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ARTICLE

Berry Marinades Enhance Oxidative Stability of Herring Fillets

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Marinating herring fillets in a 50 g/L powder of elderberry, cranberry, or black currant inhibited the oxidation of lipids and proteins and also the degradation of tocopherol. Cranberry and black currant appeared to be more efficient than elderberry in inhibiting the degradation of tocopherol and the formation of ammonium. Elderberry marinades provided the most significant color changes. The injection of fillets with a 5% salt solution resulted in significantly increased levels of carbonyls, ammonium, and biogenic amines, whereas formation of the volatile lipid compounds propanal, hexanal, 2-penten-1-ol, and 1-penten-3-ol was lowest in fillets marinated in black currant following injection of the salt solution. All marinade treatments resulted in a significantly decreased liquid holding ability, coinciding with a lower muscle pH. It is concluded that marinating herring fillets in solutions containing berry powder can enhance the quality and shelf life of the fillets and simultaneously provide the fillets with natural antioxidants beneficial for consumers.

KEYWORDS: Fish quality; marinating; natural antioxidants; shelf life

INTRODUCTION

Herring (*Clupea harengus*) is an abundant and underutilized low-priced fish species that is rich in health-promoting long-chain polyunsaturated fatty acids (PUFA). Herring is considered to be a good candidate for value addition (*1*,*2*), but the susceptibility to peroxidation of PUFA restricts the storage and processing possibilities as, besides microbial spoilage, lipid oxidation is the primary process by which sensory quality declines in muscle foods (*3*). Fresh herring typically has a mild odor, but an unpleasant fishy odor and taste can develop due to the formation of volatiles related to oxidation and microbial spoilage (*2*, *4*). Furthermore, peroxidative products, particularly aldehydes, can react with specific amino acids to form carbonyls (*5*) and protein aggregates (*6*), causing nutritional losses.

Herring is commonly eaten salted or preserved in a number of ways. Nowadays herring is often frozen before salting to kill harmful parasites. According to McLay (*7*), herring frozen within 1 day of capture and subsequent cold storage for up to 7 months at −30 °C can be used to make good marinated products. Marinades are semipreserves; the preserving principal is the combination of acetic acid and salt to impede bacterial and enzymatic processes and thereby extend the shelf life of the product. Marinating can also positively enhance textural properties and flavor (*8*).

In northern Europe, herring is commonly salted using a traditional process that can take several months (*9*). Brining by injection is a rapid method resulting in a more homogeneous salt distribution (*10*). Although salting is a good preservation method to increase the shelf life of food products, sodium chloride is a powerful muscle food pro-oxidant (*11*, *12*).

Optimizing health and performance through the diet is believed to be among the largest and most lucrative businesses throughout the world (*13*). Prevention of specific chronic health problems is an incentive for the purchase of dietary supplements, an area in which PUFA, probiotics, and superfruits are achieving particular interest (*14*). Thus, the interest in anthocyanin-rich foods and extracts has increased due to their documented health benefits (*13*).

Antioxidants can maintain quality by improving shelf life, nutritional quality, and other aspects related to quality (*15*). The high antioxidant capacity of berry extracts is particularly due to their content of different phenols, anthocyanins, and ascorbic acid (*16*). Besides health benefits related to their natural antioxidants, color attributes of berry extracts are also of interest in food processing, as color plays a vital role in the acceptability of foods. The importance of a balanced combination of PUFA and antioxidants, both for product stability and for human nutrition, was outlined by Kamal-Eldin et al. (*17*). Hence, combining fish and berries may be interesting from nutritional, sensory, and technological points of view. Nutritionally packed meals may be especially important to people with particularly high nutritional demands, for example, those who suffer from malnutrition (*18*).

The aim of this study was to evaluate the quality-related effects of marinating herring fillets in different berry extracts (cranberry, black currant, and elderberry), with particular emphasis on lipid and protein oxidation upon frozen storage. Quality tests were also performed on herring fillets that had been both salted and marinated in black currant extract.

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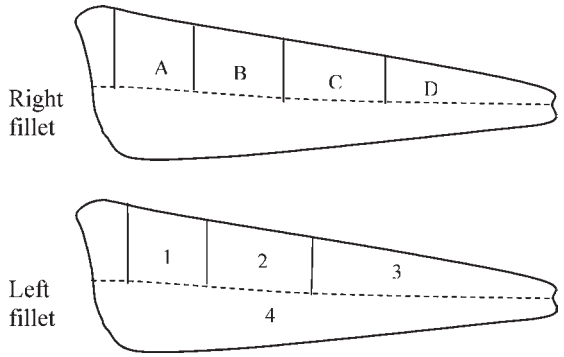

Figure 1. Schematic illustration showing the areas upon the fillet from which the analyses were conducted. Right fillet: (A) lipids; (B) tocopherols and TBARS; (C) biogenic amines; (D) carbonyls and pH. Left fillet: (1) Salt content and dry matter; (2) GC-MS; (3) water-holding capacity (pressure); (4) water-holding capacity (centrifuge). Fillet surfaces were photometrically analyzed for color.

MATERIALS AND METHODS

Fish and Treatment. Herring (*C. harengus*) was obtained from Vikomar A/S (Bud, Norway) in September 2007. Postharvest, the fish were gutted, decapitated, and frozen in a freezing funnel at −35 °C. After storage at −25 °C for 4 months, the fish were thawed at 6 °C for 48 h, filleted, and deskinned. The average weight of the gutted fish was 309 ± 32 g, and the fillet fat content averaged 11.9 ± 0.5% of wet weight (triacyl glycerols, 81.5%; polar lipids, 9.8%; and cholesterols, 4.3%). The fillets were marinated in solutions made from concentrates of either elderberry (*Sambucus nigra*), cranberry (*Vaccinium* spp.), or black currant (*Ribes nigrum*) without any pretreatment (denoted elderB, cranB, and blackC, respectively). Additionally, fillets were injected with a salt solution before they were marinated in black currant solution (denoted blackC-salted). Untreated fillets and fillets injected with the salt solution without subsequent marinating were used as reference samples (denoted control and salted, respectively). In total, 12 fillets were subjected to each treatment. Marination was performed in 10 L containers in a dark refrigerated room (6 °C) for 24 h, and injection of the salt solution (5%) was performed using a Fomaco 16/64F injection machine (Fomaco Food Machinery Co. A/S, Køge, Denmark). After marination, the fillets were vacuum-packed, stored on ice for 7 days, and then frozen at −20 °C for 6 months. Thereafter, the fillets were analyzed, or they were stored at −80 °C until required for analysis. Sectioning of the fillets for the various analyses is illustrated in **Figure 1**. Unless otherwise stated, six fillets were used for each analysis.

Fruit Powder and Salt. The following food grade spray-dried fruit concentrates were obtained from BerryPharma AG (Appenzell, Switzerland): natured-powder elderberry (code V0120014), cranberry powder (code V0-410002), and black currant powder (code V0140003). The moisture contents of the concentrates were 2.5, 2.4, and 3.2%, respectively. Marinade solutions were prepared by dissolving 50 g of berry powder per liter of tap water. The manufacturer's content analyses of the marinades, in addition to pH and antioxidant capacity, analyzed as 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method of Brand-Williams et al. (*19*), are shown in **Table 1**. The salt solutions were prepared by dissolving sodium chloride (refined salt, Norsal, CG Rieber, Ålesund, Norway) in tap water to a final concentration of 50 g/L. The berry and salt solutions were prepared 48 h prior to treatment and stored at 6 °C until used.

Salt and Water Content. Sodium chloride content in the muscle was determined as water-soluble Cl using titration with a Corning 926 chloride analyzer (Corning Medical and Scientific, Halstead, U.K.) (*20*). The water content was determined as the weight reduction (%) after 7 g of muscle had been dried overnight at 102 °C.

pH Analysis. The pH of the marinades, the thawed muscle, and the liquid lost from the fillets after thawing was analyzed using a BlueLine 21 electrode (Schott instruments, Mainz, Germany), connected to a WTW 330i pH-meter (WTW, Weilheim, Germany).

For the thin layer chromatography (TLC) analyses of lipid classes, α-tocopherol, and biogenic amines, a TLC instrument consisting of a TLC sampler 4 and a TLC Scanner 3 (CAMAG, Muttenz, Switzerland) was used.

Table 1. Industry Specifications and Determined Properties of the Berry Concentrates

	elderberry	cranberry	black currant
industry specifications[a]			
total acids,[b] g/kg	32	76	81
anthocyanins,[c] %	0.17	0.8	0.31
total polyphenols,[d] g/kg	11.2		18
analyzed values			
pH in solution[e]	4.03	2.85	3.20
DPPH, μmol of TE/g of sample	32.6	21.6	64.0

[a] BerryPharma AG, Appenzell, Switzerland. [b] As citric acid at pH 8.1. [c] As Cya-3-glu. [d] As catechin. [e] 50 g of powder/L.

Lipid Content and Lipid Classes. Fat extraction was performed according to the method of Hara and Radin (*21*), with slight modifications as outlined below. Muscle tissue (1−2 g) was extracted with 10 times the volume of hexane/isopropanol (3:2, v/v). For phase separation, 0.4 times the volume of sodium sulfate solution (0.47 M) was added, and the upper phase was transferred to a new tube and evaporated using nitrogen gas. The lipid content of the muscle was determined gravimetrically from the total extracted lipid fraction, which was then dissolved in hexane. The samples were stored at −20 °C until further analyses were performed.

The total lipids were analyzed by TLC to investigate lipid class composition. As a stationary phase, glass plates precoated with silica gel TLC plates were used (20 × 10 cm; silica gel 60; 0.20 mm layer, Merck, Darmstadt, Germany). The analyses were performed as described by Olsen and Henderson (*22*), with slight modifications according to those of Sampels (*23*).

Tocopherol Content. Content of α-tocopherol was analyzed by high-performance thin layer chromatography (HPTLC) from lipid extracts using a method modified from that of Deepam et al. (*24*). As a stationary phase, glass plates precoated with silica gel HPTLC plates were used (20 × 10 cm; silica gel 60 F_{254}; 0.20 mm layer, Merck). Prior to sample application, approximately 40 mg of lipid from each sample was saponified as described in ref *25*. After saponification, tocopherols were extracted with 2 volumes of 3 mL of hexane. The combined hexane phase was evaporated with nitrogen gas and resolved with 100 μL of chloroform. Twenty microliters of each sample was applied 10 mm from the base edge of the TLC plates in 3 mm bands with an application speed of 150 nL/s (distance between tracks was 9.4 mm). Nitrogen was used as spray gas. The tocopherols were then separated in a twin-trough chamber, 20 × 10 (CAMAG), using 25 mL of benzene/chloroform (24:2, v/v) as the mobile phase. Saturation was increased by placing a piece of dry filter paper in the chamber. Plates were removed from the chambers when they had migrated 8.9 cm from the origin, and they were subsequently air-dried at room temperature. Quantitative analysis of the separated tocopherols was performed by scanning the plates at a speed of 20 mm/s with a data resolution of 100 μm/step, a slit dimension of 5.00 × 0.45 mm, and a wavelength of 297 nm. Identification and quantification of α-tocopherol were performed by comparison with an external standard (α-tocopherol, Sigma Aldrich). For data filtering, the mode Savitsky−Golay 7 and a manual baseline correction were used.

Biogenic Amines and Ammonium. Analyses were performed by TLC following a method by Lapa-Guimaraes and Pickova (*26*). Muscle tissue (2 g) was extracted in 20 mL of 5% trichloroacetic acid. From this solution, a 2 mL aliquot was used for dansylation. Dry sodium bicarbonate was added until saturation; after that 1 mL of dansyl chloride (at a concentration of 5 mg/mL in acetone) and the samples were left to react at 40 °C for 1 h. Residual dansyl chlorides were removed by reaction with 0.5 mL of glycine (0.1 g/mL) for 20 min at 40 °C. After evaporation of residual acetone and addition of 3 mL of water, amines were extracted using three rinses of 2 mL of diethyl ether. Combined extracts were evaporated and resolved in 1 mL of ethyl acetate. Samples were applied in 4 mm bands on HPTLC plates (10 × 20 cm; silica gel 60; 0.20 mm layer, Merck) with the origin 1 cm from the plate edge. A double-development technique was used. Initially, chloroform/diethyl ether/triethylamine (6:4:1) was used to develop up to a migration distance of 10 cm from the origin; subsequently, chloroform/triethylamine (6:1) was used to develop up to 17 cm from the

Table 2. Chemical Composition and Oxidation Markers of Herring Fillets (Mean ± Standard Error; n = 6 Fillets per Treatment)[a]

		marinated			salt injected and marinated	
	control	elderberry	cranberry	black currant	control	black currant
water content, %	65.7 bc ± 0.6	67.3 ab ± 0.1	62.8 d ± 0.8	64.3 cd ± 1.5	68.5 ab ± 0.7	69.0 a ± 0.7
salt content, %	0.36 c ± 0.03	na	na	na	0.62 a ± 0.06	0.44 b ± 0.04
muscle pH	6.38 ± 0.03 a	6.18 ± 0.03 a	5.88 ± 0.11 b	5.95 ± 0.07 b	6.35 ± 0.02 a	5.95 ± 0.06 b
free fatty acids, %	4.3 ab ± 0.2	4.5 ab ± 0.1	4.1 bc ± 0.2	4.4 ab ± 0.2	3.5 c ± 0.3	4.9 a ± 0.4
α-tocopherol, μg/g	15.1 c ± 1.13	15.8 bc ± 0.15	21.5 a ± 2.76	19.8 ab ± 1.27	nd	nd
TBARS, nM/g	25.4 bc ± 3.8	22.3 bc ± 4.3	21.3 bc ± 1.8	15.9 c ± 0.7	38.3 ab ± 9.1	48.3 a ± 13.8
ammonium, μg/g	2.06 ab ± 0.03	1.40 bc ± 0.32	1.19 c ± 0.20	1.06 c ± 0.38	2.47 a ± 0.05	2.32 a ± 0.06
volatiles, ng/g						
propanal	14.4 a ± 4.9	6.9 ab ± 2.0	5.4 b ± 1.5	8.8 ab ± 0.9	6.7 ab ± 2.4	2.6 b ± 1.1
hexanal	7.6 a ± 1.3	3.4 bc ± 0.7	3.3 bc ± 0.3	3.5 bc ± 0.8	5.0 b ± 0.7	2.4 c ± 0.2
2-penten-1-ol	17.0 a ± 1.8	9.2 b ± 2.2	7.9 b ± 1.9	10.6 ab ± 1.1	7.5 b ± 2.7	5.6 b ± 0.1
1-penten-3-ol	173.8 a ± 47.1	63.8 b ± 12.7	74.5 b ± 6.3	75.1 b ± 11.4	90.1 b ± 9.1	35.8 b ± 6.4
biogenic amines, μg/g						
agmatin	0.03 a ± 0.01	0.03 a ± 0.01	0.04 a ± 0.01	0.05 a ± 0.01	0.11 b ± 0.03	0.07 ab ± 0.03
tyramine	0.11 a ± 0.00	0.12 a ± 0.02	0.09 a ± 0.02	0.08 a ± 0.02	0.03 b ± 0.00	0.03 b ± 0.00

[a] Different letters within the same row indicate significant differences between treatments (P < 0.05). na, not analyzed; nd, not detected.

origin. The plates were scanned at a wavelength of 330 nm using a K400 filter after development and drying. Identification and quantification of the different amines was achieved by comparison with external standards (Sigma-Aldrich, Germany).

Thiobarbituric Acid Reactive Substances (TBARS). Analysis of TBARS was conducted according to a method described by Miller (*27*) and slightly modified by Sampels (*23*). After reaction in darkness for 15−20 h (overnight) at room temperature (20 °C), the reaction complex was detected at a wavelength of 530 nm against a deionized water reference using a UV−visible spectrophotometer (model 918; GBC Scientific Equipment Pty. Ltd., Melbourne, Australia).

GC-MS Headspace. To identify and quantify volatile organic compounds, gas chromatography−mass spectrometry (GC-MS) was carried out according to the method described by Olsen et al. (*28*). Comparison of retention times and mass spectra of the sample peaks with those of pure standards confirmed identities of approximately 90 components. Heptanoic acid ethyl ester was used as an internal standard. Selected volatile compounds were included in the data analysis: propanal, hexanal, 2-penten-1-ol, and 1-penten-3-ol. In addition, butanal, heptanal, and nonanal were also included in the sum of alkanals.

Protein Oxidation (DNPH). Protein oxidation was estimated as carbonyl groups formed during incubation with 2,4-dinitrophenylhydrazine (DNPH) in 2 N hydrochloric acid following a slightly modified method described by Oliver et al. (*29*). Carbonyl concentration was analyzed as DNPH calculated on the basis of absorption of 21.0 mM^{-1} cm^{-1} at 370 nm for protein hydrazones. Protein concentration was measured at 280 nm in the same sample using bovine serum albumin as a standard. Protein oxidation was expressed as millimoles of carbonyls per gram of protein.

Liquid Loss. The liquid loss from coarsely chopped muscle during low-speed centrifugation at 500*g* for 10 min was analyzed in duplicate per fillet according to the method of Gomez-Guillen et al. (*30*) as modified by Regost et al. (*31*). Liquid loss was also analyzed by compressing a 4 g muscle piece to 60% of its initial height by a flat-ended cylinder (diameter = 50 mm) for 60 s, using a texture analyzer (model TA.XT2, Stable Micro Systems Ltd. (SMS), Surrey, U.K.). A black ribbon filter was folded and placed over and under the fish piece, and liquid loss was calculated as weight increase of the filter after compression as a percentage of the initial muscle weight.

Fillet Color. The fillets were photographed using a digital camera (Dolphin F145C, Allied Vision Technologies, Stratroda, Germany) in a light-proof aluminum box with standardized illumination. The camera was powered and operated through a computer as described by Folkestad et al. (*32*). The parameters L^*, a^*, and b^* were used to study the color properties of the fillet surface, where L^* is the luminance score and ranges from zero (black) to 100 (white) and a^* and b^* are chromatic scores with negative to positive values representing green to red and blue to yellow, respectively. Each fillet was analyzed four times in four different regions.

Statistical Analyses. Data were analyzed by one-way ANOVA and correlation (Pearson's correlation coefficient) analyses using the SAS program (version 9.1; SAS Institute Inc., Cary, NC). The α level was set to 5% (P < 0.05). A PLS2 plot was obtained using The Unscrambler v. 9.8 (Camo Process A/S, Oslo, Norway), where variables with values of >10 were weighted with 1/standard deviation.

RESULTS

Water, Salt, and Muscle pH. The water content averaged 66.3%, with the highest content observed in the salt-injected fillets (68.5−69%) and the lowest in the cranB fillets (62.8%) (**Table 2**). Presalting the fillets gave a slightly, but significantly, greater salt content, with a higher content in the salted (0.62%) compared with the blackC-salted fillets (0.44%) (P = 0.03). The marinated fillets had a lower pH, although the difference was nonsignificant between the control (6.38) and elderB fillets (6.18) (P = 0.06). The lowest pH values were those of the cranB, blackC, and blackC-salted samples (pH 5.88−5.95). Preinjecting the fillets with the salt solution had no effect on the muscle pH. The pH values were 6.42, 5.89, 5.49, and 5.34 in the liquid loss after thawing of the control, elderB, cranB, and blackC samples, respectively.

Free Fatty Acids, TBARS, and α-Tocopherol. Content of free fatty acids (FFA) ranged from 3.5 to 4.9% (**Table 2**). The content of FFA was significantly lower in salted fillets (3.5%) compared with the control, elderB, and blackC fillets (4.3−4.5%); however, there was no significant difference observed in comparison with the cranB fillets (4.1%). Content of FFA was highest in the blackC-salted group (4.9%), but the difference was only significant when compared with the cranB and salted samples. The average TBARS showed pronounced variation between treatments (15.9 and 48.3 nM/g of fish). The TBARS were highest for the salted and blackC-salted fillets, and these treatments also showed the greatest amount of variation between replicates (coefficient of variation (CV) = 41−49%). The lowest TBARS were observed for the blackC fillets (15.9 nM/g; CV = 8.0%), and these were 3-fold lower than presalted samples prior to marinating. The TBARS were similar for the control, elderB, and the cranB fillets (average = 21.3−25.4 nM/g; CV = 14 − 34%). The average content of α-tocopherol was 33% higher in the cranB and

blackC fillets (19.8−21.5 μg/g) compared with the control and elderB fillets (15.1−15.8 μg/g), whereas no α-tocopherol was detected in the presalted fillets (both salted and blackC-salted). The variation between replicates within each treatment was lower for FFA (CV = 2−14%) and α-tocopherol (CV = 2−22%) compared with the TBARS. The correlation between α-tocopherol and TBARS was significant ($r = -0.68$; $P = 0.002$), whereas the correlation between α-tocopherol and FFA was not significant ($P = 0.70$).

Lipid -Derived Volatiles. The lipid-derived volatiles showed a consistent pattern, with the highest contents consistently observed in the control samples (**Table 2**). The volatile component 1-penten-3-ol was the most abundant of all lipid-derived volatiles. For this component, the amount determined in the control samples was more than twice as much as that determined in the fillets marinated in berry solutions (averages of 173.8 and 71.1 ng/g, respectively). BlackC-salted samples had the lowest content of 1-penten-3-ol, but the difference was not significant when compared with elderB, cranB, blackC ($P = 0.21-0.37$), or the salted samples ($P = 0.09$). The sums of alkanals and pentenols (**Figure 2**) were significantly highest in the control samples, but a more pronounced discrimination between treatments was obtained using the sum of alkanals as opposed to the pentenols.

Protein Oxidation (DNPH). Carbonyl content was significantly greater in the salted fillets compared with all other treatments except for the control, for which the difference was nonsignificant on a 5% level ($P = 0.056$) (**Figure 2**). The numerical value of carbonyls was higher in the control samples compared with the marinated, but the difference was nonsignificant. The highest numerical variation was observed between the control and blackC ($P = 0.085$). DNPH and FFA showed a negative overall correlation ($r = -0.61$; $P = 0.008$).

Biogenic Amines and Ammonium. In addition to agmatine and tyramine, which were identified in all samples (**Table 2**), minor amounts of cadaverine and histamine were also determined in a few samples (not presented). Salted and blackC-salted samples had significantly lower contents of tyramine compared with the other treatments. Agmatine content was significantly greater in salted samples than control, elderB, cranB, and blackC samples, whereas blackC-salted had intermediate content and did not differ significantly from the other samples. Ammonium was detected in all samples, with significantly less ammonium detected in cranB and blackC samples than in control, salted, and black C-salted samples. Contents of ammonium and TBARS were positively correlated ($r = 0.63$; $P = 0.02$).

Liquid Loss. The nonmarinated samples were able to retain liquid better than the marinated samples, and salted samples had significantly lower liquid loss compared to control samples. The trend of liquid loss followed the order control < elderB < blackC < cranB, for the unsalted fillets and salted < blackC-salted for the salted fillets. Liquid loss analyzed using the centrifugation method showed a higher variation between treatments than the pressure method (**Table 3**).

Fillet Color. The fillet coloration, determined by image analysis, showed significant differences between treatments for all parameters (**Table 3**). The color parameters changed most significantly for the elderB samples (as compared with the control), where the L^* and b^* values showed particularly high changes toward a darker, bluish color. For the blackC samples, significant reductions were observed for L^*, a^*, and b^* after marinating, but the numerical changes were smaller in comparison to those observed for elderB samples. Only the b^* value changed significantly for the cranB, with the L^* and a^* values similar to those recorded for the control. Salt-injected samples showed little deviation from L^* values determined for the control, but significantly higher a^* and lower b^* values. L^*, a^*, and b^* values were similar for the blackC and blackC-salted fillets.

Multivariate Data Analysis. Partial least-squares regression (PLS-2) showed a clear discrimination between the treatments (**Figure 3**). The control samples were located almost opposite the blackC-salted samples in the correlation loadings plot, whereas the marinated fillets, which were not subjected to preinjection (elderB, cranB, and blackC), were clustered and negatively correlated to the salted samples. The different oxidation indicators were associated with different treatments, where volatile lipid compounds were clustered together with the control samples, whereas protein oxidation indicators (carbonyls, agmatine, and ammonium) were associated with the salted fillets. TBARS appeared to be associated with both salted and blackC-salted samples, and FFA and tocopherols were associated with the elderB, cranB, and blackC samples.

DISCUSSION

The average water content of the herring fillets was within the range reported by others (*33*, *34*). The significant difference of water content between treatments reflected variation in liquid-holding capacity (LHC) during freezing and thawing. The lower amount of liquid loss observed during thawing of frozen untreated fillets (control) was expected as these fillets were not soaked in liquid prior to being frozen. A clear relationship was observed between low muscle pH of the marinated fillets and increased liquid loss, probably due to effects of the configuration of the muscle proteins (*35*). It is interesting that the injection of a diluted salt solution into the fillets prior to marinating (salted and blackC-salted) gave significantly improved LHC, despite the fillet salt content increasing by only 0.1−0.3%. The lower LHC of the marinated fillets was found even for the thawed fillets, observed as higher liquid losses when exposed to pressure and low-speed centrifugation. The muscle used for the centrifugation and pressure method was sampled from the ventral and dorsal fillet part, respectively. Hence, the higher variation between treatments for the analyses performed by the centrifugation method might be due to variation in raw material properties, as composition and properties vary between sections within the same fillet (*1*).

An inverse relationship has been reported between pH and oxidation (*36*), but the fillets marinated in cranberry and those marinated in black currant had the lowest pH, TBARS, and ammonium values and also the greatest content of α-tocopherol. The pattern was more complicated for the fillets preinjected with the salt solution. For these fillets, the FFA content was significantly greatest for those subsequently marinated in black currant, and TBARS values of the blackC-salted samples also tended to be higher compared with the salted samples. In fact, preinjection resulted in triple the TBARS content of blackC-salted compared with black C samples, indicating interaction between preinjection of the salt solution and marinating in the black currant solution. TBARS values are widely used as an indication of lipid oxidation (*37*), determined as the amount of malondialdehyde formed during oxidation of fatty acids with three or more double bonds. However, additional substances may influence the results from TBARS analyses. For example, 2-alkenals and 2,4-alkadienals form pigments that absorb at the same wavelength as malondialdehyde (*38*), and also marinating gave marked color changes (particularly elderberry marinades) that may have influenced the results.

The content of α-tocopherol in the salted and blackC-salted treatments was below the detection limit of 10 μg/g, indicating that α-tocopherol was consumed during oxidation of these samples. The significantly lower FFA content in salted samples indicates that salt decreased the enzymatic lipase activity, whereas

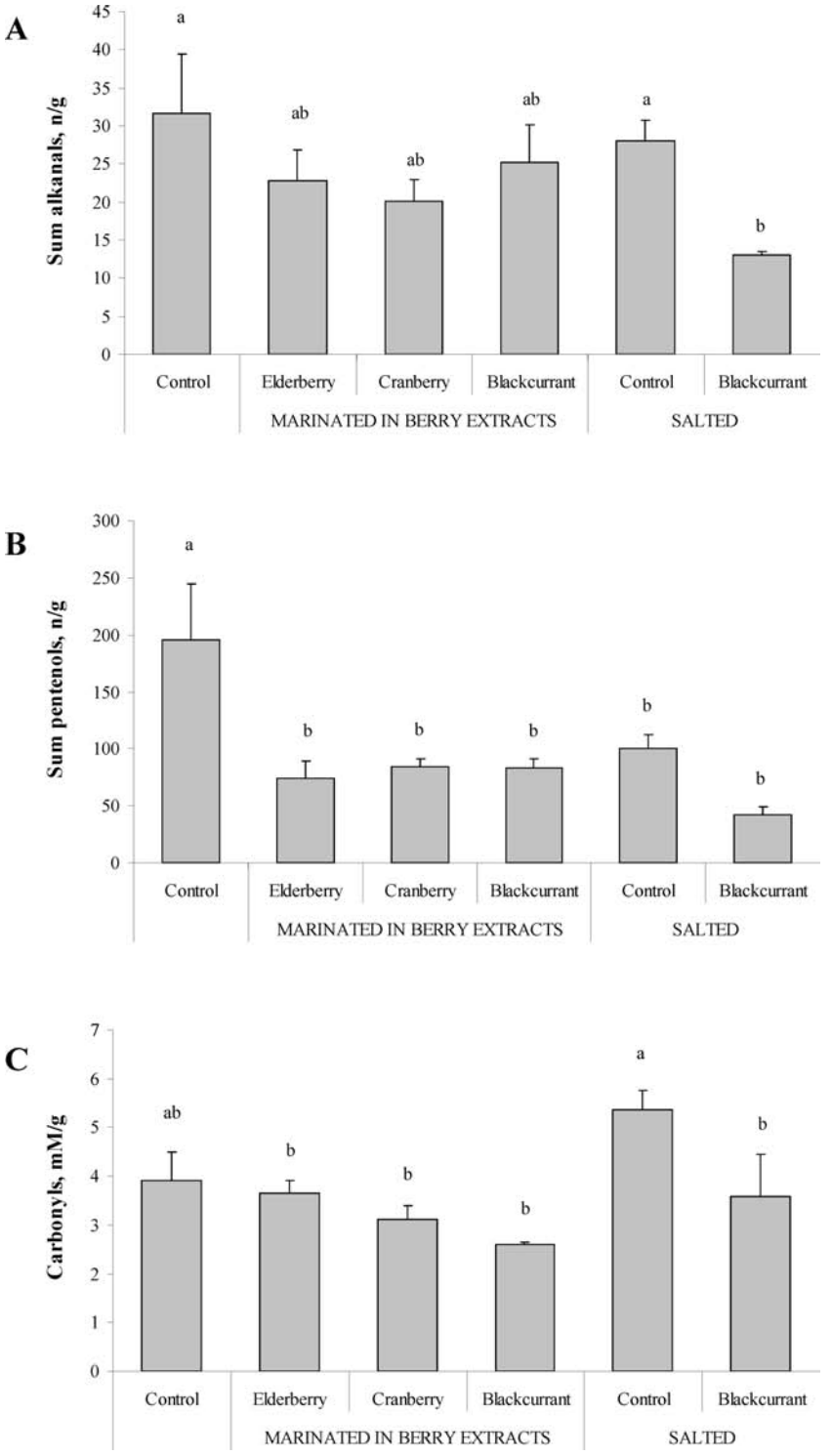

Figure 2. Lipid-derived volatiles (ng/g) identified in the headspace of herring, shown here as the sum of alkanals (**A**) and pentenols (**B**), and the protein oxidation marker, carbonyls (**C**). The sum of alkanals includes propanal, butanal, methyl-2-butanal, methyl-3-butanal, hexanal, heptanal, and nonanal, whereas the sum of pentenols includes 1-pent-3-ol and 2-pent-1-ol. Results are given as mean ± standard error. Different letters indicate significant differences between treatments.

marinating with black currant leveled out this effect. The higher content of agmatine in the salted samples in comparison with the control and the lower content of tyramine in both salted and blackC-salted samples indicate that the protecting effect of berry concentrates and salt on oxidation varies between proteins.

The antioxidant activity of the black currant powder was similar to that reported by Wu et al. (*39*) (49−100 μmol of TE/g), whereas the authors reported significantly higher antioxidant activity (147 μmol of TE/g) in elderberry compared with values found in the present study. The antioxidant level and composition of the berries will influence the antioxidative effectiveness. Additionally, the physical location of the antioxidants, the composition of the system, and interaction with other components are also important (*36*, *40*). For example, ascorbic acid and tocopherol are known to interact as antioxidants, whereby the tocopheroxy radicals are reduced back to tocopherol by ascorbic acid (*41*).

Table 3. Liquid Loss during Thawing and during Centrifugation of Intact Muscle (Pressure Method) and Surface Color Parameters of Herring Fillets (Mean ± Standard Error; $n = 6$ Fillets per Treatment)[a]

		marinated			salt injected and marinated	
	control	elderberry	cranberry	black currant	control	black currant
liquid loss, %						
pressure method, intact muscle	17.8 d ± 0.8	21.9 bc ± 0.5	28.2 a ± 2.0	23.2 b ± 1.6	18.4 cd ± 1.8	29.7 a ± 1.8
centrifugation, chopped muscle	14.8 b ± 1.2	25.3 a ± 1.7	28.8 a ± 1.7	28.7 a ± 1.5	4.8 c ± 1.4	11.0 b ± 2.3
colorimetric parameters						
L^* value	49.8 a ± 1.0	27.5 c ± 0.6	49.0 a ± 1.0	41.7 b ± 0.8	50.2 a ± 0.4	40.0 b ± 0.8
a^* value	6.8 b ± 0.4	5.6 c ± 0.1	7.4 b ± 0.3	5.8 c ± 0.2	8.9 a ± 0.2	5.6 c ± 0.1
b^*value	9.4 a ± 0.3	−1.2 e ± 0.2	6.3 c ± 0.3	1.5 d ± 0.7	8.1 b ± 0.3	0.8 d ± 0.4

[a] Different letters within the same row indicate significant differences between treatments ($P < 0.05$).

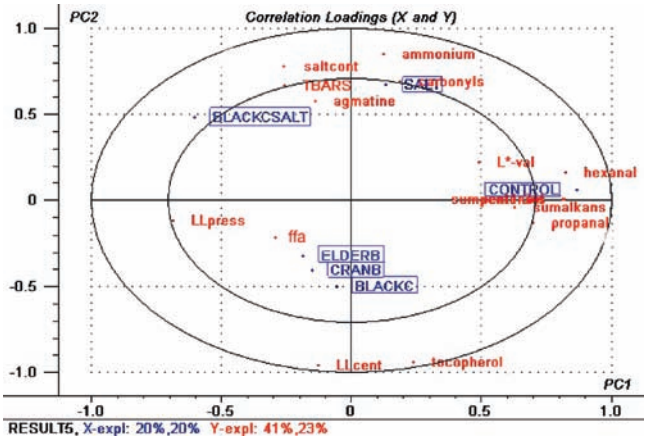

Figure 3. Correlation loadings for components 1 and 2. Treatments are framed and indicated by capital letters.

Similar interaction is also known for phenolic substances (*42*, *43*). Furthermore, Viljanen et al. (*44*) stated that the antioxidative effect is strongly dependent on the choice of raw berry material, as the antioxidant activity differs between the individual berry phenolic constituents, including anthocyanins, ellagitannins, and proanthocyanins. For example, the authors reported that lingonberry and bilberry phenolics provided the best antioxidant protection toward lipid oxidation, whereas bilberry and raspberry phenolics exhibited the best overall antioxidant activity toward protein oxidation. In bilberries and black currants, anthocyanins contributed the most to the antioxidant effect by inhibiting the formation of both hexanal and protein carbonyls.

The aroma of raw and prepared fish is the result of a complex mixture of volatile compounds, originating from many processes such as microbial enzymatic breakdown and lipid degradation (enzymic and oxidative) (*45*). In the present study, >90 different volatile substances were detected; special emphasis was given to 1-penten-3-ol and hexanal because these compounds have been identified as good markers for early lipid oxidation of food items (*46*). For instance, hexanal levels have shown positive correlation with rancid odor and unpleasant flavor (*47*), whereas 1-penten-3-ol has been considered a good marker for early oxidation of fish oil and fish rich in n-3 fatty acids (*48*). Other volatiles included were propanal, heptanal, and 2-penten-1-ol.

The content of 1-penten-3-ol was highest among the determined volatiles, but consistently lower in berry-marinated fillets. These results are partially concordant with those of Joaquin et al. (*2*), who determined propanal and 1-penten-3-ol to be the major volatile compounds in herring mince. The low level of propanal in the cranberry-marinated fillets coincided with the lowest antioxidant capacity (DPPH), but the highest content of anthocyanins of this treatment. Contrasting influences of antioxidants with regard to the formation of volatile oxidation products and also interaction between various antioxidants were reported by Olsen (*49*). The authors observed inhibited formation of 1-penten-3-ol by adding tocopherol to cod liver oil, but a binary mix of tocopherol and ascorbyl palmitate promoted formation of, for example, hexanal and inhibited formation of, for example, 2,4-heptadienal.

Preinjecting the fillets with a weak salt solution before they were marinated in black currant resulted in further decreased levels of 1-penten-3-ol and propanal, despite the fact that sodium chloride is considered to be an important muscle food pro-oxidant (*11*, *12*). It is likely that puncturing the fillets with needles during injection enabled the marinade to penetrate deeper into the muscle tissue, thus promoting the inhibiting effect against lipid oxidation. It is, however, also notable that salted samples had significantly lower contents of hexanal, 2-penten-1-ol (*Z*), and 1-penten-3-ol compared to control samples.

Ammonia, being a product of several enzymatic processes, usually forms the majority proportion of volatile amines (*50*). The proportions found in the present study were generally low in comparison to other studies. Wu and Bechtel (*51*) found ammonia values between 93 and 146 μg/g in fresh fish byproduct, and Hughes (*52*) observed levels at approximately 200 μg/g in fresh herring. Because the ammonia values for the samples treated with salt were highest, this also implies a pro-oxidative effect of salt on proteins, whereas berry-based marinades (cranB and blackC) seemed to be protective.

A wide range of analytical methods were used to elucidate the impact of marinating herring fillets in berry concentrate solutions on oxidation- and quality-related characteristics. To obtain an overview of the main patterns of variation from the data, that is, to view the main associations between *X* and *Y* variables, and also interrelationships within *X* and *Y* data, partial least-squares regression was performed. The correlation loadings plot showed a clear discrimination between treatments and also correlations between response variables. Volatiles formed from lipid oxidation were associated with the untreated control fish, whereas the berry-marinated fillets were clustered and associated with high liquid losses and high levels of α-tocopherol, but not lipid or protein oxidation products. Thus, it appears to be evident that high antioxidant levels protected the berry-marinated fillets from oxidation. The salt-injected fillets were associated with high TBARS levels and increased protein oxidation with higher levels of carbonyls, ammonium, and biogenic amines. However, subsequently marinating the fillets in black currant concentrate reduced protein oxidation. Moreover, berry marinating resulted in altered coloration. Traditionally, herring fillets are manufactured in various colored marinades (e.g., tomato sauce and wine),

but consumer acceptance of berry-marinated herring fillets needs to be elucidated.

It is concluded that marinating herring fillets in berry marinades has a protective effect toward lipid and protein oxidation, and these marinades also protect tocopherols against degradation. Berry powders can therefore enhance the quality and shelf life of herring fillets and simultaneously supply the fillets with natural health-promoting antioxidants benefitting the consumers. All berry marinades altered the fillet color, and also it is expected that flavor and odor were influenced, although such data were not included in this study. Future studies should elucidate sensory perception and microbiological evaluation of berry-marinated herring fillets, and measures to prevent high liquid losses should be considered.

ABBREVIATIONS USED

blackC, samples marinated with black currant solution; blackC-salted, samples injected with salt solution and marinated in black currant solution; cranB, samples marinated with cranberry solution; DNPH, 2,4-dinitrophenylhydrazine; DPPH, 2, 2-diphenyl-1-picrylhydrazyl; elderB, samples marinated with elderberry solution; FFA, free fatty acids; GC-MS, gas chromatography−mass spectrometry; HPTLC, high-performance thin layer chromatography; LHC, liquid-holding capacity; LLcent, LHC using centrifugation method; LLpress, LHC using centrifugation method; PUFA, polyunsaturated fatty acids; TBARS, thiobarbituric acid reactive substances; TE, tocopherol equivalents; TLC, thin layer chromatorgraphy; salted, samples injected with salt solution.

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