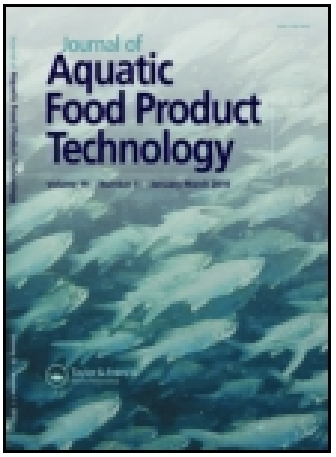

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Bioelectrical Impedance Analysis Measures of Body Composition and Condition, and Its Sensitivity to the Freezing Process

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Bioelectrical Impedance Analysis Measures of Body Composition and Condition, and Its Sensitivity to the Freezing Process

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Measuring body composition and condition are integral parameters within seafood quality assurance methods. Bioelectrical impedance analysis (BIA) has been successful in measuring body composition and condition on a variety of fish that have never been frozen, but its applicability on previously frozen fish is unknown. The objectives of this study were to measure albacore tuna (*Thunnus alalunga*) with BIA prior to and after freezing. Predictions of body composition and measures of condition (measured by phase angle) were compared in fish that were just caught and again after they were frozen and allowed to thaw. Results indicate that BIA works well on fresh fish but not after they were thawed. Correlation scores (r) between predicted and actual values of water, dry mass, fat-free mass, ash, and protein in the fresh fish were > 0.69 and < 0.17 after they were frozen. Phase angles of the fresh fish were similar to other studies ($\sim$ 24 degrees) and approached zero after they were frozen. Reactance values decreased dramatically in the frozen fish, and its usage on frozen fish may be useful to the seafood industry in determining if fish have previously been frozen.

Keywords: impedance, frozen fish, tuna, BIA sensitivity to freezing

INTRODUCTION

Seafood products are an important international commodity. Seafood products maintain their value when nutrition (i.e., body composition), taste, gastronomic delights, and sensory quality (i.e., condition/spoilage) are maintained (Huss, 1993). Understanding both composition and condition is important to seafood quality, because even a product with appropriate body composition values (e.g., high fat in tuna) can have a poor quality rating if the condition of the fish is poor (e.g., product has deteriorated due to spoilage). Being able to measure body composition and condition quickly and easily on previously frozen fish would benefit the seafood industry by improving quality assurance and marketability of fish products. Bioelectrical impedance analysis (BIA) has been shown to quickly and easily predict body composition and condition of freshly killed or anesthetized live fish, but its functionality on frozen fish is unknown (Cox and Hartman, 2005; Duncan et al., 2007; Willis and Hobday, 2008; Fitzhugh et al., 2010; Hartman et al., 2011). Research has been done using BIA to investigate how electrical measures change with freezing types (i.e., fast-frozen, slow-frozen,

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once-frozen, and twice-frozen; Vidacek et al., 2008), but models to predict body composition or condition were not made. If BIA can be used on previously frozen fish, predictions of body composition and condition could be used to enhance existing quality assurance programs that monitor seafood products.

International fishery seafood products are worth in excess of US$50 billion and when compared to terrestrial animal products, the quality of fish is more difficult to maintain (Venugopal, 2002). Quality of seafood products is affected from postmortem biochemical, physicochemical, and microbiological processes that are species specific and influenced by onboard handling and subsequent processing (Huidobro et al., 2000). Quality of the seafood product is important to the consumer and marketability of the product, therefore making assessments of seafood quality imperative and an essential discipline to guarantee safe, wholesome, and functional fisheries products (Huss, 1993, 1995). BIA usage on fresh and frozen fish may provide a means to enhance existing quality assurance programs.

Current methods of quality assurance that could be enhanced by using BIA on fresh and frozen fish include the quality index method (QIM; Huidobro et al., 2000), hazard analysis and critical control points (HACCP; Kvenberg et al., 2000). The QIM relies on weighted sensory evaluations of fish quality by trained personnel. In this, evaluations of fish quality are interpreted by the appearance, odor, flavor, and texture of the product (Venugopal, 2002; Takahashi et al., 2004). Aside from expense and training personnel, the QIM method is subjective and also species specific (Sant'Ana et al., 2011). Enhancements of QIM would be available if BIA can provide a quantitative assessment of previously frozen fish that are not species specific in a quantitative way (Cox and Heintz, 2009). For example, in the study by Cox and Heintz (2009), postmortem phase angles (measured by BIA) were shown to decrease with time after resolution of rigor mortis on adult pink salmon (*Oncoryhnchus gorbuscha*). The quantitative measure of phase angle could then be related to postmortem processes and used in quality assurance measures to determine when the product was frozen after the product was caught and killed.

Another method of quality assurance is HACCP, which sets forth ways to assure product safety by controlling food borne safety hazards (Kvenberg et al., 2000). The program addresses pathogens, filth, decomposition, pesticides, industrial chemicals, and marine biotoxins (Kvenberg et al., 2000). Using BIA on previously frozen fish would improve HACCP by providing decomposition measures and condition throughout the food chain (e.g., from fisherman to consumer). The importance of accurate measures of quality, body composition, and condition throughout the quality assurance process underscores the need to evaluate BIA on previously frozen fish. The objectives of this study were to assess BIA usage on frozen albacore tuna (*Thunnus alalunga*) by comparing body composition and conditional measures taken on fish before and after freezing and to evaluate changes in BIA measures on fish after the fish was frozen.

MATERIALS AND METHODS

To test whether BIA measures are affected by fish being frozen, measures of resistance (R) and reactance (Xc) were taken from fresh fish and from the same fish after they were frozen. Measures were used to predict body composition and calculate condition (from phase angle). Comparisons of R and Xc values, body composition, and phase angle were made between the fresh and frozen fish. Albacore tuna were collected ($n = 40$, mean length = 723 mm, mean weight = 6,599 g) by trolling on a commercial tuna boat in the Northwest Pacific ocean in July 2004. All fish were spiked, bled, measured the first time with BIA, placed on ice, and transported to the Oregon State University Seafood Laboratory and placed in a −20°C freezer. The commercial tuna boat was designated as an "ice boat" and did not have freezing capabilities, so all fish were placed on ice for the duration of the

cruise. Fish were on ice for < 7 days prior to freezing. After 2 days of being in the −20°C freezer, fish were removed from the freezer and allowed to thaw for several hours and measured with BIA a second time. Fish were allowed to thaw enough so that needle electrodes could be placed into the fish, as it was not possible for the needle electrodes to penetrate the musculature of frozen fish. One core temperature was taken from each fish by inserting a standard electronic meat thermometer approximately 7 cm into the vent of the fish. Although fish were completely frozen and then partially thawed at the time of the second measurement, fish that were measured after they were frozen will hereafter be referred to as the "frozen fish."

Measures of R and Xc were taken on the fresh and frozen fish with a tetrapolar BIA (RJL Systems, Detroit, MI, USA). The analyzer had two sets of needle electrodes (stainless, 28 gage, 12 mm; Grass Telefactor, West Warwick, RI, USA), with each set consisting of one signal and one detecting electrode. One set was placed in the anteriad dorsad region, and a second set was placed in the caudle peduncle region of the fish with the detecting electrodes of each set placed 1.0 cm proximal to the signal electrodes. Both sets of electrodes were placed to a depth of 1.0 cm into the fish. The distance between the two detecting electrodes was measured for each fish, and a current was introduced through the signal electrodes and the proximal detecting electrodes to measure R and Xc. After measures were taken on the frozen fish, individual dorsal loins were removed from the fish, vacuum-sealed, and returned to the −20°C freezer until analyzed by the Oregon State University Seafood Laboratory for body composition. Phase angles were calculated using the equation found in Cox and Heintz (2009).

For laboratory analysis of body composition, loins were thawed, homogenized, and body composition parameters were measured. Body composition parameters and methods were total body protein (TBP) by the Kjeldahl method, total body ash (TBA) in an ashing oven at 500°C, total body water (TBW) and dry weights (DW) by oven drying at 100°C, total body fat (FM) by ether extraction, and fat free mass (FFM) by mass balance equations (body mass minus FM; AOAC, 1990).

To create body composition models from BIA measures, R and Xc (from both fresh and frozen fish) were converted to common bioelectrical equations, and each set was regressed with laboratory body composition values. Bioelectrical equations included R in series (Rs) and in parallel (Rp), reactance in series (Xcs) and in parallel (Xcp), capacitance (Cf), phase angle (Pa), and impedance in series (Zs) and in parallel (Zp; Table 1). Each body composition parameter was regressed with each of the electrical equations that were converted from R and Xc measures taken from the fresh and frozen fish. The Akaike's information criterion (AIC) was used to determine which bioelectrical equation worked best for each body composition parameter. Once a model was created, the model with the best fit electrical equation was used to predict composition values. Predicted values were made from both the fresh and frozen fish models with models made from fresh and frozen BIA

TABLE 1
Equations used in both dorsal and ventral models

ID	*Equation*	*Description*
Rs	$detlen^2/R$	Resistance series
Rp	$detlen^2/[R + (Xc^2/R)]$	Resistance parallel
Xcs	$detlen^2/Xc$	Reactance series
Xcp	$detlen^2/[Xc + (R^2/Xc)]$	Reactance parallel
Cf	$detlen^2/[(1*10^{-12})/(2*3.14*50000)]$	Capacitance in farads
Pa	atan(Xc/R)*57.3	Phase angle
Zs	$detlen^2/sqrt(R^2 + Xc^2)$	Impedance in series
Zp	$detlen^2/(Xc*R)/[sqrt(Xc^2 + R^2)]$	Impedance in parallel

measurements. Comparisons were made between the (a) fresh and frozen fish raw R, Xc, and phase angle values; (b) body composition models (created from fresh and frozen measures) and laboratory measured values; and (c) predictions (from fresh and frozen models) and laboratory measured values.

RESULTS

Measures of R and Xc changed after each fish was frozen. Mean values of R were different between fresh and frozen fish, Welch $t(41.1) = -3.5$, $p = 0.001$ (Figure 1–3). Mean and standard deviations for fresh and frozen fish were 220.0 and 20.1 ohms, and 289.1 and 120.9 ohms, respectively. Mean values of Xc were different between fresh and frozen fish, Welch $t(39.0) = 91.2$, $p < 0.001$ (Figure 1). Mean and standard deviations of Xc for fresh and frozen fish were 97.5 and 6.8 ohms, and < 0.01 and < 0.01 ohms, respectively. Phase angles were significantly different between fresh and frozen fish, Welch $t(39.0) = 86.5$, $p < 0.001$; Figure 1). Mean and standard deviations of phase angle for fresh and frozen fish were 23.9 and 1.7 degrees, and < 0.01 and < 0.01 ohms, respectively. Individual fish measures of R increased in 24 of the 40 frozen fish samples, decreased in 14 of the 40 samples, and remained the same in two samples (Figure 2). Values of Xc decreased in all 40 frozen fish samples (Figure 3). Each fish was not completely thawed, and core temperatures indicated that some fish were still frozen at the core. Core temperature means and standard deviations of all the fresh and frozen fish were 28.9 and 1.6°C, and −1.3 and 1.0°C, respectively. Five of the frozen fish had core temperatures that were > 0°C.

Models and predictions of body composition parameters created with fresh fish BIA measures worked well (Figure 4). Coefficient of determination (r^2) scores between the predictions and their laboratory analyzed body composition values, ranked from highest to lowest, were TBW ($r^2 = 0.82$), FFM ($r^2 = 0.80$), TBP ($r^2 = 0.77$), TBA ($r^2 = 0.75$), DW ($r^2 = 0.69$), and TBF ($r^2 = 0.43$). Akaike information criterion ranked best candidate models as follows: TBW with Cf; TBF with Pa; and DW, FFM, TBA, and TBP with Xcs. Predictive model equations can be seen in Table 2. Prediction outcomes were distributed evenly over the 1:1 line (Figure 4).

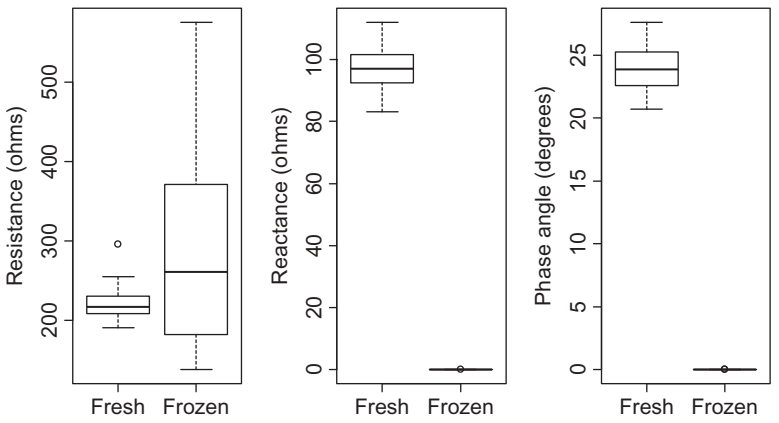

FIGURE 1 Boxplots of resistance (R) and reactance (Xc) measurements and phase angle calculations taken on 40 albacore tuna (*Thunnus alalunga*) prior to and after freezing at −20°C. Measurements were taken with bioelectrical impedance analysis.

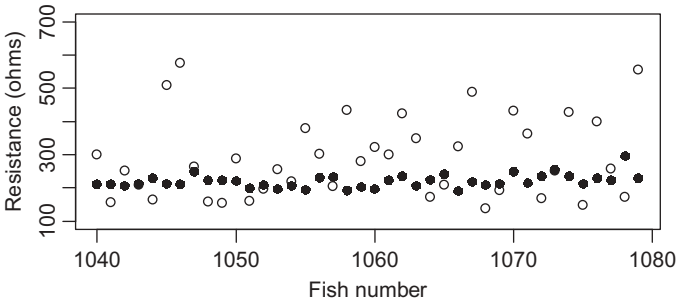

FIGURE 2 Resistance (R) values taken on 40 albacore tuna (*Thunnus alalunga*) prior to (dark circles) and after freezing at −20°C (open circles).

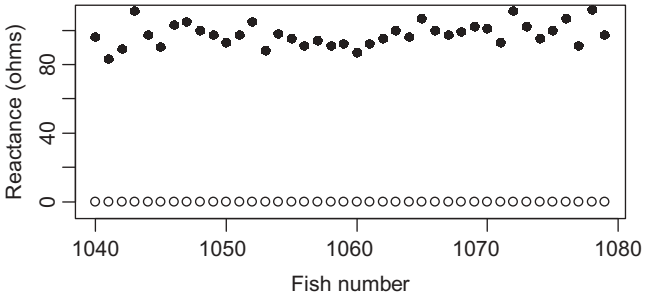

FIGURE 3 Reactance (Xc) values taken on 40 albacore tuna (*Thunnus alalunga*) prior to (dark circles) and after freezing at −20°C (open circles).

Predictions using frozen BIA measures (with models from fresh fish) did not work well (Figure 5). Predictive models made from fresh fish were the same as above that were chosen by Akaike information criterion, but predictions were calculated with BIA measures from frozen fish. Coefficient of determination scores between the prediction outcomes and their laboratory measured values, ranked from highest to lowest, were TBW ($r^2 = -0.11$), DW ($r^2 = -0.02$), TBA ($r^2 = -0.02$), TBP ($r^2 = -0.01$), and FM ($r^2 = -0.01$). Model equations from fresh fish can be seen in Table 2. Prediction outcomes were not distributed evenly over the 1:1 line (Figure 5). Predictions of TBW were underestimated, with predicted values ∼ 1,800 g; while actual values ranged from 3,000–7,000 g. All the other predicted parameters (FM, DW, FFM, TBA, and TBP) were overestimated. The largest overestimation was with TBP. The range of laboratory values for TBP was ∼ 1,200–1,900 g, while predicted values ranged from ∼ 5,000–32,000 g.

Interactions between body composition models made from frozen fish BIA measures and actual body composition values were weak. Akaike information criterion ranked candidate models as follows: TBW with Cf; FM with Cf; DW with Xcs; and FFM, TBA, and TBP with Cf. Predictive model equations made from frozen fish can be seen in Table 3. Coefficient of determination scores between the prediction models and their laboratory measured values, ranked from highest to lowest, were FFM ($r^2 = 0.16$), TBP ($r^2 = 0.16$), TBW ($r^2 = 0.10$), TBA ($r^2 = 0.06$), FM ($r^2 = 0.04$), and DW ($r^2 = 0.03$). F-statistic values for all chosen models were < 9.00.

Predictions using frozen BIA measures (with models from frozen fish) did not work. Coefficient of determination scores between the prediction outcomes and their laboratory measured values, ranked from highest to lowest, were TBP ($r^2 = 0.17$), FFM ($r^2 = 0.16$), TBW ($r^2 = 0.11$),

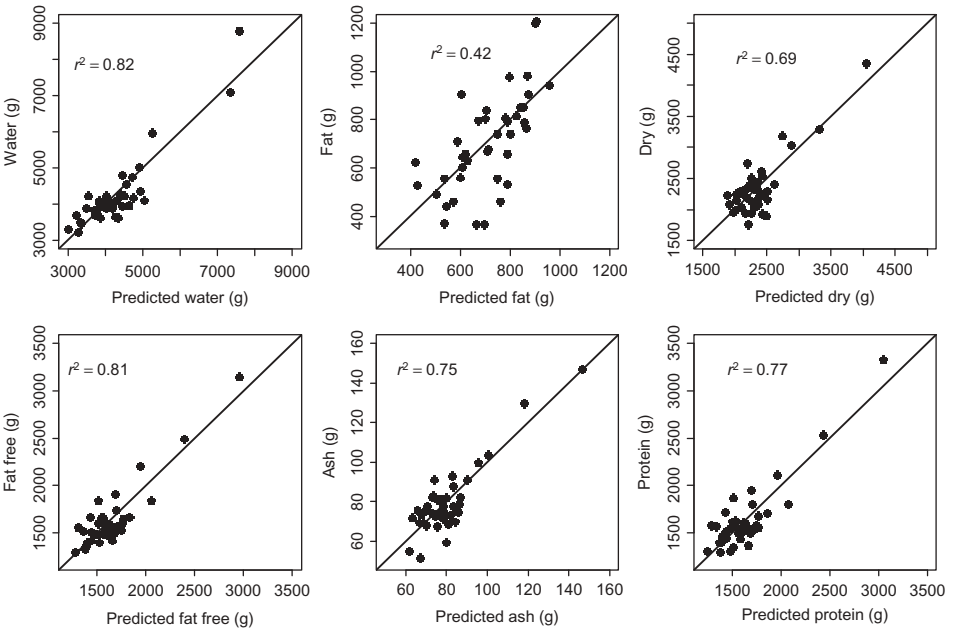

FIGURE 4 Regressions between predictions (from fresh fish measures) and actual body composition values. Models and predictions were made from 40 freshly caught albacore tuna (*Thunnus alalunga*) BIA measurements.

TABLE 2
Predictive models created from bioelectrical equations from freshly caught albacore tuna (*Thunnus alalunga*). Best fit model candidates were chosen by Akaike information criterion

Component	N	*Intercept* (SE)	*Slope* (SE)	*X*	F-*statistic* (p-*value*)	r^2
TBW	40	1.63e + 03 (2.08e + 02)	5.88e − 22 (4.42e − 23)	Cf	177.8 (0.0001)	0.82
FM	40	2.57e + 03 (339.82)	−78.01 (14.18)	Pa	30.26 (0.0001)	0.43
DW	40	246.58 (228.81)	2.24 (0.24)	Xcs	86.49 (0.0001)	0.69
FFM	40	12.94 (131.65)	1.73 (0.14)	Xcs	156.20 (0.0001)	0.80
TBA	40	−1.32 (7.60)	0.087 (0.01)	Xcs	118.1 (0.0001)	0.75
TBP	40	−74.51 (149.03)	1.83 (0.16)	Xcs	136.3 (0.0001)	0.77

TBA ($r^2 = 0.06$), and FM ($r^2 = 0.03$). Predictive model equations from frozen fish can be seen in Table 3. Prediction outcomes were distributed evenly over the 1:1 line (Figure 6).

DISCUSSION

Although BIA values were affected by frozen tissue, predictions of body composition and measures of condition using BIA values taken from fresh fish worked well. Body composition predictions and condition values of fresh fish were within the range of values found by Willis and Hobday (2008). Body composition models and condition measures of fresh fish can provide important information prior to freezing. Prior to freezing, fish must be caught, handled, and processed, and most degradation occurs during those early phases (Mazur, 1970). BIA can provide a quick and accurate

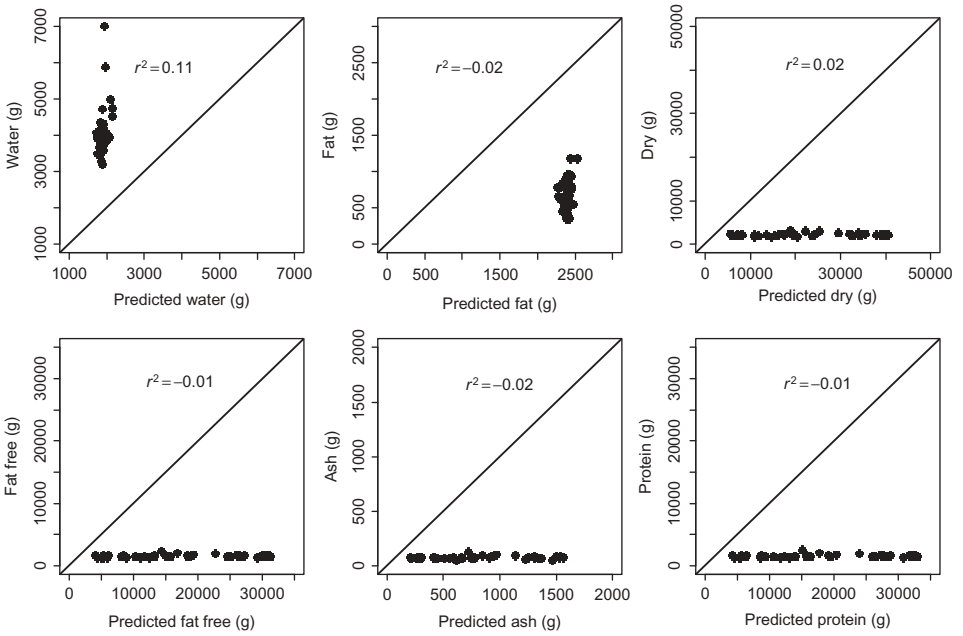

FIGURE 5 Regressions between predictions (from fresh fish models with frozen fish BIA values) and actual body composition values. Models were created from fresh caught albacore tuna (*Thunnus alalunga*), and predictions were made from the same fish after being frozen at −20°C.

TABLE 3
Predictive models created from bioelectrical equations from frozen albacore tuna (*Thunnus alalunga*). Best fit model candidates were chosen by Akaike information criterion

Component	N	*Intercept* (SE)	*Slope* (SE)	*X*	F-*statistic* (p-*value*)	r^2
TBW	40	3.53e + 03 (2.67e + 02)	1.32e − 21 (5.69e − 22)	Cf	5.42 (0.03)	0.10
FM	40	5.7e + 02 (7.68e + 01)	2.73e − 22 (1.63e − 22)	Cf	2.79 (0.10)	0.04
DW	40	2.13e + 03 (1.09e + 02)	1.45 (1.04e − 02)	Xcs	1.96 (0.17)	0.03
FFM	40	1.35e + 03 (8.59e + 01)	5.17e − 22 (1.83e − 22)	Cf	8.02 (0.01)	0.16
TBA	40	6.81e + 01 (5.42)	2.09e − 23 (1.15e − 23)	Cf	3.30 (0.08)	0.06
TBP	40	1.35e + 03 (8.77e + 01)	5.29e − 22 (1.86e − 22)	Cf	8.06 (0.007)	0.16

means to measure condition and body composition prior to freezing and packaging, and this information would be helpful throughout the food chain. Being able to quickly and easily measure body composition and condition in frozen fish would also be very informative, but freezing the tissue affected BIA measures to the point that body composition predictions and measures of condition were rejected. It was revealing that Pa and Xc measures could be used to determine whether fish have previously been frozen. Understanding the sensitivity of BIA measures to frozen and thawed tissue may provide input into how BIA may be used on fish that were previously frozen.

In our study, freezing and subsequent thawing of the tissue changed the structure and function of tissue, which caused changes in R and Xc measures. In all frozen fish, Xc values decreased to < 0.1 ohm, while some R values increased and some decreased. R values would have increased when there was a greater proportion of still frozen tissue present during BIA measurements. R values

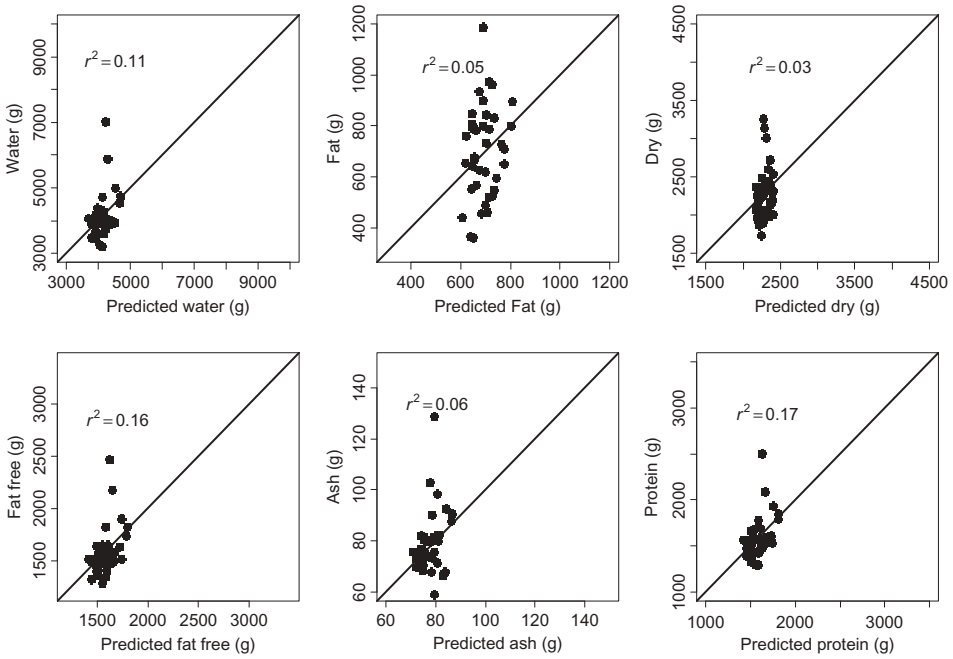

FIGURE 6 Regressions between predictions (from frozen fish measures) and actual body composition values. Models and predictions were from albacore tuna (*Thunnus alalunga*) that were previously frozen at −20°C.

would have decreased when a greater proportion of the fish was thawed out. Some of the core temperatures were above freezing, indicating that some of the tissue was thawed. The temperature was taken at one point within each fish, and it can be assumed that the proportion of thawed/frozen tissues within each fish varied. BIA measures of R and Xc were sensitive to changes within the entire volume of fish between the electrodes, so different proportions of thawed/frozen tissue were represented in the BIA measurements.

When a greater proportion of the tissue was thawed, R values decreased. When tissues are frozen, cells become dehydrated, cell membrane integrity is compromised, and intercellular and intracellular water freezes (Grimes et al., 1990). Upon thawing, compromised cell membranes cannot maintain the osmotic gradients, and macromolecules and water that previously could not enter the cell flux in. The influx of water into the compromised cell causes the cell to expand, lyse, and lose its contents to the solution outside the cell (Davalos and Rubinsky, 2004). Contents of the cell that are now mixed with the solutions outside the cells are very conductive, resulting in an increase in conductivity of the solution and a decrease in R values.

Similarly, a greater proportion of frozen tissue caused the R measures to increase. In fresh tissue, the intercellular solution of liquid water and associated electrolytes is the electrical conductor. Once tissues became frozen, electrolytes no longer carried the electrical charge, and the more highly resistive protons (H^+) carried it. The highly resistive but mobile protons greatly increased the resistance of the tissue (Eisenberg and Kauzmann, 2005). The mechanism of proton mobility which carries the charge is described by the Grotthuss mechanism (Agmon, 1995). Protons are dissociated from water and carry the charge but at a greatly reduced rate than charge movements in liquid water (which is carried by the familiar electrolytes such as Na^+ and Cl^- in solution). Protons are formed by the dissociation of water into hydronium and hydroxide ions, which makes the conductivity of ice related to the rate of the dissociation of water molecules as seen in the following equation:

$$2H_2O \leftrightarrow H_3O^+ + OH^-.$$

Textbook values of conductivity (k) of water ice at $-10°C$ are 10^{-9} ohms^{-1} cm^{-1}, an order of magnitude smaller than k for liquid water at the melting point (Eisenberg and Kauzmann, 2005). Similarly in our study, the presence of ice in the frozen fish tissue along with its low conductivity and high resistivity resulted in increases in R values.

Xc values were also affected by the freezing process. The freezing process lysed the cells causing Xc values to decrease to < 0.1 ohm. Although the freezing process resulted in the predictions of body composition not working, measures of Xc and Pa may be useful to the seafood industry as an indicator of whether a fish was previously frozen. When calculating Pa, Xc is divided by R. In the frozen fish, small Xc values (< 0.1 ohm) were divided by relatively large R values (~ 500 ohms) resulting in phase angle values that approached zero.

The reason Xc values of the tissue decreased to < 0.1 ohm was due to cell volume reductions and destruction of cell membranes. Frozen tissue is very resistive to electrical current (see Grotthuss mechanism above). With a decrease in intact cell membrane lipid bilayers, the capacitance of the system goes down, which lowers Xc values (Grimes et al., 1990). As all the fish in this study were frozen at $-20°C$, the majority of the cells would have been destroyed, which was reflected in the low Xc values. Davalos and Rubinsky (2004) used electrical impedance tomography to successfully determine human cell membrane integrity of healthy and damaged tissue due to freezing *in vivo*. Similar to their study, Xc values approached zero after freezing.

Overall, models or predictions for body composition and condition using BIA should be used on fresh fish, but measures of Xc or Pa can be used to indicate if fish tissue was previously frozen. Models of body composition and measures of condition taken from the fresh fish worked well. After the fish was frozen, measures of body composition and conditions did not work well because measures of R and Xc were sensitive to tissue changes due to freezing. Predictability of body composition and condition can be attributed to the electrical currents being affected by the (a) crystallized water ice found in the frozen tissue causing low conductance, (b) subsequent destruction of cell membranes which reduced capacitance, and (c) increase in conductivity of extracellular spaces due to the increase in electrolytes in intercellular spaces. The use of BIA to measure body composition and condition using Pa remains limited to fresh fish, but its usefulness may be expanded into other areas of freezing and cryopreservation as BIA is sensitive to changes in tissue due to the freezing process. Further research should explore R changes to fish that have been completely thawed out and also how condition measures change as fish move from a frozen state to a thawed one. The use of BIA is still in an exploratory phase but holds promise as a method that would enhance existing seafood quality assurance programs.

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