



Sensory and ATP derivative-based indicators for assessing the freshness of Atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*)

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Abstract

To estimate the shelf life of fresh fish, the processor must know the period of time between catch/harvest and arrival at the processing plant. This information is not always available, necessitating the provision of methods to estimate the time since catch or harvest. The objectives of this study were therefore to develop and/or validate sensory and ATP derivative-based methods for rapidly assessing the freshness of fish. A quality index method (QIM; raw fish) and a quantitative descriptive analysis (QDA; cooked fish) were developed and validated (against bacterial count [total viable count (TVC)] and time) for salmon (*Salmo salar*) and cod (*Gadus morhua*). The production of inosine monophosphate (IMP), inosine and hypoxanthine (Hx) and associated ratios (IMP/Hx, K_t -value or H-value) were also investigated for use as freshness markers. There was a linear relationship between QIM and TVC ($R^2 = 0.93$ for salmon and $R^2 = 0.89$ for cod), QIM and time ($R^2 = 0.96$ for salmon and $R^2 = 0.98$ for cod), QDA and TVC ($R^2 = 0.93$ for salmon and $R^2 = 0.77$ for cod) and QDA and time ($R^2 = 0.94$ for salmon and $R^2 = 0.87$ for cod), suggesting that the QIM and QDA schemes developed could be used to monitor/assess freshness. The H-value also increased linearly with TVC ($R^2 = 0.88$ for salmon and $R^2 = 0.89$ for cod) and time ($R^2 = 0.93$ for salmon and $R^2 = 0.84$ for cod). It was therefore concluded that both the QIM/QDA approach and monitoring ATP degradation, specifically expressed as the H-value, could be used as rapid methods to assess the freshness of salmon and cod arriving at the processing plant.

Keywords

Atlantic cod (*Gadus morhua*) • Atlantic salmon (*Salmo salar*) • ATP derivatives • quality index method • quantitative descriptive analysis

Introduction

Fresh seafood is perishable with a short shelf life (Ghanbari *et al.*, 2013) and approximately 10% is lost due to spoilage each year (Kulawik *et al.*, 2013; Alfaro *et al.*, 2013). Chemical and enzymic autolytic processes commence immediately following death, resulting in the loss of the 'fresh' flavours of fish. Unpleasant odours and tastes are then produced by the metabolic activities of spoilage bacteria (Schirmer *et al.*, 2009; Mørkøre *et al.*, 2010). Thus, spoilage is a complex process involving enzymatic, chemical and microbiological changes, but the latter is the primary determinant of shelf life (Anacleto *et al.*, 2011).

The quality index method (QIM) is a scheme used to assess the freshness of fish based on scoring different sensory attributes (appearance, odour and texture) during storage (Bremner, 1985). Assuming the quality index (QI) increases linearly with time, once the total score for fish at the end of their shelf life is established, the score obtained prior to this can be used to estimate the remaining shelf life (Martinsdóttir *et al.*, 2001). A similar approach, quantitative descriptive

analysis (QDA), is used to assess the sensory status of cooked fish and is often combined with the QIM assessment of the raw product by seafood specialists to achieve additional assurance of the quality of the fish (Sveinsdóttir *et al.*, 2002). Although QIM schemes have been previously developed for several fish species including pollock, plaice, haddock, turbot, dab, salmon, herring, cod and mackerel (Bonilla *et al.*, 2007; Jónsdóttir, 1992; Andrade *et al.*, 1997; Luten, 2000), more detailed schemes that use language relevant to the Irish fish processors are required (personal communication). Moreover, QDA to assess the sensory status of cooked fish is currently not available.

The autolytic processes that commence immediately post-mortem include the deamination of adenosine phosphate molecules (ATP, ADP and AMP) to inosine monophosphate (IMP) before being more slowly dephosphorylated to inosine (I) and eventually degraded to hypoxanthine (Hx; Gram and Dalgaard, 2002; Chen *et al.*, 2010). The concentration of I and Hx, expressed as the IMP/Hx ratio or the modified K_t -value $[(I + Hx)/(IMP + I + Hx) \times 100]$ or the H-value $[Hx/(IMP + I + Hx) \times 100]$,

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has been proposed as indicators of freshness for fish, but their formation varies greatly depending on the fish species and storage conditions. Several studies have demonstrated a linear relationship between Hx concentration and time in a range of fish species (Beauchat, 1973; Jahns and Rand, 1977; Dingle and Hines, 1971; Jones *et al.*, 1964; Kassemsarn *et al.*, 1963). However, a number of alternative studies have concluded that IMP (Dingle and Hines, 1971; Ehira and Uchiyama, 1974) or I (Bremner *et al.*, 1988; Murata and Sakaguchi, 1988) are better biochemical markers for evaluating freshness, although the concentrations obtained are not always linear over time (Beauchat, 1973; Jahns and Rand, 1977; Murata and Sakaguchi, 1988; Bremner *et al.*, 1988). Research is, therefore, required to determine which, if any, of these indices are suitable for use in assessing the freshness of salmon (*Salmo salar*) and cod (*Gadus morhua*). The objectives of this study were to develop and validate rapid sensory (QIM and QDA) and ATP derivative-based methods for assessing the freshness of salmon and cod arriving at the processing plant.

Materials and methods

Fish samples

Whole fresh Atlantic salmon and cod were obtained within 48 and 24 h of harvest or capture, respectively, from Connolly Fish Sales (Rathmines, Dublin 6, Ireland). The salmon and cod were of a consistent size (3–4 kg and 2.5–3 kg, respectively). The fish were transported on ice to the laboratory (Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland) within 1 h. Once on site, both the salmon and cod were stored on ice in polystyrene boxes at 2°C, for up to 10 days.

Sensory analysis

A QIM was developed for scoring attributes of the whole fish and raw fillets for both salmon (Table 1) and cod (Table 2). The fish were graded by a trained panel of 12 people, all from the Teagasc Food Research Centre (Ashtown, Dublin, Ireland). The panel underwent two 1.5-h training sessions, where they became familiar with different terminologies and descriptive language for the grading scheme. From these sessions, it was apparent what attributes needed to be included in the QIM grading scheme, i.e. skin colour, eyes, gills, texture, stiffness and mucus (whole fish) and flesh colour, odour, texture, stiffness and mucus (raw fillet). The QIM grading of the fish samples was carried out on days 0, 2, 3, 6, 8 and 10. On each of these days, the panel was presented with a whole fish and a raw fillet. There were descriptions for each attribute that related to a score of 0, 1, 2 or 3 (with the lower score indicating a fresher sample), and each panellist graded

the fish by indicating which description they thought best described the physical attribute.

QDA was carried out alongside the QIM to score the characteristics of cooked salmon (Table 3) and cod (Table 4). Similar to the QIM grading scheme, panellists were asked to grade the sensory attributes (colour, odour, taste, mouth-feel, juiciness) of the cooked fish on a scale ranging from 0 to 2, where a lower score indicated a fresher sample. Samples were steam cooked at 99°C for 7 min (Rational SCC WE 61E Electric Combi Oven, Rational, Landsberg am Lech, Germany), after which the panellists assessed the cooked fillet. Each panellist was given a small piece of cooked fish and asked to grade it. As with the QIM, a scoring sheet was provided with descriptions relating to a score of 0, 1 or 2 for each physical attribute. All panellists graded the fish by indicating which description they thought best described the physical attribute. Each QIM score was scored out of 33, and each QDA was scored out of 12. These scores were then converted to a percentage, which was used as the freshness score.

ATP derivative analysis

ATP degradation was measured on days 0, 2, 3, 6, 8 and 10. On each day, two flesh samples (5 g) were aseptically removed from the whole fish. The degradation of ATP was measured using a microplate PRECICE® K-Freshness Assay Kit following the manufacturer's methodology (Novocib, Lyon, France). This kit measures the progressive conversion of IMP, I and Hx to NADH₂, using specific dehydrogenase enzymes provided in the kit. The NADH₂ is then quantified by measuring specific absorbance at 340 nm (Multiskan GO, Thermo Fisher Scientific, Dublin, Ireland) in accordance with the manufacturer's instructions. The concentration of I and Hx was expressed as the IMP/Hx ratio or the modified K_1 -value $[I + Hx / (IMP + I + Hx) \times 100]$ or the H -value $[Hx / (IMP + I + Hx) \times 100]$.

Microbiological analysis

Microbiological analysis was carried out on days 0, 2, 3, 6, 8 and 10. On each sampling day, the fish was split into two sides. From one side, there were two samples (10 g) of inner flesh obtained on each sampling day. From the other side, the outer skin of the fish was swabbed (25 cm² surface areas) in duplicate using sterile cellulose acetate sponges pre-moistened with maximum recovery diluent [MRD; Oxoid, Basingstoke, United Kingdom (CM0733)]. Each of the meat and skin swab samples was homogenised (Pulsifier Ò PUL100E; Microgen Bioproducts Ltd, Surrey, United Kingdom) for 1 min in 90 mL MRD, and 10-fold dilution series was prepared up to 10⁻⁵. Plate count agar [PCA; Oxoid, Basingstoke, United Kingdom (CM0325)], with and without 1% NaCl, was used to estimate the total viable count (TVC; 30°C for 72 h).

Table 1. Quality index method (QIM) scheme used to evaluate the sensory characteristics of salmon (*Salmo salar*) stored at 2°C for 10 days

Parameter	Description	Score
QIM – whole salmon		
Skin colour	Shiny, bright without blemishes, iridescent pigmentation, silver	0
	Rather dull	1
	Dull, slimy, gritty, grey, lack of pigmentation	2
Mucus (gills)	Uniform, thin, transparent	0
	Little thicker, opaque	1
	Clotted, thick, yellowish	2
Slime (body)	Transparent, white	0
	Off-white	1
	Yellowish, grey-brown	2
Eye	Bright, full, clear	0
	Cloudy, dull, sunken	1
Stiffness	Firm	0
	Not quite firm	1
	Soft	2
Texture	Quick rebound from finger pressure	0
	Slow response to finger pressure	1
	Persistence of finger imprint	2
Back	Firm, full, unblemished	0
	Slightly soft	1
	Soft, mushy, blemishing, sunken	2
Belly	Firm, full, unblemished, intact	0
	Slightly soft	1
	Faded, sunken, bruised, battered, grazed	2
Blood	Bright red, not present	0
	Dull red	1
	Shadowy, brown	2
Gills	Bright red, full	0
	Brown, shrivelled	1
QIM – salmon fillets		
Colour	Orange, bright	0
	Some white, pale	1
	Overall pale	2
Texture	Firm	0
	Rather soft	1
	Very soft	2
Brightness	Transparent, bluish	0
	Opaque	1
	Milky	2
Odour	Fresh, neutral	0
	Seaweed, marine, grass	1
	Sour milk	2
Stiffness	Acetic, ammonia, offensive, unpleasant	3
	Rigour	0
Mucus	Post rigour	1
	Absent	0
	Some evidence	1
Gaping	Excessive	2
	No gaping, one longitudinal gaping at the neck part of the fillet	0
	Slight gaping, less than 25% of the fillet	1
	Slight gaping, 25%–75% of the fillet	2
	Deep gaping or slight gaping, more than 75% of the fillet	3

Table 2. Quality index method (QIM) scheme used to evaluate the sensory characteristics of cod (*Gadus morhua*) stored at 2°C for 10 days

Parameter	Description	Score
QIM – whole cod		
Skin colour	Shiny, bright without blemishes, iridescent pigmentation, silver	0
	Rather dull	1
	Dull, slimy, gritty, grey, lack of pigmentation	2
Mucus (gills)	Uniform, thin, transparent	0
	Little thicker, opaque	1
	Clotted, thick, yellowish	2
Slime (body)	Transparent, white	0
	Off-white	1
	Yellowish, grey-brown	2
Eye	Bright, full, clear	0
	Cloudy, dull, sunken	1
Stiffness	Firm	0
	Not quite firm	1
	Soft	2
Texture	Quick rebound from finger pressure	0
	Slow response to finger pressure	1
	Persistence of finger imprint	2
Back	Firm, full, unblemished	0
	Slightly soft	1
	Soft, mushy, blemishing, sunken	2
Belly	Firm, full, unblemished, intact	0
	Slightly soft	1
	Faded, sunken, bruised, battered, grazed	2
Blood	Bright red, not present	0
	Dull red	1
	Shadowy, brown	2
Gills	Bright red, full	0
	Brown, shrivelled	1
QIM – cod fillets		
Colour	White, greyish	0
	Some yellow, a little pinkish	1
	Yellow or overall pink	2
Texture	Firm	0
	Rather soft	1
	Very soft	2
Brightness	Transparent, bluish	0
	Opaque	1
	Milky	2
Odour	Fresh, neutral	0
	Seaweed, marine, grass	1
	Sour milk	2
Stiffness	Acetic, ammonia, offensive, unpleasant	3
	Rigour	0
	Post rigour	1
Mucus	Absent	0
	Some evidence	1
	Excessive	2
Gaping	No gaping, one longitudinal gaping at the neck of the fillet	0
	Slight gaping, less than 25% of the fillet	1
	Slight gaping, 25%–75% of the fillet	2
	Deep gaping or slight gaping, more than 75% of the fillet	3

Table 3. Quantitative descriptive analysis (QDA) scheme used to evaluate the sensory characteristics of cooked salmon (*Salmo salar*)

Parameter	Description	Score
QDA – cooked salmon		
Colour	Orange, bright	0
	Orange, some off-white	1
	Pale, dull	2
Odour	Fresh, seaweed odour	0
	Stronger, fishy	1
	Strong fishy, offensive, unpleasant	2
Taste	Mild, fishy, pleasant	0
	Moderately fishy	1
	Strong fishy, offensive, unpleasant	2
Mouthfeel	Dissolve, melt in the mouth, crumbly, soft	0
	Slightly chewy	1
	Chewy, rubbery, tough	2
Moist	Moist, pleasant	0
	Mildly moist	1
	Dry, very dry	2
Sticky/gluey	Does not stick to or coat the palate or the teeth	0
	Somewhat sticky	1
	Coats the palate and teeth	2

Table 4. Quantitative descriptive analysis (QDA) scheme used to evaluate the sensory characteristics of cooked cod (*Gadus morhua*)

Parameter	Description	Score
QDA – cooked cod		
Colour	White, bright	0
	Off-white	1
	Dull, dark	2
Odour	Fresh, seaweed odour	0
	Stronger fishy	1
	Strong fishy, offensive, unpleasant	2
Taste	Mild, fishy, pleasant	0
	Moderately fishy	1
	Strong fishy, offensive, unpleasant	2
Mouthfeel	Dissolve, melt in the mouth, crumbly, soft	0
	Slightly chewy	1
	Chewy, rubbery, tough	2
Moist	Moist, pleasant	0
	Mildly moist	1
	Dry, very dry	2
Sticky/gluey	Does not stick to or coat the palate or the teeth	0
	Somewhat sticky	1
	Coats the palate and teeth	2

Temperature analysis

EL-USB-2 temperature data loggers (Lascar Electronics, Whiteparish, United Kingdom) recorded the ambient temperature of the storage cold room environment, while a Testo 175T3 data logger (Testo, Lenzkirch, Germany) was used to record skin and core temperatures of the whole salmon and cod.

Data analysis

The sensory analysis (QIM and QDA) was carried out as described earlier at times $t = 0, 2, 3, 6, 8$ and 10 . At the same time intervals, the ATP derivative analysis was performed using two samples from the same fish and the TVC was obtained using four samples (two inner flesh samples and two outer skin swab samples) from the same fish. This constituted one trial, which was then repeated twice more. The equation of best fit and the correlation coefficients (R) of QIM and QDA against TVC (flesh and skin swab), storage time in ice, IMP concentration (mg/g), Hx concentration (mg/g), I concentration (mg/g), the IMP/Hx ratio, the K_1 -value (%) and the H -value (%Hx) were calculated using Microsoft® Excel 2010 (Microsoft Corporation, Redmond, WA, USA).

Results

Over the 10 days storage on ice in a chilled room set at 2°C , the average ambient temperature recorded was 1.6°C ($\pm 0.8^{\circ}\text{C}$). The average skin and core temperatures for salmon ranged between 1.5°C (± 0.9) and 2°C (± 0.8), whereas the average skin and core temperatures for cod were 1.5 (± 0.8) and 1.4°C (± 0.7), respectively. Each fish recorded a minimum temperature of 0°C for both skin and core readings. No difference in the growth of TVC grown on PCA with or without 1% NaCl was observed ($P > 0.05$), and therefore, only data obtained with 1% NaCl are presented. TVCs for salmon increased from 1.1 and 2.7 to 5.1 and 5.7 \log_{10} CFU/cm² and those for cod increased from 3.0 and 3.9 to 5.2 and 5.8 \log_{10} CFU/cm² on flesh and skin swab samples, respectively, over the 10-day storage period.

For salmon fillets, post-rigour 'stiffness', the term used to describe firmness, was the first attribute associated with a lack of freshness by the majority of panellists after 3 days and all by day 6. For whole fish, 'cloudy, dull, sunken' eyes and 'brown, shrivelled' gills are indicators of loss of freshness, with all panellists indicating the maximum score for the former at day 8 and for the 'brown shrivelled' gills at day 6. With the exception of odour (maximum QI score after 10 days), none of the other attributes reached their maximum score over the course of the experiment. Post-rigour stiffness and odour were also the attributes associated with lack of freshness in cod fillets, and these attributes both achieved the maximum

score of 1 and 2, respectively (from all panellists) by day 10. The QDA for cooked salmon suggested that colour was the most important attribute in the early indication of spoilage with seven of the 10 panellists giving it a top score after 8 days and all by 10 days, whereas for cooked cod, odour was considered the most important attribute, achieving a maximum demerit score by day 10. In contrast, most of the panellists did not consider the taste of fish to be either 'strong, fishy, offensive and unpleasant' or 'dry or very dry' until day 10. The other attributes had not reached the maximum demerit score by the end of the trial. Overall, the panel suggested that salmon was spoiled by day 10 and cod by day 8.

Regression analysis suggested a strong relationship between the QIM scores and TVC for salmon flesh ($R^2 = 0.93$) and skin swab ($R^2 = 0.95$), with approximately 10 to 14 sensory units lost for each 1 log₁₀ CFU/cm² increase in the bacterial count (Figures 1A and 1B). The corresponding R^2 values for cod were 0.89 and 0.77 with a decrease of 12 to 15 sensory units per 1 log₁₀ CFU/cm² increase in TVC (Figures 1C and 1D). Similar results were obtained for QDA scores versus time with R^2 values of 0.93 (salmon-flesh TVC), 0.94 (salmon-skin swab TVC) and 0.77 (cod-flesh TVC), although the R^2 value for cod QDA versus skin swab TVC at 0.6 suggested that the data were not as close to the fitted regression line (Figure 2). Regardless, approximately 11 to 16 sensory units were lost for each 1 log₁₀ CFU/cm² increase in TVC.

Regression analysis (Figure 3) also suggested a strong relationship between time and the QIM scores for salmon ($R^2 = 0.96$) and cod ($R^2 = 0.98$). The corresponding R^2 values for time versus QDA scores were 0.94 and 0.87 for salmon and cod, respectively (Figure 3).

After death, adenosine phosphate was immediately degraded to IMP and then more slowly to I and Hx. The IMP, I and Hx concentrations at each sampling time as well as the IMP/Hx ratio, K_1 -value and H -value, for salmon and cod are shown in Tables 5 and 6, respectively. On day 0, the concentration of IMP in the salmon samples was 2.8 mg/g, which decreased to 0.6 mg/g after 3 days before decreasing to 0.3 mg/g after 10 days. The initial Hx concentration was 1.0 mg/g, which showed an increase to 1.5 mg/g after 10 days. The concentration of I remained relatively constant (3.2 to 3.9 mg/g) throughout the trial. With the exception of H -values ($R^2 = 0.93$), the relationship between time and IMP, I or Hx concentration or between time and IMP/Hx ratio and K_1 -value was non-linear.

At time $t = 0$, the concentration of IMP in cod samples was 8.3 mg/100 g, which decreased to 0.5 mg/100 g by day 3 and thereafter remained at a residual concentration of approximately 1.0mg/100 g. The initial Hx concentration was 4.8 mg/100 g, which increased to 6.7 mg/100 g after 2 days and remained at 6 to 8 mg/100 g thereafter. I concentrations decreased from 40.6 mg/100 g at time $t = 0$ to 22.3 mg/100 g

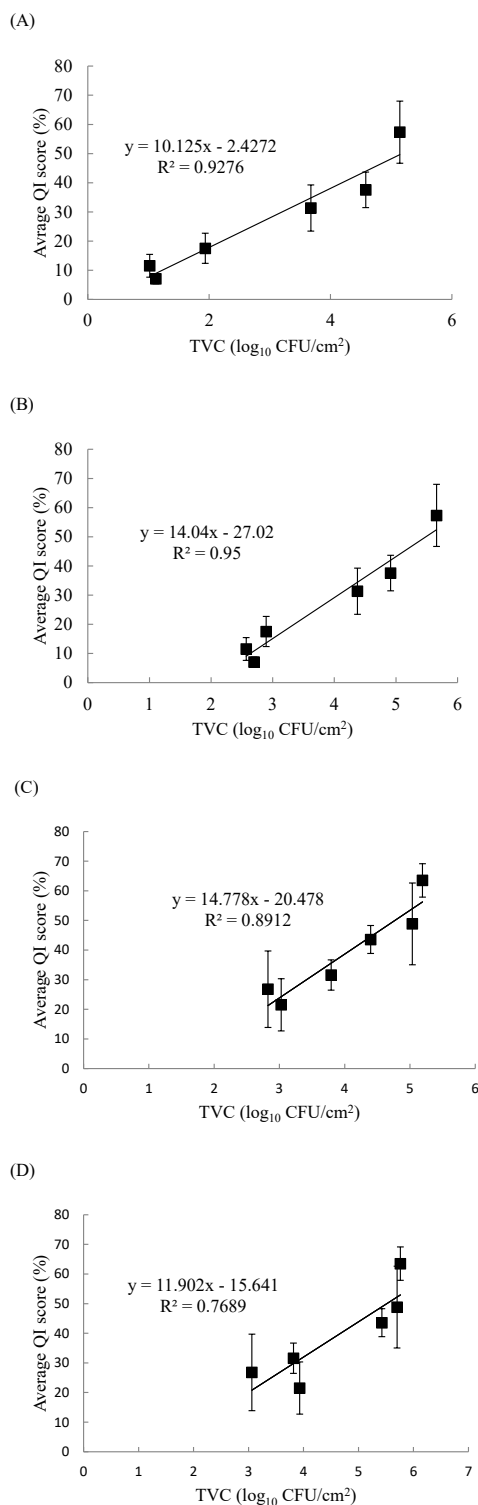


Figure 1. Relationship between the quality index (QI) score and the total viable count (TVC) for fresh salmon (*Salmo salar*) (A) flesh and (B) skin swabs and cod (*Gadus morhua*) (C) flesh and (D) skin swabs stored aerobically on ice in a chilled room at 2°C for 10 days. Each point corresponds to 24 data values.

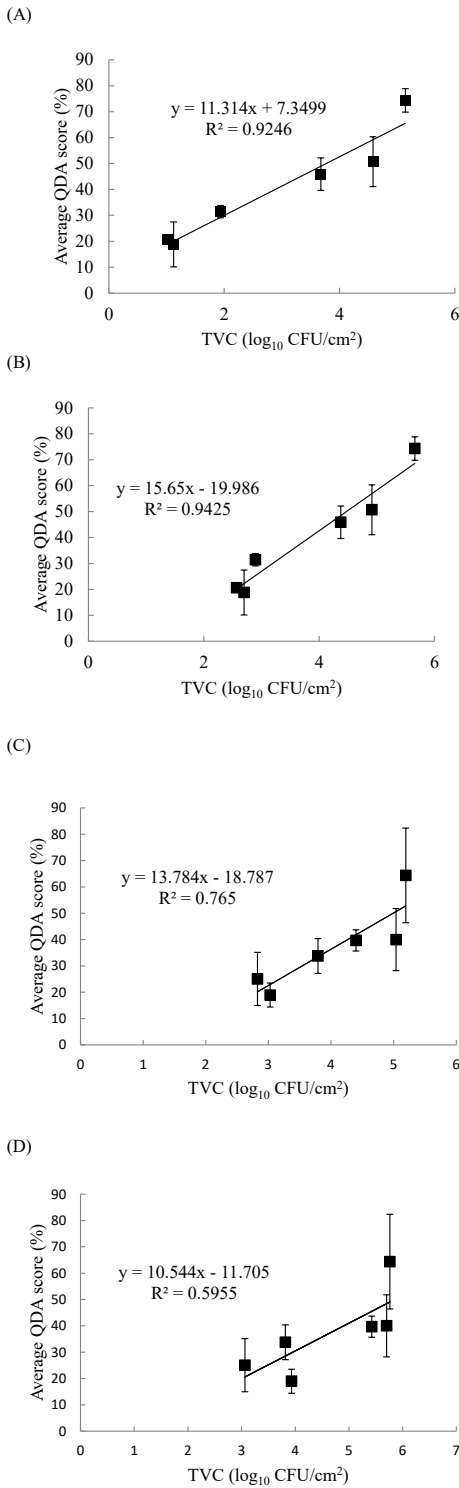


Figure 2. Relationship between the quantitative descriptive analysis (QDA) score and the total viable count (TVC) for fresh salmon (*Salmo salar*) (A) flesh and (B) skin swabs and cod (*Gadus morhua*) (C) flesh and (D) skin swabs stored aerobically on ice in a chilled room at 2°C for 10 days. Each point corresponds to 24 data values.

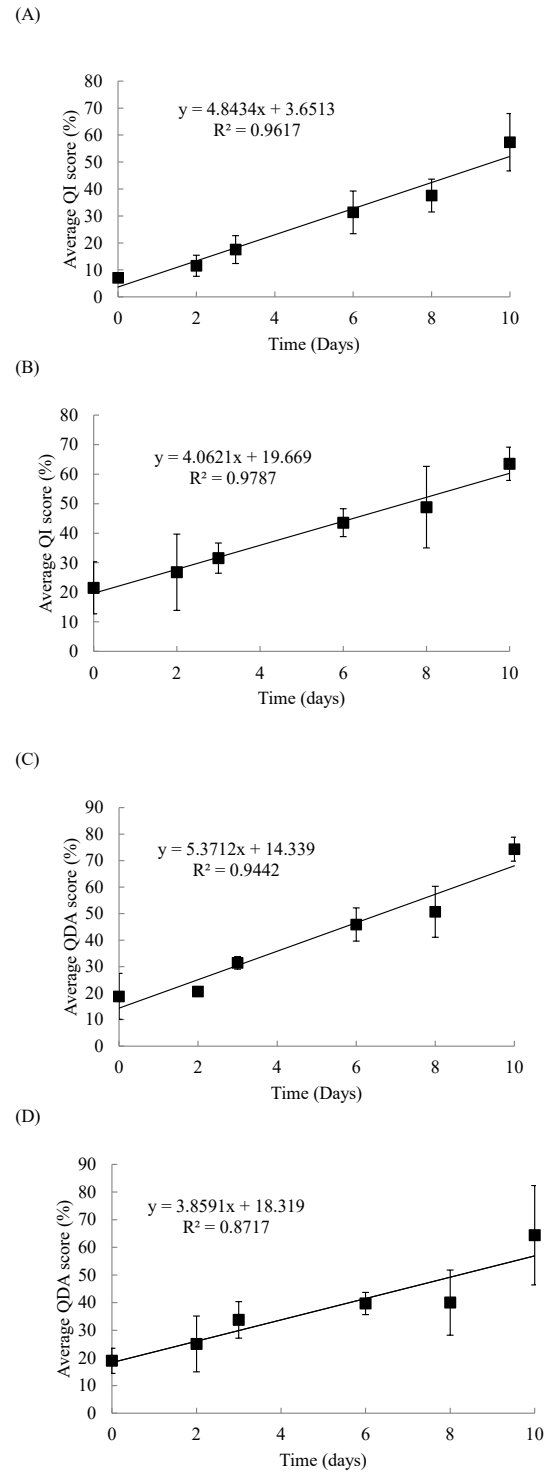


Figure 3. Relationship between the quality index (QI) score and time (days) for (A) salmon (*Salmo salar*) and (B) cod (*Gadus morhua*) and the relationship between quantitative descriptive analysis (QDA) score and time (days) for (C) salmon and (D) cod (*Gadus morhua*) stored aerobically on ice in a chilled room at 2°C for 10 days. Each point corresponds to 24 data values.

Table 5. Inosine monophosphate (IMP), inosine (I) and hypoxanthine (Hx) concentrations together with IMP/Hx ratio, K_f -value and H -value for salmon (*Salmo salar*) stored aerobically at 2°C for 10 days

Time (d)	IMP (mg/g)	I (mg/g)	Hx (mg/g)	IMP/Hx ratio	K_f -value (%)	H -value (%Hx)
0	2.8	3.6	1.0	2.8	82.2	13.5
2	1.9	3.9	1.2	1.6	72.9	17.1
3	0.6	3.8	1.2	0.5	89.3	21.4
6	0.7	3.2	1.3	0.5	86.5	25.0
8	0.6	3.2	1.3	0.5	88.2	25.5
10	0.3	3.5	1.5	0.2	94.3	28.3
R^2	0.71	0.38	0.69	0.69	0.51	0.93

$$K_f\text{-value} = I + Hx / (IMP + I + Hx) \times 100. \quad H\text{-value} = Hx / (IMP + I + Hx) \times 100.$$

Table 6. Inosine monophosphate (IMP), inosine (I) and hypoxanthine (Hx) concentrations together with IMP/Hx ratio, K_f -value and H -value for cod (*Gadus morhua*) stored aerobically at 2°C for 10 days

Time (d)	IMP (mg/100 g)	I (mg/100 g)	Hx (mg/100 g)	IMP/Hx ratio	K_f -value	H -value (%Hx)
0	8.3	40.6	4.8	1.73	84.5	8.9
2	4.3	35.6	6.7	0.64	90.8	14.4
3	0.5	34.1	6.6	0.07	98.8	16.0
6	1.1	34.1	6.0	0.18	97.3	14.6
8	0.6	27.3	7.4	0.08	98.3	21.0
10	1.0	22.3	7.8	0.13	96.8	22.1
R^2	0.57	0.91	0.65	0.53	0.53	0.84

$$K_f\text{-value} = I + Hx / (IMP + I + Hx) \times 100; \quad H\text{-value} = Hx / (IMP + I + Hx) \times 100.$$

by day 10. There was a linear relationship between time and I concentration ($R^2 = 0.91$) and H -value ($R^2 = 0.84$), but the relationship of time with IMP ($R^2 = 0.57$), Hx ($R^2 = 0.65$), IMP/Hx ratio ($R^2 = 0.53$) and K_f -value ($R^2 = 0.53$) was less well defined.

Discussion

Initial TVCs ranged from 1.1 to 3.9 \log_{10} CFU/cm², suggesting the fish were from clean water (Gram and Huss, 1996; Gram, 1992) and of good microbiological quality (Schubring, 2003; Briones *et al.*, 2010; Li *et al.*, 2017). By the end of sensory shelf life for each fish (10 days for salmon and 8 days for cod), the TVC₁₀ ranged from 5.1 to 5.8 \log_{10} CFU/cm². This is in agreement with Robson *et al.* (2007), who found seafood spoiled when the bacterial count reached 5 to 6 \log_{10} CFU/cm².

The QIM developed for salmon and cod provided a good description of the sensory changes that occurred during aerobic chilled storage, and the linear relationship between QIM scores and both TVC and time suggested that this scheme could be used to assess fish freshness. This was complemented by the QDA for cooked fish. Other studies have also reported a linear relationship between QIM score

and time for salmon (Sveinsdottir *et al.*, 2002, 2003), blackspot seabream (Sant'Ana *et al.*, 2011) and rainbow trout (Diler and Genç, 2018).

In this study, the IMP concentration decreased in the salmon (2.8 to 0.3 mg/g) and cod (8.3 to 1.0 mg/100 g) over the 10 days aerobic storage at 2°C. I levels did not change for salmon but decreased in cod from 40.6 to 22.3 mg/100 g. In contrast, only a minor increase was observed in the Hx concentration for both salmon (1.0 to 1.5 mg/g) and cod (4.8 to 7.8 mg/100 g). Similar data from other fish studies are limited and that which are available focus on Hx. Burns *et al.* (1985) found initial Hx concentrations of 0.12 and 0.15 mg/g in mackerel and cod, respectively. Whittle *et al.* (1990) reported that Hx levels increased from 2.4 to 8.8 mg/100 g in cod stored on ice for 10 days. Other studies obtained increases from 3.3 to 16.6 mg/100 g in striped bass fillets stored at 4°C for a similar time period (Karahadian *et al.*, 1997) and from 0.09 to 0.41 mg/g in salmon stored at 1°C for 20 days (Sallam, 2007). Karahadian *et al.* (1997) proposed that the loss of freshness should be expressed as an IMP/Hx ratio, K_f -value [$I + Hx / (IMP + I + Hx) \times 100$] or H -value [$Hx / (IMP + I + Hx) \times 100$] rather than monitoring the concentration of ATP derivatives. They argued that these ratios are a better indicator of freshness as they take into account the concentrations of all the ATP derivatives. In the current study, the IMP/Hx ratio, K_f -value

and H -value for salmon changed from initial values of 2.8, 82.2% and 13.5% to 0.2, 94.3% and 28.3%, respectively. The corresponding values for cod were 1.73, 84.5% and 8.9% at time $t = 0$, respectively, changing to 0.13, 96.8% and 22.1% after 10 days. Karahadian *et al.* (1997) reported a K_1 -value of 37.8% at time $t = 0$ for striped bass, which did not exceed 90% until day 9.

The pattern of increase in H -value occurred linearly in salmon and cod for both TVC and time, but the relationships between IMP/Hx ratio and K_1 -values were non-linear. Other studies on the optimum ATP derivative/ratio for monitoring fish freshness are contradictory (Bremner, 1985; Murata and Sakaguchi, 1988; Whittle *et al.*, 1990; Sallam, 2007). This was not unexpected as nucleotide degradation rates depend on a range of factors including fish maturity, muscle type, stress during capture and storage conditions (Luong *et al.*, 1992; Huss, 1995; Erikson *et al.*, 1997).

Conclusions

QIM and QDA schemes developed in this study may be used as a rapid sensory-based tool for assessing the freshness of salmon and cod. Moreover, 'cloudy, dull and sunken' eyes and brown shrivelled gills provide early indicators of loss of freshness of whole fish. The H -value may be a suitable ATP derivative ratio for assessing salmon and cod freshness, but given the conflicting reports in the literature, further studies are needed to confirm this finding.

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