COPPER INDUCED LIPID OXIDATION DURING SALTING OF COD (*GADUS MORHUA* L.)

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ABSTRACT

Yellow/brown discoloration of the muscle surface was investigated in downgraded heavily salted cod fillets from a processing plant. The results showed that discolored areas were relatively evenly distributed on the muscle surface and that lipid oxidation correlated with the copper and not the iron content, in the muscle. A model system for studying lipid oxidation and yellow discoloration of salted cod muscle was established and used for investigating the effect of including transition metals in the process. The model system confirms that copper is particularly pro-oxidative. Of the redox states, the reduced form of copper was most potent. It appears that copper concentrates in the muscle during the brining. The results also indicate that copper induced lipid oxidation was increased by low pH of the fish muscle postmortem. The model system seems well suited for detailed studies of the salting process and may be useful for developing methods which can inhibit lipid oxidation in salted products.

INTRODUCTION

Lean fish such as cod (*Gadus morhua* L.) store dietary lipids in the liver and the fat content of the edible muscle is less than 1% (Ackman 1967; Sidwell *et al.* 1978). Quality deterioration due to lipid oxidation has therefore not been regarded a problem in products based on lean fish muscle. However, such oxidation does not

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only depend on the amount of lipid present, but also on the cellular localization and the degree of unsaturation of the fatty acids (Khayat and Schwall 1983; Labuza 1971). The fatty acids in cod muscle are mostly found in the cellular membranes. The long-chain highly unsaturated fatty acid, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), which are most prone to oxidation, constitute a major portion of the fatty acids present (Khayat and Schwall 1983; Love 1988; Shahidi and Dunajski 1994). Extended frozen storage of cod fillets may lead to autoxidation of the phospholipid fraction and subsequent off-flavor formation (Hardy *et al.* 1979). Other methods of preservation, such as salting, may also reduce the acceptability of cod products due to lipid oxidation.

Heavily salted cod are traditional products from the North-Atlantic fisheries and are highly regarded as ripened fish products in many countries. Today, thoroughly washed split or filleted cod are usually pickle-salted or brine-cured for a short week and then salt ripened, i.e. kench cured in stacks, for at least 10 days. A major quality problem in such skin-on products is yellow/brownish discoloration of the flesh surface. The yellow/brown color may cover the whole muscle surface or only sections of it. Studies on salted split cod as long as fifty years ago, showed a positive correlation between the amount of copper and iron present in both the salt and muscle, and the yellow/brown discoloration of the muscle surface (Dyer 1949; Dyer and Gunnarsson 1954; Arnesen 1954; Shewan 1955). These reports did not however discuss the mechanisms or methods to prevent the discoloration other than to avoid or reduce the presence of these metals in the salt and the equipment used during processing. Due to the fairly unrefined and bulk nature of solar salt most often used, it is not always easy to comply with the limits of 0.1 and 10 ppm of copper and iron, respectively, in the salt.

The prooxidative effect of transition metals has been extensively studied using oils (Zama *et al.* 1979; Ke and Ackman 1976), specific fatty acids (Wills 1965; Liu 1970; Fisher and Deng 1977), isolated fish muscle sarcoplasmatic reticulum (Decker *et al.* 1989) or fish muscle blends (MacLean and Castell 1964; Castell and Spears 1968). However, less is known about the catalytic effect of these metals on lipids in intact or nonfractionated lean fish muscle. Blended or intact muscle tissues are more complex than pure lipid systems or isolated membranes. For example, the pH of the water fraction of seafood is an important variable to lipid oxidation by influencing the solubility of iron complexes and the oxidative properties of amino acids (Labuza 1971; Farag *et al.* 1978; Hultin 1994).

This paper reports on the discoloration, lipid oxidation and the presence of transition metals in the muscle surface of industrially produced, heavily salted cod fillets. A model system based on brining and subsequent kench curing of small cubes of fresh cod muscle is used for detailed studies of transition metal induced lipid oxidation during salting.

MATERIALS AND METHODS

Materials

Heavily salted, skin-on cod (*Gadus morhua* L.) fillets produced under traditional processing conditions, and downgraded due to yellow/brown discoloration, were obtained from a local salt fish producing plant in Northern Norway. In total, 180 fillets rejected due to yellow/brown discoloration and with a size variation between 0.3-1.4 kg, were visually examined. The distribution of the yellow color areas of the fillets was determined. Excess salt was removed and white and yellow-colored muscle surface areas from a total of 30 fillets were excised to a depth of 4 to 5 mm. The discolored and white areas were pooled separately and homogenized in a Braun Kitchen Food Processor (Braun Co. Ltd., Kronberg/TS, Germany) for 30 s. The two lots were subsequently thoroughly mixed by weight in different proportions (1 : 0, 2 : 1, 1 : 1, 1 : 2, 0 : 1) and analyzed for instrumental color, TBARS (thiobarbituric acid reactive substances), copper and iron contents.

Model System

A model system simulating normal processing conditions of salted cod was established. The cod used in these experiments were caught in February, March, June and August in the coastal waters of Vesterålen and Lofoten Islands in Northern Norway and had a round weight of 2 to 5 kg. The fish were gutted, headed and stored in ice for 2-3 days before filleting and skinning. The deboned fillets were cut into 1 - 2 cm cubes of muscle tissue. The cubes were brined for 5 to 9 days with 4 parts saturated salt solution (approximately 26% w/w NaCl in distilled water) containing different amounts of transition metals (0 - 10 ppm). Subsequently, the cubes were removed from the brine, drained for 30 s and kench cured for 15 days using solid NaCl p.a. in a weight ratio of 1 to 2. No transition metals were included during the kench curing. The temperature during the experiments was 8-10C.

Copper induced lipid oxidation was studied as described above using 400 g muscle cubes and 1600 g saturated brine for each copper concentration. To brines were added 0, 0.1, 0.5, 1.0, 3.0, 5.0 or 10 ppm Cu^{2+} from a stock solution of 10000 ppm ($CuCl_2 \cdot 2H_2O$). The pH of the fillets prior to salting were in the range of 6.58 to 6.75. The experiments were carried out twice for each copper level. Lipid oxidation (TBARS) and instrumental color of the salted muscle cubes were determined after 21 days. Additional experiments were performed using the model system with 0 or 3 ppm Cu^{2+} included during the brining period. For each concentration of copper, muscle cubes (1400 g) made from fillets with a pH prior to salting of 6.54 to 6.81, were brined with 5600 g saturated NaCl solution. TBARS, instrumental color and copper content of the muscle cubes were analyzed at different times during the 21 days of salting. An additional experiment with cod

fish having low (6.35) and high (6.98) muscle pH post mortem prior to salting, was performed to investigate the effect of post mortem muscle pH on copper induced oxidation during salting. From each cod, samples of 400 g of muscle cubes were brined with 1600 g saturated salt solution containing 1.0 ppm Cu^{2+}. Lipid oxidation (TBARS), instrumental color, muscle-pH and water content of the salted muscle cubes were determined at the end of the brining period (day 9) and at the end of the kench curing (day 21).

The effect of the redox state of copper and iron on lipid oxidation was investigated as described using 0.5 ppm of Cu^{+}, Cu^{2+}, Fe^{2+} or Fe^{3+} in the brines. The stock solutions (5000 ppm) used were CuCl, $CuCl_2 \cdot 2H_2O$, $FeCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, respectively. Brine without added transition metal was used as a control. The stock solution containing Cu^{+} was made immediately before the start of the experiment. Prior to the addition of muscle cubes, the pH of the five brines was in the range of 5.01 to 5.05. The pH of the fresh cod muscle cubes used was 6.54 to 6.65. TBARS and instrumental color were determined after the end of the brining period (day 6) and at the end of the kench curing (day 21).

Analyses

Samples were either taken at start, during the brining period of 5-9 days or at the end of the process, i.e. 21 days of salting. At each sampling, 200 g of muscle cubes (approximately 75-100 pieces) were removed and homogenized in a Braun Kitchen Food Processor for 30 s. The fish mince was either analyzed immediately or packed in plastic bags and stored for 1 - 4 weeks at -80C.

Instrumental color of the fresh or thawed salt fish mince was determined using a Minolta Chromameter, CR-200 (Minolta Camera Co., Ltd., Osaka, Japan). The detector was placed on the plastic film at four different areas of the sample recording the L* a* b* modus, obtaining a mean value.

The pH of fresh muscle tissue was measured in a 1:1 mixture of muscle homogenate and 0.15 M KCl, while the pH of salted muscle was recorded after mixing the muscle homogenate with 5 parts of distilled water. Two or three replicates of each sample were analyzed. Water content was determined by drying to constant weight at 105C.

Lipid oxidation was estimated by determining the amount of 2-thiobarbituric acid reactive substances (TBARS) in an aqueous trichloroacetic acid extract (Witte *et al.* 1970) as described previously (Dulavik *et al.* 1998). Two replicate extractions were made for each pooled sample of muscle cubes.

The concentrations of iron and copper in the muscle were determined after wet digestion; i.e. minced muscle, 8-10 g, was boiled in 25 mL diluted HCl (25%). The metals were analyzed by flame atomic-absorption spectrometry as described by Simpson and Blay (1966) using Perkin Elmer 3110 (Perkin Elmer Co. Ltd., Norwalk, CT) equipment. Standards of copper and iron (Titrisol, Merck,

Darmstadt, Germany) were also diluted in 25% (w/v) HCl. Three replicates for each pooled muscle sample were made. The average and standard deviation were calculated from these values.

Statistical analysis of the analytical data from muscle samples was performed on the software program SAS version 6.12 (SAS Institute, Cary, NC). Data were subjected to one-way analysis of variance (ANOVA) by using the general linear model procedure. Where statistical differences were noted for a measurement, differences among sample means were determined using the Tukey's Multiple comparison test. The level of significance was set at $p = 0.05$ for all tests.

RESULTS

Yellow Discolored Industrially Produced Fillets

It is known that yellow/brownish discoloration of salted cod can occur in different parts of the fillet surface. To investigate if some areas of the fillets are more prone to this quality reduction than others, the fillets were divided into four sections (loin, ventral, dorsal and tail) and the distribution of discolored areas were recorded. In total, 180 rejected fillets obtained from a salt fish processing plant were examined (Fig. 1). The yellow/brown color was relatively evenly distributed in all sections. However, it appeared that the ventral part of the fillets was more prone to discoloration than the others. Both the distribution and intensity of the yellow color varied in different fillets. Some of them were discolored in one region, others had smaller brown/yellow patches in all four sections and some of the fillets had a continuous discolored area covering more than one section. It was also observed that the depth of the discoloration, which appeared to penetrate 2-5 mm into flesh, seemed to correlate with the intensity of the yellow/brown color. No fillet gaping, pressure damages or gutting/filleting errors were associated with the yellow/brown parts.

The discoloration of industrially produced salted fillets were also studied by chemical analyses and instrumental color measurements as described in the Materials and Methods. It is shown in Fig. 2 that it is a positive correlation between increasing lipid oxidation measured as TBARS and instrumental yellow color (b*). Homogenized white surface had a b*-value of approximately +8 and a TBARS value of 7 nmol/g muscle while homogenized discolored areas had a b*-value of approximately +25 and a TBARS value of 28 nmol/g muscle. In the same samples, the reduction in lightness (L*) and the increase in red-green (a*) color were much smaller, 7 and 2.5 color units respectively (results not shown). The content of iron and copper in the blends of white and discolored salted muscle surfaces mixed in different proportions, were also analyzed and compared with the instrumental yellow color, b* (Fig. 2). The amount of copper correlated with the instrumental

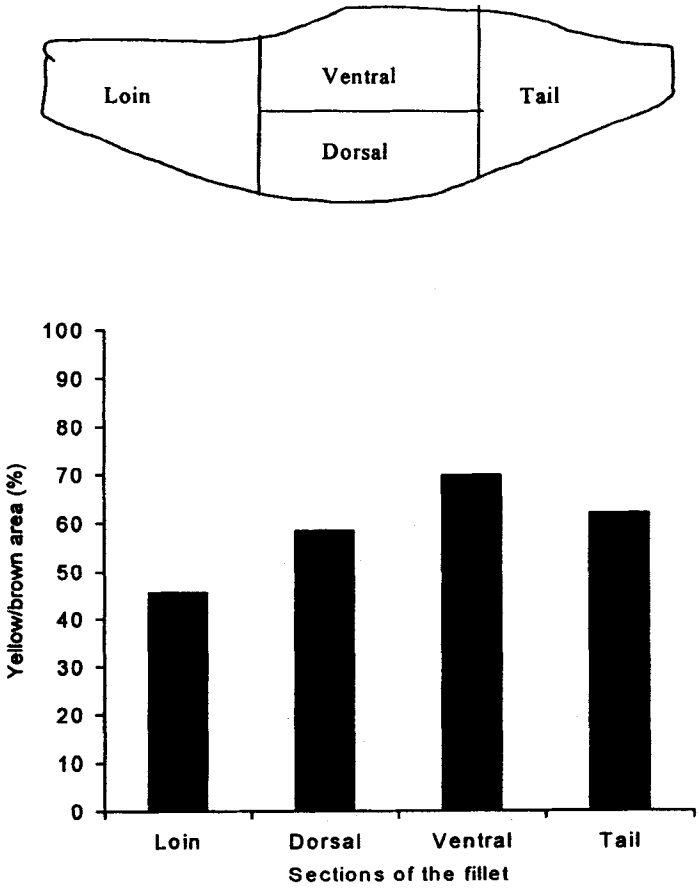

FIG.1. HEAVILY SALTED COD FILLETS (INDUSTRIAL PRODUCED) DOWNGRADED DUE TO PARTIAL DISCOLORING WERE DIVIDED INTO 4 SECTIONS: LOIN, VENTRAL, DORSAL AND TAIL AREAS
From a total number of 180 fillets, the distribution (%) of yellow/brown areas on the fillets was estimated.

yellow color and the lipid oxidation of the muscle blends. The copper concentration increased from approximately 1 ppm to 5 ppm with the increase in yellow color of the samples. However, a nonlinear relationship appears to exist between the yellow color and the copper content. The largest difference in yellow color occurred in samples with copper content ranging from 1 to 2 ppm. No correlation existed between TBARS or yellow color of the muscle blends and iron concentration which varied from around 9 to 10 ppm in all blends.

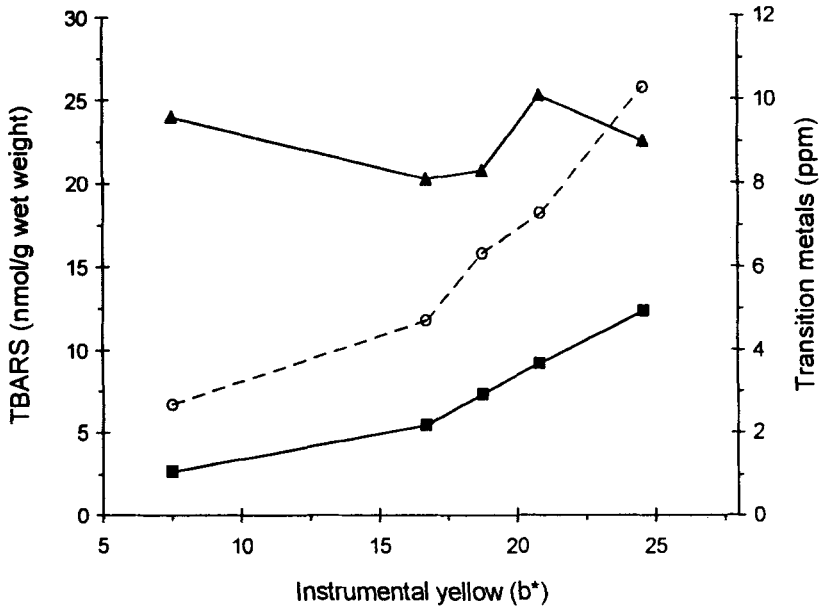

FIG. 2. THE EFFECT OF INCREASING AMOUNT (FROM 0 TO 100%) OF YELLOW/BROWN DISCOLORED MUSCLE SURFACE IN MUSCLE BLENDS ON INSTRUMENTAL YELLOW COLOR (b*), 2-THIOBARBITURIC REACTIVE SUBSTANCES (TBARS) AND CONTENT OF TRANSITION METALS
TBARS, expressed as Malondialdehyde (MDA) Equivalents, (---O---), copper (PPM) (—■—) and iron (PPM) (—▲—).

Model Systems

A model system consisting of small cubes of fresh cod muscles and brines simulating normal processing conditions was used to study metal-induced oxidation. Figure 3 shows the instrumental yellow color (b*) and TBARS of muscle tissue at the end of the kench curing when different amounts of copper (Cu^{2+}) had been included in the brines. Addition of small amounts of Cu^{2+} (>0.5 ppm) to the brine resulted in 4 to 5 times higher oxidation levels ($p<0.05$), measured both as TBARS and instrumental yellow color, in the salt-ripened muscle compared to the control. Also in this model system, a nonlinear relationship was noted between the copper concentration in the brine and the oxidation level in the muscle at the end of the kench curing (21 days of salting). The maximum instrumental recorded yellow color is approached in samples with 1 ppm Cu^{2+} added to the brine while TBARS started to level off with 3 ppm Cu^{2+} included.

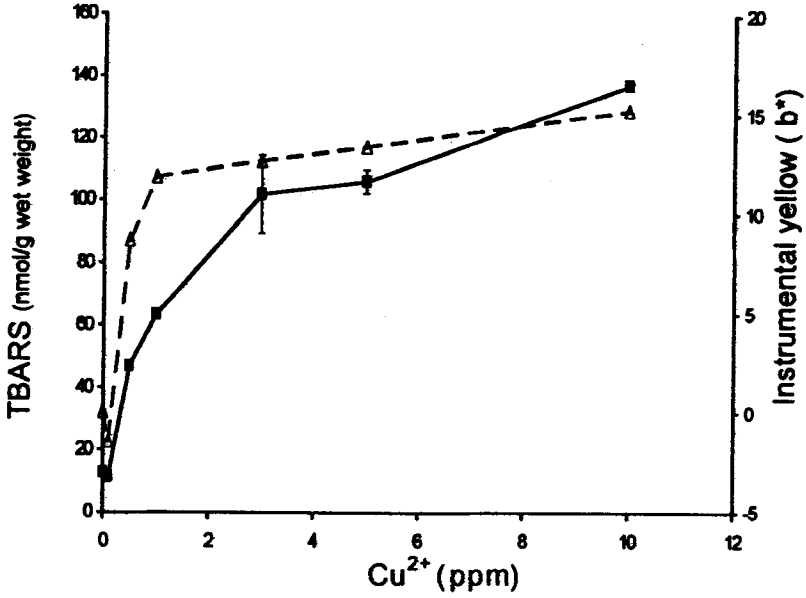

FIG. 3. LIPID OXIDATION IN SALT RIPENED COD STUDIED BY THE MODEL SYSTEM
The effect of increasing copper concentration in brines on the TBARS and instrumental yellow color (b*) of the muscle cubes at the end of the kench curing (day 21). TBARS, expressed as malondialdehyde (MDA) equivalents (—■—), instrumental yellow color, b*, (---Δ---). Error bars represent mean standard deviations for TBARS (N=7-22), only two of them visible because of small values.

Figure 4 shows the uptake of copper in the muscle and the development of lipid oxidation and instrumental yellow color in the model system during the salting process. Using 3 ppm copper in the brine, the copper concentration increased steadily in the muscle from 1.5 to 8 ppm during the first 3 days of brining (Fig. 4a). After 3 days the lipid oxidation measured as TBARS was higher than in the control and reached at the end of the kench curing approximately 115 nmol/g (Fig. 4b). The study also showed that some lipid oxidation occurred in the control samples, which had no added copper. In these samples, the TBARS increased from approximately 10 nmol/g at the start of the salting to 25 nmol/g, at the end of the kench curing (day 21). Development of yellow color could not be detected during the brining with 3 ppm copper added. However, at the end of the experiment, the difference in b*-value between the copper-brined samples and the control was about 9 (Fig. 4c).

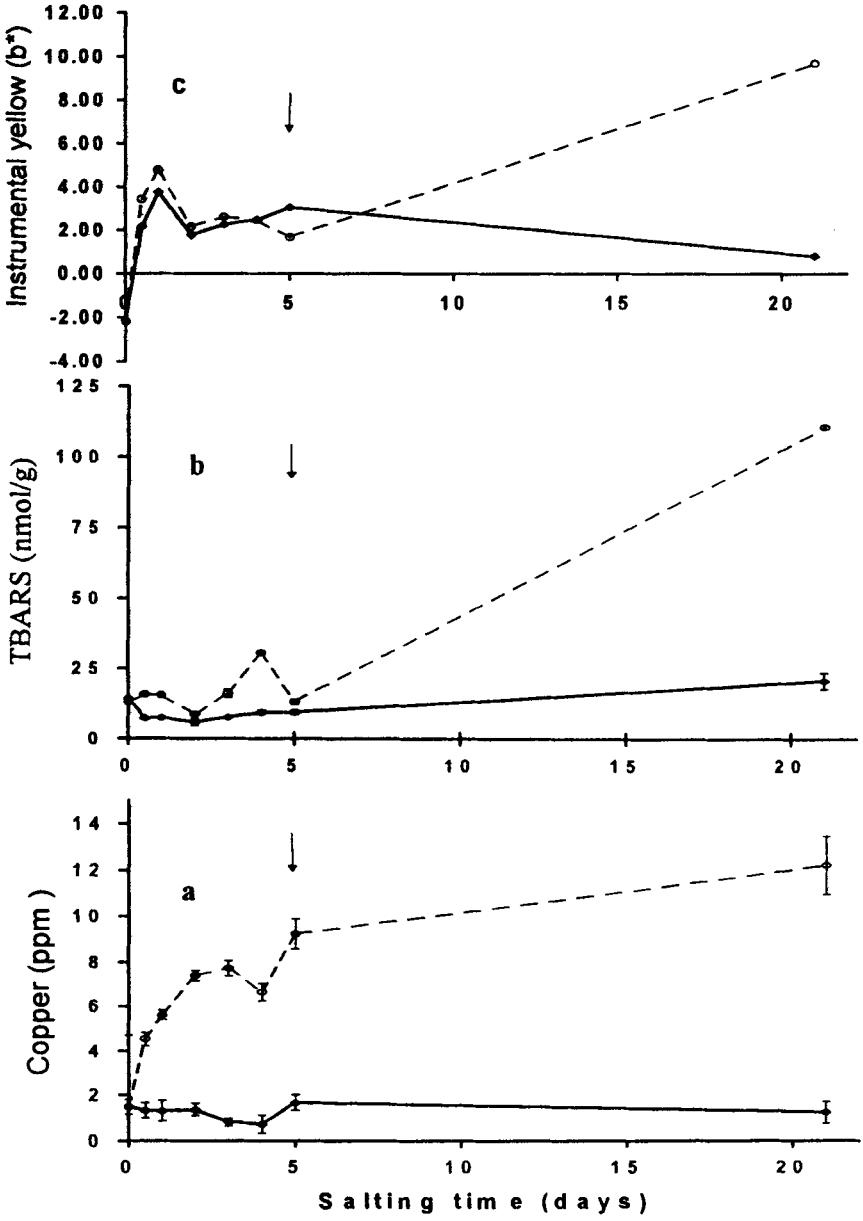

FIG. 4. LIPID OXIDATION AND COPPER CONTENT IN COD MUSCLE DURING 21 DAYS OF SALTING IN THE MODEL SYSTEM

Brines with 3 ppm copper added (--o--). Brines without added copper (—◆—). The arrows (→) indicate end of brining period and start of kench curing. (**a**): average content of copper (n=4-6) in the muscle cubes, (**b**): average TBARS expressed as malondialdehyde (MDA) equivalents (n=4-8) in the muscle cubes, (**c**): average instrumental yellow color, b*, of minced muscle cubes. Error bars in (**a**) and (**b**) represent mean standard deviations, almost not visible in (**b**) because of small values.

The model system was also used to study copper induced lipid oxidation in cod-fishes having low (6.35) and high muscle pH (6.98). Table 1 shows the instrumental yellow color (b*), TBARS, water content and muscle pH on day 9 (end of brining) and day 21 (end of kench curing). Lipid oxidation in the heavily salted product at the end of the kench curing appeared to be dependent on the pH of the fresh muscle. The fish with the low muscle pH had TBARS and instrumental, b*- value of 124.9 ± 3.4 nmol/g and 17.6 ± 1.8 while the fish with the high muscle pH had values of 80.6 ± 8.8 nmol/g and 11.9 ± 1.8, respectively. It is also shown in Table 1 that the pH of the muscle declines during the brining period of 9 days. The low pH (pH 6.35) and high pH (pH 6.98) muscles decreased in pH to 5.79 and 5.99, respectively. No further significant decrease in the pH was observed during the rest of the salting process. The water content of the muscles at the end of the brining period (day 9) was similar, approximately 69.5%. However, after finishing the kench curing (day 21), the water content of the muscle with the low initial pH was considerably lower than in the muscle with the high initial pH; 58.6 and 62.9%, respectively.

TABLE 1.
COPPER (1 PPM) INDUCED LIPID OXIDATION MEASURED AS TBARS[1] (NMOL/G WET WEIGHT) AND INSTRUMENTAL YELLOW COLOR, (b*)[1], IN COD FILLETS HAVING LOW AND HIGH MUSCLE pH[1] POST MORTEM (6.35 AND 6.98, RESPECTIVELY). WATER[1] CONTENT (%) AND MUSCLE pH[1] WERE DETERMINED AT DAY 9 AND 21 OF THE SALTING PROCESS

	SALTING TIME (DAYS):	
	9	21
High muscle pH		
Muscle pH	5.99 ± 0.16	5.96 ± 0.13
Water content	69.2 ± 0.6	62.9 ± 0.01
TBARS	21.2 ± 4.7	80.6 ± 8.8
Instrumental yellow, b*	-0.2 ± 3.7	11.9 ± 1.8
Low muscle pH		
Muscle pH	5.79 ± 0.01	5.78 ± 0.01
Water content	69.8 ± 1.0	58.6 ± 0.7
TBARS	22.9 ± 1.5	124.9 ± 3.4
Instrumental yellow, b*	1.8 ± 4.0	17.6 ± 1.8

[1] Values are average ± standard deviation (n=3-4 samples)

The ability of copper and iron in different redox states to induce lipid oxidation in intact cod muscle was investigated using the model system. With low concentration of metals (0.5 ppm), only copper, independent of redox state, was able to induce lipid oxidation as measured by TBARS and instrumental yellow color in the final product (Table 2). At the end of the brining period (day 6) only reduced copper (Cu^{+}) and reduced iron (Fe^{2+}) gave elevated TBARS values. However, no effect was observed on the instrumental yellow color at this point of the process. At the end of the kench curing (day 21) both reduced and oxidized copper had induced lipid oxidation in the salt ripened muscle. The former was particularly pro-oxidative and produced TBARS and instrumental yellow color (b*) values of more than twice those obtained by the cupric ions. In the final product, no lipid oxidation could be measured when using low concentrations of reduced or oxidized iron during the brining period.

TABLE 2.
TRANSITION METAL (0.5 PPM) INDUCED LIPID OXIDATION MEASURED AS TBARS[1] (NMOL/G WET WEIGHT) AND INSTRUMENTAL YELLOW COLOR, (b*) [1] IN COD FILLETS AT DAY 6 AND AT THE END OF THE SALTING PROCESS (DAY 21)

	TRANSITION METALS:				
	Cu^{+}	Cu^{2+}	Fe^{2+}	Fe^{3+}	Control
Day 6					
TBARS	10.1 ±0.2	7.7 ±0.3	9.4 ±0.3	7.5 ±0.2	7.7 ±0.6
Yellow, b*	-1.1 ±2.3	-1.9 ±1.2	-0.7 ±2.0	-1.2 ±0.7	-1.9 ±1.2
Day 21					
TBARS	137.8 ±6.7	57.7 ±2.1	20.4 ±0.9	21.1 ±1.6	21.5 ±2.7
Yellow, b*	15.4 ±2.1	6.8 ±0.7	2.2 ±1.4	-0.8 ±1.6	1.6 ±1.2

[1] Values are average ± standard deviation (n=3-4 samples)

DISCUSSION

Yellow Discoloration of Industrially Produced Fillets

In this paper it is shown that areas of yellow discoloration are relatively evenly distributed on the muscle surface of salted cod fillets. This indicates that external factors such as contaminants present during the processing, may be a major cause of this problem. It appears however, that the ventral section of the fillet, which includes a part of the belly flap, has a higher abundance of discoloration than other parts of the fillet. This is probably due to residues of viscera or blood, not washed away before salting, catalyzing the formation of yellow color. The observation that the yellow colour penetrates 2-5 mm into flesh is consistent with an oxygen dependent process (Lawrie 1974).

The results (Fig. 2) from the analyses of the muscle surfaces excised from the rejected salted cod fillets confirm and extend the results provided by the early reports of Shewan (1955), Dyer (1949), Dyer and Gunnarsson (1954) and Arnesen (1954) on salted split cod. A positive correlation between the copper content, lipid oxidation (TBARS) and instrumental yellow color is clearly demonstrated. It is assumed that hydroperoxide radicals and carbonyl compounds arising from oxidation of the highly unsaturated fatty acids in the cell membranes, react with free amino groups with a subsequent condensation to polymeric brown pigments as described by Pokorný (1981). Transition metal ions are important pro-oxidants in biological systems. The concentrations of iron and copper of respectively 1 and 9 ppm found in white surfaces from the salted fillets are in line with the previously reported values for fresh muscle of codfish (Sidwell *et al.* 1978; Dulavik *et al.* 1998). Although the total concentration of iron was higher than that of copper in all samples, it is clearly evident that the latter is responsible for the increase in lipid oxidation. The elevated concentration of copper seen in discolored areas of salted fillets might be due to exogenous copper present in the salt, water or equipment used during the processing. The results further indicate that properties of the muscle tissue may also contribute to the increased copper content in the muscle surface. The discolored areas are relatively large and can cover more than half of the fillet indicating that discrete copper contaminants in the solid NaCl used during the kench curing is not the cause. If dissolved copper is present in the brine, one would expect an evenly discolored muscle. Decker *et al.* (1989) have reported that the press juice of winter flounder muscle contained 1.6 μM copper and that most of this was present as low molecular weight forms. If a similar situation is found in cod, it is possible that such low molecular forms are lost to the brine during the liquid loss from the muscle in the salting process. Further investigations are required to determine the source of copper causing the increased concentration in discolored salted fillet.

Model Systems

Traditional production of salted cod fillets requires approximately three weeks. After about one week of brining in saturated NaCl solution, the fish is ripened by dry salting in stacks (kench cured) for 10 to 14 days. The model system simulating normal production of salted cod, was used to investigate the effect of copper added to the brine made of distilled water saturated with NaCl. The results (Fig. 3) from our study of the effect of increasing concentrations of Cu^{2+} on the level of TBARS in salted cod show a logarithmic curve and are basically similar to those reported for metal-induced oxidation in fresh blended cod muscle by MacLean and Castell (1964). Ke and Ackman (1976) also found that both meat and skin lipids from mackerel were particularly prone to oxidation when the copper and iron concentrations were below 5 ppm, but they could not measure any further increase

in the oxidation level when the concentration of catalyst was higher than 5 ppm. Using 1 ppm copper in the brine, we observed that the formation of yellow color approached maximum level while the amount of detectable TBARS were less than half of the maximum at the end of the kench curing. It thus appears that when 1 ppm or less copper were used, TBARS formed may have reacted with amino groups giving yellow pigmented material. When higher concentrations of copper were used, a surplus of TBARS were formed and detected in the salt ripened tissue. The results obtained with the model system agree well with the findings from the analysis of the discoloured fillets produced by the industry. In both cases, an increase in copper concentration from 1 to 2 ppm produced a relatively large increase in instrumental yellow color, but not in the amount of TBARS.

The time course study showed that when cod muscle was salted with a copper containing brine, the transition metal was rapidly absorbed by the muscle tissue. It appears, however, that the uptake of copper is more than merely an equilibrium process since the concentration of copper in the muscle, at the end of the brining period, is approximately 3 times higher than that in the brine. This might be explained by binding of positively charged copper ions to negatively charged proteins or other tissue components. The fish muscles show a 25% weight reduction during the salting process due to osmotic water loss. In muscle samples brined without added copper, a slight reduction in the copper content of the muscle was indicated, thus suggesting that copper was lost in the water expelled from the tissue. The results from the time course study also demonstrated that some lipid oxidation occurred in the fish muscle brined without copper. This can be explained by the large proportions of EPA and DHA in the cellular membranes, and the pro-oxidative effect of NaCl (Smith *et al.* 1990; Castell *et al.* 1965; Koizumi *et al.* 1981; Osinchak *et al.* 1992). The pro-oxidative effect of NaCl may be due to the reduced water activity in the muscle as reviewed by Labuza (1971), the reduction in muscle pH during the salting process, or the ability of sodium ions to replace iron-ions of cellular complexes (Hultin 1992). In most cases, the development of rancid smell and taste is regarded negatively. We suggest, however, that the slight increase in TBARS during salt ripening of cod contributes to the sensory properties required in this kind of product.

It is well known that the ultimate pH of the cod muscle influences important quality properties, such as texture (Love *et al.* 1974), fillet gaping (Love *et al.* 1972) and water holding capacity (Ofstad *et al.* 1996). Due to the seasonal variation in feed intake and spawning the post mortem pH of the cod muscle varies (Love 1979). In our preliminary study on the effect of the ultimate muscle pH post mortem, we observed that the lowest muscle pH gives the highest lipid oxidation. Castell *et al.* (1965) observed that when the pH of the cod muscle blends were adjusted and added 12% NaCl, highest lipid oxidation occurred at low pH. The mechanisms by which pH controls lipid oxidation in fish muscle is unclear. However, it has been suggested, in studies on porcine and beef meat, that this may

be the pH effect on the metal catalysts present (Owen and Lawrie 1975; Yasosky *et al.* 1984; Liu 1970).

The liquid loss during the salting process, is caused by competitive binding among water, salt and amino acid side groups. At high salt concentrations the salt-protein interaction dominates and water is lost to the environment. The approximately 4% lower water content of the kench cured muscle with lowest pH, is explained by a change in the charge of protein molecules in the direction of their isoelectric point. At this pH the protein-protein interactions are maximal and the associated, shrunken proteins exhibit minimal hydration and swelling.

The ability of copper and iron in different redox states to induce lipid oxidation in intact cod muscle was investigated using the model system. The results showed that with low concentrations of metals (0.5 ppm), only copper, induced lipid oxidation as measured by TBARS and instrumental yellow color in the final product. These results from the model system are in agreement with the industrial experience mentioned in the introduction. Ferrous iron may be inactivated either by physical interaction (chelation) or by chemical oxidation as suggested by Hultin (1992). Our findings appear contradictory to the results reported by Decker *et al.* (1989). In their work on the effect of iron and copper on lipid oxidation in sarcoplasmatic reticulum, it was observed that iron was the most effective catalyst at low concentrations (0-0.2ppm). They studied metal induced lipid oxidation in the presence of a 5 mM histidine buffer. Histidine has, however, been reported to be a strong inhibitor of copper induced hydroxyl radical formation (Rowley and Halliwell 1983). In biological systems, copper has received far less attention than iron as a pro-oxidant. But copper has been reported to be as effective or even more effective in stimulating the decomposition of peroxides (Gutteridge *et al.* 1984), causing for instance protein modification (Stadtman 1990) and formation of fluorescent lipid complexes (Gutteridge 1984).

In our model experiments, both reduced and oxidized copper induced lipid oxidation in salt-ripened muscle. The former was particularly pro-oxidative producing TBARS and instrumental yellow color (b*) values of more than twice the values obtained by the cupric ions. This may possibly be explained by direct participation of the reduced copper in a Fenton-type reaction where hydroperoxides are reduced to hydroxyl radicals or lipid alkoxy radicals. In addition, autoxidation of the metal may reduce oxygen to a superoxide radical anion, which can dismutate to hydrogen peroxide or oxidize to singlet oxygen. The latter can react directly with an unsaturated fatty acid to initiate lipid peroxidation. The oxidized metal may then react with fatty acids or a fatty acid peroxides to form fatty acid radicals or fatty acid peroxy radicals.

This paper demonstrated that copper is responsible for partial discoloration of salted cod fillets as often experienced by the industry. The model system presented in this work may be used to find ways to overcome the associated problems.

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