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RESPONSE SURFACE METHODOLOGY FOR OPTIMIZING THE MARINATION CONDITIONS DURING THE PROCESSING OF RAINBOW TROUT FILLETS

Ö. P. Can[a] and M. Ersan[*]

University of Cumhuriyet, Faculty of Engineering, Department of Food Engineering, Sivas
[*]University of Cumhuriyet, Faculty of Engineering, Department of Chemical, Sivas
Corresponding author email: gorgun7@hotmail.com, mersan@cumhuriyet.edu.tr

ABSTRACT

The objective of this study was to determine using response surface method the optimum points of the rosemary essence oil concentration along with the actual marination time in terms of colour criteria for rainbow trout fillets marinated by the addition of differing rates of rosemary essence oil. The study was conducted in three stages (first satage: 9 different experimental groups depending on the amount of rosemary oil (0.5%, 1% and 1.5%) and actual marination time (6, 12 and 24 hours), second stage: modelling was conducted with RSM and third stage: the optimum point for the model). The experimental groups of first and second stage were examined organoleptic (view, texture, colour, odor, total assessment (TA) and colour analysis (L^*, a^* and b^* values) were conducted. The samples of third stage were analyzed in terms of microbiological (total mesophile aerobe bacteria (TMAB), psycrophile bacteria (PB), lactic acid bacteria (LAB), *Enterobacteria* (EB), yeast and mould (YM)), chemical (pH total volatile basic nitrogen value (TVB-N) and thiobarbituric acid number (TBA)), organoleptic (view, texture, colour, odor, total assesment) and colour criteria (L^*, a^* and b^* values). Rosemary oil amount and actual marination time (second stage sample) determined by the design were found as 0.8% and 19 hours, respectively. L^*, a^* and b^* values determined at this point were determined as 51.1, 1.79 and 8.03 respectively. Total mezophile aerobe bacteria count reached 7.9 $\log_{10}$ cfu/g in group B (third stage sample) on 98th day.

Key words: Response surface methodology, fish fillet, rosemary oil, shelf life

INTRODUCTION

Flesh of fish is rapidly influenced by physical and environmental factors starting from the moment it was fished unless necessary measures are taken. For this reason fresh fish must be preserved with great care until it is consumed and microorganisms must be prevented from reproducing during this period (Serdaroğlu and Felekoğlu, 2005). With this purpose several methods have been developed so far, one of which is known as marinades technology. Marinades are semi-canned products which can be preserved for a limited period of time. Acetic acid and salt does not only lengthen the storage period of fillets; it also improves its taste (Cadun *et al*., 2010). Some additives can be used during marination in order to improve taste and aroma. If these additives are antibacterial and antioxidant, the organoleptic characteristics of the product will be improved and its preservation period will be extended. With this purpose usage of natural essence oils are being widely used today (Can, 2011). Essential oils are natural antimicrobial ingredients which increase the shelf-life of products when used alone or in conjunction with other preservation methods. Rosemary essence oil is used as antioxidant with food due to its phenol content (Gutierrez *et al*., 2009). Scientific studies have revealed the antibacterial, antioxidant, antiviral and improving immune system features of rosemary (Serdaroğlu and Felekoğlu, 2005). The ideal amount of rosemary oil to be added varies from one researcher to another. The organoleptic problems caused by excessive addition of rosemary oil and that insufficient addition of it does not generate expected benefits is a very little known problem by researchers. Finding the optimum values obtained for different test conditions is a critical problem. Enhancement of experimental studies by statistical and mathematical techniques is common nowadays. One of the most useful experimental design techniques to achieve this aim is the central composite design (CCD) of Response Surface Methodology (RSM), an effective and versatile methodological tool for determination of optimum levels of the processing variables for the parameters studied. In addition, the equations obtained by this methodology can be used to develop different predictive models for the tested parameters. In addition, estimated ridge analysis involved with RSM can be used to calculate the levels of these processing variables that maximize or minimize the values obtained from experimental study (Sefa Dedeh *et al.*, 2003). In this study rainbow trout fillets are marinated with rosemary oil. The optimum points of rosemary oil amount added to the marination solution and its actual marination time are determined with response surface methodology and the shelf-life of fillets marinated under these conditions is studied. One purpose of this study is to extend the shelf

life of the product using the antioxidant and antibacterial features of rosemary oil; another goal is to optimize actual marination time and rosemary oil concentration to be added to the marination solution according to colour criteria (L^*, a^* and b^* values).

MATERIALS AND METHODS

In this study fresh rainbow trout with some 30-350 g weight was used as material. The fish which were procured from Sivas fish market were taken to the laboratory and their internal organs, heads and tails were separated; paying attention to ensure uniformity in terms of thickness and length, fillets which weighed 50 grams were obtained. The study consisted of three repetitions and this process was copied in all of them. Rosemary essence oil (Herbalox® Seasoning, which can be dissolved in both oil and water) was obtained from Kalsec® and acetic acid (99% pure) was obtained from Merck. For marination of trout fillets, a marination solution was prepared which included 8% acetic acid, 4% salt with a fleet: solution ratio of 1:2. The rosemary oil added to the solution was calculated over the weight of the fleet. The study was conducted in three stages. At the first stage fillets were separated into 9 different experimental groups depending on the amount of rosemary oil (0.5%, 1% and 1.5%) and actual marination time (6, 12 and 24 hours). At the end of marination process of test samples, organoleptic (view, texture, colour, odor, total assessment (TA) and colour analysis (L^*, a^* and b^* values) were conducted. At the second stage modelling was conducted with RSM so as to determine the optimum conditions the simultaneous optimum conditions provided by rosemary oil amount and actual marination time. Five test groups were formed in 5 different points (15 h actual marination time in solution containing 1% rosemary oil, 2.5 h actual marination time in solution containing 1% rosemary oil, 28 h actual marination time in solution containing 1% rosemary oil, 15 h actual marination time in solution containing 0.3% rosemary oil, 15 h actual marination time in solution containing 1.7% rosemary oil) and organoleptic (view, texture, colour, odor, TA) and colour analysis ((L^*, a^* and b^* values) were conducted. At the third stage of the study a test group was formed depending on the optimum point for the model (rosemary oil amount and actual marination time period in marination). Taking into consideration the actual marination time period in marination for this group, two test groups were formed based on rosemary oil amount (0% rosemary oil (K) and optimum rosemary oil level (B)) and packed with vacuum package at the end of the actual marination time period in marination and examined in terms of microbiological (total mesophile aerobe bacteria (TMAB), psycrophile bacteria (PB), lactic acid bacteria (LAB), *Enterobacteria* (EB), yeast and mould (YM)), chemical (pH total volatile basic nitrogen value (TVB-N) and thiobarbituric acid number (TBA)), organoleptic (view, texture, colour, odor, total assesment) and colour criteria (L^*, a^* and b^* values). The study consisted of three repetitions.

Microbiological analysis: In order to determine the bacteria count, a 25 g fish sample was weighed under aseptic conditions, 225 ml peptone water was added, and serial dilutions were prepared and planted with the pour plate technique. TMAB and PB were determined using Plate Count Agar (PCA, Merck code 1.05463), after incubation for 48 hours at 35°C, 7°C for 10 days, respectively (Harrigan, 1998). LAB were determined using Man Rogosa Sharpe Agar (MRS, Merck), after incubation at 30°C for 72 hours in anaerobic ambience (Harrigan, 1998). For members of the family *Enterobacteriaceae* (EB), 1.0 ml sample was inoculated into 5 ml of molten (45°C) Violet Red Bile Glucose Agar (Oxoid code CM 485). After setting, a 10 ml overlay of molten medium was added and incubation was carried out at 37 °C for 24 h. The large colonies with purple haloes were counted (Harrigan, 1998). Mould and yeast count were determined using Rose Bengal Chloramphenicol Agar (25±1 °C, 5 day)

Chemical analysis: The pH value was recorded using a pH meter (Crison Basic, 20). Fish samples were thoroughly homogenized with 10 ml of distilled water and pH was measured from homogenates. TVB-N values were determined by water steam distillation device according to the method reported by Varlık *et al*. (2004), while TBA number was measured by the method given by Tarlagidis *et al*. (1960).

Sensory Analyses: In order to determine sensory quality, the samples were evaluated by 7 experienced persons using grades between 1 and 5 (1 very bad, 2 bad, 3 normal, 4 good and 5 very good) in terms of view, color, odor, texture and total assessment (TA). For this purpose the samples taken from the groups were heated in a microwave oven and immediately subjected to sensory analysis (Kurtcan and Gönül, 1978).

Color measurement: Colorimetric measurements of samples were determined in triplicate using a Colorimeter (Minolta spectrophotometer CM 3500d, Japan). The color reading includes lightness (L), redness (a) and yellowness (b). The equipment was standardized with a white color Standard. Five replicate measurements were taken for each sample, following the guidelines for color measurements from the American Meat Science Association (Hunt and Kropf, 1987).

Statistical Analyses: Statistical analyses of the data were performed in two steps as well. At the first step, Statistical analysis System (SAS) software package was utilized. Intergroup values and the values belonging to in-group days were compared. The data were subjected to

variance analysis in terms of inter-variable interactions and fix effects suitable to 3x1x3x1 factorial design in the form of "number of repetition x time of sampling x test groups x number of samples from test groups examined at a time". According to General Linear Models (GLM) procedure Fisher's Least Significant Difference (LSD) test was used. Standard deviations were calculated (SAS, 1996). Alpha values was determined as 0.05. In terms of L^*, a^* and b^* values, RSM was used in order to determine the optimum values of rosemary oil and optimum test conditions under envisaged test conditions were found (Version 7.0) and the statistical analysis of the model was performed in the form of Analysis of Variance (ANOVA). In order to find optimum test conditions, Central Composite Experimental Design (CCD) was employed and effort was paid to find the points where response functions were optimum for two input variables (Rastogi and Rashimi, 1999). For CCD independent variables are shown at cubic level and their values can be shown as -1, 0 and 1 as coded factors. With this purpose the tests required by the model were conducted and the impact of independent variables on response functions was examined; equations which connect independent variables to dependent variables in terms of real or coded variables were obtained (Eq1).

$$Y = \beta 0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_{11}^2 + \beta_{22} X_{22}^2 + \beta_{12} X_{12} \text{ (Eq 1)}$$

In the developed equation, Y represents the value of response function, β_0 represents constant number, β_1 and β_2 represent linear impact, β_{11} and β_{22} represent quadratic coefficients, and X_1 and X_2 represent actual independent variables. The compliance of the obtained equation to the model is determined with F probability value. When F value is smaller than 0.05, it is said that the obtained data are in compliance with the model. Independent variables were exchanged between X_1 Actual Rosemary oil amount (1-1.7 ml/100 ml) and X_2 Actual marination time (6-28 h). Dependent variables Y_1 represents lightness (L), Y_2 redness (a) and Y_3 yellowness (b) variables.

RESULTS

Findings belonging to the experimental samples at first stage: In the first stage of the study, fillets were classified into 9 different groups depending on the amount of rosemary oil added to the marination solution and actual marination time and findings of colour analysis for these samples are given in table 1 whereas findings of organoleptic analysis are given in table 2. When organoleptic analysis results are evaluated statistically, it was determined that the amount of actual rosemary oil and actual marination time is not significant for days within groups and between groups ($p>0.05$).

Experimental design and analysis: At the second stage of the study, colour analysis findings belonging to the points determined according to the model using RSM are given in table 3 (Version 7.0). The independent variables are found as follows: amount of rosemary oil added to the solution is 0.8% and the actual marination time of the fillets is 19 hours.

Model compliance is determined by R^2 value approaching 1. This value shows the general harmony between the test and model values. R^2 values for L^*, a^* and b^* were found as 0.9979, 0.9775 and 0.9998. Prediction R^2 (R^2_{Pred}) shows the punctual harmony between predicted model and the experiment. R^2_{Pred} values for L^*, a^* and b^* were found as 0.9878, 0.8397, 0.9992. The adjusted R^2 arranges the R^2 values for the sample size and for the number of variables in the model. R^2_{adj} values for L^*, a^* and b^* were found as 0.9964, 0.9614, 0.9997. The object of response surface methodology is to detect which experimental parameters generate signals, which are large in comparison to do noise. Adequate precision measures signal to-noise ratio, a ratio greater than 4 is desirable. Adequate precision for L^*, a^* and b^* were found as 101.99, 26.28 and 301.42. These values show that signals are adequate for the model. The lack of fit measures the failure of the model to represent data in the experimental domain at points which are not included in the regression. The value of the lack of fit for regression of Eq 2, 3, 4. is not significant for L^*, a^* and b^*. Non-significant lack of fit is good and indicates that the model equation was adequate for predicting the colour variables under any combination of values of the variables. It was concluded that independent variables X_1 ve X_2 had quadratic impact on L^*, a^* and b^* and were in compliance with the model, and that it was significant since it was smaller than F value at probability (p) 0.05. This compliance can also be concluded from high R^2 values. In addition, the equations which show the harmony of the model to the test data are given below (Eq 2, 3 4).

$$Y_1 = 48.67 - 6.30^* X_1 + 0.69^* X_2 - 0.17^* X_1^* X_2 + 5.73^* X_1 2 - 0.021^* X_2 2 \text{ (Eq 2)}$$

$$Y_2 = 5.87 - 3.48^* X_1 - 0.16^* X_2 + 0.083^* X_1^* X_2 + 0.51^* X_1 2 - 1.31.10^{-4*} X_2 2 \text{ (Eq 3)}$$

$$Y_3 = 4.34 + 2.20^* X_1 + 0.10^* X_2 + 0.016^* X_1^* X_2 + 0.34^* X_1 2 - 2.91.10^{-3*} X_2 2 \text{ (Eq 4)}$$

ANOVA test and RSM were used depending on the obtained equations and the compliance of the model with test results; as a result, the best marination conditions for independent variables were found as 0.8% for rosemary oil amount and 19 hours as optimum actual marination time for the fillets. The L^*, a^* and b^* values at this point were respectively 51.1, 1.79 and 8.03 (Fig 1). Test groups were formed taking into consideration the points determined depending on experimental design and organoleptic analysis belonging to these experimental groups is given in table 4.

Findings belonging to the experimental samples at third stage: An experimental group (group B) was formed during the third stage of the study that belongs to the values at the optimum point determined according to the design (0.8% for rosemary oil amount and 19 h as optimum actual marination time for the fillets). Microbiological analysis findings, chemical analysis findings, organoleptic analysis findings and colour criteria for this group have been given in Table 5, 6, 7 and 8 respectively.

Table 1. Results of color analysis of experimental samples

Criteria	Oil amount (%)	Time (h)		
		6	12	24
L*	0.5	50±1.57 a, z	48.9±1.13 a, z	49.1±1.1 a, z
	1	51.4±1.01 a, z	50.7±0.98 a, z	51.6±0.87 a, z
	1.5	54.2±1.21 a, z	52.5±0.16 a, z	53.3±1.17 a, z
a*	0.5	3.42±0.02 a, z	2.21±0.21 b, z	2.09±0.41 b, z
	1	1.98±0.11 a, y	1.63±0.16 a, y	1.83±0.11 a, z
	1.5	1.76±0.36 a, y	1.31±0.06 a, y	1.15±0.03 b, y
b*	0.5	6.1±0.01 a, y	6.06±0.56 a, y	6.5±0.13 a, y
	1	8.1±0.06 a, z	8.4±0.42 a, z	8.01±0.26 a, z
	1.5	9.1±0.11 a, z	10.1±0.21 a, z	9.81±0.41 a, z

Table 2. Results of sensory analysis of experimental samples

Feature	Rosemary oil amount								
	%0.5			%1			%1.5		
	Time (h)								
	6	12	24	6	12	24	6	12	24
View	4±0.52	3.8±0.12	4.2±0.16	4.5±0.52	4.2±0.41	4.4±0.64	4.1±0.41	4.6±0.56	4±0.11
Texture	3.4±0.31	3.2±0.48	4.3±0.82	3±0.28	3.8±0.64	4±0.36	3.1±0.56	3.4±0.42	4±0.26
Color	3.4±0.26	3.5±0.62	3.2±0.42	3.5±0.42	4.4±0.66	4±0.54	3.2±0.38	3.6±0.46	3.8±0.44
Odor	4.4±0.11	4±0.46	4.2±0.22	4.4±0.36	4±0.72	3.8±0.46	3±0.16	3.2±0.48	3±0.16
TA	3±0.56	3±0.22	3.2±0.46	3.8±0.26	4.2±0.53	4±0.42	3±0.11	3.6±0.48	3.2±0.26

Table 3. The central composite experimental design for two variables: Rosemary oil (% v/v) and time (h) at three levels.

Trial number	Variables		Dependent variables		
	X_1 (x_1) Actual and coded Rosemary oil amount (%)	X_2 (x_2) Actual and coded Time (h)	Y_1	Y_2	Y_3
1	1.71 (1.414)	15 (0)	55.8	1	10.42
2	0.5 (-1)	24 (1)	49.1	2.09	6.52
3	1 (0)	15 (0)	51.2	1.79	8.02
4	1 (0)	2.27 (-1)	49	2.74	7.13
5	0.29 (-1.414)	15 (0)	52.1	3.1	6.01
6	1 (0)	15 (0)	51.2	1.79	8.05
7	1(0)	15 (0)	51.2	1.79	8.05
8	1.5 (1)	24 (1)	53.3	1.15	9.81
9	1 (0)	27.73(1.414)	46.1	0.8	8.01
10	1.5 (1)	6 (-1)	54.2	1.76	9.1
11	1 (0)	15 (0)	51.2	1.79	8.05
12	0.5 (-1)	6 (-1)	50	3.42	6.1
13	1 (0)	15 (0)	51	1.79	8.02

Table 4. Organoleptic analysis findings belonging to the points determined in the design.

Feature	Rosemary oil amount				
	%0.3	%1			%1.7
	Time (h)				
	15	2.5	15	28	15
View	4.2±0.46	2±0.22	4±0.56	4.4±0.58	4.2±0.22
Texture	3.8±0.46	1±0.12	4.4±0.42	4.6±0.22	4.2±0.36
Color	3±0.16	1±0.14	3.8±0.36	4±0.12	2.8±0.18
Odor	4±0.56	1±0.22	4.4±0.22	4.6±0.16	2±0.22
Total assessment	3±0.12	1±0.24	4.6±0.8	4±0.22	3±0.26

Table 5. Microbiological analysis findings belonging to the group determined with response surface method

Microorganism		Storage time (day)								
		0	**7**	**14**	**28**	**42**	**56**	**70**	**84**	**98**
TMAB	**B**	4.3±0.01 b, z	4.8±0.02 b, z	4.4±0.01 b, y	5.1±0.04 b, z	5.4±0.02 b, y	6.6±0.06 ab, z	7.1±0.02 a, z *	7.6±0.04 a, z *	7.9±0.01 a, z *
	K	5.1±0.04 b, z	5.6±0.02 b, z	6.3±0.01 ab, z	6.1±0.01 b, z	6.9±0.03 a, z	7.4±0.04 a, z			
PB	**B**	2.4±0.02 c, z	2.9±0.01 c, z	3.6±0.01 bc, z	3.2±0.02 c, z	4.1±0.03 b, z	4.9±0.01 b, z	5.6±0.01 a, z *	6.3±0.01 a, z *	6.9±0.02 a, z *
	K	3.6±0.02 a, z	3.9±0.01 a, y	4.6±0.02 a, y	4.7±0.02 a, y	5.3±0.06 a, y	6.1±0.01 a, y			
LAB	**B**	1.8±0.01 a, z	2.1±0.02 a, z	2.8±0.02 a, z	2.3±0.02 a, z	2.1±0.01 a, z	2.6±0.01 a, z	2.3±0.01 a, z *	2.6±0.02 a, z *	2.4±0.01 a, z *
	K	2.6±0.01 a, y	3.3±0.02 a, y	3.9±0.03 a, y	4.3±0.01 a, y	4.6±0.01 a, y	4.9±0.02 a, y			
MK	**B**	2.3±0.03 b, z	2.7±0.01 b, z	2.9±0.03 b, z	3.4±0.02 ab, z	3.9±0.02 a, z	4.1±0.02 a, z	3.7±0.01 a, z *	4.4±0.01 a, z *	4.6±0.02 a, z *
	K	3.6±0.03 b, y	3.9±0.01 b, y	4.5±0.01 a, y	4.1±0.01 ab, y	4.6±0.01 a, y	5.1±0.01 a, y			

Table 6. Chemical analysis findings belonging to the group determined with response surface method

Analysis		Storage time (day)								
		0	**7**	**14**	**28**	**42**	**56**	**70**	**84**	**98**
TVB-N	B	4±0.3 b, z	7.6±0.5 b, z	9.4±0.3 b, z	10.6±0.2 b, z	12.4±0.3 a, z	14.6±0.5 a, z	20.2±0.7 a, z	22.6±0.6 a, z	24.4±0.5 a, z
	K	4.3±0.4 a, z	9.6±0.7 a, z	10.3±0.5 a, z	18.4±0.3 a, z	24.4±0.6 a, y	30.2±0.7 a, y	*	*	*
TBA	B	0.66±0.32 a, z	0.79±0.6 a, z	0.71±0.5 a, z	0.69±0.2 a, z	0.72±0.5 a, z	0.99±0.4 a, z	1.75±0.6 a, z	1.86±0.2 a, z	1.98±0.3 a, z
	K	0.86±0.3 a, z	1.1±0.5 a, z	1.6±0.4 a, z	2.7±0.5 b, z	2.9±0.6 b, z	3.1±0.3 b, z	*	*	*
pH	B	4.1±0.1 a, z	4.3±0.3 a, z	4±0.2 a, z	4.3±0.1 a, z	4.5±0.1 a, z	4.6±0.3 a, z	4.3±0.1 a, z	4.6±0.2 a, z	4.4±0.3 a, z
	K	4.1±0.1 a, z	4±0.2 a, z	3.9±0.3 a, z	4.3±0.3 a, z	4.6±0.4 a, z	4.4±0.1 a, z	*	*	*

Table 7. Sensory analysis findings belonging to the group determined with response surface method

Feature		Storage time (day)								
		0	**7**	**14**	**28**	**42**	**56**	**70**	**84**	**98**
View	B	4±0.3 [a, z]	4.6±0.5 [a, z]	4.4±0.3 [a, z]	4.6±0.2 [a, z]	4.4±0.3 [a, z]	4.6±0.5 [a, z]	4.2±0.7 [a, z]	4±0.6 [a, z]	4.4±0.5 [a, z]
	K	4±0.4	3±0.7 [a, y]	3±0.5 [a, y]	3±0.3 [a, y]	2.8±0.6 [a, y]	2.8±0.7 [a, y]	*	*	*
Texture	B	3±0.32	3.6±0.6	3.2±0.5	3±0.2	3.4±0.5	3±0.4	3±0.6	2.8±0.2	3±0.3
	K	3±0.3	3±0.5	3.6±0.4	3.2±0.5	3±0.6	2.8±0.3	*	*	*
Color	B	4±0.1 [a, z]	3.8±0.3 [a, z]	4±0.2 [a, z]	4±0.1 [a, z]	4±0.1 [a, z]	4±0.3 [a, z]	4±0.1	4±0.2	4±0.3
	K	4.4±0.1 [a, z]	4±0.2 [a, z]	3.8±0.3 [a, z]	3.6±0.3 [a, z]	3±0.4 [a, y]	3±0.1 [a, y]	*	*	*
Odor	B	4.2±0.1 [a, z]	4.4±0.3 [a, z]	4.2±0.2 [a, z]	4±0.1 [a, z]	3.8±0.1 [a, z]	4±0.3 [a, z]	4.2±0.1	4.4±0.2	4±0.3
	K	3±0.1 [a, y]	3±0.2 [a, y]	3±0.3 [a, y]	3.2±0.3 [a, z]	2.6±0.4 [a, y]	2.8±0.1 [a, y]	*	*	*
TA	B	4.2±0.1 [a, z]	4±0.3 [a, z]	4±0.2 [a, z]	4.2±0.1 [a, z]	4±0.1 [a, z]	4±0.3 [a, z]	4±0.1 [a, z]	4.4±0.2 [a, z]	4.2±0.3 [a, z]
	K	4±0.1 [a, z]	4±0.2 [a, z]	3.8±0.3 [a, z]	4±0.3 [a, z]	3.6±0.4 [a, z]	4±0.1 [a, z]	*	*	*

Table 8. Color analysis findings belonging to the group determined with response surface method

Feature		Storage time (day)								
		0	**7**	**14**	**28**	**42**	**56**	**70**	**84**	**98**
L*	B	50.9±0.3 [b, z]	55.6±0.5 [b, z]	64.4±0.3 [b, z]	60.6±0.2 [b, z]	70.4±0.3 [a, z]	74.6±0.5 [a, z]	70.2±0.4 [a, z]	72.6±0.8 [a, z]	74.4±0.6 [a, z]
	K	48.3±0.4 [a, z]	50.6±0.7 [a, z]	58.3±0.5 [a, z]	54.4±0.3 [a, z]	60.4±0.6 [a, z]	68.2±0.7 [a, z]	*	*	*
a*	B	1.6±0.32 [a, y]	1.4±0.6 [a, y]	1.1±0.5 [a, y]	2±0.2 [a, y]	2.2±0.5 [a, z]	2±0.4 [a, z]	1.8	1.8±0.2	1.6±0.3
	K	3.6±0.32 [a, z]	3±0.6 [a, z]	2.1±0.5 [a, z]	3±0.2 [a, z]	2.6±0.5 [a, z]	2.8±0.4 [a, z]	*	*	*
b*	B	8±0.1	8.3±0.3	8.4±0.2	8.2±0.1	8.5±0.1	8.6±0.3	8±0.1	8.6±0.2	9±0.3
	K	7.1±0.1	7±0.2	7.9±0.3	7.3±0.3	7.6±0.4	7.4±0.1	*	*	*

Table 1, 5, 6, 7, 8: a, b: means within a column lacking a common superscript letter are different (P<0.05); z, y: means within a row lacking a common superscript letter are different (P<0.05).

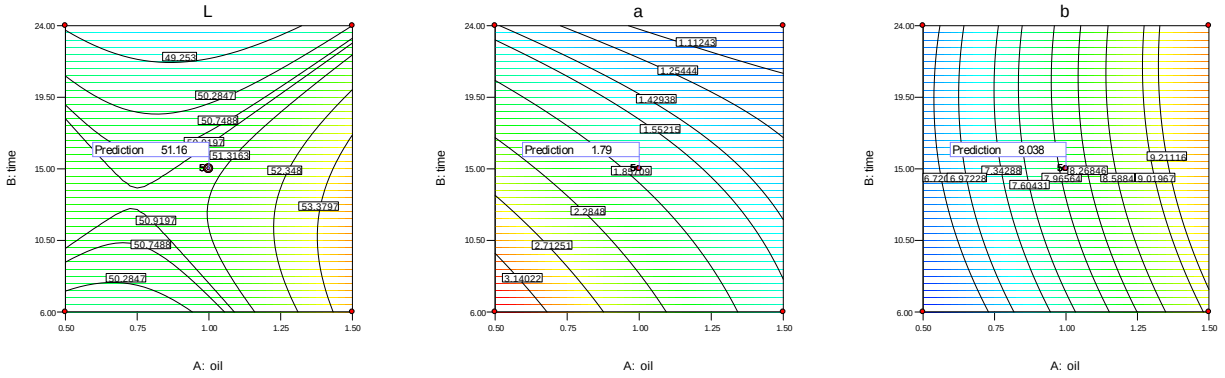

Fig.1 Response surface contour plot of L*, a*, b* showing interactive effect of rosemary oil amount (%) concentrations and time (h).

Discussion and conclusion: Organoleptic features of products on which marination process is applied play a very important role. These organoleptic features are also critical for the finalization of the marination process. Additives included in the marination solution as well as acid and salt content of marination solution are very important on organoleptic parameters. Antioxidant and antibacterial effects of the rosemary oil used in this study are reported by several authors (Burt, 2004, Gutierrez *et al*., 2009). Organoleptic features are as important as microbiological and chemical characteristics for the acceptability of a product. Therefore, the mechanisms with which rosemary oil, the microbiological and antioxidant features of which were studied before, affects the organoleptic parameters and colour criteria is studied since it is believed that the unique colour and odor of rosemary oil could be effective on these parameters. Among the experimental groups formed in the first stage of the study, it was determined that rosemary oil amount was not effective on the value of L^* but that the actual marination time decreased it. It was determined that actual marination time and amount of added oil are effective on a^* and b^* values. Candoğan *et al*. (2008), reported that actual marination time in different solutions increased a^* value whereas it decreased b^* value. When the actual marination time is examined in terms of organoleptic criteria, it is in compliance with another study. It is reported that carp fillets left to wait in brine with eugenole for different periods (6, 12 and 24 h) did not show significant difference in terms of organoleptic criteria (Can *et al*.2012). Table 3 gives the colour criteria for marinated products that belongs to the possible points determined by CCD. A high level of compliance was obtained between experimental data obtained with CCD and model values. It was observed that L^*, a^* and b^* varied in compliance with the quadratic model depending on the independent variables of the amount of oil and time . The possibility of non-compliance with the quadratic model for all three variables is less than one percent.

In the literature there are some studies for finding the best test conditions and food applications by means of applying RSM and CCD. Shan *et al*.(2010), RSM and CCD using optimum concentration and time for the hydrogen peroxide washing of common carp mince to produce acceptable kamaboko. Overall, common carp kamaboko whiteness increased but texture became weaker with as H_2O_2 concentration increased from 0.2 ml/100 ml–2.5 ml/100 ml and exposure time from 5 to 15 min. Strongest kamaboko texture was obtained at the washing condition of 0.2 ml/100 ml H_2O_2 for 10 min whereas best whiteness was obtained after treatment with 2.5 ml/100 ml H_2O_2 for 15 min. Dündar *et al*. (2012), adopted a five-factor CCD to study simultaneous effects of some processing variables such as NaCl (0–2%), fat (10–30%), ascorbic acid (0–600 ppm), cooking temperature (150–230 °C) and cooking time (5–15 min) on physicochemical properties and heterocyclic romatic amine (HAA) contents of cooked beef patties. Rosemary oil is an additive with antimicrobial effect and there are some studies putting forth that it extended shelf-life in preservation of fish flesh (Burt, 2004). In our study the number of TMAB was found to be lower in B group samples compared to K group samples. Duman *et al*. (2012), used rosemary oil in crawfish marination and emphasised that they detected a lower TMAB number compared to the control group. Cadun *et al*. (2008), reported that they detected lower TMAB in rosemary group compared to the control group in marination of rosemary-added pink deep water shrimps. The number of PB increased in both groups throughout the preservation period. The PB number determined in B group samples is determined as 1 log lower compared to K group samples. It was determined in a study the findings of which are in compliance with this paper that the number of PB in crawfish marinated with rosemary was lower compared to the control group throughout the preservation period (Duman *et al*., 2012). The *Enterobacteria* populations throughout the preservation period in B and K group samples were detected to be lower than the detectable level. This can be attributed to the presence of acetic acid and salt. Other studies emphasised that *enterobacteria* population in marinated fisheries is lower than detectable level (Cadun *et al*., 2008, Can *et al*., 2012),. LAB population remained constant in B group samples. Some in vitro studies found out that rosemary oil had partial inhibitor effect on LAB (Frenandez *et al*., 2008). In their research on antimicrobial effects of different essence oils, Viuda-Martos *et al*., (2008), stated that the most effective one on LAB was rosemary oil which was followed by sage, thyme and clove. The difference in YM population between groups K and B was found to be significant throughout the preservation period. In studies conducted in culture environment, it was determined that essence oils, waters and extracts of rosemary and thyme showed antifungal effects at different rates (Burt, 2004, Gutierrez *et al*., 2008). Rasooli and Owlia (2005), reported that thyme oil was effective on ferment-mould. Their findings are similar to the findings of this paper. TVB-N values are used as an indicator of decay of water products. TVB-N value in K group samples was found to be higher in comparison with the B group. This is believed to be the result of the fact that microbial development in rosemary added group is low. Cadun *et al*., (2008), reported that TVB-N value in rosemary added group is low. Some studies (Cadun *et al*., 2008, Serdaroğlu and Felekoğlu, 2005) emphasised that rosemary oil has antioxidant effect on fisheries. In our paper TBA value did not show much difference in B group samples throughout preservation period. Similar results are reported by other authors (Cadun *et al*., 2008, Serdaroğlu and Felekoğlu, 2005).

When B group samples and K group samples are evaluated in terms of organoleptic criteria, B group samples received higher scores in terms of colour, odor, view and TA. Some studies (Cadun *et al*., 2008, Can *et al*., 2012) found out that rosemary yielded positive results in organoleptic terms on fish and fish flesh products.

Throughout preservation period L^* and b^* values increased and a^* value decreased in both groups. It is believed that this result is caused by marination process. Similar studies were also reported by other authors (Cadun *et al*., 2008, Candoğan *et al*., 2008).

Addition of some additives for extending the preservation period of fish products is a widely used method. Several preliminary tests are being conducted in order to determine the usage ratios of these ingredients; after which, groups are being identified taking other studies into consideration. Therefore, the purpose of our study was to form less test groups and reach conclusions faster; hence, save time and labour. All these taken into account, RSM experimental design was employed in order to find optimum values in test conditions; as a result, the optimum test group was formed under required test conditions and the employability of this method was studied. Under the light of obtained findings, it was concluded that light will be shed to studies that follow.

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