Food Research International 53 (2013) 141–148

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Kinetics of quality changes in whole blue mussel (*Mytilus edulis*) during pasteurization

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ARTICLE INFO

Article history:
Received 18 March 2013
Accepted 28 April 2013

Keywords:
Area shrinkage
Cook loss
Reaction kinetics
Texture
Thermal processing

ABSTRACT

The effects of pasteurization temperatures (65 to 90 °C) on the kinetics of quality changes (cook loss, area shrinkage and texture) in whole blue mussel (*Mytilus edulis*) were investigated. The cook loss, area shrinkage and compression force increased with increasing heating time and temperature, and followed the first–order, second– and zero–order reactions, respectively. Most cook loss (30%) and area shrinkage (36%) occurred during the first 5 min of heating. A high positive correlation was found between cook loss, area shrinkage and compression force, indicating that dehydration and protein aggregation were reflected in compression force measurements, especially at higher temperatures (75 to 90 °C). The activation energy (E_a) for cook loss, area shrinkage and compression force were 55.6, 92.3 and 64.7 kJ/mol, respectively. The E_a for compression force, 64.7 kJ/mol, was lower than that reported in the literature, reflecting differences in the type and degree of protein denaturation from differences in how heat treatments were conducted.

1. Introduction

The blue mussel (*Mytilus edulis*) is widely cultivated with a global production of 207,918 tons in 2010 (FAO, 2012 http://www.fao.org/fishery/species/2688/en). Mussels are consumed raw, fresh, frozen and canned and provide a highly nutritious and relatively inexpensive source of high quality protein (60% of dry basis) (Manousaridis et al., 2005). Mussels are low in fat and cholesterol, and rich in polyunsaturated fatty acids (42–45% of the total fatty acids) (Orban et al., 2002), high in fat and water soluble vitamins and trace elements (Manousaridis et al., 2005). Due to the high amount of glycogen and free amino acids, high water activity ($a_w > 0.95$) and high pH (6.7–7.1), mussels are highly perishable (Manousaridis et al., 2005).

Mussels present a food safety concern since the whole animal is consumed including the digestive tract. These animals are very efficient filter feeders and can concentrate viruses and bacteria 1000-fold that are present in the aquatic environment (Hewitt & Greening, 2006; Love, Lovelace, & Sobsey, 2010). According to the USA National Shellfish Sanitation Program (NSSP), there are three methods to cleanse shellfish prior to consumption to reduce food borne illness risk including: 1) relay; a transfer of shellfish to approved natural waters for more than 14 days at a standard salinity and temperature range, 2) depuration in a performance verified system for more than 44 h, and 3) pasteurization by heat or high pressure (Love et al., 2010). Heat treatment is the most widely used procedure for microbial inactivation in foods generally; however, heat causes unwanted side-effects to the sensory, nutritional and functional properties of food unless its application is carefully controlled.

Both non-thermal and thermal techniques have been attempted to inactivate microbes in mussels. Manousaridis et al. (2005) used ozone (1 mg/L, 60 and 90 min) to inactivate bacteria with increasing time improving the effectiveness of the treatment. They reported the highest sensory scores for odor and taste for those mussels treated for 90 min; while application of ozone was not effective for texture retention. Depuration for 96 h resulted in a reduction of 98.7% for infectious hepatitis A virus (HAV) and 97.0% reduction in rotaviruses (Abad, Pintó, Gajardo, & Bosch, 1997), however other studies indicate that depuration may require a longer time, for example at least 5 days (De Medici et al., 2001) or 7 days (Enriquez, Frösner, Hochstein-Mintzel, Riedemann, & Reinhardt, 1992). Azanaz, Azanaz, and Ventura (2003) used chlorinated water to wash and inactivate bacteria in shucked mussel using 5 and 10 ppm chlorine solution for 3 min finding a 1–3 log reduction. Reducing pH has limited effectiveness against viruses; Hewitt and Greening (2004) reported that marinating mussels resulted in a reduction of noroviruses (NoV) and HAV after 4 weeks at pH as low as 3.75, and that feline calicivirus (FCV) was readily inactivated at this lower pH.

Boiling and steaming treatments effectively inactivate microorganisms in whole molluscan shellfish. For New Zealand greenshell mussels (*Perna canaliculus*), Hewitt and Greening (2006) showed that the differences in HAV and NoV inactivation depended upon the method of cooking, with boiling for 3 min (internal temperature of 92 °C) being more effective than steaming for 3 min (internal temperature of 63 °C) to inactivate HAV. Incomplete inactivation of HAV and rotavirus

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http://dx.doi.org/10.1016/j.foodres.2013.04.029

occurs after steaming mussels for 3 min after the shells opened (Abad et al., 1997), a conventional cooking endpoint for dishes containing shell-on mussels. Outbreaks of *Listeria monocytogenes* have been associated with smoked mussels (Baek, Lim, Lee, Min, & Kim, 2000; Brett, Short, & McLauchlin, 1998), and it is likely that HAV and NoV would also survive cold smoking and potentially some hot smoking processes unless the product is canned after smoking. F_o for in mussels at 110 °C (6.01), 116 °C (6.02), and 122 °C (6.11) have been reported (Almonacid et al., 2012).

The thermal processing of food may cause severe quality deterioration, such as degradation in color and texture, nutrient loss, cook loss (weight loss) and area shrinkage, rendering the products reducing consumer acceptance. The most significant changes in mussel during heating result from protein denaturation. Protein denaturation reduces water holding capacity, shrinks muscle fibers, and causes connective tissue degradation, subsequently leading to a harder and more compact tissue texture (Kong, Tang, Rasco, & Crapo, 2007; Ma et al., 2011; Rawson et al., 2011; Skipnes, Johnsen, Skara, Sivertsvik, & Lekang, 2011). The negative effect of thermal processing has been demonstrated in many foods such as beef (Bertola, Bevilacqua, & Zaritzky, 1994), beef and lamb (Geileskey, King, Corte, Pinto, & Ledward, 1998), pork (Du & Sun, 2005), salmon and cod (Ofstad, Kidman, Myklebust, & Hermansson, 1993), ground chicken breast (Murphy & Marks, 2000), rock cod (Franklin, Crockford, Johnston, & Kamunde, 1994), salmon (Kong, Tang, Rasco, & Crapo, 2007; Kong, Tang, Rasco, Crapo, & Smiley, 2007), and Atlantic cod (Skipnes, Ostby, & Hendrichx, 2007; Skipnes, Van der Plancken, Van Loey, & Hendrickx, 2008; Skipnes et al., 2011). Due to structural differences between shellfish and mammalian muscle, the effect of thermal processing would be more detrimental to shellfish. Paramyosin which forms the cores of the thick filaments in the adductor muscle of invertebrates is covered by a cortical layer of myosin (Paredi, Tomas, Crupkin, & Anon, 1994) which is heat stable. However, there is less connective tissue in invertebrate whole body (especially in the mantle) compared to vertebrate muscle also resulting in relatively higher textural changes resulting from protein denaturation. It may be possible to reduce these deleterious quality changes from thermal processing if a greater understanding of the kinetic parameters for thermal quality changes during pasteurization processes can be obtained with these data then used for design and optimization purposes. No systematic studies of shellfish pasteurization incorporating shucked meat quality are available. To meet these objectives, this study was designed to evaluate the kinetics of cook loss, shrinkage, color and texture changes during the pasteurization conditions in shucked blue mussel.

2. Materials and methods

2.1. Materials

Packaged fresh shelled blue mussels (*M. edulis*) were purchased from a local retailer in Pullman, WA, USA cultivated in Quilcene Bay, WA. Mussels were immediately transferred to the School of Food Science at WSU (Pullman, WA, USA), stored at −30 °C and used within 1 month. The mussels were thawed on ice, and then shucked by hand at ambient temperature and kept on ice for 15 min prior to thermal treatment.

2.2. Thermal treatments

Thermal treatments were conducted at 65, 70, 75, 80, 85 and 90 °C for various times to provide equivalent lethality and sufficient heating to inactivate norovirus and *Listeria monocytogenes* (Buckow, Isbarn, Knorr, Heinz, & Lehmacher, 2008; Gaze, Boyd, & Shaw, 2006; Gibson & Schwab, 2011) (Table 1) (n = 4).

The heating method of Kong, Tang, Rasco and Crapo (2007) and Kong, Tang, Rasco, Crapo and Smiley (2007) was used. Briefly, a single mussel (12.5 ± 5.1 g wet weight; moisture content 82 ± 0.02 g H_2O/100 g sample, n = 30) was sealed into a custom built cylindrical aluminum test cell having a 50 mm inner diameter, 8 mm inner height, and 3 mm wall thickness. Come-up time, defined as the time for the sample center temperature to reach within the 1 °C of the total temperature rise, was determined using a 0.1 mm diameter copper-constantan thermocouple (Type-T) inserted through the rubber gland in the lid of the container. The immersion length of the probe was 3 mm, thus the influence of heat conduction along the thin wire probe to the sample temperature measurement was considered to be minimal. Whole mussel was put in to the cells, and then the cells were sealed and heated in an oil bath (Model HAAKE W13, Thermo Electron Corp., Karlsruhe, Germany) at the specified temperatures using ethylene glycol as the heating medium. After heating, the sample cells were immersed into a mixed ice and water bath immediately to cool. After cooling, the mussels were removed from the cell and patted dried with a filter paper and weighed. The come-up heating times of mussel to reach at the desired temperatures are presented in Table 1. The moisture content of mussel was measured by heating the homogenized mussels at 105 °C to constant weight according to AOAC (method 952.08) (2000).

Table 1
Experimental heating conditions, come up time and cooling time for whole shucked mussel (*Mytilus edulis*).

Temperature (°C)	Heating time (min)								Come up time (min)	Cooling time (min)
65	1	5	9	13	23	33	48	63	6:50	1:08
70	1	5	7	11	19	27	35	43	6:10	1:20
75	1	4	7	10	12	14	16	20	5:40	1:40
80	1	3	5	7	9	12	15	18	5:04	1:50
85	1	2	4	5	7	9	11	13	4:23	1:57
90	1	2	3	4	5	7	9	12	3:44	2:18

2.3. Color measurement

Color (L, a, b) was measured before and after heat treatments using a Hunter colorimeter (CM-2002, MINOLTA, Osaka, Japan) with 0° viewing angle, and illumination of D_{50}. The colorimeter was calibrated against standard white ($L = 96.72$, $a = 0.11$, $b = -0.14$) and green plates ($L = 65.99$, $a = -18.77$, $b = 9.36$) before set of color measurement was taken. For each mussel, 3 measurements were performed.

2.4. Cook loss

Cook loss, was calculated as follows:

$$\text{Cook loss} = \left(\frac{\text{weight of raw sample} - \text{weight of cooked sample}}{\text{weight of raw sample}}\right) \times 100. \quad (1)$$

2.5. Area shrinkage

For sample area determination, ImageJ software Version 1.47a (National Institute of Health, USA) was used. The area of sample image was determined using the "area measurement" menu. The shrinkage ratio was calculated as:

$$\text{Area shrinkage ratio} = \left(\frac{\text{area of raw sample} - \text{area of cooked sample}}{\text{area of raw sample}}\right) \times 100. \quad (2)$$

2.6. Texture

A compression texture fixture probe was used to measure compression force (N) of the samples (Angsupanich & Ledward, 1998; Rahman, Al-Waili, Guizani, & Kasapis, 2007). The stainless steel

puncture probe (5 mm internal diameter, 35 mm probe length) was fitted to a Texture Analyser TA-XT2 (Stable Micro Systems Ltd., Surrey, UK) equipped with a 5 kg load cell. Before measurement, the raw and heated samples were allowed to equilibrate to room temperature (22 °C), which took approximately 30 min. The samples were carefully placed on the support base so that the probe was perpendicular to mussel tissue at different locations on the animal. The traveling speed for the probe was set at 1 mm/s over a distance was 5 mm. Force–time graphs were recorded and analyzed using the Texture Expert for Windows (version 1.15, Stable Micro Systems Ltd.). The compression force was defined as the peak height in the force–time profile. Four mussels were tested from each treatment (n = 4) with measurements taken at six different locations on each mussel.

2.7. Differential scanning calorimetry (DSC) analysis

DSC was performed at a heating rate of 10 °C/min over the range from 10 °C to 150 °C using a modulated differential scanning calorimeter (MDSC, Q2000, TA Instruments, Waters LLC., New Castle, DE). Empty pans were used as reference and a 2 min equilibration at 10 °C was done before each run. Following equilibration, 9–15 mg of samples were sealed into the aluminum pans (capacity 30 μL) (n = 3). The residual denaturation enthalpy (ΔH) (J/g) was defined as the area under the denaturation peak (Skipnes et al., 2008) and area calculated (Universal Analysis 2000 software, TA Instruments).

2.8. Data analysis

Generally, reaction rates for quality degradation (C) under isothermal conditions can be presented as follows (Kong, Tang, Rasco, & Crapo, 2007):

$$\frac{dC}{dt} = -k(C)^n \tag{3}$$

where k is the rate constant, C is the quality at time t, and n is the order of reaction. To find the best empirical relationship, quality data were analyzed using zero–, first– and second–order kinetic models in as Eqs. (4)–(6):

$$\text{zero} - \text{order} : C_t = C_0 - k \cdot t \tag{4}$$

$$\text{first} - \text{order} : 1n\frac{C_t}{C_0} = -k \cdot t \tag{5}$$

$$\text{second} - \text{order} : k_t = \frac{1}{c_t} - \frac{1}{c_0} \tag{6}$$

where C_0 is the initial value of the quality at time zero, C_t is the value at time t and k is the rate constant. Arrhenius equation was used to determine the degradation rate constant (k) on temperature which is described as follows:

$$k = k_0 \exp\left(-\frac{E_a}{RT}\right) \tag{7}$$

where E_a is the activation energy of the reaction (kJ/mol), R is universal gas constant (8.3145 J/mol/K), T is absolute temperature (K) and k_0 is frequency factor (per min). If Eq. (7) applies to a reaction in consideration, a plot of the rate constant on semi-logarithmic scale as a function of reciprocal absolute temperature (1/T) should yield a straight line, and the activation energy can be determined as the slope of the line multiplied by the gas constant R.

3. Results and discussion

3.1. Color change

The kinetics of color changes during the thermal processing has been applied for different aquatic foods including rockcod (Franklin et al., 1994), salmon (Kong, Tang, Rasco, & Crapo, 2007) and Atlantic cod (Skipnes et al., 2011) and for beef and lamb (Geileskey et al., 1998). However for this type of analysis to meaningfully predict treatment effects, there must be color uniformity in the raw products. In the current study, as shown in Fig. 1, color of raw mussels cultivated in the same farm and at the same time showed little uniformity. Hence kinetic data of color change were not collected.

3.2. Cook loss

Cook loss percentage shows increasing time and temperature increased cook loss significantly ($p < 0.05$) (Fig. 2). More than 30% of this loss occurred within the first 5 min at each treatment temperature. As the heating progressed, the slope gradually leveled off and the cook losses approached an equilibrium value. The temperature dependence of the rate constant (Eq. (7)), E_a and k_0 values, and coefficient of correlation are shown in Table 2. The activation energy was calculated to be 55.6 has for whole mussel, which is in the same range as that reported for other muscle foods: 37.0 kJ/mol for salmon and 54.9 kJ/mol for beef (Bertola et al., 1994; Kong, Tang, Rasco, & Crapo, 2007). The frequency factor k_0 is 5960/min was similar to that reported for salmon which was 5761/min (Kong, Tang, Rasco, & Crapo, 2007).

Thermal processing causes denaturation of muscle protein which is the primary mechanism that leads to moisture loss. Most of the cook loss was water (>85%) with some lipids and protein solids (collagen or gelatin, muscle tissue fragments and coagulated sarcoplasmic proteins) being present (Bell, Fakas, Hale, & Lanier, 2001; Kong, Tang, Rasco, & Crapo, 2007; Skipnes, Johnsen et al., 2011; Skipnes et al., 2007). Skipnes et al. (2007) showed that the cook loss was moderate during heating in the range of 40 °C to 60 °C and increased rapidly from a temperature between 60 °C and 70 °C and up to 100 °C. Most of the water in muscle is contained within myofibrils, the narrow channels between thick and thin filaments (Bertola et al., 1994; Offer, Restall, & Trinick, 1984). Heating causes denaturation of myosin and shrinkage of myofibrils and a subsequent expulsion of water (Ofstad et al., 1993). At a higher temperature, greater cook loss occurs because of higher moisture depletion (Kong, Tang, Rasco, & Crapo, 2007; Kong, Tang, Rasco, Crapo, & Smiley, 2007; Skipnes, Johnsen, et al., 2011; Skipnes et al., 2007). The cook loss for whole mussels in this study was found to be higher than in salmon (*Oncorhynchus gorbuscha*) (Kong, Tang, Rasco, & Crapo, 2007; Kong, Tang, Rasco, Crapo, & Smiley, 2007) and cod (*Gadus morhua*) (Skipnes et al., 2007) indicating that aquatic invertebrate muscle is more sensitive to thermal denaturation than that of aquatic vertebrates. Further, compared to terrestrial animal carcass muscle, fish myosins are more heat labile and more sensitive to denaturation, coagulation, degradation, or chemical changes (Paredi et al., 1994). Paramyosin, a protein found in striated muscles of invertebrates, is involved in the catch contraction of bivalves. Paramyosin forms the cores of the thick filaments in the muscle of invertebrates where it is covered by a cortical layer of myosin. In addition, because of the small amount of connective tissues in bivalve molluscs muscles the heat treatments resulted in a proportionally higher denaturation of myofibrillar protein and sarcoplasmic proteins compared to other types of muscle fiber (Paredi et al., 1994) and leading to a higher cook loss.

3.3. Area shrinkage

Area shrinkage for whole mussels increased with increasing time and temperature (Figs. 1 and 3) with 70% area shrinkage at 90 °C, and 54% shrinkage at 65 °C. Similar to cook loss, most of the area

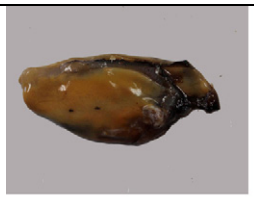
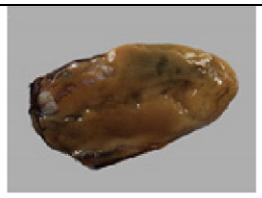
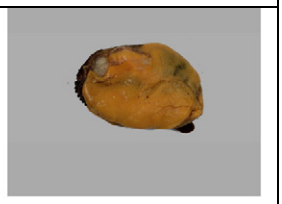
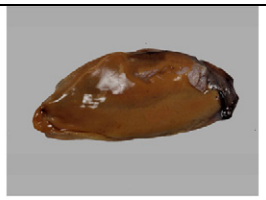
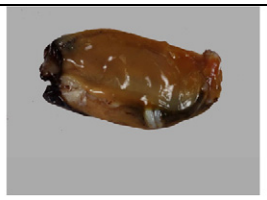
Temperature (°C)	Treatments			
65	Before heating	After 13 min of heating	Before heating	After 63 min of heating
				
75	Before heating	After 10 min of heating	Before heating	After 20 min of heating
				
85	Before heating	After 5 min of heating	Before heating	After 13 min of heating
				
90	Before heating	After 4 min of heating	Before heating	After 12 min of heating
				

Fig. 1. Visual color change and area shrinkage in whole mussel (*Mytilus edulis*) during pasteurization treatments.

shrinkage occurred during the first 5 min of heating (>36%) (Figs. 2 and 3). The rate constants: E_a and k_0 values are shown in Table 3 with activation energy of 92.3 kJ/mol and a frequency factor k_0 is 2875×10^4/min.

Heating caused denaturation of myosin and shrinkage of myofibrils, a widening of extra cellular spaces, and a subsequent expulsion of water (Ofstad et al., 1993). Area shrinkage is a result of protein denaturation and liquid loss. Protein denaturation causes shrinkage of muscle fiber diameter and sarcomere length, resulting in water soluble proteins and fats being expelled from the tissue (Bertola et al., 1994; Kong, Tang, Rasco, & Crapo, 2007; Kong, Tang, Rasco, Crapo, & Smiley, 2007) with similar trends observed for: salmon (*O. gorbuscha*) (Kong, Tang, Rasco, & Crapo, 2007; Kong, Tang, Rasco, Crapo, & Smiley, 2007), cod (*G. morhua*) and salmon (*Salmo salar*) (Ofstad et al., 1993), Atlantic cod (*G. morhua*) (Skipnes, Johnsen et al., 2011; Skipnes, Van der Plancken et al., 2008; Skipnes et al., 2007) and pork (Du & Sun, 2005).

Cook loss and area shrinkage, as anticipated, are highly correlated (Table 4). Positive linear correlations between sarcomere shrinkage and cook loss have been found in fish muscle (Ofstad, Kidman, & Hermansson, 1996), beef (Palka & Daun, 1999) and pork (Barbera & Tassone, 2006). Kong, Tang, Rasco, and Crapo (2007) found a non-linear correlation between cook loss and shrinkage in salmon meat. Degrees of shrinkage and cook loss would be anticipated to vary for different fish and anadromous migratory fishes with higher muscle fat content and stronger connective tissue would be expected to exhibit greater heat resistance than muscle from a pelagic gadoid specie such as cod. Salmon and cod muscle have different collagen contents (Kong, Tang, Rasco, & Crapo, 2007; Kong, Tang, Rasco, Crapo, & Smiley, 2007; Ofstad et al., 1993). In addition, cook loss

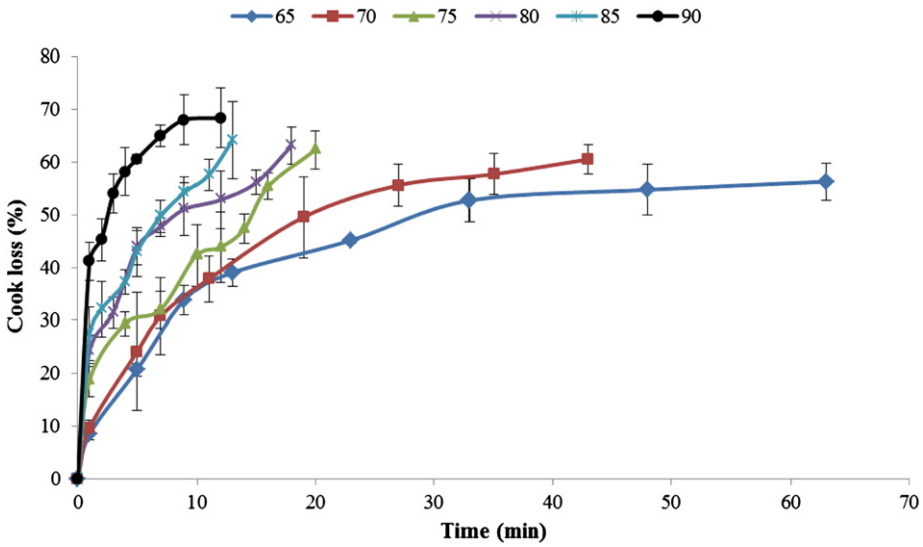


Fig. 2. The change of cook loss of whole mussel at different heating regimes, mean ± SD for four determinations.

and area shrinkage are significantly affected by sample size and shape (Kong, Tang, Rasco, & Crapo, 2007).

3.4. Texture

In current study the compression force (N) was determined as an indication of changes in textural features resulting from heat treatment. The mean compression force for raw mussel was 0.41 ± 0.11 N (n = 4, 6 readings for each shucked mussel). Heating resulted in higher compression force (Fig. 4). Lower temperatures (65, 70 and 75 °C) caused less compression force than higher treatment temperatures (80, 85 and 90 °C) suggesting that a longer time lower temperature process might result in a mussel with more acceptable sensory quality. Texture is closely correlated to water content of the muscle (Dunajski, 1979; Skipnes et al., 2011) with muscle with a higher water content being perceived as more tender. The relationship between cook loss (%) and compression force (N) (Table 4) for all temperatures is high. A peak correlating with an initial increase in compression force (N) was presented at 65 °C (9 min) and 70 °C (7 min) and less apparent at higher temperatures. This could be tied to denaturation of components within the digestive cavity of the mussel. The increase in compression observed in mussel from 75 °C to 95 °C is likely due to protein agglomeration and denaturation specifically the actin/myosin complex, upon protein denaturation.

If the strength of denatured protein aggregates is stronger than native protein aggregates, the muscle will toughen (Martens, Stabursvik, & Martens, 1982). Bhattacharya, Choudhury, and Studebaker (1993) studied the effect of hydrothermal processing on the texture of Pacific chum salmon (*Oncorhynchus keta*) at 60–100 °C and found that the hardness increased as the heating temperature increased. In another study on salmon (*O. gorbuscha*), a reduction in shear force with increasing temperature was reported at higher temperatures of 100 °C to 131 °C (Kong, Tang, Rasco, & Crapo, 2007) due to the counteraction of hardening and softening reactions at high temperatures, at which higher rapid disintegration and fragmentation of the fish muscle occurred at higher temperatures, contributing to the decrease in the measured shear force. Murphy and Marks (2000) found that with increasing temperature from 40 to 60 °C, the peak force for the ground chicken breast patties increased approximately 150%. In contrast, the peak force decreased 14.2% with increasing temperature from 60 to 80 °C. These changes in texture could be affected by changes in the soluble protein properties, myofibrillar proteins, and connective tissue in the ground chicken breast patties. Heating produced a softening of connective tissue caused by conversion of collagen to gelatin and a toughening of meat fibers caused by heat coagulation of myofibrillar proteins (Bouton & Harris, 1972). Because of the lower amount of connective tissues in mollusc muscles, even less than in finfish, less collagen is available in mussel meat to be converted to gelatin during the heat treatments that would lead to greater softness. For mussel denaturation of myofibrillar protein and sarcoplasmic proteins resulting in higher cook loss and greater compression than would be anticipated for fish muscle.

Kinetic models were used to describe experimental data, and an Arrhenius model was used to express the temperature dependence of rate constants. The rate constants, activation energy and correlation coefficient are shown in Table 5. The E_a for the mussel compression force (N) was 64.7 kJ/mol, much lower than reported activation energies of protein denaturation for salmon (70.0–100.5 kJ/mol) (Kong, Tang, Rasco, & Crapo, 2007) and protein denaturation for meat (200–600 kJ/mol) (Anglemier & Montgomery, 1976; Bertola et al., 1994). k values in this study were also lower than ones reported by Kong, Tang, Rasco, and Crapo (2007) for salmon. The increase in compression force observed for mussel might be attributed to a stronger agglomeration complex formed by mussel myofibrillar protein, specifically the actin/myosin complex, upon protein denaturation. If the strength of denatured protein aggregates are stronger than native protein aggregates, the muscle will toughen (Martens et al., 1982) in which case the most important factors would be dehydration and aggregation with protein denaturation being a lesser contributing factor. According to Table 4, there is a high correlation between compression force and cook loss, and also compression force and area shrinkage for each temperature showing the influence of the lower concentration of connective tissue and collagen in mussel meat and how this could affect texture during the heating process.

Table 2
First–order kinetic parameters for cook loss of whole mussel (*Mytilus edulis*) after heat treatment at different temperatures.

Temperature (°C)	k (min^{-1})	R^2	E_a (kJ/mol)	k_0 (min^{-1})	R^2
65	0.0131	0.82	55.6	5956	0.91
70	0.0205	0.85			
75	0.0377	0.91			
80	0.0349	0.82			
85	0.042	0.86			
90	0.0594	0.77			

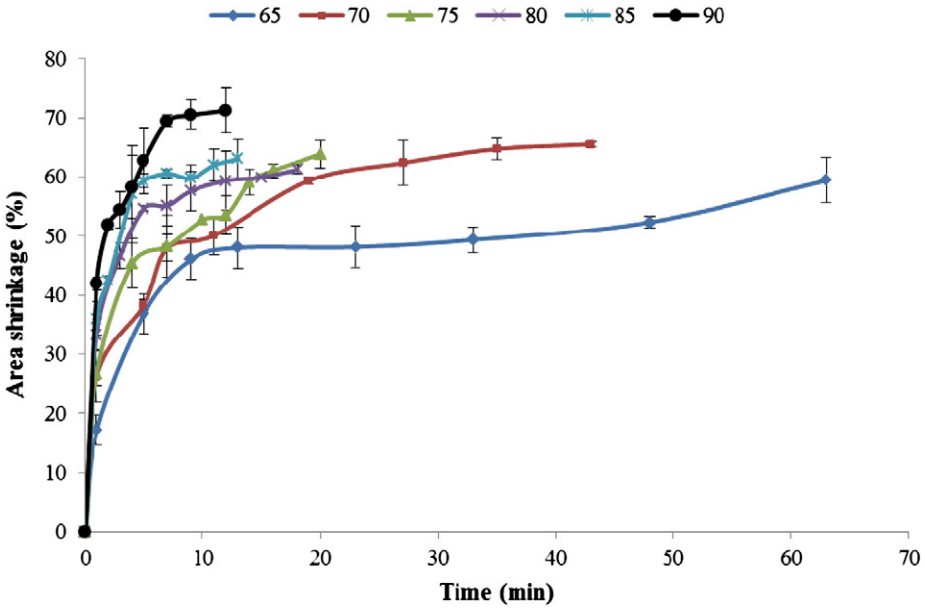


Fig. 3. The change of area shrinkage of whole mussel (*Mytilus edulis*) at different heating regimes, mean ± SD for four determinations.

3.5. DSC analysis

DSC thermograms of mussel mantle and adductor shown in Fig. 5a, b. The transitions for molluscs usually describe in terms of T_{max} because of their tissue complexity (Paredi et al., 1994). The results of fresh mussel mantle DSC thermograms showed three endothermic transitions, with T_{max} values of 79.0, 85.0, and 87.0 °C. In addition, one endothermic transition, with T_{max} value of 112 °C was observed for adductor muscle. Because of the small amount of connective tissues in bivalve mollusc muscle (Paredi et al., 1994) and fish muscle generally (Skipnes et al., 2008) the endothermic transitions can be assigned to denaturation of myofibrillar protein and sarcoplasmic proteins. The denaturation enthalpy (ΔH) which is a net value of endothermic and exothermic reactions and is correlated with the remaining content of ordered three-dimensional structures of a protein were 47.2, 6.1, and 2.4 J/g for first, second, and third peak, respectively in mussel mantle. Totally, three denaturation peaks were observed for mussel mantle. Different numbers of denaturation peak have been reported for different seafood, even for the same product. For instance, denaturation peak for cod proteins reported range between 3 and 8 when samples scanned from 0 °C to 110 °C (Hastings, Rodger, Park, Matthews, & Anderson, 1985; Skipnes et al., 2008). Each peak corresponds to denaturation of specific proteins (Hastings et al., 1985). In current study, the protein denaturation in mussel mantle started from 60 °C with steeper slope to 78 °C. Paredi et al. (1994) reported DSC for the Molina bivalve (*Aulacomya ater*) ranging between 50 and 72 °C.

DSC thermogram of mussel adductor is presented in Fig. 5b, which shows that the adductor protein denaturation was started at approximately 100 °C, and the denaturation peak was at 112 °C with the denaturation enthalpy (ΔH) of 680 J/g which shows the heat stability of adductor protein. Molluscan smooth muscles, such as the smooth bivalve adductor, exhibit a unique mechanical state called "catch state", characterized by an ability of the muscle to develop a long-lasting high tension state with little energy expenditure. These muscles are also characterized by the presence of a heat-stable protein of unknown function in amounts equal to that of myosin. In blue mussel the adductor weight to whole body weight ratio is 7.89 ± 1.27%. This protein, myorod, is localized on the surface of thick filaments together with myosin and twitchin, and it interacts in vitro with the major components of thick filaments, namely paramyosin, myosin, and twitchin (Matusovsky, Shelud'ko, Permyakova, Zukowska, & Sobieszek, 2010; Sheludo'ko, Tuturova, Permyakova, Plotnikov, & Orlova, 1999; Sheludo'ko et al., 2002). Ozawa, Watabe, and Ochiai (2010) reported the denaturation enthalpy of isolated adductor of Yesso scallop (*Mizuhopecten yessoensis*) as 2189 kJ/mol. The denaturation peak temperature T_{max} of 112 °C was higher than reported by others (T_{max} 66 °C) (Paredi et al., 1994). In current study, proteins were not individually isolated, but in other studies isolated adductor proteins have been recovered for DSC analysis (Paredi et al., 1994; Sheludo'ko, Tuturova, Permyakova, Tyurina et al., 2002; Sheludo'ko et al., 1999). For the isolated proteins a lower thermal stability has been observed compared to native protein (Paredi et al., 1994).

4. Conclusion

Cook loss increased with increasing time and temperature behaving as a first–order reaction with a high amount (30%) occurring during the first 5 min of heating. The highest area shrinkage also

Table 3
Second–order kinetic parameters for area shrinkage of whole mussel (*Mytilus edulis*) after heat treatment at different temperatures.

Temperature (°C)	k (min^{-1})	R^2	E_a (kJ/mol)	k_0 (min^{-1}) × 10^4	R^2
65	0.0001	0.73	92.3	2875	0.83
70	0.0004	0.90			
75	0.0007	0.92			
80	0.0005	0.74			
85	0.0009	0.67			
90	0.0016	0.84			

Table 4
Correlation between texture and cook loss and area shrinkage parameters.[a]

Temperature (°C)	Parameters regression (R^2)		
	CL and AS	CL and CF	AS and CF
65	0.88	0.95	0.91
70	0.99	0.96	0.97
75	0.90	0.86	0.95
80	0.90	0.90	0.97
85	0.74	0.87	0.95
90	0.95	0.94	0.94

[a] CL: Cook Loss, CF: Compression Force and AS: Area Shrinkage.

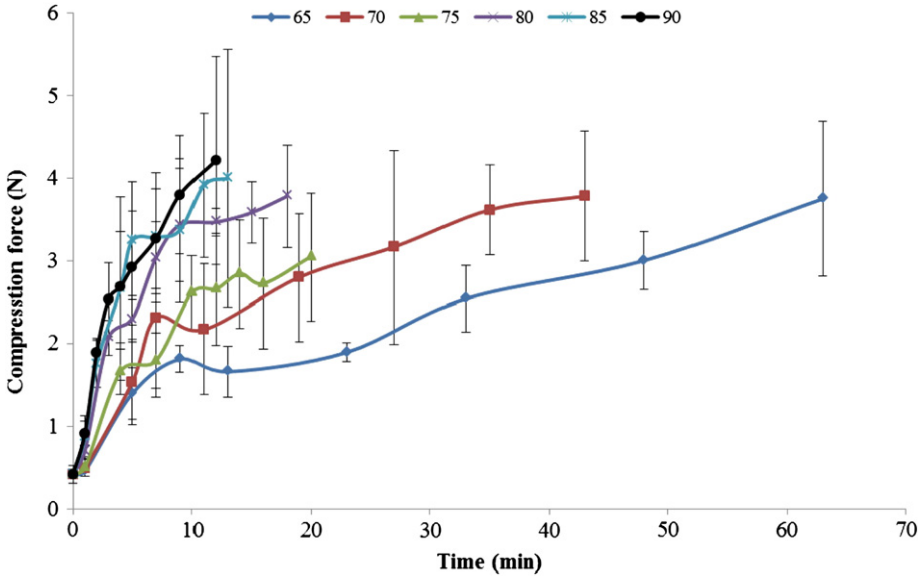


Fig. 4. The change of compression force (N) of whole mussel (*Mytilus edulis*) at different heating regimes, mean ± SD for four mussels with six readings for each.

occurred during the first 5 min of heating (36%) and followed a second–order reaction model. Textural changes were expressed as compression force (N) and were zero–order. A high positive correlation was found between cook loss, area shrinkage and compression force, indicating that dehydration, aggregation, and protein denaturation were the primary contributions to compression force, especially at higher temperatures (75 to 90 °C). Mussel meat composition is different from finfish and other meat and contains less connective tissues and collagen leading to a greater compression force during the heating treatments, because of proportionately less gelatin formation. The E_a for cook loss, area shrinkage and compression force were 55.6, 92.3, and 64.7 kJ/mol, respectively, which was lower for texture compared to other findings, showing that cook loss, and moisture depletion were more important than protein denaturation for texture changes. The results of DSC showed that due to the less connective tissue, the protein denaturation is high in whole mussel, while for adductor muscle which contains heat stable protein, T_{max} is 112 °C. The models presented in this study can be combined with microbial and virus inactivation kinetic data to optimize process parameters to produce high quality pasteurized mussel products.

Acknowledgment

This study was partially funded by USDA-NIFA 2011-68003-20096. We would like to express our sincere thanks to Frank Loring Younce, Stewart Bohnet and Dr. Roopesh Syamaladevi for technical support.

Table 5
Zero–order kinetic parameters for compression force (N) of whole mussel (*Mytilus edulis*) after heat treatment at different temperatures.

Temperature (°C)	k (min^{-1})	R^2	E_a (kJ/mol)	k_0 (min^{-1}) × 10^2	R^2
65	0.0588	0.93	64.7	5908	0.93
70	0.0668	0.85			
75	0.1506	0.92			
80	0.1557	0.78			
85	0.2303	0.83			
90	0.2636	0.88			

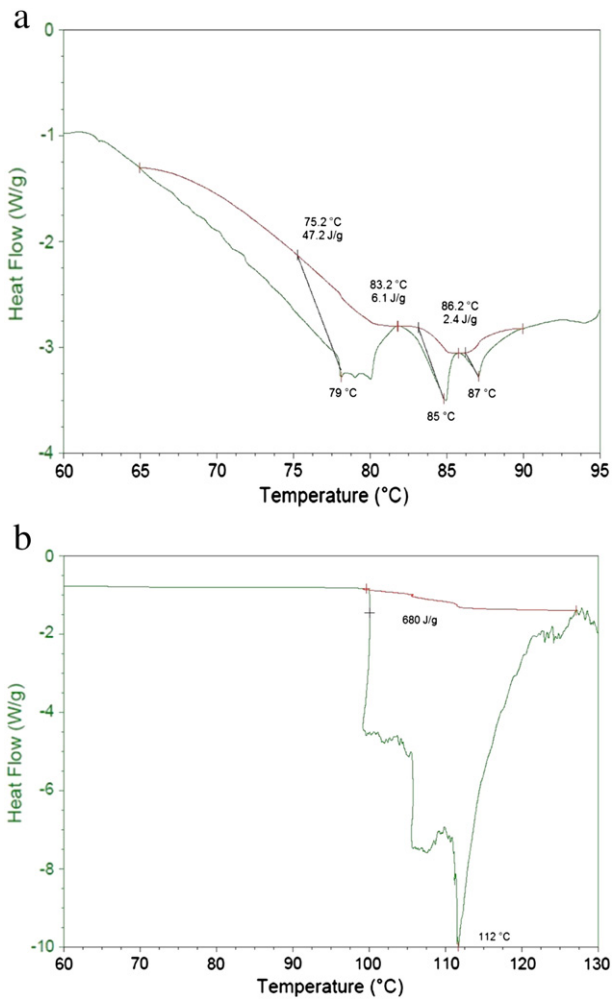


Fig. 5. DSC-thermograms for (a) mussel (*Mytilus edulis*) mantle and (b) mussel adductor at a heating rate of 10 °C/min.

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