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The effect of liquid smoking of fillets of trout (*Salmo gairdnerii*) on sensory, microbiological and chemical changes during chilled storage

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Abstract

The storage time at 4 ± 1 °C of liquid smoked fillets of trout (*Salmo gairdnerii*) produced with a new smoking technique, using a combination of liquid smoke and steaming at 2 bar pressure for 30, 45 and 60 min, was studied. Maximum total viable counts (TVC) were reached after 25 days in the samples processed for 30 min and after 48 days in those processed for 45 and 60 min. However, panellists rejected the samples long after maximum TVC was observed. The increase of TVC was also confirmed using a particle size analyser, indicating a possible direct detection of microbial growth. A reduction of about 50% in the C22:6*n* − 3/C16:0 ratio was found across the whole period of storage, indicating lipid oxidation. The hypoxanthine/inosine (Hx/Ino) ratio showed a good relationship with both TVC and sensory results. Thus, when the Hx/Ino ratio was higher than 1.3, the products had reached their maximum acceptable TVC and were approaching their rejection time by the panellists, indicating that the Hx/Ino ratio is a good indicator of the shelf-life of smoked products of trout (*S. gairdnerii*).

Keywords: Liquid smoked; Hx/Ino ratio; Pressure; TVC; Trout fillets; Particle size analyser

1. Introduction

The shelf-life of smoked fish products depends largely on the initial bacterial contamination of the raw material; on the decrease of a_w in the tissues due to brining and pre-drying, on the inactivation of putrefactive microflora due to the heat treatment, on the amount of smoke components that penetrate the product, and on the temperature, air humidity, and oxygen levels during storage (Sikorski, Haard, Motohiro, & Bonnie, 1998).

No relationship between the shelf life of smoked salmon and total numbers of bacteria has been found (Gibson & Ogden, 1986; Hansen, Gill, & Huss, 1995), which makes the use of any criteria based on total viable counts irrelevant as a quality parameter. It was found that immediately after cold-smoking of salmon fillets the aerobic plate count was 3×10^3cfu/g. The maximum acceptable microbial count has been reported 6 days after smoking but spoilage of the product was only detected by panellists after 2–3 weeks (Leroi, Jorffaud, Chevalier, & Cardinal, 1998). The authors also reported that although the aerobic plate count reached maximum acceptable levels after 6 days, the product was not completely spoiled until after 2–3 weeks of storage, despite the fact that spoilage signs were observed at 13 days. It was also reported that fish smoked for 3 days at 50–65 °C to a moisture content of 14% and stored at 79–85% relative humidity, remained at an acceptable quality for 6 months.

Leroi, Joffraud, Chevalier, and Cardinal (2001) reported that the shelf-life of cold smoked salmon was very variable (1–6 weeks) and was related to the initial Enterobacteriaceae load, which was dependant on hygienic conditions in the smokehouse. High correlations existed between the remaining shelf-life and lactobacilli count, yeast count and total volatile base (TVBN) concentration.

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Leroi and Joffraud (2000) also reported that the ability of the process to preserve fish and other products is due to the synergistic action of salting and smoking procedures. Researchers have noticed that salt concentration is the primary factor affecting the storage life of the fish product. Apart from brining time, smoking temperature and time play a key role in preservation.

Hot smoking at temperatures up to 120 °C or higher is much more effective than cold smoking, where the temperature should not rise above 30 °C. All gram-positive *cocci* predominant in smoked fish are very sensitive to heat (Pigott & Tucker, 1990).

Among potential chemical quality indicators, the development of total volatile bases (TVB-N) (Cann et al., 1984), the production of trimethylamine (TMA), the increase in volatile acids (Hansen et al., 1995), the degradation of nucleotides expressed as an increase in hypoxanthine (Cann et al., 1984; Hansen et al., 1995; Martin, Gray, & Pierson, 1978) and the oxidation of lipids (Zotos, Petridis, Siskos, & Gougoulias, 2001) have been considered. However, the origins of these chemical changes and their relationship to product quality are not well established, making their use uncertain as objective quality indicators.

Accurate shelf life information reassures consumers and enhances the quality of the specific product (Sikorski et al., 1998).Thus, the aim of this work was to assess the storage time at 4 ± 1 °C of liquid smoked trout fillets processed for 30, 45 and 60 min at 2 bar pressure through total viable count (TVC), microbial changes (using a particle size analyser), lipid oxidation, changes in adenosine triphosphate (ATP) degradation products and sensory analysis.

2. Materials and methods

2.1. Analytical reagents

Inosine (Ino), hypoxanthine (Hx) and fatty acid methyl esters (FAME) standards were purchased from Sigma Chemical Co. Ltd. (Poole, UK). All reagents used were analytical grade.

2.2. Smoking process

Farmed trout (*Salmo gairdnerii*) (24) samples was purchased from Ioannina, Greece. Immediately after arrival, fish samples were stored in ice at 0–1 °C. The average weight of the samples was 900 g to 1 kg. About 20 h after harvesting the fish were eviscerated, their heads were removed and the bodies brined in 20% sodium chloride solution for 2 h. The brine temperature was kept low (5 °C) in order to minimize microbiological contamination. Consequently, all samples were filleted and placed in drying racks for 16 h. The smoking process was conducted using a 10 L electrical commercial steamer, electronically modified in the workshop to improve its precision. Liquid smoke condensate 40 ml; (Nefeloudis SA) was added to the steamer and diluted in 2 l of tap water (2%) with a final temperature of 20 ± 2 °C. Thereafter, the smoking process continued, due to the smoky steam produced from the liquid smoke solution. Samples were placed on one level with the flesh facing the smoke liquid and processed at three different processing times: 30, 45 and 60 min. Processing time was measured from the moment the required pressure in the steamer was achieved (approximately 10–12 min). The applied steam pressure was 2 bar and the resulting temperature 115 °C. The above conditions were chosen from previous work in which these processes were found to produce high quality smoked products (Siskos, Zotos, & Taylor, 2005). After each process the liquid smoke solution was discarded and a new solution was used. After cooling the samples to room temperature, under sanitary conditions, the skin was removed from the fillets. The front part (6–8 cm) from each fillet was removed carefully, packed in polypropylene bags, stored at 4 ± 1 °C and used for sensory analysis. The remainder of the fillets was homogenised at low temperature, in order to avoid possible oxidation, mixed thoroughly and stored in polyethylene bags at 4 ± 1 °C until further analysis (Siskos et al., 2005).

2.3. Sensory analysis

Five experienced panellists, who were members of the academic staff, were used to assess the quality of trout fillets during storage. The panellists were asked to estimate firmness, smoked flavour and the presence of external moisture on the samples tested. The evaluation took place in separate booths under daylight illumination. A quality scale from 1 to 6 was used. A score of 6 was defined as extremely low firmness, intensely smoked flavoured and negligible presence of external moisture. Finally, according to their evaluation on the above variables, the panellists were asked to characterise the products as acceptable for consumption, border-line quality or unacceptable for consumption. When a sample was characterised as unacceptable by 3 out of the 5 panellists, this sample was excluded from further investigation. Sensory analysis was repeated 4 times.

2.4. ATP degradation compounds

The ATP-related compounds were determined by the method of Veciana-Norgues, Marine-Font, and Vidal-Carou (1997). The extraction procedure was performed at 4 °C. Homogenised sample (10 g) was accurately weighed into a 50 ml centrifuge tube. Perchloric acid (15 ml, 0.6 M) and a magnetic stirring bar were added. The mixture was thoroughly mixed for 10 min on a magnetic stirring plate, centrifuged for 10 min at 3000 rpm and left to separate into two phases. Perchloric acid (0.6 m, 10 ml) was added to the solid residue obtained, mixed thoroughly for 10 min and the centrifugation was repeated. After centrifugation the solid phase was discarded. The two perchloric extracts were combined a in 25 ml volumetric flask and made up to volume with 0.6 M $HClO_4$. An ali-

quot of 10 ml was adjusted to pH 6.5–6.8 with 0.1 N potassium hydroxide and left to stand for 30 min at 4 °C. Then, the aliquot was filtered to remove potassium perchlorate and made up to 25 ml with phosphate buffer (pH 7.0). The neutral extracts were finally filtered through a 0.45 μm filter before HPLC analysis using an HVLP 1300 membrane.

Mobile phase A was 0.04 M potassium dihydrogen orthophosphate and 0.06 M dipotassium hydrogen orthophosphates, adjusted to pH 7.0 with 0.1 M potassium hydroxide.

Mobile phase B was methanol.

The column used was an Ultrasphere C18, (5 μm, 250 × 4.6 mm).

The flow rate of mobile phase was 1 ml/min. The analysis was performed using a gradient program as follows:

Time (min)	0	8	9	13	0
Mobile phase A (%)	100	100	70	70	100
Mobile phase B (%)	0	0	30	30	0

2.5. Total lipid, peroxide value (PV) and FAME profiles

Total lipid content was determined by the Bligh and Dyer (1959) method, as modified by Hanson and Olley (1963). Fatty acids methylesters (FAME) were made using the method of Zotos, Hole, and Smith (1995). The method used for peroxide value was adapted from that of Lea (1952).

2.6. Total viable counts

Ten gram of each sample homogenate was diluted to 100 ml with peptone (1% in water) buffered at pH 7.5. Serial dilutions were made until 10^{-7}g/ml samples were obtained. A 1 ml aliquot of each dilution was placed in a petri dish and approximately 15 ml plate agar count was added. Each petri dish was carefully shaken, in order to achieve a homogeneous distribution of the sample. After several minutes all petri dishes were inverted and placed in an oven at 37 °C for 48 h. After 48 h of incubation, all colonies were counted, following the rules reported by Gilliland, Busta, Brinda, and Campbell (1976).

2.7. Detection of microbial changes using the Mastersizer 2000 particle size analyser

The microbial contamination due to storage was also assessed with the measurement of the particles in the samples, using the Mastersizer 2000 particle size analyser equipment. The instrument's capability has a range from 20 μm to 2000 μm. A portion of each sample (10 g) with 100 ml of distilled water was placed in a stomacher bag and treated for 1 min. The treated sample was further diluted with 900 ml of distilled water. The solution was filtered using a filter paper, in order to discard all large particles. A 600 ml portion of diluted sample was measured. Care was taken to avoid protein aggregation and thus the samples were agitated before their measurement. Each measurement was repeated six times, in order to confirm repeatability. This analysis was performed on the samples processed for 60 min and stored for 0, 25 and 62 days.

3. Results and discussion

The chemical composition of the samples was relatively stable: 68.5–69.8% moisture, 22.4–24.2% protein and 5.5–7.8% lipid, indicating that small changes should be probably expected from farmed fish.

3.1. Sensory changes due to storage

3.1.1. Firmness

The firmness of all samples was assessed as being quite low [values above the average (3) of the structured scale used] at 14 days of storage (Fig. 1). However, as the storage time increased, the products became more firm, according to the panellists. The highest increasing rate of firmness (decreasing rate of values) was observed in the samples processed for 60 min at 2 bar pressure. The trout fillets processed for 30 and 45 min at 2 bar pressure showed almost the same rate of increase of firmness during the 50 days of storage. At the same storage period, samples processed for 60 min reached the highest values in firmness (Fig. 1). At the rejection time (62 days of storage) of the samples processed for 30 and 45 min, a slight decrease in firmness was observed. A similar decrease in firmness was also observed in the samples processed for 60 min, but their firmness remained higher than the other products. However, the trout fillets processed for 60 min lost their firm-

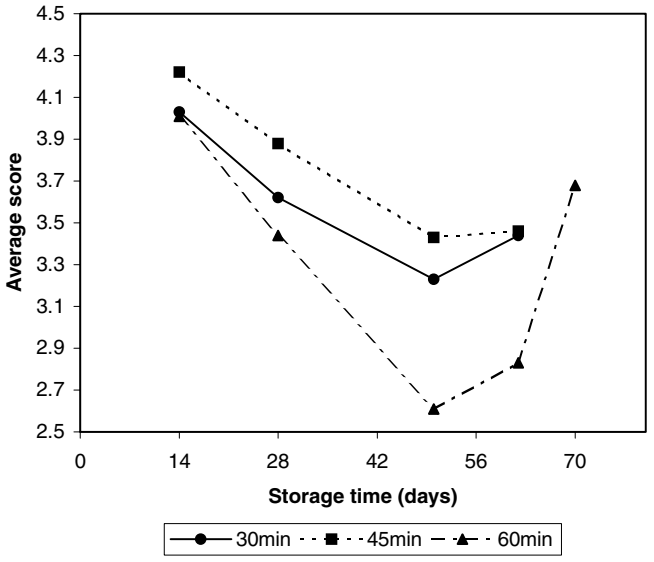

Fig. 1. Average score of firmness during storage of the trout fillets at 4 ± 1 °C. (Data are means of triplicate determinations.)

ness after 70 days storage (Fig. 1). These results indicate that protein changes occurred during storage and this was more obvious in the trout fillets processed for 60 min. They also indicate possible enzymatic protein decomposition at the end of the storage time of the products. Changes in firmness due to storage were also reported by Leroi et al. (1998).

3.1.2. Smoked flavour

The smoked flavour intensity changed in two out of the three samples examined with storage time (Fig. 2). An increase in smoked flavour intensity in the samples processed for 45 and 60 min was observed between 14 and 28 days of storage. This strong smoked flavour remained nearly constant until 50 and 62 days of storage in the trout fillets processed for 45 and 60 min respectively, thereafter a decline in smoked flavour was observed and off–flavours were detected by the panellists. It should also be noted that the smoked flavour intensity remained stable and at nearly average levels in the samples processed for 30 min throughout the entire storage period. This was probably due to the low smoked flavour intensity that these samples showed, even at the beginning of the storage time (Fig. 2).

Microbiological contamination is reported as the main factor producing off-flavours and off-odours. Development of off-flavours and odours during storage time was also reported by Lyhs et al. (2001). They reported that freshly produced "gravad" rainbow trout fillets were judged by the panellists as firm with fresh odour, and fresh rich taste, but at the rejection time the samples were described as fruity, bitter, fishy or neutral off-taste and ammonia off-odour.

3.1.3. Presence of surface moisture

The presence of external surface moisture as assessed by the panellists was almost the same in the samples processed for 60 and 45 min in the first 14 days (Fig. 3). However, the

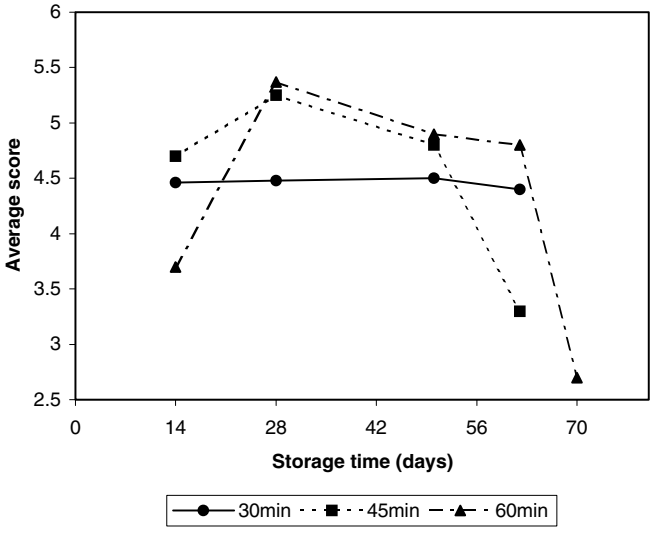

Fig. 2. Average score of smoked flavour intensity during storage of the trout fillets at 4 ± 1 °C. (Data are means of triplicate determinations.)

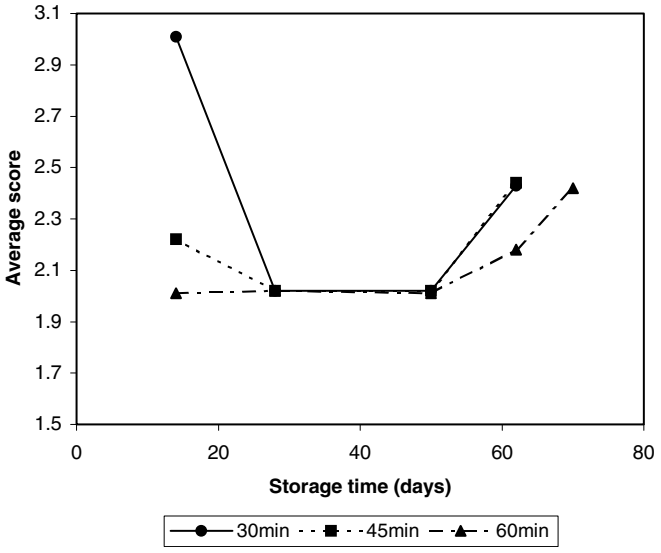

Fig. 3. Average score of external moisture presence during storage of the trout fillets at 4 ± 1 °C. (Data are means of triplicate determinations.)

samples processed for 30 min showed significantly higher external surface moisture in the same storage period. After 28 days of storage, according to the panellists, all samples had the same external surface moisture. It remained at the same level on the samples processed for 45 and 60 min but dramatically decreased on the samples processed for 30 min.

The external moisture did not show any changes during the next 22 days (50 days total storage) on all samples assessed. At the rejection time of the first two samples (62 days of storage and processed for 30 and 45 min,) the presence of surface moisture increased. However, the samples processed for 60 min showed the lowest increase compared with the other two rejected samples (Fig. 3). Finally, after 70 days of storage, the fillets processed for 60 min showed a further increase in external surface moisture, reaching the values of the other two already rejected samples. These results also indicate protein changes due to storage of the smoked trout fillets.

3.1.4. Total viable counts (TVC)

After processing, the TVC in all samples were at low levels with an average of 5.3×10^3 cfu/g, regardless of the processing time. The microbiological flora remained stable in all samples for 14 days of storage. The same initial TVC were also observed by Lyhs et al. (2001).

After 25 days of storage at 4 ± 1 °C a striking increase in microbial count was detected in the samples processed for 30 min (Fig. 4). The TVC reached at a value of 1.8×10^6 cfu/g, indicating spoilage and possible rejection of the samples (Liston & Matches, 1976). However, these samples, which were simultaneously assessed by the panellists, were not organoleptically characterised as spoiled. After 48 days of storage at 4 ± 1 °C, the TVC of the samples processed at 30 min remained stable at high levels (7×10^6 cfu/g). Additionally, high levels of microbial contamination were also

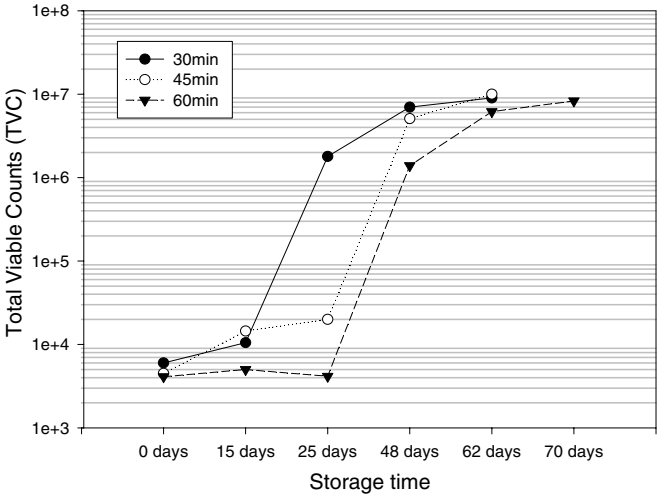

Fig. 4. Changes in TVC of the trout fillets during storage at 4 ± 1 °C. (Data are means of triplicate determinations.)

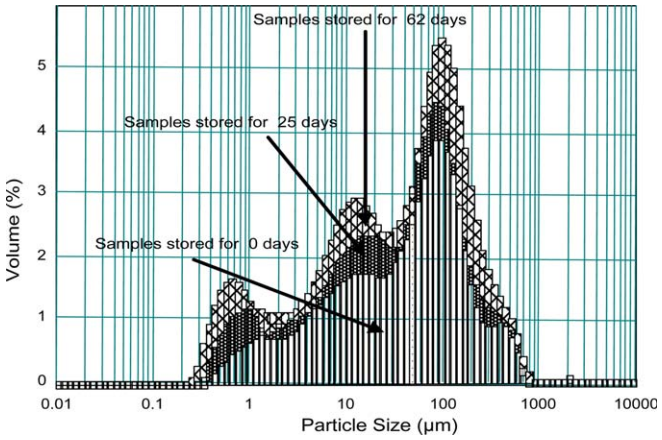

Fig. 5. Particle size differences between samples extracts stored at 4 ± 1 °C for 0, 25 and 62 days. (Data are means of six fold determinations.)

observed in the samples processed either for 45 min (5.1×10^6 cfu/g) or for 60 min (1.4×10^6 cfu/g), indicating that the storage time had reached its maximum and that the trout fillets should be rejected. However, the samples were also not rejected by the panellists. Rejection by the panellists of the samples processed at 30 and 45 min at 2 bar pressure occurred after 62 days of storage at 4 ± 1 °C, despite the fact that the maximum microbiological growth had already been reached 14 days earlier in the samples processed for 45 min and at 37 days in the samples processed for 30 min. These results indicate that spoilage sensory characteristics are generally microbiological products but their production reaches detectable levels long after maximum acceptable microbial growth. In the same period of time the microbial contamination of the samples processed for 60 min was 6.1×10^6 cfu/g and the samples were assessed as still acceptable products by the panellists. Similar results were also found by Gram and Huss (1996) investigating the maximum storage life of cold smoked salmon. Despite the fact that maximum aerobic plate count was reached after one week of storage, the product was characterised as spoiled after 2–3 weeks storage.

The trout fillets processed for 60 min at 2 bar pressure were characterised as unacceptable for consumption by the panellists after 70 days of storage, 22 days after the maximum acceptable TVC was found. These maximum TVC levels were also observed by other authors (Dondero, Cisternas, Carvajal, & Simpson, 2004; Gram & Huss, 1996; Lyhs et al., 2001).

3.1.5. Estimation of total microbial counts using the particle size analyzer (Mastersizer 2000)

As can be seen from Fig. 5, the total amount of microorganisms detected increased as storage time increased. The most characteristic change occurred in the samples stored for 62 days whereat a higher microbial count was measured. These results confirm the accumulation of microorganisms observed during storage at 4 ± 1 °C of the processed fillets and possibly introduces a new technique to detect microbial changes very quickly. Catte, Gancel, Dzierzinski, and Tailliez (1999) measured optical density (OD) at 600 nm using a Uvikon 940 spectrophotometer and reported that OD was significantly affected by liquid smoke and salt concentration. OD increased while salt and liquid smoke concentrations decreased. These results also indicate that microbiological growth affects optical density and it could be used as an indicator for microbiological spoilage.

3.2. Peroxide value (PV)

The storage time had little effect on lipid oxidation, as monitored by peroxide value (PV). Particularly, the peroxide value ranged between 15.6 meq O_2/kg and 22.5 meq O_2/kg in all samples at the beginning and at the end of storage respectively. Additionally, PV had an almost constant, increasing trend during the entire storage period in all samples, regardless of the processing time. In contrast, Medina, Satue-Gracia, German, and Frankel (1999) reported that lipid oxidation of tuna samples, as measured by PV, had a higher rate during the first 24 h at 40 °C while after this period, PV decreased significantly as a result of peroxide decomposition. Much higher levels of PV was reported by Zotos et al. (1995), indicating quite extensive lipid oxidation (PV 108 meq O_2/kg) in smoked mackerel that have been previously stored frozen.

3.3. Detection of lipid oxidation due to storage via changes in C22:6ω − 3/C16:0 ratio

The storage time significantly affected the C22:6 $n-3$/C16:0 ratio, which showed a decreasing trend, due to storage time and ranged between 0.33 and 0.15 at the beginning and at the end of storage respectively (Fig. 6). This is in contrast with the results of Rora, Regost, and Lampe (2003), who failed to detect any significant changes in fatty

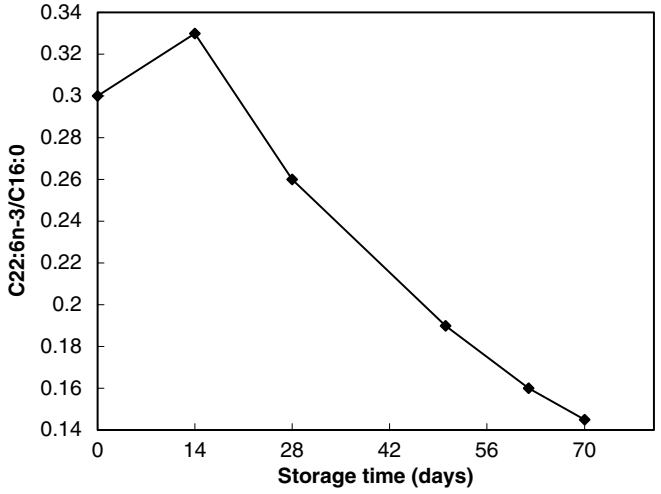

Fig. 6. Effect of storage time on C22:6*n* – 3/C16:0 ratio in all samples regardless the processing conditions. (Data are means of triplicate determinations.)

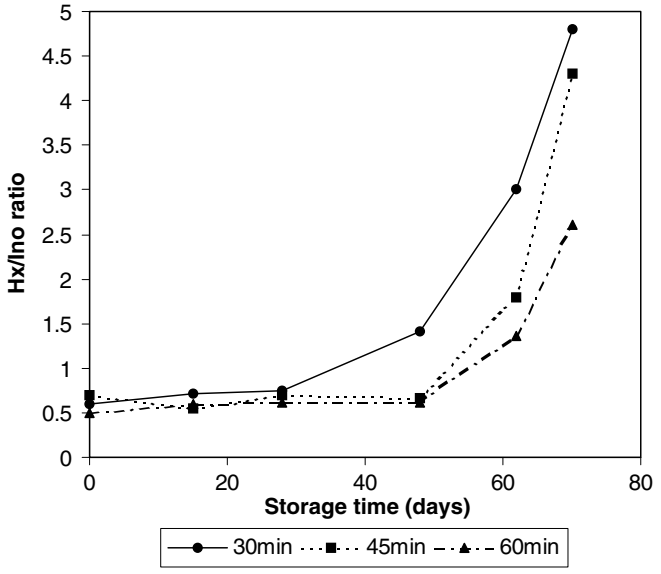

Fig. 7. Changes of hypoxanthine/inosine ratio in the trout fillets during storage at 4 ± 1 °C. (Data are means of triplicate determinations.)

acid profiles due to storage or temperature in salted and smoked salmon fillets. These results indicate an increasing rate of lipid oxidation, which was followed by the degradation of polyunsaturated fatty acids. The lipid oxidation rate as measured using the present index slightly increased (10%) in the first 14 days of storage. However, after 25 days of storage the decline of the index was 14.3% and reached 50% by the end of storage in all samples, regardless of the processing conditions (Fig. 6). It was accordingly reported that chicken frankfurters enriched with fish oil showed thiobarbituric acid (TBA) values higher than the control stored for 30 days. It was concluded that this was due to higher percentage of unsaturated fatty acids (Hong, Leblanc, Hawrysh, & Hardin, 1996).

3.4. Effects of storage on Hypoxanthine/Inosine (Hx/Ino) ratio

In this work the Hx/Ino ratio was investigated because a relationship in their changes was observed. Thus, it was found that the Hx/Ino ratio was affected by the storage period. In all samples tested, Hx production increased due to microbiological activity throughout the entire time of storage. The tested ratio of Hx/Ino remained practically stable for the first 48 days of storage for the samples processed for 60 and 45 min. This indicates low microbiological activity, which held back the degradation rate of inosine to hypoxanthine. Woyewoda, Shaw, Ke, and Burns (1986) also reported a relationship between microbiological spoilage and ATP degradation. However, in the samples processed for 30 min a small increase of hypoxanthine production was detected. At 48 days, the Hx/Ino ratio was 0.62, 0.67 and 1.42 for the samples processed for 60, 45 and 30 min respectively (Fig. 7). None of the samples tested was assessed as unacceptable for consumption during the first 48 days of storage. However, the samples processed for 30 min should have been rejected on the 25th day of storage (23 days earlier) because of their TVC values, and their Hx/Ino ratio was 1.42, confirming the microbial spoilage.

During the next 14 days of storage (62 days) two of the three samples were assessed as unacceptable for consumption. Thus, the samples processed for 30 and 45 min were not assessed as acceptable by the panellists and at the same time the ratio of Hx/Ino reached values of 3.00 and 1.80, respectively (Fig. 7). However, the samples processed for 60 min extended their shelf-life (according to panellists) above 62 days of storage. However, their microbial growth reached its maximum on the 48th day of storage and Hx/Ino ratio on the 62nd day was 1.36, confirming the microbial growth. Considering the results it could possibly be concluded that when the hypoxanthine content becomes 1.3 times more than inosine content, (Hx/Ino $\geqslant$ 1.3) the products become spoiled. At 62 days of storage the Hx/Ino ratio exceeded 1.3 for all samples. Similar relationships between microflora and hypoxanthine production have been already reported (Hansen et al., 1995; Veciana-Norgues et al., 1997).

4. Conclusions

The liquid smoked trout fillets processed for 30 min showed a storage time at 4 ± 1 °C of 25 days while those processed for 45 and 60 min up to 48 days, as assessed by the TVC values. However, the panellists rejected the samples long after maximum total viable counts were observed in all samples. The measurement of TVC via the particle size analyser (Mastersizer 2000) showed an increase of the particles throughout the storage of the trout fillets, indicating that might be a direct measurement for TVC changes.

Hypoxanthine/Inosine ratio showed a good relationship with either TVC or sensory results. When the Hx/Ino ratio became more than 1.3, the products have already reached their maximum acceptable TVC values and they were rejected by the panellists.

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