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Effect of artificial light on the lipid oxidation of whole, cooked Northern shrimp (*Pandalus borealis*) during frozen storage

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ABSTRACT

In retail, whole, cooked Northern shrimp (*Pandalus borealis*) is commonly stored in illuminated display freezers for an extended time period. The aim of the present study was to understand how and when light-induced lipid oxidation occurs. Two sizes of cooked shrimp were packed in air and stored frozen with and without artificial light for 84 days. Samples were investigated for oxidative stability (peroxide value (PV), volatile compounds, and tocopherol consumption) and color changes and free astaxanthin.

PV only increased significantly for shrimp stored with light (up to 20 meq/kg). α-tocopherol content remained unchanged during the storage period irrespective of the storage condition. Shrimp stored with light showed an increased L*-value and decreased a* and b*-values, whereas these color values did not change significantly in shrimp stored in darkness. The color changes could not be explained by changes in the free astaxanthin content as no significant changes occurred in free astaxanthin content during storage. The formation of volatile compounds depended on the shrimp size and light exposure, but the concentration of e.g. 2-methylbutanal, 3-methylbutanal and 1-penten-3-ol increased significantly more in shrimp stored in light than when they were stored without light. In conclusion, illuminated storage increased lipid oxidation in Northern shrimp.

1. Introduction

The Northern shrimp (*Pandalus borealis*) is important to the fishing industry of the North Atlantic and Artic Oceans. In 2020, 250,000 metric tonnes were caught worldwide, with Canada and Greenland as the major countries responsible for the catch (FAO, 2021). Products of this species range from whole, raw, or cooked products to cooked, peeled, and often brined products and are either sold fresh, refreshed (thawed and chilled), or frozen. Whole, cooked shrimp products are commonly sold in the Scandinavian countries as a chilled or frozen luxury commodity. Whole, cooked, and frozen shrimp are typically processed aboard fishing vessels. The typical processing starts with size sorting of the live shrimp and cooking in seawater for approximately three minutes. A subsequent cooling in fresh, cold seawater cools the products before they pass through a tunnel freezer, which individually quick freezes the products within 10 minutes (at -30 to -40 °C). After freezing, the shrimp are packed and stored frozen until arrival ashore, where they are distributed to either retail or further processed in land-based processing facilities. The frozen version of the whole, cooked shrimp is either sold in "self--serve"-freezer displays or packed in light permeable, low-density bags (Company communication, Royal Greenland Seafood A/S). The latter product has a market shelf-life of 18 months at -18 °C (Polar Seafood A/S, Product number 5032, 2022) but is often stored for several months at -25 °C before being packed for retail . During retail storage, the product is exposed to light, oxygen, and fluctuations in storage temperature, which causes undesirable changes to the products in the form of color fading, lipid oxidation (Bak et al., 1998), and textural changes (Company communication, Royal Greenland Seafood A/S).

The color changes of frozen stored shrimp have previously been shown to be caused by a reduction in the content of the pigment, astaxanthin, likely caused by the oxidation of the compound leading to degradation of astaxanthin (Bak et al., 1998; Qiao et al. 2019). The color change of frozen Northern shrimp has been reported based on a sensory assessment by Bak et al. (1998), but color changes quantified by an

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analytical methods are yet to be described.

The oxidative changes are likely caused by the oxidation of the lipids located just beneath the shell, exposed to light and oxygen. This oxidation of the lipids is expected to be the cause of the unwanted off-odors, and color changes of the meat, occurring during frozen storage. Though the quantification of lipid oxidation products (thiobarbituric acid reacting substances (TBARS)) formed in frozen stored Northern shrimp have been determined in a previous study (Bak et al., 1998), the determination and quantification of specific secondary volatile oxidation products formed during frozen storage of Northern shrimp have not yet been reported in the literature.

Oxidative deterioration not only affect lipids, but also proteins. Previous studies have reported that protein oxidation and protein denaturation of raw products of other shrimp species have led to textural changes and reduced water binding capacity. (Jin et al., 2018; Shui et al., 2021). This is most likely also the case for Northern shrimp.

The beforementioned undesirable changes in color, odor and texture of whole cooked Northern Shrimp often lead to discard of the product before the shelf life has expired. This has a negative economic impact because of loss of income from the discarded product Furthermore, discard of this particular product adds to the large amount of waste and food loss produced by the seafood industry and the rest of the value chain, where more than 30 % of the marine catch is lost (FAO, 2011). To reduce the quantity of discarded products, understanding the changes occurring to the products during frozen storage is necessary. This knowledge can in the future be used to design solutions to avoid these changes, e.g., by using alternative packaging methods or natural antioxidants.

Performing storage trials on frozen foodstuff is a long process, as freezing lowers the rate of lipid oxidation and other degradative reactions, often giving the products a practical shelf-life of up to more than a year (Hultin, 1994; James et al., 2015). Therefore, the use of stressed storage conditions, such as exposure of the products to light, has previously been used for investigations of oxidative changes in food products, as the rate of photooxidation is several times faster than autooxidation (Calligaris et al., 2016), but no storage experiments conducted in light with Northern shrimp in which primary oxidation products and secondary volatile oxidation products were measured have been reported in the literature. In the case of the whole, cooked, frozen shrimp, storage with exposure to light also mimics realistic storage conditions of the products in the retail chain.

Therefore, this study aims at providing new knowledge about the color and lipid oxidation changes occurring to two sizes of the whole, cooked Northern shrimp (*P. borealis*) during accelerated frozen storage with and without exposure to light. The color changes were measured by the content of free-astaxanthin and by CIEL*a*b* color measurements. The oxidative changes were determined by measuring primary (peroxide value, PV), secondary lipid oxidation (volatile compounds by dynamic headspace GC-MS) products, and the content of α-tocopherol. Shrimp stored without light were used as controls to determine the effect of light. This study is the first to report on volatile oxidation compounds in cooked whole Northern shrimp.

2. Materials and methods

2.1. Raw material

Northern shrimp (*Pandalus borealis*) were caught off the west coast of Greenland in January or April 2020 by Royal Greenland A/S from fishing grounds FAO 21 (Northwestern Atlantic Ocean). On board the trawler, the shrimp were boiled for approx. 3 min, chilled in seawater and individually quick frozen (IQF). IQF shrimp were packed in PE bags within cardboard boxes and stored frozen (-20 °C) during transport to and preliminary storage at the Technical University of Denmark (DTU). Two sizes of shrimp were used for storage trials: size extra-large (x-large, 60-80 pcs/kg, caught in January 2020) and medium (90-130 pcs/kg, caught in April 2020).

2.2. Packaging and storage

Shrimp were repacked in resealable, air-permeable LDPE bags (BAJ-360-140M, Brynson Packaging Ltd., UK) with 130-150 grams in each, depending on shrimp size. Samples were stored with exposure to artificial light (750 lux, 3500 K, TL-D 36W/840, Philips, Poland) or without light at -20.6±1.2 °C for 84 days. Samples were rotated each week to ensure even exposure to the light source, and every two weeks, a subset of samples in duplicate were withdrawn for analysis.

2.3. Chemical analysis

2.3.1. Sampling and sample preparation for analysis

Based on preliminary studies, whole shrimp (with head, shell, and roe) were used for all analyses. At the point of the bi-weekly sampling, liquid nitrogen was added to the frozen samples and the samples were finely chopped (Waring commercial blender 38BL41, CT, United States) prior to being stored at -80 °C until further analysis.

2.3.2. Lipid extraction and determination of oil content

The lipid content of samples was measured gravimetrically according to Bligh and Dyer (B&D) method with a reduced amount of solvent (Bligh & Dyer, 1959). All analyses were carried out in analytical replicates (n = 2).

2.3.3. Determination of fatty acid composition

Small amounts of the lipid extract obtained from B&D extraction were used to determine the fatty acid composition. The lipid extract was evaporated to dryness under nitrogen and re-dissolved in 200 µL heptane with 0.01% butylhydroxytoluene (BHT) and 100 µL toluene and 100 µL of an internal standard (2% w/v C23:0 dissolved in heptane). Boron trifluoride reagent (20 %) was added for lipid transesterification in a one-step procedure using a microwave (Multiwave 3000 SOLV, Rotor: 64MG5, Anton Paar, Graz, Austria). Samples were heated for 5 minutes at 500 W and cooled down for 10 minutes. Then samples were mixed with 1 mL saturated NaCl solution and 0.7 mL heptane with BHT (0.01%). The top layer was used for fatty acid composition analysis by gas chromatography with flame ionization detection (GC-FID; Column: DB-wax column (10 m × ID 0.1 mm × 0.1 µm film thickness, J&W Scientific, Folsom, CA, United States)) according to AOCS Official Method Ce 1b-89. A standard mix of fatty acid methyl esters (Nu Check Prep 68D, United States) was used for fatty acid identification and quantified was performed based on the internal standard. Results were reported as percentages of total fatty acids. All analyses were carried out on duplicate samples (with two analytical replicates; n = 2).

2.3.4. Determination of Peroxide Value (PV)

PV was determined on the lipid extracts using the colorimetric ferric-thiocyanate method and measured spectrophotometrically at 500 nm, as described by Shantha and Decker (1994), with few modifications as the solvent volume increased by 2%, the calibration curve was prepared with ferric chloride and PV was quantified as meq. O_2/kg oil. The measurement was performed in duplicate (n = 2).

2.3.5. Determination of volatile compounds by dynamic headspace GC–MS

Approximately 10 g of the sample was weighed and mixed with 25 mL water and 30 mg of internal standard solution (30 µg/g of 4-methyl-1-pentanol in rapeseed oil). The volatile compounds were collected on Tenax®tubes (Gerstel, GmbH & Co. KG, Germany) for 30 min at 37 °C with a nitrogen flow of 340 mL/min. After collection, the Tenax® tubes were flushed with a nitrogen flow of 50 mL/min for 20 min. The collected volatile compounds were desorbed using an automatic thermal desorber (ATD-400, PerkinElmer, Norwalk, CT, United States) at 230 °C combined with Agilent 5890 IIA model (Palo Alto, CA, United States) GC

connected to an MS HP 5972 mass selective detector. For the samples of the x-large sized shrimp, the initial oven temperature was 55 °C for 1.5 min, followed by increasing the temperature with increments at 2.0 °C/min to 90 °C, then increment at 8.0 °C/min to 230 °C, where it was held for 8 min. For the samples of the medium sized shrimp, the initial oven temperature was 60 °C for 1 min, with increments at 15.0 °C/min to 225 °C, then increment at 40 °C/min to 280 °C, where it was held for 7 min. Samples were analyzed in triplicate (n = 3). The individual compounds were confirmed by mass-spectrometry (HP 5973 inert mass-selective detector, Agilent Technologies, United States; Electron ionization mode, 70 eV, mass-to-charge ratio scan between 30 and 250). External standards (2-butanone, 2,3-pentadione, 1-penten-3-ol, 3-methyl-1-butanol, 1-pentanol, 2-penten-1-ol, 3-methyl-butanal, 2-methyl-butanal, hexanal, heptanal, 2,4-heptadienal, octanal, nonanal and decanal) were used for calibration curves (Sigma Aldrich). The standards were dissolved in ethanol and added to Tenax® tubes in concentrations ranging from 5 to 1500 ng/g and analyzed by the same method described for the two sizes of shrimp. The results are given in ng/g of shrimp.

2.3.6. Determination of tocopherol and free astaxanthin content

The tocopherol content was determined according to AOCS Method Ce 8-89 (AOCS Official method Ce 8-89, 1998). Approximately 1 g of lipid extract was evaporated to dryness under nitrogen and re-dissolved in heptane (1 mL). The extract was analyzed by HPLC–FLD (Agilent 1100 series, Agilent Technologies, CA, United States) as described by F. S. H. Lu, Bruheim, and Jacobsen (2015).

The free astaxanthin content was determined on the redissolved lipid extract by normal phase HPLC-VIS (Agilent 1100 series, Agilent Technologies, CA, United States) on a Chromsep Guard Silica (S2) column (10 mm × 2 mm). The mobile phase consisted of heptane: acetone (86:14) and was introduced at a flow rate of 1.2 mL/min. Astaxanthin was detected with a VIS-detector at 470 nm. Identification and quantification were performed using free astaxanthin standards (Roche, Basel, CH, Austria) and their calibration curves.

2.4. Hyperspectral imaging (HSI), CIEL*a*b*, and Euclidean color differences

Hyperspectral imaging (HSI) was performed with a VideometerLab 2 (VideometerLab 2, version 2.13.54, Videometer A/S, Denmark) installed with 18 channels from 375 nm to 1050 nm. Before the experiment, a series of HSI of shrimp from different production dates and storage conditions were taken to optimize the exposure time of each channel and saved as the systematic light setting for the color analysis. The system was calibrated radiometrically using a diffuse white, dark, and geometrical disc before each sampling point. Shrimp were placed in a plastic petri dish, and the HSI picture was taken using the software provided by Videometer. The HSIs were transformed into an image cube with the coordinates corresponding to the CIEL*a*b* color space, using the daylight50 correction in the Videometer software (VideometerLab 2, version 2.13.54, Videometer A/S, Denmark). To obtain the average L* (lightness), a* (+a, redness; -a, greenness) and b* (+b, yellowness; -b, blueness) coordinates for each shrimp, an in-house MATLAB script was applied (R2020b, The Mathworks Inc., MA, United States). Six shrimp were analyzed per sample (n = 6). Euclidean color differences (ΔE) between day 0 and 84 were calculated using the equation presented in ISO/CIE 11664-4:2019 (Eq. 1).

$$\Delta E = \left[\left(L_{84} - L_0^*\right)^2 + \left(a_{84} - a_0^*\right)^2 + \left(b_{84} - b_0^*\right)^2 \right]^{1/2} \quad (1)$$

2.4.1. Statistics and data treatment

Mean and standard deviations (SD) were calculated for all results, and the results were analyzed by two-way analysis of variance (GraphPad Prism Version 9.4.1, GraphPad Software, Inc., CA. United States) using storage condition (± light) and time as the main factors. The Bonferroni multiple comparison post-test was used to test the difference between treatments. The results are considered to be significant when $p \leq 0.05$. To investigate tendencies in the data, a principal component analysis (PCA) was performed using GraphPad Prism (Version 9.4.1, GraphPad Software, Inc., CA. United States) with data of oil content, PV, α-tocopherol, free astaxanthin, 14 volatile compounds, and changes in CIEL*a*b* (Δ values).

3. Results and discussion

3.1. Principal component analysis

The PCA plot shows two notable patterns in the loadings and scores; principal component (PC) 1 (explaining 44.18% of the variation in data) mainly explained the variation in data caused by storage time. PC2 (18.76%) primarily explained variation caused by the size and some variance in exposure to light for the x-large shrimp (Fig. 1). Following the PC1 of the loadings plot, shrimp stored with light were more present in the 1st and 4th quadrant, whereas shrimp without light exposure were more present in the 2nd and 3rd quadrant. Here the volatile compounds, such as hexanal and 1-penten-3-ol, and the color change measured by ΔL* correlate with samples exposed to light. This indicates that samples exposed to light are lighter in color than those stored without light and have higher levels of most volatile compounds. Following the PC2 of the loadings, smaller shrimp were only present in the 1st and 2nd quadrants, whereas larger shrimp were only present in the 3rd and 4th quadrants. Here the change in color of Δa* and Δb* were correlated with samples of smaller shrimp stored without light, whereas volatile compounds such as 2-methyl-butanal and 1-penten-3-ol were correlated with smaller shrimp exposed to light. X-large shrimp were correlated with a higher content of free astaxanthin, and samples exposed to light were correlated, amongst others, with the volatile compounds 3-methyl-butanal and 2-penten-1-ol, as well as increased peroxide values. Based on the loading plot, the contents of tocopherol and oil explain very little of the variance in the data. The following sections investigate and attempt to explain the variances in the data behind the PCA.

3.2. Chemical composition

Oil content was stable throughout the storage period with 3.2±0.3 % and 3.3±0.2 % for the medium and x-large shrimp, respectively (data not shown). Major fatty acids found were 16:0, 18:1 (n-9), 20:5 (n-3), and 22:6 (n-3) for both sizes of shrimp, the latter contributing to more than 9% of the total fatty acid content (see supplementary materials, Table S1). The composition of fatty acids is comparable to the compositions reported by Soultani et al. (2019). A higher content of saturated fatty acids was seen in the x-large shrimp (approximately 20%) compared to the medium shrimp (approximately 14 %). No noteworthy change was seen for the fatty acid composition during storage (data not shown), indicating that the lipid oxidation at levels observed in this study is not considered to be important for the nutritional composition of the shrimp.

3.3. CIEL*a*b* and Euclidean color difference

Initial CIEL*a*b* values (day 0) showed that the x-large shrimp had a higher L*-value, i.e. were lighter, and a lower a*-value, i.e., less red, than the medium-sized shrimp. This is likely caused by the larger shrimp being caught three months earlier than medium-sized shrimp, as both season and catch area could affect the color of the initial product (personal communication, Royal Greenland). The duration of frozen storage can also influence the color (Bak et al., 1998). A significant ($p<0.05$) decrease was seen over time for L*-values of both sizes of shrimp after 70 days of illuminated storage, with a significant difference ($p<0.05$) observed between storage conditions (with or without light) after 84

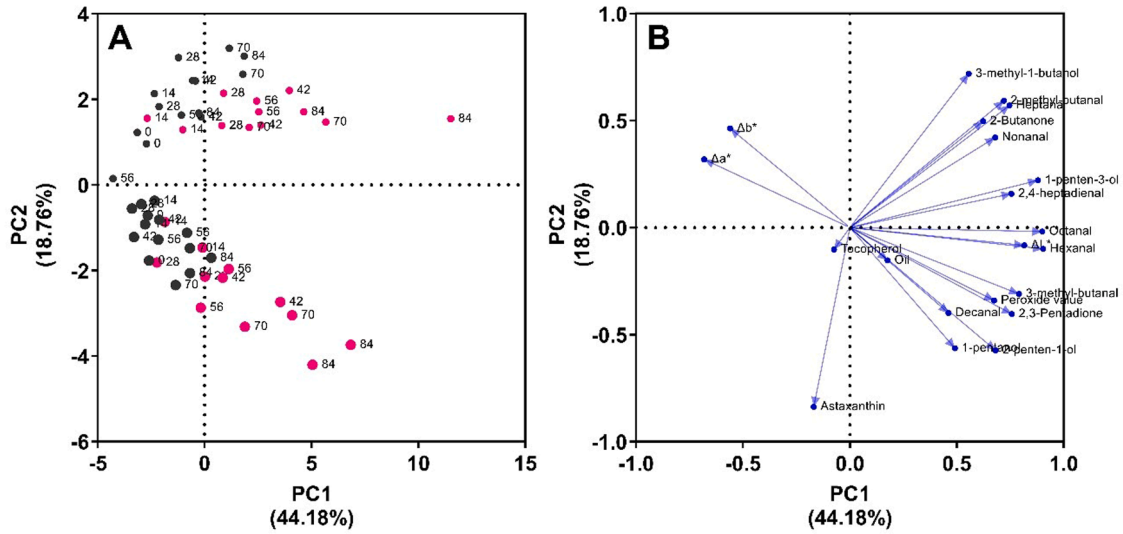

Fig. 1. PC scores (A) and loadings (B) for PCA of all data (except fatty acids) from both shrimp sizes and storage conditions. Principal components 1 and 2 explained 44.18 % and 18.76 %, respectively, of the total variation in data. Numbers indicate storage time (days), pink indicates storage with light, whereas black indicates storage without light. Small dots indicate size medium, and large dots indicate size x-large shrimp.

days. Similar differences were seen for a*-values, though only a significant difference ($p<0.05$) between storage conditions after 84 days was seen for x-large shrimp. No statistically significant ($p>0.05$) effect of time or storage conditions were noted for b*-values for either size of shrimp, though a tendency for a decrease in b*-values (yellowness) was observed. Based on the ΔE-value between day 0 and day 84 (Table 1), the exposure to artificial light caused a larger change in color compared to storage without artificial light. The difference between light and no light was slightly greater for medium-sized shrimp (ΔE =6.6) than for x-large shrimp (ΔE =6.1). The ΔE-value for storage without light was somewhat greater for x-large shrimp than for medium shrimp, which could indicate that larger shrimp change more in color than smaller shrimp during storage without light.

Increases in ΔE-values during non-illuminated storage have previously been reported for two species of cooked, frozen Mediterranean shrimp (*Parapenaeus longirostris* and *Parapandalus narval*) with ΔE-values after four months (-18 °C) of 1.15 and 0.95, respectively (Condurso et al., 2016). Changes were attributed to the degradation of astaxanthin, though the content was not analyzed. For raw, frozen *Solenocera crassicornis*, Jin et al. (2018) reported an increase in ΔE-values of 3.88 after 80 days of storage at -18 °C, with no explanation. Li et al. (2020) described that color changes, i.e., decreases in L* and increases in a* and b* values, of vacuum-packaged, boiled shrimp stored at elevated temperatures (25 and 40 °C) were caused by the Maillard reaction, lipid oxidation, phenol oxidation, and astaxanthin degradation, in decreasing order of importance. In contrast, results from cooked, frozen *Penaeus monodon* showed increases in L* and decreases in a* and b*-values by Fanning et al. (2016), which is in agreement with the color changes seen in the current study. Fanning et al. (2016) explained changes to be linked to decreases in total astaxanthin.

We hypothesize that the increase in the L*-value could be related to calcification or dehydration of the shell, which causes white spots to form during frozen storage. This white spot formation was previously shown for raw, Northern shrimp during frozen storage (Mikkelsen et al., 1997). The white spots might also be the cause for the decrease in b*-values as they lighten the shrimp, which causes the shrimp to become less yellow. However, Eymard et al. (2010) suggested that a decrease in the b*-value could be related to the content of hemeproteins, hemoglobin, and myoglobin in fish mince. These compounds are red in the reduced form, but brown in the oxidized ferric form, which could also cause the b*-value to decrease. The presence and role of hemeproteins in the oxidation of shrimp should be further elucidated. Only few and contradicting studies are reported on this issue.

Table 1
CIEL*a*b* color measurements and Euclidean color differences (ΔE) of the two sizes (medium and x-large) of Northern shrimp stored at -20 °C with and without exposure to light.

Size	Day	Condition	L*	a*	b*	ΔE
medium	0	-	58.6±2.3^{a}	24±2.2 ^{a}	24.3±1.5^{a}	-
	84	Light	65.2 ±2.4^{bx}	18.3 ±2.8^{bx}	21.9 ±2.8^{ax}	9.1
		Without light	59.9 ±2.0^{ay}	21.9 ±2.4^{ax}	24.1 ±2.0^{ax}	2.5
x-large	0	-	62.3±1.6^{a}	20.2±1.8^{a}	24.0±1.1^{a}	-
	84	Light	67.7 ±1.3^{bx}	13.1 ±1.2^{bx}	21.4±1.5^{a}	9.3
		Without light	63.1 ±1.8^{ay}	17.6 ±1.8^{ax}	22.6±3.1^{a}	3.1

^{a,b} indicates a statistically significant change compared to day 0.
^{x,y} indicates the difference between storage conditions of the same size after 84 days.

3.4. Tocopherol and free astaxanthin

α-tocopherol possesses antioxidant properties by acting as either a scavenger of free radicals or by reacting with singlet oxygen (Pazos et al., 2005; Suárez-Jiménez et al., 2016). Several studies of seafood have shown that the content of α-tocopherol decreases during the storage of both lean and fatty fish muscle (Brannan & Erickson, 1996; Pazos et al., 2005; Rodriguez et al., 2009), indicating its effectiveness as an antioxidant. Only α-tocopherol was detected in the shrimp (59±1 μg/g), and results showed no significant differences between light and dark storage nor a significant decrease over time (data not shown). This is also in accordance with the lack of variance in data explained by tocopherol in the PCA. The constant concentration of α-tocopherol indicates that this compound does not act as an antioxidant in this product during frozen storage. The precise cause of this is not known. Still, it is hypothesized that compounds formed during the cooking of the shrimp and subsequent storage, such as hydrophobic pyrroles, might act as antioxidants reducing the consumption of α-tocopherol perhaps because they are located closer to the site of lipid oxidation, but this needs to be further investigated. A study by F. S. H. Lu et al. (2014) described how the presence of hydrophobic pyrroles in krill oil could protect tocopherol

against oxidation, as pyrroles have been shown to possess antioxidative traits (F. S. H. Lu et al., 2013). Our study did not analyze the content of pyrroles. However, since it is suggested that they are formed at elevated temperatures by non-enzymatic browning and have previously been detected in other shrimp studies, it is possible that they could be present in whole, cooked shrimp.

Free astaxanthin is unstable and susceptible to oxidation (Etoh et al., 2012). However, a statistically significant ($p \leq 0.05$) difference was not seen between storage with and without light, nor was an effect of storage time seen. Furthermore, the size of the shrimp had no statistically significant ($p > 0.05$) effect on concentrations, except that a higher concentration tended to be obtained in x-large shrimp compared to medium shrimp (Fig. 2). The somewhat constant concentration of free astaxanthin indicates that color changes and decreases in a*-value (redness) and differences between storage conditions were not caused by a reduction in free astaxanthin content. However, they could be caused by changes in the concentration of ester-bound astaxanthin, which was not measured in the present study. Our results are not consistent with previously reported results by Bak et al. (1998) for *P. borealis* stored with or without light after three months at -17 °C. A significant decrease of 9 and 13 mg/kg was reported with and without light, respectively. The astaxanthin concentration was detected spectrophotometrically according to the method described by Kelly and Harmon (1972) for determinations of carotenoid content, likely giving the total astaxanthin content and perhaps explaining why a decrease occurred. Qiao et al. (2019) reported decreases in both free and astaxanthin esters during 12 weeks of non-illuminated, frozen storage of raw *Litopenaeus vannamei* and showed decreases in peak areas after 10 weeks of storage of approximately -1.6×10^7 and -1.3×10^7 for astaxanthin esters, and free astaxanthin, respectively. This shows that more esters than free astaxanthin were degraded during storage. The authors did not state the storage temperature. However, if the temperature was higher than in our study, the degradation of astaxanthin might occur at a higher rate than at the -20 °C used in our study. Therefore, a decrease in free astaxanthin might occur after the 84 days of storage in our study. A cause for the lack of decrease in astaxanthin could be that the current study measured free astaxanthin rather than astaxanthin esters or total astaxanthin, and Breithaupt (2004) reported that free astaxanthin only constitutes a minor part (2.2 %) of the total astaxanthin found in *P. borealis*. Therefore, future studies should also investigate the changes in total astaxanthin and astaxanthin esters during storage. Another possible explanation for the lack of decrease could be that the pyrroles, which may be protecting α-tocopherol against oxidation, could also protect free astaxanthin from degradation. Whereas a third explanation could be that astaxanthin is degraded slower at the wavelength of the light source used in this study compared to the light source used in other studies. Still, more investigations should be performed to elucidate this.

3.5. Peroxide value

Medium-sized shrimp exposed to light showed a steady increase in PV, though no statistically significant ($p > 0.05$) increase was seen until day 70. In contrast, no significant increase occurred for storage without light (Fig. 3A). For x-large shrimp, a significant increase in PV from day 0 was only seen after 84 days of illuminated storage, whereas a significant ($p \leq 0.05$) increase was seen between day 14 and days 42, 70, and 84 for shrimp stored under the light (Fig. 3B). No significant development of primary oxidation products (PV) was seen after 84 days of storage without light, underlining the marked effect of light on the formation of hydroperoxides. The larger surface-to-meat ratio could explain the higher increase in PV seen for medium-sized shrimp, since this would expose a greater proportion of shell and tissue to light and oxygen than would be the case for the larger shrimp. Furthermore, the larger shrimp had a higher content of saturated fatty acids (**Table S1**), which are less prone to oxidation than unsaturated fatty acids, than the medium-sized shrimp. Though we found no other studies investigating changes in PV for frozen, whole, cooked shrimp exposed to light, results have been reported for raw, frozen mud *Solenocera melantho* stored without light at -20 °C by Shi et al. (2017). This study showed an increase from 3 to 9.4 meq/kg lipid of the peeled shrimp after 12 weeks of frozen storage. This smaller increase in PV during frozen storage was likely caused by the beheading and peeling of the mud shrimp. Results from our laboratory have shown that beheading and peeling of shrimp reduce oxidation greatly (unpublished results). The effect of light (600 lux) on the storage of chilled (4 °C) shrimp oil has been investigated by Takeungwongtrakul and Benjakul (2016), who, after 40 days, found more than two times higher PV for samples stored with light and air, compared to storage in the dark. Li et al. (2020) investigated changes in boiled, vacuum-packed RTE shrimp stored at 25 and 40 °C for 50 days. Also, they found that storage in the sunlight had a pronounced effect on the production of primary oxidation products, compared to storage without sunlight, even at the relatively high storage temperatures used for storage of the RTE shrimp.

3.6. Volatile compounds

A total of 14 volatile compounds were quantified, namely 2-butanone, 2,3-pentadione, 1-penten-3-ol, 3-methyl-1-butanol, 1-pentanol, 2-penten-1-ol, 3-methyl-butanal, 2-methyl-butanal, hexanal, heptanal, 2,4-heptadienal, octanal, nonanal and decanal. These were chosen based on their importance for causing off-odors and off-flavors in seafood, as well as on their development during storage. Of the 14 quantified

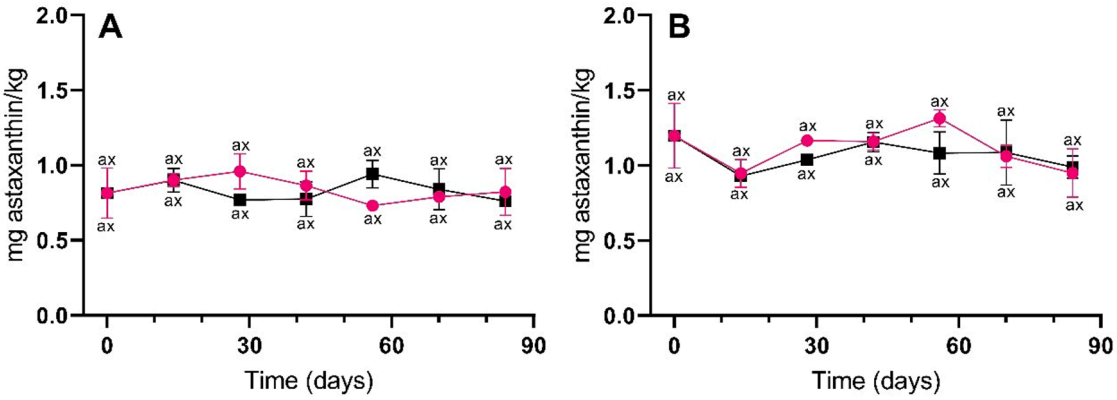

Fig. 2. Free astaxanthin content (mg/kg) of medium (A) and x-large (B) shrimp stored with (●, pink) and without (■, **black**) light for 84 days. [a] and [b] indicate statistically significant differences over time, whereas [x] and [y] indicate statistically significant differences between storage with and without light at the same sample point.

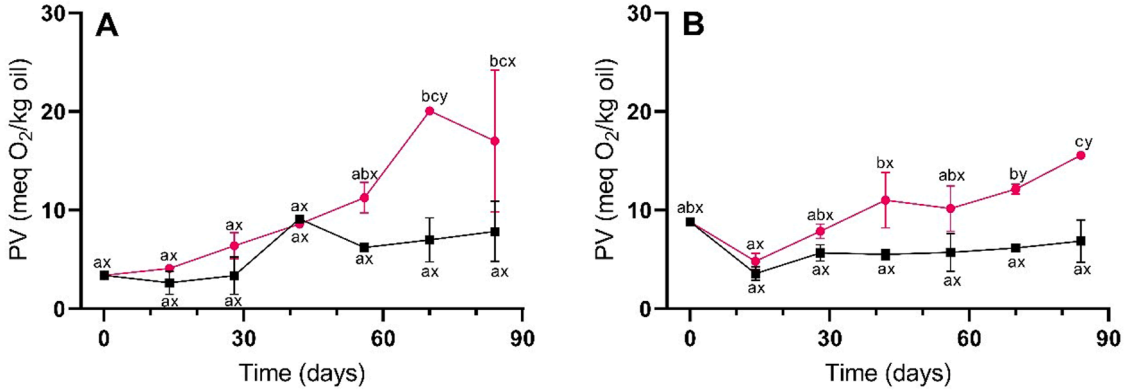

Fig. 3. Peroxide values (meq. O_2/kg oil) of medium (A) and x-large (B) shrimp stored with (●, pink) and without (■, black) light for 84 days. [a] and [b] indicate statistically significant differences over time, whereas [x] and [y] indicate statistically significant differences between storage with and without light at the same sample point.

compounds, 2-methyl-butanal, 3-methylbutanal, 3-penten-1-ol, and 2-penten-1-ol were chosen to represent the changes in secondary oxidation based on their locations in the loadings plot (Fig. 1B).

The two aldehydes 2-methylbutanal and 3-methylbutanal are both Strecker aldehydes likely produced by the non-enzymatic Strecker degradation of the free amino acids, leucine and isoleucine, often caused by protein hydrolysis in combination with lipid oxidation occurring during heating and storage (Smit et al., 2009). The presence of the two Strecker aldehydes in shrimp was also seen by Condurso et al. (2016), who described that these two aldehydes were only detected after shrimps were cooked, indicating how they are produced during the heat treatment. 2-methylbutanal and 3-methylbutanal produce odors described as cocoa or coffee-like and apple-like, respectively (Wang et al., 2022).

2-methylbutanal and 3-methylbutanal appear to explain variance for the medium and x-large shrimp, respectively (Fig. 1). Fig. 4A and B show the development of 2-methylbutanal for medium and x-large shrimp, respectively. Common for both sizes was a trend toward higher content of 2-methylbutanal throughout storage when exposed to light, compared to storage without light, though differences were not significant ($p>0.05$). The content of this volatile appears to be affected by the size of the shrimp as the content is significantly ($p<0.05$) higher at the end of illuminated storage of medium-sized shrimp than for x-large shrimp. Additionally, the effect of light appeared to be more pronounced in medium-sized shrimp. 3-methylbutanal, on the other hand, showed a tendency to be more affected by light storage in x-large shrimp than in medium shrimp though differences were not significant ($p>0.05$). Results for the medium-sized shrimp were less clear concerning the effect of light (Fig. 4C and D). Further studies should be performed to investigate the origin of Strecker aldehydes in cooked, frozen shrimp, such as protein oxidation or Maillard reactions. 2-methylbutanal and 3-methylbutanal have previously been detected in cooked, frozen stored *Parapenaeus longirostris* (large shrimp with a length of 10-15 cm), where no increases were seen during 16 months of non-illuminated storage. In contrast, the results of the same study for cooked *Parapandalus narval* (small shrimp with a length of 7 cm) showed a slight increase (Condurso et al., 2016). This could indicate how the formation of these volatile compounds depends on shrimp species and potentially by size, as also seen in our study. Furthermore, this could suggest that the formation of these volatile compounds is only increased in the presence of light.

The two alcohols 1-penten-3-ol and 2-penten-1-ol can be produced by the oxidation of polyunsaturated fatty acids (e.g., docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)) (Lee et al., 2003) and cause odors described as sweet, sharp/irritating and mushroom, respectively. Both have previously been described as important lipid oxidation products in fish oil (Lee et al., 2003). 1-penten-3-ol and 2-penten-1-ol (Fig. 4E-H) explained variance in storage time and the effect of size (Fig. 1), respectively. The content of 1-penten-3-ol increased during storage for both sizes of shrimp and both in light and darkness, with no statistically significant ($p>0.05$) difference between storage methods, indicating that the development of this alcohol was not as affected by light as other volatile compounds reported in this study. However, the content of this alcohol increased up to two times more in medium-sized shrimp than for x-large shrimp, and a significant increase was seen over time with illuminated storage for medium-sized shrimp. 2-penten-1-ol, on the other hand, increased significantly ($p\leq0.05$) more for x-large shrimp in the presence of light than for samples stored in darkness and between two and three times more than in medium-sized shrimp, where, however, no significant ($p>0.05$) effect of light was seen. 1-penten-3-ol was present in high concentrations at the end of storage (>40 ng/g) compared to the other volatile compounds for both sizes of shrimp, and a high content of 1-penten-3-ol has previously been detected in krill oil (Giogios et al., 2009; F. S. H. Lu, Bruheim, & Jacobsen, 2015). 1-penten-3-ol was previously detected in processing water from the Northern shrimp cooking process (Forghani et al., 2020). Increasing concentrations during storage at 37 °C of dried *Acetes chinensis* shrimp were observed by F. Lu et al. (2011). In contrast, decreasing concentrations were found in cooked, frozen *Parapenaeus longirostris* during storage at -18 °C (Condurso et al., 2016). 2-penten-1-ol has previously been detected in a study investigating the effect of thermal treatment on Antarctic krill meal (F. S. H. Lu, Bruheim, Ale, et al., 2015). However, we found no reports describing the formation of this volatile during the storage of shrimp products.

Among the remaining volatile compounds, the detection of the lipid-derived 2,4-heptadienal deserves further comment. Contrary to the study from Condurso et al. (2016), who did not detect 2,4-heptadienal during frozen storage of shrimp, our study found an increase for both sizes of shrimp, both with and without light. This volatile has previously been described to be responsible for the oxidized aromas of cold-stored fish, making the volatile compound's presence important for the sensory changes occurring during storage. Based on results from other studies, different pyrazines, including alkyl pyrazines, were expected to be detected during storage of whole, cooked shrimp, as these have previously been shown to be formed during heating of solutions containing N-acetylglucosamine (Lu, Bruheim, Ale, et al., 2015) and roasting of whole shrimp (Okabe et al., 2019). N-acetylglucosamine is a product of chitin hydrolysis, which is the shrimp shell's main component (Dang et al., 2018; Rødde et al., 2008), wherefore the presence of alkyl pyrazines could be expected, however, they were not detected in this study. Especially 2,5-dimethylpyrazine was expected as this has previously been detected in this product in our laboratory from samples that were more than 2 years old (unpublished data) and has been detected in boiling waters from this species (Forghani et al., 2021).

No clear effect of the two sizes of shrimp, with respect to the

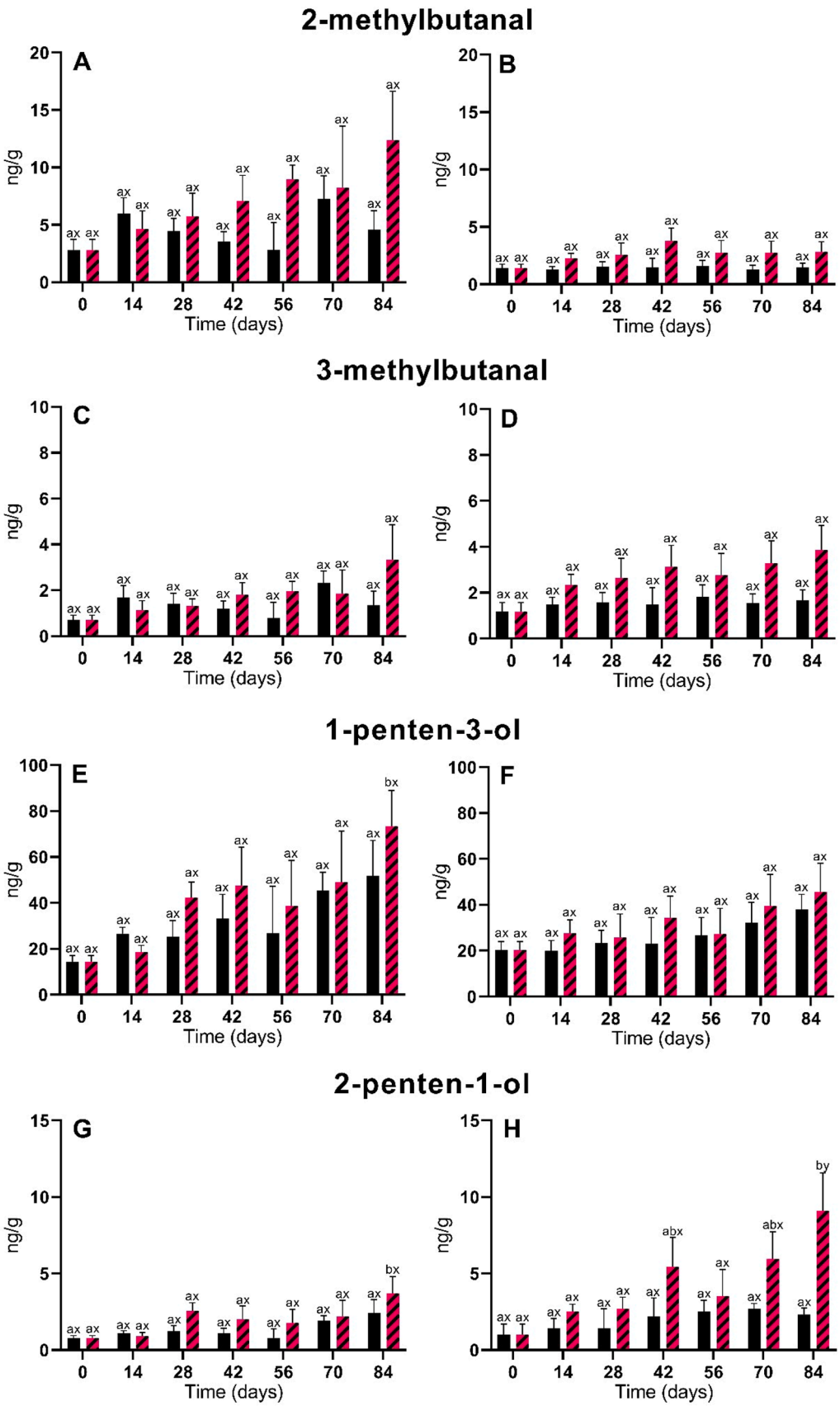

Fig. 4. Concentrations in four volatile compounds: 2-methylbutanal, 3-methyl butanal, 1-penten-3-ol and 2-penten-1-ol of medium (A, C, E, and G, respectively) and x-large (B, D, E, H, respectively) sized shrimp stored with (▨) and without (■) light for 84 days at -20 °C. ^{a}, ^{b} and ^{c} indicate statistically significant differences over time, whereas ^{x} and ^{y} indicate statistically significant differences between storage with and without light.

formation of volatile compounds, could be seen, as the concentration of some volatile compounds was increased in medium shrimp and remained somewhat constant for x-large shrimp, and vice versa. More studies should be performed to elucidate these differences.

Furthermore, the tendency of light to increase the formation of volatile compounds (Fig. 4) may be expected to become more pronounced if the storage trial had been conducted over a more extended period as hydroperoxides react with other compounds catalyzed by, for example, metals to form volatile compounds. The increased lipid oxidation (PV) seen for samples stored with light compared to dark storage is a well-known phenomenon, where light is often applied to accelerated shelf-life trials (Calligaris et al., 2016) and underlines the importance of conducting shelf-life trials under realistic storage conditions. Photooxidation is highly dependent on the presence of oxygen, wherefore storage in a modified atmosphere without oxygen, such as 100% nitrogen, has previously been shown to have a significant impact on oxidative reaction for products stored in the presence of light (Bak et al., 1998; Takeungwongtrakul & Benjakul, 2016). Furthermore, the reactions are highly dependent on the wavelength of the light, as visible light does not have the energy to produce radicals. Therefore, packaging material that does not allow for high energy wavelengths to pass through could reduce the negative effect of illuminated light storage.

4. Conclusion

It was possible to determine the effect of illuminated storage on changes in peroxide values for both sizes of shrimp, with a larger increase seen in medium-sized shrimp than in x-large shrimp. The content and increase of volatile compounds appeared to be different between the two sizes of shrimp, though most volatile compounds showed an increase in concentration during illuminated storage. No decrease was observed in the content of free astaxanthin, indicating that the color changes that occurred during this type of illuminated storage are not caused by the oxidation of free astaxanthin. No change was seen in the content of α-tocopherol during storage in any of the samples. It is hypothesized that the potential presence of pyrroles may act as antioxidants before free astaxanthin and α-tocopherol, explaining the lack of decreases in their concentrations.

The results showed that light increases the oxidation of whole, cooked Northern shrimp and that the illuminated frozen storage commonly used in retail displays greatly affects the color of the whole, cooked Northern shrimp. Therefore, protecting these products against light and oxygen during storage is important to prolong the market shelf-life.

Future studies should be done to elucidate if oxidative changes and color deterioration during frozen storage of whole, cooked Northern shrimp could be reduced by using natural antioxidants or alternative packaging methods.

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CRediT authorship contribution statement

Hanne Aarslev Jensen: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Ann-Dorit Moltke Sørensen:** Conceptualization, Methodology, Writing – review & editing. **Lisbeth Truelstrup Hansen:** Funding acquisition, Conceptualization, Writing – review & editing. **Niels Bøknæs:** Funding acquisition, Conceptualization, Resources, Writing – review & editing. **Ole Mejlholm:** Funding acquisition, Conceptualization, Resources, Writing – review & editing. **Charlotte Jacobsen:** Funding acquisition, Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships that may be considered potential competing interests: Hanne Aarslev Jensen, Niels Bøknæs, and Ole Mejlholm are employed by Royal Greenland.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2023.100369.

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