TECHNISCHE UNIVERSITÄT MÜNCHEN

WISSENSCHAFTSZENTRUM WEIHENSTEPHAN
FÜR ERNÄHRUNG, LANDNUTZUNG UND UMWELT

Lehrstuhl für Technische Mikrobiologie

Role of lipid phase composition and cell surface hydrophobicity in high pressure inactivation of *Lactobacillus plantarum* in emulsions

Thomas Anton Kafka

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

genehmigten Dissertation.

Vorsitzender:	Prof. Dr. Horst-Christian Langowski
Prüfer der Dissertation:	1. Prof. Dr. Rudi F. Vogel
	2. Prof. Dr. Ronald Gebhardt

Die Dissertation wurde am 02.03.2018 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 26.06.2018 angenommen.

Abbreviations*

°C	degree Celsius
2D	two-dimensional
ATCC	American Type Culture Collection, Manassas, Virginia, USA
A	ampere
a_w	water activity
B.	*Bacillus*
BLAST	Basic Local Alignment Search Tool (registered trademark of the National Library of Medicine, National Institutes Of Health, U.S. Department of Health & Human Services)
bp	base pair(s)
C.	*Clostridium*
cfu	colony forming units
CSH	cell surface hydrophobicity
Da	dalton
DH2O	deionized water
DNA	deoxyribonucleic acid
dNTP	deoxy-nucleoside triphosphate
e.g.	for example
em	emission
ex	excitation
EDTA	ethylenediaminetetraacetic acid
FA	fatty acid
g	gram
g	gravity
GlcNAc	N-acetylglucosamine
h	hour
HP, HPP	high pressure, high pressure processing
HHP	high hydrostatic pressure
HPT	high pressure temperature
i.e.	that is

IPB	imidazole phosphate buffer
L.	*Lactobacillus*
L	liter
LAB	lactic acid bacteria
LTA	lipoteichoic acid
M	molar (mol/L)
µ	micro (10^{-6})
m	milli (10^{-3}), meter
MALDI-TOF MS	matrix-assisted laser desorption ionization time of flight mass spectrometry
MATH	microbial adhesion to hydrocarbons
min	minute
M_r	molecular mass
MRS	de Man, Rogosa and Sharpe
MTP	microtiter plate
MurNAc	N-acetylmuramic Acid
Mw	molecular weight
N	viable counts
n	nano (10^{-9})
N_0	initial viable counts
NCBI	National Center for Biotechnology Information
nD	index of refraction measured at a wavelength of 589.3 nm for a certain material
NIST	National Institute of Standards and Technology under the U.S. Department of Commerce (Maryland, USA)
OD_x	optical density at wavelength x
p	pressure [Pa], pico (10^{-12})
PCR	polymerase chain reaction
pH	pH-value
p/T	pressure/temperature
rpm	revolutions per minute
RT	room temperature

s	second
S.	*Staphylococcus*
sd	standard deviation
T	absolute temperature
T_m	melting temperature
Taq	*Thermus aquaticus*
TMW	Technische Mikrobiologie Weihenstephan
Tris	tris(hydroxymethyl)aminomethane
TUM	Technische Universität München
UV	ultraviolet
V	volt
v/v	volume / volume
w/v	mass / volume
w/w	mass / mass
WTA	wall teichoic acid

*standard SE symbols, prefixes and abbreviations are not provided

Table of Contents

List of Figures

List of Tables

1 Introduction

In this thesis, both the role of fat and cell surface hydrophobicity (CSH) in High Hydrostatic Pressure (HHP)-mediated inactivation of spoilage-associated *Lactobacillus (L.) plantarum* and the role of wall teichoic acids (WTAs) in CSH were investigated. This chapter provides a comprehensive overview with basic knowledge, widely accepted principles, and latest scientific insights about the HHP inactivation of microorganisms, the use of emulsions as model system, the spoilage-associated genus *Lactobacillus* and CSH.

1.1 High hydrostatic pressure technology in food preservation

The consumers' demand for minimally processed, preferably "clean label" foods (without artificial additives) with freshness and flavor qualities is steadily increasing. Additionally, today´s consumers are more and more interested in convenience foods, inter alia ready-to-eat meals (RTE), which vastly reduce effort and time for preparation and thus enable consumers to maintain their work-life balance, which becomes more and more difficult in modern times. In order to meet these demands, the food industry has improved its heat preservation processes and has developed non-thermal preservation technologies, which include pulsed electric fields, oscillatory magnetic fields, irradiation, ultrasound and HHP (Bello *et al.*, 2014). The most promising preservation technology, which on the one hand has the potential to fulfil the consumers' demands for high-quality convenience foods and on the other hand, simultaneously guarantee microbiological food safety, is HHP. HHP is an emerging non-thermal food preservation technology, which, compared with conventional food preservation methods, such as pasteurization and sterilization, was proven to have relatively little effect on organoleptic properties and nutritional values of foods, while ensuring effective reduction of spoilage-associated and pathogenic microorganisms in food. Additionally, foods' shelf life is extended based on physical processing, enabling the reduction or the abandon of chemical additives (antimicrobials) such as sorbates, benzoates and nitrite, and not based on the addition of chemical additives (Glass *et al.*, 2007). Effective decontamination, and thus preservation of food, is commonly reached by subjecting packaged food to water pressures up to 600 MPa. By performing HHP treatments at initial temperatures below (or at) room temperature, value giving properties such as natural taste and flavor, vitamins, natural color and texture remain largely preserved (Cheftel, 1992, Knorr, 1993, Corbo *et al.*, 2009, Demazeau & Rivalain, 2011, Stratakos *et al.*, 2015).

A multitude of studies show that HHP can, firstly, cause a large reduction of viable microbial cells in food products and, secondly, is suitable for the treatment of temperature sensitive and convenience food products while maintaining value giving properties and thus fulfilling the consumer´s needs. These are two factors making HHP a sustainable method for serving the growing market of high quality, high priced foods (Patterson *et al.*, 2011, Stratakos *et al.*, 2015).

1.1.1 History of pressure treatments

Hite (1899) was the first person describing the application of high hydrostatic pressure to preserve food in 1899. Hite tried to find alternative preservation methods for the generally used methods sterilization, cooling and pasteurization, being all simply matters of temperature, showing either no sufficient bacterial inactivation or negative impact on milk's organoleptic properties. Hite demonstrated that HHP treatments at approx. 700 MPa can significantly increase the milk´s shelf-life and simultaneously reduce detrimental effects on organoleptic properties, observed for thermal treatments. Although this alternative preservation method was consequently already recognized in the late nineteenth century, it took almost another century until the first commercial high pressure processed foods, fruit jams in plastic cups, were available on the Japanese market in 1991 (Hori *et al.*, 1992, Tonello, 2011). Afterwards, the number of pressure treated food products steadily increased. For instance, in 1994, the French company Ulti launched pressure-pasteurized citrus juices, being the first company in Europe using HHP commercially (Tonello, 2011). In 1997, the US company Fresherized Foods began with the first industrial production of HHP treated avocado products and the Spanish company Espuña pioneered the use of HHP for the preservation of meat products, starting with the local launch of sliced cooked ham in 1998 (Grèbol, 2002).

1.1.2 Current role of HHP processing in commercial food preservation

Due to the high consumers' and retailers' acceptance of the HHP technology (Wright *et al.*, 2007), gradually more and more pressure-treated food products made their way onto supermarket shelves. As a consequence, many different pressure-treated food products are available on the market today, including seafood and fish products (~13% of HHP-treated food), vegetable products (~27%), meat products (~27%), juices and beverages (~14%) and other products like sauces, salad dressings, dips, spices, dry fruits and numerous RTE meals (~20%) (Pandrangi & Balasubramaniam, 2005, Balasubramaniam & Farkas, 2008, Tonello, 2011, Bello *et al.*, 2014, Bolumar *et al.*, 2014, Tonello-Samson, 2014, Balasubramaniam *et al.*, 2015).

In total, more than 500,000 tons of pressure-treated foods are annually produced worldwide by 265 implemented high pressure machines, representing a market of around US$ 2.5 billion (Balasubramaniam *et al.*, 2015, Elamin *et al.*, 2015).

However, several concerns hamper the more widespread use of HHP in food preservation: (I) Vegetative cells can vary largely in their barosusceptibility on species and strain level (Alpas *et al.*, 1999, Gänzle & Liu, 2015, Liu *et al.*, 2015). (II) Most bacterial endospores and some fungal ascospores are highly pressure-resistant at ambient temperatures, which are commonly used and sufficient for the inactivation of vegetative cells (Gänzle & Liu, 2015). (III) As typically applied for designing commercial thermal processes, the bactericidal effect of pressure can often be not easily predicted on the basis of D- and z-values, which are derived from log-linear models. Pressure-induced microbial inactivation is very variable, being dependent on many parameters. Thus derived models do frequently not exhibit adequate accuracy, simplicity and wide acceptance (Gänzle & Liu, 2015). (IV) The efficiency of high pressure inactivation highly depends on the food matrix and process temperature. While effects of high temperature, pH and low water activity on bacterial pressure-mediated inactivation are well examined, effects of sub-ambient temperatures and many food matrix constituents, such as fat, are not sufficiently understood (Garcia-Graells *et al.*, 1998, Smelt, 1998, Molina-Gutierrez *et al.*, 2002, Luscher *et al.*, 2004, Molina-Höppner *et al.*, 2004, Georget *et al.*, 2015). Additionally, dependent on the composition, the food matrix can hinder or promote the recovery of sublethally injured cells post pressure treatment and, thus, increase or reduce inactivation efficiency, respectively (Gänzle & Liu, 2015). Thus, to ensure sufficient bacterial inactivation, a case-by-case evaluation of the inactivation efficiency of pressure in a given food matrix is often required. (V) The present initial costs of the technology used for HHP processing are very high and use of this technology is strongly limited in terms of capacity and throughput (Balasubramaniam *et al.*, 2015). (VI) Machinery necessary for HHP processing is very complex and requires extremely high precision in its construction, use, and maintenance (Rao *et al.*, 2014).

Due to the above mentioned concerns and circumstances, in order to spread the use of HHP in food preservation and to ensure the production of safe, high quality products that do not pose any threat to consumers' health and safety and fulfil the requirements of directives and licensing authorities, additional research, providing an improved understanding of pressure-mediated cell inactivation, sublethal injury and recovery, and the interaction of pressure with intrinsic or extrinsic factors predominating in food, has to be performed.

Although it is unlikely, that HHP will replace conventional preservation and processing technologies for high volume, commodity type foods in the foreseeable future, it could either complement such methods or find its niche applications in the preservation of certain foodstuffs due to novel physicochemical and sensory properties, obtained from this technology. Moreover, it can be assumed that prospective developments in terms of pressure vessels with higher volumes and in terms of incorporation of automatized solutions in the processing lines, will increase the processing outputs and reduce the cost per kg, making this technology more accessible and attractive for some companies (Bolumar *et al.*, 2014). Additionally, in the present world aiming at a sustainable food supply, HHP could gradually become an interesting processing alternative of increasing importance, requiring shorter processing time and lower energy consumption than conventional thermal methods (Pardo & Zufía, 2012).

1.1.3 General principle of high pressure preservation of food

In general, industrial high pressure machines are operated batch (solid food products) or semi-continuous (liquid, pumpable food products) wise, applying pressure varying from 100 to 800 MPa and mild process temperatures (from 20 to 50 °C) for relatively short times (from several seconds up to several minutes) (Ting & Marshall, 2002). Appropriate processing conditions (pressure, temperature, time) have to be selected depending on the food to be treated and the microorganisms and enzymes to be inactivated (Bello *et al.*, 2014). Performing a typical HHP batch treatment for instance, food is sealed in its final package, loaded into the pressure vessel filled with pressure-transmitting fluid and pressure is increased by either pumps or by piston movement mediated volume reduction. Once the desired pressure is met, the pressure is maintained in the system without further need of energy supply. After the desired treatment time has elapsed, the pressure is released, the food packages are unloaded and the system reloaded with new food products (Ting & Marshall, 2002).

Using batch systems, recontamination is avoided since the product is processed in its final package. For semi-continuous systems, however, only suitable for liquid, pumpable foods, the product is pumped in and out of the high pressure vessel and aseptically packaged in glass bottles or gable cartons after pressure treatment, not guaranteeing undesired recontamination by post process handling of the product.

1.1.4 Basic physical principles of HHP

The effects of pressure, resulting in physicochemical changes of the pressurized sample, are governed by three general principles, the Le Châtelier's principle, the isostatic principle and the microscopic ordering principle (Daryaei & Balasubramaniam, 2012).

Le Châtelier's principle

The Principle of Le Châtelier and Braun states that if a chemical system at equilibrium is exposed to a change, the equilibrium will shift in the direction minimizing that change. Therefore, if this change is an increase in pressure, high pressure will shift the equilibrium towards the system with the lowest volume, i.e., any processes that are accompanied by a decrease in volume (e.g. chemical reactions, phase transition and changes in the molecular configuration) are favored, whereas processes leading to an increase in the total volume are inhibited by pressure (Cheftel, 1995, Butz & Tauscher, 1998).

The isostatic principle

The isostatic principle implies that pressure is transmitted quasi-instantaneously and uniformly throughout the whole sample, independent of its size and geometry (Balny & Masson, 1993, Cheftel, 1995), i.e., all parts of foods are exposed to a similar pressure intensity and uneven processing, as known for thermal treatments, is prevented.

The microscopic ordering principle

The microscopic ordering principle states that at a constant temperature, an increase of pressure results in a high degree of molecular ordering of a substance (Urrutia Benet, 2005). Therefore, pressure and temperature act antagonistically on molecular structure and chemical reactions (Balny & Masson, 1993).

1.1.5 Physicochemical changes under pressure

Applying pressure generally results in the occurrence of different physicochemical changes in the pressurized aqueous sample. Important changes, which have to be considered when applying HHP technology, include effects on temperature due to adiabatic heating, phase transition, dissociation equilibrium and the arrangement and structure of macromolecules.

1.1.5.1 Adiabatic heating

The temperature increase occurring in the sample during pressure processing, solely based on compressive work against intermolecular forces, is commonly known as adiabatic compression heating, heat of compression or adiabatic heating and plays a substantial role in food preservation. Even though no ideal adiabatic conditions were present while conducting the experiments, the term adiabatic heating is used throughout this manuscript to describe these compression-mediated heating effects occurring during the pressure build-up.

The effect of adiabatic heating can be described using basic equations, deduced from the zeroth, first and second law of thermodynamics, taking their functional relationship to temperature and pressure into account (Kessler, 2002, Reineke, 2012, Lenz, 2017). The zeroth law of thermodynamics states that if two systems are both in equilibrium with a third system, then they are in equilibrium with each other. The first law of thermodynamics is the law of conservation of energy, stating that the increase in internal energy (*U*) is equal to the total energy added to the system in form of work (*W*), heat (*Q*), and sum chemical potential (μ), i.e., the sum potential energy that can be absorbed or released during chemical reactions by a number of *N* particles of *i* different types present in the system (Job & Herrmann, 2006) (Eq. 1-1).

$$dU = dW + dQ + \sum_{i=1}^{j} \mu_i \, dN_i$$

(Eq. 1-1)

In case of HHP processes, *dW* represents the amount of volumetric work performed during running the high pressure intensifier system, and can be also expressed as:

$$dW = -pdV$$

(Eq. 1-2)

The second law of thermodynamics generally describes the tendency of natural processes to reach homogeneity of matter and energy, i.e., thermodynamic equilibrium over time. This means that any isolated system tends to degenerate into a maximal disordered state, i.e., maximum entropy (S).

In the case of HHP processing, the amount of heat energy added to the system (*dQ*) can be expressed as the temperature (T) (of the system and space where the heat comes from or goes to) multiplied by the increase of the entropy (*dS*):

$$dQ = TdS$$

(Eq. 1-3)

The equations according to the first and second law of thermodynamics can be combined to yield an equation appropriate for describing the relationship of the inner energy of a system with pressure, volume, temperature and entropy (only valid for situations where the sum chemical potential can be neglected):

$$dU = -pdV + TdS$$

(Eq. 1-4)

The usage of the first and second law of thermodynamics and the rearrangement of the Maxwell's relation (Green & Perry, 1997):

$$\left(\frac{\partial T}{\partial p}\right)_S = \left(\frac{\partial V}{\partial S}\right)_P$$

(Eq. 1-5)

enables the description of the heating and cooling during the compression and decompression as functions of thermo-physical properties of the compressible product, respectively. Taking into account the definition of the specific volume as the inverse of the density

$$v = f(p,T) = \frac{1}{\rho}$$

(Eq. 1-6)

plus the isobaric thermal expansion coefficient (α_p, 1/K) as a function of temperature and the specific volume *v*

$$\alpha_p(p,T) = \frac{1}{v} \cdot \left(\frac{\partial v}{\partial T}\right)_p$$

(Eq. 1-7)

plus the definition of the isobaric heat capacity (c_p) according to the first fundamental theorem of thermodynamics

$$c_p\,(p,T) = \left(\frac{\partial H}{\partial T}\right)_p = T \cdot \left(\frac{\partial S}{\partial T}\right)_p$$

(Eq. 1-8)

plus the definition of the compression heating coefficient (k_c) (Knoerzer *et al.*, 2010)

$$k_c = f(p,T) = \frac{\alpha_p}{\rho \cdot c_p}$$

(Eq. 1-9)

a final equation, describing the temperature increase upon physical compression (adiabatic heating rate) under adiabatic isentropic conditions, can be derived (Hoogland *et al.*, 2001)

$$\frac{dT}{dp} = \frac{\alpha_p}{\rho C_p} \cdot T = k_c \cdot T$$

(Eq. 1-10)

demonstrating that the magnitude of the temperature increase is substantially determined by the product properties compressibility, specific heat capacity (J/kg K) and density (Kg/m^3) (Ting & Balasubramaniam, 2002).

Ideal p/T-dependent adiabatic heating data of pure water is available in extensive dimensions at the NIST database (NIST, 2002) and can be utilized, in addition to mixture rules of water soluble components and correction factors (to compensate inaccuracies), for the determination of adiabatic heating rates and the valuation of maximum adiabatic heating of water-based solutions and water-based dispersions occurring during pressurization (Ardia *et al.*, 2004). In contrast to water and water-based solutions, the derivation of p/T-dependent adiabatic heating rates of complex foods is quite difficult, due to the lack of thermodynamic data (Toepfl *et al.*, 2006). However, the p/T-dependency of the compression heating coefficient (k_c) (Eq. 1-9) can be calculated based on empirical data obtained under almost ideal adiabatic conditions, enabling the prediction of maximum adiabatic heating occurring during pressurization.

Even though, the effect of adiabatic heating reached in practice, is, due to improper insulation and steady thermal equilibration, most likely less marked than adiabatic heating determined under these almost ideal adiabatic conditions, the obtained thermodynamic information can be helpful for assessing maximum possible process temperatures and for designing HHP processes.

Consequences of adiabatic heating on food processing

The thermodynamic effect of adiabatic heating, occurring during compression and decompression can be positively exploited by, on the one hand, rapidly achieving sterilization temperatures without temperature gradients throughout the product, avoiding over-processing and related losses in food quality, and, on the other hand, using the high compression-mediated cooling capacity, cooling down the food product uniformly and quasi-instantaneously (isostatic principle of pressure transmission) without any shear forces occurring inside of the product. However, it must be considered, especially for non-heated or insufficiently isolated pressure vessels, that temperature inhomogeneities do occur. In these cases, thermal equilibration occurs across the boundary of the system (heat flux to the “colder”, mostly, steel vessel wall) during pressure build-up and dwell time, resulting in possible non-uniform microbial inactivation during HHP processes (De Heij *et al.*, 2002, Ting & Balasubramaniam, 2002, Rajan *et al.*, 2006). In order to minimize thermal equilibration, either the time given to thermal equilibration must be shortened by inter alia higher compression rates, if possible, or the temperature distribution of the product as a function of time and position in the vessel has to be calculated to aid process and equipment design (De Heij *et al.*, 2002, Ardia *et al.*, 2004). Furthermore, high initial temperatures before pressure build-up and the compression heat during pressurization can lead to the occurrence of temperature peaks. Based on the composition of the treated sample and thus each constituent´s thermodynamic properties (Eq. 1-10), compression heat and the resulting temperature peaks can vary significantly.

Table 1 illustrates adiabatic heating rates of water, pressure transmitting fluids and foods, varying partly significantly in their constituents. Ranges of adiabatic heating rates demonstrate the commonly non-linear p/T-dependence of adiabatic heating rates.

Table 1: Adiabatic heating of different foods and common pressure transmitting fluid constituents. Substances were pressure treated at an initial temperature of 25 °C. Adapted from (Kesavan *et al.*, 2002, Ting & Balasubramaniam, 2002, Rasanayagam *et al.*, 2003, Ramaswamy, 2007).

Food Sample	ΔT (°C)/100 MPa
Water	~3.0
Orange juice, tomato salsa, 2% fat milk, and other water-like substances	3.0-2.6
Carbohydrates	3.6-2.6
Proteins	3.3-2.7
Linolenic acid	9.0-5.9
Soybean oil	9.1-6.2
Olive oil	8.7-6.3
Crude beef fat	~4.4
Extracted beef fat	8.3-6.3
Beef ground	~3.2
Beef fat	~6.3
Gravy beef	~3.0
Chicken fat	~4.5
Chicken breast	~3.1
Salmon	~3.2
Egg albumin	~3.0
Egg yolk	4.5-4.3
Egg whole	~3.3
Mayonnaise	7.2-5.0
Whole milk	~3.2
Tofu	~3.1
Mashed potato	~3.0
Yoghurt	~3.1
Cream cheese	4.9-4.7
Hass avocado	4.1-3.7
Honey	~3.2
Water/glycol (50/50)	4.8-3.7
Propylene glycol	5.8-5.1
Ethanol	10.6-6.8

1.1.5.2 Pressure induced phase transition

According to Le Châtelier's principle, pressure shifts equilibria towards the state with the smallest total volume. For water, occurring in various states with different densities this means that pressure modulates the temperature at which phase transition from liquid water to solid phases occurs. Bridgeman (1912) was the first scientist, presenting data on the behavior of water under pressure and its different solid phases.

Since then, various other solid phases have been discovered, especially under more extreme conditions, resulting in 16 different ice crystal and 3 amorphous structures to date (Hobbs, 1974, Luscher *et al.*, 2004, Zheligovskaya & Malenkov, 2005, Zheligovskaya & Malenkov, 2006). Next to water, also fat experiences phase transition by pressure-induced reversible changes of the phase transition temperature (shift of melting point) and crystallization behavior. Pressure increases the melting point of lipids by more than 10 °C per 100 MPa. As consequence, lipids, being liquid at room temperature, will reversibly crystallize upon pressurization, forming the denser and more stable crystals (Cheftel, 1995, Ferstl *et al.*, 2010). There, β-crystals, which have a denser and more stable crystal structure, are formed in preference to β'- and α-crystals (Cheftel, 1992).

1.1.5.3 Dissociation equilibrium and pH under pressure

One further relevant change occurring upon pressurization, which has to be considered applying HHP, is the pressure-mediated shift of the dissociation equilibrium and related changes in the pH value of aqueous systems. The dissociation equilibrium in an aqueous system is described as

$$\boldsymbol{HA + H_2O \leftrightarrow A^- + H_3O^+}$$

(Eq. 1-11)

where HA is the proton donor (acid), H_2O is water, A^- is the conjugated base, and H_3O^+ is an oxonium ion. For characterization of the dissociation equilibrium, the equilibrium constant *K* can be used, being dependent on the concentration of every molecule in the equilibrium and on the activity coefficients γ_i (correction factors). The activity coefficients in aqueous solutions can be approximately estimated by the limiting Debye-Hueckel law (Debye & Hueckel, 1923):

$$\boldsymbol{\log_{10} \gamma_i = -1.825 \cdot 10^6 \cdot z_i \cdot \sqrt{\frac{I \cdot \rho}{\varepsilon^3 \cdot T^3}}}$$

(Eq. 1-12)

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, leading to varying activity coefficients γ_i due to its pressure and temperature dependency. Since the concentration of water is very large (~55.5 M) in relation with that of a base or acid, their dissociation can be assumed to have no essential effect on the concentration of water.

Therefore, the concentration of water can be neglected in the law of mass action, resulting in the definition of the dissociation equilibrium constant K_a,

$$\boldsymbol{K_a = \frac{\gamma_{A^-} \cdot \gamma_{H_3O^+}}{\gamma_{HA}} \cdot \frac{[A^-] \cdot [H_3O^+]}{[HA]}}$$

(Eq. 1-13)

which describes the extent of dissociation of oxonium ions from an acid (proton donor). Since the dissociation equilibrium constant K_a differs for each acid and can vary in its value over a wide range, the additive inverse of its common logarithm, represented by the symbol pK_a, is often used to describe the dissociation constant K_a (Degner, 2012):

$$\boldsymbol{pK_a = -log_{10}(K_a)}$$

(Eq. 1-14)

The activity of oxonium ions (H_3O^+ ion concentration) can also vary over many degrees of magnitude, and consequently, it is common to express the activity of oxonium ions as the additive inverse of its common logarithm in a dimensional form (divided by 1 mol L^{-1}), as pH (Degner, 2012):

$$\boldsymbol{pH = -log_{10}\left(\gamma_{H_3O^+} \cdot \frac{[H_3O^+]}{1\,mol \cdot L^{-1}}\right)}$$

(Eq. 1-15)

Even though pH values are commonly used to describe the acid tolerance of microbes and the acidity of food, Eq. 1-15 demonstrated that the pH value only considers oxonium ions, completely ignoring changes of all dissociation equilibrium reaction partners of water and is thus not appropriate to describe dissociation equilibrium shifts. In order to consider changes in the concentration of all dissociation equilibrium reaction partners of water (Eq. 1-13), the p/T-dependent *pKa* value (Eq. 1-14) is more suitable for describing dissociation equilibrium shifts (Mathys *et al.*, 2008).

To account the temperature- and pressure-dependency of the dissociation equilibrium and with it, the pH value of aqueous systems, Planck (1887) has established a basic dependency of the equilibrium constant *K*, from the pressure *p* (MPa) and the absolute temperature T (K),

$$\left(\frac{d\ln(K)}{dp}\right)_T = \frac{\Delta V(p)}{R \cdot T}$$

(Eq. 1-16)

where *R* is the gas constant 8.3145 cm^3 MPa K^{-1} mol^{-1} and ΔV is the reaction volume at atmospheric pressure (cm^3 mol^{-1}), being equal to the difference of the partial volumes of products and reactants (Mathys *et al.*, 2008). By converting and integrating the Planck's equation, the pressure- and temperature-dependent changes of the acid equilibrium constant can be described as (Lenz, 2017):

$$pK_a = pK_a^0 + \frac{\lg e}{RT}\int_{p^0}^{p}\Delta V(p)dp$$

(Eq. 1-17)

1.1.5.4 Food constituents

Using the HHP technology, foods with guaranteed microbiological safety and simultaneously of high quality, showing almost no changes in their value giving properties (food taste, flavor and color), can be obtained. This phenomenon is especially ascribable to the high pressure-stability of covalent bonds, showing a negligible compressibility under pressure, commonly applied in the food industry. Due to the low compressibility of covalent bonds at pressures below 2 GPa, the covalent structure of low-molecular-weight molecules (peptides, fatty acids, saccharides, pigments, antioxidant compounds, vitamins, trace elements) and the primary structure of macromolecules (carbohydrates, proteins, lipids) are rarely affected (Gross & Jaenicke, 1994, Mozhaev *et al.*, 1994, Cheftel & Culioli, 1997, Oey *et al.*, 2008, Balasubramaniam *et al.*, 2015). Nevertheless, since pressure predominantly acts on the spatial (tertiary, quaternary and supramolecular) structures of macromolecules, impairing the non-covalent bonds (such as hydrogen, ionic and hydrophobic bonds), the commonly applied pressure in food industry is limited to the pressure height and time of application, in order to restrict non-reversible changes of especially food quality attributes (Mozhaev *et al.*, 1994). The effects of pressure on the main macronutrients (carbohydrates, proteins, lipids) and micronutrients of food, and its quality attributes are discussed in the following.

Carbohydrates

While single low-molecular sugar molecules are highly pressure-stable, macromolecular polysaccharides, commonly starches, show a high pressure sensitivity, resulting in a gelatinization upon pressure (Stute *et al.*, 1996). The pressure-mediated gelatinization of most starches is characterized by a limiting swelling (up to twice in diameter) of the starch granules, with it maintaining the granular character (Stolt *et al.*, 2000) and the loss of the birefringence under polarized light (Buckow *et al.*, 2007), and a decrease in volume of the starch suspension. Douzals *et al*. (1996) assumed that starch molecules linked with water have to occupy less volume than suspended in pure water and because of that, based on Le Châtelier's principle (1.1.4), the granule hydration would be preferential under pressure. The pressure range in which gelatinization occurs, is typical for each starch and is inter alia affected by their crystalline structure (Muhr & Blanshard, 1982, Stute *et al.*, 1996, Rubens *et al.*, 1999). Additionally, gelatinization of starch also depends on the pressure height, the pressure dwell time and the temperature (Bauer & Knorr, 2005, Rumpold, 2005).

Proteins

The effects of pressure on a protein's activity and structure was examined extensively, showing to be relatively complex by inducing reversible or irreversible changes of the protein's native structure, resulting in significant variation of the residual molecular structure (Heremans, 1982, Cheftel, 1992, Gross & Jaenicke, 1994, Mozhaev *et al.*, 1994). A detailed review, describing the changes of the protein structure under pressure and temperature was published by Knorr, Heinz and Buckow (2006). Due to the neglectable compressibility of covalent bonds (see above) and the pressure-mediated strengthening of hydrogen bonds, the primary and secondary structures (α-helix and β-sheet structures) are rarely affected by pressure (Jaenicke, 1981, Balny & Masson, 1993, Heremans & Smeller, 1998, Knorr *et al.*, 2006). Unlike covalent bonds, salt bonds and partly hydrophobic interactions, playing a crucial role in the formation of oligomeric protein structures, are strongly impaired by high pressure and thus, pressure especially acts on the protein's tertiary and quaternary structure. There, the breakdown of salt bonds and hydrophobic interactions is caused by electrostriction and alignment of water molecules close to hydrophobic groups, respectively. This illustrates that in contrast to temperature denaturation, where non-polar hydrocarbons are transferred from the hydrophobic core towards the water, pressure denaturation is based on forcing water molecules into the interior of the protein matrix (Zhang *et al.*, 1995, Nash & Jonas, 1997).

Depending on the protein concentration, the protein's structure and the applied pressure, pressure can cause the dissociation of oligomeric structures into their subunits, partial unfolding and denaturation of monomeric structures, protein aggregation and gelation (Cheftel, 1995). The reversibility of pressure-induced changes of the protein's native structure is dependent on the transgression of the pressure threshold, which depends on each protein, and the protein concentrations, which enhance irreversible protein aggregation at high concentrations (Cheftel, 1995).

Lipids

In contrast to proteins, which undergo structural changes upon pressurization, lipids (triglycerides) undergo a phase transition from the liquid to the solid state (see 1.1.5.2). The pressure-inducted phase transition of lipids is considered to have a substantial effect on the biological membranes, leading to membrane damages, which are considered to be one of the main reasons for microbial inactivation (Kato & Hayashi, 1999). The role of biological membranes on cell inactivation is discussed in more detail in section 1.1.6.2.

Micronutrients

Based on the low compressibility of covalent bonds, it is generally accepted that high pressure per se rarely impairs low molecular weight compounds and thus food value giving micronutrients, such as vitamins, pigments and antioxidant compounds remain largely preserved upon pressurization. Several authors have examined the effect of pressure on among others vitamins (B1, B6 and C), pigment content and antioxidant potential using multivitamin systems and vegetables, respectively, and could confirm the minimal effect of pressure on micronutrients in foods (Butz *et al.*, 1994, Sancho *et al.*, 1999).

Food quality attributes

The food quality attributes color, flavor, texture and nutritive value are important food characteristics for the consumers' acceptance. On the one hand, applying too high-pressure levels and/or too long pressure dwell times, undesirable changes can occur during preservation treatments and subsequent storage, minimizing the food quality and thus its value. Undesirable changes of these attributes upon pressurization, which were reported, include the development of off-colors and -flavors, loss of solubility and water-holding capacity with regard to the texture, and loss or degradation of proteins, carbohydrates, vitamins, trace elements or lipids with regard to nutritive value (Tangwongchai *et al.*, 2000, Lakshmanan *et al.*, 2005, Ludikhuyze & Hendrickx, 2006).

Reactions, which are responsible for these undesirable changes, include enzymatic and non-enzymatic browning, lipid hydrolysis or oxidation, protein denaturation (see above), hydrolysis or cross-linking, polysaccharide hydrolysis or synthesis, and degradation of natural pigments (Amanatidou *et al.*, 2000, Ludikhuyze & Hendrickx, 2006, Tintchev *et al.*, 2010). On the other hand, several studies demonstrated that HHP processing can also positively influence the mentioned food quality attributes, mainly by denaturing enzymes, which impair the food quality (Arroyo *et al.*, 1999, Shook *et al.*, 2001, Fachin *et al.*, 2002, Pandrangi & Balasubramaniam, 2005, Ludikhuyze & Hendrickx, 2006). However, since several studies were performed, showing no or contrasting effects of pressure on quality attributes of different types of foods at comparable treatment conditions (Goutefongea *et al.*, 1995, Quaglia *et al.*, 1996, Basak & Ramaswamy, 1998, Mor-Mur & Yuste, 2003), it is necessary to examine food quality attributes of the HHP-treated product to be commercialized and to adapt treatment parameters (duration and level of pressure).

1.1.6 Microbial inactivation

The inactivation of microorganisms in foods by HHP processing has been extensively researched in recent years, and it could be demonstrated that the efficiency of pressure-induced microbial inactivation depends on a variety of factors, still not fully understood to date (Dogan & Erkmen, 2004, Smiddy *et al.*, 2004, Smiddy *et al.*, 2005, Donaghy *et al.*, 2007, Klotz *et al.*, 2010). The most important factors, assumed to play essential roles in microbial inactivation, including cellular target structures for high pressure, are being discussed in this section.

1.1.6.1 Main factors affecting microbial inactivation

First of all, it was shown that high pressure sensitivity is dependent on the type of microorganism. Eukaryotes, such as yeasts and molds, were identified as the most sensitive microorganisms, being inactivated by pressure of 100 to 200 MPa (Cheftel, 1995). In general, Gram-negative microorganisms appear to be moderately sensitive to pressure while Gram-positive microorganisms, probably due to their cell wall structure, generally show a higher resistance to pressure (Shigehisa *et al.*, 1991, Smelt, 1998, Considine *et al.*, 2008, Dumay *et al.*, 2010). Significant inactivation of vegetative bacteria can be typically observed within minutes at moderate temperatures and pressures ranging between 200 and 600 MPa (Carlez *et al.*, 1994, Cheftel, 1995, Smelt, 1998, Farkas & Hoover, 2000). Notably, HHP sensitivity can vastly vary among bacterial species and even strains (Styles *et al.*, 1991, Simpson & Gilmour, 1997, Alpas *et al.*, 1999).

Endospores tend to be extremely HHP resistant, withstanding treatments of more than 1000 MPa (Smelt, 1998). In order to inactivate spores, elevated temperatures in addition to high levels of pressure are required (Balasubramaniam & Farkas, 2008). However, it must be considered that HHP can simultaneously induce germination of bacterial spores, making it necessary to run pressure cycling treatments or to apply pressure and heat sequentially, in order to ensure their complete inactivation (Sale *et al.*, 1970, Mills *et al.*, 1998, Smelt, 1998, Wuytack *et al.*, 1998). Next to the microorganism type, also the growth phase was shown to play an essential role on microorganisms' pressure sensitivity. Stationary phase cells were shown to be more pressure resistant than cells in the early growth phase (McClements *et al.*, 2001, Mañas & Mackey, 2004, Hayman *et al.*, 2007). According to inter alia Hill *et al*. (2004), the higher pressure resistance of stationary phase cells is most probably attributable to synthesis of proteins which protect against a range of adverse/stress conditions, such as oxidative stress, high salt concentrations and elevated temperatures (Robey *et al.*, 2001, Wemekamp-Kamphuis *et al.*, 2004). Besides intrinsic factors, also extrinsic factors, i.e., pH, temperature and matrix composition (see section 1.1.7) can influence HHP inactivation.

Generally, it could be observed that pressure sensitivity of microorganisms is increased at lower pH, i.e., in more acid environments (Alpas *et al.*, 2000, Koseki & Yamamoto, 2006, Ritz *et al.*, 2008, Li *et al.*, 2016). The phenomenon, that the pH of acidic solutions decreases as pressure increases, based on pressure-induced dissociation of weak acids, further promotes the effect of pressure on bacterial inactivation (Patterson, 2005). Additionally to the effect of low pH upon pressurization, a low pH environment also hampers the recovery of sublethally injured cells (Koseki & Yamamoto, 2006). The applied temperature during HHP treatment can also exert a substantial impact on microbial survival and recovery (Hogan *et al.*, 2005). While generally microorganisms show the highest pressure-resistance at temperatures close to their growth temperatures, increased inactivation can be usually observed at temperatures below or above these temperatures (Sonoike *et al.*, 1992, Gervilla *et al.*, 1997, Hayakawa *et al.*, 1998, Patterson & Kilpatrick, 1998). According to Heinz & Buckow (2015), pressure stability of microorganisms frequently appears to be maximal at the temperature range between 20 and 40 °C.

1.1.6.2 Cellular target structures for high pressure

Cellular target structures, which might be affected by pressure, their putative behavior, and their potential roles in pressure-mediated inactivation are provided below.

The general effect of pressure on biopolymers (proteins, carbohydrates) and non-polymeric biomolecules (lipids) per se can be deduced from the general pressure-mediated effects described for corresponding macromolecules present in foods (section 1.1.5.4).
It is generally proofed that the viability of vegetative microorganisms is affected by numerous pressure-induced changes, including changes of cells' morphology, the cytoplasmic membrane and the cells' metabolism by denaturation of essential cell viability maintaining enzymes (Heremans, 2001, Smelt *et al.*, 2001, Winter & Jeworrek, 2009). There, it is likely that HHP acts simultaneously on a variety of targets, and that the interplay of resulting effects leads to the final lethality of microbial cells (Hoover *et al.*, 1989, Smelt *et al.*, 2001).

Cell morphology

At increasing pressure, various morphological changes can be observed. These include inter alia cell elongation, separation of the cell membrane from the cell wall, contraction of the cell wall with the formation of pores, modifications of the cytoskeleton and strand formation, coagulation of cytoplasmic protein, and release of intracellular constituents out of the cell due to small holes in the cytoplasm (Shimada *et al.*, 1993, Molina-Höppner *et al.*, 2003).

Cytoplasmic membrane and membrane proteins

The cytoplasmic membrane is considered to be a primary target for the HHP inactivation of bacteria and it was shown that the inactivation of cells and membrane proteins strongly depends on the thermodynamic properties (fluidity, phase transition temperature, and fatty acid composition) of the membrane (Cheftel, 1995, Pagán & Mackey, 2000, Ulmer *et al.*, 2000, Gänzle & Vogel, 2001, Ulmer *et al.*, 2002, Mañas & Mackey, 2004). Based on its structure, mainly consisting of phospholipids, the cytoplasmic membrane is thought to easily undergo pressure-induced phase transition, resulting in its change from the normally fluid, liquid crystalline phase to the gel phase (Ulmer *et al.*, 2002). There, the pressure, inducing phase transition, is highly dependent on the molecular constitution of the membrane (phospholipid head group composition, degree of saturation and length of acyl chains), which substantially varies among microorganisms and their environments (Matsuki, 2015, Winter, 2015). The phase transition from the liquid crystalline to the gel phase is characterized by (I) decreased membrane fluidity and thus increased rigidity, (II) reduced conformational degrees of freedom for the acyl chains, (iii) an exothermic enthalpy change and a decrease in the partial molar volume and (IV) a lower lipid compressibility (Matsuki, 2015, Winter, 2015).

However, since an adequate membrane fluidity is crucial for the maintenance of many physiological processes (such as cell growth, cell differentiation, cell proliferation, solute transport, transmembrane signal transduction, enzyme catalysis, organization of enzymes into complexes), its obtained stiffness can have dramatic consequences, resulting in a reported impaired or even a total loss of function of membrane-bound proteins, serving functions such as an ion channel or a transporter (Chong *et al.*, 1985, Helmreich, 2002, Ulmer *et al.*, 2002, Abe, 2013, Lingfa *et al.*, 2014, Winter, 2015). Moreover, pressurization is assumed to lead to membrane permeabilization, resulting in a loss of the membrane's integrity, which is directly or indirectly essential for many physiological processes (Wouters *et al.*, 1998, Pagán & Mackey, 2000, Moussa *et al.*, 2007, Abe, 2013). These inter alia include the maintenance of ion flux and osmotic pressure, the energy production, nutrient uptake and signaling. Besides, pressure was shown to weaken the interactions between lipids and proteins and thus to cause the release of membrane-bound proteins (Ritz *et al.*, 2000, Winter, 2015).

Enzymes

Enzymes are crucial for all physiological processes, ensuring the cells' viability, and thus, pressure-induced changes in their folding and functionality strongly impair many cellular processes, including among other cell division, cellular structure, protein synthesis, DNA replication, and energy metabolism (Mota *et al.*, 2013). Although pressure-induced protein denaturation is still not fully understood, it is generally argued that pressure especially acts on the spatial structures (tertiary & quaternary) of proteins and induces unfolding by electrostriction (see 1.1.5.4). HHP can act directly, altering the proteins' properties and function or indirectly via changing the structure of the surrounding lipid phase, in turn affecting its properties and function (see cytoplasmic membrane). It is generally assumed that quaternary, multimeric enzymes, mainly maintained by pressure sensitive hydrophobic interactions, are less pressure-resistant than monomeric enzymes (Simpson & Gilmour, 1997, Wemekamp-Kamphuis *et al.*, 2004). The reversibility of pressure-induced changes is dependent on the applied pressure level and on the protein structure. According to Thakur & Nelson (1998), pressures up to 300 MPa usually induce reversible changes of proteins.

Ribosomes

Protein synthesis is accepted to be very pressure sensitive (Landau, 1967). Next to pressure-induced denaturation of enzymes, involved in protein synthesis (discussed above), also ribosomes were shown to be impaired by high pressure, and thus protein synthesis is affected simultaneously by multiple pressure-mediated effects (Abe, 2007).

In several studies has been demonstrated that ribosomes dissociate *in vitro* under high pressure and Gross *et al*. (1993) could observe that ribosome dissociation started at 40-60 MPa (Schulz *et al.*, 1976, Gross *et al.*, 1993, Gross & Jaenicke, 1994). Niven *et al*. (1999) reported that denaturation of ribosomes *in vivo* correlates with pressure-mediated cell death of *E. coli* and assumed that ribosome denaturation was caused by leakage of Mg^{2+} from the pressure-permeabilized membrane, Mg^{2+} playing an essential role in the function of ribosomes (Zitomer & Flaks, 1972).

Nucleic acids

The double-helix structure of nucleic acids (DNA, RNA) is supposed to be stable up to pressures of 1 GPa (Mozhaev *et al.*, 1994). This high pressure-stability of DNA helices is especially attributable to the covalent bonds, showing a neglectable compressibility and the hydrogen bonds, which are generally stabilized by pressure (Winter & Dzwolak, 2005). However, the condensation of nucleic acids has been reported post treatment at very high pressures and according to Chilton *et al*. (1997), HHP can cause degradation of DNA, as a result of pressure-induced release of endonucleases, normally not in contact with DNA (Mackey *et al.*, 1994, Wouters *et al.*, 1998). Even though DNA is not affected by pressures used in the food industry, its enzyme-based replication is hampered under pressure.

1.1.7 Food matrix effects on microbial inactivation

Several studies showed that the food matrix and its constituents such as carbohydrates, proteins and lipids, and physical conditions within the food can substantially influence pressure resistance of microorganisms (Simpson & Gilmour, 1997, Garcia-Graells *et al.*, 1999, Black *et al.*, 2007). The following examples will clarify that a direct extrapolation of data for microbial inactivation by HHP, obtained with buffer or physiological solutions to predict levels of inactivation in foodstuffs, will most probably give misleading results and that in most cases more severe treatments in foods are necessary to achieve the same levels of inactivation (Dogan & Erkmen, 2004, Smiddy *et al.*, 2005). For instance, for diverse foodstuffs, such as milk, dairy and meat products, it was reported that these offer a baroprotective effect to vegetative cells (Styles *et al.*, 1991, Carlez *et al.*, 1993, Raffalli *et al.*, 1994, Patterson *et al.*, 1995, Carballo *et al.*, 1997, Gervilla *et al.*, 1997, Garcia-Graells *et al.*, 1999, O'Reilly *et al.*, 2000, Hugas *et al.*, 2002, Black *et al.*, 2007). There, positive effects of food constituents on the HHP resistance of bacterial cells can be mediated by the protection of cells against damage and via their function as nutrients essential for repair (Hoover *et al.*, 1989).

Next to high amounts of proteins, which were shown to decrease HHP inactivation levels of bacterial cells, low water activity (a_w) in foodstuffs, as result of a high concentration in solutes (sugar or salt), was shown to exert a strong baroprotective effect against the HHP inactivation of microorganisms (Oxen & Knorr, 1993, Cheftel, 1995, Palou *et al.*, 1997, Simpson & Gilmour, 1997, Hauben *et al.*, 1998, Patterson, 1999, Van Opstal *et al.*, 2003, Molina-Höppner *et al.*, 2004, Smiddy *et al.*, 2004, Georget *et al.*, 2015). There, next to the solute concentration also its nature (type, ionic, non-ionic) has manifested to play a significant role in microbial inactivation (Oxen & Knorr, 1993, Patterson, 1999, Koseki & Yamamoto, 2007, Georget *et al.*, 2015). Ionic and non-ionic solutes have been argued to have a different mechanism of protection against HHP-mediated cell inactivation (Molina-Gutierrez *et al.*, 2002, Molina-Höppner *et al.*, 2004). However, although high solute concentrations in foodstuffs can exert baroprotective effects on microbial inactivation, they were reported to simultaneously hinder posttreatment recovery of sublethally damaged cells (Van Opstal *et al.*, 2003).

In contrast to the well characterized baroprotective effects of proteins and low water activity, studies investigating effects of the important food matrix parameter, fat, are scarce and conclusions drawn regarding the effect of fat on the HHP inactivation of bacterial cells are not always consistent with each other. For example, HHP inactivation studies of *L. monocytogenes* in olive oil/phosphate-buffered saline (PBS) emulsions and in cheese showed a protective effect of fat (Simpson & Gilmour, 1997, Morales *et al.*, 2006). In contrast, results from studies using liquid ultra-high temperature treated dairy cream, pasteurized milk and bovine milk indicated that the presence of fat in general does not result in decreased microbial inactivation (Raffalli *et al.*, 1994, Gervilla *et al.*, 2000, Ramaswamy *et al.*, 2009). However, the mentioned studies have in common that the effect of fat has been investigated mostly in complex food matrices. Therefore, inconsistencies concerning the effect of fat might be attributed to an interplay of adverse HHP-mediated effects exerted by different other food matrix parameters. Since more detailed studies investigating solely the effect of fat on the HHP inactivation of microorganism are completely missing, there exists a substantial lack of knowledge regarding the effect of this important food matrix parameter on the HHP inactivation of microorganisms. Executing systematic studies on the role of fat in the HHP-mediated inactivation of vegetative microorganisms could contribute to a better understanding of the role of fat and, hence, facilitate the application of HHP technology for the commercial pressurization of fatty foods while ensuring product safety.

1.2 Emulsion as model food system for fatty food

Many foods, which are commercially available and simultaneously of high relevance for the food industry, can be categorized as emulsions. Depending on phasing, these foods can be either assigned to water-in-oil (W/O)- or oil-in-water (O/W)-emulsions, the latter being much more common among commercial foods (Belitz & Grosch, 2013). Common foods, being (O/W)-emulsions include salad dressings, mayonnaise, milk, cream, ice cream, sausages and sauces like béarnaise and hollandaise, and cream and cheese sauces, being often constituent of frozen ready-meals (Darling & Birkett, 1987, Degner *et al.*, 2014). In contrast, margarine and butter, for instance, are (W/O)-emulsions (Darling & Birkett, 1987).

1.2.1 Emulsions as model system for fatty foods

In order to examine the effects of the single food constituent fat on HHP-mediated microbial inactivation, it is not sufficient to pressurize foods, showing distinct variations in terms of the parameter fat. Interfering HHP-mediated effects exerted by different other food matrix parameters cannot be excluded and will most probably attribute to inconsistent conclusions with regards to the effects of fat on microbial inactivation by HHP, as demonstrated by former studies (see 1.1.7). Following from this, minimalistic, artificial food model systems are necessary that enable the investigation of the effects of fat per se and exclude interfering effects of any kind. The use of (O/W)-emulsions as simple model system for fatty, emulsion-based foods seems legit, because (I) the majority of spoilage-relevant, emulsion-based foods are (O/W)-emulsions (see above), (II) the system can be kept simple, since oil (lipid phase), water (aqueous phase) and emulsifier are sufficient for its formation, (III) single parameters of the aqueous phase and lipid phase can be varied individually, enabling an easy adaptation to the food of interest and the investigation of single effects of each parameter and, therefore, the execution of detailed systematic studies. Parameters, which can be varied in the aqueous phase are inter alia the pH value, the protein type and concentration, and the a_w value by different types of solutes and concentrations. The main lipid phase parameters, which can be varied, are the oil- and emulsifier type, the fat content, the oil droplet size and the related fat surface.

1.2.2 Formation and stability

1.2.2.1 Emulsion formation

In general, emulsions are mixtures of at least two naturally not miscible fluids, usually comprising one hydrophobic (lipid phase) and one hydrophilic (aqueous phase) fluid.

By supplying mechanical energy in a process called emulsification, one of the two phases forms small droplets (dispersed phase) that are distributed within the other phase (continuous phase). The formation of (O/W)-emulsions or (W/O)-emulsions is dependent on phasing, i.e., whether the lipid phase is dispersed within the aqueous phase or vice versa. In food industry, the common devices used for emulsification are the high pressure homogenizer, the high-speed stirrer (e.g., ultraturrax) and the continuous scraped-surface mixer (e.g., votators) (Darling & Birkett, 1987). However, since the emulsification is accompanied with the formation of high surface tensions between the aqueous and organic liquid, the system is thermodynamically instable and both liquids seek for reducing their common contact area. Consequently, droplets of the dispersed phase start coagulating, finally leading to separation of both phases. For emulsion stabilization, commonly substances are added that either lower the interfacial tension between the immiscible liquids (emulsifier) and/or reduce motion of dispersed droplets (thickener), thus, preventing droplet aggregation after formation (Walstra, 1993, McClements, 2005).

1.2.2.2 Role of emulsifiers

Emulsifiers are of utmost importance for the formulation of stable emulsions, characterized by a uniform and stable dispersion of fat droplets throughout the aqueous phase. Their emulsification and stabilization properties are ascribable to their molecular structure, being amphiphilic molecules, possessing a hydrophilic and a hydrophobic part and thus being able to interact with hydrophilic and lipophilic substances. Consequently, on the one hand these surface-active molecules facilitate droplet breakup within the homogenizer by adsorption to the fat surfaces, reducing the interfacial tensions, and on the other hand the created layer of adsorbed emulsifier molecules reduces the risk of droplet coalescence after formation (McClements, 2005). An effective emulsifier is therefore characterized by the following criteria (Walstra, 1993, McClements, 2004, Wilde *et al.*, 2004, McClements, 2005, Jafari *et al.*, 2008): (I) the amount of emulsifier must be high enough to ensure complete covering of all formed oil droplet surfaces during homogenization, (II) the emulsifier molecules must adsorb almost immediately to fat surfaces to form a protective coating around the fat droplets before they collide with each other, and (III) the formed protective coating must prevent, by an increase of the steric and electrostatic repulsion between the oil droplets, the coalescence of the fat droplets by coming too close together. The two most common types of emulsifiers used, fulfilling these criteria, are small-molecule surfactants and dairy proteins (McClements, 2004, Degner *et al.*, 2014). In general, small-molecule surfactants consist of a polar head group and a nonpolar tail group (Stauffer, 1999, McClements, 2005, Kralova & Sjöblom, 2009).

The head group can be non-ionic, anionic or cationic, and the tail group can show variations in chain number and length, and degree of unsaturation of the chains. The small-molecule surfactants, typically used in the industry, have either one or two nonpolar tails. Compared to proteins, they are supposed to form smaller oil droplets due to a faster adsorption to oil droplet surfaces and their ability to lower the interfacial tension in a higher degree. Additionally, lower concentrations are required for stabilization of emulsions. The food-grade emulsifiers Tween® 80 and sodium caseinate, being representatives of both emulsifier types and relevant for this thesis, are briefly discussed in the following. Tween® 80 and sodium caseinate differ widely in their molecular size and are broadly used as stabilizers in the food industries, either added or naturally occurring as in the case of caseinate, which presents a part of the phosphoprotein fraction stabilizing mammalian milk (80% of the total cow's milk protein content) (Wong *et al.*, 1996, Hasenhuettl, 2008, Adheeb Usaid & Premkumar, 2014). Tween® 80 (Polysorbate 80, polyoxyethylene monooleate) is a non-ionic, low-molecular weight (O/W)-emulsifier (hydrophile-lipophile balance (HLB)-value of 15) supposed to form a thin surface layer on oil droplets in (O/W)-emulsion (Griffin, 1946, Americas, 1984, Karjiban *et al.*, 2012). In contrast, sodium caseinate typically forms thicker layers at the oil-water interface in (O/W)-emulsion. This is due to the fact that caseinate is a heterogeneous mixture of different macromolecular subunits (α_{s1}, α_{s2}, β and κ) characterized by different molecular weights (17-30 kDa) and degrees of hydrophobicity (Jollègs, 1966, Swaisgood & Fox, 1992, Wong *et al.*, 1996).

1.2.2.3 Physical stability of (O/W)-emulsions

The physical stability of an (O/W)-emulsion is dependent on the intrinsic factors emulsifier (type and concentration), particle size, density contrast, aqueous phase viscosity, pH and ionic strength. Extrinsic factors, being of high relevance for the physical stability of (O/W)-emulsions, are temperature and mechanical stress (Boode *et al.*, 1991, Klemaszewski *et al.*, 1992, Walstra, 1993, van Aken, 2002, McClements, 2004, McClements, 2005, Xu *et al.*, 2005, Degner *et al.*, 2014). The neglect or non-observance of one of these essential parameters may result in physical instability and, therefore, most probably in substantial or total phase separation within the emulsion. General mechanisms that promote the physical instability of (O/W)-emulsions are based on droplet aggregation (coalescence, flocculation), gravitational separation (creaming, sedimentation) or diffusion of oil molecules (Ostwald ripening) (Kabalnov & Shchukin, 1992, van Aken *et al.*, 2003, McClements, 2005, Tcholakova *et al.*, 2006). Coalescence and flocculation are processes, both initiated with the collision of two droplets, but while in the process of coalescence both droplets merge into a bigger droplet, they maintain their individual integrity in the process of flocculation (Figure 1).

If droplets in (O/W)-emulsions either cream or sediment depends on their densities relative to that of the aqueous phase (Figure 1). Typically, based on their lower density, oil droplets tend to move upward and accumulate at the top of the emulsion (creaming). Conversely, droplets with a higher density than the continuous phase move downward and sediment at the bottom of the emulsion (sedimentation). Ostwald ripening describes the growth of large oil droplets at the expense of small droplets due to the diffusion of oil molecules through the intervening aqueous phase. Consequently, large oil droplets continuously increase in size, whereas smaller droplets are simultaneously reduced in size, resulting in complete disappearance of small droplets over time (Kabalnov & Shchukin, 1992).

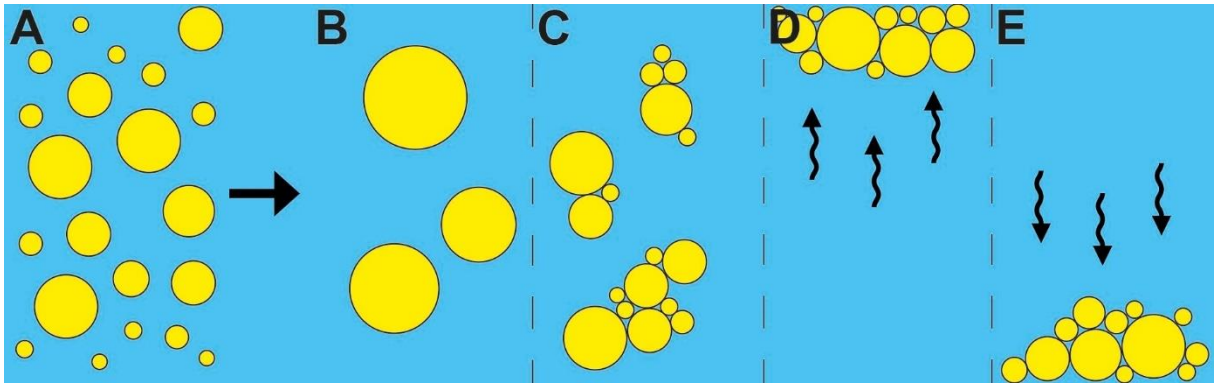

Figure 1: Schematic illustration of physical instability in (O/W)-emulsions. (A) (O/W)-emulsion with oil droplets dispersed in an aqueous phase. (B) Coalescence, collision and merger of oil droplets. (C) Flocculation, collision of oil droplet without merger. (D) Creaming, accumulation of droplets with lower densities than their surroundings at the top of the emulsion. (E) Sedimentation, accumulation of droplets with higher densities than their surroundings at the bottom of the emulsion. Adapted from Horn (2012).

1.3 The food spoilage-associated genus *Lactobacillus*

1.3.1 Genus *Lactobacillus*

The genus *Lactobacillus* belongs to the order of lactic acid bacteria (LAB) and comprises more than 170 different species and 17 subspecies that, in part, differ widely in their genomic and metabolic properties, displaying a relatively large degree of diversity (Goldstein *et al.*, 2015). Lactobacilli are Gram-positive, catalase-negative, non-sporing, rod- and cocci-shaped, facultatively anaerobic or microaerophilic bacteria. They utilize carbohydrates fermentatively and produce lactic acid as a major end-product (Hutkins, 2001). According to their metabolic pathway (Embden-Meyerhof or phosphoketolase pathway) and the resulting end-products, Lactobacilli are classified as homo- or hetero-fermentative (Marth & Steele, 2001).

Species of the genus *Lactobacillus* are ubiquitously present in humans (mouth, gastrointestinal tract, vagina) and environments, characterized by high concentrations of soluble carbohydrates, protein breakdown products, vitamins and a low oxygen tension (Aguirre & Collins, 1993, Roos *et al.*, 2005, Jespers *et al.*, 2012, Liévin-Le Moal & Servin, 2014). Isolation sources include inter alia dairy products, beverages, fermented foods, rotting vegetable material, silage and intestinal tracts of man and animal (Aguirre & Collins, 1993, Petri *et al.*, 2013). Some *Lactobacillus* strains are supposed to promote human health and are therefore used as probiotics; however, some species can also be occasional human pathogens (Aguirre & Collins, 1993, Klein *et al.*, 1998, Ljungh & Wadstrom, 2006, Salminen *et al.*, 2006, Boesten & de Vos, 2008, Lee *et al.*, 2013, Turroni *et al.*, 2014). The beneficial effect of lactobacilli to human health is inter alia promoted by their (I) occupation of mucous membranes/gastrointestinal tract and, therefore, the displacement of undesired, pathogenic parasites, (II) production of bacteriocins, inhibiting the growth of competing microorganisms, (III) production of antioxidants and, (IV) ability to immunomodulate human cells to achieve an anti-inflammatory response (Ljungh & Wadstrom, 2006, Liévin-Le Moal & Servin, 2014, Goldstein *et al.*, 2015).

Lactobacilli have been used as starter cultures in food fermentation processes for the production of diverse food products for centuries and are essential for today's food industry (Buckenhüskes, 1993, Caplice & Fitzgerald, 1999, Leroy & De Vuyst, 2004). They are used commercially in many dairy, meat and vegetable products, by being involved in ripening, fermentation and preservation processes or by being used as starter cultures (Simova *et al.*, 2008). In addition, due to their health-promoting properties, lactobacilli are advertised and sold as part of probiotic food products, such as yogurt and bacterial preparations (Sanders & Huis, 1999, Liévin-Le Moal & Servin, 2014, Auclair *et al.*, 2015). Their role in food processing and preservation is mostly based on the production of lactic acid and the ability to grow and survive in acidic environment, where other food-spoiling and pathogenic microorganisms, showing a lower acid-tolerance, are unable to proliferate (McDonald *et al.*, 1990). Some *Lactobacillus* strains produce hydrogen peroxide, bacteriocins or related substances, thus inhibiting the growth of competing microorganisms (Liévin-Le Moal & Servin, 2014). However, Lactobacilli have also been associated with food spoilage, elaborated in the following section.

1.3.2 The role of lactobacilli in food spoilage

Lactobacillus species play an important role in the spoilage of vacuum-packed, processed and fermented foods and beverages (Evans & Niven Jr, 1951, Egan *et al.*, 1980, Khalid & Marth, 1990, Aguirre & Collins, 1993, Borch *et al.*, 1996, Björkroth & Korkeala, 1997, Samelis *et al.*, 2000, Lyhs *et al.*, 2001, Chenoll *et al.*, 2006). Typically, the spoilage of foods by *Lactobacillus* species is characterized by souring, the formation of off-flavors and off-odors, discoloration and partly gas and slime production (Aguirre & Collins, 1993, Borch *et al.*, 1996, Björkroth & Korkeala, 1997). Additionally, spoilage-associated phase separation in emulsion-based food products can occur (Beveridge, 1975, Gillatt, 1991). The range of food products being spoiled by *Lactobacillus* species varies significantly, as illustrated in the following by introducing the species *L. plantarum*, *L. sakei* and *L. fructivorans*, being often associated with spoilage of food, amongst other things of fatty foods. Their general ability to tolerate low pH values down to around 3.2 (for *L. plantarum*) and their growth potential at low temperatures are important properties enabling these organisms to overcome hurdles frequently present in convenience products stored at refrigerated temperatures (McDonald *et al.*, 1990, Cebeci & Gürakan, 2003, Marceau *et al.*, 2003, Eva *et al.*, 2004, Suzuki *et al.*, 2008, Sanders *et al.*, 2015). Especially, the tolerance of very low pH values makes *L. plantarum* a target organism for the stability of many convenience products stored at refrigerated temperatures, since major pathogenic organisms that are able to grow or survive at refrigerated temperatures are inhibited at low pH values, for example, *L. monocytogenes*, *Yersinia enterocolitica* and *Campyolobacter jejuni* below pH 4.5, 4.2 and 4.9, respectively (Stern *et al.*, 1980, Doyle & Roman, 1981, Gill & Reichel, 1989, McClure *et al.*, 1989, Haddad *et al.*, 2009, Røssvoll *et al.*, 2014).

In several studies *L. plantarum* and *L. fructivorans* were reported to be among the main spoilage organisms of mayonnaise and salad dressings (Charlton *et al.*, 1934, Kurtzman *et al.*, 1971, Smittle, 1977, Smittle & Cirigcliano, 1992). *L. plantarum* was also detected in spoiled marinated herring (Lerche, 1960), cold-smoked salmon and vacuum-packed meat products (Chenoll *et al.*, 2006, Françoise, 2010) while *L. fructivorans* has been shown to be responsible for the spoilage of bottled tomato ketchup, beer and sake (Kandler, 1986, Bjorkroth & Korkeala, 1997, Suzuki *et al.*, 2008, Esmaeili *et al.*, 2015). *L. sakei* has been associated particularly with the spoilage of diverse types of meat and seafoods such as shrimps and matjes herring (Lyhs & Björkroth, 2008, Mejlholm *et al.*, 2008). The reported meat types include exemplary vacuum-packed cooked ham (Kalschne *et al.*, 2015), smoked pork loin, pariza, bacon, mortadella (Samelis *et al.*, 2000), sausages (Chenoll *et al.*, 2006), marinated fresh pork (Schirmer *et al.*, 2009), pasteurized foie gras products (Matamoros *et al.*, 2010) and vacuum-packed beef (Hernández-Macedo *et al.*, 2012, Jääskeläinen *et al.*, 2016).

1.4 CSH and its importance in bacterial localization

CSH can be assumed to play a major role regarding the localization of bacterial cells in heterogeneous, fatty foods and localization in turn can generally affect the cells' growth conditions and, potentially, their inactivation (Brocklehurst *et al.*, 1995, Parker *et al.*, 1995). For instance, direct fat surface-cell interaction could favor HHP inactivation through exposure of cells to higher temperatures caused by stronger adiabatic heating of fat compared to water. CSH can significantly vary among bacteria from highly hydrophilic to highly hydrophobic and is vastly dependent on the structure and components of the bacterial cell wall, which in turn can be subjected to changes due to the metabolic situation, growth phase, and various external factors (pH, ionic strength, growth temperature), and on additional structural elements located on the cell surface, including S-layer and fimbriae (Hazen *et al.*, 1986, van der Mei *et al.*, 1987, Van der Mei *et al.*, 1991, Martienssen, 2001, Poortinga *et al.*, 2002, Van der Mei *et al.*, 2003). In numerous reports, it is shown that cell adhesion to hydrophobic surfaces and hydrocarbon droplets increases with an increase in bacterial CSH, and consequently CSH substantially affects cell adhesion and, therefore, localization in heterogeneous systems (Rosenberg & Kjelleberg, 1986, van der Mei *et al.*, 1987, Van Loosdrecht *et al.*, 1987, Kochkodan *et al.*, 2008, Giaouris *et al.*, 2009). In general, it is further widely accepted that cell adhesion is based on an interplay of numerous acting forces, such as Brownian movement, van der Waals attraction, gravitational forces and surface electrostatic charges, and that these interactions are inter alia related to CSH (Van Loosdrecht *et al.*, 1990, Krasowska & Sigler, 2014). However, since CSH is assumed to play a crucial role in cell adhesion, studies dealing with CSH-mediated cell adhesion to oil droplets in emulsions are rare and the knowledge on this subject is still scanty, this thesis focused on the role of CSH in cell adhesion to oil droplets in emulsions (Van Loosdrecht *et al.*, 1990, Hazen, 2004, Ly *et al.*, 2006).

1.4.1 Cell wall structure of Gram-positive bacteria

Vegetative bacterial cells are surrounded by a cell wall that has multiple functions, including maintaining the cell's shape and cell integrity, and resisting internal turgor pressure. Simultaneously, serving as interface between the bacterial cell and its environment, it mediates bacterial interactions with abiotic surfaces or eukaryotic host cells (Chapot-Chartier & Kulakauskas, 2014). Dependent on the bacteria's natural environment, the cell wall can vary substantially in its structure, composition and CSH characteristics. A typical cell wall component, which can be found in all bacterial cells, is a rigid peptidoglycan (PG) layer, also known as murein, being composed of chains, which are cross-linked via oligopeptides.

These polysaccharide chains consist of alternating residues of N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) linked by a β-(1,4)-glycosidic bond (Vollmer *et al.*, 2008). Based on substantial differences in PG-layer thickness among bacteria and thus associated staining properties, as firstly described by Christian Gram (1884), the thickness of the PG-layer is utilized as primary determinant for the characterization of most bacteria as Gram-positive (PG-thickness: 30 to 100 nm, up to 40 PG-layers) or Gram-negative (PG-thickness: 7 to 8 nm, 1-3 PG-layers) (Gram, 1884, Yao *et al.*, 1999, Vollmer *et al.*, 2008, Silhavy *et al.*, 2010). Conversely to Gram-negative cells, Gram-positive cells do not have an additional outer membrane and, therefore, also no comparable periplasm (Mitchell, 1961, Beveridge, 1981, Hobot *et al.*, 1984, Beveridge & Graham, 1991, Matias & Beveridge, 2005). But, additionally to the proteins, which decorate the PG of both Gram types, Gram-positive PG is strongly modified with glycopolymers, mainly teichoic acids (TAs), which on the one hand appear to perform some of the same functions as the outer membrane, i.e., they mediate extracellular interactions, influence membrane permeability and provide additional stability to the plasma membrane, and on the other hand are supposed to serve several functions including cell adhesion to biomaterials and biofilm formation (Miörner *et al.*, 1983, Gross *et al.*, 2001, Neuhaus & Baddiley, 2003, Weidenmaier & Peschel, 2008, Swoboda *et al.*, 2010, Xia *et al.*, 2010, Brown *et al.*, 2013). Furthermore, the proteins decorating the Gram-positive PG show a high variety, some of them being analogues to proteins found in the periplasm of Gram-negative cells, and some of them being surface proteins, which are involved in cell adhesion and anchored in the thick PG-layer (Sutcliffe & Russell, 1995, Navarre & Schneewind, 1999, Van der Mei *et al.*, 2003, Dramsi *et al.*, 2008, Silhavy *et al.*, 2010). To date, the cell wall components lipoteichoic acids (LTAs), proteins with many hydrophobic side chains and lipids, are known to be associated with hydrophobic cell surfaces while proteins and polysaccharides are assumed to lend a hydrophilic cell surface (Miörner *et al.*, 1983, Hancock, 1991, Archibald *et al.*, 1993, Daffonchio *et al.*, 1995).

1.4.2 Wall Teichoic Acids (WTAs)

TAs can be classified into two groups, LTAs and WTAs, being attached to the cytoplasmic membrane or to the PG, respectively (Neuhaus & Baddiley, 2003). WTAs can constitute up to 60% of cell wall total dry weight in certain bacteria and thus form the major surface component of the bacterial cell wall (Swoboda *et al.*, 2010). WTAs have several functions, which are well summarized in the review of Xia *et al*. (2010) and Brown *et al*. (2013).

Next to functions, serving bacterial survival under disadvantageous conditions, several studies demonstrated that WTAs play an important role in cell adhesion (Gross *et al.*, 2001, Weidenmaier *et al.*, 2005, Weidenmaier & Peschel, 2008, Kohler *et al.*, 2009). However, the exact function of WTAs in cell adhesion is only superficially understood. It is not clear if WTAs directly mediate cell adhesion to abiotic and biotic surfaces by their interaction with biomaterials or if they also contribute to CSH and, therefore, consequently indirectly mediate cell adhesion. In the following, WTAs are described briefly with respect to the species *L. plantarum*, being of interest for this thesis regarding their role in CSH and, therefore, bacterial adhesion to oil droplets in emulsions.

The structures of WTAs are highly diverse and often species- and also strain-specific (Uchikawa *et al.*, 1986, Tomita *et al.*, 2009). These variations in structure can dramatically increase the intraspecies diversity of the cell wall structure and, therefore, presumably determine the major functions of WTAs and cell surface characteristics (Tomita *et al.*, 2010). WTAs are anionic glycopolymers that are covalently anchored to the MurNAC residue of PG via a disaccharide (linkage unit), mainly consisting of N-acetylmannosamine (ManNAc) (β1→4) N-acetylglucosamine (GlcNAc), with one to two glycerol-3-phosphate (Gro-P) units attached to the C4 hydroxyl of the ManNAc residue (Brown *et al.*, 2013). The glycopolymers consist of a long chain of alditol repeats (main chain), which are joined via phosphodiester bonds and extend from the Gro-P end of the linkage unit (Ward, 1981). The main chains of WTAs are often decorated by D-alanine esters associated or not with glycosyl (mainly glucose) residues (Naumova *et al.*, 2001, Neuhaus & Baddiley, 2003, Tomita *et al.*, 2009). Even though the alditol subunits of WTAs were found to vary among species and strains, WTAs of *Lactobacillus spp*. have been found to contain only glycerol in the backbone (Delcour *et al.*, 1999, Xia *et al.*, 2010). However, for *L. plantarum*, being the only exception among lactobacilli, poly(glycerol-3-phosphate) (poly(Gro-P)) and poly(ribitol-5-phosphate) (poly(Rbo-P)) WTAs have been reported (Tomita *et al.*, 2010, Bron *et al.*, 2012). The genes, involved in WTA biosynthesis, have been studied extensively in *Bacillus (B.) subtilis* and *Staphylococcus (S). aureus,* and gene homologues, which have been found in *L. plantarum* WCFS1, have been already examined in several studies (Lazarevic *et al.*, 2002, Brown *et al.*, 2008, Tomita *et al.*, 2010, Xia *et al.*, 2010). The *tag*-locus, consisting of the genes *tagD1, tagF1* and *tagF2* (*L. plantarum* WCFS1 homologues annotated as lp_0267, lp_0268, lp_0269*),* and the *tar*-locus, consisting of *tarI*, *tarJ*, *tarK* and *tarL* (*L. plantarum* WCFS1 homologues annotated as lp_1816, lp_1817, lp_1818, lp_1819), are responsible for biosynthesis of the poly(Gro-P) and poly(Rbo-P) WTA types, respectively (see Figure 2) (Bron *et al.*, 2012).

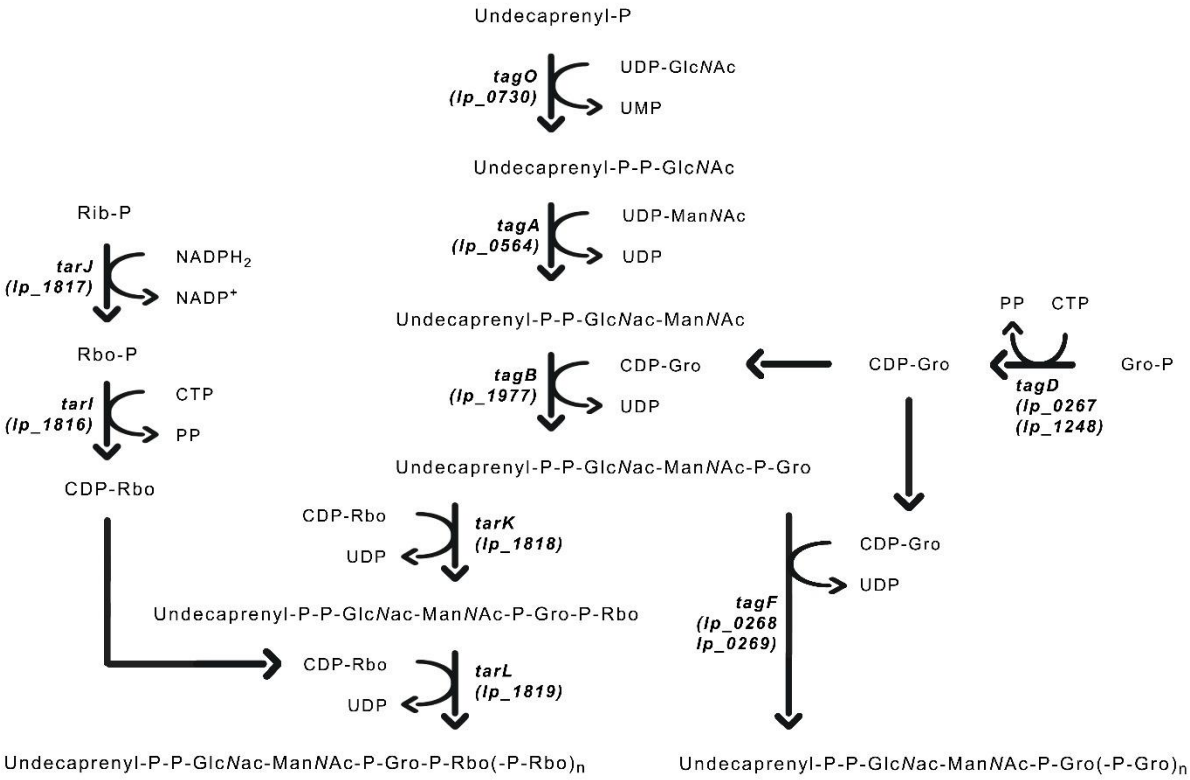

Figure 2: Biosynthesis pathways of poly(Gro-P) and poly(Rbo-P) WTAs. Numbers between brackets indicate the gene-identifiers of *tag* and *tar* homologues in the genome of *L. plantarum* WCFS1. The proteins, described in the following, are encoded by the corresponding *tag* and *tar* genes, depicted above. Independent from the WTA type, the pathway starts in the cytoplasm with the transfer of UDP-activated N-acetylglucosamine (GlcNAc) to an undecaprenyl phosphate carrier anchored in the bacterial membrane by TagO. Following, TagA transfers N-acetylmannosamine (ManNAc) from UDP-activated ManNAc to the C4 hydroxyl of the GlcNAc, forming a β-linked disaccharide (Xia *et al.*, 2010). The primase TagB couples a single phosphoglycerol (Gro-P) unit, which was CDP-activated by TagD, to the disaccharide to complete the synthesis of the linkage unit. Following, the pathways diverge. The next enzyme in the poly(Gro-P) WTA pathway is TagF, a cytidylyl transferase (oligomerase), that attaches a large, variable amount of CDP-activated Gro-P units to the linkage unit to form the Gro-P poymer (Lazarevic *et al.*, 2002). In the poly(Rbo-P) WTA pathway, the primase TarK transfers a single ribitol phosphate (Rbo-P) residue to the Gro-P (mono-, di- or tri-mer) of the linkage unit. TarJ and TarI provide CDP-activated Rbo-P from ribulose for polymerization of the Rbo-P chain, which is carried out by the oligomerase TarL (Lazarevic *et al.*, 2002, Meredith *et al.*, 2008, Xia *et al.*, 2010). Following its assembly, the lipid-linked WTA polymer, independent from its backbone, is proposed to be modified by the glycosyltransferase TagE and then transported across the cytoplasmic membrane by a two-component ABC transporter, TagGH (Xia *et al.*, 2010). Subsequently, the exported WTA polymer is coupled to PG by an transferase, which was not identified yet (Lazarevic & Karamata, 1995, Xia *et al.*, 2010). Subsequently, WTA polymers can be substituted with D-alanyl esters by enzymes encoded in the *dlt* operon (Neuhaus & Baddiley, 2003). Adapted from Bron *et al*. (2012).

The *tar*-locus is universally conserved among *L. plantarum* strains, while the *tag*-locus is only present in some strains of *L. plantarum*. Recently *L. plantarum* WCFS1 and other strains, that encode the *tag*-locus, were shown to consistently produce poly(Gro-P) WTAs, while strains that lack these genes produce poly(Rbo-P) WTAs (Tomita *et al.*, 2010). Furthermore, this observation could be proved by the deletion mutant *L. plantarum* WCFS1 ΔtagF1-F2, which was observed to perform alditol switching, producing poly(Rbo-P) instead of poly(Gro-P) WTAs (Bron *et al.*, 2012).

Additionally, a WTA deficient mutant of *L. plantarum* WCFS1 could be constructed by the deletion of the gene *tagO*, being in general the first gene in both, poly(Gro-P) and poly(Rbo-P) WTA biosynthesis pathway (Soldo *et al.*, 2002, Andre *et al.*, 2011). The authors demonstrated that disruption of the gene *tagO* completely blocks WTA production in *L. plantarum*, not being able to isolate any WTAs. The fact that *L. plantarum* WCFS1 possess the genetic capacity to produce both WTA-backbone types and that deletion mutants are available, lacking WTAs at all or producing poly(Rbo-P) instead of poly(Gro-P) WTAs, offers the possibility to study the consequences of WTA removal and WTA type on CSH of bacterial cells.

1.4.3 Determination of CSH

Bacterial CSH can only be characterized semi-quantitively by evaluating the preference for water compared to another phase, such as air or an organic solvent (Van Loosdrecht *et al.*, 1990). To date, many methods were established for the measurement of the CSH, though no method is generally accepted for determining bacterial CSH. These include inter alia contact angle measurement, microbial/bacterial adhesion to hydrocarbons (MATH/BATH), phase distribution, hydrophobic interaction chromatography (HIC) and salt aggregation (Rosenberg *et al.*, 1980, Lindahl *et al.*, 1981, Mozes & Rouxhet, 1987, Van Oss, 1995). All methods have in common that CSH is determined indirectly, partly showing inconsistencies in determined CSH due to measuring different physical interactions (Mozes & Rouxhet, 1987, Donlon & Colleran, 1993). In terms of the MATH test, the adsorption of cells to organic solvents is not exclusively based on hydrophobic interactions, it is also based on a complex interplay of Lifshitz-van der Waals, polar and electrostatic interactions and thus the determined “hydrophobicity” is not solely a result of hydrophobic interactions (Martienssen, 2001). However, the MATH test is assumed to be the most suitable method for the prediction of the adsorption behavior of microorganisms on organic solvents and, therefore, proved to be the most suitable method for this thesis, representing best the conditions present in emulsions (Martienssen, 2001). The MATH test is based on bacterial distribution in two immiscible phases due to different cell affinities to organic surfaces. The CSH is determined by measuring the absorbance of an aqueous bacterial suspension prior to the addition of a defined amount of organic solvent, followed by mixing for a fixed time, and by measuring again the absorbance in the aqueous phase after phase separation, setting the second absorbance in relation to the initial absorbance. Most commonly and also in this study, n-hexadecane is used as organic solvent, but also the use of n-octane, p-xylene and toluene was reported (Rosenberg *et al.*, 1980, Mozes & Rouxhet, 1987, Van Loosdrecht *et al.*, 1987, Busscher *et al.*, 1995).

1.5 Motivation, aim and working hypotheses

High hydrostatic pressure (HHP) processing is an emerging non-thermal food preservation technology, which, compared with conventional food preservation methods, has been proven to have relatively little effect on organoleptic properties and the nutritional value of foods without having to shorten the products' estimated shelf-life. However, although this technology has the potential to fulfil the consumers' growing demand for minimally processed, high-quality foods, its current use at the industrial level is limited to an increasing but still low number of food product categories. Among others (section 1.1.2), one major reason for this can be found in the fact that in order to ensure sufficient bacterial inactivation, a case-by-case evaluation of the inactivation efficiency of pressure in a given food matrix is commonly required. This circumstance is due to the fact that the food matrix constituents can demonstrably affect HHP inactivation and, therefore, HHP inactivation efficiency is highly dependent on the food matrix composition (section 1.1.7). While effects of food matrix constituents such as proteins, carbohydrates and inter alia salt were intensively studied, the role of the important food matrix parameter fat in HHP inactivation is not sufficiently understood. Studies, investigating effects of fat on HHP inactivation are scarce and conclusions drawn are not always consistent with each other. The reported inconsistencies can be very likely attributed to the fact that most studies were performed in complex food matrices, allowing an interplay of adverse HHP-mediated effects exerted by other food matrix parameters. Since detailed studies, investigating solely the effect of fat on the HHP inactivation of microorganism are completely missing, there exists a substantial lack of knowledge regarding the effect of fat on microbial HHP inactivation. In addition to interfering effects from other food ingredients, partially contradictory results from previous studies might reflect that fat-mediated effects on the HHP inactivation are potentially dependent on bacterial localization in heterogeneous, fatty food and direct interactions between the lipid phase and microbial cells. There, cell surface hydrophobicity (CSH) can be assumed to play a major role. However, the effect of CSH and the associated bacterial localization in food on HHP inactivation have both been fundamentally neglected so far. Among other things, this is probably due to the fact that notwithstanding countless studies our knowledge on CSH is still sketchy and its determinants are only superficially known (section 1.4). An interconnection between HHP inactivation, cell surface hydrophobicity and availability of fat has not yet been investigated but would, if existent, present a possible explanation for some of the partially contradictory results obtained in previous studies.

Against this background, the overall aim of this thesis was to contribute to closing the gap of knowledge regarding the effect of fat and fat-associated factors on HHP-mediated inactivation of spoilage-associated microorganisms in fatty foods.

In order to consider that a fat-mediated effect could be dependent on interactions between lipid phase and microbial cells, the focal points were on factors possibly influencing such interactions, that is, CSH, fat content, presence and type of emulsifier, fat surface (oil droplet size) and oil type. Since systematic data on the effect of fat and fat-associated factors in HHP inactivation do not exist, the generated data of this study should enlighten the role of fat and bacterial localization in HHP inactivation, constitute a basis for the design of future inactivation studies and facilitate the application of HHP technology to preserve fatty food without adverse effects on the products' shelf-life. In addition, new insights in terms of CSH determinants should be obtained by genome comparison of strains of the species *L. plantarum* that vary distinctly in their CSH. The identification of possible CSH-specific marker genes would contribute to the generally still deficient understanding of CSH and CSH-mediating factors and could permit the establishment of new research approaches.

The work accomplished in this thesis can be divided in three sections: the investigation (I) whether fat influences the HHP inactivation of cells of the spoilage-associated species *L. plantarum* using defined (O/W)-emulsions as simple model systems, that is, excluding additional effects of other food ingredients, (II) whether such effects are dependent on the surface hydrophobicity of bacterial cells or on the availability of fat in the model systems used, and (III) of genomes of hydrophilic and hydrophobic *L. plantarum* strains in terms of CSH-associated marker genes and their role on CSH and pressure sensitivity. This work was conducted based on following working hypotheses:

(I) Effect of fat and lipid phase parameters on HHP inactivation in (O/W)-emulsions

- Fat per se can affect HHP inactivation, and an increase in the fat content correlates with the extent of fat-mediated effects on HHP inactivation.
- The emulsifier type and, thus, the thickness of the fat-water boundary layer can have an effect on the HHP inactivation.
- The droplet size and, therefore, the fat surface area available for microbial adhesion can influence effects exerted by fat on the HHP tolerance of bacterial cells.
- Different oil types with a different fatty acid composition (saturated *versus* unsaturated) can lead to differences in the HHP inactivation.

(II) Role of CSH and bacterial localization on HHP inactivation in (O/W)-emulsions

- The presence of emulsifier in emulsifier-stabilized emulsions prevents adhesion of hydrophobic cells to the fat surface of oil droplets due to fully emulsifier-coated oil droplets. CSH-associated differences in HHP sensitivity are not dependent on direct fat surface-cell interactions.
- The absence of emulsifier in emulsifier-free emulsions facilitates the adhesion of hydrophobic cells to the fat surface of oil droplets, which leads to differences in the HHP inactivation efficiency of hydrophobic and hydrophilic strains.
- Direct fat surface-cell interaction can favor HHP inactivation through exposure of cells to locally higher temperatures caused by stronger adiabatic heating of fat.

(III) Identification of CSH-associated marker genes in genomes of the species *L. plantarum*

- Genome comparison of hydrophilic and hydrophobic strains can reveal marker genes that are characteristic for a specific CSH phenotype. The CSH of other strains of the species *L. plantarum* can be predicted upon the presence or absence of these marker genes.
- Alterations in CSH-associated marker genes in a deletion mutant strain result in pronounced physiological effects on cell surface characteristics, since interfering effects of other CSH-modulating cellular components and intraspecies biodiversity effects are excluded.

2 Materials and Methods

Standard microbiological compounds, chemicals, and supplies were acquired from different suppliers and certified for their intended use. If not specifically stated otherwise, deionized water was used for the preparation of media and buffers. All solutions required sterile were autoclaved at 121 °C for 15 min or sterilized by filtration (Millipore, 0.20 µm).

2.1 Materials

2.1.1 Chemicals

All chemicals used in this study are listed in Table 2.

Table 2: Chemicals used in this study.

Chemical	Specification	Manufacturer
6x DNA loading dye	-	Fermentas GmbH, St. Leon-Rot, Germany
Acetic acid	100%, glacial	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Acetonitrile (ACN)	≥ 99.9%	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Acetonitrile	anhydrous, 99.8%	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Agar	European agar	Becton Dickinson GmbH, Heidelberg, Germany
Ammonium chloride; NH_4Cl	≥ 99.7%	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Antifoam B emulsion	-	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Bacterial Test Standard	-	Bruker Daltonik GmbH, Bremen, Germany
Bis(2-ethylhexyl) sebacate	95%	Alfa Aesar GmbH & Co. KG, Karlsruhe, Germany
Boric acid	≥ 99,8%	Carl Roth GmbH & Co. KG, Karlsruhe, Germany

Bruker Matrix HCCA (α-cyano-4-hydroxycinnamic acid solution)	-	Bruker Daltonik GmbH, Bremen, Germany
Chloramphenicol	≥ 98.5%, Ph. Eur., for biochemistry	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Citric acid	≥ 99.5%, Ph. Eur., water-free	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
D(-)-Fructose	-	OMNI Life Science GmbH & Co. KG, Bremen, Germany
D(+)-Glucose monohydrate	for microbiology	Merck KGaA, Darmstadt, Germany
Dimidium bromide	≤ 98%	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
di-Sodium hydrogen phosphate dihydrate; $Na_2HPO_4 * 2H_2O$	for analysis	Merck KGaA, Darmstadt, Germany
di-Potassium hydrogen phosphate trihydrate; $K_2HPO_4 * 3 H_2O$	for analysis	Merck KGaA, Darmstadt, Germany
EDTA (ethylenediaminetetraacetic acid)	for molecular biology	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Ethanol, absolute	≥ 99.8%	VWR International GmbH, Heidelberg, Germany
Formic acid	98 - 100%, p.a.	Merck KGaA, Darmstadt, Germany
Glycerol	anhydrous, ultra-pure	J. T. Baker, Deventer, Netherlands
Hexadecane (n-Hexadecane)	≥ 99%	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Hydrochloric acid solution; HCl, 37%	p.a.	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Imidazole	puriss. p.a., ≥ 99.5% (GC)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
L-Asparagine monohydrate	minimum 99% (TLC)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany

L-Cysteine-HCl monohydrate	≥ 98.5%	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Magnesium sulphate heptahydrate; $MgSO_4 * 7H_2O$	ACS, Reag. Ph Eur	Merck KGaA, Darmstadt, Germany
MALDI-TOF MS bacterial test standard	-	Bruker Daltonik GmbH, Bremen, Germany
Manganese(II) sulphate monohydrate; $MnSO_4 * H_2O$	≥ 99%, p.a., ACS	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Meat extract	for microbiology	Merck KGaA, Darmstadt, Germany
Miglyol® 812	-	Caesar & Loretz GmbH, Hilden, Germany
Paraffin oil	puriss., meets analytical specification of Ph. Eur., BP, viscous liquid	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Peptone from casein	for microbiology	Merck KGaA, Darmstadt, Germany
Peptone from meat	for microbiology	Merck KGaA, Darmstadt, Germany
Polyethylene glycol 400	-	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Potassium dihydrogen phosphate, KH_2PO_4	≥ 99%, p.a.	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Rapeseed oil	-	real,- SB-Warenhaus GmbH, Düsseldorf, Germany
Sodium casein	-	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Sodium chloride; NaCl	≥ 99.5%, p.a., ACS, ISO	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Sodium hydroxide; NaOH	≥ 99%, p.a.	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Tributyrin	≥ 97%	Sigma-Aldrich Chemie GmbH, Steinheim, Germany

Trifluoroacetic acid (TFA)	≥ 99.9%	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Tris; tris(hydroxymethyl)-aminomethane	analytical grade	GERBU Biotechnik GmbH, Heidelberg, Germany
Tween® 80 (Polyoxyethylenesorbitan monooleate)	for synthesis	Merck KGaA, Darmstadt, Germany
Water	J. T. Baker®, for HPLC, electrophoresis	VWR International GmbH, Heidelberg, Germany
Yeast extract	for bacteriology	Carl Roth GmbH & Co. KG, Karlsruhe, Germany

2.1.2 Media, dilution and agar solutions

Table 3: Media, dilution and agar solutions.

Media / Buffer	Ingredients	Weight ($g\ L^{-1}$)	Remarks
mMRS	Casein peptone	10.0	Glucose/Fructose was
pH 6.2 ± 0.2*	Meat extract	5.0	autoclaved separately.
	Yeast extract	5.0	$MgSO_4 * 7\ H_2O$ and
	KH_2PO_4	4.0	$MnSO_4 * 4\ H_2O$ were
	$K_2HPO_4 * 3\ H_2O$	2.6	added after sterilization.
	NH_4Cl	3.0	
	Cystein-HCl	0.5	
	Tween® 80	1.0	
	Glucose	7.5	
	Fructose	7.5	
	$MgSO_4 * 7\ H_2O$	0.1	
	$MnSO_4 * 4\ H_2O$	0.05	
	Agar, if necessary	15.0	
CA-mMRS	see ingredients mMRS		see remarks mMRS
pH 3.5 / 4.0 / 4.5 / 6.2 ± 0.2*	Citric acid	3.84	
	L-Asparagine monohydrate	2.66	
tributyrin-mMRS Agar	see ingredients mMRS		see remarks mMRS
pH 6.2 ± 0.2*	Tributyrin	10.0	Tributyrin was added after sterilization.
LB Agar	Tryptone	10.0	
pH 7.0 ± 0.2*	Yeast extract	5.0	
	NaCl	10.0	
	Agar	15.0	
Medium 1 (DSMZ)	Peptone	5.0	
pH 7.0 ± 0.2*	Meat extract	3.0	
	Agar, if necessary	15.0	
TS+ dilution buffer	NaCl	8.5	
pH 7.0 ± 0.2*	Tryptone	14.0	
	Antifoam B Emulsion	0.1	
IPB	KH_2PO_4	0.1	
pH 6.2 ± 0.2*	$Na_2HPO_4 * 2\ H_2O$	4.45	
	Imidazole	1.7	
TBE buffer	Tris	10.8	
pH 8.0 ± 0.2*	Boric acid	5.5	
	EDTA	0.7	

*the pH was adjusted using 2 - 6 M NaOH/HCl solutions.

2.1.3 Devices

All devices used in this study are listed in Table 4.

Table 4: Devices used in this study.

Device	Model	Manufacturer
10x, 40x and 100x Objective lens	N-Achroplan 10x/0.25, N-Achroplan 40x/0.75, N-Achroplan 100x/1.25 Oil Iris	Carl Zeiss Microscopy GmbH, Munich, Germany
Balance	SI-234	Denver Instrument, Bohemia, NY, USA
Balance	SBA 52	Scaltec Instruments, Heiligenstadt, Germany
Balance	SPO 61	Scaltec Instruments, Heiligenstadt, Germany
Centrifuge	1-14	Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany
Centrifuge	6-16 K	Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany
Centrifuge	MCF-1350	LMS Consult GmbH & Co. KG, Brigachtal, Germany
Centrifuge	Rotina 380R	Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany
Colony counter	BZG 30	WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany
Colony counting imaging station	ColonyDoc-It™	Ultra-Violet Products Ltd, Upland, California, United States
Electronically controlled manual dispenser	Multipette® stream	Eppendorf AG, Hamburg, Germany
Electrophoresis system	Owl™ EasyCast™ B2	Owl Scientific Inc, Massachusetts, United States
Electrophoresis system	Owl™ A2 Großgelsystem	Owl Scientific Inc, Massachusetts, United States
Heating magnetic stirrer	ARE heating magnetic stirrer	VELP Scientifica Srl, Usmate, Italy

Heating magnetic stirrer	Wise Stir MSH-20A	©WITEG Labortechnik GmbH, Wertheim, Germany
Heating magnetic stirrer	RCT basic	IKA®-Werke GmbH & CO. KG, Staufen, Germany
High-performance dispersing machine	T25 digital ULTRA TURRAX®	IKA®-Werke GmbH & CO. KG, Staufen, Germany
High pressure intensifier system with high pressure vessel	MV2-13	Institute of High Pressure Physics, Warszawa, Poland
High pressure intensifier system	TMW-RB	Knam Schneidetechnik GmbH, Langenargen, Germany
Homogenizer	APV 1000	APV Systems, Denmark
Incubator	TC 135 S	Tintometer GmbH, Lovibond® Water Testing, Dortmund, Germany
Laminar airflow clean bench	HERA safe	Heraeus Instruments, Hanau, Germany
Laminar airflow clean bench	Kojair®, Biowizard Golden Line	KOJAIR TECH OY, Vilppula, Finland
MALDI-TOF mass spectrometer (MS)	Microflex LT	Bruker Daltonik GmbH, Bremen, Germany
Thermal cycler	Mastercycler® gradient	Eppendorf AG, Hamburg, Germany
Microplate reader	Sunrise	Tecan Deutschland GmbH, Crailsheim, Germany
Microplate reader	SpectraFluor	Tecan Deutschland GmbH, Crailsheim, Germany
Microplate reader	FLUOstar Omega	BMG LABTECH GmbH, Ortenberg, Germany
Microscope equipped with RGB camera	Axiostar plus microscope, AxioCam ICc1	Carl Zeiss Microscopy GmbH, Munich, Germany
Microwave oven	Intellowave	LG Electronics Deutschland GmbH, Ratingen, Germany

Particle sizing instrument	Mastersizer 2000	Malvern Instruments Ltd, Malvern, United Kingdom
pH electrode	InLab® Semi-Micro pH, pH 0-12	Mettler-Toledo GmbH, Gießen, Germany
pH electrode	InLab® 412, pH 0-14	Mettler-Toledo GmbH, Gießen, Germany
pH meter	Knick pH-Meter 761 Calimatic	Knick Elektronische Messgeräte GmbH, Berlin Germany
pH meter	ProLab3000	SI Analytics GmbH, Mainz, Germany
Pipettes	Pipetman (2 µL, 20 µL, 100 µL, 200 µL, 1000 µL)	Gilson International B.V, Deutschland, Limburg-Offheim, Germany
Power Supply	Power Pack P25	Biometra GmbH, Göttingen, Germany
Refractometer	Type 16650	Carl Zeiss Microscopy GmbH, Munich, Germany
Scanner	Bio-5000	Microtek, Hsinchu, Taiwan
Spectrophotometer	Novaspec II	Pharmacia Biotech, Uppsala, Sweden
Spectrophotometer	NovaspecPlus	Pharmacia Biotech, Uppsala, Sweden
Stability analyzer	LUMiFuge®	LUM GmbH, Berlin, Germany
Steam pot	Varioklav® DT 400 + Control unit DT-E	HP Medizintechnik GmbH, Oberschleißheim, Germany
Thermostatic circulator	WCR-P22	©WITEG Labortechnik GmbH, Wertheim, Germany
Thermostatic circulator	RSA	Lauda DR. R. Wobser GmbH & Co. KG, Lauda-Königshofen, Germany
Thermostatic circulator	FC 600	JULABO GmbH, Seelbach, Germany

ULT freezer	MDF-U700VX-PE	Panasonic Healthcare Co., Ltd, Gunma, Japan
UV Transilluminator	UVT-28 M	Herolab GmbH, Wiesloch, Germany
Vacuum pump	PC 3003 VARIO	VACUUBRAND GMBH + CO KG, Wertheim, Germany
Vacuum regulator	CVC 3000	VACUUBRAND GMBH + CO KG, Wertheim, Germany
Vacuum Sealing System	FoodSaver® V2860	Jarden Consumer Solutions, Oldham, United Kingdom
Vortex mixer	Vortex Genie 2	Scientific Industries Inc., Bohemia, NY, USA
Water bath	E100 LAUDA	Lauda DR. R. Wobser GmbH & Co. KG, Lauda-Königshofen, Germany
Water bath	C1 HAAKE	Thermo Haake GmbH, Karlsruhe, Germany

2.1.4 Consumables

Table 5: Consumables used in this study.

Item	Specification	Manufacturer
Cuvettes	10 x 4 x 45 mm, polystyrene	Sarstedt AG & Co., Nümbrecht, Germany
Combitips	Combitips advanced®, sterile, 1.0 mL, 2.5 mL, 10 mL	Eppendorf AG, Hamburg, Germany
Cover glasses	20 x 20 mm	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Cryo pure tubes	1.8 mL white, non-pyrogenic, non-mutagenic, non-cytotoxic	Sarstedt AG & Co., Nümbrecht, Germany
Cryo tube vials	Nunc CryoTube Vials 0.5 mL Cryobank	Thermo Fisher Scientific, Waltham, MA, USA
DNA Ladder	GeneRuler 100 bp Plus DNA Ladder	Thermo Fisher Scientific, Waltham, MA, USA
Glass pasteur pipettes	230 mm	BRAND GmbH + Co KG, Wertheim, Germany
MALDI-TOF MS stainless steel target plate	MSP 96	Bruker Daltonik GmbH, Bremen, Germany
Microscope slides	76 x 26 x 1 mm	Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany
Microtitre plates	96 well, flat base, with lid, transparent	Sarstedt AG & Co., Nümbrecht, Germany
Microtitre plates	MicroWell™ 96-well microplate, white	Thermo Fisher Scientific, Waltham, MA, USA
Petri dishes	92 x 16 mm, with ventilation cams	Sarstedt AG & Co., Nümbrecht, Germany
Pipette tips	PIPETMAN TIPS Diamond; 0.1-20 µL	Gilson International B.V, Deutschland, Limburg-Offheim, Germany
Pipette tips	1-200 µL, 100-1000 µL	Peske GmbH, Karlsruhe, Germany

Reaction tubes	200 µL, 1.5 mL, 2 mL	Eppendorf AG, Hamburg, Germany
Sterile filters	Filtropur S 0.2 and S 0.45, sterile non-pyrogenic	Sarstedt AG & Co., Nümbrecht, Germany
Sterile reagent and centrifuge tubes	15 mL, 50 mL	Sarstedt AG & Co., Nümbrecht, Germany
Syringes	single use, pyrogenfree, sterile; 10 mL, 20 mL	Dispomed Witt oHG, Gelnhausen, Germany
Test tubes	Fisherbrand™ Disposable Flint Glass Tubes with Plain End	Thermo Fisher Scientific, Waltham, MA, USA

2.1.5 Molecular biological kits, enzymes and supplies

Molecular biological kits, enzymes and supplies used in this study are listed in Table 6.

Table 6: Molecular biological kits, enzymes and supplies used in this study.

Kit / enzymes / supplies	Specification	Manufacturer
10x Incubation Mix T. Pol with $MgCl_2$	DNA amplification	MP Biomedicals, Solon, Ohio, USA
Bacterial Viability Kit L7012	SYTO® 9 (3.34 mM) used for cell staining	Thermo Fisher Scientific, Waltham, MA, USA
E.Z.N.A. Bacterial DNA Kit	DNA isolation	Omega Bio Tek Inc., Norcross, GA, USA
Lysozyme 100.000 units/mg	DNA isolation	SERVA Electrophoresis GmbH, Heidelberg, Germany
PCR Nucleotide Mix, 10 mM each	DNA amplification	Roche Diagnostics Deutschland GmbH, Mannheim, Germany
Proteinase K 20 mg/mL	DNA isolation	GERBU Biotechnik GmbH, Heidelberg, Germany
QIAGEN Genomic DNA Buffer Set	DNA isolation	QIAGEN, Hilden, Germany
QIAGEN Genomic-tip 100/G	DNA isolation	QIAGEN, Hilden, Germany
Taq DNA polymerase 5 U/µL	DNA amplification	MP Biomedicals, Solon, Ohio, USA

2.1.6 Bacterial strains

Bacterial strains used in this study were taken from the internal culture collection of the Chair of Technical Microbiology at TUM or ordered from institutions listed in Table 7.

Table 7: *L. plantarum, L. sakei and L. fructivorans* strains used in this study.

TMW	Strain	Origin	Isolation source
1.59	*L. fructivorans*	DSM 20203T	Unknown, Ernst Böcker GmbH & Co. KG
1.452	*L. fructivorans*	LTH 669, Gent LAB 681	Unknown
1.1856	*L. fructivorans*	L1	Whey
1.25	*L. plantarum*	LTH 2354, R. Vogel 1992	Raw sausage
1.1	*L. plantarum*	CTC 305	Raw sausage
1.1308	*L. plantarum*	Unknown	Unknown
1.1478	*L. plantarum*	WALA 01030879_1	Belladonna honey
1.1728	*L. plantarum*	23C	Pastry dough, Ernst Böcker GmbH & Co. KG
1.1732	*L. plantarum*	F3	Fermented Food, R. Albesharat
1.1789	*L. plantarum*	So1	Human faeces, R. Albesharat
1.1792	*L. plantarum*	Mk13	Human faeces, R. Albesharat
1.246	*L. plantarum*	Unknown	PRIMAVITA blackcurrant beverage
1.277	*L. plantarum*	C. Dieng	Palm wine
1.38	*L. plantarum*	LTH 232	Starter culture
1.409	*L. plantarum*	LTH 1870, Gent LAB 159	Sauerkraut
1.64	*L. plantarum*	DSM 20205	Unknown, Ernst Böcker GmbH & Co. KG
1.708	*L. plantarum*	CTC 51	Raw sausage
1.834	*L. plantarum*	ULICE 24-4-147	ULICE, France
1.1623	*L. plantarum*	M1r1	Breast milk
1.1594	*L. plantarum*	Unknown	Breast milk, R. Albesharat
1.9	*L. plantarum*	DSM 20174T, LTH 478	Unknown
1.2088	*L. plantarum*	P-8	Traditional fermented cow milk, W. Zhang, IMAU, China
1.2089	*L. plantarum*	16	Malt production steep water, D. van Sinderen, UCC, Ireland
-	*L. plantarum*	WCFS1	Single colony of strain NCIMB8826 from human saliva, TIFN
-	*L. plantarum*	WCFS1 ΔtagO	P. Bron et al. Microbial Cell Factories 2012, 11:123, TIFN
-	*L. plantarum*	WCFS1 ΔtagF1-F2	P. Bron et al. Microbial Cell Factories 2012, 11:123, TIFN
1.322	*L. plantarum*	Gent LAB 1019	Unknown
1.468	*L. plantarum*	Gent LAB 1158	Unknown
1.811	*L. plantarum*	Unknown	Unknown, I. Rouhsdy
1.817	*L. plantarum*	Unknown	Unknown, I. Rouhsdy
1.829	*L. plantarum*	Unknown	Unknown, I. Rouhsdy
1.835	*L. plantarum*	ULICE 26-7-161	ULICE, France
1.1237	*L. plantarum*	Lp43	Sourdough, R. Valcheva, ENITIAA
1.1204	*L. plantarum*	F14	Unknown, Ernst Böcker GmbH & Co. KG
1.1342	*L. plantarum*	LCA-9	Cargill Deutschland GmbH
1.1356	*L. plantarum*	1b	H. Ulmer, Nestle
1.1516	*L. plantarum*	Sd´1	Human faeces (Mother)
1.1609	*L. plantarum*	B0s10	Human infant faeces
1.1611	*L. plantarum*	B0s9	Human infant faeces
1.1647	*L. plantarum*	A3m5	Human faeces (Mother)
1.1662	*L. plantarum*	B4r10	Human infant faeces
1.1671	*L. plantarum*	M5m3	Breast milk
1.190	*L. plantarum*	LTH 2354	Unknown

TMW	Strain	Origin	Source
1.186	*L. plantarum*	LK 1	Unknown
SF_02	*L. plantarum*	Unknown	Raw sausage, K. Ruhland, TUM
1.1723	*L. plantarum*	BSML 1045	Unknown
1.1808	*L. plantarum*	K8	Fermented Food
1.1810	*L. plantarum*	F1	Fermented Food
1.1830	*L. plantarum*	K3	Fermented dairy
1.1831	*L. plantarum*	C4	Fermented dairy
1.13	*L. sakei*	RP3	Starter culture, Gewürzmüller GmbH
1.23	*L. sakei*	LTH 673	Raw sausage
1.151	*L. sakei*	CTC 431	Unknown
1.161	*L. sakei*	LTH 1651	Unknown
1.165	*L. sakei*	7L0201.35/ II-2	Unknown, Institute Erdmann
1.412	*L. sakei*	LTH 1768, Gent LAB 162	Sauerkraut
1.704	*L. sakei*	Sg2	Sourdough, M. Gänzle
1.1189	*L. sakei*	DSM 20017T, ATCC 15521	Unknown
1.1239	*L. sakei*	Lp46	Sourdough, R. Valcheva, ENITIAA
1.1322	*L. sakei*	INRA 23K	Unknown, Institut National de la Recherche Agronomique
1.1393	*L. sakei*	BB 3059	Salami starter culture, Blessing Biotech GmbH
1.1396	*L. sakei*	LTH 2389	Salami starter culture, Blessing Biotech GmbH
1.1399	*L. sakei*	LTH1183	Salami starter culture, Blessing Biotech GmbH
1.1407	*L. sakei*	HEIDI1	Fermented freshwater fish
1.1452	*L. sakei*	#11	BRENTA cheese
1.1474	*L. sakei*	LTH 2076	Unknown
1.1954	*L. sakei*	Nr. 51A	Gutsherrenmettwurst, A. Bantleon
2.472	*B. subtilis*	DSM 10T	Unknown

2.1.7 Synthetic oligonucleotide primers for PCR

The oligonucleotide primer sequences for all gene products used in PCR gene expression analysis are listed in Table 8. Oligonucleotides were synthesized by Eurofins (Ebersberg, Germany) and dissolved in water to a final concentration of 100 pmol μL^{-1}.

Table 8: PCR DNA primer sequences. f, forward; r, reverse; T_m, melting temperature; T_A, annealing temperature.

Gene		Primer sequence (5' → 3')	T_m (°C)	%GC	T_A (°C)	Amplification product (bp)
tagO	f	CCT AGT GGC GAC CAT GAT AC	61	55	52	320
	r	AGA CCT CCA AAG CGG CTA AC	64	55		
tagF1	f	AGG TTT ATC GCC GAC TGT TC	62	50	52	1100
	r	CGT GAA CTG TGT CCG AAA TG	61	50		
tagI	f	GCA ATG TTC CGA TGC CAA AG	62	50	50	506
	r	GGA GCT GAG TGC GTT ATA TG	60	50		
tagJ	f	ACG CCG ATG ATT TGA TCG TG	63	50	53	592
	r	ACA TCG GCT GGA ATG TCA TC	62	50		
tagK	f	AAG TGT TTG GCC CGA AGA AG	62	50	52	1012
	r	ACG ACC CGT TCG GTA TTA TG	61	50		
tagL	f	CAC AAA CGA CCA AGG AAG AC	61	50	49	389
	r	GGA GGT TCG TCT GAT AAT CC	59	50		
tarI	f	ACG TCC CGA TGC CTA AAC AG	64	55	52	507
	r	GGG CAT CAG TTA AGG CGT TG	63	55		
tarJ	f	TAC GGG TCA ACG ACC ACA AG	64	55	54	803
	r	TGC GAG TGA AGC CCA CTA AG	64	55		
tarK	f	GAT GGC CAG ATG GAT ATT CG	59	50	50	530
	r	ATA TCC CAA GGC TCC TGT AG	60	50		
tarL	f	CGG GCA CCA GTT AGC GAT AC	64	60	52	425
	r	GTC CGC CGA ATT AGC CAA CC	65	60		
07F	f	AGA GTT TGA TCT GGC TCA G	53	47	52	~1500
1507R	r	TAC CTT GTT ACG ACT TCA C	50	42	52	

2.2 Microbiological methods

2.2.1 Preparation of own culture collection

Bacterial strains were plated on mMRS agar by streak plating method. Single colonies were picked, inoculated into 10 mL mMRS medium, and grown overnight aerobically in centrifuge tubes at 30 °C. The next day, fresh mMRS medium (10 mL) was inoculated with 1% (v/v) of the overnight culture and incubated for another 24 h. The cells were harvested by centrifugation (5.000 × *g*, 5 min, RT), resuspended in 1.6 mL mMRS medium, mixed 1:1 with 80% glycerol, and stored in cryo tube vails at -80 °C. Species identity was confirmed by MALDI-TOF MS analysis (Microflex LT, Bruker Daltonik GmbH, Bremen, Germany; see 2.4.1).

2.2.2 Culture conditions

Dependent on the experimental setup, bacteria were either grown in liquid culture or on plates. Sterile mMRS medium was inoculated from the stock culture and cells were grown aerobically overnight (30 °C). *Liquid culture*: The next day, fresh mMRS medium was inoculated with 1% (v/v) of the overnight culture and further incubated at 30 °C. The cells were harvested by centrifugation (5.000 × *g*, 5 min, RT) either in their late exponential (OD_{600} of 0.8 ± 0.1) or stationary phase (24 h). For *B. subtilis* TMW 2.472, LB medium was used instead of mMRS medium. *Plate culture*: On the next day, the overnight culture was serially diluted and appropriate dilutions were plated on mMRS plates supplemented with 15 g L^{-1} agar (mMRS-agar plates) using glass beads. The plates were incubated for 72 h at 30 °C.

2.2.3 Determination of bacterial cell growth and viable cell count

2.2.3.1 Bacterial cell growth

Measuring the turbidity (= optical density, OD) of microbial cultures is a widespread method to determine the cell growth in culture. Using a photometer, the measurement is based on the amount of light reflected, scattered and absorbed by the cells. In this study, cell growth was detected by measuring the OD at 600 nm (OD_{600}) by microplate reader (Sunrise, Tecan Deutschland GmbH, Crailsheim, Germany). Therefore, mMRS or CA-mMRS medium was inoculated with 2% (v/v) of an overnight culture, 150 µL of this freshly prepared culture directly transferred to a microtiter plate and each sample overlayed with 50 mL of paraffin oil to avoid drying. The cultures were incubated at different growth temperatures and cell growth was measured after defined time periods, depending on the examined growth parameters (see 2.2.7).

Before measuring the OD_{600}, samples were shaken for 15 s with “normal” speed to stir up deposited cells.

2.2.3.2 Viable cell count

To determine the inactivation of samples after HHP treatment, viable cell count was performed. For samples in emulsifier-stabilized (O/W)-emulsion, 500 µL of sample were mixed with equal volume of an isotonic tryptone solution (TS+ buffer) supplemented with Antifoam B, followed by serial dilution using TS+ buffer. 50 µL of appropriate serial dilutions were plated on mMRS plates supplemented with 15 g L^{-1} agar (mMRS-agar plates) using glass beads. The plates were incubated for 72 h at 30 °C to allow recovery and colony formation of damaged, but viable cells. The viable cell count is presented as the average log reduction level ($log_{10}(N_0/N)$) from at least three independent experiments and error bars correspond to standard deviations.

Solid samples (bacteria stirred in emulsifier-free (O/W)-emulsions supplemented with agar) were first transferred to a vacuum bag and diluted 1:5 in TS+ buffer, subsequently the bag was vacuum sealed and the sample homogenized for 1 min, before serial dilutions and plating on mMRS-agar plates were performed.

2.2.4 Determination of growth characteristics

Using the optical densities which were obtained at 600 nm (OD_{600}) under different growth conditions, biological growth curves were derived and the growth dynamics maximum cell density (OD_{max}) and maximum specific growth rate (μ_{max}) evaluated. For determination of μ_{max} in the exponential phase, measured absorbance (OD_{600} value) was first converted to ln OD and the slopes of ln OD versus time calculated for each time interval by using following formula:

$$\mu = \frac{\ln Xt - \ln X0}{t - t0}$$

Where t = time, X = OD_{600} at time t, X_0 = OD_{600} at time t_0, µ = specific growth constant (h^{-1} or d^{-1}).

The times showing a high and constant growth rate were used for calculation of the regression line, relating time to absorbance in this time interval.

The slope of the line corresponds to μ_{max}, which was finally determined from means of μ_{max} values from at least three independent experiments.

2.2.5 Determination of CSH

CSH was determined by Microbial Adhesion To Hydrocarbons (MATH) test according to Rosenberg *et al*. (1980) with slight modifications. Bacteria were harvested in the late exponential and stationary growth phase, respectively. Cells were washed twice (5.000 × *g*, 5 min, 25 °C) with and resuspended in imidazole/phosphate buffered saline (IPB) to an OD_{600} of 0.35 to 0.4. The cell suspension (4 mL) was mixed with 0.4 mL of n-hexadecane in round-bottom glass test tubes (10 mm diameter). Following incubation at 30 °C for 10 min, the mixtures were vortexed for 2 min and then allowed to sit for 15 min at RT to ensure complete separation of the organic and aqueous phase. The absorbance of the aqueous phase was measured at 600 nm before (A_0) and after (A) the treatment with n-hexadecane using glass pipettes (Gilford 2600 UV-VIS spectrophotometer). The results are expressed as percentage absorbance of the aqueous phase after treatment with n-hexadecane relative to initial absorbance. Each value represents the mean of three independent measurements.

2.2.6 Emulsion-destabilizing potential of *Lactobacillus* species

The potential of all preselected strains to destabilize (O/W)-emulsions was evaluated by investigating their proteolytic (relevant for sodium caseinate stabilized (O/W)-emulsion) and lipolytic activity and their potential to degrade and utilize the emulsifier Tween® 80, which was used for the stabilization of most of the (O/W)-emulsions.

2.2.6.1 Proteolytic activity

The proteolytic activity of all selected strains was determined by Förster resonance energy transfer (FRET), using Pierce™ Fluorescent Protease Assay Kit (Thermo Scientific™, Waltham, MA, USA). Fluorescence properties of fluorescein isothiocyanate (FITC)-labeled casein (FTC-casein) change significantly upon digestion by cellular proteases, resulting in a measurable change in fluorescence properties. Hereby, the decrease in fluorescence quenching (= increased total fluorescence) that occurs as the FTC-casein substrate is digested into smaller fluorescein-labeled fragments, is measured. TPCK trypsin and the control strain *B. subtilis* TMW 2.472, known for its proteolytic activity, were used as positive control and FTC-casein (i.e. substrate only) was used as negative control.

The assay was performed in white 96-well microplates, using fluorescein excitation/emission filters with 485/520 nm (Gain: 300), following manufacturer`s instructions and applying IPB as working buffer.

2.2.6.2 Lipolytic activity

The lipolytic activity of selected bacterial strains was examined by plating them on tributyrin-mMRS agar (TB-mMRS) plates. Grown colonies, which secrete lipases/esterases into the extracellular space, show clear halos surrounding them, attributable to hydrolysis of tributyrin, resulting in release of butyric acid. The TB-mMRS plates were prepared as follows: mMRS medium with addition of 1.5% (w/v) agar was sterilized for 15 min at 121 °C. After cooling to ~60 °C, filter-sterilized tributyrin was added and the mixture dispersed by high-performance dispersing machine (T25 digital ULTRA TURRAX®). Immediately after dispersion, the medium was poured into petri dishes and allowed to harden. Stationary phase cultures, prepared according to 2.2.2, were serially diluted using TS+ buffer. 50 µL of appropriate serial dilutions were plated on TB-mMRS plates using glass beads, cells incubated at 30 °C and evaluated after sufficient colony growth (at least 72 h) by colony counting imaging station. *B. subtilis* TMW 2.472 was used as positive control and was incubated at 30 °C.

2.2.6.3 Degradation of emulsifier Tween® 80

The potential of the tested bacterial strains for degradation of the emulsifier Tween® 80 was determined by investigating their growth according to 2.2.3.1, using IPB with adjusted Tween® 80 concentrations of 0.1 and 1.0% (w/v) instead of mMRS. Strains were cultivated for 15 h at 30 °C and the OD_{600} was measured every 5 min with a microplate reader. Additionally, as positive control, all strains were grown in mMRS.

2.2.7 Food spoilage potential of *Lactobacillus* species

The relevance of all preselected strains regarding food spoilage was evaluated by investigating their tolerance to acidic pH, higher salinity and low temperatures.

2.2.7.1 pH tolerance

The pH tolerance of bacterial strains was examined by determining bacterial cell growth (according to 2.2.3.1) using CA-mMRS medium with adjusted pH values of 3.5, 4.0, 4.5 and 6.2.

Strains were cultivated for 96 h at 30 °C and OD_{600} was measured every 30 min with a microplate reader. Growth characteristics were determined according to 2.2.4.

2.2.7.2 Salinity tolerance

The salinity tolerance of bacterial strains was examined by determining bacterial cell growth (according to 2.2.3.1) using mMRS medium with adjusted NaCl concentrations of 5, 6, 7.5 and 10%. Strains were cultivated for 4 days at 30 °C and OD_{600} was measured every 30 min with a microplate reader. Growth parameters were determined according to 2.2.4.

2.2.7.3 Temperature tolerance

The temperature tolerance of bacterial strains was examined by determining bacterial cell growth (according to 2.2.3.1) using mMRS medium and cultivation temperatures of 4, 10 and 30 °C. Cell growth of strains cultivated at 4 and 10 °C for 9 days was measured every 24 h with a microplate reader. Cell growth of strains cultivated at 30 °C, used as positive control, was measured for 3 days every 60 min by microplate reader. Growth parameters were determined according to 2.2.4.

2.3 Molecular biological methods

2.3.1 Isolation of bacterial genomic DNA

For genome sequencing, bacterial DNA was isolated by using QIAGEN Genomic-tip 100/G (QIAGEN, Hilden, Germany) in combination with QIAGEN Genomic DNA Buffer Set (QIAGEN, Hilden, Germany) according to manufacturer's instructions applying minor modifications. Lysis time and concentration of used enzymes were adapted to the used culture volume (20 mL) and the culture properties (Gram-positive, stationary phase cells): 70 µL of RNase A (10 mg mL^{-1}), 240 µL of lysozyme (300 mg mL^{-1}) and 100 µL of Proteinase K (20 mg mL^{-1}) were used. The cell wall digestion with lysozyme and Proteinase K was extended to 4 h. Following, the lysate was incubated for 2 h with lysis buffer B2 to obtain a clear lysate. After precipitation, the genomic DNA was redissolved in 200 µL elution buffer from E.Z.N.A Bacterial DNA Kit (Omega Bio Tek Inc., Norcross, USA) and kept at 4 °C.

2.3.2 Agarose gel electrophoresis

Analysis of genomic DNA and products of PCR amplification were performed by agarose gel electrophoresis (Sambrook *et al.*, 1989). DNA samples were mixed with 6x DNA loading dye (Fermentas GmbH, St. Leon-Rot, Germany) and appropriate sample volumes and 5 µL of 100bp Plus GeneRuler DNA Ladder (Thermo Fisher Scientific, Waltham, USA) were loaded into the chambers of an 1.6% (v/v) agarose gel (agarose in 0.5x TBE buffer). The Owl™ EasyCast™ electrophoresis system (Owl Scientific Inc., Massachusetts, United States) was used to run the gels at a constant voltage of 150 V for 75 min (Power Supply Power Pack P25, Biometra GmbH). TBE buffer was used as running buffer component (Table 3). To visualize nucleic acids, gels were stained in dimidium bromide and subsequently analyzed using an UVT-28 M transilluminator (Herolab GmbH, Wiesloch, Germany) and a CCD camera (Intas-Science-Imaging Instruments GmbH, Göttingen, Germany).

2.3.3 Colony polymerase chain reaction

For specific amplification of DNA fragments, colony polymerase chain reaction (colony PCR) was performed, using bacterial colonies as DNA template (see 2.2.2, plate culture). Therefore, a single colony was dissolved in 200 µL of DH2O and 2 µL of bacterial suspension were used for the reaction mixture. All PCR reactions were carried out in a thermal cycler using the *Taq* DNA polymerase for amplification. Each set of reactions included a negative control, containing DH2O instead of template DNA and a positive control, using the universal primer set 07F/1507R for amplification of the eubacterial 16S rRNA gene (Lane, 1991). The composition of the PCR reaction mixture and the PCR program with reaction times and temperatures are listed in Table 9 and Table 10.

Table 9: Composition of PCR reaction mixture.

Master Mix (1x):	[µL]
DH2O	19.6
Taq reaction buffer + $MgCl_2$ (10x) (final conc. 1x)	2.5
dNTP mix 10 mM each (final conc. 200 µM)	0.5
Primer forward (100 pmol μL^{-1}) (final conc. 0.5 µM)	0.125
Primer reverse (100 pmol μL^{-1}) (final conc. 0.5 µM)	0.125
Taq DNA Polymerase (5 U μL^{-1}) (final conc. 0.03 U μL^{-1})	0.15
Template DNA	2.0
Total	25

Table 10: PCR cycling program.

Cycles	Steps	temperature [°C]	time [s]
1x	Initial denaturation	95	30
32x	Denaturation	95	45
	Annealing	T_m-dependent*	60
	Elongation	72	150
1x	Final elongation	72	300

*see Table 8: PCR DNA primers.

2.4 Proteomic analysis

2.4.1 MALDI-TOF MS analysis

MALDI-TOF MS (Matrix-assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry) is a spectrometric technique that allows the identification of species-specific small proteins and peptide fragments by comparing their specific masses with protein databases. In the context of this thesis, a Microflex LT MALDI-TOF mass spectrometer (Microflex LT, Bruker Daltonik GmbH, Bremen, Germany) was used to verify all used strains on species level and to perform proteomic comparisons within selected strains, using previously created strain-specific mass spectra.

2.4.1.1 Target preparation for MALDI-TOF MS analysis

According to 2.2.2, strains were grown in liquid culture or on mMRS-agar plates for generation of strain-specific mass spectra and for species verification, respectively.

To verify strains, colonies were picked from plates and spotted onto a MALDI stainless steel target plate (Bruker Daltonik GmbH, Bremen, Germany). Following, spots were overlaid with 1 µL matrix solution (10 mg mL^{-1} alpha-cyano-4-hydroxy-cinnamic acid in ACN, DH2O and TFA 50:47.5:2.5, v/v), the samples air-dried and mass spectra measurements accomplished.

To create strain-specific mass spectra, cell cultures (1 mL each) were harvested by centrifugation (13.000 rpm, 2 min), supernatant was disposed and bacterial cells were inactivated by resuspension in ethanol (70%). Proteins were extracted according to the plain cell extraction protocol of Kern *et al*. (2013). Therefore, cells were harvested by centrifugation (13.000 rpm, 2 min), supernatant was disposed and proteins were extracted using formic acid (FA, 70%), DH2O and ACN (35:15:50, v/v). Samples were centrifuged to spin down cell debris and 1 µL of the supernatant was transferred onto a stainless-steel target plate and overlaid with 1 µL matrix solution (10 mg mL^{-1} alpha-cyano-4-hydroxy-cinnamic acid in ACN, DH2O and TFA 50:47.5:2.5, v/v).

All samples were spotted in octuplicates. After air-drying of all samples, mass spectra measurements were performed.

2.4.1.2 Protein mass spectra acquisition

Specific protein mass spectra were obtained in a mass range of 2 to 20 kDa, consisting of 240 accumulated laser shots. For external mass calibration, Bacterial Test Standard was used. Analyte ionization was generated by using a nitrogen laser (λ = 337 nm) at a frequency of 60 Hz, operating in the linear positive ion detection mode under Biotyper Automation Control 2.0 (Bruker Daltonik GmbH, Bremen, Germany) and FlexControl 3.4 (Bruker Daltonik GmbH, Bremen, Germany).

2.4.1.3 Data processing

Identification of bacterial strains

Identification of all strains was carried out by matching all recorded mass spectra to microbial reference spectra by MALDI BioTyper 3.0 Software (Bruker Daltonik GmbH, Bremen, Germany). The reference database, consisting of initial 4.111 microbial reference spectra provided by the manufacturer, was supplemented with other reference entries of bacteria by TMW. Based on (I) the correlation of intensities of the matching peaks, (II) the similarity between the spectrum of the unknown microorganism and the reference spectrum, and (III) the similarity between the reference spectrum and the spectrum of the unknown microorganism, the reliability of identification was output as a log-score between 0 and 3. A log-score > 2.0 indicated a successful identification of a sample on species level and a log-score between 1.7 and 2.0 was interpreted as successful identification on genus level.

Comparative analysis of protein mass spectra

For comparative analysis of the recorded protein mass spectra from the different strains of *L. plantarum*, *L. sakei* and *L. fructivorans*, MALDI-TOF MS raw data were exported using FlexAnalysis 3.4 (Bruker Daltonik GmbH, Bremen, Germany). Based on an open sharedroot computer cluster (ATIX; http://opensharedroot.org), using a Mass Spectrometry Comparative Analysis Package (MASCAP) (Mantini *et al.*, 2010), which was implemented in octave software (Eaton & Rawlings, 2003, Eaton *et al.*, 2009), all exported protein mass spectra of each strain were pre-processed according to Usbeck *et al.* (2013) by inter alia smoothing, baseline subtraction and normalizing signal intensities.

According to Lauterbach *et al.* (2017), all single spectra of each strain were summarized to one consensus spectrum and by using the inhouse software, which is based on high-throughput multidimensional scaling (HiT-MDS) (http://dig.ipk-gatersleben.de/hitmds/hitmds.html), similarities of all consensus spectra were calculated and visualized in a Euclidean 2D plane.

2.5 Preparation and characterization of (O/W)-emulsion

In general, emulsifier-stabilized (O/W)-emulsions were prepared by adding appropriate amounts of emulsifier to oil and stirring overnight. Next, specific volumes of IPB were added to desired volumes of oil and the mixture was mixed at 23.000 rpm for 1 min by high-performance dispersing machine (IKA®-Werke GmbH & CO. KG, Staufen, Germany), followed by one- or two-stage pressure homogenization (APV Model 1000 Homogenizer, APV Systems, Denmark). As shown in Table 11, for preparation of different emulsion types, the oil and emulsifier type, their concentrations and homogenization pressure were varied.

Table 11: Used parameters for the preparation of different emulsifier-stabilized (O/W)-emulsions.

(O/W)-emulsion type	Oil	Emulsifier	Homogenization pressure	
			1. step (bar)	2. step (bar)
Small fat surface	50% (v/v) rapeseed oil	0.25% (w/v) Tween® 80	50	-
Medium fat surface (standard)	50% (v/v) rapeseed oil	1% (w/v) Tween® 80	100	20
Large fat surface	50% (v/v) rapeseed oil	2% (w/v) Tween® 80	400 (2x)	80 (2x)
Low fat	30% (v/v) rapeseed oil	1% (w/v) Tween® 80	70	-
High fat	70% (v/v) rapeseed oil	1% (w/v) Tween® 80	200 (2x)	40 (2x)
Different emulsifier type	50% (v/v) rapeseed oil	2% (w/v) sodium caseinate	100	20
Different oil type	50% (v/v) Miglyol® 812	1% (w/v) Tween® 80	70	-

Emulsifier-free (O/W)-emulsions were prepared by mixing 30 or 50% (v/v) rapeseed oil with heated (~80 °C) 70 or 50% (v/v) IPB-containing 2% (w/v) agar, respectively, mixed at 23.000 rpm for 1 min by high-performance dispersing machine, followed by two-stage pressure homogenization at 400 and 80 bar.

2.5.1 Characterization of (O/W)-emulsions prior and after HHP treatment

To examine the HHP-stability of all established (O/W)-emulsions, the droplet size and fat surface of untreated and HHP-treated (O/W)-emulsions were determined and subsequently compared. Emulsion characterization was performed by the laser particle analyzer Mastersizer 2000 (Malvern Instruments Ltd, Malvern, UK). There, the light of a focused laser beam is scattered by dispassing sample particles in particle size-dependent angles. Based on the diffraction angles and light intensities, particle sizes are calculated.

A refractive index of 1.472 nD for rapeseed oil and of 1.449 nD for Miglyol® 812, determined by a refractometer (Carl Zeiss Microscopy GmbH, Munich, Germany) at 25 °C, were used for calculations. The Mastersizer 2000 software converts the determined datasets into volume mean diameters (D(4,3), D(3,2)), Specific Surface Area (SSA) and particle-size distributions and calculates associated statistical parameters (D(v,0.1), D(v,0.5), D(v,0.9)).

All samples were HHP-treated according to 2.7 at 500 MPa/25 °C and 500 MPa/55 °C with a holding time of 5 min. Sample quantities used for measurements varied dependent on (O/W)-emulsion properties and were adjusted to gain an obscuration between 10 and 18%.

2.5.2 Characterization of emulsion stability by multisample analytical centrifugation

Phase separation stability of all used (O/W)-emulsions was investigated by multisample analytical centrifugation (LUM GmbH, Berlin, Germany). With this technique, the process of phase separation is accelerated by centrifugation and at the same time photometrically observed. The intensity of transmitted light is recorded during centrifugation as a function of phase separation position and time. The software SepView 6 was used to record the respective transmission profiles. The phase separation speed (µm s^{-1}) of all emulsion samples was analyzed by using a phase separation threshold of 15%, a measuring time frame from 1 to 700 s and a measuring range from ~190 to ~290 mm.

Samples of ~400 µL (O/W)-emulsion were applied to LUMiFuge cuvettes, which were then fixed in the analyzer. During centrifugation (2.474 rpm, 25 °C, 700 s), phase separation kinetics were acquired by measuring the transmission every 10 s.

2.6 Cell localization in emulsifier-stabilized and emulsifier-free (O/W)-emulsion

Localization of bacterial cells was examined, using an Axiostar plus microscope (Carl Zeiss Microscopy GmbH, Munich, Germany) equipped with phase-contrast optics and an epifluorescence unit. If sufficient, localization of cells was investigated by bright field microscopy. If necessary, cells were stained with SYTO® 9, a green fluorescent dye labeling all bacteria, to visualize them in oil/water mixture and (O/W)-emulsion. In these cases, epifluorescence light with the appropriate filters was used (Excitation BP 450-490 nm, Emission LP 515 nm).

Sample preparation and microscopic examination

1 mL of sample was mixed with 3 µL of SYTO® 9 and stored for 15 min in the absence of light. 3 µL of stained cell suspension were spread on a glass coverslip, which was then placed onto a glass slide. 10x, 40x and 100x objective lenses were used, giving a total magnification of 100-, 400- and 1000-fold, respectively. The 100x objective lens was used with immersion oil. Images were captured with a 1.388- by 1.038-pixel RGB camera (AxioCam ICc1, Carl Zeiss Microscopy GmbH, Munich, Germany) and processed using the software AxioVS40 V 4.8.2.0.

2.7 HHP processing

The inactivation studies as well as the adiabatic heating studies were performed in IPB. The buffer mixture IPB, consisting of the cationic buffer imidazole and phosphate buffered saline, was shown to be less pressure sensitive and thus, possible pressure-induced pH changes during HHP treatment can be minimized (Quinlan & Reinhart, 2005).

2.7.1 Inactivation studies

2.7.1.1 HHP equipment and HHP treatment

The samples were pressurized in two parallel linked 7 mL pressure vessels of the high pressure unit TMW-RB (Lenz & Vogel, 2014, Lenz *et al.*, 2015) equipped with thermostating jackets regulated by the recirculating thermostat FC 600. A mixture of 30% DH2O and 70% polyethylene glycol 400 was used as pressure-transmitting fluid. Compression and decompression rates were kept constant at 200 MPa min^{-1}. The treatment parameters pressure level, temperature and pressure holding time varied within the experiments and are therefore described in the corresponding results section.

Samples were prepared as described below, avoiding the inclusion of air as much as possible. Then, samples were placed into the preheated pressure vessels approximately 5 min before the start of the pressure treatment. Reference samples (no HHP treatment) were simultaneously incubated at the same temperature as used for the HHP treatment. After decompression, the tubes were removed from the unit and microbiological analysis was performed (see 2.2.3.2).

2.7.1.2 Sample preparation for individual treatments

For the examination of the effect of various lipid phase parameters on the HHP inactivation of *L. plantarum,* different experimental procedures were used, which are described below.

Emulsifier-stabilized (O/W)-emulsions

Different emulsifier-stabilized (O/W)-emulsions were used for studying the effect of emulsifier type, oil type, fat content and fat surface (droplet size). Therefore, strains were harvested in stationary growth phase (24 h) and washed with 10 mL of IPB (5.000 × *g*, 5 min, 25 °C). Following, the cell pellets were resuspended in the same volume of IPB, cell suspensions adjusted to cell concentrations of ~10^7 colony forming units (cfu) mL^{-1} using IPB and an appropriate (O/W)-emulsion (see 2.5), and samples dispensed in volumes of 0.6 mL in cryo tube vials with an internal thread.

Emulsifier-free (O/W)-emulsions

For the investigation of the effect of fat, based on interactions between bacterial cells and fat surface, emulsifier-free (O/W)-emulsions stabilized by agar were used. Therefore, harvested and washed cells (5.000 × *g*, 5 min, 25 °C) were diluted with IPB to 10-fold the desired concentration, cell suspensions adjusted to cell concentrations of ~10^8 cfu mL^{-1} using IPB (2% (w/v) agar) and an appropriate emulsifier-free (O/W)-emulsion, and samples (1.6 mL) subsequently filled into 2.0 mL reaction tubes.

2.7.2 Temperature control

Due to the strongly different adiabatic heating properties of fat and water, it was essential to design HHP processes in a way that enables the assessment of the effect of the fat content on microbial inactivation rather than that of different temperatures during HHP processing. Careful experimental design also facilitates the comparability of the results obtained in this thesis with results generated using different HHP equipment (Lenz *et al.*, 2015).

Although it was not fully possible to eliminate any temperature fluctuations, the mean process temperature over the entire holding time was held relatively constant regardless of the amount of fat in the model system sample. Adiabatic effects occurring in the HHP unit TMW-RB used for the inactivation experiments were compared with the adiabatic heating properties of the prepared emulsions under nearly ideal adiabatic conditions.

2.7.2.1 Temperature profiles under nearly ideal adiabatic conditions

Data acquisition

To be able to compare adiabatic effects occurring in the HHP unit TMW-RB used for the inactivation experiments with the adiabatic heating properties of the prepared emulsions under nearly ideal adiabatic conditions, temperature profiles were recorded.

For this purpose, an 8 mL pressure unit (U111, Unipress, Warszawa, Poland) with a thermostating jacket connected to a recirculating thermostat (WCR-P22, ©WITEG Labortechnik GmbH, Wertheim, Germany) was used. Bis(2-ethylhexyl) sebacate (Alfa Aesar GmbH & Co. KG, Karlsruhe, Germany) was used as pressure-transmitting fluid. Samples, including buffer (IPB), the different (O/W)-emulsion systems and pure oil were filled in a custom thin-walled PTFE tube and pressurized to 600 MPa. Sample temperature was monitored in the geometrical centre of a sample vial, using a thin type K thermocouple (U111, Unipress, Warszawa, Poland) connected (thermocouple cable and connectors, TC Direct, Mönchengladbach, Germany) to a data acquisition module (OMB-DAQ-55, OMEGA Engineering GmbH, Deckenpfronn, Germany). After equilibration of the sample temperature at either 25 or 40 °C, pressure was rapidly released with an average rate of > 100 MPa s^{-1} (data quantity can be enhanced using lower rates at around 20 MPa s^{-1}; too low rates decrease data accuracy due to an increasing influence of heat transfer between sample and environment). Temperature data was measured at a rate of 9 Hz and recorded at a rate of 3 Hz (three data points averaged) using a routine that was created with LabVIEW (National Instruments Germany GmbH, Munich, Germany). Experiments were performed in independent duplicate for each sample and equilibration temperature.

Data processing

The experimental setup allowed for minimizing, but did not completely exclude heat transfer inside the pressure vessel. To be able to consider this influence factor and the small, but inherent time lag between pressure and temperature measurements, control experiments using pure water or pure oil were performed, varying the position of the thermocouple inside and outside of the sample vail (centred, off-centred, inner and outer vial wall).

Heat transfer between the sample centre and the pressure-transmitting fluid did not play a significant role at the given pressure release rate. A MATLAB software routine (MATLAB® 2016, MathWorks Inc., Natick, USA) was used to increase the amount of data points per experiment by sub-dividing temperature curves in 10 MPa intervals, i.e., reading vectors and calculating missing data points. Data points obtained for 5 independent experiments with pure water were fitted (least-square fit; using TableCurve 2D software (Systat Software GmbH, Erkrath, Germany)), yielding 4th order polynomial equations with $p > 0.99$. A comparison of the obtained control data with available standard data for pure water (NIST, 2002) yielded another 4th order polynomial function (TableCurve 2D) for each equilibration temperature, which could be used to adjust raw data. These data, as depicted in the results section, were used to compare heating effects that can occur during pressurization under (almost ideal) adiabatic conditions of the emulsion systems used in this thesis.

2.7.2.2 Temperature control during inactivation experiments

Data acquisition

Adiabatic heating effects occurring during inactivation experiments were determined using the HHP unit TMW-RB (Lenz & Vogel, 2014, Lenz *et al.*, 2015). Pure water, buffer, liquid and solid (O/W)-emulsion model systems with different fat contents were filled into the same type of tubes as used during inactivation experiments. Since a connection of the thermocouple through the lid was not possible, one sample tube at a time was placed upside down in the center of one of the two parallel linked 7 mL pressure vessels of the HHP unit TMW-RB and a thin type K thermocouple (U111, Unipress, Warszawa, Poland) connected (thermocouple cable and connectors, TC Direct, Mönchengladbach, Germany) to a data acquisition module (OMB-DAQ-55, OMEGA Engineering GmbH, Deckenpfronn, Germany) was introduced trough the vessel bottom and used to monitor adiabatic heating in the geometrical center of a sample vial. Similar to the inactivation experiments, a mixture of 70% polyethylene glycol 400 (Roth, Karlsruhe, Germany) and 30% DH2O was used as pressure-transmitting fluid. Vessel temperature was controlled via thermostating jackets connected to a recirculating thermostat (FC 600, JULABO Labortechnik GmbH, Germany) with water as heating/cooling fluid. Temperatures were either set to 25 or 40 °C, target pressure levels were 300 and 400 MPa held for 5 min and compression and decompression rates were kept constant at 200 MPa min^{-1}. Adiabatic heating of the samples was monitored in the geometrical center of a sample vial using a thin type K thermocouple (U111, Unipress, Warszawa, Poland) connected (thermocouple cable and connectors; TC Direct, Mönchengladbach, Germany) to a data acquisition module (OMB-DAQ-55; OMEGA Engineering GmbH, Deckenpfronn, Germany).

Temperature data was measured at a rate of 9 Hz and recorded at a rate of 3 Hz (three data points averaged) using a routine that was created with LabVIEW (National Instruments Germany GmbH, Munich, Germany). Experiments were performed in independent duplicate for each sample and equilibration temperature.

Data processing

A MATLAB software routine (MATLAB® 2016; MathWorks Inc., Natick, MA, USA) was used to increase the amount of data points per experiment by sub-dividing temperature curves in 10 MPa intervals, i.e., reading vectors and calculating missing data points. Since the components and software for temperature and pressure measurement were identical to those used for the determination of adiabatic heating effects under nearly ideal adiabatic conditions, the correction function for the small-time lag between pressure and temperature measurements derived from those experiments was used to adjust raw data that have been obtained. Due to the drastically slower pressure build-up and release, this data processing had only a marginal effect. Since the actual temperature peaks occurring during inactivation experiments should be determined, no further data processing was necessary.

2.8 Genome analysis

Isolated, high molecular weight DNA (2.3.1) was sent to GATC Biotech (Konstanz, Germany) for Single Molecule Real Time (SMRT) sequencing (Eid *et al.*, 2009, McCarthy, 2010). The assemblage of the raw data was performed, using two Hierarchical Genome Assembly Process (HGAP2/3) protocols of the SMRT Analysis software (v 2.2.0 p2, Pacific Biosciences, Menlo Park, USA). Manual curation of assemblies was executed as described by PacBio online (https://github.com/PacificBiosciences/Bioinformatics-Training/wiki/Finishing-Bacterial-Genomes). The obtained assembly, using the *RS_HGAP_Assembly_3* protocol, was stated as polished assembly (fasta). The assembly resulting from the *RS_HGAP_Assembly_2* protocol was stated as draft assembly and served as control for the polished assembly. In addition, BridgeMapper (RS_BridgeMapper), a protocol implemented in SMRT Analysis, was run to check for a correct genome assembly.

By the implementation of BioPerl (http://www.bioperl.org) and the Bio::SeqIO system, the polished assembly was split into contigs. NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to test the contigs for redundancy (Altschul *et al.*, 1990, Camacho *et al.*, 2009) and the dot plot tool of Gepard 1.40 (Computational Systems Biology, University of Vienna, Austria) was used to check for overlapping ends (Krumsiek *et al.*, 2007).

Additionally, using SMRT-View 2.30 (Pacific Biosciences, Menlo Park, USA), the overlapping ends were examined for conspicuous coverage behaviour and were checked focusing on quality of mapping, since a decrease also indicated overlapping ends. Next, contigs with overlapping ends were circularised while contigs, being covered by another contig (non-sense) or redundant, were discarded. Thereby, circularisation of contigs was achieved by the introduction of an *in silico* break into the contig, followed by the circularisation itself using Minimus2 (AMOS, http://amos.sourceforge.net). Following, proper circularisation of the resulting contigs was ensured using Gepard 1.40.

In order to confirm that 100% of the initial sequence information was retained, all circularised contigs were tested using Gepard 1.40 and NCBI BLAST versus the original contigs. Subsequently, all circularised contigs as well as those for which circularisation was not possible, were merged and provided as a reference in the resequencing job by SMRT Analysis using *RS-Resequencing_1* protocol. Resequencing was repeated until an average reference consensus accordance of 100% was accomplished. The consensus sequence of the genome from the last resequencing job was downloaded and stored as fasta file. This genome fasta file was used as input for subsequent genome analysis applications, annotation and submission.

Genome annotation was achieved by submitting the genome to NCBI Prokaryotic Genome Annotation Pipeline for annotation (Angiuoli *et al.*, 2008). Submission was done as described online in detail (http://www.ncbi.nlm.nih.gov/genbank/genomesubmit). Therefore, a bioproject (PRJNA343197) was created and biosamples (SAMN05805044, SAMN05805045, SAMN05805046, SAMN05805047) for the sequenced genomes were added.

For the identification of bacterial group specific genes, the BlAst Diagnostic Gene findEr (BADGE) with standard settings was used according to Behr *et al*. (2016). Identified group specific genes as well as the WTA synthesis pathway gene equipment of all completely sequenced strains used in this thesis were checked on nucleotide and amino acid level, using nucleotide BLAST and protein BLAST with standard algorithm parameters (BLASTN / BLASTP, NCBI, (Zhang *et al.*, 2000)). Gene sequences of identified genes of interest were aligned and primers derived from conserved regions, using Clone Manager 5 (Scientific & Educational Software, Denver, USA). Primers were used for screening of bacterial strains for possible marker genes by colony PCR (2.3.3). Primer sequences and respective annealing temperatures are shown in Table 8.

2.9 Statistical analysis

To determine differences in HHP-mediated inactivation level, statistical analysis were done using Sigma Plot (Jarvis, 2016) and applying statistical tests depending on the number of groups to be compared, the normality of data and their equality of variance. The normal distribution of data and their equal variance were checked using Shapiro-Wilk and Spearman rank correlation, respectively. For the comparison of two groups consisting of normally distributed data with equal variance, the Student´s t-test was used.

For not normally distributed data or data without equal variance, the non-parametric Mann-Whitney rank sum test was used. The comparison of more than two groups was performed by analysis of variance (ANOVA) and the post-hoc Tukey test for paired comparison where ANOVA values were significant. For not normally distributed data or data without equal variance, Kruskal-Wallis one-way ANOVA on ranks was used. Groups were considered significantly different with a one-tailed p-value < 0.05.

2.10 Software

Table 12: List of software used in this thesis.

MALDI Biotyper 3.0	© Bruker Daltonik GmbH
Maldi Biotyper Automation Control 2.2	© Bruker Daltonik GmbH
flexAnalysis 3.4	© Bruker Daltonik GmbH
flexControl 3.4	© Bruker Daltonik GmbH
GIMP 2.8.10	© Spencer Kimball, Peter Mattis and the GIMP Development Team
Endnote X7.7.1	© Thomson Reuters
SigmaPlot 12.5	© Systat Software, Inc.
Adobe Acrobat Reader DC 2015	© Adobe Systems Incorporated
Mastersizer 2000 v5.60	© Malvern Instruments GmbH
AxioVS40 V 4.8.2.0	© Carl Zeiss Microscopy GmbH
ImageJ 1.50i	© Wayne Rasband, National Institutes of Health, USA
SepView® 6	© LUM GmbH
TableCurve 2D	© Systat Software GmbH
MATLAB® 2016	© MathWorks Inc.
LabVIEW	© National Instruments Germany GmbH
SMRT Analysis V 2.2.0 p2	© Pacific Biosciences of California, Inc.
SMRT-View 2.30	© Pacific Biosciences of California, Inc.
Gepard 1.40	© Computational Systems Biology, University of Vienna
Clone Manager 5	© 2016 Scientific & Educational Software

3 Results

In this thesis, above all the effect of fat on the HHP inactivation of vegetative cells was examined by performing systematic investigations with spoilage-associated *Lactobacillus* species and by using defined (O/W)-emulsions. The results of this chapter partly overlap with the results of the PhD thesis of Dominik Reitermayer, Technische Universität München (not published yet), as a result of a joint basic study diversifying into different theses.

3.1 (O/W)-emulsions as model food system

For the investigation of the effect of fat on bacterial HHP inactivation, defined (O/W)-emulsions were used as model system. For this, systematic studies with emulsions varying in lipid phase parameters were performed. On the one hand, lipid phase parameters that affect the availability of fat for bacteria were varied. These parameters were fat content (low, medium, high), emulsion-stabilizing additives (Tween® 80, sodium caseinate, agar) and fat surface (small, medium, large). On the other hand, the effect of different oil types, mainly focusing on distinct differences in fatty acid composition (saturated *versus* unsaturated), was examined. The variation of the emulsifier type, fat surface and oil type was performed under constant fat content of 50% (v/v) oil, enabling the investigation of solely the effect of the desired parameter. Besides this, to avoid the appearance of unwanted side effects due to different oil droplet sizes or fat surfaces, process parameters for the preparation of all (O/W)-emulsions were adjusted which guarantees, if not otherwise wanted, comparable median droplet sizes and thus fat surfaces.

3.1.1 Characterization of (O/W)-emulsions

The (O/W)-emulsions with varying lipid phase parameters were prepared according to chapter 2.5 and characterized with respect to volume median diameter (D(v,0.5)) (Figure 3) and the associated fat surface (Specific Surface Area (SSA)) (Figure 4) using the laser particle analyzer Mastersizer 2000. There, the characterization of the droplet size was consciously restricted to the statistical parameter D(v,0.5), which divides the population exactly into two equal halves, i.e., it reflects the diameter where 50% of the distribution is above and 50% is below and thus embodies the median droplet size. In contrast to the statistical parameters D(4,3), D(3,2), D(v,0.9) and D(v,0.1), this parameter seemed to be most suitable for the conduction of meaningful comparisons between all emulsion types.

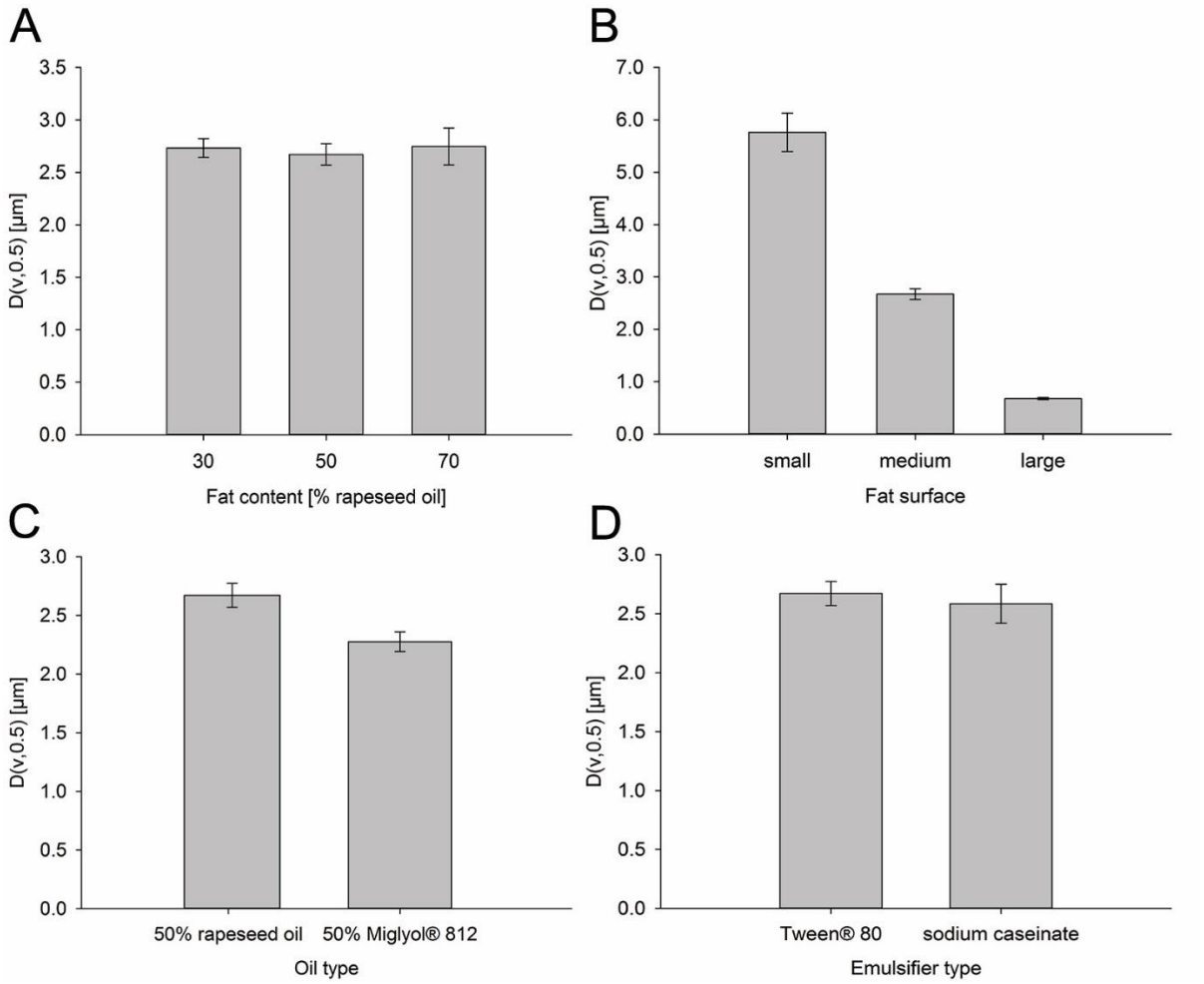

Figure 3**:** Characterization of different (O/W)-emulsion types with respect to their median droplet size. (O/W)-emulsions varying in fat content (A), fat surface (B), oil type (C) and emulsifier type (D). D(v,0.5), volume median diameter, represents median droplet size. Data presented are the mean values from at least three independent experiments and error bars correspond to standard deviations.

Both figures illustrate that the (O/W)-emulsions, which vary in fat content, oil and emulsifier types, had similar median droplet sizes (~2.6 µm) and fat surfaces (~3.7 m^2 g^{-1}), taking small measurement deviations into account. For the (O/W)-emulsions, intended to enable the investigation of a possible effect of the fat surface and thus, direct fat availability on bacterial HHP inactivation, fat surfaces of ~1.5, ~3.7 and ~10.6 m^2 g^{-1} could be set, providing a good basis for HHP studies (Appendix Table 26). Based on these results, unwanted side effects due to unintended significant differences in median droplet size and fat surface could be neglected.

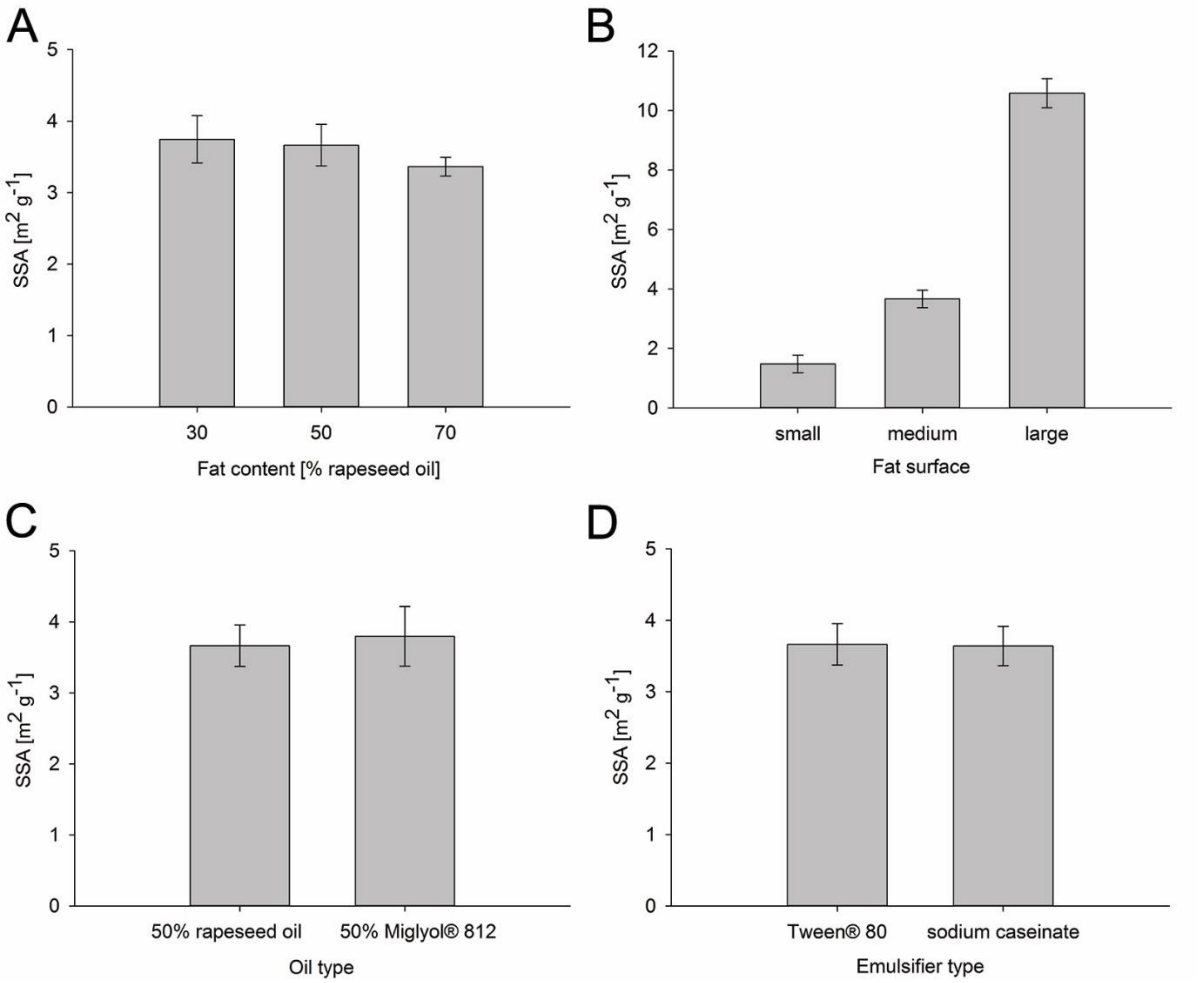

Figure 4: Characterization of different (O/W)-emulsion types with respect to their fat surface. (O/W)-emulsions varying in fat content (A), fat surface (B), oil type (C) and emulsifier type (D). SSA represents Specific Surface Area. Data presented are the mean values from at least three independent experiments and error bars correspond to standard deviations.

3.1.2 HHP stability of (O/W)-emulsions

To ensure that all (O/W)-emulsions are stable enough to serve as matrices for subsequent HHP studies, they were characterized with respect to median droplet size, fat surface and creaming velocity prior to and after HHP treatment (500 MPa/25 °C; 500 MPa/55 °C). Determination of particle size distribution was performed as described before, creaming velocity was investigated by multisample analytical centrifugation. Therefore, integral creaming kinetics of all emulsions were recorded during centrifugation (2.474 rpm, 25 °C, 700 s) and phase separation speeds were calculated (phase separation threshold: 15%; measuring time frame: 1 to 700 s; measuring range: ~190 to ~290 mm). Figure 5 summarizes the influence of pressurization on median droplet size (A) and fat surface (B) and Figure 6 on creaming velocities of all (O/W)-emulsions. Furthermore, all determined parameters are listed in detail in Table 26 (see Appendix).

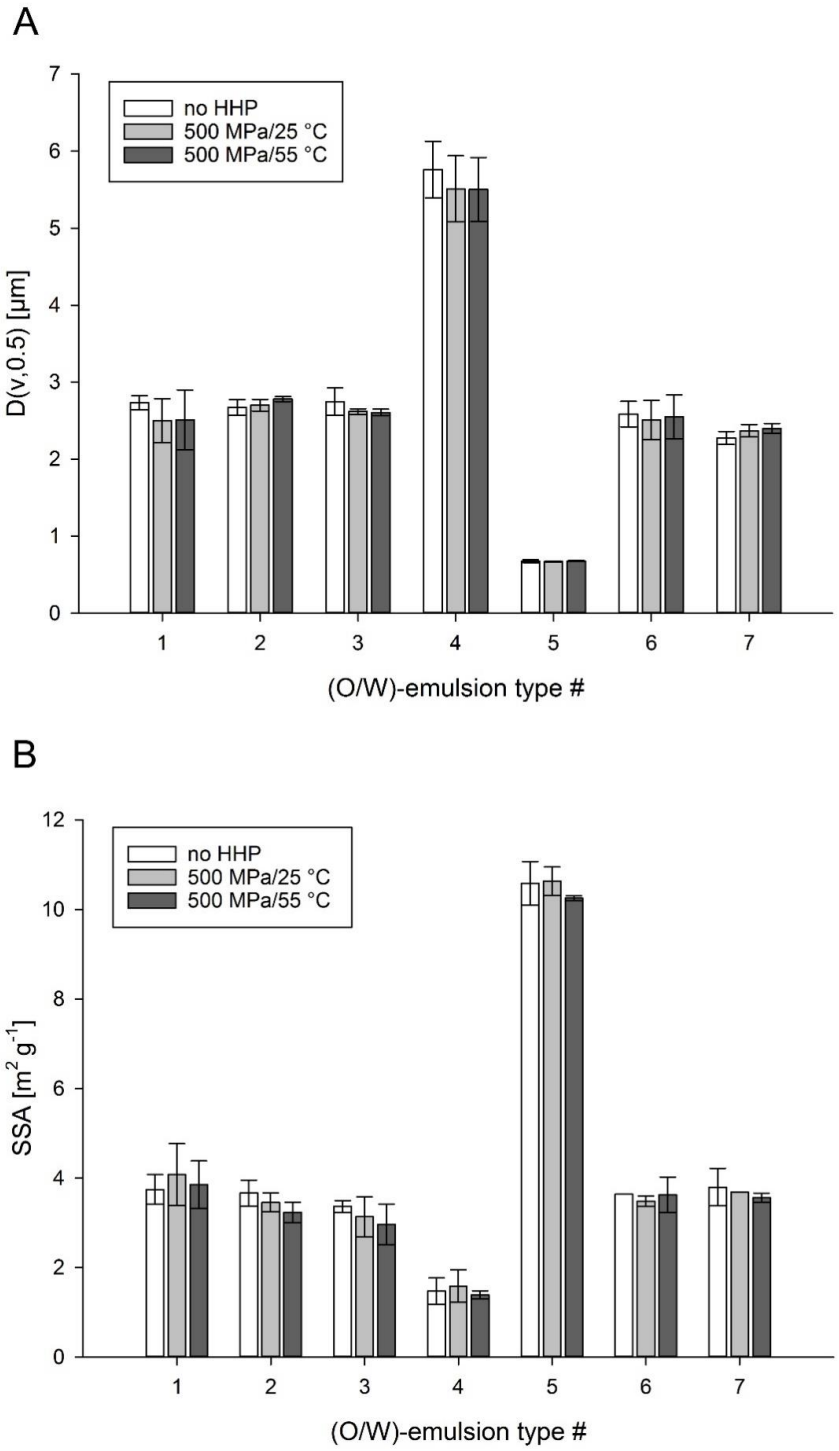

Figure 5: Median droplet size (A) and fat surface (B) of different (O/W)-emulsions prior to and after HHP treatment. (O/W)-emulsions were treated with different HPT-combinations and following their medium droplet size and fat surface were determined by a laser particle analyzer. (O/W)-emulsion type: #1, 30% rapeseed oil; #2, 50% rapeseed oil; #3, 70% rapeseed oil; #4, small SSA; #5, large SSA; #6, 50% rapeseed oil, 2% (w/v) sodium caseinate; #7, 50% Miglyol® 812. SSA represents Specific Surface Area. D(v,0.5), volume median diameter, represents median droplet size. Data presented are the mean values from at least three independent experiments and error bars correspond to standard deviations.

As Figure 5 illustrates, the behaviour of the tested emulsion properties of untreated and pressure-treated (O/W)-emulsions was quite similar and no significant effect of HHP treatment could be determined. All untreated (O/W)-emulsion contained particles with a median diameter comparable to the particles of the corresponding samples treated with 500 MPa at 25 and 55 °C. Similar to untreated (O/W)-emulsions, HHP-treated samples showed comparable fat surfaces with maximum deviation of ca. +9% (= +0.33 m^2 g^{-1}) for the emulsion with 30% (v/v) rapeseed oil (#1; 500 MPa/25 °C) and ca. -12% (= -0.40 m^2 g^{-1}) for the emulsion with 70% (v/v) rapeseed oil (#3, 500 MPa/55 °C).

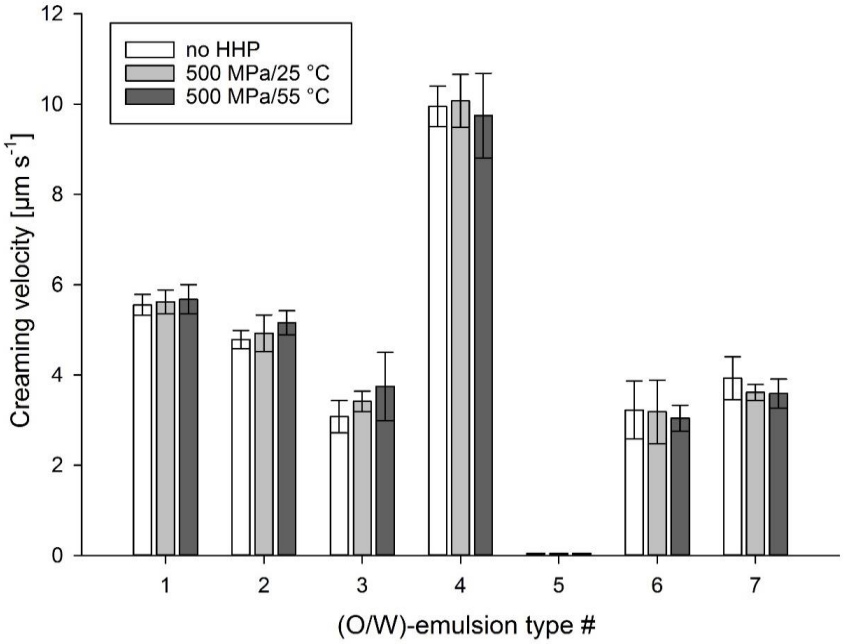

Figure 6: Creaming velocity of different (O/W)-emulsions prior to and after HHP treatment. (O/W)-emulsions were treated with different HPT-combinations and following their creaming velocities were determined by multisample analytical centrifugation. (O/W)-Emulsion type: #1, 30% rapeseed oil; #2, 50% rapeseed oil; #3, 70% rapeseed oil; #4, small SSA; #5, large SSA; #6, 50% rapeseed oil, 2% (w/v) sodium caseinate; #7, 50% Miglyol® 812. SSA represents Specific Surface Area. Data presented are the mean values from at least three independent experiments and error bars correspond to standard deviations.

In Figure 6, the creaming velocities of untreated and HHP-treated model emulsions are depicted. Obviously, the stability of all emulsions was noticeably influenced by the variation of the lipid phase parameters fat content (#1, #2, #3), fat surface (#2, #4, #5), emulsifier (#2, #6) and oil type (#2, #7), but not by the pressure treatment at the different HPT-combinations. Moreover, clear trends, showing decreasing creaming velocities (~5.6, ~4.8, ~3.1 µm s^{-1}) with higher fat content (#1, 30% (v/v); #2, 50% (v/v); #3, 70% (v/v)) as well as decreasing creaming velocities (~9.9, ~4.8, ~0.1 µm s^{-1}) with increasing fat surface (#4, 1.5 m^2 g^{-1}; #2, 3.7 m^2 g^{-1}; #5, 10.6 m^2 g^{-1}) could be observed.

The (O/W)-emulsion with Miglyol® 812 (#7, ~3.9 µm s^{-1}) appeared to be more stable by showing a lower creaming velocity than the emulsion with rapeseed oil (#2, ~4.8 µm s^{-1}) at comparable fat content. Furthermore, the replacement of the emulsifier Tween® 80 by sodium caseinate decreased creaming velocity from ~4.8 µm s^{-1} (#2) to 3.2 µm s^{-1} (#6), resulting in higher emulsion stability. Pressurization of all emulsion types led to no significant changes in creaming velocity. The maximum emulsion-destabilizing effect of pressure was determined for the (O/W)-emulsion type #3 (70% (v/v) rapeseed oil) by a change of creaming velocity from ~3.1 to ~3.4 µm s^{-1} (500 MPa/25 °C) or ~3.7 µm s^{-1} (500 MPa/55 °C), which makes a difference of up to +11% or +22%, respectively. However, considering the occurring measurement deviations (0.34 µm s^{-1}, 0.67 µm s^{-1}), the actual destabilizing effect of pressure is likely to be less pronounced.

The data presented in Figure 5 and Figure 6 demonstrate, that the impact of (O/W)-emulsion stability was clearly more affected by the varied lipid phase parameters than by HHP treatment. Due to the fact that pressure tended to induce just negligible changes in stability, all model emulsions were considered suitable for actual HHP studies.

3.2 Characterization and selection of bacterial strains

In order to retrieve detailed insights from systematic investigations, appropriate strains had to be selected. Suitable strains should vary in CSH, show a high spoilage potential and not have emulsion-destabilizing, that means no proteolytic and lipolytic activities as well as no capability to utilize Tween® 80. Therefore, 38 strains of the species *L. plantarum*, *L. sakei* and *L. fructivorans* were selected from the strain collection of the Chair of Technical Microbiology at TUM and used for strain characterization.

3.2.1 CSH of *L. plantarum*, *L. sakei* and *L. fructivorans*

CSH varies greatly among bacterial species and even among bacterial strains of one species. Assuming that CSH most likely determines the localization of a cell in (O/W)-emulsions as well as its affinity towards fat, which in turn might have an effect on the efficiency of HHP inactivation, strains with different surface characteristics should be considered.

In this thesis, CSH was determined by a modified version of the MATH test (Rosenberg *et al.*, 1980) which was the most suitable method by representing best the conditions predominant in (O/W)-emulsions.

Cells grown to their logarithmic phase showed different affinities towards n-hexadecane and were classified as highly hydrophobic (0 - 25%), moderately hydrophobic (26 - 50%), moderately hydrophilic (51 - 75%) and highly hydrophilic (76 - 100%), according to the percentage of absorbance of the aqueous phase after treatment relative to the initial absorbance (Table 13).

Table 13: Cell surface hydrophobicity of logarithmic phase cells of *Lactobacillus* species. Surface hydrophobicity was determined by MATH test.

Species	Highly hydrophobic (0 - 25%)*	Moderately hydrophobic (26 - 50%)*	Moderately hydrophilic (51 - 75%)*	Highly hydrophilic (76 - 100%)*
L. plantarum	TMW 1.25 (11%) TMW 1.277 (9%)	TMW 1.1789 (42%) TMW 1.1792 (42%) TMW 1.409 (44%) TMW 1.1623 (31%)	TMW 1.1728 (56%) TMW 1.1732 (52%) TMW 1.246 (54%) TMW 1.834 (75%) TMW 1.1594 (56%)	TMW 1.1 (98%) TMW 1.1308 (100%) TMW 1.1478 (91%) TMW 1.38 (88%) TMW 1.64 (96%) TMW 1.708 (76%) TMW 1.9 (89%)
L. sakei			TMW 1.151 (55%) TMW 1.161 (70%) TMW 1.704 (69%)	TMW 1.13 (100%) TMW 1.23 (98%) TMW 1.165 (91%) TMW 1.412 (98%) TMW 1.1189 (89%) TMW 1.1239 (91%) TMW 1.1322 (92%) TMW 1.1393 (98%) TMW 1.1396 (98%) TMW 1.1399 (90%) TMW 1.1407 (100%) TMW 1.1452 (86%) TMW 1.1474 (98%) TMW 1.1954 (86%)
L. fructivorans	TMW 1.59 (2%) TMW 1.452 (3%)		TMW 1.1856 (60%)	

*Percentage absorbance of the aqueous phase after treatment with n-hexadecane (0.4 mL volume) relative to initial absorbance.

It was found that the species *L. plantarum* shows the largest variance in CSH from highly hydrophobic (9%) to highly hydrophilic strains (100%), followed by the species *L. fructivorans* from highly hydrophobic (2%) to moderate hydrophilic strains (60%).

Consequently, strains of both species showed no species-specific CSH. In contrast, strains of the species *L. sakei* could only be identified as moderately (55%) and highly hydrophilic (100%) and thus it can be assumed that strains of the species *L. sakei* tend to generally possess hydrophilic cell surface characteristics.

3.2.2 Effect of growth phase on CSH

Since the growth phase was supposed to influence CSH, its effect on CSH was examined. Therefore, selected strains of each species that show relevant differences in surface characteristics, were additionally grown to stationary phase and analyzed by MATH test (Table 14).

Table 14: Cell surface hydrophobicity of *Lactobacillus* species in different growth phases. Strains of the species *L. plantarum*, *L. sakei* and *L. fructivorans* were grown to logarithmic and stationary phase und cell surface hydrophobicity was determined by MATH test.

	Cell surface hydrophobicity* (%)	
Bacterial strain	Log phase	Stationary phase
L. plantarum TMW 1.25	11±5.8	7±5.3
L. plantarum TMW 1.1	98±4.6	99±1.8
L. plantarum TMW 1.277	9±3.3	7±4.2
L. plantarum TMW 1.708	76±1.9	93±1.7
L. sakei TMW 1.704	69±8.7	70±3.9
L. sakei TMW 1.1239	91±7.6	98±2.8
L. sakei TMW 1.1474	98±3.8	100±4.8
L. fructivorans TMW 1.59	2±0.8	6±0.8
L. fructivorans TMW 1.1856	60±9.6	63±6.9

*Percentage absorbance of the aqueous phase after treatment with n-hexadecane (0.4 mL volume) relative to initial absorbance. Each value represents the mean of at least three independent determinations.

In general, an increase in strength of the strains' surface characteristics over cultivation time could be observed, i.e., in stationary phase hydrophobic strains showed enhanced and hydrophilic strains decreased adhesion to the organic phase (Table 14). On basis of these results, all inactivation experiments were performed using stationary phase cells, where differences in surface hydrophobicity were in principle more pronounced.

3.2.3 Biodiversity of *Lactobacillus* strains

Besides general cell surface components such as WTAs, LTAs, surface fibrils, fimbriae, oligosaccharides and S-layer, outer membrane proteins are suggested to be common bacterial CSH features (Joh *et al.*, 1999, McNab *et al.*, 1999, Van der Mei *et al.*, 2003, Tokuda & Matsuyama, 2004, Pizarro-Cerdá & Cossart, 2006, Xia *et al.*, 2010, Wang *et al.*, 2015). Assuming that outer membrane proteins, which strongly vary in amino acid composition and thus, three-dimensional conformation, significantly contribute to CSH, differences in CSH might correlate with the biodiversity on the proteomic level. In order to proof this hypothesis, MALDI-TOF-MS analysis was performed and strain-specific protein mass spectra were created. For the visualization of mass spectra similarities, dimensional reduction was done by high-throughput multidimensional scaling (HiT-MDS) and results are shown in a Euclidean 2D plane (Figure 7).

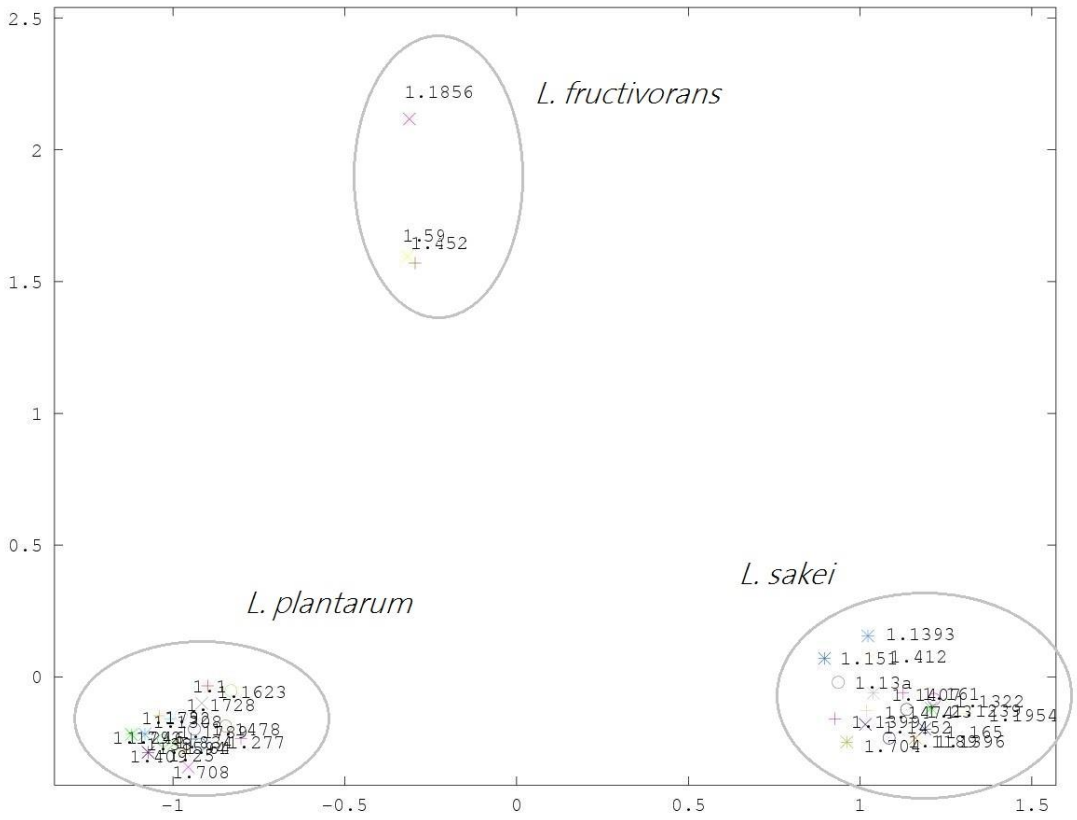

Figure 7: Multidimensional scaling (MDS) of *Lactobacillus* species based on specific protein mass spectra obtained by MALDI-TOF-MS.

Based on their species-specific protein mass spectra, a clear separation of all strains, resulting in three clusters, was observed.

Strains of the two species *L. plantarum* and *L. sakei* seem to differ slightly on proteomic level, while the protein mass spectra of the *L. fructivorans* strain TMW 1.1856 seems to vary noticeably from the protein mass spectra of *L. fructivorans* TMW 1.59 and TMW 1.452, which is shown by large distances in the 2D plane.

For the investigation of a potential correlation between the intraspecies biodiversity on proteomic level and CSH, results of the MATH test and of the species-specific HiT-MDS were combined. The results of HiT-MDS for *L. plantarum* and *L. sakei* are depicted in separate figures, showing each strain surrounded by a specific colored circle which depends on their prior determined CSH (Figure 8). As there were only three strains of *L. fructivorans*, no conclusion could be drawn from HiT-MDS (Appendix Figure 31). Although most of the strains of both species, *L. plantarum* and *L. sakei*, are positioned in the center of the 2D planes, strains with similar surface characteristics show obvious variances regarding their position and distance to each other (Figure 8(A/B)). Overall, no grouping of strains, which share similar surface hydrophobicities, could be determined in the 2D plane. Only the two *L. plantarum* strains TMW 1.25 and TMW 1.277, which both have a highly hydrophobic cell surface, share a similar position in the 2D plane. Based on these observations, no general correlation between surface hydrophobicity and biodiversity on the proteomic level could be determined.

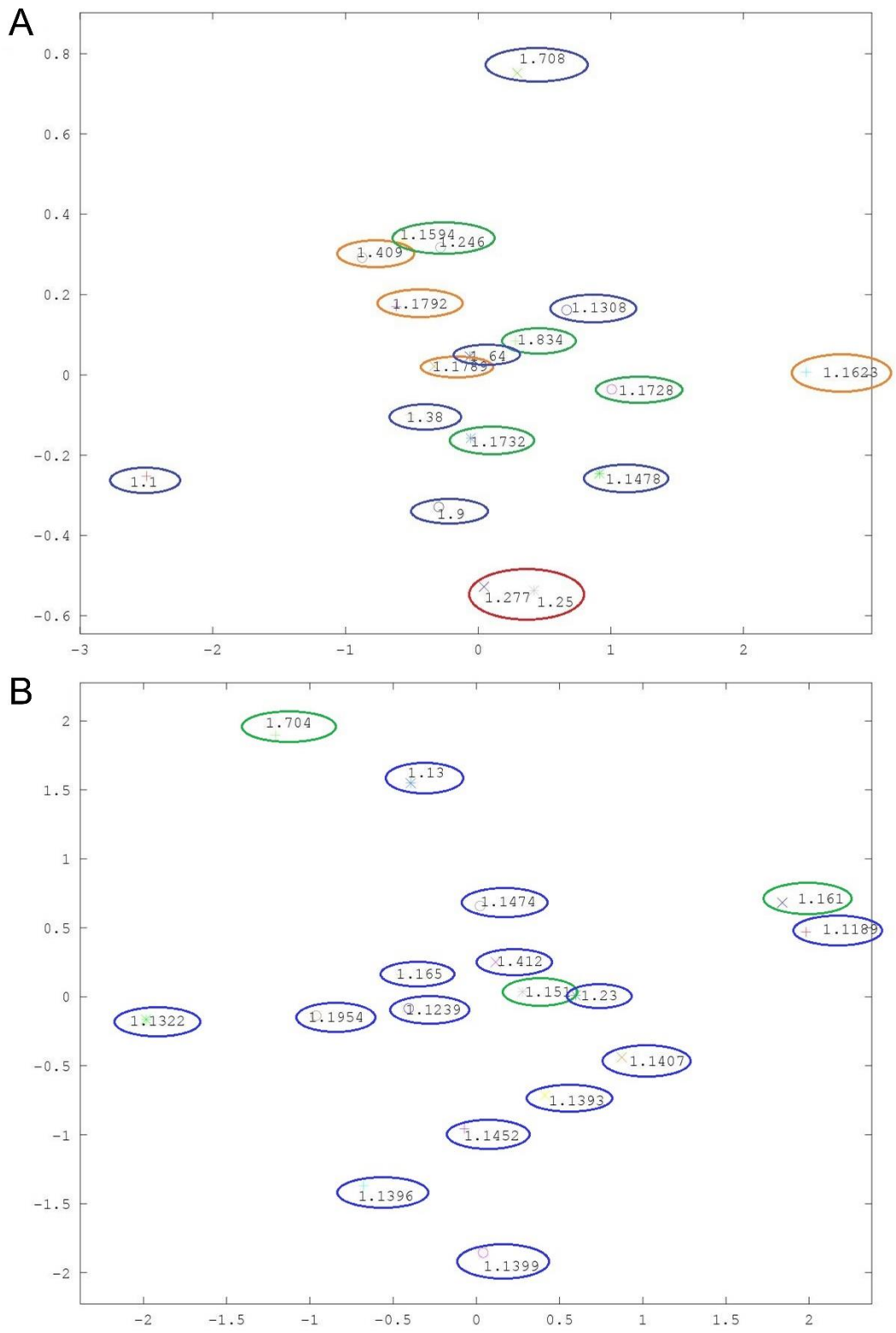

Figure 8: Multidimensional scaling (MDS) of *L. plantarum* (A) and *L. sakei* (B) based on protein mass spectra combined with cell surface hydrophobicity. Specific mass spectra were obtained by MALDI-TOF-MS based on eight replicates per strain. Surface hydrophobicity was determined by MATH test. Strains were classified as highly hydrophobic (0 - 25%), moderately hydrophobic (26 - 50%), moderately hydrophilic (51 - 75%) and highly hydrophilic (76 - 100%), depending on percentage absorbance of the aqueous phase after treatment with n-hexadecane (0.4 mL volume) relative to initial absorbance.

Based on cell surface characteristics, 2D plane position and isolation source, the initial strain collection was reduced from 38 to 14 strains (Table 15). There, a broad spectrum of CSH and differences in intraspecies biodiversity, illustrated by large variances in 2D plane position of strains, was considered.

Table 15: Preselection of *Lactobacillus* strains based on surface hydrophobicity, 2D plane position (MDS) and isolation source.

Species	Strain TMW	Cell surface hydrophobicity*	Position in 2D plane (MDS)	Isolation source
L. plantarum	1.277	Highly hydrophobic	bottom	Palm wine
	1.25	Highly hydrophobic	bottom	Raw sausage
	1.834	Moderately hydrophilic	center	ULICE, France
	1.1623	Moderately hydrophobic	right	Breast milk
	1.1478	Highly hydrophilic	marginal right	Honey (Belladonna)
	1.708	Highly hydrophilic	top	Raw sausage
	1.1	Highly hydrophilic	bottom left	Raw sausage
L. sakei	1.704	Moderately hydrophilic	top left	Sourdough
	1.151	Moderately hydrophilic	center	Unknown
	1.1239	Highly hydrophilic	central	Sourdough
	1.1474	Highly hydrophilic	marginal top	Unknown
	1.1322	Highly hydrophilic	left	Unknown
L. fructivorans	1.59	Highly hydrophobic	top left	Unknown
	1.1856	Moderately hydrophilic	center right	Whey

*Cell surface hydrophobicity defined in this study according to percentage absorbance of the aqueous phase after treatment with n-hexadecane (0.4 mL volume) relative to initial absorbance. Highly hydrophobic (0 - 25%), moderately hydrophobic (26 - 50%), moderately hydrophilic (51 - 75%) and highly hydrophilic (76 - 100%).

3.2.4 Emulsion destabilization potential of *Lactobacillus* species

In order to ensure that the strains used for HHP inactivation studies do not have the ability to destabilize or destroy (O/W)-emulsions by degradation or metabolization of essential constituents, they were checked for lipolytic and proteolytic activity and for their capability to utilize Tween® 80.

3.2.4.1 Lipolytic activity

To avoid destabilization of (O/W)-emulsions by bacterial lipoyltic activities, selected strains were grown on TB-mMRS agar plates and colonies were checked for surrounding halos, indicating bacterial production of lipases/esterases. Exemplary pictures, representing each examined species, are depicted in Figure 9. The pictures of the remaining 11 strains can be viewed in the Appendix (see Figure 32). *B. subtilis* TMW 2.472 is known for possessing lipolytic activities and was used as positive control (Figure 9D).

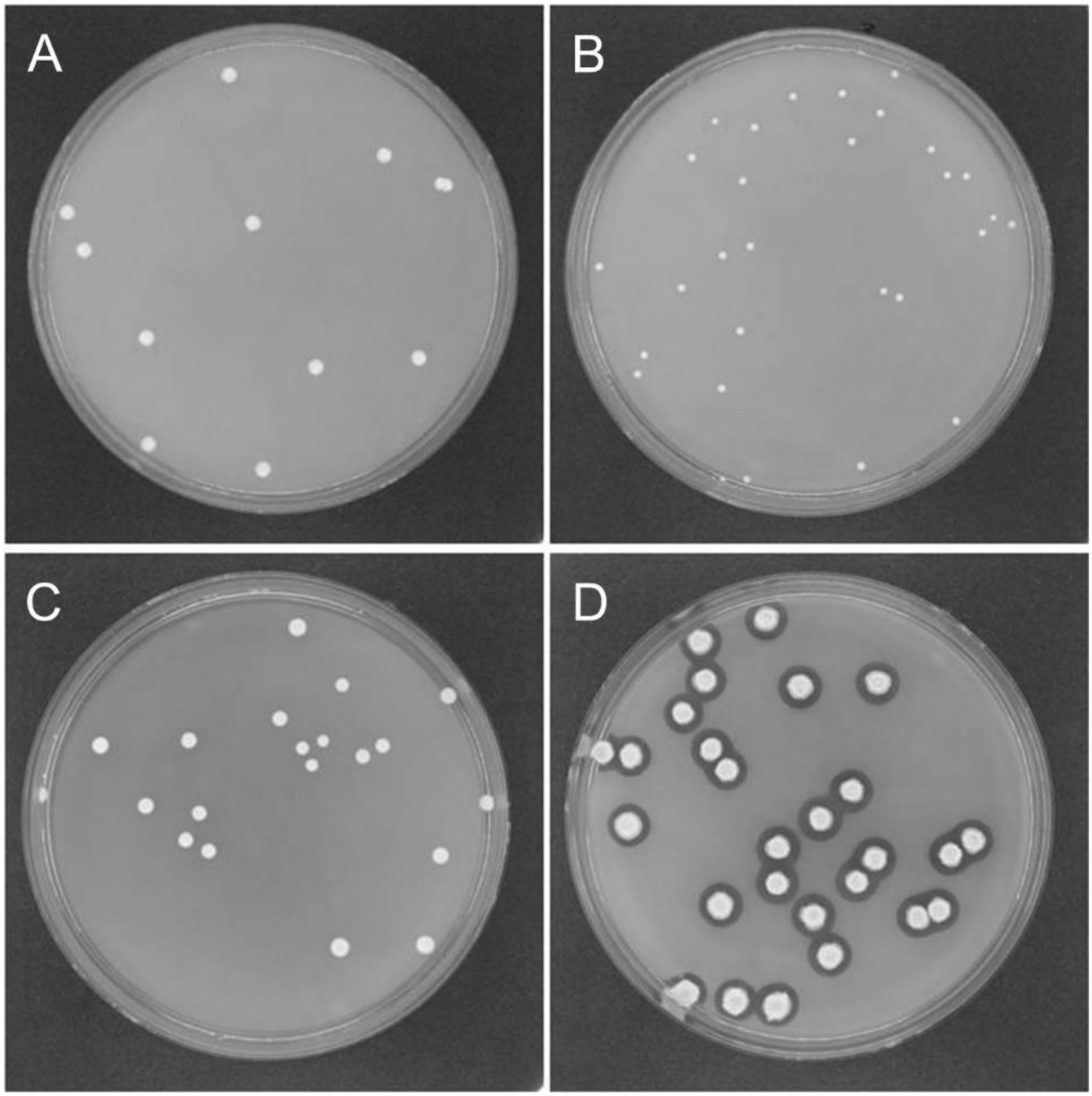

Figure 9: Examination of bacterial lipolytic activity by growth on tributyrin-mMRS agar plates. *L. plantarum* TMW 1.25 (A), *L. sakei* TMW 1.1239 (B), *L. fructivorans* TMW 1.1856 (C) and *B. subtilis* TMW 2.472 (D).

Sufficient growth of all strains could be observed by formation of clear bacterial colonies. With exception of *B. subtilis* TMW 2.472, no colony-surrounding halos could be determined for all tested *Lactobacillus* strains. Consequently, it is unlikely that the tested *Lactobacillus* strains possess emulsion-destabilizing lipolytic activity.

3.2.4.2 Proteolytic activity

To prevent destabilization of sodium caseinate-stabilized (O/W)-emulsion by bacterial proteolytic activity, all preselected strains were checked for proteolytic activity by measuring changes in fluorescence properties of the substrate FTC-casein as a result of its proteolytic digestion. Different concentrations of TPCK trypsin and the control strain *B. subtilis* TMW 2.472, known for its proteolytic activity, were used as reference proteases. The measured maximum fluorescence intensity (FI_{max}) and maximum increase in fluorescence (ΔFI_{max}) are depicted in Figure 10.

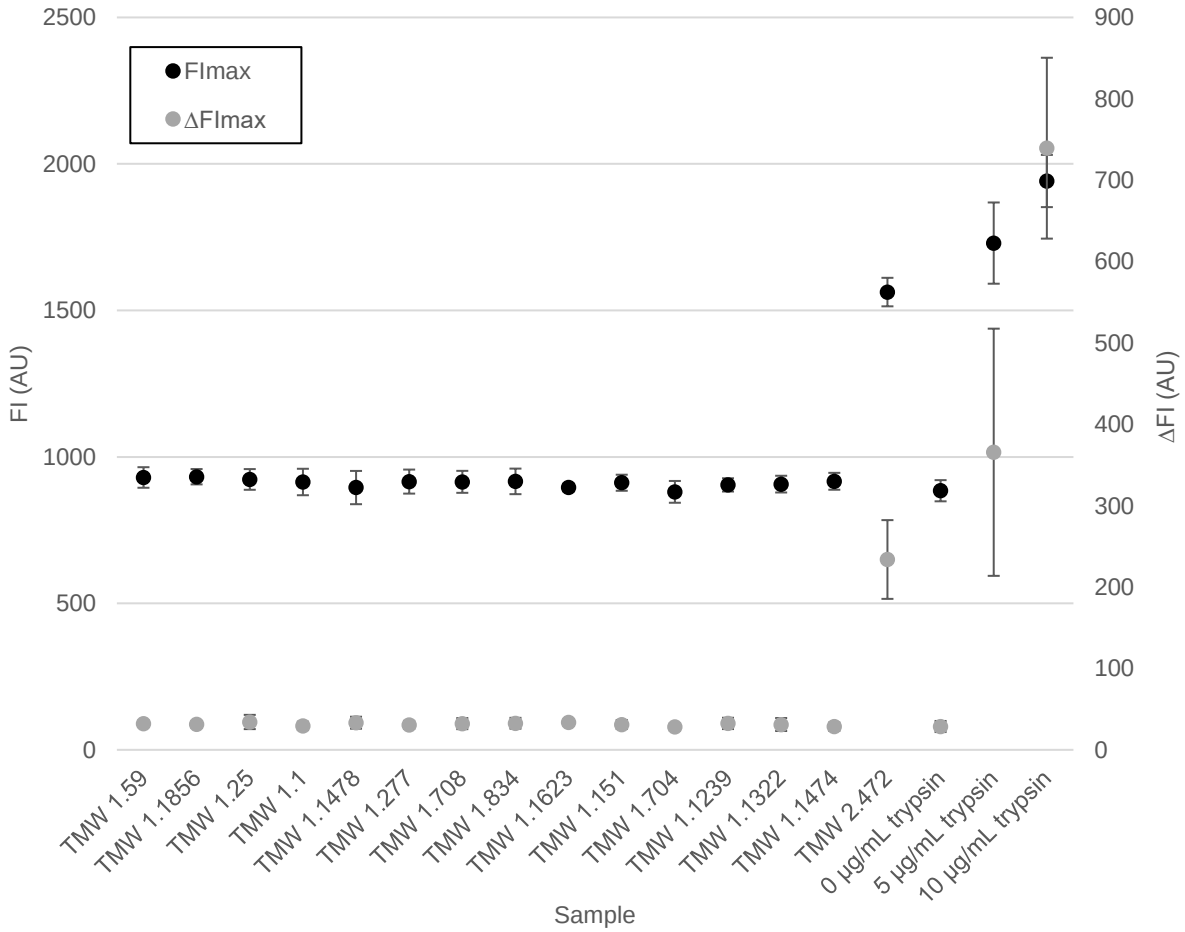

Figure 10: Determination of bacterial proteolytic activity by measuring changes in fluorescence intensity of FTC-casein. Digestion of the substrate FTC-casein results in an increase of fluorescence intensity. Fluorescein excitation/emission filters with 485/520 nm were used and the gain was set to 300. *B. subtilis* TMW 2.472 and TPCK trypsin were used as positive controls. FI_{max}, maximum fluorescence intensity; ΔFI_{max}, maximum increase in fluorescence intensity; AU, arbitrary units.

FI_{max} and ΔFI_{max} of all *Lactobacillus* strains were comparable to FI_{max} (~884 AU) and ΔFI_{max} (~29 AU) of the negative control (0 µg mL^{-1} trypsin), indicating no bacterial proteolytic activity (Appendix Table 23).

On the contrary, ΔFI_{max} increased with higher trypsin concentration (~366 AU for 5 µg mL^{-1}, ~739 AU for 10 µg mL^{-1}) and proteolytic activity of the positive control *B. subtilis* was proven by the determined ΔFI_{max} of ~234 AU.

3.2.4.3 Degradation of emulsifier Tween® 80

In order to exclude a possible destabilization of Tween® 80-stabilized (O/W)-emulsion by degradation and utilization of Tween® 80 by *Lactobacillus* strains, selected strains were grown in IPB containing 0.1% and 1.0% (w/v) Tween® 80, representing relevant Tween® 80 concentrations for this study. To ensure cells viability, strains were additionally grown in mMRS.

Table 16: Growth of *Lactobacillus* species in IPB with different Tween® 80 concentrations. Growth conditions: 30 °C, 15 h. *Lactobacillus* species were grown in mMRS as positive control. OD_{max}, maximum optical density at 600 nm. Mean values ± standard deviation are shown.

	IPB + 0.1% (w/v) Tween® 80		IPB + 1.0% (w/v) Tween® 80		mMRS	
Strain	Initial OD_{600}	OD_{max}	Initial OD_{600}	OD_{max}	Initial OD_{600}	OD_{max}
L. plantarum TMW 1.25	0.16±0.02	0.13±0.05	0.21±0.02	0.18±0.05	0.22±0.00	4.12±0.04
L. plantarum TMW 1.1	0.18± 0.00	0.25±0.05	0.21±0.01	0.22±0.02	0.21±0.02	4.71±0.04
L. plantarum TMW 1.1478	0.20±0.02	0.26±0.02	0.26±0.00	0.28±0.03	0.17±0.03	5.12±0.05
L. plantarum TMW 1.277	0.19±0.01	0.18±0.07	0.27±0.03	0.25±0.04	0.22±0.03	4.25±0.06
L. plantarum TMW 1.708	0.18±0.01	0.18±0.04	0.23±0.02	0.36±0.17	0.21±0.02	4.06±0.06
L. plantarum TMW 1.834	0.20±0.02	0.22±0.02	0.26±0.02	0.26±0.01	0.30±0.09	4.13±0.07
L. plantarum TMW 1.1623	0.24±0.04	0.29±0.06	0.23±0.00	0.27±0.04	0.18±0.01	4.37±0.13
L. fructivorans TMW 1.59	0.13±0.04	0.11±0.04	0.08±0.02	0.07±0.03	0.07±0.01	0.45±0.12
L. fructivorans TMW 1.1856	0.14±0.04	0.11±0.06	0.14±0.00	0.12±0.02	0.08±0.03	0.84±0.40
L. sakei TMW 1.151	0.06±0.07	0.11±0.08	0.15±0.02	0.15±0.03	0.12±0.00	3.10±0.17
L. sakei TMW 1.704	0.14±0.02	0.20±0.06	0.15±0.02	0.13±0.03	0.12±0.00	3.01±0.10
L. sakei TMW 1.1239	0.17±0.03	0.14±0.05	0.17±0.03	0.15±0.03	0.11±0.00	3.42±0.09
L. sakei TMW 1.1322	0.15±0.03	0.17±0.04	0.13±0.03	0.14±0.03	0.13±0.02	3.16±0.37
L. sakei TMW 1.1474	0.15±0.01	0.18±0.02	0.14±0.03	0.15±0.05	0.10±0.01	3.81±0.21

Comparing the initial OD_{600} with the maximum OD_{600} over a period of 15 h, for none of the tested strains, an increase in the optical density and thus, cell density, in IPB could be determined (Table 16). Accordingly, utilization of Tween® 80 and the related destabilization of (O/W)-emulsions by the tested strains is very unlikely.

3.2.5 Spoilage potential of *Lactobacillus* species

For further reduction of the strain collection, in a next step, bacterial strains should be selected which additionally show a high potential for spoiling food, i.e., strains which show strong growth under harsh conditions, normally used by the food industry to prevent bacterial growth and food spoilage. These conditions comprise high acidities (low pH value), low activity of water (high NaCl concentrations) and low temperatures. Bacterial growth was characterized by the growth characteristics maximum cell density (OD_{max}) and maximum growth rate (μ_{max}).

In order to investigate the pH tolerance of the preselected strains, their growth in CA-mMRS, adjusted to pH values of 3.5, 4.0, 4.5 and 6.2, which are commonly used and found in food products, was examined. The strains' tolerance to low water activity was tested by their growth in mMRS with adjusted a_w values of 0.961, 0.955, 0.948 and 0.93, using NaCl concentrations of 5%, 6%, 7.5% and 10%, respectively. The cold tolerance of *Lactobacillus* strains was examined by cultivation in mMRS at 4 and 10 °C for 9 days, using the same strains cultivated in mMRS at 30 °C for 3 days as reference. Growth curves of selected strains were derived and the growth characteristics OD_{max} and μ_{max} evaluated. Focusing on conditions showing still evaluable differences of the tested strains in pH tolerance, a_w tolerance and cold tolerance, growth at a pH of 3.5, a NaCl concentration of 7.5% (a_w = 0.948) and a temperature of 4 °C proved suitable for strain selection. The growth characteristics are summarized in Figure 11 (OD_{max}) and Figure 12 (μ_{max}). The data of all tested growth conditions are listed in Table 24 and Table 25 (see Appendix).

The maximum cell density determined for all tested *Lactobacillus* species incubated at pH of 3.5, 7.5% NaCl and 4 °C varied widely among the different species and also within each species (Figure 11).

The maximum OD_{600} of *L. fructivorans* strains at pH 3.5 and 7.5% NaCl varied the most among all species, with TMW 1.59 reaching only OD_{max} of ~0.1 and TMW 1.1856 growing up to an OD_{max} of ~1.0 and ~0.8, respectively. Reaching only maximum OD_{600} values of 0.04 for TMW 1.59 and 0.06 for TMW 1.1856, strains of the species *L. fructivorans* showed virtually no growth at 4 °C.

The maximum OD_{600} of *L. plantarum* strains varied widely at pH of 3.5, with TMW 1.1 reaching OD_{max} of ~0.2 and TMW 1.1623 growing up to OD_{max} of ~1.0. Growth at 7.5% NaCl resulted in general in more similar maximum cell densities starting from ~0.4 for TMW 1.1 up to ~0.8 for TMW 1.708.

The growth of all *L. plantarum* strains at 4 °C was strongly reduced, resulting above all in OD_{max} values from ~0.1 to ~0.2. Deviating from the other *L. plantarum* strains, TMW 1.1 reached an OD_{max} value of 0.5 which is twice as high compared to OD_{max} of most of the other strains.

The maximum OD_{600} reached by *L. sakei* at pH 3.5 varied widely among the strains from 0.03 for TMW 1.1474 to ~0.8 for TMW 1.1239, showing partly distinct variation in OD_{max} among different replicates of one sample. In the presence of 7.5% NaCl, *L. sakei* strains reached lower OD_{max} values compared to *L. plantarum* and *L. fructivorans*, showing the smallest variance in OD_{max} with values from ~0.3 for TMW 1.1474 to ~0.6 for TMW 1.1239 and as observed for growth at pH 3.5, strong variation in OD_{max} among different replicates of one sample. The maximum OD_{600} reached by *L. sakei* at 4 °C varied between 0.5 for TMW 1.704 and 0.8 for TMW 1.1474 and thus, *L. sakei* showed clearly the best growth at low temperature.

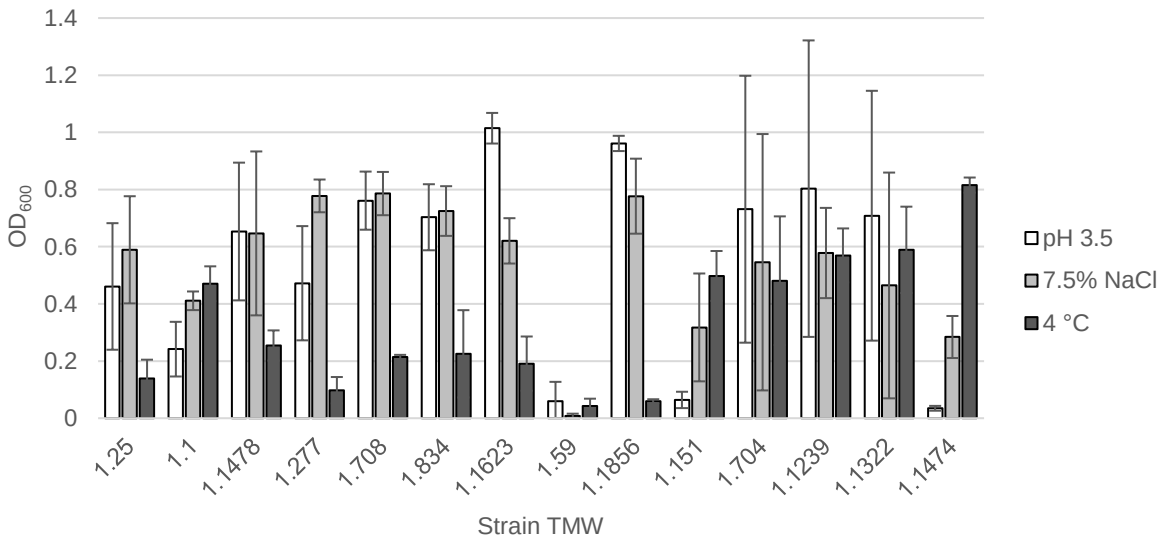

Figure 11: Maximum cell density (OD_{max}) determined for growth of different *Lactobacillus* species at low pH, reduced water activity and 4 °C. Low pH, pH 3.5. 7.5% NaCl, a_w = 0.948. OD_{600} was measured every 30 min by microplate reader for 96 h (low pH and 7.5% NaCl) or every 24 h for 9 days (4 °C) and subsequently OD_{max} was determined. *L. plantarum*: TMW 1.25, TMW 1.1, TMW 1.1478, TMW 1.277, TMW 1.708, TMW 1.834, TMW 1.1623. *L. fructivorans*: TMW 1.59, TMW 1.1856. *L. sakei*: TMW 1.151, TMW 1.704, TMW 1.1239, TMW 1.1322, TMW 1.1474.

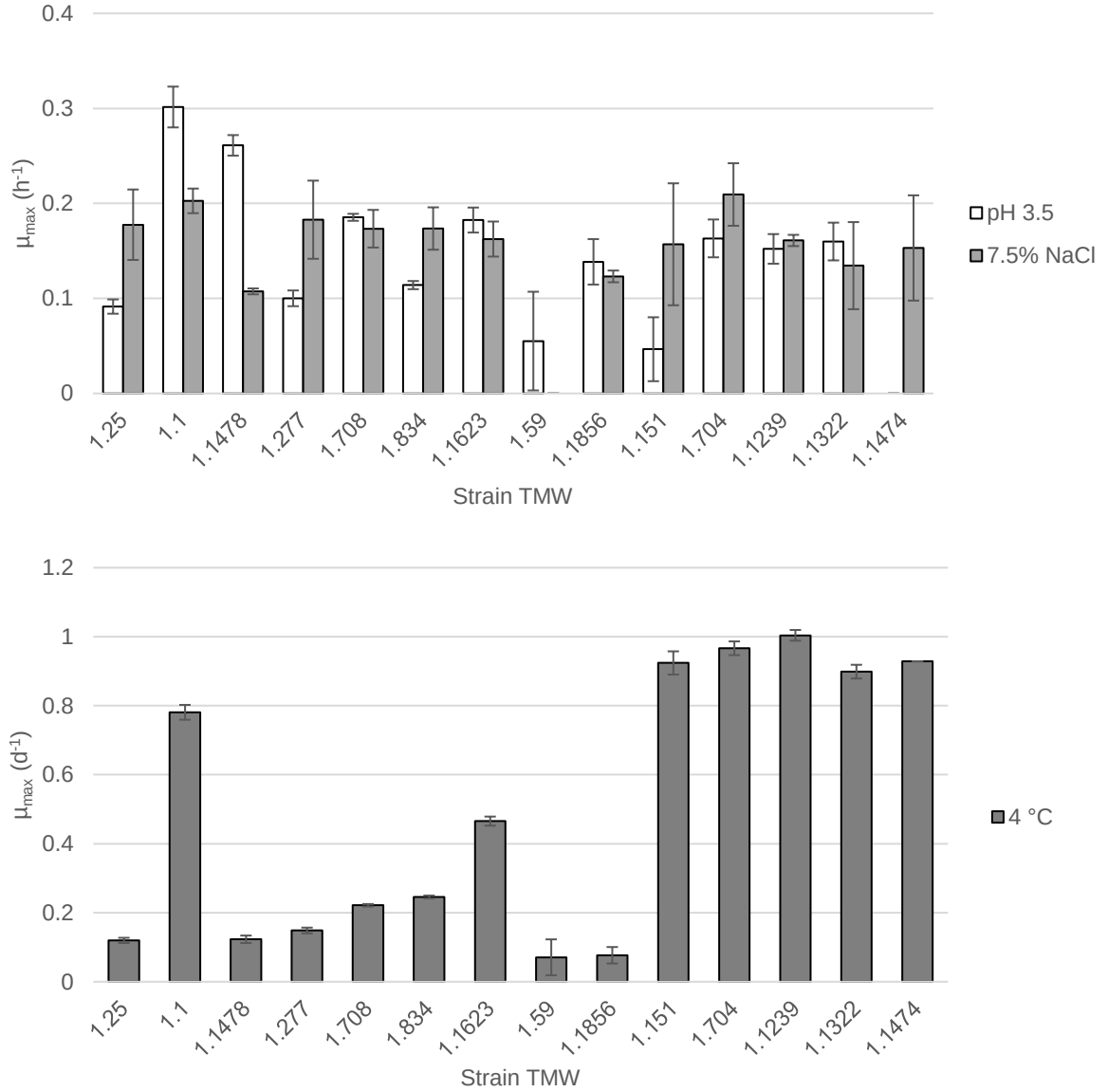

Figure 12: Maximum growth rate (μ_{max}) determined during growth of different *Lactobacillus* species at low pH, reduced water activity and 4 °C. Low pH, pH 3.5. 7.5% NaCl, a_w = 0.948. OD_{600} was measured every 30 min by microplate reader for 96 h (low pH and 7.5% NaCl) or every 24 h for 9 days (4 °C), subsequently growth curves were derived and the growth dynamic μ_{max} was evaluated. *L. plantarum*: TMW 1.25, TMW 1.1, TMW 1.1478, TMW 1.277, TMW 1.708, TMW 1.834, TMW 1.1623. *L. fructivorans*: TMW 1.59, TMW 1.1856. *L. sakei*: TMW 1.151, TMW 1.704, TMW 1.1239, TMW 1.1322, TMW 1.1474.

The maximum growth rates of the *L. fructivorans* strains corresponded well with the OD_{max} values observed at different growth conditions. While TMW 1.59 showed only very slow or even no growth at tested conditions (μ_{max} < 0.06 h^{-1}), for TMW 1.1856 μ_{max} of 0.14 h^{-1} at pH 3.5 (OD_{max} = ~1.0), 0.12 h^{-1} at 7.5% NaCl (OD_{max} = ~0.8) and 0.08 d^{-1} at 4 °C (OD_{max} = 0.06) was determined. Remarkably, in comparison to most of the strains of *L. plantarum* and *L. sakei*, TMW 1.1856 grown at pH 3.5 and at 7.5% NaCl showed in general lower maximum growth rates, but simultaneously reached mostly higher or the highest cell density, respectively.

At pH 3.5, the maximum growth rates of *L. plantarum* strains did not correspond continuously with the level of reached maximum cell densities. TMW 1.1 showed the highest μ_{max} of 0.3 h^{-1} although it reached the lowest OD_{max} of 0.24 and TMW 1.1478 had the second highest μ_{max} of 0.26 h^{-1} of all tested strains, however, it only reached a mean OD_{max} of 0.65. A similar observation for TMW 1.1 could be also made in the presence of 7.5% NaCl, again showing the highest μ_{max} (0.2 h^{-1}) while reaching the lowest OD_{max}. Contrary to the observations made for TMW 1.1 grown at pH 3.5 and 7.5% NaCl, the maximum growth rate of TMW 1.1 at 4 °C, being the highest among all *L. plantarum* strains, corresponded well with the highest observed OD_{max} of 0.47 for TMW 1.1. However, again not corresponding, TMW 1.1623, which has the second highest μ_{max} (0.47 d^{-1}) only reached a mean OD_{max} of ~0.2.

The maximum growth rates of *L. sakei* were similar to the growth rates of *L. plantarum* at respective pH and NaCl concentration, but corresponded continuously with the determined levels of maximum OD_{600} values at all growth conditions. *L. sakei* showed the highest maximum growth rates with μ_{max} reaching from 0.9 d^{-1} (TMW 1.1322) to 1.0 d^{-1} (TMW 1.1239), confirming the highest tolerance to cold among all tested species.

Selection of suitable strains for HHP inactivation studies

Suitable strains for systematic HHP inactivation studies should show substantial differences in CSH, a relevant potential to spoil food and not pose a threat to (O/W)-emulsions which are used as model system in this study. Additionally, in order to ensure a better comparability of the results achieved, selected strains should preferentially belong to the same species. Since no destabilizing effect of the tested strains on (O/W)-emulsions could be determined (3.2.4), the selection of suitable strains was accomplished based on CSH (3.2.1), spoilage potential (3.2.5), positions in Euclidean 2D plane (3.2.3) and the source of isolation (Table 15).

Both strains of the species *L. fructivorans,* TMW 1.59 and TMW 1.1856, showed high variations in CSH, but especially TMW 1.59 failed to grow under harsh conditions where most of the other strains showed good growth. Compared to *L. plantarum*, strains of the species *L. sakei* showed in general similar or slightly lower growth at low pH and at 7.5% NaCl, but revealed clearly the best cold tolerance. *L. plantarum* strains showed a generally high spoilage potential and wide variation in CSH. Since for all *L. sakei* strains moderately or highly hydrophilic surfaces were determined, strains of the species *L. plantarum* proved to be most suitable to meet all requirements.

TMW 1.25 which was isolated from raw sausage and TMW 1.277 which was isolated from palm wine revealed to be the most appropriate for representing highly hydrophobic *L. plantarum* strains.

In contrast to this, TMW 1.708 and TMW 1.1 appeared to be good representatives for hydrophilic *L. plantarum* strains, showing highly hydrophilic cell surfaces and clear differences in protein mass spectra, illustrated by distinctly different positions in Euclidean 2D plane (Figure 8A). Moreover, both strains share the same isolation source with TMW 1.25.

3.3 Localization of *L. plantarum* in (O/W)-emulsions

The localization of highly hydrophobic (TMW 1.25 and TMW 1.277) and highly hydrophilic (TMW 1.708 and TMW 1.1) strains in simple oil/water mixtures (1:1) and (O/W)-emulsions, stabilized by emulsifier or the thickener agar, was examined. This was done in order to proof whether the cells show CSH-specific adhesion to oil droplets and whether the presence of emulsifier prevents adhesion of hydrophobic cells to the fat surface.

The simple oil/water mixture was prepared by dispersing rapeseed oil in IPB. Following this, using stationary phase cells, cell concentrations of ~10^8 cfu mL^{-1} were adjusted and samples investigated by bright-field microscopy.

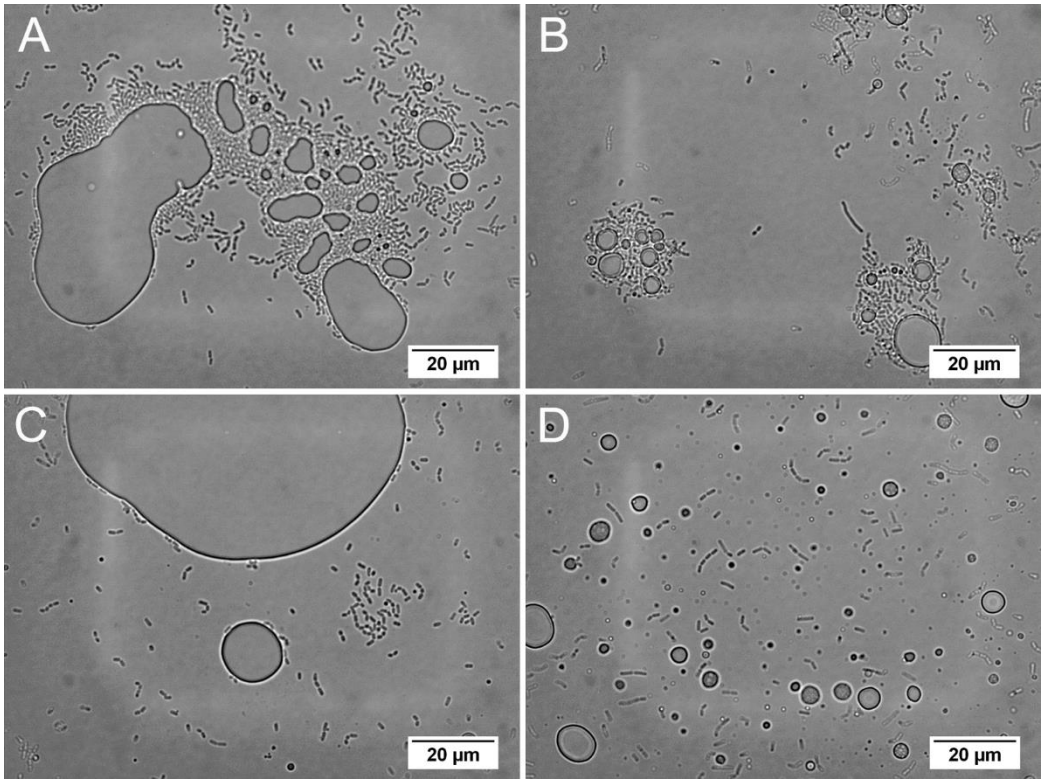

Figure 13: Localization of hydrophobic (TMW 1.25 (A), TMW 1.277 (B)) and hydrophilic (TMW 1.708 (C), TMW 1.1 (D)) strains of the species *L. plantarum* in 1:1 oil-water mixtures. Lipid phase, rapeseed oil. Aqueous phase, IPB. Magnification: 1000x.

As shown in Figure 13, the cells of both hydrophobic strains, TMW 1.25 (A) and TMW 1.277 (B) noticeably adhered to the oil droplets, whereas cells of the hydrophilic strains TMW 1.708 (C) and TMW 1.1 (D) did not show any adhesion. These observations plainly show that CSH dominantly influences cell localization in oil-water mixtures.

In contrast to this, microscopic examination of emulsifier-stabilized (O/W)-emulsion revealed homogenous distribution of oil droplets and cells. Differences in the localization of cells in emulsifier-stabilized (O/W)-emulsion, i.e., differences in adhesion behaviour depending on differences in CSH were not ascertainable (Figure 14). However, consistent with the observations made in simple oil/water mixtures, in emulsifier-free (O/W)-emulsions that were stabilized by agar, hydrophobic cells (Figure 15A/B) clearly adhered to the oil droplets, while hydrophilic cells (Figure 15C/D) did not show any adhesion. In contrast to the emulsifier-stabilized emulsion, no homogenous distribution of oil droplets was observable in the emulsifier-free emulsion.

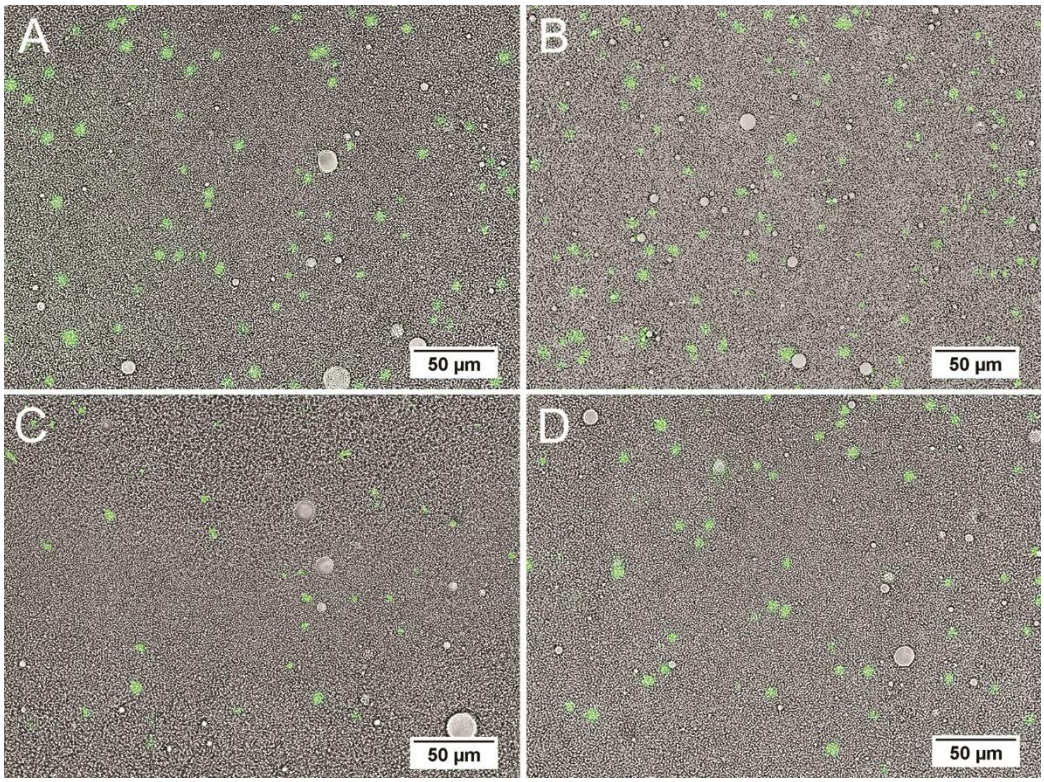

Figure 14: Fluorescence microscopy of hydrophobic (TMW 1.25 (A), TMW 1.277 (B)) and hydrophilic (TMW 1.708 (C), TMW 1.1 (D)) strains of the species *L. plantarum* in (O/W)-emulsion. 50% (v/v) rapeseed oil/IPB emulsion with 1% (w/v) Tween® 80. Cells are stained with SYTO® 9 green fluorescent dye. Magnification: 400x.

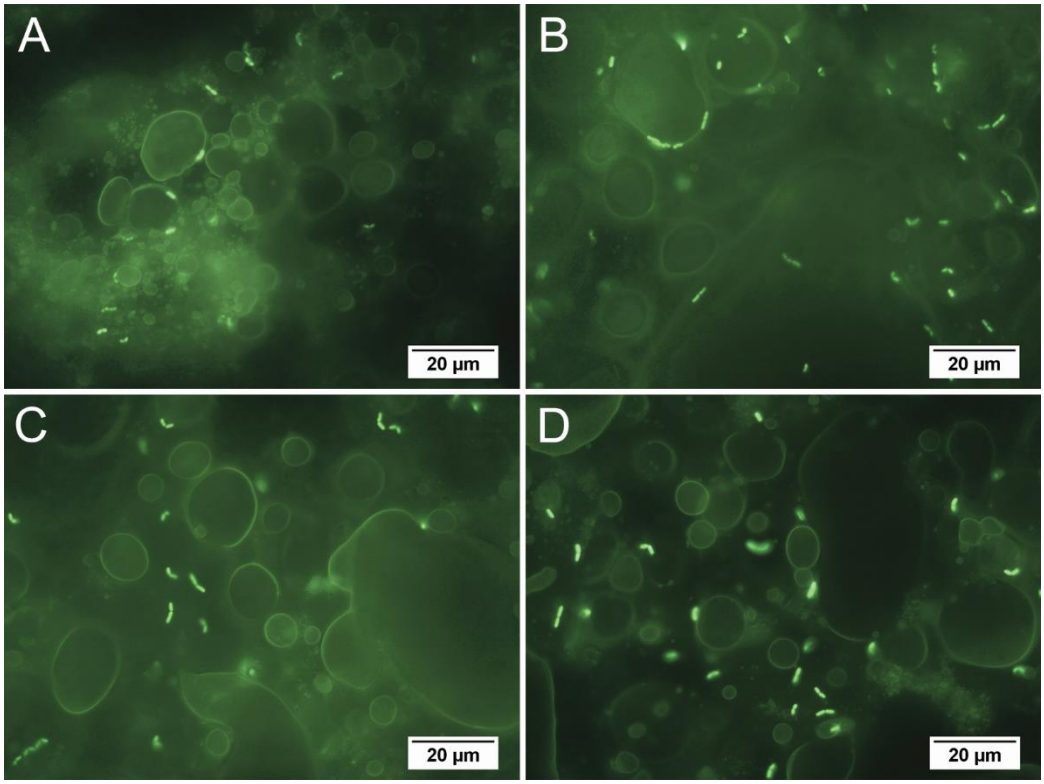

Figure 15: Fluorescence microscopy of hydrophobic (TMW 1.25 (A), TMW 1.277 (B)) and hydrophilic (TMW 1.708 (C), TMW 1.1 (D)) strains of the species *L. plantarum* in emulsifier-free (O/W)-emulsion. 50% (v/v) rapeseed oil/IPB emulsion with 2% (w/v) agar. Cells are stained with SYTO® 9 green fluorescent dye. Magnification: 1000x.

3.4 Effect of fat and bacterial surface hydrophobicity on the HHP inactivation of *L. plantarum* in emulsifier-stabilized (O/W)-emulsions

To retrieve detailed insights from systematic HHP inactivation studies in emulsifier-stabilized (O/W)-emulsions, first, preliminary experiments had to be performed. These experiments aimed to consider different adiabatic heating properties of used (O/W)-emulsions systems, in order to find appropriate HHP treatment parameters and to account a possible effect of the emulsifier per se on the HHP inactivation of *L. plantarum*.

3.4.1 Preliminary experiments

3.4.1.1 Effect of adiabatic heating during HHP treatment

Due to the extremely different adiabatic heating properties of fat and water, it was essential to determine adiabatic heating properties of all used (O/W)-emulsions systems and evaluate the efficiency of the temperature control used for HHP inactivation studies.

Thus, the assessment of the effect of the fat content and oil type on microbial inactivation rather than that of different temperatures during HHP processing should be enabled.

For this, temperature profiles of all (O/W)-emulsion types were recorded and raw data adjusted according to 2.7.2. Then, adiabatic heating effects, occurring in the temperature-controlled HHP unit TMW-RB, which was used for the inactivation experiments, were compared with the adiabatic heating properties of the prepared emulsions under nearly ideal adiabatic conditions. Figure 16 shows the comparison between standard data for pure water (NIST, 2002) and experimental data for water determined under nearly ideal adiabatic conditions. In order to consider the small time lag between pressure and temperature measurements, data was adjusted with a correction function (2.7.2.1). For both initial temperatures, 8 and 22.5 °C, the depicted temperature-pressure profiles of standard data (NIST, 2002) and experimental data resulted in a very good fit. Thus, it was assumed that the experimental setup, as well as the correction function, were appropriate for the determination and comparison of adiabatic heating properties of the (O/W)-emulsion systems under nearly ideal adiabatic conditions.

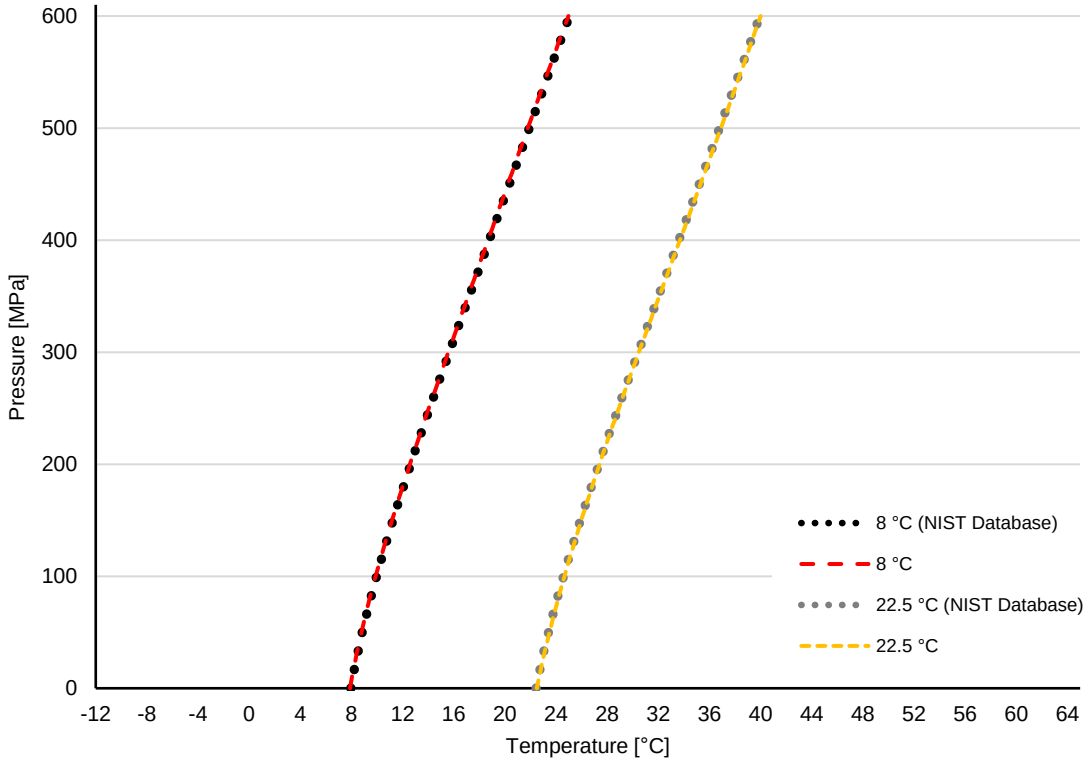

Figure 16: Comparison of data for water from the NIST (National Institute of Standards and Technology, Gaithersburg, USA) database with experimental data determined using the pressure unit U111 (Unipress, Warszawa, Poland) under nearly ideal adiabatic conditions. After equilibration of the sample temperature at either 25 or 40 °C, pressure was rapidly released with an average rate of > 100 MPa s^{-1} and temperature profiles were recorded. Recorded data was processed according to 2.7.2.1, resulting in adiabatic heating profiles of water in temperature ranges of 8 to 25 °C and 22.5 to 40 °C.

Figure 17 illustrates the effect of the fat content (0 - 100% rapeseed oil) of (O/W)-emulsions on adiabatic heating under nearly ideal adiabatic conditions. Independent of the initial temperature, adiabatic heating of (O/W)-emulsions clearly increased with higher fat content. Using an initial temperature of 25 °C, heating rates of 2.7, 3.9, 4.0, 5.4 and 6.2 °C per 100 MPa could be determined for (O/W)-emulsions with 0, 30, 50, 70 and 100% rapeseed oil, respectively. At an initial temperature of 40 °C, similar heating rates of 2.8, 3.6, 4.1, 4.9 and 5.9 °C per 100 MPa for (O/W)-emulsions with 0, 30, 50, 70 and 100% rapeseed oil were determined, respectively (Table 17).

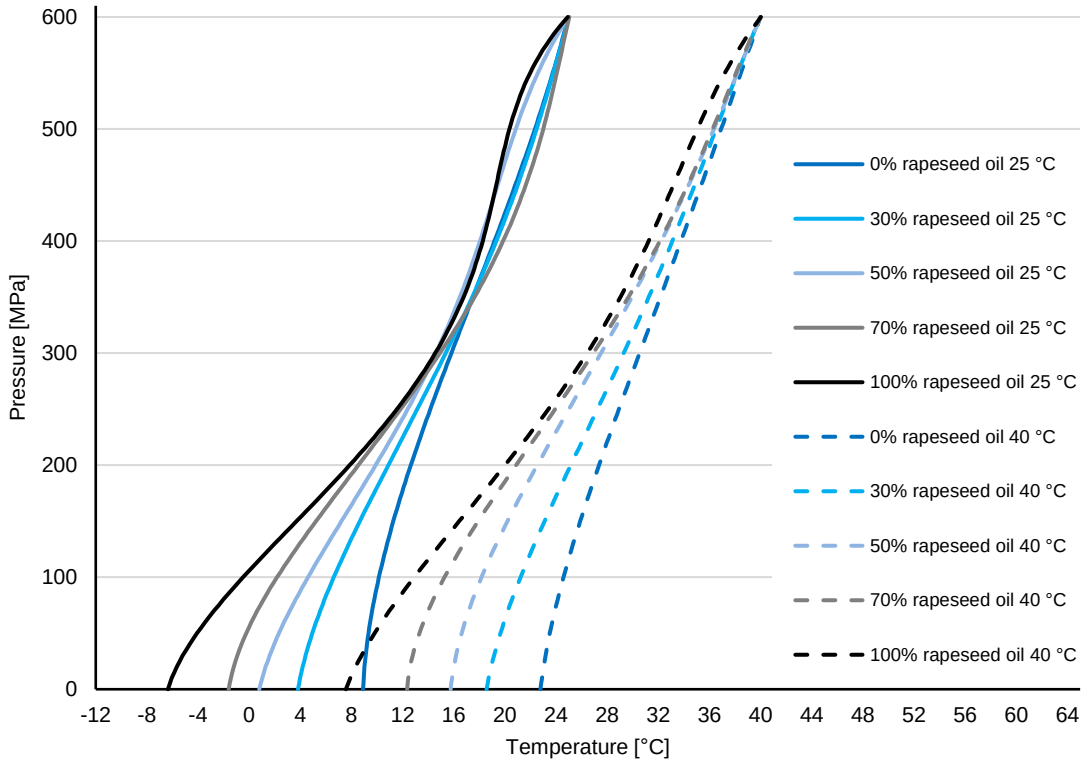

Figure 17: Adiabatic heating properties of (O/W)-emulsion systems with varying fat content under nearly ideal adiabatic conditions. Fat contents of 0 (IPB), 30, 50, 70 and 100% rapeseed oil were used. After equilibration of the sample temperature at either 25 or 40 °C, pressure was rapidly released with an average rate of > 100 MPa s^{-1} and temperature profiles were recorded. Recorded data was processed according to 2.7.2.1.

Evaluating adiabatic heating effects of (O/W)-emulsions varying in oil type, comparable temperature profiles of rapeseed oil and Miglyol® 812 at all tested fat contents could be determined (Figure 18). Using an initial temperature of 25 °C, heating rates of 4.0 °C/100 MPa and 6.2 °C/100 MPa for 50% and 100% rapeseed oil and 4.3 °C/100 MPa and 6.0 °C/100 MPa for 50% and 100% Miglyol® 812 were evaluated, respectively.

At an initial temperature of 40 °C, heating rates of 50% and 100% Miglyol® 812 were marginally higher with 4.4 °C/100 MPa and 6.3 °C/100 MPa compared to rapeseed oil with 4.1 °C/100 MPa (50%) and 5.9 °C/100 MPa (100%), respectively (Table 17).

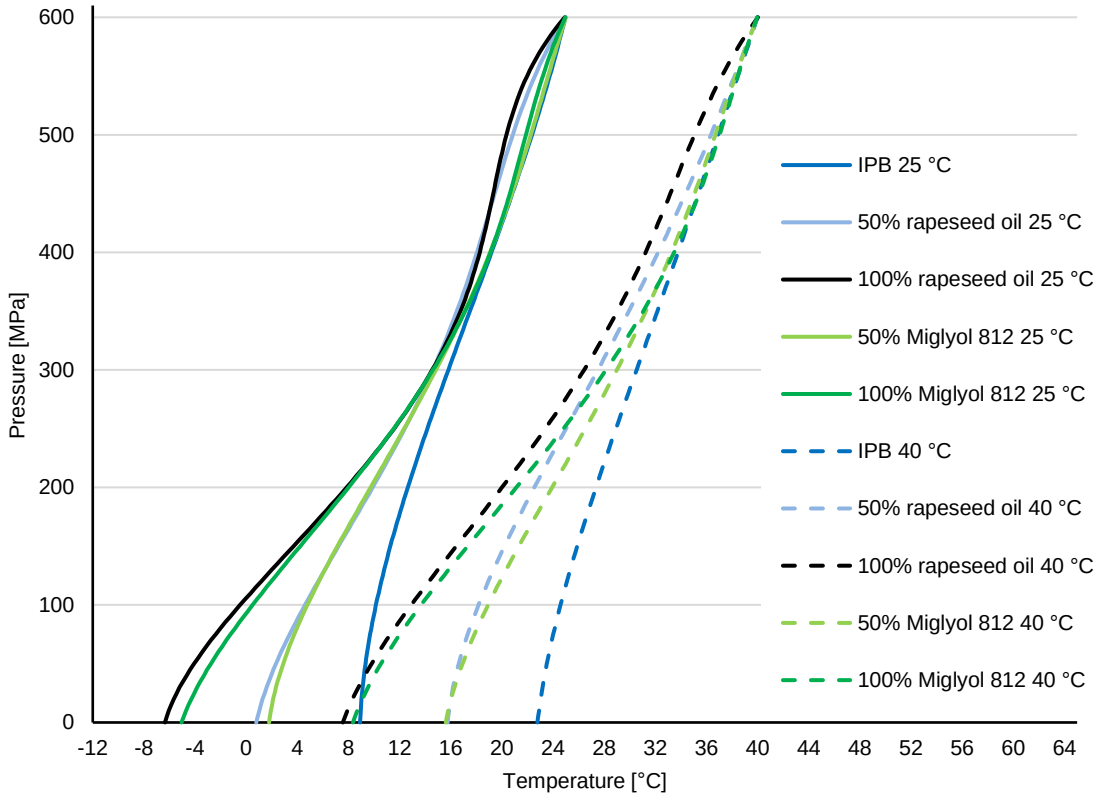

Figure 18: Adiabatic heating properties of (O/W)-emulsions varying in oil type and fat content under nearly ideal adiabatic conditions. Rapeseed oil and Miglyol® 812 were used with fat contents of 0 (IPB), 30 and 50%. After equilibration of the sample temperature at either 25 or 40 °C, pressure was rapidly released with an average rate of > 100 MPa s^{-1} and temperature profiles were recorded. Recorded data was processed according to 2.7.2.1.

Table 17: Heating rates of IPB and (O/W)-emulsions as a result of adiabatic compression under nearly ideal and real conditions.

	Initial temperature = 25 °C			Initial temperature = 40 °C	
Model system	IAH (°C/100 MPa)	RAH (°C/100 MPa) at 300 MPa	RAH (°C/100 MPa) at 400 MPa	IAH (°C/100 MPa)	RAH (°C/100 MPa) at 300 MPa
IPB	2.7	0.3	0.4	2.8	0.5
30% rapeseed oil	3.9	0.7	0.5	3.6	1.0
50% rapeseed oil	4.0	1.2	0.8	4.1	1.4
70% rapeseed oil	5.4	1.3	1.0	4.9	1.7
100% rapeseed oil	6.2	1.3	1.2	5.9	1.6
IPB + 2% (w/v) agar	2.8	0.3	0.4	2.8	0.5
30% rapeseed oil + 2% (w/v) agar	3.5	0.9	0.5	3.6	1.3
50% rapeseed oil + 2% (w/v) agar	3.8	1.2	0.6	4.2	1.6
50% Miglyol® 812	4.3	0.9	0.7	4.4	1.3
100% Miglyol® 812	6.0	1.2	0.8	6.3	1.6

Note: Temperature changes in all model systems were determined at an initial temperature of 25 or 40 °C. IAH = Ideal adiabatic heating. RAH = Real adiabatic heating.

Temperature profiles of liquid and solid (O/W)-emulsions are depicted in Figure 19. Liquid (O/W)-emulsions, stabilized by the emulsifier Tween® 80 and solid, emulsifier-free (O/W)-emulsions, stabilized by 2% (w/v) agar, showed comparable adiabatic heating rates with a maximum difference of 0.4 °C/100 MPa for the (O/W)-emulsion with 30% rapeseed oil at an initial temperature of 25 °C (Table 17).

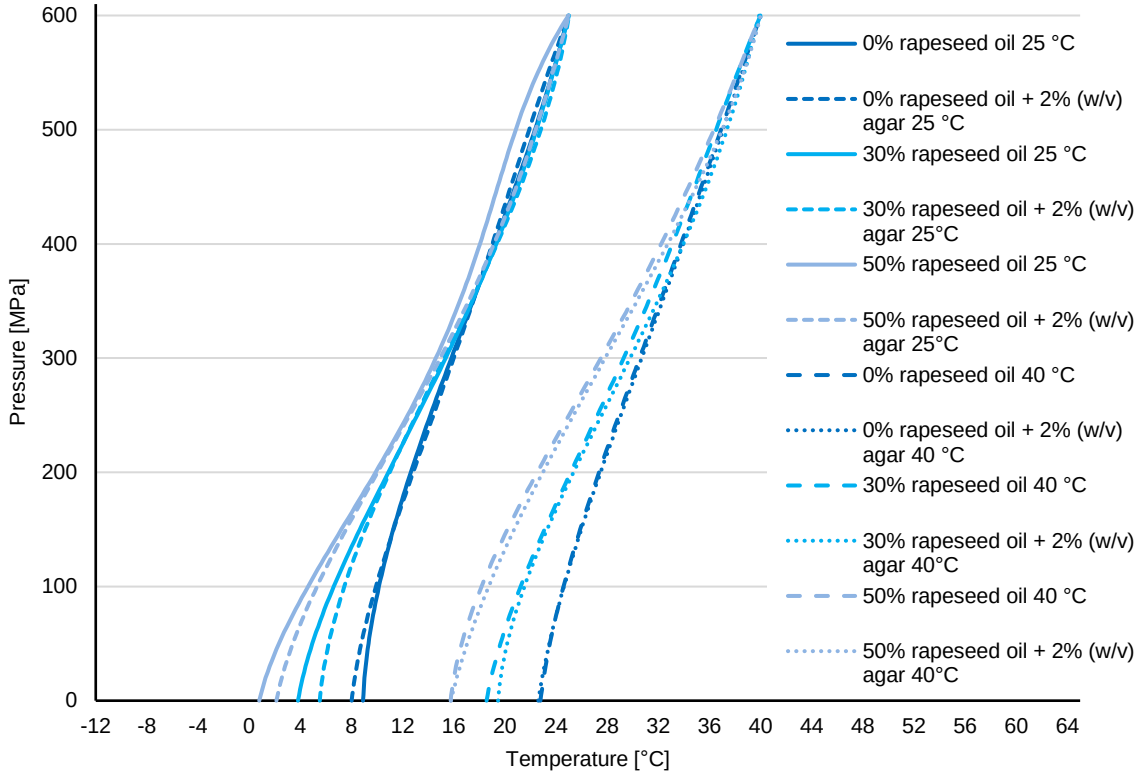

Figure 19: Adiabtic heating properties of liquid and solid (O/W)-emulsions under nearly ideal adiabatic conditions. Liquid (O/W)-emulsions were stabilized by the emulsifier Tween® 80 while solid, emulsifier-free (O/W)-emulsions were stabilized by 2% (w/v) agar. Fat contents of 0 (IPB), 30 and 50% rapeseed oil were used. After equilibration of the sample temperature at either 25 or 40 °C, pressure was rapidly released with an average rate of > 100 MPa s^{-1} and temperature profiles were recorded. Recorded data was processed according to 2.7.2.1.

Next, adiabatic effects occurring in the HHP unit TMW-RB, which was used for the inactivation experiments, were evaluated and compared with the adiabatic heating properties of the prepared emulsions under nearly ideal adiabatic conditions. Figure 20 and Figure 21 show exemplary the temperature profiles of all (O/W)-emulsions recorded during HHP treatment at 400 MPa/25 °C.

During initial pressurizing to 400 MPa, independent of the (O/W)-emulsion system, a slight increase in temperature could be observed for all model systems. This was followed by a temperature decrease, a settling at 25 °C treatment temperature during holding time and lastly a severe temperature drop due to controlled pressure-release. A summary of the heating rates, mean and maximum temperatures during HHP processing in the HHP unit TMW-RB, which was used for the inactivation experiments, is provided in Table 17 and Table 18.

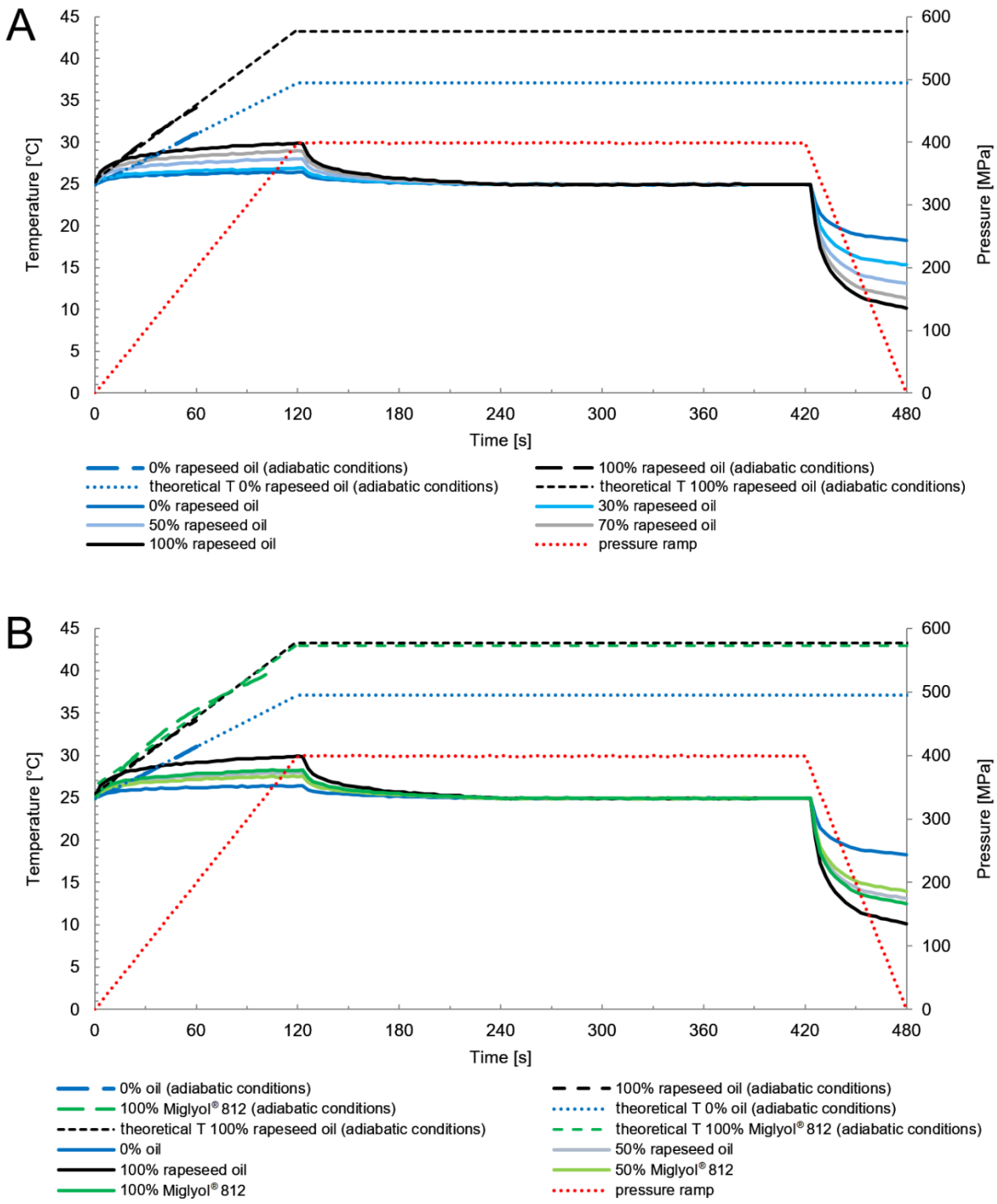

Figure 20: Temperature profiles of (O/W)-emulsions varying in fat content (A) and oil type (B) during HHP treatment under experimental conditions. HHP treatment was performed by the HHP unit TMW-RB. Temperature profiles were recorded during HHP treatment at 400 MPa/25 °C/5 min holding time and recorded data were processed according to 2.7.2.2. (A) Fat contents of 0 (IPB), 30, 50, 70 and 100% rapeseed oil were used. (B) Rapeseed oil and Miglyol® 812 were used with fat contents of 0 (IPB), 50 and 100%.

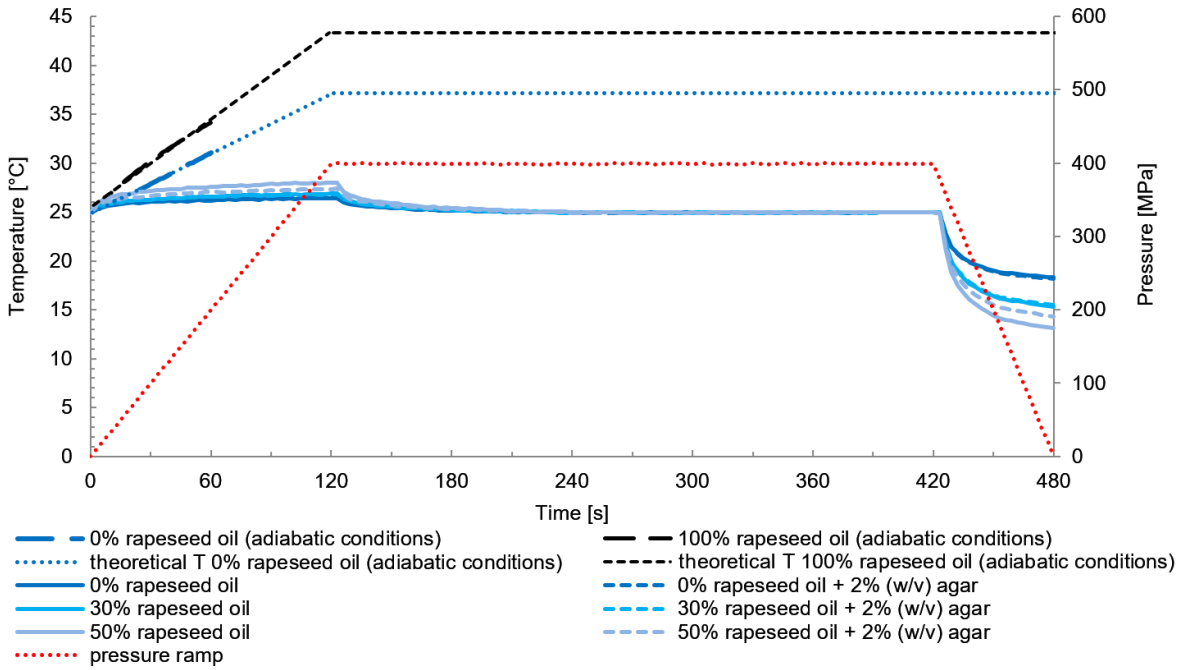

Figure 21: Temperature profiles of liquid and solid (O/W)-emulsions varying in fat content during HHP treatment under experimental conditions. HHP treatment was performed by the HHP unit TMW-RB. Temperature profiles were recorded during HHP treatment at 400 MPa/25 °C/5 min holding time and recorded data were processed according to 2.7.2.2. Liquid (O/W)-emulsions were stabilized by the emulsifier Tween® 80 while solid, emulsifier-free (O/W)-emulsions were stabilized by 2% (w/v) agar. Fat contents of 0 (IPB), 30 and 50% rapeseed oil were used.

Table 18: Mean and maximum temperatures during HHP processing of IPB and (O/W)-emulsions.

	HHP treatment at 300 MPa/25 °C		HHP treatment at 300 MPa/40 °C		HHP treatment at 400 MPa/25 °C	
Model system	T_{max} (°C)	T_{meanP}* (°C)	T_{max} (°C)	T_{meanP}* (°C)	T_{max} (°C)	T_{meanP}** (°C)
IPB	25.7	25.1	41.3	40.1	26.4	25.4
30% rapeseed oil	27.0	25.4	43.0	40.5	26.9	25.5
50% rapeseed oil	28.4	25.7	44.2	40.8	28.0	25.8
70% rapeseed oil	28.9	25.8	44.8	40.9	29.0	26.1
100% rapeseed oil	28.7	25.8	44.7	40.9	29.9	26.4
IPB + 2% (w/v) agar	25.9	25.1	41.4	40.2	26.7	25.4
30% rapeseed oil + 2% (w/v) agar	27.6	25.5	43.7	40.6	26.9	25.5
50% rapeseed oil + 2% (w/v) agar	28.5	25.7	44.7	40.9	27.4	25.6
50% Miglyol® 812	27.7	25.5	43.8	40.6	27.6	25.7
100% Miglyol® 812	28.5	25.7	44.6	40.8	28.3	25.9

Note: *Pressure build-up time of 90 s + holding time of 300 s. **Pressure build-up time of 120 s + holding time of 300 s. T_{max} = maximum temperature reached due to adiabatic compression. T_{meanP} = mean temperature during process.

Consistent with the observations under nearly ideal adiabatic conditions, higher fat content in model systems resulted in general in higher adiabatic heating of (O/W)-emulsions under real experimental conditions (see Table 17 and Table 18).

Although it was not completely possible to eliminate temperature raises due to adiabatic heating, the heating rates could be noticeably reduced by at least 61.9% for all model systems using the experimental setup for HHP inactivation studies. The highest observed heating rates were 1.7 °C/100 MPa for the (O/W)-emulsion with 70% rapeseed oil and 1.6 °C/100 MPa for 100% rapeseed oil, both at a target p/T combination of 300 MPa/40 °C. Clear differences in adiabatic heating between rapeseed oil and Miglyol® 812 as well as between liquid and solid (O/W)-emulsions were again not determinable. The mean process temperature over the entire process (defined as time pressurizing to target pressure and holding a sample at a pressure > (p_{target} – 5 MPa)) was held relatively constant regardless of the amount of fat, the oil type and the physical state of the model system sample. The measured mean temperature differences during process between samples with 0% and 100% fat were limited to 1.0 °C under all applied HHP process conditions (see Table 18). The absolute maximum temperature difference recorded between samples with and without fat was 3.5 °C for model systems with 70% and 100% rapeseed oil and a target p/T combination of 300 MPa/40 °C and 400 MPa/25 °C, respectively. On average, maximum temperature differences arising from adiabatic peaks were 2.3 °C.

The results demonstrated that the temperature control used for eliminating the occurrence of adiabatic heating effects at HHP treatments worked efficiently and thus, effects of adiabatic heating could be neglected using this experimental setup.

3.4.1.2 Inactivation kinetics

To determine appropriate HHP treatment conditions, which on the one hand lead to sufficient bacterial inactivation, but on the other hand still enable the identification of HHP inactivation influencing parameters, inactivation kinetics were run. Since the pressure sensitivity between strains within one species can vary greatly, inactivation kinetics were determined for each of the four selected strains individually, as shown in Figure 22. The pressure level was incrementally increased from 250 to 600 MPa in steps of 50 MPa at a constant pressure holding time of 5 min at 25 °C, considering high temperature-sensitivity of many fat-containing products.

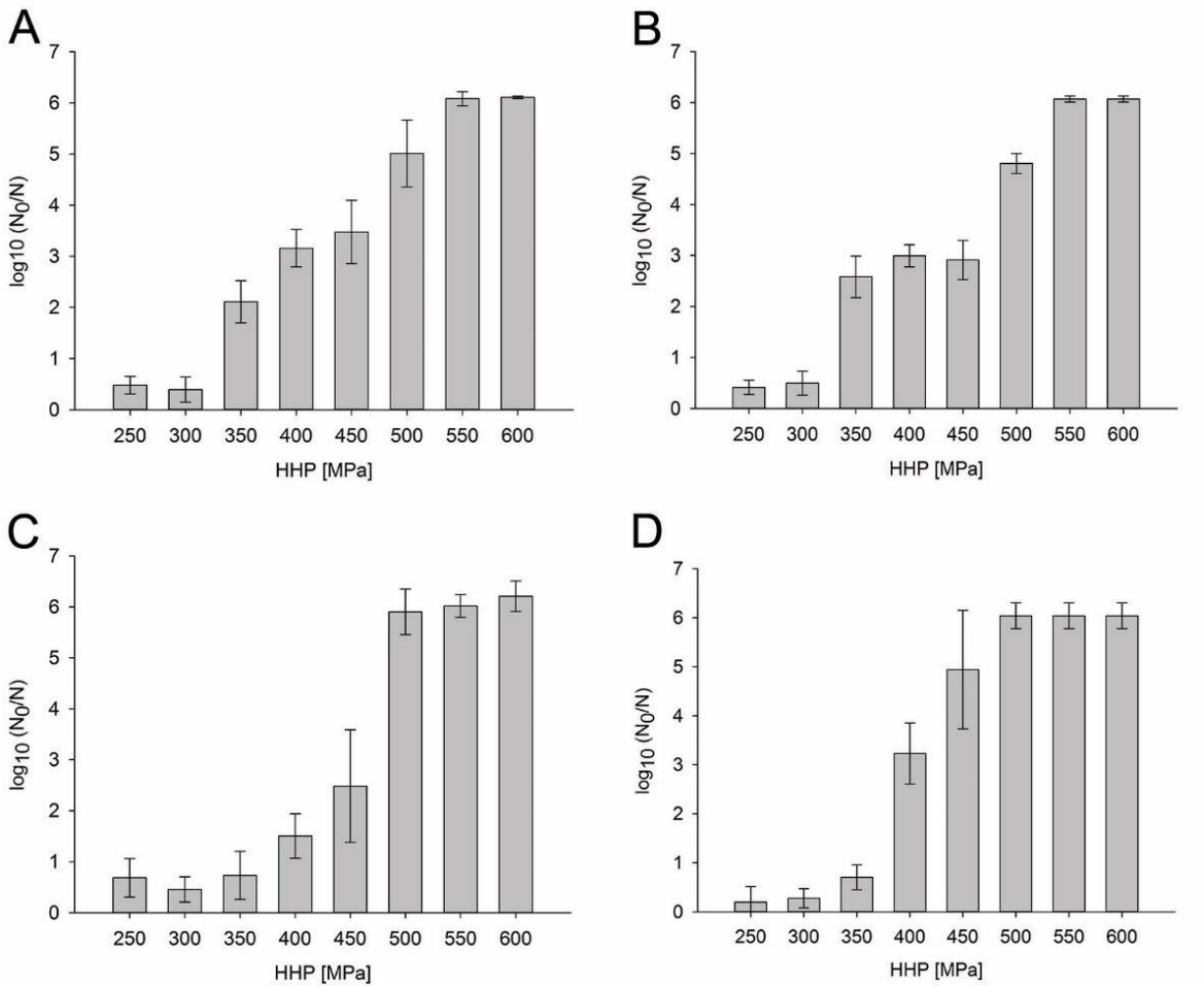

Figure 22: Inactivation kinetics of different strains of the species *L. plantarum*. The induced reduction ($log_{10}(N_0/N)$) of stationary phase cells of strains TMW 1.25 (A), TMW 1.277 (B), TMW 1.708 (C) and TMW 1.1 (D) at an inoculum of ~10^7 cells mL^{-1}, subjected to HHP treatment of 250 to 600 MPa at 25 °C for 5 min. Bacterial inactivation is stated as log reduction levels. Data presented are the mean values from at least three independent experiments and error bars correspond to standard deviations.

Independent of the tested strain, treatments at 250 and 300 MPa resulted in low cell inactivation, i.e., log inactivation levels of 0.20 to 0.69 (Figure 22, Appendix Table 27). Treatment at 350 MPa led to a clearly increased cell inactivation of the hydrophobic strains TMW 1.25 and TMW 1.277 (~1.7 and ~2.1 log higher inactivation), which was not to this extent observable for the hydrophilic strains TMW 1.708 and TMW 1.1. Applying a pressure of 400 MPa, significantly higher cell inactivation could also be observed for TMW 1.708 and TMW 1.1 with ~0.8 and ~2.5 log higher inactivation. A treatment of 450 MPa showed especially for TMW 1.708 and TMW 1.1 noticeably increased cell inactivation from 1.51 (400 MPa) to 2.49 and 3.23 (400 MPa) to 4.94 log cycles, respectively. More harsh conditions, i.e. pressure levels ≥ 500 MPa, led to an overall dramatic reduction in viable cell counts with treatments at 550 and 600 MPa resulting in even complete inactivation.

Taking into consideration the determined strain-specific differences in pressure resistance, a pressure level of 400 MPa proved to be most suitable for subsequent HHP investigations, guaranteeing substantial but not complete cell inactivation of all strains. Hereby, effects of different lipid phase parameters on HHP inactivation could be investigated.

Besides HHP treatment at 400 MPa/25 °C/5 min, subsequent inactivation studies were additionally performed at 300 MPa/25 °C/5 min and 300 MPa/40 °C/5 min. Using several HPT-combinations, a wider range could be investigated, enabling to identify a possible effect of lipid phase parameters occurring only under specific conditions. In contrast to treatments at 25 °C, treatments at 300 MPa and 40 °C led to significantly higher cell inactivation, i.e. log inactivation levels of 3.25, 3.06, 1.53 and 0.93 for TMW 1.25, TMW 1.277, TMW 1.708 and TMW 1.1, respectively (Appendix Table 29). The inactivation level found for TMW 1.708 was significantly lower than that found for the other strains, indicating that TMW 1.708 has the lowest temperature sensitivity of all tested strains.

3.4.1.3 Effect of emulsifier on the HHP inactivation of L. plantarum in aqueous suspension

To consider a possible effect of the emulsifier type (Tween® 80, sodium caseinate) and emulsifier concentration on bacterial HHP inactivation, samples with emulsifier concentrations of 0.0, 0.5, 1.0, 2.5 and 5.0% (w/v) were adjusted and subjected to 400 MPa/25 °C for 5 min. Figure 23 (detailed data is shown in Appendix Table 28) illustrates that neither for the emulsifier Tween® 80 (A) nor for sodium caseinate (B) an effect on the HHP inactivation of *L. plantarum* could be determined. As a result, it could be assumed that various concentrations and types of emulsifier, which were applied for stabilization of the (O/W)-emulsion types used in this study, have no influence on the HHP inactivation of *L. plantarum*.

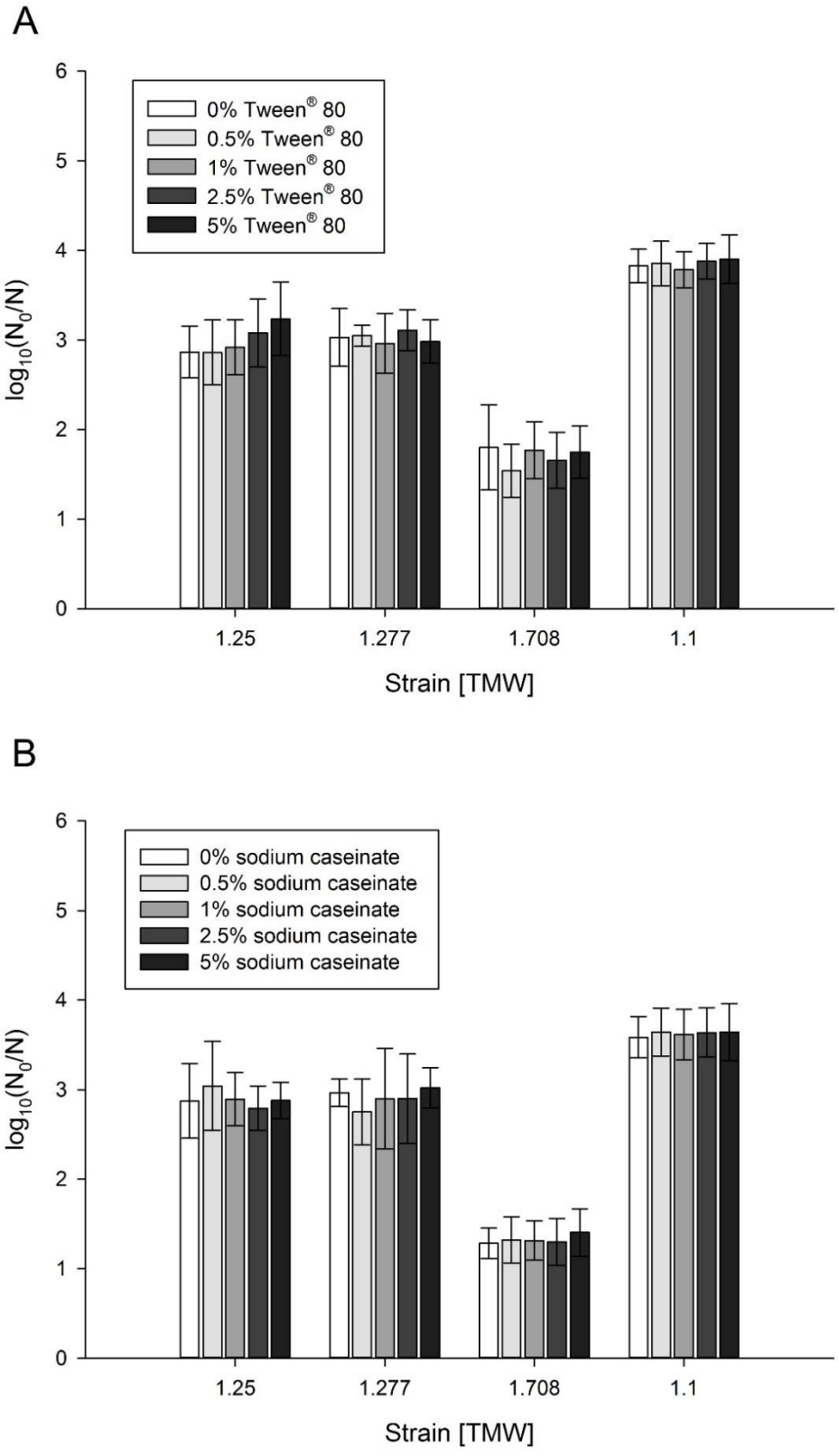

Figure 23: Effect of Tween® 80 (A) and sodium caseinate (B) on the HHP inactivation of *L. plantarum* in aqueous suspension. The HHP-induced reduction ($log_{10}(N_0/N)$) of stationary phase cells (strains TMW 1.25, TMW 1.277, TMW 1.708 and TMW 1.1) at an inoculum of ~10^7 cells mL^{-1} in the presence of different emulsifier types and -concentrations is shown (400 MPa/25 °C/5 min). Data presented are the mean values from at least three independent experiments and error bars correspond to standard deviations.

3.4.1.4 Oxidation stability of rapeseed oil and Miglyol® 812

Oxidation stability of rapeseed oil and Miglyol® 812 was examined to account a possible effect of changed oil properties, which could be caused by storage, on bacterial inactivation. For this purpose, samples of both, freshly opened as well as oil bottles, which were stored for 14 days, were sent to VFG-Labor GmbH & Co. KG (Versmold, Germany) for the examination of their oxidation stability. To determine the oxidation stability of both oil types, their aging process was accelerated by temperature increase (98 °C) and oxygen excess (6 L h^{-1}) and the time (induction time) measured until oxidation occurred. Taking into account measurement inaccuracies, comparable induction times of 12 h for fresh and 11 h for 14 days old rapeseed oil samples were determined. Regarding the fully saturated oil Miglyol® 812, for both samples comparable inductions times of > 50 h were measured. Based on these observations, changes of oil properties, i.e. oxidation of oil due to storage, could be neglected.

3.4.2 Effect of fat per se on the HHP inactivation of *L. plantarum*

After selection of appropriate HPT parameters, the effect of fat per se was determined by using Tween® 80-stabilized (O/W)-emulsions varying in fat content (0, 30, 50 and 70% (v/v) rapeseed oil) while showing comparable fat surfaces (3.1.1). Inactivation levels of all tested strains after HHP processing are summarized in Table 29 (see Appendix) and shown for treatment at 400 MPa/25 °C/5 min in Figure 24.

The pressure level of 300 MPa at 25 °C generally had a weak effect on cell counts and maximum inactivation levels were ~0.6 log, reached for the strain TMW 1.25. Using these mild temperatures, the fat content had no significant effect on HHP inactivation. However, cell inactivation at 300 MPa was markedly increased at a process temperature of 40 °C as well as in case of a HPT combination of 400 MPa/25 °C. There, the applied higher temperature or pressure resulted in comparable increases in cell inactivation of almost each strain. Only for TMW 1.1 the results were contrastive: TMW 1.1 showed the lowest temperature sensitivity but the highest pressure sensitivity among all strains, resulting in differences in log inactivation levels of ~0.6 and ~2.9, respectively. Maximum log inactivation levels at 300 MPa/40 °C and 400 MPa/25 °C target pressure were ~3.4 for TMW 1.277 (70% (v/v) rapeseed oil) and ~3.9 for TMW 1.1 (50% (v/v) rapeseed oil), respectively. Comparing the cell counts of fat-free and fat-containing samples, a clear tendency towards generally increased cell inactivation of hydrophilic (TMW 1.708 and TMW 1.1) but not hydrophobic strains with higher fat content was observed (Figure 24).

Furthermore, a significant fat-mediated increase in inactivation of TMW 1.1 (0 to 50% fat, $p = 0.032$; 0 to 70% fat, $p = 0.009$) at 300 MPa/40 °C and of TMW 1.708 (0 to 50% fat, $p = 0.048$; 0 to 70% fat, $p = 0.011$) at 400 MPa/25 °C could be determined.

The maximum difference between log inactivation levels of fat-free and fat-containing (70% (v/v) rapeseed oil) samples was 0.84 for TMW 1.1 at 300 MPa/40 °C. In general, an increase in cell inactivation with higher treatment intensities was observed.

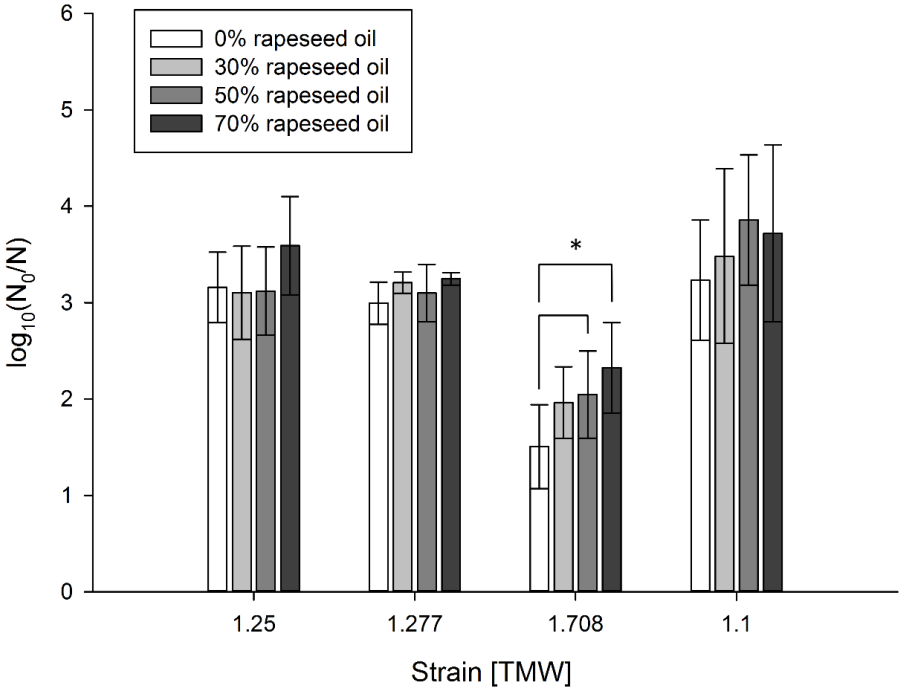

Figure 24: Effect of fat per se on the HHP inactivation of *L. plantarum* in emulsifier-stabilized (O/W)-emulsions. The induced reduction ($log_{10}(N_0/N)$) of stationary phase cells (strains TMW 1.25, TMW 1.277, TMW 1.708 and TMW 1.1) at an inoculum of ~10^7 cells mL^{-1} by HHP (400 MPa/25 °C/5 min) is shown. Data presented are the mean values from at least three independent experiments and error bars correspond to standard deviations. Asterisks indicate statistically significant differences between samples ($p < 0.05$).

3.4.3 Effect of fat/water boundary layer thickness on the HHP inactivation of *L. plantarum*

In a next step it was examined, whether the emulsifier type and thus, the thickness of the fat/water boundary layer has an effect on the HHP inactivation of *L. plantarum*. Tween® 80, a low molecular emulsifier, is assumed to form thin fat/water boundary layers, while sodium caseinate, a macromolecular emulsifier, is thought to form thick layers. Inactivation levels of all tested strains after HHP processing are shown in Table 29 (see Appendix) and for treatment at 400 MPa/25 °C in Figure 25.

Within each strain, all determined inactivation levels were comparable. Accordingly, apart from an increase in cell inactivation with higher treatment intensities, no effect of the emulsifier type on HHP inactivation could be observed. Consequently, no effect of the fat/water boundary layer thickness on the HHP inactivation of *L. plantarum* could be determined.

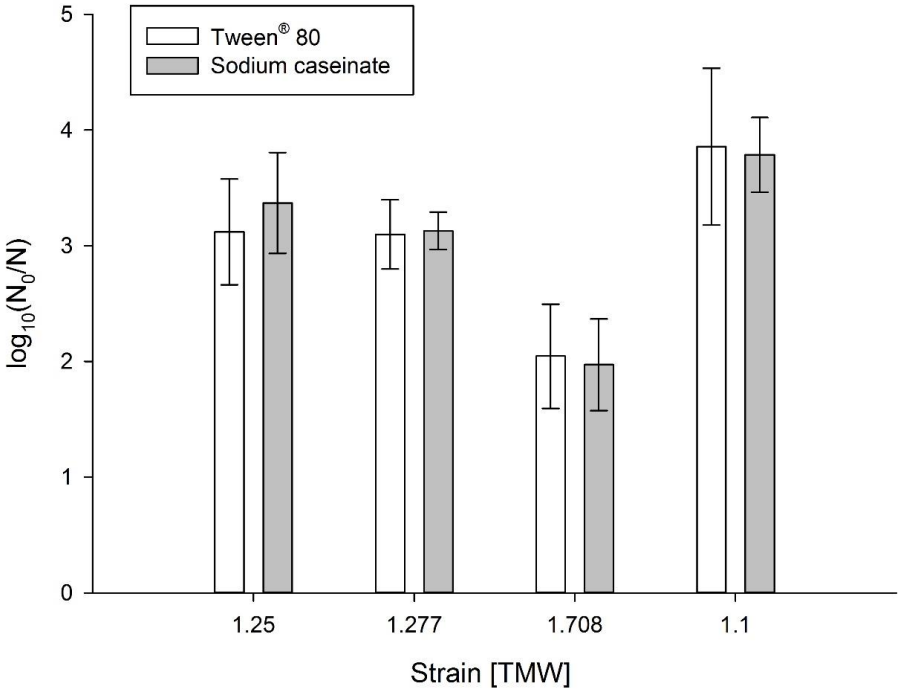

Figure 25: Effect of the emulsifier type on the HHP inactivation of *L. plantarum* in emulsifier-stabilized (O/W)-emulsions. The induced reduction ($log_{10}(N_0/N)$) of stationary phase cells (strains TMW 1.25, TMW 1.277, TMW 1.708 and TMW 1.1) at an inoculum of ~10^7 cells mL^{-1} by HHP (400 MPa/25 °C/5 min) is shown. Data presented are the mean values from at least three independent experiments and error bars correspond to standard deviations.

3.4.4 Effect of fat surface on the HHP inactivation of *L. plantarum*

Considering that an effect of fat could be based on the droplet size and, hence, fat surface potentially available for cell adhesion, the effect of different fat surfaces (1.5 $m^2 g^{-1}$, 3.7 $m^2 g^{-1}$, 10.6 $m^2 g^{-1}$) at a constant fat content of 50% (v/v) rapeseed oil on bacterial inactivation was examined.

It was found that inactivation levels of all samples were comparable among each strain and thus, the fat surface and, therefore, the droplet size appeared to have no effect on inactivation of neither hydrophobic nor hydrophilic cells in emulsifier-stabilized (O/W)-emulsions (Figure 26, Appendix Table 29).

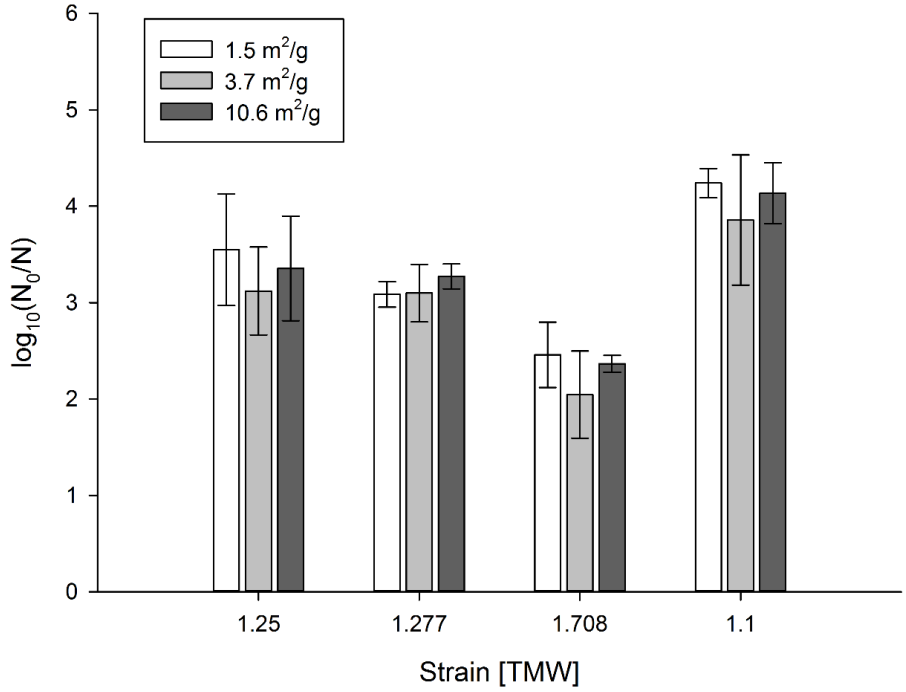

Figure 26: Effect of the fat surface on the HHP inactivation of *L. plantarum* in emulsifier-stabilized (O/W)-emulsions. The induced reduction ($log_{10}(N_0/N)$) of stationary phase cells (strains TMW 1.25, TMW 1.277, TMW 1.708 and TMW 1.1) at an inoculum of ~10^7 cells mL^{-1} by HHP (400 MPa/25 °C/5 min) is shown. Data presented are the mean values from at least three independent experiments and error bars correspond to standard deviations.

3.4.5 Effect of oil type on the HHP inactivation of *L. plantarum*

Furthermore, the effect of the oil type and thus, fatty acid composition on bacterial HHP inactivation was investigated. Rapeseed oil consists to a large extent of unsaturated fatty acids (~92.5% (w/w), Appendix Table 30), mainly oleic acid with 63.2% (w/w). In contrast to this, the synthetic oil Miglyol® 812 fully consists of short and medium chain saturated fatty acids (caprylic acid, ~55.2% (w/w); capric acid, ~44.5% (w/w)). These differences in the fatty acid composition were hypothesized to exert different fat-mediated effects on the HHP inactivation of bacterial cells.

Comparing the oil type-mediated effects on HHP inactivation, a generally lower cell inactivation was observed for Miglyol® 812 at all HPT combinations (Appendix Table 29). Maximum differences in cell inactivation between samples with rapeseed oil and Miglyol® 812 at 300 MPa/25 °C, 300 MPa/40 °C and 400 MPa/25 °C were ~0.2 log for TMW 1.277, ~0.5 log for TMW 1.708 and ~0.9 log for TMW 1.1, respectively.

A significant oil type-mediated difference in inactivation could be determined for TMW 1.277 at 300 MPa/25 °C ($p = 0.049$) and 300 MPa/40 °C ($p = 0.043$) and for TMW 1.708 at 400 MPa/25 °C ($p = 0.019$) (Figure 27).

Comparing HHP inactivation between samples with Miglyol® 812 and samples without oil, partly a tendency to slightly decreased inactivation in the presence of Miglyol® 812 could be observed (Appendix Table 29). TMW 1.25 ($p = 0.032$) and TMW 1.708 ($p = 0.038$) showed even significantly reduced cell inactivation in samples with Miglyol® 812 at 300 MPa/40 °C (unpublished data).

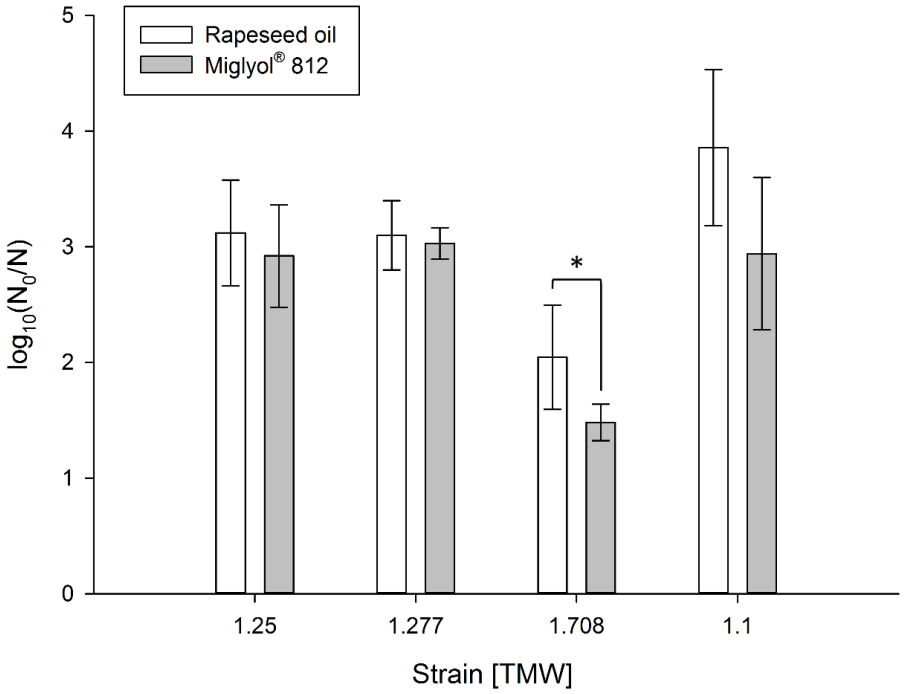

Figure 27: Effect of the oil type on the HHP inactivation of *L. plantarum* in emulsifier-stabilized (O/W)-emulsions**.** The induced reduction ($log_{10}(N_0/N)$) of stationary phase cells (strains TMW 1.25, TMW 1.277, TMW 1.708 and TMW 1.1) at an inoculum of ~10^7 cells mL^{-1} by HHP (400 MPa/25 °C/5 min) is shown. Data presented are the mean values from at least three independent experiments and error bars correspond to standard deviations. Asterisks indicate statistically significant differences between samples ($p < 0.05$).

3.5 Effect of fat and bacterial surface hydrophobicity on the HHP inactivation of *L. plantarum* in emulsifier-free (O/W)-emulsions

The presence of emulsifier was demonstrated to prevent adhesion of hydrophobic cells to oil droplets. Thus, to address the role of fat surface-cell interactions on HHP inactivation, the previously used emulsifiers were replaced by the thickener agar, resulting in solid model systems. Inactivation levels of all strains after HHP treatment at 400 MPa/25 °C/5 min are shown in Figure 28 and detailed inactivation levels are summarized in Table 31 (see Appendix).

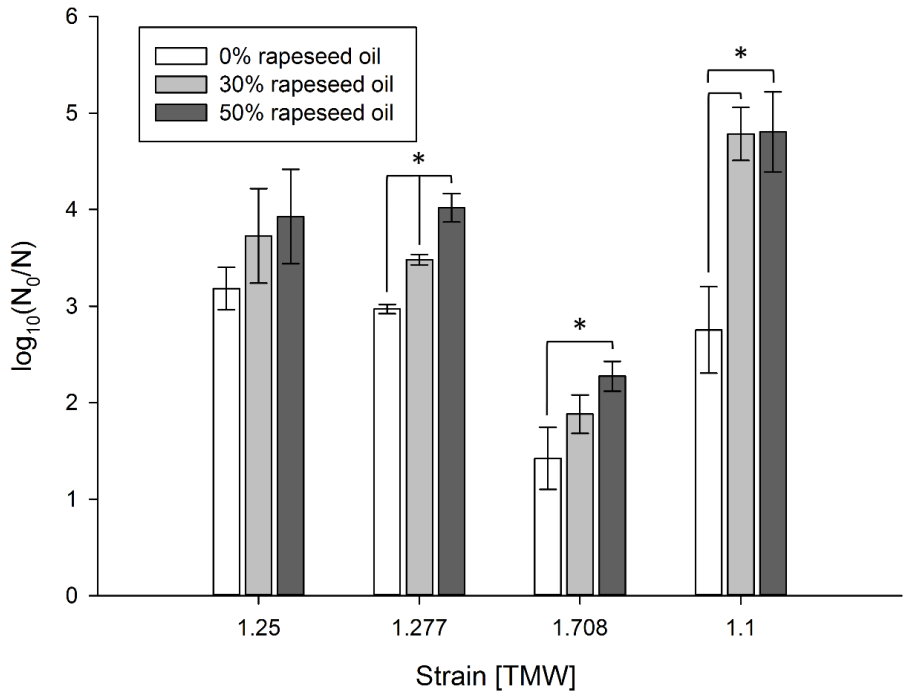

Figure 28: Effect of fat on the HHP inactivation of *L. plantarum* in emulsifier-free (O/W)-emulsions**.** The induced reduction ($log_{10}(N_0/N)$) of stationary phase cells (strains TMW 1.25, TMW 1.277, TMW 1.708 and TMW 1.1) at an inoculum of ~10^8 cells mL^{-1} by HHP (400 MPa/25 °C/5 min) is shown. Data presented are the mean values from at least three independent experiments and error bars correspond to standard deviations. Asterisks indicate statistically significant differences between samples ($p < 0.05$).

Comparing cell inactivation of samples with and without fat, a general fat-mediated increase in inactivation of both, hydrophilic and hydrophobic strains, in absence of emulsifier was observed. Significant increases in cell inactivation could be determined for TMW 1.277 and TMW 1.1 in the presence of 30% (v/v) rapeseed oil ($p = 0.002$, $p = 0.002$) and for TMW 1.277, TMW 1.708 and TMW 1.1 in the presence of 50% (v/v) rapeseed oil ($p = < 0.001$, $p = 0.011$, $p = 0.002$) (Appendix Table 31). In the presence of fat, maximum log inactivation levels reached for TMW 1.25, TMW 1.277, TMW 1.708 and TMW 1.1 were 3.93, 4.02, 2.27 and 4.81, respectively, compared to 3.18, 2.97, 1.42 and 2.75 in the absence of fat (Appendix Table 31). The maximum difference between inactivation levels of fat-containing and fat-free samples was 2.06 log for TMW 1.1 (0 - 50% (v/v) rapeseed oil), noticeably higher compared to 0.63 log for TMW 1.1 in emulsifier-stabilized (O/W)-emulsion (0 - 50% (v/v) rapeseed oil / 400 MPa/25 °C, Appendix Table 29). A tendency to higher inactivation of hydrophobic cells due to fat surface-cell interactions, which could be demonstrated in section 3.3 (Figure 15), could not be identified.

3.6 Role of WTAs in CSH and bacterial HHP sensitivity

3.6.1 Determination of CSH-related marker genes in species *L. plantarum*

In order to gain new insights regarding genes, which are associated with CSH of *L. plantarum*, genomes of hydrophilic and hydrophobic strains should be compared, potential marker genes identified and a correlation between the marker genes and CSH examined.

For the comparison of the genomes, completely sequenced genomes of *L. plantarum* strains as well as their surface characteristics (in stationary growth phase) were required. Thus, CSH of the completely sequenced *L. plantarum* strain P-8 (Zhang *et al.*, 2015) and *L. plantarum* 16 (Crowley *et al.*, 2013) was determined according to 2.2.5. Thereby, *L. plantarum* P-8 (79%) as well as *L. plantarum* 16 (80%) were identified as highly hydrophilic. To be able to compare a group of three hydrophilic with a group of three hydrophobic genomes, genomic DNA of the highly hydrophilic strain TMW 1.708 (93%) and of the hydrophobic strains TMW 1.25 (7%), TMW 1.277 (7%) and TMW 1.1623 (24%) was isolated (2.3.1), purified (2.3.2), sequenced and genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (2.8). Sequencing statistics, genome information, and accession numbers are listed in Table 19.

Table 19: Sequencing statistics, genome informations and accession numbers.

Strain	BioSample no.[a]	Accession no.[b]	Coverage (X)[c]	Size (Mb)	No. of contigs[d]	G+C Content (%)	No. of CDSs[e]
TMW 1.708	SAMN05805046	CP017374 - CP017378	250	3.24	5	44.5	2815
TMW 1.25	SAMN05805044	CP017354 - CP017362	290	3.35	9	44.3	2944
TMW 1.277	SAMN05805045	CP017363 - CP017373	247	3.40	11	44.2	2987
TMW 1.1623	SAMN05805047	CP017379 - CP017383	237	3.33	5	44.3	2919

[a]All BioSamples are part of BioProject PRJNA343197.
[b]Accession numbers are listed for all contigs of each whole genome (as a range).
[c]Average coverage of HGAP assembly.
[d]In chromosome plus plasmids and partial plasmids.
[e]CDSs, coding sequences (total) based on NCBI PGAP.

The chromosome sizes range from 3.09 to 3.14 Mb, with G+C contents of 44.6% to 44.7%. Plasmid number ranged from 4 to 10 (per strain), with G+C contents ranging from 35.0 to 55.0%. Plasmid sizes range from 0.8 to 67.9 kb, resulting in genome sizes of 3.24 to 3.40 Mb. The chromosomes encode 64 to 69 tRNAs.

Comparing the genomes of both groups on the nucleotide level by BADGE (2.8), 79 common genes could be determined in the genomes of hydrophobic strains, which are not present in the genomes of hydrophilic strains, and in turn, 17 genes in the genomes of hydrophilic strains are not present in the genomes of hydrophobic strains (Appendix Table 32 and Table 33). On protein level, 76 unique genes in genomes of hydrophobic and 20 unique genes in genomes of hydrophilic strains were found. The following bioinformatic analysis revealed genes of the WTA biosynthesis pathway as most promising genes, directly associated with the cell surface. These genes, inter alia annotated as glycerol-3-phosphate cytidylyltransferase and CDP-glycerol glycerophosphotransferase, were identified as homologues of the genes *tagD1, tagF1* and *tagF2* (*tag*-locus), which could only be identified in tested hydrophilic and were lacking in the genomes of tested hydrophobic strains. Additionally, in both, hydrophilic and hydrophobic strains, a homologue of the WTA biosynthesis pathway gene *tarK*, annotated as ribitol phosphotransferase could be identified, showing only sequence identities of 67% on nucleotide level among the two groups.

Focusing on the WTA biosynthesis pathway, all six genomes were examined regarding their WTA biosynthesis pathway gene equipment using genome information of the completely sequenced strain *L. plantarum* WCFS1 (complete and annotated genome sequence available at the NCBI database (http://www.ncbi.nlm.nih.gov/)) (Kleerebezem *et al.*, 2003) and the BLAST program on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Therewith, all genomes were analyzed in terms of WTA biosynthesis pathway genes on the nucleotide, amino acid and functional level. It was found that all genomes contain homologues of *tagO*, *tagA*, *tagB3*, *tagD2*, *tarIJKL* (*tar*-locus) and *tagGH* (Table 20). On the nucleotide as well as amino acid levels (not shown), for all homologues of *tagO*, *tagA*, *tagB3*, *tagD2* and *tagGH*, sequence identities of 99 to 100% with already described homologues of *L. plantarum* WCFS1 could be determined (Bron *et al.*, 2012). It was verified that homologues of the *tar*-loci of hydrophilic and hydrophobic strains differ by sharing gene sequence identities of only 67 to 90% (nucleotide level, Table 20) and 63 to 90% (amino acid level, not shown) and that these differences are conserved among these two groups (99% sequence similarity, 100% coverage). Additionally, as described before, it could be demonstrated that from the tested strains only hydrophilic ones contain a homologue of the additional *tag*-locus.

Table 20: Genes involved in WTA biosynthesis. Gene-identifiers indicate *tag* and *tar* homologues in genomes of *L. plantarum* strains WCFS1, P-8, 16, TMW 1.708, TMW 1.25, TMW 1.277 and TMW 1.1623. Similarities and E-values were searched for, using the nucleotide-nucleotide BLAST program on the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast). The sequences of the *L. plantarum* WCFS1 gene homologues of all listed WTA biosynthesis genes were quoted from GenBank and used as basis for bioinformatic analysis (accession no.: AL935263.2). n.a., not available.

	tagO	***tagA***	***tagB3***	***tagD2***	***tagD1***	***tagF1***	***tagF2***
WCFS1	*lp_0730*	*lp_0564*	*lp_1977*	*lp_1248*	*lp_0267*	*lp_0268*	*lp_0269*
P-8	*LBP_RS02635*	*LBP_RS02215*	*LBP_RS07545*	*LBP_RS04595*	*LBP_RS01095*	*LBP_RS01100*	*LBP_RS01105*
Query cover (%)	100	100	100	100	100	100	100
Identity (%)	99	99	99	100	100	99	99
E-value	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	*LP16_RS02850*	*LP16_RS02430*	*LP16_RS07725*	*LP16_RS04810*	*LP16_RS01135*	*LP16_RS01140*	*LP16_RS01145*
Query cover (%)	100	100	100	100	100	100	100
Identity (%)	99	99	99	100	100	99	99
E-value	0	0	0	0	0	0	0
TMW 1.708	*BIZ33_RS03070*	*BIZ33_RS02275*	*BIZ33_RS08150*	*BIZ33_RS05170*	*BIZ33_RS01080*	*BIZ33_RS01085*	*BIZ33_RS01090*
Query cover (%)	100	100	100	100	100	100	100
Identity (%)	99	100	99	100	98	99	97
E-value	0	0	0	0	0	0	0
TMW 1.25	*BIZ31_RS02960*	*BIZ31_RS02490*	*BIZ31_RS08275*	*BIZ31_RS05150*	n.a.	n.a.	n.a.
Query cover (%)	100	100	100	100	n.a.	n.a.	n.a.
Identity (%)	99	99	99	99	n.a.	n.a.	n.a.
E-value	0	0	0	0	n.a.	n.a.	n.a.
TMW 1.277	BIZ32_RS02960	BIZ32_RS02490	BIZ32_RS08075	BIZ32_RS05150	n.a.	n.a.	n.a.
Query cover (%)	100	100	100	100	n.a.	n.a.	n.a.
Identity (%)	99	99	99	99	n.a.	n.a.	n.a.
E-value	0	0	0	0	n.a.	n.a.	n.a.
TMW 1.1623	BIZ34_RS02845	BIZ34_RS02380	BIZ34_RS08290	BIZ34_RS04925	n.a.	n.a.	n.a.
Query cover (%)	100	100	100	100	n.a.	n.a.	n.a.
Identity (%)	99	99	99	100	n.a.	n.a.	n.a.
E-value	0	0	0	0	n.a.	n.a.	n.a.

	tagG	***tagH***	***tarI***	***tarJ***	***tarK***	***tarL***
WCFS1	*lp_0343*	*lp_0344*	*lp_1816*	*lp_1817*	*lp_1818*	*lp_1819*
P-8	*LBP_RS01440*	*LBP_RS01445*	*LBP_RS06845*	*LBP_RS06850*	*LBP_RS06855*	*LBP_RS06860*
Query cover (%)	100	100	100	100	100	100
Identity (%)	99	99	99	100	99	100
E-value	0.0	0.0	0.0	0.0	0.0	0.0
16	LP16_RS01480	LP16_RS01485	LP16_RS07050	LP16_RS07055	LP16_RS07060	LP16_RS07065
Query cover (%)	100	100	100	100	100	100
Identity (%)	99	99	99	100	99	99
E-value	0	0	0	0	0	0
TMW 1.708	BIZ33_RS01430	BIZ33_RS01435	BIZ33_RS07470	BIZ33_RS07475	BIZ33_RS07480	BIZ33_RS07485
Query cover (%)	100	100	100	100	100	100
Identity (%)	99	99	99	99	100	100
E-value	0	0	0	0	0	0
TMW 1.25	BIZ31_RS01455	BIZ31_RS01460	BIZ31_RS07550	BIZ31_RS07555	BIZ31_RS07560	BIZ31_RS07565
Query cover (%)	100	100	99	100	97	95
Identity (%)	99	100	73	73	67	90
E-value	0	0	4,00E-114	1,00E-176	7,00E-78	0
TMW 1.277	BIZ32_RS01455	BIZ32_RS01460	BIZ32_RS07550	BIZ32_RS07555	BIZ32_RS07560	BIZ32_RS07565
Query cover (%)	100	100	99	100	97	95
Identity (%)	99	100	73	73	67	90
E-value	0	0	4,00E-114	1,00E-176	7,00E-78	0
TMW 1.1623	BIZ34_RS01455	BIZ34_RS01460	BIZ34_RS07570	BIZ34_RS07575	BIZ34_RS07580	BIZ34_RS07585
Query cover (%)	100	100	99	100	97	95
Identity (%)	99	100	73	73	67	90
E-value	0	0	4,00E-114	1,00E-176	9,00E-77	0

3.6.2 Correlation between WTA type and CSH

In line with the findings of 3.6.1 it was hypothesized that hydrophilic strains possess a conserved WTA biosynthesis cluster that is synthesizing poly(Gro-P) WTAs while hydrophobic strains, lacking the *tag*-locus and showing conserved differences in *tar*-loci, synthesize poly(Rbo-P) WTAs (Tomita *et al.*, 2010, Bron *et al.*, 2012). In order to proof this potential correlation between the WTA type and CSH, primers were designed from sequences inside the genes of interest (homologues of *tagF1*; poly(Gro-P) WTA-associated homologues of *tar*-locus, hereinafter referred to as *tagIJKL*; poly(Rbo-P) WTA-associated homologues of *tar*-locus) by Clone Manager 5 (Scientific & Educational Software, Denver, USA) and used for screening of additional 32 bacterial strains for these genes by colony PCR (2.3.3). Then, the CSH of all additional strains was determined according to 2.2.5. The results are summarized in Table 21.

The results of the bioinformatic analysis could be confirmed by DNA amplification, resulting in the specific WTA gene patterns that were determined before (3.6.1) for the six completely sequenced *L. plantarum* strains. 36 of the tested 38 strains showed either the WTA gene pattern specific for biosynthesis of poly(Gro-P) or poly(Rbo-P) WTAs and thus, two distinct groups among strains of the species *L. plantarum* could be identified. *L. plantarum* TMW 1.1342 and TMW 1.1 showed no clear gene amplification pattern and thus could not be assigned to any of the two identified groups. However, contradicting the hypothesis that strains with hydrophobic cell surface produce poly(Rbo-P) WTAs while strains with hydrophilic cell surface synthesize poly(Gro-P) WTAs, for strains with highly hydrophobic cell surface (TMW 1.1723, SF_02, TMW 1.1830), gene patterns that are specific for the synthesis of poly(Gro-P) WTAs were determined. Moreover, the highly hydrophilic strains TMW 1.1662, TMW 1.1478, TMW 1.9 and TMW 1.1356 possess the gene equipment for the synthesis of poly(Rbo-P) WTAs. Accordingly, no correlation between the WTA type and CSH could be observed.

Table 21: WTA biosynthesis gene equipment of *L. plantarum* strains varying in cell surface hydrophobicity. Surface hydrophobicity was determined by MATH test using bacterial cells in stationary growth phase. WTA biosynthesis gene equipment of all strains was examined by colony PCR. Successfully amplified genes are marked with "+", unsuccessful amplification of genes is marked with "-". *tagIJKL* = poly(Gro-P) WTA-associated homologues of *tar*-locus. *tarIJKL* = poly(Rbo-P) WTA-associated homologues of *tar*-locus. n.as., not assignable to a WTA type.

WTA type	Strain TMW	CSH (%)*	*tagF1*	*tagI*	*tagJ*	*tagK*	*tagL*	*tarI*	*tarJ*	*tarK*	*tarL*
(Gro-P) WTA	1.1723	6±4.0	+	+	+	+	+	-	-	-	-
	SF_02	24±4.1	+	+	+	+	+	-	-	-	-
	1.1830	24±5.0	+	+	+	+	+	-	-	-	-
	1.1831	26±0.9	+	+	+	+	+	-	-	-	-
	1.835	28±4.2	+	+	+	+	+	-	-	-	-
	1.1237	31±4.4	+	+	+	+	+	-	-	-	-
	WCFS1	46±8.3	+	+	+	+	+	-	-	-	-
	1.817	49±6.0	+	+	+	+	+	-	-	-	-
	1.829	51±4.0	+	+	+	+	+	-	-	-	-
	1.322	52±5.2	+	+	+	+	+	-	-	-	-
	1.834	54±5.5	+	+	+	+	+	-	-	-	-
	1.811	62±15.7	+	+	+	+	+	-	-	-	-
	P-8	79±12.2	+	+	+	+	+	-	-	-	-
	16	80±8.5	+	+	+	+	+	-	-	-	-
	1.1204	84±5.7	+	+	+	+	+	-	-	-	-
	1.708	93±1.7	+	+	+	+	+	-	-	-	-
	1.468	94±6.2	+	+	+	+	+	-	-	-	-
	1.1808	95±5.6	+	+	+	+	+	-	-	-	-
(Rbo-P) WTA	1.25	7±5.3	-	-	-	-	-	+	+	+	+
	1.277	7±4.2	-	-	-	-	-	+	+	+	+
	1.1810	18±3.4	-	-	-	-	-	+	+	+	+
	1.1647	27±1.6	-	-	-	-	-	+	+	+	+
	1.1516	27±2.7	-	-	-	-	-	+	+	+	+
	1.1611	27±1.8	-	-	-	-	-	+	+	+	+
	1.186	31±0.8	-	-	-	-	-	+	+	+	+
	1.1623	34±4.6	-	-	-	-	-	+	+	+	+
	1.1609	36±4.5	-	-	-	-	-	+	+	+	+
	1.190	40±7.1	-	-	-	-	-	+	+	+	+
	1.1789	44±11.8	-	-	-	-	-	+	+	+	+
	1.1792	47±5.9	-	-	-	-	-	+	+	+	+
	1.409	62±17.6	-	-	-	-	-	+	+	+	+
	1.1671	72±0.2	-	-	-	-	-	+	+	+	+
	1.1662	81±0.9	-	-	-	-	-	+	+	+	+
	1.1478	84±6.6	-	-	-	-	-	+	+	+	+
	1.9	85±5.6	-	-	-	-	-	+	+	+	+
	1.1356	100±1.0	-	-	-	-	-	+	+	+	+
n.as.	1.1342	32±8.8	-	+	+	+	+	-	-	-	-
	1.1	99±1.8	+	-	+	+	-	+	-	-	-

*Percentage absorbance of the aqueous phase after treatment with n-hexadecane (0.4 mL volume) relative to initial absorbance. Each value represents the mean of at least three independent determinations.

3.6.3 Effect of WTA type on CSH of *L. plantarum* WCFS1

In order to examine a possible effect of WTAs in general on CSH in more detail, the completely sequenced wildtype strain *L. plantarum* WCFS1 as well as WCFS1 gene deletion mutants were exploited. Using deletion mutants, sharing almost identical genomes with the parental strain, other possible CSH-influencing factors could be completely excluded.

To study the effects of WTA removal, the mutant *L. plantarum* WCFS1 ΔtagO was used. Here, the single copy of *tagO* was deleted, which is the first enzyme in the WTA biosynthesis pathway and thus, essential for WTA biosynthesis (Figure 2) (Andre *et al.*, 2011). The second deletion mutant exploited, was *L. plantarum* WCFS1 ΔtagF1-F2, which lacks the genes *tagF1* and *tagF2*, necessary for the synthesis of the poly(Gro-P) backbone (Bron *et al.*, 2012). As demonstrated by Bron *et al*. (2012), the WCFS1 ΔtagF1-F2 mutant produces WTAs with a poly(Rbo-P) backbone instead of poly(Gro-P). Thus, using these three strains, the direct effects mediated by poly(Gro-P) WTAs, poly(Rbo-P) WTAs and the absence of WTAs on CSH could be examined.

The morphological characteristics and WTA gene equipment of all cells were examined by microscopic analysis and PCR amplification, respectively. Microscopic analysis revealed that cells of the wildtype strain and the WCFS1 ΔtagF1-F2 mutant showed the tendency to remain associated in short chains. This was not observable for cells of the WCFS1 ΔtagO mutant, which were showing chiefly no chains, but instead of this a tendency to cell aggregation. Additionally, these cells appeared swollen and shorter than cells of the parental and mutant strain WCFS1 ΔtagF1-F2 (data shown in Figure 29).

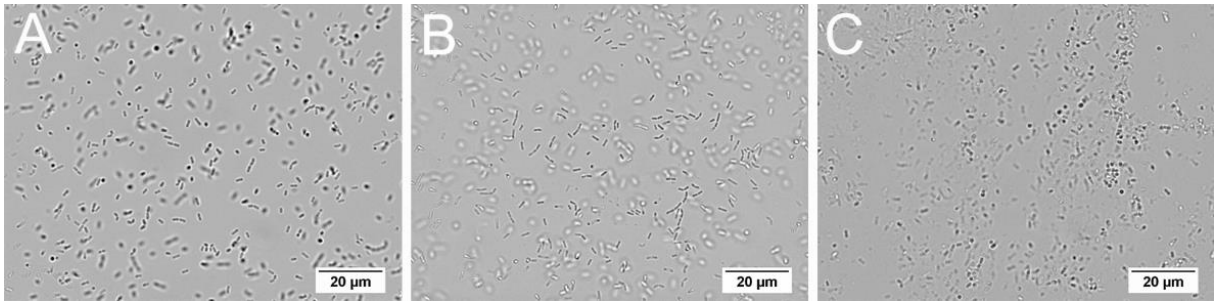

Figure 29: Microscopic analysis of *L. plantarum* WCFS1 wildtype strain and WCFS1 gene deletion mutants. (A) *L. plantarum* WCFS1; (B) *L. plantarum* WCFS1 ΔtagF1-F2; (C) *L. plantarum* WCFS1 ΔtagO.

The gene patterns obtained by PCR matched well with the theoretical gene patterns specific for poly(Gro-P)-, poly(Rbo-P)- and no WTA biosynthesis (Table 22). The identified gene pattern of the wildtype strain was representative for the biosynthesis of poly(Gro-P) WTAs, as already reported in 3.6.2. Unsuccessful amplification of the gene regions of *tagO* and *tagF1* indicate successful deletion of the mentioned genes in the corresponding deletion mutants.

Determination of CSH by MATH test revealed a moderate hydrophobic cell surface for the wildtype (46%) and highly hydrophilic cell surfaces for WCFS1 ΔtagO (77%) and WCFS1 ΔtagF1-F2 (96%) mutants (Table 22).

Table 22: WTA biosynthesis gene equipment of *L. plantarum* WCFS1 wildtype strain and WCFS1 gene deletion mutants. CSH was determined by MATH test using bacterial cells in stationary growth phase. WTA biosynthesis gene equipment of all strains was examined by colony PCR. Successfully amplified genes are marked with "+", unsuccessful amplification of genes is marked with "-". *tagIJKL* = poly(Gro-P) WTA-associated homologues of *tar*-locus. *tarIJKL* = poly(Rbo-P) WTA-associated homologues of *tar*-locus.

Strain	CSH (%)*	*tagO*	*tagF1*	*tagI*	*tagJ*	*tagK*	*tagL*	*tarI*	*tarJ*	*tarK*	*tarL*
WCFS1	46±8.3	+	+	+	+	+	+	-	-	-	-
WCFS1 ΔtagO	77±6.8	-	+	+	+	+	+	-	-	-	-
WCFS1 ΔtagF1-F2	96±2.6	+	-	+	+	+	+	-	-	-	-

*Percentage absorbance of the aqueous phase after treatment with n-hexadecane (0.4 mL volume) relative to initial absorbance. Each value represents the mean of at least three independent determinations.

3.6.4 Effect of WTA type on the HHP inactivation of *L. plantarum* WCFS1

In order to study a potential direct effect of WTAs per se and the WTA type on bacterial HHP sensitivity, stationary phase cells of the wildtype strain WCFS1 and of both WCFS1 deletions mutants ΔtagO and ΔtagF1-F2 were pressurized (500 MPa/25 °C/5 min).

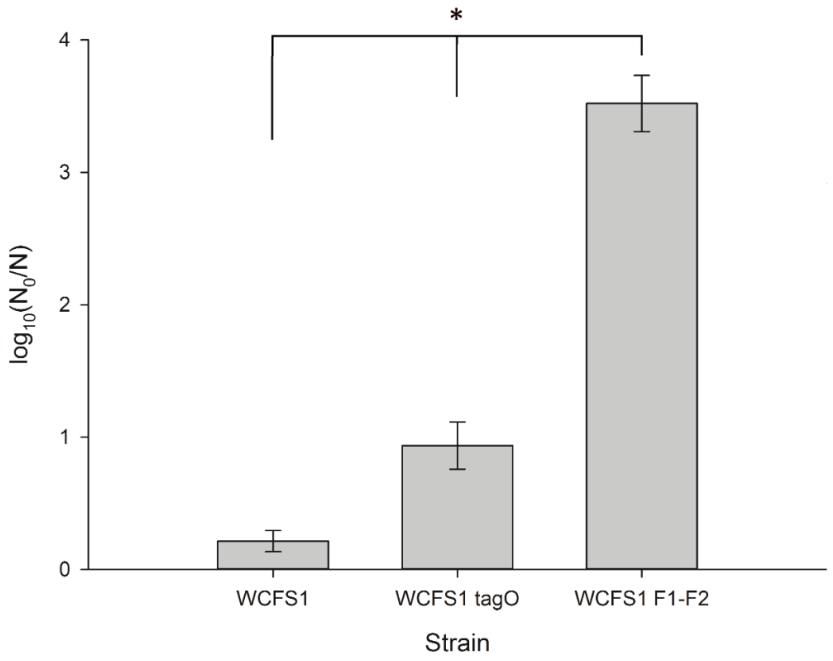

Figure 30: Effect of WTAs per se and the WTA type on the HHP inactivation of *L. plantarum* WCFS1 in IPB. The induced reduction ($log_{10}(N_0/N)$) of stationary phase cells (wild-type strain WCFS1, poly(Gro-P) WTAs; WCFS1 ΔtagO, no WTAs; WCFS1 ΔtagF1-F2, poly(Rbo-P) WTAs) at an inoculum of ~10^7 cells mL^{-1} by HHP (500 MPa/25 °C/ 5 min) is shown. Data presented are the mean values from at least three independent experiments and error bars correspond to standard deviations. Asterisks indicate statistically significant differences between samples ($p < 0.05$).

Figure 30 illustrates that the WTA type and the presence of WTAs per se had a substantial effect on the HHP inactivation of *L. plantarum* WCFS1, demonstrated by significant differences in inactivation levels ($p < 0.001$). Comparing cell inactivation of the parental strain and the deletion mutants ΔtagO and ΔtagF1-F2, the lowest inactivation level and thus highest HHP resistance could be observed for the wildtype strain with ~0.2 log (0.21±0.08), followed by the mutant WCFS1 ΔtagO with a log inactivation level of ~1.0 (0.94±0.18), which produce no WTAs at all. The mutant WCFS1 ΔtagF1-F2, synthesizing poly(Rbo-P) WTAs, showed the highest HHP sensitivity with a maximum log inactivation level of ~3.5 (3.52±0.21).

4 Discussion

Systematic studies regarding the effect of fat, lipid phase parameters and microbial localization on the HHP inactivation of *L. plantarum* in defined (O/W)-emulsion were accomplished. The presence of fat per se was demonstrated to increase or decrease the HHP inactivation of *L. plantarum*, dependent on the emulsion type and oil type. Despite of distinct intraspecies variations in the pressure tolerance of different *L. plantarum* strains, the effect of fat appears to be a complex interplay of numerous factors, however, presumably independent from the CSH and associated cell localization. Additionally, the fat surface and the fat/water boundary layer thickness have been shown to have no effect on HHP inactivation. The comparison of genomes of hydrophilic and hydrophobic strains of the species *L. plantarum* revealed genes associated with the WTA biosynthesis pathway as most promising marker genes related to CSH. Investigations regarding the role of WTAs in the development of a specific CSH phenotype showed no correlation between the WTA type and CSH, however, tested *L. plantarum* strains were proved to encode the genetic determinants for the production of either poly(Gro-P) or poly(Rbo-P) WTAs. Excluding effects that are based on intraspecies biodiversity, the presence of WTAs per se and the WTA type were observed to significantly affect bacterial CSH and pressure tolerance of *L. plantarum* WCFS1.

Based on the results of this thesis, initial working hypotheses (➢) (see section 1.5) can be refined and new theses (○) as well as new theses in a wider sense (▪) can be posted. Refined working hypotheses and new thesis are listed below. Differences between initial and refined working hypothesis are shown by highlighted key words and new, deduced thesis are indicated by italics.

(I) Effect of fat and lipid phase parameters on HHP inactivation in (O/W)-emulsions

- ➢ Fat per se can affect HHP inactivation, and an increase in the fat content correlates with the extent of fat-mediated effects on HHP inactivation.
  - ○ *The presence of rapeseed oil can increase the HHP inactivation of vegetative cells and higher fat content results in increased HHP inactivation.*
    - ▪ *Slightly higher temperatures of the overall examination system due to stronger adiabatic heating of fat can result in combination with HHP in a higher cell inactivation.*
    - ▪ *The compression heat of fat can be used consciously to reach process target temperature and thus process costs can be reduced.*

- *The presence of Miglyol® 812 can offer a baroprotective effect to vegetative cells.*
- *Pressure sensitivity can vary significantly within one species and must be considered when setting up processing regimes designed to inactivate microorganism.*
  - *HHP inactivation parameters must be adjusted to the most resistant bacteria in the food of interest in order to ensure microbial safety.*

- The emulsifier type and, thus, the thickness of the fat-water boundary layer does not have an effect on the HHP inactivation and might be neglected.
- The droplet size and, therefore, the fat surface area available for microbial adhesion does not influence effects exerted by fat on the HHP tolerance of bacterial cells and might be neglected.
- Different oil types with a different fatty acid composition (saturated *versus* unsaturated) can lead to differences in the HHP inactivation.
  - *Oil type-dependent differences in the HHP inactivation of vegetative cells are dependent on the fatty acid composition and individual fatty acids.*
    - *Unsaturated fatty acids (oleic acid, linoleic acid) might enhance the HHP inactivation of vegetative microorganisms, whereas saturated fatty acids might exert a baroprotective effect against microbial HHP inactivation.*

(II) Role of CSH and bacterial localization on HHP inactivation in (O/W)-emulsions

- The presence of emulsifier in emulsifier-stabilized emulsions prevents adhesion of hydrophobic cells to the fat surface of oil droplets due to fully emulsifier-coated oil droplets. CSH-associated differences in HHP sensitivity are not dependent on direct fat surface-cell interactions.
- The absence of emulsifier in emulsifier-free emulsions facilitates the adhesion of hydrophobic cells to the fat surface of oil droplets, which, however, does not lead to differences in the HHP inactivation efficiency of hydrophobic and hydrophilic strains.
  - *A protective effect of fat, based on fat surface-cell interaction and microbial localization in the (O/W)-emulsion, is unlikely. CSH seems to be no intrinsic pressure resistance factor.*

- *Direct fat surface-cell interaction might facilitate the interchange of unsaturated triglycerides, leading to altered membrane permeability, which results in a decreased pressure resistance.*
- *Hydrophobic cells might respond to the direct contact to fat with a change in their metabolism, which leads to an increased pressure sensitivity.*

➢ Direct fat surface-cell interaction might favor HHP inactivation through exposure of cells to locally higher temperatures caused by stronger adiabatic heating of fat.

(III) Identification of CSH-associated marker genes in genomes of the species *L. plantarum*

➢ Genome comparison of hydrophilic and hydrophobic strains can reveal marker genes that are characteristic for a specific CSH phenotype. The CSH of other strains of the species *L. plantarum* cannot be necessarily predicted upon the presence or absence of these marker genes, since effects of other cell components, which are involved in the development of the CSH phenotype, can dominate or mask the effects on CSH that are mediated by the marker genes.

- *WTAs are involved in the development of the CSH phenotype of Gram-positive cells.*
- *Strains of the species L. plantarum form two WTA type-specific groups, i.e., strains that synthesize poly(Gro-P) WTAs and strains that synthesize poly(Rbo-P) WTAs.*
- *Poly(Rbo-P) WTAs-producing strains share conserved WTA biosynthesis gene pattern, which differ from the conserved gene pattern of poly(Gro-P) WTAs-producing strains by the lack of the tag-locus and differences in nucleotide sequences of the tar-locus.*
  - *The WTA type in the cell wall of L. plantarum can be estimated by carrying out simple bioinformatic analysis or colony PCR using WTA type specific primer.*
  - *L. plantarum strains, which synthesize different WTA types, can share the same habitat.*
- *The WTA type does not correlate with a specific CSH phenotype, since intraspecies differences can conceivably mask a possible WTA type-dependent effect on bacterial CSH.*

- Alterations in CSH-associated marker genes in a deletion mutant strain result in pronounced physiological effects on cell surface characteristics, since interfering effects of other CSH-modulating cellular components and intraspecies biodiversity effects are excluded.
    - *The presence of WTAs and the WTA type have an effect on the CSH phenotype of L. plantarum WCFS1.*
        - *The absence of WTAs leads to the unmasking of cell wall proteins and polysaccharides, which convey type-specific surface characteristics and are not implicated in CSH in the presence of WTAs.*
        - *In the absence of WTAs, the CSH-influencing effect of LTAs is more determinative and leads to changes in cell surface characteristics.*
        - *Alditol-specific substitution patterns and related differences in polymer structure and cell surface charge manifest in different CSH phenotypes.*
    - *The presence of WTAs and the WTA type have an effect on the cells' pressure sensitivity.*
        - *In the absence of WTAs, LTAs partially compensate the absence of WTAs and thus, maintain a certain pressure resistance of the cells.*
        - *Alditol-switching from Gro-P to Rbo-P leads to an increased pressure sensitivity of L. plantarum WCFS1.*

4.1 Establishment of stable (O/W)-emulsion as model food system

In order to perform systematic studies to examine the effect of fat and lipid phase parameters on HHP inactivation, it had to be ensured that the established (O/W)-emulsions were pressure-stable and that the set-in microorganisms did not possess destabilizing activities. The emulsifier-stabilized (O/W)-emulsions were found to remain stable after HHP treatment at 500 MPa at 25 and 55 °C for 5 min, since no significant change in median droplet size, fat surface and creaming velocity of all established emulsions could be determined (Figure 5 and Figure 6). Comparisons with data from literature are limited, since findings regarding the high pressure-stability of emulsions are rare. In addition, comparisons are impeded by partly significant variations in the emulsion composition and HHP treatment parameters.

In line with our observations, Simpson and Gilmour (1997) found that (O/W)-emulsions (30% (v/v) olive oil) remained stable when treated at 900 MPa for 30 min. Furthermore, Karbstein *et al*. (1992) reported that (O/W)-emulsions (20% (v/v) soybean oil) tended to remain stable when treated with pressures up to 600 MPa at 40 °C. However, contrary, the latter study simultaneously demonstrated that (O/W)-emulsions with pH < 4 tended to lose their stability when treated with pressure.

Moreover, HHP has been reported to decrease emulsion stability by changing the protein conformation of emulsifiers and by pressure-mediated fat crystallization, resulting in emulsion breakdown (Darling & Birkett, 1987, Desrumaux & Marcand, 2002). In this context, studies of Boekel (1980) revealed that the emulsion breakdown is induced by fat crystals penetrating the intervening surfactant film between two oil droplets, thereby forming a lipid bridge. Due to interfacial forces, the lipid bridge is stated to grow rapidly and thus to form a strong sintered bond between the two emulsifier-coated droplets, which finally results in their coalescence (van Boekel, 1980, Darling & Birkett, 1987). Even though this thesis demonstrated that the impact of (O/W)-emulsion stability was clearly more affected by the varied lipid phase parameters than by HHP treatment (see 3.1.2), the reported findings in literature clarify that each emulsion, which is used for HHP treatments, should be checked individually for its pressure stability. In the course of this, it is recommended that special attention should be paid to the parameters pH value, fat content, median droplet size as well as type of emulsifier and oil used.

Destabilization of emulsions by microbial activities was prevented by investigating the potential of used microorganisms to degrade fat and the set-in emulsifiers Tween® 80 and sodium caseinate. Growing selected strains on TB-mMRS agar plates, no halos surrounding the grown colonies could be determined (Figure 32). Using nutrient-poorer cultivation medium (2.5 g L^{-1} casein peptone; 2.5 g L^{-1} peptone from meat; 3.0 g L^{-1} yeast extract; 10.0 g L^{-1} TB; 15.0 g L^{-1} agar) instead of the complex medium TB-mMRS, also no observations indicating bacterial lipolytic activity, could be made (unpublished data). Although the presence of lipases and esterases was already described in *Lactobacillus spp*. and especially in the species *L. plantarum*, *Lactobacillus spp*. are generally considered to be weakly lipolytic in comparison to other species (Oterholm *et al.*, 1972, Khalid & Marth, 1990, Fox *et al.*, 1993, Gobbetti *et al.*, 1996, Gobbetti *et al.*, 1997, Lopes *et al.*, 1999, Lopes *et al.*, 2002, Esteban-Torres *et al.*, 2014). Therefore, significant bacterial production of lipases and esterases, threatening emulsion stability during HHP processing, could be largely excluded.

Likewise, investigations of the growth of bacterial strains in IPB supplemented with Tween® 80 as well as the measurements of changes in fluorescence properties of the substrate FTC-casein as a result of microbial proteolytic digestion, indicated that selected strains did not possess considerable degrading activities with respect to both types of emulsifier used (Figure 10, Table 16). However, according to literature, all three, tested species are supposed to possess proteolytic activities, even though they seem so vary among the species (Spicher & Nierle, 1984, Spicher & Nierle, 1988, Kunji *et al.*, 1996, Fadda *et al.*, 1999, Sanz *et al.*, 1999, Basso *et al.*, 2004). But regardless of the stated proteolytic activity, it has to be considered that cells of *L. plantarum* were added to emulsion shortly before HHP processing and hence degradation of the emulsifier sodium caseinate is very unlikely. This assumption is promoted by the study of Khalid & Marth (1990), demonstrating that *L. plantarum* NRRL B-4004 began to hydrolyse milk protein, especially β-casein, only after 125 h of growth in sterile skim milk at 37 °C.

Further, it could be speculated that bacterial cells, dependent on their cell surface characteristics, interact with emulsifiers and therewith decrease the emulsions' stability. Ly *et al*. (2008) showed that the stability of emulsions, which were only stabilized by ionic surface-active compounds, was decreased in the presence of bacteria, when bacteria had a surface charge opposite to the one of the emulsion droplets. In addition, Ly *et al*. (2008) also observed aggregation and flocculation phenomena for emulsions stabilized with cationic surfactant, which were pronounced in the presence of more negatively charged bacteria. In line with those findings, mainly using the non-ionic emulsifier Tween® 80 in this thesis, no effect of bacterial cells on the stability of all tested emulsions could be determined.

4.2 CSH-dependent localization of spoilage-associated microorganisms in emulsifier-stabilized and emulsifier-free (O/W)-emulsions

Before the implementation of systematic HHP inactivation studies, which were focusing on the role of fat and CSH-related bacterial localization in HHP, appropriate strains were selected and CSH-specific adhesion to oil droplets examined. For this, the strains were selected according to the following criteria: (I) strains should vary distinctly in the CSH phenotype (highly hydrophilic versus highly hydrophobic), (II) strains should show a good spoilage potential and, (III) strains should not possess any significant emulsion-destabilizing activities (see previous chapter).

4.2.1 Spoilage potential of *Lactobacillus* species

The spoilage potential of selected strains of the species *L. plantarum*, *L. sakei* and *L. fructivorans* was evaluated by investigating their growth under harsh conditions that are commonly used by the food industry to prevent bacterial growth and food spoilage, i.e. growth at low pH, low a_w and refrigerating temperatures. Focusing on conditions showing evaluable differences in growth of the tested species, growth at a pH of 3.5, 7.5% NaCl (a_w = 0.948) and 4 °C proved suitable for the selection of strains with the highest spoilage potential.

Growth at 10% NaCl (a_w = 0.93) resulted in no determinable proliferation of all tested species ($\mu_{max} < 0.05\ h^{-1}$) and, therefore, this parameter was not further considered. This is not surprising, since several authors showed that *Lactobacillus* species require minimal a_w values between 0.92 and 0.94 for their growth (Blickstad, 1984, Leroy & de Vuyst, 1999, Vermeiren *et al.*, 2004, Chaillou *et al.*, 2005). Overall, the findings of this section justify the selection of the species *L. plantarum* as a good representative for relevant microorganisms that are associated with spoilage of fatty foods. Considering the species' large spectrum in CSH phenotypes (see 4.2.2), this species was assumed to be the most appropriate for the implementation of systematic HHP inactivation studies.

At large, as also described in literature, growth of all tested *Lactobacillus* species varied widely among the species and within each species, making comparisons of the tested species difficult (Figure 11, Figure 12) (Cebeci & Gürakan, 2003, Sanders *et al.*, 2015). Hence, comparisons in terms of the spoilage potential were carried out as far as possible on species level.

By reducing the pH from 6.2 to 3.5, maximum cell densities of *L. plantarum*, *L. fructivorans* and *L. sakei* were partly noticeably reduced, ranging from 0.24 to 1.01, from 0.06 to 0.96 and from 0.03 to 0.80, respectively. Maximum growth rates (μ_{max}) of *L. fructivorans* were reduced by 39.3 (TMW 1.1856) up to 86.4% (TMW 1.59). For *L. plantarum* strains, reductions ranged from 43.4 (TMW 1.1) to 82.0% (TMW 1.25) for μ_{max}. The species *L. sakei* showed reductions, ranging from 65.1 (TMW 1.1239) to 100% (TMW 1.1474) for μ_{max}. Thus, all species generally possess a pronounced pH tolerance. However, the species *L. plantarum* showed the relatively least reduction and is therefore assumed to be able to maintain pH homeostasis comparably better. The high acidity tolerance of *Lactobacillus* species, noteworthy of *L. plantarum* down to a pH of 3.2, is commonly accepted in literature. This exceptional capability can be inter alia considered as one of the main features, that are responsible for *L. plantarum* being the predominant species in spoiled foodstuff (McDonald *et al.*, 1990, Bjorkroth & Korkeala, 1997, Marceau *et al.*, 2003, Eva *et al.*, 2004).

The observed differences in acidity tolerance among several strains of one species were also reported by Cebeci and Gürakan (2003), who demonstrated that 10 out of 15 *L. plantarum* strains were able to grow at pH 3.5. These data elucidate that significant phenotypic differences between strains, as also observed for high salinity and low temperature, have to be considered when targeting lactobacilli in preservation studies. Furthermore, these observations allowed to deduce that no specific species should be considered typical, since strains of all species may cause spoilage in food products.

Investigating the salinity tolerance of all species by growth at 7.5% NaCl, largely similar growth of all species could be determined. Taking into account *L. fructivorans* TMW 1.59, showing no growth at all tested conditions, maximum cell densities of *L. plantarum*, *L. fructivorans* and *L. sakei* were noticeably reduced, ranging from 0.41 to 0.79, from 0.01 to 0.78 and from 0.28 to 0.58, respectively. The maximum growth rates were correspondingly low, ranging from 0.00 to 0.21 h^{-1}. However, it seemed like strains of the species *L. sakei* in general showed a slightly decreased tolerance compared to the other species, although growth of *L. sakei* at NaCl concentrations up to 9% was demonstrated (Chaillou *et al.*, 2005). Since all three species are inter alia active in fermented foods in which salt concentrations from 0.5 to 10.0% are common, growth was likely (Maldonado *et al.*, 2002, Lu *et al.*, 2003, Chenoll *et al.*, 2006, Plengvidhya *et al.*, 2007, Pulido *et al.*, 2007, Suzuki *et al.*, 2008).

L. fructivorans was proved to have a negligible cold tolerance, showing virtually no growth at 4 °C for both strains. *L. plantarum* strains instead, could reach maximum cell densities ranging from 0.1 to 0.47 and the species *L. sakei* turned out to possess the best cold tolerance, showing maximum cell densities up to 0.82 and maximum growth rates ranging between 0.90 d^{-1} and 1.00 d^{-1}. In line with these observations, several authors already emphasized the remarkable cold tolerance of *L. sakei*, being able to proliferate at 2 - 4 °C and, thus, being one of the most psychrotrophic species of lactobacilli (Champomier-Vergès *et al.*, 2001, Vermeiren *et al.*, 2004, Chaillou *et al.*, 2005). It is likely that the observed negligible cold tolerance of *L. fructivorans* can be ascribed to its native habitat. To date, strains of *L. fructivorans* were mainly isolated from sake and beer, both beverages usually not kept at refrigerating temperature (Suzuki *et al.*, 2008, Esmaeili *et al.*, 2015).

4.2.2 CSH and its role in localization of *Lactobacillus* species in (O/W)-emulsion

CSH was determined by a modified version of the MATH test, being one of several methods accepted and commonly used for the examination of CSH (Doyle & Rosenberg, 1995). Measuring the adsorption of cells to organic solvents, the MATH test was assumed to represent best the conditions present in emulsions (Martienssen, 2001).

However, based on the fact, that many modified versions of the original MATH test according to Rosenberg *et al*. (1980) are used, the lack of standardization is likely to contribute to inconsistencies in CSH studies, which are often discussed in literature. For example, most commonly and also in this study, n-hexadecane is used as organic solvent, but also the use of n-octane, p-xylene and toluene was reported (Rosenberg *et al.*, 1980, Mozes & Rouxhet, 1987, Van Loosdrecht *et al.*, 1987, Busscher *et al.*, 1995).

Further, the amount of organic solvent varies between ca. 0.8 - 25%, times of 10 s to 5 min and 10 to 30 min are set for phase mixing and -separation, respectively, and absorbance measurements are performed in a range from 400 to 700 nm (Rosenberg *et al.*, 1980, Fiedler & Sattler, 1992, Bunt *et al.*, 1993, Van der Mei *et al.*, 1995, Kohlweyer, 2000). It is also not defined, if the distribution ratio gives the percentage of the microorganisms that have remained in the water or that have adsorbed to the organic solvent (Dickson & Koohmaraie, 1989, Fiedler & Sattler, 1992). That is why MATH test parameters should be considered and adapted when CSH should be compared with CSH of corresponding strains of different studies. Furthermore, the MATH test, in general, only estimates bulk properties of numerous cells and interprets the cell adhesion, which is based on an interplay of Lifshitz-van der Waals, hydrophobic, polar and electrostatic interactions, as CSH (Martienssen, 2001, Goulter *et al.*, 2009). In this thesis, CSH was expressed as the percentage absorbance of the aqueous phase after treatment with n-hexadecane relative to initial absorbance and cells were classified as highly hydrophobic (0 - 25%), moderately hydrophobic (26 - 50%), moderately hydrophilic (51 - 75%) and highly hydrophilic (76 - 100%).

Investigating CSH of in total 38 strains of the species *L. plantarum*, *L. sakei* and *L. fructivorans* in exponential growth phase, the whole lower and upper limit of the possible MATH spectrum was covered. While strains of the species *L. plantarum and L. fructivorans* varied in their CSH from highly hydrophobic to highly hydrophilic or moderate hydrophilic, strains of the species *L. sakei* could be only identified as moderate and highly hydrophilic. Based on these observations, it can be hypothesized that CSH can vary substantially within a species and that CSH appears to be rather strain- than species-specific. Studies of Ly *et al*. (2006), which report distinct differences in CSH of several strains of the species *Lactococcus (L.) lactis* ssp. *lactis* subv. *diacetylactis*, strengthen this hypothesis. Investigating the CSH of selected strains in stationary growth phase, a general enhancement of their cell surface characteristics over cultivation time could be observed, that is, an enhanced diversion into either a hydrophobic or hydrophilic cell surface character with prolonged cultivation time. These results are partially similar to results of Rosenberg *et al*. (1980) and Hazen *et al*. (1986), observing stronger hydrophobicity of cells of *Serratio (S.) marcescens* and *Candida (C.) species* with increasing age, respectively.

However, in contrast to the enhanced diversification into either hydrophilic or hydrophobic surface phenotypes, CSH of the described species switched from predominantly hydrophilic (logarithmic phase) to hydrophobic (stationary phase). Contrary to these findings, Patel *et al*. (2011) who evaluated five *E. coli* strains amongst other things regarding their CSH and attachment to intact and cut fresh produce leaves, reported that all strains in logarithmic phase were significantly more hydrophobic than in stationary phase.

Although findings are partly inconsistent or even contradictory, an effect of the growth phase on CSH is obvious and has to be considered when studying CSH of microorganisms.

Using fluorescence microscopy, the localization of highly hydrophobic (TMW 1.25 and TMW 1.277) and highly hydrophilic strains (TMW 1.708 and TMW 1.1) of the species *L. plantarum* in (O/W)-emulsions was examined. It was hypothesized that hydrophobic cells would adhere to oil droplets in emulsifier-free (O/W)-emulsion and that the addition of emulsifier would prevent adhesion of hydrophobic cells to the fat surface of oil droplets. In accordance with the hypothesis, highly hydrophobic cells tended to adhere to oil droplets in the absence of emulsifier and were located at the oil-water interface, whereas highly hydrophilic cells remained in the aqueous phase without showing any adhesion to oil droplets (Figure 15). Thus, these results also corresponded well with the observations made in simple oil/water mixtures, which showed the same CSH-mediated interactions in the absence of emulsion-stabilizing agar (Figure 13). Ly *et al*. (2006) reported similar findings, investigating adhesion of hydrophilic and hydrophobic strains of the species *L. lactis* ssp. *Lactis* var. *diacetylactis* to droplets of milk cream. With that, the results are in line with the commonly accepted view in literature, that more hydrophobic cells adhere more strongly to hydrophobic surfaces, whereas more hydrophilic cells did not share this affinity, adhering more strongly to hydrophilic surfaces instead (Krasowska & Sigler, 2014). Worth mentioning, in contrast to emulsifier-free (O/W)-emulsions, the oil droplets of emulsifier-stabilized emulsions were rather small (D(v,0.5) = 2.8 µm), which impeded a differentiation between the localization of hydrophilic and hydrophobic cells, since both hydrophilic and hydrophobic cells seemed to be trapped between oil droplets. Therefore, by using this setup, differences in cell-fat surface interactions due to cell surface-specific characteristics and a prevention of direct cell-fat surface interactions by the addition of emulsifier were not detectable (Figure 14).

4.3 Effect of fat and microbial localization on the HHP inactivation of *L. plantarum* in emulsifier-stabilized and emulsifier-free (O/W)-emulsion

To date, HHP inactivation studies, which deal with the effect of the food matrix constituent fat are rare and partially contradictory (Raffalli *et al.*, 1994, Simpson & Gilmour, 1997, Gervilla *et al.*, 2000, Morales *et al.*, 2006, Ramaswamy *et al.*, 2009). In addition, most of these studies were performed in complex food matrices, which allow interfering or even concealing effects that are mediated by different food matrix constituents (see 1.1.7).

Hence, based on our previous knowledge, the effect of fat per se on the HHP inactivation of vegetative cells still remained unclear. In order to close the substantial gap of knowledge, systematic inactivation studies in model emulsion systems, investigating solely the effect of fat and fat-associated parameters, were accomplished. There, to consider an effect of fat based on fat surface-cell interactions, *L. plantarum* strains with distinct differences in CSH were used.

4.3.1 HHP inactivation in emulsifier-stabilized (O/W)-emulsion

Performing studies in buffer and defined (O/W)-emulsion model systems revealed that some of the tested strains varied significantly regarding their pressure sensitivity. Namely, strain TMW 1.1 showed the lowest temperature sensitivity but the highest pressure sensitivity among all strains. This may support the common suggestion that pressure/temperature sensitivity follow different, strain-specific mechanisms. Similarly, Pagán and Mackey (2000) showed significant differences in the HHP resistance of three strains of the species *E. coli*. Casal and Gómez (1999) reported large differences in the viability of different *Lactococcus lactis ssp. lactis* strains after pressure treatment in milk suspension and Alpas *et al.* (1999) and Patterson *et al*. (1995) demonstrated distinct differences in pressure resistance among different strains of various food-borne pathogens. These findings clarify that such variation in pressure sensitivity among bacterial species and even within one species must be considered when setting up processing regimes designed to inactivate microorganism or making recommendations for commercial processing of foods. In order to ensure microbial safety, the recommendations should be based on information obtained from the most resistant bacteria in the food of interest.

In contrast to the lipid phase parameters fat surface and fat/water boundary layer thickness, the presence of fat per se and the lipid phase parameter, oil type, had an effect on HHP inactivation.

The presence of rapeseed oil affected the HHP inactivation of both hydrophilic strains (TMW 1.708 and TMW 1.1) in emulsifier-stabilized (O/W)-emulsions. In most cases, a tendency towards an increase in HHP inactivation with higher fat content could be observed. However, a significant increase in cell inactivation could be only determined for these strains after treatments at 300 MPa/40 °C or 400 MPa/25 °C. Interestingly, no effect of rapeseed oil on the HHP inactivation of the hydrophobic strains (TMW 1.25 and TMW 1.277) could be observed. Replacing rapeseed oil by Miglyol® 812, partly a tendency to slightly decreased inactivation could be observed. At a HPT-combination of 300 MPa/40 °C, Miglyol® 812 even exerted a significant baroprotective effect on TMW 1.708 and TMW 1.25.

These findings elucidate the difficulty to define the effect of fat on HHP inactivation. On the one hand, the results of this study are partly in line with studies, reporting no effect of fat, as inter alia observed for the hydrophobic strains in emulsifier-stabilized (O/W)-emulsions with rapeseed oil (Raffalli *et al.*, 1994, Rademacher, 1999, Gervilla *et al.*, 2000, Ramaswamy *et al.*, 2009). On the other hand, the results are also partly in accordance with studies from Simpson and Gilmour (1995) and Morales *et al*. (2006), which demonstrated reduced bacterial HHP inactivation in the presence of fat, as also observed for especially TMW 1.25 and TMW 1.708 in the presence of Miglyol® 812. Noteworthy, none of these studies reported increased HHP inactivation in the presence of fat. It can be speculated that this is due to their use of mostly complex food matrices. Quite likely, most of the effects, which are mediated by different food matrix constituents, are baroprotective effects (see section 1.1.7). As a result, these effects can interfere and conceal possible fat-mediated, HHP inactivation increasing effects, as observed for rapeseed oil. However, using defined (O/W)-emulsion systems, these undesired effects were excluded, which facilitated the determination of actual fat-mediated effects and influence factors. These and possible explanations for these will be discussed in the following.

Rapeseed oil can increase HHP inactivation

A possible explanation for the determined increase in cell inactivation in the presence of rapeseed oil and with higher amounts of rapeseed oil could be a correlating increase of the emulsion's temperature due to adiabatic heating, enhancing the HHP inactivation efficiency. By shifting the ratio of IPB and rapeseed oil in favor of oil, i.e. from 100:0, 70:30, 50:50 to 30:70 (v/v), it can be assumed that the whole system heats up more by the distinctly stronger adiabatic heating of rapeseed oil compared to buffer. Even though HHP treatments were temperature controlled and thus theoretical ideal heating rates of 2.7, 3.9, 4.0 and 5.4 °C/100 MPa (at an initial temperature: 25 °C) for IPB, emulsions with 30, 50 and 70% (v/v) rapeseed oil, could be substantially reduced, respectively, a slight warming of the model systems could not be completely prevented (Table 17).

Maximum temperature deviations from the target treatment temperatures of 25 and 40 °C, which were determined during HHP processing of IPB and emulsions with 30, 50 and 70% (v/v) rapeseed oil, were +1.4 °C, +2.0 °C, +3.4 °C, +4.0 °C and +1.3 °C, +3.0 °C, +4.2 °C and +4.8 °C, respectively (Table 18).

The detected correlation between the increase in HHP inactivation and the rapeseed oil content speaks against the suggestion of Simpson and Gilmour (1995) and Morales *et al*. (2006) that local low a_w refuges and reduced a_w in the fat droplets or at their interface might are determining factors in conferring baroprotection. Indeed this suggestion is supported by the fact that several studies demonstrated a baroprotective effect of low a_w in general (Oxen & Knorr, 1993, Rendueles *et al.*, 2011). In addition, the occurrence of local low a_w refuges in (O/W)-emulsions is most likely. Though, the observed increase in the HHP inactivation of hydrophilic strains with higher fat content and thus, the occurrence of presumably more local low a_w refuges, speaks against a baroprotective effect mediated by local low a_w refuges. It cannot be excluded that local low a_w refuges indeed confer baroprotection, but other effects, simultaneously increasing cell inactivation and concealing these baroprotective effects, seem to be much more pronounced.

CSH-dependent effect of fat on HHP inactivation could inter alia be based on the exposure of cells to higher temperatures due to stronger adiabatic heating of fat

It could be speculated that competition between bacterial cells and emulsifier can occur to a certain degree. However, according to studies at hydrophobic surfaces, the removal of proteins by emulsifier, based on strong interaction between the emulsifiers and surface, is very high. This fact indicates that displacement of emulsifiers by *L. plantarum* cells, which show cell sizes of several micrometers, is very unlikely (Elwing *et al.*, 1989, Wannerberger *et al.*, 1996, Landete *et al.*, 2010). In addition to the strong interaction of emulsifiers with the hydrophobic surface of oil droplets, it has to be considered that bacterial cells were added after preparation of emulsions, which makes the adhesion of cells to the fat surface even more unlikely. Thus, it can be assumed that the oil droplets are completely covered by emulsifier and that even hydrophobic cells should not be able to directly interact with oil droplets. For hydrophilic cells, which are theoretically able to interact with the hydrophilic surface of emulsifier-covered droplets, direct contact is rather unlikely due to the hydrophilic character of their environment in the aqueous phase. The fact that microscopic analysis revealed that all tested strains were evenly distributed in emulsifier-stabilized emulsions (Figure 14), indicates that the observed effect of fat on the HHP inactivation of TMW 1.708 and TMW 1.1 is rather strain-specific and not directly associated with the CSH and related cell localization.

Still, a possible explanation for the in general observed increased cell inactivation of hydrophilic cells in the presence of rapeseed oil could be their possibly closer proximity to the hydrophilic surface of the droplets and thus exposure to slightly higher temperatures due to stronger adiabatic heating of fat. However, due to limitations in the setup used for microscopic examinations, that is, the inability to detect differences in direct microbial interactions with the fat surface, a possible effect of cell-fat surface interactions cannot be completely excluded. Hence, in order to gain new insights in cell-fat surface interactions in the presence of emulsifier and their potential role in HHP inactivation, in further investigations bacterial attachment at a single cell and at molecular level could be examined by atomic force microscopy, being a very promising tool for even measuring hydrophobic forces on the surface of single living bacterial cells (Alsteens *et al.*, 2007).

The effect of emulsifier type and fat surface on HHP inactivation is neglectable in emulsifier-stabilized (O/W)-emulsions

The reason that, besides the effect of rapeseed oil per se, no distinct effect of fat surface and fat/water boundary layer thickness in emulsifier-stabilized emulsion was observable, could be also found in a complete coverage of the oil droplet surface by emulsifiers, since varying the fat surface using appropriate amounts of emulsifier likely also resulted in a complete coverage of the oil droplet surface. This again is likely to prevent the adhesion of hydrophobic cells and, thus, possible effects on their pressure sensitivity. Since appropriate amounts of emulsifier for guaranteeing sufficient emulsion stability are either already naturally present, adjusted or completely added to emulsion-based food in food industry, complete emulsifier coverage of oil droplets is most likely. Therefore, the lipid phase parameters fat surface and fat/water boundary layer thickness can be neglected designing product-specific HHP processes for the preservation of fatty food. Moreover, the fact that no effect of both tested emulsifiers per se on HHP inactivation could be observed, supports the negligibility of the emulsifiers Tween® 80 and sodium caseinate in HHP processes (Figure 23).

Saturated and unsaturated oils mediate different effects on HHP inactivation

Studies of the effect of the oil type on HHP inactivation revealed opposing effects of fat and confirmed the hypothesis that different oil types and thus the fatty acid composition can lead to dissimilar HHP inactivation. Investigations regarding adiabatic heating of Miglyol® 812 revealed heating rates and values for T_{max} and T_{meanP}, which were nearly comparable, slightly lower to the corresponding ones of rapeseed oil. Heating rates maximally varied by 0.3 °C/100 MPa (Table 17, 50% fat content).

Maximum temperatures reached during HHP treatment showed deviations of maximum 0.7 °C and the mean temperatures during process varied not more than 0.2 °C (Table 18, 50% fat content). Following from this, adiabatic heating during HHP processing could partly contribute to the observed higher cell inactivation in the presence of rapeseed oil, though it is quite unlikely that the determined very small differences in adiabatic heating between rapeseed oil and Miglyol® 812 are responsible for the observed increased inactivation of TMW 1.708 and TMW 1.1.

Even though no clear mechanism could be identified so far, several studies indicate that some unsaturated fatty acids may enhance the HHP inactivation of microorganisms, thus being a possible explanation for the differences in cell inactivation observed for rapeseed oil and Miglyol® 812 samples. Jung *et al*. (2012) and Kruk *et al*. (2014) reported that different meat systems with added olive oil and grapeseed oil showed higher oleic and linoleic acid contents after HHP treatment and related improved microbial inactivation, respectively.

Escriu and Mor-Mur (2009) showed that *Listeria innocua* was more reduced in chicken meat mixed with olive oil (mainly unsaturated, oleic acid: 78.20 g/100 g, linoleic acid: 6.50 g/100 g, unpublished data) than in the same meat with added tallow (mainly saturated). And Rubio *et al*. (2007) evaluated the microbiological quality of three types of sausages (salchichón) with different fat compositions (control; high-oleic by addition of sunflower oil; high-linoleic by addition of soya oil) after HHP treatment, reporting that high-linoleic, but not high-oleic “salchichón” resulted in improved microbial stability. However, using a complex food matrix in combination with sunflower oil and soya oil, which show no substantial differences in fatty acid composition, i.e. for linoleic acid 63.06 g/100 g versus 61.97 g/100 g and for oleic acid 27.07 g/100 g versus 19.70 g/100 g, respectively, the statements regarding the effect of both fatty acids should be treated with caution (unpublished data). Nevertheless, these studies suggest the potential supportive role of some unsaturated fatty acids in the HHP inactivation of microorganisms in complex matrices. Consequently, it can be speculated that rapeseed oil, which mainly consists of unsaturated fatty acids, especially of oleic acid (63.20 g/100 g) and linoleic acid (19.76 g/100 g), is therefore likely to enhance HHP inactivation while Miglyol® 812, which is exclusively composed of fully saturated capric acid (44.53 g/100 g) and caprylic acid (55.21 g/100 g), is suggested to show no increasing effect on HHP inactivation (Table 30). In addition, it can be further speculated that this phenomenon is based on interchanging triglycerides of rapeseed oil with lipoproteins of cellular membrane of microorganisms, leading to altered membrane permeability, which in turn can result in increased pressure sensitivity (section 1.1.6.2).

4.3.2 HHP inactivation in emulsifier-free (O/W)-emulsion

An emulsifier-free (O/W)-emulsion, stabilized by agar, was assumed to facilitate direct adhesion of hydrophobic cells to the fat surface and thus, an effect of fat, mediated by direct fat surface-cell interaction or microbial localization could be examined. As observed before in emulsifier-stabilized emulsion, large differences regarding HHP sensitivity among the tested strains could be seen. Variations of over 2.5 log cycles in HHP sensitivity could be determined, confirming the importance to consider the variation in bacterial pressure sensitivity when setting up processing regimes designed to inactivate microorganism.

In the presence of fat, i.e. rapeseed oil, a significant increase in the HHP inactivation of three out of four strains was determined, which also correlated positively with the fat content. For the hydrophobic strain TMW 1.25 no significant increase in HHP inactivation could be ascertained, but nevertheless, a distinct increase in HHP inactivation could be detected.

Thus, in contrast to the hydrophilic strains, both hydrophobic strains showed no comparable inactivation behaviour in emulsifier-stabilized (O/W)-emulsion. Consequently, hydrophilic and hydrophobic strains showed a similar increase in HHP-mediated inactivation in the presence of rapeseed oil and hence, a protective effect of fat, based on fat surface-cell interaction and microbial localization is unlikely. These findings indicate that CSH is no intrinsic pressure resistance factor. An explanation for the increased inactivation of the hydrophobic strains could be their proximity to fat and thus exposure to slightly higher temperatures by direct fat surface-cell interactions, as observed in the absence of emulsifiers (Figure 15). As discussed before, even small temperature rises might affect cell inactivation in combination with pressure. Farther, as already mentioned before, direct fat surface-cell interaction could facilitate the interchange of unsaturated triglycerides, leading to altered membrane permeability, and it can be also speculated that the hydrophobic cells respond to the direct contact to fat with a change in their metabolism, both resulting in an increased pressure sensitivity.

Although temperature control during HHP processing was efficient, it cannot be completely excluded that slightly higher sample temperatures due to stronger adiabatic heating effects in solid emulsions, containing 30 (T_{max} = 27.6 °C, T_{meanP} = 25.5 °C) and 50% (v/v) (T_{max} = 28.5 °C, T_{meanP} = 25.7 °C) rapeseed oil, were partly responsible for the observed higher cell inactivation in the presence of fat. However, at least for the observed distinctly higher cell inactivation of all strains in solid (O/W)-emulsion, temperature peaks due to differences in adiabatic heating properties can be excluded, since no noticeable differences between maximum temperature and heating rates of the liquid and solid (O/W)-emulsions could be determined (see Table 17 and Table 18).

Still, this observation and the fact that temperatures reached in the samples consistently remained below lethal temperatures for *L. plantarum* cells make it very unlikely that adiabatic heating of fat in (O/W)-emulsion presented a major reason for higher cell inactivation in temperature controlled HHP processing.

Conclusion

The results of this thesis elucidate that the effect of fat can vary substantially, being next to pressure and temperature, dependent on various factors, not fully understood to date. It can be suggested that the effect of fat is a complex interplay of numerous factors, which inter alia include the fatty acid composition, the type of microorganism, interactions with microorganisms and other food matrix constituents and the fats' compression heat and associated temperature inhomogeneities in the food system (Gervilla *et al.*, 2000). As result, inactivation kinetics in real food systems might significantly differ from kinetics in defined model systems and, therefore, single case studies are still needed in order to ensure efficient microbial inactivation.

However, performing systematic studies regarding the role of fat in HHP inactivation, important knowledge could be generated, improving our understanding regarding the effect of fat and associated parameters on HHP inactivation. It was demonstrated that the presence of fat per se, independent from the fat surface and emulsifier, show different effects on HHP inactivation and, therefore, in order to predict inactivation in fat containing food systems, a more systematic evaluation of the role of the fatty acid composition, especially of unsaturated fatty acids is recommended and necessary. The results of this thesis, showing increased cell inactivation or no clear effect instead of decreased cell inactivation in the presence of rapeseed oil, are very promising for the food industry. By the use of rapeseed oil, which is one of the most commonly set-in fats in fatty food products, a baroprotective effect on HHP inactivation can be excluded with a high probability. Rather the property of rapeseed oil to increase HHP inactivation and, additionally, the observation that the increase in HHP efficiency correlates with higher fat content, open up a possibility to enhance inactivation efficiency by simultaneously using milder processing parameters. As a result, it is quite conceivable that negative effects of HHP on food quality can be minimized. Finally, it is obvious that the thermodynamic effect of stronger adiabatic heating of fat should be sufficiently exploited. By ensuring almost ideal adiabatic conditions, the compression heat of fat can be used consciously to reach process target temperature and thus process costs can be reduced, because active heating of the food system to process target temperature is virtually eliminated. Simultaneously, the high decompression-mediated cooling capacity can be exploited to cool down the fatty food product uniformly and quasi-instantaneously without any shear forces occurring inside of the product.

4.4 Role of WTAs in CSH and bacterial HHP sensitivity

4.4.1 The species *L. plantarum* shows intraspecies conserved differences in WTA biosynthesis pathways

In food processing industry, attachment of undesirable microorganisms to surfaces of equipment in contact with food is an entitled source of concern, since this can result in product contamination leading to serious health (pathogenic flora) and economic problems (saprophytic flora) (McFeters *et al.*, 1984, Carpentier & Cerf, 1993, Mueller, 1996, Garrett *et al.*, 2008, Mafu *et al.*, 2010). Furthermore, specific differences in cell adhesion are likely to result in different localization of bacterial cells in food, causing unequal and at worst insufficient inactivation by the applied preservation technique.

In numerous reports is shown that cell adhesion to hydrophobic surfaces and hydrocarbon droplets increases with an increase in bacterial CSH and, therefore, many studies were carried out in order to get a better understanding of CSH and with it being able to target unwanted cell adhesion (Rosenberg & Kjelleberg, 1986, van der Mei *et al.*, 1987, Van Loosdrecht *et al.*, 1987, Kochkodan *et al.*, 2008, Giaouris *et al.*, 2009). However, even though great progresses were made in recent years (1.4), we are a long way from understanding bacterial CSH and its role in cell adhesion in detail. By the comparison of genome sequences of three highly hydrophilic and three highly hydrophobic *L. plantarum* strains by BADGE, which is a new tool developed for the identification of possible marker genes by Behr *et al*. (2016), new insights in CSH-associated genes were hoped to be obtained. In the course of this, several group-specific genes could be identified. 17 genes were identified, which could be exclusively proved in the genomes of hydrophilic strains, and in turn 79 genes could be purely found in the genomes of hydrophobic strains. Bioinformatic analysis revealed for both, hydrophilic and hydrophobic strains, genes coding for various proteins, which could be identified to be associated with inter alia stress response, cell division and repair, transport, membrane and diverse enzymatic functions (Appendix Table 32 and Table 33).

However, three genes, being exclusively present in hydrophilic strains turned out to be homologues of the well characterized genes *tagD1*, *tagF1* and *tagF2 (tag*-locus*),* which are related to the WTA biosynthesis pathway (Tomita *et al.*, 2010, Bron *et al.*, 2012). Since on the one hand, WTAs form the major surface component of Gram-positive bacterial cell wall (Swoboda *et al.*, 2010), and on the other hand, several studies demonstrated that WTAs play an important role in cell adhesion, these genes were classified as the most promising genes in terms of affecting CSH (Gross *et al.*, 2001, Weidenmaier *et al.*, 2005, Weidenmaier & Peschel, 2008, Kohler *et al.*, 2009).

Further bioinformatic investigations revealed that all six genomes additionally contain homologues of the genes *tagO*, *tagA*, *tagD2*, *tagB3*, *tarIJKL* (tar-locus) and *tagGH*, and thus, each strain having a complete gene equipment necessary for WTA biosynthesis (Brown *et al.*, 2013). Though hydrophilic as well as hydrophobic strains were shown to have homologues of the *tar*-loci, marked conserved differences in nucleotide sequences among both groups could be determined in accordance with earlier studies (Tomita *et al.*, 2010). Both groups were proved to differ by sharing gene sequence identities of 67 to 90% (Table 20), as already implied for the gene *tarK* by the results of BADGE (see 3.6.1). Based on these observations it is highly likely that observed differences in the hydrophilic *tar*-locus go along with the presence of a *tag*-locus. Further, including literature, it was assumed that tested hydrophilic *L. plantarum* strains, which were proved to have the *tag*-locus, synthesize poly(Gro-P) WTAs while the tested hydrophobic strains can be regarded as strains with poly(Rbo-P) WTAs (Tomita *et al.*, 2010, Bron *et al.*, 2012).

In line with these findings it was hypothesized that hydrophilic *L. plantarum* strains in general possess a conserved WTA biosynthesis cluster, synthesizing poly(Gro-P) WTAs while hydrophobic *L. plantarum* strains, which lack the *tag*-locus, show conserved differences in the *tar*-locus and hence synthesize poly(Rbo-P) WTAs. An additional set of 32 *L. plantarum* strains was examined in terms of CSH and genes of interest (*tagF1*, *tagIJKL*, *tarIJKL*), and the hypothesized correlation between the WTA type and CSH investigated. 36 out of 38 *L. plantarum* strains were proved to encode the genetic determinants for the production of WTA variants containing either poly(Gro-P) or poly(Rbo-P) backbones and hence formed two distinct WTA type-specific groups within the species *L. plantarum* as already predicted by Tomita *et al*. (2010). *L. plantarum* TMW 1.1342 and TMW 1.1 could not be assigned to one of the two identified groups, possibly due to mutations in corresponding genes (for TMW 1.1342, gene *tagF1*) or incorrect addition of primers. It is likely that the two phylogenetic groupings of *L. plantarum* based on the WTA type can be attributed to a broadening of the intraspecies diversity of the cell wall structure in evolution of *L. plantarum* (Tomita *et al.*, 2010). Although further investigations of these strains in terms of WTA isolation and transcriptomic analysis would be meaningful in order to proof the consensus with the predicted WTA type based on determined WTA type specific gene equipment, it is highly likely that the WTA type in the cell wall of *L. plantarum* can be estimated by carrying out simple bioinformatic analysis or colony PCR using WTA type specific primer (Tomita *et al.*, 2010). Consequently, complex and time-consuming laborious preparation procedures for the prediction of the WTA type would not be necessary anymore.

Furthermore, the observation that strains, which are supposed to produce different WTA types (TMW 1.25, poly(Rbo-P) WTA; TMW 1.708 and SF_02, poly(Gro-P) WTA) were isolated from raw sausage, supports the suggestion by Tomita *et al*. (2010) that *L. plantarum* strains, which synthesize different WTA types, can share the same habitat.

However, contradicting the hypothesis that strains with hydrophobic cell surface produce poly(Rbo-P) WTAs and strains with hydrophilic cell surface synthesis poly(Gro-P) WTAs, highly hydrophobic strains (TMW 1.1723, SF_02, TMW 1.1830) were classified as poly(Gro-P) WTA and in turn, highly hydrophilic strains (TMW 1.1662, TMW 1.1478, TMW 1.9, TMW 1.1356) as poly(Rbo-P) WTA producing strains. Following from this, no correlation between the WTA type and CSH could be observed. This suggests that, even though WTAs form the major surface component of the Gram-positive bacterial cell wall, their effect on the CSH phenotype is not decisive. Instead, this finding rather supports the common assumption that CSH is a complex interplay of diverse components and their acting forces (Hazen *et al.*, 1986, van der Mei *et al.*, 1987, Van der Mei *et al.*, 1991, Martienssen, 2001, Poortinga *et al.*, 2002, Van der Mei *et al.*, 2003, Swoboda *et al.*, 2010).

In addition, this finding also points up the possibility that WTA-favored cell adhesion is more likely mediated by the direct interaction of WTAs with abiotic and biotic surfaces rather than indirectly, by contributing to CSH. Though, it has to be considered that the *L. plantarum* strains show significant differences in biodiversity, as inter alia demonstrated in this thesis by investigating a correlation between CSH and protein mass spectra (3.2.3). These intraspecies differences can conceivably mask a possible WTA type-dependent effect on bacterial CSH.

4.4.2 WTAs affect CSH and HHP inactivation

Tomita *et al.* (2010) demonstrated that the *tag*-locus encoding strain *L. plantarum* WCFS1 produce poly(Gro-P) WTAs, as also predicted in this thesis (Table 22). However, using a ΔtagO (Andre *et al.*, 2011) and a ΔtagF1-F2 mutant of *L. plantarum* WCFS1, Bron *et al*. (2012) demonstrated that the WCFS1 ΔtagO mutant is unable to produce WTAs and that the WCFS1 ΔtagF1-F2 mutant performs alditol-switching producing poly(Rbo-P) WTAs instead. Exploiting the genetic capacity of *L. plantarum* WCFS1 to produce both WTA types, the physiological consequences induced by the presence of WTAs per se and the WTA type on CSH were studied. Using isogenic deletion mutants, other CSH-influencing affects, being associated with the specie's high biodiversity, could be excluded.

The MATH test revealed the wildtype strain to possess the most hydrophobic cell surface, showing a moderately hydrophobic cell surface (46%). *L. plantarum* WCFS1 ΔtagO, which is assumed to produce no WTAs at all, showed a highly hydrophilic surface (77%) and *L. plantarum* WCFS1 ΔtagF1-F2, producing poly(Rbo-P) WTAs, showed the most hydrophilic cell surface (96%) (Table 22). Consequently, being able to exclude any effects based on intraspecies biodiversity, the presence of WTAs and the type of WTA backbone seems to indeed have an effect on CSH of *L. plantarum* WCFS1. However, since only a few studies are known that focus on the effect of the WTA type on CSH or. cell adhesion under exclusion of intraspecies biodiversity, comparisons are strongly limited (Weidenmaier *et al.*, 2005, Holland *et al.*, 2011). In addition, CSH was conceivably determined by the application of different or modified methods, making a comparison even more difficult. In terms of the observed differences in CSH between the wildtype strain and the WCFS1 ΔtagO mutant, the results contradict those observed by Holland *et al*. (2011), which show that mutation of *tagO* in *S. epidermidis* was associated with increased CSH. However, because of the fact that Holland *et al*. (2011) used a different modified version of the MATH test, direct comparisons are not meaningful (Rosenberg, 2006).

Main differences to the MATH test of this study could be inter alia found in the used buffer (1x Dulbecco's PBS) and the organic solvent (p-xylene), both variables which were described to substantially influence the results of the MATH test. On the one hand, it can be speculated that the absence of WTAs in general leads to the unmasking of cell wall proteins and polysaccharides, which convey type-specific surface characteristics and are not implicated in CSH in the presence of WTAs. Variations in side chains of surface protein, for instance, could result in different surface characteristics, possibly explaining the mentioned, contradicting results. On the other hand, a possible explanation for the altered CSH of the WCFS1 ΔtagO mutants could be found in their observed increased tendency for cell aggregation, which is in line with observations described for *B. subtilis* 168 and *S. aureus* by Soldo *et al*. (2002) and Vergara-Irigaray *et al*. (2008). The determined decrease in hydrophobicity observed in the WCFS1 ΔtagO mutants could be a consequence of this aggregation, since WCFS1 ΔtagO mutant cells could prefer to interact between themselves rather than with the organic solvent n-hexadecane. The execution of an additional hydrophobicity test, not being based on bacterial distribution between two liquid immiscible phases, for example contact angle measurement, would help to define the CSH phenotype of WCFS1 ΔtagO mutant cells in absence of unwanted, interfering aggregation.

Further, it cannot be excluded that the CSH-influencing effect emanating from LTAs, which are known for being profoundly associated with physicochemical properties of bacterial surfaces, is significantly more determinative in the absence of WTAs and thus partially responsible for the distinct changes of CSH (Sherman & Savage, 1986, Granato *et al.*, 1999, Fedtke *et al.*, 2007).

This explanation is quite conceivable, since WTAs and LTAs are suggested to have overlapping functions and are thus supposed to partially compensate for one another (Oku *et al.*, 2009, Schirner *et al.*, 2009). Moreover, there is compelling evidence that WCFS1 ΔtagO mutants showed impaired cell elongation and division, possibly influencing the cell surface characteristics. In line with several studies, microscopic analysis revealed that corresponding cells appeared swollen and shorter than cells of the wildtype strain, whereas the morphological characteristics of *L. plantarum* WCFS1 ΔtagF1-F2 strongly resembled those of the wildtype strain (Andre *et al.*, 2011, Bron *et al.*, 2012). Noteworthy, both deletion mutants showed a distinctly slower growth (unpublished data), which was also already reported for a ΔtagO mutant of *S. aureus* (Weidenmaier *et al.*, 2005). Additionally, Andre *et al*. (2011) demonstrated that a significant proportion of ΔtagO cells contained displaced septa, supporting the evidence of disturbed cell elongation and division events. However, the reported tendency of the ΔtagO mutant to remain associated in chains could not be determined, rather the already discussed tendency to cell aggregation was observable.

The distinct differences in CSH observed for the poly(Gro-P) and poly(Rbo-P) WTA producing strains, varying from moderate hydrophobic to highly hydrophilic, can be hypothesized to be attributed to alditol-specific substitution patterns and related differences in polymer structure and cell surface charge. The hydroxyls on the glycerol- or ribitol phosphate repeats of the WTA backbone are in general known to be tailored with cationic D-alanine esters and mono- or oligosaccharides (Mirelman *et al.*, 1970, Yokoyama *et al.*, 1989, Brown *et al.*, 2013). However, WTA substitution with L-lysyl or acetyl residues was also reported (Sadovskaya *et al.*, 2004). To date, the extent to which both types of tailoring occur is assumed to be amongst other things strain-specific (Jenni & Berger-Bächi, 1998, Swoboda *et al.*, 2010, Brown *et al.*, 2013). The positions in ribitol and glycerol, at which installation of substituents occur, can vary dependent on the substituent and alditol, as illustrated nicely in the study of Naumova *et al*. (2001) (Neuhaus & Baddiley, 2003, Vinogradov *et al.*, 2006). Even though the ubiquitous tailoring modification of WTAs by glycosylation and its functions are not well understood, the presence or absence of sugars was reported to affect the polymer structure (Bernal *et al.*, 2009).

Studies, investigating adhesion of *S. aureus* to artificial surfaces and host tissue proved the essential role of D-alanylation in cell adhesion by showing that the D-alanylation promote noticeably stronger cell adhesion (Gross *et al.*, 2001, Neuhaus & Baddiley, 2003, Weidenmaier *et al.*, 2005, Weidenmaier & Peschel, 2008).

There, the D-alanylation mediated enhanced cell adhesion is speculated to be based on a decrease in the net negative charge of the WTA polymers by adding positively charged amines and, therefore, by the decrease of repulsive forces that prevent bacterial adherence to abiotic and biotic surfaces (Neuhaus & Baddiley, 2003, Holland *et al.*, 2011). Consequently, alditol-specific substitution patterns can be supposed to result in the mediation of different cell surface charges and polymer structures. And these in turn are therefore a plausible explanation for the observed differences in the CSH phenotypes among poly(Gro-P) and poly(Rbo-P) WTA producing strains. Detailed studies, which analyze both types of WTA and their attached substituents (substituent type and -amount, position in alditol, number of polymer subunits, polymer structure) would be necessary in order to strengthen this hypothesis.

To this end, HHP processing revealed that the wildtype strain had the highest HHP resistance, followed by the mutant WCFS1 ΔtagO. Unexpectedly, the mutant strain WCFS1 ΔtagF1-F2 showed the highest HHP sensitivity. These results do not match with the general assumption that the absence of WTAs results in a cell wall that is more sensitive to environmental changes, as described by several authors (Hoover & Gray, 1977, Vergara-Irigaray *et al.*, 2008). However, it has to be considered that, as mentioned before, LTAs are known to be able to partially compensate the absence of WTAs (Oku *et al.*, 2009, Schirner *et al.*, 2009). This phenomenon could explain the increased, but still moderate inactivation.

Transcriptomic analysis could help to define the direct effect of the absence of WTAs on the likely increased LTA synthesis by measuring the upregulation of the involved genes (Percy & Gründling, 2014). In addition, isolation of LTAs from WTA-deficient and from WTA producing strains, followed by detailed quantitative comparisons, could give new insight in the compensation mechanism of LTAs and with it in their role in pressure resistance. It can be speculated that the compensating effect of LTAs seemed to be more pronounced, resulting in an increased pressure resistance of the mutant WCFS1 ΔtagO, than the effect of alditol switching, leading to the highest pressure sensitivity. Finally, it should be considered that the observed increased pressure sensitivity of both mutants could be a consequence of the already discussed growth or. physiological defects, most likely resulting in a more vulnerable cell wall, and hence, also increased pressure sensitivity.

Conclusion

The findings of this study sustain the complexity of the phenomenon CSH and its role on cell adhesion. It could be demonstrated that CSH is affected by WTAs per se and the WTA type, however, the effect of WTAs can be assumed to not dominate CSH. More likely, WTAs can be placed in the series of several cell surface components that are also suggested to modulate CSH (1.4). New important knowledge in terms of CSH-determinants and the role of WTAs in HHP tolerance could be generated. This knowledge opens up new perspectives in terms of CSH in general and forms a good base for further detailed studies. Promising approaches for further detailed studies are investigations in terms of (I) the compensation of lacking WTAs by LTAs and vice versa, (II) the tailoring modifications of both WTA types and their consequences on the polymer structure, and (III) the effect of WTAs per se on the structure of bacterial cell walls and related pressure sensitivity. Additionally, complete genome sequences of the *L. plantarum* strains TMW 1.25, TMW 1.277, TMW 1.1623 and TMW 1.708 were provided. Due to the fact that the newly sequenced strains were characterized in terms of various features, these genomes can be assumed to build a valuable base for future comparative analyses.

5 Summary

High Hydrostatic Pressure (HHP) processing is an emerging non-thermal food preservation technology, which has the potential to fulfill the consumers' growing demand for minimally processed, high-quality foods. However, inactivation efficiency of HHP processing of food is strongly affected by the food matrix composition, which significantly impedes generic process design approaches. Studies, investigating effects of the important food matrix parameter, fat, are scarce, and conclusions drawn are not always consistent with each other. Since detailed studies are completely missing, there exists a substantial lack of knowledge regarding the effect of fat on microbial HHP inactivation. Furthermore, effects of cell surface hydrophobicity (CSH) and bacterial localization in food on HHP inactivation were both fundamentally neglected so far. Among other things, this is probably due to the fact that the knowledge on factors modulating CSH is still sketchy and its determinants are only superficially known. Against this background, the overall goal of the present thesis was to increase the knowledge on the general role of fat in the HHP inactivation of spoilage-associated microorganisms. Since fat-mediated effects on HHP inactivation could be dependent on interactions between lipid phase and microbial cells, the focal points were on factors possibly influencing such interactions, including CSH, fat content, presence and type of emulsifier, fat surface (oil droplet size) and oil type. In addition, the aim was to identify specific marker genes that play a role in the development of different CSH phenotypes.

Systematic HHP inactivation studies, using defined (O/W)-emulsions, revealed that pressure tolerance varied noticeably among *L. plantarum* strains, which was observed to be independent from CSH. It was shown that the HHP inactivation of all tested strains in general tended to be more effective in the presence of rapeseed oil. There, the fat-mediated effect was markedly more pronounced in the absence of emulsifier. Observations in both emulsifier-stabilized and emulsifier-free (O/W)-emulsion indicated that CSH is no intrinsic pressure resistance factor. Furthermore, the HHP inactivation efficiency seemed to correlate positively with the fat content. Interestingly, the synthetic, fully saturated oil Miglyol® 812 showed no or even a slightly baroprotective effect. These determined oil type-dependent differences are likely to occur due to the dissimilar fatty acid composition of both oil types and support studies indicating that individual fatty acids can influence HHP inactivation. Both phenomena, which were observed in the presence of rapeseed oil, were additionally suggested to be, at least partially attributed to the effect of stronger adiabatic heating of fat, resulting in the exposure of cells to higher temperatures. The lipid phase parameters, emulsifier type and fat surface, did not affect HHP inactivation efficiency.

Homologues of the genes *tagD1*, *tagF1* and *tagF2* (*tag*-locus) being exclusively present in hydrophilic strains were identified as most promising marker genes associated with CSH. Since these genes are associated with the synthesis of poly(glycerol-3-phosphate) (poly(Gro-P)) wall teichoic acids (WTAs), it was hypothesized that poly(Gro-P) WTA producing strains possess a highly hydrophilic cell surface whereas strains, lacking this locus and thus synthesizing poly(ribitol-5-phosphate) (poly(Rbo-P)) WTAs, possess a highly hydrophobic cell surface. However, no correlation between the WTA type and bacterial CSH were determined. However, *L. plantarum* strains were proved to encode the genetic determinants for the production of WTA variants containing either poly(Gro-P) or poly(Rbo-P) backbones and thus to form two distinct groups within this species. Considering that intraspecies biodiversity can conceivably mask a possible WTA type-dependent effect, the genetic capacity of *L. plantarum* WCFS1 to produce both WTA-backbone types was exploited to examine the role of WTAs in the development of a specific CSH phenotype and HHP tolerance. In this context, both, the absence of WTAs and alditol switching from poly(Gro-P) to poly(Rbo-P) resulted in significantly more hydrophilic cell surfaces and substantially increased pressure sensitivity. These differences might be partially ascribable to compensation effects by lipoteichoic acids, unmasking effects of CSH-affecting cell components, and/or differences in alditol-specific substitution patterns.

Despite substantial intraspecies variations in the pressure tolerance of different *L. plantarum* strains, data presented in this study indicate that the effect of fat on HHP inactivation is the result of a complex interplay of numerous factors. Furthermore, this thesis provides systematic data for further studies and for the design of product-specific HHP processes for the preservation of fatty food. While the fatty acid composition, the overall fat content and the fats' compression heating properties are important factors that should be considered, the emulsifier type and oil droplet surface properties might be neglected. New insights regarding CSH determinants, that is, that the WTAs per se and the WTA type have distinct effects on bacterial CSH and play a role in the pressure tolerance of *L. plantarum*, redounds to a better understanding of the development of cell surface properties and cellular HHP resistance factors. This contributes to closing knowledge gaps and opens new perspectives for further research approaches.

6 Zusammenfassung

Die Hochdruck-(HHP)-Behandlung ist eine aufkommende Technologie für die nicht-thermische Lebensmittelkonservierung, die das Potenzial hat, die wachsende Nachfrage der Verbraucher nach minimal verarbeiteten, hochwertigen Lebensmitteln zu erfüllen. Die Inaktivierungseffizienz der HHP-Behandlung von Lebensmitteln wird jedoch stark von der Zusammensetzung der Lebensmittelmatrix beeinflusst, was generische Prozessdesign-Ansätze erheblich behindert. Studien, in denen der Einfluss des wichtigen Lebensmittelmatrixparameters Fett untersucht wurde, sind selten, und die dabei gezogenen Schlussfolgerungen stimmen nicht immer miteinander überein. Da detaillierte Studien nicht existieren, besteht ein erheblicher Mangel an Wissen über die Wirkung von Fett auf die mikrobielle HHP-Inaktivierung. Darüber hinaus wurden die Auswirkungen der Oberflächenhydrophobizität (CSH) und der bakteriellen Lokalisation in Lebensmitteln auf die HHP-Inaktivierung bisher weitgehend vernachlässigt. Dies ist vermutlich unter anderem dem Umstand geschuldet, dass das Wissen über Faktoren, die die CSH modulieren, noch lückenhaft ist und seine Determinanten nur oberflächlich bekannt sind. Vor diesem Hintergrund bestand das Hauptziel der vorliegenden Dissertation darin, das Wissen über die allgemeine Rolle von Fett bei der HHP-Inaktivierung von Mikroorganismen, die mit dem Lebensmittelverderb assoziiert werden, zu erweitern. Da die fettvermittelten Effekte auf die HHP-Inaktivierung von Wechselwirkungen zwischen der Lipidphase und mikrobiellen Zellen abhängig sein könnten, standen Faktoren im Vordergrund, die diese Wechselwirkungen beeinflussen können, darunter die CSH, der Fettgehalt, die Anwesenheit und Art des Emulgators, die Fettoberfläche (Öltröpfchengröße) und die Ölart. Darüber hinaus sollten spezifische Markergene identifiziert werden, die bei der Entwicklung verschiedener CSH-Phänotypen eine Rolle spielen.

Systematische HHP-Inaktivierungsstudien zeigten unter Verwendung definierter (O/W)-Emulsionen, dass die Drucktoleranz unter *L. plantarum*-Stämmen merklich, unabhängig von der CSH, variierte. Es wurde gezeigt, dass im Allgemeinen die HHP-Inaktivierung aller getesteten Stämme in Gegenwart von Rapsöl wirksamer war. Dabei war der fettvermittelte Effekt in Abwesenheit von Emulgator deutlich ausgeprägter. Die Tatsache, dass dieser Effekt sowohl in der emulgatorstabilisierten als auch in der emulgatorfreien (O/W)-Emulsion beobachtet wurde, deutet darauf hin, dass die CSH kein intrinsischer Druckresistenzfaktor ist. Darüber hinaus schien die HHP-Inaktivierungseffizienz positiv mit dem Fettgehalt zu korrelieren. Interessanterweise zeigte das synthetische, vollgesättigte Öl Miglyol® 812 keinen oder sogar einen geringen baroprotektiven Effekt.

Diese von der Ölart abhängigen Unterschiede können wahrscheinlich auf unterschiedliche Fettsäurezusammensetzungen beider Ölarten zurückgeführt werden und unterstützen Studien, die darauf hindeuten, dass einzelne Fettsäuren die HHP-Inaktivierung beeinflussen können. Beide Phänomene, die in Gegenwart von Rapsöl beobachtet wurden, könnten zudem zumindest teilweise durch den Effekt einer stärkeren adiabatischen Erwärmung von Fett erklärt werden, der zur Folge hatte, dass die Zellen höheren Temperaturen ausgesetzt waren. Die Lipidphasenparameter Emulgatortyp und Fettoberfläche zeigten keine Wirkung auf die HHP-Inaktivierungseffizienz.

Homologe der Gene tagD1, tagF1 und tagF2 (tag-Locus), die ausschließlich in hydrophilen Stämmen vorkamen, wurden als vielversprechendste, mit der CSH assoziierte Markergene identifiziert. Da diese Gene mit der Synthese von poly(glycerol-3-phosphat) (poly(Gro-P)) Zellwand-Teichonsäuren (WTAs) assoziiert sind, wurde die Hypothese aufgestellt, dass poly(Gro-P) WTA-produzierende Stämme eine stark hydrophile Zelloberfläche besitzen, während Stämme, denen dieser Locus fehlt und die somit poly(ribitol-5-phosphat) (poly(Rbo-P)) WTAs synthetisieren, eine stark hydrophobe Zelloberfläche aufweisen. Es wurde jedoch keine Korrelation zwischen dem WTA-Typ und der bakteriellen CSH festgestellt. Es wurde jedoch nachgewiesen, dass *L. plantarum*-Stämme die genetischen Determinanten für die Produktion von WTA-Varianten codieren, die entweder poly(Gro-P)- oder poly(Rbo-P)-Rückgrate enthalten und somit zwei verschiedene Gruppen innerhalb dieser Spezies bilden. In Anbetracht der Tatsache, dass die Biodiversität innerhalb der Spezies möglicherweise einen möglichen WTA-Typ-abhängigen Effekt maskiert, wurde die genetische Kapazität von *L. plantarum* WCFS1 zur Produktion beider WTA-Rückgrat-Typen genutzt, um die Rolle von WTAs bei der Entwicklung eines spezifischen CSH-Phänotyps und HPP-Toleranz zu untersuchen. In diesem Zusammenhang führten sowohl die Abwesenheit von WTAs als auch die Änderung des WTA-Rückgrat-Typs von poly(Gro-P) auf poly(Rbo-P) zu deutlich hydrophileren Zelloberflächen und einer wesentlich erhöhten Druckempfindlichkeit. Diese Unterschiede könnten teilweise auf Kompensationseffekte durch Lipoteichonsäuren, Demaskierungseffekte von CSH-beeinflussenden Zellkomponenten und/oder Unterschiede in Alditol-spezifischen Substitutionsmustern zurückzuführen sein.

Trotz erheblicher innerartlicher Unterschiede in der Drucktoleranz verschiedener *L. plantarum*-Stämme deuten die in dieser Studie präsentierten Daten darauf hin, dass die Wirkung von Fett auf die HHP-Inaktivierung auf ein komplexes Zusammenspiel zahlreicher Faktoren zurückzuführen ist.

Darüber hinaus liefert diese Arbeit systematische Daten für weitere Studien und für die Entwicklung produktspezifischer HHP-Verfahren zur Konservierung von fetthaltigen Lebensmitteln. Während die Fettsäurezusammensetzung, der Gesamtfettgehalt und die Kompressions-erwärmungseigenschaften der Fette wichtige Faktoren sind, die in Betracht gezogen werden sollten, könnten der Emulgatortyp und die Oberflächeneigenschaften der Öltropfen vernachlässigt werden. Neue Erkenntnisse über CSH-Determinanten, das heißt, dass die WTAs per se und der WTA-Typ deutliche Auswirkungen auf die bakterielle CSH haben und eine Rolle in der Drucktoleranz von *L. plantarum* spielen, führen zu einem besseren Verständnis bezüglich der Entwicklung von Zelloberflächen-Eigenschaften und zellulärer HHP-Resistenzfaktoren. Dies trägt dazu bei, Wissenslücken zu schließen und neue Perspektiven für weitere Forschungsansätze zu eröffnen.

7 References

Abe F (2007) Exploration of the effects of high hydrostatic pressure on microbial growth, physiology and survival: perspectives from piezophysiology. *Bioscience, biotechnology, and biochemistry* **71**: 2347-2357.

Abe F (2013) Dynamic structural changes in microbial membranes in response to high hydrostatic pressure analyzed using time-resolved fluorescence anisotropy measurement. *Biophysical chemistry* **183**: 3-8.

Adheeb Usaid AS & Premkumar J (2014) Emulsion and it's Applications in Food Processing - A Review. *International Journal of Engineering Research and Applications* **4**: 241-248.

Aguirre M & Collins M (1993) Lactic acid bacteria and human clinical infection. *Journal of Applied Microbiology* **75**: 95-107.

Alpas H, Kalchayanand N, Bozoglu F & Ray B (2000) Interactions of high hydrostatic pressure, pressurization temperature and pH on death and injury of pressure-resistant and pressure-sensitive strains of foodborne pathogens. *International Journal of Food Microbiology* **60**: 33-42.

Alpas H, Kalchayanand N, Bozoglu F, Sikes A, Dunne C & Ray B (1999) Variation in resistance to hydrostatic pressure among strains of food-borne pathogens. *Applied and Environmental Microbiology* **65**: 4248-4251.

Alsteens D, Dague E, Rouxhet PG, Baulard AR & Dufrêne YF (2007) Direct measurement of hydrophobic forces on cell surfaces using AFM. *Langmuir* **23**: 11977-11979.

Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ (1990) Basic local alignment search tool. *Journal of molecular biology* **215**: 403-410.

Amanatidou A, Schlüter O, Lemkau K, Gorris L, Smid E & Knorr D (2000) Effect of combined application of high pressure treatment and modified atmospheres on the shelf life of fresh Atlantic salmon. *Innovative Food Science & Emerging Technologies* **1**: 87-98.

Americas I (1984) The HLB System: A Time-saving Guide to Emulsifier Selection. *ICI Americas, Incorporated, Wilmington, Del, USA*

Andre G, Deghorain M, Bron PA, van Swam II, Kleerebezem M, Hols P & Dufrêne YF (2011) Fluorescence and atomic force microscopy imaging of wall teichoic acids in *Lactobacillus plantarum*. *ACS chemical biology* **6**: 366-376.

Angiuoli SV, Gussman A, Klimke W*, et al.* (2008) Toward an online repository of Standard Operating Procedures (SOPs) for (meta)genomic annotation. *Omics: a journal of integrative biology* **12**: 137-141.

Archibald A, Hancock I & Harwood C (1993) Cell wall structure, synthesis, and turnover. *Bacillus subtilis and other Gram-positive bacteria*: 381-410. *American Society of Microbiology*.

Ardia A, Knorr D & Heinz V (2004) Adiabatic heat modelling for pressure build-up during high-pressure treatment in liquid-food processing. *Food and Bioproducts Processing* **82**: 89-95.

Arroyo G, Sanz P & Prestamo G (1999) Response to high-pressure, low-temperature treatment in vegetables: determination of survival rates of microbial populations using flow cytometry and detection of peroxidase activity using confocal microscopy. *Journal of Applied Microbiology* **86**: 544-544.

Auclair J, Frappier M & Millette M (2015) *Lactobacillus acidophilus* CL1285, *Lactobacillus casei* LBC80R, and *Lactobacillus rhamnosus* CLR2 (Bio-K+): characterization, manufacture, mechanisms of

action, and quality control of a specific probiotic combination for primary prevention of Clostridium difficile infection. *Clinical Infectious Diseases* **60**: S135-S143.

Balasubramaniam V & Farkas D (2008) High-pressure food processing. *Revista de Agaroquimica y Tecnologia de Alimentos* **14**: 413-418.

Balasubramaniam V, Martínez-Monteagudo SI & Gupta R (2015) Principles and application of high pressure - based technologies in the food industry. *Annual review of food science and technology* **6**: 435-462.

Balny C & Masson P (1993) Effects of high pressure on proteins. *Food Reviews International* **9**: 611-628.

Basak S & Ramaswamy H (1998) Effect of high pressure processing on the texture of selected fruits and vegetables. *Journal of Texture Studies* **29**: 587-601.

Basso AL, Picariello G, Coppola R, Tremonte P, Musso SS & Luccia AD (2004) Proteolytic activity of *Lactobacillus sakei*, *Lactobacillus farciminis* and *Lactobacillus plantarum* on sarcoplasmic proteins of pork lean. *Journal of food biochemistry* **28**: 195-212.

Bauer B & Knorr D (2005) The impact of pressure, temperature and treatment time on starches: pressure-induced starch gelatinisation as pressure time temperature indicator for high hydrostatic pressure processing. *Journal of Food Engineering* **68**: 329-334.

Behr J, Geissler AJ, Schmid J, Zehe A & Vogel RF (2016) The identification of novel diagnostic marker genes for the detection of beer spoiling Pediococcus damnosus strains using the BlAst diagnostic gene findEr. *PloS one* **11**: e0152747.

Belitz H-D & Grosch W (2013) Lehrbuch der Lebensmittelchemie. Springer.

Bello EFT, Martínez GG, Ceberio BFK, Rodrigo D & López AM (2014) High pressure treatment in foods. *Foods* **3**: 476-490.

Bernal P, Zloh M & Taylor PW (2009) Disruption of d-alanyl esterification of *Staphylococcus aureus* cell wall teichoic acid by the β-lactam resistance modifier (−)-epicatechin gallate. *Journal of Antimicrobial Chemotherapy* **63**: 1156-1162.

Beveridge E (1975) The microbial spoilage of pharmaceutical products. *Microbial aspects of the deterioration of materials* **9**: 203-211.

Beveridge T (1981) Ultrastructure, chemistry, and function of the bacterial wall. *International review of cytology* **72**: 229-317.

Beveridge TJ & Graham LL (1991) Surface layers of bacteria. *Microbiological reviews* **55**: 684-705.

Bjorkroth KJ & Korkeala HJ (1997) *Lactobacillus fructivorans* spoilage of tomato ketchup. *J Food Prot* **60**: 505-509.

Björkroth K & Korkeala HJ (1997) Use of rRNA gene restriction patterns to evaluate lactic acid bacterium contamination of vacuum-packaged sliced cooked whole-meat product in a meat processing plant. *Applied and environmental microbiology* **63**: 448-453.

Black EP, Huppertz T, Fitzgerald GF & Kelly AL (2007) Baroprotection of vegetative bacteria by milk constituents: a study of *Listeria innocua*. *International Dairy Journal* **17**: 104-110.

Blickstad E (1984) The effect of water activity on growth and end-product formation of two *Lactobacillus spp.* and *Brochothrix thermosphacta* ATCC 11509 T. *Applied Microbiology and Biotechnology* **19**: 13-17.

Boesten RJ & de Vos WM (2008) Interactomics in the human intestine: Lactobacilli and Bifidobacteria make a difference. *Journal of clinical gastroenterology* **42**: S163-S167.

Bolumar T, Georget E & Mathys A (2014) High pressure processing (HPP) of foods and its combination with electron beam processing. In Pillai SD, & Shayanfar S (Eds.), *Electron Beam Pasteurization and Complementary Food Processing Technologies. Woodhead Publishing Series in Food Science Technology and Nutrition* **127**: 127-155.

Boode K, Bisperink C & Walstra P (1991) Destabilization of O/W emulsions containing fat crystals by temperature cycling. *Colloids and Surfaces* **61**: 55-74.

Borch E, Kant-Muermans M-L & Blixt Y (1996) Bacterial spoilage of meat and cured meat products. *International journal of food microbiology* **33**: 103-120.

Bridgman PW (1912) Water, in the liquid and five solid forms, under pressure. *American Academy of Arts & Sciences* **47**: 441-558.

Brocklehurst T, Parker ML, Gunning P, Coleman HP & Robins MM (1995) Growth of food-borne pathogenic bacteria in oil-in-water emulsions: II-Effect of emulsion structure on growth parameters and form of growth. *Journal of Applied Bacteriology* **78**: 609-615.

Bron PA, Van Baarlen P & Kleerebezem M (2012) Emerging molecular insights into the interaction between probiotics and the host intestinal mucosa. *Nature reviews Microbiology* **10**: 66.

Bron PA, Tomita S, van Swam II, Remus DM, Meijerink M, Wels M, Okada S, Wells JM & Kleerebezem M (2012) *Lactobacillus plantarum* possesses the capability for wall teichoic acid backbone alditol switching. *Microbial cell factories* **11(1)**: 123.

Brown S, Zhang Y-H & Walker S (2008) A revised pathway proposed for *Staphylococcus aureus* wall teichoic acid biosynthesis based on in vitro reconstitution of the intracellular steps. *Chemistry & biology* **15**: 12-21.

Brown S, Santa Maria Jr JP & Walker S (2013) Wall teichoic acids of gram-positive bacteria. *Annual review of microbiology* **67**: 313-336.

Buckenhüskes HJ (1993) Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities. *FEMS microbiology reviews* **12**: 253-271.

Buckow R, Heinz V & Knorr D (2007) High pressure phase transition kinetics of maize starch. *Journal of Food Engineering* **81**: 469-475.

Bunt CR, Jones DS & Tucker IG (1993) The effects of pH, ionic strength and organic phase on the bacterial adhesion to hydrocarbons (BATH) test. *International journal of pharmaceutics* **99**: 93-98.

Busscher H, Van de Belt-Gritter B & Van der Mei H (1995) Implications of microbial adhesion to hydrocarbons for evaluating cell surface hydrophobicity 1. Zeta potentials of hydrocarbon droplets. *Colloids and surfaces B: Biointerfaces* **5**: 111-116.

Butz P & Tauscher B (1998) Food chemistry under high hydrostatic pressure. *Special Publication - Royal Society of Chemistry* **222**: 133-144.

Butz P, Koller W, Tauscher B & Wolf S (1994) Ultra-high pressure processing of onions: chemical and sensory changes. *LWT-Food Science and Technology* **27**: 463-467.

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K & Madden TL (2009) BLAST+: architecture and applications. *BMC bioinformatics* **10**: 421.

Caplice E & Fitzgerald GF (1999) Food fermentations: role of microorganisms in food production and preservation. *International journal of food microbiology* **50**: 131-149.

Carballo J, Fernandez P, Carrascosa A, Solas M & Colmenero FJ (1997) Characteristics of low-and high-fat beef patties: effect of high hydrostatic pressure. *Journal of food protection* **60**: 48-53.

Carlez A, Rosec J-P, Richard N & Cheftel J-C (1993) High pressure inactivation of *Citrobacter freundii*, *Pseudomonas fluorescens* and *Listeria innocua* in inoculated minced beef muscle. *LWT-Food Science and Technology* **26**: 357-363.

Carlez A, Rosec J-P, Richard N & Cheftel J-C (1994) Bacterial growth during chilled storage of pressure-treated minced meat. *LWT-Food Science and Technology* **27**: 48-54.

Carpentier B & Cerf O (1993) Biofilms and their consequences, with particular reference to hygiene in the food industry. *Journal of Applied Microbiology* **75**: 499-511.

Casal V & Gómez R (1999) Effect of high pressure on the viability and enzymatic activity of mesophilic lactic acid bacteria isolated from caprine cheese. *Journal of Dairy Science* **82**: 1092-1098.

Cebeci A & Gürakan C (2003) Properties of potential probiotic *Lactobacillus plantarum* strains. *Food Microbiology* **20**: 511-518.

Chaillou S, Champomier-Vergès M-C, Cornet M, Crutz-Le Coq A-M, Dudez A-M, Martin V, Beaufils S, Darbon-Rongère E, Bossy R & Loux V (2005) The complete genome sequence of the meat-borne lactic acid bacterium *Lactobacillus sakei* 23K. *Nature biotechnology* **23**: 1527-1533.

Champomier-Vergès M-C, Chaillou S, Cornet M & Zagorec M (2001) *Lactobacillus sakei*: recent developments and future prospects. *Research in Microbiology* **152**: 839-848.

Chapot-Chartier M-P & Kulakauskas S (2014) Cell wall structure and function in lactic acid bacteria. *Microbial cell factories* **13**: S9.

Charlton D, Nelson M & Werkman C (1934) Physiology of *Lactobacillus fructivorans sp. nov.* isolated from spoiled salad dressing. *Iowa State J Sci* **9**: 1-11.

Cheftel J-C (1992) Effects of high hydrostatic pressure on food constituents. *Colloques-Institut national de la sante et de la recherche medicale colloques et seminaires*: 195-209.

Cheftel JC (1995) Review: High-pressure, microbial inactivation and food preservation/Revision: Alta-presion, inactivacion microbiologica y conservacion de alimentos. *Food science and technology international* **1**: 75-90.

Cheftel JC & Culioli J (1997) Effects of high pressure on meat: a review. *Meat science* **46**: 211-236.

Chenoll E, Macián MC & Aznar R (2006) *Lactobacillus tucceti sp. nov.*, a new lactic acid bacterium isolated from sausage. *Systematic and applied microbiology* **29**: 389-395.

Chilton P, Isaacs NS, Mackey B & Stenning R (1997) The effects of high hydrostatic pressure on bacteria. *In K Heremans (Ed), High pressure research in the biosciences and biotechnology, Belgium: Leuven University Press*: 225–228.

Chong P, Fortes P & Jameson D (1985) Mechanisms of inhibition of (Na, K)-ATPase by hydrostatic pressure studied with fluorescent probes. *Journal of Biological Chemistry* **260**: 14484-14490.

Considine KM, Kelly AL, Fitzgerald GF, Hill C & Sleator RD (2008) High-pressure processing - effects on microbial food safety and food quality. *FEMS microbiology letters* **281**: 1-9.

Corbo MR, Bevilacqua A, Campaniello D, D'Amato D, Speranza B & Sinigaglia M (2009) Prolonging microbial shelf life of foods through the use of natural compounds and non-thermal approaches - a review. *International Journal of Food Science & Technology* **44**: 223-241.

Crowley S, Bottacini F, Mahony J & van Sinderen D (2013) Complete genome sequence of *Lactobacillus plantarum* strain 16, a broad-spectrum antifungal-producing lactic acid bacterium. *Genome Announcements* **1**: e00533-00513.

Daffonchio D, Thaveesri J & Verstraete W (1995) Contact angle measurement and cell hydrophobicity of granular sludge from upflow anaerobic sludge bed reactors. *Applied and environmental microbiology* **61**: 3676-3680.

Darling D & Birkett R (1987) Food colloids in practice. *Food emulsions and foams, Woodhead Publishing Series in Food Science, Technology and Nutrition*: 1-29.

Daryaei H & Balasubramaniam V (2012) Microbial decontamination of food by high pressure processing. *Microbial decontamination in the food industry: novel methods and applications, Woodhead Publishing Ltd, Philidephia, PA*: 370-406.

De Heij W, Van Schepdael L, Van Den Berg R & Bartels P (2002) Increasing preservation efficiency and product quality through control of temperature distributions in high pressure applications. *International journal of high pressure research* **22**: 653-657.

Debye P & Hueckel E (1923) Zur Theorie der Elektrolyte. I. *Physikalische Zeitschrift* **23**: 185-206.

Degner BM, Chung C, Schlegel V, Hutkins R & McClements DJ (2014) Factors Influencing the Freeze-Thaw Stability of Emulsion-Based Foods. *Comprehensive Reviews in Food Science and Food Safety* **13**: 98-113.

Degner R (2012) *PH-Messung: der Leitfaden für Praktiker*. John Wiley & Sons.

Delcour J, Ferain T, Deghorain M, Palumbo E & Hols P (1999) The biosynthesis and functionality of the cell-wall of lactic acid bacteria. *Antonie Van Leeuwenhoek* **76**: 159-184.

Demazeau G & Rivalain N (2011) The development of high hydrostatic pressure processes as an alternative to other pathogen reduction methods. *Journal of applied microbiology* **110**: 1359-1369.

Desrumaux A & Marcand J (2002) Formation of sunflower oil emulsions stabilized by whey proteins with high-pressure homogenization (up to 350 MPa): effect of pressure on emulsion characteristics. *International journal of food science & technology* **37**: 263-269.

Dickson JS & Koohmaraie M (1989) Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. *Applied and Environmental Microbiology* **55**: 832-836.

Dogan C & Erkmen O (2004) High pressure inactivation kinetics of *Listeria monocytogenes* inactivation in broth, milk, and peach and orange juices. *Journal of Food Engineering* **62**: 47-52.

Donaghy J, Linton M, Patterson M & Rowe M (2007) Effect of high pressure and pasteurization on *Mycobacterium avium ssp. paratuberculosis* in milk. *Letters in applied microbiology* **45**: 154-159.

Dong P, Georget ES, Aganovic K, Heinz V & Mathys A (2015) Ultra high pressure homogenization (UHPH) inactivation of *Bacillus amyloliquefaciens* spores in phosphate buffered saline (PBS) and milk. *Front Microbiol* **6**: 712.

Donlon B & Colleran E (1993) A comparison of different methods to determine the hydrophobicity of acetogenic bacteria. *Journal of microbiological methods* **17**: 27-37.

Douzals J, Marechal P, Coquille J & Gervais P (1996) Microscopic study of starch gelatinization under high hydrostatic pressure. *Journal of Agricultural and Food Chemistry* **44**: 1403-1408.

Doyle M & Roman D (1981) Growth and survival of *Campylobacter fetus* subsp. *jejuni* as a function of temperature and pH. *Journal of Food Protection*® **44**: 596-601.

Doyle RJ & Rosenberg M (1995) Measurement of microbial adhesion to hydrophobic substrata. *Methods in enzymology* **253**: 542.

Dramsi S, Magnet S, Davison S & Arthur M (2008) Covalent attachment of proteins to peptidoglycan. *FEMS microbiology reviews* **32**: 307-320.

Dumay E, Chevalier-Lucia D & López-Pedemonte T (2010) High pressure and food conservation. *Comparative high pressure biology*: 85-120.

Eaton J, Bateman D & Hauberg S (2009) GNU octave version 3.0. 1 manual. *A High-level Interactive Language for Numerical Computations Boston, MA: Create Space Independent Publishing Platform*.

Eaton JW & Rawlings JB (2003) Ten years of Octave - recent developments and plans for the future. *International Workshop on Distributed Statistical Computing.*

Egan AF, Ford A & Shay B (1980) A comparison of microbacterium thermosphactum and lactobacilli as spoilage organsisms of vacuum-packaged sliced luncheon meats. *Journal of food Science* **45**: 1745-1748.

Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P & Bettman B (2009) Real-time DNA sequencing from single polymerase molecules. *Science* **323**: 133-138.

Elamin WM, Endan JB, Yosuf YA, Shamsudin R & Ahmedov A (2015) High Pressure Processing Technology and Equipment Evolution: A Review. *Journal of Engineering Science & Technology Review* **8(5)**.

Elwing H, Askendal A & Lundström I (1989) Desorption of fibrinogen and γ-globulin from solid surfaces induced by a nonionic detergent. *Journal of Colloid and Interface Science* **128**: 296-300.

Escriu R & Mor-Mur M (2009) Role of quantity and quality of fat in meat models inoculated with *Listeria innocua* or *Salmonella Typhimurium* treated by high pressure and refrigerated stored. *Food microbiology* **26**: 834-840.

Esmaeili S, Mogharrabi M, Safi F, Sohrabvandi S, Mortazavian AM & Bagheripoor-Fallah N (2015) The common spoilage microorganisms of beer: occurrence, defects, and determination - a review. *Carpathian Journal of Food Science & Technology* **7(4)**.

Esteban-Torres M, Mancheño J, de Las Rivas B & Muñoz R (2014) Production and characterization of a tributyrin esterase from *Lactobacillus plantarum* suitable for cheese lipolysis. *Journal of dairy science* **97**: 6737-6744.

Eva G, López I, Ruiz JI, Sáenz J, Fernández E, Zarazaga M, Dizy M, Torres C & Ruiz-Larrea F (2004) High tolerance of wild *Lactobacillus plantarum* and *Oenococcus oeni* strains to lyophilisation and stress environmental conditions of acid pH and ethanol. *FEMS Microbiology Letters* **230**: 53-61.

Evans JB & Niven Jr C (1951) Nutrition of the heterofermentative lactobacilli that cause greening of cured meat products. *Journal of bacteriology* **62**: 599.

Fachin D, Loey Av, VanLoeyIndrawati A, Ludikhuyze L & Hendrickx M (2002) Thermal and High-Pressure Inactivation of Tomato Polygalacturonase: A Kinetic Study. *Journal of food science* **67**: 1610-1615.

Fadda S, Sanz Y, Vignolo G, Aristoy M-C, Oliver G & Toldrá F (1999) Characterization of muscle sarcoplasmic and myofibrillar protein hydrolysis caused by *Lactobacillus plantarum*. *Applied and Environmental Microbiology* **65**: 3540-3546.

Farkas DF & Hoover DG (2000) High pressure processing. *Journal of Food Science* **65**: 47-64.

Fedtke I, Mader D, Kohler T, Moll H, Nicholson G, Biswas R, Henseler K, Götz F, Zähringer U & Peschel A (2007) A *Staphylococcus aureus* ypfP mutant with strongly reduced lipoteichoic acid (LTA) content: LTA governs bacterial surface properties and autolysin activity. *Molecular microbiology* **65**: 1078-1091.

Ferstl P, Gillig S, Kaufmann C, Dürr C, Eder C, Wierschem A & Ruß W (2010) Pressure-induced phase transitions in triacylglycerides. *Annals of the New York Academy of Sciences* **1189**: 62-67.

Fiedler S & Sattler K (1992) Hydrophobie und mikrobielle Leistung 2. Bestimmung des hydrophobiegrades oleophiler Mikroorganismen. *Zentralblatt für Mikrobiologie* **147**: 5-14.

Fox P, Law J, McSweeney P & Wallace J (1993) Biochemistry of cheese ripening. *Cheese: chemistry, physics and microbiology.* 389-438. Springer.

Françoise L (2010) Occurrence and role of lactic acid bacteria in seafood products. *Food Microbiology* **27**: 698-709.

Garcia-Graells C, Hauben KJ & Michiels CW (1998) High-pressure inactivation and sublethal injury of pressure-resistant *Escherichia coli* mutants in fruit juices. *Applied and Environmental Microbiology* **64**: 1566-1568.

Garcia-Graells C, Masschalck B & Michiels CW (1999) Inactivation of *Escherichia coli* in milk by high-hydrostatic-pressure treatment in combination with antimicrobial peptides. *Journal of food protection* **62**: 1248-1254.

Garrett TR, Bhakoo M & Zhang Z (2008) Bacterial adhesion and biofilms on surfaces. *Progress in Natural Science* **18**: 1049-1056.

Georget E, Sevenich R, Reineke K, Mathys A, Heinz V, Callanan M, Rauh C & Knorr D (2015) Inactivation of microorganisms by high isostatic pressure processing in complex matrices: a review. *Innovative Food Science & Emerging Technologies* **27**: 1-14.

Gervilla R, Ferragut V & Guamis B (2000) High pressure inactivation of microorganisms inoculated into ovine milk of different fat contents. *Journal of Dairy Science* **83**: 674-682.

Gervilla R, Capellas M, Ferragut V & Guamis B (1997) Effect of high hydrostatic pressure on *Listeria innocua* 910 CECT inoculated into ewe's milk. *Journal of food protection* **60**: 33-37.

Giaouris E, Chapot-Chartier M-P & Briandet R (2009) Surface physicochemical analysis of natural Lactococcus lactis strains reveals the existence of hydrophobic and low charged strains with altered adhesive properties. *International journal of food microbiology* **131**: 2-9.

Gill C & Reichel M (1989) Growth of the cold-tolerant pathogens *Yersinia enterocolitica*, *Aeromonas hydrophila* and *Listeria monocytogenes* on high-pH beef packaged under vacuum or carbon dioxide. *Food Microbiology* **6**: 223-230.

Gillatt J (1991) Microbial contamination of emulsion paints and its prevention. *Jocca - Surface Coatings International* **74**: 324-328.

Glass KA, McDonnell LM, Rassel RC & Zierke KL (2007) Controlling *Listeria monocytogenes* on sliced ham and turkey products using benzoate, propionate, and sorbate. *Journal of food protection* **70**: 2306-2312.

Gobbetti M, Fox P & Stepaniak L (1997) Isolation and characterization of a tributyrin esterase from *Lactobacillus plantarum* 2739. *Journal of Dairy Science* **80**: 3099-3106.

Gobbetti M, Fox PF, Smacchi E, Stepaniak L & Damiani P (1996) Purification and characterization of a lipase from *Lactobacillus plantarum* 2739. *Journal of Food Biochemistry* **20**: 227-246.

Goldstein EJ, Tyrrell KL & Citron DM (2015) *Lactobacillus* species: taxonomic complexity and controversial susceptibilities. *Clinical Infectious Diseases* **60**: S98-S107.

Goulter R, Gentle I & Dykes G (2009) Issues in determining factors influencing bacterial attachment: a review using the attachment of *Escherichia coli* to abiotic surfaces as an example. *Letters in applied microbiology* **49**: 1-7.

Goutefongea R, Rampon V, Nicolas N & Dumont J-P (1995) Meat color changes under high pressure treatment. **41**: 384-385.

Gram C (1884) Ueber die isolirte Farbung der Schizomyceten in Schnitt-und Trockenpraparaten. *Fortschritte der Medicin* **2**: 185-189.

Granato D, Perotti F, Masserey I, Rouvet M, Golliard M, Servin A & Brassart D (1999) Cell surface-associated lipoteichoic acid acts as an adhesion factor for attachment of *Lactobacillus johnsonii* La1 to human enterocyte-like Caco-2 cells. *Applied and Environmental Microbiology* **65**: 1071-1077.

Green DW & Perry RH (1997) Perry's chemical engineers' handbook. McGraw-Hill Professional, 8th edition.

Griffin WC (1946) Classification of surface-active agents by "HLB". *J Soc Cosmetic Chemists* **1**: 311-326.

Gross M & Jaenicke R (1994) Proteins under pressure. *The FEBS Journal* **221**: 617-630.

Gross M, Lehle K, Jaenicke R & Nierhaus KH (1993) Pressure-induced dissociation of ribosomes and elongation cycle intermediates. *The FEBS Journal* **218**: 463-468.

Gross M, Cramton SE, Götz F & Peschel A (2001) Key role of teichoic acid net charge in *Staphylococcus aureus* colonization of artificial surfaces. *Infection and immunity* **69**: 3423-3426.

Grèbol N (2002) Commercial use of high hydrostatic pressure in sliced cooked ham in Spain. *Progress in biotechnology* **19**: 385-388.

Gänzle M & Liu Y (2015) Mechanisms of pressure-mediated cell death and injury in *Escherichia coli*: from fundamentals to food applications. *Frontiers in microbiology* **6**: 599.

Gänzle MG & Vogel RF (2001) On-line fluorescence determination of pressure mediated outer membrane damage in *Escherichia coli*. *Systematic and applied microbiology* **24**: 477-485.

Haddad N, Burns CM, Bolla JM, Prévost H, Fédérighi M, Drider D & Cappelier JM (2009) Long-term survival of *Campylobacter jejuni* at low temperatures is dependent on polynucleotide phosphorylase activity. *Applied and Environmental Microbiology* **75**: 7310-7318.

Hancock IC (1991) Microbial cell surface architecture. *Microbial cell surface analysis*: 23-59.

Hasenhuettl GL (2008) Synthesis and commercial preparation of food emulsifiers. *Food emulsifiers and their applications*: 11-37. Springer.

Hauben K, Bernaerts K & Michiels C (1998) Protective effect of calcium on inactivation of *Escherichia coli* by high hydrostatic pressure. *Journal of Applied Microbiology* **85**: 678-684.

Hayakawa K, Ueno Y, Kawamura S, Kato T & Hayashi R (1998) Microorganism inactivation using high-pressure generation in sealed vessels under sub-zero temperature. *Applied microbiology and biotechnology* **50**: 415-418.

Hayman MM, Anantheswaran RC & Knabel SJ (2007) The effects of growth temperature and growth phase on the inactivation of *Listeria monocytogenes* in whole milk subject to high pressure processing. *International journal of food microbiology* **115**: 220-226.

Hazen K, Plotkin B & Klimas D (1986) Influence of growth conditions on cell surface hydrophobicity of *Candida albicans* and *Candida glabrata*. *Infection and immunity* **54**: 269-271.

Hazen KC (2004) Relationship between expression of cell surface hydrophobicity protein 1 (CSH1p) and surface hydrophobicity properties of *Candida dubliniensis*. *Current microbiology* **48**: 447-451.

Helmreich EJ (2002) Environmental influences on signal transduction through membranes: a retrospective mini-review. *Biophysical chemistry* **100**: 519-534.

Heremans K (1982) High pressure effects on proteins and other biomolecules. *Annual review of biophysics and bioengineering* **11**: 1-21.

Heremans K (2001) The effects of high pressure on biomaterials. *Ultra high pressure treatments of foods*: 23-51. Springer.

Heremans K & Smeller L (1998) Protein structure and dynamics at high pressure. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology* **1386**: 353-370.

Hernández-Macedo M, Contreras-Castillo C, Tsai S, Da Cruz S, Sarantopoulos C, Padula M & Dias CTdS (2012) Gases and volatile compounds associated with microorganisms in blown pack spoilage of Brazilian vacuum-packed beef. *Letters in applied microbiology* **55**: 467-475.

Hite BH (1899) The effect of pressure in the preservation of milk: a preliminary report. *West Virginia Agricultural Experiment Station* **58**: 15-35.

Hobbs PV (1974) Ice physics. *Clarendon Press*: 837.

Hobot J, Carlemalm E, Villiger W & Kellenberger E (1984) Periplasmic gel: new concept resulting from the reinvestigation of bacterial cell envelope ultrastructure by new methods. *Journal of Bacteriology* **160**: 143-152.

Hogan E, Kelly AL & Sun D-W (2005) High Pressure Processing of Foods: An Overview. *Emerging technologies for food processing*: 3-32.

Holland LM, Conlon B & O'Gara JP (2011) Mutation of *tagO* reveals an essential role for wall teichoic acids in *Staphylococcus epidermidis* biofilm development. *Microbiology* **157**: 408-418.

Hoogland H, Heij Wd & Schepdael Lv (2001) High pressure sterilization: novel technology, new products, new opportunities. *New Food*. **4(1)**: 21-26

Hoover DG & Gray R (1977) Function of cell wall teichoic acid in thermally injured *Staphylococcus aureus*. *Journal of bacteriology* **131**: 477-485.

Hoover DG, Metrick C, Papineau A, Farkas D & Knorr D (1989) Biological effects of high hydrostatic pressure on food microorganisms. *Food Technology* **43(3)**: 99-107.

Hori K, Manabe Y, Kaneko M & Sekimoto T (1992) The development of high pressure processor for food industries. *In High Pressure and Biotechnology, **224**, edited by C. Balny, R. Hayashi, K. Heremans and P. Masson. La Grande Motte: John Libbey Eurotext / INSERM*: 499-507.

Horn AF (2012) Factors Influencing the Effect of Milkbased Emulsifiers on Lipid Oxidation in Omega-3 Emulsions. *Thesis, Technical University of Denmark.*

Hugas M, Garriga M & Monfort J (2002) New mild technologies in meat processing: high pressure as a model technology. *Meat science* **62**: 359-371.

Hutkins RW (2001) Metabolism of starter cultures. *In Marth EH & Steele JL (Eds.), Applied dairy microbiology, 2nd edn.*: 207-241.

Jaenicke R (1981) Enzymes under extremes of physical conditions. *Annual review of biophysics and bioengineering* **10**: 1-67.

Jafari SM, Assadpoor E, He Y & Bhandari B (2008) Re-coalescence of emulsion droplets during high-energy emulsification. *Food hydrocolloids* **22**: 1191-1202.

Jarvis B (2016) Statistical aspects of the microbiological examination of foods. *3th edn., Academic Press.*

Jenni R & Berger-Bächi B (1998) Teichoic acid content in different lineages of *Staphylococcus aureus* NCTC8325. *Archives of microbiology* **170**: 171-178.

Jespers V, Menten J, Smet H, Poradosú S, Abdellati S, Verhelst R, Hardy L, Buvé A & Crucitti T (2012) Quantification of bacterial species of the vaginal microbiome in different groups of women, using nucleic acid amplification tests. *BMC microbiology* **12**: 83.

Job G & Herrmann F (2006) Chemical potential - a quantity in search of recognition. *European journal of physics* **27**: 353.

Joh D, Wann ER, Kreikemeyer B, Speziale P & Höök M (1999) Role of fibronectin-binding MSCRAMMs in bacterial adherence and entry into mammalian cells. *Matrix Biology* **18**: 211-223.

Jollègs P (1966) Fortschritte auf dem Gebiet der Casein-Chemie. *Angewandte Chemie* **78**: 629-637.

Jung Y-K, Jung S, Lee H-J, Kang M-G, Lee S-K, Kim Y-J & Jo C (2012) Effect of high pressure after the addition of vegetable oil on the safety and quality of beef loin. *Korean Journal for Food Science of Animal Resources* **32**: 68-76.

Jääskeläinen E, Hultman J, Parshintsev J, Riekkola M-L & Björkroth J (2016) Development of spoilage bacterial community and volatile compounds in chilled beef under vacuum or high oxygen atmospheres. *International journal of food microbiology* **223**: 25-32.

Kabalnov AS & Shchukin ED (1992) Ostwald ripening theory: applications to fluorocarbon emulsion stability. *Advances in colloid and interface science* **38**: 69-97.

Kafka T, Reitermayer D, Lenz C & Vogel R (2017) High hydrostatic pressure inactivation of *Lactobacillus plantarum* cells in (O/W)-emulsions is independent from cell surface hydrophobicity and lipid phase parameters. *High Pressure Research* **37(3)**: 430-448.

Kafka TA, Geissler AJ & Vogel RF (2017) Multiple Genome Sequences of *Lactobacillus plantarum* Strains. *Genome announcements* **5(29)**: e00654-17.

Kalschne DL, Womer R, Mattana A, Sarmento CMP, Colla LM & Colla E (2015) Characterization of the spoilage lactic acid bacteria in “sliced vacuum-packed cooked ham”. *Brazilian Journal of Microbiology* **46**: 173-181.

Kandler O (1986) Genus *Lactobacillus* Beijerinck 1901, 212^{AL}. *Bergey's manual of systematic bacteriology* **2**: 1209-1234.

Karbstein H, Schubert H, Scigalla W & Ludwig H (1992) Sterilization of emulsions by means of high pressure. *High Pressure and Biotechnology* **224**: 345-345.

Karjiban RA, Basri M, Rahman MBA & Salleh AB (2012) Structural properties of nonionic Tween® 80 micelle in water elucidated by molecular dynamics simulation. *APCBEE Procedia* **3**: 287-297.

Kato M & Hayashi R (1999) Effects of high pressure on lipids and biomembranes for understanding high-pressure-induced biological phenomena. *Bioscience, biotechnology, and biochemistry* **63**: 1321-1328.

Kern CC, Usbeck JC, Vogel RF & Behr J (2013) Optimization of matrix-assisted-laser-desorption–ionization-time-of-flight mass spectrometry for the identification of bacterial contaminants in beverages. *Journal of microbiological methods* **93**: 185-191.

Kesavan V, Balasubramaniam V, Adhikari C, Narayanaswamy G & Parulekar S (2002) Compression heating characteristics of proteins during high pressure processing. *IFT Annual Meeting, Anaheim, CA:* Abstract No. 91E-6

Kessler H-G (2002) Food and bio process engineering. *Dairy Technology, 5th edition, Verlag A*. Kessler, Munich, Germany

Khalid NM & Marth EH (1990) Proteolytic activity by strains of *Lactobacillus plantarum* and *Lactobacillus casei*. *Journal of Dairy Science* **73**: 3068-3076.

Khalid NM & Marth EH (1990) Lactobacilli - their enzymes and role in ripening and spoilage of cheese: a review. *Journal of Dairy Science* **73**: 2669-2684.

Kleerebezem M, Boekhorst J, van Kranenburg R, Molenaar D, Kuipers OP, Leer R, Tarchini R, Peters SA, Sandbrink HM & Fiers MW (2003) Complete genome sequence of *Lactobacillus plantarum* WCFS1. *Proceedings of the National Academy of Sciences* **100**: 1990-1995.

Klein G, Pack A, Bonaparte C & Reuter G (1998) Taxonomy and physiology of probiotic lactic acid bacteria. *International journal of food microbiology* **41**: 103-125.

Klemaszewski J, Das K & Kinsella J (1992) Formation and coalescence stability of emulsions stabilized by different milk proteins. *Journal of food science* **57**: 366-371.

Klotz B, Mañas P & Mackey BM (2010) The relationship between membrane damage, release of protein and loss of viability in *Escherichia coli* exposed to high hydrostatic pressure. *International journal of food microbiology* **137**: 214-220.

Knoerzer K, Buckow R, Sanguansri P & Versteeg C (2010) Adiabatic compression heating coefficients for high-pressure processing of water, propylene-glycol and mixtures - A combined experimental and numerical approach. *Journal of Food Engineering* **96**: 229-238.

Knorr D (1993) Effects of high-hydrostatic-pressure processes on food safety and quality. *Food technology* **47**: 156-161.

Knorr D, Heinz V & Buckow R (2006) High pressure application for food biopolymers. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* **1764**: 619-631.

Kochkodan V, Tsarenko S, Potapchenko N, Kosinova V & Goncharuk V (2008) Adhesion of microorganisms to polymer membranes: a photobactericidal effect of surface treatment with TiO2. *Desalination* **220**: 380-385.

Kohler T, Weidenmaier C & Peschel A (2009) Wall teichoic acid protects *Staphylococcus aureus* against antimicrobial fatty acids from human skin. *Journal of bacteriology* **191**: 4482-4484.

Kohlweyer U (2000) Isolierung und Charakterisierung eines Tetrahydrofuran abbauenden Stamms *Pseudonocardia sp*.. *Thesis, University Halle.*

Koseki S & Yamamoto K (2006) pH and solute concentration of suspension media affect the outcome of high hydrostatic pressure treatment of *Listeria monocytogenes*. *International journal of food microbiology* **111**: 175-179.

Koseki S & Yamamoto K (2007) Water activity of bacterial suspension media unable to account for the baroprotective effect of solute concentration on the inactivation of *Listeria monocytogenes* by high hydrostatic pressure. *International journal of food microbiology* **115**: 43-47.

Kralova I & Sjöblom J (2009) Surfactants used in food industry: a review. *Journal of Dispersion Science and Technology* **30**: 1363-1383.

Krasowska A & Sigler K (2014) How microorganisms use hydrophobicity and what does this mean for human needs? *Frontiers in cellular and infection microbiology* **4**: 112.

Kruk ZA, Kim HJ, Kim YJ, Rutley DL, Jung S, Lee SK & Jo C (2014) Combined effects of high pressure processing and addition of soy sauce and olive oil on safety and quality characteristics of chicken breast meat. *Asian-Australasian journal of animal sciences* **27**: 256.

Krumsiek J, Arnold R & Rattei T (2007) Gepard: a rapid and sensitive tool for creating dotplots on genome scale. *Bioinformatics* **23**: 1026-1028.

Kunji ER, Mierau I, Hagting A, Poolman B & Konings WN (1996) The proteotytic systems of lactic acid bacteria. *Antonie van Leeuwenhoek* **70**: 187-221.

Kurtzman C, Rogers R & Hesseltine C (1971) Microbiological spoilage of mayonnaise and salad dressings. *Applied microbiology* **21**: 870-874.

Lakshmanan R, Patterson MF & Piggott JR (2005) Effects of high-pressure processing on proteolytic enzymes and proteins in cold-smoked salmon during refrigerated storage. *Food Chemistry* **90**: 541-548.

Landau JV (1967) Induction, transcription and translation in *Escherichia coli*: a hydrostatic pressure study. *Biochimica et Biophysica Acta (BBA)-Nucleic Acids and Protein Synthesis* **149**: 506-512.

Landete JM, Rodríguez H, Curiel JA, de las Rivas B, López de Felipe F & Muñoz R (2010) Degradation of phenolic compounds found in olive products by *Lactobacillus plantarum* strains. *Olives and Olive Oil in Health and Disease Prevention*: 387-396.

Lane D (1991) 16S/23S rRNA sequencing. *Nucleic acid techniques in bacterial systematics*: 115-175

Lauterbach A, Usbeck JC, Behr J & Vogel RF (2017) MALDI-TOF MS typing enables the classification of brewing yeasts of the genus Saccharomyces to major beer styles. *PloS one* **12**: e0181694.

Lazarevic V & Karamata D (1995) The tagGH operon of *Bacillus subtilis* 168 encodes a two-component ABC transporter involved in the metabolism of two wall teichoic acids. *Molecular microbiology* **16**: 345-355.

Lazarevic V, Abellan F-X, Möller SB, Karamata D & Mauël C (2002) Comparison of ribitol and glycerol teichoic acid genes in *Bacillus subtilis* W23 and 168: identical function, similar divergent organization, but different regulation. *Microbiology* **148**: 815-824.

Lee I-C, Tomita S, Kleerebezem M & Bron PA (2013) The quest for probiotic effector molecules - unraveling strain specificity at the molecular level. *Pharmacological research* **69**: 61-74.

Lenz CA (2017) Effect of high hydrostatic pressure on *Clostridium botulinum* type E endospores. *Thesis, Technische Universität München.*

Lenz CA & Vogel RF (2014) Effect of sporulation medium and its divalent cation content on the heat and high pressure resistance of *Clostridium botulinum* type E spores. *Food Microbiology* **44**: 156-167.

Lenz CA, Reineke K, Knorr D & Vogel RF (2015) High pressure thermal inactivation of *Clostridium botulinum* type E endospores - kinetic modeling and mechanistic insights. *Frontiers in Microbiology* **6**: 652.

Lerche M (1960) Bombage-Ursache in Fischpräserven. *Berl Münch Tierärztl Wochenschr* **75**: 12-14.

Leroy F & de Vuyst L (1999) The presence of salt and a curing agent reduces bacteriocin production by *Lactobacillus sakei* CTC 494, a potential starter culture for sausage fermentation. *Applied and Environmental Microbiology* **65**: 5350-5356.

Leroy F & De Vuyst L (2004) Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology* **15**: 67-78.

Li H, Garcia-Hernandez R, Driedger D, McMullen LM & Gänzle M (2016) Effect of the food matrix on pressure resistance of Shiga-toxin producing *Escherichia coli*. *Food Microbiology* **57**: 96-102.

Lindahl M, Faris A, Wadström T & Hjerten S (1981) A new test based on 'salting out'to measure relative hydrophobicity of bacterial cells. *Biochimica et Biophysica Acta (BBA)-General Subjects* **677**: 471-476.

Lingfa L, Sharmila R, Nathiya T & Rajan A (2014) Role of Cell Membrane Fluidity in Living and Non-living being. *Unique Journal of Pharmaceutical and Biological sciences*.

Liu Y, Gill A, McMULLEN L & Gaenzle MG (2015) Variation in heat and pressure resistance of verotoxigenic and nontoxigenic *Escherichia coli*. *Journal of food protection* **78**: 111-120.

Liévin-Le Moal V & Servin AL (2014) Anti-infective activities of lactobacillus strains in the human intestinal microbiota: from probiotics to gastrointestinal anti-infectious biotherapeutic agents. *Clinical microbiology reviews* **27**: 167-199.

Ljungh A & Wadstrom T (2006) Lactic acid bacteria as probiotics. *Current issues in intestinal microbiology* **7**: 73-90.

Lopes MdFS, Leitão AL, Regalla M, Marques JF, Carrondo MJT & Crespo MTB (2002) Characterization of a highly thermostable extracellular lipase from *Lactobacillus plantarum*. *International journal of food microbiology* **76**: 107-115.

Lopes MFS, Cunha A, Clemente J, Carrondo MT & Crespo MB (1999) Influence of environmental factors on lipase production by *Lactobacillus plantarum*. *Applied microbiology and biotechnology* **51**: 249-254.

Lu Z, Breidt F, Fleming H, Altermann E & Klaenhammer T (2003) Isolation and characterization of a *Lactobacillus plantarum* bacteriophage, ΦJL-1, from a cucumber fermentation. *International journal of food microbiology* **84**: 225-235.

Ludikhuyze L & Hendrickx MEG (2006) Effects of High Pressure on Chemical Reactions Related to Food Quality. *Ultra high pressure treatment of foods, Hendrickx ME & Knorr D (Eds.), Kluwer Academi/Plenum Puplishers*: 167-188.

Luscher C, Balasa A, Fröhling A, Ananta E & Knorr D (2004) Effect of high-pressure-induced ice I-to-ice III phase transitions on inactivation of *Listeria innocua* in frozen suspension. *Applied and environmental microbiology* **70**: 4021-4029.

Ly M, Vo N, Le T, Belin J-M & Waché Y (2006) Diversity of the surface properties of Lactococci and consequences on adhesion to food components. *Colloids and Surfaces B: Biointerfaces* **52**: 149-153.

Ly MH, Aguedo M, Goudot S, Le M, Cayot P, Teixeira J, Le T, Belin J-M & Waché Y (2008) Interactions between bacterial surfaces and milk proteins, impact on food emulsions stability. *Food Hydrocolloids* **22**: 742-751.

Lyhs U & Björkroth JK (2008) *Lactobacillus sakei/curvatus* is the prevailing lactic acid bacterium group in spoiled maatjes herring. *Food microbiology* **25**: 529-533.

Lyhs U, Korkeala H, Vandamme P & Björkroth J (2001) *Lactobacillus alimentarius*: a specific spoilage organism in marinated herring. *International journal of food microbiology* **64**: 355-360.

Mackey B, Forestiere K, Isaacs N, Stenning R & Brooker B (1994) The effect of high hydrostatic pressure on *Salmonella thompson* and *Listeria monocytogenes* examined by electron microscopy. *Letters in Applied Microbiology* **19**: 429-432.

Mafu AA, Plumety C, Deschênes L & Goulet J (2010) Adhesion of pathogenic bacteria to food contact surfaces: Influence of pH of culture. *International journal of microbiology* **2011**.

Maldonado A, Ruiz-Barba JL, Floriano B & Jiménez-Díaz R (2002) The locus responsible for production of plantaricin S, a class IIb bacteriocin produced by *Lactobacillus plantarum* LPCO10, is widely distributed among wild-type *Lact. plantarum* strains isolated from olive fermentations. *International journal of food microbiology* **77**: 117-124.

Mantini D, Petrucci F, Pieragostino D, Del Boccio P, Sacchetta P, Candiano G, Ghiggeri GM, Lugaresi A, Federici G & Di Ilio C (2010) A computational platform for MALDI-TOF mass spectrometry data: application to serum and plasma samples. *Journal of proteomics* **73**: 562-570.

Marceau A, Zagorec M & Champomier-Vergès M-C (2003) Positive effects of growth at suboptimal temperature and high salt concentration on long-term survival of *Lactobacillus sakei*. *Research in microbiology* **154**: 37-42.

Marth EH & Steele J (2001) *Applied dairy microbiology, 2nd edn. New York, USA: Marcel Dekker Inc.*

Martienssen M (2001) Leistungen immobilisierter Mikroorganismen bei der Eliminierung von Umweltschadstoffen. *Thesis, Universitäts-und Landesbibliothek.*

Matamoros S, André S, Hue I, Prévost H & Pilet M (2010) Identification of lactic acid bacteria involved in the spoilage of pasteurized “foie gras” products. *Meat science* **85**: 467-471.

Mathys A, Kallmeyer R, Heinz V & Knorr D (2008) Impact of dissociation equilibrium shift on bacterial spore inactivation by heat and pressure. *Food Control* **19**: 1165-1173.

Matias VR & Beveridge TJ (2005) Cryo-electron microscopy reveals native polymeric cell wall structure in *Bacillus subtilis* 168 and the existence of a periplasmic space. *Molecular microbiology* **56**: 240-251.

Matsuki H (2015) How Do Membranes Respond to Pressure? *High Pressure Bioscience*: 321-343. Springer.

Mañas P & Mackey BM (2004) Morphological and physiological changes induced by high hydrostatic pressure in exponential-and stationary-phase cells of *Escherichia coli*: relationship with cell death. *Applied and Environmental Microbiology* **70**: 1545-1554.

McCarthy A (2010) Third generation DNA sequencing: pacific biosciences' single molecule real time technology. *Chemistry & biology* **17**: 675-676.

McClements D (2005) Food emulsion: principles, practice and techniques. *2nd edn. Boca Raton, Fla.: CRC Press*: 515-543.

McClements DJ (2004) Protein-stabilized emulsions. *Current opinion in colloid & interface science* **9**: 305-313.

McClements J, Patterson M & Linton M (2001) The effect of growth stage and growth temperature on high hydrostatic pressure inactivation of some psychrotrophic bacteria in milk. *Journal of food protection* **64**: 514-522.

McClure P, Roberts T & Oguru PO (1989) Comparison of the effects of sodium chloride, pH and temperature on the growth of *Listeria monocytogenes* on gradient plates and in liquid medium. *Letters in Applied Microbiology* **9**: 95-99.

McDonald L, Fleming H & Hassan H (1990) Acid tolerance of *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. *Applied and Environmental Microbiology* **56**: 2120-2124.

McFeters G, Bazin M, Mirelman D, Bryers J, Mitchell R, Caldwell D, Schubert R, Characklis W, Tanaka T & Lund D (1984) Biofilm development and its consequences. *Microbial adhesion and aggregation*: 108-124. Springer.

McNab R, Forbes H, Handley PS, Loach DM, Tannock GW & Jenkinson HF (1999) Cell wall-anchored CshA polypeptide (259 kilodaltons) in *Streptococcus gordonii* forms surface fibrils that confer hydrophobic and adhesive properties. *Journal of bacteriology* **181**: 3087-3095.

Mejlholm O, Kjeldgaard J, Modberg A, Vest MB, Bøknæs N, Koort J, Björkroth J & Dalgaard P (2008) Microbial changes and growth of *Listeria monocytogenes* during chilled storage of brined shrimp (Pandalus borealis). *International journal of food microbiology* **124**: 250-259.

Meredith TC, Swoboda JG & Walker S (2008) Late-stage polyribitol phosphate wall teichoic acid biosynthesis in *Staphylococcus aureus*. *Journal of bacteriology* **190**: 3046-3056.

Mills G, Earnshaw R & Patterson M (1998) Effects of high hydrostatic pressure on *Clostridium sporogenes* spores. *Letters in Applied Microbiology* **26**: 227-230.

Mirelman D, Beck BD & Shaw D (1970) The location of the D-alanyl ester in the ribitol teichoic acid of *Staphylococcus aureus*. *Biochemical and biophysical research communications* **39**: 712-717.

Mitchell P (1961) Approaches to the analysis of specific membrane transport. *Biological structure and function* **2**: 581-599.

Miörner H, Johansson G & Kronvall G (1983) Lipoteichoic acid is the major cell wall component responsible for surface hydrophobicity of group A streptococci. *Infection and immunity* **39**: 336-343.

Molina-Gutierrez A, Stippl V, Delgado A, Gänzle MG & Vogel RF (2002) In situ determination of the intracellular pH of *Lactococcus lactis* and *Lactobacillus plantarum* during pressure treatment. *Applied and environmental microbiology* **68**: 4399-4406.

Molina-Höppner A, Doster W, Vogel RF & Gänzle MG (2004) Protective effect of sucrose and sodium chloride for *Lactococcus lactis* during sublethal and lethal high-pressure treatments. *Applied and Environmental Microbiology* **70**: 2013-2020.

Molina-Höppner A, Sato T, Kato C, Gänzle MG & Vogel RF (2003) Effects of pressure on cell morphology and cell division of lactic acid bacteria. *Extremophiles* **7**: 511-516.

Mor-Mur M & Yuste J (2003) High pressure processing applied to cooked sausage manufacture: physical properties and sensory analysis. *Meat Science* **65**: 1187-1191.

Morales P, Calzada J, Rodríguez B, de Paz M, Gaya P & Nuñez M (2006) Effect of cheese water activity and carbohydrate content on the barotolerance of *Listeria monocytogenes* Scott A. *Journal of Food Protection*® **69**: 1328-1333.

Mota MJ, Lopes RP, Delgadillo I & Saraiva JA (2013) Microorganisms under high pressure - Adaptation, growth and biotechnological potential. *Biotechnology advances* **31**: 1426-1434.

Moussa M, Perrier-Cornet J-M & Gervais P (2007) Damage in *Escherichia coli* cells treated with a combination of high hydrostatic pressure and subzero temperature. *Applied and environmental microbiology* **73**: 6508-6518.

Mozes N & Rouxhet P (1987) Methods for measuring hydrophobicity of microorganisms. *Journal of Microbiological Methods* **6**: 99-112.

Mozhaev VV, Heremans K, Frank J, Masson P & Balny C (1994) Exploiting the effects of high hydrostatic pressure in biotechnological applications. *Trends in Biotechnology* **12**: 493-501.

Mueller RF (1996) Bacterial transport and colonization in low nutrient environments. *Water Research* **30**: 2681-2690.

Muhr A & Blanshard J (1982) Effect of hydrostatic pressure on starch gelatinisation. *Carbohydrate Polymers* **2**: 61-74.

Nash DP & Jonas J (1997) Structure of pressure-assisted cold denatured lysozyme and comparison with lysozyme folding intermediates. *Biochemistry* **36**: 14375-14383.

Naumova IB, Shashkov AS, Tul'skaya EM, Streshinskaya GM, Kozlova YI, Potekhina NV, Evtushenko LI & Stackebrandt E (2001) Cell wall teichoic acids: structural diversity, species specificity in the genus *Nocardiopsis*, and chemotaxonomic perspective. *FEMS microbiology reviews* **25**: 269-283.

Navarre WW & Schneewind O (1999) Surface proteins of gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. *Microbiology and Molecular Biology Reviews* **63**: 174-229.

Neuhaus FC & Baddiley J (2003) A continuum of anionic charge: structures and functions of D-alanyl-teichoic acids in gram-positive bacteria. *Microbiology and Molecular Biology Reviews* **67**: 686-723.

NIST (2002) Standard Reference Database 23 Version 7.0. National Institute of Standards and Technology (NIST) / American Society of Mechanical Engineers (ASME) - International Association for the Properties of Water and Steam (IAPWS); Lemmon, E. W., McLinden, M. O., Huber, M. L., 2002, Physical and Chemical Properties Devision, © by the U.S. Secretary of Commerce on behalf of The United States of America.

Niven GW, Miles CA & Mackey BM (1999) The effects of hydrostatic pressure on ribosome conformation in *Escherichia coli*: an in vivo study using differential scanning calorimetry. *Microbiology* **145**: 419-425.

O'Reilly CE, O'Connor PM, Kelly AL, Beresford TP & Murphy PM (2000) Use of hydrostatic pressure for inactivation of microbial contaminants in cheese. *Applied and environmental microbiology* **66**: 4890-4896.

Oey I, Lille M, Van Loey A & Hendrickx M (2008) Effect of high-pressure processing on colour, texture and flavour of fruit-and vegetable-based food products: a review. *Trends in Food Science & Technology* **19**: 320-328.

Oku Y, Kurokawa K, Matsuo M, Yamada S, Lee B-L & Sekimizu K (2009) Pleiotropic roles of polyglycerolphosphate synthase of lipoteichoic acid in growth of *Staphylococcus aureus* cells. *Journal of bacteriology* **191**: 141-151.

Oterholm A, Witter LD & Ordal ZJ (1972) Purification and properties of an acetyl ester hydrolase (acetylesterase) from *Lactobacillus plantarum*. *Journal of Dairy Science* **55**: 8-13.

Oxen P & Knorr D (1993) Baroprotective effects of high solute concentrations against inactivation of *Rhodotorula rubra*. *LWT-Food Science and Technology* **26**: 220-223.

Pagán R & Mackey B (2000) Relationship between membrane damage and cell death in pressure-treated *Escherichia coli* cells: differences between exponential-and stationary-phase cells and variation among strains. *Applied and Environmental Microbiology* **66**: 2829-2834.

Palou E, Lopez-Malo A, Barbosa-Canovas G, Welti-Chanes J & Swanson B (1997) Combined effect of high hydrostatic pressure and water activity on *Zygosaccharomyces bailii* inhibition. *Lett Appl Microbiol* **24**: 417-420.

Pandrangi S & Balasubramaniam V (2005) High pressure processing of salads and ready meals. *Emerging technologies for food processing, 2nd edn.*: 33-46.

Pardo G & Zufía J (2012) Life cycle assessment of food-preservation technologies. *Journal of Cleaner Production* **28**: 198-207.

Parker ML, Brocklehurst T, Gunning P, Coleman HP & Robins MM (1995) Growth of food-borne pathogenic bacteria in oil-in-water emulsions: I - Methods for investigating the form of growth. *Journal of applied bacteriology* **78**: 601-608.

Patel J, Sharma M & Ravishakar S (2011) Effect of curli expression and hydrophobicity of *Escherichia coli* O157: H7 on attachment to fresh produce surfaces. *Journal of applied microbiology* **110**: 737-745.

Patterson M (1999) High-pressure treatment of foods. *In Robinson RK, Batt CA & Patel PD (Eds.), The encyclopedia of food microbiology. New York: Academic Press.*

Patterson M (2005) Microbiology of pressure-treated foods. *Journal of applied microbiology* **98**: 1400-1409.

Patterson MF & Kilpatrick DJ (1998) The combined effect of high hydrostatic pressure and mild heat on inactivation of pathogens in milk and poultry. *Journal of food protection* **61**: 432-436.

Patterson MF, Mackle A & Linton M (2011) Effect of high pressure, in combination with antilisterial agents, on the growth of *Listeria monocytogenes* during extended storage of cooked chicken. *Food microbiology* **28**: 1505-1508.

Patterson MF, Quinn M, Simpson R & Gilmour A (1995) Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods. *Journal of Food Protection* **58**: 524-529.

Percy MG & Gründling A (2014) Lipoteichoic acid synthesis and function in Gram-positive bacteria. *Annual review of microbiology* **68**: 81-100.

Petri A, Pfannebecker J, Fröhlich J & König H (2013) Fast identification of wine related lactic acid bacteria by multiplex PCR. *Food microbiology* **33**: 48-54.

Pizarro-Cerdá J & Cossart P (2006) Bacterial adhesion and entry into host cells. *Cell* **124**: 715-727.

Planck M (1887) Ueber das Princip der Vermehrung der Entropie. *Annalen der Physik* **268**: 462-503.

Plengvidhya V, Breidt F, Lu Z & Fleming HP (2007) DNA fingerprinting of lactic acid bacteria in sauerkraut fermentations. *Applied and environmental microbiology* **73**: 7697-7702.

Poortinga AT, Bos R, Norde W & Busscher HJ (2002) Electric double layer interactions in bacterial adhesion to surfaces. *Surface science reports* **47**: 1-32.

Pulido RP, Omar NB, Abriouel H, López RL, Cañamero MM, Guyot JP & Gálvez A (2007) Characterization of lactobacilli isolated from caper berry fermentations. *Journal of applied microbiology* **102**: 583-590.

Quaglia G, Gravina R, Paperi R & Paoletti F (1996) Effect of high pressure treatments on peroxidase activity, ascorbic acid content and texture in green peas. *LWT-Food Science and Technology* **29**: 552-555.

Quinlan RJ & Reinhart GD (2005) Baroresistant buffer mixtures for biochemical analyses. *Analytical biochemistry* **341**: 69-76.

Rademacher B (1999) Hochdruckbehandlung von Milch: Untersuchungen zur Inaktivierung von Mikroorganismen und Enzymen und deren kinetische Beschreibung. *VDI-Verlag, Reihe 14, Nr. 96, Düsseldorf, Germany*

Raffalli J, Rosec J, Carlez A, Dumay E, Richard N & Cheftel J (1994) Stress et inactivation par haute pression de *Listeria innocua* introduites dans une crème laitière. *Sciences des aliments* **14**: 349-358.

Rajan S, Ahn J, Balasubramaniam V & Yousef A (2006) Combined pressure-thermal inactivation kinetics of *Bacillus amyloliquefaciens* spores in egg patty mince. *Journal of Food Protection* **69**: 853-860.

Ramaswamy HS, Jin H & Zhu S (2009) Effects of fat, casein and lactose on high-pressure destruction of *Escherichia coli* K12 (ATCC-29055) in milk. *Food and bioproducts processing* **87**: 1-6.

Ramaswamy R (2007) Thermal behavior of food materials during high pressure processing. *Thesis, The Ohio State University.*

Rao PS, Chakraborty S, Kaushik N, Kaur BP & Hulle NS (2014) High Hydrostatic Pressure Processing of Food Materials. *Introduction to Advanced Food Process Engineering, CRC Press*: 151-186.

Rasanayagam V, Balasubramaniam V, Ting E, Sizer C, Bush C & Anderson C (2003) Compression heating of selected fatty food materials during high-pressure processing. *Journal of Food Science* **68**: 254-259.

Reineke K (2012) Mechanisms of *Bacillus* spore germination and inactivation during high pressure processing. *Thesis, Universitätsbibliothek der Technischen Universität Berlin.*

Rendueles E, Omer M, Alvseike O, Alonso-Calleja C, Capita R & Prieto M (2011) Microbiological food safety assessment of high hydrostatic pressure processing: a review. *LWT-Food Science and Technology* **44**: 1251-1260.

Ritz M, Freulet M, Orange N & Federighi M (2000) Effects of high hydrostatic pressure on membrane proteins of *Salmonella typhimurium*. *International Journal of Food Microbiology* **55**: 115-119.

Ritz M, Jugiau F, Federighi M, Chapleau N & de Lamballerie M (2008) Effects of high pressure, subzero temperature, and pH on survival of *Listeria monocytogenes* in buffer and smoked salmon. *J Food Prot* **71**: 1612-1618.

Robey M, Benito A, Hutson RH, Pascual C, Park SF & Mackey BM (2001) Variation in Resistance to High Hydrostatic Pressure and *rpoS* Heterogeneity in Natural Isolates of *Escherichia coli* O157:H7. *Applied and Environmental Microbiology* **67**: 4901-4907.

Roos S, Engstrand L & Jonsson H (2005) *Lactobacillus gastricus sp. nov*., L*actobacillus antri sp. nov.*, *Lactobacillus kalixensis sp. nov.* and *Lactobacillus ultunensis sp. nov.*, isolated from human stomach mucosa. *International journal of systematic and evolutionary microbiology* **55**: 77-82.

Rosenberg M (2006) Microbial adhesion to hydrocarbons: twenty-five years of doing MATH. *FEMS microbiology letters* **262**: 129-134.

Rosenberg M & Kjelleberg S (1986) Hydrophobic interactions: role in bacterial adhesion. *Advances in microbial ecology*: 353-393. Springer.

Rosenberg M, Gutnick D & Rosenberg E (1980) Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiol Lett* **9**: 29-33.

Rubens P, Snauwaert J, Heremans K & Stute R (1999) In situ observation of pressure-induced gelation of starches studied with FTIR in the diamond anvil cell. *Carbohydrate polymers* **39**: 231-235.

Rubio B, Martínez B, García-Cachán MD, Rovira J & Jaime I (2007) The effects of high pressure treatment and of storage periods on the quality of vacuum-packed “salchichón” made of raw material enriched in monounsaturated and polyunsaturated fatty acids. *Innovative food science & emerging technologies* **8**: 180-187.

Rumpold BA (2005) Impact of high hydrostatic pressure on wheat, tapioca, and potato starches. *Thesis, Berlin Technical University.*

Røssvoll E, Rønning HT, Granum PE, Møretrø T, Hjerpekjøn MR & Langsrud S (2014) Toxin production and growth of pathogens subjected to temperature fluctuations simulating consumer handling of cold cuts. *International Journal of Food Microbiology* **185**: 82-92.

Sadovskaya I, Vinogradov E, Li J & Jabbouri Sd (2004) Structural elucidation of the extracellular and cell-wall teichoic acids of *Staphylococcus epidermidis* RP62A, a reference biofilm-positive strain. *Carbohydrate research* **339**: 1467-1473.

Sale A, Gould G & Hamilton W (1970) Inactivation of bacterial spores by hydrostatic pressure. *Microbiology* **60**: 323-334.

Salminen M, Rautelin H, Tynkkynen S, Poussa T, Saxelin M, Valtonen V & Järvinen A (2006) *Lactobacillus bacteremia*, species identification, and antimicrobial susceptibility of 85 blood isolates. *Clinical Infectious Diseases* **42**: e35-e44.

Sambrook J, Fritsch EF & Maniatis T (1989) Molecular cloning: a laboratory manual. *2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.*

Samelis J, Kakouri A & Rementzis J (2000) Selective effect of the product type and the packaging conditions on the species of lactic acid bacteria dominating the spoilage microbial association of cooked meats at 4°C. *Food Microbiology* **17**: 329-340.

Sancho F, Lambert Y, Demazeau G, Largeteau A, Bouvier J-M & Narbonne J-F (1999) Effect of ultra-high hydrostatic pressure on hydrosoluble vitamins. *Journal of Food Engineering* **39**: 247-253.

Sanders J, Oomes S, Membré J-M, Wegkamp A & Wels M (2015) Biodiversity of spoilage lactobacilli: Phenotypic characterisation. *Food microbiology* **45**: 34-44.

Sanders ME & Huis J (1999) Bringing a probiotic-containing functional food to the market: microbiological, product, regulatory and labeling issues. *Lactic Acid Bacteria: Genetics, Metabolism and Applications*: 293-315. Springer.

Sanz Y, Fadda S, Vignolo G, Aristoy M-C, Oliver G & Toldrá F (1999) Hydrolysis of muscle myofibrillar proteins by L*actobacillus curvatus* and *Lactobacillus sake*. *International Journal of Food Microbiology* **53**: 115-125.

Schirmer BC, Heir E & Langsrud S (2009) Characterization of the bacterial spoilage flora in marinated pork products. *J Appl Microbiol* **106**: 2106-2116.

Schirner K, Marles-Wright J, Lewis RJ & Errington J (2009) Distinct and essential morphogenic functions for wall-and lipo-teichoic acids in *Bacillus subtilis*. *The EMBO journal* **28**: 830-842.

Schulz E, Lüdemann H-D & Jaenicke R (1976) High pressure equilibrium studies on the dissociation - association of *E. coli* ribosomes. *FEBS letters* **64**: 40-43.

Sherman L & Savage D (1986) Lipoteichoic acids in *Lactobacillus* strains that colonize the mouse gastric epithelium. *Applied and environmental microbiology* **52**: 302-304.

Shigehisa T, Ohmori T, Saito A, Taji S & Hayashi R (1991) Effects of high hydrostatic pressure on characteristics of pork slurries and inactivation of microorganisms associated with meat and meat products. *International journal of food microbiology* **12**: 207-215.

Shimada S, Andou M, Naito N, Yamada N, Osumi M & Hayashi R (1993) Effects of hydrostatic pressure on the ultrastructure and leakage of internal substances in the yeast *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology* **40**: 123-131.

Shook C, Shellhammer T & Schwartz S (2001) Polygalacturonase, pectinesterase, and lipoxygenase activities in high-pressure-processed diced tomatoes. *Journal of agricultural and food chemistry* **49**: 664-668.

Silhavy TJ, Kahne D & Walker S (2010) The bacterial cell envelope. *Cold Spring Harbor perspectives in biology* **2**: a000414.

Simova E, Beshkova D, Angelov M & Dimitrov ZP (2008) Bacteriocin production by strain *Lactobacillus delbrueckii ssp. bulgaricus* BB18 during continuous prefermentation of yogurt starter culture and subsequent batch coagulation of milk. *Journal of industrial microbiology & biotechnology* **35**: 559-567.

Simpson R & Gilmour A (1997) The effect of high hydrostatic pressure on *Listeria monocytogenes* in phosphate-buffered saline and model food systems. *Journal of Applied Microbiology* **83**: 181-188.

Smelt J (1998) Recent advances in the microbiology of high pressure processing. *Trends in food science & technology* **9**: 152-158.

Smelt JP, Hellemons JC & Patterson M (2001) Effects of high pressure on vegetative microorganisms. *Ultra high pressure treatments of foods*: 55-76. Springer.

Smiddy M, Sleator RD, Patterson MF, Hill C & Kelly AL (2004) Role for compatible solutes glycine betaine and L-carnitine in listerial barotolerance. *Applied and environmental microbiology* **70**: 7555-7557.

Smiddy M, O'Gorman L, Sleator RD, Kerry JP, Patterson MF, Kelly AL & Hill C (2005) Greater high-pressure resistance of bacteria in oysters than in buffer. *Innovative Food Science & Emerging Technologies* **6**: 83-90.

Smittle R & Cirigcliano M (1992) Salad dressings. *Compendium of methods for the microbiological examination of foods American Public Health Association, Washington, DC*: 975-983.

Smittle RB (1977) Microbiology of Mayonnaise and Salad Dressing: A Review. *J Food Prot* **40**: 415-422.

Soldo B, Lazarevic V & Karamata D (2002) *tagO* is involved in the synthesis of all anionic cell-wall polymers in *Bacillus subtilis* 168a. *Microbiology* **148**: 2079-2087.

Sonoike K, Setoyama T, Kuma Y & Kobayashi S (1992) Effect of pressure and temperature on the death rates of *Lactobacillus casei* and *Escherichia coli*. *In Balny C, Hayashi R, Heremans K & Masson P (Eds.), High Pressure and Biotechnology, John Libbey Eurotext, Montrouge*: 297-301.

Spicher G & Nierle W (1984) The microflora of sourdough. XVIII. The protein degrading capabilities of lactic acid bacteria in sourdough. *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung* **178**: 389-392.

Spicher G & Nierle W (1988) Proteolytic activity of sourdough bacteria. *Applied microbiology and biotechnology* **28**: 487-492.

Stauffer C (1999) Emulsifiers: practical guides for the food industry. *Eagan Press, St Paul, USA, Minn, American Assn of Cereal Chemists*.

Stern N, Pierson M & Kotula A (1980) Effects of pH and sodium chloride on *Yersinia enterocolitica* growth at room and refrigeration temperatures. *Journal of Food Science* **45**: 64-67.

Stolt M, Oinonen S & Autio K (2000) Effect of high pressure on the physical properties of barley starch. *Innovative Food Science & Emerging Technologies* **1**: 167-175.

Stratakos AC, Linton M, Patterson MF & Koidis A (2015) Effect of high-pressure processing on the shelf life, safety and organoleptic characteristics of lasagne ready meals during storage at refrigeration and abuse temperature. *Innovative Food Science & Emerging Technologies* **30**: 1-7.

Stratakos AC, Delgado-Pando G, Linton M, Patterson MF & Koidis A (2015) Synergism between high-pressure processing and active packaging against *Listeria monocytogenes* in ready-to-eat chicken breast. *Innovative Food Science & Emerging Technologies* **27**: 41-47.

Stute R, Klingler R, Boguslawski S, Eshtiaghi M & Knorr D (1996) Effects of high pressures treatment on starches. *Starch-Stärke* **48**: 399-408.

Styles MF, Hoover DG & Farkas DF (1991) Response of *Listeria monocytogenes* and *Vibrio parahaemolyticus* to high hydrostatic pressure. *Journal of Food Science* **56**: 1404-1407.

Sutcliffe IC & Russell R (1995) Lipoproteins of gram-positive bacteria. *Journal of bacteriology* **177**: 1123.

Suzuki K, Asano S, Iijima K & Kitamoto K (2008) Sake and beer spoilage lactic acid bacteria - a review. *Journal of the Institute of Brewing* **114**: 209-223.

Swaisgood H & Fox P (1992) Chemistry of the caseins. *Advanced dairy chemistry-1: Proteins*: 63-110.

Swoboda JG, Campbell J, Meredith TC & Walker S (2010) Wall teichoic acid function, biosynthesis, and inhibition. *Chembiochem* **11**: 35-45.

Tangwongchai R, Ledward DA & Ames JM (2000) Effect of high-pressure treatment on the texture of cherry tomato. *Journal of Agricultural and Food Chemistry* **48**: 1434-1441.

Tcholakova S, Denkov ND, Ivanov IB & Campbell B (2006) Coalescence stability of emulsions containing globular milk proteins. *Advances in Colloid and Interface Science* **123**: 259-293.

Thakur B & Nelson P (1998) High-pressure processing and preservation of food. *Food Reviews International* **14**: 427-447.

Ting E & Marshall R (2002) Production issues related to UHP food. *In Welti-Chanes J, Barbosa-Canovas GV & Aguilera JM (Eds.), Engineering and Foods for the 21st Century. Food preservation technology series, chapter 44:* 727-738.

Ting E & Balasubramaniam V (2002) Determining thermal effects in high-pressure processing. *Food Technology* **56**: 31-35.

Tintchev F, Wackerbarth H, Kuhlmann U, Toepfl S, Knorr D, Hildebrandt P & Heinz V (2010) Molecular effects of high-pressure processing on food studied by resonance Raman. *Annals of the New York Academy of Sciences* **1189**: 34-42.

Toepfl S, Mathys A, Heinz V & Knorr D (2006) Review: Potential of emerging technologies for energy efficient and environmentally friendly food processing. *Food Reviews International* **22**: 405-423.

Tokuda H & Matsuyama S-i (2004) Sorting of lipoproteins to the outer membrane in *E. coli*. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research* **1693**: 5-13.

Tomita S, Furihata K, Nukada T, Satoh E, Uchimura T & Okada S (2009) Structures of two monomeric units of teichoic acid prepared from the cell wall of *Lactobacillus plantarum* NRIC 1068. *Bioscience, biotechnology, and biochemistry* **73**: 530-535.

Tomita S, Irisawa T, Tanaka N, Nukada T, Satoh E, Uchimura T & Okada S (2010) Comparison of components and synthesis genes of cell wall teichoic acid among *Lactobacillus plantarum* strains. *Bioscience, biotechnology, and biochemistry* **74**: 928-933.

Tonello C (2011) Case studies on high-pressure processing of foods. *Nonthermal processing technologies for food, Oxford: Wiley-Blackwell*: 36-50.

Tonello-Samson C (2014) Industrial application of high pressure processing in food industry. *Book of Abstracts, p 48; 8th International Conference on High Pressure Bioscience and Biotechnology, July 15th - 18th 2014, Nantes, France*.

Turroni F, Ventura M, Buttó LF, Duranti S, O'Toole PW, Motherway MOC & van Sinderen D (2014) Molecular dialogue between the human gut microbiota and the host: a *Lactobacillus* and *Bifidobacterium* perspective. *Cellular and molecular life sciences* **71**: 183-203.

Uchikawa K-i, Sekiawa I & Azuma I (1986) Structural studies on teichoic acids in cell walls of several serotypes of *Listeria monocytogenes*. *The Journal of Biochemistry* **99**: 315-327.

Ulmer H, Herberhold H, Fahsel S, Gänzle M, Winter R & Vogel R (2002) Effects of pressure-induced membrane phase transitions on inactivation of HorA, an ATP-dependent multidrug resistance transporter, in *Lactobacillus plantarum*. *Applied and environmental microbiology* **68**: 1088-1095.

Ulmer HM, Gänzle MG & Vogel RF (2000) Effects of high pressure on survival and metabolic activity of *Lactobacillus plantarum* TMW1. 460. *Applied and Environmental Microbiology* **66**: 3966-3973.

Usbeck JC, Kern CC, Vogel RF & Behr J (2013) Optimization of experimental and modelling parameters for the differentiation of beverage spoiling yeasts by Matrix-Assisted-Laser-Desorption/Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) in response to varying growth conditions. *Food microbiology* **36**: 379-387.

van Aken GA (2002) Flow-induced coalescence in protein-stabilized highly concentrated emulsions. *Langmuir* **18**: 2549-2556.

van Aken GA, Blijdenstein TB & Hotrum NE (2003) Colloidal destabilisation mechanisms in protein-stabilised emulsions. *Current opinion in colloid & interface science* **8**: 371-379.

van Boekel MA (1980) Influence of fat crystals in the oil phase on stability of oil-in-water emulsions. *Thesis, Wageningen Agricultural University.*

Van der Mei H, Rosenberg M & Busscher H (1991) Assessment of microbial cell surface hydrophobicity. *Microbial cell surface analysis*: 261-287.

Van der Mei H, Van de Belt-Gritter B & Busscher H (1995) Implications of microbial adhesion to hydrocarbons for evaluating cell surface hydrophobicity 2. Adhesion mechanisms. *Colloids and Surfaces B: Biointerfaces* **5**: 117-126.

Van der Mei H, Van de Belt-Gritter B, Pouwels P, Martinez B & Busscher H (2003) Cell surface hydrophobicity is conveyed by S-layer proteins - a study in recombinant lactobacilli. *Colloids and Surfaces B: Biointerfaces* **28**: 127-134.

van der Mei HC, Weerkamp AH & Busscher HJ (1987) Physico-chemical surface characteristics and adhesive properties of *Streptococcus salivarius* strains with defined cell surface structures. *FEMS microbiology letters* **40**: 15-19.

Van Loosdrecht M, Lyklema J, Norde W, Schraa G & Zehnder A (1987) The role of bacterial cell wall hydrophobicity in adhesion. *Applied and environmental microbiology* **53**: 1893-1897.

Van Loosdrecht MC, Norde W & Zehnder A (1990) Physical chemical description of bacterial adhesion. *Journal of biomaterials applications* **5**: 91-106.

Van Opstal I, Vanmuysen SC & Michiels CW (2003) High sucrose concentration protects *E. coli* against high pressure inactivation but not against high pressure sensitization to the lactoperoxidase system. *International journal of food microbiology* **88**: 1-9.

Van Oss C (1995) Hydrophobicity of biosurfaces - origin, quantitative determination and interaction energies. *Colloids and Surfaces B: Biointerfaces* **5**: 91-110.

Vergara-Irigaray M, Maira-Litrán T, Merino N, Pier GB, Penadés JR & Lasa I (2008) Wall teichoic acids are dispensable for anchoring the PNAG exopolysaccharide to the *Staphylococcus aureus* cell surface. *Microbiology* **154**: 865-877.

Vermeiren L, Devlieghere F & Debevere J (2004) Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products. *International journal of food microbiology* **96**: 149-164.

Vinogradov E, Sadovskaya I, Li J & Jabbouri S (2006) Structural elucidation of the extracellular and cell-wall teichoic acids of *Staphylococcus aureus* MN8m, a biofilm forming strain. *Carbohydrate research* **341**: 738-743.

Vollmer W, Blanot D & De Pedro MA (2008) Peptidoglycan structure and architecture. *FEMS microbiology reviews* **32**: 149-167.

Walstra P (1993) Principles of Emulsion Formation. *Chemical Engineering Science* **48**: 333-349.

Wang Z, Wang J, Ren G, Li Y & Wang X (2015) Influence of Core Oligosaccharide of Lipopolysaccharide to Outer Membrane Behavior of *Escherichia coli*. *Marine Drugs* **13**: 3325-3339.

Wannerberger K, Wahlgren M & Arnebrant T (1996) Adsorption from lipase-surfactant solutions onto methylated silica surfaces. *Colloids and Surfaces B: Biointerfaces* **6**: 27-36.

Ward J (1981) Teichoic and teichuronic acids: biosynthesis, assembly, and location. *Microbiological reviews* **45**: 211.

Weidenmaier C & Peschel A (2008) Teichoic acids and related cell-wall glycopolymers in Gram-positive physiology and host interactions. *Nature Reviews Microbiology* **6**: 276-287.

Weidenmaier C, Peschel A, Xiong Y-Q, Kristian SA, Dietz K, Yeaman MR & Bayer AS (2005) Lack of wall teichoic acids in *Staphylococcus aureus* leads to reduced interactions with endothelial cells and to attenuated virulence in a rabbit model of endocarditis. *Journal of infectious diseases* **191**: 1771-1777.

Wemekamp-Kamphuis HH, Wouters JA, de Leeuw PP, Hain T, Chakraborty T & Abee T (2004) Identification of sigma factor σB-controlled genes and their impact on acid stress, high hydrostatic pressure, and freeze survival in *Listeria monocytogenes* EGD-e. *Applied and Environmental Microbiology* **70**: 3457-3466.

Wilde P, Mackie A, Husband F, Gunning P & Morris V (2004) Proteins and emulsifiers at liquid interfaces. *Advances in Colloid and Interface Science* **108**: 63-71.

Winter R (2015) Pressure Effects on Artificial and Cellular Membranes. *High Pressure Bioscience: Basic Concepts, Applications and Frontiers, Akasaka K & Matsuki H (Eds.)*: 345-370. Springer.

Winter R & Dzwolak W (2005) Exploring the temperature-pressure configurational landscape of biomolecules: from lipid membranes to proteins. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **363**: 537-563.

Winter R & Jeworrek C (2009) Effect of pressure on membranes. *Soft Matter* **5**: 3157-3173.

Wong DW, Camirand WM, Pavlath AE, Parris N & Friedman M (1996) Structures and functionalities of milk proteins*. *Critical Reviews in Food Science & Nutrition* **36**: 807-844.

Wouters PC, Glaasker E & Smelt JP (1998) Effects of high pressure on inactivation kinetics and events related to proton efflux in *Lactobacillus plantarum*. *Applied and Environmental Microbiology* **64**: 509-514.

Wright AO, Cardello AV & Bell R (2007) Consumer Evaluations of High Pressure Processed Foods by Phenolic Compounds. *High pressure processing of foods. Blackwell Publishing, Ames, Iowa, USA:* 219-226.

Wuytack EY, Boven S & Michiels CW (1998) Comparative study of pressure-induced germination of *Bacillus subtilis* spores at low and high pressures. *Applied and environmental microbiology* **64**: 3220-3224.

Xia G, Kohler T & Peschel A (2010) The wall teichoic acid and lipoteichoic acid polymers of *Staphylococcus aureus*. *International Journal of Medical Microbiology* **300**: 148-154.

Xia G, Maier L, Sanchez-Carballo P, Li M, Otto M, Holst O & Peschel A (2010) Glycosylation of wall teichoic acid in *Staphylococcus aureus* by TarM. *Journal of Biological Chemistry* **285**: 13405-13415.

Xu W, Nikolov A & Wasan DT (2005) Shear-induced fat particle structure variation and the stability of food emulsions: II. Effects of surfactants, protein, and fat substitutes. *Journal of food engineering* **66**: 107-116.

Yao X, Jericho M, Pink D & Beveridge T (1999) Thickness and elasticity of gram-negative murein sacculi measured by atomic force microscopy. *Journal of bacteriology* **181**: 6865-6875.

Yokoyama K, Mizuguchi H, Araki Y, Kaya S & Ito E (1989) Biosynthesis of linkage units for teichoic acids in gram-positive bacteria: distribution of related enzymes and their specificities for UDP-sugars and lipid-linked intermediates. *Journal of bacteriology* **171**: 940-946.

Zhang J, Peng X, Jonas A & Jonas J (1995) NMR study of the cold, heat, and pressure unfolding of ribonuclease A. *Biochemistry* **34**: 8631-8641.

Zhang W, Sun Z, Bilige M & Zhang H (2015) Complete genome sequence of probiotic *Lactobacillus plantarum* P-8 with antibacterial activity. *Journal of Biotechnology* **193**: 41-42.

Zhang Z, Schwartz S, Wagner L & Miller W (2000) A greedy algorithm for aligning DNA sequences. *Journal of Computational biology* **7**: 203-214.

Zheligovskaya E & Malenkov G (2005) Molecular dynamics simulation of metastable ices IV and XII. *Journal of Structural Chemistry* **46**: 276-284.

Zheligovskaya EA & Malenkov GG (2006) Crystalline water ices. *Russian Chemical Reviews* **75**: 57.

Zitomer RS & Flaks JG (1972) Magnesium dependence and equilibrium of the *Escherichia coli* ribosomal subunit association. *Journal of molecular biology* **71**: 263-279.

8 Appendix

8.1 Publications and supervised student theses

8.1.1 List of publications derived from this thesis

Peer-reviewed journals

Kafka T, Reitermayer D, Lenz C & Vogel R (2017) High hydrostatic pressure inactivation of *Lactobacillus plantarum* cells in (O/W)-emulsions is independent from cell surface hydrophobicity and lipid phase parameters. High Pressure Research 37(3): 430-448.

Kafka TA, Geissler AJ & Vogel RF (2017) Multiple Genome Sequences of *Lactobacillus plantarum* Strains. Genome announcements 5(29): e00654-17.

Poster

Kafka TA, Reitermayer D, Lenz C & Vogel R (2014) Influence of surface hydrophobicity on high-pressure inactivation of *Lactobacillus plantarum*. 8th International Conference on High Pressure Bioscience and Biotechnology, HPBB 2014. 07/2014. Nantes, France.

8.1.2 Supervised student theses

Cocuzzi R. (2014) Der Einfluss von Emulgatoren auf die Hochdruckinaktivierung von Laktobazillen als relevante Verderbsorganismen für fetthaltige / emulsionsbasierte Lebensmittel.

8.2 Tables and Figures

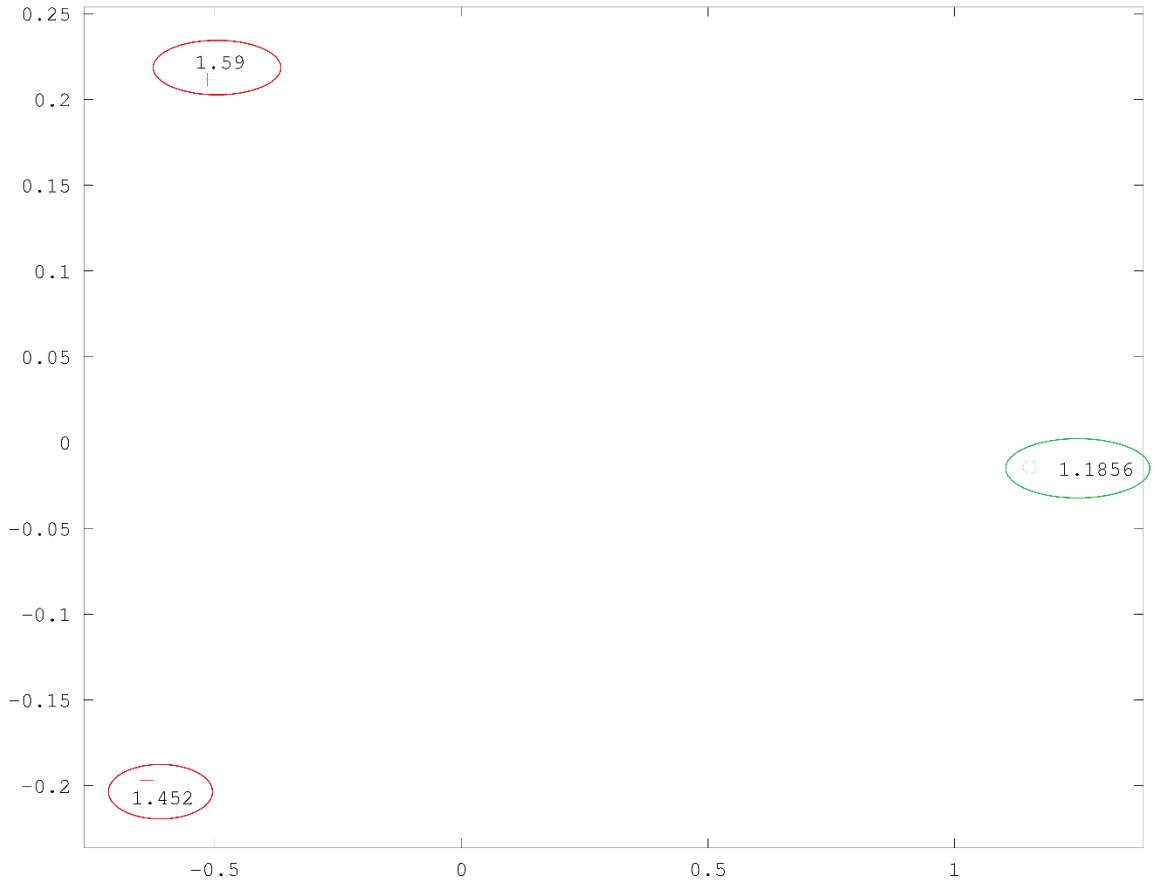

Figure 31: Multidimensional scaling (MDS) of *L. fructivorans* strains based on specific mass spectra combined with cell surface hydrophobicity. Specific mass spectra were obtained by MALDI-TOF-MS, based on 8 replicates per strain. Surface hydrophobicity was determined by MATH test. Strains were classified as highly hydrophobic (0 - 25%), moderately hydrophobic (26 - 50%), moderately hydrophilic (51 - 75%) and highly hydrophilic (76 - 100%), depending on percentage absorbance of the aqueous phase after treatment with n-hexadecane (0.4 mL volume) relative to initial absorbance.

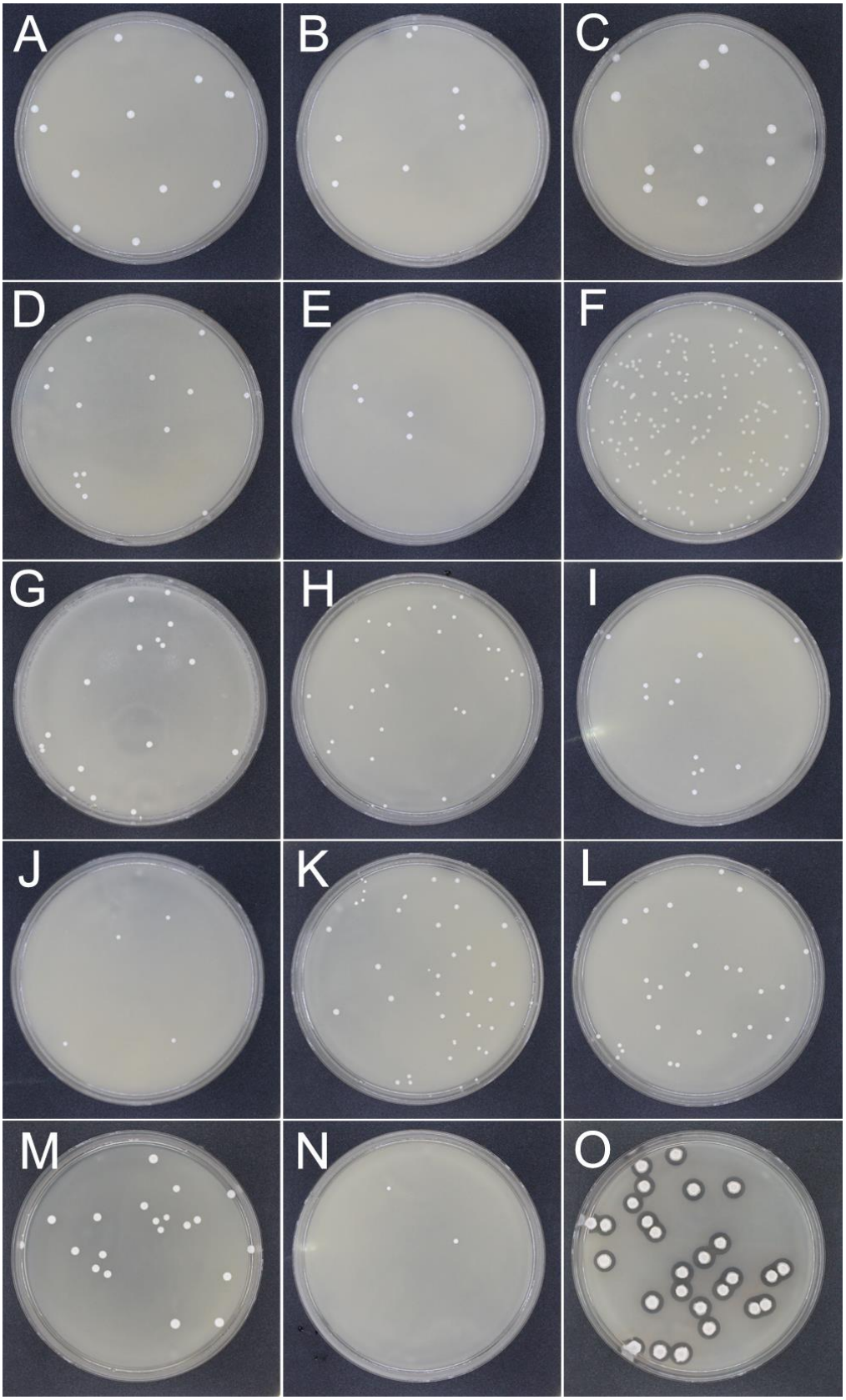

Figure 32: Examination of bacterial lipolytic activity of different species on tributyrin-mMRS agar plates. *L. plantarum*: TMW 1.25 (A), TMW 1.1 (B), TMW 1.277 (C), TMW 1.708 (D), TMW 1.834 (E), TMW 1.1478 (F), TMW 1.1623 (G); *L. sakei*: TMW 1.1239 (H), TMW 1.151 (I), TMW 1.704 (J), TMW 1.1322 (K), TMW 1.1474 (L); *L. fructivorans*: TMW 1.1856 (M), TMW 1.59 (N); *B. subtilis* TMW 2.472 (O).

Table 23: Determination of bacterial proteolytic activity by measuring changes in fluorescence intensity of the FTC-casein. Digestion of the substrate FTC-casein results in an increase of fluorescence intensity. Fluorescein excitation/emission filters with 485/520 nm were used and the gain was set to 300. *B. subtilis* TMW 2.472 and TPCK trypsin were used as positive controls. FI_{max}, maximum fluorescence intensity; ΔFI_{max}, maximum increase in fluorescence intensity; AU, arbitrary units. Mean values ± standard deviation are shown.

Sample	FI_{max} (AU)	ΔFI_{max} (AU)
L. fructivorans TMW 1.59	930±35	32±4
L. fructivorans TMW 1.1856	932±26	31±4
L. plantarum TMW 1.25	923±35	34±9
L. plantarum TMW 1.1	914±45	29±5
L. plantarum TMW 1.1478	895±57	33±7
L. plantarum TMW 1.277	916±41	31±5
L. plantarum TMW 1.708	915±38	32±7
L. plantarum TMW 1.834	916±44	33±7
L. plantarum TMW 1.1623	895±14	34±4
L. sakei TMW 1.151	912±27	31±6
L. sakei TMW 1.704	881±37	28±4
L. sakei TMW 1.1239	904±23	32±7
L. sakei TMW 1.1322	907±29	31±8
L. sakei TMW 1.1474	917±29	28±5
B. subtilis TMW 2.472	1563±49	234±48
0 µg mL^{-1} trypsin	884±36	29±7
5 µg mL^{-1} trypsin	1730±139	366±152
10 µg mL^{-1} trypsin	1942±89	739±111

Table 24: Growth of *Lactobacillus* strains at different pH values and NaCl concentrations. Growth was characterized by the growth parameters OD_{max} and μ_{max}. OD_{max} = maximum cell density measured at a wavelength of 600 nm. μ_{max} = maximum specific growth rate. Mean values ± standard deviation are shown.

	OD_{max}				μ_{max} (h^{-1})			
Strain	pH 3.5	pH 4.0	pH 4.5	pH 6.2	pH 3.5	pH 4.0	pH 4.5	pH 6.2
TMW 1.25	0.46±0.22	1.06±0.03	1.17±0.05	1.29±0.03	0.09±0.01	0.30±0.02	0.38±0.02	0.50±0.01
TMW 1.1	0.24±0.10	0.54±0.14	1.23±0.07	1.33±0.04	0.30±0.02	0.39±0.02	0.44±0.01	0.53±0.04
TMW 1.1478	0.65±0.24	1.15±0.03	1.20±0.02	1.42±0.02	0.26±0.01	0.38±0.04	0.53±0.05	0.58±0.01
TMW 1.277	0.47±0.20	1.12±0.01	1.23±0.05	1.40±0.04	0.10±0.01	0.30±0.01	0.41±0.04	0.54±0.05
TMW 1.708	0.76±0.10	1.21±0.01	1.26±0.09	1.28±0.02	0.19±0.00	0.32±0.02	0.37±0.01	0.47±0.03
TMW 1.834	0.70±0.12	1.09±0.03	1.05±0.06	1.12±0.04	0.11±0.00	0.24±0.02	0.34±0.05	0.44±0.02
TMW 1.1623	1.01±0.05	1.21±0.05	1.27±0.04	1.32±0.02	0.18±0.01	0.33±0.04	0.53±0.02	0.51±0.04
TMW 1.59	0.06±0.07	0.46±0.20	0.95±0.34	1.06±0.26	0.06±0.05	0.23±0.12	0.48±0.23	0.44±0.24
TMW 1.1856	0.96±0.03	1.37±0.02	1.34±0.05	1.24±0.02	0.14±0.02	0.26±0.04	0.24±0.01	0.23±0.05
TMW 1.151	0.06±0.03	0.06±0.03	0.71±0.19	0.71±0.18	0.05±0.03	0.09±0.05	0.20±0.06	0.30±0.13
TMW 1.704	0.73±0.47	0.42±0.51	1.06±0.30	1.01±0.29	0.16±0.02	0.23±0.04	0.26±0.03	0.49±0.15
TMW 1.1239	0.80±0.52	0.85±0.52	1.10±0.33	1.28±0.12	0.15±0.02	0.25±0.03	0.40±0.06	0.43±0.01
TMW 1.1322	0.71±0.44	0.92±0.58	1.06±0.56	1.32±0.26	0.16±0.02	0.25±0.08	0.37±0.10	0.54±0.08
TMW 1.1474	0.03±0.01	0.04±0.02	0.74±0.17	1.02±0.03	0.00±0.00	0.00±0.01	0.24±0.05	0.35±0.03

	OD_{max}				μ_{max} (h^{-1})			
Strain	5% NaCl	6% NaCl	7.5% NaCl	10% NaCl	5% NaCl	6% NaCl	7.5% NaCl	10% NaCl
TMW 1.25	1.01±0.05	0.85±0.03	0.59±0.19	0.05±0.03	0.35±0.03	0.30±0.07	0.18±0.04	0.00±0.00
TMW 1.1	1.02±0.05	0.87±0.06	0.41±0.03	0.08±0.01	0.46±0.07	0.36±0.07	0.20±0.01	0.00±0.00
TMW 1.1478	1.21±0.12	1.07±0.14	0.65±0.29	0.07±0.03	0.33±0.04	0.26±0.07	0.11±0.00	0.00±0.00
TMW 1.277	1.04±0.08	0.90±0.12	0.78±0.06	0.04±0.02	0.36±0.06	0.31±0.08	0.18±0.04	0.00±0.00
TMW 1.708	1.06±0.10	1.04±0.02	0.79±0.08	0.08±0.04	0.39±0.06	0.31±0.05	0.17±0.02	0.02±0.00
TMW 1.834	0.85±0.12	0.65±0.14	0.72±0.09	0.10±0.03	0.38±0.08	0.27±0.04	0.17±0.02	0.03±0.01
TMW 1.1623	0.91±0.20	0.84±0.04	0.62±0.08	0.06±0.03	0.36±0.05	0.26±0.02	0.16±0.02	0.00±0.00
TMW 1.59	0.51±0.44	0.27±0.45	0.01±0.01	0.02±0.02	0.05±0.05	0.09±0.10	0.00±0.00	0.00±0.00
TMW 1.1856	1.04±0.11	0.91±0.13	0.78±0.13	0.04±0.05	0.19±0.04	0.17±0.02	0.12±0.01	0.00±0.00
TMW 1.151	0.57±0.22	0.40±0.09	0.32±0.19	0.05±0.01	0.40±0.05	0.33±0.08	0.16±0.06	0.04±0.01
TMW 1.704	0.72±0.28	0.67±0.29	0.55±0.45	0.07±0.03	0.40±0.03	0.35±0.03	0.21±0.03	0.04±0.02
TMW 1.1239	0.84±0.40	0.76±0.39	0.58±0.16	0.07±0.05	0.44±0.03	0.32±0.03	0.16±0.01	0.01±0.00
TMW 1.1322	0.68±0.55	0.68±0.37	0.46±0.39	0.12±0.12	0.32±0.05	0.25±0.01	0.13±0.05	0.05±0.04
TMW 1.1474	0.71±0.10	0.55±0.12	0.28±0.07	0.04±0.02	0.38±0.06	0.29±0.08	0.15±0.06	0.00±0.00

Table 25: Growth of *Lactobacillus* strains at low temperatures. Growth was characterized by the growth parameters OD_{max} and μ_{max}. OD_{max} = maximum cell density measured at a wavelength of 600 nm. μ_{max} = maximum specific growth rate. Mean values ± standard deviation are shown.

	OD_{max}		μ_{max} (d^{-1})	
Strain	4 °C	10 °C	4 °C	10 °C
TMW 1.25	0.14±0.07	0.67±0.17	0.12±0.03	0.73±0.09
TMW 1.1	0.47±0.06	1.21±0.07	0.78±0.22	1.51±0.13
TMW 1.1478	0.25±0.05	1.13±0.07	0.12±0.04	1.27±0.15
TMW 1.277	0.10±0.05	0.59±0.16	0.15±0.01	0.80±0.09
TMW 1.708	0.21±0.01	0.65±0.04	0.22±0.05	0.99±0.04
TMW 1.834	0.23±0.15	0.74±0.15	0.25±0.06	0.88±0.06
TMW 1.1623	0.19±0.09	1.00±0.12	0.47±0.10	1.18±0.11
TMW 1.59	0.04±0.03	0.16±0.13	0.07±0.04	0.18±0.07
TMW 1.1856	0.06±0.01	0.48±0.11	0.08±0.11	0.69±0.27
TMW 1.151	0.50±0.09	0.70±0.18	0.92±0.14	2.11±0.16
TMW 1.704	0.48±0.23	0.62±0.27	0.97±0.21	2.28±0.29
TMW 1.1239	0.57±0.09	0.74±0.11	1.00±0.09	1.86±0.43
TMW 1.1322	0.59±0.15	0.88±0.23	0.90±0.14	1.58±0.30
TMW 1.1474	0.82±0.03	1.00±0.10	0.93±0.05	2.00±0.29

Table 26: Characterization of different (O/W)-emulsion types prior to and after HHP treatment. All listed emulsions were stabilized by using the emulsifier Tween® 80 with exception of emulsion type #6, which was stabilized with sodium caseinate. SSA, Specific Surface Area; D(v,0.5), volume median diameter, represents median droplet size. Mean values ± standard deviation are shown.

	Emulsion type	Treatment parameter	SSA [m^2 g^{-1}]	D(v,0.5) [µm]	Creaming velocity [µm s^{-1}]
#1	30% (v/v) rapeseed oil	-	3.7±0.33	2.7±0.09	5.6±0.23
		500 MPa/25 °C	4.1±0.69	2.5±0.28	5.6±0.27
		500 MPa/55 °C	3.9±0.53	2.5±0.39	5.7±0.32
#2	50% (v/v) rapeseed oil	-	3.7±0.29	2.7±0.10	4.8±0.20
		500 MPa/25 °C	3.5±0.21	2.7±0.08	4.9±0.41
		500 MPa/55 °C	3.2±0.22	2.8±0.03	5.2±0.27
#3	70% (v/v) rapeseed oil	-	3.4±0.13	2.7±0.18	3.1±0.36
		500 MPa/25 °C	3.1±0.44	2.6±0.04	3.4±0.23
		500 MPa/55 °C	3.0±0.45	2.6±0.04	3.7±0.75
#4	50% (v/v) rapeseed oil, small SSA	-	1.5±0.29	5.8±0.37	9.9±0.45
		500 MPa/25 °C	1.6±0.36	5.5±0.43	10.1±0.59
		500 MPa/55 °C	1.4±0.09	5.5±0.42	9.7±0.94
#5	50% (v/v) rapeseed oil, large SSA	-	10.6±0.49	0.7±0.02	0.1±0.09
		500 MPa/25 °C	10.6±0.31	0.7±0.01	0.1±0.11
		500 MPa/55 °C	10.3±0.05	0.7±0.01	0.1±0.17
#6	50% (v/v) rapeseed oil, 2% (w/v) sodium caseinate	-	3.6±0.28	2.6±0.16	3.2±0.64
		500 MPa/25 °C	3.5±0.12	2.5±0.25	3.2±0.70
		500 MPa/55 °C	3.6±0.40	2.5±0.28	3.0±0.29
#7	50% (v/v) Miglyol® 812	-	3.8±0.42	2.3±0.08	3.9±0.47
		500 MPa/25 °C	3.7±0.33	2.4±0.08	3.6±0.18
		500 MPa/55 °C	3.6±0.10	2.4±0.06	3.6±0.32

Table 27: Effect of different pressure levels on inactivation of different strains of the species *L. plantarum*. Log inactivation of strains TMW 1.25, 1.277, 1.708 and 1.1 subjected to HHP treatment of 250 to 600 MPa at 25 °C for 5 min. Initial cell count: ~7.6 log CFU mL^{-1}. Detection limit = ~6 log. Mean values ± standard deviation are shown.

	HHP (MPa)							
Strain	250	300	350	400	450	500	550	600
TMW 1.25	0.48 ± 0.17	0.39 ± 0.25	2.11 ± 0.41	3.16 ± 0.36	3.48 ± 0.62	5.01 ± 0.65	6.08 ± 0.14	6.11 ± 0.02
TMW 1.277	0.41 ± 0.14	0.50 ± 0.23	2.58 ± 0.41	3.00 ± 0.22	2.91 ± 0.38	4.81 ± 0.20	6.07 ± 0.06	6.07 ± 0.06
TMW 1.708	0.69 ± 0.38	0.46 ± 0.25	0.73 ± 0.47	1.51 ± 0.43	2.49 ± 1.10	5.90 ± 0.45	6.02 ± 0.22	6.21 ± 0.30
TMW 1.1	0.20 ± 0.32	0.28 ± 0.20	0.71 ± 0.25	3.23 ± 0.62	4.94 ± 1.21	6.04 ± 0.26	6.04 ± 0.26	6.04 ± 0.26

Table 28: Effect of Tween® 80 and sodium caseinate on the HHP inactivation of *L. plantarum* in aqueous suspension. The induced reduction ($log_{10}(N_0/N)$) of stationary phase cells (strain TMW 1.25, 1.277, 1.708 and 1.1) in the presence of different emulsifier and -concentrations by HHP (400 MPa/25 °C, 5 min) is shown. Inoculum of ~10^7 cells mL^{-1}. Mean values ± standard deviation are shown.

	Tween® 80 (% (w/v))					Sodium caseinate (% (w/v))				
Strain	0	0.5	1.0	2.5	5.0	0	0.5	1.0	2.5	5.0
TMW 1.25	2.86±0.29	2.86±0.36	2.92±0.31	3.08±0.38	3.24±0.41	2.87±0.42	3.04±0.50	2.89±0.30	2.79±0.25	2.88±0.20
TMW 1.277	3.03±0.32	3.05±0.12	2.96±0.34	3.11±0.23	2.98±0.24	2.97±0.15	2.75±0.37	2.90±0.56	2.90±0.50	3.02±0.22
TMW 1.708	1.80±0.48	1.54±0.30	1.77±0.32	1.65±0.31	1.75±0.29	1.28±0.17	1.32±0.26	1.31±0.22	1.30±0.26	1.41±0.26
TMW 1.1	3.83±0.19	3.85±0.25	3.79±0.20	3.88±0.20	3.90±0.27	3.58±0.23	3.64±0.27	3.62±0.28	3.64±0.28	3.64±0.32

Table 29: Inactivation of *L. plantarum* by HHP in different (O/W)-emulsion systems. The induced reduction ($log_{10}(N_0/N)$) of stationary phase cells (strains TMW 1.25, TMW 1.277, TMW 1.708 and TMW 1.1) at an inoculum of ~10^7 cells mL^{-1} by HHP is shown. Mean values ± standard deviation are shown. Superscript letters denote statistically significant difference among log reduction levels ($p < 0.05$).

p/T parameter combination	Strain	Fat content (% (v/v))				Emulsifier type		Fat surface ($m^2 g^{-1}$)			Oil type	
		0	30	50	70	Tween® 80	Sodium caseinate	1.5	3.7	10.6	Rapeseed oil	Miglyol® 812
300 MPa/25 °C	TMW 1.25	0.39±0.25	0.34±0.14	0.57±0.14	0.51±0.14	0.57±0.14	0.38±0.11	0.38±0.19	0.57±0.14	0.44±0.14	0.57±0.14	0.48±0.21
	TMW 1.277	0.50±0.23	0.28±0.13	0.54±0.17	0.37±0.09	0.54±0.17	0.62±0.08	0.49±0.16	0.54±0.17	0.54±0.12	0.54±0.17^{e}	0.31±0.08^{e}
	TMW 1.708	0.46±0.25	0.31±0.21	0.33±0.16	0.42±0.07	0.33±0.16	0.41±0.08	0.19±0.13	0.33±0.16	0.23±0.09	0.33±0.16	0.31±0.15
	TMW 1.1	0.28±0.20	0.23±0.11	0.26±0.19	0.33±0.05	0.26±0.19	0.31±0.11	0.16±0.08	0.26±0.19	0.20±0.08	0.26±0.19	0.27±0.12
300 MPa/40 °C	TMW 1.25	3.25±0.11	2.85±0.26	3.15±0.28	3.28±0.09	3.15±0.28	3.01±0.23	3.29±0.33	3.15±0.28	3.00±0.27	3.15±0.28	2.65±0.30
	TMW 1.277	3.06±0.23	3.05±0.12	3.25±0.15	3.43±0.16	3.25±0.15	2.94±0.56	2.90±0.59	3.25±0.15	2.90±0.50	3.25±0.15^{f}	2.91±0.22^{f}
	TMW 1.708	1.53±0.06	1.38±0.33	1.69±0.28	1.64±0.11	1.69±0.28	1.58±0.46	1.73±0.15	1.69±0.28	1.78±0.49	1.69±0.28	1.16±0.26
	TMW 1.1	0.93±0.25^{ab}	1.42±0.22	1.48±0.37^{a}	1.77±0.25^{b}	1.48±0.37	1.40±0.32	1.26±0.29	1.48±0.37	1.50±0.55	1.48±0.37	1.20±0.13
400 MPa/25 °C	TMW 1.25	3.16±0.36	3.10±0.48	3.12±0.46	3.59±0.51	3.12±0.46	3.37±0.44	3.55±0.58	3.12±0.46	3.36±0.54	3.12±0.46	2.92±0.44
	TMW 1.277	3.00±0.22	3.21±0.11	3.10±0.30	3.25±0.07	3.10±0.30	3.13±0.16	3.09±0.13	3.10±0.30	3.27±0.13	3.10±0.30	3.03±0.14
	TMW 1.708	1.51±0.43^{cd}	1.96±0.37	2.05±0.45^{c}	2.32±0.47^{d}	2.05±0.45	1.97±0.40	2.46±0.34	2.05±0.45	2.36±0.09	2.05±0.45^{g}	1.48±0.16^{g}
	TMW 1.1	3.23±0.62	3.48±0.91	3.86±0.68	3.72±0.92	3.86±0.68	3.79±0.32	4.24±0.15	3.86±0.68	4.14±0.31	3.86±0.68	2.94±0.66

Table 30: Fatty acid composition of rapeseed oil and Miglyol® 812. The fatty acid composition was determined by VFG-Labor GmbH & Co. KG (Versmold, Germany).

Fatty acid		**Rapeseed oil**	**Miglyol® 812**
saturated		(g/100 g)	(g/100 g)
Butyric acid	C 4:0	< 0.03	< 0.03
Caproic acid	C 6:0	< 0.03	< 0.03
Caprylic acid	C 8:0	< 0.03	55.21
Capric acid	C 10:0	< 0.03	44.53
Undecanoic acid	C 11:0	< 0.03	< 0.03
Lauric acid	C 12:0	< 0.03	0.18
Myristic acid	C 14:0	0.07	< 0.03
Pentadecanoic acid	C 15:0	< 0.03	0.08
Palmitic acid	C 16:0	4.55	< 0.03
Margaric acid	C 17:0	< 0.03	< 0.03
Stearic acid	C 18:0	1.95	< 0.03
Arachidic acid	C 20:0	0.55	< 0.03
Behenic acid	C 22:0	0.27	< 0.03
Lignoceric acid	C 24:0	0.05	< 0.03
monounsaturated			
Tridecenoic acid	C 13:1	< 0.03	< 0.03
Myristoleic acid	C 14:1	< 0.03	< 0.03
Pentadecenoic acid	C 15:1	< 0.03	< 0.03
Palmitoleic acid	C 16:1	0.25	< 0.03
Heptadecenoic acid	C 17:1	0.07	< 0.03
Oleic acid	C 18:1	63.20	< 0.03
Eicosenoic acid	C 20:1	1.40	< 0.03
Erucic acid	C 22:1	0.30	< 0.03
Nervonic acid	C 24:1	0.10	< 0.03
polyunsaturated			
Linoleic acid	C 18:2	19.76	< 0.03
Linolenic acid	C 18:3	7.40	< 0.03
Eicosadienic acid	C 20:2	0.09	< 0.03
Eicosatrienoic acid	C 20:3	< 0.03	< 0.03
Arachidonic acid	C 20:4	< 0.03	< 0.03
Eicosapentaenoic acid	C 20:5	< 0.03	< 0.03
Docosadienoic acid	C 22:2	< 0.03	< 0.03
Docosahexaenoic acid	C 22:6	< 0.03	< 0.03
Fatty acid groups			
saturated fatty acids		7.44	100
monounsaturated fatty acids		65.31	0
polyunsaturated fatty acids		27.25	0

Table 31: Effect of fat on the HHP inactivation of *L. plantarum* in emulsifier-free (O/W)-emulsion. The induced reduction ($log_{10}(N_0/N)$) of stationary phase cells (strains TMW 1.25, TMW 1.277, TMW 1.708 and TMW 1.1) at an inoculum of ~10^8 cells mL^{-1} by HHP is shown. Mean values ± standard deviation are shown. Superscript letters denote statistically significant difference among log reduction levels ($p < 0.05$).

		Fat content (% (v/v))		
p/T parameter combination	Strain	0	30	50
400 MPa/25 °C	TMW 1.25	3.18±0.22	3.73±0.49	3.93±0.49
	TMW 1.277	2.97±0.05^{a}	3.48±0.05^{a}	4.02±0.15^{a}
	TMW 1.708	1.42±0.32^{b}	1.88±0.20	2.27±0.16^{b}
	TMW 1.1	2.75±0.45^{cd}	4.78±0.27^{c}	4.81±0.41^{d}

Table 32: Hydrophilic strain-specific genes in *L. plantarum* identified by BADGE. In **bold**, genes of the WTA biosynthese pathway.

BADGE on nucleotide level	TMW 1.708 / locus_tag	16 / locus_tag	P-8 / locus_tag
Hypothetical protein	BIZ33_RS00200	LP16_RS00250	LBP_RS00195
Glycerol-3-phosphate cytidylyltransferase EC 2_7_7_39, *tagD1*	**BIZ33_RS01080**	**LP16_RS01135**	**LBP_RS01095**
CDP-glycerol glycerophosphotransferase EC 2_7_8_12, *tagF1*	**BIZ33_RS01085**	**LP16_RS01140**	**LBP_RS01100**
CDP-glycerol glycerophosphotransferase / glycosyltransferase, *tagF2*	**BIZ33_RS01090**	**LP16_RS01145**	**LBP_RS01105**
Putative ADP-ribosylglycohydrolase	BIZ33_RS06035	LP16_RS05645	LBP_RS05445
Ribitol phosphotransferase, *tarK*	**BIZ33_RS07480**	**LP16_RS07060**	**LBP_RS06855**
Nitrate/sulfonate/bicarbonate ABC transporter	BIZ33_RS08485	LP16_RS08070	LBP_RS07890
Nitrate/sulfonate/bicarbonate ABC transporter,permease protein	BIZ33_RS08490	LP16_RS08075	LBP_RS07895
Rod-shape determining protein	BIZ33_RS08495	LP16_RS08080	LBP_RS07900
Hypothetical protein	BIZ33_RS12060	LP16_RS11425	LBP_RS11520
GntR family transcriptional regulator	BIZ33_RS13095	LP16_RS12380	LBP_RS12445
6-phospho-beta-glucosidase EC 3_2_1_86	BIZ33_RS13100	LP16_RS12385	LBP_RS12450
PTS system, cellobiose-specific EIIC component EC 2_7_1_69	BIZ33_RS13105	LP16_RS12390	LBP_RS12455
Hypothetical protein	BIZ33_RS13110	LP16_RS12395	LBP_RS12460
Hypothetical protein	BIZ33_RS13170	LP16_RS12440	LBP_RS12490
ABC transporter ATP-binding protein	BIZ33_RS13175	LP16_RS12445	LBP_RS12495
Cro/CI family transcriptional regulator	BIZ33_RS13180	LP16_RS12450	LBP_RS12500
BAGDE on protein level	TMW 1.708 / locus_tag	16 / locus_tag	P-8 / locus_tag
Transporter	BIZ33_RS00200	LP16_RS00250	LBP_RS00195
CDP-glycerol glycerophosphotransferase EC 2_7_8_12, *tagF1*	**BIZ33_RS01085**	**LP16_RS01140**	**LBP_RS01100**
CDP-glycerol glycerophosphotransferase / glycosyltransferase, *tagF2*	**BIZ33_RS01090**	**LP16_RS01145**	**LBP_RS01105**
Hypothetical protein	BIZ33_RS01570	LP16_RS01615	LBP_RS01580
Hypothetical protein	BIZ33_RS01575	LP16_RS01620	LBP_RS01585
XRE family transcriptional regulator	BIZ33_RS01820	LP16_RS01965	LBP_RS01740
ADP-ribosylglycohydrolase	BIZ33_RS06035	LP16_RS05645	LBP_RS05445
ABC transporter ATP-binding protein	BIZ33_RS07365	LP16_RS06950	LBP_RS06745
ABC transporter permease	BIZ33_RS07370	LP16_RS06955	LBP_RS06750
LytR family transcriptional regulator	BIZ33_RS08480	LP16_RS08065	LBP_RS07885
Nitrate/sulfonate/bicarbonate ABC transporter	BIZ33_RS08485	LP16_RS08070	LBP_RS07890
Nitrate/sulfonate/bicarbonate ABC transporter,permease protein	BIZ33_RS08490	LP16_RS08075	LBP_RS07895
Rod-shape determining protein	BIZ33_RS08495	LP16_RS08080	LBP_RS07900
Hypothetical protein	BIZ33_RS12060	LP16_RS11425	LBP_RS11520
GntR family transcriptional regulator	BIZ33_RS13095	LP16_RS12380	LBP_RS12445
PTS system, cellobiose-specific EIIC component EC 2_7_1_69	BIZ33_RS13105	LP16_RS12390	LBP_RS12455
Hypothetical protein	BIZ33_RS13110	LP16_RS12395	LBP_RS12460
Hypothetical protein	BIZ33_RS13170	LP16_RS12440	LBP_RS12490
ABC transporter ATP-binding protein	BIZ33_RS13175	LP16_RS12445	LBP_RS12495
Cro/CI family transcriptional regulator	BIZ33_RS13180	LP16_RS12450	LBP_RS12500

Table 33: Hydrophobic strain-specific genes in *L. plantarum* identified by BADGE. In **bold**, genes of the WTA biosynthese pathway.

BADGE on nucleotide level	TMW 1.25 / locus_tag	TMW 1.277 / locus_tag	TMW 1.1623 / locus_tag
Hypothetical protein	BIZ31_RS01295	BIZ32_01255	BIZ34_RS01300
Transcriptional regulator	BIZ31_RS01300	BIZ32_RS01300	BIZ34_RS01305
Preprotein translocase subunit SecB	BIZ31_RS01305	BIZ32_RS01305	BIZ34_RS01305
Metal ABC transporter substrate-binding protein	BIZ31_RS01420	BIZ32_RS01420	BIZ34_RS01425
ABC transporter ATP-binding protein	BIZ31_RS01425	BIZ32_RS01425	BIZ34_RS01430
ABC transporter permease	BIZ31_RS01430	BIZ32_RS01430	BIZ34_RS01435
Ketopantoate reductase family protein	BIZ31_RS01435	BIZ32_RS01435	BIZ34_RS01440
Hypothetical protein	BIZ31_RS01650	BIZ32_RS01650	BIZ34_RS01620
Hypothetical protein	BIZ31_RS01670	BIZ32_RS01670	BIZ34_RS01640
Hypothetical protein	BIZ31_RS01770	BIZ32_RS01770	BIZ34_RS01710
Bacteriocin	BIZ31_RS01775	BIZ32_RS01775	BIZ34_RS01715
Pirin	BIZ31_RS01780	BIZ32_RS01780	BIZ34_RS01720
CPBP family intramembrane metalloprotease	BIZ31_RS01785	BIZ32_RS01785	BIZ34_RS01725
Glycosyl hydrolase family 8	BIZ31_RS03085	BIZ32_RS03085	BIZ34_RS02975
Hypothetical protein	BIZ31_RS03090	BIZ32_RS03090	BIZ34_RS02980
Hypothetical protein	BIZ31_RS03095	BIZ32_RS03095	BIZ34_RS02985
Amino acid permease / glycosyl transferase family 2	BIZ31_RS04225	BIZ32_RS03100	BIZ34_RS02990
Cellulose synthase	BIZ31_RS03105	BIZ32_RS03105	BIZ34_RS02995
Hypothetical protein	BIZ31_RS03110	BIZ32_RS03110	BIZ34_RS03000
Hypothetical protein	BIZ31_RS05925	BIZ32_RS05925	BIZ34_RS05945
Proton-efflux P-type ATPase	BIZ31_RS05945	BIZ32_RS05945	BIZ34_RS05980
DNA mismatch repair protein MutS	BIZ31_RS05960	BIZ32_RS05960	BIZ34_RS05995
Hypothetical protein	BIZ31_RS07015	BIZ32_RS07015	BIZ34_RS07035
N-acetyltransferase	BIZ31_RS07340	BIZ32_RS07340	BIZ34_RS07360
Hypothetical protein	BIZ31_RS07410	BIZ32_RS07410	BIZ34_RS07430
Ribitol phosphotransferase, *tarK*	**BIZ31_RS07560**	**BIZ32_RS07560**	**BIZ34_RS07580**
Tannase	BIZ31_RS08000	BIZ32_RS07800	BIZ34_RS08015
Hypothetical protein	BIZ31_RS08610	BIZ32_RS08410	BIZ34_RS08615
DUF4428 domain-containing protein	BIZ31_RS08615	BIZ32_RS08415	BIZ34_RS08620
Hypothetical protein	BIZ31_RS08620	BIZ32_RS08420	BIZ34_RS08625
Hypothetical protein	BIZ31_RS08625	BIZ32_RS08425	BIZ34_RS08630
Hypothetical protein	BIZ31_RS08630	BIZ32_RS08430	BIZ34_RS08635
Alpha-glucosidase / glucohydrolase	BIZ31_RS09905	BIZ32_RS09705	BIZ34_RS09875
PTS N-acetylglucosamine transporter subunit IIBC	BIZ31_RS09910	BIZ32_RS09710	BIZ34_RS09880
PTS N-acetylglucosamine transporter subunit IIABC	BIZ31_RS09915	BIZ32_RS09715	BIZ34_RS09885
PTS sugar transporter subunit IIC	BIZ31_RS09920	BIZ32_RS09720	BIZ34_RS09890
PTS mannose/fructose/sorbose transporter subunit IIB	BIZ31_RS09925	BIZ32_RS09725	BIZ34_RS09895
DegA family transcriptional regulator	BIZ31_RS09930	BIZ32_RS09730	BIZ34_RS09900
Hypothetical protein	BIZ31_RS10210	BIZ32_RS10010	BIZ34_RS10175
Lysin	BIZ31_RS10215	BIZ32_RS10015	BIZ34_RS10180
Hypothetical protein	BIZ31_RS10220	BIZ32_RS10020	BIZ34_RS10185

Hypothetical protein	BIZ31_RS10235	BIZ32_RS10035	BIZ34_RS10200
Phage tail protein	BIZ31_RS10240	BIZ32_RS10040	BIZ34_RS10205
Hypothetical protein	BIZ31_RS10245	BIZ32_RS10045	BIZ34_RS10210
Hypothetical protein	BIZ31_RS10250	BIZ32_RS10050	BIZ34_RS10215
Tail protein	BIZ31_RS10255	BIZ32_RS10055	BIZ34_RS10220
Hypothetical protein	BIZ31_RS10260	BIZ32_RS10060	BIZ34_RS10225
Hypothetical protein	BIZ31_RS10265	BIZ32_RS10065	BIZ34_RS10230
Hypothetical protein	BIZ31_RS10270	BIZ32_RS10070	BIZ34_RS10235
Hypothetical protein	BIZ31_RS10275	BIZ32_RS10075	BIZ34_RS10240
Conjugal transfer protein / hypothetical protein	BIZ31_RS10280	BIZ32_RS10080	BIZ34_RS10245
Minor capsid protein E	BIZ31_RS10285	BIZ32_RS10085	BIZ34_RS10250
Hypothetical protein	BIZ31_RS10290	BIZ32_RS10090	BIZ34_RS10255
Scaffolding protein / DUF4355 domain-containing protein	BIZ31_RS10295	BIZ32_RS10095	BIZ34_RS10260
Hypothetical protein	BIZ31_RS10300	BIZ32_RS10100	BIZ34_RS10265
Hypothetical protein	BIZ31_RS10305	BIZ32_RS10105	BIZ34_RS10270
Phage head morphogenesis protein	BIZ31_RS10310	BIZ32_RS10110	BIZ34_RS10275
Ribosomal-processing cysteine protease Prp	BIZ31_RS10315	BIZ32_RS10115	BIZ34_RS10280
Phage portal protein	BIZ31_RS10320	BIZ32_RS10120	BIZ34_RS10285
PBSX family phage terminase large subunit	BIZ31_RS10325	BIZ32_RS10125	BIZ34_RS10290
XRE family transcriptional regulator	BIZ31_RS10420	BIZ32_RS10220	BIZ34_RS10380
XRE family transcriptional regulator	BIZ31_RS10425	BIZ32_RS10225	BIZ34_RS10385
Hypothetical protein	BIZ31_RS10430	BIZ32_RS10230	BIZ34_RS10390
Aldo/keto reductase	BIZ31_RS12250	BIZ32_RS12050	BIZ34_RS12135
DUF1211 domain-containing protein	BIZ31_RS12255	BIZ32_RS12055	BIZ34_RS12140
Flavodoxin	BIZ31_RS12260	BIZ32_RS12060	BIZ34_RS12145
Glucose-1-dehydrogenase	BIZ31_RS12265	BIZ32_RS12065	BIZ34_RS12150
Sugar transporter	BIZ31_RS12270	BIZ32_RS12070	BIZ34_RS12155
Oxidoreductase	BIZ31_RS12275	BIZ32_RS12075	BIZ34_RS12160
Aldo/keto reductase	BIZ31_RS12280	BIZ32_RS12080	BIZ34_RS12165
LysR family transcriptional regulator	BIZ31_RS12285	BIZ32_RS12085	BIZ34_RS12170
Hypothetical protein	BIZ31_RS12290	BIZ32_RS12090	BIZ34_RS12175
Twin-arginine translocation pathway signal protein	BIZ31_RS12295	BIZ32_RS12095	BIZ34_RS12180
Hypothetical protein	BIZ31_RS12300	BIZ32_RS12100	BIZ34_RS12185
NAD(P)-dependent oxidoreductase	BIZ31_RS12305	BIZ32_RS12105	BIZ34_RS12190
Aldo/keto reductase	BIZ31_RS12310	BIZ32_RS12110	BIZ34_RS12195
Aldo/keto reductase	BIZ31_RS12315	BIZ32_RS12115	BIZ34_RS12200
MFS transporter	BIZ31_RS12320	BIZ32_RS12120	BIZ34_RS12205
Oxidoreductase	BIZ31_RS12325	BIZ32_RS12125	BIZ34_RS12210
BADGE on protein level	TMW 1.25 / locus_tag	TMW 1.277 / locus_tag	TMW 1.1623 / locus_tag
Hypothetical protein	BIZ31_RS01295	BIZ32_RS01295	BIZ34_RS01300
Transcriptional regulator	BIZ31_RS01300	BIZ32_RS01300	BIZ34_RS01305
Preprotein translocase subunit SecB	BIZ31_RS01305	BIZ32_RS01305	BIZ34_RS01310
Metal ABC transporter substrate-binding protein	BIZ31_RS01420	BIZ32_RS01420	BIZ34_RS01425

ABC transporter ATP-binding protein	BIZ31_RS01425	BIZ32_RS01425	BIZ34_RS01430
ABC transporter permease	BIZ31_RS01430	BIZ32_RS01430	BIZ34_RS01435
Hypothetical protein	BIZ31_RS01595	BIZ32_RS01595	BIZ34_RS01595
Hypothetical protein	BIZ31_RS01650	BIZ32_RS01650	BIZ34_RS01620
Hypothetical protein	BIZ31_RS01670	BIZ32_RS01670	BIZ34_RS01640
Bacteriocin immunity protein	BIZ31_RS01760	BIZ32_RS01760	BIZ34_RS01695
Two-peptide bacteriocin plantaricin JK subunit PlnJ	BIZ31_RS01765	BIZ32_RS01765	BIZ34_RS01705
Bacteriocin immunity protein / hypothetical protein	BIZ31_RS01770	BIZ32_RS01770	BIZ34_RS01710
Bacteriocin	BIZ31_RS01775	BIZ32_RS01775	BIZ34_RS01715
Pirin	BIZ31_RS01780	BIZ32_RS01780	BIZ34_RS01720
CPBP family intramembrane metalloprotease	BIZ31_RS01785	BIZ32_RS01785	BIZ34_RS01725
Glycosyl hydrolase family 8	BIZ31_RS03085	BIZ32_RS03085	BIZ34_RS02975
Hypothetical protein	BIZ31_RS03090	BIZ32_RS03090	BIZ34_RS02980
Hypothetical protein	BIZ31_RS03095	BIZ32_RS03095	BIZ34_RS02985
Glycosyl transferase family 2	BIZ31_RS03100	BIZ32_RS03100	BIZ34_RS02990
Cellulose synthase	BIZ31_RS03105	BIZ32_RS03105	BIZ34_RS02995
Hypothetical protein	BIZ31_RS03110	BIZ32_RS03110	BIZ34_RS03000
Hypothetical protein	BIZ31_RS05925	BIZ32_RS05925	BIZ34_RS05945
Hypothetical protein	BIZ31_RS05935	BIZ32_RS05935	BIZ34_RS05970
DNA mismatch repair protein MutS	BIZ31_RS05955	BIZ32_RS05955	BIZ34_RS05990
DNA mismatch repair protein MutS	BIZ31_RS05960	BIZ32_RS05960	BIZ34_RS05995
Hypothetical protein	BIZ31_RS07005	BIZ32_RS07005	BIZ34_RS07025
Hypothetical protein	BIZ31_RS07015	BIZ32_RS07015	BIZ34_RS07035
N-acetyltransferase	BIZ31_RS07340	BIZ32_RS07340	BIZ34_RS07360
Hypothetical protein	BIZ31_RS07410	BIZ32_RS07410	BIZ34_RS07430
Hypothetical protein / tannase	BIZ31_RS08000	BIZ32_RS07800	BIZ34_RS08015
Hypothetical protein	BIZ31_RS08610	BIZ32_RS08410	BIZ34_RS08615
DUF4428 domain-containing protein	BIZ31_RS08615	BIZ32_RS08415	BIZ34_RS08620
Hypothetical protein	BIZ31_RS08620	BIZ32_RS08420	BIZ34_RS08625
Hypothetical protein	BIZ31_RS08630	BIZ32_RS08430	BIZ34_RS08635
PTS N-acetylglucosamine transporter subunit IIBC	BIZ31_RS09910	BIZ32_RS09710	BIZ34_RS09880
PTS N-acetylglucosamine transporter subunit IIABC	BIZ31_RS09915	BIZ32_RS09715	BIZ34_RS09885
PTS sugar transporter subunit IIC	BIZ31_RS09920	BIZ32_RS09720	BIZ34_RS09890
PTS mannose/fructose/sorbose transporter subunit IIB	BIZ31_RS09925	BIZ32_RS09725	BIZ34_RS09895
DegA family transcriptional regulator	BIZ31_RS09930	BIZ32_RS09730	BIZ34_RS09900
Hypothetical protein	BIZ31_RS10210	BIZ32_RS10010	BIZ34_RS10175
Lysin	BIZ31_RS10215	BIZ32_RS10015	BIZ34_RS10180
Hypothetical protein	BIZ31_RS10220	BIZ32_RS10020	BIZ34_RS10185
DUF1617 domain-containing protein	BIZ31_RS10225	BIZ32_RS10025	BIZ34_RS10190
Hypothetical protein	BIZ31_RS10230	BIZ32_RS10030	BIZ34_RS10195
Hypothetical protein	BIZ31_RS10235	BIZ32_RS10035	BIZ34_RS10200
Hypothetical protein / phage tail protein	BIZ31_RS10240	BIZ32_RS10040	BIZ34_RS10205
Hypothetical protein	BIZ31_RS10245	BIZ32_RS10045	BIZ34_RS10210
Hypothetical protein	BIZ31_RS10250	BIZ32_RS10050	BIZ34_RS10210

Tail protein	BIZ31_RS10255	BIZ32_RS10055	BIZ34_RS10215
Hypothetical protein	BIZ31_RS10260	BIZ32_RS10060	BIZ34_RS10220
Hypothetical protein	BIZ31_RS10260	BIZ32_RS10060	BIZ34_RS10230
Hypothetical protein	BIZ31_RS10270	BIZ32_RS10070	BIZ34_RS10235
Hypothetical protein	BIZ31_RS10275	BIZ32_RS10070	BIZ34_RS10240
Hypothetical protein	BIZ31_RS10280	BIZ32_RS10080	BIZ34_RS10245
Minor capsid protein E	BIZ31_RS10285	BIZ32_RS10085	BIZ34_RS10250
Hypothetical protein	BIZ31_RS10290	BIZ32_RS10090	BIZ34_RS10255
DUF4355 domain-containing protein	BIZ31_RS10295	BIZ32_RS10095	BIZ34_RS10260
Hypothetical protein	BIZ31_RS10300	BIZ32_RS10100	BIZ34_RS10265
Hypothetical protein	BIZ31_RS10305	BIZ32_RS10105	BIZ34_RS10270
Phage head morphogenesis protein	BIZ31_RS10310	BIZ32_RS10110	BIZ34_RS10275
Ribosomal-processing cysteine protease Prp	BIZ31_RS10315	BIZ32_RS10115	BIZ34_RS10280
Phage portal protein	BIZ31_RS10320	BIZ32_RS10120	BIZ34_RS10285
PBSX family phage terminase large subunit	BIZ31_RS10325	BIZ32_RS10125	BIZ34_RS10290
XRE family transcriptional regulator	BIZ31_RS10420	BIZ32_RS10220	BIZ34_RS10380
XRE family transcriptional regulator	BIZ31_RS10425	BIZ32_RS10225	BIZ34_RS10385
Hypothetical protein	BIZ31_RS10430	BIZ32_RS10230	BIZ34_RS10390
DUF1211 domain-containing protein	BIZ31_RS12255	BIZ32_RS12055	BIZ34_RS12140
Flavodoxin	BIZ31_RS12260	BIZ32_RS12060	BIZ34_RS12145
Glucose-1-dehydrogenase	BIZ31_RS12265	BIZ32_RS12065	BIZ34_RS12150
Sugar transporter	BIZ31_RS12270	BIZ32_RS12070	BIZ34_RS12155
Oxidoreductase	BIZ31_RS12275	BIZ32_RS12125	BIZ34_RS1216
Hypothetical protein	BIZ31_RS12290	BIZ32_RS12090	BIZ34_RS12175
Twin-arginine translocation pathway signal protein	BIZ31_RS12295	BIZ32_RS12095	BIZ34_RS12180
Hypothetical protein	BIZ31_RS12300	BIZ32_RS12100	BIZ34_RS12185
Aldo/keto reductase	BIZ31_RS12310	BIZ32_RS12110	BIZ34_RS12195
MFS transporter	BIZ31_RS12320	BIZ32_RS12120	BIZ34_RS12205

9 Acknowledgements

At this point I would like to thank all those who have supported me in creating this thesis.

First of all, I would like to thank Prof. Dr. Rudi F. Vogel who gave me the opportunity to perform my PhD thesis in his department, for his support and all the motivating and fruitful discussions.

A special thanks to my supervisor Dr. Christian Lenz who has formed the basis for this thesis, always supported me and had consistently good suggestions.

Next, i would like to thank Prof. Dr. Ulrich Kulozik and the staff of the department of Lebensmittel- und Bio-Prozesstechnik at TUM, Elisabeth Eschlbeck, Mirjana Stulac and Annette Brümmer-Rolf for their expert support in the establishment of (O/W)-emulsions.

Special thanks go to Dominik Reitermayer who has shared the same project and office with me. Working with you and spending time in private life was always a pleasure for me.

A big thank you to my other office mates Tomas van Nassau and Max Maier for the many laughs, the nerve-wracking quadrocopter flights and in general the nice time we had.

Thank you Dr. Andreas Geissler for your help in bioinformatic analyzes. Thank you Maik Hilgarth for the nice experimental cooking evenings we had. I also want to thank all the other current and former members of the department of Technical Microbiology at TUM for their great helpfulness and the great atmosphere in the lab. I associate beautiful moments with each single person.

In closing, I want to thank the people I go home to.
Thank you to my siblings Christine, Jeannette, Eva and Jochen, who have supported and encouraged me over the whole PhD period and were always there for me.

I dedicate this dissertation to my mother Dr. Marie Grimm-Kafka, father Antonin Kafka and step-father Rudi Grimm. Thank you for your unconditional support, your love and the possibility to study. I learn so much from you and I emulate you.

This last word of acknowledgment I have saved for my dear girlfriend Dr. Leonie Schnell, who has been with me the last three years and has made them the best years of my life. Thank you so much!

10 Statutory Declaration

I hereby declare that I wrote the present dissertation with the topic

" Role of lipid phase composition and cell surface hydrophobicity in high pressure inactivation of *Lactobacillus plantarum* in emulsions"

independently and used no other aids than those cited. In each individual case, I have clearly identified the source of the passages that are taken word for word or paraphrased from other works.

Freising,

Thomas Kafka