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M.Sc. in Food Engineering

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**FORMULATION AND PHYSICAL CHARACTERIZATION OF
HIGH METHOXYL PECTIN AND SUNFLOWER OIL WAX
EMULSIONS**

**M.Sc. THESIS
IN
FOOD ENGINEERING**

**BY
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**M.Sc. Thesis
in
Food Engineering
Gaziantep University**

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July 2019



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ABSTRACT

FORMULATION AND PHYSICAL CHARACTERIZATION OF HIGH METHOXYL PECTIN AND SUNFLOWER OIL WAX EMULSIONS

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Pectin is a polysaccharide found on the cell walls of many plants. It is used often in food industry due to its gelling properties. Waxes are commonly used as edible coatings to enhance the water barrier properties of food products. There are many studies about emulsions, which are prepared with oil, wax and polysaccharides. This study focused on preparing emulsions with sunflower oil wax and pectin at different concentrations and characterization of the emulsions by using particle size, rheology, light microscope and low-resolution Time Domain NMR relaxometry experiments. Pectin and wax emulsions were prepared at concentrations of 5% wax and 5%, 7%, 10% pectin by using high shear homogenization. The mean particle size results of the emulsions were recorded between 1-3 μm . The optical microscope results confirmed the particle size measurements. Considering the rheology results, it was concluded that the flow behavior did not change depending on the pectin concentration. NMR spin-spin T_2 relaxation times were also measured to understand the differences between emulsions. T_2 (relaxation times) decreased, as pectin concentration increased and the change in relaxation times provided insight on the formation and stability of the emulsions. It was measured as 312.48 ± 4.70 ms for 5% pectin containing sample and 197.62 ± 1.26 ms, 115.24 ± 1.87 ms for 7% and 10%.

Key Words: Pectin, Sunflower Wax, Particle Size, Rheology, NMR Relaxometry

ÖZET

YÜKSEK METOKSİLLİ PEKTİN VE AYÇİÇEK YAĞI VAKSI EMÜLSİYONLARININ FORMÜLASYONU VE FİZİKSEL KARAKTERİZASYONU

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Pektin bazı bitkilerin hücre duvarlarında bulunan bir polisakkarittir. Jelleşme özelliğinden dolayı gıda endüstrisinde sıkça kullanılır. Vakslar yaygın olarak gıda ürünlerinin su bariyeri özelliklerini geliştirmek için yenilebilir bir kaplama olarak kullanılırlar. Yağ, vaks ve polisakkaritler ile hazırlanan emülsiyonlarla ilgili birçok çalışma bulunmaktadır. Bu çalışma, farklı konsantrasyonlarda ayçiçeği yağı vaksı ve pektin ile emülsiyonların hazırlanmasına ve parçacık boyutu, reoloji, ışık mikroskopu ve NMR relaksometre teknikleri kullanılarak bu emülsiyonların karakterizasyonu üzerine odaklanmıştır. Emülsiyonlar yüksek hızlı homojenizasyon yöntemi ile 5% vaks ve %5, %7, %10 pektin konsantrasyonunda hazırlanmıştır. Parçacık boyutu sonuçları 1-3 µm arasında kaydedilmiştir. Işık mikroskobu görüntüleri de, parçacık boyutu ölçümlerini desteklemektedir. Reoloji sonuçları göz önünde bulundurulduğunda, akışkan tipinin pektin konsantrasyonuna bağlı olarak değişmediği sonucuna varılmıştır. Emülsiyonlar arasındaki farkı anlayabilmek için NMR relaksometri ile T_2 (relaksasyon zamanı) da ölçülmüştür. T_2 (relaksasyon zamanı) pektin konsantrasyonu arttıkça azalmaktadır ve relaksasyon zamanında meydana gelen değişim, emülsiyonların oluşumu ve stabilitesi üzerine fikir vermektedir. %5 pektin içeren örnek 312.48 ± 4.70 ms ve %7, %10 için 197.62 ± 1.26 ms, 115.24 ± 1.87 ms olarak ölçülmüştür.

Anahtar Kelimeler: Pektin, Ayçiçek Vaksı, Parçacık Boyutu, Reoloji, NMR
Relaksometre



“Dedicated to my mother”

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LIST OF SYMBOLS

%:	Percentage
°C:	Degree celcius
°C/min:	Degree celcius per minute
s⁻¹:	One per second
(w/w):	Weight per weight
≥:	Equal and bigger or higher than
<:	Smaller or less than
±:	Higher or less than
mW/mg:	Milliwatt per milligram
rad/s:	Radian per second
J/g:	Joule per gram
mEq/g:	Milliequivalent per gram
a_w:	Water activity
H⁺:	Hydrogen
NaZ:	Sodium azide
O/W:	Oil in water emulsion
W/O:	Water in oil emulsion
S1:	5% P_5% SFW
S2:	7% P_5% SFW
S3:	10% P_5% SFW
S4:	5% P_5% SFW_10% SFO
S5:	7% P_5% SFW_10% SFO
S6:	10% P_5% SFW_10% SFO
S7:	SFO
S8:	5% P solution
S9:	7% P solution
S10:	10% P solution

S11:	5% P_10% SFO
S12:	7% P_10% SFO
S13:	10% P_10% SFO
S14:	SFW:SFO at a ratio of 1:2
S15:	Pure wax
T₁:	Spin-spin relaxation time
T₂:	Longitudinal relaxation time
TE:	Echo time
T_{on}:	Onset temperature
T_p:	Peak temperature
ΔH:	Enthalpy



LIST OF ABBREVIATIONS

min:	Minute(s)
m:	Meter
µm:	Micrometer
nm:	Nanometer
ms:	Millisecond
ml:	Milliliter
mg:	Milligram
Pa:	Pascal
rpm:	Revolution per minute
HMF:	5-Hydroxymethyl furfural
BPF:	Brown pigment formation
AGP:	Arabinogalactan Protein
SFO:	Sunflower oil
SFW:	Sunflower oil wax
P:	Pectin
HM:	High Methoxyl
LM:	Low Methoxyl
DE:	Degree of Esterification
FFA:	Free Fatty Acid
SFC:	Solid Fat Content
GC:	Gas Chromotography
NMR:	Nuclear Magnetic Resonance
TD NMR:	Time Domain Nuclear Magnetic Resonance
CPMG:	Carr-Purcell-Meiboom-Gill
RF:	Radio frequency
DSC:	Differential Scanning Calorimetry

et al.: et alii (and others)
etc.: etcetera
n.d.: No date
ANOVA: Analysis of Variance



CHAPTER 1

INTRODUCTION

Pectin is a heteropolysaccharide, which can be obtained from cell walls of plants. It is generally used as a thickening and gelling agent in food industry (Dickinson, 2003). There are different pectins according to their esterification and acidification ratio. It was reported that high acetylated pectin which was extracted from sugar beet was exhibited more surface-active properties than high-methoxyl and low-methoxyl pectins. Also, it could help to stabilize fine vegetable oil-in-water emulsions through thickening effect (Dea & Madden, 1986).

Wax is a lipid, which is an ester of a long chain alcohol and a fatty acid. It includes various substances such as; hydrocarbons, ketones, diketones, primary and secondary alcohols, aldehydes, sterol esters, long carbon chains (from 12 up to 38), alkanolic acids, terpenes and monoesters. All waxes are water-resistant and they are not chemically homogeneous because of these different substances. There are several kinds of waxes present such as; bee wax, carnauba wax, candelilla wax, sunflower oil wax and some synthetic waxes. Some of them are used to form emulsions and natural edible films and coatings (Lee, 1999).

When two immiscible liquids are added to each other they form an emulsion. Emulsions could be either oil-in-water or water-in-oil. They are categorized based on the dispersed and continuous phases. Since many natural or processed foods such as; milk, cream, beverages, soups, mayonnaise etc. are in the form of emulsions, knowledge of the emulsion formation and stabilization mechanisms is important for working on the food and the related industries. Also, for functional ingredients such as; colorants, preservatives, flavors, emulsions are salvaged as delivery systems.

Designing an emulsion-based delivery systems is an emerging research area. Ingredients and process are important to determine the physicochemical, sensory and biological characteristics of them.

A good emulsion production can be formed by selecting raw materials in proper types and concentrations (Bai & McClements, 2016).

There are several studies about pectin-wax based emulsions in the literature. There are several reasons of preference of this binary system. First both of them are present naturally in plants or vegetables. Also, the Food & Drug Administration approve the usage of edible films and coatings which were obtained from pectin-wax based emulsions (Panchev, Nikolova, & Pashova, 2009). Erica et.al. (2012) studied about pectin & sunflower oil wax emulsion preparation and produced an edible film from it. They focused on the effect of the concentration change of both wax and pectin. They reported that SFW change did not affect the shear stress value, but the shear stress values increased with increasing pectin concentration (Bäumler, Carelli, & Martini, 2013).

In this study, pectin-wax based emulsions were formulated and they were characterized by using different physical methods such as: particle size, optical microscopy, rheology and low-resolution TD NMR experiments. There are a few studies about pectin-wax based emulsions in the literature, but there is no a detailed report which includes the NMR characterization of these emulsions with other physical experiments. This research focused on exploring the effect of change of the pectin concentration on oil-in-water emulsions.

The aims of this project are;

- To formulate and characterize pectin-wax based emulsions,
- To explore the effect of pectin concentration on emulsions,
- To use NMR relaxometry as a tool for investigating the relation between wax and pectin in an emulsion system.

CHAPTER 2

LITERATURE REVIEW

2.1 Emulsions

Emulsions are disperse systems that contain two immiscible liquids. They include dispersed and continuous phases. The continuous phase suspends the dispersed phase droplets. Emulsions are categorized as; oil-in-water and water-in-oil. When the continuous phase is water, and the dispersed phase is oil an oil-in-water emulsion is formed and in a water-in-oil emulsion the phases are reverse. Typical oil-in-water emulsions that used in food industry include milk, mayonnaise, cream; water-in-oil emulsions are dressings, margarine and butter. Also water-soluble paints and some body lotions are examples of oil-in-water emulsions that are used in the other industries (Karbstein, & Schubert, 1995).

Emulsions are commonly formed through high-shear homogenizers that help to obtain small droplets by applying high shear stress on mixtures. Water and oil emulsions need a third component for dispersion, because their densities are different and the oil droplets tend to merge with each other that cause phase separation in mixtures. This can be prevented by using some natural or synthetic materials which are called emulsifiers or surfactants (Guzey & McClements, 2007). Emulsifiers are surface-active ingredients and during homogenization, they adsorb lipid droplets to the surface. The type of the emulsifier is very important for the formation and stability of an emulsion. There are different emulsifiers depending on the ability to form and stabilize emulsions, ease of utilization, ingredient compatibility, environmental sensitivity, as well as in their cost (Moreau, Kim, Decker, & McClements, 2003).

Emulsions can be characterized with respect to the emulsifier's nature or the system structure. This is shown in Table 2.1 (Tadros, 2013).

Table 2. 1 Classification of emulsifier types

<u>Nature of emulsifier</u>	<u>Structure of the system</u>
Simple molecules and ions	Nature of internal and external phase
O/W, W/O	Nanoemulsions
Nonionic surfactants	—
Surfactant mixtures	Micellar emulsions (microemulsions)
Ionic surfactants	Macroemulsions
Nonionic polymers	Bilayer droplets
Polyelectrolytes	Double and multiple emulsions
Mixed polymers and surfactants	Mixed emulsions
Liquid crystalline phases	—
Solid particles	—

2.1.1 Structural Classification of the Emulsion Systems

- **O/W and W/O macroemulsions:** These usually have a size range of 0.1–5 μm with an average of 1–2 μm .
- **Nanoemulsions:** These usually have a size range of 20–100 nm. Similar to macroemulsions, they are only kinetically stable.
- **Micellar emulsions or microemulsions:** These usually have the size range of 5–50 nm. They are thermodynamically stable.
- **Double and multiple emulsions:** These are known as emulsions-of-emulsions, and represented as mostly in the form of W/O/W, and O/W/O systems.
- **Mixed emulsions:** These systems consist of two different disperse droplets and they do not mix in a continuous medium.

2.1.2 Emulsion Production Methods

Homogenization is used to mix two immiscible liquids and form an emulsion. The equipments which are used for this purpose are called homogenizer. At first Auguste Gaulin introduced this machine for treating milk in 1900 and the word ‘‘homogenized’’ was first used to describe milk treated by the Gaulin machine (SPX Corporation, 2005). There are several kinds of homogenizers present now. High pressure homogenizer (microfluidizer), high shear disperser (turrax) and colloid mill are some examples of the homogenizers.

High pressure homogenizer (microfluidizer) is the most common homogenizer used in food industry especially for milk and cream. It is shown schematically in Figure 2.1. It is a continuous system which is used for emulsion production. The parts of a microfluidizer are fluid inlet, outlet, chamber and pump (McClements, 2005). In this system, mixtures or emulsions are pumped through the chamber with a high velocity and forced to pass from micro-gaps. Because of the high shear stresses and cavitation forces the size of droplets reduce (Hasenhuettl & Hartel, 2008). The pressure range of the machine is 50 to 500 bar (microfluidizer up to 1600 bar). Homogenization may be single or multiple stage. The rapid increase in pressure results in cavitation bubble collapsing and the flow turns back into a one-phase flow. Therefore, laminar and turbulent flow at entrance of valve, cavitation bubbles growth and bubble collapse in zone cause size reduction of droplets (Weiss, 2008).

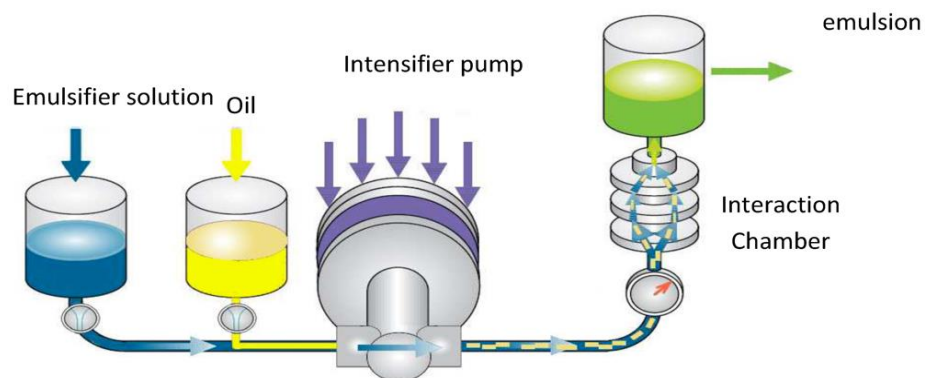


Figure 2.1 High Pressure Homogenizer (Panagiotou & Fisher, 2012)

High shear disperser which is schematically shown in Figure 2.2 is a rotor-stator system similar to colloidal mills. They have coaxial intermeshing rings with radial openings. The sample enters in the systems center and the rotor accelerates it. When it passes through the system, the sample which is a fluid is accelerated and decelerated more than one time. This results in high tangential forces. The pre-emulsion is carried through the rotor systems openings and mixes with the liquid in the gap which is between the rotor and the stator. High shear forces and turbulent flow results reducing of droplet sizes (Weiss, 2008). In this study, the high shear disperser system was used to prepare all emulsions.

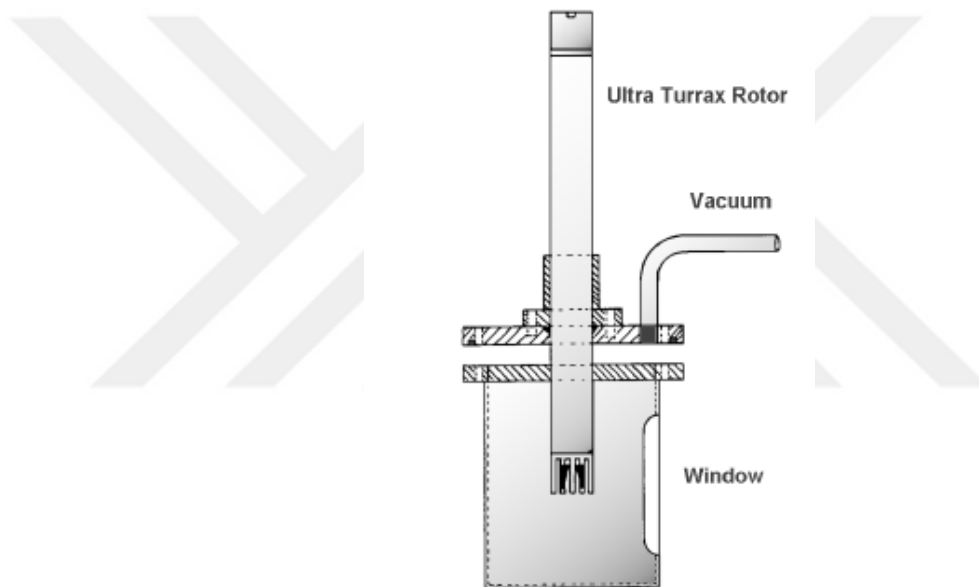


Figure 2. 2 High Shear Disperser (Turrax) (Christoph Wabel, 1998)

Colloidal mill, which is schematically shown in Figure 2.3, is a continuous system. The parts of a colloidal mill are a rotor and a stator to process emulsions. The rotor part is rotating with a high speed, but the stator is stationary. A gap is present between the rotor and stator and the emulsion is fed into it. In colloid mills, disruption of droplet occurs in a flow channel between a rotor-stator system. Due to the conical shape, the emulsions do not need an external pressure to transport. The rotating rotor applies a shear stress on the coarse emulsion particles and they get smaller. Rotator rotation speed can be set up due to desired particle size. and when

rotation speed is increased, particle size of emulsion will be decreased. Rotation increases the temperature of system and it affects the stability of emulsion, so a temperature controller is needed. (Hasenhuettl, 2008).

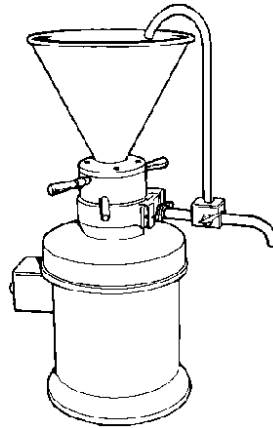


Figure 2. 3 Colloidal Mill

There are also some post-homogenization processes that are used to modify the functional properties of emulsions such as; composition, structure and physical state. Generally, these post homogenization processes have an effect on the droplets of emulsion. The potential changes on the droplets after post-homogenization techniques are shown in Figure 2.4 schematically. The droplet size can be reduced, the agglomeration of the droplets can be prevented and the stability and the uniformity of the emulsion can be achieved with these processes (Bai & McClements, 2016).

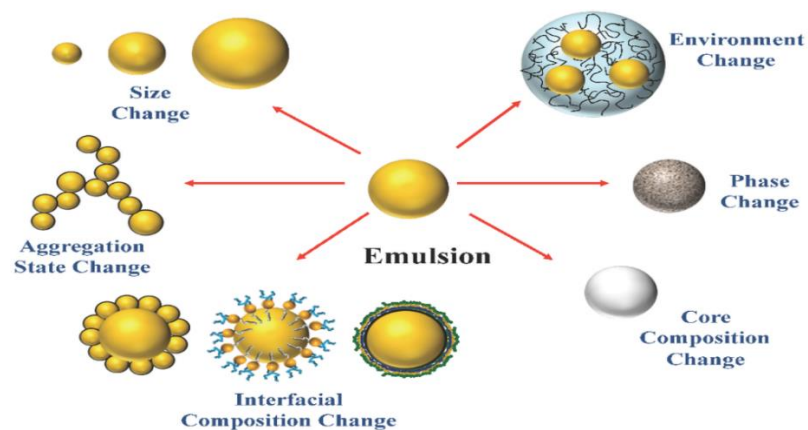


Figure 2.4 The potential changes of droplets in an oil-in-water emulsion by performing post-homogenization techniques (Bai & McClements, 2016).

2.1.3 Hydrocolloids in Emulsions

Hydrocolloids are macromolecules which are used in foods to control thickening, gelling, etc. They are hydrophilic polymers of vegetable, animal, microbial or synthetic origin and they generally contain many hydroxyl groups and mostly polyelectrolytes. Solubility, viscosity and water binding are the most important properties. Also ice recrystallization prevention, organoleptic properties and stability of emulsion are significant. Hydrocolloids can act as both stabilizers and emulsifiers in an oil-in-water emulsion according to its type (Dickinson, 2009).

There are 14 hydrocolloid gums that are known for their emulsification properties. In food applications, gum arabic, modified starches and pectin are widely used as a polysaccharide emulsifier. (Huang, Kakuda, & Cui, 2001).

Gum arabic is widely used as a stabilizer and emulsifier in food industry (Nakauma et al., 2008). It is produced naturally from *Acacia senega* tree. Benech et. al (2008) studied about the gum arabic that could be used as hydrocolloid in beverages. It was focused on the two functionality of arabic gum; a low-calorie ingredient for "light" beverage formulations and a highly performing stabilizer for liquid emulsions. The arabinogalactan protein (AGP) which is a group in arabic gum structure is crucial. The AGP can easily link up to the general structure and it holds on the oil phase of an oil-in water emulsion.

Göğüş et. al. (2000) examined the effects of some hydrocolloids and water activity on nonenzymatic browning of concentrated orange juice. They added xanthan gum and microcrystalline cellulose to the concentrated orange juice and examined the effect on the 5-hydroxymethyl furfural (HMF) accumulation and brown pigment formation (BPF). They recorded that the HMF and BPF rate were less in the concentrated orange juice with hydrocolloids than pure concentrated hydrocolloids. Also, they observed the highest HMF and BPF in the range of water activity 0.30-0.75 and the lowest was at a_w 0.80.

2.1.4 Emulsion Stability

Stability of an emulsion can be defined as the resistance of the physicochemical properties of the emulsion against changes with time. An interfacial tension is present between water and oil because of the density difference. This interfacial tension increases during emulsion formation. Increasing free energy causes thermodynamically unstable systems (Binks, Cho, Fletcher, Petsev, 1999).

For emulsions two types of stability are discussed. One of them is thermodynamic stability. Emulsions are unstable thermodynamically due to two immiscible phases being dispersed through high energy. The other one is kinetic stability. Thermodynamically unstable emulsions need an activation energy to invert to a stable form. The kinetic stability of emulsion is determined by this activation energy (Garti & Reichman, 1993).

Emulsion instability mechanisms include; flocculation, creaming or sedimentation, Ostwald ripening, coalescence and phase inversion. All mechanisms are shown in Figure 2.5, schematically.

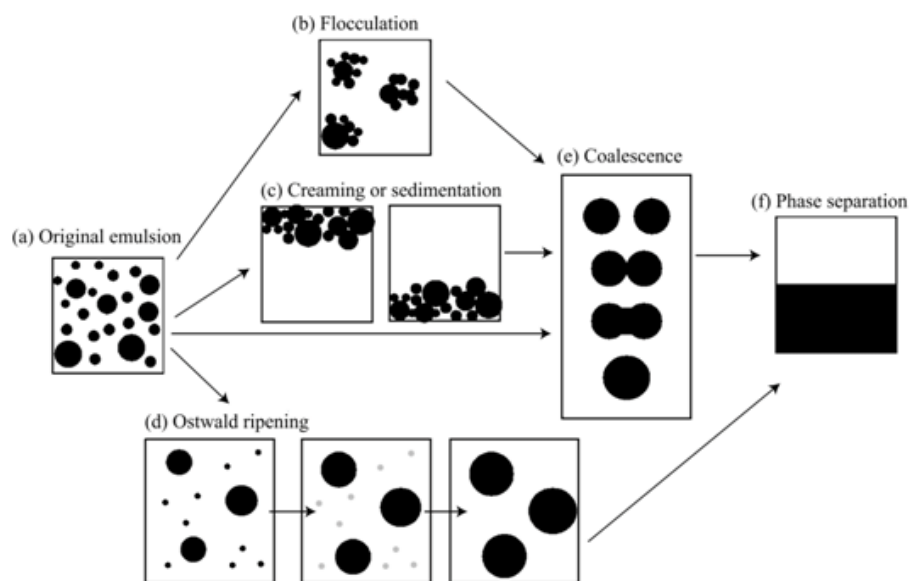


Figure 2. 5 Emulsion breakdown mechanisms schema (Salama, 2011)

Flocculation means the droplet aggregation without change on their particle size and form larger structure. This mechanism occurs because of the Van Der Waals attraction between droplets (Dickinson, Golding, & Povey, 1997). Flocculation results from both sedimentation aggregation and Brownian motion aggregation.

In sedimentation aggregation, all paths in the sedimentation are assumed as linear. Random Brownian movements cause Brownian aggregation (“Emulsion Stability and Testing,” 2011).

Creaming and sedimentation occurs from external forces which are gravitational or centrifugal. When the densities of dispersed and continuous phase are different, gravitational separation is observed. If the density of droplets (dispersed phase) are less than the medium (continuous phase), droplets move upward. The top of emulsion densifies and this formation is called creaming. On the other hand, if the droplets are denser than the medium, they move downward. The bottom of emulsion densifies and this is called sedimentation (Tadros, 2013).

Ostwald Ripening (Disproportionation) results from the solubility differences between smaller and larger droplets. Emulsions that are highly dispersed have smaller droplets with larger solubility. They disappear and their molecules move to the larger droplets with time. Also, the droplet size dissipation shifts to larger values with time (Guzey & McClements, 2007).

Coalescence is derived from fusion of two or more droplets into the larger ones and deterioration of the liquid film between droplets. If the Van Der Waals attraction of droplets is strong, it can be prevent the phase separation (Tadros, 2013).

Phase Inversion (Separation) is an exchange between continuous and disperse phase. It is a transition state which includes the other destabilization mechanisms. An O/W emulsion may invert to the W/O emulsion with time.

2.1.5 Industrial Applications of Emulsions

Emulsions are mostly used in the pharmaceutical, cosmetic and food industries. Both W/O and O/W emulsions are used, but W/O emulsions are less stable because of the high mobility of H^+ molecules of water droplets (Ushikubo & Cunha, 2014). For this reason, studies are mostly focused on O/W emulsions.

Beverages, deserts, mayonnaise and salad creams are some examples of food emulsions, also hand or face creams, lotions, hair sprays are emulsions in cosmetic and anesthetics of O/W, lipid emulsions are examples of pharmaceutical industry (Tadros, 2013).

Emulsions can be made by using one component, but the emulsions that are produced from hydrocolloids and lipids has better functionality. Some materials can be used to form an emulsion such as; oil, waxes, protein, pectin, etc. Emulsions which are formed by using these materials can also be used to form an edible film or a coating. Emulsion-based edible films and coatings can be utilized in fresh fruits and vegetables, cheese, meat, sausages and bakery products prolonging the shelf-life of them without using preservative (Galus & Kadzińska, 2015).

Fruits and vegetables are perishable foods and their shelf-life is short (Albrecht, 2007). Emulsions which contain lipids (natural waxes, paraffin) and chemical substances have an important role at this point. Edible coatings and films minimize the moisture loss (Dea, Ghidelli, Perez-Gago, & Plotto, 2012), reduce the respiration rate (Vargas, Pastor, Chiralt, McClements, & Gonzalez-Martinez, 2008), decrease enzymatic browning (Perez-Gago, Serra, & Del Río, 2006) of fruits and vegetables and thus increase the shelf life.

Emulsion-based films and coatings can also be applied to meat and meat products by foaming, spraying, brushing, dipping or rolling (Galus & Kadzińska, 2015) to prevent microbial growth.

Cheese shelf life depends on the fungal and bacterial development on its surface. Using coatings such as; whey protein isolate, glycerol, guar gum, sunflower oil together with different combinations limits the growth of pathogenic or contaminant microorganisms. Also, it reduces the water loss, hardness and color change (Martins, Cerqueira, Souza, Carmo Avides, & Vicente, 2010).

The crispy texture is important in the bakery products such as; biscuits, breakfast cereals and snacks. This property is directly related to the relative humidity of environment. Higher relative humidity causes loss of crispness and softening. Emulsified coating application that contain corn starch, methylcellulose and soybean oil at proper temperature and relative humidity to bakery products reduces the hydration kinetics (Han, 2014).

2.2 Pectin

2.2.1 Structure

Pectin is a negatively charged natural heteropolysaccharide which is commonly used due to its gelling, stabilizing and emulsifying properties in food, medication and textile industries. Pectin can be obtained mainly from apple pulp, orange crust, sunflower thalamus and sugar beet residue (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002). The powdered form image of pectin is shown in Figure 2.6. Pectin contains methyl ester at different ratios and it can dissolve in water at different neutralization level. It can form a gel with sugar and acid at proper ratios.

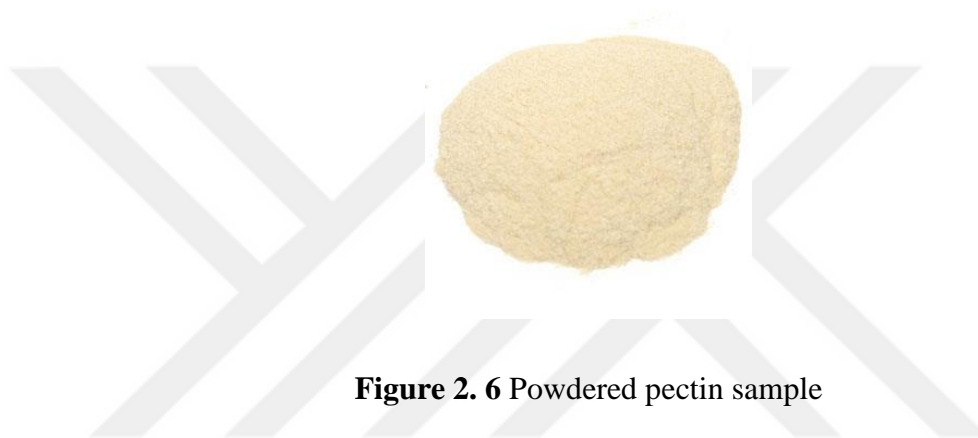


Figure 2. 6 Powdered pectin sample

Basic components of pectin (water-soluble) and pectic substances (water-insoluble) are D-galacturonic acid units that are bonded α (1-4) glycoside and partially esterified with methanol. 1.4- α -D-galacturonan, branched L-arabinose, 1.4- β -D-galactan, rhamnogalacturonan, L-rhamnan, and arabinogalactan that contain partially methyl ester group units also could be present on pectin's structure (Muhiddinovy, Khalikovy, Speakerz, & Fassihiz, 2008). The chemical structure of pectin is shown in Figure 2.7 schematically.

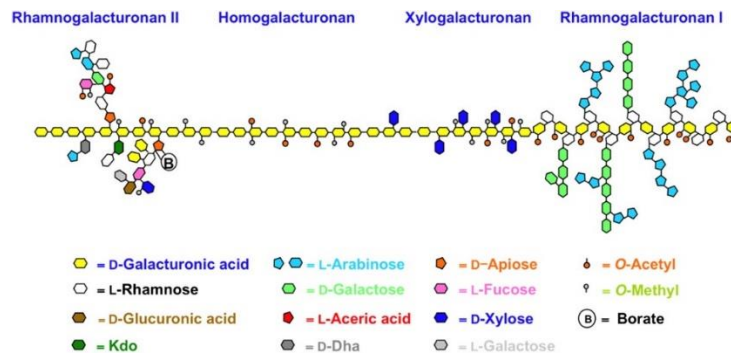


Figure 2.7 The chemical structure of pectin inn schematically ((Harholt, Suttangkakul, & Vibe Scheller, 2010)

The esterified galacturonic acid groups and the total galacturonic acid groups ratio is called as degree of esterification (DE). According to degree of esterification pectin is classified as either high methoxyl (HM) (Figure 2.8) which has above 50% DE or low methoxyl (LM) (Figure 2.9) which has below 50% DE (Grasdalen, Einar Bakøy, & Larsen, 1988). In low-methoxyl pectin, gelation results from ionic linkage via calcium bridges between two carboxyl groups belonging to two different chains in close contact with each other. In high-methoxyl pectin, the cross-linking of pectin molecules involves a combination of hydrogen bonds and hydrophobic interactions between the molecules (Thakur, Singh, Handa, & Rao, 1997).

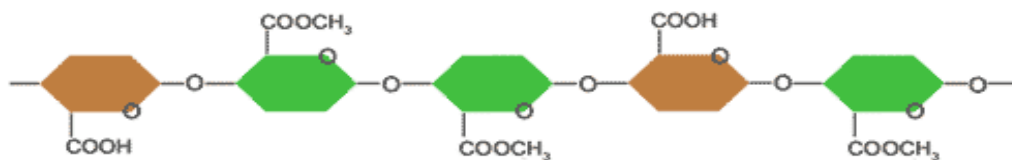


Figure 2.8 The chemical structure of high-methoxyl pectin (“IPPA: International Pectin Producers Association,” 2017)

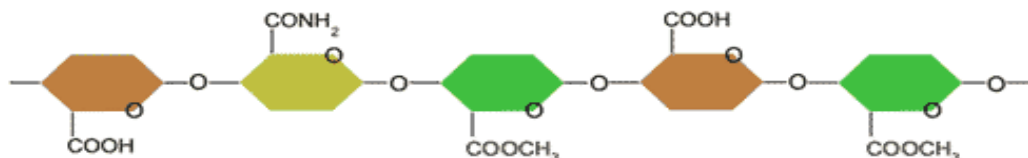


Figure 2. 9 The chemical structure of low-methoxyl pectin (“IPPA: International Pectin Producers Association,” 2017)

2.2.2 Industrial Applications of Pectin

In 1970's pectin was started to be used in food and pharmaceutical industries. Despite it was used extensively, it was not manufactured in Turkey, but was imported (Turmuçin, Ungan, & Yıldız, 1981). It was started to produce in small quantities in Mersin, Turkey recently.

Pectin was used as gelling agent and thickener in food industry. It was used generally in fruit juices to increase the yield and clarity. In 1998, Alkorta and her team focused on a different property of pectin. They studied the use of immobilization supports and continuous flow-systems (Alkorta, Garbisu, Llama, & Serra, 1998).

Akhtar et. al.(2001) focused on the emulsion stabilizing properties of pectin. They recorded that the depolymerization degree of pectin was a more important parameter than the DE value. The molecular weight of pectin influenced its efficiency. Also, they demonstrated that pectin can be used as an effective emulsifying agent in food emulsions under acidic conditions.

Also, Guo et. al. (2014) pointed the emulsifying effect of pectin to note that 'However hydrophilic capacity of macromolecules of pectin is a disadvantage to be a barrier against water, when it is used with a lipid, it can be available to form a stable emulsion with its emulsifier property'.

2.3 Waxes

2.3.1 Structure

Waxes are a class of organic compounds which consist a long chain ester of fatty acids with fatty alcohols. They are present naturally in some plants and also, can be obtained synthetically. There are many kinds of waxes present that are used in different industries (Table 2.2). A representative chemical structure of a wax is shown in Figure 2.10.

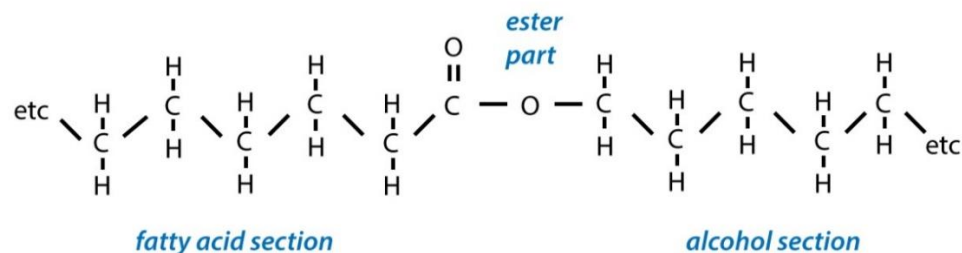


Figure 2. 10 The chemical structure of a wax

Waxes are generally used to enhance the water barrier properties of foods in the food industry. Emulsions can be obtained by mixing waxes with polysaccharides and other oils. Emulsion-based edible films can be used to prevent water loss and increase the shelf life of fruits, vegetables and meat products. Perez-Gago et. al (2006) explored the effect of the combination of whey protein concentrate and beeswax for fresh cut apples. They coated the apple pieces with edible film and they measured the browning index, color, weight loss and sensory evaluation during storage. They recorded that the combination of protein and wax could reduce the browning more than the use of a simple antioxidant.

Table 2. 2 Types of waxes and their applications (Galus & Kadzińska, 2015)

WAX	APPLICATION
BEESWAX	For consumption
CHINESE WAX	For polishes
EAR WAX	Used as a protective layer over the ear membrane
LANOLIN	For rust prevention and cosmetics
SHELLAC	Used as a wood sealant
SPERMACETI	For cosmetics and leather working
VEGETABLE	Used as a protective layer on the plant to prevent loss of water
MINERAL	Used as fine polishes
PETROLEUM	Fuels, paints, candles
SYNTHETIC	Modified waxes for use in the medical field

Also, wax-based emulsions are used in some other industries as coatings or edible films such as pharmaceutical, textile, cosmetics etc. In 1987, Bagaria et al. was focused on the mix of a wax, an emulsifying agent and water that can be useful as coating in drugs. They reported that these aqueous coating film systems could improve the thickness and uniformity of drugs (Bagaria & Lordi, 1987).

2.3.2 Sunflower Oil Wax

Sunflower oil waxes are natural components of sunflower oil which contain fatty acids and fatty alcohol ester chains as the other waxes. They are present in the hull of sunflower oil seeds and can be removed by refining (Carelli, Frizzera, Forbito, & Crapiste, 2002).

Kerr et. al. (2011) studied on the change of crystallization behavior and functional properties of anhydrous milk fat (AMF) by adding sunflower oil wax in it. They evaluated the melting behavior, microstructure of crystals, hardness and the induction times of nucleation of both pure AMF and AMF with 0.1 and 0.25 % sunflower oil wax. The researchers recorded that adding sunflower oil wax promoted the crystal growth after tempering. They reconstituted that sunflower oil wax could be used as an additive to change the physicochemical properties of low trans-fatty acid lipids.

2.3.3 Other Waxes

Besides sunflower oil wax, there are some other waxes present. The most frequent are: Jojoba which is produced from the jojoba plant; Carnauba is made from the leaves of the carnauba plant; Lanolin, made of from lamb wool. Also, Beeswax is the most used wax in industry because of its lower melting point (about 10-20 °C) (Leray, 2014). The 'wax' word is derived from an old English word 'weax' which means substances made by bees. So, beeswax is usually accepted as the reference wax (CBI Market Intelligence, 2015).

2.4 Characterization Methods of Emulsions

2.4.1 Particle Size

Particle is a relatively small or the smallest discrete portion or amount of something (Xu, 2002). Particle size plays an important role in many industries including; nanotechnology, proteins, cosmetics, polymers, fertilizers and many more. Droplet size is a determining factor for emulsion stability and also affects its viscosity and other important properties (Galus & Kadzińska, 2015).

The particles can be regular or irregular. If the particles are regular, the particle size is equally appropriate dimension. For irregular particles, there are many methods to measure particle size. In this study the Laser Diffraction Method was used to determine the particle size of high methoxyl pectin-sunflower oil wax emulsions. The laser diffraction method is based on the scattering of light by suspensions began to be implemented in the late 1970's (Syvitski, 2007). Laser diffraction method calculate the particle size distribution by measuring the angular fluctuation in light scattered intensity as a laser beam passes through a disperse particulate sample. Depending on the laser beam, large particles scatter light at small angles and small particles scatter light at large angles. It has a rapid measurement system than the other methods (Califice, Michel, Dislaire, & Pirard, 2013).

Particle size was extensively used in the food industry in different purposes. Huang et. al. (2001) measured the particle size of emulsions which were prepared 14 kinds of hydrocolloids. The emulsification properties of emulsions compared. Califice et. al. (2013) used the particle size measurement to identify the existence of two populations in the blends of differently shaped products.

2.4.2 Optical Microscopy

Optical microscope is a type of a microscope that uses visible light and a lense system to enlarge small samples. It is also called light microscope. The first light microscope was created by Zacharias Jansen in 1595. Optical microscope has two different types. Simple optical microscope that; uses only one lens for magnification, compound optical microscope that; uses many lenses to maximize magnification (Rack, 2001).

Microscopy is used to study the effect of the processing conditions and ingredients on food structure. In recent years, microstructure of foods gets more important, as it gives many information about food, structure, rheology and textural properties (Fazaeli, Tahmasebi, & EmamDjomeh, 2012).

Moss et. al. (1985) used light microscopy for understanding functional changes on the flour during milling and wheat processing. Guner and Oztop (2017) investigated the structure of multivesicular liposomes for detecting any change in physical characteristics with time.

2.4.3 Rheological Measurements

Rheology is the science of flow and deformation of matters under applied forces. Shear stress, shear rate and viscosity are the basic parameters of rheology (A Division of Sun Chemical Corporation, 1995).

Viscosity is generally defined as a measure of the resistance of a fluid to flow. But, when the molecular level is considered, viscosity can be defined as a result of interaction between different molecules in a fluid. The measurement of internal resistance which is a tangential force per unit area is called as dynamic (absolute) viscosity. The dynamic viscosity to density ratio is called kinematic viscosity (Henderson, 2004). Viscosity measurement is important in many industries, because it affects the production and transportation processes design and it gives information about the material's behavior in real.

Shear stress is a measure of a fluid's friction force, which effects on a part in the path of that fluid. Shear rate is the velocity-changing rate of the ratio of one layer of fluid passes to a contiguous layer. The shear stress and shear rate ratio gives the viscosity. Rheology is used in many industries nowadays. Especially in food industry, it gives information about physical properties of raw material, intermediate products or final products (Tabilo-Munizaga & Barbosa-Cánovas, 2005).

Rheological characterization is also used to understand the properties of emulsions. It can be used to understand the viscosity and elasticity, possibility to relax, stability in time and resistance to applied forces in deformations of emulsions (Derkach, 2009). Bäumlér et. al. (2012) prepared a pectin-wax based emulsion and detected its physicochemical characteristics to form an edible film from it. They measured the

shear stress and shear rate relationship to verify applicability of the rheological model. They evaluated a number of rheological models and recorded that, Herschel-Bulkley model was more appropriate model to explain their emulsions behavior.

Linares-Garcia et. al. (2015) studied about the viscoelastic properties and textural characterization of high methoxyl pectin (HMP) which was extracted from hawthorn. They used the rheological characterization technique to understand the elasticity and viscosity of HMP, HMPC (high methoxyl pectin in citrus 1%, at pH 3.0) and HMPH (high methoxyl pectin in hawthorn). In the light of this information, they compared the gelation behavior of these samples. According to their reports, HMPH exhibited a greater elasticity than HMPC, so it could form a better gel.

2.4.4 Low Resolution NMR Relaxometry

NMR uses radio frequency (RF) pulse to form a temporary intrusion on a sample placed into another static magnetic field. In NMR relaxometry measurement, the signal comes from whole sample. Changing proton environment can distinguish the signal coming from partition (Kirtil & Oztop, 2016).

NMR has been used extensively for characterizing the dynamic changes in food systems. It is a non-invasive method. Also, both physical and chemical properties of many foods can be obtained in a short span of time. Its basic working principle is the magnetization of spins of nucleons. It works with the odd number of nucleons. Protons coming from hydrogen atoms is usually preferred for this purpose.

There are a lot of studies that used NMR relaxometry to characterize food samples in the literature. Bryan et. al. (2002) estimated the viscosity of crude oils and crude oil emulsions by using low field NMR. In 2005, they repeated the study of estimation the viscosity of oils by adding rheological measurements. Johns et al. (2008) studied about the emulsions and their characterization with NMR. They measured the droplet size of an emulsion and quantify the molecular self-diffusion by using pulsed field gradient (PFG) technique. They reported that this technique could be used for measuring the size of the droplets in water-in-crude oil emulsions. Williams et. al. (2011) studied about the characterization of water distribution in Xanthan-Curdlan hydrogel complex using NMR.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

In this study, powdered pectin (Yantai Andre Pectin Co Ltd., China), sunflower oil wax (Koster Keunen Holland, B.V., Melting Point: 74-77, Acid Value<5) and sunflower oil (Yudum, Savola Foods, İstanbul, Turkey) were used as the main materials. Sodium azide ($\geq 99.5\%$, Sigma-Aldrich) was added to all emulsions at 0.01% to prevent microbial growth. All emulsions were prepared using distilled water.

3.2 Emulsion Preparation

Pectin solution and wax dispersion were prepared separately at a concentration of 5% (w/w). They were stirred separately at 300 rpm for 30 min at 80 °C. The temperatures were monitored using a thermometer. After the wax melted completely, pectin solution and wax-water melt were mixed with a high shear homogenizator (IKA, T18 Digital Ultra-Turrax) at 25,000 rpm for 5 min. Sodium azide was added to the emulsions to prevent microbial growth (0.1%, w/w). The emulsion was cooled to room temperature after homogenization (~ 25 °C). In addition pectin-wax emulsions, emulsions with another oil phase were also prepared. Sunflower oil was added at 10% w/w ratio (S4, S5 and S6 samples). The compositions of samples are given in Table 3.1 and 3.2 respectively. Also, the schematic diagram of emulsion preparation is shown in Figure 3.1.

Table 3. 1 The composition of pectin, wax emulsions (w/w)

SAMPLE	PECTIN (%)	WAX (%)
S1	5	5
S2	7	5
S3	10	5

Table 3. 2 The composition of pectin, wax, sunflower oil emulsions (w/w)

SAMPLE	PECTIN (%)	WAX (%)	SUNFLOWER OIL (%)
S4	5	5	10
S5	7	5	10
S6	10	5	10

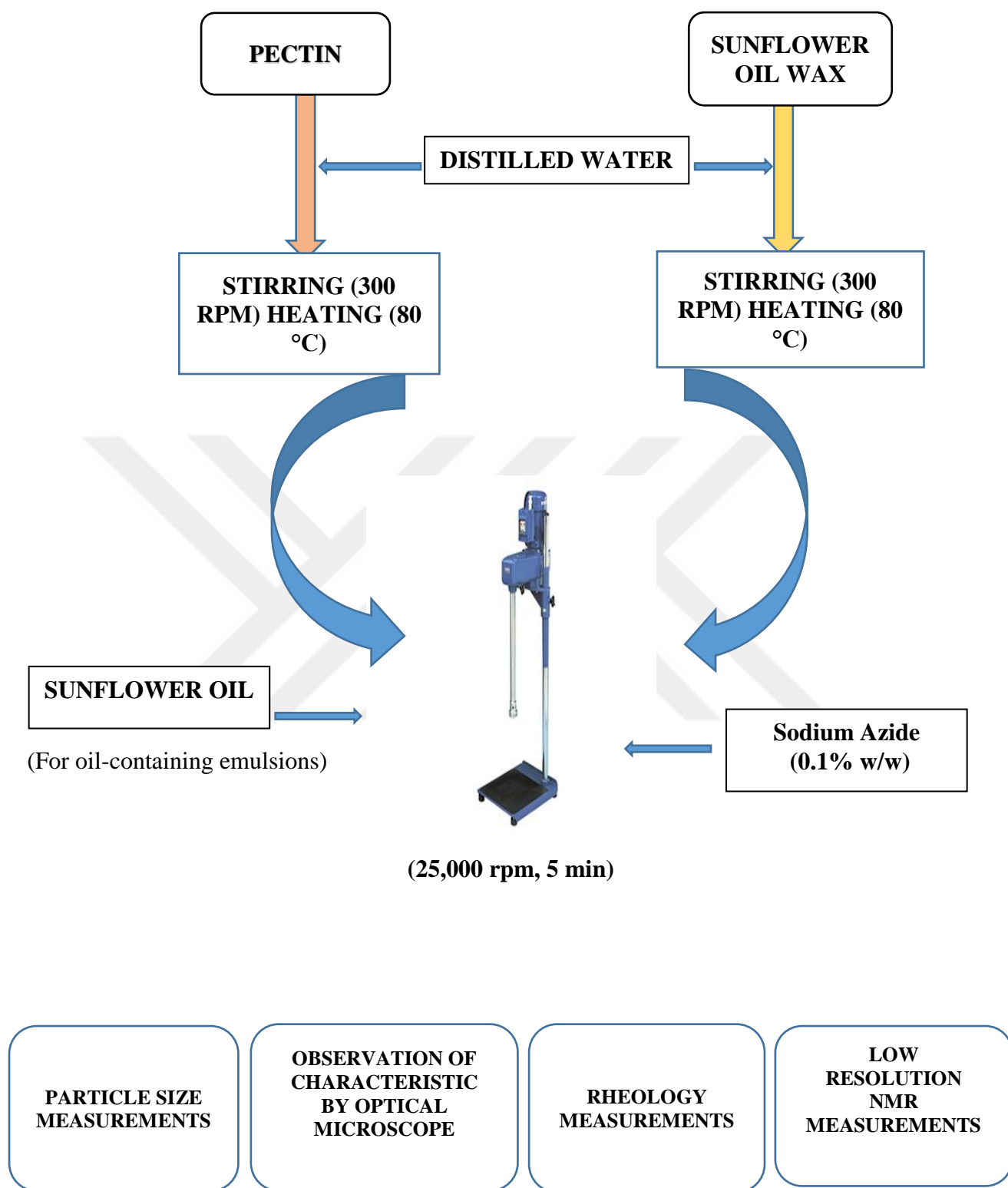


Figure 3. 1 Pectin and sunflower oil wax emulsion preparation by Ultraturrax and its characterization

3.3 Characterization of Sunflower Oil Wax

3.3.1 DSC Measurement

Differential scanning calorimetry (DSC) measures the heat flow into or from a sample as it is heated, cooled or held under isothermal conditions. The thermogram of the sunflower oil wax was plotted by using a DSC 4000 (PerkinElmer, N5370212, Waltham, USA). Approximately 10 mg of sunflower oil wax was placed on an aluminum DSC pan and sealed hermetically. The reference pan and the SFW pan were put in DSC chamber. The sample was held at 20 °C for 30 min. and then, it was heated to 120 °C at 5 °C/min. Using onset temperature (T_{on}), peak temperature (T_p) and enthalpy (ΔH) values, the melting profile of sunflower oil wax was evaluated and it was used to characterize the melting point of the wax. The DSC Thermogram of sunflower oil was shown in the Figure 3.2. SFW was also characterized by DSC and the results were found as: $T_{on} = 71.0 \text{ °C} \pm 0.6 \text{ °C}$, $T_p = 75.5 \text{ °C} \pm 0.4 \text{ °C}$ and $\Delta H = 19.402 \text{ J/g}$. Considering these findings, all samples were mixed at 80 °C to prevent crystallization of wax (Baümler et al., 2013).

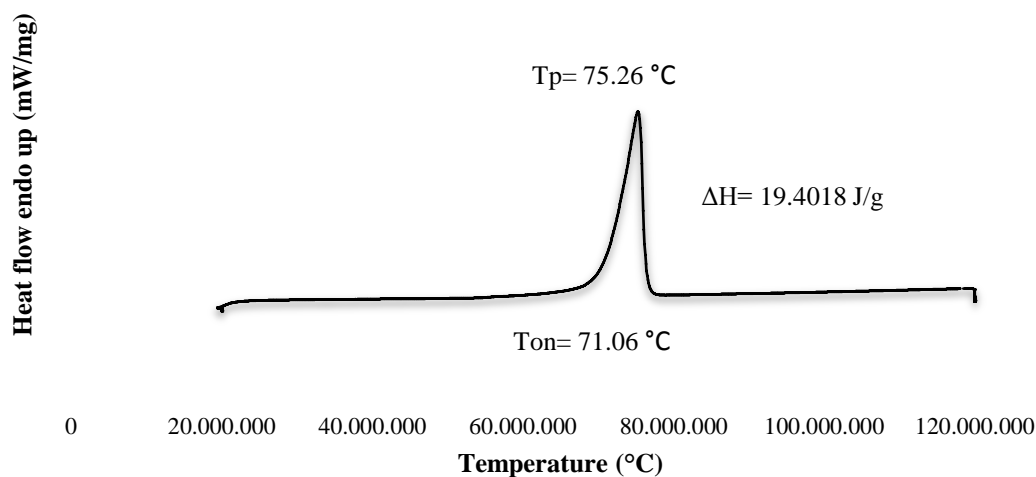


Figure 3. 2 The DSC thermogram of the sunflower oil wax

3.3.2 Water Activity Measurement

Water activity (a_w) is the partial vapor pressure of water in a substance divided by the standard state partial vapor pressure of water. The water within a product is measured both to control microbial spoilage, physical and chemical stability. 10 mg of the sunflower oil wax was placed in a water activity meter (AquaLab, Dew Point Water Activity Meter 4TE, Pullman, USA). The water activity of the sunflower oil wax was measured as 0.23.

3.3.3 The Composition and Physico-chemical Properties of Sunflower Oil Wax

The composition and the physico-chemical properties of sunflower oil wax was conducted. The free fatty acid ratio was calculated by using titration method (Trout, Estes, Friedberg, 1960); peroxide value was determined by using the official method of analysis for fats and oils (Bernal et al., 2005), color of the wax was determined by using a spectrophotometric method based on the Lovibond scale (Lowry & Tinsley, 1976), the melting point of SFW was found by using slip point test. Solid fat content which was measured by NMR (Oxford) at 20 °C, 30 °C, 35 °C, 40 °C, and 45 °C (Guthausen et al., 2004) and the composition of free fatty acids in total methyl esters ratio which was measured by a GC (Berrios, Siles, Martin, 2007). Results are given in Table 3.3.

Table 3. 3 The composition of sunflower oil wax

SUNFLOWER OIL WAX			
Parameters of Analysis	Method	Spects	Results
FFA (%)	Titration	0.4	0.07
Peroxide (meq/g)	Titration	10	7.05
Color	Lovibond 5 ^{1/4}	" Red	Red 2.2
	Colorimeter	Yellow	Yellow 20
Solid Fat Content (SFC)	NMR (OXFORD)	At 20 °C	82.3
		At 30 °C	68.7
		At 35 °C	57.2
		At 40 °C	51.5
Composition of Fatty Acids (In Total Methyl Esters)	Gas Chromotography	At 45 °C	39.8
		% w/w	
		C14:0	1.4
C16:0	9.8		
C16:1	1.2		
C18:0	4.9		
C18:1	18.6		
C18:2	44.0		
C18:3	1.3		
C20:0	4.3		
C20:1	1.0		
C21:0	0.2		
C22:0	9.7		
C22:1	0.7		
C24:0	0.1		
C26:0	0.9		
C27:0	0.2		
C28:0	1.0		
C29:0	0.3		
C30:0	0.4		

3.4 Characterization of Pectin and Sunflower Oil Wax Mixture

3.4.1 Particle Size Measurements

Particle size is a term to compare the dimensions of solid, liquid and gaseous particles. Mean particle size of pectin-wax emulsions were determined by using a light diffraction based particle size analyzer (Mastersizer 3000 Malvern, Worcestershire, UK) (Figure 3.3). It uses the technique of laser diffraction to measure mean particle size distributions from 10 nm to 3.5 mm. As the refractive index value 1.56 was used. Sample particles were assumed to be spherical and particle analyzer speed was adjusted to 2000 rpm during analyses. The volume-based D [4, 3] mean diameter was used to describe particle size.

$$\text{DeBroukere Mean: } D [4, 3] = \sum N_i D_{i4} / N_i D_{i3} \quad \text{Equation (3.1)}$$

Where D_i value is the geometric mean (the square root of upper*lower diameters) of diameters, N_i is the number of particles in emulsion with diameter D_i .



Figure 3. 3 The Malvern Mastersizer 3000 Equipment (Malvern, n.d.)

3.4.2 Optical Microscopy

The emulsions were also observed by using an inverted light microscope (PrimoVert, Zeiss, Jena, Germany). 40X-high numerical-aperture oil immersion objective lens was used. Images were observed using a microscopic camera (SONY CCD Color Digital Video C-Mount Microscope Camera, Tokyo, Japan). All sample's images were recorded using the Top View software.

3.4.3 Rheological Characterization

The rheological characterization of pectin-wax emulsions was investigated by using a dynamic rheometer (Kinexus Dynamic Rheometer, Malvern, UK). Cone-and-plate (40 mm diameter and 4° cone angle, 0.1425 mm gap) geometry was used to predict pectin and wax emulsions's flow behaviour.

The shear stress values were recorded with varying the shear rate values between 0.1 s⁻¹ to 95 s⁻¹, in a total ramp time of 2 min. Shear viscosity graphs of emulsions were plotted and the data were fit to Power Law, Newtonian models;

$$\text{Power Law Model: } \tau = K \dot{\gamma}^n \quad \text{Equation (3.2)}$$

$$\text{Newtonian Model: } \tau = \mu \frac{du}{dy} \quad \text{Equation (3.3)}$$

where τ is shear stress, K is the consistency index, $\dot{\gamma}$ is shear rate, μ is the shear viscosity of the fluid, and n is flow behaviour index.

3.4.4 NMR (Nuclear Magnetic Resonance) Relaxation Time Measurements

T_2 relaxation times of all emulsions were measured using an NMR system equipped with a permanent magnet of 0.5 T (Spin Core, Gainesville, FL, U.S.A), having a 10 mm diameter radio frequency (r.f.) coil. CPMG (Carr-Purcell-Meiboom-Gill) sequence was used to measure T_2 times. CPMG experiments was performed using a repetition delay of 3s, echo time (TE) of 2000 ms, 32 scans and number of echoes changing between 1500-3000 depending on the sample. T_2 relaxation times were calculated by using the formula;

$$S = M_0 (e^{-TE/T_2}) \quad \text{Equation (3.4)}$$

where S denotes signal, M_0 is the initial net magnetization.

NNLS (Non-Negative Least Square) Analysis

Since monoexponential fitting of the T_2 CPMG decay curve is not very exploratory especially for emulsion samples, a mathematical transformation is required to explore the multiexponential behavior. For that purpose, Prospa 3.1 (Magritek) software was used. NNLS utilizes an Inverse Laplace based routine to decompose an exponential decaying curve to its multiexponential components. The number of peaks of emulsions and their relative areas were analyzed by this method.

3.4.5 Emulsion stability

To assess the stability of emulsions, all samples were put in 10 mm sample tubes and they were centrifuged with a bench-top centrifuge (Hanil MF80, Jackjun -Dong, Korea, at 4000 rpm for 5 min.). The maximum power requirement of centrifuge is 4000 rpm (fixed angle rotor) the maximum centrifugal force is 2.469 rad/s. After centrifugation, samples were visually observed to check phase separation.

3.4.6 Statistical Analyses

The results were calculated by using the averages of at least three measurements. Mean values were reported. MINITAB (ver.16.2.0.0, Minitab Inc., United Kingdom) was used to conduct Analysis of Variance (ANOVA). Tukey test ($\alpha=0.05$) was used if significant difference was detected between the samples.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Particle Size

The laser diffraction method was used in this study and the DeBrouckere Mean Diameter (Volume Moment Mean or D [4,3] which was explained in Section 3.4.1 was reported. Mean particle size was measured for six different samples. Based on the preliminary experiments, sunflower oil wax concentration was kept constant at 5 w/w % in all samples. Three of them included 5, 7 and 10 w/w % pectin and the others include 5, 7 and 10 w/w % pectin with the addition of 10 w/w % sunflower oil. A high shear homogenizer was used to prepare emulsions and the particle sizes ranged between 1 and 3 μm . The mean particle sizes of all samples were given in Figure 4.1.

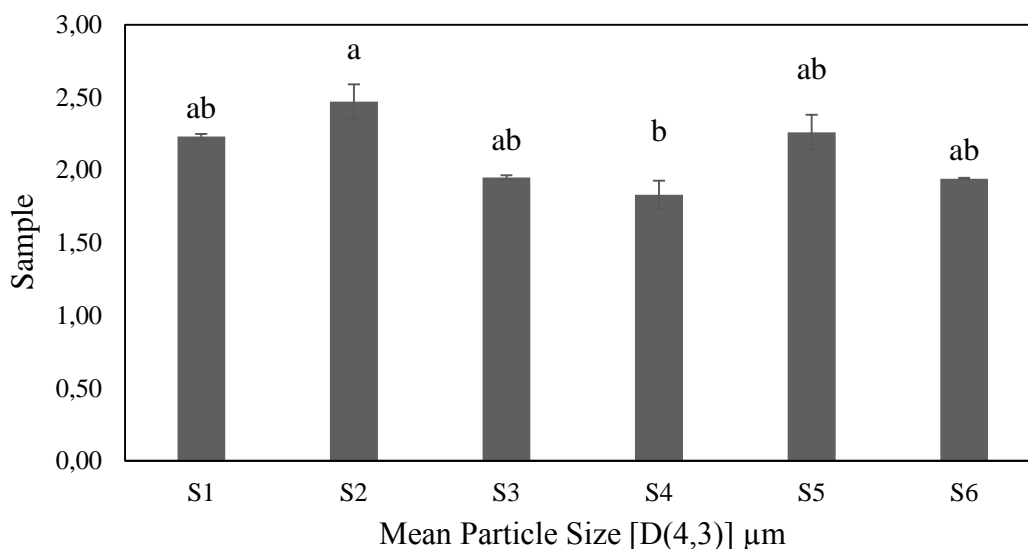


Figure 4. 1 The mean particle size measurement results of emulsions

Statistical analysis were done considering the effect of pectin concentration and sunflower oil. The results showed that, pectin concentration had a significant impact on particle size of the emulsions ($p < 0.05$) whereas addition of oil had no significant impact on the samples. Moreover, there is no synergistic effect between pectin concentration and oil addition to the emulsions ($p > 0.05$). It was shown that the sample S2 had the highest mean particle size value which was significantly different than S1 and S3 samples ($p < 0.05$) and S1 and S3 were behaving similar and S3 had the lowest mean particle size which was 1.9 μm . Erica et. al. (2012) found that increase in pectin concentration resulted in decrease on the mean particle size. Akhtar et. al. (2001) found that in the presence of protein moiety in the depolymerized pectin plays a major functional role in enhancing the emulsification properties of pectin following depolymerization. The hydrophobic proteinaceous component generates the surface activity of pectin emulsifier and acts as its strong point of anchor at the oil-water interface. It can be said that pectin started to form oil-water interface between oil and water molecules and it was competitive with wax particles for the S2 sample.

Pectin may had dominated the wax due to its high concentration in the S3 sample due to this property.

SAMPLE	PARTICLE SPAN MEAN
S1	2.305±0.05 ^a
S2	2.423±0.25 ^a
S3	1.416±0.005 ^{ba}
S4	2.283±0.8 ^a
S5	2.778±0,25 ^a
S6	1.434±0.015 ^b

Table 4. 1 The mean span measurement results of emulsions

Macroemulsions usually have a size range of 0.1–5 μm with an average of 1–2 μm and in this study, the emulsions could be categorized as macroemulsions, considering their droplet sizes. A representative mean particle size distribution plot of S3 is given in Figure 4.2. It can be seen as the emulsion could be considered to follow a monomodal behavior.

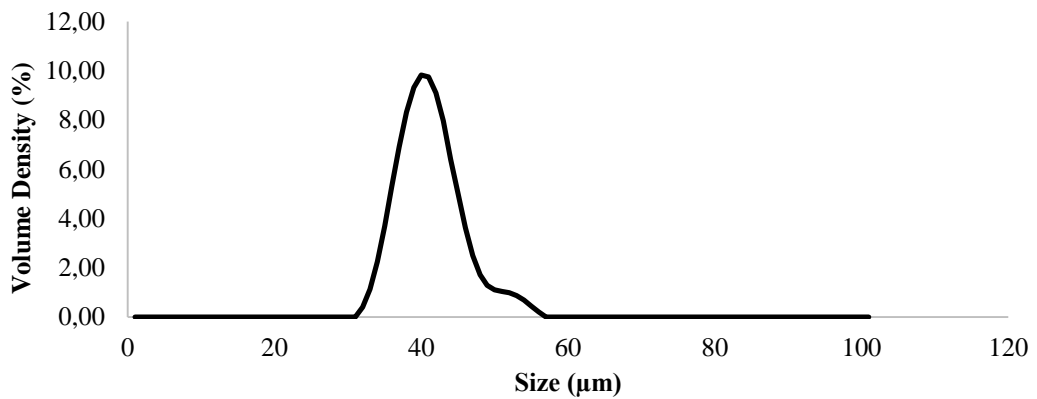


Figure 4. 2 A representative mean particle size graph of 10% pectin- 5% wax emulsion (S3).

4.2 Optical Microscope

The light microscope images are given in Figure 4.3. The light microscope images of all samples were obtained and recorded to confirm the information obtained from the results of particle size measurements. The images provided a qualitative comparison. Also, morphological properties were identified in the light of these results. The mean particle size of samples decreased, while the pectin concentration increased except S2 and S4. The images showed that the mean particle size of S4 was similar to S5 and S6, but S5 and S6 were different from each other ($p < 0.05$).

S4, S5, S6 contained 10 w/w % oil with wax and pectin. The results showed that adding oil did not affect the mean particle size. The particle size analysis and ANOVA results supported this optical microscop analysis.

The shape of droplets was assumed as spherical during particle size analysis. (Gantz, Bennett Clark, Derksen, & Small, 1990). The light microscope results confirmed this assumption.

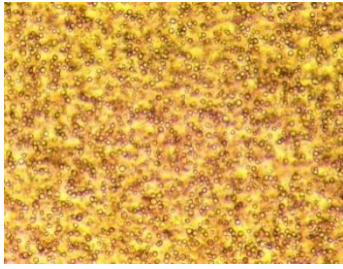
Agglomeration have occurred in some of the samples due to the heterogeneous dispersion of wax molecules in emulsions. Agglomeration could be visualized by eye (Guner & Oztop, 2017). Both microscopic images and particle size results confirmed the presence of agglomeration.



Figure 4. 3 A representative microscopic image of agglomeration for sample S5 and S6.

The light microscope images are shown in Pictures

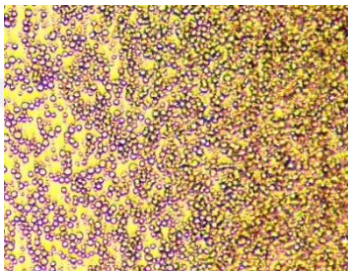
a)



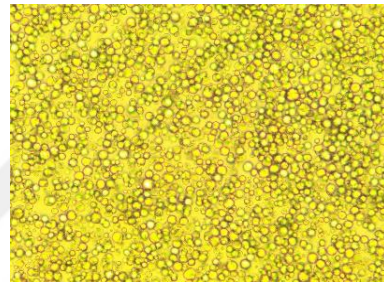
d)



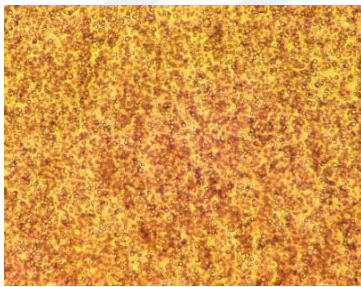
b)



e)



c)



f)



Figure 4. 4 Light microscope images of emulsions obtained at 40x magnification:

a) S1, b) S2, c) S3, d) S4, e) S5, f) S6

4.3 Rheological Characterization

The composition of an emulsion has a very significant effect on the rheological behavior. It affects both the stability and the interfacial interaction between droplets (Kim & Mason, 2017).

The main rheological behavior classification of fluids are; Newtonian and Non-newtonian. Viscosity of Newtonian fluids do not show shear rate dependency and it remains constant with increasing shear rate, but for Non-newtonian fluids, the fluid viscosity is affected and most of the time it decreases with increasing shear stress resulting in a shear-thinning behaviour (Subramanian, 2002). Concentrated emulsions generally have the properties of a Non-newtonian fluid (Pal, 1996).

In this study, six different samples that had different pectin concentration were measured. Emulsions were treated as Non-Newtonian fluids and the data were fit to the Power Law model as stated in Materials and Methods section. The k (consistency index) values and n (flow behavior) values are listed in Table 4.2.

Table 4. 2 The consistency index and flow behaviour index values of Power Law fittings of all emulsions

SAMPLE	k* (Pa.s ⁿ)	n**
S1	0.26 ± 0.11 ^d	0.96 ± 0.02 ^{ab}
S2	0.48 ± 0.07 ^d	0.96 ± 0.01 ^a
S3	9.41 ± 0.13 ^b	0.86±0.00 ^d
S4	1.11 ± 0.27 ^d	0.92±0.01 ^{bc}
S5	4.54 ± 0.45 ^c	0.90 ± 0.01 ^c
S6	20.97 ± 1.54 ^a	0.82±0.01 ^d

* k: consistency index

** n: flow behavior index

In Table A3. It is clearly shown that, pectin concentration and oil effect had significant impact on the n value of the emulsions separately (p<0.05). Moreover, the concentration of pectin and addition of oil had synergistic effect on the flow behavior of the samples (p<0.05).

When n values were considered, it was seen that S1 and S2 were closer each other and values were close to one, but S4 and S6 are significantly different from other samples ($p < 0.05$). When the studies on emulsions using pectin were examined in the literature it was seen that, these emulsions are mostly expected to display Non-Newtonian behavior (Chhabra, 2010). Particle size decreased with increasing pectin concentration of emulsions and enhanced the shear-thinning behavior of emulsions (Martini, 2013). S4 was similar to S5, whereas S3 and S6 are significantly different from S1 and S2 ($p < 0.05$). This statistical analysis showed that, pectin concentration effect started on S3, however pectin concentration and addition of oil synergistic effect was started to be observed with the sample S4. It can be seen, the shear-thinning behavior was more prominent with increasing pectin concentration. For 7% pectin-containing sample (S2) and 7% pectin and 10% oil containing sample (S5) it was obviously seen that the oil contained sample was more viscous and shear-thinning behavior was more prominent than the oil-free sample. The increased in viscosity and shear-thinning behavior of emulsions with decreased in particle size could also depend on the aggregation of particles which could be seen in the samples containing 10% pectin concentration (Figure 4.6). According to Pal et al, (2017) increase in viscosity and decrease in droplet size could be due to increase in the hydrodynamic interaction between droplets.

Flow behavior curves of S1, S2, S4 and S5 are shown in Figure 4.5. Also the flow behavior curves of S3 and S6 are shown in Figure 4.6.

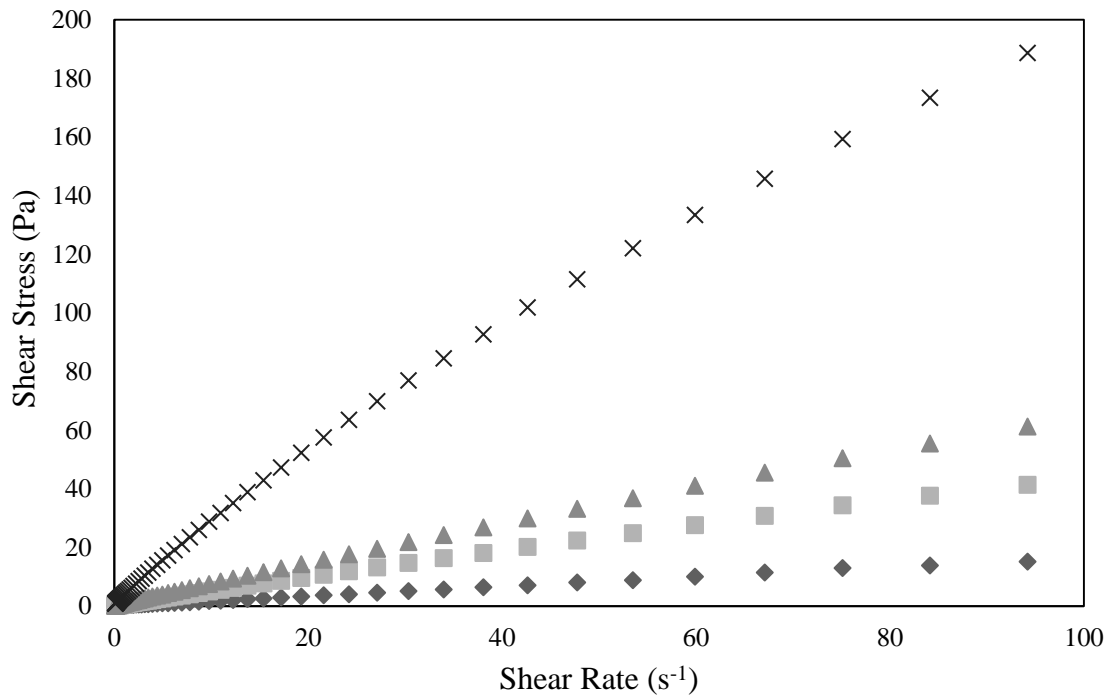


Figure 4. 5 The Power Law Model fittings: \diamond ; S1, \square ; S2, Δ ; S4, X; S5

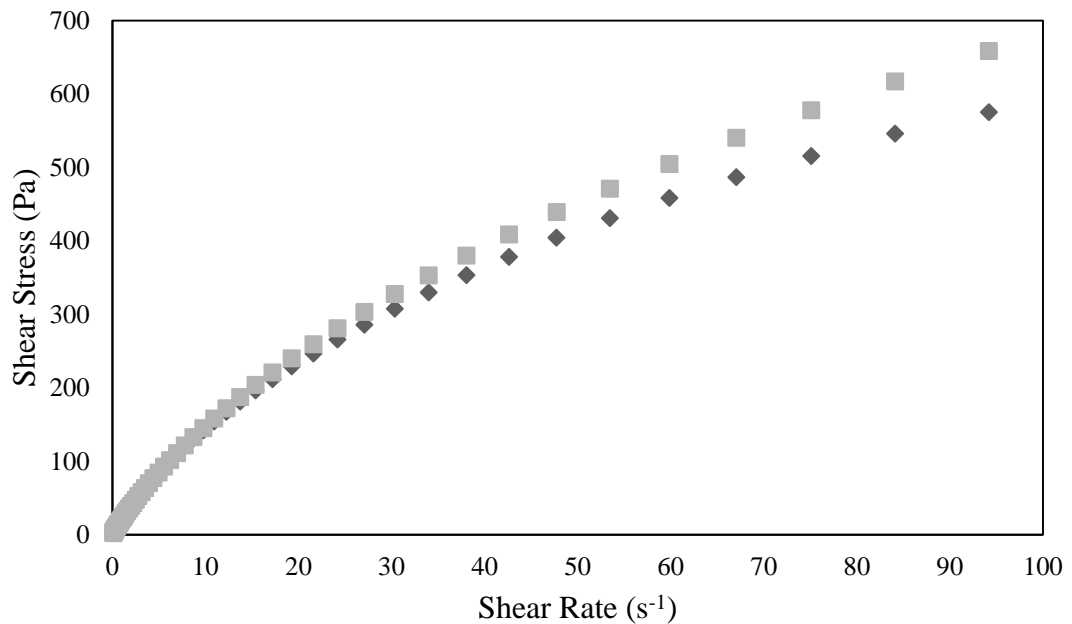


Figure 4. 6 The Power Law Model fittings: \diamond ; S3, \square ; S6

4.4 NMR (Nuclear Magnetic Resonance) Relaxometry

NMR is a non-destructive and quick method to quantify water and oil content, to explore the distribution of water and oil and other proton compartments in a variety of food samples. It relies on RF (Radio Frequency) pulses to acquire a signal. In Time domain NMR (TD-NMR) rather than chemical identification, spin-spin relaxation time (T_2) are measured and these relaxation times provides insight on the physicochemical changes that occur in a sample. Investigation of the microstructure of water-based systems is possible with Time Domain NMR experiments. The particle size measurements, evaluation of mobility states and quantification of the dispersed phase ratio could all be analyzed by using TD NMR (Kirtil & Oztop, 2016). The time domain NMR measures the mobility of H^1 atoms with an external magnetic field which is disturbed by a secondary momentary magnetic signal that is perpendicular to the primary field. Then the relaxation of the sample signal to its initial state is observed (Kirtil & Oztop, 2015) and the time constant T_2 could be calculated from the acquired signal by simple non-linear fitting procedures. .

T_2 is defined as the transverse or spin-spin relaxation time. CPMG (Carr-Purcell-Meiboom-Gill) is the most popular pulse sequence used for the measurement of spin-spin relaxation times (T_2). In this study, the spin-spin relaxation times (T_2) of all samples were measured and recorded, after they were equilibrated to room temperature. A representative T_2 plot is given in Figure 4.6. Also, a representative relaxation spectrum obtained from the decay curve through NNLS analysis is shown in Figure 4.7.

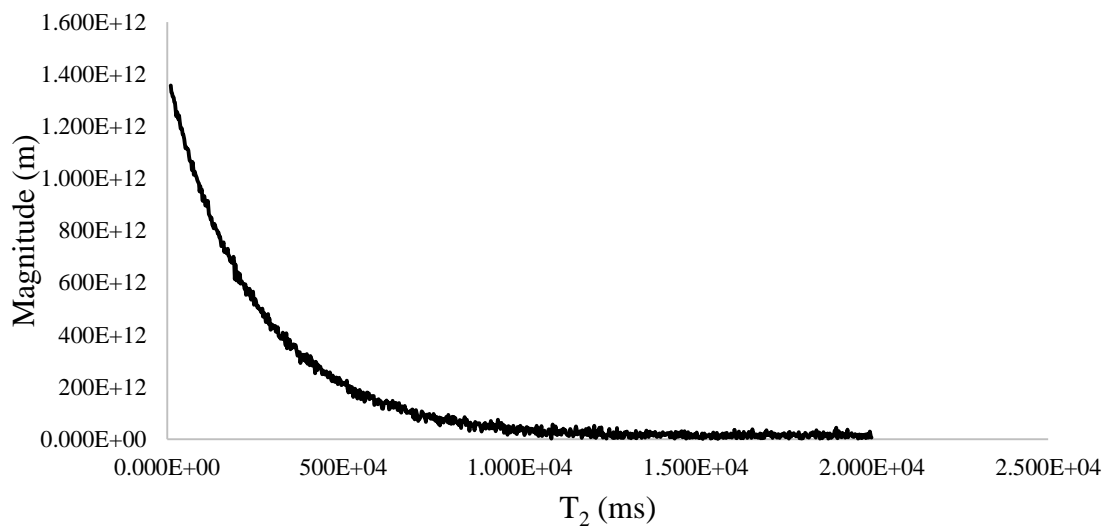


Figure 4. 7 The magnification and T_2 (relaxation time) plot of pectin-wax mixture with NMR relaxometry

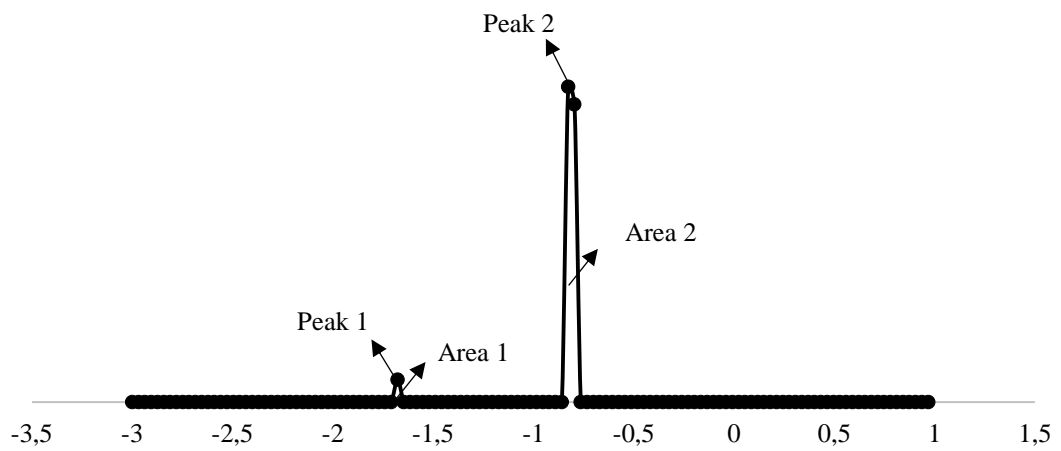


Figure 4. 8 Representative NMR relaxation spectrum of pectin-wax emulsion.

According to ANOVA results, pectin concentration and oil addition had significant effect on the samples separately ($p < 0.05$). In addition to this, there was an interaction between pectin concentration and oil effect on the emulsions ($p < 0.05$).

The measurements showed that there was an opposite effect between concentration of pectin and T_2 relaxation times. When the concentration of pectin increased, T_2 values decreased for both pectin solutions and emulsions. T_2 values of pectin solutions were respectively 355.88 ± 9.17 , 240.81 ± 2.66 , 141.14 ± 1.04 for 5, 7, 10% pectin concentration. Also, the T_2 values decreased with the addition of oil phase. T_2 value of pure wax was 36.95 ± 0.00 ms, pure SFO was 139.64 ± 3.31 ms. T_2 of S1 was measured as 312.48 ± 4.70 ms, but when sunflower oil was added to the system (S4) T_2 decreased to 268.06 ± 8.35 milliseconds with a decrease percentage of 14.

In emulsions containing sunflower oil, there was pectin-oil interaction present with the other interactions. T_2 values were shorter in S4, S5 and S6 samples when compared with S1, S2 and S3. The mobility of water is more than the oil and the T_2 value of oil is less than water (Weisenberger & Koenig, 1990). Also, T_2 values were shorter in S4, S5, S6 samples when compared with S11, S12, S13 that include wax (Table 4.3). Increasing pectin concentration, the percentages of the diminish in the intensity of T_2 values between SFO free and SFO added samples were 12 for 7% pectin containing samples and 3 at 10% pectin including samples respectively. In other words, with the increasing pectin concentration for the SFO added samples (S4, S5, and S6) the effect of oil showed a sharp decrease.

Oil not being well dispersed at low pectin concentration and oil having lower T_2 (Table 4.2) could be the reason of high decrease in T_2 at 5 and 7 % pectin concentration. On the other hand, pectin concentration of 10% resulted in lower decrease in T_2 showed that oil was well dispersed in the emulsions which was an indication that pectin was helping to disperse oil thus exhibiting surface active properties. Pectin may dominate the effect of oil with this property, because S3 and S6 samples were statistically same. Thus by looking at the T_2 relaxation times it was possible to have an idea about the emulsifying activity of a polysaccharide as also shown by other researchers (Kirtil et al, 2016).

Table 4. 3 The mean values and standard deviations of T₂ value of NMR relaxometry

SAMPLE	T ₂ (ms)
S1	312.48±4.70 ^b
S2	197.62±1.26 ^e
S3	115.24±1.87 ^h
S4	268.06±8.35 ^c
S5	174.64±8.49 ^f
S6	111.73±0.85 ^h
S8	347.77±5.59 ^a
S9	240.81±3.76 ^d
S10	141,14±1.47 ^g

Table 4. 4 The mean values and standard deviations of T₂ value of NMR relaxometry

SAMPLE	T2(ms)
S4	268.06±8.35 ^b
S5	174.64±8.49 ^d
S6	111.73±0.85 ^e
S11	380.23±11.66 ^a
S12	212.94±2.14 ^c
S13	133.41±0.63 ^e

When the relaxation spectrum was analyzed, it was observed that the relaxation behavior of emulsions could also be explained through a multiexponential relation. NMR spectra showed the presence of 3 peaks in all emulsions. In a relaxation spectrum the peaks do not specifically identify a component that is present in the sample but it refers to the protons associated with a certain compartment. The peak with shorter T₂ could attributed to the protons associated with wax whereas the longer T₂ component could be associated with water molecules strongly interacting with pectin. It was also interesting to note that addition of oil did not increase the number of peaks and it is probable that the relaxation of oil and the water that was associated with water was comparable resulting in overlap in the T₂s. Thus, they could not be identified clearly. To differentiate oil and water that was associated with pectin 2D NMR Relaxometry experiments such as T₂ experiment could be performed.

When pure wax and wax-oil mixtures compared with each other, it was observed that sunflower oil dominated the signal which came from wax. The T_2 value of pure wax was 36.95 ± 0.00 and the T_2 value of wax-oil was 142.11 ± 4.31 (Belton, Gil, Webb, & Rutledge, 2002). The results showed that the wax-oil relaxation time was very close to the pure sunflower oil value.



Table 4. 5 The mean values and standard deviations of peak values and relative areas of sample

Sample	Peak 1 (ms)	Area 1 (%)	Peak 2 (ms)	Area 2 (%)	Peak 3 (ms)	Area 3 (%)
S1	6.95±0.21 ^{ab}	7.66±1.07 ^a	18.50±0.71 ^c	4.14±0.16 ^b	315.00±7.07 ^a	88.20±0.93 ^a
S2	7.50±0.57 ^{ab}	9.96±1.07 ^a	21.00±0.00 ^{bc}	3.17±0.01 ^b	200.00±0.00 ^c	86.87±1.09 ^a
S3	6.50±0.42 ^{ab}	11.17±1.63 ^a	21.00±2.83 ^{bc}	3.03±0.44 ^b	120.00±0.00 ^d	85.81±2.08 ^a
S4	9.35±0.35 ^a	7.73±0.46 ^a	44.00±2.83 ^a	7.02±0.20 ^a	255.00±21.21 ^b	83.32±0.74 ^a
S5	8.50±0.28 ^a	7.67±0.21 ^a	28.00±0.00 ^b	4.66±0.50 ^{ab}	180.00±14.14 ^c	87.68±0.29 ^a
S6	4.60±1.84 ^b	10.53±2.91 ^a	14.50±2.12 ^c	6.80±1.42 ^a	110.00±0.00 ^d	81.98±3.35 ^a

CONCLUSION

In this study, the main purpose was to obtain stable pectin-wax-based emulsions which can be used in food industry as an edible film or a coating. Pectin and wax concentrations, presence of oil were the parameters that influence the stability and homogeneity of emulsions. For this research, 6 kinds of emulsions were prepared that they differed from their pectin concentration and oil content. Emulsions were characterized by measuring their particle sizes, rheological behaviour and T_2 relaxation times. The temperature was kept at 80 C°, before the emulsions formed considering the melting point of sunflower oil wax (75.26 C° that obtained from DSC measurement). Pectin concentrations tested were: 5, 7, and 10%. The pectin concentrations were determined considering wax concentration and pectin-wax ratio. A high shear disperser was used to homogenize the emulsions.

The mean particle sizes of emulsions decreased with increasing pectin concentration, but the mean particle size of 5% and 10% pectin contained samples were closer and 7% was significantly different ($p < 0.05$) from others. Oil addition did not affect the particle sizes of samples statistically. The optical microscopy images were parallel to the particle size results. It was determined that the rheological behavior was Non-Newtonian model for all samples. Also, the T_2 values decreased with increasing pectin concentration and when oil was added. Phase separation did not observed in any sample after centrifugation. This supported the other results.

Consequently, the overall results showed that a highly stable emulsion could be obtained from the high methoxyl pectin and sunflower oil wax. Also, pectin could act as a surfactant in emulsions between water and one or more oil phase.

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APPENDIX A
STATISTICAL ANALYSIS

1. PARTICLE SIZE ANOVA

General Linear Model: Particle Size versus Pectin Concentration; Oil Effect

Factor	Type	Levels	Values
Pectin Concentration	fixed	3	5; 7; 10
Oil Effect	fixed	2	0; 1

Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pectin Concentration	2	0,37464	0,41430	0,20715	7,13	0,017
Oil Effect	1	0,16875	0,13866	0,13866	4,77	0,060
Pectin Concentration*Oil Effect	2	0,08613	0,08613	0,04306	1,48	0,284
Error	8	0,23258	0,23258	0,02907		
Total	13	0,86209				

S = 0,170505 R-Sq = 73,02% R-Sq(adj) = 56,16%

Grouping Information Using Tukey Method and 95,0% Confidence

Pectin

Concentration	N	Mean	Grouping
7	4	2,4	A
5	6	2,0	B
10	4	1,9	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Oil

Effect	N	Mean	Grouping
0	8	2,2	A
1	6	2,0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pectin Concentration	Oil Effect	N	Mean	Grouping
7	0	2	2,5	A
7	1	2	2,3	A B
5	0	4	2,2	A B
10	0	2	1,9	A B
10	1	2	1,9	A B
5	1	2	1,8	B

Means that do not share a letter are significantly different.

General Linear Model: SPAN versus PECTIN CONC.; OIL

Factor	Type	Levels	Values
PECTIN CONC.	fixed	3	5; 7; 10
OIL	fixed	2	0; 1

Analysis of Variance for SPAN, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
PECTIN CONC.	2	4052512	4134994	2067497	26,14	0,000
OIL	1	239460	196604	196604	2,49	0,149
PECTIN CONC.*OIL	2	149708	149708	74854	0,95	0,424
Error	9	711910	711910	79101		
Total	14	5153590				

S = 281,249 R-Sq = 86,19% R-Sq(adj) = 78,51%

Unusual Observations for SPAN

Obs	SPAN	Fit	SE Fit	Residual	St Resid
5	2318,00	2816,00	198,87	-498,00	-2,50 R
6	3314,00	2816,00	198,87	498,00	2,50 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

PECTIN

CONC. N Mean Grouping

7 5 2685,6 A

5 6 2560,5 A

10 4 1424,7 B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

OIL N Mean Grouping

1 7 2342,4 A

0 8 2104,8 A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

PECTIN

CONC. OIL N Mean Grouping

5 1 2 2816,0 A

7 1 3 2777,7 A

7 0 2 2593,5 A

5 0 4 2305,0 A

10 1 2 1433,5 B

10 0 2 1416,0 B

Means that do not share a letter are significantly different.

2. RHEOLOGY ANOVA

General Linear Model: k value versus sample; oil affect

Factor	Type	Levels	Values
pectin conc.	fixed	3	5; 7; 10
oil affect	fixed	2	0; 1

Analysis of Variance for k value, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pectin conc.	2	525,32	509,00	254,50	664,34	0,000
oil affect	1	93,26	95,67	95,67	249,73	0,000
pectin*oil affect	2	60,78	60,78	30,39	79,32	0,000
Error	7	2,68	2,68	0,38		
Total	12	682,04				

S = 0,618942 R-Sq = 99,61% R-Sq(adj) = 99,33%

Unusual Observations for k value

Obs	k value	Fit	SE Fit	Residual	St Resid
12	22,0540	20,9675	0,4377	1,0865	2,48 R
13	19,8810	20,9675	0,4377	-1,0865	-2,48 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

pectin	N	Mean	Grouping
10	4	15,2	A
7	5	2,5	B
5	4	0,7	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

oil				
effect	N	Mean	Grouping	
1	6	8,9	A	
0	7	3,4	B	

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

oil				
pectin	effect	N	Mean	Grouping
10	1	2	21,0	A
10	0	2	9,4	B
7	1	2	4,5	C
5	1	2	1,1	D
7	0	3	0,5	D
5	0	2	0,3	D

Means that do not share a letter are significantly different.

General Linear Model: n value versus sample; oil affect

Factor	Type	Levels	Values
pectin	fixed	3	5; 7; 10
oil affect	fixed	2	0; 1

Analysis of Variance for n value, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pectin	2	0,0276278	0,0258901	0,0129450	117,27	0,000
oil affect	1	0,0064216	0,0060368	0,0060368	54,69	0,000
sample*oil affect	2	0,0006395	0,0006395	0,0003197	2,90	0,121
Error	7	0,0007727	0,0007727	0,0001104		
Total	12	0,0354616				

S = 0,0105063 R-Sq = 97,82% R-Sq(adj) = 96,26%

Unusual Observations for n value

Obs	n value	Fit	SE Fit	Residual	St Resid
1	0,977100	0,961350	0,007429	0,015750	2,12 R
2	0,945600	0,961350	0,007429	-0,015750	-2,12 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

pectin	N	Mean	Grouping
5	4	0,9	A
7	5	0,9	A
10	4	0,8	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

oil	ffect	N	Mean	Grouping
0	7	0,9	A	
1	6	0,9	B	

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

pectin	oil	ffect	N	Mean	Grouping
7	0	3	1,0	A	
5	0	2	1,0	A B	
5	1	2	0,9	B C	
7	1	2	0,9	C	
10	0	2	0,9	D	
10	1	2	0,8	D	

Means that do not share a letter are significantly different.

3. NMR RELAXOMETRY ANOVA

General Linear Model: T2 versus Pectin Conc.; Oil Effect

Factor	Type	Levels	Values
Pectin Conc.	fixed	3	5; 7; 10
Oil Effect	fixed	3	0; 1; 2

Analysis of Variance for T2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pectin Conc.	2	136654	135205	67602	1820,99	0,000
Oil Effect	2	12291	12291	6145	165,53	0,000
Pectin Conc.*Oil Effect	4	2071	2071	518	13,94	0,000
Error	15	557	557	37		
Total	23	151572				

S = 6,09295 R-Sq = 99,63% R-Sq(adj) = 99,44%

Unusual Observations for T2

Obs	T2	Fit	SE Fit	Residual	St Resid
5	256,260	268,063	3,518	-11,803	-2,37 R
11	162,780	174,640	3,518	-11,860	-2,38 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Pectin			
Conc.	N	Mean	Grouping
5	8	309,4	A
7	8	204,4	B
10	8	122,7	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Oil			
Effect	N	Mean	Grouping
2	6	243,2	A
0	9	208,4	B
1	9	184,8	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pectin Oil

Conc.	Effect	N	Mean	Grouping
5	2	2	347,8	A
5	0	3	312,5	B
5	1	3	268,1	C
7	2	2	240,8	D
7	0	3	197,6	E
7	1	3	174,6	F
10	2	2	141,1	G
10	0	3	115,2	H
10	1	3	111,7	H

Means that do not share a letter are significantly different.

General Linear Model: T2 versus pectin; wax effect

Factor	Type	Levels	Values
pectin	fixed	3	5; 7; 10
wax effect	fixed	2	0; 1

Analysis of Variance for T2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pectin	2	94823	100040	50020	633,01	0,000
wax effect	1	11943	11943	11943	151,14	0,000
pectin*wax effect	2	5514	5514	2757	34,89	0,000
Error	9	711	711	79		
Total	14	112992				

S = 8,88933 R-Sq = 99,37% R-Sq(adj) = 99,02%

Grouping Information Using Tukey Method and 95,0% Confidence

pectin	N	Mean	Grouping
5	5	324,1	A
7	5	193,8	B
10	5	122,9	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

wax

effect	N	Mean	Grouping
0	6	242,4	A
1	9	184,8	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

wax

pectin effect	N	Mean	Grouping
5 0	2	380,2	A
5 1	3	268,1	B
7 0	2	212,9	C
7 1	3	174,6	D
10 0	2	134,1	E
10 1	3	111,7	E

Means that do not share a letter are significantly different.

General Linear Model: peak 1 versus pectin conc.; oil affect

Factor	Type	Levels	Values
pectin conc.	fixed	3	5; 7; 10
oil affect	fixed	2	0; 1

Analysis of Variance for peak 1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pectin conc.	2	17,0467	17,0467	8,5233	12,38	0,007
oil affect	1	0,7500	0,7500	0,7500	1,09	0,337
pectin conc.*oil affect	2	9,6200	9,6200	4,8100	6,99	0,027
Error	6	4,1300	4,1300	0,6883		
Total	11	31,5467				

S = 0,829659 R-Sq = 86,91% R-Sq(adj) = 76,00%

Unusual Observations for peak 1

Obs	peak 1	Fit	SE Fit	Residual	St Resid
11	3,30000	4,60000	0,58666	-1,30000	-2,22 R
12	5,90000	4,60000	0,58666	1,30000	2,22 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

pectin

conc.	N	Mean	Grouping
5	4	8,2	A
7	4	8,0	A
10	4	5,6	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

oil

affect	N	Mean	Grouping
1	6	7,5	A
0	6	7,0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

pectin oil

conc.	affect	N	Mean	Grouping
5	1	2	9,3	A
7	1	2	8,5	A
7	0	2	7,5	A B
5	0	2	7,0	A B
10	0	2	6,5	A B
10	1	2	4,6	B

Means that do not share a letter are significantly different.

General Linear Model: area 1 versus pectin conc.; oil affect

Factor	Type	Levels	Values
pectin conc.	fixed	3	5; 7; 10
oil affect	fixed	2	0; 1

Analysis of Variance for area 1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pectin conc.	2	20,460	20,460	10,230	4,47	0,065
oil affect	1	2,727	2,727	2,727	1,19	0,317
pectin conc.*oil affect	2	2,925	2,925	1,463	0,64	0,560
Error	6	13,722	13,722	2,287		
Total	11	39,834				

S = 1,51228 R-Sq = 65,55% R-Sq(adj) = 36,85%

Grouping Information Using Tukey Method and 95,0% Confidence

pectin conc.	N	Mean	Grouping
10	4	10,8	A
7	4	8,8	A
5	4	7,7	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

oil affect	N	Mean	Grouping
0	6	9,6	A
1	6	8,6	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

pectin oil		conc.	affect	N	Mean	Grouping
10	0	2	11,2	A		
10	1	2	10,5	A		
7	0	2	10,0	A		
5	1	2	7,7	A		
7	1	2	7,7	A		
5	0	2	7,7	A		

Means that do not share a letter are significantly different.

General Linear Model: peak 2 versus pectin conc.; oil affect

Factor	Type	Levels	Values
pectin conc.	fixed	3	5; 7; 10
oil affect	fixed	2	0; 1

Analysis of Variance for peak 2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pectin conc.	2	364,50	364,50	182,25	52,07	0,000
oil affect	1	225,33	225,33	225,33	64,38	0,000
pectin conc.*oil affect	2	516,17	516,17	258,08	73,74	0,000
Error	6	21,00	21,00	3,50		
Total	11	1127,00				

S = 1,87083 R-Sq = 98,14% R-Sq(adj) = 96,58%

Grouping Information Using Tukey Method and 95,0% Confidence

pectin		conc.	N	Mean	Grouping
5	4	31,2	A		
7	4	24,5	B		
10	4	17,8	C		

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

oil
 affect N Mean Grouping
 1 6 28,8 A
 0 6 20,2 B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

pectin oil
 conc. affect N Mean Grouping
 5 1 2 44,0 A
 7 1 2 28,0 B
 10 0 2 21,0 B C
 7 0 2 21,0 B C
 5 0 2 18,5 C
 10 1 2 14,5 C

Means that do not share a letter are significantly different.

General Linear Model: area 2 versus pectin conc.; oil affect

Factor Type Levels Values
 pectin conc. fixed 3 5; 7; 10
 oil affect fixed 2 0; 1

Analysis of Variance for area 2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pectin conc.	2	5,6348	5,6348	2,8174	6,69	0,030
oil affect	1	22,0323	22,0323	22,0323	52,29	0,000
pectin conc.*oil affect	2	2,6425	2,6425	1,3213	3,14	0,117
Error	6	2,5279	2,5279	0,4213		
Total	11	32,8376				

S = 0,649089 R-Sq = 92,30% R-Sq(adj) = 85,89%

Unusual Observations for area 2

Obs	area 2	Fit	SE Fit	Residual	St Resid
11	7,80000	6,79500	0,45898	1,00500	2,19 R
12	5,79000	6,79500	0,45898	-1,00500	-2,19 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

pectin

conc.	N	Mean	Grouping
5	4	5,6	A
10	4	4,9	A B
7	4	3,9	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

oil

affect	N	Mean	Grouping
1	6	6,2	A
0	6	3,4	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

pectin oil

conc.	affect	N	Mean	Grouping
5	1	2	7,0	A
10	1	2	6,8	A
7	1	2	4,7	A B
5	0	2	4,1	B
7	0	2	3,2	B
10	0	2	3,0	B

Means that do not share a letter are significantly different.

General Linear Model: peak 3 versus pectin conc.; oil affect

Factor	Type	Levels	Values
pectin conc.	fixed	3	5; 7; 10
oil affect	fixed	2	0; 1

Analysis of Variance for peak 3, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pectin conc.	2	58067	58067	29033	248,86	0,000
oil affect	1	2700	2700	2700	23,14	0,003
pectin conc.*oil affect	2	1400	1400	700	6,00	0,037
Error	6	700	700	117		
Total	11	62867				

S = 10,8012 R-Sq = 98,89% R-Sq(adj) = 97,96%

Grouping Information Using Tukey Method and 95,0% Confidence

pectin conc.	N	Mean	Grouping
5	4	285,0	A
7	4	190,0	B
10	4	115,0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

oil affect	N	Mean	Grouping
0	6	211,7	A
1	6	181,7	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

pectin oil

conc.	affect	N	Mean	Grouping
5	0	2	315,0	A
5	1	2	255,0	B
7	0	2	200,0	C
7	1	2	180,0	C
10	0	2	120,0	D
10	1	2	110,0	D

Means that do not share a letter are significantly different.

General Linear Model: area 3 versus pectin conc.; oil affect

Factor	Type	Levels	Values
pectin conc.	fixed	3	5; 7; 10
oil affect	fixed	2	0; 1

Analysis of Variance for area 3, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pectin conc.	2	22,893	22,893	11,447	3,77	0,087
oil affect	1	20,830	20,830	20,830	6,85	0,040
pectin conc.*oil affect	2	18,302	18,302	9,151	3,01	0,124
Error	6	18,235	18,235	3,039		
Total	11	80,259				

$$S = 1,74331 \quad R\text{-Sq} = 77,28\% \quad R\text{-Sq}(\text{adj}) = 58,35\%$$

Grouping Information Using Tukey Method and 95,0% Confidence

pectin

conc.	N	Mean	Grouping
7	4	87,3	A
5	4	85,8	A
10	4	83,9	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

oil

affect N Mean Grouping

0 6 87,0 A

1 6 84,3 B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

pectin oil

conc. affect N Mean Grouping

5 0 2 88,2 A

7 1 2 87,7 A

7 0 2 86,9 A

10 0 2 85,8 A

5 1 2 83,3 A

10 1 2 82,0 A

Means that do not share a letter are significantly different.