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Effect of moist or dry heat cooking procedures on carotenoid retention and colour of fillets of rainbow trout (*Oncorhynchus mykiss*) fed astaxanthin or canthaxanthin

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ABSTRACT

Rainbow trout were pigmented with diets containing astaxanthin or canthaxanthin for 100 days, and then they were moist or dry heat-cooked. Fish fillet weight, fillet colour, and fillet biochemical contents (moisture, canthaxanthin and astaxanthin contents, and total lipid content) were analyzed. There was no significant effect of using astaxanthin or canthaxanthin on moisture, lipid or carotenoid contents of fish fillet. Giving astaxanthin or canthaxanthin to fish resulted in different hues; astaxanthin-fed fish yielded fillets that were visually more red than those of canthaxanthin-fed fish. The dry heat-cooking procedure showed the highest impact on the fillet colour. Carotenoid retention was affected by carotenoid source and cooking procedure. Canthaxanthin appeared more stable after heat processing than did astaxanthin.

1. Introduction

The pink to red colour of rainbow trout muscle is an important quality attribute for fish farmers and for consumers. This colour is caused by accumulation of carotenoids from the food, since fish are not able to synthesize them *de novo* (Goodwin, 1951). Since astaxanthin (β,β-carotene-3,3′-dihydroxy-4,4′-dione) is the major pigment of wild trout (Schiedt, Vecchi, & Glinz, 1986), both astaxanthin and canthaxanthin (β,β-carotene-4,4′-dione) are added to the feed of intensively reared rainbow trout.

The consumption of raw fish is rare in Western society and information about carotenoid contents of raw fish may be of limited value for a conclusion about their food quality. Cooking methods can have a detrimental effect on the nutrient composition (Tokur, 2007) and sensory quality (Freeman, 1999) of fish. Fish are exposed to different conditions during the cooking process which may, in turn, result in changes in their carotenoid content and may lead to colour modifications (Bhattacharya, Choudhury, & Studebaker, 1994). Carotenoids are known to deteriorate rapidly on exposure to heat (structural changes) or to light (oxidation) which results in fading, darkening or change in hue (Bauernfeind, Brubacher, Kläui, & Marusich, 1971). Traditional ovens, more than microwave ovens, are widely used at home to cook fish. Steaming, a moist heat-cooking procedure, is the most representative industrial cooking system, and it results in various food quality traits (Barbanti & Pasquini, 2005). However, this combined cooking technique is quite complex because it leads to unpredictable results due to effects of steam on meat products (Barbanti & Pasquini, 2005). While investigations into carotenoid utilization by fish are numerous (Bjerkeng, 2000), little is known about the effect of processing on the stability of carotenoids. Few controlled studies have compared the carotenoid retention and the colour of salmonid fillets that contain carotenoids such as astaxanthin and canthaxanthin (Skrede & Storebakken, 1986).

This investigation studies the effect of cooking procedures (dry and moist heat-cooking) on fillets from fish fed diets containing astaxanthin or canthaxanthin, and analyses the carotenoid retention and colour of fish fillets.

2. Material and methods

2.1. Fish and facilities

Rainbow trout (*Oncorhynchus mykiss*) from the same parental stock were obtained from the Inra experimental fish farm (Donzacq, Landes department, France). Fish were fed on a diet (the composition of which is given in Table 1) containing 100 mg of astaxanthin (Carophyll pink™, DSM, Paris, France) or 80 mg of canthaxanthin (Carophyll red™, DSM, Paris, France)/kg of diet as

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Table 1
Formula, ingredients and chemical composition of the basal diet.

Diet label			Basal (%)
Feed ingredients (g kg^{-1})			
Fish meal[a]			56
Gelatinised corn starch[b]			13.5
Crude corn starch[c]			18
Scandinavian fish oil[d]			9
Vitamin mix[e]			1.5
Mineral mix[f]			1
Sodium alginate[g]			1
Astaxanthin (mg kg^{-1}) (diet 1)[h]			100
Canthaxanthin (mg kg^{-1}) (diet 2)[i]			80
Diet chemical composition[j]	1	2	
Dry matter DM (%)	89.7	91.2	
Total lipids (% DM)	14.4	14.9	
Astaxanthin (mg kg^{-1})	98.9		
Canthaxanthin (mg kg^{-1})		80.8	

[a] Norwegian herring meal, Norse LT94, Sopropêche, 62204 – Boulogne-sur-mer, France.
[b] Amidex, Ogilvie Aquitaine, 33000 – Bordeaux, France.
[c] Descal, 40360 – Pomarez, France.
[d] Feedoil, La Lorientaise, Sopropêche, 56100 – Lorient, France.
[e] INRA 762. Vitamin mix contained the following mixed with cellulose (g kg^{-1} mix): vitamin A (500,000 IU g^{-1}), 1.5; vitamin D3 (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 (1000 mg kg^{-1}), 2.5; inositol, 50; biotin (2 mg kg^{-1}), 6.25; calcium pantothenate, 2.5; choline (50 mg kg^{-1}), 200.
[f] INRA 763. Mineral mix contained the following ingredients (g kg^{-1} mix): calcium carbonate, 215; magnesium hydroxide, 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.
[g] Alginate GF 150. Louis François Exploitation, 94100 – Saint Maur-des-Fossés, France.
[h] Carophyll® pink, DSM Nutritional product, Basel, Switzerland.
[i] Carophyll® red, DSM Nutritional product, Basel, Switzerland.
[j] Means of two independent determinations.

authorized (Council Directive No. 70/524/EEC and Council Regulation (EC) No. 1831/2003). Diets were pelleted using a steamless pelleting machine (M-Labor, Simon Heesen B.V., Boxtel, The Netherlands) through a 4.5 mm die (pellet temperature after the die ≈75 °C). Diets were stored at +4 °C prior to use. Fish were distributed at random among 2 m diameter cylindrical fibreglass tanks receiving flow-through spring water (constant temperature, 17 ± 1 °C; pH, 7.4; Cl^-, 22.5 mg/l; Ca^{2+}, 75 mg/l; dissolved oxygen, 8 mg/l) at a rate of 5 volume changes per hour. Tanks received a natural photoperiod (February to May). Fish were hand-fed twice daily (8:30, 16:30 h) to apparent satiation and complete feed ingestion was assessed visually.

At the end of the 100 day feeding period 40 fish (mean weight: 1 kg) per diet group were slaughtered, bled by cutting the gill, rapidly chilled in ice slurry, and kept in ice prior to processing. They were filleted manually the next day. Each fillet was placed individually into a plastic bag in ice, then transported to Agrotec (travel time 2 h) where they were cooked.

2.2. Processes, sampling

The following cooking treatments were used: moist heat- and dry heat-cooking. For both treatments, fish fillets were placed skin side down on individual aluminium trays, securely covered and processed in an atmospheric combination oven with revolving heat (Combi ClimaPlus® FCP, Frima GmbH, Frankfurt am Main, Germany). For moist heat-cooking, a steam generator produces hygienic steam and releases it into the cooking cabinet where it circulates at high speed. For dry heat-cooking, heating elements heat the dry air which circulates evenly throughout the interior inside the cabinet. The duration of heating for the two cooking methods (8 min for moist heat-cooking at 100 °C, and 12 min for dry heat-cooking at 180 °C, respectively) was determined as the amount of time required to reach a fish fillet core temperature of 70 °C (Johanssen, 2001). Sensors covering various measuring points of the core allowed checking of the core temperature at any time to regulate the cooking process.

Fish fillet firming was accomplished by leaving the cooked fillets in a cold room at +4 °C for 2 h (Council Regulation (EC) No. 852/2004). Total weight of the samples after cooking was measured. Fish fillets were then individually placed under vacuum in plastic pouches (Linvac 80, Linpac plastics Pontivy S.A., Pontivy, France), using a vacuum packaging machine (mod Galaxy AG 800, Multivac France sarl, Marne-la-Vallée, France). The moisture and oxygen permeabilities of this pouch were 5 g/m^2/24 h (at 25 °C, 90% relative humidity RH), and 45 cm^3/m^2/24 h (at 23 °C, 50% RH), respectively, according to the manufacturer. The packaged samples were then deep-frozen in a cryogenic freezing cabinet (Silversas™, Air Liquide S.A., Paris La Défense, France), and were stored at −20 °C in the dark until used for analyses.

2.3. Analytical methods

Analyses of fish fillet weight, fillet colour, and fillet biochemical contents (moisture, canthaxanthin, astaxanthin, total lipid) were carried out on each sample.

Muscle colour was assessed, before and after cooking, by using a chromameter (CR200, Minolta Camera Ltd, Osaka, Japan) equipped with an 8 mm dia aperture and calibrated on a white reference ceramic plate before use, as described by Choubert, Blanc, and Vallée (1997). Samples were scanned at three locations along the fillet: anterior (close to the head), mid-region, and posterior (close to the tail) sections to determine the average $L^* a^* b^*$ values as the averages of the three measurements. All measurements were expressed in colorimetric space, $L^* a^* b^*$, in accordance with the recommendations of the Commission Internationale de l'Éclairage (CIE, 1976). In this colorimetric space, "L^*" describes lightness (black = 0, white = 100); "a^*" intensity in red ($a^* > 0$), and "b^*" intensity in yellow ($b^* > 0$). The two chromatic attributes: hue angle (H (°)$_{ab}$ = arctan b^*/a^*), the hue of the colour (red = 0°, yellow = 90°) and chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) were calculated according to Wyszecki and Stiles (1967).

Chemical analyses of trout fillets were as follows: moisture after drying for 24 h at 105 °C (Oven ULE 500, Memmert gmbh, Schwabach, Germany) (AOAC, 1990), total lipids by a gravimetric method after solvent extraction (Folch, Lees, & Sloane Stanley, 1957), astaxanthin and canthaxanthin, measured spectrophotometrically after solvent extraction (Guillou, Choubert, & de la Noüe, 1993).

The carotenoid retention factor was used and weight yield and retention factors were calculated as follows (Murphy, Criner, & Gray, 1975):

$$\text{Weight yield } \% = (\text{Weight of fillet after cooking in g} / \text{weight of fillet before cooking in g}) \times 100$$

$$\text{Carotenoid retention } \% = (\text{Content of carotenoid per 100 g of fillet after cooking} / \text{Content of carotenoid per 100 g of fillet before cooking}) \times \text{Weight yield}.$$

2.4. Statistical analysis

Statistical differences between treatment groups were determined for all of the chemical and physical tests. Means were

compared by analysis of variance, ANOVA, and for change in colour parameters of cooked fish fillets by the non-parametric Kruskal–Wallis test, followed by the Tukey's multiple comparison test (Zar, 1984), using the SAS-GLM procedure in the SAS/STAT™ package (SAS, 1989). Statistical significances are indicated for $P < 0.05$.

3. Results and discussion

The proximate compositions of raw fillets from trout fed either astaxanthin or canthaxanthin are presented in Table 2. There was no significant ($p > 0.05$) effect of using astaxanthin or canthaxanthin on moisture, lipid or carotenoid contents of fish fillet. These results confirmed the finding of Scott, Rasco, and Hardy (1994) in rainbow trout and Baker, Pfeiffer, Schöner, and Smith-Lemmon (2002) in Atlantic salmon (*Salmo salar*), demonstrating a similarity in pigmentation efficacy between the two carotenoids; no difference ($p > 0.05$) occurred in the final carotenoid muscle concentration of fish of 1 kg-fed diets containing either astaxanthin or canthaxanthin. However, Storebakken et al. (1987) reported that Atlantic salmon fed the same carotenoids during the same time exhibited a greater tendency to lower carotenoid muscle concentration than did those that had ingested astaxanthin.

During cooking, components were lost from the fish fillet. This loss was in the form of vapour which was almost entirely steam and drip, which was made of liquefied fat and water. The mass loss as vapour depended only on the heat source temperature, while the drip mass and the ratio of fat to water in drip depended on the fat content of the fish fillet. Loss of both products reduced the weight of the fish fillet (Ofstad & Hermansson, 1997). Both moist and dry heat-cooked fillets were different in moisture, lipid, and carotenoid concentrations from raw fillets (Table 2). Moist cooking led to less fillet moisture loss than did dry cooking for both groups. This result is in accordance with that reported for bighead carp (*Hypophthalmichthys nobilis*) loins (Freeman, 1999) and humpback salmon (*Oncorhynchus gorbuscha*) (Gladyshev, Sushchick, Gubanenko, Demirchieva, & Kalachova, 2006). The relatively low moisture decrease observed during dry-cooking may be explained by the moderate cooking process conditions used (12 min at 180 °C, core temperature: 70 °C) compared to the more severe process conditions reported by Puwastien et al. (1999), or by Gokoglu, Yerlikaya, and Cengiz (2004). It was observed that the cooking process led to increased lipid content of fish fillet. This is not surprising since an inverse correlation with moisture and lipid content was reported earlier in fish muscle (Tokur, 2007). There were no statistically significant differences in either astaxanthin or canthaxanthin concentration in fish fillets between cooking processes. Scott et al. (1994) also observed no change in carotenoid concentrations in cooked trout fillet. However, in this study, as a result of cooking, astaxanthin concentrations decreased while canthaxanthin concentrations increased. Canthaxanthin concentration increase after cooking was due to moisture decrease. Astaxanthin concentration decrease may be due to the low stability of astaxanthin towards heat, which led to degradation of this compound, as reported for lycopene (Shi, Le Maguer, Bryan, & Kakuda, 2003). Overall, the cooking procedure showed the highest impact on the astaxanthin concentration of fish fillet, expressed as mg carotenoid/g lipid (Fig. 1). A cooking procedure × carotenoid interaction was noted ($P < 0.05$) for lipid and carotenoid fillet concentration.

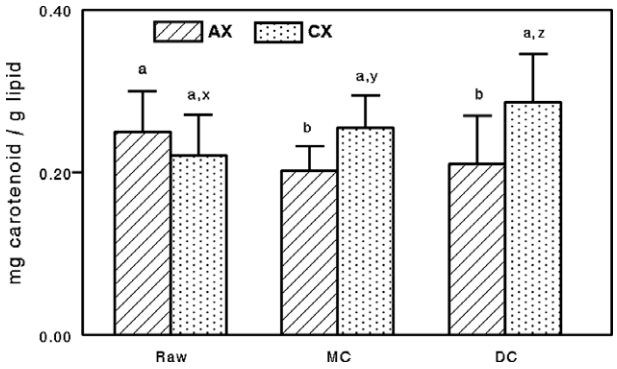

Fig. 1. Carotenoid concentration (expressed as mg carotenoid/g lipid) of raw, dry heat-(DC) and moist heat (MC)-cooked fillets from fish pigmented with diets containing astaxanthin (Ax) and canthaxanthin (Cx). Values are means ± SD, $n = 8$. Carotenoid effect: ^{a,b}for the same product (raw, dry heat (DC) and moist heat (MC) cooked fillets from fish), means with same letter are not significantly different ($P > 0.05$); Cooking effect: ^{z,y}for canthaxanthin (Cx), means with same letter are not significantly different ($P > 0.05$). For astaxanthin (Ax), means were not significantly different ($P > 0.05$).

Cooking yields for all fish groups are presented in Table 3. Variation in weight yield for all fillet samples was less than 10%. There was no significant ($p > 0.05$) effect of using astaxanthin or canthaxanthin on weight yield. But minimum weight yield values were characteristic of the dry heat-cooking treatment. No cooking procedure × carotenoid interaction was noted for weight yield. Lipid retentions were not affected by carotenoid sources but were affected ($p < 0.05$) by cooking procedure, while carotenoid retentions were not affected by cooking procedure but were affected ($p < 0.05$) by carotenoid sources. This result agrees with the trend of a loss of fat in conventional roasting, as reported by Kumar and Aalbersberg (2006) for yellowlip emperor (*Lethrinus xanthochilus*). No cooking procedure × carotenoid interactions were noted, either for lipid retention or for carotenoid retention. Canthaxanthin appeared to be more stable after heat processing (moist cooking procedure) than did astaxanthin. This is in agreement with Kanemitzu and Matsuura (1961) and Sheeman, O'Connor, Sheedy, Buckley, and FitzGerald (1998) who found an important loss of astaxanthin in salmon after heat-sterilization and smoking, respectively. However, Scott et al. (1994) reported that carotenoids are relatively stable after cooking in plastic bags by immersion in a 75 °C water bath. Loss of carotenoids could be partially associated with degradation by heat of cooking and partially with the loss into the melted fat which leached out into the drippings reported by Kumar and Aalbersberg (2006) for yellowlip emperor. In our experiment, retention of more than 100% was found for canthaxanthin.

Table 2
Proximate compositions of raw and cooked fish fillets[A].

Treatment	Astaxanthin fish fillet			Canthaxanthin fish fillet		
	Moisture (g/100 g)	Lipid (g/100 g)	Astaxanthin (mg/100 g)	Moisture (g/100 g)	Lipid (g/100 g)	Canthaxanthin (mg/100 g)
Raw	73.14 ± 1.01^{a,z}[B]	5.75 ± 1.25^{a,z}	1.41 ± 0.26^{a,z}	73.51 ± 0.84^{a,z}	5.61 ± 0.91^{a,y}	1.23 ± 0.27^{a,z}
Moist cooking	71.25 ± 0.97^{a,y}	5.92 ± 1.10^{a,z}	1.17 ± 0.16^{b,z}	71.08 ± 0.73^{a,y}	6.21 ± 1.08^{a,z}	1.56 ± 0.25^{a,z}
Dry cooking	69.26 ± 1.25^{a,x}	6.13 ± 1.13^{a,z}	1.29 ± 0.31^{a,z}	70.17 ± 0.71^{a,y}	4.98 ± 0.39^{b,y}	1.41 ± 0.27^{a,z}

[A] Values are means ± SD, $n = 8$.
[B] Carotenoid effect: within a row for the same analyte, ^{a,b}means with the same letter are not significantly different ($P > 0.05$); cooking effect: within a column, ^{z,y}means with the same letter are not significantly different ($P > 0.05$).

Table 3
Mean lipid and carotenoid retentions, and mean weight yields of cooked fish fillets[A].

Treatment	Astaxanthin fish group			Canthaxanthin fish group		
	L.R.[B] (%)	A.R.[C] (%)	W.Y.[D] (%)	L.R.[B] (%)	C.R[E]. (%)	W.Y. (%)
Moist cooking	90.6 ± 13.1^{a,z}[F]	74.7 ± 17.8^{b,z}	93.05 ± 0.70^{a,z}	96.2 ± 21.9^{a,z}	115 ± 42.8^{a,z}	93.13 ± 0.89^{a,z}
Dry cooking	81.4 ± 17.1^{a,z}	69.6 ± 19.5^{a,z}	85.04 ± 2.10^{a,y}	68.7 ± 12.0^{a,y}	91.4 ± 26.1^{a,z}	85.30 ± 2.23^{a,y}

[A] Values are means ± SD, n = 8.
[B] L.R. = lipid retention.
[C] A.R. = astaxanthin retention.
[D] W.Y. = weight yield.
[E] C.R. = canthaxanthin retention.
[F] Carotenoid effect: within a row for the same analyte, ^{a,b}means with the same letter are not significantly different ($P > 0.05$); Cooking effect: within a column, ^{z,y}means with the same letter are not significantly different ($P > 0.05$).

Table 4
Mean initial colour parameters of raw fish fillets[A].

Colour parameter	Astaxanthin fish group	Canthaxanthin fish group
L^*	40.11 ± 2.63^{aB}	40.83 ± 2.51^{a}
C^*	20.75 ± 3.04^{a}	17.67 ± 2.88^{b}
$H(^\circ)_{ab}$	54.32 ± 2.09^{b}	58.47 ± 2.30^{a}
a^*	12.03 ± 1.67^{a}	9.14 ± 1.29^{b}
b^*	16.88 ± 2.64^{a}	15.07 ± 2.68^{b}

[A] Values are means ± SD, $n = 16$.
[B] ^{a,b}Means within a row with the same letter are not significantly different ($P > 0.05$).

Retention of more than 100% carotenoid was also reported for β-carotene content of waterleaf after boiling (Renqvist, De Vreeze, & Evenhuis, 1978) and of sweet potatoes after bleaching (Chandler & Schwartz, 1988). One explanation for this increase is associated with enhanced extraction efficiency of carotenoid from the cooked samples compared with a greater difficulty in obtaining complete extraction in the raw sample (Chandler & Schwartz, 1988). Studies on coho salmon (*Oncorhynchus kisutch*) have shown that astaxanthin and canthaxanthin are bound to actomyosin by hydrophobic bonds, the hydroxyl and keto groups contributing to further stabilization of the complex (Henmi, Hata, & Hata, 1989). But, even if, up to now, this complex has not been confirmed, after cooking, a presumable change in tissue morphology occurred, allowing a greater release of carotenoid (Chandler & Schwartz, 1988).

Table 5
Changes in colour parameters of cooked fish fillets[A] expressed as difference between raw and cooked fillet.

	Astaxanthin fish fillet	Canthaxanthin fish fillet
Moist heat-cooking		
Colour parameter[B]		
L^*	−28.62 ± 1.73^{a,zB}	−29.81 ± 1.64^{a,z}
C^*	−5.49 ± 2.62^{a,z}	−4.48 ± 2.58^{a,z}
$H(^\circ)_{ab}$	−9.70 ± 1.61^{a,z}	−12.17 ± 2.49^{b,z}
a^*	0.78 ± 1.25^{b,z}	2.44 ± 1.05^{a,z}
b^*	−6.80 ± 2.40^{a,z}	−5.97 ± 2.56^{a,z}
Dry heat-cooking		
Colour parameter[B]		
L^*	−28.32 ± 2.95^{a,z}	−29.40 ± 2.16^{a,z}
C^*	−15.61 ± 4.53^{a,y}	−15.11 ± 4.60^{a,y}
$H(^\circ)_{ab}$	−12.90 ± 2.07^{a,y}	−16.94 ± 1.82^{b,y}
a^*	−2.48 ± 1.33^{b,y}	0.21 ± 1.59^{a,y}
b^*	−16.44 ± 4.44^{a,y}	−16.46 ± 4.28^{a,y}

[A] Values are means ± SD, $n = 8$.
[B] Carotenoid effect: within a row, ^{a,b}means with the same letter are not significantly different ($P > 0.05$); Cooking effect: within a column, ^{z,y}means with the same letter are not significantly different ($P > 0.05$).

Mean initial colour parameters of raw fish fillets are given in Table 4. Fillets from fish fed diets containing astaxanthin had significantly ($p < 0.01$) higher a^*, b^*, and chroma C^* values than did fillets from fish fed diets containing canthaxanthin. Similar increases in a^* and b^* values were observed in raw fillet from trout fed astaxanthin or canthaxanthin (No & Storrebaken, 1991; Scott et al., 1994). Giving astaxanthin or canthaxanthin to fish resulted in different hues; astaxanthin-fed fish showed fillets that were visually more red than were canthaxanthin-fed fish. The findings of the present study are in agreement with our previous result (Choubert, Blanc, & Courvalin, 1992). However, Skrede and Storebakken (1986) reported that in Atlantic salmon, no differentiation between the two types of carotenoids can be seen, except that caused by differences in carotenoid concentrations.

Changes in colour parameters of fish fillets after cooking are listed in Table 5. Except for a^*, all other colour parameters were decreased by the cooking process, but there was a carotenoid effect only for hue and a^* parameters. No cooking procedure × carotenoid interaction was noted for colour parameters. Whatever the carotenoid concerned, the decrease in L^* values and in b^* values during the cooking process indicate that the raw fillet decreased its brightness and lost its yellow component. This result confirmed our previous findings (Choubert et al., 1992) and also that of Bhattacharya et al. (1994) in chum salmon (*Oncorhynchus keta*). Further, fillets from trout fed the diet containing canthaxanthin showed (after the cooking procedure) a higher hue angle ($H\ (^\circ)_{ab}$) than did fillets from trout fed a diet containing astaxanthin. These results are in agreement with those of Skrede and Storebakken (1986) in Atlantic salmon.

4. Conclusion

The impact of the moist heat (steaming)-cooking procedure on colour parameters of fish fillets was lower than that of the dry heat-cooking procedure, resulting in a more pigmented product. Although hedonic sensory scoring was not used in the present study, our findings are in conformity with those of Freeman (1999), who reported that moist heat (steaming)-cooked fish had higher hedonic sensory scores than did dry heat cooked fish.

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