HIGH PRESSURE INDUCED CHANGES IN MYOFIBRILLAR FRACTION OF PORK MUSCLE

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Background

High pressure (above 100 MPa) technology offers the food industry an enormous opportunity to develop novel foods of high nutritional and sensory quality, novel texture and increased shelflife.
Pressure is well-known to exert a great influence on the properties of proteins by rearrangement and/ or destruction of noncovalent bonds such as hydrogen bonds, hydrophobic interactions, and electrostatic bonds of the tertiary structure of proteins.
The aim of present work was to assess an effect of pressurization of pork muscles on changes in the amounts of extractable myofibrillar proteins of meat.

Material and Methods:

Materials
The longissimus dorsi muscles were excised from pork carcasses about 48 hours post mortem. Half of the muscles was then subjected to high pressure treatment and the other half was untreated and use as a control.
Pressurization of the muscle
The tissue was carefully trimmed of fat and muscles were cut into pieces (20 cm x 5 cm). Samples (450g portions)were vacuum-packaged in polyethylene pouches. Each bag was transferred to a pressure vessel (h=0.3 m, D=0.10m), which was maintained at about 2°C (the space among the bags was filled with crushed ice and water), and pressure was applied at 150 or 500 MPa for 5 min. After pressurization, the samples were taken out and immediately cooled in an ice-box.
Sample preparation and protein extraction
Muscles samples were not homogenized but just cut into slices and then divided into parts; one was suspended in 0.25 M sucrose solution and the other in the saline solution and both were placed in cold room at 10° C for overnight to extract water-soluble protein (WSP) or salt-soluble protein (SSP). The concentration of saline solution corresponded to 2,6 % salt, equivalent to level commonly used in commercial processed meat products. These mixtures extracts were examined with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for protein characteristics.

SDS-Gel electrophoresis
Qualitative examination of protein extracts from pressurized meat and control meat samples was performed by SDS-PAGE electrophoresis. The method employed was similar to that described by Laemmli (1973) with 12% acrylamide slab gels. Electrophoresis was carried out in Tris-glycine buffer,pH 8,3, containing 0.1%(w/v) SDS. The samples were subjected to gel electophoresis at a constant current of 40 mA per gel. Fixation of the protein was carried out by 12% trichloroacetic acid for 1 hr and staining wth Coomassie Brillant blue R-250 (Neuhoff at al.,1988, Defaye,1995).

Results and Discussion

The results of SDS-PAGE of proteins isolated from the fresh pressurized and control muscle samples are shown in Fig. 1 and Fig. 2. The molecular weight ranges and tentative identification of the bands are also included in this figure. (Generally, identification of the minor bands proved difficult because of the lack of authentic standards.)
The slab gel system employed in this experiment could clearly distinguish qualitative composition of water-soluble protein (WSP) and salt-soluble protein (SSP) extracted from pressure treated meat as compared to the control one.
The major contractile proteins, actin and tropomyosin together with several minor protein components were readily discerned in all extracts, but myosin only in control extracts. The bands within the 80,000-160,000 Dalton region probably included the proteins α- actinin, β-actinin, C-and M-protein. The changes in the protein components of myofibrils extracted from the control (untreated) and the pressurized muscles are shown in gel patterns presented in Figures 1 and 2 for WSP and SSP respectively. In the SSP and WSP of pressurized meat minor changes in banding patterns can be seen near 70,000 and 55,00 Daltons and to more extent in region 30,000-50,000 Daltons. In region below 30,000 Daltons, it was noted the increase of band content as the pressure increased. The above noted changes in these regions were observed as disappearance of bands in region above 100,000 Daltons.
Since the above 150,000 bands has disappeared totally, and the patterns of low-molecular proteins showed an increace in intensity, it is likely that the lower polypeptide chains resulted from degradation of the myosin and other heavy proteins. Changes in the solubility or the salting out of these proteins might have contributed to these changes, but the disapperance and fainting of coincident bands in the 0.25 M sucrose extracts makes this explanation unlikely.
The contents of proteins extracted from pork muscles immediately after pressurization and from control samples, are shown in Fig.3. This shows that, while there was a trend of increasing the protein content as the salt concentration increases, the differences were not statistically significant. The amounts of the salt-soluble protein were higher than the water-soluble

protein, irrespectively of the extracts sources (pressurized meat or control). The lower quantity of proteins in the extracts from pressure treated muscles was noticed as compared to the control one. The reason for the lower amounts of proteins extracted from pressurized muscles might be ultrastructure damage of meat by pressure. These conditions could then cause change of the polypeptide chains, disruption and release shorter-chain of polypeptides to water drip.

Conclusions

The observed disappearance of high molecular myofibrillar proteins and increase of amount of low molecular protein fractions after muscle pressurisation gave the evidence of disintegration of molecular structure of the tiissue. The changes were more advanced when the applied pressure was higher.

References

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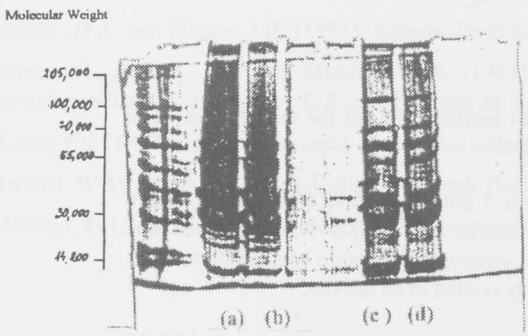

Fig. 1. SDS-PAGE patterns of water-soluble protein (WSP) extracted from pressurized and control (untreated) meat.
(a), (b) Pressurized at 150 MPa; (c), (d) control.

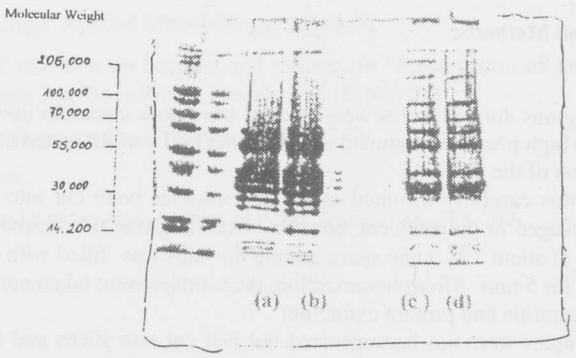

Fig. 2. SDS-PAGE patterns of salt-soluble protein (SSP) extracted from pressurized and control (untreated) meat.
(a), (b) Pressurized at 150 MPa; (c), (d) control.

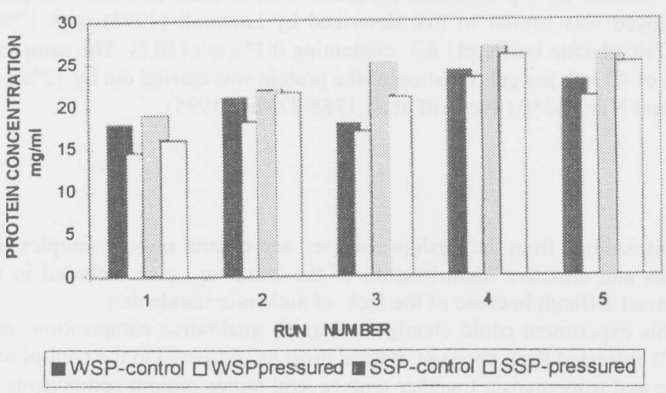

Fig.3.Influence of high pressurised treatment on protein concentration