“Citrus pectin as a hydrocolloid emulsifier: Emulsifying and emulsion stabilizing properties”

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Contents

1 General Introduction

Many food products that consumers appreciate, such as sauces, spreads or desserts, are emulsion based [1]. These food emulsions are mostly oil-in-water emulsions and thermodynamically instable. This means that eventually a separation of oil and water phase can occur which is well-known from homemade salad dressings. To overcome this issue, industrially produced food emulsions contain emulsifiers and stabilizers to improve the stability. Emulsifiers adsorb at the oil-water interface and reduce the interfacial tension of the system. Stabilizers are dissolved in the water phase in order to increase the viscosity and to reduce the mobility of oil droplets. Many substances are available that fulfil the purpose of either being a food emulsifier or stabilizer [2]. However, with both increasing awareness and changing demands of consumers, food industry constantly strives to enhance existing and furthermore develop alternative natural food ingredients. Pectin from e.g. sugar beet is a natural biopolymer that combines both emulsifying and stabilizing properties and fulfils several consumer demands: it is vegan, halal, comes from regional production, and might also be sustainably and ecologically sourced [3–5]. Production yields of sugar beet pectin, however, are limited to small industrial scales due to limited availability of the raw material. Citrus pectin can come up with the same properties as sugar beet pectin *and* is largely available because it is one of the most used gelling agents in the food industry [6, 7]. However, much less is known about its emulsifying properties [8]. Though both substances are categorized as pectins, their molecular structure differs significantly, particularly in the amount of functional groups. These differences in the molecular structure are already known to have a strong impact on the stabilizing and gelling properties of pectins. Therefore, results concerning the emulsifying properties of sugar beet pectin cannot simply be transferred to citrus pectin.

Thus, the aim of this study was to gain a deeper understanding of citrus pectin's emulsifying and emulsion stabilizing properties. These properties were studied in emulsions that were produced by high pressure homogenization. Emulsification in high pressure homogenizers is typically carried out in industry to obtain emulsions with very finely dispersed droplets. It is a highly dynamic process that requires a fast and efficient droplet stabilization [9]. Therefore, it is also a suitable process for screening substances concerning their emulsifying properties under extreme conditions [10].

In order to study the emulsifying and emulsion stabilizing properties of citrus pectin, this thesis is built up in the following way (Fig. 1.1):

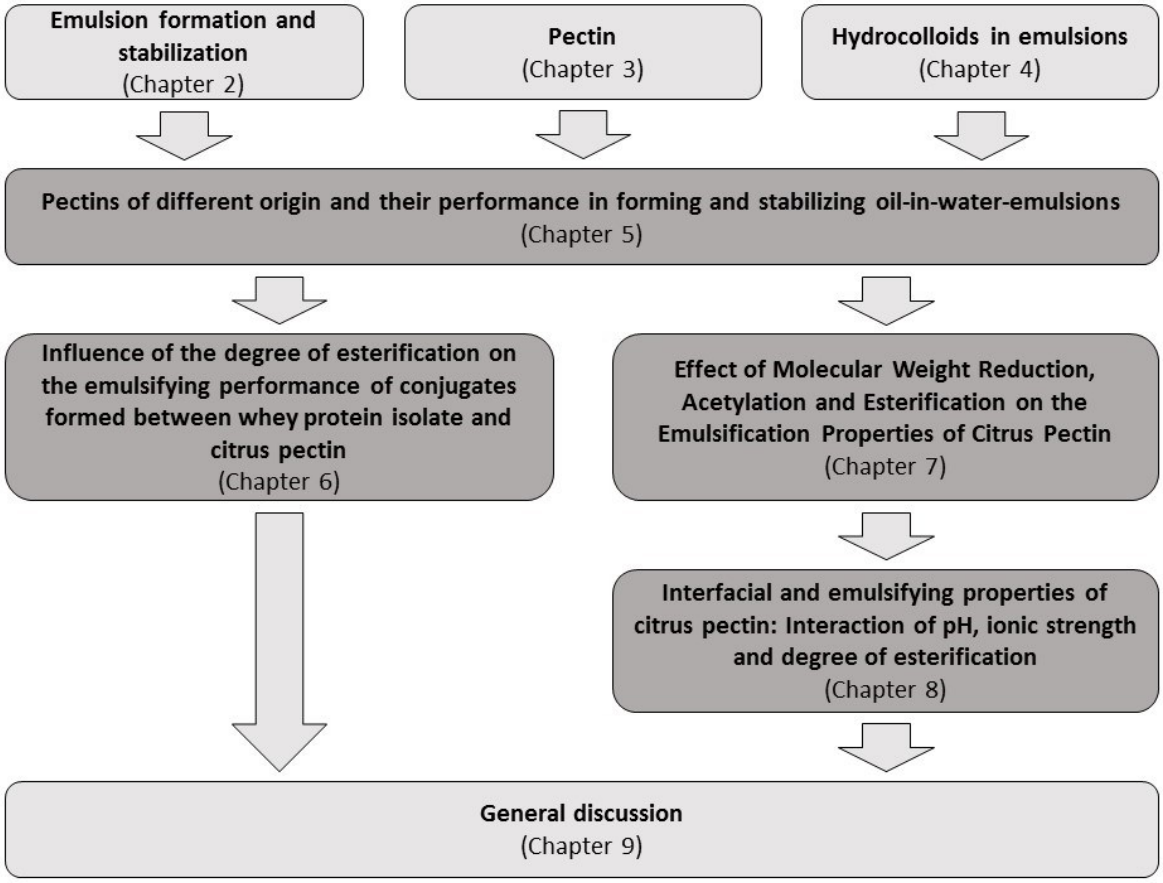

Figure 1.1: Structure of this thesis. Light grey color indicates theory and discussion chapters. Dark grey color indicates published results.

Chapter 2 will give a short overview of the emulsification process with emphasis on high pressure homogenization. Furthermore, the role of emulsifiers in the formation and stabilization of emulsions is described.

Chapter 3 gives a short review of pectin's molecular structure and industrial production in order to understand its functional properties in emulsions.

Chapter 4 outlines the behaviour of hydrocolloids in solution and at oil-water interfaces. The mechanisms by which they stabilize and destabilize emulsions are explained. These aspects are discussed in detail for pectin as one particular hydrocolloid emulsifier.

Chapter 5 reports results on the emulsifying and emulsion stabilizing properties of citrus pectin in comparison to pectins from other botanical sources. Emulsifying and stabilizing properties are studied separately. The influence of certain molecular characteristics on the emulsifying properties throughout various pectin types is investigated.

Chapter 6 will follow the question whether the emulsifying properties of citrus pectin can be enhanced by increasing its protein content. A higher protein content was achieved by covalently attaching whey proteins to citrus pectin with different degrees of esterification.

Chapter 7 investigates how the emulsifying properties of citrus pectin with a very low protein content can be modified. The degree of esterification (DE) and acetylation (DAc) as well as the molecular weight were altered with the purpose of increasing the molecule's hydrophobicity and adsorption kinetics.

Chapter 8 shows in more detail how the DE influences the polyelectrolyte character of citrus pectin. This was studied in combination with the influence of pH and ionic strength. A mechanism is suggested by which citrus pectin adsorbs at the oil-water interface at various solution conditions. Furthermore, the consequences for the interfacial, emulsifying and emulsion stabilizing properties of citrus pectin are presented.

Chapter 9 gives a summary of the findings and discusses the results comprehensively. Limitations of the reported investigations are addressed and ideas for further studies are presented.

2 Emulsion formation and stabilization

2.1 General aspects of emulsions and of the emulsification process

Emulsions are thermodynamically instable systems consisting of at least two barely miscible phases. In case of a two phase system, a disperse phase is dispersed in the form of fine droplets in a surrounding continuous phase. Depending on the relative hydrophilicity of the phases, one can distinguish between oil-in-water (O/W) and water-in-oil (W/O) emulsions. Likewise multiphase systems can exist as well that find application in food products. Water-in-oil-in-water (W/O/W) emulsions are the most important type of such systems since they are suitable for fat reduction or encapsulation of bioactives in food products [11–15]. In the following, only O/W-emulsions will be considered.

A common feature of most emulsions is their physical instability indicated by a change in droplet size over time or/and in space. Changes in droplet size are unwanted as they alter the emulsion microstructure which often negatively impacts emulsion quality and product properties [1].

The physical instability of emulsions is caused by the tendency of the disperse system to reduce its Gibbs free energy. The total differential of the Gibbs free energy of an emulsion is given by Equation (2.1) [16]:

$$dG = Vdp - SdT + \sum_k \mu_k dn_k + \sigma dA \qquad (2.1)$$

where V is the volume, p is the pressure, S is the entropy, T is the temperature, μ is the chemical potential of the individual components k, n is the amount of the components, σ is the interfacial tension and A is the interfacial area. The last term of the equation is the most relevant one for emulsion systems [1]. It shows that for a given interfacial tension the Gibbs free energy is the higher the more interfacial area exists in the system. Since emulsions with finely dispersed droplets have a particularly large interfacial area, their desire to minimize this area is also very high. Therefore, such systems readily show instability phenomena.

From Equation (2.1), it can also be seen that reducing the interfacial tension is a way to reduce the Gibbs free energy of the emulsion. This is commonly achieved by adding emulsifiers to the system. Emulsifiers are surface active substances that adsorb at the oil-water interface due to their amphiphilicity.

In order to produce an emulsion, new interface between oil and water phase needs to be created by introducing work into the system. This can be achieved by either physico-chemical or mechanical means. Several mechanical emulsification devices are available [9] such as membrane based and microfluidic devices, ultrasound sonotrodes, rotor-stator devices and high pressure homogenizers. High pressure homogenizers are very common in industrial emulsification processes due to their large throughput and their scale up possibilities. In such devices, the fluid is first compressed by a high pressure pump. Then, the compressed fluid passes a disruption unit where it expands. The energy liberated in this process causes droplet disruption.

The simplest type of a disruption unit is an orifice. However, more complex geometries such as flat valves, microfluidizers or impinging jet geometries can be found. In this thesis, only high pressure homogenization was used to produce emulsions so that explanations will also be limited to this aspect.

Figure 2.1 shows a general scheme of the mechanical emulsification process [17]. First, the continuous phase, the disperse phase and the emulsifier are premixed to produce a coarse emulsion. Then, high mechanical energy is introduced into the system to disrupt the coarse disperse phase droplets. Large amounts of new interfacial area are created which need to be stabilized immediately. If a sufficient stabilization of the interface is not achieved, the newly formed droplets will recoalescence. This phenomenon can occur to an extent that oil and water phase separate again directly after droplet breakup.

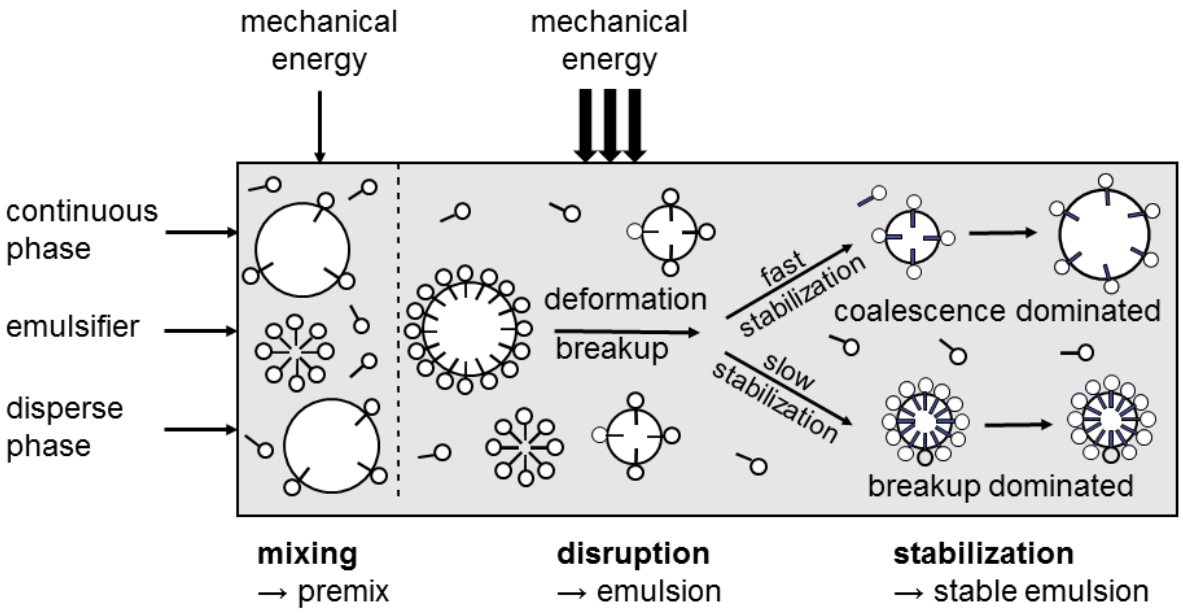

Figure 2.1: Overview of processes during mechanical emulsification according to [17].

Droplet stabilization can be reached by e.g. the adsorption of emulsifiers to the newly formed interface. Besides emulsifiers, stabilizers can be used in an emulsion to provide stability shortly after droplet breakup and during the storage of emulsions. Stabilizers are substances that are usually considered not to be surface active. However, they increase the viscosity of the continuous phase and thus reduce droplet mobility. The acting mechanism of both emulsifiers and stabilizers is not limited to droplet stabilization. As they are commonly already present in the emulsion premix, both types of substances can also influence droplet breakup. This can be either by increasing the viscosity in case of stabilizers or by changing the structure and behaviour of the interface in case of adsorbed emulsifiers. The interplay of these phenomena will be the focus of the following chapters.

2.2 Emulsification using high pressure homogenization

High pressure homogenization is one of the most often encountered processes for emulsification in industry. It is the method of choice for the creation of very finely dispersed emulsions and it can easily be scaled up. The essential elements of a high pressure homogenizer consist of high pressure pump and a disruption unit. Disruption units with a large variety of geometries are commercially available (Fig. 2.2).

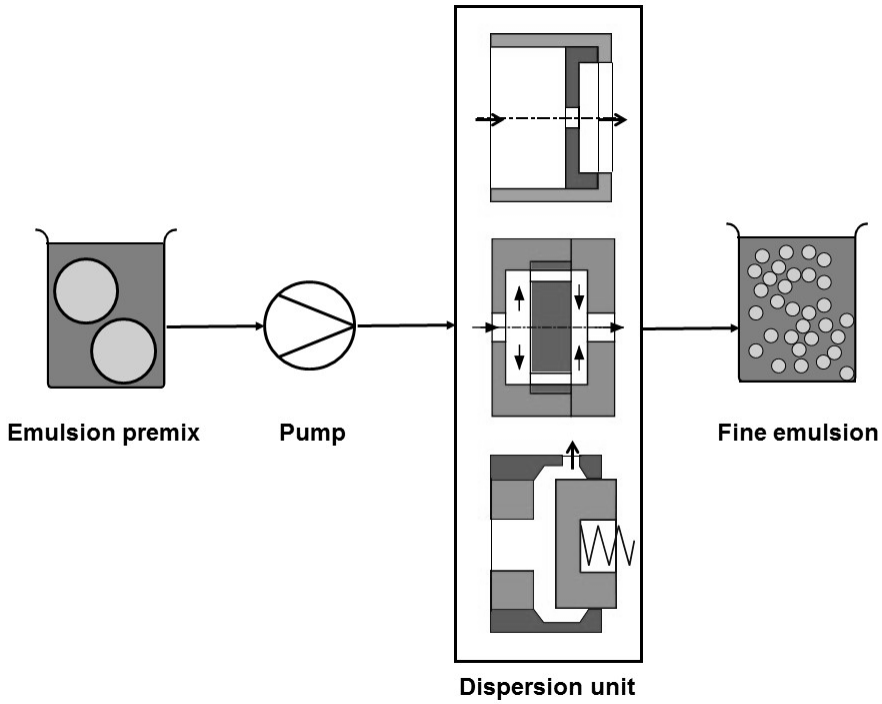

Figure 2.2: Scheme of the high pressure homogenization process. Three dispersion units are shown as examples. From top to bottom: orifice, impinging jet, flat valve geometry.

The disruption unit presents a constriction which leads to a build-up of pressure and causes the fluid to increase its speed. Different flow conditions (laminar, transitional or turbulent) will then develop depending on the fluid characteristics and the actual geometric dimensions of the disruption unit. As a result, different stresses such as shear, elongational or inertia stresses are generated which cause droplet deformation and droplet breakup in or after the disruption unit [9].

For a given disruption unit geometry, droplet breakup strongly depends on the fluid characteristics. The viscosity of the fluid is of particular importance because it can dramatically alter flow conditions [18]. Furthermore, the viscosity ratio λ plays a significant role because it influences how stresses are transmitted onto the droplet [19, 20]. The viscosity ratio λ is the ratio between the viscosity of the disperse phase η_d and the viscosity of the continuous phase η_c of the emulsion (Equation 2.2):

$$\lambda = \frac{\eta_d}{\eta_c} \tag{2.2}$$

However, in a concentrated emulsion, droplets are not surrounded by the continuous phase but by the emulsion itself. The emulsion therefore constitutes an effective medium that transmits disruptive forces onto the droplets. This is considered in the

"effective medium approach" which uses a modified effective viscosity ratio λ_{eff} (Equation 2.3) [21, 22]:

$$\lambda_{eff} = \frac{\eta_d}{\eta_{em}} \tag{2.3}$$

where η_{em} is the emulsion viscosity. In high pressure homogenizers equipped with microfluidizer geometry, it could be shown that the smallest droplet sizes can be obtained at viscosity ratios $0.1 < \lambda < 5$ [1, 23, 24]. Comparable λ_{eff} values were therefore used in the experiments reported in this thesis.

2.3 Interfacial tension at equilibrium

Droplet disruption creates large amounts of new interface in a short time. In order to reduce the interfacial tension of the newly formed interface and thus to stabilize the new droplets, emulsifiers are commonly used in emulsions. The effectiveness with which emulsifiers lower the interfacial tension depends on the type and amount of emulsifier present in the system. If a small amount of emulsifier is added to the system, the emulsifier molecules adsorb at the interface and lower the interfacial tension slightly. An increase in emulsifier concentration leads to a further reduction of interfacial tension. For small molecule emulsifiers, the interfacial tension will reach a minimum value at the so-called critical micellar concentration cmc. At this concentration, all interface is entirely covered by a monolayer of emulsifier molecules. Adding a higher amount of the small molecule emulsifier does not reduce the interfacial tension further but instead micelle formation can occur. Micelles are soluble aggregates of emulsifier molecules present in the continuous emulsion phase. Their shape and formation depends on the type of emulsifier [25].

The relationship between interfacial tension at equilibrium and emulsifier concentration in solution is qualitatively shown in Fig. 2.3. The Gibbs adsorption isotherm (Equation 2.4) presents a mathematical expression for this relationship [26]:

$$\sigma_0 - \sigma = nRT \int_0^c \Gamma(c) d\ln c \tag{2.4}$$

where σ_0 is the interfacial tension of the clean interface, σ is the interfacial tension at any given emulsifier concentration c, n is a correction term which is 1 for uncharged molecules or 2 for ionic emulsifiers, R is the ideal gas constant, T is the temperature and Γ is surface excess concentration which can be regarded as the concentration of emulsifier at the liquid-liquid interface.

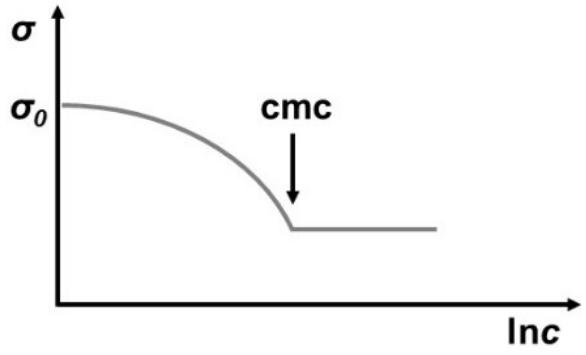

Figure 2.3: Dependence of the interfacial tension σ on the logarithm of the emulsifier concentration c. The critical micelle concentration cmc denotes the concentration at which all interface is entirely covered by emulsifier molecules.

For the actual calculation of interfacial tension values, one must also know the relationship between surface excess concentration Γ and bulk emulsifier concentration c [1]. Several adsorption isotherms with differing degree of complexity have been developed as expressions of Γ(c) [27]. The most frequently used one for liquid-liquid systems is the Langmuir adsorption isotherm (Equation 2.5) [28]:

$$\Gamma = \Gamma_{max} \frac{c}{c+\frac{1}{b}} \tag{2.5}$$

where Γ_{max} is the maximum surface excess concentration (i.e. the maximum number of adsorption sites) and b is the adsorption constant. The Langmuir adsorption isotherm is the simplest isotherm that has a physical basis. It assumes that emulsifier molecules form a monolayer at the interface, that all adsorption sites are equal and that there are no interactions amongst emulsifier molecules or with neighbouring adsorption sites [29].

For dilute systems, the Langmuir isotherm shows a nearly linear behaviour [27]. Therefore, this equation can be approximated by the Henry adsorption isotherm (Equation 2.6):

$$\Gamma = k \cdot c \tag{2.6}$$

where k is an adsorption constant [29]. The Henry isotherm is mostly used for the sorption of gases to solid surface or liquids [28]. Due to the linearity, massive simplification of further calculations is possible. Applying the Gibbs adsorption isotherm yields the surface equation of state (Equation 2.7) [27]:

$$\sigma_0 - \sigma = nRT\Gamma \tag{2.7}$$

In more concentrated systems, significant deviations from either Henry or Langmuir isotherm can occur. These deviations are due to the fact that particularly for polymeric emulsifiers the above mentioned assumptions are not fulfilled [30]. Instead, interactions between emulsifier molecules, multilayer adsorption or reorientation at the interface can be observed [31] (see chapter 8). For these cases, more complex adsorption isotherms are available.

2.4 Adsorption kinetics of emulsifiers

In fast emulsification processes such as high pressure processes it was shown that the interfacial tension at equilibrium is not necessarily a reliable measure of the effectiveness of an emulsifier. Due to the speed by which new interface is created, the dynamic interfacial tension is more relevant to these processes [32]. Therefore, the question is: How fast does an emulsifier cover newly formed interface? Or, how fast is the adsorption kinetics of an emulsifier?

One way to evaluate the adsorption kinetics of an emulsifier is by determining the effective diffusion coefficient of adsorption D_{eff} [33]. D_{eff} can be obtained from dynamic interfacial tension measurements. In these measurements, the evolution of the interfacial tension at a given emulsifier concentration over time is recorded. Then, the recorded data are interpreted in terms of mathematical models [31]. The classical model used for the interpretation of such data is the diffusion controlled adsorption model developed by Ward and Tordai [34]. It assumes that at the beginning of the adsorption process any arriving emulsifier molecule will meet an empty adsorption site at the interface. With progressing adsorption, however, the interface will become more covered. Then, arriving molecules might meet an adsorption site that is already occupied. As a result, back diffusion of molecules from close to the interface to the bulk phase will occur. The Ward-Tordai equation (Equation 2.8) reads:

$$\Gamma(t) = 2c_0\sqrt{\frac{Dt}{\pi}} - 2\sqrt{\frac{D}{\pi}}\int_0^{\sqrt{t}} c_s d(\sqrt{t-\psi}) \tag{2.8}$$

where c_0 and c_s are the emulsifier concentrations in the bulk phase and close to the interface, respectively and where t is the time, D is the diffusion coefficient and ψ is an integration variable.

Since this equation cannot be solved easily, asymptotic solutions for the short-time and long-time limit have been derived by Miller et al. [35]. The short-time approximation considers the early stage of the adsorption process in which back diffusion does not occur yet (Equation 2.9):

$$\Gamma(t) = 2c_0\sqrt{\frac{D_{eff}t}{\pi}} \tag{2.9}$$

For the interpretation of experimental data, an adsorption isotherm must now be chosen in order to relate Γ to the interfacial tension σ. Since the early stage of adsorption is considered, the emulsifier concentration at the interface can be regarded as very dilute. This allows for the use of the Henry isotherm [29]. Introducing Equation 2.7 into Equation 2.9 then yields Equation 2.10:

$$\sigma = \sigma_0 - 2nRTc_0\sqrt{\frac{D_{eff}t}{\pi}} \tag{2.10}$$

Equation 2.10 is used in chapter 8 for the interpretation of dynamic interfacial tension data. Due to the assumptions made for this equation, two conditions must be considered in order to obtain reliable measurement results. On the one hand, the time span covered by the short-time limit must be determined. This depends on the type of emulsifier under investigation. The time span of interest will be shorter for fast adsorbing emulsifiers than for slowly adsorbing ones. On the other hand, the interface

must be completely empty at the beginning of the measurement. This might require a very dynamic measurement system for fast adsorbing emulsifiers.

2.5 Dilational rheology of adsorbed emulsifier layers

Once emulsifier molecules have adsorbed onto the interface, they can form a viscoelastic film that provides a barrier against deformation. This film formation is related to both hindered droplet breakup and improved droplet stability (see chapter 2.6). It is therefore relevant to gain more detailed information about the properties of adsorbed emulsifier films. The deformation and flow properties of the adsorbed interfacial layer are the object of investigation of interfacial rheology. Depending on the type of deformation encountered by the interface, interfacial rheology can be distinguished into two main fields of study: i) shear rheology in which the interfacial area is kept constant but the shape is altered by applying a shear stress and ii) dilational rheology in which the shape of the interface is kept constant but the area varies by compression or dilation [36, 37]. Due to its relevance for both droplet breakup and stabilization, the latter will be considered in this work [38–40].

In order to evaluate the dilational rheological properties of an interface, the dilational viscoelastic modulus E* needs to be determined. This is done by sinusoidally expanding and compressing the interface (dilational strain) and monitoring the resulting changes in interfacial tension (dilational stress) [41]. The dilational viscoelastic modulus E* can then be described as (Equation 2.11):

$$E^* = \frac{d\sigma}{dA/A_0} = E' + iE'' \tag{2.11}$$

where dσ is change in interfacial tension and dA/A_0 is the relative variation of the interfacial area [42]. E* is a complex number. Its real part or storage modulus E′ describes the elastic properties, while the imaginary part or loss modulus E′′ relates to the viscous properties of the interface.

2.6 The role of emulsifiers in droplet breakup and subsequent stabilization

Generally, emulsifiers adsorbed onto the oil droplets facilitate droplet breakup. They do so by lowering the interfacial tension which is considered in the capillary number (Eq. 2.3). In case of very fast adsorbing emulsifiers, it can be assumed that the interface is entirely covered at any time during deformation. Then droplets can be treated like clean droplets but with lower interfacial tension [43]. For slower adsorbing emulsifiers such as polymers, however, several other phenomena can be observed. Upon droplet deformation, emulsifier molecules are dragged towards the tips of the droplet by the flowing continuous phase. Additional emulsifier molecules from the bulk phase cannot adsorb quickly enough so that an emulsifier depleted or even bare interface develops at the droplet equator. Consequently, an interfacial tension gradient is created which induces Marangoni forces. These forces cause emulsifier molecules to flow back into the equatorial region to compensate the different interfacial tensions. As a result the net velocity of emulsifier molecules at the interface is zero and the interface is called “immobilized”. Under such circumstances, it was observed that a

viscoelastic behaviour of the interface is induced. This can be caused by a local compression and dilation of the interface due to droplet rotation in simple shear flow or by dilation of the droplet interface in elongational flow [38, 39]. As a result, droplet breakup was found to be more difficult than would have been expected from the equilibrium interfacial tension [44].

Strong elasticity of the interface can also be induced by certain film forming emulsifiers such as proteins and polymers (see chapter 4.4) [45, 46]. In slow deforming flow fields, droplet deformation and breakup was found to be hindered as well [47, 48]. It was suggested not to treat protein covered droplets as droplets anymore, but instead as soft capsules [49]. This means that Ca_{crit} becomes practically independent of the viscosity ratio λ but is only influenced by the viscoelastic properties of the interfacial network. Which quantities are best suited to characterize the viscoelastic properties in this context has not yet been fully established [43]. However, it was suggested to entirely replace the interfacial tension σ in Ca_{crit} by the interfacial elasticity modulus G' determined by dilational measurements [50]. If the network formed at the interface is very strong and transforms the interface into a more or less rigid shell, droplet breakup is improved again although it is still more difficult than for fast adsorbing small-molecule emulsifiers [43, 45]. In this case, Ca_{crit} is modified by an effective interfacial tension that consists of the static interfacial tension and a term considering elastic interfacial properties [39, 45].

Once droplets are broken up, they need to be protected from immediate recoalescence to ensure short-term stability of the emulsion (see chapter 2.7). This can be achieved by a fast adsorption of emulsifiers onto the interface. The emulsifier molecules then reduce the interfacial tension and form a protective layer around the droplets. Furthermore, the same mechanism by which emulsifier molecules hinder droplet breakup also has a positive effect on droplet stabilization: Marangoni forces immobilizing the interface also prevent the liquid film that separates two approaching droplets from draining. Thus, coalescence is reduced. If the emulsifier molecules form a strong viscoelastic network at the interface, this might also improve droplet stability [51]. Due to the improved resistance to droplet deformation, coalescence was found to be reduced in single droplet experiments [40, 52]. The reason is that flattening of droplets that precedes coalescence is made more difficult by viscoelastic films [53]. Moreover, such films are also thought to reduce Ostwald ripening (see chapter 2.7) because they present a force that acts against droplet shrinkage [54]. However, as with the effect of elasticity on droplet breakup, only very few studies can be found that demonstrate effects in concentrated emulsions. If the adsorbed emulsifier molecules are large enough, steric effects can positively influence droplet stability (see chapter 4.4). In case of charged emulsifiers, electrostatic effects play a role as well.

2.7 Physical stability of emulsions

While the short-term stability is concerned with phenomena that take place immediately after droplet breakup (see chapter 2.6), the long-term stability covers phenomena that influence the shelf-life of emulsions. The difference lies mostly in the time-scales over which certain mechanisms act although a clear separation cannot be made [9].

The most important phenomena influencing the long-term stability of O/W emulsions are coalescence, Ostwald ripening, flocculation/aggregation and creaming/ sedimentation. As only coalescence and flocculation were observed in this thesis, these phenomena are discussed below.

Flocculation describes the sticking together of droplets upon collision. It is a reversible phenomenon and results in an apparent increase of the oil droplet size. The flocs often behave as a larger single droplet which leads to a speeding up of creaming/sedimentation. Flocculation can be reduced by reducing droplet collisions and by increasing electrostatic and steric repulsion between droplets [2]. Biopolymer bridging and depletion interactions promote flocculation [55]. These interactions are especially encountered with hydrocolloids and are described in more detail in chapter 3.4 and 8.

Once droplets collide, they do not necessarily remain as individual droplets as in flocculation. Instead, the liquid film separating two droplets can become thinner and can eventually rupture. Then, the two droplets will flow together forming one larger droplet which is described as coalescence [18]. Coalescence is an irreversible phenomenon and can be controlled in the same way as flocculation. Furthermore, for the rupture of the liquid film, the rheological properties of the interfacial layer have been described to be important [10, 40]. Thick layers with high viscoelasticity are supposed to limit coalescence.

Flocculation and coalescence are critical for the physical stability of emulsions because an (apparent) increase in oil droplet size can also facilitate creaming [56]. Moreover, strong coalescence can eventually lead to irreversible phase separation and a breaking of the emulsion.

2.8 Types of emulsifiers and stabilizers

Emulsifiers can roughly be divided into two classes: small molecule emulsifiers (or "classic" surfactants) and polymeric emulsifiers. Small molecule emulsifiers are mostly synthetically produced (e.g. Polysorbates) but derivatives from natural products (e.g. lecithin) also exist [1]. They possess one pronounced hydrophobic and one pronounced hydrophilic molecular region. The hydrophobic region of the molecule protrudes into the oil phase while the hydrophilic one protrudes into the aqueous phase. The hydrophilic part of the molecule can be ionic or non-ionic. In case of ionic emulsifiers electrostatic interactions between covered oil droplet interfaces occur. Depending on the size of the hydrophilic part, steric interactions might participate in droplet stabilization as well [2].

Small molecule emulsifiers are considered very effective emulsifiers due to their low molecular weight and volume. They show high diffusivity and are able to adsorb at the interface rapidly. This causes a fast reduction of the interfacial tension and a fast achievement of interfacial equilibrium. Small molecule emulsifiers usually adsorb in monolayers at the oil-water interface and form interfaces of comparably low elasticity [2, 57].

Most natural food emulsifiers such as proteins are polymeric emulsifiers. They are usually larger both in weight and in volume than small molecule emulsifiers.

Therefore, steric effects play a significant role for their stabilizing properties [58]. Due to their size, polymeric emulsifiers diffuse to the interface rather slowly. The molecules possess multiple hydrophobic regions which means that they also anchor with various patches at the interface. This leads to an irreversible adsorption. Furthermore, a rearrangement of the molecules at the interface can occur [59]. This rearrangement can last for several hours or even start only after several hours. Therefore, it usually takes a long time (up to several days) before an equilibrium interfacial tension is obtained [60]. Polymeric emulsifiers usually adsorb in multiple layers at the interface causing a highly viscoelastic interfacial film. Depending on their nature, polymeric emulsifiers can also possess various charged groups. In this case, electrostatic effects participate in the emulsion stabilizing properties.

Stabilizers are also mostly polymeric substances. They are usually considered not to be surface active due to their lack of pronounced hydrophobic regions. Instead, they reduce droplet mobility due to an increase in viscosity of the continuous phase. Furthermore, they can separate droplets from each other by depletion effects [60]. Typical stabilizers in food products are e.g. starch, pectin or carrageenan.

Though not as surface active as proteins or even small molecule emulsifiers, several typical food stabilizers show a significant surface active behaviour if used as sole emulsifying substance [61]. This led to the term “hydrocolloid emulsifier” for such substances [62]. Since most food stabilizers are biopolymers, hydrocolloid emulsifiers can be counted amongst the polymer emulsifiers as well. A more detailed description of their emulsifying and emulsion stabilizing properties can be found in chapter 4.

3 Pectin

3.1 Introduction

Pectin is a natural polysaccharide that is found in the cell walls of higher plants. Here, it stabilizes the plant tissues and participates in water transportation [63, 64]. Pectin has been known as a gelling agent for decennia and is also commercially available for this purpose [6]. The main sources of industrially extracted pectin are citrus, apple and sugar beet [63]. However, depending on regional availability other fruit or seeds are exploited, too [62].

Pectin's molecular structure is very complex and varies with e.g. plant source or growing conditions [8]. Besides of the raw material, extraction conditions also have a significant impact. That is why the structure of pectin in its native form, so called proto-pectin, is still not fully elucidated [65]. Very mild extraction conditions need to be applied in order to obtain nearly native material for molecular structure analysis [5]. In industrial extraction processes, harsher conditions are used in order to produce pectin with particular functionality at low cost [6]. As a consequence, significant structural changes will occur [5]. In the following, some general molecular features of pectin are described and important functional groups are explained. Furthermore, a short overview of the industrial production process is given and possible changes to the molecular structure of pectin will be pointed out. Finally, the functionality of pectin with regards to the stabilization of emulsions and to the emulsifying properties is outlined.

3.2 Molecular structure and functional groups

As a natural biopolymer, pectin has no uniform molecular structure. Actually, the term pectin covers three types of polysaccharide structures: homogalacturonan (HG), rhamnogalacturonan I (RGI) and rhamnogalacturonan II (RGII) [65]. HG is the most prevalent type accounting for about 65% of the nearly native pectin material and even more than 80% in industrially extracted pectins [64, 66]. Therefore, it is often taken as a simplification of the whole pectin structure (see Fig. 3.1) [65]. HG is a linear chain made up of α-1,4-linked galacturonic acid (GalA) residues with [63]. The carboxyl groups of the GalA monomers are partially methyl esterified at the C-6 position which provides the molecules with a certain hydrophobicity [66]. The degree of methyl esterification (DE) is a measure of the extent of the substitution. Upon extraction, pectin has a DE of about 70% but this is often altered during industrial processing in order to give the pectin particular gelling properties [6]. A reduction of the DE can be achieved by chemical (see chapter 7) or enzymatic treatment which results in different methylation patterns. While enzymatic treatment leads to a more ordered distribution of methyl ester groups (either blockwise or non-blockwise), chemical treatment leads to randomly distributed residues [67].

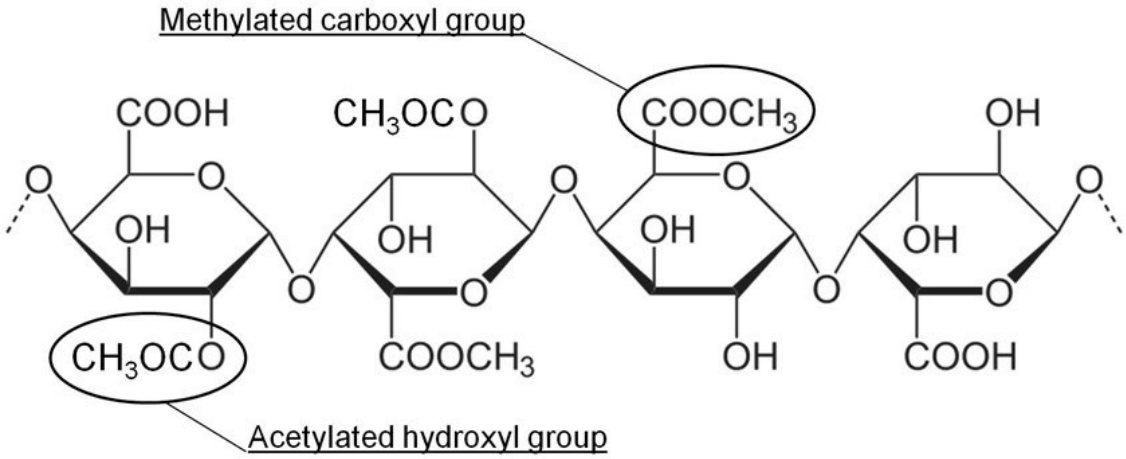

Figure 3.1: Simplified scheme of a pectin molecule - homogalacturonan with possible attached methyl and acetyl groups.

In solution, the non-methylated carboxyl groups will dissociate depending on the pH of the aqueous phase [65]. This will leave a negative charge on the GalA monomers and results in pectin showing typical features of an anionic polyelectrolyte (see chapter 4 and 8) [5]. Moreover, the GalA monomers can possess another functional group. Hydroxyl groups at the O-2 and/or O-3 position can be substituted by acetyl groups [68]. These acetyl groups also provide some hydrophobicity to the molecule and are partially responsible for the unique features of sugar beet pectin (see chapter 5) [4].

RGI consists of alternating rhamnose (Rha) and GalA monomers [67]. The rhamnose residues typically bear side chains of other neutral sugars. Enzymatic digestion of sugar beet pectin showed that cleaving the neutral sugars of RGI also resulted in a reduction of the protein content [69]. This led to the conclusion that the covalently bound protein moieties that are found with pectins are located in the RGI region, too [8]. RGII consists of mainly homogalacturonan with few insertions of rhamnose units. Short but very complexly branched side chains are attached to these rhamnose residues. However, these side chains are mostly cleaved during industrial pectin extraction [65].

Different opinions exist as to which polysaccharide structure is to be seen as the "backbone" of the molecule [67]. The most widely used model assumes HG as the linear backbone, with Rha forming distinct kinks in that backbone and the branched side chains sticking out from these kinks (Fig. 3.2) [68]. RGI and RGII do not appear in regular distances on the HG backbone but rather accumulated in particular regions. This leads to the formation of so called "smooth" and "hairy regions" along the molecule [70].

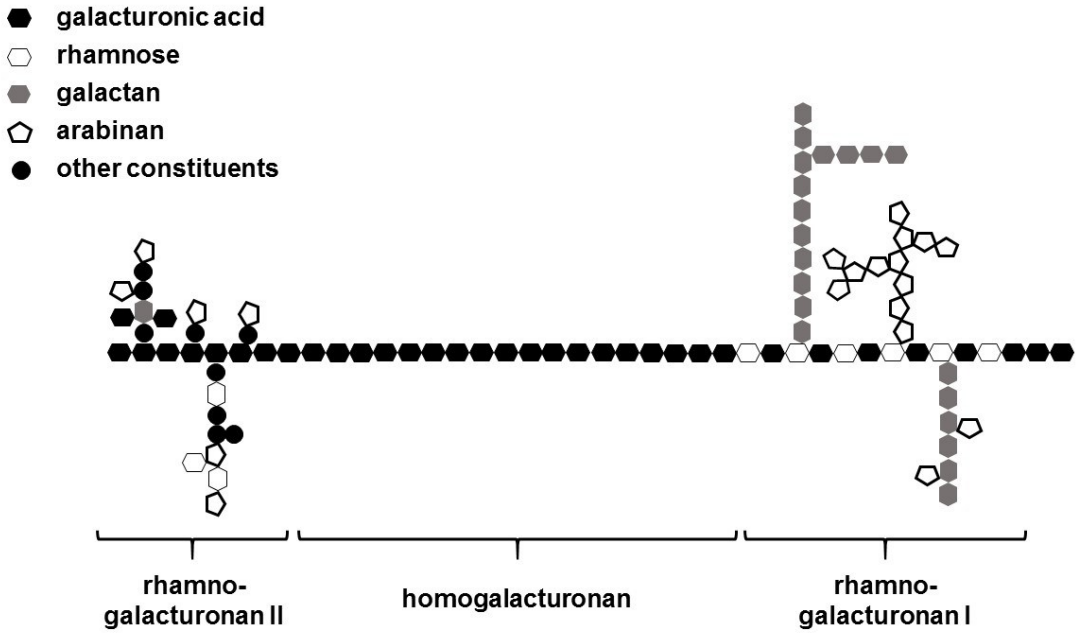

Figure 3.2: Pectin structure according to [68] with homogalacturonan backbone and RGI and RGII side chains.

The molecular weight of extracted pectic substances varies depending on the plant source, on the extraction conditions but also on the technique used for its determination [5, 71]. Nevertheless, it is generally recognized to be somewhat between 50 and 200 kDa (see chapter 7) [5].

If GalA monomers are located in the terminal position of the pectin chains, they can act as reducing sugars. That makes it possible for them to participate in further reactions such as Maillard reactions [60, 72, 73]. In this case, covalent linking to proteins takes place which leads to the formation of protein-polysaccharide conjugates of very high molecular weight (for further information see chapter 6.1).

3.3 Industrial extraction and preparation

Pectin for industrial applications is produced by first blanching, washing and drying the plant raw material [5]. This is done in order to inactivate enzymes that might degrade pectin polymers. The so prepared material can be transported and stored until further use. Pectin is extracted from the dried plant residues in acidified water. Typical extraction conditions are pH 1-3, 50-90 °C and 3-12 h. The harsher the reaction conditions are (i.e. low pH, high temperature, long duration) the higher is the pectin yield. However, these same conditions also favor depolymerization and demethylation [74]. A certain degree of degradation during acid extraction cannot be avoided so that conditions must be chosen in a way that pectin yield and quality are balanced [6]. Furthermore, the neutral sugar content of the pectin material is reduced during extraction by "trimming" of the hairy regions. It has been described that industrially extracted pectin is void of almost all RGII but a small amount of covalently bound protein can still be found [68]. Then, undissolved plant material is removed from the slurry by filtration. The resulting pectin extract can be further treated in order

to produce different pectin qualities. An acidification of the pectin extract further reduces the DE leading to low methylesterified pectins with random distribution patterns of the methyl groups. Addition of ammonia introduces amid groups into the galacturonic acid monomers creating so called amidated pectins. Finally, the pectin extract is precipitated in alcohol, washed with alcohol, dried and milled so that a fine powder is obtained.

According to EU regulation, the resulting pectin powder must have a GalA content of at least 65% to be sold as pectin with the E-number 440 [75]. Before being sold to food manufacturers, pectin powders are often standardized: Batches are blent, buffered and sugar is added in order to obtain preparations that will guarantee uniform functionality.

4 Hydrocolloids in emulsions

4.1 General aspects of food hydrocolloids

The term "hydrocolloid" is commonly used to denote substances of natural origin that are mostly used for the purpose of viscosity increase and stability control in food [76]. However, some of these substances also show significant emulsifying properties. Then, the term "hydrocolloid emulsifier" is sometimes used [62]. Hydrocolloids are often polysaccharide-based and there is some dissent as to whether proteins are supposed to be counted amongst hydrocolloids as well [60]. Whichever classification one may use, there is one feature all food hydrocolloids share – their resemblance to synthetic polymers both in basic structure and in functionality [10]. Cellulose and amylose are linear homopolymers made up entirely of glucose units and form random coils in solution. Pectin and gum arabic can be seen as examples of block copolymers [74]. Such polymers contain at least two different types of monomers that are grouped in longer sequences within the molecule. Proteins are best described as heteropolymers and they show a structural complexity that is rarely encountered with synthetic polymers. In case any of the monomers bear dissociable groups, the hydrocolloid can be regarded as a polyelectrolyte [5].

While it is possible to create synthetic polymers with very well defined structures, food hydrocolloids can come in multitude of diverse structures even from the same natural origin. This makes it difficult to predict the functional properties of a particular sample. However, certain general rules apply. Typical physicochemical features of polymers relevant for their emulsifying and emulsion stabilizing functionality and will be outlined here.

4.2 Hydrocolloids in solution

Many popular food emulsion products are water-based. In order to exploit the full potential of hydrocolloids to stabilize such emulsions, the polymers must be solvated by water. The affinity of a polymer to the surrounding medium (i.e. the aqueous phase) is described by the Flory-Huggins interaction parameter χ [59]. This parameter is a measure of the energy difference between a polymer molecule surrounded by like molecules and the polymer surrounded by the aqueous phase. A χ parameter of 0.5 indicates a so called θ-solvent meaning that the interactions between polymer molecules with each other and with the solvent are equally strong. For $\chi < 0.5$, interactions with the solvent dominate so that the aqueous phase is called a good solvent for the polymer. As a consequence, larger amounts of the polymer can be dissolved and the molecules will assume an extended shape in solution. In contrast, for $\chi > 0.5$, the surrounding medium is a poor solvent for the polymer. Less polymer can be dissolved and the individual molecules will exist in the shape of dense coils. A polymer of a given molecular weight can assume different shapes in solution depending on the solvent qualities. Therefore, the volume taken up by a polymer in solution can give an indication of the solvent quality. Measuring the hydrodynamic radius R_h of a molecule is one way to quantify this volume (see chapter 8).

Compared to neutral hydrocolloids such as amylose, the solubility of polyelectrolytes such as pectin is greatly enhanced by the presence of charged groups on the molecule. These charges also promote intramolecular repulsion and make polyelectrolytes assume a more extended conformation (see chapter 8). Moreover, this extended conformation causes polyelectrolyte molecules to already make contact with each other at very low concentrations (down to 0.1% wt). This so called entanglement results in a steep increase in bulk viscosity. Therefore, polyelectrolytes present very effective viscosity enhancers (see chapter 5) [5].

Different solvent affinities of parts and of types of hydrocolloids are at the origin of several aggregation phenomena. It can occur that a polymer is built from different monomer types and that each monomer type is characterized by a different χ parameter with respect to the aqueous medium. In this case, molecules will try to hide away the poorly solvated regions by burying them within the polymer coil. Regions of the molecules that are well solvated will be exposed by the coil. The result is a complex folding of the polymer and this is the typical behaviour of proteins in solution [59]. This mechanism is often termed hydrophobic interaction and drives micelle formation of small molecule emulsifiers.

If the concentration of a polymer in solution is high enough and regions of the monomers experience different solvabilities, molecules will aggregate by forming helix structures. In case of homopolymers, this will eventually lead to a precipitation of the polymer from solution. For block copolymers, helix formation will only occur between certain regions of the molecules which will lead to the formation of a physical gel [63].

Aggregation is not only limited to polymers of the same species. Particularly, interactions between polysaccharides and proteins – mostly by electrostatic effects – are exploited to form protein-polysaccharide complexes or coacervates with improved functionality (see chapter 6) [77]. Finally, if a second medium is offered to a polymer that presents a much better solvent to parts of the molecule, the polymer will precipitate onto this phase. This causes hydrocolloids to adsorb at liquid-liquid interfaces and it is the basis of their emulsifying properties [1].

4.3 Hydrocolloids at liquid-liquid interfaces

When a hydrocolloid adsorbs at an interface, the number of conformations available to the molecule will be restricted compared to the solution state. This causes a loss in entropy that must be compensated by the energy of adsorption in order to make permanent contact with the interface actually happen. Although the energy of adsorption of individual polymer segments might be low, the sum of the energies of all molecular contact points leads to strong adsorption [2]. Due to the multitude of polymer segments that are in contact with the oil droplet surface, hydrocolloid adsorption is usually considered to be irreversible. While desorption can theoretically take place, it is unlikely to be encountered during experimental time scales [59].

The structures that hydrocolloids build up at the interface are called loops, trains and tails. Fig. 4.1a shows a structure that is typical of homopolymers that are well solvated by water. Few and short trains but large loops and long tails occur in case of low

affinity to the oil phase. The opposite is depicted in Fig. 4.1d. A polymer where all segments show high affinity to the dispersed phase but low solvability in the continuous phase will lie flat on the oil droplet surface. Best emulsion stabilizing properties are encountered with block copolymers (Fig. 4.1 b and c). Blocks with high interfacial affinity strongly adsorb as dense trains at the interface while blocks that are better water soluble protrude into the continuous phase as large loops and tails. Any type of intermediate structure may be found as well [2].

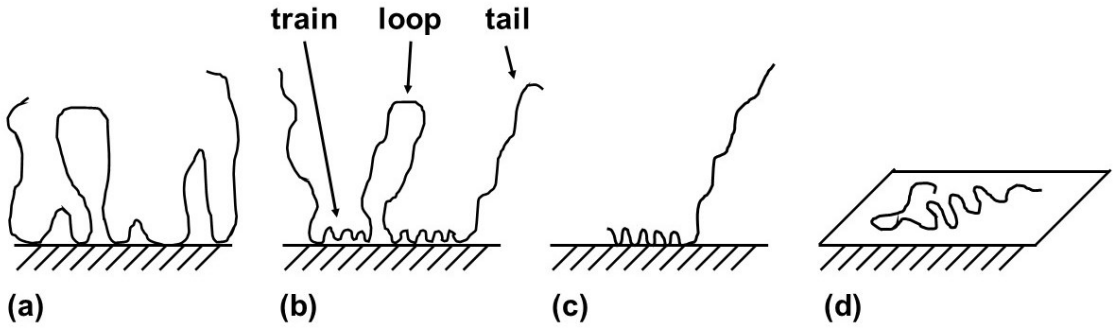

Figure 4.1: Hydrocolloid conformations at interfaces; a) random loops-trains-tails structure of a homopolymer, b) block copolymer structure (A-B-A-B-A) in which B adsorbs preferentially at the interface and A forms loops and tails, c) block copolymer B-A of which A forms a tail, d) polymer lies flat at interface (e.g. homopolymer in bad solvent).

From Fig. 4.1a it can be seen that a neutral polymer keeps a coil like, open structure when adsorbing from a good solvent. In this case the thickness of the adsorbed layer is of the same order of magnitude as R_g [59]. As the solvent quality is decreased, adsorption is enhanced (Fig. 4.1d). This means that the adsorbed hydrocolloid structure will become denser and eventually multilayer formation will occur at the interface [2]. If the concentration of the hydrocolloid at the interface approaches values similar to those at which gel formation in the bulk phase would occur, a reversible physical gel might be formed at the interface [78]. This phenomenon can be quantified by determining interfacial rheological properties (see chapter 2.5).

Polyelectrolytes show particularly strong adsorption to oppositely charged interfaces. In aqueous phases of low ionic strength, they will assume a conformation so that as many charges as possible will be exposed to the aqueous phase while less solvated segments rearrange to get into contact with the interface [1]. The resulting conformational restrictions present a loss of entropy. However, by the adsorption of the polyelectrolyte, large amounts of counterions are released from the interface so that overall a significant gain in entropy will occur. Irreversible adsorption of the polyelectrolyte is the consequence. However, the electrostatic interactions between interface and polymer can be disrupted by increasing the ionic strength of the solution. The presence of larger amounts of counterions will shield charges both at the interface and on the polymer. This will make the polyelectrolyte regain conformational entropy by further expanding into the solvent phase. If the ionic strength of the solution is high enough, the polyelectrolyte will behave as a neutral polymer and will mostly desorb if there are not any more hydrophobic blocks present within the molecule [59].

For the adsorption of proteins, the above described model of loops and trains seems to be an oversimplification. Globular proteins such as β-lactoglobulin are rather supposed to be seen as deformable particles that can interact with each other at the interface. Nevertheless, reorientation and unfolding at the interface do occur which gradually increases the adsorption energy of the protein. This process can proceed in several steps which explains the long-lasting and often non-monotonous adsorption isotherms of proteins [78].

4.4 Effects of hydrocolloids on emulsion stability

Hydrocolloids can be used in emulsions as stabilizers and as hydrocolloid emulsifiers. The main difference is whether the polymer remains mostly solvated in the continuous phase or whether it adsorbs onto oil droplets. This difference will also determine which stabilizing – or destabilizing – effects are most likely to be encountered.

4.4.1 Non-adsorbing hydrocolloids

Typically, non-adsorbing hydrocolloids are added to an emulsion as stabilizers. Their purpose is to increase the viscosity of O/W-emulsions. If the hydrocolloid is added at a concentration above its critical overlap concentration, a strong increase in viscosity of the continuous phase with increasing polymer concentration is observed. This will reduce the mobility of the oil droplets within the continuous phase. Consequently, destabilizing mechanisms that rely upon droplets being displaced will be slowed down. This applies e.g. to creaming but also to coalescence which can be limited either by decreasing the impact by which droplets collide or by slowing down film drainage (see chapter 2.7).

If conditions are chosen that promote the formation of a hydrocolloid gel, emulsion stability can be further enhanced. Gelling of the continuous emulsion phase mostly involves the formation of a yield stress. If this stress is higher than the gravitational forces experienced by the oil droplets, creaming can be entirely arrested and the oil droplets will be entrapped in the polymer network [1].

However, low concentrations of non-adsorbing hydrocolloids can also cause a destabilization of the emulsion. This phenomenon is known as depletion flocculation. Here, an osmotic pressure difference causes droplets to flocculate. When two droplets happen to be close to each other, the concentration of polymers is higher in the medium surrounding both droplets than between the two droplets. The distance between the droplets, however, is so short that polymer molecules are unable to interpenetrate. The resulting polymer concentration gradient will cause solvent molecules to diffuse out of the gap between the two droplets pulling them together and inducing flocculation [10].

4.4.2 Adsorbing hydrocolloids

When hydrocolloids are used as sole emulsifiers or when they adsorb onto an already covered oil droplet surface, other stabilizing and destabilizing effects can be observed. If the adsorbing hydrocolloids are strongly charged, electrostatic repulsion between

the covered oil droplets will occur preventing them from getting into close contact (see chapter 2.6).

If the adsorbing molecules are neutral, the dominant stabilizing effect will be steric. Two polymer covered droplets approaching each other will notice the existence of the other droplet approximately at a distance of $2R_g$. At this distance, the polymer segments protruding from the interface will start to overlap. This causes a reduction in the amount of possible conformations the polymers can assume. As a consequence, entropy is lost and the free energy of the system increases. This represents a repulsive force keeping droplets apart [59]. One precondition for this effect is that the surrounding phase represents a good solvent for the stabilizing hydrocolloid parts. In a poor solvent, protruding polymer segments will start to overlap as well. However, this will represent a gain in conformational entropy for the molecules as they are now surrounded by a better medium. This will result in an attractive force causing the droplets to flocculate [2, 59]. However, droplets will not be able to approach too closely. At shorter distances, steric repulsion will again be the dominant effect as long as polymer molecules cannot be displaced from the interface.

If the amount of adsorbing hydrocolloids in an emulsion is not enough to immediately cover all interface, bridging flocculation can occur. Under such circumstances, polymer molecules adsorb simultaneously onto different droplets in close vicinity [2]. The droplets get pulled together and the occurrence of further instability phenomena such as coalescence is facilitated (see chapter 2.7).

When adsorbed hydrocolloids interact with each other at the interface, the formation of a viscoelastic film can occur (see chapter 2.5). This is said to increase the stability of emulsions [1]. Although much research has been done both on the characterization of viscoelastic interfaces and on the stability of colloid-stabilized emulsions particularly by proteins, evidence linking interfacial viscoelasticity to emulsion stability is still sparse. A review by Erni et al. summarizes the current state of knowledge and some development has been reported since then [43]. In general, it can be said that interfacial viscoelastic films tend to reduce droplet deformation [79]. This is associated with an improved resistance against instability mechanisms that involve a change in the droplet surface area such as coalescence or Ostwald ripening [52, 54, 80–82].

4.5 Pectin as an emulsion stabilizing hydrocolloid

In food products, pectin is typically used as a gelling agent or viscosity enhancer. These properties can also be used to improve the stability of emulsions. If the viscosity of the continuous phase is high enough or the food matrix is gelled, oil droplets will be considerably restricted in their movements and can entirely come to a rest. This will suppress instability phenomena that rely upon the displacement of droplets. The viscosity and gel properties of pectin matrices depend on several intrinsic and extrinsic factors [5]. Extrinsic factors are e.g. pectin concentration, pH, ionic strength or co-solute concentration. Intrinsic factors refer to the molecular structure of the particular pectin and type and amount of its functional groups. An abundance of information can be found on the gelling mechanism and gelling conditions of individual pectin types [63, 83–85]. However, as gelled bulk phases of pectin were not within the scope

of this thesis, the information below will be limited to factors affecting the viscosity of pectin solutions.

Dilute pectin solutions behave nearly Newtonian while concentrated ones exhibit markedly shear thinning behaviour. The polyelectrolytic behaviour of pectin is particularly noticeable in dilute solutions: A reduction of pH or increase in ionic strength reduces the solution viscosity because carboxyl groups become protonated and intermolecular repulsion is suppressed [71]. At higher pectin concentrations, however, the same solution conditions increase the viscosity. This is caused by stronger molecular association and network formation when repulsive forces are reduced. The network is formed both by hydrogen bonds and by hydrophobic interactions between methoxyl groups [86].

Just like the pH, the DE of pectin can lead to an alteration of the charge distribution along the molecule. The influence of DE on solution viscosity is therefore comparable to that of pH [87]. More importantly, the flexibility of the pectin molecule in solution is altered by the amount and distribution pattern of methoxyl groups. At high DE, the molecule is more flexible and coil-like than at low DE where rigid rod behaviour dominates [88]. Furthermore, a more blockwise distribution of methoxyl groups was found to increase molecular interactions and thus to promote gel strength [67, 89].

The presence of larger amounts of acetyl groups (DAc > 15%) as commonly found in sugar beet pectin, has been shown to reduce or event prevent gel formation [90]. This is attributed to steric hindrances by the acetyl groups which make it impossible for pectin chains to associate [91]. This also points towards a less effective viscosity enhancing effect of pectins rich in acetyl groups.

Co-solutes, such as sucrose and glucose, increase the viscosity of pectin solutions by reducing the water activity and thus promoting pectin chain association as well.

4.6 Pectin as a hydrocolloid emulsifier

4.6.1 Adsorption of pectin to interface

Pectin is described to adsorb onto the oil-droplet interface according to the loops and trains model (Fig. 4.1b) [92]. Covalently bound protein moieties form anchor points at the interface while the carbohydrate chains protrude as loops and tails into the aqueous phase [93]. Atomic force microscopy (AFM) revealed that sugar beet pectin forms a less dense structure than pure protein at the air-water interface. This was attributed to the carbohydrate chains that prevent a tight packaging of the protein moieties but instead form linkages themselves [94]. More specifically, the formation of thick adsorbed pectin layers was ascribed to the neutral sugar side chains of the RG1 regions that are supposed to associate at the interface [95]. A combination of AFM with force spectroscopy showed that at low bulk concentration, SBP lies flat on the interface. At higher bulk concentrations the interface becomes rougher and a repulsive force due to steric interactions can be noted between surfaces covered with SBP [96]. Furthermore, multilayer formation of pectin at the interface was detected by Siew et al. by measuring the adsorbed layer thickness of SBP on monodisperse latex particles [93].

Electrostatic effects of adsorbed pectin layers have been reported as well and are attributed to the homogalacturonan parts [8]. Non-methylated carboxyl groups can dissociate and impose a charge onto the pectin molecule. Nakauma et al. showed that this charge is key for the emulsifying properties of SBP compared to GA [97]. However, the effect does not seem to be limited to electrostatic repulsion and a full understanding of the mechanisms is still lacking [8].

4.6.2 Role of functional groups in the emulsifying properties

The emulsifying properties of pectin are mainly attributed to the covalently bound protein moieties [68]. These are considered to form anchors that connect the molecule to the oil-water interface [92]. Evidence for this model has been found by emulsification experiments. It could be shown that the pectin fraction adsorbing onto the oil droplet surface was particularly rich in protein [98, 99]. When such emulsions were centrifuged and non-adsorbed and protein-lacking pectin from the serum layer was reused to prepare fresh emulsions, a significant increase in droplet size was observed [99]. Furthermore, sugar beet pectin that had been enzymatically cleaved of its protein moieties formed emulsions with larger droplet size and worse emulsion stability [95]. Moreover, these enzymatically treated sugar beet fractions displayed higher interfacial tension values than the original protein-rich sugar beet fraction.

Compared to the protein fraction, other hydrophobic moieties such as acetyl groups or ferulic acid have been found to be of minor importance for the emulsifying capacity of pectin. A poor correlation of the adsorbed amount of pectin with the ferulic acid content of different sugar beet pectins indicated that the ferulic acid moieties cannot be the dominant hydrophobic anchor as compared to the protein part [69]. Furthermore, ferulic acid rich pectin types did not show significantly better emulsifying properties than samples poor in ferulic acid [100].

First indications of the beneficial influence of acetyl groups on the emulsifying properties of sugar beet pectin were found by Dea and Madden [4]. Later on Endreß and Rentschler reported that sugar beet pectin with a high DAc reduced the interfacial tension more efficiently than citrus pectin with low DAc [101]. Leroux et al. chemically increased the DAc of citrus pectin up to 8% and found a reduction in the resulting emulsion droplet size [92]. Nevertheless, these droplets were still not as small as those found in the reference emulsion prepared from sugar beet pectin. In the same paper it was shown that a gradual deacetylation of sugar beet pectin did not significantly deteriorate the emulsifying properties. Therefore, Leroux et al. concluded that the presence of acetyl groups is not absolutely necessary for the formation of small droplets [92]. Keeping in mind that the protein content of the deacetylated sugar beet pectin was still more than double that of the modified citrus pectin samples one can also come to a slightly different conclusion. The DAc is of negligible importance as long as a considerable amount of much more hydrophobic protein moieties is present in the pectin sample.

Even pectin types that do not contain a siginificant amount of the above mentioned hydrophobic moeities can show reasonably good emulsifying properties [92]. This might be due to the presence of methoxyl groups and their distribution along the pectin backbone. However, evidence concerning the influence of DE on the

emulsifying properties of pectin is very scarce [8]. So far, the only explicit statement is by Akhtar et al. although they do not show any data. Apparently, in pre-experiments they found no indication of an influence of DE on the emulsifying properties which is why they did not investigate that topic further [98]. Nevertheless, it was found that pectins with a reduced DE are capable of lowering the interfacial tension more strongly [89]. This was particularly ascribed to the increased blockwise distribution of the methoxyl groups upon enzymatic treatment. In another article, Akhtar et al. showed that pectins with intermediate DE (48%) stabilized larger droplet sizes than pectins with lower or higher DE [99]. However, at the same time strong depolymerisation of the molecules took place. It is therefore hard to tell which parameter change – reduction in MW or DE – caused the observed droplet sizes.

Reducing the molecular weight (or depolymerisation) of pectin is another approach to improve the functional properties of pectins poor in protein [98, 102]. It was found that citrus pectin that experienced slight depolymerisation stabilized the smallest emulsion droplets. It was suggested that residual protein moieties were liberated due to this treatment and a better anchoring of the pectin molecules to the oil-water interface took place. However, a strong depolymerisation of citrus pectin was detrimental for emulsion stability due to reduced steric stabilization. For depolymerized and fractionated sugar beet pectin, Funami et al. found that several protein rich pectin fractions showed worse emulsifying properties than protein poor ones [69]. They concluded that the simultaneous low molecular weight of the protein rich samples either caused poor steric stabilization or altered the entire hydrophilic-lipophilic balance of the molecules. It needs to be mentioned that depolymerisation might not only alter the functionality of adsorbed pectin. It also changes the viscosity of the pectin bulk phase. This can have effects on the actual emulsification process as well (see chapter 2.2) so that superposing effects might have been observed here. Differences in viscosity upon depolymerisation were recognized by both research groups [69, 98]. However, further investigations into the effects on the emulsification process did not take place.

5 Pectins of different origin and their performance in forming and stabilizing oil-in-water-emulsions

The effectiveness of different commercially available pectin types in forming and stabilizing oil-in-water emulsions was investigated. Sugar beet pectin as well as apple and citrus pectins with different degree of methoxylation were tested. In emulsions containing small molecule emulsifiers, all investigated pectins behave similarly. They show stabilizing properties by increasing the viscosity of the aqueous phase. This also influences the effective viscosity ratio of emulsions and it results in the formation and stabilization of submicron droplets. In emulsions without small molecule emulsifiers, the investigated pectins differ in their emulsifying behavior depending on their molecular structure. The higher the amount of covalently bound protein a pectin has, the smaller the characteristic droplet size of the resulting emulsions. Pectins with intermediate degrees of esterification produce the emulsions with the largest characteristic droplet size. Furthermore, differences in the surface activity of pectins were found. Sugar beet and citrus pectins lower the surface tension more than apple pectin. Upon the addition of sucrose, an increase in surface tension is detected but only for sugar beet and citrus pectin solutions.

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5.1 Introduction

Pectin is a well-known hydrocolloid that is commonly used in food industry to gel, stabilize and texturize food products. Most of the industrially used pectins are extracted from cell walls of apple and citrus, less often also from sugar beet and grapefruit. The broad use of pectin comes from its ability to form gels with sugar (and sometimes calcium ions) under acidic conditions and to stabilize dairy proteins like caseins [6]. In the last decades, more and more effort has been put into exploring the emulsification and encapsulation properties of pectins since the consumer demand for natural food ingredients and emulsifiers increased.

When comparing the molecular features of commercially available pectins, differences depending on the origin of pectins are obvious. Sugar beet pectin (SBP) is particularly different as it has a naturally low degree of esterification (DE) and low molecular weight but is highly acetylated and contains a considerable amount of covalently bound protein [68]. SBP is not able to form gels due to the low molecular mass and high degree of acetylation (DAc). However, the acetyl groups and the protein moiety are held responsible for the remarkable emulsifying capacity of SBP [4, 92, 101]. Several studies tried to elucidate the relationship between chemical structure of SBP and its emulsifying capacity [95, 100, 103] and investigated possible uses for SBP in food products [97, 104].

Due to their low protein content and number of acetyl groups, citrus and apple pectins are considered less useful for emulsification. However, these pectins usually come with a naturally high DE and possess a higher molecular weight. To the best of our knowledge, only very few studies investigate emulsification properties of apple or citrus pectins [92, 99].

Leroux et al. focused on depolymerization of citrus pectin because preliminary studies had apparently not shown a significant influence of the variation of DE [92]. Akhtar et al. also studied depolymerized pectins and point out that the viscosity enhancing effect alone might be the reason for some of the good long term stabilities observed in pectin emulsions [99]. Although they did not study this effect further, it becomes clear that differences in the viscosity enhancing effect cannot be neglected if one wants to fully understand pectin performance in emulsions.

Pectin added as a stabilizer, increases the viscosity of the continuous aqueous phase of an emulsion and can influence the droplet size of said emulsion in several ways: First, droplet movement is reduced. On the one hand, this suppresses creaming (or sedimentation) which directly leads to an increased long term stability or shelf life of emulsions but does not influence the droplet size yet. On the other hand, however, less droplet movement also leads to decreased droplet collisions. This reduces droplet coalescence which in turn influences long term but also short term stability of emulsions [16]. The short term stability of emulsions is the ability of droplets to resist re-coalescence in an emulsification process. Improved short term stability results in smaller droplets for a fixed recipe [17]. Third, in the emulsion process, an increased viscosity due to pectin addition can also have an immediate effect on droplet breakup itself. In order to generate small droplets, stresses need to be transmitted onto the initial droplets by the surrounding medium. The quality of this transmission is

dependent on the viscosity of the aqueous phase and therefore on the viscosity ratio λ between dispersed oil phase and continuous aqueous phase [18]. For both laminar shear flow and turbulent flow conditions, droplet breakup was found to be easiest when the viscosity ratio was between 0.1 and 1 [1, 19, 20]. That means that for optimal droplet breakup the viscosity of the continuous phase needs to be equal to or up to ten times higher than the viscosity of the dispersed phase. In concentrated emulsions, however, one droplet is not only surrounded by continuous phase but rather by the emulsion itself. The "effective medium approach" therefore suggests not to use the ratio between the viscosity of dispersed phase and the continuous phase, but instead to use the ratio between the viscosity of the dispersed phase (oil) and the emulsion for such emulsions [21, 22].

We want to show in how far pectins from various origins are able to form and stabilize emulsions. Depending on their natural source, pectins have varying droplet formation and emulsion stabilizing properties. We will attempt to separate the phenomenon of droplet formation from the various emulsion stabilizing effects by investigating emulsions of the same viscosity. By this, it will be possible to compare the emulsion forming and stabilizing properties of pectins with varying type and amount of functional groups that stem from diverse plant origin.

5.2 Materials and Methods

5.2.1 Materials

Pectins from different sources and with different physicochemical characteristics were supplied by Herbstreith & Fox KG (Neuenbürg/Germany). They were chosen on the one hand to cover the full range of commercially available products and on the other hand to see differences in emulsification behavior. Sugar beet pectin (SBP), as well as citrus (CP) and apple pectins (AP) of different degree of esterification (high (HM), medium (MM) and low (LM)) were investigated. Table 5.1 shows the characteristics of the different pectins used according to supplier statements. Pure rape seed oil (from now on referred to as "vegetable oil") was purchased from FLOREAL Haagen GmbH (Saarbrücken, Germany). The food grade emulsifier Tween® 20 (T20) and lactic acid (80%) were obtained from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Sucrose was purchased from BÄKO Marken und Service eG (Bonn, Germany).

Table 5.1: Characteristics of pectins used during the investigation.

	Sugar Beet Pectin SBP	High Methylesterified Citrus Pectin HMCP	Medium Methylesterified Citrus Pectin MMCP	Low Methylesterified Citrus Pectin LMCP	High Methylesterified Apple Pectin HMAP	Medium Methylesterified Apple Pectin MMAP	Low Methylesterified Apple Pectin LMAP
Molecular Weight [kDa]	46	73	80	61	81	67	63
Protein Content [%]	4.7	2.8	2.7	3.3	1.1	1.4	1.1
Degree of Esterification [%]	58	70	52	39	71	51	39
Degree of Acetylation [%]	25	-	-	-	-	-	-

5.2.2 Preparation of pectin solutions

For the comparison of the functional properties of pectins under equal process conditions pectin solutions with equal viscosity were required. This was achieved by adding sucrose as dry matter to the solutions. Using a response surface design, in pre-experiments, pectin and sucrose concentrations were determined that combined gave equal solution viscosity for all employed pectins. The exact composition of pectin solutions can be found in Table 5.2a and 5.2b. Pectin solutions were prepared by first dissolving sucrose in distilled water at 60 °C using a magnetic stirring bar and plate. Subsequently, pectin was dissolved in the sugar solution using an Ultraturrax T-25 digital (IKA® Werke GmbH & Co. KG, Staufen, Germany) at a rotational speed of 10.000 rpm. Finally, when the solution had cooled down, the pH was adjusted to 3 using less than 0.5% v/v of lactic acid in order to avoid pectin degradation by β-elimination.

5.2.3 Preparation of emulsions

Emulsions were prepared by dispersing 30% w/w vegetable oil were dispersed into 70% w/w of continuous aqueous phase. Two types of continuous phase were used: Either a pure pectin solution or a pectin solution mixed with T20. In the latter case, 1% w/w T20 was dissolved in 69% w/w of pectin solution to give 70% w/w of continuous phase.

Vegetable oil was dispersed into the aqueous phase by first using an Ultraturrax at a rotational speed of 15.000 rpm. The oil was added over 30 s and the premix was continuously mixed for another minute.

In order to prepare fine emulsions, the premix was further homogenized in a Microfluidizer® MF 110 Y (Microfluidics Corporation, Newton, MA, USA). The emulsion was passed once at 400 bar and then a second time at 800 bar. The first pass

was done to ensure a homogenous structure before the second and decisive emulsification step. In this way, the pre-disruption of droplets leads to a viscosity ratio equal to the one of the final emulsion. Together with the balanced viscosity due to sucrose addition, it could be ensured that the viscosity ratio during droplet breakup was comparable for all emulsion compositions.

Table 5.2: Concentrations of pectin and sucrose as determined by response surface design that were used to prepare emulsions. The resulting effective viscosities and effective viscosity ratios of emulsions are given ((HPH – high pressure homogenization (emulsions prepared without T20), HPH T20 – high pressure homogenization (emulsions prepared with T20)).

Table 5.2a: Sugar beet pectin and citrus pectins.

	Sugar Beet Pectin **SBP**		High Methyl-esterified Citrus Pectin **HMCP**		Medium Methyl-esterified Citrus Pectin **MMCP**		Low Methyl-esterified Citrus Pectin **LMCP**	
Pectin [% w/w]	1.22		1.05		1.19		1.2	
Sucrose [% w/w]	19.3		10.2		12.3		12	
Viscosity and viscosity ratio	η_e [mPas]	λ_{eff} [-]	η_e [mPas]	λ_{eff} [-]	η_e [mPas]	λ_{eff} [-]	η_e [mPas]	λ_{eff} [-]
HPH	148 ± 30	0.16 ± 0.03	209 ± 50	0.12 ± 0.02	267 ± 60	0.09 ± 0.02	190 ± 20	
HPH (T20)	210 ± 10	0.11 ± 0.01	286 ± 25	0.08 ± 0.01	330 ± 60	0.07 ± 0.01	234 ± 20	

Table 5.2b: Apple pectins.

	High Methyl-esterified Apple Pectin **HMAP**		Medium Methyl-esterified Apple Pectin **MMAP**		Low Methyl-esterified Apple Pectin **LMAP**	
Pectin [% w/w]	1.19		1.19		1.18	
Sucrose [% w/w]	16.8		16.8		11.8	
Viscosity and viscosity ratio	η_e [mPas]	λ_{eff} [-]	η_e [mPas]	λ_{eff} [-]	η_e [mPas]	λ_{eff} [-]
HPH	169 ± 20	0.14 ± 0.02	218 ± 40	0.11 ± 0.02	90 ± 30	0.29 ± 0.1
HPH (T20)	261 ± 10	0.09 ± 0.01	386 ± 25	0.07 ± 0.01	191 ± 60	0.12 ± 0.01

5.2.4 Determination of viscosity ratio

All viscosities were measured with a rheometer MCR 301 (Anton Paar, Graz, Austria) equipped with Couette geometry CC-27. Rotational measurements were conducted at a temperature of 42.5 °C as this was determined to be the average processing temperature during emulsion preparation. A logarithmic shear rate profile starting at 0.1 s^{-1} and rising up to 120 s^{-1} was applied. The viscosity at 100 s^{-1} was read out and used for later calculations. The viscosity of the used vegetable oil was 23 mPa s. The

viscosity ratio λ is defined as the viscosity of the dispersed oil phase (η_{oil}) divided by the viscosity of the continuous pectin phase (η_c) as shown in Equation 5.1:

$$\lambda = \frac{\eta_{oil}}{\eta_c} \tag{5.1}$$

The effective viscosity ratio λ_{eff} is defined as the viscosity of the oil phase η_{oil} divided by the viscosity of the final emulsion η_e (Equation 5.2) and is relevant in systems where the dispersed phase concentration is larger than 5% [21, 22]:

$$\lambda_{eff} = \frac{\eta_{oil}}{\eta_e} \tag{5.2}$$

Viscosities were measured in triplicate.

5.2.5 Measurement of droplet size distribution (DSD)

The DSD was measured using a laser diffraction particle size analyzer (LS 13 320, Beckman Coulter, Inc., Miami, FL, USA). Before measuring, samples were diluted in distilled water by approximately 1:20. Measurements were accomplished using polar intensity differential scattering (PIDS) technology. Obscuration was around 45%. The optical model used also covered the Mie region. A refractive index of 1.333 and 1.47 was set for water and oil phase, respectively. The imaginary part was zero. Samples were always measured in triplicate and evaluation of scattering patterns followed the Mie model.

5.2.6 Evaluation of emulsion stability

Since creaming was not visible on any occasion, droplet size distributions were used to evaluate emulsion stability over time. For this purpose, droplet sizes of emulsions were measured again after 2 weeks. The 90th percentile of the volumetric cumulative size distribution ($d_{90,3}$) is used as a characteristic number. Over the investigated time span, emulsions were stored at ca. 6 °C.

5.2.7 Surface tension measurements

The surface tension was measured with a DCAT 11 tensiometer (Dataphysics GmbH, Filderstadt, Germany) based on the Wilhelmy plate method. Measurements were conducted in triplicate at room temperature. Pectin solutions were prepared according to the compositions in Table 5.2. Water was heated to 60 °C and pectin powder was dispersed using an Ultraturrax T-25 digital at 10,000 rpm for one minute. The solutions were cooled down to room temperature and the pH was adjusted to 3 using lactic acid. Then they were transferred into the measuring cup which was tempered to 25 °C. After the measuring plate was inserted into the surface/interface, detection of tension was immediately started.

5.2.8 Experimental design and statistical analysis

All emulsions and measurements were done in triplicate. The results of triplicate analyses were used to calculate averages and standard deviations. The data were analyzed by one-way analysis of variance and linear or quadratic regression. Significantly different mean values of variables ($p < 0.05$) were determined using

Scheffé's test. Statistical analysis was performed using the software OriginPro 9.1G (OriginLab Corp., Northampton, MA, USA).

5.3 Results and discussion

Pectin is mostly used as a stabilizing agent due to its ability to increase the viscosity of solutions. However, it can also be used as an emulsifying substance because it exhibits certain surface active properties. If an emulsion was produced with pectin as sole emulsion stabilizing substance (i.e. no surfactant added), an overlay of both stabilization and emulsification effects would occur. It logically follows that the type and amount of functional groups of different pectins influence to what extent these effects occur.

When only the emulsifying properties of different pectins are of interest, the viscosity enhancing effects still need to be accounted for. Via the viscosity ratio λ_{eff}, both droplet breakup and droplet stabilization immediately after breakup are influenced which results in different maximum droplet sizes of emulsions. Using different pectins at the same mass ratio might result in different viscosity ratios and consequently to different maximum droplet sizes. These differences in droplet size would then not result from different functional groups of pectins but they would purely be determined by the emulsification process. In order to overcome this challenge, we first produced emulsions that contain the small molecule emulsifier Tween® 20 (T20). The emulsifying effect of T20 is supposed to be dominant over that of pectin. In the so produced emulsions, it was possible to only look at the stabilizing effect of different pectins. Once the stabilizing effect of pectins was characterized, it could also be standardized in later experiments. This way, it was possible to produce pure pectin emulsions without small molecule surfactant but at comparable process conditions. This in turn made it possible to focus only on the influence of the emulsifying properties of different pectins. The influence of the type and amount of functional groups of different pectins on the emulsification properties could then be studied.

5.3.1 Influence of pectin concentration on the effective viscosity ratio and resulting droplet size distributions

Fig. 5.1 shows the droplet size distributions of emulsions that contain T20 as emulsifier and various concentrations of medium methylesterified citrus pectin (MMCP) and sucrose as stabilizing agents in the continuous phase (Table 5.3). Due to their low molecular weight, emulsifiers such as T20 (molecular weight ≈ 1.23 kDa) are usually very fast adsorbing and thus are especially suited for high pressure homogenization processes [32]. In these processes, fast adsorption to the interface has been described as the critical step for emulsion stabilization [105]. T20 also lowers the interfacial tension between water and oil phase significantly (Fig. 5.6) which helps to stabilize droplets against coalescence directly after they are broken up in the high pressure disruption unit. Therefore, in emulsions containing low molecular weight surfactants the predominant effect of pectin is a sterical stabilization of droplets that have just been broken up [55]. Furthermore, pectins increase the viscosity of the aqueous phase which reduces the mobility of oil droplets. This leads to a reduced collision frequency and

thus protects newly formed droplets against coalescence while or directly after disruption.

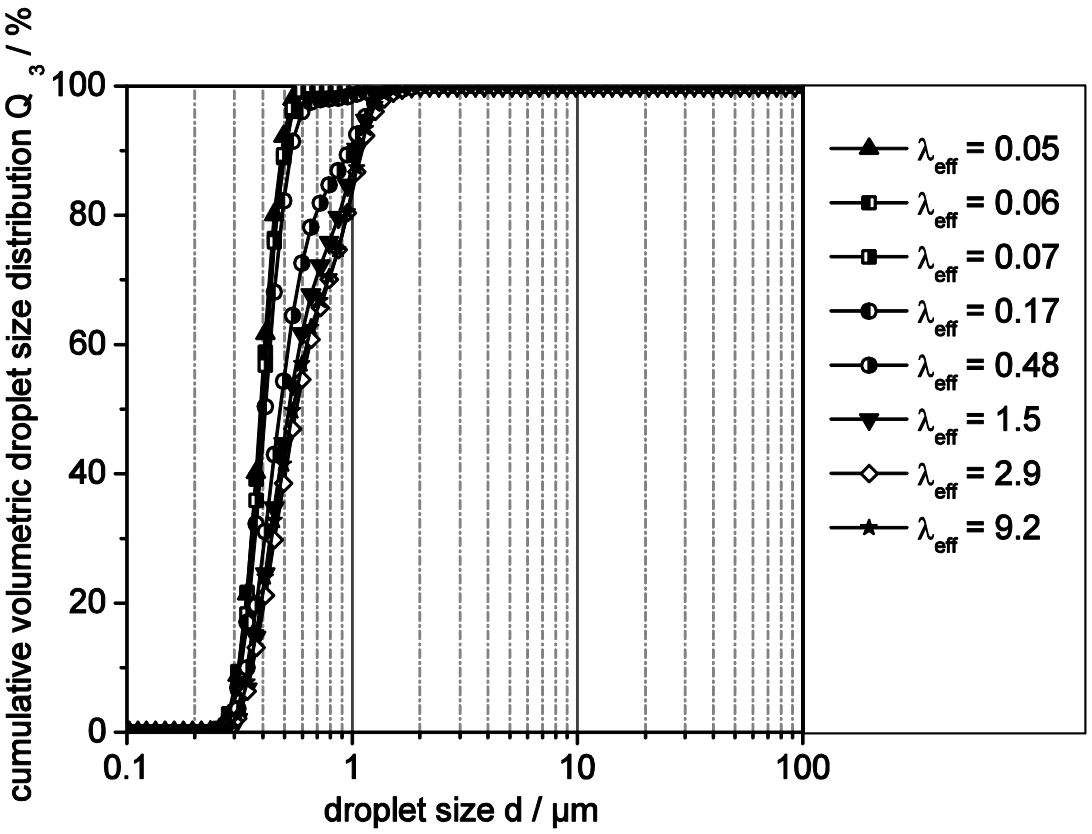

Figure 5.1: Cumulative volumetric droplet size distributions Q_3 of emulsions containing 1% T20 and various amounts of medium methylesterified citrus pectin (MMCP) and sucrose in order to obtain different effective viscosity ratios. Emulsions were processed using a Microfluidizer for two passes at 400 and 800 bar. Effective viscosity ratios λ_{eff} below 0.17 lead to vary narrow droplet size distributions with submicron droplets.

It can be seen (Fig. 5.1) that the droplet sizes of all emulsions were very small, being in the submicron range or around 1 µm. Even the emulsions that did not contain any stabilizers ($\lambda_{eff} = 9.2$) showed maximum droplet sizes $d_{90,3}$ (90^{th} percentile of the volumetric droplet size distribution Q_3) around 1 µm. However, this emulsion was also characterized by a relatively broad droplet size distribution. As the amount of pectin was increased, the viscosity of the emulsion increased, too (Table 5.3). Consequently, the effective viscosity ratio reduced. The corresponding droplet size distributions (Fig. 5.1) became narrower with a smaller amount of large droplets. Below $\lambda_{eff} = 0.17$, no change in the droplet size distribution was visible anymore. The corresponding droplet sizes therefore seem to form the lower limit reachable in the Microfluidizer under the given experimental conditions. A comparable influence of viscosity ratio on the maximum droplet size during homogenization is described in literature for both laminar and turbulent flow conditions [1, 19, 20]. Therefore, it would also be expected that at effective viscosity ratios much lower than 0.05 maximum droplet sizes would increase again. However, due to technical limitations this could not be investigated, as the liquid became too viscous to be processed.

In the investigated range of viscosity ratios, the maximum droplet size $d_{90,3}$ reduced by approximately half (1 µm at $\lambda_{eff} = 9.2$ vs. 0.5 µm at $\lambda_{eff} = 0.05$). This is a noticeable influence of viscosity ratio that could not be neglected during the experiments to follow. At equal mass ratio, pectins would influence the viscosity of their solutions differently due to their particular molecular structure. During further investigations, the influence of viscosity ratio was standardized by adjusting it at a given pectin concentration using sucrose. For $\lambda_{eff,}$ values between 0.05 and 0.17 were chosen because in this range narrow and uniform droplet size distributions with small droplet sizes were observed.

Table 5.3: Concentrations of ingredients and viscosity of emulsions prepared with Tween® 20 and MMCP for the investigation of viscosity ratio influence on droplet size distribution.

Effective viscosity ratio λ_{eff}	**0.05**	**0.06**	**0.07**	**0.17**	**0.48**	**1.5**	**2.9**	**9.2**
Pectin concentration [% w/w]	1.19	1.19	1.16	0.7	0.7	0.7	0.7	0
Sucrose concentration [% w/w]	15.3	12.3	10.9	0.7	0.35	0.14	0.07	0
Emulsion viscosity [mPas]	438	400	320	132	48	16	8	2.5

5.3.2 Influence of pectin type on emulsion stability

In order to compare the stabilizing effects of different pectin types, emulsions with T20 as emulsifier were produced at comparable effective viscosity ratio ($0.05 < \lambda_{eff} < 0.17$). The viscosity ratio was adjusted by varying the amount of sucrose added to the continuous phase. Table 5.2 shows the concentrations of pectins and sucrose and the viscosities and effective viscosity ratios of resulting emulsions after high pressure homogenization. Fig. 5.2 shows the droplet size distributions of these emulsions. For comparison, the droplet size distribution of the emulsion prepared with only T20 in the aqueous phase is also shown ($\lambda_{eff} = 9.2$, see also Fig. 5.1). Emulsions containing both different pectins and T20 displayed very small droplet sizes in the submicron range. The droplet size distributions were very narrow and resemble those in Fig. 5.1 for low λ_{eff}. It can be seen that, except for MMAP, there is no significant difference visible between the characteristic droplets sizes of emulsions prepared with different pectins and T20. Therefore, the stabilizing effect of the investigated pectins at equal effective viscosity ratio is comparable.

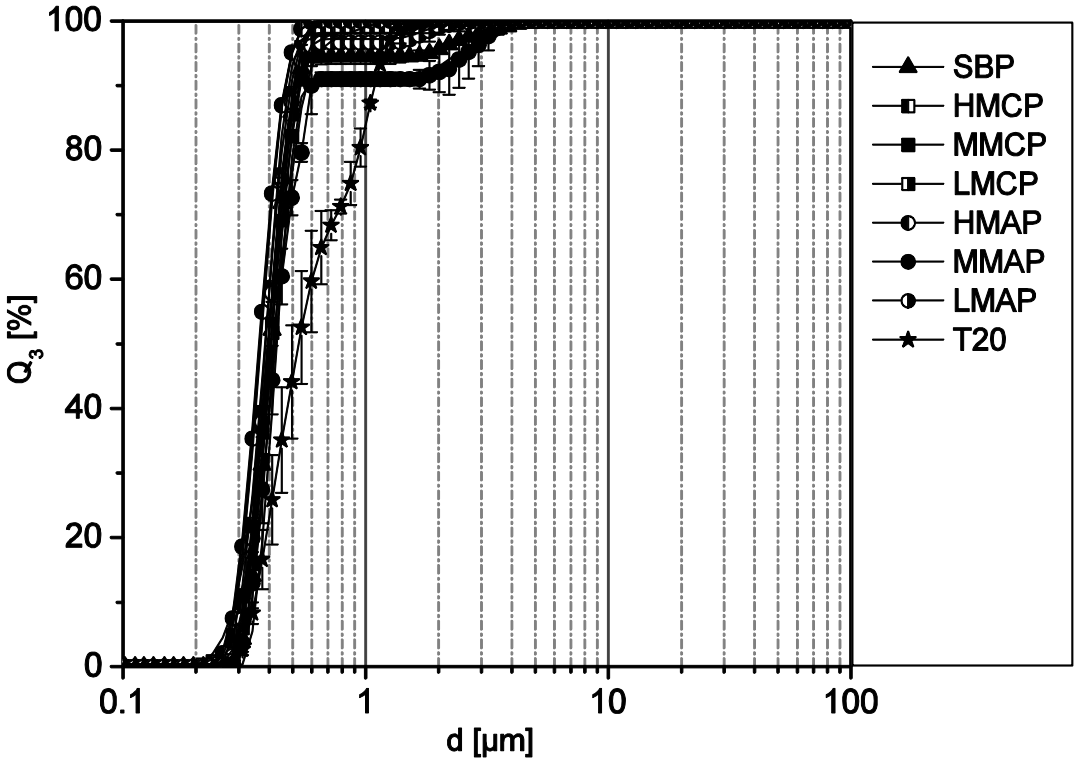

Figure 5.2: Cumulative volumetric droplet size distributions Q_3 of emulsions prepared with different pectins and added Tween® 20. For comparison, the droplet size distribution of an emulsion prepared only with T20 is shown. Emulsions were processed using a Microfluidizer for two passes at 400 and 800 bar. Droplet sizes were measured on the day of production.

5.3.3 Influence of pectin type on emulsion formation

In order to investigate only the emulsifying properties of the different pectins, the same emulsions as above have been prepared without the small molecule emulsifier. With the same effective viscosity ratio as before, stabilizing effects of pectin should stay constant as well. Only the emulsifying effects should vary and should reveal differences between individual pectins.

Fig. 5.3 shows the droplet size distribution of emulsions produced with only pectins as emulsifying substance. The concentrations of pectin and sucrose used in the emulsions are the same as for the previous experiments and can be found in Table 5.2. Firstly, all pectins were suitable for producing droplet sizes well below 20 µm. This is relevant to food applications since fat droplets become perceivable in the mouth at approximately this size [106]. Nevertheless, all the emulsions also showed droplet sizes that were larger than the ones containing T20 (compare Fig. 5.2 and Fig. 5.3). This resulted in slightly higher effective viscosity ratios (Table 5.2). At equal dispersed phase volume fraction, larger droplets lead to a lower emulsion viscosity and thus to a higher λ_{eff}. However, this is not relevant as the resulting λ_{eff} is still within the range determined in the first chapter.

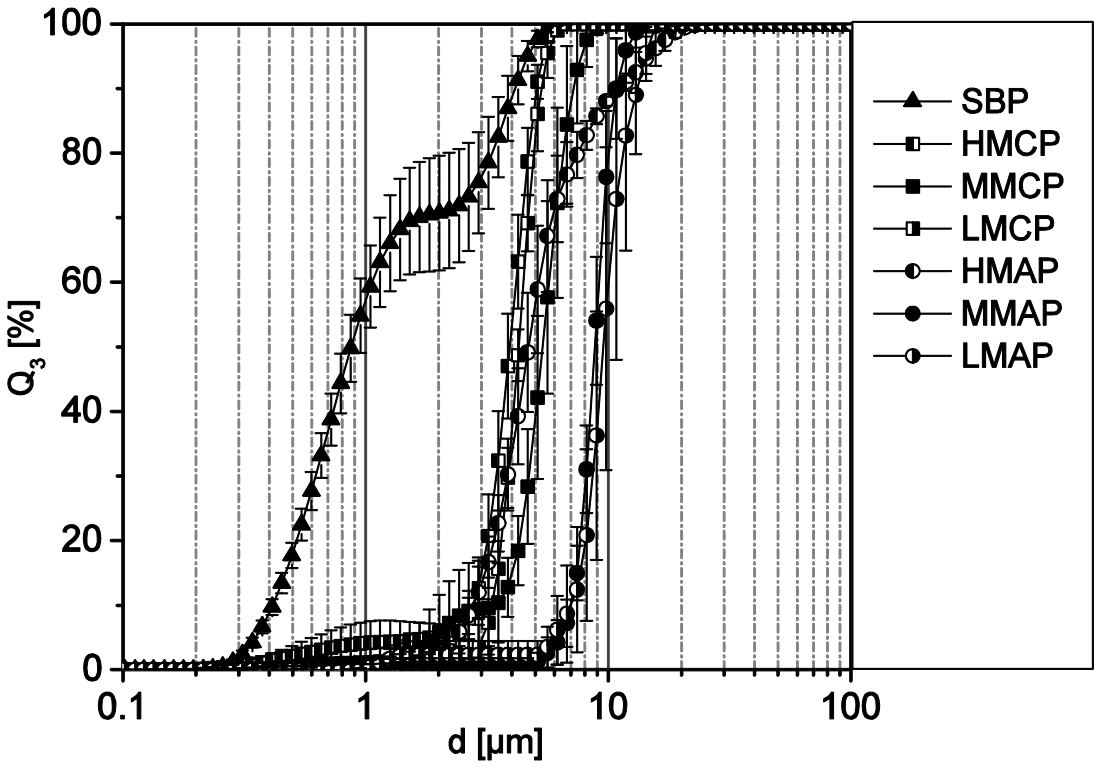

Figure 5.3: Cumulative volumetric droplet size distributions Q_3 of emulsions prepared by microfluidization without Tween® 20. Droplet sizes were measured on the day of production.

When comparing the droplet size distribution in Fig. 5.3, differences in the ability of individual pectins to form small droplets are visible: Sugar beet pectin (SBP) produced the smallest droplets, followed by the three citrus pectins and the apple pectins, but emulsions with SBP also showed much broader droplet size distributions than the other emulsions. That using SBP results in the smallest reachable droplet sizes is to be expected from literature. It has already been described as a possible hydrocolloid emulsifier before [55]. The protein moieties covalently bound to the SBP polysaccharide backbone are said to be responsible for the good emulsifying behavior [92]. Indeed, when having a closer look at the pectin properties given in Table 5.1, we can see that SBP has the highest protein content of the pectins used in this study. In order to analyze the influence of protein content on the obtained maximum droplet sizes $d_{90,3}$, the relationship between them is visualized in Fig. 5.4. The linear regression with $R^2 = 0.83$ and a slope of −2.32 indicated a strong negative correlation between obtainable $d_{90,3}$ and amount of protein. Thus, it is a possible explanation for the different characteristic droplet sizes observed between citrus and apple pectin emulsions.

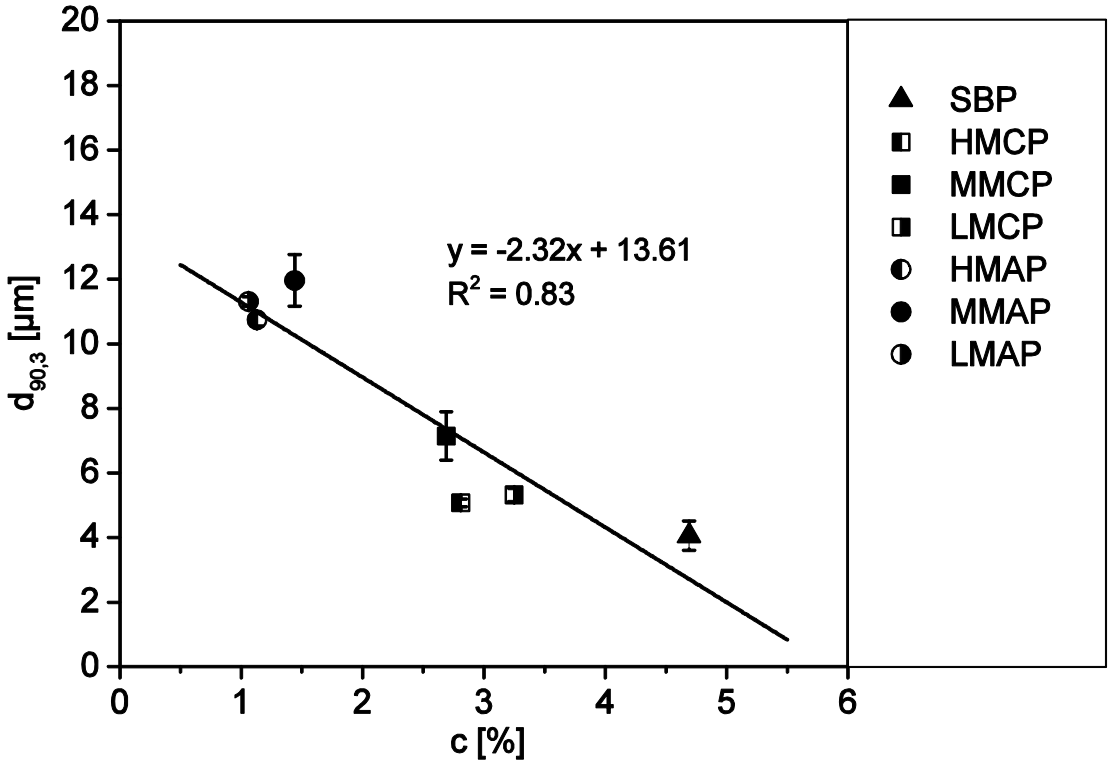

Figure 5.4: Influence of protein content c on the characteristic droplet size $d_{90,3}$ of emulsions prepared without small molecule emulsifier. The solid line shows a linear fit of the $d_{90,3}$. The linear equation and coefficient of determination are given.

While the investigated citrus pectins showed intermediate amounts of bound protein, the apple pectins possessed the lowest amounts (Table 5.1). Although the R^2 indicated a good correlation, it is also clear that not all observed differences between individual pectins can be explained by the protein content alone. Other features must also play a role, particularly for explaining the significant differences observed within certains group of pectins. Therefore, in Fig. 5.5 the relationship between characteristic droplet size $d_{90,3}$ of emulsions and degree of esterification DE of the pectins is visualized. A quadratic equation has been used to fit the droplet sizes of emulsions from citrus pectin and apple pectin, respectively. From $R^2 = 0.81$ for the citrus pectin emulsions, it can be seen that a quadratic equation describes the observed scatter reasonably well. For apple pectin emulsions, the correlation is not so strong ($R^2 = 0.45$). No significant difference has been found between the characteristic droplet size of LMAP and MMAP emulsions (see also Fig. 5.7). The reason for the parabolic scatter of droplet sizes might lie in the dominating intermolecular forces at different DE and their influence on droplet stabilization. At low DE, carboxyl groups dominate that will, to a large part, dissociate at the given pH. The resulting negative charge will lead to electrostatic repulsion that can help to stabilize formed droplets. At high DE, most carboxyl groups are methylated, leading to hydrophobic interactions between the pectin molecules. At medium DE, neither stabilizing mechanism is sufficiently present, leading to larger characteristic droplet sizes.

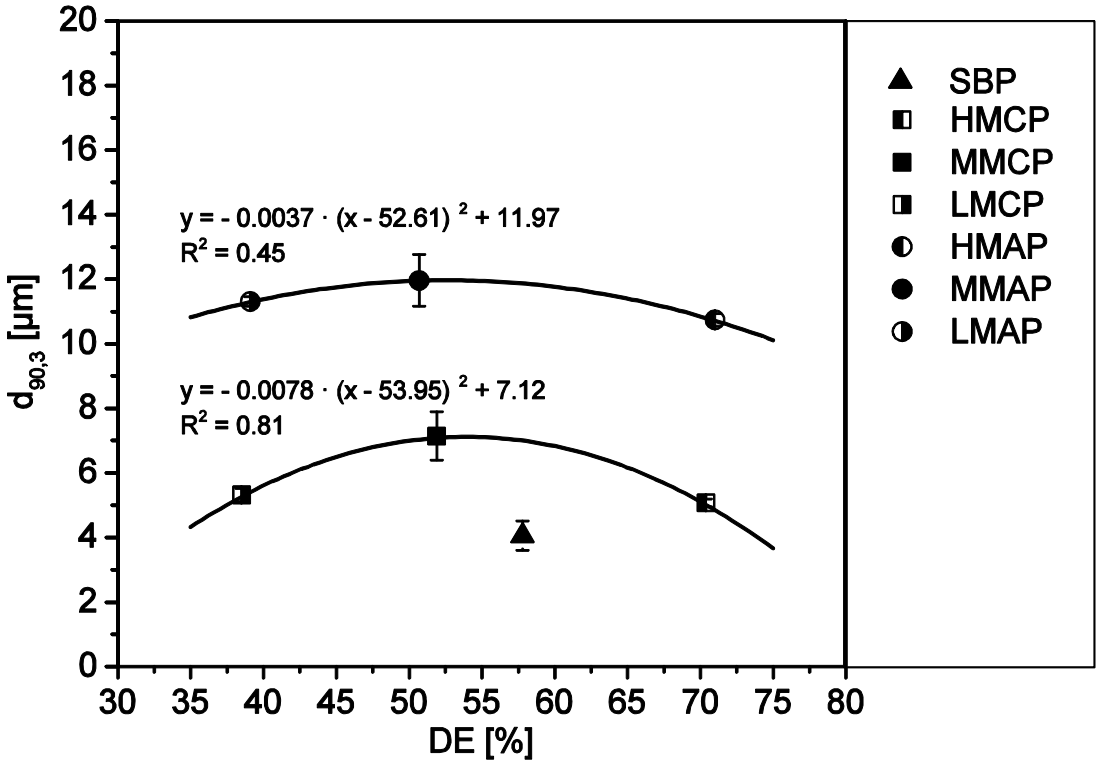

Figure 5.5: Dependence of characteristic droplet size $d_{90,3}$ of emulsions on the degree of esterification DE of pectins. Emulsions do not contain small molecule emulsifier. Quadratic equations were used to fit the $d_{90,3}$ of citrus and apple pectin emulsions. Quadratic equation and coefficients of determination are stated.

A combination of the quadratic equation used to explain the influence of DE on the characteristic droplet size, and the linear equation used to explain the influence of protein content on $d_{90,3}$ led to a fit that had an $R^2 = 0.92$ (not visualized). This showed that most of the observed variance can be explained by different values of protein content and DE. It also indicates the importance of these two functional groups for emulsion formation and stabilization with pectins. The remaining 8% of variance might be explained by other molecular features like the molecular weight or the degree of acetylation of pectins. These functional parameters also vary between the investigated pectins (Table 5.1). However, regression analysis of these parameters has not shown any obvious relations.

Moreover, from Table 5.1 it can also be seen that SBP differs strongly from apple and citrus pectins not only in the protein content or DE but also in degree of acetylation DAc and its molecular weight: SBP does not only possess more acetyl groups than the other pectins but is also a molecule with a generally lower molecular weight. The very different emulsifying behavior of SBP could therefore also be due to these other two molecular features. A positive influence of a high DAc on emulsifying properties of pectins has been described before [101]. Furthermore, SBP is likely to adsorb faster to the oil-water interface as compared to other pectins because of its lower molecular weight. Adsorption kinetics of molecules is strongly influenced by their molecular weight. This would explain why more small droplets are present in the droplet size distribution of SBP emulsions. However, the lower molecular weight is likely to result

in a lower sterical stabilization at the same time [60]. The biopolymer chains adsorbed to newly formed droplets are too short to prevent coalescence in the course of the emulsification process, particularly when fast adsorbing small molecule emulsifiers are missing.

5.3.4 Surface activity of pectins and interaction with sucrose

It was shown that both protein moieties and ester groups affect the emulsifying properties of pectins significantly. Because these functional groups influence the hydrophobicity of a pectin molecule which in turn can influence the emulsifying behavior, measurable differences in the surface activity of the investigated pectins were expected. However, sucrose which was used to set the effective viscosity ratio might influence the surface active behavior of the pectins, too. This influence of sucrose might vary depending on the functional groups of pectin that are available for interaction. We therefore measured the surface activity of five pectins that were used for emulsion production (Fig. 5.6). The surface activity of pectin solutions with and without sucrose is shown. The pectin and sucrose concentrations used to prepare the solutions were the same as in the corresponding emulsions and can be found in Table 5.3. For comparison, the surface tension of an aqueous solution of 1% T20 is shown.

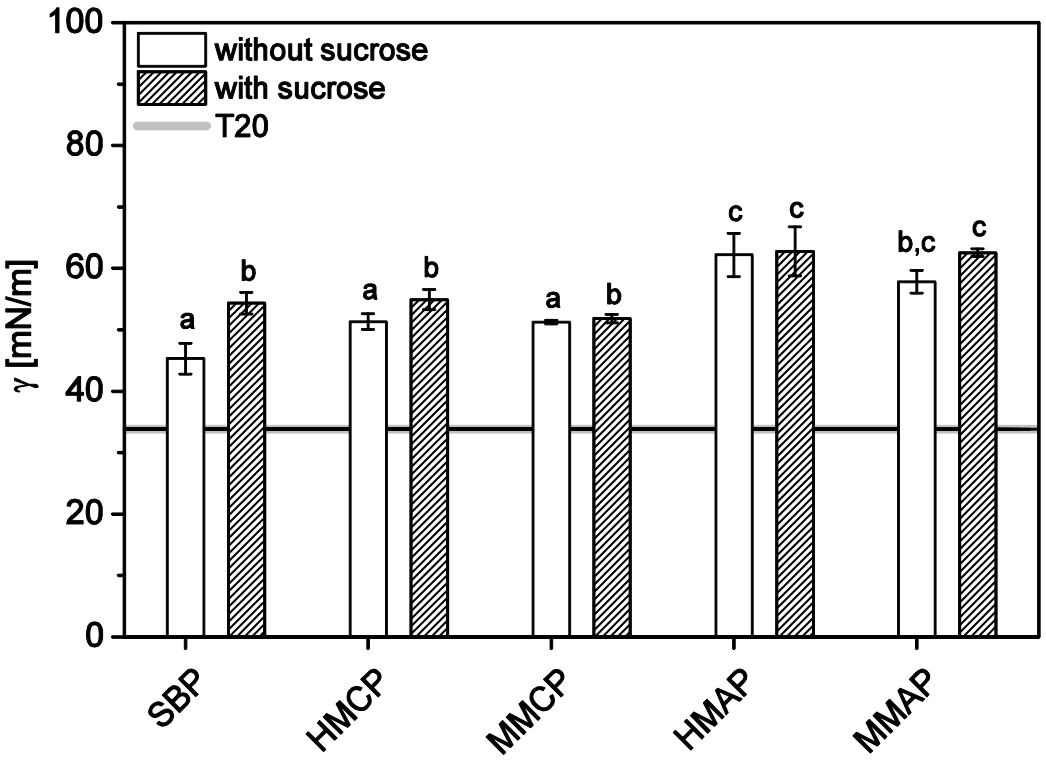

Figure 5.6: Surface tension γ of different pectin solutions with and without sucrose. The concentrations of solutions are equal to those used in emulsion preparation. Measurements were conducted at process temperature (T = 42.5 °C). The surface tension of a pure T20 solution is depicted for comparison. Different letters indicate significant differences.

All pectin solutions exhibited lower surface tension values than pure water (≈72 mN/m at 20 °C). However, T20 was able to lower the surface tension even further than any of the investigated pectins. Differences between individual pectin solutions were also visible: Apple pectin solutions with and without sucrose had significantly higher surface tension than SBP and citrus pectin solutions. SBP and citrus pectin solutions gave comparable results. Apparently, the amount of bound protein did not influence the surface tension. Otherwise, SBP solutions would have shown a lower γ. This result also shows that static surface tension measurements were not sufficient to explain the differences observed between the emulsifying behavior or SBP and CP.

It is interesting to notice that SBP and CP solutions with sucrose showed significantly higher surface tensions than those without. However, in the high pressure homogenization process, this is supposed to be of less importance compared to adsorption kinetics [105]. An explanation for the increase in surface tension by adding sucrose might lie in the induced gel formation caused by the addition of dry matter (sucrose). Pectins form gels by hydrogen bonding as well as by bonds between hydrophobic groups. These pectin–pectin chain interactions are typically altered by temperature changes [63], but are also influenced by sucrose [85]. A change in chain interaction might lead to less hydrophobic groups being available for the adsorption at the interface. Therefore, it is important to stress that the addition of dry matter does have a significant influence on the surface active behavior of pectins. So far, studies concerning the emulsifying behavior of pectins only investigated pure pectin solutions. However, this side effect of added sucrose needs to be considered and requires a more thorough investigation using dynamic tensiometry.

5.3.5 Influence of pectin type on the long term stability of emulsions

Not only is the formation of droplets during the emulsification process influenced by the viscosity enhancing effect of pectins. Also long term stability can be influenced by the emulsion stabilizing properties of pectins. We therefore investigated the change of emulsion droplet sizes over time. Fig. 5.7 displays the characteristic droplet size $d_{90,3}$ of the same emulsions measured directly after production and after two weeks. It shows that, except for SBP and HMAP, the $d_{90,3}$ of emulsions has not changed over the investigated storage time. The differences in $d_{90,3}$ initially observed for the pectin emulsions were as such still present after two weeks.

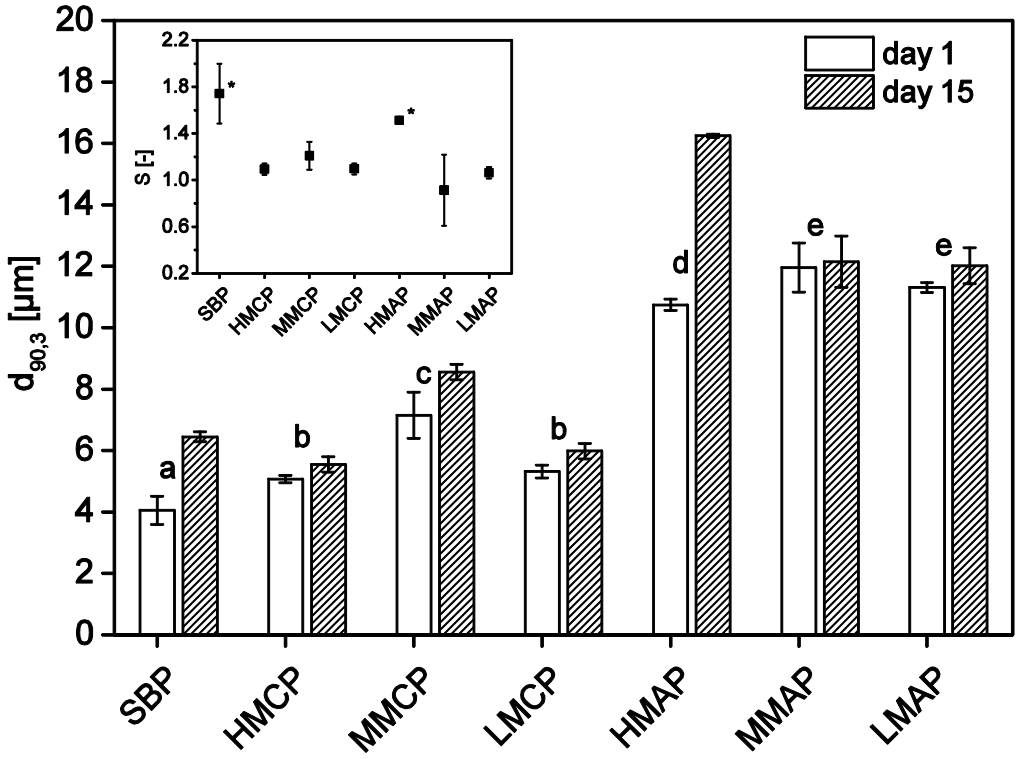

Figure 5.7: Characteristic droplet sizes $d_{90,3}$ of pectin emulsions without T20 and processed by microfluidization. The droplet sizes were measured on the day of production and after two weeks. Letters indicate significant differences for $d_{90,3}$ on both day 1 and day 15. The inset diagram shows the change of $d_{90,3}$ as the ratio S between the value determined on day 15 and day 1. Asterisks indicate significant differences.

However, for SBP and HMAP emulsions, a significant increase in the characteristic droplet size over time has been determined. This is visualized in the inset diagram: It shows the change of the characteristic droplet size S over two weeks. A value of 1 for the change S indicates that the characteristic droplet size $d_{90,3}$ does not increase over time, and the emulsions is stable. Values of around 1.8 for SBP and 1.4 for HMAP, however, indicate insufficient droplet stabilization. For SBP, the lack of long term stability might be explained by the high DAc. Acetyl groups are known to be the reason for the reduced gel forming capacity of SBP and thus might cause worse droplet stability. However, the reason for a comparable long term behavior of HMAP remains unclear.

5.4 Conclusion

Pectins from various sources can be successfully used as both stabilizers and emulsifiers. By preparing emulsions with a small molecule emulsifier, it was possible to study the stabilizing effect of the biopolymer independently of the emulsifying properties. We showed that at equal effective viscosity ratio the stabilizing effect of different pectins is comparable. At the same viscosity ratio but without small molecule emulsifier, differences in the emulsifying behavior became apparent depending on the source of pectin. These can by a large extent be explained by differences in the molecular features of pectins. The protein content and the degree of esterification were

identified to be the main parameters influencing the characteristic droplet size of pectin emulsions. A higher amount of bound protein resulted in smaller emulsion droplets. Emulsions produced from pectins with high or low DE showed smaller droplets than those produced from pectins with medium DE. However, to determine the actual mechanism, more research is needed. SBP was found to differ in various ways from the other pectins investigated. Finer yet unstable emulsions were produced. A reason for this observation could be the comparably high degree of acetylation or the low molecular weight. A general link between those parameters and droplet sizes of all emulsions could not be found but it would still be interesting to have a closer look at these features. Finally, sucrose as a dissolved substance shows an influence on the surface activity of pectins. At the sucrose concentrations required by the experimental setup, a significant increase in surface tension was observed for citrus and sugar beet pectin. The reason for this is unclear and requires more thorough investigations of the interface.

5.5 Acknowledgments

This research was conducted in the frame of the project "Allipids – A range of preventive food on the basis of healthy lipids" (FKZ 0315682). The authors would like to thank the German Federal Ministry of Research and Education (BMBF) and Projektträger Jülich for financial support of the project.

6 Influence of the degree of esterification on the emulsifying performance of conjugates formed between whey protein isolate and citrus pectin

Conjugates were prepared from whey protein isolate (WPI) and pectins in a dry heating process (80 °C, 79% RH). Citrus pectins with different degree of esterification (DE) were employed: low methylesterified (LMCP, DE = 34%), high methylesterified (HMCP, DE = 72%), very high methylesterified (VHMCP, DE = 84%). SDS-PAGE of heat treated WPI-pectin samples showed typical patterns of conjugate formation with substances of M_w > 100 kDa being detected first after 6 h of reaction time. Fluorescence intensity measurements of conjugate solutions indicated a maximum for WPI-HMCP and WPI-VHMCP after 15 – 18 hours of reaction time. However, the fluorescence intensity of WPI-LMCP solutions increased continuously reaching the highest values of the three mixtures. Zeta potential measurements of conjugate solutions exhibited the opposite behavior. A minimum was found for WPI-HMCP. The zeta potential of WPI-LMCP solutions decreased monotonously reaching values of around -50 mV. In emulsification experiments, a significant reduction of the emulsion's Sauter mean diameter $d_{3,2}$ was found when conjugates were employed at pH 5.5 and 7. The smallest droplet sizes in the hundred nanometer range were obtained for WPI-LMCP conjugate emulsions. Linking fluorescence measurements of conjugate solutions to emulsion $d_{3,2}$ revealed that 1.) the stabilizing mechanism of WPI-pectin conjugates is mostly steric, 2.) the conjugate yield is the main factor dominating emulsion droplet size and 3.) the conjugate yield is highest when low esterified pectin is used.

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6.1 Introduction

Many popular food products like mayonnaise, sauces, ice-cream etc. are emulsion based. Often, the main emulsion stabilizing substance in such products is a protein. However, protein-based emulsions are very sensitive to environmental changes, particularly to changes in pH. This sensitivity limits their applicability especially around the isoelectric point (IEP) [107]. One way to overcome this limitation is by covalently binding polysaccharides to proteins using dry heating [108–111]. Improved emulsifying properties of the resulting reaction products have been reported various times [112–115].

Conjugation of proteins with polysaccharides takes place in a Maillard like reaction and leads to the formation of molecules with higher molecular weight [110, 113]. It is reported that structural changes in the protein part are induced due to conjugation that prevent the formation of insoluble protein aggregates [73, 116]. These molecular changes are said to improve structural stability and resistance against pH changes [117]. Furthermore, conjugation changes the overall charge of the protein. Hence, the dissociation equilibrium of the conjugated protein is changed which shifts the IEP of the molecule into a more acid pH range [114, 118, 119]. This is supposed to be the reason why conjugates show improved functional properties compared to non-conjugated proteins at the IEP of the protein [120].

Several combinations of proteins and polysaccharides have been the focus of investigation [121–124]. Some authors also report on the preparation and emulsifying performance of protein-pectin conjugates [73, 108, 125]. Pectin is a hydrocolloid with reported emulsifying behavior [8]. In a previous paper, we investigated the combined influence of protein content and degree of esterification (DE) of pectins from various plant sources on the emulsion droplet size [126]. We could show that a high protein content is advantageous for pectin's emulsifying performance and that the DE of pectin does play a role, however, the exact mechanism was unclear.

Few research groups already investigated the influence of the degree of esterification (DE) of the pectin on WPI-pectin conjugate emulsifier performance. Neirynck et al. [108] compare the emulsifying performance of conjugates from WPI and high and low DE pectin. However, the high DE pectin sample that was used had been standardized meaning it was contaminated with 40% dextran according to supplier information. This makes it impossible to distinguish between the influence of pectin and dextran on conjugate formation and makes the emulsification results not suitable for the elucidation of influence of DE. Einhorn-Stoll, Ulbrich, Sever & Kunzek [73] investigated high and low DE pectin in combination with whey protein isolate (WPI) and sodium caseinate. In a combined statistical analysis no influence of DE on the emulsifying performance was found. Only when WPI conjugates were analyzed separately, a slightly improved emulsifying performance of high DE pectin conjugates was found. Here, the reason for these ambiguous results might have been the pH of emulsions. Emulsification took place at pH 7. At this pH, the remaining unconjugated WPI shows excellent emulsion stabilizing properties and might have covered the effect of conjugate formation.

We strive to establish a relationship between the DE of pectin and the emulsifier performance of the resulting WPI-pectin conjugates. Due to the lower charge of high DE pectin, we expect improved conjugate formation compared to low DE pectin [127]. This should have a direct effect on the emulsifying capacity around the IEP of the protein. However, a more hydrophilic polysaccharide, i.e. pectin with low DE, is supposed to increase the protein adsorption kinetics of the conjugated molecule [128, 129]. This was described to be due to an improved interaction of the hydrophilic part of the conjugate with the polar aqueous phase which drives the hydrophobic protein part faster towards the oil-water interface [128]. We therefore suspect that conjugates formed with a low DE pectin show superior emulsifying properties to conjugates formed with high DE pectin.

6.2 Materials and Methods

6.2.1 Materials

Whey Protein Isolate 895 was obtained from Fonterra Co-operative Group (Auckland, New Zealand). According to supplier information, the isolate typically contains 93.9% protein, 0.4% lactose and 0.3% fat. The moisture content was stated to be 4.7%. Citrus pectins with different degrees of esterification were supplied by Herbstreith & Fox KG (Neuenbürg/Württ., Germany). Pectin characteristics according to supplier information can be found in Table 6.1. For the preparation of emulsions, pure rapeseed oil (FLOREAL Haagen GmbH, Saarbrücken, Germany) was used.

Table 6.1: Characteristics of pectins used to prepare conjugates. All values are as stated by manufacturer.

		Low Methylesterified Citrus Pectin **LMCP**	High Methylesterified Citrus Pectin **HMCP**	Very High Methylesterified Citrus Pectin **VHMCP**
Molecular Weight	**[kDa]**	52	78	80
Galacturonic acid content	**[%]**	87.8	84.2	87.1
Degree of Esterification	**[%]**	34.2	71.6	83.7
Protein content	**[%]**	3.5	2.5	2.1

6.2.2 Preparation of protein-pectin conjugates

Conjugates were prepared by subjecting freeze-dried protein-pectin mixtures to a heat treatment at low water activity [125, 130]. Therefore, biopolymer solutions were prepared by dissolving first pectin then protein in water at 25 °C. The mass ratio between protein and pectin was 1:2. The mass concentration of dry matter in the solution was 2.5% w/w (low methylesterified pectin) or 5% (high and very high methylesterified pectin). The solution was given 3 – 4 h at room temperature to equilibrate. Then, the pH was adjusted to 7 using 10% w/w NaOH solution. The protein-pectin solution was deep-frozen to -18 °C within 15 min and then freeze-dried at 0.3 mbar (for 16 – 24 h) using a freeze-drier Alpha 2-4 LD plus (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). The resulting lyophilisate

was ground to particles < 25 µm in an ultra-centrifugal mill ZM 200 (Retsch, Haan, Germany) at 12,000 rpm. For each sample, approx. 12.5 g of lyophilisate powder were spread out in a Petri dish of 10 cm diameter. Petri dishes were then placed open into a climate chamber PR-15 (Thermo Tec, Rochlitz, Germany). The temperature was set to 80 °C and the relative humidity to 79%. The reaction time was varied between 1.5 and 72 h as an experimental parameter.

6.2.3 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to a modified method described by Laemmli [131] under reducing conditions. A 5% stacking gel and a 12.5% separating gel containing 0.4% SDS were used. Samples were prepared by dissolving 2 mg/ml (WPI) or 4 mg/ml (other) sample powder in demineralized water. 60 µl of each solution were mixed with 20 µl of the ready-to-use buffer Roti Load 1 (Carl Roth, Karlsruhe, Germany) and the mixtures were denaturated at 99 ± 5 °C for 5 min. Then, aliquots (20 µl) were loaded onto the gels. The gels were run in a Tris-Glycin running buffer (pH 8.3) containing 0.1% SDS at 90 V. Electrophoresis was stopped when samples reached the bottom of the gels (after 1.5 – 2 h). Proteins were stained by Coomassie Brilliant Blue R250 in 10% acetic acid. For destaining, a solution of 10% acetic acid and 25% methanol was used. To estimate the molecular weight of samples, the protein standard *Precision Plus Protein™ Dual Color* (Bio-Rad Laboratories Inc., Hercules, CA, USA) containing weight markers from 10 – 250 kDa was used.

6.2.4 Fluorescence spectroscopy

Fluorescence spectra were determined using an Infinite 200 Pro microplate reader (Tecan, Crailsheim, Germany). Biopolymer solutions were prepared by dissolving 50 mg of the sample in 20 ml demineralized water. For fluorescence excitation spectra, the emission wavelength was set to 436 nm and the excitation was scanned from 300 to 400 nm. For the emission spectra, the excitation was at 368 nm and the emission was recorded from 400 to 580 nm. Both excitation and emission slits were set to 2 nm and the average of 10 flashes was recorded.

6.2.5 Zeta potential measurements

Protein-pectin solutions were prepared by dissolving 0.25% w/v of conjugate powder in MilliQ water (25 °C) that was previously set to pH 5.5. Solutions were given at least 12 hours for hydration. The pH was measured and, if necessary, adjusted back to pH 5.5. The zeta potential was determined at 25 °C using a Zetasizer Nano ZS (Malvern Instruments GmbH, Herrenberg, Germany). Six measurements of at least twelve runs per measurement were conducted per sample.

6.2.6 Emulsion preparation

Emulsions contained 30% w/w vegetable oil as dispersed phase, 2% w/w biopolymer conjugates as emulsifier and 68% w/w demineralized water (continuous phase). For reference emulsions containing only WPI, the emulsifier concentration was limited to 0.66% as this corresponded to amount of protein present in conjugate emulsions

(protein-pectin ratio = 1:2). The emulsifier was dissolved in water using an Ultraturrax T-25 digital at 10.000 rpm. Then, the pH was adjusted at room temperature to 3, 5.5 or 7. The solution was given 30 min to equilibrate. The solutions were treated in a centrifuge (Rotanta 460R, Hettich, Bäch, Switzerland) at 10,000 g for 15 min. The supernatant was taken and the oil phase was added to prepare an emulsion premix by using the Ultraturrax at 10.000 rpm for one minute. For further homogenization, the premix was transferred to a high pressure homogenizer (Microfluidizer® MF 110 Y, Microfluidics Corporation, Newton, MA, USA). The emulsion was first emulsified at 400 bar, collected and then passed through the device a second time at 800 bar. Emulsification experiments were conducted three times per biopolymer sample.

6.2.7 Measurement of droplet size distribution

Droplet size distributions of emulsions were obtained by using a laser diffraction particle size analyzer (LS 13 320, Beckman Coulter, Inc., Miami, FL, USA) equipped with polarization intensity differential scattering (PIDS) technology. Obscuration was around 45%. The optical model used also covered the Mie region. A refractive index of 1.333 was used for the water phase and 1.47 was used for the oil phase. The imaginary part in both cases was set to zero as both phases are transparent. Emulsion samples were always measured in triplicate. Droplet size distributions were then calculated according to the Mie theory. The Sauter mean diameter $d_{3,2}$ was used as the characteristic value to describe the droplet size distribution. It is a measure for the volume specific surface area of the dispersed phase and thus corresponds to surface related properties as e.g. release of dissolved actives.

6.2.8 Statistical analysis

Statistical analysis was performed using the software OriginPro 9.1G (OriginLab Corp., Northampton, MA, USA). The results of triplicate analyses were used to calculate averages and standard deviations. The data were analyzed by one-way analysis of variance. Significantly different mean values of variables ($p < 0.05$) were determined using Scheffé's method.

6.3 Results and Discussion

6.3.1 SDS-PAGE

In order to check for the formation of high molecular weight material as an indication of conjugation, SDS-PAGE was performed on heat treated WPI-pectin mixtures. Reducing conditions were chosen, so that only covalently linked conjugates are detected. WPI-pectin conjugates are expected to have high molecular weight above 100 kDa [73, 130].

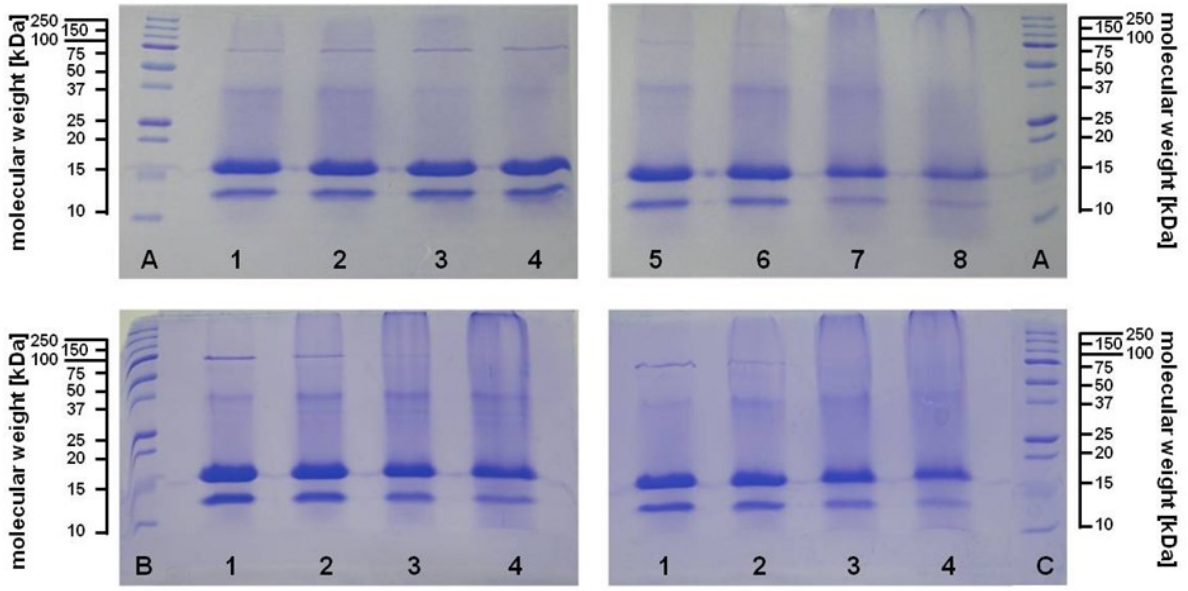

Figure 6.1: SDS-PAGE of lyophilized WPI-pectin (1:2) mixtures treated at 79% relative humidity and 80 °C for various times. Different letters refer to pectins with different DE: (A) DE = 71%, (B) DE = 84%, (C) DE = 34%. In gel (A), lanes 1 and 2 correspond to mixtures before and after lyophilization (0 h); lanes 3-8 correspond to heating times of 1.5, 3, 6, 9, 24 and 48 hours. In gel (B) and (C), lanes 1-4 correspond to heating times of 0, 6, 24 and 48 h, respectively. The lanes above the letters contain the protein standard.

Figure 6.1 shows SDS-PAGE gels of the incubated WPI-LMCP, WPI-HMCP and WPI-VHMCP mixtures. The lane above the letter corresponds to the protein standard while lanes above numbers contain the investigated samples. Freeze-drying of WPI-pectin mixtures as the initial step in conjugate preparation did not have any significant influence on the molecular weight of the protein (compare Figure 6.1, lanes A1 and A2). At the beginning, intensely colored bands were visible at 14 kDa, 18 kDa, 37 kDa and 75 kDa, corresponding to α-LA, β-LG monomeric and dimeric, and BSA, respectively (lanes A2, B1, C1) [108]. No protein containing substances with a molecular weight above 75 kDa were detected at first. Upon heat treatment, the characteristic protein bands from WPI fractions became paler as is described in literature [109, 125]. Substances with a molecular weight above 100 kDa were detected after approx. 6 h of heat treatment (Figure 6.1, lanes A5, B2, C2). These substances appeared in the form of polydispersed bands. Due to the polymeric nature of pectin, its molecular weight is not as well defined as that of whey proteins. This leads to the formation of conjugates with a broad molecular weight distribution. After 48 hours (lanes A8, B4, C4), intensive immobilized bands were seen at the gel injection point. This indicates protein rich substances which were excluded from intrusion into the gel pores because of them being too large in molecular weight and/or volume (>250 kDa) [132, 133]. SDS-PAGE of separately incubated protein and pectin did not indicate any formation of high molecular weight material (Fig. 6.2). Therefore, self-polymerization of the single substances did not take place. Only heat treatment of protein and pectin together led to the formation of high molecular weight species.

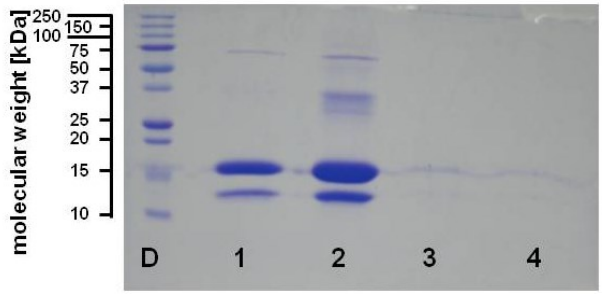

Figure 6.2: SDS-PAGE of individually investigated WPI and pectin (DE = 71%) samples. Samples were either studied without further treatment or after heat treatment for 6 h. Lane 1: WPI (after 6 h reaction time), lane 2: WPI (natural), lane 3: pectin (natural), lane 4: pectin (after 6 h reaction time). The pectin sample does not show inherent protein. WPI loses its characteristic band at 37 kDa upon heating, showing that β-LG dimers are broken up into monomers. The lane above letter D contains the protein standard.

6.3.2 Fluorescence measurements

It has been described that in the advanced stage of Maillard reaction fluorescent reaction products develop [134, 135]. Fluorescence spectroscopy can thus be used to monitor the progress of Maillard reaction [135, 136]. Using this technique, we found a maximum in fluorescence intensity at an excitation wavelength of 368 nm and at an emission wavelength of 488 nm for all protein-pectin mixtures (shown exemplarily for WPI+HMCP mixtures in Fig. 6.3). These wavelengths correspond to substances where the chromophore is a Schiff base in conjugation with an electron donating group [137].

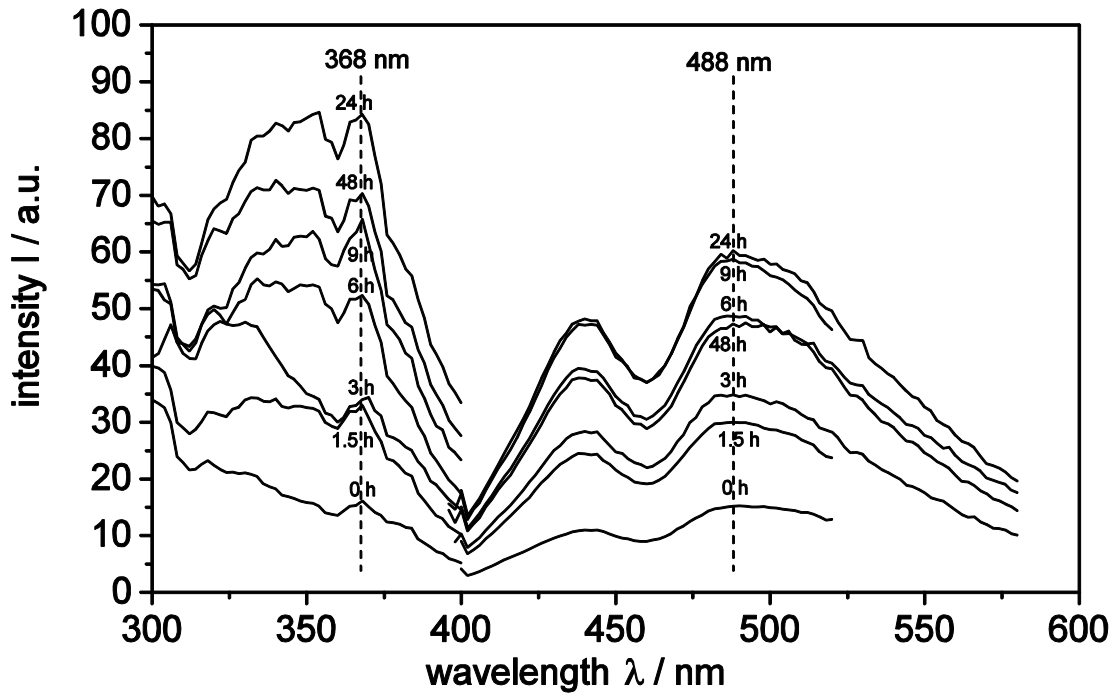

Figure 6.3: Fluorescence excitation and emission spectra of 0.25% w/v conjugate solutions. WPI+HMCP mixtures were exposed to different lengths of heat treatment as indicated in the graph. Excitation spectra: λ_{exc} = 300 to 400 nm, λ_{em} = 420 nm; emission spectra: λ_{exc} = 368 nm, λ_{em} = 400 to 520 (580) nm.

The fluorescence intensity maxima of all three WPI-pectin conjugate solutions at the mentioned wavelengths over reaction time are depicted in Figure 6.4. Solutions of WPI+HMCP and WPI+VHMCP first showed an exponential increase in fluorescence intensity that reached a maximum after about 15 to 18 hours. Then, a slower exponential decline followed. A comparable curve slope has been reported for different protein-saccharide compositions [138, 139]. The maximum was higher for WPI+HMCP than for WPI+VHMCP. However, for WPI+LMCP, no intensity maximum was found in the investigated time period. Instead, the fluorescence intensity continued to increase exponentially. After 48 hours and more, WPI-LMCP mixtures showed intensity values which were higher than those of any of the other protein-pectin mixtures. This indicates a fast and constant increase in fluorescent reaction products over the investigated reaction time.

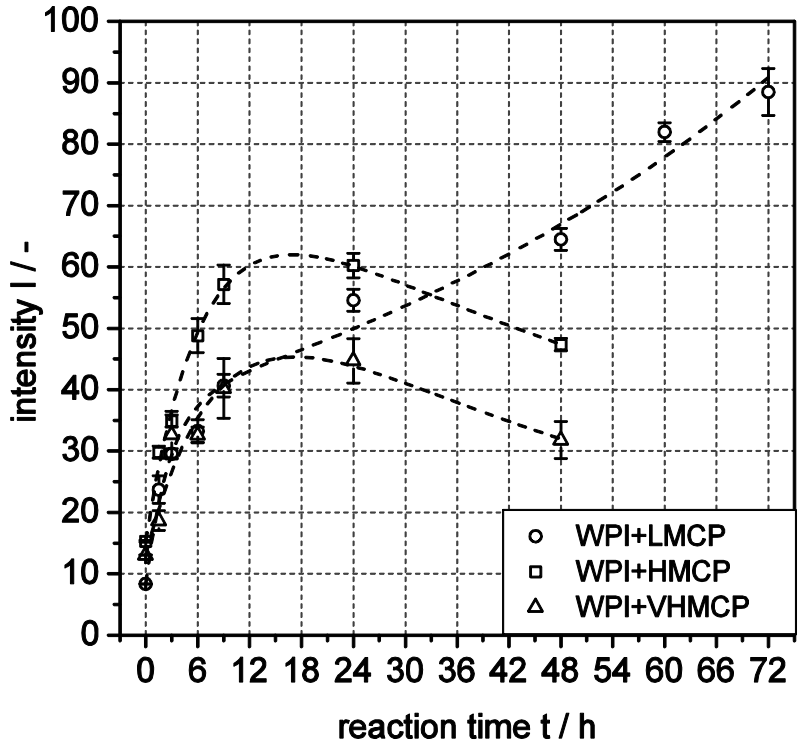

Figure 6.4: Fluorescence intensity of 0.25% w/v WPI-pectin conjugate solutions as a function of heating time. Intensities recorded at λ_{exc} = 368 nm and λ_{em} = 488 nm are reported.

These observations are very interesting as they contrast with findings reported in literature [127, 140]: Higher saccharide charge is supposed to have a negative impact on the amount of glycation and on the glycation speed. However, the same researchers also report a positive impact of lower molecular weight on the extent of glycation. When comparing the molecular weight of the citrus pectins used in the present study, it can be seen that the molecular weight of the LMCP is significantly lower than that of HMCP and VHMCP. Apparently, the positive influence of lower molecular weight predominates over the negative influence of higher negative charge on glycation for the investigated protein-pectin combinations. The different fluorescence intensity

maxima of WPI+HMCP and WPI+VHMCP cannot be explained by molecular weight according to Table 6.1.

6.3.3 Zeta potential measurements

The lower the DE the more carboxy groups are present on the pectin molecule. In aqueous solution of pH 5.5, these carboxy groups dissociate providing pectin with a negative charge. The measurable zeta potential, i.e. the amount of charge, will thus depend on the DE at a given pH. Pectins with a lower DE will exhibit a more negative ZP than pectins with a high DE. WPI also exhibits a slightly negative net charge at pH 5.5 because the IEP of the protein is only a little bit lower. Therefore, mixtures of both WPI and pectin will also show a negative ZP in solution at this pH. Conjugation will occur at the positively charged N-termini of the protein fraction resulting in a diminishment of positive charges. We therefore expect an overall reduction of ZP of WPI-pectin mixtures when conjugation takes place. Table 6.2 shows the ZP of conjugate solutions at pH 5.5. Statistically significant differences are indicated by different letters.

Table 6.2: Zeta potentials of conjugate solutions at pH 5.5 after various reaction times. Different letters indicate statistically significant differences in zeta potentials in each column.

	Zeta potential / mV		
Reaction time / h	**WPI + LMCP**	**WPI + HMCP**	**WPI + VHMCP**
0	-49.9 ± 0.8 ^{a}	-31.6 ± 0.7 ^{a}	-21.9 ± 0.2 ^{a}
1.5	-50.0 ± 0.8 ^{b}	-32.7 ± 1.0 ^{a}	-21.9 ± 0.9 ^{a}
6	-49.4 ± 1.6 ^{b}	-31.9 ± 0.3 ^{a}	-21.2 ± 0.6 ^{a}
9	-	-34.8 ± 0.6 ^{b}	-
12	-	-36.5 ± 0.8 ^{b,c}	-
15	-	-38.4 ± 0.8 ^{c}	-
24	-50.3 ± 1.3 ^{b}	-36.7 ± 0.7 ^{b,c}	-27.4 ± 0.5 ^{b}
48	-53.7 ± 1.7 ^{c}	-35.2 ± 1.0 ^{b}	-26.7 ± 1.0 ^{b}
60	-56.1 ± 1.5 ^{c}	-	-
72	-54.6 ± 1.3 ^{c}	-	-

Unheated mixtures of protein and pectins exhibited different ZP values (reaction time = 0 h). These values progressed with the DE of the pectin. The WPI+LMCP mixture showed a lower ZP (≈ -50 mV) than the WPI+VHMCP mixture (≈ -22 mV). This reflects the different amount of negative charges present on pectin molecules with varying DE. Upon heat treatment, the ZP of all conjugate solutions decreased significantly. In the case of WPI+HMCP conjugates, the zeta potential showed a minimum after about 15 hours reaction time. This might indicate a degradation of conjugates after longer reaction times. At the same reaction time, the maximum in fluorescence intensity was observed (Fig. 6.4). Since WPI+HMCP and WPI+VHMCP both showed maxima in fluorescence intensity measurements we would also expect a comparable maximum

in ZP for both conjugate types. However, there are not enough data points in the intermediate time scale to show the same effect in ZP development for WPI+VHMCP conjugates. All three conjugate types exhibited a decrease in ZP by approximately six to eight units which corresponds to a change of about 25% for WPI+VHMCP but only 10% for WPI+LMCP. This is in line with the number of charged groups in solution: VHMCP only provides a limited number of negatively charged carboxy groups so that the initial zeta potential is higher. A small reduction in the number of positively charged groups provided by WPI then leads to a stronger effect in the overall measured charge. LMCP with a large number of negatively charged groups acts almost like a buffer itself. The initial zeta potential of the mixture is already low and does not reduce much further upon progressing conjugation.

6.3.4 Emulsifying performance at pH 7

In order to test the emulsifying capacity of protein-pectin conjugates, emulsions were prepared using high pressure homogenization. At pH 7, whey protein and pectin molecules exhibit strong negative net charge so that complex formation between the two molecule types can be ruled out. Furthermore, whey protein shows excellent emulsifying properties at this pH. Any further reduction in emulsion droplet size can thus be linked to the outstanding emulsifying performance of conjugates. Figure 6.5 shows the Sauter mean diameter $d_{3,2}$ of emulsions prepared with WPI-pectin conjugates at pH 7. For comparison, the $d_{3,2}$ of emulsions prepared with only WPI at the same pH is given.

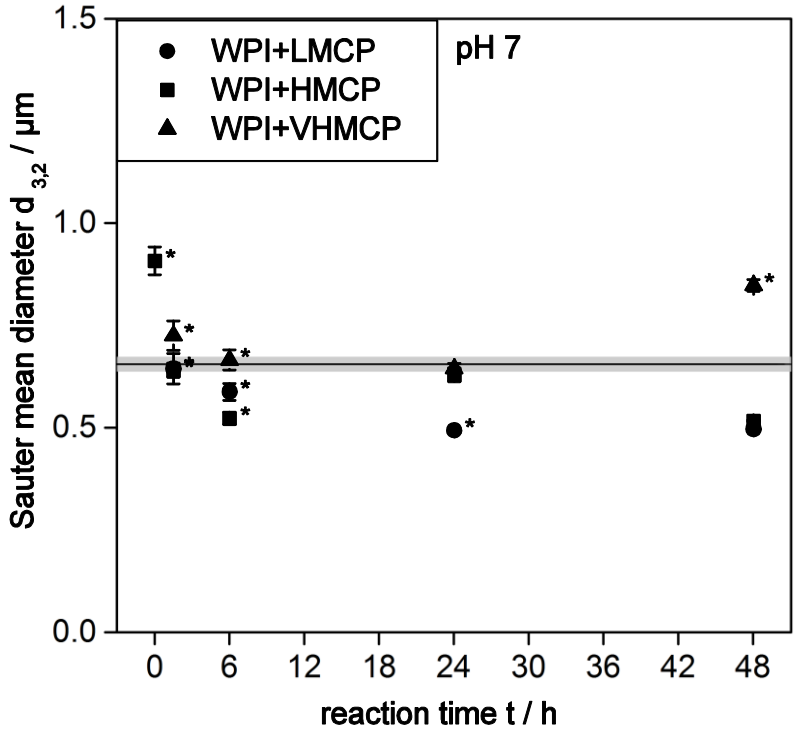

Figure 6.5: Dependence of the characteristic droplet size $d_{3,2}$ of emulsions produced at pH 7 on the reaction time of the corresponding protein-pectin mixture. The solid line and grey band mark the $d_{3,2}$ and standard deviation of a pure WPI emulsion produced at the same pH. Asterisks indicate significantly different droplet sizes.

These emulsions possessed very small mean droplet sizes of around 0.7 µm. Compared to that, emulsions prepared from conjugates showed statistically smaller $d_{3,2}$ after 6 or more hours of reaction time. This corresponds to the reaction time at which large molecular weight fractions started to be visible by SDS-PAGE. For WPI-LMCP and WPI-HMCP emulsions, a continuous decrease in $d_{3,2}$ with conjugate reaction time was observed. The smallest $d_{3,2}$ was around 0.5 µm after 48 h reaction time. In contrast, WPI-VHMCP conjugate emulsions showed a significant increase in $d_{3,2}$ at 48 h of reaction time resulting in a droplet size minimum at intermediate reaction times. Thus, the development of $d_{3,2}$ resembles that of the fluorescence measurements for WPI+VHMCP conjugates. Again, a possible explanation for this observation is the degradation of formed conjugates due to continuous heat treatment. Such minimum in droplet size was also reported by Xu et al. (2012) for WPI-sugar beet pectin conjugates, however at shorter reaction times (7 hours). The reason for this shift could be the molecular weight of the employed pectin. Sugar beet pectin is a much smaller molecule that could result in faster reaction of protein and pectin [127, 141].

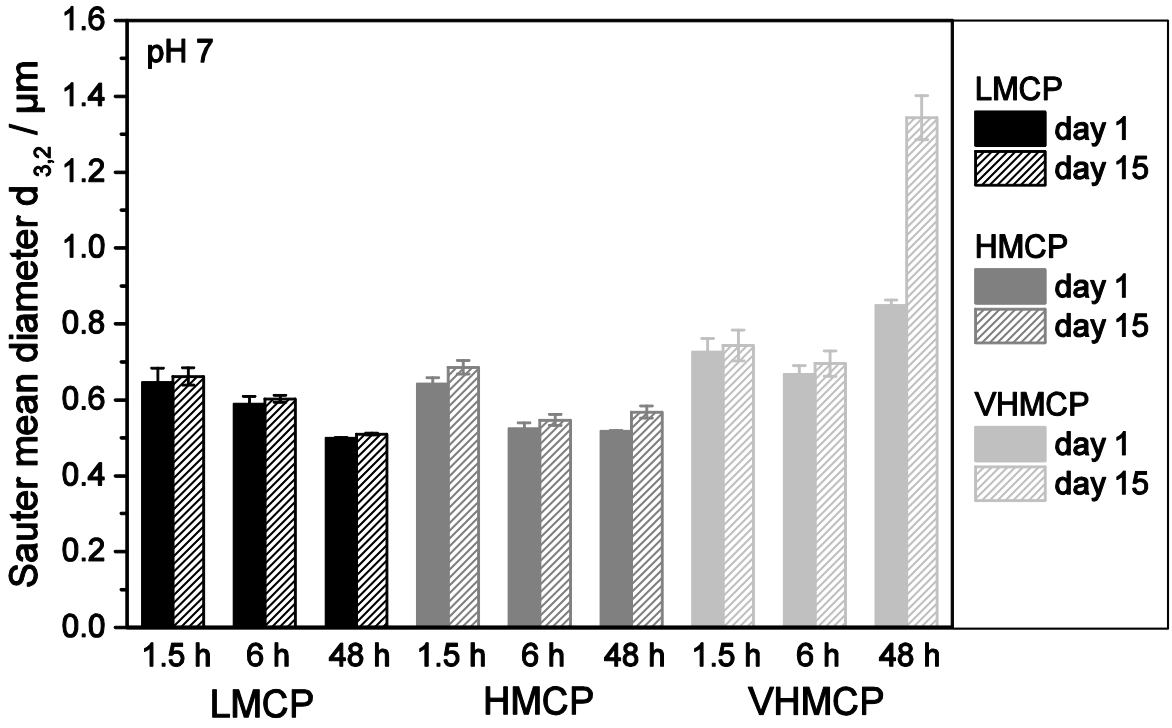

Figure 6.6: Long term stability of conjugate emulsions at pH 7. Several representative conjugate reaction times were chosen for each protein-pectin combination. The Sauter mean diameter $d_{3,2}$ at day 1 and after two weeks of storage is compared.

For the evaluation of the long term stability of emulsions, the droplet size was measured again after two weeks. Figure 6.6 shows a comparison between the Sauter mean diameter at day 1 and after two weeks of chosen emulsions. It can be seen that for most of the emulsions the Sauter mean diameter increases only very little over time. For WPI+LMCP conjugate emulsions, the droplet size even remains stable over two weeks. This can be an indication of a very good long term stability. Emulsions

produced from WPI+VHMCP (48 h reaction time), however, are an exception. Here, a strong increase in the $d_{3,2}$ over time can be observed. Already at day 1, this type of conjugates (WPI+VHMCP) had stabilized larger droplet sizes after longer reaction times. This means that not only the short term but also long term stability is worse for these conjugates. Therefore, the development of $d_{3,2}$ over two weeks is a further indication of the negative impact of long reaction times on WPI+VHMCP conjugate functionality.

6.3.5 Emulsifier performance at pH 5.5

At pH 5.5, WPI is close to its IEP. Therefore, the net charge of the protein molecules is close to zero leading to aggregation which is unfavorable for emulsion stabilization. Thus any unreacted protein will not contribute to droplet stabilization at this pH. As a reference, the Sauter mean diameter of pure WPI emulsions is given.

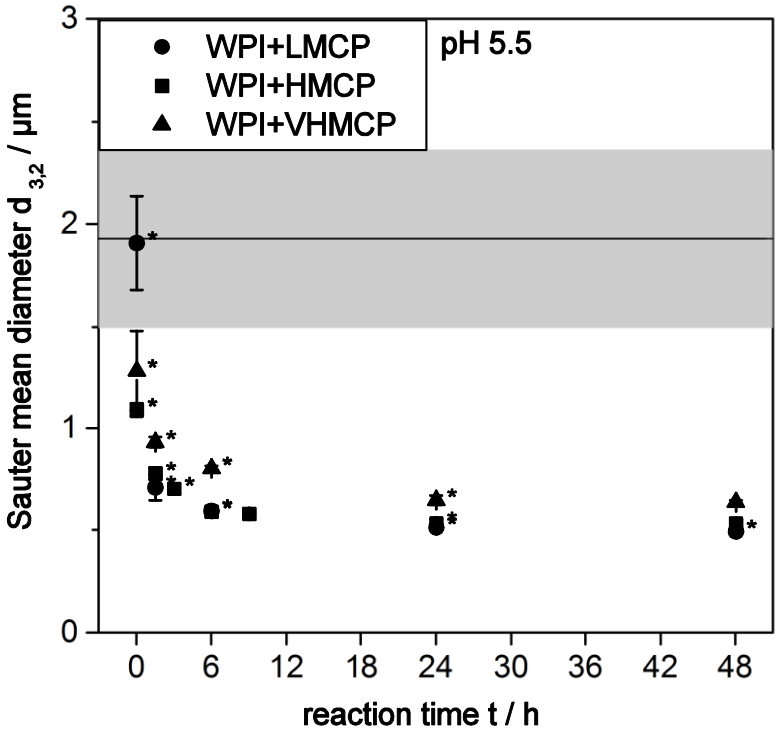

Figure 6.7: Dependence of the characteristic droplet size $d_{3,2}$ of emulsions produced at pH 5.5 on the reaction time of the corresponding protein-pectin mixture. The solid line and grey band mark the $d_{3,2}$ and standard deviation of a pure WPI emulsion produced at the same pH. Asterisks indicate significantly different droplet sizes.

The bad emulsifying performance of WPI at pH 5.5 was reflected in the large $d_{3,2}$ and standard deviation (Fig. 6.7, grey bar). Except for WPI+LMCP, untreated mixtures of protein and pectin already stabilized emulsions with a smaller $d_{3,2}$ than WPI alone. According to Neirynck et al. [132], this might have been due to electrostatic interactions between the negatively charged pectin and residual positive patches of WPI even close to the IEP. This is supposed to promote an electrostatic stabilization of oil droplets. An explanation for the unchanged droplet size in case of untreated

WPI+LMCP mixtures might be the much more negative charge of LMCP. The LMCP studied here has a much lower DE than the other two pectins and than the sugar beet pectin studied by Neirynck et al. [132]. This might have led to predominantly repulsive interactions between protein and pectin (see also Table 6.2) so that LMCP did not participate in droplet stabilization under these conditions. Emulsions prepared with any of the protein-pectin conjugates showed a significantly and strongly reduced characteristic droplet size compared to pure WPI emulsions. A gradual decrease in $d_{3,2}$ upon prolonged reaction time was observed. However, unlike at pH 7, no clear minimum was detected. Instead the droplet size seemed to level off at about 0.5 µm which corresponds to the smallest droplet size observed at pH7. The long term stability of emulsions (Fig. 6.8) correlates with these observations. All emulsion show no or only a small increase in $d_{3,2}$ over two weeks. The least change in droplet size is seen for conjugates after longer reaction times (48 h). There is no difference in stabilization behavior between any of the conjugate types.

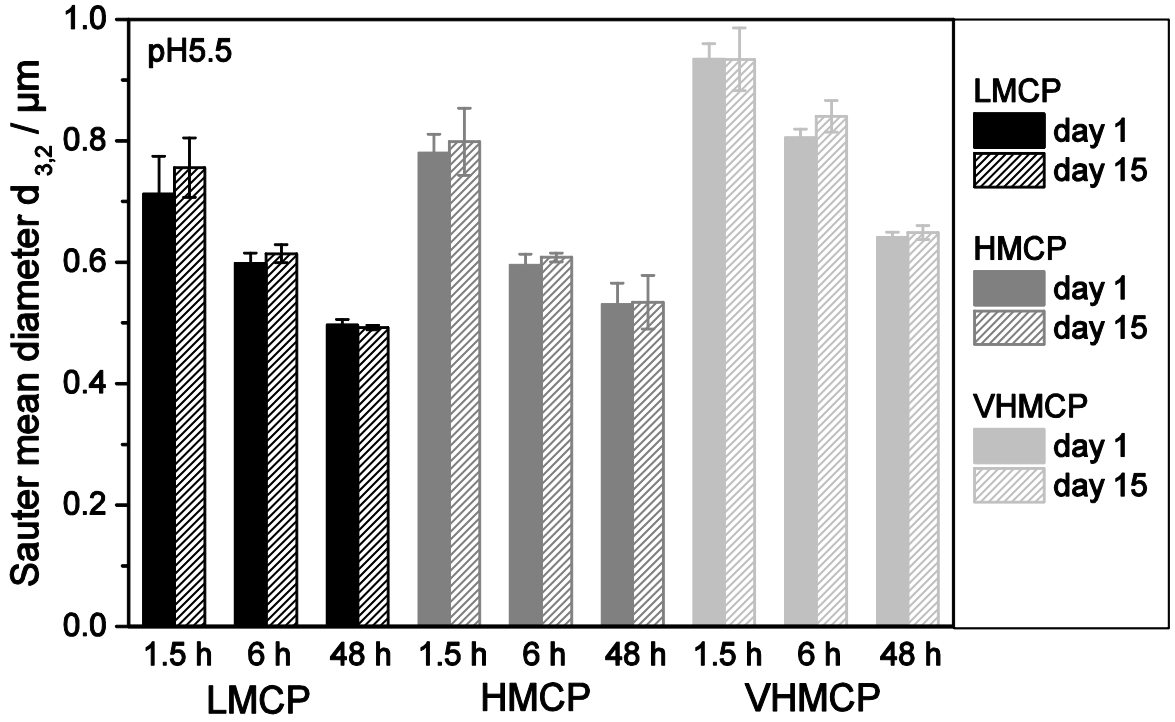

Figure 6.8: Long term stability of conjugate emulsions at pH 5.5. Several representative conjugate reaction times were chosen for each protein-pectin combination. The Sauter mean diameter $d_{3,2}$ at day 1 and after two weeks of storage is compared.

6.3.6 Influence of DE on the Sauter mean diameter of conjugate emulsions

In order to link this effect on droplet size reduction to the formation of conjugates, the $d_{3,2}$ of all emulsions is plotted versus the maximum fluorescence intensity of the corresponding conjugate solutions (Fig. 6.9). A strong decrease in the $d_{3,2}$ with increasing fluorescence intensity was observed. Differences between individual pectin types were not identified. This indicates that the mechanism of droplet size reduction

must be the same for all pectins and cannot depend on the DE of the pectin. If this had been the case, three distinct slopes – one for each DE – should have been seen.

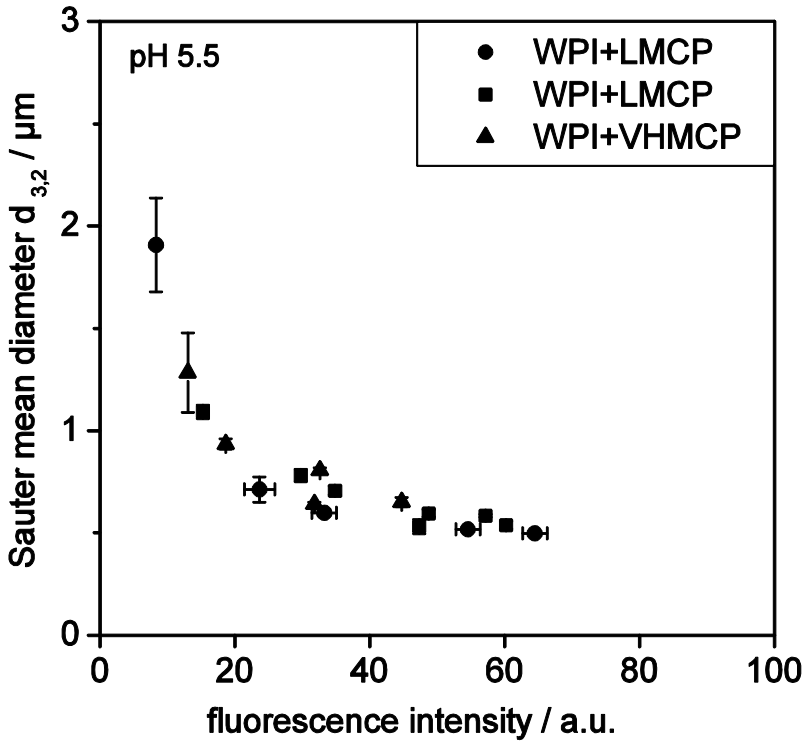

Figure 6.9: Dependence of the characteristic droplet size $d_{3,2}$ of emulsions produced at pH 5.5 on the fluorescence intensity of the corresponding conjugate solutions.

We therefore suggest that the main stabilizing mechanism is sterical stabilization resulting from the large covalently bound pectin molecules protruding into the aqueous phase. Nevertheless, electrostatic effects cannot be completely ruled out. It is accepted that a zeta potential of less than about -30 mV would lead to sufficient repulsive forces for droplet stabilization. The zeta potential measurements in Table 6.2 show that, except for WPI+VHMCP conjugates, this value was reached. To truly rule out electrostatic effects, conjugates that are composed of WPI and a pectin type with an even higher DE (> 90%) should be investigated to reduce the charge on the molecule even further. Alternatively, experiments at higher ionic strength might give an indication about the extent of electrostatic interaction. This aspect is part of ongoing research and will be covered in a further article.

Although the results show that the DE of pectin does not play a significant role in the droplet stabilizing mechanism, it does affect the obtainable droplet size indirectly. This will be explained in the following: Figure 6.4 showed distinctly different fluorescence intensity developments depending on the type of pectin. Figure 6.9 illustrated that the obtainable emulsion $d_{3,2}$ depends strongly upon the fluorescence intensity. We therefore suggest a.) that the resulting emulsion $d_{3,2}$ depends upon the conjugate yield (as reflected by fluorescence intensity) and b.) that the conjugate yield depends upon the type of the pectin (as reflected by different fluorescence intensities). This means

that at higher fluorescence intensity more conjugate is in solution which makes it possible to stabilize smaller droplets. The leveling off in droplet size (Fig. 6.9) indicates that enough active emulsifier is present in the solution to quickly stabilize the newly formed interface. The $d_{3,2}$ is now dominated by the process conditions and not determined by emulsifier concentration anymore. In order to investigate the influence of process conditions on conjugate emulsions further experiments are being conducted. Nevertheless, the following implications can already be seen now: Firstly, a low DE is advisable for WPI-pectin conjugate preparation as more emulsifying reaction products develop. The results indicate that also the molecular weight of pectin has an influence on conjugate yield that cannot be neglected. However, it needs to be kept in mind that in industrial processes of pectin preparation chemical hydrolysis is used to reduce the DE. This simultaneously causes depolymerization of the pectin molecule. A lower DE and a lower molecular weight go hand in hand for commercially available pectin. Secondly, the importance of equal yield is emphasized when assessing the emulsifying performance of protein-pectin conjugates. Different amounts of emulsifying active conjugate at the same reaction time could be the reason for sometimes contradicting results like the ones previously reported by Einhorn-Stoll et al. [73]. Thirdly, fluorescence spectroscopy is a suitable technique to predict the emulsifying performance of conjugate preparations reducing the need for tedious emulsification experiments. This could allow for a more rapid screening of conjugate preparations for emulsifying purposes.

6.4 Conclusion

In this study WPI-pectin conjugates with different DE were prepared. An improved emulsifying performance of the conjugates could be shown. Steric stabilization as the main droplet stabilizing mechanism is suggested. This is independent of the DE as all investigated pectins possessed a molecular weight that is large enough to provide sufficient steric stabilization. Furthermore, we could link the Sauter mean diameter $d_{3,2}$ of conjugate emulsions to the fluorescence intensity of conjugate solutions. Fluorescence intensity of the conjugate solutions seems to be a good measure of the conjugate yield and the emulsion $d_{3,2}$ depends on this yield, i.e. on the active emulsifier in solution. While the DE of pectin does not seem to influence the performance of the resulting conjugates, it does influence the conjugate yield. Higher yields were obtained for low methylesterified citrus pectin (LMCP). In contrast, high methylesterified citrus pectins showed reduced yields at longer reaction times. It now seems appropriate to focus research activities on finding improved reaction conditions and developing novel processes to increase the WPI-pectin yield.

6.5 Acknowlegdements

The authors would like to thank the Institute of Thermal Process Engineering and the Section of Biomolecular Separation Engineering for giving us the opportunity to conduct fluorescence spectroscopy and zeta potential measurements at their labs.

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7 Effect of Molecular Weight Reduction, Acetylation and Esterification on the Emulsification Properties of Citrus Pectin

Citrus pectin was chemically and thermally modified in order to increase the hydrophobic character of the molecule and its adsorptivity to the oil–water interface. The degree of acetylation and methylesterification was increased and the molecular weight was reduced. The emulsion formation and stabilization properties of these modified pectins were evaluated by surface and interfacial tension measurements and emulsification experiments. For the production of emulsions, a high pressure homogenizer was used. The viscosity ratio between oil and emulsion phase was adjusted by varying the amount of added sucrose. Pectins with a higher degree of methylesterification (DE) decrease the interfacial tension significantly compared to the unmodified pectin. Pectins with increased degree of acetylation (DAc), however, show higher interfacial tension values. In emulsification experiments, pectins with a reduced molecular weight do neither significantly reduce droplet sizes nor improve emulsion stability. Pectins with increased DE or DAc reduce the Sauter mean diameter $d_{3,2}$ of emulsions significantly and, in case of an DE increase, also show excellent long term stability. Their performance is also superior to sugar beet pectin under comparable experimental conditions.

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7.1 Introduction

Emulsions are of great interest to the food industry. Many popular food products (e.g. yoghurts, salty spreads, sauces) are emulsion based as this microstructure allows for improved digestibility, spreadability, mouthfeel and a pleasant texture. Moreover, emulsions offer the opportunity to encapsulate bioactives, making them a suitable delivery system in functional foods [142, 143]. This property is also used for cosmetic, pharmaceutical or agro-chemical formulations. One of the main concerns when producing emulsions is the stability of the final product. The physical stability of an emulsion describes the changes in droplet size distribution over a given time and in a determined space. Short and long term stability can be distinguished: Short term stability refers to a time scale of milliseconds to a few minutes and describes the ability of droplets to resist re-coalescence in an emulsification process. Improved short term stability results in smaller droplets for a fixed recipe [17]. Long term stability usually describes the shelf-life of a product [18]. For short term stabilization of emulsions, a surface-active "emulsifier" is needed. This amphiphilic substance adsorbs at the oil–water interface that is newly created during the emulsification process. It prevents droplets that were broken up from re-coalescing. Emulsifiers reduce the interfacial tension and therefore provide an energetic stabilization by reducing the free energy of the system. In addition, they create a steric barrier and – if being electrically charged – provide repulsive electrostatic interparticular forces [18]. "Stabilizers" or "thickeners" are often added in order to particularly increase the long term stability of emulsions. These are non-surface active, polymeric substances or hydrocolloids that increase the emulsion's viscosity. By this, droplet movement is reduced so that creaming (or sedimentation) is suppressed but also coalescence is decreased as less droplet collisions take place [16]. However, stabilizers can also have an immediate effect on droplet breakup during the emulsification process due to their viscosity enhancing properties. For the achievement of small droplets, stresses need to be transmitted onto the initial droplets by the surrounding medium. The quality of this transmission is dependent on the viscosity of the continuous phase and hence on the viscosity ratio λ between dispersed oil phase and continuous aqueous phase [18]. It was found that in laminar shear flow a single droplet is best broken up at a viscosity ratio between 0.1 and 1 [20, 144]. In concentrated emulsions, however, the phase that transmits stresses is better described by the emulsion itself. Armbruster [21] and later Jansen, Agterof and Mellema [22] proposed to use the ratio between the dispersed phase (oil) viscosity and the emulsion viscosity for such emulsions.

Due to growing consumer demand for all natural food ingredients, plant-based hydrocolloids are often chosen as stabilizers. Some of these hydrocolloids also show a certain degree of surface activity [60]. Therefore, it is an interesting concept to explore the combined effect of hydrocolloids as "emulsifier" and "stabilizer". Pectin is a hydrocolloid widely used in food industry for its gelling ability in order to stabilize and texturize food products. However, pectins can also exhibit surface active behavior [63].

The polysaccharide pectin is a heteropolymer whose backbone consists mainly of galacturonic acid monomers. These monomers can possess different functional groups

influencing the overall performance of the pectin molecules (Fig. 7.1). In citrus pectins, about 70% of the carboxyl groups of the galacturonic acid monomers are naturally methylesterified [6]. Methyl ester groups can influence the surface activity of pectin molecules as they are more hydrophobic than carboxyl groups [63]. The degree of acetylation (DAc) describes to what extent the hydroxyl groups of the galacturonic acid monomers are acetylated. The DAc of commercially available pectins can vary from below 10% (citrus and apple pectin) to up to over 25% (sugar beet pectin). The surface active behavior of sugar beet pectin has been attributed to the presence of many acetyl groups [6, 62]. Pectins may also vary in their molecular weight. Citrus pectins are of higher molecular weight than sugar beet pectin. It could be shown that the depolymerization of citrus pectins can increase their emulsifying capacity [98].

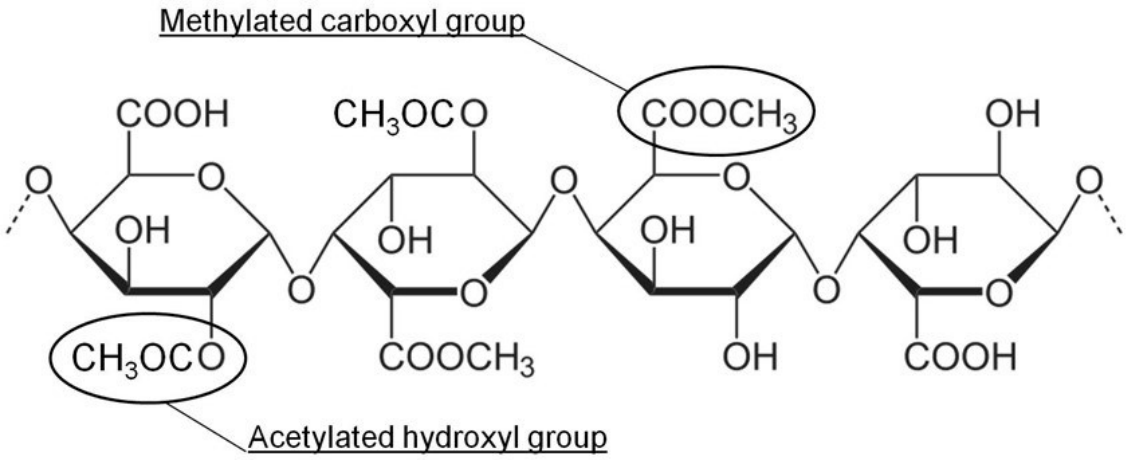

Figure 7.1: Simplified scheme of a pectin molecule with possible attached methyl and acetyl groups.

A few studies consider the influence of the molecular characteristics of pectin on its emulsifying capacity. A generally positive effect of acetyl groups on the emulsifying capacity of sugar beet pectin was shown by Dea and Madden [4]. Leroux et al. [92] only investigated depolymerization of citrus pectin because preliminary studies had apparently not shown a significant influence of the variation of DE. During the study of depolymerized pectins, Akhtar, Dickinson, Mazoyer, and Langendorff [98] realized that the viscosity enhancing effect of pectins might contribute significantly to the observed emulsion stability. However, this is not further clarified.

We therefore asked ourselves to what extent the molecular features of pectin still contribute to the emulsion forming properties when the emulsion stabilizing properties of the hydrocolloid are accounted for at the same time. In order to answer this question we chose to chemically modify pectin in order to vary its hydrophobicity. The DE and DAc of citrus pectin were changed as well as the molecular weight. To the best of our knowledge, an increase in DE for the purpose of hydrophobicity enhancement has not been investigated yet. We exclude the effect of viscosity on emulsion formation and stabilization by adjusting the viscosity ratio of the emulsions using sucrose. By this, it should possible to draw conclusions on which chemical modification is most promising for the creation of “multipurpose” pectins.

7.2 Materials and Methods

7.2.1 Materials

Highly methylesterified citrus pectin and sugar beet pectin was supplied by Herbstreith & Fox KG, Neuenbuerg/Germany. Pure rape seed oil (later on referred to as "oil") was purchased from FLOREAL Haagen GmbH, Saarbrücken, Germany. Sucrose was purchased from BÄKO Marken und Service eG, Bonn, Germany. Ethanol was obtained from Carl Roth GmbH & Co. KG, Karlsruhe, Germany. Hydrochloric acid, nitric acid, acetic anhydride and sodium hydroxide all were of analytical grade and were purchased from Merck KGaA (Darmstadt, Germany). Methanol (purity >99.85 %) was obtained from Solvadis commodity chemicals GmbH (Frankfurt, Germany).

7.2.2 Thermal Reduction of Molecular Weight

In order to produce samples with different molecular weights, pectins were thermally treated in a lab scale autoclave. Depolymerization was accomplished at the natural pH of the pectin (slightly above 3). In contrast to previous experiments described [145], higher temperatures were applied over a shorter period of time in order to reduce the necessary reaction time. 1% w/w solutions of pectin were prepared by dispersing the weighed amount of pectin powder in 60 °C warm water using an Ultra-Turrax® T-25 digital (IKA® Werke GmbH & Co. KG, Staufen, Germany). The solution was filled into a pressure-resistant flask and placed into the autoclave that had been filled with water. The autoclave was then heated to 140 °C at approx. 3.7 bar. The time during which the final temperature was kept varied in order to achieve different degrees of degradation. After the heat treatment, the autoclave was cooled down and the flask was removed. The pectin solution was mixed with three times the volume of pure ethanol in order to precipitate the pectin molecules. After 30 min, the dispersion was filtered using a nylon cloth and washed twice with pure ethanol. The alcohol insoluble substance was then dried at 60 °C for an hour and milled.

7.2.3 Methylesterification of Pectin

In order to increase the degree of esterification, a method described by Jansen and Jang [146] was chosen. Methanol is esterified with the free carboxyl groups of the galacturonic acid chain using nitric acid as a catalyst. Depending on the reaction time more and more carboxyl groups become esterified. Pectin powder was dispersed in eight times the weight amount of methanol. Then, 3.75% w/w nitric acid (65%) was added and the dispersion was stirred at room temperature using a magnetic stirrer. When the chosen time for reaction had passed, the dispersion was filtered and washed twice with pure ethanol. Finally, the alcohol insoluble substance was dried again at 60 °C for an hour and milled.

7.2.4 Acetylation with Acetic Anhydride

For increasing the DAc of pectins, the method of Babic et al. [147] developed for acetylation of tapioca starch has been adapted. Like methylesterification, acetylation of pectin is also an esterification. The reaction takes place between the hydroxyl groups

of the galacturonic acid backbone and acetic acid. A 2% w/w pectin solution was prepared at 60 °C using an Ultra-Turrax®. The solution was cooled down to 5 °C and the pH was adjusted to 8 using 10% w/w NaOH solution. The desired amount of acetic anhydride was added to the pectin solution drop by drop while ensuring that the pH remains at 8 (using NaOH solution) and the temperature remains at 5 °C (using an ice water bath). After 10 min, the reaction was stopped by reducing the pH to 3 by adding 2 M hydrochloric acid. Then, pectin was precipitated by adding three times the volume amount of pure ethanol. After 30 min, the dispersion was filtered using a nylon cloth and washed three times with pure ethanol. Finally, the alcohol insoluble substance was dried at 60 °C for an hour and milled.

7.2.5 Characterization of Pectin Samples

The characterization of pectins was accomplished on deashed samples. The molecular weight of pectin samples was determined by intrinsic viscometry using the Mark-Houwink relationship (Equation 7.1) [148, 149]:

$$[\eta] = kM^{\alpha} \tag{7.1}$$

The values of the constants $\alpha = 1.34$ and $k = 0.00014$ ml/g were taken from [150]. The degree of esterification was determined titrimetrically and corrected by the content of acetic acid [151]. The degree of acetylation is quantified by determining the amount of acetic acid in the pectin sample. For this purpose, the acetic groups bound to the galacturonic acid are hydrolyzed using a sodium hydroxide solution. This sets acetic acid free which is determined enzymatically using a UV-test kit by R-Biopharm AG (Darmstadt, Germany). The protein content was determined according to Bradford [152].

7.2.6 Measurement of Static Surface and Interfacial Tension

Surface and interfacial tension measurements were accomplished with a Dataphysics DCAT 11 tensiometer based on the Wilhelmy plate method. Measurements were conducted at 42.5 ± 0.5 °C. For surface tension measurements, 1% w/w pectin solutions were prepared. Water was heated to 60 °C and pectin powder was dispersed using an Ultraturrax T-25 digital at 10,000 rpm for 1 min. The solution was cooled down to room temperature and the pH was adjusted to 3 using hydrochloric acid. The solutions were heated again and tempered to 42.5 °C for 30 min and then transferred into the measuring cup which was tempered to the same temperature. For interfacial tension measurements, the pectin solution was covered with a layer of tempered vegetable oil. After the measuring plate was inserted into the surface/interface, detection of tension was started. Measurements were repeated three times.

7.2.7 Determination of Viscosity Ratio

Viscosities of pectin solutions and emulsions were measured with an Anton Paar Rheometer MCR 301 equipped with Couette geometry CC-27 at a temperature of 42.5 ± 0.07 °C. Rotational measurements were conducted by applying a logarithmic shear rate profile starting at 0.1 s^{-1} and rising up to 120 s^{-1}.

For the adjustment of the viscosity ratio, the emulsion viscosity was taken into account. Emulsions containing a certain amount of sucrose were prepared. The viscosity of the emulsion was measured and the viscosity ratio calculated according to following equation (Equation 7.2):

$$\lambda = \frac{\eta_{oil}}{\eta_{em}} \tag{7.2}$$

with η_{oil} as the viscosity of dispersed phase (oil) and η_{em} as the viscosity of the prepared emulsion [21]. If the viscosity ratio differed by more than 0.06 from the chosen value of 0.15 the amount of sucrose was changed and a new emulsion was produced and evaluated. More sucrose would increase viscosity and consequently reduce the viscosity ratio.

7.2.8 Emulsion Preparation

The prepared emulsions consisted of 30% w/w vegetable oil as dispersed phase and a continuous phase made up of pectin and sucrose dissolved in demineralized water. The concentration of pectin was always 1% w/w. For the preparation of the continuous phase, demineralized water was heated to 60 °C and pectin and sucrose were dissolved using an Ultraturrax T-25 digital at 10,000 rpm for 1 min. The solution was cooled down to room temperature and the pH was adjusted to 3 using hydrochloric acid. Then, oil was added and dispersed again using an Ultraturrax T-25 digital at 10,000 rpm for 1 min. The obtained pre-emulsion was transferred to a high pressure homogenizer (Microfluidizer® MF 110 Y, Microfluidics Corporation, Newton, MA, USA). The emulsion was first emulsified at 400 bar, collected and then passed through the device a second time at 800 bar.

7.2.9 Measurement of Droplet Size Distribution

Droplet size distributions were obtained by using a laser diffraction particle size analyzer (LS 13 320, Beckman Coulter, Inc., Miami, FL, USA) equipped with polar intensity differential scattering (PIDS) technology. Obscuration was around 45%. The optical model used also covered the Mie region. A refractive index of 1.333 was used for the water phase and 1.47 was used for the oil phase. The imaginary part in both cases was set to zero as both phases are transparent. Samples were always measured in triplicate. Droplet size distributions were then calculated according to the Mie theory.

7.2.10 Statistical Analysis

All emulsification experiments and measurements were done at least in triplicate. Statistical analysis was done using the data analysis software OriginPro 8.6G (OriginLab Corporation, Northampton, USA). One-way analysis of variance and Scheffé's mean separation test were performed to determine differences between samples. Significant differences are reported in terms of P-values.

7.3 Results and Discussion

7.3.1 Pectin Modification

The influence of functional groups on the emulsifying properties of citrus pectin was investigated at constant emulsion viscosity ratio. It was suspected that a higher DE and DAc of pectin would lead to a higher hydrophobicity of the molecule and thus an improved emulsifying behavior. A lower MW should increase the adsorption kinetics of the molecule and make it a more suitable substance for emulsion formation in high pressure processes. Citrus pectin was chemically modified so as to either increase the DE or DAc or to decrease the molecular weight. Only one functional parameter was supposed to be changed while the others should remain constant. In order to vary the molecular weight, pectin solutions were thermally treated. It can be noted that the longer pectin solutions were exposed to high temperatures the lower was the molecular weight obtained (Table 7.1). Therefore, depolymerization was proportional to reaction time which is in accordance with literature [145]. Heat treatment also caused a reduction in protein content from 1.9% (original pectin) to 1.4% (pectin with lowest molecular weight). The DE and DAc remain constant throughout the depolymerization process (72 ± 1 and 2.5%, respectively).

While deesterification of pectin is a common industrial procedure, an increase of DE for the purpose of enhanced hydrophobicity has not been reported yet. Jansen and Jang [146] described that the methylesterification of galacturonic acid is mostly influenced by the reaction temperature and pH and that a maximum DE of 90% can be achieved. Our results (maximum DE = 88%) support these findings. During esterification treatment, the DAc of pectins was not influenced but remained constant at 2.5%. The variation in protein content was within the margin of error of the characterization method. However, the molecular weight slightly reduced from 84 to 67 kDa with an increase in DE from 71 to 88%. This could be due to depolymerization taking place during the esterification treatment. However, increased coiling at higher DE also implies that the Mark-Houwink parameters used for calculating the molecular mass would need to be adjusted [153]. Then, it would be possible that the detected reduction in molecular weight is merely an artifact but further experiments are necessary to affirm this.

In order to increase the DAc of pectins, a method by Babic et al. [147] originally applied to tapioca starch was used. By this, it was possible to achieve DAc values of up to 14%. The molecular weight remained constant throughout the treatment (82.2 ± 2 kDa). However, a reduction of DE could be observed particularly at high DAc where the DE is by six percentage points lower compared to the original pectin. Furthermore, the harsh reaction conditions during acetylation led to a noticeable reduction of protein content from 1.9% (original pectin) to 0.6%.

Table 7.1: Characteristics of modified pectins. The experimental parameter varied during modification is shown together with resulting pectin features (ND = not determined).

Type of Modification	Molecular weight [kDa]	DE [%]	DAc [%]	Protein content [%]
Original pectin	84.3	71.1	2.5	1.9
Sugar beet pectin	46.4	57.8	24.6	4.7
MW reduction				
	75.5	70.9	2.5	1.7
	72.7	71.6	2.5	ND
	67.1	71.3	2.5	1.6
	60.8	71.0	2.5	1.6
	50.4	71.6	2.5	1.5
	47.1	72.7	2.5	1.4
	43.0	72.0	2.5	1.4
	38.5	70.9	2.5	ND
Increase in DE				
	80.6	75.2	2.5	2.0
	76.1	80.6	2.5	ND
	79.8	81.0	2.5	1.9
	73.8	83.0	2.5	1.9
	79.4	84.7	2.5	ND
	74.1	86.0	2.5	2.1
	72.2	86.8	2.5	ND
	74.6	87.4	2.5	ND
	67.9	88.2	2.5	2.0
Increase in DAc				
	82.2	70.6	4.9	0.8
	83.1	69.6	9.1	ND
	80.5	69.0	9.5	0.6
	84.6	69.2	10.5	ND
	78.6	66.5	13.5	ND
	80.2	65.3	14.0	0.6

7.3.2 Surface Activity of Modified Pectins

Surface (pectin solution/air) and interfacial tensions (pectin solution/oil) of 1% pectin solutions are summarized in Table 7.2. The measurement temperature was elevated to simulate actual emulsification process conditions. The surface/interfacial tension of demineralized water is given as a reference. Measurements showed that the system needed more than 20 h to reach equilibrium. However, the strongest decrease in tension appeared during the first few minutes. Therefore, the value detected at 10 min of measuring time is used in the following discussions. It can be seen that all pectins showed a significantly lower surface/interfacial tension than pure water. This has already been described by several authors [89, 95, 103]. Mostly, interfacial activity is attributed to the protein moiety of the pectin molecule. However, Lutz, Aserin, Wicker

and Garti [89] showed that also ester groups contribute significantly to pectin's surface active behavior. Compared to values found in literature [89, 95, 103], the here measured surface and interfacial tensions were much lower. However, this might be due to the higher measuring temperature employed in the present study.

Table 7.2: Surface (SFT) and interfacial (IFT) tension of several modifiied citrus pectins at T = 42.5 °C. Demineralized water and the original, unmodified citrus pectin are given as a reference.

Sample	SFT [mN/m]	IFT [mN/m]
Demineralized water	69.2 ± 0.3 [a]	19.8 ± 0.1 [a]
Original pectin (MW = 81 kDa; DE = 71%; DAc = 2.5%)	50.8 ± 0.7 [b]	15.4 ± 0.2 [b]
Molecular weight reduction		
MW = 73 kDa	48.6 ± 1.1 [b, c]	14.6 ± 0.2 [c]
MW = 60 kDa	47.5 ± 1.4 [c]	13.3 ± 0.2 [c]
MW = 50 kDa	46.9 ± 1.3 [c]	13.4 ± 0.6 [c]
MW = 43 kDa	46.0 ± 0.6 [c]	13.7 ± 0.3 [c]
MW = 39 kDa	48.0 ± 0.6 [b]	13.9 ± 0.1 [c]
Increase in DE		
DE = 80.6%	43.9 ± 0.3 [c]	12.3 ± 0.1 [d]
DE = 84.7%	46.7 ± 0.1 [c]	13.0 ± 0.3 [d]
DE = 86.6%	46.3 ± 0.4 [c]	13.0 ± 0.4 [c, d]
DE = 87.4%	46.7 ± 1.8 [c]	13.2 ± 0.1 [c, d]
Increase in DAc		
DAc = 9.0%	54.1 ± 0.7 [b, d]	17.9 ± 0.1 [e]
DAc = 10.5%	52.3 ± 0.2 [d]	17.5 ± 0.1 [e]
DAc = 13.5%	53.2 ± 0.2 [b, d]	17.8 ± 0.1 [e]

In general, surface and interfacial tension both behaved in the same manner. Pectins with a reduced molecular weight showed a slight decrease in surface and interfacial tension as compared with the original pectin. Depolymerization as a means to reduce surface tension has already been described by Qun and Ajun [154] for chitosan and Mazoyer, Leroux and Bruneau [102] for citrus pectin. It is speculated that depolymerization makes the proteinaceous moiety of the pectin molecule more accessible so that it can better adsorb to the oil/water interface [98]. Leroux, Langendorff, Schick, Vaishnav and Mazoyer [92] supposed that also kinetic effects might play a role. As known from polymers [153], solutions of molecular weight reduced pectins exhibit lower viscosities which allows for molecules to diffuse to the interface faster. Interestingly, a reduction of the molecular weight below 50 kDa did not lead to a further reduction of the surface/interfacial tension. There was no linear trend visible between depolymerization and interfacial tension as is in accordance

with literature [92]. However, the reasons for this remain unknown. Increasing the DE also led to reduced surface and interfacial tensions as compared to the original pectin (Table 7.2). Literature is contradictory about the influence of DE on interfacial or surface tension. While Berth, Anger, Plashchina, Braudo and Tolstoguzov [155] showed that there is no influence of DE on the interfacial tension of sodium alginate solutions, Baeza, Sanchez, Pilosof and Patino [156] saw an increase of surface pressure when the DE of propylenglycol alginate increased. Interestingly, the interfacial tension of pectins with an augmented DE was on average lower than that of depolymerized pectins. This indicates that such pectins might also show good emulsifying capacity. As compared to the original pectin, acetylation of samples led to an increased surface/interfacial tension. This effect is adverse to other results described in literature for acetylation of other biopolymers. It was shown [157] that surface tension can be lowered by an acetylation of starch. Wang, Liu and Chi [158] on the other hand deacetylated chitin and found that consequently surface tension increased.

In total, it is noticeable that amongst pectins of a certain modification type there were only minor differences in surface or interfacial tension. In general, pectin molecules are not very surface active. Furthermore, the accomplished modifications did not lead to a strong decrease in interfacial tension. Therefore, mostly insignificant differences within one type of modification were observed.

7.3.3 Viscosity Ratio

Pectins can act as both emulsifying and stabilizing agents. When studying their emulsifying properties, stabilizing effects due to viscosity increase can interfere and make interpretation of the results difficult. Therefore, the influence of differences in pectin solution viscosity during emulsion production was standardized by adjusting the viscosity ratio of emulsions using various amounts of sucrose. The viscosity ratio of all emulsions was adjusted to 0.15 ± 0.1. This value was chosen to ensure an improved droplet breakup while still giving emulsions that are liquid enough to be passed through the high pressure homogenizer. Vegetable oil showed Newtonian behavior and the viscosity was 23 ± 0.5 mPas. Pectin solutions and emulsions showed shear thinning behavior. Therefore, the viscosity at 100 s^{-1} was read out and used for comparison. Table 7.3 shows the different modified pectins, the amount of sugar in the continuous phase and the resulting viscosity ratio determined at 100 s^{-1}. It can be seen that particularly emulsions from molecular weight reduced pectins required a high amount of sucrose to obtain a viscosity that is favorable for droplet breakup. Biopolymers with lower molecular weight did not increase the viscosity of solutions that strongly. Therefore, it is necessary to add higher amounts of sucrose to improve pectin-pectin chain interactions.

Table 7.3: Overview of sucrose content required for different modified pectin emulsions to obtain a viscosity ratio of 0.15 ± 0.1.

Sample	Sucrose content [% w/w]	Viscosity ratio λ [-]
Original pectin	10	0.2 ± 0.02
Molecular weight reduction		
MW = 76 kDa - 73 kDa	10	0.2 ± 0.02
MW = 67 kDa - 60 kDa	40	0.10 ± 0.02
MW = 51 kDa - 47 kDa	45	0.12 ± 0.03
MW = 43 kDa - 39 kDa	50	0.10 ± 0.01
Increase in DE	42	0.15 ± 0.03
Increase in DAc	30	0.11 ± 0.02

7.3.4 Pectins with Reduced Molecular Weight

In order to investigate the emulsifying properties of differently modified pectins, emulsions were produced from these pectins at standardized viscosity ratio. Figure 7.2 shows the Sauter mean diameter $d_{3,2}$ of emulsions prepared from depolymerized pectins. The $d_{3,2}$ of all the emulsions was comparable. The only significant difference was found between emulsions prepared from pectin with 76 kDa and those prepared from pectins with 47 or 51 kDa. However, generally large standard deviations could be noted, particularly for emulsions from low molecular weight pectins. This indicates instability of emulsion samples. The optimum in emulsifying and stabilizing behavior that was described by Akhtar et al. [98] and Leroux et al. [92] for depolymerized pectins with a molecular weight between 50–80 kDa and at 70 kDa, respectively, could not be observed. However, it is necessary to keep in mind that the determined molecular weight might vary depending on the measurement method used and the pectin type. While Leroux et al. [92] used light scattering, Akhtar et al. [98] also employed intrinsic viscosimetry to determine the molar mass so that this result should be comparable.

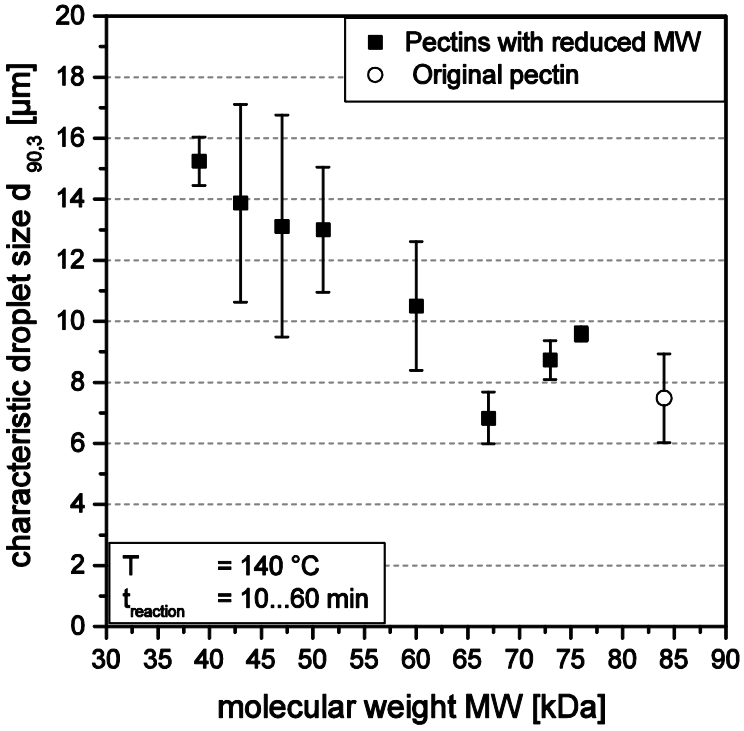

Figure 7.2: Sauter mean diameter of emulsions produced from pectins with reduced molecular weight. The d3,2 of the 76 kDa sample is significantly different from the d3,2 of samples with 47 and 51 kDa (indicated by asterisks).

As proposed by Nilsson and Bergenstahl [159], a reduced molecular weight (MW = 50–70 kDa) probably leads to a faster adsorption of pectin molecules to the droplet surface, which stabilizes newly formed droplets quicker. It is also possible that a depolymerization of pectin makes surface active groups (e.g. protein groups) better accessible so that adsorption kinetics increases [98]. Both should be favorable for relatively "fast" emulsification processes like high pressure homogenization or microfluidization [32]. Since the residence time of droplets in the droplet breakup zone is short, fast adsorbing emulsifiers are needed to quickly cover newly created droplet surface [105]. However, upon molecular weight reduction no significant reduction in droplet size could be observed. On the one hand, this might be explained by the interfacial tension that does not decrease significantly upon strong depolymerization (see Table 7.2). Liberation of protein groups therefore seems unlikely, particularly as the protein content is already very low. On the other hand, and more importantly, it can be assumed that heavy depolymerization leads to pectin chains that are too short to entangle and thus too short to successfully stabilize droplets sterically. This assumption is supported by the high concentrations of sucrose that are necessary to obtain equal viscosities with highly depolymerized pectins. Sterical stabilization is supposed to be the main stabilization mechanism [62] because high sucrose concentrations suggest that carboxyl groups are present in their undissociated form [160].

7.3.5 Emulsification Behavior of Pectins with Increased Degree of Esterification (DE)

Figure 7.3 shows the influence of DE on the Sauter mean diameter $d_{3,2}$. All pectins with increased degree of esterification showed a significantly improved emulsifying behavior compared to the original pectin ($P < 0.001$). Small standard deviations indicate that emulsions were characterized by narrowly distributed and reproducible droplet size distributions. An increase in DE by five percentage points led to a halving of the Sauter mean diameter (compared to the original pectin) and a further increase to 81% reduced the $d_{3,2}$ from 3.76 ± 0.53 µm (original pectin) to 1.22 ± 0.21 µm. However, an even higher DE did not show any significant effect anymore. These observations might be due to a stronger coiling of pectin molecules. Morris, Foster and Harding [88] showed that an increase in DE leads to citrus pectin being less extended and more coiled. This could increase the mobility of the molecule which might lead to a faster adsorption at the oil interface. Although coiling increases the flexibility of a polymer, this effect could also induce that hydrophobic groups are not easily accessible anymore. Ester groups or proteinaceous moieties could be captured in the inside of this coil which would counterbalance the positive effect of further esterification.

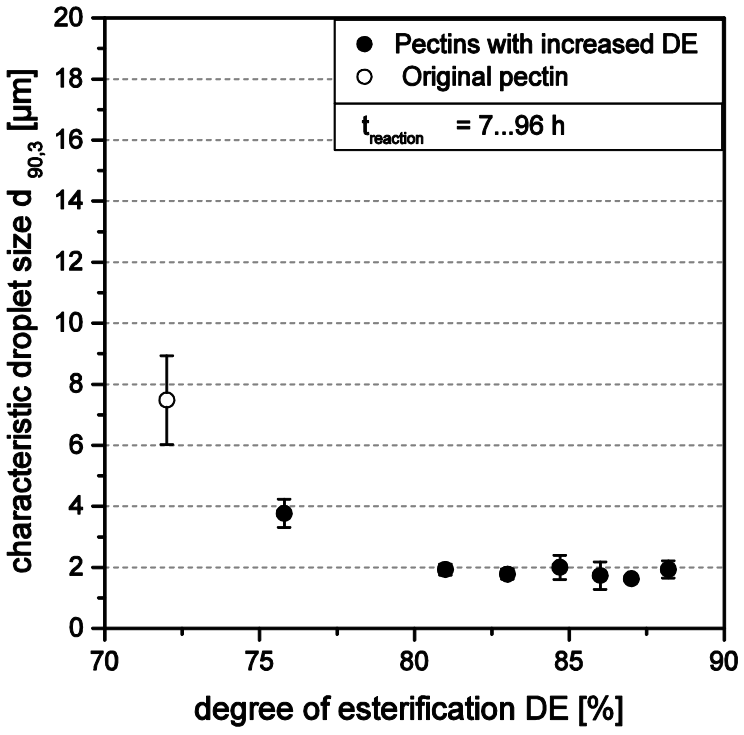

Figure 7.3: Sauter mean diameter of emulsions produced from pectins with increased degree of esterification. Asterisks indicate mean diameters that are significantly different from the rest.

When comparing Fig. 7.2 with Fig. 7.3, it can be seen that the influence of DE on droplet size was much stronger than that of molecular weight reduction. These results contradict the findings of Akhtar et al. [98] who stated that the DE is of minor importance for the emulsifying behavior of pectins. However, in their study, only

pectins with a DE from 22 to 73% were investigated. This corresponds to industrially available pectins that are deesterified to alter their gelling behavior. It therefore seems that an increase in DE above the region of commercially available pectins has much more effect on the resulting droplet size than a decrease.

7.3.6 Emulsification Behavior of Pectins with Increased Degree of Acetylation (DAc)

The influence of the DAc on the emulsifying behavior of pectins is shown in Fig. 7.4. Increasing the DAc of the original pectin first did not show any significant influence on the droplet size of emulsions ($P > 0.05$). Only when the DAc was increased to 9.5% and above, a significant droplet size reduction compared to the original pectin was found ($P < 0.001$). Then, the Sauter mean diameter $d_{3,2}$ reduced to below 3 µm.

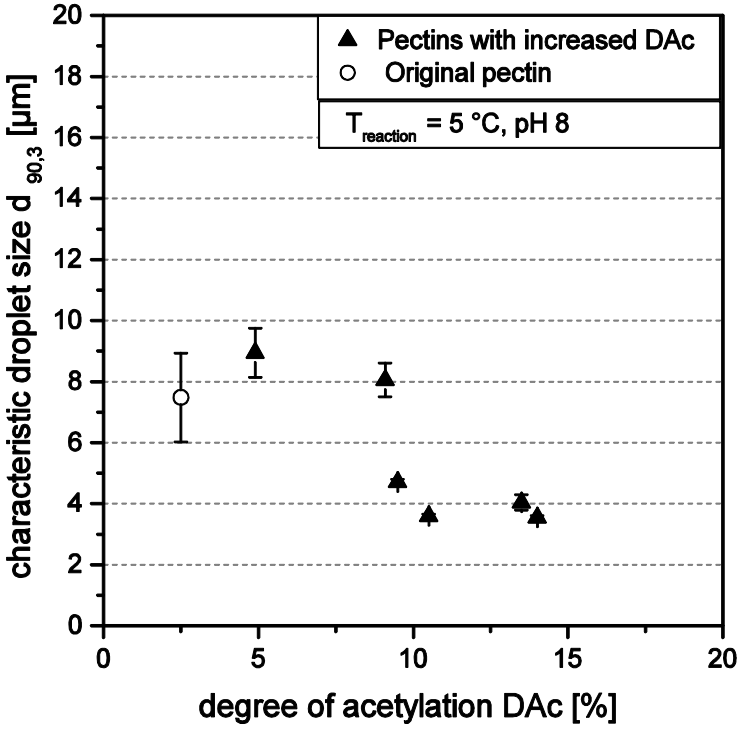

Figure 7.4: Sauter mean diameter of emulsions produced from pectins with increased degree of acetylation.

Leroux et al. [92] also showed that an increase of the DAc of citrus pectin leads to smaller droplets. Also, they deacetylated sugar beet pectin but did not observe any significant effect on the emulsifying capacity. This led them to the conclusion that the presence of acetyl groups is not necessary for obtaining a stable emulsion. However, in the mentioned study, sugar beet pectin which is naturally rich in protein was compared to citrus pectin with low protein content. Funami et al. [95] already showed that the proteinaceous moiety in sugar beet pectin is most likely the source for its good emulsifying capacity. In our experiments we observed a strong decrease in protein content (Table 7.1) when the DAc was increased which is possibly caused by the harsh

reaction conditions. The lower protein content might be the reason why a reduction of $d_{3,2}$ was not directly observed at low DAc. While only a slight improvement in emulsifying behavior due to increased acetylation took place, the droplet size enlarging effect of protein loss counteracted. A DAc of more than 9.5% was necessary to counterbalance this negative effect and to finally reduce droplet sizes below those of the original pectin (Fig. 7.4). Interfacial tension measurements support this theory. Pectins with an increased DAc exhibited higher tension values than the original pectin which might again be due to the loss of protein. This shows that interfacial tension measurements are not always a reliable method to assess a molecule's emulsifying performance. Particularly concerning hydrocolloids, Tolstoguzov [161] described that amphiphilicity is not a necessary prerequisite for emulsifying capacity. The adsorption mechanism of such polymers is also due to incompatibility effects, i.e. a gain of free energy due to separation of hydrated and non-hydrated groups. Considering the other pectin characteristics, a continuous reduction of DE during acetylation is observed which might have the same but less pronounced effect on the emulsifying behavior as the loss of protein. The decrease in DE is much less than the decrease in protein during acetylation procedure. Since the molecular weight does not change in the investigated samples an influence of it is not to be expected. It can be concluded that the DAc does play a significant role in emulsion forming capacity of pectin and that increasing the DAc is a useful means to improve the emulsifying behavior of citrus pectin. A minimum DAc of about 10% should be employed to obtain a significant improvement in $d_{3,2}$. However, it needs to be considered that the DAc cannot be increased unlimitedly. On the one hand, process handling made it difficult to achieve a DAc above 15%. On the other hand, it is arguable if such a high value is even desirable. A high DAc will reduce gel strength of the modified pectin solution and therefore will most probably have a negative effect on emulsion stability.

7.3.7 Comparative Assessment of Emulsifying and Stabilizing Performance

In order to assess the emulsifying and stabilizing performance, droplet size distributions (DSD) of emulsion prepared with chemically modified citrus pectins are compared in Fig. 7.5. From each modification type, the pectin sample performing best in emulsion formation was chosen. The corresponding DSDs are depicted together with those of the original pectin and sugar beet pectin SBP as a reference. SBP was taken for comparison because its good emulsifying properties have already been described several times [97, 104, 162].

In our experimental setup, we observed that SBP stabilized smaller droplets than the unmodified citrus pectin and the depolymerized pectin. This clearly shows that molecular weight reduction did not significantly improve the performance of citrus pectin. The pectins with an increased DAc and DE, however, excelled in the range of large droplets. In other words, the $d_{90,3}$ was smaller than that of SBP emulsions while the $d_{10,3}$was larger. This characteristic was even more dominant for the emulsions produced from pectins with an increased DE. Here, the mean droplet sizes of emulsions were nearing those of SBP emulsions with a $d_{50,3}$ of about 1 µm. In contrast to SBP, emulsions produced from DE increased pectin showed a very narrow and monomodal DSD. The bimodality and broad distribution of SBP emulsions might be

an indication for insufficient short term stabilization after droplet breakup. It could be caused by limited sterical stabilization due to the generally lower molecular weight of SBP.

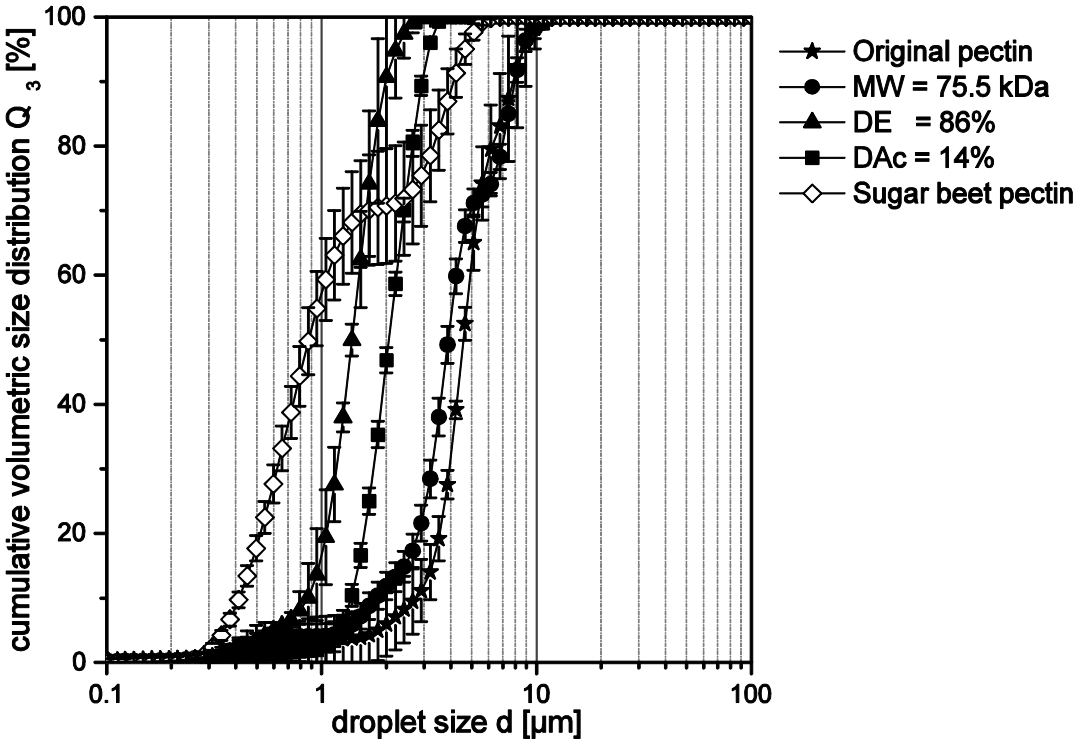

Figure 7.5: Droplet size distributions of emulsions produced from modified and unmodified citrus pectins and sugar beet pectin.

Finally, the performance of pectins to long-term stabilize oil-in-water emulsions was checked. Figure 7.6 shows the $d_{90,3}$ of the emulsions depicted in Fig. 7.5. Additionally, the DSD was checked after 2 weeks to account for any long-term changes. The Sauter mean diameter is depicted in Fig. 7.6 as well. It is obvious that the $d_{3,2}$ of the original pectin as well as the depolymerized and DE increased pectin emulsions does not increase significantly over 2 weeks of storage. The emulsions produced with DAc increased pectin as well as sugar beet pectin show a significant increase of almost 50% compared to the initial Sauter mean diameter. Here, it has to be kept in mind, that the viscosity of all emulsions was comparable since it was adjusted by varying the sucrose concentration. At low shear rates of 1 s^{-1}, the viscosity was approx. twice as high as at 100 s^{-1} for all emulsions. This means that differences in pectin solution viscosity cannot be held responsible for the differences in emulsion long term stability. Furthermore, yielding only occurs in emulsions prepared from pectins with increased DE (data not shown). It cannot explain the observed differences in stability. It is more likely that the molecular characteristics of pectins are the reason. The low storage temperature of emulsions (5 °C) favors stability due to firm gel formation. SBP does not form gels due to the high amount of acetyl groups. The same might apply to citrus pectins with increased degree of acetylation causing the poor stability of the corresponding emulsion.

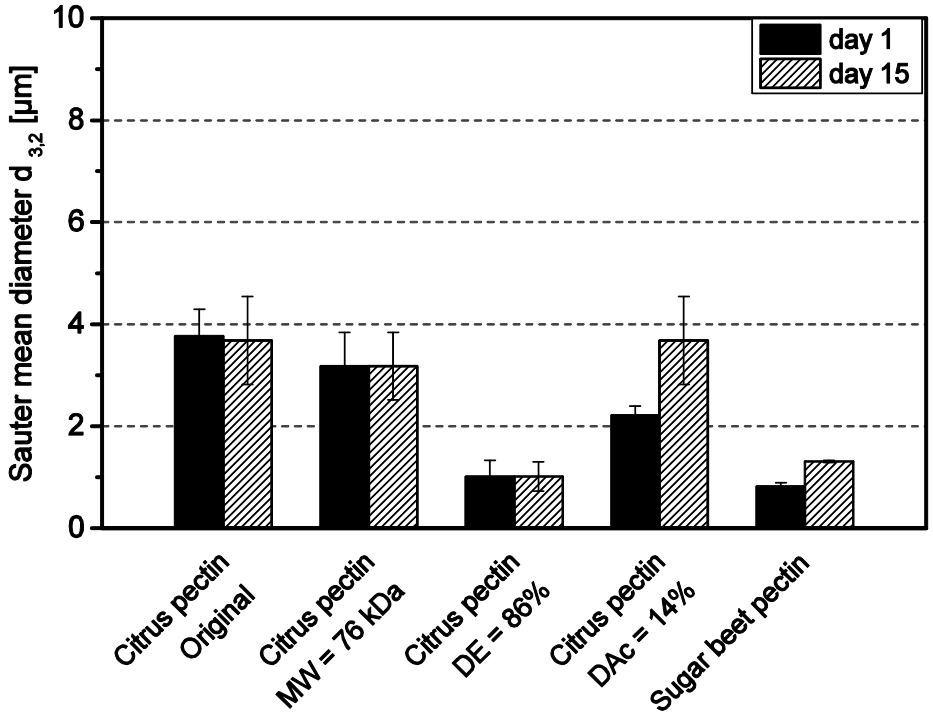

Figure 7.6: Sauter mean diameters of emulsions produced from unmodified and modified citrus pectins and sugar beet pectin. The droplet sizes were measured on the day of production and after two weeks of storage.

7.4 Conclusion

From the results it can be concluded, that the chemical modification of citrus pectin is a useful tool to improve the emulsion formation and stabilization properties. A reduction of the molecular weight of pectin does not show strong effects on the obtainable droplet size. An increase in DAc and DE significantly reduces the Sauter mean diameter. However, in case of DAc increase, the long term stability of emulsions is negatively affected. Compared to sugar beet pectin, emulsions formed with citrus pectins show monomodal droplet sizes with narrow size distributions. By choosing the right modified pectin, different droplet size ranges can be obtained. This offers the possibility to create emulsions with altered characteristics (e.g. different flow behavior, improved long term stability, etc.). Therefore, modified citrus pectins could be an interesting alternative as hydrocolloid emulsifiers.

7.5 Acknowledgements

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8 Interfacial and emulsifying properties of citrus pectin: Interaction of pH, ionic strength and degree of esterification

The interfacial and emulsifying properties of citrus pectin of different degree of esterification (DE = 55, 70 and 84%) were investigated. Ionic strength and pH were varied in order to modify the polyelectrolyte behavior of citrus pectins. The smallest hydrodynamic radius, lowest charge and fastest adsorption kinetics were found for high DE and high ionic strength at low pH. The extent of the dilational interfacial elasticity correlated with the amount and ratio of hydrogen bonds and hydrophobic interactions. The measured droplet sizes were mostly influenced by the adsorption kinetics and the salt responsiveness of citrus pectin. The faster the adsorption kinetics the smaller the resulting droplet sizes. Sodium chloride induced microgel particle formation of pectins. These microgel particles were less effective in droplet stabilization so that demulsification due to strong coalescence occurred. It was possible to counterbalance this effect by increasing the hydrocolloid emulsifier concentration.

This article has been submitted as: Schmidt, U.S., Schütz, L., Schuchmann, H.P. Interfacial and emulsifying properties of citrus pectin: Interaction of pH, ionic strength and degree of esterification.

8.1 Introduction

Citrus pectin is a gel forming polysaccharide that is widely used in the food industry as a stabilizer and viscosity enhancer [6]. Though having a rather complex molecular structure, citrus pectin can still be regarded as a biopolymer with essentially polyelectrolyte behavior [5, 66]. Pectins that are allowed for food applications consists of at least 65% galacturonic acid monomers. The degree by which the carboxyl groups of the galacturonic acid units are methylesterified is called the degree of esterification (DE). Citrus pectins with various DE are industrially prepared from citrus pulp and are commercially available. This is due to the importance of the DE for the gelling and viscosity enhancing properties of pectin [7]. Pectins with a high DE (> 50%) form a biopolymer network via hydrogen bonding and hydrophobic interactions [83]. Hydrogen bonds are formed between protonated carboxyl groups while hydrophobic interactions take place between methylester groups. It is well known that by altering the amount of carboxyl groups (i.e. DE) and the extent to which those groups are dissociated not only the network formation but also other typical polyelectrolyte properties of pectin can be manipulated [91]. For example, stronger network formation occurs at low pH and in the presence of monovalent cations [5, 163]. Furthermore, it was shown that pectin chains become more flexible at higher DE [88]. Increased molecular flexibility was also found at low pH in the presence of monovalent cations due to fewer dissociated carboxyl groups and therefore reduced electrostatic repulsion between neighboring monomers [164]. Higher ionic strength also leads to reduced hydrodynamic radii of sugar beet pectin – a different pectin type but with comparable polyelectrolyte features [165].

In spite of the extensive literature on the effects of DE, pH and ionic strength on pectin bulk properties, only little is known about their impact on the emulsifying properties of citrus pectin [8]. So far, most information can be found on the effects of cation addition to pectin emulsions. Sugar beet pectin emulsions with up to 0.1 M NaCl showed larger droplet sizes, increased instability, increased pH sensitivity and stronger interfacial adsorption [97]. They were also found to be unaffected by pH at values < 5. However, it needs to be remembered that for sugar beet pectin the emulsifying properties are mainly attributed to the significant amount of covalently bound protein [100, 166]. Low methylesterified pectin was found to reduce the interfacial tension more strongly than high esterified pectin [89]. Citrus pectin emulsions showed larger droplet sizes at pH 7 and at pH 4.7 [98]. In the same article, the group also mentions that they did not find any influence of DE on the emulsifying properties. However, recently we were able to show that an increase in DE to very high DE (> 80 %) results in a significant decrease of emulsion droplet sizes at improved long-term stability [167].

Despite the scarcity of reported evidence, one can expect effects of DE, pH and ionic strength on the interfacial and emulsifying properties of citrus pectin, from analogy with other polyelectrolytes [55]. Baeza et al. showed that propylenglycol alginate forms more elastic interfacial layers when the degree of esterification is higher [156]. Highly elastic interfacial layers were also reported for some synthetic polyelectrolytes and the importance of hydrophobic interaction for their formation was pointed out

[168]. It could also be shown that poly(acrylic acid) (a synthetic polyanion containing carboxyl groups) adsorbs more readily at the oil-water interface and lowers the interfacial tension faster at low pH and in the presence of counterions [169, 170]. Garti & Leser showed that Portulaca oleracea gum, an anionic polysaccharide, exhibits better emulsion stabilizing properties at low pH when the molecule is not charged [62].

In order to study the interfacial and emulsifying properties of citrus pectin, three citrus pectins with varying DE but otherwise comparable molecular features were investigated. The pH as well as the ionic strength of the aqueous solution were varied in order to alter the solution and adsorption properties of the pectins. Adsorption kinetics to the oil-water interface were determined and dilational rheological measurements were conducted. Finally, concentrated oil-in-water emulsions were prepared via high pressure homogenization. We will explain the resulting emulsion characteristics by the particular adsorption and interfacial properties of pectin which result from its polyelectrolyte behavior.

8.2 Materials and Methods

8.2.1 Materials

Three citrus pectins with different degrees of esterification DE (DE = 55, 70 and 84%) but otherwise comparable molecular features were supplied by Herbstreith & Fox KG Pektin-Fabriken (Neuenbürg/Germany). The molecular characteristics of pectins according to manufacturer information can be found in Table 8.1. Pure rape seed oil was purchased from Schell GmbH, Lichtenau, Germany. Florisil, hydrochloric acid, sodium hydroxide and sodium chloride were all of analytical grade and obtained from Carl Roth GmbH & Co. KG (Karlsruhe/Germany). Melamine fluoride microspheres with an average diameter of 1.04 ± 0.03 μm were obtained from microParticles GmbH (Berlin, Germany).

Table 8.1: Characteristics of the used pectin samples according to supplier information. The protein content was determined according to Bradford.

Sample		1	2	3
Degree of Esterification	[%]	84	70	55
Molecular Weight	[kDa]	73	80	79
Protein Content	[%]	2.3	1.6	2.1
Degree of Acetylation	[%]	3.4	2.7	3.2
Galacturonic acid content	[%]	88.4	83.4	86.2

8.2.2 Preparation and characterization of pectin solutions

Pectin stock solutions were prepared by dissolving 0.25% w/w of pectin powder in MilliQ water (60 °C) that was previously set to pH 2, 3 or 4 containing either 0 mM or 0.25 mM sodium chloride. Solutions were given at least 12 hours for hydration. The pH was measured and, if necessary, adjusted to the required value using HCl or NaOH solutions. The zeta potential ZP and hydrodynamic diameter R_h of pectin in solution as well as the adsorption layer thickness were determined at 25 °C using a Zetasizer Nano ZS (Malvern Instruments GmbH, Herrenberg, Germany). To obtain the ZP, six measurements of at least twelve runs were conducted per stock solution sample. R_h and adsorption layer measurements were conducted according to Siew and Williams [93]. For R_h determination, the stock solutions were diluted further to yield 10 diluted samples and were given another 12 hours for hydration. Then, three measurements of 12 runs each with 10 seconds run time were conducted per sample. Then, the detected Z-average diameter was plotted versus pectin solution concentration and extrapolated to zero pectin concentration to yield the hydrodynamic radius R_h. For determination of the adsorption layer thickness onto melamine fluoride (MF) microspheres, dilutions were prepared in such way that at the end a concentration of 10 mg/m² particle surface was reached. This was achieved in the following way: The stock suspension of MF particles had a concentration of 10% w/v. It was diluted in a solution of desired pH and ionic strength to 0.1% w/v. Then, microsphere dilution and double concentrated pectin dilution were mixed 1:1 to reach the final concentration. The mixture was given 12 hours for adsorption to take place. The thickness of the adsorbed layer was measured in the same way as the Z-average diameter.

8.2.3 Measurement of interfacial properties

The interfacial tension σ, effective diffusion coefficient D_{eff} and dilational interfacial elasticity E′ of pectin at the oil-water interface were determined using a pendant drop tensiometer PAT-1 (Sinterface Technologies GbR, Berlin, Germany). The rapeseed oil used for the measurements was purified five times according to Dopierala et al. [171]. Pectin solutions of 0.1% w/w were prepared as described above. A purified rapeseed oil droplet with a surface of 35 mm² was formed at the tip of the tensiometer capillary in the pectin solution at 25 °C. Then, the interfacial tension σ was measured. The initial slope ($t < 1000$ s) of the recorded dynamic interfacial tension data was fitted using the Ward-Tordai equation for diffusion controlled adsorption in the short-time limit ($t \rightarrow 0$) [34, 35]. From this equation, the effective diffusion coefficient D_{eff} was extracted which covers the steps of diffusion to and adsorption at an interface.

Dilational rheological properties were determined as described in [172]. After 15 h, the surface area of the oil droplet was varied sinusoidally by 1.5 mm² at a frequency of 0.01 Hz. The changes in interfacial tension over ten cycles were recorded. The interfacial dilational elasticity E′ was read out from the built-in software which directly calculates the interfacial rheological parameters via Fourier analysis. The majority of measurements was conducted once. Some random measurements were repeated and showed a reproducibility of within 3 mN/m.

8.2.4 Preparation and characterization of pectin emulsions

Pectin-based oil-in-water emulsions with a disperse phase content of 30% w/w, a pectin concentration of 1% in the aqueous phase and varying sodium chloride concentrations were prepared as previously described [126]. High pressure homogenization was done using a Microfluidizer® MF-110 EH (Microfluidics Corporation, Newton, MA, USA). The emulsion was first emulsified at 400 bar, collected and then passed through the device a second time at 800 bar. Every type of emulsion was prepared three times.

Droplet size distributions of emulsions were obtained by using a laser diffraction particle size analyzer (LS 13 320, Beckman Coulter, Inc., Miami, FL, USA) equipped with polar intensity differential scattering (PIDS) technology. Obscuration was around 45%. The optical model used also covered the Mie region. A refractive index of 1.333 was used for the water phase and 1.47 was used for the oil phase. The imaginary part in both cases was set to zero as both phases are transparent. Emulsion samples were always measured in triplicate. Droplet size distributions were then calculated according to the Mie theory. The 90^{th} percentile of the cumulative volumetric droplet size distribution $d_{90,3}$ was used as the characteristic value to describe the droplet size distribution.

Zeta potential measurements of emulsion droplets were conducted as described for pectin in solution. Emulsions were diluted 1:10 in MilliQ water of corresponding pH and ionic strength before the measurement.

Microscopy of emulsions was done using an Eclipse LV100ND (Nikon GmbH, Düsseldorf, Germany) equipped with DS-Fi1c camera in 20 and 50 fold magnification.

8.2.5 Statistical analysis

Statistical analysis was performed using the software OriginPro 9.1G (OriginLab Corp., Northampton, MA, USA). The results of triplicate analyses were used to calculate averages and standard deviations. The data were analyzed by one-way analysis of variance. Significantly different mean values of variables ($p < 0.05$) were determined using Scheffé's method.

8.3 Results and Discussion

8.3.1 Solution properties of citrus pectins

The zeta potential ZP is a measure of the charge around the pectin molecule. In Fig. 8.1 (left), ZP values of three different pectins in aqueous solutions of various pH and low ionic strength are shown. Statistical analysis showed significant differences amongst all samples. At pH 2, the ZP of all pectin samples was close to 0 mV indicating the close proximity to the apparent pK_a of the molecule. Increasing the pH to 4 resulted in a decrease of ZP values for all investigated pectins. This can be explained by the dissociation of the carboxyl groups resulting in a negative charge around the molecules. The ZP of pectin in solution did not only depend on the pH of the solution but also on the DE of the pectin. Pectin with a high DE always showed a less negative

ZP value than pectin with a lower DE: Compare e.g. -7 mV for DE84 with -21 mV for DE55 at pH4. The reason is that at higher DE the pectin sample possesses fewer carboxyl groups. Therefore, less carboxyl groups can dissociate which results in a less negatively charged molecule. Fig. 8.1 (right) shows the influence of pH on ZP values of pectin in solutions containing 0.5% w/w sodium chloride (high ionic strength). The same effects already described for low ionic strength solutions could be observed. However, the ZP was shifted towards higher values for all samples. This can be explained by the presence of positively charged sodium ions shielding the negative charge of the pectin molecule and thus reducing the measurable ZP. In correlation to the pH influence, the effect of ionic strength was stronger at pH 4 than at pH 3 (or even pH 2) as more carboxyl groups are dissociated at higher pH.

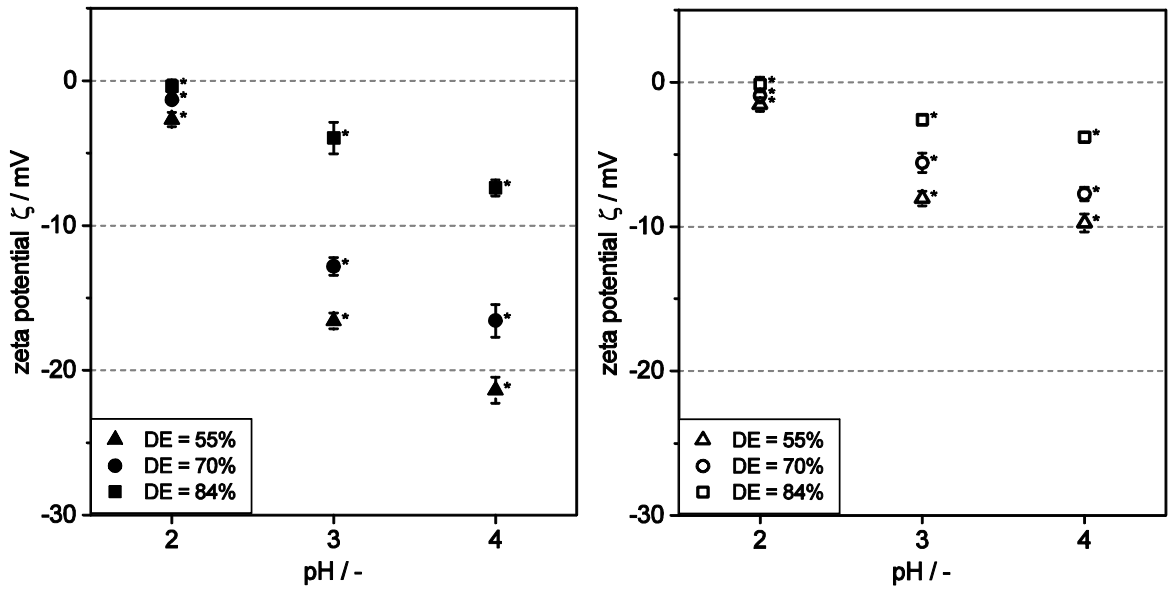

Figure 8.1: Zeta potential of pectins with different degree of esterification DE in solutions of different pH. Left: low ionic strength solutions (0% w/w NaCl), right: high ionic strength solutions (0.5% w/w NaCl). Asterisks indicate statistically significant differences.

The presence of negative charge from the citrus pectin molecules leads to electrostatic interactions. Due to the polymeric nature of pectin, not only *inter*molecular but also *intra*molecular repulsion can occur. This may lead to an increased hydrodynamic diameter R_h of the biopolymer. Therefore, the R_h of the pectin molecules in solution was determined and is shown in Fig. 8.2 (left). In low ionic strength solutions, the results are in agreement with the ZP measurements. Again, an influence of both pH and DE of the pectins was found. The R_h of pectin samples increased with increasing pH. At any given pH, the R_h of low DE pectins was larger than for high DE pectins. The presence of dissociated carboxyl groups thus led to more extended molecules due to reduced internal flexibility of the molecules [88].

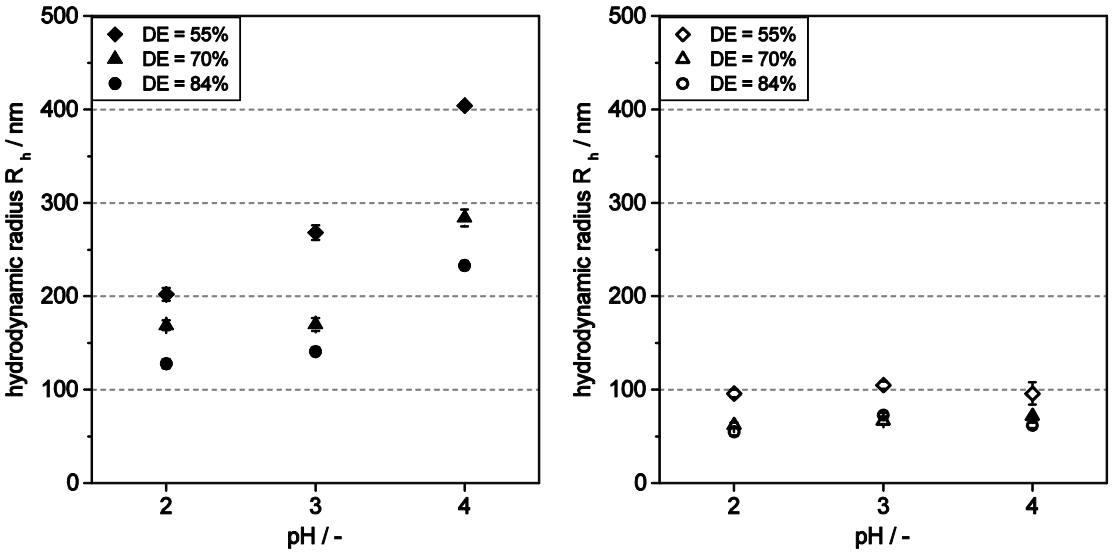

Figure 8.2: Hydrodynamic radius R_h of pectins with different degree of esterification DE in solutions of different pH. Left: low ionic strength solutions (0% w/w NaCl), right: high ionic strength solutions (0.5% w/w NaCl).

In higher ionic strength solution, however, a different behavior was observed (Fig. 8.2, right). An influence of DE could still be seen with low DE pectin showing the largest R_h values. However, there was only very little effect of the pH on the molecule size. More interestingly, a comparison of the R_h in low and high ionic strength solution revealed that the R_h was always smaller in the presence of 0.5% w/w NaCl. The largest effect could be seen at pH 4: Here, the R_h of pectin with DE = 84% was only 60 nm – a quarter of the value measured at low ionic strength. These results can be explained by a degradation of the solvent qualities of the aqueous phase. Apparently, a 0.5% w/w solution of sodium chloride presented a relatively poor solvent for citrus pectin. The polymer might thus have formed denser coils as is described for lower pH and higher ionic strength [5]. These results contradict previous findings reported in literature because Axelos et al. [173] stated that 0.1 M NaCl solutions are still good solvents for citrus pectin.

Reduced electrostatic repulsion does not only influence *intra*molecular interactions and, therefore, leads to a more compact size of the pectin molecules. It also reduces *inter*molecular repulsion which might result in a dense packing at interfaces. In order to monitor this phenomenon, the adsorption layer thickness of pectin molecules adsorbed at MF-microspheres was measured (Table 8.2). While there was only a small effect of the DE, a decrease in pH and particularly an increase in ionic strength led to a massively enlarged adsorption layer thickness. At pH 2 and 0.5% w/w sodium chloride, the measured adsorption layer was up to 1200 nm thick. This was several times the R_h of freely suspended pectin molecules in the same solution and clearly an indication of multi-layer adsorption [93, 168]. In solution without added NaCl, however, the adsorption layer was much thinner. At pH 4, the adsorbed layer thickness was smaller than the Rh of the freely suspended pectin molecules (adsorbed layer < 200 nm compared to R_h > 200 nm). This indicates that there was probably only

a single pectin layer that was spread out at the interface thus being flatter than the corresponding molecule in solution [174]. At pH 2 in low ionic strength solutions, the layer thickness was comparable to double the corresponding R_h of freely suspended pectin molecules. Again, multilayer adsorption seemed unlikely but instead a pectin monolayer with dangling loops and tails could be expected. Comparable conclusion were made by [93] for sugar beet pectin.

In summary, the solution properties of pectin are influenced by the amount of dissociated carboxyl groups. This amount is influenced by the DE of the pectin and the pH of the solution. As a result, low pH and high DE lead to less negative ZP values, small hydrodynamic radii and thicker adsorbed layers. Increasing the ionic strength of the solution enhances these effects by shielding negative charges and probably also by reducing the solvent qualities of the aqueous phase.

Table 8.2: Adsorption layer thickness of pectin with different degree of esterification DE onto melamine fluoride particles. Adsorption experiments were conducted in solutions of different pH and ionic strength. The ionic strength was varied by adding 0.5% w/w NaCl.

		Adsorption layer thickness (in nm)	
DE in %	pH	0% w/w	0.5% w/w
84	**2**	346 ± 6	1213 ± 50
70	**2**	348 ± 23	1075 ± 88
55	**2**	309 ± 8	919 ± 26
84	**4**	118 ± 34	738 ± 56
70	**4**	172 ± 3	746 ± 7
55	**4**	192 ± 25	696 ± 13

8.3.2 Interfacial properties of citrus pectins

In order to act as an efficient emulsifier, citrus pectin must be able to quickly adsorb at the oil-water interface and to quickly reduce the interfacial tension. These properties can be assessed by comparing the effective diffusion coefficient D_{eff} of adsorption for different pectin preparations. In Fig. 8.3 (left), D_{eff} of the different pectins to the oil-water interface is shown. In low ionic strength solution, a linear relationship ($R^2 = 0.99$) between D_{eff} and DE could be seen at different solution pH. Adsorption was fastest for pectin with DE = 84% (highest D_{eff} values). Increasing the pH led to a reduced D_{eff} and consequently indicated slower adsorption. This development of D_{eff} with varying DE and pH is summarized in Fig. 8.3 (right) by showing the dependency of D_{eff} on the hydrodynamic radius R_h. Both DE and pH influence the R_h of the pectin molecules as was shown in Fig. 8.2. Furthermore, the diffusion coefficient of a molecule in solution is inversely proportional to its R_h which is stated by the Stokes-Einstein equation:

$$D = \frac{k_B \cdot T}{6 \cdot \pi \cdot \eta \cdot R_h}$$

where D is the diffusion coefficient of a molecule in solution, kB is the Boltzmann constant, T is the temperature and η is the viscosity of the solution. In Fig. 8.3 (right),

this equation is given as well for comparison. It can be seen that D_{eff} strongly decreases with increasing R_h indicating an inversely proportional relationship. A good correlation with the Stokes-Einstein equation is obtained showing that the adsorption kinetics of citrus pectin is indeed diffusion controlled. However, an overall deviation towards higher D_{eff} values than expected from the Stokes-Einstein equation can be seen particularly at lower R_h values. Adsorption of pectins to the interface is thus faster than diffusion of pectin molecules in solution. This indicates that apparently also kinetic effects must play a role in the adsorption mechanism of citrus pectin.

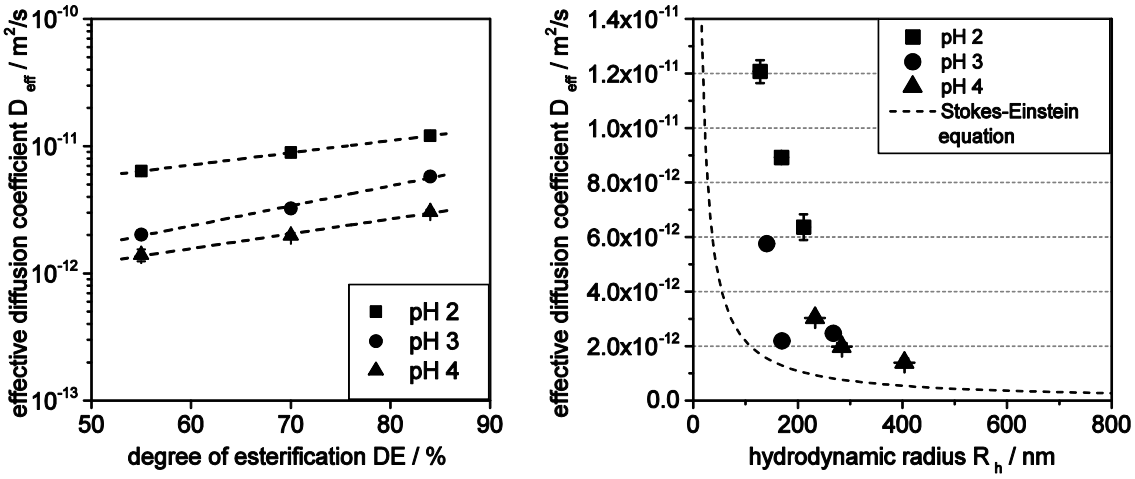

Figure 8.3: Effective diffusion coefficients D_{eff} of pectins for adsorption at the oil-water interface at 25 °C at low ionic strength (0% w/w NaCl). (left): D_{eff} depending on the DE of pectins at various pH. Dashed lines indicate linear fits with R^2 = 0.99. Diagram according to [175]. (right): D_{eff} depending on the hydrodynamic radius of pectins in solution. The Stokes-Einstein equation is given for comparison. Parameters entering this equation are chosen according to experimental setup: Measurement temperature = 25 °C, solution viscosity = 0.0089 Pas (pure water).

At higher ionic strength, it was not possible to extract reliable values of D_{eff}. This is supposed to have been due to limitations of the measurement procedure. After an oil droplet had been formed in the pectin phase, the measurement of the interfacial tension was started manually. The first detected σ values already showed a significantly lower interfacial tension (data not shown). Apparently, the interfacial tension reduction took place so rapidly that the initial stages could not be resolved by the applied measurement procedure anymore [176].

The viscoelastic properties of the oil-water interface covered with citrus pectins are evaluated by analysis of the interfacial dilational elasticity modulus E' [41]. Fig. 8.4 shows the dependence of E' on the DE. The pectin with DE = 84% did not show much change in E' upon alteration of the solution conditions. The elastic modulus of the pectin with the lowest investigated DE (55%), however, was very sensitive particularly to changes in pH. On the one hand, the E' was higher than for pectin with DE = 84% when the low DE pectin was able to form hydrogen bonds (low pH) [169, 177]. On the other hand, much lower interfacial elasticity values were detected at high pH when less hydrogen bonds could be formed. Compared to high DE pectin, low DE pectin also possessed less methoxyl groups and thus less hydrophobic interactions that might counterbalance the absence of hydrogen bonds [168]. Generally, the effect of ionic

strength on the interfacial elasticity was very small. Only at lower pH values, a slight reduction of E' upon sodium chloride addition could be noticed. This is interesting since thick adsorbed layers had been detected at high ionic strength (Table 8.2). It means that there is apparently no correlation between the interfacial elasticity and the adsorbed layer thickness.

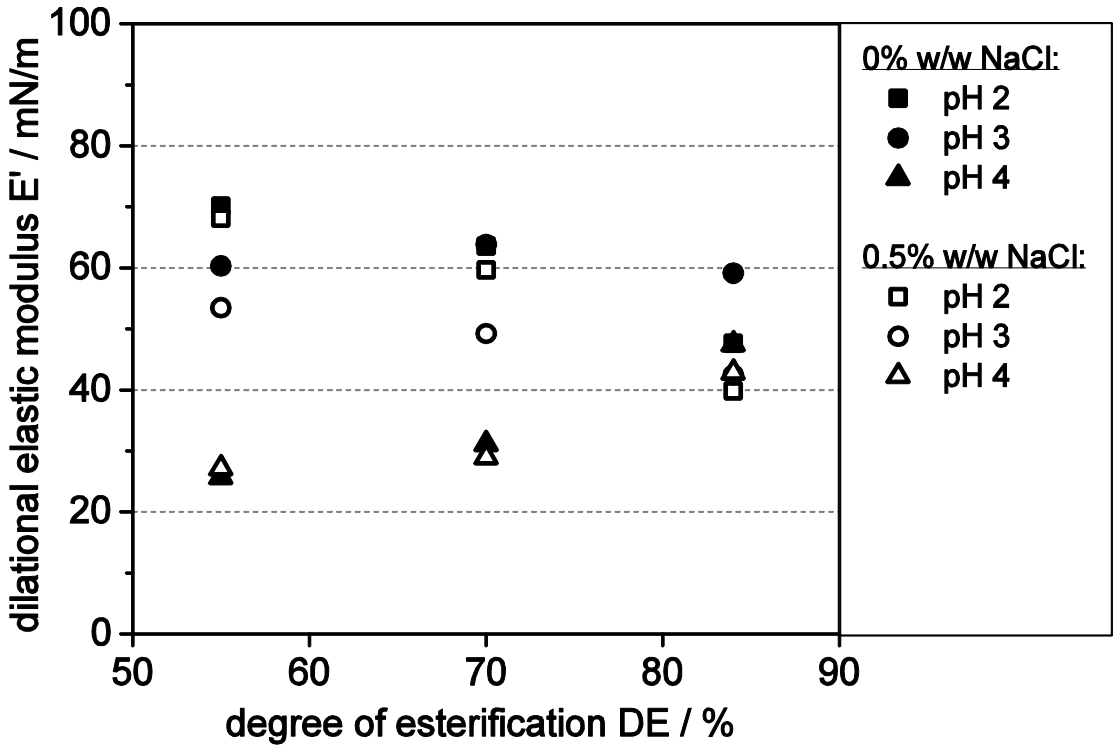

Figure 8.4: Dilational elastic modulus E' of citrus pectins at the oil-water interface determined after 15 h of adsorption time. Low ionic strength (full symbols) and high ionic strength (open symbols) conditions were investigated.

8.3.3 Emulsifying properties at low ionic strength conditions

Figure 8.5 shows the characteristic droplet sizes $d_{90,3}$ of citrus pectin emulsions prepared at low ionic strength. The $d_{90,3}$ was chosen as it represents the largest droplets found in an emulsion and it can be an early indication of occurring instabilities. Droplet sizes were found to vary between 7 and 25 μm with the largest $d_{90,3}$ values measured in emulsions prepared at pH 4. Furthermore, droplet sizes decreased with increasing DE. The droplet size seemed to follow a certain order with smaller droplets being stabilized at conditions where the pectin molecule is more hydrophobic (more methylester groups) and more protonated. Only the emulsion produced from pectin with DE = 55% at pH 2 did not follow this trend as it would have been expected to possess a smaller $d_{90,3}$. These findings are interesting as previous results showed the droplet size of sugar beet pectin stabilized emulsions is independent of pH at pH values below 5 [97]. Only above pH 5 an increase in the droplet size with increasing pH was reported [97, 98].

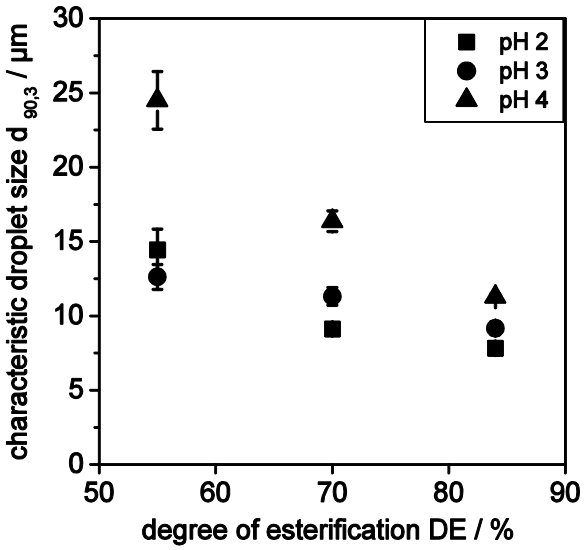

Figure 8.5: Characteristic droplet size $d_{90,3}$ of emulsions produced at low ionic strength (0% w/w NaCl added). Pectins of varying degree of esterification were used to prepare emulsions at different pH.

In order to correlate the determined droplet sizes with the interfacial properties of citrus pectin emulsions, the $d_{90,3}$ is plotted versus the D_{eff} and E′ in Fig. 8.6. It shows that the $d_{90,3}$ decreased strongly with increasing D_{eff}. This indicates that a faster adsorption of pectin at the interface led to a better droplet stabilization. In high pressure homogenization processes, a fast stabilization of the oil-water interface is necessary in order to prevent recoalescence of oil droplets [32]. The dependence of the droplet size $d_{90,3}$ on E′ seemed to be more complex (Fig. 8.6 right side). A minimum of $d_{90,3}$ at intermediate dilational elasticity values E′ of about 50 mN/m could be spotted. For both lower and higher E′, droplet sizes increased again. Particularly large droplet sizes occurred at very low E′ values. The dilational elasticity E′ is connected to droplet breakup and droplet stability although strong evidence for its influence in concentrated emulsions is still missing [43, 44, 47, 18]. Due to the hindered deformability of elastic interfaces, droplets exhibiting high E′ values are supposed to show improved resistance to droplet coalescence [40, 178]. This did not seem to be the case with the emulsions investigated in this study. Otherwise, particularly small droplet sizes should have been observed at E′ > 60 mN/m. However, dilational elasticity was also found to hinder droplet breakup in emulsions produced via a high shear apparatus [44]. This could explain the increasing droplet sizes at E′ values above approx. 50 mN/m in the present study. However, measurements at pH 4 are not in line with this explanation. For samples produced at pH 4, a reduction of the droplet size with increasing interfacial elasticity E′ could be seen.

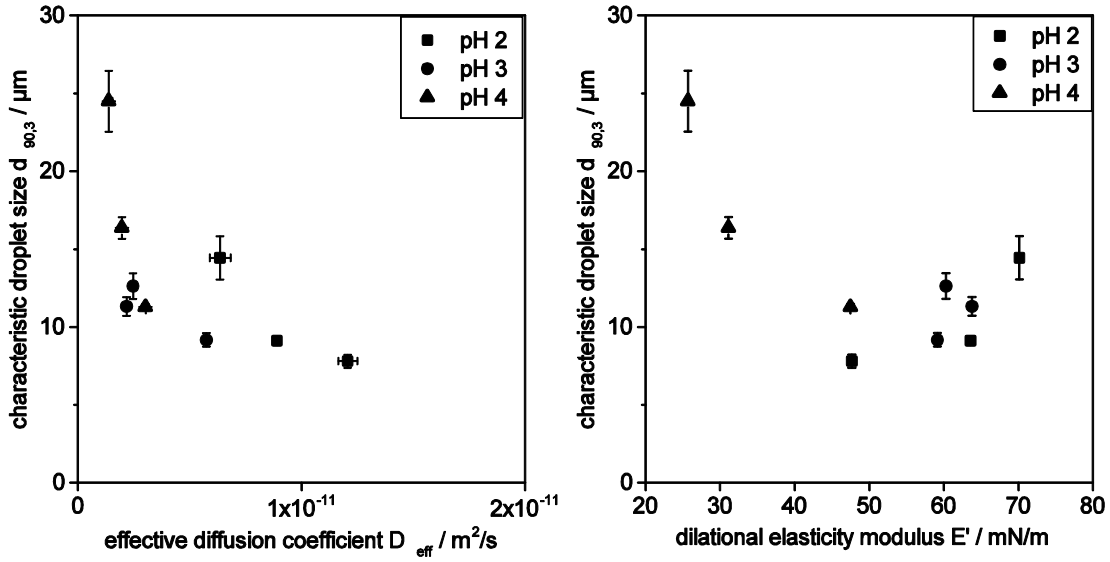

Figure 8.6: Dependence of $d_{90,3}$ on the effective diffusion coefficient D_{eff} (left) and on the dilational elasticity modulus E' (right). The $d_{90,3}$ is the characteristic droplet size of emulsion prepared by high pressure homogenization at low ionic strength (0% w/w NaCl). D_{eff} and E' were determined at the oil-water interface using oscillatory drop technique. Data points for each pH value comprise experiments with pectins with three different degrees of esterification DE.

We suggest that the origin of this difference lies in the adsorption mechanism of pectin to the oil-water interface. Table 8.3 shows the ZP values of bare, uncovered oil droplets in water that was conditioned in the same way as for the adsorption or emulsification trials. The most important information from this table is the change in sign of the ZP at higher pH.

Table 8.3: ZP values of bare oil droplets in water of different pH in the presence and absence of additional NaCl.

		pH 2	pH 3	pH 4
Without added NaCl	**(0% w/w)**	49.3 ± 12.1 mV	23.3 ± 7.9 mV	-32.7 ± 2.2 mV
With added NaCl	**(0.5% w/w)**	8.8 ± 2.9 mV	-0.7 ± 1.8 mV	-6.7 ± 4.6 mV

While at pH 2 and 3 oil droplets were charged positively, at pH 4 the charge was negative. This will have altered the interaction of the interface with adsorbing pectin molecules. At pH 3, and apparently also at pH 2, the negative charge of pectin molecules was sufficient to cause a strong electrostatic attraction between pectin molecules and the positively charged interface. The underlying mechanism is therefore mostly electrosorption [179]. However, at pH 4, both pectin molecule and interface were strongly negatively charged. As a result, a strong negative repulsion between oil droplet interface and pectin molecules took place which slowed down adsorption – hence the low D_{eff} values – and prevented strong adsorption – hence the low E' values. Adsorption could then probably only occur due to hydrophobic interactions of the methylester groups with the interface. The number of those is decreasing with decreasing DE explaining why particularly low values of D_{eff} and E'

were detected for pectin with DE = 55%. The combination of low D_{eff} and E′ led to a strongly impaired stabilization of oil droplets at pH 4 in the emulsion and large $d_{90,3}$ values were measured.

8.3.4 Emulsifying properties at high ionic strength conditions

Figure 8.7 shows the characteristic droplet size $d_{90,3}$ of emulsions prepared from citrus pectins in the presence of 0.5% w/w NaCl. The emulsions showed $d_{90,3}$ values between 10 and 20 µm at pH 2 and 3 and significantly larger values at pH 4 (up to about 50 µm). Generally, the droplet sizes in emulsions at high ionic strength were larger than in emulsions of low ionic strength.

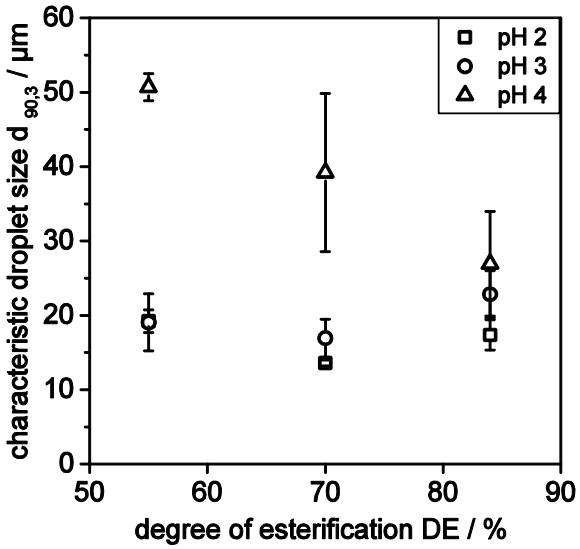

Figure 8.7: Characteristic droplet size $d_{90,3}$ of emulsions produced at low high strength (0.5% w/w NaCl added). Pectins of varying degree of esterification were used to prepare emulsions at different pH.

These results were not necessarily expected. From Figure 8.2 and 8.3, one can see that the addition of sodium chloride reduced the hydrodynamic radius and also increased D_{eff}. The higher ionic strength reduced electrostatic interactions (Table 8.3) which should have driven molecules to the interface particularly at pH 4 [170]. This also seemed to be reflected by the thick adsorption layer (Table 8.2). All features that might be connected with a decrease in droplet size and an improved droplet stabilization at low ionic strength were even more pronounced in the systems containing NaCl. This led to the assumption that also the characteristic droplet size $d_{90,3}$ should be lower. However, the opposite is the case. Therefore, we strived to identify the reasons for the deviating stabilizing properties of citrus pectins in the presence of sodium chloride.

Droplet-droplet interactions in emulsions are largely dominated by electrostatic repulsion between the oil droplets or by steric effects [9]. Since citrus pectin is a polyelectrolyte, we will first consider electrostatic repulsion. Table 8.4 shows the ZP of emulsion droplets prepared from pectins of different DE. The development of ZP values with DE and pH corresponded qualitatively to that of non-adsorbed pectin in solution (Fig. 8.1). However, at pH 4, the measured ZP values of the pectin covered

droplets were even more negative than those of pectin in solution (compare e.g. -22 mV with -41 mV for pectin with DE = 55% at low ionic strength).

Table 8.4: ZP values of emulsion droplets prepared from pectin of different DE.

		Zeta potential (in mV)	
DE in %	pH	0% w/w	0.5% w/w
84	2	-0.6 ± 1.2	-0.2 ± 1.6
70	2	-2.0 ± 1.1	-0.3 ± 0.9
55	2	-2.7 ± 0.6	-0.9 ± 0.6
84	3	-5.5 ± 1.3	-3.4 ± 0.9
70	3	-10.6 ± 2.8	-4.8 ± 1.4
55	3	-16.1 ± 3.5	-7.6 ± 0.9
84	4	-15.2 ± 1.7	-5.2 ± 1.6
70	4	-29.4 ± 2.2	-10.2 ± 1.1
55	4	-40.5 ± 3.3	-15.9 ± 1.2

Concerning droplet interactions, Table 8.4 shows that the presence of 0.5% w/w NaCl in the emulsion increased the ZP and therefore reduced the molecule charge at the oil droplet interface. While this enhanced polyelectrolyte adsorption, it could also have reduced electrostatic repulsion between oil droplets covered with citrus pectin. Comparison with Fig. 8.5, however, shows that a reduction in molecular charge did not increase the droplet size at low ionic strength. In fact, the smallest $d_{90,3}$ values were determined in emulsion systems where the ZP measurement of the pectin covered oil droplets indicated nearly zero charge (pH 2). It can thus be concluded that the main stabilizing effect in pectin emulsions was not of electrostatic but of steric nature. However, electrostatic interactions can also influence the steric stabilizing properties of polyelectrolytes. If NaCl addition makes the water phase a poorer solvent for pectin, a precipitation of pectin onto the oil droplet interface, a reduced extension of pectin molecules into the aqueous phase and ultimately strong flocculation would be expected [2, 59].

We therefore checked whether flocculation was present in the samples. Fig. 8.8 shows exemplary micrographs of emulsions created with pectin DE55 at pH4 in the presence and absence of salt. These samples were chosen because here the largest droplet size difference between low and high ionic strength systems were observed. Floc formation could be observed in the sample containing 0.5% w/w NaCl. However, there are several other unique features about the droplets in Fig. 8.8 right. On the one hand, it can be seen that the droplets with NaCl (right) were much larger than those without NaCl (left) and that their size corresponded to those measured by laser diffraction. This means that most likely droplet recoalescence occurred already shortly after breakup in the homogenizer. On the other hand, a unique "gingerbread" structure could be seen on the droplets in the right image (Fig. 8.8). Figure 8.9 shows a larger magnification of the same sample.

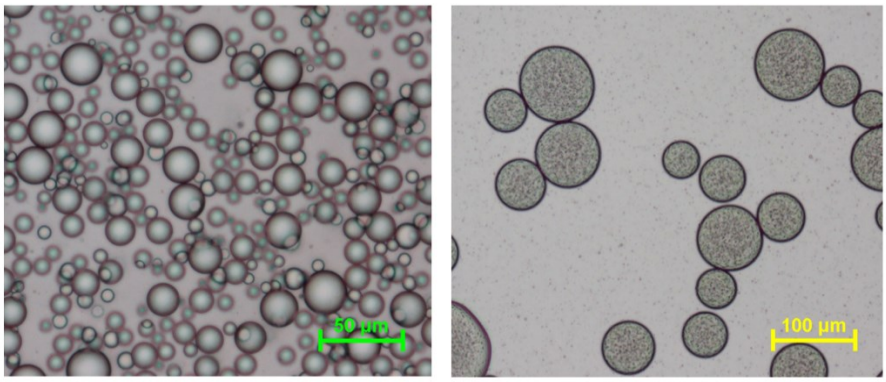

Figure 8.8: Micrographs of emulsions prepared from pectin with DE = 55% at pH 4. Left: without additional NaCl; right: with 0.5% w/w NaCl in the emulsion. NaCl had been added to the emulsion before high pressure homogenization.

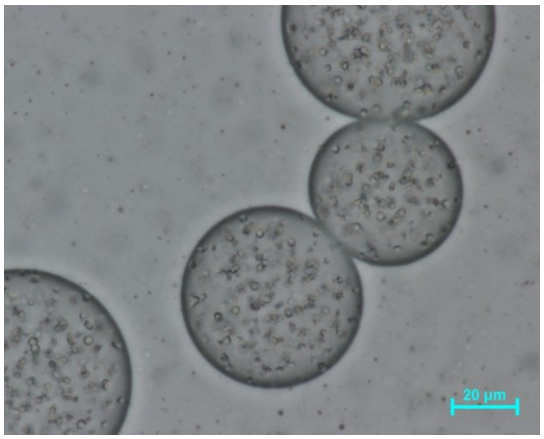

Figure 8.9: Emulsion droplets covered with microgel particles from citrus pectin. Emulsion composition: 1% w/w pectin with DE = 55%, 0.5% w/w NaCl added before homogenization.

The structures that could be seen did not have a clear phase boundary and were not perfectly spherical. We therefore suggest that the gingerbread structure was made up of pectin microgel particles. Pectin with a low DE forms strong gels in the presence of divalent cations. For monovalent cations a corresponding behavior was reported. However, we did not observe any gel formation throughout the whole bulk phase or emulsion, probably because the investigated concentrations were still too low and the DE of pectin too high. Nevertheless, the formation of tiny microgel particles seemed possible [180]. In the measurements of the adsorption layer thickness (Table 8.2), it might also have been those microgel particles that caused the thick measured layer. From the microscope image, it can be seen that the size of the microgel particles was roughly in the range of the adsorbed layer thickness for high ionic strength solutions (around 1 µm). The formation of microgel particles from chemically cross-linked apple pectin had previously been described [181]. Both microgel particles as well as oil droplet sizes reported in that study correspond to the ones given here. For the emulsions, this means the following: First, the stabilization mechanism might have been more of the Pickering type and second, the volume of hydrocolloid emulsifier might have been too low [182]. If pectin molecules were aggregated and compacted

into microgel particles, they would not have been able to broadly cover fresh interface. As a result, bare patches of oil droplets might have existed which extremely increases the coalescence probability [183]. Furthermore, bridging flocculation between the droplets had probably been induced which can also promote coalescence [182].

The addition of sodium chloride turned the aqueous solution into a poorer solvent for pectin which led to a stronger coiling of the pectin molecules. We assumed that this coiling might have resulted in a reduced volumetric emulsifier to oil ratio since pectin served as the only emulsifying agent. An increase in emulsifier concentration might thus have reduced the droplet size. In order to proof this hypothesis, emulsions with a higher concentration of pectin were prepared. At first, only the pectin concentration was increased in order to keep the ionic strength of the solution constant. Then, both pectin and NaCl concentration were increased equally in order to keep the same ratio between polyelectrolyte and counterions. The results are shown in Fig. 8.10. It can be seen that in both cases an increase in pectin concentration led to a strong decrease of the characteristic droplet size. More specifically, a doubling of the pectin concentration (2% w/w) resulted in a halving of the $d_{90,3}$. Comparing this droplet size with the one of the low ionic strength system (Fig. 8.5), it could be seen that by increasing the pectin concentration the same droplet size as in the low ionic strength system could be reached. Reducing the oil content had the same effect on the oil droplet sizes. In Fig. 8.10, the $d_{90,3}$ of an emulsion with only 5% oil at high ionic strength is depicted. Here, too, much smaller droplet sizes were achieved. This shows that by increasing the hydrocolloid emulsifier to oil ratio smaller droplet sizes can be stabilized even at higher ionic strength.

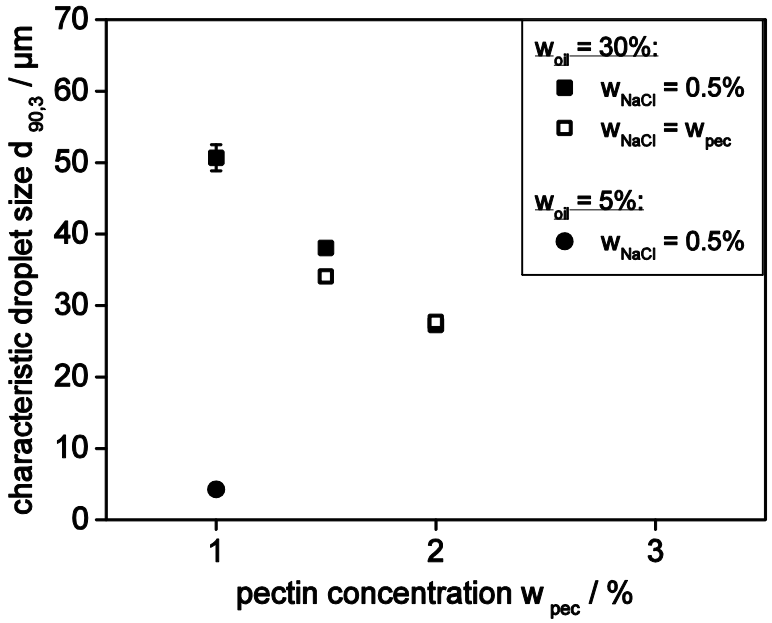

Figure 8.10: Characteristic droplet size d90,3 of emulsions prepared from pectin with DE = 55% at pH 4. The weight concentration of NaCl (full squares) or pectin+NaCl (empty squares) was varied in order to alter the emulsifier:oil ratio. The same was achieved by altering the weight concentration of oil (circle) at constant pectin+NaCl concentration.

If a stronger coiling of pectin resulted indeed in a reduced amount of available emulsifier, the question is what happens when NaCl was added to a low ionic strength emulsion *after* homogenization, whether that would induce microparticle formation on the interface and whether that would result in larger droplet sizes. In order to investigate that, a low ionic strength emulsion (DE = 55%, pH 4, 30% w/w oil) was taken and mixed with NaCl to obtain a salt concentration of 0.5% w/w in the emulsion. The droplet size of this emulsion was measured after 30 min of equilibration and was compared with the one of the original emulsion in Fig. 8.11. For better comparison, the $d_{90,3}$ of the emulsion already containing NaCl before homogenization is given as well. It can be seen that the exposure of a low ionic strength emulsion to a larger quantity of NaCl resulted in a significant increase of the $d_{90,3}$ to almost the same value as in the high ionic strength system.

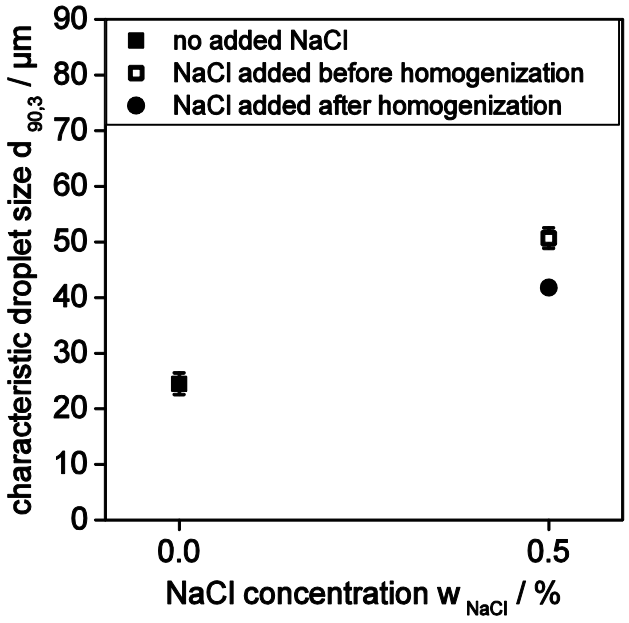

Figure 8.11: Characteristic droplet size $d_{90,3}$ of emulsions prepared from pectin with DE = 55% at pH4. The point of time at which NaCl was added to the emulsion was varied.

Reproducing this experiment under the microscope gave highly interesting results. For this, a droplet of the emulsion containing no additional salt was inspected. At a given moment, a second droplet, this time an aqueous solution of 1% w/w NaCl, was added in order to reach an overall concentration of about 0.5% w/w NaCl on the microscope slide. The sample was continuously inspected. Approximately 10 seconds after the addition of the salt solution, the first coalescence events were observed. Figure 8.12 displays a choice of three such coalescence events by showing in each case the clipping before and after the event. Several more of these events were seen in the following minutes. Of all emulsions investigated by microscopy, this was the only experimental setup in which coalescence of oil droplets was observed.

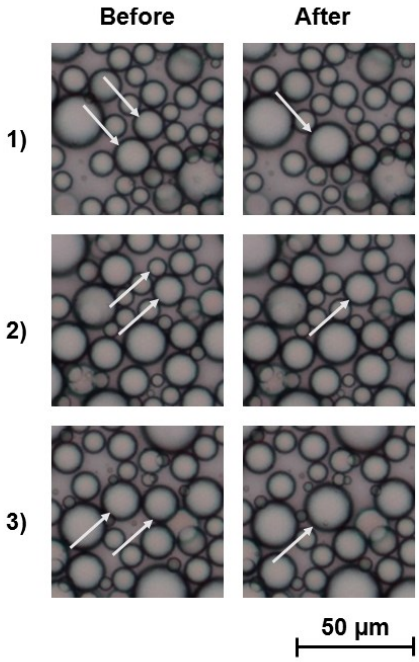

Figure 8.12: Three exemplary coalescence events taking place in a low ionic strength emulsion upon addition of NaCl. The emulsion was prepared from DE = 55% at pH 4. Obtained salt concentration upon addition of NaCl about 0.5% w/w. Pictures show the emulsion before and after the respective coalescence event. Arrows indicate droplets that will coalesce or have coalesced.

All in all, microscopy could confirm that a higher ionic strength led to an increase in oil droplet size due to coalescence. It seems plausible that this was caused by a change in conformation of pectin molecules at the interface due to an instant worsening of the solvent qualities of the aqueous phase. A comparable behavior (i.e. droplet size enlargement upon addition of a stimulus) was described for the pH responsiveness of cross-linked microgel particles from apple pectin [181]. However, these observations are not very well reflected by the dilational rheological measurements (Fig. 8.4). The main reason for this seems to be an effect of concentration. Dilational measurements were conducted at only a tenth of the pectin concentration. In order to understand these observations in terms of interfacial phenomena, further investigations are thus necessary. Therefore, dilational rheological experiments at higher concentrations should be conducted.

8.4 Conclusions

It could be shown that citrus pectins with different degrees of esterification exhibit excellent emulsifying properties and might be a suitable alternative for the production of stable food grade emulsions. Electrosorption strongly affected the adsorption of citrus pectins to the oil-water interface and that both hydrogen bonds and hydrophobic interactions influenced the interfacial elastic properties of pectin. The stabilizing mechanism by which citrus pectins acted was mainly steric. The steric stabilization was strongly influenced by the polyelectrolyte properties of citrus pectin. Pectin with a high DE was characterized by smaller hydrodynamic radii and faster

adsorption kinetics which resulted in the stabilization of the smallest observed droplet sizes. At pH 4, the surface charge of both oil droplets and pectin molecules was negative resulting in electrostatic repulsion and a much more difficult adsorption of pectin. Consequently, the largest droplet sizes were observed in this case. The 0.5% w/w NaCl solution presented a poor solvent for pectin. However, this did not result in the expected improved droplet stabilization. Due to the salt responsive behavior of citrus pectin, microgel particle formation at the oil droplet interface occurred instead. By this, oil droplets were not fully covered with the hydrocolloid emulsifier anymore which resulted in demulsification due to strong droplet coalescence. Droplet coalescence at high ionic strength could be reduced by using higher concentrations of pectin. Under these conditions, the formation of finely dispersed emulsions with microgel particles was possible. The findings bear huge implications for the industrial application of citrus pectin. On the one hand, the salt responsive behavior of citrus pectin might be exploited to create emulsions with tunable interfaces. However, it also poses challenges for the food industry. Several food products in which pectin might potentially be used as a hydrocolloid emulsifier undergo different production steps where a change in pH or ionic strength is encountered. An example might be the preparation of a salad sauce in which the emulsification step takes place before the addition of spices and conservatives. In such cases, a previously stable product might suddenly become instable due to enlarged droplet sizes and consequently faster creaming etc. This issue might be avoided by adjusting the pH to very low values (pH 2) or by choosing a pectin type that acts less salt responsive (very high DE).

8.5 Acknowledgements

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9 General discussion

Citrus pectin is a polyanionic hydrocolloid with large relevance for the food industry due to its excellent gelling and stabilizing properties [5]. Its molecular structure resembles that of a block copolymer so that it might successfully be used as a hydrocolloid emulsifier [62, 66]. Citrus pectin does not only increase the continuous phase viscosity but it also decreases the interfacial tension in an emulsion [184]. However, when evaluating the emulsifying properties of citrus pectin, the viscosity enhancing effect can pose a challenge. Highly viscous phases do not only improve emulsion stability but can also influence droplet breakup itself [20–22, 144]. Therefore, the emulsion droplet sizes that can be obtained using pectin might actually result from an overlay of both interfacial and bulk phase properties.

Therefore, in **chapter 5**, the influence of the thickening properties of citrus pectin on the emulsion droplet sizes obtained via high pressure homogenization was investigated. In these experiments, a small molecule emulsifier was added that was supposed to be the dominant interfacial active substance. By this, pectin was reduced to a pure stabilizer with viscosity enhancing effect. It could be shown that at effective viscosity ratios $0.05 < \lambda_{eff} < 0.17$ the produced emulsions all showed exactly the same very narrow droplet size distributions. This means that in this range of viscosity ratios, the emulsion viscosity did not show any difference in its effect on droplet breakup and subsequent stabilization. Therefore, in the second part of **chapter 5**, emulsions were adjusted to λ_{eff} values in the above range in order to compare only the emulsifying properties of pectins from different botanical origin. In these experiments, the importance of the protein moieties for the production of finely dispersed emulsions could be confirmed [95]. However, the results also indicated a significant effect of the degree of esterification DE on the measured droplet size. This finding is of importance because so far the DE had not been described to influence the emulsifying properties of pectin [8, 98]. The other supposedly influential molecular parameter, the molecular weight, did not show any significant effect. However, the reason for this might have been the type of investigated pectins. Compared to citrus and apple pectin, sugar beet pectin showed the most extreme values in terms of protein content and molecular weight. It was also expected to show a much higher degree of acetylation DAc. Although the exact values were not determined for citrus and apple pectin, it can be assumed that the DAc of those two pectin types is only about a fifth of the DAc of sugar beet pectin. On the whole, these unique features of sugar beet pectin might have caused the much smaller droplet sizes measured in the corresponding emulsions in this study. For citrus pectin with its lower protein content, these results meant that a modification of the molecular weight and DAc might still affect the emulsifying properties. More detailed investigations would thus be needed. Furthermore, an influence of DE was found but the mechanism behind it still needed to be clarified. Finally, from the results, one would expect better emulsifying properties of citrus pectin if it was possible to increase its protein content.

The influence of protein content is addressed in **chapter 6**. In order to increase the protein content of citrus pectin, the polysaccharide was covalently bound to whey protein isolate (WPI) in a dry heating process. Citrus pectins with different DE were

used in order to investigate the combined effects of DE and protein content. In comparison to non-conjugated citrus pectin, the WPI-pectin conjugates were expected to present block copolymers with a larger difference in the hydrophobicity of their blocks. The protein fraction should then form a strong anchor at the oil droplet interface while the polysaccharide moiety should form a long tail protruding into the continuous phase and providing steric and electrostatic stabilization. It was found that WPI-pectin conjugates showed indeed improved emulsifying properties compared to the raw material. At pH 7 – a pH value at which WPI shows excellent emulsifying properties – conjugates were able to stabilize even smaller droplets than WPI alone. The improved emulsifying properties of WPI-pectin mixtures were attributed to the presence of proteinaceous components of high molecular weight. However, no influence of the DE on the reduction of oil droplet sizes was found. Since the DE and thus the molecule charge did not influence the oil droplet size, the stabilizing mechanism was suggested to be mainly steric. This also means that all investigated pectins presented hydrophilic blocks that were large enough for a successful steric stabilization. While an influence of DE on the droplet size could not be seen, the DE did influence the yield of WPI-pectin conjugates. Citrus pectin with a low DE seemed to increase the conjugate yield. The reason for this might be the lower molecular weight of low DE citrus pectins. During the industrial processing of pectin, depolymerization occurs simultaneously to demethylation [5]. A lower molecular weight therefore means that more molecules and thus more reaction partners are available when the same pectin mass is used for conjugation. A correlation of the characteristic droplet size of emulsions with the conjugate yield showed that finer emulsions can be produced when the yield is higher. This means that the measured droplet sizes most likely depend on the amount of available emulsifier. However, effects of unreacted WPI cannot be entirely ruled out as dry heated WPI-pectin mixtures had not been purified before emulsification. In future experiments, it would therefore be interesting to separate conjugates from the protein-pectin mixture via e.g. size exclusion chromatography. Purified WPI-pectin conjugates could then be used for a deeper investigation of their interfacial and emulsifying properties. For example, it would be interesting to study their rheological behaviour at the oil-water interface. WPI is known to form a strong viscoelastic film at the interface [185]. Information on the effect of conjugation on this film would be important for understanding the improved emulsifying properties of conjugates compared to pure WPI.

So far, the production of WPI-pectin conjugates has not yet been industrially implemented. This means that such conjugates are not available in larger quantities at the moment [111]. It was thus interesting to study how the emulsifying properties of citrus pectin with naturally low protein content might be improved using industrially available processes. For this purpose, citrus pectin with a DE of 70% was used as a raw material. Then, its DE and DAc were each stepwise increased and in a third line of experiments the molecular weight was reduced. The first two modifications were supposed to increase the hydrophobic character of the molecule and thus increase pectin's affinity to the oil-water interface. Depolymerization was expected to increase the adsorption kinetics so that a faster droplet stabilization in the high pressure homogenization process might be possible. The results concerning the emulsifying properties are explained in detail in **chapter 7**. Briefly, both an increase in DE and in

DAc led to emulsions with smaller characteristic droplet sizes. The influence of DE on the emulsifying properties of citrus pectin first mentioned in chapter 5 could thus be confirmed. Above a DE of 80%, a further increase in DE did not show strong effects anymore. This might be due to steric effects that determine how pectin molecules arrange themselves at the oil-water interface. Above a certain degree of substitution, conformational aspects will make it impossible for the polymer to achieve that all hydrophobic groups will get in contact with the oil phase at the same time [186]. In case of pectin methoxylation, this critical degree of substitution is possibly 80%.

Increasing the DAc reduced the emulsion droplet size, but it was not possible to obtain the same small droplets as by increasing the DE. Although acetylation of citrus pectin raised the DAc to about 14%, it was not possible to reach the same value that was found in the commercial SBP studied in chapter 5 (DAc around 25%) due to technical limitations. A stronger acetylation to values above 14%, however, might result in even smaller droplets. Therefore, it would be interesting to use an enzymatic treatment to increase the DAc so that milder reaction conditions can be chosen [187]. Furthermore, enzymatic treatment might induce a blockwise distribution of acetyl groups while a chemical modification will only result in a random distribution. A blockwise distribution pattern of acetyl groups might be advantageous for the emulsifying properties of pectin as more distinctly hydrophobic regions can be created this way. Furthermore, such a distribution pattern has recently been confirmed for SBP [188].

In contrast to previous reports, a depolymerization of pectin did not improve the emulsifying properties [98]. Instead larger droplet sizes were measured when the molecular weight of pectin was strongly reduced. This probably originated from a reduced steric stabilization upon depolymerization. The assumed faster adsorption kinetics could not be confirmed by the experiments. It might be possible that interactions with sucrose covered the results. Sucrose was used to equilibrate the viscosity ratio as previously described in chapter 5. It promotes chain-chain interactions of pectin molecules which might have resulted in the formation of larger pectin aggregates [63, 189]. These aggregates might have behaved like larger molecules, thereby eliminating the possibly positive effects of depolymerization. This underlines the complexity of pectin's behaviour in the emulsification process and its sensitivity to changes in formulation. It also shows the necessity to investigate the emulsifying properties of sucrose-free pectin solutions.

The emulsifying properties of citrus pectin solutions free of sucrose are described in **chapter 8**. Pectins with three different DE (55%, 70%, 84%) but otherwise comparable molecular features were investigated in aqueous solutions of different pH and ionic strength. By changing the properties of the aqueous solution, the solvent qualities for pectin were supposed to change. Under poorer solvent conditions, pectin's affinity to the oil phase was expected to increase which should result in better emulsification. In order to investigate this hypothesis, the properties of citrus pectins in solution, at the oil-water interface and in actual emulsions were studied. It could be shown that by altering the solution conditions the colloidal properties of citrus pectins could be tuned. Both hydrodynamic radius and absolute value of the zeta potential were lowest for high DE, low pH and high ionic strength. These conditions correlate with a low number of dissociated carboxyl groups. At the same conditions, the fastest adsorption

kinetics at the oil-water interface and the thickest adsorption layer at the particle surface were measured. However, the elastic properties of citrus pectins at the oil-water interface showed a more complex behaviour. The pectin with the highest DE did practically not show any change in elastic behaviour upon alteration of the solution conditions. The elastic properties of the pectin with the lowest investigated DE (55%), however, were very sensitive particularly to changes in pH. On the one hand, the interfacial elasticity was higher than for pectin with DE = 84% when the low DE pectin was able to form hydrogen bonds (low pH). On the other hand, much lower interfacial elasticity values were detected at high pH when less hydrogen bonds could be formed. Compared to high DE pectin, low DE pectin also possessed less methoxyl groups and thus less hydrophobic interactions that might counterbalance the absence of hydrogen bonds. Furthermore, zeta potential measurements of pectin molecules and of the bare oil droplets revealed that at pH 4 both pectins and oil droplets are negatively charged. The resulting repulsion between both entities might also have caused the low interfacial elasticities. Moreover, these results point towards electrosorption as an important feature in the adsorption mechanism of citrus pectins.

In the emulsification experiments, smaller droplets were detected in emulsions prepared from high DE pectin at low pH. These are the same conditions that favour fast adsorption kinetics and high oil phase affinity. It also confirms that the main stabilizing mechanism of pectin is steric. If electrostatic repulsion had been the dominant mechanism, smaller droplets would have been expected at solution conditions where pectin did not show zero charge.

It could be seen that by reducing the pH and thus poorer solvent quality, pectin shows improved emulsifying behaviour. However, a stronger degradation of the solvent quality by sodium chloride addition did not reduce the droplet size further. Instead, salting out effects were seen that resulted in microgel particle formation in the sample with DE = 55% at pH 4. These microgel particles were not able to sufficiently stabilize emulsion droplets despite their high affinity to the oil phase as indicated by adsorption kinetics. It was suspected that an aggregation of pectin molecules into microgel particles caused an alteration of the ratio between citrus pectin and oil compared to the salt-free system. Therefore, the hydrocolloid emulsifier concentration was increased at a given oil concentration which indeed resulted in decrease of droplet size.

The high sensitivity of citrus pectin to changes in ionic strength could also be studied under the microscope. Upon subsequent ion addition to a salt-free emulsion, coalescence events were visually observed. It is assumed that these coalescence events occurred due to conformational changes of adsorbed pectin molecules when the solvent quality is altered. At poorer solvent conditions, pectin molecules might contract, form random coils and become flexible which might result in the appearance of bare areas at the interface. In turn, such bare areas promote coalescence. In order to better understand these observations, further studies at the oil-water interface are necessary. For example, it would be interesting to reproduce this set up in a dilational rheological experiment. Then changes in the interfacial elasticity upon sodium chloride addition could be monitored. Furthermore, it would be interesting to investigate citrus pectins with even lower DE in order to enhance microgel particle

formation. However, depolymerization occurs alongside with chemical deesterification. Therefore, the influence of molecular weight on the interfacial properties of citrus pectin should be thoroughly studied first. In order to neglect the influence of molecular weight, it would also be possible to use enzymes for the deesterification of pectin. In this context, pectin could be deesterified in a way that a blockwise distribution of methoxyl groups is created along the pectin backbone [190, 191]. A blockwise distribution of methoxyl groups has already been shown to influence the functional properties of citrus pectin concerning its gelling properties [67, 191]. The effects of a blockwise distribution on the interfacial and emulsifying properties, however, still need to be discovered [8].

Altogether, it was found that citrus pectin shows great potential as a hydrocolloid emulsifier and might be used instead of sugar beet pectin when the formulation is adjusted. The emulsifying properties of citrus pectin can be improved when the hydrophobic character of the molecule is increased. This can be achieved by increasing the protein content, the DE and to a minor extent also the DAc of the molecules. In solution, pectin was found to show typical polyelectrolyte behaviour. Although electrostatic effects play a significant role in the adsorption mechanism of citrus pectins, their stabilizing mechanism was found to be mainly steric. By properly accounting for their polyelectrolyte properties, the interfacial and emulsifying behavior of citrus pectins can strongly be altered. Due to their high sensitivity to ionic strength, citrus pectins might even be used for the production of stimuli responsive emulsions [192]. Such emulsions are very interesting for pharmaceutical applications as they allow for a targeted release of bioactives [182]. For such applications, very fine emulsion microstructures are required. These can be achieved by high pressure homogenization of citrus pectin emulsions with increased viscosity by e.g. sucrose addition. However, for a target emulsion production and subsequent controlled release properties, further studies on the interactions of citrus pectins with non-ionic cosolutes at the oil-water interface will be necessary.

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11 List of Abbreviations

AFM	Atomic Force Microscopy
AP	Apple pectin
α-LA	Alpha-Lactalbumin
β-LG	Beta-Lactoglobulin
CP	Citrus pectin
DAc	Degree of Acetylation
DE	Degree of Esterification
D_{eff}	Effective diffusion coefficient
DSD	Droplet size distribution
GalA	Galacturonic acid
GA	Gum Arabic
HG	Homogalacturonan
HM	High methylesterified
IEP	Isoelectric point
LM	Low methylesterified
MF	Melamin fluoride
MM	Medium methylesterified
O/W	Oil-in-water
PAGE	Polyacrylamide gel electrophoresis
RGI	Rhamnogalacturonan I
RGII	Rhamnogalacturonan II
RH	Relative humidity
R_h	Hydrodynamic radius
Rha	Rhamnose
SBP	Sugar beet pectin
SDS	Sodium dodecyl sulfate
T20	Tween® 20
VHMCP	Very high methylesterified citrus pectin
WPI	Whey protein isolate
ZP	Zeta potential

12 Summary and Outlook

Many food products that consumers appreciate, such as sauces, spreads or desserts, are emulsion based. These food emulsions are mostly oil-in-water emulsions and thermodynamically instable so that eventually a separation of oil and water phase can occur. To overcome this issue, industrially produced food emulsions contain emulsifiers and stabilizers to improve the stability. Many substances are available that fulfil the purpose of either being an emulsifier or stabilizer. However, with both increasing awareness and changing demands of consumers, the food industry constantly strives to enhance existing and furthermore develop alternative natural food ingredients. Citrus pectin is a natural biopolymer that is one of the most used gelling and stabilizing agents in the food industry. However, much less is known about its emulsifying properties. Pectins from different botanical origins can vary significantly in their molecular structure particularly concerning the type and amount of functional groups. These differences in the molecular structure are already known to have a strong impact on the stabilizing and gelling properties of pectins. Therefore, results concerning the emulsifying properties of one type of pectin cannot simply be transferred to another. In order to enlarge the range of available natural food emulsifiers, citrus pectin was investigated as an example concerning its emulsifying and emulsion stabilizing properties. These properties were studied in emulsions that were produced by high pressure homogenization. Emulsification in high pressure homogenizers is typically carried out in industry to obtain emulsions with very finely dispersed droplets. It is a highly dynamic process that requires a fast and efficient droplet stabilization. Therefore, it is also a suitable process for screening substances concerning their emulsifying properties under extreme conditions.

Citrus pectin is a polyanionic hydrocolloid with a molecular structure resembling that of a block copolymer. Its functional properties are controlled by the molecular weight and by the amount of several functional groups the most important of them being the covalently bound protein moieties, methoxyl groups and acetyl groups. When used as a stabilizer in emulsions, citrus pectin does not only increase the continuous phase viscosity but it also decreases the interfacial tension so that it might successfully be applied as a hydrocolloid emulsifier. However, when evaluating the emulsifying properties of citrus pectin, the viscosity enhancing effect can pose a challenge. Highly viscous phases do not only improve emulsion stability but can also influence droplet breakup itself. As a result, the emulsion droplet sizes that can be obtained using pectin might actually be caused by an overlay of both interfacial and bulk phase properties.

Therefore, the influence of the thickening properties of citrus pectin on the emulsion droplet sizes obtained via high pressure homogenization was investigated first. For this purpose, emulsions that also contained a small molecule emulsifier were prepared. The small molecule emulsifier was supposed to be the dominant interfacial active substance so that pectin was reduced to a pure stabilizer with viscosity enhancing effect. It could be shown that at effective viscosity ratios $0.05 < \lambda_{eff} < 0.17$ the produced emulsions all showed exactly the same very narrow droplet size distributions. This means that in this range of viscosity ratios, the emulsion viscosity

did not show any difference in its effect on droplet breakup and subsequent stabilization.

In the following experiments, emulsions were adjusted to λ_{eff} values in the range mentioned above in order to compare only the emulsifying properties of different pectins. Commercially available pectin types from different botanical origin were investigated: Sugar beet pectin as well as apple and citrus pectins with different degree of esterification (DE). Sugar beet pectin was chosen as a reference because its good emulsifying properties had already been described. Compared to sugar beet pectin, citrus pectin differs in most of the important molecular parameters. It has a lower protein content and degree of acetylation (DAc) as well as a higher molecular weight. In the experiments, significant differences in the emulsifying properties depending on the type and amount of functional groups were indeed found. The importance of the covalently bound protein moieties for the production of finely dispersed emulsions could be confirmed. Moreover, the results indicated a significant effect of the DE on the measured droplet size although the mechanism behind it remained unclear. This finding is of importance because so far the DE had not been described as influencing the emulsifying properties of pectin. The DAc and the molecular weight, did not show any significant influence on the obtained droplet size. From these results, two separate lines of investigation were followed. On the one hand, the question arose whether the emulsifying properties of citrus pectin can be enhanced by increasing its protein content. On the other hand, citrus pectin low in protein content should be systematically investigated concerning the remaining functional groups. This could help to both improve its emulsifying properties and understand the acting mechanism of the methoxyl groups.

In order to increase the protein content of citrus pectin, the polysaccharide was covalently bound to whey protein isolate (WPI) in a dry heating process (conjugation). Citrus pectins with different DE were used in order to investigate the combined effects of DE and protein content. In comparison to non-conjugated citrus pectin, the WPI-pectin conjugates were expected to present block copolymers with a large difference in the hydrophobicity of the blocks. The protein fraction should form a strong anchor at the oil droplet interface while the polysaccharide moiety should form a long tail protruding into the continuous phase and providing steric and electrostatic stabilization. It was found that WPI-pectin conjugates showed indeed improved emulsifying properties compared to the raw material. At pH 7 – a pH value at which WPI shows excellent emulsifying properties – conjugates were able to stabilize even smaller droplets than WPI alone. The improved emulsifying properties of WPI-pectin mixtures were attributed to the presence of proteinaceous components of high molecular weight. However, no influence of the DE on the reduction of oil droplet sizes was found. Since the DE and thus the molecule charge did not influence the oil droplet size, the stabilizing mechanism was suggested to be mainly steric. This also means that all investigated pectins presented hydrophilic blocks that were large enough for a successful steric stabilization. While an influence of DE on the droplet size could not be seen, the DE did influence the yield of WPI-pectin conjugates. Citrus pectin with a low DE seemed to create a higher conjugate yield. The reason for this might be the lower molecular weight of low DE citrus pectins. During the industrial

processing of pectin, depolymerization occurs simultaneously to demethylation. A lower molecular weight therefore means that more molecules and thus more reaction partners are available when the same pectin mass is used for conjugation. A correlation of the characteristic droplet size of emulsions with the conjugate yield showed that finer emulsions can be produced when the yield is higher. This means that the measured droplet sizes most likely depend on the amount of available emulsifier. However, effects of unreacted WPI cannot be entirely ruled out as dry heated WPI-pectin mixtures had not been purified before emulsification. In future experiments, purified WPI-pectin conjugates could be used for a deeper investigation of their interfacial and emulsifying properties.

In order to investigate how the emulsifying properties of citrus pectin with naturally low protein content might be improved using industrially available processes, citrus pectin was chemically and thermally modified. A citrus pectin with a DE of 70% was used as a raw material. Then, its DE and DAc were each stepwise increased and in a third line of experiments the molecular weight was reduced. The first two modifications were supposed to increase the hydrophobic character of the molecule and thus increase pectin's affinity to the oil-water interface. Depolymerization was expected to increase the adsorption kinetics so that a faster droplet stabilization in the high pressure homogenization process might be possible. It was found that both an increase in DE and in DAc led to emulsions with smaller characteristic droplet sizes. The influence of DE on the emulsifying properties of citrus pectin mentioned above could thus be confirmed. Increasing the DAc reduced the emulsion droplet size, but it was not possible to obtain the same small droplets as by increasing the DE. Although acetylation of citrus pectin raised the DAc to about 14%, it was not possible to reach the same value that was found in the commercial SBP sample used as a reference (DAc around 25%) due to technical limitations. In future research, enzymatic treatment might be an option because here milder reaction conditions can be chosen and a higher DAc might be reached. In contrast to previous reports, a depolymerization of pectin did not improve the emulsifying properties. Instead larger droplet sizes were measured when the molecular weight of pectin was strongly reduced. This probably originated from a reduced steric stabilization upon depolymerization. The assumed faster adsorption kinetics could not be confirmed by the experiments.

The above results showed that in citrus pectin with low protein content the DE is the most influential functional group for obtaining small emulsion droplets. More detailed investigations on the underlying mechanism were thus conducted. Citrus pectins with three different DE (55%, 70%, 84%) but otherwise comparable molecular features were investigated in aqueous solutions of different pH and ionic strength. By changing the properties of the aqueous solution, the solvent qualities for pectin were supposed to change. Under poorer solvent conditions, pectin's affinity to the oil phase was expected to increase which should result in better emulsification. In order to investigate this hypothesis, the properties of citrus pectins in solution, at the oil-water interface and in actual emulsions were studied. It could be shown that by altering the solution conditions the colloidal properties of citrus pectins could be tuned. Both hydrodynamic radius and absolute value of the zeta potential were lowest for high DE, low pH and high ionic strength. These conditions correlate with a low number of

dissociated carboxyl groups. At the same conditions, the fastest adsorption kinetics at the oil-water interface and the thickest adsorption layer at the particle surface were measured. However, the elastic properties of citrus pectins at the oil-water interface showed a more complex behaviour. The pectin with the highest DE did practically not show any change in elastic behaviour upon alteration of the solution conditions. The elastic properties of the pectin with the lowest investigated DE (55%), however, were very sensitive particularly to changes in pH. This was concluded to be due to repulsion between pectin molecules and the bare oil droplet surface at pH 4. At this pH, zeta potential measurements showed that both the pectin molecules and the bare oil droplets were strongly negatively charged. This points towards electrosorption as an important feature in the adsorption mechanism of citrus pectins.

In the emulsification experiments, smaller droplets were detected in emulsions prepared from high DE pectin at low pH. These are the same conditions that favour fast adsorption kinetics and high oil phase affinity. It also confirms that the main stabilizing mechanism of pectin is steric. If electrostatic repulsion had been the dominant mechanism, smaller droplets would have been expected at solution conditions where pectin did not show zero charge.

It could be seen that by reducing the pH and thus poorer solvent quality, pectin shows improved emulsifying behaviour. However, a stronger degradation of the solvent quality by sodium chloride addition did not reduce the droplet size further. Instead, salting out effects were seen that resulted in microgel particle formation in the sample with DE = 55% at pH 4. These microgel particles were only able to sufficiently stabilize emulsion droplets when the hydrocolloid emulsifier concentration was significantly increased.

The high sensitivity of citrus pectin to changes in ionic strength could also be studied under the microscope. On the one hand, droplets stabilized by microgel particles could be visualized. On the other hand, coalescence events were observed upon subsequent ion addition to a salt-free emulsion.

Altogether, it was found that citrus pectin shows great potential as a hydrocolloid emulsifier. Its emulsifying properties can be improved by increasing the hydrophobic character of the molecule. This can be achieved by increasing the protein content, the DE and to a minor extent also the DAc. In solution, pectin was found to show typical polyelectrolyte behaviour. Although electrostatic effects play a significant role in the adsorption mechanism of citrus pectins, their stabilizing mechanism was found to be mainly steric. By properly accounting for their polyelectrolyte properties, the interfacial and emulsifying behavior of citrus pectins can strongly be altered. Due to their high sensitivity to ionic strength, citrus pectins might even be used for the production of stimuli responsive emulsions. Such emulsions are very interesting for pharmaceutical applications as they allow for a targeted release of bioactives. For such applications, very fine emulsion microstructures are required which can be achieved by high pressure homogenization.

13 Zusammenfassung und Ausblick

Emulsionen sind thermodynamisch instabile Systeme, bei denen eine disperse Phase feinverteilt in einer kontinuierlichen Phase vorliegt. Um die Stabilität solcher Systeme zu erhöhen, werden häufig Emulgatoren und Stabilisatoren eingesetzt. Verschiedenste Stoffe stehen für diesen Zweck zur Verfügung. Mit den wachsenden Ansprüchen der Konsumenten und ihrem Wunsch nach ausschließlich natürlichen Inhaltsstoffen, besteht jedoch ein hoher Bedarf an natürlichen Emulgatoren für Lebensmittelprodukte. Citruspektin ist eines der in Lebensmitteln am häufigsten verwendeten Gelier- und Verdickungsmittel. Seine emulgierenden Eigenschaften sind jedoch nur unzureichend verstanden. Je nach pflanzlicher Herkunft gibt es große Unterschiede in der molekularen Struktur verschiedener Pektine insbesondere bezüglich der Art und Anzahl funktioneller Gruppen. Da von diesen strukturellen Unterschiede bereits bekannt ist, dass sie einen starken Einfluss auf die Gelier- und Stabilisierungseigenschaften haben, können Ergebnisse bezüglich des Emulgierverhaltens eines bestimmten Pektintyps nicht einfach auf einen anderen Pektintyp übertragen werden. Stattdessen sind eingehende Untersuchungen notwendig, um Citruspektin als möglichen alternativen Emulgator zu verwenden.

Zur Untersuchung der emulgierenden und emulsionsstabilisierenden Eigenschaften von Citruspektin wurden Emulsionen mit einem Hochdruckhomogenisator hergestellt. Der Hochdruckhomogenisations-prozess wird typischerweise verwendet, um besonders fein verteilte Emulsionen herzustellen. Es handelt sich dabei um einen sehr dynamischen Prozess, der hohe Anforderungen an den eingesetzten Emulgator stellt, da dispergierte Tropfen besonders schnell und effizient stabilisiert werden müssen. Der Prozess ist daher gut geeignet, um Emulgatoren bezüglich dieser Eigenschaften unter extremen Bedingungen zu untersuchen.

Bei Citruspektin handelt es sich um ein polyanionisches Polysaccharid, das in seiner molekularen Struktur einem Blockcopolymer ähnelt. Die funktionellen Eigenschaften von Citruspektin werden hauptsächlich durch das molekulare Gewicht sowie durch die Anzahl funktioneller Gruppen bestimmt. Hierbei sind insbesondere kovalent gebundene Protein-, Methylester- und Acetylgruppen von Bedeutung. Wird Citruspektin als emulsionsstabilisierende Substanz verwendet, kommt es nicht nur zu einer Erhöhung der Viskosität der kontinuierlichen Emulsionsphase sondern es kann auch ein Absinken der Grenzflächenspannung zwischen Öl- und Wasserphase festgestellt. Dies deutet darauf hin, dass Citruspektin evtl. auch als Hydrokolloidemulgator verwendet werden kann. Bei der Untersuchung der emulgierenden Eigenschaften von Citruspektin stellt die viskositätserhöhende Wirkung jedoch gleichzeitig eine Herausforderung dar: Eine hochviskose kontinuierliche Phase wirkt sich nicht nur auf die Stabilität der fertigen Emulsion aus, sondern kann u.U. bereits den eigentlichen Tropfenaufbruch im Hochdruckhomogenisationsprozess beeinflussen. Die beobachtbaren Tropfengrößen der mit Citruspektin stabilisierten Emulsion können daher auch durch eine Überlagerung von Grenzflächen- und Bulkphaseneffekten zustande gekommen sein.

In der vorliegenden Arbeit wurde daher zunächst der Einfluss der verdickenden Eigenschaften von Citruspektin auf die mittels Hochdruckhomogenisation erzielbaren

Tropfengrößen einer Modellemulsion untersucht. Die hierzu hergestellten Emulsionen enthielten zusätzlich einen kurzkettigen synthetischen Emulgator, der die hauptsächlich grenzflächenaktive Substanz darstellt. Dadurch konnten die emulsionsstabilisierenden Eigenschaften von Citruspektin getrennt von den emulgierenden Eigenschaften untersucht werden. Es konnte gezeigt, dass bei einem effektiven Viskositätsverhältnis von $0{,}05 < \lambda_{eff} < 0{,}17$ Emulsionen mit der gleichen engen Tropfengrößenverteilung hergestellt werden können. In der genannten Spanne von λ_{eff} weisen die verdickenden Eigenschaften von Citruspektin daher keine signifikanten Unterschiede bezüglich des Tropfenaufbruchs und der anschließenden Tropfenstabilisierung auf.

Um anschließend die emulgierenden Eigenschaften unterschiedlicher Pektine zu vergleichen, wurde λ_{eff} der Emulsionen in den folgenden Versuchen auf Werte zwischen 0,05 und 0,17 eingestellt. Verschiedene kommerziell erhältliche Pektine unterschiedlicher pflanzlicher Herkunft wurden untersucht: Apfel- und Citruspektine mit unterschiedlichem Veresterungsgrad (VEG) wurden mit Zuckerrübenpektin als Referenzsubstanz verglichen, da dessen gute emulgierende Eigenschaften bereits beschrieben waren. Zuckerrübenpektin unterscheidet sich jedoch in fast allen funktionellen Parametern stark von Citruspektin: Es hat ein niedrigeres Molekulargewicht sowie einen höheren Proteingehalt und Acetylierungsgrad (AcG). Eine Auswirkung dieser strukturellen Unterschiede auf das Emulgierverhalten der einzelnen Pektine konnte in den durchgeführten Experimenten dann auch festgestellt werden. Die positive Wirkung eines hohen Proteinanteils auf die Stabilisierung kleiner Tropfen konnte bestätigt werden. Darüber hinaus wurde ein signifikanter Einfluss des VEG auf die Tropfengröße festgestellt. Dieses Ergebnis ist von großer Tragweite, da dem VEG bisher keinerlei Bedeutung für die Emulgierwirkung von Citruspektin beigemessen wurde. Der AcG sowie das molekulare Gewicht der Pektine hatten keinen signifikanten Einfluss auf die Tropfengröße der Emulsion. Aus diesen Ergebnissen konnten zwei wichtige Fragestellungen abgeleitet werden: Zum einen stellte sich die Frage, ob sich durch eine Erhöhung des Proteinanteils die emulgierenden Eigenschaften von Citruspektin verbessern ließen. Zum anderen wurde die Notwendigkeit einer systematischen Untersuchung von Citruspektin mit geringem Proteinanteil deutlich. Der Einfluss der übrigen molekularen Parameter (VEG, AcG, molekulares Gewicht) auf das Emulgierverhalten sollte untersucht werden, wobei die Aufklärung des Wirkungsmechanismus des VEG im Vordergrund stand.

Um den Proteinanteil von Citruspektin zu erhöhen, wurde Molkenproteinisolat (engl.: whey protein isolate – WPI) durch thermische Behandlung kovalent an das Polysaccharid gebunden. Zur Herstellung dieser Protein-Polysaccharid-Konjugate wurden Citruspektine mit unterschiedlichem VEG verwendet, um gleichzeitig den Einfluss dieser funktionellen Gruppen zu betrachten. Die WPI-Pektin-Konjugate sollten in den anschließend hergestellten Emulsionen wie Blockcopolymere mit stark unterschiedlicher Hydrophobizität der einzelnen Blöcke wirken. Es wurde erwartet, dass die Proteingruppen stark an die Öltropfenoberfläche binden, während die Polysaccharidgruppen weit in die umgebende kontinuierliche Phase hineinragen. Dadurch sollte sowohl eine gute sterische als auch eine gute elektrostatische

Stabilisierung der Tropfen gewährleistet werden. Es konnte gezeigt werden, dass WPI-Pektin-Konjugate tatsächlich verbesserte emulgierende Eigenschaften im Vergleich zu den Ausgangsmaterialien aufwiesen. U.a. konnten bei pH 7 in mit Konjugaten hergestellten Emulsionen kleinere Tropfen gemessen werden als in nur mit WPI hergestellten Emulsionen. Dies ist insofern bedeutend als das pH 7 bereits einen pH-Wert darstellt, an dem WPI ausgezeichnete emulgierende Eigenschaften aufweist. Die verbesserten emulgierenden Eigenschaften der WPI-Pektin-Mischungen konnten auf das Vorhandensein hochmolekularer proteinhaltiger Komponenten zurückgeführt werden. Allerdings konnte kein Einfluss des VEG und damit auch kein Einfluss der Molekülladung auf die Tropfengröße nachgewiesen werden. Daraus wurde geschlussfolgert, dass der zugrunde liegende Stabilisierungsmechanismus hauptsächlich sterisch sein musste. Der VEG des Citruspektins zeigte einen großen Effekt bezüglich der Konjugatausbeute, welche sich bei Verwendung eines Pektins mit niedrigem VEG beträchtlich steigern ließ. Dies wurde auf das gleichzeitig niedrigere Molekulargewicht von Citruspektin mit niedrigem VEG zurückgeführt. Im industriellen Herstellungsprozess kommt es während der Reduktion des VEG von Pektin gleichzeitig zu einem gewissen Maß an Depolymerisation. Bei gleicher eingesetzter Pektinmasse bedeutete dies für die Konjugation mit WPI, dass mehr Reaktionspartner zur Verfügung standen und anscheinend mehr Konjugat gebildet werden konnte. Eine Korrelation der erzielten Emulsionstropfengrößen mit der Konjugatausbeute zeigte eine starke Reduktion der Tropfengröße bei zunehmender Ausbeute. Unterschiede in den Tropfengrößen wurden daher hauptsächlich auf die vorhandene Menge an verfügbarem Emulgator zurückgeführt. Allerdings konnte ein Einfluss von noch vorhandenem unkonjugierten WPI nicht ausgeschlossen werden, da die hitzebehandelten WPI-Pektin-Mischungen vor dem Emulgierprozess nicht aufgereinigt wurden. In weiteren Untersuchungen wäre es daher interessant, aufgereinigte Konjugatproben tiefer gehenden Analysen insbesondere bezüglich der Grenzflächeneigenschaften zu unterziehen.

Um die emulgierenden Eigenschaften von Citruspektin mit niedrigem Proteingehalt zu modifizieren, wurde ein Citruspektin mit einem VEG von 70 % verschiedenen thermischen und chemischen Behandlungen ausgesetzt. Der VEG sowie der AcG wurden schrittweise erhöht und das molekulare Gewicht wurde reduziert. Die ersten beiden Modifikationen dienten dazu, den hydrophoben Charakter des Pektinmoleküls und damit seine Affinität zur Ölphase zu erhöhen. Eine Depolymerisation von Citruspektin sollte die Adsorptionsgeschwindigkeit des Moleküls erhöhen und somit zu einer schnelleren Tropfenstabilisierung im Hochdruckhomogenisationsprozess beitragen. Es konnte gezeigt werden, dass eine Erhöhung des VEG zu Emulsionen mit kleineren Tropfen führt, sodass der oben erwähnte Einfluss des VEG auf das Emulgierergebnis bestätigt werden konnte. Auch eine Erhöhung des AcG reduzierte die Tropfengröße. Allerdings ließen sich nicht dieselben feindispersen Emulsionen wie unter Verwendung von Pektin mit erhöhtem VEG herstellen. Zwar konnte der AcG des Citruspektins auf ca. 14 % gesteigert werden. Die für Zuckerrübenpektin typischen Werte von ca. 25 % ließen sich jedoch nicht erreichen. Grund hierfür waren die harschen Reaktionsbedingungen, die bei weiterem Fortschreiten der Acetylierungsreaktion eine starke Degradation des Pektins hätten verursachen können. In weiteren Versuchen wäre es daher interessant, zu

untersuchen, ob sich der AcG durch die Verwendung geeigneter Enzyme und damit bei milderen Reaktionsbedingungen weiter erhöhen ließe. Im Gegensatz zu früheren Studien konnte kein positiver Effekt einer Molekulargewichtsreduktion nachgewiesen werden. Stattdessen wurden deutlich größere Tropfen bei Verwendung von stark depolymerisiertem Pektin gemessen. Dies wurde auf eine reduzierte sterische Stabilisierung aufgrund kürzerer Polysaccharidketten zurückgeführt. Die vermutete schnellere Adsorptionskinetik von Citruspektin konnte daher nicht bestätigt werden.

Die beschriebenen Ergebnisse zeigten, dass bei Citruspektin mit niedrigem Proteingehalt insbesondere der VEG für die Herstellung von Emulsionen mit möglichst kleinen Tropfen von Bedeutung ist. Daher wurde im nächsten Schritt untersucht, worauf der Wirkungsmechanismus des VEG beruhte. Hierzu wurden drei Citruspektine mit unterschiedlichem VEG (55 %, 70 %, 84 %) ansonsten jedoch gleichen molekularen Parametern ausgewählt und in wässrigen Lösungen mit unterschiedlichem pH-Wert und unterschiedlicher Ionenstärke untersucht. Die Lösungsmittelqualität der Wasserphase für das Pektin sollte dadurch variiert werden. Bei schlechter Lösungsmittelqualität wurde eine höhere Affinität des Pektins zur Ölphase und somit eine verbesserte emulgierende Wirkung erwartet. Untersuchungen wurden sowohl am Pektin in der Bulkphase als auch an der Öl-Wasser-Grenzfläche durchgeführt. Es konnte gezeigt werden, dass durch eine Änderung der Lösungsmittelqualität die kolloidalen Eigenschaften von Citruspektin gezielt eingestellt werden können. Für Citruspektin mit niedrigem VEG wurden bei niedrigem pH und hoher Ionenstärke sowohl der geringste hydrodynamische Radius als auch der geringste Betrag des Zetapotentials gemessen. Ursache hierfür war, dass bei den genannten Lösungsmittelbedingungen nur wenige Carboxylgruppen des Pektins dissoziert vorliegen. Unter denselben Bedingungen konnten auch die schnellste Adsorptionskinetik des Pektins an der Öl-Wasser-Grenzfläche sowie die dickste Adsorptionsschicht festgestellt werden. Die grenzflächenelastischen Eigenschaften des Citruspektins zeigten ein komplexeres Verhalten. Das Pektin mit dem höchsten VEG (= geringste Anzahl an Carboxylgruppen) wies praktisch keine Abhängigkeit der Grenzflächenelastizität von der Lösungsmittelqualität auf. Das Pektin mit dem niedrigsten VEG hingegen zeigte eine starke Sensitivität bezüglich Änderungen im pH-Wert. Dies konnte auf sich ändernde elektrostatische Wechselwirkungen zwischen Pektinmolekül und Grenzfläche zurückgeführt werden. Während bei niedrigem pH-Wert Tropfenoberfläche und Pektinmolekül entgegengesetzt geladen sind, war dies bei höherem pH-Wert nicht mehr der Fall, sodass es zu elektrostatischer Abstoßung kam. Es konnte daher nachgewiesen werden, dass Elektrosorption eine entscheidende Rolle beim Adsorptionsmechanismus von Citruspektin spielt.

In den zugehörigen Emulgierversuchen konnte gezeigt werden, dass sich besonders kleine Tropfen mit hochverestertem Pektin bei niedrigem pH stabilisieren lassen. Dies waren dieselben Bedingungen, bei denen eine hohe Adsorptionsgeschwindigkeit und eine hohe Affinität zur Ölphase, gleichzeitig aber auch eine geringe Molekülladung nachgewiesen werden konnte. Somit wurde bestätigt, dass der Stabilisierungsmechanismus von Citruspektin hauptsächlich auf sterischer und nicht auf elektrostatischer Abstoßung beruht.

Eine Verschlechterung der Lösungsmittelqualität durch Verringerung des pH-Werts führte somit zur Stabilisierung kleinerer Tropfen. Eine weitere Verschlechterung der Lösungsmittelqualität durch Erhöhung der Ionenstärke konnte dieses Ergebnis jedoch nicht weiter verbessern. Stattdessen wurden Aussalzeffekte bei der Zugabe von Natriumchlorid festgestellt. Bei pH 4 führte dies bei niedrigverestertem Pektin schließlich zur Bildung von Mikrogelpartikeln. Eine ausreichende Tropfenstabilisierung war mit diesen Mikrogelpartikeln nur bei starker Erhöhung der verwendeten Pektinkonzentration möglich. Die hohe Sensitivität von Citruspektin bezüglich der Ionenstärke ließ sich auch unter dem Mikroskop beobachten. Einerseits konnten durch Mikrogelpartikel stabilisierte Tropfen direkt visualisiert werden. Andererseits konnten nach Zugabe von Natriumchlorid zu einer Emulsion mit geringer Ionenstärken eine Vielzahl von Koaleszenzereignissen beobachten werden.

Insgesamt konnte somit gezeigt werden, dass Citruspektin ein großes Potential für den Einsatz als Hydrokolloidemulgator aufweist. Die emulgierenden Eigenschaften können durch eine Änderung des hydrophoben Charakters des Moleküls gezielt eingestellt werden. Dies lässt durch eine Erhöhung des Proteingehalts, des VEG und zu einem gewissen Maße auch durch eine Erhöhung des AcG erzielen. In Lösung weist Citruspektin die typischen Eigenschaften eines Polyelektrolyts auf. Obwohl elektrostatische Effekte eine wichtige Rolle beim Adsorptionsverhalten spielen, ist der stabilisierende Effekt von Citruspektin hauptsächlich sterischer Natur. Durch Berücksichtigung der polyelektrolytischen Eigenschaften lassen sich die Grenzflächen- und emulgierenden Eigenschaften von Citruspektin gezielt einstellen. Aufgrund seiner Sensitivität bezüglich der Ionenstärke lässt sich Citruspektin eventuell sogar zur Herstellung stimuli-responsiver Emulsionen verwenden. Solche Emulsion sind für pharmazeutische Anwendungen von besonderem Interesse, da sie die gezielte Freisetzung bioaktiver Substanzen ermöglichen. Hierfür sind feine Emulsionsmikrostrukturen notwendig, welche sich durch Hochdruckhomogenisation erzeugen lassen.

14 List of publications

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U. S. Schmidt, V. L. Pietsch, C. Rentschler, T. Kurz, H.-U. Endreß, H. P. Schuchmann (2016), *Influence of the degree of esterification on the emulsifying performance of conjugates formed between whey protein isolate and citrus pectin,* Food Hydrocolloids, 56, May, pp. 1-8

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