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Determination of astaxanthin stereoisomers and colour attributes in flesh of rainbow trout (*Oncorhynchus mykiss*) as a tool to distinguish dietary pigmentation source

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5 **Determination of astaxanthin stereoisomers and colour attributes in flesh of**
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7 **rainbow trout (*Oncorhynchus mykiss*) as a tool to distinguish the dietary**
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9 **pigmentation source**
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Abstract

The presence of carotenoids in animal tissue reflects their sources along the food chain. Astaxanthin, the main carotenoid used for salmonid pigmentation, is usually included in the feed as a synthetic product. However, other dietary sources of astaxanthin such as shrimp or krill wastes, algae meal or yeasts are also available on the market. Astaxanthin possesses two identical asymmetric atoms at C-3 and C-3' making possible three optical isomers with all-*trans* configuration of the chain: $3S,3'S$; $3R,3'S$; $3R,3'R$. The distribution of the isomers in natural astaxanthin differs from that of the synthetic product. This latter is a racemic mixture, with a typical ratio of 1:2:1 ($3S,3'S$: $3R,3'S$: $3R,3'R$), while astaxanthin from natural sources has a variable distribution of the isomers deriving from the different biological organism that synthesised it. The high performance liquid chromatographic (HPLC) analysis of all-*trans* isomers of astaxanthin was performed in different pigment sources, such as red yeast *Phaffia rhodozyma*, alga meal *Haematococcus pluvialis*, krill meal and oil, and shrimp meal. With the aim to investigate astaxanthin isomer ratios in flesh of fish fed different carotenoid sources, three groups of rainbow trout were fed for 60 days diets containing astaxanthin from synthetic source, *Haematococcus pluvialis* algae meal and *Phaffia rhodozyma* red yeast. Moreover, the distribution of optical isomers of astaxanthin in trout purchased on the Italian market was investigated. A characteristic distribution of astaxanthin stereoisomers was detected for each pigment sources and such distribution was reproduced in the flesh of trout fed with that source. Colour values measured in different sites of fillet of rainbow trout fed with different pigment sources showed no significant differences. Similarly, different sources of pigment (natural or synthetic) produced colour values of fresh fillet with no relevant or significant differences. The coefficient of distance computed amongst the feed ingredient and the trout fillet astaxanthin stereoisomers was a useful tool to identify the origin of the pigment used on farm.

Keywords:-**Introduction**

Carotenoids are widely distributed in nature and present in many plants, algae, micro-organisms and animals. Although carotenoids are largely diffused in the plant and animal kingdoms, they are synthesized *de novo* only in higher plants and protists (Goodwin, 1992). Consequently, the traceability of carotenoids in animal reflects the presence of their sources along with the food chain. Animal carotenoids mainly result from metabolic transformation (oxidation and/or reduction) of those present in the food chain. Aquatic animals constitute a considerable rich source of carotenoids, which are in many cases responsible of organoleptic characteristics considered by the consumer (bright colours in lobster, shrimp, salmon, fish eggs) (Shahidi et al. 1998, Matsumo 2001). Moreover, among the large number of pigments present in living organisms (i.e. chlorophylls, anthocyanins and porphyrins), carotenoids play an important role in protecting cells against photosensitised oxidation (Palozza and Krinsky 1992).

In farmed salmonid, astaxanthin is mainly used for pigmentation and it is usually included in the feed as a synthetic product. However, other natural dietary sources of astaxanthin such as shrimp or krill wastes, algae meal or yeasts are also available on the market. All-*trans* astaxanthin may exist as three stereoisomers: two enantiomers ($3R,3'R$ and $3S,3'S$) and a *meso* form ($3R,3'S$; $3S,3'R$). The distribution of the all-*trans* stereoisomers in natural astaxanthin differs from that of the synthetic product. This latter is a racemic mixture of three isomers, with a typical ratio of 1:2:1 ($3S,3'S$: $3R,3'S$: $3R,3'R$), while astaxanthin from natural sources has a

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variable distribution of the isomers deriving from the different biological organism that synthesised it.

Crustaceans belong to the class of marine animals that can oxidise carotenoids at the 3,3' and 4,4' position. Particularly, shrimp (*Penaeus spp.*) are able to convert by oxidative transformation dietary carotenoids such as β -carotene, zeaxanthin and canthaxanthin into astaxanthin and they are also able to absorb astaxanthin (Matsumo 1992, Schiedt et al. 1993, Sachindra et al. 2005). Similarly, in krill (*Euphausia spp.*) astaxanthin and its esters represent the major carotenoid component. A metabolic transformation to astaxanthin stereoisomers from other carotenoid sources is hypothesized as well in zooplanktonic crustaceans (Takaichi et al. 2003).

The red yeast *Phaffia rhodozyma* produces and accumulates carotenoids in lipid droplets into the cytoplasmatic membranes. Andrewes et al., (1976) reported that free astaxanthin was the most abundant carotenoid in *Phaffia* with 83-87% of the total pigment mixture. Other carotenoids such as β -carotene (2-2.5% of total carotenoids), echinenone (2-4% of total carotenoids), 3-hydroxyechinenone (3-4.5% of total carotenoids) and phoenicoxanthin (5-7% of total carotenoids) were also isolated red yeast culture. The predominant optical isomer of *Phaffia rhodozyma* unesterified astaxanthin is the 3*R*,3'*R* form.

The *Haematococcus pluvialis* microalgae mainly contains astaxanthin monoesters linked to short chain fatty acids. The astaxanthin pool of encysted *Haematococcus* is approximately 70% monoesters, 25% diesters and 5% free (Lorenz and Cysewski 2000). All of free astaxanthin and its mono and di- esters in *Haematococcus pluvialis* have predominantly the 3*S*,3'*S* form. Since commercially grown *Haematococcus pluvialis* can accumulate >30 g astaxanthin of dry matter,

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4 the alga meal represents a good source of natural astaxanthin that found an increasing use in
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6 nutrition of farmed fish (Olaizola and Huntly 2003).
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11 The distribution of astaxanthin stereoisomers has been investigated in different tissues of
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13 crustaceans (Renstrøm et al 1981, Coral-Hinostroza and Bjerkeng 2002), trout (*Oncorhynchus*
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15 *mykiss*) (Storebakken and No 1992, Bjerkeng et al. 1997, Østerlie et al. 1999,) and salmon
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17 (*Oncorhynchus spp* and *Salmo salar*) (Arai et al. 1987, Turujman et al. 1997). Astaxanthin
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19 deposition in white muscle of salmonid reflects the optical isomers ratio of dietary astaxanthin
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21 (Foss et al. 1984, Storebakken and No, 1992, Storebakken et al. 2004). On the contrary, the
22
23 distribution of astaxanthin stereoisomers in skin and posterior kidney of rainbow trout has been
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25 observed to be different from that of dietary source, indicating some selectivity in the tissue
26
27 metabolism of the optical isomers of astaxanthin. In particular Østerlie et al. (1999) found in
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29 rainbow trout fed synthetic astaxanthin an higher percentage of 3S,3'S isomer and a lower
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31 percentage of 3R,3'R isomer in kidney and skin, if compared to the percentage of optical
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33 isomers in the diet, suggesting a degree of epimerization of (3S,3'S)-astaxanthin in these tissues.
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42 High performance liquid chromatographic (HPLC) analysis of all-*trans* isomers of astaxanthin
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44 has been described in recent years by several authors (Vecchi and Müller 1979, Maoka et al.,
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46 1985, Turujman 1993, Turujman et al. 1997, Østerlie et al. 1999). The separation of optical
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48 isomers could be achieved on an achiral column after derivatization with a suitable derivatizing
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50 reagent in order to obtain the diastereoisomers, that could be easily resolved on an achiral phase
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52 column. The derivatizing reagent more used for this purpose in literature is camphenic acid
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54 chloride (Vecchi and Müller 1979, Bowen et al. 2002). Another approach is to perform direct
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56 HPLC analysis of underivatized astaxanthin on a chiral column. This latter method permits to
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4 avoid various problems encountered during the derivatization step and to reduce the analysis
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7 time to less than 15 minutes (Turujman 1993, Turujman et al. 1997).
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11 This study was designed in order to investigate the ratios of optical isomers of astaxanthin from
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13 different synthetic and natural sources used for rainbow trout pigmentation. Different
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15 ingredients such as yeasts of the genus *Phaffia* (*Phaffia rhodozyma*), *Haematococcus pluvialis*
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17 algae meal, Antarctic krill meal, krill oil and shrimp meal were analysed. Afterwards, with the
18
19 aim to investigate astaxanthin isomer ratios in flesh of fish fed different carotenoid sources,
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21 three groups of rainbow trout were fed for 60 days diets containing 60 mg kg⁻¹ of astaxanthin
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23 from synthetic source, *Haematococcus pluvialis* algae meal and *Phaffia rhodozyma* yeast.
24
25 Moreover, in order to investigate the reliability of the optical isomers distribution to distinguish
26
27 the type of pigmentation of commercially farmed trout available on the Italian market, nine
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29 specimens of pigmented rainbow trout farmed in different aquaculture plants of Northern Italy
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31 were also purchased in local retailers and analysed.
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40 **Materials and methods**

41 42 43 44 *Chemicals and reagents*

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46 Standard astaxanthin (3,3'-dihydroxy- β,β -carotene-4,4'-dione) was a gift from Hoffmann-La-
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48 Roche Inc. (Basel, Switzerland).
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51 Hexane, tetrahydrofuran (THF), methylene chloride, 2-propanol, acetonitrile, acetone,
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53 petroleum ether were LC grade and from Merck (Darmstadt, Germany); dimethyl sulfoxide
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55 (DMSO), triethylamine, sodium sulphate decahydrate and sodium sulphate anhydrous supplied
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57 by Sigma-Aldrich (St. Louis, MO, USA). Cholesterol esterase from *Pseudomonas fluorescens*
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4 used for hydrolysis of carotenoids esters was purchased from Sigma (St. Louis, MO, USA). Sep-
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6 Pak silica gel cartridges were from Waters (Milford, MA, USA).
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10 11 *Feed ingredients*

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13 Unesterified astaxanthin (Carophyll[®] pink) and canthaxanthin (Carophyll[®] red) were from
14
15 Hoffmann-La-Roche Inc. (Basel, Switzerland), microalgae meal from *Haematococcus pluvialis*
16
17 (Naturrose[®]) purchased from Cyanotech Corporation (Kailua-Kona, HI, USA) and red yeast
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19 meal (Astaxin[®]) (*Phaffia rhodozyma*) was from Igene Biotechnology Inc. (Columbia, MD,
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21 USA). Antarctic krill meal, krill oil and shrimp meal were supplied by Tepual SA (Santiago,
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23 Chile).
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31 *Fish and experimental conditions*

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33 Three outdoor raceways (L=50 X W=4 X H=1.5) were stocked each with 1900 kg rainbow trout
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35 (average weight 350 g) and were reared under normal production scale for 60 days. During the
36
37 trial, oxygen levels were always over 80% saturation and water temperature was 11±1 °C. Three
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39 pigmented diets were used in this study. Diet 1 contained Carophyll Pink (CP), diet 2 contained
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41 *Haematococcus pluvialis* algae meal (HP) and diet 3 contained *Phaffia rhodozyma* red yeast
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43 (PR). The level of inclusion in all three diets was 60 mg kg⁻¹ astaxanthin (free or esterified). The
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45 three groups of fish were fed with commercial extruded feed (41% protein, 26% fat) coated with
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47 different synthetic source. The feed ratio was set at 1% of the biomass per day. No mortalities
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49 occurred during the experiment. At the end of the trial, ten fish per group were taken and
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51 transported to the laboratory for colour measurements and astaxanthin analysis. Parallely, nine
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53 specimens of pigmented rainbow trout from unknown origin were purchased from local retailers
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55 and analysed.
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7 *Astaxanthin extraction from feed ingredients*
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9 Carotenoids from algae and yeast pigments were extracted by suspending 25 mg of sample in 5
10 mL of dimethyl sulfoxide (DMSO) and incubating in a thermostatic shaking bath at 50°C for 30
11 minutes. At the end, the sample was centrifuged at 4000 rpm for 10 minutes and the supernatant
12 collected. The obtained pellet was homogenized for 2 min using an UltraTurrax (Janke &
13 Kunkel, IKA, Staufen, Germany) in 5 mL of acetone, then centrifuged at 4000 rpm for 15
14 minutes. Acetone extraction was repeated until the organic phase resulted colourless. Similarly,
15 acetone extraction was used with Antarctic krill meal, krill oil, shrimp meal and with synthetic
16 astaxanthin source. To avoid oxidation and isomerisation of astaxanthin, all manipulations were
17 performed in diffuse light and conditioned room (20°C), under acid-free conditions and using
18 pure peroxide-free solvents (Liaaen-Jensen 1971).
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35 *Enzymatic hydrolysis of Haematococcus pluvialis extract*
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37 Three mL of *Haematococcus* acetone extract was added to 2 mL of Tris HCl (0.05 M, pH 7.00)
38 were added and put in a thermostatic bath at 37°C for 2 minutes. Then, 100 µL of cholesterol
39 esterase solution (50 unit/mL) were added and incubated in a water bath at 37°C for 30 minutes
40 with gently shaking. After the incubation 1.0 g of sodium sulphate decahydrate and 2 mL of
41 petroleum ether were added and mixed vigorously by vortex. The upper petroleum ether phase
42 was collected in a clean tube and the procedure repeated two times to make the organic phase
43 clear. Petroleum ether phase containing astaxanthin was added with sodium sulphate anhydrous
44 mixing by vortex for 30 seconds and the ethereal phase transferred in a clean tube, then dried
45 under a stream of nitrogen and reconstituted in 200 µL of hexane.
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Colour measurements in fillet

The fish were decapitated, gutted, filleted and the right fillet used for colour analysis. Colour measurements of the flesh samples were performed in the following sites: posterior to the head (neck), at the center below the dorsal fin (center) and anterior to the caudal fin (tail). Colour was measured using a Minolta Chroma Meter II Reflectance instrument (Minolta Camera Co. Ltd., Osaka, Japan). Results were recorded as a^* , b^* , L^* values where a^* describes redness, b^* yellowness and L^* lightness. Chroma values (brightness, C^*) were calculated according to the following equation: $C^* = \sqrt{a^{*2} + b^{*2}}$. Two measurements were taken on each site and the measuring head was rotated 90° between each measurement. The left fillet was packed under vacuum and stored at -20°C until astaxanthin analysis, which was performed within a few days.

Astaxanthin extraction from fish fillets

Astaxanthin was extracted from flesh according to the method of Turujman et al. (1997). Briefly, 10 g of tissue were sampled at the centre of the fillet and homogenized using an Ultra Turrax for 2 min with 10x2 mL of hexane, to remove most of lipids. The homogenate was centrifuged at 4000 rpm for 10 minutes and hexane phase was discharged. Astaxanthin was extracted from the pellet resulted after hexane extraction with 10x2 mL of acetone, following the procedure mentioned above. The acetone phase was evaporated with a rotary evaporator (Rotavapor R 110, Büchi, Switzerland) and the aqueous residue (approximately 4 mL) reconstituted in 20 mL of methylene chloride and vigorously swirled to dissolve astaxanthin. The residual water was removed with a separatory funnel and the organic phase was dried over approximately 1 g of anhydrous sodium sulfate. The methylene chloride phase containing astaxanthin was transferred in a clean tube. The amount of astaxanthin extracted was determined

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4 recording the volume and measuring the absorbance of the methylene chloride extract a 474 nm
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6 using a Jasco model V-530 spectrophotometer (Ishikawa-cho, Japan).
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10 11 *HPLC analysis of astaxanthin*

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13 The astaxanthin was purified by loading the methylene chloride phase onto a silica gel Sep-Pak
14 cartridge (Waters, Milford, MA, USA) pretreated with hexane. Astaxanthin was eluted from the
15 cartridge with 5 mL of chloroform and the eluate dried under a stream of nitrogen. Dry residue
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17 was dissolved in 200 μ L of hexane and 20 μ L were injected into the HPLC system.
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23 The HPLC system consisted of a Jasco model 980-PU pump (Ishikawa-cho, Japan), a Jasco
24 model LG-980-02 gradient unit, a Jasco model AS-950 autosampler and a Waters model 996
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26 Photodiode Array Detector (Waters, Milford, MA, USA). Chromatograms were recorded and
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28 integrated on a Millennium 32 Chromatography Software (Waters, Milford, MA, USA).
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32 Analyses were performed with a Pirkle L-leucine chiral column (Regis Technologies, Morton
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34 Grove, IL, USA). Mobile phase was hexane-tetrahydrofuran-triethylamine-acetonitrile
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36 (77:19:2:2 v/v) at a flow rate of 1.5 mL min⁻¹. Isocratic condition and ambient temperature were
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38 used, and the monitoring wavelength was 474 nm. In these conditions the retention times of the
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40 3*S*,3'*S*, meso and 3*R*,3'*R* forms were approximately 10.5, 11.5 and 12.5 minutes, respectively.
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43 Each astaxanthin extract was analyzed in duplicate.
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49 *Statistical analysis*

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51 Data are reported as mean value \pm standard error of the mean. Homogeneity of variance was
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53 confirmed and comparison between means was by one-way ANOVA. Significance was
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55 accepted at probabilities of 0.05 or less.
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For comparison of whole astaxanthin stereoisomers profile of trout fillet and the pigment ingredients of the diet, the coefficient of distance D (McIntire et al. 1969) between “reference” and “test” samples (pigment source and trout fillet, respectively) was computed.

This coefficient was originally developed to estimate the similitude of aquatic animals on the base of their fatty acids composition. Therefore, the smaller the value of the coefficient of distance D the higher the probability that trout have been fed with a diet containing that specific pigment source.

For comparison of the whole astaxanthin stereoisomers profile of “reference” (feed ingredient) and “test” (trout fillet), the coefficient of distance D (McIntire *et al.*, 1969) was computed using the following equation:

$$D_{jh} = \left[\sum_{i=1}^n (P_{ij} - P_{ih})^2 \right]^{1/2}$$

where, D_{jh} is the degree of difference (coefficient of distance) between samples j (“reference” diet) and h (“test” trout fillet), P_{ij} and P_{ih} are percentage of astaxanthin stereoisomers i in sample j and h , for each i stereoisomers. All the statistical analysis were performed by SPSS 12.0 (SPSS Inc. Chicago, Illinois).

Results and Discussion

In Figure 1 the chromatograms of a standard solution ($32 \mu\text{g mL}^{-1}$) of synthetic astaxanthin and of the extracts obtained from different sources are presented. The three peaks corresponding to 3*S*,3'*S* isomer, meso form and 3*R*,3'*R* isomer were eluted at 10.5, 11.5 and 12.6 minutes, respectively.

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4 Percentage distribution of stereoisomers was calculated from chromatograms of different
5 pigment extracts by means of relative peak area measurements. The results are presented in
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7 Figure 2. A characteristic distribution of astaxanthin optical isomers was detected for each
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9 pigment sources and such distribution was reproduced in trout fed with that source.
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11 *Haematococcus pluvialis* algae meal contained 87.6% of the 3*S*,3'*S* enantiomer, 10.1% of the
12 meso form and 2.3% of the 3*R*,3'*R* enantiomer, while the red yeast *Phaffia rhodozyma* contained
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14 1.5% of the meso form and 98.5% of the 3*R*,3'*R* enantiomer. The values of optical isomers in
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16 krill meal and krill oil were similar. In fact, 27.4% of 3*S*,3'*S* enantiomer, 21.8% of the meso
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18 form and 50.8% of 3*R*,3'*R* enantiomer were found in the krill meal whilst 35.8% of 3*S*,3'*S*
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20 enantiomer, 13.6% of the meso form and 50.6% of 3*R*,3'*R* enantiomer were found in krill oil.
21
22 On the other hand, shrimps meal contained 65.4% of 3*S*,3'*S* enantiomer, 27.9% of the meso
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24 form and 6.7% of 3*R*,3'*R* enantiomer. These results were consistent with literature values. In
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26 particular, Foss et al. (1987) analysed the content of astaxanthin stereoisomers in several species
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28 of crustaceans and demonstrated that in *Euphausia superba* (krill) the 3*R*,3'*R* isomer
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30 predominated, whereas in *Thysanoessa inermis* and in *Calanun finmarchicus*, the 3*S*,3'*S* isomer
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32 was dominant. These latter two small crustaceans are important ingredient of diets of wild
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34 salmonids, and their isomeric composition has been demonstrated similar to the isomeric
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36 composition encountered in wild salmon, in which the S-form predominated (Turujman et al.
37
38 1997). Astaxanthin isomer ratios in the flesh of fish fed different carotenoid sources is reported
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40 in Table 1. Dietary treatment influenced significantly the distribution of the optical isomers in
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42 the fillets, and the average values of the optical isomers in the flesh reflected the isomeric ratio
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44 of the dietary astaxanthin source. The astaxanthin isomers distribution in microalgae and in the
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46 corresponding trout flesh were similar and ranged from 80.0% to 87.6%, from 11.7% to 10.1%
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48 and from 2.4% to 8.3% for the 3*S*,3'*S* isomer, meso form and 3*R*,3'*R* isomer, respectively. In the
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4 red yeast and in the corresponding trout flesh the *3R,3'R* isomer predominated, ranging from
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6 98.5% to 74.9%, while the meso form and the *3S,3'S* isomer ranged from 1.5% to 10.7% and
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8 from 0% to 14.4%, respectively. In the flesh of trout fed synthetic astaxanthin, the averages of
9
10 the distribution of optical isomer were 29.6%, 48.4% and 22.0% for the *3S,3'S* isomer, meso
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12 form and *3R,3'R* isomer, respectively. Also in this case the distribution resembles that of the diet
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14 and these results were in agreement with those of Østerlie et al. (1999), who found in rainbow
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16 trout fed syntetic astaxanthin a distribution of isomers in muscle of 28.5%, 49.0% and 22.5% for
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18 *3S,3'S*, meso and *3R,3'R* isomers, respectively.
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23 Colour values measured in different sites of fillet of rainbow trout fed with different pigment
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25 sources are presented in table 2. No significant differences were registered in different parts of
26
27 the flesh. Similarly, different sources of pigment (natural or synthetic), used with the three
28
29 different diets, produced colour values of fresh fillet (table 3) with no significant differences,
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31 although there was a tendency for the CP diet to give an higher value of redness (a^*) and
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33 brightness (C^*) when compared to HP diet. This confirms the better pigmenting efficacy of free
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35 astaxanthin in comparison with esterified astaxanthin from microalgae. (White et al. 2003).
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40 Among the specimens purchased on the market (table 4), two samples showed the algal
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42 astaxhantin isomeric profile (trout 3 and 4), and three (trout 2, 5, 7) were found to contain a
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44 different pigment source that on the basis of its retention time and UV/VIS absorption spectra
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46 was reasonably recognized as canthaxanthin. Three fish samples showed to contain only
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48 synthetic astaxanthin (trout 1, 8, 9) and one (trout 6) both synthetic carotenoids: astaxanthin and
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50 canthaxantin.
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53 The coefficient of distance (table 5) computed amongst the feed ingredient and the trout fillet
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55 astaxanthin stereoisomers is a simple geometrical tool which can be useful to tentatively identify
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57 the origin of the pigment used on farm.
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4 The smallest D values registered in trout fed the Carophyll pink and trout fed microalgae were
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6 5.4 and 9.8 when computed using the synthetic astaxanthin and the microalgae as “reference”,
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8 respectively. The other D values, computed using the astaxanthin stereoisomers composition of
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10 the other analysed ingredients, were considerably higher. In trout fed the read yeast, D value
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12 (29.1) was similar to those calculated for krill meal (29.5) and krill oil (32.5).
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16 This information suggests that the coefficient of distance can be used as a general tool to explain
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18 pigment source only amongst some sources and it is particularly useful to distinguish amongst
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20 synthetic and natural sources whilst is not sensitive enough to distinguish amongst different
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22 natural astaxanthin sources, or amongst mixtures of synthetic and natural sources.
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26 Using the coefficient of distance to estimate the potential origin of the astaxanthin found in the
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28 market purchased trout, it is possible to point out that samples trout 1, trout 8 and trout 9 were
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30 with high probability fed with a diet containing a synthetic source of astaxanthin.
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33 At the best of our knowledge, this is the first time the coefficient of distance was used as a tool
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35 for the identification of the astaxanthin origin in salmonids and further information, particularly
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37 as regard the inter- and intra- population variability and the dose-response effects are requested.
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40 41 42 **References**

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Figure 1: Chromatograms of stereoisomers composition of all-*trans* astaxanthin of: (a) a standard solution of synthetic astaxanthin ($32 \mu\text{g mL}^{-1}$); (b) an *Haematococcus pluvialis* extract; (c) a *Phaffia rhodozyma* extract. HPLC conditions: Pirkle L-leucine chiral column; mobile phase hexane-tetrahydrofuran-triethylamine-acetonitrile (77:19:2:2 v/v); flow rate of 1mL min^{-1} ; monitoring wavelength 474 nm.

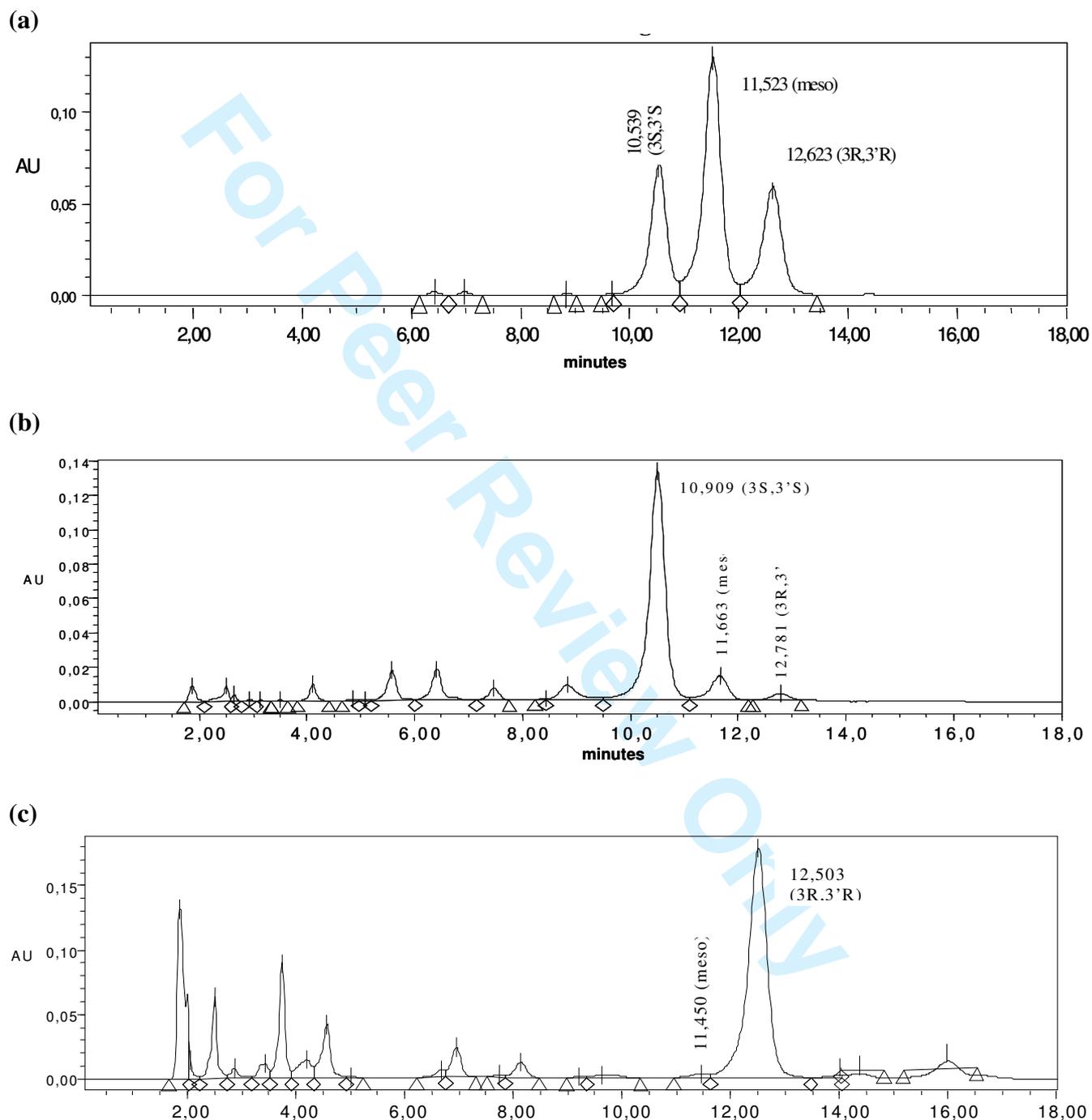
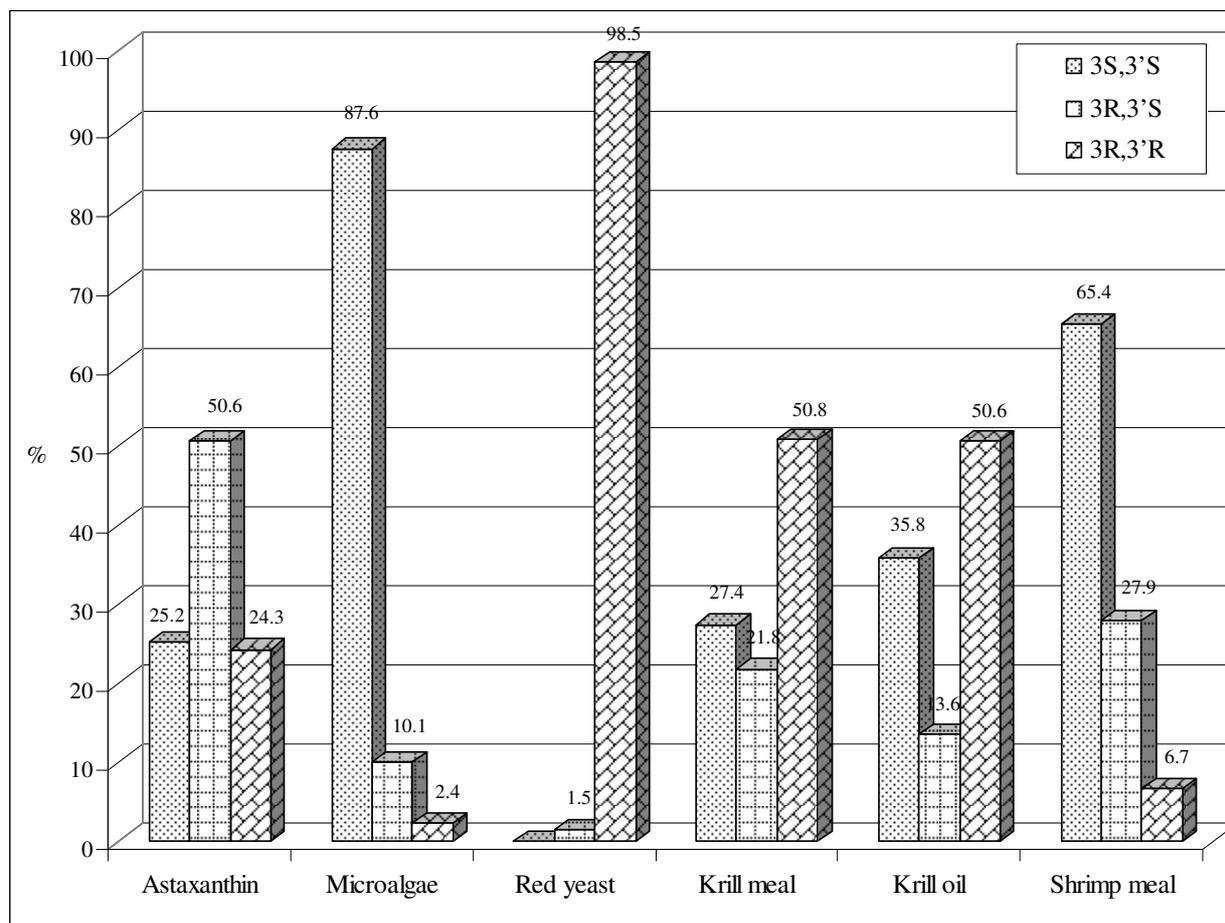


Figure 2. Percentage distribution of astaxanthin stereoisomers in pigment sources



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Table 1. Percentage distribution of astaxanthin stereoisomers in fillet of rainbow trout fed with different pigment sources (means±s.e.)

| Samples (n=10) | 3S,3'S enantiomer (%) | 3R,3'S meso (%) | 3R,3'R enantiomer (%) |
|---|-----------------------------|-----------------------|-----------------------------|
| Carophyll® pink fed trout | 29.6±3.62 | 48.4±4.26 | 22.0±7.50 |
| Microalgae (<i>Haematococcus pluvialis</i>) fed trout | 80.0±2.01 | 11.7±1.14 | 8.3±1.03 |
| Red yeast (<i>Phaffia rhodozyma</i>) fed trout | 14.4±2.33 | 10.7±2.28 | 74.9±4.52 |

Table 2. Colour values measured in three different part of fillet of rainbow trout fed with different pigment sources (means \pm s.d., n=10)

| Color value | Neck | Center | Tail | <i>P</i> ¹ |
|-------------|-----------------|-----------------|-----------------|-----------------------|
| a* | 14.8 \pm 0.74 | 14.5 \pm 0.66 | 15.8 \pm 0.33 | 0.28 |
| b* | 18.9 \pm 1.13 | 17.3 \pm 1.17 | 19.3 \pm 0.87 | 0.40 |
| L* | 44.8 \pm 1.31 | 42.3 \pm 1.42 | 43.8 \pm 1.60 | 0.48 |
| C* | 24.1 \pm 1.31 | 22.6 \pm 1.28 | 25.0 \pm 0.86 | 0.37 |

¹ Significance accepted at probabilities of 0.05 or less

Table 3. Colour values measured in fillet of rainbow trout fed with different pigment sources (means \pm s.d., n=10)

| Color value | Synthetic astaxanthin | <i>Phaffia rhodozyma</i> | <i>Haematococcus pluvialis</i> | P ¹ |
|-------------|-----------------------|--------------------------|--------------------------------|----------------|
| a* | 15.3 \pm 0.58 | 15.7 \pm 0.56 | 14.2 \pm 0.64 | 0.19 |
| b* | 18.7 \pm 1.06 | 18.6 \pm 1.04 | 18.2 \pm 1.20 | 0.95 |
| L* | 42.9 \pm 1.40 | 43.4 \pm 1.50 | 44.7 \pm 1.50 | 0.64 |
| C* | 24.1 \pm 1.18 | 24.4 \pm 1.14 | 23.1 \pm 1.28 | 0.74 |

¹ Significance accepted at probabilities of 0.05 or less

Table 4. Percentage distribution of astaxanthin stereoisomers in flesh of rainbow trout purchased on the market.

| Samples | 3S,3'S enantiomer (%) | 3R,3'S meso (%) | 3R,3'R enantiomer (%) | Presence of canthaxanthin |
|---------|-----------------------------|-----------------------|-----------------------------|---------------------------------|
| Trout 1 | 30.7 | 48.0 | 21.3 | no |
| Trout 2 | nd | nd | nd | yes |
| Trout 3 | 71.2 | 19.9 | 8.9 | no |
| Trout 4 | 68.4 | 21.8 | 9.8 | no |
| Trout 5 | nd | nd | nd | yes |
| Trout 6 | 40.5 | 26.9 | 32.6 | yes |
| Trout 7 | nd | nd | nd | yes |
| Trout 8 | 28.2 | 49.1 | 22.7 | no |
| Trout 9 | 29.8 | 48.3 | 21.9 | no |

nd = not detected

Table 5. The coefficient of distance (D value) of the astaxanthin stereoisomers amongst known and unknown trout samples and the analysed astaxanthin sources

| | Synthetic astaxanthin | Microalage (<i>H.pluvialis</i>) | Red yeast (<i>X. dendrorhous</i>) | Antartic Krill meal | Krill oil | Shrimps meal |
|---|--------------------------|--------------------------------------|---|------------------------|-----------|-----------------|
| Trout fed Carophyll® pink | 5.4 | 72.2 | 94.5 | 39.3 | 45.5 | 44.0 |
| Trout fed microalage (<i>H.pluvialis</i>) | 69.1 | 9.8 | 121.0 | 68.4 | 61.2 | 21.9 |
| Trout fed red yeast (<i>X. dendrorhous</i>) | 65.3 | 103.0 | 29.1 | 29.5 | 32.5 | 86.9 |
| Trout 1 | 6.8 | 70.9 | 95.2 | 39.6 | 45.5 | 42.7 |
| Trout 3 | 57.4 | 20.2 | 115.9 | 60.6 | 55.1 | <i>10.1</i> |
| Trout 4 | 53.9 | 23.7 | 113.8 | 58.0 | 52.9 | 7.5 |
| Trout 6 | 29.4 | 58.4 | 81.4 | 23.0 | 22.9 | 35.9 |
| Trout 8 | 3.7 | 73.9 | 93.8 | 39.2 | 45.8 | 45.7 |
| Trout 9 | 5.7 | 72.0 | 94.6 | 39.3 | 45.4 | 43.8 |

In bold font, the actual coefficient of distance amongst pigment source and trout fillet of the known samples; in italics the possible coefficient of distance amongst pigment source and trout fillet of the unknown samples.