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Effects of Organic and Synthetic Carotenoids on the Sensory Quality and Chemical Composition of Rainbow Trout (*Oncorhynchus mykiss*, W. 1792)

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Abstract: The chemical qualities and sensory properties of rainbow trout given diets containing red pepper (*Oleoresin paprika*) extract, gammarus powder, commercial astaxanthin and canthaxanthin over a 2 month period were compared to those found in the control group used for this study. Statistical differences among the groups with respect to their chemical compositions ($p<0.05$) were found. To measure these differences, the hedonic scale method was used to determine each group's sensory properties, to include general appearance (sensory properties), odour, flavour and colour. Significant changes among the groups were observed ($p<0.05$) and the resultant data revealed that increases in colour were higher in samples fed with aquaculture food substances containing canthaxanthin additives than in the coloration found in the other groups ($p<0.05$).

Key words: Rainbow trout, chemical composition, sensory quality, carotenoid, pigment

INTRODUCTION

Protein-rich foods are an important source of nutrition, particularly today in developed countries where healthy diets are highly encouraged and observed. As a major source of protein, fish is especially important for cardiovascular patients, children, pregnant women and young people because of its rich content of essential amino acids and unsaturated fatty acids (Gorga, 1998; Nettleton and Exler, 1992; Kaya *et al*., 2004).

With aquaculture products becoming increasingly more significant as natural stocks decrease, populations have been pressured in recent years to produce farm fish through the use of breeding techniques. As a result, such techniques now account for producing 41.9 million tons or 31.7% of all global fish requirements (FAO, 2001). The purpose of breeding is to produce fish with high nutritional quality, while also attracting consumers to the product itself. Particularly with trout, the colour of the fish meat is influential in determining its quality (Sigurgisladottir *et al*., 1997; Yanar *et al*., 2007). If using Salmon fish products as a benchmark for coloration, consumers mostly prefer red or pink-coloured fish (Gormley, 2008; Rounds *et al*., 1992; Skonberg *et al*., 1998). To this extent, feeding fish with carotenoid pigments is a critical factor in marketing farmed salmon (Yanar *et al*., 2007; Moe, 1990). The pink color of salmon flesh or muscles, wild or farmed, is caused by the presence of carotenoid pigments, which contain astaxanthin, canthaxanthin, *Oleoresin paprika* and gammarus (Ando *et al*., 1990; Storebakken and No, 1992; Torrissen *et al*., 1995). In addition to its pigmentation value, carotenoids possess other important benefits for human beings. They decrease the risk of cancer in some instances, cardiovascular diseases and other diseases as well (Gaziano and Hennekens, 1993).

However, feeding costs increase considerably, when adding synthetic carotenoids to the food substances used in farming fish. As reported in Norway, using an astaxanthin additive instead of *Salmo salar* (*Atlantic salmon*) increased the feeding costs at a rate of 20-25%, while increasing total production costs at around 10% (Torrissen *et al*., 1989).

Alternative sources to extract natural carotenoid have also been studied due to public concerns over the use of synthetic additives. Consequently, *Oleoresin paprika* and gammarus, rich in carotenoid, inexpensive and in abundance, have been considered as alternative sources.

Thus, the study of rainbow trout was extended for an additional 2 months to determine the specific

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differences in sensory and chemical properties, when mixing commercial astaxanthin, canthaxanthin, red pepper extract and gammarus powder in food substances for breeding. In this way, determining coloration was more efficacious.

MATERIALS AND METHODS

Experiments were conducted at Sinop Inland Waters Fish Breeding Unit at Ondokuz Mayis University. Contained in square-shaped 500 L storage tanks, rainbow trout (*Oncorhynchus mykiss*, W. 1792) as they adapted to their new environment were fed commercial trout food substances over a 15 day period.

From these tanks, 375 fish samples (154.26±1.25) were randomly selected for testing and placed in 15 round-shaped 330 L fibreglass tanks. This process was repeated 3 times. With each iteration the fish samples were fed twice a day over a 60 day period. The domestic water temperature was measured daily at 14.74±0.09°C, while the average pH value and Oxygen (O_2) content of the domestic water supply were measured at 7.99±0.02 and 5.84±0.12 mg L^{-1}, respectively.

Feeding trail: Table 1 displays 5 different diets in rations of food pellets used in the experiment, a carotenoid-free diet for the control group, a diet supplemented with 1% *Oleoresin paprika* (red pepper extract) for the second group, a diet supplemented with 5% gammarus meal for the third, a diet supplemented with 50 mg kg^{-1} commercial astaxanthin (Carophyll-pink (8%), DSM, Basel, Switzerland) for the fourth and a diet supplemented with 70 mg kg^{-1} canthaxanthin (Carophyll-red (10%), DSM, Basel, Switzerland) for the last group.

In the food rations of the 5 groups, the protein content, between 40.81 and 42.11%, contained the same energy values and fat levels (42-43%) as noted in Table 2.

The ration given to the control group had no carotenoid content. An extract of 1% red pepper (*Oleoresin paprika*) was added to the 2nd ration as a natural source of carotenoids for the 2nd group; gammarus powder, also a natural carotenoid, was added to the 3rd ration at a rate of 5% for the third; astaxanthin, a synthetic carotenoid, with an 8% Carophyll pink-content was added to the 4th ration at a rate of 50 mg kg^{-1} for the 4th and canthaxanthin, another synthetic carotenoid, with a 10% Carophyll-red content was added to the 5th ration at a rate of 70 kg mg^{-1} for the last group. The fish were fed manually twice a day at 9:00 am and 16:00 pm, *ad libitum*, according to feed demand criteria during the observation periods.

Determination of total carotenoid content in fish meat and feeding substances: Spectrophotometric methods, described by Foss *et al.* (1984) were used in determining the total carotenoid content in the fish meat. The absorbance values of the samples as against pure acetone were read on the spectrophotometer (Jasco-V-530 UV/VIS spectrophotometer) at λ_{max} (470-475) and the results were calculated in terms of the total amounts of carotenoid content in the fish meat measured in mg kg^{-1} units. The value $E_{1.1\%\ cm}$ = 1900 was used for astaxanthin and canthaxanthin in calculating these measurements (Skrede and Storebakken, 1986; Choubert and Storebakken, 1989).

AOAC spectrophotometric analysis methods, as applied by Akhtar *et al.* (1999) were utilized in the carotenoid analyses in mixed food substances that had been prepared with both natural and synthetic colour

Table 1: Rations of food substances used in the experiment (%)

Feeding material	Control	Red pepper	Gammarus	Astaxanthin	Canthaxanthin
Fish meal	40.00	40.00	39.55	40.00	40.00
Fish oil	15.14	14.91	14.51	15.14	15.14
F.F. Soy[7]	14.64	10.03	13.31	14.64	14.64
Durum flour	7.00	7.00	7.00	7.00	7.00
S.S.P.[7]	8.26	7.49	4.33	8.26	8.26
Soy pulp	7.36	12.97	9.69	7.36	7.36
Soy protein concentrate	7.00	6.00	6.00	7.00	7.00
Vitamin mix[1]	0.20	0.20	0.20	0.20	0.20
Mineral mix[2]	0.15	0.15	0.15	0.15	0.15
Choline	0.15	0.15	0.15	0.15	0.15
Dinaferm	0.10	0.10	0.10	0.10	0.10
K.B. extrati[3]	-	1.00	-	-	-
Gammarus powder[4]	-	-	5.00	-	-
Astaxanthin[5]	-	-	-	50.0 mg kg^{-1}	-
Canthaxantin[6]	-	-	-	-	70.0 mg kg^{-1}

[1]Vitamin mix: Rovimix 107 (g kg^{-1} mix); Vitamin A 8000 IU, Vitamin D3 800 IU, Vitamin E 80 mg, Vitamin K3 4.8 mg, Vitamin B1 8 mg, Vitamin B2 12 mg, Vitamin B6 8 mg, Vitamin B12 0.02 mg, Vitamin C 8 0 mg, Niasin 80 mg, Folic acid 2.4 mg, Calcium D-Pantothenate 20 mg, Biotin 0.2 mg, Inositol 120 mg; [2]Mineral mix: Remineral B fish 97 (g kg^{-1} mix); Fe 97.5 mg, Cu 18.75 mg, Mn 135 mg, Cb 0.6 mg, Zn 120 mg, 12.7 mg, Se 0.225 mg; [3]Red pepper extract: Aromeks Ltd. Co., Istanbul, Turkey; [4]Gammarus powder: Sinop, Turkey; [5]Astaxanthin: Carophyll® pink (8%), DSM, Basel, Switzerland; [6]Canthaxanthin: Carophyll® red (10%), DSM, Basel, Switzerland; [7]F.F. Soy: Full Fat Soy; S.S.P.: Sunflower Seed Pulp

Table 2: Food material contents of rations used in the experiment (dry-weight basis)

Diet groups	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude cellulose (%)	Crude ash (%)	NFE* (%)
Control	2.27	42.40	26.64	3.27	8.93	18.76
Red pepper	4.50	42.73	25.71	3.31	8.95	19.30
Gammarus	3.45	43.61	26.17	3.19	9.55	17.48
Astaxanthin	3.58	43.07	25.25	3.07	8.76	19.85
Canthaxanthin	2.41	43.13	25.85	3.05	8.71	19.26

*Nitrogen-Free Extract

additives. An extraction coefficient of acetone ($E_{1.1\%\ cm}$) was taken as 1922 in calculating the total carotenoid amounts in feed and fish samples prepared with *Oleoresin paprika* (red pepper extract) content (Lai *et al*., 1996). The value $E_{1.1\%\ cm}$ = 1900 was used in calculations for astaxanthin and canthaxanthin additives (Skrede and Storebakken, 1986; Choubert and Storebakken, 1989).

Proximate composition analysis: In both plants, the crude protein content was determined by converting the nitrogen content described in Kjeldahl's method (6.25 x) (AOAC, 1984). Lipid content and crude cellulose were calculated using AOAC (1984) methods; ash and moisture content were made by AOAC (1990) methods.

Sensory analyses: Sensory analyses were performed using ten experienced panellists. The general appearance, taste, flavour, texture, interior appearance and acceptable colouring of the fillets were determined using a 9 point hedonic scale: 9, extremely like; 8, really like; 7, moderately like; 6, slightly like; 5, neither like nor dislike; 4, slightly dislike; 3, moderately dislike; 2, really dislike and 1, extremely dislike (Özogul *et al*., 2000).

The average score for each parameter was calculated. The panel test was repeated 3 times. Then the overall average score for each parameter was calculated. The fish samples were then cooked in a microwave oven for 10 min for taste analyses and then presented to the panellists. All assessments took place in individual booths under daylight conditions. Panel tests were conducted one after the other 3 different times and a total of 10 fish samples were used for each panel. Panellists were asked to judge the colours of the fillets, i.e., gradations of pink to red, on a scale of 11-18 and annotate their responses on the associated cards.

Statistics: SigmaStat was used to categorize the data, according to an analysis of the variance ANOVA performed. Differences in mean values were then determined using Turkey's statistical analysis system (Zar, 1999) (Biostatistical Analysis 4th Edn. Prentice-hall, Englewood Cliffs, New Jersey, 929).

RESULTS AND DISCUSSION

Proximate composition: Concentrations of carotenoid in fish meat both at the beginning and end of the experiment are summarized in Table 3. As observed, the canthaxanthin group ($p<0.05$) contained the highest concentration of carotenoid, followed by oleoresin paprika, astaxanthin, gammarus and control groups, respectively.

According to a previous study, red pepper is less effective than astaxanthin sources used in feeding trout on a commercial basis (Carter *et al*., 1994; Yanar *et al*., 1997). But undesirable colours and insufficient concentrations of carotenoid were not observed in this study, as well as the tests conducted by Ingle de la *et al.* (2006). Erdem and Ergun (2000) could not obtain a sufficient concentration of carotenoid, when adding a 3-6% portion of red pepper powder to the ration. But Carter *et al.* (1994) 25 and Yanar *et al.* (1997) reported that concentrations of carotenoid achieved in their studies were sufficient. This evaluation was based on Skerede *et al*. (1990) contention that a 4.4 mg kg^{-1} concentration of carotenoid is sufficient for pink-coloured fish meat requested in the market and in a similar manner, on Torrisen *et al.* (1995), who argued that a 3-4 mg kg^{-1} concentration is sufficient for salmon products.

Yet, realistically such concentrations must be kept at a level above 4 mg kg^{-1} due to the loss of carotenoids, while being processed. Therefore, as noted above, the concentrations of carotenoid attest to the requisite values for pigmentation in the canthaxanthin, red pepper (*Oleoresin paprika*) and astaxanthin groups, consistent with Skrede and Storebakken's (1986) conclusion that a 4.4 mg kg^{-1} concentration is necessary to obtain a pink-coloured quality.

The concentration value of carotenoid in the gammarus group is rather low and close to the value of the control group ($p>0.05$). As a result of chemical analysis, sufficient pigmentation may be reached by adding red pepper extract (*Oleoresin paprika*), a natural source, to the rations, just as with synthetic sources, such as astaxanthin and canthaxanthin.

Table 4 shows the changes in chemical compositions of the fish samples with food substances with astaxanthin, canthaxanthin, *Oleoresin paprika* and gammarus additives.

According to Table 4, the highest amount of crude protein was observed in trout fed with gammarus (42.12±0.633). There were also other important differences among the groups ($p<0.05$), The amount of crude fat in

Table 3: Total concentrations of carotenoid in food substances for the 5 groups as well as in the fish samples at the beginning and end of the experiment

Groups	Control group	*Oleoresin paprika*	Gammarus	Astaxanthin	Canthaxanthin
CCF (mg kg^{-1})	-	765 (1.%)	21.54 (5%)	46.63 (50)	76.49 (70)
Initial CCM	0.86±0.01^{a}	0.86±0.01^{a}	0.86±0.01^{a}	0.86±0.01^{a}	0.86±0.01^{a}
End-of-study CCM	1.08±0.06^{a}	6.19±0.02^{b}	1.37±0.01^{a}	6.11±0.01^{b}	10.07±0.22^{c}

Each value incorporates an average±standard deviation; Values stated using different exponential letters in the same line are different in statistical meaning ($p<0.05$), CCF: Carotenoid Concentration in Feed material (mg kg^{-1}); CCM: Carotenoid Concentration in fish Meat (mg kg^{-1})

Table 4: Food substance content and carotenoid amounts (dry-weight basis) for fish samples used in the experiment

Groups	Crude protein (%)	Crude fat (%)	Crude cellulose (%)	Ash (%)	Dry weight (%)	Carotenoid (%)
Control	41.44±0.088^{b}	26.64±0.060^{d}	3.20±0.004^{c}	8.90±0.038^{b}	24.83±0.780^{b}	-
Oleoresin paprika	40.81±0.025^{a}	25.71±0.185^{b}	3.16±0.008^{c}	8.95±0.028^{b}	24.57±0,874^{a}	1.00^{c}
Gammarus	42.12±0.633^{c}	26.17±0.585^{c}	3.08±0.015^{b}	9.52±0.030^{c}	24.78±0,642^{b}	5.00^{d}
Astaxanthin	41.53±0.141^{b}	25.25±0.200^{a}	2.96±0.009^{a}	8.73±0.028^{a}	25.74±0,788^{c}	0.05^{a}
Canthaxanthin	42.09±0.004^{c}	25.85±0.215^{b}	2.98±0.075^{a}	8.72±0.038^{a}	24.59±0.409^{a}	0.07^{b}

a,b,c,d: Differences between groups expressed with different letters in the same column are important

Table 5: General appearance, internal appearance, texture, taste and odour results in fish groups fed with different diets

Groups	General appearance	Interior appearance	Texture	Taste	Odour	Average
Control	8.07±0.765	7.90±0.797	8.05±0.703	7.27±0.600	7.46±0.772	7.75
O. paprika	8.16±0.685	7.95±0.689	8.08±0.657	8.10±0.669	7.66±0.578	7.99
Gammarus	7.71±1.142	7.25±0.747	7.55±0.726	6.81±0.034	7.41±0.682	7.35
Astaxanthin	7.83±0.607	7.65±0.609	7.68±0.614	7.69±0.580	8.19±0.747	7.81
Canthaxanthin	8.09±0.004	8.18±0.348	8.38±0.345	8.02±0.706	7.99±0.248	8.33

both the control and gammarus groups were higher ($p<0.05$) than that in the other groups. The amount of cellulose in the control group was considerably higher than that in the astaxanthin and canthaxanthin groups ($p<0.05$); The amount of ash was at a maximum level in the gammarus group and the amount of dry-weight based materials in samples fed with astaxanthin was considerably higher than that in the other groups ($p<0.05$).

Yanar *et al.* (1997) detected the amounts of crude protein, crude fat and crude fibre in trout fed with red pepper extract in wet materials as 13.68, 14.13 and 19.20%, respectively. Shahidi *et al.* (1994) fed the trout with Arctic char (*Salvelinus alpinus*), astaxanthin and canthaxanthin and found the following quantities on a dry-weight basis (dwb): the amount of protein was 56.3±0.67% in the astaxanthin group; 58.1±0.34% in the canthaxanthin group, the amount of fat was 15.76±0.03% in the astaxanthin group and 19.67±0.09% in the canthaxanthin group.

The content of carotenoid varied considerably among all the groups ($p<0.05$). For example, while the control group had no carotenoid content, the highest quantity was found in the canthaxanthin group or 70 mg kg^{-1} (Table 4). Diler and Gökoglu (2004) observed the quantities of carotenoid found in trout fed over a 3 month period to be 0.65, 7.98 and 5.10 mg/100 g for the control, astaxanthin and red pepper extract groups, respectively. Torrissen and Naevdal (1984) and Peterson *et al.* (1966) calculated the amounts of carotenoid in the red pepper extract to be 50 and 3.2 mg/100 g.

Sensory analyses: Results of sensory analyses are shown in Table 5. The highest scores for general appearance, internal appearance and texture were observed in fish samples with diets containing canthaxanthin. The highest scores for taste and odour criteria were observed in the fish samples with diets containing red pepper and astaxanthin, respectively. Appearance, taste and odour scores obtained for fish samples with diets containing canthaxanthin, red pepper and astaxanthin were significantly ($p<0.05$) higher than those with other diets. Scores did not differ on texture among the fish samples with diets containing canthaxanthin and red pepper, but did differ significantly in the control group's diet. Yanar *et al.* (1997) using red pepper and astaxanthin in feeding trout, reported results more positive than those in the control group.

Astaxanthin is the main carotenoid pigment found in pink-red-coloured aquatic species. Compared to other sources of carotenoid, synthetic carotenoids are more easily absorbed and retained in the muscles of rainbow trout (Diler and Gökoglu, 2004; Torrissen and Naevdal, 1984). Since, the cost of dietary supplements of synthetic carotenoid is relatively expensive and reaches up to 10-15% of the total feeding cost, sources of natural carotenoid have been used in salmons diets. Reportedly, 90-95% of the pigments in red crab processing waste, shrimp waste and crayfish contain some form of astaxanthin. One possible use of crustacean waste materials is as a source of carotenoids, protein, lipids and other nutrients for fish grown in aquaculture (Kamata and Simpson, 1992).

Red-pepper meal is used for fish as a natural source of pigmentation (Diler and Gökoglu, 2004).This meal is added to food substances at rates of 0.5-10% as colour differences are observed over 4-6 weeks periods (Halver, 1989). Peterson *et al.* (1966) fed paprika extract to trout. Coloration was detected after 2 weeks; fairly prominent after 4 weeks.

CONCLUSION

This study substantiates that pigment substances added to trout feed influence the sensory quality in fish samples to a considerable extent. As observed, these substances changed the colours of the products and surprisingly had positive effects on their odour, especially, when using red pepper extract (*Oleoresin paprika*) additives. We may also conclude that the panellists were impressed by the colours of the fish samples. Colour characteristics are equally important as general appearance, odour and freshness in terms of affecting the prices of fishery products in the market (Torrissen *et al*., 1995).

Consequently, lower-priced natural pigment substances, such as red pepper extract (*Oleoresin paprika*) and gammarus, when compared to synthetic additives, are a greater and more important food source in fish farming.

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