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Relevance of season and nucleotide catabolism on changes in fillet quality during chilled storage of raw Atlantic salmon (*Salmo salar* L.)

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ABSTRACT

Farmed 5–6 kg Atlantic salmon were pre-rigor filleted, vacuum packed and subsequently analysed after 1, 9 and 13 days of storage at 4 °C. The study was repeated in February, April, August and October. The conversion of hypoxanthine (Hx) showed the highest seasonal variation among the nucleotide metabolites, with an inverse relationship with sea temperature at harvesting (R^2 = 0.95–0.96). The Hx content was inversely related to fresh odour and flavour (R^2 = 0.81–0.83), but positively to tenderness (R^2 = 0.87). Hence, these results suggest that salmon reared in seawater of 11–15 °C (August–October) maintain a superior sensory quality for a longer period post-mortem than salmon reared at 6–8 °C (February–April). The colour intensity increased from days 1 to 9 post-mortem, probably due to rigor contraction. The highest increase in drip loss was observed in October and the lowest in April. It is proposed that seawater temperature significantly influences the storage life of raw salmon, and that Hx is a valuable biomarker for sensory quality.

1. Introduction

Pre-rigor filleting can improve efficiency at the processing plant, as processing and purchasing of the fillet products is performed directly after slaughtering, instead of waiting several days for rigor resolution before filleting (normally 3–5 days). Previous studies using pre-rigor salmon fillets have also suggested some qualitative advantages in comparison to conventional post-rigor salmon fillets, e.g., more intense colouration, firmer texture and less gaping (Andersen, Strømsnes, Steinsholt, & Thomassen, 1994; Skjervold et al., 2001). Due to high freshness, pre-rigor processed fillets have the potential to supply the growing demand of raw fish, for example "sushi" and "sashimi". Comprehensive quality control possibilities throughout the value chain in salmon aquaculture, from farming to processing and distribution, also suit the particularly high demand for traceability and hygienic quality in raw fish products.

The overall quality perception of salmon is a multifaceted impression of product characteristics, including appearance, flavour, odour and texture. The storage temperature is considered the most important factor influencing quality and storage life of fresh fish, as both enzymatic and microbiological activity are greatly influenced by temperature (Huss, 1988). However, improved knowledge of biochemical processes occurring during storage and the relevance of these for sensory sensations is required. It is well documented that quality characteristics of salmon fillets show seasonal variations (Lavety, Afolabi, & Love, 1988; Mørkøre & Austreng, 2004; Roth et al., 2005), however no studies have focussed upon the impact of seasonal variations to changes in sensory quality and biochemical degradation during storage of raw salmon fillets.

Fresh fish generally deteriorate by one of two mechanisms; bacterial spoilage and/or autolysis (Surette, Gill, & LeBlanc, 1988). Normally, more than 90% of the nucleotides in fish muscle are purine derivatives resulting from catabolism of adenosine triphosphate (ATP) (Haard, Simpson, & Pan, 1994). Pre-slaughter crowding stress accelerates breakdown of ATP (Erikson, Beyer, & Sigholt, 1997; Mørkøre, Mazo, Tahirovic, & Einen, 2008; Sigholt et al., 1997); and in post-rigor muscle, hypoxanthine is formed as a by-product from the degradation of ATP. Certain 5′-mononucleotides, interme-

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diates in the production of hypoxanthine, are believed to be important flavour enhancers in muscle foods and a compound that falls into this category is IMP (phosphorylated inosinic acid). In contrast, hypoxanthine is suspected to be a cause of unpleasant flavours in stored fish (Foegeding, Lanier, & Hultin, 1996). The ATP catabolism to hypoxanthine is essentially caused by endogenous enzymes, however the hydrolysis of inosine and formation of hypoxanthine may also result from bacterial enzymes (Surette et al., 1988). This study focuses on post-mortem changes in sensory properties during chilled storage of raw Atlantic salmon and the biochemical origin of these changes with emphasis on nucleotide catabolism (i.e., variation of IMP, inosine and hypoxanthine). Salmon were sampled four times throughout the year to elucidate possible seasonal variation in quality development and the rate of nucleotide catabolism during storage.

2. Materials and methods

2.1. Fish material and handling

The fish used were 5–6 kg Atlantic salmon (*Salmo salar* L.), farmed at Bremnes Seashore AS at the Norwegian west coast under standard commercial conditions (density 14 kg/m^3 on average). All fish were of a similar genetic background (Salmo Breed strain) and they were fed a standard extruded dry feed (Ewos Special 1000, containing 37% protein, 34% fat; astaxanthin 20 mg/kg). The fish were starved for 12 ± 2 days before they were transported by well-boat from the rearing cage to sea cages at the processing plant quayside where they were acclimated for 2 days before slaughtering (live chilling at 2 °C for 40 min and electrical stunning). Fish were harvested four times during 2007: February, April, August, and October. The seawater temperature at the different harvesting times was 8, 6, 15 and 11 °C, respectively.

The fish were gill gut and thereafter bled in running 2 °C seawater for 60 min, prior to being gutted and filleted (skin removed, not red muscle) by machine. Thereafter, the section below and above the midline of the 3/4 foremost fillet part ('belly loin') was cut from the whole fillet (8 cm width × 25 cm length × 3.5 cm fillet height, and 390 g on average) at Salmon Brands AS. The fillet piece was packed with a liquid absorber (SuperCore® MP100, McAirlaid's GmbH & Co, Steinfurt, Germany). Packaging was performed immediately after filleting in 0.5 l high density polyester trays with a top web (PA/PEM Südpack Europa AG, Ochsenhausen, Germany). The fillet temperature at packaging was 2 °C, and the whole process from slaughtering to packaging was performed within 4 h. At each harvest, 36 packages were randomly selected from the processing line and transported overnight to Nofima Mat, Ås, Norway (temperature in the transport boxes was 1.3 ± 0.5 °C). The packages were thereafter stored at 4 °C, and the temperature in the refrigerated room was continuously monitored by logger EBI-125A/85A, Ebro Electronic (Ingolstadt, Germany). The fish were analysed on days 1, 9 and 13 post-mortem (12 packages each day). The weights of drip losses from the fish muscle were recorded after opening the packages.

2.2. Lipid content and colour measurements

The area above the lateral line was photographed by a digital camera (Dolphin F145C, Allied Vision Technologies, Stadtroda, Germany) in a light proof aluminium box with standardised illumination. The camera was placed on the top of the box, facing perpendicularly downwards and powered and operated through a computer. Each pixel within the image was represented by RGB signals from the red (R), green (G) and blue (B) channels of the camera with the RGB signals recording values between 0 (dark) and 255 (light). Results were transcribed to correspond to fillet fat content and visual colour score (RocheSalmoColourFan™), as described by Folkestad et al. (2008).

2.3. Muscle pH

Muscle pH was analysed using a 330i SET pH-metre (Wissenschaftlich-Technische-Werkstätten GmbH & Co. KG WTW, Weilheim, Germany) equipped with a muscle-electrode (Schott pH-electrode, Blueline 21 pH, WTW, Weilheim, Germany) and a temperature probe (TFK 325, WTW, Weilheim, Germany).

2.4. Adenosine triphosphate (ATP) breakdown products

Muscle sections were sampled and frozen at −80 °C until further analyses of adenosine-5′-triphosphate (ATP), adenosine-5′-diphosphate (ADP), adenosine-5′-monophosphate (AMP), inosine monophosphate (IMP), and inosine and hypoxanthine (Hx) were conducted. The samples (0.200 g) were extracted by mixing freeze-dried tissue in ice-cold 8% $HClO_4$ (2.5 ml) for 30 min before centrifugation (10 min at 11,900*g*). The supernatant (1 ml) was neutralised with 3 M K_2CO_3 (0.3 ml), centrifuged for 10 min at 11,900*g*, and finally filtrated (0.45-μm filter). ATP and degradation products were analysed by HPLC in a Waters Alliance liquid chromatograph system (2695) equipped with a photodiode array detector (2996). The analysis were performed on a XTerra MS C_{18} 5-μm column (4.6 mm × 250 mm) with a guard column XTerre MS C_{18} 5 μm (3.9 × 20 mm). The injection volume was 10 μl. The mobile phase used for the separation of nucleotides consisted of two eluents. *A*: acetonitrile and *B*: 50 mM phosphate buffer with 10 mM $CH_3(CH_2)_3]_4NBr$ (pH 7.0) as a gradient. From 0 to 13 min *A* was increased from 3% to 10%, thereafter held at 20% for 12 min, decreased to 10% for 5 min, and finally held at 3% for 10 min. The flow rate and temperature were 1.5 ml min^{-1} and 30 °C, respectively. The eluents were monitored at 254 nm and compared to Sigma external standards for each ATP related compound.

The *K*-value, a freshness index, was calculated from ATP degradation products; the percent of the sum inosine and Hx divided by the sum of ATP, ADP, AMP, IMP, inosine and Hx (Saito, Arai, & Matsuyoshi, 1959).

2.5. Instrumental texture analyses

Duplicate texture analyses were performed on each fillet where texture measurements were performed parallel to the muscle fibres on 2.5 ± 0.03 cm thick cutlets above the lateral line just anterior to the dorsal fin. The instrument used was a Texture analyser, model TA-XT2 (SMS Stable Micro Systems Ltd., Blackdown Rural Industries, Surrey, UK), equipped with a flat-ended 12.5-mm diameter cylinder (type P/0.5). The trigger force was 0.2 N and the test speed was 1 mm s^{-1}. The force–time graphs were recorded and analysed using the computer software Texture Expert for Windows (version 1.15, Stable Micro Systems). The parameters recorded were the force required to penetrate the cutlet surface (denoted as the breaking force, F_b) and the gradient on the linear part of the force–time graphs, up to the F_b (denoted as the gradient).

2.6. Sensory assessment

Twelve assessors with ⩾5 years of experience in sensory evaluation using descriptive analysis were selected and trained according to ISO 8586-1 (1993). A modified QDA method, as described in ISO 6564 (1985), was used for the evaluation. The sensory laboratory was designed according to guidelines in ISO 8589 (1988) with separate booths and electronic registration of data (CSA, Compusense Five, Version 4.6, Guelph, Ontario, Canada, 1999). Prior to

the trial, the panel was trained using raw salmon stored on ice for 1 or 12 days. They agreed on a consensus list for the profiling and on the definition of each attribute (Table 2). The assessors were trained using the correct terminology before the sensory session.

Each assessor was served three slices (5-mm thickness) from the same anatomical region from each fillet. Samples were served raw in plastic cups with a lid given a three digit code. Unsalted crackers and lukewarm water were available for rinsing the palate between samples. The assessors recorded their results at individual speed on a 15 cm non-structured continuous scale with the left side of the scale corresponding to the lowest intensity of the attribute and the right side corresponding to the highest intensity. The computer transformed responses into numbers between 1 = low intensity and 9 = high intensity. Definitions of the sensory attributes are given in Table 1.

In October, samples were also assessed by consumers selected among the staff at The Norwegian University of Life Sciences, Institute of Aquaculture Research, and Aqua Culture Protein Centre (all situated at Ås, Norway). Twelve females and 12 males tested the salmon at days 1, 9 and 13 post-mortem; different respondents were used on each occasion. The only selection criteria of test persons were that they liked and were willing to assess raw salmon. Slices from six fillet pieces (approximately 50 g) were presented to the test persons on 7 cm × 7 cm plastic trays. Fresh odour was assessed according to a five-point scale: score 1 = extremely unfresh, 2 = un-fresh, 3 = neutral, 4 = fresh, and 5 = extremely fresh.

2.7. Statistical analyses

An analysis of variance (ANOVA) using the General Linear Model implemented in the statistical software SAS software ver. 8.2 (Statistical Analysis System Institute Inc., Cary, NC, USA), was used to analyse data collected per sampling event. The day of analysis (days 1, 9, and 13) and the season of harvest were treated as explanatory variables. Significant differences among means were ranked using Duncan's multiple range tests. For the sensory data, multiple comparisons were performed using Tukey's test. Regression analyses or Pearson's product moment correlations were used to examine relationships between variables, where the level of significance was set at $P < 0.05$.

3. Results and discussion

3.1. Fat and pigment content

The average fat content of the salmon examined ranged from 15.9% to 16.9%. The content was significantly lower in October (15.9%) and April (16.1%) compared with February and August (16.9%). The average pigment content ranged from 6.4 to 6.9 mg/kg, with a significantly larger content found in April (6.8 mg/kg) and August (6.9 mg/kg), and the lowest content in February (6.4 mg/kg). The pigment content in October (6.7 mg/kg) did not differ significantly from that during other harvesting periods. The significant seasonal differences in fat and pigment content were probably of minor practical importance as the maximum variation was only 1%-unit for fat and 0.5 mg/kg for pigment content.

Table 1
Definition of the sensory attributes used in the evaluation of raw salmon, detected by a trained sensory panel.

Attribute	Definition
Odour	
Fermented	Ripening, rotten, fermented
Rancid	Oxidised fats and oils
Seawater	Briny, ocean, salt water
Fresh	Fresh/fruity and sour/sweet (frequently termed acidic)
Flavour	
Bitter	Caffeine, quinine
Seawater	Briny, ocean, salt water
Fresh	Fresh/fruity and sour/sweet (frequently termed acidic)
Texture	
Tender	Low level of chewiness
Juicy	Moist, perception of water release
Fibrous	Long particles oriented the same way
Chewing resistance	Related to cohesiveness

3.2. Muscle pH

The pH on day 1 was significantly higher in February and August (pH 6.2) than in April and October (pH 6.1). The muscle pH decreased during the storage period in February and August, reaching levels of 6.14 and 6.15, respectively. In April the pH was stable (6.13–6.14), whereas it increased significantly from 6.12 to 6.19 after 13 days of storage in October. Because the minimum pH only varied by 0.03 units between the seasonal periods (range 6.12–6.15), it is suggested that the initial glycogen level was relatively stable throughout the year, as the muscle pH reflects the conversion of glycogen to lactic acid. The significantly higher muscle pH in October probably reflects bacterial production of basic volatile components after 13 days of storage.

3.3. ATP breakdown products

The ATP and ADP content on day 1 was $\leqslant 0.01$ and $\leqslant 0.5$ μmol g^{-1}, respectively, with no significant seasonal variation. Analyses showed only traces of AMP. The IMP content was highest 1-day post-mortem, averaging 9.3 μmol g^{-1} (Fig. 1). The low ATP level on day 1 and the fast and temporary accumulation of IMP coincide with previous studies of 44 fish species, which revealed initial IMP levels of 2.8–16.6 μmol g^{-1} (median of 6.8 μmol g^{-1}; reviewed by Howgate (2006)). Thus, the IMP levels determined day 1 in the salmon examined are relatively high, ranging from 8.5 to 10.6 μmol g^{-1}. Throughout the whole storage period, the IMP content was significantly highest in April, whereas the most rapid catabolism of IMP was observed in October. The minimum IMP content after 13 days storage in October averaged 0.36 μmol g^{-1}, i.e., 4–6 times lower than at the other harvesting times (range 1.4–2.2 μmol g^{-1}). Howgate (2006) concluded that the kinetics of degradation of IMP varies both between and within species, without giving any consistent causes for the variations. The gross composition of the salmon in our study and the initial sum of ATP metabolites showed only minor variations throughout the year. Hence the seasonal variations in IMP levels were probably related to factors additional to nutritional status, for example variation in activity of autolytic enzymes.

The content of IMP was inversely related with inosine ($r = -0.83$; $P < 0.0001$) and Hx ($r = -0.71$; $P < 0.0001$). The inosine content 1-day post-mortem was significantly highest in August and October, and lowest in April. The content of inosine remained lowest in April after 13 days storage, whereas the highest level was observed in August. The Hx content on day 1 did not vary significantly among seasonal periods, however the development of Hx during storage differed significantly between seasons. The final Hx level (day 13) in the muscle was almost twice as high in April (3.3 μmol g^{-1}) when compared to that observed in August (1.7 μmol g^{-1}). The overall correlation between inosine and Hx was $r = 0.50$ ($P < 0.0001$). In October the sum of IMP, inosine and Hx decreased by 23.5%, whereas the decrease was 17–18% at the other seasonal periods. These values are relatively low, as Howgate (2006) reported a general leaching rate of 30% in 12 days.

Tropical fish generally keep longer in ice compared with fish from temperate or cold waters. Bacterial flora of fish varies with the environment, and thus psychrotrophic bacteria which are

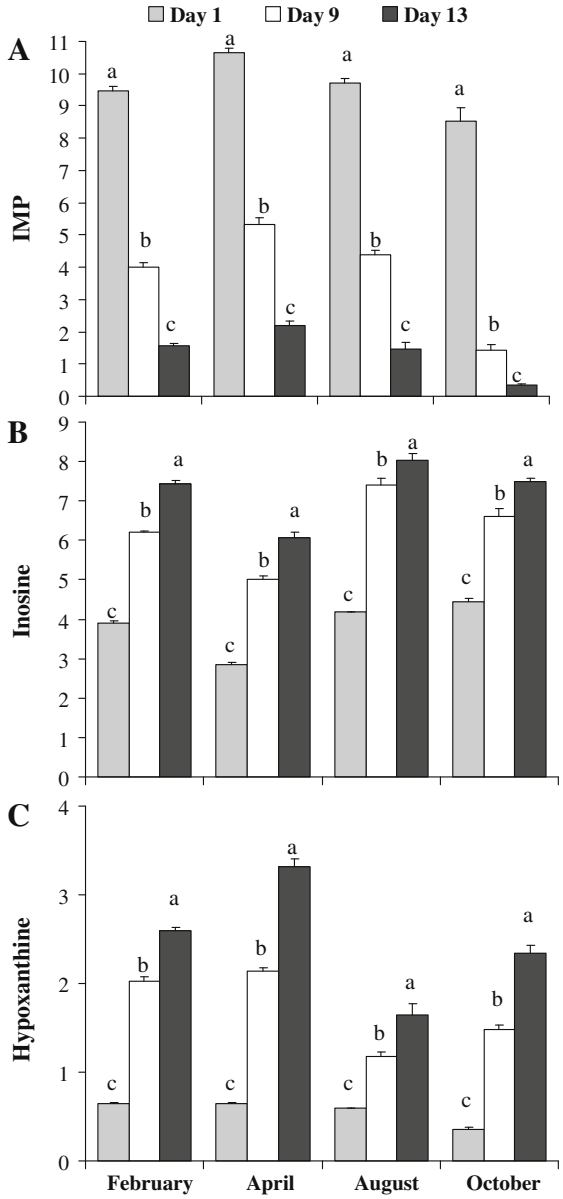

Fig. 1. Development in (A) inosine monophosphate (IMP), (B) inosine, and (C) hypoxanthine during chilled storage (4 °C) of pre-rigor fillets of vacuum packed farmed Atlantic salmon. Results are given as mean ± SE. Different superscripts denote significant variation ($P < 0.05$) during storage within seasonal period.

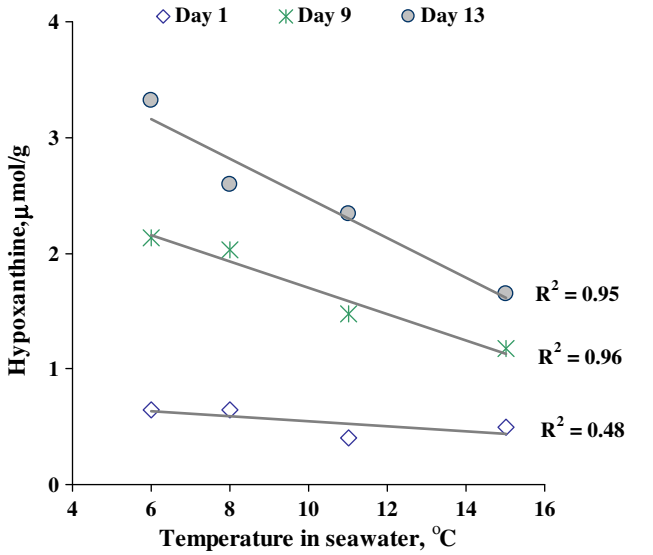

Fig. 2. The relationship between seawater temperature and hypoxanthine content (µmol g^{-1} wet muscle) in vacuum packed pre-rigor fillets of farmed Atlantic salmon. Measurements were performed after 1, 9 and 13 days of chilled storage (4 °C), respectively. Each point represents the mean of 12 individuals.

responsible for spoilage of chilled fish, form an insignificant part of the flora of tropical fish while being the dominant group of bacteria found in fish from colder waters (Huss, 1988). However, variations in seawater temperature at the time of harvesting may also influence the storage life of the same fish species, as linear regression analyses revealed a significant inverse relationship between seawater temperature and the content of Hx in the salmon muscle. The seawater temperature ranged from 6 °C in April to 15 °C in August, and after 9 and 13 days of storage the temperature explained 95–96% of the variation in Hx (Fig. 2). Some chemical changes that occur post-mortem are caused by enzymatic reactions that might take place even before microbiological actions cause any serious changes. Hence, a large decrease in temperature when chilling salmon reared at 15 °C (a decrease of 13 °C) may produce a more pronounced effect on both the microbial growth and the autolytic enzymes, when compared with the effect of chilling salmon harvested at seawater temperatures of 6 °C (a decrease of 4 °C). There is also evidence that cold acclimation enhances the capacity for lipid oxidation in salmonids (Dean, 1969; Guderley & Gawlicka, 1992; Moya-Falcón et al., 2006). It is therefore possible that biological responses to environmental temperature within the living animal may also have altered the enzymic capacity of Hx conversion. Keeping the salmon at 2 °C for 40 min before slaughtering did not seem to influence the impact of the rearing temperature on the Hx accumulation rate.

3.4. K-value

The *K*-value has been used as a freshness index of fish products for decades (Saito et al., 1959). The *K*-values 1-day post-mortem in this study were 30% on average, with a significantly lower value in April (23.9%), and a significantly higher value in October (34.8%). After 9 days of storage, the *K*-value averaged 81.3% in October, whereas the level in April was 55%, and 64% in both February and August. The final *K*-value showed an overall average of 84% after 13 days of storage with the lowest and highest value observed in April (77.9%) and October (92.9%), respectively. The final *K*-value was similar in February and August (83%). There is evidence that the *K*-values of premium quality seafood vary between fish species. For example, Kuda, Fujita, Goto, and Yano (2007) reported a *K*-value of 41% in raw shrimps for sashimi (raw eating), 9% in chub mackerel and 14% yellowfin tuna. A *K*-value of 35% was reported as a limit of acceptability in threadfin bream (Yongswawatdigul & Park, 2002), while for wild turbot a *K*-value of 75–85% was noticed on 15th day of acceptability (Özogul et al., 2005). *K*-values are also highly dependent upon the analytical method used for their determination, as Randell et al. (1999) reported *K*-values 2–3 times greater in salmon fillets when using a HPLC method compared with using a rapid paper strip method. Maximum *K*-values in fish for raw consumption may therefore be misleading and at the very least, the analytical method used for their determination should be specified.

3.5. Drip loss inside the packages

Drip loss increased significantly with storage time for each seasonal period (Fig. 3). This is consistent with a study by Rørå, Regost,

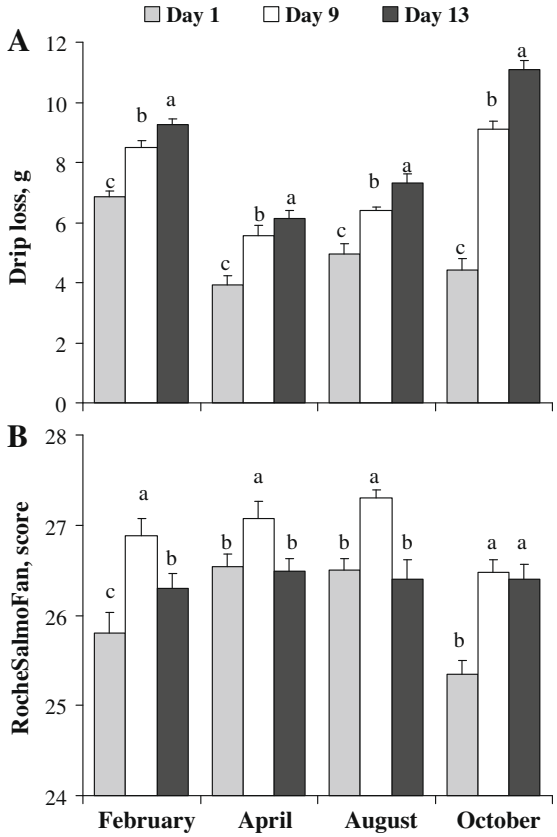

Fig. 3. Development in (A) drip loss, and (B) fillet colour (score) during chilled storage (4 °C) of vacuum packed pre-rigor fillets of farmed Atlantic salmon. Results are given as mean ± SE (n = 12). Different superscripts denote significant variation (P < 0.05) during storage within seasonal period.

and Lampe (2003) in which it was reported that storage time had a particularly strong effect on the liquid holding capacity (LHC) in comparison with other quality properties. Seasonal variations were found in both the initial drip loss and progression throughout the entire storage period. The drip loss on day 1 was lowest in April and October (3.9–4.4 g), and highest in February (6.8 g). The drip loss in April remained lowest throughout the entire storage period, whereas drip loss increased most significantly in October, reaching a final level of 11 g after 13 days of storage (i.e., 2.8% of the muscle weight). Drip loss results in a negative sensory impression, and the lost fluid also developed an unpleasant odour towards the end of the storage period. Furthermore, leaching rates of IMP and degradation products were related to liquid accumulation inside the packages, as the highest increase in drip loss was observed in October coinciding with the highest leaching rate (23.5%). Reduced LHC during storage has been attributed to denaturation of collagen (Ofstad, 1995), and changes in the state of myofibrils, in particular myosin (Mackie, 1993). Even so, efforts should be made to improve knowledge regarding the inherent and underlying factors which promote drip loss in fish. Furthermore, it is essential to develop absorbent pads with an improved ability to retain both water and oil, in order to prevent negative sensory impression by consumers due to unpleasant odour.

3.6. Visual colour score (RocheSalmoFan™)

The visual colour score of the salmon ranged from 25.3 to 27.3 (Fig. 3), thus falling within the upper level of those reported for farmed salmon (Stien, Amundsen, Mørkøre, & Nortvedt, 2006). The mean colour score increased from score 26.0 on day 1 to score 26.9 on day 9. From days 9 to 13, the colour score reached a similar level as that observed on day 1, except in October, where the colour remained stable during the period between days 9 and 13 days. The colour is strongly dependent on pigment concentration (Christiansen, Struksnaes, Estermann, & Torrissen, 1995), however it is also influenced by the muscle structure characteristics. For example Skjervold et al. (2001) reported improved colour in pre-rigor salmon fillets in comparison to their post-rigor counterparts. The favourable increase in colour intensity by 0.5–1.1 score units during the first 9 days of storage may therefore be attributed to altered shape and structure due to rigor contraction. While rigor contraction may have positively influenced the optical properties of the fillets, pigment degradation is a plausible cause for the decreased colour score from days 9 to 13.

The colour score on day 1 was significantly lower in February and October when compared with results from April and August, however colour scores were similar at all seasonal periods on day 13. The results are in concordance with unpublished results (K.-A. Rørvik, unpublished results) which show that fillet pigment in Norwegian salmon declines during the winter, and thereafter increases in response to an increase in temperature and photoperiod. The colour score was determined by image analysis, which has a very high precision (Folkestad et al., 2008). For consumers, however, it is difficult to distinguish between small variations in the colour of salmon fillets, unless side by side comparison is performed (Stien et al., 2006). Therefore, it is questionable that differences between the batches (max range = 1.2 score units) could be distinguished visually, despite the colour variation being statistically significant.

3.7. Consumer test

The odour was assessed as *fresh* to *extremely fresh* 1 day after harvesting, and even after 9 days of storage the odour was considered as *neutral* to *fresh* (Fig. 4). Males and females discriminated the odour similarly according to storage time, but females consistently described more favourable levels of freshness than males. Several studies have demonstrated that olfactory sensitivity towards particular odorants may vary according to gender, whereby women have been reported to perform better than men in certain olfactory tests (Menashe & Lancet, 2006). The present results showed that males were more critical regarding assessment of fresh odour than the females. Even after 13 days of storage, female

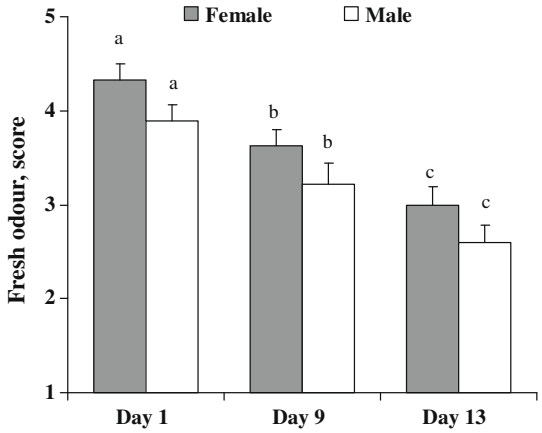

Fig. 4. Fresh odour assessed by female (n = 12) and male (n = 12) consumers during chilled storage (4 °C) of vacuum packed pre-rigor salmon fillets. Results are given as mean ± SE. Different superscripts denote significant variation (P < 0.05) during storage.

consumers assessed the odour as *neutral* whereas the males considered the odour as somewhat *un-fresh*.

The salmon was considered to be of premium quality after 9 days of storage when the Hx content averaged 1.5 μmol g^{-1} (consumer test was performed in October). The Hx content averaged 2.3 μmol g^{-1} after 13 days storage. According to Surette et al. (1988), the Hx content might not be suitable as a freshness indicator per se. Even so, the present results indicate that values $\leqslant$1.5 μmol g^{-1} may indicate extremely fresh quality of raw salmon, whereas values above 2.5 μmol g^{-1} may be considered as critical.

3.8. Texture properties

Food texture covers several related physical properties, including resistance to chewing, tenderness, fibrousness and juiciness. In particular, softness/tenderness has achieved attention as soft flesh tends to correspond to reduced acceptability by both consumers and the processing industry (Mitchie, 2001). Tenderness increased during the storage period, showing an inverse development as compared with chewiness ($r = -0.94$; $P < 0.0001$) (Table 2). However, juiciness was either stable or slightly increasing during storage despite the significant increase in drip loss. Therefore, liquid accumulation in consumer packages appears to be an odour and appearance problem rather than a problem for the sensory texture perception.

Tenderness scores tended to be higher and the score for chewiness tended to be lower on day 1 in April than compared with those obtained during other seasonal periods. Similar variations among different seasonal periods were observed for both the sensory determined tenderness and the instrumental determinations of breaking strength (F_b) ($r = -0.65$; $P = 0.03$) and force–time gradient ($r = -0.73$; $P = 0.01$). However, the variation between seasonal periods was significant when determined instrumentally, with results for breaking strength significantly lower in April and higher in August. Interestingly, the gradient showed a highly significant correlation with juiciness of ($r = -0.83$; $P = 0.002$).

Fillet texture is manifested through a complexity of events in the muscle, where a strong seasonality has been documented. For example Mørkøre and Austreng (2004) reported that seasonal period had a greater impact on texture, gaping and cupper status than dietary treatment during springtime (April vs. May) in adult Atlantic salmon. Several studies have confirmed the influence of carbohydrate dynamics on texture in fish fillets (Foegeding et al., 1996), however the present study showed no significant correlation between muscle pH and sensory assessment of texture, although the gradient determined mechanically correlated positively to the muscle pH ($r = 0.61$; $P = 0.03$). It is assumed that the lack of correlation between sensory texture assessments and pH is partly explained by the low overall variation in pH. Conversely, each of the texture attributes were correlated significantly with IMP, inosine, Hx and the *K*-value (Table 3). The correlation was consistently highest for Hx, except for chewiness which showed the highest correlation with the *K*-value. The highest correlation coefficient was seen between Hx and coarseness and Hx and tenderness, where the explanation was 87% (Table 3; Fig. 6). Therefore, Hx seems to be a valuable biochemical marker for predicting texture of chilled raw salmon. Endogenous enzymes are responsible for Hx accumulation in addition to some bacterial enzymes (Surette et al., 1988). Further studies may reveal whether there is a relationship between activity of enzymes responsible for the formation of Hx and the activity of enzymes involved in muscle degradation of salmon, for example cathepsins (Bahuaud et al., 2010). Whether autolytic activity is higher in fast growing fish with a rapid metabolic turnover is another issue to be elucidated.

3.9. Odour and flavour assessed by a trained sensory panel

Fish aroma, divided into flavour and odour, has been regarded hard to define and quantify as the sensations are so highly related

Table 2
Development in texture properties assessed by a sensory panel (scores 1–9) and instrumentally (N) during chilled storage (4 °C) of raw muscle of pre-rigor filleted Atlantic salmon.

	February	April	August	October
Sensory assessment				
Chewiness, score				
Day 1	$3.46 \pm 0.18^{a,x}$	$3.21 \pm 0.19^{a,x}$	$3.34 \pm 0.21^{a,x}$	$3.47 \pm 0.23^{a,x}$
Day 9	$2.85 \pm 0.19^{b,x}$	$2.85 \pm 0.17^{ab,x}$	$3.02 \pm 0.18^{ab,x}$	$2.70 \pm 0.21^{b,x}$
Day 13	–	$2.66 \pm 0.19^{b,x}$	$2.71 \pm 0.19^{b,x}$	$2.53 \pm 0.16^{b,x}$
Fibrousness, score				
Day 1	$2.51 \pm 0.25^{a,x}$	$2.49 \pm 0.17^{a,x}$	$2.63 \pm 0.26^{a,x}$	$2.78 \pm 0.24^{a,x}$
Day 9	$2.18 \pm 0.22^{a,x}$	$2.13 \pm 0.17^{b,x}$	$2.31 \pm 0.20^{ab,x}$	$2.33 \pm 0.24^{ab,x}$
Day 13	–	$1.97 \pm 0.19^{b,x}$	$2.15 \pm 0.20^{b,x}$	$2.10 \pm 0.17^{b,x}$
Juiciness, score				
Day 1	$4.20 \pm 0.17^{b,x}$	$4.98 \pm 0.17^{a,x}$	$4.53 \pm 0.24^{b,x}$	$4.72 \pm 0.17^{a,x}$
Day 9	$5.23 \pm 0.15^{a,x}$	$5.17 \pm 0.28^{a,x}$	$5.04 \pm 0.14^{a,x}$	$4.84 \pm 0.34^{a,x}$
Day 13	–	$5.41 \pm 0.35^{a,x}$	$4.84 \pm 0.29^{ab,x}$	$4.95 \pm 0.35^{a,x}$
Tenderness, score				
Day 1	$5.78 \pm 0.22^{b,x}$	$5.89 \pm 0.17^{b,x}$	$5.58 \pm 0.26^{b,x}$	$5.64 \pm 0.21^{b,x}$
Day 9	$6.29 \pm 0.21^{a,x}$	$6.50 \pm 0.25^{a,x}$	$6.15 \pm 0.22^{ab,x}$	$6.33 \pm 0.27^{a,x}$
Day 13	–	$6.80 \pm 0.30^{a,x}$	$6.47 \pm 0.27^{a,x}$	$6.76 \pm 0.24^{a,x}$
Instrumental analyses				
Breaking strength (N)				
Day 1	$9.27 \pm 0.46^{a,yz}$	$8.25 \pm 0.32^{a,z}$	$11.43 \pm 0.71^{a,x}$	$10.00 \pm 0.54^{a,y}$
Day 9	$7.35 \pm 0.25^{b,yz}$	$7.01 \pm 0.24^{b,z}$	$8.25 \pm 0.33^{b,y}$	$10.30 \pm 0.43^{a,x}$
Day 13	$7.95 \pm 0.66^{b,x}$	$6.76 \pm 0.32^{b,y}$	$8.54 \pm 0.58^{b,x}$	$8.90 \pm 0.50^{b,x}$
Gradient (N s^{-1})				
Day 1	$1.51 \pm 0.13^{a,y}$	$1.03 \pm 0.07^{a,z}$	$2.09 \pm 0.07^{a,x}$	$1.57 \pm 0.12^{a,y}$
Day 9	$0.79 \pm 0.04^{b,y}$	$0.69 \pm 0.04^{b,y}$	$0.85 \pm 0.05^{b,x}$	$1.27 \pm 0.08^{b,x}$
Day 13	$0.83 \pm 0.07^{b,y}$	$0.66 \pm 0.05^{b,z}$	$0.98 \pm 0.07^{b,xy}$	$1.04 \pm 0.05^{c,x}$

The data represent mean ± SE of 12 salmon. Significant differences ($P < 0.05$) between means in the same column are indicated with different superscript a and b, and between means in the same line with different superscripts x, y, and z.

Table 3
Overall correlations between ATP breakdown products and various quality related characteristics of farmed Atlantic salmon.

	IMP	Inosine	Hypoxanthine	*K*-value
Sensory analyses				
Odour				
Fermented	−0.71	ns	0.90	0.73
Rancid	−0.62	0.59	0.75	0.63
Seawater	0.82	−0.70	−0.87	−0.82
Fresh	0.88	−0.76	−0.90	−0.88
Flavour				
Bitter	−0.70	0.71	0.66	0.71
Seawater	0.80	−0.60	−0.84	−0.81
Fresh	0.85	−0.66	−0.91	−0.81
Texture				
Courseness	−0.80	−0.67	−0.93	−0.80
Chewiness	0.92	−0.77	−0.83	−0.93
Tenderness	−0.88	0.73	0.93	0.88
Juiciness	ns	ns	0.80	ns
Instrumental texture analyses				
Breaking strength	ns	ns	−0.69	ns
Gradient	0.58	−0.55	−0.64	ns
Drip loss	ns	ns	0.62	ns
Fillet colour	ns	ns	ns	ns

$P < 0.05$ for all correlations shown; non-significant correlations are denoted as "ns".

(Shamil & Birch, 1990). The present study showed significant variation in both odour and flavour between storage times, where the highest numerical variation was observed for fermented odour (score 1.7–5.7) (Fig. 5). From days 1 to 9 the fermented odour showed a generally non-significant increase by an average of 0.6 score units; however, from days 9 to 13, the increase was significant at all harvesting times (two score units on average). Rancid odour also increased during the storage period, with the highest numerical value for rancid odour recorded in February. The seawater and fresh odour decreased significantly from days 1 to 9 in April, and fresh odour also decreased significantly during the same period in February. Both seawater and fresh odour decreased significantly after 13 days of storage at all seasonal periods. Therefore there appears to be a non-linear relationship in the development of

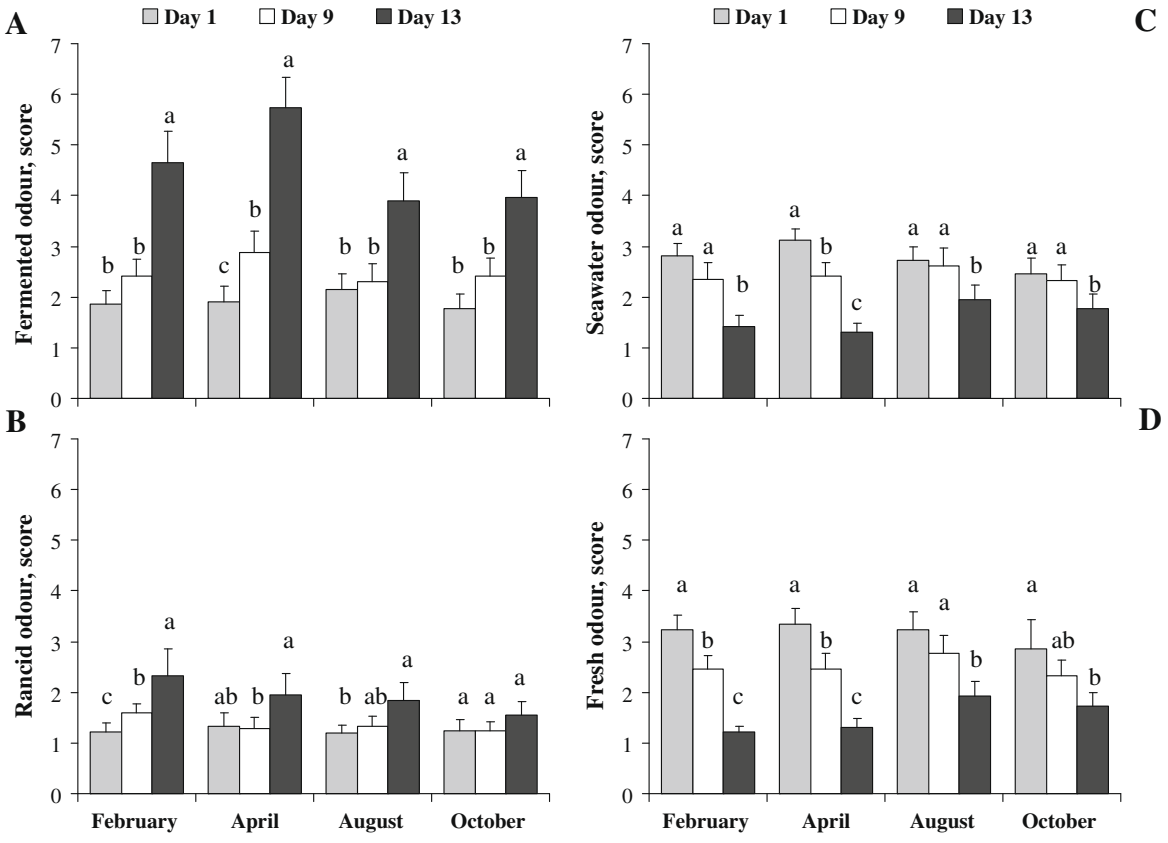

Fig. 5. Development in (A) fermented odour, (B) rancid odour, (C) seawater odour, and (D) fresh odour during chilled storage (4 °C) of pre-rigor fillets of vacuum packed farmed Atlantic salmon. Results are given as mean ± SE ($n = 12$). Different superscripts denote significant variation ($P < 0.05$) during storage within seasonal period.

Table 4
Development in flavour attributes (sensory scores 1–9) during chilled storage (4 °C) of raw muscle of pre-rigor filleted salmon slaughtered at different times of the year.

	February	April	August	October
Bitter, score				
Day 1	$3.75 \pm 0.28^{a,x}$	$3.00 \pm 0.26^{c,y}$	$4.05 \pm 0.33^{a,x}$	$3.71 \pm 0.24^{b,x}$
Day 9	$3.83 \pm 0.29^{a,x}$	$3.79 \pm 0.33^{b,x}$	$4.33 \pm 0.32^{a,x}$	$3.92 \pm 0.39^{ab,x}$
Day 13	–	$4.67 \pm 0.38^{a,x}$	$4.36 \pm 0.32^{a,x}$	$4.30 \pm 0.34^{a,x}$
Seawater, score				
Day 1	$2.92 \pm 0.25^{a,x}$	$3.10 \pm 0.22^{a,x}$	$2.95 \pm 0.35^{a,x}$	$2.79 \pm 0.31^{a,x}$
Day 9	$2.70 \pm 0.34^{a,x}$	$2.68 \pm 0.30^{a,x}$	$2.97 \pm 0.40^{a,x}$	$2.33 \pm 0.33^{ab,x}$
Day 13	–	$1.53 \pm 0.22^{b,x}$	$2.18 \pm 0.30^{b,y}$	$1.86 \pm 0.30^{b,xy}$
Freshness, score				
Day 1	$3.34 \pm 0.28^{a,x}$	$3.33 \pm 0.30^{a,x}$	$3.34 \pm 0.41^{a,x}$	$3.31 \pm 0.30^{a,x}$
Day 9	$2.73 \pm 0.30^{a,x}$	$2.72 \pm 0.35^{b,x}$	$3.13 \pm 0.44^{a,x}$	$2.53 \pm 0.37^{b,x}$
Day 13	–	$1.48 \pm 0.23^{c,y}$	$2.26 \pm 0.37^{b,x}$	$1.78 \pm 0.29^{c,xy}$

The data represent mean ± standard error of 12 salmon. Significant differences ($P < 0.05$) between means in the same column are indicated with different superscript a, b, c and between means in the same line with different superscripts x, y, and z.

unpleasant odours and loss of fresh odours during the storage period, with acceleration after 9 days of storage. Similarly, Hansen et al. (2009) observed a significant increase in unpleasant odours after 8 days of storage (1 °C) of vacuum packed raw salmon. These authors reported a concomitant shift in bacterial counts at log 5–6 cfu/g when development of unpleasant odours accelerated, indicating that off-odour may be a valuable indicator of bacterial counts.

Seawater and fresh flavour decreased whereas bitter flavour increased during the storage period of 13 days for all seasonal periods, except in August where the variation in bitter flavour was non-significant (Table 4). From days 1 to 9 there was also a decrease in fresh flavour in April and October. No flavour attribute showed significant variation in February (only analysed days 1 and 9 post-mortem).

It is believed that IMP contributes to the pleasant, fresh flavour of meat (Howgate, 2006), and that it enhances the intensity of fish flavour (Bremner, Olley, Statham, & Vail, 1988). Alternatively, the accumulation of Hx is believed to be involved in the progressive loss of desirable fresh fish flavour, such as bitter off-taste (Foegeding et al., 1996). Results from the present study showed significant positive correlations between the IMP content and the pleasant aroma attributes (fresh and seawater), whereas the correlation was negative with the unpleasant aroma attributes (fermented, rancid, and bitter). An inverse and stronger correlation was observed with Hx (Table 3), whereas no further explanation of the variation in flavour and odour was obtained by the *K*-value. The loss of pleasant aroma properties during storage was therefore most significantly attributed to the accumulation of Hx which explained 81% and 83% of the variation in fresh odour and flavour, respectively (Fig. 6). The formation of Hx may result from a combined degradation by endogenous and bacterial enzymes, however there is a need for improved knowledge regarding the correlation between formation of Hx and other deteriorating processes contributing to a decline in sensory quality.

Regardless, the Hx level appears to be a suitable indicator of the overall spoilage during storage of raw vacuum packed salmon. On day 9, the consumers rated the odour as *neutral* to *super-fresh* (determined in October). At this time, the Hx content averaged 1.5 μmol g^{-1} whereas the scores for fresh odour and flavour, assessed by the trained panel, averaged 1.8 for both properties. The relationship between seawater temperature and the Hx content on days 9 and 13 post-mortem corresponds to the equations $Hx = -0.11x\ °C + 2.8$ and $Hx = -0.17x\ °C + 4.2$, respectively. Calculations according to these equations indicate that salmon harvested in April (sea temperature 6 °C) will reach the upper limit as super-fresh before 9 days of storage, whereas salmon harvested in August (sea temperature 15 °C) will reach the same limit after 13 days of storage. From these results there is evidence to suggest that Norwegian salmon maintain sensory quality longer post-mortem when they are harvested during the summer–autumn period than during winter–spring.

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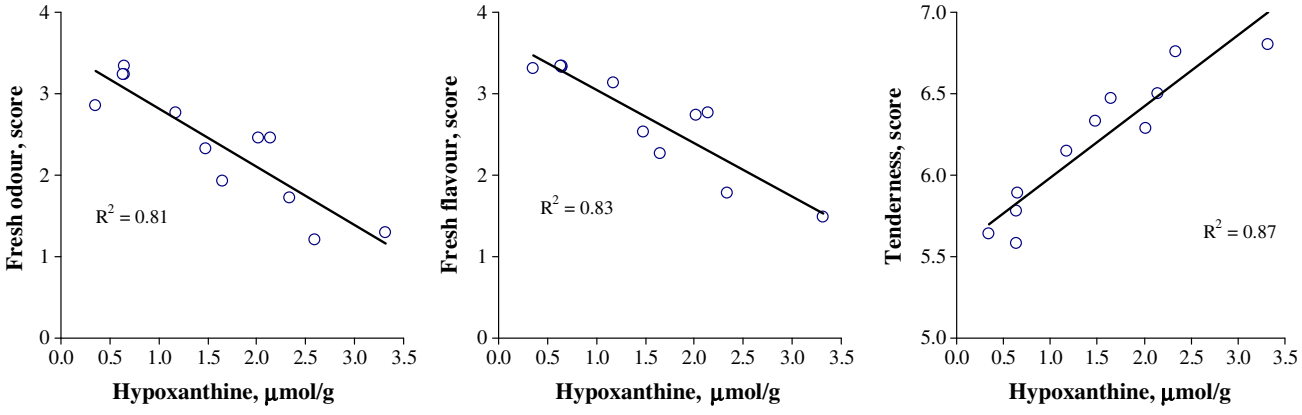

Fig. 6. Overall relationship between hypoxanthine content (μmol g^{-1} muscle) and sensory assessment (score) of (A) fresh odour, (B) fresh flavour, and (C) tenderness. Each point is the average of 12 determinations.

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