

Optimization of microwave extraction of biocompounds from black pepper using meat quality assessment

Optimización de la extracción por microondas de biocompuestos de la pimienta negra mediante la evaluación de la calidad de la carne

<https://doi.org/10.15446/rfnam.v76n2.101035>

Adeola Victor Adegoke^{1*}, Kehinde Atinuke Sanwo¹ Olajide Philip Sobukola² and Lawrence Tokunbo Egbeyale¹

ABSTRACT

Keywords:

Aqueous extract
Black pepper
GC-MS
Microwave extraction
Meat quality
Optimization

Black pepper (*Piper nigrum*) is a plant highly valued for its functional aromatic compounds. To increase bioavailability of its phytochemicals, a novel approach to extraction using microwave heating and optimization was investigated. Fixed (microwave power and particle size) and variable (heating time and solvent volume) extraction criteria were applied to obtain aqueous extracts, followed by the optimization of the quality of extracts by subjecting the extracts to meat quality test using the central composite design. Thereafter, quality indices (pH, 2-thiobarbituric acid reactive substance value, refrigeration loss, colour and cooking loss) of meat subjected to storage were determined and the outcomes were analysed using response surface methodology. A total of six optimised solutions were derived but the best extraction criteria (variable) were 87.28 min and 364.26 mL of heating time and solvent volume respectively with a desirability value of 0.624, while microwave power and particle size were kept constant. Afterwards, selected optimised extraction criteria was applied, and black pepper aqueous extract obtained was compositionally analysed using Gas Chromatography Mass Spectrometry. Remarkably, a total of 71 compounds were extracted, comprising element-based compounds such as silicon, nitrogen, sulphur and iodine. Two silicon and nitrogen-based compounds as well as one sulphur and nitrogen-sulphur based compound were obtained while seven iodine-based compounds were discovered alongside other functional compounds. This approach results in green and efficient extraction process with increased bioavailability of functional compounds. However, further modifications of extraction criteria can be applied to upscale the desirability level (score) for increased extraction efficiency.

RESUMEN

Palabras clave:

Extracto acuoso
Pimienta negra
CG-EM
Extracción por microondas
Calidad de la carne
Optimización

La pimienta negra (*Piper nigrum*) es una planta muy apreciada por sus compuestos aromáticos funcionales. Para aumentar la biodisponibilidad de sus fitoquímicos, se investigó un novedoso método de extracción mediante calentamiento por microondas y su optimización. Se aplicaron criterios de extracción fijos (potencia de microondas y tamaño de partícula) y variables (tiempo de calentamiento y volumen de disolvente) para obtener extractos acuosos, seguidos de la optimización de la calidad de los extractos sometiéndolos a una prueba de calidad de la carne mediante un diseño compuesto central. A continuación, se determinaron los índices de calidad (pH, valor de la sustancia reactiva del ácido 2-tiobarbitúrico, pérdida por refrigeración, pérdida de color y pérdida por cocción) de la carne sometida a almacenamiento y se analizaron los resultados mediante la metodología de superficie de respuesta. Se obtuvieron un total de seis soluciones optimizadas, pero los mejores criterios de extracción (variable) fueron 87,28 min y 364,26 mL de tiempo de calentamiento y volumen de disolvente, respectivamente, con un valor de deseabilidad de 0,624, mientras que la potencia de microondas y el tamaño de partícula se mantuvieron constantes. A continuación, se aplicaron los criterios de extracción optimizados seleccionados y se analizó la composición del extracto acuoso de pimienta negra obtenido mediante cromatografía de gases y espectrometría de masas. Se extrajo un total de 71 compuestos, entre los que se encontraban compuestos basados en elementos como el silicio, el nitrógeno, el azufre y el yodo. Se obtuvieron dos compuestos a base de silicio y nitrógeno, así como un compuesto a base de azufre y nitrógeno-azufre, mientras que se descubrieron siete compuestos a base de yodo junto con otros compuestos funcionales. Este enfoque dio lugar a un proceso de extracción ecológico y eficiente con una mayor biodisponibilidad de los compuestos funcionales. Sin embargo, se pueden aplicar otras modificaciones de los criterios de extracción para elevar el nivel de deseabilidad (puntuación) y aumentar la eficacia de la extracción.

¹ Federal University of Agriculture, Department of Animal Production and Health, Abeokuta, Nigeria. adegokeav@funaab.edu.ng , bumogardens@yahoo.com , egbeyalel@funaab.edu.ng 

² Federal University of Agriculture, Department of Food Science and Technology, Abeokuta, Nigeria. sobukolaop@funaab.edu.ng 

* Corresponding author

Microwave-assisted extraction is a sought-after procedure applied in industrial and homemade extractions to obtain soluble products via microwave energy. Recently, much attention has been directed to the recovery of bioactive principles from different plant parts considered waste products or of limited competitive significance between man and livestock, such as seeds, leaves, barks, pulp, rind, peels, wood and by-products. Extracts from these substances indicate that the diversity and complexities of phytochemicals constituents and the potency of natural bioactive compounds exist among polyphenols, sterols and alkaloids (secondary metabolites). To obtain sufficient yields of specific phytochemicals for effective protection or regulation of biochemical processes, the need arises for sustainable extraction procedures (Kallel et al. 2014; Aghdase et al. 2017). The reduction in operation time and solvent consumption during microwave heating increases the extraction efficiency as electrical oscillations of microwaves causes repeated rotations of aqueous molecules in the presence of electromagnetic fields that generate heat from the friction of molecules (Rodríguez, 2017; Jimenez et al. 2018). Such updated innovative strategies have resulted in extensive convenient methods of extraction with proven advantages when compared with conventional solid-liquid extraction methods (Ojalere et al. 2018a; Zin et al. 2020). Repeated experiments are often conducted to obtain information on constituents of extracts using Gas Chromatography Mass Spectrophotometry (GC-MS), but a wide range of investigations using factorial experiments can be both solvent and time consuming. Limited funds for analysis during such cost-intensive experiments can negatively affect the frequency and integrity of outcomes especially in developing economies. Therefore, a methodology that accommodates these concerns can increase man's application and utilization of beneficial phytochemicals in black pepper. Response surface methodology (RSM) allows for assessing of the impact of multiple factors and their interactions on one or more response variables (Aydar 2018). This presents a methodology for the possibility of overcoming the challenge of excessive solvent consumption and large sample sizes to produce quality extracts.

Black pepper is a crop of nutritional, medicinal and economic significance commonly sought for its desired

biological potency and flavour. Black pepper (*Piper nigrum*) is a major plant with varied applications in processing industries as products such as black pepper powder, pepper oil, and pepper aqueous are produced in response to increased quality consciousness and preference for natural flavours and medicinal properties. Agbor et al. (2006) reported that black pepper contains not only aromatic compounds but flavonoids, alkaloids, amides and lignans, and some phytochemicals extracted from high-resolution gas chromatography analysis (GC-MS) include δ -cadinol, δ -guaiene, (Z) (E)-farnesol, (E)- β -ocimene and guaiol (Pino et al. 1990). *Piper nigrum* (family Piperaceae) is a spice widely considered as "The King of spices" for it contains major bioactive phytochemicals and pungent alkaloids like piperine (1-peperoyl piperidine), safrol, pinene, sabinene, limonene, caryophyllene and linalool - compounds that contribute to its overall potency which stimulate beneficial pharmacological actions such as antihypertensive, antioxidant, antitumor, antipyretic, anti-inflammatory, anti-diarrheal, antispasmodic, hepatoprotective, antibacterial, antifungal, antiapoptotic and anti-colon toxin (Suvarna and Abdul 2019). *Piper nigrum* leaves and seeds are applied to indigenous systems of medicine in many rural communities, owing to its multidimensional influence on biological systems. In light of the diverse benefits obtainable from phytochemicals in black pepper, it becomes expedient to explore efficient extraction methods to increase the qualitative yield of black pepper extract.

The use of meat quality and RSM as assessment criteria to optimize extraction efficiency is a novel approach. This approach leverages on the rationale that the more the bioactive compounds extracted, the better the extraction process. Optimization of quality indices such as the physico-chemical and oxidative properties of meat incorporated with extracts (Insani et al. 2008; Sanwo et al. 2019) is an untapped potential for the assessment of qualitative yield generated from microwave extraction. The oxidation of lipid in meat can negatively affect the storability of many multifaceted food products as the nutrient becomes depleted and essential polyunsaturated fatty acids turns rancid as a consequence of hydrolytic and/or oxidative reactions during manufacture, processing, storage and distribution of meat leading to the generation of toxic compounds that are detrimental to consumer health (Böttcher et

al. 2015; Keller et al. 2015). On this premise, extracts containing more bioactive principles should minimize the extent of nutrient depletion and subsequent deterioration. This novel approach is notably expedient to overcome the demerits associated with other conventional techniques of extraction, such as higher extraction cost and repetitive trials; lab-intensive and time-consuming extraction, isolation and analysis; high solvent consumption and low extraction yield, which are significant drawbacks to the utilization of natural products by man (Ojalere et al. 2017; Zhang et al. 2018). This study is therefore designed to explore a novel approach to improve the quality of black pepper extracts produced from microwave extraction using meat quality assessment optimized by RSM.

MATERIALS AND METHODS

Experimental site

Microwave refluxation and major aspects of meat technological quality assessment were carried out at the Animal Product and Processing Laboratory, Department of Animal Production and Health. Extraction of the aqueous component was determined by Gas Chromatography – Mass Spectrometry (GC – MS) analysis in the Chemistry Department Laboratory, Faculty of Science, University of Lagos. Meat colour values were deduced at the Food Science Laboratory, Department of Food Science and Technology, Federal University of Agriculture, Abeokuta, but the fatty acid and lipid oxidation profile were determined at the Laboratory of Veterinary Medicine, University of Ibadan.

Experimental materials

Dried clarified pepper corms of black pepper sourced from a traditional herb–spice market in Ibadan was pulverized into a finely defined powder using an attrition mill. Black pepper powder was temporarily stored in coloured vials. Ground black pepper was sieved using a mesh and the particles were clarified using an 0.40 mm sieve, then stored in airtight container prior to further analysis. Distilled water was provided at the Feed Quality Laboratory of the Department of Animal Production and Health. Daewood (KOR6N9NC) microwave oven was used for heating aqueous black pepper.

Fresh meat from the breast muscles of carcasses of manually deboned broiler chickens (Cobb strain) weighing between 1.9–2.4 kg was sourced. Before the

slaughter of broiler chickens, ethical principles outlined by the Ethical Committee Board of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, was adhered to.

Experimental design

Central composite design (CCD) as defined by Anderson and Whitcomb (2016) was used for the experiment (process analysis and optimization procedure). 13 experimental runs were generated with 5 repetitions as the central point. Operational factors and ranges were generated from adjustments to the study of Ojalere et al. (2018a). Two variable factors – irradiation time (75–85 min) and solvent volume (360–380 mL) were applied while two fixed factors – microwave power and particle size were held constant at 320 W and 0.40 mm. Afterwards, data obtained from a qualitative assessment of extracts via meat quality assessment was optimized using response surface methodology.

Microwave refluxation procedure and extract yield calculation

13 experimental runs were generated using Design Expert software with 5 repetitions generated as central points (Table 1). 8 g of ground black pepper was mixed with 360–380 mL of distilled water per experimental run. Soaking and stirring of dissolved pepper prior to loading was carried out to achieve homogeneity and uniform hydration of the mixture placed in an irradiation-tolerant container before placement in the microwave cavity. Microwave power was fixed at 320 W. Before extraction, pre-heating was performed for 10 min at 160 W – a slight modification to the procedure established by Ojalere et al. (2017). Subsequently, the loading and unloading of the mixture and extracts from the cavity were carried out according to the procedure established by previous studies (Ojalere et al. 2017; 2018b).

Extracts obtained were cooled and placed in coloured vials prior to the soaking of chicken meat. The extraction yield was calculated by using Equation (1).

$$\text{Extraction Yield} = \frac{\text{Volume of extracts (mL)}}{\text{Volume of mixture (mL)}} * 100 \quad (1)$$

Experimental birds

20 (4-weeks old) broiler (Cobb strain) chickens reared under an intensive deep litter management system were

Table 1. Central composite design matrix used for optimizing black pepper aqueous extract.

RUN	Irradiation time (min)	Solvent volume (mL)
1	85.00	360.00
2*	80.00	370.00
3	80.00	355.86
4*	80.00	370.00
5	85.00	380.00
6	87.07	370.00
7*	80.00	370.00
8*	80.00	370.00
9*	80.00	370.00
10	80.00	384.14
11	75.00	380.00
12	75.00	360.00
13	72.93	370.00

*Midpoint repeated five times.

sourced, then further reared for four weeks. Commercial diet fed is presented in Table 2. Birds were subjected to uniform management, thereafter, birds were sacrificed and breast muscles extracted, then, weighed before soaking prior to subsequent meat quality analysis.

Table 2. Nutrient composition of commercial finisher diet.

	Composition-value
Energy (kcal (kg ME) ⁻¹)	2900
Crude protein	20.00 (%)
Fat Oil ¹	6.00 (%)
Crude fibre	5.00 (%)
Salt	0.30 (%)
Lysine	0.85 (%)
Methionine	0.35 (%)
Calcium	1.00 (%)
Available Phosphorus	0.40 (%)

DM: dry matter; ME: metabolizable energy.

Determination of microwave extraction temperature

The internal temperature of the microwave was measured for 5 min post-extraction, and temperature ranged between 37±2 °C.

Determination of meat extract uptake

20 g of raw meat from the breast muscles were weighed in triplicates and labelled; then 20 mL of extract was added to each sample and left for 30 min. The samples were subsequently removed and reweighed. The increase in weight of samples indicate the volume of extract absorbed.

Extract quality assessment

Each meat sample soaked in each extract was drained for 5 min prior to qualitative (pH, colour, refrigeration and cooking loss) investigations.

Meat pH and colour evaluation

Meat pH was measured using an Orion 230A pH meter (Thermo Fisher Scientific, Waltham, MA). Chicken meat colour was measured with a CR-400 Chroma Meter instrument (Minolta Co., Osaka, Japan) via CIE L* (lightness), CIE a* (redness), and CIE b* (yellowness) according to specification by Kralik et al. (2017).

Determination of malondialdehyde content in meat

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS). The preparatory procedure for absorbance: blank was set up following the procedure documented by Adeleye et al. (2021). Lipid oxidation was reported as milligrams

of malondialdehyde per kilogram of meat, using Equation (2) (Kim et al. 2020).

$$\text{TBARS} \left(\frac{\text{mg MDA}}{\text{kg of meat}} \right) = (\text{kg of meat absorbance of sample} - \text{absorbance of blank sample}) * 5.88 \quad (2)$$

Meat refrigeration loss

Meat samples soaked in aqueous extracts were refrigerated to determine the refrigeration loss. Each sample was

weighed prior to refrigeration, then re-weighed on day 0, 5 and 10. Refrigeration loss and loss percentages were calculated using Equations (3) and (4), respectively:

$$\text{Refrigeration loss (g)} = \text{weight before refrigeration} - \text{weight after refrigeration} \quad (3)$$

$$\text{Refrigeration loss (\%)} = \frac{\text{weight before refrigeration} - \text{weight after refrigeration}}{\text{weight before refrigeration}} * 100 \quad (4)$$

Evaluation of cooking loss

Cook loss percentages were calculated on day 10, post-refrigeration. Meat samples were allowed to drain, then weighed, wrapped in separate air-tight polythene bags and cooked in a water bath at 70 °C for 20 min, applying

slight modifications to the procedure stated by Sanwo et al. (2012).

Cooking loss was determined using Equations (5) and (6):

$$\text{Cooking loss (g)} = \text{Weight before cooking} - \text{Weight after cooking} \quad (5)$$

$$\text{Cooking loss (\%)} = \frac{\text{weight before cooking} - \text{weight after cooking}}{\text{weight before cooking}} * 100 \quad (6)$$

Gas Chromatography Mass Spectrometry of aqueous black pepper extract

Spectrometry [Gas Chromatography Mass Spectrometry (GCMS)] analysis of extract generated from the highest and best desirability was carried out. GC (Agilent 7890, USA) and MS (Agilent 5975 model, USA) had 30 mm tubular column diameter, 0.32 mm internal diameter and 0.25 µm thickness. The oven initial temperature hold was operated at 80 °C for 2 min at 12 °C min⁻¹ to a temperature of 240 °C held for 6 min. The interface temperature between GC and MS is 250 °C. A small portion of the supernatant solution was filtered using the 0.45 µm micro filter and diluted with analytical grade acetone to 1:10. One microliter of the diluted solution was then injected into the gas chromatography column at 280 °C at a helium gas velocity of 1 mL min⁻¹. The scan ranges between 50 and 500 with a spitless mode of analysis. Filtered extracts (1 µL) were diluted using analytical standard grade acetone extract 1:10 injected into the GC-MS for components identification as

described by Olalere et al. (2018a), while the bioactive components present were identified in relation to the peak area fragmentation fingerprints.

Statistical design

Data obtained from responses were analyzed using Design Expert version 13.0.5.0 (Design Expert, 2021). Mean separation at 5% level of significance was carried out according to the regression coefficients examined that were subjected to analysis of variance (ANOVA) to derive response surface and contour plots together with the coefficient of determination (R²) for each response. Numerical optimization was performed to determine the level of desirability.

RESULTS AND DISCUSSION

Quality characteristics of chicken meat soaked in black pepper aqueous extracts prepared from designed extraction combinations

Optimization of aqueous extract using chicken meat

soaked in aqueous black pepper extract subjected to irradiation time and solvent volume is presented in Table 3. Extract volume ranged between 60.42–115.13 mL, while aqueous extract uptake was between 3.15 and 17.09%. Meat pH ranged from 5.82–6.33, 6.05–6.21 and 6.39–7.23 on days 0, 5 and 10 of refrigeration storage, respectively. Meat TBARS value ranged between 0.286–2.088 and 0.11–0.351 on days 5 and 10

of storage, respectively. Loss resulting from refrigeration storage of meat samples soaked in extracts on days 5 and 10 were 9.26–18.92% and 16.4–28.72% while cook loss ranged between 30.6 and 39.73% on day 10. Meat colour parameter (L^* , a^* and b^*) ranged between 47.36–64.62, 6.12–8.77 and 14.42–21.36 respectively on day 5; 49.03–66.35, 6.86–11.45 and 13.68–23.09 respectively on day 10.

Table 3. Mean values of quality attributes of chicken meat soaked in black pepper aqueous extracts.

RUN	Time (min)	Sol Vol (mL)	Ole Ext (mL)	Ole Abs (%)	Meat pH D 0	Meat pH D 5	Meat pH D 10	TBARS D 5	TBARS D 10	Ref Ls D 5 (%)	Ref Ls D 10 (%)	L^* D 5	L^* D 10	a^* D 5	a^* D 10	b^* D 5	b^* D 10	Ck Ls D 10 (%)
1	85	360	86.80	4.11	5.89	6.16	7.23	0.40	0.20	13.54	23.88	57.38	57.64	6.77	9.36	18.12	19.33	34.60
2	80	370	71.15	3.47	6.33	6.16	6.58	0.39	0.17	12.92	26.81	64.62	54.55	7.33	11.19	20.48	17.54	34.59
3	80	355.86	81.37	17.09	6.13	6.09	6.78	0.82	0.18	15.70	28.72	57.23	56.24	6.42	9.88	15.45	17.61	30.93
4	80	370	102.49	3.99	6.08	6.13	6.78	0.30	0.18	18.26	25.68	53.92	52.34	8.03	10.17	18.44	16.74	34.42
5	85	380	91.29	7.82	5.93	6.05	6.53	0.37	0.35	9.26	24.12	51.58	54.57	7.62	6.89	16.12	14.21	38.48
6	87.07	370	93.32	7.87	5.97	6.05	6.63	0.66	0.11	10.7	26.95	52.61	55.09	7.92	7.52	18.97	17.36	34.46
7	80	370	115.13	11.33	6.34	6.14	6.61	0.38	0.19	14.56	26.15	53.71	56.87	6.74	7.34	17.13	15.36	37.14
8	80	370	92.38	4.11	6.30	6.08	6.79	0.46	0.31	12.57	31.3	52.28	50.45	6.23	7.49	14.42	15.10	31.51
9	80	370	61.91	3.47	6.16	6.12	7.19	0.29	0.19	15.82	16.4	50.18	61.85	7.92	9.64	18.52	18.27	32.50
10	80	384.14	77.28	3.15	6.21	6.07	7.05	2.09	0.31	13.31	27.82	59.17	65.74	6.12	9.54	15.91	20.79	33.31
11	75	380	108.77	4.17	5.82	6.08	7.20	0.31	0.22	12.38	20.56	63.42	64.77	8.77	11.45	21.36	23.09	31.70
12	75	360	63.26	5.73	6.09	6.21	6.90	0.34	0.24	13.6	25.66	55.51	66.35	8.63	11.14	19.73	21.42	30.60
13	72.93	370	60.42	6.42	6.25	6.05	6.39	0.37	0.27	18.92	27.28	47.36	49.03	6.22	7.44	14.59	13.68	39.73

Values are in triplicates.

Response surface and contour plots of extraction conditions optimized via meat quality assessment

The response surface and contour plots showing the interaction of irradiation time and solvent volume (variable factors) on quality indices of extracts derived is detailed in Figure 1–7. Figure 1 shows the response surface and contour plots in relation to the extract yield and solvent absorbed in meat. Volume of extract obtained was at its peak in at 338.5 mL of distilled water and 90 min of irradiation. Oleoresin uptake (%) increases as irradiation time and solvent volume increased. Extraction method employed increased bioactive compounds harvested. Notably, the temperature of operation impacted extraction efficiency. Microwave power at 320 W which is sandwiched between the 300 and 350 W employed by Olalere et al. (2018a) complemented other extraction factors with more bioactive compounds extracted. Optimal microwave irradiation energy at 320 W likely enhance molecular movement of

the electromagnetic field and the substance within the cavity, thereby, positively influencing molecular seeping of solvent into the dissolved matrix and increasing the dissolution and recovery of phytochemical compounds (Zin et al. 2020). Teo et al. (2009) expressed benefits derived from the use of closed vessel system operation. With little evaporation experienced, solvent volume is slightly sustained compared to extraction via open systems where solvent volume increases as extraction duration extends. Furthermore, dielectric conduction and refluxation of microwave cavity at lower temperature further facilitate the extraction of bioactive compounds. Such extraction method identified as green extraction method minimizes solvent consumption and operates an environmentally friendly procedure (Flórez et al. 2015). The solute: solvent ratio likewise affects microwave solvent extraction processes. Tatke and Jaiswal (2011) stressed the limitations solubility or mass transfer impose. Additionally, the quantity of black

pepper dissolved in distilled water for this study positively affect the extraction yield. Beyond 8 g, a possibility exists that an optimal value would not be attained nor additional benefits derived, possibly resulting in diluted extracts, with limited solute potency. The capacity for water to absorb energy and refluxation, thereby facilitating the uniform distribution and microwaves exposures likely aided the extraction of functional compounds in this study (Veggi et al. 2013). Similar effects results in expected extraction outcomes as irradiation from specialized microwave allows for easier and proper control of operation with operation at lower cost than pressurized extraction protocols. The effect of ratios of water to raw material can likewise affect the solute extract yield and the number of compounds obtained. In a study conducted by Passos and Coimbra (2013), rescued liquid to solid ratios did not result in higher recovery of polysaccharide but monosaccharides implying solvent volume is critical to extraction yield. Findings confirm this, as revealed by the quantity of oleoresin extracted from white and black pepper that increase as solvent increased (Olalere et al. 2017, 2018a). In this study, however, more solvent volume was used at 360–380 mL than the 120 mL reported by authors previously cited. Significant outcome may be attributed to extraction system (closed system) employed in this study. Extracts can be applied to improve shelf stability of raw products. Also, functional compounds can be isolated for medicinal or therapeutic uses. The quantity of compounds harvested using this methodology exceeds the 55 compounds found in *Piper nigrum* by Mohammed et al. (2016), attesting to the efficiency of extraction method employed.

Figure 2 shows the response surface and contour plots of the pH of meat soaked in extracts on days 0, 5 and 10 of refrigeration storage. Meat pH on day 0 decreased as irradiation time of extracts is extended and the solvent volume is lowered while generating extracts; while on day 5, meat pH reduced among samples soaked in extracts formed from higher irradiation time and 372 mL of distilled water. On day-10, meat pH decreased among samples soaked in aqueous extracts generated from increased irradiation time and solvent volume combination. Extraction process of black pepper reveal solvent volume affects meat pH. Experiment conducted affirms that meat pH is critical to quality of extract obtained. Plant molecules can absorb microwave energy causing cell disruption by internal superheating,

leading to desorption of phytochemicals and release of chemical compounds in extracts. An oscillating electric field or refluxation causes vibrations of polar molecules along with molecular friction that extends to volumetric heating, thereby causing water evaporation within cells and subsequent increased pressure that result in cell disruption (Balasubramanian et al. 2011; Tan and Muhamad 2017) and pH of resultant extract.

As seen from Figure 3, the response surface and contour plots of 2-thiobarbituric acid reactive substance count of broiler chicken meat soaked in black pepper extracts, then preserved for 5 and 10 days respectively is presented. The higher the solvent volume used for extraction, the higher the meat 2-thiobarbituric acid reactive substance (TBARs) count, especially at 371 mL in combination with 87 min of irradiation time, but on day-10, stored meat had lowered malondialdehyde value, especially when soaked in extract generated from lower solvent volume. Least meat TBARs value was recorded at 88 min of irradiation time and 367 mL of solvent volume. Extract quality affecting meat TBARs value reveal higher solvent volume increases meat TBARs (malondialdehyde) value. Though Tatke and Jaiswal (2011) explained that higher proportion of solvent volume to solid matrix resulted in higher extraction yields, however, higher moisture content and degree of unsaturation of meat lipid facilitated generation of pro-oxidants. Thus, lower solvent volume corresponds with higher extract concentration as well as generation of phenolic compounds.

Response surface and contour plots of refrigeration and cooking loss percentages obtained after soaking chicken meat in black pepper extract, stored for 5 and 10 days is shown in Figure 4. Meat refrigeration loss was suppressed for extracts generated from lower irradiation time and higher solvent volume, while on day-10, refrigeration loss was minimized at 89 min of irradiation exposure and 367 mL of solvent volume. Cooking loss of meat on day-10 post-refrigeration resulted in higher moisture loss as the irradiation time and solvent volume increased. Meat refrigeration loss was lowered among extracts generated from lower irradiation time and higher solvent volume. On the other hand, loss (%) from cooking meat incorporated with aqueous extracts on day-10 post-refrigeration had more moisture loss as the irradiation

time and solvent volume increased. According to USDA (2013), the percentage of naturally occurring water in meat varies with the type of muscle and the pH of the meat. Hence, moisture movement in and out of pore spaces of muscle cells affect the eventual moisture content of meat incorporated with extracts. Aqueous extracts contain acidic compounds that interacts with meat interstitial fat by maintaining a fairly acidic environment (pH) that fairly regulates muscle cell properties and layering, yielding meat with minimal moisture loss. Refrigeration loss of meat was fairly stabilized by aqueous extracts generated at lower irradiation time and solvent volume. Compositional analysis of black pepper by Clery et al. (2006) and Jeleń (2015) reveal black pepper contain terpenes, nitrogen-containing compounds, limonene, α - and β -pinene, methyl propanal, myrcene, 2- and 3-methylbutanal, α -phellandrene, linalool, butyric acid and 3-methylbutyric acid, altering the internal environment within meat, suppressing loose movement of water, water soluble sarcoplasmic proteins, vitamins and enzymes from interstitial spaces and muscle cells (Sanwo et al. 2019). This explains why the moisture loss was higher in meat preserved with extracts generated from higher extraction (irradiation and time) values post-cooking as the quality of bioactive principles in pepper is possibly suppressed.

The response surface and contour plots for meat lightness (L^*) of samples soaked in black pepper extract on day 5 and 10 of refrigeration storage is presented in Figure 5. Meat L^* value increased as the irradiation time and solvent volume applied to extract decreased after soaking then storing meat for 5 days; however, on day-10, higher L^* values were recorded as extraction conditions were sandwiched between 85–87 min and 361–379 mL of irradiation time and solvent volume respectively. The trend of activity of black pepper extracts on meat L^* values as storage progresses contradicts the report of Ramamoorthi et al. (2010) that storage decreased meat L^* and a^* values, possibly pointing to the effectiveness of extraction process in releasing bioactive compounds in pepper, however, that meat lightness increased with decreasing irradiation time and solvent volume indicates minimal dosage of antioxidant application favors extension of meat lightness. Falowo et al. (2014) supports this by stating that higher concentrations of antioxidants may alter expected effects through pro-oxidative action. Notably, from this study, extraction conditions sandwiched between 85–87 min

and 361–379 mL of irradiation time and solvent volume respectively in tandem with fixed extraction factors favours the preservation of meat lightness using black pepper extract.

Response surface and contour plots of redness (a^*) and yellowness (b^*) values after soaking chicken meat in black pepper extract stored in the refrigerator for 5 and 10 days is presented in Figure 6 and 7, respectively. Meat a^* value increased as irradiation time and solvent volume increased; but on day-10, as irradiation time and solvent volume lowered, meat a^* value reduced. On the other hand, meat yellowness (b^*) values were elevated for meat soaked in extracts derived from increased irradiation time and solvent volume on day-5 of storage, but on day-10, meat b^* values increased in extracts generated from lower solvent volume and fairly-constant irradiation time (91.50 min). According to Leal (2019), meat redness is perceived by consumers as an indicator of freshness and superior product quality that can further be enhanced by highly oxidizing compounds or additives. Oxidizing principles were extracted from black pepper. The antioxidant components abounded with higher irradiation time and solvent volume, though within defined set-up. The change observed on D 10 may be attributed to the kind of storage employed (refrigeration storage). Extension of the preservative potency of extracts may be achieved if storage at temperatures lower than 4 °C is employed in tandem with other preservation methodologies (active packaging) for better storage. Outcome of this study agrees with Ramamoorthi et al. (2010), whose study reveal all antioxidants increased L^* value by two units (lighter), but the irradiation dose had no effect.

Regression coefficient of responses (aqueous extract volume, meat absorption percentage, colour, loss percentage and 2-thiobarbituric acid value) as a function of the independent variables

Model regression coefficient applied for the optimization of white pepper aqueous extracts is shown in Table 4. Linear irradiation time for pH (-2727.52) on day-5; quadratic model for irradiation time and solvent volume for pH on day-5 (-1636.49 and -545.408); as well as cubic model for solvent volume were significant ($P < 0.05$) among models and coefficients assessed.

An overview of the significant models from the regression coefficient table signifies aspects extract

Table 4. Regression coefficient of response parameters of black pepper aqueous extract applied as a function of independent variable.

	Intercept (β^0)	A – Irradiation time	B – Solvent volume	AB	A ²	B ²	A ² B	AB ²	A ³	R ²
Extract vol	85.0438									0.2932
<i>P</i> -values										
Aqueous Uptake	7.38407	0.510116	-2.19552							0.3439
<i>P</i> -values		0.7187	0.1418							
Meat pH Day-0	5.99389	-0.0607484	0.140392	0.0775						0.5323
<i>P</i> -values		0.3713	0.4869	0.4180						
Meat pH Day-5	-1084.96	-2727.52*	-0.208787	-0.206716	-1636.49*	-545.408*	-0.0529289	-272.693*	-272.753*	0.4454
<i>P</i> -values		0.0459*	0.0592	0.0723	0.0459*	0.0459*	0.0651	0.0459*	0.0459*	
Meat pH Day-10	6.48239	-0.30752	-0.50227	-0.25	-0.0768621	0.125631				0.4582
<i>P</i> -values		0.4833	0.1192	0.1031	0.4722	0.2542				
TBARS Day-5	0.162038	-0.266206	0.216424	-0.00025	-0.0827086	0.388576*				0.5592
<i>P</i> -values		0.6975	0.6435	0.9991	0.6212	0.0455*				
TBARs Day 10	0.16793	-0.0279678	0.122606	0.04175	-0.00291569	0.0270869				0.4839
<i>P</i> -values		0.7866	0.1130	0.2321	0.9075	0.2999				
Meat Ref Loss Day-5	44781.3	111930	-4.49502	-2.88503	67160.9	22385.3	-0.530007	11192.7	11193.9	0.5143
<i>P</i> -values		0.2081	0.5120	0.6877	0.2081	0.2080	0.7631	0.2080	0.2081	
Meat Ref Loss Day-10	25.1522	-0.280166	1.9034	1.335	-0.111104	0.466604				0.0082
<i>P</i> -values		0.9706	0.7141	0.5880	0.9521	0.8011				
L* day 5	47.2724	-7.35124	-6.2483	-3.4275	-1.75828	2.34911				0.3980
<i>P</i> -values		0.3835	0.2840	0.2190	0.3915	0.2619				
L* day 10	50.6322	-3.2867	0.353129	-0.3725	-0.49851	3.96596				0.3589
<i>P</i> -values		0.7347	0.9572	0.9044	0.8323	0.1239				
a*-day 5	8.01647	0.842371	0.425717	0.1775	0.229543	-0.170616				0.0830
<i>P</i> -values		0.6572	0.7411	0.7699	0.6201	0.7114				
a* day 10	5.35263	-3.03489	-1.7201	-0.695	-0.564131	0.550779				0.3928
<i>P</i> -values		0.2814	0.3638	0.4326	0.4025	0.4131				
b*-day 5	18.2015	0.485757	-1.77993	-0.9075	0.14196	-0.40834				0.0800
<i>P</i> -values		0.9145	0.5680	0.5377	0.8974	0.7120				
b* day 10	15.1501	-0.730838	-3.2641	-1.6975	-0.00249913	1.83716				0.4092
<i>P</i> -values		0.8744	0.3179	0.2742	0.9982	0.1343				
Cook Loss Day-10	40.2267	5.77859	2.43323	0.695	1.34068	-1.14671				0.3755
<i>P</i> -values		0.2522	0.4656	0.6543	0.2731	0.3429				

- Significant means were separated at $P < 0.05$. TBARs – 2-thiobarbituric acid value; D – Day; Ref – Refrigeration; L -Lightness; a* - Redness; b* - Yellowness.

properties affected meat quality values presented in Table 3. On D 5, as irradiation time increases, solvent volume at 372 mL of distilled water was optimal. Since pH is a fundamental quality index for storage, it can be deduced that decline in potency of extracts

corresponds with tendency for meat pH to shift from acidity to basicity. Changes in meat pH and colour result from post mortem glycolysis and glycogen conversion into lactic acid (Honkavaara et al. 2003; Abubakar et al. 2021) and the extent of variation influences the properties

of meat. Meat soaked in acidic medium affects its hydrogen ion balance, therefore, significant changes on D 5, implies meat post-mortem changes was affected by extract properties on D 5 than D 0 and 10. *Piper nigrum* contain acidic compounds such as 2-Ethylbenzotriazole and (R)-(-)-14-Methyl-8-hexadecyn-1-ol, but overall, proportions of hydrocarbons in extracts possibly resulted in tissue alterations due to changes in muscle glycolysis with resultant decline in meat pH. Report on spices and herbs reveal these organic substances often contain high concentrations of phenolic components with an extreme H-donating activity by Falowo et al. (2014) further highlight the power of extracts. Hence, pronounced influence from

variations among extracts on D 5 signify operational extraction conditions impacted extract quality and acidity with variations in acidic strength and tissue modification on D 5. Further implications and deductions from this study indicate that generally, extraction yield increases proportionally with power, solvent volume, irradiation time and particle size until the increase becomes insignificant or the yield declines (Xiao et al. 2008).

The quadratic model for the influence of solvent volume on 2-thiobarbituric acid (TBARs) value (0.3886) on day-5. Meat TBARs value was influenced ($P<0.05$) at 0.389 on day-5 of refrigeration storage. Linear model (irradiation

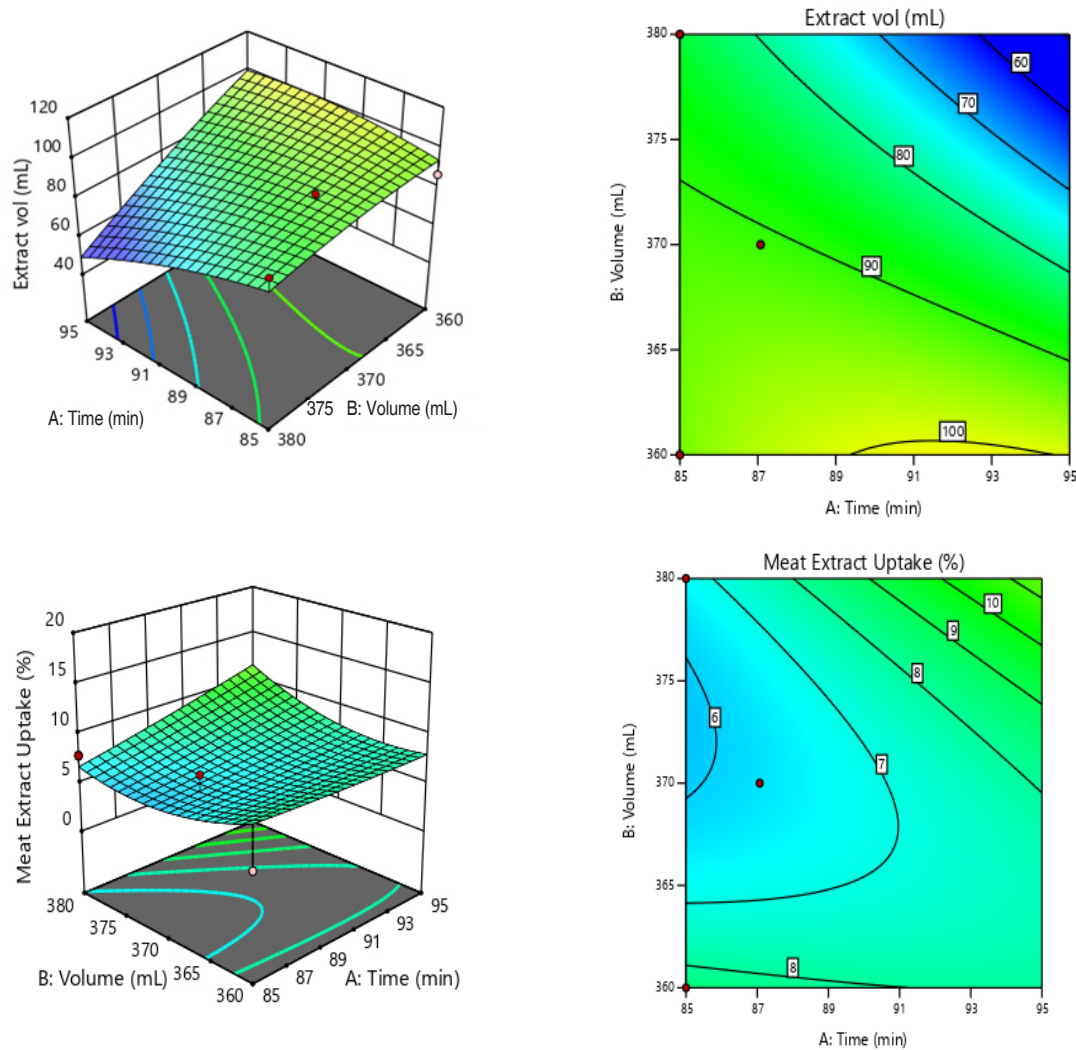


Figure 1. Response surface and contour plots of extract yield and uptake (%) of chicken meat soaked in black pepper extract.

time and solvent volume) values ranged between -7.483 and 5.788; -14.983 and 2.433 for extract volume and cook loss percentage respectively. Similarly, 2FI model and quadratic model were between -10.255 and 0.695 as well as -3.51 to 1.34 for aqueous extract volume and cook loss percentage respectively; while the quadratic model for solvent volume varied from -2.28 to 3.97 for extract volume and meat lightness respectively. Regression coefficient (R^2) ranged between 0.0830 and 0.5592 for meat lightness and malondialdehyde values on day (D) 5 of refrigeration storage. Drosou et al. (2015) explained that

the use of water as solvent under microwave extraction significantly increased the yield of polyphenols in red grape, however, this study has demonstrated that increasing solvent volume enhances extraction efficiency up till a limit and beyond this, further release of bioactive principles cannot be achieved. Therefore, increasing extraction criteria values beyond the limit will not positively enhance the extraction of polyphenols in aqueous black pepper extracts, which acts as antioxidants for quenching free radicals progressively generated beyond day 5, as meat is subjected to refrigeration storage.

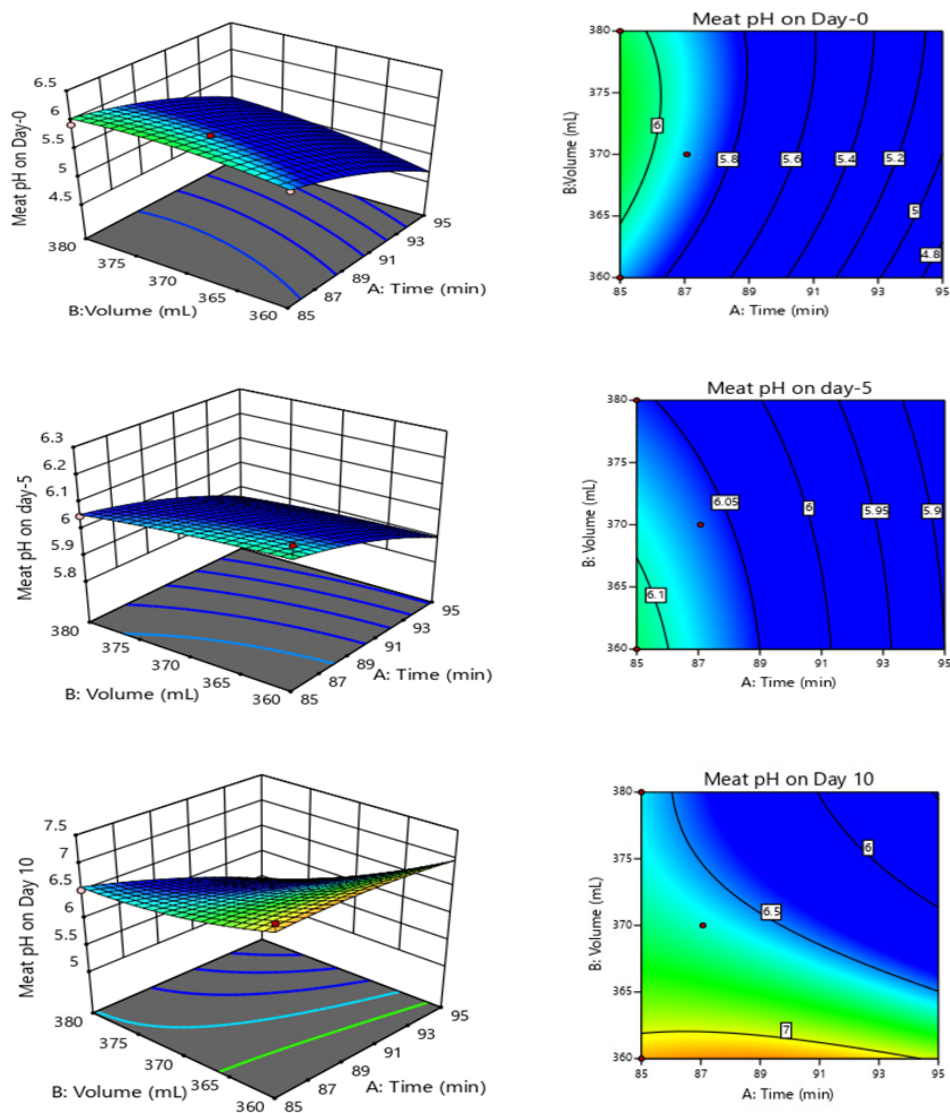


Figure 2. Response surface and contour plots of pH of meat soaked in black pepper aqueous extracts on day-0, 5 and 10 of refrigeration storage.

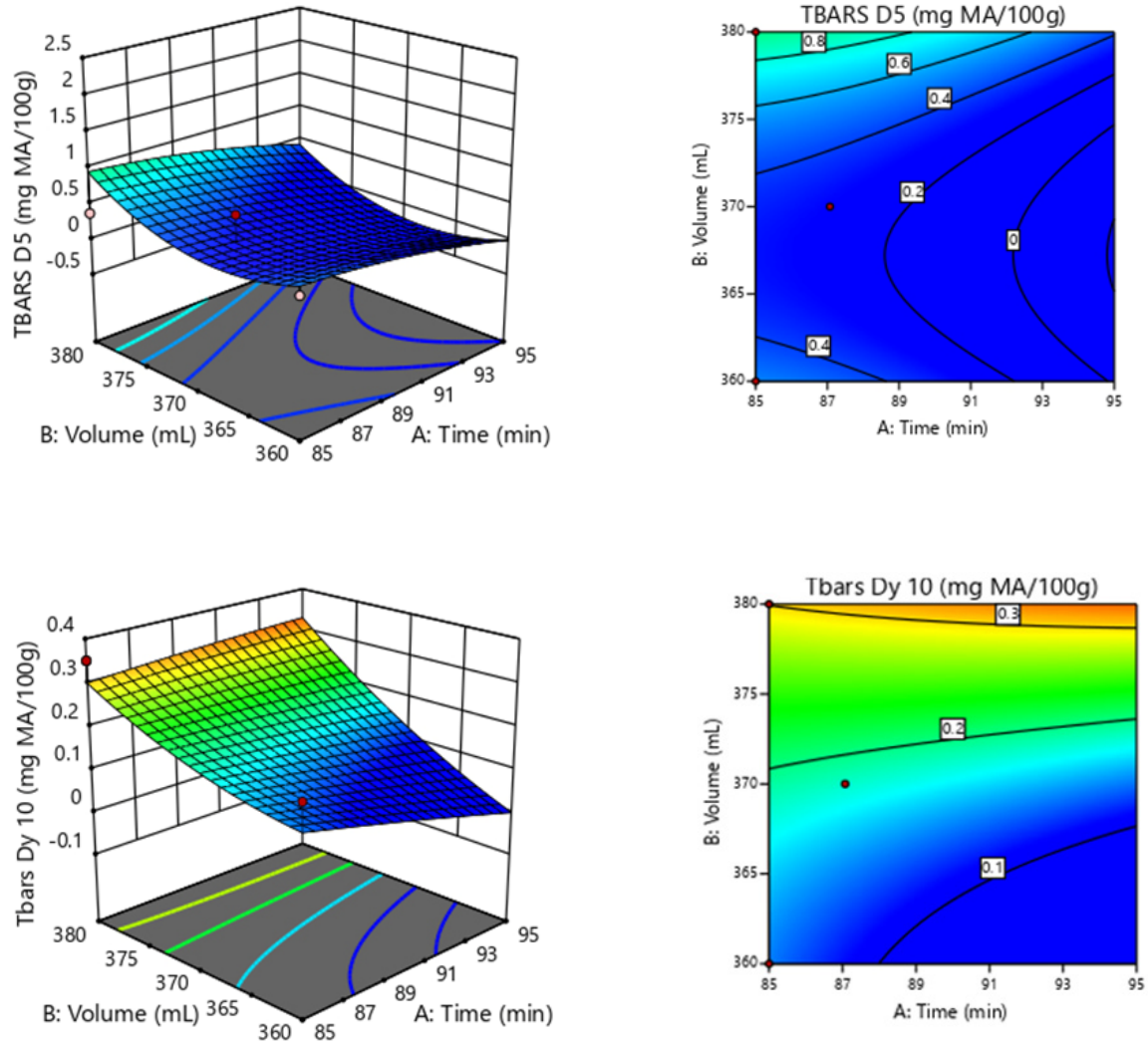


Figure 3. Response surface and contour plots of 2-thiobarbituric acid reactive substance (MDA) in meat of broiler chickens soaked in black pepper aqueous extract stored for 5 and 10 of refrigeration.

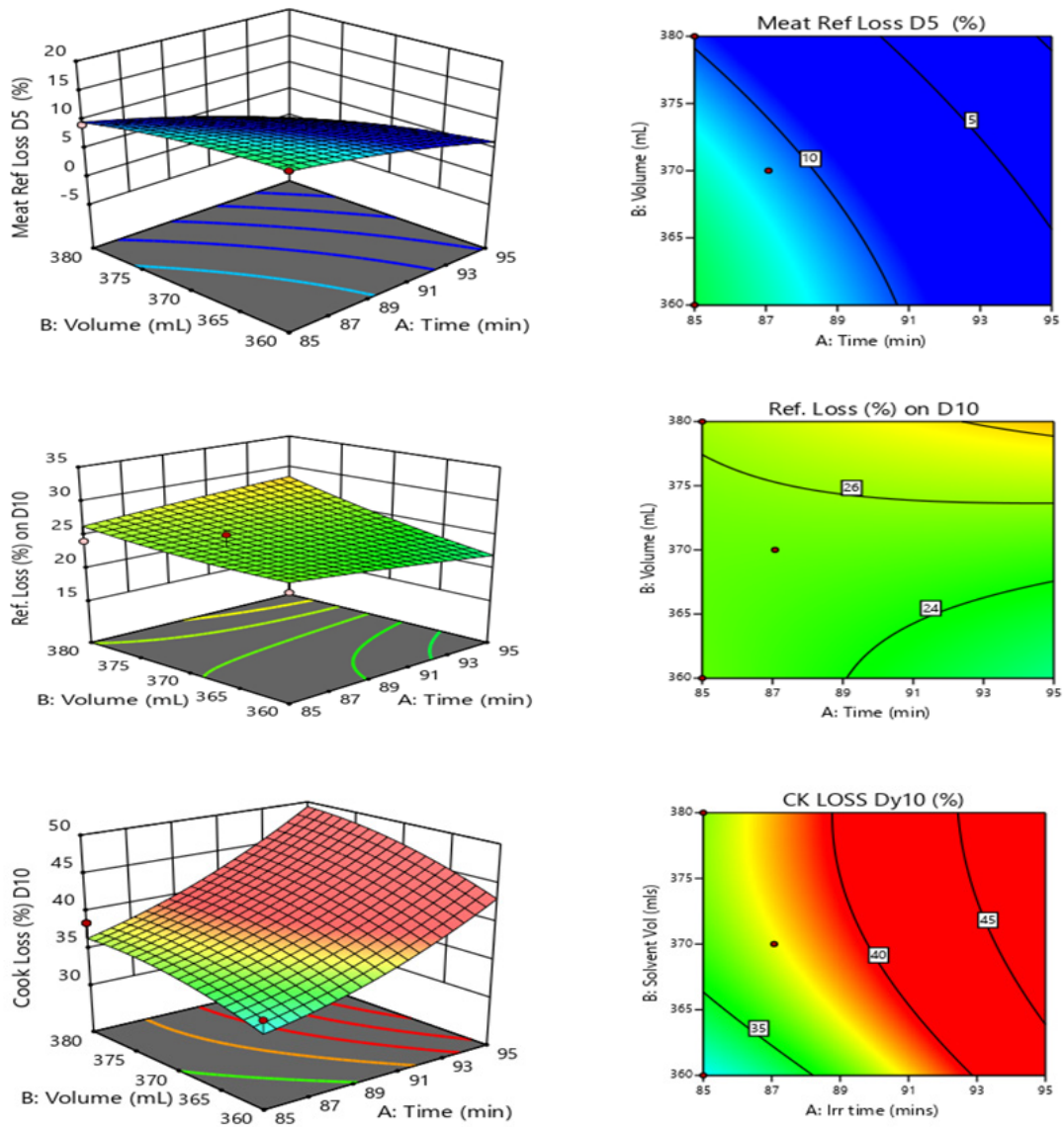


Figure 4. Response surface and contour plots of refrigeration and cook loss percentage of chicken meat soaked in black pepper aqueous extract stored for 5 and 10 days.

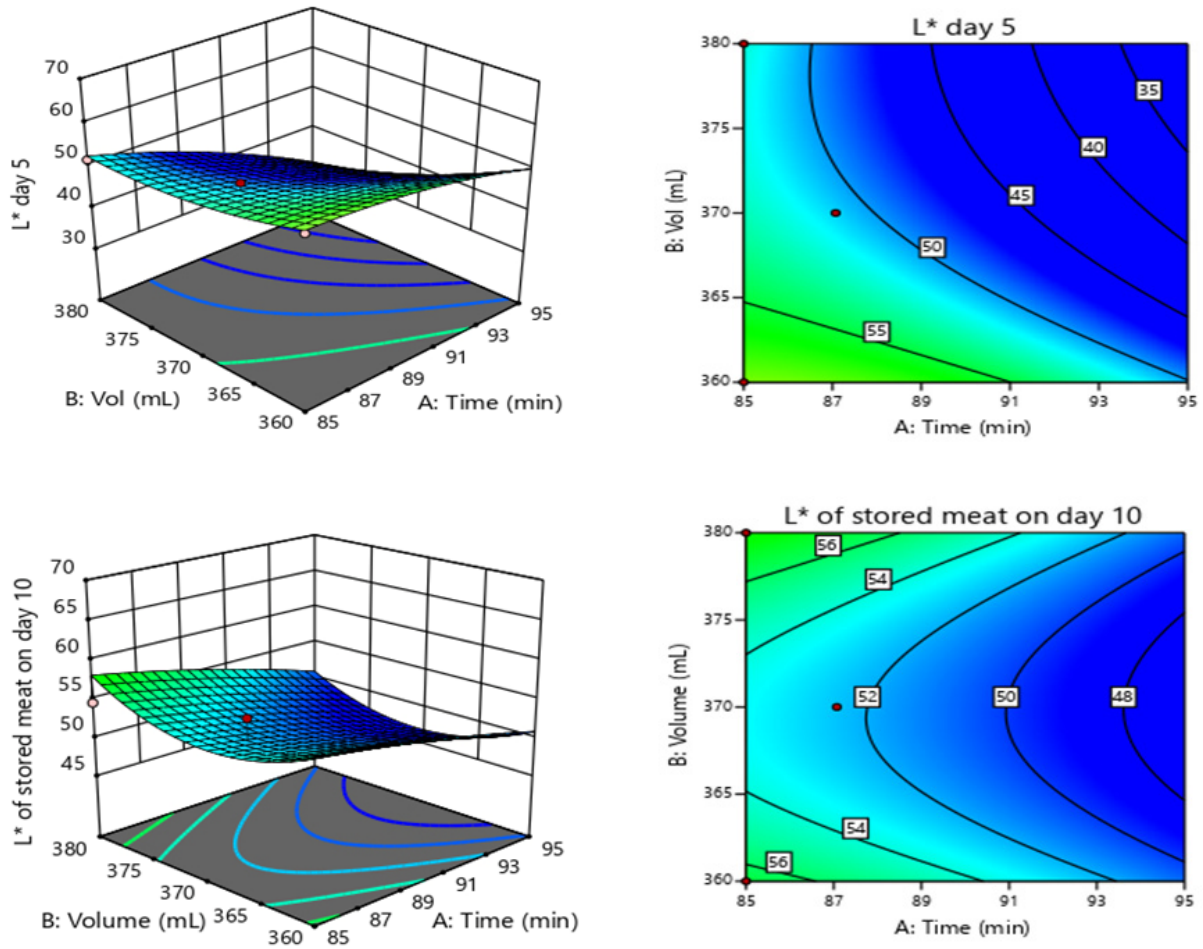


Figure 5. Response surface and contour plots of lightness value of meat soaked in black pepper aqueous extract on days 5 and 10 of refrigeration storage.

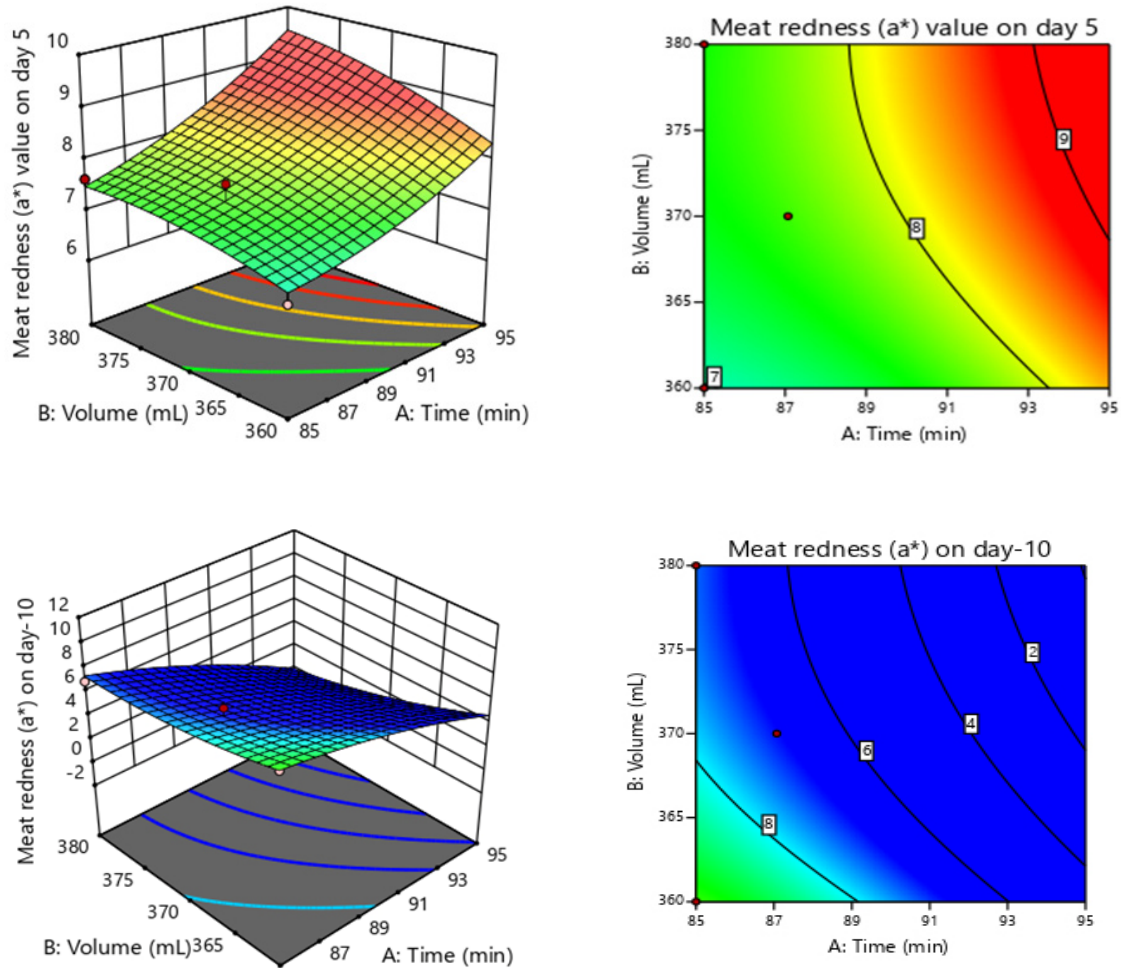


Figure 6. Response surface and contour plots of redness values of chicken meat soaked in black pepper aqueous extract on days 5 and 10 of refrigeration storage.

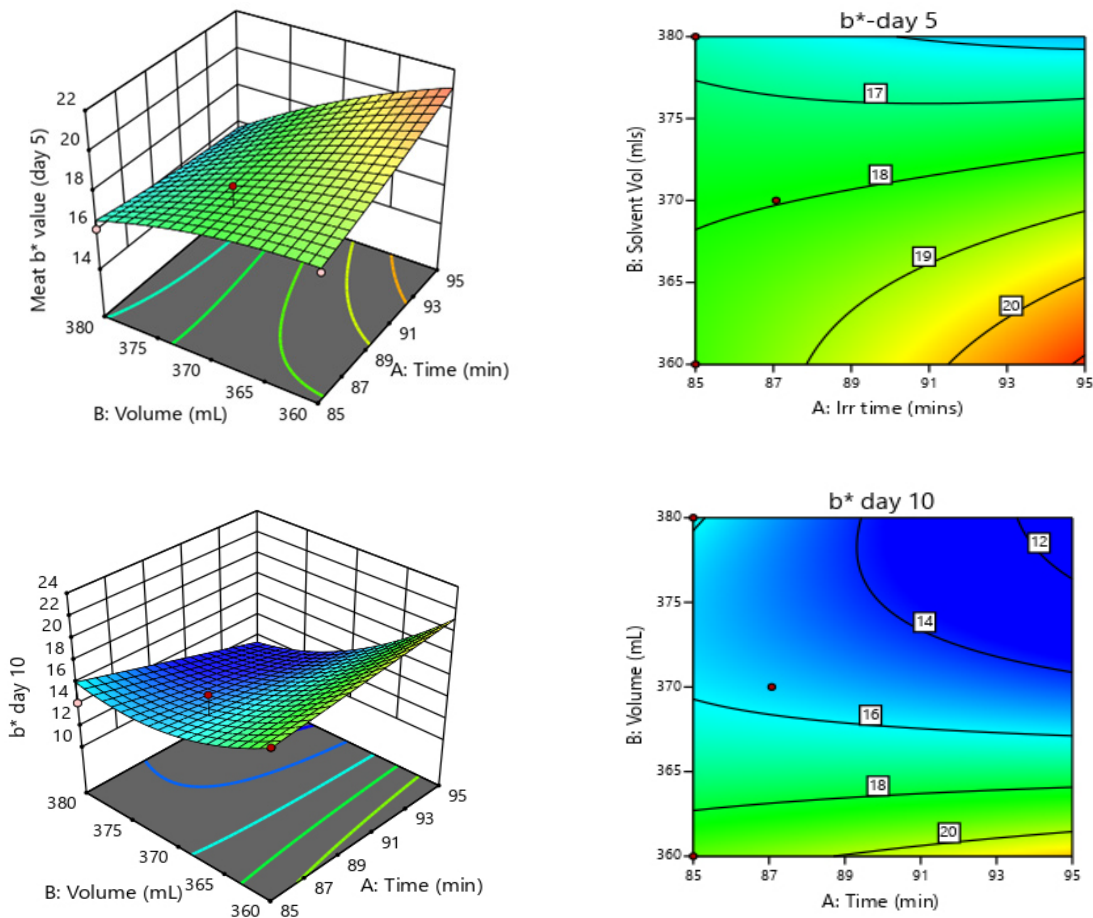


Figure 7. Response and contour plots of yellowness of meat of broiler chickens soaked in black pepper aqueous extract stored for 5 and 10 days in the refrigerator.

Criteria for optimization of production of black pepper aqueous extracts is presented in Table 5. The time for extract exposure to microwave irradiation was fixed between 85–95 min and the solvent volume was likewise fixed between 360 and 380 mL. Volume of extract to be obtained ranged between 60.42 and 115.23 mL, while extract soaked-up by meat was set to be maximized between 3.15 and 17.09. Meat pH of 5.82 was targeted for D 0 but meat pH for D 5 and 10 were minimized, ranging between 6.05–6.21 and 6.39–7.23 respectively. Meat malondialdehyde value range for D 5 was fixed between 0.286–2.088 but targeted at 0.11 for D 10. Meat lightness and redness on D 5 and 10 respectively were varied between 47.36–64.62, 49.03–66.35; 6.12–8.77 and 6.89–11.45, while cook loss of 30.60% was targeted. Refrigeration loss (%) in meat for D 5 and 10 and meat yellowness on D5 were not factored to obtain optimization outcomes.

From the suggested solutions in Table 6, six solutions were proffered. An irradiation time of 87.267 min and 364.285 mL of distilled water would result in best desirability level of 0.624, while holding solvent volume and microwave power constant, however, a similar combination of 87.245 min and 364.189 mL of distilled water can be combined as variable factors to achieve a similar effect. Other suggested solutions have desirability values of 0.623, 0.622, 0.621 and 0.612 respectively. This study has shown that irradiation time and solvent volume are critical factors that can affect the extraction of functional compounds in black pepper. Also, while the change in irradiation time of solutions suggested was close, the solvent volume affected the desirability than the irradiation time. This agrees with the findings of Jeyaratnam et al. (2016) that with increased solvent volume for extraction, a lower heat and by extension the irradiation time reduces for actual extraction as the energy requirement

Table 5. Criteria for optimization of extraction parameters.

Parameter	Goal	Limit
Irradiation time	in range	85-95
Solvent volume	in range	360-380
Extraction volume	in range	60.42-115.23
Oleoresin uptake	maximize	3.15-17.09
Meat pH D0	target	5.82
Meat pH D5	minimize	6.05-6.21
Meat pH D10	minimize	6.39-7.23
TBARs D5	minimize	0.286-2.088
TBARs D10	target	0.11
Ref. Loss D5	none	-
Ref. Loss D10	none	-
L* D5	in range	47.36-64.62
L* D10	in range	49.03-66.35
a* D5	in range	6.12-8.77
a* D10	in range	6.89-11.45
b* D5	none	
b* D10	in range	13.68-23.09
Cook loss	target	30.60

for vaporization increases. Also, from this study, the upper and lower limit for irradiation time and the extraction of white pepper with optimal desirability is between 87.245

and 87.267 min and further increment in irradiation time will not significantly increase extraction yield of bioactive compounds (Mustapa et al. 2015).

Table 6. Solutions found from optimizing aqueous black pepper extracts.

Number	Irr time	Solvent Vol	Extract vol	Oleoresin Uptake	Meat pH Day-0	Meat pH Day-5	Meat pH Day 10	TBARs D5	TBARs Dy 10	Meat Ref Loss D5
1	87.267	364.285	95.359	7.007	5.820	6.075	6.878	0.286	0.134	11.835
2	87.245	364.189	95.413	7.027	5.820	6.076	6.883	0.289	0.134	11.862
3	87.202	363.997	95.519	7.069	5.820	6.077	6.893	0.295	0.133	11.914
4	87.403	364.915	94.964	6.886	5.820	6.072	6.843	0.268	0.137	11.658
5	86.984	363.071	95.939	7.291	5.820	6.082	6.944	0.329	0.129	12.161
6	87.747	366.668	93.563	6.637	5.820	6.063	6.749	0.236	0.148	11.140

Number	Meat Ref Loss D10	L* day 5	L* day 10	a*-day 5	a* day 10	b*-day 5	b* day 10	CK Loss Dy10	Desirability
1	24.754	54.033	53.257	7.381	7.789	18.579	17.484	35.920	0.624
2	24.752	54.115	53.306	7.374	7.816	18.583	17.526	35.871	0.624
3	24.748	54.282	53.407	7.360	7.872	18.591	17.610	35.772	0.623
4	24.773	53.496	52.952	7.427	7.610	18.547	17.216	36.237	0.622
5	24.744	55.091	53.937	7.289	8.144	18.618	18.024	35.283	0.621
6	24.874	52.056	52.279	7.549	7.136	18.423	16.516	37.061	0.612

Bioactive compounds gotten from black pepper extract via optimized criteria is presented in Table 7. A total of 71 compounds were identified with a diverse range of element-based compounds such as silicon, nitrogen, sulphur and iodine. Two silicon, and nitrogen-based compounds as well as one sulphur and nitrogen-sulphur based compound were obtained, while seven iodine-based compounds were found. Palmitic acid was the major abundant component (17.05%), followed by Stearic acid (15.84%), 2-Ethylbenzotriazole (10.14%), N-Methyl-1-phenylcyclopropanamine (10.14%) and butylsemithiocarbazine (10.14%). Linoleic acid, petroselenic acid and (R)-(-)-14-Methyl-8-hexadecyn-

1-ol were 9.72 % respectively, while methyl linoleate, methyl linolealaidate and 9-cis, 11-trans-octadecadienoate were 8.55% respectively. Slightly lower yield was dibutyl phthalate and butyl isobutyl phthalate. Notably, Bis (2-ethylhexyl) phthalate content of 8.55% was present in aqueous extract. The GC-MS result for optimized black pepper extract contradicts the reports of Zhang and Xu (2015) and Olalere et al. (2018a) that white pepper contains more bioactive compounds than black pepper, thus indicating that the method of extraction employed have a strong impact on the qualitative yield of bioactive components.

Table 7. GC-MS analysis of various components in black pepper aqueous extract.

No	Component	Molecular Formula	Retention time	Area
1	Octamethylcyclotetrasiloxane (D4)	C ₈ H ₂₄ O ₄ Si ₄	3.187	0.580
2	5-Methylsalicylic acid, 2TMS derivative	C ₁₃ H ₂₂ O ₃ Si ₂	3.187	0.580
3	4-Methyldecane	C ₁₁ H ₂₄	3.409	0.850
4	2,6-Dimethylnonane	C ₁₁ H ₂₄	3.409	0.850
5	3,7-Dimethylundecane	C ₁₃ H ₂₈	3.865	1.570
6	5,7-Dimethylundecane	C ₁₃ H ₂₈	3.865	1.570
7	Tetrahydrolavandulol	C ₁₀ H ₂₂ O	4.465	0.600
8	3-Methyldecane	C ₁₁ H ₂₄	4.465	0.600
9	2-Ethylbenzotriazole	C ₈ H ₉ N ₃	4.598	10.14
10	N-Methyl-1-phenylcyclopropanamine	C ₁₀ H ₁₃ N	4.598	10.14
11	Butylsemithiocarbazine	C ₅ H ₁₃ N ₃ S	4.598	10.14
12	6-Methyldodecane	C ₁₃ H ₂₈	6.009	0.650
13	3,6-Dimethylundecane	C ₁₃ H ₂₈	6.009	0.650
14	2-Methylnonane	C ₁₂ H ₂₂	6.009	0.650
15	4,5-Dimethylnonane	C ₁₁ H ₂₄	6.564	0.830
16	2,4-Dimethylheptane	C ₉ H ₂₀	6.686	0.640
17	Sulfurous acid, butyl dodecyl ester	C ₁₆ H ₃₄ O ₃ S	6.686	0.640
18	2,6,11-Trimethyldodecane	C ₁₅ H ₃₂	6.909	1.360
19	3,7-Dimethyldecane	C ₁₂ H ₂₆	6.909	1.360
20	1-Iodoheptadecane	C ₁₆ H ₃₃ I	7.520	0.540
21	Tetracosane	C ₂₄ H ₅₀	7.520	0.540
22	1-Iodo-2-methylundecane	C ₁₂ H ₂₅ I	8.642	0.590
23	9-Methylnonadecane	C ₂₀ H ₄₂	9.264	1.22
24	Hexadecane	C ₁₆ H ₃₄	9.264	1.22
25	Tetracosane	C ₂₄ H ₅₀	9.264	1.22
26	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)] (Caryophyllene)	C ₁₅ H ₂₄	9.464	1.53

Table 7

No	Component	Molecular Formula	Retention time	Area
27	1s,4R,7R,11R-1,3,4,7-Tetramethyltricyclo [5.3.1.0(4,11)] undec-2-en-8-one	C ₁₅ H ₂₂ O	9.464	1.53
28	Heptacosane	C ₂₇ H ₅₆	9.597	0.93
29	1-Iodooctadecane	C ₁₈ H ₃₇ I	9.597	0.93
30	Tridecane, 1-iodo-	C ₁₃ H ₂₇ I	9.675	1.56
31	3,8-Dimethyldecane	C ₁₂ H ₂₆	9.675	1.56
32	2,4-di-tert-butylphenol	C ₁₄ H ₂₂ O	9.897	1.91
33	2,5-Di-tert-butylphenol	C ₁₄ H ₂₂ O	9.897	1.91
34	6,6-Diethyloctadecane	C ₂₂ H ₄₆	11.308	0.64
35	Undecane	C ₁₁ H ₂₄	11.486	0.62
36	Heptadecane	C ₁₇ H ₃₆	12.041	0.67
37	2,6,10-Trimethyltetradecane	C ₁₇ H ₃₆	12.041	0.67
38	Hentriacontane	C ₃₁ H ₆₄	12.041	1.260
39	9-Octylheptadecane	C ₂₅ H ₅₂	12.041	1.260
40	Hentriacontane	C ₃₁ H ₆₄	12.608	0.55
41	Octadecane	C ₁₈ H ₃₈	13.108	0.80
42	2-Methyltetracosane	C ₂₅ H ₅₂	13.108	0.80
43	Octacosane	C ₂₈ H ₅₈	13.586	0.67
44	Triacontane	C ₃₀ H ₆₂	13.586	0.67
45	Dotriacontane, 1-iodo-	C ₃₂ H ₆₅ I	14.030	0.98
46	3-Ethyl-5-(2-ethylbutyl) octadecane	C ₂₆ H ₅₄	14.030	0.98
47	Methyl palmitate	C ₁₇ H ₃₄ O ₂	14.408	2.80
48	Methyl 14-methylpentadecanoate	C ₁₇ H ₃₄ O ₂	14.408	2.80
49	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	14.741	6.20
50	Butyl isobutyl phthalate	C ₁₆ H ₂₂ O ₄	14.741	6.20
51	Palmitic acid	C ₁₆ H ₃₂ O ₂	14.885	17.050
52	Methyl linoleate	C ₁₈ H ₃₂ O ₂	15.996	8.55
53	Methyl (9E,12Z)-9,12-octadecadienoate (Methyl linolelaidate)	C ₁₉ H ₃₄ O ₂	15.996	8.55
54	9-cis,11-trans-Octadecadienoate	C ₁₈ H ₃₁ O ₂	15.996	8.55
55	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	16.052	4.29
56	9-Octadecenoic acid, methyl ester, (E) (methyl eladiate)	C ₁₉ H ₃₆ O ₂	16.052	4.29
57	cis-13-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	16.052	4.29
58	methyl stearate	C ₁₉ H ₃₈ O ₂	16.296	0.72
59	Ethyl heptadecanoate	C ₁₉ H ₃₈ O ₂	16.296	0.72
60	Petroselinic acid (6-Octadecenoic acid)	C ₁₆ H ₃₄ O ₂	16.452	9.72
61	(9E,12Z)-linoleic acid	C ₁₈ H ₃₂ O ₂	16.452	9.72
62	R) - (-) - 14-Methyl-8-hexadecyn-1-ol	C ₁₇ H ₃₂ O	16.452	9.72
63	Stearic acid	C ₁₈ H ₃₆ O ₂	16.719	15.84
64	Carbonic acid, decyl undecyl ester	C ₂₂ H ₄₄ O ₃	18.163	0.73

Table 7

No	Component	Molecular Formula	Retention time	Area
65	Tetracosane, 1-iodo-	C ₂₄ H ₄₉ I	18.163	0.73
66	Dotriacontane, 1-iodo-	C ₃₂ H ₆₅ I	18.163	0.73
67	(Z)-14-Methylhexadec-8-enal	C ₁₇ H ₃₂ O	19.507	0.56
68	Eicosane	C ₂₀ H ₄₂	19.507	0.56
69	Bis(2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	19.774	1.87
70	Phthalic acid, dodecyl 2-ethylhexyl ester	C ₂₈ H ₄₆ O ₄	19.774	1.87
71	Phthalic acid, hexadecyl 4-methylhept-3-yl ester	C ₃₂ H ₅₄ O ₄	19.774	1.87

CONCLUSIONS

Meat quality is a major criterion for purchase that drives consumer preference and decision. This study has demonstrated that the number of compounds extracted correspond with extraction efficiency, evidenced by the influence of extracts on meat pH and MDA count on D 5; meat lightness and eventual number of compounds extracted. Extraction conditions sandwiched between 85–87 min and 361–379 mL of irradiation time and solvent volume respectively, in tandem with other fixed extraction operational factors favors the preservation of lightness in meat, while the least meat TBARs value was recorded at 88 min of irradiation time and 367 mL of solvent volume. After optimization was performed, a total of six solutions were suggested for the extraction of aqueous black pepper, and the recommended extraction criteria had desirability level of 0.624 was for two solutions. With irradiation power and particle size fixed at 320 W and 8 g respectively, alongside 0.40 mm particle size; 87.268 min of irradiation time and 364.285 mL of solvent volume was suggested as the recommended variable extraction values, while an irradiation time of 87.245 min and 364.189 mL can be applied as effective alternative. Applying the suggested extraction conditions in a closed system, a total of 71 compounds were found which is a remarkable improvement in the extraction yield and efficiency.

REFERENCES

Abubakar A, Fitri C, Koesmara H et al (2021) Analysis of pH and cooking losses of chicken meat due to the use of different percentages of turmeric flour. IOP Conference Series: Earth and Environmental Science 667. <https://doi.org/10.1088/1755-1315/667/1/012042>

Adeleye OO, Abatan MO, Dosumu OA et al (2021) Apple cider vinegar administration on carcass characteristics and meat quality of broiler chickens. Tropical Animal Health and Production 53(6):530.

<https://doi.org/10.1007/s11250-021-02967-x>

Anderson M and Whitcomb P (2016) RSM Simplified – Optimizing Process Using Response Surface Methods for Design of Experiments. 2nd edition. Productivity Press, New York. 311 p. <https://doi.org/10.1201/9781315382326>

Agbor GA, Vinson JA, Oben JE and Ngogang JY (2006) Comparative analysis of the *in vitro* antioxidant activity of white and black pepper. Nutrition Research 26 (12):659–663. <https://doi.org/10.1016/j.nutres.2006.09.019>

Aghdase S, Vahid H and Behnam K (2017) Microwave assisted extraction of bioactive compounds from food: A Review. International Journal of Food Science and Nutrition Engineering 7 (1):19-27. <http://article.sapub.org/10.5923.j.food.20170701.03.html#Sec1>

Aydar AY (2018) Utilization of response surface methodology in optimization of extraction of plant materials. In: Silva V. statistical approaches with emphasis on design of experiments applied to chemical processes. Intech Open. London. 180p. <https://doi.org/10.5772/intechopen.73690>

Balasubramanian S, Allen JD, Kanitkar A and Boldor D (2011) Oil extraction from *Scenedesmus obliquus* Using a continuous microwave system—design, optimization, and quality characterization. Bioresource Technology 102(3):3396–3403. <https://doi.org/10.1016/j.biortech.2010.09.119>

Böttcher S, Steinhäuser U and Drusch S (2015) Off-flavour masking of secondary lipid oxidation products by pea dextrin. Food Chemistry 169:492-498. <http://dx.doi.org/10.1016/j.foodchem.2014.05.006>

Clery RA, Hammond CJ and Wright AC (2006) Nitrogen-containing compounds in black pepper oil (*Piper nigrum* L.). Journal of Essential Oil Research 18:1–3 <https://doi.org/10.1080/10412905.2006.9699369>

Design Expert (2021) Design expert version 13. Stat – Ease, Incorporation. 1300 Godward Street. North east, suite 6400 Minneapolis, MN.55413 version 13.0.5.0

Drosou C, Kyriakopoulou K, Bimpilas A et al (2015) A comparative study on different extraction techniques to recover red grape pomace polyphenols from vinification by-products. Industrial Crops and Products 75:141-149. <https://doi.org/10.1016/j.indcrop.2015.05.063>

Falowo AB, Fayemi PO and Muchenje V (2014) Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review. Food Research International 64:171-181. <https://doi.org/10.1016/j.foodres.2014.06.022>

- Flórez N, Conde E and Domínguez H (2015) Microwave assisted water extraction of plant compounds. *Journal of Chemistry Technology and Biotechnology* 90:590–607. <https://doi.org/10.1002/jctb.4519>
- Honkavaara M, Rintasalo E, Ylönen J and Pudas T (2003) Meat quality and transport stress of cattle. *Deutsche Tierärztliche Wochenschrift* 110(3):125–128.
- Insani EM, Eyherabide A, Grigioni G et al (2008) Oxidative stability and its relationship with natural antioxidants during refrigerated retail display of beef produced in Argentina. *Meat Science* 79 (3):444-452. <https://doi.org/10.1016/j.meatsci.2007.10.017>
- Jeleń H H and Gracka A (2015) Analysis of black pepper volatiles by solid phase microextraction-gas chromatography: a comparison of terpenes profiles with hydrodistillation. *Journal of Chromatography A* 1418:200–209. <https://doi.org/10.1016/j.chroma.2015.09.065>
- Jeyaratnam N, Nour AH, Kanthasamy R et al (2016) Essential oil from *Cinnamomum cassia* bark through hydro-distillation and advanced microwave assisted hydro-distillation. *Industrial Crops and Products* 92:57–66. <https://doi.org/10.1016/j.indcrop.2016.07.049>
- Jimenez JEQ, Zanin LL, Ferreira IM et al (2018) Microwave Radiation in Biocatalysis. In: Banik BK and Bandyopadhyay, D. *Advances in Microwave Chemistry*. CRC Press. Boca Raton. 534 p.
- Kallel F, Driss D, Chaari F et al (2014) Garlic (*Allium sativum* L.) husk waste as a potential source of phenolic compounds: influence of extracting solvents on its antimicrobial and antioxidant properties. *Indian Crop Production* 62:34-41. <http://doi.org/10.1016/j.indcrop.2014.07.047>
- Keller J, Camaré C, Bernis C et al (2015) Antiatherogenic and antitumoral properties Sarhad Journal of Agriculture of Opuntia cladodes: inhibition of low-density lipoprotein oxidation by vascular cells, and protection against the cytotoxicity of lipid oxidation product 4-hydroxynonenal in a colorectal cancer cellular model. *Journal of Physiology and Biochemistry* 71(3):557-587. <https://doi.org/10.1007/s13105-015-0408-x>
- Kralik G, Kralik Z, Grčević M and Hanžek D (2017) Quality of chicken meat. In: Yücel B and Taşkın T (Eds.) *Animal husbandry and nutrition*. IntechOpen, London. <http://dx.doi.org/10.5772/intechopen.72865>
- Kim HJ, Kim HJ, Jeon JJ et al (2020) Comparison of the quality characteristics of chicken breast meat from conventional and animal welfare farms under refrigerated storage. *Poultry Science* 99(3): 1788–1796. <https://doi.org/10.1016/j.psj.2019.12.009>
- Leal LN (2019) Improving meat colour and oxidative stability by antioxidant supplementation of light weight lamb diets. (Ph.D thesis). Wageningen University, Wageningen, the Netherlands. 196 p. <https://doi.org/10.18174/496702>
- Mohammed G, Omran A M and Hussein H (2016) Antibacterial and phytochemical analysis of *Piper nigrum* using gas chromatography–mass spectrum and fourier-transform infrared spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research* 8(6):977-996.
- Mustapa AN, Martín Á, Mato RB and Cocero MJ (2015) Extraction of phytocompounds from the medicinal plant *Clinacanthus nutans* Lindau by microwave-assisted extraction and supercritical carbon dioxide extraction. *Industrial Crops and Products* 74:83–94. <https://doi.org/10.1016/j.indcrop.2015.04.035>
- Olalere OA, Abdurahman NH, Yunus RM et al (2017) Comparative study of pulsed microwave and hydrodistillation extraction of piperine oil from black pepper. *IJUM Engineering Journal* 18(2):87–93. <https://doi.org/10.31436/iujem.v18i2.802>
- Olalere OA, Abdurahman HN, Yunus RM et al (2018a) Parameter study, antioxidant activities, morphological and functional characteristics in microwave extraction of medicinal oleoresins from black and white pepper. *Journal of Taibah University for Science* 12 (6):730-737. <https://doi.org/10.1080/16583655.2018.1515323>
- Olalere OA, Abdurahman NH, Yunus R et al (2018b) Evaluation of optimization parameters in microwave reflux extraction of piperine-oleoresin from black pepper (*Piper nigrum*). *Beni-Suef University Journal of Basic and Applied Sciences* 7 (4):626 – 631. <https://doi.org/10.1016/j.bjbas.2018.07.006>
- Passos CP and Coimbra MA. (2013) Microwave superheated water extraction of polysaccharides from spent coffee grounds. *Carbohydrate Polymers* 94(1):626–633. <https://doi.org/10.1016/j.carbpol.2013.01.088>
- Pino J, Rodríguez-Feo G, Borges P and Rosado A. (1990) Chemical and sensory properties of black pepper oil (*Piper nigrum* L.). *Molecular Nutrition Food Research* 34(6):555–560. <https://doi.org/10.1002/food.19900340615>
- Ramamoorthi L, Toshkov S, Stetzer A et al (2010) Effects of antioxidants on irradiated beef color. *Journal of Food Quality* 33(3): 283 - 298. <https://doi.org/10.1111/j.1745-4557.2010.00296.x>
- Rodríguez Seoane P, Flórez-Fernández N, Conde Piñeiro E and Domínguez González, H (2017) Microwave-assisted water extraction. pp. 163–198. In: Domínguez González, H, González Muñoz, M J.(Eds) *Water extraction of bioactive compounds: From plants to drug development*. Elsevier. Amsterdam, The Netherlands. 466 p.
- Sanwo KA, Iposu SO, Adegbite JA et al (2012) Quality characteristics of chevon sausage obtained from goats fed a 50% inclusion level of melon (*Colocynthis citrillus*) husk and palm (*Elaeis guineensis*) oil slurry. *Iranian Journal of Applied Animal Science* 2 (4):331–335.
- Sanwo KA, Adegoke AV, Egbeyale LT et al (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.
- Suvarna Y and Abdul Rahaman, SK (2019) Pharmacotherapeutic properties of black pepper: a systematic review. *Asian Journal of Research in Chemistry and Pharmaceutical Sciences* 7(1):293-304.
- Tan N A H and Muhamad II (2017) Optimization of Omega 3 Rich Oil extraction from elaterios-permum tapos seed by microwave assisted aqueous enzymatic extraction. *Chemical Engineering Transactions* 56:1783-1788. <https://doi.org/10.3303/CET1756298>
- Tatke P and Jaiswal Y (2011) An overview of microwave assisted extraction and its applications in herbal drug research. *Research Journal of Medicine and Plant* 5(1):21–31.
- Teo CC, Tan SN, Yong JWH et al (2009) Validation of green-solvent extraction combined with chromatographic chemical fingerprint to evaluate quality of *Stevia rebaudiana* Bertoni. *Journal of Separation Science* 32(4):613–622. <https://doi.org/10.1002/jssc.200800552>
- USDA - U.S. Department of Agriculture (2013) Water in meat and poultry. In: Food Safety and Inspection Service, <https://www.fsis.usda.gov/food-safety/safe-food-handling-and-preparation/food->

safety-basics/water-meat-poultry

Veggi PC, Martinez J and Meireles MAA (2013) Fundamentals of microwave extraction. Pp. 15-55. In: Chemat F and Cravotto G (Eds). Microwave-Assisted Extraction for Bioactive Compounds: Theory and Practice. Food Engineering Series 4, Springer, New York. 238 p.

Xiao W, Han L and Shi B (2008) Microwave-assisted extraction of flavonoids from *Radix astragali*. Separation Purification Technology 62(3):614–618. <https://doi.org/10.1016/j.seppur.2008.03.025>

Zhang QW, Lin LG and Ye WC (2018) Techniques for extraction

and isolation of natural products: a comprehensive review. Chinese Medicine 13(20):1-26. <https://doi.org/10.1186/s13020-018-0177-x>

Zhang L and Xu J G (2015) Comparative study on antioxidant activity of essential oil from white and black pepper. European Journal of Food Science and Technology 3:10–16.

Zin MM, Anucha, CB and Bánvölgy S (2020) Recovery of Phytochemicals via Electromagnetic Irradiation (Microwave-Assisted Extraction): Betalain and Phenolic Compounds in Perspective. Foods 9(7):918. <https://doi.org/10.3390/foods9070918>