PROTEIN CHANGES IN HIGH PRESSURE PASTEURIZED RAW SAUSAGE BATTER

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Background

There is a growing interest in improving the microbiological safety of batters of raw fermented sausages because fermentation and drying are able to achieve only an insufficient reduction in the number of those non-sporeforming pathogenic bacteria which have been linked already to outbreaks of certain fermented sausages or necessitated numerous recalls of contaminated meat products (Centers for Disease Control and Prevention, 1995, Sauer *et al.* 1997). Due to its ability to inactivate vegetative bacterial cells efficiently, high pressure pasteurization can be one of the alternative non-thermal procesing techniques since its advantage that it has practically no effect on small molecules and avoids thereby the formation of off-flavour. However, it has been shown also by our previous studies with minced meats that high prssure can denature proteins (Farkas et al., 2001).

Objectives

The aim of our present studies was to investigate in more detail the changes of proteins induced by high pressure pasteurization of the raw batter of a typical Hungarian fermented sausage, clarifying also the effects of sodium chloride and its curing mixture with nitrite on the high pressure-induced changes. The microbiologgical effect was evaluated with samples inoculated with *Listeria monocytogenes* as target organism.

Methods

Experimental batches of the uninoculated sausage batter with or without the regular NaCl and nitrite additive, but without addition of the lactic starter culture and similar samples but heavily inoculated with a strain of *Listeria monocytogenes* were divided into small plastic pouches and after sealing under vacuum have been subjected to a hydrostatic pressure treatment of 600 MPa for 20 min in a Stansted „Food Lab 900" high pressure rig while maintaning at about room temperature. Total aerobic viable cell counts (TVC) of non-inoculated samples and also *Listeria* counts in inoculated samples were estimated directly after the high pressure treatment, for the latter using plating on Oxford agar with *Listeria*-selective supplement. Non-microbiological testings were performed with the uninoculated samples. Colour and texture were estimated by a Minolta tristimulus colorimeter and by an SMS TAXT2i type texture analyser, respectively. Differential scanning calorimetry was done by a SETARAM „Micro-DSC III" microcalorimeter. Myofibrillar protein and sarcoplasmic protein fractions were extracted according to Offer and Moos (1973). SDS-PAGE was performed on gels of 12 % polyacrylamide containing 2 % SDS (Laemmli, 1970) and fixation and staining was done by Coomassie Blue.

Results and discussion

The HHP treatment caused somewhat more than 3 log-cycles reduction in the TVC, practically independent from the presence or absence of the curing salts. The *Listeria* counts decreased as an effect of pressure processing by 5 log-cycles in the salt-free batter and around 4 log cycles in the salt-containing samples. Discolouration as an effect of the high pressure treatment was observed was observed visually, but noted also in an increase of the tristimulus color values, namely in an increase of the lightness (L*)-value, and a decrease of the redness (a*) value (Data not shown). The discolouration was less pronounced in the nitrite-containing samples than in nitrit-less ones.

High pressure treatment reduced the softness of the samples: more in the salt containing batters than in the salt-free one. For compressing cylindric (50 mm diameter and 30 mm height) of pressurized samples to their half-height required approx. 26 % more power in the salt-free samples, approx. 50 % more in the salt containing ones, and 58 % more in those containing both salts than in the case of the respective non-pressurized samples.

DSC thermograms of the salt- free samples and those containing curing salts are shown by Figs. 1. The figure illustrates that both the pasteurizing high pressure treatment and the presence of curing salts in the batter changed drastically the DSC pattern of endotherms typical to the non-pressurized salt-free muscle tissue. In the thermogram of salt-free, non-prssurized sample the small endothermic peak between 50 and 55 °C can be attributed mainly to the heat-denaturation of myosin, and myosin subunits, the composite transition between 57 and 68 °C showing a distinct large peak and a shoulder can be considered as the heat denaturation of connective tissue (collagen and other stromal proteins) as well as sarcoplasmic proteins including various enzymes and the myoglobin, whereas the distinct peak between 72 and 78 °C is related to the denaturation of actin, actinins and troponins (Findlay and Barbut, 1990). Thermograms obtained during a second heating run of the re-cooled samples did not show any peaks in the protein denaturation range, only under 50 °C (due to the melting of the high-melting point fraction of fats), illustrating that the endothermic transitions observed over 50°C during the first DSC-run were all irreversible processes.The DSC thermograms of HHP-treated samples show a more diminished profile both as the number of heat denaturation endotherms and the total enthalpy changes are concerned.. Apparently, most of the proteins of the high-pressure treated samples were in a denatured state, except the pressure- resistant collagen. DSC thermograms of salt-containing samples showed only one large endotherm at about 65 °C in the non-pressurized sample, and a greatly diminished one at slightly less temperature in the pressurized samples.Identification of these conformation changes would require additional, more elaborate studies. The electrophoretic patterns of the salt soluble proteins (Fig. 2/a) of sausage batter showed a reduced total protein content and absence of certain protein zones of the HHP-treated samples (3, 5, 7) as compared to the untreated ones (samples 2, 4, 6). High pressure induced denaturation/aggregation of proteins caused a decrease in their solubility. However, in case of the use of SDS-PAGE solvent (Tris-buffer of pH 6.8 containing 2 % SDS and 1 % β-mecapto-ethanol) soluble proteins (B), the patterns showed that the pressure-induced aggregations were mostly reversible.

Conclusions

The non-thermal, mostly reversible denaturation/aggregation induced by the HHP treatment might result certain advantageous changes in the structure and biological activity of proteins from the point of view of nutrition.

Further work will be continued to estimate also the effect of HHP treatment on the fate of surviving microorganisms during fermentation and the quality of the fermented end-product.

Pertinent literature

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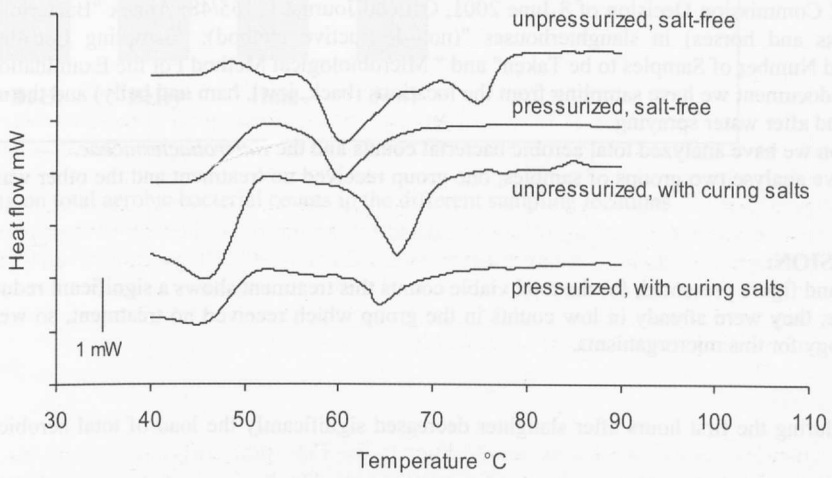

Figure 1. DSC curves of pressurized and unpressurized samples with and without curing salts

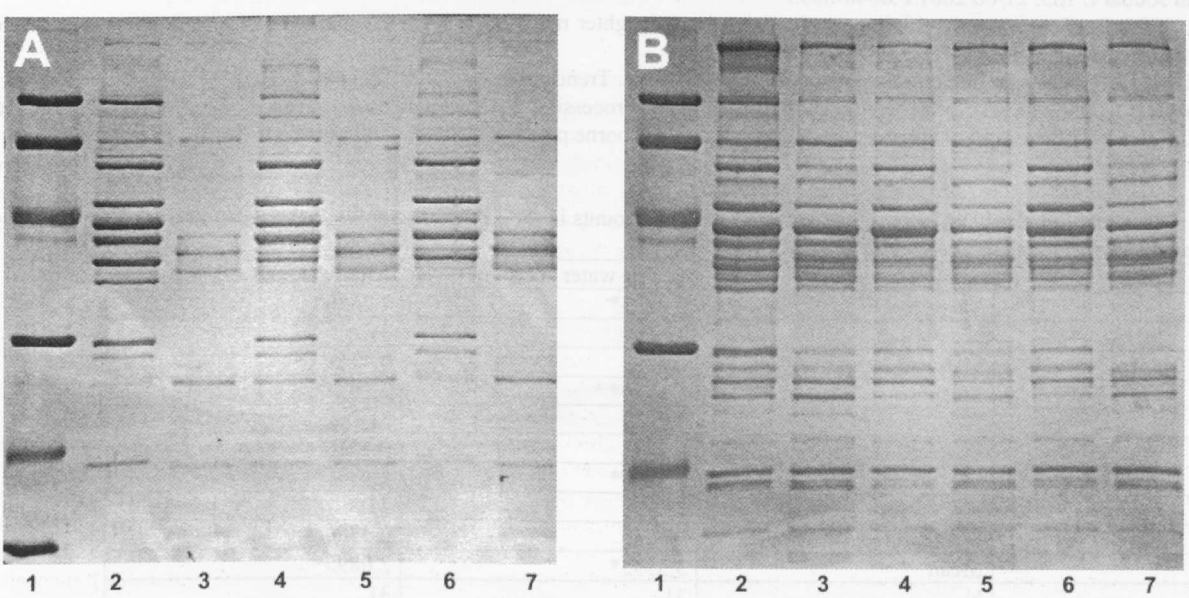

Figure 2. SDS-PAGE patterns of salt soluble (A) and SDS-PAGE solvent soluble (B) proteins of sausage batter.: 1. Standard proteins (94.0, 67.0, 43.0, 30.0, 20.1, and 14.4 kDA, respectively), 2. Untreated sample, 3. HHP sample, 4. Untreated sample with NaCl, 5. HHP sample with NaCl, 6. Untreated sample with NaCl and nitrite additives, 7. HHP sample with NaCl and nitrite additives.