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Evaluation of Occasional Nonresponse of a Washed Cod Mince Model to Hemoglobin (Hb)-Mediated Oxidation

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An emerging model to test antioxidants for application in seafoods is washed cod mince fortified with hemoglobin (Hb) as a catalyst. This system has been used to test the antioxidative activity of certain muscle extracts and some pure compounds such as BHA, BHT, TBHQ, and propyl gallate during ice storage. However, the washed cod mince model has occasionally been resistant to Hb-mediated oxidation. This has been in cases when the moisture of the model has been minimized by washes at the protein isoelectric point (pH ∼5.5) to allow for large additions of potentially antioxidative solutions. In this paper, noncontrollable and controllable factors for this intriguing occasional oxidation resistance were studied. Compositional analyses (lipid content, α-tocopherol, and lipid hydroperoxides) and structural analysis of a "normal" oxidizing model and a stable model were done to identify any differences among them. Some controllable factors related to the model preparation that were studied included different washing pH values (5.5−6.6), Hb concentrations (7.2 and 13.5 μM), final model moisture contents (75, 81, and 90%), and light exposure during ice storage (0 h, 3−4 h, or 24 h of light/day). Results revealed a 2-fold higher α-tocopherol content in the stable model than in the oxidizing model. Electron microscopy images showed a more and less disrupted myofibrillar structure in the stable and the oxidizing cod model, respectively. This indicated that "cold setting" (i.e., pre-gelation) of the stable model may have occurred and prevented Hb from diffusing freely in the model. Controllable factors that reduced lipid oxidation in the models were less Hb and lower moisture.

KEYWORDS: Antioxidant; washed; cod mince model; α-tocopherol; hydroperoxides; hemoglobin; moisture; lipid oxidation

INTRODUCTION

A considerable amount of research is being done on different aspects of antioxidants. However, there is a growing concern in the scientific community regarding antioxidant testing methods and their correlation to the claimed benefits.

The activity of antioxidants in a food system depends not only on their chemical properties, such as free radical scavenging and metal chelation, but also on factors such as physical location and interaction with other food components, as well as environmental factors such as pH and ionic strength (*1*). Many of the in vitro assays that are currently used for testing antioxidative capacity, for example, the Trolox equivalent antioxidant capacity (TEAC) and the oxygen radical absorbance capacity (ORAC), measure the free radical scavenging activity of a compound in the absence of any oxidation substrates such as lipids or proteins. They also do not have the chemical, physical, and environmental conditions expected in food products, which all in all may explain why they do not always predict how well a compound will inhibit oxidative deterioration of foods (*1*).

Within seafood research, a model that has proven to be more useful in testing antioxidants is washed cod mince (*2*−*7*). This model, which resembles well the matrix of fish muscle, has been used, for example, for studying the antioxidative activity of some aqueous muscle fractions (press juice, PJ) (*5*−*7*) of grape fibers (*8*) and of a cranberry extract (*9*). In several studies, these models have been brought to very low moisture content (∼65−75%) to allow large additions of a liquid. Dewatering has been made with pH and salt adjustments (pH 5.5, 50 mM NaCl). Occasionally these systems have not oxidized, even in the absence of antioxidants, which has been an intriguing and difficult factor to handle in ongoing research on seafood antioxidants. The reasons for the above observed phenomenon could be many, for example, the composition of the cod mince, the pH of the washing solution, the moisture content of the model system, the type and concentration of the pro-oxidant used, and the storage conditions of the model (e.g., light and temperature). The aim of this study was to identify possible noncontrollable and controllable factors responsible for the occasional lack of hemoglobin (Hb)-mediated lipid oxidation of washed cod mince. Among noncontrollable factors were initial peroxide value (PV), level of α-tocopherol, and structure.

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Among controllable factors were washing pH, moisture, Hb level, and light during storage of models.

MATERIALS AND METHODS

Fish Mince and Blood Supply. Fresh whole cod (*Gadus morhua*) was obtained from Leröy Allt i Fisk AB (Göteborg, Sweden) during the spring and autumn of 2004. The post-mortem age of the cod was 36−72 h. The light muscle was manually removed and was minced in a kitchen grinder (Ultra Power, model KSM90, KitchenAid, St. Joseph, MI) using a plate with 5 mm holes.

Hemolysate was prepared using blood obtained from rainbow trout (*Onchorhynchus mykiss*) maintained at the Department of Zoology (Göteborg University, Sweden). The fish was bled according to the method of Rowley (*10*) and kept heparinized on ice until the preparation of hemolysate.

Chemicals. Ammonium thiocyanate, barium chloride, cumene hydroperoxide, 3-aminobenzoic acid ethyl ester, heparin (sodium salt), sodium hydrosulfite, hemoglobin, and streptomycin sulfate were obtained from Sigma (St. Louis, MO). Cupric sulfate was obtained from Fisher Scientific Co. (Fair Lawn, NJ). DL-α-Tocopherol was purchased from Calbiochem (an affiliate of Merck KGaA, Darmstadt, Germany). All other chemicals used were of analytical grade.

Preparation of Washed Cod Mince Model. A known quantity (usually 600 g) of minced cod muscle was washed once with 3 volumes of cold distilled water (~4−8 °C) and twice with 3 volumes of cold 50 mM NaCl at different pH values, 5.5−6.6. During the first two washes, mince and washing solution were stirred manually for 2 min, and the mince was then leached for 15 min at 4 °C. In the first wash, the pH was not adjusted, but in the second and third washes the pH was adjusted with 1 M HCl or 1 M NaOH either to the standard value 5.5 or, in a separate trial, to pH 6.0, 6.3, or 6.6. In the first two washes, washed mince was collected by filtering the muscle suspension through a sieve. In the third wash, the mince and washing solution were homogenized for 1 min at speed 3 using an Ultra Turrax homogenizer (model T18 basic, IKA Works, Wilmington, NC). The homogenate was then centrifuged for 25 min (4 °C at 15000*g*) in a Sorvall Superspeed centrifuge (model RC-5C Plus, Kendro Laboratory Products, Stockholm, Sweden) using an SLA-1500 rotor. The supernatant was removed, and the washed mince was frozen in plastic bags at −80 °C until use.

Preparation of Hemolysate and Analysis of Hb. Hemolysate was prepared from the heparinized blood by repeated washes of the erythrocytes in 1 mM Tris buffer (pH 8, 1.7% NaCl) following the protocol of Fyhn et al. (*11*). The Hb content in the hemolysate was determined spectrophotometrically after the conversion of Hb to the CO form using sodium dithionite followed by bubbling with CO gas (*12*).

General Procedure for Preparing the Oxidation System. The mince was thawed in its plastic bag under running cold water. Excess water was then manually squeezed from the mince using a cotton towel. The final moisture of the washed cod mince was then 65−75%, and the final pH of the different models varied between 5.0 and 6.6. The squeezed model was then chopped in a small precooled mixer (Hugin, model MC-851, Coop Elektro AB, Upplands Väsby, Sweden) for 30−60 s. The larger the quantity of mince, the longer the chopping time. After the moisture content of the above pressed and chopped cod mince had been determined, the moisture content was adjusted either to the standard 81% or, in a separate trial, to 75 or 90%, by adding 50 mM phosphate buffer (pH 6.3). The pH of the remoisturized model was then checked and, if needed, adjusted to 6.3 ± 0.05 using 1 M HCl or 1 M NaOH. Hemolysate was then added to reach the required Hb concentration (usually 15 μM). Streptomycin (to reach a final value of 200 ppm on a moisture basis) was added to prevent microbial growth. Samples (20 g) were flattened in the bottom of 250 mL Erlenmeyer (E) flasks (sample thickness at the bottom of E-flasks was 5−6 mm), which were stored on ice for up to 7 days. Samples were either stored in the dark or exposed to light (900 ± 50 lx) for 3−4 h per day or 24 h per day. In standard trials, 3−4 h per day was used.

In the trial in which the effect of model moisture was tested (75, 81, and 90%), the added volume of hemolysate was adjusted so that the Hb concentration when expressed on dry matter was 65.5 μM in all three samples (75, 81, and 90% moisture with low Hb) (**Table 1**). When expressed on a moisture basis, the final Hb concentrations thereby became 21.1, 15.0, and 7.2 μM, respectively. To one of the 90% moisture models was added the same volume of hemolysate as added to the model with 81% moisture so the Hb concentration was 13.5 μM on a moisture basis. However, on the basis of total weight of model the concentration of Hb was 12.2 μM (**Table 1**). This allowed us to compare oxidation between samples with 81% moisture and 90% moisture with a fixed Hb concentration of 12.2 μM on a weight basis (**Table 1**). Because one of the two models with 90% moisture had 7.2 μM Hb and the other 13.5 μM, it was also possible to evaluate the changes in oxidation response due to different Hb concentrations.

Table 1. Moisture Content and Hb Concentration in the Different Models[a]

model	moisture (%)	Hb/moisture of model (μM)	Hb/wt of model (μM)	Hb/dry wt of model (μM)
75% moisture	75	21.1	16.0	65.5
81% moisture	81	15.0	12.2	65.5
90% moisture (low Hb)	90	7.2	6.5	65.5
90% moisture (high Hb)	90	13.5	12.2	124.5

[a] Hb is expressed on the basis of moisture content, total weight, and dry matter.

Determination of Total Lipids. Total lipid content in cod models frozen at −80 °C and from oxidation plugs taken during ice storage was determined according to the method of Lee et al. (*13*) as described by Undeland et al. (*14*). In our study the ratio of chloroform/methanol used for lipid extraction was 1:2 for cod models and 1:1 for oxidation sample plugs.

Determination of α-Tocopherol. One gram of washed cod mince was vortexed for 1 min with 5 mL of methanol (HPLC, Lab-Scan Ltd., Dublin, Ireland). The sample was then incubated at 80 °C for 30 min, followed by vortexing, sonicating, and again vortexing for 1 min each. The sample was then centrifuged for 5 min at 1600*g*, and the supernatant was used for α-tocopherol determination. An internal standard was used to check the recovery of α-tocopherol during extraction. α-Tocopherol was separated by high-performance liquid chromatography (HPLC) on a C18 column (Chromacil, EKA Chemicals, Bohus, Sweden). The mobile phase was 98% methanol water, and the flow rate was 0.4 mL/min. Peaks were detected with a Shimadzu RF-551 spectrofluorometric detector (Kyoto, Japan) using an excitation wavelength of 295 nm and an emission wavelength of 330 nm. The α-tocopherol peak was quantified against the standard DL-α-tocopherol peak.

Transmission Electron Microscopy (TEM). Samples were taken from the stable and oxidizing cod model systems directly after the adjustment of moisture and pH and the addition of Hb for TEM analysis. The samples were fixed in precooled 2% glutaraldehyde (50 mM phosphate buffer, pH 6.3) and then postfixed in 1% osmium tetraoxide (OsO_4). After that, the samples were dehydrated stepwise in ethanol and embedded in Epon/Araldite (TAAB 812), sectioned, and stained according to the method of Ofstad et al. (*15*). Sections were examined and images were taken in a TEM, LEO 906 E, at an accelerating voltage of 80 kV.

Determination of Peroxide Value (PV). One gram sample plugs were taken during ice storage by piercing a hollow plastic tube (∅ = 10 mm) onto the flattened surface in the E-flask. Sample plugs were stored frozen at −80 °C until they were subjected to lipid extraction (see method above) and PV determination using the ferric thiocyanate method of Shantha and Decker (*16*) as modified by Undeland et al. (*14*). The relative standard deviation (RSD%) of the PV estimations when six chloroform extracts from the same sample plug were analyzed was 1.1%. The RSD% values between plugs taken from the same flasks and among different flasks containing the same kind of sample in the same trial were 3.9% ($n = 6$) and 4.7% ($n = 3$), respectively.

Color Measurement. Change in the redness (a^* value) during the storage of models was monitored with a colorimeter (Minolta Chroma Meter CR-300, Minolta Corp., Ramsey, NJ) using the CIE Lab color scale (*5*). Changes in redness were recorded by measuring the redness

at the bottom of E-flasks. Measurements were done in five replicates, and an average value was used in the graphs shown.

Sensory Analysis. A small internal panel (three trained people) sniffed the headspace of the E-flasks during ice storage of the cod model samples. Care was given to recognize the intensity of rancid odor development (*17*), which was marked on a 100 mm linear scale. Zero indicated no rancid odor, and 100 indicated maximum rancid odor. A lag phase was defined as the time taken for the rancidity to reach 10 mm on the scale.

Statistics. One to six sample replicates (n) were used in each of the experiments. The number of replicates is mentioned in the respective places under Results. Each experiment was repeated two or three times, with a representative example shown as a graph. The number of analytical replicates (a) was 5 for redness analysis, 1 or 3 for PV analysis, 1 for sensory analysis, and 3 for Hb analysis. In cases when $n > 2$, results are expressed as mean ± standard deviation (SD). Student's t test was used to identify the significant difference between stable and oxidizing model in terms of initial PV, α-tocopherol, lipid content, etc.

RESULTS AND DISCUSSION

General Characteristics of the Model System. The cod model oxidation system studied consisted of washed cod mince as oxidation substrate, trout Hb as oxidation catalyst, streptomycin as antimicrobial agent, and phosphate buffer (50 mM, pH 6.3) to adjust the moisture content to 81% (physiological in fish muscle). The oxidation substrate thus mainly comprised membrane phospholipids and myofibrillar proteins. Trout Hb has been shown to efficiently oxidize these substrates in a previous study (*4*). The actual washing removes pro- and antioxidative aqueous components naturally present in cod muscle (*6*). This allows more controlled studies of individual oxidants subsequently added to the model. In a series of previous studies on the antioxidative effect of fish muscle press juices (PJ) (*5*, *6*), a low-moisture washed cod model system was developed by washes at conditions under which muscle has very low water holding (50 mM NaCl, pH 5.5). One of the aims of this paper was to determine why this low-moisture cod model occasionally did not show any oxidation response to 15 μM trout Hb. Such occasional stability to Hb has never occurred in "regular" washed cod models prepared by washing at pH 6.6.

Parts **a** and **b** of **Figure 1** show two extreme oxidation responses of extra-dry models to 15 μM Hb during ice storage. These models originated from different batches of cod, but were both prepared according to the general procedure described. One model did not show any considerable rancid odor and PV development up to 6.5 days, whereas the other model had an oxidation lag phase of only 1−2 days. Also, the rate of loss of redness (a*) was high in the oxidizing model compared to the stable model (**Figure 1c**). Because different batches of cod can vary in their composition depending on biological status, fishing area, post-mortem age etc., a first step toward explaining the oxidation differences between the models in **Figure 1** was to analyze their levels of total lipids, preformed hydroperoxides, and α-tocopherol; the latter two are in particular known to affect oxidation.

Role of Noncontrollable Factors; Initial Lipid Content, PV, α-Tocopherol, and Structure of the Stable and Oxidizing Cod Models Prepared in the Same Way. Table 2 shows the total lipid content, PV, and α-tocopherol content of the two cod models shown in **Figure 1**. The lipid contents of the oxidizing and stable models were 4.4 and 5.6% on a dry weight basis, respectively ($n = 3$), with the difference being significant ($p < 0.05$). Although there have been studies showing that the lipid content has no effect on the lipid oxidation rate (*14*), none have shown that a lower lipid content would speed oxidation.

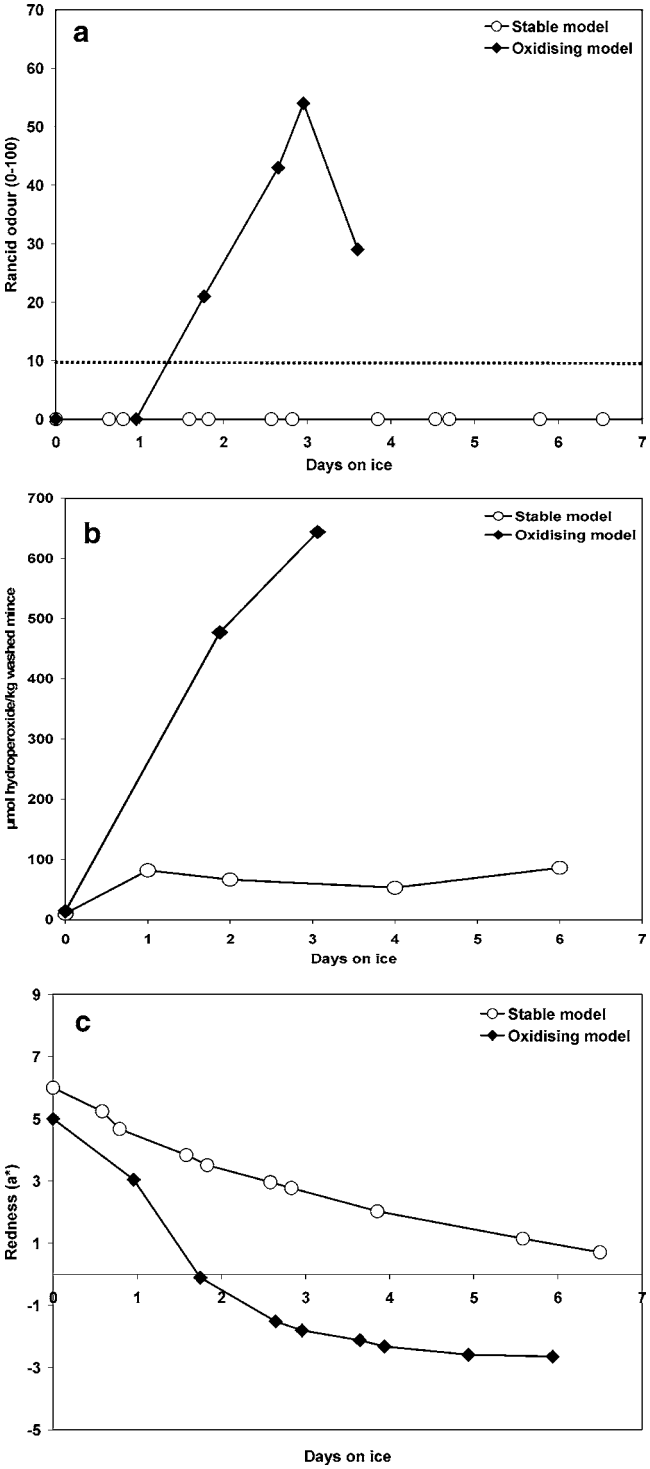

Figure 1. Hb-mediated (15 μM) development of (**a**) rancid odor, (**b**) lipid hydroperoxides, and (**c**) redness (a* value) loss during ice storage of different batches of cod mince washed once in water and then twice in 50 mM NaCl at pH 5.5. The final pH and moisture of the models were 6.3 and 81%, respectively. The dotted line in (**a**) indicates a rancid odor intensity of 10 on a scale of 100, which we used to define the rancid odor lag phase.

A factor expected to be more decisive for the rate of oxidation was the intial PV of the models. Met-Hb is known to react with peroxides, which results in the formation of compounds capable of initiating and propagating oxidation. The model that gave rise to Hb-mediated oxidation (**Figure 1**) had 124.0 μmol of hydroperoxides/kg of model (dry weight, dw, basis, $n = 3$), whereas the stable model had 213.5 μmol of hydroperoxides/

Table 2. Levels of Lipids, Hydroperoxides, and α-Tocopherol in the Stable and Oxidizing Washed Cod Models Prior to Storage[a]

model	total lipid (%) on dry wt basis	hydroperoxide (μmol/kg of model on dry wt)	α-tocopherol (mg/kg of model on dry wt)
stable cod (from spring 2004)	5.6 ± 0.3 a	213.5 ± 49.8 a	29.6 ± 2.6 a
oxidizing cod (from autumn 2004)	4.4 ± 0.0 b	124.0 ± 9.3 b	14.4 ± 0.2 b

[a] Values are expressed as mean values ± standard deviation ($n \geq 3$). Hydroperoxides were measured in a chloroform extract using the ferric thiocyanate method (*16*). α-Tocopherol was measured with HPLC in a methanol extract. Values in the same column followed by different letters are significantly different ($p < 0.05$).

kg of model (dw basis, $n = 3$). The difference in initial PV of these models was significant ($p < 0.05$). When expressed on the basis of lipid content, the levels of lipid hydroperoxides in oxidizing and stable models were 2.8 and 3.8 mmol of hydroperoxides/kg of lipid, respectively. Richards and Li (*18*) reported the lipid hydroperoxide content of unwashed cod muscle to range from 0.56 to 2.43 mmol/kg of lipid. They also reported that washed cod mince model prepared with cod mince having a higher level of preformed lipid peroxides was more susceptible to lipid oxidation during subsequent ice storage, which was different from our observation. One reason for this could be stabilization of lipid hydroperoxides by higher α-tocopherol levels in the model with higher PVs (*19*). It is also possible that the peroxide differences between our two models were not sufficient to affect the rate of lipid oxidation.

Although washing of cod mince can remove most of the endogenous antioxidants and pro-oxidants, it will not remove α-tocopherol, which is embedded in the membranes. The oxidizing model had significantly ($p < 0.001$) less α-tocopherol than the stable model, 14.4 versus 29.6 mg of α-tocopherol/kg of model, respectively (dw basis, $n = 4$), which we believe to be an important factor behind the different susceptibilities to oxidation. When α-tocopherol levels (dw basis) in cod minces used at different times for preparing models were plotted against the rancid odor lag phases obtained in the presence of 15 μM Hb, there appeared to be a clear concentration-dependent increase in the lag phases for cod minces having >20 mg of α-tocopherol/kg. Exponential regression analysis gave a curve best fit with $r^2 = 0.72$. The mechanisms by which α-tocopherol can inhibit lipid oxidation have been well documented (*20*, *21*). The fluctuations in α-tocopherol level observed between various cod batches are thought to be related to their different seasons of catch (*22*).

Cod models prepared from different batches of cod could also differ in their structural and textural properties. Already by visual observation, we sometimes noticed a different structure and texture after pressing and chopping of the freeze−thawed washed cod mince. The models that did not get oxidized had a more globular structure, whereas the models that were oxidized had a fibrous structure. To study these differences in greater detail, structural evaluation was made by TEM. **Figure 2** shows the microstructure of the stable and oxidizing cod models (panels **a** and **b**, respectively). The stable model had a more disrupted myofibrillar structure (**Figure 2a**) compared to the oxidizing one (**Figure 2b**). Furthermore, the myocommata was absent in the stable model. This indicated that the proteins of the stable model had started to "swell", maybe as an early stage of so-called "cold setting". A theory is that this swelling/gelation

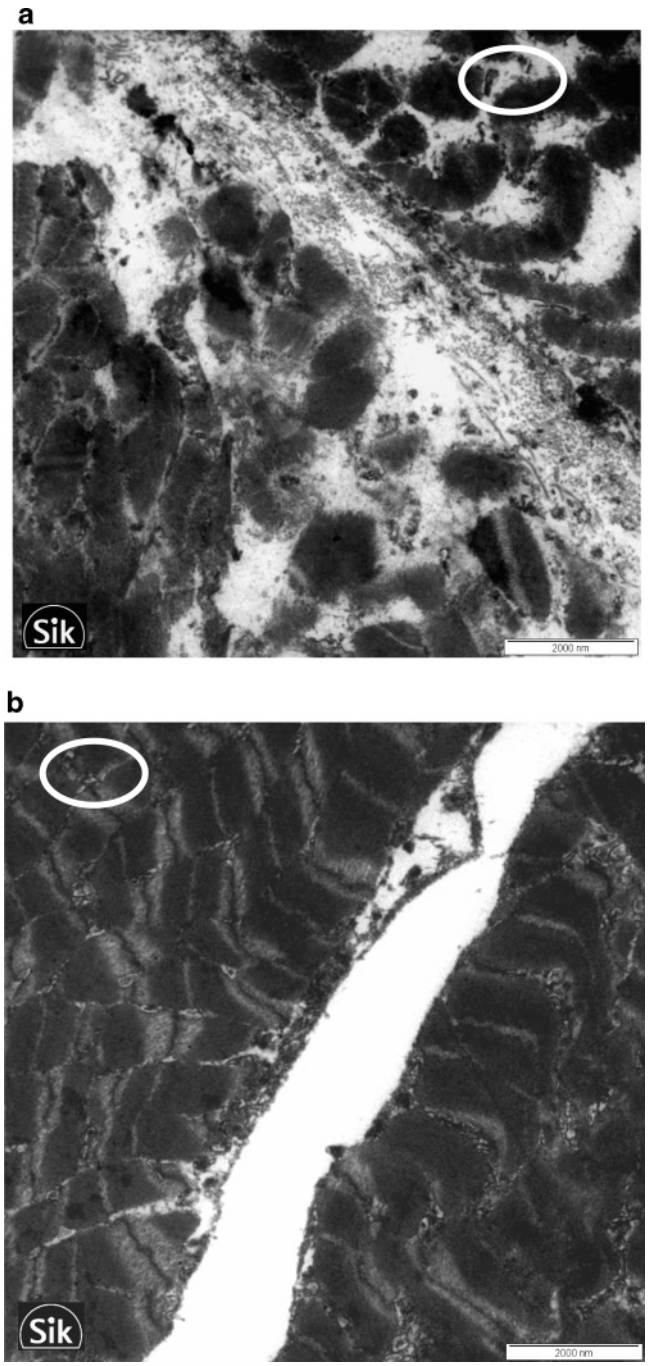

Figure 2. TEM picture of (**a**) a stable cod model and (**b**) an oxidizing cod model at 2000 nm magnification. White circles highlight examples of differences in the muscle structure of these models. Note the disruption and intactness of the myofibrillar protein network in stable and oxidized models, respectively.

physically prevented the added Hb from reaching the oxidizable membranes. Possibly, small variations, for example, initial muscle pH, washing pH, washing temperature, or moisture, during the model preparation decreased or increased the susceptibility to cold setting. It is hypothesized that washing pH has a great influence as it is difficult to get the pH exact in the region around pH 5.5.

Role of Controllable Factors; Effect of Washing pH, Moisture Content, Hb Content, and Light Exposure during Storage on Hb-Mediated Oxidation. One possible factor for occasional nonresponse of the cod model to Hb-mediated oxidation could be variation in the step when the mince is

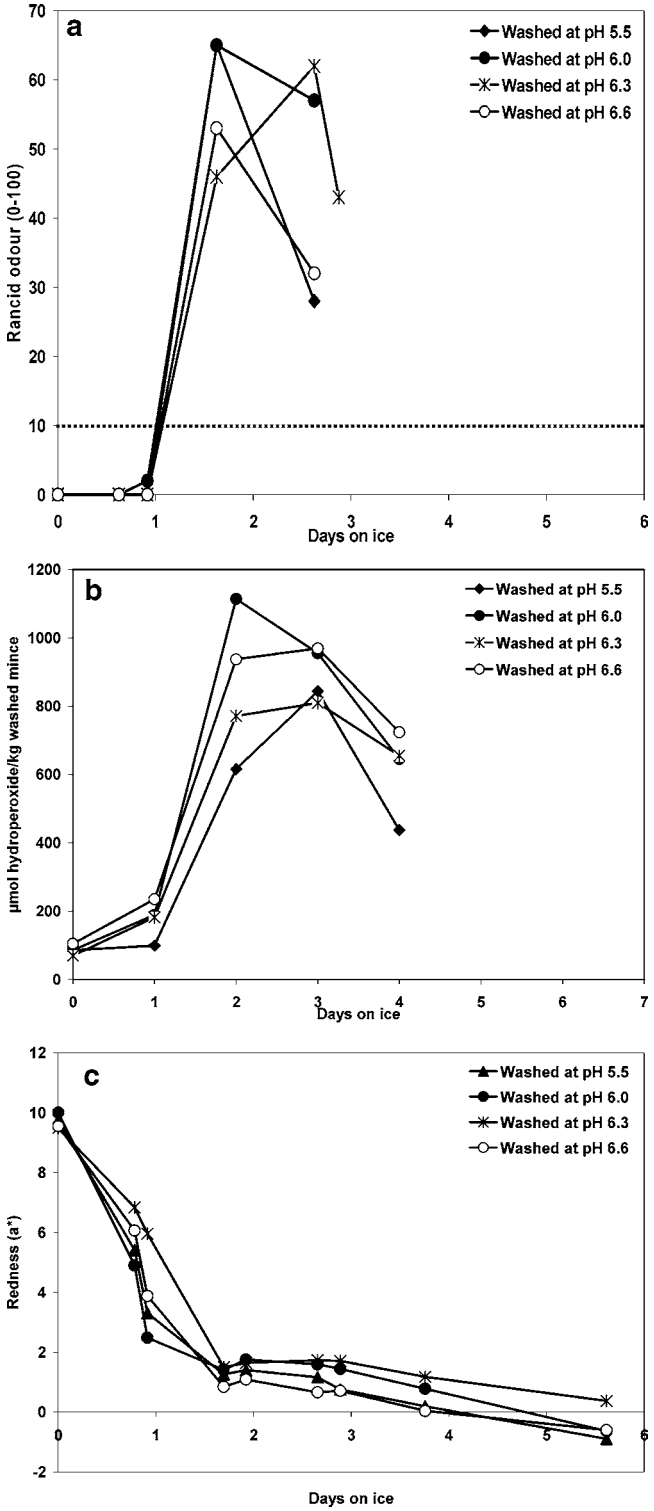

Figure 3. Hb-mediated (15 μM) development of (**a**) rancid odor, (**b**) lipid hydroperoxides, and (**c**) redness (*a** value) loss during ice storage of cod mince washed once in water and then twice in 50 mM NaCl at pH 5.5, 6.0, 6.3, or 6.6. The final pH and moisture of the models were 6.3 and 81%, respectively. The dotted line in (**a**) indicates a rancid odor intensity of 10 on a scale of 100, which we used to define the rancid odor lag phase.

subjected to pH 5.5, which is used in the second and third washing steps to minimize water binding. The effect of washing at different pH values, 5.5−6.6, on lipid oxidation was therefore tested. Panels **a**, **b**, and **c** of **Figure 3** show the development of rancid odor, PV, and redness (*a**), respectively, during ice storage of Hb-fortified cod models washed at different pH values (5.5, 6.0, 6.3, and 6.6). It can be seen that irrespective of the pH at which the cod model was washed, an oxidation lag phase of about 1 day was obtained. With regard to *a** values not much difference was found between models washed at different pH values (**Figure 3c**). However, it can be noted that after 2 days of storage on ice, when the models were the most differentiated in terms of their PVs, those models washed at pH 5.5 and 6.0 had reached the lowest and highest PVs, respectively.

Pazos et al. (*23*) studied the oxidation rate in a cod muscle microsomal model over the pH range of 5.5−8.4. They concluded that the oxidation rate is determined by two factors, a decrease in the catalytic activity of Hb at high pH versus the decrease in the susceptibility of the membranes to oxidation at low pH. The latter factor is the most pertinent to our observations. It is suggested that muscle membrane proteins can aggregate in a special way at lower pH, making the membrane lipids less susceptible for oxidation. The pH range we studied was narrower (5.5−6.6) than that of Pazos et al. (*23*), that is, 3.5−8.4, which could be one reason why we did not observe any major oxidation differences. However, in preliminary trials we also tested washing at pH 4.5 and also then no difference in oxidation was seen compared to the general washing procedure.

Richards and Hultin (*24*) studied Hb-mediated oxidation at pH 6.3 of cod models that were prepared by washing in 50 mM phosphate buffer at pH 6.0, 7.2, or 7.6. They reported a longer lag phase, or almost no oxidation, after washing at pH 7.6. These observations could support the slight difference in the PV levels at day 2 between models washed at different pH values (**Figure 3b**). It has to be kept in mind that our samples were stored at 81% moisture, whereas in the study of Richard and Hultin (*24*) the samples had 90% moisture.

This leads to our results from testing the role of moisture content of the final model for its development of Hb-mediated oxidation during ice storage. Panels **a**, **b**, and **c** of **Figure 4** show the development of rancid odor, PV, and redness (*a**), respectively, during ice storage of cod models adjusted to 75, 80, and 90% moisture; the latter was prepared at two Hb levels. Both models with 90% moisture gave rancid odor lag phases of 1 day, whereas the models with 81 and 75% moisture gave 2 and 3 day lag phases, respectively (**Figure 4a**). These results were supported by the PV data (**Figure 4b**). **Figure 4c** shows that the different initial *a** values reflect the different Hb levels obtained when the moisture was varied (7.2−21.1 μM on moisture basis). It also showed slower *a** value loss with lower moisture. A suggested reason for the higher oxidation rate with higher moisture content is an increased mobility of the Hb molecule (*25*). This is further stressed by the fact that the model with lowest moisture actually had the highest Hb content on a moisture basis: 21.1 μM Hb versus 15.0 and 7.2 μM Hb, in the 81 and 90% models, respectively. On a dry weight basis, they had, however, the same content, 65.5 μM.

The specific influence of Hb content was further tested by comparing 7.2 and 13.5 μM Hb (moisture basis) in two models with 90% moisture. The 2-fold increase in Hb concentration had no effect in terms of rancidity lag phase (**Figure 4a**,**b**). However, higher maximum rancid odor scores and PV readings were recorded in the model having 13.5 μM Hb. These observations are in line with previous observations of Undeland et al. (*14*), who saw that the increased Hb concentration increased only the oxidation intensity but not the oxidation rate of cod model systems.

It was suspected that a variation in light exposure that has existed between our experiments could be a determining factor

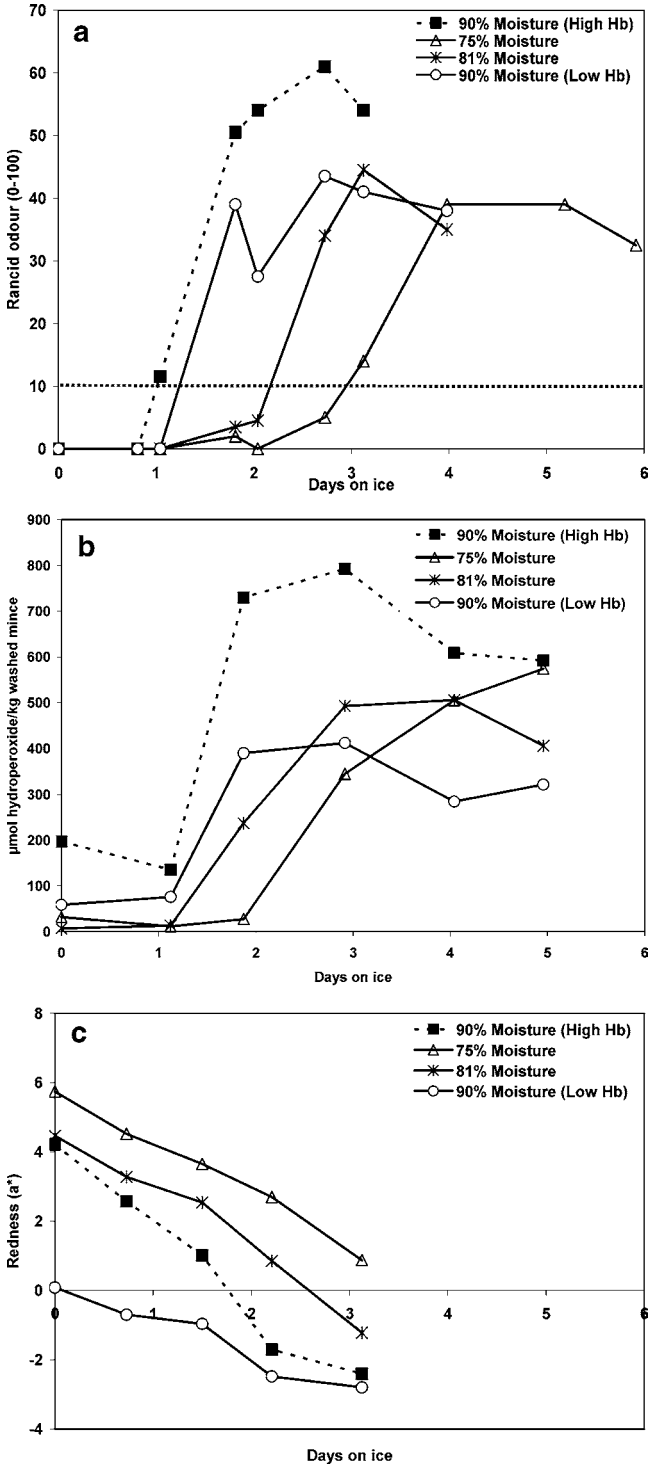

Figure 4. Hb-mediated development of (**a**) rancid odor, (**b**) lipid hydroperoxides, and (**c**) redness (*a** value) loss during ice storage of cod mince washed once in water and then twice in 50 mM NaCl at pH 5.5. Different volumes of 50 mM phosphate buffer (pH 6.3) were added to bring the final moisture level to 75, 81, or 90%. The final pH of the models was 6.3. The Hb concentration was fixed at 65.5 μM on a dry weight basis (7.2–21.1μM on a moisture basis) in all models except one of the 90% moisture models. In the latter, the volume of hemolysate added was the same as that in the 81% moisture model (giving 13.5 μM Hb on a moisture basis, see **Table 1** for further details). The dotted line in (**a**) indicates a rancid odor intensity of 10 on a scale of 100, which we used to define the rancid odor lag phase.

for oxidation of the cod models. The effect of light on Hb-mediated oxidation was evaluated by exposure of the cod models

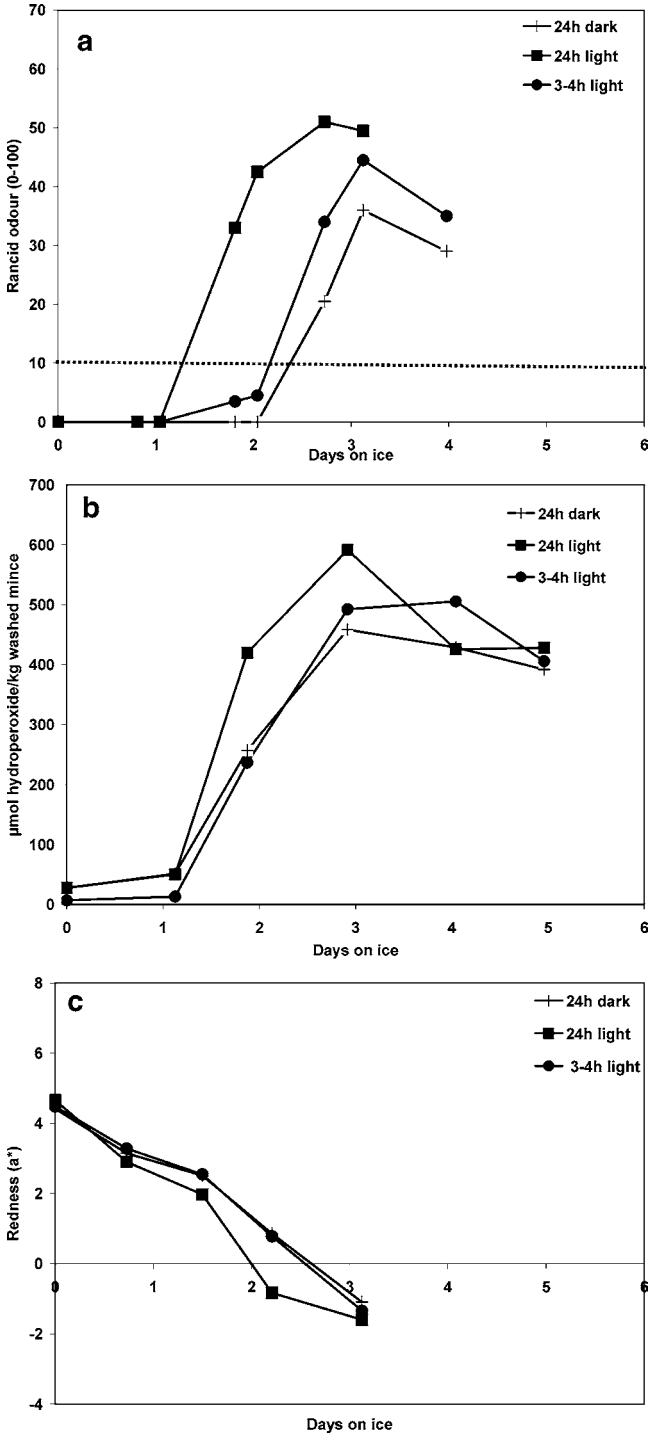

Figure 5. Hb-mediated (15 μM) development of (**a**) rancid odor, (**b**) lipid hydroperoxides, and (**c**) redness (*a** value) loss during ice storage of cod mince washed once in water and then twice in 50 mM NaCl at pH 5.5. The final pH and moisture of the models were 6.3 and 81%, respectively. Models were exposed to complete darkness or to 900 ± 50 lx of light for a period of 3–4 h or 24 h/day. The dotted line in (**a**) indicates a rancid odor intensity of 10 on a scale of 100, which we used to define the rancid odor lag phase.

either to darkness or to 900 ± 50 lx of light for 3−4 h or 24 h/day. Panels **a** and **b** of **Figure 5** show that the model exposed to full light had a 1 day shorter rancidity lag phase and a slightly higher rancidity score compared to the model exposed to darkness or to light for 3−4 hday. There was no difference in PV development except that the model exposed to light for 24 h/day had a higher PV score. **Figure 5c** shows that models

exposed to light for 24 h also had slightly higher rates of redness loss compared to the ones exposed to darkness or 3−4 h of light/day. These results indicate the small role of light in the initiation of Hb-mediated oxidation, which allows us to speculate that photo-oxidation is of limited importance in the present oxidation model.

In brief, our results indicate that the major reasons for uneven stabilities among low-moisture washed cod mince models were different α-tocopherol levels and different degrees of disruption in the myofibrillar structure. Washing cod mince within the range of pH 5.5−6.6 did not have any significant influence on the rate of Hb-mediated lipid oxidation during subsequent ice storage. The same was true for the duration of light exposure during storage and also the initial peroxide level of the model. Finally, the rate of oxidation increased with higher moisture content, and higher Hb levels gave higher maximum oxidation values.

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