QUALITY CHANGES IN AUSTRALIAN RED CLAW CRAYFISH (*CHERAX QUADRICARINATUS*) SUBJECTED TO MULTIPLE FREEZING-THAWING CYCLES[1]

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Accepted for Publication June 5, 2002

ABSTRACT

*The influence of repeated freezing-thawing (F/T, six cycles) treatment on Australian red claw crayfish (*Cherax quadricarinatus*) muscle quality was investigated. For each F/T cycle, five raw tails were analyzed for lipid oxidation, proteolysis, and thermal stability, and another five tails were cooked to determine cooking yield and tenderness (shear). Lipid oxidation occurred during repeated F/T treatment, as evidenced by a marked TBARS increase, i.e., from 0.070 mg/kg (fresh) to 1.201 mg/kg (cycle 6). While F/T induced small proteolytic and thermal stability changes, it caused a major loss in cooking yield as well as in tenderness of meat. The results showed an overall susceptibility of the main quality traits of red claw muscle to F/T abuses. Thus, to retain good eating qualities, red claw tails should not be subjected to more than three F/T cycles.*

[1] Approved for publication as journal article number 02-07-33 by the Director of the Kentucky Agricultural Experiment Station.

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Journal of Food Quality **26** (2003) 285–298. *All Rights Reserved.*

INTRODUCTION

Australian red claw crayfish (*Cherax quadricarinatus*) is a robust freshwater crustacean species native to the rivers and streams of northern Australia. They are commonly known as red claw because mature males show red marks on the outer margin of their claws. Red claw has been cultured in Australia since 1985; and in the United States research on red claw began in about 1989. Compared with native American crayfish, red claw show several important advantages, including larger potential size, higher percentage of dress-out (meat), and better tolerance of crowded culture condition (Masser and Rouse 1997).

Frozen storage greatly prohibits the growth of microorganisms on or in seafood; however, it does not prevent quality deterioration originating from chemical or physical causes (Zaritzky 2000; Jasra *et al.* 2001). Prefreezing, freezing, frozen storage, and thawing are four important phases in any consideration of frozen meat (Cassens 1994). Repeated freezing-thawing (F/T) is a very common practice at retail, at home, as well as in hotel-restaurant-institution (HRI) settings (Giddings and Hill 1978). Quality changes in crustaceans like prawn (*Machrobrachium rosenbergii*) as induced by F/T processes have been investigated (Srinivasan *et al.* 1997a, b, 1998).

Protein denaturation is one of the principal physicochemical factors responsible for quality deterioration in many frozen muscle foods (Shenouda 1980; Mackie 1993; Rahman 1999). The formation and accretion of ice crystals, and the increase in salt concentration due to the diminishment of the water phase, can cause damages to the protein structure and convert the protein into a metamorphous state (Xiong 1997). Repeated F/T processes accelerate the breakage of muscle cells due to melting and recrystallization of ice crystals, which often lead to the emancipation of enzymes from lysosomes, mitochondria, and other cellular organelles to catalyze adverse chemical reactions (Yamamoto *et al.* 1977; Hamm 1979; Benjakul and Bauer 2000). For example, when a large amount of short-chain free fatty acids are released from triacylglycerol by lipolytic enzymes, hydrolytic rancidity occurs (Refsgaard *et al.* 2000). Furthermore, lipid oxidation can be accelerated by the interaction of free fatty acids or membrane phospholipids with initiators, producing oxidative off-flavors (Sista *et al.* 1997).

Fiber shrinkage and drip loss are two common deteriorations observed in frozen and thawed muscle foods. Thawed meat is generally perceived tougher or drier than unfrozen meat. The increase in toughness of thawed meat has been attributed to myosin denaturation as well as to aggregation of myofibrillar proteins (Sikorski *et al.* 1976; Ramirez *et al.* 2000). For frozen meat stored under aerobic conditions, lipid oxidation is of particular concern. Many of the secondary lipid oxidation products (e.g., carbonyls) will cause cross-linking and

oxidation of proteins (Gardner 1979), thereby adversely affecting the texture of crustacean muscle.

Currently no information is available about the impact of repeated F/T handling on the quality of red claw meat. The present study was conducted to evaluate possible quality changes in red claw muscle following a series of F/T steps, simulating frozen storage practices often encountered at home and in food service situations. Chemical and physical reactions, including lipid oxidation, proteolysis, protein denaturation, cooking yield, and shear force, were monitored in order to identify possible causes for textural changes. Our ultimate goal was to provide information needed to estimate the shelf-life of red claw when subjected to F/T handling conditions at home, in hotel-restaurant-institution (HRI), and possibly in retail stores.

MATERIALS AND METHODS

Materials

Juvenile red claw were raised for 8 weeks to a mean live weight of 40 g in 0.02-ha ponds at Kentucky State University Aquaculture Research Center, Frankfort, Kentucky. A total of 60 red claw were randomly collected from a large sample pool. After stunning by submerging in an ice slurry, the red claw were manually processed. The tails (muscle, plus exoskeleton), averaging 10.4 $\pm$ 3.0 g in weight, were placed in iced coolers and shipped to the University of Kentucky's Food Protein Research Laboratory within 3 h of collection. On receipt, the red claw were rinsed with tap water and subjected to treatments as described below.

Freezing and Thawing

Shell-on tails were individually quick frozen in a -20C blast freezer for 2 h and subsequently packaged, without vacuum, in a large Cryovac plastic freezer bag. After storing in the -20C freezer for 1 day, all tails were thawed for 30 min in a tank filled with running tap water (18-20C). The method simulated handling practices used during foodservice operations (e.g., restaurants). The thawing water flow rate was maintained at a low level to avoid agitation. After thawing, 10 randomly selected tails were immediately placed on crushed ice for analysis. The remaining 50 thawed tails were again individually frozen in the -20C blast freezer for 2 h and stored in a plastic freezer bag at the same temperature for another day as described above. The frozen-thaw procedure, i.e., 1 day frozen storage at -20C followed by thawing in running tap water for 30 min, was repeated for six cycles.

Preparation of Samples for Analysis

For each freezing-thawing cycle, 10 randomly selected shell-on tails were analyzed. Five of them, after removal of the shell, were homogenized in a Mini Chopper (Black and Decker, Shelton, Conn.) for 30 s. Raw muscle homogenates were analyzed immediately for physicochemical properties, including lipid oxidation, proteolysis, and protein denaturation. The other five tails, with the shell on, were cooked to measure cooking yield and tenderness.

Lipid Oxidation

Lipid oxidation was measured as increases in thiobarbituric acid-reactive substances (TBARS) which were determined by using the colorimetric method described by Sinnhuber and Yu (1977). The TBARS value, expressed as mg of malonaldehyde/kg of muscle sample, was calculated using the following equation:

$$\text{TBARS(mg/ke)} = \frac{A_{532}}{w_s} \times 9.48$$

where A_{532} is the absorbance (532 nm) of the assay solution, w_s the muscle sample weight (g), and '9.48' a constant derived from the dilution factor and the molar extinction coefficient (152,000 M^{-1} cm^{-1}) of the red, TBA reaction product.

Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to detect F/T-induced proteolytic changes in red claw raw muscle. The SDS-PAGE system consisted of a 10% acrylamide separating gel and a 3% acrylamide stacking gel. SDS-PAGE was run with an SE 250 mighty Small II slab gel electrophoresis unit (Hoefer Scientific Instrument, San Francisco, Cal.) (Srinivasan *et al.* 1997a, b). Samples for electrophoresis were prepared by homogenizing 1 g of minced muscle in 100 mL of prechilled (5C) distilled, deionized water with a Polytron (Brinkman Instruments, Westbury, N.Y.) for 30 s. The homogenate was diluted 1:1 with the sample buffer containing 4% SDS, 0.125 M Tris (pH 6.8), 20% glycerol, and 10% β-mercaptoethanol. This preparation yielded a sample protein concentration of approximately 1 mg/mL, assuming a 20% protein content in raw muscle tissue. A 20 μg sample was loaded on each gel lane. Protein bands were tentatively identified by comparing their electrophoretic mobility and intensity with published results of standard muscle proteins (Porzio and Pearson 1977).

Molecular weights (MW) of unknown proteins were estimated from the regression line generated by plotting log[MW] of protein standards versus their migration distance.

Protein Denaturation

Differential scanning calorimetry (DSC) was performed to measure thermal stability of red claw muscle proteins. A model 2920 modulated DSC machine (TA Instruments, New Castle, Del.) was calibrated for temperature and baseline using indium as standard. Accurately weighed minced muscle samples (14-17 mg) were placed in polymer-coated aluminum pans (TA Instruments) and hermetically sealed. An empty sealed pan was used as reference. Samples were scanned from 10 to 100C at a heating rate of 10C/min. The enthalpy changes (ΔH) for the major thermal transitions were determined by measuring the area above the transition curves with a straight baseline constructed from the start to the end of the endotherms. Temperature at the maximum heat flow (T_{max}, i.e., temperature at peak of the endotherm) was also recorded. Both the ΔH and the T_{max} values were determined using the Universal Analysis Ver 1.2 N software supplied by the DSC Co.

Cooking Yield

For each F/T cycle, 5 randomly selected shell-on tails were cooked by immersing in boiling water (100C) for exactly 2 min. The boiled tails were immediately chilled by placing on crushed ice before further analysis. Cooking yield (%) was calculated and expressed as cooked shell-on weight divided by raw shell-on weight and multiplied by 100. After the cooking yield was measured, the tail meat was subjected to textural analysis.

Textural Analysis

A Model 4301 Instron Universal Testing Instrument (Instron Corp., Canton, Mass.) with a Warner-Bratzler shearing device attached to the load cell (100 kg capacity) was used to measure the shear force required to rupture the first intact major muscle segment from the anterior of the cooked tail meat (Srinivasan *et al.* 1997b). The tail was placed transverse to the blade, to enable cutting across the muscle fibers. The cross-head speed of the Instron was set at 20 mm/min. The first major peak (usually the highest, which represented the maximal shear force required to cut the muscle fibers) was recorded. In the present study, shear force values (kg) of individual tails were normalized based on the weights (g) of the tails, and were expressed as force per sample unit weight (kg/g) to eliminate size effects (Srinivasan *et al.* 1997b).

Statistical Analysis

Data were analyzed with the General Linear Models procedure of the Statistix 3.5 software package (Analytical Software, St. Paul, Minn.) for microcomputers. Analysis of variance (ANOVA) was performed to determine the significance of the main effects of freezing and thawing. Significant ($P < 0.05$) differences between means were identified using Least Significant Difference procedures (Sendecor and Cochran 1989).

RESULTS AND DISCUSSION

Lipid Oxidation

Malonaldehyde is a secondary product derived from hydroperoxides of oxidized lipids. Compared with fresh muscle (0 F/T cycle), the one-time F/T treatment did not generate additional TBARS ($P > 0.05$). However, the TBARS value (0.050 mg/kg at one F/T cycle) increased ($P < 0.05$) steadily to 0.679 mg/kg by four cycles, reaching 1.201 mg/kg after six cycles (Fig. 1). Lipid oxidation in muscle can be initiated through enzymic or nonenzymic pathways (Rhee 1988). Cellular organelles like microsomes and mitochondria contain enzymes and cofactors that initiate lipid oxidation (Kanner 1994). Transition metals (e.g., iron and copper) and heme proteins are major nonenzymic factors that can accelerate lipid oxidation in muscle foods (Kanner 1992). The accelerated lipid oxidation of red claw raw muscle after five F/T cycles may be due to the release of initiators from damaged cellular organelles, and additionally, to the depletion of endogenous antioxidants in the muscle.

Proteolytic Changes

No major proteolytic changes were detected in the SDS-PAGE patterns of red claw muscle after repeated F/T treatments (Fig. 2). Apparently both myosin heavy chain and actin were resistant to proteolysis. A slightly decreased band intensity in a 69 kDa polypeptide, tentatively ascribed to serum albumin (SA), was noticed after three F/T cycles. The gradual disappearance of this protein corresponded to the emergence of a new 57 kDa product, suggesting that the emerging protein may be a degraded fragment of serum albumn (Fig. 2). Processes associated with freezing and frozen storage can facilitate hydrolysis of proteins into short-chain peptides and amino acids by endogenous proteases in seafood muscle (Jasra *et al.* 2001). Repeated F/T treatments could rupture the membrane structure of cellular organelles and release proteolytic enzymes. This process may explain the detectable proteolytic changes in red claw muscle after three F/T cycles. Calpains and cathepsins, two main protease families present

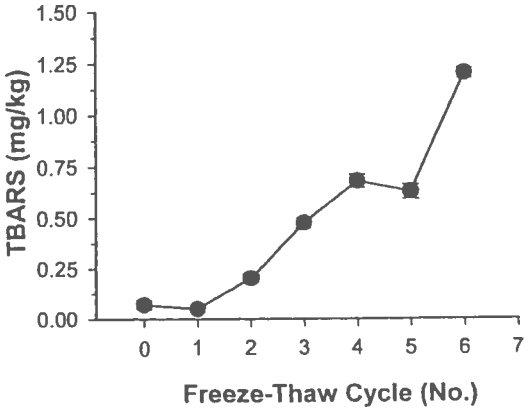

FIG. 1. FORMATION OF THIOBARBITURIC ACID-REACTIVE SUBSTANCES (TBARS) AS AN INDICATOR OF LIPID OXIDATION IN RED CLAW MUSCLE SUBJECTED TO MULTIPLE FREEZING-THAWING CYCLES

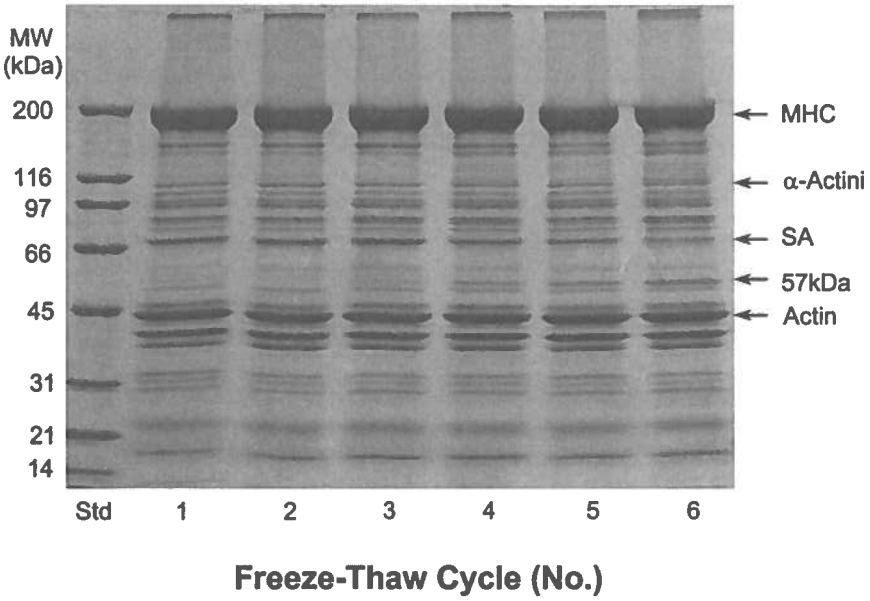

FIG. 2. ELECTROPHORETIC ANALYSIS OF PROTEOLYTIC CHANGES IN RED CLAW MUSCLE SUBJECTED TO MULTIPLE FREEZING-THAWING CYCLES
Lane "Std": protein molecular weight standards. Myosin heavy chain (MHC), serum albumin (SA) and several other selected muscle proteins are marked.

in many crustacean species (Jiang *et al.* 1992; Jiang and Chen 1999), could have played a major role in producing the observed changes. However, further studies are needed to identify the exact proteases involved.

Protein Denaturation

All muscle samples exhibited two distinct transitions (peaks I and III) and additionally, a less resolved one (II) (Fig. 3). The transition peaks I and III are typical of muscle and have been attributed to the denaturation of myosin head (or heavy meromyosin) and actin, respectively. Peak II is attributed to a combination of myosin tail, sarcoplasmic proteins and connective tissue (Stabursvik and Martens 1980).

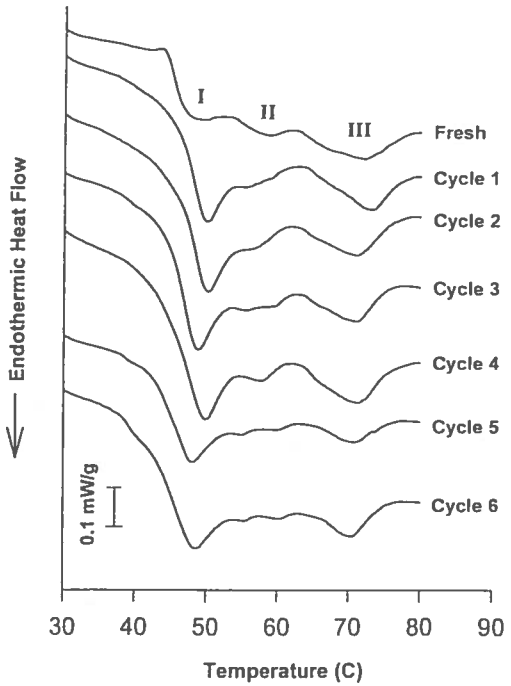

FIG. 3. DIFFERENTIAL SCANNING CALORIMETRY (DSC) OF RED CLAW MUSCLE SUBJECTED TO MULTIPLE FREEZING-THAWING CYCLES

A close examination of the individual transition peaks and the enthalpy of denaturation (ΔH) revealed some minor changes in the thermal stability of red claw muscle during multiple F/T cycles (Table 1). The T_{max} for myosin head

TABLE 1.
DIFFERENTIAL SCANNING CALORIMETRIC ANALYSIS OF RED CLAW MUSCLE SUBJECTED TO MULTIPLE FREEZING-THAWING CYCLES

Freezing-thawing cycle (No.)	Myosin (head)		Actin	
	T_{max} (C)	ΔH (J/g)	T_{max} (C)	ΔH (J/g)
Fresh	50.2±0.31[a]	0.324±0.032[a]	72.6±0.42[a]	0.260±0.031[ab]
1	50.4±0.27[a]	0.336±0.029[a]	72.5±0.31[a]	0.312±0.037[a]
2	50.4±0.33[a]	0.340±0.026[a]	70.9±0.28[b]	0.307±0.020[ab]
3	48.8±0.26[b]	0.348±0.029[a]	70.6±0.35[b]	0.246±0.035[bc]
4	49.8±0.24[a]	0.341±0.027[a]	70.9±0.27[b]	0.290±0.031[ab]
5	47.4±0.34[c]	0.321±0.030[a]	70.4±0.33[b]	0.188±0.036[c]
6	47.8±0.29[c]	0.325±0.031[a]	70.4±0.24[b]	0.204±0.028[c]

[abc] Means ± S.E. (n = 4) within the same column with different superscript letters for maximum transition temperature (T_{max}) or enthalpy of denaturation (ΔH) for myosin (peak I) and actin (peak III) differ significantly ($P < 0.05$).

decreased ($P < 0.05$) after three F/T cycles (to 48.8C from the fresh sample, 50.2C), and after six cycles, it was reduced by an additional 1C. The ΔH for myosin, however, was unaffected by the F/T treatment. For actin, its T_{max} also decreased ($P < 0.05$) with F/T after only two cycles (to 70.4C from the fresh sample, 72.6C), but no further change was noted thereafter. There was no change in ΔH for actin until the fourth F/T cycle. The results indicated that both myosin and actin were resistant to denaturation, but were destabilized upon extended F/T abuses.

A number of factors could have contributed to the decreased protein stability when red claw muscle was repeatedly frozen and thawed. Based on the results presented in Fig. 1, it appeared that oxidized lipids, including both lipid free radicals and secondary lipid oxidation products such as reactive aldehydes, could have reacted with myosin and actin to disrupt the intramolecular forces responsible for protein stability. Hydrophobic reactions with lipid compounds, as manifested in many marine products, would also contribute to protein destabilization (Shenouda 1980). Moreover, proteolytic changes (Fig. 2), albeit small, would produce a less stable protein conformation.

Cooking Yield and Textural Analysis

Cooking yield of red claw was adversely affected by the F/T treatment (Fig. 4). The values decreased from 98.7% (fresh) to 75.3% (cycle 6) ($P < 0.05$). In contrast, shear force of cooked muscle increased with F/T cycles, reaching a maximum value (0.289 kg/g) by cycle 6. Fluid exudation and fiber shrinkage caused by mechanical damage to the muscle cell membrane due to frequent water-ice phase transitions would play a significant role in reducing cooking yield and increasing Instron shear force of red claw muscle. Furthermore, as the F/T treatment cycles increased, denaturation and aggregation would lower the ability of the myofilament matrix to bind or entrap water, producing a greater cooking loss.

Lipid hydrolysis and oxidation, as well as certain endogenous enzymes, can also play an indirect, yet, important role in protein-related textural deteriorations for meat. During frozen storage, many lipolytic enzymes remain active and are responsible for producing most free fatty acids in thawed muscle tissue (Olley *et al.* 1962; Refsgaard *et al.* 2000). Yamamoto *et al.* (1977) and Hamm (1979) reported F/T abuses caused the release of enzymes from lysosomes and mitochondria. Through both hydrophobic and hydrophilic interactions, free fatty acids can bind to protein and increase protein hydrophobicity, thereby promoting hydrophobic protein-protein interaction (Xiong 1997). On the other hand, oxidized lipid products would react with proteins to form insoluble protein aggregates and protein-lipid complexes (Gardner 1979). For example, the lipid degradation products malonaldehyde (Buttkus 1970) and 4-hydroxy-2-nonenal

(Xiong 2000) were both shown to produce cross-linked myofibrillar protein aggregates. The preceding deleterious chemical changes could have a potential role in the loss of cooking yield and tenderness in F/T-treated red claw.

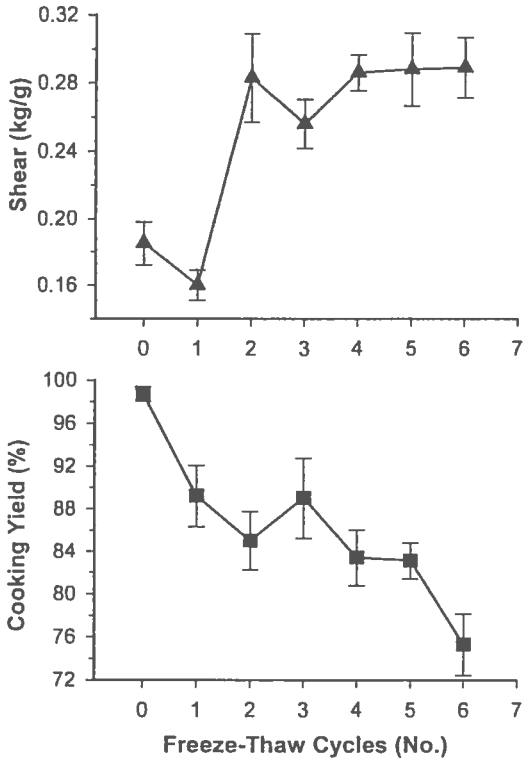

FIG. 4. COOKING YIELD AND SHEAR FORCE OF RED CLAW MUSCLE SUBJECTED TO MULTIPLE FREEZING-THAWING CYCLES

It is of interest to note that shear force of cooked red claw muscle increased rapidly ($P < 0.05$) between one and two F/T cycles; yet, no significant changes were seen for the last three cycles (Fig. 4). This phenomenon may be explained by considering two opposing effects. Tissue-toughening (i.e., due to protein denaturation and aggregation) and tissue-softening (i.e., due to proteolysis) were both likely involved during F/T cycles. As the F/T cycles continued, more proteases released from damaged organelles (i.e., lysosomes, mitochondria) would seem to dominate and off-set the counter-action of the tissue-toughening factors.

CONCLUSIONS

Exposing red claw crayfish muscle to repeated freezing-thawing up to six cycles was detrimental to the cooking yield and texture of red claw muscle under the experimental conditions used in the present study. Meat quality loss seemed related to a series of protein changes, including denaturation, aggregation, and degradation, as well as to lipid oxidation. For frozen seafood, Sinnhuber and Yu (1958) suggested TBARS values of less than 3 mg/kg to be an indicator of good quality with acceptable flavor. To ensure a good eating quality, it is advisable that frozen red claw tail meat held for retail, wholesale, or home use should undergo no more than three freezing-thawing cycles (if such practice cannot be avoided), because the TBARS (0.505 mg/kg), cooking yield (89%), and shear force (0.25 kg/g) levels obtained in this study remained within an acceptable range. Control samples undergoing only one freezing-thawing cycle on each sampling day need to be added in future work to estimate quality changes associated with freezing and storage conditions upon undergoing freezing-thawing cycles. Also, sensory analysis is required in future studies to determine consumer acceptance.

ACKNOWLEDGMENTS

This research was partially funded by a USDA 1890 Institution Capacity Building Grant awarded to Kentucky State University, and by a grant from the USDA to Kentucky State University (Project No. KYX-80-00-10A).

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