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Quality Indicators of Cultured Newfoundland Blue Mussels (*Mytilus edulis*) during Storage on Ice: Microbial Growth, pH, Lipid Oxidation, Chemical Composition Characteristics, and Microbial Fatty Acid Contents

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To devise proper strategies to evaluate and maintain the quality of cultured blue mussels (*Mytilus edulis*) during storage on ice, this study examined the potential use of microbial growth, pH, lipid oxidation, and composition characteristics as quality indicators. It also evaluated the use of individual (*i*15:0, *ai*15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0) and total bacterial fatty acid contents as a rapid method to examine the microbial quality of stored mussels. Linear regression analyses of the storage period of mussels vs various quality indicators indicated that the strongest correlation was between the storage period and microbial growth ($r = 0.973$, $p < 0.0001$) followed by the microbial fatty acid *ai*15:0 content ($r = 0.903$, $p < 0.0001$) and ash content ($r = 0.819$, $p = 0.0002$). All other correlations between the storage period and quality indicators were moderate ($r = 0.500-0.700$ and $p < 0.05$), poor ($r < 0.500$ and $p > 0.05$), or negative ($r = -0.657$ and $p = 0.027$).

KEYWORDS: Quality indicators; storage on ice; blue mussels; *Mytilus edulis*; microbial growth; pH; lipid oxidation; proximate composition; bacterial fatty acids

INTRODUCTION

Blue mussels are a favorite shellfish species consumed in North America, Europe, Japan, and China. Although frozen, vacuum-packed, pickled, smoked, and canned mussels are available in the market, most of the cultured mussels in Newfoundland or elsewhere are kept alive on ice or refrigerated (2−4 °C) until consumed. During refrigeration of fresh fish and shellfish, some microbiological, chemical, physical, biochemical, and sensory changes may occur depending on the duration and conditions of storage as well as the initial quality of the product (*1*−*4*). There is no universal method or instrument that can determine the quality of seafoods. Sensory methods are, perhaps, the most accurate quality predictors, but these are not used for routine analysis due to the need for highly trained personnel and the time-consuming procedures involved (*5*). Nonetheless, microbial, chemical, and biochemical methods are used in conjunction with sensory analysis to evaluate seafood quality (*5*−*7*). The type of analysis required by food regulations, accuracy, and the availability of instruments will determine the selection of a technique or an instrument to examine the quality of blue mussels.

Microbial growth in stored blue mussels is generally evaluated by the conventional total plate count method, which may take up to 3 days before results are available (*8*). The total plate count represents the number of bacteria that are capable of forming visible colonies at a specified temperature, and thus, high total bacterial counts (10^6-10^7 colony-forming units (CFUs)/g) may indicate poor handling, inappropriate storage conditions and transportation temperature, and old or expired seafood products (*9*). The proximate composition (protein, fat, carbohydrate, moisture, and ash) and lipid oxidation of mussels and other seafoods reflect the nutritional and flavor qualities of stored products, respectively (*10*, *11*). The TBA (2-thiobarbituric acid) test is frequently used to measure lipid oxidation in seafoods (*12*−*14*). One molecule of malonaldehyde (MA), a secondary product of lipid oxidation, reacts with two molecules of TBA to form a pink-colored complex which absorbs at 532 nm. TBA may also react with other lipid oxidation products such as 2-alkenals and 2,4-alkadienals, and thus, [TBARS] (TBA reactive substance concentration) reflects the total content of reactive aldehydes in the TBA test. Nonetheless, TBA values correlate well with sensory data (*15*).

The microbial content of seafoods must be known before the product leaves the production area. Therefore, rapid identification methods of microorganisms in seafoods are important for the aquaculture industry and public safety, and several rapid

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methods have been developed to handle large amounts of sample within reasonable time limits (*16*, *17*). Many of the newly developed testing methods may still take between 12 and 24 h (1−2 working days) because of preenrichment steps or the need to prepare pure bacterial cultures. Specific fatty acids (odd carbon number and branched chain) are found in microorganisms and thus may serve as lipid bacterial markers (*18*−*20*). Fatty acid profiles have been used not only to investigate the microbial communities in clinical and environmental samples but also to examine the occurrence of healthful fatty acids in seafoods (*21*−*23*). However, the use of lipid bacterial markers to examine the microbial content of blue mussels has not been reported. Because fatty acid content determination may require only 2−4 h, the lipid marker method may serve as the most practical method to examine the microbial quality of seafoods, including blue mussels.

Culturing of blue mussels is a fast-growing industry in Newfoundland. The blue mussel industry is expected to expand and grow further to meet the high demand of this nutritional delicacy in Europe, the United States, and elsewhere in the world. Despite rapid increases in the production of blue mussels, information about their quality changes during storage of cultured mussels on ice is scarce and fragmented in the literature. To devise proper strategies to evaluate and maintain the quality of cultured mussels, the current study was designed (1) to examine the changes occurring in microbial growth, pH, lipid oxidation, proximate composition, and fatty acid profile during storage of cultured Newfoundland blue mussels (*Mytilus edulis*) on ice and (2) using statistical analysis to select the most appropriate indices for monitoring quality changes during storage.

MATERIALS AND METHODS

Materials. Compressed air, hydrogen, and ultra-high-purity (UHP) helium were obtained from Canadian Liquid Air Ltd. (St. John's, NL, Canada). Marine agar was purchased from Becton Dickinson Microbiology Systems (Sparks, MD). Trichloroacetic acid was obtained from Fisher Scientific (Nepean, ON, Canada). Polyunsaturated fatty acid (PUFA) mixtures, namely, PUFA 1 and PUFA 3, as well as the Supleco 37 component FAME (fatty acid methyl ester) mixture and bacterial acid methyl ester mixture were acquired from Supelco Canada Ltd. (Oakville, ON, Canada). The standard fatty acid methyl ester preparation (GLC-461) was purchased from Nu-Check-Prep (Elyasin, MN). 2-Thiobarbituric acid (TBA) and 1,1,3,3-tetramethoxypropane (TMP) were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were ACS grade or better.

Sample Collection. Three different batches of Newfoundland cultured blue mussels were obtained from a processing plant 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)) and used in this study. The mussels were placed in three Styrofoam containers filled with crushed ice. The ice was replaced on a daily basis. Each container held between 90 and 100 mussels.

Sample Preparation for Analyses. Mussels (9−10) were removed on days 0, 3, 7, 10, and 14, the shells were opened, and the shell liquor and meats were collected in a beaker and subsequently homogenized for 60 s using a commercial Waring blender (Dynamic Corp. of America, New Hartford, CT). The homogenate was used for the determination of changes in microbial growth, pH, lipid oxidation, and proximate and fatty acid composition.

Microbiological Analysis. A 10 g sample of the homogenate was mixed with 90 mL of 0.1% peptone water (10 g of peptone and 5 g of NaCl in 1 L of distilled water, pH 7.2−7.3) for a further 30 s. Serial decimal dilutions from this mixture were carried out in 9 mL of 0.1% peptone water. The diluted samples (0.1 mL) were spread with a sterile glass spreader on prepoured marine agar (Becton Dickinson Microbiology Systems). Inoculated plates were incubated at 25 °C for 48 h to determine aerobic plate counts (APCs). Bacterial colonies on the agar were counted with a Quebec counter and recorded as CFUs per gram of mussel meat.

pH Measurement of the Samples. The pH of the homogenized mussel samples was determined by immersing a pH electrode into the homogenate for 60 s and observing the pH on a precalibrated Fisher Accumate pH meter, model 805 MP (Fisher Scientific, Fair Lawn, NJ).

Determination of [TBARS] as an Index of the Off-Flavor of Mussel Meat. Changes in lipid oxidation and hence off-flavor development of mussel meat were evaluated using the TBARS test as described by Siu and Draper (*24*) with minor modifications. A 5 g portion of the homogenized sample was placed in a 50 mL centrifuge tube to which 20 mL of a 10% (w/v) solution of trichloroacetic acid (TCA) and 20 mL of distilled water were added, and the mixture was vortexed (Fisher Vortex Genie 2, Fisher Scientific, Nepean, ON, Canada) at high speed for 2 min. The mixture was then centrifuged at 4000*g* for 5 min, and the supernatants were filtered through a Whatman No. 3 filter paper. A 1 mL sample 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 and cooled to room temperature, and the absorbance of the resultant colored solution was read at 532 nm with a Hewlett-Packard diode array spectrophotometer (model 8452A, Hewlett Packard Co., Palo Alto, CA). [TBARS] values (expressed as mg of malonaldehyde equivalents/kg of 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.

Proximate Composition. Moisture and ash contents were determined as described previously (*25*). The crude protein content (% nitrogen × 6.25) of blue mussels was determined by the Kjeldahl method as described previously (*28*). The total lipid content was quantified as described by Budge and Parrish (*20*). The carbohydrate content in each sample was determined by difference as follows: % carbohydrate = 100 − (% moisture + % ash + % protein + % lipid).

Fatty Acid Composition. *Preparation of FAMEs Using 6% (v/v) Sulfuric Acid in Methanol*. Blue mussel lipid extract (10 mg) was weighed into 6 mL of Chromerge-cleaned (Fisher Scientific, Fair Lawn, NJ), screw-capped, Teflon-lined, conical vials. Subsequently, 2 mL of transmethylation reagent was added to each vial, and its contents were mixed by vortexing (Fisher Vortex Genie 2) at high speed for 1 min. This reagent was prepared freshly each day by adding 6 mL of concentrated sulfuric acid to a 100 mL volumetric flask, then making the solution up to volume with spectral-grade methanol, and adding 15 mg of the antioxidant *tert*-butylhydroquinone (TBHQ). The mixture of fatty acids and reagent was incubated at 60 °C for 17 h. The vials were then cooled to room temperature, and 1 mL of distilled water was added. The FAMEs were extracted three times with 1.5 mL of *n*-hexane. A few crystals of TBHQ were added to each vial before the extraction to prevent oxidation of PUFAs. The hexane layers were separated, combined, transferred to a clean dry tube, and washed twice with distilled water. The hexane layer was separated and evaporated under a stream of nitrogen at room temperature (*26*).

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 (Hewlett-Packard, Toronto, ON, Canada) equipped with a 30 m × 0.25 mm Supelcowax-10 column (SP 2330, Supelco Canada Ltd.). The injector and flame ionization detector temperatures were both 270 °C. The oven temperature was initially 220 °C for 10.25 min, was then increased to 240 °C at 30 °C/min, and was held there for 9 min. Helium (UHP) was used as the carrier gas. The FAMEs were identified by comparing their retention times with those of authentic standard mixtures (Materials and Methods) and quantified using area normalization. The fatty acid profile was reported as the weight percentage of the total fatty acids.

Statistical Analysis. The experiments were replicated using three different batches ($n = 3$), and the homogenate of 9−10 mussels was used for different analyses per sampling day per storage container, as explained in the sample collection and preparation sections. The mean ± standard deviation was reported for each case. Prior to statistical analysis, results of microbiological analysis were transformed to log values (*27*). Analysis of variance (ANOVA) and Tukey's studentized

Table 1. Changes in Various Potential Quality Indicators during Storage of Cultured Newfoundland Blue Mussels (*M. edulis*) on Ice[a]

storage time on ice (days)	microbial growth[b]	pH	[TBARS][c]	moisture content[d]	protein content[d]	lipid content[d]	[3]Carbohydrates	[3]Ash
0	4.24 ± 0.03 a	6.42 ± 0.01 a	2.73 ± 0.33 abce	79.86 ± 0.62 a	12.64 ± 0.38 a	2.55 ± 0.04 a	3.55 ± 0.59^{abcde}	1.41 ± 0.34^{abcd}
3	5.30 ± 0.01 b	6.52 ± 0.02 bcde	2.72 ± 0.30 bc	79.98 ± 0.80 a	12.67 ± 1.22 a	2.44 ± 0.14 a	4.42 ± 1.04^{bcd}	1.48 ± 0.33^{bcd}
7	6.88 ± 0.02 c	6.57 ± 0.02 cde	3.76 ± 1.10 c	80.95 ± 0.63 a	12.02 ± 0.74 a	2.49 ± 0.25 a	2.68 ± 0.70^{cde}	1.86 ± 0.40^{cd}
10	7.72 ± 0.03 d	6.58 ± 0.02 de	5.53 ± 0.50 d	80.38 ± 0.95 a	12.27 ± 0.14 a	2.48 ± 0.07 a	2.71 ± 0.90^{de}	2.16 ± 0.09^{de}
14	8.04 ± 0.05 e	6.50 ± 0.05 e	4.19 ± 0.11 abce	80.14 ± 0.52 a	12.88 ± 0.80 a	2.44 ± 0.05 a	2.01 ± 0.70^{e}	2.53 ± 0.42^{e}

[a] Values are means from various experiments ($n = 3 \pm$ SD). Means with different letters in each column are significantly ($p < 0.05$) different from one another. [b] CFUs/g of meat. [c] Thiobarbituric acid reactive substances concentration as mg of malonaldehyde equivalents/kg of meat. [d] Proximate composition (g/100 g of wet weight).

test were performed at a level of $p < 0.05$ to evaluate the differences between mean values (*26*). Linear regression analyses were performed to establish the statistical relationships between storage days and various quality indices examined. Various statistical parameters were used to examine the strength of the examined linear relationships including the correlation coefficient (r) and level of significance (p) values. Relationships with $r < 0.5$ and $p > 0.05$ were considered to be poorly correlated, relationships with $r = 0.500-0.700$ and $p < 0.05$ were considered moderately correlated, and relationships with $r > 0.700$ and $p < 0.05$ were strongly correlated (*28*−*30*).

RESULTS

Changes in Microbial Growth, pH, Lipid Oxidation, and Proximate Composition during Storage on Ice. Changes in various potential quality indicators during storage of cultured blue mussels are summarized in **Table 1**. There was a rapid increase in total bacterial counts for 10 days in mussels stored on ice, after which the growth remained nearly constant. The initial bacterial count varied among the three batches from log 4.22 to log 4.28 (CFUs/g) at 0 days, while after 14 days of storage on ice it varied from log 7.98 to log 8.08 (CFUs/g). Initial pH values were around 6.4, thereafter increasing to about 6.5 and remaining there throughout the entire storage period. [TBARS] values increased gradually and reached a peak value of 5.53 ± 0.5 mg of malonaldehyde equivalents/kg of mussel meat after 10 days of storage on ice, thereafter declining to 4.19 ± 0.11 mg of malonaldehyde equivalents/kg of mussel meat. There were no significant ($p > 0.05$) differences in the moisture, protein, and lipid contents throughout the storage period. However, the percent ash content of mussels stored on ice was significantly ($p < 0.05$) higher for those stored for 14 days as compared to those stored for 0 or 3 days. There was no significant ($p > 0.05$) difference in the ash content among mussels stored for 7, 10, and 14 days. The carbohydrate content was significantly ($p < 0.05$) lower in mussels stored on ice after 14 days compared to mussels stored for 3 days. However, there was no significant difference ($p > 0.05$) in carbohydrate content among mussels stored for 0, 7, 10, and 14 days.

Fatty Acid Profiles during Storage. **Table 2** summarizes fatty acid profiles of cultured blue mussels stored on ice. Statistical comparison was performed on selected saturated, monounsaturated, polyunsaturated, and bacterial fatty acids that are related to human nutrition or the microbial quality of stored mussels. The content of the saturated 16:0 fatty acid (SFA) was initially (at day 0) low at about 14%, then increased to about 15−16%, and remained at this level throughout the storage period. A similar trend was observed for the total SFA content. The monounsaturated fatty acid (MUFA) 16:1$n-7$ and total MUFA contents were significantly ($p < 0.05$) higher after 7, 10, and 14 days of storage compared to those of mussels stored for 3 days. No significant ($p > 0.05$) difference was observed in the PUFA docosahexanoic acid (DHA) or the total PUFA content throughout the storage period. The content of bacterial fatty acid (BFA) *ai*15:0 in mussels increased gradually to its highest level after 3 days, but thereafter, there was no significant ($p > 0.05$) difference in its content among the stored mussels. Moreover, there was no significant ($p > 0.05$) difference in the total BFA content of the stored mussels throughout the storage period.

Linear Regression Analyses of the Storage Period on Ice vs Various Quality Indicators (Microbial Growth, pH, Lipid Oxidation, and Proximate Composition). Figure 1 and **Table 3** summarize the results of linear regression analyses of the storage period of cultured mussels vs various potential quality indicators (microbial growth, pH, lipid oxidation, and proximate composition). The strongest correlations were between the storage period and microbial growth ($r = 0.973$ and $p < 0.0001$) and ash content ($r = 0.819$ and $p = 0.0002$). All other correlation values between the storage period and the remaining quality indicators were moderate ($r < 0.700$ and $p > 0.05$), poor ($r < 0.500$ and $p > 0.05$), or negative ($r = -0.657$ and $p < 0.05$).

Linear Regression Analyses of the Storage Period on Ice vs Individual and Total Bacterial Fatty Acids. **Figure 2** and **Table 4** summarize liner regression analyses of the storage period vs individual (*i*15:0, *ai*15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, and 17:0) and total bacterial fatty acid contents. The strongest correlation was between the storage period and the bacterial fatty acid *ai*15:0 content ($r = 0.903$ and $p < 0.0001$). All other correlations between the storage period and the remaining bacterial fatty acid contents were moderate ($r = 0.500-0.700$ and $p < 0.05$) or poor ($r < 0.500$ and $p > 0.05$).

DISCUSSION

During storage of seafoods on ice, various chemical, microbiological, and sensory changes occur that lead to a reduction in the quality and consumer rejection of products (*2*, *3*). Understanding these changes may lead to the development of new techniques for maintaining the premium quality of seafoods and selection of the most appropriate indices for monitoring quality changes of seafoods during storage (*4*, *5*, *8*, *31*). Linear regression analyses are used to support the selection process. Various parameters such as the correlation value and level of significance are used to examine the strength of the linear relationships (*28*, *29*).

Various maximum acceptable microbial levels of APC (10^5-10^7) in fish and shellfish to fit human consumption have been set in different countries (*8*, *9*). However, spoilage of seafoods may not be visible until bacterial counts reach 10^8, and thus, storage studies are carried out till this level is reached (*32*). It is generally recommended that APC on PCA supplemented with 1% NaCl should not exceed 10^5 or log 5 CFUs/g (*33*). Because there is no information on the maximum acceptable levels of microbial counts on MA, log 5 counts was considered in this study to be the maximum APC acceptable level of stored

Table 2. Fatty Acid Composition (%) of Blue Mussels Stored on Ice for 14 Days ($n = 3 \pm$ SD)[a]

fatty acid	0 days of storage	SD	3 days of storage	SD	7 days of storage	SD	10 days of storage	SD	14 days of storage	SD
14:0	4.00	0.02	5.61	0.04	4.54	0.20	3.90	0.11	4.36	0.13
*i*15:0	0.45	0.08	0.56	0.01	0.48	0.05	0.44	0.01	0.45	0.01
*ai*15:0	0.00 a	0.00	0.09 b	0.08	0.17 cde	0.03	0.17 d	0.00	0.18 e	0.00
*i*16:0	0.17	0.06	0.18	0.00	0.18	0.02	0.17	0.01	0.16	0.01
*ai*16:0	0.16	0.03	0.12	0.01	0.16	0.02	0.16	0.01	0.17	0.01
16:0	14.43 a	0.60	16.29 bcde	0.04	16.73 cde	0.56	15.66 de	0.43	15.75 e	0.49
16:1*n*–7	11.95 ad	0.45	10.91 b	0.05	13.57 c	0.13	12.18 d	0.12	12.88 e	0.14
16:1*n*–5	0.38	0.03	0.44	0.00	0.42	0.01	0.36	0.01	0.39	0.01
*i*17:0	0.67	0.04	0.63	0.00	0.75	0.04	0.75	0.03	0.73	0.03
*ai*17:0	0.60	0.05	0.56	0.01	0.64	0.03	0.62	0.02	0.61	0.03
16:2*n*–4	1.02	0.06	1.06	0.21	1.05	0.01	0.99	0.00	1.01	0.00
17:0	0.39	0.03	0.25	0.22	0.41	0.02	0.44	0.02	0.43	0.02
16:3*n*–4	0.67	0.07	0.50	0.00	0.64	0.01	0.04	0.07	0.20	0.35
16:4*n*–3	0.59	0.05	0.63	0.01	0.73	0.04	1.69	0.03	1.41	0.34
16:4*n*–1	1.74	0.18	0.78	0.06	1.52	0.11	1.82	0.08	1.74	0.11
18:0	1.95	0.12	1.67	0.02	2.02	0.08	2.23	0.06	2.39	0.08
18:1*n*–9	1.33	0.06	1.10	0.01	1.26	0.21	1.51	0.02	1.59	0.02
18:1*n*–7	2.31	0.02	2.29	0.03	2.36	0.06	2.34	0.06	2.33	0.05
18:1*n*–5	0.32	0.03	0.32	0.01	0.33	0.01	0.35	0.03	0.36	0.02
18:1*n*–6	2.48	0.11	3.20	0.02	2.65	0.03	2.65	0.03	3.15	0.66
18:2*n*–4	1.37	0.01	1.28	0.05	1.30	0.02	1.32	0.02	1.39	0.06
18:3*n*–3	0.39	0.06	0.36	0.01	0.35	0.01	0.42	0.01	0.39	0.01
18:4*n*–3	4.06	0.14	3.54	0.04	3.76	0.14	3.75	0.09	3.88	0.10
20:1*n*–9	2.42	0.12	2.51	0.01	2.30	0.02	2.88	0.03	2.49	0.26
20:1*n*–7	0.92	0.08	0.84	0.00	0.79	0.01	0.98	0.07	1.09	0.04
20:2(5,11)	1.08	0.12	0.45	0.08	1.00	0.02	1.35	0.11	1.25	0.05
20:2(5,13)	0.53	0.26	0.00	0.00	0.23	0.01	0.39	0.03	0.36	0.06
20:2*n*–6	0.51	0.15	0.55	0.01	0.39	0.01	0.33	0.29	0.40	0.16
20:4*n*–6	0.82	0.03	0.87	0.37	0.91	0.04	0.83	0.08	0.80	0.02
20:4*n*–3	0.94	0.02	1.12	0.03	0.98	0.04	0.99	0.05	1.12	0.09
20:5*n*–3	20.51	0.64	22.20	0.62	21.56	1.24	18.26	0.11	18.71	0.82
22:2Δ(7,13)	1.02	0.03	0.52	0.01	0.91	0.02	1.20	0.00	0.00	0.00
22:2Δ(7,15)	0.42	0.03	0.32	0.27	0.50	0.04	0.51	0.03	0.54	0.04
22:5*n*–3	0.75	0.03	0.71	0.08	0.78	0.03	0.73	1.26	10.17	0.54
22:6*n*–3	11.05 a	0.32	11.69 a	0.33	10.42 a	0.76	10.08 a	3.13	8.06 a	1.16
Σ										
SFAs	20.38 ad	0.74	23.58 bce	0.10	23.29 cde	0.84	21.79 de	0.60	22.49 e	0.70
MUFAs	22.11 abcd	1.08	21.70 b	0.27	23.85 cde	0.62	23.42 de	0.44	24.47 e	1.31
PUFAs	45.74 a	2.01	45.79 a	2.13	45.51 a	2.43	42.88 a	5.31	49.69 a	3.80
BFAs	2.45 a	0.30	2.30 a	0.24	2.63 a	0.18	2.58 a	0.09	2.55 a	0.10

[a] Means with different letters in each row are significantly different ($p < 0.05$) from each other. SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, and BFA = bacterial fatty acids.

cultured mussels fit for human consumption. On the basis of this limited criterion, the acceptable shelf life for human consumption will only be about 3 days on ice. However, the spoilage of stored mussels continued after 3 days, reflected in the increase in bacterial counts. The strong correlation between the storage period and bacterial counts indicates the suitability of these counts as a spoilage quality indicator.

The pH has been used as an indicator of the freshness of seafoods, including mollusks, and therefore, it is not surprising that a moderate correlation is observed between this quality indicator and the storage period. Fresh and good-quality bivalves exhibit pH values varying from 6 to 7 (*31*). According to this criterion, mussels on day 0 were considered to be fresh. Bacterial fermentation of carbohydrates in seafoods may lead to production of organic acids, resulting in a reduction in pH and thus the quality (*2*, *4*, *31*). However, the observed increase in the pH of stored mussels may be explained, at least partially, by the production of alkaline compounds due to proteolytic activity or bacterial alkaline metabolites resulting from the growth of psychrotrophic bacteria during ice storage (*6*, *34*).

The TBARS test has been used frequently as a lipid oxidation indicator and thus development of off-flavor in seafoods (*12*–*15*). Data from this study ($r = 0.693$ and $p = 0.004$) also support the use of [TBARS] as a quality indicator of stored blue mussels. However, a decline in [TBARS] values after 10 days of ice storage of blue mussels should be questioned when this index is to be used as a lipid oxidation indicator beyond 10 days as it may lead to false low values (*35*, *36*). No sensory analyses were performed to determine if oxidative off-flavor was developed. However, previous studies suggest that [TBARS] values correlate well with sensory scores (*25*).

While some studies have examined changes in proximate composition in blue mussels due to processing or seasonal changes (*37*, *38*), no previous study appears to have reported changes in the proximate composition of cultured Newfoundland blue mussels during storage on ice. The proximate composition of mussels stored on ice on day 0 (1.4% ash, 79.86% moisture, 2.55% lipids, 12.64% protein, and 3.55% carbohydrate) is similar to that reported in the literature for blue mussels cultured in other countries, with some exceptions due to seasonal and reproductive cycle effects (*39*, *40*). The use of the whole mussel body, rather than specific parts, for analysis and the relatively short period of storage on ice (14 days only) may have hindered detection of marked changes in moisture, protein, and lipid contents (*41*). Furthermore, a decrease of the carbohydrate content during storage ($r = -0.657$ and $p = 0.027$) should be confirmed by actual measurement instead of obtaining the values from difference data calculations. Therefore, on the basis of this

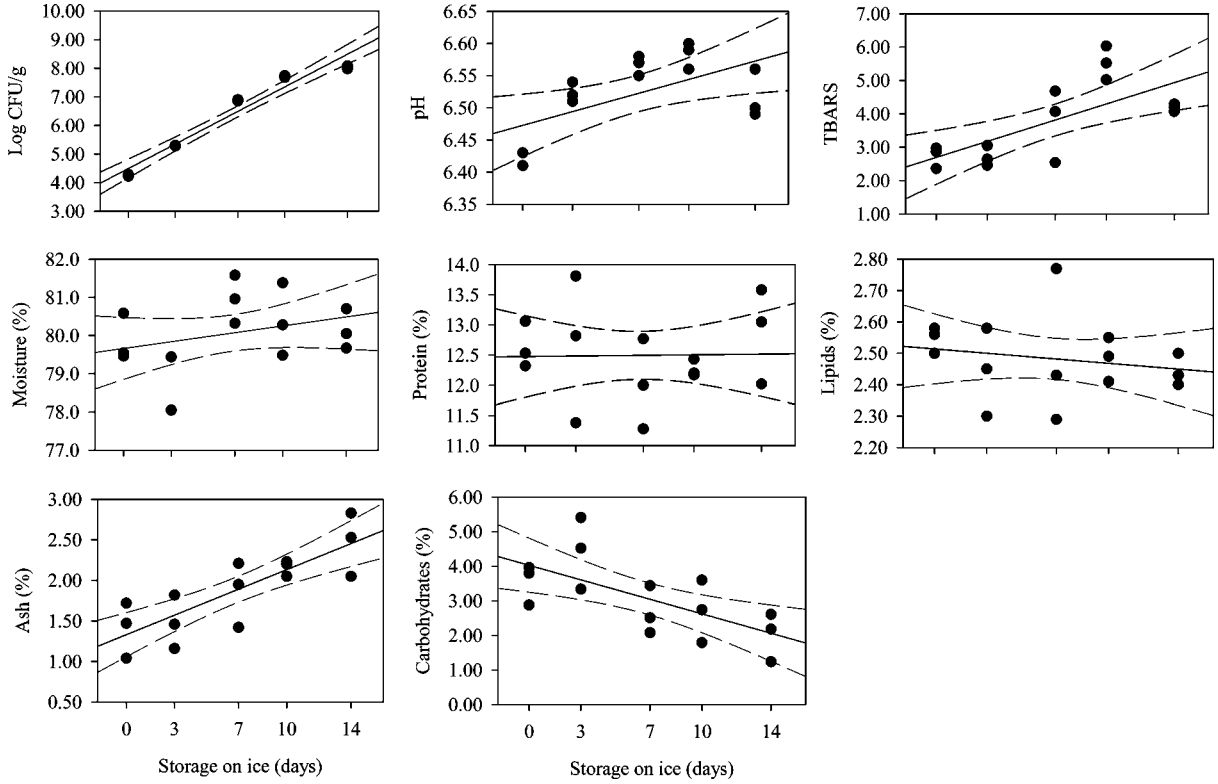

Figure 1. Linear regression lines of the blue mussel storage period on ice vs various potential quality indicators (microbial growth, pH, lipid oxidation, and proximate composition). The dashed lines indicate the confidence level (95%). TBars are in mg malonaldehyde equivalents per kg meat.

Table 3. Linear Regression Analyses of the Storage Period of Newfoundland Cultured Blue Mussels (*M. edulis*) on Ice vs Various Potential Quality Indicators (Microbial Growth, pH, [TBARS], and Proximate Composition)[a]

quality indicator during storage on ice	correlation coefficient (*r*)	level of significance (*p*)
microbial growth	0.973	<0.0001
pH	0.582	0.023
[TBARS]	0.693	0.004
moisture content	0.333	0.225
protein content	0.020	0.944
lipid content	0.196	0.483
ash content	0.819	0.0002
carbohydrate content	–0.657	0.027

[a] TBARS = thiobarbituric acid reactive substances.

study, changes in moisture, protein, and carbohydrate contents during the storage of blue mussels are not recommended as quality indicators. There was a marked increase in the ash content after 14 days of storage corresponding to a relative decrease in the content of carbohydrate and without changes in the moisture content. Others have reported changes in the ash content of blue mussels harvested in different seasons and due to processing, but have given no strong reasons for the variability (*37*, *38*). Without proper explanation to what caused the increase of the ash content during storage of blue mussels in this study, the change in the ash content is not a suitable quality indicator of stored mussels.

Statistical analyses of individual and total SFA, MUFA, and PUFA profiles of mussels stored on ice showed no major difference throughout the storage period, with some exceptions. Even though some statistical differences existed on certain storage sampling days, these would not affect the consumer's perception from a nutritional point of view and therefore the market value of the cultivated mussels. Others have reported similar observations and reached similar conclusions. For example, Xing et al. (*42*) reported no changes in the fatty acid profiles of lipid fatty fish (Atlantic mackerel) and lean fish (Atlantic cod) during storage on ice or in a freezer. In addition, when Lakshmanan (*43*) irradiated and stored Indian mackerel at 0−2 °C for 14 days, only slight differences in their lipid fatty acid composition were observed. Furthermore, Cho et al. (*44*) found that dried mussels and clams stored at 4 and 25 °C for 120 days had a slight increase in SFA content and a decrease in MUFA content and concluded that slight changes in fatty acid composition would not have major effects on consumers' acceptance or rejection of the dried products.

One interesting area that has attracted very limited attention is the use of bacterial fatty acids to examine the microbial status of refrigerated seafoods, despite the fact that these fatty acids have been used as lipid markers to examine the microbial communities in clinical and environmental studies (*18*−*22*). In addition, odd-numbered and branched-chain bacterial fatty acids have been detected in seafoods, including blue mussels (*23*). The current study, to the best of our knowledge, is the first to correlate storage time on ice (days) with individual and total bacterial fatty acid contents to find a microbial fatty acid content that can be used as a quality indicator. The bacterial fatty acid *ai*15:0 content correlated strongly with the storage period and can be a potential rapid quality indicator for stored cultured mussels. However, correlating *ai*15:0 levels with specific bacteria that contribute to spoilage of stored mussels and correlating *ai*15:0 levels with sensory analyses are among other studies required to confirm the suitability of *ai*15:0 content as a microbial quality indicator of cultured blue mussels.

ABBREVIATIONS USED

ANOVA, analysis of variance; BFA, bacterial fatty acid; CFU, colony-forming unit; FAME, fatty acid methyl ester; SFA,

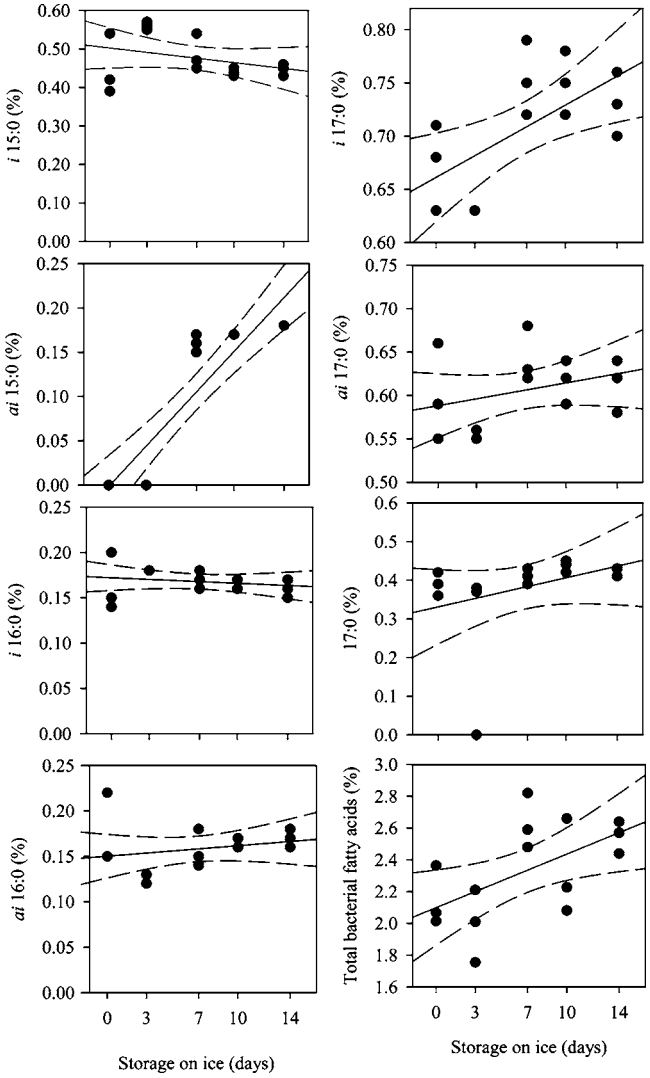

Figure 2. Linear regression lines of the blue mussel storage period on ice vs individual (*i*15:0, *ai*15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, and 17:0) and total bacterial fatty acid contents. The dashed lines indicate the confidence level (95%).

Table 4. Linear Regression Analyses of the Blue Mussel Storage Period on Ice vs Individual (*i*15:0, *ai*15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, and 17:0) and Total Bacterial Fatty Acid Contents

quality indicator during storage on ice	correlation coefficient (*r*)	level of significance (*p*)
*i*15:0 content	0.333	0.223
*ai*15:0 content	0.903	<0.0001
*i*16:0 content	0.207	0.459
*ai*16:0 content	0.227	0.415
*i*17:0 content	0.621	0.014
*ai*17:0 content	0.329	0.232
17:0 content	0.355	0.194
total bacterial fatty acid content	0.568	0.027

saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; TBARS, thiobarbituric acid reactive substance; TBHQ, *tert*-butylhydroquinone; TCA, trichloroacetic acid.

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