Ultrasonics - Sonochemistry 68 (2020) 105199

Contents lists available at ScienceDirect

Ultrasonics - Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

Structural and physicochemical properties of lotus seed starch nanoparticles prepared using ultrasonic-assisted enzymatic hydrolysis

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ARTICLE INFO

Keywords:
Lotus seed starch nanoparticles
Ultrasonic-assisted enzymatic hydrolysis
Physicochemical properties
Small-sized

ABSTRACT

Lotus seed starch nanoparticles were prepared by ultrasonic (ultrasonic power: 200 W, 600 W, 1000 W; time: 5 min, 15 min, 25 min; liquid ratio (starch: buffer solution): 1%, 3%, 5%) assisted enzymatic hydrolysis (LS-SNPs represent lotus seed starch nanoparticles prepared by enzymatic hydrolysis and U-LS-SNPs represent lotus seed starch nanoparticles prepared by high pressure homogenization-assisted enzymatic hydrolysis). The structure and physicochemical properties of U-LS-SNPs were studied by laser particle size analysis, scanning electron microscope, X-ray diffraction, Raman spectroscopy, nuclear magnetic resonance and gel permeation chromatography system. The results of scanning electron microscopy showed that the surface of U-LS-SNPs was cracked and uneven after ultrasonic-assisted enzymolysis, and there was no significant difference from LS-SNPs. The results of particle size analysis and gel permeation chromatography showed that the particle size of U-LS-SNPs (except 5% treatment group) was smaller than that of LS-SNPs. With the increase of ultrasonic power and time, the weight average molecular gradually decreased. The results of X-ray diffraction and Raman spectroscopy showed that ultrasonic waves first acted on the amorphous region of starch granules. With the increase of ultrasonic power and time, the relative crystallinity of U-LS-SNPs increased first and then decreased. The group (600 W, 15 min, 3%) had the highest relative crystallinity. The results of nuclear magnetic resonance studies showed that the hydrogen bond and double helix structure of starch were destroyed by ultrasound, and the double helix structure strength of U-LS-SNPs was weakened compared with LS-SNPs. In summary, U-LS-SNPs with the small-sized and the highest crystallinity can be prepared under the conditions of ultrasonic power of 600 W, time of 15 min and material-liquid ratio of 3%.

1. Introduction

Starch, an indispensable food nutrient, is the only carbohydrate existing in the form of granules. Its granular structure is compact and diverse. Starch is insoluble in cold water, has a poor emulsifying ability, along with poor mechanical and storage stability, therefore, the use of starch in cold water is restricted. Studies have shown that modified starch can inhibit starch retrogradation and improve stability. Our research group [1-5] has made some advances in starch research by studying the effects of ultra-high pressure, microwave irradiation, autoclaving and ultrasonic autoclaving on the structural characteristics and physicochemical properties of lotus seed resistant starch, and we have also assessed the effects of lotus seed resistant starch on the proliferation of bifidobacteria.

Modification of starch at the nanometer level to meet the requirements of industrial applications has become a major focus in the field of starch research. There is a growing interest in natural materials and nanotechnology, these materials have many characteristics such as their nanometer size, biodegradable properties, low cost, nontoxic [6], lightweight, sustainable and harmless to health or environmentally friendly, and their unique properties have led researchers to delve into the field, extracting nanosized particles from natural polysaccharides (starch [7,8] and chitin [9,10]) has been the aim. In the past few decades, a variety of technical methods have been used to prepare SNPs. The principle of SNPs preparation can be broadly divided into top-down (such as acid hydrolysis [11] or physical treatment [12]) and bottom-up methods (such as nanoprecipitation [13]). However, these methods of preparing SNPs have some disadvantages, for example, the use of chemical reagents that may pollute the environment, the average size of the particles remains quite large. In a previous study [14], it was found

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https://doi.org/10.1016/j.ultsonch.2020.105199
Received 20 December 2019; Received in revised form 2 May 2020; Accepted 28 May 2020
Available online 01 June 2020
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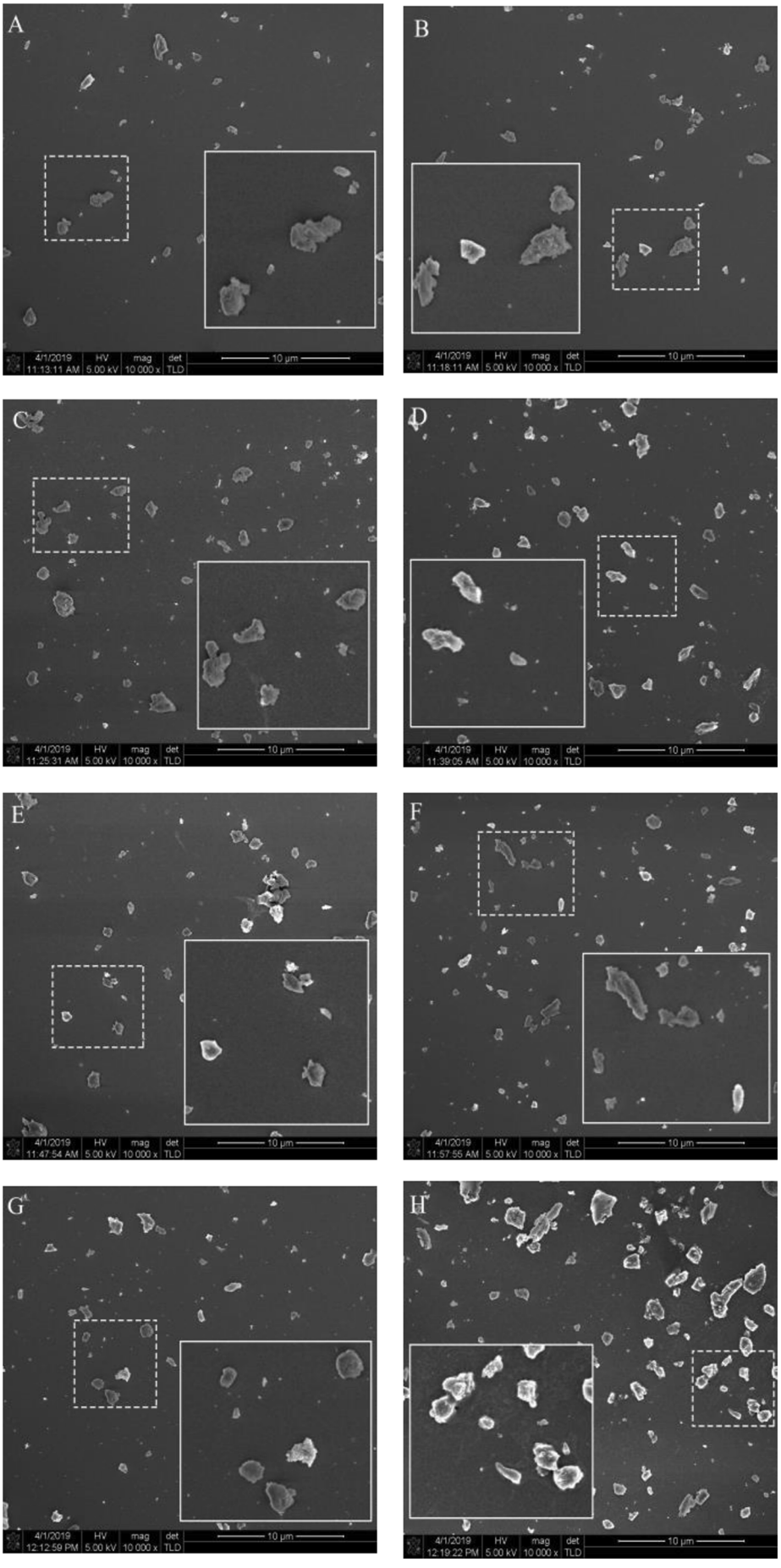

(caption on next page)

Fig. 1. Scanning electron microscope of lotus seed starch nanoparticles. The letter A represents lotus seed starch nanoparticles prepared by pullulanase, the letters B, C, D, E, F, G, H represent lotus seed starch nanoparticles prepared by ultrasonic-assisted pullulanase, ultrasonic conditions were (200 W, 15 min, 3%), (600 W, 15 min, 3%), (1000 W, 15 min, 3%), (600 W, 5 min, 3%), (600 W, 25 min, 3%), (600 W, 15 min, 1%), (600 W, 15 min, 5%), respectively. The unit "W", "min", "%" represent ultrasonic power, ultrasonic time and material-liquid ratio.

that obtaining individual nanoparticles from starch was almost impossible because of the strong tendency to produce aggregates of microparticles, the practical application of starch nanoparticles in nanocomposites has been restricted by their strong tendency to aggregate.

Ultrasound is an effective technique that meets the green goal [15], and it has the characteristics of being faster [16], milder and more environmentally friendly in degradation modification [17]. Most of the physical and chemical changes caused by ultrasound are usually attributed to cavitation effects. Acoustic cavitation causes rapid generation of bubbles in the liquid, generating tremendous pressure in a very short time (up to 20 MPa) and temperature (up to 5000 K) [18], from the final implosion when the bubble collapses. The shear forces generated by the collapse of the bubbles may break the covalent bonds in the polymer material. Ultrasonic treatment of chitosan [19], aqueous starch solutions [20] and proteins [21] is an effective method to reduce the molecular weight of these polysaccharides due to strong mechanical action and cavitation. Ultrasonic-assisted enzymatic hydrolysis is applied to nanocrystalline cellulose [22], sucrose conversion reaction [23] and delignification of sawdust [24]. It can reduce the particle size, improve the crystallinity, improve the performance of invertase, accelerate the enzymatic reaction and improve the safety, environmental protection and efficiency.

Although enzymatic hydrolysis has been frequently used in the preparation of starch nanoparticles [8,25], somehow the size of SNPs prepared by enzymatic hydrolysis was still large [26], so the practical application of starch nanoparticles in nanocomposites has been restricted by their strong tendency to aggregate. There have been no reports on the preparation of starch nanoparticles by assisted enzymatic hydrolysis under different ultrasonic conditions. The aim has been to explore the effects of different treatment conditions on the physicochemical properties of starch nanoparticles, and obtain a relationship between the preparation of starch nanoparticles by ultrasound-assisted enzymatic hydrolysis and the multiscale structure of starch nanoparticles (particle structure, aggregation structure, short-range structure, molecular chain structure).

2. Materials and methods

2.1. Materials

Lotus seed starch was isolated from lotus seeds (Green Field Fujian Co. Ltd., China). The enzyme pullulanase was purchased from Sigma-Aldrich (Sigma-Aldrich, USA). Disodium hydrogenphosphate (Na_2HPO_4), citric acid ($C_6H_8O_7$) and absolute ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All materials were used as received.

2.2. Preparation of U-LS-SNPs

2.2.1. Extraction of lotus seed starch

Lotus seed starch method that extracted by water extraction refers to Zhao [27] and it modify slightly: after being thawed naturally, the quick-frozen fresh lotus is mixed with distilled water at a liquid-to-material ratio of 1:3 to be beaten in a high-speed tissue pulverizer (Chang Zhou Xiang Tian experimental instrument factory, DS-200, China). The slurry is filtered through a 120-mesh food-grade nylon cloth and then allowed to stand at 25° C for 24 h. The supernatant is discarded and the lower filter residue is repeated with distilled water, after filter residue is washed several times to white, it adds 95% alcohol to remove oil, then it dry in an oven at 45° C for 16 h, prepare the lotus seed starch after grinding powder through a 100 mesh sieve.

2.2.2. Preparation of U-LS-SNPs

Lotus seed starch nanoparticles prepared by pullulanase using the method described by Liu et al. [28] with some modifications to the preparation procedure. Lotus seed starch was dispersed in 100 mL of disodium hydrogen phosphate (0.2 mol/L, 46.75 mL) and a citric acid (0.1 mol/L, 53.25 mL) buffer solution (pH 4.6), the starch solution was treated under different ultrasonic conditions (Microwave-ultrasonic combined extraction, XH300B, horn system, 25 ± 1KHz, Φ8mm, Beijing Xianghu Science and Technology Development Co. Ltd., China). Starch slurry was stirred vigorously in boiling water for 20 min. The temperature of starch was adjusted to 58 °C and pullulanase (100 U/g of dry starch) was added. After 8 h of incubation, the starch was heated at 100 °C for 30 min to inactivate the pullulanase. Then, 100 mL of absolute ethanol was added dropwise to the gelatinized starch solution under vigorous mechanical stirring while cooling to room temperature. The suspensions were washed several times with distilled water until neutrality and then freeze-dried to obtain short glucan chains powder. Short glucan chains powder was placed in aqueous solution (0.25% w/w) and then autoclaved at 121 °C for 30 min. The mixtures were then incubated in a water bath, which was preheated to 50 °C, and then kept for 6 h. Afterward, the mixtures were washed with distilled water several times and centrifuged at a speed of 4000 rpm for 15 min and then freeze-dried to obtain U-LS-SNPs.

2.3. Scanning electron microscopy

The sample was fixed to an aluminum column with conductive paste, and then the sample was subjected to gold treatment to attach a coating of approximately 50 nm thickness. Field emission scanning electron microscopy (Nova Nano SEM 230, FEI, USA) was used in low vacuum mode with an acceleration voltage of 10 kV.

2.4. Particle-size distribution

The particle size of U-LS-SNPs was measured with a laser particle-size meter (Mastersizer 3000, Malvern Instruments Ltd., UK). At an ambient temperature of 25 °C, the concentration of the sample was 0.01% (m/v), and the agent was ultrapure water. The dispersants and samples had a refractive index of 1.33 and 1.53 [29], respectively, and each sample was scanned three times.

2.5. X-ray diffraction

X-ray diffraction (RINT-TTR III, Rigaku, Japan) was performed at a Cu-Kα wavelength of 1.54056 Å. The scan tube voltage was 40 kV, the current was 200 mA and the scan range was $2\theta = 5°–35°$. The data acquisition step width was 0.02°.

2.6. Raman spectroscopy

Raman spectra were collected on a Lab RAM Analytical Raman microspectrograph (inVia Reflex, Renishaw, England) with a He–Ne laser source as exciting radiation ($\lambda = 632.8$ nm) and an air-cooled CDD detector.

2.7. Nuclear magnetic resonance

The molecular properties of the samples were investigated with a

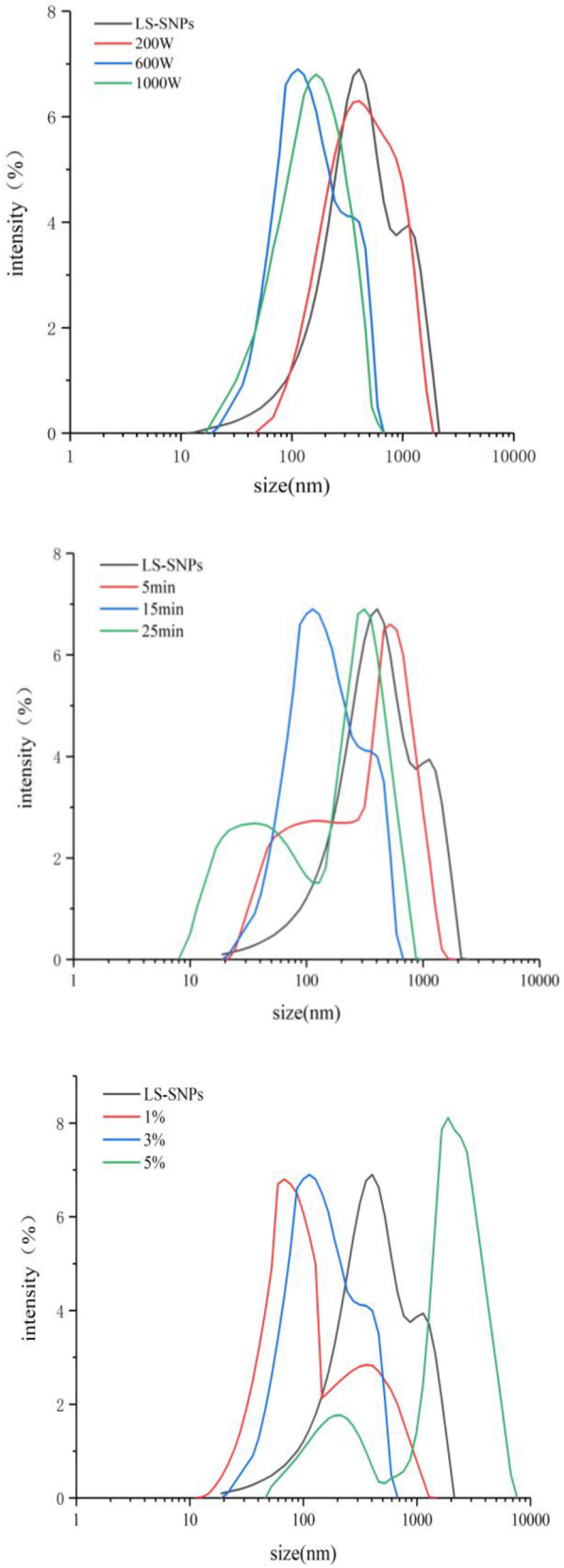

Fig. 2. Size distribution of lotus seed starch nanoparticles. The abbreviations LS-SNPs represents lotus seed starch nanoparticles prepared by pullulanase, 200 W, 600 W, 1000 W, 5 min, 15 min, 25 min, 1%, 3%, 5% represent lotus seed starch nanoparticles prepared by ultrasonic-assisted pullulanase. The unit "W", "min", "%" represent ultrasonic power, ultrasonic time and material-liquid ratio.

nuclear magnetic resonance (NMR) spectrometer (Avance III 400 WB, Bruker, Germany). The sample was scanned in an NMR spectrometer. The resonance frequency of ^{13}C was 100.62 MHz, and the double resonance probe was a 7 mm H/X CP-MAS probe. The resonance spectrum of each segment was accumulated and scanned 1000 times.

2.8. Molecular weight distributions

Analysis of sample molecular weight by gel permeation chromatography (TDA305max, Malvern Instruments Ltd., UK) was performed with 50 mmol/L lithium bromide dimethyl sulfoxide solution as the mobile phase, with helium and xenon as the source gases for laser light scattering, the refractive index of the mobile phase was set to 1.4785 [30]. The results were collected and analyzed in Astra V laser light scattering data analysis software.

2.9. Statistical analysis

The test data were statistically analyzed with DPS 9.5, and the data were analyzed by analysis of variance and expressed as mean ± standard deviation. A level of 95% ($p < 0.05$) was considered to indicate significant differences, as plotted with Origin Pro 8.5 software. All experiments were tested in parallel three times.

3. Results and discussion

3.1. Morphological structure of U-LS-SNPs

The scanning electron micrograph of lotus seed starch nanoparticles prepared by ultrasound-assisted enzymatic hydrolysis is shown in Fig. 1. Fig. 1 (A) is the lotus seed starch nanoparticles prepared by pullulanase (LS-SNPs), its surface is rough and irregular shapes, some particles are agglomerated, however, them still maintain a more complete particle morphology. The letter "B" to "H" represent U-LS-SNPs that were prepared under (200 W, 15 min, 3%), (600 W, 15 min, 3%), (1000 W, 15 min, 3%), (600 W , 5 min, 3%), (600 W, 25 min, 3%), (600 W, 15 min, 1%), (600 W, 15 min, 5%) conditions. After ultrasonic-assisted enzymatic hydrolysis treatment, the morphology of U-LS-SNPs surface has cracks and unevenness, however they still maintain the shape of particles. The reason is that the starch is first gelatinized at high temperature during the preparation of U-LS-SNPs, and then subjected to ultrasonic treatment. Ultrasonic wave produces strong stirring and mechanical shearing effects in the starch system, weakens the interaction between starch molecules and destroys the starch system, so that the pullulanase can fully contact with the starch granules and increase the damage. In addition, with the increase of ultrasonic power, time, and material-liquid ratio, there is no obvious difference in the morphology of B to H (Fig. 1).

3.2. Particle sizes of U-LS-SNPs

The particle size distribution of lotus seed starch nanoparticles prepared by ultrasonic assisted enzymatic hydrolysis is shown in Fig. 2. The particle size distribution of lotus seed starch nanoparticles (LS-SNPs) without ultrasonic treatment was 16.7–2420 nm. After ultrasonic-assisted enzymolysis treatment, the particle size distribution was significantly shifted to the left (except for treatment group 5%), indicating ultrasonic-assisted enzyme treatment further degrades the lotus seed starch nanoparticles and it has a significant effect on their size. The particle size distribution of 5% treatment group is shifted to right, the reason may be the low concentration of starch solution let distance between the molecules is large, the molecular chains are not likely to overlap and entangle with each other, however with the increase of concentration, the number of starch chains per unit volume increases, the starch chains are more likely to polymerize and twist with each other to form larger particles, resulting in an increase in the

Table 1
Size distribution of lotus seed starch nanoparticles.

Sample	D[2,3]/nm	D[3,4]/nm	D(10)/nm	D(50)/nm	D(90)/nm
LS-SNPs	334.7 ± 16.2^{b}	666.0 ± 6.2^{b}	173.7 ± 6.7^{b}	504.7 ± 6.2^{b}	1463.3 ± 11.5^{b}
200 W	331.0 ± 9.2^{b}	681.0 ± 19.5^{b}	139.3 ± 4.0^{c}	501.9 ± 2.6^{c}	1455.3 ± 30.3^{b}
600 W	135.33 ± 0.6^{d}	421.33 ± 10.5^{d}	75.0 ± 5.6^{d}	253.3 ± 3.5^{d}	587.0 ± 4.6^{e}
1000 W	133.33 ± 7.6^{d}	418.7 ± 17.6^{d}	73.3 ± 4.5^{d}	258.7 ± 3.1^{d}	593.0 ± 4.6^{e}
5 min	220.33 ± 6.4^{c}	548.0 ± 15.9^{c}	168.0 ± 11.2^{b}	414.3 ± 12.4^{c}	974.7 ± 12.7^{c}
25 min	123.33 ± 13.2^{d}	418.0 ± 14.7^{d}	84.7 ± 4.2^{d}	251.0 ± 6.6^{d}	616.7 ± 28.9^{e}
1%	135.7 ± 5.5^{d}	438.0 ± 20.7^{d}	77.0 ± 5.3^{d}	254.3 ± 4.7^{d}	726.3 ± 23.5^{d}
5%	990.0 ± 52.6^{a}	1270.0 ± 60.9^{a}	344.0 ± 13.1^{a}	1042.7 ± 50.9^{a}	5104.0 ± 100.2^{a}

Values were expressed as mean ± SD (n = 3). Different letters within columns presented significant differences ($p < 0.05$).
The abbreviations LS-SNPs represents lotus seed starch nanoparticles prepared by pullulanase, 200 W, 600 W, 1000 W, 5 min, 25 min, 1%, 5% represent lotus seed starch nanoparticles prepared by ultrasonic-assisted pullulanase. The unit "W", "min", "%" represent ultrasonic power, ultrasonic time and material-liquid ratio. (600 W, 15 min and 3% are the same sample).

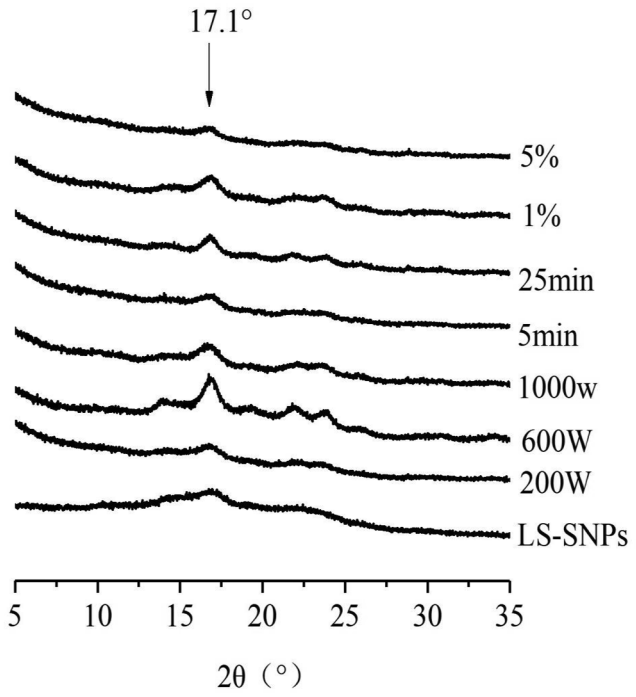

Fig. 3. X-ray diffraction of lotus seed starch nanoparticles. The abbreviations LS-SNPs represents lotus seed starch nanoparticles prepared by pullulanase, 200 W, 600 W, 1000 W, 5 min, 25 min, 1%, 5% represent lotus seed starch nanoparticles prepared by ultrasonic-assisted pullulanase. The unit "W", "min", "%" represent ultrasonic power, ultrasonic time and material-liquid ratio. (600 W, 15 min and 3% are the same sample).

viscosity of the starch solution [31].

It can be seen from Table 1, there are significant differences ($p < 0.05$) between the power group of 600 W and 200 W, the time group of 15 min and 5 min, the material-liquid ratio group of 3% and 5%, and there are no significant differences ($p < 0.05$) between 600 W and 1000 W, 15 min and 25 min, and 3% and 1%.With the increase of ultrasonic power and time, the particle size of U-LS-SNPs gradually decreases. The reason is that the ultrasonic intensity becomes larger, it weakens the interaction between starch molecules and the distribution of starch molecular chains becomes more concentrated. When the power and time continue to increase, the particle size of U-LS-SNPs does not continue to decrease, which may be because some small particles tend to adhere to large particles when the power and time continue to increase, and it occurs agglomeration [32], making U-LS-SNPs particles become larger. Considering energy consumption and economic benefits, 600 W, 15 min and 3% are the best conditions for preparing U-LS-SNPs.

3.3. X-ray diffraction

Fig. 3 is an X-ray diffraction pattern of lotus seed starch nanoparticles prepared by ultrasonic-assisted enzymatic hydrolysis. The crystalline region shows a sharp diffraction characteristic in the X-diffraction pattern, and the crystal form is complete and the grain size is large in this region; the non-crystalline region shows a diffuse diffraction characteristic in the X-diffraction pattern, which is a disordered state. U-LS-SNPs and LS-SNPs showed an obvious characteristic diffraction peak at the X-ray diffraction pattern $2\theta = 17.1°$, indicating that they are both B-type crystal forms, and no new functional group were produced after ultrasonic-assisted enzymatic hydrolysis Functional group. The relative crystallinity of lotus seed starch nanoparticles can be calculated by X-ray diffraction pattern. The crystallinity is manifested by the high degree of order (directivity) of the starch particles. Ultrasonic waves change the structural properties of the starch particles, thereby affecting the starch crystallinity. Calculate the relative crystallinity according to the method of Jia et al. [33], the relative crystallinity of LS-SNPs, 200 W, 600 W, 1000 W, 5 min, 25 min, 1%, 5% are: 65.07%, 65.11%, 74.92%, 70.22%, 65.53%, 71.50%, 72.62%, 60.02%, respectively. The relative crystallinity of lotus seed starch nanoparticles prepared by ultrasonic-assisted enzymolysis (except for the 5% treatment group) is higher than that prepared by enzymatic hydrolysis (Fig. 3). The reason is that ultrasonic treatment decomposes the starch molecular chains by shearing, which exposes a large amount of hydroxyl groups, it enhances the interaction between starch molecules and water molecules, resulting in the destruction of the starch system in the solution, which contributes to the recrystallization of the enzymatic hydrolysis of pullulanase and improves the crystallinity of lotus seed starch nanoparticles [25]. With the increase of ultrasonic power, time and material-liquid ratio, ultrasonic power has the most significant effect on the crystallinity of U-LS-SNPs. This is because when ultrasonic treatment of starch solution, a large number of small bubbles will be generated in the starch solution. When the ultrasonic power reaches a certain level, the dynamic process of bubble growth, expansion, and collapse will occur. At the moment the bubble is compressed until it collapses, a huge instantaneous pressure will be generated, which can generally be as high as tens of megapascals to hundreds of megapascals, to achieve the purpose of cutting the starch molecular chain [18]. At the same time, it promotes molecular rearrangement during the enzymatic hydrolysis process.

3.4. Structural analysis by Raman spectroscopy

The Raman spectrum of the lotus seed starch nanoparticles prepared by ultrasonic-assisted enzymatic hydrolysis is shown in Fig. 4. According to the Wiercigroch study [34], the vibration of each specific functional group was determined. Ultrasound-assisted enzymolysis preparation of lotus seed starch nanoparticles and enzymatic hydrolysis

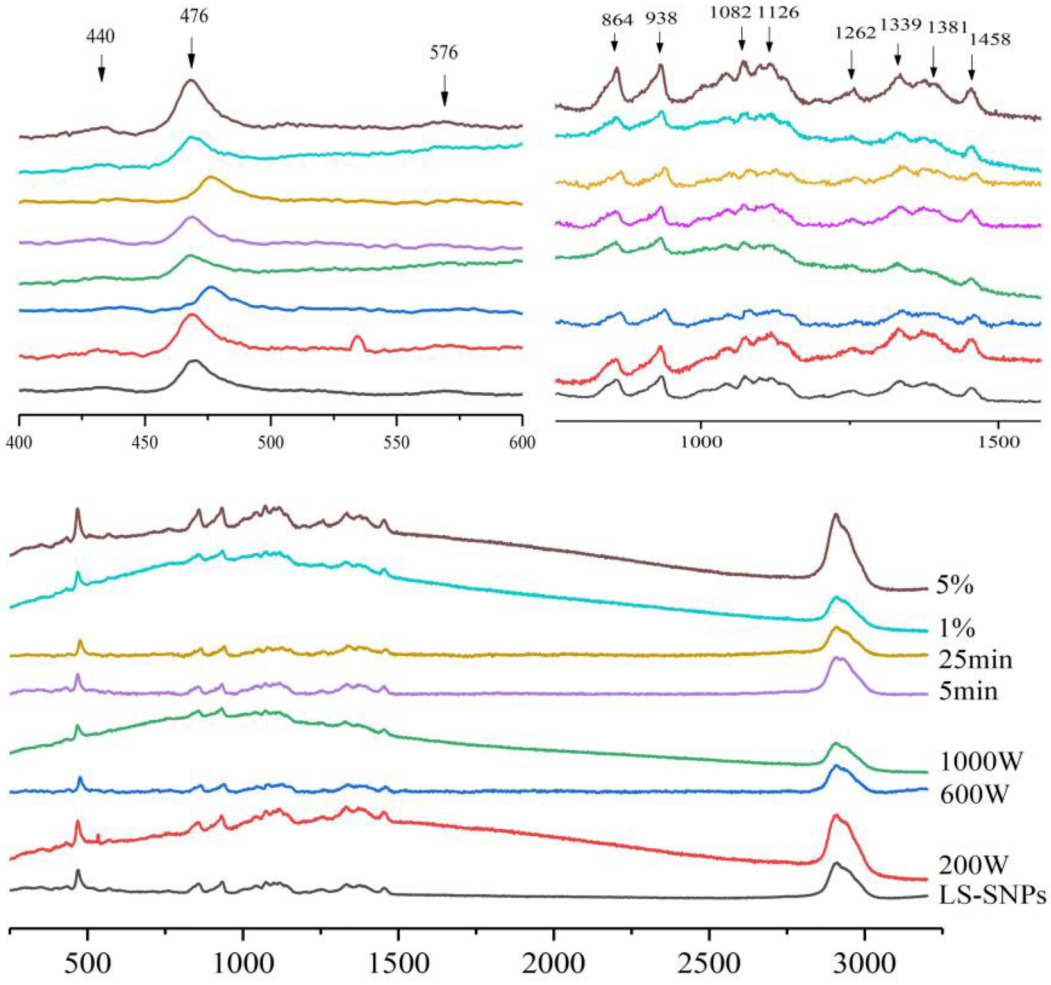

Fig. 4. Raman spectrogram of lotus seed starch nanoparticles. The abbreviations LS-SNPs represents lotus seed starch nanoparticles prepared by pullulanase, 200 W, 600 W, 1000 W, 5 min, 25 min, 1%, 5% represent lotus seed starch nanoparticles prepared by ultrasonic-assisted pullulanase. The unit "W", "min", "%" represent ultrasonic power, ultrasonic time and material-liquid ratio. (600 W, 15 min and 3% are the same sample).

preparation of lotus seed starch nanoparticles have basically the same Raman spectrum peaks, and the positions of characteristic group peaks are consistent, indicating that ultrasonic treatment has not changed the chemical constituent units of starch. The peak intensity of the lotus seed starch nanoparticles prepared by enzymatic hydrolysis is high (Fig. 4), indicating that LS-SNPs still has a non-crystalline structure, the ultrasonic-assisted enzymatic hydrolysis further destroys the disordered crystal structure and it makes the molecules order, it leads to a decrease in peak intensity, and it can be concluded that ultrasonic treatment mainly acts on the amorphous region of starch granules. Studies have shown that the strength of each characteristic peak is related to the size of starch crystal zone [35], the characteristic peak intensity of Raman spectrum at 476 cm^{-1} is significantly negatively correlated with the ordered structure of starch. The greater intensity of this characteristic peak, the lower degree of ordered structure. With the increase of ultrasonic power and time, the peak intensity of the Raman spectrum at 476 cm^{-1} increased first and then decreased. This trend is consistent with the results of X-ray diffraction pattern study. However, the 476 cm^{-1} peak intensity of 5% treatment group is higher than other treatment group. The reason may be that the starch concentration in 5% treatment group is greater than the substrate concentration of the fixed enzyme (pullulanase), which pullulanase enzymes are not fully effective.

3.5. Nuclear magnetic resonance analysis

The nuclear magnetic resonance spectrum of lotus seed starch nanoparticles prepared by ultrasonic assisted enzymatic hydrolysis is shown in Fig. 5. According to the study by Flanagan et al.[36], the chemical shift of the carbon chain backbone is distinguished: peaks of 96 to 106 ppm belong to the glucose unit C1 part, peaks of 70 to 79 ppm belong to the glucose unit C2, C3 and C5 parts, and 80 to 84 ppm belong to the glucose unit C4 part, the peak at 59 to 62 ppm belongs to the glucose unit C6 part. It can be seen from Fig. 5 that the lotus seed starch nanoparticles prepared by enzymatic hydrolysis and the lotus seed starch nanoparticles prepared by ultrasonic-assisted enzymatic hydrolysis show a bimodal structure formed by two glucose residues in the C1 region, indicating that LS-SNPs and U-LS- SNPs belong to type B starch, which is consistent with XRD results.

Studies have shown that the factors affecting crystallinity are the double helix structure, the length and content of amylopectin in the crystal region, and the interaction of the double helix structure [37]. The signal peak in the C1 region can reflect the strength of double helix structure in the starch crystal region. The higher signal peak intensity, the stronger double helix structure strength. The double helix structure strength of the lotus seed starch nanoparticles prepared by enzymatic hydrolysis is stronger than that of the lotus seed starch nanoparticles

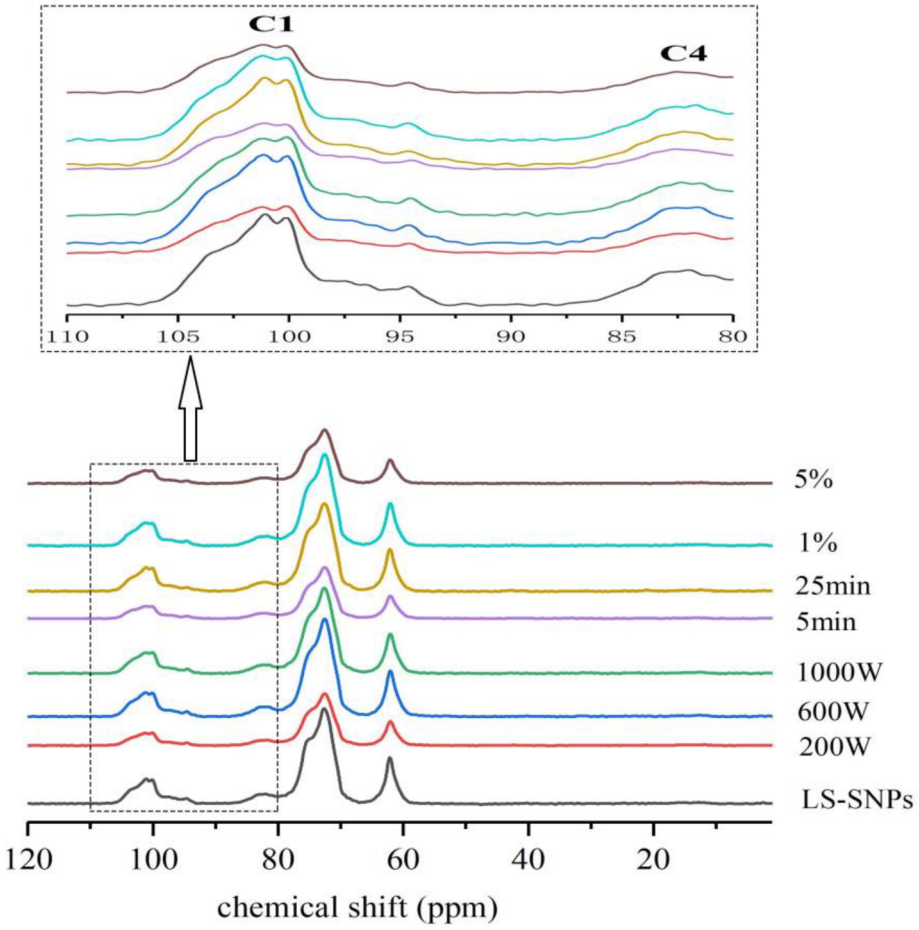

Fig. 5. Nuclear magnetic resonance spectroscopy of lotus seed starch nanoparticles. The abbreviations LS-SNPs represents lotus seed starch nanoparticles prepared by pullulanase,200 W, 600 W, 1000 W, 5 min, 25 min, 1%, 5% represent lotus seed starch nanoparticles prepared by ultrasonic-assisted pullulanase. The unit "W", "min", "%" represent ultrasonic power, ultrasonic time and material-liquid ratio. (600 W, 15 min and 3% are the same sample).

prepared by ultrasonic-assisted enzymatic hydrolysis. The reason is that the mechanical effect of ultrasonic waves breaks the hydrogen bonds between starch molecules and within the starch molecules, resulting in starch double helix structure regions extension, thereby destroying the double helix structure. With the increase of ultrasonic power, time, and material-liquid ratio, the double helix structure strength of lotus seed starch nanoparticles prepared by ultrasonic assisted enzymolysis first increased and then weakened, and the double helix strength of the treatment group (600 W, 15 min, 3%) was the strongest, the main reason is that amylose molecules can form a double helix structure [38], ultrasonic treatment is beneficial to the formation of amylose in starch solutions, but excessive ultrasonic intensity will seriously affect the crystallization behavior of amylose molecules, which will affect the formation of double helix structures.

3.6. Molecular weight distributions

Gel permeation chromatography[13,39] was used to further study the molecular weight distribution of lotus seed starch nanoparticles prepared by ultrasonic assisted enzymolysis (Fig. 6). Compared with the molecular weight distribution of lotus seed starch nanoparticles prepared by enzymatic hydrolysis (5.0×10^2Da ~ 6.3×10^6Da), the molecular weight distribution of lotus seed starch nanoparticles prepared by ultrasonic-assisted enzymolysis was significantly shifted to the left (except for 5% treatment Group), most molecular weight of the U-LS-SNPs are smaller than LS-SNPs. After ultrasonic-assisted enzymolysis treatment, the proportion of large molecular weight decreases, the proportion of small molecular weight increases, and with the increase of ultrasonic power and time, the trend of molecular weight shifting to small molecule regions is more obvious, this phenomenon can be attributed to the fact that ultrasonic treatment destroys starch molecules and shortens the molecular chain length. Qian et al. [40] used ultrasonic treatment on starch solution, the results also showed that ultrasonic waves caused the starch molecular chain to break and the chain length changed short.

The polydispersity index of the lotus seed starch nanoparticles prepared by different ultrasonic treatment conditions assisted in enzymatic hydrolysis is shown in Table 2, The greater polydispersity index, the wider molecular weight distribution. As the ultrasonic power increases, the polydispersity index decreases, indicating that the molecular weight distribution is gradually narrowing and the molecular weight distribution is more concentrated. It can be seen from Table 2 that as the ultrasonic power and time increase, the weight average molecular weight (*Mw*) gradually decreases. This is because ultrasonic waves mainly act on starch molecules through mechanical bond breaking, ultrasound causes molecular chains to break, molecular entanglement points decrease, and crystals structure are destroyed [31,40]. As the intensity of ultrasonic waves increases, the energy in the starch solution gradually accumulates. When the energy is released instantaneously, the high-frequency shear vibration and cavitation effect weaken interaction interface of gelatinization starch. The hydrogen bond between the starch molecules breaks, degrading starch [41].

4. Conclusions

The article studies the structure and physicochemical properties of lotus seed starch nanoparticles prepared by ultrasonic assisted enzymatic hydrolysis. The particle size distribution of lotus seed starch nanoparticles without ultrasonic treatment is 16.7–2420 nm. After ultrasonic-assisted enzymolysis treatment, the distribution is obviously

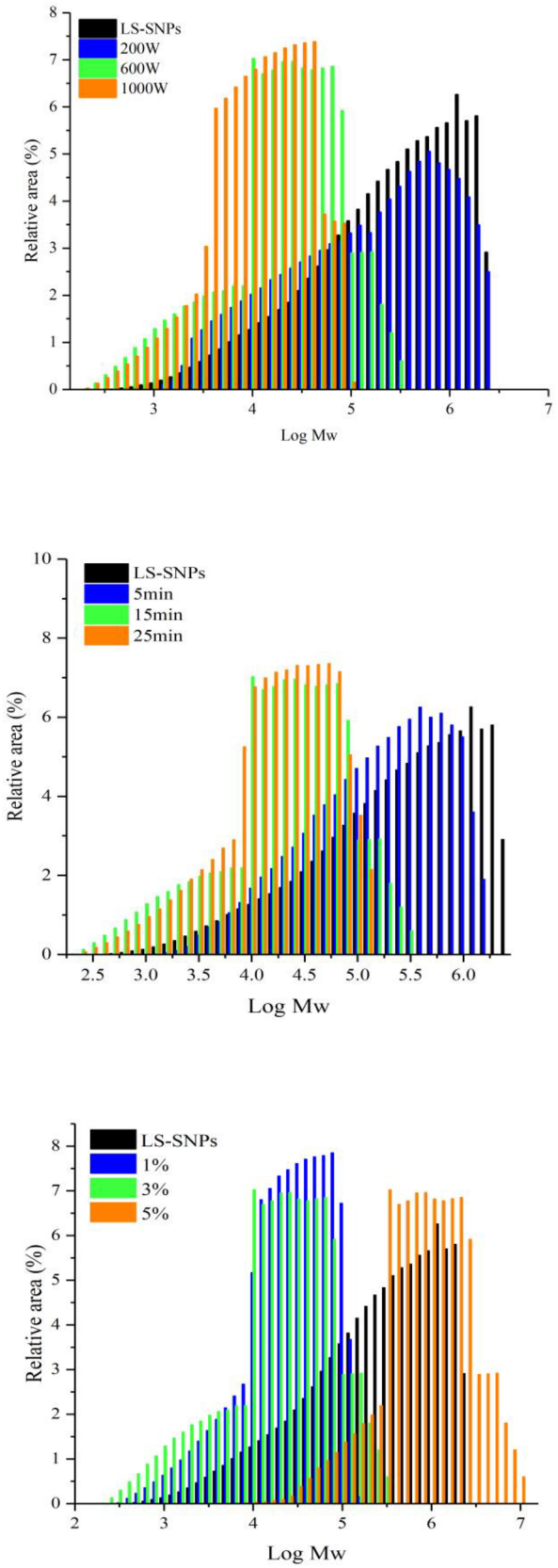

Fig. 6. Molecular weight distributions of lotus seed starch nanoparticles. The abbreviations LS-SNPs represents lotus seed starch nanoparticles prepared by pullulanase, 200 W, 600 W, 1000 W, 5 min, 15 min 25 min, 1%, 3%, 5% represent lotus seed starch nanoparticles prepared by ultrasonic-assisted pullulanase. The unit "W", "min", "%" represent ultrasonic power, ultrasonic time and material-liquid ratio.

Table 2
Molecular weight of lotus seed starch nanoparticles.

sample	*Mw* ($\times 10^5$Da)	*Mn* ($\times 10^5$Da)	Polydispersity
LS-SNPs	2.17	0.31	7.00
200 W	2.13	0.42	5.11
600 W	1.02	0.23	4.43
1000 W	1.01	0.27	3.74
5 min	1.78	0.42	4.24
25 min	1.02	0.25	4.08
1%	1.06	0.28	3.79
5%	5.61	1.27	4.42

The abbreviations LS-SNPs represents lotus seed starch nanoparticles prepared by pullulanase, 200 W, 600 W, 1000 W, 5 min, 25 min, 1%, 5% represent lotus seed starch nanoparticles prepared by ultrasonic-assisted pullulanase. The unit "W", "min", "%" represent ultrasonic power, ultrasonic time and material-liquid ratio. (600 W, 15 min and 3% are the same sample).

shifted to a small size area (except for 5% treatment group), It showed that the ultrasonic assisted enzymatic hydrolysis treatment further degraded the lotus seed starch nanoparticles, which had a significant effect on its size. With the increase of ultrasonic power and time, the starch nanoparticles prepared by the treatment group (600 W, 15 min, 3%) had the smallest particle size and the highest crystallinity. This is because ultrasonic treatment weakens the interaction between starch molecules, making lotus seed starch nanoparticles form smaller particles and destroy amorphous regions. When the ultrasonic power and time are too large, some small particles tend to adhere to the large particles, making the U-LS-SNPs particles larger. The 5% non-crystalline area of the treatment group is relatively large, which may be because the high concentration ratio of solute to solvent is greater than the substrate concentration of the immobilized enzyme, so the pullulanase cannot fully function.

5. Author statement

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors gratefully acknowledge the financial support from the FAFU Funds for Scientific and Technological Innovation (CXZX2018061), the FAFU Funds for Distinguished Young Scientists (xjq201618), and the Foundation of International Cooperation and Exchanges in Science and Technology of Fujian Agriculture and Forestry University (grant number KXGH17001).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2020.105199.

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