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Preparasi nanopartikel pati sukun: penerapan metode nanopresipitasi dan aplikasinya pada emulsi Pickering

Preparation of breadfruit starch nanoparticle: usage of nanoprecipitation method and the application in Pickering emulsion

Bovi Wira Harsanto[1,2*]

[1] Teknologi Hasil Pertanian, Fakultas Pertanian, Universitas Veteran Bangun Nusantara, Jl. Letjend Sujono Humardani No. 1, Jombor, Bendosari, Sukoharjo, Jawa Tengah
[2] Teknologi Pangan dan Hasil Pertanian, Fakultas Teknologi Pertanian, Universitas Gadjah Mada, Jl. Flora, Bulaksumur, Sleman, DIY
* Email korespondensi : boviwiraharsanto@gmail.com

ABSTRACT

Breadfruit is one of the agricultural products with high starch content (> 20%), leading to the potential for starch source. The limitation of native breadfruit starch (NBS) in the application of Pickering emulsion was the main reason for preparing breadfruit starch nanoparticles in this research. Preparation of breadfruit starch nanoparticles with the nanoprecipitation method was a time-efficient method. Therefore, this research aimed to evaluate breadfruit starch nanoparticle preparation (BSN) in the nanoprecipitation method and its application in Pickering emulsion. Investigation of size and shape, FTIR (Fourier Transform InfraRed) profile, turbidity as well as visual appearance and creaming index of Pickering emulsion was conducted to support and confirm the objective of this research. This study found that micrometer scale, high hydroxyl groups, and high turbidity of NBS were altered into the nanoscale, low hydroxyl groups, and low turbidity of BSN leading to the successful indication of preparation of breadfruit starch nanoparticle with nanoprecipitation method. The stability of Pickering emulsion improved with breadfruit starch nanoparticles, indicated by physical appearance and creaming index for 7-day storage at room temperature.

Keywords: breadfruit starch, breadfruit starch nanoparticle, nanoprecipitation, Pickering emulsion

ABSTRAK

Buah sukun merupakan salah satu hasil pertanian yang mengandung pati dalam jumlah tinggi (> 20%) sehingga berpotensi sebagai sumber pati. Keterbatasan pati sukun alami (NBS) dalam aplikasinya pada emulsi Pickering menjadi alasan utama perlunya melakukan preparasi nanopartikel pati sukun pada penelitian ini. Preparasi nanopartikel pati sukun dengan menggunakan metode nanopresipitasi merupakan metode yang efisien dari segi waktu. Oleh karena itu, penelitian ini bertujuan untuk mengevaluasi preparasi nanopartikel pati sukun (BSN) menggunakan metode nanopresipitasi dan aplikasinya pada emulsi Pickering. Peninjauan ukuran dan bentuk, profil FTIR (Fourier Transform InfraRed), turbiditas, serta kenampakan visual dan indeks creaming dari emulsi Pickering dilakukan untuk mendukung dan mengkonfirmasi tujuan penelitian ini. Hasil penelitian ini menemukan bahwa NBS yang berskala mikrometer, banyak gugus hidroksil, turbiditas tinggi dapat diubah menjadi BSN yang berskala nanometer, sedikit gugus hidroksil, dan turbiditas rendah. Hal tersebut mengindikasikan bahwa BSN dapat dipreparasi dengan baik menggunakan metode nanopresipitasi. Selain itu, stabilitas emulsi Pickering dapat diperbaiki dengan keberadaan BSN, yang diindikasikan dengan kenampakan visual dan indeks creaming yang stabil selama 7 hari penyimpanan pada suhu ruang.

Kata kunci: pati sukun, nanopartikel pati sukun, nanopresipitasi, emulsi Pickering

Introduction

Breadfruit is usually considered a low-added value food for Indonesian people (Adinugraha and Kartikawati, 2012), so diversification and innovation of breadfruit products should be done to increase the added value of breadfruit-based products. Adinugraha and Kartikawati (2012) stated that starch content in "Cilacap" Breadfruit is 20.36%, which indicates that breadfruit is rich in starch sources. Breadfruit starch is one of the underutilized starch in the food field. So, the investigation of the potency of native breadfruit starch (NBS) becomes essential to be done.

Unfortunately, native starch has a limited application in Pickering emulsion because of its large granule size (> 1 μm). The native starch can be further processed into nanometer-sized starch or starch nanoparticle (< 500 nm) to extend its application in Pickering emulsion. Tan et al. (2014) found that starch nanoparticles could be adsorbed at interface water/oil, leading to inhibition of droplet coalescence of Pickering emulsion. Ge et al. (2017) also stated that starch nanoparticles with a size of 100-220 nm could stabilize Pickering emulsion for one month. These findings can be essential information about the potency of starch nanoparticles regarding application in Pickering emulsion, not limited to the breadfruit-based starch nanoparticle (BSN).

One of the approaches to converting micrometer-sized starch into nanometer-sized starch is nanoprecipitation. Nanoprecipitation is one of the simple and fast preparation methods for making starch nanoparticle, which has proved to make nanometer-sized particles in less than 24 hours (Chang et al., 2017). Polymer concentration, such as starch concentration, becomes an essential factor in this method. De Oliveira et al. (2013) and Farrag et al. (2018) stated that higher polymer concentration leads to larger particle size because each nuclei particle has high aggregation potency. The presence of surfactants, such as Tween 80, also becomes another critical factor in the nanoprecipitation method. Some studies found that aggregation of nuclei particles could be inhibited because of steric hindrance from surfactant (Lebouille et al., 2014; Hu and McClements, 2014; Li et al., 2016).

This research aimed to prepare breadfruit-based starch nanoparticles using nanoprecipitation, which can be applied in Pickering emulsion. In this research, the parameter of size, shape, FTIR profile, and turbidity of BSN will be evaluated. Moreover, the application of BSN in Pickering emulsion will also be investigated, with Medium Chain Triglyceride (MCT) as an oil phase. Tween 80 become a surfactant in breadfruit starch nanoparticle preparation because of good dispersibility in starch suspension (Li et al., 2016).

Materials and Methods

Materials

Mature breadfruit was obtained from traditional markets and local farmers in Cilacap, Central Java, Indonesia. Ethanol absolute and Tween 80 were purchased from "MERCK", Germany. MCT oil was purchased from PT. Barco, Indonesia.

Methods

Isolation of Native Breadfruit Starch (NBS)

The first step in isolating NBS was flesh fruit peeling and washing followed by soaking flesh fruit in CaCO3 water (1% w/v) and adding excessive water to neutralize flesh fruit (pH 7). Then, wet milling of flesh fruit was conducted in a blender (Phillips, Netherlands) for 5 minutes, which resulted in a slurry. Sieving with a filter cloth was conducted to get the filtrate, followed by sedimentation for

18 hours. The resulting white starch was sun-dried for 8 hours and ground for 2 minutes leading to NBS powder. The powder was stored at ambient temperature (28oC) for analysis and as a raw material in the preparation of breadfruit starch nanoparticles.

Preparation of breadfruit starch nanoparticles (BSN)

According to Chang et al. (2017), the preparation of BSN was conducted with major modifications. NBS dispersion with a particular concentration (2 % w/v; 5 % w/v) was prepared by mixing 2 g and 5 g of NBS powder with 100 mL of distilled water. Then, the mixture was heated on a hot plate (Faithful SH-2, China) at 100^{o}C for 15 minutes under continuous stirring of 500 rpm to disperse starch breadfruit granules in the distilled water completely. Afterwards, the NBS dispersion was cooled at room temperature. On the other side, the ethanol absolute and Tween 80 with a 10:1 ratio were mix-stirred before nanoprecipitation began.

The NBS dispersion (100 mL) was added dropwise into ethanol absolute:Tween 80 mixture (100 mL), which was continually agitated with a magnetic stirrer hot plate (Faithful SH-2, China). The resulting mixture was then homogenized for 5 minutes at 6800 rpm (Ultra-Turrax Homogenizer, ULTRA-TURRAX T50, IKA, WERKE, Germany). Then, the mixture was centrifuged at 4000 rpm for 15 minutes. The precipitated BSN were obtained by removing the supernatant and rinsing 2-3 times with excessive distilled water. The BSN was cabinet dried and ground in a 200-mesh sieve. Finally, BSN powder was stored at ambient temperature for further analysis and as a raw material in the preparation of Pickering emulsion.

Characterization of NBS and BSN

The size and shape of granule/particle were observed using an optical microscope (Olympus CX21LED, Japan). Pinches of powder were placed on a microscopic slide followed by 100x magnification. Afterwards, the observed shape was measured with ImageRaster software (Informer Technologies, Inc.) to create data on granule/particle size.

FTIR profile of NBS and BSN was observed using Fourier Transform Infrared (FTIR) (Shimadzu, Jepang) with a scanning range set in 400 - 4000 cm^{-1}. The sample pellet was made by mixing 1 mg sample with 200 mg Potassium Bromide. Afterwards, the sample pellet was measured by observing the transmittance in infrared.

Meanwhile, NBS and BSN dispersion (1% w/v) was prepared by stirring for 15 minutes at room temperature, followed by measuring the absorbance of dispersion at 640 nm using a UV-Visible spectrophotometer (Genesys 10S, USA).

Preparation of Pickering emulsion

Pickering emulsion was prepared according to Ge et al. (2017) with minor modifications. The 100 mL of Pickering emulsion were prepared by mixing the BSN powder (2 gram; 4 gram), 2 mL MCT oil, and 98 mL distilled water in a glass beaker, which was continually agitated for 15 minutes at room temperature. Afterwards, Pickering emulsions were prepared using a high-speed homogenizer (Ultra-Turrax Homogenizer, ULTRA-TURRAX T50, IKA, WERKE, Germany) at 10000 rpm for 2 min at room temperature

Characterization of Pickering emulsion

The Pickering emulsion stability was characterized by creaming index (CI) based on Wang et al. (2015). Emulsions were poured into the plastic glass and stored at room temperature for seven days to monitor their CI, measured in day-1, day-3, day-5, and day-7. In addition, the visual appearance of

Pickering emulsions was characterized by capturing an image of emulsion (Canon Ixus 190, China) on day 1, day 3, day 5, and day 7.

Results and Discussion

Size and shape of NBS and BSN

Investigation of granule/particle size and shape was one of the most critical things in this research because of crucially in size alteration from native breadfruit starch (NBS) into breadfruit starch nanoparticle (BSN). Based on Figure 1, there was a size alteration of NBS (6.9 µm) into BSN 2 (530 nm) and BSN 5 (800 nm). Size reduction from NBS to BSN happened during nanoprecipitation because ethanol was added as an "anti-solvent" for breadfruit starch solvent (distilled water). This phenomenon refers to decreasing distilled water (solvent) capacity in starch dispersion, which results in the generation of starch nuclei on a nanometer scale followed by the growth of nuclei into starch nanoparticles (Chang et al., 2017). Figure 1 also showed that NBS granule was spherical and agglomerated in shape ("grape-like shape"), which differed from spherical non-agglomerated BSN.

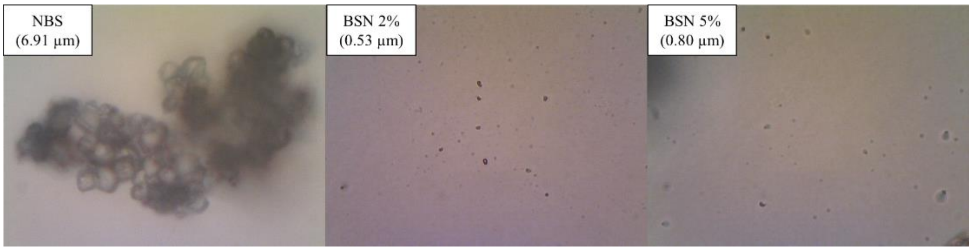

Figure 1. Optical micrograph of NBS, BSN 2%, and BSN 5% at magnification of 100x. The granule/particle size of NBS and BSN was measured using image raster software equipped at optical microscope. NBS: Native breadfruit starch; BSN 2%: breadfruit starch nanoparticle (2% w/v); BSN 5%: breadfruit starch nanoparticle (5% w/v)

The size and shape of BSN 2% were slightly different from BSN 5%. As mentioned in some studies, these findings indicated little aggregation of BSN in higher polymer concentrations. In this research, the aggregation of starch polymer might be inhibited by the presence of Tween 80, as also found by Li et al. (2016).

FTIR profile of NBS and BSN

There was a similar FTIR profile between NBS with starch from other sources (Figure 2), which showed that hydroxyl groups (OH) in 3394 cm^{-1} (El-Sheikh, 2017). OH groups might contribute to the double helix crystalline region contained in NBS.

In BSN 2% and BSN 5%, OH group intensity was decreased in wavenumber 3425.58 cm^{-1} compared to NBS (Figure 2). This phenomenon was related to the gelatinization process during an early stage of BSN preparation. The gelatinization could break the OH bonding of the starch granule and decrease OH groups (El-Sheikh, 2017). Therefore, it can be said that BSN had lower compactness than NBS. In addition, the OH group's intensity of BSN 2% was lower than BSN 5% according to the peak area at a wavenumber of 3425,58 cm^{-1} (Figure 2). The results might be associated with the higher detected starch molecule in BSN 5% compared to BSN 5%.

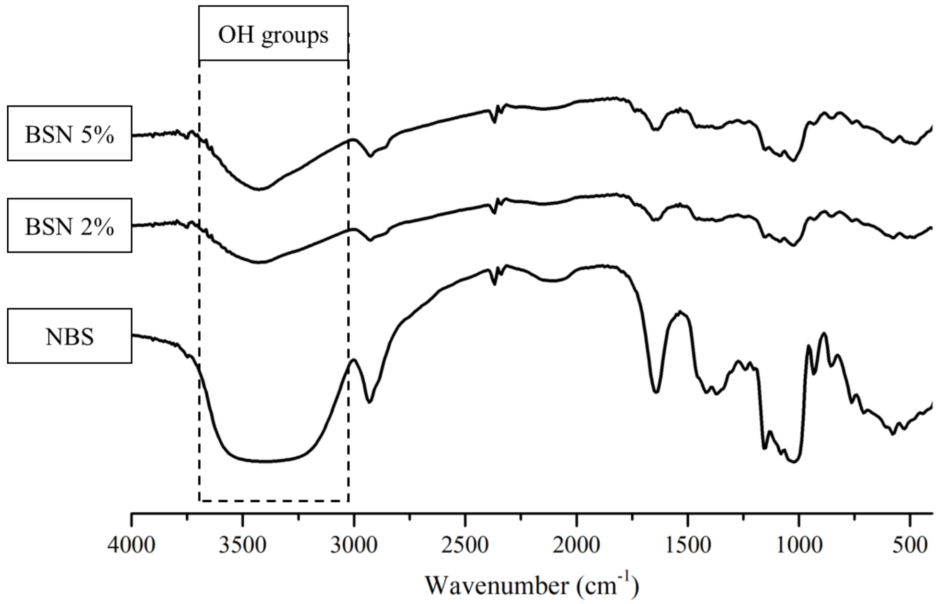

Figure 2. FTIR profile of NBS, BSN 2%, and BSN 5%. Dashes-box area represented OH-groups of starch. NBS: Native breadfruit starch; BSN 2%: breadfruit starch nanoparticle (2% w/v); BSN 5%: breadfruit starch nanoparticle (5% w/v)

Turbidity of NBS and BSN dispersion

Investigation of turbidity was related to the light transmittance of dispersion. If a dispersion contains a particle size smaller than the wavelength of visible light (400-700 nm), then visible light will be transmitted and turbidity will be low (McClements, 2010). The turbidity of the NBS granule was higher than BSN (Table 1). The results indicated that nano-sized BSN tended to transmit visible light compared to micron-sized NBS. This finding was associated with smaller particles in breadfruit starch nanoparticles compared to the wavelength of visible length (≤ 640 nm). This result could be related to the size and shape alteration of NBS into BSN (Fig. 1). Based on Table 1, the turbidity of BSN 2% was lower than BSN 5% because the size of BSN 5% was larger than BSN 2%. When visible light exposed to the dispersion, BSN 5% might absorb more visible light (wavelength of 400-700 nm) than BSN 2%.

Table 1. Turbidity of NBS and BSN using spectrophotometer UV-Vis at 640 nm

Treatment	Absorbance (640 nm)
NBS	2.19 ± 0.240
BSN 2%	0.46 ± 0.004
BSN 5%	0.77 ± 0.009

NBS: Native breadfruit starch; BSN 2%: breadfruit starch nanoparticle (2% w/v); BSN 5%: breadfruit starch nanoparticle (5% w/v)

Pickering emulsion

Pickering emulsions are emulsions stabilized by solid particles, such as starch nanoparticles. Thus, BSN was then applicated to Pickering emulsion. Medium Chain Triglyceride (MCT) oil was chosen as an oil phase, and distilled water was an aqueous phase. In this research, BSN 2% was not used in the preparation of Pickering emulsion because of the low availability of the BSN 2% sample.

Visual appearance of Pickering emulsion

The visual appearance of the emulsion was observed by capturing Pickering emulsion on day 0, day 1, day 3, day 5, and day 7.

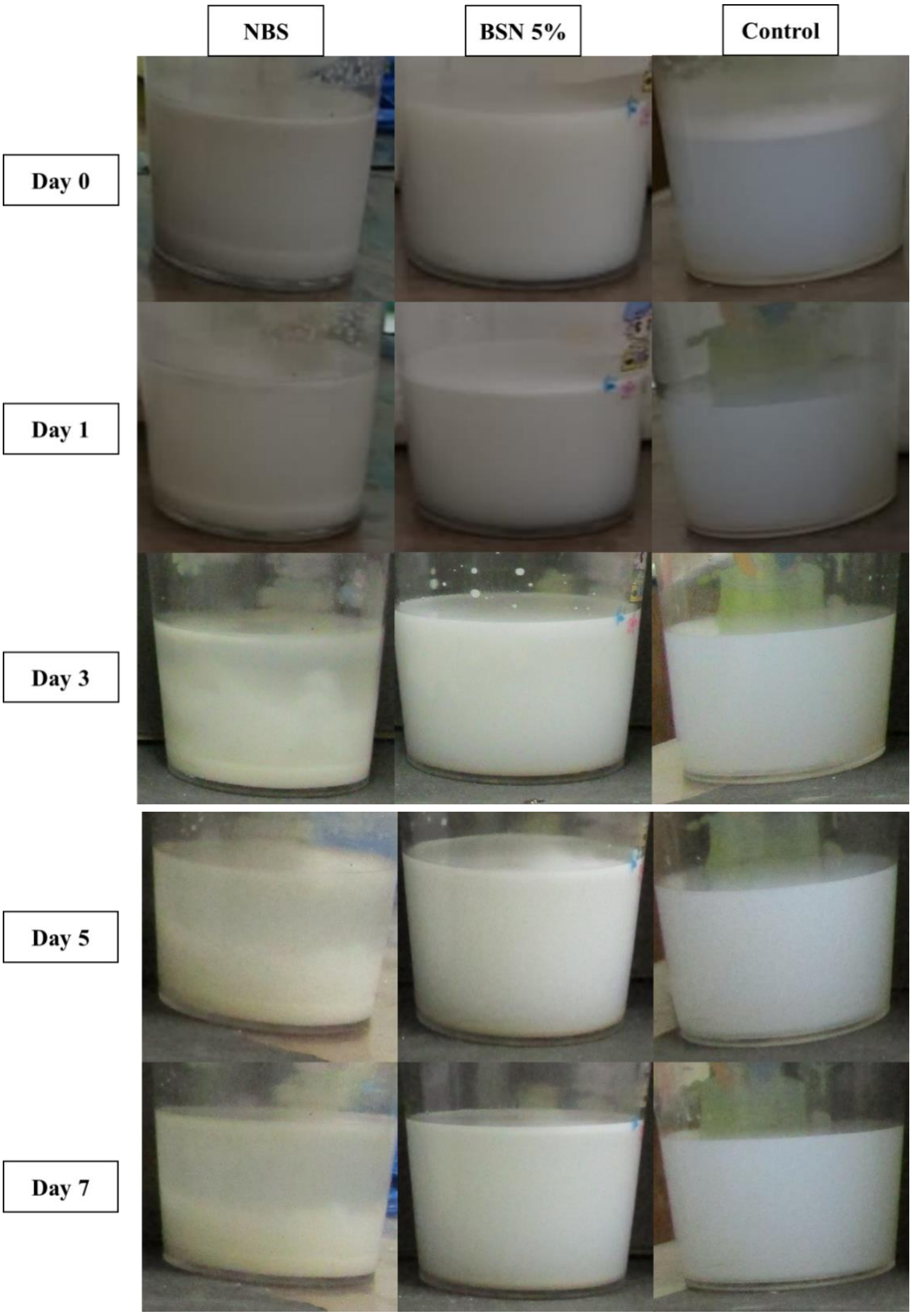

Figure 3. Visual appearance of Pickering emulsion with NBS, BSN 5%, and control at day 0, day 1, day 3, day 5, day 7. NBS: Native breadfruit starch; BSN 5%: breadfruit starch nanoparticle (5% w/v); Control: Tween 80

The observation focused on phase separation between aqueous phase and oil phase, which was marked by the presence of a layer in emulsion when capturing the image. If there is no visible layer on the emulsion, then the emulsion can be said to be stable during storage.

For seven days of storage, there were few layers in emulsion with BSN 5% (Figure 3), similar to the control sample (Tween 80 as emulsifier). The result showed that BSN 5% was a good stabilizer in Pickering emulsion. This finding referred to adsorption of BSN at the oil-water interface leading to inhibition of oil droplet coalescence. Meanwhile, the layer was seen in emulsion with NBS compared to BSN (Figure 3), indicating that BSN had better stabilizer capacity than NBS in Pickering emulsion.

Creaming index of Pickering emulsion

The creaming index of the Pickering emulsion was measured by comparing the bottom layer's height divided by the emulsion's total height. The higher the bottom layer, the higher the creaming index of Pickering emulsion, and the higher intensity of phase separation of Pickering emulsion.

Table 2 showed that the creaming index of Pickering emulsion with NBS was higher than emulsion with BSN. The result confirmed the visual appearance of Pickering emulsion (Figure 3). The finding of this research showed that BSN had stabilizer effect when applied to Pickering emulsion. Furthermore, the creaming index of Pickering emulsion with BSN 5% had a slightly different value than the control (Tween 80 as emulsifier), which was almost 0. This result indicated high emulsion stability in room storage when using BSN 5% and Tween 80. Ge et al. (2017) found that corn starch nanoparticle-stabilized Pickering emulsion could stable for 1 month of storage with creaming index value of 18%.

Table 2. Creaming Index of Pickering emulsion for 7 days storage

Treatment	Creaming Index (%)				
	Day-0	**Day-1**	**Day-3**	**Day-5**	**Day-7**
NBS	0	28.57	47.62	47.62	47.62
BSN 5%	0	0	2.27	2.27	6.82
Control (Tween 80)	0	0	0	0	0

NBS: Native breadfruit starch; BSN 5%: breadfruit starch nanoparticle (5% w/v); Control: Tween 80

Conclusion

Preparation of breadfruit starch nanoparticles by using the nanoprecipitation method could alter the properties of native breadfruit starch from micrometer-sized, agglomerated shape, high turbidity, and high compactness into nanometer-sized, non-agglomerated shape, low turbidity, and low compactness. Furthermore, starch concentration slightly affects breadfruit starch nanoparticle size in this research. Then, Pickering emulsion with MCT oil as oil phase could be stabilized by breadfruit starch nanoparticle without phase separation and low creaming index of Pickering emulsion. Therefore, Breadfruit starch nanoparticles could be a potential stabilizer material in Pickering emulsion.

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References

Adinugraha, H.A., and Kartikawati, N. (2012). Production Variasi morfologi dan kandungan gizi buah sukun. Warna Benih 13(2012): 99-106.

Chang, Y., Yan, X., Wang, Q., Ren, L., Tong, J., and Zhou, J. (2017). High efficiency and low cost preparation of size controlled starch nanoparticles through ultrasonic treatment and precipitation. Food Chemistry 227(2017): 369-375.

de Oliveira, A., Jager, E., Jager, A., Stepanek, P., and Giacomelli, F. C. (2013). Physicochemical aspects behind the size of biodegradable polymeric nanoparticles: A step forward. Colloids and Surfaces A: Physicochemical and Engineering Aspects 436(2013): 1092-1102.

El-Sheikh, M.A. (2017). New technique in starch nanoparticles synthesis. Carbohydrate Polymers 176(2017): 214-219.

Farrag, Y., Ide, W., Montero, B., Rico, M., Rodriguez-Llmazares, S., Barral, L., and Bouza, R. (2018). Preparation of starch nanoparticles loaded with quercetin using nanoprecipitation technique. International Journal of Biological Macromolecules 114(2018): 426-433.

Ge, S., Xiong, L., Li, M., Liu, J., Yang, J., Chang, R., Liang, C., and Sun, Q. (2017). Characterizations of Pickering emulsions stabilized by starch nanoparticles: Influence of starch variety and particle size. Food Chemistry 234(2017): 339-347.

Hu, K. and McClements, D.J. (2014). Fabrication of surfactant-stabilized zein nanoparticles: A pH modulated antisolvent precipitation method. Food Research International 64(2014): 329-335.

Lebouille, J.G.J.L., Stepanyan, R., Slot, J.J.M., Cohen Stuart, M.A., and Tuinier, R. (2014). Nanoprecipitation of polymers in a bad solvent. Colloids and Surfaces A: Physicochemical and Engineering Aspects 460(2014): 225-235.

Li, X., Qin, Y., Liu, C., Jiang, S., Xiong, L., and Sun, Q. (2016). Size-controlled starch nanoparticles prepared by self-assembly with different green surfactant: The effect of electrostatic repulsion or steric hindrance. Food Chemistry 199(2016): 356-363.

McClements, D.J. (2010). Emulsion design to improve the delivery of functional lipophilic components. Annual Review of Food Science and Technology 1(2010): 241-269.

Tan, Y., Xu, K., Niu, C., Liu, C., Li, Y., Wang, P., and Binks, B. P. (2014). Tryglyceride-water emulsions stabilized by starch-based nanoparticles. Food Hydrocolloids 36(2014): 70-75.

Wang, L. J., Hu, Y. Q., Yin, S. W., Yang, X. Q., Lai, F. R., and Wang, S. Q. (2015). Fabrication and characterization of antioxidant pickering emulsions stabilized by zein/chitosan complex particles (ZCPs). Journal of Agricultural and Food Chemistry 63(2015): 2514-2524.