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Effect of carotenoids from red pepper and marigold flower on pigmentation, sensory properties and fatty acid composition of rainbow trout

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Abstract

Effects of carotenoid sources on pigmentation, sensory properties and fatty acid composition of rainbow trout (*Onchorhynchus mykiss*) were investigated. The fish (120.51 ± 0.75 g) were fed with diets containing 1.8% marigold flower, 5% red pepper, 70 mg kg^{-1} commercial astaxanthin and compared with a control group for 60 days. Commercial astaxanthin provided the highest carotenoid accumulation in the fish, and this was followed by red pepper and marigold flower ($p < 0.05$). Dietary carotenoid sources did not significantly affect fatty acid composition of the fish fillets. Trout muscle coloured with commercial astaxanthin was more preferred than the others by the sensory panellists.

Keywords: Rainbow trout; Pigmentation; Sensory property; Fatty acid composition; Red pepper; Marigold flower

1. Introduction

Consumers are becoming more concerned about how fish are produced and which type of feed ingredients are used. Fish nutrition has an important impact on several parameters directly influencing the quality of fish, some of which are colour and appearance, sensory property and nutritional quality. The need for improved knowledge of fish nutrition is therefore of great importance.

The colour of salmon flesh is one of the most important quality parameters (Sigurgisladottir, Torrissen, Lie, Thomassen, & Hafsteinsson, 1997) because consumers have a preference for red or pink-coloured products of salmonid fishes (Gormley, 1992; Hatano, Takahashi, Takama, & Nakajima, 1987; Ostrander, Martinsen, Liston, & McCullough, 1976; Rounds, Clenn, & Bush, 1992; Sigurgisladottir, Parrish, Lall, & Ackman, 1994; Skonberg, Hardy, Barrows, & Dong, 1998). Therefore feeding fish with carotenoid pigments is regarded as the most important management practice for marketing of farmed salmon (Moe, 1990). Carotenoids are responsible for the typical colour of salmonid muscle, and especially astaxanthin, one of the carotenoids, is the most efficient one used for salmonid pigmentation (Ando, Osada, Hatano, & Saneyoshi, 1992; Storebakken & No, 1992; Torrisen, Hardy, & Shearer, 1989). In addition to pigmentation, carotenoids also have some other significant benefits to human beings; they decrease the risk of some cancer cases, cardiovascular diseases, and some other diseases (Gaziano & Hennekens, 1993; Mayne, 1996; Ziegler, 1989).

Fish are unable to synthesize carotenoids *de novo* (Goodwin, 1984), and these compounds must be obtained through the diet. Carotenoid pigments can be produced commercially and are commonly used for pigmentation of salmonids. However, alternative natural carotenoid sources have also been studied because of public concerns about the use

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of synthetic additives. Red pepper and marigold flower, which are cheap, abundant, and rich in carotenoid pigments, can be considered as alternative sources. While no study on marigold flowers has yet been encountered in the literature, it was shown that red pepper could be used for pigmentation of salmonids (Carter, Palafox, & Islas, 1994; Yanar, Kumlu, Çelik, Yanar, & Tekelioğlu, 1997), However, effects of these carotenoid sources on sensory properties and fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) have not yet been studied. Moreover, present knowledge is largely restricted to commercial carotenoids.

The objective of this study was to investigate changes in fatty acids, sensory properties and pigmentation of rainbow trout fed with red pepper and marigold flower, and compared with commercial astaxanthin.

2. Materials and methods

2.1. Experimental procedure

The experiment was conducted in floating net cages (1 m × 1 m × 1 m) for 60 days in the Sir Dam Lake/Turkey. Thirty rainbow trout, *Onchorhynchus mykiss* (120.51 ± 0.75 g in weight) per cage were stocked in two replicates for each experimental feed. The fish were hand-fed at 3% of their biomass twice a day for 60 days. Throughout the experiment, temperature, dissolved oxygen level and pH of the rearing water were 10.14 ± 1.91 °C, 8.49 ± 2.10 mg/1 and 7.53 ± 0.91, respectively.

2.2. Feeding trial

Four different pelleted feeds used in the experiment were: Diet CO for the control group (basal diet, no addition of carotenoids), Diet AS, supplemented with 70 mg kg^{-1} the commercial astaxanthin (Carophyll-pink, Hoffman La Roche, Switzerland), Diet RP, supplemented with 5% red pepper meal, *Capsicum annum* (containing 70 mg kg^{-1} total carotenoid) and Diet MF, supplemented with 1.8% marigold flower meal, *Tagetes erecta* (containing 70 mg kg^{-1} total carotenoid). The basal diet contained 10% crude fat, 13% crude ash, 45% crude protein, 88% dry matter, 3% crude fibre (supply was obtained from Pinar AŞ, Turkey). To moderate the dietary protein imbalance, due to plant additives between the diet groups, fish meal, including 65% protein, was added to the required level. The ingredients were first turned into a homogeneous doughy consistency by adding water and converted into pellet form by being pressed through a sieve of 4 mm holes in a grinding machine. The pellets were stored in refrigerator containers at −20 °C. Pellets were thawed before they were given to the fish.

2.3. Proximate composition analysis

In both plants, ash and moisture contents were, determined as described by AOAC (1984) methods. Lipid content was determined by the method of Bligh and Dyer (1959). Crude protein content was calculated by converting the nitrogen content, determined by Kjeldhal's method (6.25 × N) (AOAC, 1984). Crude cellulose was determined by the AOAC (1990) method (Table 1).

2.4. Carotenoid analysis

The carotenoid content of samples was extracted by the method of Torrissen and Nævdal (1984). Four fish samples were used for carotenoid analysis. The total carotenoid concentration in the flesh was determined spectrophotometrically in acetone using $E_{(1\%,1\ \mathrm{cm})} = 1900$ (Foss, Storebakken, Schiedt, Liaen, & Austreng, 1984) at 474 nm, and for both plant meals, $E_{(1\%,1\ \mathrm{cm})} = 2500$ (Schiedt & Liaaen-Jensen, 1995) at 450 nm. The total carotenoid concentrations of red pepper and marigold flower meal were determined to be 1400 and 3890 mg kg^{-1}, respectively, and these amounts were taken into account while adding to the basal diet (Table 1).

2.5. Fatty acid analysis

Lipids were extracted by the method of Bligh and Dyer (1959) and stored under nitrogen at −20 °C for further analyses. The fatty acids in the total lipid were saponified into the free form by saponification with 0.5 N methanolic NaOF, followed by esterification with 14% BF_3 (w/v) in methanol (IUPAC, 1979). Esterified samples were analysed using a Thermoquest Trace gas chromatograph equipped with a Supelco-SP-2330 fused-silica capillary column (30 m × 0.25 mm i.d., 0.20 μm film thickness of polyethylene glycol) (Supelco Inc., Bellefonte, PA, USA) and a flame-ionization detector (FID). Helium (30 ml min^{-1}) was used as the carrier gas. The samples were injected at 120 °C. After 2 min the temperature was raised at 5 °C min^{-1} to 220 °C where it was kept for 8 extra minutes. The temperatures of the injector and the detector were set at 240 and 250 °C, respectively. Fatty acid methyl esters were identified by comparing their retention times with those of the commercial fatty acid methyl ester standards. The relative concentrations of each fatty acid were expressed as percentages of their total.

Table 1
Proximate composition and total carotenoid content of natural carotenoid sources

Carotenoid sources	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Total carotenoid content (mg kg^{-1})
Marigold flower	14.17	9.35	15.3	3890
Red pepper	13.68	14.13	19.10	1400

2.6. Sensory analysis

Sensory analyses were carried out using five panellists from the faculty staff. The taste, flavour and colour acceptability of the skinless fillets were determined using a nine-point hedonic scale (1 = like a lot, 9 = dislike a lot; Meilgaard, Civille, & Carr, 1991), Panel tests were repeated three times. The average score for each parameter was calculated. For taste and flavour analyses, the fish samples (skinless fillet) were steamed for 5 min and immediately presented to the panellists. Colour analysis was conducted on raw fillets which had been cut into 5 cm squares, approximately 1.5 cm in height. Visual colour analyses were conducted in individual booths illuminated with white light. The taste and flavour analyses were conducted in a dark room to protect panellists' perceptions of taste from the influence of any colour differences. In order to reach an accurate result, panellists were provided with distilled water to clean their palates after every tasting.

2.7. Statistical analysis

Data percentage of fatty acid composition, pigment levels and weight of the fish were analysed by analysis of variance (one-way ANOVA), and any significant difference was found by Duncan's multiple comparison test (SPSS). Sensory data were analysed by a non-parametric Kruskal–Wallis test.

3. Results and discussion

3.1. Pigmentation and growth

Initial and final carotenoid contents of the fish are summarised in Table 2. Carotenoid contents of the fish fed each of the diets, supplemented with a carotenoid source, were significantly higher than those of the control group at the end of the 60 days ($p < 0.05$). Commercial astaxanthin led to the highest carotenoid accumulation in the fillet of the fish. This was followed by red pepper and marigold flower. In the current study, even though commercial astaxanthin provided better pigmentation (5.62 mg kg^{-1}) than red pepper or marigold flower (similar results were also reported on red pepper, Carter et al., 1994 & Yanar et al., 1997), the levels of carotenoid (4.75–5.27 mg kg^{-1}) obtained with red pepper and marigold flower were found to be adequate for a desired colouration in rainbow trout because a level of 4 mg kg^{-1} in the fish fillet is regarded as a minimum acceptable carotenoid concentration in marketable farmed salmon (Torrisen et al., 1989). In other studies carried out on red pepper, carotenoid contents found in the fish fillet ranged from 1.5 to 5.6 mg kg^{-1}, depending on application time and percentage of red pepper (Akhtar, Gray, Cooper, Garling, & Booren, 1999; Carter et al., 1994; Diler & Gökoğlu, 2004; Ergun & Erdem, 2000; Yanar et al., 1997).

Initial and final weights of the fish are presented in Table 2. Average initial weight of the experimental groups was 120.57 g, and it varied between 233.71 and 247.25 g at the end of the experiment ($p > 0.05$). As found in the literature (Rehulka, 2000), our study also demonstrated that growth was not affected by carotenoid sources in salmonids.

3.2. Fatty acid composition

Fatty acid composition of the rainbow trout fillets is presented in Table 3. Dominant fatty acids in all groups were myristic (C14:0), palmitic (C16:0) and stearic acid (C18:0), palmitoleic (C16;1), oleic (C18:1), linoleic (C18:2), eicosapentaenoic (C20:5) and docosahexaenoic acid (C22:6). It was determined that the total percentage of these particular acids in total fatty acids was 80%.

Although there are similarities among the distributions of fatty acids in total lipids, in general, some differences were found to exist regarding certain fatty acids: the mystric, palmitic and stearic acid proportions of saturated fatty acids in the groups fed with red pepper and marigold flower-supplemented diets were found to be higher than those in the control and astaxanthin groups ($p < 0.05$). In terms of polyunsaturated fatty acids, there were no significant differences between groups; however, eicosapentaenoic and docosahexaenoc acid rates of the groups supplemented with pigment were found to be slightly higher than those in the control group. The total polyunsaturated fatty acids of the group fed with astaxanthin supplement was the higher than those of the three other groups. The red pepper and marigold flower groups followed this, respectively. Akhtar et al. (1999) reported that oleoresin paprika and canthaxanthin supplements did not show a marked effect on the fatty acid composition of the muscle tissues of the trout, but there were some slight differences among certain fatty acids. Similarly, Sigurgisla-

Table 2
Initial and final weights and total carotenoid contents of diet groups

Diet groups	Fish weights (g)		Total carotenoid contents (mg kg^{-1})	
	Initial	Final	Initial	Final
Control	120.51 ± 0.75	245.34 ± 3.73^{a}	0.12 ± 0.16	1.47 ± 0.37^{d}
AS (70 mg kg^{-1})	120.51 ± 0.75	247.25 ± 3.28^{a}	0.12 ± 0.16	5.62 ± 0.25^{a}
RP (%5)	120.51 ± 0.75	238.42 ± 4.23^{a}	0.12 ± 0.16	5.27 ± 0.42^{b}
MF (%1.8)	120.51 ± 0.75	233.71 ± 3.37^{a}	0.12 ± 0.16	4.75 ± 0.65^{c}

Each value is a mean ± s.e. ($n = 2$ replicates). Each replicate consists in measurement of four fish (for weight data) or 30 fish (for carontenoid data). Superscripts marked with different letters are significantly different, from each other, for each sampling day ($p < 0.05$).

Table 3
Fatty acid compositions of the rainbow trout fed different carotenoid sources (% of total fatty acids)

Fatty acids	Diets			
	Control	AS	RP	MF
C12:0	0.11 ± 0.01^{a}	0.11 ± 0.01^{a}	0.11 ± 0.01^{a}	0.11 ± 0.00^{a}
C13:0	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.06 ± 0.00^{a}
C14:0	5.56 ± 0.08^{b}	5.67 ± 0.06^{b}	5.85 ± 0.06^{a}	5.92 ± 0.02^{a}
C15:0	0.67 ± 0.01^{a}	0.67 ± 0.01^{b}	0.73 ± 0.01^{a}	0.73 ± 0.01^{a}
C16.0	16.9 ± 0.06^{c}	17.6 ± 0.05^{b}	18.3 ± 0.2^{a}	18.0 ± 0.07^{a}
C17:0	0.83 ± 0.02^{b}	0.79 ± 0.00^{c}	0.86 ± 0.01^{a}	0.84 ± 0.00^{ab}
C18.0	3.51 ± 0.03^{c}	3.64 ± 0.02^{b}	3.79 ± 0.02^{a}	3.70 ± 0.04^{b}
C20:0	0.24 ± 0.04^{b}	0.29 ± 0.03^{b}	0.35 ± 0.05^{a}	0.33 ± 0.05^{a}
C21:0	0.28 ± 0.03^{a}	0.29 ± 0.01^{a}	0.27 ± 0.00^{a}	0.27 ± 0.01^{b}
C22:0	0.07 ± 0.00^{c}	0.07 ± 0.00^{c}	0.09 ± 0.00^{a}	0.08 ± 0.00^{b}
C23:0	0.14 ± 0.01^{a}	0.09 ± 0.01^{a}	0.14 ± 0.01^{a}	0.16 ± 0.01^{a}
C24.0	0.37 ± 0.01^{a}	0.36 ± 0.00^{a}	0.36 ± 0.01^{a}	0.35 ± 0.00^{a}
C14:1	0.16 ± 0.01^{a}	0.16 ± 0.00^{a}	0.13 ± 0.00^{a}	0.16 ± 0.01^{a}
C15:1	0.12 ± 0.00^{a}	0.12 ± 0.00^{a}	0.16 ± 0.00^{a}	0.13 ± 0.00^{a}
C16:1	6.88 ± 0.04^{b}	7.08 ± 0.03^{a}	6.53 ± 0.06^{c}	6.62 ± 0.01^{c}
C17:1	0.40 ± 0.00^{a}	0.38 ± 0.01^{a}	0.37 ± 0.00^{a}	0.38 ± 0.00^{a}
C18:1*n*-9t	0.41 ± 0.02^{b}	0.30 ± 0.001^{c}	0.48 ± 0.01^{a}	0.48 ± 0.01^{a}
C18:1*n*-9c	19.8 ± 0.09^{b}	20.2 ± 0.07^{a}	18.8 ± 0.09^{d}	19.0 ± 0.07^{c}
C20:1*n*-9	1.21 ± 0.01^{b}	1.23 ± 0.01^{ab}	1.27 ± 0.02^{a}	1.27 ± 0.00^{a}
C22:1*n*-9	0.16 ± 0.00^{a}	0.17 ± 0.01^{a}	0.18 ± 0.01^{a}	0.14 ± 0.02^{b}
C24:1*n*-9	0.34 ± 0.02^{b}	0.34 ± 0.01^{b}	0.40 ± 0.01^{a}	0.37 ± 0.00^{ab}
C18:2*n*-6t	0.41 ± 0.03^{a}	0.33 ± 0.00^{b}	0.30 ± 0.00^{b}	0.37 ± 0.00^{ab}
C18:2*n*-6c	5.68 ± 0.03a	5.21 ± 0.06^{c}	5.50 ± 0.05^{ab}	5.41 ± 0.03^{bc}
C20:2	0.33 ± 0.00^{a}	0.33 ± 0.00^{a}	0.34 ± 0.00^{a}	0.33 ± 0.00^{a}
C22:2	0.81 ± 0.00^{a}	0.81 ± 0.01^{a}	0.77 ± 0.01^{b}	0.77 ± 0.01^{b}
C18:3*n*-6	0.16 ± 0.00^{a}	0.14 ± 0.01^{a}	0.14 ± 0.00^{a}	0.15 ± 0.00^{a}
C18:3*n*-3	1.05 ± 0.01^{a}	1.01 ± 0.01^{a}	1.06 ± 0.04^{a}	1.02 ± 0.00^{a}
C20:3*n*-3	0.18 ± 0.01^{a}	0.19 ± 0.00^{a}	0.18 ± 0.01^{a}	0.19 ± 0.00^{a}
C20:5*n*-3	5.4 ± 0.04^{c}	5.95 ± 0.04^{a}	5.73 ± 0.02^{b}	5.75 ± 0.02^{b}
C22:6*n*-3	14.1 ± 0.03^{b}	14.6 ± 0.11^{a}	14.3 ± 0.11^{a}	14.5 ± 0.13^{a}
Saturated	28.7 ± 0.13^{c}	29.6 ± 0.1b	30.9 ± 0.24^{a}	30.5 ± 0.1^{a}
Monounsaturated	29.5 ± 0.05^{b}	29.9 ± 0.05^{a}	28.3 ± 0.17^{c}	28.56 ± 0.04^{c}
Diunsaturated	7.22 ± 0.02^{b}	6.68 ± 0.07^{b}	6.92 ± 0.17^{c}	6.88 ± 0.17^{b}
Polyunsaturated	20.9 ± 0.1^{c}	21.9 ± 0.13^{a}	21.4 ± 0.19^{b}	21.6 ± 0.03^{b}
Total lipid (%)	4.36 ± 0.15^{a}	4.03 ± 0.06^{b}	3.53 ± 0.09^{c}	4.43 ± 0.16^{a}

Superscripts marked with different letters denote significant differences ($p < 0.05$).

dottir et al. (1994) reported that commercial astaxanthin supplement did not affect lipid content and fatty acid composition of the muscle of Atlantic salmon (*Salmon salar*). In the present study, although there were generally no significant differences among the fatty acid compositions in muscle tissue, some slight differences were observed among some fatty acids, as mentioned above.

3.3. Sensory evaluations

As shown in Table 4, dietary treatment had a significant effect on the colour acceptability scores of the rainbow trout fillets, which suggests that the untrained panellists clearly perceived visual colour differences among the fillets. Raw fillets, from the dietary treatments containing astaxanthin additive, significantly had ($p < 0.05$) more acceptable colour scores than the other three dietary treatments. As expected, pinker-coloured trout muscle was preferred by the sensory panellist. Other researchers (Diler & Gökoğlu, 2004; Sigurgisladottir et al., 1994; Skonberg et al., 1998) have also reported similar results for farm-raised salmon and rainbow trout.

The reason why red pepper-supplement resulted in less visual acceptance is guessed to be that the fillet colour was lighter and the pigmentation showed a heterogeneous appearance, which is contrary to general consumer acceptance.

Raw fillets from the marigold flower-supplemented treatments received significantly lower colour preference scores than the three other dietary treatments. There occurred an undesired yellow pigmentation in the fillet of this group. Similarly, Lee (1987) reported an undesirable yellow pigment deposition in the flesh of channel catfish, which is produced by zeaxanthin and lutein.

Dietary carotenoid sources significantly affected the taste and flavour of the rainbow trout fillets in our study. The lower taste and flavour scores in fillets of rainbow trout were obtained with the control diets. The scores of the other three groups were similar ($p > 0.05$). Waagbø, Sandnes, Torrissen, Sandvin, and Øyvind (1993) have

Table 4
Sensory scores of fillets from rainbow trout fed different carotenoid sources

Sensory scores	Dietary groups			
	Control	AS	RP	MF
Colour (raw fillet)	7.29 ± 0.23^{c}	8.49 ± 0.26^{a}	7.83 ± 0.28^{b}	5.50 ± 0.14^{d}
Taste	7.39 ± 0.22^{b}	8.33 ± 0.27^{a}	8.39 ± 0.27^{a}	8.36 ± 0.21^{a}
Flavour	7.33 ± 0.18^{b}	8.48 ± 0.22^{a}	8.49 ± 0.18^{a}	8.52 ± 0.25^{a}

Superscripts marked with different letters denote significant differences ($p < 0.05$).

shown relationships between diet and fillet composition and sensory quality of Atlantic salmon with regard to dietary lipids, vitamin E and the pigment content of the feed. Josephson, Lindsay, and Stuiber (1991) examined the flavour profile of cooked salmon and identified several compounds derived from astaxanthin. It was concluded that carotenoids serve as direct precursors of salmon loaf-like flavour or as modulators of chemical reactions which convert fatty acids or other lipid precursors to a salmon aroma. Sigurgisladottir et al. (1994) reported that astaxanthin levels in the feed may affect fillet taste and texture.

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