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Hemp Flour as a Functional Ingredient for the Partial Replacement of Nitrites in a Minced Meat Model: Effect on Nutrient Composition, Antioxidant Profile and Sensory Characteristics

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Featured Application: Incorporation of hemp flour as a natural antioxidant in minced meat products to enhance nutritional quality.

Abstract: Consumers are becoming increasingly concerned about synthetic preservatives like nitrites in meat, prompting the meat industry to explore alternatives in order to lower nitrite levels. This study investigated the effects of incorporating hemp flour on the chemical and shelf-life characteristics of minced meat products with reduced nitrite content. Three types of products were prepared: HF0 (control) (0% hemp flour, 30 mg/kg $NaNO_2$), HF4 (4% hemp flour, 15 mg/kg $NaNO_2$), and HF6 (6% hemp flour, 15 mg/kg $NaNO_2$). Analyses were conducted on proximate composition, fatty acid composition, antioxidant properties, lipid oxidation, colour, texture, and sensory characteristics. The addition of hemp flour at 6% reduced moisture content and influenced ash and sodium chloride levels in minced meat products. Despite the favorable fatty acid profile of hemp flour, its inclusion did not significantly alter the composition of the products. However, it did lead to significantly lower levels of lipid oxidation and modified the antioxidant capacity. Colour attributes were affected, with a higher hemp flour content resulting in colour deterioration. Cooking loss increased with a higher hemp flour content, and the minced meat products were significantly harder. Visual and olfactory sensory evaluation indicated that there were no significant differences in most traits, suggesting consumer acceptance of hemp-flour-enriched minced meat products. Overall, this study highlights the potential of hemp as a functional ingredient in minced meat products, also exhibiting the ability to reduce lipid oxidation.

Keywords: minced meat; hemp flour; sodium nitrite; chemical composition; fatty acid composition; antioxidant profile; colour; texture; sensory characteristics

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1. Introduction

Minced meat products are very popular among consumers and account for a large proportion of consumed meat due to their relatively low price and the variety of types available [1,2]. However, minced meat products are more susceptible to oxidative rancidity than intact muscle, as grinding causes greater expose of the muscle surface to air more extensively [3]. In general, meat processing procedures such as mincing, salting, irradiation, refrigeration, freezing, and cooking affect lipid stability in meat and meat products [4,5]. Therefore, synthetic antioxidants commonly have been used to extend the shelf life of meat products and reduce spoilage [6,7].

Nowadays, consumers are increasingly seeking healthier meat options that are low in salt, fat, cholesterol, and overall calories. Additionally, there is a growing interest in incorporating bioactive components with health-promoting properties, such as carotenoids, unsaturated fatty acids, sterols, and fibre. Moreover, despite the fact that chemical additives are safe for consumers when used according to regulatory guidelines, the prevailing belief that natural compounds are inherently safer has driven the meat industry to explore plant-derived additives to reduce or eliminate the use of artificial ingredients. However, consumers also expect that these innovative meat products, despite their modified formulations, should maintain the familiar taste, appearance, and aroma of their traditionally prepared and processed counterparts [8]. In this respect, herbs and spices, fruit and vegetable extracts, and food industry by-products have been extensively explored as natural "clean label" ingredients with antimicrobial, antioxidant, texturizing, and colouring functions in meat products [6,9–11].

An emerging trend is the application of alternative novel flours from various sources to improve the characteristics and nutritional value of comminuted meat products. Products such as grape skin flour, chestnut flour, beet flour, lentil flour, oat flour, papaya flour, and hemp flour have been employed as functional ingredients in meat products [12–17], with variable results related not only to shelf life characteristics but also to sensory characteristics such as colour and texture. Regarding hemp, the effect of hemp-based additives in meat products, e.g., meatballs with hemp flour [18]; pork meat loaves with hemp seeds, de-hulled hemp seeds, hemp flour, and hemp protein [17]; pork meat burger patties with hemp seed cake [19]; and chicken meat sausage with hemp seed meal [20], has not been extensively studied. Hemp products such as seeds, oil, flour, and de-hulled hemp seeds exhibit antimicrobial and antioxidant properties and contain n-3 fatty acids, protein, and minerals [21].

The European Union approved the cultivation of hemp varieties that contain up to 0.3% THC (tetrahydrocannabinol), a psychotropic substance [22]. Finally, there is a growing interest in food products that contain hemp ingredients due to their high nutritional value. Therefore, its legalisation and increased demand are expected to increase the production of hemp worldwide. As a result, the meat industry can explore the utilisation of hemp products as an alternative food ingredient in processed meat products to improve their quality characteristics.

Nitrite is a multifunctional ingredient widely utilised in meat products, responsible for extending shelf life due to its antimicrobial properties, particularly against *Clostridium botulinum*; oxidation inhibition and its role in developing the characteristic cured colour and flavour of many products [23]. The application of sodium nitrite in meat-based products has been associated with the potential formation of carcinogenic N-nitrosamines, and therefore the application of nitrites in meat products is strictly regulated in most developed countries. Consequently, there is significant consumer demand for low-nitrite or nitrite-free products [24,25].

The aim of this study was to explore the impact of incorporating hemp flour, a natural antioxidant, on the proximate composition, fatty acid composition, antioxidant profile, and sensory characteristics of meat products. In this research, an experimental model employing reduced levels of added nitrite was utilized for refrigerated minced meat products highly susceptible to lipid oxidation.

2. Materials and Methods

2.1. Minced Meat Product Preparation, Treatments, and Storage Conditions

All ingredients were initially weighed and kept at the specified temperature until use. Specifically, pork and beef meat were stored at −18 °C for 2 days, followed by overnight thawing (2–4 °C) in a refrigerator. Subsequently, they were removed from the refrigerator and cut into small pieces. The remaining shelf-stable ingredients (sodium chloride, breadcrumbs, sodium nitrite mixed with sodium chloride, and spice mix) were kept at room temperature. The meat ingredients were minced in a cutter until a homogeneous batter was obtained. The dry ingredients were mixed with water, and the batter was thoroughly blended. Two separate batches of products were prepared for each treatment, with each batch weighing 5 kg, through two independent processes. Hemp flour was obtained from a local shop specializing in selling hemp-based food products. According to the manufacturer's information, the flour was produced in Greece from the cold-processed hemp seeds (*Cannabis sativa* L., Δ^9-tetrahydrocannabinol (THC) < 0.2%) of the Fedora 17 variety that is used in seed/grain production [26]. According to the Greek Ministry of Rural Development and Food, the production of hemp is allowed, provided that the content of the principal psychoactive constituent, Δ^9-tetrahydrocannabinol (THC), remains below 0.2% [27]. The formulation of the products with regard to their ingredients was based on the types of burger products typically sold in traditional butcher shops in Greece but without sodium nitrite addition (Table 1). Hemp flour was incorporated as a partial replacement for wheat breadcrumbs, resulting in the preparation of three types of products: HF0 (control), which did not contain hemp flour; HF4, containing 4% hemp flour; and HF6, containing 6% hemp flour.

Table 1. Ingredient composition of the minced meat products in relation to treatment.

Ingredient	HF0	HF4	HF6
Beef flank meat (g/100 g)	43.0	43.0	43.0
Pork shoulder meat (g/100 g)	43.0	43.0	43.0
Water (g/100 g)	5.5	5.5	5.5
Breadcrumbs (g/100 g)	7.0	3.0	1.0
Hemp flour (g/100 g)	0	4.0	6.0
NaCl (g/100 g)	1.3	1.3	1.3
Spice mix (black pepper, mustard, onion, nutmeg, coriander) (g/100 g)	0.2	0.2	0.2
Sodium nitrite (mg/Kg)	30	15	15

The products were shaped using a plastic burger-maker (Metaltex, Genesterio, Switzerland) to create burgers with average dimensions of approximately 10 cm in diameter and 2 cm in thickness, ensuring uniformity in size and weight. The products were carefully placed in black foam trays. All trays were overwrapped with an oxygen-permeable polyvinyl chloride film, suitable for home food storage, and displayed (under 700 lux of cool-white fluorescent illumination at 4 ± 0.5 °C and a lighting cycle of 16 h on and 8 h off) for 3 days [28].

2.2. Sample Collection for Chemical Analyses

The minced meat products intended for the determination of proximate and fatty acid compositions were collected on storage day 0. The extent of lipid oxidation and the antioxidant profile were assessed using the samples collected on storage day 2. Specimens for proximate composition were stored at 4 °C and analysed within one week of collection, while specimens designated for fatty acid composition and antioxidant profile were stored at −20 °C until analysis. The samples were meticulously hand-mixed with a spatula prior to analysis. All samples were vacuum-packaged to prevent deterioration during storage. All analyses were conducted in duplicate.

2.3. Proximate Composition of Minced Meat Products and Hemp Flour

The proximate composition of the minced meat products was analysed according to standard methods presented in AOAC [29]. Moisture content was assessed using method 950.46, which involved drying the homogenised sample in a convection chamber (ED-115, Binder GmbH, Tuttlingen, Germany) at 102 °C until a constant weight was attained. Ash content was determined utilizing method 920.153, according to which the samples were incinerated at 550 °C for 12 h in a muffle furnace (model LM 412.07, Linn High Therm GmbH, Eschenfelden, Germany) until a light-grey colour ash was obtained. Protein content was determined via method 928.08, involving nitrogen digestion and distillation apparatuses (Turbotherm type TT/12M and Vapodest type 40, Gerhardt Apparate GmbH & Co. KG, Königswinter, Germany), with the result converted to crude protein by multiplying the nitrogen content by 6.25. Fat content was assessed according to method 991.36, using extraction with petroleum ether through a Soxtherm/ Multistat type SE-416 macro-automated system (Gerhardt Apparate GmbH & Co. KG, Königswinter, Germany), with fat content calculated as the proportional difference between the sample's weight before and after solvent extraction. The content of total carbohydrates was calculated by subtracting the sum percentage of moisture, ash, protein, and fat from 100 [30]. The sodium chloride content was analysed using a variation of the 937.09 Volhard method. This involved adding an excess of a standard silver nitrate solution to the sample. The surplus silver nitrate was then titrated back using a standardised solution of ammonium thiocyanate with ferric ion as an indicator. The quantity of silver that reacted with chloride in the sample solution was determined by subtracting the excess silver from the initial silver content. Residual sodium nitrite content was determined according to the International Standard-ISO 2918(E) method [31], according to which nitrites were extracted from the sample, and the absorbance of the formed colour in the presence of sulphanilic acid and α-naphthylamine was read. The concentration of sodium nitrite, expressed in mg/kg, was calculated from a calibration curve.

The protein and fat content of the hemp flour was determined according to standard methods for cereals 979.09 and 920.85 of AOAC [29], respectively, while residual nitrite content was determined using the International Standard ISO 2918(E) method [31], as applied to the minced meat products.

2.4. Fatty Acid Composition and Nutritional Indices

Minced meat products and flour samples were thawed overnight at 4 °C. The following day, fatty acids were extracted and methylated according to the method described by O'Fallon et al. [32]. Briefly, the samples were placed in a screw-capped Pyrex tube, and aqueous KOH solution and methanol were added. The tubes were placed in a water bath (55 °C; 90 min) and vigorously shaken by hand for 5 s every 20 min to properly permeate, dissolve, and hydrolyse the samples. Tubes were cooled in iced water bath, and aqueous H_2SO_4 was added. The contents of the tubes were gently mixed by inversion, and then the tubes were placed again in the water bath (55 °C; 90 min) and shaken for 5 s every 20 min. Tubes were again cooled in iced water bath. Hexane was added, and the tubes were vortex-mixed for 5 min; following that, the tubes were centrifuged at 1100× g for 10 min. The upper phase was filtered through a 0.45 µm pore-size PVDF syringe filter, transferred into amber GC vials, and stored at −20 °C until analysed.

Fatty acid methyl ester analysis was performed on an Agilent Technologies 6890N GC (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a flame ionisation detector (FID) and an Agilent 7683 autosampler. Chromatographic separation was achieved with a fused silica capillary column (Column DB—23 0.25 mm film ID × 60 m × 0.25 mm; Agilent Model 122–2362). The injector temperature was set to 250 °C. The GC conditions were as follows: carrier gas, He; split mode injection, 50:1 (3 µL); injector and FID temperatures, 250 °C and 300 °C, respectively; and initial oven temperature, 110 °C for 6 min, increased at 11 °C per min to 165 °C, increased at 15 °C per min to 195 °C, increased at 7 °C per min to 230 °C, and held at 230 °C for 7 min. Fatty acids were characterised using three commercial standard mixtures: (a) a 37-component FAME mix (Supelco, Bellefonte, PA, USA, 47885-U), (b) PUFA-2 from animal source (Supelco, 47015-U), and (c) a blend of cis- and trans-9,11- and -10,12-octadecadienoic acid methyl esters (Sigma, St. Louis, MO, USA, O5632-250MG) as reference standards. Quantification of fatty acids was performed through peak area measurement, and the outcomes are presented as a percentage (%) of the total peak areas for all quantified acids. The fatty acids were categorised into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and unsaturated fatty acids (UFA).

The fatty acid profiles were used to determine the nutritional indices associated with healthy fat intake. The applied indices are those outlined in the recent review conducted by Chen and Liu [33] for meat products. These researchers systematically compiled information on fatty acid profiles published since 2000 to enhance the understanding of the implications and applications of diverse nutritional indices.

Polyunsaturated fatty acids/Saturated fatty acids ratio

$$\mathrm{PUFA/SFA} = \frac{\Sigma\mathrm{PUFA}}{\Sigma\mathrm{SFA}}$$

Atherogenicity Index

$$\mathrm{AI} = \frac{(\mathrm{C12:0} + (4 \times \mathrm{C14:0}) + \mathrm{C16:0})}{\Sigma\mathrm{UFA}}$$

Thrombogenicity Index

$$\mathrm{TI} = \frac{\mathrm{C14:0} + \mathrm{C16:0} + \mathrm{C18:0}}{((0.5 \times \Sigma\mathrm{MUFA}) + (0.5 \times \Sigma\mathrm{n-6PUFA}) + (3 \times \Sigma\mathrm{n-3PUFA}) + (\mathrm{n-3PUFA/n-6PUFA}))}$$

Hypocholesterolaemic/hypercholesterolaemic fatty acid ratio (h/H)

$$\mathrm{h/H} = \frac{\mathrm{C18:1n-9cis} + \Sigma\mathrm{PUFA}}{(\mathrm{C12:0} + \mathrm{C14:0} + \mathrm{C16:0})}$$

2.5. Total Phenolic Content and Antioxidant Profile

A methanolic extract was prepared for the determination of the total phenolic content of hemp flour [34]. Similarly, the total phenolic content and the antioxidant potential of the minced meat products was measured in an aqueous extract that was prepared according to the method described by Jung et al. [35]. Briefly, the samples were homogenised in distilled water using a Polytron (Kinematica AG, Littau, Switzerland model PT-MR 300). Chloroform was added, and after vigorous shaking, the lipids and the aqueous supernatant were separated. Hemp flour and minced meat product extracts were kept in aliquots in 1.5 mL Eppendorf tubes at −20 °C until they were analysed.

The total phenolic content (TPC) of the samples was determined using the Folin–Ciocalteau method [7], with the results reported as milligrams of Gallic Acid Equivalents (GAE) per g of sample. The Total Antioxidant Capacity of the samples was determined using the ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] method, following a modification of the procedure outlined by Re et al. [36]. The results were expressed as μM of Trolox equivalents (TE) per g of sample. Free radical scavenging activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, with adjustments made to the protocol described by Sanchez-Moreno et al. [37]. The results were reported as μM of Trolox equivalents (TE) per gram of sample, with Trolox serving as a water-soluble analogue of vitamin E. The Reducing Power Activity of the samples was measured using the FRAP (Ferric Reducing Antioxidant Power) method, as reported by Pulido et al. [38], with minor modifications. The results were expressed as μM of Trolox equivalents (TE) per g of sample.

2.6. Lipid Oxidation

Lipid oxidation, on storage day 2, was determined on the basis of the formation of thiobarbituric acid reactive substances (TBARS) using a modification of the method of Vyncke [39] as described by Kasapidou et al. [28]. In short, the sample was homogenised with aqueous trichloroacetic acid, including both n-propyl gallate and ethylenediaminetetraacetic acid disodium salt, using a Polytron (Kinematica AG, Littau, Switzerland model PT-MR 300). After approximately 15–20 min required for TBARS extraction, the resulting slurry was filtered. An aliquot of the filtrate was then mixed with aqueous thiobarbituric acid. Concurrently, a blank sample with trichloroacetic acid and thiobarbituric acid solutions was prepared. Samples were left overnight in the dark at room temperature.

The following day, absorbance at 532 nm was measured against the blank sample using a UV–VIS spectrophotometer (U-2800 Double Beam Spectrophotometer, Hitachi, Tokyo, Japan). TBARS were calculated using 1,1,3,3-tetraethoxypropane as a standard and reported as mg of malonaldehyde per kg of the sample.

2.7. Physicochemical Characteristics

The pH of the samples was determined using a glass electrode equipped with a built-in temperature sensor (model 5014T, Crison Instruments, Barcelona, Spain) in a pH meter (model GLP 21, Crison Instruments, Barcelona, Spain). Calibration of the pH meter was carried out following the manufacturer's guidelines, using standard buffer solutions with pH values of 4.0 and 7.0. Ten grams of sample were weighed and homogenised with 100 mL of distilled water using a Polytron (Kinematica AG, Littau, Switzerland model PT-MR 300) for 1 min. The electrode was rinsed with distilled water before testing and immersed in the meat mixture to measure the pH of the sample [40].

Water activity (a_w) was determined in intact sample specimens using a Hydrolab 3 water activity meter (Rotronic AG, Bassersdorf, Switzerland) according to manufacturer's instructions. A disposable deep cup (PS-40) was filled with the sample, and the cup was placed in the sample holder. The probe was placed on top of the sample holder, and the measurement cycle, which lasted 5 min, was initiated. Prior to measurement, the disposable sample cups, with the covers on, were placed on the same working bench as the probe to allow sufficient time for the samples to reach room temperature.

2.8. Instrumental Colour Measurement

Colour measurements were conducted on storage days 1, 2, and 3 on raw samples and on cooked samples that were prepared on storage day 2. Colour measurements were carried out using a Minolta CR-410 colorimeter (Konica Minolta Company, Osaka, Japan) with a 50 mm measuring area (aperture size) using illuminant source C and 2° standard observer angle. The light projection tube (CR-A33a, Konica Minolta, Japan) was placed over the aperture port while measurements were conducted. Before measurement, calibration was performed using a white calibration plate (Y = 93.9, x = 0.3161, y = 0.3329). Sample colour was expressed using the L* a* b* system (L* a* b* represent luminosity, redness, and yellowness respectively). Chroma (colour saturation) and hue angle values were determined according to the following equations, as reported in Kasapidou et al. [28].

$$\text{Chroma} = \sqrt{\left(a*^2 + b*^2\right)}$$

$$\text{Hue angle} = \tan^{-1}\left(\frac{b^*}{a^*}\right) \times \left(\frac{180}{\pi}\right)$$

All L*, a*, and b* values are the instrumental average of three independent measurements collected from random sites across the sample, avoiding small areas of severe discolouration.

Total colour difference (ΔE_{Lab}) between control (HF0) and samples containing hemp flour (HF4 and HF6) within the same storage day was also calculated according to the following equation:

$$\Delta E_{\text{Lab}} = \sqrt{(L*_C - L*_T)^2 + (a*_C - a*_T)^2 + (b*_C - b*_T)^2}$$

where C = Control (HF0) and T = treated samples containing hemp flour (either HF4 or HF6 treatments).

2.9. Cooking Loss and Texture Profile Analysis

The minced meat products were placed on stainless steel trays and cooked at 180 °C in a forced-air domestic oven until they reached an internal (core) temperature of 72 °C. The temperature was monitored with a digital instant-read thermometer with a stainlesssteel pointed probe, and the meat products were turned every 3 min. Cooking time and temperatures were selected to simulate domestic practice.

Cooking loss was defined as the percentage of fluids, comprising water, proteins, fats, and minerals, lost after cooking the sample [41]. This loss was calculated by measuring the differences in weight before and after cooking according to the following equation:

$$\text{Cooking loss } (\%) = \left(\frac{\text{Weight before cooking} - \text{Weight after cooking}}{\text{Weight before cooking}} \right) \times 100$$

The texture profile that simulates biting action in the mouth was determined using the Perten TVT 6700 texturometer and TexCal5® instrumental software (Perten Instruments, Hägersten, Sweden) according to a modification of method 56.01 (red meat properties) by double cycle compression. Cylinder probe 67.30.20 (P-CY20S with a 20 mm diameter) and a 10 kg load cell were employed in this procedure. The test was conducted on $2 \times 1 \times 1$ cm^3 cubes that were cut from or near the centres of the samples, as described by Zhou et al. [42]. The examined textural parameters were hardness, springiness, cohesiveness, gumminess, and chewiness. The minced meat products were cooked and allowed to cool down before being subjected to the test.

2.10. Sensory Evaluation

The raw minced meat products were assessed by an untrained student panel comprising nine members. A student panel was chosen because students frequently consume minced meat products such as burgers, meat balls, etc. Additionally, when asked, students reported being more willing to consume products containing hemp ingredients compared to senior consumers [43,44]. The products were presented monadically in a black foam tray coded with a random 3-digit number. The presentation order of samples was randomised for all the participants. Cooked products were also assessed in a similar manner. The evaluation of the products included colour, colour uniformity, overall appearance, odour, odour intensity, and overall impression and was carried out using a 7-point hedonic scale, as presented in Table 2.

Table 2. Sensory evaluation descriptors of the raw and cooked minced meat products.

Ingredient	**Hedonic Scale Descriptor**		
	1	**4**	**7**
Raw minced meat products			
Colour	Pale red	Red	Dark red
Colour uniformity	Extremely non-uniform	Normal	Extremely uniform
Overall appearance	Unacceptable	Normal	Exceptional
Odour	Unacceptable	Normal	Exceptional
Odour intensity	Extremely weak	Average	Extremely intense
Overall impression	Unacceptable	Acceptable	Exceptional
Cooked minced meat products			
Overall appearance	Unacceptable	Acceptable	Overall appearance
Colour	Extremely weak	Average	Extremely intense
Odour intensity	Unacceptable	Acceptable	Exceptional
Odour	Unacceptable	Acceptable	Exceptional

2.11. Statistical Analysis

Data were arranged according to treatment average ± standard deviation (SD). Levene's test was employed to assess the homogeneity of variances. One-way analysis of variance was used, followed by Tukey's post-hoc test in cases of homogeneity of variances. In cases where variance homogeneity was not met, the Games–Howell test was applied to assess differences between treatments. Statistical significance was determined based on p-values < 0.05 for all tests. Data analysis was performed using SPSS software (version 29.0, SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Proximate Composition of Hemp Flour and Minced Meat Products

The proximate composition of hemp flour is presented in Table 3. The protein and fat contents were found to be lower than those reported by Siano et al. [45] for flour obtained from the Fedora cultivar, with a protein content of 30.7% and that of fat of 13.6%. The nutrient content of hemp flour from the Fedora cultivar has not been extensively studied. However, in various studies, the reported protein and fat content ranges from 22.4 to 36.2 g/100 g and 4.7 to 9.1 g/100 g, respectively [46–48]. The residual sodium nitrite content of this type of hemp flour is 1.18 mg/kg, but there are no publications, to the best of our knowledge, studying the nitrite content of hemp flour that would allow comparison of the results. In general, this product's residual nitrite content is low compared to that of other food products of plant origin [49].

Table 3. Compositional characterisation of hemp flour.

Variable	Value
Proximate composition	
Protein (g/100 g)	24.5
Fat (g/100 g)	9.4
Sodium Chloride (g/100 g) [1]	0.02
Sodium Nitrite (mg/kg)	1.18
Fatty acid composition	
C16:0	9.47
C18:0	3.29
C18:1	15.84
C18:2 *n-6 cis*	52.57
C18:3 *n-6*	2.81
C18:3 *n-3*	11.88
SFA	14.39
MUFA	16.11
PUFA	68.82
Antioxidant profile	
TPC [2] (mg GAE/g)	1.10

[1] Based on declared composition on product label; [2] total phenolic content (TPC) expressed in mg of gallic acid equivalents (GAE)/g.

Table 4 shows the proximate compositions and residual nitrite levels of the minced meat products. Significant differences were observed in terms of moisture content, with samples in the HF6 treatment showing lower moisture content. The ash content also differed significantly ($p < 0.01$) between the samples in the control treatment (HF0) and those in the treatments containing hemp flour (HF4 and HF6). This difference is attributed to the markedly higher levels ($p < 0.001$) of sodium chloride in the HF0 group compared to those in the HF4 and HF6 groups. The sodium chloride content of the wheat breadcrumbs (1.5 g/100 g—the declared value) contributed to the variations in sodium chloride observed between treatments. This significant difference ($p < 0.001$) in sodium nitrite levels stems from the levels of added nitrites within the batter of the minced meat products. The content of residual nitrites from the hemp flour was negligible, given that the sodium nitrite content was 1.18 mg/kg (Table 3). The addition of 6% hemp flour lowered the sodium chloride content to approximately 15%, resulting in a healthier product [50,51] and aligning with the food reformulation efforts toward reducing sodium intake [52], considering that processed meat products comprise one of the major sources of dietary sodium intake in the form of sodium chloride [53].

Table 4. Proximate composition of the minced meat products (mean values ± SD).

Variable	Treatment			Significance
	HF0	HF4	HF6	
Moisture (g/100 g)	61.19 ± 0.69	62.33 ^{b} ± 1.82	60.02 ^{a} ± 0.71	*
Ash (g/100 g)	2.46 ^{b} ± 0.21	2.19 ^{a} ± 0.17	2.11 ^{a} ± 0.05	**
Protein (g/100 g)	14.30 ± 1.10	13.99 ± 0.37	14.87 ± 1.66	NS
Fat (g/100 g)	15.08 ± 2.36	13.78 ± 1.80	16.14 ± 2.46	NS
Carbohydrate (g/100 g)	6.96 ± 2.72	7.70 ± 3.20	6.86 ± 3.19	NS
Sodium chloride (g/100 g)	1.49 ^{b} ± 0.11	1.39 ^{b} ± 0.04	1.27 ^{a} ± 0.05	***
Sodium nitrite (mg/kg)	2.98 ^{b} ± 0.03	1.54 ^{a} ± 0.08	1.60 ^{a} ± 0.04	***

NS = Non-significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; Superscripts a and b differ at $p < 0.05$.

The inclusion of hemp flour at 6% levels resulted in a lower moisture content, a result consistent with the findings reported by Kotecka-Majchrzak et al. [18] for meat balls containing varying hemp cake levels and the results reported by Zając et al. [17] regarding pork meat loaves containing hemp flour. Additionally, there were no differences in the ash content in meat balls and meat loaves containing either hemp cake or hemp flour [17,18]. Despite hemp flour having a high protein content (24.5 g/100 g) and moderate fat content (9.4 g/100 g), (Table 3) there were no alterations in the protein and fat levels of the minced meat products, indicating that the added hemp flour levels were insufficient to affect the nutrient composition of the patties. These findings align with the results from the previously mentioned studies conducted by Kotecka-Majchrzak et al. [18] and Zając et al. [17].

As significant differences ($p < 0.05$) existed in regard to moisture content, the content of ash, protein, fat, carbohydrate, and sodium chloride was also determined on a dry matter basis (the corresponding results are not presented). The same pattern of differences between treatments was also observed when the proximate composition was expressed on a dry matter basis.

3.2. Fatty Acid Composition and Nutritional Indices

The fatty acid composition of the minced meat products, both as individual fatty acids and as different lipid classes, is presented in Table 5. Myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids were the predominant saturated fatty acids in the products across all treatments. Oleic acid (C18:1 *cis*-9) and linoleic acid (C18:2 *n-6 cis*) were the primary monounsaturated and polyunsaturated fatty acids, respectively. Significant differences ($p < 0.05$) were observed in the levels of eicosatrienoic acid (C20:3 *n-3*), arachidonic acid (C20:4 *n-6*), and lignoceric acid (C24:0); these differences could be related to product formulation, i.e., decreasing levels of breadcrumbs and increasing levels of hemp flour. Regarding lipid classes, variations were noted only in the levels of *n-3* fatty acids, which also decreased gradually with an increasing hemp flour content. Overall, there were no differences in the fatty acid profiles between treatments, indicating that the addition of hemp flour did not favourably impact the fatty acid composition of the minced meat products. The most abundant fatty acids in the hemp flour were linoleic (C18:2 *n-6*), oleic (C18:1), and linolenic acid (C18:3 *n-3*) (Table 3), and this result is in general agreement with the findings of Absi et al. [54]. Hemp flour is a defatted product; this factor, along with the relatively low added amount, could explain why the favourable fatty acid profile of hemp flour (Table 3) was not reflected in the fatty acid composition of the minced meat products.

The composition of meat products is primarily influenced by the fatty acid composition of the meat used. Similar findings were reported by Zając et al. [17] for pork meat loaves containing 5% hemp flour. However, these researchers found differences in fatty acid composition when the pork meat loaves contained either hemp protein, hemp seeds, or dehulled hemp seeds.

Table 5. Fatty acid composition (% of total identified fatty acids) of the minced meat products (mean values ± SD).

Variable	Treatment			Significance
	HF0	HF4	HF6	
Fatty acid				
C10:0	0.10 ± 0.02	0.09 ± 0.01	0.09 ± 0.01	NS
C12:0	0.28 ± 0.10	0.25 ± 0.05	0.21 ± 0.06	NS
C14:0	2.05 ± 0.25	2.08 ± 0.13	2.10 ± 0.26	NS
C14:1	0.39 ± 0.13	0.42 ± 0.08	0.43 ± 0.12	NS
C15:0	0.37 ± 0.11	0.36 ± 0.07	0.41 ± 0.09	NS
C15:1	0.17 ± 0.06	0.17 ± 0.04	0.17 ± 0.04	NS
C16:0	23.57 ± 0.40	23.77 ± 0.48	23.76 ± 0.61	NS
C16:1	2.20 ± 0.21	2.43 ± 0.18	2.27 ± 0.08	NS
C17:0	0.92 ± 0.23	0.90 ± 0.11	0.96 ± 0.17	NS
C17:1	0.46 ± 0.08	0.49 ± 0.05	0.47 ± 0.03	NS
C18:0	18.10 ± 2.38	17.29 ± 1.12	18.49 ± 1.37	NS
C18:1 *trans*	0.69 ± 0.20	0.66 ± 0.16	0.62 ± 0.23	NS
C18:1 *cis-9*	38.29 ± 2.16	39.28 ± 0.46	38.90 ± 1.15	NS
C18:1 *trans-11* (VA)	0.16 ± 0.03	0.17 ± 0.03	0.15 ± 0.03	NS
C18:2 *n-6 trans*	0.17 ± 0.05	0.15 ± 0.03	0.13 ± 0.02	NS
C18:2 *n-6 cis*	7.88 ± 1.41	7.69 ± 1.30	7.40 ± 1.35	NS
C18:3 *n-6*	0.15 ± 0.03	0.17 ± 0.02	0.18 ± 0.04	NS
C18:3 *n-3*	0.64 ± 0.11	0.60 ± 0.04	0.64 ± 0.05	NS
C18:2 *cis-9 trans-11* (CLA)	0.36 ± 0.06	0.31 ± 0.08	0.32 ± 0.07	NS
C21:0	0.60 ± 0.09	0.57 ± 0.04	0.55 ± 0.07	NS
C20:2	0.41 ± 0.08	0.40 ± 0.03	0.36 ± 0.07	NS
C20:3 *n-6*	0.22 ± 0.04	0.19 ± 0.02	0.16 ± 0.03	*
C20:4 *n-6*	0.56 ± 0.08	0.49 ± 0.05	0.42 ± 0.09	*
C20:3 *n-3*	0.15 ± 0.03	0.13 ± 0.01	0.12 ± 0.03	NS
C22:0	0.16 ± 0.07	0.12 ± 0.02	0.10 ± 0.03	NS
C20:5 *n-3*	0.21 ± 0.06	0.20 ± 0.05	0.16 ± 0.04	NS
C24:1	0.28 ± 0.10	0.27 ± 0.05	0.22 ± 0.04	NS
C24:0	0.19 ± 0.08	0.17 ± 0.03	0.10 ± 0.04	*
C22:6 *n-3*	0.17 ± 0.10	0.13 ± 0.02	Nd †	NS
Lipid class				
SFA [1]	45.77 ± 1.27	45.07 ± 0.95	46.28 ± 1.68	NS
MUFA [2]	43.34 ± 2.00	44.54 ± 0.48	43.85 ± 0.99	NS
PUFA [3]	10.89 ± 1.46	10.39 ± 1.22	9.88 ± 1.51	NS
n-3	1.15 ^{b} ± 0.16	0.99 ± 0.07	0.91 ^{a} ± 0.09	**
n-6	8.98 ± 1.35	8.68 ± 1.27	8.29 ± 1.42	NS

1 = Saturated fatty acids; 2 = monounsaturated fatty acids; 3 = polyunsaturated fatty acids; NS = Non-significant; * = $p < 0.05$; ** = $p < 0.01$; Superscripts a and b differ at $p < 0.05$; † = not determined.

The effect of hemp addition on the lipid quality nutritional indices of the minced meat products is shown in Table 6. The PUFA/SFA ratio is commonly used to assess the nutritional quality of consumed fats. This ratio suggests that polyunsaturated fatty acids (PUFA) might lower levels of low-density lipoprotein cholesterol and total serum cholesterol, whereas saturated fatty acids (SFA) tend to raise serum cholesterol levels. Therefore, a higher PUFA/SFA ratio is associated with a more beneficial effect on cardiovascular health [33], with the recommended threshold value being 0.45 [55].

Table 6. Minced meat product nutritional indices in relation to healthy fat consumption (mean values ± SD).

Index	Treatment			Significance
	HF0	HF4	HF6	
PUFA/SFA [1]	0.24 ± 0.04	0.23 ± 0.03	0.21 ± 0.04	NS
AI [2]	0.59 ± 0.04	0.59 ± 0.02	0.60 ± 0.04	NS
TI [3]	1.47 ± 0.13	1.45 ± 0.06	1.54 ± 0.10	NS
h/H [4]	3.57 ± 0.15	3.61 ± 0.07	3.55 ± 0.14	NS

1 = Polyunsaturated fatty acids/ Saturated fatty acids ratio; 2 = atherogenicity index; 3 = thrombogenicity index; 4 = hypocholesterolaemic: hypercholesterolaemic ratio; NS = Non-significant.

The Atherogenicity Index (AI) indicates the relationship between saturated fatty acids (SFA), such as lauric (C12:0), myristic (C14:0), and palmitic acid (C16:0), which are known to potentially promote atherosclerosis, and unsaturated fatty acids (UFA), recognised for their anti-atherogenic properties as they inhibit plaque formation and reduce levels of phospholipids, cholesterol, and esterified fatty acids. The Thrombogenicity Index (TI) assesses the clot-forming potential of fatty acids in blood vessels. Maintaining low values for both indices, preferably below 3 [56], is considered beneficial for human health. In the present study, the values for both the AI and TI remained below the recommended threshold and within the reported levels for beef [57], but they were lower than the reported levels for pork meat [58].

No significant differences ($p > 0.05$) were observed in the hypocholesterolemic to hypercholesterolemic (h/H) ratio among samples from the various treatments. The h/H ratio is used to depict the relationship between hypocholesterolemic and hypercholesterolemic fatty acids, with higher values being considered desirable.

Zając et al. [17] also noted that the inclusion of hemp flour had no impact on nutritional indices, such as AI, TI, and h/H, in pork meat loaves. In this study, the batter of the minced meat products comprised equal proportions of beef and pork meat, thus it would not be appropriate to evaluate the studied product's lipid quality by comparing it to similar products made solely from either beef or pork meat.

3.3. Antioxidant Profile

The effect of hemp flour addition on the antioxidant profile of minced meat products under simulated retail display conditions is presented in Table 7. As shown, there were significant ($p < 0.05$) differences in the total phenolic content (TPC), and lower levels were observed in samples on the HF4 treatment. Highly significant differences ($p < 0.001$) were also found in the total antioxidant capacity (ABTS), free radical scavenging activity (DPPH), and ferric reducing power activity (FRAP) between treatments, indicating that the addition of hemp flour modified the antioxidant capacity of the products in treatments HF4 and HF6 compared to those in treatment HF0. The variation in expression of antioxidant activity, whether measured as total antioxidant capacity, free radical scavenging activity, or ferric reducing power activity, within the same sample is attributed to the different mechanisms employed in each assay (electron transfer or mixed mode, i.e., hydrogen atom transfer and electron transfer). This is also related to the suitability of each assay for determining the antioxidant capacity of meat and meat products, as discussed in detail in the review study by Echegaray et al. [59].

Sun et al. [20] found increased radical scavenging activity in raw and cooked chicken meat sausages containing hemp seed meal. Additionally, the combined effect of phenolic compounds and nitrite is not clear. The recent review study of Ferysiuk and Wójciak [10], examining the reduction of nitrite levels in meat products through the application of plant-based ingredients, reported that this effect might be antagonistic or even pro-oxidant, and this could explain the differences in the free radical scavenging activity and ferric reducing antioxidant power values between the control (HF0) and treated samples (HF4 and HF6).

Table 7. Antioxidant profile of the minced meat products on retail display day 2 (mean values ± SD).

Variable	Treatment			Significance
	HF0	HF4	HF6	
TPC [1] (mg GAE/g)	0.68 ± 0.01	0.61 ± 0.09	0.68 ± 0.00	*
ABTS [2] (μM TE/g)	1751.96 ^{a} ± 62.83	1869.27 ^{b} ±027.12	1951.15 ^{c} ± 26.38	***
DPPH [3] (μM TE/g)	1398.23 ^{b} ± 131.87	1625.47 ^{c} ± 12.94	901.55 ^{a} ± 45.62	***
FRAP [4] (μM TE/g)	751.51 ^{c} ± 99.67	587.20 ^{b} ± 67.82	462.70 ^{a} ± 12.37	***

1 = Total phenolic content (TPC); 2 = Total antioxidant capacity; 3 = Free radical scavenging activity; 4 = Ferric reducing antioxidant power; * = $p < 0.05$; *** = $p < 0.001$; Superscripts a, b, and c differ at $p < 0.05$.

3.4. Lipid Oxidation

Hemp flour addition resulted in significantly lower ($p < 0.001$) lipid oxidation levels of the minced meat products on storage day 2 of simulated retail display (Table 8). However, increasing the hemp flour content did not further decrease oxidation levels in the minced meat products. The combination of sodium nitrite and hemp flour (treatments HF4 and HF6) resulted in lower oxidation when compared to the products containing sodium nitrite alone, i.e., treatment HF0. Kotecka-Majchrzak et al. [18] also reported lower lipid oxidation levels in meat balls containing either 4.2 or 7.4% hemp cake. Similar to our study, higher levels of hemp cake did not result in a further reduction in lipid oxidation. On the other hand, Zając et al. [17] and Sun et al. [20] reported significantly higher TBARS values in pork meat loaves containing 5% hemp flour and in chicken meat sausages containing 30–40% hemp seed meal and attributed these findings to the higher levels of polyunsaturated fatty acids as well as the loss of the antioxidant compounds present in the hemp products during processing. The antioxidant activity of hemp flour is attributed to its phenolic content. Phenolic compounds exhibit excellent free radical scavenging activity, effectively preventing lipid oxidation and extending the shelf life of meat products [60]. Hemp flour is produced from hemp seeds that have a high phenolic content and exhibit good antioxidant activity [34,61]. Additionally, hemp seeds mainly contain γ—tocopherol and lower amounts of α—tocopherol, both known for their antioxidant activity [27,62]. The effects of various plant-based additives, including extracts and powders, either alone or in combination with sodium nitrite with respect to limiting lipid oxidation in meat products has been well documented in recent reviews by Ferysiuk and Wójciak [10] and Stoica et al. [25]. It should be also noted that many of these ingredients contain naturally occurring nitrates, which can be converted into nitrites during the curing process [63]. Malondialdehyde is a relatively stable secondary product resulting from the lipid oxidation of polyunsaturated fatty acids and is therefore considered a major marker of lipid oxidation [5,64]. The threshold values at which consumers may detect rancidity are 0.5 and 2 mg of malondialdehyde/kg sample for pork and beef meat respectively [65,66]. Beef meat is more susceptible to oxidation than pork meat due to the higher content of iron and myoglobin. Based on the above, the possibility of perceiving oxidised flavour in the products from all treatments was low. Additionally, the flavour of hemp flour might mask the rancid flavour and not affect consumer acceptance.

Table 8. Extent of lipid oxidation of the minced meat products on retail display day 2 (mean values ± SD).

	Treatment			Significance
TBARS (mg/kg sample)	HF0	HF4	HF6	
	0.844 ^{b} ± 0.157	0.434 ^{a} ± 0.151	0.268 ^{a} ± 0.080	***

*** = $p < 0.001$; Superscripts a and b differ at $p < 0.05$.

3.5. Physicochemical Characteristics

The active acidity (pH) and water activity (a_w) of the minced meat products on retail display day 2 are presented in Table 9. There was a highly significant effect ($p < 0.001$) of hemp flour addition on the pH of the products, which decreased as the level of hemp flour addition increased. However, no significant differences ($p > 0.05$) were observed in relation to water activity (a_w) between treatments. Kotecka-Majchrzak et al. [18] found that adding hemp cake did not affect the pH of raw meatballs containing levels of hemp cake comparable to the added levels of hemp flour in our study, specifically 4.2% and 7.4%. Furthermore, the latter authors found that hemp cake affected water activity (a_w) in a manner similar to that observed in our study. Yuan et al. [67] reported lower pH values in frankfurter sausages containing micronised cold-pressed hemp seed cake and reduced phosphates in a dose–response manner and related this effect to the buffering effect of the proteins in the hemp seed cake. Kernel et al. [19] found that hemp seed cake increased the pH value of grilled pork burger patties and related this effect to the addition of a small amount of buffer-type compounds present in hemp. Regarding water activity (a_w), the same researchers found that the addition of hemp seed cake resulted in higher values as storage progressed. Wójciak et al. [68] similarly observed decreased values for active acidity (pH) and increased values for water activity (a_w) in minced roast beef as the inclusion level of sodium nitrite decreased.

Table 9. Active acidity (pH) and water activity (a_w) of the minced meat products on retail display day 2 (mean values ± SD).

Variable	Treatment			Significance
	HF0	**HF4**	**HF6**	
Active acidity (pH)	6.05 ^{c} ± 0.04	5.95 ^{b} ± 0.03	5.60 ^{a} ± 0.03	***
Water activity (a_w)	0.917 ± 0.010	0.922 ± 0.003	0.923 ± 0.003	NS

NS = Non-significant; *** = $p < 0.001$; Superscripts a, b, and c differ at $p < 0.05$.

3.6. Instrumental Colour Measurement

Changes in colour attributes during simulated retail display are shown in Table 10. As seen, the addition of hemp flour did not significantly affect ($p > 0.05$) the lightness (L*) and yellowness (b*) of the minced meat products on the same storage day. Kotecka-Majchrzak et al. [18] and Kernel et al. [19] reported that the addition of hemp-based ingredients resulted in a decrease in lightness, which refers to the brightness or darkness of a product. These same researchers [17,18] also found that adding hemp products to meatballs and burger patties had a limited impact on yellowness, which can be primarily related to the colour of hemp flour [20]. Regarding redness (a*), highly significant differences were observed on storage days 2 ($p < 0.01$) and 3 ($p < 0.001$). The same pattern was observed in colour saturation (Chroma) values, as expected, whereas hue angle values were less affected. The colour differences are related to the greenish-brown colour of hemp flour. As demonstrated by the colour deterioration during simulated retail display, primarily assessed in terms of redness (a*) and colour saturation (Chroma), samples from treatment HF6 exhibited greater deterioration. Lower redness (a*) values were observed for beef burgers containing levels of Moringa (*Moringa oleifera*) seed flour similar to those used in the present study, and the authors attributed this to the dilution of myoglobin [69]. Moringa seed flour and hemp flour have a similar greenish colour. Finally, with regard to the significant changes ($p < 0.05$) in hue values on both storage days 2 and 3, it was observed that the samples from the HF6 treatment and HF0 treatment had different colour descriptions [70]. Photographs of the minced meat products on display day 1 are shown in Figure 1.

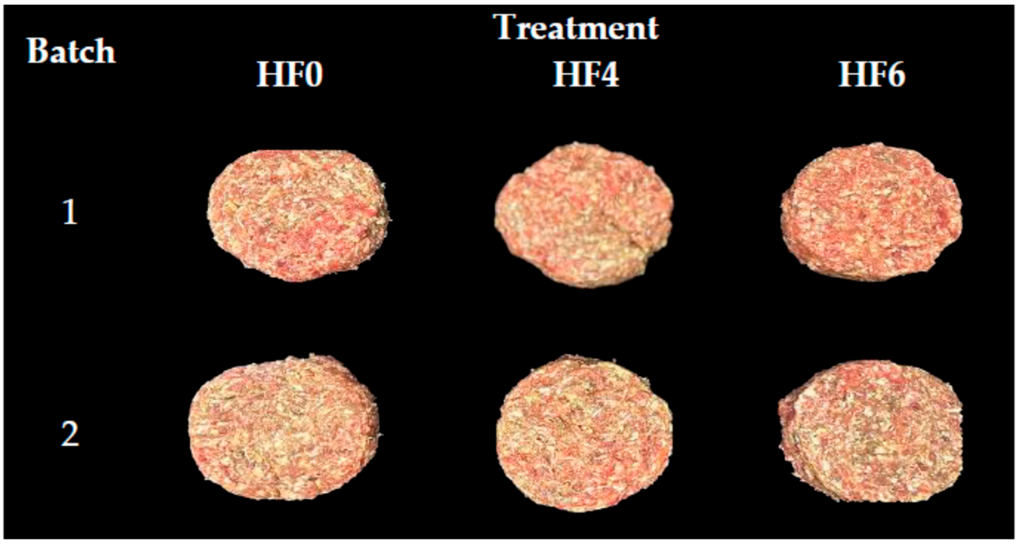


Figure 1. Minced meat products on display day 1.

Table 10. Instrumental colour changes during simulated retail display of raw minced meat products, and colour of cooked minced meat products (mean values ± SD).

Storage Day/Variable	Treatment			Significance
	HF0	HF4	HF6	
Raw minced meat products				
Lightness (L*)				
1	49.30 ±1.04	48.61 ± 1.05	49.32 ± 0.93	NS
2	49.41 ± 1.17	49.49 ± 1.47	49.24 ± 1.07	NS
3	50.52 ± 1.41	49.52 ± 1.57	51.70 ± 1.64	NS
Redness (a*)				
1	13.40 ± 2.05	14.57 ± 1.92	13.75 ± 2.35	NS
2	13.23 ^{b} ± 0.62	13.43 ^{b} ± 0.91	11.50 ^{a} ± 1.04	**
3	12.65 ^{b} ± 0.99	11.04 ^{b} ± 0.80	9.29 ^{a} ± 1.47	***
Yellowness (b*)				
1	6.13 ± 0.70	5.83 ± 0.61	5.52 ± 0.78	NS
2	5.58 ± 0.61	6.23 ± 0.73	6.03 ± 0.74	NS
3	6.23 ± 0.63	6.47 ± 0.58	6.15 ± 0.91	NS
Chroma (Saturation index)				
1	14.78 ± 1080	15.70 ± 1.89	14.85 ± 2.30	NS
2	14.38 ^{b} ± 0.51	14.82 ^{b} ± 0.91	13.00 ^{a} ± 1.07	**
3	14.11 ^{b} ± 1.01	12.81 ^{b} ± 0.72	11.20 ^{a} ± 1.20	***
Hue angle				
1	24.94 ± 4.63	21.98 ± 2.65	22.16 ± 3.75	NS
2	22.92 ^{a} ± 2.83	24.93 ± 2.89	27.70 ^{b} ± 3.20	*
3	26.25 ^{a} ± 2.43	30.43 ± 3.07	33.82 ^{b} ± 6.06	*
Cooked minced meat products—Display day 2				
Lightness (L*)	32.49 ± 6.01	40.46 ± 6.02	42.59 ± 8.42	NS
Redness (a*)	23.64 ^{b} ± 8.07	12.14 ^{a} ± 1.12	16.15 ^{a} ± 0.85	**
Yellowness (b*)	11.20 ± 2.53	8.42 ± 2.65	10.72 ± 3.84	NS
Chroma (Saturation index)	26.20 ^{b} ± 8.32	14.89 ^{a} ± 2.04	19.58 ^{b} ± 2.47	**
Hue angle	26.37 ± 4.18	34.04 ± 7.83	32.73 ± 8.86	NS

NS = Non-significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; Superscripts a and b differ at $p < 0.05$.

Since there were no significant ($p > 0.05$) differences between samples from the HF0 and HF4 treatments in terms of redness (a*) values, it can be hypothesised that there was a synergistic effect between the polyphenols present in the hemp flour and the sodium nitrate that resulted in reduced myoglobin oxidation in the samples from the HF4 and HF6 groups. Furthermore, the greater content of hemp flour in the samples from the HF6 treatment resulted in the darkening of the samples and subsequently lower redness (a*) values. The synergistic effect of polyphenols and sodium nitrites has been proposed by other researchers examining plant-based ingredients, i.e., regarding the effect of grape pomace or tea polyphenols on the colour stability of meat products [71,72].

According to Tomasevic et al. [73], total colour difference (ΔE_{Lab}) serves as an index for evaluating differences between samples from different treatment groups. A higher ΔE_{Lab} value indicates greater colour difference compared to control samples. In the present study, the ΔE_{Lab} value ranged between 2 and 3.5 for the samples from the HF0 and HF4 treatments, indicating that the colour difference was noticeable even to an inexperienced observer throughout the entire display period (Figure 2). Similarly, for the samples used in the HF0 and HF6 treatments, the ΔE_{Lab} value ranged between 2 and 3.5 on display days 1 and 2, while it exceeded 3.5 on display day 3, indicating a clear difference [19]. However, ΔE_{Lab} threshold values depend on the characteristics of a sample, and there are no precise data on colour difference threshold values for meat products [74].

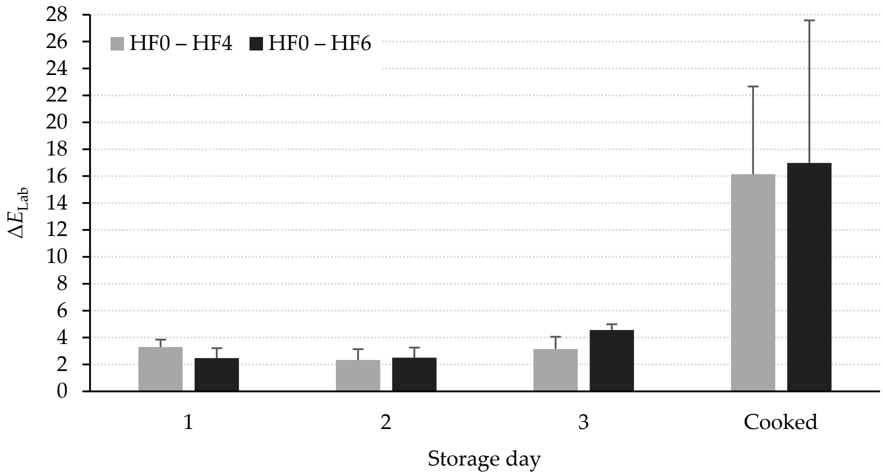


Figure 2. Total colour difference (ΔE_{Lab}) between control (HF0) and samples containing hemp flour (HF4 and HF6).

Instrumental colour evaluation of the cooked minced meat products revealed highly significant differences ($p < 0.01$) in redness (a*) and colour saturation (Chroma) values (Table 9). The effect of heat treatment on minced meat products containing hemp products is variable. The addition of hemp products resulted in lower redness (a*) values in meatballs, meat loaves and sausages [17,18,20]. However, other researchers found that hemp products did not affect redness (a*) in pork meat patties [19]. Parameters related to hemp product type and added amount influence colour differences. Chicken meat sausage samples with 10–40% hempseed meal showed significant colour changes. This was attributed to chlorophyll destruction due to protein breakdown, causing magnesium loss and generating dull green molecules, increasing product yellowness, though this was not observed in our study [20]. Kernel et al. [19] found that chlorophyll removal during lipid extraction with supercritical CO_2 may explain why heat treatment did not affect the colour of meat products. It is evident that the chlorophyll content of hemp products should be considered when incorporating hemp ingredients into the batter of minced meat products.

Total colour difference (ΔE_{Lab}) exceeded the value of 5 between samples in treatments HF0 and HF4 and also between samples in treatments HF0 and HF6 (Figure 2), showing that the control and treated samples had different colours [19].

3.7. Cooking Loss and Texture Profile Analysis

Cooking losses are presented in Table 11, and significant differences ($p < 0.001$) were observed between treatments. Samples in the HF6 treatment exhibited higher cooking losses compared to samples in the HF0 and HF4 treatments, which showed similar losses. The increased cooking losses in the samples from the HF6 treatment might be attributed to their lower sodium chloride content, resulting in reduced solubilisation of myofibrillar proteins [75]. However, unlike the findings of the present study, the addition of hempseed cake in meat balls, chicken meat sausages, and pork meat burger patties, as well as the addition of hemp flour in pork meat loaves, resulted in lower cooking losses [17–20]. Sun et al. [20] attributed reduced cooking loss to dietary fibre from hempseed meal and linked lower cooking losses in meat balls to the poor protein solubility of hemp cake. Kerner et al. [19] attributed the lower grilling losses observed in their study to the protein content of hemp-based products.

Table 11. Cooking loss and texture profile of cooked minced meat products (mean values ± SD).

Variable	Treatment			Significance
	HF0	HF4	HF6	
Cooking loss	24.14 [a] ± 3.86	23.21 [a] ± 1.61	32.76 [b] ± 0.93	***
		Texture profile		
Hardness (N)	25.81 [a] ± 0.02	38.86 [b] ± 5.95	34.97 [b] ± 5.66	***
Springiness	0.73 ± 0.01	0.65 ± 0.19	0.67 ± 0.16	NS
Cohesiveness	0.42 [b] ± 0.01	0.38 ± 0.07	0.37 [a] ± 0.04	*
Gumminess (N)	10.80 [a] ± 0.26	15.18 [b] ± 4.79	13.03 ± 3.31	*
Chewiness (N)	7.92 ± 0.24	10.72 ± 5.99	9.21 ± 4.27	NS

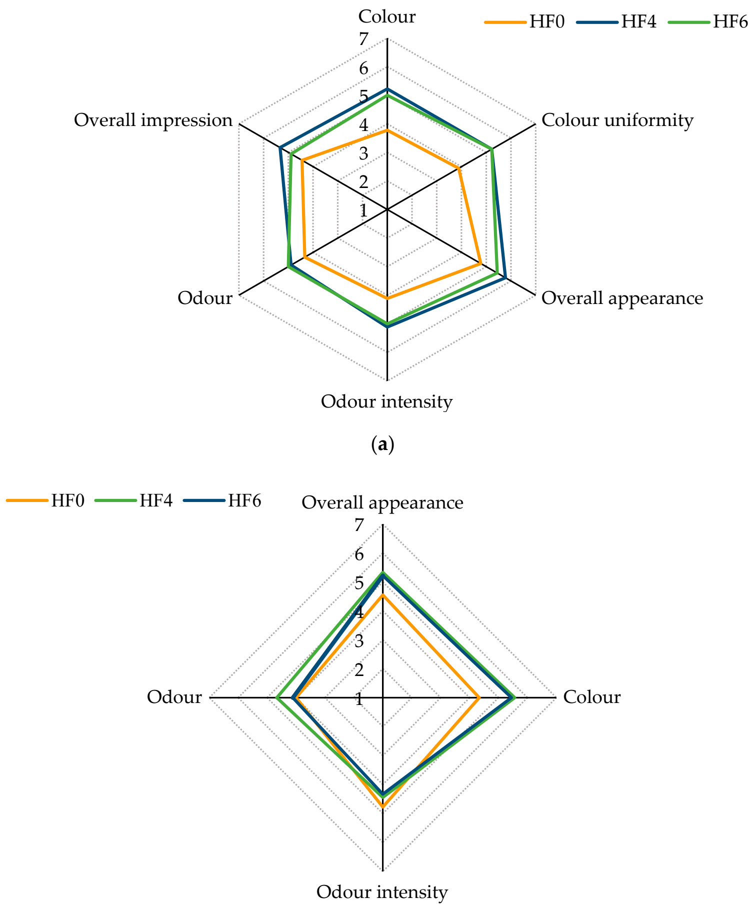
NS = Non-significant; * = $p < 0.05$; *** = $p < 0.001$; Superscripts a and b differ at $p < 0.05$.

In texture profile analysis, hardness, springiness, cohesiveness, gumminess, and chewiness are examined. The hardness value represents the peak force obtained during the initial compression of a product. Springiness, also referred to as elasticity, indicates how well a product rebounds physically after being deformed during initial compression. Cohesiveness indicates a product's ability to withstand a second deformation relative to its behaviour during the first deformation. Gumminess (hardness × cohesiveness) and chewiness (gumminess × springiness) are described as the energy required to chew a product [20,76]. As shown in Table 11, the addition of hemp flour significantly affected ($p < 0.001$) hardness, while there was a moderate effect ($p < 0.05$) on cohesiveness and gumminess. Springiness and chewiness were not affected by the addition of hemp flour ($p > 0.05$). Kotecka-Majchrzak et al. [18] reported higher but not significantly different hardness values in cooked meat balls containing increasingly higher levels of hemp cake. The same authors found that the other examined parameters, i.e., springiness, cohesiveness, gumminess, and chewiness, were not affected by enrichment with hemp seed cake. Similarly, Zając et al. [17] reported that the incorporation of hemp flour led to an increase in the firmness of pork meat loaves, while the other textural characteristics remained unaffected. On the other hand, Sun et al. [20] found that addition of increasing amounts of hemp seed meal resulted in lower hardness values in cooked chicken sausage. Additionally, the other textural characteristics were similarly affected. All the cited researchers [17,18,20] reported that changes to texture profile depend mainly on the amounts of and interactions between all components in the meat batter as well as water binding components such as protein and dietary fibre.

3.8. Sensory Evaluation

The taste panel evaluation results for visible and olfactory traits are depicted in Figure 3. For raw minced meat products, no significant differences ($p > 0.05$) were observed in any of the examined characteristics, although the samples from treatments HF4 and

HF6 scored higher compared to the control samples (HF0). Except in regard to colour, the samples from all treatments scored above the acceptability limit (score = 4) for all the examined traits. Colour exceeded the acceptability limit for the treated samples (HF4 and HF6) and was lower for the control samples (HF0). With regard to the raw products, Kernel et al. [19] reported lower scores for appearance and colour but similar odour scores for pork patties with hempseed cake compared to the control ones. Similarly, for cooked samples, the addition of hemp flour did not significantly ($p > 0.05$) affect the examined traits. Kotecka-Majchrzak et al. [18] reported higher colour intensity but lower colour uniformity values in cooked meat balls containing hemp cake in relation to the control samples. Zając et al. [17] found that pork loaves with hemp flour scored lower in appearance in relation to control samples. The sensory evaluation results suggest that the addition of hemp flour and the subsequent differences in the overall appearance did not negatively affect consumer expectations for the products. The results of the sensory evaluation in relation to the results of instrumental colour measurement corroborate the opinion held by Teixeira and Rodrigues [77] that there is a gap in understanding consumer perceptions of meat products when nitrite or nitrate is reduced or substituted with alternative ingredients. However, Zając et al. [17] reported a significant increase in consumers' willingness to buy pork meat loaves after being informed about the presence of a healthy ingredient, despite low scores for sensory characteristics. The overall sensory evaluation of the samples shows that the incorporation of hemp flour into familiar products such as minced meat products would increase consumer acceptance, despite the impact of the greenish-brown colour of hemp flour [78] and the noticeable different colour of the cooked products (total colour difference values (ΔE_{Lab}) > 10) (Figure 2) [79]. Larraín et al. [79] estimated that meat colour difference could be around a ΔE_{Lab} value in the range of 2–6 for the human eye.



(**a**)

(**b**)

Figure 3. Taste panel scores for raw (**a**) and cooked (**b**) minced meat products.

4. Conclusions

Inclusion of hemp flour did not affect the proximate composition of minced meat products, even though they contained less sodium chloride. However, the addition of hemp flour did not result in an improved fatty acid profile of the products, despite the high polyunsaturated fatty acid content of hemp flour. Nevertheless, significant variations in antioxidant capacity were observed; these were related to the significantly reduced lipid oxidation levels in minced meat products containing hemp flour. In terms of colour, noticeable changes were observed in the redness values of both the raw and cooked samples, which were attributed to the greenish-brown hue of hemp flour. Texture profile analysis showed that hemp flour addition resulted in significantly harder products. Lastly, despite receiving lower scores for sensory attributes like appearance and colour, products containing hemp flour scored higher in taste panel evaluations for visible and olfactory traits. However, achieving all the various functions of nitrites in meat products may not be feasible, making the partial replacement of nitrites in minced meat products contingent upon consumer acceptance.

In summary, the study results demonstrate that hemp flour can be effectively used as a natural ingredient with antioxidant properties in minced meat products, although some differences in sensory characteristics may arise. Further research is essential to fully explore the potential of hemp-based ingredients to reduce lipid oxidation in minced meat products and to meet consumer demands for healthier and clean label food options.

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