Quality of broiler chicken meat containing butylated hydroxytoluene, DL-α-tocopherol and microwave-extracted *Piper nigrum* on various days of refrigeration storage

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Summary

This study investigated the effects of butylated hydroxytoluene (BHT), DL-α-tocopherol (vitamin E, VitE), white pepper extract (WPE) and black pepper extract (BPE) on broiler chicken meat at 0, 5 and 10 days of storage at 4 °C. WPE and BPE were prepared to reach the desirability values 0.60 and 0.62, yielding 39 and 71 bio-compounds, respectively. Fresh meat (100 g) was apportioned to five treatment groups (each replicated three times) and treated with mixtures containing distilled water, salt, olive oil and the chosen additive (100 mg BHT, 0.033 ml VitE, 100 ml BPE or 100 ml WPE per portion, or no additive as a control). Meat pH was the lowest in the presence of VitE (7.24) and WPE (7.22) on day 10. The a^* value for redness was better preserved in the presence of BPE (9.81), WPE (9.96) and VitE (9.51) rather than the control group (8.53), but the difference was not significant. On day 5, malondialdehyde (MDA) was most reduced by BHT, followed by BPE ($P < 0.05$). On day 10, the reduction of MDA was similar between the additives. This study reveals, pepper extracts contain potent bio-compounds that can act as safe alternatives to synthetic antioxidants.

Keywords

additives; *Piper nigrum*; microwave extraction; meat colour; malondialdehyde

Meat is highly valued by humans for its nutrient-dense composition that significantly contributes to well-balanced and healthy diets. In many societies, meat is cherished as a means to achieve high quality nutrition due to high protein bio-availability (70 %) in the human body [1]. Meat can be considered a diet-based intervention for improved nutritional status, as lowered intake beyond a critical threshold may result in gross undernutrition, possibly causing cognitive and physical defects and, in the long-run, stifling of economic growth and development. Numerous studies have positively correlated animal-based foods with cognitive development, increased activity level, and normal behaviour in children due to its high bioavailability of micronutrients compared to plants [2, 3]. Poultry meat is generally characterized by low saturated fatty acid content and low associated risks of cardiovascular diseases. Contrary to the general belief that animal fat is predominantly composed of saturated fatty acids (SFA), chicken meat contains about 70 % unsaturated fats [1, 4]. Broiler chicken meat is a moderate energy source, containing highly digestible proteins with low collagen levels, good balance of unsaturated and saturated lipids, B-complex vitamins and essential micronutrients including minerals like zinc, iron and copper, thus making it a valuable source of nutrition for humans [5]. Many nutrients contained in broiler chicken meat are required to offset physiological processes, such as brain development in infants, growth in young children and women of reproductive age, hormonal signaling in pregnant and lactating women as well as maintaining health in older adults [6].

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Unfortunately, structural changes in lipid composition by radical attack during processing and storage (4 °C) can negatively interfere with the expected nutritional gains. Oxidation of meat lipids results in unfavorable changes that extend to its physical properties, while rancidity promotes the deterioration of integral muscle components, discouraging consumers from bulk or repeated purchase. To overcome this challenge, cold storage, curing, drying, salting and canning are commonly applied to preserve the nutritional and sensory qualities of meat and to minimise food wasting and sales' losses [7–9] in both low and high income societies.

In low-middle income countries, low intake of chicken meat is often caused by a limited access and poor storage facilities. On the contrary, low consumption in affluent societies is often related to concerns raised from possible risks associated with the synthetic preservatives applied to meat during storage [6, 10]. Butylated hydroxyanisole and butylated hydroxytoluene (BHT) are additives commonly employed to reduce the oxidative spoilage. However, potential health risks from toxicity of these additives prompt health-conscious consumers to seek alternatives. Synthetic vitamin E (VitE) is a conventional antioxidant with radical quenching properties. Unfortunately, increased risk of bleeding, generalized weakness, gastrointestinal upset, small bowel obstruction, pancreatitis and cholestasis are among the toxicity concerns raised [11, 12], prompting a shift in focus towards alternatives. Plant-related substances contain a lot of phytochemicals such as polyphenols, alkaloids and sterols (secondary metabolites) that act by quenching free radicals and inhibiting propagation of radical species. The study of Zhang et al. [13] demonstrated the efficacy of mulberry leaf extract in preserving meat colour and shelf life. Similarly, Adegoke et al. [14] reported the preservation of fatty acids in pork containing black pepper extract on the day 10 of refrigeration storage – alluding to its potent bioactive compounds.

White and black pepper (*Piper nigrum*) powders are often valued for their versatile bioactive properties. Black and white pepper originate from the same plant species, but they are processed differently, resulting in different chemical composition. To improve yield of bioactive compounds, updated and innovative strategies explore green technology to increase extraction efficiency [15]. Microwave extraction has yielded high proportions of polyphenols, sterols and alkaloids [9]. By producing such extracts, alternatives to synthetic additives can be developed, with guaranteed safety and antioxidant potency [16]. On this premise, this study examines the effect of BHT, VitE, black pepper extract (BPE) and white pepper extract (WPE) on the physicochemical indices (colour and pH) of broiler chicken meat across various days of storage at 4 °C. It evaluates the effect of extracts on the oxidative stability of meat on various days of storage; and examines the conservation potential of the extracts.

MATERIALS AND METHODS

Experimental site

Extraction of bio-compounds from black and white pepper (*P. nigrum*) and subsequent assessment of physicochemical properties of broiler chicken meat was carried out at the Animal Product and Processing Laboratory of the Department of Animal Production and Health of Federal University of Agriculture, Abeokuta, Nigeria (FUNAAB), while gas chromatography mass spectrometry of the extracts and the content of 2-thiobarbiuric acid reactive substances (*TBARS*) expressed as equivalent of malondialdehyde (MDA) content of stored meat were determined at an external laboratory (University of Ibadan, Ibadan, Nigeria).

Experimental materials

Dried white and black pepper corns of standard grade were obtained from a trusted market in Ibadan (Nigeria), and separately ground into fine powder with an attrition mill GX160 (Honda, Minato, Japan). Ground peppers were sieved to 0.40 mm, then stored in dark-coloured airtight containers. The distilled water was obtained from the Feed Quality Laboratory of the Department of Animal Production and Health (FUNAAB). Microwave ovens H36MOMMI (Hisense, Qingdao, China) and KOR6N9NC (Daewoo, Seoul, South Korea) were used for the extraction of bio-compounds in white and black pepper, respectively. Butylated hydroxytoluene (BHT, Loba Chemie, Mumbai, India) and vitamin E (VitE, DL-α-tocopheryl acetate, MEGA Lifesciences, Bangkok, Thailand) were obtained from the trusted stores in Ibadan and Abeokuta, Nigeria respectively. A total of twenty 4-weeks old broiler chickens (Cobb strain) from Zartech (Ibadan, Nigeria), intensively raised in a deep litter housing system, were obtained and reared intensively for additional 4 weeks. Standard commercial finisher diet (Tab. 1) was offered uniformly. Feed and water were provided ad libitum. At the expiration of rearing, birds weighed between 2.3–2.8 kg.

Afterwards, 8 birds were sacrificed following ethical guidelines established by the Animal Welfare Committee of FUNAAB. Breast muscles were extracted and weighed prior to soaking in aqueous extracts. Meat samples were enclosed in Ziploc bags and wrapped with ice packs, then placed in amber-coloured containers during the transport (1 h) from the farm to the laboratory.

Preparation of pepper extracts

White and black pepper extracts (WPE, BPE) were prepared as suggested by Design Expert12 software (Stat-Ease, Minneapolis, Minnesota, USA), using the desirability values of 0.604 and 0.624 and guided by optimization studies conducted by ADEGOKE et al. [17, 18] (Tab. 2). The sieve-size was fixed, but the microwave power, heating time and solvent volume were different between the processing of the black and white pepper. Briefly, 8 g amounts of ground white and black pepper, sieved at 0.40 mm were separately mixed with 280.00 ml and 364.29 ml of distilled water, respectively. Each mixture was thoroughly stirred in an irradiation-tolerant container prior to transfer to the microwave cavity. Power levels of 300 W and 320 W were respectively fixed and the aqueous solutions were refluxed and heated for 91.19 min and 87.27 min, respectively. Post-extraction, the probe of Mextech ST9269 thermometer (Mextech Technologies, Mumbai, India) was inserted into the microwave cavity to measure the approximate extraction temperatures that ranged between 37 ± 2 °C and 39 ± 2 °C for Daewoo and Hisense microwave ovens, respectively.

Bio-compounds in pepper extracts

The composition of the extracts was analysed via gas chromatography with mass spectrometry (GC-MS) using GC 7890 and MS 5975 (Agilent Technologies, Santa Clara, California, USA) constituted with fused capillary column Rtx-1 (Restek, Bellefonte, Pennsylvania, USA) of 30 mm tubular diameter, 0.32-mm internal diameter and thickness of 0.25-μm. A small portion of the pepper extract was sieved through a 0.45 μm micro filter. The acetone of analytical grade was mixed with the pepper extract at the ratio of 1 : 10. Afterwards, 1 μl of the solution was injected into the GC according to the procedure described by OLALERE et al. [19]. The composition of the detected compounds is shown in Tab. 3 and Tab. 4.

Experimental treatment solutions

Microwave extractions were performed twice while producing aqueous extracts of white and black pepper. Two extractions for black pepper and two for white pepper were prepared. Next, extracts were combined into one solution per pepper product. Thereafter, four experimental solutions, each consisting of three replicates were formed.

The composition of experimental treatment solutions is provided in Tab. 5. The marinating mix-

Tab. 1. Composition of commercial finisher diet fed broiler chickens.

Diet composition	Value
Crude protein [%]	20.0
Fat/Oil [%]	6.0
Crude fibre [%]	5.0
Salt [%]	0.3
Lysine [%]	0.9
Methionine [%]	0.4
Calcium [%]	1.0
Available phosphorus [%]	0.4
Metabolizable energy [MJ]	11.82

Tab. 2. Criteria for desirability values prior to production of pepper extracts.

No.	Parameter	White pepper	Black pepper
1	Extraction time [min]	91.19	87.27
2	Solvent [ml]	280.00	364.28
3	Extract [ml]	66.43	95.36
4	Extract absorbed [ml]	6.43	7.02
5	Meat pH day 0	6.00	5.80
6	Meat pH day 5	6.10	6.06
7	Meat pH day 10	6.66	6.88
8	Meat *TBARS* day 5 [g·kg^{-1}]	0.74	0.29
9	Meat *TBARS* day 10 [g·kg^{-1}]	0.23	0.13
10	Meat refrigeration loss day 5 [%]	14.8	11.8
11	Meat refrigeration loss day 10 [%]	28.4	24.8
12	Meat *L** day 5	67.39	54.03
13	Meat *L** day 10	66.39	53.26
14	Meat *a** day 5	6.94	7.38
15	Meat *a** day 10	11.99	7.79
16	Meat *b** day 5	15.32	18.58
17	Meat *b** day 10	15.82	17.48
18	Cooking loss day 10 [%]	32.0	35.9
19	Desirability	0.60	0.62

TBARS – 2-thiobarbituric acid reactive substances (expressed as gram malondialdehyde per kilogram of tissue), *L** – lightness; *a** – redness; *b** – yellowness.

Tab. 3. Gas chromatography mass spectrometry composition of aqueous white pepper extract.

No.	Component	Molecular formula	Retention time [min]	Area [%]
1	Octamethylcyclotetrasiloxane (D4)	$C_8H_{24}O_4Si_4$	3.18	2.6
2	Benzene, 1,2,3-trimethyl-	C_9H_{12}	3.39	2.1
3	Benzene, 1,2,4-trimethyl-	C_9H_{12}	3.39	2.1
4	Mesitylene	C_9H_{12}	3.39	2.1
5	3-Carene or limonene	$C_{10}H_{16}$	3.87	1.4
6	(*E*)-β-Ocimene	$C_{10}H_{16}$	3.87	1.4
7	Hendecane	$C_{11}H_{24}$	4.43	1.2
8	2-Phenylethanol	$C_8H_{10}O$	4.65	21.6
9	3-Ethyl-5-methoxy-1,3,4-oxadiazol-2(3H)-one	$C_5H_8N_2O_3$	5.04	1.1
10	α,α-Dimethylcyclopentanemethanol	$C_8H_{16}O$	5.04	1.1
11	γ-Terpinene	$C_{10}H_{18}O$	5.49	2.5
12	Terpinen-4-ol	$C_{10}H_{18}O$	5.49	2.5
13	α-Terpineol	$C_{10}H_{18}O$	5.69	2.3
14	(*L*)-α-Terpineol	$C_{10}H_{18}O$	5.69	2.3
15	α-Terpineol acetate	$C_{12}H_{20}O_2$	5.69	2.3
16	Dodecane	$C_{12}H_{26}$	5.81	1.5
17	*N*-Tridecane	$C_{13}H_{28}$	5.81	1.5
18	Heptadecane	$C_{17}H_{36}$	5.81	1.5
19	Benzaldehyde, 4-nitro-	$C_7H_5NO_3$	7.66	0.7
20	*n*-Tetradecane	$C_{14}H_{30}$	8.48	2.1
21	*n*-Pentadecane	$C_{15}H_{32}$	8.48	2.1
22	Phenol, 4-(methoxymethyl)-	$C_8H_{10}O$	8.95	0.9
23	Benzeneethanol, 4-hydroxy-	$C_8H_{10}O$	8.95	0.9
24	2,4-di-tert-Butylphenol	$C_{14}H_{22}O$	9.88	0.8
25	2,5-bis(1,1-Dimethylethyl) phenol	$C_{14}H_{22}O$	9.88	0.8
26	Cetane	$C_{16}H_{34}$	10.91	1.8
27	Hexacosane	$C_{26}H_{54}$	10.91	1.8
28	Octadecane	$C_{18}H_{36}$	13.10	2.5
29	Nonadecane	$C_{19}H_{40}$	13.10	2.5
30	Methyl palmitate	$C_{17}H_{34}O_2$	14.41	1.4
31	Dibutyl phthalate	$C_{16}H_{22}O_4$	14.74	9.4
32	Phthalic acid, butyl hexyl ester	$C_{18}H_{26}O_4$	14.74	9.4
33	*n*-Hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$	14.84	8.1
34	Cyclopentadecane	$C_{15}H_{30}$	16.36	1.1
35	1-Octadecene	$C_{18}H_{36}$	16.36	1.1
36	Cycloeicosane	$C_{20}H_{40}$	16.36	1.1
37	Octadecanoic acid (stearic acid)	$C_{18}H_{36}O_2$	16.67	10.5
38	Isooctyl phthalate	$C_{24}H_{38}O_4$	19.80	24.7
39	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	19.80	24.7

Tab. 4. Gas chromatography mass spectrometry composition of aqueous black pepper extract.

No.	Component	Molecular formula	Retention time [min]	Area [%]
1	Octamethylcyclotetrasiloxane (D4)	$C_8H_{24}O_4Si_4$	3.19	0.6
2	Bis(trimethyl-silyl)salicylic acid	$C_{13}H_{22}O_3Si_2$	3.19	0.6
3	4-Methyldecane	$C_{11}H_{24}$	3.41	0.9
4	Undecane, 3,7-dimethyl	$C_{11}H_{24}$	3.41	0.9
5	Undecane, 5,7-dimethyl	$C_{13}H_{28}$	3.87	1.6
6	1-Hexanol, 5-methyl-2-(1-methylethyl)-	$C_{10}H_{22}O$	3.87	1.6
7	Tetrahydrolavandulol	$C_{10}H_{22}O$	4.47	0.6
8	3-Methyldecane	$C_{11}H_{24}$	4.47	0.6
9	2-Ethylbenzotriazole	$C_8H_9N_3$	4.60	10.1
10	*N*-Methyl-1-phenylcyclopropanamine	$C_{10}H_{13}N$	4.60	10.1
11	Butylsemithiocarbazide	$C_5H_{13}N_3S$	4.60	10.1
12	Dodecane, 6-methyl-	$C_{13}H_{28}$	6.01	0.7
13	Undecane, 3,6-dimethyl-	$C_{13}H_{28}$	6.01	0.7
14	Nonane, 2-methyl-	$C_{10}H_{22}$	6.01	0.7
15	Nonane, 4,5-methyl-	$C_{11}H_{24}$	6.56	0.8
16	2,4-Dimethylheptane	C_9H_{20}	6.69	0.6
17	Sulfurous acid, butyl dodecyl ester	$C_{16}H_{34}O_3S$	6.69	0.6
18	2,6,11-Trimethyldodecane	$C_{15}H_{32}$	6.91	1.4
19	3,7-Dimethyldecane	$C_{12}H_{26}$	6.91	1.4
20	1-Iodohexadecane	$C_{16}H_{33}I$	7.52	0.5
21	Tetracosane	$C_{24}H_{50}$	7.52	0.5
22	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	8.64	0.6
23	Nonadecane, 9-methyl-	$C_{20}H_{42}$	9.26	1.2
24	Hexadecane	$C_{16}H_{34}$	9.26	1.2
25	Tetracosane	$C_{24}H_{50}$	9.26	1.2
26	(+)-Calarene	$C_{15}H_{24}$	9.46	1.5
27	Cyperotundone	$C_{15}H_{22}O$	9.46	1.5
28	Heptacosane	$C_{27}H_{56}$	9.60	0.9
29	1-Iodooctadecane	$C_{18}H_{37}I$	9.60	0.9
30	Tridecane, 1-iodo-	$C_{13}H_{27}I$	9.68	1.6
31	Decane, 3,8-dimethyl-	$C_{12}H_{26}$	9.68	1.6
32	2,4-di-tert-butylphenol	$C_{14}H_{22}O$	9.90	1.9
33	Phenol, 2,5-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	9.90	1.9
34	6,6-Diethylhoctadecane	$C_{22}H_{46}$	11.31	0.6
35	Undecane	$C_{11}H_{24}$	11.49	0.6
36	Heptadecane	$C_{17}H_{36}$	12.04	0.7
37	2,6,10-Trimethyltetradecane	$C_{17}H_{36}$	12.04	0.7
38	Hentriacontane	$C_{31}H_{64}$	12.04	1.3
39	9-Octylheptadecane	$C_{25}H_{52}$	12.04	1.3
40	Hentriacontane	$C_{31}H_{64}$	12.61	0.6
41	Octadecane	$C_{18}H_{38}$	13.11	0.8
42	2-Methyltetracosane	$C_{25}H_{52}$	13.11	0.8
43	Octacosane	$C_{28}H_{58}$	13.59	0.7
44	Triacontane	$C_{30}H_{62}$	13.59	0.7

Tab. 4. *continued*

No.	Component	Molecular formula	Retention time [min]	Area [%]
45	Dotriacontane, 1-iodo-	$C_{32}H_{65}I$	14.03	1.0
46	Octadecane, 3-ethyl-5-(2-ethylbutyl)-1	$C_{26}H_{54}$	14.03	1.0
47	Methyl palmitate	$C_{17}H_{34}O_2$	14.41	2.8
48	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	14.41	2.8
49	Dibutyl phthalate	$C_{16}H_{22}O_4$	14.74	6.2
50	Butyl isobutyl phthalate	$C_{16}H_{22}O_4$	14.74	6.2
51	Palmitic acid	$C_{16}H_{32}O_2$	14.89	17.1
52	Methyl linoleate	$C_{18}H_{32}O_2$	16.00	8.6
53	Methyl (9*E*,12*Z*)-9,12-octadecadienoate (methyl linolelaidate)	$C_{19}H_{34}O_2$	16.00	8.6
54	(9*Z*,11*E*)-Octadecanoic acid	$C_{18}H_{31}O_2$	16.00	8.6
55	9-Octadecenoic acid (*Z*)-, methyl ester	$C_{19}H_{36}O_2$	16.05	4.3
56	9-Octadecenoic acid, methyl ester, (*E*) (methyl eladiate)	$C_{19}H_{36}O_2$	16.05	4.3
57	13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	16.05	4.3
58	Methyl stearate	$C_{19}H_{38}O_2$	16.30	0.7
59	Ethyl heptadecanoate	$C_{19}H_{38}O_2$	16.30	0.7
60	Petroselinic acid (6-octadecenoic acid)	$C_{16}H_{34}O_2$	16.45	9.7
61	(9*E*,12*Z*)-Linoleic acid	$C_{18}H_{32}O_2$	16.45	9.7
62	(*R*)-(–)-14-Methyl-8-hexadecyn-1-ol	$C_{17}H_{32}O$	16.45	9.7
63	Stearic acid	$C_{18}H_{36}O_2$	16.72	15.8
64	Carbonic acid, decyl undecyl ester	$C_{22}H_{44}O_3$	18.16	0.7
65	Tetracosane, 1-iodo-	$C_{24}H_{49}I$	18.16	0.7
66	Dotriacontane, 1-iodo-	$C_{32}H_{65}I$	18.16	0.7
67	(*Z*)-14-Methylhexadec-8-enal	$C_{17}H_{32}O$	19.51	0.6
68	Eicosane	$C_{20}H_{42}$	19.51	0.6
69	Phthalic acid bis(2-ethylhexyl ester)	$C_{24}H_{38}O_4$	19.77	1.9
70	Phthalic acid, dodecyl 2-ethylhexyl ester	$C_{28}H_{46}O_4$	19.77	1.9
71	Phthalic acid, hexadecyl 4-methylhept-3-yl ester	$C_{32}H_{54}O_4$	19.77	1.9

tures were named VitEm, BHTm, BPEm, WPEm and the control. Treatment groups were set-up in a completely randomized design. All solutions were mixed thoroughly prior to soaking of meat. Olive oil was added to increase BHT dissolution, but was added across all treatments for uniformity. Broiler chicken meat (100 g) was apportioned to each replicate and immersed completely for 45 min. Thereafter, samples were removed, arranged in plastic trays and wrapped with packaging film.

Data collection

Treatment solution uptake

The volume of the solution absorbed after soaking the meat in the extract for 1 h was determined by expressing the difference between the weight of sample before and after the soaking as a percentage of the initial weight [18].

Meat pH

Meat pH was measured using a pH meter ATC-108A (Hanna Instruments, Woonsocket, Rhode Island, USA) by placing the electrode of the handheld pH meter deeply into the muscle. Hitherto, the pH was calibrated by placing the electrode into the buffers with pH 4 and 7, consecutively. The pH was measured on days 0, 5 and 10 of storage.

Meat colour

Instrumental analysis was carried out on meat on days 0, 5 and 10 of storage using a Miniscan XE Plus colorimeter (HunterLab, Reston, Vir-

ginia, USA) with 25 mm hole set for illumination D65, 10° standard observer angle. The colour was measured according to the scale of International Commission on Illumination (CIE). The values of L^* (lightness), a^* (redness) and b^* (yellowness) were measured. Hue angle and chroma were calculated.

Malondialdehyde content in meat

The 15 g amount of marinated chicken meat and 30 ml of distilled water were blended together for 2 min using the Solitaire Mixer Grinder (Veejays Tading, Mumbai, India). Subsequently, the ground sample was placed in a centrifuge J2-21 (Beckman Coulter, Brea, California, USA) and spinned at 3 000 ×g for 15 min. Afterwards, 20 ml of the extracting solution consisting of 0.1% propyl gallate, 0.1% ethylene diamine tetraacetic acid (EDTA) and 7.5% trichloroacetic acid was mixed with the supernatant.

Next, 1 ml of the resulting mixture and 1 ml of thiobarbituric acid (TBA) reagent were added to a labelled test tube. Samples in the test tubes were sealed and placed in a Uniscope Water Bath SM801A (Surgifriend Medicals, Okehampton, United Kingdom) for 50 min at 70 °C, prior to cooling in running water [20]. The content was transferred to a vessel suitable for spectroscopic measurement. The formed complex of MDA and TBA was measured at 532 nm using a UV-VIS spectrophotometer 3400 CX (Varian Medical Systems, Palo Alto, California, USA). Lipid oxidation was reported in grams of MDA per kilogram of meat tissue.

Statistical analysis

Data regarding meat colour, pH and MDA were arranged in a 5×3 factorial arrangement with additive and storage day designated as fixed effects and replicates as a random effect using General Linear Mixed Model of Statistical Package for Social Sciences (SPSS) version 26 (IBM, Armonk, New York, USA). Significantly different means at $P < 0.05$ were separated using Duncan Multiple Range Test of the same Statistical Package.

RESULTS AND DISCUSSION

Treatment solution uptake

The relationship between the marinade solution uptake by the meat and the presence of treatment solution is shown in Fig. 1. The absorbtion of the solution was not significantly influenced by additives ($P > 0.05$). Numerically, the highest percentage of solution uptake was observed in the control sample. This may be due to the limited presence of dissolved ions or compounds in the control.

Meat pH

The combined effect of teatment solutions (VitEm, BHTm, BPEm and WPEm) and storage days on pH of broiler chicken meat is shown in Tab. 6. Additives and storage days had a significant impact on meat pH. On day 0, BPEm had the lowest pH, though similar as WPEm and BHTm. The pH of the control was the highest on day 0 and significantly differed from BPEm, WPEm and BHTm. On day 5, samples containing VitE had the lowest ($P < 0.05$) pH, followed by the control, while BHTm was the highest, though similar as WPEm. However, on day 10, the pH of BPEm was the highest, though similar as BHTm and control, while the lowest values were detected for WPEm and VitEm.

Under normal conditions, muscle glycogen is converted into lactic acid post slaughter, resulting in reduced pH from an initial value of 6.8–7.3 to about 5.4–5.8 at rigor mortis, depending on the species of livestock and muscle fibres [21]. However, during further storage, the pH gradually increases again. The increase is attributed to the

Tab. 5. Composition of experimemntal treatment solutions.

Treatment solution	Meat [g]	VitE [ml]	BHT [mg]	BPE [ml]	WPE [ml]	Salt [g]	Olive oil [ml]	Distilled water [ml]
Control	100	–	–	–	–	1	5	100
VitEm	100	0.033	–	–	–	1	5	100
BHTm	100	–	100	–	–	1	5	100
BPEm	100	–	–	100	–	1	5	–
WPEm	100	–	–	–	100	1	5	–

VitE – vitamin E (DL-α-tocopheryl acetate), BHT – butylated hydroxytoluene, BPE – black pepper extract, WPE – white pepper extract, VitEm – Vitamin E mixture, BHTm – butylated hydroxytoluene mixture, BPEm – black pepper extract mixture, WPEm – white pepper extract mixture.

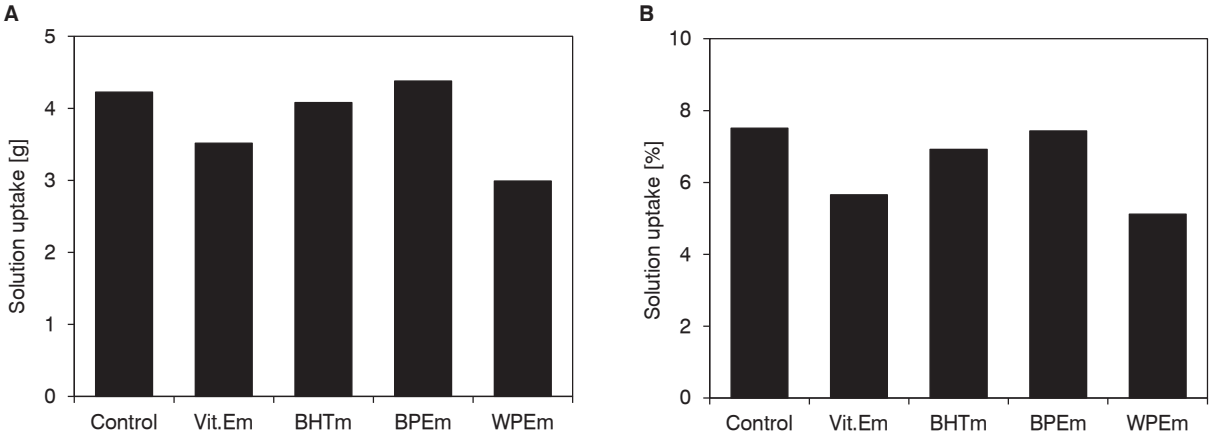

Fig. 1. Treatment solution uptake by the meat.

A – difference between the weight of sample before and after the soaking, B – solution uptake in percent.
VitEm – Vitamin E mixture, BHTm – butylated hydroxytoluene mixture, BPEm – black pepper extract mixture, WPEm – white pepper extract mixture.

Tab. 6. Combined effect of treatment solution and storage days on meat pH.

Treatment solution	Storage time		
	0 day	5 days	10 days
Control	6.78 ± 0.06^{ef}	6.91 ± 0.02^{de}	7.30 ± 0.09^{abc}
VitEm	6.66 ± 0.11^{fg}	6.66 ± 0.11^{fg}	7.24 ± 0.06^{bc}
BHTm	6.52 ± 0.14^{gh}	7.25 ± 0.25^{bc}	7.44 ± 0.09^{ab}
BPEm	6.42 ± 0.13^{h}	7.09 ± 0.07^{cd}	7.48 ± 0.04^{a}
WPEm	6.48 ± 0.21^{gh}	7.14 ± 0.19^{bc}	7.22 ± 0.04^{bc}

Values represent mean ± standard deviation. Means in the same column with different superscript differ significantly ($P < 0.05$).
VitEm – Vitamin E mixture, BHTm – butylated hydroxytoluene mixture, BPEm – black pepper extract mixture, WPEm – white pepper extract mixture.

action of microorganisms and meat endogenous enzymes, which decompose proteins and nitrogenous compounds, resulting in the release of alcaline substances. On day 0, the comparatively lower pH of BPEm samples could have been caused by the acidic compounds present in BPE, such as (*R*)-(–)-14-methyl-8-hexadecyn-1-ol or 2-ethylbenzotriazole. The further storage resulted in gradual alcalisation in all treatment groups, but WPEm and VitEm were apparently more effective than BPE in inhibiting this process as suggested by the data on day 10, though the outcome for BPEm was comparable to BHTm and the control group.

Meat colour

The influence of treatment solutions (VitEm, BHTm, BPEm and WPEm) and storage days on lightness, redness, yellowness, hue and chroma scores of meat is illustrated in Tab. 7. All the examined parameters were influenced ($P < 0.05$), excluding yellowness and chroma values of meat that were solely influenced by the storage days and not by the content of additives. The lightness L^* in BPEm samples was lowerered in comparison with other treatment groups.

Redness a^* was lower for the control and higher for BHTm than other additives, but only the difference between the control and BHTm reached the level of statistical significance ($P < 0.05$). The hue was significantly higher ($P < 0.05$) in the control compared to BHTm.

Meat containing BPE was affected by the colour of black pepper. The dark colour of black pepper is caused by enzymatic browning during processing of the *P. nigrum* fruits. These colour changes can significantly influence consumers' decision to purchase meat stored with BPE, depending on their preference. On the contrary, full maturity of the harvested berries led to higher lightness values of the WPEm samples in comparison with the BPEm samples. All the additives increased the redness values in comparison with the control, but this difference was statistically significant only in BHTm. The difference is possibly aided by the preservation of myoglobin and haemoglobin. According to RAMAMOORTHI et al. [22], high redness value results from the stabilizing effect of phenols that act as scavengers of oxidized lipid radicals. Incorporating BPE and WPE, as well as incorporating BHTm yielded numerically higher meat yellowness score than the control, but the difference was not statistically significant. Additive-treated meats had numerically lower hue

than the control, but the statistical significance was reached for BHTm only.

Meat lightness was pronounced ($P < 0.05$) on the day 5 of storage, followed by the day 0, but was significantly reduced on day 10. Meat redness value was intensified ($P < 0.05$) on day 10 as opposed to day 0 and day 5. Meat yellowness value was reduced ($P < 0.05$) on day 5 and day 10 but, was high on day 0. Meat hue was significantly ($P < 0.05$) reduced as storage days extend, while chroma intensity of meat was pronounced ($P < 0.05$) on day 0 and day 10 compared to day 5. The sole effect of storage on meat indicates increased lightness resulting from more oxyhaemoglobin on day 5, which is associated with higher molar absorptivity and lightness of meat samples. On the contrary, meat redness was elevated on the day 10, possibly from significant fraction of nitrogen dioxide existing as metastable excited state (NO^{-}) in meat. Molecules of nitrogen dioxide in such excited state emit photons that are readily dissipated in nitrogen-based bio-compounds. This agrees with the report of RAMAMOORTHI et al. [22], that increased redness can develop as a result of ferrous myoglobin derivative such as carboxymyoglobin or nitric oxide myoglobin other than oxymyoglobin. High chroma values on day 0 and day 10 possibly reflects greater lumination from purity of samples on day 0, leading to increased photon emission. On day 10, the chroma value was probably intensified by the oxidation of nitrogen-based bio-compounds

Malondialdehyde content in meat

The effect of additives and storage days on meat *TBARS* expressed as MDA content is presented in Tab. 8. Meat *TBARS* were influenced ($P < 0.05$) by both factors. On day 5, the lowest value of *TBARS* content was observed in BHTm samples, showing statistical significance ($P < 0.05$). The next lowest *TBARS* value was detected in BPEm samples ($P < 0.05$), while the control, VitEm and WPEm groups were significantly higher in comparison. On day 10, MDA

Tab. 7. Effect of treatment solution and storage days on meat colour.

	Lightness *L**	Redness *a**	Yellowness *b**	Hue	Chroma
Treatment solution					
Control	57.97 ± 5.01 ^{a}	8.53 ± 1.11 ^{b}	14.38 ± 2.47	58.94 ± 5.00 ^{a}	16.78 ± 2.23
VitEm	56.13 ± 5.47 ^{a}	9.51 ± 1.62 ^{ab}	14.37 ± 1.94	56.41 ± 5.47 ^{ab}	17.30 ± 1.93
BHTm	56.77 ± 4.51 ^{a}	10.61 ± 3.72 ^{a}	14.97 ± 2.45	55.07 ± 9.64 ^{b}	18.60 ± 3.09
BPEm	48.44 ± 7.63 ^{b}	9.81 ± 2.57 ^{ab}	14.78 ± 2.36	56.43 ± 8.82 ^{ab}	17.93 ± 2.16
WPEm	55.13 ± 4.87 ^{a}	9.96 ± 2.63 ^{ab}	14.53 ± 2.41	56.11 ± 8.79 ^{ab}	17.71 ± 2.21
Storage time					
0 days	55.72 ± 5.03 ^{b}	8.40 ± 1.31 ^{b}	16.97 ± 1.44 ^{a}	63.68 ± 3.61 ^{a}	18.97 ± 1.51 ^{a}
5 days	58.75 ± 4.73 ^{a}	8.43 ± 1.09 ^{b}	13.18 ± 1.33 ^{b}	57.32 ± 4.67 ^{b}	15.69 ± 1.12 ^{b}
10 days	50.19 ± 6.17 ^{c}	12.15 ± 2.54 ^{a}	13.70 ± 1.68 ^{b}	48.77 ± 4.83 ^{c}	18.36 ± 2.64 ^{a}

Values represent mean ± standard deviation. Means in the same column with different superscript differ significantly ($P < 0.05$). VitEm – Vitamin E mixture, BHTm – butylated hydroxytoluene mixture, BPEm – black pepper extract mixture, WPEm – white pepper extract mixture.

Tab. 8. Combined effect of treatment solution and storage days on meat malondialdehyde.

Treatment solution	*TBARS* [g·kg^{-1}]		
	0 days	5 days	10 days
Control	0.351 ± 0.001 ^{c}	0.550 ± 0.026 ^{a}	0.195 ± 0.001 ^{e}
VitEm	0.373 ± 0.024 ^{b}	0.269 ± 0.002 ^{d}	0.106 ± 0.001 ^{h}
BHTm	0.382 ± 0.003 ^{c}	0.129 ± 0.002 ^{g}	0.108 ± 0.001 ^{h}
BPEm	0.453 ± 0.001 ^{a}	0.162 ± 0.001 ^{e}	0.107 ± 0.001 ^{h}
WPEm	0.463 ± 0.001 ^{a}	0.203 ± 0.001 ^{f}	0.104 ± 0.001 ^{h}

Values represent mean ± standard deviation. Means in the same column with different superscript differ significantly ($P < 0.05$). *TBARS* – 2-thiobarbituric acid reactive substances expressed as gram malondialdehyde per kilogram of tissue, VitEm – Vitamin E mixture, BHTm – butylated hydroxytoluene mixture, BPEm – black pepper extract mixture, WPEm – white pepper extract mixture.

decreased in all the samples. Also, all meats with additives had lower MDA values than the control on day 10. Statistically identical MDA values for all antioxidant groups compared to the control confirm that BPEm and WPEm can act as potent alternatives to synthetic additives. This outcome agrees with the findings of PAGLARINI [23], where commercial plant extracts lowered the values of *TBARS* in mechanically deboned poultry meat stored at 2 °C for 10 days. Furthermore, PINDI et al. [24] showed the anti-oxidative properties of edible seaweed (reportedly rich in polyphenols) in suppressing lipid oxidation in chicken sausage stored at 4 °C for 12 days. Elevated MDA level in meat signifies vulnerability to oxidative stress. MDA levels in meat are limited by endogenous enzymatic antioxidants before and shortly after slaughter [25]. On day 0, addition of external antioxidants from pepper extracts elevated MDA in meat. However, on day 10, meat MDA was effectively suppressed by the addition of WPE and BPE, similar to conventional additives, in comparison with the control. Thus, the presented pepper extracts appear to be potent alternatives to conventional antioxidants as storage days extend. Bioactive compounds such as heptacosane; 2,4-di-tert-butylphenol, 2,5-di-tert-butylphenol and 2-methyltetracosane in BPE reportedly exert radical scavenging influence [26, 27]. Likewise, sulfurous acid, butyl dodecyl ester and phthalic acid, dodecyl 2-ethylhexyl ester are potent antioxidants in BPE. On the other hand, bis(2-ethylhexyl) phthalate in WPE was identified by KAVITHA et al. [28] in *Nocardia levis*, with reported radical quenching properties. Other compounds present in WPE, such as octamethylcyclotetrasiloxane (D4) and terpenoid alkaloids such as γ-terpinene, terpinen-4-ol, α-terpineol, as well as flavonoids such as eicodecene and octadecanoic acid contributed to the termination of radicals. On day 0, addition of VitE yielded similar result as the control. However, on day 10, the levels of MDA were lowered in the presence of VitE, similarly to other tested additives. Synthetic vitamin E is a phenolic antioxidant that acts by donating hydrogen from its hydroxyl (–OH) group to inactivate radicals, with the phenolic compound itself relatively unreactive due to its unpaired electron from the oxygen atom that has been delocalised into the aromatic ring structure for stability retention. A second route of activity facilitates the deletion of reactive oxygen/nitrogen species initiators (secondary antioxidants) by eliminating chain-initiating catalyst.

CONCLUSION

This study reveals microwave extraction of *Piper nigrum* released potent phytochemicals. WPE preserved meat colour (saturation and purity) similar as the control. Furthermore, WPE was as potent as DL-α-tocopherol in lowering meat pH on day 10 of storage. All incorporated extracts exhibited better radical quenching properties than the control, as judged by the MDA analysis. Both WPE and BPE contain bio-compounds that can act as safe alternatives to synthetic antioxidants.

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