Quasi-isothermal Analysis in a MDSC for Protein Denaturizing in Lyophilized Meat

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ABSTRACT

Meat pork consists essentially of muscle and connective tissue. Myosin constitutes 45% of the total myofibrillar protein and denature at 40-60°C, actin in about 20-25% start denaturation at 71°C and collagen in connective tissue presents complete denaturation at 70°C. Meat tenderness depends on connective tissue, which is responsible for so-called "bottom hardness" and myofibrillar proteins for “myofibrillar hardness”. Myosin, actin and collagen are the main structural proteins, so that the effect of heat influences the quality characteristics in meat.

The aim of this study was applied quasi-isothermal conditions in a MDSC calorimeter in fresh, frozen and refrozen lyophilized pork meat, to evaluate the proteins denaturation behavior taking into account the total heat flow, heat capacity, heat flow phase and out of phase Cp.

In results, total heat flow show overlapping transition temperatures for fresh and frozen meat at 55 to 63°C; refrozen samples had a similar profile at 65-75°C, with a long denaturation first step. Temperature range shows a good agreement with the reported for myosin and collagen. Reversible heat capacity shows also similar changes with a ΔCP in 1.4, 1.04 and 1.7 J/g°C for fresh, frozen and refrozen lyophilized meat samples, respectively, and the last one with a slow rate of change at 50 to 75°C. Heat flow phase shows a long lag phase in refrozen samples, taking about 150 min for decrease the angle value related to fresh and frozen samples. Furthermore, out of phase Cp indicates a maximum value for fresh and frozen samples at 150 min in 0.3 to 0.4 J/g°C and 0.1 J/g°C at 300 min for refrozen samples, indicating that the protein susceptibility to denaturation is affected by water redistribution in the last one.

Phase angle between heat flow and heating rate and out of phase Cp enables more detailed contributions for protein denaturation.

Key words: Meat; frozen; quasi-isothermal; transition temperatures; heat capacity; heat flow phase.

INTRODUCTION

Meat consists primarily of muscle and several amounts of connective tissue, as well as a small portion of epithelial and nervous tissue [1]. Since both muscle and connective tissues are primarily responsible for qualitative and quantitative characteristics of meat, any process effect study must be focused on the identification of any changes affecting these two tissues.

The myosin, that constitutes approximately 45% of the total myofibrillar protein, is the least heat stable and denatured in the temperature range from 40 - 60 °C [2]. Collagen is the main protein of connective tissue with a complete denaturing around of the 70°C. The actin is 20-25% of myofibrillar protein, is the most heat stable and starts its denaturation at 71 °C, becoming complete at 83°C.

On the other hand, the high ionic strength denatures some proteins, causing loss of muscle water retention and fibers inability to reabsorb the thawing water, which translates as loss by exudates. The protein damage often suffered during the freeze process depends on time and temperature.

DSC or MDSC using thermal analysis is a technique that has allowed determining the behaviour of materials by their transitions enthalpies and changing in heat capacity [3-10]. Thus, Brunton et al. (2006) found changes in the dielectric properties of beef muscle (bicep femoris) at temperatures of 5°C to 85°C and related them with the major structural meat protein (particularly collagen) denaturing temperature, evaluated by DSC. Their assessments on the DSC, were using a heat rate of 10º C/min, allowed to locate three thermal transitions at 59, 66 and 82° C, and related them with denaturation temperatures of myosin, collagen and actin, respectively. A relaxometry NMR and differential scanning calorimetry (DSC) study on pork, found a correlation between the denaturation myosin chains and thermally induced changes of myofibrillar water at ~ 53 - 58° C, as well as between the actin denature and the water removal from meat at ~ 80 - 82° C [11].
Assessments undertaken with the DSC at a heating rate of 1° C/min from 3°C to 90°C, allowed locating three endotherm transitions at 54, 65, and 77 °C, which corresponded to denaturation of myosin, sarcoplasmic-collagen protein and actin, respectively. Murphy et al. (1998), found three endotherm transitions in chicken breast at 53, 70 and 79° C, which placed as denaturation temperatures of myofibrillar (53° C) and sarcoplasmic (70 to 79° C) proteins compared with purified protein fractions.

The aim of this study was applied MDSC calorimeter quasi-isothermal conditions in fresh, frozen and refrozen lyophilized pork meat, to evaluate the protein's denaturation behavior, taking into account the total heat flow, heat flow phase and out of phase Cp. The two last signals were intended to relate and interpret these with that observed in the total heat flow and reversible Cp, in order to expand the founded changes as well as to view the versatility of its use to understanding the effect of heat phenomena on meat protein treatment.

MATERIALS & METHODS

The experimental tests were done with 3 samples of longissimus dorsi pork cut, castrated male from 6 months old, at 110 kg; the average weight of the rods was 3.7 kg. It was recommended that the conditions for slaughtering should have been similar. The Pork meat was purchased through a local supplier located in Mexico State.

Fresh pork meat samples were frozen and refrozen, in a convective freezer at - 25° C, and thawing for approximately 24 hrs. All samples were lyophilized for a period of 24 h at - 40° C and 0.10 mm Hg of chamber pressure in a LABCONCO freeze-dryer; fresh, frozen and refrozen pork meat samples were submitted to thermal analysis in a MDSC under quasi-isothermal conditions. Approximately, 21 mg of each hydrated sample were placed in a MDSC (TA Instruments series 2920) initially equilibrated at 50°C, modulation amplitude of ±0. 5° C/min, with 1°C increments, and 5 min equilibrated time to data storage; experimental run duration was 350min approximately. All runs were made in duplicate.

By baseline correction, according to the complete deconvolution process [9], heat flow phase and out-of-phase Cp were obtained in TA Universal Analysis software V.4.4. The MDSC calibration constants were: constant cell with Indium in 1.0908, temperature at 156.65°C and a heat capacity constant with sapphire at 1.1740.

RESULTS & DISCUSSION

Figure 1 shows a comparison of the total heat flow for the lyophilized fresh, frozen and refrozen samples. Clearly, transition temperatures are observed as well as the overlapping curves in the case of fresh and frozen meat samples. Each one of them is in temperature ranging from the 55 to 63°C, 55 to 62 and 65-75°C, with a good agreement with temperatures reported for myosin and collagen [2, 11]; the overlapping in the total heat flow profile have a transition enthalpy of 56.6 and 56.7 J/g. This samples shows that the effects of a slow freezing process are not significant since the water-protein interaction could not have great changes during the unfrozen step; otherwise when meat comes to refrozen, the transition take place almost 12°C higher than in the other two cases. For that, the meat components after refrozen could be more susceptible, and a single transition that takes a long time is observed, retarding the denaturation temperature of myosin, collagen and actin, by effect of continuous water redistribution during the unfrozen process.
These allow that a shift in the transition temperature of meat proteins is present for the refrozen process, and the greatest energy contribution may be probably for myosin, since the onset denaturing temperature in the first step is so long, ranging from 53 to 60°C. Bertram (2006) and Melendez (2002) showed that changes in this area are enabling the initial fall of total heat flow and, therefore, the lengthening of the transition temperature. Myosin molecules differ considerably in their structure of the globular heads and helical tails; Wright and Wilding (1984) showed that the three major transitions associated with the thermal denaturation of myosin in rabbit can be attributed to the different region of the molecule: helical queue, intermediate region and globular heads.

Figure 2 shows the behavior of the heat flow phase, that is the phase angle between heat flow and heating rate. This concept born considering that during an experimental run, the modulation of the heat flow does not follow the modulation of the heating rate, due to a thermal event in the sample that could be generating or absorbing a considerable amount of energy, altering the heat flow response [9]. Therefore, it is possible to assume that phase angle will be zero in those areas without present any transition and so, some alteration in the degree of change will be the effect of a kinetic or structural event in meat samples. Accordingly, we can assume that changes observed in certain areas during the analysis of fresh, frozen and refrozen samples, can be attributed to the denaturation of proteins, such as collagen, actin and myosin.

In fact, profile variations in the refrozen meat would mean that changes are taking place in the sample and directly affect the heat flow phase response, due to energy absorption, since the water-protein interaction is altered by effect of water redistribution during frozen and refrozen samples. So, the rearrangement of proteins will be towards more linked structures and major energy absorption during the heating process.

For fresh meat, the heat flow component displays a light transition at 59 to 71°C; phase angle allows locating at least seven events of interest at temperatures 55, 56, 59, 62, 64, 66 and 71°C. These temperatures are in good agreed with that reported for myosin, collagen and actin denaturation at 54, 65 and 77 °C, respectively, on pork meat [2, 11].
Figure 2. Heat flow phase for lyophilized samples.

Figure 3 shows the comparison of phase angle and $C_p^{\text{reversible}}$ signals. We observe that the reversible $C_p$ follows the profile for heat flow phase component, so that the angle phase lower point close to 60-75°C for each sample is the same as the end of fall in heat capacity ($C_p$). Regularly, this type of comparisons among phase angle and $C_p^{\text{reversible}}$ are used to study the vitrification and crystallization processes in polymers systems [9, 15, 16].

Figure 3. Reversible $C_p$ and Heat flow phase for fresh (A), frozen (B) and refrozen (C) lyophilized samples.
For lyophilized samples can be seen that in fresh and frozen meat the temperature at the end of Cp decay are in agree with a low value for the heat flow phase, however the magnitude of change in everyone is different; for fresh meat is at 1.43 J/g°C while for frozen is only 1.04 J/g°C in about 60°C. In the case of refrozen sample, reaches an order of change of 1.732 J/g°C at 75°C. Based on reversible heat capacity is closely linked with the material structure, and therefore, energy requirements to produce changes in the material, can set that effects due to the freezing of meat only cause light breaks. However, the refrozen process seems to change substantially and enhance the number of structural changes, and hence, energy requirements. Changes in the reversible Cp without phase change are related to second-order transitions such as glass transition, so that confirms that the nature of the proteins denaturation is a second order transition and there is a discontinuity in the heat capacity, which does not reach to be infinite, as a first order transition [17]. On the other hand, we can interpret that the reduction of signal in the phase angle, decrease the energy absorption due to the molecular rearrangement of protein during warming, which is an evidence of its denaturation. Refrozen lyophilized sample, initially show a significant value in the heat flow phase, whose maximum peak coincides with that seems to be a lower slope in the reversible Cp around of 50 and 60°C, range where myosin denaturation is located.

Figure 4 shows the out of phase Cp, kinetic Cp or not reversible Cp for samples studied. We see that while magnitudes of changes are from -0.4 to 0.5 J/g°C match well for the case of fresh and frozen meat with similar profiles. The magnitude order for out of phase Cp is much smaller than reversible Cp; however, this parameter is a good indicator of events such as protein denaturation by effect of low temperature applications. Refrozen samples are in good agreement with previously mentioned results, changes are observed with delays of profiles for a lower energy requirement using up to 100 minutes for the same change related to fresh and frozen samples.

**CONCLUSION**

Quasi-isothermal method in MDSC analysis, confirm the presence of important changes in meat protein for lyophilized samples after the frozen and refrozen process. Significant variations are observed in the total heat flow and reversible Cp signals in the range of 50 to 75°C. Rehydrated samples in MDSC allow to observe the denaturation reaction with a low temperature modulation and increment of 1°C during the experimental run, also the effect of continuous water mobility, particularly in case of the refrozen process. The results and temperatures range where the main changes are located agreed to the reported in several studies in the case of myosin, collagen and actin denaturation. However, shows that the myosin is mainly affected by the processes at low temperatures. On the other hand, interesting contribution provides the signal of heat flow phase and out of phase Cp, enabling a more detailed study of reacting systems in function of process time; it allows unique insight into the structure and behavior of foods, so that parameters can help to identify thermal events like meat protein denaturation, and the transition temperatures at which the heat flow is mostly affected by an event that accelerates or slows down those related with the heating rate.
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REFERENCES


