Agriculture and Food

AIMS Agriculture and Food, 8(2): 566–584.
DOI: 10.3934/agrfood.2023031
Received: 04 February 2023
Revised: 05 April 2023
Accepted: 24 April 2023
Published: 19 May 2023

http://www.aimspress.com/journal/agriculture

Research article

Analyzing the characteristics of degraded glucomannan of *Amorphophallus oncophyllus* using hydrogen peroxide and ultrasonication

Dyah H Wardhani[1,*], Hana N Ulya[1], Irvan Maulana[1], Shafira Salsabila[1], Andri C Kumoro[1], and José A Vázquez[2]

[1] Universitas Diponegoro, Chemical Engineering Department, Jl. Prof. Sudarto, SH, Tembalang, Semarang-50275, Indonesia
[2] Instituto de Investigatións Mariñas (CSIC), Grupo de Reciclado y Valorización de Materiales Residuales (REVAL), r/Eduardo Cabello, 6. Vigo, 36208, Galicia, Spain

* **Correspondence:** Email: dhwardhani@che.undip.ac.id**;** Tel: +6224720058.

Abstract: Glucomannan, one of the viscous polysaccharides, has been applied for various purposes in food industries. However, its high viscosity limits glucomannan in some applications e.g., as an injectable material and encapsulant in the spray drying method. Hence, glucomannan modification is needed to fulfill specific characteristics in such applications. This study investigated the modification of glucomannan properties under degradation treatment using hydrogen peroxide and ultrasonication in ethanol solvent. The modifications of glucomannan were conducted in a 35% hydrogen peroxide solution for 4 h and 40 kHz ultrasonication in 50% ethanol solvent. The combination of ultrasonication and oxidation significantly reduced the glucomannan viscosity, molecular weight, and swelling but increased the solubility. The oxidation, ultrasonication, or their combination approach increased carbonyl content, whiteness, and syneresis. The degradation created a coarse surface on the glucomannan particles. Interestingly, although the oxidation or the ultrasonication reduced glucomannan crystallinity, the combination of both methods increased this property. This result confirmed the synergetic treatments of the oxidation using hydrogen peroxide and the ultrasonication could effectively modify the properties of glucomannan including reducing the viscosity to the level that allowed the glucomannan to be spray-dried.

Keywords: glucomannan degradation; glucomannan oxidation; glucomannan properties; ultrasonic treatment

1. Introduction

Glucomannan, a polysaccharide extracted from the root of *Amorphophallus sp*., has been used widely as a supplement and an additive in foods, cosmetics, biotechnology and pharmaceuticals due to its biocompatibility, biodegradability and excellent gelling and film-forming ability [1]. Native glucomannan (1%) is reported to have up to 12,000 cP [2]. This high viscosity limits the glucomannan applications, such as its use as spray dryer feed and an injectable material [3]. Reducing viscosity by using a low concentration is not an appropriate approach since it could weaken the properties of spray-dried product and reduced its yield; hence, glucomannan modification is needed. Such a modification is conducted to meet the characteristics of a particular application.

Appropriate glucomannan degradation allows viscosity reduction. Apart from increasing solubility [4], degraded glucomannan was reported to have better antioxidant and probiotic activities [5]. This polysaccharide could inhibit the infection by preventing the pathogen from sticking to human cells [6].

Glucomannan degradation could be carried out via chemical, physical or biological treatment. The chemical degradations are conducted using alkalis [7,8], acids [9,10] or oxidation agents [11,12]. Jin et al. [13] and Lu et al. [14] performed γ-ray irradiation and ultrasonication as physical degradation agents for glucomannan in a non-solvent system. These degradations reduced the glucomannan molecular weight but insignificantly changed its chemical structure. β-mannanase and cellulase have been studied as biological agents for glucomannan degradation [2,15]. β-mannanase is not widely available [2]. Moreover, these enzymatic reactions were conducted in an aqueous solution which only permitted a low glucomannan concentration to avoid gelation.

Oxidation is a chemical depolymerization method using oxidants, such as sodium periodate, hydrogen peroxide, and sodium hypochlorite [12,16,17]. Hydrogen peroxide attacks the glycosidic bond of the polymer, which further reduces its molecular weight. The oxidation does not produce a toxic material for the environment [11,18].

Synergistic depolymerization has been proven to enhance polymer degradation. Pan et al. [19] improved glucomannan oxidation by combining γ-ray irradiation and hydrogen peroxide. The presence of hydrogen peroxide helped the physical degradation to occur more precisely and effectively [19]. However, the use of γ-ray irradiation required following strict regulations and using specific equipment.

Ultrasound which is used in physical degradation has been intensified to assist a carrageenan degradation process due to its effectivity [20,21]. Ultrasound decreased the molecular weight of pectin and chitosan [22,23]. This approach has also been proven to be an oxidation enhancer for the degradation of phenol and cellulose [24,25]. Combination of this oxidation method with a physical treatment could help to reduce the molecular weight effectively. However, the synergistic effect between oxidation and ultrasonication on the degradation of glucomannan has not been studied. This research aimed to study the physicochemical properties of degraded glucomannan using oxidation, ultrasonication, and their combination and compare them with those of the native glucomannan. In this research, the glucomannan was treated heterogeneously in aqueous ethanol to prevent gelation, hence allowing the occurence of higher glucomannan concentrations in the reaction. Moreover, a slightly volatile solution was selected to improve vapor pressure and led to forming more cavitations under ultrasonic treatment [26]. The cavitation bubbles collapsed during the treatment, and thus cleavaged the glycosidic bond [27]. Moreover, the presence of water in the ultrasonic system helped to generate •OH and HOO• radicals [28] which contributed to degrade many compounds [29].

2. Materials and methods

2.1. Materials

Glucomannan (91%) of the *Amorphophallus oncophyllus* tuber was bought from a local farmer in Nganjuk, East Java, Indonesia. Ethanol 96% was obtained from PT. Indo Acidatama Tbk (Solo, Central Java, Indonesia) and all other chemicals from Merck Chemical Co., Darmstadt, Hesse, Germany) were of analytical grade.

2.2. Glucomannan degradation

Glucomannan (50 g) was dispersed in 200 mL of ethanol solution (70% v/v) under 100-rpm stirring. The ethanol helped to promote the glucomannan reaction under a heterogeneous condition and prevented the gelation, thus allowing the reaction of a higher concentration of glucomannan and a higher yield. Hydrogen peroxide (30% w/w glucomannan) and copper sulfate (0.08 g) as an oxidation catalyst [11] were added to the suspension prior to ultrasonication (KW 1801033 ultrasonic cleaner, PT. Krisbow Indonesia, West Jakarta, Jakarta, Indonesia) at 40 kHz under an overhead stirrer. The suspension was filtered after 4 h of treatment and the cake was washed using ethanol (70%, 100 mL) before being dried under ambient conditions for 24 h.

2.3. Carbonyl content

The carbonyl content was determined using a hydroxylamine reagent [30]. The reagent was prepared by dissolving 25 g of hydroxylamine hydrochloride in NaOH solution (0.5 M, 100 mL) and brought to 500 mL using distilled water. The glucomannan (4 g) was dissolved in 100 mL of distilled water and boiled for 20 min. After cooling it down to 40 ℃, the solution was adjusted to pH 3.2 using 0.1 M HCl before hydroxylamine reagent (15 mL) addition. The mixture was heated at 40 ℃ for 4 h with slow shaking and titrated using 0.1 M HCl to pH 3.2. A blank sample was prepared for hydroxylamine reagent only. The carbonyl group was calculated by using Equation (1).

$$Carbonyl\ group\ (\%) = \frac{\left(V_{blank} - V_{sample}\right) \times M_{HCl} \times 100 \times 0.028}{w_{sample}} \quad (1)$$

2.4. Viscosity

Viscosity measurement was conducted using a Brookfield viscometer (RVDV II+Pro, Brookfield Engineering Laboratories, Inc, Stoughton, MA, USA). The viscosity of glucomannan solution (1%) was measured in three replications using spindles 2–7 at 100 rpm.

2.5. The viscosity average molecular weight

The molecular weight of glucomannan was analyzed based on its intrinsic viscosity [7]. Various concentrations of the glucomannan solution (0.01–0.05 g/L) were prepared at 60 ℃ for 30 min. The

flow time of the solution (t_1) in a Cannon Fenske capillary viscometer, size 100 (Schott AG, 102 Mainz, Rhineland-Palatinate, Germany) was recorded as well as the flow time of distilled water (t_2) to calculate the specific and reduction viscosities (η_{sp} and η_r) (Equations (2) and (3)).

$$\eta_{sp} = \frac{t_1 - t_0}{t_0} \tag{2}$$

$$\eta_r = \frac{\eta_{sp}}{c} \tag{3}$$

The specific and reduction viscosities were calculated for their linear equation. The intrinsic viscosity (η) was the mean of the intercepts. The Mark-Howink equation was used for the viscosity average molecular weight (M_v) identification (Equation (4)), with the values of K and a are being 5.9610^{-2} L/g and 0.73, respectively [7].

$$\eta = K \times {M_v}^a \tag{4}$$

2.6. Transparency and whiteness

For transparency determination, glucomannan solution (0.5% w/v) was prepared by stirring the glucomannan powder in distilled water for 1 h. The solution was read for its absorbance using a UV–Vis spectrophotometer (Shimadzu UV Mini 1240, Shimadzu Asia Pacific Pte Ltd., Singapore) at 680 nm [12]. Meanwhile, the whiteness of the sample powder was directly analyzed by using a colorimeter (Konica Minolta CR-400 Chroma Meter; Tokyo, Japan) without any preparation method. The obtained values of L*, a*, and b* were used for the whiteness calculation given by Equation (5).

$$Whiteness\ (\%) = 100 - \sqrt{[(100 - L^*)^2 + (a^{*2} + b^{*2})]} \tag{5}$$

2.7. Swelling and solubility

Determination of swelling and solubility was conducted based on the method of Hongbo et al. [11]. Glucomannan (0.5 g) was dispersed in 20 mL of ethanol (96%). After 30 min of stirring, the suspension was centrifuged at 3000 rpm for 30 min. The supernatant and the pellets were separated and oven-dried at 105 °C. Both the supernatant and pellets were weighed before and after drying. The swelling and solubility were calculated using Equations (6) and (7).

$$Swelling\ (\%) = \frac{w_{wet\ pellet} \times 100\%}{w_{dried\ pellet}\ (100 - solubility)} \tag{6}$$

$$Solubility\ (\%) = \frac{w_{supernatant} \times 100\%}{w_{dried\ supernatant}} \tag{7}$$

2.8. Morphology and functional groups

SEM imaging (JSM-6510 LV, JEOL, Tokyo, Japan) was used to observe the morphology of glucomannan particles. The functional groups of glucomannan were identified using IR spectra obtained via Fourier-transform infrared spectroscopy (Perkin Elmer Spotlight 200, PerkinElmer, Inc., Waltham, MA, USA).

2.9. Differential scanning calorimeter and X-ray diffraction

The change of glass transition temperature was analyzed by using a differential scanning calorimeter (DSC-60, SHIMADZU, Japan) at a heating rate of 10 ºC/min. The crystallinity of the samples was scanned using an Xpert Pro X-ray diffractometer (XRD, Malvern Panalytical, Malvern, Worcestershire, UK).

2.10. Antioxidant activity

The antioxidant activity of glucomannan was analyzed using a 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay, following the method of Lin et al. [31]. The sample solution (1%, 3 mL) was mixed with 9 mL of freshly prepared DPPH solution (0.1 mM in 70% ethanol). The solution was incubated for 30 min at 30 ºC under dark conditions. The absorbance of the solution (A_i) was measured using a UV-Vis spectrophotometer (Shimadzu UV Mini 1240, Shimadzu Asia Pacific Pte Ltd., Singapore) at 517 nm. The absorbance of the mixture of the sample (3 mL) and ethanol (9 mL) was read as A_j, while that of distilled water (3 mL) and the DPPH solution (9 mL) was read as A_0. The DPPH scavenging activity was calculated using Equation (8).

$$DPPH\ scavenging\ activity\ (\%) = \left(1 - \frac{Ai - Aj}{A_0}\right) \times 100\% \quad (8)$$

2.11. Syneresis

Syneresis of the glucomannan gel was measured using a modification of the Hu et al. [32] method. Glucomannan (0.2 g) was dissolved in 20 mL of distilled water under constant stirring for 2 h. Potassium chloride solid (0.15 g) was added to the mixture, stirred, and heated in a water bath (80 ℃) for 20 min and cooled down. The gel was weighed (m_1) and centrifuged for 15 min at 2000 rpm. The water over the gel's surface was wiped out, and the remaining gel was weighed (m_2). The syneresis degree was calculated using Equation (9) [32].

$$Syneresis\ (\%) = \frac{m_1 - m_2}{m_1} \times 100\% \quad (9)$$

2.12. Surface tension

Surface tension (γ) of the sample (1% w/v solution) was identified using the constant-drop weight method, based on the work of Morita et al. [33]. The density and volume of 30 drops of samples were

measured. The surface tension of the samples was compared with distilled water's surface tension data at the same temperature. The surface tension of the sample solution was calculated using Equation (10).

$$\gamma_s = \frac{V_s \rho_s}{V_w \rho_w} \gamma_w \tag{10}$$

2.13. Statistical Analysis

All experiments were carried out in triplicate, and the results are presented as means and standard deviations. Analysis of variance was conducted using Microsoft Excel 2019.

3. Results and discussion

3.1. Viscosity and its average molecular weight

The viscosity and its average molecular weight (M_v) of glucomannan significantly decreased after the ultrasound or/and oxidation were applied ($p < 0.05$, Figure 1a). The oxidation showed more of an effect on decreasing glucomannan viscosity and molecular weight than the sonication. During oxidation, an interaction between hydrogen peroxide and water produced hydroxyl radical through radiolysis [31,34]. Hydroxyl radical is a strong oxidation agent which allows cleavage of the carbon linkage from the hydrogen atom, which thus reforms the glucomannan structure [4]. Moreover, the acetyl group of glucomannan could be removed as an effect of the alkaline environment formation during oxidation [11], which reduced the viscosity and the molecular weight of glucomannan. However, based on the IR spectra in the following discussion (Section 3.6), the acetyl content of glucomannan was not changed significantly after the application of ultrasound or oxidation. It could be due to the weaker alkaline solution created during the oxidation compared to that of deacetylation. Wardhani et al. reported the reduction of the acetyl of glucomannan after deacetylation using a strong alkaline [35]. Figure 1a shows that the synergistic treatments of oxidation and ultrasonication dropped the viscosity below 200 cP. This condition fulfills the requirement of viscosity in spray-dried applications [2].

After sonication, a shorter glucomannan chain was produced, which reduced the chain interaction thus reducing the viscosity [36]. The viscosity of glucomannan was related to its M_v. A higher M_v glucomannan has a possibility to create more hydrogen bonds among the molecules which physically cross-linked and hinder the flow by forming a more viscous solution [4].

Meanwhile, the degradation of glucomannan under an ultrasonic treatment was due to the collapse of bubble cavitation which led to physical degradation [37,38]. When the bubbles exploded close to the glucomannan chain, they generated large shear forces which broke the polymer linkage near the midpoint of the chain [39,40]. In the combined treatment of sonication and oxidation, both physical and chemical degradation synergistically occurred at the same time, worsening the degradation and lowering the viscosity and M_v.

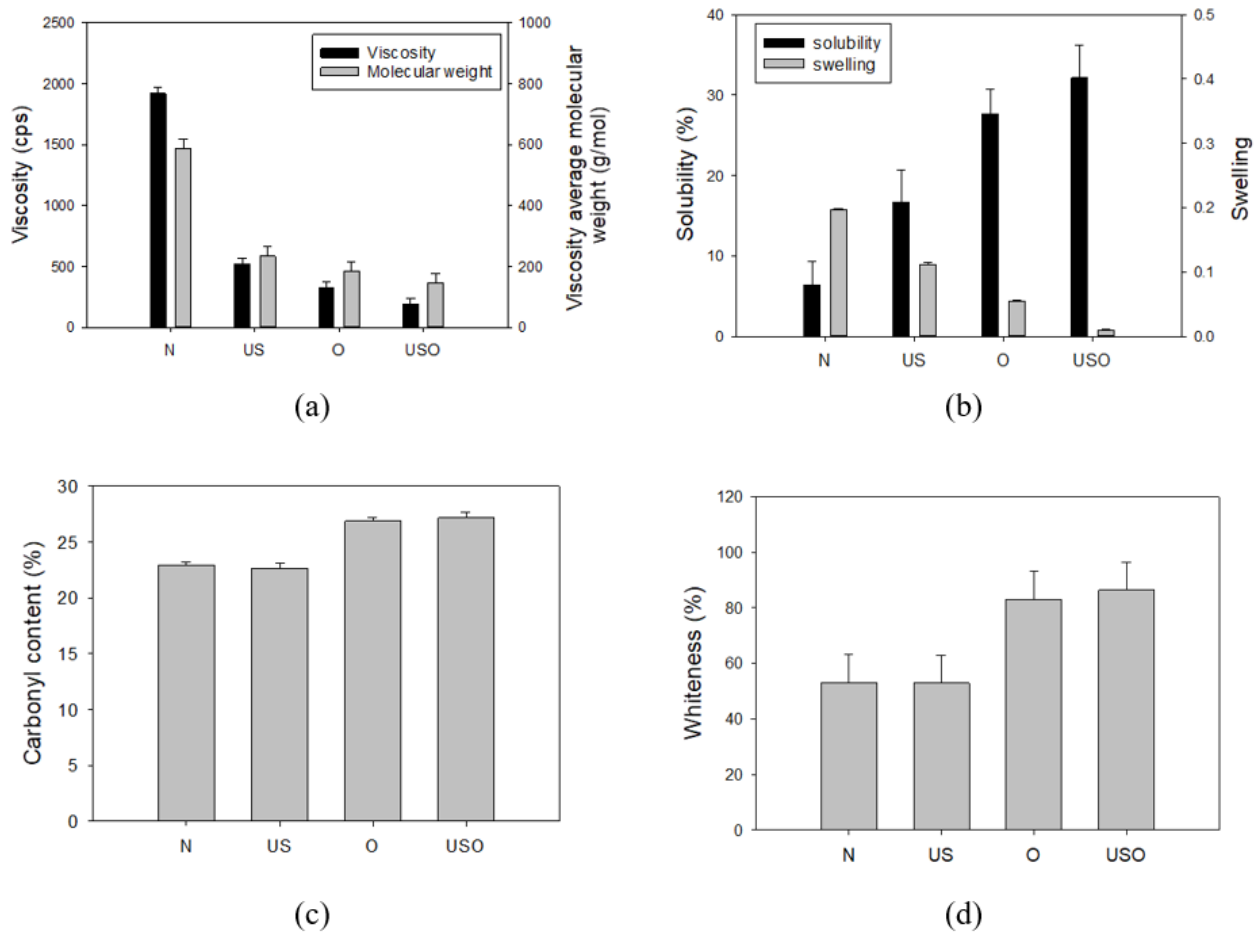

Figure 1. Physicochemical properties of native and treated glucomannans: (a) viscosity and its average molecular weight (M_v), (b) solubility and swelling, (c) carbonyl content, and (d) whiteness (N: native, US: ultrasonicated, O: oxidized, and USO: ultrasonicated-oxidized glucomannan).

3.2. Swelling and solubility

The decrease of viscosity and molecular weight led to a swelling reduction in all ultrasonic or oxidation treatments ($p < 0.05$, Figure 1b). Ebrahimi et al. [41] also found the ultrasonic application reduces the swelling of hydrogel. They also found an ultrasonic effect on starch swelling, Wang et al. [42] argued that swelling decrease could be attributed to the destruction of the granular structure of the starch, resulting in the degradation of the amylopectin chain. In this current work, the destruction of glucomannan granularity due to ultrasound, oxidation, and the combination treatment was observed as shown in Figure 2. Moreover, the oxidation also converted the hydroxyl groups to carbonyl groups which reduced the swelling power of glucomannan. Hence, the synergistic effect of ultrasound-oxidation which showed the worst granular destruction (Figure 2) led to the lowest swelling power.

On the contrary, the solubility of glucomannan was increased after the degradation ($p < 0.05$). The increase of free hydroxyl groups as the result of the cleavage of glucomannan chains after sonication eased the water binding [43]. The oxidation also increased the glucomannan solubility because it produced more porous particles. Luo et al. [44] also found friable particles after oxidation

of glucomannan. Although oxidation could also promote deacetylation of glucomannan which should decrease the solubility [11,45], the resulting alkaline ambient created by oxidation reaction was not strong enough to remove the acetyl groups. Moreover, the solubility of glucomannan was also influenced by its steric effect [46]. As a result, the solubility of oxidized glucomannan in this research increased.

3.3. Carbonyl content

Carbonyl groups were responsible for oligomer production, especially when the oxidation process was conducted in the presence of water [47]. The carbonyl contents of native and degraded glucomannan are shown in Figure 1c. The ultrasound insignificantly influenced the carbonyl content of the glucomannan ($p > 0.05$). The use of a low frequency of sonication may cause a non-visible change of carbonyl content. Chong et al. [48] only increased 0.0115% of the carbonyl content when sonicating starch at 25 kHz for 30 min. This result was in line with that of Kamble et al. [49], who reported that ultrasound promoted efficient synthesis of β-amino carbonyl compounds. However, they used a combination of sodium and p-toluene sulphonate as an aqueous hydrotropic medium to improve the solubility of organic compounds. A hydrotrope is a system that consists of a combination of hydrophilic and hydrophobic moieties. It refers to a diverse class of water-soluble surface-active compounds [49]. While, in this work, ethanol solution was used to suspend glucomannan as it was exposed to the ultrasonic treatment.

Oxidation, on the contrary, had a significant effect on the carbonyl content ($p < 0.05$). The hydrogen peroxide, as a strong oxidator, changed the hydroxyl groups of glucomannan to carbonyl groups [30]. The breakage of the main chain of glucomannan could form double bonds as part of carbonyl groups [50]. Oxidation removed the hydrogen groups from the hydroxyl and linked the carbon to oxygen which produced double-bonded carbon-oxygen as part of the carbonyl groups [51].

Physical oxidation increased the carbonyl content of glucomannan as demonstrated by Li et al. [4]. However, our results show that the combination of oxidation and ultrasound did not have a significant effect on carbonyl content compared to the lone oxidation treatment ($p > 0.05$). Koda et al. [40] found that a minimum 200 kHz frequency was required to promote the radical hydrogen generation from hydrogen peroxide using ultrasound. Meanwhile, sonication in our study was conducted at 40 kHz, far from the required minimum frequency.

3.4. Whiteness

The effects of sonication and oxidation on glucomannan whiteness are shown in Figure 1d. Ultrasonic treatment does not affect glucomannan whiteness ($p > 0.05$). This could be due to the low frequency of the ultrasound treatment; hence, less power was required to modify the glucomannan structure as shown in Figure 2. As a result, an insignificant change in color was observed between the treatment sample and the native one. Meanwhile, hydrogen peroxide was broken down into perhydroxyl radicals (OOH•) and reacted to the carbonyl group; it then attacked the C-C bond of glucomannan, which caused a bleaching effect that increased the whiteness ($p < 0.05$) [52]. Moreover, the hydrogen peroxide could also dissolve impurities, which led to a paler color of glucomannan [53]. Increasing the whiteness of a sample of the combination application of sonication and hydrogen peroxide was also reported by Abou-Okeil et al. [54].

3.5. Morphology

Figure 2 shows the surface appearance of the native and the degraded glucomannans. Sonication damaged the glucomannan's surface and created a coarser external layer. This result was supported by Li et al. [55] and Yan et al. [56], who stated that sonication did not only attack the molecular structure of glucomannan but also its physical appearance. Meanwhile, the treatment with hydrogen peroxide did not only change the glucomannan's molecular structure, but it also attacked the surface differently. More cracks were observed on the glucomannan surface following the decrease of the molecular weight and the rupture of hydrogen bonds. A similar finding was reported by Tatirat and Charoenrein [57]. More severe cracks were observed when the combination of sonication and oxidation was applied. A similar synergistic effect of irradiation and oxidation on the erosion of the particle's surface was also found by Hongbo et al. [11]. This increased damage by the combination treatment supported the previous discussion of the degradation, as explained in Section 3.1.

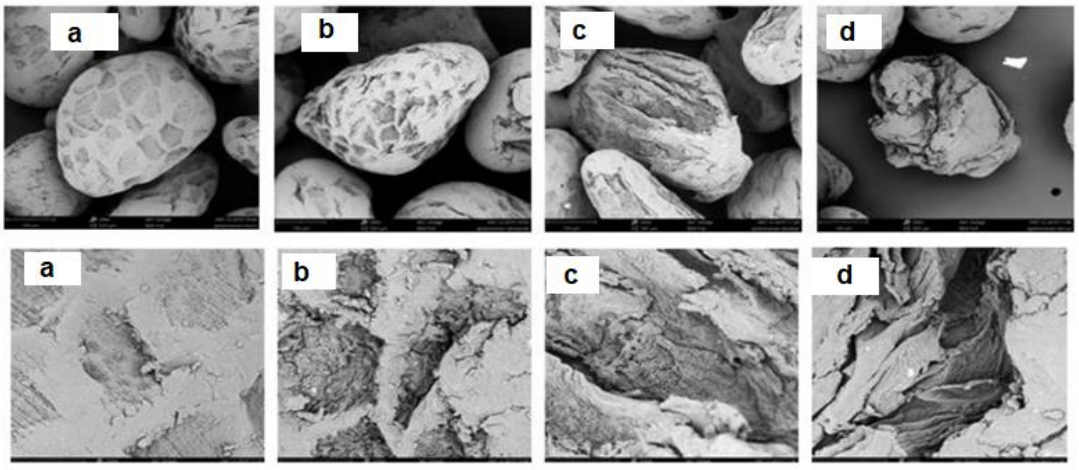

Figure 2. Morphology of (a) native, (b) ultrasonicated, (c) oxidized, and (d) combined treatment of glucomannan particle surface at 500x (top) and 2500x (bottom) magnification.

3.6. Functional groups

Overall, the functional groups of glucomannan were unchanged after sonication and oxidation were applied (Figure 3). However, these groups were present in different intensities of the spectra after the treatments. More hydroxyl groups were detected at ~3400 cm^{-1} after the treatments, as a result of broken chains of glucomannan. The C-H absorption peak was identified at ~2900 cm^{-1}. There were new peaks that appeared at ~3750 cm^{-1} as the O-H stretching peak of phenols [58] and ~2300 cm^{-1} as the effect of strengthened oxidation by sonication. Wang et al. [17] also reported a later peak in the oxidized glucomannan. No treatment affected the C=O bond of the acetyl group at ~1750 cm^{-1}, which supported the belief that the deacetylation insignificantly occurred in the oxidation process, as explained in Section 3.1. Moreover, Li et al. [4] reported that the change of C-H absorbance due to the oxidation was identified at ~1400 cm^{-1}. The peak at ~1100 cm^{-1} denoted the β-linkage in the glucomannan chain. An intensified peak was also detected at ~600 cm^{-1}, which was attributed to the hydroxyl group attached to the pyranose ring. This group was also modified as the sugar rings of the glucomannan were opened [4].

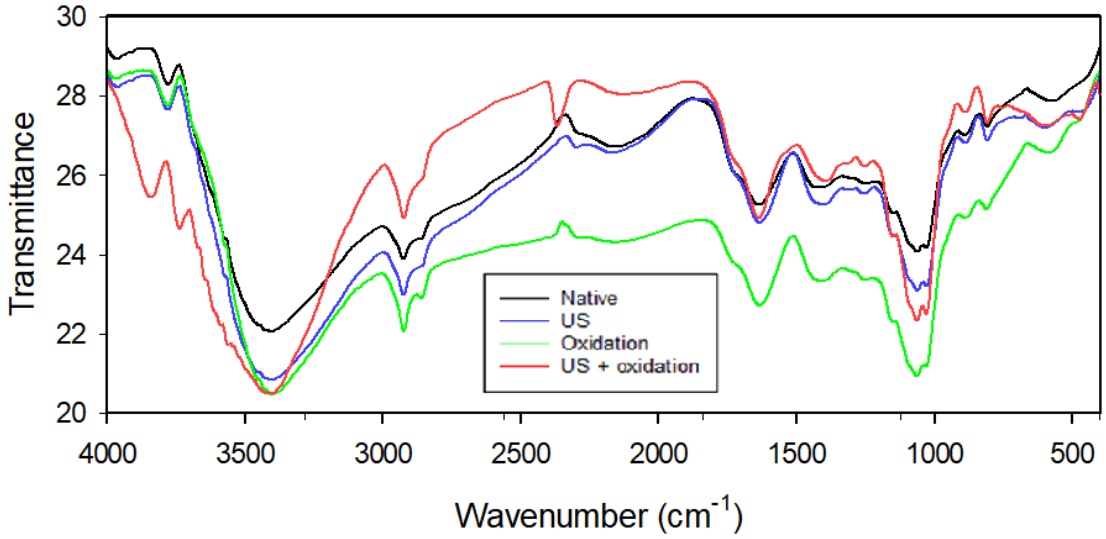

Figure 3. IR spectra comparison of native and the degraded glucomannans.

3.7. Glass transition temperature

Figure 4 shows the DSC curve of the native and the treated glucomannan. The endothermic peaks were identified from 114 to 131 ℃, while the exothermic peaks varied from 315 to 326 ℃. The endothermic peaks show moisture removal temperature by evaporation [46]. The hydroxyl groups had an important role in water molecule binding. Either sonication or oxidation treatment increased the endothermic temperature as a result of having lower molecular weights, which led to the creation of hydroxyl groups. This result supported the previous discussion of the functional group profile, as explained in Section 3.5. Higher endothermic temperature due to the depolymerization was also found by Li et al. [4]. However, the combination of sonication and oxidation lowered the endothermic temperature by ~5 ℃ compared to the native. Li et al. [46] suggested that the decrease could be due to the degree of dehydration of the sample at the preparation step.

The exothermic temperature shows the thermal decomposition temperature of glucomannan, which is related to its thermal stability. Lower exothermic temperatures were identified after the sonication and the oxidation treatments. Xu et al. [50] found that glucomannan with a lower molecular weight had lower thermal stability. The applied heat during the DSC analysis decomposed the glucomannan by breaking its chain and opening its saccharide ring [59]. Lower molecular weight glucomannans required lower temperatures to be decomposed. Luo et al. [44] stated that other than the molecular weight, the thermal stability of glucomannan was also affected by chain structure and hydrogen bonds.

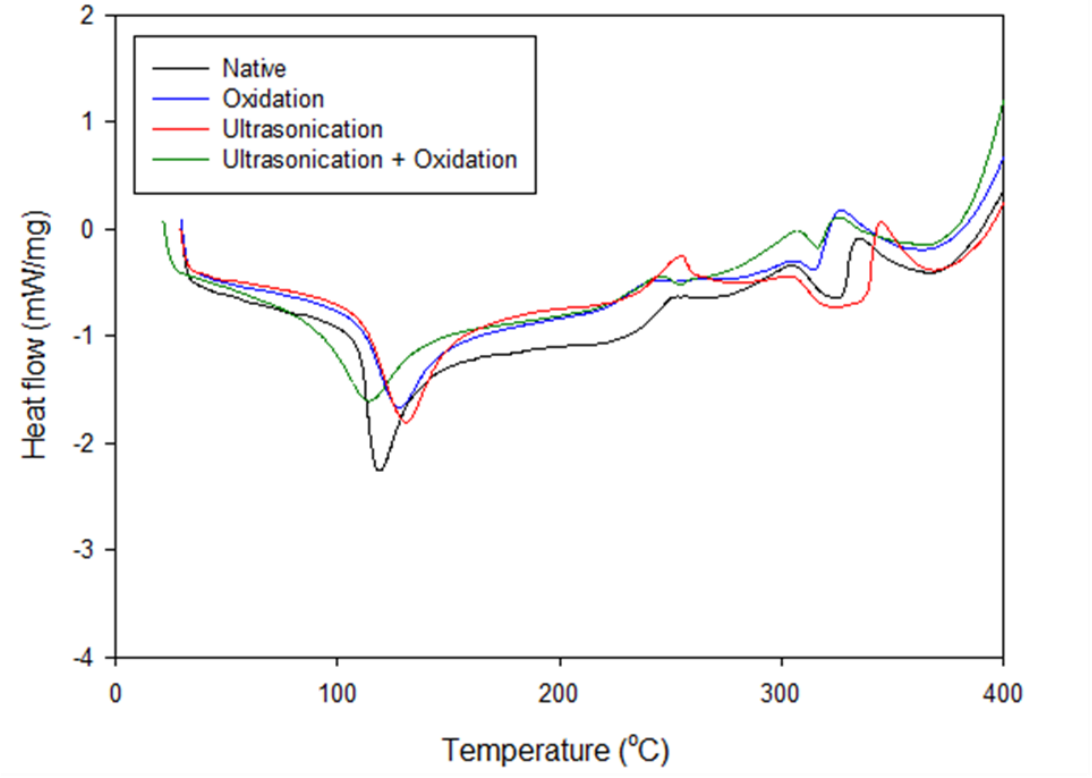

Figure 4. DSC profile of the native and the treated glucomannans.

3.8. Antioxidant activity

The antioxidant activity of the glucomannan is represented by the ability of the polysaccharide to scavenge DPPH radicals as shown in Figure 5 (top). This ability is consistent with the capability of the sample to donate hydrogen which belongs to hydroxyl groups [60]. Sonication increased the antioxidant activity because it produced glucomannan oligosaccharides with more hydroxyl groups. Yeung et al. [61] stated that the lower molecular weight of oligosaccharides affords it more active sites to bind free radicals, hence increasing the antioxidant activity. The low rise of the activity after sonication could be due to the low frequency used in this work. Interestingly, although oxidation reduced the molecular weight, the oxidation formed the carbonyl group from the hydroxyl group, which decreased the radical scavenging effect of glucomannan. A similar finding was reported by Munekata et al. [62], who found that a higher carbonyl content decreased the ability of glucomannan to prevent oxidation in meat.

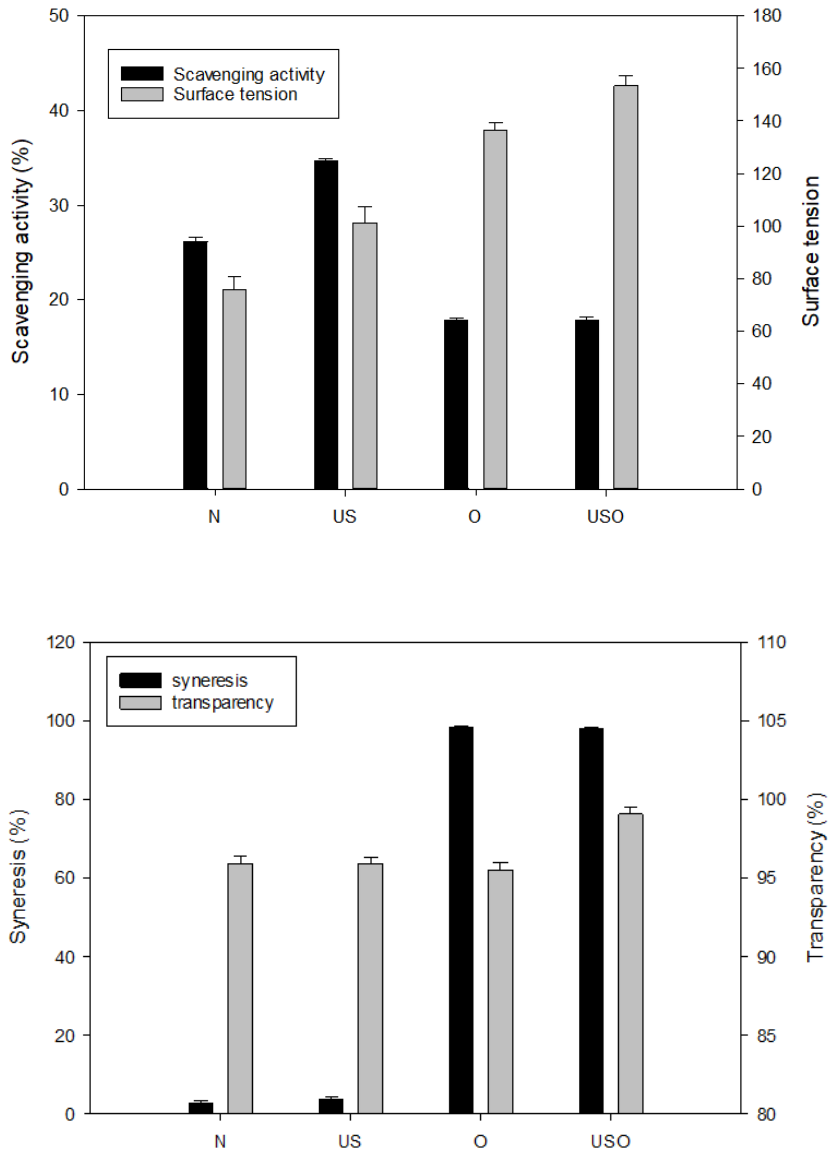

Figure 5. Comparison of native and degraded glucomannan: DPPH scavenging activity and surface tension (top); syneresis and transparency (bottom).

3.9. Surface tension

Sonication and oxidation supported the increase of the surface tension of treated glucomannan ($p < 0.05$, Figure 5 (top)). The treatments applied to the glucomannan created more hydroxyl groups, which led to an increase in the surface tension. The lower molecular weight of the polymer due to sonication increased its solubility, which led to the increase of surface tension. Arkles [63] reported that hydroxyl groups play a role in the surface tension properties of materials. Oxidation has a stronger effect than sonication in terms of increasing the surface tension, as oxidation improved the intermolecular forces of glucomannan [64]. Malviya et al. [65] found that intermolecular force was correlated with surface tension. Thus, the surface tension value for the sonication reinforcement on the oxidation process was higher than that for the sole sonication or oxidation process.

3.10. Syneresis and transparency

Syneresis indicates the amount of water expelled from the gel. A low syneresis determination represents the ability of a gel to hold the water [66]. The syneresis slightly increased as a result of the sonication (Figure 5 (bottom)). A lower chain of the polysaccharide released the water faster as explained by Lan et al. [67]. The oxidation, with or without sonication, significantly increased the syneresis. The hydroxyl group was responsible for syneresis retardation [68]. Fewer hydroxyl groups left after the oxidation could weaken the water-holding capability and thus increased the syneresis rate. In this work, the oxidation, which created more carbonyls, resulted in less capability to hold water. Bushra et al. [69] also found that the carbonyl group prevented the water associated with the starch chain. However, the gel properties of glucomannan should be set to suit its use, especially for food application [32].

The combination of sonication and oxidation improved the transparency of the glucomannan solution (Figure 5 (bottom)). The degraded glucomannan had more numbers of shorter chains that allowed light to easily pass through the solution. This condition represented a high transparency of the solution. Higher clarity of low molecular weight of glucomannan was also found by Luo et al. [44]. The presence of carbonyl groups in the glucomannan chain also supported the increase of glucomannan transparency [12]. Yang et al. [64] found that the carboxylic group attached to the oxidized starch increased the intermolecular forces and produced good integration and a homogeneous solution. However, Figure 5 (bottom) shows that the lone treatment of either oxidation or sonication did not significantly affect the glucomannan transparency ($p > 0.05$). This result suggested that shorter chains and specific functional groups were not the only influential factors in the transparency. Other factors, such as particle size and refractive indices, as well as their interaction, should also be considered [70].

3.11. X-Ray diffraction

XRD spectra of native and degraded glucomannan are presented in Figure 6. All samples showed an amorphousness characteristic with a sharp 2θ peak at ~20° [71], and other weaker peaks appeared at ~14° and ~36°. After the treatments were applied, these peaks were relatively unchanged which suggested the glucomannan stability against the treatments. Our samples showed a reduction of the M_v from 600 g/mol to 150 g/mol for 1% glucomannan. However, this difference in the M_v did not influence the diffraction peaks significantly. Luo et al. [44] reported a similar XRD spectra but with slightly different diffraction peaks between the native and the hydrolyzed glucomannan with different molecular weights. These varied diffraction peaks could be due to a significant difference in the molecular weights of their samples, in which they used 1.07×10^6, 4.6×10^5 and 1.72×10^4 Da. They found a sharper diffraction 2θ peak at 20.1° and weak shoulder peaks appeared at 13.7° and 35.2°, respectively, in all samples. Our results suggested that other factors could also contribute to the crystallinity including the shape of the nanoparticles, crystal orientation, microscale stresses and crystallographic texture, as argued by Luo et al. [44].

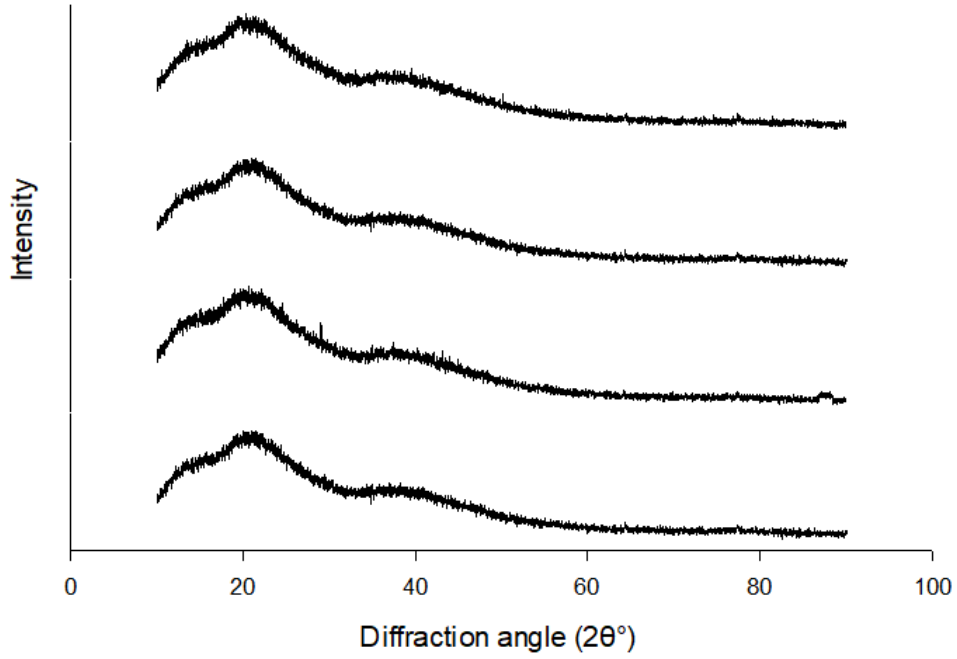

Figure 6. XRD patterns of (top to bottom): native, sonicated, oxidated, combined oxidated, and sonicated glucomannan.

Meanwhile, sonication and oxidation increased the amorphous percentage from 63.2% to 73.7% and 68.4%, respectively. Ultrasound formed cavitation-erosion, which attacked the weak crystalline region of glucomannan and increased the amorphousness, as demonstrated by Raza et al. [72]. A slight increase in amorphous percentage showed the structural rearrangement of glucomannan after the oxidation process. A similar result was reported by Lu et al. [73] for gellan gum oxidation. Meanwhile, the combination of sonication and oxidation reduced the amorphous percentage to 61.1%. Ma et al. [74] found that the synergistic work of ultrasound and hydrogen peroxide could change the physicochemical properties of copper catalysts. Moreover, ethanol as a solvent could strengthen the hydrogen linkage of glucomannan and improved its crystallinity [75]. Hence, further studies are needed to determine the effects of sonication and oxidation combinations on glucomannan crystallinity.

4. Conclusions

Lone sonication and oxidation using hydrogen peroxide, or their combination treatment modified the physicochemical properties of glucomannan. In general, those treatments reduced the viscosity, molecular weight and swelling power of glucomannan but increased its solubility. The carbonyl content, powder whiteness and gel syneresis of glucomannan increased after oxidation was applied, while the gel transparency was increased after sonication-oxidation treatment. Different effects of sonication and oxidation on antioxidant activity and thermal properties were found. However, the combined degradation did not change the functional group of glucomannan, but it physically changed the morphology of glucomannan's surface. This result confirmed, treatment of oxidation-ultrasonication could effectively modify the properties of glucomannan, including reducing the viscosity to the level that allowed the glucomannan to be spray-dried.

Acknowledgments

This work was funded by Universitas Diponegoro through RPIBT scheme-2021 (No: 185-100/UN7.6.1/PP/2021).

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Tester R, Al-Ghazzewi F (2017) Glucomannans and nutrition. *Food Hydrocoll* 68: 246–254. https://doi.org/10.1016/j.foodhyd.2016.05.017
2. Wardhani DH, Cahyono H, Ulya HN, et al. (2022) Spray-dryer feed preparation: Enzymatic degradation of glucomannan for iron nanoencapsulation. *AIMS Agric Food* 7: 683–703. https://doi.org/10.3934/agrfood.2022042
3. Yang J, Xiao JX, Ding LZ (2009) An investigation into the application of konjac glucomannan as a flavor encapsulant. *Eur Food Res Technol* 229: 467–474. https://doi.org/10.1007/s00217-009-1084-2
4. Li Y, Liu H, Xie Y, et al. (2021) Preparation, characterization and physicochemical properties of Konjac glucomannan depolymerized by ozone assisted with microwave treatment. *Food Hydrocoll* 119: 106878. https://doi.org/10.1016/j.foodhyd.2021.106878
5. Yin JY, Ma LY, Xie MY, et al. (2020) Molecular properties and gut health benefits of enzyme-hydrolyzed konjac glucomannans. *Carbohydr Polym* 237: 116117. https://doi.org/10.1016/j.carbpol.2020.116117
6. Al-Ghazzewi FH, Tester RF (2014) Inhibition of the adhesion of Escherichia coli to human epithelial cells by carbohydrates. *Bioact Carbohydr Diet Fibre* 4: 1–5. https://doi.org/10.1016/j.bcdf.2014.05.001
7. Jin W, Mei T, Wang Y, et al. (2014) Synergistic degradation of konjac glucomannan by alkaline and thermal method. *Carbohydr Polym* 99: 270–277. https://doi.org/10.1016/j.carbpol.2013.08.029
8. Wu D, Yu S, Liang H, et al. (2020) The influence of deacetylation degree of konjac glucomannan on rheological and gel properties of konjac glucomannan/κ-carrageenan mixed system. *Food Hydrocoll* 101: 105523. https://doi.org/10.1016/j.foodhyd.2019.105523
9. Su Y, Zhang M, Chang C, et al. (2022) The effect of citric-acid treatment on the physicochemical and gel properties of konjac glucomannan from *Amorphophallus bulbifer*. *Int J Biol Macromol* 216: 95–104. https://doi.org/10.1016/j.ijbiomac.2022.06.199
10. Cheng LH, Abd Karim A, Seow CC (2007) Effects of acid modification on physical properties of konjac glucomannan (KGM) films. *Food Chem* 103: 994–1002. https://doi.org/10.1016/j.foodchem.2006.09.052
11. Hongbo T, Lan W, Yanping L, et al. (2019) Effect of acidolysis and oxidation on structure and properties of konjac glucomannan. *Int J Biol Macromol* 130: 378–387. https://doi.org/10.1016/j.ijbiomac.2019.02.048
12. Chen Y, Zhao H, Liu X, et al. (2016) TEMPO-oxidized konjac glucomannan as appliance for the preparation of hard capsules. *Carbohydr Polym* 143: 262–269. https://doi.org/10.1016/j.carbpol.2016.01.072

13. Jin W, Xu W, Li Z, et al. (2014) Degraded konjac glucomannan by γ-ray irradiation assisted with ethanol: Preparation and characterization. *Food Hydrocoll* 36: 85–92. https://doi.org/10.1016/j.foodhyd.2013.09.005
14. Lu P, Guo J, Fan J, et al. (2023) Combined effect of konjac glucomannan addition and ultrasound treatment on the physical and physicochemical properties of frozen dough. *Food Chem* 411: 135516. https://doi.org/10.1016/j.foodchem.2023.135516
15. Bhaturiwala R, Bagban MA, Singh TA, et al. (2021) Partial purification and application of β-mannanase for the preparation of low molecular weight galacto and glucomannan. *Biocatal Agric Biotechnol* 36: 102155. https://doi.org/10.1016/j.bcab.2021.102155
16. Korkiatithaweechai S, Umsarika P, Praphairaksit N, et al. (2011) Controlled release of diclofenac from matrix polymer of chitosan and oxidized konjac glucomannan. *Mar Drugs* 9: 1649–1663. https://doi.org/10.3390/md9091649
17. Wang M, He W, Jin X, et al. (2015) Oxidized konjac glucomannan as a paper strength agent. *Bioresources* 10: 8089–8097.
18. Su Z, Sun F, Shi Y, et al. (2009) Effects of formulation parameters on encapsulation efficiency and release behavior of risperidone poly(D, L-lactide-co-glycolide) microsphere. *Chem Pharm Bull (Tokyo)* 57: 1251–1256. https://doi.org/10.1248/cpb.57.1251
19. Pan T, Peng S, Xu Z, et al. (2013) Synergetic degradation of konjac glucomannan by γ-ray irradiation and hydrogen peroxide. *Carbohydr Polym* 93: 761–767. https://doi.org/10.1016/j.carbpol.2012.11.075
20. Tecson MG, Abad L V., Ebajo VD, et al. (2021) Ultrasound-assisted depolymerization of kappa-carrageenan and characterization of degradation product. *Ultrason Sonochem* 73: 105540. https://doi.org/10.1016/j.ultsonch.2021.105540
21. Singh B, Sharma N (2008) Mechanistic implications of plastic degradation. *Polym Degrad Stab* 93: 561–584. https://doi.org/10.1016/j.polymdegradstab.2007.11.008
22. Ogutu FO, Mu TH (2017) Ultrasonic degradation of sweet potato pectin and its antioxidant activity. *Ultrason Sonochem* 38: 726–734. https://doi.org/10.1016/j.ultsonch.2016.08.014
23. Kang DC, Zou YH, Cheng YP, et al. (2016) Effects of power ultrasound on oxidation and structure of beef proteins during curing processing. *Ultrason Sonochem* 33: 47–53. https://doi.org/10.1016/j.ultsonch.2016.04.024
24. Aimin T, Hongwei Z, Gang C, et al. (2005) Influence of ultrasound treatment on accessibility and regioselective oxidation reactivity of cellulose. *Ultrason Sonochem* 12: 467–472. https://doi.org/10.1016/j.ultsonch.2004.07.003
25. Kidak R, Ince NH (2007) Catalysis of advanced oxidation reactions by ultrasound: A case study with phenol. *J Hazard Mater* 146: 630–635. https://doi.org/10.1016/j.jhazmat.2007.04.106
26. Vernès L, Vian M, Chemat F (2019) Chapter 12—Ultrasound and microwave as green tools for solid-liquid extraction. In: Poole CF (Ed.), *Liquid-Phase Extraction, Handbooks in Separation Science,* 355–374. https://doi.org/10.1016/B978-0-12-816911-7.00012-8
27. Yu X, Zhou C, Yang H, et al. (2015) Effect of ultrasonic treatment on the degradation and inhibition cancer cell lines of polysaccharides from *Porphyra yezoensis*. *Carbohydr Polym* 117: 650–656. https://doi.org/10.1016/j.carbpol.2014.09.086
28. Khan S, Raja MA, Sayed M, et al. (2019) Chapter 6—Advanced oxidation and reduction processes. In: Ahuja S (Ed.), *Advances in Water Purification Techniques: Meeting the Needs of Developed and Developing Countries,* 135–164. https://doi.org/10.1016/B978-0-12-814790-0.00006-5

29. Pingret D, Fabiano-Tixier AS, Chemat F (2013) Degradation during application of ultrasound in food processing: A review. *Food Control* 31: 593–606. https://doi.org/10.1016/j.foodcont.2012.11.039
30. Kuakpetoon D, Wang YJ (2006) Structural characteristics and physicochemical properties of oxidized corn starches varying in amylose content. *Carbohydr Res* 341: 1896–1915. https://doi.org/10.1016/j.carres.2006.04.013
31. Lin W, Ni Y, Wang L, et al. (2019) Physicochemical properties of degraded konjac glucomannan prepared by laser assisted with hydrogen peroxide. *Int J Biol Macromol* 129: 78–83. https://doi.org/10.1016/j.ijbiomac.2019.02.035
32. Hu Y, Tian J, Zou J, et al. (2019) Partial removal of acetyl groups in konjac glucomannan significantly improved the rheological properties and texture of konjac glucomannan and κ-carrageenan blends. *Int J Biol Macromol* 123: 1165–1171. https://doi.org/10.1016/j.ijbiomac.2018.10.190
33. Morita AT, Carastan DJ, Demarquette NR (2002) Influence of drop volume on surface tension evaluated using the pendant drop method. *Colloid Polym Sci* 280: 857–864. https://doi.org/10.1007/s00396-002-0694-z
34. Kang B, Dai Y dong, Zhang H qian, et al. (2007) Synergetic degradation of chitosan with gamma radiation and hydrogen peroxide. *Polym Degrad Stab* 92: 359–362. https://doi.org/10.1016/j.polymdegradstab.2006.12.006
35. Wardhani DH, Etnanta FN, Ulya HN, et al. (2022) Iron encapsulation by deacetylated glucomannan as an excipient using the gelation method: characteristics and controlled release. *Food Technol Biotechnol* 60: 41–51. https://doi.org/10.17113/ftb.60.01.22.7130
36. Zhu B, Xin C, Li J, et al. (2019) Ultrasonic degradation of konjac glucomannan and the effect of freezing combined with alkali treatment on their rheological profiles. *Molecules* 24: 1860. https://doi.org/10.3390/molecules24101860
37. Mason TJ, Lorimer JP (2002) *Applied Sonochemistry: The Uses of Power Ultrasound in Chemistry and Processing.* Wiley-VCH Verlag GmbH & Co. KGaA. https://doi.org/10.1002/352760054X
38. Yin JY, Ma LY, Siu KC, et al. (2019) Effects of ultrasonication on the conformational, microstructural, and antioxidant properties of konjac glucomannan. *Applied Sci (Switzerland)* 9: 461. https://doi.org/10.3390/app9030461
39. Cheng LH, Nur Halawiah H, Lai BN, et al. (2010) Ultrasound mediated acid hydrolysis of konjac glucomannan. *Int Food Res J* 17: 1043–1050.
40. Koda S, Taguchi K, Futamura K (2011) Effects of frequency and a radical scavenger on ultrasonic degradation of water-soluble polymers. *Ultrason Sonochem* 18: 276–281. https://doi.org/10.1016/j.ultsonch.2010.06.007
41. Ebrahimi R, Tarhande G, Rafiei S (2012) The study of ultrasonic degradation of superabsorbent hydrogels. *Org Chem Int* 2012: 343768. https://doi.org/10.1155/2012/343768
42. Wang M, Wu Y, Liu Y, et al. (2020) Effect of ultrasonic and microwave dual-treatment on the physicochemical properties of chestnut starch. *Polymers (Basel)* 12: 1718. https://doi.org/10.3390/polym12081718
43. Sujka M, Jamroz J (2013) Ultrasound-treated starch: SEM and TEM imaging, and functional behaviour. *Food Hydrocoll* 31: 413–419. https://doi.org/10.1016/j.foodhyd.2012.11.027
44. Luo X, Yao X, Zhang C, et al. (2012) Preparation of mid-to-high molecular weight konjac glucomannan (MHKGM) using controllable enzyme-catalyzed degradation and investigation of MHKGM properties. *J of Pol Res* 19: 9849. https://doi.org/10.1007/s10965-012-9849-x

45. Wardhani DH, Nugroho F, Aryanti N, et al. (2018) Simultaneous effect of temperature and time of deacetylation on physicochemical properties of glucomannan. *ASEAN J Chem Eng* 18: 1–8. https://doi.org/10.22146/ajche.49541
46. Li J, Ye T, Wu X, et al. (2014) Preparation and characterization of heterogeneous deacetylated konjac glucomannan. *Food Hydrocoll* 40: 9–15. https://doi.org/10.1016/j.foodhyd.2014.02.001
47. Doussin JF, Monod A (2013) Structure-activity relationship for the estimation of OH-oxidation rate constants of carbonyl compounds in the aqueous phase. *Atmos Chem Phys* 13: 11625–11641. https://doi.org/10.5194/acp-13-11625-2013.
48. Chong WT, Uthumporn U, Karim AA, et al. (2013) The influence of ultrasound on the degree of oxidation of hypochlorite-oxidized corn starch. *LWT-Food Sci Technol* 50: 439–443. https://doi.org/10.1016/j.lwt.2012.08.024
49. Kamble S, Kumbhar A, Rashinkar G, et al. (2012) Ultrasound promoted efficient and green synthesis of β-amino carbonyl compounds in aqueous hydrotropic medium. *Ultrason Sonochem* 19: 812–815. https://doi.org/10.1016/j.ultsonch.2011.12.001
50. Xu Z, Sun Y, Yang Y, et al. (2007) Effect of γ-irradiation on some physiochemical properties of konjac glucomannan. *Carbohydr Polym* 70: 444–450. https://doi.org/10.1016/j.carbpol.2007.05.011
51. Burton WG, Holman JS, Pilling GM, et al. (1995) Salters advanced chemistry: A revolution in pre-college chemistry. *J Chem Educ* 72: 227. https://doi.org/10.1021/ed072p227
52. Wuorimaa A, Jokela R, Aksela R (2006) Recent developments in the stabilization of hydrogen peroxide bleaching of pulps: An overview. *Nord Pulp Paper Res J* 21: 435–443. https://doi.org/10.3183/npprj-2006-21-04-p435-443
53. Park HJ, Kwon TY, Nam SH, et al. (2004) Changes in bovine enamel after treatment with a 30% hydrogen peroxide bleaching agent. *Dent Mater J* 23: 517–521. https://doi.org/10.4012/dmj.23.517
54. Abou-Okeil A, El-Shafie A, El Zawahry MM (2010) Ecofriendly laccase-hydrogen peroxide/ultrasound-assisted bleaching of linen fabrics and its influence on dyeing efficiency. *Ultrason Sonochem* 17: 383–390. https://doi.org/10.1016/j.ultsonch.2009.08.007
55. Li J, Li B, Geng P, et al. (2017) Ultrasonic degradation kinetics and rheological profiles of a food polysaccharide (konjac glucomannan) in water. *Food Hydrocoll* 70: 14–19. https://doi.org/10.1016/j.foodhyd.2017.03.022
56. Yan JK, Pei JJ, Ma H Le, et al. (2015) Effects of ultrasound on molecular properties, structure, chain conformation and degradation kinetics of carboxylic curdlan. *Carbohydr Polym* 121: 64–70. https://doi.org/10.1016/j.carbpol.2014.11.066
57. Tatirat O, Charoenrein S (2011) Physicochemical properties of konjac glucomannan extracted from konjac flour by a simple centrifugation process. *LWT-Food Sci Technol* 44: 2059–2063. https://doi.org/10.1016/j.lwt.2011.07.019
58. Manikandan S, Alagu Lakshmanan G, Chandran C, et al. (2016) Phytochemical screening and evaluation of tuber extract of Plectranthus rotundifolius Spreng. By GC-MS and FT-IR spectrum analysis. *Eur J Herb Med* 4: 36–40.
59. Yu H, Huang Y, Ying H, et al. (2007) Preparation and characterization of a quaternary ammonium derivative of konjac glucomannan. *Carbohydr Polym* 69: 29–40. https://doi.org/10.1016/j.carbpol.2006.08.024
60. Liu J, Xu Q, Zhang J, et al. (2015) Preparation, composition analysis and antioxidant activities of konjac oligo-glucomannan. *Carbohydr Polym* 130: 398–404. https://doi.org/10.1016/j.carbpol.2015.05.025

61. Yeung YK, Kang YR, So BR, et al. (2021) Structural, antioxidant, prebiotic and anti-inflammatory properties of pectic oligosaccharides hydrolyzed from okra pectin by Fenton reaction. *Food Hydrocoll* 118: 106779. https://doi.org/10.1016/j.foodhyd.2021.106779
62. Munekata PES, Domínguez R, Franco D, et al. (2017) Effect of natural antioxidants in Spanish salchichón elaborated with encapsulated n-3 long chain fatty acids in konjac glucomannan matrix. *Meat Sci* 124: 54–60. https://doi.org/10.1016/j.meatsci.2016.11.002
63. Arkles B (2006) Hydrophobicity, hydrophilicity and silanes. *Paint & Coat Ind Mag* 22: 114–135.
64. Yang G, Xia Y, Lin Z, et al. (2021) Physicochemical impact of cellulose nanocrystal on oxidation of starch and starch based composite films. *Int J Biol Macromol* 184: 42–49. https://doi.org/10.1016/j.ijbiomac.2021.06.009
65. Malviya R, Sharma PK, Dubey SK (2017) Antioxidant potential and emulsifying properties of kheri (*Acacia chundra, Mimosaceae*) gum polysaccharide. *Marmara Pharm J* 21: 701–706. http://dx.doi.org/10.12991/marupj.323594
66. Ako K (2017) Influence of osmotic and weight pressure on water release from polysaccharide ionic gels. *Carbohydr Polym* 169: 376–384. https://doi.org/10.1016/j.carbpol.2017.04.062
67. Lan X, Liu X, Yang Y, et al. (2017) The effect of lamellar structure ordering on the retrogradation properties of canna starch subjected to thermal and enzymatic degradation. *Food Hydrocoll* 69: 185–192. https://doi.org/10.1016/j.foodhyd.2017.02.004
68. Naknaen P, Tobkaew W, Chaichaleom S (2017) Properties of jackfruit seed starch oxidized with different levels of sodium hypochlorite. *Int J Food Prop* 20: 979–996. https://doi.org/10.1080/10942912.2016.1191868
69. Bushra M, Yun XX, Pan SY, et al. (2013) Effect of oxidation and esterification on functional properties of mung bean (*Vigna radiata (L.) Wilczek*) starch. *Eur Food Res Technol* 236: 119–128. https://doi.org/10.1007/s00217-012-1857-x
70. Schütz C, Sort J, Bacsik Z, et al. (2012) Hard and transparent films formed by nanocellulose-TiO_2 nanoparticle hybrids. *PLoS One* 7: e45828. https://doi.org/10.1371/journal.pone.0045828
71. Chen Z, Wang S, Shang L, et al. (2020) An efficient and simple approach for the controlled preparation of partially degraded konjac glucomannan. *Food Hydrocoll* 108: 106017. https://doi.org/10.1016/j.foodhyd.2020.106017
72. Raza H, Ameer K, Ma H, et al. (2021) Structural and physicochemical characterization of modified starch from arrowhead tuber (*Sagittaria sagittifolia L.*) using tri-frequency power ultrasound. *Ultrason Sonochem* 80: 105826. https://doi.org/10.1016/j.ultsonch.2021.105826
73. Lu Y, Zhao X, Fang S (2019) Characterization, antimicrobial properties and coatings application of gellan gum oxidized with hydrogen peroxide. *Foods* 8: 31. https://doi.org/10.3390/foods8010031
74. Ma X, Cheng Y, Ge Y, et al. (2018) Ultrasound-enhanced nanosized zero-valent copper activation of hydrogen peroxide for the degradation of norfloxacin. *Ultrason Sonochem* 40: 763–772. https://doi.org/10.1016/j.ultsonch.2017.08.025
75. Song Q, Wu L, Li S, et al. (2022) Aggregation of konjac glucomannan by ethanol under low-alkali treatment. *Food Chem X* 15: 100407. https://doi.org/10.1016/j.fochx.2022.100407