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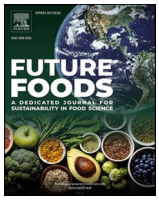

Valorization of whole herring (*Clupea harengus*) through trimming, mincing and antioxidant washing: Enhancing stability and color for food use

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ABSTRACT

Turning the valorizing of small herring (*Clupea harengus*) from primarily being a feed resource into being a valuable food ingredient requires strategies to overcome rapid lipid oxidation, dark color and processing losses. This study investigated trimming (deheading plus partial gutting or, deheading plus full gutting) and washing of mechanically separated meat (MSM) to improve oxidative stability and appearance of whole herring while maintaining maximum processing yields. Trimming reduced hemoglobin content and lipoxygenase activity gradually by up to 73% and 75% (dw), respectively, yet lipid oxidation was initiated within 1 day on ice for all samples. Washing MSM once or twice (1:3 ratio) further decreased these pro-oxidative factors but had limited effect on oxidation onset. Incorporating a rosemary-based antioxidant blend (0.5%) into the wash water delayed the start of oxidation to more than 11 days. Sufficient oxidative stability was achieved with a single antioxidant-assisted wash, which also minimized water use and processing losses of proteins and lipids. In terms of color, trimming and consecutive washes progressively increased lightness, while antioxidant-assisted washes also helped maintaining redness during storage. Correlation analysis revealed that lipid oxidation was more strongly associated with hemoglobin, lipoxygenase, and pre-formed hydroperoxides than total lipid content, highlighting these factors as important mitigation targets for the food industry. By combining targeted trimming with antioxidant-assisted washing, the oxidative stability, color, and processing efficiency of herring MSM can be improved, supporting a sustainable upcycling of small pelagic fish into nutritious food and thereby steering away from their current main use in feed production.

Introduction

Despite the high nutritional value of herring (*Clupea harengus*), it remains a marine resource which is highly underutilized for food. Herring is rich in proteins, long chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs), vitamins (e.g., D and B12), and essential micronutrients (e.g., iron, zinc, iodine, selenium, and calcium) (Abdollahi et al., 2021; Wu et al., 2022a, 2020). Still, large volumes go to feed applications, such as fish meal and fish oil production. In 2022, around seventeen million tons of small pelagic fish species, including herring, were processed into fish meal and fish oil on a global level, representing 22% of the total wild fish catch. Out of this amount, whole intact fish accounted for over 55% (FAO, 2024). For some countries, like Sweden, the feed use is even higher, and in 2024 it reached 91% of the landed small pelagic species (herring, sprat, sand eel; in declining order) out of which 87% were whole round fish (Ericson, 2025). While a main research focus in the last 10-15 years has been on food ingredient production from fish filleting side streams (Al Khawli et al., 2019; Alfio et al., 2021; Ghaly et al., 2013; Khan et al., 2022; Ling Wen Xia et al., 2024), a relatively small fraction of the total landed pelagic fish biomass, little attention has been given the valorization of whole pelagic fish such as herring. With its low carbon footprint and high availability globally, this is clearly an untapped food resource (Álvarez et al., 2018; Bianchi et al., 2022; Nisov et al., 2022, 2020).

A substantial part of the herring catches, not least in the Baltic Sea, however, consists of small-sized individuals, which are challenging to process through the traditional filleting machines used in the herring industry today. An alternative route involves developing conversion technologies utilizing whole or partially trimmed small-sized herring. Such an approach also avoids, or reduces, the substantial losses occurring during filleting (~60%) and thus, enable a higher biomass yield promoting a more sustainable use of the entire fish biomass (Alfio et al.,

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2021; Cardinaals et al., 2023; Hayes, 2023). Processing of whole fish, however, introduces several challenges compared to addressing only the fillet. During filleting, sensitive and/or pigment-enriched parts such as heads, viscera, backbones, and sometimes skins are removed, simplifying further processing (Bergman, 2015). In contrast, these parts will remain in the whole fish, leading to a highly perishable and complex raw material. For instance, intestines are rich in enzymes that could induce lipolysis and proteolysis (Luten et al., 1997), and in e.g., gills, skin and blood, the enzyme lipoxygenase (LOX) is found, which can initiate lipid oxidation (Wang et al., 2012; Wu et al., 2022a). In addition, blood-rich organs as gills, backbone, and intestinal cavity contains the highly pro-oxidative hemoglobin (Hb) (Sandblom and Gräns, 2017) which can catalyze oxidation e.g. by breaking down pre-formed hydroperoxides into free radicals. As shown by for example Richards and Hultin (2002) as well as Undeland et al., (2004), Hb is a dominant factor driving lipid oxidation in fish muscle, underlining this protein's challenging presence. Alongside oxidation, dark coloration from Hb and other pigments like eumelanin, can lower consumer acceptability of whole fish-derived food products (Cal et al., 2017; Carlsen et al., 2005; Nisov et al., 2020).

A possible route to substantially reduce levels of Hb and LOX from whole herring, and thereby stabilize the biomass towards oxidation, could be deheading (Wu et al., 2022a) with partial or full gutting, which could then be followed by mince production and potential washing for further stabilization and lightening of the color. Applying consecutive water-based washing cycles to fish mince is a well-established method in surimi production to e.g., remove sarcoplasmic proteins, improve oxidative stability, color, and gelation properties of the mince (Park, 2005). However, although multiple washing cycles (commonly 3 × 3 volumes of water) can be effective in improving the sensorial quality and stability (Hamzah et al., 2015; Somjid et al., 2021; Zhang et al., 2022), each new cycle increases water usage and can lead to an unwanted loss of protein and other nutrients (Park, 2005; Somjid et al., 2021). An alternative approach would be antioxidant-assisted washing, introducing protection against lipid oxidation early in the process, reducing the need for multiple cycles (Eymard et al., 2010; Harrysson et al., 2020; Richards and Hultin, 2002; Thongkam et al., 2023). To date, research on washed mince production from pelagic fish such as herring, sardine, or mackerel has focused mainly on clean-cut fillets as starting material (Chaijan et al., 2010, 2006; Eymard et al., 2010, 2009, 2005; Undeland et al., 1998c; Kelleher et al., 1994; Kiesvaara and Granroth, 1985; Murthy et al., 2021; Pan et al., 2018; Panpipat et al., 2023b, 2023a; Somjid et al., 2021; Thongkam et al., 2023), while to the best of our knowledge, only two studies have addressed whole round fish (Chan et al., 1995; Shaviklo and Rafipour, 2013); none comprising lipid oxidation or instrumental color analyzes, and none integrating pre-trimming as a route to lower pigment and pro-oxidant levels.

This study therefore explored the potential of whole and stepwise trimmed herring as a raw material for producing unwashed and washed MSM. For the latter, a primary aim was to optimize the mince-washing processes by evaluating how the number of washing cycles and incorporation of antioxidants into the washing solution could mitigate challenges related to dark color and lipid oxidation. Specifically, the removal of Hb and LOX was to be followed alongside delivery of antioxidants, crude composition, processing yields and ice storage stability. Our working hypothesis was that both trimming and washing would lighten the color; but that these operations *per se* would not stop lipid oxidation due to very high initial pro-oxidant levels and concomitant antioxidant wash-out (Sannaveerappa et al., 2007; Wu et al., 2020), and insufficient removal of pro-oxidants (Richards and Hultin, 2002). This research provides new insights into the utilization and valorization potential of small-sized herring or other pelagic species for direct food production, offering an alternative to the industry's current unsustainable focus on feed production.

Materials and methods

Chemicals

Methanol, acetonitrile, acetic acid, chloroform, and ammonium thiocyanate were purchased from Thermo Scientific (Fair Lawn, NJ, US). Ethanol was purchased from Solveco (Rosersberg, STH, SE). Duralox MANC-213 (hereafter referred to as Duralox) was purchased from Kalsec (Kalamazoo, MI, US), and carnosic acid and carnosol standards were purchased from Selleck Chemicals (Houston, TX, US). The remaining chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, US). All chemicals were of reagent grade or higher.

Preparation of herring raw material

Herring (*Clupea harengus*) was caught off the Limfjord, Denmark, in March of 2022 and transported directly to Ellös, Sweden. The time between catch and further processing was less than 48 h. On the processing day, herrings with a weight of <50 g were processed through a filleting machine tailor-made for small fish (FPM-200, SEAC, Sweden) producing two different trimmings; deheaded (H) and deheaded plus gutted (HG). Another part of the catch was kept as whole (W) herring. The next day, about 2 kg of each trim (W, H, HG) were transported on ice to Chalmers, separately subjected to grinding (C/E22 N, Minerva, Italy) using a final hole plate of 4.5 mm, packed in Ziplock bags, and stored at -80°C until analysis. These samples were referred to as minced reference raw materials.

Preparation of mechanically separated meat (MSM)

About 5-6 kg of fresh W, H, and HG were separately subjected to meat-bone separation (BAADER 601, BAADER, Germany) during the same day as the trimming took place. The herring was pressed through a 3 mm hole diameter perforated drum, yielding mechanically separated meat (W-MSM, H-MSM, HG-MSM). Aliquots of MSM from each trim were packed in Ziplock bags and stored at -20°C overnight until transport, on ice, to Chalmers for further storage at -80°C until analysis.

MSM washing

On the processing day, fresh W-MSM and H-MSM were also subjected to washing in one or two cycles with or without 0.5% Duralox (Wu et al., 2021b). An overview of the treatments can be seen in Table S1. The MSM-to-solution ratio used was 1:3 (w/w). Ice-cold saline washing solution was added to the MSM in a beaker kept on ice, where after the slurry was manually stirred for 5 min before dewatering of the washed MSM through 3-layer cheesecloth with an external pressure of ~0.8 kg applied. For certain treatments, half of the 1st cycle washed MSM, based on washed weight, was collected and washed in a 2nd cycle with the same procedure. Samples from filtrates and washed MSM were packed in Ziploc bags, stored at -20°C overnight, and then transported on ice to Chalmers for further storage at -80°C until start of the ice storage trial and analysis.

Ice storage trial

To monitor lipid oxidation development in MSM produced from the different herring trims, with or without subjection to the different washing treatments (Table S1), an ice storage trial was conducted according to Wu et al. (2023); who also found that the used approach could predict sample differences during frozen storage. Twenty-five grams of streptomycin sulfate (200 ppm) fortified sample were flattened and stored on ice in capped Erlenmeyer flasks. About 1 g samples were daily taken out and stored at -80°C until lipid oxidation analysis. The storage trial was conducted twice, on separate occasions, for each sample type.

Proximate composition

Total protein, total lipid, moisture, and ash content were analyzed (n=3) for all raw materials, MSM, and washed MSM. Protein analysis was performed according to the method of Lowry as modified by Markwell et al. (1978), with slight modifications in sample preparations. Before analysis about 2 g of sample was mixed with 20 ml of 1 M NaOH to solubilize the proteins, vortexed for 15 s, incubated on ice for 20 min, and homogenized at 7000 RPM (ULTRA-TURRAX T18 basic, IKA, Germany) followed by another incubation on ice for 20 min. A standard curve was constructed using bovine serum albumin in the range of 10-100 μg/ml and all samples were diluted to this range using 0.1 M NaOH. Crude lipids were analyzed according to Lee et al. (1996) as modified by Undeland et al. (2002). Ash content was gravimetrically measured by loading ~1 g of sample into porcelain crucibles, drying overnight at 105°C before heating at 550°C for 6 h in a muffle furnace (LT 3/11, Nabertherm, Germany). Moisture content was gravimetrically measured by loading 5 g of sample into metal dishes and drying at 105°C for 24 h.

Mass, protein, and lipid recovery

Total recovery of mass was calculated by dividing the weight after each processing step; trimming, mechanical separation, and MSM washing, with the starting weight of the whole herring raw material (W). According to the same principle, protein and lipid recovery was calculated by dividing the amount of protein (P) or lipid (L) in samples from each step of the processing with the total amount of protein (W_p) or lipid (W_L) in the herring raw material (Eq. 1).

$$Recovery\ (\%) = 100 \times Content_{P\ or\ L} \times weight\ of\ fraction / W_{P\ or\ L} \quad (1)$$

Total heme pigment

Sample preparation of minced raw material (W/H/HG-herring), W/H/HG-MSM, and washed MSM from W/H was performed according to Wu et al. (2020). The sample was frozen with liquid nitrogen and ground using a mortar and pestle, followed by blending into a fine powder while maintaining frozen conditions. Total heme was then measured according to Hornsey (1956). In short, heme pigment in frozen sample powder was extracted by acidic acetone and incubated in darkness, where after absorbance of the supernatant was measured at 640 nm and 700 nm using a UV-vis spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, US). Total heme was calculated as A640' = A640 - A700 using a standard curve from bovine Hb and reported as Hb-equivalents.

Lipoxygenase (LOX) activity

LOX activity of W/H/HG and W/H/HG-MSM or washed MSM thereof was measured based on extraction of the samples with phosphate buffer and using linoleic acid emulsion as a substrate, according to Wu et al. (2022). The enzyme activity unit (U/g) was defined as LOX causing an increase in absorbance of 0.001/min at 234 nm, between 0 min and 3 min of reaction at 25°C.

Carnosic acid and carnosol

The content of carnosic acid and carnosol in Duralox, Duralox-containing washing solutions, MSM-raw materials and MSM washed with Duralox were measured according to Wu et al. (2022). Samples were sonicated and extracted in methanol, whereafter the supernatant was collected for ultra-performance liquid chromatography (UPLC) analysis.

Content of carnosic acid and carnosol, were quantified using a Nexera XR UPLC system (Shimadzu, Japan), mounted with an ACQUITY UPLC BEH C18, 15 cm, 1.7 μm column, and PDA UV as detector. The following steps of the procedure were performed as described by Wu et al. (2023). Analytical standards of carnosic acid and carnosol were used for peak detection and quantification via a standard curve ranging from 12.5 to 400 ppm.

pH and ionic strength

The pH of W/H/HG-MSM and W/H-MSM washed with different solutions was measured using a pH meter (PHM210, MeterLab, Korea). The electrode (Double Pore 238400, Hamilton, USA) was directly immersed in the sample, ensuring complete submersion. After stabilization, pH readings were recorded at two different places in the sample (n=2). Conductivity of these samples were measured using a conductivity meter (CDM210, MeterLab, Korea). One gram of sample was mixed with 5 ml of DI-water, homogenized at 10 000 RPM for 60 sec (ULTRA-TURRAX T18 basic, IKA, Germany), and centrifuged at 2000 xg for 10 min. Four milliliters of liquid extract were transferred to 5 ml Eppendorf tubes and the conductivity (mS/cm) measured by immersing a 4 pole cell (CDC556T, MeterLab, Korea). Ionic strength (IS) was calculated by interpolation of a standard curve based on NaCl and expressed as NaCl-equivalents.

Color

Over the 6-11 days of ice storage trial the color of W/H/HG-MSM and W/H-MSM washed with different solutions was measured using a Minolta colorimeter (CR-400, Konica Minolta, Japan) in the CIE L*a*b* setting. A five-gram sample was placed in a small petri dish and spread into a 5-6 mm thick layer. The petri dishes were then stored with closed lids, following the same procedure as for the E-flasks in the storage trial. During the storage period, color measurements were taken by placing the petri dish on an anti-reflective glass surface (CR-A50, Konica Minolta, Japan) and positioning the colorimeter probe directly against the sample (n=5).

Lipid oxidation

Following total lipid extraction the chloroform phase was collected for peroxide value (PV) analysis as described by Larsson et al. (2007). PV was determined by reacting lipid extracts with ammonium thiocyanate and iron(II) chloride in a chloroform-methanol mixture, followed by absorbance measurement at 500 nm (Cary 60 UV-Vis, Agilent Technologies, US).

TBA-reactive substances (TBARS) were measured according to the method of Schmedes and Hölmer (1989). TBARS were analyzed in the methanol-phase of lipid extracts, clarified with trichloroacetic acid, by reacting with thiobarbituric acid under heated conditions and absorbance was measured at 532 nm.

A standard curve using cumene hydroperoxide and 1,1,3,3-tetraethoxypropane was employed for PV and TBARS determination, respectively.

Statistics

The MSM-washing trial was performed in duplicate on the same occasion. All analyses were performed in at least duplicate (n≥2) and reported as means ± standard deviation. Statistical calculations were made using SPSS software (IBM SPSS Statistics V. 28, IBM, USA). Significant differences between and within sample types were determined by One-way ANOVA (α=0.05) followed by Duncan's post-hoc multiple comparisons test (α=0.05). Spearman's correlation coefficients (α=0.05 or 0.01) were used to assess the relationships between factors (such as initial PV, prooxidants, lipid content) and secondary lipid oxidation (TBARS-rate and TBARS-level day 1), utilizing data from W/H/HG-MSM and washed MSM thereof. The TBARS-rate was determined according to Wu et al. (2022), and defined as the development rate (TBARS/day) for

those storage points which had a significantly higher TBARS values than the zero-time values (α=0.05).

Results and discussion

Proximate composition of raw materials, MSM, and washed MSM

The proximate composition of herring raw materials, MSM and washed MSM is illustrated in Table 1, both on a wet weight (ww) and dry weight (dw) basis. The latter is motivated by the significant increase in moisture content after MSM production and the first washing step, the latter creating a dilution effect. Since IS was kept constant in the washes (~153 mM NaCl-equivalents), a likely reason for raised water holding was the small increase in pH (from 6.8 to 6.9-7) and the higher relative ratio of myofibrillar proteins due to wash out of sarcoplasmic proteins (Park, 2005; Stefansson and Hultin, 1994). One and two cycles of washing increased the moisture content by 7.7% and 8.5% as well as 5.8% and 6.8% for W-MSM and H-MSM, respectively. A side study revealed that the dewatering technique applied in the present process was not as efficient as centrifugation to remove all the excess water. When W-MSM washed in saline solution was subjected to 10 min centrifugation at 2000 xg, the final moisture content of the washed MSM was just below 80%, compared to 85.9% and 85.3% using filtering through cheesecloth.

From data expressed on a dw-basis, a significant up-concentration of protein throughout the processing steps was seen. This increase was especially high, for all trims, over the MSM production step where bones, skin, and subcutaneous fat are removed, leading to an up-concentration of protein-rich muscle. This aligns well with earlier findings (Abdollahi et al., 2021). It was more difficult to explain why the protein content of HG-MSM increased compared to H-MSM. Viscera and belly flap, removed in herring filleting, was earlier reported by Wu et al. (2022b) to have the highest protein content when compared to fillet, tail and backbone, which would suggest a lowered protein content in the HG-MSM compared to H-MSM. However, the composition of herring can vary greatly with season and catch location, especially regarding lipid content (Wu et al., 2022a). The weight distribution of each part also varies with the size of the herring. As reported by Wu et al. (2022b), the head ranged between 13.7-17% of the total weight for herring sized 89-182 g, and the viscera plus blood made up 2.4-17.6%. In this study, where the average whole herring weight was 46.8 ± 3.3 g, the removal of the head reduced the total weight by 24.8% and the viscera by another 7.6%pt (Table 2). Finally, there was also a significant increase in protein content over the two washing steps, leading to a protein content of 65.8 ± 2.8 g/100 g dw and 68.9 ± 0.5 g/100 g dw for MSM from whole and deheaded herring, respectively.

Table 2
Recovery of mass, protein, and lipids from the herring raw material over the different process steps yielding mechanically separated meat and washed MSM. The total recovery data is expressed based on initial content in whole herring, whilst data within parentheses displays the recovery over the previous processing step. Data are expressed as mean (n = 2).

Treatment	Mass Recovery (ww %)	Mass Recovery (dw %)	Protein Recovery (dw %)	Lipid Recovery (dw %)
Whole				
W	100	100	100	100
W-MSM	75.5 (75.5)	67.1 (67.1)	81.1 (81.1)	43.4 (43.4)
Wa x 1 W-MSM	87.3 (115.6)	54.7 (81.5)	70.6 (87.1)	36.8 (84.8)
Wa x 2 W-MSM	79.7 (91.3)	47.9 (87.6)	66.9 (94.8)	30.1 (81.8)
Deheaded				
H	75.2 (75.2*)	75.6 (75.6*)	78.2 (78.2*)	73.6 (73.6*)
H-MSM	60.6 (80.6)	52.1 (68.9)	69.0 (88.2)	34.5 (46.9)
Wa x 1 H-MSM	67.5 (111.4)	43.6 (83.7)	60.7 (88.0)	27.4 (79.4)
Wa x 2 H-MSM	64.7 (95.9)	40.6 (93.1)	59.4 (97.9)	24.4 (89.1)
Deheaded & gutted				
HG	67.6 (89.9**)	65.4 (86.5**)	74.8 (95.7**)	37.6 (51.1**)
HG-MSM	54.6 (80.9)	46.2 (70.6)	61.6 (82.4)	23.6 (62.8)

W = whole, H = deheaded, HG = deheaded and gutted, MSM = mechanically separated meat, Wa = washed, x 1/x 2 = number of wash cycles. * Whole herring trim considered as the previous processing step. ** Deheaded herring trim considered as the previous processing step.

Along with the up-concentration of protein, the lipid content showed a declining trend over all processing steps across all herring trims, however, it was only significant between the raw material and MSM. This was likely due to the removal of skin and subcutaneous fat during the mechanical separation process. During washing, the reduction in lipid content is primarily achieved through the removal of extracellular lipid droplets, by the agitation within the washing solution that dislodges these lipids (Ingemansson, 1990). Once released from the muscle tissue, they tend to aggregate on the surface, creating a floating lipid layer due to differences in density and polarity compared to the water phase (Park et al., 1997). However, since this study applied filtration to dewater the washed MSM, the floating lipid layer was not removed, apart from lipid droplets which were small enough to pass through the used cheesecloth. This likely explains why the lipid content was not reduced as significantly in our washing process compared to other studies of surimi production where lipid level has been reduced by up to 50-70%, utilizing centrifugation or a hydraulic press in the dewatering step (Eymard et al., 2010; Somjid et al., 2021; Thongkam et al., 2023). In

Table 1
Proximate composition of herring raw material, mechanically separated meat from whole fish and from different trimmings, as well as washed MSM thereof. Data are expressed as mean ± SD (n = 3).

Treatment	Protein (g/100 g) dw	Protein (g/100 g) ww	Lipid (g/100 g) dw	Lipid (g/100 g) ww	Ash (g/100 g) dw	Ash (g/100 g) ww	Moisture (g/100 g) ww
Whole							
W	47.1 ± 1.0^{a}	11.0 ± 0.2^{c}	26.6 ± 0.2^{d}	6.2 ± 0.0^{c}	12.9 ± 0.6^{e}	3.0 ± 0.1^{g}	76.6 ± 0.2^{ab}
W-MSM	56.9 ± 0.6^{c}	11.8 ± 0.1^{de}	17.2 ± 0.8^{bc}	3.5 ± 0.2^{b}	9.8 ± 0.2^{c}	2.1 ± 0.2^{e}	79.2 ± 0.1^{c}
Wa x 1 W-MSM	60.8 ± 2.2^{d}	9.0 ± 0.2^{a}	17.9 ± 1.2^{c}	2.6 ± 0.2^{a}	9.1 ± 0.5^{abc}	1.4 ± 0.1^{c}	85.3 ± 0.6^{f}
Wa x 2 W-MSM	65.8 ± 2.8^{e}	9.2 ± 0.4^{a}	16.7 ± 0.1^{bc}	2.4 ± 0.1^{a}	8.2 ± 0.1^{a}	1.1 ± 0.0^{a}	85.9 ± 0.8^{f}
Deheaded							
H	48.7 ± 0.4^{a}	11.5 ± 0.1^{d}	25.9 ± 1.9^{d}	6.1 ± 0.4^{c}	11.1 ± 1.1^{d}	2.6 ± 0.3^{f}	76.4 ± 0.3^{a}
H-MSM	62.4 ± 0.5^{d}	12.5 ± 0.1^{f}	17.6 ± 0.9^{c}	3.4 ± 0.0^{b}	8.7 ± 0.1^{ab}	1.8 ± 0.0^{d}	79.9 ± 0.2^{cd}
Wa x 1 H-MSM	65.6 ± 1.9^{e}	10.3 ± 0.2^{b}	16.7 ± 0.7^{bc}	2.6 ± 0.1^{a}	8.7 ± 0.1^{ab}	1.3 ± 0.0^{bc}	84.5 ± 0.3^{e}
Wa x 2 H-MSM	68.9 ± 0.5^{f}	10.1 ± 0.4^{b}	16.0 ± 1.2^{bc}	2.4 ± 0.2^{a}	8.5 ± 0.2^{ab}	1.2 ± 0.0^{ab}	85.3 ± 0.5^{f}
Deheaded & gutted							
HG	53.9 ± 0.8^{b}	12.2 ± 0.2^{ef}	15.3 ± 1.2^{b}	3.5 ± 0.3^{b}	11.8 ± 0.7^{d}	2.7 ± 0.2^{f}	77.3 ± 0.2^{b}
HG-MSM	62.8 ± 1.4^{d}	12.4 ± 0.3^{f}	13.6 ± 0.8^{a}	2.7 ± 0.1^{a}	9.6 ± 0.0^{bc}	1.9 ± 0.0^{d}	80.3 ± 0.3^{d}

W = whole, H = deheaded, HG = deheaded and gutted, MSM = mechanically separated meat, Wa = washed, x 1/x 2 = number of wash cycles. Different letters within the same column indicate significant differences (one-way ANOVA followed by Duncan's test, $p<0.05$).

industrial surimi manufacturing, techniques such as screw press and rotary screen are commonly used in the dewatering and refining steps (Park, 2005; Park et al., 1997).

The ash content, which serves as an indicator of mineral content, i.e. bones (Abdollahi et al., 2021), displayed a significant decrease over the MSM production for all raw materials, where the main part of bones was separated from the herring meat. A significantly higher ash content was seen in whole herring (12.9 g/100 g dw) compared to deheaded herring (11.1 g/100 g dw) and HG herring (11.8 g/100 g dw), due to the bone-dense head still remaining. Two washing cycles of W-MSM (9.8 g/100 g dw) then significantly reduced the ash content to 8.2 g/100 g dw. Minerals present in herring MSM (Abdollahi et al., 2021), such as heme-iron, potassium, magnesium, and calcium are soluble in water and will leach out into the washing solution, more so with repeated washing cycles.

Mass, protein, and lipid recovery through mechanical separation and washing

The recovery of mass (wet weight of the material) and dry matter through the processing stages displayed a clear impact of trimming, mechanical separation, and washing cycles (Table 2). Initially, the trimming of whole to deheaded and HG herring reduced the weight by 24.8% and 32.4%, respectively. The subsequent mass recovery over the mechanical separation step was 75.5% for W-MSM (ww), which is aligned with the removal of bones and skin (Abdollahi et al., 2021). In the deheaded and HG trims, the MSM-production yielded a higher mass recovery (ww) of 80.6% and 80.9%, respectively, due to the higher proportion of muscle following head removal. However, the overall mass recovery from trimming through MSM-production was lower (60.6% and 54.6%) owed to the loss of material during head removal. During washing, the mass recovery (ww) increased after one cycle but decreased slightly again after cycle number two, the former explained by the raised water holding capacity discussed earlier (Table 1). When expressed as dry matter, the recovery over the two washing cycles was 47.9% and 40.6%, respectively, explained by the loss of e.g., water-soluble proteins and lipids.

After mechanical separation, the total protein recovery (dw) was 81.1% for W-MSM whilst significantly ($p<0.05$) lower for H-MSM (69.0%) and HG-MSM (61.6%); all based on the initial whole fish. The lower protein recovery for H-MSM and HG-MSM can be explained by the previous removal of protein-containing parts, i.e. head and viscera. The subsequent two washing cycles of W-MSM and H-MSM further reduced the total protein recovery to 66.9% and 59.4%, respectively, due to the wash-out of proteins being soluble at an IS of ~150 mmol/l (Table 4).

Lipid recovery (dw) was 73.6% and 37.6%, respectively, over the beheading and beheading plus gutting process given the significant lipid contents in head and viscera (Sathivel et al., 2003; Wu et al., 2022b, 2022a). After mechanical separation, lipid recovery was 23.6-43.4% for all trimmings, with the largest relative loss observed for W-MSM (56.6%). The results display the nature of mechanical separation in removing lipid-rich parts, such as the skin with its subdermal fat layer (Undeland et al., 1998a, 1998b). Washing further reduced lipid recovery across all materials. After one and two washing cycles, the total lipid recovery dropped to 36.8% and 30.1% for W-MSM and to 27.4% and 24.4% for H-MSM; again based on the initial whole fish.

Heme pigments in raw material, MSM, and washed MSM

As in all animals, the blood vessels in herring form a one-way circular system (Sandblom and Gräns, 2017). The heart pumps deoxygenated blood into the ventral aorta, which continues into branchial arteries supplying blood to the gills. Here, blood becomes oxygenated and then flows to the dorsal aorta which runs along the backbone and distributes blood to the body, mainly supplying the muscles and viscera. With this vascular layout, the beheading and beheading plus gutting will naturally lead to a decrease in Hb-bound heme (Sandblom and Gräns, 2017), which was also reflected in Table 3. The heme content in whole herring (148.7 μM Hb) decreased 2.2- to 3.7-fold by removing the head (to 66.1 μM Hb) and head plus viscera (to 40.0 μM Hb), respectively. As the beheading was done with a cut just behind the pectoral fin, it is however important to emphasize that the cut removed not only the gills but also the heart and liver, which are both located near the head (Sandblom and Gräns, 2017). This can explain the large difference in heme-content between whole and deheaded herring, as well as the smaller difference between deheaded and HG herring. Regarding the heme proteins, Hb is predominantly located in the red blood cells where it serves as the main oxygen transport protein (Ghirmai et al., 2022), whilst myoglobin (Mb) is primarily found in muscle tissues, including the heart. The contribution of Mb to total heme-levels in fish muscle is subject for

Table 3
Total heme pigments and LOX activity of herring raw material and over the different process steps yielding mechanically separated meat and washed MSM. Data are reported as μM Hb equiv./kg sample and enzyme activity as mU/g, on dry weight and wet weight basis, expressed as mean ± SD (n = 3).

Treatment	Heme pigments (μM Hb equiv./kg, dw)	Heme pigments (μM Hb equiv./kg, ww)	LOX activity (mU/g, dw)	LOX activity (mU/g, ww)
Whole				
W	148.7 ± 4.1^{e}	34.8 ± 1.0^{d}	597.4 ± 31.1^{e}	139.2 ± 7.2^{f}
W-MSM	225.1 ± 17.6^{f}	46.8 ± 3.7^{e}	561.3 ± 39.9^{e}	116.7 ± 8.3^{e}
Wa x 1 W-MSM	110.6 ± 24.9^{d}	16.3 ± 3.7^{c}	471.0 ± 80.2^{d}	69.2 ± 11.8^{cd}
Wa x 2 W-MSM	16.4 ± 4.7^{a}	2.3 ± 1.0^{a}	334.6 ± 22.2^{bc}	47.2 ± 3.1^{b}
Wa x 2 + AO2 W-MSM	21.4 ± 11.3^{ab}	3.1 ± 1.7^{a}	n.a.	n.a.
Wa x 1 + AO W-MSM	119.4 ± 7.9^{d}	17.6 ± 0.8^{c}	394.0 ± 31.2^{c}	57.9 ± 4.6^{bc}
Wa x 2 + AO W-MSM	18.2 ± 6.8^{a}	2.6 ± 0.7^{a}	n.a.	n.a.
Deheaded				
H	66.1 ± 2.7^{c}	15.6 ± 0.6^{c}	322.6 ± 17.8^{bc}	76.14 ± 4.2^{d}
H-MSM	85.4 ± 7.0^{c}	17.2 ± 1.4^{c}	299.2 ± 33.17^{b}	60.1 ± 6.7^{c}
Wa x 1 H-MSM	28.9 ± 2.0^{ab}	4.5 ± 0.3^{a}	197.4 ± 8.5^{a}	30.6 ± 1.3^{a}
Wa x 2 H-MSM	12.1 ± 0.0^{a}	1.8 ± 0.0^{a}	160.3 ± 15.8^{a}	23.6 ± 2.3^{a}
Wa x 2 + AO2 H-MSM	12.6 ± 4.0^{a}	1.9 ± 0.6^{a}	n.a.	n.a.
Wa x 1 + AO H-MSM	28.7 ± 0.0^{ab}	4.2 ± 0.0^{a}	159.7 ± 6.0^{a}	24.8 ± 1.0^{a}
Wa x 2 + AO H-MSM	15.4 ± 0.6^{a}	2.2 ± 0.1^{a}	n.a.	n.a.
Deheaded & gutted				
HG	40.0 ± 8.5^{b}	9.1 ± 1.9^{b}	152.2 ± 13.2^{a}	34.54 ± 3.0^{a}
HG-MSM	70.5 ± 4.3^{c}	13.9 ± 0.8^{c}	142.8 ± 13.5^{a}	28.1 ± 2.7^{a}

W = whole, H = deheaded, HG = deheaded and gutted, MSM = mechanically separated meat, Wa = washed,
x 1/x 2 = number of wash cycles, AO2 = antioxidants used in the 2nd wash cycle, AO = antioxidants in all wash cycles. n.a. = not analyzed. Different letters within dw- or ww-samples indicate significant differences (one-way ANOVA followed by Duncan's test, $p<0.05$).

debate due to the difficulty in differentiating Mb from Hb-monomers; examples of numbers reported are <5% and 35% in unbled light and dark mackerel muscle, respectively (Richards and Hultin, 2002). Mb facilitates oxygen diffusion and storage in cardiac muscle, which is crucial when oxygen supply may be limited, e.g., during intense swimming activity (Macqueen et al., 2014). Literature does not support Mb as a significant heme contributor in gills and liver, instead, Hb, due to the high presence of blood in these organs, accounts for the majority of their heme content (Sandblom and Gräns, 2017). Overall, a combination of the removal of muscle tissue (containing Mb and Hb) and blood rich parts (containing mainly Hb) lead to differences in total heme content depending on the biomass preparation method.

There was a 1.3-1.8-fold significant up-concentration of heme after mechanical separation, especially for whole herring (W-MSM) reaching 225.1 μM Hb on a dw basis (46.8 μM on a ww-basis). In teleost fish the erythropoiesis, red blood cell production, primarily occurs in the head kidney, a unique organ for these species, and not in the bone marrow as mammals (Chen et al., 2017). This can explain why the removal of bones, along with the skin, during MSM-production led to an up-concentration in heme content for all trims. The subsequent washing cycles then significantly reduced the heme content compared to the respective MSM form, with data being similar with or without added Duralox. For W-MSM, washing cycle one and two significantly reduced the heme content, on dry weight basis, in a step wise manner by ~49.0% and ~83.8%, respectively. In the case of H-MSM, the two washing cycles reduced heme content by ~66.3% and then another ~53.6% respectively, however here no significant difference was seen between washing cycles. The observed reduction is consistent with the water solubility of heme proteins.

Lipoxygenase activity in raw material, MSM and washed MSM

In herring, the enzyme group LOX is one of the main pro-oxidants, besides Hb (Fu et al., 2009; Medina et al., 1999; Samson and Stodolnik, 2001; Stodolnik and Samson, 2000; Wu et al., 2022a). Of the two main isoforms, LOX-1 has been ascribed more activity on linoleic acid with a pH-optima at approximately 9, while LOX-2 prefers arachidonic acid and linoleate, with a pH-optima at around 7 (Robinson et al., 1995; Stodolnik and Samson, 2000). Further, LOX-1 requires lipid hydroperoxides to become activated, and acts only on free fatty acids (FFA) (Damodaran et al., 2007) where it aids addition of molecular oxygen to a double bond.

A recent study by Wu et al. (2022) revealed that LOX was by far most concentrated in the head, followed by intestines, which correlates well to our findings (Table 3) where LOX activity ranked the raw material as W>H>HG and the MSMs as W-MSM > H-MSM > HG-MSM. With the removal of skin and bones over the MSM production, significant differences (on a ww-basis) were seen between W versus W-MSM and H versus H-MSM. Findings from Wang et al. (2012) ranked grass carp LOX activity in order from skin, muscle, gills, viscera and blood, which aligns with our findings from trimming and skin removal. Also Medina et al. (1999) found particularly high LOX-activity in skin of both herring and sardines.

LOX activity decreased by and between wash cycles of W-MSM, however, for H-MSM a difference in activity was only seen between non-washed and washed biomasses (Table 3). One wash cycle of W-MSM/H-MSM reduced the activity by 40.7%/49.1% and another cycle by 31.8%/22.9%, respectively on ww basis. Similar effects were observed by Aubourg et al. (2006), where soaking followed by dewatering of mackerel fillets resulted in a 44% decrease in LOX activity. The addition of Duralox in the washing solutions had no effect on LOX activity.

Initial color, pH and IS of MSM, and MSM washed with different solutions

In MSM, the initial red-to-green (a*) values were highest in W-MSM, followed by H-MSM and HG-MSM, both at similar values (Table 4). This corresponds well to the 3-fold higher levels of heme pigments in W-MSM compared to H-/HG-MSM, where the head, heart, liver and intestine had been removed (Sandblom and Gräns, 2017). W-MSM and H-MSM washed once with antioxidant initially displayed higher redness values compared to their respective non-washed MSM samples. This could be due to reduction of met-Hb (Tomoda et al., 1978) formed during the sample preparation (pre-freezing, thawing, mixing and/or packing) for the storage trial, and highlights the high sensitivity of Hb-rich fish biomasses towards oxidative changes (Richards et al., 2002). Supported by heme-data (Table 3), two washing cycles (Wa x 2 samples) clearly reduced redness compared to one cycle (Wa x 1 samples), which was one of the general targets in this and previous mince washing studies (Park, 2005). Lightness (L*) values showed a clear initial difference between non-washed and washed MSM samples. The removal of heme, especially after two washing cycles (Wa x 2), led to a slightly lighter initial color. Eumelanin, a dark and water-insoluble pigment naturally found in the eyes and intestinal cavity (Cal et al., 2017; Wibowo et al., 2022), may explain why the increase was relatively small (8%) after the second wash, despite an 84% reduction in Hb content between the first and second washing cycle. In agreement with redness values, lower lightness

Table 4
Measured pH-values, IS and initial color (L*: White to Black, a*: Red to Green, b*: Yellow to Blue) of mechanically separated herring meat and washed meat ± antioxidants. Data are expressed as mean ± SD, with n = 2 for pH and IS, and n = 5 for color values.

Treatment	pH	IS (mmol/L)	L*	a*	b*
Whole					
W-MSM	6.8 ± 0.0^{e}	240.6 ± 1.1^{f}	34.2 ± 1.6^{a}	5.5 ± 0.8^{gh}	6.0 ± 0.9^{ab}
Wa x 1 W-MSM	6.9 ± 0.1^{ef}	155.6 ± 0.3^{c}	35.2 ± 2.6^{ab}	4.7 ± 0.6^{ef}	7.7 ± 0.4^{c}
Wa x 2 W-MSM	7.0 ± 0.0^{g}	151.7 ± 0.7^{b}	40.6 ± 0.5^{d}	2.2 ± 0.3^{b}	6.4 ± 0.5^{ab}
Wa x 2 + AO2 W-MSM	6.5 ± 0.0^{b}	152.7 ± 0.6^{b}	40.4 ± 3.4^{d}	4.1 ± 0.6^{de}	6.4 ± 1.5^{ab}
Wa x 1 + AO W-MSM	6.5 ± 0.1^{bc}	157.4 ± 1.3^{c}	36.7 ± 0.9^{bc}	6.5 ± 0.2^{i}	6.8 ± 0.6^{abc}
Wa x 2 + AO W-MSM	6.4 ± 0.0^{a}	149.1 ± 0.3^{ab}	37.0 ± 1.2^{bc}	4.9 ± 0.3^{fg}	6.8 ± 0.6^{abc}
Deheaded					
H-MSM	6.9 ± 0.0^{ef}	214.3 ± 1.4^{e}	37.6 ± 2.0^{c}	4.3 ± 0.5^{e}	6.5 ± 0.7^{ab}
Wa x 1 H-MSM	7.0 ± 0.0^{fg}	158.0 ± 0.6^{c}	37.2 ± 0.7^{bc}	2.3 ± 0.2^{b}	6.4 ± 0.7^{ab}
Wa x 2 H-MSM	7.0 ± 0.1^{g}	150.0 ± 0.3^{ab}	40.0 ± 0.8^{d}	0.9 ± 0.3^{a}	5.7 ± 1.0^{a}
Wa x 2 + AO2 H-MSM	6.6 ± 0.0^{cd}	156.4 ± 0.2^{c}	40.9 ± 1.2^{d}	3.6 ± 0.1^{cd}	6.6 ± 0.7^{ab}
Wa x 1 + AO H-MSM	6.7 ± 0.0^{d}	157.0 ± 1.5^{c}	38.7 ± 1.8^{cd}	5.6 ± 0.6^{h}	6.0 ± 0.7^{ab}
Wa x 2 + AO H-MSM	6.4 ± 0.0^{a}	147.8 ± 0.6^{a}	40.3 ± 1.1^{d}	3.4 ± 0.2^{c}	6.2 ± 0.6^{ab}
Deheaded & gutted					
HG-MSM	6.9 ± 0.0^{ef}	203.6 ± 1.4^{d}	36.8 ± 0.9^{bc}	3.9 ± 0.5^{cde}	7.1 ± 0.8^{bc}

W = whole, H = deheaded, HG = deheaded and gutted, MSM = mechanically separated meat, Wa = washed,
x 1/x 2 = number of wash cycles, AO2 = antioxidants used in the 2nd wash cycle, AO = antioxidants in all wash cycles. Different letters within the same column indicate significant differences (one-way ANOVA followed by Duncan's test, $p<0.05$).

values were found for W-MSM than H- and HG-MSM. For initial yellowness (b*) values, only W-MSM washed once displayed a significant difference from all sample types.

The pH of W-MSM, H-MSM, and HG-MSM showed no significant differences, and values were between pH 6.8-6.9 (Table 4). These values were within the typical pH range for postmortem fish muscle, which usually lies between 6.2 and 7.2 due to the balance between glycolytic activity and the buffering capacity of fish proteins (Huss, 1995; Kelleher et al., 2004). For postmortem herring, pH-values are usually 6.5-7, the lower range with increasing postmortem age. Thus, the pH recorded in this study reflects short time between catch and processing. Two washing cycles with saline solution significantly increased the pH to 7.0 for both W-MSM and H-MSM which can be attributed to the removal of endogenous acids or other metabolites (Zhang et al., 2022), that become increasingly exposed during mechanical separation due to de-compartmentation. With Duralox in the washing solutions, there was a significant pH reduction to 6.4-6.7 in the washed MSM from both W-MSM and H-MSM, especially after the second wash cycle when Duralox was included in both washing cycles. This drop is primarily due to the acidic nature of e.g., ascorbic acid, citric acid and phenolic acid components in the antioxidant mix.

All washed MSMs from whole and deheaded herring displayed an IS at ~153.5 mmol/L, as a result of utilizing saline conditions in all washing solutions which correlates to an IS of 154 mmol/L. These conditions were selected to reduce the solubilization of myofibrillar proteins over consecutive wash cycles (Stefansson and Hultin, 1994), and to minimize osmotic pressure. Interestingly, the non-washed MSMs, i.e., W-MSM/H-MSM/HG-MSM showed IS values at 240.6 mmol/L, 214.3 mmol/L and 203.6 mmol/L, respectively, which represents NaCl contents of 1.4%, 1.3% and 1.2%, respectively. This increase in ionic strength can be explained by findings of Tomás et al. (2024). Their study observed a herring scale loss of ~95% during trawling, which removed the herring's ability to regulate osmolarity against the high salt content in the ocean. As a result, the IS was as high as 185.5 ± 30.2 mmol/L and full hemolysis was observed in 95% of the sampled herrings (Tomás et al., 2024). Also in the present study, no scales were observed on the herring biomass.

Lipid oxidation development in MSM as a function of trimming

Lipid oxidation products

The PV and TBARS values in Fig. 1 illustrate the development of lipid hydroperoxides and carbonyls, respectively, formed over the storage of non-washed MSM from whole, deheaded, and deheaded plus gutted herring. Both H-MSM and HG-MSM displayed a quick rise in PV on day 1, reaching 1300-1400 μM peroxide/kg, without significant differences among each other (Fig. 1A). H-MSM then declined until day 3 ($p<0.05$) while HG-MSM remained within the same level of lipid peroxides. W-MSM, with an initial PV close to H-MSM, reached a significantly lower value (860 μM peroxide/kg) than H-/HG-MSM on day 1, and then continued to develop lipid hydroperoxides until day 2 ($p<0.05$) followed by a decrease on day 3 ($p<0.05$). TBARS development (Fig. 1B) was as PV very fast, and showed similar kinetics in all three MSM-sample types, although H-MSM had the most distinct peak-value after 1 day. HG-MSM started off at significantly lower TBARS values and remained lower throughout storage.

Color development

Color data recorded over time (Fig. 3) supported developments of PV and TBARS in the sense that redness (a*) was lost due to formation of met-Hb (Tomoda et al., 1978) and/or oxidative destruction of the heme-ring (Lei et al., 2022). However, W-MSM retained its redness (a*) for a longer period during ice storage compared to H-MSM and HG-MSM ($p<0.05$, day 3 to 6), suggesting that the head, heart, liver and/or intestines contain endogenous antioxidants protecting the bright red oxyhemoglobin from being oxidized into the brownish met-Hb (Fig. 3B and E). Along with the loss of redness, lightness (L*) of H-MSM and HG-MSM increased ($p<0.05$, day 6), which beyond heme-changes could be attributed to protein coagulation at the sample surface, increasing its light reflection (Singh et al., 2022). Yellowness also increased significantly ($p<0.05$), particularly in H-MSM, but also in HG-MSM, indicating formation of yellow-brownish tertiary oxidation product as lipofuscin, via Schiff base polymerization (Chelh et al., 2007; Hamre et al., 2003). The lower yellowness in W-MSM, compared to H-MSM ($p<0.05$, day 3 to 6), again points at higher amounts of endogenous antioxidants (Wu et al., 2022b).

Influence of intrinsic factors

That hydroperoxides did not accumulate as rapidly in W-MSM as in H- and HG-MSM (Fig. 1A), despite 1.9-4.2- and 2.7-3.4-fold higher LOX-activity and Hb levels on ww-basis, respectively, could be due to the high efficiency of Hb in decomposing lipid hydroperoxides into secondary oxidation products and free radicals (Carlsen et al., 2005) which align with the pattern seen in TBARS values (Fig. 1B). However, also for TBARS data, larger differences among the samples were expected given the large differences in pro-oxidant-levels.

Especially for the peak TBARS-values, we hypothesized that W-MSM would reach even higher numbers than those documented based on the very high Hb-levels. In fact, across several earlier studies on Hb-spiked washed cod mince or minced fillets from bled or perfused trout (Ghirmai et al., 2024; Harrysson et al., 2020; Undeland et al., 2002), the ratio between Hb and TBARS has provided strikingly constant peak

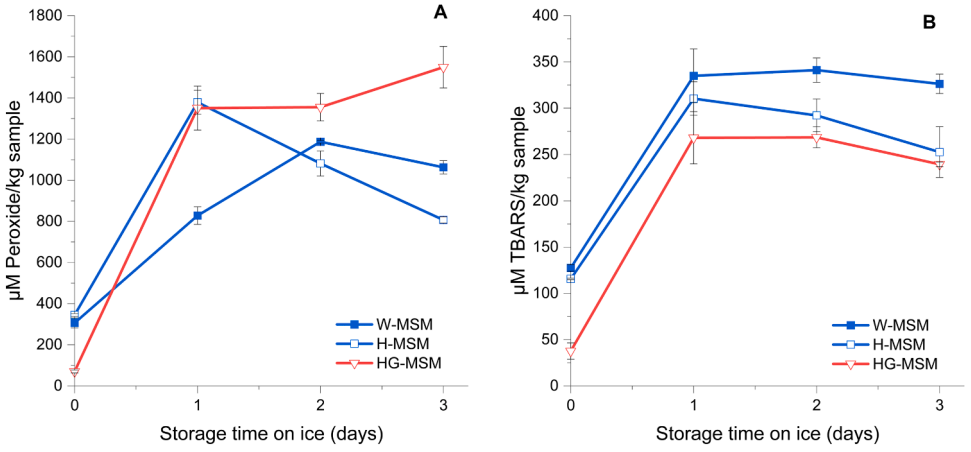

Fig. 1. PV (A) and TBARS (B) development during ice storage of mechanically separated meat from whole, deheaded and deheaded plus gutted herring. W = whole, H = deheaded, HG = deheaded and gutted, MSM = mechanically separated meat. Data are expressed as mean ± SD (n = 2).

TBARS-values (6.7-24 μM MDA equivalents/μM Hb), showing that Hb is a reactant rather than a catalyst. Here, the corresponding ratios for W-, H- and HG-MSM were 7.3, 18.1 and 19.3, thus increasing in the named order. The present samples, however, were far more complex than the clean fillets previously used and contained many different organs with unique cocktails of pro-/antioxidants. For example, endogenous antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are enriched in metabolically active tissues such as the heart and liver (Aksnes and Njaa, 1981; Hoseinifar et al., 2021; Mazeaud et al., 1979) due to their role in metabolizing oxygen and protecting against oxidative stress via detoxifying of reactive oxygen species (ROS) (Mazeaud et al., 1979). The presence of these organs in W-MSM, but not in samples derived from deheaded hearing (H/HG), could thus have counteracted the high levels of heme. Beyond cleaving hydroperoxides, heme can yield e.g., superoxide anions in reactions leading to the catalytic ferryl-/perferryl-species (Carlsen et al., 2005), revealing a role for these enzymes (Ighodaro and Akinloye, 2018).

On a general level, both PV and TBARS data agreed with our previous studies on herring backbone MSM (Wu et al., 2023, 2021a), confirming a very high susceptibility of herring tissue to lipid oxidation with a lag phase of <1 day on ice. These observations emphasize the need for protective measures to subsequently succeed in the development of high-quality food products based on herring MSM.

Lipid oxidation development in MSM as a function of washing

Lipid oxidation products

The graphs in Fig. 2 show the effect of washing treatments on lipid oxidation in W-MSM and H-MSM (data normalized to a moisture content of 80% for all samples are presented in Fig. S2). Washing in one or two cycles, stepwise reduced PV ($p<0.05$) and TBARS ($p<0.05$) values in H-MSM on day 0. In the case of W-MSM, two wash cycles were required to achieve a significant difference in 0-time values from the non-washed control. During storage, PV peaked already at day 1 in H-MSM samples, while in W-MSM, the peak appeared on day 2 for non-washed MSM, and >day 3 for single or double-washed W-MSM (Fig. 2A and C). One or two cycles gradually reduced the size of the PV-peak ($p<0.05$) for H-MSM which could not be determined for W-MSM. Also, TBARS values peaked on day 1 for all sample types except non-washed and double washed W-MSM, where the peak stretched over day 1-2 (Fig. 2B and D). The size of the peak followed the number of washes.

Color development

Regarding color changes, single-washed W-/H-MSM displayed the largest relative drop of redness (a*) over the complete storage trial ($p<0.05$), whilst all samples containing Duralox remained unchanged (Fig. 3B and E). As for non-washed samples, lightness (L*) also increased over storage ($p<0.05$, day 6), with the exception of H-MSM washed once with Duralox, which remained unchanged.

Role of intrinsic factors and washing volumes

Even though the lipid content did not change between wash cycles (Table 1), PV and TBARS values were significantly reduced ($p<0.05$), reinforcing our previous conclusion that pro-oxidants rather than lipid substrate levels are the primary drivers of lipid oxidation (Richards and Hultin, 2002). Both Hb and LOX activity decreased significantly in the washes, especially in double-washed W-MSM where 94% Hb and 40% LOX activity were removed (Table 3). Our earlier studies on cod and trout fillet minces concluded that Hb content directly influenced the extent of TBARS formation (Harrysson et al., 2020; Undeland et al., 2002). Further, in sorted herring co-products (Wu et al., 2022a), strong correlations were found between Hb levels, LOX activity and oxidation rate. In the current study, significant correlations were found between TBARS-levels and pre-formed hydroperoxides ($p<0.01$), Hb ($p<0.01$) as well as LOX activity ($p<0.05$), among which the correlation to Hb was strongest (Table S2). Aligned with this, only Hb correlated to the TBARS

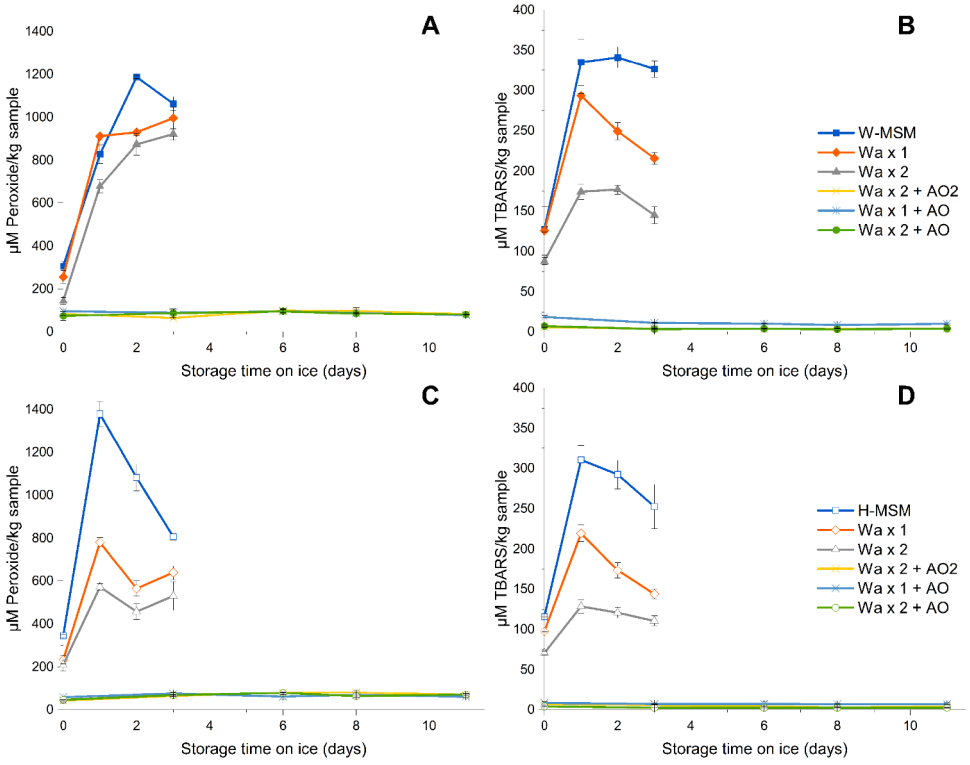

Fig. 2. PV (left panels) and TBARS (right panels) development during ice storage of mechanically separated meat and washed meat ± antioxidants thereof, from whole (A-B) and deheaded herring (C-D). W = whole, H = deheaded, MSM = mechanically separated meat,
Wa = washed, x 1/x 2 = number of wash cycles, AO2 = antioxidants added in the 2nd wash cycle, AO = antioxidants added in both wash cycles. Data are expressed as mean ± SD (n = 2).

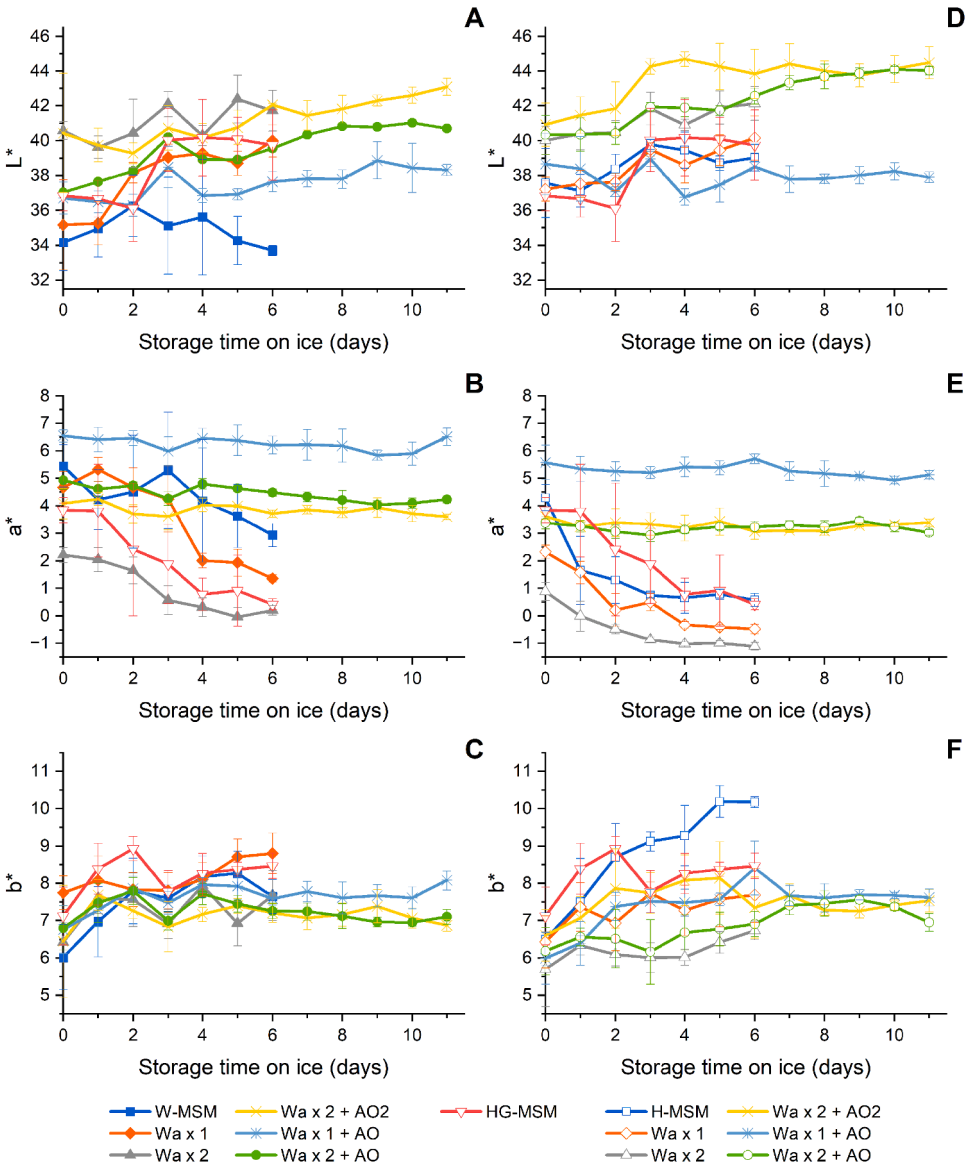

Fig. 3. Color (L*: White to Black, a*: Red to Green, b*: Yellow to Blue) development in MSM and washed MSM from whole (A-C) and deheaded (D-F) herring, and in MSM from deheaded plus gutted herring (A-F) during storage on ice. W = whole, H = deheaded, HG = deheaded and gutted, MSM = mechanically separated meat, Wa = washed, x 1/x 2 = number of wash cycles, AO2 = antioxidants added in the 2nd wash cycle, AO = antioxidants added in both wash cycles. Data are expressed as mean ± SD (n = 5).

development rate ($p<0.01$). For crude and single-washed W-MSM, peak values for TBARS only differed slightly (350 vs 300 μM) viewed against the large heme-reduction (from 46.8 to 16.3 μM Hb). The presence of Hb, even at these reduced levels after washing, aligns with findings by Richards and Hultin (2002), who observed lipid oxidation development at concentrations as low as 0.5 μM Hb in washed cod model systems. Across all our sub-studies, no significant correlation could be found between lipid oxidation and total lipids (Harrysson et al., 2020; Undeland et al., 2002; Wu et al., 2022a, 2021a).

As previously discussed, there was also a severe wash-out of LOX, especially in the first wash which reduced activities to around half. The higher moisture content of MSM samples after washing than before; ~85% vs. 79% (Table 1), could have counteracted the pro-oxidant wash-out to some extent. Raised moisture content has previously been found to promote lipid oxidation due to increased mobility of prooxidants (Barden and Decker, 2016).

It has previously been found that the volume of washing solution plays a critical role for oxidation in washed fish mince. Harrysson et al. (2020) observed a pro-oxidative effect when washing with 1 volume of solution, likely due to an imbalance between anti- and pro-oxidants, where relatively more endogenous antioxidants, like ascorbic acid, uric acid, SOD, CAT, and GPx, than pro-oxidants were washed-out. In this study, 3 volumes of washing solution were used for H-MSM ($p<0.05$) which pushed the anti- to pro-oxidant balance in favor of the former. A higher relative reduction of heme (63.8-94.3%, Table 3) than LOX (45.7-59.6%, Table 3), was found in the washes, with the resulting LOX activity in washed W-MSM/H-MSM being sufficient to contribute to the relatively high PV over day 1-3.

Antioxidant strategies to improve oxidative stability

When washing with 0.5% Duralox, which acts as a radical scavenger, metal chelator, and reductant, the oxidative potential of both LOX and Hb was effectively neutralized, preventing both the formation of lipid hydroperoxides and their subsequent breakdown into secondary oxidation products. A key component of Duralox is rosemary extract, which contains two particularly potent antioxidants, carnosol and

carnosic acid (Nieto et al., 2018; Wu et al., 2023). Carnosic acid has been reported to scavenge free radicals and donate hydrogen atoms from the phenolic ring structure, which can stabilize ROS, while carnosol has been reported to react directly with lipid radicals and to a lesser extent act as a ROS quencher (Loussouarn et al., 2017).

Although single-Duralox-washed W/H-MSM samples displayed significantly higher TBARS values than double-washed ones > 3 days, the overall very low PV and TBARS values in both sample types over up to 11 days on ice indicate that one washing cycle, when combined with antioxidants, is enough to control lipid oxidation in herring W-MSM and H-MSM. This was also confirmed by a screening for rancid odour (Fig. S1). The protective effect was maintained even under conditions that would normally promote lipid oxidation, such as lower pH (Table 4) and higher moisture content (Table 1) (Barden and Decker, 2016; Karel, 1980).

Previous studies adding Duralox directly to herring MSM or into a pre-dipping solution for the raw material (backbone) have similarly shown a very high efficacy in inhibiting lipid oxidation (Wu et al., 2021a, 2021b, 2020). Here a third way of adding Duralox was shown, which also can reduce pigmentation of treated minces. In this study, HG-MSM was not subjected to washing, as it was derived from a very clean trim with just muscle, bone and skin, i.e., no head or viscera. It also had an appealing red color, untainted by black pigments, e.g. eumelanin. However, the fact that it was very sensitive to oxidation (lag phase <1 day) motivates introducing antioxidants also to this material; which could be done via single washing (1:1-1:3), or direct mixing into the MSM (Wu et al., 2023, 2021a, 2021b). In a preliminary trial of this study, dipping and meat separation of W/H/HG herring, performed according to Wu et al. (2021), were evaluated. However, the approach provided unsatisfactory protection against lipid oxidation. It was hypothesized that the low effectiveness was due to the presence of skin on all trims during dipping, which may have hindered the penetration of the Duralox-fortified solution into the muscle tissue and that the antioxidant solution may have been removed along with the skin during mechanical separation.

The inclusion of Duralox in the washing solutions also stabilized redness over the storage trial, with an average a*-value loss of only 6% over the first 6 days compared to 64% for non-washed samples. Duralox also maintained lower b* values (Fig. 3C and F), and even though it by itself has a yellow/orange color, it did not raise the initial b* values (Table 4).

Carnosic acid and carnosol incorporation during washing with Duralox

Crude Duralox contained 6140.72 mg/kg and 1870.38 mg/kg of carnosic acid and carnosol (CA+C), respectively, which aligns with values of their sum (0.8%-0.9%) as stated by the producer Kalsec (Wu et al., 2023). These compounds were not detected (ND) in the herring raw material, produced MSM or washed MSM, indicating no cross-contamination between batches during processing. The prepared washing solution containing 0.5% Duralox had a CA+C content of 57.42 mg/kg (Fig. 4). The incorporation of CA+C reached its highest level in MSM washed twice with Duralox (Wa x 2 + AO, $p<0.05$), followed by MSM washed first with saline, and then with Duralox (Wa x 2 + AO2, $p<0.05$) and finally MSM washed once with Duralox (Wa x 1 + AO, $p<0.05$). A significant difference depending on the origin of the MSM (from whole or deheaded herring) was only seen for carnosol in Wa x 1 + AO, where it was higher for W-MSM, and for carnosic acid and CA+C in Wa x 2 + AO, where they were higher for H-MSM.

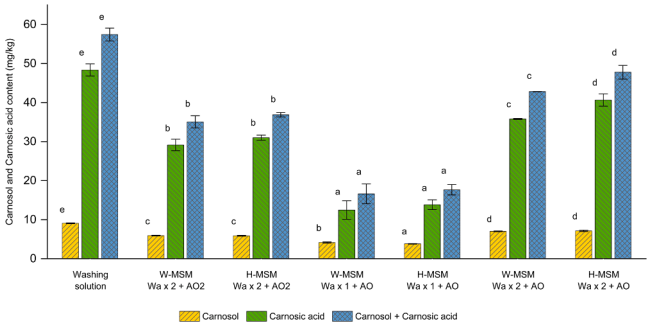

Fig. 4. Carnosic acid, carnosol, and their combined content in washing solution containing 0.5% Duralox and whole or deheaded mechanically separated meat washed with antioxidants. W = whole, H = deheaded, MSM = mechanically separated meat, Wa = washed, x 1/x 2 = number of wash cycles, AO2 = antioxidants added in the 2nd wash cycle, AO = antioxidants added in both wash cycles. Data are expressed as mean ± SD (n = 2) and different letters within each analyzed compound indicate significant differences.

It was interesting that Wa x 2 + AO2 displayed an almost two-fold increase in CA+C content compared to Wa x 1 + AO, even though both treatments only had one Duralox-wash. This could be explained by a swelling of the muscle structure after the first wash cycle, and by the fact that Wa x 2 + AO2 experienced a twofold increase in total stirring time compared to Wa x 1 + AO. This process removed both some lipids and saline-soluble proteins (Table 1) that destabilized the protein network (Hamzah et al., 2015) and likely allowed for improved antioxidant penetration.

In our previous study, where backbones were pre-dipped in 2% Duralox prior to MSM-production, the final CA+C content of the fortified MSM reached 31.7 mg/kg (Wu et al., 2023). This level fell in between MSM washed once with Duralox (16.61-17.65 mg/kg) and MSM exposed to Duralox in the second wash (35.06-36.89 mg/kg) or in both washes (42.82-47.48 mg/kg), displaying the impact of antioxidant delivery method. Even at the lower CA+C content in Wa x 1 + AO W/H-MSM (~17 mg/kg), a lag phase >11 days was however achieved (Fig. 2), implying that more diluted Duralox-solutions could be investigated. When directly mixed into minced herring by-products (head, backbone, fins, intestine, skin and residuals) 0.25% Duralox (w/w) (i.e., 21.3 mg Ca+C/kg) gave 4 days of lag phase, while adding 0.5% (42.5 mg/kg CA+C) gave >11 days (Wu et al., 2020). An important result from the current study was that the CA+C levels achieved in all our washed MSM products were well below the regulatory limit set by EFSA for CA+C at 150 mg/kg (Younes et al., 2018).

Trade-offs by trimming and by washing MSM

Washing herring MSM offers significant benefits, as shown in this study, but it also involves trade-offs that must be considered. Washing removed pro-oxidants like Hb and LOX, lightened the color, and when including Duralox, it very efficiently enhanced oxidative stability. However, these advantages come at the expense of e.g., water usage and nutrient loss (Monsen, 1988; Park, 2005). Heme iron is a crucial nutrient due to its high bioavailability, contributing to oxygen transport and various metabolic functions (Carlsen et al., 2005). Fish Hb, like other animal-sourced Hb, is more readily absorbed by the human body compared to non-heme iron from plant sources (Monsen, 1988).

One washing cycle retained approximately 77% of the original heme content, and, when including Duralox in the washing solution, extended the oxidation lag phase from <1 to >11 days on ice. Two washing cycles with Duralox indeed gave 8% higher lightness along with an up-concentration of proteins (dw-basis), but it did not provide a measurable increase in oxidative stability. Further, double washing retained only 13% of the original heme, increased the total losses of proteins and lipids throughout the process (1.13- and 1.11-fold, respectively, compared to one wash), and doubled water usage and processing time. Aqueous and lipophilic nutrients beyond current measurements are also expected to be severely diluted (Zhang et al., 2022). Thus, increasing the number of washing cycles calls for careful balancing of visual appearance, stability, nutritional properties and resource use in industrial implementations, the latter jeopardizing both environmental and economic sustainability of the process (Yin and Park, 2023).

HG-MSM was not subjected to washing as it only comprised pure muscle and thus, had a less complex composition and a lighter color. When trimming off heads, guts, and subsequently skin and bones in MSM-production, 53.8% of the dry matter, including 38.4% of the proteins and 76.4% lipids were lost (Table 2). Added to this comes the time of performing automatic beheading and gutting of very small fish. If omitting this step and directly separate meat from whole herring, double washing of the produced MSM created a loss of nearly the same amount of dry matter; 52.1%, including 33.1% protein and 69.9% lipids. Here washing time, water footprint (6.5:1, water:MSM) and micronutrient losses must however be added (Park, 2005). With a single wash of W-MSM, documented losses were smaller than in both options above, and still the required antioxidant incorporation was possible while also lightening the color. This finding is thus a key result from the present study, which presents an opportunity to improve product appearance while significantly reducing water consumption compared to conventional multi-step washing. Together with the use of minimally processed raw materials, i.e., whole or deheaded fish converted to MSM, the combined sequence reduces side streams, and minimizes the environmental footprint associated with processing. This tailored approach encourages a more sustainable and resource efficient use of underutilized fish resources, offering a route to develop nutrient dense and high-quality products from species like herring, sardine (*Sardina pilchardus*) and anchoveta (*Engraulis ringens*) where bulk catches are currently dedicated to feed rather than to direct human consumption (FAO, 2024).

Conclusion

This study demonstrated that stepwise trimming, mechanical separation, and antioxidant-assisted washing can produce high-quality mechanically separated meat (MSM) from herring raw materials which are more complex in their composition than fillets. Mechanical separation of whole, deheaded, and deheaded plus gutted herring up-concentrated protein while reducing lipid and ash content. Pre-trimming *per se* lowered pro-oxidants of MSM, but not enough to slow down lipid oxidation. Washing MSM from whole and deheaded herring further reduced pro-oxidants, increased protein concentration on a dry-weight basis, and lightened color. However, residual Hb (1.8–16.3 μM/kg) and LOX activity (23.6–69.2 mU/g) after washing were still sufficient to initiate lipid oxidation within one day, although maximum oxidation levels were reduced. Adding 0.5% of a rosemary-based antioxidant blend (citric acid, ascorbic acid, α-tocopherol, and rosemary extract) during washing was a successful strategy to both lighten color and delay the start of lipid oxidation from <1 day to >11 days, with carnosic acid and carnosol levels remaining below EFSA safety limits. One antioxidant-assisted wash was as effective as two in delaying oxidation, suggesting that additional cycles mainly benefit color rather than stability. Statistical correlation analysis reaffirmed Hb, LOX, and pre-formed hydroperoxides as key drivers of lipid oxidation in herring MSM, making them primary targets for mitigation strategies.

Processing whole small-sized herring directly into MSM removes the need for pre-trimming, avoiding equipment-intensive steps and maximizing yields of mass, protein, omega-3-rich lipids, and highly bioavailable heme iron. Even with a single antioxidant-assisted wash, up to 77% of heme iron was retained, preserving its nutritional value in line with goals for a sustainable dietary protein shift. The choice between one or two washing cycles should therefore be based on the relative importance of color improvement versus water footprint, biomass yield, and processing efficiency. These results provide flexible strategies for adapting herring MSM quality to different product specifications while promoting a more direct food use of small pelagic fish.

Altogether, this study is the first to demonstrate that a minimal processing approach combining selected trimming with variable antioxidant-assisted washes can produce oxidatively stable, lighter-colored mince from whole small pelagic fish, using less water and retaining more nutrients than conventional multi-cycle washing. Sensory quality and microbial safety remain important gaps to address for assessing consumer acceptability and shelf life, and future studies should explore these aspects alongside pilot/industrial-scale dewatering systems such as screw presses and rotary screens. By combining such aspects with the current findings, this study offers the seafood industry examples of resource efficient processing sequences that can direct herring and other underutilized fish species to direct human consumption, supporting low-trophic food system strategies.

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Ethical statement

Ethical approval was not sought for the present study because the research presented did not involve any animal or human study.

CRediT authorship contribution statement

John Axelsson: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Martin Kuhlin:** Writing – review & editing, Resources. **Ingrid Undeland:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2025.100808.

Data availability

Data will be made available on request.

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