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Predicting the contents of volatile and non-volatile amines in rainbow trout fillet during storage time via image processing technique

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RESEARCH ARTICLE

Abstract

This study describes the potential use of colorimetric parameters (L*, a*, b*, C* and h*) obtained from image processing technique (IPT) to predict the contents of spoilage indicators (histamine, total volatile nitrogen and trimethylamine nitrogen) in rainbow trout fillets (600±50 g) stored at 4±1 °C during time intervals of 0, 5, 10, 15 and 20 days. Compared to 10 days, storage for 15 or 20 days provoked major changes in the colour of fish fillets, especially the C* and h* parameters (P<0.05). L* increased significantly (P<0.05) from 82.7 (day 0) to 88.18 (day 15) and then fell to 84.37 by the end of the refrigeration period. The h* decreased (P>0.05) from 0.03 to -0.13 during the first ten days, followed by a significant (P<0.05) decline from -1.08 to -1.15 during days 15 to 20. Significant (P<0.05) multivariate regression models between spoilage indicators and colorimetric parameters (L*, C* and h*) were observed. The present study showed that IPT can be used as an auxiliary method to estimate the fillet quality.

Keywords: rainbow trout, fillet, image processing, histamine, TVN, TMA, prediction

1. Introduction

Fish, a rich source of protein with a high content of polyunsaturated acids, plays an essential role in the human diet. Indeed, the American Heart Association has recommended that fish should be included in the human diet at least twice a week in order to improve the human heart condition (Kraus *et al.*, 2000). Among all marine products, rainbow trout fillet as an invaluable source of protein, essential fatty acids and energy is in the great universal demand. This is especially true in Iran (IFO, 2009). Nowadays, fresh supply plays a very important role in all seafood and especially farmed-fish (Barat *et al.*, 2006). The necessary time intervals required to deliver the harvest to the consumer will always result in the incidence and causes of adverse changes. The existence of rich sources of protein and unsaturated fatty acids and their sensitivity to degradation has resulted in the short shelf-life of fresh fish. The shelf-life of fresh rainbow fillet preserved in ice was reported to be 10 to 12 days (Chytiri *et al.*, 2004) and only 5 days if refrigerated (Frangos *et al.*, 2010).

Fish begins to decay immediately after it is caught causing the meat to undergo adverse changes. These changes account for an approximate loss of 25% of the initial aquaculture production (Mahmoud *et al.*, 2006). Freezing is one of the best methods for fish preservation. However, because of the increasing demand for fresh rather than frozen fish, more modern and more profitable methods are implemented in order to increase shelf-life (Duun and Rustad, 2008). The most significant methods are the application of antimicrobial substances such as thymol oil (Alcicek, 2011), bitmap packaging and use of ozone gas on fish fillets (Bono and Badalucco, 2012). Qualitative amine parameters like trimethylamine nitrogen (TMA), total volatile nitrogen (TVN) and non-volatile nitrogen compounds such as histamine, which are closely related to sensory evaluation, are regarded as the most important decay indicators. This relationship is such that any increase in these parameters indicates adverse changes in odour, texture, and colour and finally the deterioration in the quality (Pons-Sánchez-Cascado *et al.*, 2006).

The sensory evaluation methods are prevalent to determine the quality of foodstuffs even though they are both time-consuming and expensive and differ from person to person. These factors ensure the motivation to develop alternative methods for the evaluation of the key features of the product more precisely and in a shorter time period. The most significant methods include the electronic nose, the electronic tongue and the computerised visual system. Image processing is another one of these methods in which various coloured spaces are used to analyse image and discolouration (Kvaal *et al.*,1998; Rehbein and Oehlenschlager, 2009). Colorimetric techniques including image processing have extensively been used in determining the colour of fish meat because of its great benefits. The colour of fresh fish fillet is dependent upon various factors including diet, killing method, bloodletting and fishing season (Rehbein and Oehlenschlager, 2009). The colour of the fillet in freshly caught fish varies between pink to light red depending upon its diet (Nollet, 2007). The dependence of varying shades of red on the amount of astaxanthin and canthaxanthin in the fish diet has already been established (Rehbein and Oehlenschlager, 2009). In recent years, several studies have taken place in order to investigate fish quality using image processing, examples of which include the determination of the amount of astaxanthin concentration in the rainbow trout fillets (Dissing *et al.*, 2011) and the extent of discolouration in the fillet of irradiated Atlantic salmon (Yagiz *et al.*, 2009). Therefore, the aim of the present study is to evaluate the relations between volatile and non-volatile amines spoilage indicators (histamine, TMA and TVN) and visualised data from image processing technique (IPT; L*, a*, b*, C* and h*) in order to predict suitable shelf-life of rainbow trout fillet during storage time at 4±1 °C and to fulfil the consumer demand.

2. Materials and methods

Sample preparation and experimental design

One hundred forty live rainbow trout with an average weight of 600±50 g was purchased from a private farm on the outskirts of Mashhad (Khorasan Razavi Province, Iran). The fish were well fed a common diet (GFT3; Beta Co. Ltd, Hormozgan, Iran) and grown in the relatively similar environmental conditions. Then, they were immediately transported to the ACECR laboratory preserved on ice according to the manual of animal ethics described by Mir-Malek *et al.* (2013), stunned by a sharp blow to the head with a wooden stick, filleted without skin and bones (left side, each fillet 165 g with dimensions 18×7 cm), randomly allocated to the coded packages (12 fillets for each storage time at three replicates) and preserved for 0, 10, 15, and 20 days (Dawood *et al.*, 1988; Rezaei *et al.*, 2007, 2010) at 4±1 °C until use. Thirty fillets were separately labelled, placed in other coded packages in order to confirm the obtained equations via back-calculation procedure.

Chemical analyses

Histamine value

After homogenising two fillets from each package with a blender (Abzar Sazan Co., Isfahan, Iran) for 75 s with 10 ml trichloroacetic acid (TCA; 5%), three portions of 75 g of homogenised fish flesh were centrifuged 4,000 rpm (1,726×*g*) for 10 min and the supernatant was stored. Extraction was repeated three times on the remaining materials with 10 ml TCA (5%). Finally, the collected solution was analysed for histamine by high-performance liquid chromatography (HPLC) apparatus (Waters 1525; Waters Company, Milford, MA, USA) equipped with a UV-detector Waters 2487 set at 254 nm. The standard histamine dihydrochloride (Fluka Biochemica, Buchs, Switzerland), methanol, chloroform, butanol, diethyl ether, n-heptane, benzoyl chloride, HCl, NaCl, and NaOH (Merck, Darmstadt, Germany) were prepared. Extraction with 5% TCA and then derivatisation with benzoyl chloride were done (Rezaei *et al.*, 2007). After addition 1 ml NaOH (2 M) and 5 µl benzoyl chloride for 20 min, 2 ml NaOH was added to stop derivatisation, centrifuged at 2,500 rpm (674×*g*) for 5 min at 4 °C. The supernatants was transformed to clean micro pipe and dried over nitrogen. Then, 200 µl methanol was added on the dried material and the mixture was filtered (0.45 microns) and finally, 20 µl supernatant was injected into the HPLC. The HPLC column was a reversed phase C18 Waters Spherisorb ODS-2 (250×4.60 mm, particle diameter 5 µm) which was supplied with a Waters Spherisorb pre-column cartridge (10 mm, length) packed with the same material (Waters).

Total volatile nitrogen value

In order to measure TVN content, 10 g of minced fillet was mixed with 2 g MgO and 300 ml distilled water. Then, the distillate was collected in a flask containing 0.3% boric acid and an indicator produced from the dissolution of methylene blue and methyl red. This solution was titrated with 0.05 N sulphuric acid solution and finally, the results explained in terms of mg of N per 100 g meat (Pezeshk *et al.*, 2011).

Trimethylamine value

TMA was measured using the AOAC procedure (AOAC, 2005). Fish meat was extracted by using a TCA solution 7.5%. One ml of the extract was dissolved in the sufficient distilled water to quadruple its volume. This volume was then increased to 18 ml by adding 1 ml of 20% formaldehyde, 3 ml potassium carbonate solution and 10 ml toluene and then shaken violently. Anhydrous sodium sulphate was subsequently added to dry any water left in the upper layers of toluene followed by 5 ml of 0.02% picric acid and finally read at 410 nm against distilled water. The results were explained with the normal distribution graph in the terms of mg of N per 100 g meat.

pH value

The pH content was measured with mixing the homogenised meat in the distilled water with a ratio of 1 to 10 using the procedure described by Mahmoud *et al.* (2006).

Colour evaluation

Photographic system

The photographic system consisted of a wooden box containing a light source (2 fluorescent bulbs) and a Canon digital camera (model 31S IXY; Canon, Tokyo, Japan). The system was designed so as to allow the smallest amount of reflection on the surface of the sample (three fillets for each replicate). The photographs were transferred to a computer by USB cable and saved in JPEG format. Image J 1.45s software (National Institute of Mental Health, Bethesda, MD, USA) was used to process the images. Camera settings are given in Table 1 (Mohebbi *et al.*, 2009).

Image processing

Because of the reliance of the photographs on the camera, they were converted into L*a*b colour space. The tristimulus L*a*b* measurement mode was used as it relates to the human eye response to colour. The L* variable represents lightness (L* = 0 for black and L* = 100 for white), a* scale represents the red/green (+a* intensity in red and -a* intensity in green). The b* scale represents the yellow/blue (+b* intensity in yellow and -b* intensity in blue). The secondary colorimetric parameters including chroma (C*) and hue angel (h*) were determined using Equation 1 (Hashemi Shahraki *et al.*, 2014; Robb *et al.*, 2000):

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{1a}$$

$$h^* = \arctan (b^*/a^*) \tag{1b}$$

The chroma is an expression of the intensity and clarity of the colour and the hue is an angular measurement where 0° indicates a red hue and 90° denotes a yellow hue. Constant hue and chroma are distorted and equally visual distances increase several-fold from purple-red to green (Rehbein and Oehlenschlager, 2009; Teimouri *et al.*, 2013). The chroma index ($(a^{*2} + b^{*2})^{½}$), also named the saturation index, represents the distance from the coordinate's origin to the determined colour point. The hue angle index (arctan (b^*/a^*)) represents the tone of colour that commonly decreases during degreening (90° represents a yellow colour, higher values indicate green and lower orange) (Hashemi Shahraki *et al.*, 2014; Macdougall, 2002).

Table 1. Camera settings for imaging.

Settings	Operation mode
Zoom	off
Flash	off
ISO	200
Aperture AV	F/5
Manual mode	15
White balance	daylight
Distance from the lens	22

Sensory evaluation

The evaluation was undertaken by seven qualified panellists from ACECR Institute (Mashhad, Iran) under identical light, temperature and humidity conditions on three fillets for each replicate. They were familiar to spoilage indicators in fish flesh. To increase the accuracy of experiment, a complementary training was scheduled on sensory spoilage of stored fresh fillets at refrigerated temperature during 21 days and colour, odour and texture of whole fillets were assessed. Finally, spoilage process was perceived completely at different intervals of 0, 3, 6, 9, 12, 15, 18 and 21 days. Also, the panellists were taught about the scale of the data range from 0 to 10 with 10 being the highest and 1 the lowest standard of quality. These conditions were stabilised for the duration of the test. Colour, odour, texture and general acceptability were the parameters evaluated by the panellists. Fresh fillet of the rainbow trout refrigerated at a temperature of 4±1 °C were allocated the highest standard of quality. Any products with a scale mark less than 6 were defined as unacceptable (Goulas and Kontominas, 2005).

Statistical analysis

After confirming the homogeneity of variance and normality of the data using Leaven and Kolmogorov-Smirnov tests, respectively, one-way ANOVA was used to compare the treatments in a completely randomised design (Zar, 1999). Duncan test was applied to compare significant differences among the treatments ($P<0.05$) with SPSS™ version 19 (SPSS Inc., Chicago, IL, USA). Kruskal-Wallis test was performed to establish any differences in the mean values related to sensory evaluation. In this regard, the changes in the contents of histamine, TMA and TVN during storage time (day) and also, relations between the contents of spoilage indicators (histamine, TMA and TVN) and colorimetric parameters (L*, a*, b*, C* and h*) were evaluated using one-variable and multivariate regression analyses with XLSTAT 2012 (Addinsoft, New York, NY, USA). Coefficients between the measured and predicted variable contents including histamine, TMA and TVN were correlated to justify the obtained equations. All measurements were carried out in triplicate and results were given as mean ± standard deviation.

3. Results and discussion

Colorimetric parameters

The values of 82.70, 7.21, and 0.03 were obtained from the colorimetric analysis of freshly used rain trout fillet for each of the parameters L*, C*, h*, respectively. As shown in Table 2, the discolouration of the rainbow trout fillet was investigated using colorimetric parameters. L* indicates the lightening of the colour of fillet during preservation. L* as a lightness parameter, had an initial value of 82.7 which significantly ($P<0.05$) rose to a peak of 88.18 by day 15, and then fell to 84.37 by end of the refrigeration period. The C* had an initial value of 7.21 which experienced no significant increase during the first ten days, followed by a significant ($P<0.05$) rise to 12.97 on day 15. This value (C*) rose to 14.02 on the last day of refrigeration, which is fairly negligible when compared to the previous five days ($P>0.05$). The h* had an initial value of 0.03 which did not change significantly until the tenth day and subsequently declined to its lowest value of -1.15 on the last day of refrigeration ($P<0.05$). There were the significantly ($P<0.05$) correlation coefficients (R) between the contents of decay indicators (histamine, TVN and TMA) and colorimetric parameters including C* (87.7, 87.9 and 84.8%, respectively) and h* (-79.8, -90.9 and -91.1%, respectively). When the contents of histamine, TVN and TMA increased, the lightness parameter L* increased gradually from 82.7 to 88.18 by day 15. However, despite the continued increase in the contents of histamine and TVN, the L* level began to fall and reached 84.34 by the end of the refrigeration period. This parameter also showed an approximately reciprocal relationship with pH.

The grade of fillet quality in the different species must be determined during the least time with the least cost. As the decay set in during the 20 day refrigeration, C* rose from 7.21 to 14.02 which was consistent with the increase in histamine, TVN and TMA contents. There was also significant change in h* value during the same period and the development of the decay and its level decreased from 0.03 to -1.15. This change into negative values indicates the reduction in the redness of the fish fillet. Denaturing of the proteins during decay which causes the dehydration of the muscles and changes the lightness of the meat surface can be mentioned among the consequences of this discolouration (Robb *et al.*, 2000). Also, myoglobin can react with TMA oxide and produce sulfhydryl compounds resulting in the green colouring (Bremner, 2002). In the present study, no green colouring was observed on fillets throughout the refrigeration period. It may indicate the existence of low amount of sulfhydryl. The lightness in rainbow trout fillet killed by electro-stimulation and refrigerated for 80 h increased to 43 from an initial value of 41, which indicated consistency with other existing research (Robb *et al.*, 2000). This amount for rainbow trout fillet refrigerated for two weeks increased from 6.33 to 10.61 (Choubert and Baccaunaud, 2006). Investigation on the C* content also indicated a similar trend and its amount increased from 1.74 to 2.49 during two weeks (Choubert and Baccaunaud, 2006). The h* value decreased from 5.4 to 3 during a 3-month refrigeration period which was consistent with the measurements taken from refrigerated rainbow trout by No and Storebakken (1991).

The discolouration of meat during refrigeration is dependent upon various factors such as polyunsaturated fatty acids, proteins and pigments, extent of unbound water and the physical structure of the meat (Chéret *et al.*, 2005). Therefore, the discolouration stemming from decay cannot be limited to any particular factor and a combination of physical, chemical and microbial factors are involved. The

Table 2. The mean (± standard deviation) values of colorimetric parameters including lightness (L*), chroma (C*) and hue (h*) in rainbow trout fillets during storage time at 4±1 °C.[1]

Day	L*	C*	h*
0	82.70 ± 0.44^{a}	7.21 ± 0.47^{a}	0.03 ± 0.0^{b}
5	85.05 ± 2.59^{ab}	6.74 ± 1.09^{a}	0.02 ± 0.11^{b}
10	85.15 ± 2.36^{ab}	7.51 ± 0.24^{a}	-0.13 ± 0.09^{b}
15	88.18 ± 3.05^{b}	12.97 ± 0.95^{b}	-1.08 ± 0.25^{a}
20	84.34 ± 1.06^{ab}	14.02 ± 1.89^{b}	-1.15 ± 1.06^{a}

[1] Different superscript letters (a-d) within columns indicate significant differences at $P<0.05$ at three replicates.

coefficients of correlation indicated that C* and h* are more highly correlated with the spoilage indicators than L*. Regression analysis of the relationship between volatile and non-volatile amines and colorimetric parameters produced multivariate predictive models with reasonable coefficients of determination. The results indicated that histamine, TVN, TMA, pH contents can be estimated from the evaluation of the colorimetric parameters. According to the coefficients of determination (r^2), these methods can be labelled as a new, quick, inexpensive technique for the evaluation of the quality of fish fillet in comparison with common laboratory methods. Standard conditions such as light source, dimensions and tools were implemented in this study as far as possible. However, in order to obtain a more complete standardised and comprehensive model, further studies is being conducted.

In the present study, data shown in Table 2 clearly indicate that something is happening between day 10 and day 15, and this is also confirmed by the statistical analyses. This suggests that colour analyses is not so useful to check the colour degradation of fish fillets stored for periods as short as 5 days (less than 10 days). In other word, considering changes in the interval from 10 to 15 days, it can be concluded that other indicators can be more accurate judgments on freshness of fillet compared with IPT. Although, further investigation is being done to evaluate the efficiency of IPT in order to sort suitable produced fillets of different species. In the present study, time intervals of 5 days were selected for 20 days to increase the statistical power of predictive models. It is considered that time intervals less than 5 days is more conservative. In this regard, time duration is one of critical parameters to evaluate the efficiency of predictive models. Because of time retardation between spoilage indices and colorimetric parameters, it is proposed that time intervals higher than 20 days with sampling times lower than 5 days is considered in the future studies. Also, type of species, body weight and muscle composition are key parameters to define predictive models of spoilage process. Based on the literature review, the present study is the first to determine the chemical parameters spoilage of rainbow trout fillet using colorimetric parameters. According to the coefficient equations obtained from this study, it can be concluded that IPT can be considered as a suitable method for estimating the fillets spoilage. Similar to most of biological experiments, there are some limitations and potential errors. The results of our study can be considered as an idea for future research on other species and is an effective step in the process based colorimetric assay instead of chemical tests. Undoubtedly, collection of large amounts of data in different species can give us more accurate conclusions in the investigation of spoilage via IPT.

Spoilage indicators and the relations with colorimetric variables

Histamine value

Histamine, a non-volatile organic nitrogen alkaline compound with low molecular weight, is one of the most important biogenic amines. As the decay sets in and the microbial load increases, bacterial decarboxylation of histidine (with the removal of the Alfa carboxyl group) leads to the creation of this substance, which can be used to determine the extent of decay in fish (Bremner, 2002; Pons-Sánchez-Cascado *et al.*, 2006). The changes in the histamine content measured at 4±1 °C during different days were shown in Table 3. There was a significantly (P<0.05) increasing trend in the histamine content of experimental fillets during a 20-day refrigeration period. The lowest amount of histamine was 1.96 mg/kg on the first day and there was no significant increase during the following ten days. However, a significant increase (P<0.05) was detected from the tenth day onwards reaching its peak at 7.98 mg/kg on day 20 (P<0.05). There was a significantly (P<0.05) quadratic regression between the histamine content during storage time (Equation 2):

$$\text{Histamine (mg/kg)} = 1.95 - 0.25\ \text{DAY} + 0.03\ \text{DAY}^2 \quad (r^2_{\text{adjusted}} = 89.1;\ P<0.05) \qquad (2)$$

Table 3. The mean (± standard deviation) values of histamine (mg/kg), total volatile nitrogen (TVN), trimethylamine value (TMA) and pH in rainbow trout fillets during storage time at 4±1 °C.[1]

Day	Histamine (mg/kg)	TVN (mg N/100 g)	TMA (mg N/100 g)	pH
0	1.96±0.24^a	13.70±0.7^a	1.03±0.3^a	6.52±0.02^a
5	1.29±0.17^a	18.10±1.79^a	3.25±0.22^b	6.44±0.03^a
10	2.42±0.16^a	36.9±3.56^b	8.22±0.43^c	6.41±0.1^a
15	4.18±0.4^b	62.9±5.46^c	13.06±0.29^d	6.54±0.04^a
20	7.98±1.71^c	103.9±8.46^d	13.12±0.34^d	6.76±0.13^b

[1] Different superscript letters within columns indicate significant differences at P<0.05 with three replicates.

The stepwise multivariate regression models between histamine content and secondary colorimetric parameters were reported as following equations:

$$\text{Histamine (mg/kg)} = 14.774 - 0.212\ L^* + 0.702\ C^* \quad (r^2_{adjusted} = 77.8;\ P<0.05) \tag{3}$$

$$\text{Histamine (mg/kg)} = 15.643 - 0.163\ L^* - 3.891\ h^* \quad (r^2_{adjusted} = 60.4;\ P<0.05) \tag{4}$$

The primary colorimetric parameters including a* and b* did not show significant differences in the model, therefore, these variables were removed from the final models. Significantly (P<0.05) correlation coefficients of 89.3, 82.3 and 79.4% were obtained between the measured and predicted histamine contents from Equation 2, 3 and 4. An increase in the histamine content in the rainbow trout fillet during the refrigeration period was observed. In the present study, the initial content of histamine (1.96 mg/100 g) can indicate the protease activity of endogenous enzymes stemming from the increase in the bacterial load reached 7.98 mg/100 g by the end of the period. Although, Rezaei *et al.* (2007) reported that the initial and final contents of histamine in rainbow trout fillet preserved in ice for 18 days were 0 and 1.6 mg/100 g, respectively. The initial histamine concentration is dependent upon factors such as the initial concentration of histidine, rate of bacterial growth, preservation conditions and temperature (Ozogul *et al.*, 2006; Rehbein and Oehlenschlager, 2009). Histamine content produced in foodstuffs is poisonous and if consumed leads to complications such as itching, flushing, and nausea (Hwang *et al.*, 2012). The maximum amount of histamine in fresh fish allowed by the US Food and Drug Administration is 100 mg/100 g (Rezaei *et al.*, 2007). None of the samples investigated in the present study reached this value. The existence of a non-linear relationship between amount of histamine and refrigeration time was confirmed by the present investigation with $r^2_{adjusted}$ equal to 89.1. This relationship can be used to estimate the amount of histamine in the rainbow trout fillet.

Total volatile nitrogen value

TVN is a compound blended from a mixture of ammonia, dimethyl amine, trimethylamine and volatile nitrogen composites that are created as a result of decomposition of other compounds. They are used as an indicator of the quality of fish meat (Rehbein and Oehlenschlager, 2009). There was a significant (P<0.05) difference between the mean amount of TVN on the first day (day 0) and the last day of refrigeration (day 20) (Table 3). The minimum amount of TVN on the first day of refrigeration was 13.7 mg N/100 g, which did not increase significantly until day 5. However, this increase became statistically significant (P<0.05) onwards and reached its peak of 103.9 mg N/100 g on day 20. The highest rate of increase in the content of TVN could be observed from day 15 onwards. This indicated an increase of 41 mg N/100 g in comparison to that of the previous day 5. There was a significantly (P<0.05) positive correlation coefficient (R=94.1%) between the contents of TVN and histamine. There was a significantly (P<0.05) quadratic regression between the TVN content during storage time (Equation 5):

$$\text{TVN (mg N/100 g)} = 13.55 - 0.09\ \text{DAY} + 0.23\ \text{DAY}^2 \quad (R^2_{adjusted}=98.3;\ P<0.05) \tag{5}$$

The appearance of higher levels of TVN appeared much sooner than changes in the colour of the fish fillets. The stepwise multivariate regression models between TVN content and secondary colorimetric parameters were reported as the following equations:

$$\text{TVN (mg N/100 g)} = 62.367 - 1.203\ L^* + 8.987\ C^* \quad (r^2_{adjusted} = 74.3;\ P<0.05) \tag{6}$$

$$\text{TVN (mg N/100 g)} = 106.978 - 1.014\ L^* - 57.047\ h^* \quad (r^2_{adjusted} = 80.3;\ P<0.05) \tag{7}$$

There were significantly (P<0.05) correlation coefficients of 84.9, 76.2 and 81.2% between the measured and predicted TVN contents from Equation 5, 6 and 7.

In the present current, the initial value of TVN in fillet was 13.7 mg N/100 g, which is an indicator of high quality fillet (Frangos *et al.*, 2010). This initial value can differ in different species of fish, for example 12.6 mg N/100 g for rainbow trout fillet (Pezeshk *et al.*, 2011) and 9 mg N/100 g for red mullet fillet (Bono and Badalucco, 2012). The TVN value was stable in the early days of refrigeration and it experienced a slow rise, which is a reason for it being considered as a weak indicator of the freshness of fish in some researches (Chytiri *et al.*, 2004). However, this content changed from day 5 onwards and possibly as a result of the increase in proteolysis of enzymatic activity and finally, the value rose to 103.9 mg N/100 g by the 20th day of refrigeration. Bacterial decomposition of meat during refrigeration is one of the factors in the formation of TVN, which explains the existence of a strong relationship between TVN and bacterial load (Alcicek, 2011; Chytiri *et al.*, 2004). The measured amount of TVN (mg N/100 g) increased from 20 to a maximum of 50 in a 9-day refrigeration period which was consistent with findings in other similar research (Frangos *et al.*, 2010). In this regard, the TVN measured at 3.5 °C during a 28-day refrigeration period reached a maximum 17.5 mg N/100 g was reported by Matejkova *et al.* (2013).The legal and acceptable amount of TVN in fish fillet was reported to be 25 mg/100 g (Pezeshk *et al.*, 2011). The amount measured in the samples in this study during the 6th and 7th days of refrigeration were found to exceed this content, hence rendering the meat unsuitable for consumption. The existence of a non-linear relationship between amount of TVN and refrigeration time can be used to estimate the amount of TVN in the rainbow trout fillet.

Trimethylamine value

TMA forms part of a volatile alkaline compound produced from the regeneration of TMA oxide stemming from the activation of bacteria such as *Shewanella putrefaciens*. Its determination can be used to indicate bacterial activity and the rate of the development of the decay (Mexis *et al.*, 2009; Rehbein and Oehlenschlager, 2009). The TMA evaluations indicated a rising trend in rainbow trout fillet (Table 3). One way ANONA indicated the existence of significant ($P<0.05$) increase between the mean amount of TMA on the first day (day 0) and the last day of refrigeration (day 20) ($P<0.05$). The minimum amount of TMA on the first day of refrigeration was 1.03 mg N/100 g, which rose to 13.12 mg N/100 g by the 15th day of refrigeration. There was a significantly ($P<0.05$) correlation between the contents of TMA with histamine (R=78.1%) and TVN (R=90.3%). There was a significantly ($P<0.05$) quadratic regression between the TMA content during storage time (Equation 8):

$$\text{TMA (mg N/100 g)} = 0.3 + 0.33\ \text{DAY} - 0.007\ \text{DAY}^2 \quad (r^2_{adjusted} = 81.1; P<0.05) \tag{8}$$

The appearance of higher levels of TMA appeared much sooner than changes in the colour of the fish fillets. The stepwise multivariate regression models between TMA content and secondary colorimetric parameters were reported as the following equations:

$$\text{TMA (mg N/100 g)} = -46.77 + 0.5\ L^* + 1.21\ C^* \quad (r^2_{adjusted} = 73.4; P<0.05) \tag{9}$$

$$\text{TMA (mg N/100 g)} = -38.63 + 0.5\ L^* - 8.17\ h^* \quad (r^2_{adjusted} = 86.0; P<0.05) \tag{10}$$

There were significantly ($P<0.05$) correlation coefficients of 88.1, 75.9 and 85.3% between the measured and predicted TMA contents from Equation 8, 9 and 10.

The initial measurement of TMA (mg N/100 g) was 1.03 which rose to 13.13 of meat by the end of the twenty-day refrigeration period. This initial value was consistent with values reported in other researches (Chytiri *et al.*, 2004). For anchovies, this value was reportedly given as 4.94 mg N/100 g. This discrepancy can be attributed to differences in species and the depth of their habitat and can determine the amount of TMA oxide in fish meat (Pons-Sánchez-Cascado *et al.*, 2006). In the early days of refrigeration, TMA content was produced at a slow rate, however, this rate increased noticeably from the fifth day onwards. That it means the parallel growth of decomposing bacteria of TMA oxide during decay leading to the creation of unpleasant odour (Ozogul *et al.*, 2006). Investigations have shown that as the fish approaches the state of being unsuitable for consumption, the production rate for TMA like that of TVN increases rapidly. Therefore, it is better to use this indicator in fish nearing total decay (Rehbein and Oehlenschlager, 2009). The maximum allowable amount of TVN in refrigerated rainbow trout fillet is 10 mg/100 g (Chytiri *et al.*, 2004). Thus, the fish samples in this study exceeded the allowable amount by the twelfth or thirteenth day. TMA forms a major part of TVN and the high coefficient of correlation between these parameters confirms this fact. The existence of a non-linear relationship between amount of TMA and refrigeration time was confirmed by the investigation with $r^2_{adjusted}$ equal to 81.1.

pH value

The pH value for muscle tissue in live fish is about 7. Because of transformations following death this value can vary from 6 to 7.1 with respect to species, fishing season, fishing method and other factors (Oraei *et al.*, 2012). The pH content of preserved rainbow trout fillets during storage time indicated a fluctuating trend (Table 3). Results indicated the existence of significant ($P<0.05$) increase between mean amount of pH on the first and the last days of refrigeration (day 20). The minimum value of pH on the first day of refrigeration was 6.52. This value continued to fall until the tenth day of refrigeration without any significant difference. However, this value began to rise significantly ($P<0.05$) thereupon and reached a peak value of 6.77. The pH value was found to be highly correlated with contents of TMA (R=54.8%), histamine (R=88.6%), and TVN (R=80%). A significantly ($P<0.05$) quadratic regression relation between the pH content during storage time was reported in the following equation (Equation 11):

$$\text{pH content} = 6.53 - 0.33\ \text{DAY} + 0.002\ \text{DAY}^2 \quad (r^2_{adjusted} = 75.7; P<0.05) \tag{11}$$

The appearance of higher levels of pH appeared much sooner than changes in the colour of the fish fillets. The stepwise multivariate regression models between pH content and secondary colorimetric parameters were reported as following equations:

$$\text{pH} = 8.013 - 0.021\ L^* + 0.036\ C^* \quad (r^2_{adjusted} = 70.0; P<0.05) \tag{12}$$

$$\text{pH} = 8.039 - 0.019\ L^* - 0.198\ h^* \quad (r^2_{adjusted} = 51.5; P<0.05) \tag{13}$$

There were significantly ($P<0.05$) correlation coefficients of 78.4, 70.9 and 67.5% between the measured and predicted pH contents from Equation 11, 12 and 13.

The initial pH value for rainbow trout fillet was 6.52 which decreased to 6.41 after a 10-day refrigeration period. The increase in the production of lactic acid from post-mortem anaerobic glycolysis might explain this reduction and reduce it to its minimum value (Bremner, 2002). This reduction might also be responsible for the weakening of tissue joints and the appearance of meet fragmentations (Bremner, 2002). The pH value began to increase after the tenth day and reached a value of 6.76 on day 20 during the present study. This increase can be related to the discontinuation of the production of anaerobic metabolic lactic acid and the production of alkaline compounds such as ammonia and TMA and other biogenic amines. These compounds generally stem from microbial activity and reduce the freshness of the fish (Oraei *et al.*, 2012). The findings are consistent with many other studies (Zhu *et al.*, 2012) and inconsistent with some researches on refrigerated rainbow trout (Haghparast *et al.*, 2010), where the pH value followed a significantly increasing pattern from the beginning of the refrigeration period. Lack of energy and glycogen reserves in the meat and the subsequent negligible production of lactic acid during post-mortem metabolism might explain this contradiction (Bremner, 2002). The maximum allowable pH value for fish meat was reported between 6.8 and 7 (Kilinc *et al.*, 2009), while the value for freshly caught fish was 6.52, which is outside this limit. Therefore, it can be said that pH alone cannot be considered as an indicator of quality and thus should only be used as an auxiliary indictor in the investigation of the quality of fish meat. In this regard, the existence of a non-linear relationship between pH value and refrigeration time was confirmed in the present study with $r^2_{adjusted}$ equal to 75.71.

Sensory evaluation

The findings indicated that the scores (colour, odour, texture and overall acceptability) of all sensory parameters gradually (P<0.05) decreased as refrigeration time increased (Table 4). Based on the plotting the regression curves (not shown data), it is quite clear that the colour, odour and texture and overall acceptability index was out of the acceptable limit after 7-8, 6, 9 and 7 days, respectively. The lowest acceptable standard was 6 on the scale of 1 to 10. The colour of the fillets was minimally acceptable by the 7th to 8th days. However, the reduction in colour quality scores of the samples was slower than those of odour. This was such that the samples could not be considered fit for consumption beyond the 6th day as far as the odour was concerned. The fillets reached their minimum acceptable texture quality by the 7th day.

4. Conclusions

Histamine, TVN, TMA and pH contents in rainbow trout fillet refrigerated at 4±1 °C for 0, 5, 10, 15 and 20 days were determined using colorimetric techniques. These parameters were first evaluated using common laboratory methods and then compared with colorimetric parameters determined by IPT. The results indicated that because of amino decay, the rainbow trout fillet loses its redness during refrigeration and gains a lighter colour. Multivariate regression analysis of the relations between chemical and colorimetric parameters showed the predictive models with high coefficients of determination (r^2). Significant correlation coefficients indicated that h* and C* values highly related to decay process. These predictive models have many benefits such as the non-requirement of laboratory materials and instruments, its simplicity and quickness and applicability of its results from the imaging of the rainbow trout fillet. However, the appearance of higher levels of TVN, TMA and pH appeared much sooner than changes in the colour of the fish fillets. The results of present study can use as primary step to design and improve electronic eye.

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Table 4. The mean (± standard deviation) scores of colour, odour, texture and overall acceptability in rainbow trout fillets during storage time at 4±1 °C.[1]

Day	Colour	Odour	Texture	Overall acceptability
0	9.50±0.17^e	9.58±0.5^e	9.38±0.77^e	9.48±0.48^e
5	7.42±012^d	6.75±0.49^d	7.88±0.79^d	7.35±0.46^d
10	4.46±0.17^c	4.13±0.74^c	5.67±0.63^c	4.75±0.51^c
15	2.40±3.1^b	0.50±0.41^b	3.50±0.73^b	2.13±0.41^b
20	0.01±1.01^a	0.02±0.01^a	0.54±0.49^a	0.19±0.16^a

[1] Different superscript letters within columns indicate significant differences at P<0.05 at three replicates.

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