Arch Lebensmittelhyg 61, 183-188 (2010) DOI 10.2376/0003-925X-61-183

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Korrespondenzadresse: nurerkan@istanbul.edu.tr

Summarv

#### Zusammenfassung

<sup>1</sup>Istanbul University, Faculty of Fisheries, Department of the Seafood Processing and Quality Control, 34470, Istanbul, Turkey; <sup>2</sup>Food Engineering Departments, Middle East Technical University, 06531, Ankara, Turkey; <sup>3</sup>Istanbul University, Institute of Science, Department of Fisheries and Seafood Processing, Bozdoğan Kemeri cad. No: 6 Işitme Engelliler Okulu Yanı Vezneciler/Istanbul, Turkey; <sup>4</sup>Food Institute, TÜBÝITAK Marmara Research Center, Gebze/Kocaeli, Turkey; <sup>5</sup>Tütün ve Alkol Piyasası Düzenleme Kurumu (TAPDK), 06520, Ankara, Turkey

# Changes in the physicochemical properties of high pressure treated rainbow trout

# Hochdruckbehandlung von Regenbogenforellen

Nuray Erkan<sup>1</sup>, Hami Alpas<sup>2</sup>, Gonca Üretener<sup>3</sup>, Arif Selçuk<sup>4</sup>, Sencer Buzrul<sup>5</sup>

Changes in the physicochemical quality of rainbow trout's High pressure (HP)treated at 220, 250 and 330 MPa, 7, 15 and 25 °C for 5 and 10 min were investigated. HP-treated rainbow trout's showed significantly increased L\* value relative to untreated rainbow trout's. Little changes in colour (a\* value) were observed, compared to untreated rainbow trout's, which did not showed b\* values. From tests of chemical properties, HP-treated rainbow trout's did not showed or showed significantly decreased thiobarbituric acid (TBA) and trimethylamine nitrogen (TMA-N) with increasing treatment pressure compared to controls. HP-treated rainbow trout samples showed significantly increased or decreased free amino acids (P < 0.05) relative to untreated trout samples. The results obtained from this study showed that the quality of HP-treated rainbow trout is best preserved at 220 MPa, 7-15-25 °C for 5-10 min and 250 MPa, 7-15 °C for 5 min.

Keywords: rainbow trout, high pressure, colour, lipid oxidation, free amino acid

Untersucht wurden physikalisch-chemische Qualitätsveränderungen von Regenbogenforellen, die mit 220, 250 und 330 MPa, bei 7, 15 und 25 °C für 5 und 10 min behandelt wurden. Die Hochdruck (HP)-behandelten Regenbogenforellenen zeigten signifikant erhöhte L \* Werte im Vergleich zu unbehandelten Regenbogenforellen. Geringgradige Änderungen der Farbe (a \* Wert) wurden, im Vergleich zu den unbehandelten Regenbogenforellen, beobachtet, jedoch nicht bei den b \* Werten. Bei der Untersuchung der chemischen Eigenschaften, zeigten die HP-behandelten Regenbogenforellen keine oder keine signifikante Zunahme von Thiobarbitursäure (TBA) und Trimethylamin-Stickstoff (TMA-N) mit wachsendem Druck bei der Behandlung im Vergleich zur Kontrollgruppe. Die HP-behandelten Proben zeigten eine erhebliche Zunahme bzw. Abnahme der freien Aminosäuren (P <0,05) im Vergleich zu den unbehandelten Proben. Die Ergebnisse dieser Studie haben gezeigt, dass die Qualität der HP-behandelten Regenbogenforellen am besten bei 220 MPa, 7-15-25 °C für 5-10 min und bei 250 MPa, 7-15 °C für 5 min erhalten bleibt.

Schlüsselwörter: Regenbogenforellen, Hochdruckbehandlung, Farbe, Lipidoxidation, freie Aminosäuren

## Introduction

Several species of freshwater fish including carp, rainbow trout are being farmed in Turkey and in other Europe countries over the last decade in order to meet the increasing demand for fresh rather than frozen fish. Of the freshwater fish species, rainbow trout (Onchorynchus mykiss) is being farmed mainly in the river waters of Northmiddle Anatolia and is sold as either whole fresh fish and in fillet in retail markets, supermarkets. Additionally, trout fillets in the form of smoked and vacuum packaged products are being exported to various European countries. Sea foods such as fish are highly perishable food products. During handling and storage, quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product. Freshwater fish are extremely perishable food commodities (Tülsner, 1994).

High pressure (HP) treatment is an alternative food preservation technology to thermal treatment or chemical preservations. There are also HP- treated fish products introduced into the market. Positive effect of HP treatment on shelf life of octopus, oyster, shrimp, mussel and raw fish have been reported (Hurdato et al., 2001; He et al., 2002; Cruz-Romero et al., 2008a; Büyükcan et al., 2009; Erkan and Üretener, 2010).

But, the effects of pressure on the structural, textural, and colour changes of sea foods are more variable compared to many other foods (Yağız et al., 2007). Visual assessment of appearance (especially colour) is one of the most important factors affecting the consumer acceptability of seafood (Cruz- Romero et al., 2008b). Some modifications in colour have been reported for fish after HP treatment (Amanatidou et al., 2000; Chevalier et al., 2001; Yağız et al., 2007).

Lipid oxidation is a major quality problem in fish (Erkan and Özden, 2008). The primary product of lipid oxidation is the fatty acid hydroperoxide, measured as peroxide value (PV). Peroxides are not stable compounds and they break down to aldehydes, ketones, and alcohols, which are the volatile products causing off flavour in products (Fernandez et al, 1997). The Thiobarbituric acid (TBA) values measure secondary products of lipid oxidation. Ohshima et al. (1993) reported an increase in both the peroxide value (PV) and TBA numbers (both measurements of lipid oxidation) of HP- treated fish muscles.

Trimethylamine nitrogen (TMA-N), have been used as freshness indicators in sea foods (Tülsner, 1994). Limited information is available on HP of seafood, and more specifically, its effect on TMA-N profile (Erkan and Üretener, 2010).

Denaturation of protein is one of the major biochemical events during HP treatment. During treatment its degradation products, amino acids and peptides, have a considerable effect on the sensory characteristics of fish. However, only limited information is available in the literature on free amino acid changes of HP-treated sea foods (Cruz-Romero et al., 2008c).

The objective of this study was to investigate the effect of different HP conditions (pressure; 220, 250 and 330 MPa, holding time; 5 and 10 min and temperature; 7, 15 and 25 °C) on some physicochemical characteristics (colour changes, TBA, TMA-N value and amino acid content) of rainbow trout.

## Materials and methods

#### Samples

Aqua cultured fresh rainbow trout were cultivated in net cages in a Turkish fish farm. A total of three kg rainbow trout were killed by immersing in ice-cold water (hypothermia) (Council Directive 86/609/EEC), packed with melted ice (2:1, fish: ice) in polystyrene boxes (Council Directive 91/493/EEC) provided with holes for drainage and were transported to the laboratory. The fish were gutted, filleted and washed. Three kilograms of samples were used for the experiment. Experiments were started about 18 hours after the death of the fish. The fish were filleted, skinned and divided portions of equal weight (15 g). The samples were covered with flexible plastic films to avoid direct contact between the samples and pressure transmitting fluid. Then they were pressurized at 220, 250 and 330 MPa at 7, 15 and 25 °C for 5 and 10 min. Immediately after HP treatment, samples were frozen to -30 °C until use for physical and chemical analysis.

### **HP** treatment

HP treatments were performed in a designed and constructed laboratory-scale unit (capacity: 30 cm<sup>3</sup>, maximum pressure: 500 MPa). Water was used as the pressure-transmitting medium. The equipment consists of a pressure chamber of cylindrical design, two end closures, a means for restraining the end closures, a pressure pump and a hydraulic unit to generate high pressure for system compression, and also a temperature control device.

The pressure vessel was made of hot galvanized carbon steel and piston was hard chrome-plated and polished to mirror finish (steel-type heat-treated special K) which was processed into the required sizes at the Electrical and Electronic Engineering Department of Middle East Technical University (Ankara, Turkey). The liquid was heated prior to pressurization to the desired temperature by an electrical heating system surrounding the chamber. Time to reach the desired pressure and also for depressurization was approximately 5–10 s for the system.

# Physical and chemical analyses

#### Physical analyses

The colour of the fish samples was determined with the help of a Konica Minolta chromo meter (model CR 400/410; Minolta, Osaka, Japan).  $L^*$  (brightness),  $a^*$  (+ a, red; – a, green) and  $b^*$  (+ b, yellow; – b, blue) values were measured. The colorimeter was calibrated using white references (CR-A44). The colour was measured on homogenates prepared from ten fish fillets. The homogenate was placed in plastic petri dishes and the colour measurement was repeated 10 times. Averages and standard deviations of  $L^*$ ,  $a^*$  and  $b^*$  values were calculated as the total colour differences. The total colour difference (E), as calculated below, was also used for evaluation,  $E = (L^{*2} + a^{*2} + b^{*2})^{1/2}$  where  $L^*$ ,  $a^*$  and  $b^*$  are the difference of the  $L^*$ ,  $a^*$  and  $b^*$  values between the treated samples and control (Gerdes and Santos Valdez, 1991).

#### Chemical analysis

The thiobarbituric acid value was determined colorimetric by the method of Erkan and Özden (2008). Trimethylamin nitrogen was determined by the method of AOAC (1998). Results of TBA and TMA-N were expressed as mg/kg muscle and mg/100 g muscle. Free amino acid content of untreated and HP treated samples was determined using the hydrolysis and derivatization technique described by Erkan et al (2010). The amino acids were determined by using a (HPLC). In this study, it was determined that cold smoked salmon contained lysine (lys), methionine (meth), isoleucine (isoleu), leucine (leu), phenylalanine (phen), valine (val), histidin (his), serine (ser), arginine (arg), cysteine (cys), tyrosine (tyr), alanine (ala), aspartic acid (asp), glutamic acid (glut), glycine (gly) and proline (prol). Amino acids were identified by comparison of their retention time with those of an authentic standard (Pierce, Amino Acid Standard Hydrolyzate, Product No: 20078 20088 20089 1800180 NCI0180, Rockford, IL, USA) and their contents were calculated on a weight basis (mg/100g).

## **Statistical analysis**

Significant differences between the samples (for colour, TBA and TMA-N analysis) were calculated by Excel XP 2003 by one-way analysis of variance (ANOVA) using a significance level of P < 0.05 by Tukey's honestly significant difference test. Calculations for free amino acid made were the mean, standard deviation, coefficients of variation in percent and F-setting the confidence level at 95 % test (Sümbüloğlu and Sümbüloğlu, 2002).

Erkan et al. (2010) reported that a\* and b\* values of fresh red mullet were not affected after treatment at 250 MPa, 3-7-15-25 °C for 5 min.

The total colour differences ( $\emptyset$ E) between samples of pressurized sea food are reported as appropriate indicators for changes in colour (Chevalier et al., 2001). Minimum ØE values were found in the following HP condition: 220 MPa, 15-25 °C for 10 min, 220 MPa, 7 °C for 5-10 min, 250 MPa, 7 °C for 5 min for rainbow trout. The total colour differences in the literature were reported as 6.6, 8.6 and 24.3 for muscles of pressurized carp fillets at 140 MPa, 4 °C for 15 and for 30 min and at 200 MPa, 4 °C for 30 min, respectively (Sequeira-Munoz et al., 2006); 5.9, 6.3, 20.3 and 24.3 for muscles of pressurized turbot fillets at 100 MPa, 4 °C for 15 and for 30 min and at 200 MPa, 4 °C for 15 and for 30 min, respectively (Chevalier et al., 2001).

The TBA values for rainbow trout samples of HP-treated samples (at 220, 250, 330 MPa, 7, 15, 25 °C for 5 min) were not significantly (p>0.05) different to those of untreated rainbow trout's; however, TBA values of HP-treated rainbow trout flesh were significantly (p < 0.05) lower than those of untreated rainbow trout samples (at 220, 250, 330 MPa, 7, 15, 25 °C for 10 min). In the literature, the TBA

**TABLE 1:** Changes in colour analysis results of unpressurized and pressurized rainbow trout.

## **Results and discussion**

Changes in the colour of rainbow trout samples were shown in Tables 1.  $L^*$  values of untreated rainbow trout samples were  $57.57 \pm 2.98$ , respectively. The pressurized rainbow trout fillets lost their transparency with an increase of the  $L^*$  values, indicative of the brightness, for both an increase of the pressure. They particularly appeared as cooked. This affected is accentuated with an increase in the pressure. The changes were attributed to the denaturation of the myofibriller and sarcoplasmic proteins. Similar results were reported for HP-treated carp (Sequeira-Munoz et al., 2006) and also for HP-treated mahi mahi, rainbow trout (Yağız et al., 2007), for HP- treated sea bream (Erkan and Üretener, 2010) and HP treated red mullet (Erkan et al., 2010).

The a\* values, normally used as an index of visual redness, did not change significantly after pressurization in rainbow trout muscle (except 250 MPa, 7 °C for 10 min). Not significantly differences (p>0.05) in b\* values were noted for rainbow trout flesh HP- treated compared to untreated rainbow trout's. It has been reported for mackerel and cod fish that the a\* values decreased after pressurization (Ohshima et al., 1993). Similarly, redness of raw cod was lost after HP treatment at ≥ 200 MPa (Angsupanich et al., 1999). Chevalier et al. (2001) observed essentially no changes in the a\* values with pressure or holding time. It has been reported for carp that the b\* values increased with pressure and with holding times at pressure levels of 140 MPa and above (Sequeira- Munoz et al., 2006).

Tem Time	perature/	7 °C/ 5 min	7 °C/ 10 min	15 °C/ 5 min	15 °C/ 10 min	25 °C/ 5 min	25 °C/ 10 min	
L*	Untreated	57.57 <sup>Aa</sup> ± 2.98*	57.57 <sup>Aa</sup> ± 2.98	57.57 <sup>Aa</sup> ± 2.98	57.57 <sup>Aa</sup> ± 2.98	57.57 <sup>Aa</sup> ± 2.98	57.57 <sup>Aa</sup> ± 2.98	
	220 MPa	65.34 <sup>Ba</sup> ± 2.42	67.01 <sup>Ba</sup> ± 1.08	67.62 <sup>Ba</sup> ± 2.72	62.76 <sup>Ba</sup> ±1.66	65.32 <sup>Ba</sup> ± 1.93	64.62 <sup>Ba</sup> ± 3.03	
	250 MPa	65.81 <sup>Ba</sup> ± 4.62	69.71 <sup>Ba</sup> ± 0.64	66.16 <sup>Ba</sup> ± 0.81	69.42 <sup>Ca</sup> ± 0.43	72.96 <sup>cb</sup> ± 2.43	68.30 <sup>Ba</sup> ± 1.53	
	330 MPa	73.99 <sup>Ba</sup> ± 5.03	70.53 <sup>8a</sup> ± 1.39	72.02 <sup>Ba</sup> ± 1.29	72.08 <sup>ca</sup> ± 3.05	75.21 <sup>ca</sup> ± 4.13	75.52 <sup>ca</sup> ± 2.32	
a*	Untreated	2.88 <sup>Aa</sup> ± 0.91	2.88 <sup>Aa</sup> ± 0.91	2.88 <sup>Aa</sup> ± 0.91	2.88 <sup>Aa</sup> ± 0.91	2.88 <sup>Aa</sup> ± 0.91	2.88 <sup>Aa</sup> ± 0.91	
	220 MPa	3.40 <sup>Aa</sup> ± 0.59	3.40 <sup>Aa</sup> ± 0.95	2.58 <sup>Aa</sup> ± 1.00	2.52 <sup>Aa</sup> ± 0.05	2.97 <sup>Aa</sup> ± 1.10	4.36 <sup>Aa</sup> ± 1.77	
	250 MPa	2.47 <sup>Aa</sup> ± 1.80	1.25 <sup>вь</sup> ± 0.34	2.84 <sup>Aa</sup> ± 0.44	2.02 <sup>Aa</sup> ± 1.14	2.22 <sup>Aa</sup> ± 1.59	2.84 <sup>Aa</sup> ± 0.69	
	330 MPa	2.96 <sup>Aa</sup> ± 0.04	1.63 <sup>Aa</sup> ± 1.27	3.49 <sup>Aa</sup> ± 0.91	1.58 <sup>Aa</sup> ± 0.78	3.97 <sup>Aa</sup> ± 2.57	2.10 <sup>Aa</sup> ± 1.00	
b*	Untreated	14.97 <sup>Aa</sup> ± 5.37	14.97 <sup>&amp;a</sup> ± 5.37	14.97 <sup>Aa</sup> ± 5.37	14.97 <sup>&amp;a</sup> ± 5.37	14.97 <sup>Aa</sup> ± 5.37	14.97 <sup>Aa</sup> ± 5.37	
	220 MPa	13.77 <sup>Aa</sup> ± 3.36	15.25 <sup>Aa</sup> ± 1.95	14.21 <sup>Aa</sup> ± 1.49	12.96 <sup>Aa</sup> ± 1.50	12.12 <sup>Aa</sup> ± 0.90	16.61 <sup>Aa</sup> ± 2.59	
	250 MPa	12.24 <sup>Aa</sup> ± 0.71	14.59 <sup>&amp;a</sup> ± 1.00	14.54 <sup>Aa</sup> ± 3.42	14.13 <sup>Aa</sup> ± 0.76	14.82 <sup>Aa</sup> ± 2.27	13.43 <sup>Aa</sup> ± 2.21	
	330 MPa	14.07 <sup>Aa</sup> ± 0.75	13.47 <sup>Aa</sup> ± 0.12	15.92 <sup>Aa</sup> ± 4.31	13.73 <sup>Aa</sup> ± 0.98	14.35 <sup>Aa</sup> ± 2.72	13.83 <sup>Aa</sup> ± 0.20	
E	Untreated	-	-	-	-	-	-	
	220 MPa	8.33 ± 0.86	9.00 ± 1.44	10.62 ± 4.31	5.63 ± 0.77	8.44 ± 0.69	6.73 ± 3.46	
	250 MPa	9.41 ± 4.84	11.72 ± 0.55	9.70 ± 3.56	11.52 ± 0.30	15.74 ± 3.73	11.19 ± 1.41	
	330 MPa	17.07 ± 5.95	12.69 ± 1.49	15.22 ± 4.79	14.14 ± 3.13	18.14 ± 2.85	17.46 ± 2.33	

\* standard deviation (n = 3); Different letters ( $^{A}$ ,  $^{B}$ ,  $^{C}$ ) in the same column indicate significant differences ( $\rho < 0.05$ ); Different letters ( $^{a}$ ,  $^{b}$ ,  $^{c}$ ) in the same line indicate significant differences (p < 0.05)

values of HP-treated fish and fish products with increasing pressure and pressure-holding times has been reported to show progressive changes (Sequeira-Munoz et al., 2006; Yağız et al., 2007). Angsupanich and Ledward (1998) reported that pressure below 400 MPa had a slight effect on lipid oxidation in cod muscle treated for 20 min at ambient temperature. However, they did not notice a significant change of the TBA number at 200 MPa. Horse makkerel and rainbow trout not seemed to be more sensitive to applied pressure in term of lipid oxidation. TBA value in HP applications are affected pressure, time and temperature. Furthermore, in lipid oxidation play a key role the content of unsaturated fats in fish species, iron compounds and myoglobin, hemoglobin and ferritin content in meat and fish (Chevalier et al., 2001). Amanatidou et al. (2000) reported that TBA values of fresh Atlantic salmon were not affected after HP treatment up to 200 MPa. Chevalier et al. (2001) favourable to find changes in TBA levels in raw turbot muscles treated at 100-140 MPa, 4 °C for 15-30 min. Sequeira-Munoz et al. (2006) reported that 100 MPa pressure 4 °C temperature 15 min time had little effect on lipid oxidation of carp fillets while 140–180 and 400 MPa, 4 °C for 15 and 30 min had significant effect on lipid oxidation. Yağız et al. (2007) reported stable TBA value compared to control samples in rainbow trout dark muscle treated at 150 and 300 MPa, room temperature for 15 min. Erkan et al. (2010) reported that TBA values of raw red mullet were not affected after high pressure treatment at 220 MPa, 3 °C for 5-10 min, 250 MPa, 7-15-25 °C for 5 min and 330 MPa, 7-15 °C for 5-10 min. Changes in TBA values of pressurized sea bream samples at 220-250 MPa, 15-25 °C for 10 min were reported as minor changes than the control (Erkan and Üretener, 2010).

was found suitable for stability of TMA-N. On the other hand, there has been little data available documenting the TMA-N content of pressurized fish in literature. TMA-N content of pressurized rainbow trout are shown in Tables 3, respectively. TMA-N content of HP-treated at 250 MPa, 7 °C for 10 min, 250-330 MPa, 15 °C for 5 min, 250-330 MPa, 25 °C for 5 min, 220-250-330 MPa, 25 °C for 10 min rainbow trout samples were found to be significantly (P < 0.05) lower than the untreated samples. The changes were attributed to the inhibition of proteolytic activity (Hernández-Andrés et al. 2005). The effect of HP treatment on colour, TBA and TMA parameters of red mullet was studied by Erkan et al. (2010). These studies indicated unchanged TMA-N content for HP treated at 220 °C MPa, 15 °C for 10 min, 220 °C MPa, 25 °C for 5 min, 250 °C MPa, 7-25 °C for 10 min, 330 °C MPa, 3 °C for 5 min. HP-induced changes in TMA-N values measured immediately after HP treatment (at 220-250-330 °C MPa, 7 and 15 °C for 5 min; at 330 °C, 3-7 °C for 10 min) in this study are in agreement with data previously reported for HP-treated sea bream samples (Erkan and Üretener, 2010).

Amino acids play an important role in human nutrition and also affect the sensory traits of food products. During the processing of foods, protein sources are treated with heat, pressure, oxidizing agents, organic solvents, alkalis and acids for a variety of reasons. Such treatments may cause modification of the nutritional value of proteins, decreasing the amino acid content through desulfuration, deamination or isomerization; reactions with lysine, methionine, cystine and tryptophan are the most susceptible to damage (Belitz and Grosch, 1999; De la Cruz-García et al., 2000). Sea foods are rich in lysine, isoleucine, leucine and valine of the essential amino acids; these amino acids

are for the development of both desirable and undesirable flavours in marine based food products. However, non-essential amino acids of sea foods, such as glutamic acid, aspartic acids also contribute to the characteristic taste and flavour of rainbow trout's (Özden, 2005).

In this study, glutamic acid, aspartic acid, lysine, leucine, arginine and valine content of untreated trout samples was found highly. Rainbow trout tissue HP-treated at at 220-250 MPa, 7-15 °C for 5-10 min and 330 MPa, 15-25 °C for 10 min did not differ significantly in the majority of amino acids compared to untreated rainbow trout tissue (Tab. 4). Amino acid contents of HP-trea-

**TABLE 2:** Changes in TBA analysis results of unpressurized and pressurized
 rainbow trout.

Temperat Time	ure/	7 °C/ 5 min	7 °C/ 10 min	15 °C/ 5 min	15 °C/ 10 min	25 °C/ 5 min	25 °C/ 10 min	
TBA (mgMDA/kg)	Untreated	2.77 <sup>Aa</sup> ± 0.15	2.77 <sup>Aa</sup> ± 0.14	2.77 <sup>Aa</sup> ± 0.15	2.77 <sup>Aa</sup> ± 0.14	2.77 <sup>Aa</sup> ± 0.15	2.77 <sup>Aa</sup> ± 0.14	
	220 MPa	2.34 <sup>Aa</sup> ± 0.07	2.23 <sup>8b</sup> ± 0.02	2.46 <sup>Aa</sup> ± 0.35	1.96 <sup>Ba</sup> ± 0.09	2.52 <sup>Aa</sup> ± 0.31	2.06 <sup>Bb</sup> ± 0.25	
	250 MPa	2.40 <sup>Aa</sup> ± 0.43	2.18 <sup>Bb</sup> ± 0.03	2.36 <sup>Aa</sup> ± 0.42	2.30 <sup>Aa</sup> ± 0.46	2.39 <sup>Aa</sup> ± 0.54	1.98 <sup>Bb</sup> ± 0.42	
	330 MPa	2.31 <sup>Aa</sup> ± 0.30	2.30A <sup>Ba</sup> ± 0.32	2.44 <sup>Aa</sup> ± 0.25	2.18 <sup>Ba</sup> ± 0.29	2.30 <sup>Aa</sup> ± 0.38	2.00 <sup>Ba</sup> ± 0.42	

\* standard deviation (n = 3); Different letters (<sup>A</sup>, <sup>B</sup>, <sup>C</sup>) in the same column indicate significant differences (p < 0.05); Different letters (<sup>a</sup>, <sup>b</sup>, <sup>c</sup>) in the same line indicate significant differences (p < 0.05)

TMA-N content is often used as a biochemical index to assess keeping quality and shelf-life of fish. In marine fish, TMA-N is formed from trimethylamine oxide (TMAO) which is a part of the non-protein nitrogen fraction of the fish flesh. TMA production is the result of bacterial enzyme activity and is the main compound responsible for an unpleasant "fishy" odour. The volatile amines trimethylamine (TMA-N) have been widely proposed as quality indicators in fish since they show a close relationship with the sensory score (Huss, 1995; Pons-Sánchez-Cascado et al., 2006). Study, we tested all the HP reconditions **TABLE 3:** Changes in TMA-N analysis results of unpressurized and pressurized rainbow trout.

Tempera Time	ture/	7 °C/ 5 min	7 °C/ 10 min	15 °C/ 5 min	15 °C/ 10 min	25 °C/ 5 min	25 °C/ 10 min	
TMA-N (mg/100 g)	Untreated	1.60 <sup>Aa</sup> ± 0.44						
	220 MPa	1.44 <sup>Aa</sup> ± 0.34	1.59 <sup>Aa</sup> ± 0.07	1.15 <sup>Aa</sup> ± 0.28	1.74 <sup>Aa</sup> ± 0.09	1.12 <sup>Aa</sup> ± 0.57	0.50 <sup>8b</sup> ± 0.14	
	250 MPa	1.51 <sup>Aa</sup> ± 0.18	0.48 <sup>8b</sup> ± 0.14	0.50 <sup>Bb</sup> ± 0.03	2.05 <sup>Ac</sup> ± 0.06	1.15 <sup>Bd</sup> ± 0.06	0.35 <sup>8b</sup> ± 0.11	
	330 MPa	1.52 <sup>Aa</sup> ± 0.13	1.81 <sup>Aa</sup> ± 0.53	0.97 <sup>Bb</sup> ± 0.18	1.73 <sup>Aa</sup> ± 0.11	1.17 <sup>Bb</sup> ± 0.07	0.95 <sup>cb</sup> ± 0.06	

\* standard deviation (n = 3), Different letters ( $^{A}$ ,  $^{B}$ ,  $^{C}$ ) in the same column indicate significant differences (p < 0.05) Different letters (a, b, c) in the same line indicate significant differences (p < 0.05)

Amino	C	220	220	250	250	330	330	220	220	250	250	330	330	220	220	250	250	330	330
acids		MPa,	MPa,	MPa,	MPa,	MPa,	MPa,	MPa,	MPa,	MPa,	MPa,	MPa,	MPa,	MPa,	MPa,	MPa,	MPa,	MPa,	MPa,
(mg/		7 °C,	7 ℃,	7 ℃,	7 °C,	7 °C,	7 °C,	15 °C,	15 °C,	15 °C,	15 °C,	15 °C,	15 °C,	25 °C,					
100g)		5 min	10 min	5 min	10 min	5 min	10 min	5 min	10 min	5 min	10 min	5 min	10 min	5 min	10 min	5 min	10 min	5 min	10 min
Lys	2111	1941	1927	1877	1933	1950	2076	1739	1813	1872	1763	1528	1932	1834	2274	2240	2039	2471	2243
	±15.99	±8.90	±2.15	±8.08	±8.17	±7.27	±18.31	±9.06	±32.94	±10.42	±6.12	±6.41	±17.64	±8.87	±28.33	±0.85	±5.14	±7.02	±5.19
Meth	683	701	786	715	623	758	832	687	711	728	728	718	729	645	836	888	746	957	735
	±1.73	±2.04	±2.17	±0.63	±2.87	±5.67	±6.18	±7.55	±7.66	±11.88	±0.02	±2.02	±5.12	±1.37	±12.14	±9.10	±3.50	±2.20	±1.92
Thre	971	932	930	901	916	1065	990	901	877	872	938	921	889	847	1122	1080	1079	1180	1007
	±3.11	±9.33	±4.30	±3.75	±3.11	±8.74	±4.16	±1.21	±20.74	±1.17	±4.61	±1.31	±6.93	±2.45	±1.80	±9.88	±0.10	±0.05	±0.65
Isoleu	1131	1188	1143	1057	1042	1309	1269	1039	1071	1039	1119	1102	1139	1061	1305	1203	1232	1374	1195
	±8.12	±9.69	±0.65	±8.50	±15.97	±2.43	±14.01	±2.26	±10.94	±2.66	±17.43	±0.80	±11.22	±4.27	±8.81	±5.63	±1.80	±5.30	±0.24
Leu	1692	1671	1637	1548	1536	1795	1774	1491	1516	1524	1607	1567	1600	1520	1902	1837	1807	2032	1793
	±5.81	±4.43	±0.65	±6.66	±11.93	±3.38	±13.19	±11.32	±9.14	±10.74	±1.10	±5.87	±2.81	±7.26	±0.75	±0.08	±3.65	±4.39	±6.84
Phen	978	1001	985	925	910	1108	1052	909	887	911	946	1064	961	917	1129	1102	1040	1215	1067
	±2.84	±3.37	±3.66	±7.29	±9.68	±4.76	±0.23	±4.78	±1.28	±4.30	±1.76	±1.60	±5.03	±4.90	±3.01	±2.65	±6.25	±1.24	±1.40
Val	1264	1350	1283	1166	1153	1511	1418	1165	1209	1146	1257	1234	1285	1196	1423	1320	1411	1496	1337
	±15.81	±8.87	±4.26	±0.14	±11.22	±3.60	±18.95	±2.57	±28.36	±4.72	±14.54	±3.55	±10.21	±6.41	±7.29	±6.80	±4.137	±2.85	±0.12
His	626	641	638	588	595	814	686	602	588	553	609	672	635	593	730	694	773	779	676
	±2.289	±1.35	±6.99	±7.77	±6.11	±6.09	±4.80	±4.06	±5.25	±0.42	±1.89	±6.36	±0.15	±3.16	±3.10	±1.32	±12.26	±1.35	±3.35
Ser	833	890	852	832	795	1650	814	851	737	795	846	787	764.02	776	989	945	1105	1019	890
	±0.65	±2.75	±6.39	±2.69	±0.084	±4.72	±1.05	±1.44	±6.25	±4.97	±6.63	±5.87	±4.09	±0.91	±3.13	±1.98	±6.59	±8.92	±5.01
Arg	1530	1466	1429	1343	1352	1429	1500	1331	1312	1285	1336	1492	1404	1354	1729	1586	1807	1752	1580
	±5.90	±2.08	±8.94	±1.70	±21.34	±14.30	±6.77	±0.41	±12.99	±6.54	±3.27	±2.61	±19.30	±5.50	±3.04	±6.76	±2.61	±3.41	±9.57
Cys	131	100	111	99	130	120	113	89	91	112	80	137	119	128	167	130	209	177	131
	±0.09	±0.77	±0.01	±2.15	±1.20	±1.02	±0.016	±0.46	±3.285	±2.03	±0.26	±2.02	±2.18	±0.10	±2.01	±1.23	±2.54	±1.35	±1.28
Tyr	808	833	818	767	750	985	867	803	764	761	803	893	801	770	948	920	969	985	872
	±5.84	±0.02	±2.66	±1.76	±0.55	±3.18	±0.38	±0.94	±1.48	±2.01	±8.49	±2.23	±0.48	±6.72	±1.48	±10.17	±1.19	±0.69	±7.34
Ala	1066	1265	1227	1126	989	1360	1355	1165	1151	1110	1078	966	1216	1080	1385	1347	1764	1506	1108
	±12.11	±8.02	±1.94	±1.71	±2.14	±10.86	±7.64	±1.25	±24.83	±5.30	±16.50	±4.29	±17.90	±10.06	±26.52	±0.57	±0.51	±3.95	±3.39
Asp	2319	2020	2109	2020	2110	2197	2269	1908	1889	1954	1863	1556	2058	1912	2472	2392	2028	2619	2463
	±10.16	±8.46	±0.18	±0.62	±4.72	±14.63	±30.41	±5.69	±28.41	±0.19	±26.01	±5.67	±10.92	±11.07	±57.34	±19.83	±7.56	±34.56	±3.23
Glut	3276	2838	2924	2789	2950	2837	3141	2634	2708	2785	2703	2288	2835	2680	3458	3312	2993	3664	3430
	±10.65	±5.55	±6.39	±12.59	±1.44	±7.29	±36.47	±2.25	±4.04	±34.14	±16.29	±4.33	±17.39	±11.37	±47.13	±11.80	±4.59	±5.90	±4.05
Gly	1014	1224	1326	996	936	1484	1112	989	946	915	1032	1128	1077	1008	1167	1154	1525	1240	1110
	±3.84	±7.83	±0.01	±1.75	±4.32	±4.02	±3.74	±8.51	±11.85	±2.50	±9.55	±1.66	±5.73	±5.17	±12.19	±3.10	±0.94	±1.50	±3.21
Prol	689	731	670	611	614	734	709	662	628	604	630	642	642	604	752	701	733	774	728
	±0.64	±6.06	±13.14	±7.12	±4.39	±1.02	±0.50	±8.18	±0.96	±4.65	±3.64	±0.87	±10.25	±4.77	±1.403	±10.19	±1.94	±0.19	±2.14

**TABLE 4:** Changes in free amino acid analysis results of unpressurized and pressurized rainbow trout (insignificant data compared to control are shown with dark colour).

ted samples were lower or higher than untreated trout samples because of protein denaturation. Denaturation of protein by HP has been well reported. Pressure, temperature and exposure of HP treatment determine degree of protein denaturation. Sendra et al. (2000) has been reported the decrease of free amino acids in cheese treated pressures in over 200-300 MPa. This change in amino acid content may cause changes in the fish's flavour and aroma, this compounds may also reflect fish product quality during storage. Amino acid changes in the HP-treated fishes that may be associated with the image of the cooked. More detailed studies should be done about it. In this study, especially at low temperatures (7 °C) with increasing pressure and holding time of changes in amino acids were determined to increase. Similar situation was observed in the press room temperature (15 °C) applications. Amino acid changes of high pressure application at 7 and 15 °C is higher than the high pressure application at 25 °C.

In conclusion, rainbow trout were subjected to HP treatments at 220, 250 and 330 MPa, 7, 15 and 25  $^{\circ}$ C for 5 and

10 min. In the selection of the best HP conditions close to control values or lower than the control colour, TBA and TMA-N values were based and are assessed together. Colour, TMA-N and TBA results indicated that HP-treated rainbow trout used in this trial had a best condition HP of at 220 MPa, 7–15–25 °C for 5–10 min and 250 MPa, 7–15 °C for 5 min.

## **Acknowledgments**

This work was supported by the TUBITAK-TOVAG projects (No: 108O668) and the Research Found of Istanbul University (Project Number BYP-2861).

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# Address for correspondence:

Assoc. Prof. Dr. Nuray Erkan Istanbul University, Faculty of Fisheries, Department of Seafood Processing and Quality Control Ordu Cad. No: 200 34470 Laleli/ Istanbul – Turkey Fax: +90 212 455 58 61 Tel.: +90 212 455 57 00/16415 E-Mail: nurerkan@istanbul.edu.tr