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Structure, texture, and sensory properties of plant-meat hybrids produced by high-moisture extrusion

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ABSTRACT

Hybrid products, in which a portion of meat is replaced with plant proteins, could be an effective solution to reduce meat consumption and its environmental impact, and provide well-balanced nutritious food. This study focused on exploring the potential of creating hybrid meat products by high-moisture extrusion from a mixture of minced beef (with 7 or 17% fat) and two different commercial pea protein ingredients. Hybrid extrudates were successfully produced from a 1:1 mixture of beef and either pea protein isolate (PI) or milled texturised pea protein concentrate (TPC). Differences in the appearance, texture and sensory properties of the hybrid extrudates depended both on ingredient properties and extrusion processing parameters (especially temperature). Extrudates with beef and PI were softer and layered, while extrudates with beef and TPC were harder and had smaller fibres. The fat content of the beef did not significantly affect the textural properties of the extrudates. The extrudates with beef and TPC had a meat-like odour and umami taste, while extrudates with beef and PI had a dominant pea-like taste and odour.

1. Introduction

The current food system is unsustainable. It generates one fourth of the global gas emissions and consumes half of habitable land and 70% of global freshwater resources (*OurWorldinData*, 2020). Among food systems, livestock production is the most inefficient. A 10% replacement of animal-based products with plant-based ones would save 176 million tons CO_2e, 38 million hectares of land, and 8.6 billion m^3 freshwater (*Blue Horizon, 2020*). This scenario would also enable the use of resources for food rather than feed.

Reduction of meat consumption by plant-meat hybrid products has been suggested as one way towards lowering the environmental impact (Baune et al., 2021). Several consumer studies have reported that a partial replacement of meat with plant-based protein is more acceptable for most of the consumers than 100% replacement of meat (Apostolidis & McLeay, 2016; Baune et al., 2021; Neville, Tarrega, Hewson, & Foster, 2017; Profeta et al., 2021). By-products of meat processing, e.g. mechanically deboned meat, can be utilised for production of plant-meat hybrids (Knoch, 2016). Additionally, blending of meat with plant-based proteins is cost-effective, it reduces calories and saturated fat, maintains high protein levels, increases fibre content, and has potential to improve the environmental footprint along with human health (Beecher, 2020).

The high-moisture extrusion process (HMEP) is a high temperature and shear-intensive process where protein unfolding, aggregation, and cross-linking, combined with a dramatic temperature drop at the cooling die, leads to formation of meat-like fibrous structures (Zhang, Chen, Kaplan, & Wang, 2022). Flavour and structural challenges are still key factors in hindering consumer acceptance of plant-based meat alternatives (Elzerman, Keulemans, Sap, & Luning, 2021). However, blending of plant-based ingredients with animal proteins can improve the protein nutritional quality (Baune et al., 2021) as well as sensory and textural properties compared to purely plant-based ingredients (Neville et al., 2017).

Legumes are popular ingredients for meat analogue production as they are excellent source of nutrients (Shanthakumar et al., 2022). Pea protein isolate (PI) is rich in protein; however, isolates are usually produced by wet fractionation, which is energy and water intensive

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Abbreviations: FAME, fatty acid methyl ester; HMEP, high-moisture extrusion processing; MUFA, monounsaturated fatty acid; OBC, oil binding capacity; PCA, principal component analysis; PI, pea protein isolate; PUFA, polyunsaturated fatty acid; RVA, rapid visco analyzer; SFA, saturated fatty acid; SME, specific mechanical energy; TPC, texturised pea protein concentrate; WBC, water binding capacity.

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process (Assatory, Vitelli, Rajabzadeh, & Legge, 2019). Therefore, protein concentrates that are produced by dry fractionation are more sustainable, but do not perform well during HMEP typically due to low protein content (<60%) and presence of other components such as starch or dietary fibre (Maningat, Jeradechachai, & Buttshaw, 2022; Schutyser, Pelgrom, Van der Goot, & Boom, 2015). Protein concentrates can be texturised and used for meat analogue production. Texturisation denaturates and crosslinks proteins as well as reduces protein solubility (Vatansever, Tulbek, & Riaz, 2020). Texturised proteins are typically used as a co-ingredient in hybrid products due to their good binding properties (Baune et al., 2021; Baune, Terjung, Tülbek, & Boukid, 2022; Neville et al., 2017).

Previous studies focusing on HMEP of mixtures of animal and plant-based proteins are limited. Knoch (2016) studied extrusion of pork and soy protein concentrate blend and observed that HMEP is a promising technology for restructuring by-products from meat processing. In a recent study, blending of soy protein isolate with 15% and 30% whey protein concentrate, led to improved anisotropic structure formation and texture in high-moisture extruded hybrid products (Wittek, Karbstein, & Emin, 2021). Also, Nisov et al. (2020) showed that HMEP of a blend of whole or gutted fish (70%) with PI (30%) resulted in a new product concept with good microbiological stability and sensory properties.

The aim of this study was to explore the potential of creating hybrid products from a blend of minced beef and two commercial pea protein ingredients by using HMEP. Hybrid extrudates were produced from a 1:1 mixture of minced beef (with 7 or 17% fat) and either PI or milled texturised pea protein concentrate (TPC). Physicochemical properties (particle size, protein solubility, viscosity, water and fat binding capacity) of the pea ingredients were analysed with the aim of gaining understanding of their behaviour in the extrusion process. The texture of the hybrid extrudates was analysed by compression and cutting tests with a Texture Analyser. The sensory properties (flavour, structure, and texture) of the extrudates were also evaluated by trained sensory panellists.

2. Materials and methods

2.1. Ingredients

The pea ingredients used in this study were PI (Nutralys® F85M, Roquette, France, protein 84.3% on dry basis, starch 0.3%, according to manufacturer: fat 9.0%) and TPC (Nutralys® T65M, Roquette, France, protein 71.4% on dry basis, starch 13.3%, according to manufacturer: fat 7.5%), which was ground before use at 10.000 rpm with an ultra-centrifugal mill (ZM 200, Retsch GmbH, Germany) using a 0.5 mm sieve. As meat ingredients, commercial minced beef with low fat content (Kunnon naudan paistijauheliha 7%, Snellman Oy Ab, Finland, according to manufacturer: 7% fat, 20% protein, 0.1% salt), and minced beef with high fat content (Naudan jauheliha, Kotimaista, HKScan Finland Oy, Finland, according to manufacturer: 17% fat, 19% protein, 0.16% salt) were used.

2.2. Characterisation of pea ingredients

2.2.1. Particle size distribution

Particle size distribution was measured from PI and milled TPC samples with Mastersizer 3000 (Malvern Instruments Ltd., UK) with an Aero S dry dispersion unit as described in Nisov, Nikinmaa, Nordlund, & Sozer (2022). Distribution was calculated with the Fraunhofer Approximation. Samples were measured in triplicate, one measurement being 5 s long and the measurement range set to 0.005–5000 μm.

2.2.2. Protein content, solubility, and starch

The total protein content was analysed from PI and from milled TPC with a Protein/Nitrogen Analyser (rapid MAX N exceed, Elementar Analysensysteme GmbH, Langenselbold, Germany) with a conversion factor of 6.25. The total protein content was determined based on the nitrogen (N) content according to the AACC (1995) crude protein combustion method 46–30.01, in triplicate. Additionally, starch content was evaluated from PI and TPC with a Total Starch Assay kit (AA/AMG, Megazyme, Ireland) according to the instructions provided by the manufacturer. Starch content was evaluated in triplicate.

Protein solubility samples were prepared as described in Pöri, Nisov, and Nordlund et al. (2022), with slight modifications. Shortly, 40 mg/g PI and TPC suspensions were prepared in Milli-Q water, mixed for 5 min, and centrifuged at 10.000×*g* at 20 °C for 15 min. The supernatant was collected and stored at −20 °C until it was analysed with a Protein/Nitrogen Analyser as described above. Protein solubility was calculated as the ratio of the protein content in the supernatant to the protein content in the suspension before centrifugation. Protein solubility was calculated as the average of quadruplicate measurements.

2.2.3. Water and oil binding capacity

The water binding capacity (WBC) of PI and milled TPC was analysed in excess water as described in Silventoinen, Kortekangas, Ercili-Cura, and Nordlund et al. (2021). Shortly, 1 g of PI or TPC was mixed with distilled water (10 mL) and shaken manually for 5 s at 10 min intervals for 30 min. The suspension was centrifuged (Heraeus Multifuge X1R, Thermo Fisher Scientific, Germany) at 2.000×*g* for 10 min, the supernatant poured away and the leftover pellet was weighed. Water binding was expressed as g water retained in the pellet per g sample dry matter. The analysis was performed in triplicate.

Oil binding capacity (OBC) was analysed in triplicate from PI and TPC according to AACC (2009) 56–11.02 method, with slight modifications. Water was replaced by oil and sample amount of 2.5 g was used instead of 5 g. Shortly, 2.5 g of sample was mixed with rapeseed oil (25 g) and vortexed for 5 s in 5 min intervals for 20 min. The suspension was centrifuged (1.000×*g*, 15 min) and the supernatant was poured away. The sediment was weighed and OBC was expressed as g oil retained per g sample dry matter.

2.2.4. Viscosity (RVA)

Viscosity was measured in triplicate from PI and milled TPC with a Rapid Visco Analyser (RVA) super 4 (Newport scientific, Australia). First, a 150 mg/g suspension was prepared to distilled water according to dry matter content (total volume of 28.5 g). The suspension was tempered in RVA for 15 min at 25 °C, and then heated to 99 °C at a rate of 10 °C/min. Then the suspension was tempered at 99 °C for 5 min, cooled back to 25 °C at a rate of 10 °C/min, and finally kept at 25 °C for 5 min. Mixer speed was set to 160 rpm. Initial viscosity after 1 min and 15 min of mixing at 25 °C, viscosity after 5 min at 99 °C, and final viscosity at the end of the measurement were recorded from the viscosity curve.

2.3. High-moisture extrusion processing

Hybrid meat extrudates were produced with a lab-scale twin-screw extruder (Process11 Hygienic, Thermo Scientific, Germany) equipped with a long cooling die (5 × 20 × 250 mm). The screws were co-rotating with a diameter of 11 mm (L/D 40:1). Torque, die temperature, and the temperature of the sample before entering the cooling die i.e., melt temperature, were recorded during extrusion (Table 1). The optimal temperature profile for PI was adapted from Nisov et al. (2020). The aim was to use same profile for all samples, but as TPC-containing samples did not show structure formation at the same temperatures as PI, higher temperatures were chosen (Table 1). Water feed was adjusted according to visual observations. Cooling die temperature, screw speed, and flour feed were fixed parameters in the extrusion (Table 1). Reference extrudate was made with PI (referred now on as PI_ref) and rest of the extrudates were mixtures of beef (low or high fat) and PI or TPC, mixed in 1:1 ratio in a food processor (CombiMax 600, Braun GmbH, Germany)

Table 1
Conditions of the high-moisture extrusion processing. (PI: pea protein isolate, TPC: texturised pea protein concentrate, T: temperature, Hifat: high fat content; Lowfat: low fat content; Ref: reference; SME: specific mechanical energy).

Sample	Temperature profile from die to feeder (°C)	Screw speed (rpm)	Flour (g/h)	Water (mL/h)	Melt T (°C)	Die T (°C)	Cooling die T (°C)	Torque (N)	SME (kJ/kg)
PI_ref	125 150 150 135 100 80 70 60	250	250	295	125	125	30	0.8	302
PI_hifat	125 150 150 135 100 80 70 60	250	250	77	125	125	30	0.7	264
PI_lowfat	125 150 150 135 100 80 70 60	250	250	107	125	125	30	0.7	264
TPC_hifat	145 160 160 145 100 80 70 60	250	250	78	143	145	30	0.7	264
TPC_lowfat	145 160 160 145 100 80 70 60	250	250	75	143	145	30	0.7	264

(referred now on as PI_hifat, PI_lowfat, TPC_hifat, and TPC_lowfat). The formed extrudates were vacuum packed and frozen at −20 °C. Dry matter contents were measured in triplicate with a moisture analyser (MB120, OHAUS Corporation, USA) from thawed extrudates homogenised with a Bamix® hand blender. The thawed extrudates were also examined at 1× magnification with a stereomicroscope (ZEISS SteREO Discovery.V8, Carl Zeiss AG, Germany). Images were taken from the surface of the intact extrudates and from the fractured area after bending the extrudates in the longitudinal direction. The specific mechanical energy (SME) was calculated according to Osen, Toelstede, Wild, Eisner, and Schweiggert-Weisz (2014).

2.3.1. *Fat content and fatty acid profile of extrudates*

Analysis of the fat content was based on the NMKL 160 method (*NMKL 160, 1998*) in duplicate. Shortly, homogenised extrudates were hydrolysed (HydroTherm, Gerhardt GmbH, Germany) with 4 mol/L hydrochloric acid and fat was extracted (Soxtherm, Gerhardt GmbH, Germany) with petroleum ether solvent. The solvent was evaporated, and the extracted fat amount was weighed.

Fatty acid profile was analysed by first extracting the fat from homogenised extrudates by hexane-iso-propanol (3:2, v/v) mixture. The mixture was centrifuged, and the supernatant was moved to a tube. Solvent was evaporated in a 70 °C heating block under nitrogen flow. Fatty acid methyl esters (FAMEs) were prepared from the extracted fat as described by Bannon et al. (1982). The prepared FAMEs were then analysed with an Agilent 7890B gas chromatograph (Agilent Technologies Inc., USA) equipped with a flame ionization detector (FID). The FAMEs were separated on an Agilent CP-SIL 88 capillary column (50 m × 0.25 mm i.d. with 0.2 μm film). Hydrogen was used as a carrier gas with 90 kPa constant pressure. The oven temperature program was the following: from 70 °C (hold 1 min) to 160 °C with 10 °C/min, to 190 °C with 3 °C/min and to 240 °C with 10 °C/min (hold 10 min). The injector and detector temperatures were 250 °C. Injected sample volume was 1 μl in a 1:80 split ratio. FAMEs were identified based on FAME reference mixtures and literature data.

2.3.2. *Textural analysis of extrudates*

Cutting and compression force was measured from the extrudates similarly as described in Saldanha do Carmo et al. (2021), with a Texture Analyser (TA.XTplus, Stable Micro Systems Ltd., UK). Thawed extrudates were tempered to room temperature and test pieces cut into squares (1.7 × 1.7 cm). Cutting force was measured in cross-sectional (C) and longitudinal (L) directions according to the flow of the material in the cooling die, by using a knife blade (HDP/BS). The test speed was set to 2 mm/s, and maximum cutting force was determined for 8–10 replicate samples in both directions. The texturisation degree was calculated as ratio of the cutting force in the two measured directions (L/C) (Chen, Wei, Zhang, & Ojokoh, 2010). For compression measurements, a 45 mm aluminium cylindrical probe was used. The test speed was set to 1 mm/s and the target strain to 50%. Maximum compression force was determined from 8 to 10 replicate samples.

2.3.3. *Sensory evaluation of extrudates*

The sensory attributes of the extrudates were characterized with generic descriptive analysis. The sensory panel consisted of eight trained assessors. Written informed consents were obtained from the assessors prior to the evaluation. The base sensory lexicon was formulated by four trained panellists who were not part of the main panel. Previous sensory profiling studies done in the organization (Nisov et al., 2020) were used as a basis. The base lexicon was then provided along with all samples for the training session for the eight panellists divided in two groups. During training, the two groups refined the base attribute list, discussed the intensity ranges of the samples, and decided on reference samples for the intensities. The resulting sensory profile had nine attributes (pea odour, meat stock odour, meat's frying fat odour, umami, pea flavour, toughness, fattiness, fibrousness, and breakability). Five reference products and one set of reference images were also included for further specifying the attribute types and for anchoring the 0–10 line scales.

The microbiological quality of the extrudates was analysed against the typical microbes in meat products. No growth was observed and thus the microbiological quality was considered acceptable. The extrudates and reference products were served in 50 mL transparent closed plastic cups with 3-digit codes. The frozen extrudates were first cut into 4 cm slices and thawed for 30 min at ambient temperature. Three slices were then placed in each closed plastic cup, the samples were heated for 15 min at 50 °C, and further cooled for 15 min in the plastic cups before serving. Wheat toast bread and water were used as palate cleansers. The extrudates were evaluated in duplicate in a complete block design with a Latin square serving order randomization. The data were collected with Compusense five 5.6 (Compusense Inc., Guelph, Canada).

2.3.4. *Statistical analysis*

SPSS version 26 and 28 (IBM Corp, USA) were used for statistical analyses. The limit of the statistical significance was $p < 0.05$. The descriptive sensory data was analysed with a two-way mixed model analysis of variance (ANOVA) with samples as the fixed factor and the assessors as a random factor. Textural data was analysed with one-way ANOVA. Tukey's HSD was used as the post hoc test. The principal component analysis (PCA) model containing the sensory profiling attributes, absolute fatty acid contents (approximated as relative fatty acid composition multiplied with the lipid content) and instrumental texture measurements were created with The Unscrambler version 10.5.1 (CAMO Software AS, Norway) with averaged, autoscaled data.

3. Results and discussion

3.1. *Characteristics of pea ingredients*

Initial particle size of pea ingredients were similar (Table 2). Although the particle size of the protein ingredients doesn't have an essential impact in fibrous structure formation (Osen et al., 2014), it affect WBC, solubility, and viscosity of the ingredients and may thus indirectly affect their performance during HMEP. Particle size also plays an important role in the flow behaviour of a powder in the feeder of the extruder (McGuire, Siliveru, Ambrose, & Alavi, 2022).

The protein solubility of both PI and TPC was relatively low at their native pH (~7.5) (Table 2), which is in agreement with previous studies (Osen et al., 2014; Wang, Bhirud, & Tyler, 1999). The low solubility of the ingredients is expected to be a result of protein denaturation taking place during the thermomechanical treatments involved in their

Table 2

Properties of the pea ingredients. (PI: pea protein isolate; TPC: texturised pea protein concentrate; WBC: water binding capacity; OBC: oil binding capacity) (Replicates: particle size, water binding, oil binding and viscosity were done in triplicate, and protein solubility in quadruplicate).

Sample	Particle size D50 (μm)	Protein solubility (%)	WBC g/g	OBC g/g	Viscosity (mPa s)			
					Initial	15 min/25 °C	5 min/99 °C	Final
PI	109 ± 0	26 ± 1	4.2 ± 0.1	1.5 ± 0.0	2675 ± 113	745 ± 7	119 ± 1	446 ± 6
TPC	119 ± 2	12 ± 0	2.6 ± 0.3	1.5 ± 0.0	265 ± 22	277 ± 18	172 ± 5	898 ± 25

production process. It is generally considered that protein solubility should be limited for structure formation to place in HMEP (Geerts, Dekkers, van der Padt, & van der Goot, 2018). For example, in the study of Geerts et al., 2018, it was shown that fibrous structure formation by HMEP from a blend of soy protein and soy flour was possible only after toasting the blend at 150 °C, which decreased its nitrogen solubility index from about 90% to below 40%.

The WBC results showed that PI had higher WBC than TPC (Table 2). The WBC of PI was quite close to the previously reported value (5.0 mL/g) for the same commercial PI (Osen et al., 2014). Wang et al. (1999) reported that texturisation increased the WBC of dry fractionated pea protein concentrate from 1.0 to 2.1 g H_2O/g. WBC has been shown to increase with an increasing degree of protein denaturation, and thus with a decrease in protein solubility (Akharume, Aluko, & Adedeji, 2021; Ma et al., 2011; Quinn & Paton, 1979). WBC is also affected by presence of other compounds than protein in the matrix, such as starch or dietary fibre (Aguilera, Esteban, Benitez, Molla, & Martin-Cabrejas, 2009), however, the higher WBC of PI in the present study was most likely related to its high protein content (Ma et al., 2022). In the study of Geerts et al., 2018, a WBC of around 6 g/g of a toasted soy protein-soy flour blend was associated with fibrous structure formation, whereas without toasting it was about 1 g/g and no anisotropic structure formation took place.

The OBC results showed that PI and TPC had the same OBC (Table 2). Osen et al. (2014) reported an OBC of 1.7 mL/g for the same commercial PI as in the present study. Another, less denatured PI had a lower OBC of 1.3 mL/g at pH 7.5. The OBC of pea ingredients is not to the same extent affected by the protein content as WBC (Ma et al., 2022).

There was a clear difference in the viscosity profiles of PI and TPC (Table 2). PI showed a very high initial viscosity which decreased during the 15 min tempering at 25 °C, and even more during tempering at 99 °C. However, PI viscosity increased during the cooling phase, thus final viscosity was 446 mPa s. On the other hand, TPC had a quite low initial viscosity, which increased slightly during the 15 min tempering at 25 °C. At the end of 99 °C tempering, TPC's viscosity had decreased but as the cooling phase started, viscosity started to increase rapidly and final viscosity of TPC was 898 mPa s.

A similar difference in viscosity profiles was reported by Osen et al. (2014) for two different commercial PI products. They showed that the high initial viscosity was linked with high WBC, low solubility, and complete protein denaturation of the material. In this study, the difference between initial viscosity of the PI and TPC could be related to the higher protein content and WBC of PI, which already at 25 °C forms a highly viscous network due to hydration and swelling of the PI particles. It seems that the formed network is broken down rather easily during further mechanical shearing. The TPC dispersion was much less prone to changes during the initial mixing phase at 25 °C. The higher final viscosity of TPC could be caused by re-association of the starch molecules present in the material (Pelgrom, Boom, & Schutyser, 2015), although it is possible that the starch was already partly gelatinised in the texturisation process. The starch content of PI was very low. The final viscosity after heating and cooling in the RVA may give an indication of the ability of the material to solidify (e.g. by gelation) after heating and cooling in the extrusion process, although the ingredient concentration and heating temperature were much lower in the RVA measurements than during actual HMEP. Fixation of the phase separated structure by solidification in the cooling die can affect the formation of anisotropic structures in extrusion (Tolstoguzov, 1993).

3.2. Characteristics of extrudates

3.2.1. Fatty acid profile

Fatty acid profile and total fat content was evaluated from the extrudates (Tables 3 and 4). As expected, the lowest fat content (~4%) was measured from PI_ref sample, which was 100% plant-based extrudate and the highest fat contents (~9%) were measured from the PI_hifat and TPC_hifat extrudates (Table 4). Statistical differences were observed between the extrudates, however, high fat hybrid extrudates were in the same post hoc group. Differences between fat contents were mostly related to fat content of the extruded mass and fat loss during extrusion as it was dripping from the sides of the cooling die.

Generally, there were clear differences between the fatty acid contents of the samples. Compared to PI_ref, the addition of beef increased the contents of palmitic, palmitoleic, stearic, and oleic acid in the hybrid extrudates (Table 3). Hybrid extrudates had almost equal amount of saturates fatty acids (SFA) (35–38%), while PI_ref had only 19%. Palmitic, stearic, and myristic acid comprised 89–95% of all SFAs. The amount of monounsaturated fatty acids (MUFAs) in the hybrid extrudates varied between 39 and 46%, and PI_ref had 26%. Over 50% of PI_ref's fatty acids were polyunsaturated fatty acids (PUFAs), as expected. Generally, plant proteins are richer in PUFAs than animal

Table 3

Relative contents (%) of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA), and ratio of omega fatty acids of the extrudates. (PI: pea protein isolate; TPC: texturised pea protein concentrate; Hifat: high fat content; Lowfat: low fat content; Ref: reference; n.d., not detected).

Fatty acid	PI_ref	PI_hifat	PI_lowfat	TPC_hifat	TPC_lowfat
Saturated Fatty Acids total	19.4 ± 3.1	38.4 ± 6.1	35.2 ± 5.6	38.6 ± 6.2	35.2 ± 5.6
Myristic acid (C14:0)	*0.3 ± 0.1*	*2.2 ± 0.2*	*1.9 ± 0.2*	*2.2 ± 0.2*	*1.8 ± 0.2*
Palmitic acid (C16:0)	*12.2 ± 1.2*	*21.8 ± 2.2*	*20.3 ± 2.0*	*21.8 ± 2.2*	*20.2 ± 2.0*
Stearic acid (C18:0)	*4.9 ± 0.5*	*12.3 ± 1.2*	*10.7 ± 1.1*	*12.5 ± 1.2*	*10.8 ± 1.1*
other SFA	*2.0*	*2.1*	*2.3*	*2.1*	*2.4*
Monounsaturated Fatty Acids total	26.3 ± 4.2	45.1 ± 7.2	41.3 ± 6.6	46.0 ± 7.4	39.1 ± 6.3
Palmitoleic acid + isomers (C16:1)	*0.3 ± 0.1*	*4.4 ± 0.4*	*3.5 ± 0.4*	*4.6 ± 0.5*	*3.4 ± 0.3*
Oleic acid + isomers (C18:1)	*25.6 ± 2.6*	*39.1 ± 3.9*	*36.3 ± 3.6*	*39.6 ± 4.0*	*34.6 ± 3.5*
other MUFA	*0.4*	*1.6*	*1.5*	*1.8*	*1.1*
Polyunsaturated Fatty Acids total	53.6 ± 8.6	13.9 ± 2.2	21.0 ± 3.4	12.7 ± 2.0	23.2 ± 3.7
Linoleic acid (C18:2n-6)	*44.8 ± 4.5*	*11.3 ± 1.1*	*16.9 ± 1.7*	*10.1 ± 1.0*	*18.5 ± 1.8*
α-linolenic acid (C18:3n-3)	*8.8 ± 0.9*	*2.2 ± 0.2*	*3.6 ± 0.4*	*2.2 ± 0.2*	*4.3 ± 0.4*
other PUFA	*n.d.*	*0.4*	*0.5*	*0.4*	*0.4*
Omega-3 fatty acids	8.8 ± 1.4	2.3 ± 0.4	3.7 ± 0.6	2.3 ± 0.4	4.4 ± 0.7
Omega-6 fatty acids	44.9 ± 7.2	11.5 ± 1.8	17.1 ± 2.7	10.2 ± 1.6	18.7 ± 3.0
Fatty acids Omega-6/Omega-3 ratio	5.1	5.0	4.6	4.4	4.2

Table 4
Dry matter (%), total fat content (%), compression (N), cutting force (N), and texturisation degree results of the hybrid meat extrudates. Different superscript letters (a, b, c, and d) indicate a significant difference ($p < 0.05$) between the samples (Tukey's HSD). (C: cross-sectional, L: longitudinal, PI: pea protein isolate, TPC: texturised pea protein concentrate; Hifat: high fat content; Lowfat: low fat content; Ref: reference) (Replicates: dry matter was measured in triplicate, fat content measured in duplicate, compression and cutting forces were measured from PI_hifat, PI_lowfat, and TPC_hifat with 8 replicates, and from PI_ref and TPC_lowfat with 10 replicates) $p < 0.05$

Sample	Cutting force (N)		Texturisation degree	Compression (N)	Dry matter (%)	Total fat (%) as is
	L	C	L/C			
PI_ref	27 ± 2^b	11 ± 3^b	2.7 ± 1^b	76 ± 8^a	46 ± 1^a	4.2 ± 0^a
PI_hifat	12 ± 1^a	6.9 ± 3^a	1.9 ± 1^a	78 ± 10^a	52 ± 1^c	9.1 ± 0^d
PI_lowfat	12 ± 1^a	6.9 ± 1^a	1.8 ± 0^a	89 ± 11^a	46 ± 0^a	6.1 ± 0^c
TPC_hifat	29 ± 3^b	19 ± 1^c	1.6 ± 0^a	111 ± 9^b	54 ± 0^d	8.9 ± 0^d
TPC_lowfat	28 ± 3^b	19 ± 2^c	1.5 ± 0^a	118 ± 10^b	49 ± 0^b	5.4 ± 0^b

proteins (Langyan et al., 2021). The addition of beef reduced the PUFA content with over 30% compared to the reference. The low-fat hybrids had a PUFA content near 20%, while the high-fat hybrids had only 13–14% PUFA content. The PUFA content reduction in hybrid samples was expected as meat contains usually high amounts of SFAs (~40%) but has low amounts of PUFAs (Wood, Enser, Richardson, & Whittington, 2007). The higher the fat content was in beef; the higher reduction was seen in PUFA content. Linoleic and α-linolenic acid were the most abundant PUFAs (>96% of all PUFAs).

3.2.2. *Macrostructure*

Clear macro-structural differences could be observed between the hybrid extrudates (Fig. 1). Extrudate made with PI was more cracked on the surface, had a layered structure resembling myomere structures in fish (Listrat et al., 2016), and also crumbled easily. When the outer and inner structures of extrudates were compared, it could be seen that the addition of beef made the PI hybrid extrudates more compact with a homogenous internal structure, which was observed after bending of the extrudates in the longitudinal direction (Fig. 1). In the case of fish-PI hybrids, Nisov et al. (2020) observed that the structure of the hybrids was weaker compared to 100% PI extrudate as the fibrous structure formation at 125 °C was only due to PI. They speculated that with higher extrusion temperatures the fish fibre aligment could be enhanced and additionally PI would form a stronger protein network. In this study, the results are similar with PI hybrids, probably also due to fibrillation of PI at 125 °C. However, the fibre formation was hindered due to the lubricating effect of fat, as shear stress was reduced (Kyriakopoulou, Keppler, & van der Goot, 2021; Kendler, Duchardt, Karbstein, and Emin, 2021), which is in-line with lower torque and calculated SME values (Table 1). Nisov et al., 2020 reported that low torque may indicate that the protein melt inside the barrel has a lower resistance towards the shearing as a result of weaker interactions between proteins.

The TPC-containing hybrid extrudates consisted of fine protein fibres with well-designed thin laminated layers (Fig. 1). TPC was extruded with higher temperatures compared to PI, and with higher extrusion temperatures, pea proteins formed a stronger protein network and probably aligment of beef proteins was also enhanced, which improved the structure of the TPC hybrid extrudates (Nisov et al., 2020). Additionally, Osen et al. (2014) showed that with increasing extrusion temperatures, pea proteins macrostructure became more homogenous and by tearing the extrudate, the fine fibres could be observed.

Structure formation mechanism during HMEP is mainly based on protein denaturation and alignment of molecular structure in the direction of flow based on the current common knowledge on HMEP. However, knowledge on the exact mechanism is not well-known. In this study, PI and TPC were already denatured prior to HMEP, therefore structuring due to further denaturation was less likely to occur as also stated in Murillo, Osen, Hiermaier, and Ganzenmüller (2019). Under shear flow process, the hot protein melt and water mixture decomposes into two immiscible phases described as spinodal phase separation in polymer physics (Badalassi, Ceniceros, & Banerjee, 2003). Both spinodal phase separation and thermodynamic incompatibility have been introduced to influence the formation of fibrous structures during HMEP, especially for proteins which were already aggregated prior to further processing with HME (Murillo et al., 2019; Zhang, Chen, et al., 2022).

3.2.3. *Cutting and compression strength*

The cutting results clearly showed that the force required to cut the extrudates was higher in longitudinal direction than in cross-sectional direction (Table 4). This indicated that fibres were aligned more in cross-sectional direction in the extrudates. The cutting forces between PI and TPC-containing hybrid extrudates differed significantly according to the post hoc test (Table 4). The lower cutting forces for PI-containing

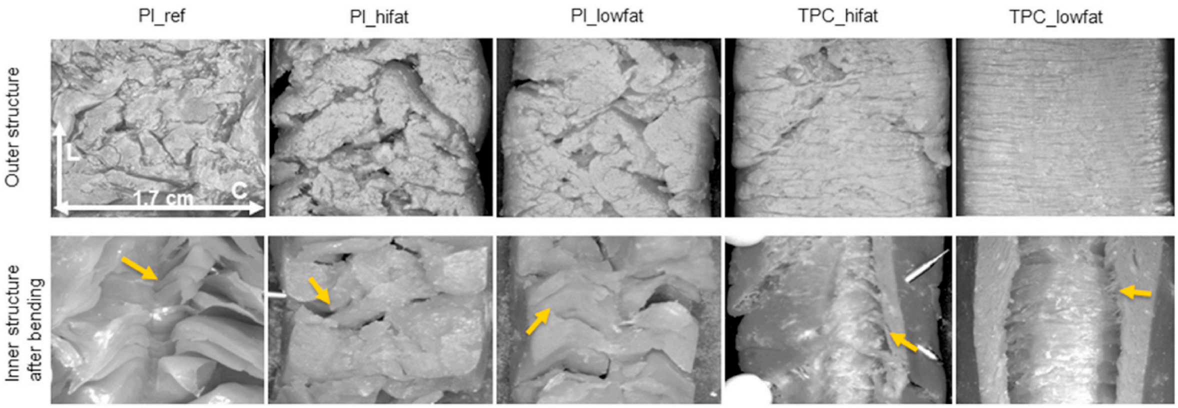

Fig. 1. Stereo microscopy images of the hybrid extrudates with 1× magnification taken from the outer structure (top view) and from the inner structure after bending the extrudate. Width of the extrudates was about 1.7 cm. Yellow arrows indicate the presence of the inner layers and fibrous structures. (C: cross-sectional; Hifat: high fat content; L, longitudinal; Lowfat: low fat content; PI: pea protein isolate; Ref: reference; TPC: texturised pea protein concentrate). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

extrudates were expected as Fig. 1 also showed that the surface of the extrudates was highly cracked in cross-sectional direction, indicating the extrudates to be prone to fracturing. Similar observations were also made by Zhang, Zhao, et al. (2022) for gluten extrudates. The higher cutting force results of TPC hybrid extrudates supported the hypothesis of formation of a stronger fibrous structure at higher extrusion temperatures. Additionally, Knoch (2016) showed that with higher extrusion temperatures cutting force increased in soy-pork hybrid extrudates as the extrudates became firmer. Osen et al. (2014) also observed that higher extrusion temperatures correlated positively with the cutting strength of PI extrudates. It seemed that the fat content of beef did not effect on the cutting results as the cutting force of PI_hifat did not differ significantly from that of PI_lowfat. A similar trend was observed for TPC_hifat and TPC_lowfat samples.

The texturisation degree value, which is the ratio between longitudinal and cross-sectional cutting forces, was used as an indicator to predict fibrous structure formation (Chen et al., 2010). The highest texturisation degree was obtained for PI_ref, with a L/C value of 2.7 (Table 4). The hybrid extrudates also showed relatively high texturisation values of 1.5–1.9, similar to those reported for boiled chicken meat (1.6) (Chiang, Loveday, Hardacre, & Parker, 2019). Kendler et al. (2021) studied the effect of vegetable oil addition (0, 2, 4, and 6%) on extrusion and observed that increasing oil content made the anisotropic structure less evident in gluten-based meat analogues. However, the same study showed that the addition of vegetable oil more towards the end of the extruder instead of barrel entrance increased the degree of anisotropy. It is good to highlight here that in this study; the lipids introduced during extrusion were fat parts of the minced beef and could only be fed to the extruder through the feed hopper after mixing with pea proteins. Recently, HMEP of PI together with stearic acid led to well-defined anisotropic structures as compared to typical unsaturated fatty acids found in vegetable oils (e.g. oleic/linoleic acid) (Chen, Zhang, Liu, Li, & Wang, 2023). Stearic acid was found to weaken the hydrogen bonds within proteins and promote conversion of α-helix and β-sheet to the β-turn and random coil structures at the cooling die. This further weakened the protein-protein interaction forces and resulted in formation of loose and flexible anisotropic fibrous structures between protein aggregates triggered by presence of hydrophobic interactions and hydrogen bonds (Chen et al., 2023).

Extrudates with PI were softer and required less compression force (76–89 N) compared to extrudates with TPC (111–118 N) (Table 4). The harder structure of TPC-containing extrudates was expected as they had a well-organized, more compact structure consisting of densely packed thin fibres based on visual observation (Fig. 1). As explained in the previous section, the authors hypothesized that this structural and textural difference associated with TPC hybrid extrudates were more associated with higher extrusion temperatures and phase separation. The dry matter content of the extrudates varied between 46 and 54%, however, no clear relation was found between the dry matter and the measured textural properties (Table 4). Textural properties of the hybrid extrudates were more related to the type of pea ingredient used.

3.2.4. *Sensory properties*

The hybrid extrudates had different sensory properties depending on their ingredients and their fat content (Fig. 2, Table 5). In general, there were 3 main sample groups based on the sensory properties: 1) PI only, 2) beef and TPC, and 3) beef and PI. The TPC-containing samples had more intense meat-like sensory properties, such as a more intense meat stock odour and umami taste but were also tougher. Kaleda et al. (2020), reported that texturisation of pea-oat protein blend reduced the amount of volatile compounds due to high temperatures during the dry extrusion process. In this study, TPC was a texturised protein and probably during pre-texturisation process there was a partial loss of volatile flavour compounds, making TPC have a milder flavour profile compared to PI, thus, meaty odour and umami taste were observed more clearly from the TPC hybrid extrudates. The PI-containing samples had more intense pea odour and flavour and were more breakable. Comparison of the three PI-containing samples demonstrated that the addition of beef increased the meaty odour and reduced the perceived beany flavour. However, this change was achieved already with the lower fat content. Both the respective low-fat and high-fat samples for both PI and TPC were similar in their sensory properties, since they were always in the same Tukey's post hoc group (Table 5). For example, there were no statistically significant differences in the fatty mouthfeel.

According to PCA model, SFAs and MUFAs were positively correlated with meat-like odour properties. Previously, SFAs and MUFAs such as palmitoleic, stearic, and oleic acids have been reported as indicator fatty acids for meaty flavour (Song et al., 2017). This is due to the specific

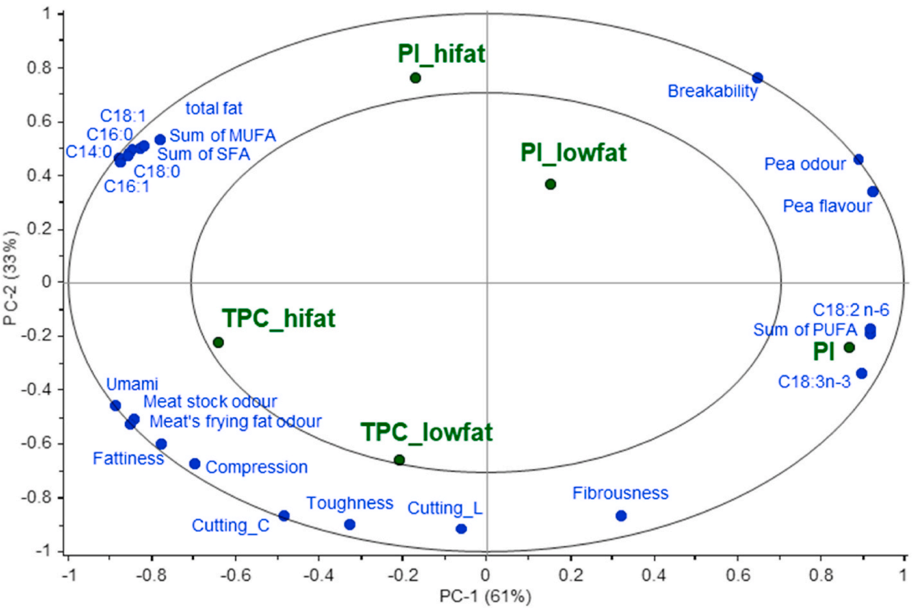

Fig. 2. Principal component analysis (PCA) model containing the sensory profiling attributes, instrumental texture measurements, and fatty acid contents. (SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; PI: pea protein isolate; TPC: texturised pea protein concentrate; L: longitudinal; C: cross-sectional; Hifat: high fat content; Lowfat: low fat content; Ref: reference).

Table 5

Sensory profiling results of the hybrid meat extrudates. The ANOVA p-values and product effect size estimates, averages, and Tukey's post hoc groups are shown. (PI: pea protein isolate; TPC: texturised pea protein concentrate; Hifat: high fat content; Lowfat: low fat content; Ref: reference) Each attribute was measured from 16 replicates. $p < 0.05$

Attribute	ANOVA		PI_ref	PI_hifat	PI_lowfat	TPC_hifat	TPC_lowfat
	p-value	partial η2					
Pea odour	0.001	0.59	5.7 ± 1.8^{a}	4.3 ± 1.6^{b}	4.6 ± 1.2^{ab}	1.9 ± 1.9^{c}	2.2 ± 2.2^{c}
Meat stock odour	0.001	0.80	0.7 ± 0.6^{c}	2.0 ± 1.3^{b}	2.3 ± 1.2^{b}	4.7 ± 1.7^{a}	4.8 ± 1.4^{a}
Meat's frying fat odour	0.001	0.63	0.2 ± 0.3^{c}	1.4 ± 1.1^{b}	1.1 ± 0.8^{b}	3.9 ± 2.7^{a}	3.7 ± 2.5^{a}
Umami	0.001	0.53	2.6 ± 1.5^{b}	3.7 ± 1.5^{b}	3.5 ± 1.2^{b}	5.5 ± 2.2^{a}	5.3 ± 1.7^{a}
Pea flavour	0.001	0.56	4.3 ± 1.8^{a}	3.0 ± 1.5^{b}	3.2 ± 1.2^{b}	1.9 ± 1.6^{c}	1.9 ± 1.4^{c}
Toughness	0.001	0.49	4.1 ± 1.6^{ab}	3.4 ± 1.4^{b}	3.1 ± 1.0^{b}	4.7 ± 0.9^{a}	4.9 ± 0.7^{a}
Fattiness	0.066	0.26	2.6 ± 1.4	2.7 ± 1.3	3.0 ± 1.1	3.9 ± 1.7	3.7 ± 1.5
Fibrousness	0.014	0.35	7.9 ± 1.4^{a}	5.5 ± 1.7^{c}	5.8 ± 1.5^{c}	7.1 ± 1.5^{b}	7.2 ± 1.7^{b}
Breakability	0.001	0.90	7.9 ± 2.2^{a}	8.5 ± 0.9^{a}	8.1 ± 1.3^{ab}	3.2 ± 2.0^{b}	3.3 ± 1.8^{b}

lipid degradation and the resulting volatile compounds such as octanal and γ-butyrolactone from the degradation of stearic acid (Resconi, Escudero, & Campo, 2013). Additionally, Wang et al. (2022) reviewed flavour challenges in plant-based meat alternatives and discussed that extrusion temperatures above 120 °C may inactivate lipid-modifying enzymes and prevent formation of unwanted flavour reactions, for example in legumes.

Instrumental cutting forces correlated positively with sensory toughness and fibrousness, and negatively with sensory breakability (Fig. 2). The panellists evaluated TPC hybrid extrudates to be tougher and PI extrudates to be more breakable and similar conclusions could be drawn from the textural measurement results. According to sensory the panel, there were significant differences between fibrousness and breakability between PI and TPC hybrids, even though the instrumental texturisation degree was not statistically different (Tables 4 and 5). As explained in Chen et al. (2023) in the previous section, the elevated levels of saturated fatty acids in hybrid extrudates might have resulted in formation of more flexible aggregate structures, which favoured anisotropy. Instrumental textural measurements have been previously observed to correlate with sensory evaluated texture (firmness and elasticity) of extrudates made from faba bean concentrate (Saldanha do Carmo et al., 2021).

4. Conclusions

In this study, co-extrusion of raw beef and pea proteins enabled the development of hybrid meat extrudates. The properties of the obtained hybrid extrudates were dependent on the properties of pea ingredient (PI vs TPC) and the extrusion process conditions. PI-containing hybrid extrudates had a layered and fractured structure, whereas TPC-containing hybrid extrudates had a more distinct fibrous structure and stronger texture. The hybrid extrudates with TPC had a less pronounced pea-like and a more distinct meat-like flavour than the ones with PI, due to the pre-texturisation of TPC, which reduced the amount of volatile compounds.

Both pea ingredients were considered suitable for HMEP in terms of their protein content, low solubility, and high WBC, however, the presence of starch in TPC may have enhanced phase separation and fibrous structure formation in the extrusion process. In the future, the co-extrusion process of raw beef and plant proteins could enable the utilisation of beef side-streams for hybrid meat extrudate production and create new business opportunities for the food industry.

CRediT authorship contribution statement

Pinja Pöri: Writing – original draft, Validation, Formal analysis, Investigation, Visualization. **Heikki Aisala:** Sensory evaluation, Writing – review & editing. **Julia Liu:** Sensory evaluation, Writing – review & editing. **Martina Lille:** Conceptualization, Validation, Writing – review & editing, Supervision, Funding acquisition. **Nesli Sozer:** Conceptualization, Validation, Writing – review & editing, Supervision, Funding acquisition.

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.

Data availability

The data that has been used is confidential.

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