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Meat analogues from a faba bean concentrate can be generated by high moisture extrusion

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ABSTRACT

The main objective of this study was to investigate the feasibility of production of meat analogues from a faba bean protein concentrate (obtained from dry fractionation) using high-moisture extrusion (HME). The impact of the temperature, ratio between water and product feed rate and product feed rate on the physicochemical properties of the meat analogues during high-moisture extrusion (HME) was studied. The functional, textural, and sensory properties were also assessed. The impact of the moisture content was generally higher than the impact of the temperature and the feed rate within the responses evaluated. The different extrusion conditions tested did not influence the relative distribution of the oligosaccharides. Meat analogues produced with temperatures ranging between 130 and 140 °C, ratio between water and product feed rate of 4 and feed rate of 11 rpm (1.10 Kg/h) had the most positive sensory and textural properties judged by a good bite-feel (firmness) and elasticity in line with attributes associated with this product category. Of oligosaccharides analysed the content of verbascose was the highest α-galacto-oligossacharide and the content of the oligosaccharides is significant higher in the extracts of the heat-treated material compared to the starting material.

1. Introduction

Currently, agronomic, environmental and public health issues are promoting the reduction of animal protein consumption (Dekkers et al., 2018). Plant proteins constitute a more sustainable and healthier option and can supply us with an extensive diversity of sources, such as legumes (Asgar et al., 2010). Legumes belong to the family of Leguminosae that are subdivided in pulses (faba beans and peas) and oilseeds (soy), differing in the content of carbohydrates and lipids stored in the seeds (Neacsu et al., 2016). Pulses are low in fat and represent valuable sources of vitamins (i.e. B_{12}), minerals (i.e. iron, zinc, magnesium, calcium) and phytochemicals, positive for human health (Frohlich et al., 2014).

To replace meat, two main concepts exists: meat substitutes and meat analogues. Meat substitutes are plant material blended with meat, leading to reduced meat intake. Meat analogues are plant protein products or ingredients similar to meat in its functionality, being alike in appearance, when fried or cooked. There are several to produce meat analogues depending on the type of texture that one wants to achieve (Riaz, 2011). Extrusion technology is currently the most employed and established practical technique in the food industry (Assatory et al., 2019) to produce different food products (Philipp et al., 2017) starting from various types of protein enriched ingredients or mixtures. Moreover, it is possible to differentiate between two classes of meat analogues. Extrusion with a feed moisture content below 40% is named as low-moisture extrusion (LME), while extrusion performed with a feed moisture content above 40% is considered high-moisture extrusion (HME) (Akdogan, 1999). The HME enables to obtain a non-expanded fibrous products to mimic texture and mouthfeel of meat-products (Hood-Niefer, 2017). Meat analogues produced through HME from soybean protein are the most common but recently, issues related to deforestation (Heusala et al., 2020) and consumer awareness about potential harmful health effects (Sukalingam et al., 2015) play an important role in holding back the use of soybean protein. Hence the exploitation of alternative and more sustainable resources, such as pulses is seriously considered. However, there are few studies concerning the use of pulses (pea and lupin protein) for the production of meat analogues using HME (Osen et al., 2014; Palanisamy et al., 2019). Commercial pea protein isolates have been texturized by a HME process (moisture content of 55%) at a process temperature ranging between 120 °C and 160 °C and low screw rotation speed (SRS) (Osen et al., 2014). Similarly, a fibrous meat analogue from lupin proteins have been produced using a temperature

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Abbreviations

DOE	design of experiments
DSC	differential scanning calorimetry
FCD	face-centred design
FPF-FB	faba bean protein concentrate
FR	product feed rate
HME	high-moisture extrusion
HMMA	high-moisture meat analogue
HZ4	temperature in heating zone four of the extruder
LMW CHO	low-molecular weight carbohydrates
MA	meat analogue
MC_{MADOE}	moisture content of meat analogues
OBC	oil binding capacity
PCA	principal component analysis
RSM	response surface methodology
RVA	rapid visco analyser
SME	specific mechanical energy
SRS	screw rotation speed
T_{out}	surface temperature of the MADOE product measured at the outlet of the cooling die
T_{MADOE}	temperature of the MADOE product at the middle/central point of the meat analogue measured by an external probe
WBC	water binding capacity
WFR	water feed rate
WFR/FR	ratio between water and product feed rate

between 135 and 180 °C, a moisture content between 47 and 68% and a high SRS (Palanisamy et al., 2019).

A valuable, sustainable protein-rich and unexplored alternative raw material is faba bean (*Vicia faba L.*), that can offer diversity to the consumers. Faba beans are low in fat (1–2%), rich in fibre (7–9%), polyphenols and other non-nutrient compounds. Their basic composition of nutrients is considered beneficial for the health, avoiding chronical and degenerative diseases, including Parkinson's disease. Faba beans are a good source of lysine and its high fibre content may play an important role in the hypoglycaemic activity (Multari et al., 2015). Besides its nutritional values, faba bean has many environmental advantages, which make it a useful and versatile crop. It can fix atmospheric nitrogen and grow and develop at extreme climatic conditions (Chapagain and Riseman, 2015; Link et al., 2010; Multari et al., 2015). Only few studies exist concerning the impact of extrusion on the properties of whole faba bean. Masoero et al. (2016) studied the effect of extrusion on the nutritional value of pulses, including faba beans. It was concluded that extrusion increased starch digestibility of faba beans (Masoero et al., 2016). In another study conducted by Adamidou et al. (2011), the impact of extrusion preconditioning and drying temperatures on the chemical composition and antinutritional factors of faba beans was assessed (Adamidou et al., 2011). Results showed that the non-starch polysaccharides (NSP) values were slightly reduced by processing faba beans. The protein content of faba bean flours normally ranges from 20% to 41% dm (Cloutt et al., 1987). Using dry fractionation (milling and air classification) the protein content in faba bean concentrate increase 2 to 3 times to 55 and 65% dry matter (Cloutt et al., 1987; do Carmo et al., 2020).

The objective of the present work was to study meat analogues production through HME of a faba bean protein concentrate. The impact of selected extrusion process parameters on the technical performance of the ingredient, as well as the effect of extrusion on the functional, sensorial, and textural properties of the products were studied. To our knowledge, this is the first study concerning the production of a meat analogue constituted solely from a faba bean protein concentrate obtained through a dry-fractionation process.

2. Materials and methods

Materials

The protein concentrate from faba beans (FPF-FB) was obtained by dry milling and air-classification (Skjelfoss Korn AS, Hobøl, Norway). The composition of the fine protein fraction FPF-FB was 63.5% dry matter (dm) of protein (Dumas, $N \times 6.25$), 9.8% dm of non-starch polysaccharides - NSP (Englyst et al., 1994), 3.5% dm of starch (Megazyme, Wicklow, Ireland), 7% dm of low molecular weight oligosaccharides (see below), 3.6% dm of fat and 5.2% dm of ash. The fat and ash content were determined according to Ballance et al. (2018). The identified constituents add up to 93% of the dry matter content. The lacking material balance (7%) can be attributed to lignin (not determined in the NSP fibre analysis) and/or residual water not accounted for in some analysis. Analytical grade reagents and standards used for analysis were bought from regular suppliers.

High-moisture extrusion (HME) of FPF-FB to produce meat analogues – experimental design

The production of meat analogues based on a faba bean protein concentrate was explored via Response Surface Methodology (RSM). High-moisture extrusion was performed following a Face-Centred Design (FCD), varying three factors: raw material feed rate (FR), ratio between water feed rate (WFR) and FR (WFR/FR) and temperature in the last zone of the extruder – HZ4 (Table 1). The WFR/FR entering the extruder was selected in order to investigate different moisture levels. Higher values of WFR/FR correspond to higher moisture levels of the FPF-FB inside the extruder. The product feed rate (FR) was varied with the purpose to subject the mass inside the extruder to different residence times, as well as to obtain different degrees of filling in the extruder chamber at a constant screw rotation speed (SRS). A liquid pump (Watson-Marlow 530 Du, Falmouth, Cornwall, England) was used to feed water at a variable WFR. A vertical volumetric feeder type was used to feed the FPF-FB into the extruder at different FR. The range of process conditions to be studied were selected on pre-experiments (data not shown) and other studies performed with analogous products (Osen et al., 2014; Palanisamy et al., 2019). Seventeen experiments were performed, including three central points to check the reproducibility of the extrusion process. The FR varied from 9 to 13 rpm (corresponding approximately to 0.95 kg/h and 1.30 kg/h, respectively), WFR/FR varied from 3 to 5 and HZ4 varied from 120 °C to 140 °C (Table 1). The WFR was adjusted accordingly, which varied from 14.8 to 35.4 mL/min. The FPF-FB was processed using a laboratory, co-rotating twin-screw extruder (KETSE 20/40 Brabender GmbH and Co. KG, Duisburg, Germany) described previously by do Carmo et al. (2019). From the feeding zone to the die, the screw configuration (constant for all experiments) was constituted by 12 forward conveying elements (fce): 5×30 mm, 4×20 mm, 2×30 mm, 1×20 mm; 1 forward conveying kneading block (fckb) of 45° after water addition, 3×30 mm fce, 4×20 mm fce, 1×30 mm 45° fckb, 1×10 mm 45° fckb, 1×10 mm reverse conveying element (rce), 1×30 mm fce, 1×10 mm rce, 1×30 mm fce, 1×10 mm rce, 1×30 mm 45° fckb, 1×20 mm 45° reverse kneading block (rkb), 1×15 mm fce, 3×20 mm fce and 1×30 mm fce. The temperature profile was kept constant at 40/80/115 ^{0}C in heating zone 1, 2 and 3 of the extruder, while the last heating zone (HZ4) was established according to the design of experiments (DOE) (Table 1). The last zone of the extruder (melting zone) was varied because it has shown impact on the product texture properties of pea protein meat analogues (Osen et al., 2014) and based on another study that evaluated the impact of the last temperature zone on the product properties of lupin meat analogues (Palanisamy et al., 2019).

The SRS was kept constant at 900 rpm, established in preliminary trials (data not shown). At the end (die exit) of the extruder, a modular cooling die with 3 separated cooling-zones (Brabender GmbH & Co. KG,

Table 1
Face-Centred Design used for extrusion experiments (process conditions) and respective dependent process responses (measured variables).

Sample	Operating conditions HZ4 [°C]	FR [rpm]	WFR/FR	Measured variables SME [KJ/kg]	P [bar]	DL [%]	TP [kg/h]	T_{MADOE} [°C]	T_{out} [°C]	RT [s]
MADOE-1	120 (−1)	9 (−1)	3 (−1)	326.7	41.9	21.4	1.9 ± 0.1	20.61 ± 0.31	20.8	380.7
MADOE-2	140 (+1)	9 (−1)	3 (−1)	267.8	2.58	17.4	2.2 ± 0.2	29.41 ± 2.71	22.3	379.1
MADOE-3	120 (−1)	9 (−1)	5 (+1)	148.5	1.60	13.1	2.8 ± 0.1	26.31 ± 0.54	21.9	308.9
MADOE-4	140 (+1)	9 (−1)	5 (+1)	135.2	1.06	12.3	2.8 ± 0.1	27.12 ± 0.26	22.1	302.9
MADOE-5	120 (−1)	13 (+1)	3 (−1)	216.1	3.24	18.4	3.1 ± 0.2	28. 47 ± 1.41	22.1	265.6
MADOE-6	140 (+1)	13 (+1)	3 (−1)	216.3	3.45	20.0	3.3 ± 0.5	35.14 ± 4.08	25.5	270.4
MADOE-7	120 (−1)	13 (+1)	5 (+1)	115.5	1.05	13.2	3.6 ± 0.2	32.35 ± 1.26	23.6	205.5
MADOE-8	140 (+1)	13 (+1)	5 (+1)	96.30	1.41	12.6	4.8 ± 0.4	46.80 ± 5.47	26.4	216.5
MADOE-9	120 (−1)	11 (0)	4 (0)	174.8	2.35	15.4	2.9 ± 0.3	27.48 ± 1.22	21.9	274.7
MADOE-10	140 (+1)	11 (0)	4 (0)	145.3	1.42	13.6	3.0 ± 0.2	32.8 ± 3.94	23.3	279.0
MADOE-11	130 (0)	11 (0)	3 (−1)	235.1	2.55	17.4	2.9 ± 0.3	25.2 ± 2.25	21.4	319.4
MADOE-12	130 (0)	11 (0)	5 (+1)	118.3	1.59	13.1	3.5 ± 0.2	32.5 ± 2.07	23.6	251.9
MADOE-13	130 (0)	9 (−1)	3 (−1)	194.8	1.89	14.7	2.5 ± 0.2	24.79 ± 1.63	21.3	340.0
MADOE-14	130 (0)	13 (+1)	4 (0)	148.8	2.55	16.2	3.7 ± 0.1	34.67 ± 1.38	24.2	236.1
MADOE-15	130 (0)	11 (0)	4 (0)	172.0	2.19	15.3	2.7 ± 0.3	28.52 ± 3.62	22.4	276.1
MADOE-16	130 (0)	11 (0)	4 (0)	165.0	2.17	15.4	3.1 ± 0.3	28.97 ± 1.82	22.6	281.0
MADOE-17	130 (0)	11 (0)	4 (0)	162.8	2.24	15.3	3.1 ± 0.2	30.10 ± 2.23	22.6	284.8

MADOE-*X* = meat analogue produced at condition (X), HZ4 = temperature in zone 4 of the extruder, FR = feed rate of the FPF-FB, WFR/FR = ratio between water feed rate and FPF-FB feed rate, SME = specific mechanical energy, *P* = pressure, DL = drive load, TP = throughput, T_{MADOE} = temperature of the MADOE product at the middle/central point of the meat analogue measured by an external probe, T_{out} = surface temperature of the MADOE product measured at the outlet of the cooling die, RT = average retention time.

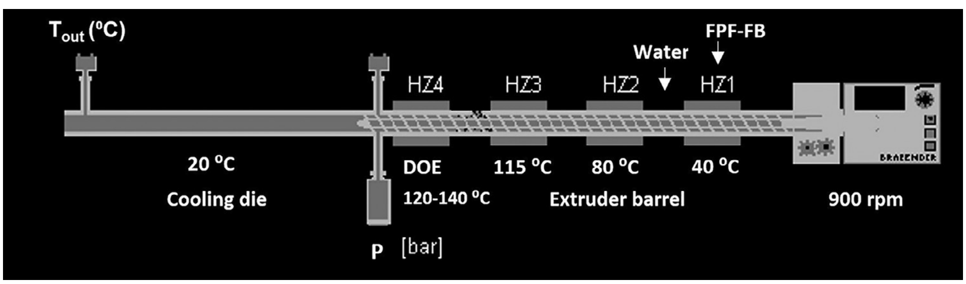

Fig. 1. Extruder set-up for the production of the meat analogue products (MADOE)
FPF-FB: Faba bean protein concentrate; DOE: Design of Experiments; P: Pressure; T_{out}: Surface temperature of the MADOE product measured at the outlet of the cooling die; HZ1: Heating zone 1; HZ2: Heating zone 2; HZ3: Heating zone 3; HZ4: Heating zone 4.

Duisburg, Germany) with dimensions of 30 × 5 × 300 mm (w x h x l) was connected. Thermostat-regulated circulating water at 20 °C was used as a cooling medium (Fig. 1). The extruded samples (meat analogues) were collected during the steady state of the process and packed in plastic containers. One part of the samples was frozen at −40 °C and another part was stored refrigerated at +4 °C until further analysis. Subsamples were also frozen and subjected to freeze drying as a basis for subsequent composition analysis.

2.3. Measured dependent process variables

The throughput/mass flow of each extruded sample was determined by weighting the material collected per minute. The drive load (DL) was recorded for all samples. These values were then utilized to calculate the specific mechanical energy (SME) for all process conditions. The SME was calculated as described by do Carmo et al. (2019). Moreover, the process pressure, the temperature of the MADOE product at the middle/central point of the meat analogue measured by an external probe (T_{MADOE}) and the surface temperature of the MADOE product measured at the outlet of the cooling die (T_{out}) were also recorded. The mean residence time (RT) represents the time that the protein concentrate mass spent inside the extruder. It was calculated as the ratio between the extruder's effective volume (m^3) and the volumetric flow rate of the proteinaceous mass (m^3/s) inside the extruder, considering the average density. The density was determined by measuring the mass of a known volume of the premix suspension (slurry) entering the inlet zone of the extruder. An assumption has been made that the elevated pressure and temperature in the barrel do not affect statistically significantly the melt density (Willett, 1996).

2.4. Heat-treatment of the starting material (FPF-FB) using the rapid visco analyser (RVA)

A heat-treated FPF-FB (referred as RVA sample) was produced under controlled conditions, using a rapid visco analyser (RVA-4, Newport Scientific, Australia), following the AACC Method 76–21 STD 2 (AACC, 2009). This sample was produced as a control in order to better evaluate the impact of extrusion on the chemical composition of the FPF-FB. Briefly, 4 g of raw material (FPF-FB) was mixed with 25 mL of water. The sample was stirred at 960 rpm and heated at 50 °C for 10 s. Then, it was stirred at 160 rpm at 50 °C for 60 s, heated to 95 °C for 60 s, held at 95 °C for 150 s with a stirring of 160 rpm and finally cooled down to 50 °C. The obtained heat-treated FPF-FB was freeze dried using a Christ Gamma 1–16 LSCplus freeze-drier (Martin Christ Gefriertrocknungsanlagen GmbH, Germany), further milled using a RETSCH ZM 100 mill (Retsch GmbH Haan, Germany) through a 0.5 mm screen and kept until further analysis.

Characterization of the meat analogues produced by HME

Thermal analysis by differential scanning calorimetry (DSC)

The FPF-FB, RVA and a representative freeze-dried meat analogue sample (MADOE-10) were analysed by DSC to evaluate their thermal properties according to Ai et al. (2016) using DSC (DSC123e, METTLER Toledo, Stockholm) with minor modifications. Indium was used as calibration and a sealed empty pan as a reference. Measurements were performed in medium pressure crucibles sealed hermetically (7 mm diameter, ME-26,929, METTLER) with pin. Briefly, 30 mg of samples (ground to 0.5 mm) and 60 μL of MQ water were added to the crucible. Then, the samples were heated from 18 °C to 120 °C (heating rate of 5 °C/min).

The onset temperature, the maximum peak, the outset temperature of the endothermic peaks and the enthalpy (J/kg), were given by the software STARe. The analysis was done in duplicates.

Colour properties

Colour measurements of the raw material and meat analogues were performed using the MINOLTA colorimeter (CR-400 Minolta Japan) and obtained with the software SpectraMagic NX. The L* (lightness), a* (green - red) and b* (blue - yellow) values were recorded. Calibration was done with illuminant D65. Six measurements were taken in different surface zones of each sample and the final L*, a*, b* values were expressed as their average. The browning index (BI) was determined following Wani & Kumar (2016).

Functional properties: cooking yield (CY), water binding capacity (WBC) and oil binding capacity (OBC)

Cooking yield (CY) of the extrudates was determined as the water uptake during cooking according to Palanisamy et al. (2018). Measurements were done in triplicates. WBC and OBC of the FPF-FB and the freeze-dried milled MADOE samples were determined according to the AACC method 56–20 (AAOC, 2000) and Tehrani et al. (2017), respectively. Measurements were done in triplicates.

Textural properties

The texture properties (elasticity and firmness) of the meat analogues were measured using a TA.XTplus texture analyser (Stable Micro Systems Ltd., Godalming, UK). The samples were thawed at 4 °C overnight and equilibrated at room temperature for at least 2 h before texture analysis. The measurements were acquired and treated by the Exponent Connect software (Texture Technologies Corp., Hamilton (MA), USA). The samples were subjected to cutting and compression tests according to Palanisamy et al., 2018, and to a tensile test according to Bast et al., 2015, with minor modifications. During the cutting test, 2 × 2 cm (*l* x *w*) square shaped samples were cut using a HDP/BSK blade set with knife, with a test speed of 2 mm/s. Samples were evaluated transversal (longitudinal strength, F_L) to the direction of the extrudate flow out from the extruder. For each sample and for each condition, measurements were taken in triplicates. The compression test was performed on 3 × 3 × 0.5 mm (*l* x *w* x *h*) strips using a P/35 probe with a pre-test speed of 1 mm/s, a test speed of 0.5 mm/s and a post-test speed of 0.5 mm/s. Moreover, samples were compressed to 50% of original thickness. Six measurements were performed for each sample. The elasticity was calculated based on the compression test results. For the tensile test, samples were cut in a dumbbell-shape and were pulled with a A/TG probe at a test speed of 1.50 mm/s until they break. Samples were tested at least in triplicates both transversally and longitudinally to the direction of the flow from the extruder (Bast et al., 2015). The anisotropic index (AI) was calculated by the ratio between the maximum peak forces given by the transversal and the longitudinal tensile measurements, according to Bast et al. (2015).

Sensory evaluation

For practical reasons, to reduce the number of samples being tasted by the trained panel, nine representative samples (MADOE-1, MADOE-2, MADOE-6, MADOE-8, MADOE-9, MADOE-10, MADOE-11, MADOE-14 and MADOE-15) of different product texture properties were assessed for the sensory descriptive analysis. The sensory laboratory in which the sensory analyses were conducted followed the ISO 8589 (ISO, 2010). The sample were analysed by a trained sensory panel using descriptive sensory profiling (DSP) according to Generic Descriptive Analysis (GDA) (Lawless and Heymann, 2010). The panel comprised nine selected and trained assessors/judges at Nofima - Norwegian Institute for Food, Fisheries and Aquaculture Research, Norway. The assessors were selected by their sensory abilities as described by ISO 8586:2012 (ISO, 2012) and were trained, controlled and tested regularly. The sensory panel was calibrated with a pre-test before the main test. For the test, red light was used in the lab for all samples to eliminate appearance differences. The samples were served steamed in a convection oven at 100 °C for 30 min without using any vacuum-bags, in pieces of 4 × 2.5 × 0.5 cm size. Each judge was served with one piece at a temperature of 20 °C ± 2 °C. During pre-trials test, they agreed upon a list of 22 attributes (Table 1 in SM), 6 in aromas, 10 in taste/flavour and 6 in texture. During analysis, 9 samples were evaluated in two replicates. A randomized order was used to serve samples and replicates. A 15 cm non-structured continuous line scale was used to assess the sensory attributes, from the lowest intensity (left side of the scale) to the highest intensity (right side of the scale). EyeQuestion (Logic8, Holland) was utilized for recording the data.

Moisture content (MC)

The MC of the raw material (FPF-FB) and the meat analogues (MADOE) was analysed by drying at 105 °C according to ACCCI Method 44–15.01 (AACCI,1995, StPaul. MN).

2.8. Analysis of low-molecular weight carbohydrates (LMW-CHO)

In short, freeze dried material were grinded in a Retch laboratory mill to a particle size < 0.5 mm and 50 mg material subjected to extraction by adding 9 mL 56% (v/v) ethanol and 1 mL melibiose (1 mg/mL) and left for shaking at 50 °C for 1 hr in a 10 mL centrifugation tube. Samples were then subjected to centrifugation (4000 rpm) and an aliquot was diluted 1:20 by water, filtrated through 0.22 μm and analysed by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) following a method adapted from Helgerud et al. (2016) used to analyse the LMW-CHO. Glucose (Glc), fructose (Fru), sucrose (Suc), raffinose (Raf), stachyose (Sta) and verbascose (Verb) were used to calculate individual response factors relative to melibiose and for identification of the chromatographic peaks. Moreover, in order to exclude the possible effect of the freeze-drying treatment on the oligosaccharide's composition, the dry faba bean protein fraction (FPF) was suspended in water, frozen and freeze dried (FPF-FD) and this sample was also analysed. The centre points of the DOE (MADOE 15–17) were used for the analysis of the LMW-CHO in order to work with a more representative sample of all samples of the DOE. Moreover, these process parameters (centre points) were amongst the best process conditions.

2.9. Statistical analysis

The results from the DOE were treated using Minitab® 18.1. Analysis of variance (ANOVA) and Fischer's least significance difference (LSD) test were performed using Minitab Ltd. The sensory data was analysed using ANOVA and Tukey tests after being recorded with EyeQuestion (Logic8, Holland).

3. Results and discussion

Modelling of high-moisture extrusion (HME) of FPF-FB to produce meat analogues

The production of meat analogues through HME of a FPF-FB followed a Face Centred Design (FCD) and the results obtained for the dependent process variables (SME - specific mechanical energy, DL - drive load, pressure, throughput, T_{out} - surface temperature of the MADOE product measured at the outlet of the cooling die, and T_{MADOE} - temperature of the MADOE product at the middle/central point of the meat analogue measured by an external probe are present in Table 1. MADOE-15,16 and 17 are the central points of the design of experiments (DOE), allowing to verify the repeatability of extrusion process, through the coefficient of variation (CVR). The SME of the central points were used to calculate the CVR of the process, which resulted to be 2.9%. The linear and quadratic effects of the independent process variables (HZ4, FR, WFR/FR) and their interactions on the responses evaluated were estimated (Table 2 in SM). The theoretical values predicted by the model and the experimental data showed a good agreement. Only for the lightness (L^*), low values of R^2 and R_{adj}^2 were identified (lack of fit). Furthermore, 84–99% of the responses evaluated were explained by the respective models (Table 3 in SM).

As observed in Fig. 2 and in the SM (Table 2), the WFR/FR (moisture content) had a major effect on the measured variables/properties of the MADOE's. Higher moisture content within the tested range corresponded to higher values of throughput, T_{out}, T_{MADOE}, MC_{MADOE} - moisture content of meat analogues, b* - yellowness, WBC – water binding capacity and OBC – oil binding capacity. On the other hand, a decrease on the drive load, SME, pressure, AI - anisotropic index, firmness and elasticity was observed with increasing moisture content.

The SME and drive load, SME and pressure and pressure and drive load had a high correlation between them (Fig. 6 in SM) as also previously identified by the authors in prior work (do Carmo et al., 2019).

The SME varied from 96.3 to 326.7 kJ/kg. During high moisture extrusion of other pulses, higher SME values were achieved using comparable process conditions (Ai et al., 2016; Osen et al., 2014; Palanisamy et al., 2019). An increase of water content (higher WFR/FR) and temperature (HZ4) which is translated in lower mass viscosity led to a decrease on the drive load and consequently on the SME (Fig. 2a). The drive load varied between 12.3 to 21.4% (Table 1). Lower drive load, results in lower SME to keep constant the screw rotation speed (SRS), as also reported from previous studies (Ai et al., 2016; Osen et al., 2014; Palanisamy et al., 2019). Pressure values varied from 10.5 to 41.9 bar (Table 1) and was significantly affected by the WFR/FR. An increase in the feed rate led to an increase in pressure when the temperature in zone 4 (HZ4) was high. Higher WFR/FR (higher water content) and lower viscosity promote the decrease in pressure, as also reported by Bock and Deiters (2017) and Palanisamy et al. (2019). The throughput varied from 1.9 to 4.8 kg/h amongst the conditions tested and increased with high moisture content or high feed rate. Also, when both the temperature in zone 4 and the feed rate increased, the throughput also increased. Moreover, the surface temperature of the MADOE product measured at the outlet of the cooling die (T_{out}) increased with increasing temperature, feed rate and moisture content, ranging from 20.8 to 26.4 °C. The temperature of the MADOE product at the middle/central point of the meat analogue measured by an external probe (T_{MADOE}) was increased with high feed rate (lower residence time). The lower residence time in the extruder and consequently in the cooling die, led to a higher temperature of the meat analogues exiting the cooling die, resulted in a less efficient cooling of the meat analogues. The T_{MADOE} ranged between 20.6 and 46.8 °C.

Moisture content, colour, and texture properties of the meat analogues

The moisture content of the meat analogues (MC_{MADOE}) varied from 46.0 to 65.5% and was changed while varying the WFR/FR of the process. The impact of the process conditions on the colour and texture properties of the meat analogues was evaluated and results are shown in Table 2. The meat analogues (MADOE) produced at higher moisture content (higher WFR/FR) led to lighter and yellowish products, while samples processed at higher temperature led to higher redness, especially at lower moisture content. An increase in redness can be explained by the Maillard reaction from the interaction between a carbonyl group from reducing sugars and amine groups from proteins when subjected to high temperatures (Damodaran, 1996). The browning index was significantly affected by the temperature in zone 4 especially when the moisture content was kept low. This can indicate that browning reactions were promoted by higher temperature and lower water content as also referred by Palanisamy et al. (2019).

Table 2
Physical properties of the meat analogues.

Sample	Operating conditions			Physical properties							
					Textural properties			Colour properties			
	HZ4 [°C]	FR [rpm]	WFR/FR	MC_{MADOE} [%]	E	Firmness [N]	AI	L*	a*	b*	BI
FPF-FB	n.a.	n.a.	n.a.	9.74 ± 0.94^{n}	n.a.	n.a.	n.a.	92.68 ± 0.63^{a}	−1.24 ± 0.04^{j}	11.66 ± 0.23^{hi}	12.1 ± 0.3^{i}
MADOE-1	120 (−1)	9 (−1)	3 (−1)	46.0 ± 0.12^{m}	0.365 ± 0.054^{abc}	30.26 ± 4.51^{cd}	2.88	44.88 ± 0.42^{g}	0.61 ± 0.05^{fg}	11.43 ± 0.21^{ij}	29.7 ± 0.5^{h}
MADOE-2	140 (+1)	9 (−1)	3 (−1)	47.8 ± 0.31^{l}	0.304 ± 0.110^{de}	31.45 ± 3.18^{c}	0.79	42.38 ± 0.88^{j}	2.44 ± 0.10^{a}	15.51 ± 0.68^{b}	48.9 ± 1.9^{a}
MADOE-3	120 (−1)	9 (−1)	5 (+1)	60.8 ± 0.13^{c}	0.180 ± 0.030^{g}	8.64 ± 0.23^{kl}	2.95	47.55 ± 0.32^{d}	0.57 ± 0.02^{fgh}	15.01 ± 0.19^{c}	37.8 ± 0.7^{de}
MADOE-4	140 (+1)	9 (−1)	5 (+1)	61.2 ± 0.10^{b}	0.211 ± 0.025^{fg}	11.53 ± 0.57^{jk}	1.81	45.54 ± 0.33^{fg}	1.04 ± 0.06^{d}	12.96 ± 0.11^{g}	34.4 ± 0.7^{g}
MADOE-5	120 (−1)	13 (+1)	3 (−1)	50.6 ± 0.24^{k}	0.388 ± 0.044^{ab}	32.15 ± 5.20^{c}	3.56	43.28 ± 0.44^{ij}	0.42 ± 0.01^{hi}	11.05 ± 0.42^{j}	29.5 ± 1.2^{h}
MADOE-6	140 (+1)	13 (+1)	3 (−1)	46.3 ± 0.11^{m}	0.407 ± 0.034^{a}	44.57 ± 2.97^{a}	1.46	44.72 ± 2.63^{gh}	2.20 ± 0.07^{b}	14.35 ± 2.88^{d}	41.2 ± 6.2^{bc}
MADOE-7	120 (−1)	13 (+1)	5 (+1)	65.5 ± 0.34^{a}	0.118 ± 0.041^{h}	6.97 ± 0.20^{l}	1.83	52.17 ± 0.28^{b}	1.29 ± 0.11^{c}	16.84 ± 0.18^{a}	39.9 ± 0.5^{cd}
MADOE-8	140 (+1)	13 (+1)	5 (+1)	61.1 ± 0.09^{bc}	0.336 ± 0.044^{bcd}	20.33 ± 2.25^{gh}	0.90	49.51 ± 0.48^{c}	1.37 ± 0.11^{c}	17.02 ± 0.33^{a}	43.2 ± 1.0^{b}
MADOE-9	120 (−1)	11 (0)	4 (0)	57.7 ± 0.22^{e}	0.334 ± 0.023^{bcd}	22.17 ± 2.76^{fg}	3.96	43.22 ± 0.49^{ij}	0.40 ± 0.03^{hi}	11.16 ± 0.10^{j}	29.7 ± 0.3^{h}
MADOE-10	140 (+1)	11 (0)	4 (0)	54.0 ± 0.19^{gh}	0.418 ± 0.032^{a}	26.97 ± 0.36^{de}	2.29	46.81 ± 1.16^{de}	0.79 ± 1.30^{e}	11.98 ± 1.69^{h}	30.2 ± 2.9^{h}
MADOE-11	130 (0)	11 (0)	3 (−1)	51.0 ± 0.31^{j}	0.409 ± 0.041^{a}	39.35 ± 2.56^{b}	3.19	45.92 ± 0.57^{ef}	0.46 ± 0.04^{ghi}	13.04 ± 0.30^{g}	33.4 ± 1.0^{g}
MADOE-12	130 (0)	11 (0)	5 (+1)	58.5 ± 0.21^{d}	0.256 ± 0.016^{ef}	14.11 ± 0.32^{ij}	2.31	46.78 ± 0.32^{de}	0.69 ± 0.02^{ef}	14.39 ± 0.18^{d}	36.8 ± 0.9^{ef}
MADOE-13	130 (0)	9 (−1)	4 (0)	54.8 ± 0.09^{f}	0.280 ± 0.022^{de}	14.54 ± 2.23^{ij}	2.74	46.01 ± 0.30^{ef}	0.65 ± 0.06^{f}	13.53 ± 0.23^{ef}	35.1 ± 0.8^{fg}
MADOE-14	130 (0)	13 (+1)	4 (0)	52.8 ± 0.28^{i}	0.315 ± 0.034^{cd}	26.20 ± 1.26^{def}	3.59	45.50 ± 0.99^{fg}	0.49 ± 0.04^{ghi}	13.47 ± 0.20^{ef}	35.2 ± 1.7^{fg}
MADOE-15 (C)	130 (0)	11 (0)	4 (0)	53.6 ± 0.14^{gh}	0.306 ± 0.033^{de}	23.23 ± 0.77^{efg}	3.78	43.75 ± 0.78^{hi}	0.34 ± 0.03^{i}	11.08 ± 0.23^{j}	29.1 ± 0.9^{h}
MADOE-16 (C)	130 (0)	11 (0)	4 (0)	54.9 ± 0.27^{fg}	0.295 ± 0.043^{de}	17.90 ± 2.99^{hi}	3.97	45.94 ± 0.56^{ef}	0.47 ± 0.02^{ghi}	13.32 ± 0.15^{fg}	34.1 ± 0.8^{g}
MADOE-17 (C)	130 (0)	11 (0)	4 (0)	54.2 ± 0.11^{g}	0.317 ± 0.039^{cd}	26.63 ± 2.70^{de}	3.38	46.11 ± 0.46^{ef}	0.55 ± 0.05^{fgh}	13.75 ± 0.21^{e}	35.5 ± 1.0^{fg}
Mean ± SD (C)	130 (0)	11 (0)	4 (0)	54.2 ± 0.65	0.306 ± 0.011	22.59 ± 4.40	3.71 ± 0.30	45.27 ± 1.32	0.45 ± 0.11	12.72 ± 1.43	32.90 ± 3.4

MADOE-X = meat analogues produced at different conditions (X), HZ4 = temperature in zone 4, FR = feed rate of the FPF-FB, WFR/FR = ratio between water feed rate and FPF-FB feed rate, FPF-FB= fine protein fraction from faba beans, MC_{MADOE} = moisture content of the final product, E = elasticity, Firmness = max force of longitudinal cutting, AI = anisotropic index, L* = lightness, a* = redness, b* = yellowness, BI = browning index, SD = standard deviation, (C) = Central points.
n.a.: not applicable. Means with different letters are significantly different ($p<0.05$).

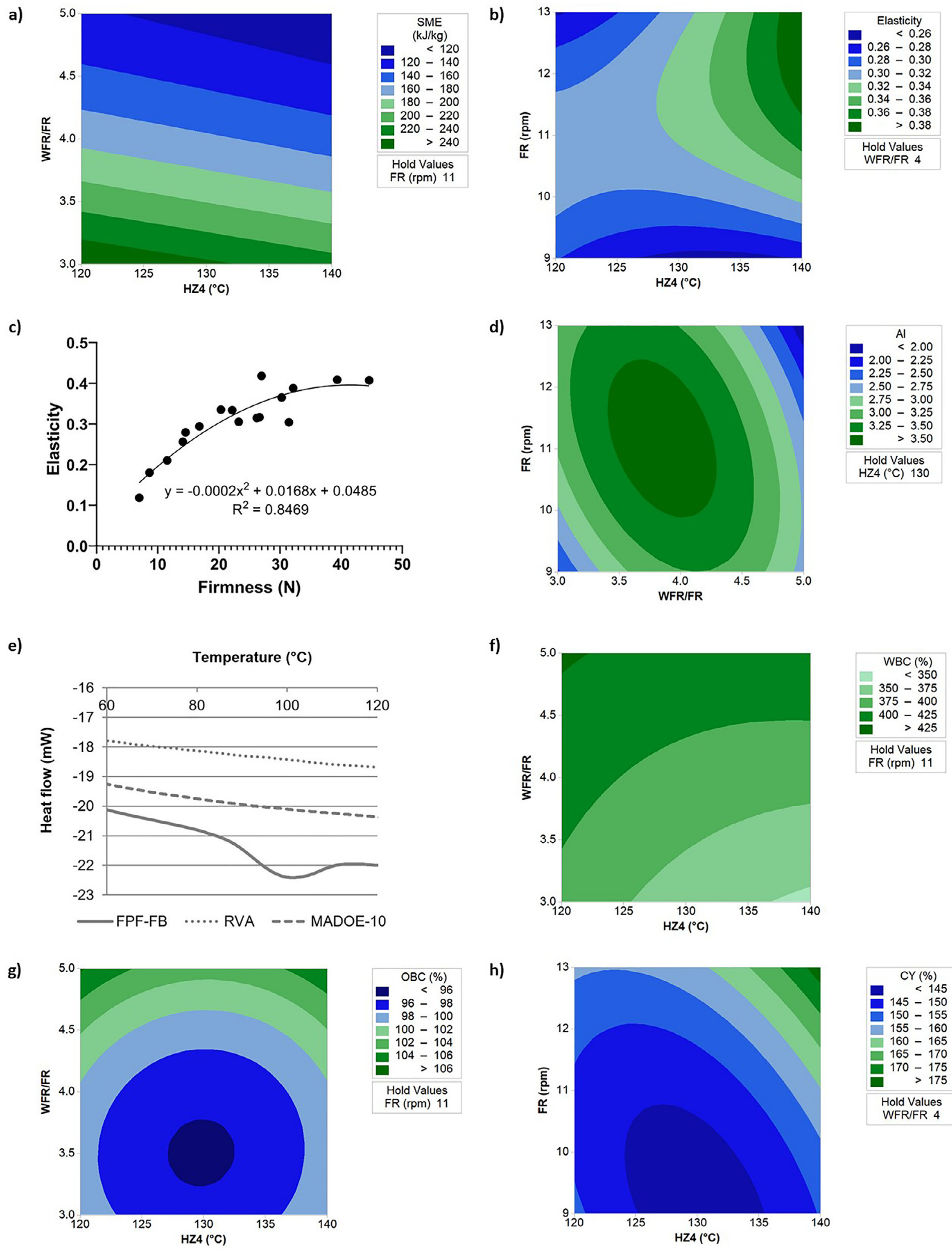

Fig. 2. (a) Response contour plot (CP) fitted to the SME as a function of temperature in zone 4 (HZ4) and ratio between water feed rate and FPF-FB feed rate (WFR/FR); (b) CP fitted to the Elasticity as a function of HZ4 and feed rate (FR); (c) Polynomial correlation between Elasticity and Firmness of the meat analogues; (d) CP fitted to the AI as a function of WFR/FR and FR; (e) DSC heating scan of FPF-FB, RVA sample and an extruded sample (MADOE-10); (f) CP fitted to the WBC as a function of HZ4 and WFR/FR; (g) CP fitted to the OBC as a function of HZ4 and WFR/FR; (h) CP fitted to the CY as a function of HZ4 and FR.

Texture is one of the most important characteristics for the consumer acceptance meat analogue products (Palanisamy et al., 2018). The main challenge when exploiting high-moisture extrusion to produce meat analogues is to successfully resemble whole-muscle fibrous structure found in meat. This, indeed, cannot be achieved with low moisture extrusion process (Riaz, 2011). In the present study, cutting, compression and tensile tests were performed to measure textural properties of the products, namely firmness, elasticity, and anisotropy. The maximum force (cutting test) needed to move a knife to cut the product was measured to determine its firmness (Dekkers et al., 2018), and according to Palanisamy et al. (2018) it could represent an indirect measurement of texturization. Also, cutting force closely resemble the maximum force required during the biting action or cutting action by a knife during consumption. These are some of the first qualities considered by consumers (Smewing, 2016). Elasticity was determined upon compression test and was useful to define the extent of deformation of the extrudates related to the structural organization of the product, as considered by Palanisamy et al. (2018). The results are presented in Table 2 and showed that all process parameters significantly affected firmness and elasticity but only the temperature in zone 4 significantly affected the anisotropic index (Table 2 in SM). The water content (WFR/FR) appeared to affect texture the most. At high level of WFR/FR (higher MC) all the values of firmness and elasticity decreased amongst the samples, as also observed by Lin et al. (2000). In accordance with this study, water plays a major role on texture properties, rather than the types of protein bonds formed during extrusion. High water content was responsible for loose and soft products, because of inefficient texturization. Moreover, Palanisamy et al. (2018) concluded that higher values of firmness and elasticity can be related to the formation of a stronger and denser network. As referred by these authors, higher elasticity was obtained which was translated in more compact structures, with numerous and small open spaces between fibres. Such a structural model appeared to be less likely to be deformed irreversibly during compression. Accordingly, in the present study a correlation between elasticity and firmness was observed (Fig. 2c): at higher elasticity, firmness increased. At higher temperature and higher feed rate (lower residence time), elasticity and firmness also increased. Chen et al. (2010a), working with extrusion of soy protein isolates at a wide moisture range (28–60%), observed that lower residence time contributes to an increase in product firmness, as well as chewiness and tensile strength (Chen et al., 2010b). Anisotropy can be described as the presence of preferred orientation of the dimensional features in the structure (Hilliard, 1967). A good correlation (R^2=0.95) was found between the anisotropic index and input process parameters, namely temperature in zone 4 (HZ4), feed rate (FR) and ratio between water feed rate and feed rate (WFR/FR), indicating that different process conditions resulted in different anisotropic structuring amongst the samples. The anisotropic index was significantly decreased at high temperature as well as at high moisture content and high feed rate (short residence time) (Fig. 2d). Moreover, a correlation between the anisotropic index and the SME was found (Fig. 7 in SM). At lower and higher levels of energy input, mainly caused by differences in mass viscosity, the product anisotropy decreased, being higher for intermediate SME values. For instance, sample MADOE-6 showed the lowest anisotropic index and the highest values of firmness and SME (Table 2). This correlation suggests that a strong network was formed, but with lack of anisotropy (lengthwise orientated fibers). Similar results were obtained by Osen et al. (2014) with pea proteins. They observed that longitudinal cutting force (firmness) increased significantly with temperature and multi-layered structure started to form above 120 °C. However, when reaching temperatures close to 160 °C, even if the hardness was maximum, the product showed a more homogeneous structure with no parabolic pattern but rather predominantly lengthwise orientated fibers. On the other hand, sample MADOE-8 required the lowest level of energy input (SME) and showed low anisotropic index too. The lack of strong anisotropic network in this case could be mainly attributed to the high-water content and feed rate. The resulting mass became particularly fluid and spent very little time in the extruder, leading to a non-efficient protein denaturation, as previously described (Palanisamy et al., 2019).

Water-binding capacity (WBC), oil-binding capacity (OBC) and cooking yield (CY)

Since the water-binding capacity (WBC) varies according to the number of polar sites interacting with water (Osen et al., 2014) it is a property that is influenced by the primary structure, conformation state, hydrophilicity of a food protein and the presence of carbohydrates (Tehrani et al., 2017). Upon protein denaturation during extrusion processing (high temperature, high pressure conditions), the hydrophilic groups can be more exposed in the surface of the molecule, leading to increased WBC (Osen et al., 2014). Indeed, extrusion process improved the water binding capacity (WBC) of all MADOE, as shown in Fig. 2f/Table 4 in SM. In comparison with the non-extruded faba bean protein concentrate (FPF-FB), the extruded products exhibited significantly higher WBC: from 82.5% in the FPF-FB up to 444.2% in the meat analogues (MADOE). In fact, protein denaturation during high-moisture extrusion was confirmed by DSC. The DSC graph for the FPF-FB, RVA and MADOE-10 samples are shown in Fig. 2e. One endothermic peak (denaturation temperature) was observed at 100.5 °C for the FPF-FB. For the processed samples (RVA and MADOE-10), no peaks were detected, meaning that proteins were fully denatured during the process. Similar results were observed by Ai et al. (2016), working with low-moisture extrusion of common bean protein powders (Ai et al., 2016).

Temperature (HZ4) and moisture content (WFR/FR) significantly affected the water binding capacity (WBC) of the meat analogues (Table 2 in SM). WBC increased with higher moisture content but decreased with increasing temperature. Such a behaviour disagrees with other results in the literature (Chen et al, 2010). Tehrani et al. (2017) linked higher temperature and lower moisture content with higher degree of denaturation (Tehrani et al., 2017). In their study, the resulting higher viscosity and friction in the barrel, led to more severe shear stress and thus higher protein denaturation. These differences in process effect on WBC could be caused by differences in protein composition in the raw materials (pea, lupin, and soya). Differences in protein composition could promote unique denaturation and aggregation patterns, resulting in diverse water binding capacity in the final product.

The oil binding capacity (OBC) depends on protein surface hydrophobic groups. It can be modified during extrusion process due to protein denaturation (Tehrani et al., 2017). Normally, the native proteins exhibit lower OBC than the denatured forms, because of structural folding (Osen et al., 2014). In the present study this was not verified. Extrusion process had a significant effect in reducing the OBC of FPF-FB (from 116.1% to 93.1%). Similar results were obtained by Ai et al. (2016) and Zabalza et al., (2000) for common bean and kidney bean proteins, working with low-moisture extrusion (LME) (Ai et al., 2016; Alonso et al., 2000). According to their studies, a decrease in surface hydrophobicity is due to the occurrence of denaturation and aggregation together. In aggregation there are interaction between hydrophobic groups which lead to an overall decrease in surface hydrophobicity lowering the OBC of the extrudates. In the present study, OBC values of the meat analogues ranged from 93.1 to 105.5% (Table 4 in SM). As shown in Fig. 2g and in the Table 2 in SM, the moisture content (WFR/FR), as well as the interaction between the temperature in zone 4 (HZ4) and FR, had significant effect on the OBC. OBC was higher with higher water content and decreased when the temperature and the feed rate increased together.

Cooking yield (CY) has been correlated with the water uptake during cooking, and differences can be explained by both chemical and physical modification of the proteinaceous matrix during extrusion (Palanisamy et al., 2018). CY values varied from 145.13 to 191.44% and was significantly affected by the temperature and feed rate. When the temperature and feed rate raised, independently, CY increased too.

Table 3
Low-molecular weight carbohydrates (LMW-CHO) contents of FPF-FD (non-extruded), RVA (heat-treated at atmospheric conditions) and MADOE (extruded meat analogues).

	LMW-CHO composition LMW carbohydrates (mg/100 g dm)					
Sample	Glucose	Fructose	Sucrose	Raffinose	Stachyose	Verbascose
FPF-FD	50.2 ± 1.9^a	0.3 ± 0.5^a	768.0 ± 44.6^a	150.7 ± 0.3^a	1257.8 ± 48.9^a	4673.9 ± 113.7^a
RVA	39.7 ± 0.8^b	2.0 ± 0.4^b	1375.9 ± 7.6^b	195.7 ± 3.0^b	1742.2 ± 7.0^b	5079.7 ± 18.8^b
MADOE (C)	8.7 ± 1.0^c	6.9 ± 0.3^c	1308.0 ± 11.4^c	220.6 ± 6.1^c	1618.6 ± 20.6^c	4952.1 ± 60.2^c

FPF-FD= Freeze-dried fine protein fraction of faba bean, RVA = sample from the Rapid Viscosity Analysis, LMW-CHO = low molecular weight carbohydrates content.
The standard error deviations were done in triplicates.

Similar results were obtained by Palanisamy et al. (2019) with lupin proteins. When the two parameters varied together, the positive effect of the feed rate increase on the CY was observed only at higher temperature level. Also, when product feed rate and the moisture content increased together, CY increased too (Table 2 in SM). An increase in the CY can be correlated with a weaker protein network (lower firmness) which allows more water uptake (Palanisamy et al., 2018). The moisture content had a significant negative effect on the cooking yield only at low feed rate, meaning that at high residence time, an increase in water leads to a reduction in the cooking yield. In the last case, probably a more compact protein network (higher firmness) was created and thus a reduction of free space for water penetration (Palanisamy et al., 2018).

Sensory perception and correlation with variables measured instrumentally

The impact of extrusion on two instrumental textural properties was evaluated, namely the firmness and elasticity. These properties were in turn correlated with the sensory perception evaluated by the panel. Thirteen of the twenty-two attributes evaluated were significantly different within the samples tested (Table 5 in SM). Fig. 3 shows the sensory space characterized by the nine samples taken into consideration the attributes evaluated. The attributes measured instrumentally were overimposed in the map as supplementary variables. Mostly the textural and the flavour characteristics separated the samples in the perceptual space. Samples extruded at high moisture content: MADOE-9, MADOE-14 and MADOE-8 were associated to the attributes raw grain flavour, raw grain odour and juiciness. On the other side, samples extruded at low moisture content: MADOE-2, MADOE-11 and MADOE-6 were sensory described as gummy, firm, granular and fibrous in texture, and with bean flavour. The firmness and elasticity measured instrumentally were linked to this region of the map (left side). Moreover, the MADOE extruded at lower temperature and lower moisture content (MADOE-1) was associated with fracturability in texture and sulphur odour. Samples processed at higher temperature and higher moisture content (MADOE-10 and MADOE-15) sit middle-way in the perceptual space, associated with and moderate firmness, juiciness, granularity, gumminess and fracturability. More information on the correlation between sensory and instrumental parameters can be found in Table 6 of the SM. The firmness measured sensorially and instrumentally were very well correlated. Regarding other chemical evaluations (cooking yield, WBC and OBC), a statistically significant correlation between the WBC and the sensory parameters (firmness, juiciness, fibrousness and gumminess) was found. Regarding the cooking yield and OBC, no correlations were found (Table 6 of the SM). Generally, samples obtained at extreme conditions of moisture content or temperature were not considered good for consumption (both sensorially and texturally), according to their sensory descriptions. On the contrary, samples extruded at a combination of medium-high temperature (130–140 °C) and moderate moisture content (WFR/FR of 4) at a fixed feed rate of 11 rpm (1.10 Kg/h) had better profiles, both sensorially and texturally. Meat quality is normally characterized by attributes such as firmness, tenderness, and juiciness, as well as typical colour and flavour (FAO, 2014). Amongst all the significant attributes considered in this study, fibrousness, firmness, gumminess and juiciness seem to be the most important for the product characterization. Meat, and therefore meat analogues, should appear tender and moderately firm, rather than soft or too hard. Soft meat products are perceived negatively, whereas excessively firm products could require a higher biting force, resulting harder to chew by the consumer and gummy. Another main factor considered when consuming meat analogues is the mouthfeel and fibrous texture similar to meat (Palanisamy et al., 2018). Without the fibrous structure, the product could be perceived as hard and dry or mushy and wet (Smewing, 2016). Overall, high elasticity combined with fibrousness, moderate firmness and juiciness are the final product conditions which should be aimed for. Therefore, the instrumental and sensory profiles help in highlighting the best processing parameters, that were achieved at a HZ4 between 130 and 140 °C, WFR/FR of 4 and a FR of 11 rpm (1.10 Kg/h).

LMW-CHO composition of the meat analogues

The effect of extrusion on the LMW-CHO content of the meat analogues was analysed and results are presented in Table 3. The contents of LMW carbohydrate in the ethanol extracts of the starting materials and the heat-treated samples are visualized in the chromatogram (Fig. 4). The reduction of glucose is evident upon heat treatment, attributed to glucose taking part in Maillard reactions upon heating (Björck and Asp, 1983; Singh et al., 2007). Traces of fructose are detected (< 7 mg/100 g) but no attempts were done for an accurate quantification.

Calculated on the basis of dry weight the samples are dominated by the higher raffinose series of oligosaccharides in addition to sucrose. The extracts of the FPF-FD starting material was mainly composed of verbascose (4.7% dm), stachyose (1.3% dm) and sucrose (0.8% dm). Raffinose (0.15% dm) was the lowest abundant oligosaccharide present in the FPF-FD. Other studies of whole faba bean or derived concentrates reported the same trend. Verbascose was the highest α-galacto-oligossacharide followed by stachyose and raffinose (Bhatty and Christison, 1984). It is evident that the content of the oligosaccharides is significant higher in the extracts of the heat-treated material (RVA and MADOE) compared to the starting material. There is also a small, but significant difference between the oligosaccharides quantified in RVA extracts as compared with extruded materials (MADOE). We however suspect this difference to be an analytical artefact, e.g. that the extractability introduced by the heat treatment, rather than the total content of the oligosaccharides, were altered. The observation that the characteristic non-digestible oligosaccharides did not change in relative amounts during extrusion (MADOE) and RVA processing (Fig. 8 in SM) support this. Our selected conditions for analysis were based on a quantitative extraction study of plant materials (Johansen et al., 1996) and our previous experiences with potato (Helgerud et al., 2016) and other plant materials. Quite some different extraction procedures for similar plant materials are found in the literature, with a large span the ratio between volume of the extraction liquid and the sample added, the actual extraction solvent, the temperature, the time, the use or lack of internal standards and the chromatographic system. An increase of detected sugars after extru-

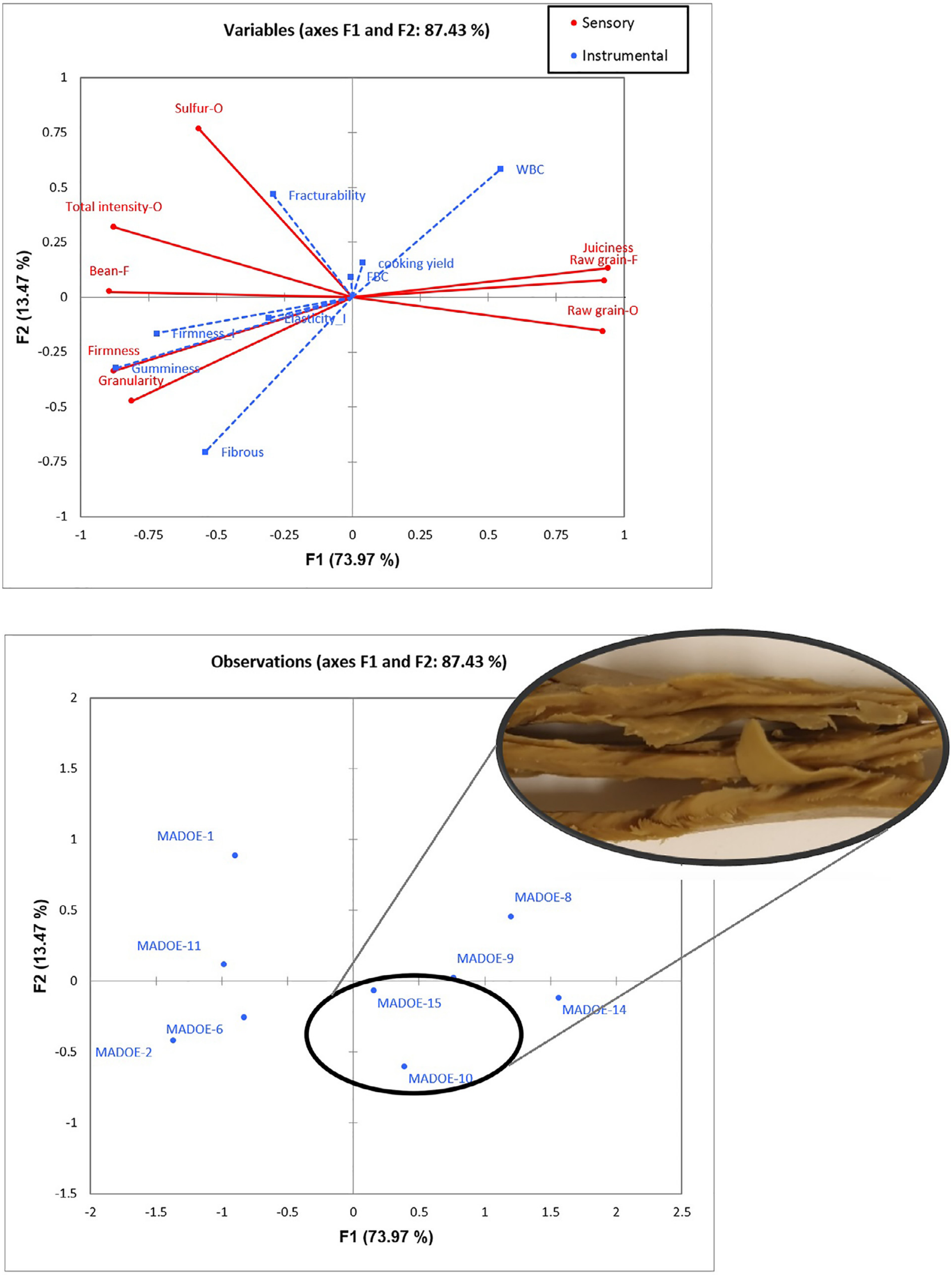

Fig. 3. Correlation of sensory variables (in red) and instrumental texture (in blue) data via Multiple factor analysis (MFA): (a) sample plot, (b) variable plot. Instrumental data are overimposed as supplementary variables on the map. F1 & F2 are the first and second PC in the weighted final PCA.

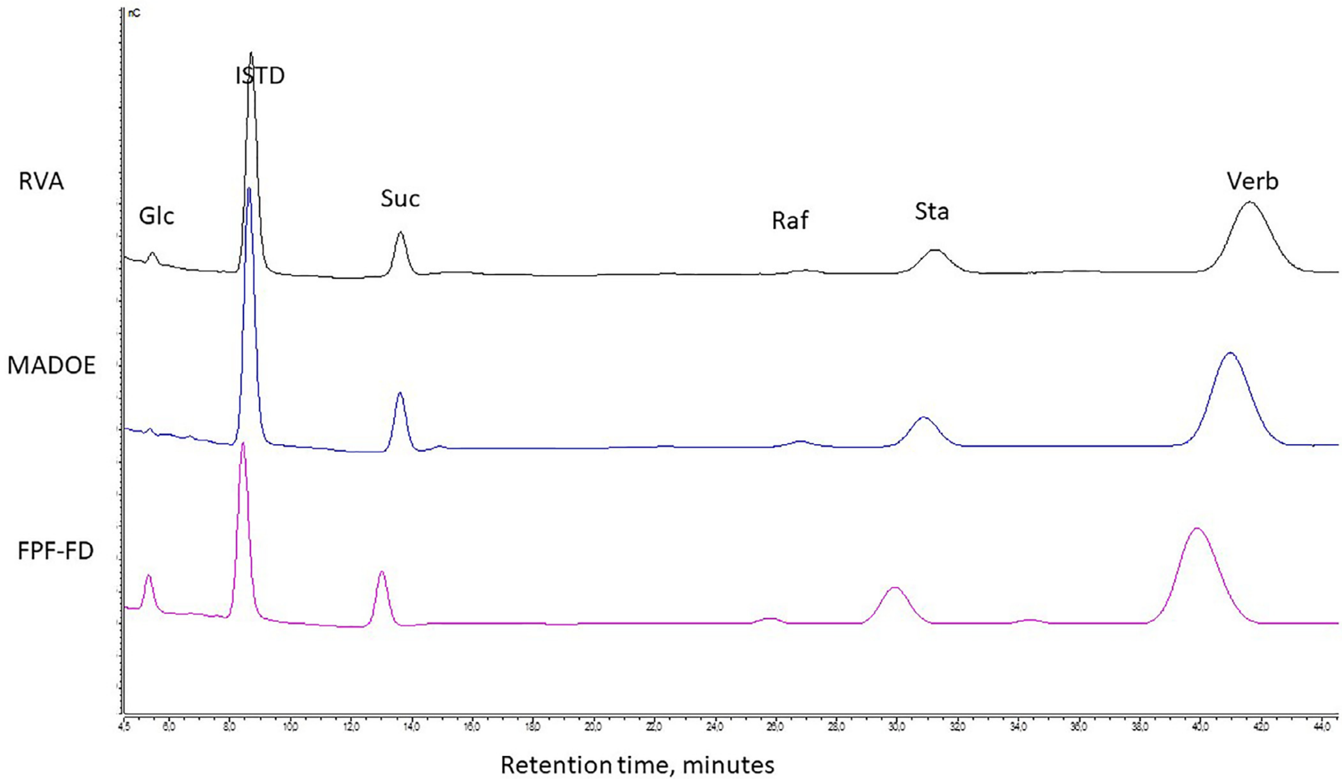

Fig. 4. The distribution of the sugars in the starting material (FPF-FB) and in a representative sample from the extrusion (MADOE). The arrow indicates the occurrence of a small amount of fructose, whereas Glc, Suc, Raf, Sta and Verb indicates the peaks of Glucose, Sucrose, Raffinose, Stachyose and Verbascose, respectively. Melibiose was used as an internal standard (ISTD).

sion of similar materials have been reported previously (Arribas et al., 2019; Morales et al., 2015), suggesting release of oligosaccharides from other macromolecules or simply a modification of the food matrix. On the opposite, a decrease in soluble sugars upon extrusion has also been reported (Berrios et al., 2002). We suggest that apart from a reduction of reducing sugars due Maillard reactions, an observed small increase or decrease in oligosaccharides solely upon extrusion could be an issue introduced by the sample preparation in the analytical procedure.

Conclusion

This study shown that it is possible to produce meat analogues solely from a protein concentrate of faba beans produced by dry fractionation that is suited for a meat-like product. Optimum parameters with temperature (HZ4) ranging between 130 and 140 °C, WFR/FR ratio of 4 and feed rate at 11 rpm (1.10 Kg/h) enables products with a fibrous structure. At these process parameters, the meat analogues had good bite-feeling, good elasticity/firmness, and positive sensory attributes in line with attributes present in this product category. Moreover, the SME varied between 145.3 and 172 kJ/kg. Amongst the responses evaluated, the impact of the moisture content was greater than the impact of the temperature and the feed rate. Higher protein denaturation translated by the higher water binding capacity (WBC) was promoted at high extrusion moisture content. Moreover, a correlation between the WBC and the firmness (measured instrumentally and sensorially), juiciness, fibrousness and gumminess was found. The varying browning index of the meat analogues enabled to conclude that browning reactions occurred during extrusion and were promoted by higher temperatures (HZ4) and low moisture contents (low WFR/FR) and related a low quantity of available reducing sugars, but not involving the more abundant oligosaccharides.

Declaration of Competing Interest

The authors declare that they have no conflict of interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2021.100014.

References

AACC, 2009. AACC Method 76-21.02. General Pasting Method for Wheat or Rye Flour or Starch Using the Rapid Visco Analyser, 11th ed. AACC.

AAOC, 2000. Method 56-20. Hydration Capacity of Pregelatinized Cereal Product. AAOC Chemists AAoC (Ed.), Approved Methods of the AACC (10th).

Adamidou, S., Nengas, I., Grigorakis, K., Nikolopoulou, D., Jauncey, K., 2011. Chemical composition and antinutritional factors of field peas (Pisum sativum), chickpeas (Cicer arietinum), and Faba Beans (Vicia faba) as affected by extrusion preconditioning and drying temperatures. Cereal Chem. 88 (1), 80–86. doi:10.1094/CCHEM-05-10-0077.

Ai, Y., Cichy, K.A., Harte, J.B., Kelly, J.D., Ng, P.K.W., 2016. Effects of extrusion cooking on the chemical composition and functional properties of dry common bean powders. Food Chem. 211, 538–545. doi:10.1016/j.foodchem.2016.05.095.

Akdogan, H., 1999. High moisture food extrusion. Int J Food Sci. Technol. 34 (3), 195–207. doi:10.1046/j.1365-2621.1999.00256.x.

Alonso, R., Orúe, E., Zabalza, M.J., Grant, G., Marzo, F., 2000. Effect of extrusion cooking on structure and functional properties of pea and kidney bean proteins. J. Sci. Food Agric. 80 (3), 397–403 10.1002/1097-0010(200002)80:3<397::AID–JSFA542>3.0.CO;2-3.

Arribas, C., Cabellos, B., Cuadrado, C., Guillamón, E., Pedrosa, M.M., 2018. The effect of extrusion on the bioactive compounds and antioxidant capacity of novel gluten-free expanded products based on carob fruit, pea and rice blends. Innovat. Food Sci. Emerg. Technol. 52, 100–107. doi:10.1016/j.ifset.2018.12.003.

Asgar, M.A., Fazilah, A., Huda, N., Bhat, R., Karim, A.A., 2010. Nonmeat protein alternatives as meat extenders and meat analogs. Comprehens. Rev. Food Sci. Food Saf. 9 (5), 513–529. doi:10.1111/j.1541-4337.2010.00124.x.

Assatory, A., Vitelli, M., Rajabzadeh, A.R., Legge, R.L., 2019. Dry fractionation methods for plant protein, starch and fiber enrichment: a review. Trends Food Sci. Technol. 86, 340–351. doi:10.1016/j.tifs.2019.02.006.

Ballance, S., Knutsen, S.H., Fosvold, Ø.W., Wickham, M., Trenado, C.D.-T., Monro, J., 2018. Glyceamic and insulinaemic response to mashed potato alone, or with broccoli, broccoli fibre or cellulose in healthy adults. Eur. J. Nutr. 57 (1), 199–207.

Bast, R., Sharma, P., Easton, H.K.B., Dessev, T.T., Lad, M., Munro, P.A, 2015. Tensile testing to quantitate the anisotropy and strain hardening of mozzarella cheese. Int. Dairy J. 44, 6–14. doi:10.1016/j.idairyj.2014.12.006.

Berrios, J.D.J., Camára, M., Torija, M.E., Alonso, M, 2002. Effect of extrusion cooking and sodium bicarbonate addition on the carbohydrate composition of black bean flours. J. Food Process. Preserv. 26 (2), 113–128. doi:10.1111/j.1745-4549.2002.tb00856.x.

Bhatty, R.S., Christison, G.I., 1984. Composition and nutritional quality of pea (Pisum sativum L.), faba bean (Vicia faba L. spp. minor) and lentil (Lens culinaris Medik.) meals, protein concentrates and isolates. Qualitas Plantarum Plant Foods Hum. Nutr. 34 (1), 41–51. doi:10.1007/BF01095071.

Björck, I., Asp, N.G., 1983. The effects of extrusion cooking on nutritional value - a literature review. J. Food Eng. 2 (4), 281–308. doi:10.1016/0260-8774(83)90016-X.

Bock, J.E., Deiters, P., 2017. Laboratory-scale extrusion: open the black box of extrusion processing for improved product development. Cereal Foods World 62 (4), 151–155. doi:10.1094/CFW-62-4-0151.

Chapagain, T., Riseman, A., 2015. Nitrogen and carbon transformations, water use efficiency and ecosystem productivity in monocultures and wheat-bean intercropping systems. Nutrient Cycling in Agroecosystems 101 (1), 107–121. doi:10.1007/s10705-014-9647-4.

Chen, F.L., Wei, Y.M., Zhang, B., 2010a. Characterization of water state and distribution in textured soybean protein using DSC and NMR. J. Food Eng. 100 (3), 522–526. doi:10.1016/j.jfoodeng.2010.04.040.

Chen, F.L., Wei, Y.M., Zhang, B., Ojokoh, A.O., 2010b. System parameters and product properties response of soybean protein extruded at wide moisture range. J. Food Eng. 96 (2), 208–213. doi:10.1016/j.jfoodeng.2009.07.014.

Cloutt, P., Walker, A.F., Pike, D.J., 1987. Air classification of flours of three legume species: fractionation of protein. J. Sci. Food Agric. 38 (2), 177–186. doi:10.1002/jsfa.2740380209.

Damodaran, S., 1996. Amino acids, peptides, and proteins. In: Food Chemistry, pp. 412–413.

Dekkers, B.L., Boom, R.M., Goot, A.J.Van Der, 2018a. Structuring processes for meat analogues. Trends Food Sci. Technol. 81 (August), 25–36. doi:10.1016/j.tifs.2018.08.011.

Dekkers, B.L., Hamoen, R., Boom, R.M., Jan, A., Goot, V.Der, 2018b. Understanding fiber formation in a concentrated soy protein isolate - Pectin blend. J. Food Eng. 222, 84–92. doi:10.1016/j.jfoodeng.2017.11.014.

do Carmo, C.S., Silventoinen, P., Nordgård, C.T., Poudroux, C., Dessev, T., Zobel, H., Holtekjølen, A.K., Draget, K.I., Holopainen-Mantila, U., Knutsen, S.H., Sahlstrøm, S., 2020. Is dehulling of peas and faba beans necessary prior to dry fractionation for the production of protein- and starch-rich fractions? Impact on physical properties, chemical composition and techno-functional properties. J Food Eng 278, 109937. doi:10.1016/J.JFOODENG.2020.109937.

do Carmo, C.S., Varela, P., Poudroux, C., Dessev, T., Myhrer, K., Rieder, A., Zobel, H., Sahlstrøm, S., Knutsen, S.H., 2019. The impact of extrusion parameters on physicochemical, nutritional and sensorial properties of expanded snacks from pea and oat fractions. LWT, 108252 doi:10.1016/J.LWT.2019.108252.

Englyst, H.N., Quigley, M.E., Hudson, G.J., 1994. Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatography, high-performance liquid chromatography, or spectrophotometric measurement of component sugars. Anal. Chem. 119 (July), 1497–1509.

Frohlich, P., Boux, G., Malcolmson, L., 2014. Pulse ingredients as healthier options in extruded products. Cereal Foods World 59 (3), 120–125. doi:10.1094/CFW-59-3-0120.

Helgerud, T., Knutsen, S.H., Afseth, N.K., Stene, K.F., Rukke, E.O., Ballance, S., 2016. Evaluation of hand-held instruments for representative determination of glucose in potatoes. Potato Res. 59 (2), 99–112.

Heusala, H., Sinkko, T., Sözer, N., Hytönen, E., Mogensen, L., Knudsen, M.T., 2020. Carbon footprint and land use of oat and faba bean protein concentrates using a life cycle assessment approach. J. Clean. Prod. 242, 118376. doi:10.1016/j.jclepro.2019.118376.

Hilliard, J.E., 1967. Determination of structural anisotropy. In: Elias, H. (Ed.), Stereology. Springer, Berlin Heidelberg, pp. 219–227.

Hood-Niefer, S., 2017. Insights into extrusion of protein. Cereal Foods World 62 (4), 148–150. doi:10.1094/CFW-62-4-0148.

ISO, 2010. International Standard ISO 8589: Sensory Analysis: General Guidance for the Design of Test Rooms. ISO.

ISO. (2012). ISO 8586:2012 Sensory analysis — General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors.

Johansen, H.N., Glitsø, V., Bach Knudsen, K.E., 1996. Influence of extraction solvent and temperature on the quantitative determination of oligosaccharides from plant materials by high-performance liquid chromatography. J. Agric. Food Chem. 44 (6), 1470–1474.

Lawless, H.T., Heymann, H., 2010. Descriptive analysis. In: Sensory Evaluation of Food: Principles and Practices. Springer, New York, pp. 227–257. doi:10.1007/978-1-4419-6488-5_10.

Lin, S., Huff, H.E., Hsieh, F., 2000. Texture and chemical characteristics of soy protein meat analog extruded at high moisture. J. Food Sci. 65 (2), 264–269. doi:10.1111/j.1365-2621.2000.tb15991.x.

Link, W., Balko, C., Stoddard, F.L., 2010. Winter hardiness in faba bean: physiology and breeding. Field Crops Res. 115 (3), 287–296. doi:10.1016/j.fcr.2008.08.004.

Masoero, F., Pulimeno, A.M., Rossi, F., Masoero, F., Pulimeno, A.M., Rossi, F., Masoero, F., Pulimeno, A.M., & Rossi, F. (2016). Effect of extrusion, espansion and toasting on the nutritional value of peas, faba beans and lupins. 10.4081/ijas.2005.177

Morales, P., Berrios, J.D.J., Varela, A., Burbano, C., Cuadrado, C., Muzquiz, M., Pedrosa, M.M, 2015. Novel fiber-rich lentil flours as snack-type functional foods: an extrusion cooking effect on bioactive compounds. Food Funct. 6 (9), 3135–3143.

Multari, S., Stewart, D., Russell, W.R., 2015. Potential of fava bean as future protein supply to partially replace meat intake in the human diet. Comprehens. Rev. Food Sci. Food Saf. 14 (5), 511–522. doi:10.1111/1541-4337.12146.

Neacsu, M., McBey, D., Johnstone, A.M., 2016. Meat reduction and plant-based food: replacement of meat: nutritional, health, and social aspects. In: Sustainable Protein Sources, pp. 359–375. doi:10.1016/B978-0-12-802778-3.00022-6.

Osen, R., Toelstede, S., Wild, F., Eisner, P., Schweiggert-Weisz, U., 2014. High moisture extrusion cooking of pea protein isolates: raw material characteristics, extruder responses, and texture properties. J. Food Eng. 127, 67–74. doi:10.1016/j.jfoodeng.2013.11.023.

Palanisamy, M., Franke, K., Berger, R.G., Heinz, V., Töpfl, S., 2019. High moisture extrusion of lupin protein: influence of extrusion parameters on extruder responses and product properties. J. Sci. Food Agric. 99 (5), 2175–2185. doi:10.1002/jsfa.9410.

Palanisamy, M., Töpfl, S., Aganovic, K., Berger, R.G., 2018. Influence of iota carrageenan addition on the properties of soya protein meat analogues. LWT - Food Sci. Technol. 87, 546–552. doi:10.1016/j.lwt.2017.09.029.

Philipp, C., Oey, I., Silcock, P., Beck, S.M., Buckow, R., 2017. Impact of protein content on physical and microstructural properties of extruded rice starch-pea protein snacks. J. Food Eng. 212, 165–173. doi:10.1016/j.jfoodeng.2017.05.024.

Riaz, M.N., 2011. Texturized vegetable proteins. In: Handbook of Food Proteins, pp. 395–418. doi:10.1016/B978-1-84569-758-7.50015-0.

Singh, S., Wakeling, L., Gamlath, S., 2007. Retention of essential amino acids during extrusion of protein and reducing sugars. J. Agric. Food Chem. 55 (21), 8779–8786. doi:10.1021/jf071769z.

Smewing, J., 2016. Substituting your meat at mealtimes. Stable Micro Syst. 1–13.

Sukalingam, K., Ganesan, K., Das, S., Thent, Z.C., 2015. An insight into the harmful effects of soy protein: a review. Clin. Ter. 166 (3), 131–139.

Tehrani, M., Ehtiati, A., Sharifi Azghandi, S., 2017. Application of genetic algorithm to optimize extrusion condition for soy-based meat analogue texturization. J. Food Sci. Technol. 54 (5), 1119–1125. doi:10.1007/s13197-017-2524-9.

Wani, S.A., Kumar, P., 2016. Effect of extrusion on the nutritional, antioxidant and microstructural characteristics of nutritionally enriched snacks. J. Food Process. Preserv. 40 (2), 166–173. doi:10.1111/jfpp.12593.

Willett, J.L., 1996. The effect of density on melt viscosity calculations. Starch - Stärke 48 (4), 154–156. doi:10.1002/star.19960480406.