HIGH PRESSURE AND TEMPERATURE INTERACTION ON PORK MEAT PROTEIN DENATURATION: A CALORIMETRIC STUDY

G. Villamonte, C. Guyon, L. Pottier and M. de Lamballerie

ONIRIS, Food Process Engineering, UMR CNRS GEPEA 6144, CS 82225, 44322 Nantes Cedex 3, France

Abstract – The impact of high pressure (200, 350 and 500 MPa) and processing temperature (20, 40 and 60 °C) on protein denaturation of pork meat was evaluated. Meat protein denaturation was analyzed by differential scanning calorimetry. Denaturation of meat proteins was observed above 200 MPa at 20 °C. Nevertheless, high pressure promotes protein denaturation from 200 MPa when the processing temperature increased to 40 °C. Likewise, moderate temperature (60 °C) and high pressure (350 – 600 MPa) induced more than 80 % of meat protein denaturation. In contrast to myofibrillar and sarcoplasmic proteins, high pressure treatment hindered the thermal denaturation of collagen at 60 °C.

Key Words – DSC, temperature, High pressure.

I. INTRODUCTION

Functionality of proteins in meat products is determined by their conformational properties. High pressure processing affects meat quality by means of protein denaturation. The relationship between structural changes of proteins and textural properties on beef muscle after high pressure processing at different temperatures was previously reported [1, 2]. In fact, combining high pressure and temperature treatments enables different textures on meat products [1, 3]. Meat protein denaturation plays an important role which is not fully understood. Dissociation, solubilization, aggregation or gelation of meat proteins can be induced by high pressure processing without thermal treatment [4-6]. High pressure affects non covalent interactions present on secondary, tertiary and quaternary structures of proteins [7] and denatures protein at non denaturing temperatures [8]. In contrast to thermal denaturation, high pressure processing maintains hydrogen bonds of protein structure. The aim of this study was to determine the effects of high pressure treatment and temperature processing on the thermal behavior of pork meat proteins.

II. MATERIALS AND METHODS

Sample preparation

Ground meat (diameter: 4.5 mm) was prepared from pork muscle (*biceps femoris*). Muscle (pH: 5.60 ± 0.08) was obtained from Large white sow 96 hours after slaughter. The samples were vacuum packaged in polyamide/polyethylene bags (La Bovida, Paris, France) and deep-frozen. The freezing had no effect on meat protein denaturation as verified by the differential scanning calorimetry. Before treatment, meat samples were thawed overnight at 4 °C. Samples were treated or not at different pressure and temperature conditions. For heat treatment, the samples were heat-treated in a water bath (20, 40 or 60 °C) for 6 minutes. Temperature of sample center was checked by a thermocouple. Samples were cooled for 30 minutes at room temperature before analysis. Untreated sample at 20 °C was considered as control.

Pressure treatment

Samples were pressure-treated from 200 to 500 MPa for 6 min in a 3 L high pressure pilot unit (ACB, Nantes, France) equipped with a water jacket and a temperature regulator device (Julabo, Seelbach, Germany). The temperature of transmitting fluid (water) in the vessel was regulated at 20 °C, 40 °C or 60 °C (±2 °C). The level of pressure was reached at 3 MPa/s and then released in few seconds (< 2s).

Differential scanning calorimetry (DSC)

Samples were analyzed using Micro DSC VII (Setaram, Caluire et Cuire, France). 900 mg of meat sample was weighted into a Hastelloy vessels and water was used as a reference sample. Vessels were heated from 20 °C to 110 °C at a rate of 0.5 °C/min. Three replicates were analyzed. Total

denaturation enthalpy (ΔH, J/g) was calculated by the area under the transition curve from 40 °C to 80 °C. Enthalpy values were normalized to dry-matter content obtained from desiccation at 105 °C. The temperature of denaturation was estimated from the peak temperature of the thermal transition. Deconvolution was performed by Peak fit 4.0 software (AISN Software, Oregon, USA). Gaussian functions were assumed for analysis of the single thermal transition of proteins.

Statistical analysis

Statgraphics 5.1 software (StatPoint Technologies, Virginia, USA) was used for data analysis. Analysis of variance ($p<0.05$) was applied to evaluate the effects and interactions of high pressure and temperature. The enthalpy of denaturation was included in the analysis and the missing values were considered as zero. Significant differences between treatments were obtained by using the test of Tukey with a confidence level of 5%.

III. RESULTS AND DISCUSSION

Figure 1 shows the thermograms of meat proteins that were or not treated by high pressure at 20 °C. Thermograms of untreated pork muscle (0.1 MPa) presented five thermal transitions. The 1st and 4th endothermic peaks (51 °C and 67 °C) were mainly considered to myosin domains [9]; however, it also includes minor myofibrillar proteins and actomyosin complex. The 5th thermal transition (71 °C) corresponded to actin [9]. Collagen and sarcoplasmic proteins were reported as the 2nd transition (59 °C). The 3rd thermal transition was a fraction of sarcoplasmic proteins (temperature of denaturation 63 °C) [9].

Total denaturation enthalpy of pork meat proteins

Table 1 shows the enthalpy and temperature of denaturation of pork meat proteins after the pressure treatment. A control at atmospheric pressure shows that for treatment up to 40 °C, no modification of the total denaturation enthalpy is observed whereas a treatment at 60 °C decreases the total denaturation enthalpy by more than 50%. High pressure processing modified the thermal behavior of pork meat proteins. These changes were pressure-dependent. At 20 °C, treatments at 200 MPa did not produce main modifications (figure 1). Increasing processing temperature (from 20 to 40 or 60 °C) altered the thermograms of meat proteins (data not shown). Increasing processing temperature to 40 °C promotes pressure-denaturation at not denaturing pressure level (≥ 200 MPa).

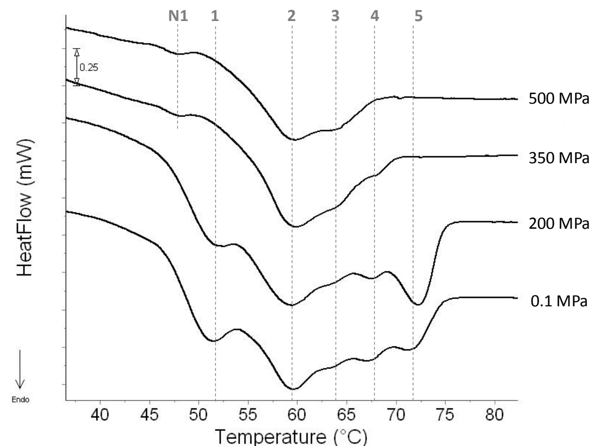

Figure 1: Thermograms of pork meat proteins without or after high pressure processing at 20 °C (N1: Pressure-induced structure, 1: Myosin domain, 2: Collagen and sarcoplasmic proteins, 3: Sarcoplasmic proteins, 4: Myosin domain, 5: Actin).

Combining high pressure (≥ 200 MPa) and thermal treatment (60 °C) promotes further meat protein denaturation (table 1). An additional thermal transition N1 was identified in samples treated at 350 and 500 MPa for processing temperatures at 20 and 40 °C. After pressure treatments (350-500 MPa) at 60 °C no endothermic peak N1 was detected. However, at 60 °C, an endothermic peak N2 was observed at atmospheric pressure and 200 MPa (table 1).

Myofibrillar proteins

The 1st thermal transition of myosin was completely denatured after heat treatment at 60 °C whatever the pressure level. Pressure-induced denaturation of myosin was identified from 350 MPa at 20 °C (table 1). Pressure treatment slightly increased the temperature of denaturation of the 1st thermal transition. The susceptibility of myosin to pressure denaturation at 200 MPa could be due to the changes on the contribution of entropic and enthalpic forces stabilizing protein structure.

Table 1: Enthalpy and temperature of denaturation of pork meat proteins at different high pressure and temperature conditions.

Pressure (MPa)	T (°C)	Enthalpy (J/g MS)								Temperature of denaturation (°C)						
		Total	N1	N2	1	2	3	4	5	N1	N2	1	2	3	4	5
0.1	20	10.16^{a}	-	-	2.86^{a}	2.96^{a}	1.74^{a}	1.37^{a}	1.22^{a}	-	-	51.2$^{a.b}$	59.1$^{a.b}$	63.3$^{a.b}$	66.6^{a}	70.9^{a}
	40	10.13^{a}	-	-	2.51^{b}	2.87^{a}	1.72^{a}	1.57^{a}	1.47$^{a.b}$	-	-	51.0^{a}	58.8^{a}	62.9^{a}	66.6^{a}	71.9^{b}
	60	4.57$^{c.d}$	-	0.13^{a}	-	-	1.11$^{c.d}$	1.41^{a}	1.92^{c}	-	42.9^{a}	-	-	63.7$^{a.b}$	66.8$^{a.b}$	72.8^{c}
200	20	10.32^{a}	-	-	2.32^{b}	2.99^{a}	1.85^{a}	1.60^{a}	1.56^{b}	-	-	51.5^{b}	59.0$^{a.b}$	63.0^{a}	67.2$^{b.c}$	72.1$^{b.c}$
	40	8.62^{b}	-	-	1.81^{c}	2.71$^{a.b}$	1.57$^{a.b}$	1.32^{a}	1.22^{a}	-	-	52.5^{c}	58.9$^{a.b}$	63.6$^{a.b}$	67.5$^{c.d}$	71.5$^{a.b}$
	60	2.43$^{e.f}$	-	0.07^{b}	-	0.82^{e}	0.58^{e}	0.96^{b}	-	-	42.9^{a}	-	61.1^{c}	63.6$^{a.b}$	67.3^{c}	-
350	20	5.12^{c}	0.62^{a}	-	-	2.51$^{a.b.c}$	1.49$^{a.b.c}$	0.50^{c}	-	47.9^{a}	-	-	59.6^{b}	63.5$^{a.b}$	67.8^{d}	-
	40	4.54$^{c.d}$	0.84^{b}	-	-	1.85$^{c.d}$	1.86^{a}	-	-	47.5^{a}	-	-	59.8^{b}	64.0^{b}	-	-
	60	0.82^{g}	-	-	-	0.80^{e}	-	-	-	-	-	-	60.6^{c}	-	-	-
500	20	3.81^{d}	0.54^{a}	-	-	2.07$^{b.c.d}$	1.21$^{b.c.d}$	-	-	47.5^{a}	-	-	59.5$^{a.b}$	63.7$^{a.b}$	-	-
	40	3.38$^{d.e}$	0.52^{a}	-	-	1.84$^{c.d}$	1.03^{d}	-	-	47.5^{a}	-	-	59.6^{b}	63.7$^{a.b}$	-	-
	60	1.44$^{f.g}$	-	-	-	1.44$^{d.e}$	-	-	-	-	-	-	59.0$^{a.b}$	-	-	-

The 4th transition corresponding to another myosin domain was not affected by temperature at ambient pressure. High pressure induced total denaturation of this component at 500 MPa. However, at processing temperature of 60 °C, the 4th thermal transition of myosin became susceptible to pressure denaturation at 200 MPa. The temperature of denaturation slightly increased with pressure and temperature. Below 500 MPa, effect of high pressure on denaturation of myosin domain depended on temperature of treatment.

At atmospheric pressure, the enthalpy of denaturation of actin (5th transition) increased significantly with treatment temperature (table 1) which can be explained by the dissociation of the actomyosin complex that could increase the concentration of the protein. At 200 MPa and 20 °C, the denaturation enthalpy increased about 29% (table 1) which can be explained by the dissociation of actomyosin under high pressure. The 5th transition disappeared above 350 MPa. A slight increase of the temperature of denaturation was observed with increasing temperature. Actin is thermostable and barosensitive. Moreover, the interaction between high pressure and temperature provoked that the actin became thermosensitive at low levels of high pressure.

The interaction between high pressure and temperature has a synergistic effect for denaturing myofibrillar proteins with an exception on the case of actin since a stabilization at 200 MPa and 20 °C was described.

Collagen and sarcoplasmic proteins

Collagen and sarcoplasmic proteins were significantly affected by high pressure and temperature on a different way compared to myofibrillar proteins. The 2nd thermal transition corresponding to collagen and sarcoplasmic proteins and 3rd thermal transition of sarcoplasmic proteins were only partially denaturated at 500 MPa and 20 °C.

The proteins corresponding to the 2nd transition were completely denaturated at 60 °C (0.1 MPa). At 40 °C, the susceptibility of the 2nd endothermic peak increased. The collagen and sarcoplasmic proteins became partially denaturated at 350 MPa. At 60 °C, the degree of collagen and sarcoplasmic proteins denaturation was reduced by increasing the pressure level. It is generally accepted that the collagen is relatively unaffected by pressure [10] since it is primarily stabilized by pressure-insensitive hydrogen bonds. However, high pressure treatment at 500 MPa at 20 °C had a significant effect on these proteins by denaturing about 30%. Interaction between high pressure and temperature was denoted by the stabilizing effect of high pressure at 60 °C compared to total denaturation of proteins at 0.1 MPa. For all

treatments tested, the denaturation temperature of the 2nd transition remained stable (table 1).

The processing temperature of 60 °C affected the 3rd thermal transition of sarcoplasmic proteins and this modification was enhanced with high pressure. The 3rd thermal transition was partially denatured at 500 MPa without differences between 20 and 40 °C. The 5th endothermic peak disappeared after a treatment at 60 °C and 350 MPa or 500 MPa. Interaction between high pressure (200 MPa) and temperature (60 °C) was showed with 67% of denaturation (table 1). The temperature of denaturation of the 3rd thermal transition did not vary according to pressure/temperature conditions.

IV. CONCLUSION

Myofibrillar proteins are strongly affected by high pressure; in particular, the denaturation of the 1st domain of myosin was complete for pressure upper than 350 MPa. Calorimetric results confirm that high pressure induces depolymerization, denaturation and aggregation of myofibrillar proteins [5, 11-12]. On the contrary, collagen and sarcoplasmic proteins are less sensitive to high pressure treatment. Moreover, considering collagen, high pressure hampers its thermal denaturation observed after a 60 °C treatment.

Pressure and temperature have a synergistic effect on protein denaturation especially at high temperature (> 60 °C). Then, the extent of protein denaturation and the profile of meat proteins involved can be modulated. Therefore, combining high pressure and temperature treatments could allow the development of meat products with new textures and potentially without additives.

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