Quality of Pigmented (Astaxanthin and Canthaxanthin) Rainbow Trout *(Oncorhynchus mykiss)* Fillets Stored under Vacuum Packaging during Chilled Storage

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Shelf life and color stability of muscle of rainbow trout fed ketocarotenoids (astaxanthin and canthaxanthin) stored at 4 °C under vacuum-packaging conditions were studied by bacterial enumeration of mesophilic (TVC), psychrotrophic (TPC), and total coliforms (TC), TBARS (2-thiobarbituric acid reactive substances), color measurements (CIELCH), and carotenoid quantification by HPLC. An increase of TBARS values and microbial development was observed at 5 days when a loss in astaxanthin and canthaxanthin concentration occurred. A second decrease in canthaxanthin levels took place at 11 days when a maximun in TBARS values (5.9 ± 1.7 nmol of TMP/mg of lipid) was also observed. Decrease in carotenoid concentrations was not reflected by color parameters. Evidence indicated that astaxanthin is more stable than canthaxanthin under vacuum packaging at 4 °C. On the basis of a TVC limit of acceptability of 10^6 microorganisms/g, the shelf life of rainbow trout fillets, stored under vacuum packaging at 4 °C, was 5 days.

Keywords: *Rainbow trout; vacuum packaging; color stability; carotenoid; shelf life*

INTRODUCTION

Pigmentation of rainbow trout is due to ingested carotenoids (André, 1926) because fish are unable to synthesize these carotenoids de novo. In intensive rearing systems carotenoids are added to the feed in the form of industrially synthesized compounds. Pigmentation of cultured salmonids affects overall consumer product acceptance as well as variations in ultimate product price.

Stability of the deposited carotenoids in muscle during shipping, storage, and cooking is critical to ensure optimal acceptance of aquaculturally raised products. Deterioration of carotenoids in frozen stored rainbow trout muscle has been reported by Chen et al. (1984), Pozo et al. (1988), and Anderson et al. (1990) and in salmon muscle by Lusk et al. (1964). However, No and Storebakken (1991) have reported that vacuum-packed rainbow trout fillets could be held for at least 6 months at −20 and −80 °C without significant carotenoid loss, although frozen storage at −80 °C is considered impractical under industrial conditions due to the great energy expenditure.

Fresh fish products are highly susceptible to spoilage from postmortem microbial growth and biochemical endproducts (e.g., enzymes) resulting from the microbial growth or combinations of both. The effect of microbial activity on fresh seafood proteins results in pronounced off-flavor and off-odor and production of fatty acid hydroperoxides, precursors of rancid flavor substances. These effects lead to a short shelf life and often heavy economic losses in fish and other seafood products (Reddy et al., 1992). The deterioration of carotenoids also can be attributed either to nonenzymatic degradation, for example, by light, heat, or oxygen, or to enzymatic deterioration, for example, by lipoxygenase, peroxidase (Krinsky, 1989).

With increased volumes of aquacultured products, the impact of quality on market conditions must be better understood. Knowledge of stability during storage of deposited carotenoids is critical to ensure optimal quality of marketed salmonids. To keep prepacked fresh pigmented rainbow trout products safe and of good quality during storage, vacuum packaging may be necessary. Ice and mechanical refrigeration are the most common means of retarding microbial and biochemical spoilage in freshly caught seafood during distribution and marketing. Modified atmosphere packaging (MAP), a technologically viable method, has been used as a supplement to ice or mechanical refrigeration to reduce losses and extend the storage shelf life of fresh seafood products (Reddy et al., 1992). In MAP, normal air is replaced with various gas mixtures to regulate microbial activity and/or retard deterioration of the product. Another MAP process is vacuum packaging, which also is used to preserve food products (Wilhelm, 1982; Genigeorgis, 1985).

The objective of this investigation was to study the shelf life and stability of coloration during chilled storage of vacuum-packed rainbow trout muscles. Instrumental color analyses, combined with pigment content, were used to assess changes in carotenoids and color during storage. Moreover, the relationship between 2-thiobarbituric acid reactive substances (TBARS) and microbial development and carotenoid stability also was investigated.

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MATERIALS AND METHODS

Feeding and Rearing. The experiment was carried out at the INRA (Institut National de la Recherche Agronomique) experimental fish farm of Donzacq (Landes Department, France). Fifty-six rainbow trout (*Oncorhynchus mykiss*) from the same parental stock, with a mean initial weight of 170 g, were placed into two concrete tanks (2 $m^2 \times 0.40$ m depth) set in parallel rows. Each tank was gravity-fed to maintain a constant temperature of 17 ± 1 °C spring water (pH 7.4, Cl^- 22.5 mg L^{-1}, Ca^{2+} 75 mg L^{-1}, at a rate of 7 volume changes per hour). A natural photoperiod was followed. Fish were hand-fed twice a day ad libitum during 8 weeks with a dry pelleted diet and reached a final weight of 260 g. The formulation of the feed was fish meal (35%), soybean oil cake (30%), pregelatinized starch (25%), fish oil (6%), mineral mix (Labbé et al., 1993) (1%), vitamin mix (Labbé et al., 1993) (1.5%), and sodium alginate (1.5%). Astaxanthin (Carophyll pink, 5% astaxanthin, Hoffmann-La Roche Basel, Switzerland) or canthaxanthin (Carophyll red, 10% canthaxanthin, Hoffmann-La Roche) was added as gelatin-stabilized beadlets. The feed contained 100 mg of astaxanthin/kg of diet or 80 mg of canthaxanthin/kg of diet according to EC legislation. Feed was pelleted using a dry, steamless pelleting machine (Simon-Heesen B.V., Boxtel, The Netherlands) fitted with a 4 mm diameter die. The pelleted diets were allowed to dry for 1 h at ambient temperature and were stored at 4 °C. At the end of the feeding period the fish were sacrificed by a blow to the head.

Packaging and Storage Condition. Trout were deskinned and filleted. The head and tail were removed by cutting fillets in front of the anal fin and halfway between the head and the front of the back fin. All left lateral muscles were vacuum packed (Multivac A300, Multivac, Wolfertschwenden, Germany). Low permeable plastic laminate film was used for packaging (BB4L Cryovac, Grace S.A., Barcelona, Spain). The fillets were stored at 4 °C, and samples were removed for color, chemical, and microbiological analyses at 1, 3, 5, 7, 11, and 15 days of storage. Five packs were opened each observation day to carry out the different analyses.

Analytical Methods. *Microbiological Assays.* Ten grams for each sample was diluted 10-fold in peptone water (PW) and homogenized for 30 s in a Masticator (IUL GmbH, Königswinter, Germany). The homogenates were further diluted in PW for the different microbiological analyses. In all experiments, total viable counts (TVC) were performed by pour plating with plate count agar (PCA) (Cultimed, Panreac S.A., Barcelona, Spain), and plates were incubated at 30 °C for 3 days (Pascual Anderson, 1992a). Total psychrotrophic counts (TPC) were done using the pour-plating method on PCA at 6.5 °C for 10 days (Pascual Anderson, 1992b). Total coliforms (TC) were enumerated on pour plates prepared with Violet Red Bile agar (VRBA; Cultimed, Panreac S.A.) at 37 °C for 24 h (Pascual Anderson, 1992c). Plates were counted and the counts expressed as log colony-forming units per gram of muscle.

Color Analysis. Color was measured in CIELCH color space. Measurements were made using a spectrochromameter (CM-508-i, Minolta Camera Co., Ltd., Osaka, Japan) with an 8 mm diameter measuring area with respect to CIE (1976) reference standard illuminant D_{65} and observer angle 10°. Before measurements, the apparatus was calibrated with a white plate reference standard (CM-A21, Minolta Camera Co.). The measurement of the color attributes was carried out by applying the spectrochromameter onto three points of fish muscle intact: neck, back, and tail parts. Lightness (L^*), redness (a^*), and yellowness (b^*) values were calculated from the absorbance spectra in the range 400−700 nm at 20 nm intervals. Each color value was calculated by averaging three measurements. From a^* and b^* values the hue [$H(^\circ)_{ab} = tg^{-1}b^*/a^*$] and the chroma [$C^*_{ab} = (a^{*2} + b^{*2})^{1/2}$] were calculated according to the method of Wyszecki and Stiles (1967) using the computer software Chroma Control S (CM-1 release 1.20, Minolta Camera Co.).

Carotenoids. All carotenoid analyses were carried out in duplicate on homogenized flesh, and the general precautions for isolation and handling of carotenoids (Fiasson et al., 1969) were followed. Carotenoid concentrations were determined by HPLC (Guillou et al., 1993) using a pump (2150, LKB, Bromma, Sweden) and a photodiode array detector (990, Waters, Bedford, MA). The analytical column (150 × 3.9 mm i.d. stainless steel tube) was packed with C_{18} reversed phase material of 4 μm particle size (Waters). Elution was performed with an isocratic solvent: acetonitrile/dichloromethane/methanol/water/propionic acid (71:22:4:2:1, by volume) at 0.3 mL/min. All solvents used were of HPLC grade (Panreac Química S.A., Barcelona, Spain) and filtered through a 0.45 μm nylon membrane filter (Scharlau S.A., Barcelona, Spain) before use. External standard in solutions of known concentrations was used for peak identification and quantification. The detection of carotenoids was carried out at 480 nm for astaxanthin and at 472 nm for canthaxanthin (Guillou et al., 1993). Data were computed (990 software, Waters).

TBARS. Fish samples (neck, back, and tail) were homogenized and analyzed using a modification of the thiobarbituric acid (TBA) method (New, 1990). One gram of sample of this homogenate was homogenized (Heldolph RZR-1, Selecta, Barcelona, Spain) with 3 mL of distillated water (DW). To 100 μL of this homogenate, containing ~1 mg of lipids, were added 100 μL of BHT solution (0.22 g in 100 mL of absolute ethanol), 1.5 mL of glycine buffer (2.23 g in 100 mL of DW), and 1.5 mL of TBA reagent (0.5 g of TBA and 0.3 g of sodium dodecyl sulfate in 100 mL of DW). Samples were stirred and heated in a boiling water bath for 30 min and then immediately cooled in an ice bath. One milliliter of glacial acetic acid followed by 2 mL of chloroform was added to the samples, and the mixture was vortexed vigorously and centrifuged (type 7000577, Selecta) at 2000*g* for 20 min.

This method is based on the spectrophotometric measurement of a red chromophore formed by the reaction of TBA with secondary products from lipid oxidation of unsaturated fatty acids with malondialdehyde (MDA). The supernatant was read at 532 and 450 nm (Bird et al., 1983) using a UV−vis spectrophotometer (PU8745 Philips S.A., Cambridge, England). A blank with DW and a standard solution curve of tetramethoxypropane (TMP) in DW were treated similarly as the samples. TBARS were expressed as nanomoles of TMP per milligram of lipid.

Exudate. Exudation losses were evaluated from the loss of weight of the muscles expressed as a percentage of initial fish weight (Pastoriza et al., 1996).

Statistical Analyses. Data were subjected to one-way analysis of variance (ANOVA) and Student t test using SPSS program for PC (Narusis, 1995) to study the effect of storage time on pigment retention, TBARS, microbial development, and CIELCH color values.

Table 1. Bacterial Counts[a] of Pigmented Rainbow Trout Fillets during Vacuum Packaging at 4 °C

storage time (days)	TVC[b]	TPC[b]	TC[b]
1	3.1 ± 0.8	1.3 ± 0.0	1.3 ± 0.0
3	4.7 ± 0.6	1.9 ± 0.2	1.3 ± 0.0
5	5.5 ± 1.1*	2.0 ± 0.2*	5.6 ± 0.4*
7	6.5 ± 1.0*	2.3 ± 0.2*	6.6 ± 0.5*
11	8.2 ± 0.3*	5.9 ± 0.1*	8.5 ± 0.0*
15	9.3 ± 0.1*	6.0 ± 0.1*	8.4 ± 0.1*

[a] Log microbial counts. [b] Values are expressed as means of six independent determinations (three samples for astaxanthin and three samples for canthaxanthin) ± SD. An asterisk (*) indicates statistical significance ($P < 0.05$).

RESULTS

Microbiological Changes. Microbial counts on rainbow trout fillets stored at 4 °C under vacuum packaging are summarized in Table 1. Each value represents the mean value of six samples (three fillets for astaxanthin and three fillets for canthaxanthin).

Table 2. Changes in Color Parameters (CIELCH) of Pigmented Trout Fillets with Astaxanthin during Vacuum Packaging at 4 °C

storage time (days)	L^* [a]	C^* [a]	$H(°)_{ab}$ [a]
1	4.3 ± 3.6	15.3 ± 3.3	61.3 ± 6.3
3	44.8 ± 1.0	18.1 ± 2.3	64.3 ± 2.0
5	43.0 ± 1.1	15.8 ± 2.3	60.7 ± 3.5
7	43.5 ± 3.6	14.1 ± 1.2	62.4 ± 7.0
11	50.5 ± 5.5*	17.2 ± 6.6	60.4 ± 6.7
15	51.8 ± 03.4*	16.7 ± 6.9	55.1 ± 3.0

[a] Values are expressed as means of five independent determinations ± SD. An asterisk (*) indicates statistical significance ($P < 0.05$).

Table 3. Changes in Color Parameters (CIELCH) of Pigmented Trout Fillets with Canthaxanthin during Vacuum Packaging at 4 °C

storage time (days)	L^* [a]	C^* [a]	$H(°)_{ab}$ [a]
1	45.6 ± 3.7	10.9 ± 3.6	44.6 ± 12.4
3	47.4 ± 1.5	13.1 ± 4.2	43.9 ± 3.7
5	46.3 ± 2.1	14.7 ± 5.4	41.0 ± 3.7
7	47.4. ± 2.1	12.4 ± 1.6	39.7 ± 3.4
11	47.7. ± 1.2	9.8 ± 3.9	44.7 ± 5.8
15	48.1 ± 1.8	10.2 ± 2.5	44.1 ± 1.5

[a] Values are expressed as means of five independent determinations ± SD. An asterisk (*) indicates statistical significance ($P < 0.05$).

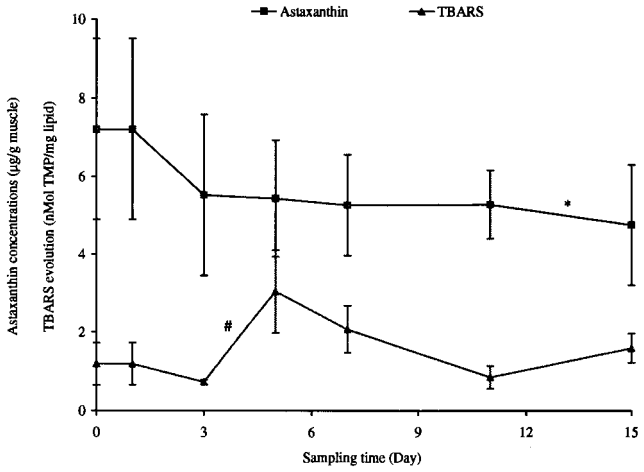

Figure 1. Muscle astaxanthin concentration (μg/g of muscle) evolution and means for development of TBARS (nmol of TMP/mg of lipid) under vacuum packaging during chilled storage (mean ± SD, $n = 5$): (*) significant differences ($P < 0.05$) in astaxanthin content; (#) significant differences ($P < 0.05$) in TBARS evolution.

TVC values of stored samples showed significant increases ($P < 0.05$) after 5 days. TPC and TC followed a pattern similar to that of TVC. Storage time accelerated bacterial growth in fish. Significant differences ($P < 0.05$) were noted for TVC, TPC, and TC development during storage.

Storage Effects on Color Stability. The muscle of fish fed astaxanthin or canthaxanthin was colored with variable intensity. Tables 2 and 3 show the effect of chilled storage length on color parameter changes. No significant differences were noted for chroma (C^*) and hue [$H(°)_{ab}$] with increasing storage. However, significant differences ($P < 0.05$) for lightness (L^*) between days 5 and 11 were observed.

Changes in Carotenoid Content. Figures 1 and 2 show the effect of length of chilled storage on changes

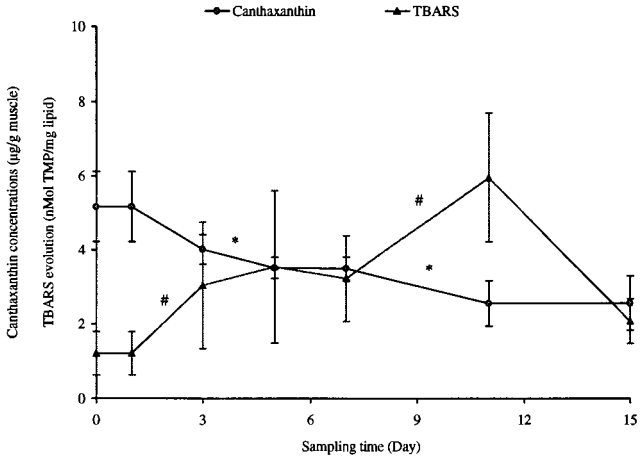

Figure 2. Muscle canthaxanthin concentration (μg/g of muscle) evolution and means for development of TBARS (nmol of TMP/mg of lipid) under vacuum packaging during chilled storage (mean ± SD, $n = 5$): (*) significant differences ($P < 0.05$) in canthaxanthin content; (#) significant differences ($P < 0.05$) in TBARS evolution.

in carotenoid concentration. A significant loss ($P < 0.05$) in astaxanthin concentration (34%) was noted between the first day of storage and 15 days of storage. At the beginning of the experiment, a notable amount of astaxanthin (7.2 μg of astaxanthin/g of muscle) was measured in the flesh. At 5 days of storage, astaxanthin concentration decreased by 25%. In contrast, canthaxanthin evolution showed a marked decrease until 5 days after storage (32%) followed by a second decrease at 11 days (49%).

Lipid Oxidation. The TBARS values in muscle tissue after storage of fish fed astaxanthin (Figure 1) showed no significant differences at 3 days ($P > 0.05$). At 5 days after storage, TBARS amounts increased rapidly and were significantly higher ($P < 0.05$) than those found at 3 days after storage. TBARS values decreased until 15 days of storage.

In muscle tissue of fish fed canthaxanthin (Figure 2) an increase in TBARS values took place at the same time (5 days after storage) as in muscle of fish fed astaxanthin. Nevertheless, the highest TBARS values were found at 11 days (5.9 nmol of TMP/mg of lipid) in flesh of trout fed canthaxanthin. TBARS levels of canthaxanthin-fed trout muscles were higher than those of astaxanthin-fed trout muscles.

The decrease of astaxanthin and canthaxanthin content was observed at the same time when microbial development and TBARS values increased. Canthaxanthin decrease was more marked than that of astaxanthin. The first decrease at 5 days of canthaxanthin content was similar to that of astaxanthin, but a notable second decrease in canthaxanthin level was observed at 11 days corresponding to a maximum of TBARS values. An important exudate loss was observed in vacuum-stored rainbow trout fillets after 72 h. Exudate loss increased throughout storage, with values increasing from 2.6 to 4.4% (Figure 3).

DISCUSSION

Considerable color variations were observed among neck, back, and tail parts in spectrochromometer measurements. These differences are due to variation in carotenoid concentrations along the fish. However, Yamazaki et al. (1983) did not find significant differ-

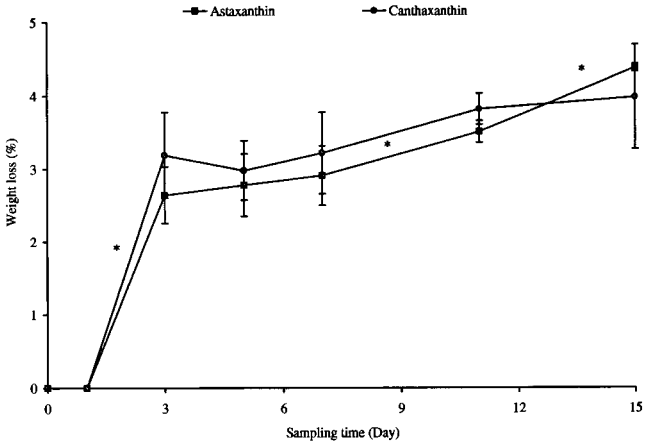

Figure 3. Weight loss of pigmented (astaxanthin and canthaxanthin) rainbow trout fillets packed under vacuum during storage at 4 °C (mean ± SD, $n = 5$): (*) significant differences ($P < 0.05$).

ences in astaxanthin content among back, belly, and tail of silver salmon (*O. kisutch*). The functioning of chromometers is comparable to the principle of color perception used by the human eye. Instrumentally measured color is also highly correlated with values from sensory tests (i.e., appearance) (Skrede et al., 1990a) and color cards (Skrede et al., 1990b; Choubert et al., 1997). Instrumental color measurements and sensory panelist assessment of discoloration in beef steaks demonstrated similar responses (Chan et al., 1995).

A loss in carotenoid content was observed in trout fillets stored under vacuum packaging at 4 °C during storage time. Nevertheless, the decrease of canthaxanthin (49%) is more important than that of astaxanthin (25%) because this loss is marked by a second decrease at 11 days. Vacuum packaging was effective in short-term preservation of red coloration for astaxanthin but not for canthaxanthin-pigmented trout at refrigeration temperatures. Temperature of storage and oxygen concentration both are considered important in determining the rate of loss of carotenoid pigments during processing and storage of seafood products (Lusk et al., 1964). Our results show that the carotenoid decrease is not reflected by chroma (C^*) color value. This may be explained by the fact that astaxanthin or canthaxanthin concentration was determined in homogenized flesh from the fillets and not in the surface layer.

TBARS values were used to measure oxidative rancidity as well as freshness of the fish studied (Kolakowska and Deutry, 1983). TBARS increase is considered to be an indication of onset of oxidative rancidity in fresh fish (Sweet, 1973). The TBA test probably is the most extensively used chemical method for semiquantitative estimation of lipid oxidation in foods (Sorensen and Jorgensen, 1966). TBARS values found in the flesh of fish fed canthaxanthin were higher than those of fish supplied with astaxanthin at 5 days. However, the important TBARS increase at 11 days for canthaxanthin exceeds levels found for astaxanthin. The increase of TBARS resulted from reactions of fish lipids leading to an accumulation of lipid secondary oxidation products such as MDA. The higher TBARS levels were attributed to an increased production of secondary oxidation products. Carotenoids are very efficient antioxidants (Burton and Ingold, 1984), and as an antioxidant, astaxanthin functions in protecting lipid tissue from peroxidation.

If 10^6 microorganisms/g concentrations are considered the TVC limit of acceptability (Pastoriza et al., 1996), shelf life of rainbow trout fillets was 5 days when stored under vacuum packaging at 4 °C. On the basis of the results of the present investigation the increase of TBARS values and microbial development took place at 5 days and at the same time astaxanthin and canthaxanthin content decreased; thus, these two phenomena may be related.

In addition, the second increase of TBARS values in muscles pigmented with canthaxanthin values occurred at 11 days when an important decrease in canthaxanthin content occurred. Carotenoids are bleached as the result of complex autoxidation reactions (Tannenbaum et al., 1993). On the other hand, microbial development took place at 5 days when carotenoid content in muscle decreased. Bala et al. (1977) reported a concomitant decrease in oxymyoglobin content with increased bacterial load and suggested that bacteria may cause oxymyoglobin oxidation. O'Keefe and Hood (1982) hyphothesized that increased oxygen consumption, which might be initiated by bacterial growth, may lower partial oxygen pressure sufficiently to enhance oxymyoglobin discoloration. However, Bevilacqua and Zaritzky (1986) provided data that failed to support this hyphothesis.

The oxygen transmission rate (OTR) of the packaging film itself is important for the development of secondary oxidation products from the lipids in rainbow trout fillets during freezer storage (Christophersen et al., 1992). The OTR of the plastic film used is currently used for food packaging. No prevention of lipid oxidation was found in vaccum packaging in rainbow trout since astaxanthin, play as a "super vitamin E" (Miki, 1991, 1996). This result is in agreement with those of Huang et al. (1992) for TBARS production during iced storage of fillets from channel catfish (*Ictalurus punctatus*) using vacuum packaging.

Exudation is a consequence of a lower water-holding capacity of fish proteins and a lower muscle pH (Cheftel and Cheftel, 1976). Lipid oxidation products such as MDA may cause cross-linking of proteins, which decreases their solubility and their water-holding capacity (Know et al., 1965). Our results show that vacuum packaging at 4 °C was ineffective for pigmented rainbow trout fillets. The exudate present and carotenoid losses result in an undesirable product. To delay bacterial growth and rancidity development, lower storage temperatures should be investigated. In addition, future research should focus on procedures to reduce temperature or to use MAP with high levels of carbon dioxide.

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