Influence of microwave treatment on the structure and functionality of pure amylose and amylopectin systems

Yuyue Zhong [a], Yu Tian [a], Xingxun Liu [b], Li Ding [a], Jacob Judas Kain Kirkensgaard [c,d], Kim Hebelstrup [e], Jean-Luc Putaux [f], Andreas Blennow [a,*]

[a] *Department of Plant and Environmental Sciences, University of Copenhagen, DK-1871, Frederiksberg C, Denmark*
[b] *Lab of Food Soft Matter Structure and Advanced Manufacturing, College of Food Science and Engineering / Collaborative Innovation Center for Modern Grain Circulation and Safety / Key Laboratory of Grains and Oils Quality Control and Processing, Nanjing University of Finance and Economics, Nanjing, 210023, China*
[c] *Department of Food Science, University of Copenhagen, DK-1958, Frederiksberg C, Denmark*
[d] *Niels Bohr Institute, University of Copenhagen, Copenhagen, Denmark*
[e] *Section for Crop Genetics and Biotechnology, Aarhus University, Flakkebjerg, Forsøgsvej 1, 4200, Slagelse, Denmark*
[f] *Univ. Grenoble Alpes, CNRS, CERMAV, F-38000, Grenoble, France*

ARTICLE INFO

ABSTRACT

Pure granular amylose (AM) and pure granular amylopectin (waxy) starch (AP) granules have the high nutritional value in food industry. Effects of microwave treatment (400 W/g DW, 1–8 min) on the structure and properties of transgenic AM granules and AP granules were investigated in direct comparison. Microwave treatment, especially during the first 3 min, decreased the molecular weight of molecules in both the AM and the AP samples. The crystallinity of AM starch initially increased from 15.6% to 20.6%, which was associated with the formation of new V_h-type crystals. After that, crystallinity decreased alongside to 11.3% with the complete disruption of B-type crystals. In contrast, the crystallinity of AP starch initially decreased from 18.9% to 10.8% followed by an increase to 20.0%. Upon prolonged treatment of AM granules, the resistant starch and water solubility was significantly increased. Our data demonstrate notable different microwave-dependent reorganization patterns for pure granular AM and AP molecules as native granular systems, which is helpful to the improvement of functionality of these two starches.

1. Introduction

Starch is a natural biopolymer found as energy reserve in our most important crops including cereals, tubers, roots, fruits, and seeds, and has been applied in different industries, such as food, drug delivery, and composite biodegradable plastics. Starch has different levels of structure, including amylose and amylopectin molecules (~0.1 nm range), crystalline and amorphous lamellar structure (~10 nm range), alternating amorphous and semi-crystalline growth rings (~0.1 μm range) and starch granules (1–100 μm range) (Zhong et al., 2019). Native starch has limited industrial utilization due to a number of undesirable properties, such as water insolubility at room temperature, retrogradation and syneresis. Generally, physical/chemical/enzymatic modifications of starch improve important properties like suppressed or stimulated retrogradation and increased paste clarity and stability and thus is of major industrial interest within food, feed, paper, and textile applications (Blennow, 2018; Maniglia, Castanha, Le-Bail, Le-Bail, & Augusto, 2020; Vanier, El Halal, Dias, & da Rosa Zavareze, 2017).

Microwave treatment has gained in importance as an efficient processing protocol for clean starch modification due to its ability to achieve more uniform heating, safe handling, ease of operation, low maintenance and high efficiency in modifying starch structure to improve its functional properties (Chandrasekaran, Ramanathan, & Basak, 2013; Yang et al., 2017). However, the mechanisms of microwave-induced molecular and physical alterations are still obscure and await direct comparative testing, especially for pure granular AM and AP systems.

Microwave treatment, through its high-frequency electric fields, typically induces micro movements and frictions of starch molecules (Fan et al., 2013) thereby converting electromagnetic energy into thermal energy and generating concurrent internal and external heating (Galema, 1997). It has been reported that microwave treatment can

---

* Corresponding author.
  E-mail address: abl@plen.ku.dk (A. Blennow).

increase the resistant starch (RS) content (Mutlu, Kahraman, & Özt) and gelatinization temperature of normal starch (Yang et al., 2017), and decrease the viscosity of maize pure amylopectin (AP, so-called waxy maize starch) granules (Yang et al., 2017) by inducing the disruption and rearrangement of the starch molecules (Yang et al., 2017; Zhong et al., 2019). Existing data suggest that starch molecules located in amorphous regions of the starch granule were affected initially, followed by effects on the crystalline regions (Chandrasekaran et al., 2013; Yang et al., 2017).

High-amylose (HAM) starch has drawn attention mainly due to its high RS content and excellent mechanical properties and these starch types therefore attain high value in vastly different areas, such as for bioplastics films, coating and functional food products (Guan et al., 2011; Zhong, Liu, Qu, Blennow, et al., 2020). Just like for normal starch, long-term microwave treatment of HAM starch can increase its gelatinization transition temperatures and the gelatinization temperature range, decrease swelling power, water solubility, syneresis and gelatinization enthalpy (Luo, He, Fu, Luo, & Gao, 2006). Interestingly, the structural stability of HAM starch can be improved after only a rather short-time microwave treatment (400 W g^{-1}, 1 min), reflected as an increase in peak viscosity and RS content, which is suggested to be an effect of aggregation of cleaved AM chains (Zhong et al., 2019). With further treatment, the structural stability of the starch is decreased as indicated by a decrease in peak viscosity and RS content, which suggests major disordering of the crystalline regions (Zhong et al., 2019). Hence, by controlling the microwave treatment time and applied energy, the functionality of HAM starch can be altered correspondingly according to the requirements.

The structural and functional alterations of waxy starch (AP) (Yang et al., 2017), low-AM starch (Luo et al., 2006), and HAM starch (Zhong et al., 2019) resulting from microwave irradiation have been previously investigated. For AP maize starch granules, the process entailed an initial disruption of the amorphous regions followed by its destruction (Yang et al., 2017). However, the effect of microwave treatment of AM in its native, granular state remains to be investigated. Such data will gain understanding on how microwaves affect AM molecules in their pure granular state, which constitutes a much more dynamic, but also partly stable aggregated system, than AP. The recent staggering developments in crop bioengineering have now permitted the generation of very high AM contents (Blennow et al., 2013; Hebelstrup, Sagnelli, & Blennow, 2015) and a pure AM barley mutant was previously generated by suppressing all starch branching enzymes (Blennow et al., 2013; Carciofi et al., 2012; Hebelstrup et al., 2015), providing the possibly to investigate the role of AM more precisely (Goldstein et al., 2016). AM chains are cleaved and aggregated during the microwave heating in HAM starch thereby increasing the granular stability (Zhong et al., 2019). We hypothesize that the aggregation level of AM molecules in a pure AM granular system is higher than that of a HAM starch system, without the hindering effect of AP molecules. By this approach, the functionality (i.e., higher resistant starch content) of AM can be further improved.

We anticipate that pure amylose and pure amylopectin derived from engineered crops can be game changers for future demanding applications and hypothesized that such systems respond differently against microwave treatment. Therefore, we investigated the effects of such treatment at different time points (1–8 min) on pure granular barley AM as compared to pure granular maize AP by collecting data at different structural levels, rheological properties and amylolytic digestibility. We chose waxy maize starch granules as the pure AP system since the effect of microwave treatment on this starch type is very well characterized (e. g. Yang et al., 2017; Luo et al., 2006). Pure AP starches have substantial similarity in structure across waxy barley and waxy wheat (Vamadevan & Bertoft, 2018). In contrast, the difference between waxy starch and high-amylose starch is more remarkable (Zhong, Liu, Qu, Blennow, et al., 2020). Our data provide fundamental and direct comparative information on how AM and AP in their native, granular system are

differently affected by microwave irradiation. Such information is helpful to understand the structural changes of AM and AP granular starches under microwave irradiation, thereby further improving industrial functionality of these two starch types.

2. Materials and methods

2.1. Materials

AP granules from maize (*Zea maize*) (provided by Sigma, St. Louis, MO, USA) and AM granules extracted from a transgenic barley line with all starch branching enzymes (SBEs) suppressed (Carciofi et al., 2012) were purified by alkaline method and used in this study. The purity of starches was tested by the total starch assay kit (Sigma–Aldrich), with the purity higher than 95% (Table S1). The lipid content and protein content are both lower than 1%. α-1,6-glucosidic acting debranching enzymes, isoamylase (E-ISAMY, 280 U mL^{-1}), and pullulanase (E-PULKP, 650 U mL^{-1}), were supplied from Megazyme (Ireland). Pancreatin (Cat. No. P7545) and amyloglucosidase (Cat. No. A7095) were from Sigma (St. Louis, MO, USA).

2.2. Microwave treatment

Short-term microwave treatment was performed as described (Zhong et al., 2019). Specifically, the moisture content of starches was measured by heating at 120 °C for 48 h. AM or AP (2.0 g, dry weight) was spread on glass petri dishes as a uniform layer, followed by injecting an appropriate amount of distilled water into the glass petri dishes by a syringe to increase the sample moisture content to 30% (w/w) with 3 h slow stirring. The weight and moisture contents were specifically set at intermediate level for easily controlling the treatment process and the comparability with the reported HAM starch systems (Zhong et al., 2019). The petri dishes were sealed and placed in the center of a microwave oven (frequency 2450 MHz, Amana Refrigeration Inc., IA, USA) with the output power of 800 W, i.e., 400 W g^{-1} DW. Treatment was performed for 1, 2, 3, 4, 6, and 8 min and a uniform treatment was secured by automatic rotation. The color gradually turned yellow from 4 min indication partial. The treated samples were collected and dried at 30 °C for 24 h prior to further analysis to decrease the humidity of samples to the similar level of native raw starches. According to the time (x) of microwave treatment, the samples were referred to as APx or AMx. For each time point, six batches were prepared, and three of them were randomly selected, pooled and mixed.

2.3. Size-exclusion chromatography (SEC) and iodine-binding capacity

The molecular structure of both native and debranched samples was analyzed by a size-exclusion chromatography – triple detector array SEC-TDA (Viscotek, Malvern, UK) instrument equipped with tandem GS-520HQ/GS-320HQ Shodex columns attached to a TDA302 detector array (Zhong et al., 2021). Samples (5 mg) were dissolved in 20 μL 2M sodium hydroxide (NaOH) in 4 °C overnight for gentle gelatinization of starch by alkaline, diluted to 5 mg mL^{-1} and incubated at 80 °C for 5 h. The samples were diluted to 1 mg mL^{-1}, centrifuged at 20,000 *g* for 5 min and 50 μL supernatant injected onto the SEC-TDA system. Elution was performed using ammonium formate (10 mM) at a flow rate of 0.5 mL mL^{-1} and column temperature of 60 °C. Debranching of gelatinized samples (AP and AM were gelatinized starch dispersion in screwed tubes by heating at 99 °C and 130 °C (with high-pressure) for 1 h, respectively) was performed by adding 2 μL isoamylase and 2 μL pullulanase and incubating 5 mg mL^{-1} in sodium acetate buffer (pH 4.0) at 40 °C for 3 h. Complete debranching was verified by the point of insignificant increase in reducing ends (Doner and Irwin, 1992). Samples were diluted to 1 mg mL^{-1} before injecting to the SEC-TDA system. Linear monodisperse pullulan standards (P50, P200, Sigma Aldrich) were used for instrument calibration and permit calculation the absolute molecular weight and

hydrodynamic radii (R_h) of the native samples.

The iodine complexation of samples was analyzed using 5 mg sample powder dissolved in 0.75 mL of 4 M NaOH under vigorous stirring overnight, where after 2.25 mL MilliQ water was added. Finally, 10 μL sample was mixed with 200 μL of 10-times diluted Lugol solution (pH = 2) before measuring the absorbance at 550 and 620 nm (Carciofi et al., 2012).

2.4. High-performance anion exchange chromatography-pulsed amperometric detection (HPAEC-PAD)

Enzymatic debranching was as described in section 2.3. AM or AP (10 μL, 5 mg mL^{-1}) was injected onto a CarboPac PA-200 column attached to an HPAEC-PAD (Dionex, Sunnyvale, CA, USA) system. Peak integration and detector response were performed as described (Blennow, Bay-Smidt, Wischmann, Olsen, & Møller, 1998).

2.5. Wide-angle X-ray scattering (WAXS)

Following conditioning of the samples at a humidity chamber with 90% relative humidity by saturated KCl for 72 h, the samples were analyzed using a SAXSLab instrument (JJ-X-ray, Copenhagen, Denmark) equipped with a 100 XL + microfocus sealed X-ray tube (Cu-K$_\alpha$ radiation, Rigaku, The Woodlands Texas, USA) and a 2D 300 K Pilatus detector (Dectris Ltd, Baden, Switzerland) (Zhong et al., 2021). The radially averaged intensity I is given as a function of the scattering angle 2θ in the angular range 5–30° using 40 mA current, 40 kV voltage, and 0.1542 nm wavelength Cu Kα radiation. The relative crystallinity was calculated as described (Brückner 2000; Goldstein et al., 2016). The amorphous background scattering was estimated by a 30-cycle iterative smoothing algorithm (MATLAB, Natick, Massachusetts, USA), and the relative crystallinity was calculated:

Relative Crystallinity = Area of Peaks/Total Area

where the areas were numerically integrated using built in MATLAB functions (Brückner, 2000; Goldstein et al., 2017).

2.6. Swelling power and water solubility

Swelling power and water solubility were determined as described (Wang & Copeland, 2012). AM or AP (40 mg mL^{-1} DW) was heated at 60 °C for 1 h, followed by cooling to 20 °C and centrifugation at 13,000 g for 20 min. The supernatant was collected and dried at 120 °C for 24 h, and the water solubility (%) was calculated as percent of the weight of the dried supernatant of the original dry weight (40 mg). Swelling power (g/g) was calculated from the ratio of the weight of the precipitate and the original weight of the sample.

2.7. In vitro digestion

The digestion of raw samples and retrograded samples (starch was gelatinized and stored at 4 °C for 1 day) was analyzed with minor modifications (Zhong et al., 2019). The gelatinization of AP and AM starches were conducted by heating at 99 °C and 130 °C for 1 h, respectively. AM or AP (100 mg), 5 mL water and 10 mL sodium acetate buffer (0.1 M, pH 5.2) were mixed by vortexing for 5 min, followed by equilibrating the mixture at 37 °C for 30 min. The reaction was initiated by adding 2.5 mL sodium acetate buffer containing 18.75 mg pancreatin and 13.4 μL amyloglucosidase into the mixture. Aliquots (0.1 mL) were taken at 20 and 120 min and the reaction terminated by adding 1 mL 95% ethanol. Released glucose content was analyzed by the Megazyme GOPOD kit. The samples digested in 0–20 min were defined as rapidly digested starch (RDS), and the starch digested in 20–120 min was defined as slowly digested starch (SDS). The remaining residues were subtracted from RDS and SDS and defined as RS.

2.8. Rheology

The dynamic rheological analysis was analyzed from gelatinized (8%, w/v) starch gels by a Discovery HR-3 Rheometer (TA Instruments, New Castle, UK) at 25 °C. The gelatinization was performed as mentioned in section 2.3. Frequency sweeps were carried out from 0.01 to 100 Hz. Rheological parameters including storage modulus (G′), loss modulus (G″), loss tangent (G′/G″, tan δ), and the modulus of complex viscosity (η*) were calculated.

2.9. Statistical analysis

WAXS analyses were performed once for each sample and all other experiments were performed in triplicates. Differences were analyzed using one-way analyses of variance (ANOVAs) followed by Duncan's test ($p < 0.05$) in SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Microwave-assisted molecular degradation

The molecular size distribution as analyzed by SEC-TDA of the native (not debranched) samples (Fig. 1A) showed a shift of retention volume of both the AP and the AM to higher values with the increased microwave treatment time, indicating a microwave-induced molecular scission of both types of glucan. The molecular weight (Mw) and molecular size (R_h) of AP and AM both showed sigmoidal decays and for both AP and AM, the sharp drop occurred between 3 and 4 min (Table 1). The AP sample also showed a similar decrease in iodine complexation ratio (620 nm/550 nm) (Table 1), demonstrating that the iodine complexation of AP and its Mw concomitantly decreased. In contrast, the iodine complexation of AM decreased slowly during the entire treatment period, indicating that the decrease in Mw of AM had less effect on iodine complexation. The chain length distribution (CLD) of the side chains analyzed by SEC of debranched samples (Fig. 1B), demonstrated that the CLDs of AP after debranching only had a minor decrease, whereas CLDs of the AM samples displayed a more drastic shift of the peak position to higher elution volumes.

3.2. Chain length distribution (CLD) of AP and AM side chains

HPAEC-PAD data (Fig. 2A) revealed further chain distributional details. Specifically, chains with DP > 16 were degraded to shorter chains with DP < 16, peaking at DP 11 produced after 1 min microwave treatment, whereas no more significant variation of AP CLD was found with further treatment (Fig. 2B). AM also exhibited some side chains with the peak at DP 12 after debranching, showing that some molecules in AM had partial branches (Fig. 2A). AM granules are generated by suppressing all SBEs during starch biosynthesis pathways (Carciofi et al., 2012), and 'amylose-like' material is produced during the amylopectin biosynthesis (Zhong, Liu, Qu, Li, Blennow, & Liu, 2020). We suggest that the branches in AM originate from the 'amylose-like' material. It is clear that, with increased microwave treatment time, the range of CLD in AM became narrower starting at DP 1–60 ending at DP 1–30, which implies a preferential degradation of long side chains. For AM, the microwave treatment led to significant increases in the relative content of maltooligosaccharides, i.e. chains with DP < 10 (Fig. 2B).

3.3. Crystalline dynamics of the microwave-treated samples

The change in crystalline allomorphs as deduced from WAXS data (Fig. 3) showed that all AP granules exhibited the typical signature of the A-type allomorph with two strong peaks at 2θ around 15 and 23°, and an unresolved doublet at 2θ = 17 and 18° (Cheetham & Tao, 1998). Microwave treatment resulted in an initial decrease in intensity of all reflections, especially for the peak at 2θ = 23° in samples 0–3 min,
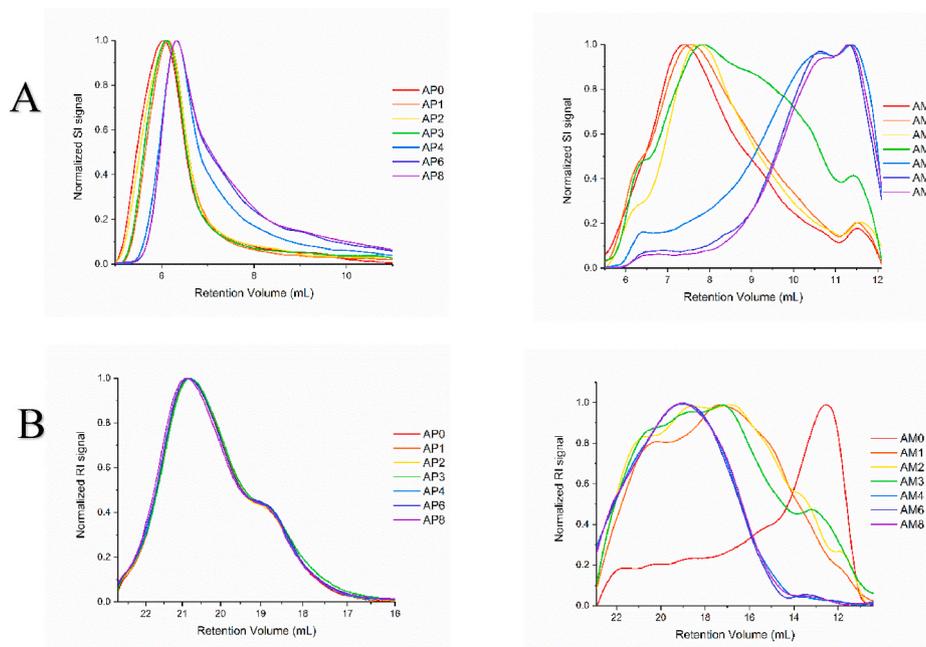
Fig. 1. SEC profiles of native AP and AM microwave treated for different time periods (A), and SEC profiles of debranched samples (B). Abbreviations as in the main text.

Table 1
Molecular weight (Mw), hydrodynamic radii (R_h), and iodine-binding capacity (absorbance at 620 nm/550 nm) of microwave treated AM and AP (n = 2).

Treatment time (min)	AP			AM		
	Mw $\times 10^7$	R_h (nm)	Iodine binding capacity	Mw $\times 10^7$	R_h (nm)	Iodine binding capacity
0	17.7 ± 0.9[a]	85.9 ± 6.9[a]	1.27 ± 0.01[a]	9.6 ± 1.2[a]	30.5 ± 1.2[a]	0.65 ± 0.00[a]
1	17.3 ± 0.9[a]	80.3 ± 6.3[b]	1.25 ± 0.02[ab]	8.4 ± 1.3[b]	27.5 ± 1.0[b]	0.65 ± 0.00[a]
2	12.6 ± 0.9[b]	72.4 ± 3.4[c]	1.22 ± 0.02[b]	5.9 ± 0.4[c]	26.8 ± 0.3[bc]	0.64 ± 0.00[b]
3	1.7 ± 0.0[c]	35.7 ± 1.4[d]	1.14 ± 0.01[c]	3.0 ± 0.1[d]	25.9 ± 0.7[c]	0.64 ± 0.00[b]
4	0.9 ± 0.1[d]	34.1 ± 0.2[e]	0.80 ± 0.01[d]	0.5 ± 0.1[e]	13.9 ± 0.4[d]	0.64 ± 0.00[b]
6	0.9 ± 0.1[d]	34.7 ± 0.1[e]	0.60 ± 0.02[e]	0.1 ± 0.0[f]	9.6 ± 0.0[e]	0.63 ± 0.00[c]
8	0.8 ± 0.0[d]	31.9 ± 0.6[f]	0.59 ± 0.01[e]	0.1 ± 0.0[f]	8.3 ± 0.0[f]	0.63 ± 0.00[c]

All data are means ± standard deviation. Values with different letters in the same column are significantly different at $p < 0.05$.

revealing damage in the crystalline regions, which has been documented also for normal starch granules (Oyeyinka, Umaru, Olatunde, & Joseph, 2019). Interestingly, we found that the intensity of the AP granular peaks increased again upon further treatment (4–8 min) which is supposedly associated with the strengthened reaggregation capacity of degraded AP molecules (Zhong et al., 2021). In contrast, the initial AM granules displayed a combination of B-type (at 2θ = 17°, 22°, and 26°) and V_h-type (at 2θ = 8°, 13°, 15°, and 20°) allomorph (Fig. 3), as reported (Carciofi et al., 2012). Increased treatment time from 0 to 2 min weakened the intensities of peaks at 2θ of 22° and 26° and increased the

intensity of peak at 2θ of 20°, which demonstrates the disruption of the B-type allomorph and concomitant formation of the V_h-type allomorph by amylose and endonegous uncomplexed lipids. However, further treatment (2–8 min), resulted in the decreased intensity of the V_h-type allomorph, reflecting a destruction of this allomorph possibly due to the degradation of amylose or the degradation of the lipids which in turn induce a destabilization of the V-type allomorph.

Correspondingly, for AP, we observed that the relative crystallinity decreased initially (0–3 min), followed by an increase (3–8 min) with increased microwave treatment. By contrast, the relative crystallinity of AM granules initially increased (0–2 min) and then decreased (2–8 min). Although the microwave treatments induced a molecular degradation in both AP and AM granules, significant differences between these two polysaccharides were identified. AP molecules form ordered crystalline structures due to the ability of its short side chains to form double helices that assemble in a parallel manner to form 9 nm repeated lamellar structures in the starch granule (Bertoft, 2017). Short-time microwave-assisted degradation of AP granules, i.e. 1-3 min, possibly induced the partial collapse of these crystalline lamellae and the unwinding of double helices. However, a longer microwave treatment of AP granules further degraded this type of glucan into small molecules, which became more flexible. Hence, the high-frequency microwave electric fields can lead to micro-movements of these degraded AP molecules as suggested (Fan et al., 2013) resulting in molecular reorganization of double helices. In contrast, pure AM granules are not capable to build such ordered structures, as evident from the irregular morphology and disordered lamellar structure of AM granules (Carciofi et al., 2012; Shaik, Carciofi, Martens, Hebelstrup, & Blennow, 2014, 2016; Goldstein et al., 2016). However, the low molecular size of AM granules as compared to AP granules makes it more mobile, and thus AM molecules can form new crystals in the first 2 min through microwave-assisted molecular movements. It should be noted that the original B-type crystals were also disrupted during this process. A reorganization from the original weak V_h/B-type to a stronger, mainly B-type, allomorph was identified in acid-hydrolyzed (lintner) starch samples (Goldstein et al., 2016). Further degradation of AM molecules was possibly related to the production of very small maltooligosaccharides that potentially prevent the formation of large crystalline structures (Goldstein et al., 2016).
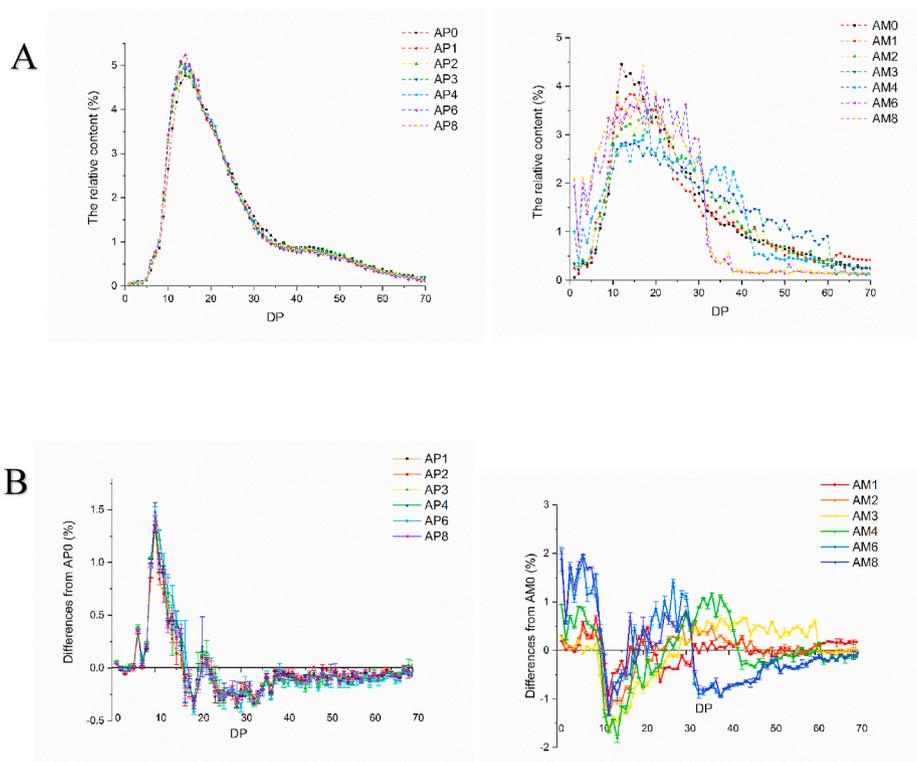
Fig. 2. Chain length distribution profiles as determined by HPAEC of debranched AP and AM microwave treated for different time periods (A), and AP chain length distribution difference plots from corresponding controls AP0 and AM0 (B), respectively. Abbreviations as in Fig. 1.

The effects of microwave treatment on altering the crystalline allomorph have also been reported for the potato starch system (Lewandowicz, Fornal, & Walkowski, 1997) and for amylomaize V (2010). In these cases, the WAXS profile changed from B-type to A-type, due to the partial vaporization of the water molecules in the central channel of the B-unit cell and their subsequent reorganization (Braşoveanu & Nemţ). However, no such transformation was found in microwave-treated A-type starch granules, like waxy and normal maize starches (Luo, 2006; Yang et al., 2017) and normal wheat starch (Lewandowicz, Jankowski, & Fornal, 2000). Hence, the microwave treatment has only the capability to induce a conversion from B- to A-type and not *vice versa* except after complete dissolution of amylose (Nishiyama et al., 2010).

3.4. Swelling power and water solubility

Increased treatment time suppressed the swelling power for both AP and AM (Fig. 4), which is consistent with previous studies on natural (mixed AM/AP) starch samples (Oyeyinka et al., 2019; Zeng et al., 2016). It has been suggested that the transformation of crystalline type and decreased granular crystallinity were the main reasons for the decreased swelling power of microwave-treated starch (Oyeyinka et al., 2019). However, the WAXS profiles show that the crystalline pattern of AP samples was not changed by the treatment and the crystallinity was increased in the 3–8 min period, and thus, the decreased swelling power observed for AP was likely attributed to other effects. We therefore suggest that decreased swelling power was mainly caused by molecular degradation as previously suggested (Keeratiburana, Hansen, Soontaranon, Blennow, & ; Zhong et al., 2021). After microwave treatment, molecules in AM and AP samples were cleaved into different smaller segments, which readily aggregated, decreasing space to bind water. In comparison to AP, AM had lower swelling power all the time points. Such an effect can possibly also originate from the formation of a network surrounding the swollen granules formed by leached AM molecules during heating inhibiting the further swelling (Tester & Morrison,

1990). Therefore, microwave-treated AP and AM can have high potential in food products that require low swelling such as starch noodles (Lii & Chang, 1991).

Native AM had a lower water solubility than native AP (Fig. 4). Microwave treatment has been documented to generate soluble chains mainly composed of leached AP and short AM chains (Green, Blankenhorn, & Hart, 1975; Jackson, Waniska, & Rooney, 1989). The AM sample contained low amounts of short AM chains (Fig. 1A) leading to a low water solubility (Fig. 4). However, the microwave treatment led to an increased water solubility in both AM and AP by molecular degradation producing small soluble maltooligosaccharides. This increase was notably higher in AM than in AP (Fig. 4), an effect likely due to the higher degradation degree of AM samples (Fig. 1A) leading to leakage (Green et al., 1975; Jackson et al., 1989).

Our data can be compared to the minor effects in swelling power and water solubility observed in maize starches with different AM contents after microwave treatment when using a much lower energy (1 W g^{-1}), but somewhat longer treatment time of 20 min. Such treatment can be expected to have much lower degradation levels in comparison to our study, which was also observed.

3.5. Amylolytic digestibility

For granular AP, up to 3 min of treatment resulted in increased RDS content and decreased SDS and RS contents (Table 2), likely related to damage of crystalline granule regions (section 3.3), which can increase their enzymatic susceptibility. This effect is in agreement with previous data (Emami, Perera, Meda, & Tyler, 2012). However, a continued treatment increased the amylolytic resistance, i.e., the RDS content decreased, while the SDS content recovered to some degree (Table 2), which likely is an effect of the aggregation of AP fragments forming new crystals (Fig. 3). Starch is an important ingredient in food industry, and most starch-based foods, including bread, pasta and noodles, are stored for longer periods before selling, which affects the quality of the
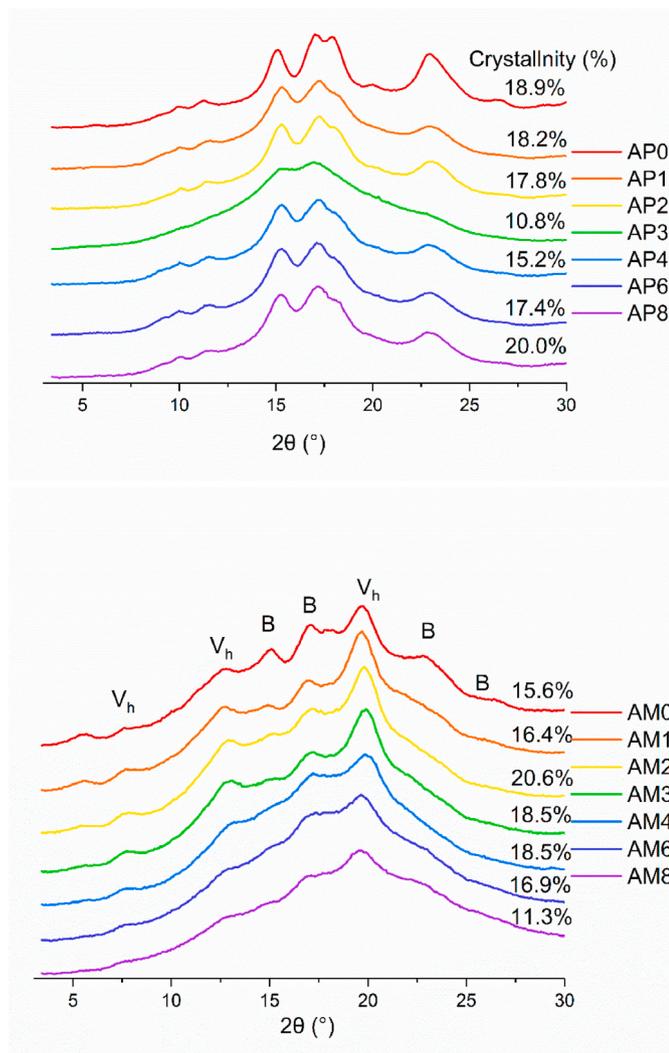
Fig. 3. Effects of crystallinity and crystalline allomorphs of AP and AM as determined by WAXS. Abbreviations as in Fig. 1.
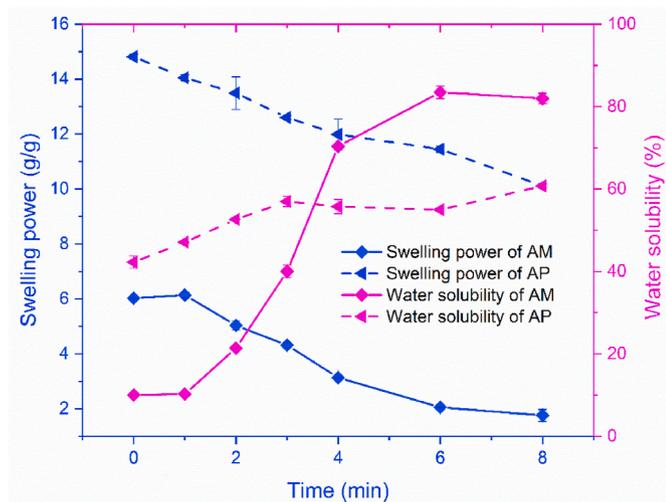
Fig. 4. Swelling power and water solubility of AP and AM microwave-treated for different time periods.

Table 2

In vitro digestion parameters of native AP and AM treated with microwave irradiation for different time periods (n = 2).

Treatment time (min)	AP			AM		
	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
0	44.7 ± 1.5[f]	25.3 ± 0.2[b]	30.0 ± 1.2[a]	32.2 ± 0.7[c]	29.9 ± 0.5[a]	37.9 ± 1.2[d]
1	52.4 ± 0.3[e]	24.6 ± 1.0[c]	22.9 ± 1.4[b]	42.0 ± 0.9[b]	22.2 ± 0.7[b]	35.8 ± 0.3[e]
2	57.2 ± 1.1[d]	23.4 ± 0.7[d]	19.4 ± 1.7[c]	48.7 ± 1.1[a]	22.0 ± 0.7[b]	29.2 ± 0.4[f]
3	66.6 ± 1.0[a]	13.5 ± 0.7[f]	19.9 ± 1.9[c]	47.4 ± 1.1[a]	22.6 ± 0.5[b]	30.0 ± 1.6[f]
4	62.5 ± 0.5[b]	12.9 ± 1.1[g]	24.7 ± 1.5[b]	41.7 ± 0.1[b]	5.7 ± 1.2[c]	52.6 ± 1.0[c]
6	60.0 ± 0.4[c]	18.1 ± 1.5[e]	21.9 ± 1.1[b]	33.1 ± 0.6[c]	1.0 ± 0.0[e]	65.9 ± 0.6[b]
8	56.7 ± 0.1[d]	21.3 ± 1.6[a]	22.0 ± 1.7[d]	29.6 ± 0.8[d]	2.6 ± 0.7[d]	67.9 ± 0.1[a]

All data are means ± standard deviation. Values with different letters in the same column are significantly different at *p* < 0.05.

product, like, for example, the staling of bread. Hence, how structure and nutrition change during staling is important. Due to the fact that starch-based foods are normally eaten in two days by consumers, we set a one-day retrogradation experiment. The degree of digestibility of retrograded starch is mainly related to differential arrangement of starch chains. Interestingly, our data showed that, for AP, the RS content increased within the first 3 min of treatment, followed by a reduction from the fourth min and onwards. These data indicate that the low levels of AP molecular degradation can accelerate the reorganization of AP molecules and the formation of aggregated double-helical structures, possibly by decreasing the degree of branching, increasing the amounts and improving the rearrangement of linear segments (Zeng et al., 2016; Yang et al., 2017), in agreement with the previous findings (Zeng et al., 2016). Our data suggest that the further degradation of AP did not result in additional reassociation of chains, possibly due to the weak association capacity of the shorter linear segments in these samples.

For granular AM, although new V_h-type crystals were formed during the disruption of B-type crystals during the first 2 min of treatment, it seems that damage of the crystalline regions still resulted in a loss of structural stability and disordering of the granules, thereby increasing its amylolytic susceptibility, as demonstrated by increased RDS and decreased SDS and RS (Table 2). With further microwave treatment, the RDS content dropped and the RS content rapidly increased, i.e., the amylolytic resistance increased. Such increase in RS was possibly attributed to the aggregation of highly mobile, small, hydrated AM molecules. Although most crystals were disrupted, the remaining residues formed by such small AM molecules were highly resistant, possibly due to their smaller surface area. Hence, our data suggest that the digestibility of AM granules is not directly associated with the crystallinity or the amounts of V_h-type crystals but more likely with the molecular weight and re-association capacity of starch molecules. Microwave-treated AM granules subsequently subjected to hydrothermal treatment and retrogradation had limited effects on the contents in RDS, SDS, and RS for the mildly (4 min) microwave-treated granules, showing that the degradation level observed during this period had an insignificant effect on reorganization and degradability of AM (Table 3). However, from the fourth minute, the RS content increased dramatically and RDS and SDS contents decreased, indicating that AM molecules rearranged more efficiently with decreased molecular size.

Hence, we found that in a microwave-heated native granular system, both AM and AP underwent an initial increase in amylolytic susceptibility (RDS) whereafter it decreased down to the level of native starch (AM) or slightly above this level (AP). Such increase in amylolytic susceptibility is related to the erosion of granular structure (Zhang, Chen,

Table 3

In vitro digestion parameters of retrograded AP and AM treated with microwave irradiation for different time periods (n = 2).

Treatment time (min)	AP			AM		
	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
0	56.8 ± 0.1[b]	30.5 ± 0.2[b]	12.8 ± 0.3[e]	19.4 ± 0.2[c]	35.8 ± 0.9[a]	44.8 ± 1.1[c]
1	62.0 ± 0.4[a]	20.7 ± 0.2[e]	17.3 ± 0.6[d]	17.2 ± 0.0[d]	36.4 ± 1.1[a]	46.3 ± 1.1[c]
2	55.8 ± 0.6[c]	23.7 ± 0.2[c]	20.5 ± 0.8[c]	19.6 ± 0.3[c]	33.3 ± 0.6[b]	47.1 ± 0.9[c]
3	53.1 ± 0.5[d]	21.8 ± 0.2[d]	25.1 ± 0.3[a]	22.5 ± 0.2[a]	33.5 ± 0.7[b]	44.0 ± 0.9[c]
4	52.8 ± 0.3[d]	24.2 ± 0.7[c]	23.0 ± .04[b]	21.1 ± 0.3[b]	30.2 ± 0.2[c]	48.8 ± 0.6[c]
6	57.7 ± 0.2[b]	20.7 ± 0.2[e]	21.6 ± 0.4[c]	13.5 ± 0.2e	22.5 ± 0.4[e]	64.0 ± 0.6[b]
8	43.5 ± 0.3[e]	44.1 ± 0.4[a]	12.3 ± 0.0[e]	6.3 ± 0.1[f]	26.5 ± 0.2[d]	67.0 ± 0.3[a]

All data are means ± standard deviation. Values with different letters in the same column are significantly different at $p < 0.05$.

Liu, & Wang, 2010). However, further microwave treatment increased the enzymatic resistance of both AM and AP. Such an effect is supposedly due to further destruction of the crystalline structures providing increased flexibility for mobility and re-association of amylose and amylopectin molecules in the granules (Zhong et al., 2019). As expected, AM granules showed a higher amylolytic resistance (high RS) than AP (high SDS) at this stage, due to the stronger re-association capacity of amylose molecules than amylopectin molecules (Zeng et al., 2016). The effect of microwave treatment on amylolytic resistance of retrograded AP and AM was different. Initially the enzymatic resistance of AP increased due to the degradation of amylopectin molecules (Table 3) which possibly resulted in the decrease of branching degree and the increase of linear segments (Yang et al., 2017; Zeng et al., 2016). However, our data show that a further treatment resulted in decreased RS content (Table 3), implying that the low capacity of smaller amylopectin segments to reassociate and form complexes with low amylolytic resistance. Instead, for AM, the RS content started to increase from the sixth minutes when the Mw of AM molecules was sufficiently small to allow for reorganization of the chains.

3.6. Rheological characteristics

The rheological behavior of starch gels depends on the nature of soluble and re-associated molecules, as starch granules are fully disrupted following previous heating. The rheology of AP was not altered by a mild (1–2 min) microwave treatment, i.e., all rheological parameters including storage modulus (G′), loss modulus (G″), loss tangent (G′/G″, tan δ), and the modulus of complex viscosity (η*) for AP0, AP1 and AP2 were similar (Fig. 6). With further treatment, G′, G″, and η* of AP started to decline, reflecting a general decrease of the strengths of the AP gels (Franck, 2004, pp. 1–17; Sun, Sun, Wang, Sánchez-Soto, & Schiraldi, 2018). Samples treated for 3–4 min exhibited higher tan δ than the original granules, indicating the formation of a plastic gel system (Franck, 2004; Sun et al., 2018). Although 5–6 min of microwave treatments showed low tan δ at low frequency, they had the highest increase in tan δ with increased frequency, hence showing the strongest deformability of the AP granules. Therefore, the molecular degradation of AP by microwave irradiation decreased the gel strength, but increased the plastic behavior and deformability at longer treatment times.

The G′ of the AM gels initially increased (Fig. 5), showing that microwave irradiation can also increase the strengths of AM gels, although G′ started to decrease with further treatment, possibly due to the weak capacity of highly degraded AM molecules on forming gel network. Treatment for 2 and 3 min resulted in higher G″ than the original and

mildly treated granules indicating that longer treatment generated viscoelastic systems with higher energy dissipation (viscosity or plasticity) under deformation. However, G″ decreased below the value of the original sample on extensive treatment (6- and 8-min treatments) demonstrating a very low viscosity. The value of tan δ was similar in all AM samples, except for the 8 min sample, suggesting that the microwave irradiation had a limited effect on the elastic and plastic behavior of the AM gels. Just as for the G′ and G″, the complex modulus η* initially increased (0–3 min) and then decreased with longer (3–8 min) microwave treatment. Hence, the strength of AM gels can be increased by a 1–2 min microwave treatment, while longer treatment times continuously decreased the strengths of AM gels.

3.7. The comparison of the effects of microwave treatment on the structure of AP and AM

Our data for pure granular AM and AP systems suggest that short-term (1 min) microwave irradiations at moderate (400 W g^{-1}) regimes mostly affect the crystalline parts for both systems (Fig. 6). For a granular AP system treated at relatively low (160 W g^{-1}) irradiation energy, it has been shown that the amorphous region is first affected, followed by changes in the crystalline parts (Yang et al., 2017). Our data suggest that for relatively high irradiation energy (400 W g^{-1}), the disruption of the amorphous regions could not be monitored and only the effects on the crystalline parts were recorded. Specifically, the microwave treatment resulted in the degradation of starch molecules, thereby inducing a rearrangement of the crystalline structure while the granule integrity was preserved (Fig. S1). However, due to their molecular and physical differences, AP and AM displayed different modifications in their crystalline structure.

Highly branched AP molecules, also characterized by having a large molecular size, can build an ordered coherent backbone structure thereby contributing to an ordered crystalline structure and a regular granular morphology. Therefore, when pure AP molecules are cleaved and disrupted by microwave irradiation, the crystallinity is decreased. However, when AP molecules are further degraded, the cleaved segments, with much lower molecular size, gain in mobility these can migrate under the high-frequency electric fields and reaggregate resulting in recovered crystallinity.

In contrast, small-size AM molecules are normally present in the normal starch granules. Although pure AM molecules also generated granules in barley, the granules were irregular and highly aggregated and with no lamellar peak (Goldstein et al., 2016), demonstrating a disordered internal structure of AM. Therefore, as AM molecules were degraded by microwave irradiation, the B-type crystalline allomorph was quickly disrupted, demonstrating the instability and strain of AM within these granules. An interesting effect is the formation of V$_h$-type crystals, which is likely attributed to the high flexibility of AM molecules, especially degraded AM segments, within the granular matrix. Hence, we suggest a new possibility of high gelatinization temperature typical for HAM starch. Due to the high AM content, new and more stable crystals are formed during heating, thereby increasing the thermal resistance of AM. However, upon further treatment, B-type crystals are completely destroyed and partial V$_h$ crystals are also disrupted, which is mainly associated with the further cleavage of AM molecules.

Using the same microwave power (400 W g^{-1}), high AM (50%) starch underwent similar degradation and aggregation of the AM fraction after a 1-min microwave treatment (Zhong et al., 2019). However, in the HAM starch system, the molecular degradation did not further proceed with further microwave treatment, although the crystalline disordering increased (Zhong et al., 2019). This indicates a protecting role of AP in the HAM system which is not present in the pure AM system. A similar effect was found in AP in this study and a previous study (Yang et al., 2017), showing a high degradation resistance of starch containing AP. Hence, we suggest, under the microwave treatment, the degradation in starch granules is increased with the increase of amylose
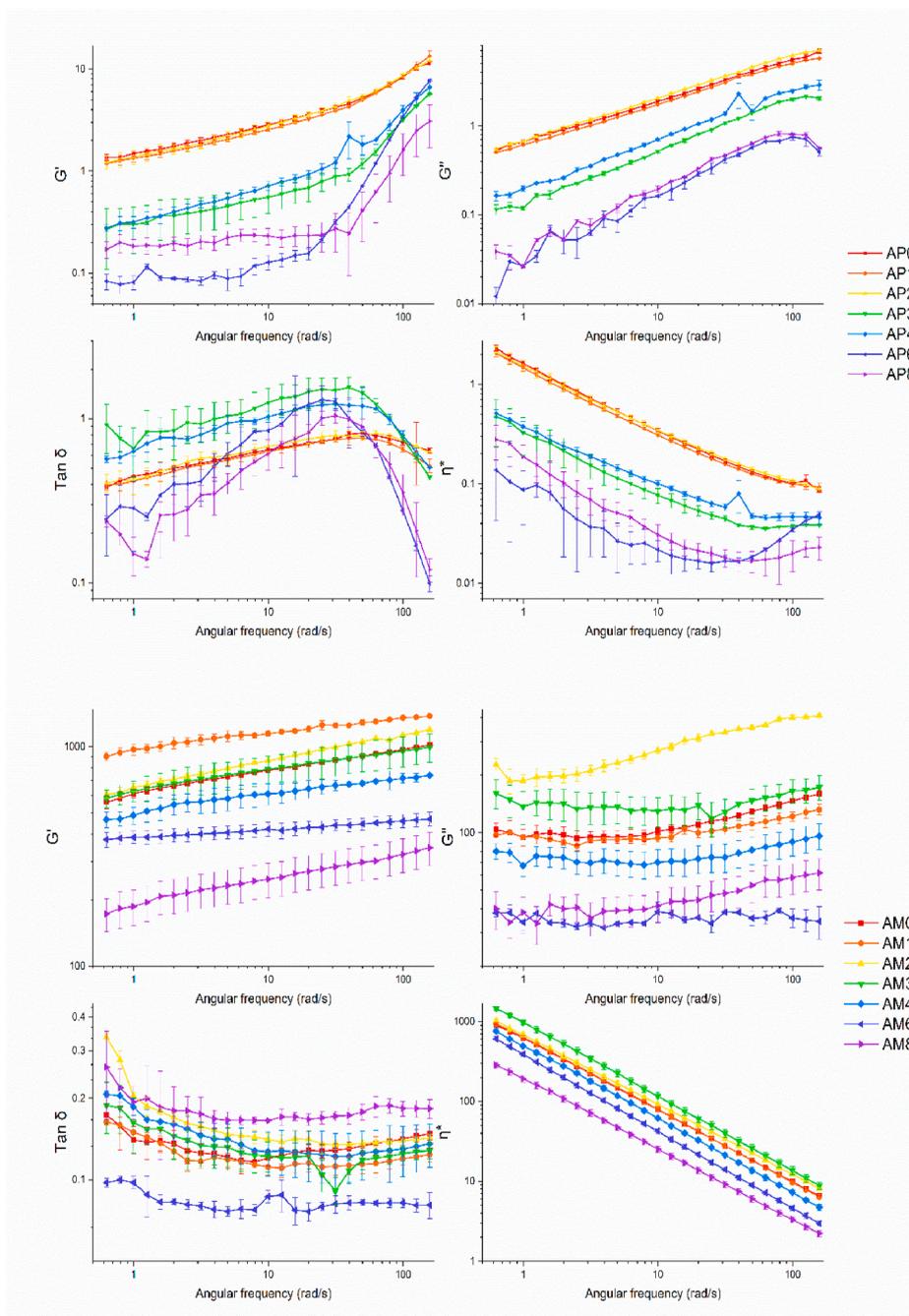
Fig. 5. Rheological characteristics of AP and AM treated for different time periods. Abbreviations as in Fig. 1.

content in such granules, due to the greater protection effect of amylopectin on granular stability. However, the increase of amylose content also results in the increase the mobility of linear small segments, thereby leading to the re-association of these segments and affecting the crystalline type. However, it worth mentioning that the amylolytic digestibility of starch granules under the microwave treatment is not linearly correlated with the crystallinity. Instead, we suggest that the Mw of degraded starch molecules plays a more important role on the amylolytic resistance of starches. However, the underlying mechanism is not fully explained in this study and need to be further explored in the future.

3.8. The effects of microwave treatment on the functionality of pure AP and AM systems

Our data demonstrate that a microwave treatment can increase the water solubility and decrease the swelling power of both AP and AM separately. The effect on the water solubility was the most dominant. For AP, treatment weakened the strength of AP gels (3–4 min treatment), but increased the plastic behavior and deformability (6–8 min treatment). In contrast, short-time microwave treatment of AM increased the gel strength, whereas its effect on the elastic and plastic behavior of the gel was limited. Initially, the amylolytic susceptibility was increased for both AP and AM and then it decreased as an effect of re-association of the glucan fragments produced during treatment. As expected, re-associated AM molecules were more resistant to amylase hydrolysis than re-associated AP molecules, as demonstrated by the increase in RS
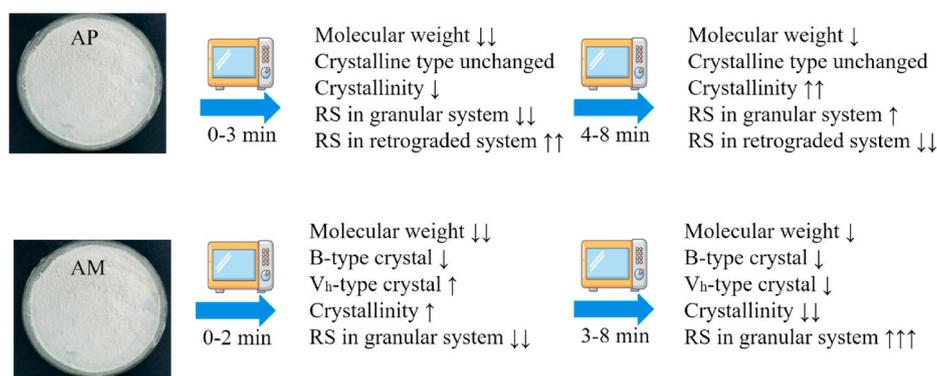
Fig. 6. How the structure and digestibility of AM and AP granular starches changed by microwave heating.

in the AM system and the increase of SDS in the AP system. The effect on amylolytic resistance was highly related to the treatment time. The enzymatic resistance of AP increased within the first 3 min to reach a maximum, while the enzymatic resistance of AM started increasing from the fourth minute whereafter the resistance kept increasing with treatment time.

4. Conclusion

The effects of relatively high-energy microwave treatment on the structure and functional properties of native granular pure AM and AP systems were compared. The treatments resulted in the degradation of AM and AP molecules in both samples. The crystallographic data confirmed the high level of AM degradation and aggregation and the formation of V_h-type crystalline allomorph in the AM granular system during the first 2 min of microwave treatment, as demonstrated by the increase in crystallinity. However, the RS content decreased during the period, implying that AM re-aggregation without involving AP (like for a typical starch granule) was not capable of stabilizing the granular structure. With further treatment, AM degraded the granular AM system but the crystallinity started decreasing, showing that the crystalline regions of AM were mainly damaged by the microwave irradiation. In contrast, the crystallinity in the AP granular system first decreased followed by an increase upon further irradiation, reflecting that in contrast to AM, AP underwent a different structural change. Hence, our study demonstrated specific and dynamic structural changes of AM and AP granular systems under microwave treatment and their different changes in molecular and physical characteristics. Different experimental conditions including microwave power, moisture content and sample weight, will affect the outcome of the product structures. However, we suggest that the structural transformation process of AM and AP granular starches under microwave irradiation follows the same process as shown in this study. Hence, the information is helpful for industries to further improve the functionality, like enhancing resistant starch content and improving water solubility and gel strength of AM and AP by controlling the extent of microwave treatment.

CRediT authorship contribution statement

Yuyue Zhong: Conceptualization, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. **Yu Tian:** Writing – original draft, Investigation. **Xingxun Liu:** Writing – original draft, Investigation. **Li Ding:** Conceptualization, Investigation. **Jacob Judas Kain Kirkensgaard:** Formal analysis. **Kim Hebelstrup:** Resources. **Jean-Luc Putaux:** Writing – original draft, Investigation. **Andreas Blennow:** Resources, Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2021.106856.

References

Bertoft, E. (2017). Understanding starch structure: Recent progress. *Agronomy, 7*(3), 56.
Blennow, A. (2018). Chapter 4 - starch bioengineering. In M. Sjöö, & L. Nilsson (Eds.), *Starch in food* (2nd ed., pp. 179–222). Woodhead Publishing.
Blennow, A., Bay-Smidt, A. M., Wischmann, B., Olsen, C. E., & Møller, B. L. (1998). The degree of starch phosphorylation is related to the chain length distribution of the neutral and the phosphorylated chains of amylopectin. *Carbohydrate Research, 307*(1), 45–54.
Blennow, A., Jensen, S. L., Shaik, S. S., Skryhan, K., Carciofi, M., Holm, P. B., et al. (2013). Future cereal starch bioengineering: Cereal ancestors encounter gene technology and designer enzymes. *Cereal Chemistry, 90*(4), 274–287.
Braşoveanu, M., & Nemţanu, M. R. (2014). Behaviour of starch exposed to microwave radiation treatment. *Starch - Stärke, 66*(1–2), 3–14.
Carciofi, M., Blennow, A., Jensen, S. L., Shaik, S. S., Henriksen, A., Buléon, A., et al. (2012). Concerted suppression of all starch branching enzyme genes in barley produces amylose-only starch granules. *BMC Plant Biology, 12*(1), 223, 223.
Chandrasekaran, S., Ramanathan, S., & Basak, T. (2013). Microwave food processing—a review. *Food Research International, 52*(1), 243–261.
Cheetham, N. W. H., & Tao, L. (1998). Variation in crystalline type with amylose content in maize starch granules: An X-ray powder diffraction study. *Carbohydrate Polymers, 36*(4), 277–284.
Doner, L. W., & Irwin, P. L. (1992). Assay of reducing end-groups in oligosaccharide homologues with 2, 2′-bicinchoninate. *Analytical Biochemistry, 202*(1), 50–53.
Emami, S., Perera, A., Meda, V., & Tyler, R. T. (2012). Effect of microwave treatment on starch digestibility and physico-chemical properties of three barley types. *Food and Bioprocess Technology, 5*(6), 2266–2274.
Fan, D., Ma, W., Wang, L., Huang, J., Zhang, F., Zhao, J., et al. (2013). Determining the effects of microwave heating on the ordered structures of rice starch by NMR. *Carbohydrate Polymers, 92*(2), 1395–1401.
Franck, A. (2004). *Understanding rheology of structured fluids*. Book of TA instruments.
Galema, S. A. (1997). Microwave chemistry. *Chemical Society Reviews, 26*(3), 233–238.
Goldstein, A., Annor, G., Putaux, J.-L., Hebelstrup, K. H., Blennow, A., & Bertoft, E. (2016). Impact of full range of amylose contents on the architecture of starch granules. *International Journal of Biological Macromolecules, 89*, 305–318.
Green, M. M., Blankenhorn, G., & Hart, H. (1975). Which starch fraction is water-soluble, amylose or amylopectin? *Journal of Chemical Education, 52*(11), 729.
Guan, S., Wang, P., Liu, H., Liu, G., Ma, Y., & Zhao, L. (2011). Production of high-amylose maize lines using RNA interference in sbe2a. *African Journal of Biotechnology, 10*(68), 15229–15237.
Hebelstrup, K. H., Sagnelli, D., & Blennow, A. (2015). The future of starch bioengineering: GM microorganisms or GM plants? *Frontiers of Plant Science, 6*, 247.

Jackson, D., Waniska, R., & Rooney, L. W. (1989). Differential water solubility of corn and sorghum starches as characterized by high-performance size-exclusion chromatography. *Cereal Chemistry, 66*(3), 228–232.

Keeratiburana, T., Hansen, A. R., Soontaranon, S., Blennow, A., & Tongta, S. (2020). Porous high amylose rice starch modified by amyloglucosidase and maltogenic α-amylase. *Carbohydrate Polymers, 230,* 115611.

Lewandowicz, G., Fornal, J., & Walkowski, A. (1997). Effect of microwave radiation on physico-chemical properties and structure of potato and tapioca starches. *Carbohydrate Polymers, 34*(4), 213–220.

Lewandowicz, G., Jankowski, T., & Fornal, J. (2000). Effect of microwave radiation on physico-chemical properties and structure of cereal starches. *Carbohydrate Polymers, 42*(2), 193–199.

Lii, C. Y., & Chang, Y. H. (1991). Study of starch in Taiwan. *Food Reviews International, 7*(2), 185–203.

Luo, Z., He, X., Fu, X., Luo, F., & Gao, Q. (2006). Effect of microwave radiation on the physicochemical properties of normal maize, waxy maize and amylomaize V starches. *Starch Staerke, 58*(9), 468–474.

Maniglia, B. C., Castanha, N., Le-Bail, P., Le-Bail, A., & Augusto, P. E. (2020). Starch modification through environmentally friendly alternatives: A review. *Critical Reviews in Food Science and Nutrition*, 1–24. https://doi.org/10.1080/10408398.2020.1778633

Mutlu, S., Kahraman, K., & Öztürk, S. (2017). Optimization of resistant starch formation from high amylose corn starch by microwave irradiation treatments and characterization of starch preparations. *International Journal of Biological Macromolecules, 95,* 635–642.

Nishiyama, Y., Mazeau, K., Morin, M., Cardoso, M. B., Chanzy, H., & Putaux, J. L. (2010). Molecular and crystal structure of 7-fold V-amylose complexed with 2-propanol. *Macromolecules, 43*(20), 8628–8636.

Oyeyinka, S. A., Umaru, E., Olatunde, S. J., & Joseph, J. K. (2019). Effect of short microwave heating time on physicochemical and functional properties of Bambara groundnut starch. *Food Bioscience, 28,* 36–41.

Shaik, S. S., Carciofi, M., Martens, H. J., Hebelstrup, K. H., & Blennow, A. (2014). Starch bioengineering affects cereal grain germination and seedling establishment. *Journal of Experimental Botany, 65/9,* 2257–2270.

Shaik, S. S., Obata, T., Hebelstrup, K. H., Fernie, A. R., Mateiu, R. V., & Blennow, A. (2016). Starch granule re-structuring by starch branching enzyme and glucan water dikinase modulation affects caryopsis physiology and metabolism. *PloS One, 11*(2), Article e0149613.

Sun, M., Sun, H., Wang, Y., Sánchez-Soto, M., & Schiraldi, D. A. (2018). The relation between the rheological properties of gels and the mechanical properties of their corresponding aerogels. *Gels, 4*(2), 33.

Tester, R. F., & Morrison, W. R. (1990). Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. *Cereal Chemistry, 67*(6), 551–557.

Vamadevan, V., & Bertoft, E. (2018). Impact of different structural types of amylopectin on retrogradation. *Food Hydrocolloids, 80,* 88–96.

Vanier, N. L., El Halal, S. L. M., Dias, A. R. G., & da Rosa Zavareze, E. (2017). Molecular structure, functionality and applications of oxidized starches: A review. *Food Chemistry, 221,* 1546–1559.

Wang, S., & Copeland, L. (2012). Phase transitions of pea starch over a wide range of water content. *Journal of Agricultural and Food Chemistry, 60*(25), 6439–6446.

Yang, Q., Qi, L., Luo, Z., Kong, X., Xiao, Z., Wang, P., et al. (2017). Effect of microwave irradiation on internal molecular structure and physical properties of waxy maize starch. *Food Hydrocolloids, 69,* 473–482.

Zeng, S., Chen, B., Zeng, H., Guo, Z., Lu, X., Zhang, Y., et al. (2016). Effect of microwave irradiation on the physicochemical and digestive properties of lotus seed starch. *Journal of Agricultural and Food Chemistry, 64*(12), 2442–2449.

Zhang, J., Chen, F., Liu, F., & Wang, Z. W. (2010). Study on structural changes of microwave heat-moisture treated resistant Canna edulis Ker starch during digestion in vitro. *Food Hydrocolloids, 24*(1), 27–34.

Zhong, Y., Keeratiburana, T., Kirkensgaard, J. J. K., Khakimov, B., Blennow, A., & Hansen, A. R. (2021). Generation of short-chained granular corn starch by maltogenic α-amylase and transglucosidase treatment. *Carbohydrate Polymers, 251,* 117056.

Zhong, Y., Liang, W., Pu, H., Blennow, A., Liu, X., & Guo, D. (2019). Short-time microwave treatment affects the multi-scale structure and digestive properties of high-amylose maize starch. *International Journal of Biological Macromolecules, 137,* 870–877.

Zhong, Y., Liu, L., Qu, J., Blennow, A., Hansen, A. R., Wu, Y., et al. (2020a). Amylose content and specific fine structures affect lamellar structure and digestibility of maize starches. *Food Hydrocolloids, 108,* 105994.

Zhong, Y., Liu, L., Qu, J., Li, S., Blennow, A., Seytahmetovna, S. A., et al. (2020b). The relationship between the expression pattern of starch biosynthesis enzymes and molecular structure of high amylose maize starch. *Carbohydrate Polymers, 247,* 116681.