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Extractabilities of astaxanthin and protein from muscle tissue of Atlantic salmon (*Salmo salar*) as affected by brine concentration and pH☆

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Abstract

Carotenoids are associated with proteins in muscle tissues of salmonid fishes. The extractabilities of astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) and protein from minced muscle of Atlantic salmon (*Salmo salar*) were investigated using different brine concentrations (0–6 M) and different pHs (4–8) to discover whether they contribute to poor colouration of salmonid fish muscle. The salmon had been fed a diet, containing 55 mg kg^{-1} astaxanthin from *Xanthophyllomyces dendrorhous*, for 12 weeks, and contained 3.9 ± 0.03 mg carotenoids kg^{-1} muscle. The plot of ratios between extracted astaxanthin and protein was bell-shaped with a maximum at pH 7, and was considerably lower in brine (1–6 M NaCl) than in distilled water. Regardless of the NaCl-concentration, the highest extractabilities of protein and astaxanthin were found at pH 6–7. Extractabilities of protein and astaxanthin were highest for NaCl concentrations of 1–4 M. The total amount of proteins extracted with brine correlated well with extracted astaxanthin ($R^2 = 0.82$). The amounts of astaxanthin extractable from the mince were in the range 1.6–7.4 and 5.9–21.3% of total carotenoid content, in distilled deionised water and 1.0 M brine, respectively. In conclusion, the use of excess water during dry salting, may dilute salt at the surface and partly contribute to discoloration of salmon products, whereas protein–astaxanthin extraction is expected to be less during injection and brine salting due to the high salt concentration.

Keywords: Astaxanthin; Carotenoid; Protein; Extractability; Atlantic salmon; pH; NaCl

1. Introduction

Atlantic salmon (*Salmo salar*) farming is a large industry in Norway, and 423,000 metric tons were produced in 2000 (Norwegian Seafood Export Council, 2002). An estimated 40–50% of the amount produced reaches the consumer as a cold-smoked product (Borch & Aaker, 1997). The characteristic red colour of wild Atlantic salmon muscle is mainly due to the presence of astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione). Astaxanthin and canthaxanthin (β,β-carotene-4,4′-dione), Fig. 1, are the most commonly used carotenoids for muscle pigmentation of salmonid fishes (Bjerkeng, 2000). Astaxanthin is unesterified in the muscle (Henmi, Iwata, Hata, & Hata, 1987), the geometrical all-*E* isomer predominating (>90% of total astaxanthin; Bjerkeng, Følling, Lagocki, Storebakken, Olli, & Alsted, 1997; Henmi, Hata, & Takeuchi, 1990a), and it is believed to be bound non-specifically to the hydrophobic pocket in the actomyosin protein-complex by weak interactions (Henmi et al. 1987; Henmi, Hata, & Hata, 1989; Henmi, Hata, & Hata, 1990b; Henmi, Hata, & Takeuchi, 1991).

Muscle proteins are often classified as sarcoplasmic proteins, myofibrillar proteins (myosin, actin and actomyosin), and connective-tissue or stromal proteins (collagen) (Rodger & Wilding, 1990; Skaara & Regenstein, 1990). The sarcoplasmic proteins are soluble in water or dilute salt solutions, whereas the myofibrillar proteins are soluble at salt concentrations >0.3M (Hultin, Feng,

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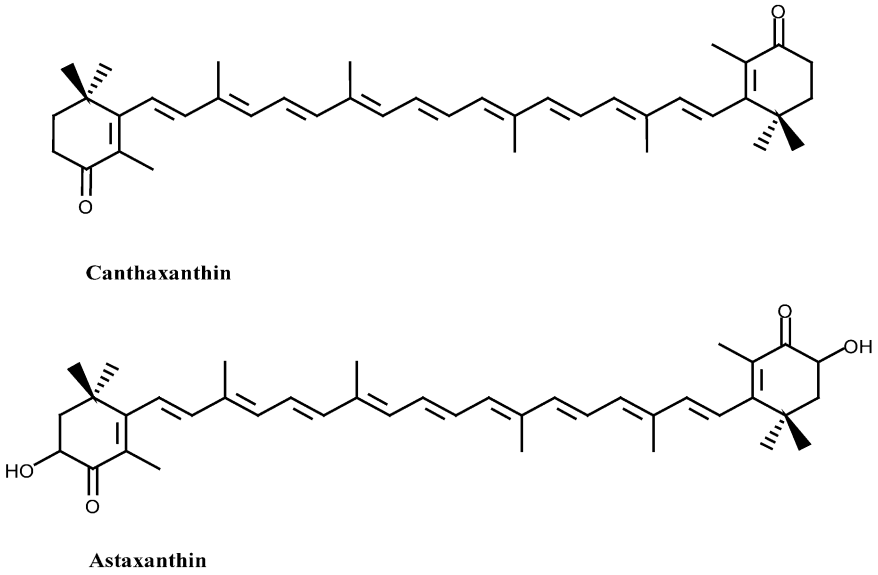

Fig. 1. Structures of all-*E*-astaxanthin and all-*E*-canthaxanthin.

& Stanley, 1995). Myosin and actomyosin require high salt concentration for extraction (Hultin et al., 1995; Stefansson & Hultin, 1994). Nevertheless, reports indicate that some of the myofibrillar proteins are water-soluble (Hennigar, Buck, Hultin, Peleg & Vareltzis, 1988; Lin & Park, 1996; Wu, Atallah & Hultin, 1991).

Cold-smoking of Atlantic salmon includes salting and drying, and the products typically contain between 2.0% and 3.9% salt in the water phase (Hansen, Gill, & Huss, 1995; Hansen, Røntved, & Huss, 1998). The salt is usually added by drysalting, brine salting or injection-salting.

Occasionally, severe discoloration of smoked salmon is observed, possibly caused by detrimental processing conditions, as is often indicated by its superficial nature. During processing, the smokehouses use several different processing methods, and the information on the effects of these processing conditions on different quality parameters (e.g. colour) is scarce. Process parameter settings employed during injection-salting may adversly affect product yield and fillet gaping during processing of Atlantic salmon fillets (Birkeland, Skåra, Bjerkeng, & Rørå, 2003). Furthermore, different processing conditions may include excessive use of water to remove surplus salt, extensive residence time of the salted fillets in water to adjust the salt content and to induce a more homogeneous salt distribution in the muscle, high residence time covered with salt during dry salting, extensive brine immersion or injection of brine at a high pressure before smoking. These processing conditions may cause extraction of different salt- and water-soluble proteins. Extraction of protein–carotenoid complexes during processing might cause surface discoloration, and lead to a smoked product of reduced quality. Previous investigations have shown that both salting time and brine concentration have significant effects on instrumental colour measurements (CIE $L^*a^*b^*$) during dry- and brine-salting (Birkeland & Bjerkeng, unpublished). We therefore investigated the extractability of astaxanthin and protein from minced muscle of Atlantic salmon using different brine concentrations up to 6 M NaCl in the pH range 4–8 to evaluate to what extent these factors could contribute to discoloration.

2. Materials and methods

2.1. Materials

Twenty frozen, skinned fillets of Atlantic salmon (*Salmo salar*; mean final weight ca. 1600 g) were obtained from AKVAFORSK, and transported on ice to NOR-CONSERV. The fish had been fed a diet containing 55 mg kg^{-1} astaxanthin from *Xanthophyllomyces dendrorhous* (formerly *Phaffia rhodozyma*) for 12 weeks. After thawing, the fillets were ground and blended into a homogeneous mince using a blender (Robot Coupe Blixer 5 Plus R5A, Vicennes, France), speed 3000 rpm. Batches of mince (approximately 100 g) were stored in sealed plastic containers at −70 °C until they were analysed.

2.2. Proximate analyses

The crude protein content was determined according to the Kjeldahl method (International Standard 5983, 1979) using a conversion factor of 6.25, and the crude fat content according to the method of Bligh and Dyer (1959). Determination of the total carotenoid concentration was performed according to Norwegian

Standard NS 9402 (1994). Triplicate analyses were performed on all samples.

2.3. Extraction of water-soluble proteins

Extraction of water-soluble proteins was performed after modification of the methods of Anderson and Ravesi (1968) and Licciardello, Ravesi, Lundstrøm, Wilhelm, Correia, and Allsup (1982). All work with protein extraction was performed on ice-cooled samples and all solutions used were kept cold (approx. 5 °C). Samples of minced fillet (10 g) were accurately weighed into a centrifuge bottle (200 ml) and distilled deionised water (150 ml; MODULAB® ModuPure™, Reagent Grade Water System, Continental® Water Systems Corporation, San Antoinio, Texas, US) was added. The mixture was stirred vigorously, using a magnet stirrer for 2 min, and pH adjusted to the desired level by addition of HCl or NaOH using a pH-meter (Beckman pHI 72 Meter with Combination Electrode and Automatic Temperature Compensation, Beckman Instruments Inc., Fullerton, California, USA). The suspension was then extracted with an Ultra-Turrax macerator (13000 rpm; Ultra Turrax T25, Janke & Kunkel GMBH & Co. KG, Staufen, Germany) for 30 s, and centrifuged at 8000 × *g* for 20 min (Sorvall®, RC-5C PLUS, Sorvall Products L.P., Newtown, Connecticut, USA). Proteins remaining in the supernatant after centrifugation were defined as soluble protein. The supernatant was filtered through glass wadding and transferred to a graduated flask (150 ml), and the volume adjusted with distilled deionised water. The extracts containing the water-soluble proteins were stored in sealed plastic containers at −12 °C until analysed for total protein content and carotenoid concentration.

2.4. Extraction of salt-soluble proteins

NaCl-solutions (150 ml) of selected concentrations (1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 M, respectively) were added to the remaining precipitate, and the procedure described above repeated. In the range of maximum protein extractability, a second series of extractions was performed using 1 M NaCl solution in the pH range from 6.25 to 7.50 with increments of 0.25 in pH. This was done to gain some more detailed information on the extraction of astaxanthin in this pH range relevant for processing of smoked salmon. The extracts containing salt-soluble proteins were stored in sealed plastic containers at −12 °C until analyses of total protein content and carotenoids.

2.5. Quantitative determination of total protein content in the protein extracts

The frozen protein extracts were thawed in a refrigerator overnight, and total protein content determined according to a modified BIO RAD DC Protein Assay (Bio-Rad Laboratories, Hecules, California, US). A stock solution of bovine serum albumin (BSA, 1 mg ml^{-1}, crystallized and lyophilized bovine albumin, Sigma Chemical Company, St. Louis, US) was prepared and a standard-curve, ranging from 0 to 450 μg BSA ml^{-1}, was constructed. Quadruplicate samples (20 μl) of blank (distilled deionised water), standard and protein-extract were applied to the micro-wells by using a multipipette (Finnpipette Digital MCP 5–50 μl, Labsystems Oy, Helsinki, Finland). The protein extracts were diluted with distilled deionised water to fit to the standard-curve whenever necessary. Reagent A (35 μl, alkaline copper tartrate solution), containing 20 μl Reagent S (sodium dodecylsulphate) per ml, was applied to each micro-well. Finally, Reagent B (300 μl, dilute folin reagent) was added to each micro-well by using a multipipette (Finnpipette Digital MCP 50–300 μl, Labsystems Oy, Helsinki, Finland). After reacting for 20 min, the samples were measured spectrophotometrically using a microplate absorbance reader (KC-Junior Microplate Absorbance Reader Elx800, Bio-Tek Instruments Inc., Winooski, US) at 750 nm.

2.6. Quantitative determination of astaxanthin in the protein extracts

The astaxanthin concentration of the protein extracts was determined after modification of the method of Wathne, Bjerkeng, Storebakken, Vassvik, and Odland (1998). The protein extract was transferred to a centrifuge bottle (200 ml) and methanol (15 ml) containing 1,6-di-*t*-buthyl-*p*-cresol (BHT, 500 mg l^{-1}) as an antioxidant was added. The sample was extracted using an Ultra-Turrax macerator (13,000 rpm) for 1 min. After settling for 10 min in the dark the sample was centrifuged at ca. 1500 × *g* for 10 min. The water-phase was decanted off, and chloroform (50 ml) was added and the extraction procedure repeated twice. An aliquot of the chloroform-phase (75.0 ml) was removed under reduced pressure and the sample was dissolved in the HPLC mobile-phase (acetone/*n*-hexane/methanol 20:80:1). In an effort to assure complete extraction of astaxanthin from the protein extracts, the chloroform-phase was subjected to a third extraction with chloroform (50 ml) in the experiment investigating the pH range 6.25–7.50.

The extract was filtered into an HPLC sample vial through a 0.45-μm syringe filter (Alltech Associates, Inc., State College, PA, USA). The astaxanthin content was determined by isocratic HPLC (Perkin-Elmer Turbochrom LC Plus System, Shelton, CT, USA), using a Spherisorb S5-CN Nitrile column (Waters, Milford, MA, USA; length = 250 mm; internal diameter 4.6 mm; particle size 5 μm). All-*E*-astaxanthin (Hoffmann-La Roche, Basel, Switzerland) was used as an external

standard ($E_{1\%,\ 1\ cm} = 2100$, $\lambda_{max} = 470$ nm; Britton, 1995). Concentrations were calculated based on integrated chromatogram peak areas.

3. Results

3.1. Analyses of raw material

The total carotenoid content of the minced Atlantic salmon muscle was 3.9±0.03 mg kg^{-1}, and the total protein and lipid contents were 20.4±0.03 and 9.7±0.1%, respectively. The major carotenoid was astaxanthin, and the astaxanthin metabolite idoxanthin (3,3′,4′-trihydroxy-β,β-carotene-4-one) comprised less than 3% of total carotenoids.

3.2. Extraction of proteins and astaxanthin with distilled deionised water

A sharp decline in the amount of extracted water-soluble proteins was observed in the pH-range 4.0 (96.1±18.7 mg g^{-1} mince) to 6.0 (20.0±3.7 mg g^{-1} mince), followed by an increase in extractability in the range from pH 6.0 to pH 8.0 (40.1±3.6 mg g^{-1} mince), (Fig. 2) Similarly, the extractability of astaxanthin decreased in the pH-range 4.0 (0.25±0.26 mg kg^{-1} mince) to pH 5.0 (0.036±0.03 mg kg^{-1} mince), followed by an increase in extractability from pH 5.0 to 8.0 (0.29±0.16 mg kg^{-1} mince), (Fig. 2). At most, 7.5% of the total carotenoid content was extracted using distilled deionised water. The correlation between extracted water-soluble proteins and astaxanthin was $R^2 = 0.47$.

During the second extraction of the mince with distilled deionised water, the protein extractability was highest at pH 4.0 (37.9±2.5 mg g^{-1} mince), (Fig. 3a). Low levels of extracted proteins were found at pH 5.0, and extractability increased slightly during increase of pH to 8.0. The ratio between extracted astaxanthin and protein increased steadily from pH 5 and peaked at pH 7, (Table 1). When brine was used (1–6 M), the ratio between extracted astaxanthin and protein was considerably lower, and rather constant over the pH range 4–8.

3.3. Extraction of proteins with brine

The amounts of salt-soluble proteins were highest at pH 6.0 or 7.0 for all the investigated brine concentrations, (Fig. 3a). For brine concentrations 1.0, 2.0 and 6.0 M there was an increase in extractability from pH 4.0 to 6.0, and a subsequent decrease in extractability from pH 6.0 to 8.0. For brines in the range 3.0–5.0 M there was an increase in extractability in the pH range 4.0–7.0, followed by a

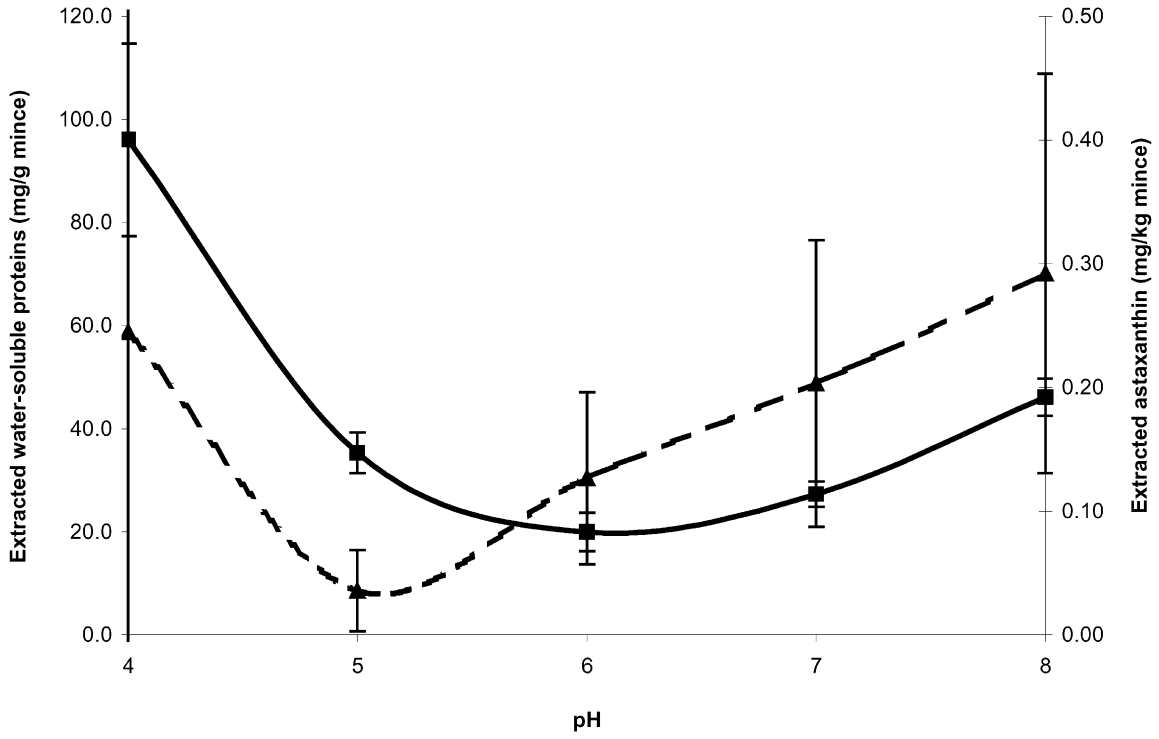

Fig. 2. Extracted proteins (mg g^{-1} mince) and astaxanthin (mg kg^{-1} mince) in distilled/deionised water at pH 4.0, 5.0, 6.0, 7.0 and 8.0. –■– = extracted water-soluble proteins, - -▲- - = extracted astaxanthin.

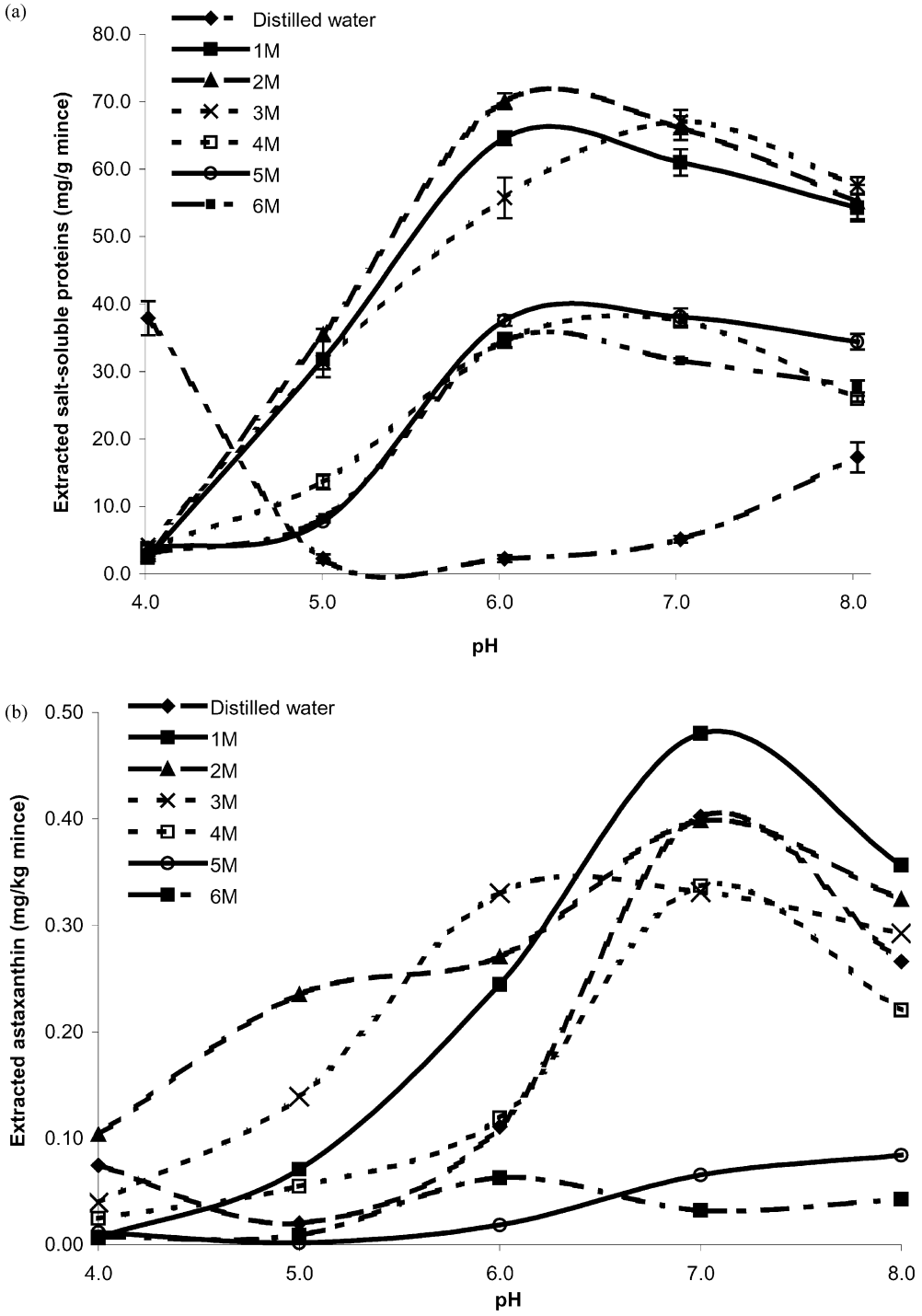

Fig. 3. (a). Extractable proteins (mg g^{-1} mince) in brine of various concentrations and pH. Extraction was performed in distilled/deionised water, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 M brine and at pH 4.0, 5.0, 6.0, 7.0 and 8.0. (b). Extractable astaxanthin (mg kg^{-1} mince) in brine of various concentrations and various pH values. Extraction was performed in distilled/deionised water, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 M brine and at pH 4.0, 5.0, 6.0, 7.0 and 8.0.

decrease in extractability from pH 7.0 to 8.0. At brine concentrations in the range 1.0–3.0 M, there was a higher protein extractability than with brine concentrations in the range 4.0–6.0 M. The highest amount of proteins extracted (70.1 ± 1.0 mg g^{-1} mince) was found at pH 6.0 using 2.0 M brine.

3.4. Extraction of astaxanthin in brine

Regardless of the brine concentration, the highest astaxanthin extractability appeared at pH 7.0, (Fig. 3b). The maximum extractability of astaxanthin (0.48 ± 0.07 mg kg^{-1} mince) was observed for 1 M brine, and 12%

Table 1
Ratio between extracted astaxanthin (mg kg^{-1} mince) and extracted salt soluble protein (mg g^{-1} mince)×100

PH	DD-water	Ratio×100					
		1 M	2 M	3 M	4 M	5 M	6 M
4	0.20	0.29	3.68	0.93	0.66	0.30	0.24
5	0.90	0.22	0.66	0.44	0.40	0.02	0.11
6	4.92	0.38	0.39	0.59	0.34	0.05	0.18
7	7.83	0.79	0.60	0.49	0.90	0.17	0.10
8	1.54	0.66	0.59	0.51	0.85	0.25	0.15

of the total carotenoid content of the mince was solubilised. Lowest extractability (0.03±0.01 mg kg^{-1} mince) was observed for 6 M brine, in which 0.8% of the total carotenoid content was solubilised.

The correlation between solubilised proteins, (Fig. 3a), and astaxanthin, (Fig. 3b), in brine was in the range $R^2 = 0.70$–0.91. No linear correlation was observed between extracted proteins and astaxanthin in distilled and deionised water.

3.5. Extraction of proteins and astaxanthin in distilled deionised water and 1.0 M brine

The amount of proteins extracted by distilled deionised water and 1.0 M brine, respectively, ranged from 24.2±0.7 to 42.8±1.3 and from 63.5±1.2 to 86.7±5.4 mg g^{-1} mince (Table 2). After extraction in distilled deionised water, an increase in protein extractability was observed from pH 6.25 to pH 7.50 while, after extraction in 1.0 M brine, protein extractability varied with pH, but a general increase from pH 6.25 to pH 7.50 was observed.

In distilled deionised water and 1.0 M brine, the amounts of extractable astaxanthin ranged from 0.06±0.01-0.29±0.07 mg kg^{-1} mince and from 0.23±0.01 to 0.83±0.12 mg kg^{-1} mince, respectively, (Fig. 4). In both distilled deionised water and 1.0 M brine a slight decrease in astaxanthin extractability from pH 6.25 to pH 6.50 was observed, followed by an increase in extractability from pH 6.50 to pH 7.50. The correlations (R^2) between extracted proteins, (Table 2), and astaxanthin in distilled and deionised water and 1.0 M brine, (Fig. 4), were 0.84 and 0.64, respectively. The proportion of extractable astaxanthin of total carotenoid content in the mince was in the range 1.6–7.4 and 5.9–21.3% in distilled and deionised water and 1.0 M brine, respectively.

Table 2
Extracted proteins (mg g^{-1} mince) in distilled deionised water and 1.0 M brine at various pH values

pH	Extracted proteins	
	Distilled deionised water	Brine (1.0 M)
6.25	24.2±0.7	68.0±2.7
6.50	26.1±1.2	63.5±1.2
6.75	28.0±0.7	86.7±5.4
7.00	34.6±3.0	83.9±1.4
7.25	38.2±0.6	80.8±5.0
7.50	42.8±1.3	78.2±0.3

3.6. Total amounts of extractable proteins and astaxanthin

The total amount of water- and salt-soluble proteins combined, was in the range from 89.6±0.7 to 121±1.6 mg g^{-1} mince, (Fig. 5). The lowest and highest extractabilities were observed at pH 6.50 and 7.50, respectively. The same pattern was observed for the total amount of extractable astaxanthin (combined astaxanthin extracts from distilled deionised water and 1.0 M brine). At pH 6.50, 0.29±0.01 mg astaxanthin kg^{-1} mince was extracted and, at pH 7.50, 1.12±0.17 mg astaxanthin kg^{-1} mince was extracted. At the most, 29% of total carotenoid content was solubilised. The correlation between the total amount of extracted proteins and total amount of extracted astaxanthin was rather high ($R^2 = 0.82$).

A relatively high correlation ($R^2 = 0.85$) between total carotenoid content (mg kg^{-1} mince) in the precipitate after protein extraction and total amount of extracted proteins (mg g^{-1} mince) was observed. A relatively high correlation ($R^2 = 0.54$) between total carotenoid content in the precipitate and total amount of extracted astaxanthin (mg kg^{-1} mince) was also observed.

4. Discussion

The two main classes of carotenoid-protein complexes are the carotenoproteins in which xanthophylls (typically astaxanthin) are stoichiometrically bound to proteins with non-covalent bonds and the carotenoid-lipo(glyco)proteins in which carotenoids are non-stoichiometrically dissolved in the lipid component (Goodwin, 1984). It is not yet clear whether the actomyosin–astaxanthin complex belongs to any of these classes. However, it has been shown that astaxanthin can form complexes with actomyosins from different fish species, but the ratio of astaxanthin to protein ranges from 0.32 to 1.01 mg astaxanthin g^{-1} protein in carp and common mackerel, respectively, actomyosin from salmonid fish species being intermediate (Henmi et al., 1990b). Actomyosins with high surface hydrophobicity combine well with astaxanthin, and this indicates that the association with actomyosin is by non-specific hydrophobic binding (Henmi et al., 1990b). Astaxanthin is the most commonly used carotenoid in Norwegian salmon farming, and the most efficient carotenoid used for pigmentation

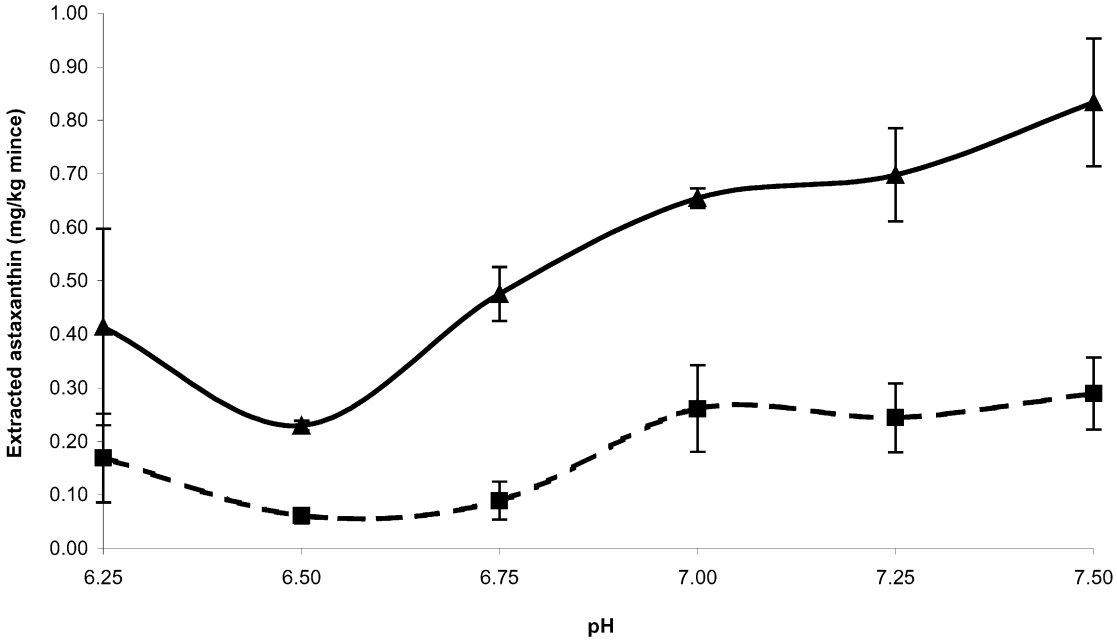

Fig. 4. Extracted astaxanthin (mg kg^{-1} mince) in distilled/deionised water and 1.0 M brine in the pH-range 6.25–7.50. - -■- - = distilled/deionised water, –▲– = 1.0M brine.

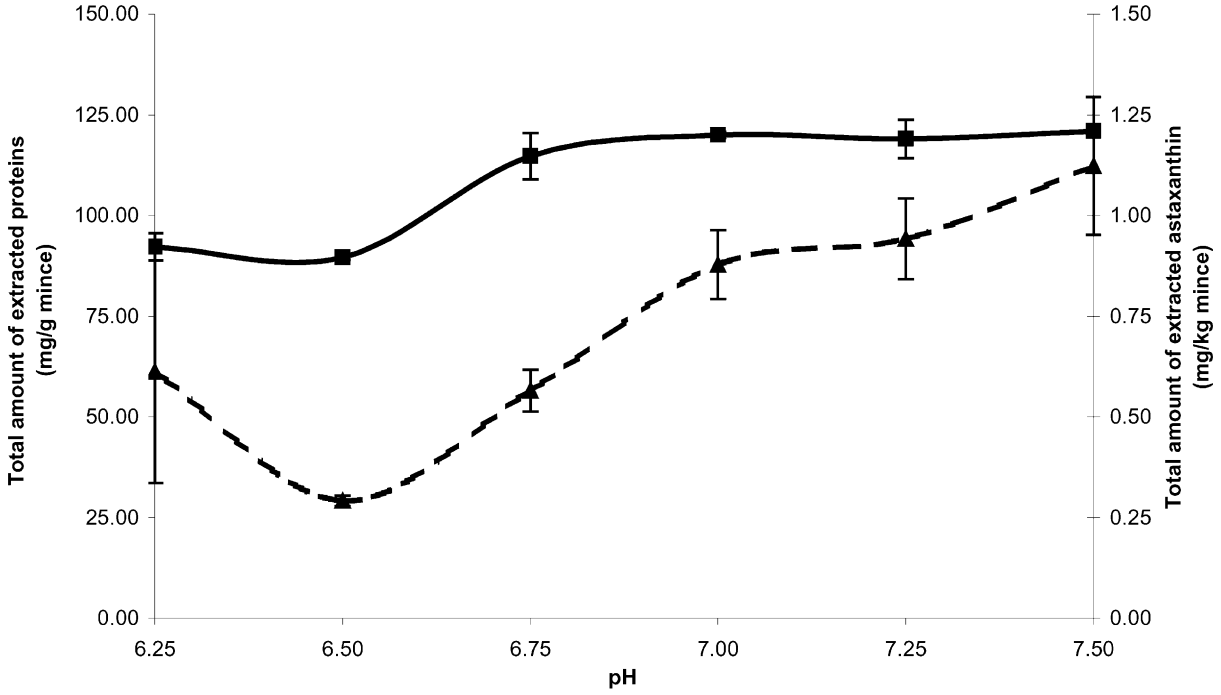

Fig. 5. Total amount of extracted proteins (water-soluble + salt-soluble; mg g^{-1} mince) and astaxanthin (water fraction + brine fraction; mg kg^{-1} mince) in 1.0 M brine. Extractions were performed at pH 6.25, 6.50, 6.75, 7.00, 7.25 and 7.50. –■ – = total amount of extracted proteins, - -▲- - = total amount of extracted astaxanthin.

of rainbow trout (Torrissen, Hardy, & Shearer, 1989; Storebakken & No, 1992). However, recent reports indicate that astaxanthin and canthaxanthin are equally efficient carotenoid sources for muscle pigmentation in Atlantic salmon (Baker, Pfeiffer, Schöner, & Smith-Lemmon, 2002; Buttle, Crampton, & Williams, 2001). Whether these species differences are related to properties of the binding proteins in the muscle, or to absorption, deposition and metabolism rates, requires future investigation.

A rational design and control of meat processing require a thorough understanding of the mechanism causing the changes in water retention and protein solubility that accompany treatment with NaCl (Knight

& Parsons, 1988). In the first extraction with distilled and deionised water, (Fig. 2) extractable astaxanthin and proteins, had minima at pH 5 and 6, respectively. During the second extraction with distilled and deionised water protein, (Fig. 3a), solubility was low in the pH-range from 4 to 7, whereas maximum extractable astaxanthin was found at pH 7, (Fig. 3b). Thus, the ratio between extracted astaxanthin and protein was very high at pH 7. After washing out sarcoplasmic proteins and endogenous salt, the solubility of cod muscle myofibrillar proteins at low ionic strength was found to be low in the pH-range 5–6.5 (Stefansson & Hultin, 1994). Similarly, Lin and Park (1998) found very low solubility of Chinook salmon myosin in the pH-range 4–7. The ratio between extracted astaxanthin and extracted protein was 2.5–6 times higher in water that in any of the brines in the present experiment. This might indicate that a sarcoplasmatic protein that binds relatively much astaxanthin compared with the other treatments was extracted with distilled deionised water at pH 7. A candidate protein may be albumin that is highly water-soluble close to neutral pH, and 40% of the extravascular fraction may be present in the muscle tissues (cf. Peters, 1996). Also, Atlantic salmon albumin is expressed in muscle tissues (Byrnes & Gannon, 1990), and astaxanthin has been shown to combine with bovine serum albumin (Henmi et al., 1991), and serum albumin has been indicated as a major transport protein of astaxanthin in Atlantic salmon plasma (Aas, Bjerkeng, Storebakken, & Ruyter, 1999). Work is in progress to more closely determine the nature of the proteins associated with astaxanthin.

The protein extractability was rather similar for NaCl concentrations above 1 M at any pH. The highest solubility of proteins was observed at pH 6 and 7. Similar results have been reported for cod muscle (Stefansson & Hultin, 1994). Myofibrillar proteins have different properties, depending on intrinsic, environmental and processing factors (Cofrades, Careche, Carballo, & Colmenero, 1993). At increasing salt concentration, protein solubility is first increased (salting-in), rendering the proteins less stable with the increasing salt concentrations, followed by a decreased solubility (salting-out) upon further addition of salt (Creighton, 1993; Stefansson & Hultin, 1994). Salts that increase the solubility of proteins also tend to denature them (Arakawa, Bhat, & Timasheff, 1990), and the susceptibility of muscle myofibrillar proteins to the denaturing action of sodium chloride is especially acute in fish (Hultin et al., 1995). The denaturing action of sodium chloride on muscle myofibrillar proteins might be the cause of the decreasing solubility at high salt concentrations (Kelleher & Hultin, 1991). Sarcoplasmic (Hultin et al., 1995) and myofibrillar protein (Hultin et al., 1995; Lefevre, Fauconneau, Ouali, & Culioli, 2002; Rodger & Wilding, 1990; Skaara & Regenstein, 1990) solubility also depend on pH, and small differences in pH can alter solubility markedly (Parsons & Knight, 1990; Stefansson & Hultin, 1994). Minimum solubility is usually observed close to the isoelectric point, where the proteins have zero net charge and the protein–water interactions are replaced by protein–protein interactions (Lin & Park, 1998). Solubility increases at pH values farther away from this region (Creighton, 1993; Lefevre et al., 2002), and the solubility in the isoelectric region might be altered by salt that cause salting-in and salting-out effects (Skaara & Regenstein, 1990). In the present experiment we used frozen raw material, which may lead to denaturation of actomyosin into actin and myosin (cf. Mackie, 1993). Regardless of NaCl-concentration, the highest extractability of astaxanthin was found at pH 7 in the present experiment. In the brine extracts the ratio between astaxanthin and protein was fairly constant at all pH levels investigated. This suggests that astaxanthin was associated with the extracted proteins in an equal quantitative ratio and further suggests that this may be myosin. At most, ca. 10% of total carotenoid content was solubilised.

The correlation between the extracted amount of protein and astaxanthin was high in the pH range between 6.25 and 7.50 and using 1 M NaCl solution ($R^2 = 0.72$).

Grabowska and Hamm (1979) found that the major contributors to the increased protein extraction in 1.0 M NaCl are large proteins, mainly a complex of actin and myosin, and it is well known that concentrations of NaCl around 1 M cause high dissociation of protein assemblies (salting-in). Reduced extraction of myosin occurs because myosin is salted out of solution at high salt concentrations (Grabowska & Hamm, 1979). During injection, pockets of brine can be formed (Freixanet, 1993; Xargayo, Lagares, Fernandez, Ruiz, & Borrell, 2002), and the tissue adjacent to the pockets might tend to be selectively depleted of sarcoplasmic proteins as the brine moves through into the surrounding tissue, with a corresponding accumulation of these proteins elsewhere (Knight & Parsons, 1988). In dry-salted salmonid fishes, the use of excess water may dilute salt at the surface and partly contribute to discoloration. Protein–astaxanthin extraction is expected to be less during injection and brine salting, due to the high salt concentrations. It is therefore suggested that extensive use of water to remove salt and immersion of the fillets in water, to dilute and to induce homogeneous salt distribution in muscle during smoke processing, is kept to a minimum to avoid extraction of carotenoid–protein complexes.

5. Conclusion

Our results indicate that sarcoplasmic proteins of Atlantic salmon have relatively high concentrations of astaxanthin, and that brine extraction by itself is not

responsible for complete bleaching of the surface carotenoids of salmon fillets. However, considerable amounts of astaxanthin were extracted, along with proteins, near neutral pH, at brine concentrations up to 4 M. Thus, colouration of dry salted salmon fillets for smoke processing may be affected if large amounts of water are used and prolonged exposure to diluted brine occurs, whereas the high salt concentrations used during injection and brine salting may prevent astaxanthin–protein extraction. A water-soluble astaxanthin–protein complex apparently was extracted at neutral pH. Therefore, future studies should focus on the nature of protein–astaxanthin associations in the muscle tissues of salmonid fishes.

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