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QUALITATIVE YIELD OF WHITE PEPPER EXTRACT OPTIMIZED BY MICROWAVE EXTRACTION AND MEAT QUALITY ASSESSMENT – AN ALTERNATIVE APPROACH

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Key words: microwave extraction, Piper nigrum extracts, optimization, meat quality, bioactive compounds.

Abstract

This study experiments the application of closed microwave extraction on aqueous white pepper–guided by two fixed [microwave power (300 W) and sieve size (0.40 mm)] and two variable [irradiation time (75–85 min) and solvent volume (280–300 mL)] factors in a central composite design. Extracts generated were optimized via meat quality assessment. From responses generated post-optimization, twelve solutions were proffered. Five solutions had highest desirability value of 0.604. Extraction criteria for recommended desirability require microwave power of 300 W, ground white pepper screened at 0.40 mm, irradiation time of 91.19 min and 280 mL of solvent volume (distilled water), but the other four solutions all require 280 mL of solvent volume and 91.151, 91.131, 91.241 and 91.091 min of irradiation time respectively. Gas Chromatography Mass Spectrometry (GC-MS) analysis of the recommended extract had a remarkable yield of forty-one (41) compounds. This green extraction procedure shows promise for future extractions.

Introduction

High solvent volume and extended extraction periods characterize traditional extraction processes that most times result in low quality yield.

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Microwave extraction have been reported as an efficient technique to obtain bioactive compounds from simple and complex herbal mixtures (Laroze et al. 2008, Neuchter et al. 2004). Closed and open microwave heating systems have been explored with significant yield, safe processes and better outputs (Olalere et al. 2017). Microwave extraction in controlled setup prevents thermo-chemical degradation of heat-sensitive phenolic compounds (Olalere and Gan 2020)–an advantage in the field of therapeutics, agriculture and medicine.

Plants contain natural antioxidants and are of considerable interest for the development of new medicines (Amal Belakredar et al. 2021). Bio-active components in Piper species have served therapeutic functions (Barh et al. 2013). White pepper (*Piper nigrum*) corm commonly found in Western countries is a ripe berry devoid of its outer skin (Agbor et al., 2006). Piper species are traditionally consumed to treat ailments or alternatively applied as preservative, insecticide or anti-microbial (Aziz et al. 2015, Gasparetto et al. 2017). Compositional analysis of oleoresin extracted by Olalere et al. (2018) yielded 31 bioactive compounds and approaches to increase extraction yield is desirable.

Application of meat quality assessment as an optimization tool is a new approach to increase extraction yield with less repetitive processes under environmentally friendly conditions. This procedure leverage on a rationale that the higher the antioxidants in extracts, the likelier the effectiveness of extraction. Therefore, an assessment of meat quality as it influences the quality of extract produced is based on the knowledge that oxidation of lipids post-slaughter can negatively affect the quality of fresh meat (Insani et al. 2008, Trout 2003). This study therefore uses an alternative approach to optimize the qualitative yield of white pepper extract via microwave extraction and meat quality assessment. The study aims to generate criteria for extracting white pepper; to optimize extraction outcomes using meat quality evaluations and to evaluate extraction efficiency using GC-MS (Gas Chromatography – Mass Spectrometry) analysis.

Materials and Methods

Experimental site

Microwave extraction was performed at the Laboratory of Feed Quality, Department of Animal Production and Health, Federal University of Agriculture, Abeokuta. Meat colour and thiobarbituric acid reactive substance value were measured at Laboratory of Food Science and Technology

and Laboratory of Veterinary Medicine; Federal University of Agriculture, Abeokuta. Compositional analysis (GC-MS) of extract with the best suggested desirability was performed at the Laboratory of Chemistry, Faculty of Science, University of Lagos.

Experimental design

Two numerical factors (independent variables): irradiation time (75–85 min) and solvent volume (280–300 mL) were varied alongside two fixed factors (microwave power and sieve size were held constant at 320 W and 0.40 mm respectively) to generate thirteen experimental runs. Central Composite Design (CCD) was employed for process analysis, while response surface methodology was applied for the optimization phase as described by ANDERSON and WHITCOMB (2016). Thereafter, Gas Chromatography and Mass Spectrometry analysis of the optimized extract with the best desirability was performed accordingly.

Sourcing and preparation of test materials

Dried white pepper corm was sourced from a renowned herb market and pulverized into finely defined powder using an attrition mill. Powdered sample was clarified using 0.105 mm sieve prior to storage in air – tight amber coloured vials. Hi-sense (H36MOMMI) microwave was used for extraction process, while distilled water was used as solvent.

Twenty-five (4-weeks old) broiler chickens intensively raised under deep litter management system for five (5) weeks were subjected to uniform management. Commercial diet fed is shown in Table 2. Afterwards, the birds were sacrificed and the breast muscles were extracted and weighed prior to use.

Ethical guideline. Ethical guidelines were strictly adhered to prior to slaughter following the established guidelines established by the Animal Welfare and Ethics Committee of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta.

Microwave extraction procedure. Thirteen (13) experimental runs were proposed by Design Expert software (5 repetitions generated as centre point). Eight (8) grams of ground white pepper was dissolved in each run comprising 275.86–304.14 mL of distilled water. Solute was stirred until homogeneity and uniformity was attained. The of mixture was placed in an irradiation-tolerant container before placement in the microwave cavity. Pre-heating of the cavity was performed for 15 min at 100 W. Afterwards, 300 W power was set and extraction was performed following designed sug-

gestions (Table 1). Subsequently, loading and unloading of mixture and extracts from the cavity was carried out according to procedure established by previous studies (OLALERE et al. 2017 2018). Next, extracts obtained were cooled and stored in coloured vials prior to meat quality assessment.

Extract yield calculation. The percentage yield of extraction is expressed as follows:

$$\text{Extraction Yield} = \frac{volume\ of\ extracts\ [\text{ml}]}{volume\ of\ mixture\ [\text{ml}]} \cdot 100.$$

Gas chromatography mass spectrophotometry of aqueous white pepper extract. Gas Chromatography Mass Spectrophotometry analysis of extract suggested desirability was performed. Filtered extract (1 μL) was diluted using an analytical standard grade acetone extract at 1 : 10 ratio and injected into the column for components identification according to OLALERE et al. (2018). Compounds identified in relation to the peak area fragmentation fingerprints were recorded.

Statistical design

Data obtained from responses were analysed using Design Expert version 12.0.3.0 (D_x 12, 2019). Mean separation at 5% level of significance was carried out by subjecting regression coefficients to Analysis of Variance (ANOVA) to obtain coefficient of determination (R^2) for each response. Numerical optimization was carried out to ascertain the level of desirability.

Table 1

Central composite design matrix for extraction of aqueous white pepper

RUN	Irradiation time [min]	Solvent volume [mL]
1	2	3
1	90.00	304.14
2*	90.00	290.00
3	95.00	300.00
4	85.00	300.00
5*	90.00	290.00
6*	90.00	290.00
7	82.93	290.00
8*	90.00	290.00
9	85.00	280.00
10*	90.00	290.00
11	97.07	290.00

cont. table 1

1	2	3
12	95.00	280.00
13	90.00	275.89

Explanations: *****midpoint repeated five times

Table 2

Nutrient composition of commercial finisher diet fed broiler chickens

Ingredient (DM)	Composition
Energy [Kcal]	2900
Crude protein [%]	20.00
Fat/Oil [%]	6.00
Crude fibre [%]	5.00
Salt [%]	0.30
Lysine [%]	0.85
Methionine [%]	0.35
Calcium [%]	1.00
Available Phosphorus [%]	0.40

Explanations: DM – dry matter, Kcal – kilocalories, % – percentage

Data collection

Extract uptake. Fifteen (15) grams of meat from the breast was weighed out in triplicates. Fifteen (15) mL of extract was added to each replicate and soaked for 30 min. Samples were subsequently removed and reweighed after 5 min. Increase in weight of samples indicate the volume of extract absorbed, and weight change was expressed as a percentage of the initial weight of meat before soaking. Afterwards, qualitative evaluations were carried out on meat samples that contain extracts.

Determination of microwave internal temperature. An LCD digital thermometer (MEXTECH) (St-9283B) probe was inserted into the microwave (Hi-sense H36MOMMI) cavity for 5 min post-extraction, and temperature range of 39 ±2^{o}C was recorded.

Evaluation of extract quality using meat quality analysis

pH assessment. Measurement of pH of meat was carried out with an ATC pH meter (Hanna Instruments) as described by KIM et al. (2009). Measurements were repeated on d 5 and 10. Colour measurements were determined using Chroma meter model – CR-400 (Konica Minolta, Tokyo, Japan). Colour categorization was based on 2 points on each meat sample.

Refrigeration loss [%]. Refrigeration loss percentage of meat samples containing extracts was evaluated:

$$\text{Refrigeration loss } [\%] = \frac{weight\ before\ refrigeration\ -\ weight\ after\ refrigeration}{weight\ before\ refrigeration} \cdot 100$$

Refrigeration loss percentages on d 0, 5 and 10 were recorded.

Cooking loss [%]. On day-10, post refrigeration loss analysis, meat cooking loss was determined. Samples were allowed to drain, then cooked in water bath at 65°C for 30 minutes to calculate the cooking loss percentage. After cooking, the residual moisture was allowed to drain, then weighed as follows:

$$\text{cooking loss } = weight\ before\ cooking - weight\ after\ cooking$$

$$\text{cooking loss } [\%] = \frac{weight\ before\ cooking - weight\ after\ cooking}{weight\ before\ cooking} \cdot 100$$

Oxidative rancidity measurement of meat samples containing extracts. Each meat sample (5 g) containing extract was homogenized in 15 ml of distilled water. Sample homogenate (5 ml) was transferred to a test tube and the lipid oxidation was measured as thiobarbituric acid reactive substances (TBARS) (BIDLACK et al. 1973), using an absorbance standardized at 532 nm.

$$T\text{BARS mg}\frac{\text{MDA}}{\text{kg}} of\ meat = (\text{kg of meat absorbance of sample} - \text{absorbance of blank sample}) \cdot 5.88$$

Result

Quality characteristics of chicken meat incorporated with white pepper aqueous extracts

The result for optimization of white pepper extract using broiler chicken meat is presented (Table 3). Extract volume from extraction process yielded 41.08–136.96 mL, while aqueous uptake by meat was between 0.4 and 20.33%. Meat pH of soaked samples on d 0, 5 and 10 ranged between 5.61–6.33%, 6.05–6.25% and 6.43–7.19% respectively. Meat TBARS (malondialdehyde value) ranged from 0.277–0.97, and 0.157–0.397 on day (d) 5 and 10 respectively. Meat refrigeration loss were 9.97–19.83% and 18.19–33.74% respectively on d 5 and 10, while cook loss was between 17.54 and 40.23%. Meat L*, a* and b* ranged between 59.25–76.81, 5.19–11.24 and 13.12–18.75 respectively on d 5 and 61.32–72.71, 8.44–14.53 and 14.82–17.76 respectively on d 10 of storage (4°C).

Table 3

Mean values of quality indices of chicken meat incorporated with white pepper extracts

RUN	Time [min]	Sol. Vol. [mL]	Aq. Ext. [mL]	Ext. Yield %	Ext. 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*5	L*10	a*5	a*10	b*5	b*10	Ck ls d 10 [%]
1	90.00	304.14	116.25	37.24	8.97	6.33	6.15	6.97	0.34	0.32	19.83	30.26	71.49	66.72	8.03	12.97	17.09	14.53	31.30
2	90.00	290.00	127.98	42.95	7.49	6.16	6.18	6.99	0.57	0.25	15.82	27.23	67.74	67.89	11.24	14.38	16.30	13.26	32.36
3	95.00	300.00	85.88	27.88	2.33	6.33	6.10	6.43	0.97	0.18	15.36	24.72	70.97	65.87	9.49	8.48	18.66	16.00	37.5
4	85.00	300.00	136.96	44.47	6.11	6.17	6.25	6.80	0.40	0.18	18.25	32.3	76.81	72.71	8.31	9.65	18.75	15.88	34.82
5	90.00	290.00	118.58	39.79	10.15	6.02	6.18	6.44	0.43	0.22	19.19	27.42	68.46	67.86	8.61	9.80	18.67	16.78	38.57
6	90.00	290.00	122.9	41.24	7.63	6.20	6.20	6.63	2.82	0.30	17.15	30.31	86.88	68.61	5.19	14.53	14.96	15.23	25.93
7	82.93	290.00	109.28	36.67	20.33	6.12	6.11	6.50	0.39	0.31	19.07	33.74	67.32	68.65	6.38	9.29	16.77	17.30	36.14
8	90.00	290.00	75.70	25.40	7.17	5.98	6.09	6.90	0.67	0.31	16.25	26.73	70.76	66.96	6.03	8.44	16.60	14.82	36.84
9	85.00	280.00	69.00	23.96	0.40	6.29	6.05	6.51	0.28	0.30	11.33	27.61	76.28	68.96	6.70	10.75	14.70	17.76	17.54
10	90.00	290.00	76.18	25.56	9.04	6.15	6.09	7.19	0.39	0.40	15.49	23.89	67.59	71.02	5.22	11.93	14.72	16.65	29.38
11	97.07	290.00	59.27	19.89	14.36	6.20	6.10	6.69	0.36	0.27	9.97	18.19	69.4	68.57	7.41	12.33	17.32	16.12	35.53
12	95.00	280.00	60.92	21.15	8.16	5.61	6.09	6.43	0.58	0.21	15.31	28.62	59.25	69.79	6.68	11.57	13.12	17.61	40.23
13	90.00	275.86	41.08	14.47	7.10	6.20	6.07	6.57	0.63	0.16	14.20	30.53	65.64	61.32	7.37	11.12	17.48	14.97	31.72

Explanations: Ext .– extract; Abs – absorbed; Vol. – volume; TBARS – thiobarbituric acid reactive substances; Ref. – refrigeration; L* – Lightness; a* – redness; b* – yellowness; Aq. – aqueous; Ck. – cook; Ls – loss

Figure 1–7 shows the trend of influence irradiation time and solvent volume exert on meat quality. Figure 1 shows the response surface and contour plots derived from extract yield and meat solvent absorption. Extract yield was reduced as irradiation time and solvent volume increased. The volume of extract absorbed increased among extracts generated with 293 and 300 mL of distilled water and 85 min of irradiation time. Figure 2 shows the response surface and contour plots of pH of meat soaked in extracts on day (d) 0, 5 and 10 of refrigeration storage. Extracts produced from 91.25 min and 287.5 mL of irradiation time and solvent volume respectively lowered meat pH on d 0, but on d 5, pH was least at 85.5 min and 280.5 mL of time and solvent combination respectively. On d 10, meat pH of 6.5 was recorded for extracts produced with 282 mL and 85.5 min of solvent volume and irradiation time respectively. Figure 3 presents the response surface and contour plots for 2-thiobarbituric acid reactive substance value of meat of broiler chickens containing aqueous extract on d 5 and 10 of refrigeration storage. The TBARs on d 5 was lowered in sample containing extract generated below or beyond 85 and 95 min of irradiation, though highest at 90.5 min and 285 mL of irradiation time and solvent volume respectively; while oxidative rancidity measured reduced as exposure to irradiation extends beyond 95 min and solvent volume lowered beyond 280 mL of distilled water.

Response surface and contour plots of refrigeration and cook loss percentages of chicken meat soaked in white pepper aqueous extract on d 5 and 10 is presented (Figure 4). Refrigeration loss [%] of meat on d 5 was minimal for meat containing extract produced between 85.5 and 94.75 min of irradiation exposure as well as 282 and 284 mL of solvent volume; though on d 10, a combination of 287 mL of solvent and 94 min of irradiation exposure along with other set criteria yielded extracts that highly lowered meat pH.

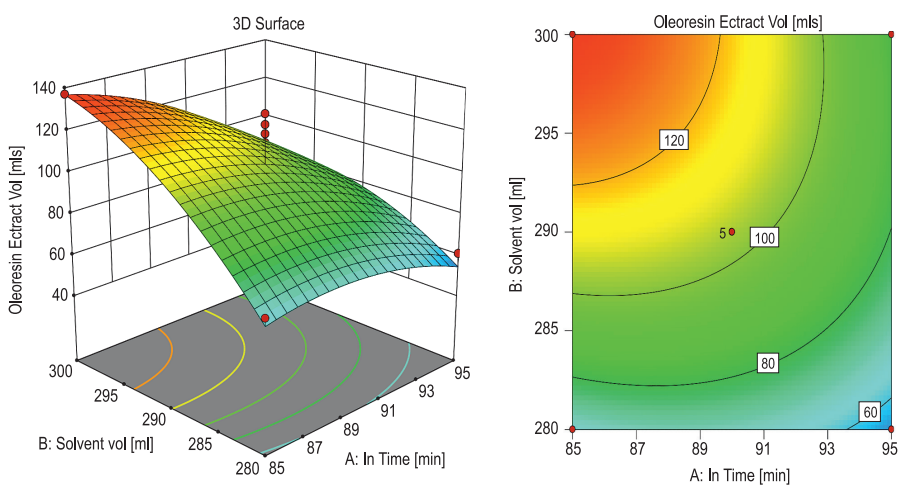

Fig. 1. Response surface and contour plots for extract yield and meat aqueous extract absorption

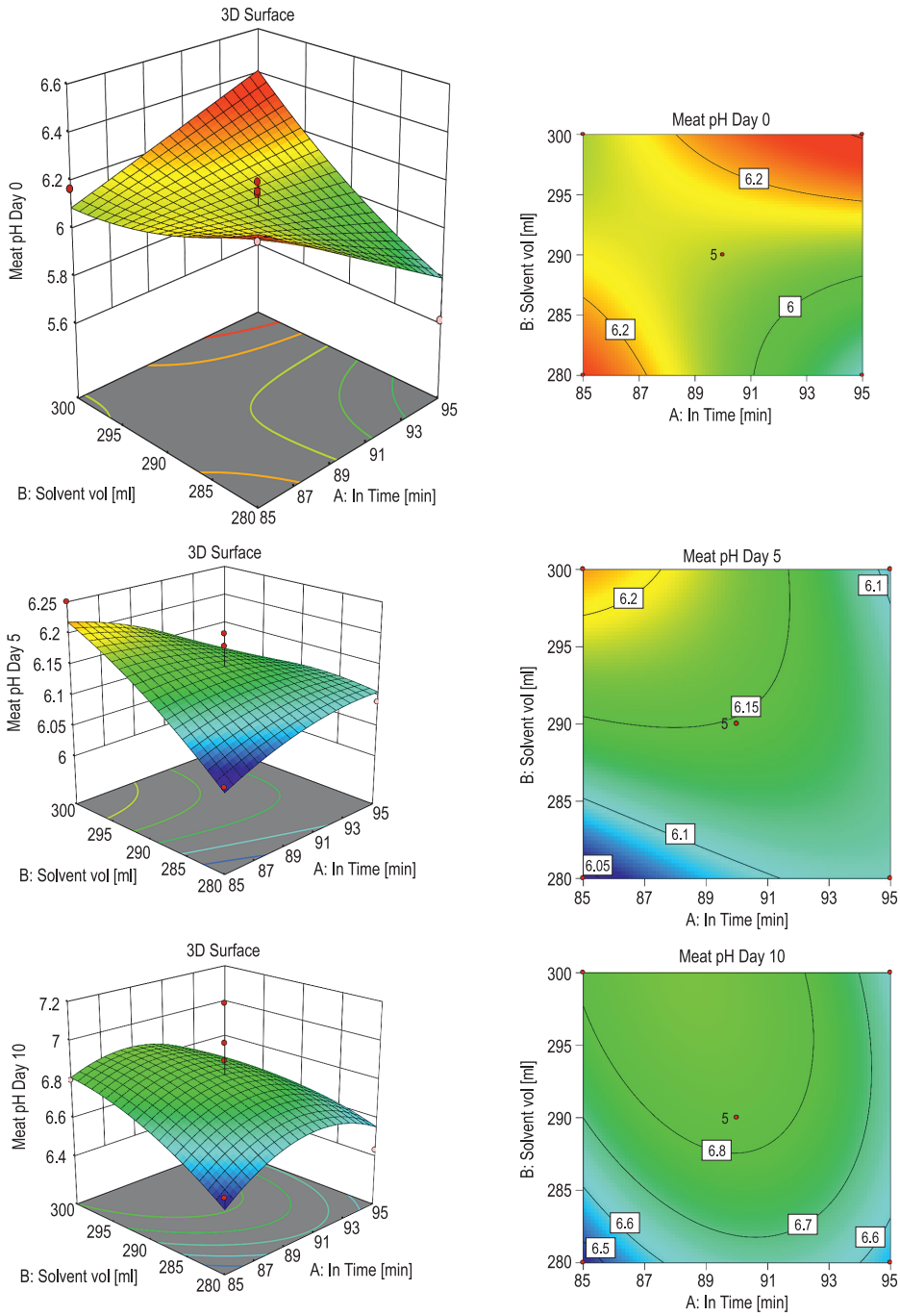

Fig. 2. Response surface and contour plots of pH of meat containing white pepper aqueous extract on d 0, 5 and 10 of storage (4ºC)

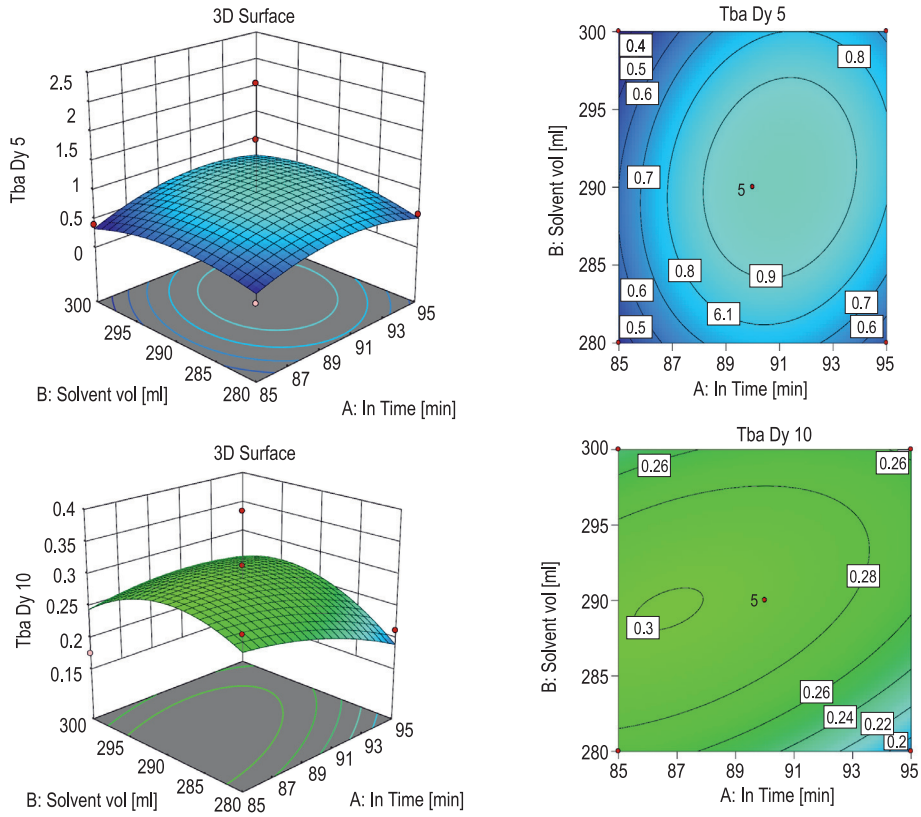

Fig. 3. Response surface and contour plots of 2-thiobarbituric acid value of meat containing extract on d 5 and 10 of refrigeration storage

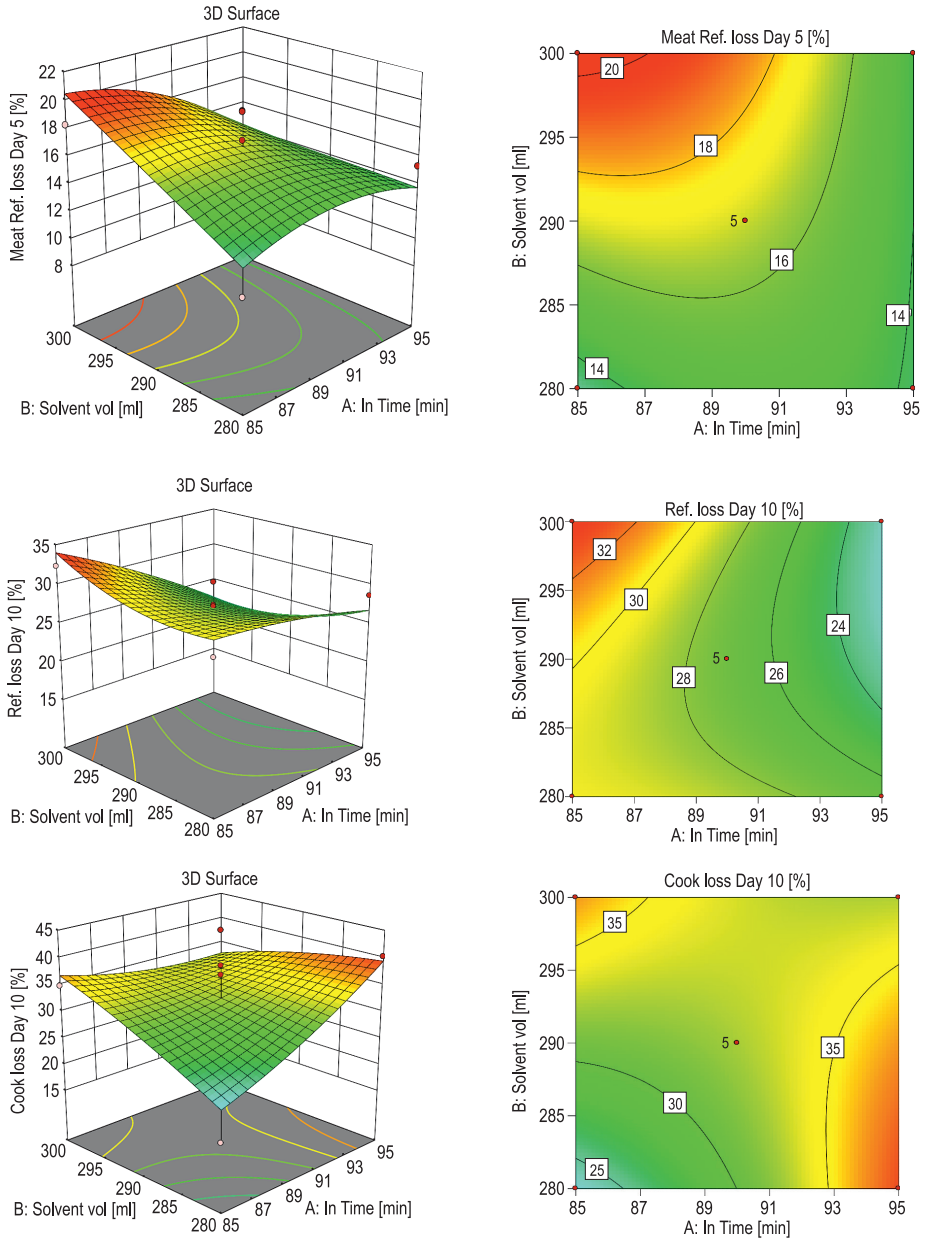

Fig. 4. Response surface and contour plots of refrigeration and cook loss of meat containing extract on d 5 and 10 of refrigeration storage

Cook loss [%] was very minimal for samples soaked in extract generated from 86.5 min and 282 mL of irradiation time and solvent volume respectively. Response surface and contour plots for Lightness (L^*) of meat containing extract on d 5 and 10 of refrigeration storage is shown (Figure 5).

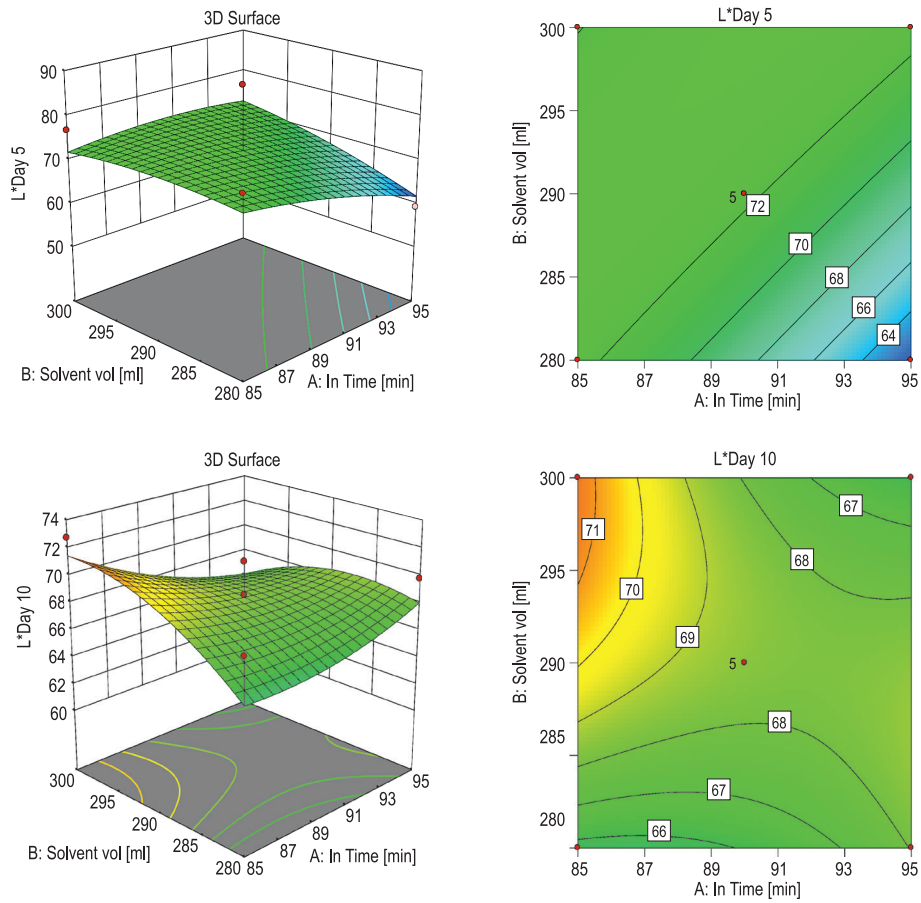

Fig. 5. Response surface and contour plots for L^* values of meat preserved with white pepper aqueous extract on d 5 and 10 of refrigeration storage

Meat L^* peaked among samples soaked in extracts produced from a combination of 90 min and 290 mL of irradiation time and solvent volume on d 5, but on d 10, samples soaked in aqueous extracts generated from 294 mL and 85.5 min of solvent volume and irradiation time respectively had the highest lightness value. Display of response surface graphs and contour plots to reveal redness (a*) value of meat after soaking in white pepper aqueous extract on d 5 and 10 is illustrated in Figure 6.

Extract generated from 94 min and 299 mL of irradiation time and solvent volume, alongside other set extraction criteria preserved meat redness, but on d 10, 91.5 and 94.5 min of irradiation time and 286 mL of solvent volume, alongside other factors yielded extracts with desirable redness, while yellowness (b^*) value of stored meat (Figure 7) was greater among samples preserved with extract generated with higher solvent volume alongside 90 min of irradiation, however, on d 10, b^* was highest among samples stored with extracts from 285.5 mL and 86.5 min of solvent volume and irradiation time respectively.

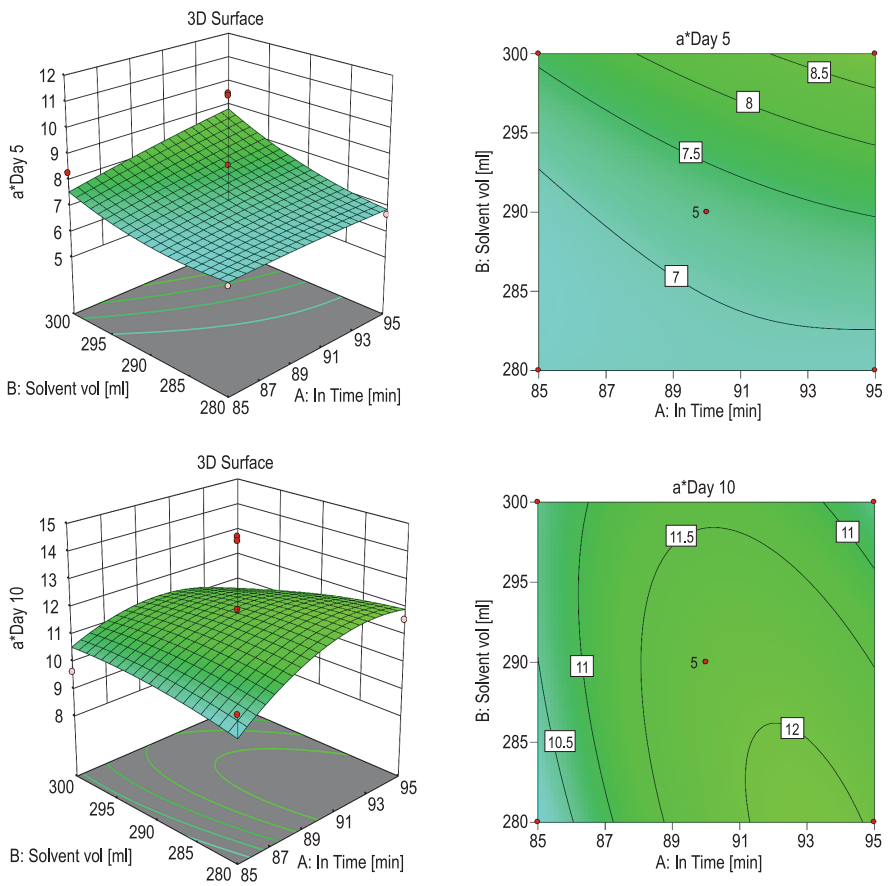

Fig. 6. Response surface and contour plots for a^* value of meat containing white pepper aqueous extract on d 5 and 10 of refrigeration storage

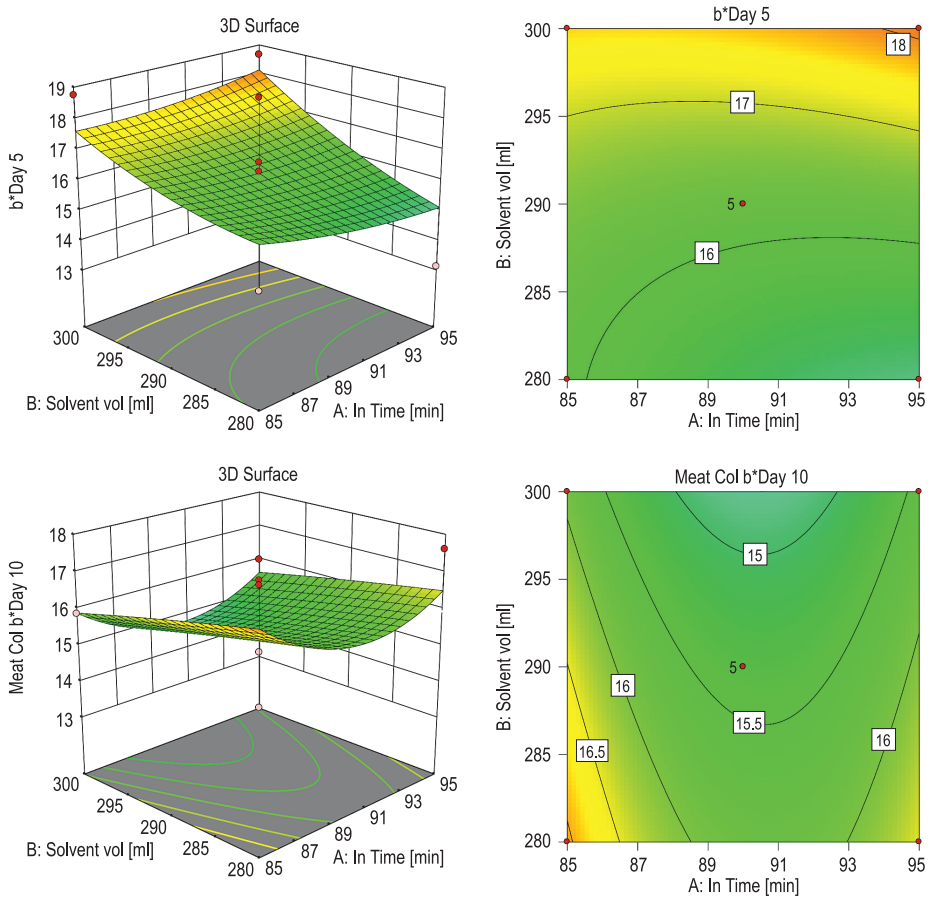

Fig. 7. Response surface and contour plots for b* value of broiler chicken meat containing white pepper aqueous extract on d 5 and 10 of refrigeration storage

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

Regression coefficient of models utilized for optimization procedure of white pepper aqueous extract is presented (Table 4). Significant ($p < 0.05$) parameters such as linear model for meat refrigeration loss percentage [%] on d 10; pH and refrigeration loss % on d 5 as well as 2FI (factor interaction) model for meat pH on d 0 were shown. Range of linear irradiation value was between -17.68 to 3.06 for aqueous extract volume and cook loss on d 10 were respectively documented.

Table 4

Regression coefficient of response parameters of white pepper aqueous extract applied as a function of independent variable

Specification	Intercept (β^0)	A – Irradiation time	B – solvent volume	AB	A^2	B^2	A^2B	AB^2	R^2
Aqueous Extract Vol	104.268	-16.2356	**24.9033***	-10.75	-8.3165	-11.1215	–	–	0.7539
p-values	–	0.0569	**0.0101***	0.3219	0.3129	0.1893	–	–	–
Aqueous Uptake	8.296	-0.557857	0.315572	-2.885	2.4145	-2.2405	–	–	0.4218
p-values	–	0.7575	0.8610	0.2788	0.2361	0.2683	–	–	–
Meat pH d 0	6.102	0.0282843	0.0459619	**0.21***	0.000875	0.053375	0.104038	-0.158284	0.6870
p-values	–	0.6336	0.4473	**0.0131***	0.9843	0.2625	0.2443	0.1010	–
Meat pH d 5	6.148	-0.0155178	**0.0403921***	-0.0475	-0.01775	-0.01525	–	–	0.6577
p-values	–	0.3631	**0.0391***	0.0733	0.3340	0.4023	–	–	–
Meat pH d 10	6.83	-0.0226624	0.106961	-0.0725	-0.1525	-0.065	–	–	0.3862
p-values	–	0.8108	0.2791	0.5915	0.1628	0.5274	–	–	–
TBARs d 5	0.9756	0.1048	0.0127651	0.067	-0.268987	-0.213737	–	–	0.1557
p-values	–	0.7235	0.9655	0.8725	0.4073	0.5062	–	–	–
TBARs d 10	0.2952	-0.0173817	0.0104914	0.023	-0.014225	-0.040225	–	–	0.2954
p-values	–	0.5416	0.7101	0.5673	0.6394	0.2088	–	–	–
Meat ref. loss Day-5	16.78	-1.47242	**1.8665***	-1.7175	-1.30625	-0.05875	–	–	0.6680
p-values	–	0.1019	**0.0485***	0.1646	0.1635	0.9461	–	–	–
Ref. loss d 10	27.116	**-3.57013***	0.0510203	-2.1475	-0.542375	1.67262	–	–	0.7392
p-values	–	**0.0072***	0.9588	0.1549	0.6118	0.1455	–	–	–
Cook loss d 10	32.616	3.06342	1.7445	-5.0025	1.322	-0.8405	–	–	0.4922
p-values	–	0.1710	0.4138	0.1216	0.5587	0.7079	–	–	–
L*d 5	72.286	-2.49105	2.56539	2.7975	-1.37175	-1.26925	–	–	0.2959
p-values	–	0.3642	0.3510	0.4662	0.6336	0.6588	–	–	–
L* d 10	68.468	-0.765392	0.933344	-1.9175	0.825375	-1.46962	–	–	0.5510
p-values	–	0.3935	0.3042	0.1513	0.3910	0.1476	–	–	–
a*d 5	7.258	0.32708	0.669173	0.3	-0.057125	0.345375	–	–	0.1613
p-values	–	0.6666	0.3883	0.7791	0.9437	0.6714	–	–	–
a*d 10	11.816	0.493651	-0.196713	-0.4975	-0.83175	-0.21425	–	–	0.1644
p-values	–	0.5837	0.8255	0.6945	0.3968	0.8228	–	–	–
b* d 5	16.25	-0.111523	1.12981	0.3725	0.183125	0.303125	–	–	0.3174
p-values	–	0.8723	0.1351	0.7055	0.8059	0.6853	–	–	–
Meat b* d 10	15.348	-0.212347	-0.514032	0.0675	0.951625	-0.028375	–	–	0.4253
p-values	–	0.6617	0.3055	0.9211	0.0980	0.9562	–	–	–

Explanations: * – significant model terms; Irr – irradiation; TBARs – 2-thiobarbituric acid value; d – day; Ref. – refrigeration; L* – lightness; a* – redness; b* – yellowness

Also, linear model for solvent volume ranged between -0.197 and 26.57 for meat L* and aqueous absorption values on d 10 of storage respectively, similarly as 2FI model for aqueous extract volume and meat pH on d 0. Meat quadratic model was not significant ($p > 0.05$) but ranged between -8.317 and 2.415 for extract volume and absorption percentage, while quadratic model for solvent volume for extract volume and refrigeration loss were -11.122 and 1.67 on d 10 of storage. R^2 values (coefficient of determination) was 0.1557 for TBARs on d 5 and 0.7392 for refrigeration loss on d 10.

Responses suggested for the optimization of *Piper nigrum* aqueous extract assessment

A total of twelve (12) solutions were proffered by RSM (Table 5), with a desirability range of 0.587–0.604 or 58.7–60.4%. To prepare extract of suggested desirability, 91.19 min of irradiation time and 280 ml of solvent volume were conditioned alongside 300 W of microwave power and *P. nigrum* powder screened at 0.40 mm.

Gas chromatography-mass spectrometry of optimized white pepper aqueous extracts

The GC-MS result for optimized white pepper aqueous extract of best desirability is reported (Table 6). A total of 41 compounds were identified. One silicon and sulphur-based compound was gotten. Stearic acid, iso-octyl phthalate and Bis (2-ethylhexyl) phthalate were present in high proportion than all other compounds, followed by 2-phenylethanol–a terpenoid. Dibutyl phthalate present was moderately abundant alongside *Phthalic acid and butyl hexyl ester. Next is palmitic acid, then* Octamethylcyclotetrasiloxane (D4) and terpenoid alkaloids such as γterpinene, Terpinen-4-OL and α-terpineol. Flavonoids such as eicodecene and octadecanoic acid were present. As shown in Figure 8, least quantity of 0.74 for p-nitrobenzaldehyde pale in area compared to highest value of 24.65 for isoctyl phthalate and bis (2-ethylhexyl) phthalate.

Table 5

Solutions for numerical optimization of responses using white and black pepper aqueous extract on broiler chicken meat

Number	Time	Solvent vol.	Aqueous extract vol.	Ext. Abs.	Meat pH D 0	Meat pH D 5	Meat pH D 10	TBARS D 5	TBARS D 10	Meat ref. loss D 5	Ref. loss D 10	Cook loss D 10	L* D 5	L* D 10	a*- D 5	a* D-10	b* D 5	Meat Col b* D 10	Desirability
1	91.188	280.000	66.470	6.429	5.995	6.099	6.661	0.743	0.234	14.839	28.369	32.022	67.117	66.385	6.937	11.987	15.319	15.821	0.604
2	91.151	280.000	66.539	6.404	5.997	6.099	6.661	0.744	0.234	14.842	28.381	31.958	67.161	66.374	6.937	11.983	15.322	15.820	0.604
3	91.139	280.000	66.561	6.396	5.998	6.099	6.661	0.744	0.235	14.843	28.385	31.938	67.175	66.370	6.937	11.981	15.323	15.819	0.604
4	91.241	280.000	66.370	6.466	5.993	6.099	6.661	0.742	0.234	14.835	28.351	32.114	67.054	66.402	6.937	11.993	15.314	15.823	0.604
5	91.091	280.000	66.650	6.363	6.001	6.098	6.662	0.745	0.235	14.846	28.401	31.854	67.232	66.356	6.937	11.975	15.326	15.818	0.604
6	90.960	280.000	66.883	6.276	6.007	6.098	6.662	0.746	0.236	14.854	28.444	31.629	67.385	66.317	6.937	11.958	15.337	15.815	0.603
7	91.450	280.000	65.952	6.618	5.982	6.100	6.660	0.737	0.232	14.816	28.279	32.482	66.802	66.469	6.937	12.016	15.298	15.833	0.603
8	91.507	280.000	65.836	6.660	5.979	6.100	6.659	0.736	0.231	14.810	28.260	32.582	66.733	66.487	6.937	12.022	15.294	15.836	0.603
9	90.771	280.000	67.200	6.156	6.017	6.097	6.662	0.749	0.238	14.861	28.505	31.306	67.604	66.262	6.937	11.931	15.353	15.813	0.603
10	91.611	280.000	65.613	6.741	5.973	6.101	6.658	0.733	0.230	14.798	28.223	32.768	66.605	66.522	6.937	12.032	15.286	15.842	0.603
11	91.838	280.000	65.104	6.921	5.962	6.102	6.656	0.727	0.228	14.768	28.141	33.174	66.322	66.600	6.936	12.050	15.270	15.859	0.600
12	92.660	280.000	62.971	7.661	5.919	6.104	6.641	0.693	0.219	14.615	27.827	34.696	65.250	66.912	6.932	12.090	15.218	15.954	0.587

Explanations: Ext. – extract; Irr – irradiation; TBARS – 2-thiobarbituric acid reactive substances; Day – D; Ref. – refrigeration; Ab – absorption; L* –- lightness; a* – redness; b*– yellowness; Vol. – volume; Col – colour

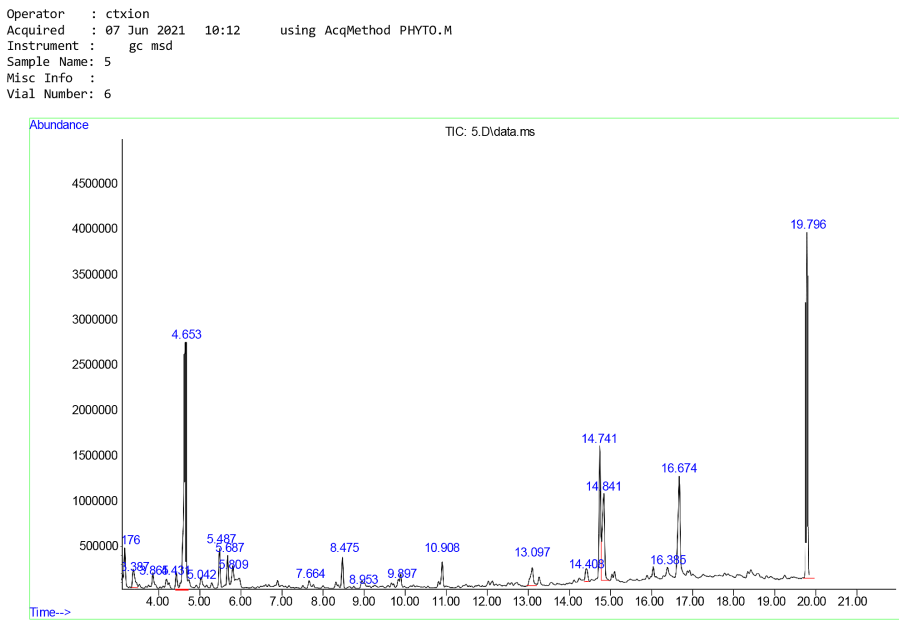

Fig. 8. Spectrometry of white pepper exctact (suggested desirability)

Table 6

Gas chromatography mass spectrometry of components of white pepper aqueous extract

Component	Molecular formula	Retention time [min]	Area [%]
1	2	3	4
Octamethylcyclotetrasiloxane (D4)	$C_8H_{24}O_4Si_4$	3.176	2.57
Hemellitol	C_9H_{12}	3.387	2.09
Pseudocumene	C_9H_{12}	3.387	2.09
Mesitylene	C_9H_{12}	3.387	2.09
3-Carene or limonene	$C_{10}H_{16}$	3.865	1.42
(E)-ß-ocimene	$C_{10}H_{16}$	3.865	1.42
Hendecane	$C_{11}H_{24}$	4.431	1.17
2-phenylethanol	$C_8H_{10}O$	4.653	21.60
α-terpineol	$C_{10}H_{18}O$	5.042	1.07
3-Ethyl-5-methoxy-1,3,4-oxadiazol-2 (3H)-one	$C_5H_8N_2O_3$	5.042	1.07
α, α-Dimethylcyclopentanemethanol	$C_8H_{16}O$	5.042	1.07
γ-terpinene	$C_{10}H_{18}O$	5.487	2.46

cont. table 6

1	2	3	4
Terpinen 4 OL	$C_{10}H_{18}O$	5.487	2.46
α-terpineol	$C_{10}H_{18}O$	5.687	2.25
(L)-alpha-Terpineol	$C_{10}H_{18}O$	5.687	2.25
a-Terpineol acetate	$C_{12}H_{20}O_2$	5.687	2.25
Dodecane	$C_{12}H_{26}$	5.809	1.52
N-Tridecane	$C_{13}H_{28}$	5.809	1.52
Heptdecane	$C_{17}H_{36}$	5.809	1.52
p-nitrobenzaldehyde	$C_7H_5NO_3$	7.664	0.74
n-tetradecane	$C_{14}H_{30}$	8.475	2.09
n-pentadecane	$C_{15}H_{32}$	8.475	2.09
p-(methoxymethyl)phenol	$C_8H_{10}O$	8.953	0.89
4-(2-hydroxyethyl) phenol	$C_8H_{10}O$	8.953	0.89
2,4-di-tert-butylphenol	$C_{14}H_{22}O$	9.879	0.81
2,5-bis(1,1-dimethylethyl) phenol	$C_{14}H_{22}O$	9.879	0.81
Cetane	$C_{16}H_{34}$	10.908	1.77
Hexacosane	$C_{26}H_{54}$	10.908	1.77
Octadecane	$C_{18}H_{36}$	13.097	2.46
Nonadecane	$C_{19}H_{40}$	13.097	2.46
Methyl palmitate	$C_{17}H_{34}O_2$	14.408	1.40
Dibutyl phthalate	$C_{16}H_{22}O_4$	14.741	9.42
Phthalic acid, butyl hexyl ester	$C_{18}H_{26}O_4$	14.741	9.42
Dibutyl phthalate	$C_{16}H_{22}O_4$	14.741	9.42
n-Hexadecanoic acid (palmitic acid)	$C_{16}H_{22}O_2$	14.841	8.13
Cyclopentadecane	$C_{16}H_{32}O_2$	16.358	1.12
1-Octadecene	$C_{15}H_{30}$	16.358	1.12
Cycloeicosane	$C_{18}H_{36}$	16.358	1.12
Octadecanoic acid (stearic acid)	$C_{20}H_{40}$	16.674	10.50
Isooctyl phthalate	$C_{18}H_{36}O_2$	19.796	24.65
Bis (2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	19.796	24.65

Discussion

From the response surface and contour plots results, it could be deduced that extract yield increases as irradiation time and solvent volume increases. This agrees with TUSHAR et al. (2017), who observed increase in the yield of oil as contact time and solvent volume increased; possibly due to increased movement or kinetics among particles accompanied by refluxation within the microwave cavity. Also, the volume of extract absorbed by meat increased as irradiation time and solvent volume increased since microwave heating at the designed conditions generally limit thermal decomposition of soluble compounds (VENTURA et al. 2017), thus increasing the availability of solute. The data obtained for meat pH for all days of refrigeration storage showed that the application of higher irradiation time and solvent volume resulted in production of extract of high acidity but the potency was short-lived compared with least pH values of 6.05 and 6.50 on d 5 and 10 generated using 85.5 min. In a study conducted by ISMAIL-SUHAIMY (2021), it was observed that increase in extraction time and microwave power caused a decrease in flavonoid yield; implying lower extraction time favors extraction of bioactive and thermal-stable compounds. Notably, those values were obtained using almost the same solvent volume (281 and 282 mL respectively) as this study, though lower than the 287.5 mL required to generate extracts that best-lowered meat pH on d 0. The same trend was observed for meat TBARs value on d 5, refrigeration loss, cooking loss and meat lightness value. These parameters were best minimized at 85.5 min of irradiation time, while refrigeration loss was best-minimized for samples containing extracts generated from 86.5 min on d 5 of storage. On the contrary, meat L^* was increased at 85.5 min of extraction. From the studies of CHEN (2015) and HABEEBULLAH et al. (2020), selectivity of extraction, biological strategies present and increased bioactive components obtained provides a wide range of active principles that minimizes the extent of spoilage. Meat a^* and b^* values were higher after soaking in extracts generated from higher extraction time (92.5–95 min) and solvent volume, yet, it was observed that as days of storage progressed, lower irradiation time and solvent volume yielded higher a^* and b^* values.

From the regression analysis table, increased extract volume correlates to increased exposure of absorbing surface to irradiation. Meat pH was significantly affected on d 10 possibly from the combination of irradiation time and solvent volume at low levels. As exposure to irradiation and extraction time extend, an associated risk of degeneration of thermolabile constituents exists (AL-HARAHSHEH and KINGMAN 2004). Therefore,

beyond threshold, extract obtained will be of low quality. This supports the report published by OLALERE et al. (2018), whose study reveal oleoresin harvested decreased beyond 120 min of irradiation exposure. Similarly, meat quality on d 10 reveal TBARs was minimal among samples containing extracts prepared from irradiation time beyond 95 min and solvent volume below 280 mL. Thermal and chemical degradation by hydrolysis, transesterification, or oxidation products controlled by rapid heating is induced by microwaves subjected to limited water content (CHAN et al. 2011, FERHAT et al. 2006, SOZMEN et al. 2012). SHASHIKANT and MAYUR (2019) affirm this by stating that the moisture/water present in heated matrix can strongly influence microwave absorption as it supports compound extraction by modifying the polarity of solvent or water applied.

Result obtained reveal irradiation time and solvent volume significantly affected meat pH on d 0 of refrigeration. White pepper extract generated using 286 mL of distilled water and 92 min of irradiation produced extracts that resulted in lowered meat pH. Extraction conditions highly favour increased acidity, corresponding to lowered pH in meat. More solvent volume effectively increased meat pH on d 5 of refrigeration. KLONT (2005) and MARTINS et al. (2018) explained that post mortem metabolism (glycolysis) and glycogen conversion into lactic acid yields highest quality products that tend to fall within a pH range of 5.7 and 6.0. Both the rate and extent of reduction of meat pH post-mortem influences meat quality characteristics. Increase in solvent volume reduces acidity levels of white pepper aqueous extract that subsequently affect meat pH value. BHATTARAI et al. (2013) reported that concentration dependence of molar volumes appears to be negligible over the entire concentration range if the molar volume remains constant. The pH of meat influences its water holding capacity (WHC)–a quality parameter closely related to product yield and quality. If lower pH values result from lower solvent volume, then the posit above indicate lower solvent volume should translate into lower refrigeration loss in meat. In this study, refrigeration loss decreased as the irradiation time increased. OSMIĆ et al. (2019) reported that excessive time and temperature of extraction negatively influence yield of total phenolics, flavonoids and anti-oxidant compounds of sage extracts. This implies that increased irradiation time likely had negative impact on the yield of bioactive substances from *P. nigrum*. RAMAN and GAIKAR (2002) affirmed that high microwave powers of 300 and 450 W increased solvent loss by 16–20%.

Conclusion

From this study, criteria to optimize extraction of functional compounds in white pepper require eight grams of white pepper; 300 W of microwave power; sieve size of 0.40 mm; 91.188 min of irradiation time and 280 mL of solvent volume. Though a total of twelve solutions were suggested, a desirability value of 0.604 was recommended for five solutions, but the GC-MS analysis of the best suggested extract reveal forty-one compounds were present–a remarkable improvement compared to outcomes of microwave extraction reported. Bio-compounds such as 2-phenylethanol, bis (2-ethylhexyl) phthalate and iso-octyl phthalate are compounds with the highest amounts extracted as seen from the GC-MS analysis carried out.

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