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Development of a mathematical protocol to graphically analyze irreversible changes induced by high pressure treatment in fish muscle proteins

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ABSTRACT

The aim of this study was to develop a mathematical protocol (MP) to graphically analyze the irreversible changes induced by high pressure treatment in fish muscle protein. The MP was validated by thermal analysis data (DSC) from pressurized samples of *Seriolella violacea* muscle tissue between 200 and 500 MPa for 2 s to 10 min. The MP included methodologies to obtain the thermodynamic parameters of protein denaturation and building 3-D plots from representative thermograms. Results obtained from the application of the MP showed that the pressurization of muscle tissue provoked that the total denaturation enthalpies values diminished, these changes were more evident for treatments >450 MPa. The irreversible denaturation of actin in the pressurized samples was observed for any treatment condition >300 MPa and greater than 3 min. The proposed MP is a powerful tool to improve data analysis from DSC when muscle tissue samples are subjected to pressurization.

1. Introduction

Chemical and physical factors can induce protein denaturation (Clarkson et al., 2016; Larrea-Wachtendorff et al., 2015; Stumpe and Grubmüller, 2007; Totosaus et al., 2002). Temperature (heat treatment, frozen) and pressure are the main physical factors that are studied (Espinosa et al., 2017; Pucci and Rooman, 2017; Cheng et al., 2017; Jantakoson et al., 2012; Fernandez-Martin, 2007; Zamri et al., 2006; Ma and Ledward, 2004; Lambelet et al., 1995). When proteins are exposed to a heat treatment, the secondary, tertiary, and quaternary protein structures are maintained through weak bonds being hydrogen bonds the most labile. On the other hand, when proteins are exposed to a pressure treatment, hydrophobic and electrostatic interactions are the most vulnerable and hydrogen bonds are largely unaffected by pressure (Galazka and Ledward, 1998). The high hydrostatic pressure (HHP) technology has been widely used in the field of food processing because of several advantages over traditional food preservation methods (Üretener, 2010; Dzwolak et al., 2002). It has long been known that conventional thermal processes affect the appearance, flavors, and nutritional value of foods. On other hand, HHP maintains the organoleptic properties, vitamins and nutrient of foods. Additionally, this process eliminates food pathogens and extends the shelf life of food (Huang et al., 2017; Wang et al., 2016; Rastogi, 2013). Food response to pressurization is difficult to predict because it is necessary to take into account the combined effect of three parameters, *i)* pressure level, *ii)* pressure holding time, and *iii)* temperature (Erkan et al., 2011). Other parameters, such as sample size, state of *rigor*, species nature, pH, chemical composition, in among others, are important factors to consider during pressurizing of meat, fish and seafood (Sun and Holley, 2010; Yagiz et al., 2007, 2009). If the purpose of the pressurizing is the bacteria inactivation, temperature and pressurization rate must be carefully selected (Ahn and Balasubramaniam, 2007). Pressure level can lead to significant conformational changes that influence protein functionality (Colmenero, 2002; Messens et al., 1997; Mozhaev et al., 1996; Macfarlane and McKenzie, 1976) and pressure-induced protein unfolding is a slow process that can occur over timescales of seconds to minutes to hours (Sarupria et al., 2010).

Changes induced by HHP on proteins normally can be studied by differential scanning calorimetry (DSC). Two important thermodynamic parameters can be obtained through protein thermal analysis by DSC, denaturation temperature (T_m) and denaturation

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enthalpy (ΔH). In addition, total denaturation enthalpy (ΔH_T) involving all the peaks in each thermogram is also a frequently determined thermodynamic parameter. Denaturation temperature and denaturation enthalpy of each independent protein of a complex system such as muscle tissue is difficult to determine. Total denaturation enthalpy in muscle tissue thermograms is normally estimated as the area under the DSC transition line using a straight line connecting the beginning and end of each thermogram from approximately 30 to 80 °C (Matos et al., 2011; Skipnes et al., 2008; Chapleau et al., 2004; Chevalier et al., 2001). A similar method is used for different individual peaks, that is, a straight line connecting the beginning and end of each peak which constitutes each thermogram (Matos et al., 2011; Chapleau et al., 2004). An alternative method to determine thermodynamic parameters of protein denaturation is DSC thermogram deconvolution. Deconvolution has been originally applied to protein heat capacity curves (Freire, 1994), but it is possible to use it to determine the number of thermal transitions during thermal denaturation and the thermodynamic parameters for each of those transitions in DSC thermogram analysis (Chapleau et al., 2004; Rossi and Schiraldi, 1992). Thermogram deconvolution has been perceived as a better analytical tool for understanding both the dynamics and patterns of protein deterioration (Badea et al., 2012). Deconvolution has provided good results for egg proteins (Rossi and Schiraldi, 1992) and isolated myofibrillar proteins (Chapleau et al., 2004), but its use in samples of whole muscle tissue has not been yet investigated.

Several DSC applications show the influence of HHP on seafood and other muscle foods (Larrea-Wachtendorff et al., 2015; Qiu et al., 2014; Jantakoson et al., 2012; Fernandez-Martin, 2007; Zamri et al., 2006; Schubring, 2005; Schubring et al., 2003; Chevalier et al., 2001; Angsupanich et al., 1999; Angsupanich and Ledward, 1998). Unfortunately, thermal analysis of a complex system by DSC, such as muscle tissue, also produces complex thermograms (Schubring, 2005; Chevalier et al., 2001); a better understanding of pressurization effects on protein thermal stability has been limited. The methodology currently being used to analyze changes in protein thermal stability first involves organizing the thermograms of both treated and untreated samples in two-dimensional graphical representations (2-D plots) and then comparing to find differences between them. Only the most characteristic thermogram for each physical treatment condition (among several experimentally obtained) are used to build such 2-D plots; it would therefore be very interesting to establish a methodology to use the information of all of the experimentally obtained thermograms to build a representative thermogram of the entire process.

In any typical 2-D plot, the x-axis shows thermal analysis temperature while the y-axis shows differential heat flow (Larrea-Wachtendorff et al., 2015; Schubring, 2005; Chevalier et al., 2001; Angsupanich et al., 1999). The DSC software normally displays experimental thermograms in graphically spaced diagrams so that they do not overlap; however, this spacing is not governed by an axis that is correlated with the intensity of an applied treatment. Therefore, it is a complex task to determine the changes induced by the treatment applied on protein thermal stability using 2-D plots. To build 3-D plots from the experimentally obtained data is an interesting option to clearly understand the changes in the treated samples. In these proposed 3-D plots, the x-axis shows the intensity of applied treatment (i.e., temperature, pressure level, or pressure holding time) while the y- and z-axes show thermal analysis temperature and differential heat flow, respectively. Data on a uniform mesh are required to build a 3-D plot, but it is often impractical or impossible to obtain experimental data for all the specific points at which values are desired. One way to solve this problem is to apply a mathematical approximation technique such as the Renka and Cline algorithm (3-D interpolation algorithms). This technique can interpolate data from a non-uniform mesh to a set of rectangular grid points (Renka and Cline, 1984). There is currently no evidence of building both representative thermograms and 3-D plots from thermal analysis experimental data (DSC) to analyze changes produced by a physical treatment in proteins.

It is necessary to explore new techniques of experimental data analysis which provide muscle tissue thermal analysis. The aim of this study was to develop a mathematical protocol (MP) to graphically analyze the irreversible changes induced by high pressure treatment in fish muscle protein. The proposed MP included developing methodologies to obtain the thermodynamic parameters of protein denaturation, building representative thermograms, interpolation of representative thermograms data, and building three-dimensional graphical representations of representative thermograms (3-D plots).

2. Materials and methods

2.1. Mathematical protocol

2.1.1. Determination of thermodynamic parameters

In this research study, DSC thermogram deconvolution was applied to determine two thermodynamic parameters of protein denaturation, temperature and enthalpy from both treated and untreated samples. Total denaturation enthalpy was also considered. In the proposed mathematical protocol, each thermogram was considered as a function (Q) that depends on thermal analysis temperature or time. Thus $Q(T)$ and $Q(t)$ represented thermograms in terms of temperature or time, respectively. In addition, the behavior of function Q is strongly dependent on the configuration (isothermal phase temperature and time and dynamic curve heating rate) of thermal analysis and the intensity at which the samples were previously treated. The following constant parameters of thermal analysis were considered in the present study: isothermal phase temperature (T_{iso}, °C), time (t_{iso}, s) and dynamic curve heating rate (ν , °C min^{-1}). The intensity of the physical treatment was established by two parameters. For example, these two parameters in the pressurization treatment were pressure holding time (t_P, s) and pressure level (P, MPa).

Fig. 1 displays a flowchart of the mathematical protocol proposed in the present study. Before performing the muscle tissue thermogram deconvolution process, it was first necessary to export the dataset (thermal analysis temperature and differential heat flow) from the thermograms obtained by DSC (Fig. 1, stage I). The STARe evaluation software (v.10.01, Mettler-Toledo AG, Schwerzenbach, Switzerland) was used for that purpose and the procedure is described as follows: The dataset of each thermogram was exported to a two-column table on a Microsoft Office Excel 2010 worksheet using the STARe evaluation software. The first column in each exported table contained the independent variable, thermal analysis temperature (T, °C) while the second column contained the dependent variable, differential heat flow (q, mW). Two new columns were then included on the worksheet from the recently exported data where the first new column was thermal analysis time (t, s) computed from thermal analysis temperature (T, °C) according to Equation (1), where 60 (s min^{-1}) is the time conversion factor and T_{iso}, ν, and t_{iso} were previously described.

$$t\,(s) = \left[\frac{T\,(^{\circ}C) - T_{iso}(^{\circ}C)}{\nu\,(^{\circ}C\ min^{-1})} * 60\ \left(s\ min^{-1}\right)\right] + t_{iso}(s) \qquad (1)$$

The second new column was differential heat flow per gram of sample (Q, W g^{-1}) computed from differential heat flow (q, mW) using Equation (2) where 0.001 (W mW^{-1}) is the heat flow conversion factor and m is the weight (g) of muscle tissue analyzed in

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