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The Effects of Extracts from Buckwheat Hulls on the Quality Characteristics of Chicken Meatballs during Refrigerated Storage

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Featured Application: Buckwheat hulls are a valuable source of natural antioxidants and may be used as an additive in the production of meat products.

Abstract: Buckwheat hulls, due to their high content of phenolic compounds, could be used as a promising food ingredient, the use of which would reduce the waste generated during the production of buckwheat groats. The aim of this study was to investigate the influence of buckwheat hull extracts on the quality of chicken meatballs. Meatballs were produced according to three different treatments: without extracts (Control) and with water (WE) and ethanolic (EE) extracts of buckwheat hulls. The phenolic compositions of the extracts were analysed and their effects on the colour, lipid oxidation, microbiological and sensory qualities of the chicken meatballs were studied. The ethanolic extract of buckwheat hulls was characterised by a total polyphenolic content more than double that of the water extract. Rutin was the major phenolic compound identified in the extracts, with the ethanolic extract containing more than four times as much rutin as the water extract. Oxidative changes in lipids in the meatballs prepared with extracts of buckwheat hulls occurred more slowly than those in Control. This was indicated by lower TBARS values and the longest fat induction time. The results suggested that, although the ethanolic extract of buckwheat hulls was characterised by a higher content of polyphenolic compounds compared to the water extract, both additives showed similar antioxidant activities in chicken meatballs during 14 days of refrigerated storage.

Keywords: antioxidant activity; buckwheat hulls; chicken meatballs; phenolic compounds

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1. Introduction

During the production and storage of meat products, a variety of processes occur, including lipid oxidation, which can negatively affect colour, flavour, aroma, texture and nutritional value, thereby significantly reducing shelf-life. Poultry meat products are particularly susceptible to oxidative changes due to their high concentrations of unsaturated fatty acids [1,2]. Antioxidants, usually synthetic, are used to reduce these adverse changes. However, due to consumers' aversion to synthetic additives and their search for so-called 'clean label' products, natural antioxidants derived from plant raw materials are of increasing interest among food manufacturers [3–7]. In the food industry, the 'clean label trend' is one of the fastest growing trends, aiming to improve the health quality of food but without compromising its safety and other characteristics [8].

There are many substances in plants that exhibit strong antioxidant activity, and phenolic compounds are particularly valuable, including the following: phenolic acids, flavonoids, tannins, stilbenes and lignans [9,10]. Some natural antioxidants from plants are already used in the meat industry, e.g., herbs and spices, such as oregano, rosemary, thyme, sage, ginger and mint [5,11,12]. A number of studies have shown that lipid oxidation in meat products could be significantly reduced by adding extracts from various plant parts, including leaves, stems, roots, fruits and seeds [1,5,13–17]. The use of extracts can facilitate standardisation of the quality of finished products and reduce the risk of microbiological contamination. It is also important that raw materials, such as hulls, skins, peels, seeds and marc, which are waste products from the fruit and vegetable, fat and cereal industries, can be used to produce extracts [7,18–22].

The production of buckwheat groats generates by-products, such as bran and husks [23]. Buckwheat hulls have already found industrial use, as pillow fillers and as ingredients in biodegradable packaging, among other uses. However, due to their contents of bioactive components, buckwheat hulls may be used as promising food ingredients. Buckwheat hulls, in addition to their high fibre content, may be a valuable source of phenolic compounds [23]. Buckwheat hulls have been shown to have higher phenolic contents and antioxidant properties in comparison to buckwheat groats. While buckwheat groats mainly contain rutin and isovitexin, buckwheat hulls contain orientin, isoorientin, vitexin and quercetin, in addition to the two flavonoids [24–27]. Salejda et al. [28] showed that the addition of ground buckwheat hulls at a level of 1–3% improved the nutritional value of frankfurters but unfortunately also caused changes in the technological and sensory qualities of these products. The use of crushed plant materials can cause changes in sensory characteristics, especially colour and taste [5,29]. These changes are more pronounced in poultry meat products with low natural colour intensity [3,30]. It has been reported in the literature that plant extracts had lesser effects on the sensory qualities of meat products [14,16]. Studies by numerous authors have described the antioxidant activities of buckwheat hull extracts [23,26,27,31]. However, there are few studies in which buckwheat hull extracts have been used to reduce lipid oxidation in meat products. Therefore, the aim of the present study was to determine the influence of buckwheat hull extracts on selected quality characteristics of chicken meatballs during refrigerated storage. The novelty of these studies is the presentation of the application of buckwheat hull extracts as natural antioxidants in the production of poultry meat products. It was also checked how these additives affect the sensory and microbiological qualities of the final products, which, to the best of our knowledge, has not been the subject of a publication so far.

2. Materials and Methods

2.1. Materials

The raw materials (chicken thigh meat and pork jowl) for the production of chicken meatballs were purchased from a local meat producer. Ground buckwheat hulls were obtained directly from an organic food distributor (Bio Planet, Leszno, Poland). The chemicals and solvents were purchased from Merck (Darmstadt, Germany), Sigma-Aldrich (Poznań, Poland) and POCh (Gliwice, Poland).

2.2. Preparation of Extracts

Water and ethanolic extracts of buckwheat hulls were obtained by batch extraction according to the method of Zwolan et al. [16]. A universal extraction system in an automated Soxhlet apparatus (B-811, Büchi Labortechnik AG, Flawil, Switzerland) was used. Ground buckwheat hulls (40 g) were used for the preparation of each extract and were distributed into 8 extraction thimbles. As solvents, distilled water and ethanol (70 mL of ethanol in 100 mL of solution) were used. The buckwheat hulls in thimbles were extracted for 15 cycles with 150 mL of suitable solvent, all the while maintaining the boiling point of the solvent. The obtained portions were combined and filtered (with Whatman No. 1 filter paper). Then, the solvent was separated in a rotary evaporator (Rotovaporator R-205,

Büchi Labortechnik AG, Flawil, Switzerland) at 60 °C, at a vacuum pressure of 72 mbar for the water extract and of 175 mbar for the ethanolic extract and at a flask rotation speed of 75 rpm. The process was carried out until 40 g of concentrated extract was obtained, which corresponded to the weight of the hulls used.

2.3. UPLC Analysis of Phenolic Compounds in Extracts

The phenolic compound analysis for the extracts was carried out using an Acquity Ultra Performance LC system (UPLC), which was equipped with a binary solvent manager (Waters Corporation, Milford, MA, USA), a UPLC BEH C18 column (100 × 2.1 mm, 1.7 µm; Waters Corporation, Milford, MA, USA) and a Q-Tof Micro mass spectrometer (Waters, Manchester, UK) with an electrospray ionization (ESI) source operating in negative and positive modes. The mobile phase consisted of 0.1% formic acid, *v/v* (solvent A) and 100% acetonitrile (solvent B). During the extraction and determination of phenolic compounds, the procedures described by Oszmiański et al. [32] were followed. The retention times and spectra were compared with those obtained for pure standards. The results are expressed as milligrams per gram of dry matter (mg/g_{dm}).

2.4. Product Formulation

A simple formulation was used to prepare a base meat batter for the chicken meatballs as follows: 75% chicken thigh, 10% de-skinned pork jowl, 5% egg mass, 10% wheat bun (hydrated 1:1). The addition of salt (1.2%) and pepper (Kamis, Poland, 0.1%) was calculated with reference to the weight of the base batter. Using this base meat batter, three treatments were prepared: Control (without the addition of buckwheat hull extract), WE (with the addition of water extract of buckwheat hulls) or EE (with the addition of ethanolic extract of buckwheat hulls). The extract addition was at a level of 1.2 g/100 g of batter. The amounts of added extracts were set at a maximum level that did not impair the sensory characteristics of the products (results not shown).

2.5. Product Processing and Storage

Chicken thigh meat and pork jowl were minced in a laboratory grinder with 4.5 mm holes. All ingredients were mixed in a laboratory mixer (Kenwood Major type KM 800, Kenwood Ltd., Havant, England, Type K agitator) for 5 min until evenly distributed. After forming 40 ± 1 g meatballs, they were frozen in a freezer at a temperature of −18 °C (1 h) to consolidate the shape. Then meatballs were baked in a combi oven (at 180 °C, without humidification, until the geometric center reached 80 °C). After cooling, the meatballs were vacuum packed, with a Multivac C200 packaging machine (under a pressure of 100 mBar; Multivac Sepp Haggenmüller GmbH & Co. KG, Wolfertschwenden, Germany) into bags of multilayer film (PE/PA, 75 µm thick) and stored under cooling conditions (from 2 °C to 6 °C) in the absence of light for 1, 7 and 14 days. The experiment was repeated three times, in three independent experimental series.

2.6. Lipid Oxidation Analysis

2.6.1. TBARS Determination

The values of thiobarbituric-acid-reactive substances (TBARSs) were assayed using the method described by Shahidi [33]. Absorbance was measured at 532 nm in a spectrophotometer (CampSpec M501, Spectronic Camspec Ltd., Leeds, UK) against a blank containing 5 mL of 10% trichloroacetic acid and 5 mL of 2-thiobarbituric acid. TBARS values were calculated from a standard curve and expressed in mg of MDA (malondialdehyde) per kg of meatballs.

2.6.2. DSC Analysis

The measurement of the oxidative stability of the fat extracted from meatballs was carried out using the isothermal mode of a DSC instrument (Q20, TA Instruments, New Castle, DE, USA) coupled with a high-pressure cell. Extraction of fat from samples was

performed according to the method of Boselli et al. [34]. DSC measurement was performed according to the method proposed by Wirkowska-Wojdyła et al. [35]. The samples of extracted fat of 3–4 mg were weighed into an open aluminum pan and placed in the sample chamber under an oxygen atmosphere with an initial pressure of 1400 kPa and an oxygen flow rate of 100 mL/min. The isothermal temperature for each sample was 120 °C. The oxidative induction time was obtained from PDSC (pressure differential scanning calorimetry) curves. From the resulting PDSC exotherms, the times to reach the peak maximum (τ_{max}) were determined and used for assessment of the oxidative stabilities of the samples.

2.7. Colour Measurement

The L*, a* and b* colour parameters were determined in the CIE L*a*b* colour space with cross-sections of meatballs directly after the products were cut, using a Minolta colourimeter (CR-200; Konica Minolta, Tokyo, Japan). A D65 illuminant, with a measuring head hole 8 mm in diameter and a 2° standard observer angle, was used. The device was calibrated on a white calibration plate (L* 97.81, a* −0.45, b* +1.88). Total colour differences (ΔE) between meatballs with added extracts and the control products (for individual storage times) were calculated as follows:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

2.8. Sensory Evaluation

Sensory evaluation of chicken meatballs was carried out in a sensory evaluation laboratory, meeting the requirements of Polish Standard PN-EN ISO 8589:2010 [36]. The meatballs were evaluated by a well-trained panel of 10 individuals (5 women and 5 men). The panelists assessed the samples using 10 cm-length unstructured line scales: one end of the scale represented the extremely acceptable status of the selected sensory attributes of meatballs and the other end extremely unacceptable status. The following attributes were evaluated: the colour of the cross-sectional area, odour, taste and texture. In addition, the overall sensory quality of the individual meatball treatments was determined on the basis of all the differentiators evaluated and their proportions. The samples prior to the evaluation were kept at room temperature for about 20 min for temperature equilibration.

2.9. Microbiological Analysis

Samples were prepared according to PN-EN ISO 6887-2:2017 [37]. Microbiological analyses included total plate counts (TPCs) of aerobic mesophilic microorganisms (30 °C for 48 h) on Plate Count Agar medium (BTL, Łódź, Poland) [38], lactic acid bacteria (LAB; 30 °C for 72 h) on MRS medium (de Man, Rogosa and Sharpe Agar; Bio-Rad Laboratories, Inc., Hercules, CA, USA) [39], psychrotrophic bacteria (4 °C for 10 days) on Plate Count Agar medium (BTL, Łódź, Poland) [40] and Enterobacteriaceae (37 °C for 24 h) on Violet Red Bile Glucose Agar medium (BTL, Łódź, Poland) [41]. All the bacterial counts were presented as $\log_{10}$ of colony-forming units per gram of product (log cfu/g).

2.10. Statistical Analysis

Data was expressed as means ± standard deviations. The results were statistically analysed using Statistica 13.3PL (TIBCO Software Inc., Palo Alto, Santa Clara, CA, USA). All experiments were performed in three repetitions (n = 3). One-way analysis of variance (ANOVA) and Tukey's HSD test allowed for the determination of statistically significant differences ($p < 0.05$).

3. Results and Discussion

3.1. Phenolic Compounds Identified in Buckwheat Hull Extracts

As presented in Table 1, eight phenolic compounds were identified in the buckwheat hull extracts. In detail, one phenolic acid and seven flavonoids and their derivatives were detected. The ethanolic extract of buckwheat hulls had more than twice the total polyphenolic content of the water extract. The observed differences between the contents of individual polyphenols in the ethanolic and water extracts were significant ($p < 0.05$). Rutin was the main phenolic compound identified in the buckwheat hull extracts, with more than four times as much in the ethanolic extract as in the water extract (accounting for about 67% and 35% of all identified phenolic compounds, respectively). In the ethanolic extract of buckwheat hulls, the following were still present in higher amounts: hyperoside (10.7%) and isoorientin (7.5%), and in the water extract, vitexin (27.3%) and isoorientin (12.6%). Direct comparison of the polyphenolic contents of buckwheat hull extracts with other data in the literature was difficult, as large differences were observed in the qualitative and quantitative compositions of extracts presented by other authors [14,25–27,31]. Such differences could be related to the genotypic and environmental differences between species, sample preparation procedures and methods of determination [6].

Table 1. Phenolic compounds (mg/g_{dm}) in buckwheat hull extracts.

Compounds	Water Extract of Buckwheat Hulls	Ethanolic Extract of Buckwheat Hulls	λmax (nm)	MS-MS	Retention Time (min)
Caffeic acid hexoside	$1.37^{a} \pm 0.01$	$2.54^{b} \pm 0.01$	325	341/179	2.81
Orientin	$0.82^{a} \pm 0.04$	$2.14^{b} \pm 0.02$	269/346	447/357/327	6.07
Isoorientin	$2.52^{a} \pm 0.02$	$3.35^{b} \pm 0.01$	255/348	447/357/327	6.22
Vitexin	$5.45^{b} \pm 0.03$	$0.42^{a} \pm 0.01$	267/337	431	6.76
Rutin (quercetin-3-rutinoside)	$6.96^{a} \pm 0.02$	$30.06^{b} \pm 0.02$	264/348	609/431/301	6.85
Hyperoside (quercetin-3-galactoside)	$1.96^{a} \pm 0.01$	$4.78^{b} \pm 0.01$	254/354	463/301	7.02
Isoquercitrin (quercetin-3-glucoside)	$0.57^{a} \pm 0.01$	$1.29^{b} \pm 0.01$	255/350	463/301	7.13
Luteolin-7-glucoside	$0.34^{b} \pm 0.01$	$0.27^{a} \pm 0.01$	253/342	447/285	7.59
Sum of identified phenolic compounds	19.99	44.85			

^{a,b} Different letters within a row indicate that mean values are significantly different at $p < 0.05$.

Reports in the literature highlight the fact that the compositions of extracts vary according to the solvents used [23,27]. No single solvent can extract all phenolic compounds, as they have different polarities and solubilities. The most commonly used solvents for extraction are acetone, methanol, ethanol and their aqueous mixtures, or water alone [11]. More phenolic compounds can be extracted by aqueous organic solvents than absolute organic solvents [31,42,43]. Lapornik et al. [44] showed that more phenolic compounds can be extracted with the use of alcohol (70% ethanol or 70% methanol) than water. The usage of water in the solvent may increase the polarity, cause the plant materials to swell and thus make it easier for the solvent to penetrate the plant material matrix [27,45].

3.2. Lipid Oxidation of Chicken Meatballs

TBARS index values and induction times were measured to determine the effectiveness of buckwheat hull extracts in inhibiting the lipid oxidation of fats during the refrigerated storage of the vacuum-packed chicken meatballs (Table 2).

Table 2. Evolution of TBARS values and induction times of chicken meatballs during refrigerated storage.

Sample	TBARS (mg MDA/kg)			Induction Time (min)		
	1 [1]	7	14	1	7	14
Control	$0.65^{a\,x} \pm 0.05$	$0.85^{b\,y} \pm 0.08$	$0.88^{b\,y} \pm 0.05$	$12.22^{a\,x} \pm 0.34$	$11.43^{a\,x} \pm 0.52$	$11.22^{a\,x} \pm 0.72$
WE	$0.55^{a\,x} \pm 0.10$	$0.68^{a\,b\,y} \pm 0.12$	$0.64^{a\,y} \pm 0.09$	$13.23^{b\,x} \pm 0.30$	$12.98^{b\,x} \pm 0.27$	$12.43^{a\,b\,x} \pm 0.53$
EE	$0.56^{a\,x} \pm 0.07$	$0.55^{a\,x} \pm 0.10$	$0.56^{a\,x} \pm 0.06$	$13.61^{b\,x} \pm 0.43$	$13.49^{b\,x} \pm 0.47$	$12.74^{b\,x} \pm 0.29$

[1] Sampling at 1, 7 and 14 days of storage, respectively. [a, b] Different letters within a row indicate that mean values are significantly different at $p < 0.05$. [x, y] Different letters within a row indicate that mean values are significantly different at $p < 0.05$.

Both buckwheat hull extracts exerted inhibitory effects on lipid oxidation in chicken meatballs, although with differentiated intensity. On day 1, no significant ($p > 0.05$) differences were observed between samples; however, it could be noted that TBARS values in the chicken meatballs made with buckwheat hull extracts (WE and EE) were lower than in Control. On day 7, significantly ($p < 0.05$) lower TBARS values were found only for EE and on day 14 for both WE and EE compared to Control. Although no significant ($p > 0.05$) differences were found between EE and WE, it could be seen that, during storage, lipid oxidation occurred more slowly in EE than in WE. In the case of EE, TBARS values remained at the same level for 14 days of storage, and on the last day were lower than in Control by approximately 36%. However, in WE, after 7 days of storage, TBARS values increased significantly ($p < 0.05$), and on the last day of storage were lower than in Control by only 27%.

Studies using extracts from the roots of *Scutellaria baicalensis* [17] and *Nigella sativa* seeds [16] also showed higher efficiencies of ethanolic extracts in inhibiting lipid oxidation in chicken meatballs compared with water extracts. The ethanolic extract of *Scutellaria baicalensis* roots was characterised by better antioxidant properties than the ethanolic extract of *Nigella sativa* seeds. On the 14th day of storage, meatballs prepared with the ethanolic extract of *Nigella sativa* seeds had TBARS values about 20% lower than the control product, while meatballs prepared with *Scutellaria baicalensis* root extract had TBARS values about 50% lower. The authors explained the higher effectiveness of the ethanolic extract in reducing lipid oxidation by reference to its higher content of polyphenolic compounds. However, a higher content of phenolic compounds in extracts does not always determine a proportionally higher antioxidant potential [46]. Despite the fact that, in the present study, more than twice as many polyphenolic compounds were extracted from the ethanolic extract of buckwheat hulls as from the water extract (Table 1), the differences in antioxidant activity in the finished product (represented by TBARS values) were not significant ($p < 0.05$). Studies in the literature report that the antioxidant activities of buckwheat hull extracts may be influenced not only by the flavonoids present but also by other compounds that may not have been extracted by the solvents used, as well as by interactions between the individual compounds [27]. It is noteworthy that in all variants of chicken meatballs the levels of MDA after 14 days of storage were lower than the limit reported by Selani et al. [18], according to which meat products can be considered well-preserved in regard to oxidative changes, of less than 3 mg MDA/kg product.

The oxidative stability of lipids extracted from chicken meatballs can also be characterised by the induction time determined on the basis of the PDSC technique, which makes it possible to study the stability of fats under elevated temperature conditions, in this case 120 °C, i.e., conditions close to the practical heat treatment of meat. The lower the induction time, the less stable the fat is, indicating the lower antioxidant activity of the extract. Fat extracted from chicken meatballs made with buckwheat hull extracts was characterised by a longer induction time than the fat extracted from the Control. For the fat extracted from the EE treatment, these differences were significant ($p < 0.05$) for each of the analysed times. In contrast, for fat with WE, the significance of the differences was only marked after 1 and 7 days of storage, confirming the higher antioxidant potential of the ethanolic

extract. A reduction in induction time was observed during storage, but these differences were not significant ($p > 0.05$), regardless of the meatball treatment. The results of the PDSC measurements for the oxidative stability of the fat extracted from the chicken meatballs are consistent with the results of the TBARS index determinations. The lower TBARS index values corresponded to longer induction times. According to us, this demonstrates the effective inhibition of lipid oxidation processes by buckwheat hull extracts.

3.3. Colour of Chicken Meatballs

The addition of buckwheat hull ethanolic extract resulted in a significant ($p < 0.05$) reduction in the value of the a* colour parameter of chicken meatballs compared to the values measured for Control, regardless of the storage time considered (Table 3). Lower values for this parameter were also observed in comparison with WE, although significant ($p < 0.05$) differences occurred only on days 1 and 14 of storage. However, there was no significant ($p > 0.05$) effect of the addition of extracts of buckwheat hulls on the L* and b* parameters of meatballs. Moreover, there was no significant ($p > 0.05$) effect of storage time on the above-mentioned parameters.

Variation in the a* colour parameter of chicken meatballs made with ethanolic extract of buckwheat hulls did not affect the total colour difference parameter (ΔE). The ΔE values obtained (<2.0) indicate that the difference in the colour of chicken meatballs made with extracts compared to Control may only be apparent to a person experienced in recognising colour nuances. The effects of the addition of plant extracts on the colour of meat products were differentiated, depending, for example, on the source from which the extract was obtained or the form and the amount added to the product [4,13,16,47,48]. However, colour changes induced by the addition of extracts are generally smaller compared to the addition of plant materials in their native form, i.e., dried and crushed (powdered) [16,28,30].

3.4. Sensory Attributes of Chicken Meatballs

Analysis of the sensory evaluation results showed no significant ($p > 0.05$) effect of the addition of extracts of buckwheat hulls on selected quality characteristics of chicken meatballs (Table 4), regardless of storage time.

Over time, there was a trend towards lower scores for all chicken meatball sensory quality traits considered. However, a significant ($p < 0.05$) effect of storage time was found only for the odours of WE and EE and the overall desirability for WE. It should be emphasised that an important element in the production of meat products enriched with plant-derived ingredients is the maintenance of the appropriate sensory characteristics at a level acceptable to the consumer [49]. In the present study, this criterion was met, and the products prepared with extracts achieved high acceptability, comparable to that of the Control. Other studies [50] have shown that the use of ethanolic plant extracts characterised by specific aromas and tastes, such as rosemary extract, can worsen the aroma and taste of poultry meatballs. However, dried rosemary was evaluated by a sensory panel similarly to herbal seasoning and positively influenced the above-mentioned sensory characteristics of the product.

Table 3. Evolution of colour parameters (L*a*b*) of chicken meatballs during refrigerated storage.

Sample	L*			a*			b*			ΔE		
	1 [1]	7	14	1	7	14	1	7	14	1	7	14
Control	$66.83^{a\,x} \pm 0.45$	$67.73^{a\,x} \pm 0.34$	$67.12^{a\,x} \pm 0.82$	$6.53^{b\,x} \pm 0.43$	$7.26^{b\,x} \pm 0.44$	$7.14^{b\,x} \pm 0.14$	$10.45^{a\,x} \pm 0.15$	$9.73^{a\,x} \pm 0.24$	$10.34^{a\,x} \pm 0.47$	-	-	-
WE	$66.26^{a\,x} \pm 1.01$	$66.64^{a\,x} \pm 1.86$	$67.49^{a\,x} \pm 0.56$	$5.90^{b\,x} \pm 0.40$	$6.31^{a\,b\,x\,y} \pm 0.63$	$7.07^{b\,y} \pm 0.28$	$9.71^{a\,x} \pm 0.67$	$10.36^{a\,x} \pm 0.32$	$10.04^{a\,x} \pm 0.28$	1.1 ± 0.5	1.6 ± 0.6	0.5 ± 0.2
EE	$66.69^{a\,x} \pm 0.22$	$67.09^{a\,x} \pm 0.87$	$66.51^{a\,x} \pm 1.33$	$4.75^{a\,x} \pm 0.34$	$5.87^{a\,x} \pm 0.56$	$5.86^{a\,x} \pm 0.51$	$10.52^{a\,x} \pm 0.15$	$10.33^{a\,x} \pm 0.62$	$10.82^{a\,x} \pm 0.28$	1.8 ± 0.5	1.6 ± 0.7	1.5 ± 0.2

[1] Sampling at 1, 7 and 14 days of storage, respectively. [a, b] Different letters within a row indicate that mean values are significantly different at $p < 0.05$. [x, y] Different letters within a row indicate that mean values are significantly different at $p < 0.05$.

Table 4. Evolution of sensory attributes of chicken meatballs during refrigerated storage.

Sample	Colour			Odour			Taste			Texture			Overall Desirability		
	1 [1]	7	14	1	7	14	1	7	14	1	7	14	1	7	14
Control	$7.6^{a\,x} \pm 1.2$	$5.9^{a\,x} \pm 1.4$	$6.2^{a\,x} \pm 1.2$	$7.0^{a\,x} \pm 1.5$	$6.5^{a\,x} \pm 1.4$	$6.5^{a\,x} \pm 1.1$	$7.4^{a\,x} \pm 1.1$	$7.0^{a\,x} \pm 1.5$	$7.1^{a\,x} \pm 1.1$	$8.0^{a\,x} \pm 1.2$	$7.5^{a\,x} \pm 0.9$	$7.8^{a\,x} \pm 1.3$	$7.5^{a\,x} \pm 1.4$	$7.5^{a\,x} \pm 1.1$	$7.3^{a\,x} \pm 1.2$
WE	$7.2^{a\,x} \pm 1.6$	$6.2^{a\,x} \pm 1.4$	$6.2^{a\,x} \pm 1.1$	$7.2^{a\,y} \pm 1.2$	$6.7^{a\,x\,y} \pm 1.2$	$5.7^{a\,x} \pm 1.5$	$7.3^{a\,x} \pm 1.2$	$6.9^{a\,x} \pm 1.5$	$6.4^{a\,x} \pm 1.2$	$8.0^{a\,x} \pm 1.2$	$7.3^{a\,x} \pm 1.0$	$7.7^{a\,x} \pm 1.0$	$7.7^{a\,y} \pm 0.9$	$7.4^{a\,x\,y} \pm 1.1$	$7.0^{a\,x} \pm 0.5$
EE	$7.2^{a\,x} \pm 1.6$	$6.2^{a\,x} \pm 1.3$	$6.3^{a\,x} \pm 1.4$	$7.3^{a\,y} \pm 1.3$	$6.3^{a\,x\,y} \pm 1.3$	$5.9^{a\,x} \pm 1.1$	$6.7^{a\,x} \pm 1.6$	$7.2^{a\,x} \pm 0.9$	$7.1^{a\,x} \pm 1.2$	$7.7^{a\,x} \pm 1.3$	$6.9^{a\,x} \pm 1.4$	$7.3^{a\,x} \pm 1.1$	$8.0^{a\,x} \pm 1.2$	$7.5^{a\,x} \pm 1.1$	$7.2^{a\,x} \pm 0.9$

[1] Sampling at 1, 7 and 14 days of storage, respectively. [a] Different letters within a row indicate that mean values are significantly different at $p < 0.05$. [x, y] Different letters within a row indicate that mean values are significantly different at $p < 0.05$.

3.5. Bacterial Counts for the Chicken Meatballs

The application of extracts of buckwheat hulls did not significantly ($p > 0.05$) affect the counts of TPC and psychrotrophic bacteria in chicken meatballs on days 1 and 7 of storage. In contrast, on day 14, in meatballs made with added extracts (WE and EE), the numbers of these bacteria were significantly ($p < 0.05$) lower than in the Control. In the case of LAB, the inhibitory effect of the extracts was lesser. A significant ($p < 0.05$) decrease in the number of bacteria belonging to this group compared to the Control was only found in EE on day 7 of storage. Significantly ($p < 0.05$) higher bacterial counts for all groups were found on day 14 in all meatballs in comparison to the bacterial count determined on day 1. In all meatball treatments, no Enterobacteriaceae in 0.1 g were detected (Table 5). A significant increase in total aerobic microorganisms was also found during the refrigerated storage of chicken meatballs produced with the addition of rosemary preparations [50], chicken meatballs prepared with water and ethanolic extracts [17] and chicken patties made with fruit peel powders added [7]. The maximum limit for meat products of 5 log cfu/g [51] for TPC and psychrotrophic bacteria was exceeded on day 14 of refrigerated storage. Thus, it can be concluded that microbial deterioration, rather than lipid oxidation, was the limiting factor of the shelf-life of chicken meatballs in the present study.

Table 5. Evolution of microbial counts of chicken meatballs during refrigerated storage.

Sample	TPC (Total Plate Count)			Psychrotrophic Bacteria			LAB (Lactic Acid Bacteria)			Enterobacteriaceae		
	1 [1]	7	14	1	7	14	1	7	14	1	7	14
Control	$2.59^{a\,x} \pm 0.11$	$4.33^{a\,x} \pm 0.17$	$5.99^{b\,y} \pm 0.08$	$1.82^{a\,x} \pm 0.10$	$4.99^{a\,y} \pm 0.07$	$5.91^{b\,z} \pm 0.22$	$2.28^{a\,x} \pm 0.22$	$3.26^{b\,x} \pm 0.10$	$4.70^{a\,y} \pm 0.19$	ND	ND	ND
WE	$2.44^{a\,x} \pm 0.17$	$4.35^{a\,x} \pm 0.21$	$5.79^{a\,y} \pm 0.14$	$1.77^{a\,x} \pm 0.10$	$4.93^{a\,y} \pm 0.09$	$5.32^{a\,z} \pm 0.11$	$2.22^{a\,x} \pm 0.36$	$3.11^{a\,b\,x} \pm 0.14$	$4.65^{a\,y} \pm 0.07$	ND	ND	ND
EE	$2.32^{a\,x} \pm 0.24$	$4.24^{a\,x} \pm 0.13$	$5.69^{a\,y} \pm 0.11$	$1.62^{a\,x} \pm 0.22$	$4.87^{a\,y} \pm 0.07$	$5.19^{a\,z} \pm 0.06$	$2.09^{a\,x} \pm 0.25$	$2.98^{a\,x} \pm 0.12$	$4.59^{a\,y} \pm 0.13$	ND	ND	ND

Data (log cfu/g) are the means of two replicates and three repetitions. [1] Sampling at 1, 7 and 14 days of storage, respectively. [a, b] Different letters within a row indicate that mean values are significantly different at $p < 0.05$. [x, y, z] Different letters within a row indicate that mean values are significantly different at $p < 0.05$. ND: not detectable.

According to literature reports, phenolic compounds found in plant extracts not only have antioxidant properties but also show antimicrobial activity [17,19,52]. However, their influence on the inhibition of the development of microorganisms in meat products is not always noticeable [17].

4. Conclusions

New trends in food preservation point to the need to reduce the use of synthetic additives and replace them with natural additives of plant origin. Increasingly, waste raw materials are being used for this purpose, which, as in the case of buckwheat hulls, can be a valuable source of phenolic compounds that exhibit antioxidant properties. Our results show the potential use of buckwheat hull extracts as effective natural antioxidants in vacuum-packed chicken meatballs. Although the ethanolic extract of buckwheat hulls had more than twice the polyphenolic content of the water extract, both additives showed similar antioxidant activities (represented by TBARS values and induction times) in chicken meatballs during 14 days of refrigerated storage. The use of extracts of buckwheat hulls in the meatballs did not result in their differentiation with respect to sensory quality, and their effect on colour was minor ($\Delta E < 2$).

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