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Texturized vegetable protein from a faba bean protein concentrate and an oat fraction: Impact on physicochemical, nutritional, textural and sensory properties

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A B S T R A C T

Texturized vegetable protein (TVP) from a blend of faba bean protein concentrate and an oat beta-glucan rich fraction was produced by low-moisture extrusion to combine nutritional benefits of both ingredients. The effect of extrusion conditions (temperature in zone 6 (HZ6), feed rate (FR) and moisture content (MC)) on physicochemical, nutritional, textural, and sensory attributes was studied. Overall, effect of the FR and MC of the blend showed greater impact on TVP properties rather than the temperature. TVPs produced at 28.5%, 5 Kg/h and 150^{0}C and at 30.8%, 4 Kg/h and 160^{0}C for MC, FR and HZ6, respectively, presented improved properties to be further formulated into a meat analogue product. The beta-glucan content of TVP (5.6g/100g dm) was high enough to reach >1 g beta-glucan per serving in a final food product (e.g., vegan burger), which qualifies for the health claim "reduces low-density lipoprotein (LDL)-cholesterol" approved by the European Food Safety Authority. The combination of these ingredients resulted simultaneously in an improved composition of essential amino acids and increased the protein quality of the blend as the calculated digestible indispensable amino acid score (DIAAS) improved from 72.2 (beta-glucan rich fraction alone) or 76.1 (faba bean protein concentrate alone) to 90.2 (blend).

1. Introduction

A protein shift from animal to plant-based sources is needed to supply food to the growing population worldwide. The production of animal proteins such as meat have a significant negative impact on the environment. Moreover, excessive consumption of red meat is associated with higher risk of development of different diseases (Baune et al., 2021). Plant-based alternatives already commercialized are generally obtained from wheat gluten or soy owing to their exceptional capacity to create structures similar to meat. Soy is the most explored plant protein processed by extrusion cooking (Ferawati et al., 2021). However, their allergenicity, environmental, agricultural and sustainability concerns have encouraged the search for other protein sources, for instance pulses and cereals (Kyriakopoulou et al., 2019).

Pulses like field peas and faba beans are locally produced in Norway and can be an alternative to soya and reduce negative environmental footprints in food production (Ferawati et al., 2021). Pulses are valuable sources of protein, carbohydrates, dietary fiber, B-vitamins, and minerals. In terms of the amino acid profile, pulses have high quantities of lysine which is a limiting amino acid in cereals and low quantities of methionine, cysteine and tryptophan (Bessada et al., 2019; Sá et al., 2020). Faba bean (*Vicia faba L.*) is an unexplored alternative protein source. The protein content of whole seeds normally varies between 26 and 31 % dm. After dry fractionation, a protein concentrate with 60–

Abbreviations: a*, Redness; b*, Yellowness; BG28, 28% oat beta-glucan fraction; BI, Browning index; CCRD, Central Composite Rotatable Design; DOE, Design of experiments; DIAAS, Digestible indispensable amino acid score; DL, Drive load; DT, Dry texturization; FBPC, Faba bean protein concentrate; FR, Feed rate; HZ, Heating zone of the extruder barrel; HZ6, Temperature in heating zone six of the extruder barrel; L*, Lightness; LME, Low-moisture extrusion; LMW-CHO, Low-molecular weight carbohydrates; MC, Moisture content; NSP, Non-starch polysaccharides; OBC, Oil binding capacity; P, Pressure; PC, Principal component; PCA, Principal component analysis; STARTMIX, The dry mixture of the oat ingredient (BG28) and the faba bean protein concentrate (FBPC) prior to extrusion processing; RSM, Response Surface Methodology; SUPM, Supplementary material; SEI, Sectional Expansion Index; SME, Specific Mechanical Energy; SRS, Screw Rotation Speed; TP, Throughput; TPA, Texture profile analysis; TVP, Texturized vegetable protein; WT, Wet texturization; WFR, Water feed rate; WFR/FR, Ratio between water feed rate and blend feed rate; WHC, Water holding capacity; WSI, Water solubility index; ΔE, Total colour change.

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65% dm of protein is normally obtained (Saldanha do Carmo et al., 2021).

Cereals are also a valuable source of protein (Mäkinen et al., 2017) and the blend of pulses and cereals is a possible strategy to develop foods with a balanced nutritional composition (Petterson, 2011). Among different cereals, oat has the highest protein content (15%-20%), soluble fiber (beta-glucan), unsaturated fatty acids, and antioxidants (Lásztity, 1998).

Plant proteins are usually texturized by extrusion technology into crucial ingredients for meat analogues (Kyriakopoulou et al., 2019). Two concepts exists for the production of meat analogues: Dry-texturization (DT), low-moisture extrusion (LME) or dry-extrusion in which the moisture content is up to 40% and wet-texturization (WT), high-moisture extrusion (HME) or wet-extrusion in which the moisture content is above 40% (Akdogan, 1999; Zhang et al., 2022). In LME the raw-materials are transformed into texturized vegetable proteins (TVP) that requires to be rehydrated before further formulation into a final meat analogue product (Riaz, 2011).

Both LME and HME are flexible operating processes. However, smaller companies cannot afford to set up a factory for HME in which more resources are needed (i.e. cooling die) than for LME (already implemented/installed in several companies) to produce snacks, etc. LME has been used to produce TVPs not only focused on research but also for commercial purposes. The benefits of using LME are the continuous procedure, good production capability and reasonably good energy efficiency (Schimd et al., 2022). HME of pulses to produce meat alternatives, including faba bean has already been explored previously (Ferawati et al., 2021; Ramos-Diaz et al., 2022; Saldanha do Carmo et al., 2021). Concerning LME of faba beans to produce textured vegetable protein (TVP), there are no studies in the literature. Dry texturization to produce TVP (moisture content between 26 and 35%) of a pea protein isolate (81.5% dw) was investigated by Beck et al. (2017), including structural modifications on the secondary structure of protein and formation of an expanded network. A pea protein concentrate obtained by milling and air-classification was used to produce TVP (moisture content between 24.0 and 30.5%) (Wang et al., 1999). After rehydration, the TVP had a meat like texture, resembling commercially soy protein TVP. A soy protein isolate mixed with wheat gluten and corn starch was used to produce TVP (30% moisture content) (Samard et al., 2019). A spongy structure was obtained when wheat gluten was incorporated. Concerning cereals, wheat gluten has been extruded to produce TVP (Samard and Ryu, 2019), oat protein concentrate (30%) has been mixed with a pea protein concentrate, pea protein isolate and soy protein isolate to produce TVP (20-35% moisture content) (De Angelis et al., 2020). As previously reported, it was possible to generate meat analogues using HME from a faba bean protein concentrate with good sensorial and textural attributes at temperatures between 130 and 140^{0}C, feed rate of 1.1 Kg/h and a ratio between water feed rate and the blend feed rate of 4 (Saldanha do Carmo, et al., 2021). Moreover meat analogues from a blend of faba bean and pea proteins produced by high moisture extrusion have been also successfully generated (Ferawati et al., 2021).

This is the first study of LME to produce TVP using a blend of faba bean protein concentrate and an oat beta-glucan fraction. Response Surface Methodology (RSM), namely a central composite rotatable design (CCRD) was used to evaluate the effect of selected extrusion process parameters on the physicochemical and technofunctional properties of the ingredients. Furthermore, the textural and sensory properties of the TVP that are to be further utilized in the formulation of plant-based meat analogue products are described. The impact of combining faba bean protein with a *beta*-glucan enriched oat fraction on the nutritional quality of the produced extrudates was evaluated by calculating the digestible indispensable amino acid score (DIAAS). This is a measure of protein quality and evaluating the beta-glucan content of the extrudates and potential food products produced thereof in relation to the health claims from the European Food Safety Authority (EFSA) for oat beta-glucan on reduction of LDL-cholesterol (EFSA, 2009, 2010, EFSA, 2011), which requires a beta-glucan content of 1 g per serving.

2. Materials and methods

2.1. Materials

The faba bean protein (65% protein) concentrate (FBPC) was purchased from Vestkorn (F65X) (Tau, Norway). Oat fiber containing 28% beta-glucan and 23% protein, SWEOAT® Bran BG28 was purchased from Naturex (Bua, Sweden). A commercial sample of texturized protein from a faba bean concentrate (F6501M) was used as reference and was purchased from Vestkorn (Tau, Norway). Reagents and standards (analytical grade) were acquired from regular suppliers.

2.2. Low-moisture extrusion (LME) of FBPC and BG28 (STARTMIX)

The raw-material mixture (STARTMIX) before extrusion was prepared by mixing 80% of FBPC and the 20% BG28 in a Stephan Mixer (A. Stephan U. Sohne, Hamelin/Wesser, Germany) to guarantee the ingredients were homogeneously blended. They were mixed at the maximum speed of the equipment during 1 min prior to the extrusion trials. Response Surface Methodology (RSM) was used to study the production of TVP by LME using a twin screw extruder following a Rotatable Central Composite Design (RCCD), varying 3 factors: (1) Feed rate of the STARTMIX (FR): to study different residence times and level of packing of the blend in the extruder barrel; (2) Ratio between the feed rate of water and STARTMIX feed rate (WFR/FR): to investigate different moisture contents (MC) of the STARTMIX; greater WFR/FR corresponds to greater moisture contents; and, (3) Temperature in zone 6 (last) of the extruder – HZ6. The water was fed at a variable feed rate (WFR) by a Watson-Marlow 530 Du pump. The STARTMIX was fed into the extruder at different feed rates using a gravimetric feeder (Brabender GmbH and Co. KG, Duisburg, Germany). Published work with similar raw-materials (de Angelis et al., 2020; Kaleda et al., 2021) and pre-trials (data not presented) was the basis to decide on the low and high values of extruder conditions to be investigated. In total, 17 trials (including 3 central points for reproducibility evaluation) were conducted. The STARTMIX feed rate (FR) varied from 3.32 to 6.68 Kg/h, the WFR/FR from 0.26 to 0.46 (corresponding to target moisture contents between 28.5 and 38.4%) and HZ6 varied from 133.2°C to 166.8°C.

The STARTMIX was extruded using a laboratory co-rotating twin-screw extruder (TSE) KETSE 20/40 (Brabender GmbH and Co. KG, Duisburg, Germany) with a screw diameter of 2 cm and a length of 40 cm (L/D ratio of 20:1), as described earlier by Saldanha do Carmo et al. (2019). The screw configuration (SC) was kept constant. From the first sector of the extruder to the die exit of the TSE, the SC was established by 13 forward conveying elements (fce): 1×40 mm, 1×20 mm, 9×30 mm, 1×15 mm, 1×30 mm; 1 tooth mixing element with eight teeth, 3 tooth rows and 20 mm length; 8×20mm fce; 1×30mm conveying kneading block (fckb) of 45°; 1×10 mm reverse conveying element (rce); 1×20 mm fce, 1×30 mm 45° fckb, 1×10 mm rce; 1×20 mm fce, 1×20 mm fckb of 45°; 1×20 mm reverse kneading block (rkb) of 45°, 1×10 mm rce; 1×20 mm fce, 1×30 mm fckb and 1×30 mm fce. From heating zones 1-5 of the extruder, the temperature was kept fixed at 40/80/100/130/30 ^{0}C, respectively, whereas the heating zone 6 (HZ6-last) was established according to the CCRD. The screw rotation speed (SRS) and the die diameter remained constant at 900 rpm and 3mm respectively, based on pre-trials (data not shown). A steady speed of 150 rpm was set in the cutter (Brabender, GmbH and Co. KG, Duisburg, Germany) attached at the die exit. When the steady state of the process conditions was achieved, the TVP samples were collected and allowed to cool down and were further dried at 50°C for 2h in a baking oven (Revent International AB, Uplands Väsby, Sweden). Aliquotes were cooled down, packed in plastic boxes until further analysis or frozen for a subsequent sensory assessment. For some analysis, part of the TVP samples were milled (RETSCH

ZM 100 mill, Retsch GmbH, Haan, Germany) with a 0.5 mm sieve and stored at 4^0C.

2.3. Dependent process responses

The mass flow/throughput, the pressure, the drive load and the specific mechanical energy of each TVP sample was obtained according to Saldanha do Carmo et al. (2019).

2.4. Characterization of extrudates produced by LME

2.4.1. Bulk density (BD) and sectional expansion index (SEI)

The BD (g/cm^3) was determined by weighing 20 g of milled extrudates in a graduated cylinder and recording the volume occupied (method adapted from Wang et al. 1999). The BD was presented as the average of 3 measurements. The extrudate diameter was measured after extrusion (before drying) for all 17 samples at least in triplicates using a calliper at different locations of the extrudates. The SEI was determined according to Alvarez-Martinez et al. (1988) and Horvat and Schuchmann (2013).

2.4.2. Colour properties

The colour properties of the STARTMIX and the milled TVP samples were evaluated with the colorimeter (CR-400 Minolta Japan), according to the methods described by Saldanha do Carmo et al., (2021).

2.4.3. Functional properties: Oil Binding Capacity (OBC), Water Holding Capacity (WHC) and Water Solubility Index (WSI)

OBC, WHC and WSI of the FBPC:BG28 mixture (STARTMIX) and the milled TVP samples were determined according to Setia et al. (2019) and Kaleda et al. (2021), with some modifications. Briefly, 1g of milled sample was suspended in 10 mL of distilled water or rapeseed oil. The samples were kept vertically for 30 min, vortexing for 10s at time intervals of 5 min. The suspension was then subjected to centrifugation at 3000g for 15 min at room temperature. The OBC and WHC was calculated by dividing the weight of the retained water/bound oil over the dry weight of the initial powder. The WSI was determined according with Kaleda et al. (2021) by collecting and freeze drying of the water supernatant. The WSI was expressed as the % of dried material in the supernatant to the initial material weight. Measurements were done in triplicates.

2.4.4. Textural properties

The textural attributes of the TVP samples were evaluated using a TA.XTplus texture analyser (Stable Micro Systems Ltd., Godalming, UK) and were obtained88 and processed by the Exponent Connect (Hamilton, USA) software. Texture profile analysis (TPA) using a 36 mm flat probe (P36-R) and a 5 Kg load cell was used to analyse the samples. Prior to the analysis, the samples were re-hydrated, following to the method described by Kaleda et al. (2021) with some modifications. The TVP samples were compressed twice by 60% of original thickness at a speed of 1mm/s and holding time of 5 s between compressions. Three measurements were performed for every TVP sample. The hardness, cohesiveness, springiness, chewiness, resilience, and gumminess were determined with the software.

2.5. Nutritional composition of ingredients, STARTMIX and TVP samples

2.5.1. Proximate composition

The moisture air oven method (44.15.01) was used to determine the moisture content of the ingredients, the blend formulation (STARTMIX) and TVP samples (AACC, 1995). The protein content of these samples were measured by combustion (Dumas, N × 6.25). The non-starch polysaccharide (NSP) was determined by the method of Englyst et al. (1994), as described by Saldanha do Carmo et al. (2019). A Megazyme kit according to the method AOAC 995.16 (2000) was utilized to evaluate the β-glucan content. Values for total fat in BG28 and FBPC were provided by the supplier and corresponding values in ingredient mix and TVPs were calculated based on the 80:20 % (w/w) of FBPC to BG28. Non-digestible oligosaccharides were determined by HPAEC-PAD, as earlier described (Saldanha do Carmo et al., 2022). Available carbohydrates were calculated based on the sum of glucose, sucrose, and fructose (by HPAEC-PAD) and starch (by Megazyme kit).

2.5.2. Amino acid composition and calculated DIAAS values

The amino acid composition of BG28, FBPC, STARTMIX and center point TVPs (DT-MA-15) was measured by HPLC after hydrolysis in 6M HCl for 22h, at 110 C, derivatisation with 6-Aminoquinolyl-N-Hydroxysuccinimidyl Carbamate (AQC) and quantification with HPLC and fluorescence detection (Cohen and Michaud, 1993). The content of tryptophan was measured after barium hydroxide hydrolyses of the protein and calculated according to Miller (1967). The amino acid composition of FBPC and BG28 was used to calculate DIAAS values for the ingredients and the mixture (80:20 w/w) following the method described by the Food and Agricultural Organization of the United Nations (FAO) report on Dietary protein quality evaluation in human nutrition (Consultation, 2011). The true ileal amino acid digestibility for each essential amino acid in the calculations were estimated based on literature data. For BG28 values for dehulled oats determined in growing pigs (Cervantes-Pahm et al., 2014) were used. For faba beans *in vivo* data from rats of true ileal digestibility of amino acids were available. There, the true protein digestibility of faba bean proteins was measured *in vivo* in a rat assay (Nosworthy et al., 2018) and was the highest among the different bean varieties tested. Therefore, the true ileal amino acid digestibility of FBPC was estimated by using true ileal amino acid digestibility values for broad beans measured in growing pigs (Han et al., 2020).

2.6. Sensory evaluation

Eleven samples with different product texture properties (DT-MA-2, DT-MA-6 to DT-MA-15) were selected (to represent an array of different sensory characteristics) and assessed for quantitative descriptive analysis according to ISO 13299:2016 (ISO, 2016). The laboratory used for the sensory evaluation followed the ISO 8589 (ISO, 2010). A trained sensory panel (tested and calibrated regularly) analysed the samples utilizing descriptive sensory profiling (Lawless and Heymann, 2010) according to GDA - Generic Descriptive Analysis. The trained assessors (eight nominated) at Nofima, Norway constituted a panel and were selected as explained by ISO 8586:2012 (ISO, 2012). The assessors are regularly trained, controlled, and tested. A preliminary test before the formal test was used to calibrate the sensory panel. At the lab, red light was used to eliminate colour differences during the sensory evaluation of all samples. The samples were prepared by mixing 30 g of dried sample with 0.5 L of tap water in a plastic bag which was sealed, 30 min before serving. The bag was immediately placed in a water bath holding +60°C for 10 min. After soaking, the water was strained off and the samples lightly dried with kitchen paper. Each judge was served with 3 g of sample (dry matter content) at 21°C ± 1°C. The judges agreed upon a list of twenty-one attributes during the pre-trials (Table 1 in SUPM-Supplementary Materials), regarding odour, taste/flavour, and texture. In the formal analysis, 11 samples were assessed in two replicates. During serving of samples, a balanced presentation order was applied to serve the TVP samples and replicates (William's Latin Square design). The sensory attributes were evaluated using an unstructured scale from 1 (the lowest intensity) to 9 (the highest intensity). To record the data, EyeQuestion (Logic8, Utrecht, Holland) was used.

Table 1
Nutrient composition of raw materials, raw material mix (STARTMIX) and TVP center point (DT-MA-15) in g/100 g dry matter.

	BG28	FBPC	STARTMIX	DT-MA-15
Protein	23	65	56.6	56.6
Fat	5.0	4.5	4.6	4.6
NSP	44.6	10.9	16.8	17.8
beta-glucan	28	0.7	5.6	5.6
available carbohydrates	9.0	8.0	8.2	8.2
Glucose, fructose, sucrose	1.9	1.9	1.8	1.6
non-digestible oligosaccharides	0.7	6	4.7	4.2
Amino acid composition				
Alanine	1.3	2.6	2.2	2.2
Arginine	1.6	6.2	5.4	5.3
Aspartic acid	1.9	6.6	5.5	5.5
Cysteine	1.0	0.8	0.9	0.8
Glutamic acid	4.4	10.0	8.7	8.7
Glycine	1.4	2.5	2.3	2.3
Histidine	0.5	1.6	1.4	1.3
Isoleucine	0.9	2.6	2.2	2.2
Leucine	1.7	4.6	3.9	3.8
Lysine	0.9	3.7	3.2	3.2
Methionine	0.4	0.5	0.5	0.4
Phenylalanine	1.1	2.4	2.3	2.3
Proline	1.3	2.7	2.4	2.4
Serine	1.3	3.0	2.6	2.6
Threonine	0.8	2.2	1.9	1.9
Tryptophan	0.3	0.6	0.5	0.5
Tyrosine	0.9	1.9	1.6	1.4
Valine	1.2	2.8	2.4	2.4
DIAAS*	72.2 (lysine)	76.1 (methionine)	90.2 (methionine)	

* DIAAS values were determined based on the amino acid composition of BG28 and FBPC using literature data for true ileal amino acid digestibility obtained in pigs fed de-hulled oat (Cervantes-Pahm et al., 2014) or broad beans (Han et al., 2020).

2.7. Statistical analysis

Minitab® 21.1, from Minitab Ltd. (UK) was used to treat the data from the CCRD and to determined significant differences (ANOVA and Fischer's least significant difference) among TVP samples produced at different extruder conditions. ANOVA and Tukey tests were used for statistical analysis of the sensory descriptive data. In order to explore sensory and instrumental/analytical correlations, Multiple factor Analysis (MFA) and linear correlations were processed using XLStat 2015, Addingsoft (USA).

3. Results and discussion

3.1. Nutritional composition of ingredient mix and TVP

The nutritional composition of raw materials, raw material mix (STARTMIX) and TVP (center point DT-MA-15) is presented in Table 1. Extrusion of the raw material mix had only a very minor impact on the nutritional composition. There was a slight decrease in sugars (glucose, fructose, sucrose) and non-digestible oligosaccharides (NDO) during extrusion. This may be related to partial hydrolysis of NDO and subsequent reduction of reducing sugars in Maillard reaction as described previously (Saldanha do Carmo et al., 2019). Extrusion had no impact on the amino acid composition of the STARTMIX. However, the combination of BG28 (20%) with FBPC (80%) improved the nutritional quality of the mix and TVPs due to the complementary amino acid composition of oat and faba bean proteins. The calculated DIAAS values (based on literature data for true ileal amino acid digestibility in pigs) increased from 72.2 (BG28 alone) or 76.1 (FBPC alone) to 90.2 (mix). Not surprisingly lysine was the first limiting amino acid in BG 28, while methionine was the first limiting amino acid in FBPC and the STARTMIX. To further improve the nutritional quality of TVP, an even higher proportion of oat protein containing ingredient could be used. The STARTMIX and TVP also contained 5.6 g beta-glucan and 56.6 g protein per 100 g dry matter (Table 1). TVP is one of the main ingredients in plant-based meat alternatives often supplying 80-100% of the total protein in these products. The median protein content of plant-based meat analogue burgers on the Swedish market was 14 g per 100 g final product (Bryngelsson et al., 2022). Assuming the TVP from this study would supply 80% of the protein in this burger, the final burger would also contain 1.1 g beta-glucan per serving (100 g), which qualifies for the health claim (EFSA) for oat beta-glucan on reduction of LDL-cholesterol (Pomeroy et al., 2001).

3.2. Impact of process parameters on the measured dependent process parameters

The preparation/production of TVP was investigated and optimized using a CCRD (Table 2) varying three factors: HZ6, FR and WFR/FR (MC). The results of the SME, DL, P and TP (dependent process responses) are presented in Table 2. SME results of three center points of the CCRD (DT-MA-15, DT-MA-16 and DT-MA-17) were utilized to estimate the CVR (coefficient of repeatability variation) of the LME, which was calculated to be 2%. The CVR was low indicating a good repeatability.

The effects of the process variables on the responses evaluated are shown in Table 2 of the Supplementary Materials (SUPM). The response data obtained showed a good agreement with the predicted values. Moreover, 76.7-99.8% of the data were described by the models (Table 3 in SUPM). For the density, springiness, gumminess, WHC, OBC and WSI, low R^2 and R_{adj}^2 were observed (a lack of fit). The fitted response surfaces are presented in Fig. 1 and Fig. 1–4 in SUPM.

The WFR/FR (moisture content) and the feed rate (FR) were the process parameters that mostly influenced the dependent process responses (SME, DL, P and TP). As previously reported for LME and HME of other ingredients (Saldanha do Carmo et al., 2019, 2021), a linear correlation between P and DL (R^2=0.7) was found (Fig. 2). When the DL increased, the P increased too.

A very significant negative impact ($p < 0.001$) of the FR and WFR/FR (MC) on the SME was observed. When the FR and MC increased independently, the SME decreased. This can be explained by the lower force (viscosity decreases) needed to push the mixture towards the die at higher MC creating less friction between the dough and the screws and extruder shaft (Chen et al., 2010). The higher FR leads to a decreased in SME (reduced product viscosity) because the same mechanical energy is available for greater feed rate/mass (Bock and Deiters, 2017). On the other hand, when both the FR and the MC were increased, the SME also increased. The HZ6 (temperature) also had a significant impact on SME ($p = 0.002$). When the HZ6 increased, the SME decreased due to lower product viscosity and lower torque/drive load (Chen et al., 2010). A quadratic significant effect of the FR ($p = 0.007$) indicated that the SME can be described by a four-dimensional concave surface. In the present work, the SME varied from 244 to 558 Wh/Kg with the highest value obtained at 140^{0}C, FR of 4 Kg/h, a target MC of 30.8% and SRS of 900 rpm. In a study conducted by De Angelis et al. (2020) regarding the production of TVPs from dry-fractionated pea and oat proteins, SME values varied between 63 and 357 Wh/Kg for a soy isolate and an oat protein mixture (70:30% w/w) and for a pea and oat protein mixture (70:30% w/w), respectively (De Angelis et al., 2020). The highest value achieved in the study conducted by De Angelis et al. (2020) compared to the present study might be explained by the low MC levels used. The highest SME reported by De Angelis et al. (2020) was obtained at a moisture content of 20%, a temperature of 140^{0}C, a SRS of 800 rpm, and a FR of 5.65 Kg/h which was below the SME values obtained in the present study. Chen et al. (2010) reported values between 1111 and 1389 Wh/Kg at a moisture level between 28-36%, a SRS of 160 rpm, temperatures of 140–160^{0}C and a FR of 1.2 Kg/h for a extruded soybean protein isolate. The lower values achieved in the present study might be probably

Table 2
CCRD and measured/calculated process responses.

Sample	Process conditions			Target MC	Process responses			
	HZ6 (^{0}C)	FR (Kg/h)	WFR/FR		SME (Wh/Kg)	P (bar)	DL (%)	TP (Kg/h)
DT-MA-1	140.0	4.00	0.30	30.8	557.67	24.4 ± 0.7	35.5 ± 0.9	4.20 ± 0.06
DT-MA-2	160.0	4.00	0.30	30.8	520.40	20.6 ± 1.3	34.9 ± 0.7	4.36 ± 0.14
DT-MA-3	140.0	6.00	0.30	30.8	397.72	26.9 ± 0.7	38.1 ± 0.8	6.68 ± 0.09
DT-MA-4	160.0	6.00	0.30	30.8	384.46	22.1 ± 1.0	36.4 ± 0.9	6.35 ± 0.07
DT-MA-5	140.0	4.00	0.42	36.6	344.58	19.2 ± 1.1	30.3 ± 0.9	4.98 ± 0.03
DT-MA-6	160.0	4.00	0.42	36.6	341.73	17.0 ± 1.7	30.3 ± 0.7	5.00 ± 0.02
DT-MA-7	140.0	6.00	0.42	36.6	267.72	21.4 ± 0.5	32.4 ± 1.0	7.34 ± 0.14
DT-MA-8	160.0	6.00	0.42	36.6	258.97	19.5 ± 0.3	31.5 ± 0.5	7.20 ± 0.23
DT-MA-9	133.2	5.00	0.36	33.8	389.78	22.9 ± 0.8	34.1 ± 0.9	5.58 ± 0.15
DT-MA-10	166.8	5.00	0.36	33.8	360.65	17.5 ± 0.8	33.1 ± 1.1	5.70 ± 0.02
DT-MA-11	150.0	3.32	0.36	33.8	483.63	19.3 ± 0.7	32.0 ± 1.3	3.97 ± 0.02
DT-MA-12	150.0	6.68	0.36	33.8	290.69	22.7 ± 1.0	36.9 ± 1.2	8.30 ± 0.73
DT-MA-13	150.0	5.00	0.26	28.5	509.48	23.9 ± 1.4	38.8 ± 1.4	5.38 ± 0.12
DT-MA-14	150.0	5.00	0.46	38.4	244.02	17.6 ± 0.6	28.8 ± 1.1	6.27 ± 0.08
DT-MA-15 (C)	150.0	5.00	0.36	33.8	377.71	20.1 ± 0.7	34.1 ± 0.7	5.76 ± 0.08
DT-MA-16 (C)	150.0	5.00	0.36	33.8	365.25	19.6 ± 1.0	33.4 ± 0.8	5.73 ± 0.18
DT-MA-17(C)	150.0	5.00	0.36	33.8	364.71	21.8 ± 1.7	33.6 ± 1.0	5.80 ± 0.25

Temperature in zone 6 of the extruder (HZ6), ratio between water feed rate and STARTMIX feed rate (WFR/FR) and Feed rate of STARTMIX (FR), moisture content (MC), throughput (TP), drive load (DL), specific mechanical energy (SME), pressure (P)

Table 3
Impact of extrusion on physical properties of the texturized products.

Sample	Process conditions				Density (g/cm^3)	SEI	Colour properties				
	HZ6 (^{0}C)	FR (Kg/h)	WFR/ FR	Target MC (%)			L*	a*	b*	ΔE	BI
STARTMIX	**-**	**-**	**-**	**-**	$\mathbf{0.62 \pm 0.009^{a}}$	**-**	$\mathbf{93.8 \pm 0.04^{a}}$	$\mathbf{-0.48 \pm 0.03^{h}}$	$\mathbf{9.857 \pm 0.13^{j}}$	**-**	$\mathbf{10.46 \pm 0.17^{j}}$
DT-MA-1	140.0	4.00	0.30	30.8	0.48 ± 0.003^{fg}	1.9 ± 0.63^{cd}	76.4 ± 0.23^{j}	3.06 ± 0.10^{c}	24.01 ± 0.23^{c}	22.70 ± 0.32^{bc}	39.86 ± 0.62^{bc}
DT-MA-2	160.0	4.00	0.30	30.8	0.49 ± 0.006^{ef}	3.1 ± 1.19^{ab}	76.0 ± 0.37^{jk}	3.51 ± 0.14^{b}	23.90 ± 0.17^{cd}	23.06 ± 0.41^{ab}	40.38 ± 0.67^{b}
DT-MA-3	140.0	6.00	0.30	30.8	0.49 ± 0.006^{ef}	3.0 ± 0.38^{ab}	78.7 ± 0.14^{h}	2.37 ± 0.08^{e}	23.59 ± 0.08^{defg}	20.59 ± 0.16^{ef}	37.04 ± 0.29^{de}
DT-MA-4	160.0	6.00	0.30	30.8	0.50 ± 0.004^{de}	2.7 ± 0.32^{b}	79.5 ± 0.02^{efg}	2.09 ± 0.02^{fg}	23.47 ± 0.05^{fg}	19.90 ± 0.04^{gh}	36.13 ± 0.10^{ef}
DT-MA-5	140.0	4.00	0.42	36.6	0.48 ± 0.003^{g}	1.1 ± 0.12^{e}	77.6 ± 0.68i	2.79 ± 0.17^{d}	24.84 ± 0.22^{a}	22.28 ± 0.65^{c}	40.34 ± 0.95^{b}
DT-MA-6	160.0	4.00	0.42	36.6	0.48 ± 0.003^{g}	1.4 ± 0.21^{cde}	79.2 ± 0.26^{fgh}	2.38 ± 0.09^{e}	23.80 ± 0.20^{cde}	20.43 ± 0.32^{fg}	37.19 ± 0.57^{d}
DT-MA-7	140.0	6.00	0.42	36.6	0.50 ± 0.006^{cde}	1.3 ± 0.19^{de}	78.8 ± 0.27^{h}	2.84 ± 0.08^{d}	24.42 ± 0.28^{b}	21.19 ± 0.40^{de}	38.95 ± 0.74^{c}
DT-MA-8	160.0	6.00	0.42	36.6	0.48 ± 0.003^{fg}	1.4 ± 0.13^{cde}	80.8 ± 0.12^{b}	2.10 ± 0.06^{fg}	22.98 ± 0.16^{i}	18.64 ± 0.20^{j}	34.63 ± 0.38^{hi}
DT-MA-9	133.2	5.00	0.36	33.8	0.50 ± 0.004^{de}	1.5 ± 0.24^{cde}	79.0 ± 0.78^{gh}	2.36 ± 0.29^{e}	23.71 ± 0.47^{cdef}	20.46 ± 0.91^{fg}	37.09 ± 1.53^{de}
DT-MA-10	166.8	5.00	0.36	33.8	0.51 ± 0.007^{b}	1.6 ± 0.22^{cde}	80.4 ± 0.23^{bc}	1.93 ± 0.06^{g}	23.06 ± 0.14^{i}	18.97 ± 0.27^{ij}	34.81 ± 0.41^{ghi}
DT-MA-11	150.0	3.32	0.36	33.8	0.52 ± 0.004^{b}	1.4 ± 0.25^{de}	77.7 ± 0.32^{i}	2.41 ± 0.08^{e}	23.43 ± 0.24^{fgh}	21.29 ± 0.19^{d}	37.40 ± 0.36^{d}
DT-MA-12	150.0	6.68	0.36	33.8	0.51 ± 0.019^{bcd}	2.0 ± 0.51^{c}	80.9 ± 0.13^{b}	1.97 ± 0.01^{fg}	23.00 ± 0.02^{i}	18.59 ± 0.08^{j}	34.50 ± 0.05^{i}
DT-MA-13	150.0	5.00	0.26	28.5	0.49 ± 0.009^{efg}	3.4 ± 0.91^{a}	75.8 ± 0.15^{k}	3.92 ± 0.08^{a}	24.43 ± 0.10^{b}	23.56 ± 0.12^{a}	41.89 ± 0.23^{a}
DT-MA-14	150.0	5.00	0.46	38.4	0.51 ± 0.004^{bc}	1.2 ± 0.04^{de}	80.2 ± 0.05^{cd}	2.38 ± 0.04^{e}	23.58 ± 0.17^{efg}	19.52 ± 0.12^{hi}	36.22 ± 0.30^{ef}
DT-MA-15 (C)	150.0	5.00	0.36	33.8	0.51 ± 0.006^{bcd}	1.4 ± 0.14^{cde}	79.7 ± 0.25e	2.15 ± 0.07^{f}	23.26 ± 0.26^{ghi}	19.66 ± 0.36^{h}	35.75 ± 0.63^{fg}
DT-MA-16 (C)	150.0	5.00	0.36	33.8	0.51 ± 0.010^{bc}	1.7 ± 0.25^{cde}	79.7 ± 0.14^{de}	2.10 ± 0.09^{fg}	23.27 ± 0.12^{ghi}	19.65 ± 0.19^{h}	35.71 ± 0.34^{fgh}
DT-MA-17(C)	150.0	5.00	0.36	33.8	0.51 ± 0.004^{bc}	1.6 ± 0.20^{cde}	79.5 ± 0.18^{ef}	2.09 ± 0.06^{fg}	23.12 ± 0.17^{hi}	19.65 ± 0.24^{h}	35.52 ± 0.43^{fghi}

Temperature in zone 6 of the extruder (HZ6), ratio between water feed rate and STARTMIX feed rate (WFR/FR) and Feed rate of STARTMIX (FR), moisture content (MC), Sectional expansion index (SEI), Lightness (L*), redness (a*), yellowness (b*), total colour change (ΔE) and browning index (BI).

attributed to a higher shear screw configuration used in this case (not described in the publication of Chen et al. 2010). Using an extruder with higher capacity (30 Kg/h) a pea protein isolate was texturized by LME at moisture content levels of 26–35%, SRS of 400–700 rpm and barrel temperatures of 130-170^{0}C and the highest SME achieved was 90 Wh/Kg at 700 rpm and 137^{0}C (Beck et al., 2017). The low values achieved compared to the present study can possibly be attributed to a lower-shear force used and the raw-material properties (higher fat content) that lubricates the extruder screws and barrel reducing the mechanical shear (Riaz and Rokey, 2011). TVPs were produced using blends of pea protein isolates and oat protein by Kaleda et al. (2021) at moisture levels between 25 and 35%, temperatures between 135 and 170^{0}C and SRS between 200 and 700 rpm. SME values between 38 and 290 Wh/Kg were achieved which were lower compared to the present study. Lower SME is possibly attributed to higher fat contents of the raw-material blends (11.1–15.5 %).

The DL varied from 28.8% (23.0 Nm torque) to 38.8% (31.1 Nm torque). Equally to the SME, a very significant impact of the WFR/FR (MC) on the DL was observed. When the MC increased, both DL and SME decreased. A linear correlation was found between the DL and the MC ($R^2 = 0.8$). The moisture reduces the friction during extrusion, acting as a lubricant thus reducing the drive load. Moreover, when the FR increased (very significant impact), the DL increased too. The P varied from 17.0 to 26.9 bar. Moreover, an increase on the WFR/FR (MC), the HZ6 or the FR independently led to increased P (very significant impact). De Angelis et al. (2020) reported values of torque between 12.8 and 24.1 Nm and P between 16.3 and 38.1 bar during LME of mixtures of a pea protein concentrate, pea protein isolate, oat protein and soy isolates (De Angelis et al., 2020). The higher values of P and DL reported in comparison with the results obtained in the present work can possibly be related with the lower moisture content (20%) assessed in the study, causing higher friction in the extruder barrel and less lubrication. Beck et al. (2017) reported higher values of P between 46 and 68 bar for LME using a pea protein isolate with 81.5 % dm protein content. The TP varied from 4.0 to 8.3 Kg/h and was affected significantly by the MC and the FR which were increased when these two variables increased independently.

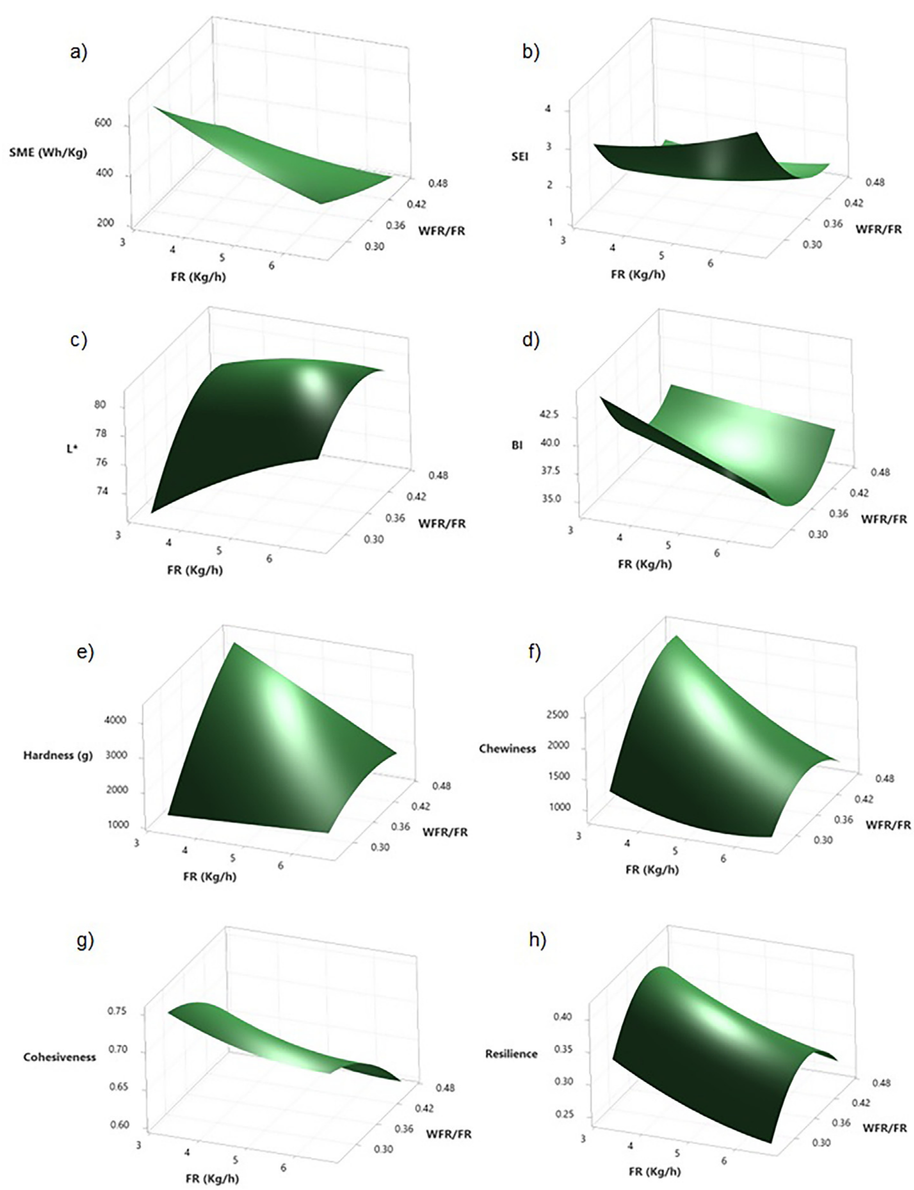

Fig. 1. Response surfaces fitted to: (a) Specific Mechanical Energy (SME) as a function of feed rate of STARTMIX (FR) and ratio between water feed rate and STARTMIX feed rate (WFR/FR); (b) Sectional expansion index (SEI) as a function of FR and WFR/FR; (c) Lightness (L*) as a function of FR and WFR/FR; (d) Browning index (BI) as a function of FR and WFR/FR; (e) Hardness as a function of FR and WFR/FR; (f) Chewiness as a function of FR and WFR/FR; (g) Cohesiveness as a function of FR and WFR/FR; (h) Resilience as a function of FR and WFR/FR.

3.3. Impact of process parameters on the density, sectional expansion index (SEI) and colour properties

The results of density, SEI (before drying of the extrudates) and colour properties are presented in Table 3. The density is normally linked with the expansion of the extrudates (Pitts et al., 2014). In the present work, the density decreased from 0.62 down to 0.48 upon extrusion processing. However, the density remained relatively constant for some process conditions tested. Beck et al. (2017) reported constant density values for SME's above 72 Wh/Kg for LME of pea protein isolate which is also the case of the present study for SME values between 244 and 558 Wh/Kg. As suggested by Beck et al. (2017) this can indicate a similar pore size distribution and cell wall structure among the extrudates.

Higher moisture content and the increase of both FR and the HZ6 at the same time led to less expanded products. On the other hand, higher FR led to higher expansion of the extrudates. A correlation was found between the target MC and the SEI (R^2=0.86). When the MC increased (less viscous mass inside the extruder), the SEI decreased (Fig. 2). In a previous study conducted by Saldanha do Carmo et al. (2019), this correlation was also observed, during extrusion of pea protein, pea starch and oat fiber. The highest SEI was obtained for DT-MA-13 (3.36), DT-MA-2 (3.08) and DT-MA-3 (3.03), produced at lower moisture contents (28.5-30.8%).

The FR and the MC (WFR/FR) had a major impact on the BI and a* (redness). When these two parameters increased independently, the BI and a* decreased. On the other hand, an increase of these two variables independently (very significant impact), led to an increased L* (lighter products). Wang et al. (1999) reported similar results for TVP from a pea protein concentrate. The b* (yellowness) and browning index (BI) were significantly affected by the HZ6, which were decreased with an increase of this variable. Moreover, when the HZ6 increased (significant impact), the lightness increased (lighter products). Wang et al. (1999), Saldanha do Carmo et al. (2019, 2021) reported the contrary: when HZ6 increased, the L* decreased. Moreover, a linear correlation (R^2=0.70) was found between the L* and the SME. With increasing SME, the lightness decreased (darker products were obtained). These results indicates that the effect of the SME was higher than the effect of the HZ6 on the lightness and browning index, suggesting that the mechanical energy, shear forces and friction of the raw-material between screws and the barrel had the greatest impact on these parameters as also reported by Rausch (2009). In general, higher SME led to darker products and higher

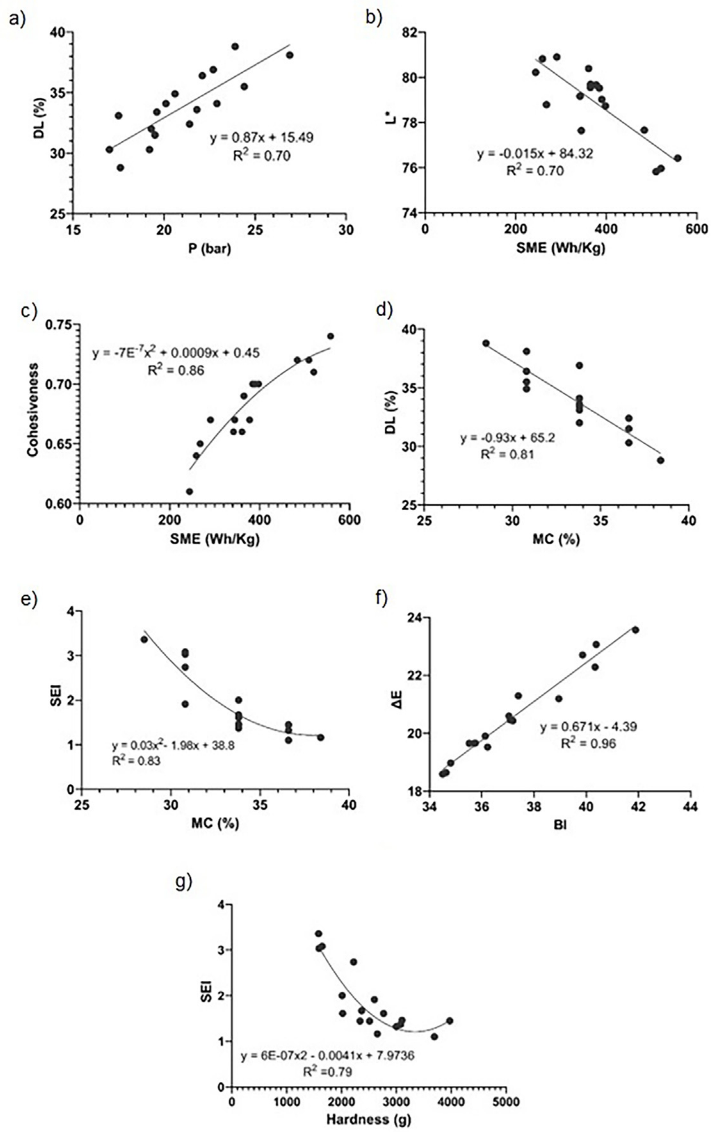

Fig. 2. Correlations between: (a) Drive load (DL) and Pressure (P); (b) Lightness (L*) and Specific Mechanical Energy (SME); (c) Cohesiveness and SME; (d) DL and target moisture content (MC); (e) Sectional Expansion Index (SEI) and target MC; (f) Colour differences ΔE and Browning index (BI); g) SEI and Hardness.

browning index, suggesting that browning reactions were not promoted by the temperature. When operating with HZ6 above 150^0C, a six-order polynomial correlation (R^2 = 0.91) was found between the SME and the BI. As also reported by Saldanha do Carmo et al. (2019), the samples that showed to be darker and that had higher browning index were produced at the highest SME values and showed higher expansion. Overall, the colour differences / changes (ΔE) were significantly affected by all extrusion processing parameters evaluated. When the HZ6, FR and WFR/FR (moisture content) increased independently, the ΔE decreased. A quadratic significant effect of the WFR/FR ($p = 0.006$) indicated that the ΔE can be described by a four-dimensional concave surface. Moreover, the ΔE were mainly driven by Maillard reactions (Fig. 2), that can be confirmed by the high linear correlation ($R^2 = 0.96$) between the ΔE and the BI.

3.4. Impact of process parameters on the WHC, OBC and WSI

The succulence and juiciness of meat are linked to their capacity to hold water (WHC) or to hold oil (OBC) (Asgar et al., 2010; Kaleda et al., 2021). These properties depend on the composition and processing (change of microstructure) of the raw materials. In STARTMIX, protein was the main constituent (56.6% dm). Generally, the protein side chains bind to water through hydrogen bonds and the non-polar side chains binds to oil. Moreover, starch and fiber binds to water (Mazaheri Tehrani et al., 2017; Saldanha do Carmo et al., 2020). The protein denaturation and aggregation are linked with the amount of soluble components in water assessed through the WSI (Kaleda et al., 2021). The results of the WHC, OBC and WSI of the non-extruded blend (STARTMIX) and dry texturized extrudates are presented in Table 4.

The WHC increased with extrusion processing from 275% (STARTMIX) to 502 % (DT-MA-5) processed at low HZ6, low FR and high MC (36.6 %). Samard and Ryu (2019) reported WHC values between 323% and 522% for dry-texturized mung bean protein isolate and soy protein isolate, respectively. De Angelis et al. (2020) obtained WHC from 93% for dry-fractionated pea protein and 646% for soy protein isolate before extrusion processing, which was in agreement with the values obtained for the STARTMIX. The WHCs obtained in the present work enabled WHCs between 407 and 502 % after extrusion processing. An increased WHC (hydrophilic groups are more open to the molecule surface) is due to protein denaturation (Osen et al., 2014). The higher values obtained compared to the ones reported in the literature cited here, can be related to the higher WHC of the BG28, which is due both to its high fiber content and the specific fiber composition (high content of beta-glucan).

The OBC is also related with capillarity interactions (Du et al., 2014), which means that higher expansion and porosity leads to higher capacity to bind oil. However, this was not verified in the present study. In

Table 4
Water holding capacity (WHC), water solubility index (WSI) and oil binding capacity (OBC) of BG28, FBPC, STARTMIX, TVPs produced and TVP F6501 faba bean commercial product.

Sample	Process conditions			Target MC	Technofunctional properties		
	HZ6 (^{0}C)	FR (Kg/h)	WFR/FR		WHC (%)	OBC (%)	WSI (%)
BG28	-	-	-	-	**1052 ± 43.8**	**nd**	**2.45 ± 0.43**
FBPC	-	-	-	-	**191.7 ± 0.4**	**nd**	**62.21 ± 0.37**
STARTMIX (FBPC:BG28)	-	-	-	-	**274.8 ± 2.8^{j}**	**223.4 ± 0.9^{b}**	**49.67 ± 0.06^{a}**
DT-MA-1	140.0	4.00	0.30	30.8	452.7 ± 4.3^{bc}	211.9 ± 0.4^{efgh}	20.05 ± 0.37^{c}
DT-MA-2	160.0	4.00	0.30	30.8	456.7 ± 17.4^{b}	213.1 ± 0.1^{de}	20.02 ± 0.13^{c}
DT-MA-3	140.0	6.00	0.30	30.8	454.9 ± 3.3^{b}	211.1 ± 0.6^{fgh}	19.18 ± 0.65^{d}
DT-MA-4	160.0	6.00	0.30	30.8	461.3 ± 1.8^{b}	210.9 ± 1.6^{gh}	18.35 ± 1.61^{fgh}
DT-MA-5	140.0	4.00	0.42	36.6	502.2 ± 8.4^{a}	227.5 ± 1.3^{a}	16.57 ± 1.25^{k}
DT-MA-6	160.0	4.00	0.42	36.6	439.3 ± 23^{cde}	215.4 ± 0.1^{c}	18.23 ± 0.03^{ho}
DT-MA-7	140.0	6.00	0.42	36.6	416.4 ± 3.5^{ghi}	224.0 ± 0.7^{b}	17.65 ± 0.75^{j}
DT-MA-8	160.0	6.00	0.42	36.6	406.6 ± 3.2^{i}	215.5 ± 0.6^{c}	18.32 ± 0.65^{gh}
DT-MA-9	133.2	5.00	0.36	33.8	426.0 ± 3.8^{efgh}	213.7 ± 0.8^{d}	18.84 ± 0.82^{de}
DT-MA-10	166.8	5.00	0.36	33.8	424.4 ± 4.3^{fgh}	213.1 ± 0.4^{de}	18.73 ± 0.42^{ef}
DT-MA-11	150.0	3.32	0.36	33.8	438.6 ± 5.3^{de}	212.9 ± 0.2^{de}	18.7 ± 0.17^{efg}
DT-MA-12	150.0	6.68	0.36	33.8	429.7 ± 9.3^{efg}	212.5 ± 0.9^{defg}	18.46 ± 0.87^{efgh}
DT-MA-13	150.0	5.00	0.26	28.5	451.9 ± 6.2^{bcd}	213.7 ± 0.4^{d}	20.95 ± 0.44^{c}
DT-MA-14	150.0	5.00	0.46	38.4	414.8 ± 5.5^{hi}	212.6 ± 1.6^{def}	17.93 ± 0.04^{ij}
DT-MA-15 (C)	150.0	5.00	0.36	33.8	424.0 ± 2.2^{fgh}	212.1 ± 0.6^{defgh}	18.8 ± 0.59^{de}
DT-MA-16 (C)	150.0	5.00	0.36	33.8	433.0 ± 0.8^{ef}	210.5 ± 0.7^{h}	18.62 ± 0.66^{efg}
DT-MA-17(C)	150.0	5.00	0.36	33.8	429.0 ± 5.4^{efg}	211.2 ± 2.3^{fgh}	18.59 ± 2.29^{efgh}
VestKorn F6501M (CR)	-	-	-	-	191.7 ± 0.4	399.3 ± 3.7	20.65 ± 0.26

Means that do not share a letter are significantly different ($p<0.05$); nd: not determined; CR: Commercial reference.

general, lower expansion led to higher OBC. The OBC of the TVPs varied from 210% (DT-MA-16) to 227% (DT-MA-5). The OBC of the STARTMIX was 223%. No significant differences were found between the STARTMIX and the TVPs produced at low HZ6 (140^{0}C), high FR (6Kg/h) and high MC (36.6%) (DT-MA-7). However, when the FR was decreased (4 Kg/h), a significant increase in the OBC was obtained (DT-MA-5). This can be due to the higher residence time of the STARTMIX inside the extruder and higher SME. In general, the OBC decreased upon extrusion. The OBC depends on protein hydrophobic group at the protein surface (Mazaheri Tehrani et al., 2017). A decrease in the hydrophobicity at the surface can be possibly explained by the existence of aggregation and denaturation simultaneously (Ai et al., 2016). The slightly higher OBC of the TVP DT-MA-5 can probably be associated with a higher degree of denaturation and aggregation (confirmed by the lowest WSI) promoted at higher residence time and higher MC. De Angelis et al. (2020), found OBCs between 102 and 166% for the raw blends and OBC between 106 and 185% for the TVPs. Also in this study, the OBC varied very little compared to the variation of the WHC. The lower values of OBC were attributed to the use of dry-fractionated pea fine fraction that didn't enable large modifications in terms of functionality of the meat analogues compared to blends containing protein isolates (de Angelis et al., 2020). In the present study the FBPC was produced by dry-fractionation, but the blend also contained an oat beta-glucan rich fraction that also contains 23 % protein. The higher OBC and WHC values obtained in the present study can probably be related to the oat ingredient showing that BG28 can give an improvement in the functionality of the final meat analogues in terms of texture and functional properties. In practical terms, higher WHC will help to retain more water in the formulation of the final meat analogues leading to juicier products.

In the present work, the WSI decreased from 49.7% (STARTMIX) to 16.6% for TVP (DT-MA-5). Kaleda et al. (2021) reported a decrease in the WSI upon extrusion of a combination of pea protein and oat protein concentrates (70:30% w/w) and an increase of the WSI for blends of 50:50 and 30:70, due to an increase of the carbohydrate content. In the present work, all TVPs showed higher WHC and lower WSI and OBC. Commercial reference (VestKorn F6501M) had lower WHC, higher OBC and similar WSI compared to the TVP produced in the present work. Altogether, these results means that a protein network was formed agreeing with Samard et al. (2019) and Alonso et al. (2000) for texturization of plant protein meat analogues and pea and kidney proteins, respectively.

3.5. *Impact of process parameters on the textural properties*

The textural properties of the hydrated TVPs were evaluated using texture profile analysis (TPA). The texture profile is particularly important for understanding consumer acceptance of the final meat analogue products. The results of the measured attributes are summarized in Table 5.

The hardness corresponds to the maximum force needed to compress the hydrated TVPs (Kaleda et al., 2021) and it is defined as the force required to compress the sample using teeth (De Angelis et al., 2020). The hardness was calculated as the maximum force of the first compression. The hardness varied from 1582 g (15.51 N) for DT-MA-13 to 3973 g (38.96 N) for DT-MA-6. Moreover, the hardness decreased with increasing FR. On the other hand, when the MC increased, the hardness also increased. Chen et al. (2010) produced soy protein isolate TVPs, and an increase in the moisture content led to a sharp decrease on the hardness and chewiness. This difference can be due to the dramatic increase in the moisture content from 28% to 60%. Samard et al. (2019) for a TVP produced from a mixture of corn, starch, SPI and wheat gluten, reported values of hardness between 2000 and 4000 g, without and with wheat gluten, respectively, being in line with what was obtained in the present work. Kaleda et al. (2021), reported values between 4 and 81 N for TVPs produced from diferent mixtures containing pea and oat protein alone or in combination. This is similar to the results obtained in the present work. Overall, higher hardness corresponds to a denser network (lower pressure at the die, thus less expansion), as also found by Kaleda et al. (2021). The same trend was observed in the present work (Fig. 2). De Angelis et al. (2020) reported higher hardness values of 27 N for TVPs produced from protein isolates of pea, soya and oat than those produced from pea protein concentrate (13.55–18.33 N). In the present work, a dry-fractionated FBPC was used and higher values of hardness were also achieved (39 N), which were produced at high HZ6 (160^{0}C), low FR (4 Kg/h) and high MC (36.6%). These observations indicate that it is not needed to use a protein isolate to obtain higher hardness values. The texture parameters were more dependent on the process conditions than on the type of process to obtain the protein ingredient.

Table 5
Textural properties of extrudates measured instrumentally (TPA results).

Sample	Process conditions				Textural properties					
	HZ6 (^{0}C)	FR (Kg/h)	WFR/FR	Target MC (%)	Hardness (g)	Springiness	Cohesiveness	Gumminess	Chewiness (g)	Resilience
DT-MA-1	140.0	4.00	0.30	30.8	2599 ± 218.1^{def}	0.982 ± 0.024^{bc}	0.741 ± 0.020^{a}	1929 ± 209.7^{cd}	1893 ± 196.2^{bc}	0.40 ± 0.03^{ab}
DT-MA-2	160.0	4.00	0.30	30.8	1646 ± 167.3^{i}	1.006 ± 0.060^{ab}	0.709 ± 0.030^{ab}	1167 ± 120.1^{ij}	1293 ± 139.7^{fgh}	0.31 ± 0.03^{gh}
DT-MA-3	140.0	6.00	0.30	30.8	1588 ± 111.0^{i}	0.976 ± 0.005^{bcd}	0.696 ± 0.019^{bcde}	1105 ± 68.44^{j}	1102 ± 78.5^{gh}	0.31 ± 0.01^{fgh}
DT-MA-4	160.0	6.00	0.30	30.8	2221 ± 282.2^{gh}	0.960 ± 0.029^{bcd}	0.702 ± 0.019^{bcd}	1560 ± 193.7^{fg}	1494 ± 153.7^{def}	0.33 ± 0.01^{fgh}
DT-MA-5	140.0	4.00	0.42	36.6	3694 ± 203.4^{a}	0.954 ± 0.038^{bcd}	0.671 ± 0.038^{defg}	2478 ± 205.7^{a}	2363 ± 207.0^{a}	0.39 ± 0.03^{bc}
DT-MA-6	160.0	4.00	0.42	36.6	3973 ± 146.7^{a}	0.920 ± 0.042^{cd}	0.656 ± 0.012^{fgh}	2633 ± 86.76^{a}	2447 ± 224.2^{a}	0.37 ± 0.02^{cde}
DT-MA-7	140.0	6.00	0.42	36.6	3001 ± 226.5^{bc}	0.937 ± 0.030^{bcd}	0.651 ± 0.009^{gh}	1952 ± 120.6^{cd}	1830 ± 138.0^{bc}	0.34 ± 0.01^{ef}
DT-MA-8	160.0	6.00	0.42	36.6	2337 ± 126.3^{fg}	0.978 ± 0.033^{bc}	0.635 ± 0.021^{hi}	1486 ± 127.5^{fgh}	1456 ± 169.1^{def}	0.32 ± 0.03^{fgh}
DT-MA-9	133.2	5.00	0.36	33.8	3102 ± 31.27^{b}	0.954 ± 0.018^{bcd}	0.704 ± 0.012^{bc}	2168 ± 57.59^{bc}	2075 ± 4.070^{b}	0.39 ± 0.02^{abc}
DT-MA-10	166.8	5.00	0.36	33.8	2020 ± 138.1^{h}	0.997 ± 0.002^{abc}	0.657 ± 0.001^{fgh}	1328 ± 88.54^{hij}	1329 ± 121.0^{efg}	0.32 ± 0.01^{fgh}
DT-MA-11	150.0	3.32	0.36	33.8	3080 ± 192.7^{b}	1.087 ± 0.159^{a}	0.724 ± 0.019^{ab}	2227 ± 119.6^{b}	2408 ± 214.9^{a}	0.42 ± 0.02^{a}
DT-MA-12	150.0	6.68	0.36	33.8	2012 ± 137.2^{h}	0.953 ± 0.013^{bcd}	0.666 ± 0.020^{efgh}	1342 ± 120.6^{ghi}	1280 ± 132.3^{fgh}	0.30 ± 0.01^{hi}
DT-MA-13	150.0	5.00	0.26	28.5	1582 ± 145.4^{i}	0.956 ± 0.048^{bcd}	0.716 ± 0.025^{ab}	1133 ± 130.1^{ij}	991.9 ± 13.54^{h}	0.27 ± 0.02^{i}
DT-MA-14	150.0	5.00	0.46	38.4	2652 ± 242.2^{de}	0.891 ± 0.032^{d}	0.612 ± 0.024^{i}	1621 ± 143.4^{f}	1444 ± 142.6^{def}	0.29 ± 0.02^{hi}
DT-MA-15 (C)	150.0	5.00	0.36	33.8	2511 ± 50.90^{defg}	0.982 ± 0.027^{bc}	0.673 ± 0.018^{cdefg}	1691 ± 79.29^{ef}	1661± 118.1^{cd}	0.34 ± 0.02^{efg}
DT-MA-16 (C)	150.0	5.00	0.36	33.8	2366 ± 97.10^{efg}	0.970 ± 0.012^{bcd}	0.694 ± 0.007^{bcde}	1643 ± 81.52^{f}	1634 ± 30.80^{cde}	0.34 ± 0.001^{def}
DT-MA-17(C)	150.0	5.00	0.36	33.8	2767 ± 239.6^{cd}	0.984 ± 0.011^{bc}	0.690 ± 0.009^{bcdef}	1909 ± 170.6^{de}	1878 ± 147.6^{bc}	0.38 ± 0.01^{bcd}
VestKorn F6501M (CR)	-	-	-	-	1701 ± 27.6	0.921 ± 0.053	0.604 ± 0.049	1013 ± 96.00	902.4 ± 82.68	0.28 ± 0.03

Temperature in zone 6 of the extruder (HZ6), ratio between water feed rate and STARTMIX feed rate (WFR/FR) and Feed rate of STARTMIX (FR), moisture content (MC); VestKorn Texturized protein (VestKorn F6501M); Commercial reference (CR). Means with different letters are significantly different ($p<0.05$)

Springiness describes the recovery of the TVPs after deformation (Kaleda et al., 2021) and defines to what extent the sample recovers after a second compression to its initial height (De Angelis et al., 2020). The springiness was expressed as the ratio between the height of the sample at the start of the second compression and the height of the sample at the start of the first compression. The springiness varied from 0.89 (DT-MA-14) to 1.09 (DT-MA-11). The results of springiness were not significantly affected by the process parameters tested, but increased slighlty with increasing SME. Kaleda et al. (2021) reported statistically significant decrease in springiness when the protein concentration was decreased and obtained values of springiness between 0.82 and 0.99 during production of TVPs from of oat and pea protein blends. De Angelis et al. (2020) reported lower springiness values between 0.72 and 0.87 for meat analogues based on blends of pea, oat, soy concentrates and isolates. The lower springiness values compared to the ones obtained in the present work can be possibly explained by the lower temperatures used in the work of De Angelis et al. (2020).

The cohesiveness indicates the strenght of the network formed (Kaleda et al., 2021) and it is defined as the quantity of sample that stands together during chewing (De Angelis et al., 2020). The cohesiveness results were expressed as the area of work during the second compression divided by the area of work during the first compression. A high correlation between the extruder conditions tested and the cohesiveness was found (R^2 = 0.96). The cohesiveness varied from 0.61 (DT-MA-14) to 0.74 (DT-MA-1) being significantly affected by all processing parameters studied. When the HZ6, FR and MC increased independently, the cohesiveness decreased, meaning that a less strong network was formed. A high correlation (Fig. 2) was also found between the SME and the cohesiveness (R^2 = 0.86) which could be described by a second order polynomial equation. When the SME increased, a stronger network was formed. An average cohesiveness of 0.64 was reported by Kaleda et al. (2021) for pea and oat protein TVPs. Moreover, this average cohesiveness decreased with decreasing protein content. Less protein-protein interactions were established leading to lowest cohesiveness. In the present work, the protein content was kept constant throughout the experiments. A stronger network and protein-protein interations were promoted at higher SME's. De Angelis et al. (2020) reported values of cohesiveness from 0.54 to 0.62 for blends of pea, oat and soya, which was lower than the ones achieved in the present work.

The gumminess is a mechanical textural characteristic associated to the cohesiveness of a tender product. It is connected to the effort needed to desintegrate the product in the mouth to a ready form for swallowing and it is defined as the product of hardness times cohesiveness (Pons and Fiszman, 1996). The gumminess varied from 1105 (DT-MA-3), produced at low HZ6, high FR and low MC) and 2632 (DT-MA-6, produced at higher temperature, low FR and high MC).

The resilience is how strong and fast is the recovery (Kaleda et al., 2021) and was calculated by dividing the upstroke energy of the first compression by the downstroke energy of the first compression. The resilience varied between 0.27 (DT-MA-13) and 0.42 (DT-MA-11). Kaleda et al. (2021) reported values of resilience in average of 0.43 and 0.46. For a mixture tested containing similar protein content (Blend D, 63.5% dm) of that of STARTMIX (57.1 % dm), the resilience reported was around 0.36 being in agreement with the results obtained in the present work.

Chewiness represents the energy needed to chew the hydrated TVPs (Kaleda et al., 2020) and it is defined as the force required to masticate the sample before swallowing (De Angelis et al., 2020). The chewiness was determined multiplying the gumminess by the springiness. The chewiness varied from 992 g (9.7 N) for DT-MA-13 to 2447 g (24 N) for DT-MA-6 and was significantly influenced by the FR and the MC. When the FR increased, the chewiness decreased and when the MC increased, the chewiness also increased. Samard and Ryu (2019), reported values of chewiness between 215 and 2677 g for TVPs of peanut protein isolate and soy protein isolate, respectively. Kaleda et al. (2020) reported values of chewiness around 17 N for TVPs of pea and oat protein, being in line with the results obtained in the present work. De Angelis et al. (2020) reported values of chewiness between 5.25 N and 14.77 N for pea, oat and soy protein blends. In the present work, higher values were obtain (up to 24 N). This can be probably due to different chemical compositions of the ingredients. For instance, due to higher amount of carbohydrates, that contributes to build texture (starch gelatinization), leading to more expanded products, thus a more porous structure and less chewiness (Gomez and Aguilera, 1984).

As a reference, Wee et al. 2018 reported values of resilience, cohesiveness and springiness of cooked chicken of 0.36, 0.71 and 0.79, respectively. The springiness of the TVPs produced in the present work were slightly lower than that of boiled chicken but all the other values assessed were similar. However, extremely springy foods can also be perceived as hard, cohesive and rubbery which could be complicated to masticate (Kaleda et al., 2021; Wee et al., 2018). In general, all extruder parameters had a strong effect on the textural properties measured. The

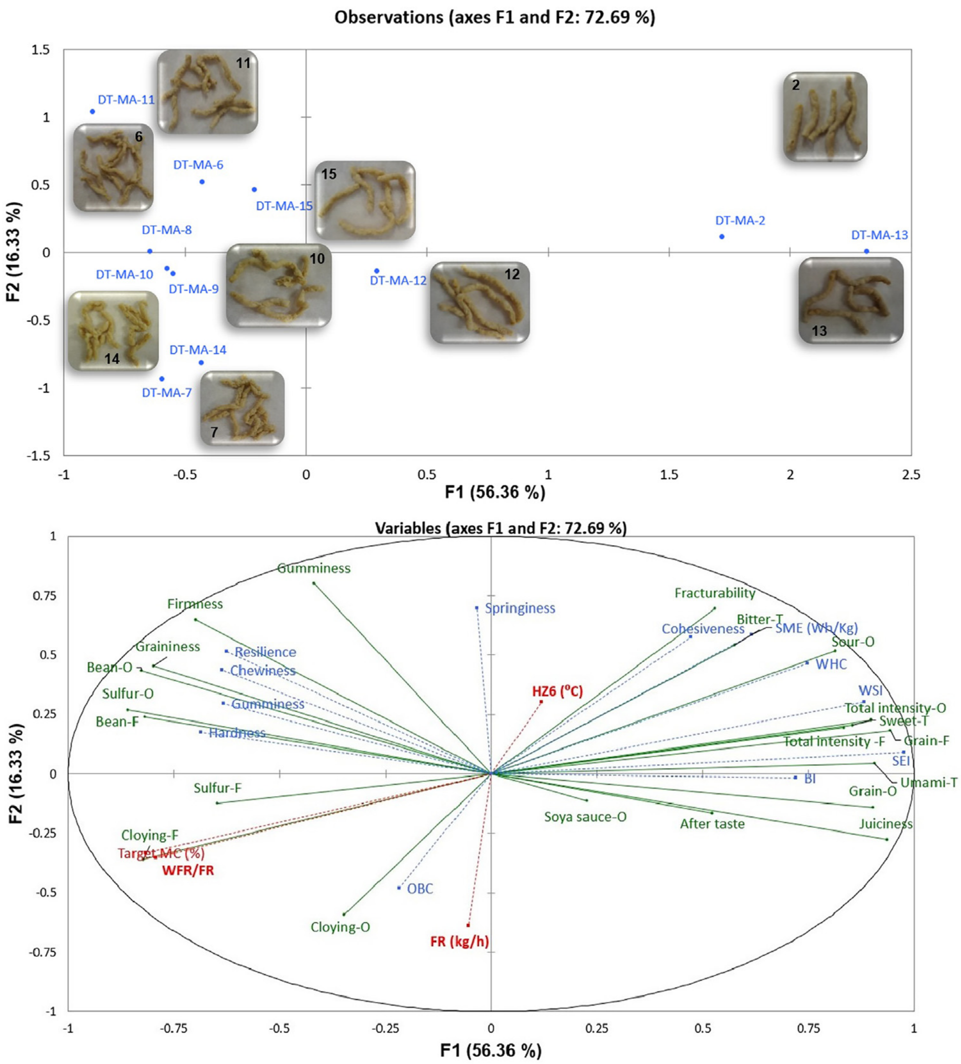

Fig. 3. Correlation between the processing parameters (in red), the physicochemical parameters measured instrumentally/analytically (in blue) and the sensory variables assessed by the panel (in green) via Multiple factor analysis (MFA). The data obtained instrumentally/analytically are overimposed as supplementary variables. F1 and F2 correspond to the first and second principal component in the final weighed PCA. Pictures correspond to the extrudates after soaking (further details in SM). Pictures of the extrudates before soaking were presented in SM.

commercial faba bean TVP sample, VestKorn (F6501M), had similar texture parameters as obtained for samples DT-MA-2 and DT-MA-13.

In this study, the characterization of the attributes of the intermediate ingredients (TVP) were conducted. In future studies, when the focus will be the final application (meat analogues), consumer controlled studies will be performed in the overall acceptability of the target final product.

3.6. *Sensory perception assessed by the panel and correlation with the instrumental texture*

The sensory properties of the 11 selected samples were analysed and the results are reported in Fig. 3. Moreover, the textural and some physicochemical properties measured analytically were correlated with the properties evaluated by the panel, which were overimposed as supplementary variables (Fig. 3). Fourteen of the twenty-one sensory attributes evaluated were found to be significantly different (Table 4 in SUPM) among the TVP samples tested, namely: total intensity of odour, sour odour, grain odour, bean odour, total intensity of flavour, sweet taste, umami taste, bean flavour, grain flavour, firmness, juiciness, graininess, gumminess and fracturability.

Fig. 3 show that 56.4 % of the variation is explained by the principal component 1 (F1) which show the most important differences (representing the MC), while 16.3 % of the variation is explained by the principal component 2 (F2) which show the second most important differences (representing the FR). The closer a sample is to a sensory/texture/physicochemical attribute or extruder variable in the plot, the higher the intensity of that attribute has been measured in the respective sample.

The eleven samples evaluated by the panel were characterized in the sensory space presented in Fig. 3. Some physicochemical and textural properties measured instrumentally were correlated with the sensory attributes assessed by the panel and were overimposed in the perceptual map (Fig. 3). Samples DT-MA-6, DT-MA-8, DT-MA-9, DT-MA-10, DT-MA-11 and DT-MA-15 sit on the upper left side of the map and had less odour intensity, average sour and grain odours and the highest bean odour. Moreover, they presented average flavour intensity, the least sweet and umami tastes, average grain flavour and the highest bean flavour. Regarding the texture properties accessed by the panel, these samples had higher firmness, lower juiciness, higher graininess, higher gumminess and average fracturability. Concerning the texture attributes evaluated instrumentally, these samples presented higher chewiness, hardness, resilience and gumminess and medium cohesiveness and springiness.

Sample DT-MA-12, DT-MA-7 and DT-MA-14 sit mid-way and in the bottom left side of the map and were produced at high FR (5-6.68 Kg/h) and higher moisture levels (33.8%-38.4%), presenting less sourness and flavour intensity, average bean flavour, less grain odour and flavour. Relating the textural attributes evaluated by the panel, they presented average juiciness and graininess, less gumminess and average firmness.

Regarding the texture attributes evaluated instrumentally, these samples presented average chewiness, hardness, resilience, gumminess and springiness and lower cohesiveness.

Samples produced at low moisture levels sit on the right side on the map: DT-MA-13 (28.5%) and DT-MA-2 (30.8%) and had the highest overall odour intensity mainly due to the contribution of grain and sour odours. Moreover, these samples also presented higher flavour intensity corresponding to a higher sweet and umami taste and grain flavour. Furthermore, these samples presented the least bean odour among all samples tested (Table 4 in SUPM). The bean odour is usually not pleasant for consumers (Kyriakopoulou et al., 2021; Roland et al., 2017). Higher expansion also helped removing undesirable volatiles, such as, from the faba bean protein concentrate and introduced Maillard products. Moreover, the addition of 20% of oat beta glucan fraction and the use of the right extrusion processing parameters (considering that the oat content was kept constant in all samples) might have promoted to mask/smooth the bean odour, leading to an improvement of the sensory profile of the extrudates DT-MA-13 and DT-MA-2, that were produced at higher HZ6 (150–160^{0}C) and lower MC (28.5–30.8 %). Also, a synergistic effect between the FBPC and the BG28 could have promoted a higher grain odour and taste perception hiding the bean odour and flavour. Stronger Maillard reactions have promoted a higher sweet taste (confirmed by the highest browning indexes determined among all samples). Moreover, these samples were produced at higher SME, lower MC, average FR presenting higher expansion, higher browning index, higher WHC and higher WSI.

De Angelis et al. (2020), reported similar findings for TVP from a blend of pea concentrate and oat protein (70:30% w/w) with 20% moisture content, a protein content of 54.7 %, feed rate of 5.65 Kg/h, a SRS of 800 rpm, a temperature profile of 40 (HZ1)-70-130-150-140-140 (HZ6) and a SME of 357 Wh/Kg.

Regarding the texture attributes evaluated by the sensory panel, DT-MA-2 and DT-MA-13 were the less firm (hard) together with sample DT-MA-12, having the highest juiciness, least graininess, average gumminess and the highest fracturability. With regards to the texture attributes evaluated instrumentally, these samples had highest cohesiveness, average springiness and lower hardness, chewiness, gumminess and resilience.

The MC is linked to the WHC and juiciness of meat, which is one of the most desirable attributes of this product (Cornet et al., 2021). Food with high springiness is more complicated to chew and break down in the mouth (Wee et al., 2018). Moreover, the chewiness depends on the moistness and hardness of the product (Kaleda et al., 2021). Bitterness and aftertaste are undesirable attributes in a meat analogue (Kaleda et al., 2021). A homogeneous structure of the final meat analogue products can be difficult to achieve if the TVP are too hard (De Angelis et al., 2020).

A good correlation between the attributes measured by the panel and instrumentally/analytically were observed. Detailed information can be found in Table 5 of the SUPM. The juiciness assessed by the panel, was negatively correlated with the hardness, gumminess, chewiness and resilience measured instrumentally. If the juiciness of the TVP is low, the use of water binding agents is needed in the final formulations of plant-based meat alternative (Sha and Xiong, 2020). Therefore, high levels of hardness, gumminess, chewiness, and resilience are not desirable in a meat analogue product. On the other hand, the graininess determined sensorially was positively correlated with these texture parameters referred above. The gumminess evaluated by the panel, was positively correlated with the gumminess, chewiness and resilience measured instrumentally. Moreover, the firmness was positively correlated with the gumminess, chewiness and resilience. These results gave important input for which suitable ranges of springiness, cohesiveness, gumminess, chewiness, and resilience can be targeted for sensory acceptability.

Overall, TVPs produced at a combination of moisture content of 28.5 %, a HZ6 of 150^{0}C and a FR of 5Kg/h or a moisture content of 30.8%, HZ6 of 160^{0}C and FR of 4Kg/h were the selected best processing parameters, due to the positive sensorial/textural and physicochemical attributes for this category of products.

4. Conclusion

A TVP based on a blend of a faba bean protein concentrate and an oat beta-glucan rich fraction was successfully produced by low-moisture extrusion. Moisture content and feed rate had the strongest impact on the physicochemical, textural and sensorial properties of the TVP. Lower moisture content (28.5–30.8%) together with HZ6 between 150 and 160^{0}C and FR between 4 and 5 Kg/h were the selected processing parameters that led to TVPs with improved physicochemical / techno-functional properties, such as, higher SEI, WHC, WSI, browning index and juiciness. At these conditions, the produced TVPs presented higher flavour and odour intensities, namely higher grain, umami and sweet tastes and the least bean odour among all samples tested. Texturally, it led to less hard, gummy, chewy and resilient TVPs with medium springiness and cohesiveness. A good correlation between the parameters measured sensorially and instrumentally/analytically were obtained. The TVPs produced are intended ingredients for the formulation of plant-based meat analogue and, further sensorial, and textural studies will be required in the target final product. The BG28 in the blend provided enough beta-glucan in the TVP for final products (e.g. vegan burgers) to bear a health claim related to reduction of LDL-cholesterol, which can be used if providing 1g oat beta-glucan per serving. The combination of FBPC with BG28 also improved the protein quality of the TVP by improving the amino acid composition. The calculated DIAAS score improved from 72.2 (BG28 alone) or 76.1 (FBPC alone) to 90.2 in the STARTMIX with methionine as the first limiting amino acid in FBPC and the STARTMIX and lysine as the first limiting amino acid in BG28. Overall, the blend of FBPC and BG28 constitute an interesting sustainable and healthy alternative to soy and gluten alternatives to produce TVP with desirable techno functional, nutritional, sensorial and textural attributes to develop a meat analogue product.

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Ethical statement regarding sensory evaluation

All research activities related to human subjects were regulated under the Research Ethics Act 2017, and carried out in agreement with guidelines from national and international advisory bodies, conventions and agreements (cf. National Committee for Research Ethics in Science and Technology (NENT), National Committee for Research Ethics in Social Sciences and the Humanities (NESH), the European Group on Ethics in Science and New Technologies (EGE)/European Commission, the International Committee of Medical Journal Editors, and ICMJE (the Vancouver Convention). Nofima complies with ethical principles and Applicable international, EU and national law (in particular, EU Directive 95/46/EC).

Declaration of Competing Interest

The authors declare that they have no conflict of interests.

Data availability

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

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2023.100228.

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