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Stability of astaxanthin and canthaxanthin in raw and smoked Atlantic salmon (*Salmo salar*) during frozen storage

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The objective of the present study was to establish the effect of frozen storage and smoking on the stability of astaxanthin and canthaxanthin in farm raised salmon. Salmon samples were obtained from two sea farms where they had been maintained on commercial feeds containing either astaxanthin or canthaxanthin. The results showed that there was no significant change in visual colour score or carotenoid content of astaxanthin-fed fish during frozen storage for up to 12 weeks. However, smoking fish from this group after frozen storage for 6 and 12 weeks significantly decreased pigment content from an initial level of 9.39 ± 0.23 to 7.99 ± 0.08 and 7.26 ± 0.07 mg kg^{-1}, respectively. Furthermore, Hunter L and a* values of raw fish were affected at 6 weeks but not at 12 weeks of storage. Significant changes were measured in canthaxanthin-fed fish stored for up to 12 weeks at −20°C. Visual colour scores decreased and carotenoid content of the flesh decreased from 10.6 ± 0.27 to 4.36 ± 0.2 mg kg^{-1}. Hunter L values increased, while a* values decreased. Smoking canthaxanthin-pigmented fish had no significant effect on the carotenoid content.

INTRODUCTION

In recent years the production of farmed Atlantic salmon (*Salmo salar*) has increased substantially (Heen, 1994). With this increase in production, effective processing and frozen storage has become necessary. The quality of any processed or frozen product must, however, be maintained. One of the most important quality attributes for the consumer acceptability of many foods is colour. In salmonids (salmon and trout), a uniform pink colour is traditionally considered to indicate a high quality product, making satisfactory pigmentation critical for their consumer acceptance. However, similar to other animals, salmonids have no *de novo* synthesis of carotenoids and cultivated fish must obtain them from the diet.

The main pigment of wild salmon is astaxanthin, and only smaller amounts of canthaxanthin, β-carotene, lutein, tunaxanthin and zeaxanthin are found (Simpson *et al.*, 1981; Torrissen *et al.*, 1989). The concentration of astaxanthin in salmon and trout has been found to vary between ~3 and 37 mg kg^{-1} (Torrissen *et al.*, 1989, Storebakken and No, 1992). Cultured salmon and trout are typically reared on diets containing either astaxanthin or canthaxanthin. Both pigments are available in synthetic form and both are currently permitted within the EU for inclusion in feeds up to 80 mg kg^{-1} canthaxanthin and 100 mg kg^{-1} astaxanthin (EC Feed Additive Directive 70/524). Scientific trials show that commercial astaxanthin and canthaxanthin are absorbed, transported and deposited in the same way as the carotenoids consumed by wild fish (Schiedt *et al.*, 1985; Storebakken *et al.*, 1985; Foss *et al.*, 1987; Mori *et al.*, 1989; Bjerkeng *et al.*, 1990; Liaaen-Jensen and Storebakken, 1990). Astaxanthin is, however, more effectively absorbed and deposited than canthaxanthin. (Foss *et al.*, 1984, 1987; Schiedt *et al.*, 1985; Storebakken *et al.*, 1987; Choubert and Storebakken, 1989; Torrissen, 1989). Generally, feeds for maturing Atlantic salmon are supplied with 50–100 mg kg^{-1} of pigment for 6–18 months depending on the length of the final growing phase and the intended size of the fish at harvest (Putnam, 1991). The cost of this is substantial and can be responsible for between 10 and 15% of the total

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feed cost (Torrissen *et al.*, 1990). Thus, the flesh pigmentation of salmonids is of economic importance in fish farming and knowledge of the stability of the deposited carotenoids during processing and storage is critical. Salmonid products are exposed to different conditions during processing and storage which may, in turn, result in changes in the carotenoid content and which may lead to colour modifications. While investigations into the absorption, deposition and utilisation of various carotenoids are numerous, little is known about the effect of storage and processing on their stability. The aim of the present study was to establish the effect of processing and frozen storage on the stability of astaxanthin and canthaxanthin in commercially raised Atlantic salmon.

MATERIALS AND METHODS

Experimental design

This study was carried out using sixty Atlantic salmon (*Salmo salar*): two groups of 30 fish each. The fish were obtained from two fish farms and had been raised on commercial diets containing either astaxanthin or canthaxanthin. The whole, ungutted fish were kept on ice during transportation from the farms. Following gutting, two fillets were taken from each fish, of which, one remained raw, while the other fillet from each fish was smoked using a standard commercial procedure. Ten raw and ten smoked fillets from each group were analysed at this point. The remaining 20 raw and 20 smoked fillets were vacuum packed, placed into light-proofed boxes and frozen and stored at −20°C. After 6 and 12 weeks frozen storage, 10 smoked fillets from each group were removed for analysis. At the same time 10 raw fillets from each group were removed and thawed. A section of raw flesh from each fillet was removed for analysis. The remaining fillet was then smoked and analysed.

Colour assessment

Visual assessment of the raw flesh of both groups of salmon was carried out under standardised conditions using the ROCHE 'Colour Card' system (F. Hoffmann-La Roche). This system was developed in order to help characterise and classify the pigmentation of raw salmon flesh. In the system, the colour of the flesh is compared to a range of reference colours on a colour card. The number of the reference colour on the card most similar to the colour of the fish determines the colour 'score'. There are two cards in the ROCHE system, one of which is used on salmon fillets (colour score range, 11–18), while the other is used for sliced salmon (colour score range, 1–8). The identities of the samples were not known to the analyst.

In addition, instrumental colour analysis was performed on the raw salmon flesh using a Minolta colorimeter (CR-300 series). Hunter L, a* and b* values were measured, where L represents lightness, a* redness and b* yellowness (Hunt, 1977). Three readings were taken along the thickest section of each fillet.

Carotenoid analyses

The concentration of carotenoids in the raw and smoked salmon samples were determined by HPLC, following extraction with acetone. Samples (3.0 g) were homogenised with an equal weight of anhydrous sodium sulphate and extracted with three 10 ml aliquots of acetone. The combined extracts were filtered, the solvent evaporated under nitrogen (< 50°C) and the residue redissolved in 5 ml n-heptane. This solution was then transferred onto a silica S-Pak cartridge (Waters, Millipore Corporation, Milford, MA, USA). The cartridge was eluted with 10 ml 20% diethyl ether in n-heptane and 10 ml ethanol. The ethanol elute was evaporated under nitrogen and the residue was redissolved in 1 ml of the mobile phase, which consisted of 20% ethyl acetate and 80% methanol:water (9 + 1). HPLC separation of the samples was carried out on a Nucleosil 5 C18 (25 cm × 4 mm i.d.) reverse phase column and using a Shimadzu LC-6A Liquid Chromatograph, equipped with a 20 μl loop. Detection was achieved with a Shimadzu SPD-10AV UV-visible wavelength detector at 470 nm and chromatograms were recorded with a Shimadzu C-R6A integrator. External standards of known concentration were used for peak identification and quantification (Christophersen *et al.*, 1989).

Statistics

Results from the quantitative analysis of the raw and smoked salmon samples and results from the different colour analyses were subjected to analysis of variance and Tukey's Test (Snedecor, 1956).

RESULTS

The two groups of fish used in this experiment were obtained from different commercial fish farms, did not have identical genetic backgrounds, and were exposed to different feeding regimes, environment, and slaughtering conditions. Thus, it was not possible to directly compare results obtained from the two groups. The mean weight ± SE of fish in each group was 2.25 ± 0.05 kg (astaxanthin-fed) and 2.26 ± 0.03 kg (canthaxanthin-fed).

Table 1 shows the mean scores from the visual assessment of fillets from astaxanthin- and canthaxanthin-fed salmon prior to storage, and after frozen storage for 6 and 12 weeks. The initial mean ± SE score for astaxanthin fed fish was 16.2 ± 0.20, while that of canthaxanthin fed fish was 16.8 ± 0.20. There was no significant difference in the visual score assessment of

Table 1. Scores from visual assessment (ROCHE system) of the flesh colour of astaxanthin- and canthaxanthin-fed Atlantic salmon during frozen storage (−20°C) for 6 and 12 weeks

	Storage time (weeks)		
Feed group	0 (mean ± SE)[a]	6 (mean ± SE)[a]	12 (mean ± SE)[a]
Astaxanthin	16.2 ± 0.20[a]	15.7 ± 0.15[a]	15.4 ± 0.22[a]
Canthaxanthin	16.8 ± 0.20[a]	15.3 ± 0.15[b]	14.0 ± 0.26[c]

[a]Values represent a mean value with standard error of 10 individually assessed fish.
[a,b,c]Significantly different at $p < 0.05$

the astaxanthin-fed fish after frozen storage for 6 (15.7 ± 0.15) and 12 (15.4 ± 0.22) weeks. However, the visual scores for canthaxanthin fed fish were significantly different after 6 (15.3 ± 0.15) and 12 (14.0 ± 0.26) weeks.

The colour of the raw salmon flesh was characterised by tristimulus colorimetric parameters. The changes in Hunter L, a* and b* values of raw fillets from astaxanthin-fed fish during frozen storage for up to 12 weeks are shown in Fig. 1. Prior to storage, values of L, 36.3 ± 0.25, a*, 6.73 ± 0.12, and b*, 8.97 ± 0.14, were

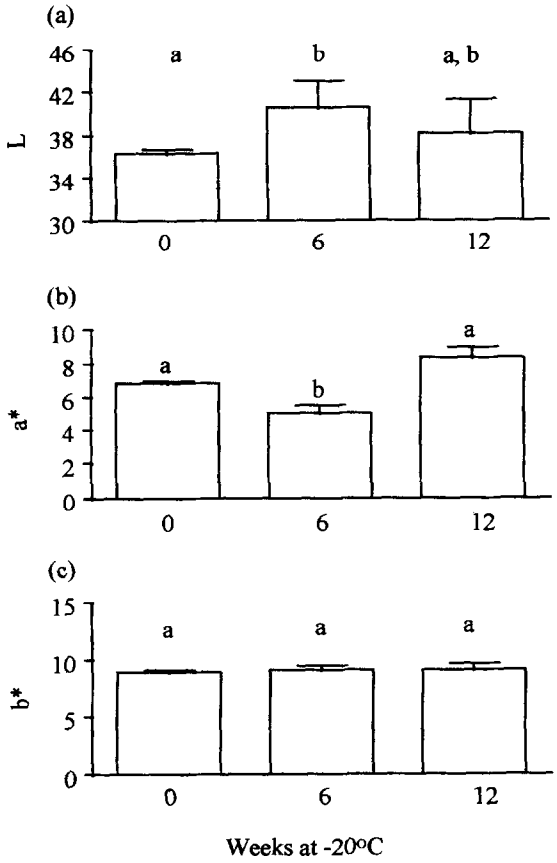

Fig. 1. Effect of frozen storage at −20°C for 12 weeks on Hunter (a) lightness, L; (b) redness, a* and (c) yellowness, b* values of raw fillets pigmented with astaxanthin. [a,b]Significantly different ($p < 0.05$).

measured. The L value of astaxanthin-fed fish increased significantly ($p < 0.05$) at 6 weeks storage (40.6 ± 2.31). However, there was no significant difference in lightness after 12 weeks storage. After 6 weeks frozen storage, mean a* value (4.98 ± 0.50) was significantly ($p < 0.05$) lower than fresh fish or those stored for 12 weeks. However, no significant difference in a* value was measured after 12 weeks (8.39 ± 0.50) compared to fresh fish. Frozen storage had no effect on b* value of astaxanthin-fed fish after 6 (9.04 ± 0.35) or 12 weeks (9.10 ± 0.48). Christophersen *et al.* (1992) reported a similar lack of effect on b* value in astaxanthin-pigmented trout. Hunter L (37.9 ± 0.29), a* (5.25 ± 0.21), and b* (9.24 ± 0.21) values were measured prior to storage of canthaxanthin-fed fish. During frozen storage for up to 12 weeks, the b* value decreased only marginally (8.39 ± 0.17). However, the L value increased significantly ($p < 0.05$) after only 6 weeks (39.2 ± 0.57), while the a* value decreased significantly ($p < 0.05$) after 12 weeks storage (3.84 ± 0.31).

As would be expected, the main carotenoid found in the flesh of the astaxanthin-fed fish was identified as astaxanthin (9.39 ± 0.23 mg kg^{-1}; Table 2), while the main pigment found in the flesh of the canthaxanthin-fed fish was canthaxanthin (10.6 ± 0.27 mg kg^{-1}; Table 3). Trace amounts of astaxanthin (< 0.01 mg kg^{-1}) were also present in the flesh of the canthaxanthin–fed fish. However, these levels were too low to have any significant influence on the colour of the flesh.

There was no significant difference between astaxanthin concentration of raw flesh measured prior to storage (9.39 ± 0.23 mg kg^{-1}) and that after frozen storage for 6 (9.18 ± 0.07 mg kg^{-1}) or 12 (8.98 ± 0.14 mg kg^{-1}) weeks. Smoking raw fresh salmon did not significantly affect the concentration of astaxanthin. However, the process of smoking had a significant influence on the astaxanthin concentration of frozen salmon flesh. When raw astaxanthin–fed fish was held in frozen storage 6 or 12 weeks, levels of astaxanthin decreased from 9.18 ± 0.07 mg kg^{-1} to 7.99 ± 0.08 mg kg^{-1} (6 weeks frozen storage), and from 8.98 ± 0.14 mg kg^{-1} to 7.26 ± 0.07 mg kg^{-1} (12 weeks frozen storage) when the raw defrosted fish was smoked.

There was no significant difference ($p > 0.05$) in the astaxanthin content between raw astaxanthin-fed salmon and raw astaxanthin-fed salmon stored frozen for 6 weeks and then smoked. However, raw fish, after 12 weeks frozen storage and then smoked, contained significantly ($p < 0.05$) less astaxanthin than fresh raw fish.

The canthaxanthin contents of the flesh of canthaxanthin-fed fish are shown in Table 3. A significant reduction in the levels of canthaxanthin was measured in raw flesh during frozen storage of up to 12 weeks. Levels of canthaxanthin decreased from 10.6 ± 0.27 mg kg^{-1} prior to storage, to 8.30 ± 0.19 mg kg^{-1} after 6 weeks and to 4.36 ± 0.20 mg kg^{-1} after 12 weeks. The process of smoking did not affect the concentration of canthaxanthin in flesh of salmon.

Table 2. Pigment concentration (mg kg^{-1}) of Atlantic salmon fed astaxanthin

	Storage time (weeks)		
	0 (mean ± SE)[a]	6 (mean ± SE)[a]	12 (mean ± SE)[a]
Raw	9.39 ± 0.23[a,1]	9.18 ± 0.07[a,1]	8.98 ± 0.14[a,1]
Stored frozen then smoked	—	7.99 ± 0.08[a,2]	7.26 ± 0.07[a,2]
Stored smoked	8.54 ± 0.21[a,1]	7.60 ± 0.07[a,2]	7.38 ± 0.09[a,2]

[a]Values represent a mean value with standard error of 10 individually assessed fish.
[a]Means in the same row followed by the same superscript are not significantly different ($p < 0.05$).
[1,2,3]Means in the same column followed by a different superscript are significantly different ($p < 0.05$).

Table 3. Pigment concentration (mg kg^{-1}) of Atlantic salmon fed canthaxanthin

	Storage time (weeks)		
	0 (mean ± SE)[a]	6 (mean ± SE)[a]	12 (mean ± SE)[a]
Raw	10.6 (0.27)[a,1]	8.30 (0.19)[b,1]	4.36 (0.20)[c,1]
Stored frozen then smoked	—	6.10 (0.09)[a,1]	3.62 (0.18)[b,1]
Stored smoked	9.26 (0.31)[a,1]	7.55 (0.25)[a,1]	4.38 (0.14)[b,1]

*Values represent a mean value with standard error of 10 individually assessed fish.
[a,b,c]Means in the same row followed by a different superscript are significantly different ($p < 0.05$).
[1,2]Means in the same column followed by a different superscript are significantly different ($p < 0.05$).

DISCUSSION

Several studies have shown that the pigment stability of salmonids during frozen storage is uncertain. Ingamansson *et al.* (1993) found that the astaxanthin content of rainbow trout (*Oncorhynchus mykiss*) fillets from fish fed on diets supplemented with synthetic astaxanthin decreased significantly after 8 months storage at −15°C. Christophersen *et al.* (1992) measured the astaxanthin content of rainbow trout fillets over a storage period of 180 days at −18°C and found a slight decrease in pigment concentration during that time. However, synthetic astaxanthin and canthaxanthin were found to be stable in rainbow trout fillets after 180 days at −20°C (No and Storebakken, 1991), and for up to 180 days at −18°C, −28°C and −80°C (Scott *et al.*, 1994). These latter studies (No and Storebakken, 1991; Scott *et al.*, 1994) suggest that both astaxanthin and canthaxanthin are stable during storage.

Results from the present study have shown that canthaxanthin levels decreased considerably during frozen storage. This loss in colour was also visually verified by the decrease in 'ROCHE' colour card score. The eye is relatively insensitive to increases of pigment above a certain level. Several reports have recommended or suggested levels of flesh carotenoids over which visual assessment of pigment is unreliable. Foss *et al.* (1984) considered that visual assessment is unreliable in rainbow trout if the carotenoid concentration is greater than 6 mg kg^{-1}. A report from 'Roche Products' (Anon., 1988) considered that differences in pigment concentration cannot usually be distinguished visually in salmon flesh at carotenoid concentrations above 7 mg kg^{-1}. Levels of astaxanthin in salmon flesh in this study were well in excess of 7 mg kg^{-1}. However, levels of canthaxanthin were considerably below this after 12 weeks storage. Thus, canthaxanthin-pigmented fish should only be stored for a maximum of 6 weeks. Feeding surplus pigment, particularly in the case of canthaxanthin-pigmented fish, may act as a form of buffer against fading if the fish are stored frozen. However, there is no commercial advantage to the farmer in producing fresh salmon with excessive amounts of pigment, as a stronger colour is not perceived by the eye of the consumer.

The process of smoking had a negative effect on the carotenoid concentration of fillets pigmented with astaxanthin, but this effect was not evident in fresh fillets and only in frozen fillets.

In conclusion, astaxanthin and canthaxanthin were affected in slightly different ways by frozen storage and smoking of salmon fillets. Canthaxanthin-fed fish seem to be better for smoking although, when frozen, they lose colour more rapidly than astaxanthin-pigmented fish fish. If farmed salmon production is to continue expanding, traditional as well as new markets will have to be exploited. In this respect, there will be a need to produce capable of undergoing a variety of treatments without losing the characteristic colour associated with good quality salmon. A combination of astaxanthin and canthaxanthin in the diet may be required to produce fish capable of undergoing frozen storage and/or smoking.

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