diego Moretti reports financial support was provided by Swiss National Science Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary information

Supplementary information to this article (SI-A, -B and -C) can be found online at https://doi.org/10.1016/j.foodres.2025.117636.

Data availability

All relevant data are made available in the supplementary material.

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