Thesis book

Investigation of beef ageing, development of the ageing technology

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1. Introduction

Protein intake in adequate amounts and quality is necessary for building up the cells of the human organism and for their appropriate functioning. In absence of proteins the organism is not able to protect itself against deleterious effects, the immune system gets weaker, becomes susceptible to various illnesses. Meat is one of our most important protein sources that contains all essential amino acids.

In the last four decades consumption of meat, especially of red meat, continuously decreased in Hungary. One of the reasons are the often mentioned negative opinions about nutrition-physiological aspects of red meat, and the mass diseases, like foot and mouth disease or BSE, sweeping over the livestock in West Europe in the previous years, and their food safety assessment decreased beef consumption all over Europe and in Hungary as well.

Preparation of beef is more challenging for a housewife than preparation of chicken meat or pork and it is more time consuming, too (SZENTE, SZIGETI, SZAKÁLY 2009). Roasting technique of beef steak is not well known among Hungarian consumers, beef is most often cooked as beef-stew. Tenderness is one of the most important quality factors of beef influencing consumer acceptance. It depends not only on the age of the animal but on the state of aging of beef. Consumers often attribute the long preparation time to the age of the animal, but it is more influenced by the state of aging.

Aging means storage for a longer time than necessary for the cooling of carcasses which promotes extension of the effect of proteolytic enzymes. Tendering of beef starts after rigor mortis was released, and this process can last even for a few weeks. The process is hard to control and it is strongly influenced by the tissue composition, feeding, species, effects before and after slaughter, rate of cooling (SØRHEIM et.al 2001). Although aged beef is more and more often available on the Hungarian market, aging technology is not used in Hungarian practice. Aging of the hanging carcass after devisceration at the temperature of the environment (even at + 20°C) for 1 - 2 days to accelerate aging was a known procedure in the old slaughtering technology. Nowadays food safety rules stricktly regulate cooling conditions after slaughter, and long term beef aging is not used either in the households or in the gastronomy (lack of knowledge, room and refrigeration capacity).

The practice of steak roasting is spreading among the Hungarian gourmets. Steak appears as a special dish on barbecue parties in summertime. Lately aged, tender beef became popular on the Hungarian market, too, and import (Argentine, Irish) aged beef pieces are already available, but their high price limits their general use.

Based on the foreign aging practice dry and wet aging processes are distinguished. During dry aging carcasses are hoisted and aged without packaging. However, during the long time beef becomes pale, its surface dryes out and the chances of microbial spoilage is high. Wet aging means aging of meat in package. Losses are less and the risk of microbial contamination is less, too.

The aim of the thesis was to monitor the effects of complex physical, chemical and biochemical processes during aging and to develop an aging technology which makes aging of beef faster and easier and can be implemented in the Hungarian meat industry as well.

2. Objectives

The aim of the thesis was to monitor the effects of complex physical, chemical and biochemical processes during aging. The investigations were aimed at:

- The effects of aging temperatures of 0 – 1°C and + 5°C, respectively, on the physical, chemical and microbiological state, on the protein structure of beef striploin and whether the process of aging and tendering can be monitored by organoleptic analysis.
- Differences as a result of aging beef striploin at 0 - 1ºC and + 5ºC, respectively, in the physical, chemical, microbiological characteristics, protein structure and membrane permeability
- Suitability of SDS PAGE for monitoring the aging phases of beef striploin, which protein molecules become visible by electrophoresis by changing the parameters of sample and gel preparation
- Acceleration of aging process by the application of active ultrasound, what is the effect of ultrasonic treatment on texture, protein structure and membrane permeability.

3. Materials and methods

Raw material of the investigations was beef strip loin to monitor the aging processes in beef because beef striploin is suitable for steak roasting and it is a relatively homogenous raw material. Beef samples for the aging experiments were obtained directly from the cutting plant 24 hours after slaughter.

Quarter carcasses came from young Hungarian Simmenthal bulls kept under the same feeding and breeding conditions at a Hungarian livestock farm. Their age ranged between 14 and 26 months. Cutting and deboning of the hind quarter carcasses was done in the plant. Deboned beef striploin samples were cut in 40 cm pieces and individually vacuum packed. Weight of the samples varied between 2600 and 4300 g.

Aging experiment

Investigation of beef aging

In designing the first series of investigations I took into consideration that to produce a microbiologically safe product low temperature is necessary. Samples were put for 27 days in a refrigerator at 0 – 1 °C. Since by increasing temperature the enzymes promoting tenderness are activated, aging experiments were performed at + 5 °C as well. Temperature fluctuation in the refrigerator was measured by RFID tags with temperature sensors. Temperature fluctuation during aging was 0 – 1 °C. Measurements on the same day were performed in triplicates.

The following investigations were performed:

- pH
- Color
- Texture (TPA, WB)
- Organoleptic quality
- Microbiological state
- Drip loss
- Changes in protein structure (PAGE-ELFO)
- Changes in protein structure (DSC)
- Changes in ion efflux.

Samples were packed in 70 μm thick EVOH foil.

Treatment of samples by active ultrasound

Two cm thick striploin samples were individually vacuum packaged and put into the ultrasonic equipment filled with 8 °C water. Weight of the samples varied between 160 – 180 g. Active Ultrasound Laboratory type (400W/20kHz) high intensity ultrasonic mechanical system was used for the treatment of samples.

The ultrasonic head is changeable. During the treatments the biggest (5 cm diameter) head was used. Beef samples were treated by 20 kHz frequency, 3 W/cm^2 intensity ultrasound for 60 and 90 min, respectively.

Following treatment, samples were placed in 0 - 1℃ and + 5℃ refrigerators, respectively. The following parameters were measured during the aging experiments:

- Changes in texture (TPA, WB)
- Changes in protein structure (PAGE-ELFO)

- Changes in structure (DSC)
- Changes in ion efflux.

Methods

Determination of pH of raw meat samples

Electrochemical determination of pH is based on the potential difference between the two electrodes (reference and measuring electrodes). Potential of the reference electrode (usually saturated calomel electrode) is independent of the pH of the given sample, and the potential of the measuring (indicator) electrode is proportional to the H+ concentration of the sample. pH meters measure the intensity of the electric current produced by the potential difference between the calomel and the glass electrode. The two electrodes are fit in one combined glass electrode, thus only one electrode is used in the measurements.

Color measurement

Minolta CR-200 type colorimeter was used to measure the color changes on the surface of the samples during aging. Measurement is based on the principle of additive color mixing, that is every color can be produced as a mixture of three lights with different wavelengths.

Determination of drip loss

Drip loss was determined by weighting the weight of beef samples at the beginning and at the time of the investigations.

Organoleptic analysis

Tenderness of steak made of aged beef can be relished and sensed the most. In Hungary only few know the technology of preparation and the quality criteria of a nice steak. Organoleptic analysis was performed by a 10-member panel. Preference assessment of properties characteristic to steaks (crispness, juiciness, tenderness, taste, overall impression) was made. The highest score for each characteristic was 5 points.

Two cm slices of samples were roasted according to NATTRESS és JEREMIAH (2000) to medium.

Measurement of changes in texture

Samples were 2 cm thick and weighted 210 – 180 g. Heat treatment of samples for texture analysis was performed in slices at 67°C for 15 min in vacuum packed form (HWANG et.al 2004).

Temperature of samples during texture measurement was 24 °C, since temperature greatly influences the texture characteristics of samples. Texture measurements were carried out by a Stable Micro System (SMS) texture analyser.

Texture analysis by texture profile analysis (TPA)

Following heat treatment and cooling 2 cm high cylinders with 1,25 cm diameter were cut for texture profile analysis (TPA). The cylinders were compressed to 70% of their original height. Six-seven cylinders were cut from each slice so the number of parallel measurements was 14-16.

Texture analysis by Warner - Bratzler shear device

After heat treatment and cooling 2 cm broad strips were cut from the slices and the strips were cut through by a Warner-Bratzler shear blade set. Three strips were cut from each slice and the strips were cut into 3 cm pieces. This way number of parallel measurements was 9 – 12.

Microbiological investigations

The following microbiological measurements were performed: anaerobic total viable cell count (MSZ 3640/4-86), lactic acid bacteria count (MSZ EN ISO 15214: 2005), *E. coli* count (MSZ 3640-18: 1979), *Pseudomonas* count (MSZ 3640-7: 1980), and number of mesophilic aerobic and facultative anaerobic bacteria (MSZ EN ISO 4833:2003) according to the EU directive 2073/2005 EK and 4/1998.(XI.11.) EüM decree.

Determination of membrane permeability of beef

Changes in memebrane permeability were determined by a Labvig OE-420 type instrument at the Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics.

Cells of the instrument were filled with 3 cm^3 of 0,3M mannitol solution and 0,36 és 0,4 g of samples (control and treated) were put into each cell. Ion efflux was determined as the change in the current intensity (I, μA), that is in conductivity, at ambient temperature. Thus changes in the dynamics of the semipermeability of the membrane could be characterized.

Polyacrylamide gel electrophoresis

Gels were run in a Bio-Rad Mini-PROTEAN 4 Cell apparatus. Gels were digitalized by a Gel Doc XR Scanner and evaluated by the Quantity One (ver. 4.6.8.) software. The investigation was performed according to the LAEMMLI (1970) method.

After running gels were stained by 0,24% Comassie Brillant Blue R 250 (50% methanol) for 1 hour then gels were washed in a solution containing 7 % methanol and 9% acetic acid.

Investigation of protein status by Differential Scanning Calorimetry

Differential scanning calorimeter (DSC) is detecting the heat flow. Sample and reference material are spatially separated and they are heated at the same time according to the same heating programme. Detected values are recorded in a PC. The programme has two functions:

- Records and evaluates data.
- Shape of the curves is used for identification of the given material while the size of the area below the curve is used for quantitative evaluation, from which transition heat can be calculated.

DSC analyses of control and treated samples were performed in a „MicroDSC III" type microcalorimeter (SETARAM, France).

Statistical programmes, methods

Results were processed by MS-Excel programme, mathematical-statistical evaluation was performed by SPSS (ver 11.) and MS-Excel programmes, respectively, at 95% probability level. Results were analysed by one-compound analysis of variance (probability of mean identity at 95% significance level, LSD).

4. Results and discussion

In the last four decades consumption of beef continuously decreased in Hungary, which was affected – among others - by the negative opinions about nutrition-physiological aspects of red meat. Tenderness is one of the most important quality attributes which determine the palatability of meat, that is influenced not only by the age of the animal but by the state of aging of meat, too.

Process of meat aging is the complexity of biochemical changes when characteristic taste and proper texture are developed as a result of the activity of proteolytic enzymes. Time needed for

these processes is strongly time-dependant. In the last few years, by the change in the taste and demands of a certain group of consumers, and the barbecue parties coming into fashion, there is a growing market demand for aged beef. Use of aging technology is limited to certain regions of the carcass, which are suitable for aging. One of the most suitable types of beef for aging is striploin.

Food safety reasons require cooling of carcasses after slaughter, thus as a consequence aging technology has to be changed.

In the present work aging experiments were carried out at 0 - 5 °C temperature interval with vacuum packed beef to fulfil food safety requirements. Aging in vacuum packaging is advantegous from more viewpoints. Any meat plant can easily implement it, because of easy handling, its good technological feasibility, and vacuum packaging ensures anaerobic conditions.

The aim of the present work was to study the effects of complex physical, chemical and biochemical processes during beef aging, to obtain deeper insight in the processes taking place, and to develop an aging technology which makes processing of aged beef easier and more rapid and can be implemented by meat processing plants. To reach this goal, I chose the investigation of active ultrasound which might be an aging technology safely realizable by meat plants.

Process of beef aging was examined in two temperature intervals during the aging experiments. Changes in color, pH, drip loss, texture and microbiological status were measured during aging. Calorimetric characteristics of striploin, evolution of the rate of ion efflux in the fibres and changes in the quantity and quality of detectable proteins were monitored. Processes during aging and the process of tenderizing were accelerated by active ultrasound.

Color is one of the most important quality attributes of meat influencing consumer acceptance. Changes in lightness (L*), red-green hue (a*) and blue-yellow hue (b*) were detected during aging at the lower and higher aging temperatures. Aging process of meat can be well characterized by the changes in pH after rigor mortis. During pH decrease the values change from the physiological pH of 7,2 to pH ,5-5,6. According to my findings pH of beef striploin practically didn't change by the end of aging. Change in the water holding capacity of meat is an important quality factor for the meat industry. During the used aging peocess, amount of drip loss didn't exceed 2,5 – 3 % by the 27^{th} day after slaughter at neither of the aging temperatures.

Microbiological status of fresh meat can not be neglected by food safety viewpoint. Number of lactic acid producing bacteria decreased at the lower aging temperature. Total anaerobic cell count increased during aging, but it didn't exceed the food safety limit at the lower aging temperature while at the higher aging temperature 10^6 TVC was reached on the 15^{th} day.

Processes occurring during aging of beef affect the technological behaviour of meat. Thus detection of the changes in the main protein fractions was very important. SDS-PAGE was used for this purpose. Calorimetric characteristics of striploin were monitored by DSC method. DSC measurements were performed to determine the peak temperature of protein denaturation of meat proteins and the enthalpy (ΔH) of denaturation that provides quantitative information.

After treatment with active ultrasound there was an increase in the number of low molecular weight protein fractions detected by SDS-PAGE, which shows the action of the proteolytic enzyme system. The ultrasound destroyed the membrane structure and thus protelytic enzymes could easily release.

Number of protein fractions decreased in samples treated for 90 min. Since protein content didn't change during aging, a part of the proteins aggregated and remained in the wells of the stacking gel. Amount of myoglobin (17 kDa) and desimin (55 kDa) decreased with the progress of aging days. According to the densitograms, no changes could be detected from the 9^{th} day on. Color intensity of the protein bands of samples aged at higher temperature was higher mostly in case of low molecular weight (40 – 17 kDa) proteins.

In the assessment of aging processes changes in the membrane were detected. Permeability of the membrane changes during aging and storage. The measurements proved that this change could be monitored by the examination of the rate of ion efflux during aging. Amount of ion efflux in samples aged at lower temperature increased during aging that proved the increase in membrane permeability during aging. Membrane permeability of samples aged at higher temperature was higher, too.

The measurements proved that the use of 3 W/cm^2 ultrasound increased the ion efflux of beef striploin. Magnitude of ion efflux increased proportionally with the increase of treatment time.

Detection of textural changes played an important role during aging experiments. I proved that 14 – 17 days are needed to obtain proper tenderness of beef under low temperature (0 - 1°C) aging conditions. Aging time necessary for the development of proper texture at higher aging temperature (+5 °C) shortened to 7 – 8 days. I proved the temperature-dependence of the process in the previously mentioned temperature range. If the membranes were injured in post mortem state, then enzymes were released first from the lysosomes then, after the injury of the cell membrane, from the cells themselves. These enzymes exerted proteolytic effect thus promoting the development of tenderness. By the application of active ultrasound (3 W/cm^2) a part of the membrane was injured, rate of the ion efflux increased, thus not only a mechanical effect contributed to the tenderness but the appropriate enzymes were released and made meat more tender. Beside the objective texture analyses subjective organoleptic analysis was also performed. Organoleptic analysis is an important tool also in the assessment of consumer preference in Hungary.

During the use of active ultrasound such mechanical effects are exerted on the sample that injure the membrane system thus promoting the efficiency of the multicatalytical enzyme system. These effects contribute to the process of tenderization and the mechanical waves affect the muscle structure as well.

In summary, I proved that treatment by 3 W/cm^2 intensity ultrasound favourably affected the texture of beef striploin and it may be introduced to meat plants as well.

5. Theses (new scientific results)

1. I established that at aging of vacuum packed beef striploin 8 days are needed for the development of proper texture at +5 °C. To achieve the same texture twice as much time is needed at 0 – 1°C.
2. I proved that changes in protein composition during aging of vacuum packed beef striploin at 0 – 1°C and +5°C, respectively, can be detected by SDS-PAGE method.
3. Structure of membranes is injured by the use of 3 W/cm^2 active ultrasound, magnitude of ion efflux increases. Magnitude of ion efflux increases proportionally with increasing treatment time.
4. I established treatment by active ultrasound causes textural changes. Firmness of beef striploin treated by 3 W/cm^2 intensity ultrasound for 60 min then aged in vacuum packaging at 0 - 1ºC decreased to the third compared to that of the control sample.
5. I established that number of proteins detectable by SDS-PAGE method decreased to a qurter in samples treated by 3 W/cm^2 ultrasound for 90 min by the end of aging. Intensity of proteins detectable by SDS-PAGE method in beef striploin aged in vacuum packaging at +5°C was higher, aging temperature has a significant effect.
6. I established that calorimetric characteristics of beef striploin aged in vacuum packaging at 0 – 1-°C and +5°C, respectively are not changed.

List of publications

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IF articles

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2. Marhahús érlelésének innovatív fejlesztése új érlelési technológia kidolgozása – **TESCO** 2006

3. Hűtő- és fagyasztó pultok, ill. szigetek hőkamerás feltérképezése – **TESCO** 2006

4. Hűtőlánc élelmiszerbiztonsági feltételeinek innovatív fejlesztése a hűtött élelmiszerek idő- és térbeli hőmérsékletprofiljának monitorozása – **TESCO** 2006

5. Előhűtött csomagolt és csomagolatlan sertés húsok hőmérséklet változásának vizsgálata különböző tartózkodási hőmérsékleteken (12 és 20°C) – **TESCO** 2006

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10. Csomagolás nélküli előhűtött húsok, húskészítmények és saláták hűtőpulti tárolási módjának innovatív fejlesztése a fizikai és mikrobiológiai állapotjellemzők javítására – **TESCO** 2007

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13. Védőgázos darált marhahúsminták védőgáz összetételének és mikrobiológiai állapotának vizsgálata – **TESCO** 2007

14. Pulyka kábításának innovatív fejlesztése, paramétereinek optimálása a kábultság és a húsminőség tekintetében – **SÁGA** 2007

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