Available online at www.elixirpublishers.com (Elixir International Journal)

Food Science

Elixir Food Science 131 (2019) 53337-53343

ISSN: 2229-712X

Influence of Some Local Spices on Chemical and Antioxidant Properties of Hot-Smoked African Catfish *(Clariasgariepinus)*

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ARTICLE INFO

Article history:

Received: 24 April 2018;

Received in revised form: 19 June 2019;

Accepted: 29 June 2019;

Keywords

Spices,
Catfish,
Optimization,
Chemical and Antioxidant Properties,
Preservative.

ABSTRACT

Fish constitutes a very important component in the diets of both man and animals as it provides the much needed protein and other nutrients for a healthy living however, it is prone to rapid putrefaction and spoilage. In this study, local spices were applied on hot-smoked African catfish for its preservative tendencies using Response Surface Methodology of Design Expert 6.0.8.Five independent variables consisting of soaking times of 0.0, 7.50, 15.00, 22.50 and 30.0 min; soaking temperatures (30, 35, 40, 45, 50^{o}C); garlic, ginger and turmeric with concentrations of 0.0, 2.50, 5.00, 7.50 and 10.0 g/100ml respectively were investigated. Predictive models for the response variables were developed as a function of process variables. A second-order polynomials obtained to predict the response variables were all significant ($p<0.05$) with good correlation coefficients (R^2) between 0.869 and 0.999 showing that the models can be used to navigate the design space. Selection of the best (optimum) combination of garlic, ginger and turmeric concentrations, extraction temperature and extraction time of 7.29 g/100ml, 7.50 g/100ml, 2.5 g/100ml, 38.65 ^{o}C and 7.51 min respectively produced optimized responses in spiced smoked catfish with CP, ash, TP, FRAP, MC, fat and FFA of 65.20%, 8.59%, 2.45 mg/100 g, 20.24 mg/100 g, 2.20%, 11.17% and 0.72% respectively. This successfully established the synergistic effect of local spices on the chemical and antioxidant properties of the hot-smoked catfish.

1. Introduction

Fish forms a major part of human diet, and serves as a source of high quality protein, essential vitamins and healthful polyunsaturated fatty acids (Özpolatet al., 2014).Fish is the most valuable and most consumed aquatic food item by man. Fish makes up about 60% of world protein supply and developing countries derive more than 30% of their annual protein from fish (Tyokumbur and Awobode, 2014). In Africa, over 17.5% of the animal protein comes from fish while in Nigeria; fish constitutes over 40% of animal protein intake of the people (Daramolaet al., 2014) and 37% of protein of all sort consumed (Agbabiakaet al., 2012). Nigeria is highly endowed with both freshwater and marine fishes; however, catfishes appear to be more commercially important among the available freshwater fishes.

African catfish is of great commercial importance in Nigeria, because it is the most common freshwater fish widely cultivated and consumed among Nigerians (Olaifa et al., 2004; Ogundiranet al., 2007). Due to poor preservative culture, processing methods and lack of appropriate fish preservation facilities, fish spoils so easily between 16-36 h after harvest (Daramolaet al., 2014). To avoid this, fish farmers and fishermen sell their products at ridiculously low prices leading to poor economic status of these peasants. Also, about 50% of the total fish caught in Nigeria is lost to spoilage due to lack of appropriate preservation (Tyokumbur and Awobode, 2014). In order to avoid fish spoilage, they are smoked after harvesting.

Smoking of fish is one of the most ancient processing technologies. About 60% of fish caught in Nigeria inland waters are preserved by smoke curing (Daramolaet al., 2014). Smoking has been used for centuries for preservation, and is still widely used for this purpose among several communities in the third world (Ward, 1995; Daramolaet al., 2014). Smoking permits lengthy preservation by removing moisture, which aids bacteriological and enzymatic spoilage. Traditionally, smoked fish has a limited shelf-life and several studies have been conducted on the potential of spice in organoleptic enhancement.

Spices are known for their nutritional and medicinal values. The utilization of combinations of more than two spices, variation of temperature and time of soaking in the spice extract before smoking have not been widely exploited in Nigeria. Studies on synergetic influence of different spices on nutrient profiles are scanty. Hence, this study therefore, sought to evaluate the synergetic influence of ginger, garlic and turmeric on the chemical and antioxidant properties of hot-smoked catfish with the application of Response Surface Methodology (RSM).

2. Materials and Methods

Life adult catfishes were purchased from Owena in Ondo State, while ginger, garlic and turmeric were purchased from a local market in Ado Ekiti, Nigeria. All reagents used were of analytical grade.

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2.1Methods

2.1.1 Preparation of Samples

Dried ginger, garlic and turmeric samples were thoroughly cleaned and further dried in the cabinet drier at 50 oC until constant weight were obtained (Dinstel, 2013). The dried spices were milled separately using coffee mill and sieved to pass through a mesh size of 250 µm before packaging in air tight polythene bags until needed for experiment. The levels of concentrations (0, 2.50, 5.00, 7.50, 10.00 g/100 ml) of each of the spices were combined randomly using the Design Expert 6.0.8. The cleaned eviscerated whole fish was soaked in the spice extract, at the specified contact time and temperature following the Coded level and real values for the fractional CCRD experimental design Table 1. The proportion of catfish to spice extract used was 1:4. The spiced soaked catfish was allowed to drain for about 5min before smoking in a smoking kiln with firewood, at a temperature of 80 ^oC for 7 h (Abdel-Hamied*et al*., 2009). The spiced smoked catfish was cooled and stored at ambient temperature (30 ± 2°C) until needed for analysis. The spiced smoked catfish were analyzed for Moisture Content, Crude Protein, Fat, Ash, Free Fatty Acids, Total Phenols, and Ferric Reducing Antioxidant Power.

2.1.2 Experimental design

The effects of the five independent variables; X_1 (garlic), X_2 (ginger), X3 (turmeric), X_4 (Extraction temperature) and X_5 (Extraction time) on the dependent (responses) variables; Y_1 (Moisture Content, MC), Y_2 (Crude Protein, CP), Y_3 (Fat), Y_4 (Ash), Y_5 (Free Fatty Acids, FFA), Y_6 (Total Phenols, TP) and Y_7 (Ferric Reducing Antioxidant Power, FRAP) were evaluated using the RSM as summarized in Table 2 in coded terms. Models were generated and fitted to a second order polynomial equation while regression analysis and analysis of variance at ($p<0.05$) level of significance were used to determine fitness of the models. Design – Expert (version 6.0.8) (Stat-Ease Inc., Minneapolis, U.S.A) was used for the experimental design and data analysis.

Table 1. Coded level and real values for the fractional CCRD experimental design

Independent variable	Coded variable	Coded levels and real values				
		-2	-1	0	+1	+ 2
Garlic (g/100 ml)	X_1	0.0	2.5	5.0	7.5	10.0
Ginger (g/100 ml)	X_2	0.0	2.5	5.0	7.5	10.0
Turmeric (g/100 ml)	X_3	0.0	2.5	5.0	7.5	10.0
Extraction Temperature (^oC)	X_4	30	35	40	45	50
Extraction Time (min)	X_5	0.0	7.5	15	22.5	30.0

Table 2. Experimental design and results obtained for chemical and antioxidant properties

[a]Run no	Input/independent variables[b]					Response/dependent variables[c]						
	X_1	X_2	X_3	X_4	X_5	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7
1	2	0	0	0	0	2.65	58.74	16.61	7.00	1.13	1.69	9.9
2	1	-1	-1	-1	1	2.99	63.78	13.39	8.77	0.85	1.78	17.42
3	1	-1	-1	1	1	3.82	62.6	18.48	8.95	0.85	2.02	12.16
4	0	2	0	0	0	2.73	55.27	16.26	9.23	0.85	2.85	36.9
5	-1	-1	-1	-1	-1	3.35	64.52	17.72	8.66	0.85	1.3	9.49
6	0	0	0	-2	0	2.65	65.18	20.18	7.09	0.85	1.13	0.79
7	0	0	0	0	0	2.91	64.52	15.61	9.26	0.85	0.93	2.92
8	0	0	0	0	-2	2.33	61.71	18.69	7.74	1.13	0.9	10.17
9	0	0	-2	0	0	2.77	64.38	12.30	9.56	0.85	1.47	5.96
10	-1	-1	-1	-1	-1	3.03	61.14	21.61	8.99	0.85	0.53	3.42
11	-2	0	0	0	0	3.35	65.51	13.54	8.32	0.85	1.59	15.2
12	-1	-1	-1	-1	-1	2.65	57.11	19.21	8.43	1.13	2.32	12.61
13	0	-2	0	0	0	3.19	64.30	10.12	8.38	1.13	1.7	7.5
14	-1	-1	-1	1	-1	2.32	55.25	19.04	8.16	0.85	1.59	6.55
15	-1	-1	-1	-1	1	2.50	55.93	21.52	6.97	1.42	2.26	11.66
16	0	0	0	0	0	3.29	67.28	12.71	8.29	1.42	2.34	36.94
17	-1	-1	-1	-1	1	3.77	50.62	22.42	7.54	0.81	1.78	16.78
18	-1	-1	-1	1	1	3.1	58.74	21.23	7.62	1.13	0.77	9.17
19	-1	-1	-1	1	1	3.2	59.57	19.60	7.59	1.42	0.67	27.97
20	-1	-1	-1	-1	1	2.87	60.45	22.84	8.55	0.85	1.59	19.95
21	-1	-1	-1	1	1	2.59	57.22	19.56	7.36	1.42	1.32	8.9
22	0	0	0	0	-2	2.91	55.3	26.37	7.01	1.13	0.89	15.69
23	-1	-1	-1	1	1	2.87	64.49	16.34	7.43	1.42	1.37	12.61
24	0	0	2	0	0	4.01	53.97	21.42	8.68	0.85	1.72	10.94
25	-1	-1	-1	1	-1	4.98	64.5	10.03	9.14	0.85	1.91	32.77
26	0	0	0	2	0	3.18	60.88	14.12	7.28	0.85	1.41	23.67
27	-1	-1	-1	1	-1	3.22	68.14	11.17	7.82	0.85	1.58	19.73
28	-1	-1	-1	1	1	3.27	65.61	11.82	8.26	0.85	1.7	19.68

[a]Did not necessarily correspond to the order of experiment.

[b]X_1=Garlic, X_2= Ginger, X_3= Turmeric, X_4 = Soaking temperature, X_5 = Soaking time

[c]Y_1 = Moisture content, Y_2 = Crude protein, Y_3 = Fat, Y_4 = Ash, Y_5= FFA, Y_6 = Total phenol, Y_7 =FRAP

These responses were in the range:2.32-4.78%, 50.62-68.14%, 10.03-26.37%, 6.97-9.65%, 0.81-1.42%, 0.53-2.85 mg/g and 0.79-36.94 mg/g for moisture, crude protein, fat, ash, FFA, total phenol and FRAP respectively.

2.1.3 Chemical Analysis of Smoked Catfish

Chemical analyses conducted on the nutrient composition of the smoked catfish were Moisture Content, Crude Protein, Fat, Ash, Free Fatty Acid, Total Phenol and Ferric Reducing Antioxidant Power using standard procedures of AOAC (2005).

2.1.4 Optimization procedure and validation of data

Numerical optimization techniques were used on the multiple responses subject to maximum CP, Ash, TP and FRAP and minimum MC, fat and FFA as constraints. The best Spiced Smoked Catfish was selected from the series of suggested optimized product species input variables of the Design Expert software and were used to experimentally re-produce the desired smoked spiced catfish.

2.1.5 Sensory Evaluation

The optimized, unspiced (control) and commercial samples of catfish were presented in coded forms to 75 panellists. The samples were evaluated using a 7-point hedonic scale ranked between extremely like and extremely dislike for 1 and 7 points scale respectively. Each sample was assessed for taste, texture, appearance, after taste, aroma and general acceptability in clean foil paper. Each sample assessment by the panellists were preceded with mouth wash and rinsing to have unbiased judgement.

2.1.6 Statistical Analysis

Statistical analysis of all experiments carried out in the laboratory was done using Analysis of Variance (ANOVA) in Design Expert software (version 6.0.8), while sensory evaluation results were analysed using SPSS (version 20).

3. Results and Discussion

3.1 Moisture Content of Spiced Smoked Catfish

The relationship between the independent variables and moisture content (Table 2) is described by Equation 1. The significant model terms were X_3 (turmeric), X_4 (soaking temperature) and interaction terms for garlic and ginger (X_1X_2), garlic and temperature (X_2X_4), ginger and time (X_2X_5), temperature and time (X_4X_5).

$$\text{Moisture (coded)} = +0.47-0.023X_1 - 0.019X_2+0.061X_3 + 0.018X_4-9.892X_5+0.012X_1X_2-0.043X_1X_3-0.022X_1X_4+0.020X_1X_5+2.094X_2X_3+ 0.074X_2X_4+5.834X_2X_5-0.035X_3X_5-8.000X_3X_5+ 1.989X_4X_5 \quad (1)$$

Analysis of variance for each variable showed that a significant effect ($p<0.05$) was found for moisture content for which X_3, X_4, X_1X_2, X_1X_5, X_2X_3, X_2X_4,X_2X5 and X_4X_5 exhibited positive effect while negative interaction terms was seen for X_1, X_2, X_1X_3, $X1X_4$, X_3X_5 from the equation. The positive interaction terms of garlic, ginger, turmeric, temperature and time reduces the moisture content. The moisture content ranged from 2.32% to 4.78 with an average value of 3.08%. The average moisture content of spiced smoked catfish was lower than the value (6.95%) reported by Oluborode *et al.* (2014). Oyelese, (2014) reported that the moisture content of fish subjected to different treatments decreased after smoking from 6.95 to 4.93%. The lower values recorded in this present study might be attributed to the effect of the spice addition and possibly the difference in thecondition of smoking. The moisture content of smoked catfish is of great importance during storage, the lower it is the better the storability.

3.2 Crude Protein of Spiced Smoked Catfish

The relationship between the independent variables and crude protein is summarizedin Equation 2. The positive interaction terms signify a synergistic effect and a negative sign signifies an antagonistic effect. The negative quadratic effect of temperature and time implies that further increase in extraction temperature and time has the tendency to reduce the protein content of the spiced smoked catfish.

$$\text{Crude protein (coded)} = +1.92- 0.017X_1- 0.013X_2-8.082X -3.729X_4- 0.022X_5 - 6.833X_1^2- 0.011X_22- 6.981X_32-5.166X_42- 0.017X_52- 0.012X_1X_2- 0.015X_1X_3- 0.012X_1X_4-9.992X_1X_5- 0.011X_2X_3- 7.868X_2X_4- 0.020X_2X_5- 0.015X_3X_4+ 0.012 X_3X_5- 6.570X_4X_5 \quad (2)$$

The highest crude protein recorded in the spiced smoked catfish was 68.14%. Previous studies reported protein content (62.14%) in smoked catfish (Aladetoun *et al.*,2009). The crude protein ranged from 50.62%to 68.14% with average of 60.95%. The protein content in this research was found to be higher than the protein content reported by Aladetoun *et al.* (2009) for smoked catfish without spices. Increase in the crude protein content of the spiced smoked catfish shows that the spices contributed positively to the protein content (Ndimele *et al.,* 2011). This is in line with the report of Pannevis (2003), indicating that smoked *Clarias gariepinus* is a good source of protein.

3.3 Fat content of Spiced Smoked Catfish

The relationship between the independent variables and fat indicated that spices have the potential of reducing fat content in the smoked catfish as shown in Equation 3.

$$\text{Fat(coded)}=+17.23+0.93X_1+1.39X_2+0.68X_3-3.34X_4+1.69X_5+0.52X_1^2-0.067X_2^2+0.11X_3^2+2.08X_4^2+2.85X_5^2+0.73X_1X_2+2.51X_1X_3+0.96X_1X_4-0.18X_1X_5+0.85X_2X_3-.55X_2X_4+1.49X_2X_5 -2.09X_3X_4-2.75X_3X_5+0.80X_4X_5 \quad ...(3)$$

The fat content of the spiced smoked catfish reduced from 26.37 to 10.03% with an average value of 17.28%. The average value reported in this study (falls within the same range (17.16 to 39.01%) of value reported by Adefemi (2010). According to Bankole (2013) and Olapade*et al.* (2011), *Clariasgariepinus* belong to relatively low fat fish species.

3.4 Ash content of Spiced Smoked Catfish

The relationship between independent variables and ash content is shown in Equation 4 and Figure 1. The significant model terms were X_2 (ginger), X_3 (turmeric), X_4 (time), interaction of X_2X_4 (ginger and temperature), X_3X_4 (turmeric and temperature), X_4X_5 (temperature and time) and quadratic term ofX_3^2 (turmeric and ginger) exhibited a positive effect.

$$\text{Ash (coded)} =+1.14 - 0.018X_1+ 0.010X_2 + 4.883X_3 + 3.458X_4 -0.011X_5 -8.697X_1^2+ 6.675X_2^2+ 0.018X_3^2- 0.015X_4^2-0.013X_5^2- 0.018X_1X_2- 0.012X_1X_3 - 4.071X_1X_4 - 4.841X_1X_5-0.011X_2X_3+8.466X_2X_4+0.012X_2X_5+2.453X_3X_4-1.636X_3X_5+0.012X_4X_5 \quad ...(4)$$

This indicates that increasing these independent variables will increase the ash content in the smoked fish. As shown in Figure 1, addition of spices especially ginger increased the ash content. Ash content of the smoked fish may be caused by different factors, such as fish species, smoking method, smoking time, temperature and spice concentration (Adegunwa*et al.* 2013). The highest value of ash recorded was 9.56%. The high value of the ash obtained could be tied to the mineral content of the spice extract applied, which agrees with the report of Turan and Sonmez (2007). Also increase in ash content may be due to loss of moisture during smoking.

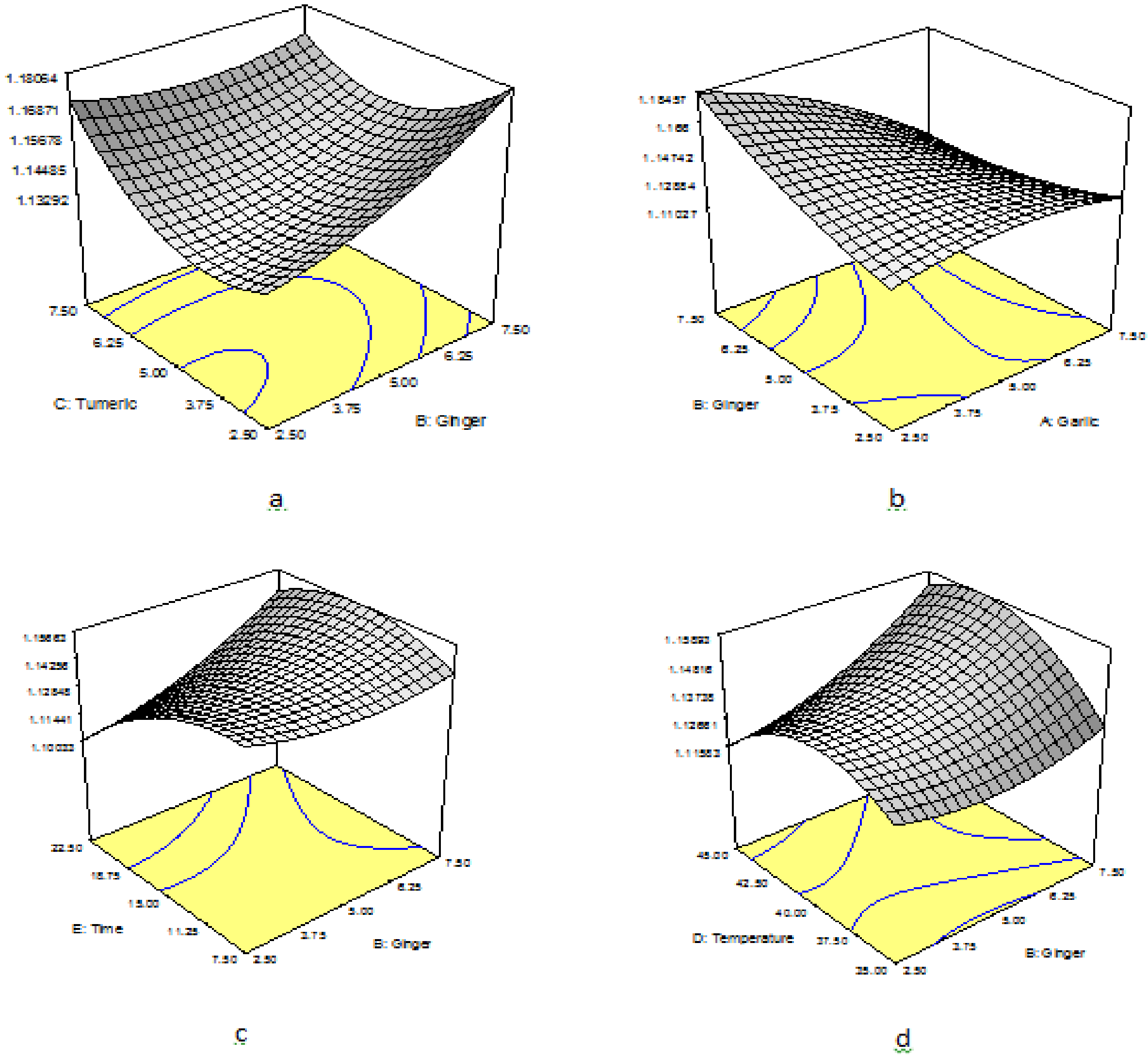

Figure 1. Effect of the interaction of variables on the ash content of spiced smoked catfish: (a)ginger and turmeric (b) garlic and ginger (c) ginger and time (d) ginger and temperature.

3.5 FFA content of Spiced Smoked Catfish

The model showing the relationship between the independent variables and FFA is expressed in Equation 5.

$$FFA\ (coded) =+ 0.87 + 0.061X_1 - 0.089X_2 - 6.029\text{-}003X_3 + 9.363X_4 + 6.029X_5 + 0.027X_1^2 + 0.027X_2^2 - 8.235X_3^2 - 8.235X_4^2 + 0.062X_5^2 + 0.13X_1X_2 + 0.049X_1X_3 + 0.098X_1X_4 + 0.093X_1X_5 + 0.20X_2X_3 - 0.057X_2X_4 - 0.062X_2X_5 + 0.022X_3X_4 + 0.027X_3X_5 - 0.022X_4X_5 \quad (5)$$

The negative sign in front of the factors are important parameters responsible for reducing the FFA. The FFA in the spiced smoked catfish reduced from 1.42 to 0.81% with average of 1.01%. The average value recorded was lower than the value (2.12%) reported by Eyo (2001). According to Clucas and Ward (2006) various concentration of spices used in smoked catfish inhibit FFA production. The low FFA recorded in this study suggests the potential of ginger and turmeric as antioxidants to inhibit the synthesis of FFA.

3.6 Total Phenol content of Spiced Smoked Catfish

The relationship between the independent variables and Total phenol is described by Figure 2. A significant effect ($p<0.05$) was found for the total phenol. The interaction terms, quadratic terms with positive signs in Equation 6 increases the total phenol in the smoked catfish.

$$(Phenol\ coded) = -0.048 + 0.027X_1 - 0.012X_2 - 1.644X_3 - 0.043X_4 - 4.449X_5 + 0.066X_1^2 + 0.063X_2^2 + 0.062X_3^2 + 9.586X_4^2 - 0.017X_1X_2 - 4.228X_1X_3 - 0.060X_1X_4 - 0.058X_1X_5 + 0.039X_2X_3 - 0.050X_2X_4 - 0.093X_2X_5 + 0.036X_3X_4 + 4.952X_3X_5 - 0.088X_4X_5 \quad (6)$$

The relatively high total phenol may be attributed to the various phenolic compounds present in the spices used in smoked fish (Srinivasan, 2014). The phenol content was between 0.53 to 2.85 mg/g with an average value of 1.53 mg/g which is higher than the total phenol content (1.37 mg/g) reported by Kumolu–Johnson and Ndimele (2011) for fresh ginger treatment on smoked catfish. Spice (ginger, garlic, onion etc) are edible plant materials that exhibit antioxidant, antiseptic and bacteriostatic properties. They are added to food to delay onset of deterioration, such as rancidity and also function as seasonings to foods as well as impart flavour to the foods (Abel–Hamied*et al*.,2009).

3.7 FRAP content of Spiced Smoked Catfish

The model showing the relationship between the independent variables and FRAP is expressed in Equation 7. Positive sign in front of the factors signifies a synergistic effect, while negative sign signifies an antagonistic effect. The positive factors are important parameters responsible for increasing the antioxidant content of the smoked catfish.

$$(FRAP\ coded) = + 1.11 + 1.500X_1 + 0.13X_2 + 0.033X_3 + 0.053X_4 + 0.047X_5 + 0.014X_1X_2 - 0.050X_1X_3 - 0.071X_1X_4 - 0.015X_1X_5 + 0.096X_2X_3 + 0.024X_2X_4 - 0.041X_2X_5 + 0.078X_3X_4 + 0.098X_3X_5 - 0.056X_4X_5 \quad ...(7)$$

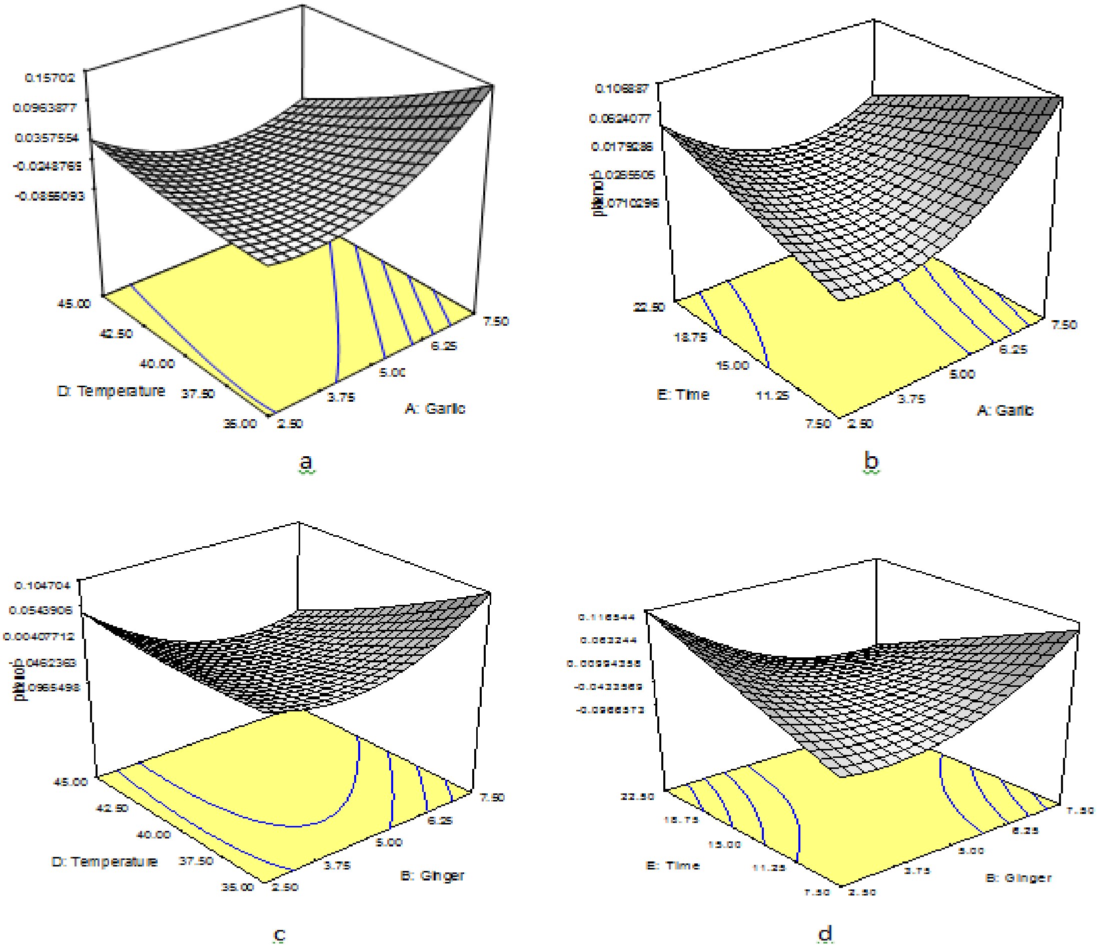

Figure 2. Effect of independent variables on Phenol content of Spiced Smoked Catfish.

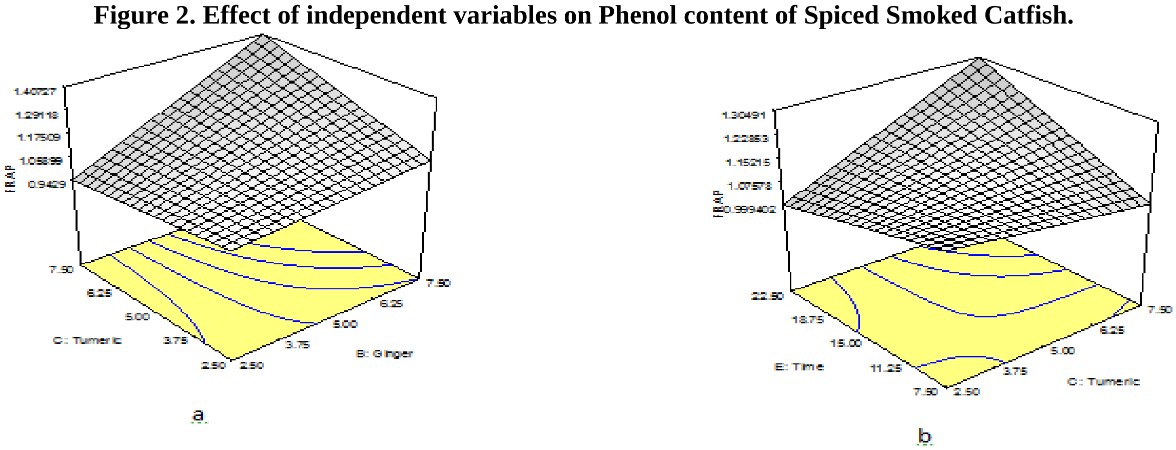

Figure 3. Effect of interaction of variables on the FRAP content of Spiced Smoked Catfish (a) ginger and turmeric (b) turmeric and time.

The high value of FRAP 36.94 mg/g may be linked to the antioxidant properties of the spices used in this study (Asnaashari*et al.*, 2014). However, the low value (0.79 mg/g) observed is as a result of low temperature that did not activate the reducing power. As shown in Figure 3, both ginger and turmeric, turmeric and time increased the FRAP content of the smoked catfish. The FRAP in hot-smoked catfish was much higher than that of smoked mackerel (*Scomberscombrus*) fish (4.05 mg/g) (Iheagwara, 2013).

3.8 Optimization

Applying the desirability function with the goal of maximizing responses of interest and minimizing the ones with adverse effect on the spiced smoked catfish qualities, the optimum smoked catfish was produced from combination of the following parameters; garlic, 7.29 g/100 ml; ginger, 7.50 g/100 ml; turmeric, 2.5 g/100 ml; extraction temperature, 38.65 ^{o}C; and immersion time, 7.51 min. The responses of the predicted values for optimized spiced smoked catfish and the actual optimized spiced smoked catfish parameters are presented in Figure 4. The alignment of the values to the 45^{o} line shows that the models used are predictive in nature and are comparable to the experimented optimized smoked catfish. Also, the high correlation coefficients obtained (R^2= 0.9994) shows that the observations from the predicted and actual are very close.

3.9 Sensory attributes of the optimized, control and commercial smoked catfish

The results of the sensory attributes of the optimized, control and commercial smoked catfish are presented in Table 3. The smoked catfish samples were ranked in order of preference with the most preferred (extremely like 1) and the least preferred (extremely dislike 7).

The optimized smoked catfish had the best scorings in aroma, taste, aftertaste and overall acceptability in the sensory attributes evaluated. This implies that the optimized smoked catfish had better sensory attributes and is better preferred by the panellist in terms of aroma, taste, aftertaste and overall acceptability than the control and commercial catfish. Organoleptically, the general pattern of consumer preference for the products indicates that optimized sample was most acceptable by the panellists. This may be attributed to the seasoning effect of the spices used in the fish which have been reported to enhance the overall organoleptic properties of food (Devi *et al.*, 2011).

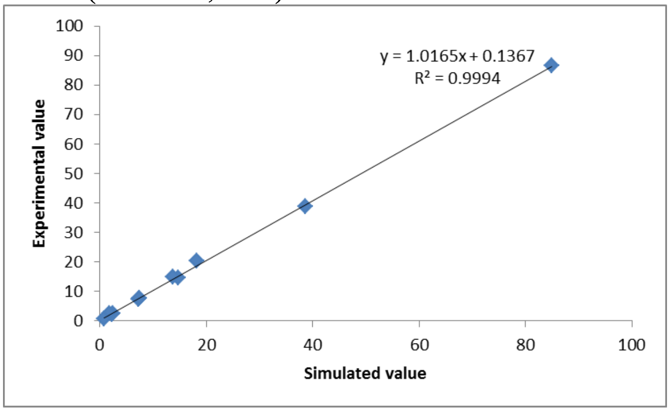

Figure 4. Graph of Simulated Optimised Sample against actual Experimental Values.

Table 3. Mean sensory attributes of the optimized, control and commercial smoked catfish.

Parameter	Optimized spiced smoked catfish	Control smoked catfish	Commercial catfish
Appearance	7.46	7.44	7.03
Aroma	7.51	7.44	6.07
Texture	7.24	6.77	6.87
Taste	7.47	7.03	6.84
Aftertaste	7.50	6.8	6.71
Overall acceptability	7.54	7.09	6.90

4. Conclusion

The importance of introducing spices into fish smoking for preservation, improved organoleptic properties and antioxidant enriching was demonstrated. Operating parameters of temperature and time was found to play significant role in attaining a higher level of product quality measured by the chemical and oxidative properties. The application of Response surface methodology RSM has brought optimum level of variables (garlic, ginger, turmeric, extraction temperature and time) in producing a wholesome hot- smoked catfish. Thorough cleaning of sample catfish enabled the molecular migration of characteristic properties of spices into the fish on soaking. The chemical characteristics of the spiced soaked, hot-smoked catfish showed that spiced catfish samples are richer in protein, ash and antioxidant properties, and hence improved their organoleptic properties. Consequently, the antioxidant properties of fat and protein rich resources can be improved with the addition of local spices.

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