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Enhancing the Integration of Protein-Rich Oat Waste Material into Meat Formulations

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Abstract: The objective of this study was to modify a protein-rich by-product, generated during β-glucan production, to render it appropriate for incorporation into meat products. Additionally, the study sought to assess the quality of a prototype meat product containing oat additives, depending on its concentration. Through hydrolyzation, its solubility was enhanced, making it suitable for broader applications in food products. With an average protein content of 52% and fat content of 6%, the pure hydrolysate exhibited a notable ferric ion reduction, as well as metal chelating properties. In meat formulations, the hydrolysate was integrated at concentrations of 1%, 2%, and 3%, relative to the meat mass. Following cooking and subsequent storage for 21 days, assessments were conducted every 7 days to evaluate colour retention, texture, and oxidation status. At concentrations of 2% to 3% (equivalent to 2–3 g/100 g), the hydrolysate significantly enhanced colour stability, while concurrently fostering oxidation. Notably, cohesiveness and resilience were augmented, with no discernible impact on hardness. The application of oat protein hydrolysate, particularly at 2–3 g/100 g, serves as a viable strategy for enhancing colour stability in meat formulations. However, its pro-oxidative effects necessitate supplementation with antioxidants to mitigate potential deterioration in the final product.

Keywords: nitrite alternative; oat protein hydrolysate; colour; quality

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1. Introduction

The concept of the circular economy is growing in popularity, and there are more and more examples of reducing, reusing, and recycling on a global scale [1]. Incredible amounts of waste are generated during food product, food additive, or diet supplement and natural pharmaceutical manufacturing. There are already many good examples of waste management in agriculture production, such as using part of the bovine hide left over from leather production for collagen casing manufacturing [2], crop waste for feeds, and biofuel production, etc. [3]. Nonetheless, there is still room for more modifications. One such recycling possibility may be reprocessing by-products from oat β-glucan syrup production. Oats are a well-known cereal widely used in human diets. Its high β-glucan content (4–8% [4]) is beneficial for human health, which is demonstrated by its ability to decrease blood cholesterol, lower the risk of cardiovascular diseases, prevent diabetes, and reduce obesity [5]. The methods of obtaining β-glucan syrups may vary. One example of obtaining β (1–3) β (1–4) glucans is described in a United States patent (US20060122149A1). The extraction is conducted using alkaline solutions, and after separating the insoluble part, alcohol is used to precipitate β-glucans. After extraction, the insoluble material is treated as waste. The final extraction yields of extracted β-glucans, ranging from 30–90%, depend on the extraction method and the β-glucan content [6]. Therefore, the amounts of the waste material are considerable. As the methods vary, the quality of the waste material is also variable. In the research conducted by Aktas-Akyildiz et al. [6], in which an enzymatic method was proposed, the raw material was characterised as containing 23.7–25.1% of protein in the dry matter. Depending on the type of oats and the enzyme applied, the

amount of protein in the extract varied from 5.1 to 12.1% of dry matter, which means that the rest of the proteins remained undissolved.

Based on our current knowledge, the residue remaining after β-glucan syrup production is typically utilized as a bulk material in animal feed manufacturing. To enhance the economic sustainability of this production method, it could also find application in the food industry as a basic filler/extender or an additive, potentially offering additional quality-improving functions. Oat protein is already acknowledged as a premium grain protein with high nutritional value, suitable for the production of meat substitutes. Furthermore, it has the capacity to form stable gels when subjected to heat [7,8]. Oat protein exhibits low solubility within the pH range typically encountered in meat products (4.0–6.0), irrespective of ionic strength, as indicated by NaCl concentration [9]. Various successful approaches have been suggested for chemically, physically, and enzymatically modifying oat proteins to enhance their functional properties [10]. Each type of modification can impart slightly varying properties to the resulting product [7,11]. Protein hydrolysates and bioactive peptides derived from food proteins, owing to their active properties, can serve as natural food additives with diverse functionalities. Moreover, generating protein hydrolysates from waste materials in the food industry could offer a means of waste management [12–14].

The presence of fat in meat products poses an issue regarding lipid oxidation. In the absence of antioxidants, this process can result in flavour degradation and a rise in the levels of harmful oxidation by-products [15]. The utilization of plant-derived additives is becoming increasingly prevalent in the meat industry [16]. Consumers now seek products that offer pleasing sensory qualities, extended shelf life, and minimal additive content. Meeting these demands poses a challenge for producers, prompting exploration into a variety of natural substances that could serve as effective, cost-efficient, and readily available additives [17]. Additionally, there is a growing need to integrate protein extenders into meat products to reduce meat consumption, leading to the emergence of hybrid products [18].

Based on our previous research regarding soy protein hydrolysates [19], it was assumed that enzymatic modification may reveal the antioxidant and binding properties of the oat by-product. To the best of our knowledge, oat protein hydrolysates from oat by-products have not been used as additives in meat products. Hence, the objective of this study was twofold: firstly, to modify the residual product remaining after β-glucan syrup production, with the aim of creating a water-soluble functional product suitable for use as a meat additive; secondly, to assess the impact of incorporating this product into the meat formulation on the quality of the resulting meat product.

2. Materials and Methods

2.1. Materials

The oat protein was donated from a local retailer (Polfeed, Krakow, Poland). It was a dried by-product (moisture 5.00 ± 0.08%) left over from β-glucan syrup production.

2.2. Oat Protein Hydrolysate Production and Characterisation

2.2.1. Oat Protein Hydrolysate Production

Oat protein hydrolysate (OPH) was prepared using the same hydrolysis conditions as those described by Tkaczewska et al. [19]. The oat by-product was ground in a laboratory mill (MPM MMK-06), and the raw material prepared in this way was mixed with distilled water at a 1:10 ratio. The suspension was stirred with a magnetic stirrer (MR, Heidolph, Germany) at 350 rpm for 15 min. Based on data from the literature, the enzyme preparation showing potential to produce protein hydrolysates with antioxidant activity was chosen; this was a food-grade enzyme: Alcalase® (Novozymes, Bagsværd, Denmark). Hydrolysis conditions were a pH 8.0 and a temperature of 65 °C. The pre-prepared suspension was heated to the set temperature, and the pH was adjusted using 1 M NaOH. The addition of enzyme preparations was 2% for protein content. Hydrolysis was carried out for 180 min. During the first 15 min, the pH was continuously monitored and corrected with 1 M NaOH

or 1 M HCl. Then, the pH was controlled every 15 min. The reaction was terminated by maintaining the hydrolysates at 95 °C for 15 min, and the samples were centrifuged at 4000× *g* for 15 min at 15 °C. The obtained supernatant was lyophilised (freeze-drying condition: vacuum 0.5 mBar, condenser temperature −55 °C). The hydrolysis process was carried out in triplicate.

2.2.2. Proximate Composition and Degree of Hydrolysis of OPH

The proximate composition of the oat by-product and protein hydrolysate was evaluated according to AOAC methods (protein 979.09; fat 920.39; moisture 925.09; ash 923.03, with carbohydrate content calculated based on the other components) [20]. The degree of hydrolysis was estimated by formal titration using exactly the same method as that described by Noman et al. [21].

2.2.3. Electrophoresis (SDS–PAGE) of OPH

Sodium dodecyl sulphate polyacrylamide gel electrophoresis of the hydrolysate was performed as described by Laemmli [22] using Mini-PROTEAN (Bio-Rad, Hercules, CA, USA) precast gels. The applied molecular weight markers were the Precision Plus Protein™ Unstained Protein Standards (6.5–130 kDa; Bio-Rad, USA). Separation was performed in 15%, separating acrylamide gels, and then staining was carried out in accordance with the standard Coomassie procedure (Bio-Rad, USA).

2.2.4. Amino Acid Profile and Free Amino Acid Content in OPH

The amino acid profile and free amino acid analysis was performed according to the same method as we described in our previous study [19].

For the analysis of free amino acids, the hydrolysate solution (100 mg/10 mL of 0.1 M HCl) underwent ultrasonic treatment in a Polsonic ultrasonic bath (Palczyński, Warsaw, Poland) for 20 min. Subsequently, the sample underwent centrifugation at 3000× *g* for 10 min at 4 °C, and 5 mL of the resulting supernatant was combined with 5 mL of trichloroacetic acid solution (400 g/L). This mixture was vortexed, cooled in an ice bath for 10 min, and then centrifuged at 20,000× *g* for 10 min. The resulting 10 mL of free amino acid solution was then derivatized by combining it with 70 µL of borate buffer (with a pH ranging from 8.2 to 9.0) and 20 µL of 6-aminoquinolyl-N-hydroxysuccinimidylcarbamate (AQC) dissolved in an acetonitrile solution (3:1 m/v). The standards were prepared following the same procedure as that used for the samples.

For total amino acid analysis, 30 mg of the sample was hydrolysed using 4 mL of 6 M HCl and 15 µL of phenol at 110 °C for 24 h. The hydrolysis process occurred in a nitrogen-sealed environment. The resulting hydrolysate was then filtered using a syringe filter with a pore diameter of 45 µm and dried under a continuous stream of nitrogen. These prepared samples were appropriately diluted and then derivatized by mixing 10 µL of sample with 70 µL of boron buffer (with a pH ranging from 8.2 to 9.0) and 20 µL of AQC in a concentration of 3 mg of ACQ per 1 mL of acetonitrile. The standards were prepared using the same method used for the samples.

Analysis was conducted using the Dionex Ultimate 3000 HPLC system from Thermo Scientific, (Waltham, MA, USA), equipped with an LPG-3400 SD 4-channel gradient pump, a WPS 3000 TSL auto-sampler, and an FLD 3400RS 4-channel fluorescent detector. Separation was achieved using a Nova-Pak C18, 4-µm (150 mm × 3.9 mm) column from Waters, Milford, MA, USA. The elution process consisted of acetate-phosphate buffer (A) and a mixture of 60:40 acetonitrile/water (B). The separation temperature was maintained at 37 °C, and detection parameters were set at an excitation of 250 nm and an emission at a 395 nm wavelength. Quantitative analysis utilized a one-point calibration method with analytical standards (50 pmol for each concentration).

2.2.5. Antioxidant Properties of OPH

FRAP, metal chelating ability, and DPPH method were evaluated, as described by Tkaczewska et al. [19].

2.3. Assessing the Possibility of Using an OPH as a Meat Additive

2.3.1. Meat Sample Production

Meat formulations were prepared using pork meat (10% fat, 19% protein, 70% water) obtained from a local retailer (Lidl Polska, Krakow, Poland). Each formulation contained meat, salt, and water. The control (C) was prepared without any other additive. The oat protein hydrolysate constituted 1%, 2%, and 3% of meat mass in the 1H, 2H and 3H samples, respectively. The lyophilisate hydrolysate was dissolved in water, mixed with other ingredients, and all the mass was homogenised for 3 min using Robo Coupe (Vincennes, France). The meat batter was stuffed into plastic tubes and cooked in a hot water bath (90 °C) for 30 min. After cooking, the samples were cooled, and the fluid was removed. The samples were stored in closed containers (the same in which they were cooked) at 4 °C for 21 days, and texture, colour, and TBARS values were analysed every 7 days.

2.3.2. pH Analysis

A pH analysis was conducted in raw batters using the Elmetron Cp-505 electrode (Zabrze, Poland). The instrument was calibrated using standard phosphate buffers (pH 4.0 and 7.0) and corrected for temperature. The samples were diluted with distilled water in a 1:1 ratio, and the pH was measured. Each measurement was performed in duplicate.

2.3.3. Cooking Losses

To determine the cooking losses, the samples were weighed before and after cooking and cooling, with fluid removal. Weight loss was calculated and presented as a % of raw sample mass.

2.3.4. Product and Batter Moisture, Protein, and Fat Content

Moisture (no. 950.46), protein (no. 981.10) and fat (no. 991.36) content was analysed in each raw and cooked batter, based on AOAC methods [20].

2.3.5. Colour Analysis

The colour parameters (L*, a*, and b* coordinates in a CIELab system) of the cooked meat samples were measured using the CM-3500d Konica Minolta spectrophotometer (Osaka, Japan). Black and white enamel was used to calibrate the instrument according to the manufacturer recommendations. The measurement parameters were: reflectance mode, illuminant D, and 10° observer angle. All the samples were ground before the analysis. Five measurements were performed for each sample.

2.3.6. Texture Profile Analysis (TPA)

A TA-XT2 texturometer (Stable Micro Systems Ltd., Surrey, UK) was used to determine the texture profile of the samples. The meat samples were removed from the plastic tubes after cooking and cooling. The samples (15 mm × 15 mm) were analysed cold (5–8 °C). The double compression test was performed under the following conditions: 50% contraction, pre-test speed of 5 mm/s, 2 mm/s test speed, and post-test speed of 5 mm/s. Six readings were taken for each sample.

2.3.7. TBARS (Thiobarbituric Acid Reactive Substances) Analysis

TBARS analysis was conducted as described by Zajac et al. [23]. Initially, 10 g of the sample was homogenized with 34.25 mL of cold (4 °C) 4% perchloric acid and 0.75 mL of butylated hydroxytoluene in ethanol. After homogenization, the mixture was filtered through Whatman No. 1 filter paper (GE Healthcare, Little Chalfont, UK) into a 50 mL

volumetric flask and adjusted to 50 mL using 4% perchloric acid. Subsequently, 5 mL of the filtrate was transferred to a 20 mL vial containing 5 mL of a TBA (thiobarbituric acid) water solution (0.02 mol/L). The vial was sealed and heated in a water bath at 100 °C for 1 h. Absorbance was then measured at 532 nm against a blank comprising 5 mL of 4% perchloric acid and 5 mL of the TBA reagent. TBARS values were determined as mg of malonaldehyde per kg of sample and calculated by multiplying the absorbance values by the K coefficient of 5.5. All the measurements were conducted in triplicate.

2.4. Statistical Analysis

The statistical analysis was performed on three independent experimental batches using Statistica v 13.0 software (Tibco, Palo Alto, CA, USA). The analyses were performed in triplicate in all of the tests, except for TPA and cooking losses tests, in which 6 and 12 readings, respectively, were taken for each variant. The Shapiro–Wilk test was conducted to determine the normality of the results. For the results which did not fit the normal distribution model, a Box–Cox transformation was conducted (except for 'adhesiveness', in which negative numbers were noted and the Yeo-Johnson transformation was applied). The two-way ANOVA test was conducted when the effects of OPH addition and storage time were tested as fixed effects. Tukey's test, at a significance level of $p < 0.05$, was performed to determine statistically significant differences between treatments (various levels of OPH addition), storage time (1, 8, 15, and 22 days), and their combined effects. The results are presented as mean values ± standard errors.

3. Results

In our research plan, we established two primary objectives. The first objective was to modify the protein-rich by-product to render it suitable for use as an additive in meat processing. Ideally, the additive should function as both an additional protein source and an antioxidant. To achieve this, we conducted enzymatic hydrolysis, retaining only the soluble fractions. We then proceeded to characterize the hydrolysate, and the results are presented as follows.

3.1. Characterisation of Oat Protein Hydrolysate Properties

Raw material obtained from β-glucan extraction and the hydrolysed material were analysed. Fat, protein, ash, moisture, and carbohydrate content, as well as the antioxidant properties, are presented in Table 1. The molecular weight distribution of oat by-product protein hydrolysates was monitored via tricine-SDS-PAGE (Figure 1). A relatively low level of protein hydrolysis was observed in the electrophoretic pattern.

Table 1. Proximate composition of the oat sample and hydrolysate and antioxidant properties of the hydrolysate (mean values ± standard errors).

	Moisture	**Fat**	**Protein**	**Ash**	**Carbohydrates**
Raw material	5.00 ± 0.08	10.64 ± 1.17	42.18 ± 1.48	6.87 ± 0.90	35.07 ± 3.67
Hydrolyzed	0.50 ± 0.02	6.11 ± 0.79	52.00 ± 3.29	1.82 ± 0.16	40.31 ± 1.06
Antioxidant properties					
	FRAP μM Trolox/mg liophylysate		Metal chelating ability [%]		DPPH [%]
Hydrolyzed	18.26 ± 0.15		59.15 ± 2.78		1.23 ± 0.47

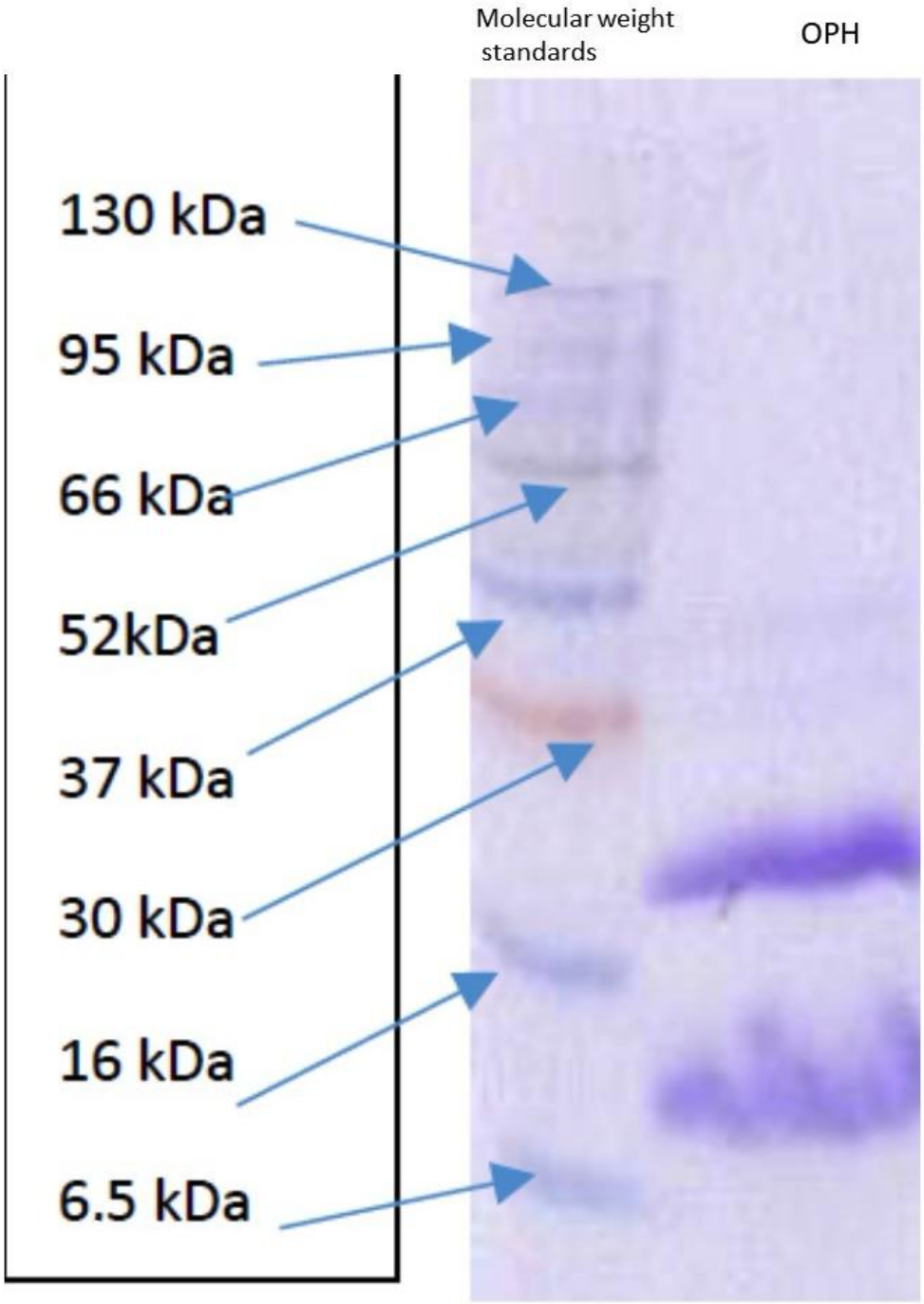

Figure 1. SDS-PAGE protein profile of oat by-product protein hydrolysates obtained using Alcalase. The first line displays the protein standard, while the second line represents the sample applied to the gel (OPH).

In Table 2, the amino acid profile and free amino acid content in protein hydrolysates from oat by-products are shown. The fundamental amino acids in the oat protein hydrolysate were found to be glutamic acid and leucine, constituting approximately 23.77% and 8.46% of the overall amino acids, respectively.

Table 2. Amino acid profile and free amino acid content in protein hydrolysates from oats by-products.

	Amino Acid Profile [%]	Free Amino Acids [mg/100 g]
Aspartic acid	8.42 ± 0.02	7.43 ± 0.45
Serine	4.99 ± 0.01	19.83 ± 1.21
Glutamic acid	23.77 ± 0.03	32.74 ± 1.99
Glycine	5.21 ± 0.02	10.87 ± 0.66
Histidine	2.43 ± 0.00	23.55 ± 1.43
Arginine	7.05 ± 0.05	9.76 ± 0.59
Threonine	3.54 ± 0.01	12.23 ± 0.74
Alanine	5.35 ± 0.18	16.62 ± 1.01
Proline	5.83 ± 0.02	22.78 ± 1.39
Cysteine	0.09 ± 0.00	0.00 ± 0.00
Tyrosine	3.60 ± 0.00	11.03 ± 0.67
Valine	6.21 ± 0.00	32.35 ± 1.97
Methionine	1.58 ± 0.02	2.13 ± 0.13
Lysine	4.25 ± 0.00	44.02 ± 2.68
Isoleucine	4.43 ± 0.00	58.36 ± 3.55
Leucine	8.46 ± 0.00	49.43 ± 3.01
Phenylalanine	4.78 ± 0.09	4.09 ± 0.25
Sum of amino acids (mg/100 g of hydrolysates)		357.23 ± 21.76

3.2. Assessing the Possibility of Using OPH as a Meat Additive

The second objective of our research was to assess the quality of model meat products and determine the optimal amount required to achieve an antioxidant effect. Various quality parameters were tested to comprehensively characterise the entire product. The most surprising results were observed in relation to meat colour and its stability during storage.

The obtained results are presented in the following section.

3.2.1. Product and Batter Moisture, Protein and Fat Content, pH, and Cooking Yields

The dry matter and fat content in both the raw batters and the cooked samples, as well as cooking yields, were analysed to determine the differences caused by OPH addition. The results are presented in Table 3. A total of 2 and 3% of OPH inclusion in meat batter improved cooking yields. Fat content decreased beginning with the application of 1% OPH. The pH of the meat batters increased with OPH addition, although the differences were not statistically significant.

Table 3. Dry matter and fat content of raw and cooked samples, pH of batters, and cooking yields (mean values ± standard errors).

Variant		pH		Dry Matter [%]		Fat [%]		Cooking Yield [%]	
C	Raw	6.28	±0.21	26.82 ^{2}	±0.24	10.33 ^{2}	±0.16		
	Cooked			33.76 ^{a}	±0.09	13.69 ^{a}	±0.05	90.63 ^{c}	±0.20
1H	Raw	6.37	±0.27	26.87 ^{2}	±0.15	9.49 ^{2}	±0.22		
	Cooked			31.97 ^{b}	±0.15	12.12 ^{b}	±0.15	91.89 ^{bc}	±0.27
2H	Raw	6.54	±0.17	27.15 ^{2}	±0.14	9.57 ^{2}	±0.30		
	Cooked			30.47 ^{c}	±0.01	10.82 ^{b}	±0.13	92.71 ^{b}	±0.36
3H	Raw	6.66	±0.15	29.36 ^{1}	±0.51	11.38 ^{1}	±0.70		
	Cooked			31.18 ^{bc}	0.04	11.93 ^{b}	±0.05	93.74 ^{a}	±0.18

Values in the same column with different letters indicate significant differences between cooked samples ($p < 0.05$). Values in the same column with different numbers indicate significant differences between raw samples. Results are expressed as mean ± standard error. In the case of dry matter, only cooked samples were compared. The labels 1H, 2H, and 3H represent samples with 1, 2, and 3% of the oat protein hydrolysate added, respectively; C—Control sample.

3.2.2. Changes in Colour Parameters of Meat Samples

The samples containing OPH exhibited higher redness parameters, which increased during the storage time in all the samples with OPH, in contrast to the control sample (Table 4). All the samples with OPH were darker and slightly more yellow. The most significant differences were noted between the control and the 3H sample. The effect was visible, as presented in Figure 2, and confirmed by ΔE calculations.

Table 4. Changes in colour parameters of meat samples during storage (mean values ± standard errors); ΔE value calculated using the Control as a standard.

Variant	Storage Duration [Days]	L*		a*		b*		ΔE
C	1	65.70 ^{ab}	±0.35	1.30 ^{cde}	±0.04	11.73 ^{d}	±0.17	
	7	65.90 ^{a}	±0.37	0.88 ^{fgh}	±0.06	12.00 ^{cd}	±0.13	
	14	65.43 ^{ab}	±0.70	0.66 ^{h}	±0.13	12.62 ^{abc}	±0.26	
	21	65.44 ^{ab}	±0.47	0.83 ^{gh}	±0.04	12.72 ^{abc}	±0.22	
1H	1	64.68 ^{abcd}	±0.31	1.49 ^{abcd}	±0.04	11.98 ^{cd}	±0.15	1.04
	7	65.09 ^{abc}	±0.28	1.16 ^{def}	±0.03	12.17 ^{bcd}	±0.11	0.86
	14	63.94 ^{abcde}	±0.65	1.05 ^{efg}	±0.11	12.73 ^{abc}	±0.22	1.49
	21	64.39 ^{abcde}	±0.52	1.15 ^{efg}	±0.05	12.90 ^{ab}	±0.21	1.10
2H	1	63.77 ^{abcde}	±0.26	1.66 ^{ab}	±0.05	12.32 ^{abcd}	±0.12	1.97
	7	64.26 ^{abcde}	±0.25	1.37 ^{bcde}	±0.04	12.39 ^{abcd}	±0.09	1.72
	14	63.11 ^{cde}	±0.55	1.29 ^{cde}	±0.07	13.00 ^{a}	±0.22	2.42
	21	63.65 ^{bcde}	±0.60	1.50 ^{abc}	±0.03	13.06 ^{a}	±0.16	1.91
3H	1	62.92 ^{cde}	±0.27	1.79 ^{a}	±0.07	12.60 ^{abc}	±0.10	2.82
	7	63.61 ^{bcde}	±0.16	1.48 ^{abcd}	±0.06	12.64 ^{abc}	±0.04	2.38
	14	62.31 ^{e}	±0.53	1.59 ^{abc}	±0.09	13.15 ^{a}	±0.20	3.26
	21	62.56 ^{de}	±0.44	1.69 ^{ab}	±0.07	13.09 ^{a}	±0.12	3.00

Values in the same column with different letters indicate significant differences ($p < 0.05$). Results are expressed as mean ± standard error. The labels 1H, 2H, and 3H represent samples with 1, 2, and 3% of the oat protein hydrolysate added, respectively; C—Control sample.

Figure 2. Cross-section of meat samples. The labels 1H, 2H, and 3H represent samples with 1, 2, and 3% of the oat protein hydrolysate added, respectively; C—Control sample.

3.2.3. Texture Profile Analysis of Meat Samples

The texture profile parameters in samples with and without OPH are presented in Table 5. There were no significant differences in adhesiveness or springiness. Samples with 2 and 3% of OPH were characterised by higher cohesiveness and resilience when compared to the Control sample. The hardness and chewiness of both the Control sample and the 3H sample increased during storage. A similar trend was observed in 1H and 2H; however, these differences were not statistically significant. The other texture parameters remained unaffected.

Table 5. Texture parameter changes in meat samples during storage (mean values ± standard errors).

Variant	Storage Duration	Hardness [N]		Adhesiveness [ns]		Springiness [ns]		Cohesiveness		Chewiness		Resilience	
C	1	41.55 ^{d}	±1.28	−27.00	±6.76	0.83	±0.01	0.62 ^{c}	±0.02	21.20 ^{d}	±0.78	0.27 ^{e}	±0.01
	8	53.70 ^{abc}	±2.23	−23.95	±6.28	0.81	±0.03	0.64 ^{c}	±0.02	28.01 ^{bcd}	±1.61	0.29 ^{de}	±0.01
	15	54.85 ^{abc}	±3.47	−44.18	±11.44	0.86	±0.01	0.68 ^{abc}	±0.01	31.97 ^{abc}	±2.03	0.32 ^{bcde}	±0.01
	22	56.94 ^{ab}	±1.33	−35.08	±6.36	0.85	±0.01	0.65 ^{bc}	±0.03	31.40 ^{abc}	±1.68	0.29 ^{de}	±0.02
1H	1	43.05 ^{cd}	±2.53	−25.64	±7.42	0.81	±0.04	0.69 ^{abc}	±0.01	24.11 ^{cd}	±1.79	0.32 ^{bcd}	±0.01
	8	59.32 ^{a}	±2.30	−44.22	±5.60	0.85	±0.01	0.67 ^{bc}	±0.01	33.62 ^{ab}	±1.08	0.31 ^{cde}	±0.01
	15	55.28 ^{abc}	±3.03	−55.57	±9.93	0.85	±0.03	0.71 ^{abc}	±0.02	33.28 ^{abc}	±1.98	0.33 ^{abcd}	±0.01
	22	53.99 ^{abc}	±1.65	−27.65	±6.23	0.89	±0.01	0.71 ^{ab}	±0.02	34.23 ^{ab}	±1.29	0.34 ^{abc}	±0.01
2H	1	46.75 ^{bcd}	±2.70	−38.62	±6.21	0.84	±0.03	0.72 ^{ab}	±0.01	28.17 ^{bcd}	±1.73	0.35 ^{abc}	±0.00
	8	57.37 ^{a}	±2.37	−42.21	±6.71	0.85	±0.02	0.71 ^{abc}	±0.01	34.80 ^{a}	±1.66	0.34 ^{abc}	±0.00
	15	52.58 ^{abcd}	±1.97	−21.68	±6.68	0.90	±0.01	0.73 ^{a}	±0.00	34.44 ^{ab}	±1.52	0.35 ^{abc}	±0.01
	22	51.46 ^{abcd}	±2.61	−34.14	±6.50	0.88	±0.02	0.73 ^{ab}	±0.01	33.05 ^{abc}	±1.50	0.36 ^{ab}	±0.01
3H	1	44.09 ^{cd}	±1.86	−49.48	±5.68	0.85	±0.03	0.73 ^{a}	±0.01	27.37 ^{bcd}	±1.39	0.36 ^{ab}	±0.01
	8	56.37 ^{ab}	±1.52	−45.92	±6.14	0.87	±0.01	0.72 ^{ab}	±0.01	35.48 ^{a}	±0.86	0.35 ^{ab}	±0.00
	15	48.17 ^{abcd}	±3.13	−20.31	±7.05	0.87	±0.04	0.74 ^{a}	±0.01	31.64 ^{abc}	±2.80	0.37 ^{a}	±0.01
	22	56.56 ^{ab}	±1.78	−48.82	±6.71	0.87	±0.01	0.74 ^{a}	±0.00	36.32 ^{a}	±0.84	0.37 ^{a}	±0.00

Values in the same column with different letters indicate significant differences ($p < 0.05$). Results are expressed as mean ± standard error. [ns]—no significant differences were found between samples. The labels 1H, 2H, and 3H represent samples with 1, 2, and 3% of the oat protein hydrolysate added, respectively; C—Control sample.

3.2.4. TBARS Changes of Meat Samples during Storage

The TBARS values of all the variants are presented in Figure 3. The obtained values were comparable on the first day of the analyses, and increased during storage. The values were higher in products containing OPH when compared to the Control.

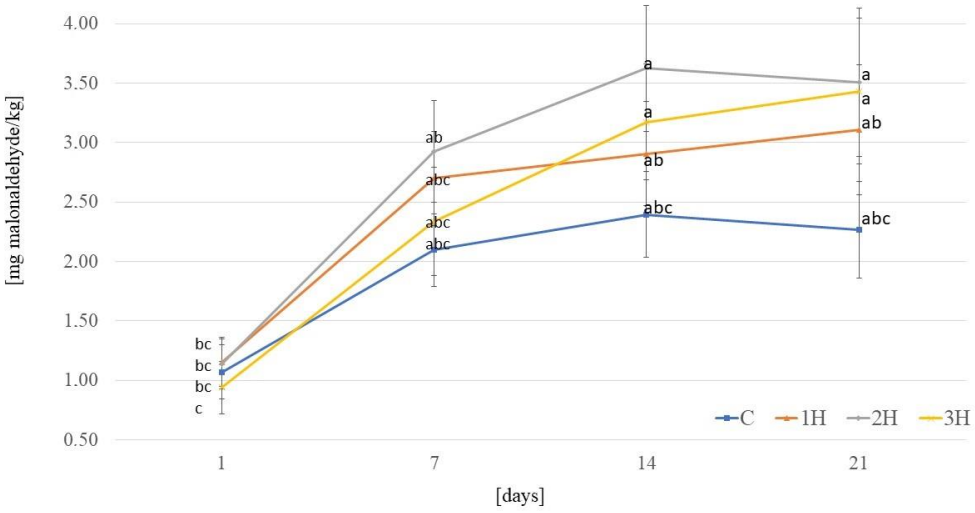

Figure 3. TBARS results for meat samples containing various levels of the oat protein hydrolysate. Values with different letters indicate significant differences ($p < 0.05$). The labels 1H, 2H, and 3H represent samples with 1, 2, and 3% of the oat protein hydrolysate added, respectively; C—Control sample.

4. Discussion

According to Sterna et al. [24], the protein content in oat grain depends on its variety, and it ranges from 9.7 to 17.3%, with the highest levels noted in the naked oat variety. This is important information for producers whose aim is to obtain protein hydrolysates. As mentioned in the Section 1, the material left over from β-glucan extraction typically contains 23.7–25.1% of protein. The material used in our study showed an increased protein content (42%). The hydrolysis allowed us to obtain a higher protein content in the final product (52%). However, it was not as high as that obtained by other researchers [25], mainly because in our case, we did not isolate the protein before the hydrolysis. Higher protein content results from the fact that after hydrolysis, we used only soluble fractions, discarding the pellet consisting of fat and carbohydrates, as well as mineral substances. The proportions of the basic constituents changed. Oat protein has garnered interest in recent years due to its distinct composition and pleasant sensory attributes compared to those of legumes and oil seeds [26]. The protein contains globulins, albumins, glutelins, and prolamins. Additionally, oats are gluten free and rich in sulphur-containing amino acids [27]. From a practical standpoint, the higher protein content enables more efficient production of the hydrolysate. Hydrolysis changed the proportions of the basic component contents (Table 1). The oat raw material contained more fat and mineral substances, which decreased after the hydrolysis. According to Zheng et al. [10], hydrolysis can induce significant changes in protein structure, affecting the amino acid pattern, protein conformation, and molecular weight profile.

Literature data on the subject show that modification by enzymatic hydrolysis is highly dependent on the nature of the protein and the degree of hydrolysis, which must be controlled to avoid excessive proteolysis leading to undesirable effects impairing functionality [28,29]. The degree of OPH hydrolysis was quite low. After 3 h of hydrolysis, the DH was only 12.06 ± 1.01%. The oat protein hydrolysates exhibited faint bands around 37 and 22 kDa (Figure 1), which likely represent the acid and basic polypeptide oat globulin, respectively [30]. Furthermore, there were noticeable wide bands in the hydrolysates within the weight range between 10–20 kDa. This suggests that the basic polypeptide of oat globulin, approximately 22 kDa in size, was partly broken down into smaller pieces. Overall, the electrophoretic profiles of oat protein hydrolysates indicated significant shifts towards lower molecular weight peptides, suggesting their potential nutritive value and biological significance [10].

Plant-derived peptides could be thought to exhibit antioxidant activity [31], with certain amino acids acting as metal chelators and hydrogen/electron donors through their interaction with free radicals, thereby stopping radical chain reactions or preventing their formation [32]. The hydrolysis process can also be well-reflected by measuring the amount of produced free amino acids [33]. The results are consistent with previous literature data in which it has been stated that glutamic acid and leucine are the most abundant amino acids in oat protein [10].

Generally, free amino acids are not effective antioxidants. However, other researchers [34,35] found that hydrophobic amino acids could express significant performance in regards to the functional properties of food proteins. These amino acids present in protein hydrolysates can exhibit excellent antioxidant properties and may be incorporated into other foods as supplements. Typically, the antioxidant activity of protein hydrolysates is not determined by individual amino acids alone, but by their combination with peptides [36]. The OPH obtained in this study exhibited a considerable content of free hydrophobic amino acids, especially isoleucine (58.36 mg/100 g) and leucine (49.43 mg/100 g).

The antioxidant properties of protein hydrolysates contributed to various combinations of selected free amino acids and peptides [37,38]. Plant protein hydrolysates proved to have a positive antioxidant effect; however, their activity depends on the source, the degree of hydrolysis, and the concentration [39]. The results of our study (Table 1) indicate that the protein hydrolysate exhibited strong metal chelating activity (59.15 ± 4.81%). This was

confirmed in other studies on oat protein hydrolysates This property seems to be especially important in terms of meat applications, as meat naturally contains a highly prooxidative agent as an iron ion [40]. The OPH exhibited low DPPH radical scavenging activity (1.23 ± 0.81%) and high ferric ion reducing activity (18.25 µM Trolox/mg OPH ± 0.27). These results were lower compared to those obtained for the soy husk hydrolysate obtained in our previous study [19]. However, our preliminary studies on OPH showed promising colour improving properties; therefore, the full study was prepared to verify the effect of OPH on other quality and technological parameters of meat samples.

Assessing the Possibility of Using OPH as a Meat Additive

Water holding capacity (WHC) is one of the most important properties of meat and meat products. It is described as the ability to hold the water (its own or added) within the muscle fibres or in an emulsion. Therefore, increased WHC is a desirable property worth improving [41]. All the additives used as fillers or extenders in the meat industry must meet specific requirements, including exhibiting a neutral colour, taste, and smell; a high water holding capacity; or emulsifying properties. If it is to be used for ham injection, the additive must additionally be soluble. For this reason, OPH was tested for its binding- and yield-promoting abilities.

The increased pH of meat leads to an increase in water holding capacity in the formulations, which is proportional to the amount of added OPH. These results are in agreement with those obtained from previous studies in which a higher pH enhanced water holding capacity and firmness [41]. However, in sausages prepared with a plasma hydrolysate, the opposite effect could be observed. The pH of the batters decreased, along with the plasma concentrations, and changes in yields were not noted [42]. Another reason for the increase in WHC could simply be an addition of proteins, which usually promotes water retention within the samples; however, the opposite effect may occur, as it did in our previous study [43]. In the trial, an increased protein content (achieved by hemp protein implementation) did not increase WHC because of the edestin properties. The cooking yields for the Control, 1H, 2H, and 3H were 90.6%, 91.9%, 92.7%, and 93.7%, respectively. A close analysis of the effect of OPH in terms of water and fat binding revealed that water binding increased with OPH concentration, but apparently, fat binding was not affected. All the samples exhibited a comparable fat content, regardless of the OPH concentration. By comparing the actual change between the raw and cooked samples, calculated as a percentage of fat in the dry matter, it can be observed that more fat was retained in the Control. Similar results were achieved in our previous study, in which we used a soybean protein hydrolysate as a meat additive [19]. The analysis of cooking loss showed that samples with soybean protein hydrolysates retained more water and less fat compared to the control samples. This may have resulted from the fact that along with the protein hydrolysate, additional proteins were introduced, and the amount of fat was proportionally lower.

The colour differences were noted between samples. According to Stokes et al. [44], differences in ΔE values below 2.15 are not discernible. Therefore, in our case, only 3% of the OPH concentration would be effective. However, we were already able to see the difference at 2%. Altmann et al. [45] noted 30% of correctly perceived differences between products at ΔE = 1, which proves that ΔE values close to 2.0 could definitely be distinguished by consumers. One of the most important results is that the a* parameter was stable during the storage period in 2H and 3H, in contrast to 1H and the Controls, in which the a* parameter had already decreased within the first 7 days of storage. On the other hand, redness, calculated as the a*/b* ratio, also dropped in the 3H sample during storage, as well as in all the other samples, but it was much more significant in 3H. These results indicate that OPH could be used in meat processing as a substance for supporting the pink colour formation in the "nitrite free" products or in those with a lower nitrite content. This quality seems to be especially important, considering the latest changes in European Union Regulation 1333/2033 [46], in which a significant decrease (i.e., 80 vs. 150 ppm of nitrite in a large group of cured products) will be required by the October 2025. These observations led us

to a further investigation regarding the cause of such a phenomenon. As plants naturally contain nitrates, which could contribute to meat colour formation, the nitrite and nitrate content was analysed in both the OPH and in the meat samples. Additionally, we tested the samples for the presence of the nitroso pigment.

We suspected that even low nitrite/nitrate concentrations could have reacted with myoglobin, forming nitroso hemochromogen during heating. In this case, nitrites/nitrates would not be present, as opposed to the stable nitroso pigment. All the results were negative, which indicates that the nitrogen-involving reactions did not occur. Although the pink colour of meat is usually discussed in the context of myoglobin and its forms [47], some other factors, such as polyphenols or amino acid content, may play role in colour formation [48,49]. Furthermore, zinc protoporphyrin IX could play a role, as was suggested by Wakamatsu et al. [50]. Nonetheless, this pink effect is worth further investigation.

The samples were comparable on the first day of storage, and all of them were oxidised during the storage process, which is typical for meat products [19,40]. Thus, various strategies are applied to inhibit the process to the highest extent. Recent trends indicate that natural additives, preferably of plant origin, are accepted by consumers [51]. The results of our study show that OPH not only inhibited meat oxidation, but even increased the process. Analysing the trends of TBARS increase in all the samples, it may be concluded that the higher addition of OPH promotes oxidation in meat samples. However, due to strong batch-to-batch variations, those differences were not statistically significant. Similar results were obtained in our previous study, in which soy husk protein was used [19].

Interestingly, the positive oxidation inhibiting effects of oat protein hydrolysates on meat samples have been presented by other researchers [25,52]. Yet, this study is the second hydrolysate we used in meat preparation, each showing oxidation enhancing properties, which is in contrast with the literature data. On the other hand, in another research, in which plasma hydrolysate was added to sausage, peroxide values increased significantly, and TBARS values increased slightly. The highest TBARs levels were noted in the sample with a 2.5% concentration of the hydrolysate, but the differences were not statistically significant [42]. As was previously mentioned, hydrolysis may lead to different results, depending on the extension and general condition of the process. Oxidation control can be achieved through the utilization of antioxidant additives, which can be either natural or synthetic. A diverse range of compounds, including phenols, flavonoids, tannins, lignans, tocopherols, and carotenoids, offer different modes of action in this regard. Ascorbic acid and rosmarinic acid are among the commonly used additives in the meat industry [16,51,53]. Nevertheless, these interactions might lead to unforeseen outcomes, underscoring the importance of conducting thorough investigations. It is possible that sodium chloride present in the OPH, due to HCl and NaOH used during hydrolysis, may be an important factor for increasing oxidation in the samples [54]. It is difficult to verify whether the hydrolysates used in other studies were obtained with considerable amounts of sodium chloride, as it is usually not mentioned in the description of the methods. Moreover, some polyphenols present in the OPH may also be significant. The pro-oxidant activity of flavonoids is believed to be proportional to the number of hydroxyl groups, especially in the B ring [55]. According to Masuda et al. [56], the presence of ortho- or para-substituted polyphenol structures was observed to promote the oxidation of oxymyoglobin. Additionally, certain polyphenols containing transition metals can cause prooxidant properties, as these have the ability to reduce metal ions involved in redox-cycling, thereby facilitating the production of hydroxyl radicals through Fenton and Fenton-like reactions [57]. Another contributing factor could be the natural presence of iron in oats [58], which when combined with the iron present in meat, accelerates the oxidation. This aspect of the research will be further explored in subsequent investigations.

It is established that proteins play an important role in the process of emulsified sausage production. The batters are considered oil-in-water emulsions, in which fat globules covered with protein layers are immersed in a myofibrillar protein gel matrix. Heating allows the formation of a stable gel, in which disulfide bridges are formed between fat

and protein [59]. Additional proteins (both animal and plant) usually increase the stability of meat emulsions, as well as the hardness of the meat products [60]. Among the most popular plant proteins used in the meat industry are soy proteins, along with others such as wheat, peas, etc., which possess gelling properties and allow for the improvement of the water holding capacity and texture of meat products. Proper quality meat is evaluated in terms of WHC and gel-formation; however, meat defects occur quite often. Plant proteins may support meat protein functionality, working with them to retain moisture and assure proper texture [61]. Incorporating unknown additives into a meat system may result in unexpected results [62].

Although the observations made during the performance of these tests led us to conclude that hardness increased along with OPH addition, the differences were negligible and not statistically significant. Hardness increased in all the samples along with storage time, probably due to drying, despite the containers being closed. OPH improved the cohesiveness and resilience of the meat samples, which were especially visible in samples containing 2 and 3% of OPH. This may be explained by the presence of carbohydrates, part of which were fibres (data not published). This may also suggest the occurrence of plant and meat protein interactions. Gao et al. [63] stated that no chemical interactions between salt-soluble meat proteins and carrageenan or soy protein isolate mixtures were detected in the formulations. Nonetheless, this additive may have changed the conformation of meat proteins, activating functional groups and forming additional disulfide bonds. These changes may have strengthened the gel. It has also been suggested that the myosin light chain could be protected by the additive preventing its destruction during thermal treatment and increasing gel stability. The same effect was achieved in our research via OPH. Small molecules obtained due to hydrolysis may have the ability to penetrate the system and improve the structure. OPH could also change a proportion of α-helices to β-sheets of meat proteins, as such an outcome was achieved in research on potato and meat protein mixtures [62]. The formation of β-sheets by unfolding α-helices was proved to be essential in protein network formation [64].

5. Conclusions

The enzymatic hydrolysis of the by-product obtained from β-glucan production improved solubility, making it useful in meat processing. The pure hydrolysate exhibited moderate antioxidant activity. Despite our expectations, the most significant limitation of the results was that it encouraged oxidation in meat formulations. This phenomenon requires further investigation. The hydrolysate did not affect hardness, but it improved the cohesiveness and resilience of the meat prototype. The key finding of our experiment was the observation that when added to a meat emulsion, the oat by-product had a beneficial effect on colour, particularly with regard to the a* parameter, which is responsible for redness. These improvements in quality could be applied in the manufacturing of nitrite-free meat products, offering benefits to both the meat industry and consumers. This could potentially result in the availability of safer meat products on the market. The findings hold promise for the introduction of oat protein hydrolysates into the meat industry, pending further exploration of consumer acceptance and resolution of the oxidation issue.

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