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PJST Vol. 32 (2) Mar.
2024

ISSN 0128-7680
e-ISSN 2231-8526

PJST Vol. 32 (2) Mar. 2024

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Application of the Ultrasonic Method to Produce Starch Nanoparticles from Cassava Starch

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ABSTRACT

Starch nanoparticles have the potential to be developed as a cassava starch derivative. The research aims to obtain the optimal process conditions (ultrasonic process time and starch concentration) to produce starch nanoparticles with the best characteristics. The treatment variables used in this study were the duration of the ultrasonication process (30, 60, and 90 minutes) and the starch concentration (1%, 2%, and 3%). The results showed that the ultrasonication process time and starch concentration affected the yield, particle size and distribution, polydispersity index, optical characteristics (transmittance), and clarity score of starch nanoparticles. Ultrasonic process time of 90 minutes and starch concentration of 3% will produce starch nanoparticle products with a yield of 13.68%, particle size $\leq$ 100 nm of 23.6%, average particle size of 230.8 nm with polydispersity index of 0.581, transmittance value of 61.27%, and a solution clarity score of 3.80 (not clear). Tapioca-based SNPs can be developed solely with ultrasonic method to simplify the process.

Keywords: Cassava starch, starch nanoparticles, ultrasonic

INTRODUCTION

Starch is a natural, renewable, biodegradable polymer many plants use to store energy. Starch is the second most abundant biomass in nature and is found in staple crop commodities such as rice, corn, wheat, cassava, and potatoes (BeMiller & Whistler, 2009). The primary potential source of starch in Indonesia is cassava starch obtained from cassava extraction (Zukryandry et al., 2022). Based on data

from the Food and Agriculture Organization (FAO) in 2012, Indonesia is the world's third exporter of tapioca, followed by Thailand and Vietnam (Hidayat et al., 2021). According to BPS-Statistics of Lampung Province (2022), Indonesia's cassava production in 2021 will be 19,341,233 tons, and Lampung Province, with a production of 6,683,758 tons, is the main producer of cassava in Indonesia (34.5%).

Starch nanoparticles (SNPs) have the potential to be developed as a tapioca derivative product. SNPs are nano-sized starch derivative products (one billionth of a meter, 10-9 meters) with a size range of 1–100 nm (EFSA Scientific Committee, 2011). The process of modifying starch into starch nanoparticle products has many advantages, including increasing stability, chemical reactivity, flowability, opacity, and mechanical strength (Zhu et al., 2007), improving the sensory characteristics of the product (Sharma et al., 2013), and enhancing encapsulation ability for bioactive components (Ezhilarasi et al., 2013).

Despite their potential, the development of SNPs based on tapioca is relatively limited and is mostly developed from corn starch (Le-Corre et al., 2010; Kim et al., 2013; Kumari et al., 2020) and rice starch (Zuo et al., 2012). Compared to corn and rice starch, cassava starch (tapioca) is a more economical source in Indonesia. The development of SNPs based on tapioca will increase the added value of the tapioca industry.

The manufacture of SNPs can be carried out using various methods, namely, acid hydrolysis (Le-Corre et al., 2010), enzymatic hydrolysis (Le-Corre et al., 2010), high-pressure homogenization (Liu et al., 2016), gamma irradiation (Garcia et al., 2011; Lamanna et al., 2013), a combination of acid hydrolysis and ultrasonication (Kim et al., 2013; Goncalves et al., 2014), and ultrasonication (Haaj et al., 2013). The research results by Haaj et al. (2013) showed that SNP products could be prepared solely with the ultrasonic method, simplifying the manufacturing process.

According to Jambrak et al. (2010), the ultrasonication process to produce SNPs can be carried out using an ultrasonic probe or a bath system. Compared to an ultrasonic system bath, using an ultrasonic system probe will be more effective with a shorter processing time (Bonto et al., 2021) and produce SNP products with better characteristics (Haaj et al., 2013). This study aims to obtain optimal process conditions (ultrasonic process time and starch concentration) to produce cassava starch-based starch nanoparticles with the best characteristics (yield, distribution and particle size, transmittance, and clarity).

MATERIALS AND METHODS

Equipment

The main tools used are Ultrasonication probe Biomaisen type MSUCD 650, UV-Vis single beam spectrophotometer Aelab type AE-S60-4U, and Particle Size Analyzer (PSA) Malvern Zetasizer Nano ZS type.

Starch Nanoparticle (SNP) Formation

The formation of SNPs from cassava starch was modified from the method of Haaj et al. (2013) by preparing 50 ml of cassava starch solution with concentrations according to treatment (1%, 2%, and 3%). The probe temperature is set below 40ºC, kept constant by adding ice, and the process frequency is set at 20 kHz. The probe used has a diameter of 6 cm with an ultrasonic power of 650 W. The ultrasonication process is then carried out with the duration of the ultrasonication process according to the treatment (30, 60, and 90 min). The solution resulting from the sonication process was then filtered using 1-micron Whatman filter paper and tested for yield and characteristics.

Yield Analysis

The yield is the percentage of the dry weight of the SNP product divided by the initial weight of the starch raw material, with the following Equation 1:

$$Yield\ (\%) = \frac{mass\ of\ SNP\ (gram)}{mass\ of\ initial\ starch\ (gram)} \times 100\% \qquad (1)$$

Analysis of Particle Size

The distribution and size of SNPs were analyzed using a particle size analyzer (PSA) with the dynamic light scattering (DLS) method that utilizes infrared scattering. The SNP solution sample was put into the PSA cuvette. Infrared scattering was fired at the sample so that the sample would react to produce Brownian motion (random motion of the particles). The tool then analyzes this random motion, where the smaller the particle size, the faster the movement.

In addition to the distribution and size of SNPs, the polydispersity Index (PI) value, a measure of molecular mass distribution in the sample, could also be obtained. The PI value indicates the level of confidence in the size of the particles dispersed in a solution. The smaller the polydispersity value, the better the particle size distribution confidence level in the starch solution. Conversely, if the polydispersity value is higher, then the particles present in the sample are not uniform and unstable and would quickly flocculate.

Transmittance Analysis

Samples of SNP solution resulting from the sonication process of various treatments were put into the spectrophotometer cuvette. Analysis was conducted by placing a cuvette into a UV-Vis spectrophotometer with a 450–800 nm wavelength range. The results obtained were then recorded in the form of transmittance percentage values.

Clarity Analysis

Observation of the clarity of the SNP solution was carried out after being left for 2 hours (Haaj et al., 2013). The sensory test was carried out using 10 panelists using a hedonic score of 1–5 (score 1 = very unclear; score 2 = not clear; score 3 = not clear enough; score 4 = clear; score 5 = very clear).

RESULT AND DISCUSSION

Yield of Starch Nanoparticles

The yield of SNPs at various ultrasonic process times and starch concentrations is presented in Table 1. The test results in Table 1 show that the ultrasonication process, with a duration of 90 minutes and 3% starch concentration, will produce SNP products with higher yields (13.68%) than other treatments. The higher yield of this SNP indicates that increasing the ultrasonication process time to 90 minutes and increasing the starch concentration to 3% will cause the breakdown of starch molecules into nano-sized to become more intensive. The yield of SNPs using the ultrasonic method (13.68%) is relatively the same as the acid hydrolysis method (15%) but lower than the combined acid and ultrasonic hydrolysis method, which can reach 78% (Kim et al., 2013).

The longer the ultrasonic process, the more intensive the degradation process of starch molecules. According to Czechowska-Biskup et al. (2005), the ultrasonic application will cause the degradation of starch molecules caused by mechanochemical effects. The more intensive the starch degradation process, the smaller the granule size.

The increase in SNP yields up to 3% starch concentration, indicating that up to 3% starch solution concentration, the starch degradation process was still occurring intensively. A different opinion was conveyed by Haaj et al. (2013), which stated that the ultrasonication process without chemical treatment was effective at low concentrations (1%–2%). The conditioning of starch in the form of an aqueous solution, not a suspension, is based on the

Table 1

Yield of SNP at various ultrasonication process times and starch concentrations (mean ± SD, n =3)

Treatment	SNP yield (%)
Processing time 30 minutes, starch concentration 1%	11.94 ± 0.02
Processing time 30 minutes, starch concentration 2%	13.18 ± 0.20
Processing time 30 minutes, starch concentration 3%	13.33 ± 0.18
Processing time 60 minutes, starch concentration 1%	12.02 ± 0.11
Processing time 60 minutes, starch concentration 2%	13.37 ± 0.17
Processing time 60 minutes, starch concentration 3%	13.56 ± 0.21
Processing time 90 minutes, starch concentration 1%	12.32 ± 0.23
Processing time 90 minutes, starch concentration 2%	13.66 ± 0.24
Processing time 90 minutes, starch concentration 3%	13.68 ± 0.05

results of Czechowska-Biskup et al. (2005), which showed that the process of degradation of starch molecules was more effective in aqueous/solution conditions.

Distribution and Particle Size of SNP

Distribution, particle size, and PI of SNPs at various ultrasonic process times and starch concentrations are presented in Table 2 and Figure 1. The results in Figure 1 show the percentage of SNP particle size at various particle sizes continuously using a particle size analyzer (PSA). In contrast, the results in Table 2 show the particle size in various particle size groups ($\leq$100 nm, 101-1000 nm, and > 1000 nm).
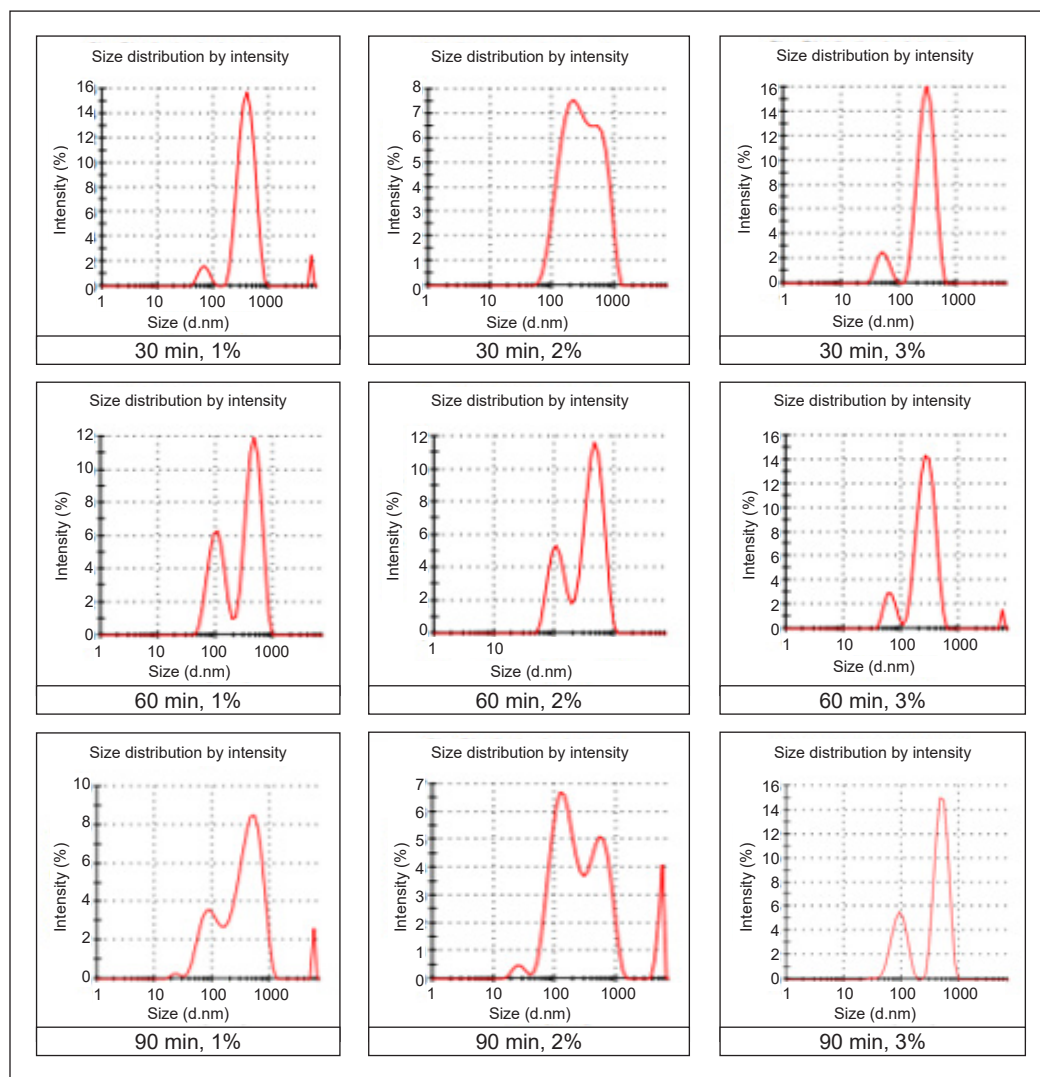
Figure 1. Distribution of various SNP sizes at various ultrasonication process times and starch concentrations

Table 2

Particle size distribution per size group and polydispersity index of SNPs at various ultrasonication process times and starch concentrations

Treatment	SNP Particle Size				Polydispersity index
	≤ 100 nm (%)	101 – 1000 nm (%)	> 1000 nm (%)	Average (nm)	
Processing time 30 minutes, starch concentration 1%	6.30	93.70	0.00	501.50	0.47
Processing time 30 minutes, starch concentration 2%	7.60	91.50	0.90	419.90	0,47
Processing time 30 minutes, starch concentration 3%	11.00	89.00	0.00	470.20	0.46
Processing time 60 minutes, starch concentration 1%	12.00	86.40	1.60	429.60	0.51
Processing time 60 minutes, starch concentration 2%	16.70	83.30	0.00	355.00	0.47
Processing time 60 minutes, starch concentration 3%	22.90	77.10	0.00	333.70	0.34
Processing time 90 minutes, starch concentration 1%	20.10	76.80	3.10	430.30	0.50
Processing time 90 minutes, starch concentration 2%	22.30	69.70	8.00	422.90	0.58
Processing time 90 minutes, starch concentration 3%	23.60	76.40	0.00	230.80	0.58

Most SNPs are 101 to 1000 nm in size showing, that the sonication process is quite effective in reducing the size of starch particles (Figure 1). According to Boufi et al. (2018) and Zuo et al. (2012), the ultrasonic method was able to damage and reduce the size of starch granules. The research results in Table 2 also show the presence of particles with a diameter of more than 1000 nm with a small intensity. Particles with a size of more than 1000 nm are thought to be starch particles that have agglomerated into a larger size. According to Jambrak et al. (2010), with changes in temperature and longer storage time, nanoparticles can agglomerate into larger sizes.

The results in Table 2 show that the ultrasonic process of starch with a concentration of 1%–3% for 30–90 minutes will produce SNP products with a diameter range of 230.80 nm to 501.50 nm and a PI value range of 0.34–0.58 nm. The lowest PI was shown in the sonication time of 60 minutes with a starch concentration of 3% with a PI of 0.34 and an average particle size of 333.70 nm. The low PI indicates that the particle size dispersion of SNP is homogeneous and evenly distributed. A PI value greater than 0.70 indicates a very wide distribution of particle sizes so that sedimentation is likely to occur.

The results showed that the ultrasonication process, with a duration of 90 minutes and 3% starch concentration, would produce SNP products with a particle size of less than 100 nm, which was higher (23.6%) than the other treatments. The results also showed that

the longer the sonification process and the higher the starch concentration, the higher the percentage of SNP particles less than 100 nanometers in size. It indicates that ultrasonication can break down starch granules into smaller sizes. The phenomenon of acoustic cavitation by ultrasonic waves causes starch particles to break into nano-sized pieces (Czechowska-Biskup et al., 2005). The increase in the percentage of SNP particle size in line with the increase in concentration up to 3% also shows that at a starch concentration of up to 3%, the cavitation process, which causes the breakdown of starch granules into nano-sized, still occurs effectively. The increase in the cavitation process, in line with the increase in starch concentration in the formation of SNPs, was also reported by Jambrak et al. (2010).

Starch Nanoparticles Transminttance Values

The transmittance value of SNPs at various ultrasonic process times and starch concentrations are presented in Table 3 and Figure 2. The results show that the ultrasonication process, with a duration of 30 minutes and 1% starch concentration, will produce SNP products with the highest transmittance values (86.38%). Conversely, the ultrasonication process time of 90 minutes and 3% starch concentration will produce SNP products with the lowest transmittance value (61.27%).

Ultrasonic process time of 90 minutes and concentration of 30% (Table 2, Figure 2) will produce SNPs with the lowest transmittance value compared to other treatments. The

Table 3

Transmittance values of SNPs at various ultrasonic process times and starch concentrations (mean ± SD, n =3)

| Treatment	Transmittance (%) at wavelength (nm)					Average transmittance (%)
	450	500	600	700	800	
Processing time 30 minutes, starch concentration 1%	85.31 ± 0.08	85.62 ± 0.04	86.78 ± 0.13	86.60 ± 0.65	88.34 ± 0.16	86.53 ± 0.20
Processing time 30 minutes, starch concentration 2%	75.17 ± 0.11	76.55 ± 0.13	77.37 ±0.44	77.96 ±0.42	80.16 ± 0.05	77.44 ± 0.14
Processing time 30 minutes, starch concentration 3%	66.72 ± 0.32	67.47 ± 0.64	69.27 ± 0.23	69.97 ± 0.34	72.00 ± 1.63	69.09 ± 0.52
Processing time 60 minutes, starch concentration 1%	82.37 ± 0.64	82.30 ± 0.36	83.25 ± 0.43	83.20 ± 0.19	83.83 ± 0.55	82.99 ± 0.34
Processing time 60 minutes, starch concentration 2%	69.65 ± 1.06	70.78 ± 0.40	72.58 ± 0.64	73.71 ± 0.18	76.88 ± 0.40	72.72 ± 0.30
Processing time 60 minutes, starch concentration 3%	62.74 ± 0.38	63.56 ± 0.51	64.76 ± 0.40	64.72 ± 0.13	66.48 ± 0.27	64.45 ± 0.15
Processing time 90 minutes, starch concentration 1%	78.97 ± 0.48	78.51 ± 0.30	78.16 ± 0.12	78.12 ± 0.43	78.47 ± 0.27	78.45 ± 0.29
Processing time 90 minutes, starch concentration 2%	64.91 ± 0.48	65.86 ± 0.65	67.27 ± 0.19	68.40 ± 0.28	70.22 ± 0.24	67.33 ± 0.31
Processing time 90 minutes, starch concentration 3%	58.29 ± 0.25	59.41 ± 0.41	61.09 ± 0.11	62.23 ± 0.23	64.23 ± 0.10	61.05 ± 0.22
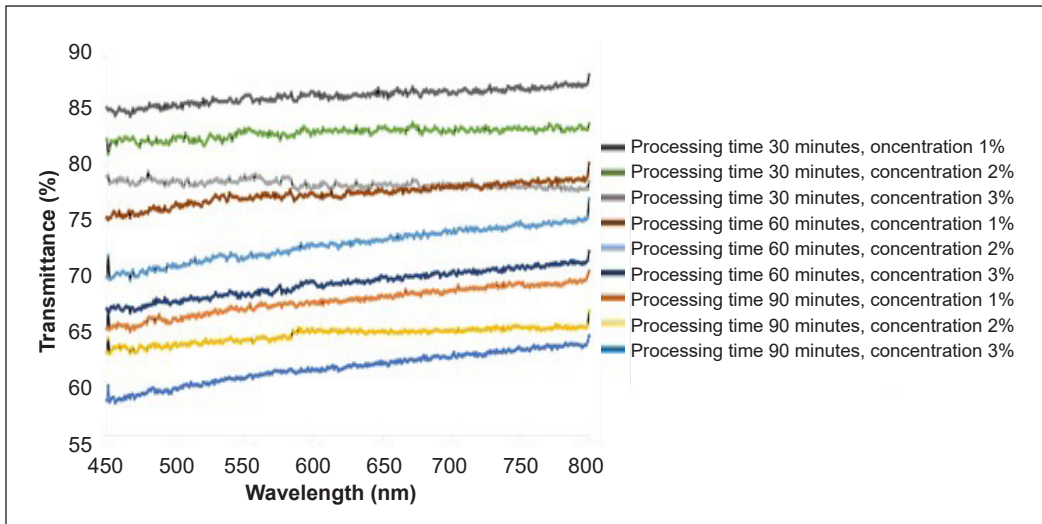
Figure 2. SNP transmittance curves for various ultrasonication process times and starch concentrations

lower transmittance value of the SNP is strongly related to the size of the SNP particles. The smaller the SNP particle size is, the more difficult it is for the starch particles to precipitate and the lower the transmittance value is. On the other hand, the larger the SNP particle size is, the faster the particles settle and the greater the transmittance value is. Changes in the transmittance of SNPs and a decrease in particle size were also reported by Bel Haaj et al. (2013) on SNP formation in corn starch. According to Haaj et al. (2013), SNPs with a size of more than 10 μm will precipitate quickly.

Starch Nanoparticles Clarity Score

The clarity scores of SNPs at various lengths of the ultrasonication process are presented in Table 4 and Figure 3. The results show that the 90-minute ultrasonication process and 3% starch concentration will produce SNPs with the lowest level of clarity compared to other treatments. The lower clarity of the SNP is strongly related to the size of the SNP particles and their solubility. The smaller the SNP particle size, the lower the clarity of the SNP solution because the nano-sized SNP particles will dissolve and have difficulty settling even though they have been left for 2 hours. The increase in SNP solubility with the smaller particle size is mainly related to the increase in the porosity of starch granules (Sujka, 2017). Changes in the level of clarity of SNP solutions, along with a decrease in particle size, were also reported by Jambrak et al. (2010) and Kim et al. (2013) on SNP formation in corn starch.

The decrease in the clarity score is also directly proportional to the decline in the transmittance value. The smaller the particle size, the lower the transmittance value and the clarity score. If a solution is passed by light, there will be a scattering of dissolved

particles, which causes a reduction in transparency. It is closely related to the size of the particles dispersed in the solution. In solutions containing nano-sized granules, these are soluble so that the scattering effect becomes more significant, reducing the transmittance value of the solution and its clarity score.

Table 4
SNP clarity scores at various ultrasonic process times and starch concentrations (mean ± SD, n =10)

Treatment	SNP clarity score (%)
Processing time 30 minutes, starch concentration 1%	3.80 ± 0.13
Processing time 30 minutes, starch concentration 2%	3.60 ± 0.20
Processing time 30 minutes, starch concentration 3%	3.10 ± 0.27
Processing time 60 minutes, starch concentration 1%	2.90 ± 0.22
Processing time 60 minutes, starch concentration 2%	2.80 ± 0.08
Processing time 60 minutes, starch concentration 3%	2.70 ± 0.07
Processing time 90 minutes, starch concentration 1%	2.40 ± 0.13
Processing time 90 minutes, starch concentration 2%	2.20 ± 0.09
Processing time 90 minutes, starch concentration 3%	2.10 ± 0.21

Score description: 1 = very unclear; 2 = not clear; 3 = not clear enough; 4 = clear; 5 = very clear
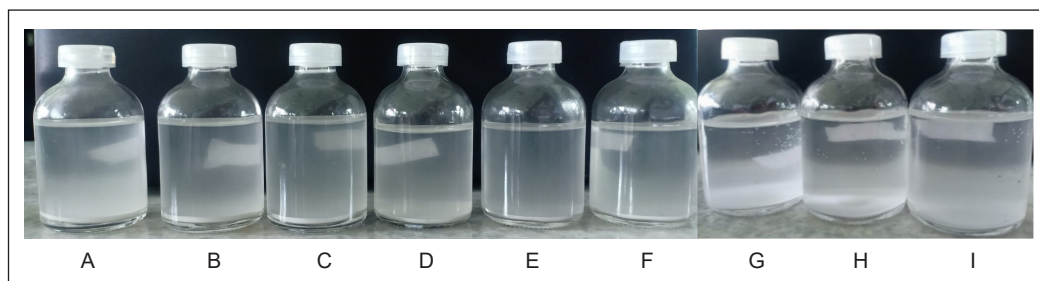
Figure 3. Clarity of SNP solutions at various ultrasonic process times and starch concentrations (A = 30 min, 1%; B = 30 min, 2%; C = 30 min, 3%; D = 60 min, 1%; E = 60 min, 2%; F = 60 min, 3%; G = 90 min, 1%; H = 90 min, 2%; I = 90 min, 3%)

CONCLUSION

Ultrasonic process time and starch concentration affect the yield, particle size and distribution, polydispersity index, optical characteristics (transmittance), and SNP clarity score. Ultrasonic process time of 90 minutes and starch concentration of 3% will produce SNP products with a yield of 13.68%, particle size ≤ 100 nm of 23.6%, average particle size of 230.8 nm with polydispersity index of 0.581, transmittance value of 61.27%, and a solution clarity score of 3.80 (not clear).

Tapioca-based SNPs can be developed solely with ultrasonic method to simplify the process. Further research is needed to improve the yield of SNPs based on tapioca.

ACKNOWLEDGEMENTS

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