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Healthy consumption of seafood by cooking at the right temperature and time: Proven by rich physical and chemical parameter support

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Abstract: Monitoring certain factors both before and after cooking makes it possible to ensure the healthfulness of the food. The most significant chemical and/or physical changes that take place when food is heated are those caused by denaturing proteins. This study's goal is to assess the degree of protein denaturation (PD) in rainbow trout (*Oncorhynchus mykiss*) fillets that have been prepared using the most common time and temperature combinations. A few physicochemical characteristics, including area shrinkage, cooking loss, L*, a*, and b* values, thiobarbituric acid reactive substance, pH, total volatile basic, differential scanning calorimeter, texture profile analysis, and Fourier transform infrared investigations, were observed in fish flesh (cooked and raw samples) in this context.

Except for pH, there were statistically significant differences ($p<0.05$) within the treatment temperature groups. The results showed that short-term, low-temperature treatments had the best effects on the metrics measuring area shrinkage, cooking loss, and protein degradation. Despite the fact that deterioration was being watched in all groups based on the circumstance (time and/or temperature), it was determined that PD was developing faster at higher temperatures and protein coagulation became more obvious.

Keywords: Healthy eating, FTIR, DSC, protein degradation, fish, fillet

INTRODUCTION

Fish and other seafood are fundamental nutritional ingredients for a healthy life that are enjoyed globally. Besides, the high degree of spoilage of these products has led to the development of a prevalent variety of preservation, processing, and analytical techniques in this sector. At the same time, since healthy nutrition has become a philosophy of life in our century, the importance of cooking food in a healthy way has also emerged. In line with this trend, interest in baking foods with traditional methods is also increasing. Parallel efforts continue at the same pace in seafood, researchers from different disciplines (gastronomy, aquaculture producers, aquaculture engineers, etc.) come together and conduct research. Due to the unique characteristics of fish meat, the cooking stage becomes more important.

Although the purpose of different baking methods is to give the fish different aromas, it has become a priority in order to increase durability and reduce the rate of nutrient loss during baking.

Tasty foods are prepared through a number of processes in order to meet the nutritional needs of people. In one of these processes, foods, including those in the industrial field, are subjected to baking, known as controlled heat treatment, before they are made available for consumption. Conventional baking methods allow heat transfer through conduction, convection, and radiation. Of these methods, steaming and dry-heat baking are the most preferred conventional methods (Devine and Dikeman, 2014).

Meat and meat products, as well as aroma formation, some physical (brittleness, color, size, etc.), chemical (protein and lipid denaturation), and microbiological changes caused by the meat quality, affect the nutritional value. One of the most important of these events is protein denaturation, which is considered the first stage of changes in protein oxidation reactions (Bastıoğlu et al., 2011).

With its high essential fatty acid-amino acid, mineral substance, and vitamin content, seafood is one of the foods with high nutritional value, which has an important function for the development of children as well as for adult people to lead a healthy life. In this study, physical and chemical studies

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were used to investigate the impact of generally favored temperatures and cooking durations on rainbow trout fillet quality. In addition to the classical methods targeting protein denaturation, the integration of techniques that are considered quite new in the food sector has been taken into consideration, and it is aimed at establishing the infrastructure for what can be done for high quality criteria in future studies.

In this work, rainbow trout fillets were baked at two different temperatures and times that are often employed in dry heat (oven baking), and protein denaturation in the fillets was examined using a variety of assays. For this purpose, besides the classical methods used at protein denaturation, the integration of the new techniques in the food sector is taken into consideration, and it is aimed to create the infrastructure for the ones that can be done for high quality criteria in future studies.

MATERIALS AND METHODS

Experimental Design and Cooking procedure

Rainbow trout which was grown under routine conditions in the Atatürk University Fisheries Faculty Fresh Water Fish Production and Treatment Center, was used as fish material in the research. The fillets obtained from the marked sized rainbow trout were transferred to the Atatürk University Fisheries Faculty Seafood Processing Laboratory. The baking process was started on the fillets by taking into consideration the commonly used baking temperatures and times. The fillets, which were stored at 4 ºC were heat treated (cooked) in the oven (convection oven) at 180 ºC (30 min and 20 min), and at 200 ºC (20 min and 30 min).

Physical/chemical analyses [pH, cooking loss, area shrinkage, color, TBARS: thiobarbituric acid reactive substance, TVB-N: total volatile basic-nirogen, DSC: Differential Scanning Calorimeter, TPA: texture profile analysis, and FTIR: Fourier Transform Infrared] were done on the fillets before and after baking (Bayram, 2019).

pH

10 g raw and cooked fish flesh samples were taken and distilled water (100 ml) was added then pH values were measured in homogenized mixture.

Cooking loss

The weights of raw and cooked fillets are weighed with precision scale (Scaltec, SBA 41) and cooking loss is calculated proportionally with the formula of Ovissipour et al. (2017).

Cooking loss= ((final weight-first weight) ÷first weight) ×10

Area shrinkage

Area shrinkage of raw and cooked fillets were determined by scale and area shrinkage ratios were calculated proportionally (Ovissipour et al., 2017).

Color measurement

In the color measurement of samples, colour [value of L*, a* and b*] were examined by using Minolta (CR-200, Minolta Co, Osaka, Japanese) calorimeter. The color values are measured based on three-dimensional color measurement according to the criteria of CIELAB (Commision Internationele de I'E Clairage).

In this criterion, these values show color intensity as; (L*); L*=0; L*=100 white (darkness / clearance); (a*); +a* = red, -a* = green and (b*); +b*=yellow, -b* = blue (Bayram, 2019; Atamanalp et al., 2021).

Texture profile analyze

Texture Analysis Device (England, Stable Micro System, model TA-XT plus) with 75 mm probe was used to determine the TPA (textural properties test) of the samples taken from fish fillets. Without removing the skin, 30 mm length, width, and approximately 12 mm in height samples were taken from single fillet of each fish. From the obtained TPA curve; cohesiveness, flexibility, hardness, adhesion, gum and chewiness parameters were measured.

Determination of Total volatile basic nitrogen (TVB-N)

Minor modifications were done in the method given by Malle and Tao (1987). The TVB-N value was calculated by taking into account the H_2SO_4 solution spent in titration (Bayram, 2019; Atamanalp et al., 2021).

Determination of Thiobarbituric Acid Reactive Substance (TBARS)

TBARS is an important biomarker of the lipid peroxidation process strongly associated with lipid degragation levels. In this investigation, the method given by Lemon (1975) was used to determine the value of TBARS and the TBARS value was calculated as µmol Malonaldehit /kg tissue.

Differential scanning calorimeter (DSC)

For the DSC analysis, approximately 10 mg of tissue were taken from fish flesh samples and weighed in aluminum DSC containers (40 µL) and the containers were hermetically sealed. Sample containers placed in DSC (using empty container as reference) were heated from 20 °C to 90 °C at a heating rate of 5 °C / min. Enthalpy and temperature parameters of the thermal changes of proteins were monitor from the provided thermograms. The DSC-60 Plus (Japanese) was calibrated using indium (ΔH: 28,5 j/g; T: 156.6 ºC) for temperature and heat flow prior to analysis (Akköse, 2017).

Fourier transform infrared (FTIR)

A FTIR spectrometer (PerkinElmer precisely) with the specular reflection principle was used for both groups of samples from fish fillets and different time / temperature heat-treated fillets (1 cm x 0.5 cm x 0.2 cm). The samples were placed in direct contact with the ATR crystal cell, and the spectrum was taken from 4000 to 400 cm^{-1}. For each sample, 10 spectra were collected at room temperature (nearly 22 °C) (Ovissipour et al., 2017).

RESULTS AND DISCUSSION

Food is an important source for sustaining human life in healthy conditions. It is also known to the consumer that food safety is as vital as food sustainability (Sridhar et al., 2021). Global aquaculture production is estimated to reach 201 million metric tons in 2030 (Lundebye et al., 2021). Baking in the oven is one of the traditional cooking methods used since ancient times and is still preferred more frequently. In this study, physical and chemical changes and protein degradation with DSC were examined by applying different temperatures to rainbow trout at different times with this cooking method. In our study, it was observed that undesirable loss rates and protein degradation increased with high cooking temperatures. Since the new analysis methods that emerged with the development of technology give faster and more reliable results about product quality, the possibilities of their use in the food sector should definitely be investigated. As a matter of fact, our findings from protein denaturation and physical changes revealed that it is a safe, high-quality product that can be used to eliminate cooking errors. It has also been revealed that the selected temperature values in our study are not suitable for the analysis of protein denaturation with DSC, and lower temperature ranges should be tried.

pH

It is known that pH is an important factor in determining the quality of seafood and increasing its endurance after the process. In our study, pH was found to be significant ($p < 0.05$) in raw samples and statistical differences were not significant ($p > 0.05$) in heat treated samples (Table 1).

Table 1. pH measurements of raw and baking fillets with different temperature applied at different times (Mean±Standard deviation)

Group	Before heat process*	After heat process ^{NS}
180 ºC / 20 min.	6.30±0.29^{a}	6.05±0.12^{a}
180 ºC / 30 min.	6.29±0.29^{a}	6.15±0.11^{a}
200 ºC / 20 min.	6.21±0.29^{ab}	6.13±0.04^{a}
200 ºC / 30 min.	6.19±0.29^{b}	6.14±0.10^{a}

*p<0,05, NS: Not significant, there is no statistical difference between the values shown in the same column with the same letter (a, b).

In the raw fillet samples, the highest pH value was found as 6.30±0.29 and the lowest value was 6.19±0.29. These differences are depending on factors such as fish species and habitat, muscle type, nutritional status, rigor process, storage conditions and the buffering capacity of meat (Chaijan et al., 2013).

At the end of the heat treatments, the highest pH was obtained at 180 ºC during 30 min group (6.15±0.11) and the lowest value at 180 ºC during 20 min group (6.05±0.12). Our current research pH values and changes of the raw and cooked fillets, are consistent with previous studies which done with rainbow trout (Alak et al., 2010; Alak et al., 2011).

Öz et al. (2007) reported that the pH value of the heat-treated rainbow trout increased but Kotan (2015) research findings had showed that pH value decreased as a result of baking the same fish species in the oven (180 ºC / 10 min). Our study findings are compatible with Kotan (2015), but at the different with Öz et al. (2007) is thought to be affected by the baking technique and duration.

Cooking loss and area shrinkage results

The initial and post-baking weight measurements of the fillets with different baking temperature and times are carried out and the % changes are presented in the Table 2. Temperature and duration were statistically significant between groups ($p < 0.05$). The results demonstrated that cooking loss increased significantly ($p < 0.05$) due to the time and temperature of the heat treatment. The area shrinkages were determined to be statistically significant ($p < 0.05$) in raw and cooked (different time and temperature) filets and presented in Table 2. The highest area shrinkage was obtained as 40.23±4.93 in 200 ºC /30 min. cooked filets, and the lowest value was as 24.84±3.33 in 180 ºC / 20 min group fillets.

Table 2. Cooking loss and area shrinkage results of raw and baking fillets with different temperature applied at different times (Mean±Standard deviation)

Group	Cooking loss (%)*	Area shrinkage (%)*
180 ºC / 20 min.	15.37±5.69 [ab]	24.84±3.33[c]
180 ºC / 30 min.	17.96±4.90 [ab]	33.07±8.75[ab]
200 ºC / 20 min.	13.61±5.04 [b]	29.53±9.65[bc]
200 ºC / 30 min.	20.22±5.13[a]	40.23±4.93[a]

*p<0,05, there is no statistical difference between the values shown in the same column with the same letter (a, b).

Considering the results of these studies, the reason why these losses do not rise to very high levels is that fish meat contains high levels of myofibrillary protein and connective tissue (Ovissipour et al., 2013). Again, as known, more than 85% of the baking loss is moisture, while the rest forms lipids, muscle fragments, and some byproduct (aggregated sarcoplasmic proteins, collagen or gelatin etc) (Kong et al., 2007a; Skipnes et al., 2008; Skipnes et al., 2011; Ovissipour et al., 2013). Baking of meat results in denaturation of proteins, loss of water/lipid and shrinkage. The most important point in the application of heat treatment is the last temperature of the center (the coldest point) and how long it remains. Our findings are consistent with previous studies done with various fish species (Kong et al., 2007a; Kong et al., 2007b; Skipnes et al., 2011; Ovissipour et al., 2013; Cao et al., 2016).

When the sources of variation of our study were taken into consideration, it was observed that the cooking loss rate of 180 ºC and 200 ºC was more in 30 min group. In comparison of the cooking loss rate of the two different temperatures for 30 minutes, it was determined that the loss of 200 ºC was higher. This can be attributed to a reduction in the water holding capacity due to excess water loss of the fillets exposed to high temperatures for a long time.

The process that is effective in area shrinkages; is explained by the loss of moisture due to changes in the functional properties of the protein and as a result of the muscle protein denaturation and collection by heat treatment (Ovissipour et al., 2013; Cao et al., 2016). Also during the heat treatment, the diameter of the muscle fibers and the length of the sarcomere may bereduced due to the discarded water, soluble proteins, lipids and PD (Ovissipour et al., 2017). Numerous studies have been found to be related to protein denaturation of baking and area shrinkages and parallel to our study data (Barbera and Tassone, 2006; Kong et al., 2007a,b; Ovissipour et al., 2013; 2017). In addition to this, it has been reported that the fish meat is narrowed and thickening of the vertical section accompanied by protein denaturation (Liu et al., 2013).

In the description of the area shrinkages in the cooked samples, the breakdown of hydrogen bonds at high temperature and the shortening of the collagen molecule by opening and loosening of the fibrillary structure (shrinkage of up to one-third of the first length) is considered.

Color measurement results

The results of the color values (L*, +a*, and +b*) of the raw and different time / temperature cooked filets are given in Table 3.

L*, +a*, and +b* values of the heat treatment groups were 67,85-79,13, 0,40-1,36, and 16,28-22,42, respectively. When L (brightness) values are examined, it is seen that the lowest values are 67,85 in 200 ºC/ 30 min group and the highest (77,18) in 180 ºC / 20 min. group. High temperature application for long time affected the brightness of the materials negatively.

The highest +a value (1,36) was obtained in 200 ºC / 30 min. group and the lowest (0,40) was in 180 ºC /20 min. group. Haard (1992) was met with a similar situation in one of his researches, and reported that, due to denaturation of proteins, carotenoids and sugar between the oxidation of the fish proteins was attributed to the mallard reaction.

When the + b (yellow) data were examined, it was found that the lowest value (16.28) in 200 ºC / 20 min and, the group of 200 ºC/30 minutes was reached the highest value (22.42). During the baking of rainbow trout fillets, the color of the fish meat was masked by a rapid increase in L* value and a* sharp decrease in a value ($p < 0.05$). As a known condition, the formation of L*, a* and b* values caused by temperature can disrupt the colour balance in the cooked samples. Studies done with Pacific salmon (*Oncorhynchus keta*) (Bhattacharya et al., 1994) and pink salmon (Kong et al., 2007a) reported similar trends with our results. Whitening is the result of rapid denaturation of both proteins and carotenoid oxidation, while the browning phase is related to the Maillard reaction between sugars, fish proteins and amines (Haard, 1992). In this study, the determined browning phase is thought to be caused by high test temperatures. Because browning is usually related to process temperature and time (Whistler and Daniel, 1990; Kong et al., 2007a).

Table 3. Color measurements of raw and baking fillets with different temperature applied at different times (Mean±Standard deviation)

L*	**Raw fillet**	37.10±2.82[d]
	180 ºC / 20 min.	77.18±5.15 [ab]
	180 ºC / 30 min.	71.36±7.48 [bc]
	200 ºC / 20 min.	79.13±3.95 [a]
	200 ºC / 30 min.	67.85±7.35 [c]
a*	**Raw fillet**	2.62±1.93 [a]
	180 ºC / 20 min.	0.40±0.64 [b]
	180 ºC / 30 min.	0.58±1.07 [b]
	200 ºC / 20 min.	0.58±0.99 [b]
	200 ºC / 30 min.	1.36±1.18 [ab]
b*	**Raw fillet**	7.06±2.38 [d]
	180 ºC / 20 min.	19.28±1.91 [b]
	180 ºC / 30 min.	19.03±1.55 [b]
	200 ºC / 20 min.	16.28±1.82 [c]
	200 ºC / 30 min.	22.42±0.44 [a]

$p<0,05$, there is no statistical difference between the values shown in the same column with the same letter (a, b).

Texture profile results

The data of the textures of the raw and cooked filets (different time / temperature) are given in Table 4. Kong et al. (2007a) gave the most suitable peak temperature of the tissue for optimum baking time for the pink salmon as 100 and 131.1 °C. Since the temperature of protein denaturation in fish muscle is reported to be between 40 and 80 °C (Skipnes et al., 2008), tissue changes are probably due to the denaturation of myofibrillary proteins and the gelation of collagen (Kong et al., 2007a). Myosin and actin are very important due to the low collagen content in gelation for fish muscles (Skipnes et al., 2011). In this study, the hardening phase time was decreased with increasing temperature, and at higher temperatures, protein denaturation rate was determined to be faster than protein collection. This situation is consistent with other findings of the study (FTIR and DSC). Again, the temperature applied during baking can change the physical properties of the fish meat by affecting the protein denaturation. Especially the deterioration of myosome is more effective in this process (Sahin and Sumnu, 2001; Liu et al., 2013).

Our data showed that different cooking treatments significantly reduced elasticity and gummy. Textural structure of fish meat (due to myofibrillar proteins) does not show a high texture strength as high as meat and chicken meat and especially processed products have been reported to affect the textural properties of protein and lipid quality (Dinçer et al., 2017). According to Kerr et al. (2005); some tissue modifier situations act on the binding and stiffness of proteins.

Table 4. Texture results of raw and baking fillets with different temperature applied at different times (Mean±Standard deviation)

	Groups				
Texture					
	Raw filet	**180°C/20 min**	**180°C /30 min**	**200°C /20 min**	**200°C /30 min**
Hardness (N)*	8.45±0.97^{a}	7.60±0.35^{a}	12.37±2.93^{b}	9.15±1.63^{a}	18.38±3.28^{c}
Stickiness (N.s)^{NS}	0.68±0.39^{a}	0.52±0.33^{a}	0.18±0.13^{a}	0.56±0.23^{a}	0.28±0.29^{a}
Flexibility ^{NS}	0.27±0.35^{a}	0.12±0.03^{a}	0.11±0.03^{a}	0.11±0.01^{a}	0.11±0.01^{a}
Cohesiveness ^{NS}	0.29±0.05^{a}	0.35±0.06^{a}	0.33±0.03^{a}	0.34±0.04^{a}	0.33±0.03^{a}
Elasticity (mm)*	2.61±0.46^{c}	1.98±0.33^{a}	2.04±0.53^{ab}	1.68±0.18^{a}	2.37±0.27^{bc}
Gummy (N)*	2.43±0.18^{a}	2.65±0.46^{a}	4.12±0.96^{b}	3.14±0.67^{ab}	6.23±1.40^{c}
Chewiness (N.mm)*	6.34±1.12^{ab}	5.32±1.46^{a}	8.02±0.72^{b}	5.18±0.69^{a}	14.68±3.11^{c}

*p<0,05, NS: Not significant, there is no statistical difference between the values shown in the same column with the same letter (a, b).

TVB-N and TBARS results

TVB-N analyze is a chemical method used to determine the quality and freshness of seafood (Çetinkaya et al., 2014). TVB-N value changes depending on many factors, such as fish species, the hunting season, degree of maturity, sex, age, processing technology and storage time.

While the average TVB-N value of the raw fillets was 9.18 ± 1.69 mg / 100 g, this value showed an increase due to the time and temperature. The highest value was found in 200 ºC /20 min group as 17.64±1.70mg/100 g and at 200 ºC/ 30 min group as 18.76±1.69 mg/100 g.

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In this study, TVB-N measurements were made in the raw and different time / temperature heat treated filet samples. Total volatile basic nitrogen (TVB-N) value in fish is an important criterion in determining freshness. Lipid oxidative products lead to protein oxidation. In addition, lipid oxidation and protein oxidation can occur independently or together. TVB-N; is a nitrogen-containing group of compounds originating from protein degradation by enzymes and bacteria, including NH3, amines (Duan et al., 2018).
Kolsarıcı and Özkaya (1998) reported that cold and hot smoking of rainbow trout, caused changes in TVB-N values. They also found that temperature can increase TVB-N levels in microbial and enzymatic activation. The high amounts of TVB-N obtained in our study can be explained by the applied heat levels.

The TVB-N changes caused by different applications were determined as significant at $p < 0.05$ level and the differences are presented in Table 5.

Table 5. TVB-N and TBARS values of raw and baking fillets with different temperature applied at different times (Mean±Standard deviation)

Group	TVB-N (mg/100g)*	TBARS (µmol malonaldehyde /kg tissue)*
Raw filet	9.18±1.68^{b}	0.95±0.29^{c}
180 ºC / 20 min.	13.72±1.67^{ab}	1.89±0.56^{bc}
180 ºC / 30 min.	16.95±1.69^{a}	2.60±0.83^{b}
200 ºC / 20 min.	17.64±1.70^{a}	2.01±0.55^{bc}

*p<0,05, there is no statistical difference between the values shown in the same column with the same letter (a, b).

The number of thiobarbituric acid (TBA) is a method used in meat and meat products to determine the aggravation caused by autoxidation in lipids. The oxidative reaction during the storage of meat and meat products in cold or frozen conditions results in the formation of various oxidation products. These products are hydroperoxide, peroxide, aldehyde and ketones, respectively. The most important aldehyde formed is malonaldehyde (MA) (Yeo and Shibamoto, 1992). This aldehyde is an important parameter in determining the degree of oxidation. The amount of MA determined by TBA analysis is directly proportional to the oxidation of meat.

In this study, changes in the TBARS values of the fillets exposed to different temperature / time applications were determined. This change in TBARS values of raw/heat treated fillets was shown in Table 5 and found significant at $p < 0.05$ level. The mean TBARS value of raw filets was found as 0.95±0.29 µmol malonaldehyde/kg tissue. The highest TBARS value after baking was obtained as 5.54±2.26 µmol malonaldehyde/kg tissue in 200 ºC - 30 min group.
Secondary oxidation products were evaluated in order to determine the course of the lipid oxidation process. TBARS is one of the classical methods of monitoring secondary products of lipid oxidation (Qiu et al., 2016). In TBARS; it is well known that oxidants such as radicals form protein and lipid damage in processed aquatic products, especially fish containing excess polyunsaturated fatty acids, are critical for shortening shelf life (Duan et al., 2018).

The TBARS values of the samples cooked 30 min. at 180 ºC and 200 ºC were found to be higher compared to the shorter periods baking of the same temperatures. Our study was tried to cook with the same technique in the same fish species. This result coincides with the effect of heat treatment on the TBARS value. Similarly, it is known that TBARS is lower in raw meat and reactive compounds formed by baking react with various compounds such as protein and amino acids found in meat to increase TBARS value (Meinert et al., 2007; Sanchez del Pulgar et al., 2012; Dominguez et al., 2014).
In this study, the increase in lipid oxidation at high temperature for long periods of baking was found as marginal. When the formation of secondary oxidation products was evaluated, it was concluded that TBARS value was below the acceptable limit value (2-2.5 mg MDA/ kg meat) in all cooked samples except for high temperature long-term application, this situation could not be defined as increased lipid oxidation (Al-Sagir et al., 2004).

Thermal stability results

The thermal properties of the raw and heat treated (different time-temperature) fillets are given in Table 6. In the thermograms obtained by thermal analyzes, three different peaks were determined for the control sample (raw fillet) and these peaks were respectively myosin (T_1, 〖ΔH〗_1), and sarcoplasmic proteins with collagen (T_3, 〖ΔH〗_3) and actin (T_4, 〖ΔH〗_4) was taken into consideration in the denaturation (Table 6).

Table 6. DSC results of control and different temperature/time applied groups

Group	1. peak		2. peak		3. peak	
	Temperature (C)	**ΔH (J/g)**	**Temperature(C)**	**ΔH (J/g)**	**Temperature (C)**	**ΔH (J/g)**
Raw fillet	41.83	1.11	65.93	0.08	75.65	0.49
	41.59	0.76	65.68	0.07	75.05	0.25
180 ºC/20 min	-	-	-	-	-	-
180 ºC /30 min	-	-	-	-	-	-
200 ºC/ 20 min	-	-	-	-	-	-
200 ºC /30 min	-	-	-	-	-	-

However, in the cooked samples myosin, actin and sarcoplasmic proteins with no longer observed that the peaks of collagen denaturation. In order to estimate the degree of protein denaturation of fish meat during baking, the activation energy must be known. Peak temperature (Tmax) and heating rate (b) are two parameters used in the DSC method and indispensable for interpretation (Liu et al., 2013).

For DSC analysis, there is no data in rainbow trout so it is defined as similar temperature ranges with reference to the data of Skipnes et al. (2008) and Liu et al. (2013) in different fish species.

In this respect, the myosin (40-50 °C) and actin (70-80 °C) proteins were taken into account and in the prediction of the degree of protein denaturation, the concentration of myosin and actin detected during heating for the denaturation of these two proteins was focused.

As is known, there is a connection between reduction in tissue, increase in proteolytic activity and myosin denaturation. The deterioration of tissue and high temperature are thought to have negative effects in the absence of peaks (Sahin and Sumnu, 2001).

The temperature in protein denaturation of the tissue is considered to be the main cause, since the physical properties of fish meat during the baking process are partially damaged by protein denaturation (Liu et al., 2013). When consider the effects of different cooking time/temperature on the protein degradation, it is not surprising that temperature enthalpy is decreased and eventual coagulation to the cooking samples.

TBARS level, other important parameters, showed a significant increase under high temperature exposure compared to the control and the other treated groups.

The enthalpy values that cannot be taken can be explained by a decrease in the thermal stability of proteins. In our study, the TVB-N results, which are examined as validation parameters, are in parallel with the DSC results. The amount of TVB-N, which increases with temperature, is directly proportional to the increase in compounds such as amine and ammonia. The increase in the amount of compounds such as amine and ammonia in the medium strengthens the result of protein denaturation.

Fourier transform infrared (FTIR)

In this study, wave motion measurement of raw and cooked (different time-temperature) fillets was determined by FTIR readings and the changes were given in Figure 1.

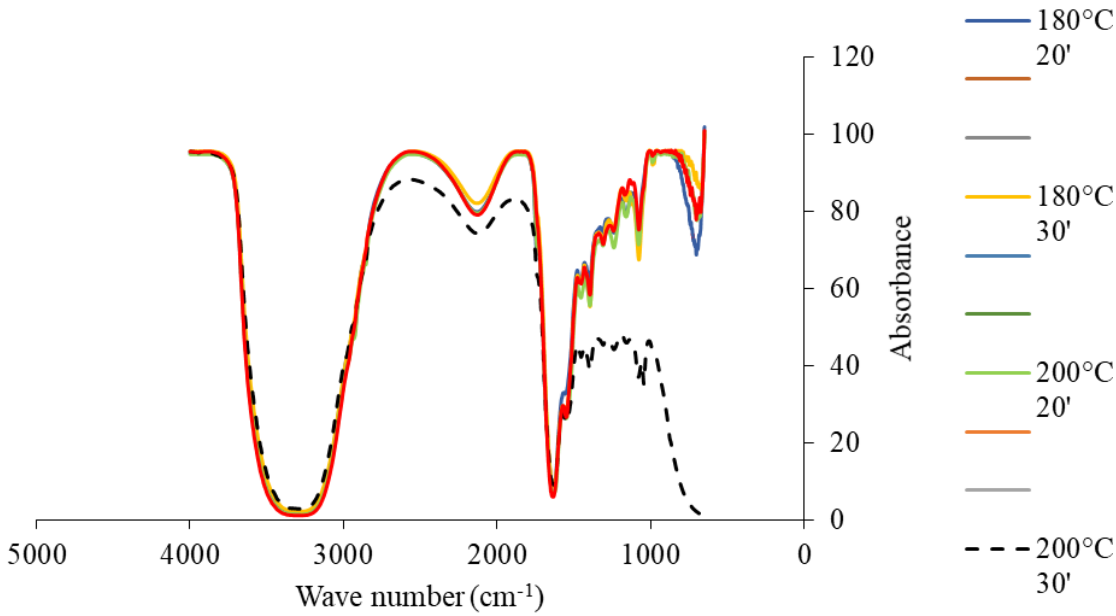

Figure1. FTIR results of raw and baking fillets with different temperature applied at different times

Due to the high-water activity in fish fillets, there were no significant differences in the FTIR graphs of raw and heat-treated groups. In this case, it is also believed that the amine groups formed depending on the temperature have an effect. The presence of amine groups and high-water activity as a known condition make it difficult to read the other bonds on the spectrophotometer in the graphs.

Amid I (1700-1600 cm^{-1}) in the Myofiber spectrum is known as the most dominant and most useful group in studies. This structure is also widely used to study the secondary structure of protein. In this common use, hydrogen bonding patterns, dipole-dipole interaction and susceptibility to the geometry of the polypeptide backbone are quite important (Carton et al., 2009; Astruc et al., 2012). The changes with the effects of heating or salting in Amid I spectrum were examined in various muscles (Carton et al., 2009; Ojagh et al., 2011; Astruc et al., 2012). In this study, the highest absorbance read in the fillets of the original spectrum was recorded as values of 3271.71 cm^{-1} - 3288.04 cm^{-1}, and recorded as the indicating the presence of amine groups. This absorbance gave increase/decrease frequencies depending on the amount of water, heating temperature and time.

The determined minimum frequencies, sample-layer structures in the heated samples can be attributed to the increased aggregation at the intermolecular level in the cooked fillets (Carton et al., 2009; Ojagh et al., 2011). The same trend may also be related to changes in intramolecular antiparallel β-layer structures in heated samples (Ovissipour et al., 2017).

When the graphs were examined in general terms, functional groups were determined according to the energy values, with 2127.83 cm^{-1} and 2127.31 cm^{-1}, ether groups defining bonds were determined with high rate in all graphs.

Although multiple confirmation tests were used to determine denaturation in our study, it should be supported by different studies (different fish species, different processing technologies, different environmental conditions and different temperatures).

There is a need for new researches in order to increase the studies on the loss of basic components with different cooking techniques and to reduce the lipid and protein denaturation that occur during cooking and storage to the minimum or to observe the optimum.

CONCLUSION

The food and food processing industry worldwide requires novel products and novel processes to customize products. In this study, it was determined that different temperature/time applications in the oven caused changes of fillets and protein degradation in rainbow trout. This study demonstrates the ability of low temperature and time to protect rainbow trout fillet from quality damage induced by high temperature exposure.

Our findings from protein denaturation and physical changes have shown that it can be used to improve safety and high-quality products and improve baking errors. In our study, it was also revealed that protein denaturation of the selected temperature values was not suitable for DSC analysis and that lower temperature ranges should be tried. In this study,

although multiple verification tests are used to determine denaturation should be supported with different studies (different fish species, different processing technologies, different ambient conditions and different temperatures). Considering all data we can say that;

1.In our study, it was observed that the highest pH value was 6.30±0.29 and the lowest value was 6.19±0.29 in the untreated samples. As a result of heat treatment applications, the highest pH was found to be 6.15±0.11 at 180 °C/30 min and the lowest value was 6.05±0.12 at 180 °C/20 min.

2.Considering the sources of variation, it was determined that the rate of weight loss at 180 °C and 200 °C was higher in 30 minutes. In the comparison of two different temperatures, it was determined that the weight loss rates were higher at high temperatures.

3.The surface area losses of the fillets cooked raw and cooked at different temperatures/times were found to be statistically significant. The highest surface area shrinkage was 40.23±4.93% at 200 °C/30 min. The lowest loss in cooked fillets is 24.84±3.33% at 180 °C/20 min. obtained in cooked fillets. Another remarkable point in this parameter is that the maximum surface area shrinkages are determined in long time periods.

4.The range of color values of the heat treatment groups are L*; 67.85-79.13, a*: 0.40-1.36, and b*: 16.28-22.42, respectively. When the L* (brightness) values are examined, the lowest is seen at 67.85 at 200 °C in 30 minutes, and the highest at 77.18 at 180 °C in 20 minutes. High temperature application for a long time adversely affected the gloss of the materials. The highest +a* value was determined at 1.36 to 200 °C in 30 minutes and the lowest at 0.40 to 180 °C in 20 minutes. Finally, when the +b* value was examined, it was seen that it reached the lowest value with 16.28 in 20 minutes at 200 °C and the highest value with 22.42 in 30 minutes at 200 °C.

5.It had been observed that the applied temperatures and times cause changes for each parameter (hardness, stickiness, flexibility, cohesiveness, elasticity, gumminess and chewiness) on the texture, especially hardness, elasticity, gumminess and chewiness parameters.

6.While the average TVB-N value of raw fillets was 9.18±1.69 mg/100 g, this value increased depending on time and temperature as a result of cooking. The highest values were found as 17.64±1.70 mg/100 g in the 200 °C/20 min group and 18.76±1.69 mg/100 g in the 200 °C/30 min group.

7.In the TBARS value, the average TBARS value of the raw fillets was 0.95±0.29 µmol Malonaldehyde/kg tissue, and the highest TBARS value after cooking was 5.54±2.26 µmol Malonaldehyde/kg tissue at 200 °C/30 min.

8.Three different peaks were determined for the control sample (raw fillet) in the thermograms obtained as a result of the thermal analysis, however, the peaks of the targeted proteins could not be read because protein denaturation (denaturation of collagen with myosin, actin and sarcoplasmic proteins) occurred in the cooked samples.

9.In FTIR measurements, it was determined that the high water ratios and amine contents in both raw samples and cooked fillets prevented the appearance of small molecule bonds by making some shadows.

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CONFICT OF INTEREST

The authors declare no competing interests

ETHICAL APPROVAL

Ethics committee approval is not required.

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CONSENT FOR PUBLICATION

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