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„Flavoring System for a Low Fat Margarine“

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1 Introduction

1.1 The role of fat in food products

The role of fat in food is double-edged. On the one hand dietary fat is a major risk factor for a positive energy balance which leads to obesity and many obesity associated diseases as hypertension and diabetes (DAMJANOVI and BARTON, 2008). On the other hand fat strongly enhances the sensory properties of a food product; fat positively affects appearance, texture, mouth feel and flavor which interact to form flavor perception (GONZÁLES-TOMÁS et al., 2008).

A better understanding of the link between fat intake and health is changing consumer habits. Subsequently, there has been a growing demand for fat-reduced products. However, fat free food often has poor sensory properties which significantly lower consumer acceptance. Much effort has been made to develop foods that are tasty and fat-reduced; with little success though.

The modified release of flavor components is one factor that strongly contributes to the different oral perception of low-fat products. The aim of this work was to find an adequate flavoring system for low-fat margarine to reduce the sensory losses that go along with fat reduction.

1.2 Margarine

Margarine was invented in the middle of the 19th century to substitute butter cheaply. The basic ingredients are a vegetable oil blend, water, salt, colour, acid, flavor, preservatives and surface-active molecules. Regarding microstructure margarine is an oil-in-water emulsion with a typical average droplet size of 5-10 μm . Nowadays margarine has become a self-contained product with several advantages compared to butter: Margarine can be varied in many aspects; controlled levels of cholesterol, fat, saturated- and trans- fatty acids, phytosterols and vitamins make margarine a food to meet nutritional demands easily.

Margarine production involves three basic steps: emulsification, crystallisation of the fat phase and plastification of the crystallised emulsion: The aqueous phase is added to a mixture of oil blend and emulsifier under constant stirring. Afterwards the emulsion is cooled in scraped-wall heat exchangers where crystallisation of the fat phase starts. Plastification takes place in subsequent working units (GUNSTONE, 2008).

Fat reduced margarines have become popular in the last few years. However, fat reduction often goes along with unsatisfying sensory properties. Apart from textural deviations (e.g. a mouth-feel) fat-reduced margarines have a different flavor profile and thus a different oral perception compared to full fat products. The elimination or mitigation of these disparities might contribute to the fabrication of low-fat margarine with satisfying sensory properties.

1.3 Flavor

The flavor of a food is the product of all sensory impressions caused by the stimulation of receptors in the mouth and in the nose. The most important flavor stimuli are volatile and non-volatile molecules that are delivered by the food matrix before and while eating. Non-volatile components are perceived by taste buds on the tongue. Five types enable us to distinguish between sweet, sour, bitter, salty and umami. A further type of non-volatile components are trigeminal stimuli that cause sensations like burning, cooling, prickling etc. In contrast, there is a countless amount of volatile components that are sensed by receptors in the nose. These volatile components are responsible for the aroma of a food. Beside taste and aroma, food properties as texture contribute to the perceived flavor of a food. In the end the flavor of a food is a combination of taste, aroma and mouth-feel.

Before eating flavor impression arises from volatile components that are emitted to the air. While eating, the perceived flavor results from volatile components that stimulate receptors in the nasal cavity, as well as from non-volatile components causing excitations on the tongue. The actual oral perception rests upon the type and concentration of the present molecules; furthermore, it is governed by additional factors such as the environmental matrix, the precise location of the flavor

molecules and physical and chemical interactions with other molecules (e.g. proteins, emulsifiers). In the end, flavor perception is a hardly predictable, highly complex process with many influencing variables. One factor that has a strong impact on the release of flavor components and is of great importance for this work is the fat content of a food (MCCLEMENTS 1998).

1.4 The role of fat in flavor release

Fat acts as a solvent for lipophilic aromatic substances and therefore plays an important role in flavor release. Lipophilic flavor molecules are well dissolved in the fat phase of a food and are consequently slowly released. Accordingly, the perceived aroma intensity of full-fat foods builds up gradually and dissipates slowly. In contrast, the perceived aroma intensity of fat reduced food products is strong at the beginning, but not very long lasting. The amount of fat that holds the flavor molecules back is smaller; thus, the concentration of lipophilic aroma compounds in the fat phase is comparable higher. The higher concentration of flavor components finally entails an accelerated release of hydrophobic components. This is schematically illustrated with a time/intensity diagram in Fig. 1. In contrast, the release of hydrophilic aroma components seems to be hardly influenced by the fat content of the food matrix (WEEL et al., 2004).

Taken together, fat reduction accelerates the release of (hydrophobic) flavor components and leads to changes in flavor perception which is one reason for the different oral perception of full and low-fat foods. In conclusion, an appropriate modulation of flavor release is a supposable tool to create low-fat products with sensory properties more similar to those of full-fat products.

1.5 Flavoring system for a low-fat margarine

Conventional butter flavors that are commonly used in low-fat margarine offer no satisfying results in terms of flavor release. Thus, the aim of this work was to create a flavoring system for a low-fat margarine that reduces the sensory losses

accompanied by fat reduction. The overall aim was to modulate the release of hydrophobic flavor components and thereby simulate a fat matrix. This could contribute to gain low-fat margarine with similar sensory properties as a full fat version.

Basically, there are two ways to modify aroma release: The use of flavor modulating ingredients (e.g. cyclodextrins) or the encapsulation of flavor molecules within emulsion based delivery systems.

1.5.1 Flavor modulation with cyclodextrins

The three types of cyclodextrins namely α -, β - and γ -cyclodextrin that are composed of six, seven or eight (1,4)-linked glycosyl units (DEL VALLE, 2004). The β -CD molecule is shown in Fig. 2. 7 D-glucose monomers are linked by α -(1,4) bonds. Thus, the molecule possesses hydrophobic cavities and external hydrophilic faces. This structure enables β -CD to form inclusion complexes with other molecules (e.g. flavor molecules). The binding energy varies with the structure of the guest molecules; they can either be tightly or loosely associated to the cyclodextrin molecule (REINECCIUS et al., 2004).

KANT et al. (2004) studied the release of volatile compounds from β -CD to examine its potential to adapt flavor release in low-fat products. When β -CD was added to fat free yoghurt, the release of a lemon flavor became more similar to that of full fat yoghurt. The study shows the ability of β -CD to bring the flavor profile of a low-fat product closer to that of a full fat product.

SZENTE and SZEJTLI (2004) expect that encapsulation of active food compounds within β -CD prolongs shelf life of food products because the molecules are protected against oxidation, light-induced decomposition, heat degradation and evaporation.

1.5.2 Flavor modulation with emulsions

Emulsions consist of two mutually immiscible fluids (typically oil and water), with one of them finely dispersed within the other. To retard phase separation a suitable emulsifier is required. According to the distribution of the oil and the

aqueous phase, emulsions belong either to the oil-in-water (O/W) or the water-in-oil (W/O) type. Fig. 4 illustrates the different emulsion types. Water-in-oil emulsions (e.g., margarine, butter and spreads) consist of water droplets dispersed within an oil phase, whereas oil-in-water emulsions (e.g., mayonnaise, milk, creams, soups and sauces) consists of oil droplets dispersed within water. If the emulsions themselves are dispersed within an aqueous phase water-in-oil-in-water (W/O/W) or oil-in-water-in-oil (O/W/O) emulsions are formed. The droplets are called the dispersed phase; the liquid that surrounds them is called the continuous phase. According to their droplet size emulsions can further be divided into turbid macro- (0.5–100 μm), and transparent micro- and nano-emulsions (10-100 nm) (MCCLEMENTS 1998. VACLAVIK et CHRISTIAN, 2007). These emulsion types have different properties; some of them are listed in Table 1. Fig 3 illustrates emulsion based systems that can be used for the encapsulation and delivery of flavor compounds.

1.5.2.1 Emulsifier

Emulsifiers are amphiphilic which means that they combine hydrophilic and lipophilic properties in one single molecule. Thus, emulsifying molecules can form an interfacial film between two immiscible liquids. Consequently, emulsifiers show the ability to stabilize emulsions. Emulsions consist of two fluids with distinct polarities that normally tend to separate and exist as two separate layers. A meta-stable emulsion, where the phases do not separate for a certain period of time, requires the use of a suitable emulsifier. An emulsion is formed when energy is supplied to this system (e. g., by homogenizing or stirring). Oil and water are mixed; as a result droplets are formed. The required energy (W) is proportional to the surface augmentation (SA) of emulsion droplets and the surface tension (δ) (equ. 1). The present emulsifier lowers the interfacial tension and therefore decreases the amount of energy needed to disrupt the particles. To this end the presence of emulsifier favors the formation of smaller molecules. Moreover, the freshly formed droplets are covered with a thin layer of amphiphilic molecules where the hydrophilic groups reach into the water phase, whereas the lipophilic groups orient towards the oil phase. Subsequently, the emulsifier acts as a connector between the two immiscible phases and prevents the emulsion droplets from coalescence and aggregation. As the surface area of the emulsion droplets becomes larger with a decreasing droplet

size, more emulsifier is needed to create emulsions with smaller particles. Incompletely covered particles will coalesce with each other and lead to larger droplets accompanied by phase separation. Consequently, larger molecules (e.g., proteins) are more efficient emulsifying agents because they comprehensively extend over the entire surface area.

$$W = SA \cdot \delta \quad (\text{equ. 1})$$

The resulting emulsion type depends on the nature of the emulsifying agent. To practicably categorize emulsifiers due to their hydrophilic and hydrophobic character the HLB (hydrophilic/lipophilic balance) system has been invented. The HLB scale is a support to find the most suitable emulsifier for a special liquid system. It ranges from 1 to 20 and describes the amount of hydrophilic functional groups within a molecule. Emulsifiers with a low HLB level (3-6) have few hydrophilic groups and are therefore more lipophilic; consequently they lower the surface tension of the oil phase and thus support the formation of water-in-oil emulsions because the water phase exhibits a higher surface tension and forms droplets. Hydrophilic emulsifiers with a high HLB (8-18) lead to the formation of oil-in-water emulsions. Generally, an emulsion is optimally stabilized if the emulsifier and the continuous phase have a similar HLB value because then they mix exhaustively (VACLAVIK et CHRISTIAN, 2007).

Gum Arabic

Gum Arabic is a commonly used stabilizing agent for beverage O/W emulsions with very good emulsifying properties. It derives from the bark exudate of *Acacia senegal*. Thus, it is a naturally occurring, surface-active polysaccharide–protein complex (DICKINSON and MCCLEMENTS 1995). Polysaccharides act as thickening and/or gelling agents that control the network-structure and rheology of the aqueous continuous phase. They inhibit gravity-induced creaming and therefore retard phase separation during storage. Overall emulsion stability may be improved by synergistic

interactions of proteins and polysaccharides (LEAL-CALDERON et al., 2007). This is supported by DICKINSON (2008) who showed that protein-polysaccharide complexes have very good emulsifying and emulsion stabilizing properties, especially in cases where the protein alone is poorly soluble. Unlike other polysaccharides Gum Arabic is not predominantly hydrophilic, because it has non-polar groups (MCCLEMENTS 2007). The surface-active properties of Gum Arabic derive from hydrophilic arabinogalactan blocks that are bound to a hydrophobic polypeptide backbone. This arabinogalactan-protein complex is instrumental in its ability to surround the emulsion particles with an interfacial layer and prevent their aggregation. Problems that are associated to the use of Gum Arabic are the price, high quantity required and an unsteady quality. A combination of different types of Gum Arabic in one formulation increases stability caused by synergistic effects (CHANMAI and MCCLEMENTS, 2002) (NAKAUMA et al., 2008). CHO and PARK (2003) investigated the stabilizing properties of Gum Arabic and maltodextrine in a double emulsion. The aqueous phases of the simple emulsions were stabilized with various ratios of Gum Arabic and maltodextrine (from 0:10 to 5:5). It turned out that the emulsion stability index did not increase beyond a ratio 2:3. Consequently, Gum Arabic can be mixed with maltodextrine at a ratio 2:3 without stability losses. In contrast, too low levels of Gum Arabic (1:4 or less) significantly reduced emulsion stability.

NAKAUMA et al. (2008) studied the emulsifying and emulsion stabilizing properties of three hydrocolloids (sugar beet pectin, soybean soluble polysaccharide and Gum Arabic) in O/W emulsion. The emulsifying activity of the hydrocolloids was attributed to the protein moieties that are linked with the polysaccharide structures. The emulsion stabilizing properties are based on the carbohydrate regions. Sugar beet pectin was shown to have the best emulsion stabilizing properties.

PGPR

Polyglycerol Polyricinoleate (E476) is composed of polyglycerol esters of polycondensed fatty acids. PGPR is strongly lipophilic and thus has a HLB value of 1. It is widely used in chocolate and confectionary industry (Anonymous, Palsgaard, 2009).

1.5.2.2 Emulsification

Emulsions are fabricated by dispersing two immiscible fluids. Several emulsification techniques can be used in this regard. Conventional emulsification devices are dispersing machines, colloid mills and high pressure homogenizers. Usually the aim of emulsification is to produce emulsion droplets that are as small as possible because to a certain extent emulsion stability increases with decreasing droplet size.

Microfluidizer

A microfluidizer is a high-energy emulsification method that uses a particle-particle collision to produce small droplets. The coarse emulsion is separated into two emulsion jets that collide with each other in the reaction chamber under high pressure. The occurring shear forces provide a very fine emulsion with a homogenous particle size distribution. A microfluidizer can be run with different kinds of reaction chambers. Relevant to this work were the Y- and the Z-reaction chamber.

JAFARI et al. (2007) produced oil-in-water emulsions via microfluidization and observed a tendency of the emulsion droplets to re-coalesce if the processing pressure passed over 40-60 MPa. Furthermore, they came to the conclusion that repeated processing cycles also contributed to re-coalescence.

CHO and PARK (2003) used a microfluidizer to produce O/W/O multiple emulsion for flavor encapsulation and subsequent dehydration. The microfluidized emulsions were more stable than the ones prepared with a homogenizer and had a more homogenous particle size distribution. The double emulsion particles retained 71% of the encapsulated flavor during the dehydration process.

The actual way to increase the amount of energy depends on the conception of the used machine. In case of a microfluidizer the energy input can be varied by increasing the processing pressure, recirculation the emulsion several times or by the choice of the reaction chamber (MCCLEMENTS 1998).

In order to avoid an over-processing and re-coalescence of the emulsions it is important to investigate adequate processing pressures.

1.5.2.3 Emulsion Stability

Emulsion stability describes the ability of an emulsion to resist structural changes over time. Emulsions are meta-stable systems; which means that the phases naturally separate as a function of time. Fig. 6 illustrates the instability mechanism of an O/W emulsion. The oil droplets that are dispersed within the aqueous phase have a lower density and thus a tendency to move upwards. The single particles tend to reunion because this is the state with the lowest energy. Single particles stick together and form bigger aggregates. The process where droplets stick together and finally form one single droplet is known as coalescence; coalescence in turn favors the creaming of the oil phase. Among others this context can be described by the help of Stokes' Law. Stokes' Law describes the factors that contribute to phase separation. Consideration of these factors facilitates the creation of stable emulsions.

The speed, at which a particle moves (V) in an ideal liquid, depends on several forces acting upon it. These forces are the densities of the two media (ρ_1, ρ_2), shear viscosity (η) and gravity (g). Furthermore, the radius of the particle (r) is substantial for the effect of gravitational forces.

$$V = \frac{(\rho_1 - \rho_2)gr^2}{18\eta} \quad (\text{equ. 2})$$

Viscosity

Stokes' law indicates that a high viscosity (η) of the continuous phase decelerates the velocity at which the dispersed particles move. This refers to upward and lateral movement of the emulsion particles. Accordingly, the reduced tendency to move upwards prevents gravitational separation; moreover, the collision-rates of the particles is reduced which also prevents coalescence. Thickening agents such as gums, pectin or gelatin augment the viscosity of the dispersed phase (VACLAVIK et CHRISTIAN, 2007). On the other hand, a higher viscosity inhibits the formation of local turbulences during homogenization and deteriorates particle disruption and

finally increases droplet size (MCCLEMENTS 1998). To this end, a higher viscosity increases emulsion stability but also impedes particle size reduction.

Density Difference

The speed and the direction, at which a particle moves, depend on the densities of the two media. The bigger the density difference the more pronounced is the movement of a particle. The direction of the movement is affected by the polarities of the dispersed and the continuous phase. An oil droplet in an oil-in-water emulsion has a lower density as the surrounding liquid; subsequently, it moves upwards and creams at the surface. In contrast, a water droplet in a water-in-oil emulsion has a comparably higher density and will sediment to the ground (MCCLEMENTS 1998).

Droplet size

According to Stokes' law the speed at which a particle moves is proportional to the square of its diameter. Large droplets will easily move, especially if the density differences are very pronounced. Small droplets, in contrast, are less affected by gravitational forces; thus, they have a smaller tendency to move. As a result creaming and sedimentation are less distinct in emulsions with a small particle size. Consequently, a reduction of the average droplet diameter increases emulsion stability (VACLAVIK et CHRISTIAN 2007. MCCLEMENTS 1998). This though, is only valid for a certain range of particle size. It was recognized that the context between emulsion stability and particle size is not necessarily linear. A too small average particle size favors coalescence of the emulsion droplets and in the end contributes to phase separation. As mentioned above droplet size plays an important role in emulsion stability. Moreover, it has an impact on the appearance and viscosity of the emulsion. To create emulsions with desired properties, it is important to know the factors that make a contribution to droplet size.

To specify the quality of an emulsion more precisely it is important to know the average particle size and the particle size range. Because two emulsions that have the same average particle size can remarkably differ from each other with respect to the particle distribution. Emulsions with a low average particle size and a

narrow range are expected to be more stable than emulsions that have the same average particle size and a wider range, even though it has a higher number of smaller particles; because the emulsion that has a wider range contains bigger particles that are more likely to sediment or cream.

The minimum size of emulsion droplets depends on the properties and amount of the present emulsifier. A decreasing particle size goes along with a higher surface area. If there is insufficient emulsifier to entirely cover the whole surface, there is no separating layer between the droplets; thus, the droplets will tend to coalesce. Moreover, an emulsifier reduces the interfacial tension between the water and the oil face. This facilitates the disruption of the single droplets and contributes to a smaller droplet size at a certain energy input.

equ. 3 describes the context between droplet size (r_{min}), droplet concentration (dispersed phase volume fraction; Φ), concentration of emulsifier (c_s) and the excess surface concentration of the emulsifier at saturation (Γ_{sat}). According to the equation a reduction of particle size is accompanied by a higher droplet concentration. To cover all particles with an interfacial layer, more emulsifier is needed. In other words, the formation of smaller droplets is inevitably linked to a higher emulsifier concentration. Moreover, droplet size can further be minimized at equal concentration by choosing an emulsifier with a smaller Γ_{sat} . Nevertheless, particle size is a multivariable problem that is influenced by additional factors; consequently, the achieved particle size is normally bigger than the theoretically achievable limit. Whether the theoretically possible droplet size and the factual droplet size considerably deviate from each other also depends on the energy input (MCCLEMENTS 1998).

$$r_{min} = \frac{3 \cdot \Gamma_{sat} \cdot \Phi}{c_s} \quad (equ. 3)$$

Provided that there is enough emulsifier to entirely coat the droplet surface, particle size can be minimized by supplying more energy to the system. A higher energy input is linked to a smaller average particle size in the final emulsion. Indeed,

this rule of thumb seems to lose its validity beyond moderate processing pressures; thus, there seems to be no linear function between particle size and energy input. Several studies suggest that too high pressures have an inverse effect and lead to a re-increase of particle size due to an over-processing of the emulsion.

JAFARI et al. (2007) produced oil-in-water emulsions via microfluidization and observed a tendency of the emulsion droplets to re-coalesce if the processing pressure passed over 40-60 MPa. Furthermore, they came to the conclusion that repeated processing cycles also contributed to re-coalescence.

Properties of the continuous and dispersed phase

The composition and ratio of oil and the water phase has a strong impact on droplet size and emulsion stability.

Due to their origin, oils differ in their chemical structure and can furthermore contain different amounts of surface active components (e.g. free fatty acids, monoacylglycerols, diacylglycerols). These variations can have a meaningful impact on the surface tension of the emulsion phases and consequently on the smallest achievable droplet size (MCCLEMENTS 1998).

Beside a reduction of particle size and a higher viscosity of the continuous phase the rate of gravitational separation can theoretically further be minimized by increasing the volume of the dispersed phase; thus, by increasing the dispersed-phase volume fraction. The dispersed-phase volume fraction is equal to the volume of emulsion droplets divided by the total volume of the emulsion. In short it describes the concentration of emulsion droplets within the dispersed phase. A high dispersed-phase volume fraction reduces the movement of emulsion droplets because they are close-packed (MCCLEMENTS 1998). However, this assumption requires that each of the emulsion droplets is entirely covered by a layer of emulsifying agent to avoid coalescence. If this is not the case the closeness of the particles might lead to coalescence.

1.5.2.4 Double emulsions

Double emulsions consist of more than one dispersed phase. The structure of double emulsions is shown in Fig. 4 and Fig 3. Water-in-oil-in-water (W/O/W) emulsions consist of a W/O emulsion that is dispersed within water. In contrast, oil-in-water-in-oil (O/W/O) emulsions are composed of an O/W emulsion that is dispersed in oil.

Due to the bigger interface double emulsions and are thermodynamically more unstable than simple emulsions (VAN DER GRAAF et al., 2005). Nevertheless, double emulsions offer a big variety of technological opportunities: The fat-content of food can be lowered by loading the fat phase with water (LOBATO-CALLEROS et al., 2008). Double emulsions contribute to the delayed release of chemical substances (e.g. flavor molecules, vitamins) that are implemented in the droplets (FECHNER et al., 2007); they have taste masking and protecting effects on functional ingredients (KHAN et al., 2006) and can be used as carriers for functional components. Molecules can be encapsulated within any of the three phases; thus, multiple emulsions serve as delivery systems for one or more functional components that might adversely affect each other if they were present in the same phase (WEISS et al., 2006).

BRÜCKNER et al. (2007) investigated storage stability and flavor release of a spray dried W/O/W emulsion where the inner aqueous phase contained an aqueous solution of a hydrophilic aromatic compound. W/O/W emulsions were stable during storage and released a sufficient high amount of flavor and were therefore regarded to be suitable encapsulation systems.

Influence of surfactants on double emulsion stability

As mentioned the stability of an emulsion crucially depends on the type and concentration of the used emulsifier. Double emulsions contain (at least) two surfactants of opposite solubility. One is lipophilic (low HLB value) and one is hydrophilic (high HLB). In the case of a W/O/W emulsion the lipophilic surfactant stabilizes the interface of the inner W/O emulsion. The hydrophilic emulsifier

stabilizes the external interface of the oil globules. The stability of the final double emulsion depends on the combination of these two surfactants.

KAWAKATSU et al. (2001) tested Span 20 (sorbitan laurate), Span 80 (sorbitan monooleate) and tetraglycerol polyricinoleate (TPGR) as emulsifying agents for W/O/W emulsions. No stable double emulsions were produced with Span 20 and Span 80 due to a low stability of the W/O coarse emulsion caused by large water droplets. TPGR was found to be a suitable emulsifier for W/O/W emulsions. The internal water droplets were stable at a high TPGR concentration. However, a high TPGR concentration favored coalescence of the internal oil droplets.

GARTI and BISPERINK (1998) showed that a high concentration of lipophilic surfactant and a low concentration of hydrophilic surfactant positively affected the stability of W/O/W emulsions. An exaggerated concentration of hydrophilic emulsifier promotes interactions between the inner and the outer water phases which results in release of the entrapped water.

These findings are supported by FICHEUX et al. (1998) who studied two phenomena that were responsible for the instability of W/O/W double emulsions: Coalescence of the water droplets with each other and coalescence of the inner water droplets with the globule interface, accompanied by an escape towards the external phase. A high concentration of the hydrophilic surfactant contributed to the release of the internal water. They drew the conclusion that the concentration of the hydrophilic surfactant must be moderate in order to obtain a stable W/O/W emulsion.

1.5.2.5 Multilayer emulsions

A multilayer oil-in-water emulsion (M-O/W) consists of oil droplets dispersed in an aqueous medium. Unlike a conventional emulsion each droplet is surrounded by a coating composed of emulsifier and biopolymer molecules. It is possible to obtain multilayer emulsions with multiple layers if two or more biopolymers are added to the emulsion (MACCLEMENTS et al., 2007). The structure and formation of multilayer emulsions are shown in Fig 3 and Fig. 5.

SHAW et al. (2008) and KLINKESORN et al. (2005) showed that a multilayer emulsion consisting of lecithin and chitosan can be used for the delivery of omega-3 fatty acids in functional foods. It was also shown that the multilayer emulsion could be spray dried to facilitate its use in food products.

1.5.2.6 Solid lipid particles

Solid lipid particles are structurally equal to conventional emulsion. The difference between the two emulsion types is that the oil phase of solid lipid particles is fully or partly solidified. Solid lipid particles are formed by homogenizing oil and water in the presence of a hydrophilic emulsifier. The emulsion is tempered subsequently so that some or all of the lipid molecules crystallize. Solid lipid particles are suited for the controlled delivery of hydrophobic functional components. For example a bioactive lipid could be trapped within a solid lipid phase that is designed to melt at a particular temperature. The main limitation of this method is that the solid particles must be prepared at higher temperatures which could have a negative impact on heat sensitive ingredients (MACCLEMENTS et al., 2007). The structure of solid lipid particles is shown in Fig 3.

1.5.2.7 Filled hydrogel particle emulsion (O/W/W-emulsion)

A filled hydrogel particle emulsion is an oil-in-water emulsion where the oil droplets are surrounded by hydrogel. They can be used for the delivery of hydrophilic and hydrophobic components. Filled hydrogel particles can be used for the delivery of hydrophilic and hydrophobic functional components. Hydrophobic molecules can be trapped within the oil phase and hydrophilic molecules can be encapsulated within the hydrogel (MACCLEMENTS et al., 2007). The structure of filled hydrogel particle emulsion is showed in Fig 3.

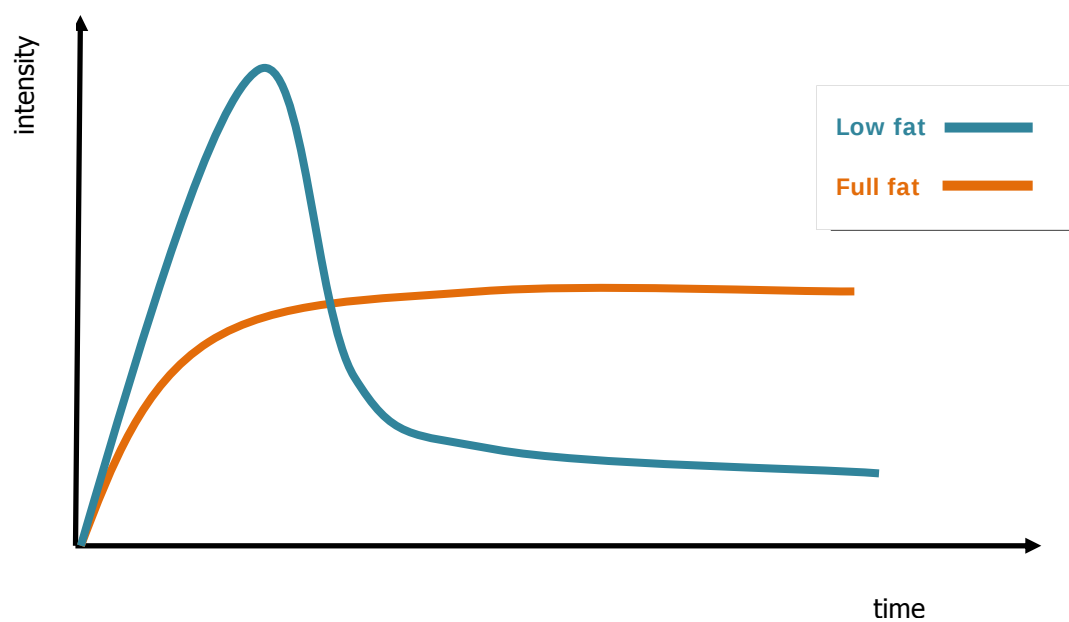


Fig. 1: Time/intensity diagram of flavor in full and low fat products

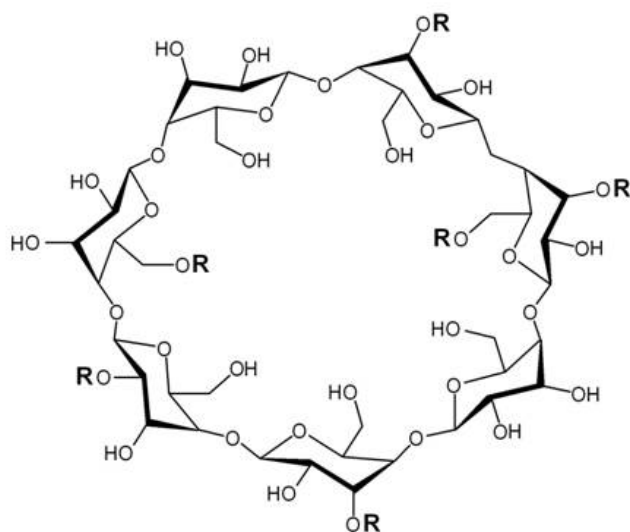


Fig. 2: Structure of β -cyclodextrin

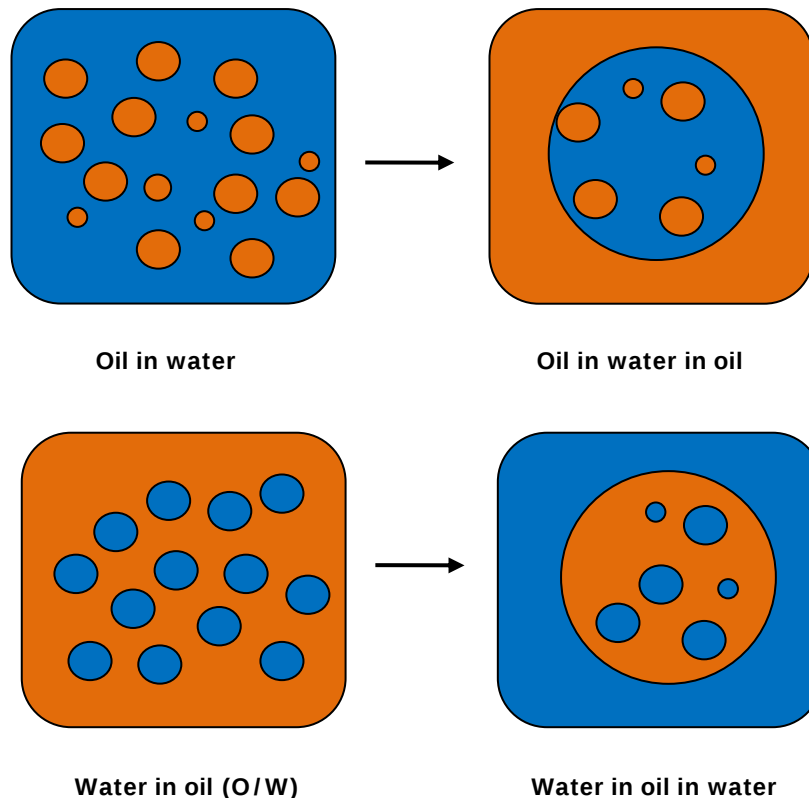


Fig 3: Different emulsion based delivery systems (WINDHAB et al., 2005).

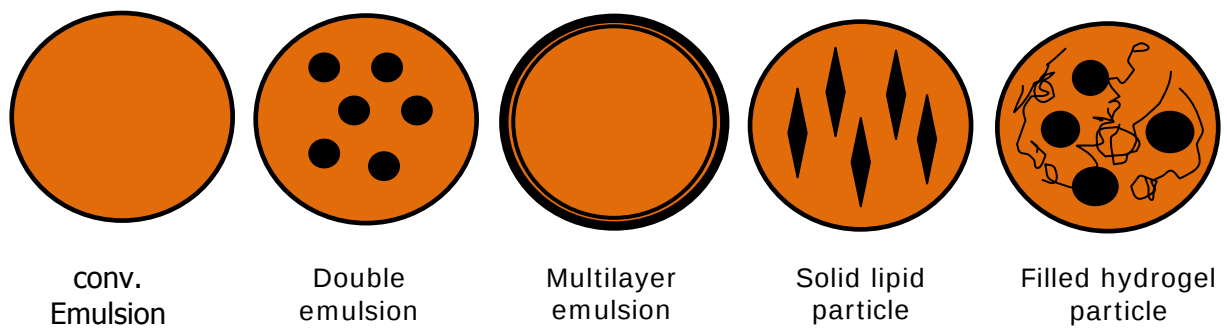


Fig. 4: Structure of different emulsion types

Property	Macroemulsion	Microemulsion	Nanoemulsion
Appearance	Formulation-dependent	Transparent to milky	Transparent
Preparation methods	Classic homogenization	High energy (pressure)	Low-energy emulsification
Surfactant load	Fairly low	Medium (<10%)	Fairly high (10-20%)
Droplet size	0.5–100 μm	100–1000 nm	10–100 nm
Thermodynamic stability	Unstable; kinetically stable	Unstable; kinetically stable	Stable

Table 1: Properties of different emulsion types (WINDHAB et al., 2005)

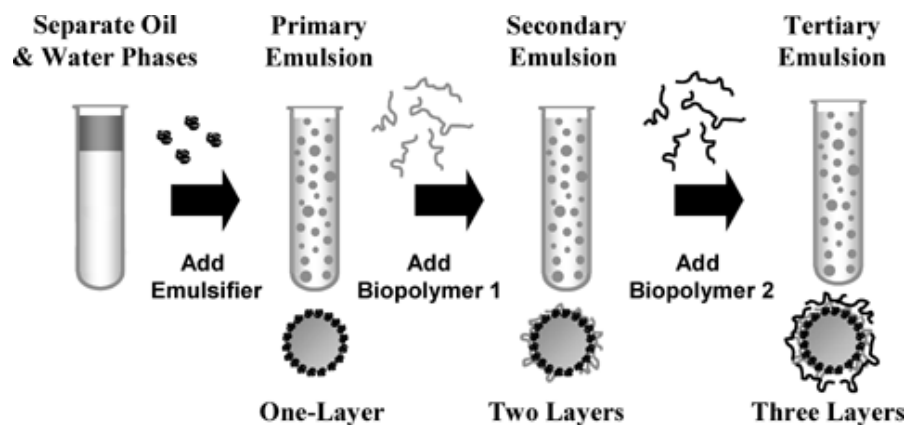


Fig. 5: Formation of multilayer emulsions (MACCLEMENTS et al., 2007)

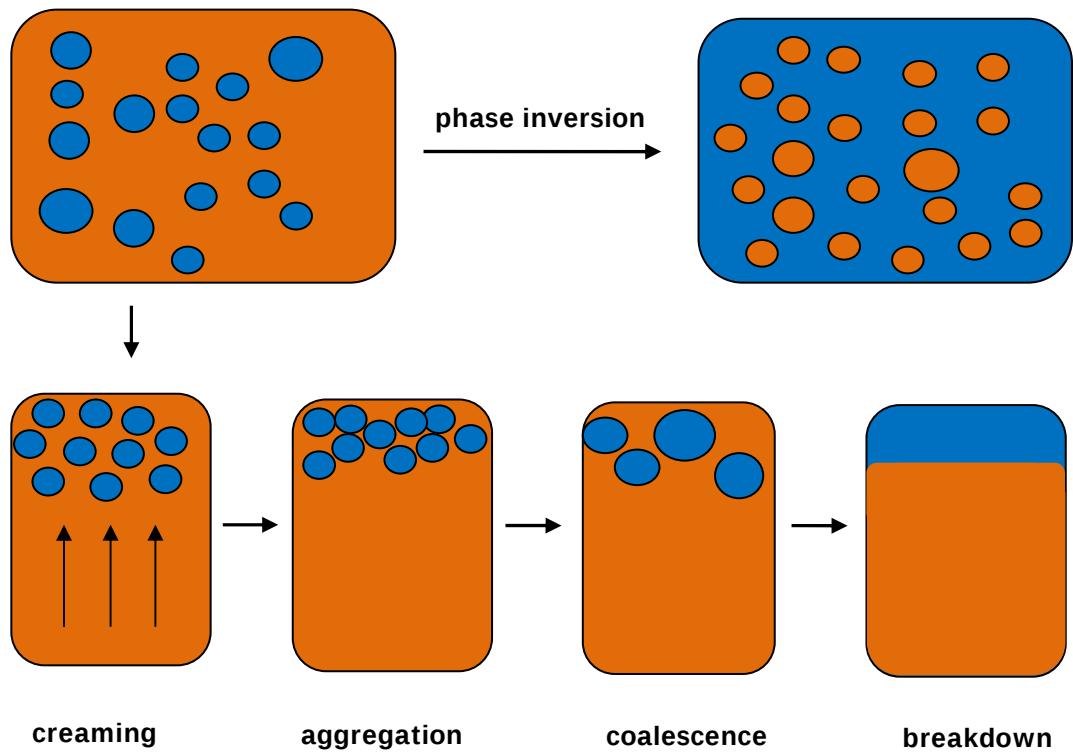


Fig. 6: Instability mechanism of emulsions

2 Aim of the work

The principal idea behind the project was that a modulation of flavor release could mitigate the sensory losses that go along with fat reduction. Theoretically, a slower flavor release can bring the sensory perception of low-fat margarine closer to that of a full fat margarine. Literature research showed that emulsions could be appropriate flavor modulating agents in margarine.

The overall aim of the project was to create stable flavor emulsions basing on three common solvents. The investigation of appropriate formulas and processing conditions should entail the development of stable flavor emulsions (Fig. 7).

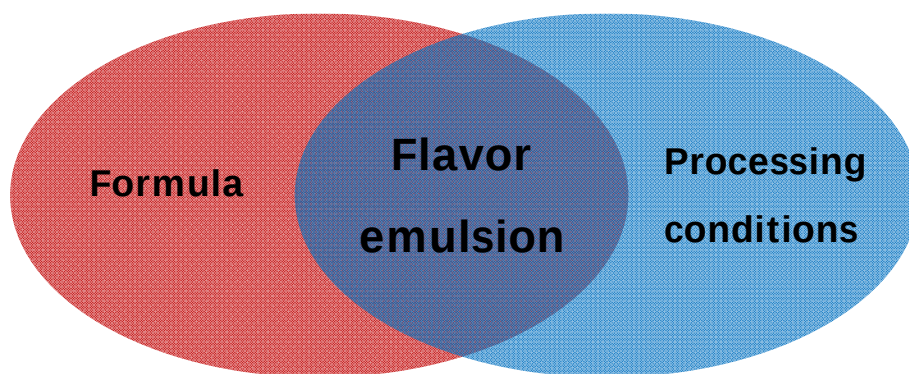


Fig. 7: Focus of practical work

3 Materials and methods

3.1 Raw material

All raw materials were stored at 4 °C.

3.1.1 Flavors

Three flavors that are composed of equal qualities and quantities of flavor components and solely differ from each other regarding to the solvent are used in this work. The flavors were delivered by Symrise A.G. 831788 bases on triacetin and is called "flavor TA" in the experimental protocol. 831789 bases on plant triglyceride. It is called "flavor PTG" in the experimental protocol. 831790 bases on propylene glycol; it is called "flavor Propy" in the experimental protocol.

3.1.2 Stabilizers and emulsifiers

A mixture of Gum Arabic 100325 and Gum Arabic 158296 at equal parts is used for all trials. Gum Arabic is mixed at equal parts with maltodextrine. Gum Arabic and maltodextrine were delivered by Symrise A.G. PGPR (Polyglycerol Polyricinoleate; E476) derived from Palsgaard.

3.1.3 Sunflower oil

The sunflower oil "Sonnin 10020" was purchased from Walter Rau, Neusser ÖL und Fett AG, Germany.

3.2 Emulsion preparation

3.2.1 Preparation of the coarse O/W simple emulsion

Gum Arabic and maltodextrine were weighed in on the laboratory scale (*Sartorius GP 5202; d: ± 0.01 ; Germany*). A 1:1 blend of Gum Arabic 100325 and Gum Arabic 158294 was used for the trials. Distilled water was weighted in a beaker glass. The mixture of Gum Arabic and maltodextrine was added to the beaker glass spoon by spoon under constant stirring with the Ultra-Turrax (*Ultra-Turrax T50, Janke & Kunkel IKA-Labortechnik*) at 8000 rpm. The solution was mixed up to homogeneity. In the trials where Flavor Propy was part of the aqueous phase, Gum Arabic and maltodextrine were dissolved in distilled water; Flavor Propy was added to the solution afterwards. To prepare the W/O emulsion the required amount of lipophilic liquid was slowly added to the aqueous phase under constant stirring with the Ultra-Turrax at 8000 rpm. The oil phase consisted either of sunflower oil, Flavor PTG or Flavor TA. When the oil phase was fully added to the aqueous phase the coarse simple emulsion was mixed for less than 30 seconds because a further disruption of the emulsion particles was accomplished with the microfluidizer as described in 3.2.4.

3.2.2 Preparation of the double emulsions of the O/W/O type

The emulsions of the O/W/O type were prepared with the microfluidized emulsions of the O/W type. PGPR was weighted into a beaker glass with a laboratory scale and diluted with the required amount of lipophilic solvent. The solution was mixed with the Ultra-Turrax at 1000 rpm. A required amount of O/W emulsion was slowly added to the PGPR-solution under constant stirring at 4000 rpm with the Ultra-Turrax. The coarse double emulsion was then processed with the microfluidizer as described in 3.2.4.

The O/W emulsion was fairly viscous; thus, considerable amounts of emulsion adhered to the beaker glass. To make sure that the volume of dispersed phase was not too low, following method was used: The calculated amount of O/W emulsion

was weighted into the beaker glass. Subsequently, the beaker glass was emptied as comprehensively as possible. The amount of emulsion that remained in the beaker glass was regarded to be constant. Accordingly, the baker glass was put onto the scale, tared and filled with the calculational amount of emulsion.

3.2.3 Preparation of the double emulsion of the W/O/W type

A certain amount of PGPR was weighted into a beaker glass on the laboratory scale, diluted with lipophilic solvent and mixed with the Ultra-Turrax at 1000 rpm up to homogeneity. Hydrophilic solvent was weighted into a beaker glass and slowly added to the lipophilic phase under constant stirring with the Ultra-Turrax at 8000 rpm. When the hydrophilic phase was fully added to the oil phase the coarse simple emulsion was mixed for less than 30 seconds because a further disruption of the emulsion particles was accomplished with the microfluidizer. The W/O emulsion was processed with the microfluidizer as described in 3.2.4. To prepare the double emulsion certain amounts of water and Gum Arabic and maltodextrine were weighted into two beaker glasses. Gum Arabic and maltodextrine were added to the water spoon by spoon under constant stirring with the Ultra-Turrax at 8000 rpm. The coarse double emulsion was then processed with the microfluidizer.

Subsequently, the O/W emulsion was slowly dispersed within the aqueous solution. The W/O emulsion was fairly viscous; thus, considerable amounts of emulsion adhered to the beaker glass. To make sure that the volume of dispersed phase was not too low following method was used: The calculated amount of W/O emulsion was weighted into the beaker glass. Subsequently, the beaker glass was emptied as comprehensively as possible. The amount of emulsion that remained in the beaker glass was regarded to be constant. Accordingly, the baker glass was put onto the scale, tared and filled with the calculational amount of emulsion.

3.2.4 Microfluidization

Fig. 8 shows a picture of the microfluidizer (*Microfluidizer M-110 P, Microfluidics, USA*) including all relevant components. Before working, the pipeline of the microfluidizer was cleaned with approximately 300 mL ethanol. Ethanol was filled into the holding tank (A). The microfluidizer was switched on with button D. Processing started when button E was pulled. Switch F was adjusted according to the processing pressure before working. Setting 10K was used for processing pressures up to 50 MPa, setting 20 K for processing pressures from 50 to 120 MPa and setting 30K was used beyond 120 MPa. Settings were only changed if the microfluidizer was switched off. The processing pressure was adjusted with button C during and/or before microfluidization. The pressure gauge (B) showed the actual processing pressure. Ethanol residues were rinsed with water. To avoid air admittance it was important to make sure that the holding tank was never empty and the processed liquids were bubble-free. After cleaning the microfluidizer was de-aerated. A thin metal stick was used to press the non-return valve that was located beneath the holding tank inside the pipeline. The emulsion was filled into holding tank when the holding tank was clean and dry. As the diameter of the reaction chamber was 100 μm or less, it was important that the emulsions contained no foreign matter or big particles. The microfluidizer was cooled with water when the processing pressure went up beyond 120 MPa to avoid temperature raise in the product. After the pressure was regulated, ten to twenty passes of the microfluidized emulsion were discarded to make sure that the emulsion was treated with the desired pressure and residue water was entirely eliminated from the pipeline. After processing the microfluidizer was cleaned with ethanol.

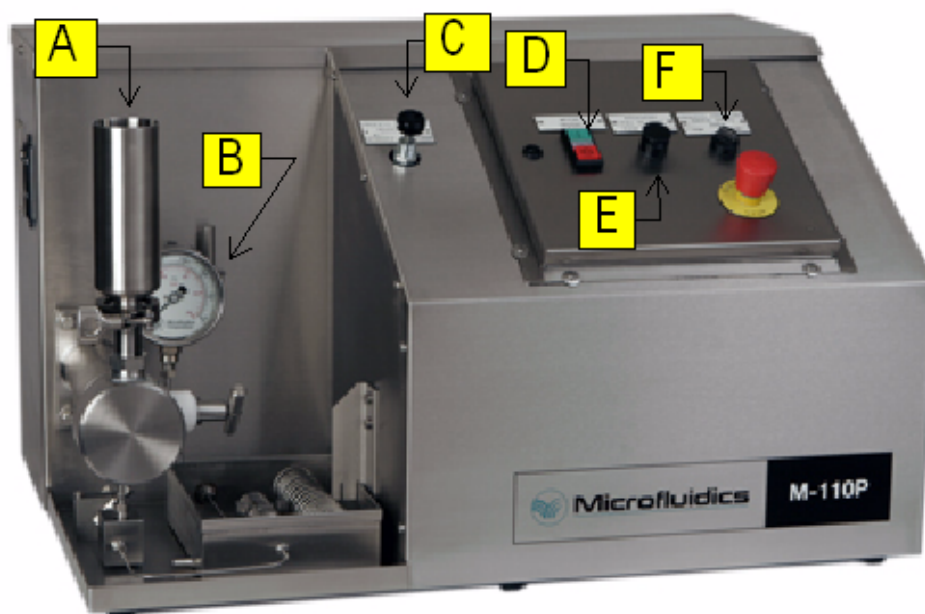


Fig. 8: Microfluidizer M-110P

3.3 Analytical methods

3.3.1 Image analysis

The emulsions were analyzed with an image analyzer that consisted of a microscope (*Olympus BX 51, Olympus, Japan*) a camera (*ColorView III, Olympus, Japan*) and a software (*Cell^F, Olympus, Japan*). The image analyzer offered the opportunity to get an overview of the particle size and the particle size distribution of the flavor emulsions. Slides were photographed and statistically analyzed.

To get more informative pictures the hydrophilic emulsions were dissolved in water and the hydrophobic emulsions were dissolved in sunflower oil. The dissolved emulsions were applied on a microscope slide and covered with a coverglass. The coverglass was dropped with immersion oil. The slides were most meaningful at hundredfold magnification. The exposures were photographed; images that seemed to show a representative cross section were chosen for statistical analysis. The diameter of approximately 100 representative particles was manually measured with the Cell^F software. As the number of particles that underwent statistical analysis was

limited to a cross-section, one cannot exclude that the factual average particle size of the emulsions was over- or underestimated.

Fig. 9 and Fig. 10 give a short introduction into the steps that were most relevant to the work: the measurement of emulsion particles and generation of an excel file containing all relevant data. Fig. 9 shows how particle diameters can be measured. When button $\leftarrow \rightarrow$ is selected the distance of two points can be determined. The window on the left side lists the already collected data. The marked button in Fig. 10 generates an excel file of the already measured particle sizes. This excel file can be used for further analysis with the STATGRAPHIC centurion XV software.

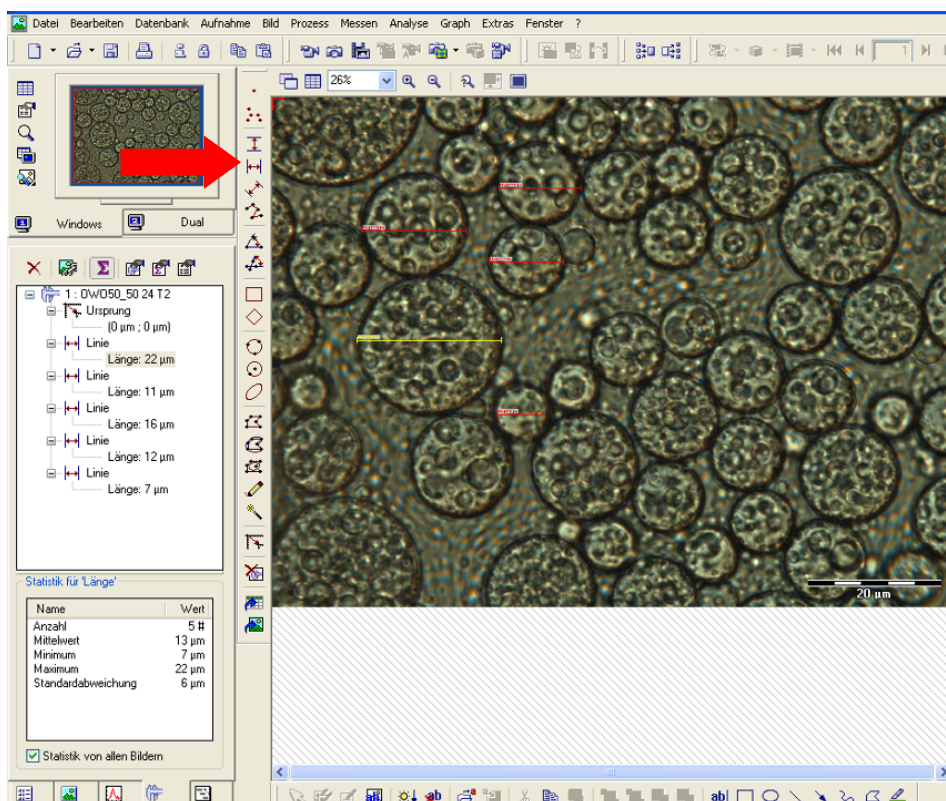


Fig. 9: Measurement of particle sizes with Cell^F software.

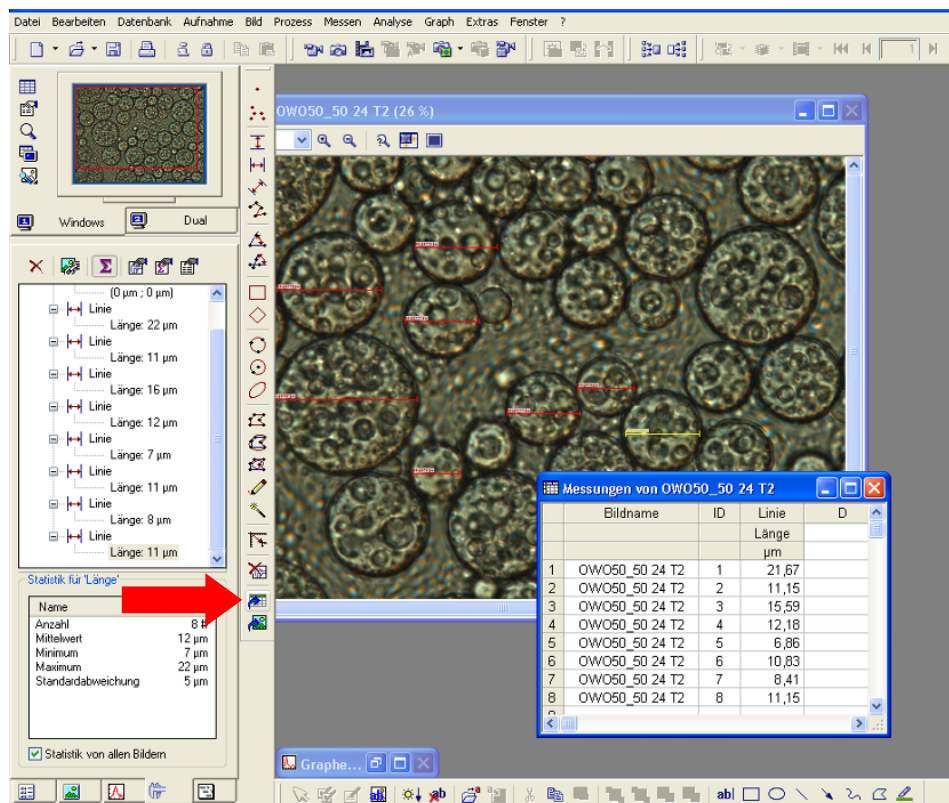


Fig. 10: Statistical function of Cell^F software.

3.3.2 Solubility tests

To solubility of an emulsion corresponds to the hydrophilicity of the (outer) continuous phase; in other words W/O/W and O/W emulsions are hydrophilic whereas O/W/O and W/O emulsions are lipophilic. Consequently, the results of solubility tests can exclude at least two emulsion types and are a tool to check whether phase inversion Fig. 6 occurred.

Solubility tests are performed by mixing approximately 1g of the emulsion with approximately 20 g of water or oil in a beaker glass. Emulsions that are hydrophilic are regarded to be W/O/W or O/W; emulsions that are lipophilic are regarded to be O/W/O or W/O.

3.3.3 Emulsion stability

3.3.3.1 Heat treatment

To study the impact of higher temperatures on emulsion stability the emulsions are heated from 25 to 95° C. Each temperature step is held for five minutes.

Approximately 10 mL of the emulsion are filled into a glass test tube with screwtop. The glass tubes are placed in a tube stand and heated in a water quench. Putative changes are optically registered after each step.

3.3.3.2 Centrifugation

To estimate storage stability the emulsion samples are centrifuged. According to Stokes' Law particle sedimentation is proportional to gravity. As gravity is augmented in a centrifuge phase separation is enhanced. Thus, centrifugation acceleratedly simulates what happens during storage.

The variable testing parameters are time and rounds per minute (=rpm). According to literature there is no standard method for emulsion stability tests with centrifuges. The testing conditions must be adapted to the desired stability of the emulsion sample. In literature centrifugation times range from 10-30 min; the rounds per minute range from 1000-5000. As the emulsions should be stable for at least six months at room temperature both time and rpm were set fairly high. Finally, the emulsion samples were centrifuged for 30 minutes at 3000 rpm.

The emulsion samples were centrifuged (*Centrifuge 5810. Eppendorf, Germany*) after processing with the microfluidizer or the Ultra-Turrax. 20 mL plastic centrifuge tubes with screwtop were filled with two passes of microfluidized emulsion or a corresponding volume of ultra-turraxed emulsion. The tubes were centrifuged at 3000 rpm for 30 minutes. Emulsions that showed no phase separation were stored at room temperature as described in 3.3.3.3. Emulsions that exhibit phase separation were rejected.

3.3.3.3 Storage test

The storage stability of the emulsion samples was studied by storing 20 mL plastic tubes filled with the microfluidized or ultra-turraxed emulsion samples at room temperature or cool storage (6°C) for six months.

3.3.3.4 Evaluation of emulsion stability tests

The emulsions were checked for signs of instability after the stability tests. In case of storage tests the emulsions were surveyed regularly.

The emulsions were examined with respect to sedimentation, ring formation and phase separation. If sedimentation or ring formation was detected the emulsion samples were regarded as unstable and were rejected.

3.4 Statistical methods

Emulsion droplet size was statistically analyzed with the software STATGRAPHICS centurion XV. "Two-sample Comparison" was chosen for dependent samples; for example to study the differences between the first and the second passage at equal pressures. In contrast, "Multiple-Sample Comparison" was used for independently prepared samples. Collectives that contained more than two samples were analyzed with the function "Multiple-Sample Comparison". However, results of the generated t-tests were only valid in case of homoskedasticity. Homoskedasticity means that the variances of the samples are equal. If the variances are unequal a t-test delivers no meaningful results; in such case only the medians and distributions were analyzed. All tests were accomplished on the 95% confidence level.

ANOVA analysis was used to detect whether the single samples of a collective differed from each other. Subsequently, the differences were more precisely analyzed with a multiple range test. The multiple range test compared all samples of the collective with each other, explored which samples differ from each other and generated homogenous groups according to these results. Samples that were within the same homogenous group did not differ from each other. The method that was

currently used to discriminate putative differences among the means is Fisher's least significant difference (LSD) procedure with a 5% error rate.

4 Results and Discussion

4.1 Composition of O/W and O/W/O emulsions

4.1.1 Introduction

The aim of this trial was to estimate the composition of O/W and O/W/O flavor emulsions. Stable emulsions should be prepared with all used flavors (831788, 831789 and 831790). Stability tests and image analysis gave an overview of emulsion stability, particle size and particle size distribution.

4.1.2 Experimental protocol

The emulsions were prepared as described in chapter 3.2.1 and 3.2.2; with the difference that the simple emulsions were stirred for 10 minutes with the Ultra-Turrax at 8000 rpm because the microfluidizer was not yet used for this trial. Flavor PTG and flavor TA were used as the dispersed phase for simple emulsions. Flavor Propy formed the continuous phase. shows the composition of the O/W emulsions. To investigate the best ratio of dispersed and continuous phase five types of emulsions that differed with respect to the ratio of continuous and dispersed phase were prepared. The ratios of continuous and dispersed phase were 4:1, 2:3, 1:1, 3:2 and 1:4. The PGPR concentration in the continuous phase was constantly kept at 5%. No double emulsions were prepared with the emulsions where the continuous phase contained propylene glycol purely; because the phases had separated shortly after preparation of the simple emulsions.

The emulsions were analyzed with the image analyzer as described in chapter 3.3.1 and heated as described in chapter 3.3.3.1.

Table 2: Composition of the O/W emulsions

Emulsion based on:	Plant triglyceride	Plant triglyceride		Plant triglyceride
		Propylene glycol		Triacetin
Maltodextrine and Gum Arabic (g)	100	100	100	100
Propylene glycol (g)		233.3	116.6	
Plant triglyceride (g)	111.1	111.1	111.1	111.1
Triacetin (g)				
Water (g)	233.3		116.7	233.3
Sum:	444.4	444.4	444.4	444.4

4.1.3 Results & Discussion

The simple emulsion where propylene glycol was used undilutedly was not stable; thus, no double emulsions were prepared out of this emulsion.

The Box-and-Whisker-Plot in Fig. 11 gives an overview of the four double emulsions where the simple emulsion consisted of flavor PTG only and the simple emulsion itself. It displays that the double emulsion that consisted of one part continuous phase and four parts dispersed phase had the biggest average particle size. Moreover, it shows that the average particle size was decreasing with increasing amount of continuous phase. The particle size of the O/W simple emulsion was comparable to the O/W/O emulsions that contained a high volume of continuous phase.

The relationship between the volume of continuous phase and particle size is illustrated in Fig. 12. The amount of continuous phase is expressed as the ratio of continuous and dispersed phase. Thus, a high ratio means a high amount of continuous phase. The graph clearly shows that a high volume of continuous phase was linked to smaller particles; a low volume, in contrast, was linked to bigger particles. The graph illustrates a noticeable increase in particle size when the volume of continuous phase in the O/W/O emulsion was 40% or less. This is also shown in Fig. 13, Fig. 14 and Fig. 15. The photographs show the double emulsions with a ratio of 3:2, 2:3 and 1:4. Obviously, particle size decreased with increasing amount of continuous phase.

To find out whether the differences between the particle sizes of the five emulsions were significant, statistical analysis was performed as described in chapter 3.4. The ANOVA analysis revealed a statistically significant difference between the means of the 6 samples ($\alpha:0.05$; $p:<0.0000$). The differences were further specified with a multiple range test. The emulsions were assorted to homogenous groups according to their particle size. The results of the multiple range test are listed in Table 3. The O/W/O emulsion where the volume of continuous phase was biggest had the smallest particle size. It was in the same homogenous group with the double emulsion that consisted of three parts continuous phase and two parts dispersed phase and the simple emulsion that provided the basis for all double emulsions. Consequently, the three emulsions did not differ from each other with respect to their particle size. As particle size is a relevant factor in emulsion stability the results indicate that the emulsions that belong to the homogenous group A are more stable than the others. As there is no statistical difference between the emulsions of one homogenous group and the flavor content of the emulsion should be as high as possible the ratio 3:2 (sunflower oil: O/W emulsion) was chosen for further experiments.

Trials that were performed afterwards confirmed that a higher continuous phase concentration contributed to emulsion stability. In chapter 4.10 and 4.11 the ratios of continuous and dispersed phase were either 1:1 or 3:2. In both trials the emulsions that contained less continuous phase were less stable than the ones where the continuous phase concentration was higher. A bigger particle size, caused by a smaller continuous phase concentration is a putative explanation for these results.

Anyway, the minimal achievable particle size of an emulsion is a multivariable problem that is also influenced by other parameters such as emulsifier concentration. As the emulsifier concentration was kept constant in the continuous phase, the concentration was variable in the entire emulsion. Consequently, the impact of emulsifier concentration was not evaluated in this experiment. However, this is a parameter that should be studied more closely.

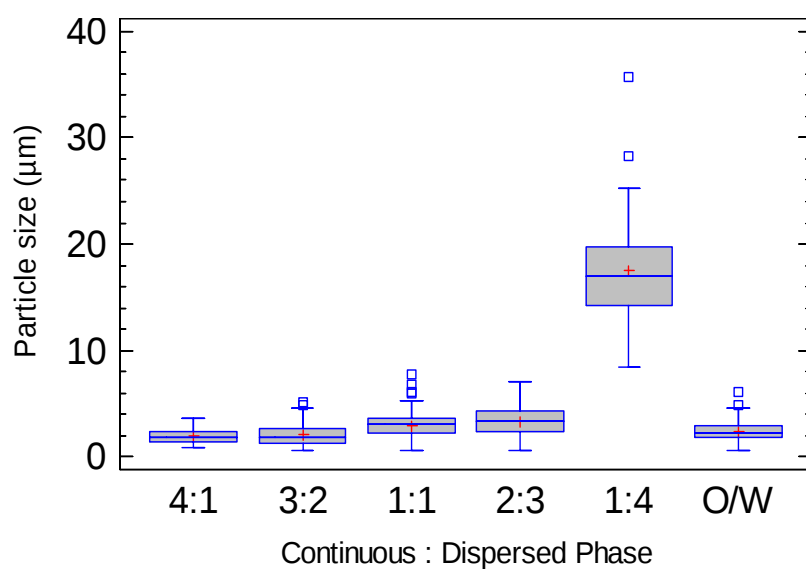


Fig. 11: Box and Whisker Plot of O/W and O/W/O emulsions. Data derived from the emulsion based on plant triglyceride.

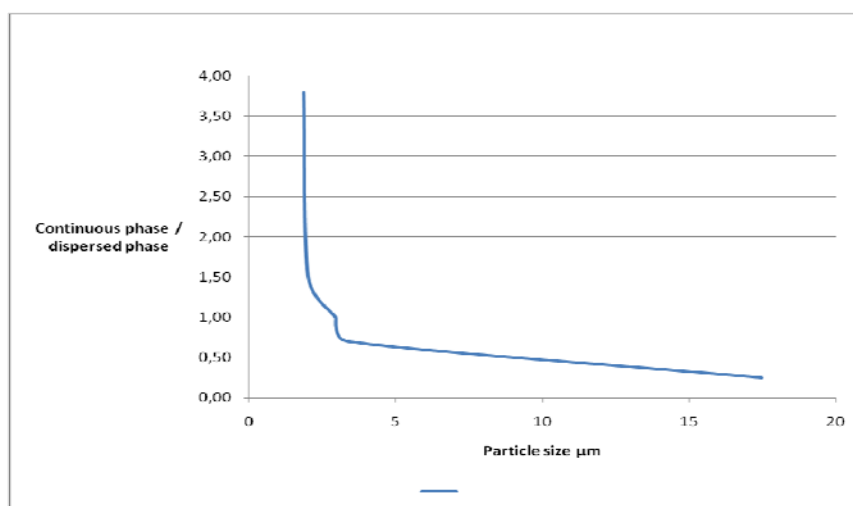


Fig. 12: Effect of the ratio of continuous and dispersed phase on particle size

Table 3: Homogenous groups and average particle size of the emulsions.

Data derived from the emulsion based on plant triglyceride.

Sunflower Oil : O/W Emulsion	Average Particle Size (μm)	Homogenous Groups
4 : 1	1.87	A
3 : 2	2.02	A
1 : 1	2.95	B
2 : 3	3.36	B
1 : 4	17.47	C
Simple Emulsion	2.37	A

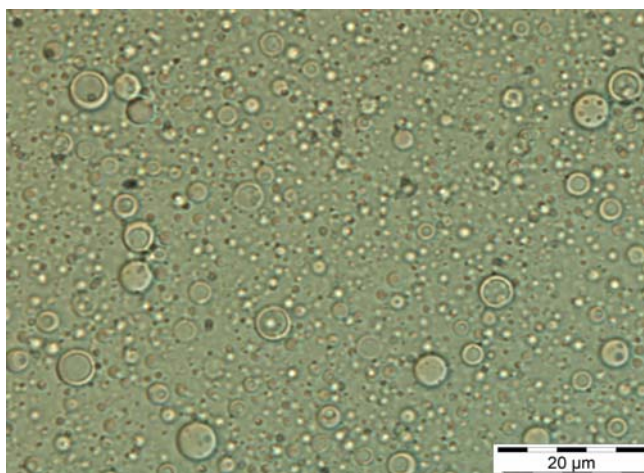


Fig. 13: O/W/O emulsion; sunflower oil: O/W $\triangleq$ 3:2

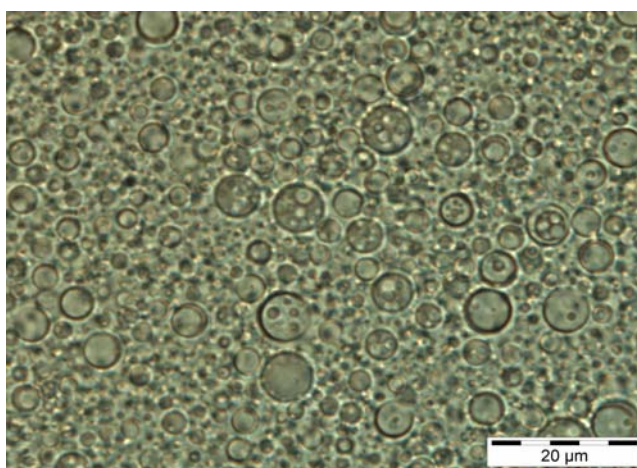


Fig. 14: O/W/O emulsion; sunflower oil: O/W $\triangleq$ 2:3

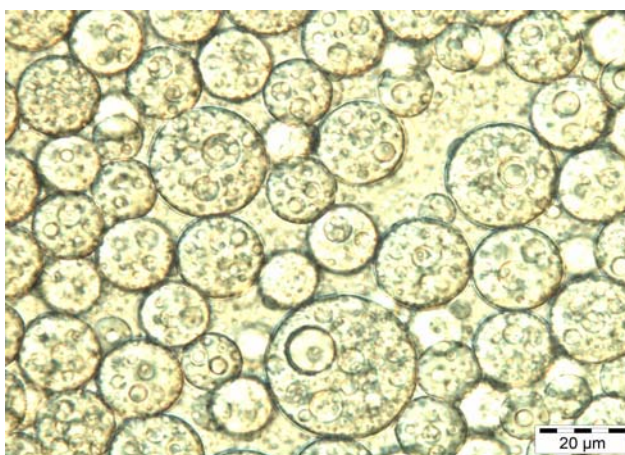


Fig. 15: O/W/O emulsion; sunflower oil: O/W $\triangleq$ 1:4

4.2 Processing pressure for O/W simple emulsions

4.2.1 Introduction

The aim of this trial was to decrease particle size of an O/W emulsion and to find out to which extent particle size can be reduced by a high pressure emulsification device.

4.2.2 Experimental procedure

The emulsions were prepared as described in chapter 3.2.1 and 3.2.2. 150 g (± 0.01 g) maltodextrine and Gum Arabic were mixed with 350 g (± 0.01 g) distilled water; 166.6 g (± 0.01 g) flavor PTG formed the dispersed phase. The coarse emulsion was passed through the microfluidizer as described in 3.2.4 at pressures that ranged from 10-200 MPa. The Z-reaction chamber was used in this trial. The samples were analyzed with an image analyzer as described in chapter 3.3.1. One emulsion did not undergo microfluidization to study the performance of the microfluidizer. The Ultra-turraxed sample was only mixed shortly.

4.2.3 Results & Discussion

The Box-and-Whisker Plot in Fig. 16 gives an overview of the eleven emulsions that were prepared in this trial and shows that the particle sizes of the single emulsions were at close quarters. It also illustrates that the average particle size and the particle size range became smaller between 10 and 100 MPa and that this tendency did not continue at higher pressures; particle size increased beyond 140 MPa.

Fig. 18 displays the particle size distributions of all emulsions that were produced in this trial. Particles beyond 8 μm were not considered because they did not remarkably affect the statement. The figure specifies the observations of the Box-and-Whisker Plot and shows that the reduction of average particle size was accompanied by a narrower particle size distribution. The illustration shows that the

particle size distribution became narrower up to 80 MPa. The emulsions that were prepared at low pressures or with the Ultra-Turrax had more particles bigger than 3 μm than emulsions that were processed at 60 or 80 MPa. The emulsion that was prepared at 80 MPa had the narrowest range of all. This is of great importance because the number of big particles that have a tendency to sediment becomes smaller when the range decreases; accordingly, a narrow particle size distribution contributes to emulsion stability. These results suggest that pressures of 80 MPa are best suited for producing emulsions with a small average particle size and a narrow particle size distribution. Fig. 18 also shows that the particle size distribution enlarged at higher pressures which had a negative effect on emulsion stability. Beyond 100 MPa the average particle size and the particle size distribution deteriorated. This suggests that higher pressures led to an over-processing of the emulsion and were detrimental to emulsion stability. To find out which processing pressure led to the best results and which processing pressures were already too high, frequency histograms of the emulsions that were processed at 60, 80, 100 and 120 MPa are displayed in Fig. 18. An increase of processing pressure from 60 to 80 MPa led to a particle size reduction and a narrower particle size distribution. The emulsion that was processed at 80 MPa had the highest number of particles below one μm . An increase of processing pressure from 80 to 100 MPa increased the average particle size and particle size distribution and thus deteriorated the emulsion. The emulsion that was processed at 120 MPa delivered better results. This is quite remarkable because these results did not fit the tendency of increasing particle sizes with increasing processing pressure. However, the test was repeated under the same conditions (chapter 4.3); the results changed marginally. Also in chapter 4.3 the emulsion that was processed at 80 MPa was best; followed by the emulsion that was processed at 120 MPa. The two trials are more closely compared in 3.3. To this end it remains unclear why the emulsions that were processed at 120 MPa delivered results divergent from the tendency in two independent experiments.

The effect of processing pressure of particle size is more directly illustrated in Fig. 19; it plots the average and the median of particle size against the processing pressure. The median and the average values decreased up to 80 MPa; an application of higher pressures led to an increase in particle size. Consequently,

higher processing pressures deteriorated the emulsions; even though the particle size at high pressures was smaller compared to very low processing pressures.

To find out whether these observations were relevant, statistical analysis was performed as described in 3.4. The ANOVA analysis revealed a statistically significant difference between the twelve samples at the 95.0% confidence level ($p:0.0000$). The differences were more precisely analyzed with a multiple range test. Emulsions that belong to the same homogenous group do not statistically differ from each other. Table 4 lists the results of the multiple range test including a summarizing statistic. The emulsions are assorted in ascending order due to the average particle size. The simple emulsion that was prepared with the Ultra-Turrax had the biggest particles; it is the only one that belongs to the homogenous group F. Consequently, a processing with the microfluidizer reduced particle size and led to a more finely dispersed emulsion at any processing pressure. The emulsions that were prepared at 80 or 120 MPa had the smallest average particle size and belonged to one same homogenous group. All other emulsion did not belong to the homogenous group A and were consequently significantly bigger.

The results suggest that moderate processing pressures of 80 MPa are most suitable for emulsion processing because the resulting emulsions have a low average particle size and a narrow particle size range. Higher pressures increased particle size compared to moderate pressures but delivered better results than very low pressures. The microfluidizer ameliorated the coarse simple emulsion that was shortly mixed with the Ultra-Turrax at any applied pressure.

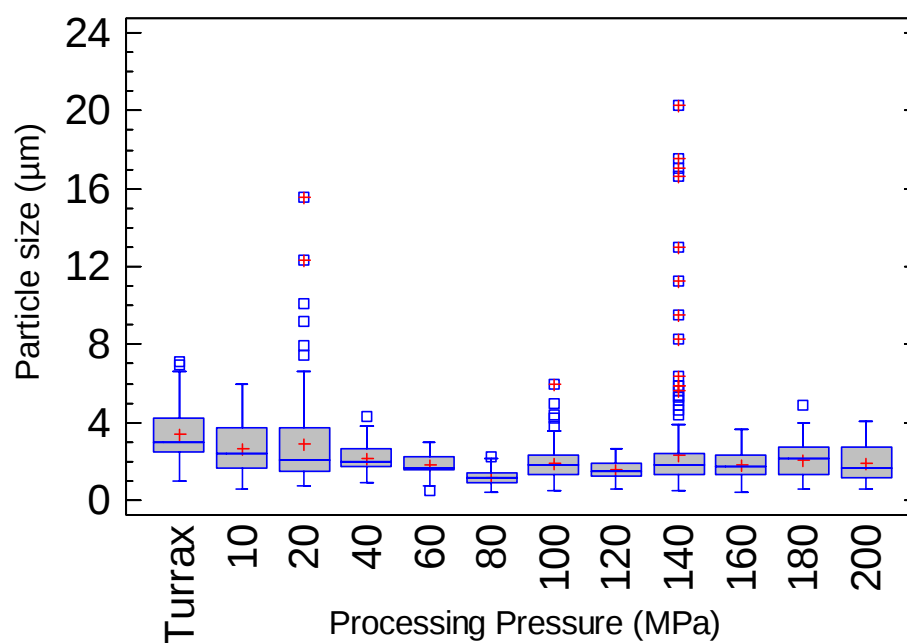


Fig. 16: Box-and-Whisker Plot of the simple emulsion

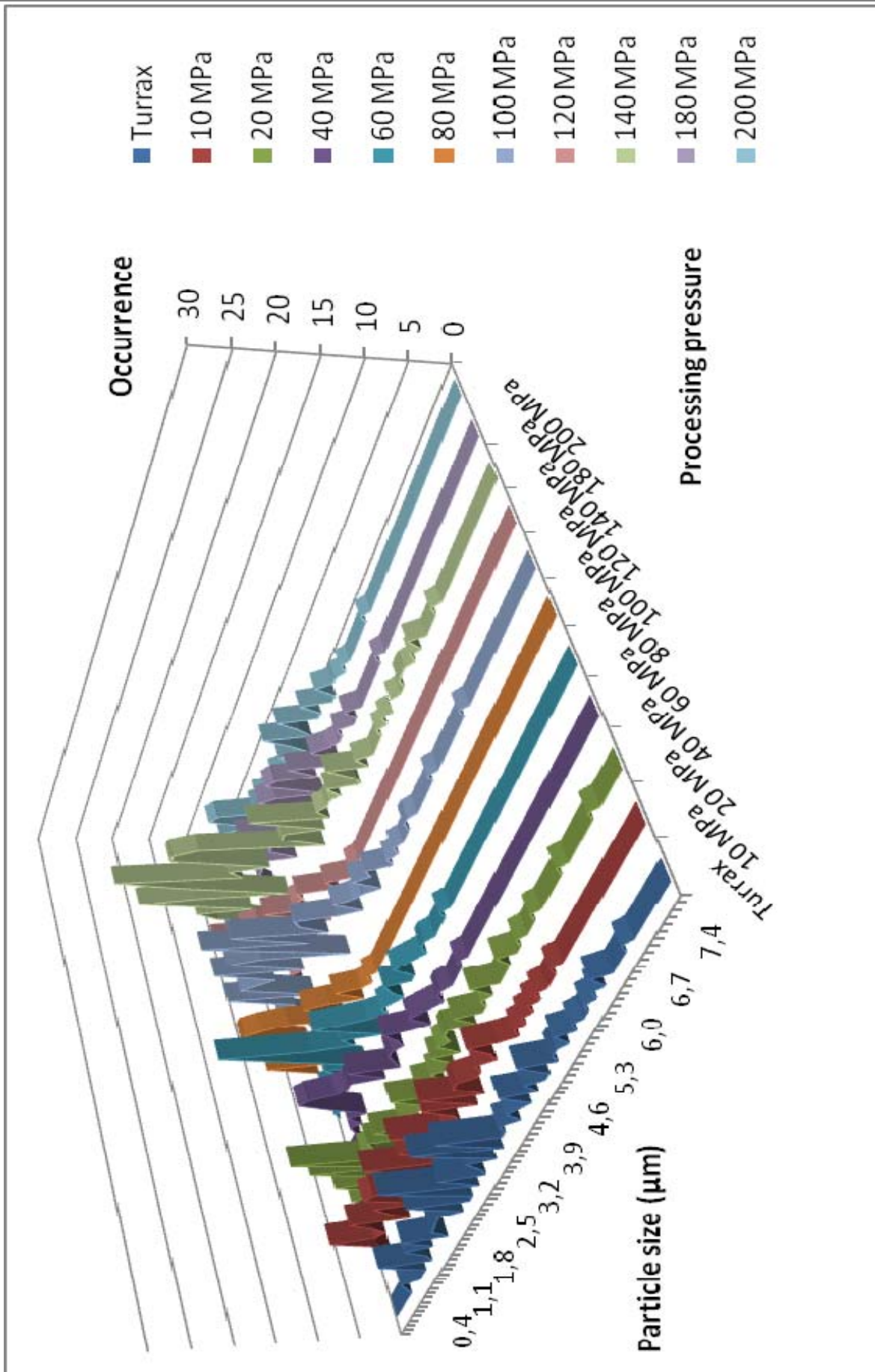


Fig. 17: Particle size distributions of O/W emulsions that were processed at different pressures

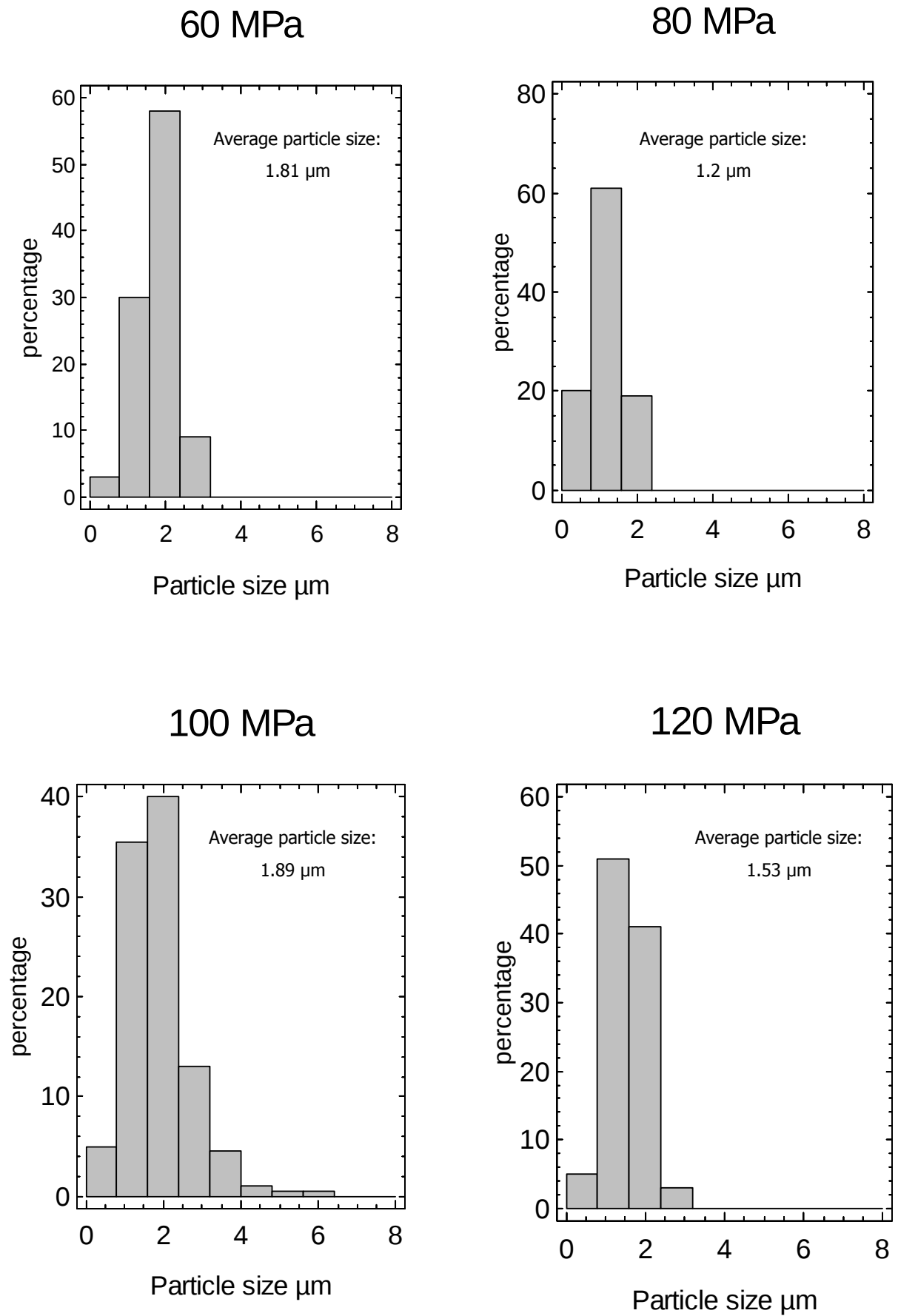


Fig. 18: Frequency Histograms of emulsions that were processed at 60, 80, 100 and 120 MPa

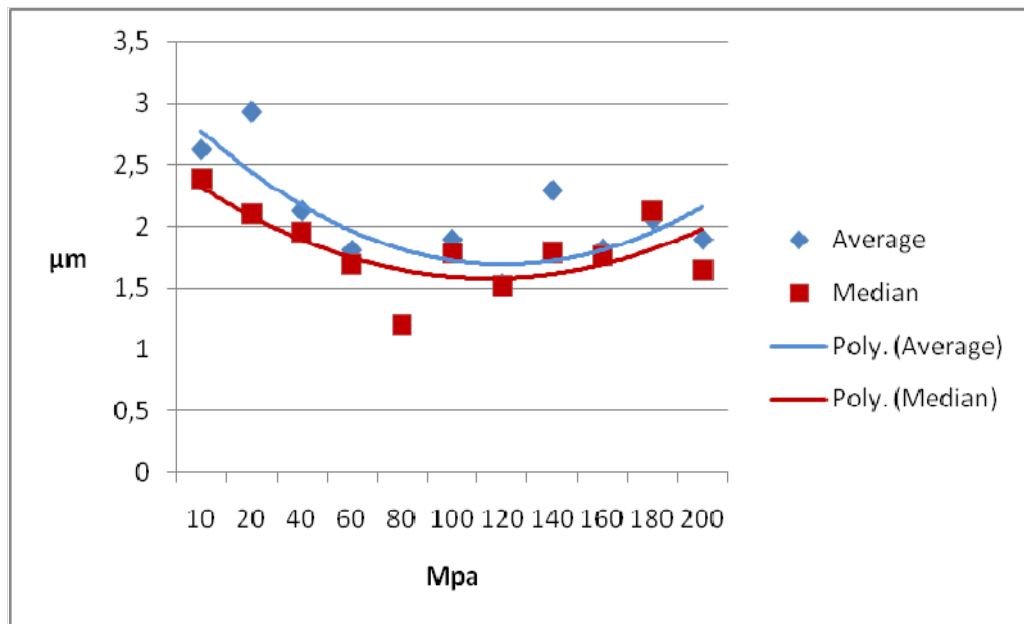


Fig. 19: Relationship between processing pressure and particle size

Table 4: Results of the multiple range test including a summarizing statistic

Increasing particle size ↓	Processing Pressure (MPa)	Average particle size (µm)	Minimum (µm)	Maximum (µm)	Range (µm)	Standard deviation	Coeff. of variation (%)	Homogeneous Groups					
	80	1.2	0.41	2.26	1.85	0.41	34.63	A					
	120	1.53	0.58	2.68	2.1	0.46	29.91	A	B				
	60	1.81	0.51	3.01	2.5	0.52	28.72		B	C			
	160	1.82	0.44	3.65	3.21	0.73	40.18		B	C			
	200	1.89	0.59	4.05	3.46	0.9	47.59		B	C			
	100	1.89	0.51	5.98	5.46	0.81	42.51			C			
	180	2.06	0.59	4.84	4.25	0.86	41.74			C	D		
	40	2.13	0.92	4.32	3.4	0.68	31.74			C	D		
	140	2.29	0.51	20.28	19.77	2.33	101.54				D		
	10	2.63	0.58	5.94	5.36	1.25	47.42				D	E	
	20	2.94	0.74	15.57	14.83	2.37	80.81				D	E	
	Ultra-Turrax	3.37	1.02	7.16	6.13	1.42	42						F

4.3 The influence of multiple passages on particle size

4.3.1 Introduction

Theory revealed that the particle size of an emulsion can be reduced by supplying more energy. Chapter 4.2 already dealt with the impact of processing pressure on the average particle size and the particle size range. The aim of this trial was to find out whether repeated passes of the emulsion through the microfluidizer led to smaller emulsion particles or a narrower particle size distribution.

4.3.2 Experimental procedure

The simple emulsions were prepared as described in chapter 3.2.1 and 3.2.2. The emulsion formula was the same as in 4.2. Nine of ten coarse emulsions were passed through the microfluidizer at pressures that ranged from 40-200 MPa. A small amount of the emulsion was taken to determine putative changes after the second run. The rest of the emulsion was recirculated a second time each with the same pressure. The samples were analyzed with an image analyzer as described in chapter 3.3.1. The collected data was appraised with statistical methods as described in chapter 3.4.

4.3.3 Results & Discussion

The Box-and-Whisker-Plot in Fig. 20 gives an overview of all samples that were produced in this trial. It shows that particle size was reduced through a second for most emulsions and that the particle size range could be reduced through a second passage.

Fig. 21 illustrates the changes of the median and average values of particle size after the first and after the second run. The median particle size was smaller for all emulsion except for 80 and 120 MPa. A second run increased the average particle size in these two emulsions; the difference at 120 MPa was marginal.

Table 5 lists the changes in the average particle sizes and the particle size range caused by the second run. The emulsions are assorted in ascending order to the particle size. It is quite remarkable that the two emulsions (processing at 80 and 120 MPa) that had the smallest average particle size and the most homogenous distribution after the first run exhibited worse results after the second passage. All other emulsions exhibited a smaller average particle size and a narrower distribution after the second run. The exact numbers are absolutely and proportionally listed in the table. To explore whether the observed changes were significant the data was statistically analyzed as described in 3.4.

The ANOVA analysis revealed a statistically significant difference between the means of the 16 samples at the 95.0% confidence level ($p:0.0000$). The differences were more precisely analyzed with a multiple range test. Table 6 lists the result of the multiple range test including a summarizing statistic. In the table the emulsions are assorted in ascending order to the average particle sizes. The emulsions that were processed one time at 80 MPa or two times at 40 or 60 MPa belonged to the homogenous group A; accordingly, they had the smallest average particle size and did not statistically differ from each other. Emulsions that were treated with high pressures had a big average particle size; irrespective of the number of passages and belonged to the homogenous groups G, H; I and J. To this end, Table 6 displays that small average particle sizes and narrow particle size distributions were obtained at moderate pressures and high pressures increased particle size and deteriorated the emulsions; consequently, it supports the finding of 4.2 that came to the same conclusion.

The results of statistical analysis including results of Table 5 and Table 6 are separately discussed for each pressure in the chapters 4.3.3.1 to 4.3.3.8.

Fig. 30 visualized the particle size distributions of all emulsions that were prepared in this experiment.

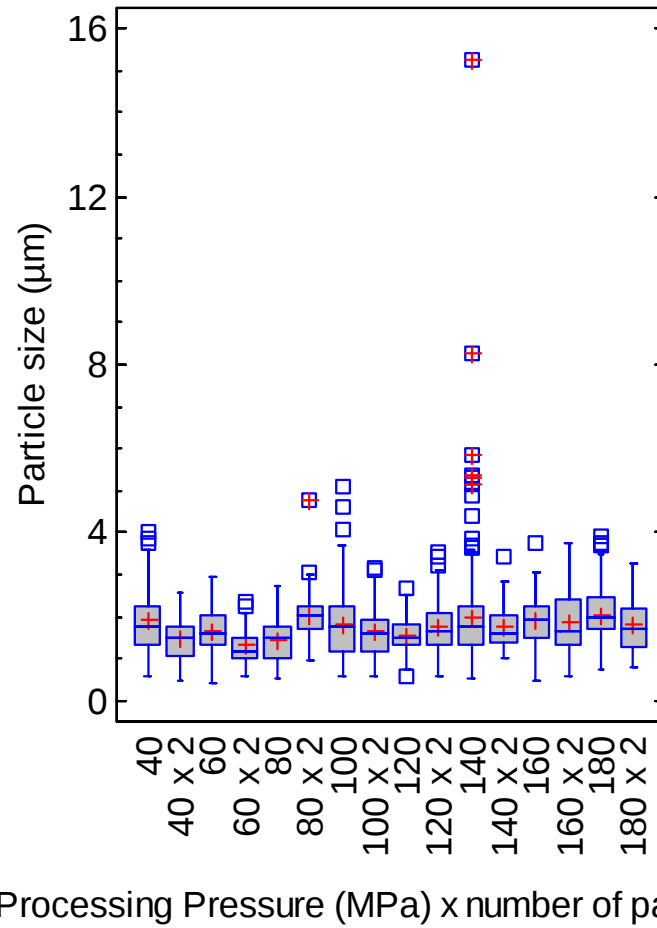


Fig. 20: Box-and-Whisker-Plot of the simple emulsions. Comparison of the first and

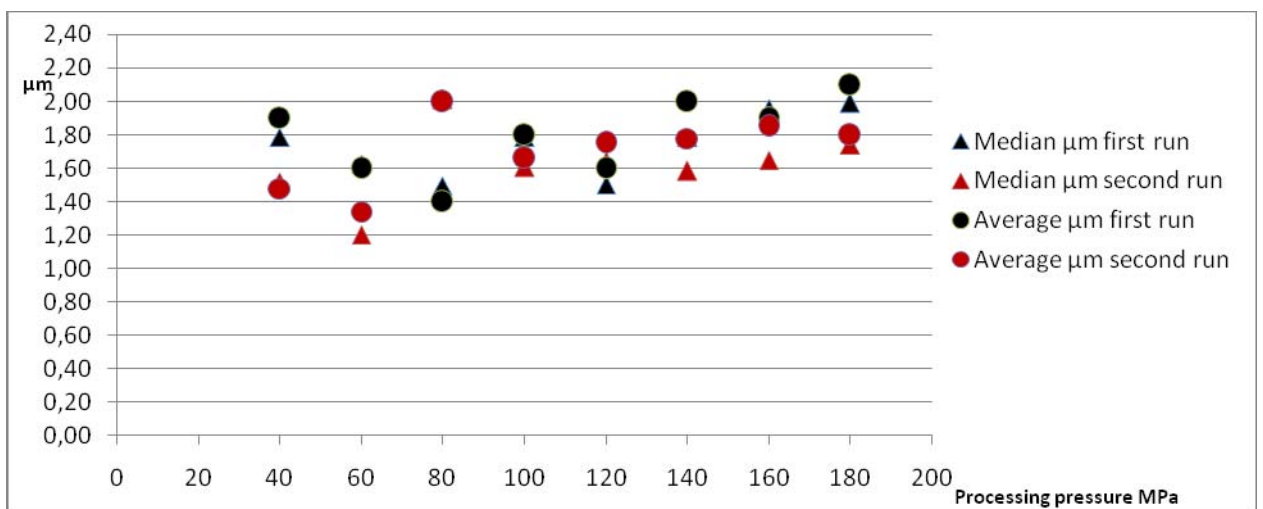


Fig. 21: Median values of particle size after the first and after the second run

Table 5: Changes in particle size and range after the first and second passage

Increasing particle size ↓	Processing pressure (MPa)	Average particle size (μm) 1 st run	Average particle size (μm) 2 nd run	Difference μm	Difference %
	80	1.44	2.02	-0.58	40.4
	120	1.57	1.75	-0.18	11.5
	60	1.66	1.34	0.33	-19.7
	100	1.83	1.66	0.16	-8.9
	40	1.91	1.48	0.43	-22.7
	160	1.91	1.85	0.06	-3.2
	140	1.98	1.78	0.20	-10.3
	180	2.08	1.80	0.29	-13.9
	Processing pressure (MPa)	Range (μm) 1 st run	Range (μm) 2 nd run	Difference μm	Difference %
Increasing particle size ↓	120	2.08	2.96	-0.88	42.4
	80	2.24	3.79	-1.55	69.2
	60	2.50	1.79	0.72	-28.6
	180	3.18	2.50	0.68	-21.4
	160	3.27	3.16	0.11	-3.5
	40	3.44	2.10	1.34	-38.9
	100	4.50	2.56	1.94	-43.1
	140	14.77	2.41	12.37	-83.7

Table 6: Summarizing statistic

Processing pressure (MPa)	Average particle size (μm)	Standard deviation	Coeff. of variation (%)	Minimum	Maximum	Range	Homogenous groups									
600 x 2	1.34	0.42	31.79	0.58	2.37	1.79	A									
800	1.44	0.47	32.46	0.52	2.76	2.24	A	B								
400 x 2	1.48	0.45	30.26	0.47	2.57	2.10	A	B	C							
1200	1.57	0.41	26.39	0.58	2.66	2.08		B	C	D						
1000 x 2	1.66	0.55	33.35	0.58	3.14	2.56			C	D	E					
600	1.66	0.53	32.12	0.44	2.94	2.50			C	D	E	F				
1200 x 2	1.75	0.60	34.08	0.58	3.54	2.96				D	E	F	G			
1400 x 2	1.78	0.58	32.42	1.03	3.44	2.41			C	D	E	F	G	H	I	J
1800 x 2	1.80	0.58	32.57	0.80	3.30	2.50					E	F	G		I	
1000	1.83	0.81	44.30	0.58	5.08	4.50					E	F	G	H	I	
1600 x 2	1.85	0.74	39.75	0.58	3.75	3.16						F	G	H	I	
400	1.91	0.70	36.66	0.58	4.02	3.44							G	H	I	J
1600	1.91	0.56	29.32	0.46	3.73	3.27							G	H	I	J
1400 bar	1.98	1.28	64.40	0.51	15.28	14.77								H		J
800 bar x 2	2.02	0.57	28.20	0.98	4.77	3.79								H	I	J
1800	2.08	0.68	32.71	0.74	3.92	3.18										J

4.3.3.1 One and two passages at 40 MPa

Due to heteroskedasticity (α : 0.05; P : 0.0003) no t-test was performed. The medians differed from each other after the first and the second run (α : 0.05; p : 0.0001). Fig. 22 displays the particle size distributions after both passages and illustrates that the average particle size and the particle size distribution improved through a second passage. This is underlined by Table 6 that displays the observation numerically. The second run dropped the range by 38.9% and the average particle size by 22.7%. Thus, the second run significantly contributed to a more homogenous emulsion with smaller particles.

After the second run the emulsion that was processed at 40 MPa was the second-best regarding the average particle size and range. Only the emulsion that was prepared at 60 MPa exhibited better results after each passage. A comparison of the two emulsions revealed equal variances and means (α : 0.05; p : 0.7056; 0.0878); however, the medians differed (α : 0.05; p : 0.0474).

Fig. 30 shows the particle size distributions in context with the other emulsions.

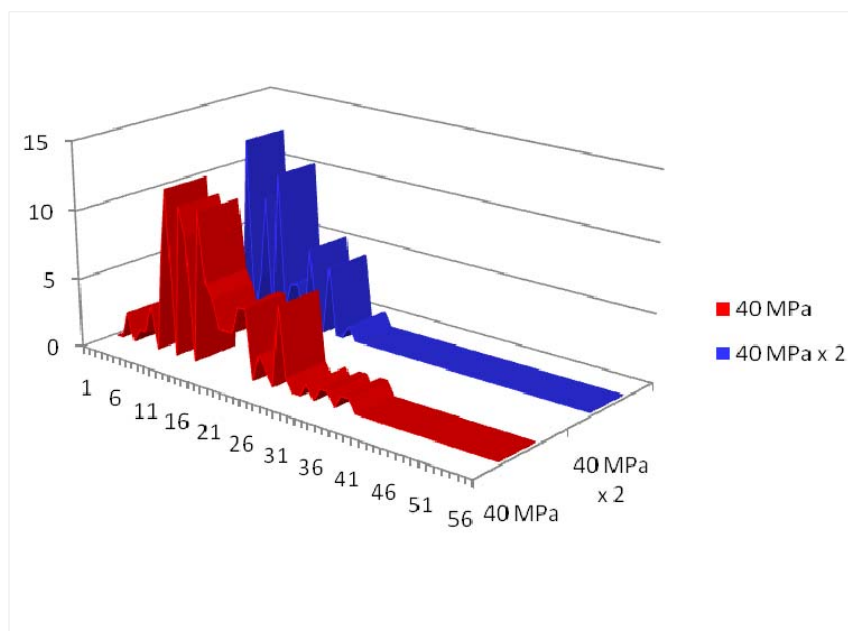


Fig. 22: Particle size distribution of 40 MPa and 40 MPa x 2

4.3.3.2 One and two passages at 60 MPa

Due to homoskedasticity (α : 0.05; p : 0.0630) a t-test was performed. The drop of the average particle size by 19.7% through the second run was statistically significant. Because the means and the medians of the two samples differed from each other significantly (α : 0.05; p : 0.0001; p : 0.0003). Fig. 23 displays the particle size distributions after both runs and illustrates that the average particle size and the particle size distribution improved through a second passage. Table 6 supports this observation numerically.

The emulsion that was prepared at 60 MPa two times had the smallest particles and the narrowest particle size distribution of all samples. The second run dropped the range and the average particle and therefore contributed to a more homogenous emulsion with smaller particles. These results suggest that pressures of 60 MPa are sufficiently high to produce emulsions with small, finely dispersed particles.

It clearly shows that the emulsion was more homogeneously distributed than the other emulsions.

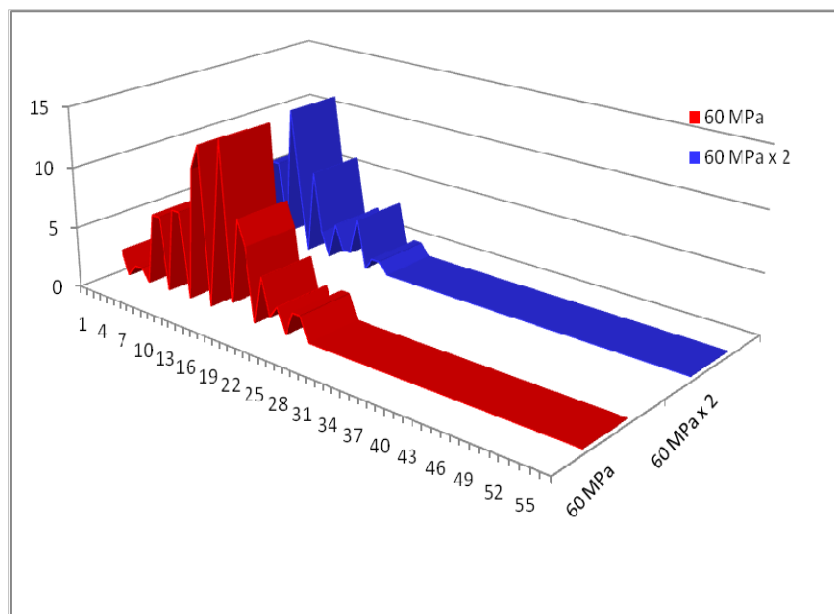


Fig. 23: Particle size distribution of 60 MPa and 60 Mpa x 2

4.3.3.3 One and two passages at 80 MPa

The emulsion that was processed at 80 MPa exhibited significantly larger particles and a wider particle size distribution after the second run. This difference refers to the means and the medians of the two samples. (α : 0.05; p : 0.0000; p : 0.0000).

After the first run the emulsion had the smallest average particle size and the second-smallest range; however, this changed after the second run. The average particle size and the range increased by 40% respectively 70%. Fig. 24 illustrates the particle size distribution of the emulsion after the first and second passage. Fig. 30 shows it in context with the other emulsions. Both bespeak the decline after the second run.

An over-processing of the emulsion during the second run is a putative explanation for this deterioration. This assumption is in accordance with literature [JAFARI et al. (2007)] stating that too high pressures or too many passages are detrimental to emulsion stability. However, the image analysis that was performed to determine the average particle size is a further source of errors; one cannot exclude that particle sizes have been over- or underestimated.

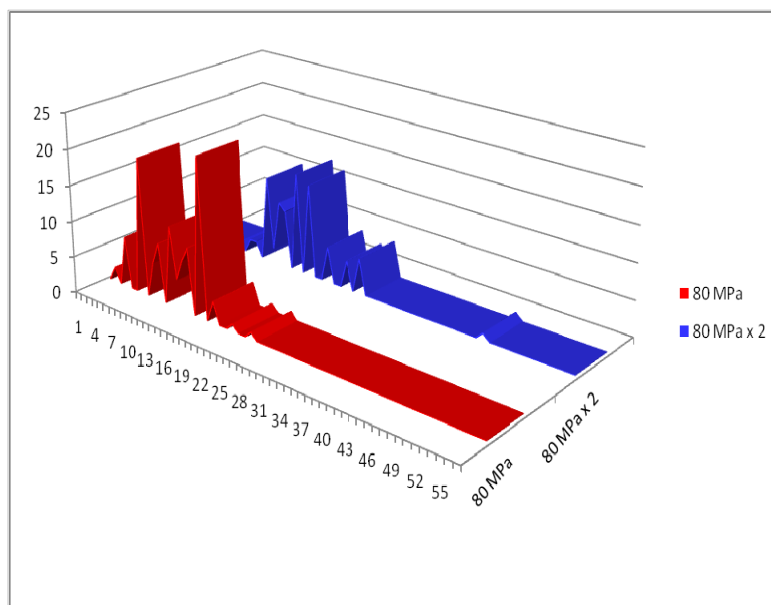


Fig. 24: Particle size distribution of emulsions that were prepared at 80 MPa.

4.3.3.4 One and two passages at 100 MPa

Heteroskedasticity (α : 0.05; P : 0.0000) disqualified a t-test. The medians of the samples were equal after the first and the second run (α : 0.05; p : 0.0815). Thus, the drop of particle size by 8.9% in the second run was not statistically significant. However, the range dropped by 43% which strongly suggests that the emulsion became more homogenous, even though the reduction of particle size was marginal. Fig. 25 illustrates the particle size distribution after the two runs. It clearly shows the narrower range after the second run, but also visualizes the inhomogeneous structure after the first pass. This clearly puts the amelioration into perspective. Even though the improvement after the second run was proportionally distinct, the factual range was only averagely narrow.

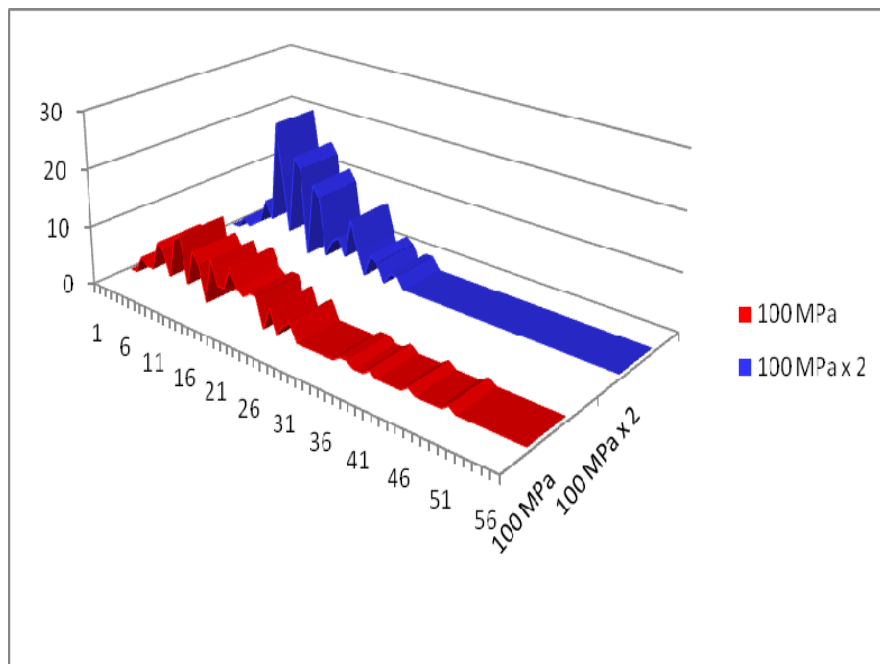


Fig. 25: Particle size distribution of emulsions that were prepared at 100 MPa.

4.3.3.5 One and two passages at 120 MPa

A second run at 120 MPa deteriorated the results. Table 6 shows that the emulsion was second best after the first passage. Generally, this observation does not fit the tendency of increasing particle sizes with increasing processing pressures, because the emulsions that were processed at 100 and 140 MPa delivered worse results. However, two independently performed experiments (described in the chapters 4.2 and 4.3) came to these results. The average particle size increased by 11.5% the range by 42% in the second run; nevertheless, the medians did not differ significantly. (α : 0.05; p : 0.1625). The average particle size was 1.57 μm after the first and 1.75 μm after the second run which is negligible.

Fig. 26 and Fig. 30 exemplify the changes concerning the particle size distributions. Both visualize a remarkably wider distribution after the second run.

It is quite remarkable that the two emulsions that had the smallest average particle size and the most homogenous distribution after the first run deteriorated after the second passage. Even though, the changes were significant in only one case.

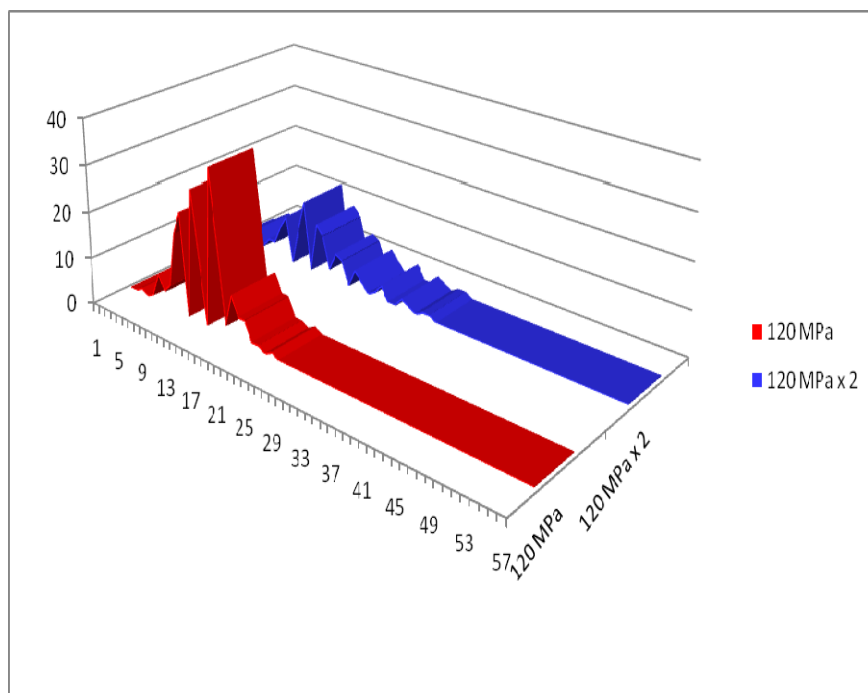


Fig. 26: Particle size distribution of emulsions that were prepared at 120 MPa.

4.3.3.6 One and two passages at 140 MPa

The samples were heteroskedastic (α : 0.05; p : 0.000). There was no difference between the medians and the distributions of the samples (α : 0.05; p : 0.3524 p : 0.3524). Thus, the drop of the average particle size by 10.3% was not significant.

The particle size distribution dropped by 83.7% which is visualized in Fig. 27. However, after the first pass the emulsion that was prepared at 140 MPa had the widest particle size distribution of all; consequently this amelioration must be seen in context to the inhomogeneous initial situation. Table 5 and Table 6 show that the amelioration after the second passage were moderate compared to other emulsions of this experiment.

In conclusion, the results of this trial are contradictory: On the one hand they indicate that high processing pressures promote over-processing and formation of bigger particles; on the other hand a second run with the same pressure increased homogeneity.

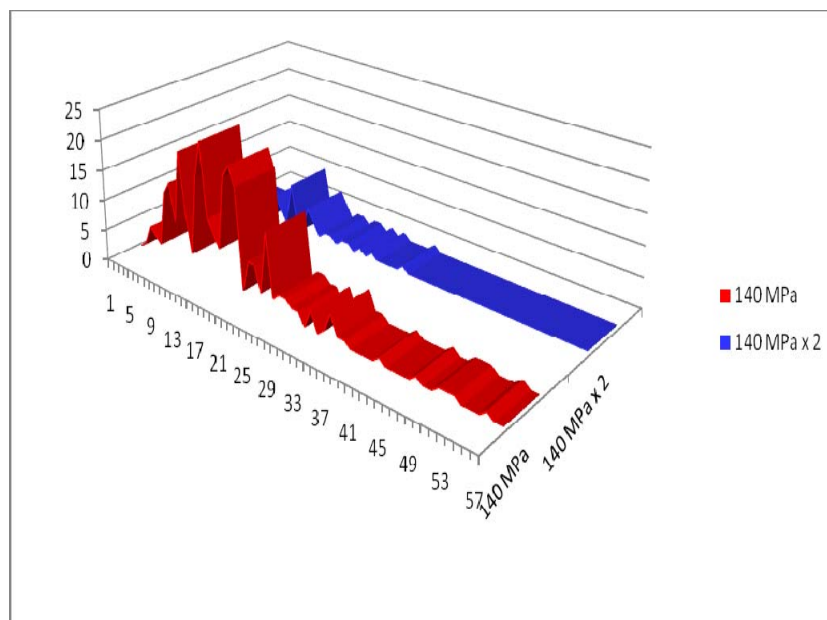


Fig. 27: Particle size distribution of emulsions that were prepared at 140 MPa.

4.3.3.7 One and two passages at 160 MPa

There was no difference between the standard deviations, means and medians of the samples (α : 0.05; p : 0.1601; p : 0.7149; p : 0.4618). Consequently, the second run did not lead to statistically relevant changes. The differences between the first and the second run referring to the average particle sizes and their distributions are listed in Table 6. Both trials led to emulsions with an average particle size of approximately 1.9 μm ; thus, the results at 160 MPa were moderate. The table also displays the homogenous groups that were generated in the multiple range test. All emulsions that were processed with pressures up to 120 MPa had a big average particle size and a wide particle size distribution. This result underlines the findings of 4.2 where it was stated that too high pressures increase particle size and lead to a wider distribution.

Fig. 28 displays the distribution after the first and the second run.

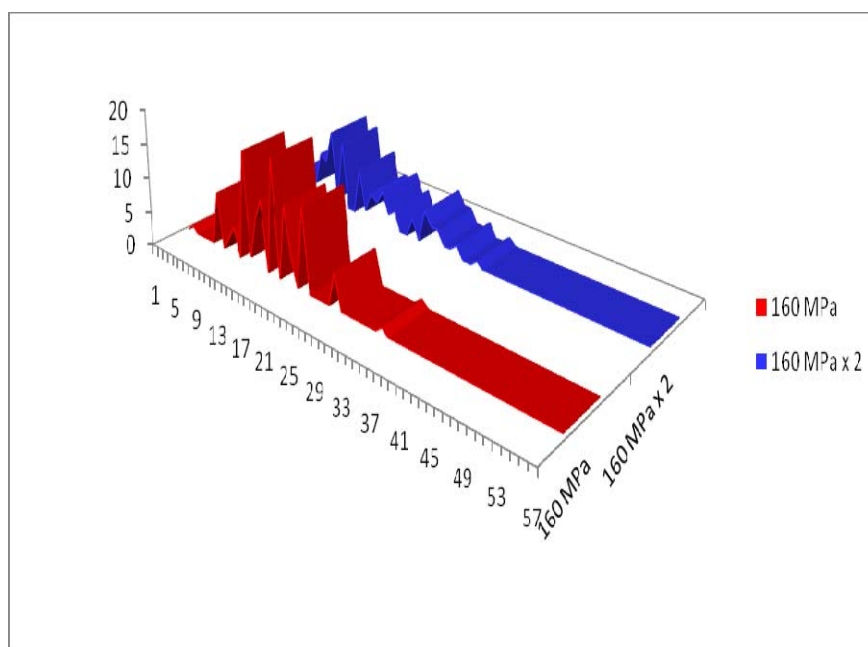


Fig. 28: Particle size distribution of emulsions that were prepared at 160 MPa.

4.3.3.8 One and two passages at 180 MPa

There was no statistical difference between the standard deviations of the two samples (α : 0.05; p : 0.122715). The means, medians and distributions of the two samples differed statistically (α : 0.05; p : 0.0012; p : 0.0019; p : 0.0002).

The summarizing statistic in Table 6 shows that the results improved after the second passage. The second run dropped the range of particles by 21.4% and the average particle size by 13.9% which was significant. Anyway, the factual particle size was large compared to samples that were produced at moderate pressures after the first and the second run. Moreover, the particle size distributions were relatively wide compared to samples where less pressure was applied. These results indicate that high processing pressures have an inverse effect and do not contribute to emulsions with smaller particles and a narrower particle size distribution.

Fig. 29 displays the particle size distributions after both passages.

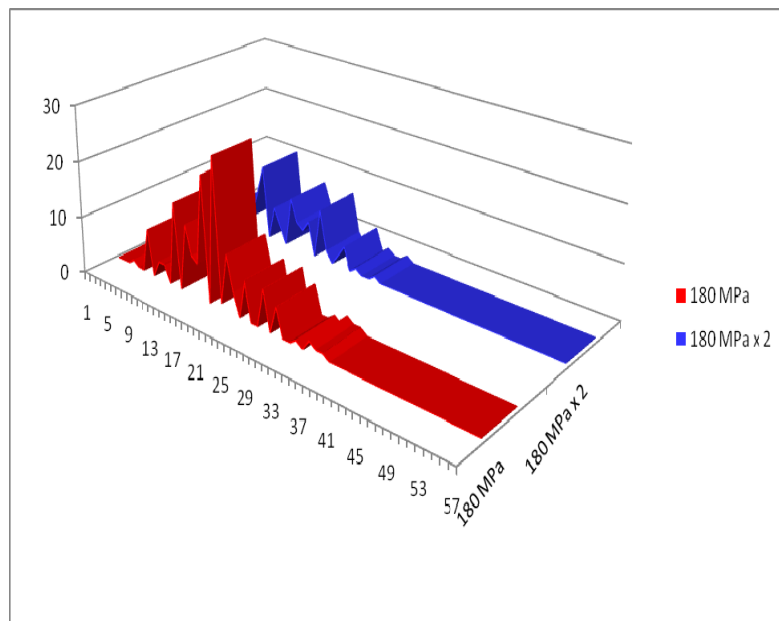


Fig. 29: Particle size distribution of emulsions that were prepared at 180 MPa.

4.3.3.9 Comprehensive conclusion

A second passage of the O/W emulsion through the microfluidizer led to a further particle size reduction that was accompanied by a narrower particle size distribution in six of eight emulsions. The differences were significant for the emulsions that were processed at 40, 60 and 180 MPa. The emulsions that were processed at 80 and 120 MPa were the most homogenous ones with the smallest particles after the first run. A second passage deteriorated the results. This suggests that emulsions that already have a small average particle size and a narrow distribution after the first run, cannot further be improved by a second run.

The smallest particles were obtained by one passage at 80 MPa or two passages at 40 or 60 MPa. Whereas two passages at 60 MPa was best, followed by one passage at 80 MPa. To find out whether there was a significant difference between the three samples the ANOVA was performed; it detected no differences between the means of the three samples (α : 0.05; p : 0.2272; F : 1.49). Consequently, the emulsions did not statistically differ from each other with respect to the average particle size. However, two passages at 40 or 60 MPa led to a narrower particle size range which is listed in Table 6.

Finally, a second run leads to product losses, is more time and cost intensive and furthermore did not lead to significantly smaller particles. The observed narrower range that went along with a second passage was moderate; consequently, there are hardly any arguments to recommend multiple passages with a microfluidizer for this formulation. Thus, the results indicate that one passage at 80 MPa is well suited for producing emulsions with a small particle size and a homogenous distribution.

Furthermore, the trial showed that too high processing pressures have an inverse impact on the average particle size and the particle size distribution. Emulsions that were prepared beyond 120 MPa averagely had bigger particles and a more inhomogeneous structure.

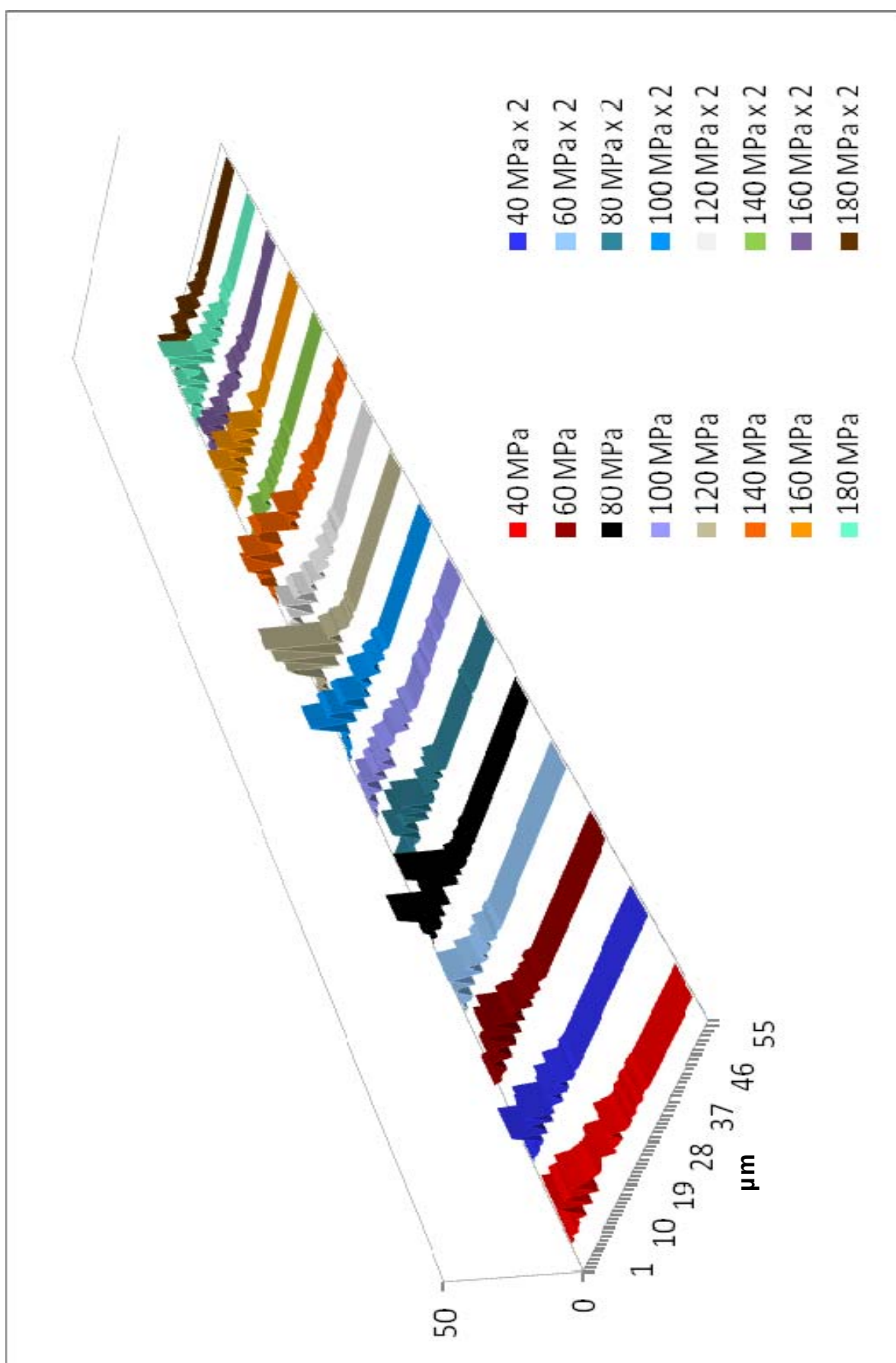


Fig. 30: Particle size distributions of all emulsions

4.4 Reproducibility of particle sizes

4.4.1 Introduction

Eight samples of the chapters 4.2 and 4.3 were prepared at equal conditions; to find out whether the production of emulsions via micro-fluidization entails reproducible results, these eight samples of the two trials were compared.

4.4.2 Results and Discussion

Fig. 31 pictures the particle sizes of the emulsions prepared in chapter 4.2 and 4.3 based on their average and mean values. The figure shows that the values for some processing pressures are at close quarters, whereas others differ ocularly. The results for the average particle size and the medians were similar in both trials for 60, 100 and 120 MPa. Fig. 32 and Fig. 33 separately illustrate the average particle sizes and medians of particle size of both experiments. The trendlines reveal that both trials had the same tendency; particle size decreased up to 80 respectively 100 MPA; consequently, an application of higher pressures went along with an increase in particle size in 4.2 and 4.3. Thus, the results of two independent trials suggest that too high processing pressures increase the average particle size and that moderate pressures between 60 and 120 MPa deliver better results regarding the average particle size. Generally, the average and median values were closer in chapter 4.3; this shows that the emulsions were more homogenously distributed in this trial.

It is quite remarkable that the results for 100 and 120 MPa are similar in both trials, because the results do not fit in the above mentioned tendency of increasing particles with increasing pressure. To this end, it remains unclear whether one processing pressure was overestimated in both experiments; and if, which one it was. JAFARI et al. (2007) came to the conclusion that pressures beyond 60 MPa have an inverse effect on particle size. This is not in accordance with the results of the two trials because a processing at 60 and 80 MPa delivered the best results in terms of particle size. So the effect of processing pressures might be formula dependent. However, as too high pressures were found to have a negative effect, it seems more likely that the average particle sizes at 120 MPa have been rated too low in both experiments and that the inverse effect of high processing pressures starts

beyond 80 MPa. This seems self-evident because the particle sizes were bigger at 100 and 140 MPa and the results of 120 MPa are out of this line.

The summarizing statistic in Table 7 lists the results of both trials; the processing pressures were assorted in ascending order. The table shows that pressures of 60, 80 and 120 MPa led to small average particle sizes in both experiments. As mentioned it is likely that the average particle sizes at 120 MPa were estimated too low in both experiments. The likeness of medians and average values underlines a homogenous distribution which is an important point concerning emulsion stability.

The single processing pressures are separately discussed below.

Table: 7: Sumarizing statistic of the chapters 4.2 and 4.3

Processing Pressure	Chapter	Average (μm)	Median (μm)	Minimum (μm)	Maximum (μm)	Range (μm)	σ*(μm)	C. var ** (%)
40 MPa	4.2	2.13	1.96	0.92	4.32	3.4	0.68	31.74
	4.3	1.91	1.79	0.58	4.02	3.44	0.7	36.66
60 MPa	4.2	1.81	1.69	0.51	3.01	2.5	0.52	28.72
	4.3	1.66	1.62	0.44	2.94	2.5	0.53	32.12
80 MPa	4.2	1.2	1.2	0.41	2.26	1.85	0.41	34.63
	4.3	1.42	1.49	0.52	2.47	1.95	0.45	31.41
100 MPa	4.2	1.89	1.78	0.51	5.98	5.46	0.81	42.51
	4.3	1.83	1.79	0.58	5.08	4.5	0.81	44.3
120 MPa	4.2	1.53	1.51	0.58	2.68	2.1	0.46	29.91
	4.3	1.57	1.5	0.58	2.66	2.08	0.41	26.39
140 MPa	4.2	2.29	1.79	0.51	16.75	17	2.33	101.54
	4.3	2.01	1.79	0.72	20.28	19.56	1.51	75.06
160 MPa	4.2	1.82	1.76	0.44	3.65	3.21	0.73	40.18
	4.3	1.91	1.95	0.46	3.73	3.27	0.56	29.32
180 Mpa	4.2	2.06	2.13	0.59	4.84	4.25	0.86	41.74
	4.3	2.08	1.99	0.74	3.92	3.18	0.68	32.71

*...standard deviation

**...coefficient of variation

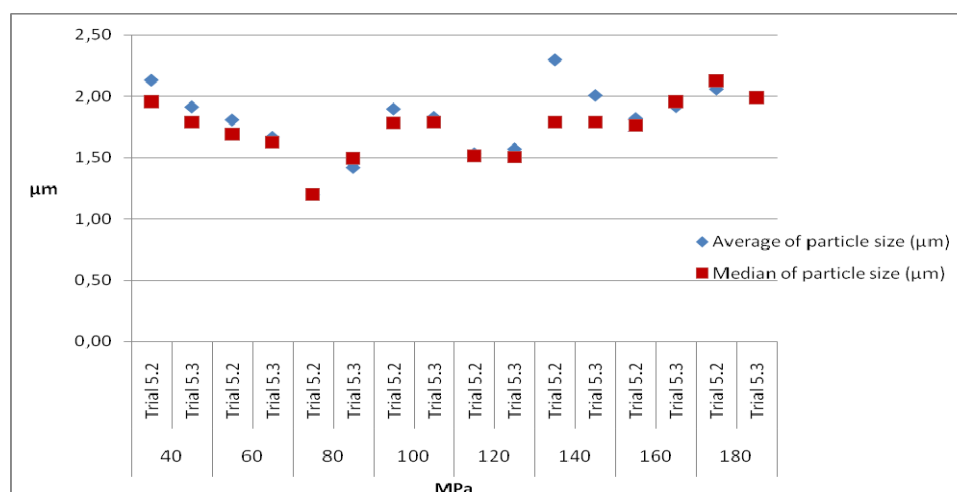


Fig. 31: Average and mean values of the emulsions prepared in chapter 4.2 and 4.3

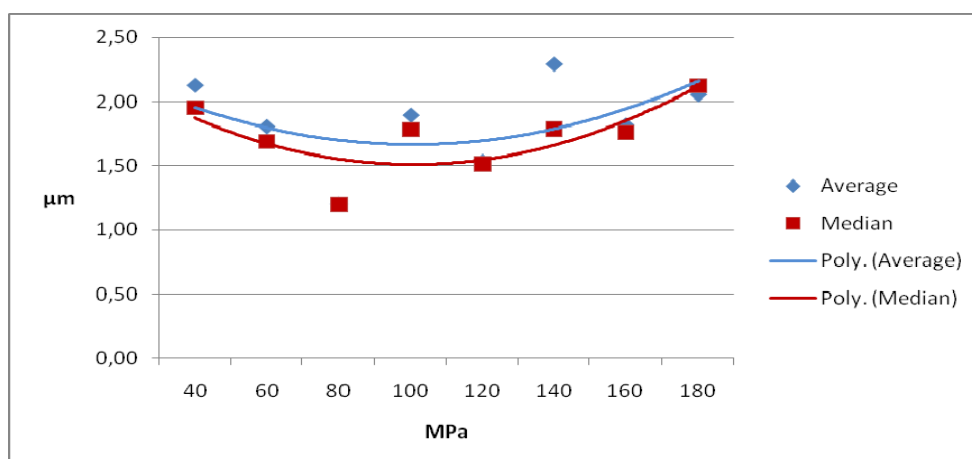


Fig. 32: Average and median of particle sizes in chapter 4.2.

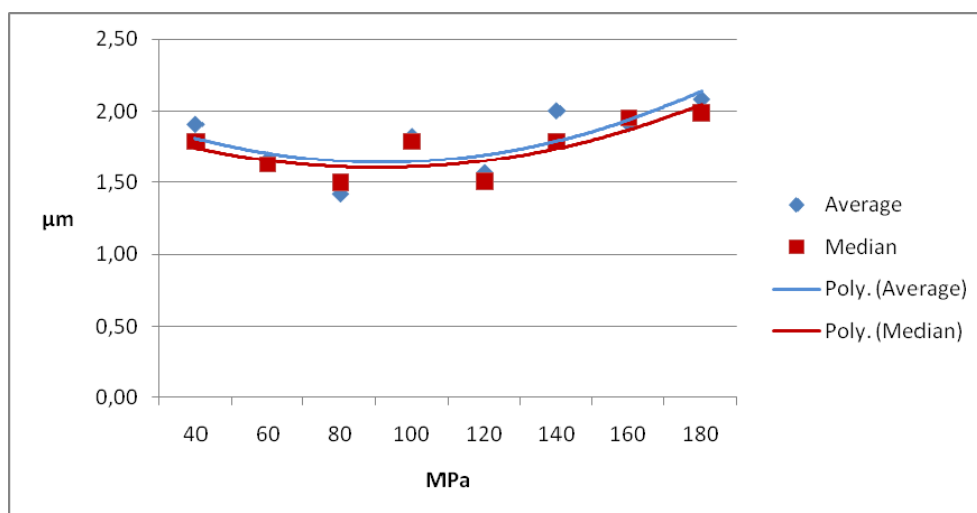


Fig. 33: Average and median of particle sizes in chapter 4.3

4.4.2.1 40 MPa in chapter 4.2 and 4.3

The two samples differed significantly regarding the means, medians and distributions (α : 0.05; p : 0.0253; 0.0082; 0.0091); the results were not reproducible. Table 7 lists the summarizing statistic of the two samples that includes all relevant data. The table shows that the differences between the two trials regarding mean and medians were approximately 0.2 μm which is no grave difference.

Fig. 34 displays the distributions of the two emulsions visually. The emulsion in trial 4.3 had more particles below one and two μm and fewer particles beyond three μm which resulted in a significantly lower average particle size. The emulsion had an almost equal range but the minimum and maximum values were set lower in trial 4.3.

Table 7: Summary statistic of emulsions prepared at 40 MPa in chapter 4.2 and 4.3

Statistics	Particle size (μm) Chapter 4.2 40 MPa	Particle size (μm) Chapter 4.3 40 MPa
Mean	2.13	1.91
Median	1.96	1.79
Minimum	0.92	0.58
Maximum	4.32	4.02
Range	3.40	3.44
Standard deviation	0.68	0.70
Coeff. of variation	31.74%	36.66%

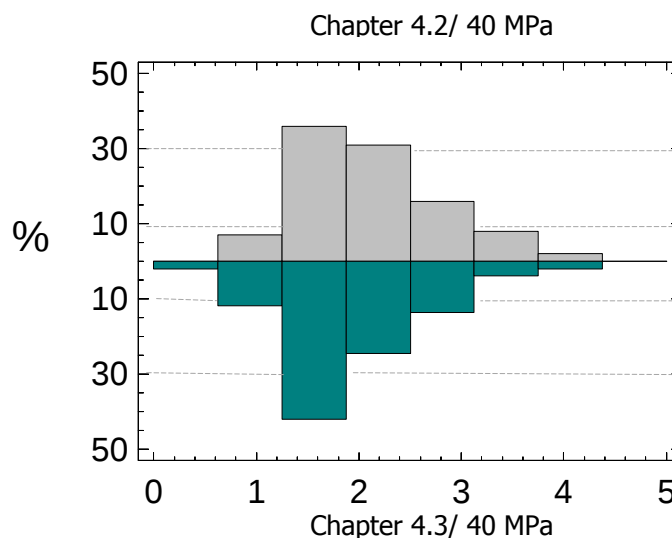


Fig. 34: Frequency histograms of the chapters 4.2 and 4.3. Processing at 40 MPa.

4.4.2.2 60 MPa in chapter 4.2 and 4.3

As there were no significant differences between the standard deviations, means and medians (α : 0.05; p : 0.7613; 0.0592. 0.0722) the two samples prepared in trial 4.2 and 4.3 at 60 MPa were significantly equal; consequently, the results were reproducible.

Table 8 shows the summarizing statistic of the two samples. The minimum and maximum levels were almost equal. The frequency histogram in Fig. 35 illustrates that the distributions were unequal; which was significant (α : 0.05; p : 0.0297). The emulsion that was produced in trial 4.3 exhibited more particles between 1 and 1.5 μm and therefore had a smaller average particle size. The results in 4.2 were more normally distributed.

Table 8: Summarizing statistic of emulsions prepared at 60 MPa in chapter 4.2 and 4.3

Statistics	Particle size (μm) Chapter 4.2 60 MPa	Particle size (μm) Chapter 4.3 60 MPa
Mean	1.81	1.66
Median	1.69	1.62
Minimum	0.51	0.44
Maximum	3.01	2.94
Range	2.50	2.50
Standard deviation	0.52	0.53
Coeff. of variation	29%	32%

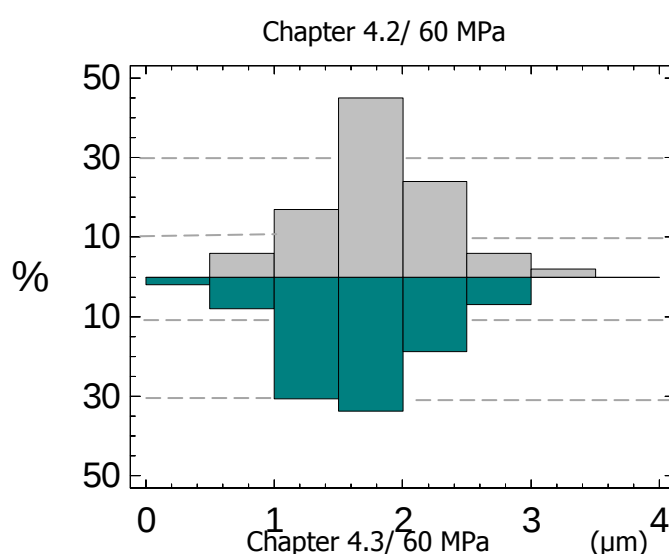


Fig. 35: Frequency histograms of chapter 4.2 and 4.3. Processing at 60 MPa.

4.4.2.3 80 MPa in chapter 4.2 and 4.3

The data was homoskedastic (α : 0.05; p : 0.2643). The means, medians and distributions were significantly unequal (α : 0.05; p : 0.0002; 0.0006; 0.0001). Thus, the samples of the two trials differed; irrespective of equal processing conditions.

Fig. 36 displays a frequency histogram of both emulsions. Table 9 contains relevant statistical data. Emulsions processed at 80 MPa had the lowest maximum levels and the lowest range in both trials; moreover, the means and the medians for each emulsion were similar which evidences a homogenous distribution in both trials. From that point of view a processing of simple emulsions at 80 MPa is recommendable, even though the results were statistically not reproducible.

Table 9: Summarizing statistic of emulsions prepared at 80 MPa in chapter 4.2 and 4.3

Statistics	Particle size (μm) Chapter 4.2 80 MPa	Particle size (μm) Chapter 4.3 80 MPa
Mean	1.20	1.44
Median	1.20	1.49
Minimum	0.41	0.52
Maximum	2.26	2.76
Range	1.85	2.24
Standard deviation	0.41	0.47
Coeff. of variation	34.63%	32.46%

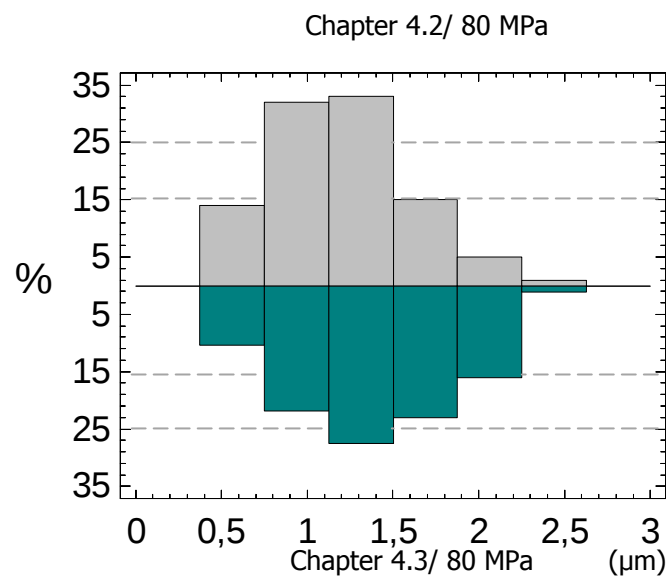


Fig. 36: Frequency histograms of chapter 4.2 and 4.3. Processing at 80 MPa.

4.4.2.4 100 MPa in chapter 4.2 and 4.3

Standard deviations, means, medians and distributions of the two samples were significantly equal (α : 0.05; p : 0.9401; 0.4745; 0.4653; 0.3893). Consequently, the same results were obtained in both experiments.

Table 10 lists the summarizing statistic of the two samples. The means and medians for each emulsion were similar; thus, both emulsions were distributed homogenously. The frequency histogram in Fig. 37 underlines this; one can see that most particles were located between 1 and 2.5 μm . Furthermore the figure illustrates that there were only marginal differences between the two emulsions.

Table 10: Summarizing statistic of emulsions prepared at 100 MPa in chapter 4.2 and 4.3

Statistics	Particle size (μm) Chapter 4.2 100 MPa	Particle size (μm) Chapter 4.3 100 MPa
Mean	1.89	1.83
Median	1.78	1.79
Minimum	0.51	0.58
Maximum	5.98	5.08
Range	5.46	4.50
Standard deviation	0.81	0.81
Coeff. of variation	42.51%	44.30%

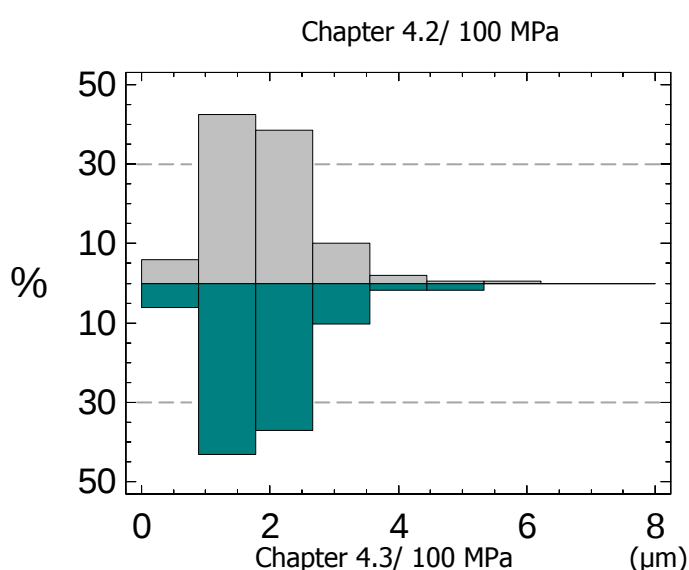


Fig. 37: Frequency histograms of chapter 4.2 and 4.3. Processing at 100 MPa.

4.4.2.5 120 MPa in chapter 4.2 and 4.3

There were no significant differences between the standard deviations, means, medians and distributions of the samples (α : 0.05; p 0.2753; 0.978825; 0.9785; 0.3512). Thus, the samples were equal.

Table 11 shows a summarizing statistic. The frequency histograms in Fig. 38 illustrate an even distribution. The average particle size was comparably low both times. As already mentioned, it is likely that the values for the particle sizes have been underestimated in both trials because the results do not fit the observation that particle sizes re-increase if exaggerated processing pressures are applied to the emulsions. Furthermore, the particle sizes were higher at 100 and 140 MPa.

Table 11: Summarizing statistic of emulsions prepared at 120 MPa in chapter 4.2 and 4.3

Statistics	Particle size (μm) Chapter 4.2 120 MPa	Particle size (μm) Chapter 4.3 120 MPa
Mean	1.53	1.57
Median	1.51	1.50
Minimum	0.58	0.58
Maximum	2.68	2.66
Range	2.10	2.08
Standard deviation	0.46	0.41
Coeff. of variation	29.91%	26.39%

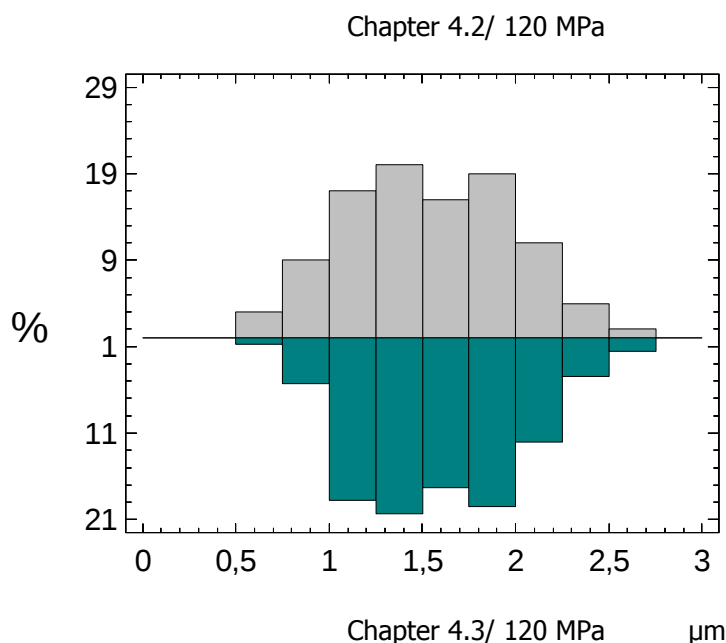


Fig. 38: Frequency histograms of chapter 4.2 and 4.3. Processing at 120 MPa.

4.4.2.6 140 MPa in chapter 4.2 and 4.3

The data was heteroskedastic (α : 0.05; p : 0.000). The standard deviations and coefficients of variation were relatively high in both trials. However, there were no significant differences concerning the medians and the distributions (α : 0.05; p : 0.5529; 0.2050) of the two emulsions. Table 12 lists a summarizing statistic of the two samples. 39 displays a frequency histogram of the respective emulsions which shows that the samples were equally distributed. Compared to other emulsions, the average particle size was fairly high in both trials; this could be the result of an over-processing due to exaggerated processing pressures.

Table 12: Summarizing statistic of emulsions prepared at 140 MPa in chapter 4.2 and 4.3

Statistics	Particle size (μm) Chapter 4.2 140 MPa	Particle size (μm) Chapter 4.3 140 MPa
Mean	2.29	2.01
Median	1.79	1.79
Minimum	0.51	0.72
Maximum	17	20.28
Range	16.57	19.56
Standard deviation	2.33	1.51
Coeff. of variation	101.54%	75.06%

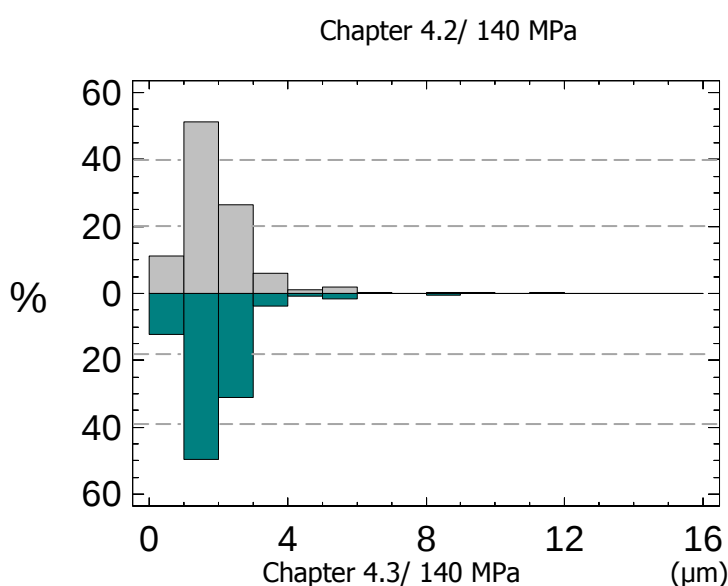


Fig. 39: Frequency histograms of chapter 4.2 and 4.3. Processing at 140 MPa.

4.4.2.7 160 MPa in chapter 4.2 and 4.3

The data was heteroskedastic (α : 0.05; p : 0.0071). The distributions differed significantly (α : 0.05; p : 0.2752); the medians were significantly equal (α : 0.05; p : 0.1030).

Table 13 lists the summarizing statistic which shows that the means, minimum and maximum values and the range were similar in both trials. The frequency histograms in Fig. 40 illustrate that the emulsion of chapter 4.2 had more small particles than the emulsion of chapter 4.3. In the end the average particle sizes did not differ drastically, even though the distributions were unlike. Generally, the average particle size was fairly high.

Table 13: Summarizing statistic of emulsions prepared at 160 MPa in chapter 4.2 and 4.3.

Statistics	Particle size (μm) Chapter 4.2 160 MPa	Particle size (μm) Chapter 4.3 160 MPa
Mean	1.82	1.91
Median	1.76	1.95
Minimum	0.44	0.46
Maximum	3.65	3.73
Range	3.21	3.27
Standard deviation	0.73	0.56
Coeff. of variation	40.18%	29.32%

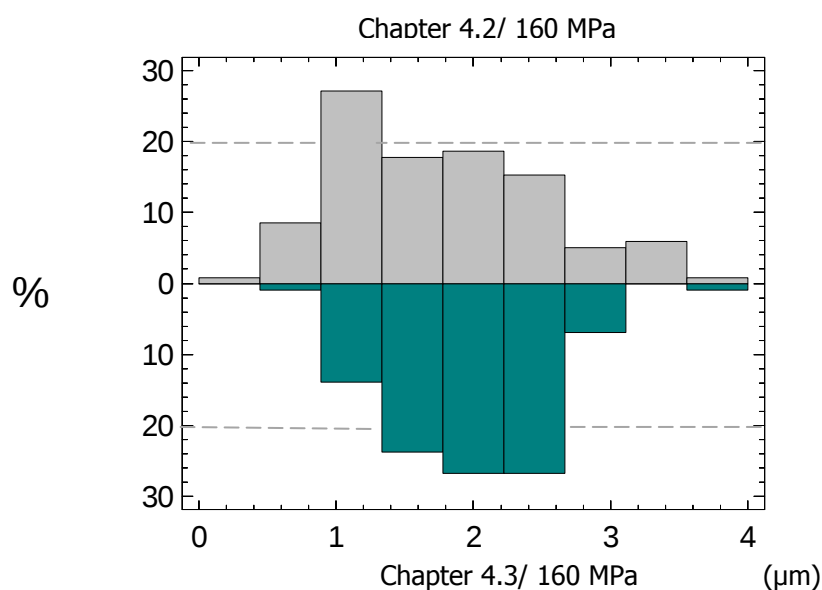


Fig. 40: Frequency histograms of chapter 4.2 and 4.3. Processing at 160 MPa.

4.4.2.8 180 MPa in chapter 4.2 and 4.3

The data was heteroskedastic (α : 0.05; p : 0.0132; 0.0005). The medians and the distributions were significantly equal (α : 0.05; p : 0.7540; 0.0972). Table 14 summarizes the samples statistically. The frequency histograms in Fig. 41 illustrate that the emulsion of chapter 4.2 had a wider range and more big particles than the emulsion of chapter 4.3. Compared to emulsions that were prepared at milder conditions, the average particle size was rather high.

Table 14: Summarizing statistic of emulsions prepared at 180 MPa in chapter 4.2 and 4.3

Statistics	Particle size (μm) Chapter 4.2 180 MPa	Particle size (μm) Chapter 4.3 180 MPa
Mean	2.06	2.08
Median	2.13	1.99
Minimum	0.59	0.74
Maximum	4.84	3.92
Range	4.25	3.18
Standard deviation	0.86	0.68
Coeff. of variation	41.74%	32.71%

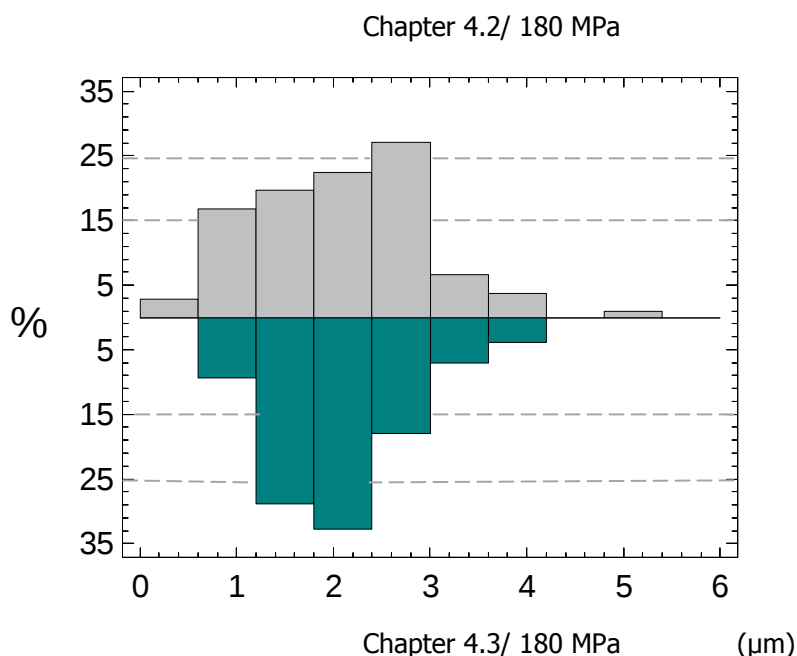


Fig. 41: Frequency histograms of chapter 4.2 and 4.3. Processing at 180 MPa.

4.4.2.9 Comprehensive conclusion

The statistical tests have partly detected significant differences between equally prepared emulsions, whereas some results were reproducible.

Table 16 lists the results of the multiple range test that analyzed the differences and similarities of the samples. Each homogenous group contains emulsions that are similar and do not differ from each other statistically. All emulsions that were prepared at the same pressure shared at least one homogenous group and are consequently similar. Table 16 also summarizes the results of the previous statistical test of the paired emulsion samples prepared in chapter 4.2 and 4.3 with respect to means, medians, standard deviations and distributions. All samples except the ones that were prepared at 40 MPa had equal medians. All samples with equal variances exhibited equal means. To this end, the processing of O/W emulsions with a microfluidizer was satisfactorily reproducible; there were no grave differences between samples that were prepared at equal conditions in two independent experiments.

The comparison of the two trials underlines the statement that moderate pressures contribute to the formation of emulsions with a small average particle size and a homogenous distribution, whereas too high pressures increase particle size and the particle size range; this in turn has a negative effect on emulsion stability. In both trials the best results were obtained at pressures that ranged from 60-120 MPa. As already mentioned it is thinkable that the particle sizes of the emulsions that were processed at 120 MPa have been estimated too low and that the particle size is factually bigger. One putative reason is the manual measurement of particle sizes; as the number of particles that underwent statistical analysis was limited to a cross-section, one cannot exclude that the factual average particle size of the emulsions was over- or underestimated. The pictures that were chosen for analysis were naturally fragmentary; thus, it is possible that unrepresentative cross-sections have been chosen.

Table 15: Summary of statistical tests and homogenous groups of the chapters 4.2 and 4.3. The table summarizes the result of the previous statistical tests. = means that the statistical parameters did not differ from each other. ≠ means that there was a significant difference between the emulsions.

Pressure (MPa)	Chapter	Summary of statistical tests*				Homogenous groups**						
		Mean	Median	Standard.dev	Distribution	A	B	C	D	E	F	G
40	Chapter 4.2	≠	≠	=	≠						X	X
	Chapter 4.3								X	X	X	X
60	Chapter 4.2	=	=	=	≠			X	X	X		
	Chapter 4.3						X	X	X			
80	Chapter 4.2	n.e***	=	≠	=	X						
	Chapter 4.3					X	X					
100	Chapter 4.2	=	=	=	=				X	X	X	
	Chapter 4.3								X	X		
120	Chapter 4.2	=	=	=	=		X	X				
	Chapter 4.3						X	X				
140	Chapter 4.2	n.e***	=	≠	=							X
	Chapter 4.3									X	X	X
160	Chapter 4.2	n.e***	=	≠	=				X	X		
	Chapter 4.3								X	X	X	X
180	Chapter 4.2	n.e***	=	≠	=					X	X	X
	Chapter 4.3										X	X

*summary of the chapters...4.4.2.1-4.4.2.8

**...data from multiple range test

***...data was not evaluated (n.e.) because standard deviation was unequal

4.5 Processing pressure and particle size - Coloring of the inner oil phase

4.5.1 Introduction

Chapters 4.2, 4.3 and 4.4 describe the context between processing pressure and particle size. The trials show that particle size decreased up to 80 MPa and higher pressures have an inverse effect. However, these results only refer to simple emulsions. As double emulsions are expected to have better flavor encapsulating properties it is essential to study the impact on processing conditions on the qualities of double emulsions. The aim of this trial was to find out in which way the processing pressure of the simple emulsion determines the structure of the respective double emulsion.

Furthermore, this trial dealt with the question whether the generated emulsions factually were double emulsions of the O/W/O type. Such concerns arose in the course of previous image analyzing of double emulsions. The pictures gave reason to believe that not all droplets were O/W droplets. Actually, the photographs confirmed the presence of double emulsion droplets; however, there were also particles that did not have a typical double-emulsion-droplet structure and rather looked like simple emulsion droplets. This can be seen in Fig. 44 and Fig. 45. Theoretically, double emulsions are sensitive to shear forces; accordingly, it is thinkable that too rough processing conditions destroyed the primal structure and triggered phase inversion. This fact and the previously-observed, untypical droplets indicated that the dispersed phase of the final double emulsion consisted of O/W and water droplets. To get information about the factual composition of the double emulsion, it was prepared in two variations: The first time the inner oil phase consisted of flavor PTG, the second time the flavor was colored with β -carotene to visualize the distribution of the inner oil phase in the microscope.

4.5.2 Experimental procedure

The emulsions were prepared as described in chapter 3.2.1.1 and 3.2.1.2. 150 g maltodextrine and 350 g (± 0.01 g) distilled water made up the aqueous phase. One time the oil phase consisted of 166.67 g (± 0.01 g) flavor PTG. The second time 10% β -carotene were added to the flavor PTG. The simple emulsions were prepared at pressures that ranged from 40 to 180 MPa in 20 MPa steps. The sunflower oil that formed the outer oil phase contained 5% PGPR. The double emulsions were processed at 5 MPa. The Y-reaction chamber was used in this trial. The colored and the uncolored samples were analyzed with an image analyzer as described in chapter 3.2.3. The uncolored double emulsions were centrifuged as described in chapter 3.2.5. The collected data was appraised with statistical methods as described in 3.3.2.

4.5.3 Results and discussion

4.5.3.1 Centrifugation

All samples exhibited slight sediments. There were no observable differences between the samples. These results are in accordance with image analysis that did not detect any differences. A putative reason for the partial instability of the sample is a lack of emulsifying or stabilizing agents. Consequently the concentration of Gum Arabic, maltodextrine and PGPR was augmented in following trials.

4.5.3.2 Statistical test

The ANOVA did not reveal any significant differences between the eight samples (α : 0.05; p : 0.0572). Thus, the processing pressure of the simple emulsion had no effect on the particle size of the respective double emulsion. These results indicate that there is no need to process the simple emulsion with pressures beyond 40 MPa.

Table 16 lists a summarizing statistic of all samples. Fig. 46 visualizes the particle size distributions for all double emulsion that were produced in this trial. Even though there was no significant difference referring to the average particle size, the distributions of the single emulsions partly differ from each other considerably. A look at the particle size range reveals that the emulsions that were processed at 60 and 100 MPa had the narrowest range whereas other emulsion exhibited large particles as well. However, there is no recognizable trend that is correlating to the processing pressure of the simple emulsion.

4.5.3.3 Interpretation of image analysis

As mentioned it was unclear whether the confected emulsions were of the O/W/O type. Previously performed image analyzes had clearly illustrated the presence of double emulsion particles but they are also showed countless droplets that looked like simple emulsion particles. This fact arose the question whether those particles were small O/W particles or un-encapsulated water droplets. Uncolored microscope pictures allowed no further statements because one could not distinguish between the water and the oil phase. Solubility tests proved that the emulsions were lipophilic, thus they could only be O/W/O or W/O emulsions because other emulsion types (that consist of two or three phases) have a hydrophilic continuous phase and are therefore hydrophilic. To visualize the inner structure of the emulsions, the inner oil phase was colored with β -carotene. β -carotene is a lipophilic molecule and must therefore allocate in the oil phase. The colored O/W emulsion is shown in Fig 42. It consisted of orange lipid particles that were dispersed in a transparent aqueous phase. Fig. 43, Fig. 44 and Fig. 45 show double emulsions that were prepared out of colored simple emulsions. There is a noteworthy contrast between the outer continuous phase and the colored inner oil phase. One can clearly see the yellow particles in the transparent medium. This indicated that no phase inversion had occurred. In case of phase inversion the inner and the outer oil phase would have entirely mixed to form the continuous phase of a W/O emulsion and there would not have been a contrast between the two phases. Another fact that accounted for the presence of O/W/O emulsions was that each yellow particle must have been

surrounded by an aqueous layer, because the continuous phase was lipophilic too; if the interfacial layer had lacked, the two oil phases would have simply mixed. To this end it is safe to say that the confected emulsions were O/W/O emulsions.

In terms of emulsion stability it is surely better to have smaller droplets to reduce sedimentation. The big O/W droplets contain more water than the smaller ones; the oil droplets inside must be surrounded by an aqueous medium because otherwise they would immediately coalesce. High water content goes along with increasing density; a big density difference between the emulsion phases favors phase separation. The water content is least if one oil droplet is surrounded by a thin aqueous layer. In such case the density difference between this droplet and the continuous phase is small which contributes to emulsion stability because such particles will hardly sediment. One was to further reduce particle size, is to use more emulsifier.

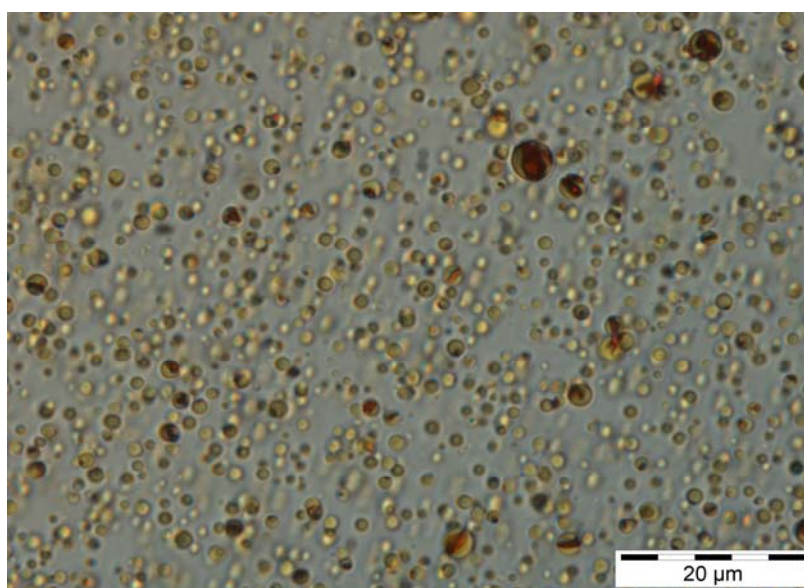


Fig 42: O/W emulsion processed at 80

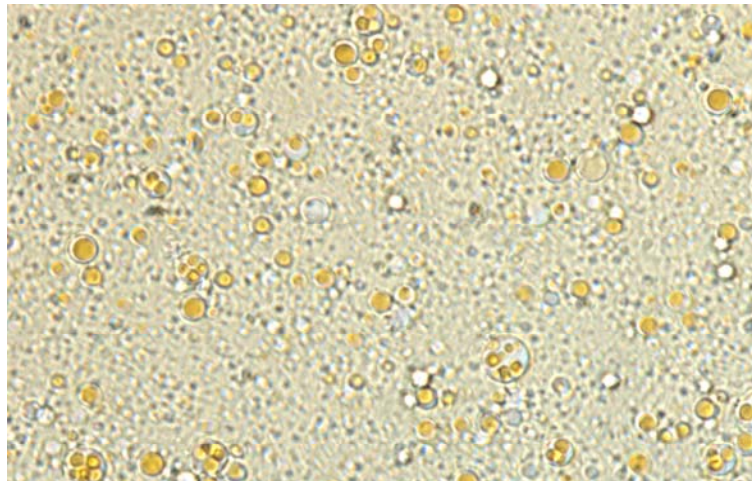


Fig. 43: *O/W/O emulsion. Processing pressure simple emulsion: 80 MPa. Processing pressure double emulsion: 5 MPa*

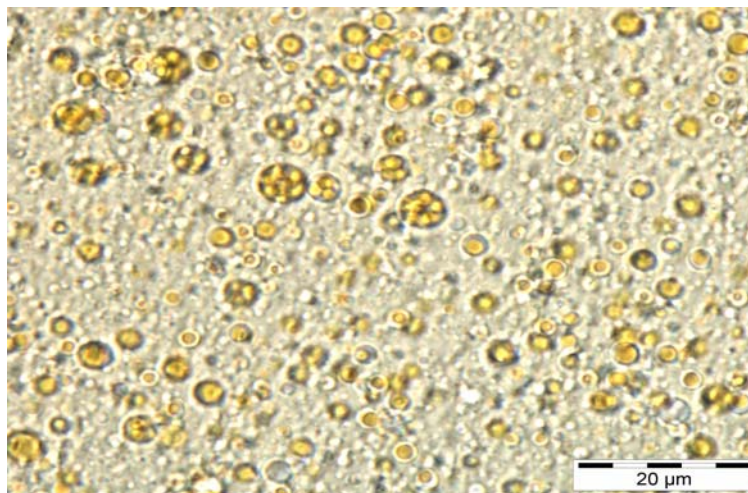


Fig. 44: *O/W/O emulsion. Processing pressure simple emulsion: 100 MPa. Processing pressure double emulsion: 5 MPa*

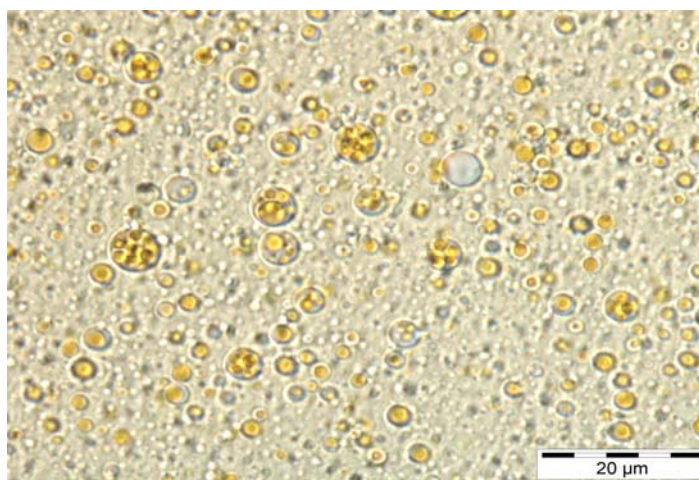


Fig. 45: *O/W/O emulsion. Simple emulsion was processed at 120 MPa. Double emulsion was processed at 5 MPa*

Table 16: Summarizing statistic of O/W/O emulsions

Processing Pressure (MPa)		Average (μm)	Median (μm)	Minimum (μm)	Maximum (μm)	Range (μm)	Standard deviation	Coeff. of variation
O/W	O/W/O							
40	5	2.72	2.45	1.16	9.07	7.91	1.68	61.90%
60		2.22	2.27	0.62	4.5	3.88	0.75	33.86%
80		2.24	2.12	0.73	5.47	4.74	0.94	41.93%
100		2.23	2.18	0.87	4.5	3.62	0.82	36.72%
120		2.46	2.08	0.89	8.01	7.12	1.36	55.36%
140		2.91	2.66	0.75	6.83	6.08	1.3	44.64%
160		2.22	1.96	0.58	6.87	6.29	1.15	51.92%
180		2.24	1.92	0.93	7.46	6.53	1.21	54.06%

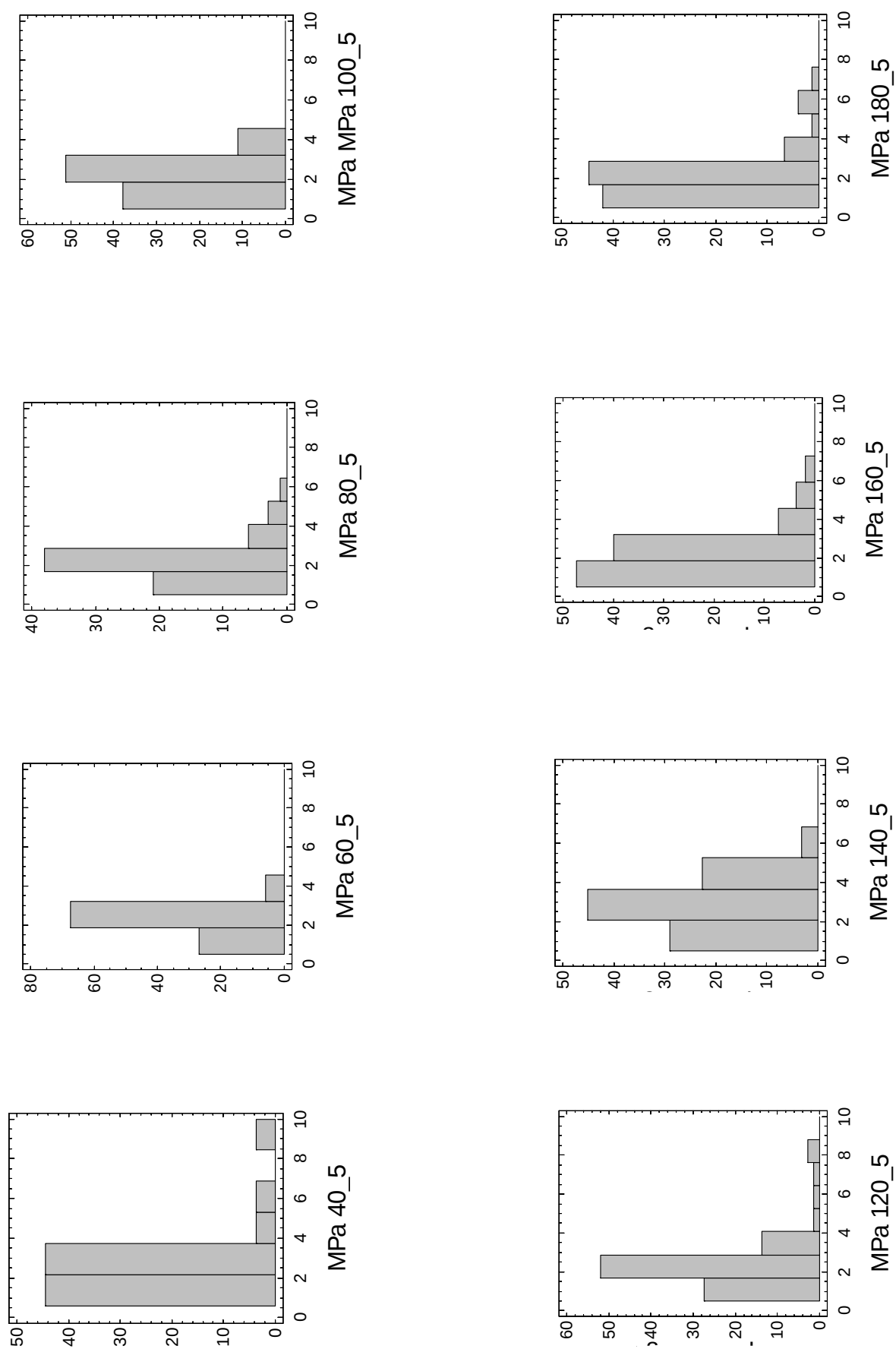


Fig. 46: Frequency of particle size in %.

4.6 Processing pressure and storage stability

4.6.1 Introduction

The aim of this trial was to study the impact of double emulsion processing pressure on storage stability of O/W/O emulsions. The experiment was conducted with the formula of trial 5.1; the emulsifier concentration in the double emulsion was raised from 5 to 10%, to increase emulsion stability.

4.6.2 Experimental procedure

The emulsions were prepared as described in chapter 3.2.1 and 3.2.2. 150 g (± 0.01 g) maltodextrine and Gum Arabic and 350 g (± 0.01 g) distilled water made up the aqueous phase. The oil phase consisted of 166.67 g (± 0.01 g) flavor PTG. The simple emulsion was processed with the microfluidizer at 120 MPa. Subsequently, double emulsions of the O/W/O type were prepared. 100 g (± 0.01 g) of the simple emulsion were dispersed in 150 g (± 0.01 g) sunflower oil that contained 10% PGPR. The double emulsions were processed at pressures that ranged from 5 to 40 MPa in 5 MPa steps. The emulsions were centrifuged as described in 3.3.3.2 and subsequently stored at room temperature as described in 3.3.3.3 for six months.

4.6.3 Results and discussion

There were no observable differences between the samples after centrifugation. All prepared emulsions were stable and did not show ring formation or sedimentation.

After the centrifuged samples had been stored at room temperature for six months some differences could be explored. The double emulsions that had been processed with pressures up to 20 MPa were more inhomogeneous; they exhibited sedimentation. The double emulsions that were processed at below 20 MPa exhibited no visible phase separation. However, they had a rancid smell due to the sunflower oil that was used as outer continuous phase.

This experiment has shown that it was possible to create stable O/W/O emulsions. It was observed that a combination of 120 MPa for the simple emulsion and 15 MPa or less for the double emulsion lead to stable flavor emulsions. This observation goes along with the results of literature research, revealing that double emulsions were sensitive to shear forces and should therefore be prepared under mild conditions.

One occurring problem was the rancid smell of the emulsions at the end of the storage test. This breakdown of the sunflower oil could be avoided by using appropriate antioxidants or replacing the sunflower oil by a more stable agent.

However, the flavor content of the emulsions was only 10%. Further trials will show whether flavor content can be augmented without stability losses.

4.7 Influence of processing pressure and number of passages of the simple emulsion on double emulsion stability

4.7.1 Introduction

The previous trial has shown that it was possible to create stable O/W/O emulsions under certain conditions. The aim of this experiment was to explore other processing conditions that led to stable emulsions and to reproduce or improve the already obtained results. It was studied whether higher or lower pressures and repeated passes through the microfluidizer increased emulsion stability.

4.7.2 Experimental procedure

The emulsions were prepared as described in chapter 3.2.1 and 3.2.2. 150 g (± 0.01 g) maltodextrine and Gum Arabic and 350 g (± 0.01 g) distilled water made up the aqueous phase. The oil phase consisted of 166.67 g (± 0.01 g) flavor PTG. The simple emulsions were passed through the microfluidizer at 80, 120 and 180 MPa as described in 3.2.4. One part of the already processed emulsions was passed through the microfluidizer a second time; each with the same pressure. The Y-reaction chamber was used in this trial. Subsequently, double emulsions of the O/W/O type were prepared. 100 g (± 0.01 g) of the simple emulsion were dispersed in 150 g sunflower oil that contained 10% PGPR. Approximately 10 g of four double emulsions (2x800, 2x1200, 1x1800 and 2x1800 MPa) were filled into a test tube and centrifuged before the rest was processed with the microfluidizer to find out whether double emulsions could also be prepared with an Ultra-Turrax. All double emulsions were then processed at 10 and 20 MPa. The emulsions were centrifuged as described in 3.3.3.2 and subsequently stored at room temperature as described in 3.3.3.3 for six months.

4.7.3 Results & Discussion

The double emulsion samples showed no observable differences after processing and after centrifugation. All prepared emulsions were stable and did not

show ring formation or sedimentation. After six months of storage at room temperature some differences could be detected: All samples where the simple emulsion had been processed at 80 MPa exhibited slight sedimentation; in comparison, the emulsions where the simple emulsion had been processed at 120 MPa seemed to more homogenous. The emulsions were comparable to the ones prepare in trial 5.6. The sample where the microfluidizer was not used for the double emulsion, showed a distinct ring formation. All other samples did not remarkably differ from each other; they were all stable. 180 MPa delivered results comparable to 120 MPa. One of the emulsions where no microfluidizer was used for the double emulsion showed a ring formation (1x180 MPa) the other one did not. However, the differences between the samples prepared at 80, 120 or 180 MPa were marginal.

In the end, it is difficult to say how and to which extend the processing pressure of the simple emulsion influenced double emulsion stability. One thing that can be said with a certain security is that more passages had no beneficial effect. As there were hardly any differences between the single samples none of the pressures can be preferred instead of the others.

The processing pressure of the simple emulsion had no detectable effect on long term emulsion stability in this trial. Further experiments will qualify or falsify this statement.

4.8 Impact of simple emulsion flavor content on double emulsion stability

4.8.1 Introduction

Previous trials have shown that it was possible to create stable flavor emulsions basing on plant triglyceride. However, the flavor content was only 10%. The aim of this experiment was to maximize the flavor content of the O/W/O emulsion to get a marketable product. The impact on double emulsion flavor content on storage stability should be studied.

4.8.2 Experimental procedure

The emulsions were prepared as described in chapter 3.2.1 and 3.2.2. Flavor PTG was used as the dispersed phase of the simple emulsion. To investigate the impact of flavor content on emulsion stability six emulsions with variable flavor content in the O/W emulsion were produced; the ratios of continuous and dispersed phase were varied. The continuous phase was a 30% solution of Gum Arabic and maltodextrine. The respective ratios and the corresponding flavor concentrations are listed in Table 17. The simple emulsions were processed with the microfluidizer at 120 MPa as described in 3.2.4 The Y-reaction chamber was used in this trial. Double emulsions were prepared with all simple emulsions. To investigate the impact of emulsifier concentration on double emulsion stability the outer lipophilic phase contained either 5 or 10% PGPR. The double emulsions were composed of 100 g (± 0.01 g) simple emulsion and 150 g sunflower oil and PGPR. The double emulsion samples were processed with the microfluidizer at 10 and 20 MPa to confirm the results of former trials.

The simple emulsions that contained most flavor (emulsion five and six) were unstable after processing with the microfluidizer. To collect information about the impact of simple emulsion stability on the stability of the final double emulsion, the unstable emulsions were not rejected; they were also used as basic material for double emulsions. The two phases of the respective emulsions were re-emulsified with the Ultra-Turrax at 8000 rpm up to homogeneity before double emulsion

preparation. The emulsions were centrifuged as described in chapter 3.3.3.2 and subsequently stored at room temperature as described in chapter 3.3.3.3 for six months.

Table 17: Flavor content of the simple emulsions. Flavor PTG formed the dispersed phase; thus, a higher volume of dispersed phase increased flavor content.

O/W Emulsion	Continuous phase: dispersed phase	Flavor content (%)	
		Simple emulsion	Double emulsion*
Simple emulsion 1	1:3	25	10
Simple emulsion 2	2:5	28.6	11.4
Simple emulsion 3	1:2	33.3	13.3
Simple emulsion 4	2:3	40	16
Simple emulsion 5	1:1	50	20
Simple emulsion 6	6:5	54	21.6

* Continuous phase: dispersed phase: 1:1.5

4.8.3 Results & Discussion

The given PGPR concentrations always refer to the continuous phase of the double emulsion; they are not converted to the entire double emulsion.

4.8.3.1 Double emulsion flavor content 10%

All double emulsion samples were stable after processing. All samples that contained 10% flavor were stable after centrifugation. The double emulsion that contained only 5% PGPR and was processed at 10 MPa showed a slight ring formation. However, the differences between the single samples were slight. These results are in accordance with trial 4.6 and 4.7. After six months of storage at room temperature the samples that contained 5% PGPR exhibited sedimentation. The sedimentation seemed to be more distinct in the samples where the double emulsion had been processed at 20 MPa; the double emulsion where the double emulsion had been processed at 10 MPa had a more homogenous appearance.

The double emulsions that contained 10% PGPR were stable. They showed no sedimentation or ring formation. The emulsion had the same formula as the double emulsions in trial 4.6 and 4.7. Equal samples that were produced in the three trials all looked alike; this fact supports the reproducibility of the experiments.

It should be considered that the samples that underwent the storage test had already been centrifuged before. This procedure is a considerable stress for the emulsion. Thus, it is thinkable that the samples that contain less emulsifier could be more homogenous if they are not additionally stressed via centrifugation.

4.8.3.2 Double emulsion flavor content 11.4%

All samples that contained 11.4% flavor were stable after centrifugation. The double emulsion that contained only 5% PGPR and was processed at 10 MPa showed a slight ring formation. All other emulsions showed no signs of instability. Again the differences between the single samples were marginal. After six months of storage at room temperature the differences had become more distinct. The double emulsions that contained 5% PGPR exhibited sedimentation which again was more pronounced in the emulsion that was processed at 20 MPa. The emulsions that contained 10 % PGPR showed neither sedimentation nor ring formation. These results suggest that double emulsions that contain 11.4% PGPR are stable at room temperature for six months.

4.8.3.3 Double emulsion flavor content 13.3%

Principally, the results were the same as in chapter 4.8.3.1 and 4.8.3.2. The double emulsions that contained five 5% PGPR showed a ring formation after centrifugation and were less homogenous than the emulsions that contained 10% PGPR. After six months of storage at room temperature the double emulsions that contained 5% PGPR exhibited sedimentations. The emulsions that contained 10 % PGPR showed no signs of instability. These results suggest that double emulsions that contain 13.3% PGPR are stable at room temperature for six months.

4.8.3.4 Double emulsion flavor content 16%

The results after centrifugation were the same as above, the emulsion showed neither sedimentation nor ring formation after centrifugation. After six months of storage at room temperature, some differences could be detected. Again the samples that contained 5% PGPR showed sedimentation. The emulsions that contained 10% PGPR were more homogenous but they showed a ring formation on the top of the test tube. This finding states that a flavor content of 16% in the O/W/O emulsions leads to changes in storage stability compared to double emulsions that contain less flavor. However, the emulsion samples had been centrifuged before they were stored; consequently, it is possible that all samples would have been stable after six months without centrifugation.

4.8.3.5 Double emulsion flavor content 20 and 21.6%

The simple emulsions that provided the basis for the O/W/O emulsion were not stable after processing with the microfluidizer at 120 MPa. The samples were re-emulsified with the Ultra-Turrax.

All samples were stable after processing. After centrifugation the samples that contained 5% PGPR exhibited a slight ring formation. The emulsions did not remarkably differ from the ones that based on stable simple emulsions. However, after the storage test the samples were more inhomogeneous than the other ones. All samples exhibited sedimentation; it was more pronounced in the samples that contained 5% PGPR.

4.8.3.6 Comprehensive conclusion

The conclusion that can be drawn from this trial is that a flavor content of 16% has no negative impact on the stability of O/W/O flavor emulsions. Each set of emulsions substantiates that a higher emulsifier concentration increases double emulsion stability. All samples that contained 5% PGPR were less stable after the storage test; even though there were no big differences after centrifugation. Moreover, the trial showed that double emulsions that had been processed at 20

MPa where less stable than the samples processed at 10 MPa. This became more distinct with increasing flavor content.

It is quite remarkable that the double emulsions that based on unstable simple emulsions were not completely unstable and, at least after centrifugation, comparable to the ones that based on stable emulsions. This suggests that the properties of the simple emulsion have little impact on the stability of the final double emulsion. This finding goes along with the results of trial 4.7 where the processing pressure of the simple emulsion seemed to have no impact on double emulsion stability.

Finally, it should be kept in mind that all emulsion samples had been centrifuged before they underwent the storage test. This procedure is a considerable stress for the emulsion. Thus, it is thinkable that the samples that contain less emulsifier could be more homogenous if they are not additionally stressed via centrifugation and that the overall flavor content can further be increased.

To this end, the results of this experiment state that a stable flavor emulsion with a total flavor content of 16% can be produced. A PGPR content of 10% and a processing of the double emulsion at 10 MPa seem to be most contributory to emulsion stability.

4.9 O/W/O flavor emulsions based on flavor Propy and sunflower oil

4.9.1 Introduction

In former trials it was possible to create stable flavor emulsions that based on flavor PTG. The aim of this experiment was to create stable flavor emulsion of the O/W/O type where flavor Propy made up the continuous phase of the simple emulsion. The formula based on the one used in chapter 4.1. The continuous phase of the double emulsion contained 10% PGPR because the previous trial had shown that a higher emulsifier concentration increased stability.

4.9.2 Experimental procedure

The emulsions were prepared as described in chapter 3.2.1 and 3.2.2. Flavor Propy made up the aqueous phase of the simple emulsion; sunflower oil formed the dispersed phase of the simple emulsion and the continuous phase of the double emulsion. Table 18 lists the composition of the simple emulsion. The simple emulsion was passed through the microfluidizer at 80 MPa as described in 3.2.4. The Y-reaction chamber was used in this trial. Double emulsions were prepared by dispersing 100 g (± 0.01 g) of the simple emulsion within 150 g (± 0.01 g) sunflower oil that contained 10% PGPR. The double emulsions were processed at 10, 20, 30, 40 or 50 MPa. The emulsions were centrifuged as described in 3.3.3.2 and subsequently stored at room temperature as described in 3.3.3.3 for six months.

Table 18: Composition of the simple emulsion based on flavor Propy

Ingredients (g)	Mass (g)
Gum Arabic and maltodextrine	75
Flavor Propy	100
Distilled water	75
Sunflower oil	83.3

4.9.3 Results & Discussion

All prepared double emulsions were stable after processing and after centrifugation. There were no evident differences between the single samples. After six months of storage at room temperature there were observable differences between the samples. However, all samples showed slight sedimentations. The double emulsion that was prepared at 30 MPa was found to be the most homogenous followed by the one that was processed at 20 MPa. This result is controversial with previous trials, where mildly processed double emulsions were obviously more stable. Pressures of 10, 40, 50 and 60 MPa led to distinct sedimentations; 10, 50 and 60 MPa were found to be worst.

It is important to mention that the storage test was conducted with already centrifuged samples. It is entirely conceivable that the samples could be stable if they were not centrifuged. Another point in this context is storage temperature. As the sedimentations were only slight, it is imaginable that the double emulsions basing on flavor Propy could be homogenous at lower storage temperatures.

4.10 O/W/O flavor emulsions based on flavor Propy and PTG and sunflower oil

4.10.1 Introduction

The aim of the experiment was to create a stable flavor emulsion of the O/W/O type with flavor Propy and flavor PTG.

In chapter 4.9 it was possible to produce double emulsions where the inner continuous phase consisted of flavor Propy that where stable after centrifugation. Slight sedimentations that could be explored at the end of the storage test, gave reason to believe that the emulsion could be stable at more favorable storage conditions. However, the flavor content was only 10%. To maximize the flavor content of the emulsion, and generate a merchantable product flavor PTG formed the dispersed phase of the simple emulsion instead of sunflower oil.

As a lower amount of continuous phase in the double emulsion is a further way to increase the overall flavor content and additionally the amount of encapsulated flavor the impact of the dispersed phase volume fraction on emulsion stability was studied too.

4.10.2 Experimental procedure

The emulsions were prepared as described in chapter 3.2.1 and 3.2.2. The oil phase of the simple emulsion consisted of flavor PTG. The continuous phase consisted of propylene glycol and distilled water at equal parts. Table 19 shows the composition of the simple emulsion. The simple emulsion was passed through the microfluidizer at 80 MPa as described in 3.2.4. The Y-reaction chamber was used in this trial. 100 g (± 0.01 g) of the simple emulsion were either dispersed in 100 or 150 g (± 0.01 g) sunflower oil that contained 10% PGPR. The double emulsions were processed at 10, 20 or 30 MPa. The emulsions were centrifuged as described in 3.3.3.2 and subsequently stored at room temperature as described in 3.3.3.3 for six months. To exclude phase inversion the double emulsion samples underwent a solubility test as described in 3.3.2 after centrifugation and after the storage test.

Ingredients (g)	Mass (g)
Gum Arabic and maltodextrine	75
Flavor Propy	80
Distilled water	95
Flavor PTG	83.3

Table 19: Composition of the simple emulsion basing on flavor Propy and flavor PTG

4.10.3 Results & Discussion

The double emulsion samples did not exhibit noticeable differences after processing and after centrifugation. All samples were homogenous and insoluble in water; thus, phase inversion was excluded.

After six months of storage at room temperature the samples featured clear differences: The double emulsion that consisted of 100 g simple emulsion and 150 g sunflower oil that was processed at 20 MPa was the most homogenous; it showed neither sedimentation nor ring formation. All other samples exhibited sedimentations which were most distinct in the double emulsions that contained a lower volume of continuous phase and were processed at 20 or 30 MPa. These results suggest that the flavor content of a O/W/O emulsion can be augmented from 10 to 14% without stability losses, if the dispersed phase of the simple emulsion is formed by flavor PTG; the continuous phase of the simple emulsion is a mixture of propylene glycol and distilled water, the ratio of both phases is 1:4 and the ratio of dispersed and continuous phase for the double emulsion is 2:3.

However, it is within the realms of possibility that the double emulsions that contain less continuous phase could be stable at lower storage temperatures and without previous centrifugation. A lower volume of continuous phase is desirable for two reasons: Firstly, the volume of encapsulated flavor is higher; consequently, a higher amount of flavor is released more slowly which should increase long-lastingness of the flavor impression. Secondly, the overall flavor content of the emulsion is higher if no lipophilic agent is found that successfully replaces sunflower oil in the continuous phase of the double emulsion.

4.11 O/W/O flavor emulsions based on two flavors and sunflower oil – Processing pressure and stability

4.11.1 Introduction

The aim of this trial was to study the impact of processing pressures and different ratios of continuous and dispersed phase in the double emulsion on storage stability.

4.11.2 Experimental procedure

The emulsions were prepared as described in chapter 3.2.1 and 3.2.2. The oil phase of the simple emulsion consisted of flavor PTG. The aqueous phase was a 1:1 blend of distilled water and flavor Propy. The composition was the same as in trial 5.10. Table 19 lists the composition of the simple emulsion. The simple emulsion was passed through the microfluidizer at 80 MPa as described in 3.2.4. The processing pressures ranged from 40 to 180 MPa and were increased in 20 MPa steps.

To find out whether simple emulsions could also be prepared with the Ultra-Turrax 400 g (± 0.01 g) of the coarse simple emulsion were not processed with the microfluidizer. The 400 g of coarse simple emulsion were dispersed within 600 g of sunflower oil that contained 10% PGPR. Approximately, 10 g were taken for stability tests; the rest was passed through the microfluidizer at different pressures.

The emulsions that were processed with the microfluidizer were also dispersed within sunflower oil that contained 10% PGPR. The ratio of dispersed and continuous phase was 2:3. The double emulsions were then processed at pressures that ranged from 10 to 90 MPa.

The emulsions were centrifuged as described in 3.3.3.2 and subsequently stored at room temperature as described in 3.3.3.3 for six months.

4.11.3 Results & Discussion

All samples were stable after processing and directly after centrifugation. After three weeks of storage at room temperature, first differences could be explored. All double emulsions that were processed with pressures beyond 50 MPa showed

precipitate at the bottom of the test tube. Thus, the samples were rejected. Too high pressures seemed to negatively affect double emulsion stability. There were not yet differences between the other samples. After six months of storage at room temperature the differences between the emulsions became more distinct. The results that were obtained after the storage test are discussed in chapter 4.11.3.1 and 4.11.3.2.

4.11.3.1 Ratio dispersed and continuous phase 2:3

The samples where the simple emulsion had been processed between 40 and 120 MPa and the processing pressure for the double emulsion was 10 MPa were stable after six months at room temperature. They exhibited neither sedimentation nor ring formation. All other samples showed sedimentation.

These results suggest that the processing pressure of the simple and the double emulsion have an impact on double emulsion stability. This was not detected in former trials because the processing pressures did not exceed 120 MPa and a decrease in storage stability was observed at 140 MPa and higher.

4.11.3.2 Ratio dispersed and continuous phase 1:1

None of the samples was homogenous after storage at room temperature for six months. All samples showed sedimentation which was more pronounced when the double emulsion was treated with higher pressures. The samples where the simple emulsions were processed at 10 MPa exhibited least sedimentation. Compared to the samples of trial 4.1 the sedimentation was more pronounced.

4.11.3.3 Comprehensive conclusion

The trial showed that the results of trial 4.1 were reproducible; stable flavor emulsions that contained flavor Propy and flavor PTG could be prepared a second time. Furthermore, the experiment showed that the processing pressure of the simple and the double emulsion have an impact on double emulsion stability. This was not observed in former trials because the pressures were not elevated to that extend. The trial demonstrates that too high processing pressures of the simple

emulsion (140 MPa and higher) reduce double emulsion stability. Furthermore, it was shown that too high processing pressures of the double emulsion also reduce emulsion stability.

As there were no observable differences between the stable samples it is recommendable to process the simple emulsion at 40 MPa. Higher processing pressures are not expected to have any advantages. In contrary, emulsion stability decreased at higher pressures; thus it is reasonable to choose lower pressures.

In terms of emulsion formula the results indicate that a lower volume of continuous phase of the double emulsion leads to unstable emulsions. According to this, the ratio of dispersed and continuous phase of the double emulsion should be at least 2:3 to obtain emulsions that are stable at room temperature for six months.

4.12 O/W/O flavor emulsions based on flavor Propy and flavor PTG

4.12.1 Introduction

The trials 4.8. 4.9. 4.1 and 4.11 have shown that it was possible to create stable flavor emulsions that contained flavor PTG, flavor Propy or a combination of both. The maximum flavor content to maintain stability was 16%. To further increase the flavor content the sunflower in the outer continuous phase should be replaced by flavor PTG in this experiment. Furthermore, the impact of processing pressure and the ratio of continuous and dispersed phase should be studied.

4.12.2 Experimental procedure

The emulsions were prepared as described in chapter 3.2.1.1, 3.2.1.2 and 3.2.1.2. The oil phase of the simple emulsion consisted of flavor PTG. The aqueous phase was a 1:1 blend of distilled water and flavor Propy. The composition was the same as in trial 5.10. Table 19 lists the composition of the simple emulsion. The processing pressures for the simple emulsions ranged from 20 to 160 MPa and were increased in 20 MPa steps. Double emulsions were prepared by dispersing the simple emulsion into flavor PTG that contained 10% PGPR. The double emulsions were processed with the microfluidizer at 10. 20 and 30 MPa. The emulsions were centrifuged as described in 3.3.3.2 and subsequently stored at room temperature as described in 3.3.3.3 for six months.

4.12.3 Results & Discussion

All samples were stable after processing; directly after centrifugation all samples exhibited sedimentations and some showed a ring on the top of the test tube. After five days of storage at room temperature the phases of all emulsion had completely separated.

To study whether these results could be drawn back to any mistakes in execution the trial was repeated under the same conditions; finally, the results of the two experiments did not differ.

It was not possible to produce stable flavor emulsions of the O/W/O type where the outer continuous phase consisted of flavor PTG.

Anyway, the emulsions that were prepared in trial 4.10 and 4.11 differ from the unstable emulsion with respect to the flavor PTG in the outer continuous phase. Thus, it can be deduced that the flavor in the outer phase was responsible for emulsion instability. Two ways to solve this problem and finally increase the overall flavor content of flavor emulsions could be to replace the solvent PTG by any other agent that forms stable emulsions with PGPR or to find a different emulsifier that stabilizes solvent PTG.

4.13 Double emulsions of the W/O/W type

4.13.1 Introduction

To investigate whether it was possible to create stable emulsions of W/O/W type with the provided raw materials, the following experiment was conducted: Two types of simple emulsions based on oil and water or oil and flavor Propy were stabilized with 5, 10 or 15% of PGPR to estimate the required amount of emulsifier. Subsequently, the simple emulsions were dispersed in water stabilized with Gum Arabic and maltodextrine.

4.13.2 Experimental procedure

The emulsions were prepared as described in chapter 3.2.3. 5, 10 or 15% of PGPR were dissolved in 210 g ($\pm$ 0.01 g) of sunflower oil. The solution was mixed with an Ultra-Turrax at 4000 rpm up to homogeneity. The continuous phase consisted of 70 g distilled water or flavor Propy. The simple emulsions were passed through the microfluidizer at 120 MPa. The Y-reaction chamber was used in this trial. Double emulsions were prepared by suspending the simple emulsions in a 30% solution of Gum Arabic and maltodextrine at a ratio 3:2. The double emulsions were passed through the microfluidizer at 30 MPa as described in 3.2.4.

4.13.3 Results & Discussion

The double emulsions were dissolved in water to check whether they could be of the W/O/W type. None of the six samples was water soluble; all were hydrophobic. Consequently, they were not of the W/O/W type. The results of the solubility test suggest that the produced emulsion was a W/O emulsion. A possible explanation for this is that the PGPR in the continuous phase of the simple emulsion is a more effective emulsifier than the Gum Arabic in the continuous phase of the double emulsion. PGPR forms a W/O emulsion whereas Gum Arabic stabilizes O/W emulsions. When the simple emulsion was dispersed in the second aqueous phase, redundant PGPR might have augmented the present dispersed phase; thus, no W/O/W emulsion was formed.

BONNET et al. (2009) report that they were able to produce stable, food grade W/O/W emulsions with PGPR and sodium caseinate (Span 80) for the encapsulation of magnesium. The simple emulsion was prepared with 30% PGPR in the oil phase, after emulsification the W/O emulsion was diluted with oil to decrease the PGPR concentration to 5%. Consequently, they had less redundant emulsifier in the oil phase that could affect the formation of a double emulsion. This, the use of a different emulsifier and a different emulsification process are explanations for the successful creation of stable W/O/W emulsion.

5 Conclusions

5.1 Processing conditions for flavor emulsions

5.1.1 Processing pressure of simple emulsions

The relationship between processing pressure and particle size and particle size distribution was studied. The observed relationship was not linear. An increase of processing pressure went along with a smaller average particle size at moderate pressures. Particle size reduced up to 80 MPa. In contrast, processing pressures beyond 120 MPa led to bigger particles and thus had an inverse effect on the average particle size. This observation goes along with literature; theory has shown that too excessive pressures lead to over-processing, increased particle size and finally decreased stability. This tendency was observed in two independent experiments.

However, it was also demonstrated that the processing pressure and accordingly the particle size of the simple emulsion had a marginal effect on the properties of the final double emulsion. Variable, moderate processing pressures of the simple emulsions did not lead to detectable differences in the final double emulsions when the processing pressure for the double emulsion was kept constantly low. Processing pressures of the simple emulsion at 140 MPa decreased double emulsion stability in one experiment. This can be disregarded because the optimal processing pressures of simple emulsions are below 140 MPa.

To this end moderate processing pressures seem reasonable; furthermore it is questionable whether a high pressure emulsification device is needed to produce storage stable emulsions.

5.1.2 Processing pressure of double emulsions

The processing pressure of the double emulsion had a meaningful impact on storage stability of the final double emulsion. Double emulsions that were processed at pressures beyond 30 MPa were less stable than emulsions that were processed

with lower pressures. A processing of the double emulsion at 10 or 20 MPa led to the most stable emulsions.

5.1.3 The influence of the number of passages

It was shown that a second passage of O/W emulsions through a microfluidizer effectively reduced particle size and contributed to a more homogenous distribution at all pressures. Even though particle size reduction was significant in most cases, a second passage seems unnecessary in practice because the final average particle size did not significantly differ from emulsions that were processed only once. Thus, one passage achieved similar results. Quite interesting was that a second emulsification step deteriorated emulsion that already had a small particle size and a narrow distribution after the first passage. This suggests that the minimal achievable particle size for one formulation cannot be decreased by supplying more energy to the emulsion neither by higher pressures nor by multiple passages.

5.2 Formulation

5.2.1 Formulation and storage stability

The experiments that aimed at maximizing the flavor content showed that a too high flavor concentration decreased emulsion stability, especially when the flavor was present in the outer continuous phase of the double emulsion.

The flavor content of the simple emulsion that was used as basic ingredient for the double emulsion could be increased up to 40% without stability losses of the double emulsion when flavor PTG was used. In this case the resulting O/W/O flavor emulsion had an overall flavor content of 16% and was stable at room temperature for six months. Emulsions that contained more flavor in the dispersed phase of the simple emulsion or had a lower volume of outer continuous phase were less stable and exhibited sedimentations after six months of storage at room temperature. It

was shown that a too low volume of outer continuous phase remarkably reduced storage stability at room temperature.

Two solvents could be successfully combined in one emulsion to increase the flavor content. A storage stable emulsion that contained flavor PTG in the dispersed phase of the simple emulsion and flavor Propy in the continuous phase of the simple emulsion could be prepared. This emulsion had an overall flavor content of 20%. Any of the stable O/W/O flavor emulsions contained sunflower oil as the outer continuous phase; it was not possible to replace sunflower oil by flavor without stability losses. Simple emulsions that were dispersed within flavor PTG were only stable for a few days.

Double emulsions of the W/O/W type could not be prepared with the used raw material.

6 Abstract

Fat reduced margarines often have poor sensory qualities. One reason for the sensory unattractiveness is the enhanced release of lipophilic flavor components which negatively affects flavor perception. Full fat margarine has a long-lasting flavor impression. In contrast, low-fat margarine typically has an initial flavor-boost which quickly abates, due to the lower fat volume that holds the flavor components back insufficiently. Theoretically, a slower or less intense release of lipophilic flavor components can mitigate the differences between full- and low-fat margarine.

The aim of this work was to develop stable flavor emulsions for low-fat margarine to encapsulate butter flavor and thus mitigate the sensory losses that are accompanied by fat reduction. The work comprised the evaluation of formulas and processing conditions leading to marketable flavor emulsions.

The work showed that it was possible to create sufficiently stable O/W/O flavor emulsions that based on propylene glycol and plant triglyceride with an overall flavor content of 16%. Higher flavor contents reduced storage stability and could therefore not be realized.

The flavor emulsions were processed with a microfluidizer which is a high pressure emulsification device. Experiments that aimed at evaluating appropriate processing conditions came to the conclusion that too high processing pressures of simple and double emulsions negatively affect emulsion stability. Nevertheless, the optimal conditions are formula-dependent and can not be generalized.

Zusammenfassung

Die sensorischen Eigenschaften von fettreduzierter Margarine sind oftmals wenig zufriedenstellend, was unter anderem daran liegt, dass lipophile Aromakomponenten auf Grund der reduzierten Fettmatrix schneller freigesetzt werden. Im Gegensatz zu Vollfettmargarine, bei der die Aromafreisetzung gleichmäßig erfolgt, weist fettreduzierte Margarine einen anfänglichen „Flavor-boost“ auf. Theoretisch kann eine langsamere oder weniger intensive Aromafreisetzung

dazu beitragen, dass sie sensorischen Unterschiede zwischen Vollfettmargarine und fettreduzierter Margarine weniger gravierend ausfallen.

Das Ziel dieser Arbeit war es stabile Aromaemulsionen herzustellen, um so die Freisetzung des Aromas zu verzögern und die sensorischen Einbußen, die mit einer Fettreduktion einhergehen, zu mildern. Die Arbeit umfasste die Entwicklung von geeigneten Rezepturen und Prozessbedingungen für die Herstellung von vermarktbarsten Emulsionen.

Es gelang lagerstabile O/W/O Emulsionen aus Propylenglykol und pflanzlichen Triglycerid mit einem Aromagehalt von 16% herzustellen. Höhere Gehalte an Aroma reduzierten die Lagerstabilität.

Die Aromaemulsionen wurden mit einem Microfluidizer hergestellt. Es konnte gezeigt werden, dass der ideale Prozessdruck rezepturabhängig ist, dass aber generell zu hohe Drücke zu einer reduzierten Stabilität führen.

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Ich habe mich bemüht, sämtliche Inhaber der Bildrechte ausfindig zu machen und ihre Zustimmung zur Verwendung der Bilder in dieser Arbeit eingeholt. Sollte dennoch eine Urheberrechtsverletzung bekannt werden, ersuche ich um Meldung bei mir.

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Education

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Publications

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