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Modification of Quinoa Starch by High Hydrostatic Pressure and Ultrasonication and Assessment of Its Physicochemical Properties

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ABSTRACT: Quinoa starch was extracted and treated with different technologies to modify its properties. High hydrostatic pressure (HHP) at 250, 350, and 500 MPa at 20 and 40 °C and ultrasonication at 100% power with a constant pulse for 15 min were applied as modification methods. Following the treatments, functional, rheological, morphological, and structural analyses were carried out. Time domain (TD) NMR relaxometry experiments confirmed that HHP treatment caused less granule swelling of starch. NMR relaxation spectra of quinoa starch revealed mainly two distinct proton populations. Results have demonstrated that HHP treatment had a strong modification effect on quinoa starch compared to US and provided a new type of modified quinoa starch that can be used as an instant food product and thickening agent with the feature of retaining the granular shape and reducing swelling. The results were also confirmed by FTIR, XRD, SEM, and DSC analyses.

KEYWORDS: *TD-NMR relaxometry, high hydrostatic pressure (HHP), ultrasonication (US), quinoa starch, starch modification*

1. INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a seed recognized as a *pseudocereal* which is a broadleaf plant containing valuable energy sources due to its starch content. The quinoa seeds are small, oval shaped and flat seeds, which are usually pale yellow but can range from white to black or pink to red color. It is a nutritionally balanced food that contains high amounts of protein, carbohydrate, essential fatty acids, bioactive compounds, and minerals. Moreover, quinoa does not contain gluten, so it can be a perfect gluten-free alternative for celiac patients.[1−3]

Starch is the major component of quinoa, which comprises more than 50% of the dry weight.[1,4] Quinoa starch (QS) granules, which are generally located in the perisperm of the quinoa seed, have relatively small size (0.4−2 μm) and polygonal shape.[1] QS has relatively low amylose content ranging from 4−25%.[1,3] The amylopectin of QS is high and has a unique chain length distribution with larger number of short chains and smaller number of long chains compared to other cereals.[1] These unique features of QS provide several functional properties including gelatinization at relatively low temperatures compared to amaranth, normal and waxy starches, pasting at relatively low temperatures, having maximal viscosity compared to normal and waxy starches and outstanding freeze−thaw stability.[3] These functional properties make QS a valuable ingredient for food formulations.

Modifications of quinoa starch have been shown to extend the range of its functionalities.[3,5] Starches are frequently changed physically and chemically to enhance the proportion of resistant starch, reduce viscosity, and speed up the gelatinization process. This helps to solve the problem of native starch's instability under processing circumstances.[6] In this study, as one of the physical modification methods, high hydrostatic pressure (HHP) has been selected since it has a great potential to obtain improved properties in terms of paste and gel texture, enhanced film formation, adhesion, emulsion stabilization and freeze−thaw stability.[7]

High hydrostatic pressure (HHP) treatment is an attractive nonthermal technique in food treatment and preservation applications. It is widely used to change the physicochemical structure of food macromolecules.[7,8] It was shown that, in the presence of excess water, HHP treatment resulted in nonthermal gelatinization of the native starch granules and degradation of crystalline regions in the starch granules. In addition to the botanical origin and genotypes of the starch, experimental conditions such as pressure level, holding time, starch concentration and temperature parameters used during the HHP treatment also affected the degree of HHP induced starch gelatinization.[8] It was observed that HHP induced gelatinization limited amylose leaching to the liquid phase by providing the development of amylose-lipid complexes or double helices with amylose and outer branches of amylopectin, which resulted in reduced starch swelling.[8]

The second technology used for modification was ultrasonication (US). During the ultrasonication procedure, a liquid sample is exposed to ultrasonic waves between 20 and 10 MHz, which causes agitation. Sound waves that move through liquid mediums generate cavitation. When cavitation bubbles burst, the cell wall is damaged as a result of the significant

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temperature and pressure changes that occur. US can also be used to modify the properties of starch. The ultrasonic treatment was found to destroy the crystalline region in starch granules of tapioca starch and cornstarch at higher amplitude or sonication time. The ultrasound induced starch modification was mainly associated with the cavitational force experienced by the starch molecule during ultrasonication.[9,10]

Time domain (TD) Nuclear Magnetic Resonance (NMR) relaxometry is a noninvasive, nondestructive and fast method, which has gained popularity in food science. The basic concept behind NMR technology depends on the measurement of the relaxation times (*longitudinal (T_1) and transverse (T_2) relaxation times*) of the protons by getting signals after a RF pulse is applied and removed. When these T_1 and T_2 relaxation curves are decomposed, proton pools could be identified which gives information about the status of water/oil within a sample.[11,12]

In the literature, there are studies in the field of HHP and US induced starch modifications, but little information is available in the field of HHP and US treated quinoa starch that has further been analyzed by TD NMR relaxometry. The objective of this study was to examine the functional rheological, structural and morphological properties of HHP and US treated quinoa starch and observe the modifications through TD NMR relaxometry experiments. As complementary studies to TD-NMR, swelling/solubility, particle size, FTIR and SEM measurements were also conducted to confirm the results. In addition to HHP and US treated quinoa starch samples, the starch was fully gelatinized by heat treatment and considered as the control gelatinized starch to make further comparison.

2. MATERIALS AND METHODS

2.1. Starch Isolation. Quinoa seeds were bought from a local market in Ankara, with the brand name as "Ala Çiftçi". For the isolation of quinoa starch, the water steeping method was used as described in the literature.[13] Quinoa seeds were steeped in water at a ratio of 1:6 at room temperature for 24 h with intermittent stirring. Then, using a lab scale blender, wet milling was applied for 2 min. Following wet milling, the slurry was filtered through 100 mesh size sieves, and the obtained filtrate was centrifuged at 1850*g* for 15 min by an MF 80 General Centrifuge (Hanil, Incheon, South Korea). The supernatant and the yellowish starch cake were removed carefully. The obtained starch cake was resuspended in water and centrifuged, and the yellowish layer was removed again after each centrifugation by repeating this procedure four times for the purification of starch. The recovered purified starch was dried at 40 °C in a hot air oven for 6 h. Then, powder quinoa starch (QS) samples were obtained by grinding and stored in an airtight container for further analysis. Extra purity analysis was not performed for starch.

2.2. Modification of the Starch. *2.2.1. High Hydrostatic Pressure (HHP) Treatment.* Quinoa starch suspensions were generated by mixing quinoa starch with distilled water at a concentration of 10% (w/v). The ratio was determined based on the preliminary studies.[6] The suspension was stirred gently using a magnetic stirrer (Multi Hot Plate Stirrer, Wisd, DAIHAN Scientific Co., Ltd., Korea) for 5 min and was sealed in 25 mL sterile polyethylene cryotubes so that no gas was left in the tube. A high hydrostatic pressure apparatus of type 760.0118 was used for HHP treatment. The apparatus consists of a cylindrical pressurization chamber with a water and glycol mixture as the pressure transmitting medium that has been filled into the chamber. The pressurization chamber has a capacity of 100 mL, a length of 153 mm and an inner diameter of 24 mm. Prepared QS suspensions were pressurized at pressure levels of 250, 350, and 500 MPa at 20 and 40 °C for 5 min. Due to the short pressure release time, the pressurization time reported in this study did not include the pressure increase and release times (less than 20 s). After HHP treatment, samples were centrifuged at 1700*g* for 5 min (using an MF 80 General Centrifuge from Hanil, Incheon, South Korea), freeze-dried for about 48 h, and then ground to powder for additional analyses.

2.2.2. Ultrasound (US) Treatment. Quinoa starch suspensions were prepared at a concentration of 5% (w/w) based on the preliminary studies.[11] The suspension was stirred gently using a magnetic stirrer (Multi Hot Plate Stirrer, Wisd, DAIHAN Scientific Co., Ltd., Korea) for 5 min. A probe-type ultrasonicator equipped with the MS73 probe (Bandelin Sonopuls HD 3100, Bandelin electronic GmbH & Co. KG, Berlin, Germany) was used to provide the US treatment for 15 min at 100% power with constant pulse while the subject was submerged in freezing water. In US experiments different parameters were not tested, as preliminary results showed that this combination showed some changes on the samples. Following US treatment, samples were centrifuged at 1700*g* for 5 min (MF 80 General Centrifuge, Hanil, Incheon, South Korea), freeze-dried for about 48 h and ground to powder form for further analyses.

2.2.3. Fully Gelatinized Starch Preparation. Conventional heat treatment was used to obtain the fully gelatinized (control gelatinized) quinoa starch samples, which were prepared by heating 10% quinoa starch suspension for 30 min in a water bath (GFL 1086, Burgwedel, Germany) at 90 °C. After heat treatment, the material was centrifuged (MF 80 General Centrifuge, Hanil, Incheon, South Korea) at 1700*g* for 5 min, freeze-dried for almost 48 h, and pulverized to powder form for additional analyses.

2.3. Analysis of the Starch. *2.3.1. Measurement of Swelling Power (SP) and Water Solubility Index (WSI).* Water solubility index (WSI, %) and swelling power (SP, g/g) were calculated.[3] Quinoa starch suspensions of 4% were prepared in a centrifuge tube and heated for 30 min at 90 °C in a shaking water bath (GFL 1086, Burgwedel, Germany). Each suspension was then cooled and centrifuged at 1500*g* for 30 min (MF 80 General Centrifuge, Hanil, Incheon, South Korea). After centrifugation, the precipitate was weighed (W_s), and the supernatant was dried at 100 °C for 24 h until constant weight was achieved (W_1). WSI and SP were calculated by the following equations:

$$\mathrm{WSI}\left(\frac{\mathrm{g}}{100\ \mathrm{g}}\right) = [\text{Weight of the soluble material in the supernatant, } W_1\ (\mathrm{g},\ \text{dry basis})]/[\text{Weight of the starch sample, } W_0\ (\mathrm{g},\ \text{dry basis})] \times 100$$

$$\mathrm{SP} = \text{Weight of the precipitate, } W_s\ (\mathrm{g},\ \text{dry basis})]/[\text{Weight of the starch sample, } W_0\ (\mathrm{g},\ \text{dry basis}) \times (100 - \mathrm{WSI})] \times 100$$

2.3.2. Rheological Measurements. Rheological measurements were carried out using the cup and bob geometry of the rheometer (Kinexus lab+, Malvern Instruments Ltd., Worcestershire, UK). For the study, 10% (w/w) quinoa starch suspensions were prepared, and the cup was filled with 20 mL of the prepared suspension.[10] Using the following formula, the shear stress values of the samples with increasing shear rates (from 0.1 to 100 s^{-1}) were measured and fitted to the power law:

$$\tau = k\gamma^n$$

$$\ln \tau = \ln k + n \ln \gamma$$

where n = flow behavior index, k = consistency index ($Pa{\cdot}s^n$), τ = shear stress (Pa), and γ = shear rate (s^{-1}).

2.3.3. Particle Size Measurements. Particle size distributions of quinoa starch samples were analyzed by a particle analyzer based on a laser diffraction (Mastersizer 3000, Malvern Instruments Ltd., Malvern, UK). Samples were subjected to stirring during measurements. Measurements were done in the 1% to 25% obscuration range for "irregular shape" particles. The starch refractive and absorption

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Table 1. Water Solubility Index (WSI, %), Swelling Power (SP, g/g), T_2 Relaxation Times, Particle Size, Rheological Properties (Flow Behavior Index (n), Consistency Index (K)), and Thermal Properties of Quinoa Starches[a]

Treatment	Water solubility index (WSI, %)	Swelling power (SP,g/g)	T_2(ms)	$D[4,3]$ (μm)	n	K (Pa·s^n) × 10^{-3}	T_0(°C)	T_p(°C)	ΔH (J/g)	DG (%)
Control	43.10^{c}	22.40^{bc}	65.96^{ab}	13.97^{e}	0.76^{ab}	1.16^{a}	48.19	59.57	0.82	0%
HHP - 250 MPa, 20 °C	44.00^{b}	23.05^{b}	70.11^{ab}	18.40^{ed}	0.77^{ab}	0.86^{b}	54.48	65.61	0.72	12%
HHP - 250 MPa, 40 °C	43.30^{bc}	23.24^{b}	74.12^{ab}	19.33^{ed}	0.78^{ab}	0.81^{c}	56.62	66.25	0.26	69%
HHP - 350 MPa, 20 °C	41.00^{d}	21.70^{bc}	90.87^{a}	17.23^{dc}	0.76^{b}	1.07^{d}	53.12	64.38	0.51	37%
HHP - 350 MPa, 40 °C	37.75^{e}	17.57^{d}	100.86^{a}	36.37^{c}	0.79^{a}	1.01^{e}	55.08	62.89	0.53	35%
HHP - 500 MPa, 20 °C	12.55^{g}	11.17^{e}	547.35^{c}	48.40^{ba}	0.93^{c}	0.61^{f}	59.03	67.1	1.85	−126%
HHP - 500 MPa, 40 °C	11.85^{g}	11.13^{e}	546.35^{c}	53.53^{ba}	0.98^{d}	0.44^{g}	57.03	64.62	2.18	−166%
US - 15 min	36.10^{f}	19.12^{cd}	91.49^{a}	21.00^{ed}	0.78^{ab}	1.09^{h}	54.11	64.53	0.73	11%
Control gelatinized (90 °C)	58.00^{a}	38.32^{a}	22.71^{b}	55^{a}	0.99^{d}	0.43^{g}	45.43	54.39	0.47	43%

[a]Values are an average of triplicate observations, and values with different letters are significantly different (*$p < 0.05$). Control (0.1 MPa, 25 °C); HHP, high hydrostatic pressure (250 MPa, 20 and 40 °C; 350 MPa, 20 and 40 °C; 500 MPa, 20 and 40 °C); US, ultrasonication (100% power, 15 min); Control gelatinized, heat treatment (90 °C); $D[4,3]$, volume-weighted mean diameter; T_0, onset temperature; T_p, peak temperature; ΔH, enthalpy (J/g); DG, degree of gelatinization.

index values were taken as 1.51 and 0.1, respectively. The refractive index of water was used as 1.33.[14] $D[4,3]$ values were computed using the instrument software.

2.3.4. FTIR Analysis. Using an IR Affinity-1 spectrometer with an attenuated total reflectance (ATR) attachment (Shimadzu Corporation, Kyoto, Japan), the FTIR spectra of the samples of quinoa starch were acquired. The measurements were collected over a wavelength range of 500−4000 cm^{-1} with 32 scans. The mean of the scans of each sample was reported in the spectrum including absorbance vs wavelength plots of quinoa starch samples.

2.3.5. DSC Analysis. Using a Differential Scanning Calorimeter DSC 4000 (PerkinElmer, Waltham, MA, U.S.A.) at METU Central Laboratory (Ankara, Turkey), the gelatinization properties of the quinoa starch samples were evaluated. Pure nitrogen gas was applied through the DSC 4000 system at a flow rate of 19.8 mL/min in a hermetically sealed aluminum pan. An empty aluminum pan was used as the reference for all measurements. The samples were heated from 20 to 80 °C with a heating rate of 10 °C/min. The gelatinization degrees of the samples were calculated by using the following formula:[15]

$$\text{Degree of gelatinization (DG) (\%)} = \left(1 - \frac{\Delta H_{\text{treated}}}{\Delta H_{\text{native}}}\right) \times 100$$

where ΔH_{native} is the enthalpy of native untreated sample and $\Delta H_{\text{treated}}$ is the enthalpy of treated sample.

2.3.6. XRD Analysis. For X-ray Diffraction experiments, a Rigaku Ultima-IV X-ray Diffractometer (Japan) at 40 kV and 30 mA at METU Central Laboratory (Ankara, Turkey) was used. Data were collected between 4 and 70 °C with the 2θ range. Crystalline peaks were analyzed using Origin software (Version 9.0). Based on the following formula given by refs 16 and 17 the crystallinity degree (CD) of the samples was calculated:

$$\text{CD} = \frac{I_c}{I_c + I_a}$$

where I_c is the crystalline phase's integrated intensity and I_a is the amorphous phase's integrated intensity.

2.3.7. Nuclear Magnetic Resonance (NMR) Relaxometry Measurements. For TD-NMR relaxometry experiments, the 0.5 T (22.40 MHz) low-field benchtop 1H NMR equipment (SpinCore Technologies, Inc., Gainesville, USA) with a 10 mm radio frequency coil was used. Quinoa starch suspensions with 10% (w/w) were placed into the cylindrical 10 mm tubes. The spin−spin relaxation times (T_2) of quinoa starch samples were measured using the Carr−Purcell−Meiboom−Gill (CPMG) sequence. Parameters were chosen as *1000 μs echo time (TE), 512 number of points, 300 kHz spectral width, number of echoes changing between 1000 and 5000, repetition delay of 3 s, and 32 scans.* The received NMR signals were evaluated with MATLAB and fitted to mono- and biexponential models in order to get T_2 relaxation curves. Additionally, Non-Negative Least Square (NNLS) analysis of the multiexponential behavior of T_2 relaxation curves was carried out (PROSPA, Magritek Inc., Wellington, New Zealand). T_2 relaxation spectrum was obtained by 1D-NNLS analysis.[18]

2.3.8. SEM Analysis. Scanning electron microscopy (SEM) images were obtained at the Scanning Electron Microscopy Laboratory, Metallurgical and Materials Engineering Department, Middle East Technical University (METU), Turkey. Samples of quinoa starch were coated with a thin coating of Au-Pd (6−11 nm; 10 mA; 40 s) at room temperature, and the analysis was conducted using a scanning electron microscope at various magnifications (SEM, Quanta SC7620, England).

2.3.9. Statistical analysis. To compare the results of the analyses, analysis of variance (ANOVA) with Tukey's multiple range test was employed. For $p < 0.05$, differences were considered significant.

3. RESULTS AND DISCUSSION

3.1. Water Solubility Index (WSI) and Swelling Power (SP). SP and WSI values of quinoa starch samples are given in Table 1 for the nine types of starches which were exposed to HHP (250, 350, and 500 MPa at 20 and 40 °C for 5 min), US (5 min), and heat treatment (control gelatinized) with untreated samples provided as control. The results show that the WSI values of the quinoa starch decreased significantly with HHP and US treatments ($p < 0.05$). However, HHP treatment was found to have more effect on the WSI ($p < 0.05$). For the HHP treated samples, the results showed that HHP treatment of 250 MPa at 20 and 40 °C did not result in a significant change, while pressure levels above 350 and 500 MPa showed a significant difference for WSI. This could be explained by the fact that HHP treated starch granules could stay intact or partially disintegrate resulting in restricted amylose leaching and thus a decrease in the WSI values of the starch.[9] For quinoa starch samples treated with US, the reason for the decrease in the WSI compared to control sample could be the inadequacy of US treatment time duration to destroy the starch granule completely. This resulted in less amylose leaching and a decrease in the WSI value of the starch. The WSI of the quinoa starch gelatinized by heat treatment (90 °C) was also found to increase significantly ($p < 0.05$), which can be linked to the leaching of water-soluble components by complete destruction of the starch granule.[2]

According to Table 1, SP values decreased significantly as with HHP and US treatments ($p < 0.05$). For the HHP treated

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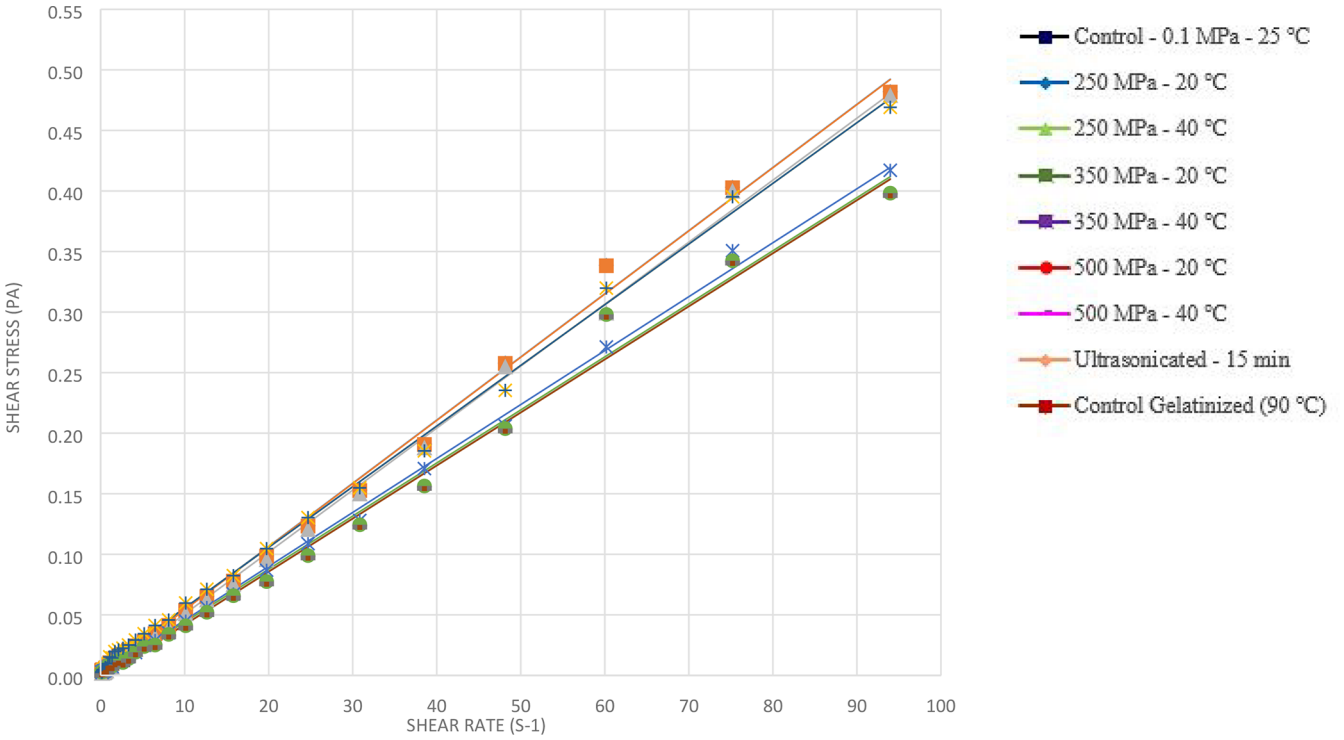

Figure 1. Variation of shear stress with shear strain of untreated [control (0.1 MPa, 25 °C)] and treated quinoa starch with HHP (high hydrostatic pressure) at 250 MPa, 20 °C; 250 MPa, 40 °C; 350 MPa, 20 °C; 350 MPa, 40 °C; 500 MPa, 20 °C; 500 MPa, 40 °C; US (ultrasonication) at 100% power, 15 min; and control gelatinized (by heat treatment at 90 °C).

samples, the results showed that HHP treatment of 250 MPa at 20 and 40 °C and 350 MPa at 20 °C did not have a significant effect on SP values. Similarly, HHP treatment of 350 MPa at 40 °C and US treatment did not show significant changes in SP values ($p < 0.05$). However, the SP value of the quinoa starch gelatinized by heat treatment (90 °C) showed the highest SP value, which indicates the existence of a higher amount of damaged starch than starches treated with HHP and US. This can be attributed to amylose leaching because amylose restricts swelling by strengthening the internal network within the granules.[14] Thus, it was hypothesized that with further HHP application up to 500 MPa, SP and WSI values decrease because of the decrease in leaching of water-soluble components such as amylose with HHP treatment. In addition, formation of amylose-lipid complexes stabilized the granule structure and thus reduced the SP and WSI values. Moreover, US treatment caused less amylose leaching and thus an insignificant decrease in the SP value of quinoa starch due to inadequacy of the US treatment time duration to destroy the starch granule completely.[19]

3.2. Rheological Measurements. Shear stress/shear rate profiles for the untreated, HHP and US treated, and heat gelatinized (control gelatinized) quinoa starch suspensions are given in Figure 1. Under the current experimental conditions, the quinoa starch samples showed non-Newtonian and shear thinning behavior as the viscosities of the samples decreased with the increasing shear rate.[6] Shear stress and shear rate data were fitted by a power law model. The power law constants k and n are given in Table 1. n (flow behavior index) values of the samples increased with the increased pressure and did not show any significant change with high hydrostatic pressure (HHP) treatment up to 500 MPa and ultrasonication (US) ($p > 0.05$). HHP treatment at 500 MPa, 40 °C and heat treatment did not result in a significant change in n values of the quinoa starch samples either ($p > 0.05$). A decrease in k (consistency index) values was observed with increasing pressure and ultrasonication. However, k values did not show any significant change with high hydrostatic pressure (HHP) treatment at 500 MPa at 40 °C and heat treatment ($p > 0.05$). The apparent viscosity of the starch samples decreased with increasing pressure. Heat treatment also decreased the apparent viscosity. The results showed that HHP treatment at 500 MPa at 20 and 40 °C resulted in a lower degree of shear-thinning in quinoa starch samples due to the increase in the n value by approaching to $n = 1$. The increase in the n value might be related to the limited amylose leaching and improved granule integrity of HHP treated quinoa starch at 500 MPa.[6] Control gelatinization by heat treatment resulted in an increase in the n value of quinoa starch indicating less pseudoplastic behavior. In the literature, Xu et al. found that the n value increased with increasing temperature, indicating reduced pseudoplasticity, while it was stated in another study that the n value decreased by increasing the heating temperature, resulting in more pseudoplastic behavior.[20] In this study, the reason for the increase in the value of control gelatinized quinoa starch might be related to the structure and composition of starch and the ghost structures, which stayed after gelatinization as remnants.[6] The results of rheological measurements are in agreement with other studies by Li and Zhu,[6] who reported that pressure increase (at 500 and 600 MPa) caused a decrease in the apparent viscosity and consistency index value of the starch. In another study conducted on the ultrasound effect on corn starch, it was reported that ultrasound treatment (at maximum power and intensity (400 W, 73 W cm^{-2})) decreased the apparent viscosity of the starch samples.[10]

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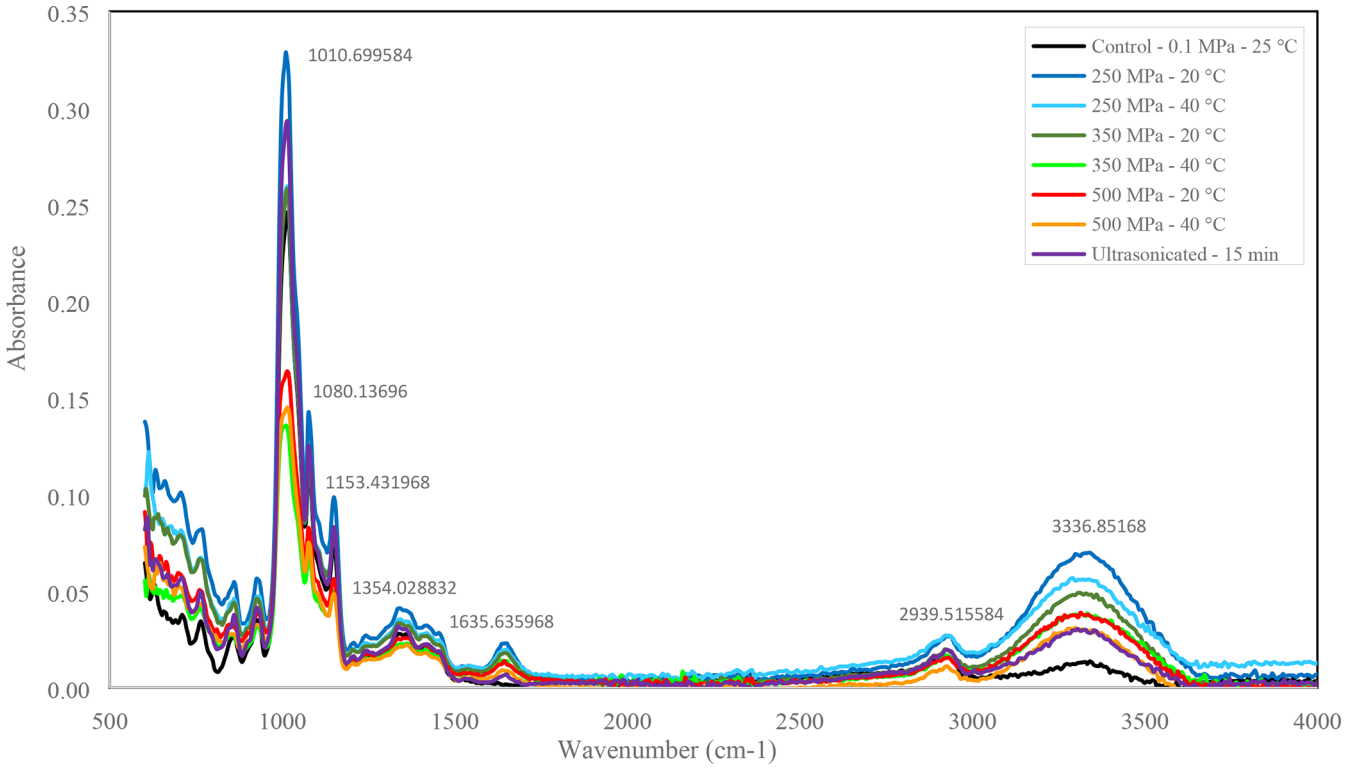

Figure 2. FTIR spectra of untreated [control (0.1 MPa, 25 °C)] and treated quinoa starch with HHP (high hydrostatic pressure) at 250 MPa, 20 °C; 250 MPa, 40 °C; 350 MPa, 20 °C; 350 MPa, 40 °C; 500 MPa, 20 °C; 500 MPa, 40 °C; US (ultrasonication) at 100% power, 15 min).

3.3. Particle Size. The average particle size distribution of quinoa starch granules is given in Table 1. $D[4,3]$ represents the volume mean diameter. The particle size distribution revealed an apparent increase in the volume mean diameter with HHP treatment ($p < 0.05$). The volume mean diameter of the quinoa starch granules increased from 13.96 to 53.53 μm following HHP treatment at 500 MPa, 40 °C. Heat treatment also resulted in similar particle size distribution with HHP treatment at 500 MPa. Particle size increases after HHP treatment have also been reported in the literature. Guo et al.[15] reported that the particle size of lotus seed starch increased after treatment with ultrahigh pressure at 400 MPa. This was associated with the aggregation of gelatinized starch. After HHP treatment, the outer shell of the starch granule was destroyed, and the inner section swelled, leading to aggregation of the modified granules, thus resulting in an increase in the particle size of starch granules. US treatment did not result in any significant increase in the $D[4,3]$ value of the quinoa starch ($p > 0.05$).

3.4. FTIR Spectra. To identify the major functional groups present in the untreated, HHP-treated and US-treated quinoa starch samples, Fourier transform infrared (FTIR) spectra are illustrated in Figure 2. The starch samples showed strong adsorption bands at 3337, 2940, 1636, 1354, 1153, 1080, and 1011 cm^{-1}. The broadest band observed at 3337 cm^{-1} was associated with the O−H stretching vibration. For the modified starches treated with HHP and US, the intensity of the peak at 3337 cm^{-1} was higher than the untreated quinoa starch. This increase in peak intensity was attributed to the increase in the number of functional groups associated to the peak. It was observed that as the pressure was increased, the intensity of the peak decreased, indicating that the pressure increase resulted in limited amylose leaching and formation of amylose-lipid complexes that would destroy the ability of the starch to retain bound water (or indicating lower crystallinity of the sample). The results were in agreement with the previous results. It was stated that the increase in pressure caused a decrease in the peak intensity at 3330 cm^{-1}.[2] Thus, the O−H stretching bands of the modified starches were higher than those of untreated starches.

In the obtained FTIR spectra, another peak was detected at 2940 cm^{-1} which was related with C−H stretching[21] and the intensity of the peak increased with pressure increase. The pressure at 500 MPa, 40 °C decreased the peak intensity, suggesting that pressure increase weakened the C−H vibrations. Similar results were stated by Kızıl et al.,[21] and it was stated that the peak between 2800 and 3000 cm^{-1} was related with CH_2 deformation and the ratio of amylose to amylopectin affected the intensity of the peak.

The intensity of the peak at 1636 cm^{-1} was known to result from O−H bending of water molecules (water absorbed in the amorphous regions of starch).[22] It was stated that the peak at 1673 cm^{-1} was affected by the change in the crystallinity of the starch, and the intensity of this peak became weaker as the crystallinity of the starch increased. Fang et al.[22] stated that the peak observed at 1640 cm^{-1} was related with the tightly bound water content of the starch. Therefore, the peaks observed at 1636 cm^{-1} could be considered in relation with O−H group bending. It was observed that the peak intensity decreased with increasing HHP and US treatments. This result also correlated with T_2 values of NMR measurements that will be explored in the latter section.

The intensity of the peak at 1354 and 1153 cm^{-1} showed an increase up to 350 MPa, 40 °C and a decrease after 350 MPa, 40 °C. The peak at 1344 cm^{-1} was related with CH_2 bending modes. It was also stated that the vibrations related to the

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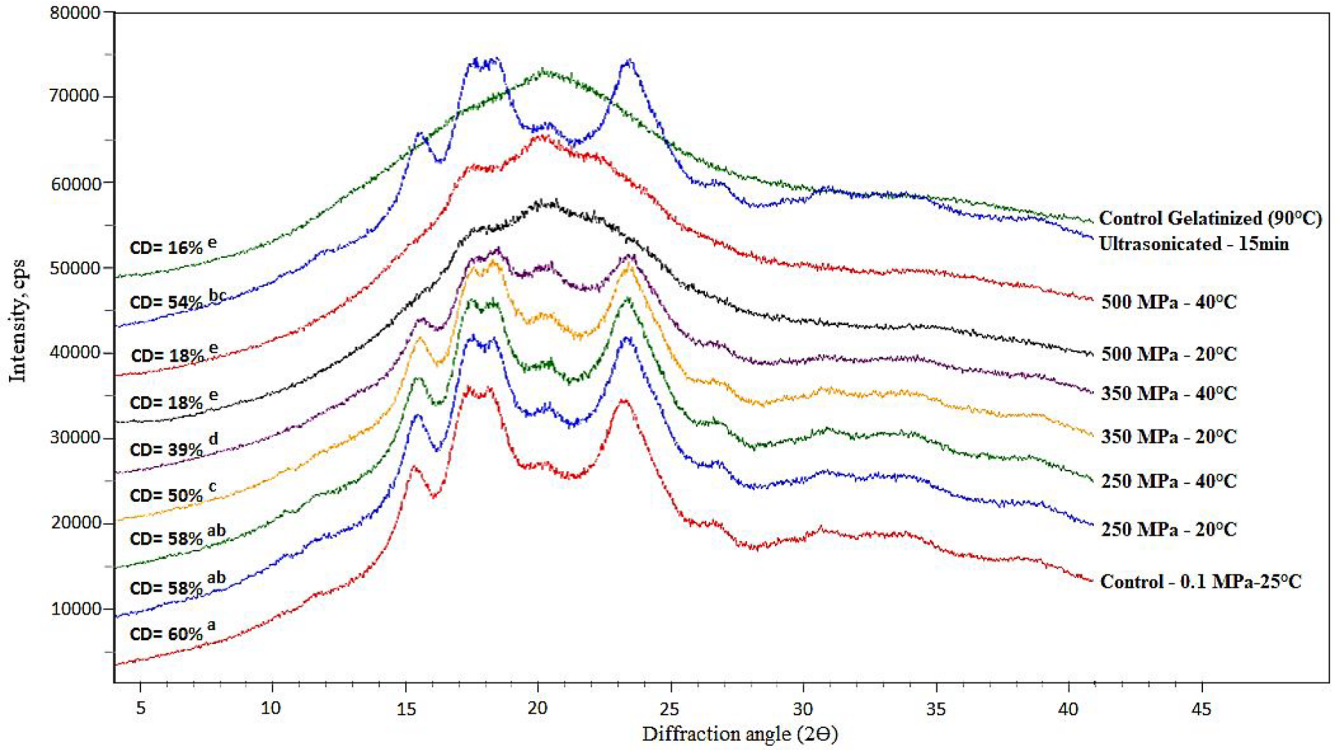

Figure 3. X-ray diffraction patterns and degree of crystallinity (CD) of untreated [control (0.1 MPa, 25 °C)] and treated quinoa starch with HHP (high hydrostatic pressure) at 250 MPa, 20 °C; 250 MPa, 40 °C; 350 MPa, 20 °C; 350 MPa, 40 °C; 500 MPa, 20 °C; 500 MPa, 40 °C; ultrasonicated at 100% power, 15 min; and control gelatinized (by heat treatment at 90 °C).

bending and deformation related to C and H atoms might be tracked between 1500 and 1300 cm^{-1}. The peak at 1153 cm^{-1} was originated from C−O and CH_2 stretching.[22]

A sharper peak was observed at 1011 cm^{-1} indicating C−O−C stretching.[22] It was explained in the previous study that the peak observed between 1060 and 990 cm^{-1} could be associated with the strain deformations of the C−O−C and flexion of the OH and related with the characteristic of the polysaccharides.[22] The peak at 1000 cm^{-1} was stated as water sensitive and attributed to intramolecular H bonding of OH groups and attributed to the crystallinity in the starch granule.[23] It was also stated that high pressure caused destruction of the crystalline structure of lentil starch granule.[2] The observations obtained by FTIR spectroscopy were also consistent with the NMR and SEM results.

3.5. Thermal Properties. The thermal properties of the native and modified starches are displayed in Table 1. It was shown that the onset temperature of quinoa starch increased with HHP and US treatments. However, it decreased by heat treatment. Several studies in the literature have shown that HHP decreased the gelatinization temperature and the enthalpy change (ΔH) of starches such as lotus seed starch, rice starch, wheat starch and barley starch.[24] On the other hand, HHP has been shown to increase the onset (T_0) and peak temperature (T_p) of potato and high-amylose maize starch.[25] This was explained by the loss of less stable crystalline structures and the emergence of amylose-lipid complexes after HHP treatment. It was considered that the amylose-lipid complex possessed a high melting temperature, which resulted in an increase in the gelatinization temperature of starch with higher lipid contents treated with HHP.[25] The degree of gelatinization (DG) values of native and modified quinoa starches are displayed in Table 1. They increased up to HHP treatment of 350 MPa pressure at 20 °C, showing the gelatinization of starch at that pressure level. However, they decreased with HHP treatment of 350 MPa pressure at 20 °C. This decrease was associated with the emergence of amylose-lipid complexes and the loss of less stable crystalline structures during HHP treatment. It may also be attributed to the retrogradation of starch at HHP treatment of 350 and 500 MPa pressure levels due to the unconscious storage of the samples prior to the measurement. HHP treatment at 250 MPa at 20 °C and US treatment did not show a significant difference in DG values. Heat treatment at 90 °C also increased the DG significantly.

3.6. X-ray Diffraction Patterns. The X-ray diffraction (XRD) patterns of untreated, HHP and US treated, and heat gelatinized quinoa starch samples are presented in Figure 3. Untreated quinoa starch demonstrated a doublet at 2θ values of 17.1° and 18° and singlets at 2θ values of 15.3° and 23.15°, which exhibits an A-type X-ray diffraction pattern. A weak peak was observed at a 2θ value of 20.16°, which was attributed to the amylose-lipid complex. No significant changes in XRD patterns were observed for quinoa starch after HHP treatment of 250 MPa at 20 and 40 °C, 350 MPa at 20 °C and US treatment, expressing that the starch crystalline structure was not damaged under these conditions. This was consistent with the previous results of the functional properties of quinoa starch at these parameters. After HHP treatment of 350 MPa at 40 °C, the decrease of the diffraction intensity of quinoa starch started. At HHP treatment of 500 MPa at 20 and 40 °C and heat treatment, the peaks became more broader and larger due to turning of the doublet peak at a 2θ value of 17.1° into a single peak and disappearance of other peaks. This was associated with the conversion of the A-type crystalline structure to the B-type crystalline structure, indicating

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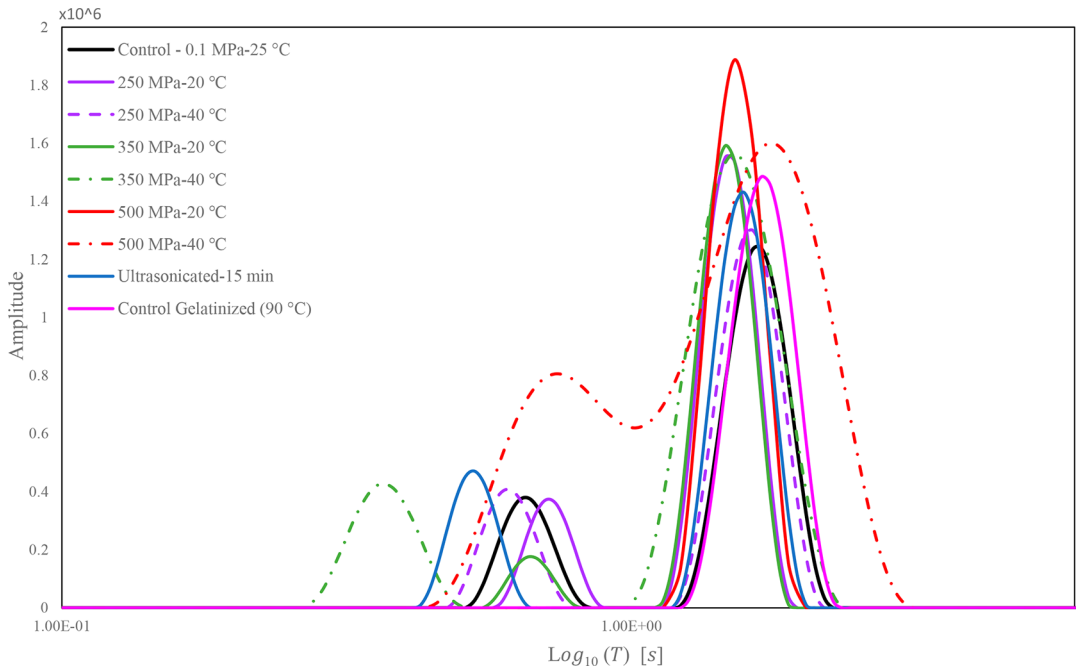

Figure 4. T_2 relaxation spectrum of quinoa starch slurries soaked with distilled water for control (0.1 MPa, 25 °C) and HHP treated at 250 MPa, 20 °C; 250 MPa, 40 °C; 350 MPa, 20 °C; 350 MPa, 40 °C; 500 MPa, 20 °C; 500 MPa, 40 °C; ultrasonicated at 100% power, 15 min; and control gelatinized (by heat treatment at 90 °C).

destruction of the internal crystalline structure and starch gelatinization. Furthermore, as pressure increased, the peak at a 2θ value of 20.16° became more prominent. This might be attributed to the high pressure encouraging the development of amylose-lipid complexes.

The degree of crystallinity (CD) values are also demonstrated in Figure 3. The results showed that the CD values of quinoa starch decreased with HHP, US and heat treatments. It was observed that HHP treatment of 250 MPa at 20 and 40 °C and US treatment did not result in a significant change in the CD values of quinoa starch, while pressure levels above 350 and 500 MPa showed a significant difference for the CD values of quinoa starch. The CD of the heat treated quinoa starch was also found to decrease significantly ($p < 0.05$). Thus, it was concluded that HHP treatments of 350 MPa at 40 °C and 500 MPa at 20 and 40 °C and heat treatment were sufficient to destroy the internal crystalline structure of the quinoa starch, while HHP treatments of 250 MPa at 20 and 40 °C, 350 MPa at 20 °C and US treatments were inadequate enough.[26]

3.7. NMR Relaxometry. *3.7.1. Spin−Spin T_2 Relaxation Time Measurements.* T_2 relaxation time values of quinoa starch samples which were exposed to HHP and US treatment are given in Table 1. It was observed that there was an increasing trend in T_2 relaxation time values of quinoa starch samples which were exposed to HHP and US treatment. The T_2 value of quinoa starch increased with both treatments, while HHP treatment showed a higher increase of the T_2 values of quinoa starches ($p < 0.05$) at 500 MPa. However, the quinoa starch samples that were fully gelatinized by heat treatment had shorter T_2 values. The increase in the T_2 value of HHP treated quinoa starches could be mainly related to the mechanism of HHP induced starch gelatinization which affects the crystalline and supramolecular structures of starch granules.[9,27] Application of HHP to starch samples resulted in a conversion of the A-type crystalline structure to the B-type crystalline structure which favored interhelical water, leading to better H-bonds. This caused less double helix dissociation as compared with heat induced gelatinization of starch, resulting in poor amylose leaching and thus less granule swelling of starch.[28] Amylose-fatty acid complexes formed during HHP induced gelatinization could also result in restriction of granule swelling.[25] Moreover, another reason behind the increase in the T_2 value of HHP treated quinoa starches was related to the shear force applied during stirring in the conventional heat gelatinization of starch.[9] For US treatment, it was observed that there is not a significant increase in the T_2 values of quinoa starch samples when compared to HHP treatment up to 500 MPa at 20 °C and control gelatinization with heat treatment, while there is a significant decrease when compared to HHP treatment at 500 MPa at 20 and 40 °C. The reason behind this could be related to the inadequacy of the time duration of US treatment in order to destroy the starch granule completely resulting in reduced amylose leaching and thus reduced granule swelling.[11]

3.7.2. Water Proton Transverse Relaxation Time Distributions. To explore the effect of modifications on water compartmentalization in the starch granule, T_2 relaxation spectra were also analyzed by non-negative-least-squares (NNLS) analysis. Figure 4 shows representative T_2 relaxation spectra for the quinoa starch samples. Untreated quinoa starch exhibited mainly two distinct proton populations (peaks). The peak with lower T_2 value was associated with an environment with lower mobility, the interior rigid part of the granule. This could be explained by the slow exchange between the water inside and outside of the granules. The peak with higher T_2 value, on the other hand, was attributed to the more mobile environment, which was the bulk water content in the exterior of the granule. There was a fast diffusive exchange between hydroxyl protons in the amylopectin and amylose molecules in the bulk water of external space.[29,30] HHP treatment up to 500 MPa and US treatment caused only minor changes in the T_2 relaxometry spectra. However, pressure treatment at 500 MPa

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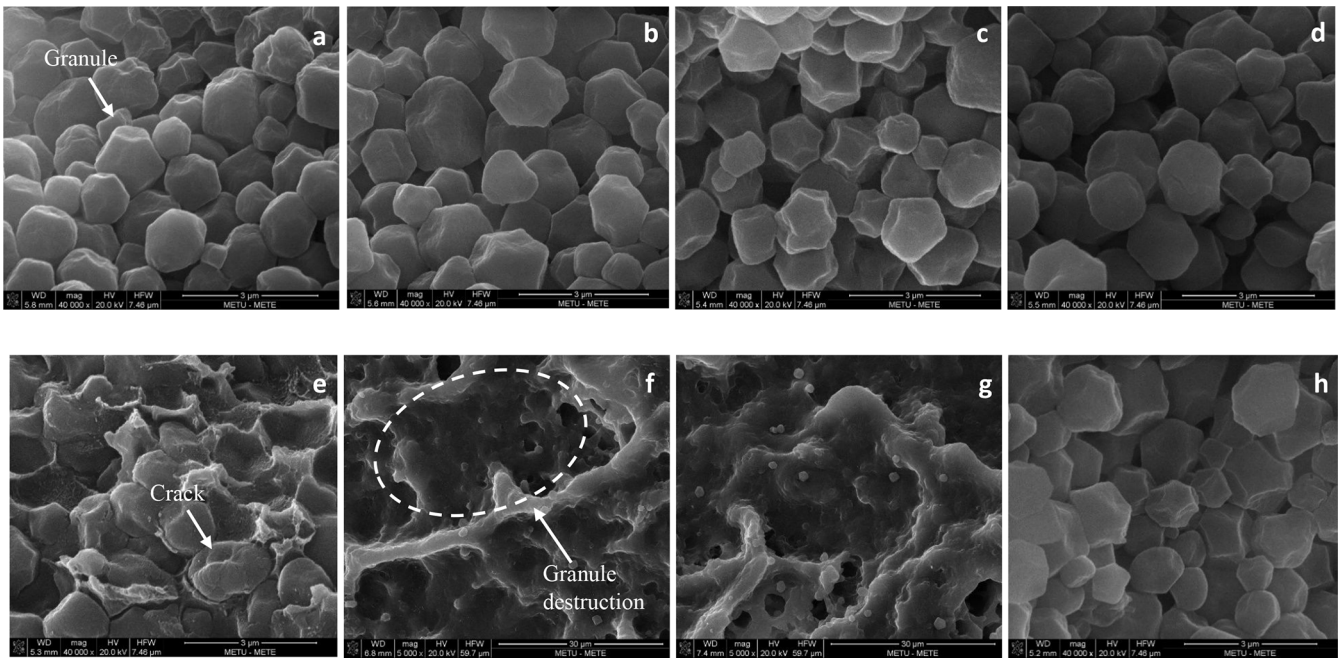

Figure 5. Scanning electron microscopy (SEM) images of untreated [control (0.1 MPa, 25 °C)] (a) and quinoa starches treated with high hydrostatic pressure (HHP) at 250 MPa, 20 °C (b); 250 MPa, 40 °C (c); 350 MPa, 20 °C (d); 350 MPa, 40 °C (e); 500 MPa, 20 °C (f); 500 MPa, 40 °C (g); ultrasonicated (US) at 100% power, 15 min (h).

resulted in significant changes in the proton mobility of the quinoa starch. The area of the peak with higher T_2 value increased while the area of the peak with lower T_2 value decreased (a lower fraction), resulting in them merging as one peak. This was consistent with the high pressure swelling of the starch granule.[31] HHP treatment at 500 MPa seemed to enhance the mobilization of the amorphous region.

As can be seen in Figure 4, fully gelatinized quinoa starch exhibited one peak. Heat induced gelatinization increased the diffusive exchange of water between interior and exterior regions of the granule. The reason behind this phenomenon was related with the destruction in the granule structure during heat treatment. The heat treatment applied for fully gelatinization of starch resulted in destruction of the structural barriers, which led to a faster diffusive exchange of water molecules between compartments in the granule. As a result of this structural change, the area of the peak representing the protons coming from a less mobile environment decreased or the peaks merged as one peak, representing one water compartment in the granule after granule breakdown. These findings were in good agreement with the water fractions in the compartments of the starch granule.[30] NMR relaxometry results were found to be consistent with the results obtained from FTIR spectra.

3.8. Morphological Structure. Scanning electron microscopy images of untreated and HHP and US treated quinoa starches are shown in Figure 5. The untreated native starch granules have polygonal shape. The starch granule's surface was smooth and free of cracks. It was evident that the starches treated at 250 MPa, 20−40 °C and 350 MPa, 20 °C did not show any significant change as compared to the native starch. The quinoa starch samples treated with US displayed similar surface structure with the native starch. In previous studies, small fissures and depressions were observed on the surface of potato and wheat starch granules treated with US for 30 min at a frequency of 20 kHz and power of 170 W.[31] It was consistent to observe a smooth surface structure of US treated quinoa starch when compared with US treatment with higher time duration.

At 350 MPa, 40 °C, morphological changes such as crack formation began to appear. HHP treated samples especially at 500 MPa showed granule destruction due to the gelatinization of quinoa starch. Furthermore, the observations presented in Figure 5 were consistent with morphological and FTIR results since the gelatinization effect of HHP treatment damaged the starch granule. In the literature, similar observations were also reported for different starches.[2,32]

4. CONCLUSION

In this study, the physicochemical characteristics of quinoa starch exposed to high hydrostatic pressure (HHP) and ultrasonication (US) were investigated. The swelling power and water solubility index of quinoa starch decreased with both treatments; however, HHP caused a significant decrease at 500 MPa. The apparent viscosity of the starch samples decreased with increasing pressure and US treatment. Both treatments resulted in an increase in the $D[4,3]$ value of the quinoa starch, but HHP treatment caused a significant increase in the volume mean diameter ($D[4,3]$). FTIR results also revealed consistent results supporting that HHP treatment led to strong changes while US treatment caused minor changes on quinoa starch. DSC and XRD results revealed the destruction of the crystalline structure by HHP treatment of 350 MPa at 40 °C and of 500 MPa at 20 and 40 °C and heat treatment for control gelatinization. SEM results also showed the intense effect of HHP treatment on quinoa starch, especially at the 500 MPa pressure level. Time domain (TD) NMR results showed that both treatments resulted with an increase in T_2 values, while HHP treatment caused a strong increase in T_2 values ($p < 0.05$) at the 500 MPa pressure level. NMR relaxation spectra of quinoa starch revealed mainly two distinct proton populations. HHP treatment at the 500 MPa pressure level

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caused faster exchange of proton populations compared to US treatment. Thus, it was supported that HHP and US treatments had a modification effect on quinoa starch, while HHP treatment at 500 MPa caused the strongest effect on quinoa starch by providing a new type of modified quinoa starch that can be used as an instant food product and thickening agent with the feature of retaining the granular shape and reducing swelling. Moreover, NMR relaxometry was proved as a useful tool to investigate the quinoa starch water interactions subjected to different treatments.

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Notes

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REFERENCES

(1) Abugoch James, L. E.Quinoa (*Chenopodium quinoa* Willd.): Composition, Chemistry, Nutritional, and Functional Properties. In *Advances in Food and Nutrition Research*; Taylor, S. L., Ed.; Academic Press, 2009; Vol. *58*, Chapter 1, pp 1−31). DOI: 10.1016/S1043-4526(09)58001-1.

(2) Ahmed, J.; Thomas, L.; Arfat, Y. A.; Joseph, A. Rheological, structural and functional properties of high-pressure treated quinoa starch in dispersions. *Carbohydr. Polym.* **2018**, *197* (May), 649−657.

(3) Li, G.; Wang, S.; Zhu, F. Physicochemical properties of quinoa starch. *Carbohydr. Polym.* **2016**, *137*, 328−338.

(4) Lindeboom, N.; Chang, P. R.; Falk, K. C.; Tyler, R. T. Characteristics of starch from eight quinoa lines. *Cereal Chem.* **2005**, *82* (2), 216−222.

(5) Li, G.; Zhu, F. Effect of high pressure on rheological and thermal properties of quinoa and maize starches. *Food Chem.* **2018**, *241*, 380−386.

(6) Pei-Ling, L.; Xiao-Song, H.; Qun, S. Effect of high hydrostatic pressure on starches: A review. *Starch/Staerke* **2010**, *62* (12), 615−628.

(7) Kim, H. S.; Kim, B. Y.; Baik, M. Y. Application of ultra high pressure (UHP) in starch chemistry. *Critical Reviews in Food Science and Nutrition* **2012**, *52* (2), 123−141.

(8) Yang, Z.; Chaib, S.; Gu, Q.; Hemar, Y. Impact of pressure on physicochemical properties of starch dispersions. *Food Hydrocolloids* **2017**, *68*, 164−177.

(9) Jambrak, A. R.; Herceg, Z.; Šubarić, D.; Babić, J.; Brnčić, M.; Brnčić, S. R.; Gelo, J. Ultrasound effect on physical properties of corn starch. *Carbohydr. Polym.* **2010**, *79* (1), 91−100.

(10) Manchun, S.; Nunthanid, J.; Limmatvapirat, S.; Sriamornsak, P. Effect of ultrasonic treatment on physical properties of tapioca starch. *Advanced Materials Research* **2012**, *506*, 294−297.

(11) Kirtil, E.; Oztop, M. H. 1H Nuclear Magnetic Resonance Relaxometry and Magnetic Resonance Imaging and Applications in Food Science and Processing. *Food Engineering Reviews* **2016**, *8* (1), 1−22.

(12) Ozel, B.; Dag, D.; Kilercioglu, M.; Sumnu, S. G.; Oztop, M. H. NMR relaxometry as a tool to understand the effect of microwave heating on starch-water interactions and gelatinization behavior. *LWT - Food Science and Technology* **2017**, *83*, 10−17.

(13) Jan, K. N.; Panesar, P. S.; Singh, S. Process standardization for isolation of quinoa starch and its characterization in comparison with other starches. *Journal of Food Measurement and Characterization* **2017**, *11* (4), 1919−1927.

(14) Guo, Z.; Zeng, S.; Lu, X.; Zhou, M.; Zheng, M.; Zheng, B. Structural and physicochemical properties of lotus seed starch treated with ultra-high pressure. *Food Chem.* **2015**, *186*, 223−230.

(15) Bitik, A.; Sumnu, G.; Oztop, M. Physicochemical and Structural Characterization of Microfluidized and Sonicated Legume Starches. *Food and Bioprocess Technology* **2019**, *12* (7), 1144−1156.

(16) Demirkesen, I.; Campanella, O. H.; Sumnu, G.; Sahin, S.; Hamaker, B. R. A Study on Staling Characteristics of Gluten-Free Breads Prepared with Chestnut and Rice Flours. *Food and Bioprocess Technology* **2014**, *7* (3), 806−820.

(17) Ribotta, P. D.; Cuffini, S.; León, A. E.; Añón, M. C. The staling of bread: An X-ray diffraction study. *European Food Research and Technology* **2004**, *218* (3), 219−223.

(18) Unal, K.; Alpas, H.; Aktas, H.; Oztop, M. H. Time domain (TD)-NMR relaxometry as a tool to investigate the cell integrity of tomato seeds exposed to osmotic stress (OS), ultrasonication (US) and high hydrostatic pressure (HHP). *Journal of Food Science and Technology* **2020**, *57* (10), 3739−3747.

(19) Zhu, F.; Li, H. Effect of high hydrostatic pressure on physicochemical properties of quinoa flour. *LWT* **2019**, *114*, 108367.

(20) Xu, F.; Zhang, L.; Liu, W.; Liu, Q.; Wang, F.; Zhang, H.; Blecker, C. Physicochemical and structural characterization of potato starch with different degrees of gelatinization. *Foods* **2021**, *10* (5), 1104.

(21) Kizil, R.; Irudayaraj, J.; Seetharaman, K. Characterization of Irradiated Starches by Using FT-Raman and FTIR Spectroscopy. *J. Agric. Food Chem.* **2002**, *50* (14), 3912−3918, DOI: 10.1021/jf011652p.

(22) Fang, J. M.; Fowler, P. A.; Tomkinson, J.; Hill, C. A. S. The preparation and characterisation of a series of chemically modified potato starches. *Carbohydr. Polym.* **2002**, *47* (3), 245−252.

(23) Warren, F. J.; Gidley, M. J.; Flanagan, B. M. Infrared spectroscopy as a tool to characterise starch ordered structure - A joint FTIR-ATR, NMR, XRD and DSC study. *Carbohydr. Polym.* **2016**, *139*, 35−42.

(24) Katopo, H.; Song, Y.; Jane, J. L. Effect and mechanism of ultrahigh hydrostatic pressure on the structure and properties of starches. *Carbohydr. Polym.* **2002**, *47* (3), 233−244.

(25) Kugimiya, M.; Donovan, J. W.; Wong, R. Y. Phase Transitions of Amylose-Lipid Complexes in Starches: A Calorimetric Study. *Starch - Stärke* **1980**, *32* (8), 265−270.

(26) Wang, J.; Zhu, H.; Li, S.; Wang, S.; Wang, S.; Copeland, L. Insights into structure and function of high pressure-modified starches with different crystalline polymorphs. *Int. J. Biol. Macromol.* **2017**, *102*, 414−424.

(27) Yang, Z.; Swedlund, P.; Hemar, Y.; Mo, G.; Wei, Y.; Li, Z.; Wu, Z. Effect of high hydrostatic pressure on the supramolecular structure of corn starch with different amylose contents. *Int. J. Biol. Macromol.* **2016**, *85*, 604−614.

(28) Yang, Z.; Gu, Q.; Hemar, Y. In situ study of maize starch gelatinization under ultra-high hydrostatic pressure using X-ray diffraction. *Carbohydr. Polym.* **2013**, *97* (1), 235−238.

(29) Tananuwong, K.; Reid, D. S. DSC and NMR relaxation studies of starch-water interactions during gelatinization. *Carbohydr. Polym.* **2004**, *58* (3), 345−358.

(30) Tang, H. R.; Godward, J.; Hills, B. Distribution of water in native starch granules - a multinuclear NMR study. *Carbohydr. Polym.* **2000**, *43* (4), 375−387.

(31) Sujka, M.; Jamroz, J. Ultrasound-treated starch: SEM and TEM imaging, and functional behaviour. *Food Hydrocolloids* **2013**, *31* (2), 413−419.
(32) Okur, I.; Ozel, B.; Oztop, M. H.; Alpas, H. Effect of high hydrostatic pressure in physicochemical properties and in vitro digestibility of cornstarch by nuclear magnetic resonance relaxometry. *Journal of Food Process Engineering* **2019**, *42* (6), 1−10.

https://doi.org/10.1021/acsfoodscitech.2c00230
ACS Food Sci. Technol. 2023, 3, 31−40