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Application of fractional factorial design to improve hot smoked Nile Perch (*Lates niloticus*) quality

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Abstract

The aim of this research was to study the parameters influencing moisture, salt and total phenol content of hot smoked Nile Perch and to compare the influence of processing conditions on its proximate composition and sensory quality. The effect on moisture, salt and total phenol content was investigated using 2_{VI}^{7-3} fractional factorial design with seven factors and two levels: X_1 for brine concentration, X_2 for brining time, X_3 for preliminary smoking-drying time, X_4 for smoking time, X_5 for hot smoking time, X_6 for smoking temperature and X_7 for hot smoking temperature. A Multiple Linear Regression Analysis was performed to fit the mathematical model to the collected data and the model tested using analysis of variance (ANOVA). Results of ANOVA indicated the good accuracy and highest significance of the mathematical model. The range of total protein and lipid content was 17.96 to 34.34% and 0.87 to 4.43%, respectively. The range of general acceptability of smoked Nile Perch was 4.6 to 8.2 based on 9-point hedonic scale. Based on the results obtained, including the overall acceptability, proximate composition and smoking criteria of finished product, samples produced with the following conditions: brining at 4% for 270min, drying at 30°C for 30min and the smoked time/ temperature cycles following: 30°C/120min; 50°C/240min and 80°C/240min was the best and most accepted. The results derived from this study indicate that the fractional factorial design is a useful screening tool for improving industrial smoking process of Nile Perch.

1. Introduction

Smoked fish is one of the most nutritious food due to its high content in unsaturated fatty acids, vitamins, minerals and essential amino acids (Arvanitoyannis and Kotsanopoulos, 2012). In France, smoked salmon is a highly valued ready-to-eat product and its consumption has increased considerably in the last decade (Gallart-Jornet *et al.,* 2007). France is the single largest market for smoked salmon in Europe with annual sales estimates at 25000 – 30000tons and a sale value growing at 4.7% per year (Rora *et al.,* 2004). Hence, the smoked salmon industry is an important and vital sector in the Europe economy. Indeed, salmon seems to be the most investigated smoked fish but a lot of other fishes among which is the Nile Perch (Marc *et al.*, 1998) can also be processed by this technique. The Nile perch is a large freshwater fish found extensively in the rivers and lakes of Africa. It is a very popular food not only due to its sensory attributes (firm texture and white flesh) but also to its high nutritional values. With a relatively high protein level, its flesh reveals the high content of omega-3 fatty acids, which is important for cardiovascular diseases prevention (Werimo, 1996). Despite the potential that Nile Perch presents as an important source of income, the only processing technology locally applied to give it an added value is smoking, but smoking technology of this fish is not well developed (Marc *et al*., 1998).

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Smoking technology is one of the oldest food preservation methods, which is increasingly used nowadays to impart particular organoleptic characteristics to fishes then for fish preservation (Pszczola, 1995; Varlet *et al*., 2007)**.** The fish smoking process can be divided into two basic categories: cold smoking and hot smoking. Generally, both smoking methods are done in three stages: brining, drying and smoking. The cold smoking process is referred to the smoking of the product at a temperature below 28°C, therefore nutrients structure is preserved. In hot smoking, the product temperature may reach up to 70–100°C, leading to product cooking which is generally more suitable for direct consumption. However, the exposure of wet fish surface directly to high temperature is the most likely cause of sensory value and nutrient loss, and can produce a hard coating that prevents moisture from migrating to the surface for proper drying and limit the penetration of phenolic compounds which has considerable importance for the preservation and organoleptic properties of the smoked products (Kjällstrand and Petersson, 2001). This phenomenon is known as "case hardening". To avoid "case hardening", The smoking industry now applies the "progressive cooking-smoking", which uses increasing time/ temperature smoking cycles (Knockaert, 2002). Upon the type of fish and regional preferences for a particular product, the hot smoking of fish requires three, four or five steps in addition to the step of salting and drying. Each of the above steps has a different purpose and operating conditions (Rahman, 2007). According to Knockaert (2002), these steps are preliminary drying-smoking, cooking-smoking, and heating/cooking smoking. Preliminary drying-smoking (30°C) is the removal of surface moisture, leading to a protein coating (pellicle) on each piece of fish so that it accepts an even smoke deposit. The second step (60°C) involves the conditions where smoke is deposited evenly on the surface of each piece to ensure good flavor, color, and surface preservation. The final smoking step, 90/100°C is required to allow the cooking and exudation of smoked fish fat. Usually, these three cycles require 8–12 h. Cycles of 4 h or less are possible with thin and lightly smoked products (Hilderbrand, 1992). The industrial specifications for finished smoked fish in the French market generally recommend a moisture content lower than 65% in the fish flesh (Cardinal *et al*., 2001). Additionally, hot smoked fish requires at least 3.5% water phase salt (WPS) to prevents the production of toxins by some bacteria like *Clostridium botulinum* (Hilderbrand, 1992). However, due to differences found in consumer preferences from countries, the smoking industry must adapt their production parameters (salting time, salt level, and time/temperature smoking cycles) in order to respect the market demand depending on regional preferences and to satisfy their requirement for profit (Cardinal *et al.,* 2004). The hot smoking fish requires skill and experience to produce a high-quality end product, because the transport mechanisms of salt, water, and deposition of smoke on the fish surface are governed by several factors involved during the smoking process, which can interact on quality and shelf-life of the finished product as well as its nutritional and sensory qualities. Thus, numerous researches has been conducted on the effects of smoking on the nutritional quality of many seafood species (Cardinal *et al*., 2001; Goulas and Kontominas, 2005; Cardinal *et al.,*2006; Aba and Ifannyi, 2013; Teklu and Lema, 2015) but no reference concerning smoked Nile Perch has been found in literature. To obtain a better understanding of the industrial process applications, it is important to use the basic concepts of research in food engineering related to modeling. A practical solution proposed to solve this problem is to use a fractional factorial design, which includes the performing of a limited number of experiments, and that has proven effective in solving many industrial problems (Montgomery, 2013). The purpose of this experimental approach is to gather the largest possible amount of information with the smallest number of experiments, including main effects (influence of primary factors) and joint effects (interactions) of several primary factors.

The aim of this work is to study the effects of seven smoking process parameters on moisture, salt and total phenol content of smoked Nile Perch, as well as the influence of different smoking conditions on its nutritional and sensory quality.

2. Materials and methods

2.1 Raw material and sample preparation

A total of 20 fresh Nile Perch (*Lates niloticus*) of 3.5 - 4 kg from both sex was purchased from Lake Mbakaou fishing site, in Adamawa Region of Cameroon, in November and December 2015. The fishes were transported in an ice box, directly to the Food Science Laboratory of the National High School of Agro-Industrial Sciences (ENSAI) in Cameroon where they were washed, cleaned, eviscerated, and manually filleted and cleaned before being subjected to brining operation.

2.2 Brining

Brine was prepared in a stainless-steel container by dissolving refined NaCl in distilled water. The brine was vigorously stirred and stored overnight in a refrigerator for complete dissolution of the salt and temperature stabilization (4°C). The fillets (about 200g each trimmed manually) were immersed in brine at a ratio of 1:10 (w/w) and kept in a refrigerator (4°C) for a determined time (Figure 1). After brining, each fillet was rinsed for approximately 20 seconds in cold distilled water and placed on trays in the refrigerator for 1 h.

2.3 Hot smoking procedure

The smoke equipment used for the smoking process of the fillets was a Matindex Air Conditioned (Model 74560, Lamurax Germany) with 0.5 m/s airspeed. A generator produced smoke by pyrolysis (400°C) of moistened (20%) hardwood sawdust (*Lophira alata*). Brined fish fillets were put on trolley grids, and the smoking process started with pre-drying in the smoking oven for 30 min at 30°C, without any smoke thereafter the fish fillets were subjected to smoking with increasing temperature in order to determine smoking time/temperature cycles (Figure 1). The smoked fish fillets were then cooled at room temperature for 2h and stored in polyethylene bag.

2.4 Proximate analysis

The proximal composition of smoked fish fillet was performed in triplicate using AOAC methods (AOAC, 2000). Moisture content was determined by oven drying of 5 g of minced smoked fish fillet at 105°C until constant weight. Total crude protein (Nitrogen content x 6.25) was determined from 1 g smoked sample. Total lipids were extracted from a 5 g sample of the minced smoked fish fillets using n-hexane as solvent. Results were expressed as g/100 g of smoked sample.

2.5 Salt and total phenol content

Sodium chloride content in smoked fish samples was determined using Mohr's method described by Karl *et al*. (2002). The phenolic content in smoked fish was quantified by the Folin-Ciocalteu's phenol method using UV-visible spectrophotometer at 760 nm as described by Marc *et al*.(1998) with the following modifications: To 5.0 g of ground sample was added 50 mL of 95% ethanol and the phenolic compound extracted for 30 min with a magnetic stirrer and filtered into a 100mL flask using Whatman paper No 1. The same sample was subjected to a second extraction using 40 mL of 95% ethanol and both

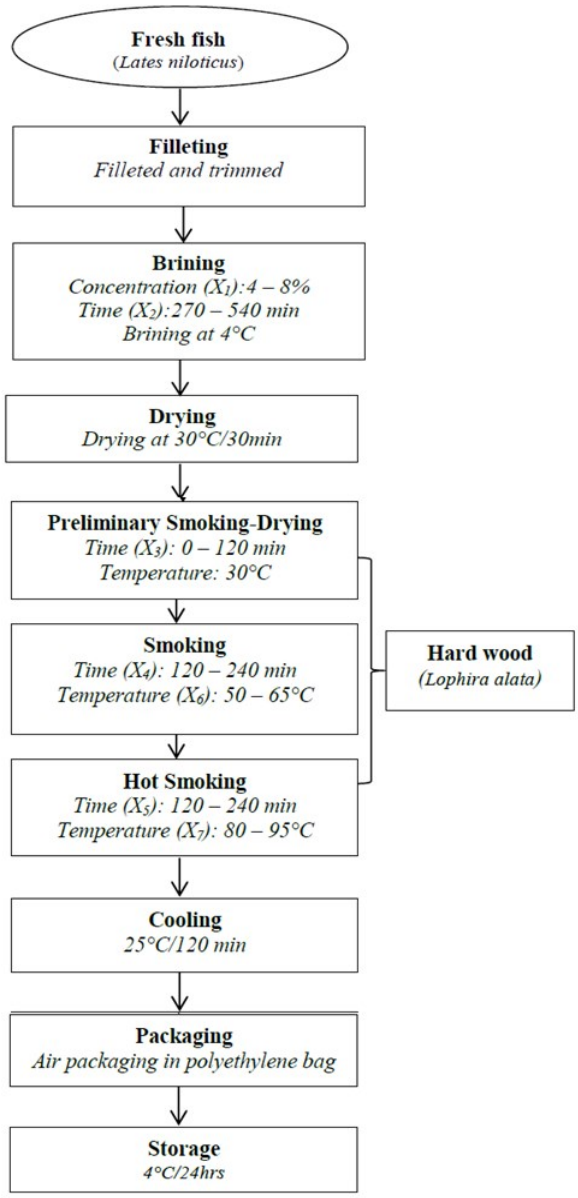

Figure 1. Flow diagram of the production of Hot Smoked Nile Perch (*Lates niloticus*)

extracts mixed. The total volume was adjusted to 100mL using 95% alcohol, homogenized and centrifuged at 6000 x *g* for 5 minutes. 0.5 mL of the supernatant was added to 4.5 mL of distilled water and 0.5 mL of Folin Ciocalteu reagent (10%) in a test tube. The mixture was incubated for 3 minutes at room temperature before adding 0.5 mL of 10% sodium carbonate then mixed and incubated for 40 min in the dark at room temperature. The absorbance was read at 760nm. The total phenol content was expressed as mg equivalent gallic acid /100g smoked fish using the standard curve of gallic acid.

2.6 Sensory evaluation

Organoleptic characteristics of smoked fish mainly texture, taste, odor, color, and overall acceptability was evaluated by fifteen panelists, students (both sex) of the

Department of Food Science and Nutrition of ENSAI. Samples were removed from the refrigerator, held for 30 min at room temperature and presented randomly with different tree digit code at each panelist. The consumers expressed their degree of liking or disliking using a nine-point hedonic scale ranging from "like extremely: 9" to "dislike extremely: 1" (Poste *et al.,* 1991).

2.7 Experimental design

A two-level fractional factorial design 2_{VI}^{7-3} for 20 experiments, including 16 experiments for the 2_{VI}^{7-3} design (seven factors with two levels) and 4 additional replicates at the center point was used for this study. The tested factors were: X_1 = brine concentration, X_2 = brining time, X_3 =preliminary smoking-drying time, X_4 = smoking time, X_5 = hot smoking time, X_6 = smoking temperature and X_7 = hot smoking temperature. The real and coded value of each parameter is presented in Table 1. The choice of the levels of variable was based on the preliminary experiments and on the literature.

Table 1. Factors, their coded levels and actual values as used in the design

Independent variables	Symbol	Real values of coded levels		
		-1	0	1
Brine concentration (%)	X_1	4	6	8
Brining time (min)	X_2	270	405	540
Preliminary smoking-drying time (min)	X_3	0	60	120
Smoking time (min)	X_4	120	180	240
Hot smoking time (min)	X_5	120	180	240
Smoking temperature (°C)	X_6	50	57.5	65
Hot smoking temperature (°C)	X_7	80	87.5	95

The first-order model with interaction terms proposed for each response variable (y_i) was based on the multiple linear regression method. The empirical model in terms of coded factors was:

$$y_i = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i<j}^{k} \sum \beta_{ij} x_i x_j + \varepsilon$$

where y_i is the predicted response (% moisture, salt, and total phenol content), x_i are the coded values of the factors, β_0 is a constant, β_i is the main effect coefficients for each variable and β_{ij} is the interaction effect coefficients between two variables, and ε represent the noise, curvature or error observed in the response.

The Statistica software (version 7.0.0) was used to perform statistical analysis and response surface. The goodness of fit of the model and significance of each regression coefficient was evaluated by regression analysis and ANOVA.

3. Results and discussion

3.1 Evaluation of fitted model

Two levels were attributed to each variable as indicated in Table 1 and twenty experiments were conducted to determine how the seven variables influence moisture, salt and total phenol content of smoked Nile Perch obtained. Factorial fractional methodology is an empirical modeling technique used to evaluate the relationship between a set of controllable experimental factors and observed results. The moisture, salt and total phenol content of smoked Nile Perch from the experimental design at each experimental point are summarized and listed in Table 2. The results were analyzed using analysis of variance (ANOVA). The regression equations (1, 2 and 3) obtained give respectively, the moisture, salt and total phenol content of smoked Nile Perch, as a function of different variables. Table 3 (a-c) summarized the estimated effects and coefficients of each factor of the corresponding empirical model at 95% confidence level. The *p*-values were used as a tool to check the significance of each of the coefficients. The smaller the *p*-value, the more significant was the corresponding coefficient. For a 95% confidence levels, the *p*-value should be less than or equal to 0.05 for the effect to be statistically significant. Only the significant terms are included in each equation, which is valid only for coded units:

$$Y_{Mo} = 66.020 - 1.324X_1 - 1.30X_3 - 1.607X_4 - 2.406X_5 - 2.333X_6 - 0.945X_7 - 1.426X_1X_6 \quad (1)$$

$$Y_{NaCl} = 3.993 + 0.783X_1 - 0.000X_2 + 0.406X_4 + 0.347X_5 - 0.157X_6 + 0.734X_7 - 0.587X_1X_2 + 0.395X_1X_6 + 1.080 \quad (2)$$

$$Y_{TP} = 42.266 + 9.700X_6 - 9.035 \quad (3)$$

*Factor X_2 (brining time) and X_6 (Smoking temperature) are not significant, however, due to their significant interactions in X_1X_2 and X_1X_6 respectively they were included in the model (2)

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Table 2. Moisture, Salt and total phenol content of hot smoked Nile Perch as function of different smoking conditions

		Independent variables							Responses		
Run Order	Samples	Brine concentration	Brining time	Preliminary smoking-drying time	Smoking time	Hot smoking time	Smoking temperature	Hot smoking temperature	Moisture content (%)	Salt content (¨%)	Phenol content (mg/100g)
1	A	1	-1	1	1	-1	-1	1	65.57 ± 0.06^{cdef}	6.27 ± 0.12^{j}	31.17 ± 0.76^{ab}
2	B	-1	-1	-1	1	-1	1	1	67.76 ± 1.93^{hi}	2.43 ± 0.06^{a}	50.00 ± 4.92^{fg}
3	C	1	1	1	-1	1	-1	-1	68.33 ± 0.32^{i}	3.22 ± 0.04^{b}	29.33 ± 1.04^{ab}
4	D	-1	-1	-1	-1	-1	-1	-1	72.65 ± 2.15^{k}	2.12 ± 0.03^{a}	32.83 ± 1.58^{b}
5	E	1	-1	1	-1	-1	1	-1	64.43 ± 0.56^{c}	4.16 ± 0.42^{d}	45.67 ± 3.33^{ef}
6	F	-1	1	-1	1	1	-1	1	65.30 ± 0.15^{cde}	5.41 ± 0.09^{h}	34.17 ± 1.44^{bc}
7	G	0	0	0	0	0	0	0	$65.88\pm0.07d^{efg}$	4.70 ± 0.37^{ef}	38.17 ± 1.26^{cd}
8	H	-1	1	1	-1	-1	-1	1	70.12 ± 0.05^{j}	4.44 ± 0.29^{de}	27.83 ± 0.57^{a}
9	I	-1	-1	1	1	1	-1	-1	64.94 ± 0.14^{cd}	3.08 ± 0.1^{b}	38.17 ± 4.07^{cd}
10	J	-1	1	1	1	-1	1	-1	$66.17\pm0.76d^{efg}$	3.03 ± 0.03^{b}	48.05 ± 2.29^{fg}
11	K	1	-1	-1	1	1	1	-1	59.65 ± 0.57^{b}	5.26 ± 0.06^{gh}	48.33 ± 4.25^{fg}
12	L	1	1	1	1	1	1	1	52.44 ± 0.08^{a}	6.81 ± 0.29^{k}	78.50 ± 4.00^{h}
13	M	1	1	-1	1	-1	-1	-1	72.61 ± 0.38^{k}	2.90 ± 0.32^{b}	34.17 ± 1.53^{bc}
14	N	1	-1	-1	-1	1	-1	1	66.44 ± 0.11^{efgh}	5.76 ± 0.03^{i}	33.01 ± 1.01^{b}
15	O	-1	-1	1	-1	1	1	1	64.87 ± 0.75^{cd}	2.87 ± 0.03^{b}	51.83 ± 1.76^{g}
16	P	0	0	0	0	0	0	0	65.54 ± 0.10^{cdef}	5.17 ± 0.06^{gh}	31.02 ± 0.59^{ab}
17	Q	0	0	0	0	0	0	0	$65.91\pm0.06d^{efg}$	5.43 ± 0.14^{h}	31.18 ± 1.04^{ab}
18	R	0	0	0	0	0	0	0	65.12 ± 0.15^{cde}	5.00 ± 0.03^{fg}	32.33 ± 0.57^{ab}
19	S	-1	1	-1	-1	1	1	-1	66.94 ± 0.88^{fgh}	2.31 ± 0.25^{a}	50.17 ± 3.75^{fg}
20	T	1	1	-1	-1	-1	1	1	67.23 ± 0.06^{ghi}	3.82 ± 0.17^{c}	42.83 ± 0.76^{de}

$^{a,b,c....k}$ Values in each column with different superscripts are significantly differents ($p < 0.05$): Duncan Test

Table 3. Estimated Effect and coefficients of the empirical model for; (a) moisture content; (b) salt content; (c) phenol content

(a)

	Effect	Std.Err.	t(8)	p	Coeff.	Std.Err.
Mean/Interaction	66.01954	0.251552	262.4489	0.000000	66.01954	0.251552
Curvature	-0.81798	1.124974	-0.7271	0.487890	-0.40899	0.562487
(1)Brine concentration	-2.64792	0.503104	-5.2632	0.000762	-1.32396	0.251552
(3)Preliminary smoking-drying time	-2.60371	0.503104	-5.1753	0.000848	-1.30185	0.251552
(4)Smoking time	-3.21312	0.503104	-6.3866	0.000212	-1.60656	0.251552
(5)Hot smoking time	-4.81237	0.503104	-9.5654	0.000012	-2.40619	0.251552
(6)Smoking temperature	-4.66610	0.503104	-9.2746	0.000015	-2.33305	0.251552
(7)Hot smoking temperature	-1.89002	0.503104	-3.7567	0.005570	-0.94501	0.251552
1 by 3	-0.96721	0.503104	-1.9225	0.090767	-0.48360	0.251552
1 by 5	-1.14754	0.503104	-2.2809	0.051996	-0.57377	0.251552
1 by 6	-2.85163	0.503104	-5.6681	0.000471	-1.42582	0.251552
1 by 7	-1.22555	0.503104	-2.4360	0.040820	-0.61277	0.251552

(b)

	Effect	Std.Err.	t(8)	p	Coeff.	Std.Err.
Mean/Interaction	66.01954	0.251552	262.4489	0.000000	66.01954	0.251552
Curvature	-0.81798	1.124974	-0.7271	0.487890	-0.40899	0.562487
(1)Brine concentration	-2.64792	0.503104	-5.2632	0.000762	-1.32396	0.251552
(3)Preliminary smoking-drying time	-2.60371	0.503104	-5.1753	0.000848	-1.30185	0.251552
(4)Smoking time	-3.21312	0.503104	-6.3866	0.000212	-1.60656	0.251552
(5)Hot smoking time	-4.81237	0.503104	-9.5654	0.000012	-2.40619	0.251552
(6)Smoking temperature	-4.66610	0.503104	-9.2746	0.000015	-2.33305	0.251552
(7)Hot smoking temperature	-1.89002	0.503104	-3.7567	0.005570	-0.94501	0.251552
1 by 3	-0.96721	0.503104	-1.9225	0.090767	-0.48360	0.251552
1 by 5	-1.14754	0.503104	-2.2809	0.051996	-0.57377	0.251552
1 by 6	-2.85163	0.503104	-5.6681	0.000471	-1.42582	0.251552
1 by 7	-1.22555	0.503104	-2.4360	0.040820	-0.61277	0.251552

(c)

	Effect	Std.Err.	t(13)	p	Coeff.	Std.Err.
Mean/Interaction	42.2658	1.674544	25.24016	0.000000	42.26576	1.674544
Curvature	-18.0691	7.488788	-2.41282	0.031324	-9.03455	3.744394
(3)Preliminary smoking-drying time	3.1555	3.349088	0.94220	0.363269	1.57776	1.674544
(4)Smoking time	6.2485	3.349088	1.86574	0.084800	3.12426	1.674544
(5)Hot smoking time	6.3283	3.349088	1.88955	0.081325	3.16414	1.674544
(6)Smoking temperature	19.3997	3.349088	5.79254	0.000063	9.69986	1.674544
(7)Hot smoking temperature	2.7635	3.349088	0.82514	0.424173	1.38174	1.674544

Table 4. Analysis of variance (ANOVA) for suggested linear model of; (a) moisture content; (b) salt content; (c) phenol content

(a)

	SS	df	MS	F	p
Main effects	290.474	6	48.4124	47.82	0.0000
Curvature	0.5353	1	0.53527	0.52868	0.487890
(1)Brine concentration	28.0460	1	28.04603	27.70104	0.000762
(3)Preliminary smoking-drying time	27.1172	1	27.11719	26.78362	0.000848
(4)Smoking time	41.2965	1	41.29647	40.78849	0.000212
(5)Hot smoking time	92.6358	1	92.63581	91.49631	0.000012
(6)Smoking temperature	87.0900	1	87.08996	86.01868	0.000015
(7)Hot smoking temperature	14.2887	1	14.28865	14.11289	0.005570
1 by 3	3.7420	1	3.74197	3.69594	0.090767
1 by 5	5.2674	1	5.26741	5.20261	0.051996
1 by 6	32.5273	1	32.52725	32.12714	0.000471
1 by 7	6.0079	1	6.00789	5.93399	0.040820
Residual Error	8.0996	8	1.01245		
Lack of Fit	7.692	5	1.5383	11.31	0.037
Pure Error	0.408	3	0.1360		
Total SS	346.6535	19			

S = 1.00621 PRESS = 79.9884 R^2 = 97.66% (prod)- R^2 = 76.93% Adj-R^2 = 94.45%
SS: Sum of squares; df: degree of freedom; MS: Mean square; F: F test; p: *p*-value

(b)

	SS	df	MS	F	p
Main effects	24.2984	7	3.47120	18.11	0.000
Curvature	3.73514	1	3.735139	19.48246	0.001686
(1)Brine concentration	9.79843	1	9.798432	51.10854	0.000054
(2)Brining time	0.00000	1	0.000002	0.00001	0.997769
(3)Preliminary smoking-drying time	0.93625	1	0.936255	4.88350	0.054457
(4)Smoking time	2.63726	1	2.637264	13.75595	0.004853
(5)Hot smoking time	1.92230	1	1.922303	10.02672	0.011430
(6)Smoking temperature	0.39382	1	0.393819	2.05416	0.185601
(7)Hot smoking temperature	8.61032	1	8.610321	44.91137	0.000088
1 by 2	5.50565	1	5.505649	28.71742	0.000457
1 by 6	2.49814	1	2.498137	13.03026	0.005662
Residual Error	1.72546	9	0.191718		
Lack of Fit	1.4423	6	0.24038	2.55	0.237
Pure Error	0.2832	3	0.09439		
Total SS	37.76278	19			

S = 0.437856 PRESS = 15.8907 R^2 = 95.43% (pred)- R^2 = 57.92% Adj-R^2 = 90.35%
SS: Sum of squares; df: degree of freedom; MS: Mean square; F: F test; p: *p*-value

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(c)

	SS	df	MS	F	p
Main effects	1892.14	5	378.43	8.43	0.001
Curvature	261.194	1	261.194	5.82171	0.031324
(3)Preliminary smoking-drying time	39.829	1	39.829	0.88775	0.363269
(4)Smoking time	156.176	1	156.176	3.48098	0.084800
(5)Hot smoking time	160.188	1	160.188	3.57040	0.081325
(6)Smoking temperature	1505.398	1	1505.398	33.55354	0.000063
(7)Hot smoking temperature	30.547	1	30.547	0.68086	0.424173
Residual Error	583.252	13	44.866		
Lack of Fit	549.01	10	54.90	4.81	0.111
Pure Error	32.24	3	11.41		
Total SS	2736.585	19			

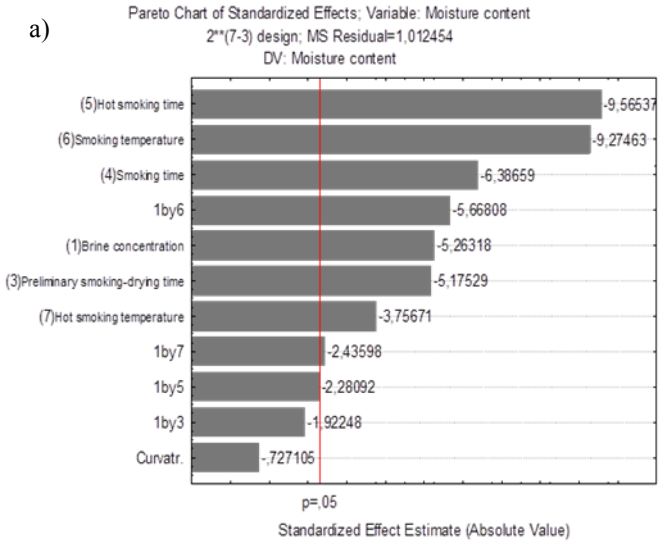
S = 6.69818 PRESS = 1815.90 R^2 = 78.69% (pred)- R² = 33.64% Adj-R^2= 68.85%
SS: Sum of squares; df: degree of freedom; MS: Mean square; F: F test; p: *p*-value

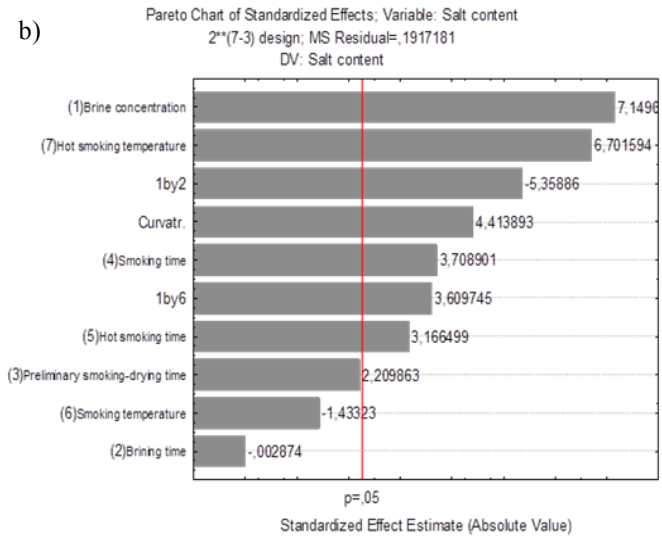
Where Y_{Mo}, Y_{NaCl} and Y_{TP} are the responses of moisture, salt and total phenol content, respectively, and X_1, X_2, X_3, X_4, X_5, X_6 and X_7 are the coded values of the test variables X_1 = brine concentration, X_2 = brining time, X_3 = preliminary smoking-drying time, X_4 = smoking time, X_5 = hot smoking time, X_6 = smoking temperature and X_7 = hot smoking temperature, respectively.

The goodness of fit of these mathematical models was checked by criteria at 95% confidence level. These criteria include the coefficient of determination (R^2), adjusted-R^2 and predicted-R^2. The corresponding analysis of variance (ANOVA) of each model is presented in Table 4 (a-c). The coefficient of determination (R^2) measures the proportion of total variability explained by the model. It is suggested that for a good fit model R^2 should be close to 1 and should be at least 0.8. A value greater than 0.8 indicates aptness/ correctness of the model and the R^2 value less than 0.8 usually indicate an insufficiently precise description of the experimental data (Teklu and Lema, 2015). In this study, the coefficient of determination (R^2), which was found to be 0.9766, 0.9543 and 0.7869 respectively for moisture, salt, and total phenol content indicates that respectively 97.66, 95.43 and 78.69% of the variability in the response can be explained by these respective models. This shows that the equations (1 and 2) are suitable models to describe the response of the experiment. However, a large value of R^2 does not always imply that the regression model is a good one, because the value of R^2 always increases with the addition of a new variable to the model, regardless of whether additional variable is statistically significant or not (Onsekizoglu *et al.*, 2010). Thus, it is preferred to use the adjusted-R^2 to evaluate the model adequacy since it is adjusted for the number of terms in the model, that is, the number of factors. The adjusted-R^2 should be over 0.9 indicating a high degree of correlation between the observed and predicted values (Shahabadi and Reyhani, 2014). In this study, the value of adjusted determination coefficient (Adj-R^2) which was found to be 0.9445, 0.9035 and 0.6885 respectively for moisture, salt, and total phenol content indicates a high significance of the model 1 and 2, but not for the model 3, which shows that the regression model explained the process well. So, it can be seen in Figure 3 (a) and Figure 3 (b) that values of moisture and salt content predicted by the model 1 and 2 are almost perfectly adjusted to the right. Predicted -R^2 measures the amount of variation in new data explained by the model. Generally, a number closer to 1 is preferred (Onsekizoglu *et al*., 2010). The Predicted R^2 was 0.7693, 0.5792 and 0.3364 respectively for moisture, salt, and total phenol content.

3.2 Effects of model factors and their interaction on each response

These results can be graphically discussed in the Pareto Chart (Figure 2), which displays the magnitudes of the effects from the results obtained. The effects are sorted from largest to smallest and all factors which are assigned by the sign (+) or (-) have a positive or negative effect, respectively in the corresponding response.





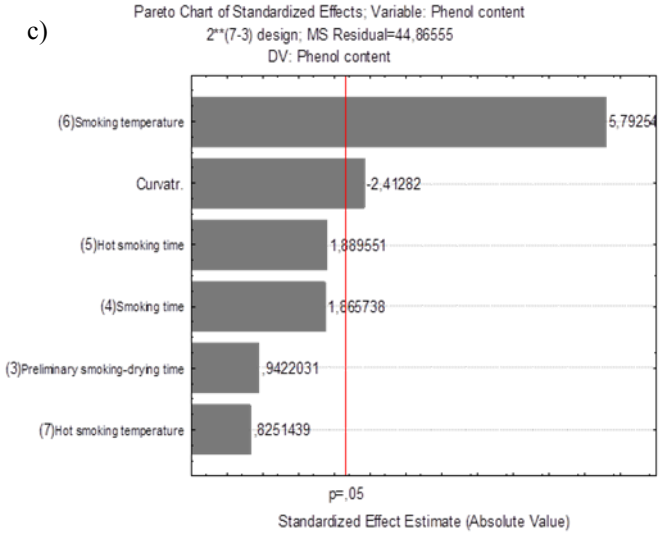
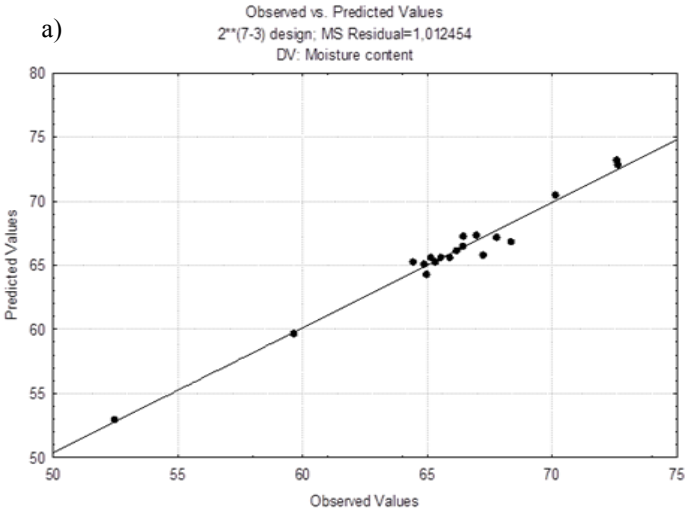
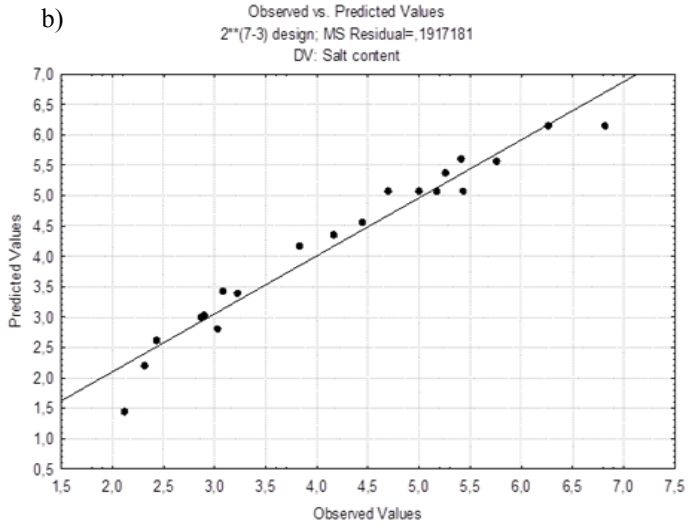


Figure 2. Pareto's chart of standardized effects for variables using the responses: (a) moisture content; (b) salt content; (c) phenol content.





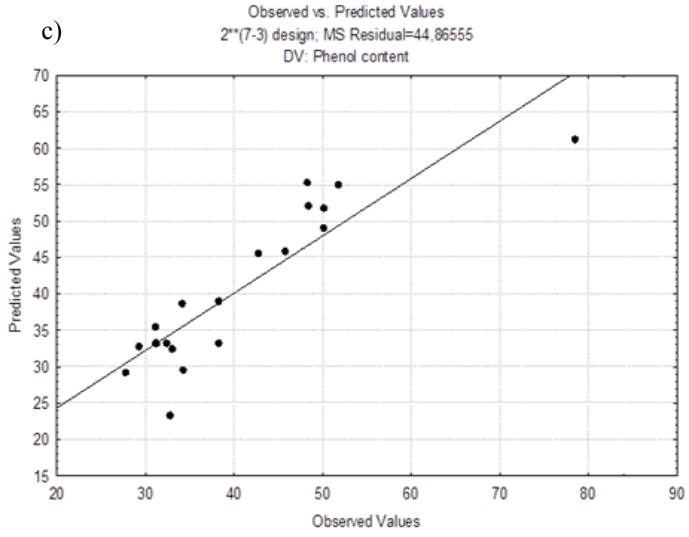


Figure 3. Comparison plot between experimental values and predicted values of; (a) moisture content; (b) salt content; (c) phenol content, using Equation 1, 2 and 3, respectively.

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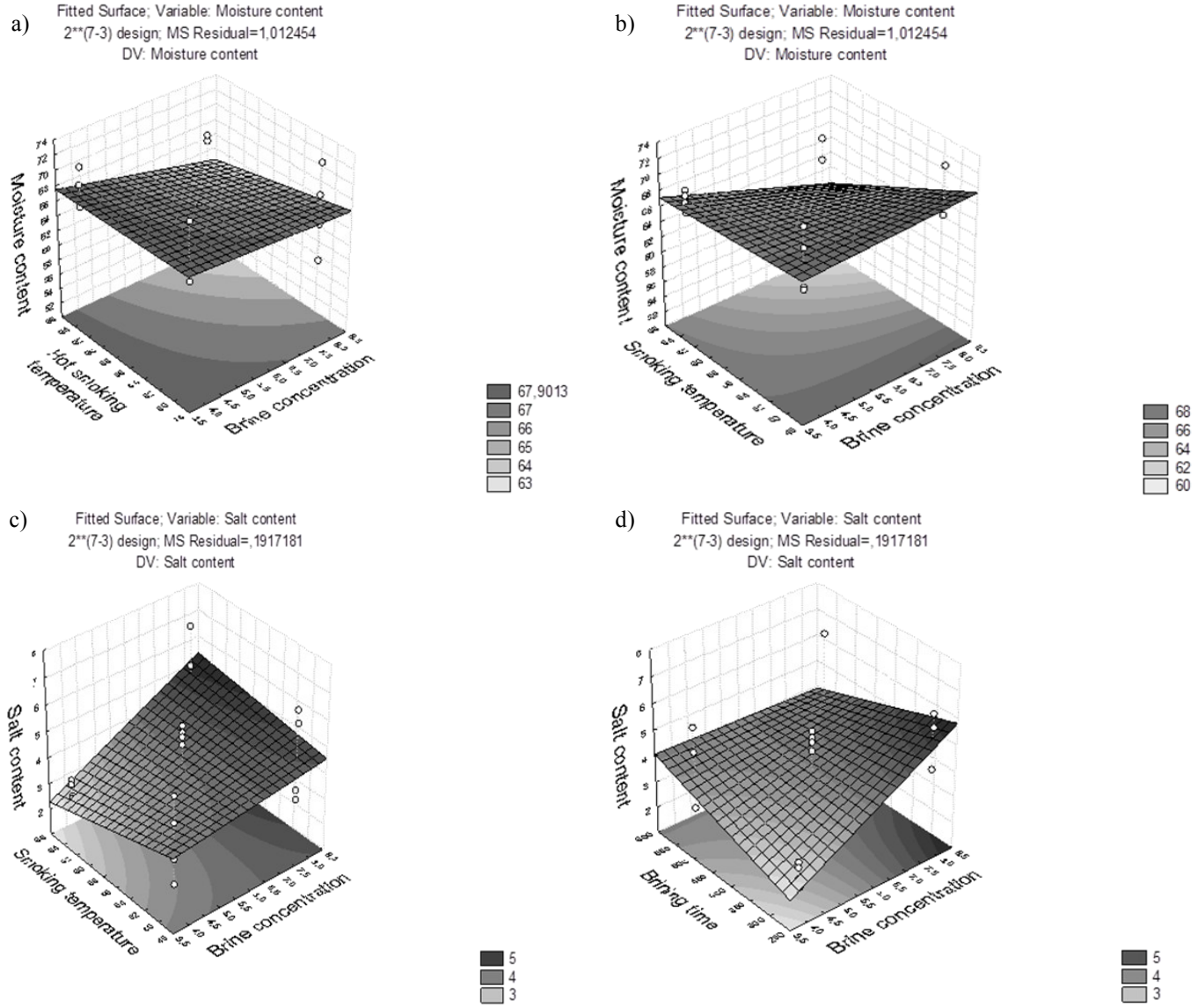

Figure 4. Responses surfaces and contour plots showing the effect of the interaction between; (a) brine concentration-hot smoking temperature on moisture content; (b) brine concentration-smoking temperature on moisture content; (c) brine concentration-smoking time on salt content; (d) brine concentration-brining time on salt content

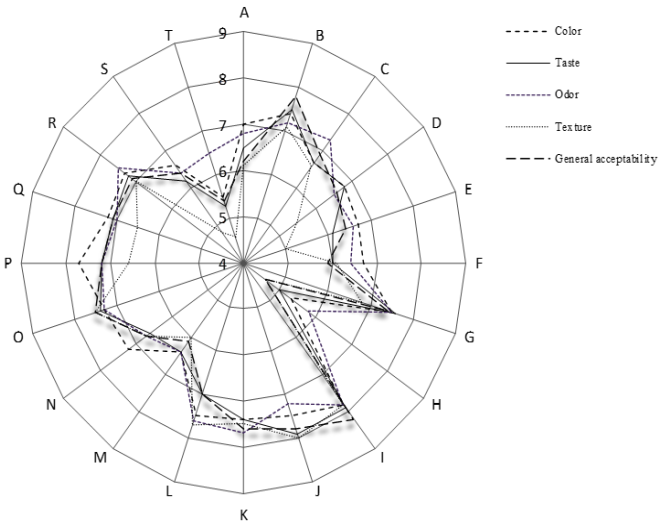

Figure 5. Radar chart of sensory evaluation of hot smoked Nile Perch as function of different smoking conditions

As can be seen in Figure 2(a), the increase in time and temperature in all smoking steps influenced negatively, in a statistically significant way, the water content of smoked fish fillet. The magnitudes of the effects, decreases in the order X_5, X_6, X_4, X_1, X_3 and X_7, indicating that the hot smoking time (X_5) and smoking temperature (X_6) affected moisture content most strongly than the other factors. As reported by Aba and Ifannyi (2013) processing parameters (time, temperature) are the main contributors for a lower moisture content of smoked fish. Indeed, increasing the temperature increases the vapor tension of water so the dehydration of the fish, and thereby increases water loss during the smoking process.

Figure 2(b) reveals that the increase in brine concentration and hot smoking temperature influenced positively, the salt content of smoked fish fillet.

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The magnitude of the effects on the salt content is higher for X_1, indicating that the brine concentration affected salt content of smoked fish more strongly than any other factor. That could be attributed to the fact that, during brining the uptake of salt by the muscle and the loss of water from it occurs simultaneously, due to the differences in the osmotic pressure between muscle cells and the brine (Gallart-Jornet *et al*., 2007).

Concerning the total phenol content (Figure 2(c)), the magnitudes of the effects on the total phenol content decreased in the order X_6, X_5, X_4, X_3 and X_7. Accordingly, operating smoking temperature (X_6) affected positively, the total phenol content more strongly than did any of the other parameters. A study by Chan *et al.* (1975) concerning smoked mackerel, using smoke produced from moist hickory sawdust, showed that concentrations of phenolic compounds in fish muscle increased when temperature increased up to 75°C and then decreased as the product surface dried.

One of the main advantages of factorial design methodologies is their ability to reveal the interaction between the input parameters under study. The best way to visualize these interactions on the dependent variables is to draw 3D surface response plots with the contour plots of the model, which were done by varying two variables within the experimental range and holding the other constant (middle value). Figure 4(a-d) shows the 3D surface plots of all the significant interaction effects confirmed by ANOVA. Figure 4 (a, b) shows the 3D surface plot of moisture content at varying temperature and brine concentration. From these figures, it can be observed that water content in smoked Nile Perch.

decreased with the increase of both brine concentration (X_1) -smoking temperature (X_6) and brine concentration (X_1) – hot smoking temperature (X_7). For example, in Figure 4(b), at a lower smoking temperature of 50°C and lower brine concentration of 4%, the moisture content was about 68%. Whereas at a higher smoking temperature of 65°C and higher brine concentration of 8%, the moisture content decreased to 60%. On the other hand, the salt concentration of smoked fish increased with brine concentration and temperature to reach 4.75% and 5.5% at 50°C and 65°C respectively (Figure 4c). This could be due to the fact that the increase of temperature led to dehydration of the product linked to water evaporation, which resulted in an increase in the NaCl concentration per unit weight of product flesh. Figure 4d shows the effect of brine concentration and brining time on the salt content.

This figure shows that at a low level of brine concentration, an increase in brining time increases salt content in smoked flesh fillet. On the contrary, at a high level of brine concentration, an increase in brining time decreases salt content. In fact, in many foodstuffs, the brining process generally led to salt gain and water loss. Nevertheless, Graiver *et al.* (2006) found that in the flesh, water movement (co- or counter-current) depended on the NaCl concentration of the brine solution. At very long contact times, that is, under equilibrium conditions, flesh treated with increasing concentrations of NaCl showed important modifications. For NaCl concentrations in the solution ranging between 5 to 200 g/L the tissue gained water and incorporated high amount of solutes ("salting in"). Salt has, in fact, been shown to cause a significant displacement of water from the outside to the inside of the myofibrillar matrix (Bertram *et al.,* 2001). According to Cheng and Sun (2008), an increase in water holding capacity and hydration in salted muscle fibers in low brine concentration are generally attributed to enhanced electrostatic repulsion between myofibrillar filaments. They suggested that Cl^- ions bound to the filaments and increased the electrostatic repulsive force between them, the protein structure matrix unfolded and the swelling occurred causing the filament lattices to expand for water entrapment. The maximum water uptake is generally observed for NaCl concentrations ranging between 7 and 10% which is the consequence for a high weight gain as reported by Graiver *et al*. (2006). This phenomenon could be the reason of an apparent decrease in the salt concentration in the salted Nile perch fish fillets at 8% brine.

3.3 Protein content

In Table 5, the protein content of smoked Nile Perch ranged from 17.96 to 34.34% with sample D having the lowest value while sample L had the highest value. These lowest and highest values are significantly different from other treatments ($P<0.05$). The lowest (52.44%) or highest (72.65%) moisture content recorded respectively for sample L and sample D contributes to the highest or lowest crude protein value. Generally, after hot smoking, the protein content of the fillet will be higher due to increasing dry matter content per unit weight. This is mainly due to the removal of water during the smoking process (Steffens, 2006). In support of present findings, Jittinandana *et al.* (2002); Koral *et al.* (2010) have reported the protein content of 27.42 and 20.55% in hot smoked *Oncorhynchus mykiss* and hot smoked *Sarda sardas* respectively. However, Adeyemi

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Table 5. Protein and lipid content of hot smoked Nile Perch as function of different smoking conditions

		Independent variables							Responses	
Run Order	Samples	Brine concentration	Brining time	Preliminary smoking-drying time	Smoking time	Hot smoking time	Smoking temperature	Hot smoking temperature	Lipid (¨%)	Protein (¨%)
1	A	8	1	-1	1	1	-1	-1	2.60±0.08^{g}	22.55±0.46b^{cd}
2	B	4	-1	-1	-1	1	-1	1	0.90±0.09^{a}	26.11±1.51^{fg}
3	C	8	1	1	1	-1	1	-1	1.96±0.05^{e}	24.19±0.29^{e}
4	D	4	-1	-1	-1	-1	-1	-1	3.75±0.26^{i}	17.96±1.3^{a}
5	E	8	1	-1	1	-1	-1	1	1.73±0.04^{d}	26.62±0.41^{fg}
6	F	4	-1	1	-1	1	1	-1	4.43±0.11^{j}	22.47±0.32^{bc}
7	G	6	0	0	0	0	0	0	1.72±0.01^{d}	25.93±0.68^{fg}
8	H	4	-1	1	1	-1	-1	-1	0.97±0.01^{ab}	22.09±1.9^{b}
9	I	4	-1	-1	1	1	1	-1	2.65±0.08^{g}	27.26±0.23^{g}
10	J	4	-1	1	1	1	-1	1	2.39±0.03^{f}	26.57±0.70^{fg}
11	K	8	1	-1	-1	1	1	1	2.55±0.05^{g}	28.75±0.57^{h}
12	L	8	1	1	1	1	1	1	3.20±0.12^{h}	34.34±0.24^{i}
13	M	8	1	1	-1	1	-1	-1	0.87±0.10^{a}	21.69±0.20^{b}
14	N	8	1	-1	-1	-1	1	-1	1.19±0.02^{c}	23.50±0.56^{cde}
15	O	4	-1	-1	1	-1	1	1	1.12±0.09^{bc}	29.02±0.71^{h}
16	P	6	0	0	0	0	0	0	1.71±0.08^{d}	26.72±0.06^{fg}
17	Q	6	0	0	0	0	0	0	1.71±0.02^{d}	25.61±0.21^{f}
18	R	6	0	0	0	0	0	0	1.75±0.01^{d}	26.22±0.15^{fg}
19	S	4	-1	1	-1	-1	1	1	1.96±0.04^{e}	23.82±0.31^{de}
20	T	8	1	1	-1	-1	-1	1	2.38±0.10^{f}	22.80±0.22b^{cd}

$^{a,b,c...I}$ Values in each column with different superscripts are significantly differents ($p<0.05$): Duncan Test

et al. (2013) have reported a high protein content of 55.57% in hot smoked *Trachurus trachurus.*

3.4 Fat content

The fat content of the smoked fish fillet ranged between 0.87% (sample M) and 4.43% (sample F) (Table 5). Significant ($p<0.05$) differences were observed in the fat content between sample F and all the other samples. It is also observed that sample L, despite its lowest water content, had a lipid content of 3.2%, which was significantly lower ($p<0.05$) than that of sample F which had undergone less severe processing. This could be due to exudation of fat in smoked fillets at high temperature, resulting in low lipid content. In addition, oxidation during salting and smoking may also occur (Goulas and Kontominas, 2005; Guizani *et al.,* 2014). Similar results (0.7% to 2.0%) has been shown by Kabahenda *et al.* (2011) in hot smoked juvenile perch.

3.5 Sensory evaluation

Results of sensory evaluation based on a 9-point hedonic scale of smoked Nile Perch fish fillets are shown in Figure 5. In general, the average score of general acceptability, color, odor, texture and taste of the samples are significantly different ($p<0.05$) and were between "neither like nor dislike 5" to "like very much 8" in 9-point hedonic scale for taste, color, texture and general acceptability except for odor that was between "like slightly 6" to "like very much 8" in 9-point hedonic scale. In general, sample H received the lowest score with respect to odor, texture, taste, color and general acceptability probably due to it's both higher moisture and salt content. For the same sensory attributes (odor, texture, taste, color and general acceptability), sample I, with its firm texture and golden brown color, was most preferred by the panelist. The reasons for this high score are numerous but mainly come from its processing parameters. The process of color development in smoked fish begins with the carbonyls being absorbed into the surface. The carbonyls then react with amino groups in the fish and follow a similar path of reactions as in the Maillard browning reaction (Varlet, Prost and Serot, 2007). This group of reactions is enhanced as the temperature and dryness of the product are increased. The phenolic compounds play a prominent role in flavor development. Guaiacol is the phenolic primarily associated with smoke flavor, and syringol is the phenolic primarily associated with smoke aroma (Maga, 1988; Varlet, Prost and Serot, 2007). One of the most important quality characteristics of smoked fish flesh is its muscle texture. In hot-smoked foods, texture changes are mainly due to the denaturation of proteins by heat (Gill *et al.,* 1992). In addition, the water content of smoked fish flesh strongly influences its texture, with lower water contents producing firmer products (Li *et al.,* 1998).

4. Conclusion

The evaluation of significant factors affecting the moisture, salt and total phenol content of smoked Nile Perch was performed using two-level fractional factorial design. In addition, the influence of different smoking conditions resulting from the experimental design was evaluated on the proximal composition and sensory characteristics of smoked Nile Perch. The results showed that all the factors (brine concentration, preliminary smoking-drying time, smoking time, hot smoking time, smoking temperature and hot smoking temperature) have a negative and significant effect on water content except the brining time. On the other hand, all the factors (brine concentration, preliminary smoking-drying time, smoking time, hot smoking time and hot smoking temperature) have a positive and significant effect on salt content except the brining time and smoking temperature. However, for total phenolic compound, only the smoking temperature has the most statistical significant effect in the chosen range. Different smoking conditions have a great influence on proximate composition content (Protein and lipid) and sensory quality (color, texture, odor, taste and overall acceptability) of the smoked fish fillet. The best smoking condition is, therefore, one that produces a final product with both best overall sensory acceptability and nutritional properties to a level that is beneficial to consumers, and a final product with good smoking criteria (moisture, salt, total phenol content). Thus, brining at 4%/270 min, drying at 30°C/30min and the smoked time/temperature cycles following: 30° C/120min; 50°C/240min and 80°C/240min is the best process condition for hot smoking Nile Perch (*Lates niloticus*).

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