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Élelmiszertudományi Kar

THESIS

EFFECTS OF THE USE OF HIGH HYDROSTATIC PRESSURE TECHNOLOGY ON THE MICROBIOLOGY AND OTHER QUALITY PARAMETERS OF SOME SELECTED FOODSTUFFS

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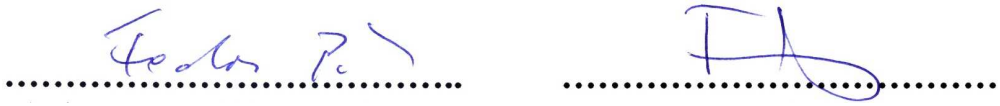
The applicant met the requirement of the PhD regulations of the Corvinus University of Budapest and thesis is accepted for the defence process.



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Introduction

In recent years, there is an increasing consumer demand for minimally processed, high quality and microbiologically safe foods. This has stimulated the food industry to find new methods to satisfy this demand. Among new food preservative technologies the attention directed towards the non-thermal food processing methods as the elimination of the unfavourable effects of heat treatments is expected. High hydrostatic pressure (HHP) is viewed as one of the more promising non-thermal methods for food preservation. In general, high pressure inactivates microorganisms, modifies biopolimers, including enzyme inactivation, protein denaturation and gelation, modifies the physico-chemical properties of water, while leaving nutritional values, colour and flavour components largely unaffected. Since the pressure changes in foods are instantaneous and uniform, the process is independent of the volume and the shape of the food. The efficiency of pressure treatment is influenced by the pressure level, treatment time and temperature, pressurization/decompression rate and heat distribution inside the vessel. Moreover, parameters as pH, water activity, starting temperature and the composition of food also have an effect on the result of high pressure processing. Even though HHP was first used for foods about 100 years ago, there was little progress made in applying this technology for commercial purposes until 1990, when the first commercially manufactured products became available in Japan. In the past 20 years, there have been a number of significant technological developments and advances in processing equipment and also in describing the effects of high pressure on microorganisms. There are already a number of different types of food that have been treated by high pressure. Fruit products were among the first pressure treated foods to be introduced to the markets in Japan. Nowadays, several pressure treated foods, particularly fruit juices, purees, jams, yoghurts and other products are already commercially available. These foods also rely on factors (low pH, low temperature) other than pressure treatment alone to provide microbiologically safe and stable products.

Despite the detailed information that can be derived from studies on isolated food components, it is still impossible to predict all the complex networks of interactions among the various constituents found in real food systems. Therefore experiments should be conducted on real foods in order to identify the nature of the treatment-induced modifications and their influence on the properties of the food under investigation.

Objectives

Implementation of high hydrostatic pressure technology to the food industry offers a wide field of research.

In the present study, the possible use of high hydrostatic pressure treatment was investigated in case of some selected foods. Microbiological state and other parameters, influencing the shelf life and consumer preference were investigated at the chosen treatment parameters.

Materials and methods

The selected materials and the chosen examinations were the following:

- Deboned turkey meat - raw material of several products of the food industry - was pressure treated at 200 MPa, 20 min at room temperature, than stored at 4ºC for 15 days. Microbiological experiments were performed, which included the enumeration of the total viable cell counts, the enterobacteriaceae cell counts and the most probable number of coliforms and *E. coli*. Since deboned turkey meat is exposed to several prooxidant effects during processing, the changes of lipid oxidation and cholesterol oxidation were also investigated.
- The easily perishable chicken liver was pressure treated at 200 MPa, 300 MPa, and 400 MPa for 20 min at room temperatures, then microbiological (total viable cell counts, enterobacteriaceae, coliforms, *E. coli*), lipid oxidation and cholesterol oxidation investigations were carried out. The latter proved to be important because of the high cholesterol content of chicken liver. The examinations were completed with the measurements of changes in the colour parameters.
- Minced beef - having high consumer preference – was pressure treated at 200 MPa, and 300 MPa for 20 min at room temperature than stored at 4ºC for 15 days. Microbiological (total viable cell counts, coliforms, *E.coli*) and protein denaturation (DSC) examinations were made, changes in colour parameters were determined because of the discoloration experienced in case of red meats.
- Effects of pressure treatments of fresh bovine milk at 200 MPa, 400 MPa, 600 MPa, 800 MPa for 5 min at room temperature were compared to the effects of heat treatment. Samples were stored at 4ºC for a week. Changes in the total viable cell counts were followed, protein denaturation (PAGE) was examined, colour measurements were made, and changes in odour components were analysed.
- Hen egg – widely used food component because of its functional properties – was pressure treated at 300 MPa, 400 MPa, and 500 MPa, for 5 min at room temperature. Examinations of

protein denaturation (DSC) and colour measurements were performed as a part of an extensive study.

- Strawberry is a high value raw material of the food industry, component of many products, having limited shelf life and may only keep in the refrigerator for a couple of days. Strawberry samples were pressurized at 400 and 600 MPa for 5 min at room temperature, than stored 4ºC for 2 days. Minimally processed fruits must maintain freshlike characteristics while providing a convenient shelf life and assuring safety. In the present study, *Enterococcus faecalis* was used as a gram positive surrogate microorganism. *Enterococcus faecalis* is a widely spread microorganism, can be found from soil to natural waters and highly resistant against hydrostatic pressure. The objectives of this work were to investigate whether the whole fresh strawberries are suitable for pressurization, and the potential of pressure treatment to reduce the number of *Enterococcus faecalis* on the fresh berries. The combined effects of pressure (100-600 MPa), temperature (-20ºC, 4ºC, 20ºC), and pH (7.2, 4.5) on *Enterococcus faecalis* culture were also studied, and the degree of injury and inactivation were determined.

The samples were pressure treated in a FoodLab 900 (Stansted Fluid Power Ltd., U.K.) type laboratory pressure vessel.

Plate count agar was used to enumerate the aerobic mesophiles and the enterobacteriaceae cells count. Coliforms and *E. coli* were counted by MPN technique in liquid medium.

Lipid oxidation was examined by measuring TBA and following changes in the formation of cholesterol oxidation products. The thiobarbituric acid reactive substances were estimated according to Newburg and Concone. Cholesterol oxidation products were separated by thin layer chromatography, the measurement of the individual products was carried out by enzymatic method.

DSC analyses of pressure treated minced beef and egg white samples were performed on a MicroDSC III (SETARAM) microcalorimeter to follow the changes in protein fractions. Since DSC did not bring clear results in case of milk samples, the changes in protein structure was studied by gel electrophoresis, where protein fractions were separated by native PAGE.

The colour changes resulted by pressure treatments was measured by using a Minolta CR-200 instrument, and expressed as CIE Lab L* (lightness), a* (redness), b* (yellowness).

The changes in odour parameters of milk samples caused by various pressure and heat treatment were analysed by electronic nose (AppliedSensor), which profiles the headspace volatiles over and around the sample, producing fingerprints for each sample.

Results

Microbiological examinations

The microbial effects of high hydrostatic pressure treatment were investigated in case of mechanically deboned turkey meat, chicken liver, minced beef meat, and fresh bovine milk. Among microbiological examinations, the total aerobic mesophile counts was enumerated in every sample. Enterobacteriaceae count was determined in case of mechanically deboned turkey meat and chicken liver, while the most probable number of coliforms and *E. coli* was performed in deboned turkey meat, chicken liver and minced beef meat samples.

As a direct effect of 200 MPa pressure treatment of deboned turkey meat, 1-log reduction in total plate count was observed, while enterobacteriaceae, coliforms and *E. coli* decreased below the detection level. During 15 days storage at 4˚C, the investigated microba populations increased in control samples, but no changes were detectable in pressure treated samples.

The microbial examinations of pressure treated chicken liver showed that increasing pressure level resulted in increased microbial inactivation. While in case of deboned turkey the enterobacteriaceae and coliforms were eliminated by 200 MPa pressure treatment, chicken liver needed 300-400 MPa treatment to ensure the same result. The observed difference could be originated from the different starting microflora (liver was more contaminated) or the different composition of deboned turkey and chicken liver.

Pressure treatment at 200 and 300 MPa caused 2-log reduction in the microflora of minced beef meat. During 15 days storage at 4˚C, the surviving microbial cells were able to grow and the shelf life of minced beef samples didn't last for two weeks. After pressure treatments, the number of coliforms ($6*10^2$ in untreated samples) decreased under the detection level and didn't show growth during 15 days storage. Since coliforms were not detectable in untreated samples during chilled storage, their lack is presumably not the effect of pressure treatment on the first place, but probably the result of their sensitivity to antibacterial metabolism products made by lactic acid bacteria growing in the vacuum-packaged samples during storage.

In case of milk samples, with the increase of pressure level, an increase in the inactivation of mesophile aerob cell counts was detected. While 200 MPa pressure treatment didn't cause significant loss in the total aerobic mesophile count, 400 MPa, 600 MPa, 800 MPa treatments increased the microbial inactivation with several orders of magnitude. During one-week storage at 4˚C the growth of the microflora was delayed, the self-life of samples treated at 400 MPa, 600 MPa, and 800 MPa proved to be longer than heat treated samples.

The suitability of high pressure treatment on the whole fresh strawberry was investigated, pressure resistance of *Enterococcus faecalis* was studied in case of different pH and temperature

combination. The *Enterococcus faecalis* employed in the present study was particularly pressure resistant, our previous examinations have shown that it was capable of surviving on strawberry at refrigeration temperature despite the acidic conditions (pH 3.6). The short time treatment was vital in these experiments, since earlier tests proved that during short time treatment strawberries keep their favourable physical qualities. In strawberries, pressurized at 400 and 600 MPa for 5 min, the number of inoculated *Enterococcus faecalis* showed 5-6 log reduction. After pressure treatments, samples were stored at 4˚C for 2 days, but no recovery was detected in any sample. At neutral medium (pH 7.2) *Enterococcus faecalis* cells proved to be pressure resistant, only 600 MPa pressure treatment could achieve 4-log reduction of viable cell counts, which showed an increase during storage, the proportion of injured cells decreased. This result suggests that after pressure treatment, a repair mechanism was turned on, decreasing the amount of injured cells. Under acidic condition (pH 4.5), *Enterococcus faecalis* cells become more sensitive to pressure, inactivation could be achieved at lower pressure (400 MPa), the sublethally injured cells could not recover during storage. Comparing the experiments performed at neutral and low pH, the results suggest that the sublethaly injured proportion of cells, surviving the pressure treatment, are unable to repair the pressure damage in conditions of low pH. The frozen samples haven't shown significant viability loss, only about 1-log decrease was achieved even after 600 MPa pressure treatment.

Lipid oxidational examinations

The effects of high pressure treatment on lipid oxidation were examined on mechanically deboned turkey and chicken liver. Measurement of lipid oxidation processes is of great importance in case of deboned meats in general, as it is exposed to several prooxidant effects during processing. Chicken liver has high cholesterol content so it is necessary to follow lipid oxidation in case of this material as well. In both cases TBA values were measured and changes in the formation of cholesterol oxidation products were followed. High hydrostatic pressure treatment accelerated lipid oxidation in the investigated materials. TBA value of chicken liver showed an important increase after 400 MPa pressure treatment, in case of deboned turkey TBA value increased considerably after 200 MPa pressure treatement already. It could be explained by the processing method of the deboned turkey, or its fat content (9,16%). In tendency, changes of the quantity of choleserol oxidation products in the investigated samples were similar to the TBA changes observed. High hydrostatic pressure treatment induced increased production of cholesterol oxidative products. In case of deboned turkey meat, 200 MPa pressure treatment increased the amount of total cholesterol oxidation products. Among the cholesterol derivatives, 7α-hydroxicholesterol, a 7β-hydroxicholesterol és a 7-ketocholesterol were detected. In chicken liver samples 400 MPa pressure treatment caused an

increase in the amount of total oxysterines, apart from the above products, cholestan- 3β, 5α, 6β-triol and cholesterol- 5α, 6α-epoxid were detected.

Protein denaturation examinations

Protein denaturation, caused by high hydrostatic pressure treatment, was investigated in case of minced beef, egg white and fresh bovine milk. DSC analyses were performed to follow the changes in protein fractions of pressure treated minced beef and egg white samples. The endothermic peaks of the major protein fractions were well identifiable. The denaturation, occurred in the protein fraction of minced beef as an effect of 300 MPa high pressure treatment, manifested as a gradual decrease of the endothermic peaks of actin and miosin. The DSC thermogram of egg white sample, treated at 500 MPa, was almost entirely flattened out, endothermic peak of conalbumin was not detectable, while ovalbumin showed minimal heat-denaturation during DSC-scanning. Since DSC did not bring clear results in case of milk samples, the changes in protein structure was studied by gel electrophoresis, where protein fractions were separated by native PAGE. The electrophoretic pattern of milk samples showed that increased pressure level caused increased denaturation of certain protein fraction of milk. Casein and α-lactalbumin content seemed to be unchanged with the increasing pressure. The two isomers of β-lactoglobulin seemed to be the most responsive to pressure. The intensity of the β-lactoglobulin bands was decreasing and the less mobile β-lactoglobulin-A denatured first.

Colour measurements

The changes of the colour parameters of pressure treated chicken liver, minced meat, milk, egg white and egg yolk samples were measured.

L* values of pressurized chicken liver samples increased considerably in each case, discoloration was visible. The a* and b* values increased at a lesser degree.

Drastic colour changes occurred in pressure treated minced beef samples, L* and b* values increased at a large extent. The formed colour was similar to cooked meat due to oxymioglobin-metmioglobin transformation.

Examination of pressure treated egg white showed that after 400 MPa and 500 MPa treatments L* value increased considerably, while b* decreased at large extent. At these pressure levels the main protein fractions of egg white, conalbumin and ovalbumin, partly or completely denaturated, these changes were observed in change of colour as well. All the three colour parameters of egg yolk samples showed significant decrease compared to the untreated samples.

Milk colour was not affected by hydrostatic pressure processing at a large extent. A small decrease of L* and b* values was observed and a slight increase of a* value occurred with pressure increase.

Electronic nose measurement

Electronic nose measurments and their chemometric analysis resulted in a distinct separation of chemosensor responses amongst the different samples, suggesting that some odour changes of milk has occurred.

Summary and recommendations

It can be ascertained that high hydrostatic pressure treatment efficiently decreased the number of microorganisms in each food sample used in this research. In case of mechanically deboned turkey and chicken liver the pressure-induced lipid oxidation may limit the usefulness of high pressure technology. Studies shall be continued to investigate the possible control of pressure-induced lipid oxidation by antioxidants. The modification of the colour of chicken liver and minced beef could be hamper the commercialization of these pressurized products. In case of egg white and minced beef, the partially or complete non-thermal denaturation of protein fractions could lead to the change of functional properties, which would make possible to develop new types of products. Amongst the protein fractions of milk, only β-lactoglobulin seemed to be pressure sensitive, which could play a role in the making of allergenic free dairy products. The results of the work carried out with strawberries showed that high pressure treatment can improve the safety and the quality of strawberry, as a minimally processed product. The fact, that microbial injury can occur at lower than lethal pressures, can lead to combination with other treatments. As the injured cells proved to be able to recover under favorable conditions, the effect of storage time cannot be underestimated in high pressure studies. The sensitization of cells to acid prior pressure treatment allows *Enterococcus faecalis* to be inactivated by lower HHP treatments that would be necessary for neutral conditions. This study suggest that commercially-practicable short-time pressure process can be used to inactivate a highly pressure resistant *Enterococcus faecalis* in low-pH foodstuffs, but the appropriate storage conditions have to be determined to achieve satisfactory result.

As a summarized, general conclusion, it can be stated, that high hydrostatic pressure treatment as a food processing technology in practice, requires optimization in case of every single product type. The method is microbiologically efficient and quality protective, but further researches are necessary to prevent changes in lipid oxidation and regeneration of sublethally injured microorganisms in pressure treated foods during storage.

Regarding the further researches required, and the current cost and capacity limit of the high hydrostatic pressure technology, it is unlikely to replace conventional thermal processing, but it could offer commercially feasible alternatives in the case of novel food products with improved functional properties, that cannot be attained by conventional heating.

Thesis (New scientific results)

1. The sublethaly injured proportion of *Enterococcus faecalis* cells, surviving the pressure treatment, are unable to repair the pressure damage in conditions of low pH.
2. It can be stated that even at low pressure levels, high hydrostatic pressure treatment accelerated lipid oxidation and induced increased production of cholesterol oxidative products in mechanically deboned turkey meat and chicken liver.
3. It was revealed, that high hydrostatic pressure treatment at the chosen treatment conditions caused undesirable colour changes in chicken liver and minced beef.
4. It was observed that pressure treated milk samples were well separated and distinguishable from untreated ones by electronic nose measurement, according to their odour components.

List of publications:

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