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Meat Analogues from Pea Protein: Effect of Different Oat Protein Concentrates and Post Treatment on Selected Technological Properties of High-Moisture Extrudates

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Abstract: The effect of using oat protein concentrates from different sources on the techno/functional properties of pea protein high-moisture extrudates (HME) have been investigated. The oat protein fractions were commercially produced by wet milling (OP) or produced by dry fractionation (OA) in our pilot plant facilities. The texture cutting force of HME was significantly increased by the inclusion of OP and had higher anisotropy, while addition of OA did not influence the texture. Addition of both oat sources to the pea mixture led to higher water and oil binding ability on both initial dry ingredients and extrudates. The color of the meat analogues was significantly affected by the type of oat added. CLSM image analysis of the extrudates showed that the type of oat used influenced fiber alignment and apparent porosity of the protein network. Post-extrusion treatment with water at 80 °C/20 min of the extrudates significantly reduced the cutting strength of the meat analogues and significantly caused a shift toward a lighter color. The reduction of texture strength of extrudates with OP rendered it similar to cooked chicken samples, while extrudates with OA showed a more resilient fiber strength to the hot water treatment.

Keywords: high-moisture extrusion; oat protein concentrates; pea protein; microstructure; texture; color; dry fractionation

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1. Introduction

The increasing demand for nutritious and tasty plant-based processed products is pushing the food industry to continue to develop solutions for substituting processed or unprocessed meat products with meat analogues [1]. Two attractive plant categories in this field are pulses (such as soy, peas, and beans) and cereals (such as oats), which are good sources of complementary essential amino acids and other nutritional components, and have proteins suitable for technological applications after processing, including fractionation and texturization [2].

High-moisture extrusion (HME) is a technology for processing plant-based proteins into a fibrous meat-like structure [3,4]. The process consists of mechanical shearing of the materials with more than 40% moisture under sufficient pressure and heat treatment (>110–140 °C depending on the raw material) [5,6]. This causes the unfolding of the globular proteins, followed by their aggregation and realignment. The fibrous texture is formed in the cooling die where the temperature is significantly reduced (<100 °C) [3] and crosslinking of the protein molecules occurs [3,7]. To achieve a fibrous structure, it is essential that the total protein concentration in the raw material or combined ingredients is 50–70 wt% [3,8]. Protein concentrates from pulses and cereals have gained interest over the

last few years as new ingredients in alternative meat applications due to their functional similarity to soy protein glycinin [9].

The application of pea protein in meat analogues has recently increased due to its health benefits such as its antioxidant capacity and ability to modulate intestinal bacteria [10]. Research has shown that pea protein concentrate or isolate can be used in high-moisture extrusion (HME) to create a fibrous meat-like structure [5,11,12]. However, the properties of pea protein present some challenges related to the strength, anisotropy, and functionality of the fibers formed [10]. To address these issues, various approaches have been attempted, including mixing pea protein with other materials such as ingredients containing gluten or polysaccharides [13–15].

Low-moisture extrusion (LME) of oats has been previously reported with a focus on oat bran or oat flour as the raw material. LME products have a moisture content of less than 40% and are primarily produced as snacks or texturized vegetable protein (TVP) [16,17]. Oat protein concentrates (OPC) can be obtained through wet milling or dry fractionation. As described by De Angelis, et al. [18], Lantmännen produces PrOatein®, an oat protein concentrate using a combination of dry and wet milling, where dehulled oats are dry milled to separate the oat flour from the bran, which is enzymatically treated with alpha-amylase in hot water to enhance protein separation. The suspension is decanted first to obtain the soluble protein and fiber in the solution, which are heat treated to denature the protein, then decanted and centrifuged to separate the solid protein from the soluble fiber. Solids rich in oat protein are then roller dried to produce the oat protein concentrate powder, characterized with high fat and protein contents of 13–15% and 52–55%, respectively [19]. Although this non-conventional fractionation process does not utilize chemicals or involve any pH-adjustment substances as in conventional wet fractionation for isolates production, there is a significant amount of water used and the protein state is altered due to the heat treatment. Dry fractionation, alternatively, is a more environmentally friendly solution for producing protein concentrates as it does not utilize water [20]. However, for oats, the materials have to be subjected to an initial defatting procedure; otherwise, the air classification procedure fails. Dry fractionation preserves the protein's native state as it does not involve the heat treatment and improves powder functionality [21]. However, the protein's purity is lower [20,22]. De Angelis, Kaleda, Pasqualone, Vaikma, Tamm, Tammik, Squeo and Summo [18] conducted a study on blends of an oat protein concentrate (PrOatein®) and pea protein isolate and concentrates using LME to produce TVP. The use of this blend not only improved the amino acid profile, but also enhanced the sensory and functional properties of the extrudates compared to the mixture of oat and pea protein concentrates. In sensory and texture tests, the combination of the three ingredients resulted in reduced hardness, chewiness, and elasticity of the extruded material. While a few studies have explored the use of oat protein concentrates in high-moisture extrusion [23,24], there is a lack of research on the effect of including OPC from various sources and compositions, such as variations in fat content and pre-processing conditions. Immonen, Chandrakusuma, Sibakov, Poikelispää and Sontag-Strohm [24] examined the impact of blending destarched oat protein concentrates with pea protein isolates in high-moisture extruded products and discovered that the inclusion of destarched oat protein, even at a low concentration of 30% protein in a blend, can create a more fibrillar structure at extrusion temperatures above 140 °C. Destarched oat protein reduced the cutting strength compared to extrudates produced from pea protein isolate. To the best of the authors' knowledge, the effect of incorporating oat protein concentrates from various sources and compositions, such as fat content and differences in pre-processing conditions, has not yet been studied.

The aim of this research was to study the effect of mixing different oat protein concentrate sources (produced by enzymatic wet milling or dry fractionation) on the techno-functional properties of pea protein-based meat analogues produced by high-moisture extrusion. Additionally, we explored the effect of hot water treatment (HWT) as a post-extrusion processing step on the texture and color of the extrudates. To the best of the authors' knowledge, no previous studies have examined the impact of HWT as a post treat-

ment on the properties of high-moisture extrudates. For benchmarking purposes, boiled chicken breast fillets were included in the study. We hypothesized that the addition of oat protein concentrates could enhance the functionality of pulse-based extrudates, leading to products with a more diverse amino acid profile.

2. Materials and Methods

2.1. Materials

The raw materials used comprised pea protein concentrate (PPC) (AMN 55% protein, 35% carbohydrates, and 3.8% fat on a dry weight basis (dm) (AM Nutrition, Stavanger, Norway) and pea protein isolate (PPI) (78.6% protein, 6.8% carbohydrates, and 7.8% fat, IsoPro80, ABS Food, China). Commercial oat protein concentrate PrOatein® (OP) was obtained from Lantmännen Biorefineries (Kimstad, Sweden). The proximate composition was 54.4% protein, 24.7% carbohydrate, and 18.7% fat (dm). Whole grain oat variety 'Active' was obtained from Lantmännen (Stockholm, Sweden). The latter was further processed at Nofima to produce the oat protein concentrate with 56% protein content (OA) via the dry fractionation method adapted from do Carmo, Silventoinen-Veijalainen, Zobel, Holopainen-Mantila, Sahlstrøm and Knutsen [22]. Briefly, the oat grains were non-heat treated, dehulled, and mechanically flattened to flakes in a Ferrell-Ross flaking mill (Ferrell-Ross, Hereford, TX, USA). SC-CO_2 extraction of lipids was performed on a pilot scale in a multi-use SFE plant with a pressure vessel of 5 L (SciMed, Stockport, UK). Oat flakes were defatted using SC-CO_2 with a flow of 200 mL CO_2/min, pressure of 350 bar, and a temperature of 45 °C. The defatted oat flakes were pin milled at 20,000 rpm and air classified to obtain a fine fraction with 56% *w*/*w* protein content, 31% native starch, 2% non-soluble polysaccharides (NSP), and 6% total fat (dm) (Hosokawa Alpine 100 UPZ, Hosokawa Alpine, Augsburg, Germany). The mixtures used for high-moisture extrusion were 50% PPC: 50% PPI (PP), 45% PPC: 45% PPI: 10% OP (PP_OP), 45% PPC: 45% PPI: 10% OA (PP_OA) targeting 66 ± 2% protein content on a dry basis.

2.2. High-Moisture Extrusion

Production of high-moisture extruded protein was performed in a pilot scale using a co-rotating twin-screw extruder (KETSE 20/40 Brabender GmbH and Co. KG, Duisburg, Germany) with a screw diameter of 20 mm each and a length of 400 mm (L/D ratio of 20:1). The screw configuration setup, adapted from do Carmo, et al. [25], contained 12 forward conveying elements (fce) from the feeding zone to the die, 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 extruder was connected to a liquid peristaltic pump (Watson-Marlow 530 Du, Falmouth, Cornwall, England) which was used to feed water at a variable feeding rate (WFR). A vertical gravimetric feeder (Brabender GmbH and Co., KG, Duisburg, Germany) was used to feed the powders into the extruder at different mass feeding rates (FR). A 600 mm cooling die was connected to the extruder with internal dimensions of 600 × 25 × 7 mm (L × W × H), and a Julabo 600F water bath (Julabo Gmbh, Seelbach, Germany) set at 30 °C controlled the temperature of the cooling die. Table 1 shows the set parameters for the samples collected. Extrudates were then collected, allowed to cool down, packed in plastic containers, and stored frozen until further analysis.

Table 1. Material chemical composition, extrusion mass feeding rate (FR), water feeding rate (WFR), screw rotation speed (SRS), extruder barrel heating zones (Z), and cooling die temperature during processing.

Material [1]	PP	PP_OA	PP_OP
FR (Kg/h)	2	2	3
WFR (Kg/h)	2.3	2.3	1.9
Target moisture (%)	64	64	52
SRS (rpm)	600	600	500
Heating Zone (°C)			
Z1	40	40	40
Z2	80	80	80
Z3	130	130	130
Z4	150	145	145
Measured Cooling die (°C)	40	40	40
Composition %			
Protein (dm)	64.8 ± 0.0	64.8 ± 1.4	66.1 ± 0.5
Fat	5.3 ± 0.1	4.1 ± 0.1	6.5 ± 0.1
Moisture *	54.2 ± 1.4	54.8 ± 1.2	39.7 ± 0.3
GFS [2]	1.1 ± 0.0	1.3 ± 0.1	1.0 ± 0.0
NDO [3]	4.7 ± 0.0	4.8 ± 0.4	4.0 ± 0.0

[1] PP (50% PPC: 50% PPI), PP_OP (45% PPC: 45% PPI: 10% OP), PP_OA (45% PPC: 45% PPI: 10% OA). [2] GFS: glucose, fructose, and sucrose; [3] NDO: non-digestible oligosaccharides (raffinose, stachyose, verbascose). * Moisture content in HME.

2.3. Compositional Analysis

Protein content was measured using the Dumas N combustion procedure (AOAC, 990.03) [26] and values were calculated based on the standard conversion factor N × 6.25. This fits well with the compositional data sheets supplied from the commercial supplier and is therefore used for a relative comparison of the blends. Total fat content was measured by extraction of fat with petroleum benzine before and after hydrolysis with hydrochloric acid following the EC 152/2009 method for sampling and analysis [27]. Moisture content was determined by an oven method 44–15.01 according to AACC [28]. For low-molecular weight carbohydrates analysis, sample extraction was carried out by adding 9.5 mL ethanol (50% v/v) to 50 mg sample, and 0.5 mL of melibiose (0.02%) as an internal standard. Samples were incubated at 50 °C for 1 h under continuous shaking and then they were centrifuged at 4000 rpm. Thereafter, an aliquot was diluted 1:20 with water and filtered through a 0.22 µm Millex GV filter (Merck, Darmstadt, Germany) and then analyzed by high-performance liquid chromatography as described by do Carmo, Knutsen, Malizia, Dessev, Geny, Zobel, Myhrer, Varela and Sahlstrøm [25]. Compositional values of commercial products were used from the supplier datasheets.

2.4. Water and Oil Absorption Capacity

Water absorption capacity (WAC) and oil absorption capacity (OAC) of the raw materials were measured following AACC methods [29] and adapted from [30]. The respective extrudates were also measured for WAC and OAC following an adapted method by Kantanen, et al. [31] and Lin, et al. [32]. The extrudates with dimensions of 25 × 25 × 7 mm^3 (length × width × height) were dried in an airflow convection oven (Revent, Sweden) at 40 °C overnight. Dried samples were weighed (g) and then immersed in 40 mL deionized water or rapeseed oil at 50 °C for 18 h. Thereafter, the samples were removed from oil or water and weighed again (g). The WAC or OAC was calculated as the percentage of weight gained after hydration. Each sample was measured in triplicate. The samples from WAC and OAC were also used for color measurements (see Section 2.6).

2.5. Cooking Yield

To measure the cooking yield, sample pieces of thawed extrudates with dimensions of 25 × 25 × 7 mm^3 (length × width × height) were immersed in water at 80 °C for

20 min and drained for 10 min at room temperature [33]. The yield was calculated as the percentage gain in weight of the extrudates after this treatment. The hot water treated extruded samples were also used to study the subsequent effect on texture (see analysis Section 2.7).

2.6. Color Properties

The surface color of extruded materials before and after WAC or OAC test (50 °C) was measured using a digital color imaging system (DigiEye, VeriVide Ltd., Leicester, UK) as described by [34]. The extruded material was cut into 25 × 25 × 7 mm^3 (length × width × height) dimensions, placed in a white plastic cup in a lightbox with standardized daylight (CIE D65), and three measurements were taken for each sample. The L^* (lightness), a^* (green–red), and b^* (blue–yellow) values were obtained. Total color change (ΔE) was used to represent the color change between the processed extrudates and the post water or oil treated extrudates (effect of post processing). A modified ΔE Equation (1) from Altan, et al. [35] was used to calculate the total change for each sample. The subscript (e) indicates the measurement of the extrudates, and the subscript (c) indicates the measurement of the extrudates after treatment in water or oil.

$$\Delta E = \sqrt{(L_c^* - L_e^*)^2 + (a_c^* - a_e^*)^2 + (b_c^* - b_e^*)^2} \quad (1)$$

2.7. Texture Properties

The fiber strength of the extrudates, was evaluated by a Warner–Bratzler Shear Force test before and after further immersion in hot water, in the longitudinal (L) and transversal (T) directions, as described by Ferawati, Zahari, Barman, Hefni, Ahlström, Witthöft and Östbring [5] using TA.XTplus texture analyzer (Stable Micro Systems Ltd., Godalming, UK). Sample pieces with dimensions of 25 × 25 × 7 mm^3 (length × width × height) were kept at room temperature and positioned onto a heavy-duty platform (HDP/90). The amount of force needed by a knife blade to move at a cross speed of 1.5 $mm.s^{-1}$ to penetrate the sample at full depth was measured. As the full depth of the samples varied between pre-cooked (7 mm) and cooked (8 or 9 mm) samples, the values were accounted for hardness of the extrudates and are presented in Newton force (N). At least 3–5 replicates were used for statistical analysis. The anisotropic index (AI) was calculated according to Wittek, et al. [36] as the ratio of transversal cutting to the longitudinal cutting force.

2.8. Confocal Laser Scanning Microscopy

Longitudinal and transversal sections of the extrudates were dual stained for protein-rich and fat-rich domains with fluorescein 5-isothiocyanate (FITC; Sigma-Aldrich, St Louis, MO, USA) and Nile Red (Nile Blue A Oxazone, Sigma-Aldrich), respectively. The section samples were carefully placed on an object glass, and confocal laser scanning microscopy (CLSM) was performed with an inverted Leica TCS SP5 microscope (Leica Microsystems CMS GmbH, Mannheim, Germany) equipped with Ar/DPPS laser with excitation wavelengths of 488 nm for protein and 633 nm for fat, and emission wavelengths of 509–589 nm for protein and 651–739 nm for fat. An oil immersion objective (HCX PL APO lambda blue, Leica Microsystems CMS GmbH) with 40× magnification was used. A representative digital image of each sample with 1024 × 1024 resolution was acquired (Leica Confocal Software version 2.00, Leica Microsystems CMS GmbH, Krefeld, Germany).

To characterize the anisotropic microstructural descriptors of the CLSM images, quantitative image analysis was adapted according to the procedure described by Clemons, et al. [37]. The OrientationJ plugin (ImageJ database) was used to characterize the fiber orientation in the samples based on structure tensor analysis in a local neighborhood of the region of interest. The local orientation and anisotropy of the CLSM images were quantified by the dimensionless coherency coefficient [37] provided that a value of 1 corresponds to a structure that has one dominant fiber orientation (anisotropy) and value of 0 corresponds to a complete isotropic structure (no defined dominant orientation). With this descriptor, it

was possible to differentiate the CLSM anisotropic microstructural characteristics of the samples not only qualitatively, but also quantitatively.

2.9. Statistical Analysis

Significant effects of treatments on the variables (measured characteristics) were evaluated using ANOVA at $p < 0.05$, and the mean values were compared using a Tukey pairwise comparison test. As the Tukey test can be very conservative at 95% confidence interval for heterogeneous HME samples, trends were considered at $0.1 < p < 0.05$. Pearson correlations were tested at 95% confidence level and significant correlations were declared at $p < 0.05$. All statistical data were processed using Minitab® 21.3.1 software.

3. Results

3.1. Production Setup and Characterization of the Meat Analogues

The processing parameters used for each of the protein mixtures and their chemical composition are presented in Table 1. During PP_OP sample processing using the same settings as for PP and PP_OA, an increase in lubrication coupled with a reduction in the mass flow exiting the die was observed. This is most probably due to the relatively higher fat content compared to the other samples. Gradual changes to the parameters were performed: the feed rate was increased to fill the shafts, the water feed rate was reduced, and the screw rotational speed (SRS) was lowered, until a consistent product was obtained. This results in PP_OP extrudates having relatively lower total moisture content as compared to the rest of the samples.

3.2. Techno-Functional Properties of Raw Materials and High-Moisture Extrudates

The properties of the raw material and their respective extrudates related to techno functionality and appearance are presented in Table 2.

Table 2. Properties of high-moisture extrudates: water absorption capacity (WAC), oil absorption capacity (OAC), cooking yield, and color measurements.

Material				PP	PP_OP	PP_OA
WAC %			RM	169.7 ± 0.1 [b]	212.3 ± 0.2 [a]	167.5 ± 0.5 [c]
			HME	85.2 ± 19.4 [a]	112.6 ± 1.0 [a]	115.7 ± 8.9 [a]
OAC %			RM	129.7 ± 0.2 [b]	132.9 ± 0.2 [c]	128.6 ± 0.1 [a]
			HME	0.8 ± 0.5 [a]	2.8 ± 1.2 [a]	0.9 ± 0.7 [a]
Cooking Yield %				128.2 ± 3.3 [a]	128.0 ± 0.6 [a]	130.0 ± 4.0 [a]
HME Colour	HME		*L**	51.4 ± 0.7 [b]	43.3 ± 0.9 [c]	55.5 ± 1.2 [a]
			*a**	10.3 ± 0.4 [a]	9.4 ± 0.3 [a]	7.4 ± 0.8 [b]
			*b**	26.5 ± 0.8 [a]	18.4 ± 0.6 [b]	25.5 ± 1.8 [a]
			Yellowness	58.2 ± 1.1 [a]	49.9 ± 0.6 [c]	54.7 ± 2.9 [b]
	Water-treated *		*L**	67.1 ± 1.2 [a]	65.7 ± 1.3 [a]	61.5 ± 1.5 [b]
			*a**	8.6 ± 0.5 [a]	8.1 ± 0.0 [b]	5.4 ± 0.9 [b]
			*b**	21.7 ± 2.0 [a]	21.7 ± 0.2 [ab]	20.7 ± 0.9 [ab]
			Yellowness	44.5 ± 3.2 [a]	44.9 ± 0.7 [a]	45.2 ± 1.8 [a]
	Oil-treated *		*L**	38.2 ± 1.1 [a]	39.6 ± 1.5 [a]	39.7 ± 1.6 [a]
			*a**	11.7 ± 1.2 [ab]	10.4 ± 0.4 [ab]	13.4 ± 2.3 [a]
			*b**	14.0 ± 2.1 [b]	14.7 ± 0.9 [b]	16.2 ± 1.3 [b]
			Yellowness	44.1 ± 3.7 [a]	44.9 ± 1.2 [a]	47.7 ± 2.9 [a]

[a–c] Means that do not share a superscript within a row are significantly different ($p < 0.05$). PP (50% PPC: 50% PPI), PP_OP (45% PPC:45% PPI:10% OP), PP_OA (45% PPC: 45% PPI: 10% OA). RM: raw material; HME: high-moisture extrudates; *L** = lightness; *a** = redness; *b** = yellowness. * Water or oil-treated measurements were performed on samples from WAC and OAC tests.

The different ability of the raw material and respective extrudates to hydrate or absorb water in a non-destructive method shows that at the raw material level, the addition of OP to the pea mixture significantly increased the powder's WAC and the extrudates as compared to OA addition or pea alone. On the extruded material level, the addition of

oat showed a slight tendency to increase the WAC. A good correlation (r = 0.72) was found between the WAC of the raw material and its respective HME. Extrudates with OP (PrOatein®) showed a tendency for higher oil absorption capacity (OAC) as compared to pea alone and pea with OA.

The inclusion of OA or OP did not significantly improve the cooking yield compared to the reference (PP). Overall, the extrudates show stability throughout the post treatment similar to the consumer's preparation of chicken, which involves cooking in water at a minimum of 80 °C for 20 min [38,39].

3.3. Color Profile

The type of oat concentrate added in the starting material dramatically influences the color of the HME. The addition of OA to pea gave a similar *L** value that significantly improved the lightness of the extrudates as compared to the control pea only samples. On the contrary, the addition of OP significantly reduced the light color of the pea extrudates leading to the darker color of the products. The green to red range in color was also significantly affected by the addition of OA and resulted in lower values to the control and the PrOatein®-based product. The blue to yellow color value showed a rather significant similarity between the control and defatted oat products.

After immersing the extrudates in water or in oil (from WAC and OAC tests), the color was measured (Table 2) and the total change in color (Δ*E*) was calculated (Figure 1). All post-treated extrudates showed statistical differences from the untreated ones, especially those subjected to water. PP_OP was significantly the most affected by treatment with water, and not highly affected by the oil treatment. The change in color when heated in water was driven by a significant increase in *L** value which indicates an increase in lightness (toward white) in color. While the change in color when heated in oil was driven by a significant reduction in *L** value which indicates a shift toward a dark color.

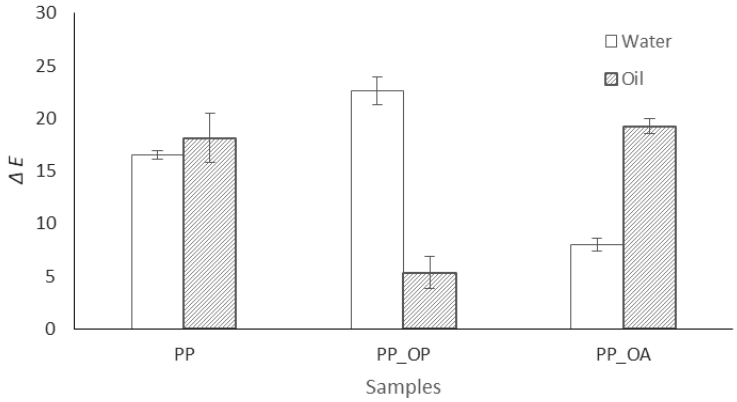

Figure 1. Total change in color (Δ*E*) of high-moisture extrudates due to the water and oil treatment. PP (50% PPC: 50% PPI), PP_OP (45% PPC: 45% PPI: 10% OP), PP_OA (45% PPC: 45% PPI: 10% OA).

3.4. Texture Properties of HME

Figure 2a shows the force required to cut the high-moisture extrudates in transversal and longitudinal directions. The transversal cutting is against the flow direction of the material within the cooling die and indicates the formation of an anisotropic structure. While the addition of only 10% of OP (PP_OP) leads to a significant increase in their cutting force as compared to the pea only extrudates (PP), the OA addition at the same concentration did not significantly influence the pea cutting force. PP_OP also achieved higher strength as compared to the chicken samples.

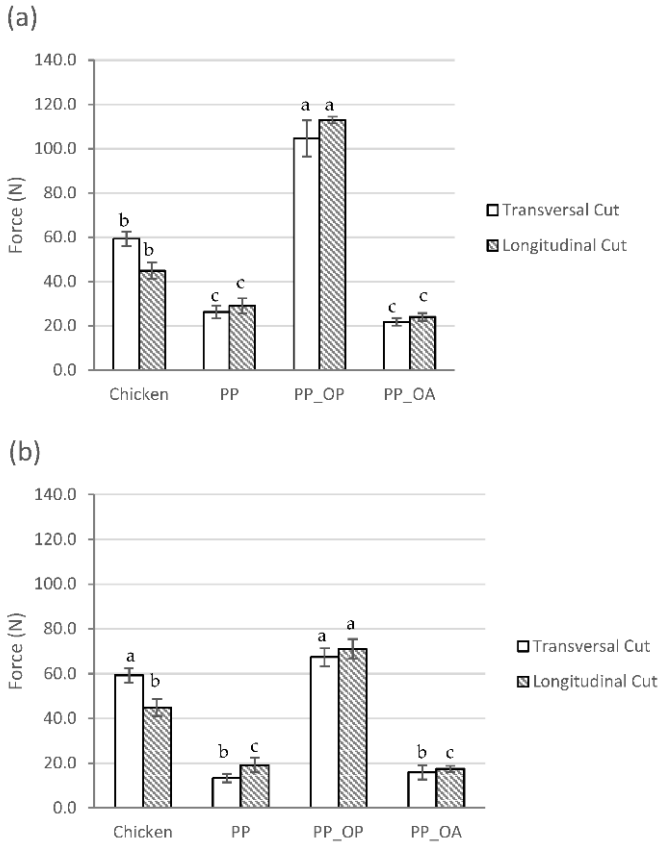

Figure 2. Cutting force (N) of (**a**) high-moisture extrudates and (**b**) hot water treated HME in transversal and longitudinal directions. PP (50% PPC: 50% PPI), PP_OP (45% PPC: 45% PPI: 10% OP), PP_OA (45% PPC: 45% PPI: 10% OA). The chicken samples were measured after the water treatment at 80 °C for 20 min and the data were used for statistical comparison with both the pre- and post-treated extrudate samples. $^{a–c}$ Means that do not share a superscript within the same cutting direction are significantly different ($p < 0.05$).

The anisotropic index in Figure 3 reflects the degree of fiber alignment in the protein structure, a particular characteristic in meat fibers. The addition of OP relatively increased the AI of pea, while the addition of OA did not influence the strength of the fibers as compared to the reference PP.

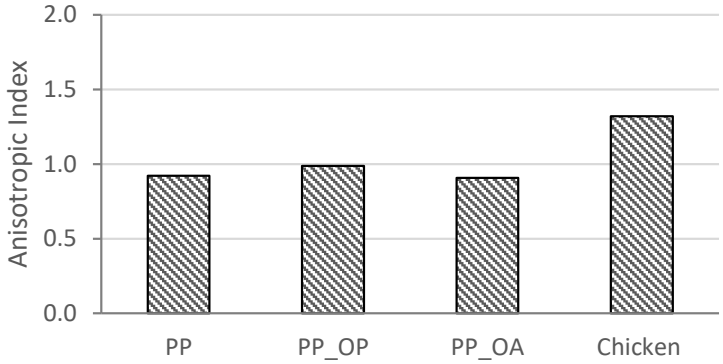

Figure 3. Anisotropic index of the high-moisture extrudates (ratio between transversal and longitudinal cutting force). PP (50% PPC: 50% PPI), PP_OP (45% PPC: 45% PPI: 10% OP), PP_OA (45% PPC: 45% PPI: 10% OA).

The cutting force was also measured for the hot water treated extrudates (Figure 2b). In general, the cutting force was significantly reduced for all extrudates in the T- and L-directions. The average percentage reduction in hardness for PP, PP_OP, and PP_OA in the T-direction was 49, 36, and 27%, respectively and in the L-direction was 34, 37, and 27%, respectively. HWT PP_OP increased softness and rendered their texture strength as significantly similar to the boiled chicken. While the average reduction in hardness in both cutting directions due to HWT is small for PP, the addition of OP to the pea mixture gave a large reduction in hardness compared to OA which gave only a small reduction in hardness for PP_OA.

3.5. Microstructure of HME

The microstructure of the extrudates in the transversal and longitudinal directions is presented in Figure 4. In the CLSM images, three main colors can be distinguished: red (protein mesh), green (fat fraction), and black (unstained fractions). The latter represents air (or water) pockets embedded between the lamellar planes of the material. The conducted image analysis allowed us to quantify the level of anisotropy of the fiber alignment through the calculated coefficient of coherency (Table 3).

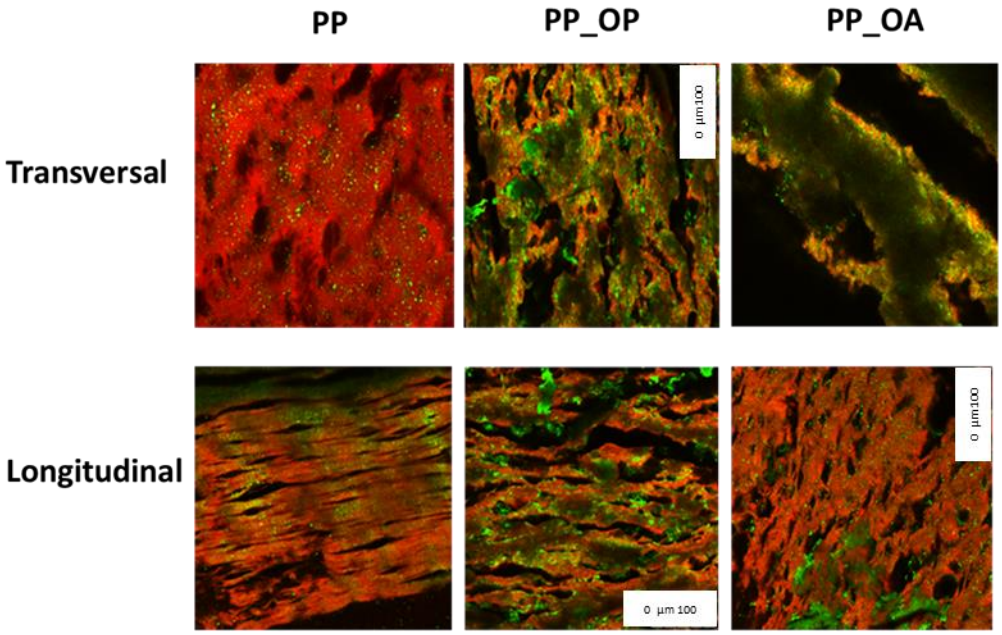

Figure 4. CLSM images and microstructure of high-moisture extrudates measured in the longitudinal and transversal sections. PP (50% PPC: 50% PPI), PP_OP (45% PPC: 45% PPI: 10% OP), PP_OA (45% PPC: 45% PPI: 10% OA). Red stains refer to protein and green stains refer to fat.

Table 3. Microstructural characteristics of high-moisture extrudates determined by CLSM image analysis.

Material	PP	PP_OP	PP_OA
Coefficient of coherency (-)	0.5 ± 0.0^{a}	0.3 ± 0.0^{b}	0.2 ± 0.0^{c}
Median mesh pore area (um^2)	73.4 ± 2.0^{c}	112.3 ± 6.9^{b}	132.9 ± 4.0^{a}
Protein mesh intersection density $\times 10^{-3}$ (int/um^2)	2.8 ± 0.2^{b}	4.1 ± 0.5^{a}	4.3 ± 0.9^{a}

[a–c] Means that do not share a superscript within a row are significantly different ($p < 0.05$). PP (50% PPC: 50% PPI), PP_OP (45% PPC: 45% PPI: 10% OP), PP_OA (45% PPC: 45% PPI: 10% OA).

Confocal images in Figure 4 revealed that PP has visually more protein alignments in the longitudinal section as compared to the rest of the sample, which is also confirmed by the high value of the coherency coefficient (0.504). Hence, a denser protein structure is also

observed as evidenced by the lower values of mesh pore area and number of intersections per unit area (Table 3).

The images derived from OP-containing exudates in Figure 4 had a higher intensity of green staining due to the increased fat content in the mixture. The fat phase was distributed both as small droplets and large fat pools through the sample. The addition of OP and OA created a more porous structure (higher values of protein mesh pore area and intersection density) and reduced the degree of fiber alignment manifested by a lower coefficient of coherency (Table 3).

4. Discussion

4.1. Effect of Oat Addition on Raw Material Techno Functionality

Water absorption capacity depends on several factors, including protein structure, hydrophilicity, and conformation, which are different between cereals and pulses [40]. The ability of OP to absorb more water than the OA (Table 2) could be linked to several factors including the variety of oats, composition, and the difference in heat treatment history during powder production. Native and gelatinized oat starch do not absorb water as much as degraded starch (maltodextrins-containing materials). While OA contains 31.2% native starch and 2.1% NSP with 0.5% beta-glucan (internal analysis), OP contains up to 24% degraded starch or maltodextrin and up to 3% beta glucan, wherein they both absorb water [19]. Unfolding of the native protein structure due to the heat treatment favors reorientation of the hydrophilic sites toward the surface and increases their ability to bind with water [11]. Production of OP included at least two steps of heat treatment to separate the protein from the bran via an enzymatic treatment, followed by protein denaturation to separate the solid protein phase from soluble fibers by decanting, and finally, roller drying to transform them into powder. OA, on the other hand, followed a heat-free production process which preserved a larger state of native protein in the material. Differences in the protein state could explain the impact on the raw materials of WAC. Similarly, PP_OP showed an increased ability to adhere to oil, which could also be attributed to the heat-treatment history of the OP fraction. An excessive high-heat treatment causes protein denaturation, which favors higher OAC as compared to undenatured protein where hydrophobic sites become exposed to the protein surface, allowing for more oil binding ability [11,41]. The PP_OP higher tendency to absorb oil indicates strong polarity of their protein structure formation as compared to PP and PP_OA. This could also be evidenced by the originally high fat content (>15%) in PrOatein® (OP) products. It could also indicate that OP contains more of the lipid binding protein tryptophan than OA. The process to produce OP probably preserves tryptophan in comparison to dry fractionation [42].

4.2. Techno-Functional Properties of HME

Although the extrudates did not have significant differences in their WAC or OAC, Table 2 shows a clear trend ($0.1 < p < 0.05$) that demonstrates the addition of OPC increased the WAC of the extrudates, and the addition of OP increased the OAC in PP_OP. The effect of heat treatment on WAC has been contradictory in literature. A positive correlation was reported where increasing the barrel temperature increases the WAC of extrudates [43,44], and a negative correlation was reported [25,30]. The contradiction was explained by the different proteins composition and aggregation behavior between the raw materials and highlighted moisture content as a key factor. The test in these studies was, however, performed on milled extrudates which probably allow for a more effective surface area for water penetration. The heat treatment during the extrusion process causes protein unfolding and starch degradation in the material, which most probably led to an increase in WAC of PP and PP_OA extrudates, making them comparable to PP_OP extrudates and minimizing the differences observed between the raw materials.

Cooking yield measures the ability of the extrudates to uptake additional water. It reflects the structural stability of the product when subjected to a high-temperature treatment (80 °C/20 min) for a short time under conditions resembling chicken meat preparation,

and can indicate sensory attributes such as juiciness [12,45]. The different oat sources did not significantly impact the PP cooking yield as shown in Table 2. The current results are slightly lower than the previously published data on the cooking yield of pea-based extrudates (138–140% for pea protein concentrates and isolates, respectively) [12,33]. However, the experimentation parameters should be considered critically for such conclusion.

4.3. Effect of Oat Addition on HME Color

PP results in Table 2 are within the color range recently published by Rekola, et al. [46], where L^*, a^*, and b^* values were 47.6, 9.3, and 21.4, respectively for PPI and 56.5, 6.8, and 32.3, respectively for PPC. The addition of OP or OA significantly impacted the color profile of the extrudates, where PP_OP samples shifted toward a darker color. This could be attributed to the higher fat content in this mixture, making it more susceptible to lipid oxidation during the extrusion process. OA addition, on the other hand, reduced the red color of the extrudates, which is commonly reported as a result of the Maillard reaction catalyzed by the high temperature in the extruder barrel.

Immersing the extrudates in warm water proved to bring a positive impact on total color change in the extrudates toward higher L^* values, as shown in Table 2. A combination of heat and water can contribute to protein solubilization, removal of other constituents in the water, or rearrangement of the fibrous network, allowing for absorbing less light, and thus appearing lighter in color. Color changes with ΔE values of 1 to 2 units between the two samples can be sufficient for the human eye to indicate a difference in color [47,48]. This depends on the intensity of the color where the higher intensity color may require ΔE values of 4 units [48]. The extrudates showed at least ΔE values of 5 units between each other due to the post treatment as shown in Figure 1. The water treatment also reduced the significant variation in L^* between the samples as shown in Table 2, resulting in similar lightness, except for PP_OA. It is worth mentioning that the color of the extrudates from the cooking yield test was also measured, and the effect on color change validated the impact of WAC treatment. Similar changes in color are observed in chicken meat products after cooking (80–95 °C/20 min) [49]. Fletcher, Qiao and Smith [49] reported that cooking reduces the variation in color of meat products, and dark meat has been observed to be most affected. The water from WAC and HWT appeared yellowish, which is probably due to the solubilized compounds including proteins.

4.4. Effect of Oat Addition on HME Texture and Relation to Microstructure

The cutting strength represents the force associated with the consumer's first bite. Higher values for cutting force indicate a hard texture. The PP and PP_OP results in Figure 2a are in line with the literature, where HME from PPI or PPC has a lower hardness profile compared with the boiled chicken samples, and the addition of PrOatein® (OP) to pea protein increased the fibrousness of extruded materials as its ratio increased in the blend [5,50]. The high fat content in the PP_OP sample likely indirectly contributes to the enhanced cutting strength. This is possibly due to the fat lubricating effect on the extruder barrel which reduces the material shear and moisture requirements, resulting in a relatively firmer texture profile with lower moisture content of around 40%. Although PP_OA hardness did not significantly differ from PP, it is worthwhile to note that it was visually observed that PP_OA had flow instability at the exit of the cooling die, and the fibers appeared differently than PP extrudates. Nevertheless, they still formed good quality fiber.

Anisotropy is one of the prerequisites for product functionality and meat-like experience during mastication. A very fibrous product typically has a higher anisotropic index compared to the non-fibrous product. None of the plant-based meat analogues achieved a similar AI to the boiled chicken samples as shown in Figure 3, which may indicate a lower degree of fiber layering and transversal alignment. However, fibers oriented in parallel direction or shorter crosslinked fibers may reduce the anisotropic index as the cutting plane might fall between the adjacent layers. It is important to note that the anisotropic value determined by the cutting tests quantifies fiber alignment and does not directly

measure hardness or compactness. Nevertheless, a more in-depth investigation is needed to understand this relationship.

Microstructure images in Figure 4 showed that PP has visually a denser protein structure with more fiber alignments in the L section, which is confirmed by their high coherency coefficient value (0.504) as compared to PP_OA and PP_OP which showed a more porous structure and lower coherency coefficient value, as shown in Table 3. Similar observations were reported on the increased protein alignment of pea protein isolates in the longitudinal section [11,12].

In this study, the degree of fiber alignment or the density of protein structure does not necessarily reflect the cutting strength of the extrudates, and no statistically significant correlation was found between the cutting force and structural parameters quantified. PP_OP had the highest cutting force, yet it showed a relatively more porous structure as compared to PP. On the other hand, PP_OA samples with even less dense structure demonstrated a low cutting force, but this might be related to the small fat droplets and air voids that were homogeneously dispersed through the protein matrix. Similarly to the suggestions raised by Clemons, Bradshaw, Toshniwal, Chaudhari, Stevenson, Lynch, Fear, Wood and Iyer [37], one may hypothesize that both bubbles and fat droplets may act as a structure weakening element that can form and propagate micro cracks in the protein matrix. Moreover, it must be outlined that PP_OP after post treatment which had a similar hardness to chicken, but little difference in longitudinal to transversal cutting force, does not necessarily indicate poor fiber development but rather poor fiber alignment (anisotropy) as evident from the report on the coefficient of coherency as shown in Table 3.

Analysis of the obtained data suggests that the increased porous structure of PP_OA contributed to their tendency to increase in WAC compared to PP and PP_OP. The pore sizes and intersection density both demonstrated a strong positive linear correlation with WAC (R2 = 0.93 and 0.99, respectively). However, no clear correlation was found between the structural parameters and OAC and cooking yield. All these results indicate that it is difficult to relate textural (cutting force) and functional properties (WAC, OAC, cooking yield) to a single specific structural characteristic of the meat analogues.

4.5. Effect of Post Treatment on HME Texture

The average reduction in hardness as an effect of the HME post treatment was measured as the lowest for PP_OA as shown Figure 2b. This suggests that OA addition led to the formation of more stable fiber strength against the hot water treatment, making it a more predictable physical attribute during the post-processing treatment compared to the other samples. Palanisamy, Töpfl, Aganovic and Berger [33] also reported a significant decrease in cutting force in T- and L-directions of soy meat analogues with different matrices after water cooking. This type of post-processing influence on the physical properties of HME is scarce in literature; therefore, it is challenging to compare the results with other studies.

5. Conclusions

The production of high-moisture extrudates from pea proteins (concentrate and isolate) combined with oat protein concentrates obtained by wet milling or dry fractionation was successful, although variations in fat levels influenced processing parameters and final product attributes. CLSM images clearly revealed fibrous-like structures in all samples. Both oat protein sources had noticeable effects on the microstructure, fibrousness, and color of the extrudates, even with just a 10% inclusion in any preparation. Dry-fractionated oat protein with peas mixture demonstrated a more randomly oriented and coarsely connected protein network (limited fiber alignment) as compared to pea proteins alone, yet the functional and hardness properties did not differ from peas alone and were comparable to boiled chicken hardness. Wet-milled PrOatein® addition to pea proteins created a more porous protein network structure, increased WAC, OAC, and the hardness of the extrudates as compared to peas alone or boiled chicken. The cutting force increased with the degree of fiber alignment but did not correlate with the anisotropic index, which highlights the

complexity of understanding the texture. The post-extrusion hot water treatment of the extrudates induced changes toward a lighter color and created a softer texture especially for products with PrOatein®, while products with dry-fractionated oat showed the least change in cutting force, indicating the formation of stable and resilient fibers. We have shown that dry-fractionated oat proteins can be used to enhance the amino acid profile of pea protein extrudates without negatively influencing their functional and textural properties. On the other hand, wet-milled oat protein requires some process optimization or simply can serve to deliver new products with other characteristics. Further research is needed to understand how structure and fiber alignment are interconnected. The best method for techno-functional oat protein concentrate production will remain dependent on the intended food application and can be further tailored. Factors such as protein or starch heat-treatment history during fractionation are crucial parameters.

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Abbreviations

FR	Mass Feeding rate
GFS	Glucose, Fructose, and Sucrose
HME	High-moisture extrusion
HWT	Hot water treatment
LME	Low-moisture extrusion
NDO	Non-digestible oligosaccharides (raffinose, stachyose, verbascose)
NSP	Non-starch polysaccharides
OAC	Oil absorption capacity
OA	Oat protein concentrate—oat variety Active
OP	Oat protein concentrate—PrOatein®
PPC	Pea protein concentrates
PPI	Pea protein isolates
SRS	Screw rotation speed
WAC	Water absorption capacity
WFR	Water feeding rate

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