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High moisture extrusion of pea protein isolate to mimic chicken texture: Instrumental and sensory insights

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ABSTRACT

High moisture extrusion processing (HMEP) is gaining attention in the plant-based food industry to obtain meat-like textures from different vegetal protein sources. The aim of this study was to evaluate the impact of extrusion process parameters (water feeding rate, barrel temperature and screw speed) on the texture and moisture content of high moisture extrudates (HME) from pea protein isolate using response surface methodology. Sensory textural profile of selected HME was compared with those of cooked chicken breast and a commercial extruded meat analogue sample. Extrusion process parameters had a significant effect on the characteristics of the final product. Barrel temperatures between 145 °C and 165 °C and a water feed rate between 53% and 57% produced HME with an instrumental texture similar to cooked chicken breast, 165 °C and 55% water feed being the conditions that produced the HME with the highest meat-like appearance and sensory fibrousness.

1. Introduction

Consumer demand for alternative protein sources that are nutritious and impact the environment less than meat products is on the rise. In Europe, almost 46% of consumers are already reducing their meat consumption and 39% intend to reduce it in the future (Smart Protein Project, 2021). However, barriers such as price, availability when eating out and lack of information make the dietary change towards a plant-based diet difficult to effectuate (Perez-Cueto et al., 2022). This circumstance presents an opportunity for innovation in the food sector, where meat analogues are starting to gain attention. Meat analogues are food products made from non-meat ingredients, eaten as a replacement for meat products. They aim to mimic their appearance, mouthfeel and flavour and they have more potential to attract non-vegetarian consumers than conventional plant products (Fiorentini, Kinchla, & Nolden, 2020), thereby facilitating the transition to a plant-based diet.

Meat analogues are found in different formats simulating sausages, nuggets, meatballs, mince, whole-muscle and burger meat, the latter being the most represented (Andreani et al., 2023). Mimicking muscle meat poses a challenge in terms of formulation and texture, and High Moisture Extrusion Processing (HMEP) is being studied as an approach to achieve this. HMEP is a thermo-mechanical process that consists of mixing, extrusion-cooking and cooling stages. Raw materials (or dry protein formula) are mixed with water using a twin-screw extruder to be later passed through a cooking barrel with high pressure, shearing force and high temperature. Last, the cooked mixture passes through a long and narrow cooling die where the proteins are aligned and texturised (Ryu, 2020). The use of this technology allows HME with a fibrous texture resembling animal muscle fibres to be achieved (Zhang, Chen, Kaplan, & Wang, 2022) which can be further used to elaborate meat product analogues that simulate the structure of whole-cut or pulled chicken, pork or beef. Although other characteristics such as nutritional aspects are important when attempting to replace meat completely, achieving a meat-like texture is crucial as it is known that meat alternatives that mimic meat texture (and taste) have the highest chance to be accepted by consumers (Michel, Hartmann, & Siegrist, 2021).

Despite the research conducted so far on HMEP, the formation of the fibrous structure in the extruder is still often described as a black box process (Schmid, Farahnaky, Adhikari, & Torley, 2022). During the HMEP, extrusion conditions play a very important role in the texturisation of the HME (Ryu, 2020). Cooking temperature has been shown to influence the formation of fibrous layered structures in HME from soy (Wittek, Zeiler, Karbstein, & Emin, 2021), pea (Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014) and fava bean protein (Ferawati et al., 2021). Water to protein ratio and screw speed have also been described as having a significant effect on the textural properties of HME and they all contribute synergically to the final outcome, meaning that their effect on the characteristics of the final product should be studied

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in combination (Schmid et al., 2022). The properties of the used protein source also affect the texturisation of the HME. Soy and wheat gluten, which are currently used as principal ingredients in commercial plant-based meat analogues (Andreani et al., 2023), are able to form fibrous structures using HMEP (Chiang, Loveday, Hardacre, & Parker, 2019; Wittek et al., 2021). However, both sources are allergenic, and soybean has been related to deforestation issues (Gasparri, Grau & Gutiérrez Angonese, 2013) and to potential negative health effects (Sukalingam, Ganesan, Das, & Thent, 2015). Wang, van den Berg, et al. (2022) recently obtained highly fibrous extrudates with similar instrumental hardness and chewiness to cooked chicken breasts from pea protein isolates (PPI) by HMEP. This, added to its nutritional profile and low potential for allergenic responses (Nowak-Wegrzyn, Sampson, Wood, & Sicherer, 2003), positions pea protein as an interesting substitute for soy and wheat gluten. Several works regarding HMEP conditions of pea protein isolates (PPI) have already been published (Ferawati et al., 2021; Osen et al., 2014; Zahari et al., 2021), including different instrumental methodologies for evaluating texturisation. However, instrumental texture analysis alone is not sufficient to make conclusions in comparison to real meat texture. Sensory analysis is important for a better characterisation of meat analogues, since sensory properties are the main determinants of product acceptance by the consumer (Ballco & Gracia, 2022). Recently, some work has been done on sensory analysis of low-moisture PPI extrudates (de Angelis et al., 2020) and of HME from PPI and wheat blends (Richter et al., 2024), both of which evaluated several mouthfeel textural attributes. Nevertheless, deeper investigation on sensory evaluation including additional perceptual modalities would allow a more complete understanding of the overall textural perception in comparison to meat, as consumers' expectations toward a food product start through visual perception and can also be influenced by hand-feel touch cues (Pramudya & Seo, 2019).

The aim of this study was to evaluate the impact of HMEP parameters (barrel temperature, screw speed and water feed rate) on the texture and moisture content of high moisture PPI extrudates, identifying those products with better chicken meat structure mimicking. Texture-related sensory evaluation including visual, tactile and mouthfeel perceptual modalities was performed on the HME with better instrumental texture and compared to cooked chicken breast and a commercial extruded soy-based analogue.

2. Materials and methods

2.1. Materials and sample preparation

Pea protein isolate (PPI) (PISANE™ M9, Cosucra, Belgium), with 86 ± 2% of protein content on dry matter and bottled water (Font Vella, Aguas Danone SA, Spain) were used to produce the HME samples, as described in Section 2.2.

Cooked chicken breasts were used as reference samples and were prepared according to Chiang et al. (2019), with some modifications. In brief, three breasts were packed individually in plastic bags (HT3000 Barrier Bag ®, Sealed Air, Charlotte, NC, USA) and cooked in a heated water bath set at 77 °C until they reached an internal temperature of 75 °C. Chicken exudate was eliminated, and chicken reference samples cooled down at room temperature (22 °C ± 2 °C) for at least 1 h. A commercial extruded soy-based analogue (Bocados Originales, Foods for Tomorrow SL, Spain) was also evaluated.

2.2. Extrusion conditions and experimental design

HMEP was performed using a laboratory-scale co-rotating and intermeshing twin-screw extruder (Process 11, Thermo Fisher Scientific Inc., Waltham, MA, USA) (Fig. 1). The extruder barrel is divided into eight elements, including seven zones that can be heated and cooled independently (Z2-Z8), and an additional heated zone in the die adapter (Zdie). The temperatures in heating zones Z2 to Z4 were fixed at 50, 90 and 110 °C, respectively, and Z_{die} at 100 °C. The temperature in heating zones Z5 to Z8 was always the same and varied according to the experiment. A cooling die (H 4 mm x W 19 mm x L 125 mm) supplied by a refrigerated bath circulator set at 20 °C ("KISS K6", HUBER, Peter Huber Kältemaschinenban AG, Offenburg, Germany) was assembled on the end of the extruder outlet. A breaker plate was placed between the die zone and the cooling die. It consists of a perforated steel disk that homogeneously distributes pressure and aligns the flow before entering the cooling stage (Cornet et al., 2022) and is also known to help initiate protein alignment (Akdogan, 1999). A peristaltic pump (Reglo ICC Pump, ISMATEC, IDEX Health and Science LLC, Oak Harbor, WA, USA) and a vertical Volumetric MiniTwin Feeder with agitator (Brabender Technology GmbH, Duisburg, Germany) were used to feed water and PPI, respectively, into the extruder at different rates.

The effect of extrusion conditions on the HME samples elaborated with PPI was explored by response surface methodology. A central composite design (with orthogonal axial value, α = 1.287) with two

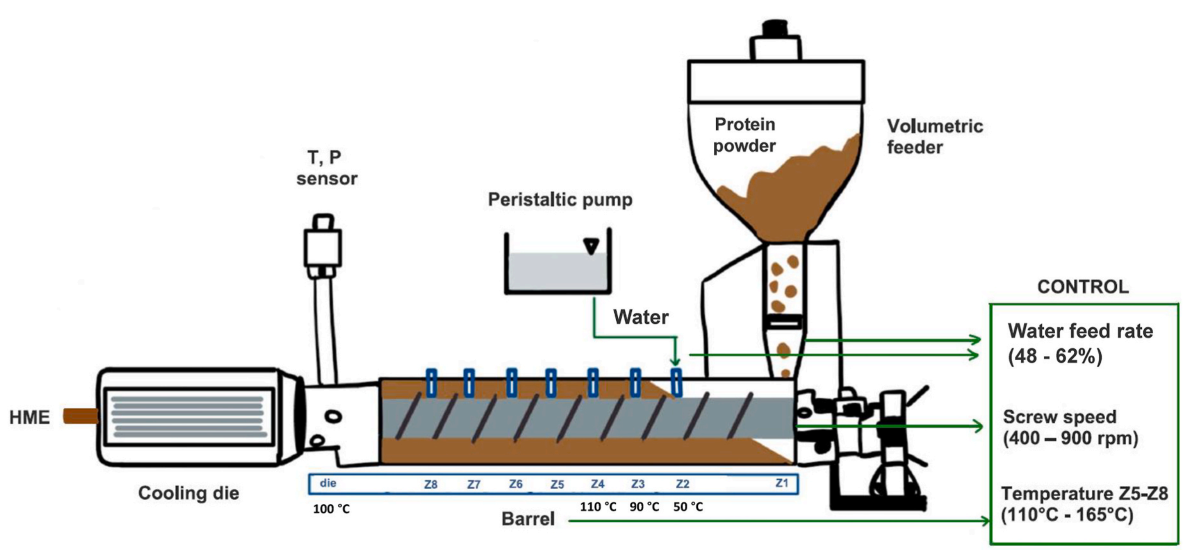

Fig. 1. Schematic view of the extruder and extrusion conditions used. HME: high moisture extrudate; T: temperature; P: pressure.

central points (C) was applied with three varying factors: water feed rate (from 48 to 62%), temperature in heating zones Z5 to Z8 (from 110 to 165 °C) and screw speed (from 400 to 900 rpm) (Table 1). While the water feed rate varied, the global feeding rate was kept constant (approximately 0.96 kg/h), and PPI feed rate was varied accordingly. The range of extrusion conditions included in this study were selected based on preliminary trials (data not shown). Sixteen experiments were performed by triplicate (batches).

Once the extrusion temperature was stable at the set value in each experiment, HME was elaborated and samples were collected and cut manually, sealed in plastic bags, and stored frozen at −18 ± 2 °C for a maximum of one week until further physicochemical analysis.

2.3. Physicochemical characterisation

2.3.1. Moisture content

Chicken reference, commercial and HME samples (previously thawed at 4 °C overnight) were cut into small pieces and moisture content was determined by drying at 103 ± 2 °C until a constant weight was reached, according to AOAC Official Method 950.46 (AOAC, 1990). All analyses were performed in duplicate.

2.3.2. Instrumental texture analysis

Textural properties were measured using a TA.HD*plusC* texture analyser (Stable Micro Systems Ltd., UK) and a 5 kg load cell. HME samples were thawed at 4 °C overnight, manually cut into H 4 mm x W 19 mm x L 50 mm strips and tempered at 22 °C ± 2 °C for at least 2 h before the analyses. Chicken reference and commercial samples were sliced at 4 ± 0.5 mm with a slicer machine (GP 350 EUROCORT AYERBE, Navarra, Spain) and cut manually to a width of 19 mm and a length of 50 mm to reproduce the size of the HME samples. Data were acquired and treated using the 6.1.16.0 version of Exponent Stable Micro Systems software (Stable Micro Systems Ltd., UK). Samples were subjected to shear and puncture tests and for each sample from each of the three batches, six parallel extrudates were measured (total n = 18).

In the shear test, the HME samples were cut perpendicular to the direction of the extrudate flow using a Warner-Bratzler blade set with a rectangular slot blade, at a probe speed of 2 mm/s. The same test was applied to the chicken reference samples, which were cut perpendicular to the direction of the chicken muscle fibres, and to the commercial samples. Values for shear force, area and gradient were recorded as parameters that have been used extensively to study meat tenderness, compressibility, and firmness (Voisey & Larmond, 1977). Shear force (expressed in N) is the force needed to shear through the sample and can also be interpreted as an indirect measurement of texturisation (Palanisamy, Töpfl, Aganovic, & Berger, 2018). Shear area (N.mm) is the area under the curve and represents the total energy required to cut the sample. Gradient (N/mm) was recorded as the curve slope from 20% to 80% of the maximum force.

For the puncture test, samples were penetrated 3 mm in the same direction used for the shear test, using a 5 mm Ø probe at a speed of 5 mm/s. Values for puncture force, area and gradient were recorded, which have also been extensively used to evaluate the hardness or toughness of varied food samples (Bourne, 1966). In this test, puncture force (expressed in N) is the force required to puncture the sample and is related to hardness. The area under the curve (N.mm) is the mechanical work needed to reach the rupture point and is related to the work of penetration. Gradient (N/mm) was recorded as the curve slope from 10% to 30% of the maximum force.

Table 1
Central composite design of the extrusion experiments for the three batches.

Sample	T Z5-Z8 [°C]	Screw speed [rpm]	Water feed [%]
HME-1	116	844	60
HME-2	116	456	60
HME-3	116	844	50
HME-4	116	456	50
HME-5 (C)	138	650	55
HME-6	138	650	48
HME-7 (C)	138	650	55
HME-8	138	900	55
HME-9	138	400	55
HME-10	138	650	62
HME-11	110	650	55
HME-12	159	456	60
HME-13	159	844	60
HME-14	159	456	50
HME-15	159	844	50
HME-16	165	650	55

2.4. Texture-related sensory evaluation

Two HME samples with similar instrumental texture to the chicken reference samples were selected and evaluated by six trained assessors (ISO 8586:2003), together with the chicken and the commercial samples. Descriptive sensory analysis sessions were conducted in a sensory laboratory (ISO 8589:2010) under red light to reduce the effect of colour difference between samples. All samples were prepared by manually pulling them apart into shreds, simulating pulled meat and to homogenise their appearance, and were served at 20 ± 2 °C on transparent Petri dishes (Corning ® SB93-101, NY, USA).

The generation of descriptors was carried out by means of three prior sessions, in which different small groups of trained assessors (n = 4) assessed the different HME, chicken reference and commercial samples and agreed, by consensus, on the most relevant attributes to be assessed. Nine texture-related attributes were finally retained and classified into three groups: visual texture, where meat-like appearance of the samples was evaluated; tactile texture, which included fibrousness, breakability, and cohesion; and mouthfeel texture, in which hardness, gumminess, mouthfeel fibrousness, moisture sensation and pastiness were assessed. All attributes are described in Table 2.

All samples were evaluated in six different sessions (two sessions per batch) and were coded with three random numbers and presented to the assessors, balancing the first-order and the carry-over effects (Macfie, Bratchell, Greenhoff, & Vallis, 1989). A non-structured scoring scale (Amerine, Pangborn, & Roessler, 1965) was used, with a score of 0 representing absence of the descriptor and 10 high intensity of the attribute.

Table 2
Definition of visual, tactile and mouthfeel texture-related attributes evaluated during sensory analysis.

Attribute	Definition
Visual Perception	
Meat-like appearance	Visible resemblance to meat, without contemplating colour
Tactile Perception	
Fibrousness	Appreciation of elongated fibres when separating the sample longitudinally with the fingers
Breakability	Breaking degree of fibers when bending the sample transversally with the fingers
Cohesion	Resistance to breakage when stretching the sample manually from the extremes
Mouthfeel perception	
Hardness	Force required to bite the sample during the first bite
Gumminess	Energy required to disintegrate the sample to a state ready for swallowing
Fibrousness	Perception of fibres during mastication and manipulation with the tongue against the palate
Moisture sensation	Sensation of moisture in the mouth during chewing and manipulation with the tongue against the palate
Pastiness	Degree to which the sample turns into a paste, similar to a flour and water mixture, after chewing

2.5. Statistical analysis

Linear regression using the standard least squares method was applied to model the effect of extrusion conditions on the physicochemical properties (moisture content and instrumental texture) of HME using JMP 16 software (JMP Statistical Discovery LLC, NC, USA). Only significant terms ($p < 0.05$) and main factors involved in these were included in the models. Response surface regression models of shear test parameters were used to build contour plots and identify extrusion conditions that produce HME with a texture similar to the reference samples. Before model fitting, the predictive variables were scaled by transforming each range from −1 to 1.

Physicochemical and sensory results were analysed using XLSTAT v2020.1 software (Addinsoft, Paris, France). ANOVA was used to evaluate physicochemical and sensory data. Sample nature (the CCB, CSMA and two HME selected samples) was considered a fixed effect in both cases, and the assessor and tasting session as additional fixed effects for the sensory data. Mean values were compared by Tukey test. An additional non-parametric ANOVA (Kruskal–Wallis) was previously carried out for sensory data, based on the non-normal distribution of the test variables. Since the non-parametric test provided results similar to those obtained by the parametric ANOVA, the latter option was kept. Principal Component Analysis (PCA) was performed over the mean values of physicochemical and sensory data for the CCB, CSMA and two selected HME samples.

3. Results and discussion

3.1. Physicochemical characterisation

The physicochemical properties of the chicken reference, commercial and HME samples elaborated at different process conditions are shown in Table 3. The moisture content of the HME samples ranged from 49.5% to 64.5% and increased with water feed rate, as expected. Significant differences were observed between different HME samples ($p < 0.05$). Although all the HME samples had a lower moisture content than the chicken reference and commercial samples (around 69%), the use of higher water feed rate hindered the texturisation of HME (results not shown). Target moisture contents higher than 70% in HMEP have been reported to result in less firm and undesirably soft extrudates as the protein concentration is lower and fewer protein–protein interactions can be established, reducing the number and density of fibres formed (Schmid et al., 2022). In line with the results obtained, Osen et al. (2014) found that HME from pea protein isolate with fibrous structure was obtained with a moisture content of 55%, which was also lower than the moisture content of the chicken samples. The fact that HME optimum water content is often less than in real meat could be explained because most of the water in muscle is held within the structure of the muscle and muscle cells but is not bound per se to protein (Huff-Lonergan & Lonergan, 2005). This high percentage of water in meat is known as entrapped (or immobilized) water (Fennema, 1985). The structure of HME, however, may not have the ability to hold the same amount of entrapped water than meat.

The HME samples elaborated under different conditions showed significant textural differences for both puncture and shear test parameters. Shear area and gradient parameters of shear test were the textural parameters that provided most information on the variation between samples. The shear test was therefore considered more adequate than the puncture test for texture analysis of HME in this study. Other authors have also stated that shear tests are more reliable than puncture tests for detecting variations in meat tenderness (Cavitt, Meullenet, Gandhapuneni, Youm, & Owens, 2005).

Analysis of variance results showed that HME-12, HME-13 and HME-16 presented no significant differences with respect to chicken reference ($p > 0.05$). This fact suggests that they could be the most meat-like samples.

Table 3
LS means of physicochemical properties of the high moisture extruded samples elaborated using different extrusion conditions defined by the central composite design, and of the reference and commercial samples (n = 3).

Sample	Extrusion conditions			Moisture content	Puncture test textural properties			Shear test textural properties		
	T Z5-Z8 (°C)	Screw speed (rpm)	Water feed (%)	(%)	Force (N)	Area (N. mm)	Gradient (N/mm)	Force (N)	Area (N. mm)	Gradient (N/mm)
HME-1	116	844	60	62.3 ^{b,c}	6.36 ^{c}	11.08 ^{b}	3.77 ^{c,d}	5.04 ^{c}	15.49 ^{e}	2.54 ^{d,e}
HME-2	116	456	60	61.6 ^{c,d}	7.55 ^{b,c}	11.42 ^{b}	4.52 ^{c,d}	5.58 ^{b,c}	19.46 ^{e}	2.87 ^{d,e}
HME-3	116	844	50	52.8 ^{g,h}	11.87 ^{a,b,c}	19.64 ^{a,b}	9.86 ^{a,b}	6.91 ^{b,c}	18.45 ^{e}	5.94 ^{a,b}
HME-4	116	456	50	52.1 ^{h,i}	12.01 ^{a,b,c}	15.63 ^{b}	9.80 ^{a,b}	7.50 ^{b,c}	20.83 ^{e}	5.86 ^{a,b,c}
HME- (C)	138	650	55	56.8 ^{f}	12.40 ^{a,b,c}	20.31 ^{a,b}	7.28 ^{b,c}	8.46 ^{b,c}	32.97 ^{e}	4.09 ^{b,c,d}
HME-6	138	650	48	49.5 ^{i}	19.10 ^{a}	34.06 ^{a}	13.37 ^{a}	11.15 ^{b}	33.27 ^{e}	7.26 ^{a}
HME-8	138	900	55	56.6 ^{f}	12.45 ^{a,b,c}	20.68 ^{a,b}	7.61 ^{b,c}	8.68 ^{b,c}	35.23 ^{d,e}	4.18 ^{b,c,d}
HME-9	138	400	55	56.5 ^{f}	12.79 ^{a,b,c}	20.75 ^{a,b}	7.48 ^{b,c}	6.80 ^{b,c}	27.44 ^{e}	3.83 ^{c,d,e}
HME-10	138	650	62	64.5 ^{b}	9.69 ^{b,c}	14.65 ^{b}	3.95 ^{c,d}	5.13 ^{c}	21.92 ^{e}	2.59 ^{d,e}
HME-11	110	650	55	57.6 ^{e,f}	10.52 ^{b,c}	18.83 ^{a,b}	6.86 ^{b,c}	5.62 ^{b,c}	17.81 ^{e}	3.49 ^{d,e}
HME-12	159	456	60	60.0 ^{c,d,e}	15.57 ^{a,b}	22.88 ^{a,b}	6.06 ^{b,c,d}	7.47 ^{b,c}	42.12 ^{c,d,e}	3.25 ^{d,e}
HME-13	159	844	60	59.5 ^{d,e}	13.98 ^{a,b,c}	19.57 ^{a,b}	5.09 ^{c,d}	7.64 ^{b,c}	46.45 ^{c,d,e}	2.95 ^{d,e}
HME-14	159	456	50	50.4 ^{h,i}	11.19 ^{a,b,c}	12.63 ^{b}	3.79 ^{c,d}	18.86 ^{a}	136.98 ^{b}	4.53 ^{b,c,d}
HME-15	159	844	50	49.6 ^{i}	10.87 ^{a,b,c}	13.22 ^{b}	3.80 ^{c,d}	23.09 ^{a}	181.58 ^{a}	5.77 ^{a,b,c}
HME-16	165	650	55	55.6 ^{f,g}	8.77 ^{b,c}	11.51 ^{b}	3.60 ^{c,d}	8.53 ^{b,c}	75.54 ^{c}	1.97 ^{e}
Chicken		–		68.8 ^{a}	7.26 ^{b,c}	8.40 ^{b}	1.92 ^{d}	9.90 ^{b,c}	69.55 ^{c,d}	2.84 ^{d,e}
Commercial		–		69.1 ^{a}	8.55 ^{b,c}	7.80 ^{b}	2.28 ^{d}	11.49 ^{b}	76.76 ^{c}	3.50 ^{d,e}
RMSE				*0.914*	*2.790*	*0.652*	*1.480*	*1.945*	*5.825*	*0.697*

[a-i] LS Means without a common letter within the same column are significantly different ($p < 0.05$). HME: high-moisture extrudate; T Z5-Z8: temperatures at barrel zones 5 to 8; C indicates central point.

3.2. Effect of HMEP conditions on physicochemical characteristics

Table 4 summarises the response surface regression models fitted with scaled predictive variables for moisture content and textural parameters of the HME samples. Temperature and water feed rate (either individually or their interactions) had a significant effect on the moisture content and on all the textural parameters. Screw speed had no significant effect on any studied textural parameter, although its interaction with temperature and water feed influenced moisture content. According to the literature, the effect of screw speed on HME texture parameters remains unclear. Samard, Gu, and Ryu (2019) found that difference of 50 rpm in screw speed had a minor effect on texture characteristics of HME elaborated with soy protein isolate in combination with gluten and corn starch. Zahari et al. (2021) studied a wider screw speed variation (from 500 to 900 rpm) at moisture contents between 60% and 70% and found that lowering the screw speed resulted in increased hardness at a same moisture level, relating it with increased residence time, higher shearing impact and increased creation of new bonds. However, Ferawati et al. (2021) observed that screw speed affected the hardness and cutting strength of yellow pea and fava bean HME with a moisture content of 70%, but not at moisture contents between 66% and 69%. During high moisture extrusion, the alignment of protein molecules and their linkage via intermolecular bonds is expected. Higher water content might be interfering the contact between protein molecules and delaying the linkage between them, requiring more time of residence to achieve a certain texturisation of the protein. However, at lower moisture contents, such as in our study (≤60%), the protein-protein linkage could be occurring earlier and not be significantly affected by the screw speed in the studied range (400–900 rpm).

The regression model for moisture content presented a high coefficient of determination ($R^2 = 0.86$) and could allow the optimisation of the extrusion conditions in relation to this parameter. However, HME with similar moisture content to chicken reference and commercial samples cannot be obtained under the studied condition ranges because textural characteristics would be negatively affected if water feed rate was increased, as discussed in Section 3.1. Increasing moisture content by post-HMEP treatments (such as a seasoning process) could therefore be explored as an alternative to increase this parameter.

Regarding the regression models of puncture test parameters, they all presented coefficients of determination between 0.32 and 0.69, meaning that other factors may be affecting puncture characteristics and that the puncture test is not the most suitable method to define optimal extrusion conditions, as also mentioned previously in Section 3.1. However, regression models of shear force, area and gradient had higher coefficients of determination ($R^2 = 0.71$, $R^2 = 0.79$ and $R^2 = 0.79$, respectively) and were used to investigate texture behaviour affected by the interaction of the most significant extrusion conditions (water feed rate and barrel temperature).

Fig. 2 shows the effect of water feed rate and barrel temperature on shear force (A), area (B) and gradient (C). Higher extrusion temperatures and higher protein content resulted in harder HME samples (higher shear force and area). Chen, Wei, Zhang, and Ojokoh (2010) also described an increase in hardness and chewability of HME from soy protein isolate at lower moisture content and higher extrusion temperature. Shear gradient values also increased with lower water feed rate, meaning that samples with a higher protein content also show higher resistance to deformation.

According to the predictive models, shear force, area and gradient similar to the chicken reference (broad outlined areas in Fig. 2) can be achieved simultaneously only by applying barrel temperatures between 145 and 165 °C and water feed rate between 53 and 57% (shaded areas in Fig. 2). These extrusion conditions were considered optimal to elaborate the most meat-like HME samples in terms of instrumental texture, as they allowed shear characteristics similar to those of chicken reference to be obtained. Few studies have explored the potential of HMEP of PPI in comparison to chicken meat before. Wang, van den Berg, et al. (2022) obtained HME from PPI with instrumental hardness similar to chicken meat by HMEP at 160 °C and with chicken-like instrumental chewiness at 140 °C, both elaborated with a water feed of 60% and at 200 rpm. Ferawati et al. (2021) also obtained HME from yellow pea with chicken-like instrumental texture by HMEP at 150 °C with a target moisture content of 68%. All findings share extrusion temperatures similar to the optimal found on the present work. However, it is the global combination of the different extrusion parameters (as well as other factors such as total flow rate and extruder dimensions) that affect the texture of HME.

Table 4
Centered regression coefficients ± standard error and (p-value) of the response surface regression models of each physicochemical property studied for the HME samples.

Term	Moisture content (%)	Puncture force (N)	Puncture area (N.mm)	Puncture gradient (N/mm)	Shear force (N)	Shear area (N.mm)	Shear gradient (N/mm)
Intercept	57.22 ± 0.410 *(<0.001*)*	13.21 ± 0.727 *(<0.001*)*	21.45 ± 1.515 *(<0.001*)*	7.77 ± 0.415 *(<0.001*)*	8.92 ± 0.371 *(<0.001*)*	34.098 ± 4.394 *(<0.001*)*	4.015 ± 0.214 *(<0.001*)*
WFR	6.50 ± 0.424 *(<0.001*)*	−1.84 ± 0.733 *(0.016*)*	−2.74 ± 1.527 *(0.080)*	−2.47 ± 0.418 *(<0.001*)*	−4.32 ± 0.607 *(<0.001*)*	−27.16 ± 4.705 *(<0.001*)*	−2.01 ± 0.180 *(<0.001*)*
SS [rpm]	0.51 ± 0.400 *(0.214)*	NS	NS	NS	NS	NS	NS
T [°C]	−0.68 ± 0.400 *(0.094)*	1.32 ± 0.692 *(0.064)*	0.126 ± 1.449 *(0.931)*	−1.53 ± 0.394 *(<0.001*)*	3.81 ± 0.574 *(<0.001*)*	44.17 ± 4.448 *(<0.001*)*	−0.29 ± 0.170 *(0.093)*
WFR * SS	NS	NS	NS	NS	NS	NS	NS
T * WFR	NS	3.94 ± 1.152 *(0.001*)*	6.62 ± 2.400 *(0.009*)*	3.36 ± 0.657 *(<0.001*)*	−4.68 ± 0.969 *(<0.001*)*	−46.55 ± 7.517 *(<0.001*)*	NS
T * SS	−1.77 ± 0.613 *(0.006*)*	NS	NS	NS	NS	NS	NS
WFR ^{2}	NS	NS	NS	NS	NS	NS	1.21 ± 0.325 *(<0.001*)*
T ^{2}	NS	−3.45 ± 1.299 *(0.011*)*	−8.15 ± 2.705 *(0.004*)*	−2.94 ± 0.740 *(<0.001*)*	NS	28.40 ± 7.969 *(<0.001*)*	−0.92 ± 0.307 *(0.004*)*
SS ^{2}	−1.78 ± 0.740 *(0.021*)*	NS	NS	NS	NS	NS	NS
R^2	0.86	0.41	0.32	0.69	0.71	0.79	0.79
RMSE	1.810	3.131	6.523	1.785	2.501	19.385	0.745

* Indicates significant effect ($p < 0.05$); SS: screw speed; T: temperature; WFR: water feed rate; NS: not significant and not included in the model.

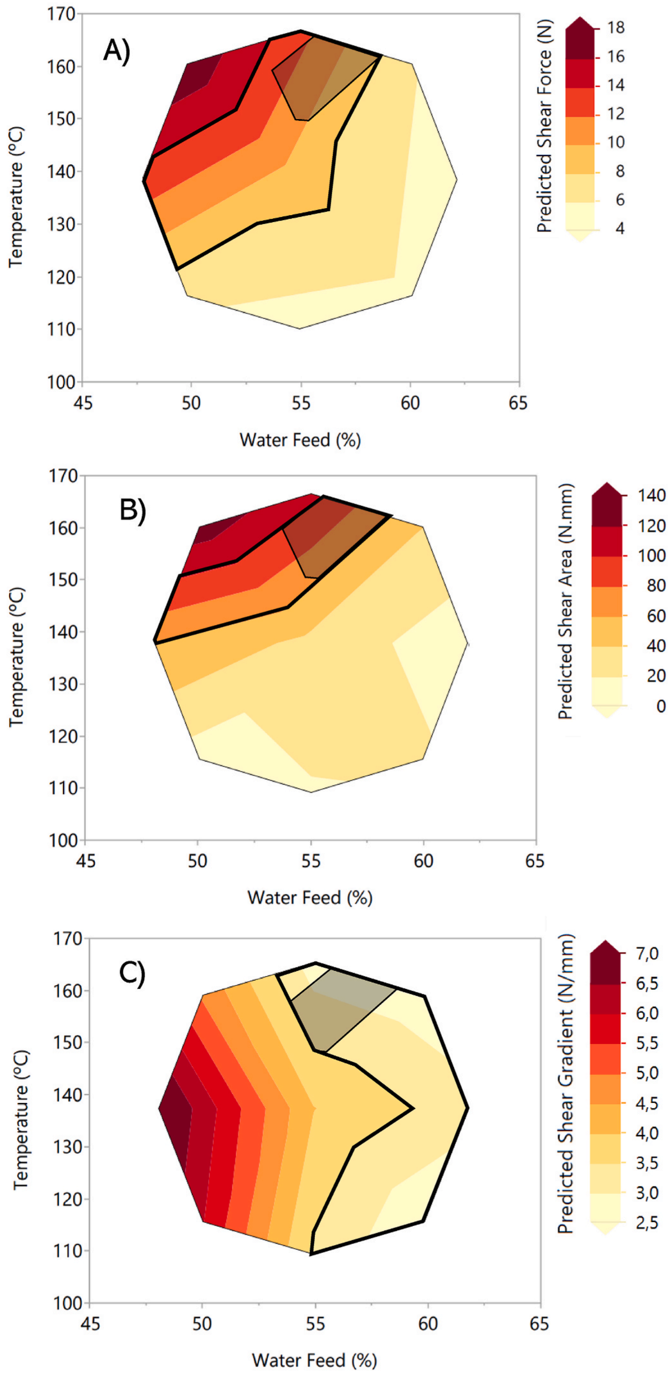

Fig. 2. Response contour plot of barrel temperature as a function of water feed rate fitted to shear force (A), area (B) and gradient (C). Broad outlined areas correspond to chicken reference values for each parameter and shaded areas indicate similar values to chicken reference for all parameters simultaneously.

3.3. *Sensory texture profile of selected HME, chicken reference and commercial samples*

3.3.1. *Selection of samples for sensory analysis*

HME-12, HME-13 (both produced at 159 °C, 60% water feed) and HME-16 (165 °C, 55% water feed) were elaborated under the conditions previously described as optimal (or close) and were selected for having the instrumental textural characteristics most similar to the chicken reference samples ($p > 0.05$), as discussed in Section 3.1. Although all the samples were produced using conditions close to the defined optimal, HME-12 and HME-13 were discarded due to their lack of homogeneity. During their elaboration, die temperature was not constant and was higher than under other conditions (data not shown), probably due to the higher water content, as reported previously by (Saldanha do Carmo et al., 2021). As there was a more drastic drop in temperature before entering the cooling die, compositional and structural changes may have occurred in the final product, which were reflected in an irregular appearance of the samples. For these reasons, only HME-16 was selected out of the three for inclusion in the sensory analysis. HME-14 (159 °C, 50% water feed), which was also elaborated at high temperature but had a lower water content, was additionally chosen to be included instead.

3.3.2. *Sensory evaluation*

The results for the sensory descriptive texture analysis of the selected HME, chicken reference and commercial samples are shown in Table 5. Images of the four evaluated samples are shown in Fig. 3. Chicken reference showed the highest meat-like appearance (8.9 in a scale out of 10) and, although both HME samples and the commercial analogue presented significantly different appearance, the scores for the HME samples were closer to the chicken reference than the commercial samples. Regarding tactile texture, the chicken reference samples were the most fibrous (8.8 in a scale out of 10). Although the HME samples scored significantly lower for tactile fibrousness than the chicken samples, both were more fibrous than the commercial samples. Moreover, chicken samples scored 1.3 and 6.0 for tactile breakability and cohesiveness, respectively. The commercial sample showed no significant differences ($p > 0.05$) for tactile breakability but presented a higher cohesiveness than chicken. Both HME samples were more breakable when folded and less cohesive than the chicken or commercial samples. Breakability of HME from PPI has been previously evaluated by Pöri, Aisala, Liu, Lille, and Sozer (2023), who also reported high scores for this attribute on PPI HME (7.9 in a scale out of 10).

For mouthfeel hardness, chicken reference scored 5.6. While the commercial sample and HME-16 (165 °C, 55% water) showed similar results, HME-14 (159 °C, 50% water) was scored significantly higher ($p < 0.05$). This could be related to its lower moisture content (50.4%). In addition, PPI has been described to generate HME with harder sensory texture than other protein sources (de Angelis et al., 2020). In relation to gumminess, the chicken reference was the least gummy (2.3), followed by the commercial analogue and lastly both HME samples, which were the gummiest. This aspect could be improved in future studies by adding oil to the formula of the extruded samples, as it is known to reduce the chewiness (which is related to gumminess) of certain high-moisture extruded proteins (Wang, Zhang, et al., 2022).

Like for tactile fibrousness, the chicken reference was the sample that presented the highest mouthfeel fibrousness. Again, both HME samples

Table 5
Texture-related sensory evaluation scores (scale 1–10) of the selected HME, the chicken reference and the commercial extruded soy-based analogue samples ($n = 36$).

	HME-14	HME-16	Reference	Commercial
Visual				
Meat-like appearance	5.6 [b]	6.4 [b]	8.9 [a]	4.1 [c]
Tactile				
Fibrousness	4.6 [c]	6.5 [b]	8.8 [a]	1.7 [d]
Breakability	6.4 [a]	3.9 [b]	1.3 [c]	0.9 [c]
Cohesion	2.7 [d]	4.8 [c]	6.0 [b]	8.2 [a]
Mouthfeel				
Hardness	7.2 [a]	5.9 [b]	5.6 [b]	5.9 [b]
Gumminess	6.3 [a]	5.8 [a]	2.3 [a]	3.9 [b]
Fibrousness	5.0 [b]	4.7 [b]	6.9 [a]	3.1 [a]
Sense of moisture	2.4 [d]	3.7 [c]	5.2 [b]	7.0 [a]
Pastiness	3.5 [b]	3.6 [b]	5.0 [a]	2.0 [c]

[a-d] Mean values containing different letters in the same row are significantly different ($p < 0.05$).

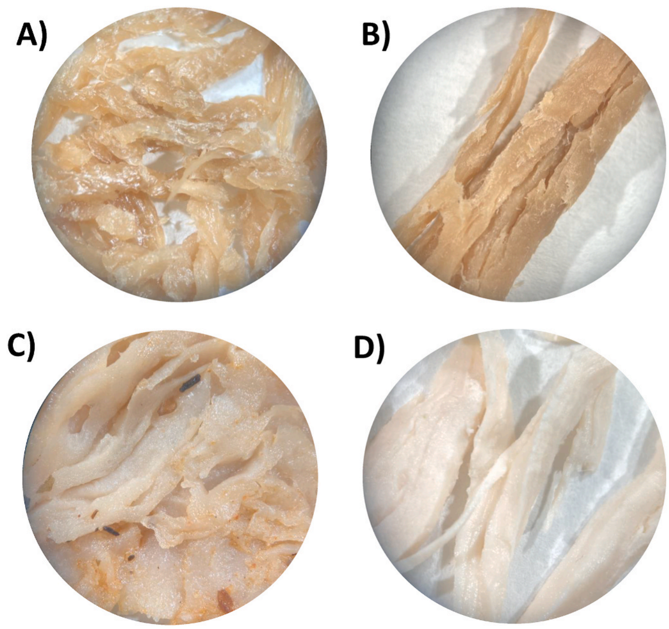

Fig. 3. Manually shredded samples for the sensory evaluation: HME-14 (A), HME-16 (B), chicken reference (C) and commercial extruded soy-based meat analogue (D).

presented a more similar fibrousness with the chicken sample than with the commercial analogue. In terms of moisture sensation, both the chicken and commercial samples were distinguished for having a higher score than the HME samples as they had a significantly higher moisture content (approximately 69%), and there was also the additional influence of the seasoning in the case of the commercial analogue.

Although all the samples scored in the lower half of the scale for pastiness, the chicken reference samples were the pastiest, followed by the HME samples and the commercial analogue, which presented the lowest score.

Globally, the texture evaluation of HME samples from the sensory analysis was in line with the physicochemical analysis, showing that HME-16 is more similar to the chicken reference than HME-14. However, more differences between HME samples and chicken were evidenced here. Additionally, and contrarily to the physicochemical results, the commercial soy-based analogue was the sample that diverged the most from the chicken reference sensory profile. This highlights the importance of sensory analysis when evaluating this kind of products, putting in evidence that although instrumental texture methodologies can be useful as a first step to characterise texturisation of HME, it is far from being sufficient on its own to reach conclusions.

Moreover, evaluations from visual, tactile and mouthfeel modalities lead to different conclusions, suggesting that all three perception modalities can influence the overall acceptance of the sample in comparison to meat. It is known that although the overall appearance of meat analogues should resemble familiar meat products to set positive expectations (Fiorentini et al., 2020), there are factors that can mask textural and other type of flaws and can improve the perception of the product, such as colour, shape, seasoning and meal context (Elzerman, Hoek, van Boekel, & Luning, 2011). However, when perceived liking after consumption is lower than expected, which may occur when the visual cues misrepresent the taste, odour, texture and flavour of the product, a disconfirmation of expectations occurs (Deliza & MacFie, 1996), meaning that a more meat-like sensory texture profile (HME-16) will better satisfy consumers' global expectations.

3.4. Relationship between physicochemical and sensory properties

Fig. 4 shows the results of the PCA for the physicochemical and sensory data of the four evaluated samples (chicken reference, HME-14 (159 °C, 50% water), HME-16 (165 °C, 55% water) and commercial soy-based analogue). The first two dimensions of the PCA explained 88.54% of the variance. All instrumental shear textural parameters had a positive influence on the first principal component (F1), as well as on sensory hardness, gumminess and breakage when bending. Shear force and area presented a positive correlation with mouthfeel hardness ($r = 0.995$ and $r = 0.944$, respectively). Pori et al. (2023) also found a positive correlation between instrumental shear forces (both transversal and longitudinal) with sensory toughness and mouthfeel on HME from plant-meat hybrids. Moisture content, moisture sensation and cohesion had the opposite effect on the first component. This corroborates previous works that have described a negative correlation between texture attributes and moisture content (Lin, Huff, & Hsieh, 2002). Conversely, the second principal component (F2) was positively influenced by meat-like appearance, pastiness, and tactile and mouthfeel fibrousness, which were all also positively correlated with each other.

Chicken reference is represented in the first quadrant of the biplot. On one hand (shown by F1 axis), it is moister, more cohesive, less breakable, less hard, and less gummy than the rest of samples. On the other hand (shown by F2 axis), it is more fibrous, pasty, and meat-like. Regarding the first axis, the commercial analogue has a similar profile to the chicken sample. Oppositely, HME-14 is situated furthest away, being dryer, less cohesive, harder, gummier and more breakable. On the second axis, however, the commercial analogue is the sample that is furthest away from the reference (chicken), being less fibrous and meat-like. HME-16 is the only sample that presented intermediate values for both principal components and has the closest overall profile to chicken for F1 and F2 components.

4. Conclusions

Extrusion temperature and water feed rate have a significant effect on the moisture content and textural characteristics of HME, whereas screw speed has no significant effect on most of the studied parameters. Extrusion temperatures between 145 and 165 °C and water feed rate between 53 and 57% are the optimal conditions to obtain HME from PPI with instrumental texture similar to chicken. Additionally, sensory analysis has shown to provide valuable information on the overall textural perception of the product, which is crucial for future consumer acceptance. HME from PPI with the best meat-like appearance and sensory fibrousness in comparison to cooked chicken breast can be achieved at extrusion conditions of 165 °C and 55% water feed rate. Although a breakthrough towards obtaining an optimal textured HME from only pea protein isolate and water was achieved, further studies on seasoning and the addition of oil and other ingredients in the initial formula should be carried out to improve textural characteristics, especially to reduce gumminess and to increase the sense of moisture.

Ethical statement

Ethical approval for the involvement of human subjects in this study was granted by the Ethical Committee of the Institute of Agrifood Research and Technology (IRTA), registration number CCSC 24–2022. Each participant provided a written informed consent to take part in the study.

CRediT authorship contribution statement

Clara Barnés-Calle: Formal analysis, Investigation, Methodology, Writing – original draft. **Grau Matas:** Formal analysis, Investigation, Writing – review & editing. **Anna Claret:** Formal analysis, Investigation, Writing – review & editing. **Lluis Guerrero:** Formal analysis,

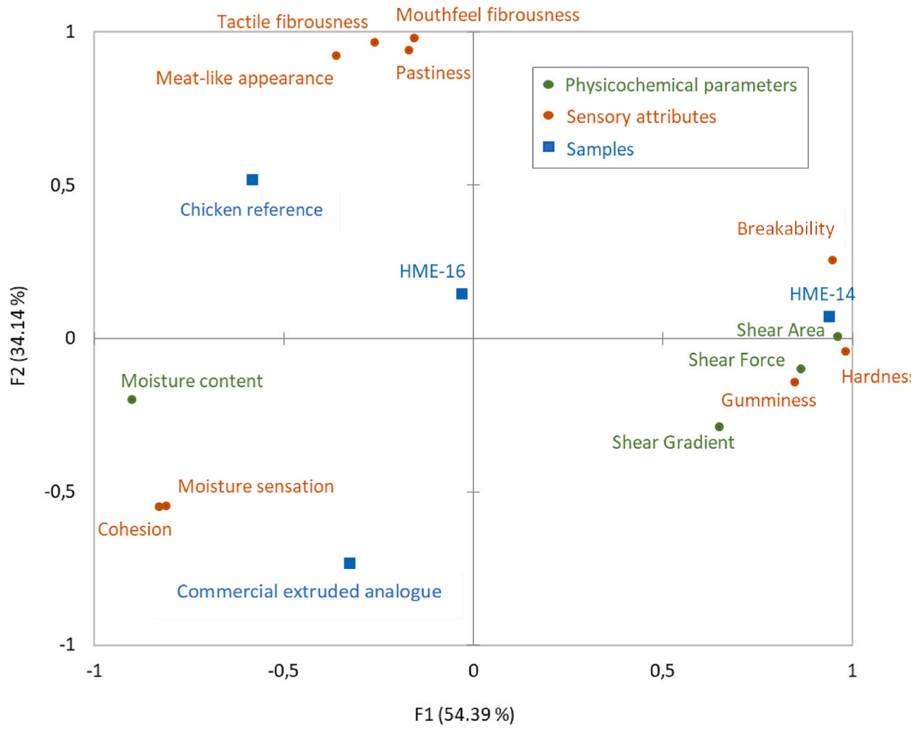

Fig. 4. Sensory and physicochemical correlations of the four evaluated samples (HME-14, HME-16, chicken reference and commercial extruded soy-based analogue).

Investigation, Writing – review & editing. **Elena Fulladosa:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. **Pere Gou:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no conflict of interests.

Data availability

Data will be made available on request.

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