British Biotechnology Journal
15(3): 1-15, 2016, Article no.BBJ.27867
ISSN: 2231–2927, NLM ID: 101616695

SCIENCEDOMAIN *international*
www.sciencedomain.org

Optimizing Salting and Immersion Time in Liquid Smoke of Farmed Gilthead Sea Bream (*Sparus aurata*) Fillets Using Response Surface Methodology

Maria Makri[1*], Xanthe Douvi[1] and Giorgia Ioannidoy[2]

[1]Department of Aquaculture and Fisheries, Technological Educational Institute of Western Greece, Nea Ktiria 30200, Messolonghi, Greece.
[2]SAO SA Fish Farm Commerce and Processing Company, 60, Riga Feraiou Street 262.21, Patras, Greece.

Authors' contributions

This work was carried out in collaboration between all authors. Author MM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors XD and GI managed the analyses of the study. Author GI managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2016/27867
Editor(s):
(1) Cosmas Nathanailides, Department Aquaculture & Fisheries, TEI of Epirus, Greece.
Reviewers:
(1) Alex Augusto Gonçalves, Federal Rural University of Semi Arid, Brazil.
(2) Michael Lokuruka, Karatina University, Kenya.
(3) Rewaida Abdel-Gaber, Cairo University, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/15804

Original Research Article

Received 23rd June 2016
Accepted 9th August 2016
Published 16th August 2016

ABSTRACT

Aims: To produce smoked gilthead sea bream fillets using the liquid smoke dipping method during brining and optimize the brining-smoking process using the response surface method.
Study Design: The experimental design was a two-level Central Composite Design that included 14 runs divided into two blocks.
Place and Duration of Study: Department of Fisheries and Aquaculture Technology, Technological Educational Institute of Western Greece, Messolonghi, Greece, between April 2015 and December 2015.
Methodology: Second order polynomial models for yield, moisture content, salt content, water

**Corresponding author: E-mail: mmakri@teimes.gr;*

activity, pH, redness index and instrumental texture parameters of salted and smoked with liquid smoke gilthead sea bream filets were developed as a function of salt brine content (from 8.7 to 19.3% (w/w)) and immersion time (from 30 to139 minutes) in brining solutions that contained 10% w/w liquid smoke. A numerical optimization was used to find the optimum values for salt brine content and immersion time. For the confirmation of the models, smoked gilthead sea breams were prepared using the optimal settings of the factors. The yield, salt content, water content, water activity, redness index and the textural parameters were determined. The results were statistically compared to the values predicted by the mathematical models. A consumer panel evaluated the smoked gilthead sea breams prepared using the optimal settings of the factors. The proximate composition of the smoked gilthead sea bream was also determined.

Conclusion: Within the range of brine salt content and immersion time used in the present work, the response surface model analysis and the model derived from the numerical optimization method proved useful in determining the optimum settings for salting and smoking with the liquid smoke of gilthead sea bream fillets. The optimal conditions for salt brine content and immersion time were 15.867% (w/w) and 109.975 minutes, respectively. Under optimal conditions, yield, water and salt content, water activity, pH, redness index, maximum shear force and work of smoked gilthead sea bream were 79.73%, 61.99%, 3.81% 0.94, 5.55, 0.41, 1269.1 (g*) and 3659.8 (g* sec), respectively. The water, protein, lipid and ash content of the smoked product were 61.87%, 20.89%, 12.43% and 3.56%, respectively, and the consumer panel highly accepted it.

Keywords: Liquid smoke; gilthead sea bream; salting; optimizing.

1. INTRODUCTION

Gilthead sea bream (*Sparus aurata*) is one of the most important fish species farmed in Greece. However, in recent years, the increased supply of gilthead sea bream caused prices to decline [1]. In certain, also, periods of the year, there is a plentiful supply of fresh fish in the market, which causes a further decline in the prices [2]. There is, therefore, need to look for the development of value-added products of gilthead sea bream for commercial or industrial use. Such products should fulfil consumers' demands and make fish farming industries more profitable. VASILIADOU et al. [3] suggested that smoked gilthead sea bream could be such a product.

Smoking is a traditional processing method that gives particular flavour, aroma, texture and colour to the smoked fish [4]. Liquid smoke has several advantages over traditional smoking. Smoked fish with liquid smoke contain fewer quantities of the carcinogenic polycyclic aromatic hydrocarbons (PAHs) compared to those smoked with conventional methods [5]. Also, several common food-borne pathogens have shown sensitivity to liquid smoke in vitro and food systems [6]. Moreover, liquid smoke has the advantage over the traditional smoking methods of lowering costs and less environmental damage [7]. Thus, liquid smoke has the potential for use as an alternative to the traditional smoking process. It has been tested in various sea foods, including salmon [8], trout [4,7,9,10], mackerel [11], anchovy [12], swordfish [13] and various species of bivalve molluscs [14-16].

Immersion in a brine solution is the most common method of salting fish that are going to be smoked. Brine solutions can also be used to apply liquid smoke onto the fish tissue [11]. Salt functions as a flavour enhancer; improves the texture and has a noticeable preservative effect to the smoked fish [17]. In published studies, the salting process presents large variation regarding the salt brine content and immersion time [12,18].

Response surface methodology (RSM) is a statistical method used for developing, improving, and optimizing processes when several factors influence the response of interest. The objective of RSM is to build a mathematical model that precisely describes the overall process [19]. It has been used to optimize processes in several kinds of seafood such as sausages from minced mullet [20], surimi from mechanically recovered fish muscle [21], smoked mussels [16] and smoked catfish [22].

Although the effects of traditional smoking on quality parameters of gilthead sea bream have been investigated [3], no reference concerning the use of liquid smoke for the production of smoked gilthead sea bream has been found in the literature. Therefore, the aim of this work was to produce smoked gilthead sea bream fillets using the liquid smoke dipping method during brining and optimize the brining-smoking process using the RMS method.

2. MATERIALS AND METHODS

2.1 Fish

Gilthead sea breams (average weight and length 356 g and 251 mm, respectively) were offered by a fish farming company located in Western Greece (Sao SA, Patra, Greece). Fish were packed in an insulated polystyrene container with flaked ice and delivered to the Technological Educational Institute of Western Greece in Messolonghi on the next day of their harvesting. At the laboratory, the fish were washed, eviscerated and filleted manually. Then, the fillets were wrapped individually in aluminium foil, placed in plastic bags and vacuumed. Afterwards, the fillets were stored frozen at -80℃ until use. The mean weight of the skinned on fillets was 65.0±3.8 g (average± S.D.).

2.2 Processing of Gilthead Sea Bream Fillets with Liquid Smoke

The vacuum packed fillets were allowed to thaw in a refrigerator chamber at 2℃ overnight (12 hours). The thawed fillets were weighed and soaked in separate containers containing the pre-chilled at 2℃ brining/smoking solution [named from now on immersion solution]. The immersion solution contained 20% (w/w) of commercial saccharose, 20% (w/w) of sodium chloride, 0.0070% (w/w) of colorant E-102 and 10% (w/w) of liquid smoke. The salt brine content and immersion time for each run are shown in Table 1. The liquid smoke was a commercially available, water-soluble liquid smoke condensate (Papadimitriou SA, Sindos, Greece). The fish to brine ratio was 1:2, and brine temperature was 2℃. After brining and smoking, the fillets were dried in an oven with forced air circulation at 35℃for 60 min, smeared with sunflower and cooked at 90℃ for 60 min, to an internal temperature of 70℃ at the end of the process. The internal temperature of the smoked product was monitored using K-type thermocouples (0.5 mm, Comark Instruments, U.K) and a recording thermometer (Comark KM1242, Comark Instruments, UK). The smoked fillets were cooled at 2℃ overnight and then they re-weighted for processing yield determinations. Then, the smoked fillets were sliced into two portions as follows: a) the anterior portion, which was used for the chemical and colour determinations, and b) the middle-tail portion that was used for the instrumental textural determinations.

2.3 Physicochemical Analyses

Yield was calculated following the formula:

$$\text{Yield} = \{(\text{weight of raw fillet in gramme}) \times (\text{weight of cooked fillet in gramme})^{-1}\} \times 100$$

A portion of fish muscle (2g) was used for moisture determination according to the method of AOAC 1995 [23]. Salt content in samples was determined by the Mohr method [24]. Water activity (aw) measurements were made using the Novasina apparatus, model Lab master-aw (Novasina AG, Switzerland). The pH was measured according to the method of VARELTZIS et al. [25]. Readings were taken using a Chrison Basic 20 model pH-meter (Crison Instruments SA, Spain) at ambient temperature. Colour measurements were carried out using a Handerlab Miniscan EZ Meter (Hunter Associates Laboratory, Inc., USA). The instrument was standardized against a white and black tile before each measurement. Each measurement was repeated three times for each sample. a∗ and b∗ values were measured, and redness index (RI) was calculated using the equation $RI=axb^{-1}$[4]. The shear force and work of smoked sea breams were measured using a universal testing machine (Stable Micro System, Model TA-XT Plus, Texture Exponent, Surrey, UK) equipped with a modified 4-bladed Kramer shear cell. The cross-head speed was 3 mm sec^{-1}; the cutting distance was 48 mm, and the load cell was 50 kg. The proximate composition and the aqueous phase salt of the smoked sea bream fillets that were prepared using the optimal salt brine content and immersion time were determined. The residue from the moisture determination was heated at 550℃ for 24 hours for ash determination. Crude protein of the sea bream burgers was analyzed by the Kjeldahl method [23]. Total fat content (%) was determined from 4 g freeze dried sample using petroleum ether and a Soxtherm S-360D extraction unit (Gerhardt, Germany). The aqueous phase salt was calculated following the formula:

$$\text{Aqueous phase salt} = \{\%NaCl / (\%NaCl + \%H_20)\} \times 100 \text{ [9]}.$$

Table 1. Design matrix for smoked sea bream

Blocks	Runs	Salt Brine Content g/100 g		Immersion Time minutes	
		Coded values	Actual values	Coded values	Actual values
Block 2	1	0	12.9	0	75
Block 2	2	0	12.9	1.41	139
Block 2	3	0	12.9	0	75
Block 2	4	1.41	19.3	0	75
Block 2	5	0	12.9	0	75
Block 2	6	0	12.9	-1.41	11
Block 2	7	-1.41	6.5	0	75
Block 1	8	-1	8.4	1	120
Block 1	9	1	17.4	1	120
Block 1	10	-1	8.4	-1	30
Block 1	11	0	12.9	0	75
Block 1	12	0	12.9	0	75
Block 1	13	0	12.9	0	75
Block 1	14	1	17.4	-1	30

2.4 Experimental Design and Statistical Analysis

All statistical analyses, generation of response surfaces, optimization, verification and contour plots were accomplished using the Expert Design version 9 (Stat-Ease Inc., Minneapolis, USA) statistical software. The experimental design was a two-level Central Composite Design that included 14 runs divided into two blocks. The coded and actual values of each run are shown in Table 1. A response surface methodology was used to analyze the effect of the two factors (A and B) on the responses (Y) using the second-order polynomial response surface with the corresponding interactions:

$$Y= b_o + b_1A + b_2B +b_{12}AB +b_{11}A^2 +b_{22}B^2 \quad (1)$$

Where Y is the corresponding response variable; A and B are the codified factors (A=salt brine content, and B= immersion time), and b_1 and b_2 are linear, b_{12} is interaction, and b_{11} and b_{22} are quadratic coefficients of the model. A stepwise methodology was followed to determine the significant terms in Equation (1). The significances of all coefficients in the equations were judged statistically at a probability (P) of 0.05. Adjusted and prediction R^2, prediction error sum of squares (PRESS), and adequacy precision were used to confirm the adequacy of the models [19]. Prediction R^2 comparable to adjusted R^2, a low PRESS value and adequacy precision higher than 4 suggest that the model as fitted is adequate to predicting. For these experiments, forty-two fish were used, that were obtained in two blocks of twenty-one fish. For each run, the physicochemical properties of six fillets were analyzed.

A numerical optimization was used to find the optimum values for salt brine content and immersion time. For the confirmation of the models, two batches of six smoked sea breams were prepared using the optimal settings of the factors. The yield, salt, and water content, aw, RI and the textural parameters were determined as described in Materials and Methods section. Finally, results were statistically compared to the values predicted by the mathematical models.

2.5 Sensory Evaluations

A consumer panel of sixty-three university students and other people evaluated smoked sea breams prepared using the optimal settings of the factors. For this purpose, a total number of 42 fillets were processed as described above. Sensory evaluations of saltiness, texture, flavour and acceptability were performed following the sensory scales described by VASSILIADOU et al. [3]. Saltiness and texture of the smoked sea bream were scored using a seven-point scale: Saltiness, 7 for an excessively salty product, 1 for no salt; texture, 7 for an extremely tough and dry product, 1 for an extremely soft, watery product. The flavour and acceptability of the smoked sea bream were scored using a five-point scale: Flavour, 5 for an extremely tasty product, 1 for a tasteless product; Acceptability, 5 for a highly acceptable product and 1 to the unacceptable product. According to these sensory scales, the limit of the acceptability was defined between 3 and 5 for saltiness texture, flavour, and acceptability.

3. RESULTS AND DISCUSSION

3.1 Statistical Analysis on Model Fitting

The results of the physicochemical parameters of gilthead sea bream fillets subjected to different levels of brine concentration and immersion time are presented in Table 2. The factors and the responses were fitted to the following coded equations:

$$\text{Yield}=+82.32-1.86*A-1.76*B \tag{2}$$

$$\text{Water Content}=+64.67-1.51*A-1.34*B-1.25*AB \tag{3}$$

$$\text{Salt Content}=+2.98+0.71*A+0.56*B+0.26*AB-0.34*B^2 \tag{4}$$

$$\text{Water Activity [aw]}=+0.96-0.010*A-9.251E-003*B-5.333E-003*AB \tag{5}$$

$$\text{pH}=+5.71-0.21*B \tag{6}$$

$$\text{Redness Index [RI]}=+0.38+0.018*A+0.43*B-0.026*B^2 \tag{7}$$

$$\text{Max Shear Force}=+1060.69+111.91*A+173.30*B \tag{8}$$

$$\text{Shear Work}=+3063.31+240.67*A+563.44*B \tag{9}$$

The analyses of variance showed that the models and the coefficients were significant ($P < 0.05$). The models as fitted explain 72.44, 83.04, 96.09, 99.49, 73.42, 86.98, 83.51 and 93.20% of the variability in the yield, water content, salt content, water, aw, pH, RI, maximum shear force, and work, respectively. All models possessed no significant [$P>0.05$] lack of fit values, low PRESS, prediction R^2 comparable to fitted R^2, and adequacy precision higher than 4 (Table 3). Therefore, the models as fitted provided an adequate approximation to the true system.

3.2 Effect of Salt Brine Content and Immersion Time on Physicochemical Parameters of Smoked Gilthead sea Bream Fillets

The level of b1 and b2 values (Equation 2) indicates the higher linear negative effect of salt brine content followed by that of immersion time on the yield of smoked sea bream. This result suggests a decrease in yield with an increase of these two factors and can be attributed to the changes in water content of the smoked sea bream, as will be discussed later in this study. It appears that the yield reaches optimal conditions at approximately 13% (w/w) salt brine content and 80 minutes immersion time (Fig. 1). In this study, the mean filleting yield was 36.6% of the weight of whole sea breams, and that of the smoked sea bream ranged from 86.7 to 79.4% of the initial weight of the fillets. Thus, the overall processing yield of the smoked sea bream fillets ranged from 31.7 to 29.1%. By contrast to the results of the present study, VASILIADOU et al. [3] report an average overall processing yield of 37% for hot smoked gilthead sea breams that were processed without a head, guts, skin, and tail. However, the processing yield of fish after smoking depends on their size and biological condition, the form of the treated samples (e.g., whole fish or fillets) and the technology applied [26].

The level of b1 and b2 values (Equation 3) indicates the higher negative linear effect of salt brine content followed by that of immersion time on the water content of smoked sea bream. This result suggests a decrease in water content with an increase of these two factors. The interaction coefficient (b12) of salt brine content and immersion time has, also, a negative effect. In the contour plot (Fig. 2), it is observed that the water content of the smoked sea bream reaches its minimum at approximately 17% (w/w) salt brine content and 110 minutes immersion time.

The level of b1 and b2 values (Equation 4) indicates the higher positive effect of salt brine content followed by that of immersion time on the salt content of the smoked sea bream. This result shows an increase of the salt content of smoked sea bream with an increase of these two factors. The interaction coefficient (b12) of salt brine content and immersion time has, also, a positive effect, whereas the quadratic coefficient (b22) indicates an adverse effect of immersion time. In the contour plot (Fig. 3), it is observed that the salt content of the smoked sea bream reaches its maximum at approximately 17% (w/w) salt brine content and 96 minutes immersion time. JITTINANDANA et al. [9] observed similar results for smoked trout and CORZO et al. [22] for smoked catfish with those found in the present study for water and salt content of smoked sea bream. When a fish muscle is brined, water diffuses out of muscle and salt diffuses from

Table 2. Data of physical, chemical and textural properties of smoked sea bream processed with different levels of salt brine contents and immersion times in the brining salting solution

Std	Block	Run	A: Salt brine content	B: Immersion time	Yield	Water content	Salt content	Water activity (aw)	pH	Redness index (RI)	Max shear force	Shear work
			% [w/w]	minutes	%	% [w/w]	%[w/w]				g*	g*x sec
12	Block 2	1	12.9	75	84[1]	63.73	2.94	0.957	5.70	0.366	1065.25	3004.94
11	Block 2	2	12.9	139	78.29	63.49	2.97	0.945	5.52	0.386	1229.95	3594.55
14	Block 2	3	12.9	75	83	64.97	2.99	0.957	5.70	0.379	1149.66	3141.39
9	Block 2	4	19.3	75	79.36	61.69	3.85	0.942	5.62	0.405	1310.24	3396.89
13	Block 2	5	12.9	75	81	64.49	3.31	0.957	5.49	0.403	1069.6	3140.19
10	Block 2	6	12.9	11	84.60	67.78	1.56	0.971	6.12	0.249	759.336	2237.16
8	Block 2	7	6.5	75	85.05	66.21	1.79	0.970	5.65	0.327	829.8	2521.03
3	Block 1	8	8.4	120	82.20	65.99	2.32	0.962	5.55	0.400	1146.13	3547.57
4	Block 1	9	17.4	120	80.59	60.63	4.24	0.930	5.49	0.420	1343.67	3927.41
1	Block 1	10	8.4	30	86.6	65.80	1.62	0.970	5.93	0.327	875.334	2290.12
6	Block 1	11	12.9	75	81.59	64.61	3.00	0.956	5.82	0.395	1028.41	3267.61
5	Block 1	12	12.9	75	81.20	65.80	3.01	0.956	5.62	0.397	1057.31	3072.39
7	Block 1	13	12.9	75	83.64	64.72	2.88	0.955	5.81	0.376	1091.33	3148.06
2	Block 1	14	17.4	30	81.35	65.44	2.48	0.959	5.92	0.344	893.62	2597.01

[1] *Mean from six independent measurements*

Table 3. Statistics of models

Statistics	Yield	Water content	Salt content	Water activity [aw]	pH	Redness index (RI)	Max shear force	Shear work
	%	% [w/w]	% [w/w]				g*	g*xsec
Lack of Fit	0.7132	0.2719	0.4280	0.2379	0.7672	0.4388	0.0731	0.1444
Adjusted R-Squared	0.7244	0.8304	0.9609	0.9949	0.7342	0.8698	0.8351	0.9320
Predition R-Squared	0.5570	0.6727	0.9223	0.9901	0.5573	0.7431	0.6628	0.8722
Prediction Error Sum of Squares [PRESS]	30.18	14.57	0.61	1.661E-005	0.20	6.456E-003	1.331E+005	4.069E+005
Adequacy Precision	10.832	12.975	25.580	81.944	12.623	17.144	14.493	23.910

brine into muscle, due to the differences in concentration and osmotic pressures among inter-muscles and salting agent [27,28]. Thus, the overall result is the enrichment with sodium chloride and partial dehydration of fish muscle.

The level of b1 and b2 values (Equation 5) indicates the negative linear effect of salt brine content and immersion time on aw of smoked sea bream. The mentioned effect suggests that the water activity of the smoked sea bream decreases with the increase of salt brine content and immersion time. The interaction coefficient (b12) of the salting parameters has a small negative effect. In general, raw fish may contain a variety of pathogenic for humans microbes and parasites [29-34]. Consumption of infected fish may cause serious disease in humans unless they are properly processed and preserved [34]. Microbial growth is retarded by salt levels which reduce water activity (aw) of fish muscle to about 0.97 or less. Smoked fish with salt contents greater than 3.5% in their water phases will usually have such a value of water activity although many factors can cause variation. A water activity of less than 0.85 is necessary to make products stable at room temperature, and a value of aw of about 0.75 or less is needed to inhibit mold growth [35]. In the contour plot (Fig. 4), it is observed that the water activity of smoked sea bream reaches its minimum at approximately 14% (w/w) salt brine content and 94 minutes immersion time.

The level of b2 value (Equation 6) indicates the negative linear effect of immersion time on pH. This result suggests that pH of smoked sea bream decreases with the increase of immersion time. The result is mainly due to the high acidity of the liquid smoke used in the present study [13]. The mentioned effect is shown in Fig. 5 by the shape of parallel lines. Minimal values of pH are approached at an immersion time of approximately 100 minutes.

The level of b1 and b2 values (Equation 7) indicates the higher linear positive effect of immersion time followed by salt brine content on RI of smoked sea bream. The result suggests that the red tone (higher a* values) of the smoked sea bream becomes more intense with an increase of salt brine content and immersion time. As expected, the amount of the absorbed from the fish liquid smoke, which had a dark red colour, increases with immersion time. Also, as was mentioned earlier in this paper, the water content of sea bream muscle decreases with increases of both salt brine content and immersion time, which causes condensation of the absorbed liquid smoke in sea bream muscle giving a more intense reddish tone to the finished product. The quadratic coefficient (b_{22}) indicates a negative effect of immersion time on RI. Colour measurements of smoked fish can be of great interest for producers or retailers due to the impact of consumer purchase. Previous studies on smoked fish have shown that

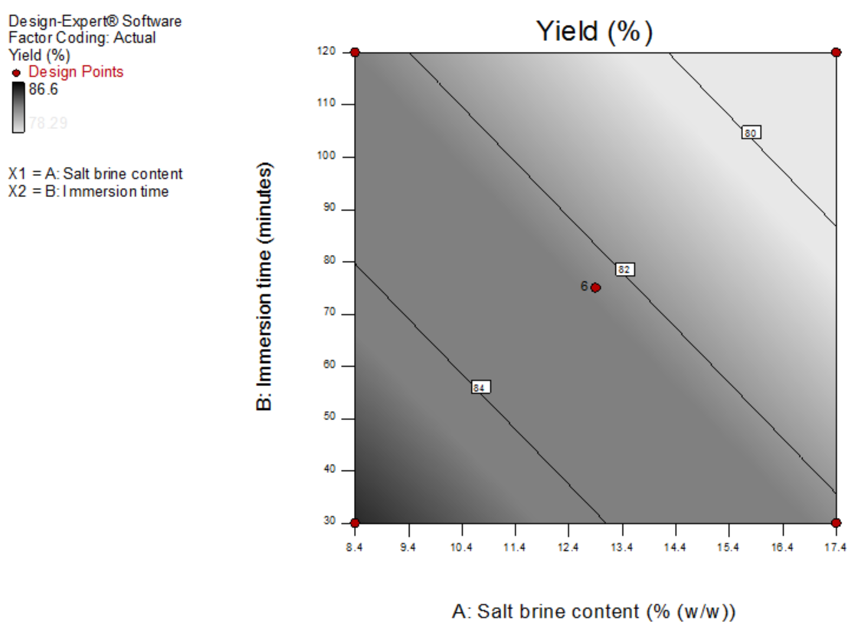

Fig. 1. Contour plot of yield of smoked sea bream

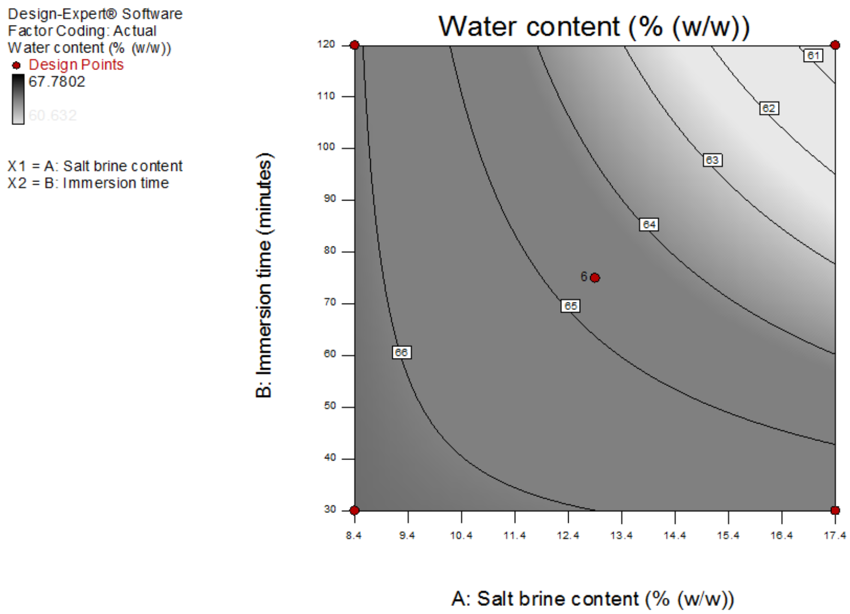

Fig. 2. Contour plot of water content of smoked sea bream

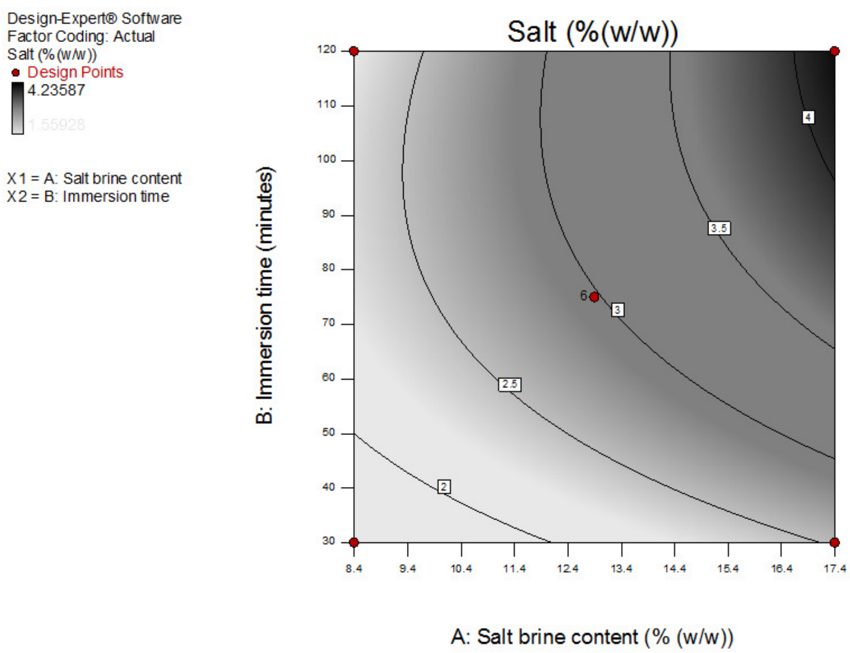

Fig. 3. Contour plot of the salt content of smoked sea bream

the colour of the products is related to the pigment received in the diet, the fat content of the fish and the smoking technology applied [36]. In the contour plot (Fig. 6), it is observed that smoked sea bream reaches maximum redness at approximately 13% (w/w) salt brine content and 75 minutes immersion time.

The level of b_1 and b_2 values in Equations 8 and 9 suggest a positive linear effect of brine concentration and immersion time on maximum shear force (i.e. hardness/softness values) and shear work (i.e. toughness/tenderness values) of smoked sea bream. Furthermore, the effect of immersion time is higher than that of salt

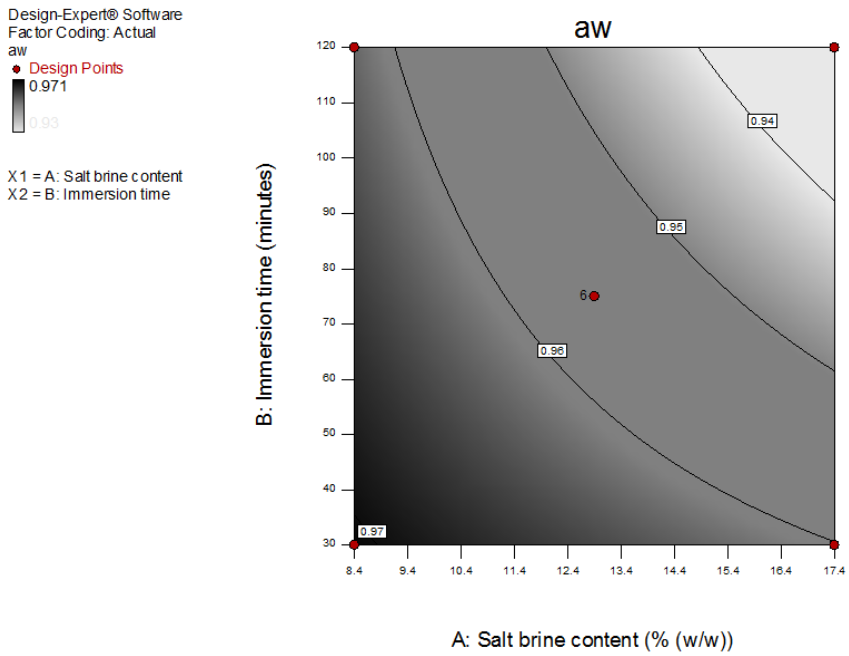

Fig. 4. Contour plot of water activity (aw) of smoked sea bream

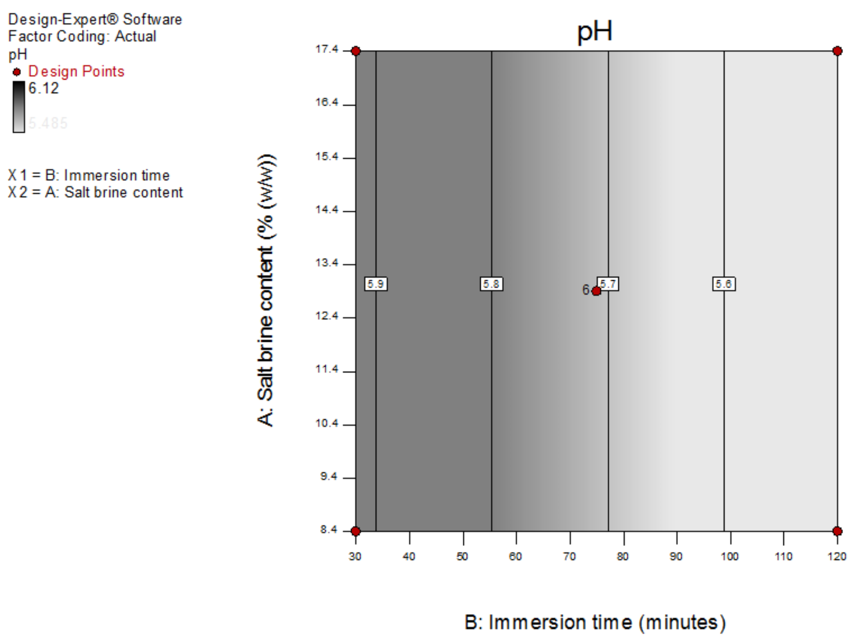

Fig. 5. Contour plot of pH of smoked sea bream

brine content in both responses. These results suggest that the smoked sea bream fillets are harder and tougher as salt brine content and immersion time increase. These effects are shown in Figs. 7 and 8 by the shape of parallel lines which approach their maximal values above levels of salt brine content 14.4% and immersion time 100 minutes for maximum shear force, respectively, and of salt brine content 10.4% and immersion time 90 minutes for shear work, respectively. Salt brine content and duration of brining had a major effect on the texture of smoked rainbow trout [9]. Changes in instrumental texture of salmon treated with liquid smoke flavourings are also reported by Martinez et al. [8]. Fish species with less water content are harder in texture than those with more water content [37]. The texture of smoked fish is, mainly, determined from the state of myofibrillar proteins that is primarily related to the salt concentration in the water phase of fish [38]. Interaction of fish myofibrillar protein with salt may cause protein denaturation, which causes changes in texture and reduced water holding capacity [39]. Altogether, the changes in the texture of smoked sea bream of the present study can be attributed to the decreasing moisture and increasing salt content in the water phase of the smoked fillets as salt brine content and immersion time were increasing.

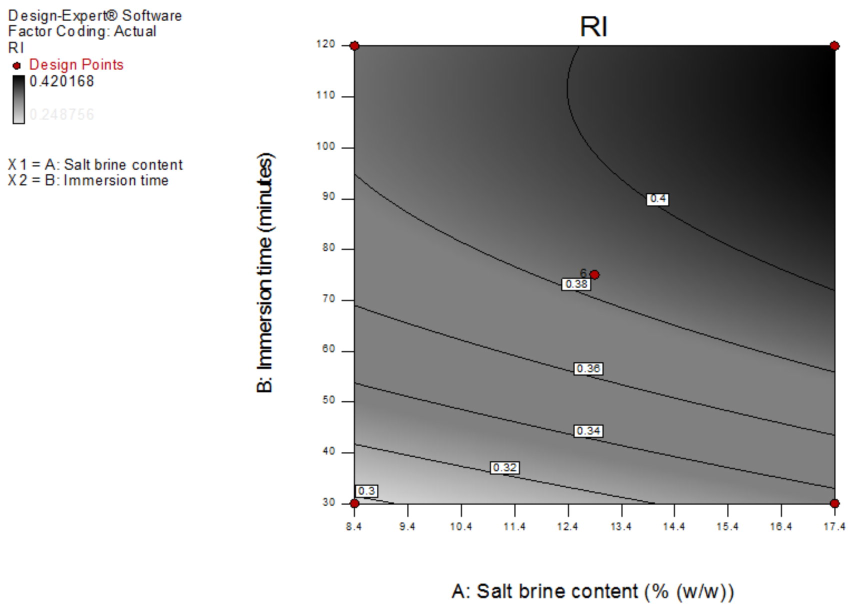

Fig. 6. Contour plot of redness index (RI) of smoked sea bream

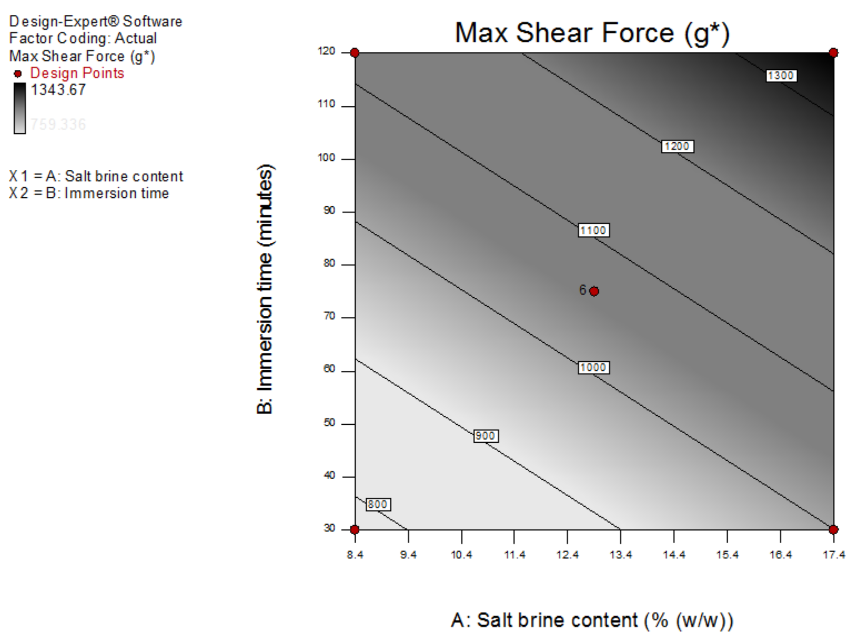

Fig. 7. Contour plot of maximum shear force of smoked sea bream

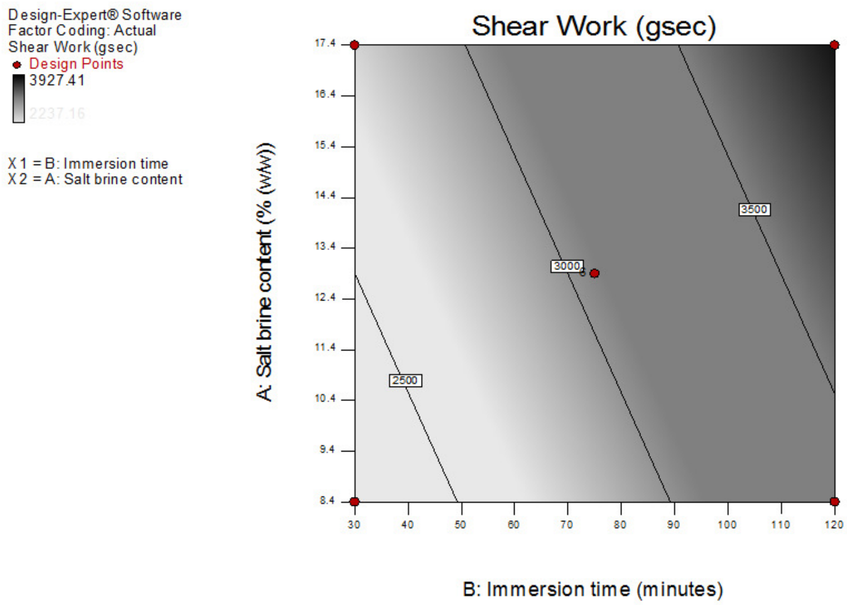

Fig. 8. Contour plot of shear work of smoked sea bream

3.3 Numerical Optimization and Verification of the Models

Table 4 shows the optimization criteria for each factor and response. The optimum combination of salt brine content and immersion time were 15.867% (w/w) and 109.975 minutes, respectively, with an overall desirability D = 0.606. Under optimal combination, yield, water, and salt content, water activity [aw], pH, redness index, maximum shear force and work of smoked gilthead sea bream were 79.73%, 61.99%, 3.81%, 0.94, 5.55, 0.414, 1269.1 (g*) and 3659.8 (g* sec), respectively (Table 4).

For the verification of the models, the optimum combination of the factors above was used to prepare smoked sea breams, and all the response variables of this product were determined. The experimental means of the responses were as follows: yield 80.28%, water content 61.87%, salt content 3.90%, water activity (aw) 0.94, pH 5.39, redness index 0.392, maximum shear force 1.331.21 g*, and work 3706.86 g* sec. The experimental values of each response were compared with those predicted by the models. Both sets of values and the confirmation prediction intervals are presented in Table 5. Since the average values from the

Table 4. Criteria and output for numerical optimization of smoked sea bream

Criteria	Goal	Lower Limit	Upper Limit	Output of Factors and Responses[1]
A: Salt brine content	is in the range	8.4	17.4	15.864
B:Immersion time	is in range	30	120	109.975
Yield [%]	Maximize	78.29	86.6	79.73
Water content (%)	Minimize	60.632	67.7802	61.99
Salt content (%)	Maximize	1.55928	4.23587	3.81
aw[2]	Minimize	0.93	0.971	0.939
pH	Minimize	5.485	6.12	5.55
RI[3]	is in range	0.248756	0.420168	0.414
Max Shear Force (g*)	is in range	759.336	1343.67	1269.10
Shear Work (g* sec)	is in range	2237.16	3927.41	3659.76
Desirability				0.606

[1]Factors= Salt brine content and Immersion time; Responses= yield, water, and salt content, water activity (aw)[2], pH, redness index[3], maximum shear force and work

Table 5. Predicted and experimental physical chemical and textural properties of smoked sea bream processed using salt brine content 15.86% and immersion time 109.97 minutes

Response	Predicted	Experimental mean	95% PI [2] low	95% PI high
Yield (%)	79.73	80.28	77.39	82.06
Water content ([1]n=2, %)	61.99	61.87	60.42	63.56
Salt (n=2, %)	3.81	3.90	3.48	4.13
aw (n=2)	0.939	0.940	0.94	0.94
pH (n=2)	5.55	5.39	5.37	5.73
RI	0.414	0.392	0.38	0.45
Max Shear Force (n=2, g*)	1269.10	1331.21	1131.72	1406.48
Shear Work (n=2, g* sec)	3659.76	3706.86	3409.07	3910.45

[1] n= number of replicates [each replicate contained 6 fillets], [2]PI= Prediction interval

confirmation experiments were within the confirmation prediction intervals, the models were confirmed. Furthermore, such a product has aqueous phase salt content equal to 5.60% (w/w). An aqueous phase salt of 5% (w/w) is required for complete protection of smoked with liquid smoke fish products against the growth of Clostridium botulinum at temperatures between 3℃ and 10℃ [40]. Thus, the optimum combination of the brine salt content and immersion time proposed in the present study can be used by industry to control the formation of Clostridium botulinum toxin in smoked with liquid smoke sea breams provided that the products be preserved at temperatures between 3℃ and 10℃.

3.4 Proximate Composition and Sensory Assessment of Smoked Gilthead Sea Bream

The results for the proximate composition of the raw fish and smoked product are presented in Table 6. The proximate composition of raw gilthead sea breams is similar to that reported by KYRANA et al. [41] for the farmed gilthead sea bream. The water content of smoked sea bream was equal to 61.87%, and less than the value of 65% that is recommended as the maximum value in industrial smoked products [42]. Salting, drying and cooking reduced the moisture content and consequently increased the protein, fat and ash contents of the smoked product. Similar observations are recorded for other smoked fish, including traditionally smoked sea bream [3] and smoked with liquid smoke anchovy [12].

The mean scores of the sensory attributes of the smoked sea bream were close to the "best" scores of every one of the sensory properties (Table 6) Thus; the consumer panel highly accepted the smoked sea bream. These results are similar to those reported by VASSILIADOU et al. [3] for the smoked sea bream using the traditional method. However, smoking of fish using liquid smoke presents several advantages over the traditional smoking methods, the most important of which are reported in the introduction of the present study. Thus, liquid smoke has the potential for use as an alternative to the traditional smoking process for the production of smoked sea bream.

Table 6. Proximate composition and sensory attributes of smoked sea bream processed using salt brine content15.86% and immersion time 109.97 minutes

Proximate composition	Raw gilthead sea bream	Smoked product
Water content	73.52±0.86[1]	61.87±2.75
Protein content	19.98±1.05	20.89±1.05
Fat content	3.93±0.83	12.43±0.79
Ash	1.37±0.07	3.56± 0.21
Sensory attributes		
Saltiness		4.38±0.65[2]
Texture		4.25±0.62
Flavour		4.32±0.90
Acceptability		4.15±0.71

[1] Mean value ± standard deviation [n= 3], [2]Mean value ± standard deviation [n= 63]

4. CONCLUSION

Second order polynomial models for yield, moisture content, salt content, aw, pH, redness index and instrumental texture parameters of salted and smoked with liquid smoke gilthead sea bream filets were developed as a function of salt brine content and immersion time in brining solutions that contained 10% w/w liquid smoke. Within the experimental range, response surface methodology and the numerical optimization method proved useful in determining the optimum settings for salting and smoking with the liquid smoke of gilthead sea bream fillets. The optimal conditions for salt brine content and immersion time were 15.867% (w/w) and 109.975 minutes, respectively. Under optimal conditions, yield, water and salt content, water activity (aw), pH, redness index, maximum shear force and work of smoked gilthead sea bream were 79.73%, 61.99%, 3.81% 0.94, 5.55, 0.41, 1269.1 (g*) and 3659.8 (g* sec), respectively. The water, protein, lipid and ash content of the smoked product were 61.87%, 20.89%, 12.43% and 3.56%, respectively, and the consumer panel highly accepted it.

ETHICAL CONSIDERATION FOR THE ANIMAL USE

The fish used in the present study were raised and harvested following the common methodology used by the Greek fish companies.

COMPETING INTERESTS

Authors have declared that there are no competing interests.

REFERENCES

1. AQUAMEDIA. Available:http//www.aquamedia.org/1/4/122
2. Food and Agricultural Organization (FAO); 2012. Available:http//www.globefish.org/1/4/2012
3. Vasiliadou S, Ambrosiadis I, Vareltzis K, Fletouris D, Gavriilidou I. Effect of smoking on quality parameters of farmed gilthead sea bream (*Sparus aurata* L.) and sensory attributes of the smoked product. Eur Food Res Technol. 2005;221(3-4):232-236.
4. Siskos I, Zotos A, Taylor KDA. The effect of drying, pressure and processing time on the quality of liquid-smoked trout (*Salmo gairdnerii*) fillets. J Sci. Food Agr. 2005;85(12):2054-2060.
5. Lingbeck JM, Cordero P, O'Bryan CA, Johnson MG, Ricke SC, Crandall PG. Functionality of liquid smoke as an all-natural antimicrobial in food preservation. Meat Sci. 2014;97(2):197-206.
6. Holley RA, Patel D. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiol. 2005;22(4):273-292.
7. Dimitriadou D, Zotos A, Petridis D, Taylor AKD. Improvement in the production of smoked trout fillets (*Salmo gairdnerii*) steamed with liquid smoke. Food Sci. Technol Int. 2008;14(1):67-77.
8. Martinez O, Salmerón J, Guillén MD, Casas C. Textural and physicochemical changes in salmon (*Salmo salar*) treated with commercial liquid smoke flavourings. Food Chem. 2007;100(2):498-503.
9. Jittinandana S, Kenney PB, Slider SD, Kiser RA. Effect of brine concentration and brining time on quality of smoked rainbow trout fillets. J. Food Sci. 2002;67(6):2095-2099.
10. Hattula T, Elfving K, Mroueh UM, Luoma T. Use of liquid smoke flavouring as an alternative to traditional flue gas smoking of rainbow trout fillets (*Oncorhynchus mykiss*). LWT - Food Sci. Technol. 2001;34(8):521-525.
11. Chatzikyriakidou K, Katsanidis E. Effect of liquid smoke dipping and packaging method on the keeping quality of raw and cooked chub mackerel (*Scomber japonicus*) fillets. J. Aqua. Food Prod. Technol. 2012;21(5):445-454.
12. Alçiçek Z, Zencir Ö, Çelik Çakiroğullari G, Atar HH. The effect of liquid smoking of anchovy (*Engraulis encrasicolus*, l. 1758) fillets on sensory, meat yield, polycyclic aromatic hydrocarbon (PAH) content, and chemical changes. J. Aqua. Food Prod. Technol. 2010;19(3-4):264-273.
13. Muratore G, Mazzaglia A, Lanza CM, Licciardello F. Effect of process variables on the quality of swordfish fillets flavored with smoke condensate. J Food Pro Pres. 2007;31(2):167-177.
14. Alçiçek Z, Balaban MÖ. Characterization of green shelled mussel meat. Part I: Quantification of color changes during brining and liquid smoke application using image analysis. J. Aqua. Food Prod. Technol. 2015;24(1):1-14
15. Alçiçek Z, Balaban MÖ. Characterization of green lipped mussel meat. Part II: Changes in physical characteristics as a result of brining and liquid smoke application. J. Aqua. Food Prod. Technol. 2015;24(1):15-30
16. Petridis D, Zotos A, Kampouris T, Roumelioti Z. Optimization of a steaming with liquid smoke smoking process of Mediterranean mussel (*Mytilus galloprovincialis*). Food Sci Technol Int. 2013;19(1):59-68.
17. Thorarinsdottir KA, Arason S, Bogason S, Kristbergsson K. The effects of various salt

concentrations during brine curing of cod. Int. J. Food Sci. Technol. 2004;39(1):79–89.

18. Yanar Y, Çelik M, Akamca E. Effects of brine concentration on shelf-life of hot-smoked tilapia (*Oreochromis niloticus*) stored at 4℃. Food Chem. 2006;97(2): 244-247.
19. Myers RH, Montgomery DC. Response surface methodology: Process and product optimization using designed experiments. John Wiley and Sons, Inc., New York; 2002.
20. Daley LH, Deng JC. Determining the optimal ranges of factors affecting the sensory acceptability of a minced mullet sausage. J Food Sci. 1978;43(5):1497-1500.
21. Fogaça FHS, Trinca LA, Bombo ÁJ, Silvia Sant'Ana L. Optimization of the surimi production from mechanically recovered fish meat (MRFM) using response surface methodology. J Food Qual. 2013;36(3): 209-216.
22. Corzo O, Bracho N, Rodríguez J, Arias JM. Optimizing salting and smoking of catfish (*Bagre marinus*) using response surface methodology. J Aqua Food Prod Technol; 2015. (In press). Available:http://dx.doi.org/10.1080/10498850.2013.855286
23. AOAC. Official methods of analysis of the association of official analytical chemists. Virginia: Patricia Cunniff; 1995.
24. Treadwell FP, Hall WT. Analytical chemistry. New York: John Wiley and Sons, Inc; 1928.
25. Vareltzis K, Zetou F, Tsiaras I. Textural deterioration of chub mackerel (*Scomber japonicus collias*) and smooth hound (*Mustellus mustellus* L.) in frozen storage in relation to chemical parameters. Leensm.- Wiss u.-Technol. 1988;21:206-211.
26. Arvanitoyannis I, Kotsanopoulos K. Smoking of fish and seafood: History, methods and effects on physical, nutritional and microbiological properties. Food Bioprocess Technol. 2012;5(3):831-853.
27. Raoult-Wack AL. Recent advances in the osmotic dehydration of foods. Trends Food Sci. Technol. 1994;5(8):255–260.
28. Yao Z, Le Maguer M. Osmotic dehydration: An analysis of fluxes and shrinkage in cellular structure. Trans. ASAE. 1996;396: 2211–2216.
29. Abo-Rahma Y, Abdel-Gaber R, Kamal Ahmed A. First record of Anisakis simplex third-stage larvae (Nematoda, anisakidae) in European hake merluccius *Merluccius lessepsianus* in Egyptian water. J Parasit Res. 2016;1-8.
30. Abdel-Gaber R, Abdel-Ghaffar F, Bashtar A-R, Morsy K, Saleh R. Interaction between the intestinal parasite Polyonchobothrium clarias (Cestode: Ptychobothriidae) from the African sharptooth catfish *Clarias gariepinus* and heavy metal pollutants in an aquatic environment in Egypt. J Helminthol. 2016;8:1-11.
31. Abdel-Ghaffar F, Abdel-Gaber R, Bashtar AR, Morsy K, Mehlhorn H, Al Quraishy S, et al. *Hysterothylacium aduncum* (Nematoda, Anisakidae) with a new host record from the common sole *Solea solea* (Soleidae) and its role as a biological indicator of pollution. Parasitol Res. 2015;114:32.
32. Abdelsalam M, Abdel-Gaber R, Mahmoud MA, Mahdy OA, Khafaga NIM, Warda M. Morphological, molecular and pathological appraisal of *Callitetrarhynchus gracilis plerocerci* (*Lacistorhynchidae*) infecting Atlantic little tunny (*Euthynnus alletteratus*) in Southeastern Mediterranean. J Adv Res. 2015;7(2):317–326.
33. Abdel-Gaber R, Abdel-Ghaffar F, Bashtar A-R. Taxonomical and Phylogenetic characterizations of a new intestinal parasite *Proenenterum myripristiae* (*Digenea, Lepocreadiidae*) infecting pinecone soldier fish *Myripristis murdjan* (*Beryciformes, Holocentridae*) from red sea, Egypt. Intr J Cur Res. 2015;7(12): 23496-23503.
34. American Pablic Health Association. Compedium of methods for the microbiological examination of foods. fourth editionedided by Frances Pouch Downers Keith ITO Washington dc; 2011.
35. U.S. Public Health Services.Food Code. Food Processing Criteria Annex 6; 2013. Available:http//www.fda.gov/downloads/Food/GuidanceRegulation/UCM252416.pdf (Accessed 20/05/2016)
36. Cardinal M, Gunnlaugsdottir H, Bjoernevik M, Ouisse A, Luc Vallet J, Leroi F. Sensory characteristics of cold-smoked Atlantic salmon (*Salmo salar*) from European market and relationships with chemical, physical and microbiological measurements. Food Res Int. 2004;37(2):181-193.

37. Dunajski E. Texture of fish muscle. J Text Stud. 1980;10(4):301-318.
38. Barat JM, Rodríguez-Barona S, Andrés A, Fito P. Influence of increasing brine concentration in the cod-salting process. J Food Sci. 2002;679(5):1922-1925.
39. Gallart-Jornet L, Barat JM, Rustad T, Erikson U, Escriche I, Fito P. Influence of brine concentration on Atlantic salmon fillet salting. J. Food Eng. 2007;80(1):267–275.
40. Food and Agricultural Organization (FAO). Standard for smoked fish, smoke-flavoured fish and smoke-dried fish Codex Stan 311. 2013;21:8. Available:http//www.fao.org/input/download/standards/CXS_311e.p (Accessed 19/07/2016)
41. Kyrana VR, Lougovois VP, Valsamis DS. Assessment of self - life of maricultured gilthead sea bream (*Sparus aurata*) stored in ice. International J Food Sci Technol. 1997;32(4):339-347.
42. Cardinal M, Knockaert C, Torrissen O, Sigurgisladottir S, Morkore T, Thomassen T, et al. Relation of smoking parameters to the yield, color and sensory quality of smoked Atlantic salmon (*Salmo salar*). Food Res Int. 2001;34:537–550.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15804