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IMPLICATIONS OF BUTYLATED HYDROXYTOLUENE AND OPTIMIZED *PIPER NIGRUM* EXTRACTS APPLICATION ON QUALITY OF STORED PORK

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Abstract

Implications of butylated hydroxytoluene (BHT) and optimized *Piper nigrum* (White and Black Pepper) aqueous extracts (WPAE and BPAE) incorporation on fatty acid profile and physico-chemical indices of pork subjected to refrigeration storage was investigated. To produce optimized *Piper nigrum* aqueous extracts, desirability values of WPAE and BPAE were upscaled from 60 and 62% to 71 and 97% respectively using Design Expert$_{12}$, resulting in the yield of 65 and 87 bio-compounds. Fresh pork (100 g) was apportioned to four treatment groups (each replicated four times), comprising the Control (0% antioxidant); BHT (100 mg of BHT); BPAE (100 ml of BPE) and WPAE (100 ml of WPE), then soaked for 30 min prior to refrigeration storage [day (d) 0, 5 and 10] in a completely randomized design. All parameters examined were influenced ($p < 0.05$) except additive effect on pork pH. Pork preserved with BPAE and WPAE minimized deterioration of fatty acids (FA) such as oleic, palmitoleic, total mono unsaturated fatty acid (MUFA) and poly unsaturated fatty acid (PUFA) than 0% antioxidant. BPAE had the highest MUFA: SFA, and alongside BHT, had significantly ($p < 0.05$) low index of atherogenicity. BPAE and WPAE had lesser cooking loss percentages than the Control. Additives X Storage day's effect reveal BPAE yielded least refrigeration loss (%) among samples evaluated and had extensive protective benefits. BPAE had the lowest milligram malondialdehyde per gram tissue on d 10 of storage. Extract from BP may potentially contain compounds that could offer enhanced meat preservation and promote consumer health.

Keywords: optimized *Piper nigrum* aqueous extracts, BHT, pig meat, fatty acid profile, oxidative stability, cooking and refrigeration losses, 2-thiobarbituric acid reactive substance

INTRODUCTION

Nowadays, most consumers of meat are favorably disposed to meat products of higher nutritional value irrespective of the substance applied to limit deterioration or the storage employed, yet, health-conscious consumers are concerned of the role dietary sources play in the occurrence of diet-related diseases in humans (Beriain *et al.*, 2018). Meat is a delicacy cherished for the peculiar sensations experienced by consumers from mastication as it contributes to healthy diets and transfer of balanced-profile proteins. On worldwide basis, meat supply is projected to expand to meet consumer demand, expectedly reaching 377 Mt by 2031. Generally, pork consumption is projected to increase to 129 Mt in the next ten years (OECD/FAO, 2022). Consumers are attracted to pork due to its lower price and product consistency. In several tropical countries, favorable relative prices have positioned pork and poultry as choice meats to meet rising demand from the middle class. Aside its quality protein profile,

its B complex vitamins content such as vitamin B12 and mineral nutrients like zinc, selenium, iron and phosphorus makes it a desired choice (Pereira and Vincente, 2013; Bohrer, 2017). Pork, though commonly consumed contains less unsaturated fatty acid (UFA) compared to poultry. Muzolf-Panek, and Kaczmarek (2021) further explained that beef and pork contain significantly less poly unsaturated fatty acid (PUFA) and lower value for PUFA/UFA index compared to chicken meat.

Fatty acids (FA's) are important structural components of cells and biological membranes that are built into lipids derived components such as phospholipids, sphingolipids and lipoproteins. FA's contribute significant roles in fluid regulation in the human body by providing energy for membrane constituents of cells and influencing physiological activities in humans (Brown and Marnett, 2011; Dennis and Morris, 2015). FA's are likewise considered essential as cellular lipid mediators. In spite of numerous benefits that can be derived from FA's, oxidation of PUFA's during storage, processing and cooking significantly limit nutritional benefits humans derive. Additionally, food quality and shelf life are adversely impacted when unsaturated fats are oxidized. Reduced nutritional quality can translate into poor health for consumers, depending on the concentration of rancid substances in products. Fresh meat kept in the refrigerator (≤ 4 °C) is prone to oxidative and microbial spoilage. Psychrotrophic and facultative anaerobes can grow rapidly by depleting glucose and subsequently amino acids (Ling, 2015; Osman and Faruk, 2015; Wang *et al*., 2023) Quality preservation of meat therefore entails spoilage limitation by minimizing the extent of lipid oxidation and microbial proliferation.

Processing methods commonly applied during short term storage of meat include chilling, refrigeration and cooking. These methods are employed to conserve nutrients in meat as consumers are nourished with safe and quality products. While benefits of refrigeration and cooking are well documented, shrinkage of meat occurs, causing significant impact on meat quality and nutrient pool, though, the latter is notably critical in suppressing and destroying foodborne pathogens (Kondjoyan *et al*., 2014) Refrigeration and cooking significantly affect protein quality via structural and textural changes. The extent of evaporation of cellular moisture, rate of destruction of cell membranes, loss of cellular fibres and connective tissues as well as myofibrillar proteins contribute to the overall quality of stored meat (Pathare and Roskilly, 2016). Meat processors employ strategies to minimize *Postmortem* disruption of polypeptides in tissue though the disruption is essential for the development of flavour and eating qualities of meat. These disruptions initiates lipid oxidation, which unregulated, alters the nutritional, physico-chemical (colour and texture) and sensorial properties (taste and aroma) of meat, causing off-flavour development that culminates into unacceptable taste and poor product quality (Lima *et al*., 2013). Consequently, the search for safe substances and effective strategies to minimize loss of nutrients in meat is currently investigated.

There is an urgent need to develop safe and effective procedures to extract and isolate bioactive compounds since increased extraction efficiency results in the production of safe additives that are of significance to meat farmers and retailers. Biological activities of *Piper nigrum* (*P. nigrum*) seed and extracts have been explored and investigated. Piper species (white and black) are traditionally consumed to treat ailments or alternatively applied as antioxidant, preservative, insecticide or anti-microbial (Aziz *et al*., 2015; Gasparetto *et al*., 2017; Zhang *et al*., 2018). Extraction of organic compounds from very different types of matrixes using microwave technology has resulted in increased extraction efficiency and subsequent release of bio-active phytochemicals (Duarte *et al*., 2014). Currently, different kinds of solvents are used to access bio-active compounds in *P. nigrum* with varying yield and quality, however, the application of alcoholic extracts of plants directly on meat can result in significant shrinkage as well as global environmental implications; prompting the need for green methodology. The study, therefore, investigate the potency of butylated hydroxytoluene and *P. nigrum* extracts on quality of stored pork. It aims to prepare *P. nigrum* extracts using microwave heating in an environmentally friendly way, to compositionally identify bio-compounds in *P. nigrum* extracts using Gas Chromatography Mass Spectrometry (GC-MS), to describe the effect of prepared extracts on physico-chemical properties of pork during short term [24 h (d 0), 5 and 10 days] storage and to examine the potency of *P. nigrum* extracts on pork FA and lipid on d 10 of storage (4 °C).

MATERIALS AND METHODS

Materials

Fresh pork from the thigh (*Biceps femoris*) of the hind limb of a mixed breed pig of internal pH of 5.8 (24 h *postmortem*) was sourced from a local market. Distilled water was obtained from the Laboratory of Animal Product and Processing, Department of Animal Production and Health. Butylated hydroxytoluene [2,6-Di-tert-Butyl-p-Cresol (99% purity) by Loba Chemie] was gotten from a reputable store. Dried peppercorns of black and white pepper were sourced from a reputable local herb market, separately grinded using an attrition mill (industrial grinding mill Gx200 petrol engine, India). Afterwards, powdered peppers were clarified using an 0.40 mm sieve (Adegoke *et al*., 2013; Adegoke *et al*. submitted), then sealed and stored in air-tight amber colored vials.

Generation of Extraction Criteria for the Production of Piper Nigrum Aqueous Extracts

Extraction parameters were generated by modifying production constraints from data provided [Adegoke *et al.* (2023); submitted data] by Design Expert Software. Post-upscale of previous data, 97% desirability was achieved after setting the following targets. Extraction time was set to range between 85 and 95 min; solvent volume was fixed between 360 and 380 mL; extract volume was set between 60.42 and 115.15 mL; extract absorbed was defined as maximized between 3 and 7 mL; pH on d 0 and 5 were targeted at 5.85 and 6.05 respectively; pH on d 10 was set to range between 6.37 and 7; meat 2-thiobarbituric acid reactive substance (TBARS) value on d 5 and 10 was targeted at 0.3 and 0.11 mg malondialdehyde (MDA) g^{-1} tissue respectively, while cooking loss was set to range between 30.6 and 41%.

To achieve 71% desirability for white pepper extract, Design Expert software was employed. Extraction time was fixed between 85.2 and 95 min; solvent volume was fixed to range between 280 and 295 mL; extract volume was set as minimized at 41 mL; extract absorbed was set as maximized between 0.4 and 6.3 mL; pH on d 0 and 5 were defined as targeted at 5.61 and 6.05 respectively; pH on d 10 was set as minimized at 6.6; and 2-thiobarbituric acid reactive substance (TBARS) values on d 5 and 10 were set as minimized, and targeted at 0.277 and 0.230 mg (MDA) g^{-1} tissue respectively; while cooking loss was fixed at 17.54%. Afterwards, extraction conditions in Tab. I were proffered. Subsequently, ground *P. nigrum* powders were screened with a 0.40 mm sieve. Thereafter, eight grams was separately weighed per pepper and dissolved in 404.21 and 414.21 mL of distilled water for white and black pepper respectively. Each mixture was thoroughly stirred in microwave-tolerant containers and placed in heating cavities of Samsung (GE109MST) and Daewood (KOR6N9NC) microwave ovens respectively, then subsequent heating at 300 and 320 W was performed for 85.27 and 86.91 min to produce white and black pepper aqueous extracts (WPAE and BPAE). Afterwards, containers were unloaded and allowed to cool prior to evaluation of bio-compounds extracted via Gas Chromatography Mass Spectrometry (GC-MS) analysis.

Analysis of Bio-Compounds in Optimized Aqueous Extracts

Spectrometry (GCMS analysis) of optimized *P. nigrum* extracts generated was carried out. An Agilent Technologies Network GC system was set up comprising GC (Agilent 7890A, USA) and MS (Agilent 5975C model, USA) with column model Agilent technologies HP5MS of 30 m length, 0.32 mm internal diameter and 0.25 µL thickness. Agilent Technologies GC injector of initial temperature of 80 °C was held for 2 min at 12 degrees per min, and

I: *Criteria for desirability values prior to production of white and black pepper aqueous extracts*

Number	Pepper extract	Ext Time (min)	Solvent vol (mL)	Extract vol	Extract Absorbed	Meat pH d 0	Meat pH d 5	Meat pH d 10	TBARS d 5	TBARS d 10	Cook loss % d 10	Desirability
1	White	85.273	280.000	65.997	5.698	6.284	6.057	6.587	0.275	0.308	18.997	0.713
2	Black	86.908	364.206	95.229	7.014	5.850	6.081	6.887	0.301	0.137	35.620	0.971

Ext – extraction; TBARs – 2-thiobarbituric acid value (MDA); d – day; Ref – Refrigeration; Desirability was upscale from Adegoke *et al.* (2023); Adegoke *et al.* (submitted)

raised to 240 degrees for 6 min. Little portion of the supernatant solution was sieved through a 0.45 µm micro filter and acetone of analytical grade was added at 1:10. Subsequently, a microliter of solution was injected into the GC according to procedure reported by Adegoke *et al.* (2023). Afterwards, spitless mode of analysis was carried out using scan ranges between 50 and 500, followed by identification of compounds according to the apex fragmentation fingerprints area for extracts of black (retention time of 4.56–20.40 min) and white pepper (retention time of 3.33–20.40 min).

Experimental Treatment Groups

Two repetitions (microwave extraction) were performed to constitute each *P. nigrum* group. A total of four experimental groups, each consisting four replicates were formed. Treatments comprised the Control (100 g of Pork + 0% antioxidant + 1 g of NaCl + 5 mL of Olive oil + 100 mL of distilled water), BHT [100 g of Pork + 0.1 g of BHT (Naveena *et al*., 2008) + 1 g of NaCl + 5 mL of Olive oil + 100 mL of distilled water], WPAE [100 g of pork + 5 mL of olive oil + 1 g of NaCl + 100 mL of white pepper extract (WPE)] and BPAE [100 g of pork + 5 mL of Olive oil + 1 g of NaCl + 100 mL of black pepper extract (BPE)] groups outlaid in a Completely Randomized Design. All compositions were thoroughly mixed prior to pork insertion. Olive oil was added to aid the dissolution of BHT, while its inclusion across all replicates was carried out to maintain uniformity. Next, one hundred grams (100 g) of pork apportioned to each replicate was completely immersed for 30 min. Afterwards, immersed samples were removed and placed in soft disposable plastic trays wrapped with packaging film and placed in refrigerator in preparation for subsequent quality evaluations across storage (4 °C) days.

Data Collection

Meat Fatty Acid Profile

On d 10 of storage (±4 °C), lipid was extracted from pork according to method established by Folch *et al.* (1957). In total, 10 g of pork was cut out, blended and homogenized. Chloroform: methanol (2 : 1, v/v) mixture was added to blended meat at 40 mL: 2 g. Samples were incorporated at 1 µL in split mode (20 : 1). Separation was performed using chromatograph equipped with a flame ionization detector and GC column [HP-88 (Agilent Technologies, USA) (30 m × 0.25 µm × 0.25 mm BPX-70 fused silica capillary column)]. Initial column temperature was held at 115 °C for 2 min at 10 °C/min after which temperature was increased to 200 °C held at a rate of 18.5 min at 60 °C min^{-1}, and raised to 245 °C for 4 min. Injector and detector temperature was 250 °C. Helium gas was passed through the column at 1.0 flow rate, 14.6 pressure and velocity of 29. Injector temperature was held at 300 °C, while hydrogen flow occurred at 40.0 but air flow at the detector was 184.0. Further flow of helium gas occurred at 45.0 through a temperature of 300 °C. Absorbance of concentration of different standard solutions specific to fatty acid and sample benzene extracts were measured using a spectrophotometer according to wavelength identified for each fatty acid. Area under each peak was identified by fatty acid methyl esters that were compared with standard AOCS (1998) Ce1f-96. Result was presented in mg/100 g of tissue. Relationship between the addition of the main proatherogenic saturated and antiatherogenic unsaturated fatty acids were computed to obtain pork index of atherogenicity as reported by Ulbricht and Southgate (1991):

$$IA = \frac{(C12{:}0 + (4\ X\ C14{:}0) + C16{:}0)}{\Sigma MUFA + \Sigma(n-6) + \Sigma(n-3)}.$$

Meat pH

To calibrate the pH meter (model pH – 108A by ATC Hanna, India), the probe of the pH meter was inserted into buffer 7, quickly cleaned, then inserted into buffer 4. Afterwards, the electrode of the standardized hand-held pH meter was inserted deeply into pork containing additive to obtain the initial pH. The procedure was repeated on day (d) 5 and 10.

Meat Refrigeration Loss

Samples containing additives were re-weighed after 24 h (d 0), d 5 and 10 to determine weight change, which was subsequently expressed as a percentage of the initial weight.

Refrigeration weight loss was calculated as follows:

$$Refrigeration\ loss\ (\%) = \frac{weight\ before\ refrigeration - weight\ after\ refrigeration}{weight\ before\ refrigeration}.$$

Meat Cooking Loss

After determining refrigeration loss percentage on d 10, samples were weighed, wrapped in separate air-tight polythene bag and cooked in a digital water bath (Uniscope Sm801a, Surgifriend Medicals, England) at 70 °C for 30 minutes (Sanwo *et al*., 2019). Cooking loss percentage was calculated as follows:

$$Cooking\ loss\ (\%) = \frac{Weight\ before\ cooking - Weight\ after\ cooking}{weight\ before\ cooking} \times 100.$$

Meat Malondialdehyde Content

On d 10 of refrigeration storage, ten grams (10 g) of pork sample was cut out per replicate

and blended with 20 mL of extracting solution (7.5% trichloroacetic acid + 0.1% ethylenediamenetetraacetic acid + 0.1% propyl gallate) for 30 s using a tissue homogenizer. Afterwards, mixture was decanted for 1 min and filtrate (using muslin cloth) was centrifuged [Merlin 503 by Spectra Scientific Limited, UK) at 2000 rpm for 15 min. Next, 1 mL of supernatant extract was added to 1 mL of TBA reagent in labelled test tubes. The test tubes (larger) were slightly sealed (to allow air escape) and placed in water bath [Uniscope Laboratory Water Bath (Model SM801A) by Surgifriend Medicals, England] at 70 °C for 40 min, then cooled with running water. Aliquots were afterwards transferred to 96 well plates and absorbance of pink chromogen formed against blank was measured at 532 nm using a UV-VIS spectrophotometer (Helios, Thermo-spectronic, Cambridge, UK). Results were presented as malondialdehyde equivalents (mg MDA/g tissue of pork).

Statistical Analysis

Data obtained from physico-chemical and fatty acid profile assessments were subjected to One-way analysis of variance (ANOVA) in a Completely Randomized Design. Data for additives × storage days was outlaid in a 4 × 3 factorial arrangement with additives and storage days as fixed effects and the replicates as a random effect; and analysed using the General Linear Mixed Model (GLMM) of Statistical Package for Social Sciences (SPSS) version 26 (2019). Significant means at 5% level of significance were separated using Duncan Multiple Range Test (DMRT) of the same Statistical Package.

RESULTS

GC-MS analysis for optimized WPAE and BPAE yielded 65 and 87 bio-compounds respectively (Tab. II and III). WPAE contained 22 nitrogen-based compounds, consisting of one fluorine-nitrogen based compound, one bromine-nitrogen based compound, four nitrogen-sulphur based compounds

II: *Gas chromatography mass spectrometry of bio-compounds in white pepper aqueous extract*

S/N	Compound	Molecular formula	Area
1	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3(trimethylsiloxy)trisiloxane	$C_{11}H_{32}O_4Si_4$	1.03
2	Cyclohexene, 4-methyl-1-(1-methylethenyl)	$C_{10}H_{18}$	1.06
3	2-Methyl-6-(5-methyl-2-thiazolin-2-ylamino) pyridine	$C_{10}H_{13}N_3S$	1.06
4	Benzaldehyde, 2-methoxy-	$CH_3OC_6H_4CHO$	1.06
5	1-Piperidinecarboxaldehyde	$C_6H_{11}NO$	1.09
6	1-(2-Adamantylidene) semicarbazide	$C_{11}H_{17}N_3O$	1.13
7	5,6-Epoxy-2,2-dimethyl-3-heptyne	$C_9H_{14}O$	1.13
8	2,4-Decadienamide, N-isobutyl-, (E, E,)	$C_{14}H_{25}NO$	1.18
9	8-Oxabicyclo [3.2.1] oct-6-en-3-one, 2,4-dimethyl	$C_9H_{12}O_2$	1.18
10	1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy) tetrasiloxan-1-ol	$C_{12}H_{34}O_5Si_4$	1.21
11	Hexasiloxane, tetradecamethyl-	$C_{14}H_{42}O_5Si_6$	1.22
12	1-(2-Adamantylidene) semicarbazide	$C_{11}H_{17}N_3O$	1.31
14	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	1.32
15	2-Amino-4-methylphenol, 2TMS derivative	$C_{13}H_{25}NOSi_2$	1.32
16	Salicylic acid, 2TMS derivative	$C_{13}H_{22}O_3Si$	1.32
17	Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	1.39
18	6-Anilino-2-(m-tolyl)-1,3,5-thiadiazine-4-thione	$C_3H_2N_2S_2$	1.41
19	Ethylene glycol, 2-methoxybenzoate	$C_{10}H_{12}O_2$	1.41
20	Benzene, 2,4-diethyl-1-methyl-	$C_{11}H_{16}$	1.53
21	Pyrido[2,3-d] pyrimidine, 4-phenyl-	$C_{13}H_9N_3$	1.53
22	N-Methyl-1-adamantaneacetamide	$C_{13}H_{21}NO$	1.53
23	Caryophyllenyl alcohol	$C_{15}H_{26}O$	1.55
24	Thiophene, 2,3,4-trimethyl-	$C_7H_{10}S$	1.55
25	2-Acetylcyclopentanone	$C_6H_{10}O$	1.55

S/N	Compound	Molecular formula	Area
26	(-)-Spathulenol	$C_{15}H_{24}O$	1.55
27	(3-Methylphenyl) phenylmethanol	$C_{14}H_{14}O$	1.55
28	2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl) but-2-en-1-ol	$C_{14}H_{24}O$	1.55
29	Trimethylsilyl [2-(4-chlorophenyl)-4-phenyl-1,3-thiazol-5-yl] acetate	$C_{25}H_{24}ClNO_2SSi$	1.68
30	2,5-Dihydroxybenzoic acid, 3TMS derivative	$C_{16}H_{30}O_4S$	1.68
31	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]	$C_{12}H_{36}O_4Si$	1.68
32	3-Trimethylsilyloxystearic acid, trimethylsilyl ester	$C_{24}H_{52}O_3Si_2$	1.94
33	1,1,1,5,7,7,7-Heptamethyl-3,3-bistrimethylsiloxy) tetrasiloxane	$C_{13}H_{39}O_5Si_6$	1.94
34	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_6Si_5$	2.04
35	4-Ethylbenzamide	$C_9H_{11}NO$	2.06
36	Phthalic acid, isobutyl 2-methoxyethyl ester	$C_{15}H_{20}O_5$	2.06
37	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene- [1ar-(1a. alpha.,4a. alpha.,7.beta., 7a.beta.,7b.alpha.)]	$C_{15}H_{24}O$	2.24
38	(3aS,4R,7R)-1,4,9,9-Tetramethyl-5, 6,7,8-tetrahydro-4H-3a,7-methanoazulene	$C_{15}H_{22}$	2.24
39	[3-(4-Methoxyphenyl)-4,5-dihydro-1,2-oxazol-5-yl] methanol	$C_{10}H_{13}NO_3$	2.24
40	2-Ethylacridine	$C_{15}H_{13}N$	2.24
41	Tris(tert-butyldimethylsilyloxy)arsane	$C_{18}H_{45}AsO_3Si_3$	2.24
42	Benzamide, N-(1,1-dimethylethyl)-4-methoxy	$C_{12}H_{18}N_2O_2$	2.53
43	1,2-Benzisothiazole-3-propanoic acid	$C_{10}H_9NO_2S$	2.53
44	(E)-5-(Benzo[d][1,3] dioxol-5-yl)-1-(piperidin-1-yl)pent-2-en-1-one	$C_{17}H_{21}NO_3$	2.53
45	Pentasiloxane, dodecamethyl-	$C_{12}H_{36}O_4Si_5$	2.59
46	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	$C_{16}H_{48}O_7Si_8$	2.59
47	3-Isopropoxy-1,1,1,7,7,7-hexamethy l-3,5,5-tris(trimethylsiloxy)tetrasiloxane	$C_{18}H_{52}O_7Si_7$	2.59
48	l- (+)-Lactic acid, tert-butyldimethylsilyl ether	$C_{15}H_{34}O_3Si_2$	3.05
49	Allyl(2-butoxy) dimethylsilane	$C_9H_{20}OSi$	3.05
50	Chloromethyl cyanide	C_2H_2ClN	3.05
51	Benzene, 1,2-dichloro-	C_6H_4Cl	3.99
52	Benzene, 1,3-dichloro-	$C_6H_4Cl_2$	3.99
53	4-Methylnaphtho[1,2-b] thiophene	$C_{13}H_{10}S$	5.77
54	Benzene, 1-fluoro-4-(2-phenylethenyl)	$C_{14}H_9F$	5.77
55	Tacrine	$C_{13}H_{14}N_2$	5.77
56	Furane-2-carbohydrazide, 5-phenylethynyl	$C_{13}H_{10}N_2O_2$	7.83
57	Butylphosphonic acid, butyl 5-methoxy-3-methylpentyl ester	$C_{14}H_{31}O_4P$	7.83
58	1,2-Diborane (4) diamine, 1,2-diethyl-N, N, N', N'-tetramethyl	$C_8H_{22}B_2N_2$	7.83
59	Caryophyllene	$C_{15}H_{24}$	8.68
60	Tetrasiloxane, decamethyl-	$C_{10}H_{30}O_3Si_4$	10.01
61	Arsenous acid, tris(trimethylsilyl) ester	$C_9H_{27}AsO_3Si_3$	11.80
62	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	11.80
63	Oxime-, methoxy-phenyl-	$C_8H_9NO_2$	13.73
64	Silane, dimethyl (dimethyl (but-2-en yolky)silyloxy) (but-2-enyloxy)	$C_{12}H_{26}O_2Si$	13.73
65	Oxazolidine, 3-phenyl-	$C_9H_{11}NO$	13.73

III: *Gas Chromatography Mass Spectrometry of bio-compounds in black pepper aqueous extract*

S/N	Compound	Molecular formula	Area
1	Ethyl 4-hydroxymandelate, 2TMS derivative	$C_{16}H_{28}O_4Si_2$	0.49
2	3-Hydroxymandelic acid, ethyl ester, di-TMS	$C_{16}H_{28}O_4Si_2$	0.49
3	p-Trimethylsilyloxyphenyl-bis(trimethylsilyloxy)ethane	$C_{17}H_{34}O_3Si_3$	0.49
4	4-(4-Methylphenylamino)pyrido [3,2-c] pyridazine	$C_{14}H_{12}N_4$	0.49
5	3-Amino-7-nitro-1,2,4-benzotriazine 1-oxide	$C_7H_5N_5O_3$	0.49
6	trans-3-Ethoxy-b-methyl-b-nitrostyrene	$C_{11}H_{13}NO_3$	0.49
7	1-Piperidinecarboxaldehyde	$C_6H_{11}NO$	0.54
8	3,3-Dimethylpiperidine	$C_7H_{15}N$	0.54
9	Caprolactam	$C_6H_{11}NO$	0.54
10	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	0.59
11	N-(Trifluoracetyl)-O,O',O''-tris (trimethylsilyl)epinephrine	$C_{20}H_{36}F_3NO_4Si_3$	0.59
12	N-(Trifluoroacetyl)-N,O,O',O''-tetrakis(trimethylsilyl) norepinephrine	$C_{22}H_{42}F_3NO_4Si_4$	0.59
13	Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl	$C_{10}H_{16}$	0.60
14	1,3,7-Octatriene, 3,7-dimethyl-	$C_{10}H_{16}$	0.60
15	. beta. –Ocimene	$C_{10}H_{16}$	0.60
16	2-Propenoic acid, 2-methyl-3-(4-nitrophenyl)	$C_{10}H_9NO_4$	0.60
17	Piperonal	$C_8H_6O_3$	0.63
18	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	$C_{14}H_{42}O_6Si_7$	0.63
19	3-Amino-2-phenazinol ditms	$C_{18}H_{25}N_3OSi_2$	0.63
20	Ar-tumerone	$C_{15}H_{20}O$	0.67
21	3-Methyl-2-butenoic acid, 3-phenyl-2-propenyl Ester	$C_{14}H_{18}O_2$	0.67
22	(Z, Z)-. alpha. –Farnesene	$C_{15}H_{24}$	0.73
23	1-(2-Adamantylidene) semicarbazide	$C_{11}H_{17}N_3O$	0.73
24	1,7-Octadiene, 3,6-dimethylene-	$C_{10}H_{14}$	0.73
25	[1,2,4] Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester	$C_{10}H_{12}N_4O_3S$	0.77
26	Pyrrolo[2,3-g]indazole-7,8-dione, 1,6-dihydro-	$C_9H_5N_3O_2$	0.77
27	Camphene	$C_{10}H_{16}$	0.77
28	Diazoprogesterone	$C_{21}H_{30}N_4$	0.79
29	Bicyclo[3.1.0]hexane, 6-methylene-	C_7H_{10}	0.79
30	1H-Pyrazole-1-acetamide,4-iodo-N-(phenylmethyl)	$C_{12}H_{12}IN_3O$	0.90
31	gamma. –Neoclovene	$C_{15}H_{24}$	0.90
32	1H-3a,7-Methanoazulene,octahydro-1H-3a,7-Methanoazulene, octahydro-	$C_{16}H_{26}O_2$	0.90
33	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	1.05
34	Phenol, 3,4-dimethoxy-	$C_8H_{10}O_3$	1.05
35	3-Amino-2,6-dimethoxypyridine	$C_7H_{10}N_2$	1.05
36	1-((1S,3aR,4R,7S,7aS)-4-Hydroxy-7-isopropyl-4-methyloctahydro-1H-ind en-1-yl) ethenone	$C_{15}H_{26}O_2$	1.13
37	((1R,4S,5R)-1-Methyl-4-(prop-1-en-2yl) spiro[4.5]dec-7-en-8-yl) methanol	$C_{15}H_{24}O$	1.13
38	Phenol, 4-methyl-2-nitro-	$C_7H_7NO_3$	1.13
39	Cyclopentylsilane	C_5H_9Si	1.20
40	Cyclohexanol, 4-methoxy-	$C_7H_{12}O_2$	1.20
41	3-Octadecanone	$C_{18}H_{36}O$	1.20

S/N	Compound	Molecular formula	Area
42	2-Fluoro-4-methylpyridine	C_6H_6FN	1.35
43	2-Acetylcyclopentanone	$C_7H_{10}O_2$	1.35
44	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	$C_{13}H_{39}O_5Si_6$	1.47
45	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	1.47
46	Oxazepam, 2TMS derivative	$C_{15}H_{11}ClN_2O_2$	1.47
47	6,7-Dimethyl-1,2,3,5,8,8a-hexahydronaphthalene	$C_{12}H_{18}$	1.51
48	Sospathulenol	$C_{15}H_{24}O$	1.51
49	Benzene, 1-(1,5-dimethyl-4-hexenyl	$C_{15}H_{22}$	1.59
50	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S- (R*, S*)]	$C_{15}H_{24}$	1.59
51	Cedrene	$C_{15}H_{24}$	1.59
52	gamma. – himachalane	$C_{15}H_{24}$	1.59
53	2,5-Dihydroxybenzoic acid, 3TMS derivative	$C_{16}H_{30}O_4Si$	1.88
54	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_6Si_5$	1.93
55	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	1.98
56	4-Hydroxy-3-methylacetophenone	$C_9H_{10}O_2$	1.98
57	Cycloheptasiloxane, tetradecamethyl	$C_{14}H_{42}O_7Si_7$	2.38
58	Trisiloxane,1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]	$C_{12}H_{36}O_4Si$	2.38
59	Pentasiloxane, dodecamethyl-	$C_{12}H_{36}O_4Si_5$	2.38
60	8-Methyl-4-azafluorenone	$C_{19}H_{14}N_2$	2.46
61	2-Amino-9-fluorenone	$C_{13}H_9NO$	2.46
62	4-Amino-9-fluorenone	$C_{13}H_9NO$	2,46
63	2-Bromo-2'-methoxyacetophenone	$C_9H_9BrO_2$	2.66
64	Ethylene glycol, 2-methoxybenzoate	$C_{10}H_{12}O_3$	2.66
65	2-Methoxy-N'-[4-(trifluoromethyl)pyridin-2-yl]benzohydrazide	$C_7H_6F_3NO$	2.66
66	2-Phenyl-3-(2-furyl)-propenal	$C_{13}H_{10}O_2$	2.77
67	2-furancarboxaldehyde, 5-[2-phenylethenyl]	$C_{13}H_{10}O_2$	2.77
68	Phosphonous acid, phenyl-, diethylester	$C_{10}H_{15}O_2P$	2.77
69	2-Naphthalenecarboxylicacid,8-ethenyl-3,4,4a,5,6,7,8,8a-octahydro5-methylene	$C_{18}H_{22}O_2$	3.20
70	Caryophyllene oxide	$C_{15}H_{24}O$	3.20
71	(-)-Spathulenol	$C_{15}H_{24}O$	3.20
72	6,6-Dimethyl-2-vinylidenebicyclo [3.1.1] heptane	$C_{11}H_{16}$	3.50
73	Caryophyllene	$C_{15}H_{24}$	3.50
74	trans-. beta. –Ocimene	$C_{10}H_{16}$	3.50
75	Dibenzothiophene, 4-methyl-	$C_{13}H_{10}S$	4.31
76	Benzene 1-fluoro-3-(2-phenylethen yl)- (E)		4.31
77	Dibenzothiophene, 3-methyl-	$C_{13}H_{10}S$	4.31
78	Hexane, 1-chloro-5-methyl-	$C_7H_{15}Cl$	5.03
79	(3R,4aS,8aS)-8a-Methyl-5-methylene-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene	$C_{15}H_{22}$	5.03
80	6,7-Dimethyl-1,2,3,5,8,8a-hexahydr 1] hept-2-ene	$C_{10}H_{18}$	5.03
81	Tris(tert-butyldimethylsilyloxy)arsane	$C_{18}H_{45}AsO_3Si_3$	10.28
82	Arsenous acid, tris (trimethylsilyl) ester	$C_9H_{27}AsO_3Si_3$	10.28
83	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	10.83

S/N	Compound	Molecular formula	Area
84	1-Chloromethyl-4-(1,1-diethylpropy l) benzene	$C_{15}H_{23}Cl$	10.90
85	Thiophene, 2-heptyl-5-propyl-	$C_{14}H_{24}S$	10.90
86	Furane-2-carbohydrazide, 5-phenylethynyl	$C_{13}H_{10}N_2O_2$	10.90
87	Piperine	$C_{17}H_{19}NO_3$	24.11

IV: *Main effect of additives and storage days on physio-chemical parameters of stored pork*

Parameters	Additive					Storage days			
	Control	BHT	BPAE	WPAE	SEM	0	5	10	SEM
pH	5.95	6.00	5.92	5.87	0.06	5.01^{c}	6.30^{b}	6.50^{a}	0.05
REF loss (%)	8.39^{b}	9.14^{b}	7.72^{b}	12.71^{a}	0.51	3.39^{c}	9.83^{b}	15.24^{a}	0.44
Cooking loss (%)	39.77^{a}	36.99^{ab}	36.34^{b}	36.06^{b}	0.60	0	0	0	0

$^{a, b, c}$ – means on the same row with different superscript differ significantly ($p < 0.05$)
BHT – Butylated hydroxytoluene; WPAE- white pepper aqueous extract; BPAE- black pepper aqueous extract; REF – refrigeration; SEM – standard error of mean

V: *Interactive effect of additive and storage days on pH and refrigeration loss of stored pork*

Additive	Control			BHT			BPAE			WPAE			
Day	0	5	10	0	5	10	0	5	10	0	5	10	
pH	5.22^{c}	6.24^{b}	6.40^{ab}	5.31^{c}	6.31^{ab}	6.41^{ab}	4.82^{d}	6.39^{ab}	6.57^{ab}	4.71^{d}	6.26^{b}	6.64^{a}	0.11
REF Loss (%)	2.34^{h}	9.34^{ef}	13.50^{bc}	2.68^{h}	9.65^{def}	15.08^{b}	2.46^{h}	8.61^{fg}	12.10^{cd}	6.11^{g}	11.73^{cde}	20.28^{a}	0·80

$^{a, b, c, d, e, f, g, h}$ – means on the same row with different superscript are significantly ($p < 0.05$) different
BHT – Butylated hydroxytoluene; WPAE – white pepper aqueous extract; BPAE – black pepper aqueous extract; REF – refrigeration; SEM – standard error of mean

and one nitrogen-boron-based compound. It also contains silicon-based compounds (22) and sulphur containing compounds (6) that comprise one sulphur-silicon based compound. Four chlorine-based compounds comprising 2 chlorine-nitrogen based compounds were found. Arsane containing compounds (2) and phosphorus-based compound (1) were likewise extracted. Area of yield ranged from 1.03–13.73% with 3-Ethoxy-1,1,1,5,5,5-hexamethyl-3(trimethylsiloxy)trisiloxane least and Oxime-, methoxy-phenyl-, Silane, dimethyl (dimethyl (but-2-en yolky) silyloxy) (but-2-enyloxy) and Oxazolidine, 3-phenyl- highest. For BPAE, twenty-five nitrogen-containing compounds were present, with chlorine-nitrogen (1), nitrogen-sulphur (1), fluorine-nitrogen (1), iodine-nitrogen (1) and fluorine-nitrogen-sulphur-chlorine-based compound (1) found. Six sulphur-based compounds were gotten as well as three chlorine-containing compounds. One phosphorus-based and bromine-based compound were respectively seen. A total of nineteen silicon-based compounds were harvested, while two arsane-containing compounds were extracted. Piperine was the most abundant bio-compound harvested, spanning an area of 24.11%. Compositional analysis of extracts revealed rich diversity of element-based compounds. This is a significant upgrade to outcomes reported by Olalere *et al.* (2018) and Adegoke *et al.* (2023) that had 31 and 71 bio-compounds respectively.

Main effect of storage days and additives (0% antioxidant, BHT, and *P. nigrum* extracts) on pork pH, refrigeration (REF) loss and cooking loss is shown (Tab. IV). Sole effect of additives and storage days was significant ($p < 0.05$), except pH. Pork pH was numerically highest among meat samples containing BHT but lowest among WPAE samples. Weight loss (%) from refrigeration was increased when WPAE was added to pork than 0% antioxidant, BHT and BPAE additives. Cooking loss (%) of pork containing *P. nigrum* extracts was reduced compared to 0% antioxidant group.

From parameters assessed (Tab. IV), both pH and refrigeration loss (%) were affected ($p < 0.05$) exclusively by refrigeration storage days. The same statistical significance was identified for meat pH and refrigeration loss percentage. As days of refrigeration storage extended, the pH and weight loss increased ($p < 0.05$), with highest loss recorded on d 10.

The interaction effect of additives and storage days on pork pH and refrigeration loss is documented (Tab. V). All parameters examined were influenced ($p < 0.05$). On d 0, both *P. nigrum* groups had lower pH than Control and BHT. Peak pH was recorded for WPAE on d 10. Refrigeration loss (%) increased across storage days and treatment. Loss (%) from

VI: *Influence of aqueous additives on fatty acid profile pork on d 10 of storage (4 °C)*

Parameters (mg/100 g tissue)	Control	BHT	BPAE	WPAE	SEM
Lauric (12:0)	2.65^{b}	1.98^{c}	2.73^{b}	3.15^{a}	0.11
Stearic (18:0)	7.70^{b}	6.80^{c}	7.85^{b}	8.28^{a}	0.15
Palmitic (16:0)	3.33^{b}	2.53^{c}	3.50^{ab}	3.74^{a}	0.13
Myristic (14:0)	0.90^{a}	0.28^{c}	0.21^{c}	0.48^{b}	0.07
Butyric (4:0)	1.18^{a}	0.73^{b}	1.18^{a}	1.33^{a}	0.07
Caprylic (8:0)	1.85^{b}	1.48^{c}	1.90^{b}	2.28^{a}	0.08
Valeric (5:0)	1.30^{ab}	0.85^{c}	1.15^{b}	1.55^{a}	0.08
SFA	18.89^{b}	14.65^{c}	18.47^{b}	20.85^{a}	0.62
Oleic (18:1; ω-9)	3.73^{c}	2.83^{d}	4.08^{b}	4.40^{a}	0.16
Palmitoleic (16:1; ω-7)	0.35^{b}	0.50^{ab}	0.70^{a}	0.60^{a}	0.05
MUFA	4.08^{b}	3.33^{c}	4.78^{a}	5.00^{a}	0.18
MUFA: SFA	1.76^{c}	1.85^{bc}	2.09^{a}	1.93^{b}	0.04
Arachidonic (20:4; ω-6)	3.95^{b}	4.60^{a}	3.95^{b}	4.53^{a}	0.10
Linoleic (18:2; ω-6)	3.63^{b}	2.88^{c}	4.13^{a}	4.35^{a}	0.15
PUFA	7.58^{c}	7.48^{c}	8.08^{b}	8.88^{a}	0.35
PUFA: SFA	4.01^{c}	5.11^{a}	4.37^{b}	4.26^{bc}	0.12
UFA	11.70^{c}	10.80^{d}	12.90^{b}	13.90^{a}	0.10
UFA: SFA	6.19^{d}	7.37^{a}	5.63^{b}	5.37^{c}	0.11
IA	8.68^{a}	5.40^{b}	5.72^{b}	7.27^{ab}	0.47

$^{a, b, c, d}$ – means on the same row with different superscript differ significantly ($p < 0.05$)
BHT – Butylated hydroxytoluene; BPAE – Black Pepper Aqueous Extract; WPAE – White Pepper Aqueous Extract; MUFA – Monounsaturated fatty acid; SFA – Saturated fatty acid; UFA – Unsaturated fatty acid; PUFA – Polyunsaturated fatty acid; SEM – standard error of mean; IA – index of atherogenicity

WPAE samples was highest across treatment groups on d 0, but lesser in BPAE on d 5 and 10 though similar ($p < 0.05$) as the Control.

Fatty acid profile of pork containing aqueous additives is presented in Tab. VI. Saturated fatty acid (SFA) in meat include lauric, stearic, palmitic, butyric, myristic, caprylic, valeric fatty acid. Monounsaturated and polyunsaturated fatty acids (MUFA and PUFA) present were oleic and palmitoleic; linoleic and arachidonic acid respectively. All fatty acid indices measured were influenced ($p < 0.05$), but pork N3: N6 ratio was not affected. An identical statistical significance was observed across treatment groups for lauric and stearic acid, with WPAE highest and BHT lowest. The control group contained more myristic acid than BHT and BPAE-preserved pork. WPAE contained more palmitic acid than BHT and Control, though similar as BPAE. Butyric acid was higher among Control, WPAE and BPAE than BHT. Identical superscript was assigned to caprylic and total SF acid values. Pork containing WPAE had more SF acids than Control and BPAE; while both latter groups had more than BHT. BHT samples had less valeric acid than WPAE. Also, oleic acid was reduced in WPAE, BPAE, Control and BHT respectively ($p < 0.05$), while palmitoleic acid was high in WPAE and BPAE than the Control. Identical superscript was assigned linoleic acid and total MUFA. Pork containing BHT had lesser MUFA than the Control and *P. nigrum* groups. Also, MUFA: SFA ratio was reduced ($p < 0.05$) in the Control compared to BPAE that was highest. Arachidonic acid was higher in BHT and WPAE samples than BPAE and the Control, while linoleic fatty acid was reduced in BHT samples than the Control, but highest among WPAE and BPAE groups. Pork PUFA was more in meat containing WPAE than BPAE, however, both Control and BHT had identical total PUFA values, though least. Pork PUFA: SFA proportion was least for the Control but highest in BHT. Statistically identical superscripts were recorded for oleic acid and UFA, with BPAE highest but BHT lowest. Pork UFA: SFA was least in the Control, followed by WPAE, then BPAE but BHT was highest. Meat index of atherogenicity (IA) was low in BHT and BPAE compared to the Control but WPAE was intermediate ($p < 0.05$).

The 2-thiobarbituric acid reactive substance (TBARs) of pork soaked in aqueous additives stored under refrigeration condition for 10 days is revealed (Tab. VII). Pork TBARs was significantly ($p < 0.05$) affected. WPAE had the highest TBARs, followed by the Control, but BPAE was lowest.

VII: *Oxidative status of pork containing aqueous additives on d 10 of refrigeration storage*

Parameter	Control	BHT	BPAE	WPAE	SEM
TBARS (mg MDA/g tissue)	5.385[b]	4.930[c]	3.455[d]	7.015[a]	0.328

[a, b, c, d] – means on the same row with different superscript differ significantly ($p < 0.05$)
BHT – Butylated hydroxytoluene; WPAE – white pepper aqueous extract; BPAE – black pepper aqueous extract; MDA – malondialdehyde; TBARS – 2 thiobarbituric acid reactive substance; SEM – standard error of mean

DISCUSSION

Increase in meat pH across storage (4 °C) days, solely due to refrigeration was influenced by end products released from the metabolism of materials from protein origin alongside protein degradation facilitated by the proliferation of psychrotrophic microorganisms (Rey *et al.*, 1976). Biogenic amines formed from amino acid decarboxylase action of microbes elevate pH during storage (4 °C) by complementing contributory activity of peptides, dipeptides, and free amino acids produced. This process in turn changes the structural composition of pork due to changes in myofibrils and sarcolemma linkages. Once degraded, the connection between myofibrils and the cell membrane is lost, thus creating a free-rein of water movement and subsequent water loss from the cells of stored meat (Straadt *et al.*, 2007).

Lower refrigeration loss in BPAE may be attributed to the combined activity of phosphate and dissolved salt (NaCl). Puolanne and Ruusunen (1983) and Offer and Knight (1988) explained that phosphate markedly enhance water-holding properties of NaCl at 1.0 to 1.5% as a result of the cleavage of actin and myosin bonds within the muscle by phosphates, thus facilitating the enlargement of filament lattice within meat (Warner, 2017). The water holding capacity of pork muscle was affected, evidenced by a decline in pH among BPAE samples. Also, 1-Chloromethyl-4-(1,1-diethylpropy l) benzene, Hexane, 1-chloro-5-methyl-, 2-Bromo-2'-methoxyacetophenone, 1H-Pyrazole-1-acetamide,4-iodo-N-(phenylmethyl), N-(Trifluoracetyl)-O,O',O''-tris (trimethylsilyl) in BPAE alongside compounds such as Chloromethyl cyanide, Benzene, 1,2-dichloro-; Benzene, 1,3-dichloro-; Benzene, 1-fluoro-4-(2-phenylethenyl), 4-Methylnaphtho[1,2-b] thiophene, 2-Methyl-6-(5-methyl-2-thiazolin-2-ylamino) pyridine play supportive role by restricting the permeability of muscle cells against higher permeability of muscle membrane.

Pork endo-, peri- and epimysial collagens are generally affected after cooking, causing shortening of actin and myosin filament, reduced muscle cell diameter and increased release of juice in meat that comprise of fat and water. In the control group, degradation of protein subunits results in disruption of myofibril as less pork juice is entrapped within protein structures by capillary forces. Reduced water holding capacity of pork in the control group increased the cooking loss of meat (Lorenzo *et al.*, 2015; Botinestean *et al.*, 2018).

Rapid decline in pH of pork from combined activity of additive and refrigeration storage on d 0 among WPAE and BPAE samples may be attributed to rise in hydrogen ion concentration, primarily as a result of dissociation of weak acids. Some phytochemicals such as 3-Hydroxymandelic acid, ethyl ester, di-TMS; 2-Propenoic acid, 2-methyl-3-(4-nitrophenyl); 3-Methyl-2-butenoic acid, 3-phenyl-2-propenylEster, Arsenous acid, tris (trimethylsilyl) ester; 2-Naphthalenecarboxylicacid,8-ethenyl-3,4,4a,5,6,7,8,8a-octahydro5-methylene and Phosphonous acid, phenyl-, diethylester in BPAE; as well as Salicylic acid, 2TMS derivative; 2,5-Dihydroxybenzoic acid, 3TMS derivative; Phthalic acid, isobutyl 2-methoxyethyl ester; 3-Trimethylsilyloxystearic acid, trimethylsilyl ester and 1,2-Benzisothiazole-3-propanoic acid in WPAE are weak organic acids that can tilt meat pH towards acidity. Munteanu and Apetrei (2021) explained that the extent of hydrogen ion donation by phytochemicals depends on several factors such as the chemical structure, concentration and pH of the solution. Hence, hydrogen-rich phytochemicals can act as proton donors (donate hydrogen ions), lowering pork pH by increasing the concentration of hydrogen ions (H+). Also, subsequent rise in pH among WPAE and BPAE preserved pork like other groups on d 5 and 10 suggests loss in strength of acids as storage days extends. Jokanović and Cvetković (2009) identified oxidation of weak acids and anaerobic degradation of organic acids as possible causes of rise in pH as storage extends; and on d 10, all samples were affected in similar ways by storage conditions. On d 10, refrigeration loss was lowered among BPAE samples than other groups possibly due to the rich matrix of organic acid present. While the strength of organic acids lowered during storage likely from dilution effect of hygroscopic pull of moisture available from salts present, it could however be deduced that the power of concentration influences to a larger degree the quality of produce stored. The diminishing influence of organic bio-compounds is similar to loss of strength of ascorbic acid during storage (Lisiewska *et al.*, 2003). Additionally, the presence of fluorine-nitrogen based compound, bromine-nitrogen based compound, nitrogen-sulphur based compound, sulphur containing compounds and abundantly rich hydrogen-based compounds likely modified the initial pH of pork, causing a decline, though not significant. This may have resulted in significant subtle shrinkage of the myosin head, causing changes in myofibrillar space

and subsequent weight during refrigeration. Meat with low pH, increased reduction in intra-fibrillar space corresponds with increased loss of attraction between the protein and water molecules. This is commonly induced in meat marinated in acidic conditions (Huff-Lonergan and Lonergan, 2005).

Reports have detailed the significance of fatty acid to man's diet. Mensink (2013) explained that low SFA in the diet lowers blood total serum and LDL cholesterol concentrations. Nowadays, generally acceptable dietary compositions tend towards lowering SFA in man's diet while increasing the UFA content. The result obtained tends to favour the application of BHT on pork, however, careful assessment of the interrelationship between the SFA and total MUFA and PUFA for BHT may reveal some likely implications on cardiovascular health of consumers. In this study, pork MUFA and PUFA proportions were conserved by the radical quenching or/and antimicrobial potency of organic additives. Though WPAE had more influence on conservation of oleic fatty acid, similar amounts of palmitoleic and total MUFA as BPAE was documented. Pork soaked in WPAE and BPAE with higher proportions of UFA suggests that the mode of action of bio-compounds extends beyond its antioxidant protection. Microbes deteriorate fatty acids, especially lipolitic bacteria, which left uncontrolled, could catalyze hydrolysis of fat, resulting in the production of aldehydes and acids. Microorganisms in the genera of Alteromonas, Achromobacter, Flavobacterium, Pseudomonas, Staphylococcus and Yeast are lipolytic. Research has shown that lipolytic microbes can hydrolyze triglycerides from extracellular lipases produced using lipases like triacylglycerol and acyl hydrolase enzymes to speed up the hydrolysis process (Chandra *et al*., 2020). Piperine – the principal bio-active abundant in BPAE is capable of altering the permeability of bacterial cell wall composed of high level of lipid material. Wenxue *et al.* (2018) examined the influence of black pepper on Staphylococcus aureus and E coli species and reported that 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7β,7a, β,7βα.)] - found in the chloroform extract of black pepper degraded bacterial cell walls and membranes alongside the inhibition of protein synthesis. Similarly, 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene- [1ar-(1a. alpha.,4a. alpha.,7. beta., 7a. beta.,7b. alpha.)] in WPAE possibly stifled microbial growth by the release of intracellular transaminases; causing degradation of cell wall and membranes of fat-decomposing lipolytic bacterial species. Higher proportion of MUFA and MUFA: SFA, especially among BPAE samples, followed by WPAE is desirable as diets with favorable cardiovascular protection and an improved lipid profile is sought after by meat consumers (Leah *et al.*, 2011). MUFA: SFA proportion is more important in assessing the quality of dietary lipids consumed than the PUFA: SFA ratio, which is substantiated by the recommendation of World Health Organization that balanced proportions of dietary fats (SFA: MUFA: PUFA) at 1: 1.5: 1 is recommended (Grover *et al*., 2021). From this recommendation, BPAE and WPAE samples appear appealing than others.

From the perspective of PUFA/SFA index - commonly used to examine the role diet plays on cardiovascular health; BHT-incorporated pork appears healthier for human consumption since a higher ratio corresponds to healthier influence, but as Chen and Liu (2020) explained, not all of the main classes of PUFA positively contribute to the prevention of arterial or cardiac diseases, and not all SFA's elevate serum cholesterol. The findings of Dietschy (1998) showed that C12:0 (lauric), C14:0 (stearic), and C16:0 (palmitic) may contribute to the elevation of serum cholesterol concentration by limiting the activity of low-density lipoprotein receptors but C18:1 n-9 cis (oleic acid), increases the activity of low-density lipoprotein receptors (LDLRs) and decreases the cholesterol concentration in serum. Maragoni *et al.* (2020) points to dietary intake of linoleic acid (LA, C18:2 n-6) as inversely correlated with arterial or cardiac diseases. Thus, the proportion of anti-cardiac to pro cardiac ratio for BHT, BPAE and WPAE according to Chen and Liu (2020) is implicative. Anti-cardiac [(C18:1+ C18:2) BHT samples (0.571), BPAE samples (0.821) and WPAE samples (0.875)] to pro-cardiac [(C12:0+C14:0+C16:0) BHT (0.479), BPAE (0.644) and WPAE (0.737)] ratio equals 1.19: 1.27: 1.19. This implies that consuming pork stored with WPAE may result in low exposure to arterial plaque development similar as BHT-incorporated meat, though without any safety concern or risk to health, yet, better than both, BPAE samples potentially least exposes consumers to the development of arterial plaque.

Least TBARs in BPAE may be due to rich phytochemicals of biological significance in black pepper extract. (-)-Spathulenol in BPAE is a sesquiterpenoids alkaloid with antioxidant capacity. Similarly, Cyclotrisiloxane, hexamethyl- is a major bio-compound found in seaweed with antioxidant properties (Adedapo *et al*., 2011). Other compounds such as. beta. –Ocimene, Ar-tumerone, Caryophyllene, trans-. beta. –Ocimene and piperine are few radical quenching compounds from the abundant pool of phytochemicals extracted. Radical quenching phytochemicals (phenols, alkaloids, sesquiterpenoids, terpenes and other compounds) in medicinal plants exhibit redox quenching properties by adsorbing and neutralizing free radicals including singlet and triplet oxygen or by decomposing peroxide in cell, tissue and meat (Adedapo *et al*., 2011; Moyo *et al*., 2022). The absence of piperine in WPAE likely limited the potent expression of WPAE as an anti-oxidant on d 10 of storage.

CONCLUSION

Pork containing BPAE is desirable for its minimal oxidative rancidity and lower moisture loss – a significance to meat consumers and processors. While BPAE and WPAE had higher and highest UFA respectively, this study has showed that extract from BP may potentially contain compounds that could offer enhanced meat preservation as shown by MUFA: SFA and TBARs values for BPAE-preserved pork. Though this study investigated the benefits of aqueous extracts up till d 10, a more potent means for limiting deterioration may be explored such as a combination of updated storage technologies alongside application of optimized extract.

Disclosure statement

The authors report there are no competing interests to declare.

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REFERENCES

ADEDAPO, A., JIMOH, F. and AFOLAYAN, A. 2011. Comparison of the nutritive value and biological activities of the acetone, methanol and water extracts of the leaves of *Bidens pilosa* and *Chenopodium album. Acta Poloniae Pharmaceutical Drug Research*, 68(1): 83–92.

ADEGOKE, A. V., SANWO, K. A., SOBUKOLA, O. P., EGBEYALE, L. T. and EKUNSEITAN, O. Qualitative yield of white pepper extract optimized by microwave extraction and meat quality assessment – an alternative approach. *Polish Journal of Natural Sciences* (Accepted Jan. 17, 2024).

ADEGOKE, A. V., SANWO, K. A., SOBUKOLA, O. P. and EGBEYALE, L. T. 2023. Optimization of *microwave* extraction of biocomponds in black pepper using meat quality assessment. *Revista Facultad Nacional de Agronomia Medellin*, 76(2): 10347–10368.

AOCS. 1998. *Official Methods and Recommended Practices of the AOCS*. 5th Edition. Champaign, USA: American Oil Chemists' Society, 1200 p.

AZIZ, D. M., HAMA, J. R. and ALAM, S. M. 2015. Synthesising novel derivatives of piperine from black pepper (*Piper nigrum L.*). *Journal of Food Measurement and Characterization*, 3: 324–331. https://doi.org/10.1007/s11694-015-9239-2

BERIAIN, M. J., GÓMEZ, I., IBÁÑEZ, F. C., SARRIÉS, V. and ORDÓÑEZ, A. I. 2018. Chapter 1—Improvement of the Functional and Healthy Properties of Meat Products. In: *Handbook of Food Bioengineering*. Amsterdam, The Netherlands: Elsevier, pp. 1–74.

BOHRER, B. M. 2017. Review: Nutrient density and nutritional value of meat products and non-meat foods high in protein. *Trends Food Science Technology*, 65: 103–112. https://doi.org/10.1016/j.tifs.2017.04.016

BOTINESTEAN, C., KEENAN, D. F., KERRY, J. P. and HAMILL, R. M. 2016. The effect of thermal treatments including sous-vide, blast freezing and their combinations on beef tenderness of m. semitendinosus steaks targeted at elderly consumers. *LWT*, 74: 154–159.

BROWN, H. A. and MARNETT, L. J. 2011. Introduction to lipid biochemistry, metabolism, and signaling. *Chemical Reviews*, 111: 5817–5820. https://doi.org/10.1021/cr200363s

CHANDRA, P., ENESPA, S. R. and ARORA, P. K. 2020. Microbial lipases and their industrial applications: a comprehensive review. *Microbial Cell Factories*, 19: 169. https://doi.org/10.1186/s12934-020-01428-8

CHEN, J. and LIU, H. 2020. Nutritional indices for assessing fatty acids: a mini-review. *International Journal of Molecular Sciences*, 21(16): 5695. https://doi.org/10.3390/ijms21165695

DENNIS, E. A. and NORRIS, P. C. 2015. Eicosanoid storm in infection and inflammation. *Nature Reviews Immunology*, 15: 511–523. https://doi.org/10.1038/nri3859

DIETSCHY, J. M. 1998. Dietary fatty acids and the regulation of plasma low density lipoprotein cholesterol concentrations. *Journal of Nutrition*, 128: 444–448. https://doi.org/10.1093/jn/128.2.444S

DUARTE, K. C., JUSTINO, I. L., GOMES, A., TERESA, ROCHA-SANTOS, S. M. and ARMANDO, C. D. 2014. Green analytical methodologies for preparation of extracts and analysis of bioactive compounds. *Analysis of Marine Samples in Search of Bioactive Compounds*, 65(4): 69–70.

FAYE, A., HOMER, R. D., REEVES, R. R. and BROOKS, A. J. M. 1991. Characterization of the nickel rich extract from the nickel hyperaccumulator Dichapetalum gelonioides. *Phytochemistry*, 30(7): 2141–2145.

FOLCH, J., LEES, M. S. and STANLEY, G. H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biology Chemistry*, 226: 497–509.

GASPARETTO, A., CRUZ, A. B., WAGNER, T. M., BONOMONI, T. J., CORREA, R. and MALHEIROS, A. 2017. Seasonal variation in the chemical composition, antimicrobial and mutagenic potential of essential oils from Piper cernum. *Industrial Crops and Products*, 95: 256–263. http://doi.org/10.4025/actascibiolsci.v43i1.53534

GROVER, S., KUMARI, P., KUMAR, A., SONI, A., SEHGAL, S. and SHARMA, V. 2021. Preparation and quality evaluation of different oil blends. *Letters in Applied NanoBioScience*, 10: 2126–2137.

HUFF-LONERGAN, E. and LONERGAN, S. M. 2005. Mechanisms of water-holding capacity of meat: The role of post-mortem biochemical and structural changes. *Meat Science*, 71: 194–204. Printed with permission from Elsevier.

IBM. 2019. *Statistical Package for Social Sciences* (2019). IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.

JOKANOVIĆ, M. R. and CVETKOVIĆ, B. R. 2009. Effect of preservation method and storage condition on ascorbic acid loss in beverages. *Acta Periodica Technologica*, 40: 1–7. https://doi.org/10.2298/APT0940001C

KONDJOYAN, A., KOHLER, A., REALINI, C. E., PORTANGUEN, S., KOWALSKI, R., CLERJON, S., GATELLIER, P., CHEVOLLEAU, S., BONNY, J-M. and DEBRAUWER, L. 2014. Towards models for the prediction of beef meat quality during cooking. *Meat Science*, 97(3): 323–331.

LEAH, G. G., HARRIS-JANZ, S. P. J. and JONES, H. 2011. Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. *Lipids*, 46(3): 209–228. http://doi.org/10.1007/s11745-010-3524-y

LIMA, D. M., RANGEL, A., URBANO, S., MITZI, G. and MORENO, G. M. 2013. Oxidação lipídica da carne ovina. *Acta Veterinaria Brasilica,* 7(1): 14–28.

LING, T. 2015. Oxidation of Polyunsaturated Fatty Acids and its Impact on Food Quality and Human Health. *Advances in food technology and nutritional sciences*, 1(6): 135–142. http://dx.doi.org/10.17140/AFTNSOJ-1-123

LISIEWSKA, Z., SŁUPSKI, J. and ZUCHOWICZ, E. 2003. Effect of temperature and storage period on the preservation of vitamin C, thiamine and riboflavin in frozen dill (*Anethum graveolens* L.). *Electronic Journal of Polish Agriculture Universities*, 6: 1–7.

LORENZO, J. M., CITTADINI, A., MUNEKATA, P. E. and DOMÍNGUEZ, R. 2015. Physicochemical properties of foal meat as affected by cooking methods. *Meat Science*, 108: 50–54.

MARANGONI, F., AGOSTONI, C., BORGHI, C., CATAPANO, A. L., CENA, H., GHISELLI, A., LA VECCHIA, C., LERCKER, G., MANZATO, E., PIRILLO, A., RICCARDI, G., RISÉ, P., VISIOLI, F. and POLI, A. 2020. Dietary linoleic acid and human health: Focus on cardiovascular and cardiometabolic effects. *Atherosclerosis*, 292: 90–98. https://doi.org/10.1016/j.atherosclerosis.2019.11.018

MENSINK, R. P. 2013. Fatty Acids: Health effects of saturated fatty acids. In: *Encyclopedia of human nutrition*. 3rd Edition. Academic Press, pp. 215–219. https://doi.org/10.1016/B978-0-12-375083-9.00101-X

MOYO, B., OYEDEMI, S., MASIKA, P. and MUCHENJE, V. 2012. Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. *Meat Science*, 91: 441–447.

MUNTEANU, I. G. and APETREI, C. 2021. Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences*, 22(7): 3380.

MUZOLF-PANEK, M. and KACZMAREK, A. 2021. Chemometric Analysis of Fatty Acid Composition of Raw Chicken, Beef, and Pork Meat with Plant Extract Addition during Refrigerated Storage. *Molecules (Basel, Switzerland)*, 26(16): 4952. https://doi.org/10.3390/molecules26164952

NAGY, K. and TIUCA, I-D. 2017. Importance of fatty acids in physiopathology of human body. In: CATALA, A. (Ed.). *Fatty Acids*. IntechOpen. http://doi.org/10.5772/67407

NAVEENA, B. M., SEN, A. R., VAITHIYANATHAN, S., BABJI, Y. and KONDAIAH, N. 2008. Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. *Meat Science*, 80: 1304–1308.

OECD/FAO. 2022. OECD-FAO Agricultural Outlook. *OECD Agriculture statistics* [database]. https://www.oecd-ilibrary.org/agriculture-and-food/data/oecd-agriculture-statistics_agr-data-en

OFFER, G. and KNIGHT, P. 1988. The structural basis of water-holding in meat. Part 1: General principles and water uptake in meat processing. *Chemistry*, 4: 23.

PATHARE, P. B. and ROSKILLY, A. P. 2016. Review article: Quality and Energy Evaluation in Meat Cooking. *Food Engineering Review*, 8: 435–447. https://doi.org/10.1007/s12393-016-9143-5

PEREIRA, P.M.D. and VICENTE, A.F.D. 2013. Meat nutritional composition and nutritive role in the human diet. *Meat Science*, 93(3): 586–592.

PUOLANNE, E. and RUUSUNEN, M. 1983. Ein¯uss des Salzzusatzes auf das WasserbindungsvermoÈgen der verschiedener Fleischsortimente in BruÈhwurst. *Fleischwirtschaft*, 62: 238–239.

REY, C. R., KRAFT, A. A., TOPEL, D. G., PARRISH, F. C., JR. and Hotchkiss, D. K. 1976. Microbiology of pale, dark and normal pork. *Journal of Food Science*, 41: 111–116. https://doi.org/10.1111/j.1365-2621.1976.tb01114.x

SANWO, K. A., ADEGOKE, A. V., EGBEYALE, L. T., ABIONA, J. A., SOBAYO, R. A., OBAJULUWA, O. V. and ABDULAZEEZ, A. O. 2019. Meat quality and lipid profile of broiler chickens fed diets containing turmeric (*Curcuma longa*) powder and cayenne pepper (*capsicum frutescens*) powder as antioxidants. *Journal of Agricultural Science and Environment*, 19(1&2): 73–91.

STRAADT, I. K., RASMUSSEN, M., ANDERSEN, H. J. and BERTRAM, H. C. 2007. Aging-induced changes in microstructure and water distribution in fresh and cooked pork in relation to water-holding capacity and cooking loss - a combined confocal laser scanning microscopy (CLSM) and low field nuclear magnetic resonance relaxation study. *Meat Science*, 75: 687e695.

ULBRICHT, T. L. V., SOUTHGATE, D. A. T. 1991. Coronary Heart Disease: seven dietary factors. *The Lancet*, 338(8773): 985–992.

WANG, D., XIAO, H., LYU, L., CHEN, H. and WEI, F. 2023. Lipid oxidation in food science and nutritional health: A comprehensive review. *Oil Crop Science*, 8(1): 35–44.

WARNER, R. D. 2017. The Eating Quality of Meat—IV Water-Holding Capacity and Juiciness. In: *Lawrie's Meat Science*. Woodhead Publishing Series in Food Science, Technology and Nutrition, pp. 419–459. https://doi.org/10.1016/B978-0-08-100694-8.00014-5

WENXUE, C., LAN, Z., WEIJUN, C., YUEYING, H. and HAIMING, C. 2018. Effects of black pepper (*Piper nigrum* l.) chloroform extract on the enzymatic activity and metabolism of *Escherichia coli* and *Staphylococcus aureus*". *Journal of Food Quality*, 9635184. https://doi.org/10.1155/2018/9635184

ZHANG, Q-W., LIN, L. G. and YE, W. C. 2018. Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine*, 13: 20. https://doi.org/10.1186/s13020-018-0177-x

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