Research Article

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Oxidation–reduction potential and lipid oxidation in ready-to-eat blue mussels in red sauce: criteria for package design

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Abstract

BACKGROUND: Ready-to-eat in-package pasteurized blue mussels in red sauce requires refrigerated storage or in combination with an aerobic environment to prevent the growth of anaerobes. A low barrier packaging may create an aerobic environment; however, it causes lipid oxidation in mussels. Thus, evaluation of the oxidation–reduction potential (*Eh*) (aerobic/anaerobic nature of food) and lipid oxidation is essential. Three packaging materials with oxygen transmission rate (OTR) of 62 (F-62), 40 (F-40) and 3 (F-3) $cm^3\ m^{-2}\ day^{-1}$ were selected for this study. Lipid oxidation was measured by color changes in thiobarbituric acid reactive substances (TBARS) at 532 nm (TBARS@532) and 450 nm (TBARS@450).

RESULTS: Significantly higher ($P < 0.05$) TBARS@532 was found in mussels packaged in higher OTR film. TBARS@450 in mussels packaged with F-62 and F-40 gradually increased during refrigerated storage (3.5 ± 0.5 °C), but remained constant after 20 days of storage for mussels packaged with F-3. The *Eh* of pasteurized sauce was not significantly affected ($P > 0.05$) by OTR and remained negative (< −80 mV) during storage. Negative *Eh* values can support the growth of anaerobes such as *Clostridium botulinum*. The headspace oxygen concentration was reduced by about 50% from its initial value during pasteurization, and then further declined during storage. The headspace oxygen concentration was higher in trays packaged with higher OTR film.

CONCLUSION: Mussels packed with high OTR film showed higher lipid oxidation, indicating that high barrier film is required for packaging of mussels. Pasteurized mussels must be kept in refrigerated storage to prevent growth of anaerobic proteolytic *C. botulinum* spores under temperature abuse.

Keywords: blue mussels; oxygen transmission rate; polymer packaging; pasteurization

INTRODUCTION

Blue mussels (*Mytilus edulis*) are bivalve molluscs that are considered a high-value food. They are high in protein (almost 60% dry basis), low in fat (2.5% wet basis), and low in cholesterol.[1] Mussels are favored shellfish in European countries, Japan, China and North America. They are consumed steamed, boiled, smoked, shucked and ozonated.[2–4] However, fresh mussels are highly perishable, even under refrigerated conditions. The shelf life is further limited by high pH, water activity ($a_w > 0.95$), glycogen content and free amino acids.[5] Thus, to increase the shelf life of mussels, proper processing and storage is essential.

Pasteurization is a milder heat treatment process designed to inactivate microbes of public health significance. Since the heat-resistant spores are not inactivated during pasteurization, refrigerated storage (below or equal to 4 °C) is required for extended shelf-life. For low-acid food such as seafoods (pH > 4.6), a pasteurization value of $P^{7}_{90°C} = 10$ min is needed to inactivate non-proteolytic, psychrotrophic *Clostridium botulinum* type E.[6–9]

For in-package thermal pasteurization of mussels, it is necessary to develop polymeric packaging with an appropriate oxygen transmission rate (OTR). For pasteurized products, an in-package aerobic environment and or a refrigeration temperature of 4 °C is required to reduce the risk of the anaerobic growth including proteolytic *C. botulinum*.[10] However, the growth rate of pathogenic spore-forming anaerobes (*Clostridium* spp.) increases by several folds at temperatures above 10 °C and anaerobic conditions.[11] Oxidation–reduction potential (*Eh*) can be used to characterize aerobic or anaerobic conditions in packaged, thermally processed food products.[12] Oxidation reduction or redox potential reflects the ability of a biological or chemical system to oxidize (lose electrons) or reduce (gain electrons). An oxidized substance loses electrons, while another substance is reduced by accepting those electrons.[13] The aerobic condition is needed to prevent or delay the growth anaerobes when the food undergoes any form of temperature abuse. Since the OTR of the packaging films may

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affect the aerobic environment and thereby *Eh* of food due to dissolved oxygen, it is necessary to investigate the influence of OTR on the *Eh* of packaged food. However, oxygen may decrease the quality of mussels due to chemical changes during storage. Mussels can be chemically spoiled by lipid oxidation and further increases in the presence of oxygen. Lipid oxidation is often analyzed by measuring malonaldehyde (MDA) content, a secondary lipid oxidation product, which is expressed as thiobarbituric reactive substances (TBARS). In the traditional TBARS assay, a reaction between thiobarbituric acid (TBA) and sample extract produces a red pigment with an absorbance maximum at 532 (TBARS@532). However, reaction of alkanals and other aldehydes is also possible which forms a yellow pigment with the highest absorbance at 450 nm.[14] In the past, TBARS at 532 nm and 450 nm as a measure of lipid oxidation was conducted for extruded meat products[15] and freeze-dried meat.[16] TBARS has also been considered as one of the major reasons for the development of warmed-over flavor (WOF) in cooked meat during refrigerated storage.[17,18] Rhee *et al.*[18] reported that a cardboard-like flavor in cooked refrigerated meat increases with increasing TBARS content. The level of headspace oxygen may be affected by packaging films with different OTRs, which change the conditions for lipid oxidation and *Eh*. Thus, the overall objective of this study was to understand the influence of lid film OTR on changes in the headspace oxygen, *Eh* and TBARS at 532 and 450 nm in pasteurized mussels during refrigerated storage. This study also examined the pH of mussels and sauce and hardness of the mussels.

MATERIALS AND METHODS

Sample preparation and packaging

For this study, fresh blue mussels (*Mytilus edulis*) with shell were purchased in bulk from a local retailer (Safeway, Pullman, WA, USA) and immediately stored in a −30 °C chamber in the Food Science and Human Nutrition building at Washington State University, Pullman, WA. Mussels were used within 3–4 days of storage. Before preparation, mussels were thawed on ice and washed in normal tap water. The meat was recovered (average weight = 10 ± 2.5 g, $n = 20$) from the shell. Approximately 200 ± 5 g of mussels was added to each tray with sauce, as described below.

Three multi-layer polymeric films with three different oxygen transmission rates (OTRs) were acquired from film manufacturers and used as a lid material to seal the trays. The first film had a nine-layer (mLLDPE/LLDPE/LLDPE/tie/Nylon/tie/LLDPE/LLDPE/mLLDPE; thickness = 110 µm) coextruded structure with OTR of 62 $cm^3\ m^{-2}\ day^{-1}$ at 0% RH, 23 °C. The second film consisted of five layers (LDPE/tie/Nylon/tie/LDPE; thickness = 90 µm) with an OTR of 40 $cm^3\ m^{-2}\ day^{-1}$ at 0% RH, 23 °C. The third film had a three-layer (PET/tie/EVOH-PP, thickness = 105 µm) structure with an OTR of 3 $cm^3\ m^{-2}\ day^{-1}$ at 23 °C, 0% RH. Three films were designated as F-62 (OTR = 62 $cm^3\ m^{-2}\ day^{-1}$), F-40 (OTR = 40 $cm^3\ m^{-2}\ day^{-1}$) and F-3 (OTR = 3 $cm^3\ m^{-2}\ day^{-1}$). Films F-62, F-40, and F-3 had a water vapor transmission rate (WVTR) of 3.88, 4.34, 1.14 g $m^{-2}\ day^{(-1)}$ at 100% RH and 37.8 °C. Approximately 70 g of red tomato sauce, 3 g of salt, and 1.5 g of paprika were homogenized with a blender and added to the 200 g of mussels (mussel meat with exudate). This was vacuum sealed (200 mbar; sealing condition: 185 °C for 4 s dwell time) in a 10-ounce Rexam tray (Silgan Plastic Food Containers, Union, MO, USA) (nearly impermeable to oxygen; inner dimensions: 14.5 cm × 10 cm × 3 cm) using a vacuum sealer (MULTIVAC T-200; Multivac Inc., Kansas City, MO, USA). Due to the thickness of the sheets (1.05 mm) used in the manufacturing of the trays, the tray walls were considered to be impermeable to oxygen. The oxygen transmission through the lid was taken into account. Trays were prepared in duplicate for each treatment. A total of 36 trays was prepared for this study ($N = 36$). In this study, the trays packaged with aluminum foil were not used with an intention to create little or more aerobic environment by allowing oxygen transfer through lid films.

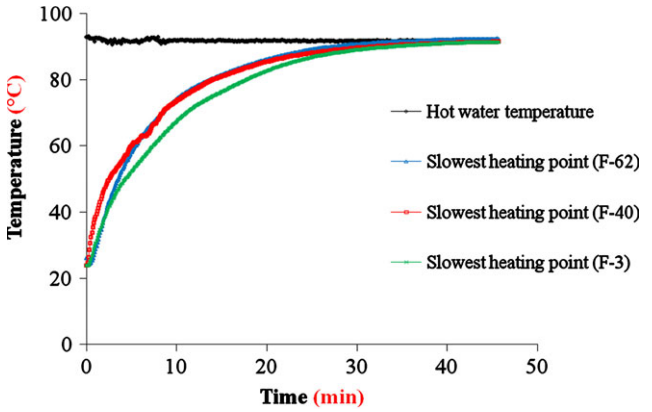

Figure 1. Time–temperature profile of slowest heating region inside packaged trays during pasteurization in hot water at 90 °C.

Thermal pasteurization

Thermal pasteurization of the packaged mussels was carried out by immersing trays in hot water in a steam-jacketed kettle, and the water temperature was maintained at 93 °C. During processing, the center temperature (slowest heating point, 1.5 cm from tray bottom) of tray was monitored by a T-type thermocouple (TMQSS-032U-6, 0.032 inch outer diameter, 6 inch long; Omega Engineering, Stamford, CT, USA). The tip of the thermocouple was inserted into one of the mussels in the tray to monitor the temperature rise in the sample. The time–temperature data ($n = 2$) were collected by a data logger system connected to the thermocouples. The pasteurization value $P^{z}_{T_{ref}}$ describes the intensity of pasteurization of a food system and the $P^{7}_{90°C} = 10$ min is designed for 6-log reduction of psychrotrophic, non-proteolytic *Clostridium botulinum* type E spores. The pasteurization value can be calculated as follows:

$$P^{z=7°C}_{T_{ref}=90°C} = \int_0^t 10^{[T(t)-T_{ref}]/z}\,dt \qquad (1)$$

where $T(t)$ is the temperature rise of the center of the tray with respect to time, T_{ref} is the reference temperature, and the z value of the psychrotrophic nonproteolytic *C. botulinum* is 7 °C.[7] The trays were immediately cooled to room temperature by running cold water at 23 °C for 15 min. Immediately after processing (0 day), two trays were analyzed, and the remainder was stored at 3.5 ± 0.5 °C in the dark. An additional thermometer was used to monitor the temperature throughout storage. The mussel samples were analyzed at 0, 10, 20, 30, 45 and 60 days of storage.

The time for slowest heating region inside the tray packaged with F-62 and F-40 to achieve desired lethality ($P^{7}_{90°C} = 10$ min) was 35 min, while it was 37 min for the tray packaged with F-3 (Fig. 1).

Headspace oxygen

The headspace oxygen concentration in the packaged trays was measured with a non-invasive oxygen analyzer (Oxysense GEN

III 300 series system; OxySense, Las Vegas, NV, USA). An Oxydot (O2xyDot®) was attached to the surface of the film in contact with the headspace inside the trays, and at one of the corners of the lid prior to sealing the trays. The dot was illuminated by blue light from a LED sensor. The blue light was absorbed by the dot and the sensor captured the emitted red light. The trays were taken out of storage and equilibrated at room temperature (23 °C) for the measurements. The Oxydot (O2xyDot®) has the ability to sense the temperature and waited till it showed 23 °C during measurements. The Oxydots can measure the oxygen concentration at temperatures ranging from 0 to 50 °C. Before measurement, the dots were calibrated against standard values provided by the manufacturer. The headspace oxygen concentration (% O_2) was measured before and after processing, and during refrigerated storage. These experiments were repeated three times in duplicate for each type of packaging (number of trays, $n = 6$). Additionally, the initial headspace volume in the tray after vacuum sealing was measured using water displacement technique. The amount of oxygen in the headspace was calculated using the ideal gas law and expressed in grams.

Oxidation–reduction potential

The oxidation–reduction potential (*Eh* values in mV) of the sauce was determined using a pre-calibrated platinum ring redox electrode with a 3 mol L^{-1} KCl reference electrolyte (InLab® Redox Pro; Mettler Toledo, Columbus, OH, USA) connected to a pH meter (Seven Go pH meter; Mettler Toledo). Measurements were performed *in situ*, immediately after opening the tray. *Eh* values at six different locations inside the tray (number of trays, $n = 2$) were collected until they reached a stable value, as indicated by the instrument. The measurement took 60 to 120 s. The Eh_7 (*Eh* at pH 7) value of food was calculated algebraically after including the pH correction factor of 59 mV/pH at 25 °C[12] as:

$$Eh_7 = Eh - 59\,(7 - \mathrm{pH}) \tag{2}$$

where *Eh* and pH are the measured *Eh* and pH of the sauce, respectively.

Lipid oxidation

Lipid oxidation was determined by a modified 2-thobarbituric acid reactive substance (TBARS).[19] Whole mussel samples (number of mussels, $n = 6$) were homogenized in deionized (DI) water (1:10 w/v) using a Polytron PT 2500E (Kinematica, Bohemia, NY, USA) in a 50 mL tube for 30 s at 10 000 rpm. Butylated hydroxyanisole (BHA) (50 μL g^{-1} wet mussel, 7.2% in ethanol) (Acros Organics, Thermo Fisher Scientific Inc., Bridgewater, NJ, USA) was added to wet mussels before homogenizing in DI water. Thiobarbituric acid (TBA) (reagent grade; J.T. Baker, Avantor Performance Materials, Center Valley, PA, USA), trichloroacetic acid (Fisher Scientific, Pittsburgh, PA, USA), hydrochloric acid (J.T. Baker, Avantor Performance Materials) (TBA-TCA-HCl) stock solution was prepared by mixing 15% w/v TCA, 0.375% w/v TBA, and 0.25 mol L^{-1} HCl. The mixture was heated slightly for total dissolution of the TBA. One milliliter of mussel homogenate was taken in duplicate from each sample, mixed thoroughly with 2.5 mL of TBA-TCA-HCl solution in a 15 mL of centrifuge tube, and placed in a hot water (95 °C) bath (ISOTEMP 215; Fisher Scientific, Pittsburgh, PA, USA) for 20 min to develop the color. The mixture was immediately cooled down in an ice-water bath for 10 min, and centrifuged in a Beckman centrifuge (Beckman J2-HS; Beckman, Brea, CA, USA) at 10 °C and 2968 × *g* for 15 min. The absorbance of the resulting supernatant was measured at 532 and 450 nm using a UV–visible spectrophotometer (Ultrospec 4000; Amersham Pharmacia Biotech., GE Healthcare Life-sciences, Pittsburgh, PA, USA) against a blank containing 1 mL of DI water with 2.5 mL of TBA-TCA-HCl solution. Additionally, a separate kinetic cell study with only mussel tissue was performed to determine the potential matrix effect of the sauce on the TBARS assay. The aluminium kinetic cell had an 8 mm height, 50 mm inner diameter, and 3 mm wall thickness. Mussels were bisected longitudinally into two equal parts. One half was analyzed for TBARS after heat treatment (90 °C for 10 min, come-up time 1.55 s) and the other was analyzed without heat treatment. The absorbance values were expressed as TBARS@532 and TBARS@450 as a measure of oxidation. The amount of MDA (corresponding to the TBARS@532 nm) was determined from a standard curve prepared from the MDA standard (OXI-TEK TBARS analysis kit; Enzo Life Sciences, Farmingdale, NY, USA). This was expressed as TBARS (μmole MDA g^{-1} of wet mussel) only.

Lipid oxidation kinetics

A 'logistic equation' was employed to simulate lipid oxidation kinetics to consider individual reaction steps of free radical chain reaction that involves initiation, propagation, termination.[20] The logistic equation[20] can be written in differential form:

$$\frac{\mathrm{d}C}{\mathrm{d}t} = kC\left(1 - \frac{C}{C_{\max}}\right) \tag{3}$$

where C is the concentration of oxidation products (TBARS) at time t, C_{max} is the maximum concentration of C at the end of lipid oxidation process, k is the reaction rate constant. It is noticeable that the $1 - C/C_{max}$ term in the Eqn (3) approaches 1 when $C << C_{max}$ yielding a first order reaction; when $C = C_{max}$, the term $1 - C/C_{max}$ becomes zero which indicates the end of the process ($dC/dt = 0$). The integral form of the Eqn (3) can be written as:

$$kt = a - \ln\left(C_{\max}/C - 1\right) \tag{4}$$

where $a = \ln(C_{max}/C_0 - 1)$ and C_0 is the concentration at $t = 0$.

A non-linear regression analysis (quasi-Newton) was performed to determine the k and a values in the Eqn (4) using MATLAB R2013a software (MathWorks, Natick, MA, USA).

Overall cook/weight loss

The pasteurized mussels were washed gently by dipping them into normal DI water and then dried on blotting paper for 1 min. The overall cook loss (just after pasteurization) and weight loss of the mussels (during storage) were calculated as follows:

$$\text{overall cook/weight loss}\,(\%) = \frac{w_i - w_f}{w_i} \times 100 \tag{5}$$

where w_i is the initial weight and w_f is the final weight.

pH

The pH of mussels and sauce was measured during 60 days of refrigerated storage. The mussels were dipped into DI water for 15 s to wash the sauce layer from the mussel surface. The mussel samples (number of mussels, $n = 6$) were homogenized in fresh distilled water (1:2 w/v) using a Polytron PT 2500E at 6000 rpm for 2 min. The pH of the homogenate was measured

Table 1. Average of log reduction of headspace oxygen in trays ($n = 6$) during refrigerated storage

	Headspace oxygen concentration (V, %)			$\log(V/V_0)$		
Storage (days)	F-62	F-40	F-3	F-62	F-40	F-3
Non-pasteurized	19.1 ± 0.11	18.9 ± 0.22	18.6 ± 0.12	–	–	–
0	9.2 ± 0.64	9.0 ± 0.64	9.1 ± 0.57	0^{a}	0^{a}	0^{a}
10	3.3 ± 0.64	2.60 ± 0.30	1.9 ± 0.01	0.45 ± 0.05^{b}	0.54 ± 0.02^{c}	0.67 ± 0.03^{d}
20	0.6 ± 0.08	0.2 ± 0.01	0.2 ± 0.04	1.22 ± 0.04^{e}	1.58 ± 0.02^{h}	1.61 ± 0.04^{h}
30	0.3 ± 0.01	0.2 ± 0.03	0.1 ± 0.04	1.47 ± 0.05^{g}	1.61 ± 0.03^{h}	1.87 ± 0.10^{i}
45	0.4 ± 0.02	0.2 ± 0.01	0.1 ± 0.02	1.37 ± 0.05^{f}	1.64 ± 0.02^{h}	1.83 ± 0.04^{i}
60	0.3 ± 0.01	0.3 ± 0.01	0.2 ± 0.01	1.43 ± 0.01^{fg}	1.46 ± 0.05^{g}	1.63 ± 0.04^{h}

V_0 and V represent the headspace oxygen concentration at 0 day and during storage, respectively.
Values are given as mean ± SD and compared along rows and columns.
Values with different superscript letters are significantly different ($P < 0.05$). The values reported are $\log(V/V_0)$.

by immersing the pH electrode in the sample. The pH value was obtained until the value becomes stable as indicated by the pH meter (FE 20 pH meter; Mettler Toledo). For each tray, the pH of the sauce (mixture of red tomato sauce, salt, and paprika plus mussel exudates following pasteurization) was measured by direct immersion of the pH electrode in six different locations in the sauce ($n = 2$) (a total of 12 measurements on each storage day). The pH electrode was calibrated with a buffer solution of pH 4 and 7 before the original measurements were taken.

Hardness

The hardness of the mussels was analyzed according to the method of Ovissipour *et al.*[5] A texture analyzer, TA-XT2 (Stable Micro Systems Ltd, Guildford, UK) equipped with a stainless steel puncture probe (35 mm length and 5 mm diameter, flat tip), was used. The traveling speed was set at 1 mm s^{-1} for a distance of 5 mm. The trays were equilibrated at room temperature (23 °C) and the mussels were taken out of the tray just before measurement. The peak compression force (N) was obtained by analyzing the force–time graph using Texture Expert software for Windows (version 1.15, Stable Micro Systems Ltd). The measurement was taken at four different locations on each mussel, and eight randomly selected mussels ($n = 8$) were analyzed for each treatment.

Statistical analysis

The experimental data analysis involved a completely randomized design (CRD) with a two-way ANOVA test. Fisher's least square difference (LSD) method with a significance level of $\alpha = 0.05$ was employed. A two-way ANOVA test ($P < \alpha$) was employed to determine the interaction between storage time and packaging type. Mean values and standard deviation (SD) from means were reported. All data analysis was performed with commercial statistical software SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Headspace oxygen

Headspace oxygen plays a critical role in lipid oxidation of packaged food products.[21,22] The measured initial headspace oxygen before pasteurization was 19.1, 18.9, and 18.6% for trays packaged with F-62, F-40, and F-3, respectively (Table 1). The initial headspace volume in the trays packaged with F-62, F-40, and F-3 was 14 ± 1, 15 ± 1, and 15 ± 1 mL, respectively. The calculated available initial amount of oxygen in the headspace would be

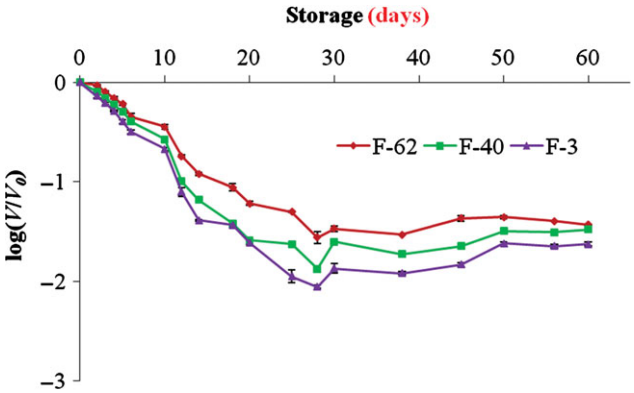

Figure 2. Reduction of headspace oxygen concentration in packaged trays during refrigerated storage ($n = 6$) (V_0 and V denote the oxygen headspace concentration at 0 day and during storage, respectively).

6.91×10^{-4} g, 7.36×10^{-4} g, and 7.24×10^{-4} g for F-62, F-40 and F-3, respectively. After pasteurization, a significant reduction in headspace oxygen occurred. The headspace oxygen decreased to 9.2, 9.0, and 9.1%, which was almost 50% reduction from its initial value (Table 1). One possible cause of decline in headspace oxygen is the consumption of oxygen by mussels and sauce during thermal pasteurization. Headspace oxygen further decreased to 0.3, 0.2, and 0.1% after 30 days of storage for trays packaged with F-62, F-40 and F-3, respectively. The primary reason for such decline may be the dissolution of headspace oxygen into the aqueous fraction of pasteurized food. The average value of log reduction [$\log(V/V_0)$] of headspace oxygen with respect to 0 day is presented in Table 1. The average log reduction of headspace oxygen after 30 days of refrigerated storage was 1.47, 1.61 and 1.87 for F-62, F-40 and F-3, respectively. No significant changes were observed between 25 to 60 days of storage (Fig. 2). The reduction was significantly higher ($P < 0.05$) in trays packaged with F-3 than that of F-40 and F-62 (Fig. 2), indicating that the OTR of the film had a significant effect on headspace oxygen concentration inside the packaged food, both post-processing and during storage.

Oxidation–reduction potential

The *Eh* value of non-pasteurized sauce fell in range of 51.5–57.7 mV (Table 2). The high *Eh* value of the food may be attributed to low pH value, such as fruit juices.[23] The tomato sauce used in this study was acidic (approximate pH = 4) to slightly acidic

Table 2. Oxidation–reduction values of sauce during refrigerated storage (n = 2)

Film		Storage (days) NP	0	10	20	30	45	60
F-62	*Eh*, mV	51.5 ± 2.8^{g}	-97.5 ± 9.9^{abc}	-105.3 ± 14.6^{abc}	-92.8 ± 11.1^{abc}	-103.3 ± 11.3^{bc}	-96.0 ± 15.8^{ab}	-99.3 ± 10.5^{abc}
F-40	*Eh*, mV	54.5 ± 5.3^{g}	-89.7 ± 10.9^{a}	-103.8 ± 7.7^{bc}	-97.5 ± 10.1^{abc}	-106.0 ± 10.7^{c}	-95.8 ± 9.7^{abc}	-103.0 ± 11.1^{abc}
F-3	*Eh*, mV	57.7 ± 8.8^{g}	-129.5 ± 8.1^{d}	-124 ± 22.6^{e}	-139.3 ± 16.1^{de}	-137.7 ± 13.2^{de}	-159.5 ± 8.8^{f}	-168.8 ± 17.5^{f}
F-62	Eh_7, mV	-125.5 ± 2.8^{g}	-209.6 ± 9.9^{d}	-206.7 ± 14.6^{bcd}	-194.9 ± 5.5^{abcd}	-204.8 ± 11.3^{cd}	-200.4 ± 15.8^{abc}	-198.5 ± 10.5^{abcd}
F-40	Eh_7, mV	-122.5 ± 5.3^{g}	-190.6 ± 10.9^{ab}	-195.3 ± 7.7^{abc}	-192.5 ± 10.1^{abc}	-197.5 ± 10.7^{abcd}	-187.3 ± 9.7^{a}	-191.5 ± 11.1^{ab}
F-3	Eh_7, mV	-119.3 ± 8.8^{g}	-246.7 ± 8.1^{e}	-238.3 ± 22.6^{e}	-254.6 ± 16.1^{e}	-255.8 ± 13.2^{e}	-271.9 ± 8.8^{f}	-282.9 ± 17.5^{f}

Values in rows and columns are given as mean ± SD and compared along rows and columns.
Values with different superscript letters are significantly different ($P < 0.05$).
NP, non-pasteurized.

(pH to 5.5), depending upon its composition. After pasteurization, the *Eh* value dropped to a range of −89.7 to −129.5 mV. This indicates that the composition of the sauce changed from an oxidized state (positive) to a reduced state (negative) due to heat treatment and the diffusion of higher pH-soluble components from mussels into tomato sauce. The mussels and sauce were packaged at 200 mbar vacuum, which would have removed some dissolved oxygen during packaging. Heating may have also expelled dissolved oxygen from the sauce. Additionally, pasteurization altered the pH and overall composition of sauce. Increase in pH can also be associated with decreasing *Eh*. According to the Nernst equation, hydrogen ion concentration or pH significantly affects the *Eh*. Every unit of decrease in pH results in an increase in *Eh* value by 59 mV. It should be noted that food components such as redox substances, pH, ratio of reductant and oxidant, salt content, poising capacity (foods' resistance to change their redox potential), availability of oxygen, type of packaging, and microbial activity determines the redox value of food.[12,13,24]

No significant change ($P > 0.05$) in *Eh* values was observed throughout the storage period, indicating a well-poised system (Table 2). The *Eh* value of the sauce system packaged with F-3 was significantly lower (−129.5 mV) than either F-62 (−97.5 mV) or F-40 (−89.7 mV) at 0 day, which may be due to a little higher processing time for F-3 (37 min). However, food composition plays a major role in determining the *Eh* of the system. The Eh_7 values were also calculated using Eqn (2), and the values for the non-pasteurized sauce were further lowered to −125.5, −122.5, and −119.3 mV for F-62, F-40, and F-3, respectively. The corresponding values decreased to −209.6, −190.6, and −246.7 after heat processing (Table 2). Montville and Conway[12] reported that the Eh_7 value for canned foods ranged from −18 to −438 mV. They observed that only four out of 26 canned foods showed aerobic conditions (positive redox values) even when exposed to the atmosphere for 24 hr at 4 °C. Foods might have low or negative oxidation–reduction potential, whether they are packaged in air or exposed to air. Thus, air does not always make the food aerobic.[12,13] One major finding from their study reveals that foods inoculated with *C. botulinum* and with higher redox potential do not favor the formation of toxins. However, toxin formation was observed in the same foods with lower *Eh* or Eh_7 values.[12]

The growth of different groups of microorganisms depends on a certain range of redox values: aerobes, +500 to +300 mV; facultative anaerobes +300 to +100 mV; and anaerobes, +100 to −250 mV or lower.[24,25] In our study, Eh/Eh_7 values after processing were always negative, regardless of the OTR of the films studied, indicating a favorable environment to support the growth of anaerobes such as *Clostridium*. Lund *et al.*[26] attempted to correlate the partial pressure of oxygen in the medium to the redox potential of the medium on the growth of *C. botulinum* type E. They concluded that the relationship depends on the chemical nature of the medium. A recent study was conducted to investigate the effect of anoxic (<0.5%), micro-oxic (6–8%) and oxic (20%) conditions on growth of anaerobic spore-forming *Clostridium perfringens* in beef and nutrient broth at 7 °C and 22 °C.[11] After 5 days of storage at 22 °C, *C. perfringens* counts in beef samples increased by 4–5-log and 1-log under anoxic and micro-oxic condition whereas reduction in counts (>1-log) was observed under oxic condition. However, the anaerobic spore-former *C. perfringens*, when inoculated at 1-log CFU g^{-1}, could not survive any of the oxygen environments at 7 °C after 14 days of storage irrespective of growth medium. When inoculated at higher concentration (2-log CFU g^{-1}) in beef, it survived with 1-log reduction from its initial value only under anoxic condition at 7 °C. Similar results were also found for broth at 7 °C but it also survived under micro-oxic condition with 1-log reduction.[11] In the present study, storage temperature was 3.5 °C which is much more severe than 7 °C. Therefore, it can be concluded that growth of anaerobic spore-formers such as *Clostridium* spp. is prohibited even the oxygen concentration in the trays reduced from 9.0% (micro-oxic) to 0.6% (anoxic) after 20 days of storage. The anoxic condition in the headspace and negative redox potential of the medium both are favorable for growth of anaerobic spore-forming bacteria. Thus, temperature abuse (>10 °C) must be avoided.

Oxygen is a strong oxidizing agent. Our study showed that the oxidation–reduction potential did not change significantly ($P > 0.05$), even though the oxygen was rapidly consumed by the food during storage (Fig. 2). This strongly indicates that the food is 'well-poised'.

Lipid oxidation

In our study, incubation of raw mussel (non-pasteurized) homogenate and TCA-TBA-HCl assay developed a pink color ($\lambda_{max} = 532$ nm). However, a yellow pigment ($\lambda_{max} = 450$ nm) was observed during analysis of pasteurized mussels. This is possibly due to the reaction with aldehydes,[16] amines, and other oxidizing compounds in mussels. A similar phenomenon was observed in a kinetic cell study, in which a pink color was observed for unprocessed mussel samples while the cooked sample (the other half) developed a yellow tinge.

A few studies report the yellow color instead of the pink color during the TBARS assay for lipid oxidation, including extruded

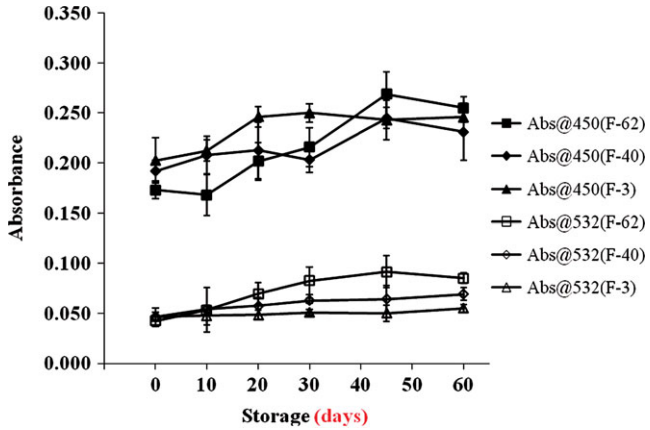

Figure 3. Absorbance values of lipid oxidation of mussel TBARS analysis at 532 and 450 nm over the storage period ($n = 6$).

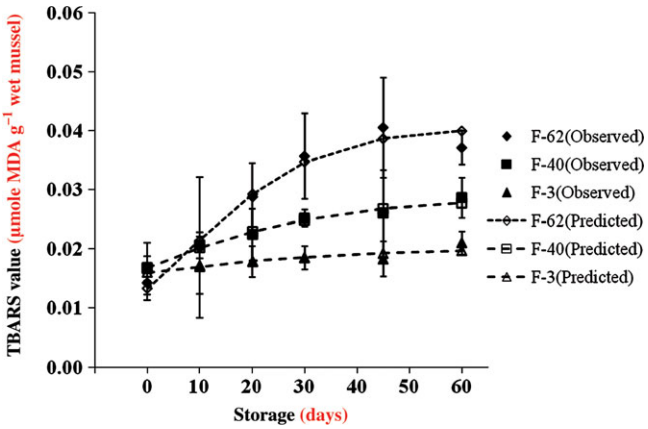

Figure 4. Lipid oxidation kinetics of pasteurized mussels during 60 days of refrigerated storage.

meat product[15] and freeze-dried beef.[16] For beef, the yellow color was attributed to the reaction between aldehydes and thiobarbituric acid (TBA).[16,27] The initial value of TBARS@532 for raw mussels ranged from 0.104 to 0.108, with an almost 55–60% decrease in absorbance values for pasteurized mussels (0.104 to 0.042 for F-62, 0.106 to 0.047 for F-40, and 0.108 to 0.048 for F-3). At the same time, a 155–220% increase in TBARS@450 from its initial value (corresponding to the unprocessed mussel) was observed (0.059 to 0.173 for F-62, 0.06 to 0.192 for F-40, and 0.08 to 0.203 for F-3). Wilkinson *et al.*[16] also noticed similar phenomenon for freeze-dried beef (TBARS@450 > TBARS@532) whereas fresh beef showed completely opposite behavior (TBARS@450 < TBARS@532).

Lipid oxidation (TBARS@532 and TBARS, μmole of MDA g^{-1} of wet mussel) was significantly higher ($P < 0.05$) in packaged mussels with higher OTR film (F-62), followed by lower OTR films (F-40 and F-3) (Fig. 3). The TBARS@450 of mussels packaged in F-62 and F-40 showed an increasing trend during storage, whereas in trays packaged with F-3, this remained almost constant after 20 days of refrigerated storage (Fig. 3). The TBARS value of mussel reached 0.037 ± 0.003 for F-62, 0.029 ± 0.003 for F-40, 0.021 ± 0.002 for F-3 μmole g^{-1} of wet mussel after 60 days of storage. The TBARS content at 532 nm for raw mussel was 3.38–3.54 mg of MDA equivalent kg^{-1} of mussel which is a little higher than the reported value of 2.73 mg of MDA equivalent kg^{-1} of raw mussel.[1] After 60 days of storage, the highest TBARS for pasteurized mussels would be 0.83 mg of MDA equivalent kg^{-1} (packed in F-62). In good quality fish, the TBARS content varies from 5 to 8 mg MDA kg^{-1}, and correlates well with sensory properties of product.[28] However, no sensory evaluations were performed to correlate TBA@532 and TBA@450 to oxidative rancidity developed in mussels.

The TBARS value (μmole MDA g^{-1} of wet mussels) was fitted to Eqn (4). The R^2 values obtained were 0.97, 0.95, and 0.92 for F-62, F-40, and F-3, respectively. The obtained reaction rate constants (k) were 0.084 day^{-1}, 0.051 day^{-1} and 0.024 day^{-1} for the mussels packaged with F-62, F-40 and F-3, respectively. The predicted and observed values are shown in Fig. 4. Results demonstrated that the film OTR and the reaction rate constant (k) follow a linear relationship (Fig. 5). The reaction rate constant (k) was found to be higher for mussels packed with a higher OTR film. The logistic type mathematical equation also simulated the lipid oxidation kinetics of several food systems, such as egg yolk, cooked pork meat, frozen

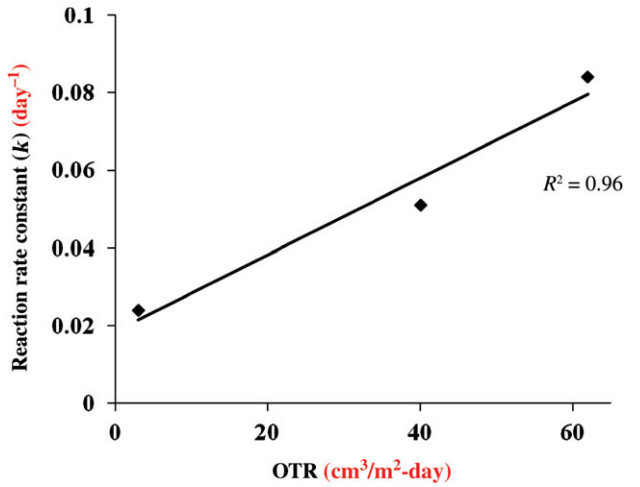

Figure 5. Relationship between packaging film OTR and reaction rate constant (k) at 3.5 ± 0.5 °C.

mackerel fillets, reconstructed beef and pork nuggets, raw poultry meat[20] and oil emulsions.[29]

Lipid oxidation depends on the availability of oxygen, presence of light and transition metals, storage temperature, fatty acid composition, and degree of unsaturation in lipids. In the current study, mainly oxygen was responsible for lipid oxidation in the product as trays were stored in dark and refrigerated storage. Lipid oxidation was higher in the trays with higher headspace oxygen. Under refrigerated temperatures, the solubility of oxygen in liquid media increases (e.g. oxygen solubility in water: 2.3×10^{-8} at 0 °C and 9.8×10^{-9} mol kg^{-1} Pa^{-1} at 50 °C). Thus, lipid oxidation may also be driven by dissolved oxygen as temperature falls. A study on soybean oil stored at 55 °C showed that volatile formation in the oil is significantly influenced by the amount of dissolved oxygen.[30] However, these results may not be comparable with our results, since oxygen solubility in oil is higher than that of the aqueous phase. For example, at 20 °C, oxygen is 5.4 and 7.9 times more soluble in olive oil and sunflower oil compared to water, respectively.[31] The dependence of lipid oxidation on the oxygen partial pressure has also been investigated for oils[32,33] and powders.[34] A system with more than 4–5% oxygen concentration can be considered a high-oxygen environment for lipids at room temperature.[16] In our study, although the oxygen concentration

Table 3. The pH value of mussels ($n = 6$) and sauce ($n = 2$) during refrigerated storage

	pH of mussel		
Storage (days)	F-62	F-40	F-3
Non-pasteurized	6.4 ± 0.0^{j}	6.7 ± 0.2^{k}	6.4 ± 0.2^{j}
0	5.7 ± 0.0^{a}	5.5 ± 0.10^{b}	5.1 ± 0.0^{gh}
10	5.3 ± 0.0^{de}	5.3 ± 0.1^{ef}	5.1 ± 0.0^{i}
20	5.1 ± 0.0^{g}	5.3 ± 0.1^{f}	5.0 ± 0.0^{i}
30	5.3 ± 0.0^{def}	5.6 ± 0.1^{b}	5.0 ± 0.0^{hi}
45	5.4 ± 0.0^{cd}	5.5 ± 0.1^{b}	5.0 ± 0.1^{hi}
60	5.4 ± 0.0^{d}	5.5 ± 0.0^{bc}	5.0 ± 0.0^{hi}
	pH of sauce		
	F-62	F-40	F-3
Non-pasteurized	4.0 ± 0.0^{k}	4.0 ± 0.0^{k}	4.0 ± 0.0^{k}
0	5.1 ± 0.0^{g}	5.3 ± 0.0^{de}	5.0 ± 0.0^{j}
10	5.3 ± 0.0^{e}	5.5 ± 0.0^{b}	5.1 ± 0.0^{hi}
20	5.3 ± 0.0^{e}	5.4 ± 0.0^{c}	5.1 ± 0.0^{i}
30	5.3 ± 0.0^{e}	5.5 ± 0.0^{b}	5.0 ± 0.01^{j}
45	5.2 ± 0.0^{f}	5.5 ± 0.0^{b}	5.1 ± 0.01^{ghi}
60	5.3 ± 0.0^{3}	5.5 ± 0.0^{b}	5.1 ± 0.01^{ghi}

Values are given as mean ± SD and compared along rows and columns. Values with different superscript letters are significantly different ($P < 0.05$).

was about 9% at the beginning of storage, it decreased to < 5% after 5 days of storage. In this context, direct comparison may not be applicable, since refrigerated condition slows down oxidation processes.

Overall cook/weight loss

The overall cook loss was calculated using Eqn (5) as 65.8 ± 0.2 %, 72 ± 1.2 %, 75 ± 2.3 % of the initial weight of the mussels packaged in trays of F-62, F-40 and F-3, respectively (data not shown) at the start of storage (0 day). Our results agree with earlier values obtained by Ovissipour *et al.*[5] who reported a cook loss of around 70% after 10 min of heating at 90 °C. No significant change ($P > 0.05$) in the overall weight of mussels was observed during storage.

pH

Raw mussels are a low-acid food (pH > 4.6) with pH values of 6.7 and 6.4 (Table 3). These values accord with the reported initial pH value of blue mussels, which is 6.4 (Khan *et al.*[1]) and 6.8.[35] The initial pH of the sauce (mixture of red tomato sauce, salt, and paprika) was 4.00, which is slightly acidic. After pasteurization, pH of the mussels packaged with F-62, F-40 and F-3 decreased to 5.7, 5.5 and 5.1, respectively. However, the sauce pH increased by almost 1 – 1.3 unit (Table 3), reflecting heat-induced diffusion during pasteurization. These results accords with those of other studies, which found a sharp decrease in initial pH of Mediterranean mussels (by 3 units) after hot-smoking[36] and saury fillet (by 2 units) after marination.[36] Filling media (aqueous medium or oil) can significantly alter the quality, creating losses in water, amino acids, proteins, minerals, vitamins, and fatty acids in fish muscle.[37] After pasteurization (0 day), the pH of mussels and sauce packed in trays with F-62 and F-40 differed significantly ($P < 0.05$) (Table 3), reaching equilibrium after 30 days of storage. It was also evident that the pH of mussels packed in F-62 and F-40 increased after 20 days of storage, with almost no changes in mussel pH for F-3. However, no significant ($P > 0.05$) change was observed in pH of mussels and sauce packed in trays with any of the three films during storage (Table 3).

Table 4. The peak compression force (N) of whole mussel during 60 days of refrigerated storage ($n = 8$)

	Compression force (N)		
Raw mussel/storage	F-62	F-40	F-3
Raw mussel	0.88 ± 0.20^{gh}	0.98 ± 0.17^{gh}	0.75 ± 0.15^{h}
Storage, days			
0	1.99 ± 0.65^{bcd}	2.10 ± 0.75^{bc}	1.61 ± 0.22^{de}
10	1.80 ± 0.28^{cde}	2.37 ± 0.65^{ab}	1.41 ± 0.35^{ef}
20	1.66 ± 0.34^{de}	2.61 ± 0.56^{a}	1.20 ± 0.22^{fg}
30	1.64 ± 0.40^{de}	2.21 ± 0.42^{abc}	1.50 ± 0.24^{ef}
45	2.09 ± 0.38^{bc}	2.32 ± 0.58^{ab}	1.68 ± 0.38^{de}
60	1.80 ± 0.30^{cde}	2.10 ± 0.43^{bc}	1.65 ± 0.33^{de}

Values are given as mean ± SD and compared along the rows and columns.
Values with different superscript letters are significantly different ($P < 0.05$).

Hardness

The mean hardness value for raw, non-pasteurized mussels ranged from 0.75 ± 0.15 to 0.98 ± 0.17 N (Table 4). Ovissipour *et al.*[5] reported the mean compression force for raw mussel to be 0.41 ± 0.11 N. This difference due to variations in the size and shape of the raw mussels tested. Upon pasteurization, the compression force increased more than two times from its initial values (0.88 to 1.99 N, 0.98 to 2.10 N, and 0.75 to 1.61 N). Ovissipour *et al.*[5] found compression values close to 4.00 N after 10 min of processing at 90 °C in a kinetic cell. Our compression values were lower, due to filling media (sauce in this particular case), the amount of samples, and the heat distribution and penetration (slow heating) patterns inside the trays. Larsen *et al.*[38] correlated the hardness of cooked King salmon to sensory properties. The salmon fillets were subjected to various cooking processes (poached, pan fried, microwaved, oven baked, steamed, and deep fried) until the internal temperature of fillets reached 70 °C. They reported that the average hardness value increased from 0.70 N (raw fillets) to 0.99, 0.93, 1.45, 1.21, 1.93 and 2.52 N for poached, steamed, microwaved, oven baked, pan fried and deep fried samples, respectively. Sensory analysis revealed that consumers liked salmon fillet that has middle range of textural hardness with baked ones liked most followed by pan fried fillets. The texture of deep fried (most intense cooking method) and poached (least intense cooking method) samples were not favored by the consumers. Salmon fillets and mussel texture are not truly comparable, also cooking processes are different. However, texture of mussels can be accepted by consumers on the basis that hardness of pasteurized mussels (1.61 – 2.10 N) is comparable to the baked (1.21 N) or pan fried (1.93 N) fillets.

Agglomeration and denaturation of the mussel protein (actin-myosin complex) in cooking temperatures from 75 to 95 °C toughened the mussels, and thereby increased the compression force.[5] An increase in firmness due to muscle toughening and subsequent loss in the water-holding capacity of the fish muscle as a result of heating was reported by several authors.[39,40]

Wattanachant *et al.*[41] observed a significant ($P < 0.05$) increase in shear force of chicken mussel after heating at 50 and 80 °C. In another study on thermal processing of salmon, two different force peaks were observed after 5 min and 60 min heating at 121 °C.[38] This was attributed to the denaturation of the most of the protein during the first 5 min of heating, and later, the formation of coagulated sarcoplasmic proteinaeous aggregates between myofibrils after 60 min of heating, respectively.[38]

The hardness value of the mussel remained almost constant, with no significant ($P > 0.05$) changes over 60 days of storage. The presence of salt in the sauce medium could help in maintaining texture throughout the storage period. In a study reported in the literature, no significant difference ($P > 0.05$) in textural properties was noticed for pickled fish-roes (containing 12% salt w/w) even after 6 months of storage at 28 °C.[42] However, no significant ($P > 0.05$) increase in compression value was observed.

CONCLUSIONS

Lipid oxidation causes a drastic reduction in headspace oxygen level after the pasteurization process. Further decline in headspace oxygen concentration was observed during the 60 day storage period (3.5 ± 0.5 °C). The oxidation–reduction potential of sauce changed from aerobic (positive *Eh*) to anaerobic conditions (negative *Eh*) following pasteurization. During the storage at 3.5 ± 0.5 °C, there was a negative oxidation–reduction potential for sauce, regardless of the type of packaging film. The lipid oxidation can be measured at 450 nm and 532 nm as the TBARS assay color changed from pink to yellow after pasteurization. The lipid oxidation and its reaction rate constant were higher for trays packaged with higher OTR films. Our findings demonstrate that mussels should be packaged with lower OTR (higher barrier) film to minimize chemical changes during storage. Additionally, mussels that are pasteurized in the package should be kept under refrigerated storage without temperature abuse to prevent the growth of anaerobic spore forming bacteria such as proteolytic *C. botulinum*.

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