THESES OF THE DOCTORAL DISSERTATION

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Budapest

2019

SZENT ISTVÁN UNIVERSITY

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EFFECT OF HIGH HYDROSTATIC PRESSURE ON PROTEIN STRUCTURE AND PHYSICOCHEMICAL CHARACTERISTICS OF LIVESTOCK PRODUCTS

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2019

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The candidate has fulfilled all the conditions prescribed by the doctoral school of Szent István University, the comments and suggestion at the thesis workshop were taken into consideration when revising the thesis, so the dissertation can be submitted to a public debate.

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1. INTRODUCTION AND OBJECTIVES

The consumption of livestock products is as old as the human history and part of our daily diet. Consumption of them is beneficial in many different ways. They are rich in proteins, minerals, fats and essential amino acids. Consuming them helps the human body to function optimally. Our society today is increasingly aware of the concept of healthy nutrition and healthy food, therefore it is essential that the food industry also tries to meet these needs. The application of minimal processing technologies is becoming more widespread across the world, in case of livestock products as well.

Based on literary knowledge each livestock product reacts with different sensitivities to high hydrostatic pressure treatment. Since proteins play an important role in the development of both organoleptic and techno-functional properties in livestock products, I would like to examine the changes in the structure of the proteins as well as the changes in the physicochemical properties of the products in order to be able to choose the optimal technology parameters and to better understand the background and effects of this minimal processing preservation method (HHP). In my thesis I have subjected different livestock products to one of this minimal processing technology, the high hydrostatic pressure treatment. The properties of the different livestock products may change due to the HHP treatment. The extent of the change may depend on the parameters of the treatment, the species of animal and the different body area/muscles from the product was made. The main objective of my research was the examination of changes (physico-chemical, protein structure) of livestock products (beef sirloin, beef blood, pork loin, chicken breast, eggs and milk) due to high hydrostatic pressure treatment. The matrix of the treatment parameters was the same in case of each product (except milk), the food products were treated from 100 MPa up to 600 MPa (5 minutes) in 100 MPa increments. Samples without HHP treatment served as control.

In the case of the studied livestock products my aim was to trace and examine the changes in the proteins as a result of the high hydrostatic pressure treatment. For this purpose of my experiment I used electrophoretic, spectrophotometric and thermodynamic techniques (IEF, SDS-PAGE, NATIV-PAGE, CE, determination of the relative proportion of total pigment and myoglobin forms, DSC) to study the effects of the treatments on proteins and their structure, and the physico-chemical properties of the products such as color, pH and dynamic viscosity.

2. RESULTS

Colour measurement results:

It can be concluded that during my experiments in the case of meat products (beef sirloin, pork loin, chicken breast), due to the high hydrostatic pressure treatment both of the L *, a* and b* colour values significantly changed compared to the control samples (ANOVA). In case of each meat products can be seen as their colours are lightened, the L* values increased. For beef sirloin, L* values especially showed a more than 30% increase in data after 300 MPa pressure treatment, which treatment level also can be considered as a treshold. In case of beef sirloin, pork loin and chicken breast, the 400, 500 and 600 MPa pressure treatments no longer significantly altered the L* values of the samples. There was a substantial increase in b* yellow-blue color values after pressure treatment level of 300 MPa and above, however no for the a* red-green color values, thus the coloration of the samples was shifted towards the yellow color range in all three types of meat. In the case of beef blood, the L* color values are significantly decreased by pressure treatments, especially at 300, 400, and 500 MPa, which resulted a darker coloration of the samples. These results were different from the meat samples, where we could see that the L* values increased significantly (the colour of the samples became lighter). For the color components of a* red-green and b* yellow-blue, the measured results show a decrease from 300 MPa and above treatments compared to the control sample, which change can be observed well even by the human eye. In the case of liquied egg white it can be concluded that although the HHP treatment causes a significant change in the colour of the samples, the sudden change of the color data occurs only at higher pressure level values (400 MPa, but mainly 500 and 600 MPa), which also shown by the calculated ΔE* colour difference value. For the results of the liquied whole egg colour measurement can be stated that all three color components were significantly affected by the high pressure treatment. For the b* yellow-green color component increasing pressure levels resulted a decreasing tendency values, indicating that liquied whole egg has increasingly lost from its yellow color intensity. Regarding milk color measurement it can be established as Tukey test homogeneity test shows that there is no significant difference in L* values between raw milk and pasteurized milk and L * values for sterilized milk and 300-600 MPa pressure-treated samples represent the same group. In case of the a* and b* values the results of raw, pasteurized and pressurized milk are very close to each other, only sterilized milk shows difference in the range.

pH measurement results:

Regarding the change in the pH value of livestock products after HHP treatments, it can be concluded that the treatments caused a significant change in the pH values of each kind of products compared to the control samples, however, these changes occurred at different pressure levels. In general, there was an increase in the data of beef sirloin, pork loin, chicken breast, liquid egg white and liquid whole egg, while data are decreased in case of beef blood and milk.

Dinamyc viscosity results:

Dinamyc viscosity measurements have been performed on products that are in liquid state such as beef blood, liquid egg white, liquied whole egg and milk. It was observed in each case of the studied products that the dinamyc viscosity values of the samples increased with higher pressure level treatments. This increase was the most intensive for beef blood and liquied egg products, while the smallest difference was measured for milk. In the case of 600 MPa pressure treatment of liquied egg white and the 500 and 600 MPa treatment of liquied whole egg resulted in the formation of such a gel structure which could not be measured with the rheometer.

Spectrophotometric results:

During the determination of total pigment content, especially the myoglobin concentration in the pressure treated beef sirloin, pork loin and chicken breast samples were not changed comperad to the untreated samples. From the results it can be concluded that with the applied measurement method the state of the given protein is not a determining factor in the measurement of the amount of heme-containing components, because the heme component in denatured state can also be detected. Concerning the relative proportion of myoglobin forms of beef sirloin it can be stated, that the pressure treatment resulted a decrease in the oxymoglobin (OMb) ratio, while at the same time the ratio of metmioglobin (MMb) increases. Deoxymioglobin (DMb) ratio triples at 600 MPa pressure treatment compared to 450 MPa level. In case of 600 MPa pressure treatment the relative ratio of methmioglobin increased from 20% to about 45%.

Electrophoretic measurement results:

It was found that the HHP treatment affects different proteins and groups of proteins with different extent. Myoglobin in the beef sirloin changes due to HHP treatment only at higher pressure levels of 500 and 600 MPa (its native state and solubility), meanwhile changes in the

same protein begin at 400 MPa incase of pork loin. The pressure treatments of beef sirloin on different levels did not cause any significant changes in the measured carnosine and ancerin content according to the applied capillary electrophoresis method. In addition, based on the SDS-PAGE resolution and densitometry data of myofibrillar proteins in beef, at 300 MPa and above (400, 500, 600 MPa), the protein intensity values were reduced by at least 50% as a result due to the change in protein solubility. In chicken breast 400, 500, and 600 MPa pressure treatment resulted the appearance of degradation products and aggregates compared to the control sample, which can be detected by electrophoretic techniques. In case of beef blood and liquied egg products (milk also in some measurements), my experiments have shown that proteins in liquid state are more resistant to pressure treatment, their denaturation and aggregation mainly happen at higher pressure levels (500 MPa and mostly 600 MPa). In case of liquid egg protein and liquid whole egg I found that the pressure treatment of 100, 200, 300, 400 and 500 MPa did not cause significant changes in the electrophoretic separation patterns of the egg products and in the denaturation enthalpy datas compared to the control samples. Changes in protein solubility and native state are observed mainly after 600 MPa pressure treatment.

Thermodynamic measurement results:

By increasing the levels of pressure treatments, the value of denaturation enthalpy was reduced, in other words the amount of protein what still can be denatured was reduced in each case of the examined livestock products. However, the extent of the decreases was different in case of the different kind of food products. It can be stated that in the three examined meat (beef sirloin, pork loin, chicken breast) the myofibrillar proteins (myosin, actin) involved in muscle building are more sensitive against pressure treatment than connective tissue and sarcoplasmic proteins. The peak detected on the beef blood thermogram (68ºC - 70ºC) is albumin, which makes up most of the blood proteins, significant decreases were observed in both peak temperature and denaturation enthalpy compared to the control sample at pressure treatments of higher levels such as 500 MPa and 600 MPa. In case of liquid egg white and liquid whole egg products the 100-500 MPa pressure treatments do not yet caused a significant change in the denatured amount of protein, but 600 MPa pressure treatments reduced the denaturation enthalpy by nearly one third.

2.2 NEW SCIENTIFIC RESULTS

Theses of electrophoretic measurements:

1. In case of myoglobin which belongs to the group of sarcoplasmic proteins, I determined that by electrophoretic separation (isoelectric focal position, SDS-polyacrylamide gel electrophoresis and NATIV-polyacrylamide gel electrophoresis) that pressure treatments of 100, 200, 300 and 400 MPa (5 minutes) did not change the electrophoretic separation of myoglobin from beef sirloin (Longissimus dorsi) compared to the control sample. Changes in solubility and native state of this protein were observed mainly due to 500 and 600 MPa pressure treatments.
   Csehi B, Szerdahelyi E, Pásztor-Huszár K, Salamon B, Tóth A, Zeke I, Jónás G, Friedrich L, Changes of protein profiles in pork and beef meat caused by high hydrostatic pressure treatment. ACTA ALIMENTARIA HUNGARICA 45:(4) pp. 565-571. (2016)

2. I have demonstrated by electrophoretic separation (isoelectric focusing, SDS-polyacrylamide gel electrophoresis and NATIV-polyacrylamide gel electrophoresis) of the myoglobin in pork loin (Longissimus dorsi) that high pressure treatments of 100, 200, 300 MPa (5 minutes) did not change the electrophoretic separation of myoglobin compared to the control sample, however pressure treatments of 400, 500 and 600 MPa for 5 minutes caused a loss of native state of myoglobin and a significant decrease in its solubility.
   Csehi B, Szerdahelyi E, Pásztor-Huszár K, Salamon B, Tóth A, Zeke I, Jónás G, Friedrich L, Changes of protein profiles in pork and beef meat caused by high hydrostatic pressure treatment. ACTA ALIMENTARIA HUNGARICA 45:(4) pp. 565-571. (2016)

3. I have established that based on SDS-polyacrylamide gel electrophoresis separation and densitometry data of myofibrillar proteins of Longissimus dorsi (in case of extraction with 0.7 M NaCl solution) due to 300 MPa and above levels of high pressure treatment (400, 500 and 600 MPa; 5 minutes) the protein intensity values were reduced by at least 50% as a result of changes in protein solubility. Therefore the 300 MPa (5 minutes) pressure treatment can be considered as a threshold based on SDS-PAGE and color measurement results.

4. In case of imidazole dipeptides (carnosine and anserine) in beef sirloin (Longissimus dorsi), I found by the applied capillary electrophoresis method that the pressure treatments (from 100 to 600 MPa, 5 minutes) did not cause a significant change in the carnosine and anserine content ($P > 0.005$).

5. I have concluded that in case of chicken breast (Pullum pectus) the high pressure treatments of 400, 500 and 600 MPa (5 minutes) resulted the appearance of degradation products and aggregates compared to the control sample, observed by electrophoretic separation (isoelectric focusing, SDS-polyacrylamide gel electrophoresis and NATIV-polyacrylamide gel electrophoresis).

6. I have found that in case of beef blood, liquied eggs products and partly in case of milk, when the proteins are dissolved in liquid medium they are more resistant against high pressure treatment, denaturation and aggregation occurs mainly at higher pressure treatment levels (500 MPa and mostly 600 MPa).

Thesis of electrophoretic and thermoanalytical measurements:

7. In case of liquid egg white and liquid whole egg I have determined by electrophoretic separation and thermodynamic measurements (SDS-polyacrylamide gel electrophoresis, NATIV-polyacrylamide gel electrophoresis and DSC) that pressure treatments of 100, 200, 300, 400 and 500 MPa (5 minutes) did not cause a significant change in the electrophoretic separation of the liquid egg products and denaturation enthalpy data compared to the control samples. Changes in protein solubility and in its native state are observed mainly due to 600 MPa pressure treatment.

3. CONCLUSIONS AND RECOMMENDATIONS

The experiments clearly showed that, like microorganisms, the different livestock products have different "sensitivities" to high hydrostatic pressure treatment. In my work I have focused on the physicochemical properties and proteins of the examined products, since the role of proteins is decisive in these products and the changes in protein structure and physico-chemical parameters may affect the techno-functional properties in many cases. I concluded that the high pressure treatments affect the different proteins and groups of proteins in a different extent. The myoglobin of beef sirloin changes only due to higher levels of HHP treatment 500 and 600 MPa (its native state and solubility), meanwhile changes of the myoglobyn in pork loin begin already by 400 MPa treatment. In case of chicken breast protein aggregates and fragments appear due to pressure treatments of 300 MPa and above, which can be detected by electrophoretic techniques. In case of beef blood, liquid egg products and in case of some measurements of milk, my experiments also established that when the proteins are dissolved in liquid medium they are more resistant against pressure treatment, denaturation and aggregation mainly happen at higher pressure levels (500 and 600 MPa). However, the causal background of these measurements is not always fully understood, therefore the use of additional measurement techniques could help to obtain a clearer picture of the effects of the examined technology.

For the practical application and development of HHP treatment it is essential to define the recommended thresholds of the treatment, therefore based on the measured results, my maximum pressure threshold recommendations are as follows:

- Beef Striploin: 400 MPa, 5 min
- Pork Loin: 300 MPa, 5 min
- Chicken breast: 300 MPa, 5 min
- Beef Blood: 400 MPa, 5 min
- Liquid Egg White: 400 MPa (500 MPa depending on the aim of use), 5 min
- Liquid Whole Egg: 400 MPa (500 MPa depending on the aim of use), 5 min
- Milk: 500-600 MPa, 5 min

4. PUBLICATIONS RELATED TO THE THESIS

IF publications:

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