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Full Length Research Paper

Use of response surface methodology (RSM) to investigate the effect of carp (*Cyprinus carpio*) fillets cooked at different temperature and oil amount

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This study was performed to investigate the effect of time and oil content on live microorganisms by periodically measuring the central temperature in heat treatment applied to carp fillets treated with different oil ratios and to determine the quality criteria of fillets during preservation period. Fillets were classified into five groups according to oil ratios, they were treated and the total number of mesophile aerobic bacteria (TMAB) was determined by measuring central heats of fillets in periods suitable to design. Furthermore, after the heat treatment, the fillets were stored at +4°C and examined microbiologically, chemically, statistically and sensory evaluation of preservation period was done. The effect of baking period and amount of oil added to the fillets on central temperature of the fillets and TMAB were investigated by response surface methodology based on Box-Behnken experimental design, by Box-Behnken experimental design adapted to R_1 (central temperature) quadratic model (R^2= 0.8482), and R_2 (TMAB) linear model (R^2=0.79).

Key words: Carp fillet, sensory quality, microbiological and chemical quality, oil amount, RSM.

INTRODUCTION

Today, one of the most important problems that humanity faces is malnutrition. While living standards of some societies have been increasing as parallel to the rapidly developing technology of our era, significant amount of world population suffers from hunger and many people die as a consequence in undeveloped countries (Emblem, 2000). As parallel to the increase in population, production of animal proteins must be increased. Increasing fish production by effective use of marine and inland water potentials is one of the best alternatives in meeting the protein deficit (Emblem, 2000). Fish has high protein content and it is also rich in terms of vitamin and mineral content. It has a high digestibility, high oil acid ratio and low calorie (Arslan, 2002). Fish is a good food source in terms of essential amino acids. As fish can be freshly consumed in different ways (fried, baked, steamed, etc.), it can also be consumed by being turned into various fish products (Varlık et al., 2004). Various technological processes such as smoking, marinating, and freezing are applied to preserve fish for longer periods (Varlık et al., 2004). Heat treatment applied to fisheries is one of these preservation methods. It provides inactivation of microorganisms which causes degradation in fish and increases preservation period. In addition, it gives flavor and aroma to fish (Rosnes et al., 1999). Resistance of microorganisms to heat is directly related to the structure of the food. Salt concentration, pH and amount of oil can be counted among these factors. It is known that the amount of oil causes heat resistance of microorganism to increase (Erol, 2007).

Preserving food by applying heat treatment is one of the oldest methods of preservation. While applying heat treatment, factors such as the type of food, nutrition content, additives and time should be taken into account. Long-term heat treatment applications may cause negative

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Table 1. The composition of experimental samples.

Group	Control	A	B	C	D
Salt	+	+	+	+	+
Oil (%)	0	5	10	15	20

effects on food constituents. The extension of preservation period of *Cyprinus carpio* by applying heat treatment and increasing fish consumption as a convenience food were aimed in this study. *C. carpio* is not a favorite fish for consumption. However, it is raised too much in Turkey. If its consumption as a convenience food can be provided, it is considered that this might contribute to the economy. Moreover, it is also considered that the study might be an alternative to the frying. Frying can cause serious problems that endanger human health but still it is highly preferred as a consumption method. By combining conventional baking method with oil addition in this study, it was attempted to produce an alternative method. Oil amount was changed in the study and heat treatment was applied. The purpose of changing oil content is that if the fillets were treated with sauces enriched by spices, these sauces may contain different amount of oil. In addition, heating period and temperature applied to fish is very important. Fish contains essential amino acids which are very important for our nutrition. Excess heat can give good results in terms of microbial safety but it may also cause nutritional value to decrease. It is known that nutrient composition increases resistance of microorganisms to heat.

This study was performed to determine the heat treatment applied to *C. carpio* fillets containing various amounts of oil and to specify quality criteria during preservation. Mathematical modeling can be used to investigate factors affected during the cooking of foods. Prediction models for quality change can be created by applying kinetic models to experimental data. Connecting quality parameters to cooking methods allows for the optimization of the cooking process as a whole. For example, Haiqing et al. (1999) created a model that successfully predicted transient temperature and moisture distributions during convection cooking of chicken patties. From these prediction models, conclusions can be made on the optimal time-temperature combination for this specific food product. This applies to the effect of processing conditions on food quality attributes. One of these techniques which has the highest usability among others is response surface methodology (RSM) (Yu-long and Han, 2005; Açıkel et al., 2010). In this study, by using RSM, the effects of heat treatment and various oil amount applied to same amount of fish on the total number of aerobic bacteria were investigated. Accordingly, the optimization of experimental conditions and the determination of the optimum amount of oil to be added were attempted. The baking treatments of fish preservation are very old technique and RSM is used on optimization of processing. But, both baking treatment technology and RSM with optimization together has not been used lately in fish fillets. To the authors' knowledge there is no optimization study concerning *C. carpio* fillets. This study aimed at increasing the product quality by optimization of oil amount and heat treatment period.

MATERIALS AND METHODS

C. carpio carpio L., 1758 fished from Keban Dam Lake (Turkey, in Semtember 2011) were immediately transported to the laboratory under cold chain. The study comprised three trials and in each trial, the fishes were 10 kg. The fishes from Keban Dam lake was brought to the laboratory in cold chain. The heads of the fishes were cut, the fishes were skinned and their internal organs were removed. Then, from approximately 50 g weight and 6 x 6 x 4 cm (length x thickness) size, 40 fillets were prepared after the fishes were cleaned from muscles and bones, for one trial. The fillets obtained were water washed and then drained.

Preparation of experimental samples

Dry-salting was applied to the fillets (3%, over fish weight). The fillets were classified into 5 groups as; Control group: 0% oil; Group A: 5% oil; Group B: 10% oil; Group C: 15 % oil and Group D: 20% oil (amount of oils were calculated considering fish weight) (Table 1). The control group fillets were put on a tray in a way that they had no contact to each other. Oil containing groups were also put on a tray and the same amount of oil calculated before was applied onto the fillets and the remaining oil was poured onto the tray (size 48 x 52 cm) before cooking. The tray was placed horizontally at 19 cm above the bottom of the oven (just in the middle of the oven). The amount of baking samples per batch was 12 fillets (the groups cooked separately). Then, the fillets were baked for 55 min in an conventional oven (Arcelik MF 2009, 38 x 50 x 54 cm (height x width x depth) size) whose temperature was adjusted to 150°C. Central temperatures of the fillets were measured by a K-type thermocouple (HI 9057 KJT, Hanna Instruments, Portugal). The sensory appearance of the product during baking was also taken into account. Total number of mesophilic aerobic bacteria was periodically measured during baking. In addition, total number of mesophilic bacteria was also measured in different periods by RSM statistical method. After baking was finished, top of the tray was covered by an sterile aluminum folio and the tray was cooled in deep freeze until the central temperatures of the fillets decreased to +4 °C and then the fillets were packed under aseptic conditions (each samples one for sterile gloves and pens used to prevent cross contamination, under bunsen burner) then with vacuum. The vacuumed fillets were preserved at +4°C and then microbiological, chemical and sensory evaluations were done at the 0th, 7th, 14th, 21st, 28th, 35th and 42nd days. The study was repeated three times and the results were evaluated by taking the average of three values.

Microbiological analyses

Microbiological analyses were performed in two steps. At the first step, it was done during baking of the fillets whereas it was performed periodically at the second step during the preservation. The samples were taken in periods chosen according to the method described above (RSM) and total number of mesophilic aerobic bacteria. Plate count agar (Oxoid CM 325) was utilized. The plates

were incubated at 35°C for 48 h and the colonies formed were counted (Harrigan and Mccance, 1976).

At the second step, on the 0th, 7th, 14th, 21st, 28th, 35th and 42nd days of preservation, plate count agar (PCA) (at 30±1°C for 72 h), plate count agar (PCA) (at 7±1°C for 7 days), violet res bile glucose agar (VRBGA) (at 37±1°C for 24 h), tributyrin agar (at 30±1°C for 3 days), iron agar (at 15±1°C for 3 days) were used for counting mesophilic aerobic bacteria, psychrophile bacteria, *enterobacteria*, lipolitic bacteria and H_2S producing bacteria, respectively (Harrigan and Mccance, 1976).

Chemical analyses

Total volatile basic nitrogen (TVB-N) amounts were determined by water steam distillation device according to the method reported by Varlık et al. (2004), total number of thiobarbituric acid by the method proposed by Tarlagidis et al. (1960), number of peroxide by modified Wheeler method (Varlık et al., 1993) and free oil acid amount according to the method specified by Yetim (2002).

Sensory analyses

In order to determine sensory quality, the cooked fish samples were evaluated by 7 experienced persons (selected according to ISO 8586-1 standard) using grades between 1 and 5 (1 very bad, 2 bad, 3 normal, 4 good and 5 very good) in terms of color, smell, taste, texture and general inclination. Cooked fish fillets were prepared by steaming for 10 to 20 min at 98°C (Ojagh et al., 2010).

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 3 x 1 x 3 x 1 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 (Anonymous, 1996). Alpha values was determined as 0.05. At the second step, experimental design was done by using response surface methodology (RSM).

Experimental design

Response Surface Method (RSM) based on Box-Behnken factorial design examines the relationship between input variables and one or more output variables. RSM provides design by the help of polynomials matched to the data obtained by designed experiments. It is the combination of statistical techniques and mathematical models used in analysis of problems in case there are many variables affecting the method variable. It is commonly used in formulating a new product, enhancement of design, process design and process development. In this study Box–Behnken factorial design with two factors and two levels, a total of 17 runs were used to optimize the range and levels of chosen variables.

Even though second order polynomials are usually used in complex system modeling in RSM, it is also possible to use higher order polynomials:

$$R = \beta 0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i<j}^{k} (\beta_i X_i X_j) + \sum_{i=1}^{k} (\beta_{ii} X_i X_i) \quad (1)$$

Formation of response surface model is realized by the prediction of β coefficients shown above. Prediction of these coefficients is possible by least squares regression. Design Expert 7.0.0 (trial version, Stat Ease Inc.,Minneapolis, USA) computer program was used for determination of the coefficients of Equation 1.

RESULTS

Microbiological analysis results

Microbiological analysis results during storage of samples

Total numbers of mesophilic aerobic bacteria, psycrophyle bacteria, *enterobacteria*, lipolythic bacteria and H_2S producing bacteria were determined on raw fillets (before grouping) as 6.36 $\log_{10}$ cfu/g, 3.23 $\log_{10}$ cfu /g, 1.8 $\log_{10}$ cfu /g, 2.8 $\log_{10}$ cfu /g and 2.1 $\log_{10}$ cfu /g, respectively. In microbiological analysis done during preservation total numbers of mesophilic aerobic bacteria, *Enterobacteria*, lipolythic bacteria and H_2S producing bacteria were determined below <10 cfu /g in all groups on all sampling days.

Chemical analysis results

The findings from chemical analysis are presented in Table 2. As the samples were assessed in terms of TVB-N, the difference among the groups and in-group days was statistically insignificant ($p>0.05$). The number of TBA showed an increase during the preservation period. The difference between Group control and A (5% oil) were found to be statistically significant ($p<0.05$) starting from the 14th day of preservation. The highest PV values among the samples belonged to Group D (20% oil) and the difference between in-group days was statistically significant ($p<0.05$). There was no significant change in FFA value determined on the samples ($p>0.05$) during preservation period.

Sensory analysis results

Sensory analysis findings are given in Table 3. As color criteria was assessed, it was found that the difference between Groups A (5% oil) and B (10% oil) (from 28th day) was statistically significant ($p<0.05$). Comparing the experimental samples in terms of taste, it was found that the differences among Group control, A (5% oil) and B (10% oil) and the difference between Groups C (15% oil) and D (20% oil) were statistically significant ($p<0.05$). Moreover, Group C (15% oil) and D (20% oil) got the lowest grades. Group D (20% oil) got the lowest grades in terms of general liking. On the last day of preservation period, the difference between Group D (20% oil) and the other groups was found to be statistically significant ($p<0.05$).

Table 2. Results of chemical analysis of carp filets stored at 4°C.

Analysis	Group	Storage time (day)						
		0	7	14	21	28	35	42
TVB-N(mg N/100 g)	Control	12.03±0.06^{a,z}	11.56±0.04^{a,z}	12.24±0.03^{a,z}	13.28±0.07^{a,z}	14±0.09^{a,z}	14.12±0.06^{a,z}	14.16±0.06^{a,z}
	A	12.03±0.03^{a,z}	12.14±0.11^{a,z}	10.22±0.13^{a,z}	12.12±0.09^{a,z}	11.56±0.08^{a,z}	14.12±0.11^{a,z}	14±0.9^{a,z}
	B	12.03±0.9^{a,z}	12.56±0.13^{a,z}	11.98±0.18^{a,z}	12.22±0.11^{a,z}	13.34±0.13^{a,z}	14.22±0.9^{a,z}	14±0.13^{a,z}
	C	12.03±0.16^{a,z}	12.56±0.11^{a,z}	11.42±0.09^{a,z}	12.22±0.12^{a,z}	12.56±0.1^{a,z}	14.12±0.11^{a,z}	14.28±0.13^{a,z}
	D	12.03±0.11^{a,z}	12.56±0.16^{a,z}	12.22±0.11^{a,z}	14.12±0.16^{a,z}	14±0.4^{a,z}	14.18±0.8^{a,z}	13.48±0.12^{a,z}
TBA (mg MDA/kg)	Control	0.15±0.09^{b,z}	0.27±0.16^{b,z}	0.83±1.1^{a,y}	0.96±0.09^{a,y}	0.92±0.9^{a,y}	1.02±1.1^{a,y}	1.11±0.16^{a,y}
	A	0.15±0.01^{b,z}	0.33±0.04^{b,z}	0.92±0.1^{a,y}	0.83±0.31^{a,y}	0.89±0.03^{a,y}	1.14±0.12^{a,y}	1.23±0.11^{a,y}
	B	0.15±0.01^{b,z}	0.42±001^{b,z}	1.02±0.3^{a,zy}	1.56±0.18^{a,z}	1.83±0.21^{a,z}	1.92±0.16^{a,zy}	2.9±0.13^{a,z}
	C	0.15±0.03^{b,z}	0.56±0.02^{b,z}	1.33±0.21^{ab,z}	2.21±0.09^{a,z}	2.59±0.1^{a,z}	2.98±0.12^{a,z}	2.93±0.21^{a,z}
	D	0.15±0.01^{c,z}	0.82±0.03^{c,z}	1.64±0.19^{b,z}	2.38±0.21^{a,z}	2.63±0.16^{a,z}	3.01±0.14^{a,z}	3.46±0.18^{a,y}
PV (milimol O_2/kg)	Control	1.17±0.21^{b,y}	1.3±0.19^{b,y}	1.21±0.14^{b,y}	1.37±0.16^{b,y}	2.63±0.13^{a,y}	2.86±0.21^{a,y}	3.21±0.18^{a,y}
	A	1.4±0.12^{b,z}	1.57±0.24^{b,y}	1.92±0.13^{b,zy}	2.76±0.22^{a,zy}	3.33±0.19^{a,z}	3.5±0.12^{a,z}	3.86±0.14^{a,zy}
	B	1.51±0.16^{b,z}	1.87±0.56^{b,z}	2.21±0.18^{ab,z}	3.12±0.31^{a,z}	3.5±0.21^{a,z}	3.98±0.36^{a,z}	4.53±0.22^{a,z}
	C	1.42±0.21^{b,z}	1.98±0.42^{b,z}	2.19±0.21^{ab,z}	3.21±0.18^{a,z}	3.51±0.24^{a,z}	4.17±0.23^{a,z}	4.98±0.24^{a,z}
	D	1.46±0.18^{c,z}	2.26±0.21^{c,z}	4.01±0.42^{ab,z}	3.98±0.22^{b,z}	4.13±0.18^{a,z}	5.01±0.42^{a,z}	5.26±0.33^{a,z}
FFA (% oleik acid)	Control	0.67±0.11^{a,z}	0.71±0.13^{a,z}	0.75±0.18^{a,z}	0.86±0.12^{a,z}	0.73±0.22^{a,z}	0.81±0.22^{a,z}	0.86±0.21^{a,z}
	A	0.75±0.13^{a,z}	0.83±0.16^{a,z}	0.76±0.22^{a,z}	0.92±0.14^{a,z}	0.88±0.19^{a,z}	0.72±0.13^{a,z}	0.88±0.18^{a,z}
	B	0.89±0.21^{a,z}	0.85±0.12^{a,z}	0.66±0.11^{a,z}	0.53±0.21^{a,z}	0.75±0.21^{a,z}	0.68±0.26^{a,z}	0.83±0.26^{a,z}
	C	0.82±0.16^{a,z}	0.56±0.13^{a,z}	0.76±0.19^{a,z}	0.74±0.19^{a,z}	0.82±0.19^{a,z}	0.41±0.11^{a,z}	0.93±0.18^{a,z}
	D	0.91±0.18^{a,z}	0.69±0.21^{a,z}	0.89±0.13^{a,z}	0.61±0.21^{a,z}	0.56±0.14^{a,z}	0.98±0.18^{a,z}	0.80±0.14^{a,z}

Each value is the mean of three samples taken from two replicate experiments (n: 3 x 2: 6), Error bars show m± SD ; 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).

RSM results

Heat treatment was applied to *C. carpio* fillets for 55 min. Since the fillets were baked at the end of this period, 55 min were chosen as limit value for experimental design (Table 4). The amount of oil was changed between 0 and 20%. Total number of mesophilicic aerobic bacteria and central temperature values were examined for the experimental conditions proposed by RSM. In this way, the time period at which the total number of mesophilicic bacteria was under detectable limit (<10) was determined for optimum experimental conditions. Moreover, by using experimental design, equations relating the number of micro-organism and central temperature to input variables were derived.

According to the summary of variance (ANOVA) results, R^2_{adj} was found as 0.8021. F value (13.97) used to justify the model and probability values (P<0.005) were acceptable in terms of model conformity. The variation of central temperature with baking time and the amount of oil added fits to the third order model and is given by Equation 2.

$$R_1=57.05+46.75x_1-37.01x_2-1.24x_1x_2-7.39x_2^2+7.61x_2^2+26.30x_1^2x_2+0.50x_1x_2^2-8.67\ x_1^3+9.57x_2^3 \quad (2)$$

Where, R_1 represents central temperature (°C), x_1 stands for time (min) and x_2 denotes amount of oil added (%).

In the equation above, *Cyprinus carpio* fillets were affected in first, second and third order by central temperature and amount of oil added. Besides, time and oil amount (second order) affects together. The variation of central temperature

Table 3. Results of sensory analysis of carp filets stored at 4°C.

Feature	Group	Storage time (day)						
		0	7	14	21	28	35	42
Colour	Control	4.62±0.1^{a,z}	4.37±0.1^{a,z}	4.68±0.2^{a,z}	3.12±0.3^{a,z}	2±0.1^{b,y}	1±0.3^{c,y}	1±0.3^{c,y}
	A	4.56±0.2^{a,z}	4.42±0.2^{a,z}	4.26±0.3^{a,z}	4.12±0.1^{a,z}	4.16±0.3^{a,z}	3±0.1^{b,z}	2.16±0.3^{b,z}
	B	4.42±0.4^{a,z}	4.16±0.1^{a,z}	4.16±0.2^{a,z}	3.86±0.1^{a,z}	3±0.1^{b,z}	3.1±0.3^{b,z}	2±0.1^{c,z}
	C	4±0.1^{a,z}	3.82±0.3^{a,zy}	3.56±0.1^{a,y}	3±0.3^{a,z}	2±0.1^{b,y}	1±0.3^{c,y}	1±0.1^{c,y}
	D	3.8±0.1^{a,z}	3.14±0.2^{a,y}	3.16±0.4^{a,y}	3±0.3^{a,z}	1±0.1^{b,x}	1±0.3^{b,y}	1±0.4^{b,y}
Odour	Control	4.68±0.1^{a,z}	4.14±0.1^{a,z}	4.21±0.3^{a,z}	3±0.1^{a,z}	2±0.6^{b,y}	2±0.1^{b,y}	1±0.1^{c,y}
	A	4.56±0.1^{a,z}	4.42±0.16^{a,z}	4.14±0.3^{a,z}	4±0.1^{a,z}	3.52±0.11^{a,z}	3±0.1^{a,z}	2±0.1^{b,z}
	B	3±0.1^{a,y}	2.9±0.11^{a,y}	3.04±0.3^{a,z}	3.56±0.1^{a,z}	3±0.3^{a,z}	3±0.1^{a,z}	2±0.5^{b,z}
	C	3±0.1^{a,y}	3.12±0.3^{a,zy}	2.98±0.2^{a,y}	2.56±0.4^{a,y}	2.2±0.11^{a,y}	2±0.1^{a,y}	1±0.1^{b,y}
	D	2.5±0.08^{a,x}	2.51±0.3^{a,y}	2.43±0.3^{a,y}	1.9±0.17^{a,y}	1.62±0.6^{a,x}	1±0.1^{b,x}	1±0.1^{b,y}
Taste	Control	3±0.5^{a,z}	2.98±0.6^{a,zy}	3±0.6^{a,z}	2.92±0.1^{a,z}	2±0.1^{b,y}	2±0.3^{b,z}	2±0.6^{b,z}
	A	3.46±0.1^{a,z}	3.13±0.21^{a,z}	3±0.1^{a,z}	3±0.1^{a,z}	3.12±0.1^{a,z}	2±0.1^{b,z}	2±0.6^{b,z}
	B	3.93±0.7^{a,z}	3.56±0.5^{a,z}	3.42±0.11^{a,z}	3.13±0.1^{a,z}	3±0.1^{a,z}	2±0.1^{b,z}	2±0.1^{b,z}
	C	2.75±0.3^{a,y}	2.42±0.1^{a,y}	2.96±0.3^{a,zy}	2.56±0.5^{a,zy}	2.48±0.2^{a,zy}	1±0.1^{b,y}	1±0.1^{b,y}
	D	2.75±0.7^{a,y}	2.75±0.3^{a,y}	2.56±0.5^{a,y}	2.32±0.3^{a,y}	2.12±0.1^{a,y}	1±0.1^{b,y}	1±0.1^{b,y}
Texture	Control	3.56±0.3^{a,z}	3.21±0.6^{a,z}	3±0.11^{a,z}	2±0.1^{b,z}	1±0.1^{c,z}	1±0.1^{c,z}	1±0.1^{c,z}
	A	3.42±0.3^{a,z}	3.13±0.1^{a,z}	3.2±0.5^{a,z}	2.56±0.6^{a,z}	2.42±0.3^{a,z}	2±0.1^{b,z}	2±0.1^{b,z}
	B	3.11±0.1^{a,z}	3±0.3^{a,z}	2.8±0.5^{a,z}	2.2±0.2^{b,z}	2.98±0.11^{a,z}	2.5±0.6^{ab,z}	2±0.1^{b,z}
	C	3±0.6^{a,z}	3±0.6^{a,z}	2.21±0.6^{b,z}	2.98±0.3^{a,z}	2.22±0.6^{b,z}	2.5±0.5^{ab,z}	2±0.1^{b,z}
	D	3.2±0.2^{a,z}	3.3±0.6^{a,z}	2.42±0.6^{b,z}	3±0.11^{a,z}	3±0.1^{a,z}	3±0.3^{a,z}	3±0.1^{a,z}
Total assesment	Control	4±0.6^{a,z}	3.92±0.6^{a,z}	3.56±0.1^{a,z}	3.72±0.4^{a,z}	3±0.3^{a,z}	2±0.3^{b,y}	2±0.6^{a,z}
	A	4.2±0.1^{a,z}	4±0.1^{a,z}	3.98±0.5^{a,z}	3.56±0.4^{a,z}	3.12±0.3^{a,z}	3±0.5^{a,z}	2±0.4^{a,z}
	B	3.96±0.1^{a,z}	3.56±0.5^{a,z}	3±0.11^{a,z}	2±0.4^{b,y}	2±0.1^{b,y}	2±0.1^{b,y}	2±0.4^{a,z}
	C	3.83±0.3^{a,z}	3.98±0.2^{a,z}	2.83±0.1^{a,z}	2.21±0.1^{ab,y}	2±0.1^{b,y}	2±0.1^{b,y}	2±0.1^{a,z}
	D	3±0.3^{a,y}	2.22±0.1^{b,y}	2±01^{b,y}	1±0.1^{c,x}	1±0.1^{c,x}	1±0.1^{c,x}	1±0.1^{b,y}

Each value is the mean of three samples taken from two replicate experiments (n: 3 x 2: 6), Error bars show m± SD ; a, b, c: means within a column lacking a common superscript letter are different (P<0.05); z, y, x: means within a row lacking a common superscript letter are different (P<0.05).

by oil amount and baking time is shown by 2D and 3D graphs (Figures 1 and 2). Figure 3 shows the conformity between the experimental results obtained and the results expected by RSM. Accordingly, it can be concluded that the experimental data fits to the model.

According to the summary of variance (ANOVA) results, R^2_{adj} was found as 0.7539. F value (25.51) used to justify the model and probability values (P<0.005) were acceptable in terms model conformity.

The variation of the number of microorganism with baking time and the amount of oil added fits to linear model and is given by Equation 3.

$$R_2 = 3.70 - 3.42x_1 + 0.99\ x_2 \quad (3)$$

Where, R_2 represents total mesophilic aerobic bacteria.

Table 4. The variation in central temperature and the number of microorganism for the design obtained by Box-Behnken design.

Run	Time (min)	Group	Central temparature value (R_1)	Number of microorganism (R_2)
1	27.5	D	40.6	4.86
2	55	Control	96.1	*
3	41.25	A	94	*
4	0	Control	15.9	6.36
5	13.75	B	28.9	6.43
6	0	D	15.9	6.36
7	0	B	15.9	6.36
8	55	D	90.1	*
9	27.5	Control	94	*
10	27.5	C	38.6	5.72
11	55	B	92	*
12	27.5	A	85	4.50
13	41.25	C	73.7	3.46
14	13.75	C	27.4	6.07
15	41.25	B	74	1.47
16	13.75	A	51	5.63
17	27.5	B	38.7	5.66

*$\log_{10}$ cfu/g <10

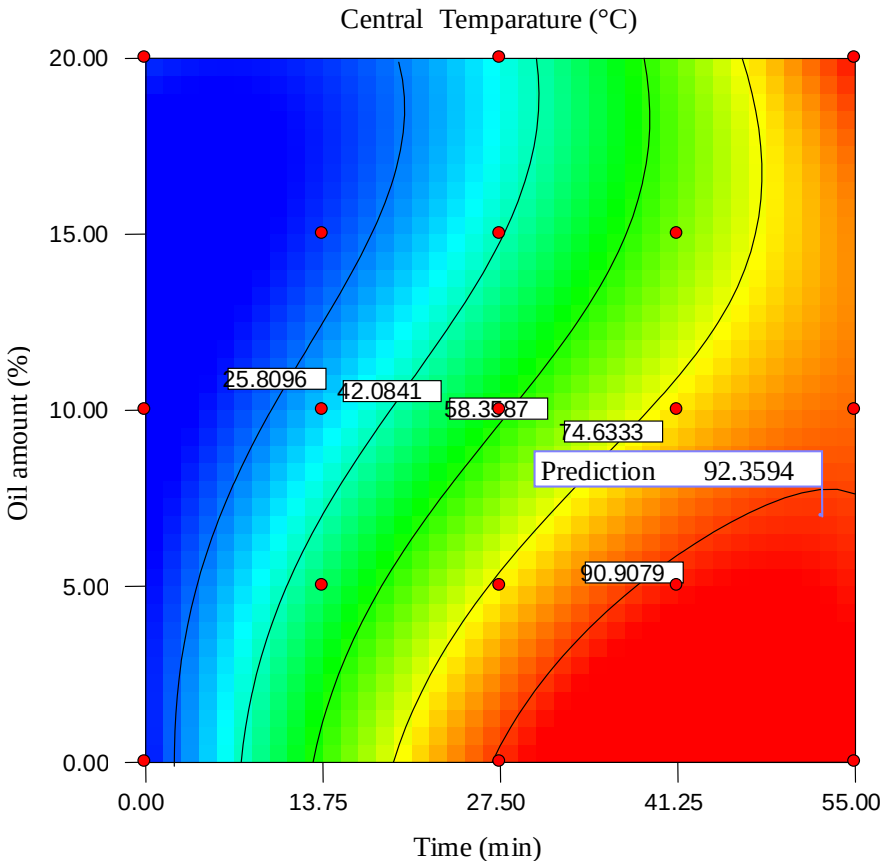

Figure 1. Response surface graph (2D) for R_1 model which shows the variation of central.

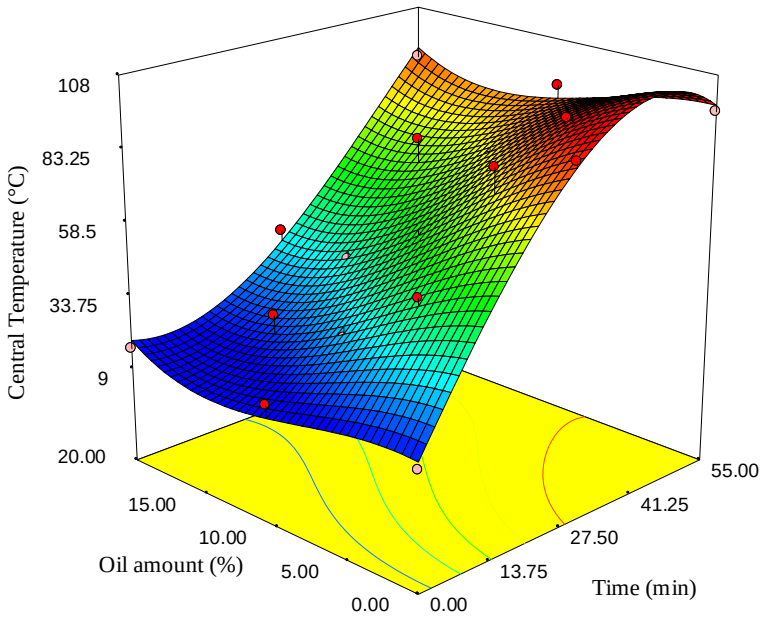

Figure 2. Response surface graph (3D) for R_1 model which shows the variation of central.

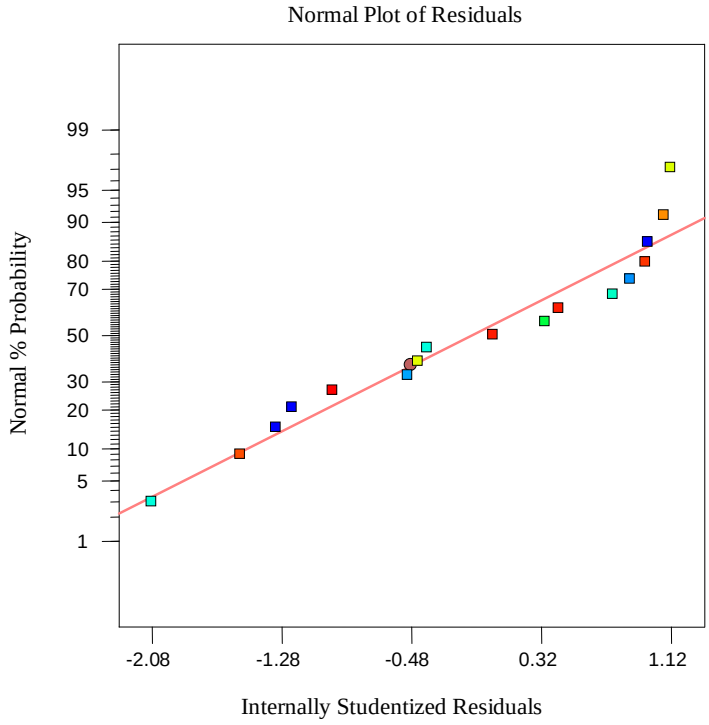

Figure 3. The conformity of experimental results and expected results by RSM.

It can be seen from Equation 3 that the number of microorganism was linearly affected by the time and oil amount. The variation of number of microorganism by oil amount and baking time is shown by 2D and 3D graphs. Accordingly, as the amount of oil added to the fillets increased, the time for number of mesophilicic bacteria decreased below the detectable limit gets longer (Figures 4 and 5). Figure 6 shows the conformity between the

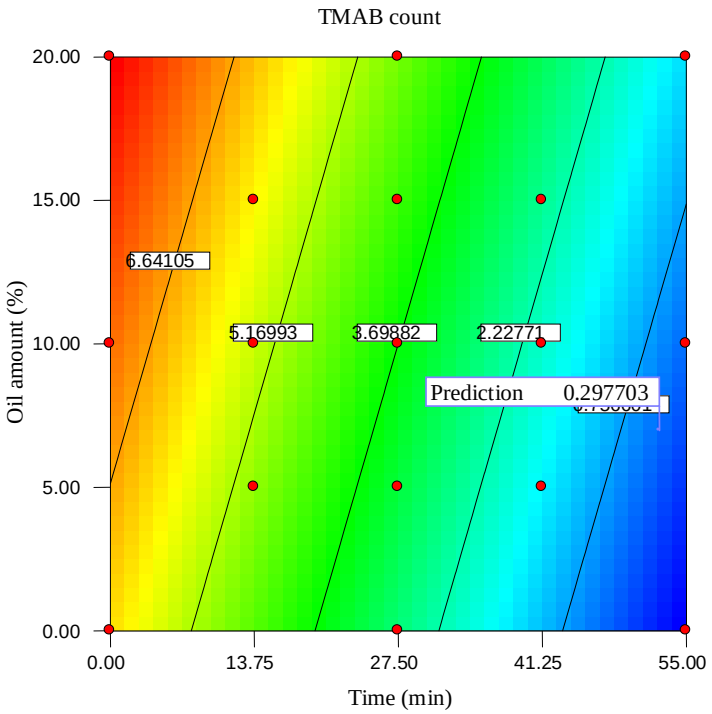

Figure 4. Response surface graph (2D) for R_2 model which shows the variation of total number of mesophilicic aerobic bacteria with the amount of oil added to the fillets and baking time.

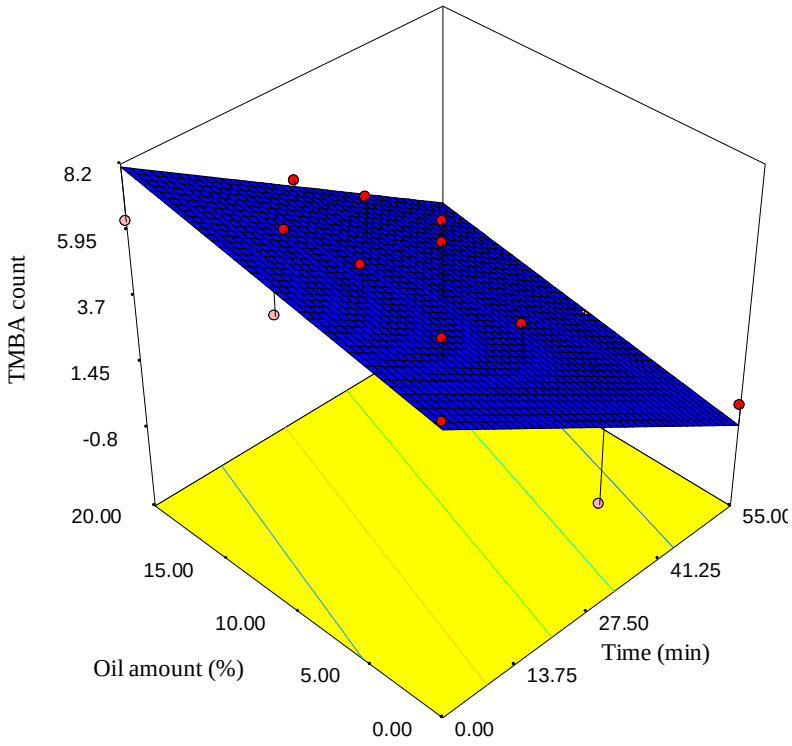

Figure 5. Response surface graph (3D) for R_2 model which shows the variation of total number of mesophilicic aerobic bacteria with the amount of oil added to the fillets and baking time.

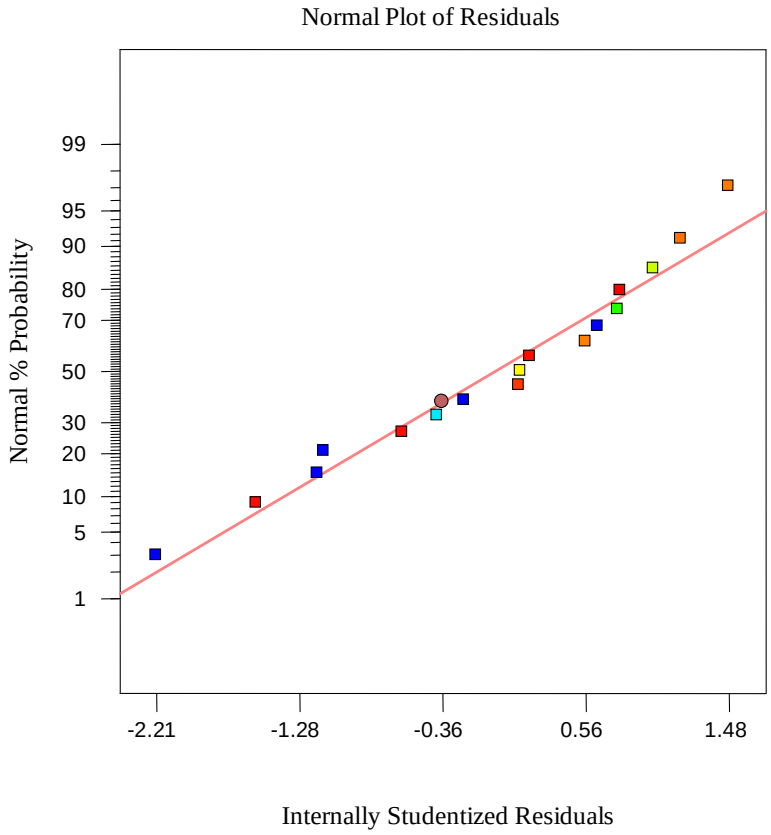

Figure 6. The conformity of experimental results and expected results by RSM.

experimental results obtained and the results expected by RSM. According to the figure, it can be concluded that the experimental data fits to the model. As central temperature and total mesophilicic bacteria were assessed, the optimum baking time and the amount of oil were determined as 52.42 min and 6.98%, respectively. The central temperature at optimum point was 92.35°C and total number number mesophilicic bacteria was determined as 0.29 log10 cfu/g.

DISCUSSION

Rosnes et al., (1999) applied thermal process at 70°C for 15 mins after they vacuumed sauced trout fillets and preserved them at 4 and 10°C. At the end of day 42, researchers found the total number of mesophilic aerobe bacteria to be < 1 $\log_{10}$ kob/g of fillets kept at 4°C. Although, the method applied was different in this study, the mesophilic aerobic bacteria count detection was <1 $\log_{10}$ cfu/g 42 days. Bergslien (1996) preserved trout fillets at 2°C to which 10 minof thermal process was applied at 65°C. On day 7 of preservation, the total number of mesophilic aerobe bacteria was found to be above 5 $\log_{10}$ cfu/g. However, even the value determined in fresh fillet (4.16 $\log_{10}$ kob/g) in the study was lower than this value, which was found as <10 kob/g on day 7 of the study. Simpson et al. (1994) first applied heat treatment to sauced spaghetti and sauced meat at 65°C (for 71 and 105 min) and at 75°C (for 37 and 40 min) and then preserved them at +5 and +15°C and investigated the total number of mesophilic aerobic bacteria and lactic acid bacteria. They reported that the samples preserved at +5°C kept their quality for more than 35 days, but they observed sensory degradation (acidity) on 14th on the samples preserved at 15°C. It is considered that this might be caused by breeding of microorganism that is hurt by heat treatment and reactivated again at the preservation temperature.

Salmon fillets were treated by heat at 90°C for 3.3 mins and they were preserved at 2°C for 345 days (Gonzalez et al, 2005). It was determined that the number of psychrophile bacteria on raw fillets (5 $\log_{10}$ cfu/g) decreased below 1 $\log_{10}$ cfu/g. As the product obtained by the same way preserved at 10°C, it was also determined that the number of psychrophile bacteria was under 1 $\log_{10}$ cfu/g. Although temperature and heating time are lower, findings of the study match with those of the current study. In the same study (Gonzalez et al., 2005), while the numbers of mesophilic aerobic bacteria and mesophilic anaerobic bacteria were 4.4 $\log_{10}$ cfu/g and 4.3 $\log_{10}$ cfu/g, after heat treatment and preservation

period, it was found as 1.5 $\log_{10}$ cfu/g. However, it is higher than our findings in the current study. Gonzalez et al. (2004) treated olive oil and salt added salmon fillets by heat at 65°C for 5 min, at 90°C for 10 and 15 min, and then preserved them at 2 and 10°C. The number of mesophilic aerobic bacteria determined on raw fillet as 4.77 $\log_{10}$ cfu/g was found to be 2 $\log_{10}$ cfu/g after 45 days on the fillets treated by heat at 90°C for 10 min. This finding differs from ours. It is believed that this difference can be explained by the difference in method, applied temperature and additives included in the fillets. Sardines were marinated and then put into jars and treated by heat for 20 min after their central temperature reached 70°C, and the samples were preserved at 4°C for 6 months and the total number of mesophilic aerobic bacteria, psychrophile aerobic bacteria, lactic acid bacteria, yeast and fungus were determined. It was reported that the number of microorganisms listed above were determined as lower than 10 cfu/g (Kılınç and Çaklı, 2005). This study is in agreement with out findings although it yielded partially different results.

Central temperatures of meatballs produced by mince during cooking were measured. It was reported that the central temperature of meatballs containing 20% oil was lower than the other group at the same period (Jeong et al, 2007). This result matches with our findings.

In another study, salami samples containing 9 and 0% olive oil were treated by heat and total number of mesophilic aerobic bacteria and *enterobacteria* were determined in certain periods by measuring central temperatures. Central temperature values were found very close to each other. The authors emphasized that the total number of mesophilic aerobic bacteria of the group containing 0% oil at 45th min was 1 log higher than the one containing 9% oil. Even though the product and the method were different from that of the current study, the results support the findings of the current study (Ayadi, 2009).

Determination of total volatile basic nitrogen (TVB-N) used in assessing the freshness of fish and fish products is an important parameter. It was reported that TVB-N value increases as parallel to preservation period of fish products. Huss (1988), reported the allowable limit value as 30 to 40 mg/100 g. In the current study, TVB-N values did not exceed the reported limit; TVB-N value was within consumable limits for sauced and heat treated *C. carpio* fillets.

In determination of the degree of lipid oxidation, peroxide value and thiobarbituric acid analysis were used. TBA value which is one of the most important criteria of degradation in fish is a result of lipid oxidation. However, peroxide value giving the amount of hydroperoxides generated at the initial stage of oxidative degradation is more commonly used. In the current study, TBA value increased for all samples but still remained below the consumable limit value (7 to 8 mg MDA/kg). It is considered that the reason for this increase might be that the heat treatment oxidized the oils and thus formed malonaldehyde. In their study, Weber et al. (2008) baked silver catfish fillets and identified increases in TBA values. Except group D (20% oil), the fillets had TBA values 3 below mg MDA/ kg and found ideal for consumption (Weber et al., 2008). PV values of fillets increased during preservation period and the highest values were determined in D group samples. High TBA value recorded for group D (20% oil) samples might support this result. It was found that the increases in PV values were parallel to increase in oil content. This might be explained by the increase in PV values of the fast added depending on the heat treatment and thus increasing the PV value of the product.

Free fatty acids (FFA) are shaped by the lipolysis of triglyceride and phospholipids. Increasing amount of free fatty acid in food composition is one of the factors that accelerate the oxidation. Lipid oxidation is one of the most important factors limiting preservation period (Pearson et al., 1983). No significant changes were observed in FFA values in our study (Table 2). It is considered that this situation might be shaped by the inactivation of enzymes in the fillets by the effect of heat. These results are in agreement with those of Al-Saghir et al. (2004) , who observed a decrease of FFA in Salmon fillets, steamed or pan-fried, either with or without different types of oil. Chantachum et al. (2000) also observed a lower FFA content in oil prepared from tuna heads, by heating at 95°C.

RSM can be used successfully in the determination of optimum condition in food technology. Kahyaoglu and Kaya (2006), applied heat treatment to sesame seeds at different temperature ranges (120 to 180°C) for different periods. They optimized experimental conditions depending on the seed hardness and moisture content. Sevimli et al. (2005) optimized cake baking conditions by RSM by using halogen lamp-microwave combination. Kilicceker and Kurt, (2010) investigated the optimization of the most suitable coating material by RSM on sensory quality of pearl mullet fillets. However, there is no study concerning *C. carpio*. In our study, it was showed that amount of oil and baking time fit to third order model for central temperature of the fillets and linear model for number of microorganisms. By these models, the optimum values of 52.42 min for baking time and 6.98% for amount of oil were obtained. Central temperature at the optimum point was 92.35°C and total number of mesophilic aerobic bacteria at this temperature was found as 0.29 $\log_{10}$ cfu/g. Thus, RSM was successfully applied for *C. carcio* fillets.

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