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Colour changes of fillets of rainbow trout (*Oncorhynchus mykiss* W.) fed astaxanthin or canthaxanthin during storage under controlled or modified atmosphere

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Abstract

Rainbow trout were fed diets containing two levels of lipids (9 g/100 g and 24 g/100 g) associated with two keto-carotenoid pigments (80 mg of astaxanthin or of canthaxanthin/kg of diet) for 4 months. After slaughter colour stability of fillets was studied during a 4-week storage at +4 °C under controlled (CA) and modified (MA) atmospheres under 100% air, 60:40 N_2–CO_2 mix and 60:40 air–CO_2 mix. Fillets from fish fed high fat level diets showed higher chroma and higher a^* and b^* colour parameters than those from fish fed low fat level diets. Storage time increased lightness and hue angle in CA but only lightness under MA. After storage at +4 °C lightness of fish fillets stored under MA were lower ($P<0.05$) than those stored under CA. Carotenoid source resulted in differences in chroma and hue angle of fish fillet stored under CA and MA. Dietary lipid levels resulted in differences in chroma under CA. Under CA the lower ($P<0.05$) differences between stored-initial values was for N_2–CO_2 and the higher ($P<0.05$) for air. Under MA, air–CO_2 and N_2–CO_2 gave similar results for L^*, C^* and $H(^\circ)_{ab}$. Our experiment demonstrated that colour parameters of fish fillets reacted differently according to gas mixture and storage time.

Keywords: Trout; *Oncorhynchus mykiss*; Controlled atmosphere; Modified atmosphere; Colour stability; Astaxanthin; Canthaxanthin

1. Introduction

Quality of rainbow trout flesh is partially determined by its colour which is influenced by the carotenoid pigment content (Francis, 1995). Rainbow trout flesh pigmentation is caused by keto-carotenoids (astaxanthin (AX) and canthaxanthin (CX) that fish cannot synthesize de novo. Carotenoids are of dietary origin: in the wild, trout find carotenoids in their preys but in intensive fish culture AX or CX are added to the diet. These keto-carotenoids contain a conjugated carbon–carbon double bonds system, responsible for their colour. The high number of conjugated double bonds may be subject to oxidation in air which leads to discoloration of the carotenoid (Liaaen-Jensen, 1971).

In rainbow trout, fat deposition reflect by its importance and its composition dietary lipids (Toyomizu, Kawasaki, & Tomiyasu, 1963). They play an important role in the fish quality determination on a nutritional and an organoleptic point of view (Greene & Selivonchick, 1987). As carotenoid pigments are lipid soluble compounds, to increase dietary fat would increase carotenoid absorption and then pigment deposition in trout flesh. In fact results are not so clear. If Spinelli (1979) noted that fat addition to rainbow trout diet increased the AX amount in fish muscle, this result was not observed by Seurman, Martinsen, and Little (1979) and Torrissen (1985). Moreover, Abdul Malak, Zwingelstein, Jouanneteau, and Kœnig (1975) and Choubert and Luquet (1983) reported a negative effect of the dietary lipid amount on the fish flesh pigmentation. On the other hand CX digestibility in rainbow trout increased with increasing dietary lipid level (Torrissen, Hardy, Shearer, Scott, & Stone, 1990).

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Fish flesh is highly perishable due to its autolitic enzymes and its high post-mortem pH changes that favours bacterial growth (Ward & Baj, 1988). Fish is traditionally stored under refrigeration in air (Stammen, Gerdes, & Caparaso, 1990). However, in combination with good refrigeration and handling conditions, modified atmosphere packaging is an increasingly preservation technique for significantly improving fish fillet quality and reducing wastage (Day, 2001). Packaging of fish fillet under controlled (CA) or modified (MA) atmosphere involves the replacement of air by other gases, usually CO_2, N_2, and O_2, alone or in combination. The aim is to inhibit physical, chemical, and microbiological changes that lead to fish fillet deterioration (Statham, 1984). The basic difference between CA and MA systems is that gas levels are strictly maintained all times under CA system, whereas gas mixture is flushed into the system once and changes with time and product in the MA system (Brecht, 1980).

The objective of our study was to investigate the colour stability of trout fillets during cold storage in CA or MA using a 60:40 N_2–CO_2 mix and a 60:40 air–CO_2 mix and 100% air as control. Trout were fed AX and CX associated with high or low dietary lipid levels. Colour stability was monitored by instrumental colour measurements and keto-carotenoid content analyses.

2. Materials and methods

2.1. Fish and diets

Sixty rainbow trout (*Oncorhynchus mykiss* W.) were reared at the INRA experimental fish farm in Donzacq (Landes Dept.). Fish were fed for 4 months on diets (the composition of which is given in Table 1) containing two levels of lipids (low = LE and high = HE) combined with two carotenoid pigments: AX or CX. Diets were designated as: LECX, HECX, LEAX, HEAX. At the end of the feeding period fish (mean weight: 1 kg) were slaughtered, weighed and individually put in plastic bags in ice, then transported to AGROTEC (travel time # 2 h).

Table 1
Formulas, ingredients and chemical composition of the experimental feeds[a]

Diet label	HE	LE
Feed Ingredients (g/100 g)		
Fish meal	58	58
Gelatinised wheat starch	20	13.5
Crude wheat starch	0	24
Fish oil	19	1.5
Vitamin mix[b]	1	1
Mineral mix[c]	1	1
Sodium alginate	1	1
Synthetic canthaxanthin[d]	+	+
or Synthetic astaxanthin[e]	+	+
Feed chemical composition		
Dry matter DM (g/100 g)	95.44	95.08
Total lipids (g/100 g DM)	23.80	8.70
Canthaxanthin (mg kg^{-1} DM)	72.1	73.8
or Astaxanthin (mg kg^{-1} DM)	70.6	75.5

[a]Diets were pelleted using a steamless pelleting machine (M-Labor, Seemon-Heesen, Boxtel, The Netherland) through a 4.5 mm dye. Pellets were allowed to dry in a drying cabinet (Bulkit, Monclar, France) at 38 °C for 4 h and were stored at +4 °C prior to use.

[b]INRA 762. Vitamin mix contained the following diluted in cellulose (g kg^{-1} mix): vitamin A (500,000 IU g^{-1}), 1.5; vitamin D_3 (100,000 IU g^{-1}), 1.5; vitamin E (500 IU g^{-1}), 6; vitamin K, 0.25; thiamin, 0.75; riboflavin, 1.5; pyridoxine, 0.75; nicotinic acid, 8.75; vitamin C, 25; folic acid, 0.25; vitamin B12 (1,000 mg kg^{-1}), 2.5; inositol, 50; biotin (2 g/100 g), 6.25; calcium pantothenate, 2.5; choline (50 g/100 g), 200.

[c]INRA 763. Mineral mix contained the following ingredients (g kg^{-1} mix): calcium carbonate, 215; magnesium hydroxyde, 124; KCl, 90; ferric citrate, 20; KI, 0.4; NaCl, 40; calcium hydrogen phosphate ($CaHPO_4$), 500; copper sulfate, 3; zinc sulfate, 4; cobalt sulfate, 0.2; manganese sulfate, 3.

[d]Carophyll® red, DSM Nutritional Products Europe Ltd. (formerly F. Hoffmann-La Roche), Basel, Switzerland. Supplementation level: 80 mg astaxanthin kg^{-1} of feed.

[e]Carophyll® pink, DSM Nutritional products Europe Ltd. (formerly F. Hoffmann-La Roche), Basel, Switzerland. Supplementation level: 80 mg astaxanthin kg^{-1} of feed.

2.2. Experimental design

The processing plant was kept at constant temperature (+4 °C). Fifteen fish per diet group were filleted manually and cut into portions with six such portions obtained per fish fillet for CA test and three for MA test. Each portion was individually tagged with an inox numbered label and weighed. Pure-grade gases (Air Liquide, Jouy-en-Josas, France) were used and gas-mixing were performed through a multiple ways mixing apparatus (AGROTEC conception). The following gas mixtures were used: 100% air as control, a 60:40 N_2–CO_2 mix, and a 60:40 air–CO_2 mix.

- *CA test*: Fish portions (20 per box) were distributed at random in Plexiglas tubular boxes (capacity: 9 l, dia. = 160 mm, L = 450 mm) and stored in the dark in a cold room (+4±0.2 °C). The gas mixtures were strictly maintained in a 10±1 l h^{-1} flow rate during the whole experiment. The gas composition was constant and continuously controlled throughout the storage.
- *MA test*: Fish portions (2 per tray) were packaged at random in white polystyrene trays (PEHD-565, Dynopack, Lundsiden, Norway) with a pad of absorbent paper each with two portions with about 70% filling degree (fish to gas ratio) that is 1:1.5 v/v. Each tray was first air evacuated, flushed with gas mixture and wrapped with a shrinkable plastic film (Dynoseal GPO 1570, Soplaril, Arras, France) using a vacuum packaging machine (mod Galaxy AG 63, Multivac France sarl, Marne-la-vallée, France) equipped with built-in vacuum pump and gas flush. The oxygen barrier of the film was 5 cm^3 m^{-2} 24 h^{-1} at 23 °C, 1 atm pressure, and 95% relative humidity. Trays were stored in a cold room (+4 °C), in the dark, during the whole experiment.