Ayşe KORKUT

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ENCAPSULATION OF OMEGA-3 FATTY ACIDS INTO STARCH NANOPARTICLE STABILIZED PICKERING EMULSIONS

A THESIS
SUBMITTED TO THE DEPARTMENT OF
ADVANCED MATERIALS AND NANOTECHNOLOGY
AND THE GRADUATE SCHOOL OF ENGINEERING AND SCIENCE
OF ABDULLAH GUL UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER

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SCIENTIFIC ETHICS COMPLIANCE

I hereby declare that all information in this document has been obtained in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

Ayşe KORKUT

REGULATORY COMPLIANCE

M.Sc.thesis titled **"Encapsulation of Omega-3 Fatty Acids into Starch Nanoparticle Stabilized Pickering Emulsions**" has been prepared in accordance with the Thesis Writing Guidelines of the Abdullah Gül University, Graduate School of Engineering & Science.

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ACCEPTANCE AND APPROVAL

M.Sc. thesis titled "**Encapsulation of Omega-3 Fatty Acids into Starch Nanoparticle Stabilized Pickering Emulsions**" and prepared by Ayşe KORKUT has been accepted by jury in the Advanced Materials and Nanotechnology Graduate Program at Abdullah Gül University, Graduate School of Engineering & Science.

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Graduate School Dean

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ABSTRACT

ENCAPSULATION OF OMEGA-3 FATTY ACIDS INTO STARCH NANOPARTICLE STABILIZED PICKERING EMULSIONS

Ayşe KORKUT
M.Sc. in Advanced Materials and Nanotechnology
Advisor: Assoc. Prof. Dr. Kevser KAHRAMAN
January 2021

The main purpose of the thesis is to produce starch nanoparticles to be used as an emulsion stabilizer. In the first part of the thesis, starch nanoparticles were produced via acid hydrolysis and the starch nanoparticles were characterized in terms of morphological properties and size, crystallinity and structural properties. Pickering emulsions were prepared in two different oil fractions (Φ0.6 and Φ0.8) with different oils (sunflower and corn oil). To determine the starch nanoparticle which provides the best emulsion stability, emulsions were prepared with addition of 2% (mg starch/g emulsion) starch nanoparticles. Emulsions were stored for 30 days at room conditions and phase separation was visually examined. The most stable emulsion was prepared with corn oil at Φ0.6 oil fraction when the starch nanoparticle (%2) produced with a 1:3 starch:H_2SO_4 ratio and 3 days hydrolysis (1:3 (3)) was used as stabilizer. In the second part of the thesis, omega-3 fatty acids were encapsulated in Pickering emulsions. Flaxseed oil was selected as the omega-3 source. The emulsions were prepared using flaxseed oil at a Φ0.2 oil fraction with the addition of 3% starch nanoparticles (1: 3 (3)). The emulsions were stored for 15 days at 25±1°C. Changes in the emulsions during storage were examined in terms of physical stability, peroxide number, pH, particle size, and zeta potential. Pickering emulsions stabilized with starch nanoparticles to encapsulate omega-3 fatty acids made flaxseed oil more resistant to primary oxidation.

Keywords: starch nanoparticle, emulsion, omega-3, flaxseed oil, oxidation

ÖZET

OMEGA-3 YAĞ ASİTLERİNİN NİŞASTA NANOPARTİKÜLLERLE STABİLİZE EDİLMİŞ EMÜLSİYONLAR İÇİNE ENKAPSÜLE EDİLMESİ

Ayşe KORKUT
İleri Malzeme ve Nanoteknoloji Yüksek Lisans Programı
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Ocak 2021

Bu tez çalışmasının temel amacı emülsiyon stabilizatörü olarak kullanılacak nişasta nanopartiküllerini üretmektir. Tezin ilk bölümünde asit hidrolizi ile nişasta nanopartikülleri üretilmiş ve nişasta nanopartikülleri morfolojik özellikler ve boyut, kristalinite ve yapısal özellikler açısından karakterize edilmiştir. Pickering emülsiyonlar, iki farklı yağ fraksiyonunda (Φ0.6 ve Φ0.8) ve farklı yağlarla (ayçiçeği ve mısır yağı) hazırlanmıştır. En iyi emülsiyon stabilitesini sağlayan nişasta nanopartikülünü belirlemek için emülsiyonlar %2 nişasta nanopartikülü (mg nişasta/g emülsiyon) ilavesiyle hazırlanmıştır. Emülsiyonlar, oda koşullarında 30 gün süreyle depolanmış ve faz ayrımı olup olmadığı gözlenmiştir. En stabil emülsiyon, Φ0.6 yağ fraksiyonunda mısır yağı ile 1:3 nişasta:H_2SO_4 oranı (1:3 (3)) ile 3 gün hidrolize edilmiş nişasta nanopartikülü (%2) kullanılarak hazırlanmıştır. Tezin ikinci bölümünde, omega-3 yağ asitleri Pickering emülsiyonlar içine enkapsüle edilmiştir. Omega-3 kaynağı olarak keten tohumu yağı seçilmiştir. Emülsiyonlar, %3 oranında nişasta nanopartikülleri (1:3(3)) ilavesiyle Φ0.2 yağ fraksiyonunda hazırlanmıştır. Emülsiyonlar 25±1°C' de 15 gün depolanmış ve depolama sırasında emülsiyonlardaki değişiklikler fiziksel stabilite, peroksit sayısı, pH, partikül boyutu ve zeta potansiyeli açısından incelenmiştir. Omega-3 yağ asitlerinin enkapsüle edilmesi için kullanılan nişasta nanopartikülleri ile stabilize edilen Pickering emülsiyonlar, keten tohumu yağını birincil oksidasyona karşı daha dirençli hale getirmiştir.

Anahtar kelimeler: nişasta nanopartikül, emülsiyon, omega-3, keten tohumu yağı, oksidasyon

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List of Abbreviations

ζ	Zeta
ALA	Alpha-linoleic (α- linoleic) acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FT-IR	Fourier Transform Infrared Spectrophotometer
PUFA	Polyunsaturated fatty acids
RS	Resistant starch
SEM	Scanning Electron Microscope
SNC	Starch Nanocrystals
XRD	X-Ray Diffractometer

Chapter 1

1. Introduction

Emulsions are defined as “thermodynamically unstable heterogeneous systems in which two or more liquid phases, that are completely or partially immiscible, are dispersed in each other”. Emulsions are generally composed of oil and water phases and they can be examined in two groups according to the distribution of these two phases. The emulsions formed when the oil particles are dispersed in water phase are called “oil-in-water (o/w) emulsions”, and the emulsions in which water droplets are dispersed in the oil medium are called “water-in-oil (w/o) emulsions” [1]. Milk can be given as an example of an “oil-in-water emulsion”, whereas margarine is an “oil-in-water emulsion”. In milk, the fat phase forms small droplets within the water phase. Margarine contains droplets of water in a mixture of fat and vegetable oils. The schematic illustration of “oil-in-water (o/w)” and “water-in-oil (w/o)” emulsions are given in Figure 1.1.

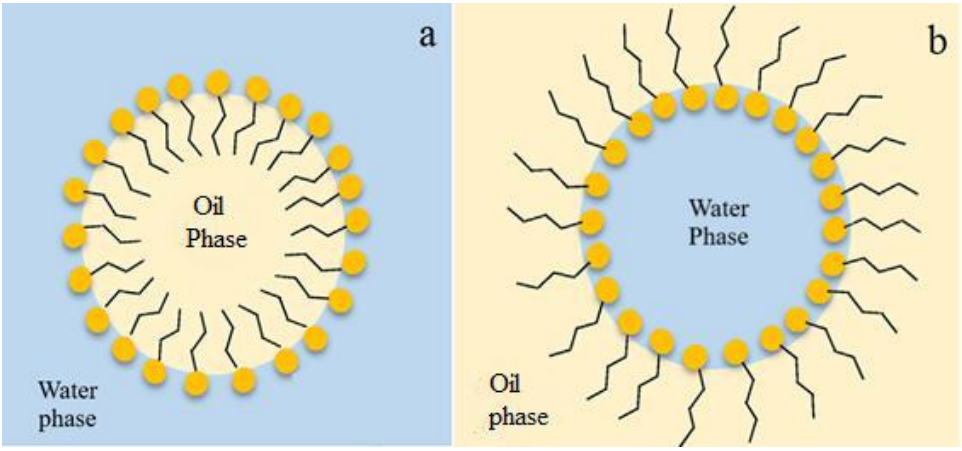

Figure 1.1 Schematic illustration of emulsions stabilized with surfactants; a) oil-in-water (o/w) emulsion, b) water-in-oil (w/o) emulsions. Reprinted from Mohamed et al. [2].

Emulsions are unstable thermodynamic systems as their components have affinity to separate and then decrease their interfacial energy. Emulsions stability can be achieved by using surfactants and emulsifiers. While surfactants stabilize emulsions by

reducing the interfacial tension between the continuous and dispersed phases, emulsifiers such as hydrocolloids and proteins provide stability by forming a steric interface film. The structure of a surfactant is composed of a polar (hydrophilic) head and non-polar (lipophilic) tail as seen in Figure 1.2.

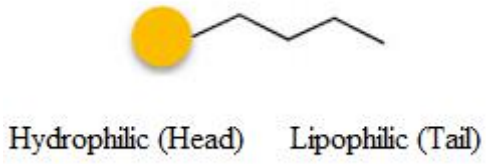

Figure 1.2 Schematic illustration of a surfactant; Reprinted from Mohamed et al. [2]

Many types of synthetic surfactants and emulsifiers are available commercially to obtain stable emulsions during shelf life. However, due to the prejudice against non-natural synthetic surfactants and emulsifiers, researches are carried out on the production of emulsifiers from natural sources. Nowadays, the interest on the natural emulsifiers to stabilize the emulsions increases gradually because of low risk on food products and consumer friendly-utilization [3], [4]. Natural emulsifiers consist of solid particles and provide better stability in emulsions in addition to its low toxicity in food grade [5]. Emulsions stabilized with such solid particles are defined as "Pickering emulsions" [6], [7]. Solid particles are used in emulsions as an intermediate hydrophobicity adsorbed intensely at the interface. These particles have a steric restriction against the aggregation of emulsion droplets. Pickering emulsions can be prepared as "oil-in-water (o/w)" and "water-in-oil (w/o)" emulsions. Figure 1.3 illustrates the shape of the droplets forming the Pickering emulsions. In Pickering emulsions, the size of solid particles used as stabilizing agents should be smaller than the size of emulsion droplets (sub-micron, ~100 nm). Some natural particles such as starch, chitin, cellulose and proteins nanoparticles can be used as Pickering emulsion stabilizer [7]–[9].

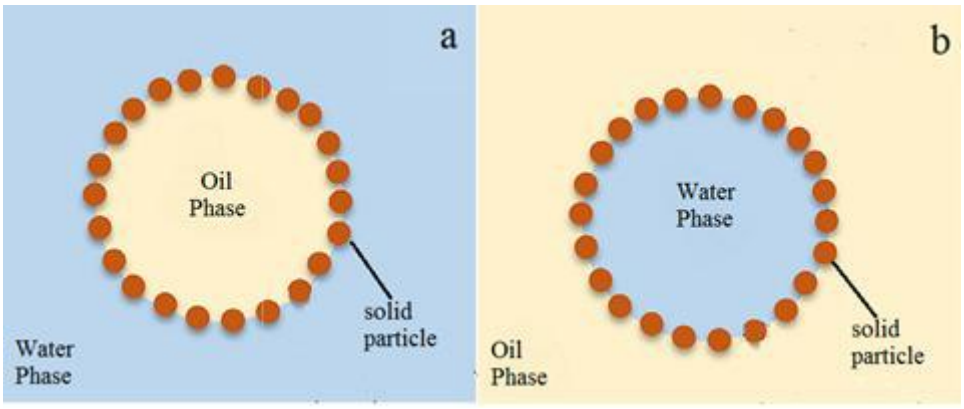

Figure 1.3 Schematic illustration of Pickering emulsions; a) o/w Pickering emulsion (stabilized with hydrophilic solid particles), b) w/o Pickering emulsions (stabilized with hydrophilic solid particles). Reprinted from Mohamed et al [2].

Depletion of fossil energy resources and increasing environmental concerns has also increased the demand for products produced from renewable and sustainable non-petroleum sources in many areas. This has led to the coming together of two areas such as nanotechnology, which enables the development of novel materials, and the production of biomaterials, which allows the use of environmentally friendly raw materials. Nanotechnology is a field that has been applied in many sectors such as information, security, medicine, cosmetics, energy, textile, environment and food and is used in research and development studies for industrial product improvement. In the food industry, nanotechnology is used in "packaging, food monitoring, controlled release of additives and bioactive compounds". Nanoparticles can also be used in foods to modify their functional properties [10]. Nanotechnology applications have been accelerated since nanoscale materials have many unique and advantageous properties. [7]–[9]. Starch, a renewable, biodegradable and non-toxic material that is widely found in nature, has attracted considerable attention in the production of nano materials in recent years. In addition to the fact that starch is an abundant and easily obtainable resource in nature, it has started to attract attention especially in the production of nano materials in recent years due to its positive effects such as biodegradability and no toxicity.

Starch is one of the biopolymers commonly found in nature and synthesized by plants to store the energy they obtain through photosynthesis. Starch is a polysaccharide formed by the polymerization of α-D-glucose units [11] (Figure 1.4) and consists of two types of glucose polymer "amylose, that is mostly linear" and "amylopectin, that is

highly branched". Most starches have 20-25% amylose; but there also are some exceptions. Some starches have higher amylose content than the regular ones and are called as "high amylose containing" starches. Some others have 100% amylopectin and called as "waxy starches" [11].

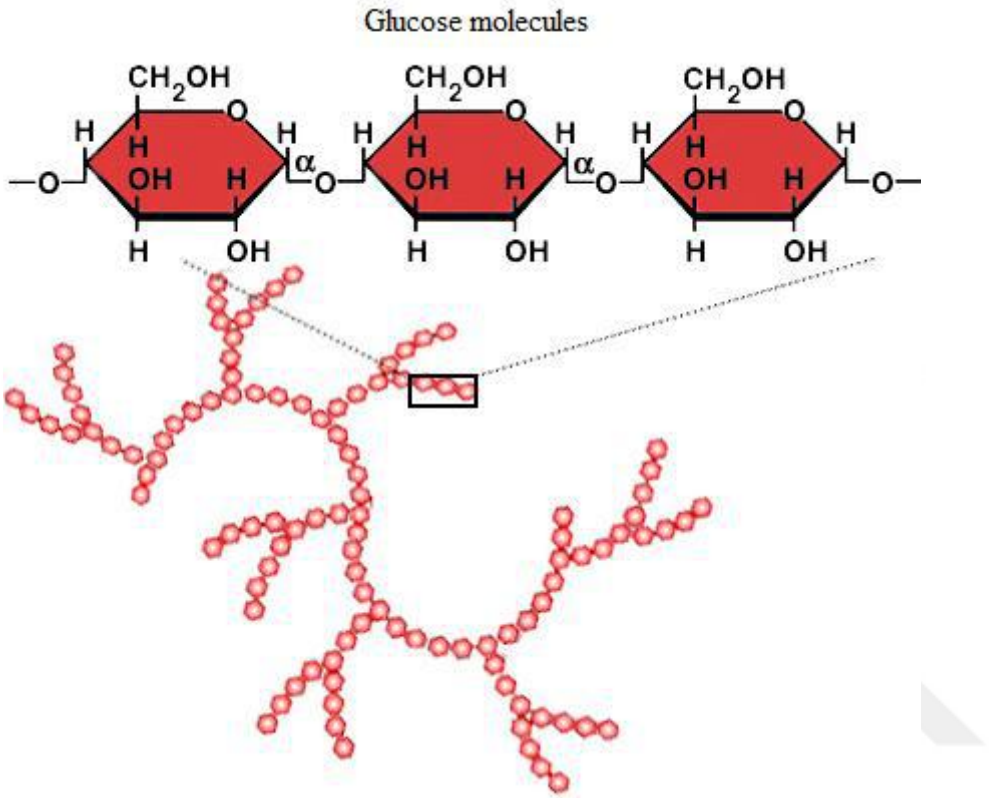

Figure 1.4 Structure of starch [12].

Chemical structures of amylose and amylopectin are shown in Figure 1.5. Amylose consists of long chains of α-D-glucopyranosyl residues linked between their their 1- and 4-positions (α 1→4 glycosidic links). Amylose chains are not completely linear; they also contain a small amount of branching. Amylopectin is larger than amylose and as in amylose the glucose molecules linked each other with α 1→4 glycosidic links. But there are also some α 1→6 glycosidic linkages between glucose molecules, which makes the molecule branched.

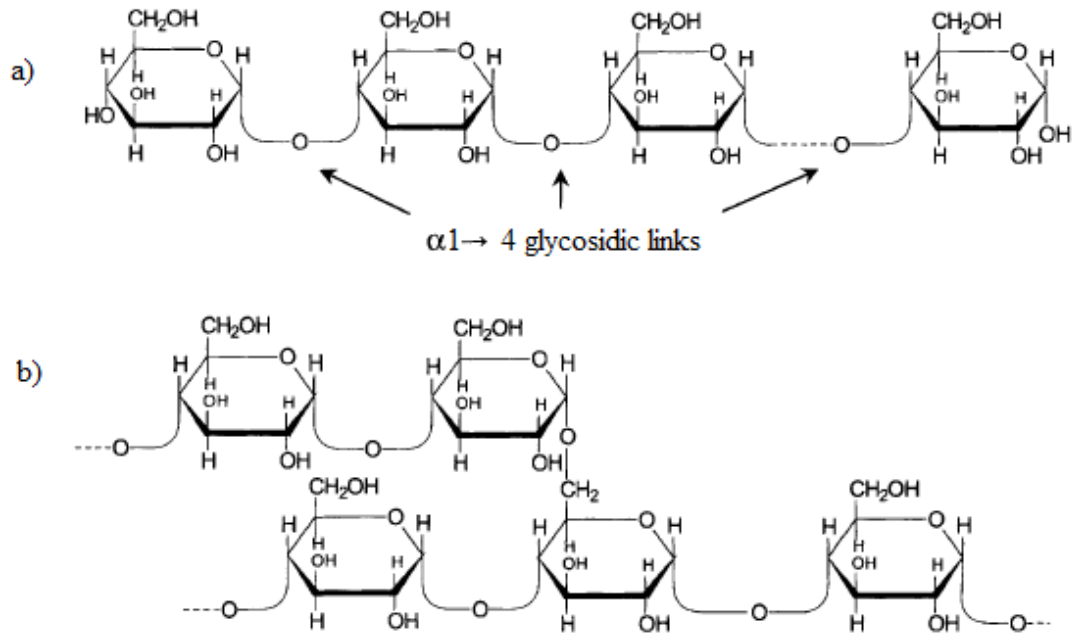

Figure 1.5 Chemical structure of a) amylose and b) amylopectin [13].

A starch granule (2-100 µm) has a multi-scale structure (Figure 1.6 [14]). The granule includes growth rings that are 120-500 nm in size. These growth rings are composed of blocklets that have size as 20-50 nm. The blocklets consist of 9 nm in size amorphous and crystalline lamellae. Last, these lamellae contain 0.1-1 nm amylopectin and amylose chains [14].

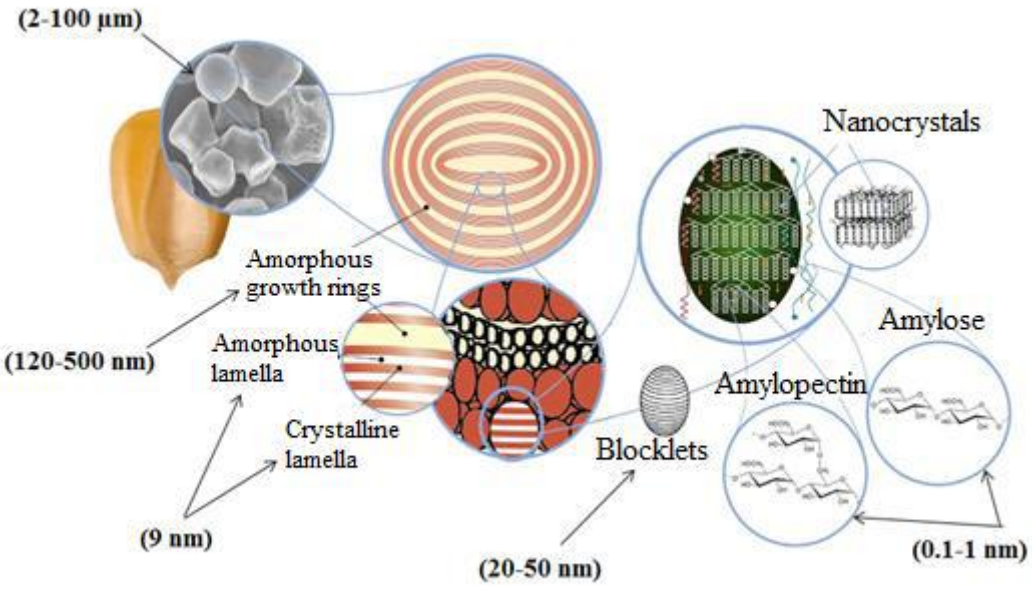

Figure 1.6 Detailed structure of a starch granule. Reprinted from Le Corre and Angellier [14].

There are several hydroxyl groups in the starch nanoparticle structure. Although, the crystalline amylopectin, which is the branched polymer, is resistant to acid hydrolysis; amylose, which is the linear polymer of starch is susceptible to hydrolysis. Therefore, while starch nanoparticles show hydrophilic properties due to the hydroxyl groups, they also show hydrophobic properties due to their crystalline regions. Starch nanoparticles/nanocrystals is formed due to the disruptive effects of acid hydrolysis on the semi-crystalline structure of starch molecule [14]. Emulsifiers must have both hydrophobic and hydrophilic regions. In other words, the most important feature of emulsifiers is that they are wettable in both phases forming the emulsion. Due to its hydrophobic and hydrophilic feature, starch nanoparticles have a potential to be used as a Pickering emulsion stabilizer.

The demand for functional foods having high nutritional value, low artificial ingredients and good organoleptic features is increasing. This has led to an increase in research on encapsulation in the food industry as encapsulation can be used to improve the stability of sensitive compounds [15]. Encapsulation is the one of the techniques, which prevents the substances from the effects of ambient conditions by coating them with a substance called as wall material and is used in food industry to overcome food instability and degradation during shelf life.

Among the functional foods products, the ones containing omega-3 fatty acids have gained importance. Omega-3 fatty acids are defined as essential fatty acids consisting 50-60% of α-linolenic which gets transformed into long chain "polyunsaturated fatty acids (PUFAs)" such as "eicosapentaenoic acid (EPA)" and "docosahexaenoic acid (DHA)" [16], [17]. These PUFAs have positive effects on the human health such as improving the brain and nervous system, lowering the hypertension and cholesterol [16]–[18]. Also PUFAs are effective materials on the reducing the risk of some cancers (colon, prostate and breast) [19]. The intake of the PUFAs is also important for the eye health and injury reparation. They control diabetes [20], [21] and helps developing strong memory [16].

In nature, omega-3 fatty acids are found in some food materials such as fish, walnut oil, algal oil, flaxseed oil and dark plants. Among these omega-3 fatty acid sources, flaxseed is one of the inexpensive and plentiful sources that omega-3 fatty acids can be derived from. However, flaxseed oil is highly susceptible to the lipid oxidation during shelf life and production because of its high PUFAs composition. As stated above, encapsulation protects the substances from ambient conditions and used in

many areas as well as food industry. In the light of these acknowledges we hypothesed that encapsulation of flaxseed oil derived omega-3 fatty acids using Pickering emulsions, may protect the flaxseed oil against lipid oxidation.

There are various studies in the literature in which starch nanoparticles were used as Pickering emulsion stabilizers [7], [22], [23]. Ge et al. [7] produced starch nanoparticles using sulfuric acid from waxy corn starch containing 100% amylopectin and examined their use as emulsion stabilizers. Gong et al. [22] emphasized that starch nanoparticles obtained from hydrolysis of corn starch using sulfuric acid performed better emulsion stability than that of the control starch sample. In another study, Haaj et al. [23] prepared Pickering emulsions that were stabilized using starch nanoparticles from waxy maize starch hydrolyzed with two different acids (HCl and H_2SO_4). The crystalline region of the starch is more resistant to acid hydrolysis. On the other hand, acid sensitive parts (mainly the amorphous parts) are broken down into their monomers during long acid hydrolysis and removed by washing after hydrolysis. Therefore, the main handicap of using regular amylose containing starches is the low yield while producing the starch nanoparticle. Hence, most of the studies in the literature are conducted with 100% amylopectin containing waxy starches to increase the yield.

In this thesis, one of our aims was to produce starch nanoparticles, to be used as a Pickering emulsion stabilizer, with high yield. In accordance with this purpose, we hypothesized that, instead of using waxy starches, using a starch source that is highly resistant to digestion may also result in starch nanoparticles with high yield. “The starch and starch degradation products that cannot be digested in the small intestine are defined as resistant starch (RS)” [24]. RS can be classified in five types; “RS1 is the physically inaccessible starch”, “RS2 is the granular starch”, “RS3 is the retrograded starch”, “RS4 is the chemically modified starch” and “RS5 is the amylose-lipid complex”. Among these resistant starches, RS4, the chemically modified one can be produced using different methods such as oxidation, etherification, esterification and cross-linking. Cross-linking can be performed in the presence of chemical reagents, which are in charge for the formation of ether or ester linkages within the starch molecule. It is well known that cross-linked starches are the highest resistant starch containing types in the literature [25]. In accordance with our hypothesis, for the preparation of starch nanoparticles we have chosen a high RS containing commercial cross-linked RS4 sample which was produced using a regular amylose containing wheat starch as the starch source.

This thesis consists of two parts. In the first part, Chapter 2, we have investigated the reaction conditions to produce starch nanoparticles with high yield. Then the potential of the nanoparticles to be used as a Pickering emulsion stabilizer was also analyzed. In the second part of the thesis, Chapter 3, we have used starch nanoparticles to encapsulate omega-3 fatty acids in flaxseed oil Pickering emulsions and investigate the potential protective effect of these emulsions against oxidation.

Chapter 2

2. Preparation of Pickering Emulsions Stabilized by Starch Nanoparticles

2.1. Introduction

Emulsions are defined as “heterogeneous systems consisting of two or more fully or partially immiscible liquids in which one of the liquid phases (dispersed phase) are dispersed in the other (continuous phase)” [26]. Emulsion applications are common in the food, agriculture, cosmetics and pharmaceutical industries. Emulsion systems are used in the creation of many food formulations such as margarine, mayonnaise, ice cream, sausage, chocolate, etc. A physically stable emulsion is expected to show no separation in the dispersed phase during its shelf life, to flow easily and to take its initial form homogeneously with a little agitation [27]. Emulsion stability can be defined as “the ability of an emulsion system to resist changing properties over time”. An emulsion system can become unstable during storage. Instability may result in changes in the organization of the molecules within the system, while chemical instability can cause changes in the types of molecules present.

The instability of emulsions can be prevented by using synthetic surfactants and emulsifiers such as hydrocolloids and proteins. While surfactants stabilize emulsions by reducing the “interfacial tension” between the phases of emulsion, emulsifiers such as hydrocolloids and proteins provide stability by forming a steric interface film. There are also solid particles in nano- and micro- sizes that play an important role in the stabilization of emulsions. Emulsions prepared using these solid particles are defined as "Pickering emulsions". It has been stated that Pickering emulsions provide better stabilization than the emulsions stabilized using surfactants/hydrocolloids [4], [26], [28]. The solid particles in the structure of these emulsions strongly surround the droplets by adsorbing on the interface of the two phases that make up the emulsion, due

to their good wettability properties [29], [30]. In addition to its positive effects on stability, the use of smaller amount of particles (0.04-1%) are sufficient for Pickering emulsion preparation compared to the ones of surfactant/emulsifier required for the regular emulsion formation.

Biological interactions and irritant properties of surfactants limit their use, especially in the food, cosmetic and pharmaceutical industries. Therefore, interest in emulsion stabilizers obtained from natural sources instead of synthetic surfactants has increased in recent years. Starch, a renewable, biodegradable and non-toxic material that is widely found in nature, has attracted considerable attention in the production of nano materials in recent years. In native starch, amylose and the branched parts of amylopectin are amorphous, however the linear parts of amylopectin are crystalline. While amylopectin is responsible for the crystalline regions of starch polymer, amylose polymer includes many hydroxyl groups in the structure. Starch has a multiscale structure [14]. While the size of individual starch granule changes between 2 to 100 μm, there are also different regions in its structure that have different size in nanoscale. In addition to the fact that starch contains different nanoscale polymers in its structure, its bioavailability, low cost and being a plant-sourced material enables starch to be evaluated as a potential in nanomaterial production [31].

In general, there are two approaches, as “top-down” and “bottom-up”, for the synthesis of nanomaterials. Top-down approach refers to cutting of a bulk material and it can be carried out two different ways as physical treatment and hydrolysis. Hydrolysis can be done with two different techniques such as enzymatic and acidic [32]. On the other hand, bottom-up approach refers to building up of a material from the bottom: “atom by atom” or “molecule by molecule”. This approach is also carried out with different techniques such as electrospraying, electrospinning and nanoprecipitation [32]. Both approaches can be used to produce starch nanoparticle. In the literature, there are studies related to starch nanoparticle production using “acid hydrolysis, enzymatic hydrolysis, high pressure homogenization, ultrasonication, reactive extrusion, gamma irradiation and nanoprecipitation” [32]–[35]. Acid hydrolysis has been widely used for the production of starch nanoparticles because of its simplicity and ease to control. There is a big potential of starch nanoparticles for industrial applications. Researches has been done for their utilization as a composite material, packaging material and drug carrier [32]. They also have potential to be used as emulsion stabilizer due to their chemical feature. While starch nanoparticles show hydrophilic properties as they

contain many hydroxyl groups in their structure, they also show hydrophobic properties due to their crystalline regions. Emulsion stabilizer has to be wettable in both phases (oil and water) forming the emulsion, therefore there is a great potential of starch nanoparticles to stabilize emulsions. There are several studies investigating the potential use of starch nanoparticles as stabilizers in Pickering emulsions [22], [23], [36]. The crystalline regions of the starch are more resistant to acid hydrolysis than the amorphous parts. During acid hydrolysis, amorphous parts are removed while crystalline parts remain, therefore to achieve high yields, most of the researches are carried out with waxy starches that have 100% amylopectin.

In this part of the thesis, one of our aims was to increase the yield of the starch nanoparticle to be used as an emulsion stabilizer. Instead of using waxy starch we have searched other starch sources that may result in high yield during starch nanoparticle production. Resistant starch (RS) is defined as "the starch and starch degradation products that cannot be digested in the small intestine" [24]. RS4 is one of the RS types that is chemically modified with oxidation, etherification, esterification and cross-linking. There is a commercially available RS4 starch, which is highly resistant to digestion. We hypothesized that due to its chemical structure, using RS4 may result in high starch nanoparticle yield. To the best of our knowledge, this is the first time that RS4 starch sample is used for the preparation of starch nanoparticles to be used as emulsion stabilizer.

2.2. Materials and Methods

2.2.1. Chemicals

RS4 produced from wheat starch was supplied from MGP Ingredients, Inc. (Kansas, USA). Native wheat starch was also used for comparison and purchased from Smart Kimya Ltd. Şti. (İzmir, Turkey). Sunflower oil (Vera; Gaziantep, Turkey) and corn oil (Çiğdem; Adana, Turkey) was purchased from a local store. The other chemicals used in this part of the thesis were sulfuric acid (Merck, Germany) and sodium hydroxide (Merck, Germany).

2.2.2. Production of Starch Nanoparticle

For the first part of the thesis, it was aimed to determine the starch nanoparticle production conditions to achieve the best emulsion stability. For this purpose, firstly starch nanoparticles were produced using acid hydrolysis according to the method of Angellier et al. [33]. For the production of starch nanoparticles 150 g of starch sample (native wheat starch and RS4) was mixed with various amount of sulfuric acid solution (H_2SO_4, 3.16 M). The hydrolysis conditions and starch:H_2SO_4 ratios (g/ml) are shown in Table 2.1. Starch samples were hydrolyzed at 50°C for 1-5 days using a round bottom flask magnetic stirrer equipped with a heater (Heidolph, Germany) (Figure 2.1). At the end of the hydrolysis time, the solution was centrifuged (Beckman Allegra X-30R, USA) at 4000 rpm for 10 min and supernatant was decanted. Starch precipitate was mixed with 20 ml of deionized water and starch suspension was neutralized with NaOH (5 M).

Table 2.1 Hydrolysis conditions of native wheat starch and RS4

Sample ID*	Hydrolysis time (day)	Hydrolysis temperature (°C)	Starch (g)	H_2SO_4 (ml)
1:4 (1)	1	50	20	80
1:3 (3)	3	50	20	60
1:4 (3)	3	50	20	80
1:5 (3)	3	50	20	100
1:7(3)	3	50	20	140
1:4 (5)	5	50	20	80

*The ratio in the sample ID is the starch:H_2SO_4 ratio in g/ml. The numbers in the parenthesis shows the hydrolysis time

Figure 2.1 Hydrolysis set up

The neutralized starch suspension was subjected to dialysis procedure to remove impurities such as free sulfuric acid residue and salt. Figure 2.2 shows the schematic representation of the dialysis process. The neutralized starch suspension was poured into a 10 cm long dialysis bag (Sigma D9402, USA) to dialyze against deionized water. The filled dialysis bag was placed in a beaker that was filled with deionized water. The dialysis process was carried out for 5 days on a magnetic stirrer with continuous stirring at 400 rpm (Velp, Italy). During the process the water in the beaker was renewed daily. At the end of dialysis, suspension was freeze dried (Labconco, USA). These samples were named as "starch nanoparticles" in the rest of the thesis.

The yield of the samples after acid hydrolysis was calculated as the percent ratio of dry solids after freeze drying based on the initial weight of starch using the Eq. 2.1.

$$Yield(\%) = \frac{final\ weight\ after\ drying\ (g)}{initial\ weight\ (g)} \quad (2.1)$$

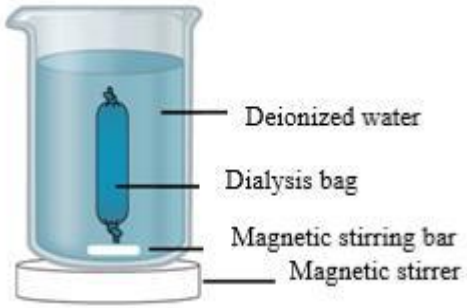

Figure 2.2 Schematic representation of dialysis process

2.2.3. Characterization of Starch Nanoparticle

2.2.3.1. Scanning Electron Microscopy (SEM) Analysis

Scanning electron microscope (SEM) was used to analyze the effect of acid hydrolysis on the morphology of the starch nanoparticles produced using RS4 [37]. Prior to analysis, dry samples were coated with gold for 4 min using a Sputter Quorum Coater. The samples were viewed using a Zeiss GeminiSEM 300 (Germany) analyzer under 2kV analyzing conditions. RS4 was also analyzed as a control starch sample.

2.2.3.2. Indication of Crystallinity

X-Ray diffractometer (Bruker AXS D8 advance model, Germany) was used to investigate the crystalline structure of the starch nanoparticles produced using RS4 [37]. Measurements were performed with two parallels (Cu-Kα radiation, λ=1.54056A˚) and specific voltage 40 and current as 30 mA. The position was set to 2Θ = 5-40°. RS4 sample was also analyzed as a control sample.

2.2.3.3. Structural Identification

Fourier Transform Infrared Spectrophotometer (FT-IR, Thermo Nicolet Avatar 370) was used to determine the structural features of the starch nanoparticles produced using RS4. Measurements were done in the wavelength range of 4000-400 cm^{-1}. Each sample was screened 32 times. RS4 sample was also analyzed as a control sample.

2.2.4. Selection of the Starch Nanoparticle to Achieve the Best Emulsion Stability

To select the starch nanoparticle to achieve the best emulsion stability, Pickering emulsions were prepared using the starch nanoparticles produced at Section 2.2.2. Two different oils (sunflower and corn oil) were used as the oil phase. Emulsions were prepared at both Φ0.6 and Φ0.8 oil fractions for each oil type. In brief, 2% (w/w) starch nanoparticle was mixed with water in a 20 ml glass bottle and sonicated for 15 sec at 98% amplitude (Bandelin 3100, Germany). Then oil was added to starch-water suspension and sonicated for 75 sec for homogenization. During homogenization, bottles were kept into ice bath to prevent overheating. Emulsions were stored in glass bottles at room condition for 30 days. The components of emulsions prepared in this part of the thesis are shown in Table 2.2. The physical stability of the emulsions was observed visually during storage.

Table 2.2 Components of emulsions prepared with constant nanoparticle ratio

Oil Fraction (Φ)	Oil Type		Starch Nanoparticle ID*	Starch Nanoparticle Ratio % (w/w)
0.6	Sun flower	Corn	1:4 (1)	2
0.6	Sun flower	Corn	1:3 (3)	2
0.6	Sun flower	Corn	1:4 (3)	2
0.6	Sun flower	Corn	1:5 (3)	2
0.6	Sun flower	Corn	1:7 (3)	2
0.6	Sun flower	Corn	1:4 (5)	2
0.8	Sun flower	Corn	1:4 (1)	2
0.8	Sun flower	Corn	1:3 (3)	2
0.8	Sun flower	Corn	1:4 (3)	2
0.8	Sun flower	Corn	1:5 (3)	2
0.8	Sun flower	Corn	1:7 (3)	2
0.8	Sun flower	Corn	1:4 (5)	2

*The ratio in the sample ID is the starch:H_2SO_4 ratio in g/ml. The numbers in the parenthesis shows the hydrolysis time.

2.2.5. Selection of the Starch Nanoparticle Ratio (w/w, %) and Oil Fraction (Φ) to Achieve the Best Emulsion Stability

In this section it was aimed to determine the starch nanoparticle ratio and oil fraction (Φ) to achieve the best emulsion stability. For this purpose, the starch nanoparticle produced using RS4 at a starch:H_2SO_4 ratio of 1:3 (g/ml) and hydrolysis time of 3 days (Sample ID; 1:3(3)) was used for the preparation of Pickering emulsions. Corn oil was used as the oil phase and emulsions were prepared at different oil fractions (Φ) using various starch nanoparticle ratio. Oil fractions (Φ) and starch nanoparticle ratio (w/w) are shown in Table 2.3. The emulsions were prepared using the same procedure described in Section 2.2.4. These emulsions were also stored at room conditions for 30 days and the physical stability of the emulsions were observed visually during storage.

Table 2.3 Components of emulsions prepared with Sample 1:3 (3) and corn oil

Oil Fraction (Φ)	Starch Nanoparticle Ratio* (w/w, %)
0.2	0
0.2	1
0.2	2
0.2	3
0.4	0
0.4	1
0.4	2
0.4	3
0.6	0
0.6	1
0.6	2
0.6	3

*mg starch nanoparticle/10g water+oil, w/w

2.3. Results and Discussion

2.3.1. Effect of Hydrolysis Conditions on the Yield and Characteristics of Starch Nanoparticle

2.3.1.1. Starch Nanoparticle Yield

Starch nanoparticle yield produced using native wheat starch and RS4 (Fibersym) with acid hydrolysis (3.16 M H_2SO_4, 50°C, 300 rpm) are shown in Table 2.4 and Table 2.5. Table 2.4 shows the yield of native wheat starch and RS4 samples hydrolyzed with H_2SO_4 at a starch:H_2SO_4 ratio of 1:4 (g/ml) for different time intervals (1, 3 and 5 days). The yield of the wheat starch samples hydrolyzed at a starch: H_2SO_4 ratio of 1:4 (g/ml) for different time intervals (1, 3 and 5 days) were significantly ($p<0.05$, paired t-test) lower than that of RS4. The highest yield for native wheat starch (17.3%) and RS4 (56.0%) were achieved for the samples hydrolyzed for 1 day at a starch:H_2SO_4 ratio of 1:4 g/ml (1:4 (1)). The increase in hydrolysis day caused a decrease in the hydrolysis yield for the RS4 samples. The yield decreased from 56.0% to 30.0%, when the hydrolysis day increased from 1 to 5. Unfortunately, similar trend was not observed with the wheat starch.

Table 2.5 shows the yields of the native wheat starch and RS4 samples hydrolyzed at constant hydrolysis time (3 days). The highest yield (5.9%) for the wheat starch samples hydrolysed for 3 days was achieved for the sample hydrolyzed with a starch:H_2SO_4 ratio of 1:5 (g/ml) (1:5 (3)). The yield of the wheat starch samples

hydrolyzed at various starch: H_2SO_4 ratios at constant hydrolysis time (3 days) were significantly ($p<0.05$, paired t-test) lower than that of RS4.

The highest yield (36.0%) for RS4 samples hydrolyzed for 3 days were achieved for the sample hydrolyzed at a starch:H_2SO_4 ratio of 1:3 (g/ml) and 1:4 (g/ml). The increase in the H_2SO_4 ratio above 4 decreased the yield independent from reaction time for the RS4 samples. The yields of the RS4 samples hydrolyzed for 3 days at a starch:H_2SO_4 ratio of 1:5 (g/ml) and 1:7 (g/ml) were 17% and 18%, respectively. As the yield of the samples produced using native wheat starch was significantly lower ($p<0.05$, paired t-test) than that of the ones produced using RS4 samples, RS4 was selected for the production of starch nanoparticles in the rest of the thesis.

Table 2.4 Yield of samples hydrolyzed with H_2SO_4 at a starch:H_2SO_4 ratio of 1:4 (g/ml) for different time intervals (1, 3 and 5 days)

Sample ID*	Hydrolysis temperature (°C)	Hydrolysis time (day)	Initial starch (g)	Starch Source	
				Wheat Starch Yield (%)	RS4 Yield (%)
1:4 (1)	50	1	20	17.3	56.0
1:4 (3)	50	3	20	3.7	36.0
1:4 (5)	50	5	20	6.3	30.0

*The ratio in the sample ID is the starch:H_2SO_4 ratio in g/ml. The numbers in the parenthesis shows the hydrolysis time

Table 2.5 Yields of samples hydrolyzed with various starch:H_2SO_4 ratio at constant hydrolysis time (3 days)

Sample ID*	Hydrolysis temperature (°C)	Hydrolysis time (day)	Initial starch (g)	Starch Source	
				Wheat Starch Yield (%)	RS4 Yield (%)
1:3 (3)	50	3	20	5.3	36.0
1:4 (3)	50	3	20	3.7	36.0
1:5 (3)	50	3	20	5.9	17.0
1:7 (3)	50	3	20	1.6	18.0

*The ratio in the sample ID is the starch:H_2SO_4 ratio in g/ml. The numbers in the parenthesis shows the hydrolysis time

Amylose and amylopectin are the polymers of starches structure. Most of the starches contain 20-25% amylose [11]. Among these polymers, amylose is susceptible to acid hydrolysis and can be degraded during the process and removed with washing. Amylopectin is the more acid resistant part of the starch. Due to this phenomena most of the researches for the production of starch nanoparticles are conducted using waxy

starches which contain 100% amylopectin [33], [38]. Angellier et al. [33] optimized the preparation conditions of starch nanocrystals within the shortest time and the highest yield and obtained starch nanocrystals with a yield of 15.7% after 5 days hydrolysis using 3.16 M H_2SO_4. Kim et al. [38] reported that an extended period of acid hydrolysis (longer than 5 days) caused low yields (less than 20%). There are no studies available in literature for starch nanoparticles produced by acid hydrolysis of RS4. The yield of hydrolysis of RS4 sample (56%, 36%, 30%) was found as higher than the yield of hydrolysis of the waxy maize starch when compared the literature results. The RS4 sample used in this study was produced commercially from wheat starch using cross linking agents that creates ether or ester linkages within the starch molecule. This chemical feature of RS4 seems to protect it from acid hydrolysis.

2.3.2. Starch Nanoparticle Characterization

2.3.2.1. Scanning Electron Microscopy (SEM)

SEM images of RS4 and the starch nanoparticles produced using RS4 are illustrated in Figure 2.3. The RS4 granules were seen round in shape with smooth surfaces (Figure 2.3a). There were deep hallows in the center of the granules. The sizes of granules varied between 5-20 μm. The similar observations were reported in the literature for RS4 samples produced from wheat starch [39], [40]. Figure 2.3b shows the SEM image of the sample hydrolyzed with a starch:H_2SO_4 ratio (g/ml) of 1:4 for 1 day (1:4 (1)). This sample had a similar size and granular form with the RS4 shown in Figure 2.3a. There were no noticeable differences in the granular structure and size, which can be due to the insufficient hydrolysis. As the granule size of the Sample 1:4 (1) was 5-20 μm, this sample cannot be evaluated as starch nanoparticle.

Figure 2.3c shows the SEM image of Sample 1:3 (3) that was produced with a starch: H_2SO_4ratio (g/ml) of 1:3 for 3 days. The shape and size of the granules of Sample 1:3 (3) were different than that of the RS4 (Figure 2.3a) and Sample 1:4 (1) (Figure 2.3b). The granule size of Sample 1:3 (3) were dramatically low (<50 nm) and this sample can be considered as “starch nanoparticle”. While RS4 and Sample 1:4 (1) were seen as individual intact granules, starch nanoparticles were found in aggregates. In the literature the sizes of starch nanoparticles were also found to be quite low (<200 nm) compared to wheat starch [31], [41], [42].

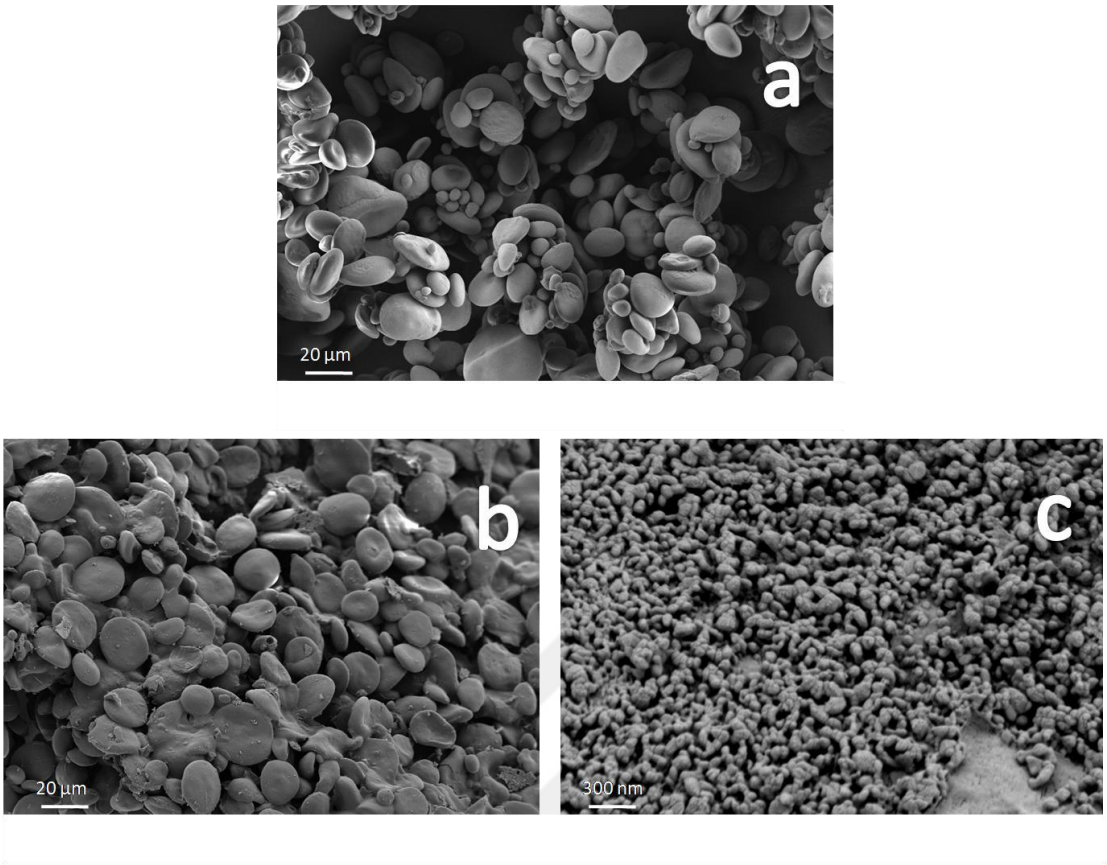

Figure 2.3 SEM images of the samples. a) Resistant starch, b) Sample 1:4 (1), c) Sample 1:3 (3). The ratio in the sample ID is the starch:H_2SO_4 ratio in g/ml. The numbers in the parenthesis shows the hydrolysis time

Figure 2.4 shows the SEM images of the Samples 1:4 (3), 1:5 (3), 1:7 (3) and 1:4 (5). All of the starch granules were observed as very fine particles (<50 nm) similar to 1: 3 (3) sample (Figure 2.3c). Except the Sample 1:4 (5) (Figure 2.4d), all samples were found in aggregates with round in shape. The images showed that the size of starch granules of the 3 days hydrolyzed samples decreased less than 50 nm regardless from the acid ratio. It could be easily seen 3-day hydrolysis at 50 °C was sufficient for the reduction of the size of starch granules. As can be seen from Figure 2.3 and 2.4, while RS4 granules were found independently from each other, starch nanoparticles were present as aggregated granules. The presence of starch nanoparticles in clusters has been observed in many studies in the literature [43]. The reason for the clustering of starch nanoparticles in this way was shown as the new hydrogen bonds formed as a result of the interaction of hydroxyl groups on the surface of the nanoparticles with each other [44]. No significant difference in shape and size was observed among the nanoparticles obtained by 3 days hydrolysis of RS4 at various starch: H_2SO_4 ratio.

The SEM image of the sample hydrolyzed at a starch:H_2SO_4 ratio (g/ml) of 1:4 for 5 days (1:4 (5)) is also illustrated in Figure 2.4d. In terms of granular size and shape, this sample was different from other samples hydrolyzing for 3 days. The starch nanoparticles were detected in the surface as uniform. Different from 3-day hydrolyzed samples, clusters or aggregates were not observed with 5-day hydrolyzed samples. On the other hand, the size of starch granules were <20 µm. The reason of this situation could be long hydrolysis period.

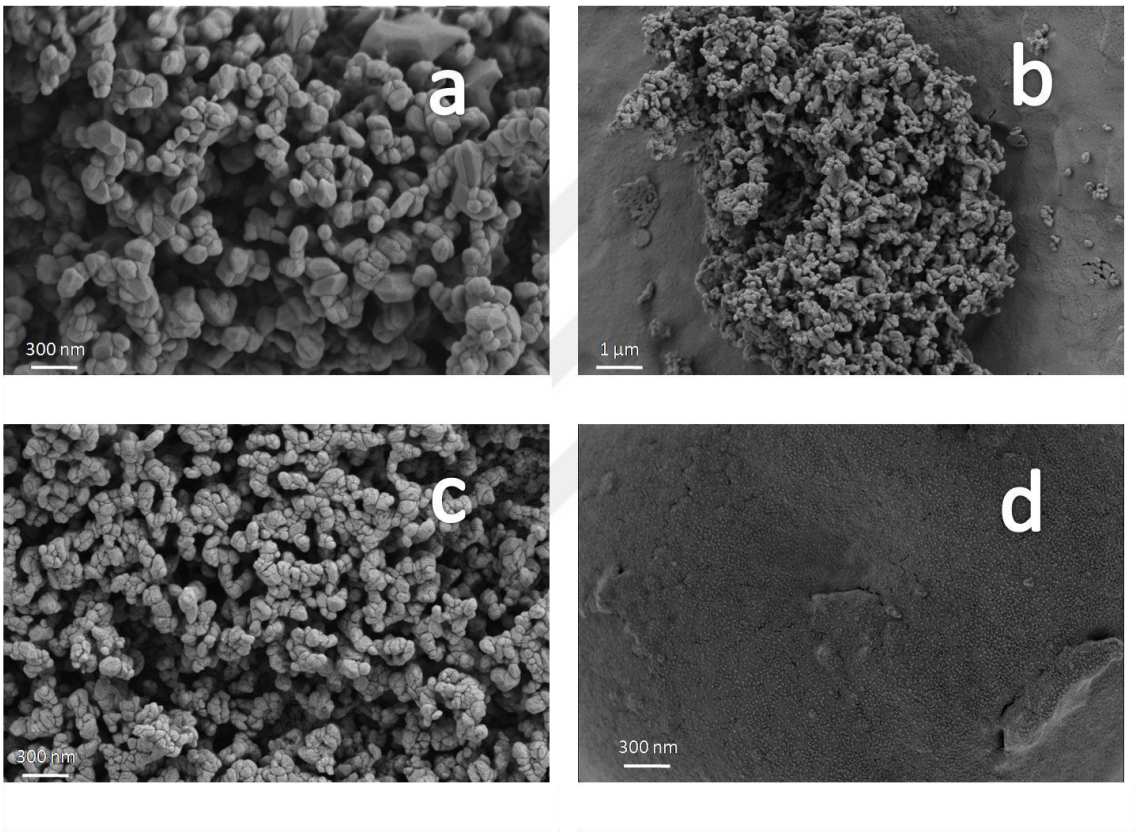

Figure 2.4 SEM images hydrolyzed RS4 samples. a) 1:4 (3), b) 1:5 (3), c) 1:7 (3) and d) 1:4 (5). The ratio in the sample ID is the starch:H_2SO_4 ratio in g/ml. The numbers in the parenthesis shows the hydrolysis time

2.3.2.2. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectrums of the RS4 and starch nanoparticles are shown in Figure. 2.5. The small peak seen at nearly 3650 cm^{-1} wavelength indicates the free water entry on the surface of starch samples [45]. The presence of stretching vibrations at 3600 cm^{-1} verifies the existence of O-H bond (Figure 2.5) [46]. Each of the starch nanoparticles and RS4 has a sharp peak at 2963 cm^{-1}. The absorption band seen at 2963 cm^{-1}

indicates the presence of C-H stretching vibration. Similar results were also given in the literature [47]–[49]. The small peak seen at 1647 cm^{-1} indicates the bound water existing in the granule [49]. The peaks at 1248 cm^{-1} and 1395 cm^{-1} are the bending vibrations of O-H and the C-H bending vibrations, respectively. As can be seen from Figure 2.5, noticeable difference was not observed between the native resistant starch (RS4) and starch nanoparticles in terms of bonds. As the starch nanoparticles had peaks at the same absorption bands with their natural counterpart (RS4) indicate that the general starch structure is preserved after hydrolysis. While acid hydrolysis did not cause any change in the general starch structure, it only reduced the granule size of starch samples (Figure 2.3 and 2.4).

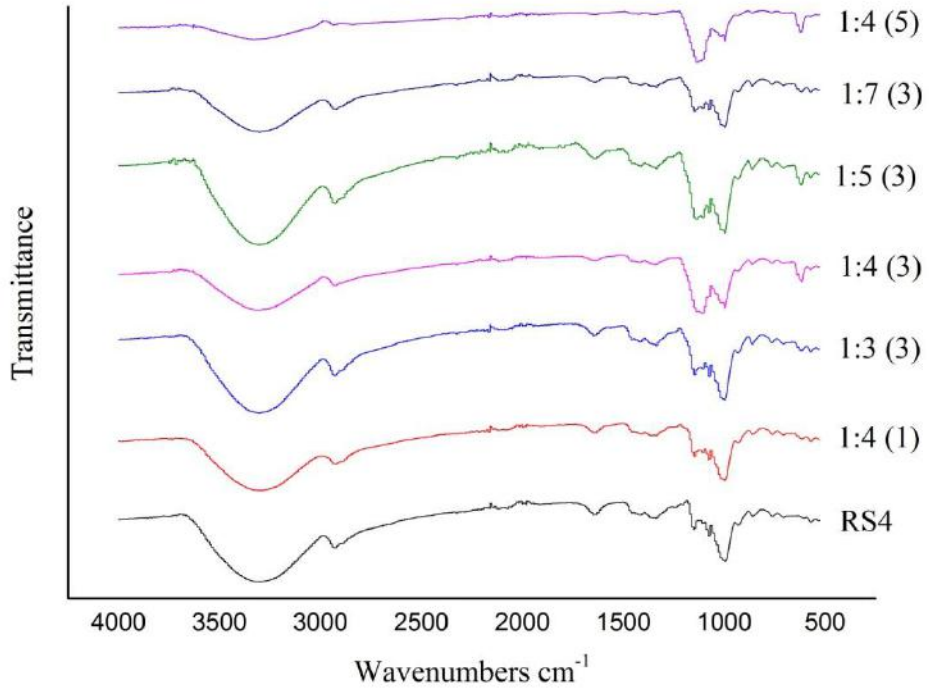

Figure 2.5 Fourier transform infrared spectroscopy (FT-IR) spectrums of samples. The ratio in the sample ID is the starch:H_2SO_4 ratio in g/ml. The numbers in the parenthesis shows the hydrolysis time

2.3.2.1. X-Ray Diffraction (XRD)

The X-ray diffractograms of resistant starch and starch nanoparticles are illustrated in Figure 2.6. Starch can be categorized in three groups (A, B and C type starches) according to its crystalline feature [50]. The starches having A type crystalline

structure are cereal starches and give specific peaks in at "2Θ = 15°, 17°, 18° and 23°" [24]. The RS4 samples had peaks at 14.9°, 16.9°, 18.0° and 23.2° (2Θ) diffraction angle (Figure 2.6). In addition, samples named as 1:4 (1) and 1:3 (3) had peaks same as RS4. As seen in Figure 2.6, these three samples had the peaks similar with the A type starches. Although the samples named as 1:4 (3), 1:5 (3), 1:7 (3) and 1:4 (5) had the A type crystalline starch peaks, they had also different peaks in diffraction pattern. According to the XRD analysis report, the peaks that were seen bigger than 2Θ= 30° were reported as the polyaniline. Therefore these starch samples were not indicated as A type crystalline form starches.

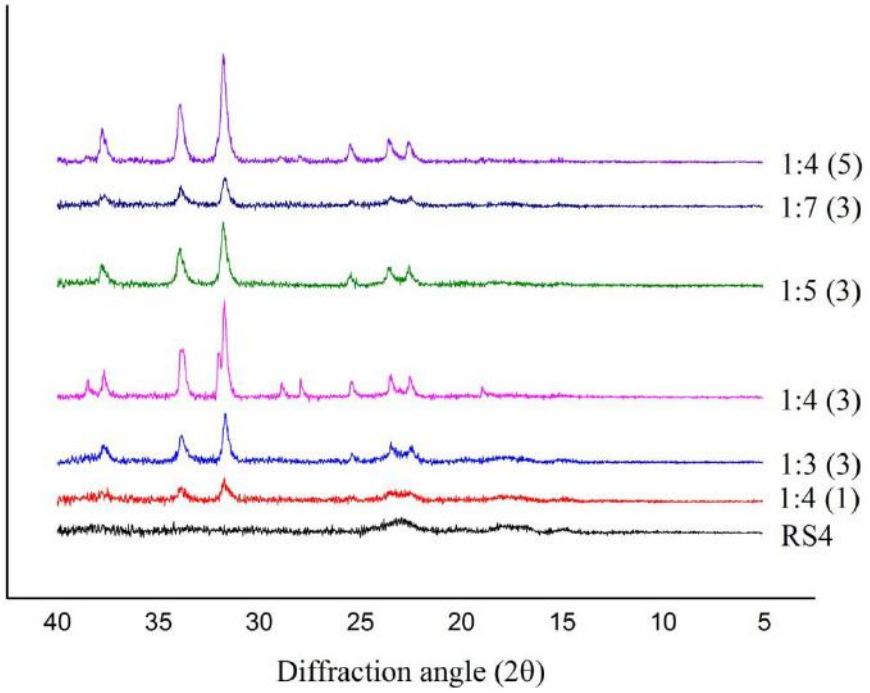

Figure 2.6 X-ray diffractograms of samples. The ratio in the sample ID is the starch:H_2SO_4 ratio in g/ml. The numbers in the parenthesis shows the hydrolysis time

On the other hand, the crystallinity of the samples varied between 56.4% and 77% depending to the hydrolysis condition (starch: H_2SO_4 ratio (g/ml) and hydrolysis time). While RS4 had 54.0% crystallinity, the hydrolysis of this sample caused an increase in the crystallinity of the samples (Table 2.6). The highest crystallinity (77.0%) was achieved for the sample hydrolyzed with a starch:H_2SO_4 ratio of 1:4 (g/ml) for 5 days.

Crystalline regions in starch granules are more resistant to acid hydrolysis than amorphous regions. So crystalline parts inside the starch granule can be isolated in the

presence of dilute sulfuric or hydrochloric acid [32]. In this study acid hydrolysis with dilute H_2SO_4 selectively acted on amorphous regions and it provided highly crystalline starch nanoparticles.

Table 2.6 Crystallinity of the RS4 and starch nanoparticles samples

Sample ID*	Crystallinity %
RS4 (Fibersym)	54.0
1:4 (1)	56.4
1:3 (3)	60.6
1:4 (3)	76.5
1:5 (3)	70.9
1:7 (3)	66.8
1:4 (5)	77.0

The ratio in the sample ID is the starch:H_2SO_4 ratio in g/ml. The numbers in the parenthesis shows the hydrolysis time

2.3.3. Effect of Hydrolysis Conditions of the Starch Nanoparticles on the Stability of Pickering Emulsions

Physical stability is a critical factor for emulsions as it evaluates shows whether or not a product is suitable for its future use. Phase separation is not desired for none of the emulsion-based product. [51]. The physical stability of the emulsions prepared using the starch nanoparticles produced at Section 2.2.4 was detected by visual observation during 30 days of storage at room conditions. Initial and final photographs (after 30-day storage) of the emulsions prepared with sunflower oil are illustrated in Figure 2.7. At initial, all samples that were prepared using sunflower oil had nearly same white cream appearance (Figure. 2.7 (a and c)). Due to the sonication effect, all tubes were seen as an emulsion form independently from the oil fraction. After 30-day storage, appearance of the emulsions changed dramatically. For the Φ0.6 oil fraction, there were only three samples that were stable at the end of the storage (Figure 2.7b). The first sample was separated completely to its oil and water phases expected, because it was only oil-water mixture. A separation was also observed with the second sample which was prepared with RS4 (Figure 2.7b, 2nd sample). There was a slight/ partial separation at the bottom of the 3rd sample which was prepared using 1:4 (1) sample. As stated above (Section 2.3.2.1) due to its size distribution, this sample was not considered as starch nanoparticle. Stable emulsions were observed with the 4th, 7th and 8th samples produced using 1:3 (3), 1:7 (3) and 1:4 (5), respectively (Figure 2.7b). When the sunflower oil

fraction changed to Φ0.8, there were not any stable samples after 30-day storage (Figure 2.7d).

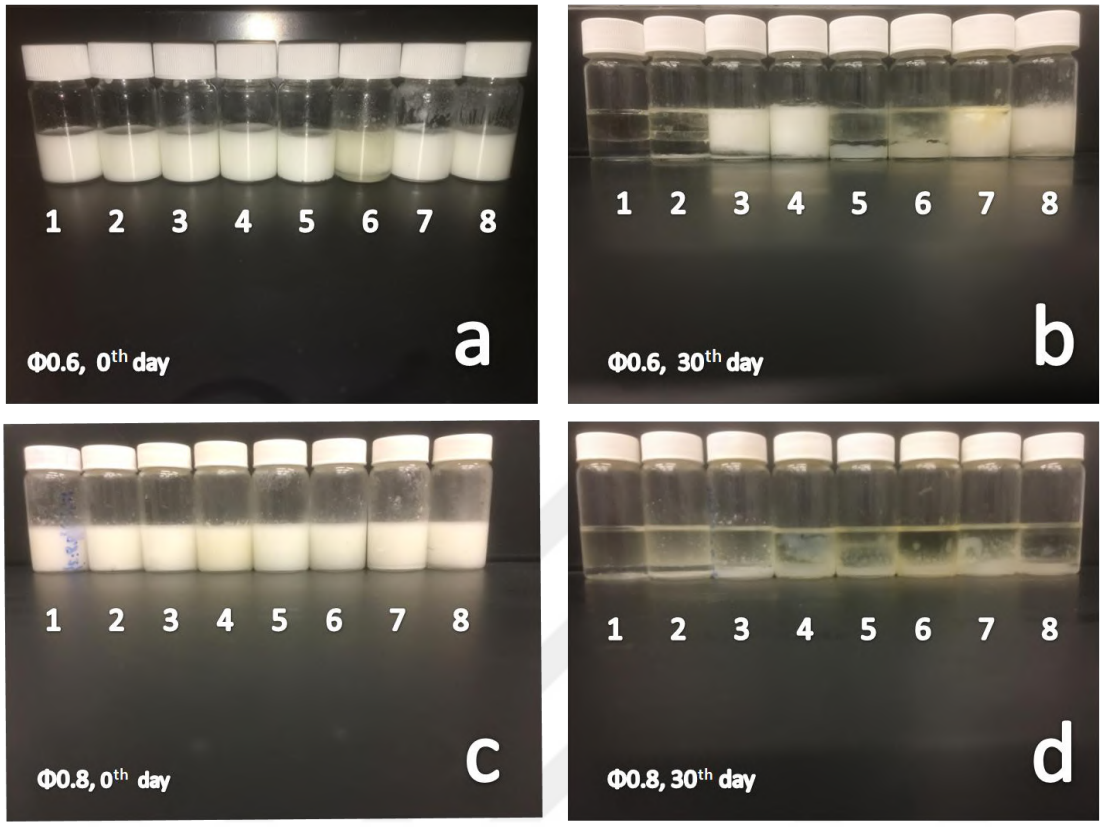

Figure 2.7 Emulsions prepared with sunflower oil. a) Φ0.6 oil fraction at 0th day, b) Φ0.6 oil fraction at 30th day, c) Φ0.8 oil fraction at 0th day, d) Φ0.8 oil fraction at 30th day. 1: [no starch, only oil and water], 2: [2% RS4 (w/w)], 3: [2% 1:4 (1) (w/w)], 4: [2% 1:3 (3) (w/w)], 5: [2% 1:4 (3) (w/w)], 6: [2% 1:5 (3) (w/w)], 7: [2% 1:7 (3) (w/w)], 8: [2% 1:4 (5) (w/w)].

Initial appearance and final photographs (after 30-day storage) of the emulsions prepared with corn oil are illustrated in Figure 2.8. Four samples (numbered as 4, 5, 6 and 7) prepared with corn oil at an oil fraction of Φ0.6 protected their stability after 30-day storage (Figure 2.8b). When the oil fraction was changed to Φ0.8, the physical stability of emulsions prepared with corn oil deteriorated similar to the ones prepared with sunflower oil (Fig 2.7d). None of the samples prepared using corn oil (at an oil fraction of Φ0.8) was stable after 30-day storage. According to the Figure 2.7d and 2.8d, it can be easily detected that Φ0.8 oil fraction was not suitable for the emulsions independently from oil type. There are several studies about Pickering emulsions stabilized by starch nanoparticle. Ge et al. [7] studied the characterization of the Pickering emulsions stabilized by various starch nanoparticles (corn, tapioca, sweet

potato, and waxy corn starch) for 1-month storage. They determined the critical oil fraction of emulsions stabilized using corn starch nanoparticles as Φ0.5. They indicated that poor stability was measured for the emulsions with oil fractions that exceed the critical point. In another studies, Tan et al. [52] illustrated that emulsions prepared with oil fractions from Φ0.1 to Φ0.6 had long term stability, while the ones prepared with the oil fraction higher than Φ0.7 were unstable during 1 month.

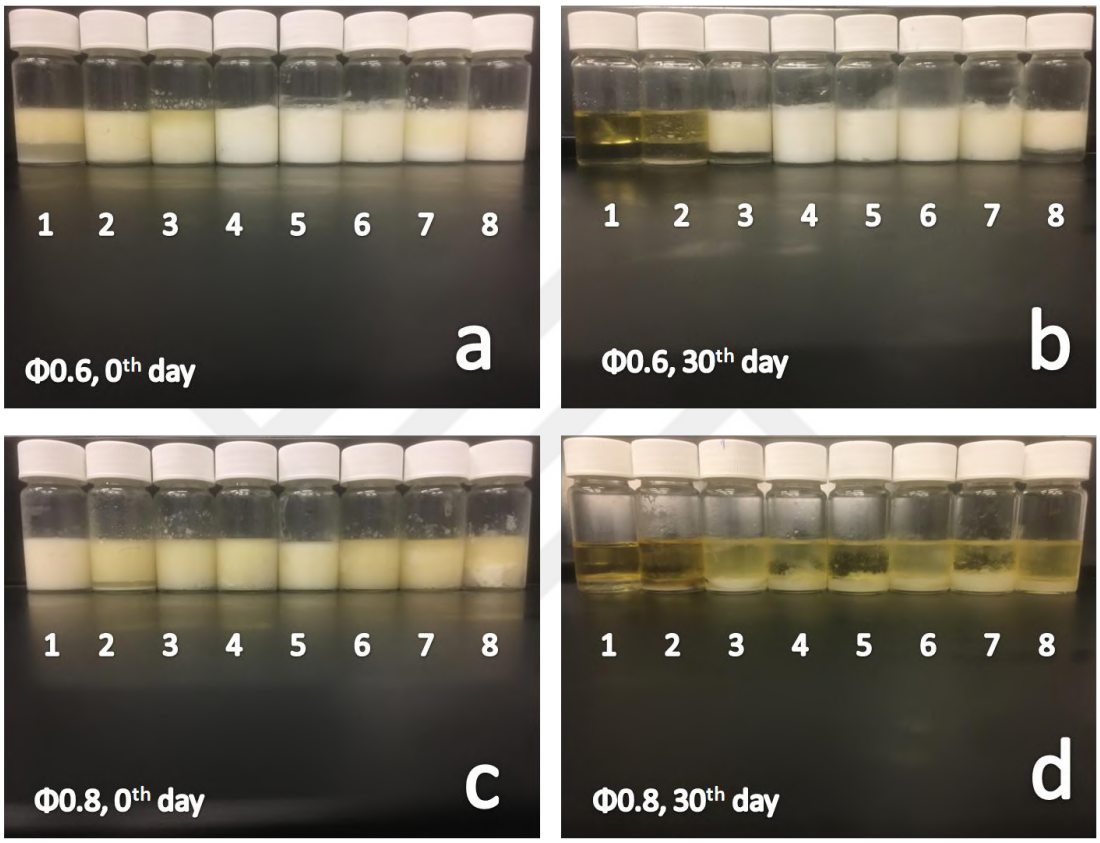

Figure 2.8 Emulsions prepared with corn oil. a) Φ0.6 oil fraction at 0th day, b) Φ0.6 oil fraction at 30th day, c) Φ0.8 oil fraction at 0th day, d) Φ0.8 oil fraction at 30th day. 1: [no starch, only oil and water], 2: [2% RS4 (w/w)], 3: [2% 1:4 (1) (w/w)], 4: [2% 1:3 (3) (w/w)], 5: [2% 1:4 (3) (w/w)], 6: [2% 1:5 (3) (w/w)], 7: [2% 1:7 (3) (w/w)], 8: [2% 1:4 (5) (w/w)].

The emulsions prepared using 1:3 (3), 1:7 (3) and 1:4 (5) samples were stable for both oil types at Φ0.6 oil fraction (Figure 2.7b and 2.8b). In addition, the emulsions prepared using corn oil was more stable than the ones prepared using sunflower oil. Among these nanoparticles, the one named as 1:3 (3) had the lowest starch:H_2SO_4 ratio (g/ml) and hydrolysis time. Therefore, to determine the optimum starch nanoparticle

concentration and oil fraction to achieve the best emulsion stability 1:3 (3) sample and corn oil was used for the next part of thesis.

2.3.4. Effect of Starch Nanoparticle Ratio (w/w, %) and Oil Fraction (Φ) on the Stability of Pickering Emulsions

Quantity of encapsulating agent (surfactant or emulsifier) is a very important factor to stabilize the emulsion [16]. Encapsulation agent in insufficient concentration causes active substance to be shared adjacent and irreversible bridge droplets in emulsions [53]. Nevertheless, excess of the encapsulating agent, above essential amount to cover oil droplet, might increase its surface load and have negative effects on the emulsion properties [54].

In order to find the optimum starch nanoparticle (encapsulating agent/emulsifier) concentration and oil fraction to achieve the best stability, a series of emulsion were prepared with different starch nanoparticle concentration and oil fraction.

The emulsions prepared using 1:3(3) at different starch nanoparticle ratio (1, 2 and 3%, mg/g) and corn oil fractions (Φ0.2, Φ0.4 and Φ0.6) are shown in Figure 2.9. The first sample for all oil fractions was prepared with only corn oil and water. As can be seen from Figure 2.9b, d, f, these first samples were separated into oil and water phase as expected. The phase separation occurred a few hours after the sonication (Figure is not shown). The emulsion samples prepared using various amount (1, 2 and 3% (w/w)) of 1:3 (3) had a similar appearance (white and creamy) at initial (Figure 2.9a, c and e). Among the emulsions prepared at Φ0.2 oil fraction; only the ones prepared with 2% and 3% starch nanoparticle were stable (Figure 2.9b). Phase separation was detected at the bottom of the emulsion sample prepared with 1% (w/w) starch nanoparticle. Similar observation was made with the samples prepared at Φ0.4 oil fraction. The stability of the samples prepared with Φ0.6 seemed to be lower than the ones prepared with Φ0.2 and Φ0.4 oil fractions. There was only one stable emulsion after 30-day storage (2% starch nanoparticle, Figure 2.9f). The studies about visual observation of stability of Pickering emulsions stabilized by various nanoparticles were available in literature [7], [51], [55]–[57]. Ge at al. [7] showed that increase in the cream volume of emulsions occurs with the increasing corn starch nanoparticle concentration for the same oil fraction.

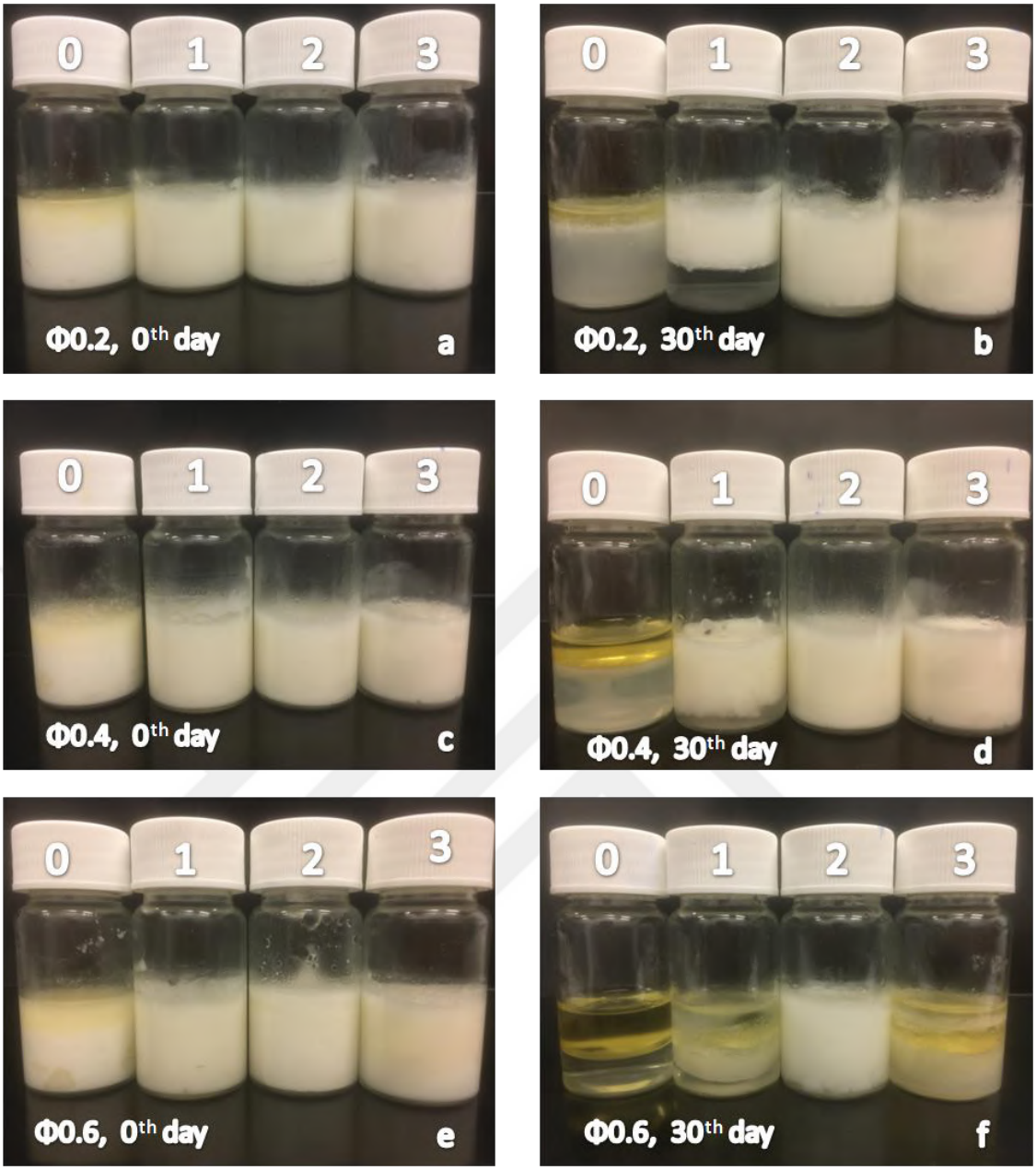

Figure 2.9 Pickering emulsions of corn oil with 1:3 (3) RS4 nanoparticles; a) Φ0.2 oil fraction at 0th day, b) Φ0.2 oil fraction at 30th day, c) Φ0.4 oil fraction at 0th day, d) Φ0.4 oil fraction at 30th day. 0: [0% starch nanoparticle (w/w)], 1: [1% starch nanoparticle (w/w)], 2: [2%starch nanoparticle (w/w)], 3: [3% starch nanoparticle (w/w)].

According to the 30 days storage observations, 1% RS4 nanoparticle (1: 3 (3)) was not sufficient for the stability of emulsions for all fractions (the 2nd bottles of Figure 2.9b, d, f which were named as "1").

For the emulsions prepared with 2% RS4 nanoparticle seems to be stable for all oil fractions after 30-day storage, however there were slight phase separation at the bottom of the samples with an oil fraction of Φ0.2 and Φ0.6. The emulsions prepared

using 3% RS4 nanoparticle at an oil fraction of Φ0.2 and Φ0.4 were stable after 30-day storage. Among the stable emulsions, the one with the lowest oil fraction is the 4th bottle of Figure 2.9b. This sample was prepared using 3% RS4 nanoparticle at an oil fraction of Φ0.2. Therefore, it was decided to prepare the emulsions with the addition of 3% (w/w) RS4 nanoparticles at Φ0.2 oil fraction for the next part of the thesis.

Chapter 3

3. Preparation of Stable Flaxseed Oil Pickering Emulsions as a Delivery System of Omega-3 Fatty Acids

3.1. Introduction

It has been stated in many studies that “Omega-3 fatty acids” found in oily fish, flax seeds, nuts such as walnuts and dark colored vegetables have a positive effect on health. It is stated in the literature that “omega-3 fatty acids are essential not only for normal growth and development, but also for their positive effects on heart, brain, eye, joint and skin health” [18], [58], [59]. In addition to these, there are various studies showing that omega-3 fatty acids have effects in the avoidance of some diseases which are defined as coronary heart diseases, hypertension, diabetes and arthritis [59], [60]. Besides, it has been stated that omega-3 fatty acids help to reduce the triglyceride level in blood serum [59]. Many studies are illustrated that adequate intake of omega-3 fatty acids during pregnancy affects positively on the retina and general health of the fetus [58], [61].

Omega-3 fatty acids are subjected in the class of polyunsaturated fatty acids (PUFA). It contains multiple double bonds in its structure. Alpha-linolenic (α- linolenic) acid (ALA, 18:3(n-3)), which is in the omega-3 fatty acid group, is an essential fatty acid that cannot be synthesized by the human body but should be taken from outside due to its benefits to the human body [62]. Other omega-3 fatty acids of nutritional importance are “long-chain metabolites of ALA”, “eicosapentaenoic acid (EPA, 20:5(n-3))” and “docosahexaenoic acid (DHA, 22:6(n-3))”.

Adequate omega-3 intake is possible by consuming foods enriched with omega-3 fatty acids in order to achieve the positive health effects. Although there are plenty of

foods enriched with omega-3 fatty acids, there are technical difficulties associated with their manufacture, transport, storage, bioavailability, and sensory acceptability. The physical and chemical properties of omega-3 fatty acids limit their application as a potential food ingredient. Due to the unsaturated double bonds in its structure, omega-3 fatty acids become more sensitive to the oxidation. Oxidation is a type of food spoilage that causes a decrease in the nutritional value and food quality. Oxidation of the double bonds in the structure of omega-3 not only negatively affects the bioavailability of omega-3, but also causes the emergence of hydroperoxides, aromas and odors that are not accepted by consumers [59].

Flaxseed, whose Latin name *Linum usitatissimumis* means "very useful", has been grown since the ancient times [17]. Nowadays, flaxseed has been the center of diet and disease studies due to its positive effects on the health deal with some of its biologically active materials in the structure. Flaxseed is rich in omega-3 fatty acids: α-linolenic acid (ALA), soluble and insoluble fibers, proteins and antioxidants [16], [63]–[66]. Goyal et al. [17] mentioned that the "popularity of flaxseed increase recently according to positive effects on health such as decreasing cardiovascular diseases, reduce risk of cancer, particularly of the mammary and prostate gland, anti-inflammatory activity, laxative effect, and alleviation of menopausal symptoms and osteoporosis".

Two types of flaxseed are available in nature as "(1) brown" and "(2) yellow or golden". The nutritional characteristics and fatty acids composition are the same for both types. However, there is one exception for the type of yellow flaxseed called solin (trade name Linola), which includes lower omega-3 fatty acids [67]. The brown one is generally used as a component in paints, varnish, fiber and cattle feed [68], [69]. Edible forms of flax are whole flaxseeds, milled flax, roasted flax and flaxseed oil. With respect to the physical structure flaxseed is defined as a multicomponent system and it contains bio-active plant substances such as "oil, protein, dietary fiber, soluble polysaccharides, lignans, phenolic compounds, vitamins (A, C, F and E) and minerals (P, Mg, K, Na, Fe, Cu, Mn and Zn)" [70], [71]. Flaxseed is recommended as a rich plant source of the omega-3 fatty acid [72], [73]. Flaxseed oil includes low amount of saturated fatty acids (9%), moderate amount of mono saturated fatty acids (18 %), and high amount of polyunsaturated fatty acid (73 %) [17], [63]. The majority of fatty acids form α-linolenic acid ranging from 39.0% to 60.4%, followed by oleic, linoleic, palmitic and stearic acids, respectively [17], [74]. Although flaxseed oil naturally contains antioxidants such as tocopherols and beta-carotene, subjecting flax seed to processes

such as extraction and purification makes flaxseed oil more susceptible to oxidation [17]. The bioavailability of ALA changes with respect to the type of flax ingested (the bioavailability of ALA is greater when it was consumed in oil form than the bioavailability of milled or whole ones) [17], [75].

Flaxseed oil that is evaluated as a rich source of "essential fatty acids (EFAs), linoleic acid (n-6) and α-linolenic acid (n-3)", control prostaglandins synthesis and hence helps injury healing process. Lack of EFAs causes phrynoderma or toad skin, horny eruptions on the limbs and poor injury healing, etc. [17].

Methods have been developed that allow the use of essential fatty acids in food, which have high nutritional value but are very sensitive to environmental conditions. Encapsulation, one of these methods, prevents small solid particles from being affected by ambient conditions by coating them with a substance called as wall material. The use of bioactive food ingredients such as vitamins, antioxidants and enzymes by encapsulating with nano technological applications enables the formation of foods with advanced product formulation such as functional food.

Encapsulation is generally used in food industry to overcome food instability and their performance in a last food submission. Encapsulation is necessary in some cases to solve the problems about physical or chemical instability, physical incompatibility of ingredients in each other, adverse relations of the ingredient with other components of the food formulation or releasing of flavor or bioactive components [76].

The food materials are available in industries that are suitable for encapsulation. These are categorized as "(a) flavorings (e.g. sweeteners, seasonings, spices), (b) acids and bases (e.g. citric acid, sodium bicarbonate), (c) lipids (oils or fats), (d) food additives (e.g. preservatives, pigments), (e) minerals (e.g. magnesium and salts) (f) vitamins (e.g. D and K) and (g) colors" [76]–[78]. Encapsulation of bioactive components especially omega-3 fatty acids [66], [72], [79], plant phytonutrients oils [78] and probiotic bacteria [80], [81], has great interest, due to the health benefits. The factors affecting the encapsulation stability are; the chemical structure, molecular weight, polarity and volatility of the food components and its reaction with the matrix material. Microencapsulation reduces the oxidation sensitivity of omega-3 fatty acids, making them more moderate to heat and production conditions. In practice, encapsulation can be done by different methods such as spray drying, freeze drying, fluid bed coating. In most of the methods, emulsion is prepared as a pre-treatment. In

order to encapsulate lipophilic substances, especially omega-3 fatty acids, an oil/water emulsion is required.

There are various studies in the literature for the encapsulation of omega-3 fatty acids. Heinzelmann et al. [82] used emulsions prepared with sodium caseinate for the microencapsulation of omega-3 fatty acids. In another study where egg white powder was used as an emulsifier, omega-3 fatty acids was microencapsulated by spray drying and freeze drying methods [83]. In various studies, gum arabic, lactose and maltodextrin have also been shown as matrix materials used in encapsulating omega-3 fatty acids [84], [85].

As mentioned above, emulsion preparation is one of the methods used in encapsulation of lipophilic materials. Within the scope of this thesis, flax seed oil was used as a source of omega-3 fatty acids. However flaxseed oil is highly susceptible to the lipid oxidation because of its high PUFA composition. To overcome this problem, it was aimed to prevent oxidation by preparing a stable flaxseed oil Pickering emulsion stabilized with starch nanoparticles to be used as a delivery system of omega-3 fatty acids.

3.2. Materials and Methods

3.2.1. Chemicals

RS4 was supplied from MGP Ingredients, Inc. (Kansas, USA). Flaxseed oil was supplied as freshly cold pressed form Eminel Bitkisel Ürünler (Kayseri, Turkey) and used as a source of omega-3 fatty acids. The chemicals used in this study were: sulfuric acid (Merck, Germany), sodium hydroxide (Merck, Germany), hexane (Merck, Germany), acetic acid (Merck, Germany), chloroform (Merck, Germany), soluble starch (Merck, Germany), potassium iodine (Sigma-Aldrich, USA) and sodium thiosulfate (Sigma-Aldrich, USA).

3.2.2. Determination of the Fatty Acid Composition of Flaxseed Oil

Fatty Acid Composition of the cold pressed flaxseed oil used in this thesis was determined by TÜBİTAK MAM using the standard method (IUPAC IID19 Solid and liquid oils, 1992) [86].

3.2.3. Omega-3 Encapsulation into Pickering Emulsions Using Starch Nanoparticle

For the preparation of Pickering emulsions of flaxseed oil, starch nanoparticle produced using RS4 at a starch:H_2SO_4 ratio of 1:3 (g/ml) and hydrolysis time of 3 day (Sample ID; 1:3(3)) was used. The details of the production were given in Section 2.2.2. After some preliminary experiments, Φ0.2 was selected as the flaxseed oil fraction. Pickering emulsions were prepared at Φ0.2 (flaxseed oil fraction) with 3% (w/w) starch nanoparticle according to the principle that was detailed at Section 2.2.4. Emulsions and flaxseed oil were stored at 25±1°C in a test cabinet (Nüve TK120, Turkey) for 15 days for further analysis.

3.2.4. Determination of Physical Stability of Emulsions

Besides visual observation, stability of the emulsions was also determined using the method of Kumar et al. [66]. Emulsions (10 ml) were poured into glass test tubes (97 mm height, 15 mm internal diameter) and were examined for 3, 6, 9, 12 and 15 days (25 ±1°C, Nüve TK120, Turkey). The alteration in the height of serum phase showed the separation of emulsions and physical stability of emulsions was calculated using Eq. (3.1);

$$Stability\ \% = 100 \times \frac{H}{Ho} \tag{3.1}$$

where, Ho and H are the initial and final heights (mm) of emulsions, respectively.

3.2.5. pH of Emulsions

pH of the emulsions (10 ml) were determined using a pH meter (Starter 2100 Ohaus, USA).

3.2.6. Particle Size Distribution and Zeta-(ζ) Potential

Zetasizer (Malvern, UK) was used for the article size distribution of the emulsions. One ml of emulsion was dissolved in 99 ml of deionized water and homogenized with ultrasound bath for 5 min for the removal of gas bubbles (Wisd WUC- AO3H, Korea). The temperature of Zetasizer was maintained at 25°C during the measurement and refractive index was selected as 1.35. Also, the polydispersity index (PDI) was measured as the ratio of the square of particle size standard deviation to mean

particle size. Three measurements were done and the mean value was recorded. The results of particle size distribution were expressed as diameter (nm) vs. intensity (%).

3.2.7. Determination of Peroxide Value

Oxidative stability of emulsions was determined after the extraction of flaxseed oil with hexane. Briefly, 50 ml of emulsion was mixed with 50 ml of hexane and vortexed (Heidolph Multi Reax, Germany) at 1875 rpm for 25 min. Emulsion lost its physical form at the end of mixing and the hexane-oil mixture was separated by using a separating funnel. The remaining mixture after extraction was mixed with 25 ml hexane once again and vortexed for 25 min. Same procedure was applied to hexane-oil mixture to separate oil. Hexane was evaporated under fume hood to obtain oil.

The peroxide value of the extracted oil was analyzed using the AOAC official method 965.33 [87]. Oil (2 g) was mixed with 25 ml of acetic acid-chloroform solution (3:2, v/v) and shaken to dissolve. One ml of saturated iodine solution was added to mixture and kept at dark for 5 min. At the end of the time, 75 ml of distilled water and 1 ml of 1% starch solution was added to mixture. The final blue solution was titrated slowly with 0.01 N sodium thiosulphate ($Na_2S_2O_3$) with continuous shaking until the yellow color occurred and blue color disappeared. The peroxide value was calculated using the Eq. 3.2;

$$Peroxide\ Value\left(\frac{millieqvtperoxide}{kgoil}\right) = \frac{S \times M \times 1000}{oil\ weight} \quad (3.2)$$

where S is ml of $Na_2S_2O_3$ (blank corrected) and M is normality of $Na_2S_2O_3$.

3.3. Results and Discussion

3.3.1. Fatty Acid Composition of Flaxseed Oil

The fatty acid composition of flaxseed oil is shown in Table 3.1. According to the results, it can be seen that the flaxseed oil used in this thesis was rich in omega-3 fatty acids (48.55%): α-linolenic acid (ALA) (48.52%) and short chain polyunsaturated fatty acids (PUFA). Among the lipids in flaxseed oil, α-linolenic acid is the major fatty acid (48.52%) followed by oleic (19.47%), linoleic (15.86%), palmitic (5.59%) and stearic acids (3.98%) (Table 3.1). There are many studies are available in literature that indicate

the flaxseed oil as an omega-3 fatty acid source [17], [66], [88], [89]. Based on the results of analysis, flaxseed oil can be considered as an omega-3 source.

Table 3.1 Fatty acid composition of the flaxseed oil

Fatty acids	Results (%)
Myristic acid (C14:0)	0.05
Pentadeconoic acid (C15:0)	0.02
Palmitic acid (C16:0)	5.59
Palmitoleic acid (C16:1)	0.07
Heptadeconoic acid (C17:0)	0.05
Stearic acid (C18:0)	3.98
Oleic acid (C18:1n9c)	19.47
Linoleic acid (C18:2n6c)	15.86
Arachidic acid (C20:0)	0.4
γ-Linolenic acid (C18:3n6)	0.17
cis-1-Eicosenoic acid (C20:1)	0.11
α- Linolenic acid (C18:3n3)	48.52
cis-11,14,17-Eicosadienoic acid (C20:2)	0.06
Behenic acid (C22:0)	0.09
cis-11,14,17-Eisatrieonic acid (C20:3n3)	0.03
Lignoseric acid (C24:0)	0.06
Omega-3	48.55
Omega-6	16.03

3.3.2. Physical Stability of the Flaxseed Emulsions

Physical stability for the emulsions is defined as the capability to resist the separation of the phases [66], and the emulsions are held at a particular condition for a defined time interval to evaluate of the physical stability. Table 3.2 shows the physical stabilities of the flaxseed oil emulsions during 15-day storage. Emulsions were physically very stable (100%) for the first 9 days at 25±1°C. There was a slight separation of the emulsions for the samples stored for 12 and 15 days. However, the difference between the emulsion stability of the samples was not significant ($p>0.05$). Similar results were available in literature for the emulsions of flaxseed oils. Goyal et al. [16] determined no separation of phases in flaxseed oil emulsions stabilized with whey protein concentrate during 28 days. Tonon et al. [79] produced the flaxseed oil emulsions at various oil fractions (10-30% oil) using gum arabic and observed no separation for 24 h storage. In another study, Carneiro et al. [72] reported that a slight separation was observed 24 h after the production of flaxseed oil emulsions stabilized with maltodextrin.

Table 3.2 Physical stability of the flaxseed oil emulsions

Time (day)	Stability (%)
0	100.0 a
3	100.0 a
6	100.0 a
9	100.0 a
12	96.9 a
15	97.8 a

Values having the same letters are not significantly different ($p>0.05$)

3.3.3. Changes in the pH of the Flaxseed Oil Emulsions

Table 3.3 illustrates the pH values of the emulsions during 15-day storage period. The pH decreased from an initial value of 6.65 to 5.60 after 15-day storage at 25±1°C. The lowest pH (pH 4.80) was determined for the samples stored at 9 day. But the decrease in the pH did not change in direct proportion with the increase in the storage day. After the 9^{th} day, the pH of the emulsions increased to 5.01 and 5.60 for the 12^{th} and 15^{th} days, respectively.

Electrostatic stability of the emulsions is dependent on the pH, due to alteration in the charge on the surface or the Zeta (ζ)-potential of the emulsions that varies with the pH [90]. The decrease in the pH of the emulsion may cause a decrease in the net negative charge, but this decrease was not adequate to illustrate some physical separation during 15-day storage period. Similar results were also reported by Kumar et al. [66]. They observed that the pH of the buttermilk concentrate flaxseed oil emulsion decreased during storage without any phase separation. In another study, Hu et al. [51] examined effects of pH of the emulsions on the physical stability and reported that low pH (2.9-3.0) causing the low stability while the ones produced at pH 4-9 was unaffected during storage of 2 months. Surface charge on the emulsions changes due to the pH and ionic strength of the emulsions. The pH of the emulsions cannot directly give a suggestion about the characteristics of the emulsions. pH is an important parameter in the thickness and the structural organization of biopolymer molecules in terms of interfacial layer of emulsion [53].

Table 3.3 pH values of the emulsions during storage

Time (day)	pH
0	6.65
3	6.47
6	5.74
9	4.80
12	5.01
15	5.60

3.3.4. Changes in the Size Distribution and Zeta (ζ)-Potential of Flaxseed Oil Emulsions

The size of the emulsions droplets are the essential properties for emulsion-based food products for the determination of its shelf life, appearance, texture, and flavor. The fraction of particles of emulsions in different size classes are evaluated by the particle size distribution. Particle size distribution changes with depending on the various factors such as quantity of encapsulating agent, emulsification technique and components of the emulsion [17].

The alterations in the size distribution of flaxseed oil emulsions during the storage at 25±1°C are illustrated in Figure 3.1. During 15-day storage period, particle size (z-average) of flaxseed oil emulsions increased up to 9 days (Figure 3.1). The polydispersity index (PDI) of the emulsions changed from 0.63 to 0.61. When the PDI value is greater than 0.7, particles have wide distribution due to the more heterogeneous nature [91], which increases the probability of aggregation of the emulsion droplets. The z-average of the flaxseed oil emulsions at the 0^{th} day was 424.1 nm. The highest respective z-average was reached on the 9^{th} day, while the minimum values were determined for 0^{th} day of storage. After 12 days storage, the z-average values of the emulsions decreased to 620.1 nm and emulsions also had tendency to decrease at the 15^{th} day. The final measurement at the end of the 15 days was determined as 583.1 nm. Studies about the particle size of emulsions are available in the literature. Kumar et al. [66] prepared flaxseed oil emulsion using buttermilk concentrate and reported the z-average as 403.4 nm at zero day. They also indicated that z-average value steadily increased during 15 days of storage. Wang et al. [92] measured the z-average as 700-1300 nm for the emulsions of soybean oil that stabilized with using flaxseed protein. In addition to that, smaller z-average values (135 nm) was reached by the Kentish et al. [93] for the flaxseed oil emulsions that prepared under high pressure (100 MPa) stabilized with tween 40 surfactant (a synthetic emulsifier). Tonon et al. [79] prepared

the flaxseed oil emulsions with using three different stabilizing agents as whey protein, gum arabic and modified starch. Although emulsions stabilized with modified starch had the largest particle size with the widest distribution than the ones prepared with the other wall materials, the emulsions had the best stability.

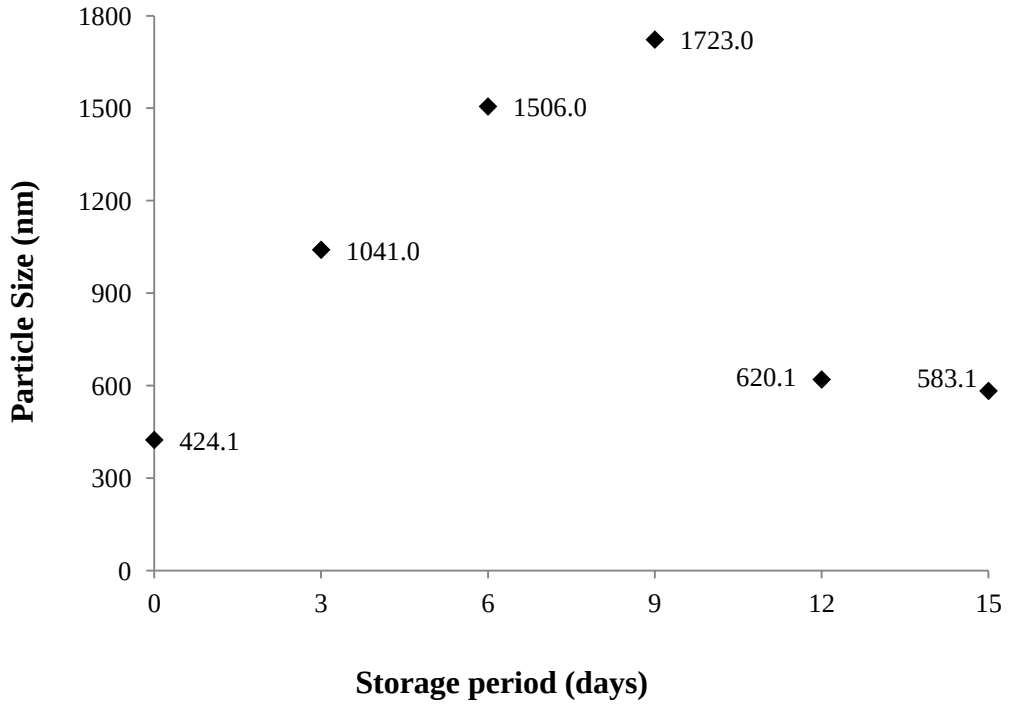

Figure 3.1 Effect of storage on the particle size of flaxseed oil emulsions

Zeta (ζ)-potential is defined as "the potential variation between the dispersion medium and the motionless layer of fluid attached to a dispersed particle". The ζ-potential is "a scale of the amount of the electrostatic repulsion/attraction between particles" and is an elementary parameter that affect the stability [94]. When the particles have ζ-potential value in the range of between +30 mV and -30 mV, they are defined as less stable [95]. On the other hand, particles that has ζ-potential values as higher than +30 mV and lower than -30 mV considered as particles with high ζ-potential values. The charge on droplet can affect the rheological properties of an emulsion. The emulsion with high ζ-potential (negative or positive) are electrically stable, on the other hand the ones with low ζ-potential have a tendency to coalesce or flocculate [16]. Since, high ζ-potential causes the increasing in the electrostatic repulsions between the particles that cause the decrease on the Vander Waals forces.

These forces are responsible for the agglomeration which leads to particles to be larger [96].

ζ-potential value of the emulsions on different days of storage at the 25±1°C are presented in Figure 3.2. ζ-potential values decreased from -59.0 to -61.2 mV during storage period up to 6 days. The flaxseed oil emulsion had the highest (-59 mV) and lowest (-61.2 mV) ζ-potential values on the 0 day and 3rd, 6th day, respectively. Jeong et al. [94] found the ζ-potential of the resistant starch nanoparticles varying between -37.7 and -43.3 mV and indicated that the most stable nanoparticle was having the ζ-potential of -43.3 mV. The electrical characteristics of emulsion droplets can be managed with the selection of particular emulsifier types. For example stabilization of droplets made using polysaccharide emulsifiers have a tendency for a negative charge (for example, gum arabic, modified starch, and beet pectin) [90]. Ge et al. [7] used various starch nanoparticles to stabilize the Pickering emulsions and recorded the ζ-potential values between -14.5 and -17.4 mV. Another study about ζ-potential of emulsions, flaxseed oil encapsulation by buttermilk solids, were indicated that ζ-potential varying between -22.33 to -21.47 mV for the 15 storage period [66]. Khalloufi et al. [97] determined around -50 mV ζ–potential on their study about emulsions which were stabilized with mixture of whey protein isolate and flaxseed gum.

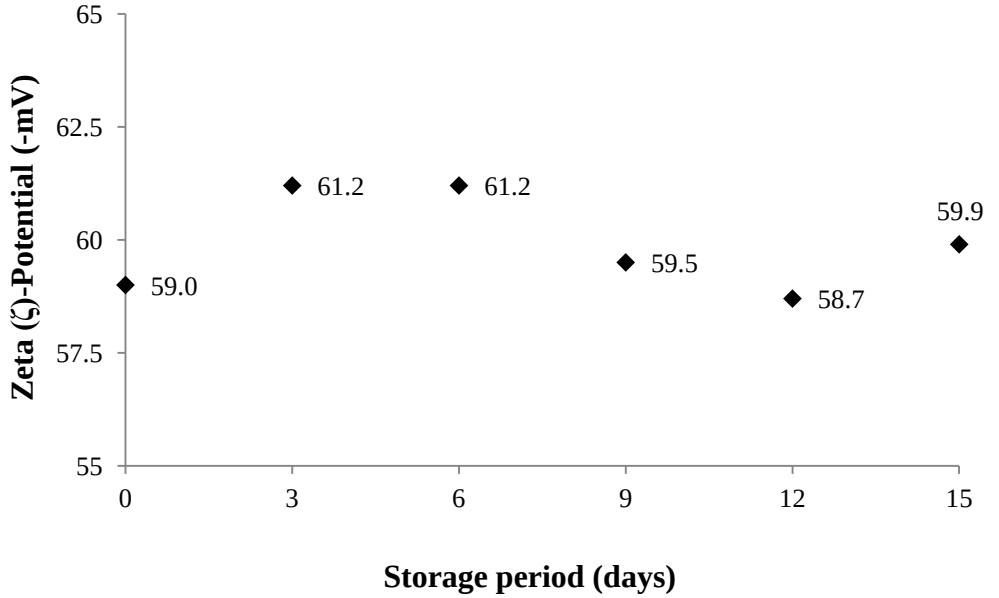

Figure 3.2 Effect of the storage on the zeta potential of flaxseed oil emulsions

3.3.5. Changes in the Peroxide Value of Flaxseed Oil during Storage

The peroxide value of the emulsions and the native flaxseed oil was determined to analyze the primary oxidation products (lipid hydroperoxides). Although the formation of hydroperoxides throughout the primary oxidation of oils are in a colorless and odorless form, they affect the quality of the product in negatively [66]. Flaxseed oil is very sensitive to oxidation because of its rich composition of ALA (Section 3.3.1). ALA (C18:3) is twenty times more susceptible to oxidation than oleic acid (C18:1) [98].

Peroxide value of the native flaxseed oil and encapsulated flaxseed oil in the form of emulsions are shown in Table 3.4. Both samples (native flaxseed oil and encapsulated oil) had same peroxide value at the initial storage time. The flaxseed oil at the initial time of the storage had the lowest peroxide value (0.98 meq peroxide/kg oil) and the peroxide value increased with an increase in the storage time. Peroxide value of the native flaxseed oil increased from 0.98 to 2.41 meq peroxide/kg oil during 15 days storage at the 25±1°C. This increase was significant ($p<0.05$) for some of the samples. There was a minor increase in the peroxide value of the samples stored for 6, 9 and 12 days, however the increase was not significant ($p>0.05$). The highest peroxide value was achieved with the flaxseed oil sample stored for 15 days. The increase in the peroxide value of the native flaxseed oil throughout the storage period indicates the primary oxidation.

Similar to the physical stability values of the flaxseed oil emulsions (Table 3.2), the peroxide value of the emulsions stored for 15 days did not change significantly ($p>0.05$). Encapsulated flaxseed oil into emulsion is more stable than native flaxseed oil against oxidation. Similar to our results, Goyal et al. [16] also reported that the peroxide value of emulsified flaxseed oil was lower than the native one. They indicated that the peroxide value of native flaxseed oil increased 44.26% of its initial value, while the ones encapsulated into emulsions only increased 20.93%. Peroxide value of flaxseed oil in emulsions (0.99 meq/kg oil) were below the upper value (15 meq peroxide/kg) stated by Codex Alimentarius Commission [99] for the cold-pressed and virgin oils.

Table 3.4 Peroxide values of the native and emulsified flaxseed oil during storage

Time (day)	Peroxide Value (meq peroxide/ kg oil)	
	Flaxseed oil (Native)	Flaxseed oil (Encapsulated)
0	0.98 d	0.98a
3	1.23 c	0.98 a
6	1.46 b	0.99 a
9	1.47 b	0.98 a
12	1.48 b	0.99 a
15	2.41 a	0.99 a

Means with different letters within each column are significantly different ($p<0.05$)

Chapter 4

4. Conclusion and Future Prospects

4.1. Conclusions

In the first part of the thesis, starch nanoparticles were produced from native wheat starch and RS4 using acid hydrolysis method and their potential to be used as Pickering emulsion stabilizers were investigated. As the yield of the wheat starch samples (1.6-17.3%) were very low compared to the RS4 samples (17.0- 56.0%), starch nanoparticles prepared from RS4 samples were used for Pickering emulsion experiments. Firstly, to investigate the effect of hydrolysis conditions of the starch nanoparticles on the stability, Pickering emulsions were prepared using starch nanoparticles (2%) at both Φ0.6 and Φ0.8 oil fractions for sunflower and corn oils. Among these emulsions, the most stable ones were prepared using corn oil and the most stable emulsion (Φ0.6) was prepared using the starch nanoparticle (2%) produced with the hydrolysis of RS4 for 3 days at a starch:H_2SO_4 ratio of 1:3 (1:3 (3)). The effect of starch nanoparticle ratio (w/w%) and oil fraction (Φ) on the stability of Pickering emulsions were also investigated. For this purpose, corn oil was used as the oil phase and emulsions were prepared at different oil fractions (Φ0.2, Φ0.4 and Φ0.6) using various starch nanoparticle (1:3 (3)) ratios (0, 1, 2 and 3%). The most stable emulsion was achieved at Φ0.2 oil fraction by using 3% starch nanoparticle (w/w) ratio.

In the second part of the thesis, as an emulsion stabilizer. Pickering emulsions of flaxseed oil were prepared at Φ0.2 flaxseed oil fraction with 3% (w/w) starch nanoparticle that was produced at a starch:H_2SO_4 ratio of 1:3 (g/ml) for 3 days hydrolysis (Sample ID; 1:3 (3)). The flaxseed oil emulsions were physically stable for a storage period of 15 days at 25±1°C. While the peroxide value of the flaxseed oil stored for 15 days increased significantly, the peroxide value of the emulsions did not change significantly.

We can conclude that starch nanoparticles can be used as emulsion stabilizers and Pickering emulsions stabilized using starch nanoparticle protects the lipid oxidation of

flaxseed oil and increase its stability during storage. The most important finding is that the emulsion systems can be created using starch nanoparticles, which is a natural source, which will lead a decrease in the use of synthetic emulsifiers.

4.2. Societal Impact and Contribution to Global Sustainability

Many consumer products such as “food, nutritional supplements, pharmaceutical, personal care, and cosmetic products” are prepared using emulsions. Emulsions are defined as “thermodinamically unstable systems that have a tendency to separate back into their components during storage”. To produce products with long shelf life, emulsions should be stabilized using emulsifiers. Most of the emulsifiers used in the food industry are artificial (synthetic), and some of them are derived from animal sources. Depletion of the resources and increasing environmental concerns have also increased the demand for the products produced from renewable and sustainable sources in food production, as in many areas. The demand for natural ingredients in food products is also increasing due to the increase in the consumer awareness of the importance of healthy eating. Therefore, food producers are trying to substitute synthetic emulsifiers with the natural ones.

Starch is one of the biopolymers commonly found in nature and synthesized by plants. It is a polysaccharide formed by the polymerization of α-D-glucose units and consist of two types of polymers; “amylose” and “amylopectin”. Most of the carbohydrates, as well as starch, are not good emulsifiers as they are mainly consisting of polar monosaccharides. Their chemical structure should be modified somehow to increase their ability to stabilize emulsions. One of the modification methods is acid hydrolysis. The amorphous part of starch is susceptible to acid hydrolysis, on the other hand the crystalline part is more resistant. During acid hydrolysis, the amorphous part (mainly the amylose and the linear parts of amylopectin) is converted to smaller parts and removed from the media with washing. The remaining parts, which can be also called as “starch nanoparticles” have both hydrophobic and hydrophilic parts, which make them possible stabilizers for emulsions.

The emulsion systems can be used as delivery systems for some bioactive components, which have positive health effects. Omega-3 fatty acids are essential fatty acids for human health and should be taken from the diet. However, they are

unsaturated fatty acids which can be easily oxidized in the presence of oxygen and light. Lipid oxidation is an important problem that deteriorates the food quality. It negatively effects the shelf life of food products as well as decreases the nutritive value. Besides, as a result of lipid oxidation a wide range of oxidation products that have “mutagenic, carcinogenic and cytotoxic properties” which are produced. These products can be considered as a risk factor for human health. Due to the beneficial health of omega-3 fatty acids, they should be incorporated into food products. However, they need to be protected against oxidation during processing and storage. Encapsulation technology is one of the methods that protects omega-3 fatty acids and allow their delivery into food products.

In this thesis we have produced nanoparticles with high yield using starch which is an abundant source in the nature. We have used these nanoparticles as emulsion stabilizer in Pickering emulsions as a delivery system of omega-3 fatty acids. With this thesis we successfully overcome the oxidation problem of omega-3 fatty acids by preparing a stable flaxseed oil Pickering emulsion stabilized with starch nanoparticles. We believe that this thesis will make a contribution in terms of sustainability since we have produced a plant-based emulsifier. In addition, in this thesis omega-3 fatty acids, which have positive effects on health, are encapsulated using a newly plant based emulsifier which makes the thesis. this thesis will also shed light on studies on good health and well being. In addition, since omega-3 fatty acids, which have positive effects on health are encapsulated this thesis will also shed light on studies on health and well-being.

4.3. Future Prospects

For the future work, model food systems based on Pickering emulsions have to be prepared to better investigate the effect of starch nanoparticles on the physical and chemical stability of emulsions. In this thesis we have analyzed the primary oxidation products. Secondary oxidation products can also be analyzed to better understand the influence of starch nanoparticles on the protective effect of lipid oxidation of omega-3 fatty acids.

BIBLIOGRAPHY

[1] D. J. McClements, "Food Emulsions: Principles, Practices, and Techniques", CRC Press, (2004).

[2] A. I. A. Mohamed, I. A. Hussein, A. S. Sultan, and G. A. Al-Muntasheri, “Use of organoclay as a stabilizer for water-in-oil emulsions under high-temperature high-salinity conditions,” J. Pet. Sci. Eng., vol. 160, pp. 302–312, (2018).

[3] C. Qiu, R. Chang, J. Yang, S. Ge, L. Xiong, M. Zhao, M. Li, “Preparation and characterization of essential oil-loaded starch nanoparticles formed by short glucan chains,” Food Chem., vol. 221, pp. 1426–1433, (2017).

[4] D. Marku, M. Wahlgren, M. Rayner, M. Sjöö, and A. Timgren, “Characterization of starch Pickering emulsions for potential applications in topical formulations,” Int. J. Pharm., vol. 428, no. 1–2, pp. 1–7, (2012).

[5] Y. Chevalier and M. A. Bolzinger, “Emulsions stabilized with solid nanoparticles: Pickering emulsions,” Colloids Surfaces A Physicochem. Eng. Asp., vol. 439, pp. 23–34, (2013).

[6] E. Dickinson, “Food emulsions and foams: Stabilization by particles,” Curr. Opin. Colloid Interface Sci., vol. 15, no. 1–2, pp. 40–49, (2010).

[7] S. Ge, L. Xiong, M. Li, J. Liu, J. Yang, R. Chang, C. Liang, “Characterizations of Pickering emulsions stabilized by starch nanoparticles: Influence of starch variety and particle size,” Food Chem., vol. 234, pp. 339–347, (2017).

[8] H. N. Liang and C. he Tang, “Pea protein exhibits a novel Pickering stabilization for oil-in-water emulsions at pH 3.0,” LWT - Food Sci. Technol., vol. 58, no. 2, pp. 463–469, (2014).

[9] Y. Tan, K. Xu, C. Liu, Y. Li, C. Lu, and P. Wang, “Fabrication of starch-based nanospheres to stabilize pickering emulsion,” Carbohydr. Polym., vol. 88, no. 4, pp. 1358–1363, (2012).

[10] C. Moraru, C. Panchapakesan, Q. Huang, P. Takhistov and J. Kokini, “Nanotechnology: A New Frontier in Food Science ,” Food technology, (2003).

[11] E. Šárka and V. Dvořáček, “Waxy starch as a perspective raw material (a review),” Food Hydrocoll., vol. 69, pp. 402–409, (2017).

[12] Starch: Foods, Digestion, Glycemic Index, https://www.nutrientsreview.com/carbs/polysaccharides-starch.html (Accessed: 24-Jan-2021).

[13] R. C. M. Paula and J. F. Kennedy, Food: The Chemistry of its Components, vol. 18, no. 3. (1992).

[14] D. Le Corre and H. Angellier-Coussy, “Preparation and application of starch nanoparticles for nanocomposites: A review,” React. Funct. Polym., vol. 85, pp. 97–120, (2014).

[15] M. Fathi, Á. Martín, and D. J. McClements, “Nanoencapsulation of food ingredients using carbohydrate based delivery systems,” in Trends in Food Science and Technology, vol. 39, no. 1, Elsevier, pp. 18–39, (2014).

[16] A. Goyal, V. Sharma, N. Upadhyay, A. Singh, S. Arora, “Development of stable flaxseed oil emulsions as a potential delivery system of ω-3 fatty acids,” J. Food Sci. Technol., vol. 52, no. 7, pp. 4256–4265, (2015).

[17] A. Goyal, V. Sharma, N. Upadhyay, S. Gill, and M. Sihag, “Flax and flaxseed oil: an ancient medicine & modern functional food,” J. Food Sci. Technol., vol. 51, no. 9, pp. 1633–1653, (2014).

[18] A. P. Simopoulos, “Omega-3 fatty acids in health and disease and in growth and development,” Am. J. Clin. Nutr., vol. 54, no. 3, pp. 438–463, (1991).
[19] J. S. Truan, J. M. Chen, and L. U. Thompson, “Comparative effects of sesame seed lignan and flaxseed lignan in reducing the growth of human breast tumors (MCF-7) at high levels of circulating estrogen in athymic mice,” Nutr. Cancer, vol. 64, no. 1, pp. 65–71, (2012).
[20] K. Prasad, S. V. Mantha, A. D. Muir, and N. D. Westcott, “Protective effect of secoisolariciresinol diglucoside against streptozotocin-induced diabetes and its mechanism,” Mol. Cell. Biochem., vol. 206, no. 1–2, pp. 141–150, (2000).
[21] H. Adlercreutz, “Lignans and human health,” Critical Reviews in Clinical Laboratory Sciences, vol. 44, no. 5–6. Crit Rev Clin Lab Sci, pp. 483–525, Sep-(2007).
[22] B. Gong, W. Liu, X. Chen, H. Tan, X. Zhang, H. Wang, D. Yu, G. Li, Z. Song, “Stabilizing alkenyl succinic anhydride (ASA) emulsions with starch nanocrystals and fluorescent carbon dots,” Carbohydr. Polym., vol. 165, pp. 13–21, (2017).
[23] S. B. Haaj, W. Thielemans, A. Magnin, and S. Boufi, “Starch Nanocrystal Stabilized Pickering Emulsion Polymerization for Nanocomposites with Improved Performance,” ACS Appl. Mater. Interfaces, vol. 6, no. 11, pp. 8263–8273, (2014).
[24] X. S. Sha, ZJ. Xiang, L. Bin, L. Zing, Y. Jiao, S. Kun, “Preparation and physical characteristics of resistant starch (type 4) in acetylated indica rice,” Food Chem., vol. 134, no. 1, pp. 149–154, (2012).
[25] A. Korkut and K. Kahraman, “Production of cross-linked resistant starch from tapioca starch and effect of reaction conditions on the functional properties, morphology, X‑ray pattern, FT-IR spectra and digestibility,” J. Food Meas. Charact., no. 0123456789, (2021).
[26] X. Zhai, D. Lin, D. Liu, and X. Yang, “Emulsions stabilized by nano fi bers from bacterial cellulose : New potential food-grade Pickering emulsions,” Food Res. Int., vol. 103, no. July 2017, pp. 12–20, (2018).
[27] A. Domb and W. Khan, "Biodegradable Polymers as Drug Carrier Systems," Crc Press, (2013).
[28] M. Matos, A. Laca, F. Rea, O. Iglesias, M. Rayner, and G. Gutiérrez, “O/W emulsions stabilized by OSA-modified starch granules versus non-ionic surfactant: Stability, rheological behaviour and resveratrol encapsulation,” J. Food Eng., vol. 222, pp. 207–217, (2018).
[29] F. Liu, J. Zheng, C. Huang, C. Tang, and S. Ou, “Food Hydrocolloids Pickering high internal phase emulsions stabilized by protein-covered cellulose nanocrystals,” Food Hydrocoll., vol. 82, pp. 96–105, (2018).
[30] A. Pandey, M. Derakhshandeh. S. Kedzior, B. Pilapil “Role of interparticle interactions on microstructural and rheological properties of cellulose nanocrystal stabilized emulsions,” J. Colloid Interface Sci., vol. 532, pp. 808–818, (2018).
[31] A. Korkut and K. Kahraman, “Nişasta Nanokristali Üretimi ve Karakterizasyonu,” Eur. J. Sci. Technol., no. 17, pp. 471–476, (2019).
[32] H.-Y. Kim, S. S. Park, and S.-T. Lim, “Preparation, characterization and utilization of starch nanoparticles,” Colloids Surfaces B Biointerfaces, vol. 126, pp. 607–620, (2015).
[33] H. Angellier, L. Choisnard, S. Molina-Boisseau, A. Patrick Ozil, and Alain Dufresne, “Optimization of the Preparation of Aqueous Suspensions of Waxy Maize Starch Nanocrystals Using a Response Surface Methodology,”

Biomacromolecules, vol. 5, pp. 1545–1551, (2004).
[34] D. Liu, Q. Wu, H. Chen, and P. R. Chang, “Transitional properties of starch colloid with particle size reduction from micro- to nanometer,” J. Colloid Interface Sci., vol. 339, no. 1, pp. 117–124, (2009).
[35] Q. Sun, M. Gong, Y. Li, and L. Xiong, “Effect of retrogradation time on preparation and characterization of proso millet starch nanoparticles,” Carbohydr. Polym., vol. 111, pp. 133–138, (2014).
[36] C. Li, P. Sun, and C. Yang, “Emulsion stabilized by starch nanocrystals,” Starch/Staerke, vol. 64, no. 6, pp. 497–502, (2012).
[37] J. Xie, C. Hse, C. F. De Hoop, T. Hu, J. Qi, and T. F. Shupe, “Isolation and characterization of cellulose nanofibers from bamboo using microwave liquefaction combined with chemical treatment and ultrasonication,” Carbohydr. Polym., vol. 151, pp. 725–734, (2016).
[38] J. H. Kim, D. H. Park, and J.-Y. Kim, “Effect of heat-moisture treatment under mildly acidic condition on fragmentation of waxy maize starch granules into nanoparticles,” Food Hydrocoll., vol. 63, pp. 59–66, (2017).
[39] mgp ingredients: https://www.mgpingredients.com/sites/default/files/2018-03/Fibersym (Accessed: 02-Dec-2020).
[40] L. U. Thompson, C. C. Maningat, K. Woo, and P. A. Seib, “In vitro digestion of RS4-type resistant wheat and potato starches, and fermentation of indigestible fractions,” Cereal Chem., vol. 88, no. 1, pp. 72–79, (2011).
[41] H.-Y. Kim, J. H. Lee, J.-Y. Kim, W.-J. Lim, and S.-T. Lim, “Characterization of nanoparticles prepared by acid hydrolysis of various starches,” Starch - Stärke, vol. 64, no. 5, pp. 367–373, (2012).
[42] A. Dufresne, “Crystalline starch based nanoparticles,” Curr. Opin. Colloid Interface Sci., vol. 19, no. 5, pp. 397–408, (2014).
[43] J. Saeng-on and D. Aht-Ong, “Production of starch nanocrystals from agricultural materials using mild acid hydrolysis method: Optimization and characterization,” Polym. from Renew. Resour., vol. 8, no. 3, pp. 91–116, (2017).
[44] M. Mariano, A. Mukurumbira, E. O. Amonsou, A. Dufresne, and J. J. Mellem, “Microstructure, thermal properties and crystallinity of amadumbe starch nanocrystals,” Int. J. Biol. Macromol., vol. 102, pp. 241–247, (2017).
[45] N. Da Silva Miranda Sechi and P. T. Marques, “Preparation and physicochemical, structural and morphological characterization of phosphorylated starch,” in Materials Research, vol. 20, pp. 174–180, (2017).
[46] A. León, P. Reuquen, C. Garin, R. Segura, P. Vargas, “FTIR and raman characterization of TiO2 nanoparticles coated with polyethylene glycol as carrier for 2-methoxyestradiol,” Appl. Sci., vol. 7, no. 1, pp. 1–9, (2017).
[47] B. A. Ashwar, A. Gani, A. Shah, and F. A. Masoodi, “Physicochemical properties, in-vitro digestibility and structural elucidation of RS4 from rice starch,” Int. J. Biol. Macromol., vol. 105, pp. 471–477, (2017).
[48] F. Gao, D. Li, C. H. Bi, Z. H. Mao, and B. Adhikari, “Preparation and characterization of starch crosslinked with sodium trimetaphosphate and hydrolyzed by enzymes,” Carbohydr. Polym., (2014).
[49] H. Peng, H. Xiong, and S. Wang, “Soluble starch – based biodegradable and microporous microspheres as potential adsorbent for stabilization and controlled release of coix seed oil,” pp. 693–702, (2011).
[50] N. W. H. Cheetham and L. Tao, “Variation in crystalline type with amylose content in maize starch granules: An X-ray powder diffraction study,” Carbohydr. Polym., vol. 36, no. 4, pp. 277–284, (1998).

[51] Y. Q. Hu, S. Yin, J. Zhu, J. Qi, J. Guo, L. Wu, “Fabrication and characterization of novel Pickering emulsions and Pickering high internal emulsions stabilized by gliadin colloidal particles,” Food Hydrocoll., vol. 61, pp. 300–310, (2016).
[52] Y. Tan, K. Xu, C. Niu, C. Liu,Y. Li, P. Wang “Triglyceride-water emulsions stabilised by starch-based nanoparticles,” Food Hydrocoll., vol. 36, pp. 70–75, (2014).
[53] E. Dickinson, “Milk protein interfacial layers and the relationship to emulsion stability and rheology,” Colloids and Surfaces B: Biointerfaces, vol. 20, no. 3., pp. 197–210, (2001).
[54] D. J. McClements, “Protein-stabilized emulsions,” Current Opinion in Colloid and Interface Science, vol. 9, no. 5. pp. 305–313, (2004).
[55] AACCI, AACC Approved Methods of Analysis, 11th ed. St. Paul, MN: The Association, (2000).
[56] M. V. Tzoumaki, T. Moschakis, V. Kiosseoglou, and C. G. Biliaderis, “Oil-in-water emulsions stabilized by chitin nanocrystal particles,” Food Hydrocoll., vol. 25, no. 6, pp. 1521–1529, (2011).
[57] M. Jo, C. Ban, K. K. T. Goh, and Y. J. Choi, “Influence of chitosan-coating on the stability and digestion of emulsions stabilized by waxy maize starch crystals,” Food Hydrocoll., vol. 94, no. March, pp. 603–612, (2019).
[58] W. E. Connor, “Importance of n−3 fatty acids in health and disease,” Am. J. Clin. Nutr., vol. 71, no. 1, pp. 171S-175S, (2000).
[59] P. Kaushik, K. Dowling, C. J. Barrow, and B. Adhikari, “Microencapsulation of omega-3 fatty acids: A review of microencapsulation and characterization methods,” J. Funct. Foods, vol. 19, pp. 868–881, (2015).
[60] J. A. Tur, M. M. Bibiloni, A. Sureda, and A. Pons, “Dietary sources of omega 3 fatty acids: public health risks and benefits,” Br. J. Nutr., vol. 107, no. S2, pp. S23–S52, (2012).
[61] B. Koletzko, C. Agostoni, S. Carlson, T. Clandinin, G. Hornstra, M. Neuringer, “Long chain polyunsaturated fatty acids (LC-PUFA) and perinatal development,” Acta Paediatr., vol. 90, no. 4, pp. 460–464, (2007).
[62] S. Chatterjee and Z. M. A. Judeh, “Microencapsulation of fish oil,” Lipid Technology, (2016).
[63] M. Tańska, B. Roszkowska, M. Skrajda, and G. Dąbrowski, “Commercial Cold Pressed Flaxseed Oils Quality and Oxidative Stability at the Beginning and the End of Their Shelf Life,” J. Oleo Sci, vol. 65, no. 2, pp. 111–121, (2016).
[64] S. Ivanov, T. Rashevskaya, and M. Makhonina, “Flaxseed additive application in dairy products production,” Procedia Food Sci., vol. 1, pp. 275–280, (2011).
[65] B. D. Oomah, “Flaxseed as a functional food source,” J. Sci. Food Agric., vol. 81, no. 9, pp. 889–894, (2001).
[66] R. Kumar, L. Sabikhi, G. Rathod, and N. Chaudhary, “Storage Studies of Flaxseed Oil Encapsulated by Buttermilk Solids,” Food Bioprocess Technol., vol. 13, no. 8, pp. 1392–1404, (2020).
[67] J. C. P. Dribnenki, S. F. McEachern, Y. Chen, A. G. Green, and K. Y. Rashid, “2149 Solin (low linolenic flax),” Can. J. Plant Sci., vol. 87, no. 2, pp. 297–299, (2007).
[68] J. Faintuch, L. A. Bortolotto, P. C. Marques, J. J. Faintuch, J. I. França, and I. Cecconello, “Systemic inflammation and carotid diameter in obese patients: Pilot comparative study with flaxseed powder and cassava powder,” Nutr. Hosp., vol. 26, no. 1, pp. 208–213, (2011).
[69] K. K. Singh, D. Mridula, J. Rehal, and P. Barnwal, “Flaxseed: A potential source

of food, feed and fiber," Crit. Rev. Food Sci. Nutr., vol. 51, no. 3, pp. 210–222, (2011).
[70] C. K. O. Dzuvor, J. T. Taylor, C. Acquah, S. Pan, and D. Agyei, "Bioprocessing of functional ingredients from flaxseed," Molecules, vol. 23, no. 10, pp. 1–18, (2018).
[71] M. Rubilar, C. Gutiérrez, M. Verdugo, C. Shene, and J. Sineiro, "Flaxseed as a source of functional ingredients," Journal of Soil Science and Plant Nutrition, vol. 10, no. 3. Sociedad Chilena de la Ciencia del Suelo, pp. 373–377, (2010).
[72] H. C. F. Carneiro, R. V. Tonon, C. R. F. Grosso, and M. D. Hubinger, "Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials," J. Food Eng., vol. 115, no. 4, pp. 443–451, (2013).
[73] S. K. Gebauer, T. L. Psota, W. S. Harris, and P. M. Kris-Etherton, "n-3 Fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits," in American Journal of Clinical Nutrition, vol. 83, no. 6, (2006).
[74] M. A. Pellizzon, J. T. Billheimer, L. T. Bloedon, P. O. Szapary, and D. J. Rader, "Flaxseed Reduces Plasma Cholesterol Levels in Hypercholesterolemic Mouse Models," J. Am. Coll. Nutr., vol. 26, no. 1, pp. 66–75, (2007).
[75] J. A. Austria, M. Richard, N. Melanie, N. Mirna, L. Andrea, "Bioavailability of Alpha-Linolenic Acid in Subjects after Ingestion of Three Different Forms of Flaxseed," J. Am. Coll. Nutr., vol. 27, no. 2, pp. 214–221, (2008).
[76] M. A. Augustin and Y. Hemar, "Nano- and micro-structured assemblies for encapsulation of food ingredients," Chem. Soc. Rev., vol. 38, no. 4, pp. 902–912, (2009).
[77] B. F. Gibbs, S. Kermasha, I. Alli, and C. N. Mulligan, "Encapsulation in the food industry: A review," Int. J. Food Sci. Nutr., vol. 50, no. 3, pp. 213–224, (1999).
[78] A. Madene, M. Jacquot, J. Scher, and S. Desobry, "Flavour encapsulation and controlled release - A review," Int. J. Food Sci. Technol., vol. 41, no. 1, pp. 1–21, (2006).
[79] R. V. Tonon, R. B. Pedro, C. R. F. Grosso, and M. D. Hubinger, "Microencapsulation of Flaxseed Oil by Spray Drying: Effect of Oil Load and Type of Wall Material," Dry. Technol., vol. 30, no. 13, pp. 1491–1501, (2012).
[80] R. Puupponen-Pimiä, A. Aura, K. Oksman "Development of functional ingredients for gut health," Trends Food Sci. Technol., vol. 13, no. 1, pp. 3–11, (2002).
[81] C. P. Champagne and P. Fustier, "Microencapsulation for the improved delivery of bioactive compounds into foods," Current Opinion in Biotechnology, vol. 18, no. 2. Curr Opin Biotechnol, pp. 184–190, (2007).
[82] K. Heinzelmann, K. Franke, J. Velasco, and G. Márquez-Ruiz, "Microencapsulation of fish oil by freeze-drying techniques and influence of process parameters on oxidative stability during storage," Eur. Food Res. Technol., vol. 211, no. 4, pp. 234–239, (2000).
[83] K. Taguchi, K. Iwami, F. Ibuki, and M. Kawabata, "Oxidative Stability of Sardine Oil Embedded in Spray-dried Egg White Powder and Its Use for n-3 Unsaturated Fatty Acid Fortification of Cookies," Biosci. Biotechnol. Biochem., vol. 56, no. 4, pp. 560–563, (1992).
[84] Yasumasa Minemoto, and Shuji Adachi, and R. Matsuno, "Comparison of Oxidation of Methyl Linoleate Encapsulated with Gum Arabic by Hot-Air-Drying and Freeze-Drying," J. Agric. Food. Chem., (1997).

[85] K. Heinzelmann and K. Franke, “Using freezing and drying techniques of emulsions for the microencapsulation of fish oil to improve oxidation stability,” Colloids Surfaces B Biointerfaces, vol. 12, no. 3–6, pp. 223–229, (1999).
[86] F. and D. International Union of Pure and Applied Chemistry Commission on Oils, “Standard Methods for the Analysis of Oils, Fats and Derivatives,” p. 151, (1992).
[87] AOAC, “Peroxide Value of Oils and Fats,” AOAC Offical Methods 965.33, vol. 41, no. 5. pp. 38–43, (2000).
[88] R. V. Tonon, C. R. F. Grosso, and M. D. Hubinger, “Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying,” Food Res. Int., vol. 44, no. 1, pp. 282–289, (2011).
[89] S. K. Gebauer, T. L. Psota, W. S. Harris, and P. M. Kris-Etherton, “n-3 Fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits,” in American Journal of Clinical Nutrition, vol. 83, no. 6, (2006).
[90] D. J. McClements, E. A. Decker, and J. Weiss, “Emulsion-Based Delivery Systems for Lipophilic Bioactive Components,” J. Food Sci., vol. 72, no. 8, pp. R109–R124, (2007).
[91] K. J. Sreeram, M. Nidhin, R. Indumathy, and B. U. Nair, “Synthesis of iron oxide nanoparticles of narrow size distribution on polysaccharide templates,” Bull. Mater. Sci., vol. 31, no. 1, pp. 93–96, (2008).
[92] B. Wang, D. Li, L. J. Wang, and N. Özkan, “Effect of concentrated flaxseed protein on the stability and rheological properties of soybean oil-in-water emulsions,” J. Food Eng., vol. 96, no. 4, pp. 555–561, (2010).
[93] S. Kentish, T. J. Wooster, M. Ashokkumar, S. Balachandran, R. Mawson, and L. Simons, “The use of ultrasonics for nanoemulsion preparation,” Innov. Food Sci. Emerg. Technol., vol. 9, no. 2, pp. 170–175, (2008).
[94] O. Jeong and M. Shin, “Preparation and stability of resistant starch nanoparticles, using acid hydrolysis and cross-linking of waxy rice starch,” Food Chem., vol. 256, pp. 77–84, (2018).
[95] A. Barhoum, M. L. García-Betancourt, H. Rahier, and G. Van Assche, “Physicochemical characterization of nanomaterials: Polymorph, composition, wettability, and thermal stability,” in Emerging Applications of Nanoparticles and Architectural Nanostructures: Current Prospects and Future Trends, pp. 255–278, (2018).
[96] M. Ahmad, A. Gani, I. Hassan, Q. Huang, and H. Shabbir, “Production and characterization of starch nanoparticles by mild alkali hydrolysis and ultra-sonication process,” Sci. Rep., vol. 10, no. 1, pp. 1–11, (2020).
[97] S. Khalloufi, M. Alexander, H. Douglas Goff, and M. Corredig, “Physicochemical properties of whey protein isolate stabilized oil-in-water emulsions when mixed with flaxseed gum at neutral pH,” Food Res. Int., vol. 41, no. 10, pp. 964–972, (2008).
[98] E. A. Decker, C. C. Akoh, and R. S. Wilkes, “Incorporation of (n-3) fatty acids in foods: Challenges and opportunities,” J. Nutr., vol. 142, no. 3, (2012).
[99] Codex Alimentarius, “Standard for Named Vegetable Oils Codex Stan 210-1999,” in Codex Alimentarius, pp. 1–13, (1999).

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SELECTED PUBLICATIONS

1) **Korkut, A.,** Kahraman, K., "Production of cross-linked resistant starch from tapioca starch and effect of reaction conditions on the functional properties, morphology, X-ray pattern, FT-IR spectra and digestibility". Journal of Food Measurement and Characterization, https://doi.org/10.1007/s11694-020-00764-y, (2021)

2) **Korkut, A.**, Kahraman, K., "Nişasta Nanokristali Üretimi ve Karakterizasyonu". Avrupa Bilim ve Teknoloji Dergisi, 17, 471-476, (2019)

3) Severcan, S.Ş., **Korkut, A**., Kahraman, K., "Nanocellulose as an ingredient in food industry", International Turkic World Congress on Science and Engineering, Niğde, Türkiye. (Sözlü Sunum) (June 17-18, 2019)