Preparation of EO water

Acidic electrolyzed water (pH: 2.7; ORP:1150 mV; free chlorine: 60 ppm) was generated at 9-12 V direct current (dc) for 15 min using a two-compartment batch scale electrolysis apparatus (Super Oxseed Labo, Electrolyzed Water Generator, Aoi Electronic Corp., Kannami, Shizuoka, Japan), with the anode and cathode sides of the chamber divided by an ion exchange diaphragm according to our previous studies (Ovissipour et al., 2015; Al-Qadiri et al., 2016 a,b; Shiroodi et al., 2016). Commercially available neutral electrolyzed water (NEW) (Aquaox Disinfectant 275) was provided from Aquaox LLC. (Dillsburg, PA, U.S.A.) with active hypochlorous acid (275 ppm) which was generated electrochemically by electrolysis of a dilute sodium chloride solution passing through an electrolytic cell at neutral pH. For treating the Atlantic salmon fillets the NEW was diluted to obtain a solution with free available chlorine content of 60 ppm, a pH of 6.8, and an ORP of 786 mV.

The ORP and pH were measured using a pocket-sized redox meter (HI 98201, HANNA® Instruments, Ann Arbor, Michigan, USA) and a pH meter (FE20, Mettler-Toledo, Columbus, OH, USA), respectively. The free chlorine concentration of the EO water was measured with a DPD assay (Colorimeter™ Analysis System, Hach Co., Loveland, CO, USA) according to the manufacturer instructions.

Fourier transform infrared spectroscopy (FTIR)

Salmon muscles blocks were prepared from the raw and heat treated samples at different times and temperatures ($1\times0.5\times0.2$ cm^2). FT-IR spectral features of each individual salmon muscle sample were collected using Thermo Nicolet Avatar 360 FT-IR spectrometer (Thermo Electron Inc., San Jose, CA, USA). The salmon muscle sample were placed in direct contact with the ATR ZnSe

crystal spectra taken between 4000 to 400 cm^{-1}. Ten spectra were collected at room temperature (ca. 22°C) for each sample in triplicate with thirty spectra for each sample in total, at different locations on the surface of the salmon muscle. For each spectrum 32 scans were averaged with the spectra resolution of 4 cm^{-1}.

Atlantic Salmon Protein Denaturation

One of the major concerns about the electrolyzed water application in food processing and sanitation is the impact of the electrolyzed water on the quality and nutritional value of the final products. In this study, we applied FTIR combined with chemometrics to study the secondary structure of Atlantic salmon protein exposed to different electrolyzed water at different temperatures.

In order to study the effect of different treatments on protein structure of Atlantic salmon, amide I (1700-1600 cm^{-1}) which is the most dominant band in myofiber spectrum was focused for studying the secondary structure of protein because of its sensitivity to hydrogen-bonding patterns, dipole-dipole interaction, and geometry of the polypeptide backbone (Astruc et al., 2012; Carton et al., 2009). Amide I is assigned to C=O stretching vibration, and minor in-plane N-H bending and C-N stretching vibration. Many researchers used amide I region to study the effect of different processing on secondary structure of protein, such as brined Atlantic salmon (Carton et al., 2009; Bocker et al., 2008), pressurized and heated (40°C) Atlantic salmon (Ojagh et al., 2011), cooked pork (Bertram et al., 2006; Wu et al., 2006), aged beef (Kirschner et al., 2004), heated beef (Astruc et al., 2012), and pasteurized Atlantic salmon (Ovissipour et al., 2017).

In current study, amide I in original spectra had the maximum absorbance at 1638 cm^{-1} in raw Atlantic salmon, which was shifted to lower frequencies by increasing the temperature and applying electrolyzed water. The results showed that, the peak was shifted significantly to 1630 cm^{-1} in samples treated at 65°C using AEW ($P < 0.05$). The lowest frequencies for water, AEW and NEW were observed at 65°C, however the frequency for samples treated with water as control and NEW was higher than AEW. In addition, the peak area and the peak intensity decreased significantly ($P < 0.05$), and the minimum peak area and intensity were related to the samples which were treated at 65°C by AEW. The two-way ANOVA analysis showed that the peak area and peak intensity, significantly influenced by temperature, the solutions, and their interaction. However, the NEW had less impact on the peak intensity and area compared to AEW, which is due to the type of chlorine, lower pH and higher ORP of the AEW.

In order to improve the spectra in amide I region to focus on secondary structure of the protein, second derivative was provided. Second derivative spectra revealed several bands including 1695 cm^{-1}, 1682 cm^{-1}, 1668 cm^{-1}, 1660 cm^{-1}, 1651 cm^{-1}, 1631 cm^{-1}, 1625 cm^{-1}, 1618 cm^{-1}, 1611 cm^{-1}, which was similar to other researcher's findings for the Atlantic salmon (Bocker et al., 2008; Ojagh et al., 2011; Ovissipour et al., 2017). The band at 1651 cm^{-1} is related to the α-helical structures which are present to 90% of the myosin protein and shows protein loop structures (Kirschner et al., 2004; Bertram et al., 2006; Bocker et al., 2008). The highest intensity for this peak in all treatments, was related to the samples treated with deionized water and NEW, and the lowest peak intensity was observed in samples treated by AEW. In addition, this peak, increased by increasing the temperature which showed the protein denaturation and unfolding increased by increasing the temperature of heating and lower pH of the AEW. Other researchers also reported that by increasing the temperature the intensity of this peak is decreasing and tend to be diminished at

higher temperatures (Wu et al., 2006; Ojagh et al., 2011; Ovissipour et al., 2017). The intensity of the peak around 1618 cm^{-1}, increased by increasing the temperature and the greatest peak intensity at each temperature was related to the samples which were treated by AEW. The peak around 1618 cm^{-1} is assigned to the β-sheet structures and showed the increasing of protein aggregation in samples at the intermolecular level (Carton et al., 2009; Ojagh et al., 2011; Ovissipour et al., 2017). The same results were observed at 1682 cm^{-1}, which is related to changes in intramolecular antiparallel β-sheet structures in thermally treated samples, particularly in those which were exposed to AEW, which is in agreement with other researches reports (Carton et al., 2009; Ojagh et al., 2011; Ovissipour et al., 2017). To study the variance of the myofiber spectra for treated samples, Principal component analysis (PCA) was applied. The PCA results for samples treated with deionized water, NEW and AEW at different temperatures (20, 50, 55, 60, and 65°C) is presented in Figure 1. The results showed that nearly 63% of the variance was explained by PC1, and 20% was explained by PC2. The PCA results showed that the samples which were subjected to AEW, were discriminated from the other samples, and the deionized and NEW samples were similar. Hence, AEW had highest impact on the quality of the Atlantic salmon compared to NEW at each temperature. It has been reported that the temperature of the protein denaturation strongly depends on pH (Heyes and El-Sayed, 2001). At pH, lower than 3, protonation of some of the negative carboxylic amino acids occurs which changes the hydrophobicity of certain regions of the protein and alters the polar bonds in both intrahelical and interhelical regions (Heyes and El-Sayed, 2001; Furlan et al., 2007). Significant decrease in α-helical structure and increase in β-sheet groups, due to the low pH have been reported by other researchers (Furlan et al., 2007; Saguer et al., 2013). In addition, the effect of temperature on protein denaturation depends on the ionic strength of the solutions (Saguer et al., 2013). It has been shown that higher ORP and low pH cause

high ionic strength in electrolyzed water solution (Pangloli and Hung, 2011). Since, in this study, the AEW pH and ORP were 2.7 and 1150 mV, respectively, more protein denaturation was observed in AEW treated samples at higher temperatures compared to NEW with the pH and ORP of 6.8 and 786 mV.

Other than low pH in AEW, the form of chlorine also has impact on spectra. Due to the lower pH, the free available chlorine is mainly in the form of Cl_2, which can strongly reduce the peak intensity compared to hypochlorous acid in NEW (Xue et al., 2012).

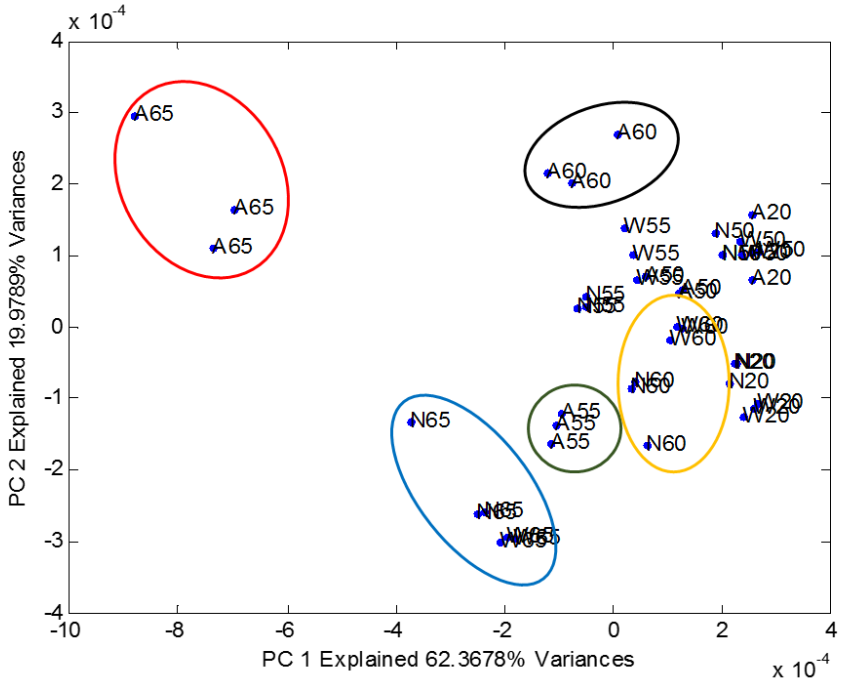

Figure 1: The PCA for Atlantic salmon muscles treated by water, AEW and NEW at different temperatures