High hydrostatic pressure- temperature modeling of Frankfurters batters- mechanisms, salt reduction, applications

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The LORD is my shepherd; I shall not want. He maketh me to lie down in green pastures: he leadeth me beside the still waters. He restoreth my soul: he leadeth me in the paths of righteousness for his name's sake. Yea, though I walk through the valley of the shadow of death, I will fear no evil: for thou art with me; thy rod and thy staff they comfort me. Thou preparest a table before me in the presence of mine enemies: thou anointest my head with oil; my cup runneth over. Surely goodness and mercy shall follow me all the days of my life: and I will dwell in the house of the LORD for ever.

Psalm 23

Dedicated to my family

Zusammenfassung

Der Einfluss kombinierter hydrostatischer Hochdruck-Temperaturbehandlung auf die Strukturmodifizierung sowie auf die funktionellen und sensorischen Eigenschaften von Brät für Frankfurter Würstchen wurde untersucht. Verschiedene Kombinationen von Prozess- und Rezepturparametern wurden analysiert um dadurch wichtige Mechanismen in der Brätstruktur zu definieren. Der Prozess der Löslichkeit sowie des Löslichkeitsgrades der Fleischproteine insbesondere des Myosin wurden als der Hauptprozess erkannt, welcher die höchste signifikante Wirkung auf die Funktionalitätseigenschaften des Brätes aufwies. Der maximale Löslichkeitsgrad wurde bei allen Rezepturen, in Abhängigkeit zur Behandlungszeit, bei 200 MPa/ 40°C beobachtet. Die Prozesse der Proteinlöslichkeit und Proteinaggregation wiesen einen signifikanten Effekt auf die Struktur sowie auf die funktionellen und sensorischen Eigenschaften nach ein- und zweischrittiger Hochdruck-Temperaturbehandlung auf.

Der Effekt des Hochdruckgradienten (PG= 40 MPa/s und PG= 2.5 MPa/) wurde untersucht und es wurde festgestellt, dass dieser einen entscheidenden Einfluss auf das Proteinnetzwerk sowie auf die funktionellen Eigenschaften des Brätes hat, welche bei niedrigem PG (2.5 MPa/s) als Resultat der Bildung einer sekundären Matrix, parallel zur Hauptmatrix verbessert wurden. Dieses Phänomen wurde mittels Raster-Elektronen-Mikroskopie (REM) visualisiert. Die Bildung der Sekundärmatrix wurde in Abhängigkeit zur Ionenstärke sowie am Gehalt löslicher Proteine und dessen Aggregationsgrad in der löslichen Phase festgestellt. Nach SDS-PAGE-Analysen wurden die Proteine, die in der löslichen Phase für die Löslichkeits-, Aggregations- und Gelbildungsprozesse verantwortlich waren, definiert: Myosin S-1, S-2, N-terminal, C-terminal, die leichten Myosinketten und Aktin. Besonders wichtig für die Bildung der Sekundärmatrix waren die S-1 Kopf-Domänen: N- und C- terminale.

Der Gehalt von Proteineinheiten kleiner als 6 kDa (unterhalb der SDS-PAGE Nachweisgrenze) stieg bei einem Behandlungsdruck bis zu 200 Mpa an. Bei gleichem Druck und längeren Haltezeiten wurde eine Verschiebung des Proteinpeaks zum höher molekularen Bereich beobachtet. Bei 300 MPa war dieser Prozess intensiver, was zu der Behauptung führt, dass die Aggregationsprozesse bei einem Druck ab 200 MPa beginnen und sich mit steigendem Druck bis 300 MPa weiterentwickeln.

Eine weitere Strukturverbesserung resultierend in der Festigkeitssteigerung wurde nach einer zweischrittigen Behandlung, im ersten Schritt im Bereich von 100-300 MPa bei einer Haltezeit von 1min und 5 min, bei 10°C und 40°C im zweiten Schritt bei 600 MPa/ 240 s beobachtet. Dieses war auch mittels REM zu sehen, die Matrix des Frankfurter-Brätes war aufgeblähter, homogener mit einer besser entwickelten Sekundärmatrix. Eine Verbindung zwischen dem Haltezeiteffekt und des Gehaltes löslicher Proteine auf der einen Seite und

eine erhöhte Intensivierung von Protein-Protein-Interaktionen auf der anderen wurde festgestellt.

Um die Möglichkeit der Salzreduktion festzustellen, wurde ein Vergleich der funktionellen und sensorischen Eigenschaften bei konventionellen und hochdruckbehandelten Frankfurter Würstchen durchgeführt, wobei nur minimale Unterschiede festgestellt wurden. Nach der chemischen Bestimmung des NaCl-Gehaltes in der löslichen Phase wurde eine Steigerung des NaCl-Gehaltes wie folgt konventionell (thermisch), < hoch PG (40 MPa/s), < niedrig PG (2.5 MPa/s) und < zweischrittiger Behandlung bei einem maximalen Druck von 200 MPa/ 60s/ 40°C für den ersten Schritt festgestellt.

Ein hypothetischer Mechanismus von Wasser-Protein-Interaktionen bei einer Hochdruck-Temperaturbehandlung wurde erarbeitet. Mittels dieses Modells wurden wichtige Zusammenhänge des kombinierten Hochdruck-Salzeffektes auf die Proteinmoleküle, die zum Verbesserung der Proteinlöslichkeit beitragen, erklärt. Die Disruption des Myosinkopfes wurde als entscheidend für die Steigerung der Anzahl der S-1 Domänen sowie der Anzahl andere kleinerer Proteinmolekülbruchteile im Vergleich zu den thermisch behandelten Frankfurter Würstchen erkannt. In einem weiteren Modell wurden die P-T-Löslichkeits-, Aggregations- und Gelbildungs-Intervalle aufgrund der Ergebnisse erstellt.

Des Weiteren wurde eine Struktur- und Funktionalitätseigenschaftsverbesserung von Frankfurter Würstchen mittels Putenseparatorenfleisch hergestellt mittels Hochdruck-Temperatur-Behandlung erreicht.

Auf Basis der untersuchten Strukturierungsprozesse bei Frankfurter Würstchen weist die kombinierte Hochdruck-Temperatur-Behandlung großes Potenzial für die Entwicklung innovativer Fleischprodukte (mit reduzierten Salzgehalt und verbesserten funktionellen Eigenschaften) sowie für Optimierung der Herstellungstechnologie von Frankfurter Würstchen bei der Reduktion der Prozesszeiten und Energiekosten (Kosten für thermische Behandlung) auf.

Abstract

The impact of high pressure/temperature treatment on structure modifying and functional sensory properties of frankfurters batter was investigated. Various combinations of process and recipe parameters were realized in order to determine main structure processes. Degree of solubilization of meat proteins, particularly of myosin, was identified to be a key process with significant effect on the batter structure properties. The maximum solubilization level was detected at 200 MPa/40 °C for all analyzed recipes, which was found to be treatment time dependent. Protein solubilization and aggregation level were found to have significant effect on the structure, functional and sensory properties after studying one and two steps pressure/temperature treatment.

The impact of pressurizing gradient- PG (PG= 40 MPa/s and PG= 2.5 MPa/s) was investigated and estimated to have a significant effect on the protein network and functional properties, respectively. These were improved at low PG (2.5 MPa/s) as a phenomenon of secondary network building parallel to the main matrix. This was observed by using scanning electron microscopy (SEM). Batter secondary-structure characteristics were found to be ionic-strength dependent, which was related to the amount of soluble proteins as well as their aggregation level in the aqueous phase. According to the SDS-PAGE analysis, the major role in the solubilization, aggregation and gelation processes taking part in the aqueous phase was due to the myosin S-1, S-2, N-terminal, C-terminals, the MLC and actin during the high pressure-temperature treatment. Especially important for the secondary network formation because of their excellent binding capacity were found to be the S-1 head domains: N- and C-terminals.

Protein units smaller than 6 kDa (under the SDS-PAGE detection limit) were seen to increase up to 200 MPa analyzed by HPLC. At 200 MPa and longer treatment time a shift of protein peaks in the higher molecular weight range, which was related to the process of protein aggregation, was detected. At 300 MPa this process was significantly intensified, defining the range of aggregation onset at 200 MPa and development above 300 MPa.

Further structure improvement, including firmness increase, was found after a two-step HP-T treatment with a first “solubilization” step at 100-300 MPa /1min and 5min/ 10°C, 40°C and second “structure formation” step at 600 MPa/ 240 s, compared to the one-step processing. This structural improvement was visualized by SEM as the frankfurters matrix was more swollen and homogenous and with better developed secondary network. A correlation between the treatment time effect on the matrix in the first pressure step and its dependency on the level of solubilized proteins, supposed to increase protein-protein interactions, was detected.

In order to reduce salt content, a comparison of conventional (2% NaCl) and reduced-sodium (1% NaCl) HP-T treated batters was investigated and similar functional and sensory

properties were found. Chemical analysis of NaCl content in the aqueous phases showed an NaCl increase for the batter samples as follows conventional (thermal)< high PG< low PG < two steps treated batters, with a maximum at 200 MPa for 60 s and 40°C for the first step of the two steps treatment.

Hypothetical mechanisms of water-protein interactions under high pressure/temperature conditions were elaborated. According to this model, important relationships of the combined salt/high pressure impact on the protein molecule to the protein solubilization improvement were found. The myosin head high pressure induced disruption was suggested to be crucial to the increase of S-1 domains as well as other smaller protein units compare to the thermal treated batters. Regions of solubilization, aggregation, and gelation on the basis of estimated results were defined and presented in a p-T landscape.

Improvement of structure and functional properties (WHC, and in this case decrease of sausage firmness) of mechanically deboned turkey frankfurters was realized after HP-temperature treatment.

According to the studied frankfurters'structurizing processes, HP-T processing has great potential for developing innovative meat products (salt-reduced sausages with improved functional properties) and optimizing technology by reducing process and thermal treatment times.

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List of Abbreviations

A	Helmholtz Energy (kJ kg^{-1})
ADP	Adenosine diphosphate
ANS	1-anilino-8-naphthalene sulphonate
ATP	Adenosine triphosphate
D	Denaturated state
Deoxy-Mb	Deoxymyoglobin
DIL	Deutsches Institut für Lebensmitteltechnik (German Institute of Food Technologies)
ΔE^2	Total color difference
FD	Fat droplets
FFCTFM	Fat-free-connective-tissue-free-meat
G	Gibbs free energy (kJ kg^{-1})
G'	Storage modulus
$\Delta H°$	Standart enthalpy
HMM	Heavy meromyosin
HP	High pressure
HPP	High pressure processing
HP-T	High pressure- temperature
I	Ion strength
IAPWS	International Association for the Properties of Water and Steam
IW	Immobilized water
K	Equilibrium constant
K_a	Acid dissociation constant
K_w	Ion product of water ((mol $kg^{-1})^2$)
L*	Lightness
LMM	Light meromyosin
LE	Linear elasticity
LMBG	German Food and Commodity Goods Law
MDM	Mechanically deboned meat
Met-Mb	Metmyoglobin
N	Naturated state
NIST	National Institute of Standards and Technology
NOMb	Nitric oxide myoglobin
Oxy-Mb	Oxymyoglobin
P	Pressure
P_D	Denaturated protein
PG	Pressure gradient
P_N	Native protein
Q	Thermal energy (J)
RI	Refractive index
RR	Resonance Raman
S	Entropy (kJ kg^{-1} K^{-1})
S-1, S-2	Subfragment-1, -2
SDS-PAGE	Sodium dodecylsulfate polyacrylamide gel electrophoresis
SEC-HPLC	Size exclusion chromatography-high-performance liquid chromatography
SEM	Scanning Electron Microscopy
T	Temperature (°C)

TN-T, TN-I, TN-C	Troponin–T, I, C
U	Internal energy (kJ kg^{-1})
UV	Ultraviolet
V	Volume (m^3)
W	Wight
WHC	Water holding capacity
a*	Redness
b*	yellowness
c_p	Specific heat (kJ kg^{-1} K^{-1})
f	frequency
k	Empirical parameter
n	Empirical parameter
q	Heat energy (kJ kg^{-1})
w	Volumetric work (kJ kg^{-1})
z_i	Number of elementary charges of the ion i
α	Significant level
α_p	Isobaric expansion coefficient (K^{-1})
β_T	Isothermal compressibility (MPa^{-1})
ε	Relative static permittivity
η	Dynamic viscosity (μPa s or MPa s)
λ	Thermal conductivity (mW m^{-1} K^{-1}or W m^{-1} K^{-1})
χ	Total number of protein molecules
ϕ	Numbers of molecule aggregated at a certain point of the gelation process
ψ	Numbers of molecule aggregated at a certain point of the gelation process

1 Introduction and Objective of Work

Almost 100 years after the pioneer work of Hite (Hite 1899), the first industrial application of high pressure processing (HPP) was realized. The scientific interest of HPP in the last two decades has increased and logically has led to more industrial applications in various fields of the food industry (Tonello 2010). The great increase in industrial applications since the year 2000 demonstrates the efficiency and the competitiveness of the HP- food processing (Figure 1.1). Parallel to the industrialization of HPP, intensive further equipment development (seals improvement) and energy costs optimization (volume increase up to 420 L, parallel working vessels- Tandem, NC-Hyperbaric) has been made. Presently, there are over 150 HPP equipments worldwide in use with a total food production of 250,413 tons, 90,315 tons of which are meat products (calculated for 2009, Tonello, C. 2010). The mild character of the HPP provides a great opportunity for developing novel food products of superior nutritional and sensory quality. The advantages of HPP of foods compared to the conventional thermal treatment, such as preservation at moderate temperature, retaining the fresh character of the foods, treatment of packed products, and immediate pressure effect inside the matrix without any delay, promises a lot of future application opportunities.

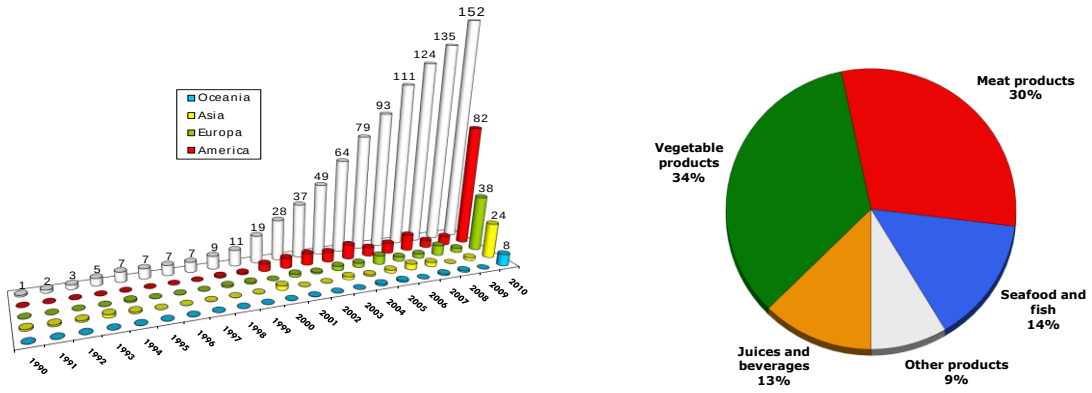


Figure 1.1: Number of industrial HPP equipments in the food industry and their field specification (Tonello 2010)

In general, industrial HPP meat applications are performed in order to extend shelf life at low and medium temperatures, where inactivation of relevant pathogen microorganisms at pressures above 400 MPa is possible (Buckow and Heinz 2008).

In addition to microorganisms inactivation, pressure affects the processes of meat denaturation, solubilization, aggregation and gelation (Yamamoto, Hayashi et al. 1993; Cheftel and Culioli 1997; Jiménez Colmenero 2002; Iwasaki, Noshiroya et al. 2006; Sikes, Tobin et al. 2009). Pressure induced gels provide generally smoother, more glossy, less firm, and more elastic gels with improved water holding capacity, compared to thermally induced gels (Cheftel and Culioli 1997; Jiménez Colmenero 2002).

HP-pretreatment of conventional thermal processing is also reported to improve functionality of sausage batters (Suzuki and Macfarlane, 1984, Sikes *et al.* 2009).

Suzuki and Macfarlane (1984) and Sikes et al. (2009) report the effect of processing pressure on batter structure (gelation) different from that of the thermal processing. This fact provides a great potential of modifying meat-processed products such as batter, affecting their functional properties by HP and creating innovative products. Today, new innovations can be a significant determining factor in one's ability to take advantage of the market.

Frankfurters originated more than 100 years ago and now enjoy worldwide popularity. They belong to the group of classical raw-cooked meat products. The stage after comminuting and mixing of the mass (raw process phase) is known as a batter.

Frankfurters sausage batter can be defined as a poly-dispersed system consisting of a liquid continuous phase (water and soluble proteins, ions), a dispersed liquid phase (fat droplets) and a dispersed solid phase (non-solved muscle fiber particles, connective tissue, spices). With respect to rheological properties, sausage batters show visco-elastic behavior that may result in known effects, such as the Weissenberg-effect and swelling. Conventionally, denaturation processes, e.g., transforming batter to sausage, are induced by heating to a core temperature of approx. 72°C. Alternatively, structure change processes can be caused by application of high hydrostatic pressures (HP) or combined high pressure- temperature (HP-T) treatment.

During the course of this work, essential mechanisms of HP and HP-T texture and functional properties modification of Frankfurter batters have been studied. Systematic studies to identify the mechanisms of solubilization, structure modification, proteins disruption, aggregation and gelation depending on the temperature, treatment time, pressurizing gradient, one step, two steps treatment and salt content have been performed. Detailed studies of meat batter protein solubilization under different process and recipe variations have been carried out. Determination of the maximum solubilzation level of particular meat proteins and protein subunits, as well as their participation in the aggregation and gelation processes, was of great importance for modifying sausage structure.

Possibilities of salt reduction after high pressure processing without any negative effects on the sausage batter structure and sensory properties have been tested. Hypothetic mechanisms of the high pressure effect on the meat batter proteins, and the mechanism of high pressure aggregation and gelation in the aqueous phase have been developed.

Industrial relevance and comparison to the conventional treatment of HP-T denaturated pork and MDM turkey Frankfurters have been investigated. The potential of successful industrial application of texture modified and NaCl reduced HP-temperature formed (denaturated) sausages have been verified.

2 Literature Review

2.1 Meat proteins

The muscle consists of a mass of contractile fibers that generally lie parallel to the long axis (Figure 2.1). These fibers are bound together in a network of connective tissue, which merges at each end to form tendons and adhesions connected directly or indirectly to bone. A muscle fiber normally has a diameter of 20-100 μm. The length can vary from a few millimeters to tens of centimeters. Each muscle fiber contains 1000- 2000 elongated, thread-like structures called myofibrils which are 1-2 μm in diameter and exhibit cross-striations or alternating light and dark zones (Regenstein and Regenstein 1984). These zones arise from two sets of interdigitating filaments, the thin and thick filaments, that overlap and slide past one another to cause shortening during muscle contraction (Cohen 1975).

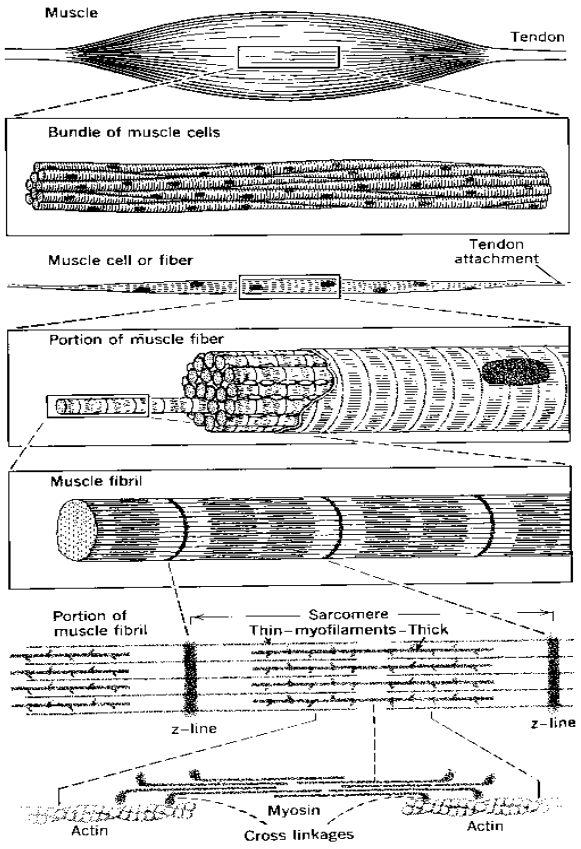


Figure 2.1: Muscle structure (Shimek and Kim 2012)

The sarcomere, the basic contractile unit of muscle, is that portion of the myofibril between two adjacent Z-disks. The Z-disk is the boundary for the sarcomere and is oriented perpendicular to the thin filaments and the long axis of the myofibril. The thin filaments of the

sarcomere, composing the I-band, are isotropic; i.e., they do not refract birefringent light. They have an 8 nm diameter and are composed of actin, tropomyosin and troponin in a 7:1:1 molar ratio in rabbit muscle (Potter 1974). The thick filaments called A-bands are anisotropic and refract birefringent light (McCormick 1994). They are about 1.5 μm long and 12-16 nm in diameter, containing 200-400 molecules of myosin which form cross bridges with actin and include the ATPase activity. The A-bands are bipolar and possess projections, heads that bind to actin along either end of the filament.

Meat proteins are divided into three groups based in their solubility characteristics: sarcoplasmic proteins, myofibrillar proteins and stromal proteins.

2.1.1 Sarcoplasmic proteins

Sarcoplasmic proteins are soluble at an ionic strength of 0.1 M or less at neutral pH. They constitute 30-35 % of total protein in skeletal muscle and contain at least 100-200 different proteins (Goll, Robson et al. 1977). They have a relatively small molecule size, appear globular or rod-shaped in structure, and have low viscosity (Asghar, Samejima et al. 1985). Myoglobin is the most important sarcoplasmic protein responsible for the meat color, which is often related with the product quality (Kijowski 2001). It depends not only on the quantity of myoglobin, but also on the chemical state of the myoglobin molecule (Howell and Lawrie 1984).

2.1.2 Myofibrillar proteins

Myofibrillar proteins are the largest fraction of muscle tissue proteins and constitute the myofibril. They are intermediate in solubility and have high water binding and emulsifying capacity, 95% and 70-90% of the total, respectively. Myofibrillar proteins are responsible for the textural properties of processed meat products (Yasui, Ishioroshi et al. 1980; Asghar, Samejima et al. 1985). The properties of Myofibrillar proteins are listed in (Table 2.1)

The myofibrillar proteins of skeletal muscle are composed of actomyosin (15-20 % actin and 50-55 % myosin), known also as contractile proteins, and of six regulatory proteins. The six are tropomyosin (5-8 %), troponin (5-8 %), α- actinin (2-3 %), β- actinin (0.5-1 %), component C (2-3 %) and M-line proteins (3-5 %). The regulatory proteins control actin-myosin interaction and regulate assembly of individual myofibrillar proteins into filaments.

Myofibrils consist of organized arrays of thick and thin myofilaments as shown in (Figure 2.1).

Table 2.1: Properties of myofibrillar proteins according to (Goll, Robson et al. 1977).

Protein	*% of Myofibril by wt*	*Mol wt (gm/mole)*	*Subunit polypeptide composition*
Myosin	50-58	475,000	200,000 D –two
			20,000 – one
			19,050- two
			16,500- one
Actin	15-20	41,785	41,785- one
Tropomyosin	4-6	70,000	35,000- one
			32,000- one
Troponin	4-6	72,000	30,503- one (TN-T)
			20,864- one (TN-I)
			17,846one (TN-C)
C-protein	2.5-3	140,000	140,000- one
α- actinin	2-3	206,000	103,000- two
β- actinin	$<$1	70,000	
M- protein	3-5	160,000	160,000- one
Paramyosin	2-30	220,000	110,000- two

2.1.2.1 Myosin

The major contractile protein is myosin. Myosin is a large fibrous molecule (~500 kDa), which resembles a thread (2 nm x160 nm) with two globular heads (19 nm long) attached at one end to what is generally referred to as the tail (Bailey 1982). It is composed of two large subunits- myosin heavy chains (MHC) and four small subunits- myosin light chains (MLC). The two heavy chains form the rod portion and a large part of the myosin head. The two light chains are located in each of the myosin heads (Bechtel 1986).

Substantial information about the structure and function of myosin has been gained from the study of fragments obtained after treating the myosin molecule with proteolitic enzymes such as papain, trypsin and chymotrypsin (Lowey, Slayter et al. 1969).

The myosin molecule consists of two main proteinase-susceptible regions. A region of the tail that lacks the α-helical conformation is susceptible to proteolysis by trypsin, which cleaves the molecule into a heavy meromyosin (HMM) fragment (350 kDa) containing both heads and a portion of the molecule and light meromyosin (LMM) fragment (150 kDa) consisting of the tail (Figure 2.2). HMM has ATPase activity and actin binding ability, while LMM has self-assembly properties. HMM and LMM differ in their amino acid composition. HMM has a

relatively higher content of cysteine, phenylalanine, tryosine and tryptophane than LMM (Kominz, Hough et al. 1954).

Under controlled conditions, the HMM fragment can be cleaved further into two separate heads known as subfragment-1 (S-1) and a short segment of the myosin tail known as subfragment-2 (S-2). S-1 and S-2 subfragments have a molecular weight of 115 kDa and 60 kDa respectively (Young, Himmelfarb et al. 1965). It is suggested that S-1 may play a critical role in the functionality of myosin in processed muscle foods due to its excellent binding capacity (Borejdo and Assulin 1980; Borejdo 2002).

Myosin contains a large amount of aspartic acid and glutamic acid residues as well basic residues histidine, lysine, and arginine (Harrington 1979). The isoelectric point of myosin is ~5.3. By processing conditions where the pH value is around 6, the myosin molecule will be negatively charged and have the ability to bind water (Harrington 1979). Salt will further enhance the water-binding ability of myosin by increasing the effective net negative charge and by breaking ionic bonds, causing molecular swelling and water uptake (Acton *et al.*, 1983). The functionality of myosin in processed meat products will be reviewed in the protein gelation chapter (2.3.3).

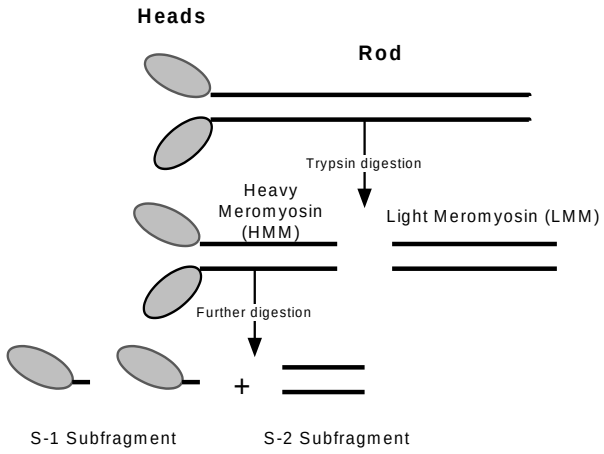


Figure 2.2: Generation of Light Meromyosin (LMM), Heavy Meromyosin (HMM), S-1 subfragment, S- 2 subfragment (Bechtel 1986).

The structure of myosin S-1 is illustrated in Figure 2.3. The head of S-1 consists of a seven-stranded beta-sheet and a C-terminal tail containing the regulatory light chain (magenta) and the essential light chain (yellow). The proteolytic fragments of S1 are color coded as follows: 25 K (N-terminal), *green;* 50 K, *red;* and 20 K (C-terminal) *blue* (Rayment, Rypniewski et al. 1993). The 50 K fragment spans two domains: the 50 K upper domain and the 50 K lower domain or actin-binding domain (grey). Part of the 50-kDa and 20-kDa fragments form the actin-binding site, whereas part of the 50-kDa and 25-kDa fragments of S1 form the ATP-binding site. The ATP-binding site is about 4 nm from the actin-binding site. The ATP-binding site has the sequence GLY-GLU-SER-GLY-ALA-GLY-LYS-THR, which is similar to the

sequences found in the active sites of other ATPases. The ATP-binding site was identified as a pocket. There is a cleft in the upper part of the head that extends from under the ATP-binding site to the actin interface. Both portions, above and below the cleft, are involved in the actin binding. When ATP binds to S-1, the pocket most likely closes and the cleft widens disturbing the S-1-actin binding, or in other words, the ATP dissociates S-1 from actin. When ATP is hydrolyzed by S-1 to ADP and P_i, actin recombines with S-1. The accompanying structural changes are the narrowing of the cleft and opening of the ATP-binding pocket. These subtle changes are called conformational changes that play a key role in the mechanism of muscle contraction.

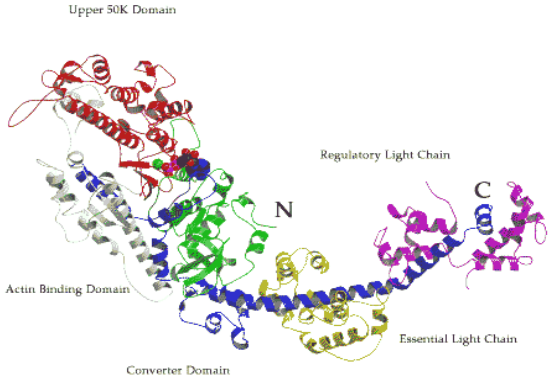


Figure 2.3: Ribbon representation of the structure of S-1 sub fragment (Geeves and Holmes 1999).

2.1.2.2 Actin

Actin is the major constituent of the thin myofilaments and accounts for 22% of the myofibrillar protein (Yates and Greaser 1983). Actin exists in globular and fibrous form. Globular actin, known also as G-actin, is composed of 376 amino acid residues with a molecular weight of 42 kDa (Kijowski 2001). Under physiological conditions, G-actin molecules polymerize into a 6 nm wide double-stranded, right handed helical structure (fibrous actin or F-actin) (Huxley, 1963; Steiner *et al.*, 1952). F-actin is produced from globular actin solutions upon increasing the ionic strength (0.1 M KCl), and the polymerization is believed to take place in at least four reversible steps (Skaara and Regenstein 1990). First - activation of actin monomers (Cooper, Buhle et al. 1983); second - nucleation (formation of oligomers); third - elongation; and fourth - annealing (Kondo and Ishiwata 1976). The F-actin forms the backbone of the thin filament and also provides binding sites for tropomyosin and troponin complex which regulates the activity of myosin ATPase. F-actin comes into contact with the myosin heads of the thick filaments under presence of Ca^+ which lead to ATP breakdown and results in a muscle contraction (Bechtel 1986). Actin alone does not have any binding property in a model system (Fukazawa,

Hashimoto et al. 1961; Samejima, Hashimoto et al. 1969). However, in the presence of myosin, actin exerts a “synergistic effect”, thereby considerably complementing the binding characteristics of myosin. This improvement was thought to be due to the formation of the actomyosin complex in the system. Adding of F-actin to myosin preparations induce strong gels when the myosin-actin weight ratio is 2.7 (Yasui, Ishioroshi et al. 1980).

2.1.3 Stromal proteins

Stromal proteins are also known as connective tissue proteins. To this group belong collagen, elastin and the cell membrane lipoproteins. They exhibit a fibrous structure. In the tissues, collagen is the most prevalent quantitatively.

Collagen

Collagen is found widely distributed in the body and comprises 20-25% of the total protein. It is the principal protein in bone, tendon and skin. Its molecule is built from three helically twisted polypeptide chains, which are stabilized by intermolecular and intramolecular bonds. It is a rigid protein 300 nm in length, 1.5 nm in diameter and approximately 300 kDa. The entire molecule consists of about 3000 amino acids.

Collagen fibers are not dissolved by dilute acid or alkali solutions or by concentrated solutions of certain neutral salts and nonelectrolytes unless they have been previously denaturated by heat or urea. Collagen is characterized by undergoing a sharply defined thermal shrinkage at a given temperature, which is characteristic of the species at a given age. Prolonged heat treatment above the thermal shrinkage temperature converts collagen to soluble gelatin. Collagen is also characterized by inter- and intramolecular cross-links, of which the amount increases with the animal’s age (Pearson and Gillett 1999).

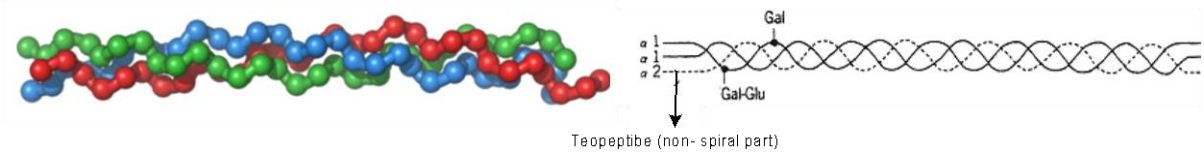


Figure 2.4: Ribbon model of collagen triple helix, collagen molecule.

Meat toughness is mostly related to collagen. With the age of the animal more covalent bonds inside and between collagen molecules are formed. This contributes significantly to the meat toughness (Asghar, Samejima et al. 1985). Stromal proteins possess no gelation ability, so that only coagulation above 80°C is observed (Ziegler and Acton 1984). Intermolecular cross-linking occurs between collagen molecules in such higher-ordered structures, and these links serve to increase the strength of tissue and to ensure temperature stability (Pearson and Gillett 1999).

2.2 Raw- cooked sausages–Frankfurters

Frankfurters belong to the group of “all meat” classical raw-cooked meat products, made from muscle meat, animal fat and water/ice as main components, as well as small amounts of non-meat ingredients. These are products of relatively high quality and good nutritional value. They are known as raw-cooked because of the two phases of their preparation. In the first phase the product components are processed raw by comminuting and mixing (Heinz and Haeutzinger 2007). Frankfurters batter can be defined as a poly-dispersed system consisting of a liquid continuous phase (water and soluble proteins, ions), a dispersed liquid phase (fat droplets) and a dispersed solid phase (non dissolved muscle fiber particles, connective tissue, spices). In the second phase (cooking) the frankfurters batter (visco-elastic poly-dispersed system), due to protein denaturation after heat treatment (76°C/30 min), converts into a solid state so the sausages become shape stable (Figure 2.5). The cooking process forms not only a firm-elastic texture typical for ready-to-eat raw-cooked products, but also provides a certain degree of bacterial stability (Bindrich, Tintchev et al. 2009).

2.2.1 Frankfurters batter product flow

As previously written, the product flow of the raw-cooked sausages consists of two main phases. The raw phase of processing is based on the steps of grinding, chilling, chopping and stuffing. They are described in detail in chapter 3.1, where the standardization of model batter used in this work is performed. The second phase contains the steps of batter thermal treatment (smoke and cook process steps), where the formation of the final structure takes part. In Figure 2.5, in addition to the product flow, native to gel transformation of the batter structure is shown (Whiting 1988). Alternatively, the thermal treatment steps could be removed by a single combined HPP/ temperature step. Therefore the denaturation of the protein matrix resulting in an increase in hardness could be in this case due to the combined pressure temperature treatment (as shown in red in Figure 2.5).

It is well known that HPP causes protein denaturation, the level of which increases with increasing pressure (Cheftel and Culioli 1997; Buckow *et al.* 2007).

One of the main goals of this work was to study the mechanism of the Frankfurters sausages formation under pressure and temperature, and to determine the key process parameters affecting the optimal functional properties. The advantages of the high HPP or HP-temperature processing (compared to the conventional thermal treatment), with possible industrially beneficial ramifications, can be defined as follows: 1- pressure affects the whole product simultaneously, without a delay effect; 2- adiabatic heating caused by pressurization

(about 18 °C for 600 MPa with a starting temperature, T_{start}, of 5°C, differing according to the initial temperature) can have an additional effect for texturizing the product (see Figure 2.14); 3- process of pressure denaturation is significantly faster than thermal denaturation (Buckow *et al.* 2007; Tintchev *et al.* 2007); 4- possible structure improvement (WHC, firmness), caused indirectly by a pressure-induced increase of soluble proteins (Sikes *et al.* 2009; Iwasaki *et al.*2006); 5- inactivation of pathogen microorganisms within 5 min (Buckow *et al.* 2007); 6- possible reduction of thermal energy (costs) and 7- possible reduction of NaCl content (Sikes *et al.* 2009; Iwasaki *et al.*2006).

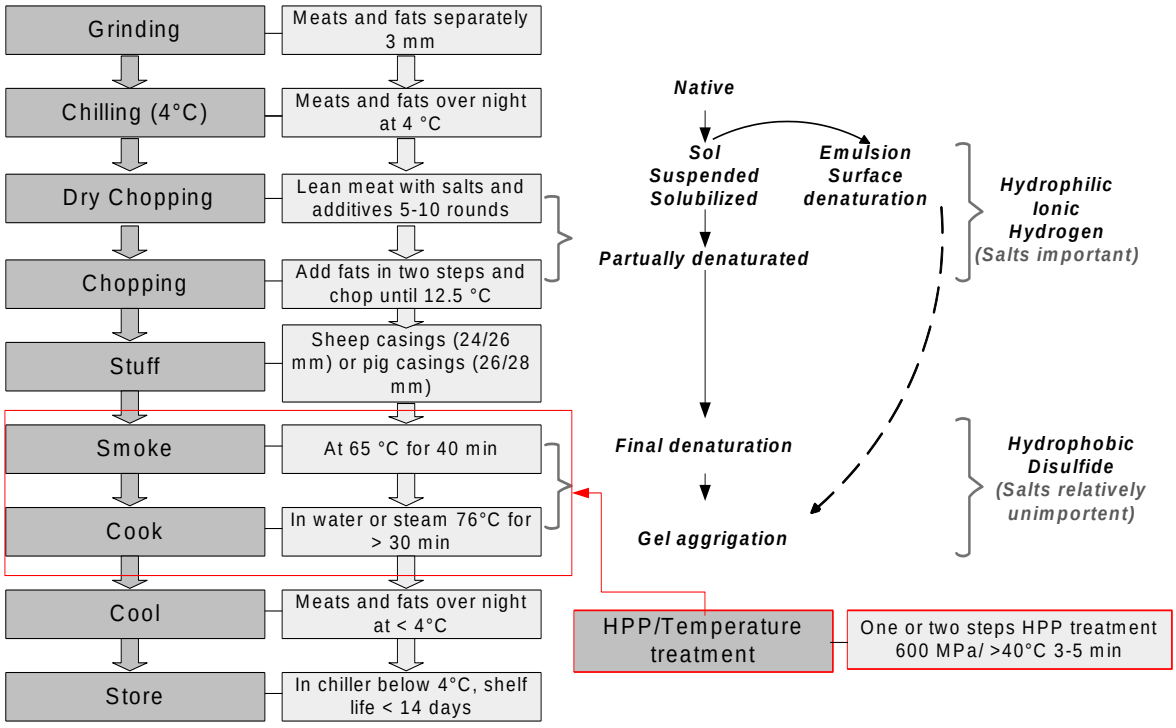


Figure 2.5: Frankfurters product flow combined with state and protein interactions changes during the production process. Transformation from native to gel aggregated state is presented according to Whitining, R.C, 1988.

2.2.2 Mechanism of raw-cooked (Frankfurters) structure formation

The process of transformation from native (raw material) to gel (sausage) stage can be performed as four main steps mechanism (Figure 2.6) (Heinz, G. and Hautzinger 2007). Each step is important for the final quality of the product, affecting the functional properties of the sausage. Gel formation with optimal texture is dependent on many factors such as protein concentration, ionic strength, pH, heating temperature and rate, as well as postmortem history of the muscle and its type (Asghar, Samejima et al. 1985; Gordon and Barbut 1992; Pietrasik and Li-Chan 2002).The first phase of the structure formation is the condition after dry chopping, where salts and lean meat are already mixed. Through further

chopping after the addition of water, the tiny muscle fibers are cut into small fragments, providing greater protein surface for contacting with water and salts. The main goal of this phase is by physical (chopping) and chemical (salting) treatment to maximize the amount of meat proteins to be extracted and dissolved. The solubilization of the meat proteins plays a major role for the WHC in the frankfurters sausages. Solubilization of myosin, especially, is of great importance. Most of the muscle fiber fragments swell through water incorporation and become gelatinous or solubilized; others stay unchanged as water may remain loosely bound between the fragments. In the B phase, the base network is mainly built from myosin proteins and fractions (Figure 2.6- B); after the myosin network is built by continued chopping, fat is added (the stage between the second and the third phase). In the whole step of chopping (Figure 2.6 A-C), the insalting process is of great importance, as hydrophilic, ionic, and hydrogen interactions take place. The impact of salts on the water binding will be presented in detail in the next chapter.

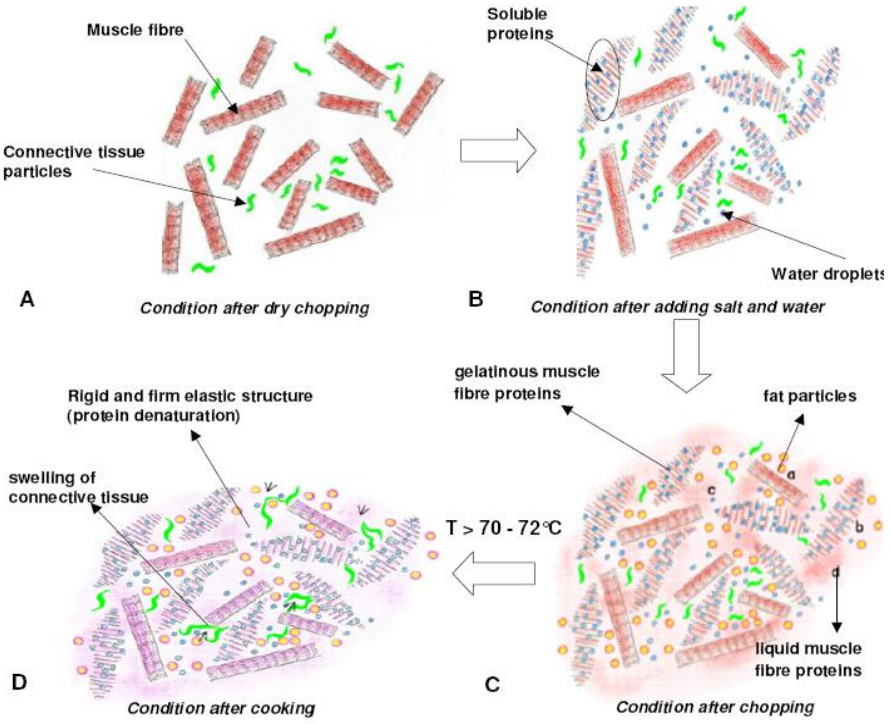


Figure 2.6: Stages of frankfurters network building modified according to Heinz and Hautzinger (2007), A- condition after dry chopping; B- chopping after water addition (building of matrix based mainly on myosin from the lean meat); C- condition after chopping with fat addition (batter with immobilized fat and water droplets); D- condition after heat coagulation/denaturation.
Fat particles-yellow coated by protein -bright red.
connective tissue- green.

The mass in the third phase (Figure 2.6- C) is defined as an aqueous protein phase or “matrix”, where small fat globules are dispersed. The fat globules are immobilized in the matrix and their coalescence prevented by two mechanisms: first through physical forces-

“coating”, and second through entrapping in the viscous protein (Heinz, G. and Hautzinger 2007). The emulsion, after the chopping and before the thermal treatment steps, is commonly known as sausage batter. In the batter, myosin is neither in true solution nor denaturated (Whiting, R.C 1988). The last phase presents the transformation of raw sausage batter into a formed sausage through heat (core temperature not lower than 70-72°C), where the sausage structure becomes shape stable (Figure 2.6- D) (Bindrich 2009). The protein network structure becomes firm and elastic through protein denaturation and the connective tissue particles swell and become softer.

As the temperature increases during heat processing, hydrogen bonds weaken, protein-water interactions decrease, and hydrophobic protein-protein interactions increase (Figure 2.5) (Whiting, R.C 1988).

2.3 Meat batter functional properties

The aspects of protein functionality and interactions are summarized in Figure 2.7. The factors influencing the functional properties can be divided into intrinsic and environmental. The intrinsic factors are composition of proteins, conformation of proteins, mono- or multi-components, homogeneity and heterogeneity. Environmental factors are water, pH, temperature, oxidizing/ reducing agents and lipids, flavors, and sugars. Other important factors affecting the functional properties of proteins are the process treatment factors: such as heating, drying, physical modification, chemical modification, and pressurization (Kinsella and Srinivasan 1981).

The interactions and the aspects of protein functionality are presented in Figure 2.7. Many of the protein properties are reflected in interactions with water as demonstrated by sorption, viscosity, gelation, solubility, emulsifying, surfactant and rheological properties, including textural and sensory.

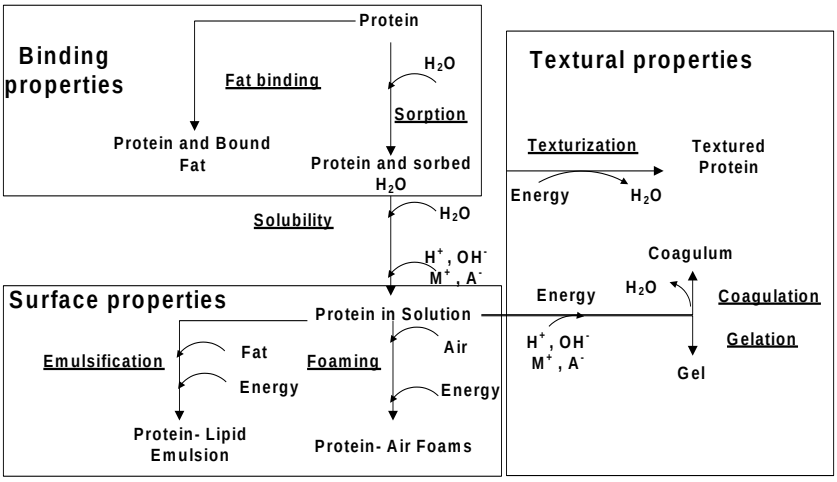


Figure 2.7: Aspects of protein functionality and its interrelationships (Phillips and Beuchat 1981).

Gelation of myofibrillar proteins has a major role of manufacturing processed meats such as row-cooked sausages (i.e. Frankfurters or Wiener). It is mostly induced by input of energy into the matrix, resulting in its formation.

Absorption of water and swelling change the hydrodynamic properties of food systems affecting viscosity and flow characteristics. Viscosity is useful in evaluating the thickening potentials in fluids and batter-type foods. It is influenced by solubility and swelling of proteins. Protein solubility is influential in imparting a “body” to aqueous solutions and in the formation and stability of emulsions (Figure 2.8).

The functional properties of Frankfurters batter are related to the level of protein solubilization as well as water and fat immobilization in the batter matrix as shown in Figure 2.8. The level of protein solubilization depends on firmness, elasticity, and viscoelasticity, discussed in more detail in chapter 4.1. These are also dependent on the level of immobilized water as well as the type of bound water such as firm bound, weak bound and free water. The capability of the batter matrix to bind water is related to the functional property of juiciness, which is of significant importance for the quality of the product and the consumer’s acceptance.

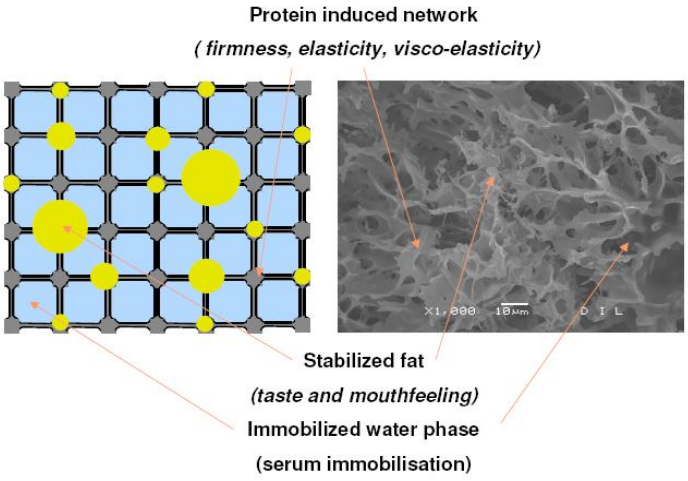


Figure 2.8: Elements of frankfurters structure and their effect on the batter functional properties (Bindrich 2008).

2.3.1 Matrix-Water-Interactions

Water holding capacity (WHC) of meat and meat products is the ability to absorb and retain water during mechanical treatment (chopping, coarse grinding, comminuting, stuffing), thermal treatment, subsequent transportation, and storage (Xiong 1997). WHC influences the

quality of meat and meat products as juiciness, tenderness, and taste. The juiciness is related to the impression of moisture running out of the meat that is reached after pressure applied by the teeth, determined by sensory testing (Zayas 1997).

Physicochemically, the water in meat and meat products is present either in bound or free states. The bound water is tightly associated with proteins through charged groups and dipolar sites on the protein surface. Its amount is primarily influenced by the amino acid composition of proteins. The free water is held by capillary and surface tension forces and is highly dependent upon the protein structure. There are two major types of forces that contribute to WHC in meat: polarity, including surface charges, and capillary effects

The interactions between water molecules and hydrophilic groups from the protein side chains occur via hydrogen bonds. The hydrophilic groups responsible for the fast binding of water are of two types. One type includes the polar groups of the side chains of protein, such as carboxyl-, amino-, hydroxyl-, and sulphydryl groups. The other type is undissociated carbonyl-, and imido-groups of the peptide bonds, where binding of water is due to the dipolar character of water (Hamm 1960).

WHC of meat proteins (Frankfurters batter proteins) are affected by protein concentration, pH, temperature, presence of hydrophilic polysaccharides, lipids and salts, rate and duration of heat treatment as well as storage conditions. The parameters affecting processing of Frankfurters will be briefly presented in the following chapters.

2.3.1.1 Influence of pH on WHC

The value of pH in protein solution affects the charged groups of the protein molecule. Minimum water retention can be observed in the isoelectric region (pH=5), where the net protein charge is zero and the protein-protein interactions are maximal (Grau, Hamm et al. 1953 ; Niinivaara and Ryyniinen 1953; Niinivaara and Pohja 1954). The normal pH of meat is in the basic range and small changes in meat pH may cause significant changes in WHC. Protein molecules behave as zwitterions carrying a net positive or negative charge depending on their isoelectric point and on the pH of the environment (Messens, Van Camp et al. 1997). According to the “zwitterions” theory, the swelling effect of acids and bases on protein gels is due to a cleavage of the electrostatic cross linkages between the peptide chains of the protein:

Proton donator (acid)

$R\text{-}COO^- \qquad {}^+NH_3\text{—}R' + H^+A \rightarrow R\text{—}COOH \qquad {}^+NH_3\text{—}R' + A$

Proton acceptor (Rayment, Rypniewski et al. 1993).

$R\text{-}COO^- \qquad {}^+NH_3\text{—}R' + B \rightarrow R\text{—}COO^- \qquad {}^+NH_2\text{—}R' + H^+B$

According to the reactions above, “counter charges” are eliminated by addition of base or acid and the protein net charge is increased. The effect of acid or base on the swelling can be described as a repulsion between same charged groups so that a space between the peptide chains results, where more water can penetrate these species (Hamm 1960).

2.3.1.2 Influence of salts (ionic strength) on WHC

Salts increase ionic strength in the batter aqueous phase so that the physical and chemical properties are affected. These cause structural changes of myofibrils such as: swelling of myofibrils caused by charge repulsion between myofilaments; depolymerization of myofilaments; and dissociation of actin from myosin or actomyosin from the myofibril structure, leading to extraction of myosin, actin and other meat proteins. These effects are maximum at an ionic strength of about 1.0 M NaCl (Xiong 1997). Further increase of the ionic strength resulted in a decrease of bound water and was related to the ,,salting out” effect involving strong binding of water by the salt and consequent dehydration of the proteins.

The effect of NaCl on water retention is related to the attraction of Na^+ and Cl^- ions to positively and negatively charged groups (Schellmnn 1953). The mechanism of diffusion and water immobilization is shown in Figure 2.9. Salt ions dissolved in water are attracted to the oppositely charged ions from the charged side chains ($–COO^-$ or $– NH_3^+$) and become immobilized together with their surrounding water layers. By moving the ions and water in between the protein chains of the myofibers, the attractive forces of the ions in the protein side chains become weaker and the myofibers swell due to molecular movement (Honikel 2010). The level of WHC after addition of NaCl depends on the pH. Protein ability to retain water is higher at pH above the isoelectric point compare to the acid range. This phenomenon is discussed in detailed by Hamm, R. 1960 and is related to the higher protein net charge in the basic range. This results in repulsive forces between negatively charged groups, which cause opening of the protein molecule, as water-protein interactions are more intensive. In the acidic range, the binding of anions reduces the electronic repulsion between positively charged groups. The hydrating effect of the anion depends on how tightly it is bound (Zayas 1997).

Alkaline phosphates positively influence the WHC through rising of the pH-value and increasing ionic strength, causing a molecule repulsion effect as shown in Figure 2.9. This solubilization improvement is related to the phosphates functioning to dissociate the actomyosin complex. Polyphosphates and NaCl influence the binding strength of myosin

heads to actin, which leads to dissociation of actomyosin. Alkaline phosphates increase pH about 0.3 units (Zayas 1997).

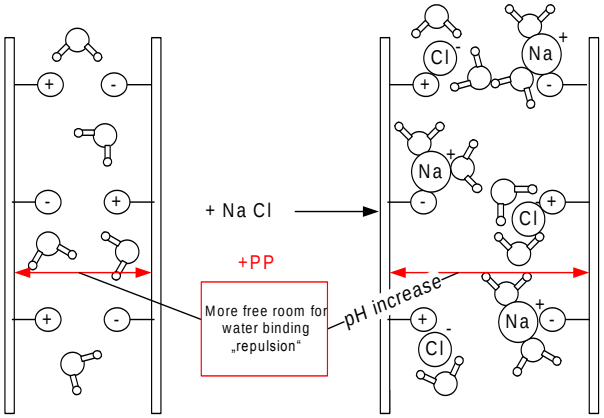


Figure 2.9: Mechanism of ion binding to the charged groups protein groups (Ternes 1995); Effect of Polyphosphate and pH (Honikel 1986).

For the production of raw-cooked sausages, combined effects of NaCl and polyphosphates on the meat protein solubilization process are usually used. For the conventional Frankfurters recipe these salt contents are 2 % NaCl and 0.3 % polyphosphate (Heinz, G. and Hautzinger 2007).

2.3.2 Protein Solubility

Protein solubility is defined as the amount of protein that goes into solution or colloidal dispersion under specified conditions and is not sedimented by moderate centrifugal forces. Thus solubility at saturation represents an equilibrium between the solid and soluble phases (Skaara and Regenstein 1990).

Solubility is affected by pH, temperature and ionic strength. Minimum protein solubility is usually observed in the protein isoelectric region, but it may be altered by the effect of salt. This can alter the electrostatic, hydration and water structuring effects, resulting in an enhanced solubility (salting-in effect) or insolubilization (salting-out effect) (Hamm 1960).

Protein solubility is most important for the manufacture of processed meat products including comminuted, restructured and formed meats. Many of the functional properties and the quality of these products are dependent to the protein solubility process (Kinsella 1976).

Protein solubility can be considered as a prerequisite step for the processes of gelation, emulsification, binding and texturization. It is influenced by the level of comminuting, structure of myofibrils, pH and the ionic strength. On the basis of solubility in aqueous solvents the muscle proteins are divided into three major classes: sarcoplasmatic (most

soluble), myofibrillar (soluble in dilute salt solutions), and stroma proteins (least soluble) (Goll, Robson et al. 1977).

Myosin, as the most important protein with regard to the functional properties, is soluble at high ionic strength (Γ>0.3 M) and is insoluble at low ionic strength. Maximum solubilization of myosin is reached at an ionic strength of 1.0 M (Offer and Trinick 1983).

Protein solubility is affected by a sensitive balance between repulsive and attractive intermolecular forces, and proteins are soluble when electrostatic repulsion between proteins is greater than hydrophobic interactions (Zayas 1997). Ionic strength above 0.5 M (approximately 2% NaCl) is widely used in processed meats to provide the necessary solubilization to produce products with desirable functional properties (Heinz, G. and Hautzinger 2007).

2.3.3 Gelation

Gel is a form of matter between a solid and a liquid, consisting of strands or chains cross-linked to create a continuous network immersed in a liquid medium. Gelation of muscle proteins is responsible for the physical and chemical stabilization of fat and water in comminuted meat products, which contributes to desirable binding characteristics, texture and appearance of the products (Acton, Ziegler et al. 1983; Asghar, Samejima et al. 1985; Smith 1988; Gordon and Barbut 1992).

Structure stability of processed meats is mostly caused by protein denaturation connected with changes of the native protein structure. Denaturing of proteins is defined as a rearrangement of the polypeptide chains from a native to a more disordered arrangement (Heinz and Haeutzinger 2007). Protein properties can be explained by a shift in the equilibrium between these states. Protein denaturation can be represented as a reversible transition between native and denaturated states N $\leftrightarrow$ D and the equilibrium constant K can be defined from equation (2.1):

$$K = \frac{Denatured\ \ state}{Native\, state} \qquad (2.1)$$

The equilibrium constant is dependent on external variables such as temperature, pressure and solvent composition. This can be physically interpreted as changes of standard enthalpy (ΔH°) and standard volume (ΔV). Detailed presentation of the denaturation mechanisms is shown in chapter 2.4.4.

Gels formed from the salt- extracted myofibrillar proteins at the junction of meat particles are responsible for texture improvement (emulsion stability and tenderness) of cooked sausage products (Fukazawa, Hashimoto et al. 1961; Macfarlane, Schmidt et al. 1977).

The relationship between muscle protein structures and physical properties of the gels are difficult to be established because of the fact that muscles are very complex polymers and can undergo major structural changes during meat processing even by small variations of the processing parameters.

Despite the difficulty in precisely predicting gel properties from the protein native structure, many of the gel properties can be related to the structure of “intermediates,” i.e., denaturated proteins and their polymers or aggregates. The multilevel process of protein gelation involves the following steps:

$$\chi P_N \xrightarrow[(denaturation)]{heating} \chi P_D \xrightarrow[(aggregation)]{heating} (P_D)_{\phi,\psi} \xrightarrow[(cross-linking)]{heating/cooling} (P_D)_{\phi} - (P_D)_{\psi} - \cdots \quad (2.2)$$

where χ is the total number of protein molecules, ϕ and ψ ($\phi + \psi + \ldots = \chi$) are the numbers of molecules aggregated at a certain point of the gelation process, P_N is the native protein, and P_D is the denaturated protein. The final gel state corresponds to aggregates of partly and fully denaturated proteins (Xiong 1997).

The meat protein denaturation/gelation commonly results after heating treatment as shown in equation (2.2).

Because of the globular structure of the sarcoplasmatic protein they have poor gelling ability.

In their early study, (Hamm and Deatherage 1960) defined the major changes of ground beef which take part during heat treatment between 20-65 °C. They reported an unfolding of protein chains combined with formation of new salt and hydrogen bonds in the range 30-40°C. At 40°C, a strong denaturation process starts, continuing up to 50°C, resulting in the formation of new stable cross linkages. A decrease in the amount of negatively charged groups with increasing temperature was also observed. Continued formation of new cross linkages occurred between 50- 55°C, and at about 65 °C the denaturation process was almost but not complete finished (Hamm and Deatherage 1960).

For denaturation (gelation process), the meat proteins myosin and actin are of significant importance. Their denaturation can affect and modify the gelling properties of meat and processed meat structure.

Myosin can form two completely different gel structures, depending on ionic strength. Fine stranded gel structures are formed at low ionic strength (0.25 M KCl), and coarsely aggregated gel structures are formed at high ionic strength (0.6 M KCl) (Hermansson, Harbitz et al. 1986). They found that the fine stranded structure had a higher rigidity than the

coarsely aggregated structure. Stranded myosin gels were formed from turbid solutions and the aggregated gels from clear solutions.

The mechanism of myosin thermal gelation consists of the entangling of tails after helix-coil transitions (Samejima, Ishioroshi et al. 1981). Initially, intermolecular coagulation of denaturated myosin heads occurs, causing myosin aggregation in daisy-wheel shaped oligomers (Yamamoto 1990; Sharp and Offer 1992).

Denaturation can also be induced during high pressure treatment, since the protein structure is affected by rearrangement upon compression (discussed further in chapter 2.4.4) (Cheftel and Culioli 1997; Jiménez Colmenero 2002). Estimation of the differences between thermal, pressure, and combined pressure-temperature denaturation/ gelation of the Frankfurters meat proteins, as well as their functional properties, is the main goal of the present thesis. Gelation of meat proteins in batter after HP- and HP-T treatment is reviewed in chapter 2.4.5.5.

2.3.4 Emulsification

Comminuted meats belong to the group of macro emulsions (0.01-0.1 μm) resembling oil-in-water emulsion in certain aspects. A sausage emulsion cannot be considered as a typical oil-in-water emulsion like mayonnaise. This emulsion is more correctly called a meat batter, which more appropriately describes its nature as a multiphase system (Xiong 1997).

The stabilization of meat batters is based on two main mechanisms. The first mechanism is the physical entrapment of fat globules in the protein matrix formed largely via protein-protein interactions. In the second mechanism, fat globules are stabilized by an interfacial film (membrane) that surrounds them. Scanning electron microscopy allows for the visualization of the coating mechanism on the fat globules surface (Figure 2.10- C). Proteins forming the coating membrane are connected via cross-linkages with the protein matrix in the continuous phase, resulting in a stabilized three dimensional microstructure of meat batter (Theno and Schmidt 1978). The emulsification process is significantly affected by the extraction or solubilization of salt soluble proteins during grinding and chopping (Pearson and Gillett 1999).

The relative emulsifying capacity of different muscle proteins in 0.3 M and 0.6 M NaCl solutions is found to be as follows: myosin>actomyosin>sarcoplasmatic proteins>actin (Hegarty, Bratzlar et al. 1963; Galluzzo and Regenstein 1978).

The unbalanced distribution of amino acids in different segments of myosin, i.e., a prevalence of the hydrophobic residues in the head region or HMM S-1 sub fragment and a predominance of polar groups in the tail or LMM portion, makes myosin an ideal emulsifier.

Furthermore, myosin has a high length-to-diameter ratio (roughly 40:1), a structure that is conducive to strong protein-protein interaction and molecular flexibility at the interface (Xiong 1997).

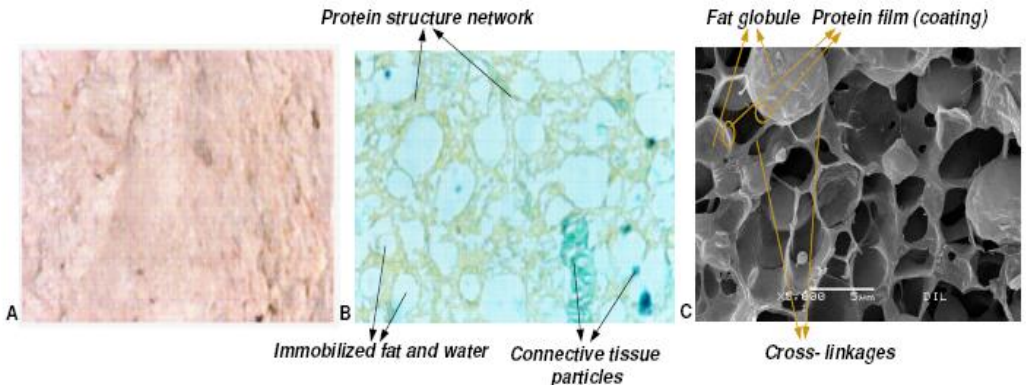


Figure 2.10: A- Frankfurter's batter; B- Microscopic image; C- Scanning electron microscopic (SEM) image

The emulsion system is commonly stabilized by temperature, inducing changes in the myosin conformation due to denaturation, which may involve formation of disulfide bonds.
In batter formulae consisting of a high content of connective tissues, a collagen membrane, identical to the myosin coating, covers the fat particles. However, by heat processing, the collagen shrinks, converts to gelatin and drains from the fat surface, resulting in an uncoated fat particle and a droplet of gelatin gelatine solution. Therefore it could lead to poor quality products with a fat cap at the top of the sausage and a jelly pocket at the bottom (Pearson and Gillett 1999).

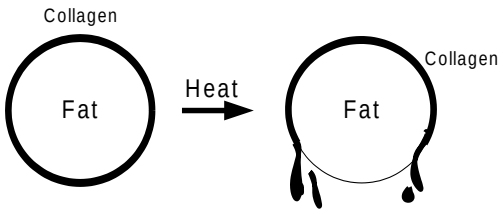


Figure 2.11: Schematic representation of fat globule coated by a collagen film converting to gelatin after heat treatment (Pearson and Gillett 1999).

Taste and mouth feeling depends on the level and homogeneity of stabilized fat. Water binding, fat binding, and emulsification of raw batter are closely related to the textural properties.

2.4 High hydrostatic pressure

The first application of high hydrostatic pressure for food preservation was done by Hite in 1899. He reported around 4 days of extended shelf life for milk after high pressure treatment at 600 MPa for one hour holding time. Bridgman reported in 1914 about the influences of pressure on egg albumin. Years later the interest of high pressure treatment on foods increased significantly leading to research intensification in the last two decades. As a logical consequence, the first industrial application was implemented. The potential of high pressure as a mild preservation process for foods was proved and a significant increase in industrial equipment was seen (over 150) (Tonello, C 2010). After successful realization of the first HPP products on the market, a great scientific interest in various fields of the food industry was awakened. Today, there is an increase in the number of institutions worldwide investing in high pressure research on foods, determining not only microorganism inactivation but also the mechanisms of food structure modifications. Generally, HPP is used industrially for extension of shelf life by microbial pathogens inactivation. The mechanism of inactivation is based on structural changes at the cell membranes and inactivation of enzymes, which control the metabolism (Knorr and Heinz 2001). The present technical limit of industrial high pressure equipment allows pressures up to 800-900 MPa. A significant inactivation (-5 $\log_{10}$) of vegetative microorganisms, yeasts and viruses even at low temperatures occurs at pressures above 400 MPa (Buckow and Heinz 2008). The combined HPP-effect of inactivation and structure modification could offer future challenges and interesting possibilities for developing superior food products. Therefore a detailed knowledge about base HPP and HP-T -structure modifying processes is needed.

2.4.1 Thermodynamics of high hydrostatic pressure

Hydrostatic pressure can be generated by the addition of free energy, e.g. heating at constant volume or by mechanical volume reduction. It can be expressed as the change of internal energy- U at constant entropy- S. For a closed hydrostatic system the change of the internal energy U can be described by the first law of thermodynamics (equation (2.3):

$$dU = dw + dq \tag{2.3}$$

where dw is the amount of volumetric work and dq the amount of heat energy. The heat term is related to the temperature and entropy such that

$$dq = TdS \tag{2.4}$$

The specific work of compression can be expressed as

$$W_{compr.} = -\int Vdp \tag{2.5}$$

so that equation (2.3) can be written as

$$dU = -\ pdV + TdS \tag{2.6}$$

where the internal energy is a function of Volume (V) and entropy (S) changes.

Pressure-temperature relationships of the system can be expressed, using as the following equations

$$dH = Vdp + TdS \tag{2.7}$$

$$dA = -SdT - pdV \tag{2.8}$$

$$dG = Vdp - SdT \tag{2.9}$$

Where H (S, V) is enthalpy, and A (T, V) and G (T, p) are the Helmholtz and Gibbs free energy functions.

According to the thermodynamic theory, the characteristic of proteins can be presented in a p-T plane as an elliptic phase diagram. Mathematically this kind of shape originates from the fact that second order terms give a significant contribution to the ΔG, which is the difference in free energy between the denaturated and the native state according to equation (2.17) (Heremans and Smeller 1998).

2.4.2 Effect of pressure on water

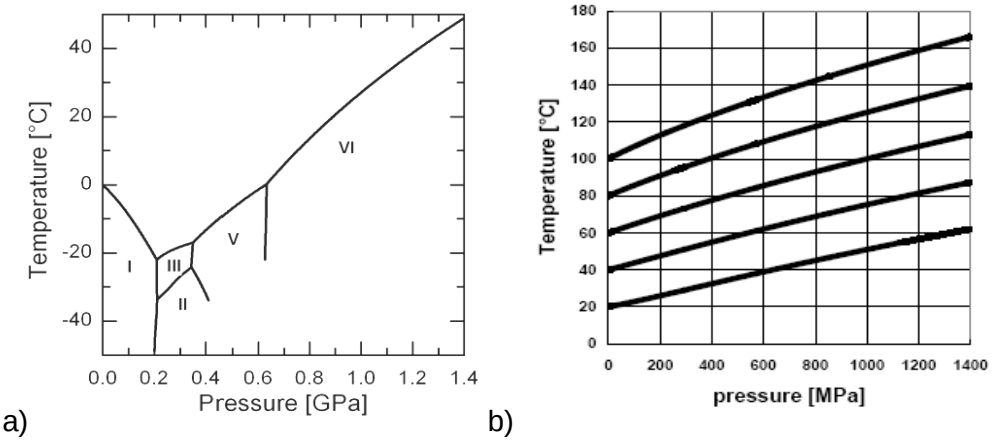
2.4.2.1 Physical properties of water under pressure

The effects of pressure on water as a main component of the food matrix as well as a common pressure transmitting medium in the HPP-food industry are of great importance. For optimizing the HPP and operating the controlling treatment conditions, the effects of HPP on water such as water adiabatic heating and water dissociation must be known. Significant for this research was the liquid water state under different high pressure-temperature combinations. Bridgman (1912) found that water under different high pressure-temperature conditions forms ice with diverse crystalline structures and densities respectively (Figure 2.12-a). At present, 12 crystalline structures and two amorphous states are known.

Thermodynamic properties of water are listed in the “International Association for the Properties of Water and Steam” (IAPWS) as well as in the database from the “National Institute of Standards and Technology” (NIST) Based on these data, Mathys and Knorr (2008) extrapolated up to 1400 MPa and presented in a p-T landscape the relationships for

some water parameters such as specific volumetric work, density, internal energy U, enthalpy H, Helmholtz free energy, Gibbs free energy G, entropy S, isobaric heat capacity c_p, thermal expansion coefficient α_p, isothermal compressibility β_T, thermal conductivity λ, and dynamic viscosity η. Increasing pressure results in a volume contraction of 10 %, 17% and 23% at 400 MPa, 800 MPa and 1400 MPa, respectively (Mathys, Kallmeyer et al. 2008).

In an adiabatic system, water compression results in a temperature increase. Water is commonly used as a pressure-transmitting medium in the HPP-food industry and its temperature increase directly affects the process conditions. The rate of temperature increase with pressure increase depends on the initial temperature. At low starting temperatures the increase in temperature due to compression is significantly lower (approx. 2 K / 100 MPa) than at higher starting temperatures (up to 5 K / 100 MPa at T>80°C) (Buckow 2006).



a) b)

Figure 2.12: a) Phase diagram of water according to (Bridgman 1912); b) Adiabatic heating of water caused by compression (Ardia 2004).

2.4.2.2 Dissociation reactions of water under high pressure

Pressure increases the ionic product of water by 10 to 100-fold at 1000 MPa depending on the temperature. Water dissociation under pressure is driven by a water “electrostriction” phenomenon: water molecules rearrange in a more compact manner around electric charges (Cheftel and Culioli 1997). The dissociation reaction

$$2H_2O \Leftrightarrow H_3O^+ + OH^- \qquad (2.10)$$

has a ΔV of –22 ml per mole of dissociated water, where two water molecule react to produce a hydronium ion (H_3O^+) and a hydroxide ion (OH^-) (Heremans, Van Camp et al. 1997).

For reactions in water or diluted aqueous solutions the molarity of water is practically constant and is omitted from the acidity constant expression by convention. The resulting equilibrium constant is called the dissociation constant or ion product of water (K_w). This can be expressed by the follow equation

$$K_w = K_a[H_2O] = K_{eq}[H_2O] = [H_3O^+][OH^-] \quad (2.11)$$

where

$[H_3O^+]$ is the molarity of hydrogen (hydronium) ion

$[OH^-]$ is the molarity of hydroxide ion

K_{eq} is the equilibrium constant, defined by

$$K_{eq} = \frac{[H_3O^+][OH^-]}{[H_2O]^2} \quad (2.12)$$

K_a is the acidity constant, defined by

$$K_a = K_{eq} \times [H_2O] = \frac{[H_3O^+][OH^-]}{H_2O} \quad (2.13)$$

At standard ambient pressure and temperature, 0.1 MPa and 25 °C, $K_w=1.0x10^{-14}$ and pure water dissociates to molarities of hydroxide and hydronium ion $[H_3O^+] = [OH^-]$.

Because the dissociation constant K_w varies with temperature, it is often represented by the additive inverse of its common logarithm, represented by the symbol pK_w and expressed with the follow equation

$$pK_w = -\log_{10} K_w = -\log_{10}([H_3O^+].[OH^-]) \quad (2.14)$$

At Standard Ambient Temperature and Pressure (SATP) $pK_w=-\log_{10}(1.0x10^{-14})=14.0$. The pK_w value depends on the temperature, so that as temperature increases pK_w decreases. Knorr and Mathys (2008) presented the dissociation equilibrium shift as a negative logarithm of the ion product dependent on pressure and temperature (Figure 2.13). During the dissociation equilibrium shift the pH value changes (equation (2.15), while the concentration remains constant.

The hydrogen ion concentration is mostly given in terms of the pH, where

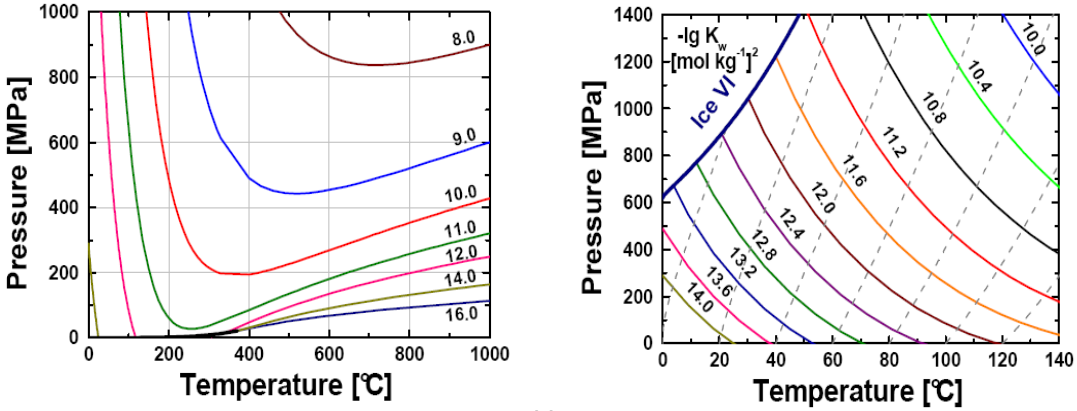
$$pH = \log_{10} \frac{1}{H_3O^+} = -\log_{10}(H_3O^+) \qquad (2.15)$$

with the concentration of H_3O^+ in mol l^{-1}.

The activity coefficients in aqueous solutions can be estimated by the Debye-Hueckel law (Debye and Hueckel 1923) according to the equation

$$\log_{10} \gamma_i = -1.825.10^6.z_i.\sqrt{\frac{I.\rho}{\varepsilon^3.T^3}} \qquad (2.16)$$

where z_i is the number of elementary charges of the ion i; I is the ion strength; and ε is the relative static permittivity.



a) b)

Figure 2.13: Dissociation equilibrium shift (negative logarithm of the ion product K_w) in pure water a) p-T conditions up to 1000 MPa and 1000°C; b) up to 1400 MPa and 140°C with adiabatic lines (--) (Knorr and Mathys 2008).

2.4.3 Adiabatic heating of different food matrixes and food components

As already mentioned in chapter 2.4.2.1, compression of water leads to adiabatic heating of 2°C and 5°C / 100 MPa at low and high (T>80°C) initial temperatures, respectively (Buckow 2006).

Adiabatic heating of ~3°C for water, ~3.2°C for salmon, ~4.5 for chicken fat, ~6.3 for beef fat, ~8.7 for olive oil, ~9.1 for soy oil per 100 MPa compression at 25°C initial temperature is reported (Ting, Balasubramaniam et al. 2002). According to them the effect (temperature increase) due to adiabatic heating of fats and oils were significantly higher.

Adiabatic heating in the sausage matrix (white sausage, Bavarian type) was studied by Maslak 2007 (TU-Münhen, Germany, personal communication) at the Berlin University of

Technology. He measured temperature profiles in white Bavarian sausage (in the sausage) and in water (pressure transmitting medium) during HPP at different initial temperatures (Figure 2.14). Maslak found an adiabatic heating dependency, similar to that reported by Buckow (2006), namely that the temperature increase due to adiabatic heating is higher by HPP performed at higher initial temperatures. Adiabatic heating of 3°C / 100 MPa for 5°C initial temperature, 3.33°C,/ 100 MPa for 15°C initial temperature and 3.48°C / 100 MPa for 20°C initial temperature (Figure 2.14-a, b, c) was estimated. Comparison of adiabatic heating in the sausage core and in the pressure medium (water) showed that the temperature increase profile in the sausage core during HPP was about 2.5-3°C higher than in the water (due to the fat content as reported by Ting *et al.* 2002).

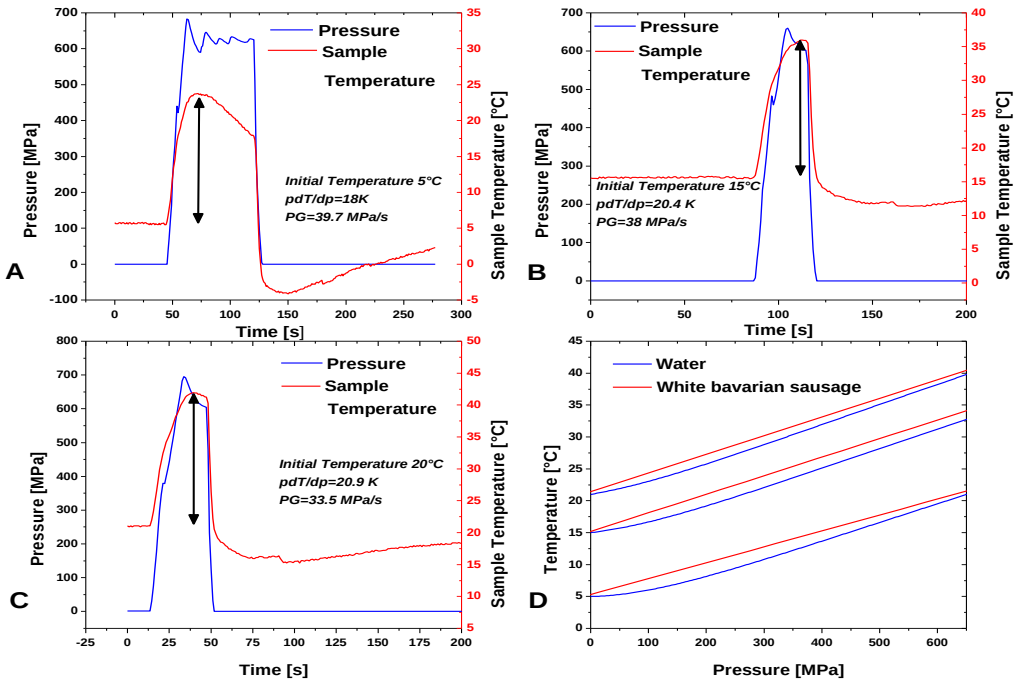


Figure 2.14: Adiabatic heating of white sausage by different initial temperatures a) 5°C initial temperature, b) 15°C and c) 20°C initial temperature; d- adiabatic heating- comparison water and sausage measured in the sausage core (Dominik Maslak, 2007 TU-Münhen, Germany, personal communication).

For his measurements, sausages underwent pressure gradients (PG) of 33.5-39.7 MPa/s (Figure 2.14-d). This PG correlates with the PG used in the present work (40 MPa/s). Because of the similarity of the sausage recipes and PG used in this study and that from Maslak, the levels of adiabatic heating from Figure 2.14 were used for calculating process temperature during the pressure treatment in the present scientific work. After reaching the process pressure level, homogeneity of the process temperature (initial temperature+adiabatic heating) depends on the cooling effect of the vessel walls. Temperature homogeneity can be affected by the vessel size, insulation from the vessel

walls, or the level of the vessel tempering. The problem of determining the homogeneity of the process temperature, especially in the high temperature range, with regard of spore inactivation is until now not industrially solved (Mathys 2008). Lab and industrial scale equipment operating at high temperatures is currently under development (Tonello, C. 2010, NC Hyperbaric, Spain, personal communication).

After pressure release, cooling occurs (dependent on the pressure release rate) until the initial temperature, or lower, is reached (Mathys 2008).

For combined pressure-temperature processing, the adiabatic heating can be observed as a positive effect causing the temperature to increase homogenously in the product. Another positive effect is that this increase could be done within some seconds so that thermal energy and processing time could be reduced.

2.4.4 Effect of pressure on proteins

Change in the protein behavior under high pressure is based on Le Chatelier`s principle: under pressure the equilibrium shifts towards the state, which occupies a smaller volume. In his pioneering work Bridgman 1912, studying the protein denaturation of egg white found that after high pressure at 7 kbar a similar outlook but not identical to cooked egg was obtained.

Protein behavior as a function of high pressure and temperature can be expressed as an elliptic stability phase diagram Figure 2.15- a. It is based on the systematic experimental kinetic studies of Suzuki, K. 1960 as well as the thermodynamic studies by Hawley (1971). It is presented in a two state thermodynamic formalism suggesting a transition between two protein states (Mozhaev. 1996). However, there exists another theory, which says that there are some pre-denaturation transitions enabling the denaturation process to be described as a step-wise process Figure 2.15- c. These pre-denaturation transitions are called intermediate or molten globule states; (Gordon and Barbut 1992; Jonas and Jonas 1994; Ptitsyn and Uversky 1994; Cléry, Renault et al. 1995).

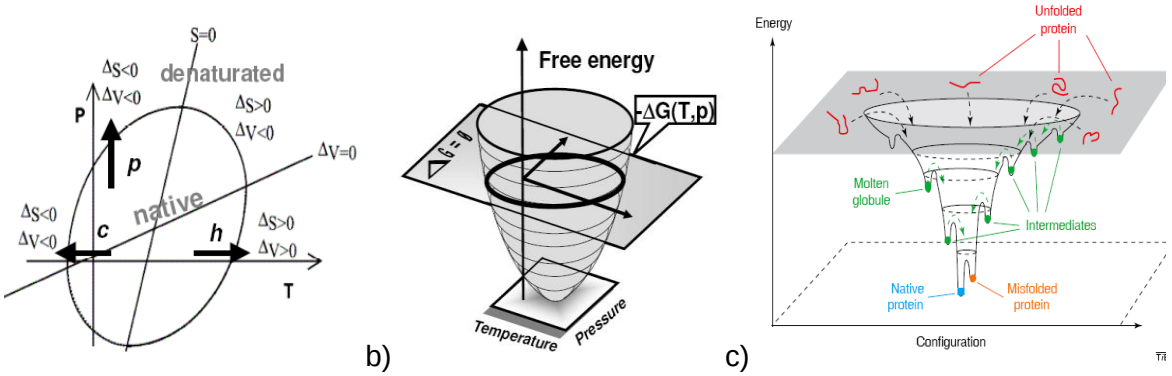


Figure 2.15: Elliptical temperature-pressure stability phase diagram (Heremans 2005) after (Suzuki 1960) and (Hawley 1971); b) three-dimensional free energy landscape (Heinz and Knorr, 2002) and c) (Radford 2000).

In Hawley's two-distinct-states theory (native and denaturated), the Gibbs free energy between the two states is defined as

$$\Delta G = G_{denaturated} - G_{native} \tag{2.17}$$

Integration of equation 2.9 using a Taylor series expansion up to second order terms results in equation (2.18) (Smeller 2002).

$$\begin{aligned}\Delta G = \Delta G_0 + \Delta V_0 (p - p_0) - \Delta S_0 (T - T_0) + (\Delta\beta / 2) + (p - p_0)^2 \\ - (\Delta c_p / 2T_0)(T - T_0)^2 + \Delta\alpha (p - p_0)(T - T_0)\end{aligned} \tag{2.18}$$

Where Δ denotes the change of the corresponding parameter during unfolding.
The slope of the phase transition line in an arbitrarily chosen point of the ellipse can be written in the form of the Clausius- Clapeyron equation:

$$\frac{\partial T}{\partial p} = -\frac{\frac{\partial \Delta G}{\partial p}}{\frac{\partial \Delta G}{\partial T}} = \frac{\Delta V_0 + \Delta\beta (p - p_0) + \Delta\alpha (T - T_0)}{\Delta S_0 - \Delta\alpha (p - p_0) + \Delta Cp \frac{T - T_0}{T_0}} \tag{2.19}$$

The transition line which describes the equilibrium border between native and denaturated states of proteins is defined by ΔG=0. That means that folded and unfolded proteins have the same Gibbs free energy. The slope of this transition line can be obtained using equation (2.19).
Shape, size and orientation of the elliptic transition line are defined by six thermodynamic parameters ($\Delta\beta$, $\Delta\alpha$, ΔC_p, ΔV_0, ΔS_0, ΔG_0). In spite of the complexity of the model, some influence of these parameters can be predicted.
For example, a decrease of ΔC_p broadens the ellipse along the temperature axis, as a decrease in $\Delta\beta$ does the same in the along the pressure axis. Furthermore, $\Delta\alpha$ predominantly determines the orientation of the ellipse.
According to the proteins phase diagram, several denaturation mechanisms exist: heat denaturation, cold denaturation (reviewed in detail by (Privalov 1990), pressure denaturation, and combined pressure- temperature denaturation (Heremans and Smeller 1998). The elliptic shape of the diagram can vary according to order of the terms order. Using the Clausius- Clapeyron equation, the ΔV=0 curve for T_{max} as well as the ΔS curve for p_{max} is

linear (Figure 2.15). This means that at maximum pressure and temperature the native state is stable so that the slope of the ellipse is zero (Smeller 2002).

The Hawley theory is based on the assumption that only two protein states without any intermediate states are possible (Smeller 2002). According to its thermodynamic denaturation description, the denaturation could be a reversible process (Heremans and Smeller 1998). However denaturated proteins tend to build gel, which is stabilized by a network of intermolecular hydrogen bonding, which in turn prevents refolding to the native state. Therefore, this means that the irreversibility is not included in this model.

A schematic model of the pressure increase impact on the protein structure is shown in Figure 2.16. This model shows changes of the protein states according to the changes of the protein structure and is similar to that presented by Smeller (2002).

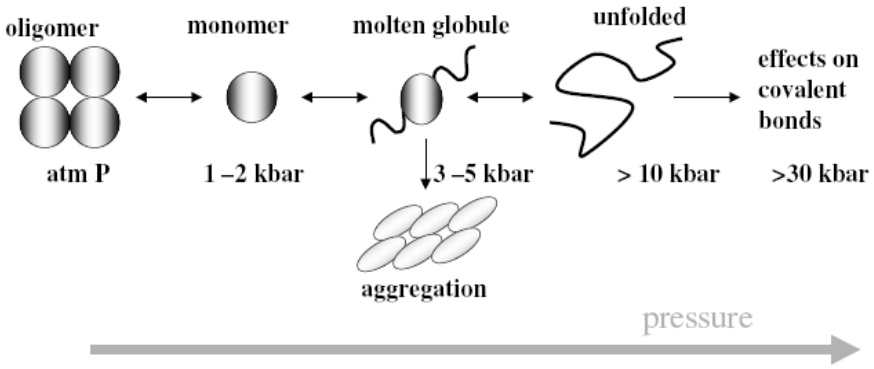


Figure 2.16: Protein states order of events depending on pressure increase (Ando 2003).

According to this the intermediate structure that appears during the refolding of a pressure denaturated protein can also be regarded as a destabilized state of the proteins, which is prone to aggregation. The intermediate states correlates with three dimensional free energy landscape (Figure 2.15-b, c (Heinz and Knorr, 2002, Radford 2000).

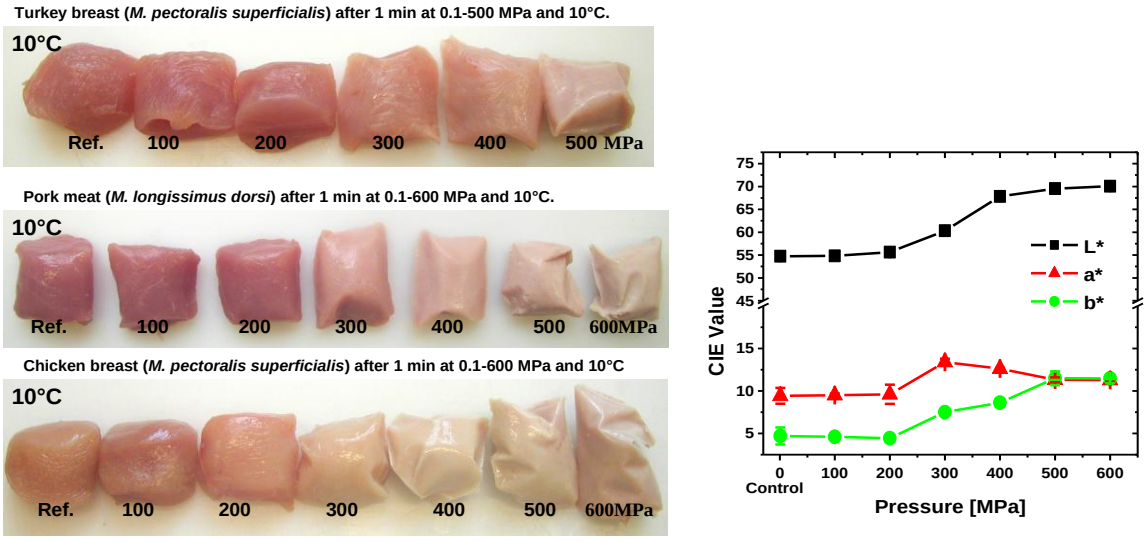
2.4.5 Effect of pressure on meat proteins

There are significant differences in appearance and textural properties between pressure- and heat-induced gels. These differences can modify novel HP- functional properties of meat products. In this chapter, high pressure effect on meat proteins will be briefly discussed. HP-protein changes can affect the processes of denaturation, solubilization and gelation (Cheftel and Culioli 1997; Jimenez Colmenero 2002).

2.4.5.1 High pressure effect on myoglobin

Pressurization may cause unwanted side-effects including color or texture changes of meat. Such changes have a negative impact on the marketability of pressure-treated products since consumers judge the quality of food on the basis of the color. The pressure-based discoloration is due to denaturation of myoglobin. Discoloration of red muscle from pork and beef was found also at pressures of 400-600 MPa and 5-10°C (Carlez, Veciana-Nogues et al. 1995). Structural changes of Mb in solution induced by high pressure have previously been studied using infrared and UV-Vs absorption spectroscopy by (Cheftel and Culioli 1997).

Meat discoloration through pressure processing appears to result from (1) a 'whitening' effect in the range 200-350 MPa, due to globin denaturation and/or to heme displacement or release, and (2) oxidation of ferrous myoglobin to ferric metmyoglobin, at or above 400 MPa (Cheftel 1995). Both degradation phenomena appear to depend more on critical pressure thresholds than on time, since they took place within 2 to 5 minutes after the target pressure is reached (Carlez, Veciana-Nogues et al. 1995). Photographic evidence of this can be seen in Figure 2.17 where drastic discoloration of pork meat was detected after only 1 min treatment time at 400 MPa.



a) b)

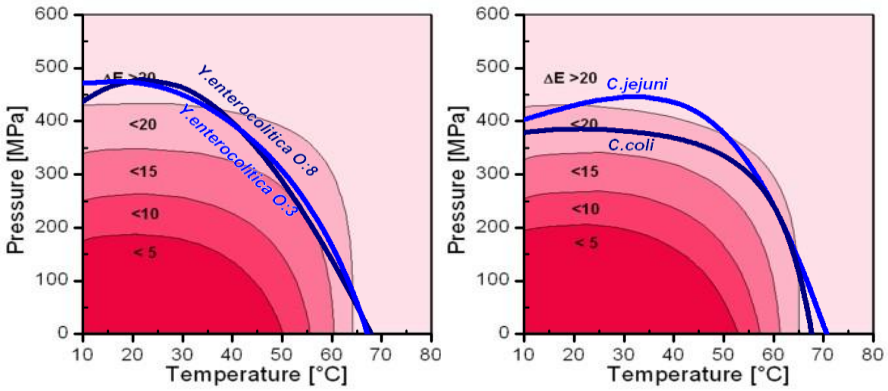
Figure 2.17: a) Changes of native pork and poultry meat by HPP 0-600 MPa after 1 min treatment and 10 °C start temperature b) Changes of the CIE color parameter L*, a*, b* of pork meat at 10 C° and 0.1-600 MPa for 1 min (Tintchev, F. *et al.* 2010)

Minor effects are already visible at 300 MPa for pork and turkey and 200 MPa for chicken meat. For pork meat these are presented with the help of the L*a*b* color space system, where L* are the lightness, a* are the redness, and b* are the yellowness) values in Fig. 1-b. An increase in lightness (L*) for all three meats and no significant change in the redness (a* value) of pork meat was observed (Tintchev, F. *et al.* 2010).

Figure 2.18 -a, b presents the inactivation kinetics of *Y. enteroclolitica* in pork meat as well as *Campilobacter coli* and *Campilobacter jejunii* in a chicken meat slurry (Buckow and Heinz 2008). These are combined with the kinetics of discoloration of pork and turkey meat based on the total color difference according to equation (2.20).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (2.20)$$

This presents the square mean of differences of the three CIE color parameters. Therefore, a 5-log inactivation leads to a significant discoloration of pork and turkey fresh meat.



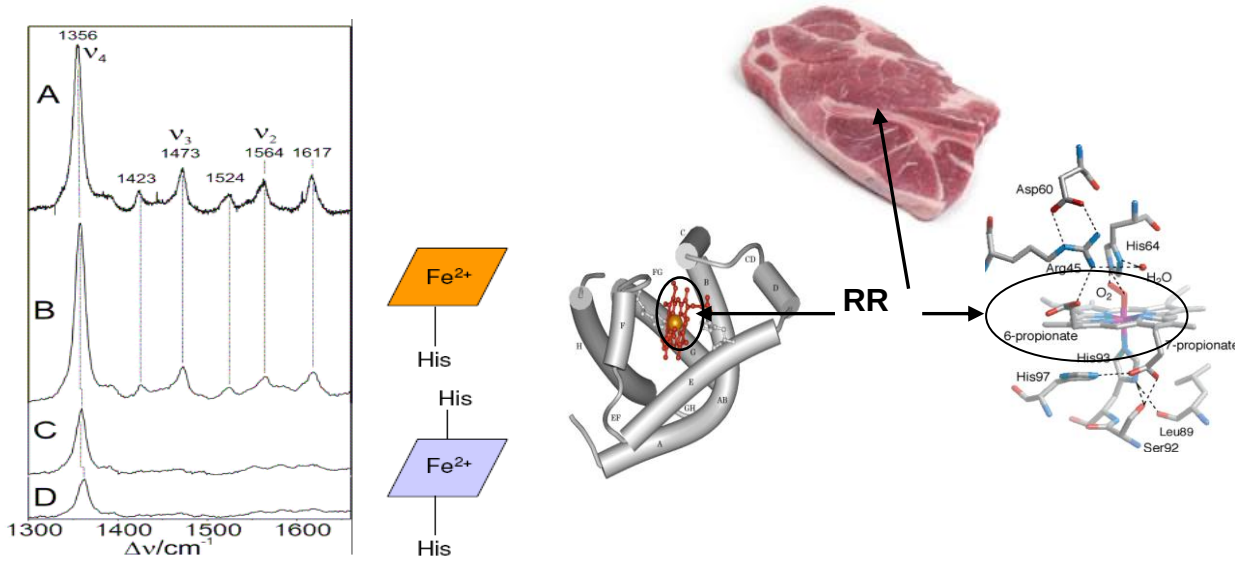
a) b)

Figure 2.18: p-T-landscape for 5 log inactivation after 1 min treatment time of a) *Y. enteroclolitica* in pork meat*, b*) *Campilobacter coli* and *Campilobacter jejunii* (in meat chicken slurry) combined with pT-landscape of color changes presented by ΔE value as an indicator of the total color difference ΔE (Buckow, Tintchev et al. 2007; Tintchev, Buckow et al. 2007)

Besides the UV-Vs absorption spectroscopy an alternative method such as Resonance Raman spectroscopy could be performed.

Resonance Raman (RR) spectroscopy can provide molecular information about the chromophores of pressurized food, which are responsible for the color changes and can explain the discoloration mechanism. This technique is particularly suitable to probe Mb in tissues since it can selectively probe the vibrational spectrum of the haeme by excitation in resonance with an electronic transition, while the remainder of the protein and meat matrix does not contribute to the spectrum (Figure 2.19- a).

RR spectroscopy was used to directly characterize structural changes in the haeme pocket of Mb in nontreated and pressurized pork meat (Figure 2.19.) The results, which are compared with those obtained for Mb in solution, contribute to the understanding of the pressure-induced processes of Mb in intact meat tissues and in solution (Tintchev, F. *et al.* 2010).



a) b)

Figure 2.19: a) Resonance Raman (RR) spectroscopy measurement made directly in the chromophores in the meat tissue; b) RR spectra of (**a**) deoxymyoglobin in solution, (**b**) at ambient pressure directly in meat tissue, (**c**) after 600 MPa for 10 min, and (**d**) 700 MPa for 1 min directly in the meat tissue (Tintchev, Wackerbarth et al. 2010).

RR spectroscopy thus can differentiate between the myoglobin species: deoxymyoglobin (Deoxy-Mb), oxymyoglobin (Oxy-Mb), and metmyoglobin (Met-Mb) by the frequencies of so-called marker bands in the region between 1300 and 1700 cm^{-1}. In reduced deoxy-Mb, the frequency is observed at 1356 cm^{-1}, whereas in the oxidized oxygen-free form, Met-Mb, this mode shifts up to 1370 cm^{-1}. Both in deoxy-Mb and met-Mb, the haeme iron is in a high-spin configuration with the imidazole residue of histidine 93 as the proximal ligand. The distal position remains vacant in the deoxy-Mb form whereas a water molecule occupies the sixth coordination site in met-Mb. These configurations are reflected by the spin- and coordination marker bands above 1450 cm^{-1}, particularly of the mode ν_3, which are found at 1473 and 1481 cm^{-1} in deoxy-Mb and met-Mb, respectively. Oxygen binding to the ferrous state induces a low spin configuration, in which the electron density is partially transferred to the empty antibonding orbitals of the ligand such that the ν_4 frequency shifts up to 1377 cm^{-1}. The other marker bands, ν_3 and ν_2, are found at higher frequencies such that, altogether, the analysis of this spectral region allows for an unambiguous identification of the Mb state.

After high pressure treatment, distinct spectral changes are observed in the RR spectrum (Figure 2.19-b). The overall RR intensity decreases, accompanied by changes in the frequencies and intensities of the marker bands. The ν_4 envelope shows a slight up shift of the peak maximum from 1356 cm^{-1} to 1359 and 1363 cm^{-1} in spectra of meat previously pressurized at 600 and 700 MPa, respectively. Moreover a closer inspection of the region between 1420 and 1620 cm^{-1} reveals the intensity decrease of the bands at 1473 (ν_3) and 1564 cm^{-1} (Heremans and Smeller) which are replaced by bands at 1493 and 1581 cm^{-1}. These frequencies of the marker bands (ν_4, ν_3 and ν_2) are typical for the modes of a ferrous

heme in a six coordinated low spin configuration. The nature of the second axial ligand is not clear *a priori*. A possible candidate is histidine 64 which is located in the distal part of the heme pocket. Indeed, the RR spectral parameters of the non-native 6cLS ferrous Mb form resemble those of the bis-histidine-coordinated 6cLS ferrous cytochrome (Oellerich, Wackerbarth et al. 2002). Such a pressure-induced bis-coordinated heme species of Mb has in fact been previously discussed by (Zipp and Kauzmann 1973; Ognumola, Kauzmann et al. 1976).

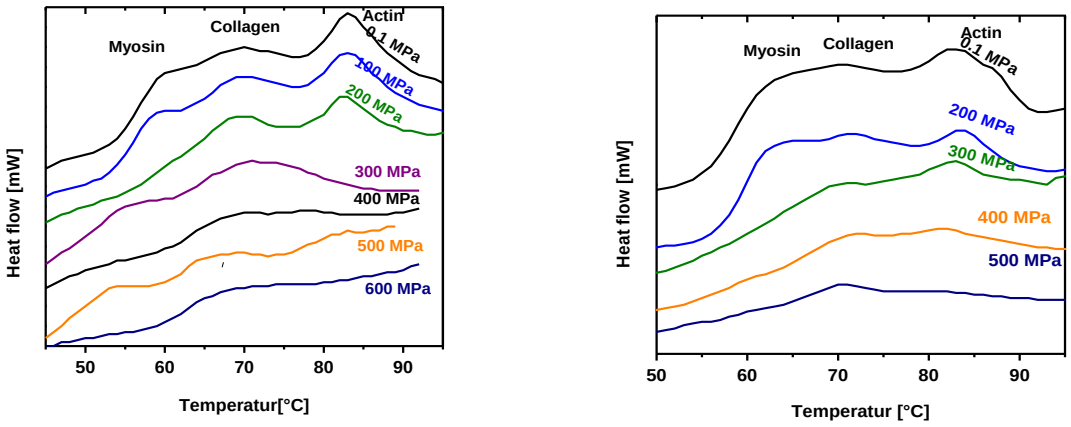
The Resonance Raman spectra studies demonstrated that high pressure-treatment of fresh meat induces the formation of a non-native ferrous myoglobin species (Tintchev, Kuhlmann et al. 2009). The non-native myoglobin species contribute to the color/appearance of the pork meat, since this species is not able to catalyze oxidation of the meat matrix (as iron is in the ferrous state).

2.4.5.2 High pressure effect on meat proteins-denaturation

The effect of high pressure on the myofibrillar proteins plays a significant role in modifying the functional properties of meat products. High pressure processing affects denaturation, solubilization, aggregation, tissue disintegration, as well as gelation (Cheftel and Culioli 1997, Comenero, J. 2002). In this chapter, a short review of the available literature on high-pressure denaturation of meat proteins is presented.

Protein denaturation under pressure is a complex phenomenon induced by the disruption of hydrophobic bonds, sulfphydryl interactions, and salt bridges. High pressure acts by altering the balance of intermolecular and solvent-protein interactions (Chapleau, Mangavel et al. 2003).

Meat myofibrillar proteins are pressure sensitive, and increasing pressure leads to progressive protein denaturation, as shown in Figure 2.20.



a) b)

Figure 2.20: Differential scanning calorimetry profiles of a) pork and t b) turkey meat proteins (Buckow *et al.* 2007, Tintchev, F. *et al.* 2007).

Muscle protein denaturation can be analyzed using differential scanning calorimetry (DSC). Normally, pork meat gives three endothermic transitions: myosin (~59°C), a mixture of connective tissue/sarcoplasmic protein (~66°C), and actin (~78°C), although these endothermic transitions may vary due to differences in pH and ionic strength (Zamri, Ledward et al. 2006). The evolution of ΔH_T with increasing pressure is shown in Figure 2.21-a. The total denaturation enthalpy difference of chicken, turkey and pork proteins decrease with increasing pressure, representing the level of protein denaturation (Tintchev, F. *et al.* 2007, Buckow *et al.* 2007). Similar observations are reported by Chapleu *et al*. (2003) who studied the protein structure of myofibrillar proteins in a phosphate buffer (pH 6.0, containing 0.6 M KCl). The decrease in total denaturation enthalpy starts at 200 MPa with the disappearing of the myosin peak, which correlates to a moderate increase in hardness. Increase of hardness after HPP in the range of 200-400 MPa was found for cod muscle and beef muscle (Angsupanich and Ledward 1998; Ma and Ledward 2004). Pressure treatment at 300 MPa leads to further flattening of the actin peak, which together with the myosin denaturation results in hardness increase showed in Figure 2.24. The denaturation continues until 600 MPa where the minimum of the total enthalpy and maximum denaturation is reached (Tintchev, F. *et al.* 2007, Buckow *et al.* 2007). Similar denaturation behavior was observed by Sikes et al. (2009), studying 1% NaCl mince beef meat and batters up to 400 MPa.

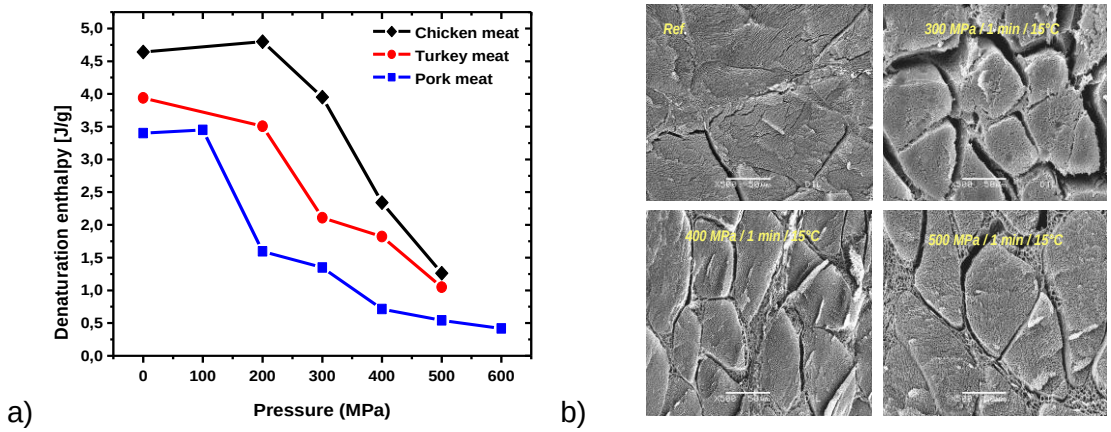


Figure 2.21: a) Denaturation enthalpy of chicken, turkey and poultry fresh meat b) Structural changes of pork fresh meat at different pressures for 1 min holding time studied by SEM (Tintchev, F. *et al.* 2007, Buckow *et al.* 2007)

SEM micrographs of the structural changes of pork meat are presented in Figure 2.21- b. At 300 MPa HPP for 1 min, disintegration due to denaturation of the meat matrix was visible. This resulted in larger free spaces between the myofibril bundles and led to increased hardness and drip loss (Figure 2.24). With increasing pressure, there is an increase of

protein denaturation and a simultaneous gelatinization process, which induces protein swelling and results in better WHC and hardness decrease, as observed by the samples treated at 400 and 500 MPa (Figure 2.21- b), where no gaps were visible (Tintchev, F. *et al.* 2007, Buckow *et al.* 2007).

2.4.5.3 High pressure effect on solubilization of meat proteins-

As a consequence of depolymerization, pressure induces an increase in the solubilization of myofibrillar proteins (Cheftel and Culioli 1997). A significant increase of solubilised myofibrillar proteins from *ovine m. longissimus dorsi* in saline solutions was observed (Macfarlane 1974). Iwasaki et al. (2006) reported about protein solubilization from chicken myofibrils suspension in 0.2 M NaCl with pH 6.0 as well as from 1-2 % NaCl pork patty analyzed by SDS-PAGE. Solubilization of myosin, C-protein, α-actinin, tropomyosin and troponins was observed to increase up to 200 MPa. With further pressure increase up to 300 MPa a small decrease of band intensity was observed. Based of these observations dissociation of both thin and thick filaments was indicated. Iwasaki et al. (2006) also reported an increase in protein concentration of the supernatant of pressurized chicken myofibrils, as listed in Table 2.2.
Similar results are reported by Sikes *et al.* 2009. As a consequence of the enhanced amount of soluble proteins, increased interactions between protein constituents and water occurred. These pressure-treated homogenates exhibited less drip loss during subsequent cooking, which resulted in a firmer and more cohesive final product (Cheftel and Culioli 1997).

Table 2.2: Protein concentration of the supernatant of pressurized chicken myofibrils (Iwasaki, Noshiroya et al. 2006)

Pressure (MPa)	Protein concentration (mg/ml)
0.1	0.82 ±0.01
100	0.94 ±0.01
200	1.80 ±0.07
300	1.26 ±0.01

In one other study, (Macfarlane and McKenzie 1976) have found that the solubilization of proteins was dependent on pH, temperature, and both salt type and concentration, so that pressurization was very effective for the solubilization process above pH 3.6 and 0.2 M salt. Furthermore they have found that pressure holding time and temperature also have an influence on the solubilization at treatment times longer than 5 min and low temperatures,

since more soluble proteins are obtained. Suzuki et al. (1991) reported solubilization starting at 150 MPa, increasing with HPP increase and reaching its maximum at 300 MPa. Solubilization of actin, tropomyosin, troponin C as well as M- protein was reported to start at 100 MPa pressure, whereas solubilization of myosin heavy chains required higher pressure levels (300 MPa) (Macfarlane 1974).

Besides myosin solubilization, pressure induced solubilization of collagen could have an impact on gelation in batter containing high amount of connective tissue (for example poultry MDM batters). Collagen thermal stability reduction and a significant increase in heat solubility of collagen after HPP up to 500 MPa were reported. These correlated with a significant reduction of meat toughness by pressure treatment up to 300 MPa, which suggests that HPP may have caused physical changes in the intramuscular connective tissue (Ichinoseki, S. et al. 2006).

2.4.5.4 High pressure effect on aggregation of meat proteins

The pressure effect on aggregation of myosin molecules was studied in detail by Yamamoto *et al.* (1993). Their mechanism of the myosin HP- aggregation is shown in Figure 2.22. In their model, the intramolecular or intermolecular head-to-head interaction takes place after exposure to relatively low pressure or during an early stage of exposure to high pressure. The intramolecular interaction results in the formation of one-headed myosin molecules, and the intermolecular association produces clusters, in which heads of the molecules are loosely bound.

One-headed myosin and also small oligomeric species still retain the capability for oligomerization through intermolecular head-to-head interaction. Increasing pressure and extending duration of exposure causes packing of heads, resulting in the formation of daisy-wheel shaped oligomers (Yamamoto, Hayashi et al. 1993). In the same study they found a relationship between pressure-treatment time and the increase of myosin oligomers. At 210 MPa oligomer aggregations increased progressively from 25.4 % after 5 min up to 64.4 % after 30 min of treatment time. This was related with a monomer myosin decrease from 26.8 % after 5 min to 1.1 % after 30 min of treatment time, respectively. These changes correlated to the increased turbidity of the myosin solution, which led to the suggestion that irreversible aggregation of myosin molecules occurred during HPP.

Similar observations were reported by Iwasaki *et al.* (2006), studying the morphological change of pressurized chicken myofibrils and chicken bundle (0.2 M NaCl; pH 6.0). They observed HPP structure thickening at 100 MPa, structure disruption at 200 MPa, and aggregation at 300 MPa.

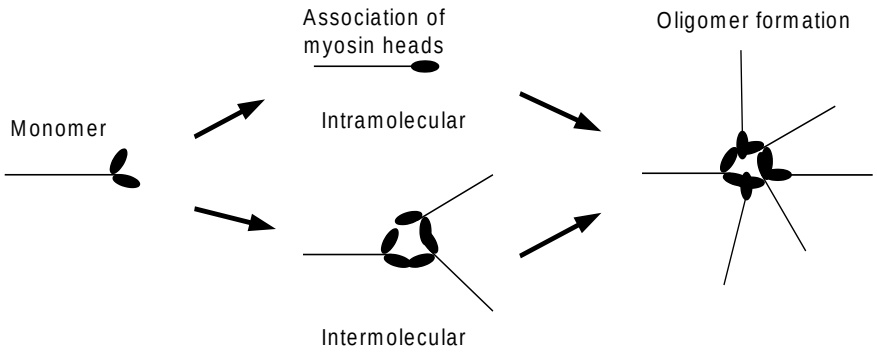


Figure 2.22: Schematic diagram for the formation of oligomeric species of myosin molecules by HPP (Yamamoto, Hayashi et al. 1993).

The head-head packing of the myosin molecule is due to the hydrophobic interactions, which under pressure according to Le Chatelier's principle rearrange themselves into the most efficient packing shown in Figure 2.23), which in this case seems to be the hydrophobic packing (clump) (Ando 2003). Therefore the peptide chains must orient their hydrophilic amino acids towards the outside of the molecule. The hydrophobic amino acids are simultaneously buried inside the molecule to exclude water from this core (Kilara and Harwalkar 1996).

The exclusion of water from contact with hydrophobic residues causes a large increase in entropy and the close packing of protein interiors leads to low enthalpy (Privalov and Khechinashvili 1974).

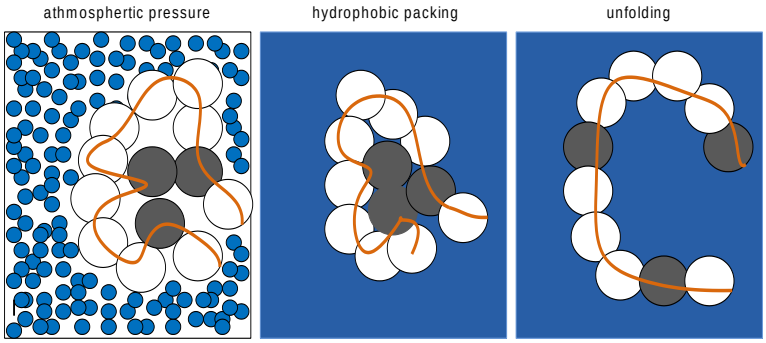


Figure 2.23: Volume minimization under high hydrostatic pressure (Ando 2003).

The pressure induced myosin aggregates are similar to those that are heat induced (Yamamoto 1990). However upon heating, gel formation of monomeric myosin started with aggregation of myosin heads and formation of daisy-wheel shaped oligomers similar to Figure 2.22. The molecule tails, extending radially from the cluster (daisy-wheel formations) are entangled to form a network structure. This subsequent linkage through entangling of tails is considered to be a helix-coil transition (Samejima, Ishioroshi et al. 1981), which according to Yamamoto, K. et al. (1993) does not occur during high pressure treatment.

Yamamoto suggested that the tail portion of the myosin molecule is not affected by HPP and supposed that this could be a possible reason why myosins do not form a gel by pressurization alone. The same conclusion was also made by Chattong et al. (2009). They reported that combined pressure and heat needs to be applied to obtain efficient protein aggregation and gelation, where a heat induced helix-coil transition can occur and the myosin molecules could interact through the entanglement of the tails.

The differences between heat and pressure induced gels could be caused by differences in the mechanism of denaturation. High pressure induced gels are based on the protein volume decrease, while the heat denaturation of proteins is caused by the violent movement of molecules, leading to destruction of non-covalent bonds. The pressure induced mechanism is associated with the rearrangement of water molecules surrounding amino acid residues, which could be an explanation for observed increasing glossiness and transparency of pressure induced gels (Okamoto, Kawamura et al. 1990).

For better understanding of pressure induced gelation, the pressure effect of the myosin head and tail portion must be studied in detail. Iwasaki and Yamamoto (Iwasaki and Yamamoto 2003) found, by measuring intrinsic and ANS fluorescence intensity up to 400 MPa, that the most pressure-sensitive portion of the myosin molecule was the head. The polarity around the tryptophan residue of the myosin head was increased and the hydrophobic cores were exposed to the molecule surface. In contrast, the tail in the helical polypeptide chain was partially dissociated. They also found that pressure-induced small-sized aggregates did not grow into larger formations under constant pressure, but amino acid residues did appear on the molecule surface. Small size aggregates grew to larger aggregates in the decompression process.

In another study (Iwasaki, Yamamoto et al. 2002) studied the structural changes in chicken myosin S-1 at HPP by analyzing the ATP-induced fluorescence, ε-ADP fluorescence, Acto-S-1 ATPase, and SDS-PAGE. They concluded that pressure-induced structural changes of S-1 begin at about 150 MPa, which after pressurization above 250-300 MPa led to a loss of the intrinsic structures of ATPase and actin binding sites. Furthermore, after pressure treatment, the appearance of three S-1 domains with 27 kDa (N-terminal), 50 kDa and 20 kDa (C-terminals) was reported. However, above 300 MPa, a density decrease of the domain characteristic SDS-PAGE bands and the appearance of three novel bands (fragments) between 75 kDa and 50 kDA and 50 kDa and 27 kDa were observed. The appearance of the three novel fragments was found to correlate with a turbidity increase, where a maximum turbidity at 210 MPa/ 5 min was reported in the study of Yamamoto et al. (1993).

The scientific reports reviewed thus far, discussing the mechanism of myosin aggregation under pressure, correlate and complement each other. From the reviewed literature, a complete model can be obtained to describe changes in the myosin molecule as well as

reconfiguration of its structure during high pressure treatment. Head to head interactions started above 70 MPa (Yamamoto, Hayashi et al. 1993). In the range of 70-300 MPa, an increase is seen in the surface hydrophobicity of bovine myofibrillar proteins in a phosphate buffer with pH=6 containing 0.6 M KCl. This was combined with an increase of the total denaturation enthalpy of myosin, as studied by DSC (Chapleau, Mangavel et al. 2003). Above pressures of 250-300 MPa, there appears to be a loss in the intrinsic structure of the S-1 domains related with the decrease of band density at 300 MPa analyzed by SDS-PAGE (Iwasaki, Yamamoto et al. 2002). All these observations lead to the conclusion that the changes taking part in the myosin head (S-1) cause an increase in hydrophobicity. The unfolded molecules subfragments and subunits rearrange themselves in small-volume configurations conformations (one- and two-headed myosin aggregations bound by head-to-head interactions). The mechanism of the aggregation of myosin molecules is primarily dependent on the salt content, pH value as well as type of the myosin solution and concentration (Hermansson, Harbitz et al. 1986).

2.4.5.5 High pressure effect on gelation of meat proteins

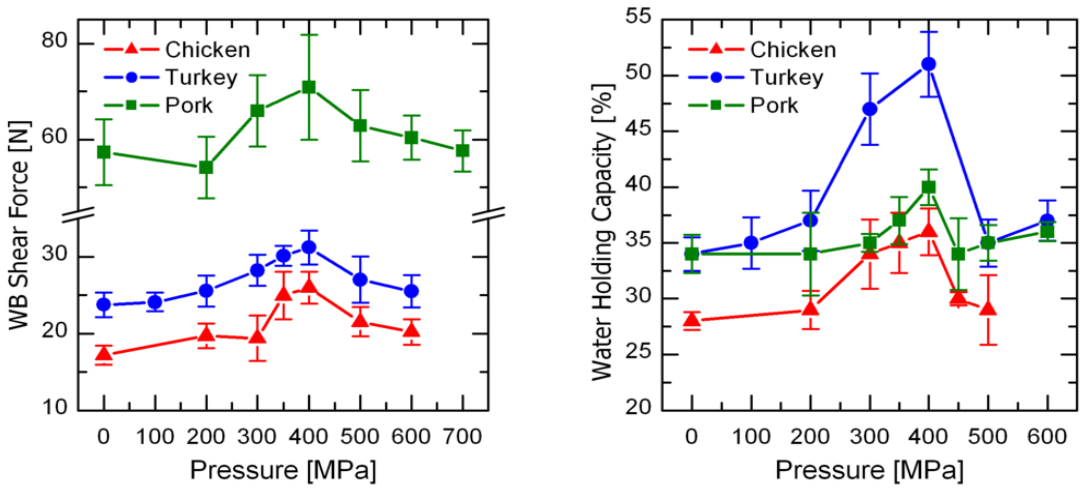
Generally, high-pressure induced gels were observed to have a different appearance (smoother, more glossy) and different functional properties (less firm, more elastic) compared to thermally treated gels (Cheftel and Culioli 1997). Cheftel and Culioli also reported pressure-assisted depolymerization of actin and actomyosin.

Similarly to heat-induced gelation, pressure-induced gelation depends on the protein system, structural disintegration, pH, and ionic strength, as well as on the HPP conditions (pressure level, treatment time, and pressurizing temperature) (Jiménez Colmenero 2002). In his review, Jeménez Colmenero (2002) classified the meat gelation process in several categories according to the meat system (raw or preheated) and according to the pressure-temperature combinations. Pressurization at low temperatures, less than 30-35°C, leads to and increased gel strength with increasing pressure (Ko, Tanaka et al. 1990; Okamoto, Kawamura et al. 1990; Carballo, Fernandez et al. 1996; Fernandez-Martin, Fernandez et al. 1997; Tintchev, Buckow et al. 2007).

Pressurized temperature treatment above 40 °C limits the gel process of the meat system (Jiménez Colmenero 2002). Different kinds of batter, heated to 60-80 °C at pressures in the range of 200-600 MPa and with treatment times to 30 min, formed softer gels, but with improved WHC compared to only thermally treated batters (Chung, Gebrehiwot et al. 1994; Fernandez-Martin, Fernandez et al. 1997; Okazaki, Ueda et al. 1997; Colmenero, Fernández et al. 1998; Chattong and Apichartsrangkoon 2009). Jeménez Colmenero suggested two possible mechanisms that limit the firmness in the myosystems. First, the pressure

processing partially preserves the proteins from thermal denaturation, and second, proteolytic activity increases, causing some myofibrillar protein breakdown and the formation of various molecular fragments. In this case, pressure-treated gels are less rigid with improved WHC than thermal-treated gels.

Descriptions for gelation processes in chicken, turkey, and pork meat are reported by Tintchev et al. (2007). Observations of a shear force and drip loss increase of pork meat, at pressures up to 400-450 MPa, (denaturation and disintegration processes) and ambient starting temperature (Figure 2.24). At higher pressures, a decrease of measurable shear force and drip loss to values comparable with the control sample was detected. These results, combined with the structural changes that occur during pressure treatment up to 600 MPa, leads to the conclusion that some structural changes took place in the range of 450-500 MPa. That structural improvement could be related to a possible gel formation, positively affecting firmness and WHC.



a) b)

Figure 2.24: a) Warner-Bratzler shear force values and b) water holding capacity of chicken, turkey, and pork meat at a starting temperature of 25°C and pressurized at 0.1-700 MPa for 1 min (Tintchev, F. *et al.* 2007, Buckow *et al.* 2007).

Pressure assisted gels under mild heating are formed at pressures above 200 MPa (Yamamoto, Miura et al. 1990; Angsupanich, Edde et al. 1999).

Gels with significant improvement of the functional properties (WHC, firmness, gumminess, chewiness) could results after the conventional heat treatment with a HP pre-treatment step in the low pressure range (100-300) (Macfarlane, McKenzie et al. 1984; Suzuki and Macfarlane 1984) (Iwasaki, Noshiroya et al. 2006; Sikes, Tobin et al. 2009).

2.4.5.6 High pressure effect on the myofibrillar protein structure

The effects of high pressure on the myofibrillar protein structure were reported in detail by Chapleau et al. (2003). This study analyzed myofibrillar protein in a phosphate buffer with pH 6.0 and 0.6 M KCl. The primary structure of the proteins, due to the absence of an effect on covalent bonds, was not found. HPP induced irreversible changes in the secondary structure at pressures above 300 MPa (Smith, Galazka et al. 2000; Chapleau, Mangavel et al. 2003). The weakest non-covalent interactions between amino acid residues that support the tertiary structure were first destabilized and then replaced by protein-water interactions at high pressure (Mozhaev, Heremans et al. 1996).

Increase of ANS binding under pressure is used as an indicator of molten globule formations. This can be analyzed by ANS-fluorescence intensity. Using this technique, the pressure effect on the surface hydrophobicity was found to increase at pressures above 100 MPa, indicating that the hydrophobic core of the protein become more exposed (Chapleau, Mangavel et al. 2003). An ANS fluorescence intensity of S-1 increase up to 350 MPa was also observed by Iwasaki et al. (2003). He concluded that during the compression the three-dimensional structure volume of the myosin molecule decreases due to collapse of hydrogen intermolecular bonds and disruption of the formation of hydrogen bonds among free water and amino acid residues in the myosin molecule. The myosin molecule was collapsed, resulting in the formation of small size aggregates, which is also seen from the mechanism described in (Figure 2.22) by Yamamoto et al (1993).

The same forces stabilize the quaternary structure as the tertiary structure. In the native state, myosin polymers are bound by their tails to form filamentous aggregates (Chapleau, Mangavel et al. 2003). Pressure could dissociate this aggregation and promote a new type of aggregation according to the mechanism of Yamamoto et al. (1993), shown and already discussed in Figure 2.22.

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3 Materials and Methods

3.1 Standard model batter

3.1.1 Frankfurters model batter

A typical Frankfurters recipe has been used. Ingredients for a 5 kg batch are: 1.25 kg pork meat II (neck), 1 kg grade pork meat III (shank meat), 1.6 kg grade pork meat IV (back fat), 1.15 kg ice, 100 g pickling salt (99.6 % NaCl and 0.4 % $NaNO_2$ from Suprase, Hengelo, Netherlands), and 15 g diphosphate (AVO- Werke August Beisse GmbH). Pork meat was sorted out according to Table 3.1.

Model batter (frankfurters recipe)				
Pork meat	***Additives***	***BEFFE* (%)***	***Fat (%)***	***ICE (%)***
1.25 kg/ 5 kg batter S II Type- pork neck meat	20 g/kg Pickling salt	8.94	31.57	1.15 kg/5 kg batter
1 kg/ 5 kg batter S III Type- pork shank meat	3 g/kg phosphate			
1.6 kg/ 5 batter S IV Type- pork back fat				

Table 3.1: Preparation of model meat batter based on frankfurters recipe for 5 kg batch product BEFFE* (German)- FFCTFM- fat-free-connective-tissue-free-meat

Barrow pork meat was used 24 hours after slaughter. Pork meat was supplied from the Diekmann G.H. slaughterhouse in Essen, Oldenburg, Germany. It was chilled at 4 °C before the grinding process. The pH value of the meat was between 5.7- 5.9.

Subsequently, the meat was cut into small pieces. For the grinding process a meat grinder (Mado Model MEW 603, Dornhan, Germany) with two knives, one kidney plate, and two grinder plates was used (with hole sizes 16 mm and 3 mm) (Figure 3.1-a, b). The temperature after the grinding process was in the range of 9-11°C. Chopping where the lean meat was placed into a six-knife bowl cutter followed the grinding process (Alexander model N20, Remscheid, Germany (Figure 3.1-c) with the salt and phosphate to mix the whole batch.

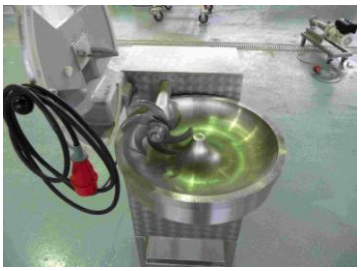
a)  b)  c) 

Figure 3.1: a) Grinder cutting set with plates and knives (system "Unger"); b) grinder (Mado Model MEW 603); c) six-knives bowl-cutter (Alexander Model N20)

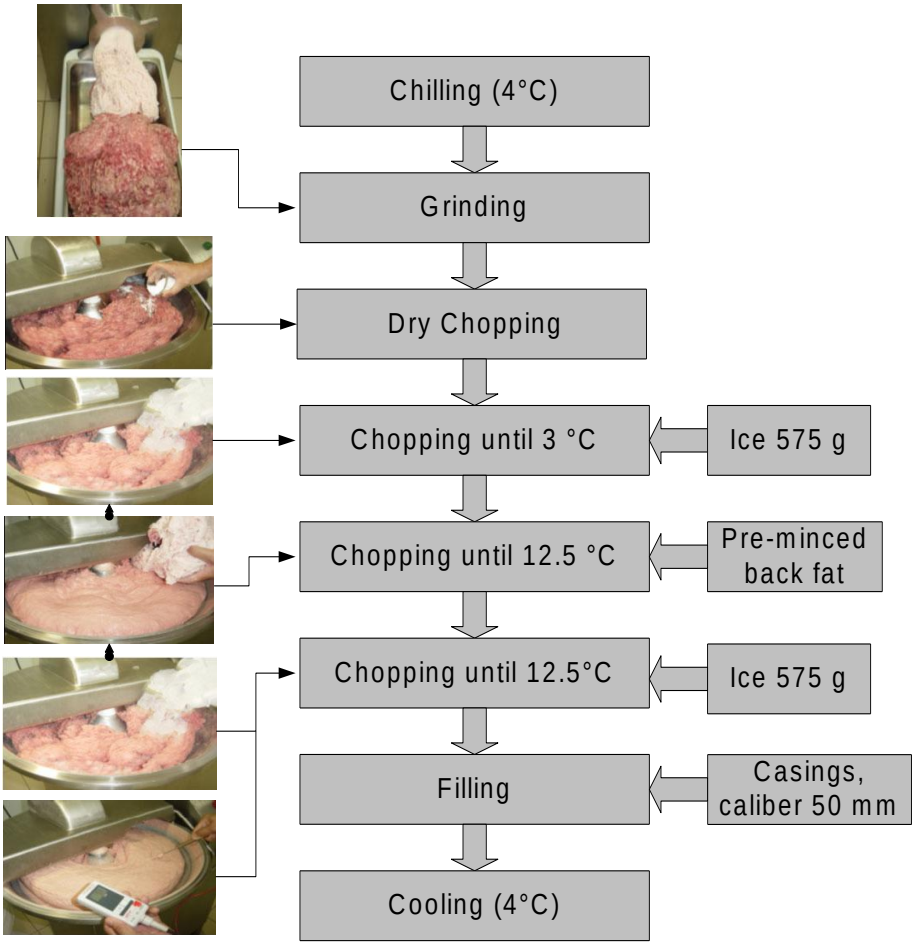


Figure 3.2: Standard batter preparation (production flow)

The chopping process was done in eight steps (Figure 3.2):

Step1 : The lean meat portion is pre-minced and kept chilled (2°C).

Step 2 : The lean meat is placed in the bowl cutter with the salt and additives for the whole batch. The mixture is chopped for 30s without ice (dry chopping).

Step 3 : Ice is then added (575 g). The chopping continued at a fast bowl chopper speed until a mixture temperature of 3°C is reached.

Step 4 : Fat (pre-minced and chilled) is added and chopped at high speed until batter temperature of 12.5°C is reached.

Step 5 : The second amount of ice (575 g) is added and chopped until a final batter temperature of 12.5°C is reached.

Step 6 : Batter is filled into casings (caliber 50 mm) using a filling machine (piston stuffer). The casings are filled to maximum capacity to avoid surface wrinkles and air pockets in the final product.

Step 7 : Filled sausages are chilled to 4 °C. The final batter acidity of the standardized batter was pH 6.0- 6.2

To guarantee reproducibility, a uniform initial state of the sausage batter before HP-T treatment was needed. Testing was done to determine how many days after slaughtering a batch of raw material could be processed to sausage batter and how long after preparation the sausage batter can be considered as stable, with respect to rheological properties. For this purpose, sausage batter rheological properties were assessed using the oscillation test. The oscillation analysis is very sensitive for detection of structural changes resulting in changes in rheological properties. Firmness parameter k was calculated according to the storage modulus-frequency function (discussed later and shown in equation 3.23).

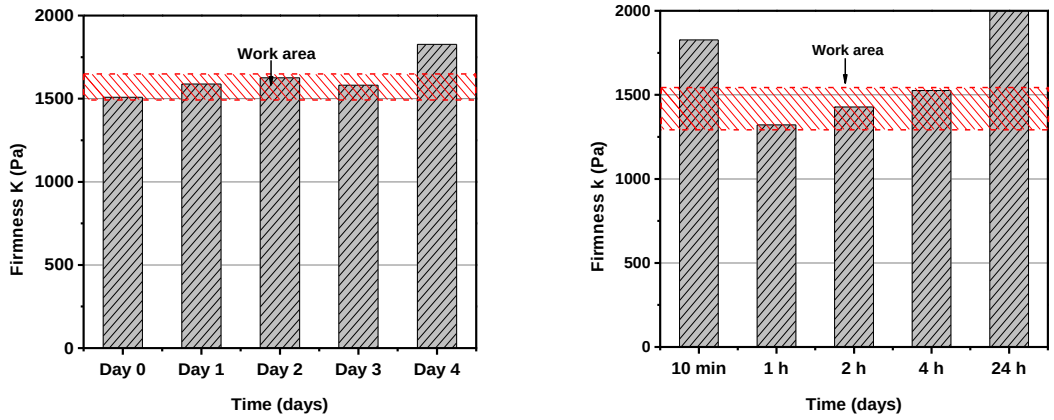


Figure 3.3: a) Firmness parameter of batter batches produced on different days after slaughtering; b) Firmness of batter batches as function of time after preparation (measured by oscillation test)

After determining that the reproducibility of the batter samples could be evaluated according to the firmness parameter k, it was found that the raw material starts to affect the properties of sausage batter if used up to 4 days after slaughtering, as shown in Figure 3.3- a. Furthermore, it was estimated that a batch of sausage batter should be used no less than 45 min and no later than 4 hours after preparing, as shown in Figure 3.3- b. Outside of this time frame, spontaneous structure formation processes occur which significantly influence material properties.

After batter preparation, it was found that the firmness decreased after 1 hour to a measure of 1300 Pa, and increased again significantly after 24 hours of chilled storage, as shown in Figure 3.3- b. A firmness range of 1300-1550 Pa, which corresponded to a period of time 1-3 hours after batter preparation, was defined as being the range for acceptable workability.
In the control recipe, 2% salt and 0.3% phosphate was added. To study the influence of reduced salt and phosphate content on the batter structure after high pressure-temperature treatment, modifications to the batter recipe were examined. These modifications are listed in Table 3.2.

Table 3.2: Batter recipes

Control recipe	***Reduced NaCl content***	***Reduced Phosphate content***	***Reduced NaCl and Phosphate content***
2% NaCl, 0.3% Phosphate	1% NaCl, 0.3% Phosphate	2% NaCl, 0.2% Phosphate	1% NaCl, 0.15% Phosphate
	0.5% NaCl, 0.3% Phosphate	2% NaCl, 0.1% Phosphate	

3.1.2 Turkey MDM meat batter

Mechanically deboned turkey meat was supplied commercially (Heidemark Mästerkreis GmbH & Co. KG, Alhorn, Germany). Preparation of the model turkey batter was done according to the process flow from Figure 3.2, starting from the dry chopping process. Three different recipes, according to Table 3.3, were done: 1- without wheat fiber; 2- with added wheat fiber (160 g); and 3- with added wheat fiber (160 g) and 10 % vegetable oil (840 g).

Table 3.3: Preparation of model turkey MDM batter for 5 kg batch product

Ingredients	***Weight [g]***
Raw materials:	
Turkey MDM	6.000 g
Ice	2.000 g
Fillers:	
Without wheat fiber (recipe 1)	
Wheat fiber (recipes 2 and 3)	160 g
Additives:	
Nitrite curing salt	120 g
Phosphate	18 g
Ascorbic acid	30 g
Vegetable oil (recipe 3):	840 g
Seasonings	50 g
Monosodium glutamate (E-621)	30 g

3.1.3 Performance of batter preparation, HPP and batter analysis.

Model batter was prepared according to the production flow from Figure 3.2. Model frankfurters batters were prepared according to Table 3.1 for pork frankfurters and according to Table 3.3 for MDM turkey batter. Batter recipes with reduced ion concentration were prepared according Table 3.2. Samples were heated to the core initial temperature in a water bath for about 15 min. Different combinations of high pressure- pressure treatments were carried out according to Table 3.4 and Table 3.5.

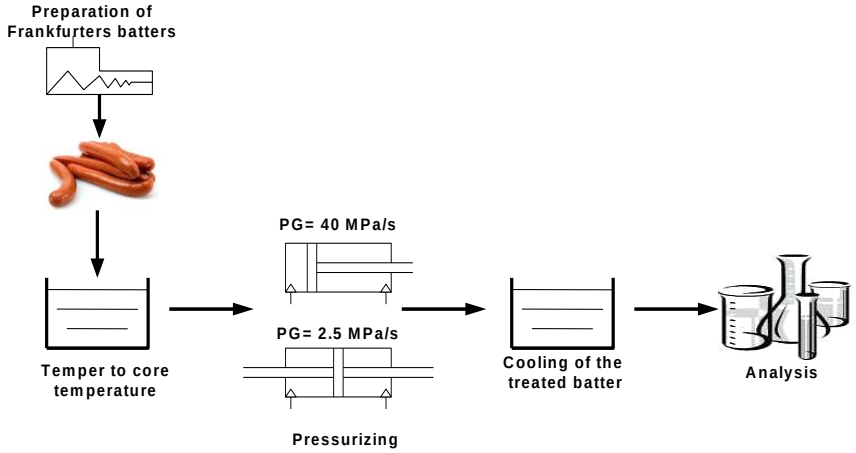


Figure 3.4: Sample treatment performance

Processing of the samples was done according to Figure 3.4. After batter preparation, the samples were heated in a water bath to the desired initial core temperature. Within 3 hours after batter preparation, the samples were then pressurized. Next, pressure treated samples were cooled in ice to prevent further denaturation processes. After cooling, different analysis methods were performed.

3.2 High pressure equipment

High-pressure processing of sausage batter was carried out in an industrial scale HP system (NC-Hyperbaric, model Wave 6000/55, Burgos, Spain) which capable of a pressure build-up time, or pressurization gradient (PG), of 2.5 MPa/s. (Uhde High Pressure Technologies, model HDS 08-0003, Hagen Germany) In pilot equipment from the same company, a pressurization gradient of 40 MPa/s is possible. NC – Hyperbaric is an industrial system with a horizontal vessel layout, a vessel volume of 55 L, vessel length of 2000 mm, and diameter of 200 mm. The high pressure generation of NC equipment is based on an indirect

compression method with one built-in intensifier, which is usual for industrial systems. Indirect compression uses a high pressure intensifier to pump a pressure medium from a reservoir into the high pressure vessel (Figure 3.6).

a)  b) 

Figure 3.5: a) NC-Hyperbaric, model Wave 6000/55, Burgos, Spain; b) Uhde High Pressure Technologies, model HDS 08-0003, Hagen, Germany

It operates on the principle of pressure intensification through a double-effect piston and works in a single stage from 0 up to 600 MPa. The operating pressure of the system of 40 - 600 MPa is built up by water as a pressure-transmitting medium.

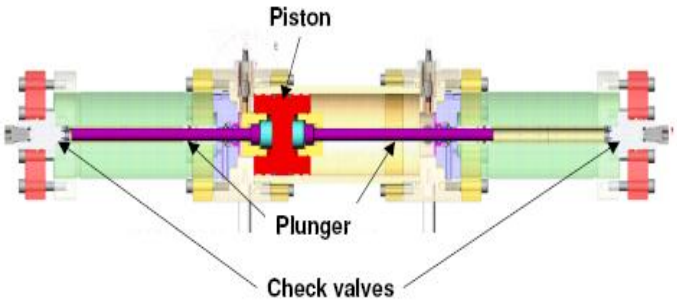
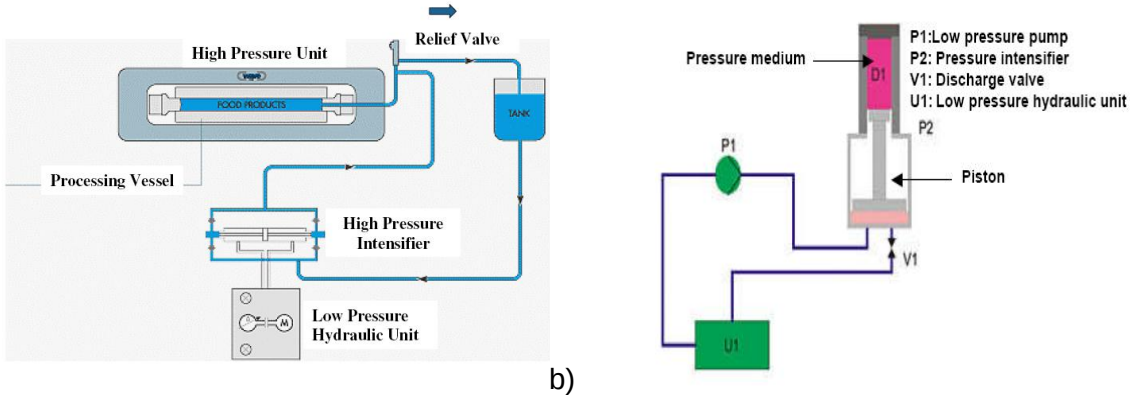


Figure 3.6: Intensifier- intensification through a double-effect piston

The high pressure chamber of the preservation unit is made of age-hardened stainless steel, pre-stressed by high strength wire winding. The manufacturing process of the chamber guarantees that the interior is always under compression, thereby enormously increasing its fatigue life. The loading and unloading of product containers is automated. The low-pressure filling system uses an independent circuit from the high pressure one, without the presence of valves that are subject to high pressure. There are two independent pumps, which are responsible for filing/draining the chamber and for feeding the intensifiers. The productivity of the NC Hyperbaric equipment can be calculated as a function of vessel fill percentage; pressure level, and pressure hold time. For example, for a treatment cycle with a vessel fill percentage of 60 % at 600 MPa for 5 minutes holding time, the product capacity is 179 kg/h, which means 5.4 cycles per hour.



a) b)

Figure 3.7: a) Indirect compression (NC, Hyperbaric), b) direct compression (UHDE)

Uhde HPP pilot equipment is a labor scale designed to pressurize small batches for scientific research. The system consists of a high pressure vertical vessel with an integrated pressure intensifier and mobile frame. For pressure generation, a direct compression method is used. The large diameter end of the piston is driven by a low-pressure pump, which allows very fast compression with a pressure gradient (PG) of 40 MPa/s. The maximum operating pressure of the equipment is 800 MPa. The volume of the vessel is 3 l with diameter of 98 mm and length of 330 mm. Loading and unloading of product, as well as filling of water to be used as the pressure medium, are not automated and have to be done manually.

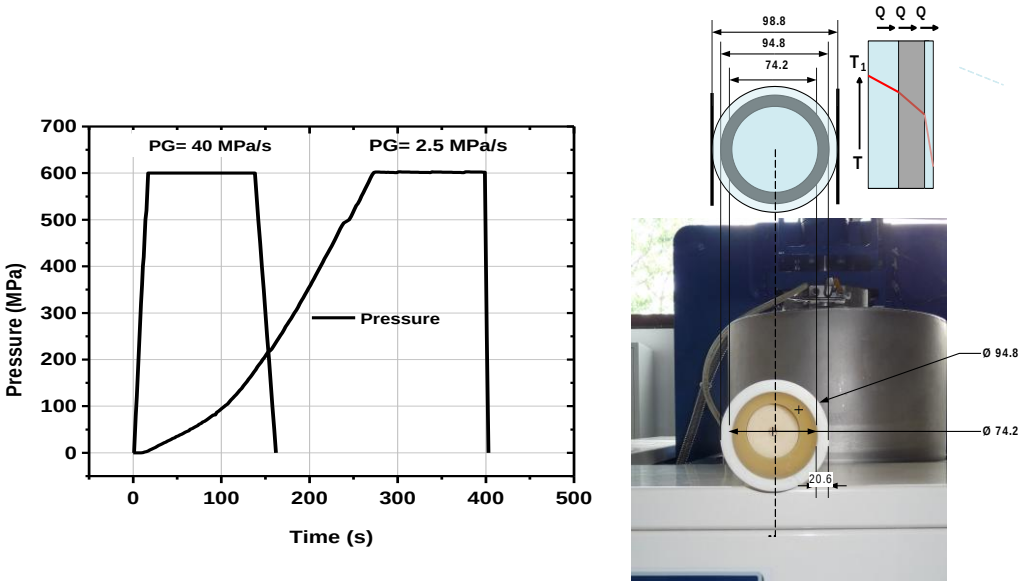


Figure 3.8: High pressure build-up rate- pressurization gradient (PG)

Adiabatic heating occurs during compression of all compressible materials, due to inner friction that occurs under extreme pressures. This compressive adiabatic heating was used to obtain the process temperature for modeling the p-T effects on the firmness, WHC, and color. To reach a uniform and constant temperature during the holding time of a high

pressure treatment, a Teflon liner filled with heated water was used. The rate of heat loss can be express with the Newton's law of cooling.

$$\frac{dQ}{dt} = h \cdot A(T_1 - T_2) \tag{3.21}$$

where Q is the thermal energy in joules, h is the heat transfer coefficient, *A* is the heat transfer surface area, T_1 is the water temperature, and T_2 is the vessel temperature. The rate of the convective heat transfer can be calculated using the following equation:

$$q = hA(T_1 - T_2) \tag{3.22}$$

A pressure of 600 MPa in the UHDE pressure equipment can be reached in about 15 s, corresponding to a pressure build-up rate or pressurizing gradient (PG) of 40 MPa/s. The treatment time is measured after reaching the desired pressure. Measuring the temperature profile inside the vessel was not possible. The process sample temperature was based on the dependency observed by Maslak (personal communication, 2010) (Figure 2.14). According to his observations, the adiabatic heating was dependent on the initial temperature, with an increase of about 0.33°C° per 10°C dT. That means that a batter sample with an initial temperature of 50°C would have adiabatic heating of 4.5°C/100 MPa, which after pressurizing up to 600 MPa would reach a temperature of 77°C (Figure 3.9).

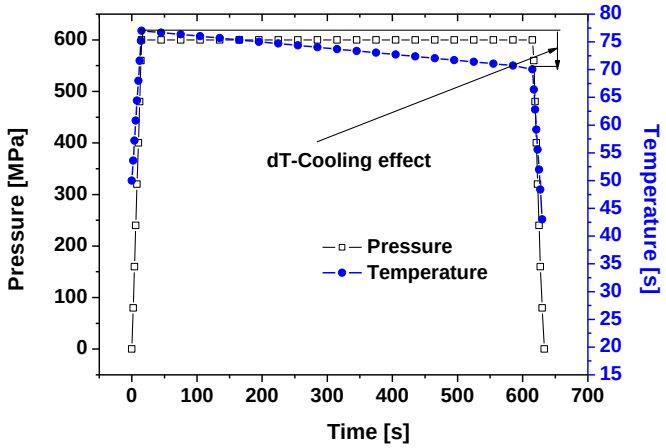


Figure 3.9: Pressure- temperature profile during pressure build-up rate, pressure holding time and decompression (UHDE- pressure equipment).

The process temperature was calculated as the mean value of the temperature difference between the holding time start temperature and the holding time end temperature ($T_{process} = \frac{T_{after\ adiabatic heating} + T_{end\ of\ holding time}}{2}$).

The cooling effect was calculated using equation (3.22) and was different depending on the initial temperature, the level of adiabatic heating and the treatment time. Therefore during the treatment, the decrease in temperature was calculated from the temperature difference for each pressure temperature combination as a function of the holding time. Functional properties of the batter at the process mean temperature for each pressure/temperature/holding time combination were used in the model.

3.2.1 Treatment variables

Experiments were carried out according to a factorial plan with hydrostatic pressure, sausage batter temperature before pressurization (ϑ), pressure treating time (t), and pressurization gradient (PG) as cause variables, and with empirical parameters of oscillation function, water holding capacity, and sausage batter color as effect variables.

3.2.1.1 Treatment variables for estimating pressure-temperature landscape

The pressure-temperature landscape (p-T diagram) is the most concise way of presenting the combined effects of pressure and temperature. The treatment variables used for obtaining the functional properties relationship as functions of pressure and temperature are shown in table 3.4.

Table 3.4: Treatment variables for obtaining p-T landscape

	Pressure (MPa)	***0.1- 600 MPa***
Variables	Pressurizing time	1-600 s
	Pressurizing gradient (PG)	2.5 MPa/s; 40 MPa/s
	Product initial temperature	10-50 °C
Ion concentrations	NaCl	(2%; 1%; 0.5 %)
	Phosphate	(0.3%; 0.2%; 0.1%)

3.2.1.2 Treatment variables used for estimating protein solubilization

Solubilization of meat proteins was studied using the treatment variables according to Table 3.5. Solubilization in the low pressure range 100; 200 and 300 MPa for 1; 5; 10 minutes holding was studied (step 1- Figure 3.10). At this first step, sausage batter with reduced NaCl and Phosphate content was obtained so that the dependency of the solubilization of meat proteins on pressure level, holding time, temperature, as well as NaCl could be studied.

Table 3.5: Treatment variables for obtaining protein solubilization levels (step1) and effect of solubilization on the final matrix (step1+2)

Variables	Pressure Step 1	100; 200; 300 MPa
	Pressure Step 2	600 MPa
	Pressurizing time Step 1	1; 5; 10 min.
	Pressurizing time Step 2	4 min.
	Pressurizing gradient (PG)	40 MPa/s
	starting temperature	10; 40 °C
Ion concentrations	NaCl	(2%; 1%; 0.5 %)
	Phosphate	(0.3%; 0.2%; 0.1%)

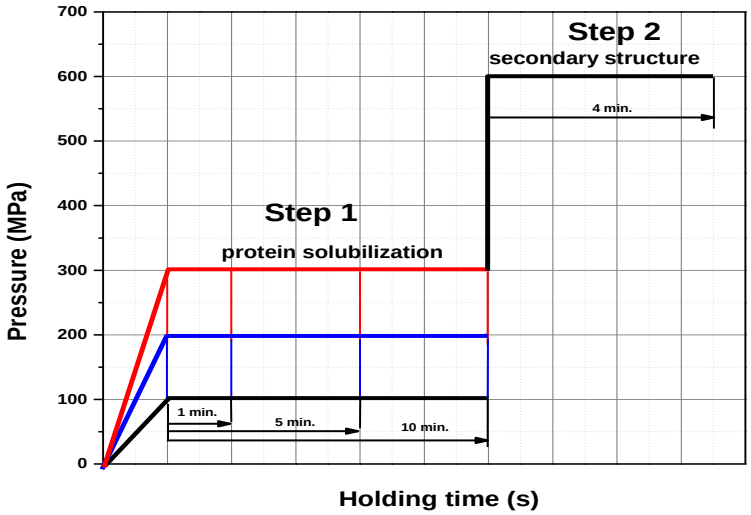


Figure 3.10: High pressure profile with two steps: first step to induce protein solubilization, and second step to modify the secondary sausage batter structure

The second step was done at 600 MPa as shown in Figure 3.10. For the two-step treatment, two different temperatures were used, 10 and 40°C. A low temperature of 10 °C was used to investigate only the high pressure treatment influence of protein solubilization in the range 100- 300 MPa, as well as the subsequent structure modification at 600 MPa. A temperature of 40 °C was used to investigate the combined pressure-temperature effect on solubilization and secondary structure formation.

3.3 Analytical Methods

The determined parameters after the high pressure/temperature treatment, along with the analytical methods used to obtain them, are listed in Table 3.6.

Table 3.6: Determined parameters and analytical methods used to obtain them.

Parameter	Method
Storage modulus	Oscillatory test
Firmness	Oscillatory test
Maximal elasticity	Oscillatory test (Amplitude sweep)
Drip loss	Modified method according to Hamm (1972)
Cook loss	Modified method according to Hamm (1972)
Protein content	HPLS analysis
Protein content	Kjeldahl analysis
Qualification of soluble proteins	SDS- Page
Color	L*a*b*-values (Photometric)
NaCl content	Chemical (Titrimetric)
NaCl taste	Sensoryl Analysis
Juiciness	Sensory Analysis
Firmness	Sensory Analysis

3.3.1 Oscillatory tests

3.3.1.1 Characterization of network firmness

The oscillatory test is an appropriate non-destructive technique to characterize firmness of sausage batter structure. Storage modulus, G', represents the strength which is necessary to reversibly stretch internal structural elements (Figure 3.11- b). The storage modulus–frequency function was measured (AR 2000, TA Instruments) using a parallel-plate geometry (Figure 3.12- b), which can be fitted to an empirical power law function as shown in Figure 3.11- a (Bindrich 2008).

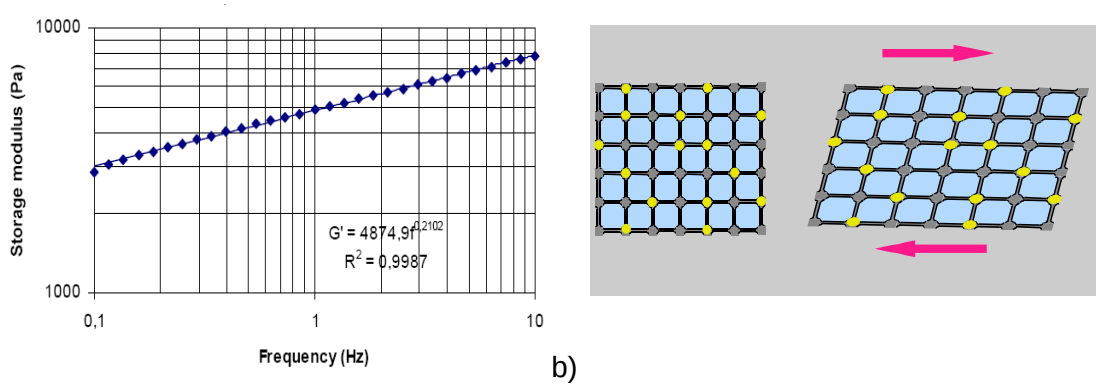


Figure 3.11: a) Storage modulus-frequency function of sausage batter; b) Reversible deformation of a parallel-plate geometry (Bindrich 2008)

The visco-elastic behavior of model (frankfurters) batter corresponds to the Maxwell model, which contains a Hookean spring in series with a Newtonian dashpot according to Figure

3.12-a. Changes of rheological properties are a result of structure modifications and/or changes of the physico-chemical state of the internal network.

The storage modulus-frequency function was evaluated according to the power law function (3.23), where the coefficients are used to characterize changes of rheological properties.

$$G' = k.f^{n} \qquad (3.23)$$

Where *G'* is the storage modulus, *f* is the frequency, and *k* and *n* are empirical parameters. The parameter *k* corresponds to the firmness of the visco-elastic material while *n* represents the viscous component in the un-destroyed-structure state.

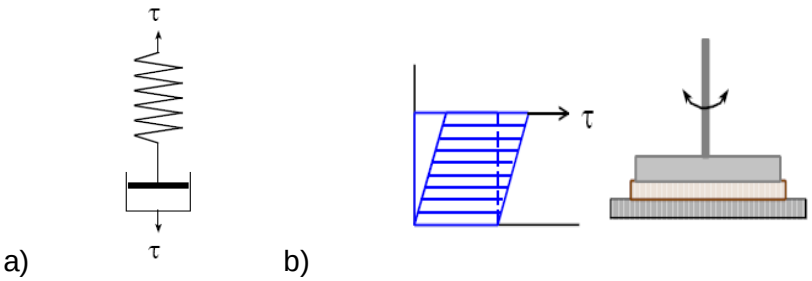


Figure 3.12: a) Maxwell- model of visco-elastic behavior; b) Parallel plate geometry.

For studying temperature-induced changes in rheological behavior, such as gelatinization and denaturation, a time sweep conducted in conjunction with a controlled change in temperature up to 80°C at 1 Hz was carried out.

3.3.1.2 Characterization of linear elasticity: Amplitude sweep

During an amplitude sweep the amplitude of shear stress (deformation) is varied while the frequency is kept constant. Measured G' as a function of deformation is plotted in Figure 3.13. The region where G' is constant, which means that the sample is undisturbed, is called linear-elastic. The structure is disturbed when G' starts to decrease, at a deformation near 0.0015.

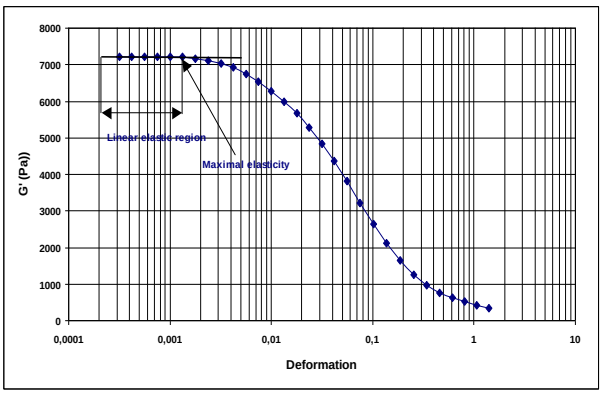


Figure 3.13: Characterization of linear elasticity using amplitude sweep. G' is plotted against the deformation.

3.3.2 Characterization of water holding capacity (WHC)

Determination of WHC was performed by the methods of drip loss, cook loss, as well as purge loss as a control of the cook loss

3.3.2.1 Drip loss

The ability of sausage to retain water (sometimes called serum) was determined on the basis of the amount of drip loss resulting from samples with weight of 1g between double layers of filter paper sheets being treated with pressure of 19.62 kPa for 5 minutes according to the modified method of Hamm (Hamm 1972). The percentages of drip loss were calculated according to equation (3.24).

$$Drip\,loss\,(\%) = \frac{W_1 - W_2}{W_1} 100 \qquad (3.24)$$

Where W_1 is weight before and W_2 after cooking

3.3.2.2 Cook loss and Purge loss

Samples (30 g) packed in plastic bags were cooked for 20 min fully submerged in a water bath at 80 °C. After cooking, the samples were cooled in running tap water for 30 min. Free water was removed from the surfaces of the samples with an absorbent paper towel. They were then weighed and the percentage of cook loss was calculated according equation (3.25).

$$Cook\,loss\,(\%) = \frac{W_1 - W_2}{W_1}\ 100 \qquad (3.25)$$

Where W_1 is the initial weight before pressurization and W_2 is the weight after pressurization. Purge losses were determined by weighing the meat juice left in the plastic bags, and was used as a check to the cook loss.

3.3.3 Batter network structure

Sausage batter structure was investigated by Scanning Electron Microscopy (SEM). Volume elements of edge length of 1.5 mm were frozen in super-cooled liquid N_2 and broken. Free water was removed by sublimation. Sample surfaces were sputtered with gold and images were obtained by cryo- SEM (JEOL JSM- 6460, Japan) at -180°C. The SEM apparatus is shown in Figure 3.14.

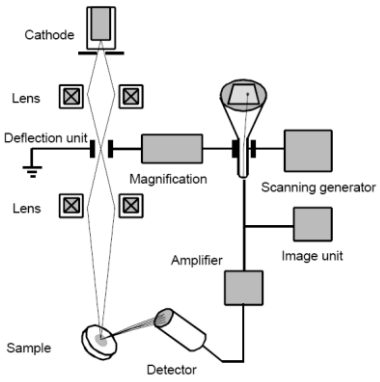


Figure 3.14: Scanning electron microscopy apparatus

3.3.4 Qualitative and quantitative analysis of batter protein solubility

Sausage batter (28g) was diluted with bi-distilled H_20 (15 ml) in order to achieve a total protein content of 1% - 5% (w/w). After preparation, the batter dispersion was allowed to equilibrate under mild agitation for 1 hour at 20°C. After equilibration, samples were centrifuged at 18,000 g for 20 minutes at 10 °C (Sorvall Ultra centrifuge; OTD Combi, Du Pont Company, Wilmington Delaware, USA). Up to three distinct phases could be recovered after centrifugation (Figure 3.15).

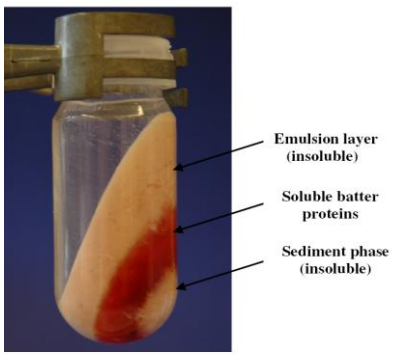


Figure 3.15: Phases recovered after centrifugation of batter

The insoluble matter formed sediment at the bottom of the tube and an emulsion was also formed. The subnatant phase between the sediment and emulsion layers contained the

soluble matter. The aqueous phase was carefully piped out, chilled, and stored in preparation for measurement of the total protein content according to Kjeldahl (3.3.4.1), SDS-PAGE analysis (3.3.4.3) and HPLC analysis (3.3.4.2).

3.3.4.1 Total protein count according to Kjeldahl

The method according to Kjeldahl is the most common method in determining the total protein content. First, it requires that the total nitrogen be determined in a protein-containing sample. The method is laid down in § 64 of the German Food and Feed Code (LFGB, formerly § 35 of the German Food and Commodity Goods Law LMBG). The organically combined nitrogen is transformed into ammonia sulphate by digesting the substance with concentrate of sulphuric acid. The ammonia produced when excess base is added is carried over by distillation, collected in a saturated boric acid solution and subsequently titrated with 0.1 M hydrochloric acid. The raw protein content is determined by multiplying the measured nitrogen content by 6.25.

3.3.4.2 HPLC analysis

High performance liquid chromatography HPLC analysis was performed using a Waters 2695 Alliance Separations Module equipped with a Waters 2414 Refractive Index (RI) Detector (WATERS, Milford MA, USA). The protein content was determined by SEC-HPLC. An isocratic flow with pure aqueous phosphate buffer (0.15 M) was used. Analyses were carried out at room temperature at a flow rate of 0.5 mL/min using a Superdex 200 10/300 column (10 * 300 mm, 13µ, GE HEALTHCARE, Munich, Germany). Peak detection was performed using UV at 214 nm. Quantification of total protein content has been done using the Kjeldahl analysis for several samples, and calculation of total peak area.

3.3.4.3 SDS-PAGE analysis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to qualify soluble proteins. Commercially available “Criterion Precast “gels (4 to 20%) were used for all experiments (BioRad, München, Germany). The Bio- Rad Mini Protean electrophoresis cell (BiorRad, München, Germany) at voltages of 100 mA and 60mA was used. A buffer solution of 12.1g Tris, 7.5g Glycin, 1 g SDS per 1000 ml bi- distilled H_20 was used. Samples were pre-treated according to the Laemmli method (Laemmli 1970). The gels were stained with 80 ml Coomassie blue-R350 and distained in a 200 ml solution containing methanol/acetic acid/bi- distilled H_20 (30:10:60, v/v/v).

The molecular weight of each band was estimated by comparing the migration distance with that obtained for standard proteins of known molecular weight. A SigmaMarker^{TM} Wide Molecular Weight Range Standard (MW 6500-205000 D Sigma-Aldrich, Steinheim, Germany) was migrated alongside the other samples. After fixation and drying, each gel was scanned and analyzed with an image-analysis software (UN-SCAN-IT gel, Version 6.1, Gel and Graph Digitizing Software, Silk Scientific Inc, Utah, USA).

Quantification of protein band by image analysis

The program used to analyze the high-resolution image of the gel is especially designed for the quantitative analysis of electrophoretic bands. The intensity of the staining of a band is expressed as a volume, obtained by multiplying the pixel intensity of the image by the surface area of the band. This band volume is proportional to the area under the curve on the obtained electrophoretogram. The quantification of the bands is dependent on the settings applied during image analysis of the scanned gel image, so the standardization of settings was important. The key parameters used are summarized below.

Lane detection: Automatic lane detection was used with the number of tiers set on 1. If necessary, a manual adjustment of lane edges was carried out as appropriate, as well as adjustments to account for lane deformation and band grimaces.

Background removal: The subtraction method used is called “rolling ball” and the radius was set at 1000. This method was found to be the most efficient to account for variations in background intensity within as well as between lanes

Band detection: Only a manual marking of each individual band was found to provide an accurate result. The software automatically set the position of band edges

The calculated volume for each band in each lane was given in tabular form in the program.

3.3.5 Color measurements

Color measurements of meat have been performed using a colorimeter CM- 700d/ 600d from (Konica Minolta Sensing, Langenhagen, Germany) utilizing the specular component mode that includes diffuse and specular reflection. In this way, the color can be evaluated independent of the surface structure. Illumination was performed by D65 (standard illuminant defined by the International Commission on Illumination), which is intended to represent average daylight and has a correlating color temperature of approximately 6500 K. The results are expressed within the L*a*b* color space which is based on the uniform distribution of colors and is very close to human perception of color. L* is the luminance or lightness component, ranging from 0 to 100, and the parameters a* (from green to red) and b* (from blue to yellow) are the two chromatic components, which range from -60 to +60.

3.3.6 Determination of chloride content in the soluble phase

The preparation of the soluble phase was done as already described in chapter 2.3.4. The determination of salt content (chloride) in the soluble phase of the frankfurters batter was done according to the standard method summarized in § 64 of the German Food and Feed Code (LFGB, formerly § 35 of the German Food and Commodity Goods Law LMBG).

3.3.7 Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed using a MDSC 2920 (TA Instruments, Eschborn, Germany). Samples of about 20 mg were inserted into a standard aluminum pan, which was then hermitically sealed and heated from 10-120 °C with a rate of 10 K/min. Untreated samples were used as reference. The total denaturation enthalpy ($\Delta H_{T'}$) was determined as the peak area above base line. Enthalpy was expressed in J/g.
The heat flow rate to the sample depends on whether the process is exothermic or endothermic and can be obtained according to equation (3.26).

$$\frac{dQ}{dt} = c_p \cdot \frac{dT}{dt} + f(t,T) \qquad (3.26)$$

Where *dQ/ dt* is the heat flow rate measured in W, c_p is the specific heat capacity ($J.g^{-1}/K$), *dT/dt* is the heating rate measured in Ks^{-1}, and *f(t,T)* is a function capturing other time dependent parameters.

3.3.8 Texture measurements

To measure texture, a Warner-Bratzler shearing blade with a thickness of 3.21 mm, length of 125 mm was assembled to a TA.XT2® Texture Analyzer (Stable Micro Systems, Surrey, England). It shears or cuts through the sample with a test speed of 1 mm/sec. The computer software was set to plot force versus time during testing and the results were expressed as the maximum peak force (cutting force in N) required to shear through the sample. Samples of MDM turkey sausages were prepared with a length of 20 mm and diameter of 20 mm. This test method incorporates compression of samples beneath the blade, tension in the adjoining batter matrix, and shearing of the sausages. Mean value of the cutting force was obtained by measuring of 5 similar samples from a particular sausage.

3.3.9 Sensory analysis

The sensory analysis was done according to the paired comparison test. The method is laid down in § 64 of the German Food and Feed Code (LFGB, formerly § 35 of the German Food and Commodity Goods Law LMBG) and correspond to ISO 5495.

Sample preparation:

Pressure-temperature treated samples were compared to conventional thermally treated samples. Pressure treatment was performed at 600 MPa and initial core temperature of 30-50 °C. Thermally treated samples were done according to the standard recipe, whereas pressure treated samples with reduced sodium content of 1% NaCl were also performed. Pork and poultry (Turkey) MDM Frankfurters were analyzed. After preparing (cutting and labeling) the samples (pressure and temperature treated), they were cooled at 4°C.

Performing the analysis:

The difference between pairs of samples was evaluated using 8 selected assessors to answer the following questions:

Which sample is saltier?

Which sample is juicier?

Which sample is softer?

Analysis of results:

There are two possible forms of the sensory analysis test. The first is concerned with the detection and the determination of the direction of specified differences between two products, and the second is concerned with a preference for one of them. In both cases, a null hypothesis can be made. In statistical terms, this is expressed by stating that for each assessor participating in the test, the probability is the same for either A or B being designated as having the greater intensity (or being preferred), i.e., $P_A=P_B=1/2$. Where

$$P(X = x / p, n) = P_{n,\,p}(x) = \binom{n}{x} p^x q^{n-x} = {}_nC_x p^x q^{n-x} = \frac{n!}{x!(n-x)!} p^x q^{n-x} \quad (3.27)$$

where x= 0,1,2…n

From equation (3.27), it follows that

$$(p+q)^n = \sum_{x=0}^{n} \binom{n}{x} = p^x q^{n-x} = 1 \quad (3.28)$$

Where

p+q=1⇔1/2+1/2=1

Scoring, significance level:

When the results of a test are analyzed, there are two possible conclusions. The null hypothesis is rejected or not rejected.

In this work, two significant levels are used α=0.05 (5%) or α=0.01 (1%). It is important to note that, for the purposes of this study, the null hypothesis can be rejected at the “5 % level”, but it cannot be rejected at the “1 % level”.

4 Results and discussion

4.1 Rheological characterization of meat batter during storage depending on salt content

Rheological characterization of batter during storage time was necessary for defining the initial quality of the studied batter samples. Analyzing their visco-elastic and structural properties as well as their optimal treatment point (pressure/temperature) was of great importance in assuring the maximum accuracy of the study.

The impact of storage duration was found to depend on the ion concentration (NaCl and phosphate content), and in fact, a significant relation between batter firmness and salt concentration was found, as shown in Figure 4.1. Batters with reduced NaCl (0.5 %) content were found to be significantly firmer than conventional batter (with 2 % NaCl). A possible explanation for this effect is suggested to be the influence of the ionic strength, affecting protein solubility and firmness. A significant tendency of batter rheological behavior (firmness profiles) during the storage time and specifically to the different batter recipes was detected.

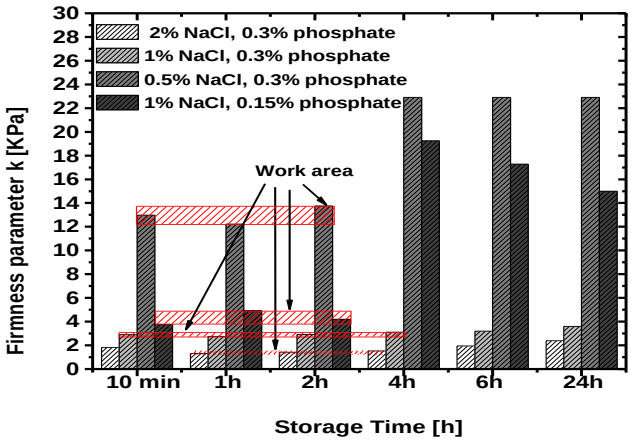


Figure 4.1: Gel firmness of frankfurters pork batter as a function of storage time for various NaCl concentrations.

An increase in firmness and network building capacity with increasing storage time was observed. After 4 hours of storage an abrupt firmness increase for the low sodium gel (0.5 NaCl, 0.3 phosphate) was detected. Similar rheological behavior was also reported in the literature (Hammer 2001; Euring, Grupa et al. 2009).

According to the firmness behavior during the storage time, work areas with similar initial parameter (firmness) for each recipe were defined (Figure 4.1). Batters less than 4 hours old having similar rheological character and functional properties were used for the HP-

temperature treatment. Storage of frankfurters batter longer than the 4th and up to 24th h caused some firmness changes (Figure 4.1). Profile of firmness parameter increased for 2% and 1% NaCl, stable firmness profile for 0.5% NaCl and decrease for 1% NaCl, 0.15% phosphate recipes were measured estimated.

The work area (or the time interval after batter preparation where pressure-temperature processing was performed, assuring similar initial properties) for reduced phosphate recipes was defined as shown in Figure 4.2.

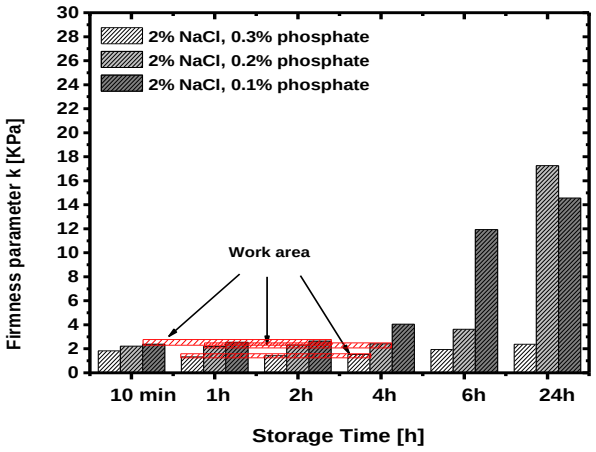


Figure 4.2: Gel firmness of frankfurter pork batters as a function of storage time for various phosphate concentrations.

According to the results the reduction of NaCl content was found to have a greater impact on the batter firmness and the functional properties of frankfurters batter compared to reduce phosphate content batters.

4.2 Influence of ion concentration on the Gel firmness, Drip and Cook loss at 300 and 600 MPa and different temperatures

4.2.1 Pressure- temperature treatment at low PG= 2.5 MPa/ s and high PG= 40 MPa/s on conventional and NaCl reduced frankfurter (pork) batters

An investigation of the effect of pressurization gradient (PG) on gel firmness, drip loss and cook loss of batters with diverse ion concentrations (combinations of NaCl and phosphate) was performed. Pressure levels at 300 and 600 MPa for 10°C, 30°C and 40°C initial temperatures and a holding time of 240 s were used. Pressurization was carried out at low PG- 2.5 using industrial high pressure equipment (NC, Burgos, Spain). Analysis of the

measured data showed that rheological behavior and WHC of sausage batter was significantly dependent on all considered cause variables and their interactions (Figure 4.3, Figure 4.4 and Figure 4.5).

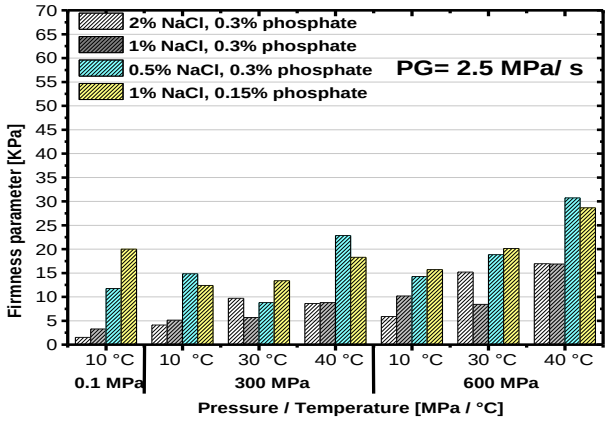


Figure 4.3: Gel firmness of frankfurters pork batter for different NaCl contents as a function of temperature after pressurizing at PG= 2.5 MPa/s to the desired set pressure for a 240s holding time.

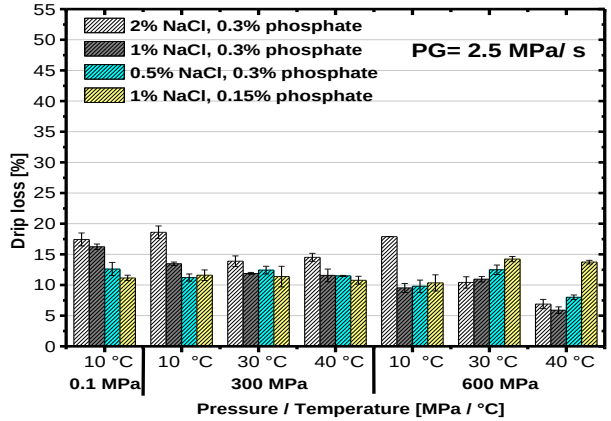


Figure 4.4: Influence of reduced NaCl on WHC expressed as Drip loss by combined HP-T treatment, plotted as a function of temperature after pressurizing at PG= 2.5 MPa/s to the desired set pressure for a 240s holding time.

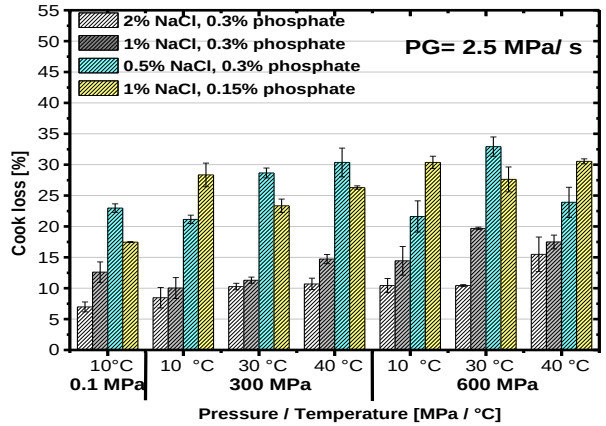


Figure 4.5: Influence of reduced NaCl on WHC expressed as Cook loss by combined HP-temperature treatment, plotted as a function of temperature after pressurizing at PG= 2.5 MPa/s to the desired set pressure for a 240s holding time.

To isolate the effect of high pressure, a low initial temperature of 10°C was used. The combined pressure- temperature was carried out at 30°C and 40°C. As expected, batter firmness increased with increasing pressure, additionally firmness also increased with increasing temperature. Maximum batter firmness for all recipe combinations was found at 600 MPa pressure and 40°C (Figure 4.3). Temperature was observed to amplify the pressure induced effects.

Minimum drip loss was observed at 600 MPa and 40°C, except for recipe with 1% NaCl, 0.15 phosphate (Figure 4.4). Cook loss maximum was observed at 600 MPa and 30 °C for the 1% and 0.5% NaCl content batters, and at 600 MPa and 40°C for the 2% NaCl content batter and for the 1% NaCl, 0.15 phosphate batters.

The impact of high PG (40 MPa/s) on firmness, drip loss and cook loss was estimated using the pilot scale UHDE, model HDS 08-0003, Hagen, Germany equipment and presented in Figure 4.6, Figure 4.7 and Figure 4.8.

The maximum firmness was found at 600 MPa and 40°C for 2% and 1% NaCl as well as 1% NaCl, 0.15 phosphate, and at 600 MPa and 30°C for 0.5% NaCl (Figure 4.6). Decrease of drip loss with increasing pressure was detected, with a minimum observed at 600 MPa and 40°C (Figure 4.7). The maximum cook loss correlated with the maximum firmness at 600 MPa and 40°C for 2% and 1% NaCl, as well as 1% NaCl, 0.15 phosphate, and at 600 MPa and 30°C for 0.5% NaCl (Figure 4.8).

The PG effect on batter firmness at low (Figure 4.3) and high (Figure 4.6) pressurization rate were compared. Batters with 2% NaCl and 1% NaCl, 0.15% phosphate at low PG were significantly firmer at 600 MPa and 40°C than at high PG. Conversely, batters with reduced 0.5% NaCl were significantly firmer at high PG for all pressure-temperature combinations, with a maximum at 600 MPa and 30°C. The effect of PG was most clearly seen in the reduced 0.5% NaCl content batter, where the in-salting effect did not play as major a role as that of HP and PG. After HP treatment at low PG a positive effect of drip loss reduction for all recipes was detected (Figure 4.4 and Figure 4.7). The similar by more distinct reduction effect was observed for the cook loss parameter for the reduced NaCl batters (Figure 4.5, Figure 4.8).

In summary, low PG was found to have a positive effect by improving functional properties (WHC and firmness).

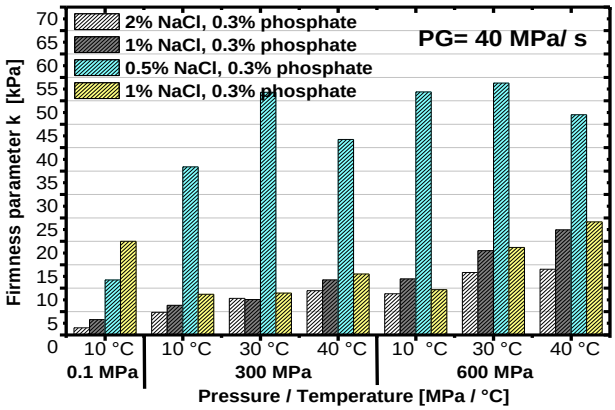


Figure 4.6: Gel firmness of frankfurters pork batter for different NaCl contents as a function of temperature after pressurizing at PG= 40 MPa/s to the desired set pressure for a 240s holding time.

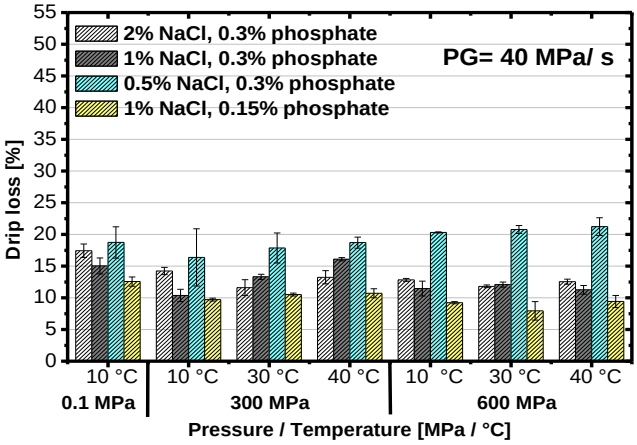


Figure 4.7: Influence of reduced NaCl on WHC expressed as Drip loss by combined HP-T treatment, plotted as a function of temperature after pressurizing at PG= 40 MPa/s to the desired set pressure for a 240s holding time.

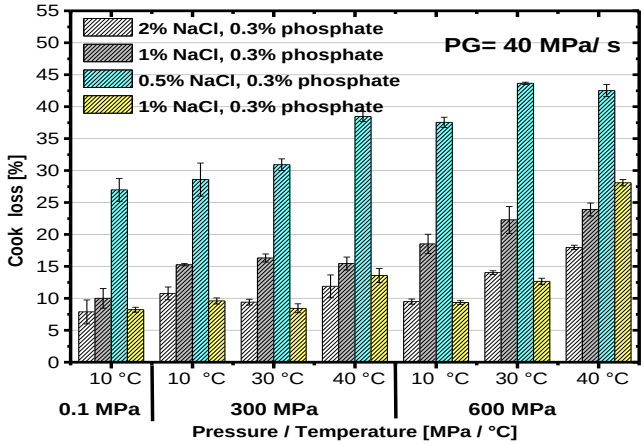


Figure 4.8: Influence of reduced NaCl on WHC expressed as Cook loss by combined HP-temperature treatment, plotted as a function of temperature after pressurizing at PG= 40 MPa/s to the desired set pressure for a 240s holding time.

4.2.2 Pressure- temperature treatment at low PG= 2.5 MPa/ s and high PG= 40 MPa/s on conventional and phosphate reduced frankfurter (pork) batters

Besides NaCl, phosphate content also plays an important role in the determination of batter functional properties. The impact of phosphate content on firmness, drip loss and cook loss of pressure-temperature treated batters was examined. The effect of pressurization gradient (PG) was studied in a manner similar to that of reduced NaCl content, using the same treatment conditions.

Firmness of pork batters at low PG= 2.5 for all recipes was increased with increasing pressure and temperature with a maximum at 600 MPa and 40°C except for the batter with 0.2% phosphate content. For this case, a maximum firmness at 600 MPa and 30°C was found (Figure 4.9). The same firmness behavior was observed at PG= 40 MPa (Figure 4.12). A little drip loss improvement was detected for all recipes at higher pressures for both the 2.5 and 40 MPa/s pressure gradients. The improvement was higher at PG= 40 MPa/s (Figure 4.10 and Figure 4.13).

The Improvement in the cook loss behavior was observed at high pressures for a PG of 2.5 MPa (Figure 4.11). Contrarily, a higher cook loss was found with increasing pressure at PG= 40 MPa/s (Figure 4.14).

In comparing phosphate-ion reduction (chapter 4.2.2) to NaCl-ion reduction (chapter 4.2.1,), it was found that NaCl-reduced batters had firmer structure and better WHC (reduced drip loss) for both PGs. Cook loss of NaCl-reduced batters were higher compared to the phosphate reduced batters.

Summarizing the results from the reduced-ion pressure-temperature treated batters, the closest behavior to the control recipe was estimated for the 1% NaCl batters at low PG (2.5 MPa/s). These had similar firmness, less drip loss and comparable cook loss after the HP-T treatment. Combined reduction of NaCl and phosphate by 50 % (1% NaCl and 0.15 % phosphate) according to the conventional recipe led to firmer batters with very poor water binding.

Improvement in the rheological properties of pork batters and chicken myofibrils after pressurizing and then heating was found by (Iwasaki, Noshiroya et al. 2006). Sikes et al. (2009) also reported improvement in the textural properties of pressurized and cooked beef batters with reduced NaCl. According to these authors, a reduction of NaCl without negative effects on the functional properties of batters was possible. It was proposed that HPP induced an increase in solubilization, resulting in an improvement in the batter matrix. This hypothesis is studied in detail the following chapters using various types of methods and analyses, measuring the quantitative and qualitative changes of the meat proteins as well as structure of the batter matrix (studied by SEM).

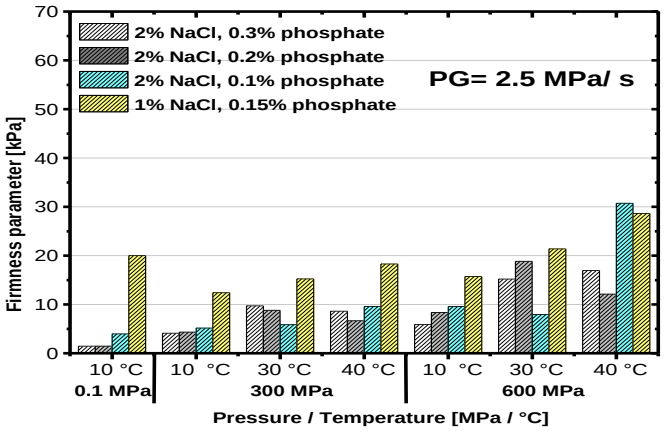


Figure 4.9: Gel firmness parameters of frankfurter pork batters at different phosphate contents as a function of temperature with a PG= 2.5 MPa/s to different maximum pressures.

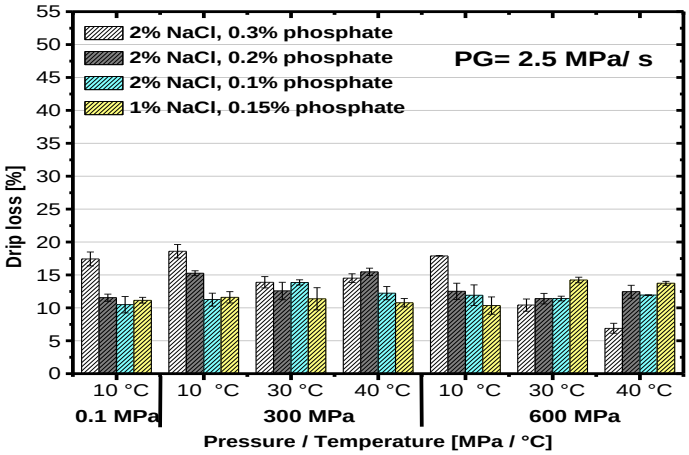


Figure 4.10: Influence of reduced phosphate content on WHC expressed as Drip loss by combined HP-T treatment, plotted as a function of temperature after pressurizing at PG= 2.5 MPa/s to the desired set pressure for a 240s holding time.

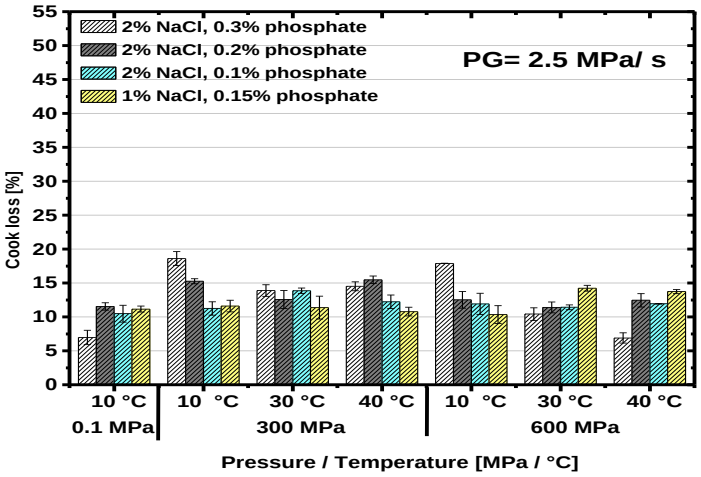


Figure 4.11: Influence of reduced phosphate content on WHC expressed as Cook loss by combined HP-temperature treatment, plotted as a function of temperature after pressurizing at PG= 2.5 MPa/s to the desired set pressure for a 240s holding time.

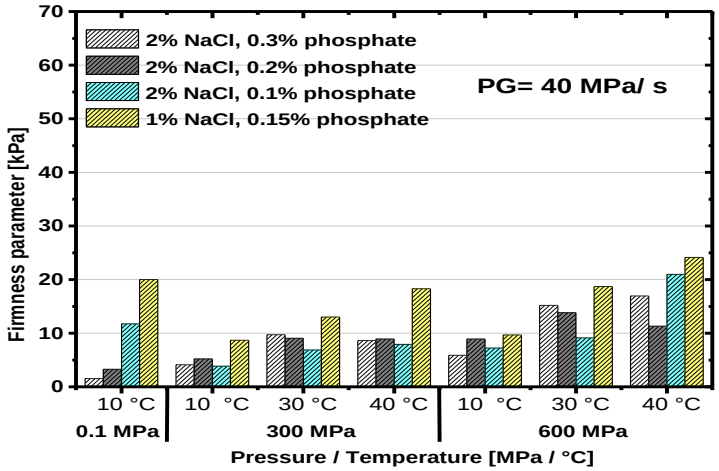


Figure 4.12: Gel firmness parameters of frankfurter pork batters at different phosphate contents as a function of temperature with a PG= 40 MPa/s to different maximum pressures.

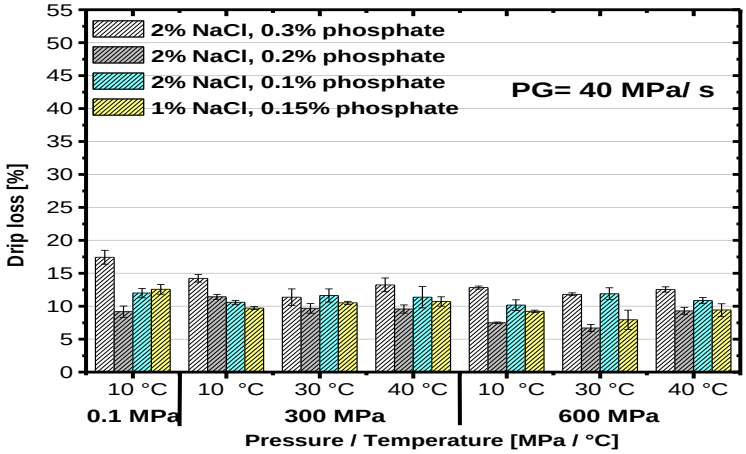


Figure 4.13: Influence of reduced phosphate content on WHC expressed as Drip loss by combined HP-T treatment, plotted as a function of temperature after pressurizing at PG= 40 MPa/s to the desired set pressure for a 240s holding time.

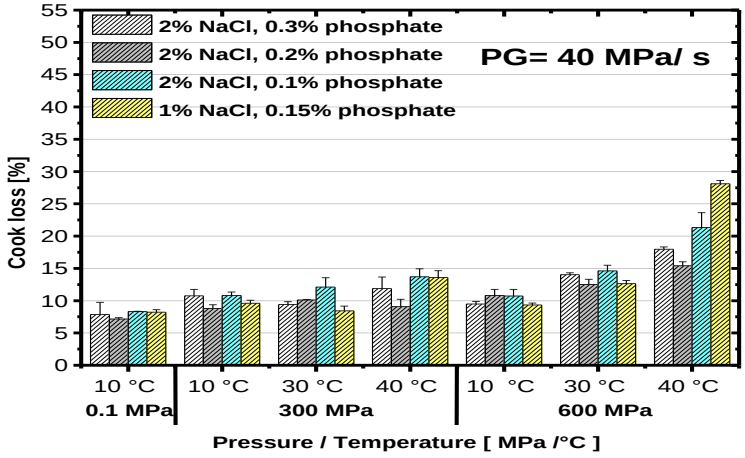


Figure 4.14: Influence of reduced phosphate content on WHC expressed as Cook loss by combined HP-temperature treatment, plotted as a function of temperature after pressurizing at PG= 40 MPa/s to the desired set pressure for a 240s holding time.

4.3 Influence of NaCl and PG on maximum elasticity at 300 and 600 MPa and different temperatures

In addition to the functional parameter of firmness, drip loss and cook loss, the parameter of maximum elasticity is important for better understanding of the visco-elastic behavior under treatment conditions. In Figure 4.15, linear elastic ranges are shown for 2% and 1% NaCl batters after different HP-temperature/PG treatment combinations. The values of maximum elasticity can generally be defined as the point where irreversible changes and structure breakdown occur. This is indicated in the figure as the deformation that results in a change in G'.

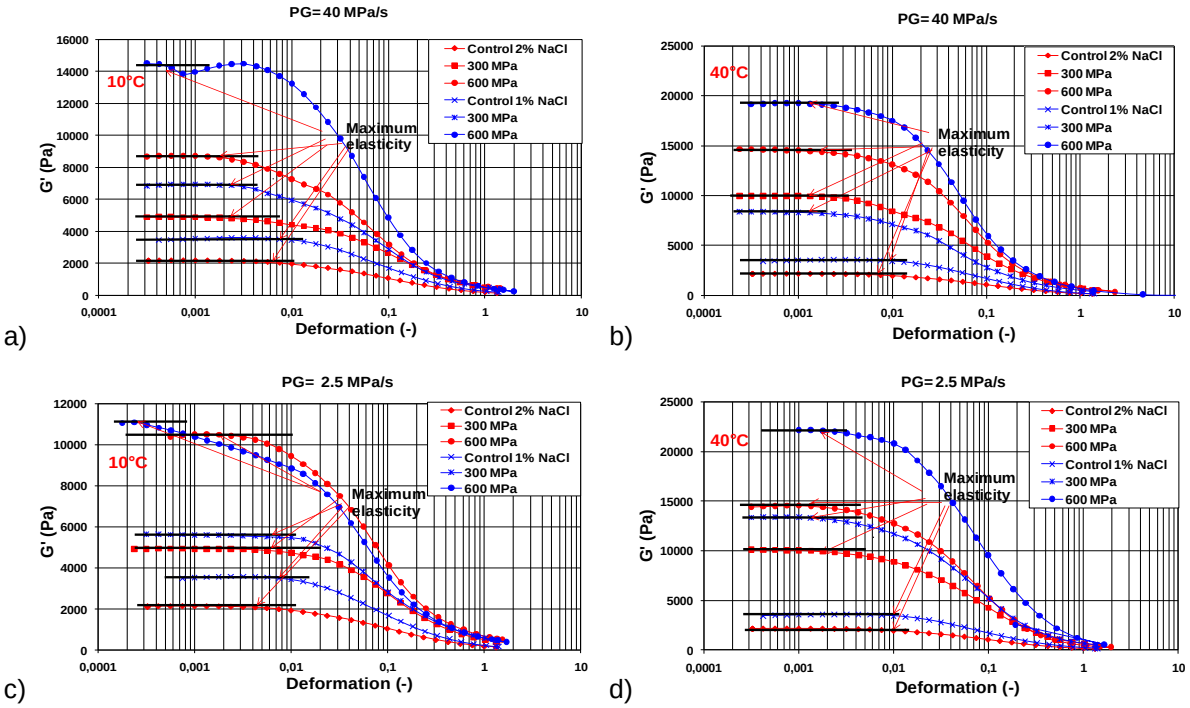


Figure 4.15: Determination of the maximum elasticity at 300 and 600 MPa for 240 s treatment time at high PG (40MPa/ s) and a) 10°C and b) 40°C initial temperature; and at low PG (2.5 MPa/s) and c) 10°C and d) 40°C initial temperature.

The linear elastic region decreased with increasing pressure as some structure changes related to protein denaturation took place. It was found that the maximal elasticity at 10°C was higher at 300 MPa and lower at 600 MPa, similar to samples treated at 40°C. On the basis of these observations, it could be assumed that the pressure in the range of 300 MPa additionally enhanced the denaturation (disintegration process). With increased pressurization, the denaturation process is believed to be accompanied by improved gelation. With regard to the PG, significant differences at 300 MPa were found, as samples at low PG possessed higher values of maximal elasticity.

4.4 Texture changes comparison of thermal and HP-T treated batters according to cutting force measurements performed by texture analyzer

Texture changes of conventional and reduced NaCl have been studied by using cutting force applied to 0.188 m^2 cylinder surface and measured by texture analyzer TA.XT2 type. Thermally treated batters were treated at 80°C for 20 min, whereas for the HP-T treated batters treatment time of 240 s was used. For the HP-T treatment maximal equipment pressure of 600 MPa at 40°C, 50°C and PG= 2.5 MPa/s for obtaining sausage stable structure have been performed. According to the measurements, thermally treated samples were significantly firmer compared to the pressure treated ones (Figure 4.16). This was in agreement with the results from the sensory analysis discussed in chapter 4.16, where HP-temperature treated samples were softer and juicier than thermal samples.

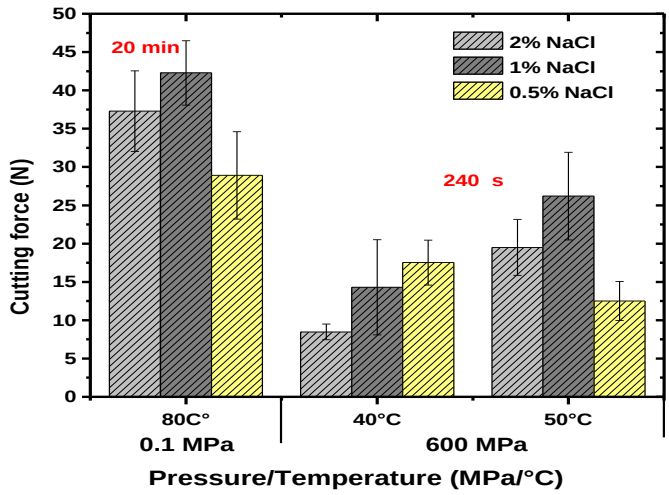


Figure 4.16: Cutting force texture measurements-comparison between thermal and HP-T treated batters after 20 min and 240 s treatment time.

With increasing temperature increased the firmness of the HP- treated Frankfurters as the highest value was detected for the 1% NaCl recipe. According to the results a positive effect of the higher temperature on the batter firmness at this pressure level was obtained. This effect could be explained with the additional impact of temperature on the structure improvment. This improvement can be related to building of more cross-linkages, where increased protein-protein was suggested. The structure improvement through pressure treatment is discussed in detail in the following chapters, using scanning electron microscopy images. Therefore, to obtain sausages with similar firmness compared to thermally treated sausages, higher initial temperatures above 50 °C are needed. Other possible means for

achieving structure hardening include the addition of different fillers by modifying the sausage recipe.

4.5 Impact of HP-T on batter color

4.5.1 Impact of HPP (300 and 600 MPa) on batter color for recipes with different NaCl and temperatures

The combined effects of high pressure (300, 600 MPa) and temperature (10-40°C) treatment on the color of frankfurter batters (for the three NaCl content recipes), after 240 s treatment time, have been studied. Color changes are presented using the L*a*b* color space system. According to that system, values of L* (lightness), a* (redness), and b* (yellowness) parameters for the different batters were analyzed. The results are shown in Figure 4.17.

Generally, it was determined that batter color was stable after HP- temperature treatment with only small changes, on the order of 0.5-2 units. These changes were found to be minimal compared to a previous study of the color of HP-treated raw pork meat, where a significant increase of L* up to 20 units was found (Tintchev et al 2010). In this case (frankfurter batter), it is suggested that Myoglobin (Mb) was stabilized by building a complex formation of nitric oxide myoglobin (NOMb). This complex formation seemed to be pressure-temperature stable (up to 40°C initial temperature).

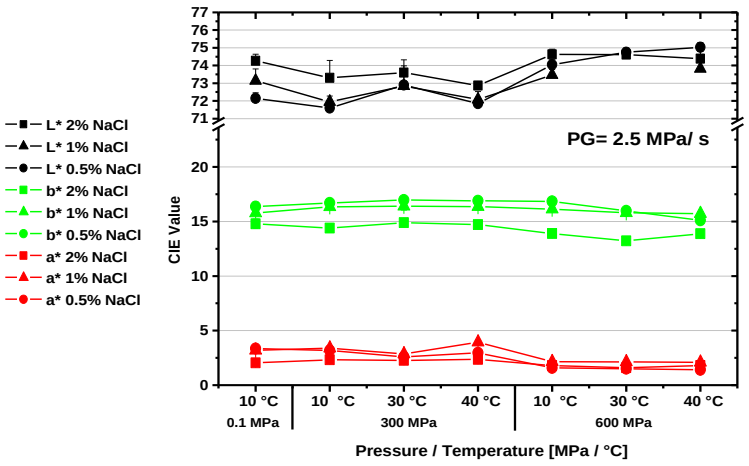


Figure 4.17: Changes of CIE color parameters L*, a*, and b* of frankfurters batter at 0.1, 300, 600 MPa and 10 – 40°C after 240 s treatment time.

At 300 MPa, a small decrease was observed in L* of about 0.5 – 1 units for all recipes, followed by a little increase at higher pressures of 600 MPa. The increase of lightness parameter L* was maximum for the 0.5% NaCl recipe, which confirmed the stabilizing effect

of nitrite under these conditions. For the 1% and 2% NaCl batters, L* increased about 0.5- 1 units compared to the unpressurized sample. An analysis of the b* (yellowness) parameter found that only small changes of 1-2 units occurred at 600 MPa for the 0.5 and 2% recipes, while the 1% NaCl recipe stayed almost unchanged. Small but not significant changes in the a* (redness) parameter at 600 MPa for all NaCl content combinations were also observed.
In summary, the color changes resulting from these pressure-temperature treatment conditions were found to be very small compared to the untreated samples, and therefore it can be assumed that are no significant negative changes from the consumer's point of view.

4.5.2 Impact of HPP (0.1-600 MPa) on batter color for recipes with different NaCl at 40°C

The impact of HPP over the whole pressure range up to 600 MPa, at 40°C and 240 s treatment time, has been examined. The changes of CIE values were compared to the conventional treatment of Frankfurters at 0.1/ 80°C and 20 min.

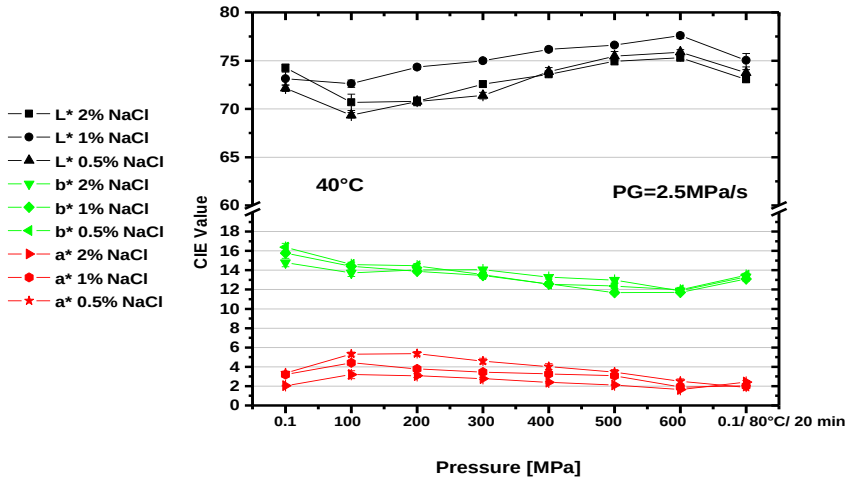


Figure 4.18: Changes of CIE color parameters L*, a*, b* of frankfurters batter HP- treated up to 600 MPa at 40°C after 240 s treatment time.

With increasing pressure increased the L* parameter with maximum value at 600 MPa. This increase was most distinctive for the 1% NaCl batters. The b* parameter was found to decrease with increasing pressure for all batters recipes. According to the measurements an increase of the a* parameter was found in the 100-300 MPa pressure range. This was followed at higher pressures above 300 MPa up to 600 by a decrease until reaching values similar to the control samples. A comparison between the conventional thermal treatment

and the HP-temperature treatment showed some small changes. L*, a*and b* values were similar to the control samples, whereas a small decrease of the L* value and an increase of the b* value compare to the highest pressure treatment 600 MPa/40°C were detected.

4.6 Effect of combined high pressure-temperature treatment and PG on batter structure depending on NaCl content

To investigate the mechanisms of structure modifications of frankfurters batter depending on pressure, temperature, PG and NaCl content, scanning electron microscope micrographs were taken (Figure 4.19). Two control samples (untreated batter structure), one with 2% NaCl and 0.3% phosphate, and the other with reduced 0.5% NaCl and 0.3% phosphate, were compared to the thermally treated samples. Two pressure-temperature combinations based on the maximal differences of the functional properties, such as firmness, drip loss, and cook loss, were chosen based on the analysis in chapter 4.2, namely the pressure-temperature treatments at 300 MPa and 10°C (low P-T-impact) and 600 MPa/40°C (high P-T-impact). SEM- micrographs were found to be a very helpful tool for visualizing and analyzing the primary and secondary meat protein matrix, fat emulsification, and water binding.

SEM micrographs are presented for two magnification levels (10 µm and 5 µm length of bar). Micrographs were taken at both pressurizing gradients, with PG= 40 displayed in the first and third columns of Figure of 4.19, and PG= 2.5 displayed in the second and fourth columns. Comparing the control samples of 0.5% and 2% NaCl batter in Figure 4.19- a and Figure 4.19- c some differences in the protein matrix were observed. The matrix of the reduced-NaCl batter was found to be rougher than that of the conventional batter. Furthermore, the batter network contained a lower number of gaps (expressing sublimated water) and higher structure density. However the matrix of the conventional salted batter (2%NaCl) exhibited a good structure, where more gaps were homogeneously arranged (Figure 4.19- c, d). Swelling of the batter matrix was observed at 300 MPa/ 10°C for the 240 s treatment time as shown in Figure 4.19 e-l. The matrix treated at low PG was more swollen (Figure 4.19- f, g, h, l) compared to the matrix treated at high PG (Figure 4.19- e, I, g, k). The network of the conventional recipe (2% NaCl) was more swollen (Figure 4.19- g, h, k, l) as more water was supposed to be immobilized (see the gap in Figure 4.19- l) compared to the batter with reduced NaCl (0.5 % NaCl) (Figure 4.19- e, f, I, g). These structure modifications could explain the behavior of the firmness, drip loss, and cook loss parameters, discussed in chapter 4.2.1., where the functional properties of the 0.5% NaCl batter at low PG were significantly improved.

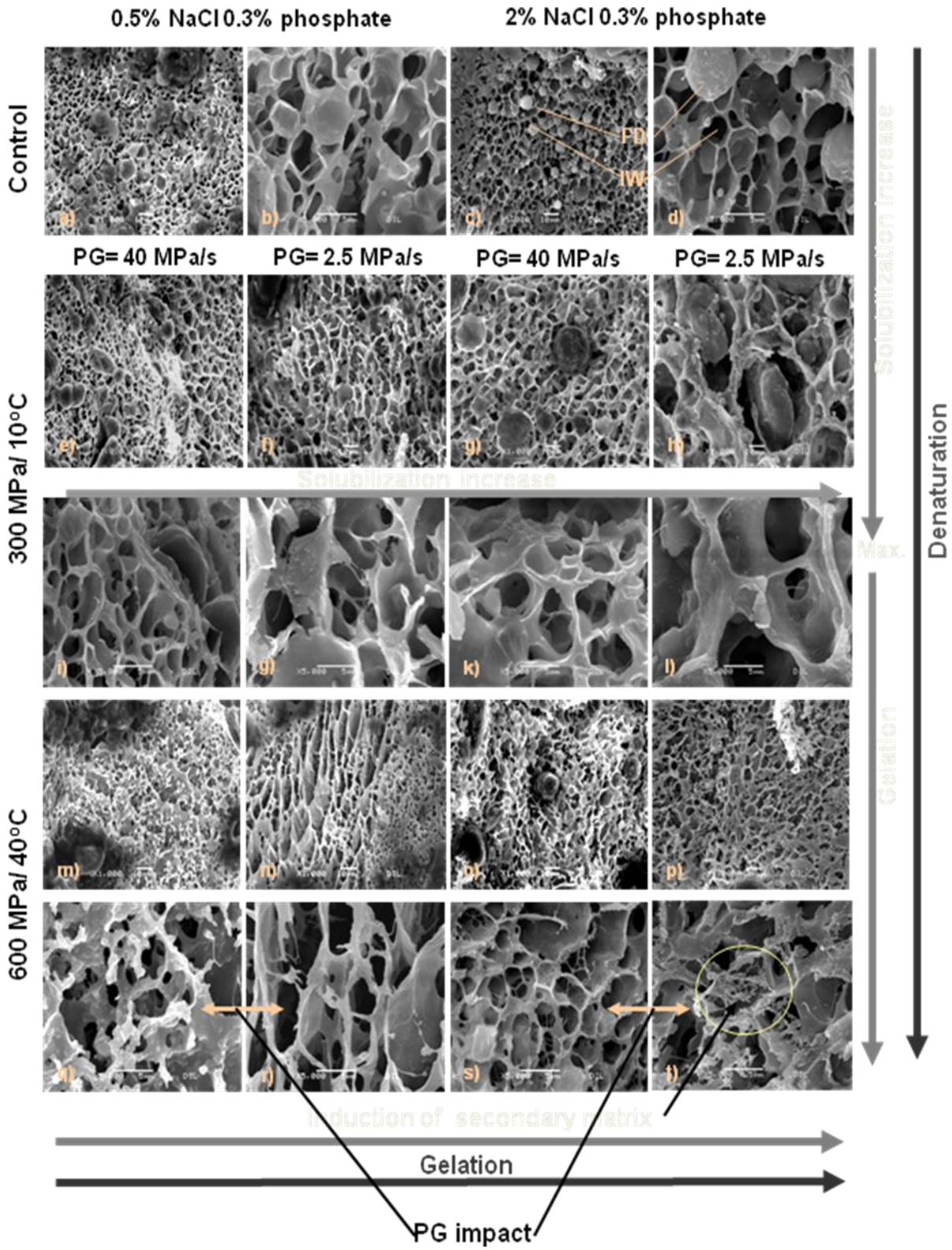


Figure 4.19: SEM micrographs of frankfurters batter structure of control samples at 0.5 % NaCl, 0.3 phosphate (a,b); 2 % NaCl, 0.3 phosphate (c,d); and treated at two pressure- temperature combinations 300 MPa/ 10°C (e-l), and 600 MPa/ 40°C (m-t) for 240 s. The impact of PG =40 MPa/s is shown in rows I, III, and 2.5 MPa/s in rows II and IV. Two magnification levels are presented: bar of length 10 µm (a, c rows II and IV), and bar of length 5 µm (b, d rows III and V).
FD- fat droplets
IW- immobilized water

The process of gelation was clearly visible at 600 MPa/ 40°C for the 240 s treatment (Figure 4.19- m-t). The formation of a secondary network parallel to the main matrix was observed (Figure 4.19- t). This was composed of anetwork of fine strands, suggested to be a result of networking of small aggregate conformations. Therefore the low PG impact was expressed in a noticeably extended secondary network, which was not visible in batters treated with high PG. This additional network surface extension enhances the possibility for more water molecules to be immobilized in the protein network and consequently for more protein-water interactions to occur. This network is suggested to be formed by soluble proteins, which migrated in the aqueous phase due to the combined salt-pressure effect. The denaturation of the soluble proteins in the aqueous phase resulting in a 3-D structure has often reported in the literature after HPP (Chapleau *et al.* 2003). Formation of a secondary network was not found in the batter matrix treated with PG of 40 MPa/s (Figure 4.19- q), which was rougher compared to the low-PG matrix (Figure 4.19- r). These PG-dependent observations correlate with the significant firmness and WHC differences already discussed in chapters 4.2.1-2. Based on the SEM- observations and the results in chapter 4.2.1, a relationship between the secondary matrix and its surface area improvement was found. The dependency of solubilization of meat proteins as well as the aggregation mechanism on the secondary network formation and the functional properties of batter under various HP-T/ holding time/ionic strength conditions will be discussed later in this work. According to the observed resulting changes in the functional properties and structure, it is concluded that HP-T processing in the higher pressure ranges of 500-600 MPa could be relevant for a possible industrial application.

In order to improve structure firmness and WHC, combined P-T treatments at 600 MPa and higher temperatures in the range of 40°C, 50°C and 60°C for 4 min were performed and compared to the control batter and the conventional thermally treated batter. Simulating sausage production in industrial conditions, the industrial NC-Hyperbaric (Burgos, Spain) equipment with low PG of 2.5 MPa/s was used.

As shown in Figure 4.20 the temperature increase was found to have a great impact on the matrix as aggregation and subsequent gelation were improved. After treatment at 600 MPa/ 40 °C/ 4 min, the parallel secondary matrix was already improved (visible). Furthermore, improvements continued at temperatures above 50 °C as the difference from the conventional batter structure was reduced. Moreover, the batter matrix at 60°C was extremely swollen, where its structure was more voluminous compared to the thermally treated batter. The thickness (swollenness) of the gel indicates WHC improvement, resulting in a smoother and more elastic structure compared to the thermally treated one, as reported in the literature (Cheftel and Culioli 1997). Based on this result, it could be concluded that significant gelation improvement would occur with increasing temperature at higher pressure

levels. Besides the WHC improvement, a firmness increase could also result with increasing temperature, possibly due to increased protein-protein interactions as well as greater secondary matrix surface (4.2, 4.17).

Gels with better WHC, less rigidity, and a smother and more glossy finish result after heating under high pressure conditions, or pressure treatment prior to heating (Okamoto, Kawamura et al. 1990; Cheftel and Culioli 1997; Fernandez-Martin, Fernandez et al. 1997; Jimenez-Colmenero, Cofrades et al. 1998).

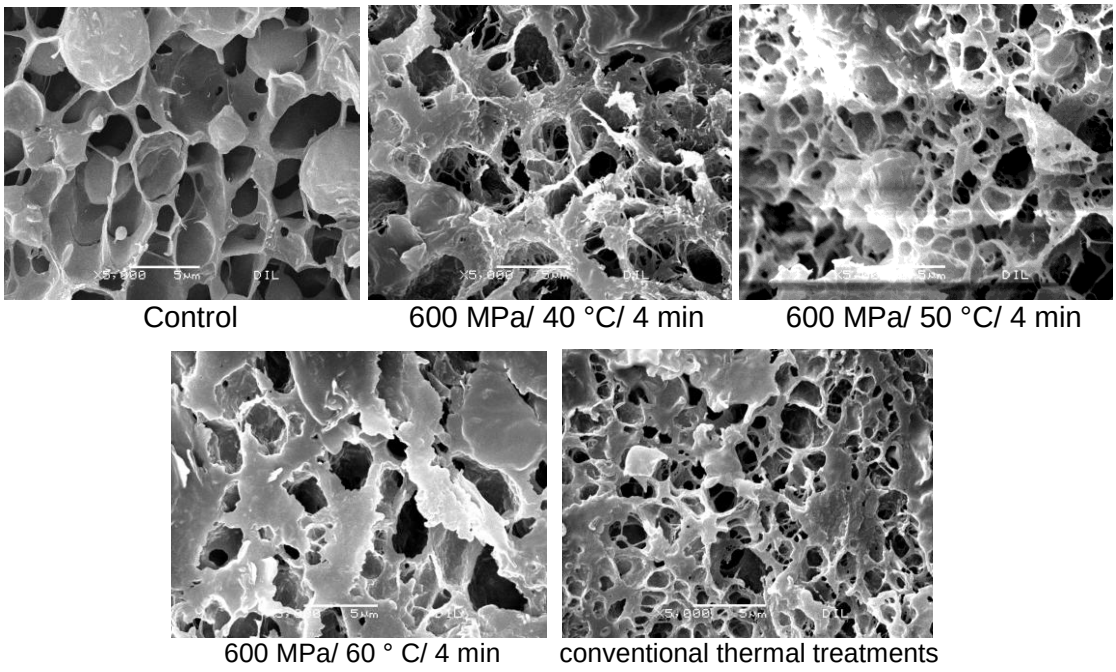


Figure 4.20: SEM micrographs of frankfurters batter structure (2% NaCl, 0.3 phosphate) treated at 600 MPa/ 40°/ 4 min, 50°C, 60°C and PG= 2.5 MPa/ s in comparison to control and thermally treated samples.

Iwasaki, T. *et al.,* 2006, reported that HPP caused a three-dimensional structure of meat protein suspensions. They have detected that the myofibrils after treatment with 100 MPa looked thicker, and at 200 MPa the structure of the myofibrils was disrupted. In their study, they found changes in the microstructure in heated-pressurized and heated myofibrillar gels. The pressure-treated gel was found to form a finer structure after heating. The pressure-temperature induced gels, after pressurization to 200 MPa, were more elastic and with decreased gel strength. These gels have a strand-type gel according to (Hermansson, Harbitz et al. 1986), who studied the formation of two types of Gels from bovine myosin. They concluded that all fine-strand myosin gels were formed from turbid solutions and aggregate gels from clear solutions. Furthermore, the conditions required for the formation of strand- type myosin gels were already present before the heat treatment, and the strands

were made up of myosin filaments at certain pH and ionic strength combinations, which produced turbid solutions.

After high-pressure treatment of isolated bovine myofibrillar proteins, new matrices are formed reminiscent of a molten globule state (Chapleau, Mangavel et al. 2003).

The results presented in this chapter were found to be in agreement with those previously reported. These were performed to give additional information about the HP-T/ionic strength impact on the network. Fine strands resulted from myosin light chains or myosin subunits migrating in the aqueous phase. These formations are believed to denaturate and swell under higher pressures or temperatures (chapter 4.9). The protein migration could be explained by some hypothetical mechanisms. First, more active myosin side chains could result after denaturation of the actin-myosin complex as well as the myosin molecule itself. Second, an ionic strength increase could be caused by the increase of the ion product during HPP. Other interesting alternatives could be the possible disruption of the myosin molecule, as well as the repulsion of molecule chains due to the increase of water penetration resulting from the compression.

Yamamoto *et al.* (1993) reported that either an intramolecular association of two heads in a single molecule or an intermolecular head-to-head association of myosin takes place upon HPP. Furthermore, they found that pressure treatment, itself, up to 210 MPa, did not induce gelation. After heating, an aggregation of myosin heads first takes place, followed by the formation of daisy-wheel shaped oligomers. Therefore gel matrices at higher pressures (Figure 4.19 and Figure 4.20) could be a result of similar daisy-wheel shaped aggregates interacting with each other through entanglement of the tails, which results after heat-induced helix-coil transitions (Chattong and Apichartsrangkoon 2009).

4.7 Denaturation of frankfurters batter at 600 MPa depending on increasing temperatures measured by Deferential Scanning Calorimetry (DSC)

The impact of denaturation, as a further explanation of the processes of structure formation (as shown in Figure 4.20) was studied thermoanalytically by Differential Scanning Calorimetry (DSC). The DSC method can give some quantitative and qualitative information of the denaturation of proteins, measuring the denaturation.

Normally, a pork meat thermogram shows three endothermic transitions associated with myosin (59°C), sarcoplasmatic and connective tissue proteins (66°C), and actin (78°C) giving ΔH_T= 3.6 J/g (Tintchev, Buckow et al. 2007). Comminuting of sausage batters leads to destabilization of myosin and actin caused by mechanical stress in the presence of NaCl,

which results in only one endothermic peak at 68°C and reduction of ΔH_T= 1.08 J/g (Figure 4.21) (Fernandez-Martin, Fernandez et al. 1997).

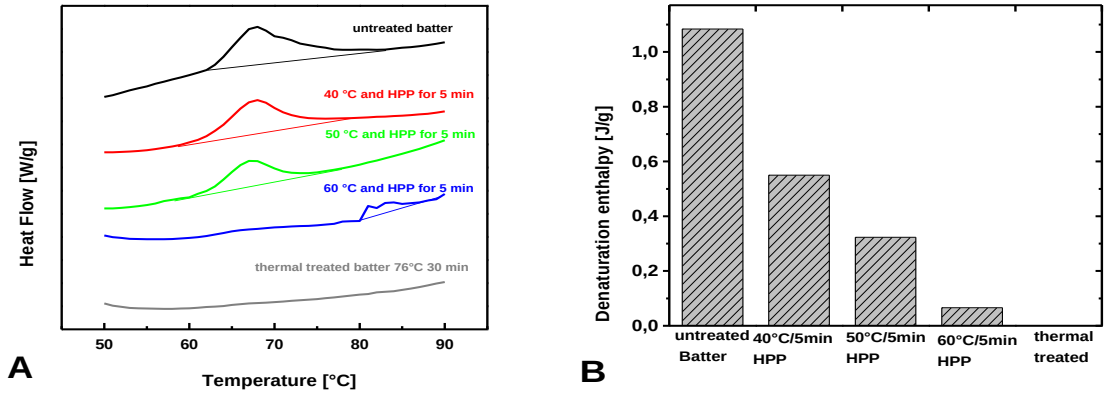


Figure 4.21: a) DSC profiles of: untreated and pressurized meat batters at 600 MPa and 40, 50, 60°C for 4 min and thermally treated (76°C/ 30 min); b) heat flow values of: untreated and pressurized meat batters at 600 MPa at 40, 50, 60°C for 5 min and thermally treated (76°C/ 30 min).

Pressurization of pork batter (in this case 2% NaCl, 0.3 phosphate) treated by 600 MPa/ 40°C/ 5 min/ 2.5 MPa/s further decreases denaturation enthalpy ΔH_T= 0.55 J/g, which decreases with increasing temperature as follow, ΔH_T= 0.32 J/g and ΔH_T= 0.066 J/g at 50°C and 60 °C initial temperature, respectively. However the highest value of denaturation resulted from a thermal treatment of 76 °C for 30 min. Some new compounds and a shift of the endothermic peak were found at 600 MPa and 60°C, which can possibly be explained by some new structural formations and protein modifications. This batter protein endothermic behavior correlates completely to the microstructure changes observed in Figure 4.20, where the structure modification and the major temperature impact are clearly visible.

Results generated in this chapter are in agreement with those reported by von Fernandez-Martin et al. (1997) and Sikes et al. (2009), who observed similar meat protein denaturation behavior.

4.8 Defining of total protein count in the aqueous phase according to Kjeldahl

Measurements of total nitrogen by Kjeldahl analysis were performed for determination of the total meat protein content in the supernatant. The measurements were done for batters with 2% and 1% NaCl in the recipe at the low pressure range 0- 300 MPa for temperatures 10°C-40°C as well as at the maximal pressure level of 600 MPa for the same temperatures. The

results of the measurements are presented in percents from the supernatant, obtained from 28g batter diluted with 15ml bi-distilled H_2O after centrifugation. Some of the results were directly determined using the Kjeldahl method while other were calculated according to the total protein area measured by the HPLC analysis. The results of the total protein count in the supernatant are shown in Table 4.1.

Table 4.1: Total protein count in the batter supernatant measured and calculated in % according to the Kjeldahl method

Pressure (MPa)	***Temperature (°C)***	***Total protein count in the supernatant (%)***	
		2%NaCl, 0.3 phosphate	***1%NaCl, 0.3 phosphate***
Control	***10***	1.0	0.8
	10	2.1	1.9
100	***30***	2.3	2.1
	40	2.4	2.2
	10	2.8	2.6
200	***30***	2.9	2.7
	40	3	2.8
	10	2.4	2.3
300	***30***	2.3	2.2
	40	2.1	2.0
	10	1.7	1.6
600	***30***	1.5	1.5
	40	1.3	1.3

After HP treatment at 100 MPa and 10°C and 4 min treatment time, the total protein count in the aqueous phase was double that of the control sample. The temperature impact induced additionally the process of solubilization up to 200 MPa where solubilization maximum at 40 °C was noted. Pressures above 300 MPa led to decrease of the soluble proteins leading to the conclusion that some protein changes start at this pressure level. Treatment at maximum pressure of 600 MPa resulted in a significant decrease of soluble proteins due to the denaturation process, which has its' maximum at this pressure level as already mentioned in the previous chapter. The differences of the total protein count between these two recipes were minimal. This observation can explain the fact of the relative similar functional properties already discussed in chapter 4.2. For the formation of the secondary matrix not only the total protein count but also the particular constellation of the proteins, especially those with good gelling properties, is important. Therefore protein quantitative and qualitative study of the batter soluble proteins will be performed in the next chapter.

4.9 Defining of solubilization and denaturation pressure treatment intervals; comparison to thermal treatment studied by SDS-PAGE

Pressure treatment causes an increase in the soluble proteins in myosin emulsions as a function of the ionic strength (Macfarlane 1974; J. J. Macfarlane 1976; Macfarlane and McKenzie 1976; Macfarlane, McKenzie et al. 1984; Iwasaki, Noshiroya et al. 2006; Sikes, Tobin et al. 2009).

In order to define solubilzation and denaturation levels of frankfurters batter, pressure-temperature (200- 600 MPa/ 10°C) and thermal (55°C- 75°C) treatments for 5 min holding time were performed (Figure 4.22). Solubility and denaturation were analyzed by SDS-PAGE detecting proteins with molecular weight in the range 6.5-205 kDa, where characteristic bands of meat proteins and their densities give qualitative and quantitative information of changes induced under certain treatments. The results after HPP were more intensive compared to those after thermal treatment, clearly indicating a significant increase of soluble protein fraction (Figure 4.22).

Myofibrillar myosin molecules have a molecular weight of 475 kDa based on subunits of 200 kDa (myosin heavy chains) and 20 kDa, 19.050 kDa, 16.5 kDa (myosin light chains). Myosin subunits can be also divided into fast-twitch myosin (heavy chains- 200 kDa, LC-1- 25 kDa, LC-2- 18 kDa, LC-3- 16 kDa) and slow-twitch myosin (heavy chains- 200 kDa, LC-1- 27.5 kDa, LC-2- 19 kDa (Weeds 1980). Myosin can also be split into two functional fragments, light meromyosin with molecular weight of 150 kDa and heavy meromyosin with MW of 350 kDa. Furthermore, meromyosin can be split into S-1 (115 kDa) (sub fragment 1) and S-2 (60 kDa) (sub fragment 2- S-2). S-1 is especially important for the functionality of myosin in processed meat products due to its excellent binding capacity (Borejdo and Assulin 1980; Borejdo 2002). Furthermore, S-1 is composed of three domains with molecular weights of 27 kDa (N-terminal), 50 kDa and 20 kDa (C-terminal) (Iwasaki, Yamamoto et al. 2002). Maximum solubilization of both S-1 domains with molecular weight of 50 kDa and at 27 kDa, as well as S-2, was found to be at 200- 300 MPa (Figure 4.22 and Figure 4.23). Similar solubilization behavior with a maximum at 200 MPa was detected for actin (43 kDa). Pressures above 300 MPa led to a density decrease indicating that denaturation took place. In most methods, tropomyosin is found in the 34-36 kDa range as a doublet (Salm, Forrest et al. 1983 Baumann, 1984). Tropomyosin, according to the results in Figure 4.22, seems to be pressure and thermal stable. After a maximum at 300-400 MPa, only a small density decrease for Tropomyosin was detected. Therefore it should not be relevant in regards to the pressure or pressure-heat gelation. According to (Yasui, Ishioroshi et al. 1982), the regulatory proteins tropomyosin and troponin have no influence on the heat-induced gelation.

Myosin heavy chains were not detected after HPP in the supernatant. According to the solubilization and denaturation behavior of the batter proteins, the S- 1 domains, S-2 sub fragment, actin and MLC, are all important for the building of the gel matrix at higher pressures and temperatures.

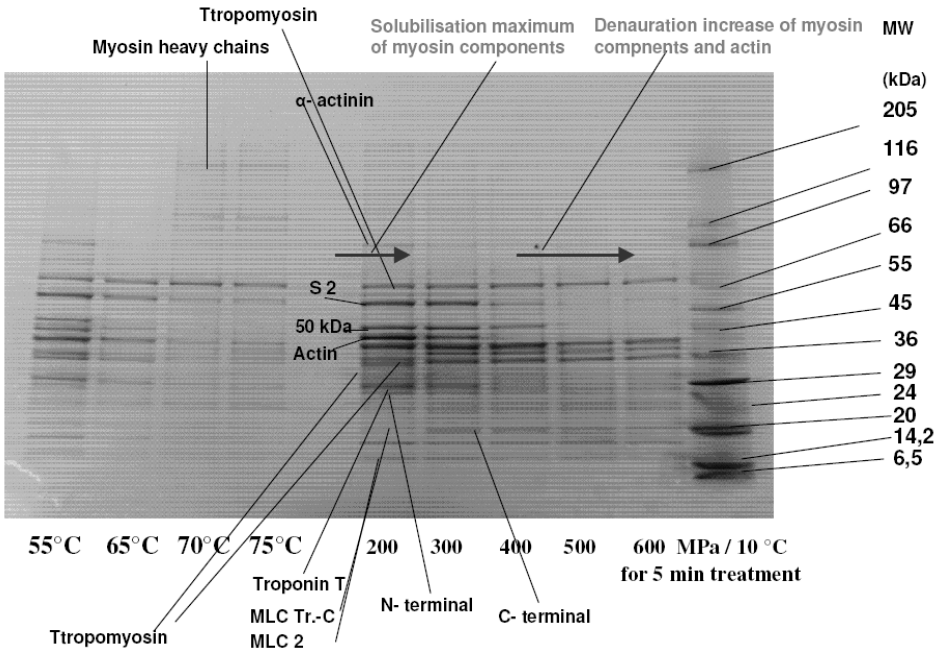


Figure 4.22: SDS-PAGE pattern of solubilized fraction of pressure and temperature treated sausage batter after 5 min treatment time (2 % NaCl, 0.3 phosphate).

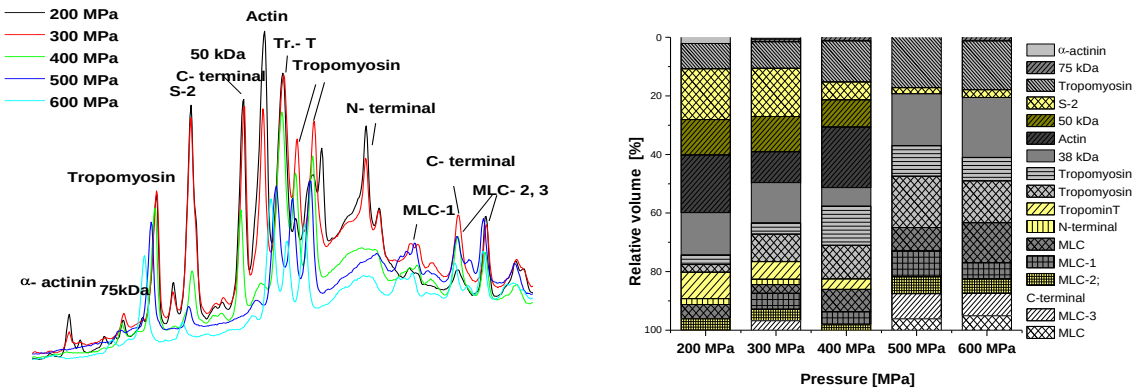


Figure 4.23: a) Density profile of SDS gels and b) Relative volume for different treatment pressures.

This is particularly true for the myosin low molecular weight subunits and actin, which increased at about 200- 300 MPa and denaturated at higher pressures. The mechanism must differ from the thermally treated batter, based on the functional properties and matrix microstructure (Yamamoto, Hayashi et al. 1993). The binding sites of ATP and actin are

located in the junctions of 25-50 kDa and 50-20 kDa, respectively (Mornet, Pantel et al. 1979; Hozumi and Muhlrad 2002). Therefore, their solubilization and aggregation behavior under pressure are essential for modifying batter functionality. The hydrophobicity of myosin increases with pressure up to 250 MPa (Yamamoto, Hayashi et al. 1993). Further pressure increase leads to a decrease in helix content, which takes place in the pressure range of 250- 300 MPa, stopping the hydrophobicity increase (Yamamoto, Yoshida et al. 1994). The binding ability of S-1 did not change up to 200 MPa, but decreased at pressures above 300 MPa (Iwasaki, Yamamoto et al. 2002). This shows that the structure of actin binding sites is changed by pressure above 300 MPa, due to the structural alteration of the actin-binding site in S-1.

The results from Figure 4.22 and Figure 4.23 are in agreement with the literature and could be summarized to conclude that significant changes, related to the solubilization and aggregation processes of myosin subunits and actin, occur in the range of 100-300 MPa.

4.10 Quantitative and qualitative SDS-PAGE analysis of meat protein solubility referring to different HP-t/ holding time and batter recipe variants

For estimating the dependency of the maximum solubilization range on the HP-T conditions and the NaCl content of the batter recipe, various combinations of these parameters were performed. Two initial temperatures (10°C and 40°C) in combination with a range of pressures (100-300 MPa) have been used. Cold pressurization at 10°C was used to measure only the pressure effect, and a second medium temperature level at 40°C was used to measure the combined pressure- temperature impact. In Figure 4.24, SDS-PAGE of supernatants from centrifuged frankfurter batters with three different NaCl contents in their initial recipes, namely 2%, 1%, and 0.5%, under various P/T/holding time combinations are shown. A clear tendency for a decrease in band density with decreasing NaCl content was observed. Therefore, it is suggested that the impact of NaCl content on the HP- solubilization process could play a significant role by furthering the processes forming the batter structure.

Upon analyzing the band densities, it appears that 2% and 1% NaCl supernatants exhibit maximum solubilization in the range of 300 MPa/ 40°C for 1 min treatment time. In this processing range, a visible tropomyosin and troponin-T maximum was detected. According to the literature, and as shown in Figure 4.22 and Figure 4.23), these two meat proteins are not significantly relevant to the gel building batter properties (Yasui *et al.* 1982). However according to Figure 4.22, and to other literature, the maximum number of myosin subunits was expected in the range of 100-200 MPa (Yamamoto, K. *et al.* 1993; Iwasaki, T. *et al.* 2002).

For 0.5 % NaCl content batters, the maximum solubilization was found at 200 MPa/ 10°C and 10 min holding time. For this recipe a clear dependency on the treatment time was difficult to determine, except at 200 MPa/ 10°C for all recipes.

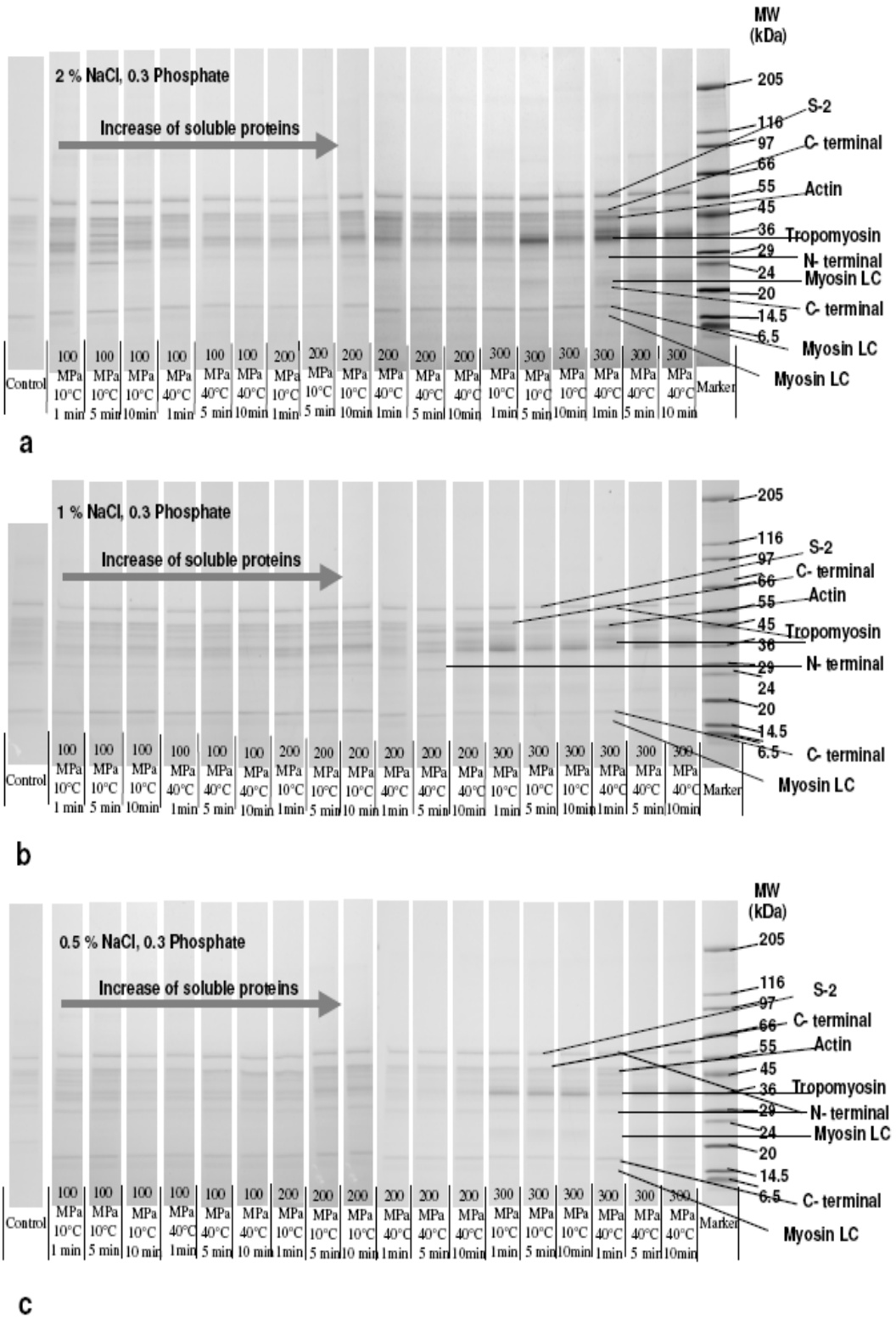


Figure 4.24: SDS-PAGE pattern of solubilized fraction of pressure/ temperature treated sausage batter a) 2 % NaCl, 0.3 phosphate; b) 1% NaCl, 0.3 phosphate; c) 0.5 % NaCl, 0.3 phosphate. Myosin LC- light chains.

4.10.1 SDS-PAGE analysis of soluble meat proteins of conventional batter (2 % NaCl, 0.3 phosphate) at pressures of 100-300 MPa, and depending on temperature and treatment time

A detailed SDS-PAGE solubilization analysis was performed for batters undergoing several different treatment patterns. The results are shown in Figure 4.25.

A significant effect on protein solubilization was observed after HPP at the lowest performed treatment pressure level (in this case 100 MPa) even at low temperature and short treatment, compared to the control sample. No myosin heavy chains were found in the supernatant after any of the treatments. Therefore, it was concluded that mechanical (cutting, grinding) and chemical (in-salting) damage occurred after the batter preparation process. To isolate the pressure effect on the solubilization measurements at a constant temperature of 10°C for 1 min and for 5 min are presented (Figure 4.25-a, c). To determine the combined pressure-temperature effect, a higher temperature of 40°C was used (Figure 4.25-b, d).

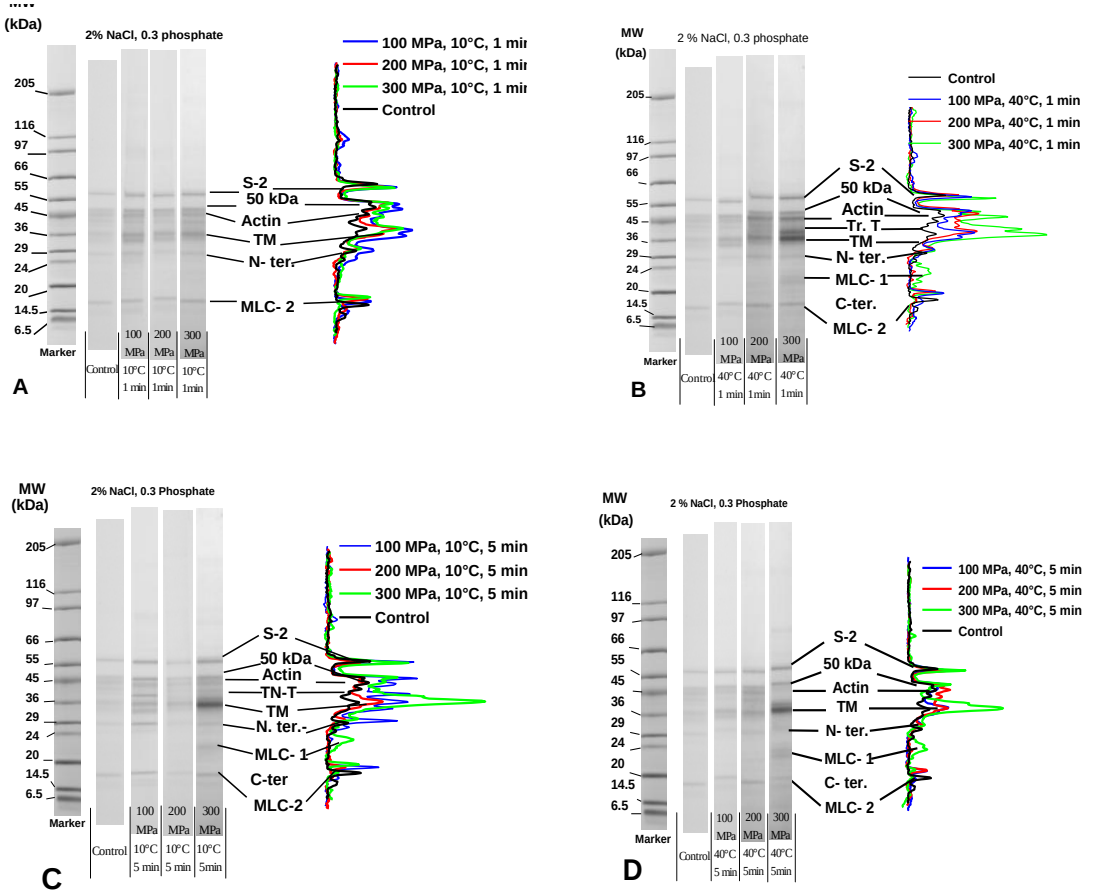


Figure 4.25: a) SDS-PAGE pattern and quantification surface peaks of solubilized fraction depending on pressure increase at a) 10°Cand b) 40°C, for 1 min holding time (2 % NaCl, 0.3 phosphate) and at c) 10°C and d) 40°C for 5 min holding time (2 % NaCl, 0.3 phosphate).

Myosin light chains, sub fragments and domains were detected in the control as well as in the pressure treated samples. An increase in solubilization was found even after 100 MPa and 10°C for 1 min, compared to the control (Figure 4.25-a). No significant increase in solubilization with further pressure increase was observed. Except for tropomyosin, similar results were also found after treatment at 10°C and 5 min holding time (Figure 4.25-c).

A clear tendency of increasing solubilization of proteins with increasing pressure was seen after treatment at 40°C (Figure 4.25-b, d). The solubilization maximum for tropomyosin, S-2, MLC-1, and actin was found after HPP at 300 MPa. However, the maximum solubilization for the MLC-2, N- terminal, and C-terminals at 50 kDa and 20 kDa was detected to be at 200 MPa.

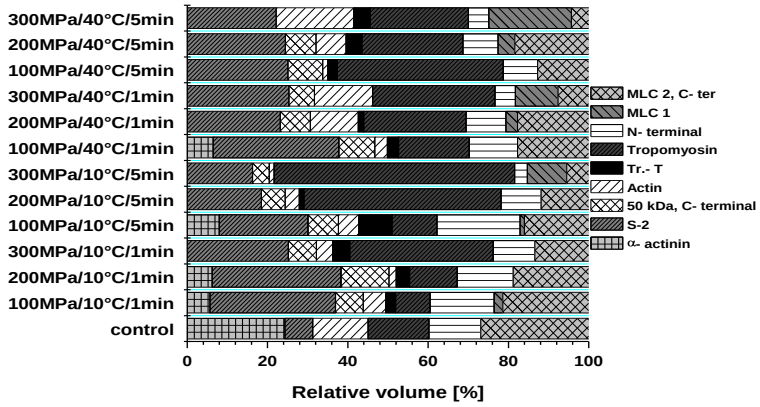


Figure 4.26: Relative volumes of (2% NaCl, 0.5 phosphate batter) soluble proteins after various pressure/ temperature/ treatment time combination.

By comparing Figure 4.25-b and Figure 4.25-d .it was found that the majority of band density increase occurred after 1 minute, as seen by comparing the samples of 5 min treatment time. Possible explanations for this are the formation of aggregations with molecular weight higher than 205 kDa, or the disintegration and cleavage of protein molecules into subunits and sub fragments smaller than the SDS-PAGE detecting range (>6.5). These two possibilities as functions of processing time will be presented in the following chapters, followed by the HPLC analysis of supernatant protein behavior and their possible influence on the functional properties of batters after two-step HPP. The relative meat protein volume in the supernatant for each treatment is presented in Figure 4.26.

With regards to the optimal functional properties of batter, and based on the discussed results, the best parameters for achieving optimal protein composition was found to be at 200 MPa/ 40° for 1 min.

4.10.2 SDS-PAGE analysis of soluble meat proteins 100-300 MPa of NaCl reduced batter (1 % NaCl, 0.3 phosphate) depending on temperature and treatment time

The pressure-temperature solubilization of reduced NaCl batters (1% NaCl, 0.3 phosphate) was studied and presented in Figure 4.27. Generally the band densities of this recipe were lower compare to that for 2% NaCl. In all treatment combinations, solubilization increases with increasing pressure, with a maximum at 300 MPa/ 40 °C and 1 min treatment time (Figure 4.27- B). A large increase of actin and tropomyosin was found, as well as the appearance of MLC-2, but also a little decrease of MLC- 1. According to Figure 4.27, an increase in the solubilization of actin, tropomyosin, and MLC- 1 with increasing pressure was observed, with a maximum at 300 MPa. The amount of MLC- 2 was found to be maximised at 100 MPa or 200 MPa without a significant difference between them, which corresponds to the results of Iwasaki, T. *et al.* (2006), who found that myosin content in the supernatant increased up to 200 MPa.

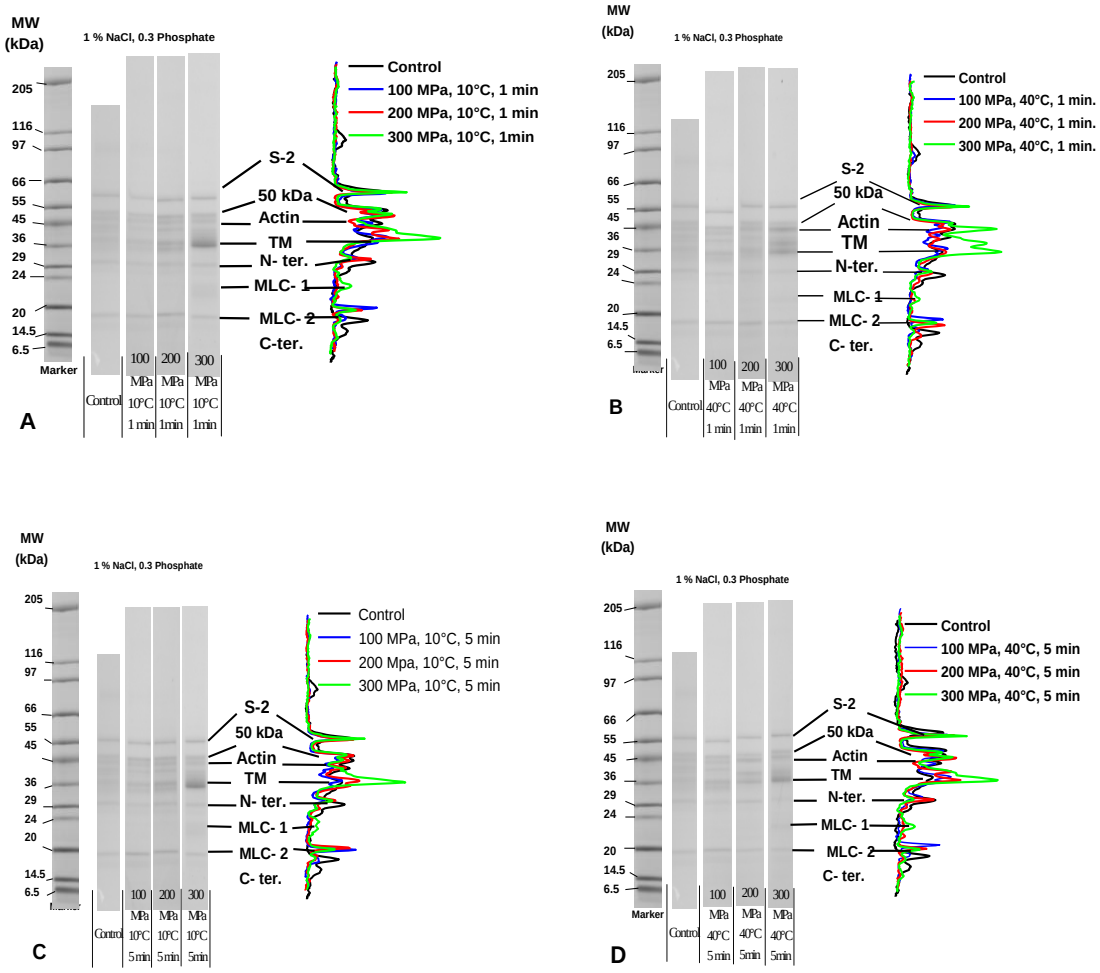


Figure 4.27: a) SDS-PAGE pattern and quantification surface peaks of solubilized fraction depending on pressure increase at A) 10°C and B) 40°C for 1 min holding time (1% NaCl, 0.3 phosphate) and at C 10°C and D) 40°C for 5 min holding time (1% NaCl, 0.3 phosphate).

Percentage distribution of the relative volume of soluble proteins for each sample was calculated using image-analysis software (UN-SCAN-IT gel, Version 6.1, Gel and Graph Digitizing Software, Silk Scientific INC, Utah, USA) and presented in Figure 4.28.

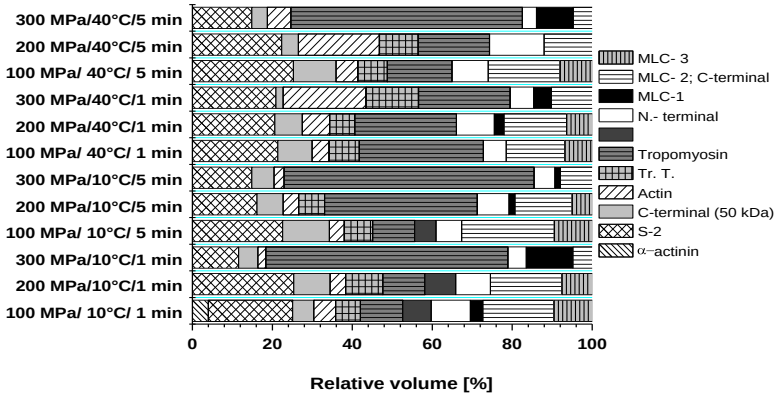


Figure 4.28: Relative volume of 2% NaCl, 0.5 phosphate batter soluble protein after various pressure/ temperature/ treatment time combination.

4.10.3 Quantitative and qualitative SDS-PAGE analysis of soluble meat proteins at 300 and 600 MPa depending on the temperature

In order to explain the changes of the functional properties (firmness and WHC) after pressure- temperature treatment, already discussed in chapter 4.2, SDS- PAGE analysis was performed at 300 and 600 MPa (results shown in Figure 4.29). After analyzing the characteristic bands, the solubility and denaturation processes were noted to be dependent on temperature.

According to that analysis, a solubilization maximum was found for the 300 MPa and 30°C for 2% NaCl and 300 MPa and 40°C for 1% NaCl conditions. The HPP in the higher temperature range (30°C, 40°C) led to additional solubilization (as shown in Figure 4.29 and Figure 4.30-A, B). Further improvement in solubilization was not observed after HPP at 600 MPa, except for tropomyosin. The peaks and bands density in Figure 4.30 and Figure 4.29, as well as the relative protein volume in Figure 4.31, confirm the observations of Figure 4.22 and Figure 4.23, namely which proteins are involved in the formation of the secondary matrix due to the denaturation. Therefore for the formation of the secondary structure the proteins S- 2, C- terminals (50 kDa and 20 kDa), actin, N- terminal, and MLC were responsible. These were supposed to form aggregations due to the strong hydrophobic properties of the myosin head subunits in the 100-300 MPa pressure range. The firmness increase, as well as the WHC improvement, is suggested to be due to a further network development, where the unfolding of the aggregates break down the hydrophobic interaction by creating cross-linkages.

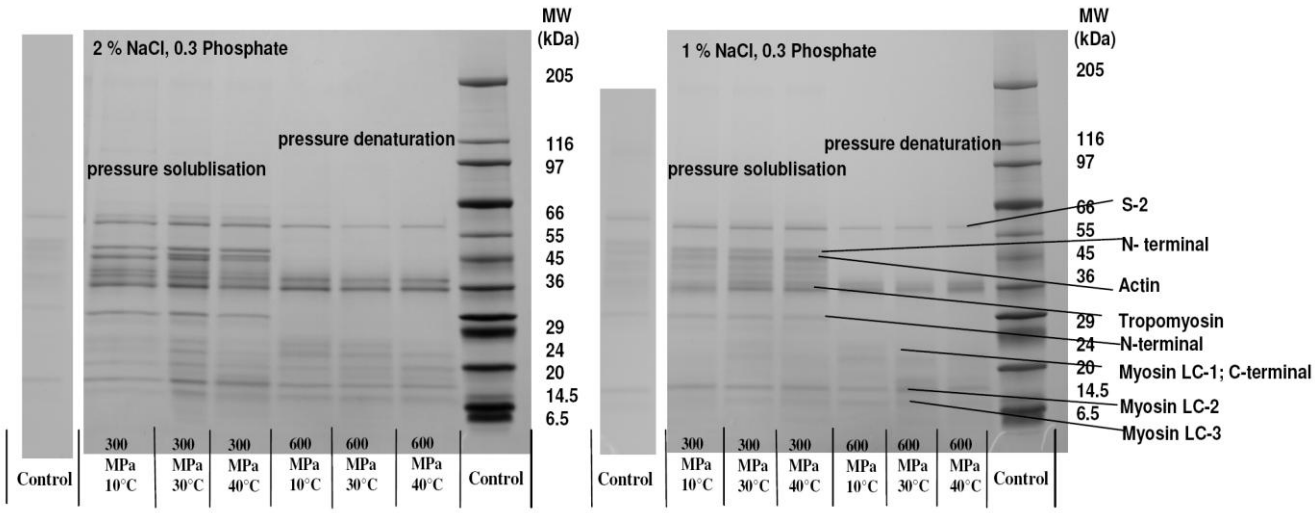


Figure 4.29: SDS-PAGE pattern of the solubilized portion of pressure-temperature treated batters. Solubilization (300 MPa) and denaturation (600 MPa) occurred with increasing temperature 10°C-40°C for samples with 2% and 1% NaCl content.

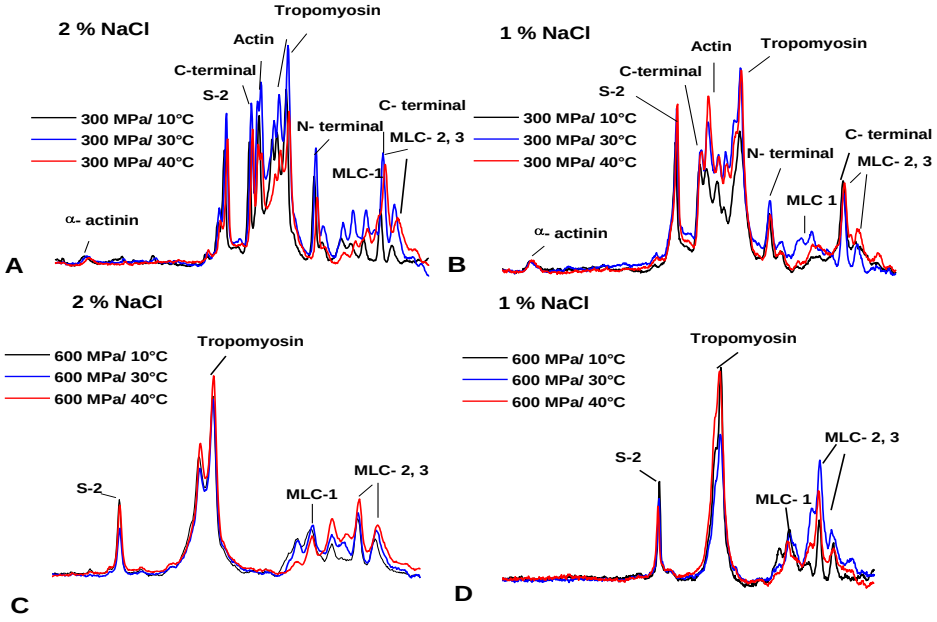
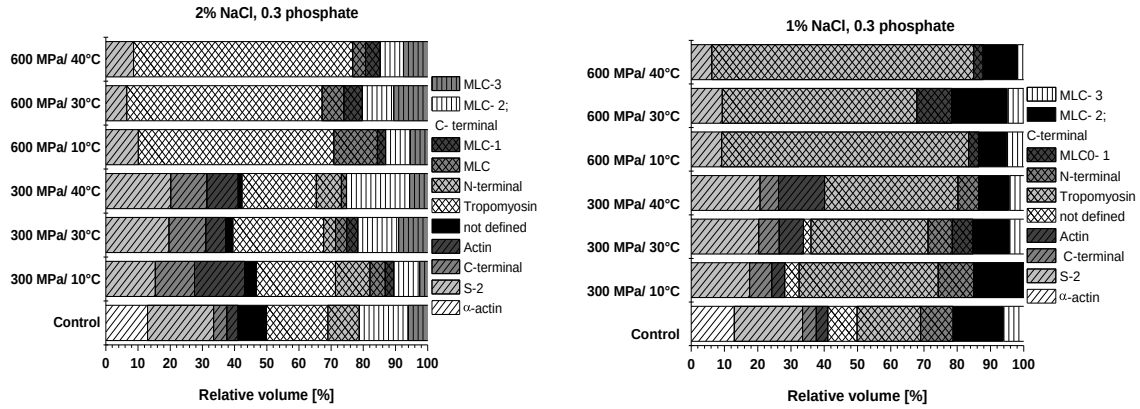


Figure 4.30: SDS-PAGE quantification surface peaks of the solubilized portion of pressure-temperature treated batters. Solubilization occurred at 300 MPa (A- 2% NaCl and B-1% NaCl) and denaturation at 600 MPa (C- 2% NaCl and D- 1% NaCl).



a) b)

Figure 4.31: Relative volume of soluble protein after 300 MPa and 600 MPa and 10- 40°C of a) 2% and b) 1% NaCl.

4.10.4 Quantification of soluble meat protein compression 200 MPa and 300 MPa depending on temperature and treatment time

As already discussed, the maximum solubilization and the start of aggregation processes, according to the experimental observations and the literature, is supposed to occur in the range of 200-300 MPa. The estimated solubilization range in the course of this work was shown in (Figure 4.24), where also the impact of treatment holding time was studied. A comparison was made between HPP at 200 MPa and 300 MPa, with holding time up to 10 min and temperature at 10°C and 40°C for batter with 1% NaCl content, as shown in Figure 4.32. Prediction of the maximum protein solubilization for samples treated with different holding times is of great importance. Therefore, the dependency on treatment time of the solubilization process was studied. Controlling the solubilization through treatment time was suggested to increase the possibility of batter structure modification and optimize the process for industrial application.

The density profiles of the supernatants pattern at 10°C and 200 MPa for all treatment times were very similar, where only a small increase in the proteins responsible for the secondary matrix were noted (Figure 4.32- A). For the same temperature at 300 MPa, an increase in the density profiles with increasing holding time was observed, with a maximum after 10 min treatment time (Figure 4.32- C).

At 40 °C and 200 MPa pressure treatment, an increase in density profiles with increasing holding time was found with a maximum after 10 min treatment time. In contrast, a density decrease with increasing holding time was observed at 300 MPa (Figure 4.32- D) for all proteins except for Tropomyosin.

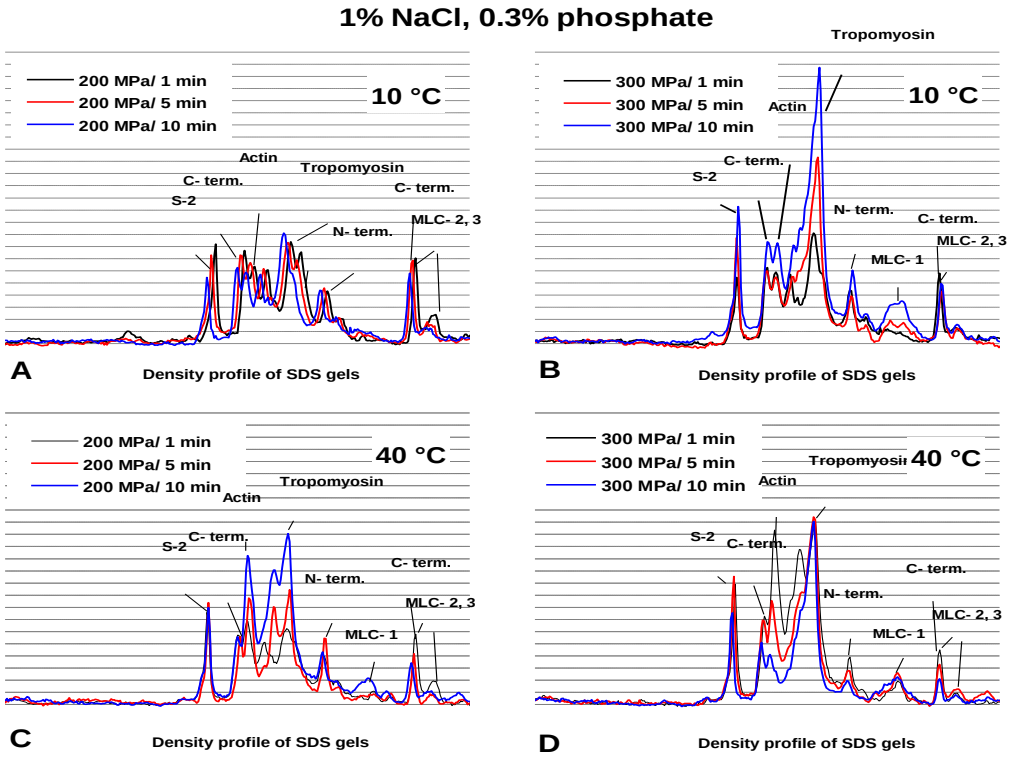


Figure 4.32: SDS-PAGE quantification surface peaks of solubilised fraction of pressure-temperature treated batters depending on treatment time. Solubilisation at 200 MPa (10°C– A and 40°C- C) and at 300 MPa (10°C– B and 40°C- D) for 1% NaCl, 0.3% phosphate.

The relative volumes of the density peaks from Figure 4.32 are presented in Figure 4.33. According to the results in Figure 4.32 and Figure 4.33, it can be concluded that the maximum solubilization occurred after HPP at 300 MPa/ 10 min treatment time and10°C (Figure 4.32-B). Further pressure increase at this temperature realized as a second pressure step, provided the gel with poor functional properties (firmness and WHC), as discussed in chapter 4.2. For the combined pressure-temperature structure modification (firmness improvement) inside the pressure vessel, a starting temperature of 40°C is more relevant. The possibility of improving the WHC by pressurization at 10°C is realizable only when followed by heat treatment, where the increase of soluble proteins can positively affect the aggregation and matrix formation (gelation). According to the results, HPP at 40°C initial temperature provides two possible options for obtaining maximum solubility; treatment at 300 MPa for 1 min, and treatment at 200 MPa for 5 min. Because the SDS-PAGE analysis is limited to a particular molecular weight range, a general conclusion on the maximum solubilization process was suggested to be made after an additional HPLC analysis. This was needed for obtaining information from a wider molecular weight range, as described in the next chapter.

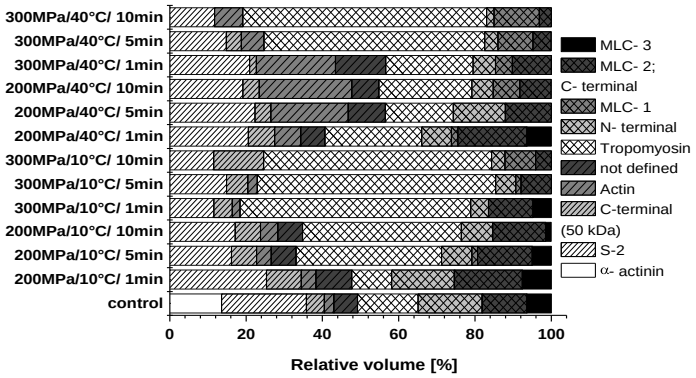


Figure 4.33: Relative volume of soluble proteins in 1% NaCl batter after treatment with 200 MPa and 300 MPa and 10-40°C.

4.11 Quantitative and qualitative analysis of batter protein solubility studied by HPLC analysis

Quantitative and qualitative analysis of batter aqueous phase using SDS-PAGE analysis was limited to only the molecular weight range 6.4 kDa- 205 kDa. Therefore HPLC analysis was performed to observe protein changes outside this range. It is difficult to identify particular proteins using HPLC analysis. However it is useful for obtaining information about the changes of protein sub-fragments smaller than 6.4 kDa, which result after tissue disintegration and pressure-induced protein molecule damage. Another aspect obtainable with this method is the aggregation range, where the soluble proteins, their sub-fragments, and sub-units start to group (aggregate).

HPLC profiles of the soluble protein fraction, after high pressure processing (100-600 MPa) at 40°C and 5 min treatment time, are shown in Figure 4.34. A clear trend in the behavior of the protein units or sub-units was observed. After pressure treatment with 100 MPa, a large number of sub-units were observed in the molecular range under 6.4 kDa, which were undetectable by SDS-PAGE. In this range the total content of protein sub-units was found to reach its maximum at 200 MPa. Therefore, this pressure level was defined as the maximum solubilization level, where the meat protein molecules were maximally damaged (solubilized or disrupted). This was proven by observations of the peaks at the next pressure level- 300 MPa, where the amount of small soluble protein sub-fragments (under 6.4 kDa) was significantly reduced. Furthermore, this reduction was combined with an increase of protein fragmentations, detected as a shift in the protein peaks to the range of higher molecular weight (above 240 kDa). This led to the conclusion that at 300 MPa, aggregations of small molecular protein sub-fragments transformed into bigger conformations. These observations correlate with the results reported by Yamamoto *et al* (1993). They found an aggregation process at 210 MPa. However they did not experiment at higher pressure levels.

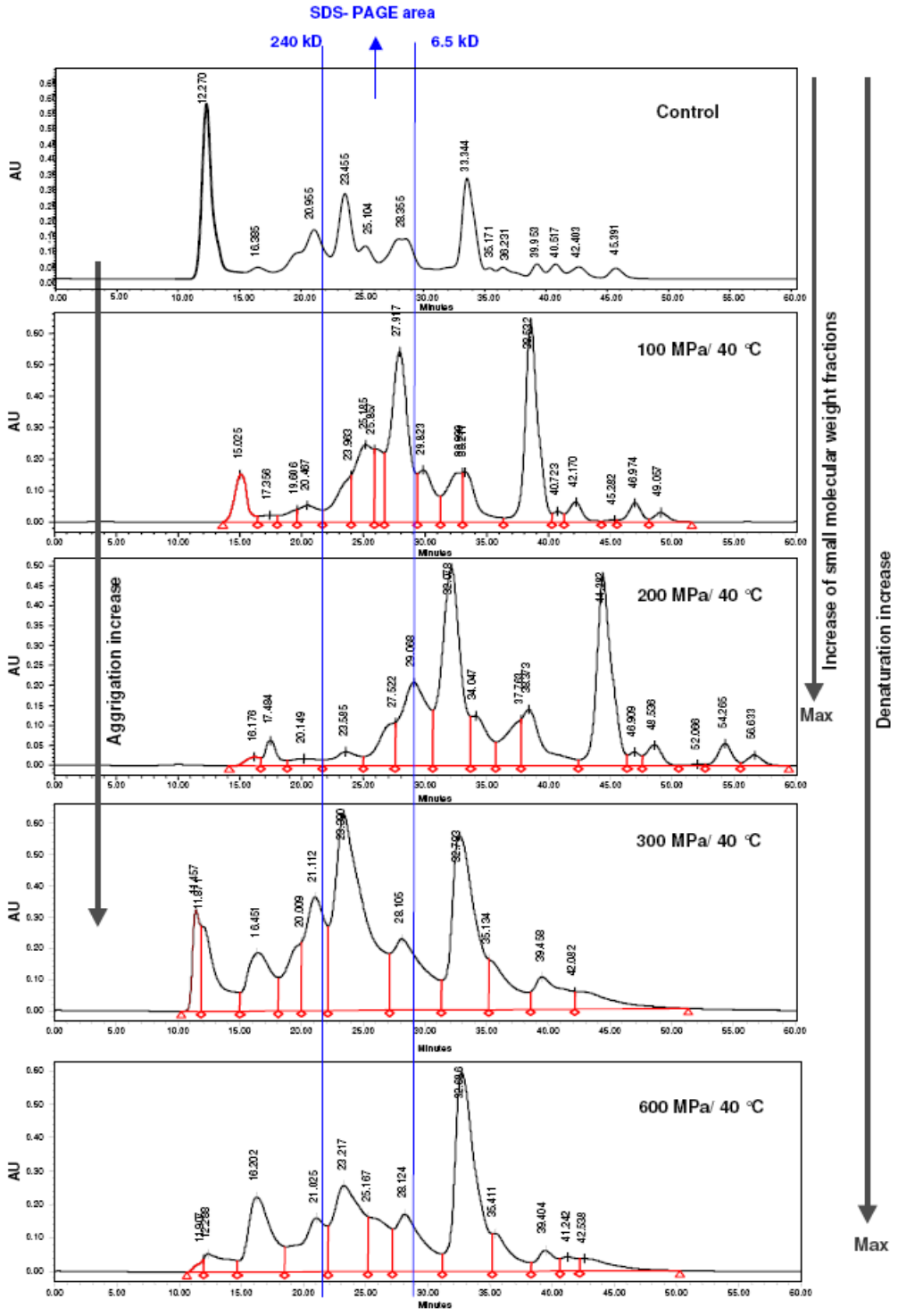


Figure 4.34: HPLC profiles of batter soluble proteins at different high pressure treatments at 40 °C initial temperature after 5 min treatment time.

At HPP of 600 MPa and 40°C, a decrease of proteins peaks was generally observed. This can be explained by the network building process that takes place during the treatment. As already shown and discussed in Figure 4.19, Figure 4.21 and Figure 4.29, maximum denaturation (gelation) and network formation occurred at these processing conditions. The

agglomeration that began building at 300 MPa (the first stage of secondary network building) was transformed into batter matrix that was swollen and well formed.

Table 4.2: Peak area of high pressure-treated batters at 100- 600 MPa/ 40 °C and 5 min treatment time measured by HPLC analysis

Pressure (MPa)	***Molecular weight above 240 kDa area x10 ^{7}***	***Molecular weight 240-6.4 kDa area x10 ^{7}***	***Molecular weight under 6.4 kDa area x10 ^{7}***
control	6.36	5.12	4.44
100	2.03	11.51	7.95
200	0.9	4.51	16.
300	11.8	14.89	9.65
600	6.18	8.27	9.34

HPLC analysis for determining the effect of treatment time on the soluble batter proteins was performed at a constant 200-MPa pressure level and temperatures of 10°C and 40°C. Two different treatment times of 1min and 10 min were used. According to the results presented in Figure 4.35, an interesting trend was observed. Protein profiles of batters treated longer were shifted to the higher molecular range. This suggests that the pressure treatment time could play a significant role in the level of protein aggregation.

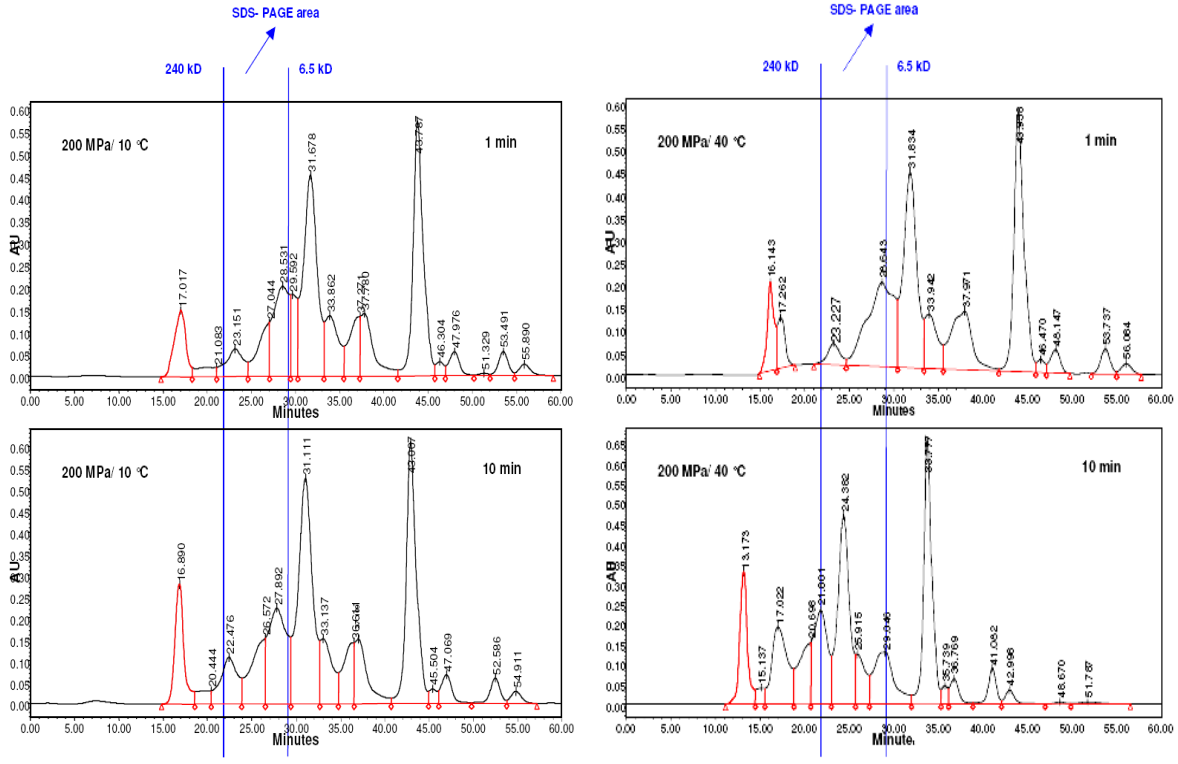


Figure 4.35: HPLC profiles of batter soluble proteins at 200 MPa at 10°C and 40°C initial temperature after 1 min and 10 min treatment times.

Table 4.3: Peak area of high pressure treated batters at 200 MPa; 10°C and 40 °C; 60 s and 600 s treatment time

Pressure (MPa)	***Molecular weight above 240 kDa area x10 ^{7}***	***Molecular weight 240-6.4 kDa area x10 ^{7}***	***Molecular weight under 6.4 kDa area x10 ^{7}***
200/10°C/60 s	1.61	10.2	9.90
200/10°C/600 s	2.2	12.4	10.5.
200/40°C/60 s	2.3	10.4	10.08
200/40°C/600 s	4.6	11.33	5.8

Comparing the protein profiles at the two temperature levels, it was noted that the shift was significantly distinct for the supernatants at higher temperature (40°C). Therefore it was concluded that at constant HPP and higher temperatures, the aggregation process was additionally induced. Improvement of aggregation (growing) through longer treatment times could have a significant effect on the functional properties of batter processed in a two steps HPP, as discussed in the next chapter.

A similar trend was also observed by Yamamoto *et al.* (1993), who detected an increase of dimmer, trimmer and oligomer myosin aggregates with increasing pressure.

4.12 Influence of ion concentration (reduced NaCl) of two step pressurization on the functional properties (firmness and WHC)

As already discussed, protein solubilization depends on the various pressure/temperature/ holding time combinations.

This chapter aims to determine how HPP in two steps, performed according to Figure 3.10, can affect batter functional properties. The first step, in the pressure range of 100-300 MPa, has been performed to induce soluble protein in the aqueous phase. The second HP-step was performed after completing the solubilization (first step) by raising the pressure to 600 MPa and then holding for 240 s. The main purpose of the second HP-step was to produce a HP-temperature gelation effect on the network. The impact of the additionally extended solubilization in the two steps HPP on firmness and WHC will be presented in this chapter.

To define the effect of pressure alone on the matrix formation, a low initial temperature (10°C) was used. The results are presented in Figure 4.36 and Figure 4.38. The impact of combined pressure-temperature treatment was studied at 40 °C initial temperature (Figure

4.39 and Figure 4.41). The two-step treatment was compared to the one-step treatment for the both PG (2.5 MPa/ and 40 MPa/s).

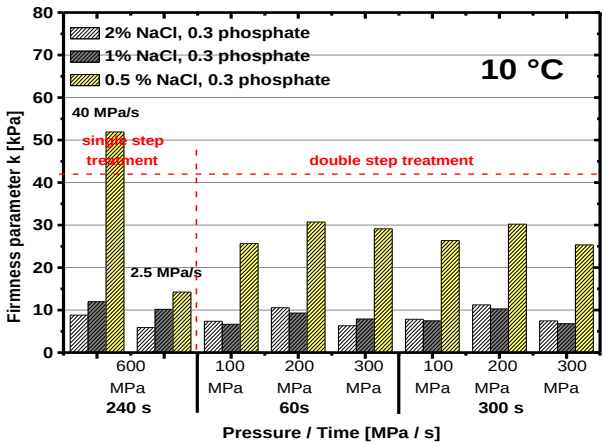


Figure 4.36: Batter firmness for one-step and two-step HPP at 10 °C (second step at 600 MPa/ 240s).

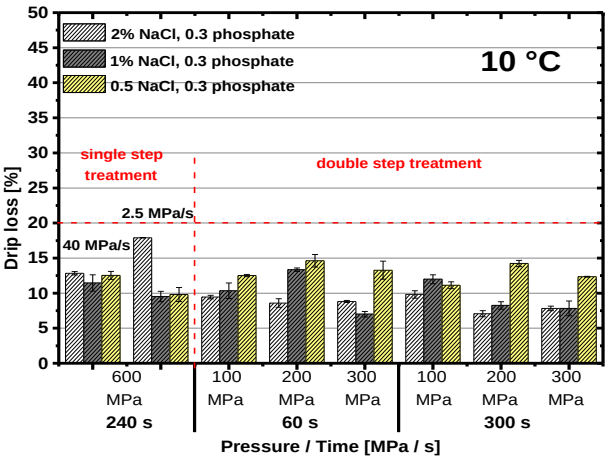


Figure 4.37: Drip loss comparison for one step/ two steps HPP at 10 °C, second step at 600 MPa/ 240s).

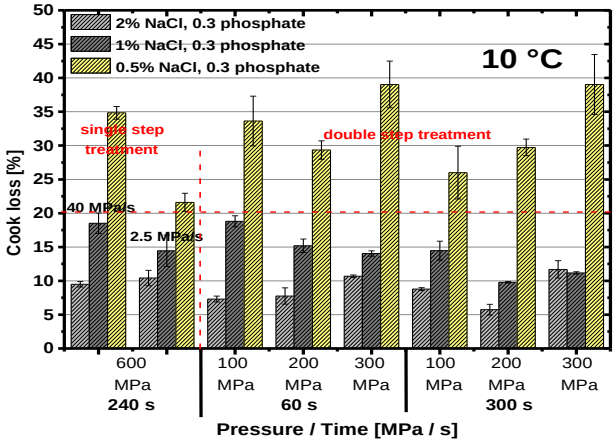


Figure 4.38: Cook loss comparison one step/ two steps HPP at 10 °C second step at 600 MPa/ 240s).

Batter with 2% NaCl content showed maximum firmness after the two-step treatment at 10°C initial temperature, with the first step being done at 200 MPa/ 300s (Figure 4.36). Furthermore, the WHC presented as drip and cook loss at this treatment combination was significantly improved, compared to the other treatments and recipes (Figure 4.37 and Figure 4.38). For the reduced 1% NaCl recipe, maximum firmness was found after a one-step pressure treatment at PG=40 MPa/s. In this case, it is suggested that the high level of structure firmness was due to a low content of soluble proteins, resulting in rougher structure (Figure 4.19- q, r) with poor WHC, expressed as higher drip and cook loss (Figure 4.37 and Figure 4.38). A similar firmness value for the same recipe was found after two steps (first step- 200 MPa/ 300 s) treatment. The HP-treatment WHC (drip loss and cook loss) was significantly improved compared to the high PG treatment (Figure 4.37 and Figure 4.38). The recipe with reduced sodium content (0.5 NaCl) was found to have the highest WHC combined with a low firmness value for the one step treatment and low PG. The firmness parameter observed after combined HP- temperature processing at 40°C for both one step and two steps treatment (Figure 4.39), was significantly higher compared to the low temperature samples. Maximum firmness and minimum cook loss for the 2% and 1% NaCl-content batters were found at 200 MPa/300s for the first step treatment (Figure 4.39 and Figure 4.41). Similar to the results at 10°C, batters with 0.5% NaCl showed better WHC and lower firmness at 40°C, after one step pressurization and low PG, compared to the other treatments.

Summarizing the results, positive effects on the functional properties, with two-step pressurization for both temperature levels, were found for the 1% and 2% NaCl recipes, where pressure levels and holding times played a major role. Based on these observations, optimal treatment (maximum firmness, minimum WHC) after the two-step treatment with the first step at 200 MPa/ 300 s was estimated. A clear relationship was found between the functional properties under the processing conditions and the maximal solubilization of myosin fragments in this range. The improvement in solubilization is suggested to affect the level of aggregation and the gelation in the second HP-step. The mechanism of aggregation of myosin fragments in the first step was assumed to be similar to that reported by Yamamoto et al. (1993). The significant firmness increase in the batter matrix after first step treatment at 200 MPa with increasing duration of that HP-step correlated with the HPLC analysis observations (chapter 4.11), maximal elasticity analysis (chapter 4.13), SEM-structure analysis (chapter 4.14), and the analysis of NaCl content in the supernatant (chapter 4.15). According to all these analysis the suggestion that the level of aggregation (growing), which was found to be treatment time dependent, can affect the functional properties of firmness, WHC, elasticity as well as batter structure. That assumption is also confirmed by the observations of Yamamoto et al. (1993), finding aggregations to be growing

with increasing the treatment duration at 210 MPa. During the process of aggregates growth a process of protein-protein increase instead of protein-water interactions is supposed to occur.

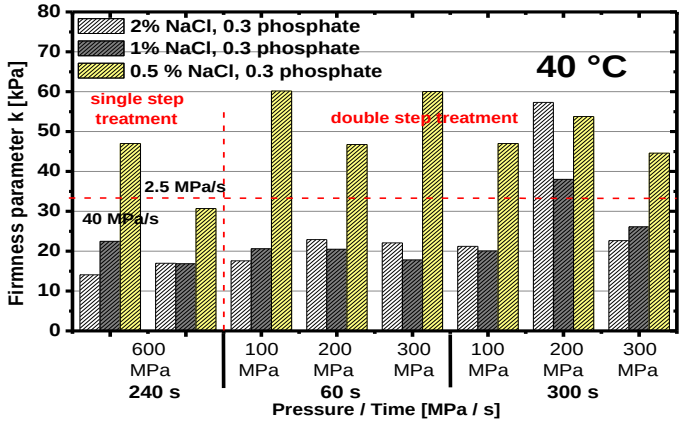


Figure 4.39: Batter firmness one step/ two steps HPP at 40 °C initial temperature (by second step 600 MPa/ 240s).

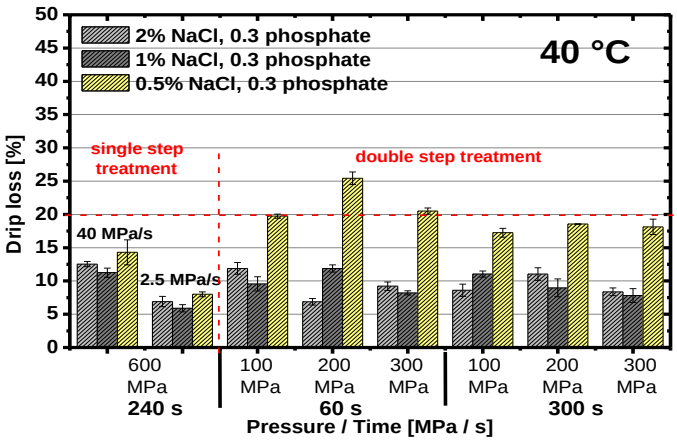


Figure 4.40: Drip loss comparison one step/ two steps HPP at 40 °C initial temperature (by second step 600 MPa/ 240s).

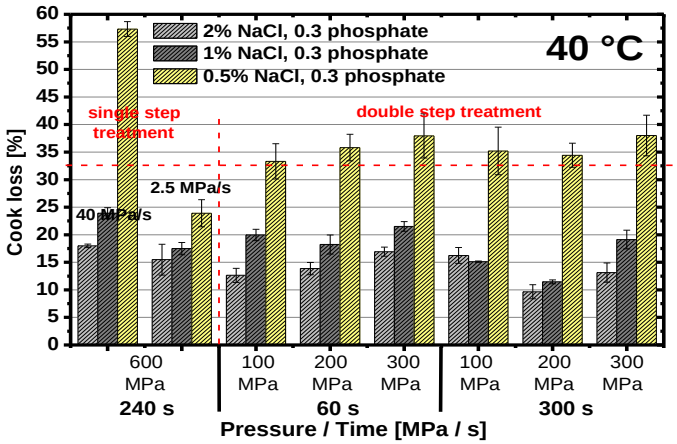


Figure 4.41: Cook loss comparison one step/ two steps HPP at 40 °C initial temperature (by second step 600 MPa/ 240s).

For the 0.5% NaCl the optimal HP- treatment was found to be occurring at one step pressure treatment after slow compression expressed in maximal WHC and relative low firmness.

4.13 Impact of one step and two steps HP- temperature treatment on the maximal elasticity

Maximal elasticity or the linear elastic (LE) range was obtained for various HP-temperature treatments of samples by performing amplitude sweep analysis. Analyzing the maximal elasticity range and the functional properties parameters was needed to make the knowledge of the structure-forming mechanisms, which takes part in the batter structure after HP-temperature treatment more complete.

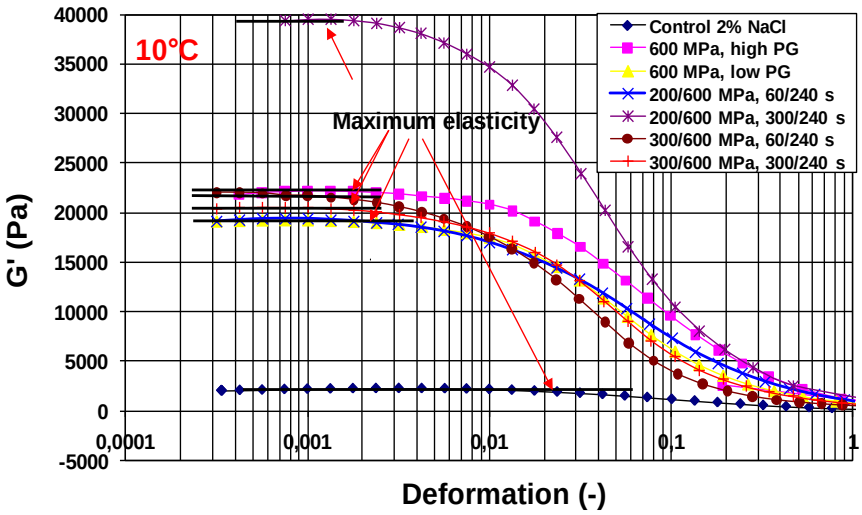


Figure 4.42: Maximal elasticity of one step and two steps HP- temperature treated batter at a) 10°C and b) 40°C- initial temperature.

Decrease of the linear elastic region such as maximal elasticity was observed after HP-temperature treatment of frankfurters batter (Figure 4.42 Figure 4.43). This decrease was more distinct after the two steps treatment correlating with measurements of firmness increase shown in Figure 4.36 and Figure 4.39. A tendency of LE- range decrease in dependency of PG and the steps of the HPP was found to be as follows: control>high PG>slow PG>two steps HPP. Minimum linear elasticity was found to occur after the two steps treatment at 200/600 MPa for the treatment time of 300/240 s was applied. The decrease of LE-range or deformation limit was more distinct at higher temperatures of 40°C Figure 4.43 and Figure 4.44.

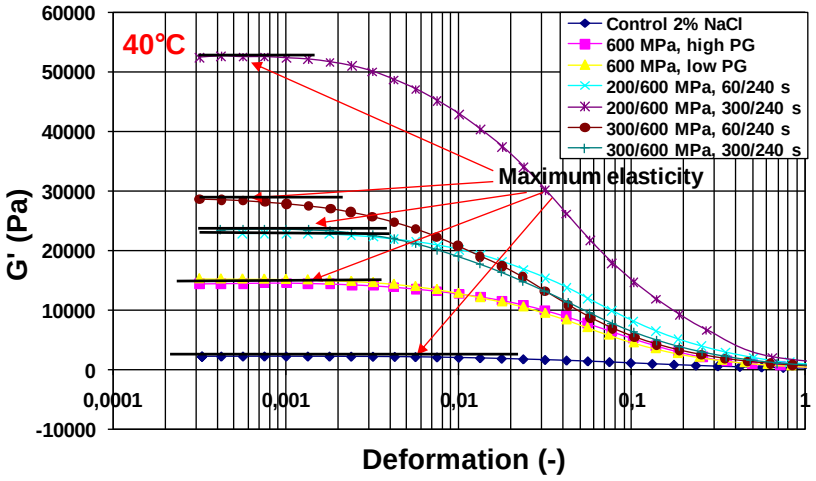
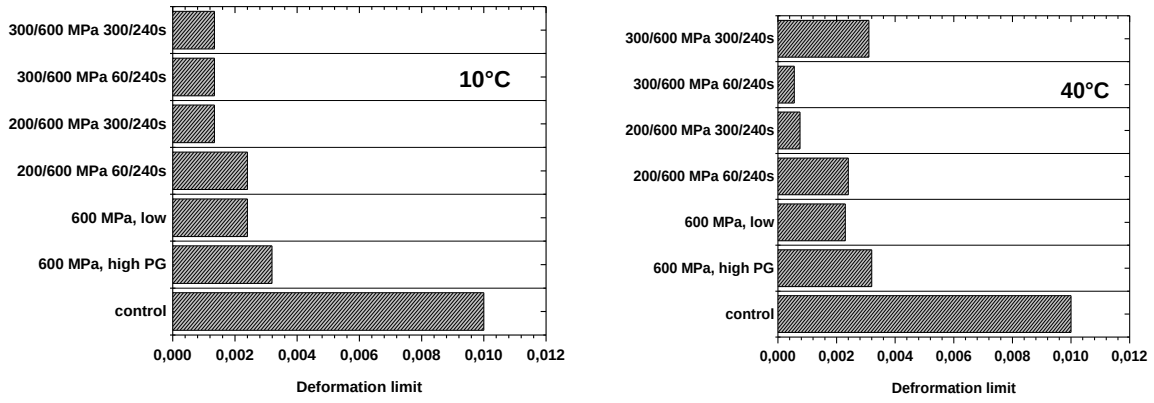


Figure 4.43: Maximal elasticity of one step and two steps HP- temperature treated batter at 40°C-initial temperature.

This minimum was found corresponding with the firmness maximum observed in Figure 4.36 and Figure 4.39 and discussed in chapter 4.12. Summarizing the results from chapter 4.12 and these from the current chapter it could be concluded that gels, that were derived after two steps treatment are firmer and less elastic than these after one step treatment. This firmness increase and loose of elasticity did not lead to significantly poorer WHC (Figure 4.40, Figure 4.41).



a) b)

Figure 4.44: Deformation limit of one step and two steps HP- temperature treated batter at a) 10°C and b) 40°C-initial temperature.

4.14 Comparison one step- two steps HPP and thermal treatment studied by SEM

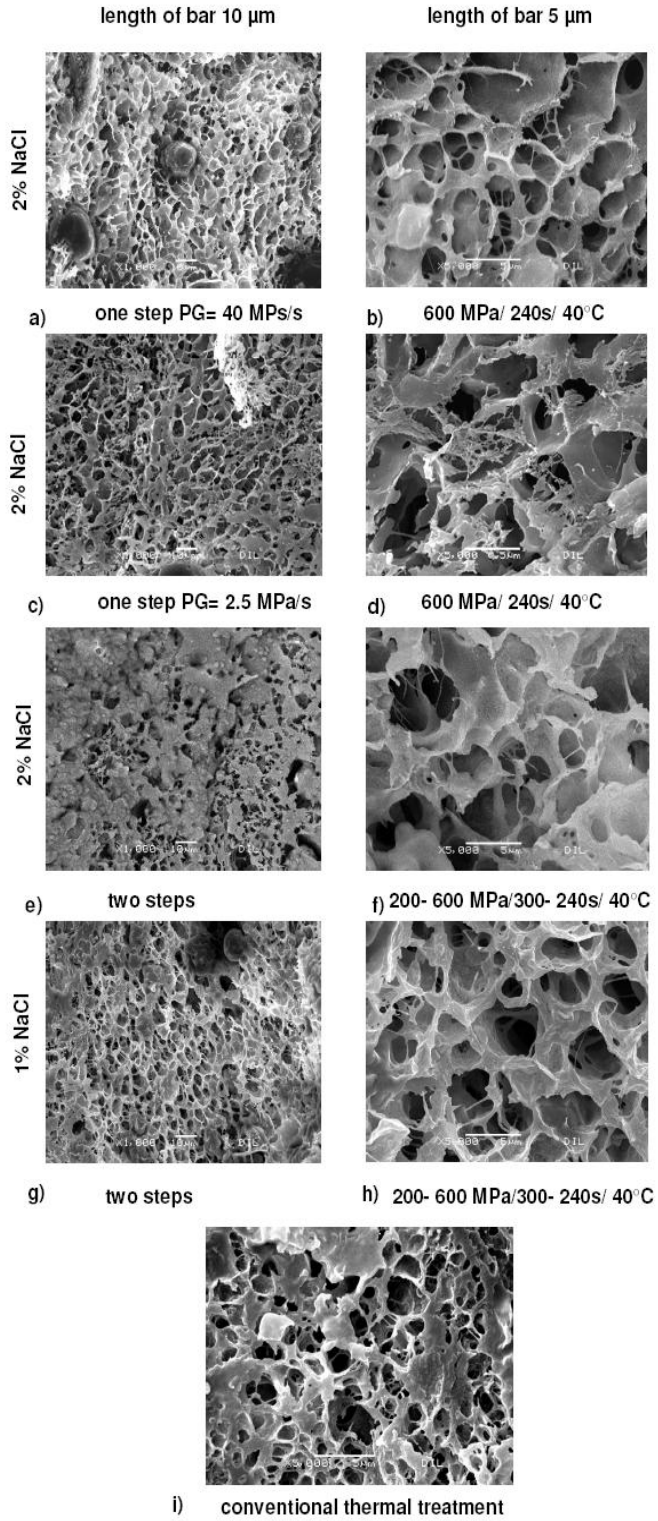


Figure 4.45: SEM micrographs of frankfurters batter structure (2% NaCl, 0.3 phosphate) treated at 600 MPa/ 4 min/ 40°- one step treatment at PG of 40 MPa/s and PG of 2.5 MPa/s (c,d); two steps treatment at 200 MPa/300 s/40°C first step for 2% NaCl content (e,f) and 1% NaCl (g,h) and thermal treatment (i).

Observation of the structural changes after one and two steps HP-temperature treatments in order to give additional explanation of the changes in the functional properties were performed by SEM. One-step pressurization (600 MPa/240 s/40°C) of frankfurters batter was compared to two steps treatments (200-600 MPa/ 300 s- 240 s for 2% and 1% NaCl) as well as to the conventional thermal treatment (Figure 4.45). Visible structure differences such as improved matrix swelling and gelation were found. The matrix of the two steps treatment (Figure 4.45- c, d, e, f, g, h) was significantly more swollen and thicker than the one step treatment matrix (Figure 4.45- a, b, c, d). These changes in the main and in the secondary matrix could explain the improvement of the functional properties showed in Figure 4.39, Figure 4.40 and Figure 4.41. The significant firmness increase for 2% and 1% NaCl content recipes in Figure 4.39 could be caused by an increase of protein-protein interactions, which resulted after an enhancement of the aggregation of soluble proteins.

The thermal treated matrix was found to be less swollen compared to the one- and two steps- HPP, which can be explained with the better WHC, derived by the improvement of water immobilization of the pressure treated batters.

4.15 Estimation of Salt content in the soluble phase

4.15.1 After one step pressure- temperature treatment in dependence on pressurization gradient (PG)

After analyzing the positive effects of HPP on protein solubilization and WHC improvement it was suggested that an eventual salt reduction of the conventional recipe could be possible. Therefore, a chemical analysis of NaCl content in the aqueous phase and a sensory analysis for contemplating the consumer's acceptance have been performed.

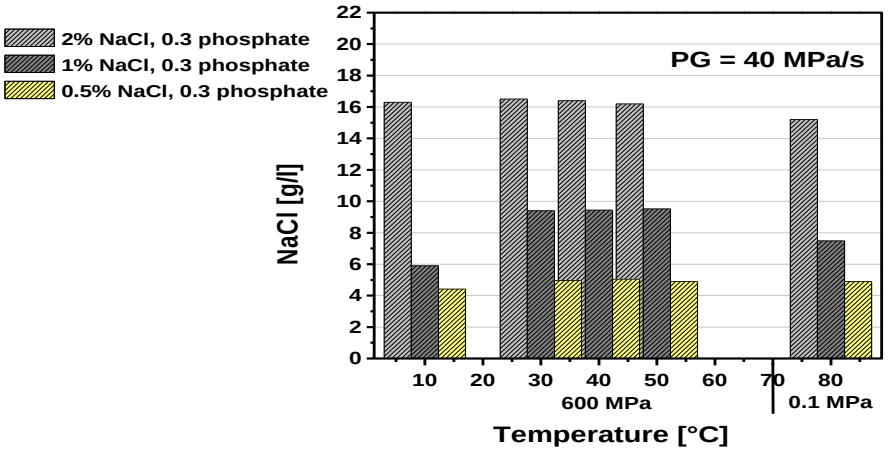


Figure 4.46: Salt content in the soluble phase at 600 MPa and different temperatures compared to the thermal treatment (0.1 MPa and 80 °C 20 min.) by high PG= 20 MPa/s.

One step pressure- temperature treatments at low and high PG as well as two steps treatment were compared to the conventional thermal treatment (Figure 4.46, Figure 4.47, Figure 4.48 and Figure 4.49).

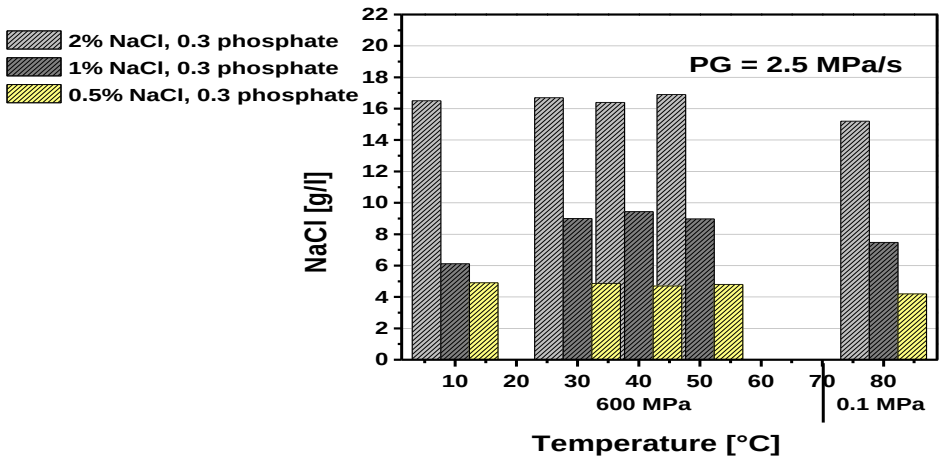


Figure 4.47: Salt content in the soluble phase at 600 MPa and different temperatures compared to the thermal treatment (0.1 MPa and 80 °C for 20 min.) by low PG= 2.5 MPa/s.

Increases of NaCl content in the aqueous phase were found for all HP- treatments compared to the conventional treatment. This was more distinct for the 1% NaCl recipe at temperatures above 30°C, for which a maximum was found to be at 40°C for PG = 2.5 and at 50°C for PG= 40 MPa/s (about 2 g/l more than the thermal treated). For 2% NaCl content recipe showed that the increase was maximum 1.5 g/l (at 600 MPa/50°C/2.5 MPa/s). For 0.5 % NaCl content recipe minimal increase with about 0.7 g/l (at 600 MPa/50 °C/ 2.5 MPa/s) was measured. The impact of PG after one-step pressure treatment at 600 MPa and 10°C-50°C initial temperature were found to be insignificant (Figure 4.46, Figure 4.47).

4.15.2 After two steps pressure-temperature treatment in dependence on initial temperature and treatment time

For estimating the effect on NaCl content in the aqueous phase of two steps HP-T treatments, with first steps in the range of 100-300 MPa and two treatment times (60 s and 300 s) at low and medium initial temperature 10°C, 40°C were studied (Figure 4.48, Figure 4.49). The second (denaturation step) was done at 600 MPa/240 s. The amount of NaCl after two steps treatment was compared to the one step treatment and the conventional treatment.

An additional increase of NaCl in the soluble (aqueous) phase was found after two steps treatment compare to the one step and the thermal treatment. It was significant (higher than 2% and 0.5 NaCl batters) for the reduced 1% NaCl content batters with a maximum at 200 MPa after 60 s treatment at 10° C and 40 °C (Figure 4.48, Figure 4.49).

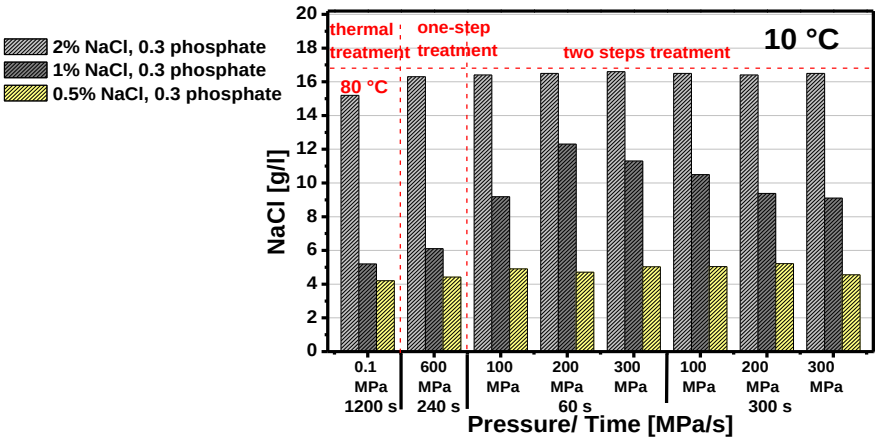


Figure 4.48: NaCl content in the soluble phase comparison one step to two steps HPP at 10 °C (by second step at 600 MPa/ 240s) and to the thermal treatment (at 0.1 MPa and 80 °C for 20 min.).

For the recipes with 2% and 0.5% NaCl the increases were minimal compared to the one step treatment and significant (for some process combinations) compared to the thermal samples. The maximum was found at 300 MPa/60 s/10°C (for 2% and 0.5% NaCl); 100 MPa/60 s/10°C (for 0.5% NaCl) and at 300 MPa/60 s/40°C (for 2% NaCl) respectively.

In general at low 10°C initial temperature the NaCl content increase was slightly higher than at 40 °C. In regard to the treatment time after 60 s, NaCl concentration was higher that after 300 s pressurizing. The increase of NaCl content is supposed to be somehow influenced by the solubilization level.

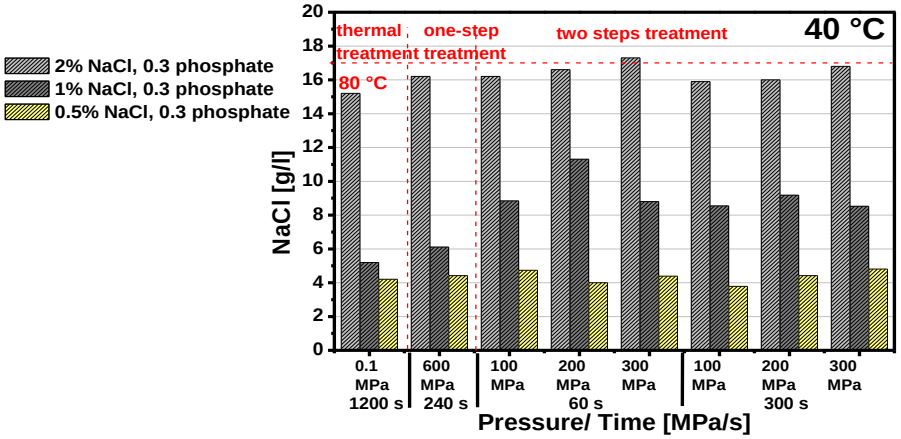


Figure 4.49: NaCl content in the soluble phase comparison one step to two steps HPP at 40 °C (by second step 600 MPa/ 240s) and to the thermal treatment (at 0.1MPa and 80 °C for 20 min).

According to the results in this chapter, it was estimated that HPP induced the increase of NaCl content in the soluble phase. This increase was found to be dependent on the initial NaCl content in the batter recipe. The most significant NaCl increases, compared to the thermally treated samples, were found for 1% NaCl after both one- and two-step treatment. This phenomenon is supposed to be related to the level of soluble proteins as well as their level of aggregation in the aqueous phase. In the batter supernatants with more small protein fragments aggregates it is assumed more salts are detected. This assumption is based on the hypothesis that the small protein aggregates could provide more active charged groups, attracting more salt ions, which was successfully detected by the analysis. Growing of the protein aggregates was supposed to reduce the free-charged groups, and subsequently also reduce the attracted salt ions. It was suggested that this growing would lead to more stable conformations in the main and the secondary matrix, reflecting in the reduction of ionic salt interactions in the aqueous phase.

The maximum of NaCl found after HPP with first step at 200 MPa/ 60 s confirmed the conclusions made in chapters 4.10 and 4.11. Namely that the most of the structure changes are due to the level of solubilization and aggregation processes, which take part in the HPP range of 200 MPa. The increase of the holding time at this pressure level showed obvious changes in the functional properties (firmness increase chapter 4.12) related to the reduction of the NaCl content.

The analysis of the results for the salt reduced samples showed that the effect of NaCl increase in the aqueous phase was higher for 1% NaCl samples. This correlated with the functionality properties and structure improvement found for this recipe, where these after HPP were similar to conventional recipe (2% NaCl). Therefore the pressure effect was more distinct for 1% NaCl batters. For batters with 2% NaCl content this effect was less detectable. This could be explained with a particular saturation level in the batters or with the higher protein solubilization caused by the higher ionic strength. For the reduced 0.5% NaCl content batters this minimal NaCl increase could be explained with the fact that no secondary network building according to the SEM observations (chapter 4.6) took place. Therefore the salts are expected to be involved in interactions in the main matrix caused by the fact of insufficient salts in the emulsion. But still, the positive pressure effect for this recipe was also visible.

The level of NaCl increase in the aqueous phase after HPP was supposed to have influence on the sausage taste so that an additional sensory test was needed.

4.16 Sensory analysis of frankfurters– comparison heat and high pressure/ temperature treated sausages

Sensory analysis using the paired comparison test according to ISO 5495 as described in material and methods chapter 3.3.9 have been carried out. Conventionally (thermally) treated frankfurters were compare to HP- treated at 600 MPa and 30°C, 40°C and 50°C. High pressure-temperature treatment was performed at the NC- Hyperbaric, Burgos, Spain equipment with low PG= 2.5 MPa/s. For the HP-T treated samples the conventional (2% NaCl) and the NaCl reduced (1% NaCl) were used. The samples were compared by different parameters such as firmness, juiciness and saltiness. Sensory assessors, as part of this analysis, were obtained to determinate the differences between two samples and the specifications of these differences.

Table 4.4: Sensory analysis- comparison thermal treated and HP- thermal treated differ in salty taste

HP-Temperature Treatment [MPa/ °C] NaCl content [%]	*Thermal treated Sample is saltier*	*HP- T Sample is saltier*	*No difference*	*Level of significance*
600 MPa/ 40°C 1% NaCl content	7		1	α= 0.5
600 MPa/ 50°C 1% NaCl content	6		2	not significant
600 MPa/ 50°C 2% NaCl content	1	6	1	not significant

Sensory analyses differ in salty taste presented in Table 4.4, which shows that samples treated using the conventional (thermal) treatment were significantly (≤ 0.05 p level) saltier than the pressurized samples (600 MPa/ 40°C and 1% NaCl content). At 600 MPa/ 50°C and 1% NaCl no significant difference was found, whereas 75% from the assessors defined the thermal treated samples as saltier. Half of them noted that these differences were minimal and two of them did not show any difference. The 2% NaCl batter distinguish no significant (≤ 0.05 p level) difference but 75% of the assessors found that the HP treated was saltier and only one the opposite.

Batter juiciness analyses showed that HP- temperature treated batters were significantly (≤ 0.01 p level) juicier than thermal treated samples (Table 4.5). Furthermore it was noted from almost all of assessors that juiciness was related with slimy, elastic and more voluminous structure.

Table 4.5: Sensory analysis- comparison thermal treated and HP- thermal treated differ in juiciness

HP-Temperature Treatment [MPa/ °C] NaCl content [%]	*Thermal treated Sample is juicier*	*HP- T Sample is juicier*	*No difference*	*Level of significance*
600 MPa/ 40°C 1% NaCl content		8		α= 0.1
600 MPa/ 50°C 1% NaCl content		8		α= 0.1
600 MPa/ 50°C 2% NaCl content		8		α= 0.1

Significant firmness (≤ 0.01 p level and ≤ 0.05 p level) was found after thermal treatment compared to HP treatment of 600 MPa/ 40°C/ 1% NaCl and 600 MPa/ 50°C/ 2% respectively (Table 4.6).

No significance was found at 600 MPa/ 50°C/ 1% NaCl even though 75% of the answers defined thermal treated batter as firmer.

Table 4.6: Sensory analysis- comparison thermal treated and HP- thermal treated differ in firmness

HP-Temperature Treatment [MPa/ °C] NaCl content [%]	*Thermal treated Sample is firmer*	*HP- T Sample is firmer*	*No difference*	*Significance Level*
600 MPa/ 40°C 1% NaCl content	8			α= 0.1
600 MPa/ 50°C 1% NaCl content	6	2		not significant
600 MPa/ 50°C 2% NaCl content	7	1		α= 0.5

Based on the results of the sensory analysis it could be concluded that after pressure treatment the sausages from the various combinations were significantly juicier and softer. At higher temperature and lower NaCl content a little firmness increase started. Furthermore were the thermal treated batters were with 75 % saltier compared to HPP-batters (600 MPa/ 50°C and 1% NaCl), which according to the assessors' comments were only slightly saltier.

In general, the results correlate with these estimated in the previous chapters, namely the improvement of WHC, due to prior solubilization improve (chapter 4.10), building of secondary matrix (chapter 4.6) as well as increase of NaCl in the aqueous phase (chapter 4.15).

4.17 P-T landscapes of functional properties of frankfurters batter

Models describing the effect of pressure and temperature on the functional properties, such as drip loss and cook loss, as well as the color of frankfurters batter were empirically fitted using statistical program (Table Curve 3D v3 Statistical Package, Systat Software Inc., Richmond, CA, USA).

4.17.1 Drip loss p-T landscapes of frankfurters batter depending on NaCl content and treatment time

Due to the natural character of batter matrix and possible minimal differences in protein content in the sausage composition, pH and preparation deviations in the modeling were expected. The complexity of the processes, taking part under different pressure/temperature/holding time conditions, has established the non- linear behavior of the water holding capacity. The WHC parameter of drip and cook loss were found according to the results of the current work and the research to be functions of several processes (denaturation, solubilization, protein molecule disruption, aggregation and gelation). These are presented in different combinations for every P/T/treatment time processing affecting the functional properties. Kinetics modeling (often used to present microbial inactivation) of the drip and cook loss parameters was extremely difficult. This was provoked from high mean deviations and the non-linear behavior of the parameters. According to the above-mentioned facts and the elaborateness of the eventual resulted models, no accurate interpretation of the results was expected. Therefore, an empirical fitting of cook and drip loss was chosen to be performed for every single treatment time. Pressure-temperature impact on drip loss in the range of 0.1-600/10- 60 °C for 3 different holding times 60 s, 300 s and 600 s in quasi isobaric/ isothermal (PG= 40 MPa/s) conditions was investigated (Figure 4.50). Drip loss models were obtained by empirically fitting using the Cosine series bivariate order 4 equation. According to the modeling some trends in the drip loss (WHC) depending on NaCl contents and treatment time under pressure–temperature combinations were estimated. Batters with reduced 1% NaCl were found to have a higher drip loss compared to the conventional recipe. This trend was more distinct for the 0.5% NaCl batters. The tendencies of the drip loss behavior for all recipes to decrease in the range of 200 MPa was observed to be enhanced at higher temperatures (< 50C).

The minimum in the range of 150-200 MPa and higher temperatures was concluded to be related to the maximal protein solubilization, which in combination with temperature above 50°C led to enhanced improvement of the secondary and the main matrix, resulting in better WHC. In the pressure range of 300–450 MPa increase of drip loss, which was reduced at higher temperatures for most of the models. This effect could be explained with the matrix

transformation in this range and because of the fact that the gelation is not complete. WHC pressure improvement is generally expected at pressure levels above 450 MPa (Tintchev, Buckow et al. 2007).

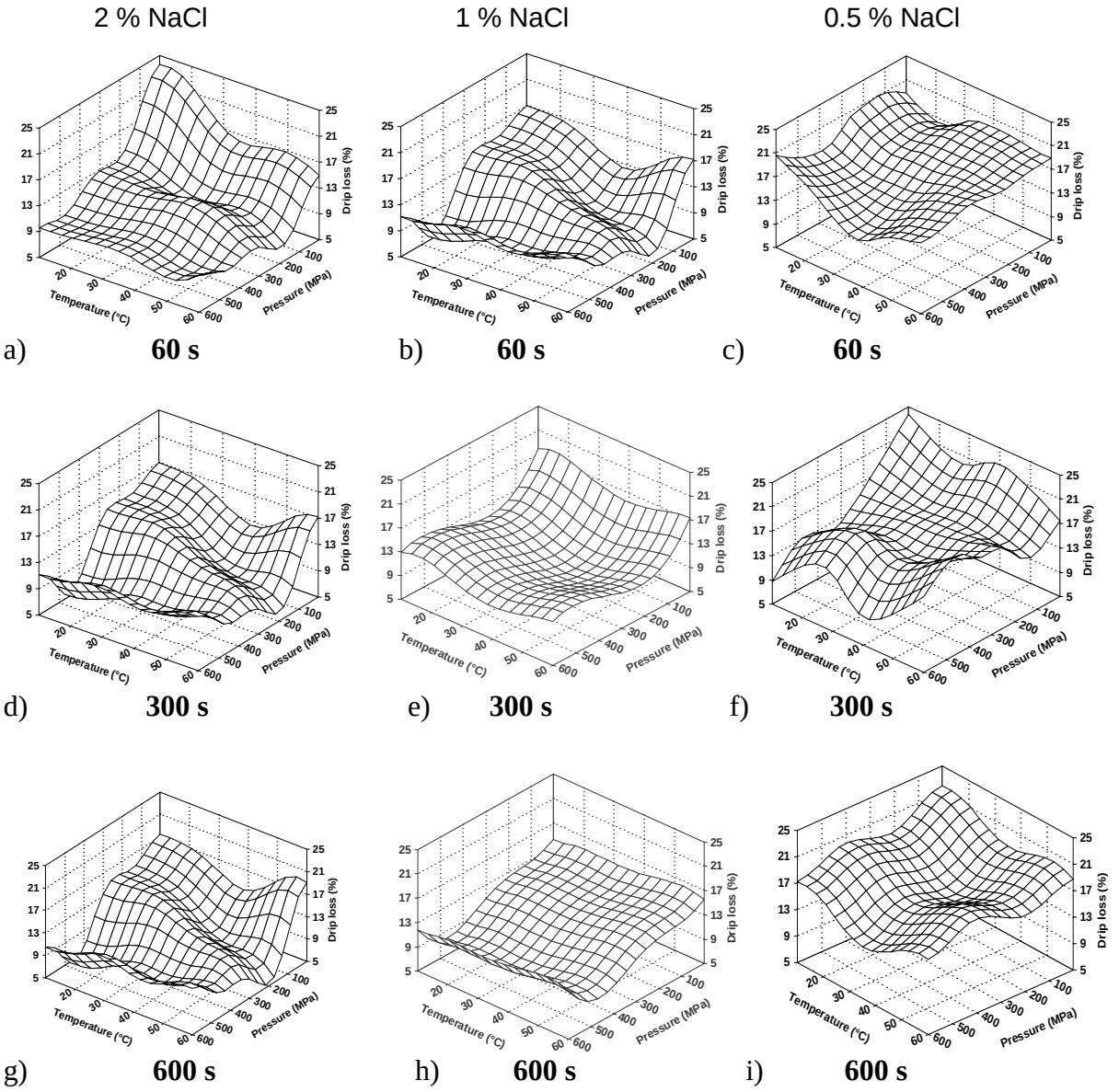


Figure 4.50: Drip loss p-T landscapes of frankfurters batter depending on NaCl content 2% NaCl- column I- a) 60 s (R^2=0.74); d) 300 s (R^2=0.95); g) 600 s (R^2=0.90);; 1% NaCl- column II- b) 60 s (R^2=0.64); e) 300 s (R^2=0.68); h) 600 s (R^2=0.72); 0.5 NaCl- column III- c) 60 s (R^2=0.82); f) 300 s (R^2=0.84); i) 600 s (R^2=0.69)

The results of drip loss improvement reported by Tintchev, F. *et al.* 2007 as shown in Figure 2.24- b estimated for raw pork and poultry meat correlated with these shown in (Figure 4.50). Therefore, after HP-treatment at higher pressures above 450 MPa drip loss generally decreases. This effect was synergistically enhanced with increasing temperature.

It has to be contemplated that the PG= 40 MPa/ s can assure an extremely short pressure build up time so that protein solubilization during pressurizing is minimal. Therefore matrix

with optimal drip loss in the range of 150-200 MPa at higher temperatures was logical according to the observations of Macfarlane et al. (1984) and the current study respectively. Summarizing the results of this chapter, it can be concluded that drip loss is minimal (maximal WHC) at 200 MPa and above 500 MPa pressure treatments at temperatures higher than 40 °C.

4.17.2 Cook loss p-T landscapes of frankfurters batter depending on NaCl content and treatment time

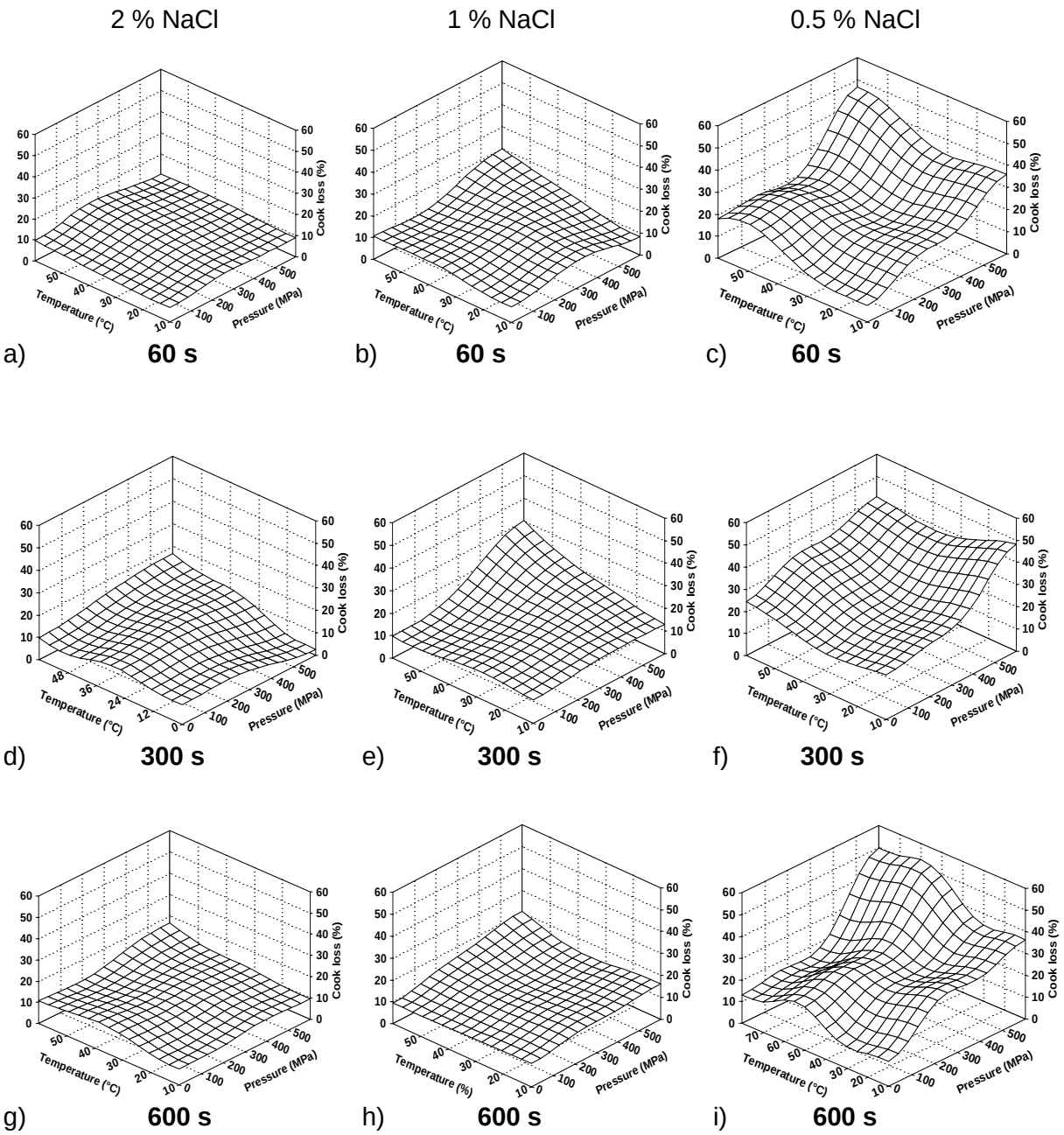


Figure 4.51: Cook loss p-T landscapes of frankfurters batter depending on NaCl content. 2% NaCl- column I- a) 60 s (R^2=0.64); d) 300 s (R^2=0.72); g) 600 s (R^2=0.56);' 1% NaCl- column II- b) 60 s (R^2=0.63); e) 300 s (R^2=0.70); h) 600 s (R^2=0.70); 0.5 NaCl- column III- c) 60 s (R^2=0.73); f) 300 s (R^2=0.61); i) 600 s (R^2=0.75)

Cook loss or WHC of pressure treated batters with different NaCl contents after batter cooking for 20 min was studied. Cook loss models were obtained after empirically fitting using the Cosine series bivariate order 4 equations (Figure 4.51). Cook loss was found to increase with reducing the NaCl content in the recipe. This was for the 1% NaCl recipe minimal, but for recipes with 0.5 % NaCl it was significantly higher. At low temperatures cook loss was only slightly higher with increasing pressures for the 2% and 1% NaCl, which was enhanced at higher temperatures (Figure 4.51- a, b, d, e, g, h). In contrast to this, pressure induced cook loss increased at low temperatures for the 0.5 % NaCl recipes. This was additionally more affected at higher temperatures resulting in significant cook loss increase (Figure 4.51 – c, I). Similar results were reported from (Iwasaki, Noshiroya et al. 2006), who studied cooking loss of pork batters in the range of 0-400 for unpressurized and pressurized batter with 1- 2% NaCl.

4.17.3 Drip loss p-T landscapes of frankfurters batter (conventional recipe) depending on PG and treatment time

In order to obtain the PG impact on the drip loss, the models were obtained by empirically fitting of the Cosine series bivariate order 4 equations (Table Curve 3D v3 Statistical Package, Systat Software Inc., Richmond, CA, USA). As already discussed in chapter 4.2 observing the pressure levels at 300 MPa and 600 MPa a major role for the functional properties and particularly on tne WHC expressed in drip and cook loos was found to be played by the PG parameter. Frankfurter batters have been HP-temperature treated with PG= 40 MPa/s and PG= 2.5 MPa/s for the whole possible pressure range up to 600 MPa in process temperature combinations up to 60 °C for three different treatment times (Figure 4.52). The significant improvement of the WHC after treatment with low PG was clearly observed for all treatment times. However it was more enhanced at higher pressures and temperatures. The small drip loss increase at 300-400 MPa after the optimal solubility range of 200 MPa for the batters with low PG was almost invisible compared to the batters treated with high PG. Drip loss at 200 MPa, 600 MPa and at temperatures of 50 °C, 60 °C and PG= 2.5 MPa/s was found to be almost similar. In contrast, after comparison of the batters treated with high PG under the same treatment conditions, higher drip loss values were observed at pressures of 600 MPa. Therefore the improving effect in the range of 200 MPa (optimum solubilization according to chapters 4.10 and 4.11) and higher temperatures through the low PG were reached (compensate). However after 600 s treatment time the WHC improvement due to the PG- impact was decreased especially for the 200 MPa and 600 MPa at temperatures higher than 40°C.

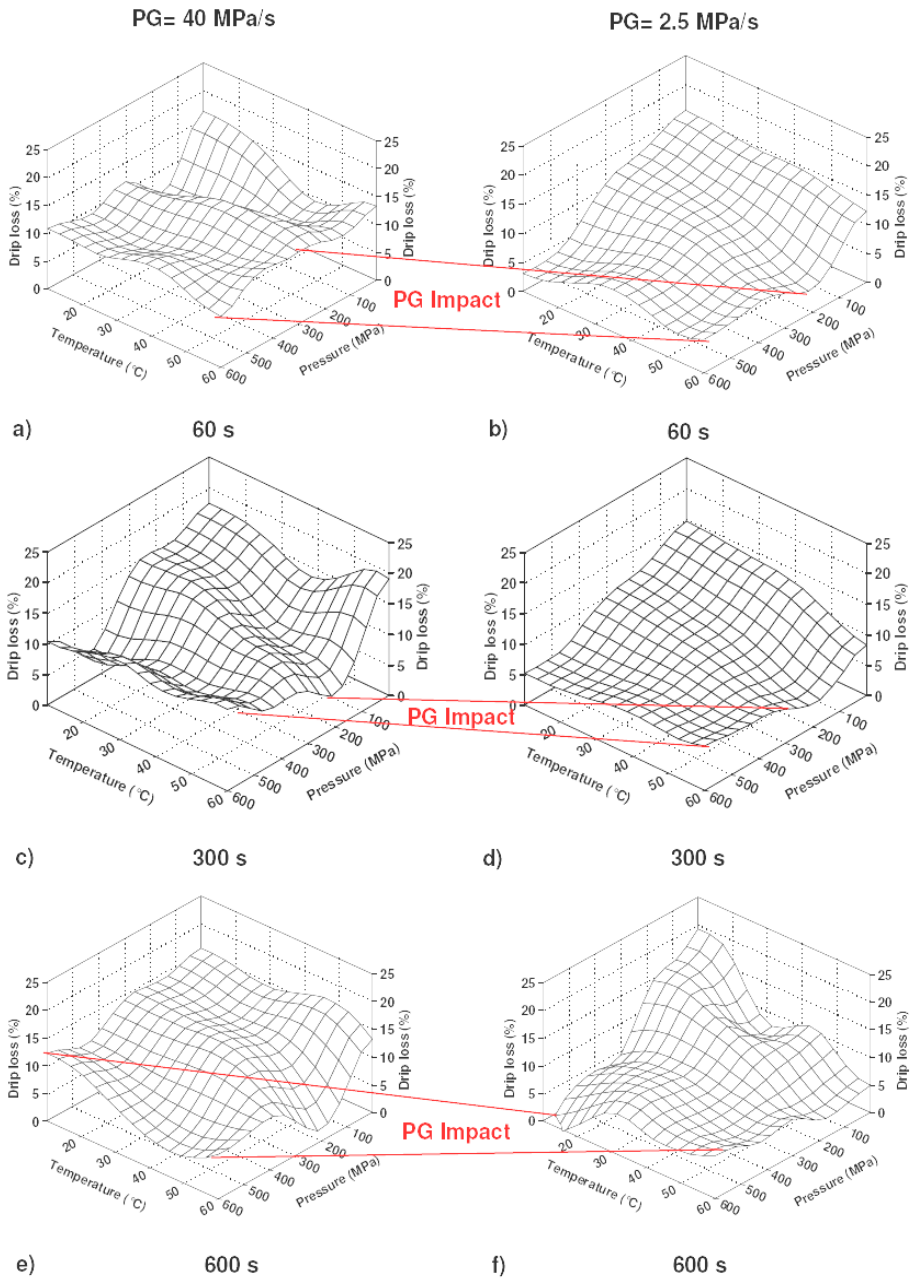
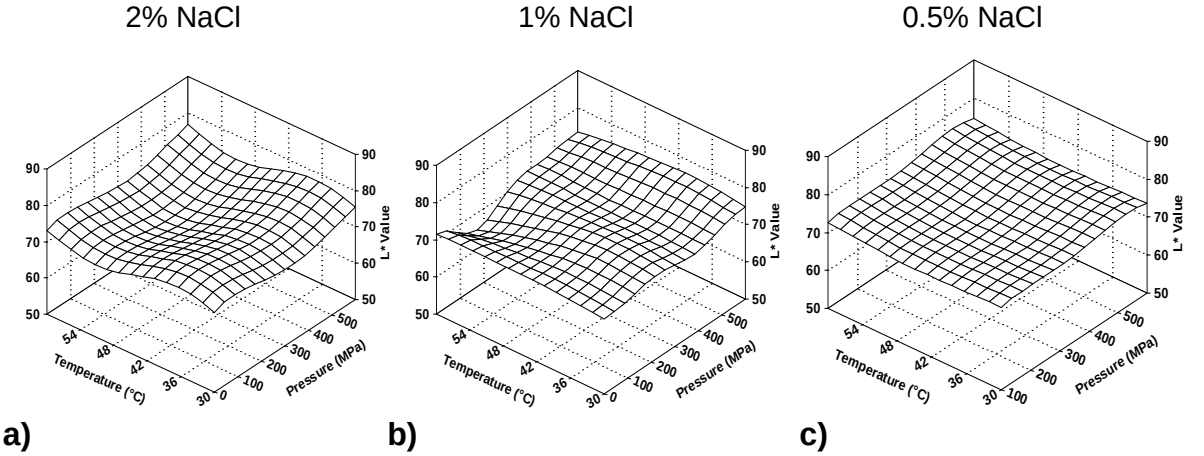


Figure 4.52: Drip loss p-T landscapes of frankfurter batters according to the conventional recipe (2 % NaCl, 0.3 % phosphate depending on PG content (PG= 40 MPa/s- column1- a (R^2=0.77), c (R^2=0.90), e (R^2=0.84); PG= 2.5 MPa/s - column 2- b (R^2=0.90), d (R^2=0.64), f (R^2=0.88) and treatment time of 60 s, 300 s, 600 s.

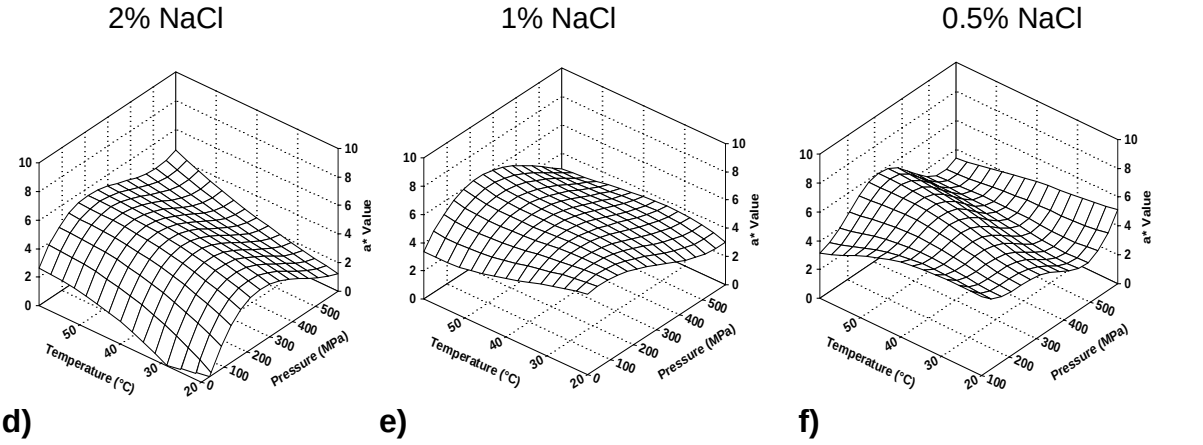
4.18 P-T landscapes of frankfurters batter color parameters

Empirically the p-T impact on of color parameters (L*, a*, b*) was derived by fitting of the experimental data performed by Table Curve 3D v3 Statistical Package, Systat Software Inc., Richmond, CA, USA. Experimental data was obtained for different p-T combinations for 240 s treatment time and PG = 40 MPa/s and showed in Figure 4.53.

L* parameter



a* parameter



b* parameter

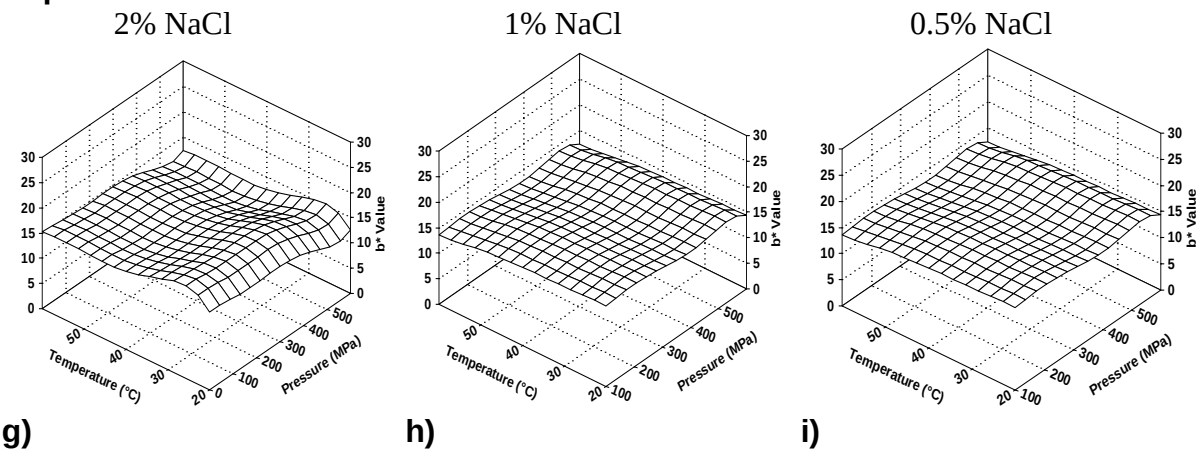


Figure 4.53: P-T landscapes color changes of the CIE L*, a*, b* parameters after 240 treatment and PG= 40 MPa/s. Lightnes parameter L*- Row I- a) 2% NaCl (R^2=0.98); b) 1% NaCl (R^2=0.97); c) 0.5% NaCl (R^2=0.82); redness parameter a*- Row II- d) 2% NaCl (R^2=0.71); e) 1% NaCl (R^2=0.57), f) 0.5% NaCl (R^2=0.92); yellowness parameter b*- Row III- g) 2% NaCl (R^2=0.73); h) 1% NaCl (R^2=0.92); I) 0.5% NaCl (R^2=0.92)

Experimental data of the L*- parameter has regressively been fitted to a 5-order polynomial (4.1):

$$\ln (L^*) = A_0 + A_1 p + A_2 p^2 + A_3 p^3 + A_4 p^4 + A_5 T + A_6 T^2 + A_7 T^3 + A_8 T^4 + A_9 T^5 \qquad (4.1)$$

where the parameters A_0, A_1, A_2, A_3, A_4, A_5, A_6, A_7, A_8 and A_9 were estimated by regression analysis using pressure in (MPa) and temperature in (°C).

According to the estimated models for the lightness color parameter L* no significant differences by comparison of the particular recipes with 2%, 1% and 0.5% NaCl was detected (Figure 4.53- a, b, c). A slight lightness increase with increasing pressure and temperature was observed.

Experimental data of the a*- parameter has regressively been fitted to a third- order polynomial (4.2):

$$\ln(a^*) = A_0 + A_1 p + A_2 T + A_3 p^2 + A_4 T^2 + A_5 pT + A_6 p^3 + A_7 T^3 + A_8 pT^2 + A_9 p^{2T} \qquad (4.2)$$

The redness parameter a* increased for all recipes up to pressures of 200-300 MPa (Figure 4.53- d, e, f). This was followed at higher pressures above 300 MPa up to 600 by a decrease until reaching values similar to the control samples.

The experimental data of the b* parameter has been fitted to a 5-order polynomial (4.3):

$$\ln(b^*) = A_0 + A_1 p + A_2 p^2 + A_3 p^3 + A_4 p^4 + A_5 p^5 + A_6 T + A_7 T^2 + A_8 T^3 + A_9 T^4 + A_{10} T^5 \qquad (4.3)$$

The yellowness- b* parameter was found to decrease with increasing pressure for all batters recipes. Therefore salt content was not a significant factor for the yellowness parameter according to the regression models.

On the basis of the estimated models it can be assumed that the changes of the three color parameters L*, a* and b* for the three particular recipes with 2%, 1% and 0.5% NaCl were very minimal. Therefore the color of the frankfurters batters under the investigated conditions was found to be stabilized also by the recipes with reduced salt content (NaCl). The color differences between thermally and high pressure treated batters was found to be also not significant (chapter 4.5). These led to the conclusion that pressure-temperature treatment of sausages even with reduced salt content has no negative effect on color.

4.19 Possible industrial applications. Effect of p-T on turkey mechanically deboned meat (MDM) sausage

4.19.1 Firmness reduction of poultry (MDM) frankfurters- recipe development, estimation of best treatment parameter

Frankfurters that are formed through HPP have generally more elastic and softer structure compared to thermally treated samples. Firmer structure, closer to the firmness of thermal treated batters, can be obtained after HP-temperature treatment at higher initial temperatures above 50°C but anyway this structure is also elastic and soft. These elastic properties were due, as already discussed, to the induced solubilization and building of secondary structure. Another reason for the enhanced softness and elasticity of the batters was the composition of meat proteins in the pork batter and especially the myosin content in the aqueous phase. Therefore for industrial application of pork frankfurters the firmness of the HP-treated sausages has to be increased to be as similar as possible to the conventional (thermal) sausages, which are known to the consumers. One possible way to improve the structure firmness is the optimization of the recipe based on changes of the main components or using different additives (proteins, emulsifiers). Another possibility could be the optimization of the process parameters (grinding gradient, cutting grade, cutting rate, pH and temperature).

Searching for other possible application fields, some analysis of pressure/temperature treated frankfurters made from mechanically deboned poultry meat (MDM) were carried out.

Mechanically deboned poultry (MDM) meat is added mostly in comminuted meat products such as frankfurters and hotdogs. In most European countries the use of MDM meat is limited because of the low customers' acceptance as well as the short shelf life due to higher contamination hazards this meat may cause. By contrast, in some Asian countries based on their religious, political and economical specifics, the industrial application of MDM is of great interest.

Sausages (hotdogs and frankfurters) made from mechanically deboned meat (MDM) without added binders and fillers are known to have very firm structure and bad WHC. This is caused by the increased content of non-salt soluble connective tissue (collagen, elastin) and synergistically affected through the lower myosin content. After analyzing the effect (improvement) of solubility and WHC of pork frankfurters batters it was supposed that a similar positive HPP effect on the MDM turkey batters could be reached.

MDM turkey batters were prepared according the recipe in Table 3.3 but without the addition of any binders. Samples were treated at 600 MPa and 40 °C starting temperature for 5 min holding time in the NC-hyperbaric, Burgos, Spain equipment (Figure 4.55). Pressure and convenience treated (25 min at 80 °C) samples were compared by estimating the cutting force and firmness parameter using texture analyzer as described in chapter 3.3.8.

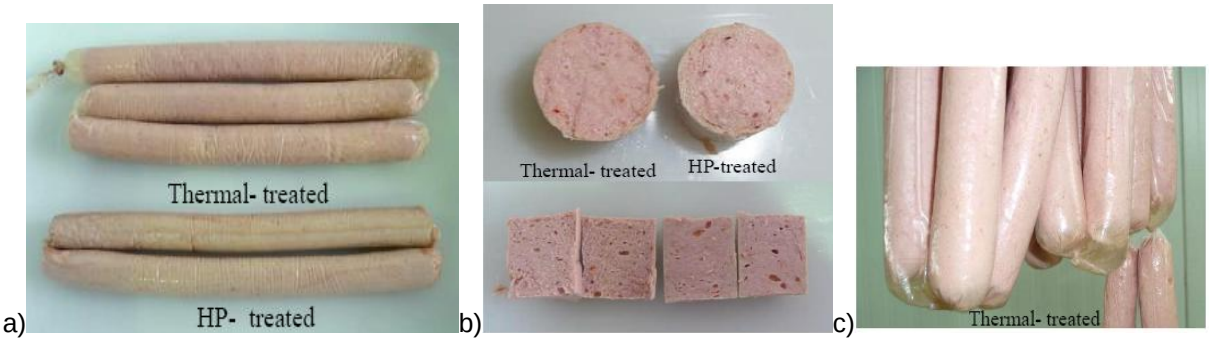


Figure 4.54: a) Thermal and high pressure treated sausages; b) thermal and high pressure treated sausage structures; c) purge loss after cooking.

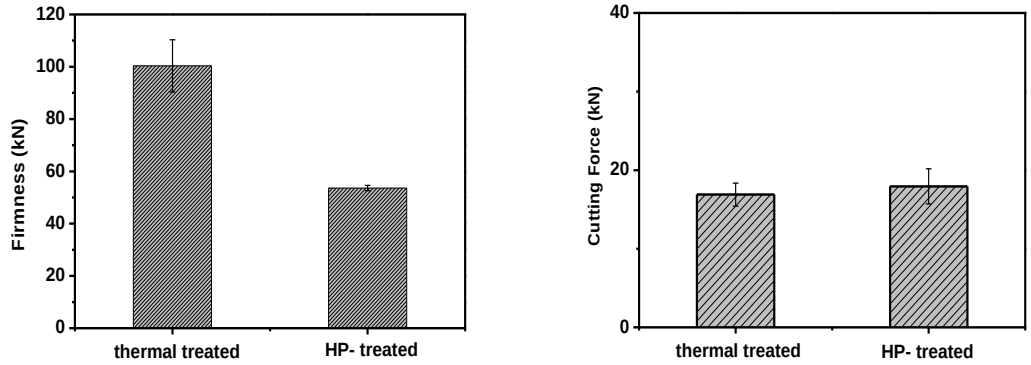
The structure of HP-treated sausage was obviously more homogeneous (Figure 4.55- b). Significantly higher purge loss was detected after thermal treatment comparing to HP-temperature treated samples, where no separation of purge loss was found (Figure 4.55- a). The poor water immobilization properties after thermal treatment supposedly was to be due to the thermal shrinkage of collagen (Ziegler and Acton 1984; Pearson and Gillett 1999). The shrinkage results in uncoated fat particles and bad immobilization batter properties (according to the mechanism in Figure 2.11). Therefore, the final products were of poor quality with fat cap at the top of the sausage as well as a jelly pocket at the bottom.

Texture analysis measurements of the batter cutting force and firmness parameter are presented in (Figure 4.54). As it is shown, the firmness of thermal treated samples was twice as high as HP-treated samples (Figure 4.54- a). However, no significant difference was found between the cutting force parameter of thermal and HP- treated samples (Figure 4.54- b).

For the improvement of frankfurters' functional properties made from MDM turkey meat some optimizations of the recipes have been performed. 2% wheat fibers were added to the MDM poultry batters in the recipe (Table 3.3). This led to firmness decrease of the conventional thermal treatment, which in this case was the desired positive effect (Figure 4.56- b). Further decrease of firmness and cutting force was observed after the combined HP- temperature treatment was performed (Figure 4.56). The firmness decrease, which occurred after the addition of wheat fibers was suggested to be caused after their gelatinization took place under temperature and HP processing. It was reported that after HP-treatment and medium temperature (in the range of 30- 40 °C) maximum gelatinization of wheat starch take parts (Bauer and Knorr 2005).

Similar observations were reported also by (Douzals, Maréchal et al. 1996), who found that irreversible starch gelatinization started above 300 MPa. According to other reports the gelatinization process could be shifted at lower pressures and higher temperatures (Bauer and Knorr 2005). Therefore HP-T treatment of MDM batters containing wheat fibers in their

recipe was found to have a positive effect in regard of WHC improvement and firmness reduction. The effect of firmness reduction was more enhanced after a further optimization of the recipe caused by an addition of 10 % plant oil. Increasing of pressure and temperature, which led to firmness increase, may be due to further formation of cross linkages.



a) b)

Figure 4.55: a) Firmness of thermal and HP-treated sausages; b) Cutting force of thermal and HP-treated sausages.

Parallel to the gelatinization of wheat fibers some HP-structural changes of collagen took place (Ichinoseki, Nishiumi et al. 2006). They reported that HHP tenderization (shear force decrease) of bovine intramuscular connective tissue, correlated with a reduction of collagen thermal stability. They have also observed a significant increase of soluble collagen, which was redoubled after treatment at 500 MPa. The results reported by Ichinoseki *et al.* 2006 and the observations of the current research confirmed, that significant changes occurred in the very compact und thermal resistant collagen molecule after HPP was applied.

Besides the positive collagen and wheat fibers changes, solubilization and gelation of the small myosin sub-units contribute to the matrix improvement.

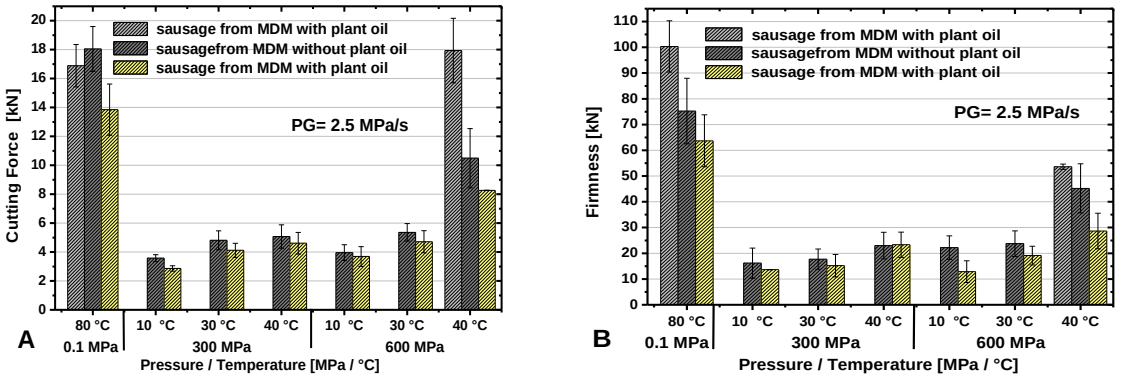


Figure 4.56: a) Cutting force and b) Firmness of temperature-pressure treated MDM poultry sausages according to the recipes (with and without plant oil).

4.19.2 WHC analysis (drip and cook loss)

The changes of WHC presented as drip and cook loss of MDM turkey batters are shown in Figure 4.57- a, b. Drip and cook loss minimum was found after the conventional thermal treatment. According to the Figure 4.54 and Table 4.8 drip and cook loss were expected to be at their worst after the thermal treatment. It was suggested that these minimal values were due to poor water immobilizing properties of the thermal treated samples (as water was already separated as a purge before the analysis). Therefore a representative comparison to the thermal treated samples was in this case not possible. A small drip and cook loss increase up to 30 °C and improve (decrease) above at 40°C after HPP was detected.

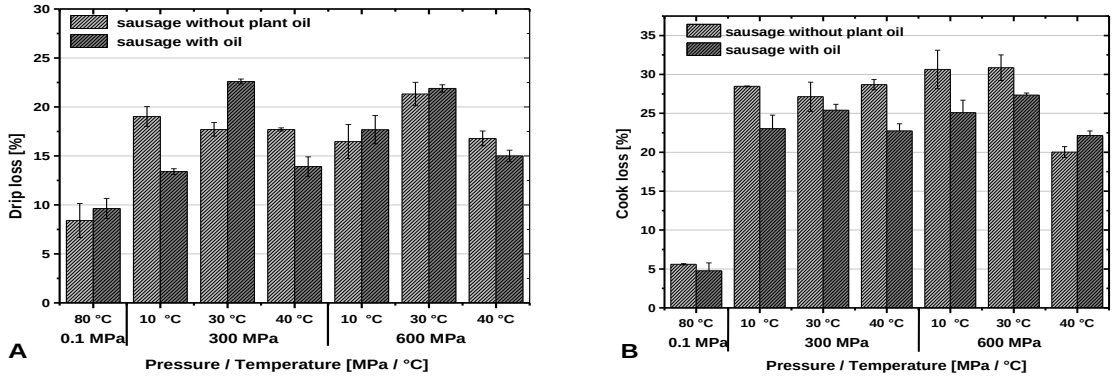


Figure 4.57: a) Drip loss and b) Cook loss of temperature-pressure treated MDM poultry sausages according to the recipes (with and without plant oil).

4.19.3 Sensory analysis

Based on the product development of the MDM poultry, processed with HP, medium initial temperature of 40 °C and cold smoking improved frankfurters sausages (with 2% wheat fibers and 10% plant oil) were prepared These were presented at the Seminar Meat and processed meat–innovative product design concepts (October, 2009). Product acceptance according to the meat experts was found to be very positive. Representative data of sensory analysis estimating the functional properties saltiness, juiciness and firmness similar to the pork batters were performed and presented in Table 4.7, Table 4.8 and Table 4.9. For this sensory analysis, two different batter recipes, one with 2% NaCl, 0.3 phosphate content for the thermal treatment, and one with reduced 1% NaCl, 0.3 phosphate for the HP-temperature treatment, were used. This was done so that sausages undergoing pressure processing and salt reduction can be compared by sensory analysis to the conventional treatment.

Table 4.7: Sensory analysis- comparison thermal treated and HP- thermal treated of MDM- poultry differ in salty taste

HP-Temperature Treatment [MPa/ °C] NaCl content [%]	*Thermal treated Sample is saltier [2% NaCl]*	*HP- T Sample is saltier [1% NaCl]*	*No difference*	*Level of significance*
600 MPa/ 30°C	5		2	not significant
600 MPa/ 40°C	4		3	not significant
600 MPa/ 50°C	4	1	2	not significant

According to Table 4.7 no significant difference between thermal with 2% NaCl and HP-temperature treated samples with reduced 1% NaCl was found. Based on these results a reduction with about 100% or 75% of NaCl for an eventual industrial application could be possible. No significant difference when estimating the batter juiciness was found. But still 85.7 % of the assessors found HP- temperature treated samples to be juicier.

Table 4.8: Sensory analysis- comparison thermal treated and HP- thermal treated of MDM poultry differ in juiciness

HP-Temperature Treatment [MPa/ °C] NaCl content [%]	*Thermal treated Sample is juicier [2% NaCl]*	*HP- T Sample is juicier [1% NaCl]*	*No difference*	*Level of significance*
600 MPa/ 30°C	1	6		not significant
600 MPa/ 40°C		6	1	not significant
600 MPa/ 50°C	1	6		not significant

No significant difference (≤ 0.05 p level) of the firmness parameter was estimated but 85.7 % of the assessors noted the thermal treated samples were firmer Table 4.8.

Table 4.9: Sensory analysis- comparison thermal treated and HP- thermal treated of MDM-poultry differ in firmness

HP-Temperature Treatment [MPa/ °C] NaCl content [%]	*Thermal treated Sample is firmer [2% NaCl]*	*HP- T Sample is firmer [1% NaCl]*	*No difference*	*Significance Level*
600 MPa/ 40°C	6	1		not significant
600 MPa/ 50°C	6		1	not significant
600 MPa/ 50°C	6	1		not significant

4.19.4 Microstructural changes of HP- temperature and conventional treated MDM batters

The functional and sensory changes discussed in the previous chapter have their logical explanation in the microstructure batter changes (mechanisms), which resulted after thermal and pressure- temperature treatment. Increase of the juiciness and saltiness as well as decrease of firmness (in this case these were desireded effects) were related to some microstructure changes analyzed by SEM and showed in Figure 4.58. When using different magnification levels some visible differences were found. Generally batter matrix was found to be not as homogeneous as the pork frankfurters matrix. The MDM turkey batter matrix was found to be rougher and separated as different regions could be defined. Regions with finer network were also detected. These were assumed to be built from myosin or myosin sub-units. Small bone particles, which resulted from the separation process, were also detected. Their part in the MDM turkey meat is normally about 1%. The main matrix was formed from a mixture of connective tissue (collagen), small content myofibrilar proteins and wheat fibers.

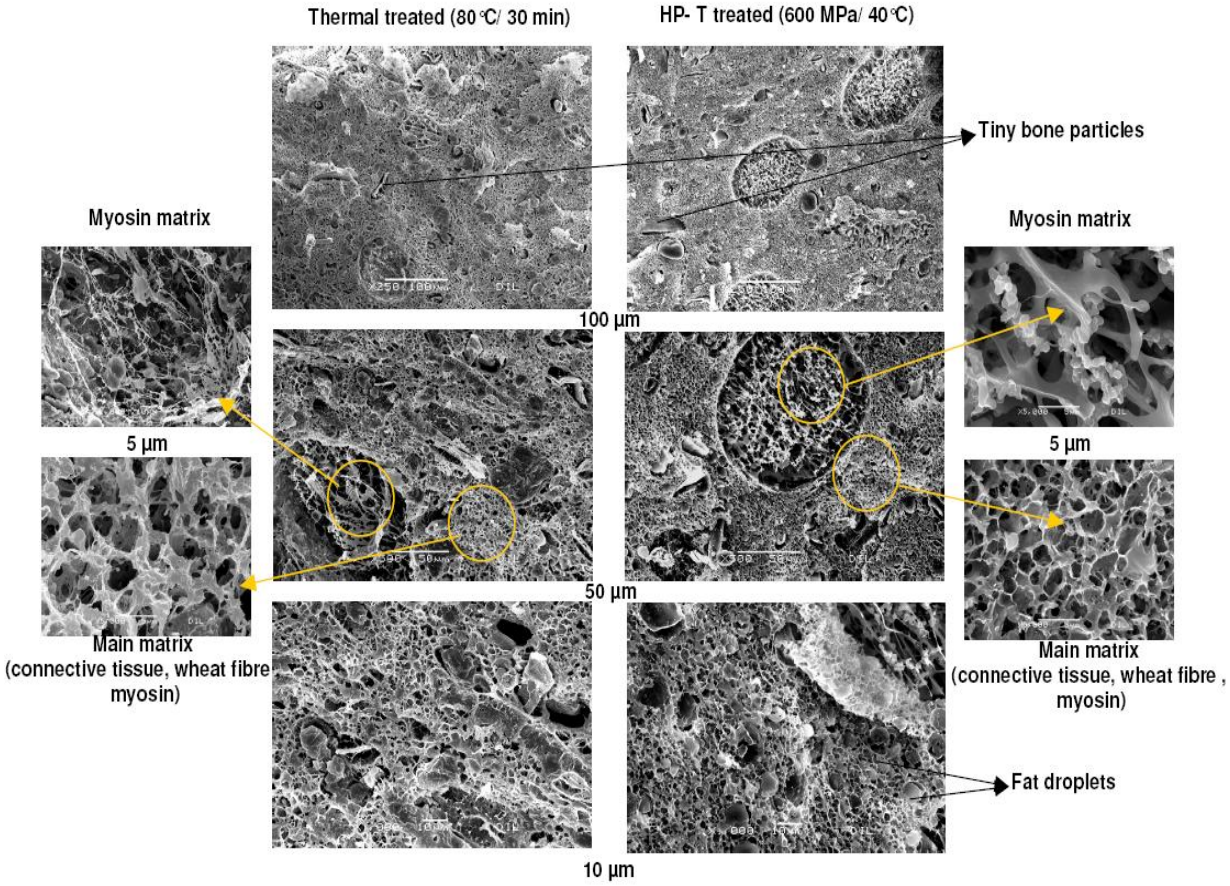


Figure 4.58: Batter microstructure of MDM poultry analyzed by (SEM). Comparison between conventional thermal treatment (80°C/ 30 min) and HP-T treatment (600 MPa/ 40°C).

After HP- temperature processing an improvement of the main matrix was observed. It was more homogenous, finer and with more gaps (sublimated water). The increase of gaps in the matrix resulted in improvement of water immobilization as more water is held by capillary forces. . A significant improvement in the myosin region was also observed which was better developed than the network built from connective tissue.

4.19.5 Color (L*a*b*) analysis- comparison HP- temperature and conventional (thermal) treated MDM sausages

Impact of HP- temperature treatment on the color of MDM turkey frankfurters as important quality parameter was estimated. Recipes with and without the addition of plant oil after combined HP- temperature and conventional (thermal) treatment were analyzed using L*a*b*color space system Figure 4.59. Small increase of L* (lightness) and decrease of a* and b* values with increasing pressure were observed. The recipe with 10 % plant oil was found to have higher L*and lower a* value, which can be explained with the differences in the light reflectance. After comparison of HP-temperature treated samples at 600 MPa and the conventional (thermal treatment) no significant color changes were found. Thus no negative color effects were found after application of high pressure treatment of MDM turkey sausages.

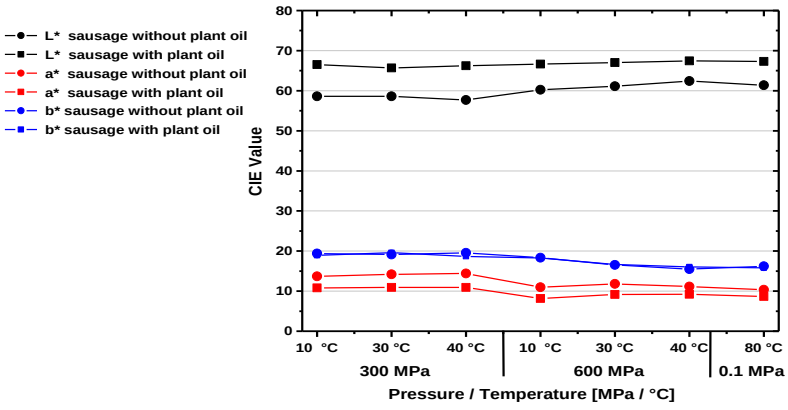


Figure 4.59: Changes of the CIE color parameter L*, a*, b* of MDM turkey HP- temperature and thermal treated (recipes with and without plant oil).

5 Conclusions and outlook

In the course of this work, new contributions to the understanding of batter structure modification (Frankfurter type) have been investigated:

- Impact of pressurization gradient- PG on processes of structure modification, solubilization, aggregation, and gelation, such as its effect on the functional (WHC) properties, has been studied.
- Impact of two-step HP-temperature treatment on batter structure modification has been investigated.
- Impact of the secondary protein matrix build in the aqueous phase and its dependence on treatment parameters and salt content has been analyzed.
- Definition of meat proteins, responsible for the building of the secondary protein network, has been made. The major role of the myosin sub-fragments (especially the head domains) by the secondary network formation has been analyzed and discussed.
- Detailed study of the solubilization range and its optimum in dependence of HP-temperature combinations, treatment time and NaCl content has been reported.
- Process parameter intervals defining solubilization, aggregation and gelation areas, as well as their relationships to structure and functional properties were estimated.
- HP-temperature improvement of turkey MDM-frankfurters has been investigated.
- Possibility of pickling salt reduction after HP-temperature processing through comparison of chemical, rheological, textural, sensory and structural analyses.

Due to the natural character of the meat products, it is generally difficult to obtain samples with identical initial functional properties. Therefore, an optimal pressure-temperature processing interval for receiving batters first needed to be defined in order to provide identical rheological properties after batter preparation. During storage, some tendencies for change of the visco-elastic behavior of batters, according to their ionic strength (salt content), were observed. Batter structure was found to be stable (to have identical initial properties measured by oscillation test) between an hour and 4 hours after batter preparation. During longer storage, after the 4^{th} hour, a progressive increase in firmness was detected. Firmness parameter k varied according to the batter recipe, where higher ionic strength batters were found to be softer and more elastic. Dynamic oscillation measurements performed during temperature increase detected higher G' values for high salt content batters. Hence a clear relationship between batter ionic strength and changes in batter structure properties during

storage and heating was found, as high ionic strength batters become softer and more elastic.

Tests were performed to show the impact of fast (high PG) and slow (low PG) compression on functional properties by comparison to established processing techniques using pilot (direct compression, UHDE) and industrial (indirect compression, NC-Hyperbaric) equipment Two pressure levels, 300 MPa and 600 MPa, as well as low (10°C) and medium (40°C) temperatures, were investigated using conventional and salt-reduced batters. The pressure gradient PG was shown to have a significant impact on batter functional properties for all recipes. Batters processed at low PG were firmer and with improved WHC. This improvement was more apparent for salt reduced batters (1%, 0.5% NaCl) at low PG (2.5 MPa/s), while the functional properties of 1% NaCl batters were found to be similar to those prepared according to the conventional recipe (2% NaCl). To better explain this phenomenon, Scanning Electron Microscopy for selected treatments and recipes was performed. Detailed observations of batter structure showed significant differences depending on ionic strength, pressure level and PG. The structure of low-NaCl content batters (0.5%) was found to be rougher and denser than the batters containing 2% NaCl. Pressurization up to 600 MPa resulted in a significant structure improvement compare to the 300 MPa pressure treated batters. For batters treated at low PG, this improvement was also more distinct at high NaCl content, believed to be due to the formation of a secondary protein network parallel to the main structure network for the 2% NaCl batter. The overlaid additional network located in the aqueous phase was suggested to be responsible for the 3D look of pressure treated meat gel, observed by SEM and also reported by other scientists (Chapleau *et al.* 2003, Cheftel and Culioli 1997, Jimenez Colmenero 2002).

For the 0.5% NaCl reduced batters no secondary network formation at low PG was detected. However, they did exhibit a finer structure compared to the batters treated at high PG. Therefore, in addition to the PG, the NaCl content was also concluded to have a significant impact on batter network properties.

Formation of the secondary network was due to an HP-induced increase of soluble meat proteins migrated into the aqueous phase. Through aggregation and gelation at higher pressures and temperatures, the increased availability of soluble meat proteins was responsible for the formation of the parallel network. The additional network formation was suggested, according to the SEM images and the functional properties analyses in chapters 4.2 and 4.6, to be the main reason for the functional properties improvement found in batters treated at low PG. Analysis of the results appears to support the existence of a PG-dependency on the amount and configuration of soluble proteins.

Identification of the particular solubilization and gelatinization (denaturation) pressure-temperature ranges was of great importance for understanding the HP-structure modifying

process. Therefore SDS-PAGE analyses of the soluble proteins for the whole pressure range up to 600 MPa for 5 min have been performed. Solubilization was found to increase at pressures up to 300 MPa as its maximum was protein specific. At about 200-300 MPa, a decrease of the SDS-PAGE band intensities caused by aggregation or denaturation started and increased progressively with increasing pressure. Based on these observations from the SDS-PAGE analysis the hypothesis was verified; namely, that the HPP- induced increase of soluble meat proteins in the aqueous phase was responsible for the formation of the secondary parallel network.

After a detailed study, the meat proteins, which take part in the secondary network and their maximal solubilization level, were defined. For the different batter recipes, HP- temperature generated solubilization was performed in the region 0.1-300 MPa for various treatment times. According to the results, myosin sub-fragments- S-1, S-2, N- and C- terminal; MLC, and actin were found to be involved in the secondary network formation. For the myosin molecule fragments and sub-units, the maximum solubilization was detected at 200 MPa/ 40°C. For some MLC and actin, the maximum was at 300 MPa. The process of solubilization exhibited more enhancement at higher temperatures and longer treatment times. After HPLC analysis of the soluble proteins, performed in addition to the SDS-PAGE analysis for visualizing protein changes in a broader spectrum, some interesting tendencies were observed. The amount of low molecular weight soluble proteins ($>$6 kDa), which were not detectible with the SDS-PAGE analysis, was found to be maximum at 200 MPa. Further pressure increase up to 300 MPa led to a protein peaks shift to higher molecular weights ($<$230 kDa), attending a parallel decrease of the small molecular proteins (under 6 kDa). According to the SDS-PAGE and HPLC observations the increased amount of myosin sub-fragments and unidentified small protein units led to the conclusion that an HP- disruption of the meat proteins molecules takes place parallel to the solubilization process. These two processes were detected to have their maximum at pressure treatments of about 200-300 MPa pressure. Furthermore, it was found that the process of aggregation of the disrupted and solubilized protein sub-units started at 300 MPa.

The effects over the whole range of the process parameter were required to be known to determine the optimal batter structure. Therefore, according to the results discussed in the current work, as well to other scientific reports (Yamamoto *et al.* 1993, Cheftel and Culioli 1997, Chapleau *et al.* 2003, Ando 2003, Iwasaki and Yamamoto 2003, Jimenez Colmenero 2002), a hypothetical mechanism of water binding and solubilization caused by water-protein interactions was developed. This was based on the ion-binding mechanism of Honikel 1986, combining the effect of HPP and salts (Figure 5.1). By the conventional “in salting” mechanism, water binding and solubilization are positively affected through the ionic strength increase. The HPP seems to induce synergistically the water-protein interactions. It is

proposed in this work that the HPP-salt impact depends on a combination of processes occurring during HPP, as described below:

The process of **Myosin denaturation** started at about 100 MPa and finished at about 300 MPa (Figure 2.20). The myosin unfolding results in quaternary and tertiary structure disruption as well as small changes in the secondary structure (Chapleau, Mangavel et al. 2003). Therefore, an increase of free side chains (more charged groups) occurs, leading to intensification of water-protein interactions and improved binding.

Opening of the protein molecule normally provoked by the repulsing effect of phosphates is proposed to be promoted through penetration, by adding more water (-ΔV) into the same volume. The level of molecules opening is assumed to be pressure dependent, as shown in Figure 5.1.

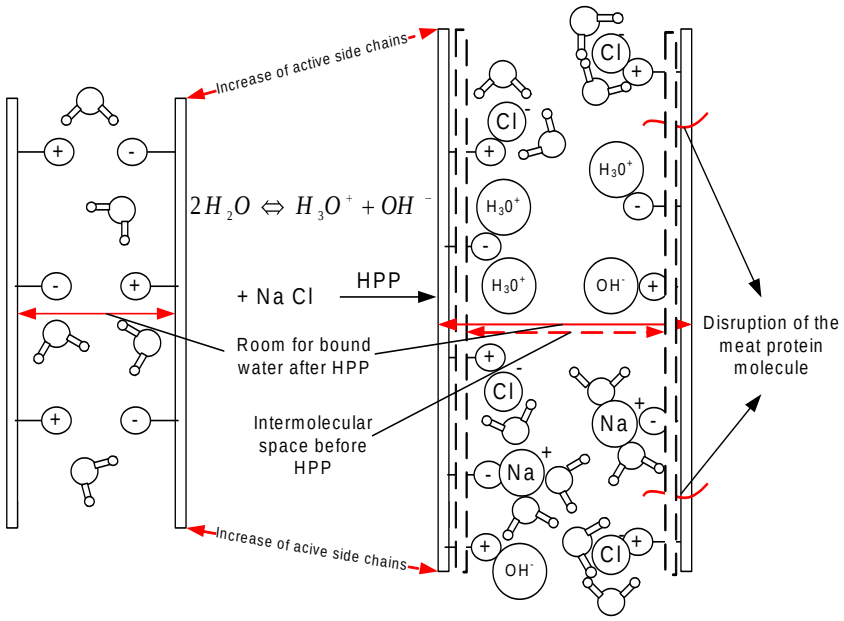


Figure 5.1: Effect of high pressure and salts (NaCl and PP- polyphosphate) on the mechanism of water binding of meat proteins

Parallel to the HP-induced process of myosin denaturation, dissociation of the actomyosin complex occurs. The process of **actomyosin complex dissociation** is normally caused by phosphate in the conventional batter preparation.

The process of **Ionic strength increase,** caused by water dissociation during HPP, could additionally enhance the solubilization effect on the meat proteins discussed in chapter 2.4.2.2. As a result, hydronium and hydroxide ions can be attracted to the protein charged groups (Figure 5.1).

HPP induced molecule disruption of meat proteins, and particularly of myosin, observed by SDS-PAGE and HPLC analysis played a key role affecting protein solubilization and

increasing the protein amount (especially the amount of sub-fragments and sub-units from the primary meat proteins) in the aqueous phase.

A Hypothetical mechanism for changes in the myosin molecule through batter preparation and HPP of frankfurters, based on the results from this work, and additionally confirmed from other scientific reports (Yamamoto *et al.* 1993, Ando 2003, Iwasaki and Yamamoto 2003), is presented in Figure 5.2. After the chemical, mechanical and pressure-temperature strain during processing, no intact myosin molecules in the supernatant were found. Instead, a disruption of the myosin molecule into myosin fragments, similar to an enzymatic effect, was found. The amounts of the three S-1 myosin domains (N- terminal and C-terminal- 50 kDa and 20 kDa), the regulatory and essential light chains, as well as actin, were all increased. The maximum amounts were found in the range 150-250 MPa. Due to the high hydrophobic character of the myosin head domains, the beginnings of agglomeration, depending on the HPP-holding time, occurred (Chapter 4.9). Hydrophobic packing of protein sub-fragments decreases caused by pressure through the system volume decrease, where a transformation to a more stable energetic level in this pressure range takes part.

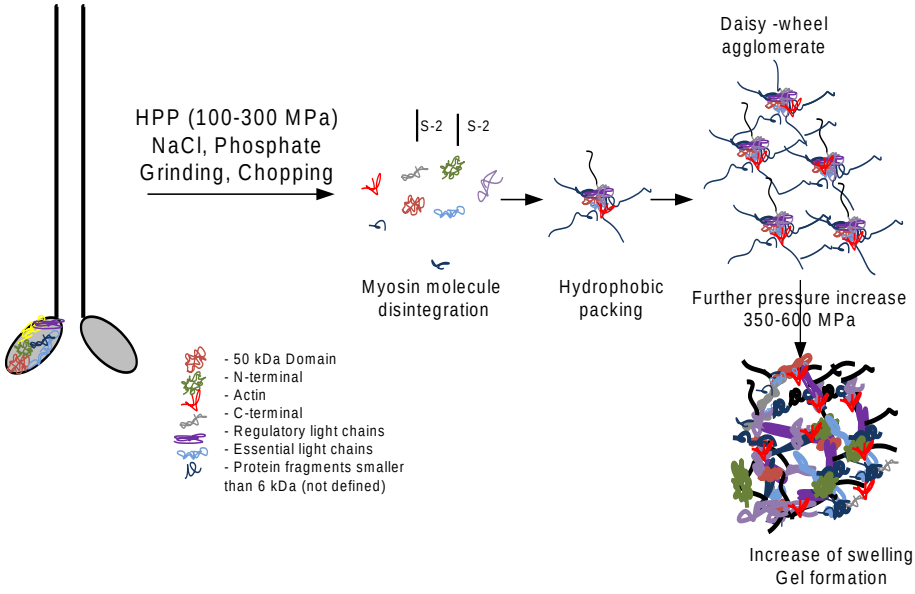


Figure 5.2: Hypothetical mechanism of secondary structure formation during high pressure-temperature treatment based on the Yamamoto's mechanism of hydrophobic packing and formation of daisy-wheel conformations.

The aggregation mechanism is supposed to be similar to the daisy-wheel formation already explained by Yamamoto *et al*. 1993. The difference to Yamamoto's mechanism was based on the fact that agglomerations of smaller proteins (S-1 sub fragments) proceeded, as the integrity of the myosin molecule was significantly disrupted. These aggregations, caused mostly by hydrophobic interactions, could be defined as the first stage of the secondary-matrix network formation. Further network formation results from the participation of the

regulatory and the essential light chains as well as actin, S-2 sub fragments and other unidentified protein. Actin is also believed to affect the gel properties since it is responsible for the viscous element of the system (Stone and Stanley 1992).

The exact configuration of these aggregates and the detailed protein-protein interactions are unknown. With increasing pressure, the agglomerations themselves (induced from the increased swelling) develop in a protein network, which under certain conditions form gel structures. Pressure above 300 MPa leads to more unfolded structures due to breaking of hydrophobic bindings. This is in agreement with the observations of Capleau *et al*. (2003) showing that this unfolding starts at pressures over 300 MPa. The resultant structure is more spread (efficient packing), so that more water may be immobilized by capillary forces. According to Iwasaki and Yamamoto (2003), pressure induced gelation of myosin occurs only by head to head interactions where tail-to-tail interactions, which taking part in the heat-induced gelation, are not involved. However, their conclusions were made based on experimentation at pressures up to 400 MPa. Therefore, contributions due to tail-to-tail interactions as well as tail entangling at higher pressures, and especially at higher temperatures (above 40°C), should not be excluded.

The processes of batter protein solubilization, aggregation, denaturation and gel building after 240 s (industrial relevant preservation treatment time) can be summarized in a p-T-diagram (Figure 5.3).

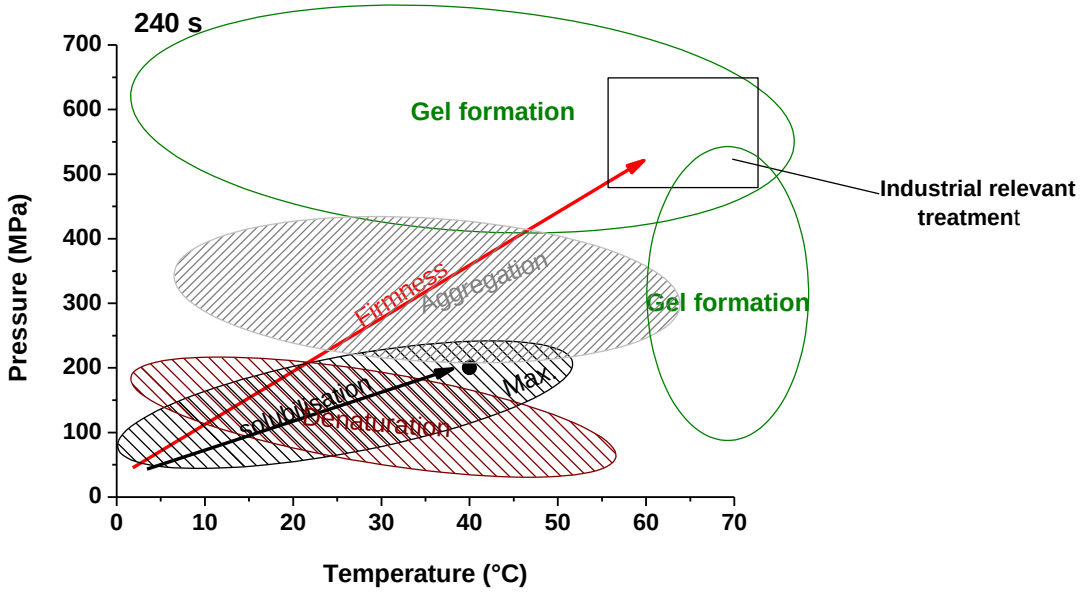


Figure 5.3: Hypothetical p-T ranges of myosin solubilization, aggregation and gelation presented after HPP of 240 s, summarizing observations of this work and other scientific reports.

The range of myosin solubilization (or molecule disruption) can be defined to take part in the range of 0-300 MPa (see chapters 4.10 and 4.11) with a maximum at 200 MPa/40°C. The solubilization range corresponds to the myosin denaturation area 0-200 MPa (see Figure 2.20). After disruption of the myosin molecule due to denaturation, solubilization or disintegration begins to occur, marking the start of the aggregation process at about 300 MPa (chapters 4.9, 4.10, and 4.11). This finding correlates with the results of Iwasaki and Yamamoto, K. (2002). In this work, however, the aggregation process is shown to continue up to higher pressure levels, where the gelation process begins. This according to the results obtained at pressures higher than 400 MPa (see Figure 2.24 Figure 4.49 and Figure 4.50). Gel building is possible at lower pressures and higher temperatures (above 60°C). According to the p-T landscapes of WHC in chapter 4.16, pressure-induced gels with good functional properties can be also achieved at low pressures and higher temperatures (above 60°C).

Meat-protein denaturation, measured by total denaturation enthalpy, was found to increase linearly with increasing pressure and temperature, resulting in increased firmness (see Figure 2.20, Figure 2.21, Figure 4.22). Pressure-temperature forming of Frankfurters sausages, after 240-s holding time and with improved functional properties and improved microorganism inactivation, could be achieved at pressures above 500 MPa and initial temperatures higher than 40°C. These processing parameters are in industrial relevant ranges. As already discussed, the processes of solubilization, aggregation and gelation are enhanced, resulting in an improved sausage structure at low PG. Therefore, a slow compression rate (low PG) should be preferred for industrial applications.

Investigations of the improvement in batter structure and functional properties, after HPP in two steps, have been performed. The effect of the first (solubilization) step for various process parameters (100-300 MPa/10°, 40°C/1-5 min) on the final batter structure have been studied in detail. The second pressure step, providing structure formation and microorganism inactivation, was done at 600 MPa for 240 s. Optimal treatment parameters with regard to WHC and firmness were estimated for the first step to be 200 MPa/40°C after 5 min treatment time.

Impact of low and high PG as well as a comparison between one- and two-step treatments is summarized in Figure 5.4, where a clear structure behavior tendency is noted. Gel-structure development was improved after low PG and two-step treatment. A strong relationship was identified between structure and the improvement in functional properties. The dependency of treatment duration in the range of 100-300 MPa on structure modification was related to the increase of soluble proteins. The visible structural volume increase (swelling) was suggested to be caused by the increase of water-protein bindings.

Generally, it is known that pressure-induced gels are smoother, more elastic, more transparent and softer than thermal-induced gels, even when performed at higher

temperatures (above 40°C initial temperature). The differences between the gels can be well explained with the effects and phenomenon already discussed in this work as follows:

The better transparency of pressure-induced gels is suggested to be caused by increased water-protein interactions. Improved softness could be related to the disruption of the intact myosin molecules and to the gelation caused by aggregations of smaller molecular protein fragments and sub-units. Another reason for the improved softness could be the increased amount of water compressed inside of the protein matrix, preventing the building of cross linkages.

A comparison between one-step and two-step treatments at 200 MPa/40°C showed an increase in firmness without a negative effect on the WHC. This firmness increase was dependent on treatment time and was greater after 5 min holding time of the first pressure step. This Interesting phenomenon could be explained with a possible relationship to the protein agglomeration level at the first step. More protein-protein instead of protein-water interactions in the gel formation could be the reason. Yamamoto et al. (1993) reported similar observations, where they noted an increase of oligomers and decrease of monomers, dimers and trimers during longer treatment times. Therefore the balance between interactions (water-protein, protein-protein), which seem to be treatment-time dependent, could play a key role for modifying batter structure. More investigations are needed in the range of 200 MPa under various temperature conditions and treatment times.

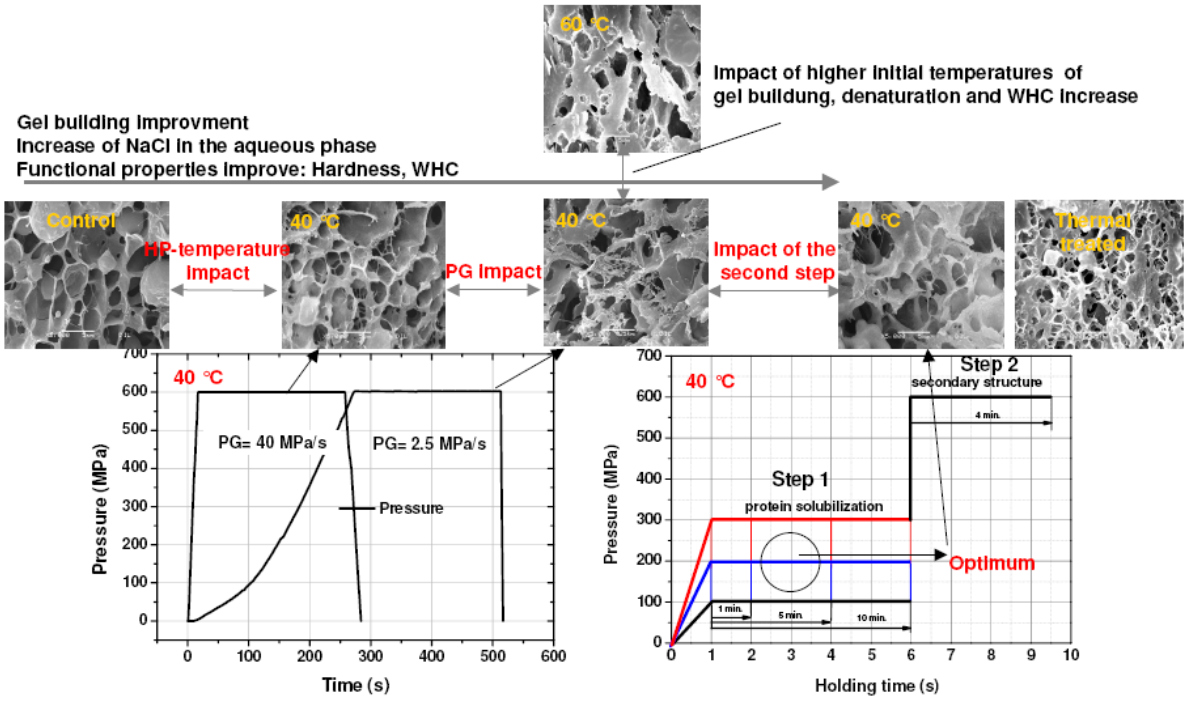


Figure 5.4: Modification of structure and functional properties of Frankfurters batter depending on the processing parameter (PG, T and treatment steps); magnification level of SEM images- 5μm length of bar.

Investigations with regard to salt reduction showed that a reduction of NaCl without any negative effect on the functional properties was possible. As already discussed, the explanation for this phenomenon is related to the pressure-induced water dissociation (increase of water activity) as well as the myosin-molecule cleavage. Therefore, the “in-salt” effect of the conventional treatment was partially compensated by the impact of pressure treatment.

Chemical analysis of NaCl content in the batter supernatants showed a NaCl increase corresponding to processing, which can be summarized as follows: high PG < low PG < two-step treatment. Maximum NaCl content was found after the two-step treatment (200 MPa/600 MPa/40°C) with a 60-s treatment time for the first step, and 240 s for the second step. Increasing the treatment time to 300 s for the first step led to a NaCl decrease in the supernatant of the final batter matrix. This phenomenon can be explained with a possible relationship between non-aggregated and aggregated protein fragments, since an increase in agglomerations was found to be holding-time dependent (chapter 4.15). This observation was supported by an increase in the firmness after 300-s treatment time, as well as a little but not insignificant WCH decrease.

Investigations of combined 50 % reduction of NaCl and phosphate led to batters with poor functional properties. Batters or sausages with reduced phosphate content could be also possible by keeping the NaCl content unchanged or minimally reduced.

According to sensory testing, the salt taste of the 2% NaCl batter after HP-T treatment was more intense compared to the conventional treatment. Comparison between 2% NaCl/conventional treatment and 1% NaCl/HPP-temperature treatment showed only a little difference, where the 1% NaCl sausages tasted almost as salty as the conventional sausages. Therefore, NaCl reduction from 2% to 1.2% NaCl is absolutely possible with regards to taste and improved functional properties.

Industrial relevance

Preservation and forming (denaturation, structure modification) of Frankfurters sausages in one process step could be industrially possible. However HP-temperature sausages are softer more elastic, and with improved WHC. Therefore, more research and development of structure firmness optimization, without a negative effect on the WHC is needed. Optimization of sausage production parameters, such as grinding and cutting, as well as the use of additives to induce more cross linking (transglutaminase and egg white, more connective tissue etc.) could be an option. As already shown, a two-step treatment processing led to a significant firmness increase. For more detailed understanding of that improvement, and for even further optimization, more investigation into the two-step HP-T processing are needed, especially with the first step in the range of 200 MPa. Investigations

at higher temperatures, in order to reduce the holding time at 200 MPa while realizing maximal structure improvement, could also be an option.

Another possible application for improved functional properties of frankfurters is high-pressure pretreatment prior to a conventional process, as reported by Suzuki and Macfarlane (1984) and Sikes *et al.* (2009).

For processing of MDM meat (or meat containing large amounts of connective tissue), HPP could be a helpful tool for disruption of the strong protein-protein collagen interactions leading to improvements in structure-immobilizing properties.

Great advantages could be obtained in the HP-temperature sausage processing by reduction of the process time, as the thermal treatment step for forming and preservation should be unecessary. Elimination of a thermal step can lead to eventual reductions in process cost. The characterization of pressurized sausages as an innovative and superior food product can be enhanced by improved salt reduction. Therefore, improvement in sausage quality should correspond with the world's trends and expectation of salt reduction.

In summary, HP-temperature treated sausages may improve the competitiveness of meat companies by producing salt-reduced sausages with better functional properties and extended shelf life, all with reduced process flow.

Curriculum Vitae and List of Publications

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Education

Since 07/2007	External PhD. Student at the German Institute of Food Technologies (DIL- Quakenbrueck), Prof. D. Knorr (TU-Berlin), Dr. V. Heinz (DIL) High hydrostatic pressure- temperature modeling of Frankfurters batters- mechanisms, salt reduction, applications
12/06/2007	Dipl.-Ing. Food Technology, Berlin University of Technology Title of thesis: Effect of combined high pressure-temperature treatments on the sensory and microbial quality of pork and poultry meat
2001- 2007	Studies of. Food Technology at Berlin University of Technology.
12/10/2003	Master of Food Technology University of Food Technology,Plovdiv, Bulgaria
1995- 2003	Studies of Food Technology at Plovdiv University of Food Technology (meat and milk technology)
Date and place of birth	Date and place of birth: 10. February 1978, Gabrovo, Bulgaria

Fields of interest

improvement of innovative processes for the food industry

structure modification of meat and fish products

process design and optimization

product design- novel meat product

Eidesstattliche Erklärung

Ich erkläre an Eides Statt, dass die vorliegende Dissertation in allen Teilen von mir selbsständig angefertigt wurde und die benutzten Hilfsmittel vollständig angegeben worden sind.

Weiter erkläre ich, dass ich nicht schon anderweitig einmal die Promotionsabsicht angemeldet oder ein Promotionseröffnungsverfahren beantragt habe.

Veröfentlichungen von irgenwelchen Teilen der vorliegenden Dissertation sind von mir wie folgt vorgenommen worden.

Duderstadt, 06.03.2013

Filip Tintchev

List of Publications

Journal articles (peer reviewed)

Tintchev, F., Bindrich, U., Toepfl, S., Heinz, V. und Knorr, D. (2012). High hydrostatic pressure/temperature modeling of frankfurter batters using two steps treatment. Innovative Food Science and Emerging Technologies. (submitted manuscript).

Tintchev, F., Bindrich, U., Toepfl, S., Strijowski, U., Heinz, V. & Knorr, D., High hydrostatic pressure/temperature modeling of frankfurter batters, Meat Science (2013), doi:10.1016/j.meatsci.2013.02.012

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