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Pickering Emulsions Stabilized by Starch Nanoparticles

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Abstract

Emulsions are mixtures of immiscible liquids where one liquid is dispersed in another. They are thermodynamically unstable due to high surface energy between the phases, which is not desired in the shelf life of a product. This instability can be controlled using emulsion stabilizers. Due to consumers' negative opinions on artificial materials, there is a great interest in the production of natural emulsion stabilizers. Recent studies focus on Pickering emulsions that are stabilized by solid particles, which offer better stability, biological compatibility, environmental friendliness, lower cost, and toxicity. As an abundant, renewable, biodegradable and non-toxic material, starch has gained attention for its potential use as emulsion stabilizer. Moreover, in recent years starch nanoparticles (SNPs) have gained popularity as emulsion stabilizers. The main purpose of this study is to produce SNP with high yield to be used as an emulsion stabilizer. In the first part of the study, wheat starch and cross-linked wheat starch were hydrolyzed with H_2SO_4 at various starch:H_2SO_4 ratio and different time intervals. Cross-linked wheat starch resulted in significantly ($p<0.05$) higher yield compared to the native wheat starch. SNPs were characterized in terms of their morphology and size. After characterization of SNPs with Scanning Electron Microscopy (SEM), X-Ray diffractometer (XRD) and Fourier Transform Infrared Spectrophotometer (FT-IR), Pickering emulsions were prepared with two different oil fractions (Φ0.6 and Φ0.8) and emulsions were stored for 30 days at room conditions visually examine phase separation. SNP sample produced at a starch: H_2SO_4 ratio of 1:3 and 3 days hydrolysis (1:3 (3)) showed the best emulsion stability without any separation during the storage. The results of the present study indicated that SNPs are promising emulsion stabilizers that can be used in various industries.

Keywords: Emulsion stability, FT-IR, Pickering emulsion, SEM, Starch nanoparticle, XRD

1.Introduction

Emulsions are defined as the mixture of immiscible liquids in which one liquid (disperse phase) is dispersed in another liquid (continuous phase) (Rayees et al., 2024). An 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, which can be named as a stable emulsion (Domb and Khan, 2013). However, emulsions are thermodynamically instable due to the high surface energy between two phases, which is not desired in the shelf life of a product. The instability of emulsions can be prevented by using surface active substances (Rayees et al., 2024). Recent studies focused on emulsions stabilized with solid particles which are defined as Pickering emulsions (Pan et al., 2023). Pickering emulsions have many advantages over conventional emulsions such as better stabilization, biological compatibility, environmental friendliness, low cost, low toxicity (Cassani et al., 2023; Li et al., 2022; Lv et al., 2023; Marku et al., 2012; Matos et al., 2018; Ming et al., 2023; Shabir et al., 2023; Zhai et al., 2018; Zhou et al., 2023). Due to these advantages Pickering emulsions can be widely applied in many fields such as food, agriculture, pharmaceutical,

cosmetics, and petrol extraction (Cahyana et al., 2022; Ming et al., 2023). Interest in natural emulsion stabilizers has increased in recent years due to the biological interactions and irritant properties of surfactants.

Starch, a renewable, biodegradable and non-toxic material that is widely found in nature, has attracted considerable attention due to its possible utilization as emulsion stabilizer. Starch is a polysaccharide formed by the polymerization of α-D-glucose units. It has two macromolecules in its structure, a) amylose is mainly linear and the glucose molecules are linked each other with α-1,4 glucosidic bonds, b) amylopectin is the branched chains consisting of short amylose groups linked to each other with α -1,6 glucosidic bonds (Šárka and Dvořáček, 2017). Depending on the source, starch generally contains around 20–30% amylose and 70–80% amylopectin, (Marta et al., 2023). 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 (Le Corre and Angellier-Coussy, 2014). The size of individual starch granule changes between 2 to 100 µm depending on the source and there are also different regions in its structure that have different size in nanoscale. In addition to having 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 (Korkut and Kahraman, 2019).

There are two methodologies for nanomaterial synthesis; "top-down" and "bottom-up". "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 hydrolysis and acidic hydrolysis (Kim et al., 2015). On the other hand, "bottom-up approach" is building up of a material from the bottom. This approach is also carried out with different techniques such as electrospraying, electrospinning and nanoprecipitation (Kim et al., 2015). Both approaches can be used to produce starch nanoparticles (SNPs). In the literature, there are studies related to SNP production using "acid hydrolysis, enzymatic hydrolysis, high pressure homogenization, ultrasonication, reactive extrusion, gamma irradiation and nanoprecipitation" (Angellier et al., 2004; Kim et al., 2015; Liu et al., 2009; Sun et al., 2014). Acid hydrolysis has been widely used to produce SNPs because of its simplicity and ease to control. There is a big potential of SNPs for industrial applications, i.e. utilization as a composite material, packaging material and drug carrier (Kim et al., 2015). The studies showed that modification of starch size from micro- to nano- size resulted in enhanced functional properties such as formation of stable Pickering emulsions (Gong et al., 2017; Haaj et al., 2014; Marta et al., 2023). While SNPs show hydrophilic properties as they contain many hydroxyl groups in their structure, they also show hydrophobic properties due to their crystalline regions. Emulsion stabilizers must be wettable in both the oil and water phases of the emulsion; thus, SNPs possess significant promise for emulsion stabilization as they have both hydrophobic and hydrophilic properties.

Amylose: Amylopectin ratio may vary from 17% to 38% depending on the starch source. On the other hand, there are some cereal starches containing 100% amylopectin which are named as waxy starches (Koksel et al., 2023). Amylose is susceptible to acid hydrolysis and can be degraded during the acid hydrolysis process and removed with washing. Therefore, the main handicap of using regular amylose containing starches is the low yield while producing the SNP. On the other hand, amylopectin is the more resistant to acids. Hence, most of the SNP production studies in the literature are conducted with 100% amylopectin containing waxy starches to increase the yield (Angellier et al., 2004; Kim et al., 2017). Nevertheless, the yield of SNP production from waxy starches did not exceed 20%, according to the literature. In this study, we

aimed to produce SNPs, to be used as a Pickering emulsion stabilizer, with high yield. Phosphorylation is a starch cross-linking method and used for chemical modification in food-grade starch production. Crosslinking occurs by creating intramolecular and/or intermolecular connections by bonding a phosphate between hydroxyl groups in starch molecules in the amorphous region (Landerito and Wang, 2005; Shukri and Shi, 2017). In accordance with this purpose, we hypothesized that the amorphous regions of the cross-linked starches, which are more compact due to the phosphate bonds, can become resistant to acid hydrolysis and degradation, preventing their removal in the washing process, which may also increase SNP yield.

In the first part of the study, we used commercially available cross-linked wheat starch to produce SNP. Different acid hydrolysis conditions were studied to achieve the highest SNP yield. The SNPs were characterized in terms of their morphology, crystallinity and structural features using Scanning Electron Microscopy (SEM), X-Ray diffractometer (XRD) and Fourier Transform Infrared Spectrophotometer (FT-IR), respectively. In the second part of the study, the SNP having the highest production yield was used for Pickering emulsion formation and the emulsions were analyzed in terms of their stability.

2. Materials and Methods

Materials

Cross-linked wheat starch and native wheat starch was supplied from MGP Ingredients, Inc. (Kansas, USA) and Smart Kimya Ltd. Şti. (İzmir, Türkiye), respectively. Sulfuric acid and sodium hydroxide were purchased from Merck, Germany. Corn oil (Çiğdem; Adana, Türkiye) was supplied from a local store.

Production of Starch Nanoparticle (SNP)

Starch nanoparticles (SNPs) were produced using acid hydrolysis according to the method of (Hélène Angellier et al., 2004). Cross-linked wheat starch was mixed with sulfuric acid solution (H_2SO_4, 3.16 M) at various hydrolysis conditions and starch: H_2SO_4 ratio (g/ml). To understand the effect of hydrolysis time on the SNP production yield, starch: H_2SO_4 ratio was kept constant (1:4, g/ml) and hydrolysis was conducted for 1, 3 and 5 days. Another set of SNPs were prepared to investigate the effect of starch: H_2SO_4 ratio on the yield. For this purpose, starch samples were mixed with H_2SO_4 at a ratio of 1:3, 1:5 and 1:7, and subjected to hydrolysis for constant time (3 days). Starch samples were hydrolyzed at 50°C at the stated time using a round bottom flask magnetic stirrer equipped with a heater (Heidolph, Germany). 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). The neutralized starch suspension was poured into a 10 cm long dialysis bag (Sigma D9402, USA) to dialyze against deionized water to remove impurities such as free sulfuric acid residue and salt. The filled dialysis bag was placed in a beaker 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). To investigate the effect of cross-linking on the SNP yield native wheat starch was also used for SNP production. 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. 1

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

Characterization of Starch Nanoparticles

Scanning Electron Microscopy (SEM) Analysis

Scanning electron microscope (SEM) was used to analyze the effect of acid hydrolysis on the morphology of the samples (Xie et al., 2016). 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.

Crystallinity Analysis

X-Ray diffractometer (Bruker AXS D8 advance model, Germany) was used to investigate the crystalline structure of the samples (Xie et al., 2016). 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°.

Structural Identification

Fourier Transform Infrared Spectrophotometer (FT-IR, Thermo Nicolet Avatar 370) was used to determine the structural features of the samples. Measurements were done in the wavelength range of 4000-400 cm^{-1}. Each sample was screened 32 times.

Selection of the Starch Nanoparticle to Achieve the Best Emulsion Stability

To select the SNP to achieve the best emulsion stability, Pickering emulsions were prepared using corn oil at Φ0.6 and Φ0.8 oil fractions. In brief, 2% (w/w) of SNP was mixed with water in a 20 ml glass bottle and sonicated for 15 sec at 98% amplitude (Bandelin 3100, Germany). Corn oil was added to starch-water suspension and sonicated for 75 sec for homogenization. During homogenization, bottles were kept in ice bath to prevent overheating. Emulsions were stored in glass bottles in room condition for 30 days to observe physical stability.

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

After the selection of the SNP to achieve the best emulsion stability, we aimed to select the SNP ratio and oil fraction (Φ) that was needed to achieve the best emulsion stability. For this purpose, SNP produced at a starch: H_2SO_4 ratio of 1:3 (g/ml) and hydrolysis time of 3 days (1:3(3)) was used to prepare the Pickering emulsions. Emulsions were prepared at different corn oil fraction values (Φ0.2, Φ0.4, Φ0.6) using various SNP ratio (1%, 2%, 3%, w/w). Emulsions were prepared using the same procedure described above. These emulsions were also stored in room conditions for 30 days and the physical stability of the emulsions were observed visually during storage.

Statistical Analysis

All experiments were performed in duplicates. The results were analyzed using a one-way analysis of variance. When significant ($p < 0.05$) differences were found, the least significant difference (LSD) was used. Paired t-test were used to determine the differences among means.

3. Results and Discussion

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

3.1. Starch Nanoparticle Yield

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 cross-linked wheat starch (Table 1). The highest yield for native wheat starch (17.3%) and cross-linked wheat starch (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 cross-linked wheat starch samples. The yield decreased from 56.0% to 30.0%, when the hydrolysis time increased from 1 to 5 days.

Table 1. Yield for starch nanoparticle (%) prepared at constant starch:H_2SO_4 ratio (1:4, g/ml) and different hydrolysis time (1, 3 and 5 days)

Sample ID*	Hydrolysis time (day)	Yield (%)	
		Wheat Starch	Cross-linked wheat starch
1:4 (1)	1	17.3^a ± 0.57	56.0^a ±1.41
1:4 (3)	3	3.7^c ± 0.35	36.0^b ± 2.12
1:4 (5)	5	6.3^b ± 0.64	30.0^b ± 2.12

**The ratio in the sample ID is the starch:H_2SO_4 ratio in g/ml. The number in the parenthesis shows the hydrolysis time (day).*
Different letters in the same column indicates that the values are significantly different ($p<0.05$)

Table 2 shows the yield of the samples hydrolyzed at constant hydrolysis time (3 days). The highest yield for the wheat starch samples hydrolyzed for 3 days was achieved for the sample hydrolyzed with a starch: H_2SO_4 ratio of 1:5 (g/ml) (1:5 (3)) (5.9%). 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 cross-linked wheat starch.

Table 2 Yield for starch nanoparticle (%) prepared at various starch: H_2SO_4 ratio and constant hydrolysis time (3 days)

Sample ID*	Yield (%)	
	Wheat Starch	Cross-linked wheat starch
1:3 (3)	5.3^a ± 0.28	36.0^a ± 0.14
1:4 (3)	3.7^b ± 0.35	36.0^a ± 2.12
1:5 (3)	5.9^a ± 0.28	17.0^b ± 0.28
1:7 (3)	1.6^c ± 0.28	18.0^b ± 0.56

*The ratio in the sample ID is the starch:H_2SO_4 ratio in g/ml. The number in the parenthesis shows the hydrolysis time (day).
Different letters in the same column indicates that the values are significantly different ($p<0.05$)

The highest yield was 36.0% and achieved for the cross-linked wheat starch samples hydrolyzed for 3 days at a starch: H_2SO_4 ratio of 1:3 (g/ml) and 1:4 (g/ml). The increase in the starch: H_2SO_4 ratio above 1:4 decreased the yield independent from reaction time for the cross-linked wheat starch samples. The yield values of the cross-linked wheat starch 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 cross-linked wheat starch was

significantly higher ($p<0.05$, paired t-test) than that of the ones produced using native wheat starch, cross-linked wheat starch was selected to produce SNPs in the rest of the study. Angellier et al. (2004) optimized SNP production conditions and obtained SNP with a yield of 15.7% after 5 days hydrolysis using 3.16 M H_2SO_4. Kim et al. (2017) 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 SNP production with higher yields. The yield of SNP production achieved in our study using cross-linked wheat starch was found to be the highest yield in the literature.

3.2. Starch Nanoparticle Characterization

3.2.1. Scanning Electron Microscopy (SEM)

SEM images of some of the SNPs produced using cross-linked wheat starch and its counterpart (cross-linked wheat starch) are illustrated in Figure 1. The cross-linked wheat starch granules were seen round in shape with smooth surfaces (Figure 1a). There were deep hollows in the center of the granules. The size of granules varied between 5-20 µm. Similar observations were reported in the literature for cross-linked starch samples produced from wheat starch (Thompson et al., 2011). Figure 1b 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 cross-linked wheat starch shown in Figure 1a. 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 SNP.

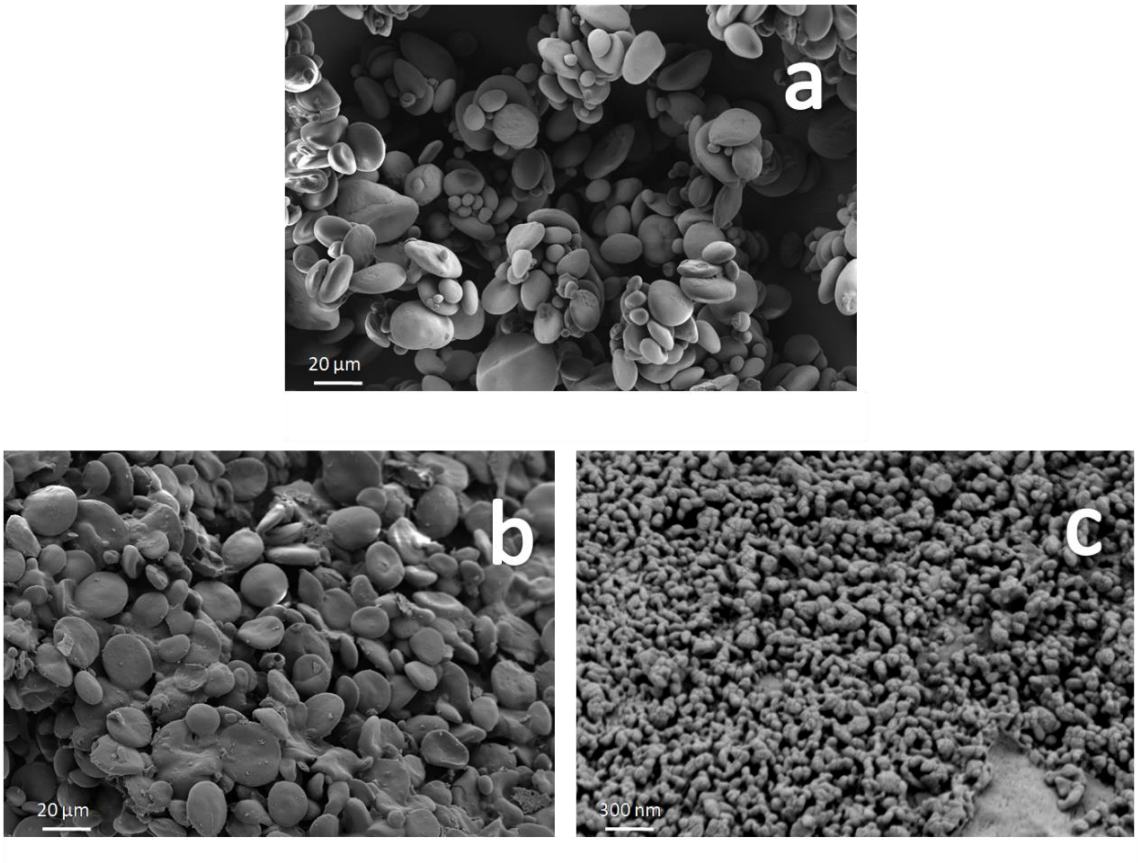

Figure 1. SEM images of a) Cross-linked wheat starch, b) Sample 1:4(1), c) Sample 1:3 (3). The ratio in the sample ID is the "cross-linked wheat starch: H_2SO_4 ratio" in g/ml. The number in the parenthesis shows the hydrolysis time (day).

Figure 1c shows the SEM image of Sample 1:3 (3) that was produced with a starch: H_2SO_4 ratio (g/ml) of 1:3 for 3 days. The shape and size of the granules of Sample 1:3 (3) was different than that of the cross-linked wheat starch (Figure 1a) and Sample 1:4 (1) (Figure 1b). The granule size of Sample 1:3 (3) was dramatically low (<50 nm) and this sample can be considered as SNP. While cross-linked wheat starch and Sample 1:4 (1) were seen as individual intact granules, SNPs were found in aggregates. In the literature the sizes of SNPs were also found to be quite low (<200 nm) compared to wheat starch (Dufresne, 2014; Kim et al., 2012; Korkut and Kahraman, 2019).

Figure 2 shows the SEM images of the Samples 1:4 (3), 1:5 (3), 1:7 (3) and 1:4 (5). All the starch granules were observed as very fine particles (<50 nm) similar to 1: 3 (3) sample (Figure 1c). Except the Sample 1:4 (5) (Figure 2d), all samples were found in aggregates. The images showed that the size of starch granules which were 3 day-hydrolyzed decreased less than 50 nm regardless of 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 1 and 2, while cross-linked wheat starch granules were found independently from each other, SNPs were present as aggregated granules. The presence of SNPs in clusters has been observed in many studies in the literature (Saeng-on and Aht-Ong, 2017). The reason for the clustering of SNPs in this way was shown as the new hydrogen bonds formed because of the interaction of hydroxyl groups on the surface of the nanoparticles with each other (Mariano et al., 2017). No significant difference in shape and size was observed among the SNPs obtained by 3 days hydrolysis of cross-linked wheat starch at various starch: H_2SO_4 ratio.

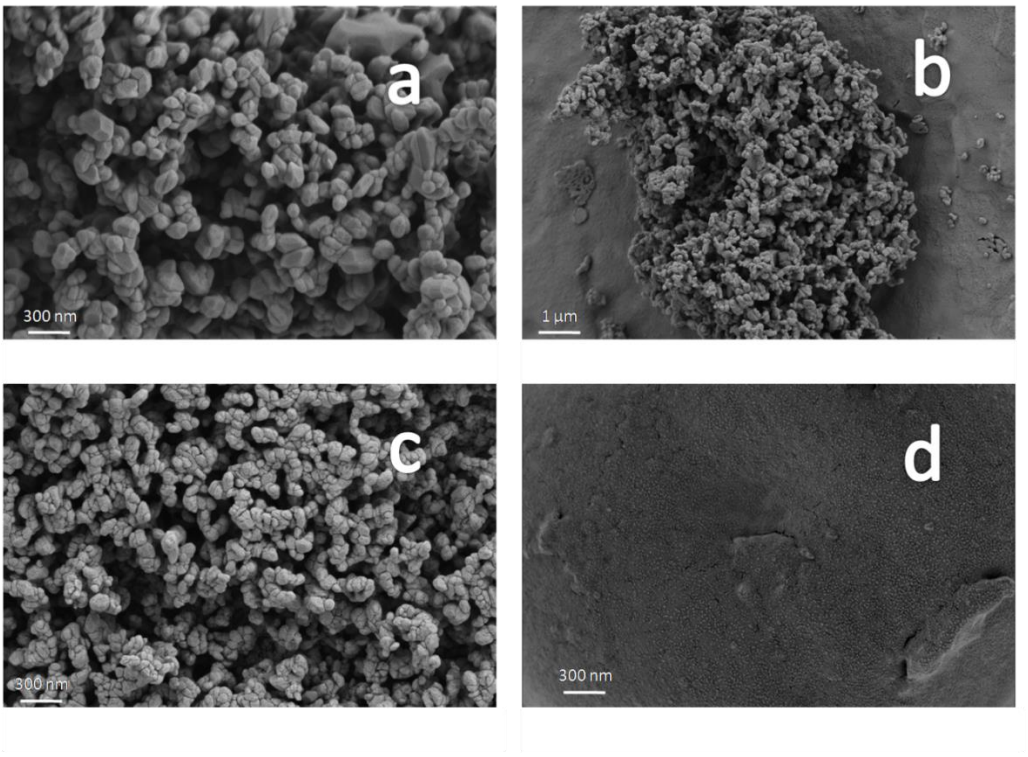

Figure 2. SEM images of a) 1:4 (3), b) 1:5 (3), c) 1:7 (3) and d) 1:4 (5). The ratio in the sample ID is the "cross-linked wheat starch: H_2SO_4 ratio" in g/ml. The number in the parenthesis shows the hydrolysis time (day).

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 illustrated in Figure 2d. In terms of granular size and shape, this sample was different from other samples hydrolyzed for 3 days. The SNPs 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 was <20 µm. The reason for this situation could be the long hydrolysis period.

3.2.2. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectrums of the cross-linked wheat starch and SNPs are shown in Figure 3. The small peak seen at nearly 3650 cm^{-1} wavelength indicates the free water entry on the surface of starch samples (Da Silva Miranda Sechi and Marques, 2017). The presence of stretching vibrations at 3600 cm^{-1} verifies the existence of O-H bond (Figure 3) (León et al., 2017). All of the samples had 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 (Ashwar et al., 2017; Gao et al., 2014; Peng et al., 2011). The small peak seen at 1647 cm^{-1} indicates the bound water existing in the granule (Peng et al., 2011). 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 3, noticeable difference was not observed between the cross-linked wheat starch and SNPs in terms of bonds. SNPs having peaks at the same absorption bands with cross-linked wheat starch indicate that the general starch structure is preserved after hydrolysis. On the other hand, there are some small peaks in some samples (e.g. 1:5(3)) that are absent in others such as 1:4 (5), probably due to the hydrolysis effect. However, it can be said that acid hydrolysis did not cause substantial changes in the general starch structure; however, it only reduced the granule size of starch samples (Figure 1 and 2).

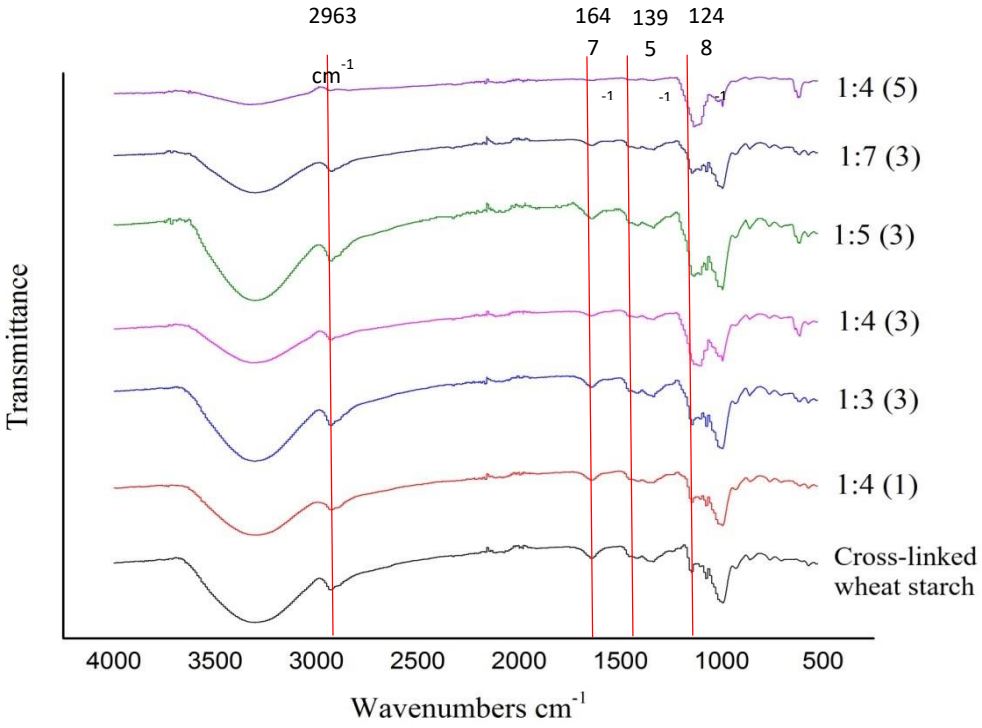

Figure 3. Fourier transform infrared spectroscopy (FT-IR) spectrums of the samples. The ratio in the sample ID is the "cross-linked wheat starch: H_2SO_4 ratio" in g/ml. The number in the parenthesis shows the hydrolysis time (day).

3.2.3. X-Ray Diffraction (XRD)

The X-ray diffractograms of cross-linked wheat starch and SNPs are illustrated in Figure 4. Starch can be categorized in three groups (A, B and C type starches) according to its crystalline feature. The starches having A type crystalline structure are cereal starches and give specific peaks in at "2θ = 15°, 17°, 18° and 23°" (Sha et al., 2012). The cross-linked wheat starch had peaks at 14.9°, 16.9°, 18.0° and 23.2° (2θ) diffraction angle (Figure 4). In addition, samples named as 1:4 (1) and 1:3 (3) had peaks same as cross-linked wheat starch. As seen in Figure 4, these three samples had peaks similar to 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.

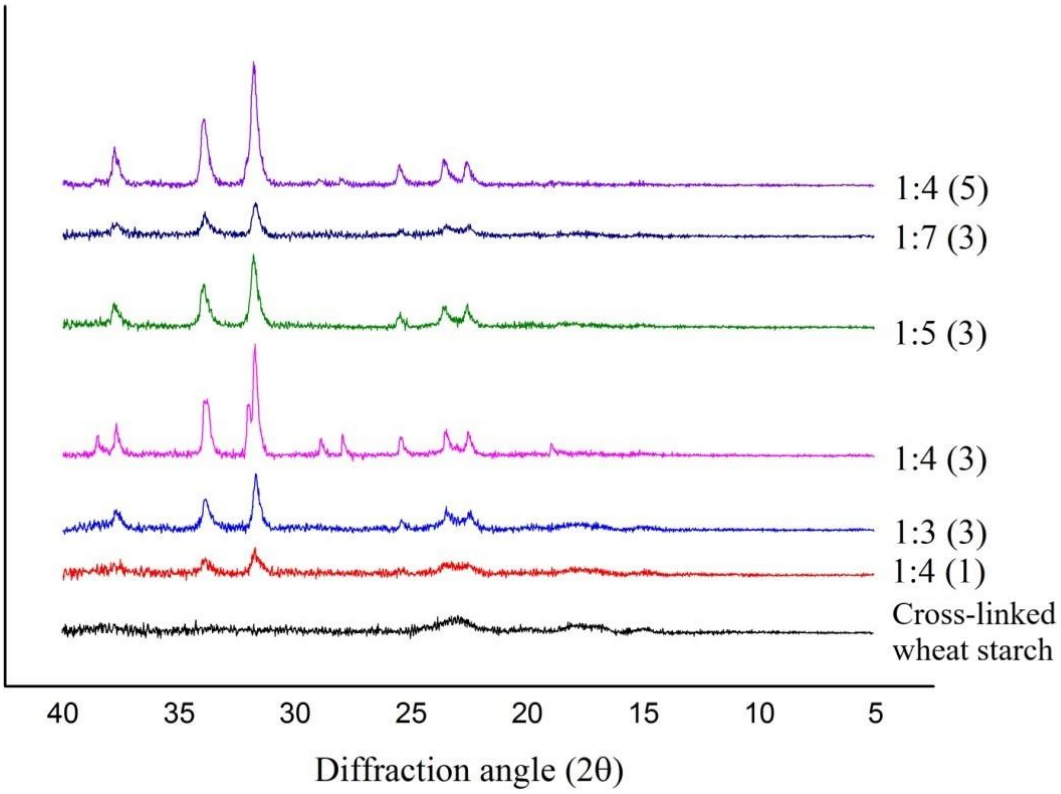

Figure 4. X-ray diffractograms of samples. The ratio in the sample ID is "cross-linked wheat starch:H_2SO_4 ratio" in g/ml. The number in the parenthesis shows the hydrolysis time (day).

On the other hand, the crystallinity of the samples varied between 56.4% and 77% depending on the hydrolysis condition (starch:H_2SO_4 ratio (g/ml) and hydrolysis time). While cross-linked wheat starch had 54.0% crystallinity, the hydrolysis of this sample caused an increase in the crystallinity of the samples (Table 3). 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.

Table 3. Crystallinity of the cross-linked wheat starch and SNPs

Sample ID*	Crystallinity %
Cross-linked wheat starch	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 number in the parenthesis shows the hydrolysis time (day).

Crystalline regions in starch granules are more resistant to acid hydrolysis than amorphous regions. Therefore, crystalline parts inside the starch granule can be isolated in the presence of dilute sulfuric or hydrochloric acid (Kim et al., 2015). In this study acid hydrolysis with dilute H_2SO_4 selectively acted on amorphous regions and it provided highly crystalline SNPs.

3.2.4. Effect of Hydrolysis Condition of the Starch Nanoparticles on the Stability of Pickering

Emulsions

Physical stability is a critical factor for emulsions as it shows whether a product is suitable for its future use or not. Phase separation is not desired for none of the emulsion-based products (Hu et al., 2016). The physical stability of the emulsions prepared using the SNPs was detected by visual observation during 30 days of storage in room conditions. Initial appearance and final photographs (after 30-day storage) of the emulsions prepared with corn oil are illustrated in Figure 5. 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 5b). When the oil fraction was changed to Φ0.8, the physical stability of emulsions prepared with corn oil deteriorated. None of the samples prepared using corn oil (at an oil fraction of Φ0.8) was stable after 30-day storage. There are several studies about Pickering emulsions stabilized by SNPs. Ge et al. ((2017)) studied the characterization of the Pickering emulsions stabilized by various SNPs (corn, tapioca, sweet potato, and waxy corn starch) for 1-month storage. They determined the critical oil fraction of emulsions stabilized using corn SNPs as Φ0.5. They indicated that poor stability was measured for the emulsions with oil fractions that exceed the critical point. In another study, Tan et al. (2014) 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 for 1 month.

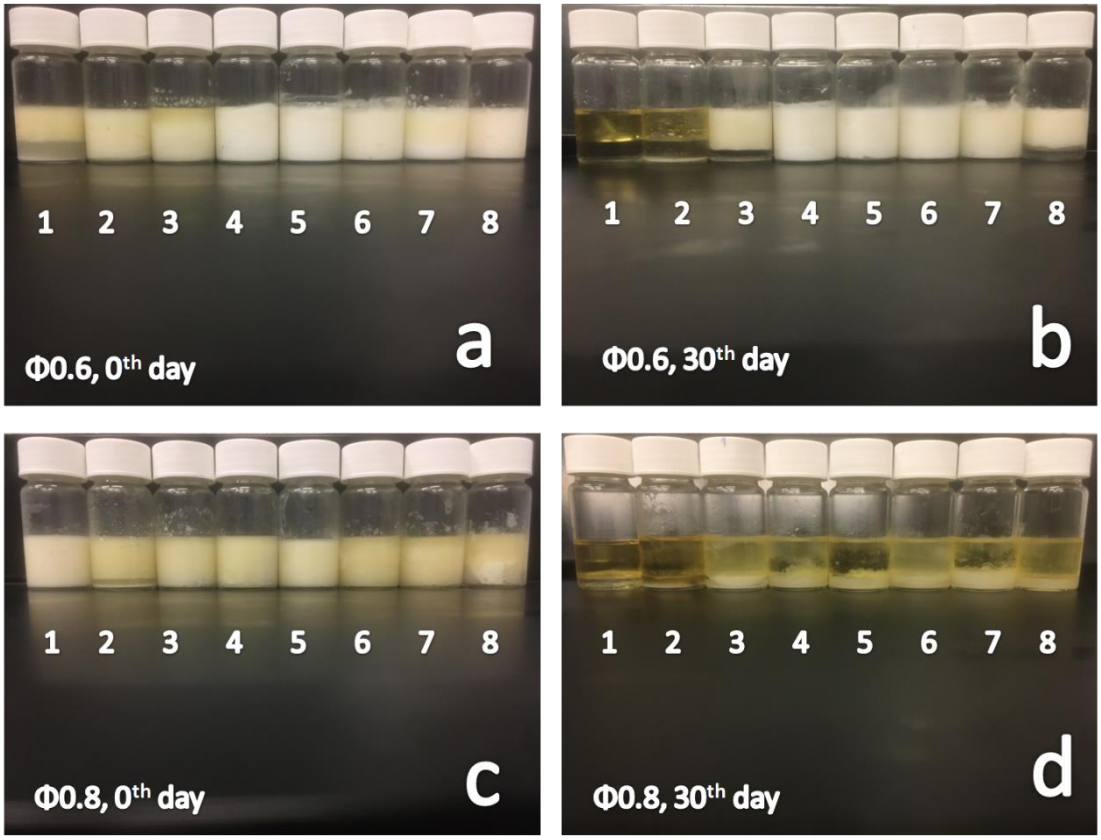

Figure 5. Emulsions prepared with corn oil. a) Φ0.6 oil fraction at 0th day, b) Φ0.6 oil fraction at 30th day, c) Φ0.8 oil fraction at 0th day, d) Φ0.8 oil fraction at 30th day. 1: [no starch, only oil and water], 2: [2% cross-linked wheat starch (w/w)], 3: [2% 1:4 (1) (w/w)], 4: [2% 1:3 (3) (w/w)], 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 ratio in the sample ID is the "cross-linked wheat starch:H_2SO_4 ratio" in g/ml. The number in the parenthesis shows the hydrolysis time (day).

The emulsions prepared using 1:3 (3), 1:7 (3) and 1:4 (5) samples were stable at Φ0.6 corn oil fraction (Figure 5b). 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 SNP concentration and oil fraction to achieve the best emulsion stability, 1:3 (3) sample was used for the next part of the study.

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 emulsions (Goyal et al., 2015) from an economic point of view. Encapsulation agent used in insufficient concentration causes active substance to be shared adjacent and irreversible bridge droplets in emulsions (Dickinson, 2001). 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 (McClements, 2015).

In order to find the optimum SNP concentration and oil fraction to achieve the best stability, a series of emulsions were prepared with different SNP concentration and oil fraction. The emulsions prepared using 1:3(3) with different SNP ratios (1, 2 and 3%, mg/g) and corn oil fractions (Φ0.2, Φ0.4 and Φ0.6) are shown in Figure 6. The first sample for all oil fractions (named as "0") was prepared with only corn oil and water. As can be seen from Figure 6b, 6d,

6f, these first samples were separated into oil and water phase as expected. Indeed, the phase separation occurred a few hours after the sonication (Picture is not shown). The emulsion samples prepared using various amount of 1:3 (3) (1, 2 and 3% (w/w)) had a similar appearance (white and creamy) at initial (Figure 6a, 6c and 6e). Among the emulsions prepared at Φ0.2 oil fraction; only the ones prepared with 2% and 3% SNP were stable (Figure 6f). Phase separation was detected at the bottom of the emulsion sample prepared with 1% (w/w) SNP. 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% SNP, Figure 6f).

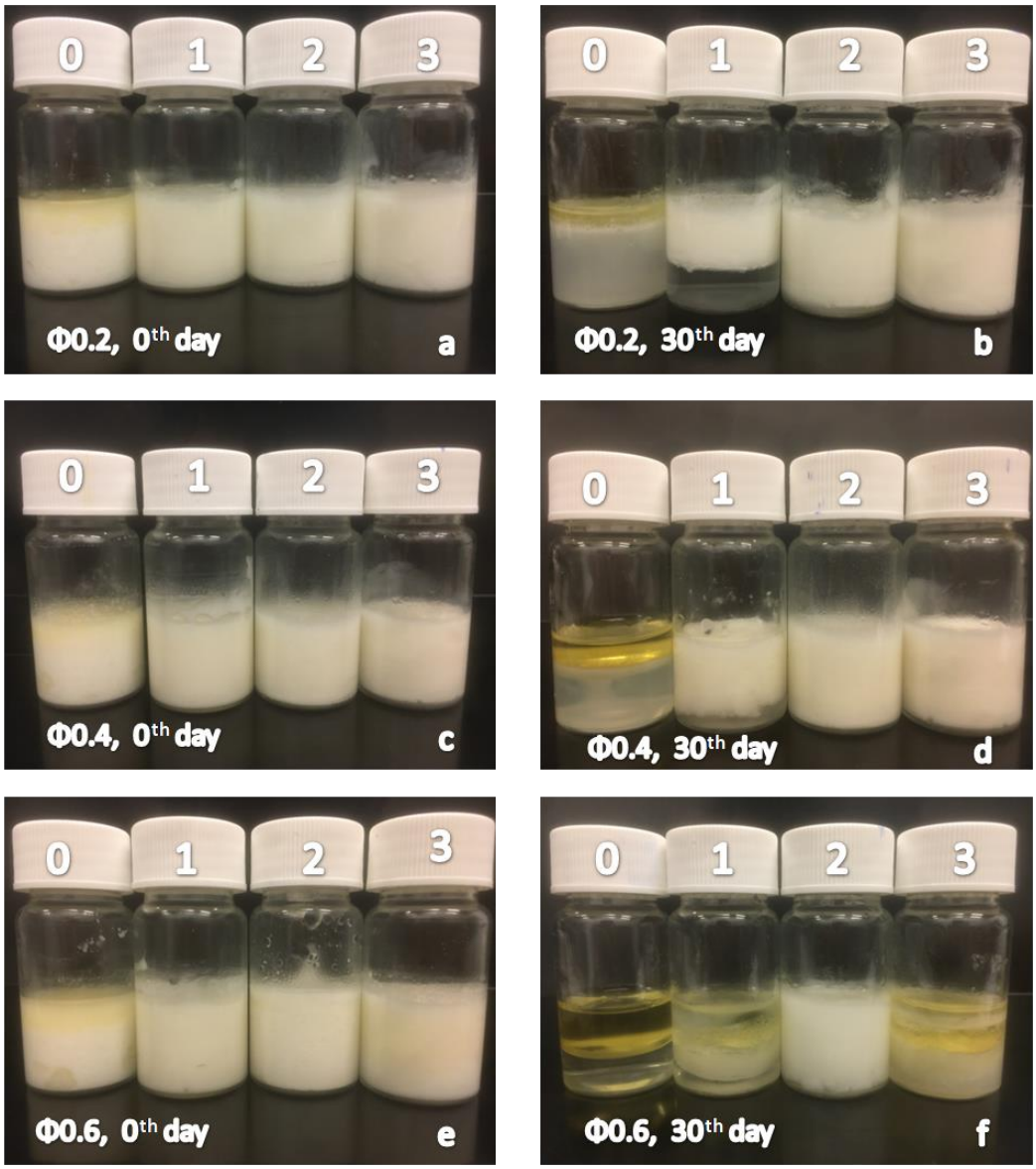

Figure 6. Pickering emulsions of corn oil with sample 1:3 (3); a) Φ0.2 oil fraction at 0th day, b) Φ0.2 oil fraction at 30th day, c) Φ0.4 oil fraction at 0th day, d) Φ0.4 oil fraction at 30th day. 0: [0% SNP (w/w)], 1: [1% SNP (w/w)], 2: [2% SNP (w/w)], 3: [3% SNP (w/w)]. The ratio in the sample ID is the "cross-linked wheat starch: H_2SO_4 ratio" in g/ml. The number in the parenthesis shows the hydrolysis time (day).

Studies on visual observation of Pickering emulsions stabilized by various nanoparticles were available in literature (Ge et al., 2017; Hu et al., 2016; Jo et al., 2019; Tzoumaki et al., 2011). Ge at al. (2017) showed that increase in the cream volume of emulsions occurs with the increasing corn SNP concentration for the same oil fraction. According to the 30 days storage observation, 1% SNP (1: 3 (3)) was not sufficient for the stability of emulsions for all fractions (the 2nd bottles of Figure 6b, 6d, 6f which were named as "1"). The emulsions prepared with 2% SNP 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% SNP 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 fourth bottle of Figure 6b (named as "3"). This sample was prepared using 3% SNP at an oil fraction of Φ0.2.

4. Conclusion

In the first part of the study the effects of hydrolysis conditions, mainly starch: H_2SO_4 ratio and hydrolysis time on the starch nanoparticle (SNP) production yield were evaluated. During hydrolysis with acid, amorphous regions of the starch (amylose) are removed. As the amylose content of most starches is lower compared to the crystalline parts (amylopectin), the yield for SNP production is relatively low. In this study, we have used a starch source which was produced by phosphorylation (cross-linked wheat starch) to produce SNP. The results showed that the possible intra- and intermolecular bonds created in the starch structure with the phosphorylation process resulted in resistance to acid hydrolysis and excessive degradation. Therefore, by using cross-linked wheat starch the removal of the starch nanoparticles during hydrolysis was prevented and SNP yield increased. In the second part of the study the SNPs were used to stabilize the Pickering emulsions, and the emulsions were evaluated in terms of their stability during storage for 30 days. The highest emulsion stability was achieved with the SNP produced at a 1:3 starch: H_2SO_4 ratio and 3 day-hydrolysis (1:3 (3)). It can be concluded that apart from their emulsion stabilizer property, due to the high production yield SNPs produced using cross-linked wheat starch might be an important source of emulsion stabilizer to be used in a wide range of industry.

Conflict of Interest Statement

No potential conflict of interest was reported by the authors.

Author Contribution Statement

AK: Data collection, investigation, formal analysis, and writing
KK: Supervision, conceptualization, methodology, review and editing

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