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Fisheries and Aquaculture Journal

Research Article

The Effects of Different Stunning Techniques on Meat Quality of Brown Trout (*Salmo Trutta Fario*)

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ABSTRACT

This study was conducted to assess the effects of various stunning methods on meat quality traits of *Salmo trutta fario*. A total of seventy five fish (1 year old, female/male) were exposed to one of the five stunning procedure: iced water, electrical shock, carbondioxide (CO_2) saturated water, percussive stunning and asphyxia. Highest initial fillet pH was measured in CO_2 group's meat on the first day of the trial and the lowest initial pH was belonged to asphyxia, but the difference among groups was not statistically significant ($p>0.05$). Significant relation was determined between pH and storage period of the fillets ($p<0.01$). No significant relation was obtained between killing methods and L* value of the meat. Killing method and storage period did not affect the water holding capacity and pH of the fillets. However; carbondioxide was thought to be the best method for better total volatile base nitrogen (TVBN) values; for better thiobarbituric acid-reactive substances (TBARS) of the meat, iced water method should be preferred.

Keywords: Brown trout; Iced water; Electrical shock; CO_2 saturated water; Percussive stunning; Asphyxia; Meat quality

INTRODUCTION

Despite of the increase in number of human population, there has been a very clear decline of the food sources on the World. On the other hand, rising of the obesity as a global problem and increasing in consciousness of the mankind, obviously have induced a trend in attributing importance to healthy and balanced nutrition [1]. Therefore, the demand for seafood, which known as an valuable food resource for healthy populations, has increased, recently [2,3]. Since, seafood includes many nutritional elements in a fine balanced and amount; with high quality protein; is known as a very valuable meat source [4,5]. However, because of high nutritional traits; lipid, protein and non-protein nitrogen compounds; it serves as an perfect substrate for microorganisms and they leads to the putrefaction, maybe before reaches to the consumers, in many conditions [3,6,7].

For example, if pre-slaughter period includes many stressors, the onset of the rigor mortis procedure shortens. Shorter procedure causes a rapid decline in ATP and glycogen reserves, in muscle tissues. Handling and processing procedures of the fish that passed the rigor, bring out the meat quality losses. Hence, pre-rigor period should be as short as to cover all processing steps (remove of internal organs, washing, icing, and packaging). The processing of the fish, after rigor, gives rise to, undesirable changes in physical, freshness and marketing ability traits and shortens the shelf life of the products [8,9]. *S. trutta* is one of the most important fish species due to its aquaculture potential, economic value and wide consumer demand and skin color is an important commercial trait in fish farming, given that this phenotype influences consumer acceptance, thereby determining the commercial value that fish can reach [10,11]. Killing method is one of the important pre-slaughter factors affecting the quality of the fish products and post-portem meat traits. When fish are killed rapidly, stress can be reduced and this improve both welfare and meat quality [12,13].

A wide range of analytical methods has been used to characterize the quality changes that occur to a muscle food during commercial sterilization processes. Color, texture, and cook loss are among the most frequently used quality indicators. Area shrinkage, caused by heat-induced protein denaturation and the resultant shrinkage of the muscle fibers are also important cooking quality of muscle foods [14]. The instrumental color measurement usually uses the L^*, a^*, b^* scale in which primary parameters are lightness (L^*), redness (a^*), and yellowness (b^*). Conventionally, the color values are measured by a colorimetric or spectrophotometric method, involving reflected light from a sample surface. Recently, the quality of aqua cultured fish fillets was widely studied also by measurement of chemical

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Received: December 19, 2018; **Accepted:** February 20, 2019; **Published:** February 28, 2019

Citation: Genc M, Alak G, Atamanalp M, Sabuncuoglu N, Kocaman EM, Ceylan ZG, et al. (2019) The Effects of Different Stunning Techniques on Meat Quality of Brown Trout (*Salmo Trutta Fario*). Fish Aqua J 10:264. doi: 10.35248/2150-3508.19.10.264

pH, water holding capacity (WHC), TVB-N (Total volatile base nitrogen) and TBARS (Thiobarbituricreactive substances).

In the present study, five killing methods were applied to *S. trutta fario* trouts and some meat and color traits of the fish were examined and compared within methods. In the scientific literature, according to the best of knowledge, no study examining the relation between stress and meat traits has been performed on *S. trutta fario* species.

MATERIALS AND METHODS

Experimental animals

Seventy five *S. trutta fario* fish (1 year old, female/male) were obtained from the breeding unit in Ataturk University Fisheries Faculty's Research center. Each group designed with 15 fish stocking. Water temperature was (11.5 ± 1.5)°C, dissolved oxygen level was 9.1 mg/L and pH was 7.4, during the study. Water was distributed to the tanks with a minimum flow of 0.5 L/min per kg of fish. The breeding and the research procedures were in compliance with CCAC guidelines. For five killing methods, equal numbers of about 180 g fish were distributed randomly, to each group.

Killing methods

Iced water: Traditional process, which 2/3 of the water tank filled with ice (0-2) °C, was used [15].

Carbon dioxide (CO_2) method: Water was saturated with CO_2, for 2 hours and the saturation maintained during the experiment. The fish were kept within CO_2 saturated water minimum 4 minutes and as soon as the stunning was diagnosed, the fish were decapitated.

Electrical shock: Steel rod electrodes were floored with 2.5 cm intervals, horizontally. After fish transported to the water tank, 175 V electric current was applied for 1.5 seconds then the animals were decapitated.

Percussive stunning: Fish were percuted on the head, by using a steel hammer, after taken out from the water [16], and were decapitated.

Asphyxia: The fish were taken out from the water and waited for the asphyxia. After the struggle ended the animals were decapitated.

Chemical studies of the fillets were as below

pH: Muscle pH was measured post-mortem 0, 3rd, 6th, 9th days, by using pH meter (WTW Inolab) after homogenization of 10 g meat within 100 ml distillate water, as described by Gokalp et al. [17].

Color measures: Color of the fillets was determined by using a colourimeter (Minolta Chroma Meter CR-400, Konica Minolta, Osaka, Japan) The L^* variable represents lightness (L^*=0 for black, L^*=100 for white), the a^* scale represents the red/green, $+a^*$ intensity in red and a* intensity in green and the b^* scale represents the yellow/blue, $+b^*$ intensity in yellow and b^* intensity in blue [18].

WHC (Water holding capacity): 5 g to 10 g of whole homogenized fish fillets were dried after mixing with sea sand at 105°C to constant weight (at least 12 h). The water content was calculated in duplicate and expressed in percentage as reported by Schroder [19].

TVB-N and TBARS: Thiobarbituric acid-reactive substances (TBARS) and TVB-N (Total volatile base nitrogen) were performed as described by Lee et al. [20,21].

Statistics: The impact of the killing method and storage period of the fillets on the pH, L^*, a^*, b^*, WHC, TVB-N and TBARS values were analysed by two-way analysis of variance (ANOVA). All statistical analyses were performed using the SPSS software package SPSS [22].

RESULTS

Highest fillet pH was measured in CO_2 group meat, on the first day of the examination and the lowest was belonged to asphyxia, however the difference among groups was not statistically significant ($p>0.05$), (Table 1). Besides, very significant relation was calculated between pH and storage period of the fillets ($p<0.01$).

pH value of the fillets decreased until 3rd day of the storage, then increased in all experimental groups, except for asphyxia one. In asphyxia group, pH increased on 3rd and 6th days, after it began to decline. No significant relation was observed between killing methods and L^* value of the meat ($p>0.05$), (Table 2). Besides, L^* value was not affected by storage period of the fillets ($p>0.05$).

Redness (a^*) value of the fillets was not affected by the killing methods however, the value changed slightly iced water, CO_2 and electric shock groups, with increasing in the storage period days ($p<0.05$), (Table 3).

Table 1: pH values of the fillets according to killing methods and storage period.

	Storage time				
Killing method	0 day	3rd day	6th day	9th day	Total
Iced water	6.453	6.397	6.563	6.663	6.519
CO_2	6.587	6.337	6.477	6.68	6.53
Asphyxia	6.43	6.603	6.673	6.57	6.569
Electrical shock	6.57	6.403	6.51	6.48	6.491
Percussive stunning	6.547	6.463	6.54	6.59	6.535
Total	6.517bc	6.449c	6.553ab	6.597a	6.529
SEM	-	-	0.057	-	-
			P		
Killing method	-	-	0.421ns	-	-
Storage time	-	-	0.002**	-	-

ns: non-significant; **: $p<0.01$

Table 2: Lightness (L^*) values of the fillets according to various killing methods and storage period.

	Storage time				
Killing method	0 day	3rd day	6th day	9th day	Total
Iced water	49.413	55.24	52.173	51.28	52.027
CO_2	55.467	54.717	51.597	49.697	52.869
Asphyxia	48.737	49.41	47.473	54.937	50.207
Electrical shock	50.017	52.14	50.767	60.293	53.304
Percussive stunning	45.393	52.65	61.783	52.88	53.177
Total	49.805	52.831	52.813	53.817	52.317
SEM	-		3.203	-	
			P		
Killing method	-		0.638ns	-	
Storage time	-		0.286ns	-	

ns: non-significant

Yellowness (b*) value of the fillet groups did not changed with killing methods, significantly (p>0.05), (Table 4).

Yellowness (b*) value of the fillets, from asphyxia and electrical shock groups, increased until 9th day of the storage period. Value b* from the fillets belonging percussive stunning group increased until 6th of the period, then decreased, whereas the value was determined highest on the 3rd day, then decreased until 9th day of the storage period (p<0.05). According to the results, killing method and storage period did not affect the water holding capacity of the fillets (Table 5).

According to the first day analyse, lowest TVB-N value was belonged to CO_2 group, and the asphyxia group had the highest value (p<0.01), (Table 6). On the 6th day of the storage period the lowest TVB-N value was obtained in electrical shock; whereas the lowest level was belonged to the CO_2 group, on the 9th day of the storage (p<0.001).

Total volatile base nitrogen (TVB-N) level of the fillets, linearly increased by increasing storage period time (Figure 1).

Lipid oxidation of the fillets value (TBARS) was lowest in iced water group, on the first day of the analyse; and it was the highest in those CO_2 ones. Electric shock group had higher value than those other methods on 3rd, 6th and 9th storage days analyses (p<0.05), (Table 7).

TBARS value of the fillets was linearly increased with increasing storage time, except for asphyxia and CO_2 groups.

Table 3: Redness (a*) values of the fillets with regard to various killing methods and storage period.

	Storage time				
Killing method	0 day	3rd day	6th day	9th day	Total
Iced water	0.943	6.543	2.687	0.85	2.756
CO_2	3.153	2.06	6.213	2.07	3.374
Asphyxia	1.94	3.01	6.637	3.303	3.723
Electrical shock	4.773	1.073	4.46	4.487	3.698
Percussive stunning	1.06	1.787	2.873	2.51	2.058
Total	2.374b	2.894b	4.574a	2.644b	3.122
SEM	-		1.191	-	
			P		
Killing method	-		0.243ns	-	
Storage time	-		0.025	-	

Table 4: Yellowness (b*) values of the fillets with regard to various killing methods and storage period.

	Storage time				
Killing method	0 day	3rd day	6th day	9th day	Total
Iced water	2.903	8.72	4.113	3.417	4.788
CO_2	6.063	7.21	5.657	3.517	5.612
Asphyxia	4.02	4.527	5.777	5.837	5.04
Electrical shock	3.507	5.5	7.16	13.23	7.349
Percussive stunning	1.557	5.887	7.93	4.027	4.85
Total	3.610b	6.369a	6.127a	6.005a	5.528
SEM	-		1.602	-	
			P		
Killing method	-		0.152ns	-	
Storage time	-		0.011	-	

Table 5: WHC (Water Holding Capacity) values of the fillets according to various killing methods and storage period.

	Storage time				
Killing method	0 day	3rd day	6th day	9th day	Total
Iced water	0.993	0.992	0.992	0.991	0.992
CO_2	0.992	0.99	0.99	0.991	0.991
Asphyxia	0.993	0.993	0.994	0.991	0.993
Electrical shock	0.994	0.993	0.99	0.992	0.992
Percussive stunning	0.993	0.991	0.992	0.991	0.992
Total	0.993	0.992	0.992	0.991	0.992
SEM	-		0.001	-	
			P		
Killing method	-		0.230ns	-	
Storage time	-		0.594ns	-	

ns: non-significant

Table 6: (TVB-N) values of the fillets with regard to various killing methods and storage period (mg/100 g).

	Storage time				
Killing method	0 day	3rd day	6th day	9th day	Total
Iced water	12.656	13.104	14	16.016	13.863c
CO_2	12.264	12.992	13.832	15.456	13.614cd
Asphyxia	13.272	13.832	15.176	17.64	14.867a
Electrical shock	12.32	13.216	13.664	15.568	13.580d
Percussive stunning	13.048	13.384	14.392	16.688	14.291b
Total	12.586d	13.211c	14.243c	16.110a	14.037
SEM	-		0.128	-	
			P		
Killing method	-		0	-	
Storage time	-		0	-	

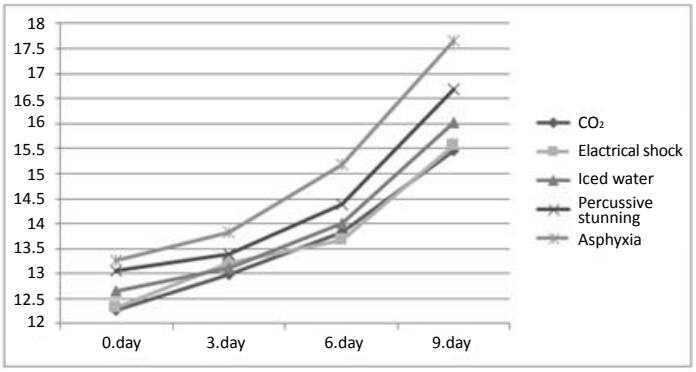

Figure 1: Changing of the fillets' TVB-N levels according to storage period duration.

DISCUSSION

There is a close relation between pre mortem endocrine acute kept reaction and post mortem biochemical processes in the animals, kept for food. It is highly important to examine the effect of the stressors on the color, composition and optimal storage conditions of the seafood products [5]. In the present study, pH, color, WHC, TVB-N and TBARS traits of the trout fillets were compared with regard to five killing methods. Depending on the increased pre-mortem stress, inclined muscular activity affects the level of the anaerobic glycolysis and ATP demolition and this induces the discharge of the energy reserves and production of lactic acid, then pH decreases and rigor mortis develops earlier [9]. At that time,

Table 7: Lipid oxidation (TBARS) values of the fillets with regard to various killing methods and storage period (µmolMA/kg).

	Storage time				
Killing method	0 day	3rd day	6th day	9th day	Total
Iced water	1.541	2.584	3.355	4.352	2.909c
CO_2	2.856	2.539	3.717	4.307	3.336ab
Asphyxia	2.539	2.357	3.4	4.488	3.072bc
Electrical shock	1.813	2.901	4.624	4.941	3.474a
Percussive stunning	1.768	2.629	3.581	4.397	3.188bc
Total	1.978a	2.598b	3.720c	4.488d	3.196
SEM	-		0.152	-	
			P		
Killing method	-		0.002	-	
Storage time	-		0	-	

the durability of fillets reduces, softness, paleness and deformation occur and the quality gets worse [23,24]. The measure of the pH in seafood is a valuable tool for determining the freshness of the product [5].

Post-mortem pH of the fillets changes according to the species, season and breed of the fish, diet composition, it feeding type, fish activity at the time of the catching and other stress conditions and ranges between 6.0-7.1 [25,26]. The previous researches concluded that, in sudden death conditions, progress of the rigor mortis and the changes of the compounds regarding to energy metabolism had always lower velocity; besides, the storage period was also longer than those struggled ones [8,10,12,23,27]. Ruff et al. [10] recorded that, for minimising pre-mortem stress, the fish should be killed after percussive stunning of the turbot (*Scophthalmus maximus L*). Marx et al. [28] stated that on 0 and 3rd day's post-mortem, pH level was found to be lowest in CO_2 group, when compared to percussive and electric shock stunning. Kiessling et al. [27] pointed to lowest pH level, in CO_2 stunned groups, because of strong muscular contraction and high glycogen demolition of the Atlantic salmon (*Salmo salar L*) meat.

Stien et al. [29] declared that, stressed fish had lower pH than those nonstressed ones but, storage period did not affect the pH results, statistically. Hultmann et al. [30], found out lowest muscular pH in stress exposed group than control ones, on post mortem 5th day and the researchers added that the pH of the fillets decreased with progressing of the storage period of the Atlantic cod (*Gadus morhua*) fillets. In this research, killing method did not affect the pH of the fillets. Contradicting results may arise from the species, body weight, diet, and breed and pre mortem environmental conditions of the fish. Hisar et al. [3] reported the pH of the Bonito fish (*Sarda sarda*) during storage period, as follows: 5.99 ± 0.04, 5.98 ± 0.09, 6.02 ± 0.11 ve 6.18 ± 0.15, for 0, 3rd, 6th, 9th days, respectively and showed the increase with increasing days of storage. Sengor et al. [5,31] declared the important increase in pH of the scad (*Trachurus trachurus L*) fillets during storage in refrigerator conditions, and admitted that the pH had exceed the acceptable level.

Similar results were obtained in the present research, showing that the storage period had important effect on pH of the fillets and it should be taken into account as an indicator for the stored meat quality. Rancidity and putrefaction appear not only via changing of the smell sensation, but also softening and discoloration of the meat product [31]. Consumers generally prefer light pink and light red colors in fish meat and pay more for it [32].

In the case of stress conditions, researchers declared that a^* and L^* values changed significantly, especially in high water temperatures [33]. On the contrary, Kiessling et al. [27], concluded that in Atlantic salmon which subjected to CO_2 (high stress) or iso-eugenol anesthesia (low stress), the fillets from CO_2 ones had slightly higher a^* and b^* values than those exposed to low stress. According to Hultmann et al. [30] yellowness in stressed fish were higher than those non stressed group of the Atlantic cod (*Gadus morhua*); Kiessling et al. [27] declared that CO_2 stunning did not affect color of the meat both in fresh and frozen fillets of the Atlantic salmon (*Salmo salar L*); Lefevre et al. [23] stated a decrease in lightness and yellowness of the rainbow trout fish, exposed to asphyxia.

In the present research killing methods had not significant effect on lightness, redness and yellowness values of the fillets ($p>0.05$); lightness values were similar during 0 day to 9 days of storage, however redness had the peak value on 6th day, whereas the yellowness was significantly increased on 3rd day and maintained high until the end of the storage period ($p<0.01$). L^*, a^*, b^* values were largely within the ranges, reported before on various fish species. Comparison with other studies should, however, be made with care as Stien et al. [29] showed that determination of fish fillet color using different colorimetric instruments; L^*, a^*, b^* resulted in considerable variation in the values. Besides, tank conditions, diet composition, other pre-mortem factors may have significant effect on the results of the trials.

WHC is one of the characteristics of the meat and water is preferred to be high level in the tissues and is of great importance both to the industry and the consumer. Hultmann et al. [30], stated that WHC of the fillets obtained from pre-slaughter stressed fish, had lower WHC than those of controls. Kiessling et al. [27], declared no effect of the CO_2 killing method on WHC of both fresh and frozen fillets. Higuera et al. [25] recorded no significant changes of the WHC during 18 day storage period, with only 5% loss weight. In a previous study, on trout's, WHC of the fillets from CO_2 killing method was determined to be lower than those killed via electric shock and percussive stunning methods [28]. In the present research, WHC of the fillets were not changed by both killing methods and storage period ($p>0.05$). WHC of meat is a sensitive parameter and affected by numerous factors; from mineral composition of the diet to humidity level of the storage unit. Besides, the sample size of this research may not be enough to determine the differences of WHC among trial groups.

TVB-N analyse is commonly used indicator for determining the quality of the meat. Higuera et al. [25], TVB-N of ray fish during 18 day of storage and on 3rd day, the researchers obtained very high level for TVB-N and pointed out that, after 3rd day of storage, the fish should not be consumed by human, because of health concerns. However, Chytiri et al. [34] could not find out an important increase in TVB-N on the rainbow trout's until 18th day of storage. Sengor et al. [5], stated that on the 7th day of storage, the TVB-N of the fillets from *Trachurus trachurus L.* exceeded the acceptable level, also, Demirci et al. [31] declared that, that fillets obtained from the same fish species became stale on 8th day. According to another research, studied on Rainbow trout's fillet, it is stated that, if the TVB-N concentration exceed above 30 mg/100 g, it should not be consumed [35]. Hisar et al. [3] pointed out the important increase in TVB-N levels during storage period in Bonita fish.

In present research, highest TVB-N was measured in the fillets, killed

via asphyxia method and TVB-N increased with increasing storage period. On 9th day of the storage highest TVB-N was obtained in asphyxia killed fish as 17.640 mg/100 g and it is concluded that, that level was not harmless for the human consumption. The TVB-N values are affected even by species, catching season, region and sex of the fish. The level of TVB-N in freshly caught fish was reported to range between 5 mg and 20 mg/100 g muscle [36]. When compared to previous findings, present study was thought to be similar to those, however, there found important differences among researches, with regard to number of the storage days that the fillets considered as 'fresh'. TVB-N is thought to be a good tool for progressive times, since it increases with increasing bacterial activity, but may not be a good indicator for early period changes within the fillets.

In the present research, TBARS level was changed with killing methods and the highest level was measured in electrical shock group and followed by CO_2. The TBARS results of percussive stunning and asphyxia methods were statistically equal and the lowest TBARS was measured in iced water group. The value, was always highest for all 3rd, 6th and 9th days of the storage period in electrical shock group, when compared to those other groups ($p<0.05$), (Table 7) and always increased with increasing storage period. The present results confirm that electrical shock caused faster lipid oxidation, as showed by increased TBARS. Similar to the present results, Hisar et al. [3] declared an important increase in TBARS value, with progressing storage period. The other research was concluded that, catching method and stress level were reported to affect the TBARS [37].

CONCLUSION

According to this study, TBARS was thought to be the most sensitive parameter, for determining the slaughter stress and storage period in *S. trutta fario* and it is known that TBARS has important effect on the pH and color traits (especially on a^* and b^* values) of the fillets. Many factors, such as body size, growth rate and fat content and diet composition, tank density, crowding, pre mortem activity, survival time, tank and storage temperature, age of the animal and the season may have significant effects on quality of the product. Besides, onset and resolution of the process of rigor mortis is known to have important effect on meat quality of the slaughter animals. Some of the results of this research support the hypothesis of a negative effect of pre mortem stress on fish fillet quality and storage safety.

Pre-mortem handling and stunning method influences the process of rigor mortis and post-mortem traits of the fillets. In terms of quality, pH of the fillets were not influenced by the killing method, but, CO_2 method was the best method for better TVBN values, however, for better TBARS, iced water was. Although electrical shock was significantly affected the yellowness of the fillets, with regard to meat quality, no superiority was found among the killing methods, in this research. Taking into account the increased consumer's awareness of welfare and food quality issues on cultured species, further studies, are need to understand the effects of numerous pre-mortem and post-mortem conditions on the animal welfare and food safety issues.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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