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MRI Max Rubner-Institut

Advantages of high pressure treatment of long- and short-term ripened raw fermented sausages

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Agenda

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- High hydrostatic pressure treatment (HPT) in general
- Effects of high hydrostatic pressure on food components
- HPT of short-term ripened Tea sausage
- HPT of long-term ripened Salami
- Summary

HPT in general
Industrial application

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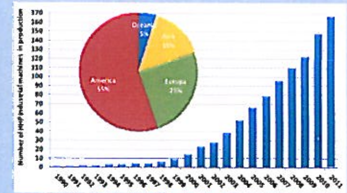
- Evolution of HPP industrial machines approx. 167 HP facilities worldwide
  → 25% in Europe



Source: Hiperbaric, 2012

- → 30% in Meat processing industry

Source: F. Purroy et al., 2012

HPT in general
Objectives

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- Preservation of food, cosmetics and pharmaceuticals
- Inactivation of microorganisms and enzymes
- Controlled denaturation of proteins
- Controlled gel formation of polysaccharides/starches
- Controlled change of the phase conditions of fats

⇒ New approach to food design

- Manufacturing minimally processed food
- high nutritive value and freshness of food
- long shelf life without preservatives

HPT in general
Advantages

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- Increase in product safety
- Processing and preservation of products in final package
- No thermal damage of the products
- Minimal changes in sensory quality and vitamin content
- Pressure has no spatial and time gradient
- No need for preservatives

HPT in general
Active principles

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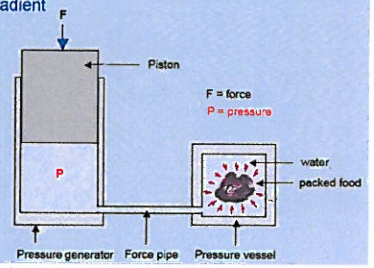
- *Prerequisites:*
  - flexible, water-/compression-proof package
  - water containing food
- *Principles*
  - consistent effectiveness over the product
  - pressure has no spatial and time gradient

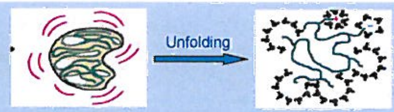


HPT in general
Basic principles

- Le Chatelier's Principle
  Chemical reactions which result in a decrease in total volume (negative activation volume) are enhanced by pressure, and vice versa.
- Isostatic rule
  The high pressure process is volume independent. Therefore pressure is instantaneous and uniform throughout the pressure vessel.
- Electrostriction
  Pressure leads to increased ionization, because water molecules arrange more compact around electric charges. This results in more or less pronounced negative and reversible pH shifts dependent on the chemical nature of the buffer.
- Compression energy
  Energy input during pressurization is very small compared to thermal processes. Therefore no chemical reactions involving covalent bonds are observed.
- Heat of compression
  Pressurization is accompanied by a uniform temperature increase.
  Food components have specific heat of compression values (e.g., water ~3 °C/100 MPa, fats and oils ~6 °C to 8 °C/100 MPa).
  Heat of compression values of water and water-like substances (proteins, carbohydrates) increase with increasing initial temperature.
  Heat of compression of fats and oils does not change with initial product temperature.

Effects of HHP on food components
Proteins

- Denaturation of proteins



- Pressure-induced reactions come along with volume reduction
  - dissociation processes
  - formation of hydrogen bridges or hydrophobic compounds
- Factors influencing pressure-induced changes of proteins:
  pH, ion concentration, treatment temperature, protein concentration, additives, and storage conditions (Pfister et al., 2000)
- Noticeable changes in protein structure
  - unfolding of peptide chains above 200 MPa
  - changes in secondary structure at ambient temperature not below 700 MPa (Balny & Mason, 1993)
- Colour changes in meat
  - denaturation of globular myoglobin fractions from > 200 MPa ➡ bright colour
  - > 400 MPa: partial oxidation of Fe^{2+} to Fe^{3+} along with denaturation of globin ➡ grey to brownish colour (Cheah & Ledward, 1996, Pfister et al., 2000)

Effects of HHP on food components
Enzymes

- The enzymatic pattern of muscle meat has an influence on the mode of action of HP treatment
- Changes in structure lead to changes in enzyme activity
- Lysosomal proteases:
  - Hydrolysis of myofibrillar proteins
    at > 300 MPa: enzyme release - increased activity of cathepsin D
- Sarcoplasmatic calpains:
  - Hydrolysis of proteins of the Z-line
    Ca^{2+} activated protease – decline of activity (Schneider, 2004)

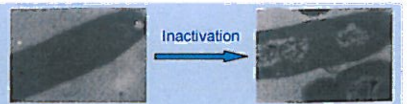
Effects of HHP on food components
Lipids

- Change of phase conditions of fats and water

- Cooperative effect with denaturation of proteins (Wada, 1992)
- Release of Fe ions enhances catalysis of oxidation processes
- Interactions between agents, influence of additives (Krzikalla, 2008)
- Degree of oxidation depending on water content of food (Pfister et al., 2000)
- Formation of free radicals with increasing pressure and treatment time accelerates lipid oxidation (Bragagnolo et al., 2006)
- Fat hydrolysis influenced by water content and activity of lipases
- Release of Ca^{2+} cations from sarcoplasmatic reticulum based on structure loss of myofibrils leads to weakness in meat texture (Okamoto et al., 1995)

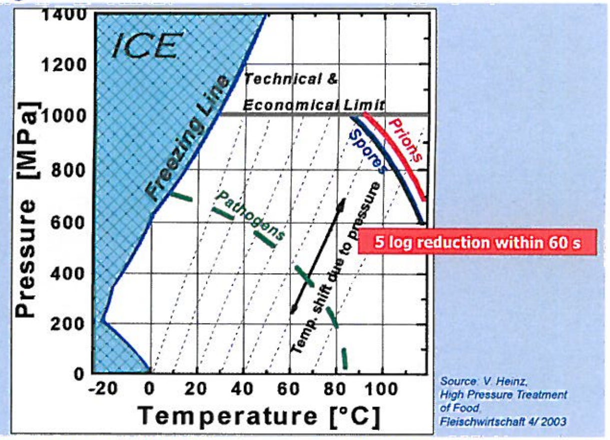
Effects of HHP on food components
Microorganisms

- Inactivation of microorganisms and enzymes resulting in the Preservation of food, cosmetic and pharmaceutical products



- Various effects of high hydrostatic pressure can be grouped into:
  - cell membrane-related effects
  - pressure-induced cellular changes
  - biochemical aspects
  - effects on genetic mechanisms
  - cellular morphology is altered by pressure
  - cell division slows with increasing pressures
  - Pressure intensity, temperature, treatment time
  - Food matrix, type and morphology of microorganismens (Cheftel, 1995; Cheftel & Culioli, 1997; Lopez-Caballero et al., 1999)
- Pressure sensitivity of microbes
  - Gram negative bacteria (Pseudomonades, *Salmonella* spp, *Yersinia enterocolitica*, *Vibrio parahaemolyticus*) > Yeasts > complex Viruses > Moulds > Gram positive bacteria (*Listeria monocytogenes*, *Staphylococcus aureus*) (Cheftel, 1995; Lopez-Caballero et al., 1999)
- Inactivation of *Listeria* required 600 MPa at 20 °C for 10 min, and of *Salmonella* – 300 MPa at 17 °C for 10 min (Begonya Marcos et al., 2005)

Effects of HHP on food components
Microorganisms



Source: V. Heinz, High Pressure Treatment of Food, Fleischwirtschaft 4/ 2003

HPT of short-term ripened raw sausage
Spreadable fermented sausages

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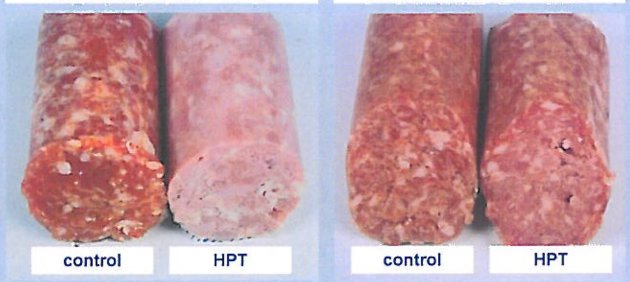
Before fermentation	After fermentation
HPT at 400 MPa, T = 20 °C	HPT at 600 MPa, T = 20 °C

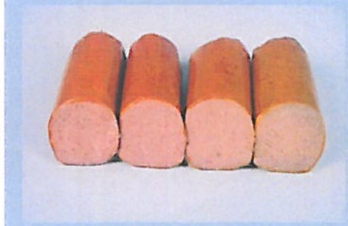


HPT of short-term ripened Tea sausage
Spreadable fermented sausages

MRI

HP treated Tea sausage
24 days after manufacture / 18 days after HPT

Contr. 400 600 800

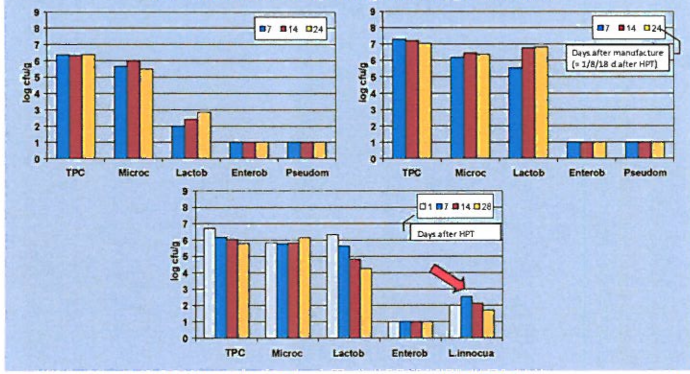


Contr. 600 800

HPT of short-term ripened Tea sausage
Effects on microorganisms

MRI

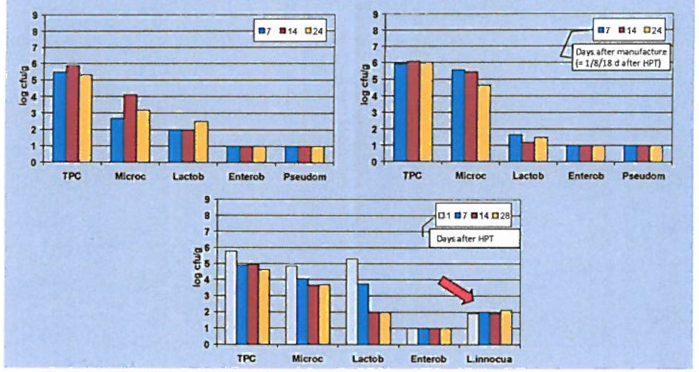
Plate counts after HPT of Tea sausage
HPT at 20, -5 and 0 °C; storage at 7 °C, p = 600 MPa



HPT of short-term ripened Tea sausage
Effects on microorganisms

MRI

Plate counts after HPT of Tea sausage
HPT at 20, -5 and 0 °C; storage at 7 °C, p = 800 MPa



HPT of short-term ripened Tea sausage
Summary

MRI

Sensory traits and quality

- HPT at +20 °C – unacceptable regarding colour, texture, aroma
- HPT at –5 and 0 °C – acceptable product up to 600 MPa

Microbiological safety

- HPT at –5, 0 and 20 °C – inactivation of spoilage bacteria
- Spoilage bacteria as well as surrogates of pathogenes with plate count of 10^6 cfu/g were inactivated
- at 20 °C starting temperature and/or 800 MPa pressure intensity, a significant reduction in LAB was found

HPT of short-term ripened Tea sausage
Summary

MRI

Physico-chemical parameters

- Water activity and peroxid no.
  - low sensitivity against HPT
- Colour:
  - becomes bright and pale
  - L* value increased
  - a* and b* values decreased
- Hardness:
  - noticeable increase due to HPT
- pH:
  - increased with pressure intensity
  - relation with increasing number of viable LAB assumed
- Lipid oxidation:
  - Acid no.: lower in HP treated samples
  - TBARS: concentration increases with pressure intensity

HPT of long-term ripened Salami
Dry-fermented raw sausages

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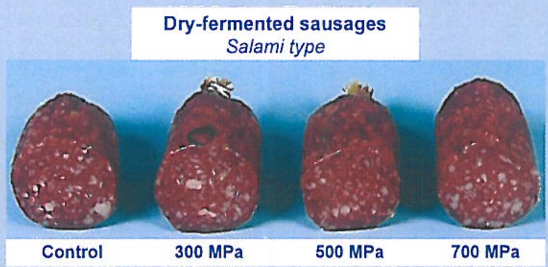

HPT of long-term ripened Salami
Materials and methods

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Recipe:
- 40 % beef
- 40 % pork
- 20 % backfat
- 26.0 g/kg nitrite curing salt
- 2.0 g/kg sugar
- 0.5 g/kg starter culture
- 0.3 g/kg sodium ascorbat
- 5.0 g/kg seasonings

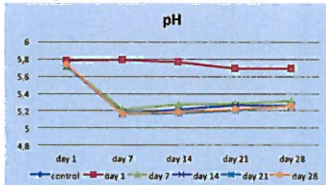
HPT at T = 20 °C; p = 600 MPa; t = 10 min

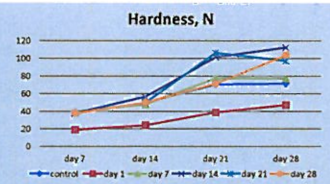
HP application during ripening:
at days 1, 7, 14, 21 and 28

- Effects of HPT on fermentation and ripening of dry-fermented sausage
  - technological criteria (pH, a_w, hardness)
  - microbiological status
  - cured colour formation
  - lipid oxidation
- HP-induced changes storage of dry-fermented sausage
  - microbiological
  - oxidative
  - sensory

HPT of long-term ripened Salami
Physico-chemical results

MRI



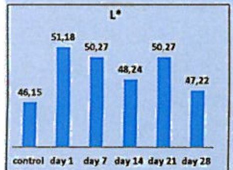


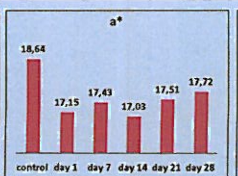
Influence of HP on the course of water activity (not shown), pH, and hardness during ripening of dry-fermented sausages HP treated at different periods of ripening (p = 600 MPa, T = 20 °C, t = 10 min)

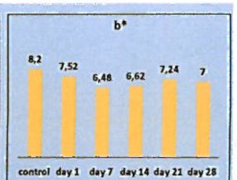
- HPT at the first day of ripening prevented lactic acid formation and thus pH drop
- HPT at a later time had no influence on fermentation and acidification
- Texture formation corresponded to the development of the pH value
- HPT after 14 days of ripening caused a noticeable increase in hardness in the final product

HPT of long-term ripened Salami
Effect on colour values

MRI





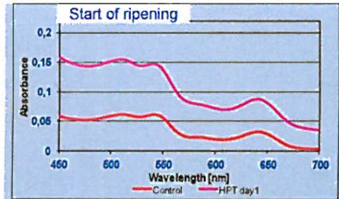


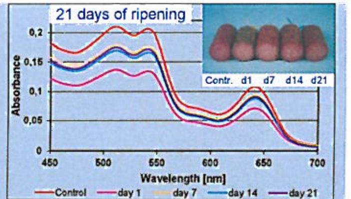
Influence of HP on colour values during ripening of dry-fermented sausages HP treated at different periods of ripening (p = 600 MPa, T = 20 °C, t = 10 min)

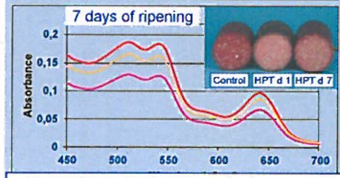
- HPT at the beginning of ripening resulted in strongest changes of product colour
- L* value was most affected resulting in a slight increase in brightness of colour of sausages
- HPT is recommended to be applied at the end of ripening

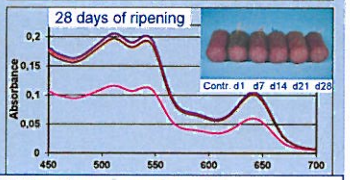
HPT of long-term ripened Salami
Effects on cured colour formation

MRI





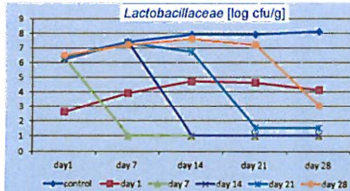


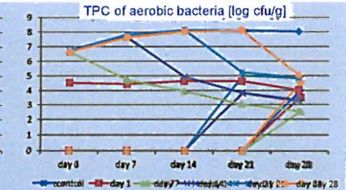


- HPT at the beginning of ripening increased NO-Mb formation compared to control
- HPT at the beginning of ripening inhibited cured colour formation during subsequent ripening
- all other HP treatments showed similar NO-Mb formation at the end of ripening

HPT of long-term ripened Salami
Effects on microbiology

MRI





Influence of HP on bacterial counts during ripening of dry-fermented sausages HP treated at different periods of ripening (p = 600 MPa, T = 20 °C, t = 10 min)

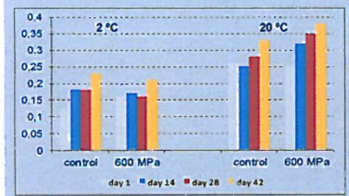
- except for pressurization at day 1 and 28, HPT stopped growth of *Lactobacillaceae*
- course of TPC of aerobic bacteria mainly corresponded to behaviour of *Lactobacillaceae*
- at the end of ripening, reduction of *Lactobacillaceae* in HPT samples ranged from 3-5 log cycles
- independent of treatment, Micrococci reached a plate count of 2 to 5 cfu/g at the end of ripening

HPT of long-term ripened Salami
Changes during storage

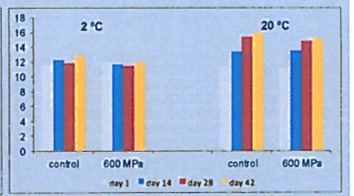
MRI

Sliced raw fermented sausages
(a_w value 0.91; 600 MPa, 20 °C, 10 min; storage at 2 °C and 20 °C)

TBARS [mg MDA/kg]



Acid number



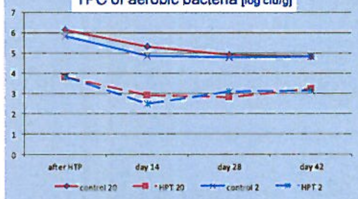
- minimal differences between control and HP treated samples concerning acid number
- slight, but steady increase of TBARS values
- significantly higher values at 20 °C compared to storage at 2 °C with all samples

HPT of long-term ripened Salami
Changes during storage

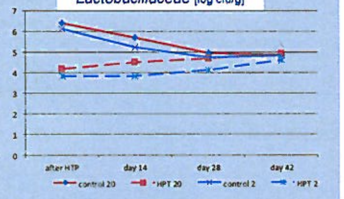
MRI

Sliced raw fermented sausages
(a_w value 0.91; 600 MPa at 20 °C for 10 min at end of ripening; storage at 2 °C and 20 °C)

TPC of aerobic bacteria [log cfu/g]



Lactobacillaceae [log cfu/g]



- Reduction of TPC of aerobic bacteria and Lactobacilli by 2 log cycles immediately after HPT
- TPC decreased by about 1 log cycle during storage
- Storage temperature had almost no influence on TPC
- *Lactobacillaceae* count of control samples dropped down during storage, while that of HP samples increased

Summary

MRI

- HPT of long- and short-term ripened salami type sausages should be done at the end of the ripening period.
- Based on chemical parameters (TBARS and ANo.), HPT induced slight increase in lipid oxidation during storage of raw sausage.
  However, the sensory quality of HP treated samples was similar to that of control samples.
- HPT is an additional hurdle concerning the microbiological stability of dry-fermented sausages.
  However, it did not increase their overall quality.
- The effect of high hydrostatic pressure on the molecular structure of food stuff and on product associated microbial contaminants is versatile and hardly predictable.
  - Each product should be tested individually to ascertain relevant HPP parameters – for determining the optimum pressure-temperature-time combination to be applied (temperature, duration and temporal progression, pressure intensity etc.)
  - Technological processing conditions might have to be adjusted (recipe, process parameters, packaging and storage conditions)

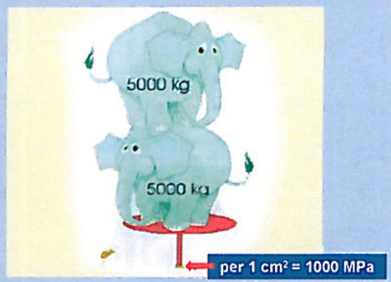
Summary

MRI

- HPP offers good prospects for dry-fermented raw sausage and raw dry-cured meats
  - if a "zero-tolerance policy" for *Listeria monocytogenes* would be introduced
  - HPP – a prerequisite to keep this market segment going (for safety reasons)
- Another important aspect: products are subjected to HP treatment in the final consumer package in order to avoid recontamination
- The status of the foodstuff prior to treatment has an influence on the course of the changes during storage
  - lower-quality products cannot be improved by means of HPP
  - any statements about what influence the status of the product after HPP may have on changes during storage are not recommended
  - consequently, it is important to provide evidence of the changes caused by storage in the interest of consumers.
- Classical analytical methods are hardly suitable to prove HP treatment of products
  - HPP does not result in any specific changes
  - HP treated products are substantially equivalent to meat products produced using conventional technology.

Thank you ...

MRI



... for your attention!

Literature References

Balasubramaniam, V.M. and D. Farkas (2008): High-pressure Food Processing. Food Sci. Tech. Int. *14*, 5, 413–418

Balny, C. and P. Mason (1993): Effects of high pressure on proteins. Food Rev. Int. *9*, 611–628

Baron, A., J.-M. Dénes, Chr. Durier (2005): High-pressure treatment of cloudy apple juice. European Commission Project FAIR-CT96-1113, Final Report, INRA, France.

Begonya, M., T. Aymerich, M. Dolors Guardia and M. Garriga (2007): Assessment of high hydrostatic pressure and starter culture on the quality properties of low-acid fermented sausages. Meat Sci. *76* (1), 46–53.

Beresford, T. and C. Lane (2000): High Pressure Processing of Dairy Foods. Research report, The Dairy Products Research Centre Moorepark, Fermoy, Co. Cork, Ireland

Bragagnolo, N., B. Danielsen and L.H. Skibsted (2006): Combined effect of salt addition and high pressure processing on formation of free radicals in chicken thigh and breast muscle. Eur Food Res Technol *223*, 669–673.

Buckow, R., A. Sikes, R. Tume (2013). Effect of High Pressure on Physicochemical Properties of Meat. Critical Reviews in Food Science and Nutrition *53* (7), 770–786.

Butz, P. (2007): Einfluss der Hochdruckbehandlung auf chemische Veränderungen von Peptiden in Lebensmitteln. Mitteilungsblatt der Fleischforschung Kulmbach *46*, Nr. 176, 117–118.

Chapleau, N., C. Mangavel, J.-Pierre Compoint, M. de Lamballerie-Anton (2004): Effect of high-pressure processing on myofibrillar protein structure. J Sci Food Agric *84*, 66–74

Cheah, P.B. and D.A. Ledward (1996): High pressure effects on lipid oxidation in minced pork. Meat Science *43*, 123–134

Cheah, P.B. and D.A. Ledward (1997): Inhibition of metmyoglobin formation in fresh beef by pressure treatment. Meat Science *45*, 411–418.

Cheftel, J. C. (1995): Review: High-pressure, microbial inactivation and food preservation. Food Sci. Technol. Int. *1*, 75–90.

Cheftel, J. C. and J. Culioli (1997): Effects of High Pressure on Meat: A Review. Meat Sci. *46* (3), 211–236.

Chen, X., C.-G. Chen, Y.-Z. Zhou, P.-J. Li, F. Ma, T. Nishiumi, A. Suzuki (2014). Effects of high pressure processing on the thermal gelling properties of chicken breast myosin containing k-carrageenan. Food Hydrocolloids 40, 262–272

Dederer, I. und W.-D. Müller (2007): Hochdruckinduzierte Veränderungen bei schnittfester Rohwurst während der Reifung und Lagerung. Mitteilungsblatt der Fleischforschung Kulmbach *46*, Nr. 176, 127–134.

Delgado, F.J., J. Delgado, J. González-Crespo, R. Cava, R. Ramírez (2013): High-pressure processing of a raw milk cheese improved its food safety maintaining the sensory quality. Food Sci. Tech. Int. *19*, 6, 493–501.

Fernandez Garcia, A. et al. (2002): Enzyme-Substrate Specific Interactions: In Situ Assessments Under High Pressure. S. 189–192. In: R. Hayashi (Hrsg.) Trends in High Pressure Bioscience and Biotechnology: Proc. First International Conference on High Pressure Bioscience and Biotechnology

Fischer, S. (2007): Auswirkungen der Prozessführung und der Milieubedingungen auf qualitative Parameter hochdruckbehandelter frischer Bratwurst. Mitteilungsblatt der Fleischforschung Kulmbach *46*, Nr. 176, 119–126.

Fischer, S. und F. Schwägele (2007): Hochdruckbehandlung bei Fleischerzeugnissen – technologische Nutzung der Auswirkungen dieses innovativen Verfahrens auf die chemisch-physikalischen, sensorischen und mikrobiologischen Produktparameter. Schlussbericht AiF 14256N, Bundesforschungsanstalt für Ernährung und Lebensmittel (BFEL), Kulmbach

Literature References

Garriga, M., Mª T. Aymerich, M. Hugas (2002). Effect of high pressure processing on the microbiology of skin-vacuum packaged sliced meat products: cooked pork ham, dry cured pork ham and marinated beef loin. Final Report, Monells

Hanjun, M. und D.A. Ledward (2013). High pressure processing of fresh meat – Is it worth it? Meat Science *95*, 4, 897–903

Heinz, V. und F. Kortschack (2002): Method for modifying the protein structure of PrP(sc) in a targeted manner. Patent WO 02/49460

Kröckel, L. und W.-D. Müller (2002): Inaktivierung von Bakterien in vakuumverpacktem Brühwurstaufschnitt: orientierende Versuche mit hohen hydrostatischen Drücken. Fleischwirtschaft *82*, 9, 121-124.

Krzikalla, K. E. (2008). Hochdruckinduzierte Veränderungen von Lebensmittelinhaltsstoffen. Dissertation, Technische Universität Berlin

Lautenschläger, R., I. Dederer, N. Vostrikova (2013). High pressure processing of Frankfurter-type sausages with added antioxidants. Are there synergistic or antagonistic effects? (unpublished results)

Lautenschläger, R. and I. Dederer (2013): High pressure processing of meat products. Report of the Max Rubner-Institut (MRI) of 19 September 2013 requested by the Federal Ministry of Food, Agriculture and Consumer Protection

Lautenschläger, R. (2007): Einfluss unterschiedlicher Temperaturen bei der Hochdruckbehandlung von streichfähiger Rohwurst. 42. Kulmbacher Woche.

Lautenschläger, R. und W.-D. Müller (2006). Auswirkung der Hochdruckbehandlung von Nürnberger Bratwurst in Schutzatmosphärenpackungen auf sensorische, mikrobiologische und verpackungstechnische Parameter. Abschlussbericht zum Forschungsprojekt (Projektpartner: Bundesforschungsanstalt für Ernährung und Lebensmittel, Standort Kulmbach und Kupfer & Sohn GmbH & Co. KG, Heilbronn)

Lopez-Caballero, M. E., J. Carballo and F. Jimenez-Colmenero (1999). Microbiological changes in pressurized, prepackaged sliced cooked ham. J. Food Prot. *62* (12), 1411–1415.

Ma, H.-J., D.A. Ledward (2004): High pressure/thermal treatment effects on the texture of beef muscle. Meat Science *68*, 347–355.

Marcos, B., J.P. Kerry, A.M. Mullen (2010). High pressure induced changes on sarcoplasmic protein fraction and quality indicators. Meat Science *85*, 115–120.

Müller, W.-D. und I. Dederer (2008): Untersuchungen zur Haltbarmachung von Brühwurstkonserven durch Hochdruck- und Wärmebehandlung. Fleischwirtschaft *88*, 2, 99–102.

Münch, S. (2005). Einfluss der Hochdruckbehandlung auf die Bildung von Cholesteroloxiden in Brühwurstaufschnitt. Mitteilungsblatt der Fleischforschung Kulmbach *44*, Nr. 168, 107–114

N.N. (2011): Kinetics of Microbial Inactivation for Alternative Food Processing Technologies – High Pressure Processing. U.S. Food and Drug Administration, Ph. 1-888-INFO-FDA (1-888-463-6332).

Okamoto, A., A. Suzuki, Y. Ikeuchi, and M. Saito (1995). Effects of high pressure treatment on Ca^{2+} release and Ca^{2+} uptake of sarcoplasmic reticulum. Bioscience Biotechnology and Biochemistry *59*, 266–270

Pfister, M.K.-H. et al. (2000). Der Einfluss der Hochdruckbehandlung auf chemische Veränderungen in Lebensmitteln. Eine Literaturstudie. Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin. Berlin (BgVV-Hefte) 3, 17–22.

Pfister, M., und L.I. Dehne (2001) High Pressure Processing – Ein Überblick über chemische Veränderungen in Lebensmitteln. Deutsche Lebensmittel-Rundschau *97*, 7, 257–268.

Literature References

Purroy, B.F., C. Tonello, R. Peregrina, C. de Celis (2011): Industrial high pressure processing of avocado products: emerging trends and implementation in new markets. Proceedings VII World Avocado Congress 2011, Cairns, Australia. 5 – 9 September 2011

Rastogi, N.K. (2013). Recent Developments in High Pressure Processing of Foods. SpringerBriefs in Food, Health, and Nutrition, 67–88.

Schneider, C. (2004): Hochdruckbehandlung von Fleisch und Fleischerzeugnissen. (oral presentation)

Serfert, Y. (2002). Versuche zum Einfluss hohen hydrostatischen Druckes auf die Folsäurederivate 5-Methyl-Tetrahydrofolat, 5-Formyl-Tetrahydrofolat und Tetrahydrofolat in Modelllösungen und Orangensaft. Diploma Thesis, FH Bernburg, Germany

Simonin, H., F. Duranton and M. de Lamballerie (2012). New Insights into the High-Pressure Processing of Meat and Meat Products. Comprehensive Reviews in Food Science and Food Safety *11*, 285–306.

Speroni, F., N. Szerman, S.R. Vaudagna (2014): High hydrostatic pressure processing of beef patties. Effects of pressure level and sodium tripolyphosphate and sodium chloride concentrations on thermal and aggregative properties of proteins. Innovative Food Science and Emerging Technologies *23*, 10–17.

Varela-Santos, E., A. Ochoa-Martinez, G. Tabilo-Munizaga, J.E. Reyes, M. Pérez-Won, V. Briones-Labarca, J. Morales-Castro (2012). Effect of high hydrostatic pressure (HHP) processing on physicochemical properties, bioactive compounds and shelf-life of pomegranate juice. Innovative Food Science and Emerging Technologies *13*, 13–22

Velázquez-Estrada, R.M., M.M. Hernández-Herrero, B. Guamis-López, A.X. Roig-Sagués (2012): Impact of ultra high pressure homogenization on pectin methylesterase activity and microbial characteristics of orange juice. A comparative study against conventional heat pasteurization. Innovative Food Science and Emerging Technologies *13*, 100–106.

Villamonte, G., H. Simonin, F. Duranton, R. Chéret, M. de Lamballerie (2013). Functionality of pork meat proteins. Impact of sodium chloride and phosphates under high-pressure processing. Innovative Food Science and Emerging Technologies *18*, 15–23.

Voigt, D.D., F. Chevalier, J.A. Donaghy, M.F. Patterson, M.C. Qian, A.L. Kelly (2012). Effect of high-pressure treatment of milk for cheese manufacture on proteolysis, lipolysis, texture and functionality of Cheddar cheese during ripening. Innovative Food Science and Emerging Technologies *13*, 23–30.

Wada, S. (1992). Quality and lipid change of sardine meat by high pressure treatment. in: High Pressure and Biotechnology. C. Balny, R. Hayashi, K. Heremans, P. Masson) ed. Colloque INSERM/John Libbey Eurotext Ltd, Montrouge, France. 235–238.

Winter, R. and Chr. Jeworrek (2009): Effect of pressure on membranes. Soft Matter *5*, 3157–3173. The Royal Society of Chemistry