Inhibition of Hemoglobin-Mediated Oxidation of Regular and Lipid-Fortified Washed Cod Mince by a White Grape Dietary Fiber

ISABEL SÁNCHEZ-ALONSO,[†] JAVIER BORDERÍAS,[†] KARIN LARSSON,[‡] AND
INGRID UNDELAND*,[‡]

Department of Science and Technology of Meat and Meat Products and Fish and Fishery Products,
Instituto del Frío (CSIC), José Antonio Nováis No. 10. E28040, Madrid, Spain, and Department of
Chemical and Biological Engineering, Food Science, Chalmers University of Technology,
SE-41296, Göteborg, Sweden

The effectiveness of a white grape dietary fiber concentrate (WGDF) against hemoglobin-mediated oxidation of washed cod mince, with and without 10% added herring oil, was evaluated during ice storage. WGDF was added at two different levels: 2 and 4% based on final weight. An ethanol extract with the ethanol extractable polyphenols (EPP) and the ethanol-extracted grape dietary fiber residue were also tested as antioxidants in the washed cod mince. The addition of WGDF to the model system completely and significantly ($p \leq 0.05$) inhibited the hemoglobin-mediated development of rancid odor during the entire ice storage (10 days). Thiobarbituric acid reactive substances (TBARS) development and red color (a^*) losses supported the sensory data. Controls fortified with 10% herring oil oxidized at the same rate as oil-free controls and were also significantly stabilized by 2% WGDF. The EEP of the WGDF had the same high antioxidant capacity as whole WGDF. The ethanol-extracted fiber residue significantly ($p \leq 0.05$) reduced maximum oxidation values obtained for controls by $\sim$50% and extended the oxidation lag phase by 1 day ($p \leq 0.05$). Thus, this study showed great potential of WGDF to prevent rancidity in fish, mainly due to EPP but also due to the ethanol-extracted dietary fiber residue itself.

KEYWORDS: Grape; dietary fiber; lipid oxidation; antioxidant; hemoglobin; model system; washed cod; neutral lipids; polyphenols

INTRODUCTION

Lipid oxidation negatively affects flavor, odor, color, texture, and the nutritional value of muscle seafood during storage. The addition of antioxidants during processing of seafood has emerged as an effective methodology to minimize such quality deterioration (*1*−*3*). Although synthetic antioxidants have been widely used (*4*), there is nowadays a growing interest to replace such compounds by naturally occurring antioxidants (*5*). Along with this, there has been increasing interest within the industry in using plant extracts to minimize or retard lipid oxidation in various food products. Examples are some natural phenolic compounds in extracts of green tea, rosemary, and extra virgin olive oil, which have proven to be effective in preventing rancidity in many lipid-containing systems such as fish muscle (*6*−*8*).

Recent research has stressed that vinification byproducts are particularly rich in a wide range of polyphenols (*9*, *10*). There are many references in the literature to the composition and antioxidant properties of grape polyphenols (*11*, *12*). It has been reported that many of the compounds present in grapes (anthocyanidins/anthocyanins, flavonoids, and phenolic acids) have antioxidant activities similar to some synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene (*13*). Some studies have been published on the effectiveness of grape polyphenols in delaying lipid oxidation in minced fatty fish muscle during frozen storage (*14*, *15*). It was also recently reported that grape dietary fiber concentrates obtained from white or red grape byproducts have high antioxidant effects in frozen stored minced horse mackerel muscle (*16*, *17*). The grape dietary fiber concentrates are considered natural products that combine the beneficial effects of dietary fiber and antioxidant properties through the presence of natural antioxidants (*18*). It would be of great importance to deepen the knowledge on their antioxidative mechanisms in fish and also on how they work under different conditions, for example, fat content and storage.

Hemoglobin (Hb) is one of the strongest endogenous catalysts of fish muscle lipid oxidation (*19*). Oxygen can be bound to the ferrous iron (oxy-Hb), or the iron binding site can be vacant (deoxy-Hb). An important reaction related to the ability of Hb

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* To whom correspondence should be addressed. Tel: +46-31-772 38 20. Fax: +46-31-772 38 30. E-mail: undeland@chalmers.se.
† Instituto del Frío (CSIC).
‡ Chalmers University of Technology.

to stimulate lipid oxidation is Hb autoxidation (*19*). Met-Hb is greyish-brown and, thus, causes discoloration in addition to catalyzed lipid oxidation. Recent research in a washed cod mince system with or without added menhaden oil (7.5 and 15%) revealed that Hb from trout gave rise to the same intensity of rancid odor, loss of red color, and thiobarbituric acid reactive substances (TBARS) increase in all three systems. The level of Hb proved to be much more important than the total lipid content for rancidity development (*20*). Thus, to retain high sensory quality of fish during storage, all natural compounds or extracts effectively targeting Hb-mediated lipid oxidation at different lipid levels would be of great interest for the industry.

The aim of this study was to evaluate whether the addition of white grape dietary fiber concentrate (WGDF) could prevent Hb-mediated oxidation of the membrane lipids of washed cod mince during ice storage. To simulate how Hb-mediated oxidation of high-fat fish would respond to the WGDF, triacylglycerols in the form of crude herring oil were in some experiments added to the washed cod mince prior to adding the WGDF. The antioxidant properties of different extracts from WGDF, an ethanol extract and the ethanol-extracted dietary fiber residue, were also tested as antioxidants in the washed cod mince model system.

MATERIALS AND METHODS

Chemicals. All of the chemicals used were analytical grade and were obtained from Panreac Química S.A. (Barcelona, Spain), Sigma-Aldrich Co., or Merck (Darmstadt, Germany).

Fish and Fish Blood Supply. Fresh cod fish (*Gadus morhua*) was obtained from Leröy Allt i Fisk AB (Göteborg, Sweden). The fish was transported in ice to the laboratory where it was filleted within 24 h after catch. The white muscle was manually separated and trimmed so that all of the dark muscle, cartilage, and vein remains were removed. The initial pH value of the filleted cod was 6.60 ± 0.05. Fish blood was obtained from rainbow trout (*Onchorhynchus mykiss*) kept at the Department of Zoology (Göteborg University, Sweden). Oil was prepared from fresh whole herring (*Clupea harengus*) obtained from Paul Mattsson AB (Ellös, Sweden). The postmortem age of the herring was 36 h at the time of arrival in our lab.

Washed Minced Cod Model System. The washed cod model system was prepared according to Wetterskog and Undeland (*21*). Cod white muscle was minced using a kitchen grinder (Ultra Power, model KSM90, KitchenAid, St. Joseph, MI) fitted with a plate with holes having 4.7 mm diameter. The pH value of the minced cod muscle was 6.54 ± 0.02. The mince was washed once in cold distilled water at a 1:3 mince to water ratio (w/w) by stirring manually with a plastic rod for 2 min. Then, the mixture was leached for 15 min and was dewatered in a sieve. The procedure was repeated twice replacing water by 50 mM phosphate buffer (pH 6.6). In the third wash, the mixture was homogenized with a polytron (Ultra Turrax, IKA Werks, Intermed Labassco) for 1 min and 15 s at 14000 rpm speed prior to the 15 min leaching. It was then centrifuged at 15000*g* for 25 min at 4 °C in a Sorvall Superspeed centrifuge (model RC-2B, Instrument AB Lambda, 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. The final washed cod model system had a pH of 6.59 ± 0.01 and a moisture content of $84.3 \pm 0.1\%$.

Bleeding of Fish and Preparation of Hemolysate. Trout were anesthetized and bled as described by Rowley (*22*). Hemolysates were prepared from the whole blood according to Fyhn et al. (*23*) by washing the red blood cells four times in 1 mM Tris (pH 8) containing 290 mM NaCl and then lysing them in 1 mM Tris (pH 8). To quantify the Hb levels in the hemolysates, the method of Brown (*24*) was adapted as described by Richards and Hultin (*25*).

Fish Oil Separation. Whole deheaded herring were minced. The mince (200 g) was put in polypropylene centrifuge bottles. Centrifugation was done for 30 min at 11000 rpm at room temperature. The oil supernatant was recovered.

WGDF. White grape pomace (peels and seeds) (*Vitis vinifera* var. *Airén*) obtained from a winery (Vinícola de Castilla, S. A., Manzanares, Ciudad Real, Spain) was processed following a patented procedure (*26*), freeze-dried, milled to a particle size less than 0.25 mm, and stored at −20 °C. The final product named WGDF consisted of the following: protein, 7.31%; ash, 3.5%; oil, 5.50% [63.62% linoleic acid ($C_{18:2\omega6}$), 17.19% oleic acid ($C_{18:1\omega9}$), 10.49% palmitic acid ($C_{16:0}$), 5.49% stearic acid ($C_{18:0}$), 0.89% vacenic acid ($C_{18:1\omega7}$), and 2.32% linolenic acid ($C_{18:3\omega3}$)]; soluble polyphenols, 7.85%; moisture, 1.82%; insoluble dietary fiber, 53.26%; and soluble dietary fiber, 23.01%. Its antioxidant capacity was 4.13 Trolox mequiv/g of WGDF measured by an electrochemical method developed by Alonso et al. (*27*), which is a modification of Miller's method.

Preparation of Ethanol Extract and Fiber Residue from White Grape Dietary Fiber. Soluble or extractable polyphenols (EPP) are low or intermediate molecular mass phenolics that are extracted from WGDF using different solvents as ethanol. An ethanol extract was here prepared by adding 100 mL of ethanol to 1.6 g of WGDF. The mixture was homogenized in a Sorwall mixer for 1 min and then was put in a shaking water bath at 60 °C for 2 h. After the solid particles (dietary fiber and associated compounds) filtered away, the extract was concentrated by rotary evaporation (48−50 °C) to obtain a light green residual powder. The concentrate was resolubilized in 10 mL of ethanol and was frozen at −80 °C. The ethanol-extracted fibers (fiber residue) left in the filter paper were also collected and stored at −80 °C until use. The antioxidant capacity of ethanol extract was 4.07 Trolox mequiv as measured with the same method as was used for the WGDF (*27*). The fiber residue had no antioxidative capacity when it was extracted again with ethanol and the ethanol extract with the same method was tested (*27*). The main EPP as identified with high-performance liquid chromatography−diode array detection−mass spectrometry (*27*) appeared to be flavonoid (catequins, epicatequins, and quercetin) and gallic acid (unpublished data).

Measurement of Moisture Content and pH. The moisture content of the model system and WGDF was determined on 2 g samples using an HA300 moisture balance (Precisa HA 300, Zurich, Switzerland), and the pH was measured using a Ross Sure-Flow pH electrode (model 8165 combination epoxy electrode, Orion, Beverly, MA).

Determination of Total Lipids. The total lipid content in cod models was determined with chloroform:methanol (1:2) as described by Undeland et al. (*20*).

Preparation of Oxidation System. The washed mince was thawed in a sealed plastic bag under running cold water, and its pH was then adjusted from pH 6.6 to 6.3 by dropwise addition of 0.5−1 M HCl and/or 0.5−1 M NaOH followed by manual stirring with a stainless steel spatula. Into some washed cod mince samples, 10% (w/w) of herring oil was manually stirred to simulate fatty fish.

Selected samples of washed cod mince, with or without 10% herring oil, were fortified with WGDF to reach 2 and 4% based on final sample weight. It should be stressed that the 4% level was only included in a limited number of experiments, without the inclusion of TBARS analyses. Prior to addition, the WGDF was dispersed in the cold phosphate buffer, which was used to adjust the final moisture to the desired levels (see below). These concentrations of WGDF were chosen on the basis of earlier studies where WGDF was effective in delaying lipid oxidation in unwashed minced fish muscle (*16, 17, 28*). The WGDF solution was manually stirred into the cod model for 2 min.

To some of the oil-free samples, an amount of the EPP was added to reach a polyphenol content equivalent to that found in the 2% WGDF fortified samples (∼1.8 mg of EPP per gram of final sample). The ethanol-extracted grape fiber residue, which contained NEPP (nonextractable polyphenols and associated compounds), was also added to oil-free samples at 2% based on final sample weight.

Streptomycin and hemolysate were finally added to each of the samples to reach levels in the moisture fraction of 200 ppm and 20 µM Hb, respectively. Both streptomycin and hemolysate were stirred in manually for 2 min each using stainless steel spatula. In controls, the hemolysate was replaced by the same amount of 50 mM phosphate buffer. The final moisture of oil-free samples was ∼85%, and of samples with herring oil, it was ∼76.6%. The samples (final weight of ∼25 g) were then flattened out with an L-shaped stainless steel spatula

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in the bottom of a 250 mL screw-capped glass Erlenmeyer flask, resulting in a sample thickness of ~5−6 mm. For all trials, the capped sample bottles were stored on ice in darkness for up to 10 days. Samples were analyzed at the beginning of the experiment and then every 12 h.

Color Measurement. Changes in redness ($a*$) of the cod mince samples were measured during ice storage of the Erlenmeyer flasks with a colorimeter (Minolta Chroma Meter CR-300, Minolta Corp., Ramsey, NJ) using the CIE Lab color scale. Standardization of the instrument was done using a white Minolta calibration plate with a D_{65} illuminant and 2° observer. The colorimeter was connected to a personal computer, and SpectraMatch software (Minolta Corp.) was used to handle the measurements and to store the data. Measurements were done by pressing a probe (Ø = 0.5 cm) against the bottoms of the Erlenmeyer flasks. Three replicate measurements were done at different locations of the bottom, and an average value was used in further calculations.

Peroxide Value (PV) Analysis. During storage of samples, 1 g sample "plugs" were taken at regular intervals for the analysis of PV. The samples were picked from the bottom of the flasks using a hollow cylinder with a diameter of ~1 cm so that a constant surface-to-volume ratio was achieved. Samples were wrapped individually in aluminum foil and stored at −80 °C until analysis. Lipid extraction and PV analysis were done as reported by Undeland et al. (*20*). Results are expressed as μmol peroxide/kg sample. The relative standard deviation (RSD%) for the PV method was 1.1% based on six replicate analyses of the same chloroform extract.

TBARS Analysis. The procedure to recover the "sample plugs" was the same as in the PV analysis. TBARS analysis was performed according to the method of Lemon (*29*), after extraction of the 1 g of sample with 6 mL of 7.5% trichloroacetic acid solution (TCA) containing 0.1% propyl gallate and 0.1% ethylenediaminetetraacetic acid. Quantification was done according to a standard curve based on 1,1,3,3-tetraethoxypropane. Results are expressed as μmol TBARS/kg of sample. The RSD% for the TBARS method was 1.3% based on four replicate analyses of the same TCA extract.

Sensory Analysis. Once to twice daily, a small internal panel (3−4 trained people) sniffed the headspace above the samples by uncapping the Erlenmeyer flasks to detect rancid odor (*30*). The intensity of the rancid odor was judged according to a scale from 0 to 100, with 0 indicating no smell, 10 slightly rancid, 50 medium rancid, and 100 maximum rancid. A reference oil sample that during training was agreed upon as slightly rancid was presented during each sensory session to anchor the slightly rancid point on the scale. This sample was prepared by diluting a very oxidized fish oil into an almost neutral vegetable oil. Samples were presented in a completely dark room under red lights to negate color differences.

Statistics. Each sample type was replicated between two and five times (thus, $n = 2$−5), mostly with the replicates distributed over independent storage experiments using different batches of washed cod mince and oil. This was since the use of sensory analysis limited the possible number of sample replicates within each storage experiment. The 1 g sample plugs taken out daily from the sample flasks were analyzed in triplicate (thus, $a = 3$) for PV and TBARS. Color was measured at at least three spots under the sample flasks ($a = 3$), and at least three persons smelled the sample headspace at each sampling point ($a = 3$). An average value of the replicate analyses was used in calculations of sample variation and significance testing.

To significantly differentiate between oxidation data obtained from different storage points or from different sample types, data were compared with t tests. Differences among mean values obtained from the compositional analyses of model systems were tested by analysis of variance, and significance levels were obtained with Tukey's HSD multiple range test. The software used was SPSS (version 14.0 for windows). Differences were regarded as significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

Characteristics of the Model System Used. Whole fish muscle is very complex in its composition, which makes it difficult to evaluate the influence from different factors on lipid oxidation. The use of washed minced cod muscle as a model system provides a matrix that has the structure of muscle, that is, with intact myofibrillar proteins and membranes but virtually free of endogenous triacylglycerols and pro- and antioxidants. Different levels of these components can then be added back to these model systems in a controlled manner, making it possible to evaluate their effects on the fish matrix.

The washed minced cod model system used here contained ~0.7% lipids (w/w), essentially all as endogenous membrane phospholipids. It has been speculated that the total lipid content in fish is decisive for the rate of lipid oxidation to occur in the fish muscle. However, a fairly recent study showed that adding up to 15% triacylglycerols in the form of menhaden oil did not alter the rate or intensity of Hb-catalyzed oxidation of washed cod mince containing about 0.7% membrane lipids (*20*). In this study, we repeated this kind of trial to investigate the effect of lipid content on the effect of WGDF as an antioxidant against Hb-mediated oxidation. The lipid content was varied by adding 10% of herring oil to selected samples.

Antioxidative Effects of WGDF. Two and 4% of WGDF (w/w) were used to test the role of this grape-derived byproduct against the development of lipid oxidation in the washed cod mince (with and without 10% added oil) during ice storage. Both of these levels of WGDF were previously shown to be effective in delaying lipid oxidation in minced horse mackerel muscle during frozen storage (*17*). Also, these amounts of dietary fiber are technically and sensorically possible to include in restructured fish products (*28, 31*). However, the lower percentage (2%) was better evaluated by a sensory panel (*31*), which is why we focused the current study more on washed cod mince with 2% WGDF.

During storage on ice, no significant changes in rancid odor were detected in the washed cod mince in the absence of added Hb (**Figure 1A**). When 20 μM Hb was added to the model system, moderate to strong rancid odors were detected within 1 day of ice storage in samples both with and without 10% added oil. The rate of rancid odor development was thus not altered by the addition of 10% herring oil to the washed cod mince. However, the rancid odor intensity became significantly higher in samples without oil ($p \leq 0.05$). In all oxidized samples, the rancidity intensity started declining after 2−3 days. The addition of WGDF to the samples at 2 or 4% completely and significantly ($p \leq 0.05$) inhibited Hb-mediated development of rancid odor during the entire ice storage (10 days). PV and TBARS were determined in the same samples as on which sensory analyses were conduced (**Figure 1B,C**). The development of PV in control samples with Hb was fast and showed a maximum value at day 2 of ice storage, both with and without added oil (**Figure 1B**). Contrary to the sensory data, the sample without added oil gave significantly ($p \leq 0.05$) lower maximum PVs than the sample with oil. The sample without added Hb did not show any PV development throughout the study. When WGDF was added to oil-free samples, the initial PV became higher than without WGDF, but PV development was significantly ($p \leq 0.05$) delayed. During the 9 days of ice storage, there was, however, a certain development of PV in samples with both 2 and 4% WGDF, up to 306 and 507 μmol peroxides/kg sample, respectively.

In oil-containing samples, the 2% WGDF gave even higher initial PVs and there was a significant PV development between day 1 and day 3. However, still, the PV development in this sample was significantly reduced as compared to the corresponding oil-containing control.

The WGDF itself contains different pigmented substances as carotenoids that could cause interferences with the PV
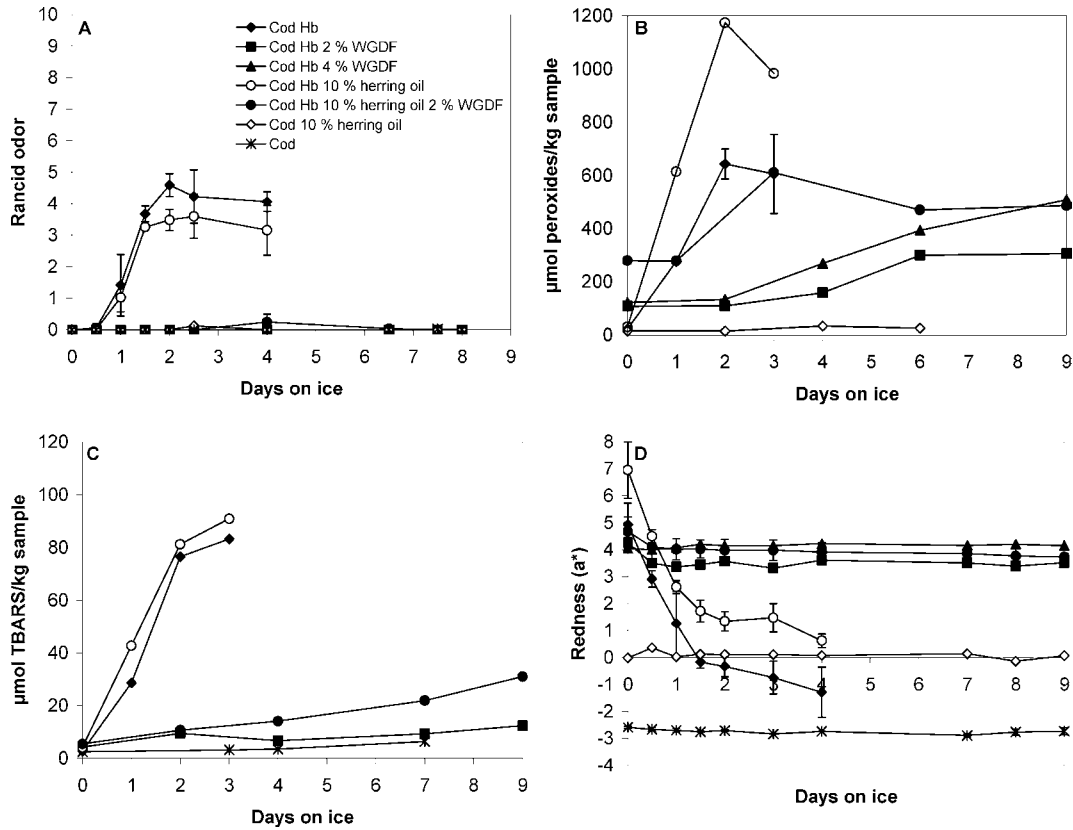
Figure 1. Hb-mediated (20 μM) changes in (**A**) rancid odor, (**B**) lipid peroxides, (**C**) TBARS, and (**D**) redness (*a**) of washed cod mince with and without 10% added herring oil and with and without 2 and 4% WGDF. Note that TBARS data are lacking for the 4% WGDF sample. Samples were stored on ice and in darkness. The majority of the points in the graphs are average numbers obtained from two replicate samples ($n = 2$), each analyzed three times ($a = 3$). The oil-free control sample with Hb was, however, tested four times (thus, $n = 4$). Error bars illustrate the data span between the sample replicates (highest value − lowest value/2). Controls without Hb and the 4% WGDF sample were only run once and thus lack error bars.

method, and thus, that could be a reason for the elevated PVs already from the start in samples with WGDF. That oil together with WGDF gave even higher zero-time values could indicate that the compounds that interfere with the PV method are oil soluble. Because of the mentioned interferences, PV was excluded during further experiments.

TBARS development reflected the sensory data better than the PV (**Figure 1C**). TBARS developed quickly in control samples containing Hb, and the rate and extent of TBARS formation were not significantly altered by the addition of herring oil. These results are, together with the sensory data, concordant with those reported by Undeland et al. (*20*) studying the addition of 7.5 and 15% commercial menhaden oil into washed cod mince. When 2% WGDF was added to oil-free and oil-containing samples, the TBARS increase was significantly ($p \leq 0.05$) reduced in both cases. Only a very vague increase was seen, especially in samples with oil and WGDF. Thus, from **Figures 1A,C**, it is seen that the addition of WGDF was slightly more effective in inhibiting rancid odors and TBARS development than PV development during 9 days of ice storage. This could indicate an ability of the WGDF to prevent peroxide breakdown into volatiles. Furthermore, the antioxidative effect was slightly more pronounced in low-lipid samples (**Figure 1B,C**).

During ice storage of the samples, changes in redness color (*a**) due to Hb oxidation were measured as an additional indicator of lipid oxidation (**Figure 1D**). During the ice storage, the control samples without Hb did not show any significant change in redness values both with and without herring oil added. When Hb was added, the sample redness given by the

Hb molecule itself decreased significantly ($p \leq 0.05$) during the first 3 days of storage in samples both with and without oil. This indicated met-Hb formation. Samples with 2 and 4% added WGDF did not show any significant changes in redness during the storage both with and without added herring oil. It should be mentioned that the WGDF themselves gave a reddish color to the samples, which could mask some potential Hb changes. However, the measured changes in redness supported very well the sensory and chemical data from the same samples in that redness faded only when oxidation products developed.

Some studies have been published recently addressing the antioxidant properties of grape dietary fiber concentrates when added to minced horse mackerel muscle during frozen storage (*16, 17*). These studies reported intense antiradical activity [measured as H-donating activity by DPPH, ferric reducing ability (FRAP), and 2,2′-azinobis(3-ethyl-benzthiazoline-6-sulfonic acid) methods] in fish samples with grape dietary fiber added. It was also found that the grape dietary fibers retarded the formation of peroxides, conjugated dienes, and TBARS in the minced fish muscle during frozen storage. Previous papers (*32−35*) have shown that WGDFs have a rich qualitative and quantitative polyphenolic profile. These data are often correlated with the antioxidant activity of WGDF polyphenol extracts (EPP). Thus, the antioxidant protection of the washed cod minced muscle by WGDF obtained in our studies may be a result of its polyphenol content. The grape polyphenols in WGDF can be divided into two groups: nonextractable polyphenols (NEPP; polymeric proanthocyanidins and high molecular weight hydrolyzable tannins) and EPP [mainly flavonoid derivatives: flavonols (quercetin) and flavan-3-ols

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(catechin and epicatechin, monomers, and compounds of different polymeric degrees and glycosilated forms), and phenolic acids (gallic acid)]. Usual ranges of EPP and NEPP in grape antioxidant dietary fiber concentrates are 1−9 and 15−30%, respectively (*18*). In the human body, EPP appear to be absorbed from the digestive tract and produce systemic effects, while NEPP are not absorbed and quantitatively recovered in feces (*36*). In vitro antioxidant properties of EPP, for example, from wine, have been widely reported (*37−39*). Hagerman, Rield, and Jones (*40*) reported that NEPP (tannin−protein complex) retained at least half the activity of the free tannin to inhibit oxidative damage in vitro. However, no studies have previously been reported about the antioxidant capacity of grape dietary fiber residue (NEPP and associated compounds) in food systems.

Antioxidative Effects of the EPPs and the Fiber Residue When Tested Separately. To study the separate antioxidant effect of the EPP and the fiber part (including NEPP) from WGDF, an ethanol extract of WGDF was prepared and compared with the ethanol-extracted fiber residue. The ethanol extract was added to the low lipid washed cod mince model at a polyphenol level equivalent to that found in samples with 2% WGDF. The ethanol-extracted fiber residue was added at a 2% weight basis. In this study, a control was also prepared to study the effect of ethanol alone on lipid oxidation. To compensate for the higher moisture level in samples with ethanol, a second control with the same amount of extra buffer was also included (final moisture, 87%). Results from this trial are shown in **Figure 2A,C**. Sensory, TBARS, and redness data for controls and WGDF-fortified samples were very similar to those shown in **Figure 1A,C,D**. However, **Figure 2 A,C** also showed that the EPP, just like whole WGDF, completely and significantly ($p \leq 0.05$) inhibited rancid odor, TBARS, and redness loss. Although there was a slight masking of rancid odors in ethanol-containing control samples (**Figure 2A**), TBARS and redness data showed that ethanol alone did not significantly inhibit lipid oxidation. These results indicate that an EPP extract of WGDF possesses the same antioxidant capacity as crude WGDF. The ability of different grape flavonoids to inhibit lipid oxidation in unwashed fish mince has been documented before (*14, 15*). Flavonoids act as antioxidants by donating electrons to free radicals, thereby stopping radical chain reactions (*41*). Pazos et al. (*14*) reported that an optimal combination of the right degree of procyanidin polymerization and % galloylation in polyphenols, as in WGDF, may explain the high antioxidant efficacy of grape polyphenols in frozen fish muscle. Hu et al. (*42*) reported higher inhibition of lipid oxidation in grape seed extract rich in oligomeric procyanidins as compared to their monomeric counterparts in algal oil-in-water emulsions. Besides free radical scavenging activities, grape flavonoids may also delay lipid oxidation by chelating metals (*41, 43, 44*). Galloyl groups in flavonoids have iron chelating properties in vitro and are believed to interfere with iron absorption in vivo (*45*). Moreover, flavonoids retain their free radical scavenging activities also after forming complexes with iron ions (*46*). Kanner et al. (*38*) reported a high antioxidant effect of wine phenols in a lipid peroxidation model system consisting of a buffered carotene−linoleate mixture with myoglobin as the oxidation catalyst. It was discussed that the effect was due to the ability of wine phenolics to reduce the catalytic ferryl myoglobin form (*38*). Lee et al. (*47*) reported a high effectiveness of certain cranberry fractions, similar to those found in WGDF, in inhibiting Hb-mediated lipid oxidation in washed cod mince during ice storage. Flavonols and phenolic acids were
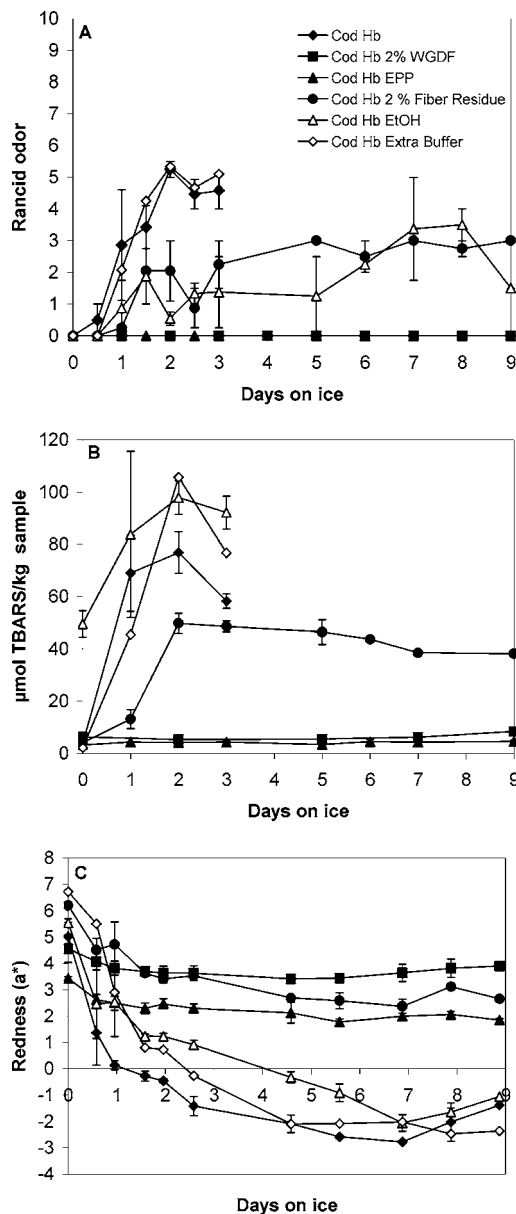
Figure 2. Hb-mediated (20 μM) changes in (**A**) rancid odor, (**B**) TBARS, and (**C**) redness (*a**) of washed cod mince with and without WGDF (2% ww), EPP (an amount equal to that found in the 2% WGDF sample), and the ethanol-extracted WGDF residue (2% ww). Samples were stored on ice and in darkness. The final moisture content of samples with EPP, ethanol, and extra buffer was ∼87.6%. The other samples had ∼85% moisture. The majority of the points in the graphs are average numbers obtained from two replicate samples ($n = 2$), each analyzed three times ($a = 3$). Error bars in panels **A**, **B**, and **C** illustrate the data span between the two sample replicates (highest value − lowest value/2). The control with extra buffer was only run once and thus lack error bars.

more effective than oligomeric polyphenols (e.g., proanthocyanidins), and quercetin was tentatively identified as the most effective compound. The results were ascribed the flavonols ability to orient readily into membrane bilayers (*48*), the site where alkoxyl and heme radicals are produced (*49*). It was discussed that the oligomeric polyphenols are too bulky to orient in such a way. The study also showed that isolated fractions of cranberry extract were more effective antioxidants than the crude freeze-dried cranberry extract itself.

Figure 2A,B also shows that the fiber residue resulting from the ethanol extraction significantly ($p \leq 0.05$) lowered maximum values for rancid odor and TBARS (by ∼50%) and significantly

($p \leq 0.05$) extended the oxidation lag phase (by ~1−2 days) as compared to controls. Thus, these results indicate that the antioxidant properties of WGDF are due mainly to the EPP fraction but that there is some effect also when the EPP fraction was removed (**Figure 2**). The latter could be due to the NEPP content or due to the dietary fiber matrix itself. The NEPP in the dietary fibers is mainly condensed tannins of high molecular mass, some of which are in the free form and some that are bound to proteins and the fiber (*50*, *51*). The NEPP are thought to have a lower antioxidant capacity than EPP due to their linkage to cell walls of the dietary fiber matrix (*18*).

The main constituent of the fiber fractions of grape pomace is Klason lignin (*50*). That dietary fibers like this can chelate bivalent ions has been repeatedly shown by others (*52−56*). Although the interaction mechanism between the dietary fiber and the metal cations is not well-known, it seems to be due to an entrapment process into the matrix and/or the yielding of a complex (*52*, *57*, *58*). The metal chelating properties of both the WGDF flavonoids and/or the dietary fiber are especially important in fish minces where heme proteins from blood and dark muscle are blended with the much cleaner light muscle. However, the dietary fiber itself could also have some other way of action, for example, trapping water, which could affect the lipid oxidation rate negatively by lowering the sample moisture. More studies are necessary to understand the antioxidant properties of whole fiber residue from WGDF.

To summarize, this study showed that adding 2−4% whole WGDF, or an ethanol extract thereof, to a lean or fatty minced fish product appears to be a promising way of preventing rancidity during ice storage. Interestingly enough, the polyphenol-stripped fiber part of the WGDF also had fairly good antioxidative properties. In particular, the fiber part reduced the maximum oxidation values obtained.

ABBREVIATIONS USED

EPP, extractable polyphenols; FRAP, ferric reducing ability; Hb, hemoglobin; NEPP, nonextractable polyphenols; PV, peroxide value; SD, standard deviation; TBARS, thiobarbituric acid reactive substances test; WGDF, white grape dietary fiber concentrate.

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