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Freshness assessment of European eel (*Anguilla anguilla*) by sensory, chemical and microbiological methods

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Abstract

Freshness assessment of European eel (*Anguilla anguilla*) stored in ice and in boxes without ice at 3 ± 1 °C was assessed by sensory, chemical (total volatile basic nitrogen (TVB-N), thiobarbituric values (TBA), peroxide value (PV), free fatty acid (FFA), and pH) and microbiological (total viable counts, TVC) methods. The limit for sensory acceptability of eel stored in ice was ~12–14 days, and ~5–7 days at 3 ± 1 °C. TVB-N level of about ≥10 mg TVB-N 100 g^{-1} flesh could be regarded as the limit of acceptability. PV values and the release of FFA increased during storage in ice and at 3 ± 1 °C but the increases were greater at 3 ± 1 °C. Values of pH showed no statistically significant ($P > 0.05$) changes for eel stored in ice and at 3 ± 1 °C. Water losses of fillets stored at at 3 ± 1 °C were higher ($P < 0.05$) than those stored in ice. TBA values were found to fluctuate under both storage conditions. This study shows that sensory analysis of eel correlated well with microbiological analysis. The acceptability of eel decreased as TVB-N, FFA, PV and TVC values increased.

Keywords: European eel; Freshness indicators; Fish quality

1. Introduction

Eels are generally classified as warmwater fish and 19 species, including subspecies of the *Anguilla* genus, are distributed throughout the world (Arai, 1991). There are four species which are commercially important. These are *Anguilla anguilla* in Europe, *Anguilla japonica* in the Far East, *Anguilla rostrata* in North America and *Anguilla australis* in Australia and New Zealand. Eels are usually processed before retailing and process techniques include smoking, jellying, pickling and *kabayaki* for the Japanese market. Eels (*A. Anguilla*) are an economically important fish species along the eastern and southern coasts of Turkey. The market demand for fresh eel has increased markedly due to its aroma and high flesh yield. In addition, the increase in demand from European countries has resulted in the exporting of wild eel. Therefore, the study of freshness quality of eel is of interest to retailers and consumers.

Freshness is the most important attribute when assessing the quality of fish. Sensory characteristics of whole fish are clearly visible to consumers and sensory methods are still the most satisfactory for assessing the freshness quality since they give the best idea of consumer acceptance (Connel, 1995). Non-sensory methods, using biochemical, physical and microbiological analyses, are also used to assess the freshness quality of fish (Gill, 1992). Biochemical and physical methods measure the concentrations of breakdown products from bacterial or enzymatic activity. A number of spoilage indicators have been used, including total volatile basic nitrogen (TVB-N), trimethylamine (TMA) and

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formation of biogenic amines (Botta, Lauder, & Jewer, 1984a, 1984b; Hebard, Flick, & Martin, 1982; Mietz & Karmas, 1978), whereas nucleotide degradation product ratios (such as hypoxanthine, K, K_i values) have been used as freshness indicators (Saito, Arai, & Matsuyoshi, 1959; Karube, Matsuoka, Suzuki, Watanabe, & Toyama, 1984; Luong & Male, 1992).

Lipid oxidation is a major quality problem. It leads to the development of off-flavour and off-odours in edible oils and fat-containing foods, called oxidative rancidity (Nawar, 1996; Hamilton, 1994). Eel fillets are rich in polyunsaturated fatty acids which are susceptible to peroxidation. Because of their high degree of unsaturation, they are less resistant to oxidation than other animal or vegetable oils (Nawar, 1996). Free radicals react with oxygen to produce fatty acid peroxides. The fatty acid peroxides are free radicals which can attack another lipid molecule, resulting in peroxide and a new free radical (Hamre, Lie, & Sandnes, 2003). 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. PV and thiobarbituric values (TBA) are the major chemical indices of oxidative rancidity (Melton, 1983a, 1983b; Rossell, 1989). TBA value measure secondary products of lipid oxidation. TBA consists mainly of malondialdehyde as a representative of aldehydes. The oxidation process can also lower nutritional quality and modify texture and colour (Lie, 2001).

There are studies on the effects of slaughtering methods on the quality of raw and smoked eels (Vishwanath, Lilabati, & Bijen, 1998; Morzel & van de Vis, 2003) and on quality and welfare of eel (van de Vis et al., 2001). However, there is limited information on the shelf life and freshness quality of eel. The objectives of this study were to investigate the shelf life and freshness quality of eel stored in ice and in boxes without ice (3 ± 1 °C) in terms of sensory, chemical (TVB-N, TBA, PV, free fatty acid (FFA), and pH) and microbiological (total viable counts, TVC) methods.

2. Materials and methods

2.1. Sample preparation and storage of eels

Eels purchased from a local fish processing company were one-day post capture on arrival at the laboratory in ice. Eels (average weight: 228.5 ± 21.98 g) were gutted, washed and divided into two lots in ice. One lot was stored in ice at a fish-to-ice ratio of 2:1 (w/w), the second lot was stored in boxes without ice. All boxes were then stored in a refrigerator (3 ± 1 °C) for up to 19 days. Sensory and chemical analyses were performed on days 1, 5, 8, 12, 15 and 19 whereas PV and FFA were analysed on days 2, 6, 9, 13, 16 and 20 after extraction of fat. Data were obtained using three fish which were minced for each sampling.

2.2. Proximate analysis

The eel fish samples were analysed in triplicate for proximate composition: lipid content by the Bligh and Dyer (1959) method, moisture content by AOAC (1990) method, total crude protein by Kjeldhal method (AOAC, 1984), and ash content by AOAC (1990) method.

2.3. Analytical methods

The TVB-N content of eel was determined according to the method of Antonocopoulus (1973) and expressed as mg TVB-N per 100 g eel muscle. The value of TBA was determined according to Tarladgis, Watts, and Yonathan (1960) in eel fillets to evaluate the oxidation stability during storage and the results expressed as TBA value, milligrammes of malondialdehyde per kg flesh. FFA analysis, expressed as % of oleic acid was done by the AOAS (1994) method. PV, expressed in milliequivalents of peroxide oxygen per kilogramme of fat, was determined according to AOAS (1994). The pH of eel fillets was determined using a pH meter (315i, Germany). The sample was homogenised in distilled water in the ratio 1:10 (w/v) and the measurement was done by pH meter. The water-holding capacity (WHC) of raw sample was determined as ''centrifuge drip'' in each fish sample. About 5g of fish, without skin and bones, were weighed into dry clean centrifuge tubes and centrifuged at 3000 rpm for 30 min at −4 °C. Water-holding capacity was calculated on a wet weight basis as $100 \times (1 - S/V)$, where S is the weight of the expelled water, V is the initial weight of sample (Del Valle & Gonzales-Inigo, 1968).

2.4. Sensory analysis

For sensory analysis, triplicate samples, from each of the two storage conditions, were taken at regular intervals. Sensory analysis was assessed using the Tasmanian Food Research Unit scheme (Branch & Vail, 1985) with modifications for eel. Table 1 shows the modified Tasmanian Food Research Unit freshness assessment scheme. This sensory assessment approach evaluates freshness by giving demerit points according to certain aspects of general appearances (e.g. skin, slime, eyes, belly, odour). Each assessment was carried out by a minimum of six trained panellists Panellists were asked to state whether or not the fish were acceptable. This was

Table 1
Modified Tasmanian Food Research Unit freshness assessment scheme for gutted eels

Score	0	1	2	3
Appearance				
Dorsal skin	Very Bright, clear contrast	Bright, less contrast	Slightly dull	Dull
Abdominal skin	Shining colour, white	Slightly yellowish	Yellowish, slightly shrinkage	Yellow-brown
Skin slime	Absent	Slightly slimy	Slimy	Very slimy,excessive
Firmness	Very stiff and firm	Fairly stiff and firm	Fairly soft	Soft or very soft
Eyes				
Clarity	Clear	Slightly Cloudy	Cloudy	
Shape	Normal	Slightly Sunken	Sunken	
Iris	Visible	Not Visible		
Belly cavity				
Stains	Opalescent	Greyish	Yellow-brown	
Blood	Red	Dark red	Brown	
Flesh odour	Fresh water fish	Neutral, milky	Fishy	Spoilt
Total demerit points				

*Sum of score is from 0 to 24.

used to determine the shelf life of the eel. The acceptable shelf life was found to correspond to a demerit score of 10 ± 2.

2.5. Microbiological analysis

Samples, from each of three different eel fish (triplicate) stored under the two different storage conditions, were taken to estimate TVC. Ten grammes of fish muscle were mixed with 90 ml of Ringer solution and then stomached for 3 min. Further decimal dilutions were made up to 10^{-8} and then 0.1 ml of each dilution was pipetted onto the surface of plate count agar plates, in triplicate. They were then incubated for 2 days at 30 °C.

2.6. Statistical analysis

For data analysis, standard deviation and ANOVA were used. Significance of differences was defined at $P < 0.05$. Statistical comparison was based on three samples for each specific storage time.

3. Results and discussion

3.1. Sensory assessment

Fig. 1 shows freshness scores obtained from gutted eel stored in ice and in boxes without ice from day 1 to day 19. Demerit points increased in both conditions with a higher increase for the fish stored in boxes without ice. On the whole, the appearance score increased with storage time, indicating the progressive loss of freshness in both iced and without-ice storage conditions. The appearance of eel stored in boxes without ice was poorer than in ice. Skin of eel stored at 3 ± 1 °C were drier than that of eel in ice. The appearance of eyes of eel in ice was more cloudy than that of eel stored in boxes without ice, indicating that eyes of eel were not a good criterion for ice storage to assess the freshness of fish. The limit for acceptability of eel stored in ice was ~12–14 days, and in boxes without ice, ~5–7 days. Although the initial sensory scores for the two storage conditions were the same on day 1, these scores for fish stored in boxes without ice were significantly higher than for fish stored in ice at day 8, and day 12 ($P < 0.05$). The acceptable shelf life was found to correspond to a demerit score of 12 for ice after 12 days of storage and 8 in boxes without ice after 5 days of storage. The storage life of fish is affected by the initial microbial load of the fish, and storage temperature (Church, 1998).

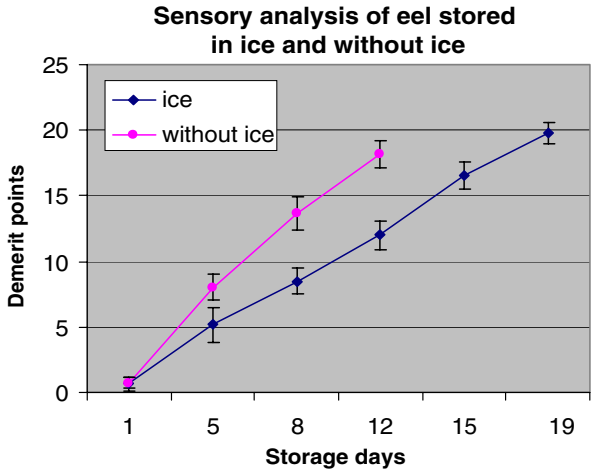

Fig. 1. Sensory assessment of European eel stored in ice and without ice (3 ± 1 °C).

Table 2
Proximate analysis (%) of European eel

	Protein	Fat	Moisture	Ash
Eel	17.5 ± 0.83	20.86 ± 0.82	60.12 ± 0.40	1.05 ± 0.11

Data are expressed as means ± standard deviation ($n = 3$).

3.2. Chemical assessment

The proximate composition of the sample on day 1 is shown in Table 2. The fat content of eel was high (% 20.86), causing fast deterioration of its desirable flavour. Vishwanath et al. (1998) found 10.74% of lipid in fresh *Monopterus albus* (mud eel fish).

TVB-N concentrations of eel stored under the two different storage conditions are shown in Fig. 2. At the beginning of storage, the TVB-N value was 6.96 mg/100 g flesh for eel stored in both ice and boxes without ice. The TVB-N values rose to 103 mg TVB-N/100 g flesh by the end of the storage period for eel stored in boxes without ice and 19.4 mg TVB-N/100 g for eel stored in ice. Significant differences ($P < 0.05$) were found in TVB-N levels after 5 days of storage between the storage conditions. Eel stored in boxes without ice deteriorated more rapidly than did fish stored in ice (Fig. 2). The TVB-N values were 12.4 mg TVB-N/100 g for eel stored in ice and 22.6 mg TVB-N/100 g for eel stored in boxes without ice when the eels were rejected by panellists after 15 and 8 days of storage, respectively. The level of TVB-N in freshly caught fish is generally between 5 and 20 mg N per 100 g muscle. However, the levels of 30–35 mg N per 100 g muscle are considered the limit of acceptability for ice-stored cold water fish (Huss, 1988; Connel, 1995). In the present study, the TVB-N level of about ≥10 mg TVB-N/100 g flesh could be regarded as the limit of acceptability for iced European eel. The TVB-N values of eel stored in ice remained below (19.4 mg/100 g), the upper limit of acceptability throughout the entire storage period. TVB-N is produced mainly by bacterial decomposition of fish flesh, and higher level of TVC of gutted eel after 8 days of storage in boxes without ice (6.7 log cfu g^{-1}) could account for the TVB-N values of eel. Based on the results obtained from this study, TVB-N could be used as an indicator of eel quality, as shown in a variety of fish, such as European hake (Perez-Villarreal & Howgate, 1987), Atlantic cod (Botta et al., 1984a, Botta, Lauder, & Jewer, 1984b), sardine (Ababouch et al., 1996; Özogul, Polat, & Özogul, 2004).

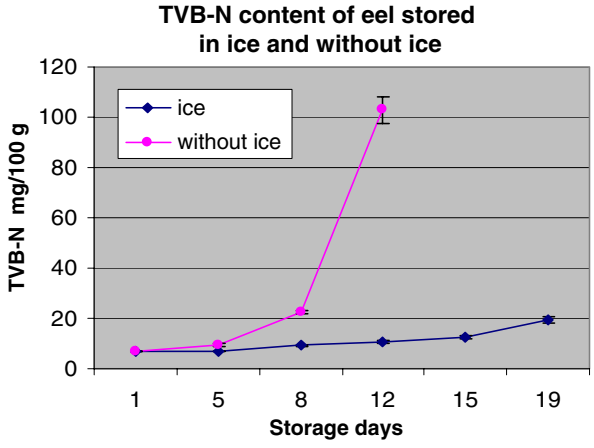

Fig. 2. Changes in TVB-N value of European eel stored in ice and without ice (3 ± 1 °C).

Mean pH measurements over the period of storage in ice and in boxes without ice are shown in Tables 1 and 2, respectively. Low pH is used as indicator of stress at the time of slaughtering of many animals. Low initial pH is associated with higher stress before slauhgtering (Azam, Mackie, & Smith, 1989; Marx, Brunner, Weinzierl, Hoffmann, & Stolle, 1997; van de Vis et al., 2001). This is caused by the depletion of energy reserves, mainly glycogen, with the production of lactate. In this current study, the low initial pH values may indicate that fish were subjected to stress during slaughtering. Similar pH values were obtained by Morzel and van de Vis (2003) and van de Vis et al. (2001) who studied slaughtering methods on quality of raw and smoked eels and on quality and welfare of eel, respectively. For smoked eel, pH values were found to be stable over time.

The relatively low pH levels, at the beginning of the storage period, also reflected the good state of the eel. Values of pH showed no statistically significant ($P > 0.05$) changes for eel stored in ice and in boxes without ice during the entire period of storage. The increase in pH after 5 days in eel stored in ice, and 8 days in eel stored in boxes without ice, was associated with the spoilage of fish. Kyrana and Lougovois (2002) found similar pH values for European sea bass over the period of iced storage. However, Papadopoulos, Chouliara, Badeka, Savvaidid, and Kontominas (2003) found higher pH values (>7) for gutted and ungutted sea bass stored in ice. Post mortem pH varies from 6.0 to 7.1, depending on season, species and other factors (Simeonidou, Govaris, & Vareltzis, 1998).

Water-holding capacity of eel stored in ice was not significantly ($P > 0.05$) different from that of eel stored in boxes without ice, whereas water lossess in eel fillets stored in boxes without ice were higher than in eel stored in ice.

Lipid deterioration limits shelf life of oily fish such as eel and herring (Hamre et al., 2003; Morzel & van de Vis, 2003). Glycerides, glycolipids and phospholipids are hydrolysed by lipases to free fatty acids, which then undergo further oxidation to produce low moleculer weight compounds, such as aldehydes and ketones (Hamilton, Kalu, Prisk, Padley, & Pierce, 1997). These compounds are responsible for off-flavour and off-odour and taste of fish (Toyomizu, Hanaoka, & Yamaguchi,

1981). In addition, FFA and their oxidation products would have an effect on muscle texture and functionality since they interact with myofibrillar proteins and promote protein aggregation (Pacheco-Aguilar, Lugo-Sánchez, & Robles-Burgueño, 2000). In the present study, the release of FFA increased ($P < 0.05$) during storage of eel stored in ice and in boxes without ice but higher increase in eel in boxes without ice. Initial values ranged from 0.59 to 0.57 (expressed as % of oleic acid) while final values ranged from 1.79 to 1.60 for eel stored in ice and in boxes without ice, respectively. These results indicate that there is a relationship between FFA release and loss of freshness.

Shelf life of oily fish species is limited due to the oxidation of lipid. An increase in the PV during storage of eel was observed. There were significant differences ($P < 0.05$) in PV values between two storage conditions on days 8 and 12. Initial PV values were 5.19 meq/kg for eel stored in ice and 5.28 meq/kg for eel stored in boxes without ice (Tables 3 and 4). The maximum values were 19.7 meq/kg for eel stored in ice and 21.6 meq/kg for eel held in boxes without ice. Initial PV values were found to be 0.8–1.2 for herring (Smith, Hardy, McDonald, & Temoleton, 1980) and 27.6 for fresh sardine (Cho, Endo, Fujimoto, & Kaneda, 1989).

The TBA index is widely used as a indicator of degree of lipid oxidation. Nishimoto, Suwetja, and Miki (1985) reported, for mackerel, 4 and 27 mg malonaldehyde (MA)/kg muscle for good and low quality fish, respectively. Although the TBA values in this study were found to be quite low for the two different storage conditions, the values of TBA for gutted eel samples were higher than for eel stored in boxes without ice throughout the storage period. Auburg (1993) rseported that TBA values may not give actual rates of lipid oxidation since malonaldehyde can interact with other components of fish such as nucleosides, nucleic acid, proteins, amino acids of phospholipids and other aldehydes which are end-products of lipid oxidation. This interaction can vary with fish species. Although peroxide value and TBA value are commonly used to measure rancidity, they do not actually show the level of freshness quality (Melton, 1983a, 1983b).

3.3. Microbiological assessment

Microbial counts on eels kept in ice and in boxes without ice are shown in Fig. 3. There was an increase in total viable counts over the period of storage. Bacteria grew more quickly in eels kept in boxes without ice than in those kept in ice. There were significant differences ($P < 0.05$) in total viable count of fish stored in

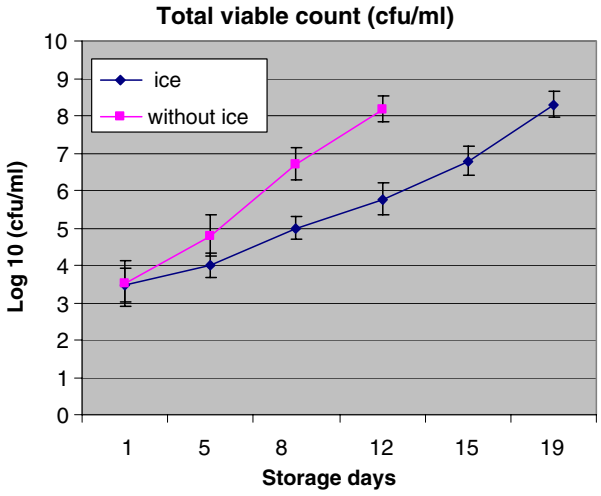

Fig. 3. Changes in TVC of European eel stored in ice and without ice (3 ± 1 °C).

Table 3
Changes in pH, water-holding capacity (WHC), free fatty acids (FFA), peroxide value (PV), thiobarbituric acid (TBA) value, in European eel over the period of iced storage

Days in ice	pH	WHC (%)	FFA (% of oleic acid)	PV (meq/kg)	TBA (mg MA kg^{-1})
1	6.03 ± 0.03	13.33 ± 1.46	0.59 ± 0.04	5.19 ± 0.12	0.07 ± 0
5	6.14 ± 0.01	12.81 ± 1.45	0.68 ± 0.1	5.58 ± 0.41	0.08 ± 0.01
8	6.37 ± 0.23	12.28 ± 1.22	0.77 ± 0.32	3.12 ± 0.62	0.07 ± 0.02
12	6.44 ± 0.03	12.18 ± 0.48	1.07 ± 0.23	15.8 ± 1.64	0.04 ± 0.01
15	6.79 ± 0.03	12.01 ± 0.72	1.61 ± 0.91	19.7 ± 0.84	0.06 ± 0.02
19	6.84 ± 0.26	11.77 ± 1.21	1.79 ± 0.11	4.06 ± 3.64	0.08 ± 0.01

Table 4
Changes in pH, water-holding capacity (WHC), free fatty acids (FFA), peroxide value (PV), thiobarbituric acid (TBA) value, in European eel over the period of storage at 3 ± 1°C

Days at 3 ± 1°C	pH	WHC (%)	FFA (% of oleic acid)	PV (meq/kg)	TBA (mg MA kg^{-1})
1	6.09 ± 0.05	12.45 ± 1.2	0.57 ± 0.04	5.28 ± 0.25	0.07 ± 0.02
5	6.04 ± 0.13	11.84 ± 2.49	1.05 ± 0.2	5.89 ± 0.3	0.10 ± 0.02
8	6.16 ± 0.06	11.55 ± 1.11	1.49 ± 0.51	16 ± 0.55	0.13 ± 0.02
12	6.65 ± 0.01	11.04 ± 1.30	1.60 ± 0.38	21.6 ± 2.73	0.08 ± 0.01

ice and in boxes without ice on days 5, 8 and 12. Similar results were reported by Randell, Hattula, and Ahvenainen (1997) for rainbow trout, and by Wilhelm (1982) for rockfish, salmon, trout and croaker. To achieve microbiological benefit, the storage temperature of the product should be as low as ice storage. If 10^6 microorganisms/g are considered the TVC limit of acceptability, the shelf life of eel was approximately 13–14 days for ice and 6–7 days for fish kept in boxes without ice. This conclusion implies that sensory analysis of eel correlated well with microbiological analysis. The results of chemical analysis show that fish started to spoil after 5 days because of bacterial activity, whereas lipid oxidation was apparent only after 8 days.

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