Thermograms of the combined High Hydrostatic Pressure and Sous-vide treated \textit{Longissimus dorsi} of pork

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Abstract. In this work, slices of \textit{Longissimus dorsi} of pork was used as raw material to establish the effects of the sous-vide technology and the high hydrostatic pressure treatments (and their combinations) on meat. The state of the proteins in meat has a very important effect on several quality parameters of the product, such as weight loss, water holding capacity, organoleptic properties. Therefore it is important to follow and analyse the denaturation of the protein content during food processing. The samples were cooked sous-vide (60 °C, 5-480 minutes) or pressurized (100-600 MPa, 5 minutes, room temperature). Also two steps treatments were studied combining both technologies, applying high hydrostatic pressure treatment (300 or 600 MPa, 5 minutes, room temperature) after or previous to sous-vide cooking (60 °C, 30 minutes). The changes in the condition of meat proteins were followed by a differential scanning calorimeter. The DSC curves were analysed using the unit’s own software where denaturation heat was determined. Thermograms show through the change of the sample’s protein state the dissimilar effect of the treatments. Using the Polar Qualification System -previously proved to be effective with NIR measurements- the spectral information was reduced to a two dimensional polar co-ordinate system where each DSC curve is represented by a “quality point”. As a new experiment the applied PQS data reduction method compared to the traditional thermal analysis data processing gave us less information on the differences of our samples although the results are promising as we were able to detect the same trends and characteristics.

1. Introduction

At the development of modern food processes, it is important to ensure the microbiological stability of the product with only the slightest damage in the quality parameters. This can be achieved by applying the principle of hurdle technology [1]. The lethality of low temperature heat treatment could be enhanced by combining it with other physical treatments. Sous-vide cooking as a mild heat treatment and high hydrostatic pressure technology seem to be promising for this purpose. The sous-vide technology is already applied not only in small scale but in the catering industry as well [2]. The optimal time-temperature parameters are highly dependent on the raw material [3]. It results juicy, perfectly cooked products with minimal nutrient loss and high organoleptic value [4]. With keeping the heat treatment at a minimal level, we face several microbiological problems [2]. The application of HHP as a secondary treatment could be a solution only in the case if it is not altering the excellent properties of the original sous-vide product. Only a few studies can be found in literature investigating the effects of the combined treatment of sous-vide and high hydrostatic pressure. Pineapple [5], fish [6,7] and chicken meat patties [8] were investigated previously. In meat, proteins are the second most important constituents after water. The state of the proteins in meat products has a very important role
and affects several quality parameters of the product, such as weight loss, water holding capacity, sensory properties. The protein components of the meat react differently in the case of heat induced denaturation as well as at the pressure induced changes [9,10]. The Differential Scanning Calorimeter can detect the kinetic changes of the proteins [11] and the preserved quality of the meat [12,13]. Therefore this work presents a detailed study on the behaviour of the proteins in pork under different one and two step food processing technologies. The aim also was to analyse the power of the PQS data transformation method using the DSC curves as spectral information.

2. Materials and methods
   2.1. Sample preparation
   The whole pork chops (Longissimus dorsi) were purchased at a wholesale market (m= 3.78 – 3.93 kg). After being prepared to be free of surface fat, ligament and connective tissue each meat was cut into slices (2 cm) transversally to the fiber direction. The weight of each sample was 60-70 g. Samples were vacuum sealed in 90 µm PA/PE plastic pouches using a Multivac C100 V.S. machine. All treated samples were kept frozen at -24 °C as the DSC measurements took several days.

   2.2. Sous-vide treatment (SV)
   Heat treatment was carried out in a water bath (Labor Műszeripari Művek LP507/1). Samples were cooked sous-vide at 60 °C, the treatment time was 5, 15, 30, 60, 120, 240 or 480 minutes. Samples were cooled in iced water immediately after cooking.

   2.3. High hydrostatic pressure treatment (HHP)
   Meat samples were pressurized at 100, 200, 300, 400, 500, or 600 MPa for 5 minutes at room temperature in a RESATO FPU-100-2000 HHP unit (Resato International B.V., the Netherlands). The pressure transmission fluid was glycol-oil mixture (Resato PG fluid, Roden, Holland). The pressure build-up rate was 100 MPa/minute. Build-up and decompression times were not included in the treatment time. The initial temperature of the pressure transmission fluid was 18.5 °C, the adiabatic temperature change of the system (samples and the pressure transmission fluid) was under 14 °C.

   2.4. Combined treatments
   Following the hurdle principle, we used both technologies. Heat treatment was carried out at 60 °C for 30 minutes and high hydrostatic pressure was set at 300 and 600 MPa for 5 minutes. The combinations were: heat after pressure treatment and pressure after heat treatment at both pressure levels. Treatment parameters were determined by previous heat transfer calculations and practical experience for the sous-vide cooking and two borderline pressure values were chosen for the high hydrostatic pressure treatment. The 300 MPa is around the entry treatment level where protein denaturation and microbiological inactivation is already observed while the 600 MPa is a generally applied pressure value in the food industry [10]. As the PA/PE pouch packaging of the raw material is suitable for both technologies cross-contamination is avoided during treatments.

   2.5. DSC analysis
   The changes in the state of meat proteins were followed by a SETARAM micro DSC III differential scanning calorimeter. The scanned temperature range was 20-95 °C at a heating rate of 1 °C/minute. The DSC curves were analysed in the 35-90 °C range using the unit’s own Calisto software. The total denaturation enthalpy of each meat sample was determined. The enthalpy is calculated with integration – it is equal with the area in between the DSC curve and the baseline.

   2.6. PQS data analysis
   The recorded DSC curves were analysed using the PQS32 Evaluation Software (MetriNIR Research, Development and Service Co., Budapest, Hungary). This data reduction method was primarily used with NIR measurements [14-16] and was previously applied with promising results to analyse DSC
data of cocoa butter and its constituents [17]. In PQS the spectrum is plotted in a two-dimensional polar co-ordinate system (X-Y). The quality points – the center of gravity of the polar spectrum- is obtained from the DSC thermograms in the range of 35-90 °C. The properties of a sample can be characterized by the location of its quality point in the polar quality plane. The distance between them can describe the similarity, the smaller the value the more the samples are identical and vice-versa.

3. Results and discussion
The shape of the DSC curves characterize well the sample and its composition. The untreated (raw) pork sample has three well distinguished peaks. The myosin protein group can be found at around 50-55 °C, the sarcoplasm proteins and collagen at around 60-65 °C and the actin at 70-75 °C. Just after the 5 minute heat treatment, proteins denatured below the temperature of 55-60 °C, while the other components remain intact. As the treatment time is increased more important protein quantities are damaged or denatured even in the ranges higher than 60 °C. After 480 minutes nearly all protein groups are affected by the heat. (Figure 1.a)

![Figure 1](image)

*Figure 1.* DSC thermograms of the sous-vide cooked at 60 °C (a), the high hydrostatic pressure treated for 5 minutes at room temperature (b), and the combined treated (c) meat samples (SV = sous-vide cooked at 60 °C for 30 minutes, HHP300 = 300 MPa high hydrostatic pressure treated for 5 minutes at room temperature, HHP600 = 600 MPa high hydrostatic pressure treated for 5 minutes at room temperature.

In the case of high hydrostatic treatment the most important observation is that the HHP treatment has effect on different protein groups than the heat treatment induced denaturation. We can also observe that there is a borderline between 200 and 300 MPa. Below this pressure value the treatment has practically no effect on the meat samples. Ongoing from 300 MPa denaturation can be seen at the myosin and actin groups. The collagen and sarcoplasmic proteins are still stable and the pressure has only a slight effect on them. (Figure 1.b)
On the results of the combined treatments we have to remark that the order of the treatments has a very important role concerning the protein state of the samples. The application of the heat treatment in combination with the pressure treatment is still resulting a partly denatured meat sample. This could be observed also at 600 MPa but when the lower pressure level (300 MPa) was used the difference between the order of the treatments is much more important. (Figure 1.c)

The calculated enthalpy values are in accordance with the visual observations as it can be seen on the diagrams. (Figure 2.a,b,c)

The measured enthalpy values were 2.795 J/g at the raw sample, while at the sous-vide treated samples we measured 1.847; 1.243; 1.055; 0.892; 0.459; 0.287; 0.143 J/g at the 5, 15, 30, 60, 120, 240, 480 minute samples respectively that results a decrease in accordance with treatment time. (Figure 2.a) The enthalpy of the pressure treated samples were 2.671; 2.315; 1.068; 0.902; 0.796; 0.489 J/g at 100, 200, 300, 400, 500, and 600 MPa samples respectively. (Figure 2.b)

To compare the two preservation methods (heat and pressure) the 300 MPa (5 minutes) pressure treated sample needed approximately the same denaturation enthalpy as the 30 minute heat treatment at 60 °C while they show very different characteristics. The pressure treated sample in physical appearance is very similar to the raw meat while the sous-vide sample shows the typical signs of cooked meat (not shown). The measurement of the combined treated samples resulted the most important data of this study. When the pressure treatment was applied previous to sous-vide cooking the enthalpy values show no difference between the two pressure levels. In the reverse order (heat treatment followed by the pressure treatment) the pressure value determined the result.

![Figure 2](image)

**Figure 2.** Denaturation enthalpy values of the sous-vide cooked at 60 °C (a), the high hydrostatic pressure treated for 5 minutes at room temperature (b), and the two step combined treated (c) meat samples (SV = sous-vide cooked at 60 °C for 30 minutes, HHP300 = 300 MPa high hydrostatic pressure treated for 5 minutes at room temperature, HHP600 = 600 MPa high hydrostatic pressure treated for 5 minutes at room temperature).

The PQS quality points in the X-Y polar co-ordinate system are able to visualize the major changes in the protein state effectively. (Figure 3.a) The raw sample is well separated from the cooked ones on the X axis, while the cooked samples show a vertical linear pattern as the Y value increase the treatment time was longer so the heat impact was more important. The 5 minute heat treatment is already well separated from the raw sample indicating the fast structural changes in the protein groups. The next four treatment times (15, 30, 60, 120 minutes) form a subgroup showing the similarities already observed on the DSC curves above (Figure 1.a). In between this group the PQS show no difference. This result is derived from the similar forms of the curves with the same well defined peaks. The 240 and 480 minute samples are on the same axis as all the heat treated ones but as they contain mostly denatured proteins they are well separated in the polar quality plane. The apparent
contradiction that the 480 minutes cooked sample is next to the raw sample in the X-Y polar space is due to the PQS data transformation as it’s curve is more “balanced” with the three main peaks therefore it is similar in this polar space to the 480 minute cooked sample’s curve that has no significant peak. That results a nearer position to the raw sample than the ones treated for shorter time and having only one but well defined peak.

![Figure 3](image_url)

**Figure 3.** PQS quality points in the polar quality plane of the sous-vide samples cooked at 60 °C (a), the pressure treated samples (b), and the combined treated (c) meat samples.

The co-ordinates of the HHP treatments similarly to the literature data and the above seen spectra show two well defined subgroups separated by the X axis values. (Figure 3.b) The raw, the 100 MPa and the 200 MPa treatments are in the first group with minimal or no changes in the protein state. The more important pressure value treatments (300, 400, 500 and 600 MPa) are forming the second group, where denaturation already altered the protein structure. The results of the combined treatments show new scientific data. The differences due to the order of the two minimal processing procedures can be observed. By all means, the 300 MPa HHP-treated sample is the nearest to the raw meat as it underwent only a mild treatment and most of their proteins were kept in a native state. The position of the quality points of the simple (one step heat or pressure) treatments reflect their dissimilar character. The combined treated samples form a subgroup well separated from the single treatments but the distance within the group is not so important. (Figure 3.c)

4. Conclusions

Based on the results it can be concluded that both data processing methods were capable of distinguishing the different pork samples treated by a single technology at different levels or the combinations of sous-vide treatments and HHP processes. Using the DSC’s own software only one data, the enthalpy value was calculated for each sample. The PQS method used the DSC curves like spectral information and positioned the samples in the X-Y polar space according to the structural changes of the sample’s protein content. These changes could be monitored objectively using the PQS data transformation. With this unsupervised data processing method groups could be formed where similar characteristics are observed. The results of this experimental work can help in the process design and also point on the importance of the treatment order of the combined minimal processing technologies.

Acknowledgments

The authors wish to thank for -making the HHP treatments possible- for TÁMOP 4.2.1/B/09/1/KMR/-2010-0005 program of the National Development Agency, Hungary.

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