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Effects of mechanical handling, storage on ice and ascorbic acid treatment on lipid oxidation in cultured Newfoundland blue mussel (*Mytilus edulis*)

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Abstract

Lipid oxidation of cultured blue mussels (*Mytilus edulis*) during mechanical handling and storage on ice was investigated. Furthermore, the exposure of blue mussels to ascorbic acid (Asc) as an antioxidant and its effects on lipid oxidation of sample was monitored. Thiobarbituric acid reactive substances (TBARS) of stored mussels were significantly ($p < 0.05$) higher than those of the fresh mussels. Mechanical handling of mussels, which includes washing, sorting and packaging, for up to 1 h did not affect their oxidative status significantly ($p < 0.05$). Furthermore, exposing live mussels to specific concentrations of Asc retarded lipid oxidation significantly ($p < 0.05$) during storage on ice for only 5 days, after which the Asc became a pro-oxidant.

Keywords: Cultured blue mussel; *Mytilus edulis*; Mechanical handling; Storage on ice; Ascorbic acid; Antioxidant; Pro-oxidant

1. Introduction

The omega-3 ($n-3$) polyunsaturated fatty acids (PUFA) are critical components of cell membranes, and are therefore important in the structure, dynamics and control of membrane-associated biochemical functions; their major source for human consumption are seafoods. Epidemiological, clinical and nutritional studies have demonstrated that consumption of seafoods has beneficial effects in the alleviation of various clinical disorders, including cardiovascular disease, diabetes, obesity, arthritis, asthma, depression, hyperactivity and some types of cancer (Shahidi, 1998). The main PUFA present in seafoods, including blue mussels, are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Budge & Parrish, 2003).

Lipid oxidation in seafoods contributes to the loss of freshness and $n-3$ PUFA, depending on the storage period and temperature, natural antioxidants, type of chemical or physical treatment and packaging (Bell, McEvoy, Tocher, & Sargent, 2000; Bragadottir, Palmadottir, & Kristbergsson, 2004; Gieseg, Cuddihy, Hill, & Davison, 2000; Khalil & Mansour, 1997). The application of synthetic and natural antioxidants to control lipid oxidation in seafoods is well established. Frigg, Parbucki, and Ruhdel (2000) studied the effects of a tocopherol dietary supplement on oxidative stability of trout fillets and found that supplementation effectively controlled lipid oxidation. Similarly, Akhtar, Gray, Booren, and Garling (1998) were able to control lipid oxidation in rainbow trout during refrigeration and freezing using surface application of tocopherol and oleoresin rosemary. Gaitlin, Bai, and Erickson (1998)

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incorporated natural (α-tocopheryl acetate) and synthetic antioxidants (ethoxyquin and butylated hydroxytoluene) in the feed to control lipid oxidation in channel catfish. In the latter study, only the natural antioxidants provided additional protection against lipid oxidation. Tang, Sheehan, Joe Buckely, Morrissey, and Kerry (2001) examined the antioxidant activity of tea catechins and α-tocopherol on lipids of fresh minced red meat, fish and poultry stored for 10 days at 4°C and found that antioxidant treatment controlled lipid oxidation in the stored meats. More recently, Undeland, Hultin, and Richards (2003) used aqueous extracts from some seafood muscles, which contained antioxidants, to inhibit lipid oxidation in cod muscle membranes.

L-Ascorbic acid (Asc) is an effective antioxidant that can help to maintain iron- and copper-containing enzymes in their required reduced form. It can scavenge free radicals in biological systems, including lipids, thus prevent their oxidative damage. Ascorbic acid acts synergistically with tocopherols to reduce lipid peroxyl radicals and regenerates tocopherols for further antioxidant actions. Meanwhile, Asc can also exhibit pro-oxidant properties depending on its concentrations and presence of metal ions in the system (Halliwell & Whiteman, 1997; Yen, Duh, & Tsai, 2002). In 1993, a new technique was developed by Thed, Erickson and Shewfelt to introduce Asc to aquaculture fish species. In this technique, fish were kept in a temperature-controlled water tank and exposed to various concentrations of Asc for a specific time, then harvested and frozen to evaluate the effectiveness of Asc in controlling lipid oxidation during storage. This technique has been used to control lipid oxidation in Channel cat fish (Thed & Erickson, 1994) and Norwegian herring fillets (Hamre, Lie, & Sandnes, 2003).

Polyunsaturated fatty acids constitute about 50% of the total lipids in Newfoundland cultured blue mussels (*Mytilus edulis*) (Budge & Parrish, 2003). Despite this high content of PUFA in mussels, very little is known about lipid oxidation during mechanical handling or storage on ice and almost no information is available about the effects of Asc treatment on lipid oxidation of blue mussels. Therefore, the objectives of this study were to examine the effects of storage on ice, mechanical handling and Asc treatment on lipid oxidation of blue mussels. Other variables that can be affected by Asc treatment such as pH, antioxidant content and fatty acid composition were also monitored.

2. Materials and methods

2.1. Materials

Commercial size mussels (approximately 3 years old) were obtained from two processing (washing, sorting and packaging) plants, Fortune Harbor (FH) and Charles Arm (CA) in Notre Dame Bay, Newfoundland, Canada. Compressed air, hydrogen and UHP helium were obtained from Canadian Liquid Air Ltd. (St. John's, NL). Trichloroacetic acid was obtained from Fisher Scientific (Nepean, ON). PUFA 1, PUFA 3, Supleco 37 component FAME mixture and bacterial acid methyl ester mixture, from Supelco, Canada Ltd. (Oakville, ON), GLC-461 standard fatty acid methyl ester preparation from Nu-Check-Prep, (Elyasin, MN). 2-Thiobarbituric acid (TBA), 1,1,3,3-teteramethoxypropane (TMP), L-ascorbic acid (ACS grade), L-ascorbic acid (SigmaUltra), sodium ascorbate and 14% BF_3–methanol were obtained from Sigma Chemical Co. (St. Louis, MO). Reagents, standards and buffers required for photochemiluminescence measurements of ascorbic acid and tocopherol were obtained from Analytik Jena USA (Delaware, OH). All other chemicals were ACS grade or better.

2.2. Effect of mechanical handling

Three different batches (100 mussels from each batch) of mussels were collected from CA and FH plants during mechanical handling (washing, sorting, and packaging), (first at the beginning (0 h)), second at the middle (after 30 min) and third at the end (after 1 h). TBARS analysis was carried out for use as an index of lipid oxidation.

2.3. Effect of storage of mussels on ice

Mussels, obtained as described in the previous section, from CA and FH were distributed in small bags (about 35 mussels per bag) with a wide mesh size. The bags were stored in a plastic container (70 cm length × 35 cm width × 25 cm height) filled with crushed ice (10 cm height). Mussels were separated from the ice by a plastic bag (70 cm length × 35 cm width). TBARS analysis was carried out on days 0, 3, 7, 10 and 14.

2.4. Effect of ascorbic acid treatment

Twenty-five mussels were placed in a plastic container filled with 10 L filtered seawater (FSW) and held at 2 °C for 24 h for acclimation. The water was replaced with 9 L FSW and mussels were left for another 2 h. Specific amounts of sodium ascorbate (NaAs), ACS grade ascorbic acid and ultrapure ascorbic acid were then dissolved in 1 L of FSW. The prepared solutions were poured into the containers (one solution per container). The final concentration for each type of Asc was 0.01 M. Mussels were exposed to various types of Asc for 24 h. The mussels were then removed from water and stored on ice for 10 days as described above. Controls were run with mussels without any Asc treatment. Each treatment

was replicated three times. The mussels were homogenized and analyzed on days 0, 5 and 10 for pH, fatty acid composition, TBARS, ascorbic acid and tocopherol determination as described below.

2.5. Sample preparation for analysis

Mussel shells (5–6) were opened and the shell liquor and meats collected in a beaker placed in container filled with crushed ice and subsequently homogenized for 60 s with a commercial Waring blender (Dynamic Corporation of America, New Hartford, CT). All subsequent analyses were performed on this homogenate.

2.6. pH measurements

The pH of filtered sea water and homogenized mussel samples was determined by immersing a pH electrode into water or the homogenate for 60 s and observing the pH on a pre-calibrated Fisher Accumate pH meter model 805 MP (Fisher Scientific, Fair Lawn, NJ).

2.7. Determination of 2-thiobarbituric acid reactive substances as an index of lipid oxidation

Changes in oxidative state and hence possible off-flavor development of mussel meat were evaluated using direct 2-thiobarbituric acid reactive substances (TBARS) test as described by Siu and Draper (1978) with some modifications. Five grams of the homogenized sample were placed in a 50 mL centrifuge tube to which 20 mL of a 10% (w/v) solution of trichloroacetic acid (TCA) and 20 mL distilled water were added and vortexed (Fisher Vortex Genie 2, Fisher Scientific, Nepean, ON) at high speed for 2 min. The mixture was then centrifuged at 4000*g* for 5 min and the supernatants filtered through a Whatman No. 3 filter paper. One milliliter of a 0.01 M aqueous solution of 2-thiobarbituric acid and 4 mL of the filtrate were mixed. The mixture was heated in a boiling water bath for 25 min, cooled to room temperature, and the absorbance of the resultant colored solution was read at 532 nm using a Hewlett–Packard diode array spectrophotometer (Model 8452A, Agilant Co., Palo Alto, CA). TBARS values (expressed as mg malonaldehyde equivalents/kg mussel meat) were calculated by multiplying the absorbance readings by a factor of 10.2, which was obtained from a standard line prepared using 1,1,3,3-tetramethoxypropane as a precursor of malonaldehyde.

2.8. Determination of fatty acid composition

Fatty acid composition was determined as described by Budge and Parrish (2003). In this method, fatty acid methyl esters (FAME) of lipid extracts of blue mussels were prepared using 14% BF_3–MeOH mixture. Lipid extracts were dissolved in 0.5 mL hexane and 1.5 mL 14% BF_3–MeOH mixture (Supelco, Oakville, ON). The samples were flushed with nitrogen and heated for 90 min at 85 °C. After cooling to room temperature, FAME were extracted three times with a mixture of 2 mL hexane and 2 mL water, followed by centrifugation at 2000*g* for 2 min. Hexane layers, which contained FAME were separated, combined, and evaporated under a stream of nitrogen at room temperature.

2.9. Analysis of fatty acid methyl esters by gas chromatography

Fatty acid methyl esters, prepared as described previously, were analyzed with a Hewlett–Packard 5890 II gas chromatograph (Agilant, Palo alto, CA) equipped with a $30\,m \times 0.25\,mm$ Supelcowax-10 column (SP 2330, Supelco Canada Ltd., Oakville, ON). The injector and flame ionization detector temperatures were both 270 °C. The oven temperature was initially 220 °C for 10.25 min and then increased to 240 °C at 30 °C/min and held there for 9 min. Helium (UHP) was used as the carrier gas. The FAME were identified by comparing their retention times with those of authentic standard mixtures (PUFA 1, PUFA 3, Supleco 37) component FAME mixture and bacterial acid methyl ester mixture; Supelco, Canada Ltd. (Oakville, ON) and GLC-461 Nu-Check-Prep. (Elyasin, MN), quantified using area normalization and each fatty acid reported as percentage of the total fatty acids.

3. Photochemiluminescence detection of water-soluble (ascorbic acid) and lipid-soluble (α-tocopherol) antioxidants in mussels

3.1. Extraction of ascorbic acid (Asc) from blue mussels

Approximately 2 g of the homogenate were mixed with 5 mL of ice-cold 10% TCA and the mixture vortexed using a Fisher Vortex Genie 2 (Fisher Scientific, Nepean, ON) at high speed for 2 min. The mixture was centrifuged at 4000*g* for 5 min and the supernatant filtered through a Whatman No. 3 filter paper. The antioxidant capacity of Asc was determined in the filtrate.

3.2. Determination of ascorbic acid and α-tocopherol in blue mussels

Ascorbic acid in seawater and blue mussels (expressed as Asc equivalent/L of seawater or g meat) and tocopherol in mussel lipid extract (expressed as μmol Trolox equivalent/g meat) were assayed by a photochemiluminescence (PCL) method with a PHOTOCHEM® instrument (Analytik Jena USA, Delaware, OH).

The assay mixture for Asc measurement consisted of 1.5 mL of reagent 1 (diluent), 1 mL of reagent 2 (buffer, pH 10.5), 0.025 mL of photosensitizer (luminol) and 0.05, 0.01, 0.015, 0.02 and 0.025 mL reagent 4 (Asc standard solution) or sample solution (0.01 or 0.015 mL) of an appropriate dilution of the seawater or blues mussels filtrate (2.12.1) (Amarowicz, Raab, & Karamac, 1999). The lag phase generated was used to calculate Asc content using PCLsoft software v 3.1 (Analytik Jena USA, Delaware, OH). The assay mixture for tocopherol measurements consisted of 2.3 mL reagent 1 (diluent), 0.2 mL reagent 2 (buffer, pH 10.5) 0.025 mL of photosensitizer (luminol), and 0.05, 0.015, 0.02, 0.025, and 0.03 mL reagent 4 (Trolox standard solution) or mussel lipid extract (0.1 mL of an appropriate dilution of the extract) (Popov & Lewin, 1996). Tocopherol occurrence in blue mussels was calculated using a standard curve and expressed as Trolox equivalents using PCLsoft® v 3.1 (Analytik Jena USA, Delaware, OH).

3.3. Statistical analysis

All experiments were replicated three times. Mean ± standard deviation was reported for each case. Analyses of variance (ANOVA) and Tukey's studentized test were performed at a level of $p < 0.05$ to evaluate the significance of differences between mean values (Freund, Williams, & Perles, 1988).

4. Results

4.1. Effect of mechanical handling on lipid oxidation in blue mussels

There were no significant ($p > 0.05$) differences in TBARS values among the three batches of sample collected from FA or CA mussel processing plants (data not shown). Average TBARS values of mussels obtained from FH and CA were 2.18 ± 0.03 and 1.3 ± 0.01 mg malonaldehyde equivalents/kg meat, respectively.

4.2. Effect of storage on ice on lipid oxidation in blue mussels

There was a gradual increase in TBARS values of stored mussels from CA and FH up to day 7, after which TBARS values started to decline (Fig. 1). TBARS values of FH mussels were significantly ($p < 0.05$) higher than those of CA mussels throughout the storage period.

4.3. Effect of Asc treatment on pH values of seawater and blue mussels

The pH of filtered seawater without blue mussels was near neutrality at the beginning (0 time; 7.34 ± 0.02 and after 24 h 7.37 ± 0.02), whereas the pH of seawater with blue mussels decreased from 7.35 ± 0.02 at 0 h to

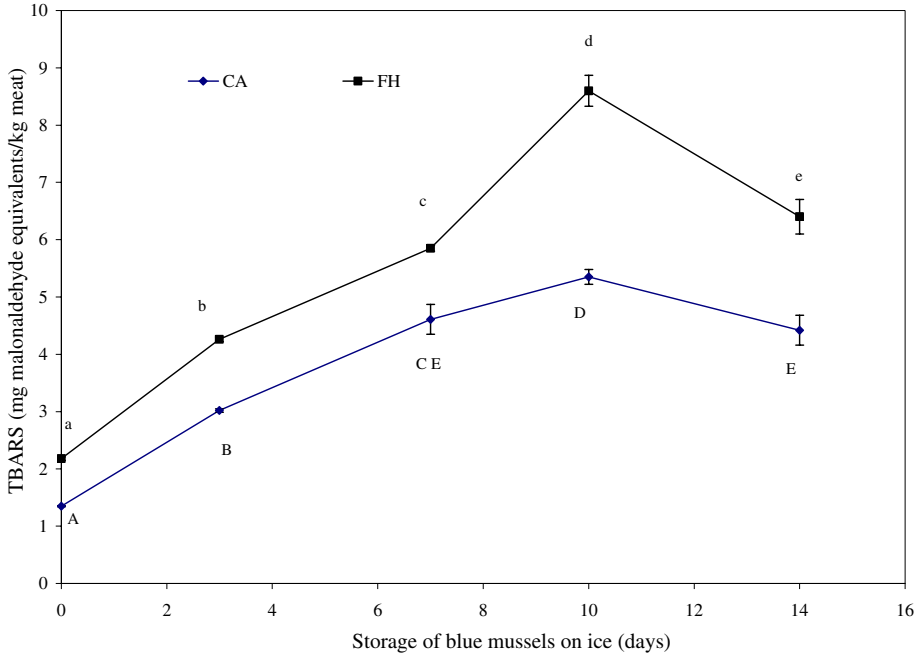

Fig. 1. Thiobarbituric acid reactive substances (TBARS) values (mg malonaldehyde equivalents/kg meat) of blue mussels obtained from two aquaculture sites Charles Arm (CA) and Fortune Harbor (FH), and stored on ice for 14 days (mean ± SD, $n = 3$). Means for each set with different letters (a, b, c or A, B, C) are significantly ($p < 0.05$) different from each other.

7.16 ± 0.02 after 24 h. After exposure of mussels for 24 h to NaAs at cold room temperature, pH increased from 6.77 ± 0.02 at 0 h to 7.14 ± 0.01. The pH of filtered seawater decreased considerably after the addition of ACS grade Asc to 4.06 ± 0.01, but after 24 h increased to 4.25 ± 0.01. Similarly, the addition of ultrapure grade Asc reduced the pH of filtered seawater containing mussels to 3.94 ± 0.04, but this value increased to 4.19 ± 0.01 after 24 h. No significant ($p < 0.05$) differences were observed among the pH values of all treated mussels during storage on ice (data not shown).

4.4. Effect of Asc treatment on lipid oxidation in mussels stored on ice

The TBARS values for mussels with or without Asc treatment were not significantly ($p < 0.05$) different from one another on day 0 (Fig. 2). However, after 5 days of storage on ice, all blue mussels exposed to Asc had significantly ($p < 0.05$) lower TBARS values compared to the control. The lowest TBARS values were for mussels exposed to NaAs followed by mussels exposed to ACS grade Asc and finally mussels exposed to ultrapure grade Asc. This indicated that NaAs initially was more effective than other treatments in controlling lipid oxidation on ice stored mussels. However, a reverse trend was observed after 10 day of storage, when TBARS values for all treatments were significantly ($p < 0.05$) higher than those of the control. This indicated that all Asc forms eventually had a pro-oxidant activity on the lipids of stored mussels. NaAs followed by ACS grade Asc then ultrapure Asc exposures exhibited the highest pro-oxidant activity.

4.5. Ascorbic acid in seawater and blue mussels after their treatments

Ascorbic acid in filtered seawater was initially 10,000 μmol (Asc equivalent/L of seawater) for all treatments, but decreased considerably after 24 h to 1192 ± 54.44 μmol/L for NaAs, 906 ± 95.25 μmol/L for ACS grade and 658 ± 89.06 μmol/L for ultrapure grade. There were no significant ($p < 0.05$) differences in Asc in stored mussels among the various treatments at 0 day and after 5 and 10 days (Fig. 3). However, Asc in NaAs treated mussels decreased from an initial concentration of 35.19 ± 8.27–13.19 ± 3.9 μmol/g meat after 10 days of storage; in ACS grade Asc treatment, it decreased from 33.13 ± 9.21 μmol to 16.06 ± 0.89 μmol/g meat and in ultrapure grade Asc treatment, it decreased from 27.38 ± 4.9 to 18.63 ± 4.5 μmol/g meat.

4.6. Tocopherol content in blue mussels after the ascorbic acid treatment

There were no significant ($p > 0.05$) differences in tocopherol content (expressed as μmol Trolox equivalent/g meat) among mussels treated with various forms

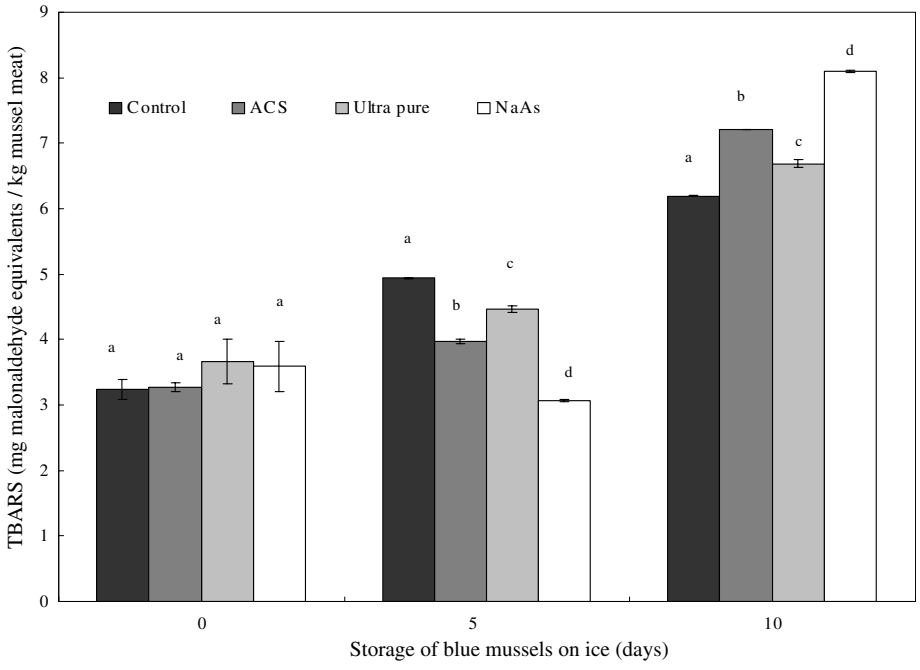

Fig. 2. Thiobarbituric acid reactive substances (TBARS) values (mg malonaldehyde equivalents/kg meat) of mussels treated with 10,000 μmol/L of various grades and forms of Asc and stored on ice for 10 days (Mean ± SD, $n = 3$). Means for each day with different letters are significantly ($p < 0.05$) different from each other.

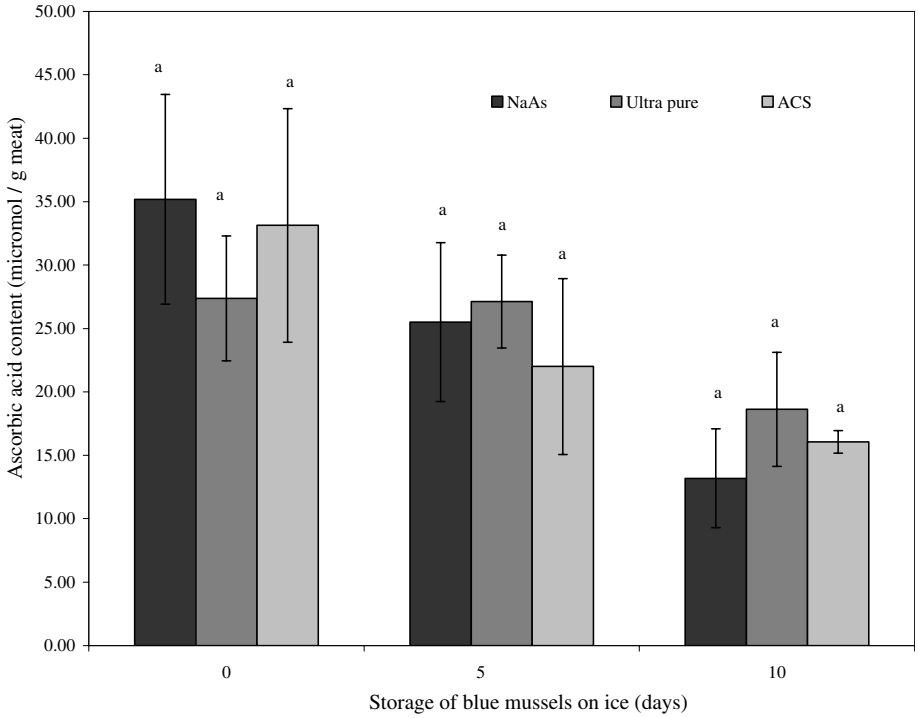

Fig. 3. Ascorbic acid content (μm/g meat) in mussels treated with 10,000 μmol/L of various grades and forms of Asc and stored on ice for 10 days (Mean ± SD, $n = 3$). Means for each day with different letters are significantly ($p < 0.05$) different from each other.

of Asc at each sampling day throughout the storage period (Fig. 4). Data in this figure, however, shows significant ($p < 0.05$) reduction in tocopherol content in all treated samples after 5 and 10 days of storage. For example, tocopherol in ACS grade treated mussels decreased from 0.32 ± 0.04 μmol Trolox equivalent/g meat

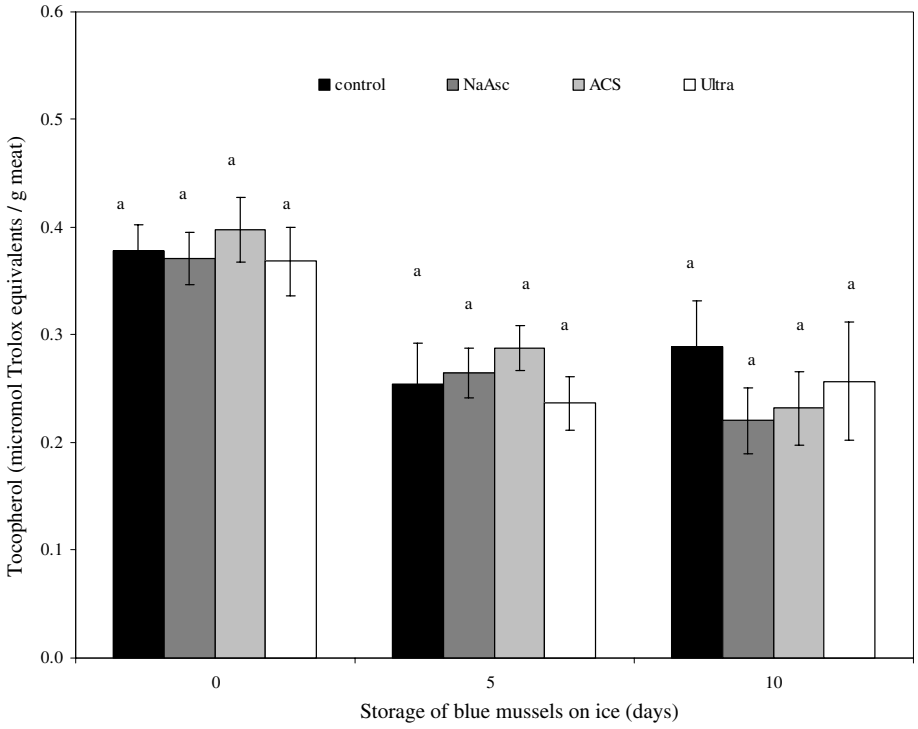

Fig. 4. Tocopherol content (μm Trolox equivalent/g meat) in mussels treated with 10,000 μmol/L of various forms and grades of Asc and stored on ice for 10 days. (Mean ± SD, $n = 3$). Means for each day with different letters are significantly ($p < 0.05$) different from each other.

on day 0 to 0.26 ± 0.04 μmol Trolox equivalent/g meat after 10 days of storage. Similar trends were observed with other treatments (see Fig. 5).

4.7. Fatty acids profiles of blue mussels after the ascorbic acid treatments

The content of 16:0 fatty acid the in the control was significantly ($p < 0.05$) higher than that in the treated mussels at each sampling day throughout the storage period. Similarly, control and ACS grade Asc treated mussels contained higher ($p < 0.05$) amounts of the monounsaturated fatty acid 16:1$n-7$ throughout the storage period than did other treated mussels. Eicosapentaenoic acid (EPA; 20:5$n-3$) was the major polyunsaturated fatty acid detected in the control and Asc treated mussels (Table 1). The initial amount of EPA in the Asc treated mussels was significantly ($p < 0.05$) higher than that in the control on day 0, after which there were no significant ($p > 0.05$) differences among all treated mussels with the exception of the EPA content in ACS grade Asc treated mussels that was higher ($p < 0.05$) than in other treatments after 5 days of storage (data not shown). No significant ($p > 0.05$) differences were observed in major saturated, monounsaturated, and polyunsaturated fatty acids and the total SFA, MUFA and PUFA in mussels treated with various Asc forms and grades during their storage on ice for 10 days with few exceptions that do not affect the overall discussions or conclusions reached here (Table 1).

5. Discussion

Mussels removed from their natural marine habitat are under constant stress due to inadequate change in their natural environmental, both the temperature and anaerobiosis. Various methods and techniques are used to measure stress in live blue mussels (Bayne & Thompson, 1970). Measurement of thiobarbituric acid reactive substance (TBARS) is commonly carried out to evaluate oxidative stress in seafoods (Bell et al., 2000; Frigg et al., 2000; Hamre et al., 2003). Antioxidants also affect the oxidative status of seafoods, hence their measurement was also included in this study (Bell et al., 2000; Gieseg et al., 2000; Undeland, Hall, & Lingert, 1999).

Examination of TBARS values from both plants suggests that their mechanical handling did not adversely affect the oxidative status of the mussels. This can be explained, at least partially, by the short period of time used in processing and immediate packing on ice. If larger batches of mussels are to be handled, requiring several hours of mechanical handling, further oxidative stress related studies should be conducted.

The TBARS values of mussels stored on ice from both plants for 14 days increased gradually up to 10 days, and thereafter declined. This decline in TBARS values after 10 days of storage should be considered when this index is used for monitoring lipid oxidation beyond 10 days of storage as it may lead to false low values (Shahidi & Spurvey, 1995; Shahidi & Pegg, 1993). The relatively large standard deviation among TBARS values of mussels during the later period of storage

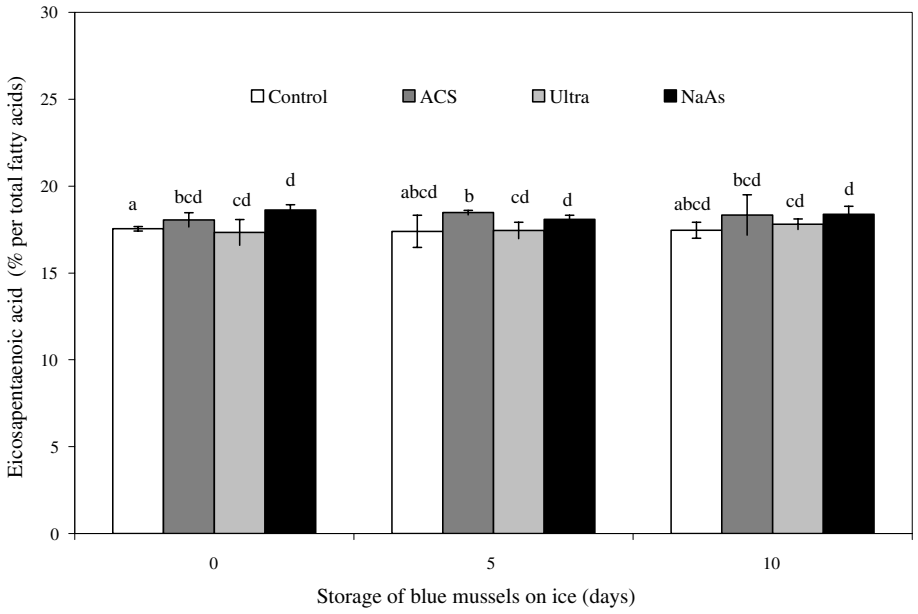

Fig. 5. Major polyunsaturated fatty acid, eicosapentenoic acid (20:5 $n-3$) (% of total fatty acids) of mussels treated with 10,000 μmol/L of various forms and grades of Asc and stored on ice for 10 days (Mean ± SD, $n = 3$). Means for each day with different letters are significantly ($p < 0.05$) different from each other.

Table 1
Fatty acid composition (%) of blue mussels treated with various types of ascorbic acid and stored on ice for 10 days

Fatty acid	Control		ACS		Ultra		NaAs	
	%	SD	%	SD	%	SD	%	SD
14:0	2.91	0.29	2.59	0.16	2.86	0.02	2.49	0.07
*i*15:0	0.54	0.02	0.46	0.03	0.51	0.00	0.31	0.27
*ai*15:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15:00	0.26	0.06	0.18	0.01	0.28	0.00	0.29	0.13
*i*16:0	0.12	0.10	0.00	0.00	0.17	0.00	0.06	0.10
*ai*16:0	0.05	0.09	0.00	0.00	0.07	0.06	0.03	0.05
16:0	17.70[a]	0.74	15.66[a]	1.04	16.89[a]	0.16	16.60[a]	0.48
16:1*n* − 7	8.84[a]	0.42	9.74[a]	0.64	8.53[a]	0.07	8.32[a]	0.21
16:1*n* − 5	0.33	0.02	0.34	0.02	0.29	0.03	0.22	0.19
*i*17:0	0.82	0.00	0.60	0.05	0.75	0.01	0.76	0.01
*ai*17:0	0.35	0.16	0.53	0.22	0.33	0.13	0.42	0.07
16:2*n* − 4	0.53	0.06	0.56	0.05	0.44	0.01	0.56	0.08
17:0	0.76	0.01	0.20	0.34	0.68	0.01	0.70	0.01
16:3*n* − 4	2.75	0.60	2.52	0.13	3.29	0.06	2.80	0.06
16:4*n* − 3	0.34	0.02	0.08	0.14	0.31	0.04	0.31	0.01
16:4*n* − 1	0.09	0.15	0.06	0.10	0.24	0.05	0.08	0.14
18:0	3.81	0.24	2.47	0.16	3.27	0.04	3.72	0.10
18:1*n* − 9	0.36	0.05	0.36	0.02	0.32	0.01	0.23	0.20
18:1*n* − 7	1.81	0.06	1.92	0.12	1.65	0.02	2.36	0.05
18:1*n* − 6	3.22	0.17	2.87	0.18	3.19	0.03	3.04	0.06
18:1*n* − 5	0.41	0.07	0.41	0.03	0.40	0.01	0.39	0.04
18:2*n* − 6	1.59	0.11	1.69	0.13	1.48	0.01	1.45	0.01
18:2*n* − 4	0.30	0.04	0.38	0.01	0.32	0.06	0.34	0.02
18:3*n* − 6	0.38	0.08	0.55	0.03	0.36	0.05	0.45	0.04
18:3*n* − 3	1.09	0.05	1.14	0.07	1.04	0.01	1.10	0.03
18:4*n* − 3	3.33	0.29	3.86	0.22	3.33	0.04	3.43	0.10
18:4*n* − 1	0.38	0.03	0.58	0.03	0.35	0.01	0.37	0.02
20:0	0.00	0.00	1.10	0.07	0.93	0.03	1.00	0.05
20:1*n* − 9	3.31	0.15	2.82	0.19	2.91	0.02	3.14	0.09
20:1*n* − 7	1.36	0.04	1.29	0.09	1.31	0.09	1.29	0.03
20:2 Δ 5,11	1.95	0.10	1.44	0.76	1.77	0.09	1.89	0.05
20:2 Δ 5,13	0.48	0.03	0.65	0.20	0.47	0.10	0.50	0.02
20:2*n* − 6	0.60	0.05	0.97	0.28	0.55	0.05	0.62	0.02
20:3*n* − 3	0.42	0.04	0.45	0.03	0.35	0.00	0.27	0.24
20:4*n* − 6	1.35	0.02	1.24	0.07	1.36	0.05	1.31	0.03
20:4*n* − 3	0.12	0.22	0.13	0.23	0.32	0.00	0.12	0.20
20:5*n* − 3	17.46[a]	0.46	18.34[a]	1.16	17.81[a]	0.30	18.38[a]	0.46
22:2 delta 7,13	2.01	0.11	2.12	0.12	1.76	0.01	1.93	0.02
22:2 delta 7,15	0.15	0.26	0.70	0.38	0.61	0.37	0.90	0.55
21:5*n* − 3	0.25	0.43	0.21	0.36	0.40	0.35	0.18	0.32
22:5*n* − 6	0.00	0.00	0.00	0.00	0.27	0.00	0.08	0.14
22:5*n* − 3	1.13	0.09	0.62	0.54	1.03	0.02	1.22	0.46
22:6*n* − 3	13.55	0.24	13.08	0.86	13.42	0.32	14.48	0.92
Σ								
Saturated	24.68[a]	1.33	22.00[a]	1.44	24.23[a]	0.25	24.1[a]	0.83
Monounsaturated	19.64[a]	1.13	19.75[a]	1.39	18.6[a]	0.3	18.99[a]	1.01
Polyunsaturated	50.25[a]	3.3	51.37[a]	5.77	51.28[a]	1.9	52.77[a]	3.78

Values are mean of three determinations with the standard deviations (SD). ACS and Ultra; ACS grade and Ultrapure ascorbic acid; NaAs, sodium ascorbate.

indicates that some mussels could have been very weak or dead due to high bacterial load, while others were resistant.

The pH of filtered seawater used in this study was close to neutrality at 0 h and after 24 h of mussels treated with various forms and grades of Asc. The decrease in pH values after the addition of NaAs, ACS and ultrapure Asc can be explained by dissociation of acid salts and acids in the aqueous medium (Anonymous a&b, 1983). Addition of 5 g of Asc to 1 L of water (pH = 7) can reduce the pH to 3, while addition of 50 g to 1 L of water will decrease the pH to 2 (Anonymous, 1983a). In the current study, 2 g of NaAs/L was used to obtain 0.01 M of NaAs and 1.76 g per liter was used to obtain 0.01 M of ACS and ultrapure grades Asc solutions. Buffering capacity of filtered seawater may not be

effective in resisting change in pH due to Asc addition. Acetic acid was used to control the pH of seawater during the addition of sodium ascorbate (Thed & Erickson, 1994). The pH of all treated and control mussels was about 6.5, except for ultrapure grade Asc treated mussels which was around 6.4 and is an anomaly.

The balance between endogenous pro- and antioxidants as well as exposure to physical, chemical and environmental variables determines the susceptibility of seafoods to oxidative damage (Bell et al., 2000; Erickson, 1997; Gieseg et al., 2000; Undeland et al., 1999). In this study, an attempt was made to increase the balance of water-soluble antioxidants through physical treatment with various forms and grades of Asc. This technique has been successfully used to control lipid oxidation during freezing of Channel catfish for up to six months (Erickson, 1997; Thed, Erickson, & Shewfelt, 1993). In a recent study, however, exposing of herring fish to 0.2 g Asc/L in tanks or the fishing vessel was ineffective in controlling lipid oxidation throughout 14 weeks of storage in freezers (Hamre et al., 2003). Spraying the same fish with 20 g Asc/L after harvest and filleting did control lipid oxidation for the first 9 weeks of storage, after which the fillets were unaffected by the treatment. This indicates that many variables, including concentration, application technique and storage period play an important role in the effectiveness of Asc as an antioxidant in seafoods. The current study, to the best of our knowledge, is the first to report the use of water-soluble natural antioxidants to control lipid oxidation in live bivalves stored on ice. Asc and its salts are highly susceptible to oxidation in aqueous solution, especially at high pH (Anonymous, 1983 a&b). This may explain, in part, considerable decrease in the antioxidant capacity of NaAs, ACS and ultrapure grades Asc in filtered seawater after 24 h of treatment of mussels.

The Asc absorbed by blue mussels exhibited antioxidant activity in controlling lipid oxidation during the first 5 days of storage. Thereafter, they become pro-oxidant. The strongest ($p < 0.05$) antioxidant activity on day 5 was observed for NaAs followed by ACS grade Asc and then by ultrapure grade Asc. This can be explained by the fact that Asc and tocopherol antioxidant capacities were not significantly ($p < 0.05$) lower on day 5 of storage of the control and treated mussels. After 10 days of storage, the reverse trend was observed and NaAs was more ($p < 0.05$) pro-oxidant followed by ACS grade Asc and finally ultrapure grade Asc. This trend of pro-oxidant activity corresponded with the reduced levels of antioxidant capacities of Asc and tocopherol in stored mussels, an observation that has also been reported in other studies (Brannan & Erickson, 1996; Yen et al., 2002). In addition, the loss of Asc is greater at refrigeration or ice than freezing storage temperatures. The Asc can act synergistically with tocopherols to protect against lipid oxidation up to certain limits and at specific Asc to tocopherol ratios and concentrations. Therefore, changes in these conditions may actually accelerate oxidation rather than inhibiting it (Bell et al., 2000; Gieseg et al., 2000; Undeland et al., 1999). Another explanation for pro-oxidant activity of Asc is the presence of iron in mussels. Asc at certain concentrations and in the presence of other water-soluble antioxidants such as glutathione peroxidase can keep the iron in the hemoglobin and myoglobin (both considered as potential pro-oxidants in seafoods) in the reduced form (Halliwell & Whiteman, 1997; Ramannathan & Das, 1992; Richards, Stephen, & Hultin, 1998). However, a reduction in Asc, glutathione and other endogenous antioxidants, as well as the presence of free iron or other minerals, due to prolonged storage and cell death may enhance lipid oxidation (Ramannathan & Das, 1992; Romeo, 1997; Yen et al., 2002). In fact, the iron–ascorbate system is used in biological systems to initiate lipid oxidation in vitro (Halliwell & Whiteman, 1997).

Exposing mussels to various grades and forms of Asc did not change, to any significant ($p < 0.05$) level, the major saturated, monounsaturated and polyunsaturated fatty acids as well as their total amounts in stored mussels. Although significant ($p < 0.05$) differences were observed in the 16:0 and 16:1$n-7$, they were not sufficient to affect acceptance or rejection of the treated mussels.

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