Effects of sonication with acetic acid on functional properties of maize starch

By

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Declaration

I declare that the dissertation herewith submitted for the degree MSc Food Science at the University of Pretoria has not previously been submitted for a degree at any other university or institution of higher education.

Mojalefa Jonas Matsoso
April 2020

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ABSTRACT

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Supervisor: Prof M.N Emmambux

Degree: MSc Food Science

Starch is chemically or physically modified to improve functional properties for wider applications. The objective of this work is to chemically and physically modify starch with a combination of sonication and acetic acid. Native starch was sonicated at 382 W/cm^2 in distilled water, 5.16 M and 10.16 M acetic acid for 0, 3, 5 and 10 days, sonicated for 1 h each day. The experiment was a 2 (sonication level) x 3 (acetic acid concentration) x 4 (time interval) factorial. The treated and untreated starch were analysed for pasting properties, gel strength, water solubility index, water absorption index, Fourier transform infrared (FTIR), thermal properties with differential scanning calorimetry (DSC), X-ray diffraction, thermal properties, microstructure using light microscopy and scanning electron microscopy analyses.

Starch sonicated in water had the highest peak, breakdown and setback viscosity as compared to starch that was sonicated in both concentrations of acetic acid and non-sonicated starches in acetic acid and water. Pasting temperature of all treated starches were lower than pasting temperatures of native and non-sonicated starch in water. Water solubility index and water absorption index at 90 ºC for starch sonicated in 5.16 M and 10.16 M increased from day 0 to day 3, and decreased from 5 days to 10 days. Scanning electron microscopy revealed that starch granules sonicated in acetic acid were partially eroded and had pits on the surface, suggesting that these pits increase surface area for increase water absorption and peak viscosity. Starch sonicated in 10.16 M had the formation of new absorption peak when analysed with FTIR that was associated with the acetyl group. Due the possible incorporation of acetyl group, firmness of the starch gels decreased with increasing number of days for both sonicated and non-sonicated starches and this incorporation did not affect the stickiness of the gel.

There were no notable changes in A type molecular arrangement that was displayed by all treated starches. By comparing 0, 3, 5 and 10 days, starch sonicated in both acetic acid concentrations had relatively high relative crystallinity and non-sonicated in the same acetic acid concentration and native starch. Gelatinisation endotherm of sonicated starch both acetic acid concentrations decreased for 3 days and increased from 5 to 10 days while the enthalpy of non-sonicated starches decreased with increasing days of treatment. There were no obvious changes in shape and size of sonicated and non-sonicated starch in both 5.16 M, 10.16 M and water for 3, 5 and 10 days as determined by SEM. The maltese crosses reduced gradually from 3 to 10 days however, profound change was more observed in starch sonicated in both acetic acid concentrations (5.16 M and 10.16 M) than in non-sonicated starches with and without acetic acid. The combination of sonication with acetic acid had the potential to chemically and physically modify starch and be use as “clean label”.

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1. Introduction

Starch is relatively inexpensive and widely available, as such, it is biomaterial used in the food and non-industries. Starch has at least two important features that makes it a promising biomaterial for industrial uses. Firstly, it is susceptible to both chemical and enzymatic modification that allows the innovation and development of starch with new features and functionalities. Secondly, starch is biodegradable environment friendly (Shogren, Doane, Garlotta, Lawton & Willett, 2003; Goheen & Wool, 1991). However, these two features limits wider application of starch in food industries and other non-food application.

Starch can also be modified by using physical methods as well. Physical methods are those that does not use chemicals (except water) to hydrolyse starch. These methods includes sonication (Bel Haaj, Magnin, Pétrier & Boufi, 2013), microfludier/extructer (Song, Thio & Deng, 2011), high pressure homogenisation (Shi, Li, Wang, Li & Adhikari, 2011), high-energy ball milling (Lin, Qin, Hong & Li, 2016) and media milling (Chen, Shen & Yeh, 2010). All these mentions methods require high energy to modify starch, as such, starch would gelatinize during the process. This makes physical methods very hard to control to maintain original features of starch as compared to chemical methods.

Acid hydrolyses is one of the most used methods to modify starch chemically. Strong acids such as sulphuric acid and hydrochloric acid are used for hydrolysis to produce chemically modified starch. Chemical methods are not environmentally friendly and do not follow the principle of "green chemistry". Acetic acid is less harsh to the environment since it can be removed from the solution via distillation to be reused. As such, the objective of this study was to investigate and evaluate the effect of sonication as physical method, at low power, in combination with acetic acid, with the aim of modifying starch physically and chemically.

2. Literature review

In this chapter, literature review on sonication as a type of physical method used modify starch. Furthermore, acid hydrolyses is also reviewed as chemical method used to modify starch to widen starch application.

Starch is a primary source of energy for humans and other animals. It is found in most parts of a plant, from roots (including tuber) to leaves. In a native form, starch exists in a form of granules that differ in size and shape depending on botanical origin (Zhang, Guo, Feng, Wang, Wang & He, 2016; Geera, Nelson, Souza & Huber, 2006). Starch granules consist mostly of two polysaccharides, amylose and amylopectin. Native starch has limited uses in food and other industries. However, it has at least two important features that make it a promising polymer for future industrial uses. Firstly, it is susceptible to both chemical and enzymatic modification that allows the innovation and development of starch with new features and functionalities. Secondly, starch is biodegradable in soil and water and further degrades into sugars and organic acid, making it environmentally friendly. Native starch has been modified to be applicable for industrial use.

There are four ways in which starch has been modified; chemical modification, physical modification, enzymatic modification and genetic modification (Table 2.1). This review only focuses on chemical and physical modifications.

Table 2. 1: The combination of chemical and physical modifications of starch.

Major findings	**Type of modification**	**References**
Starch was acetylated with decreased in relative crystallinity. Decrease in swelling power, peak viscosity and gelatinisation enthalpy.	Combination of chemical and physical modification; Microwave with glacial acetic acid.	(Zhao, Li, Xu, Jing, Gou, Yu, Zheng & Li, 2018)
Starch granules formed a discontinuous compact fibrous structure having many surface cracks. A-type was transformed to A + V type pattern with decrease in crystallinity. Increased in resistance starch content.	Enzymatic modification; Combination of β–amylase and transglucosidase.	(Shah, Masoodi, Gani & Ashwar, 2018)
Stearic acid increased paste viscosity and resulted in formation of type II amylose-lipid complexes while irradiation reduced pasted viscosity and formed type I amylose-lipid complexes.	Combination of chemical and physical modification; Gamma irradiation with stearic acid.	(Ocloo, Minnaar & Emmambux, 2016)
Ultrasound reduced time in liquefaction process and increased dextrose equivalent during saccharification process. Decrease in relative crystallinity and starch granules was notched and had grooves on the surface.	Combination of enzymatic and physical modification; Ultrasound treatment followed by enzymatic treatment.	(Li, Li & Zhu, 2018)

Increase in carboxyl and carbonyl contents. Swelling power decreased in oxidised sago and tapioca starches but increased in oxidised maize starch.	Chemical modification; Oxidation of ozone gas.	(Chan, Bhat & Karim, 2009)
Starch was hydrolysed into nano-sized particles. Starch granules were eroded and broken.	Physical modification; High power ultrasonication	(Bel Haaj *et al.*, 2013)
The extent in hydrolysis was higher in amaranth starch than waxy starch. Relative crystallinity increased with increasing days.	Chemical modification; Sulphuric acid	(Sanchez De La Concha, Agama-Acevedo, Nuñez-Santiago, Bello-Perez, Garcia & Alvarez-Ramirez, 2018)

2.1 Overview of structure and properties of starch

Starch exits in the form of semi-crystalline granules that consists of two major chemical components (Fig 2.1); amylose and amylopectin. Both these polysaccharides consist of chains of D-glucose residues that are linked by α-(1, 4) linkages and are further interconnected by α-(1, 6) glycosidic bonds. Amylose is essentially linear with α-(1,4) linkages and few branches (Hizukuri, Takeda, Yasuda & Suzuki, 1981). While amylopectin is branched with short chains that are linked by α-(1, 4) linkages that are interconnected by α-(1, 6) linkages.

As mentioned above, starch granules occur in the form of semi-crystalline which is made up of the amorphous and crystalline regions. The growth rings in the starch granules are made up the altering amorphous and crystalline shells that have a thickness ranging between 100 and 400 nm (Pérez & Bertoft, 2010). The short chain of amylopectin form double-helices, which crystallise and contribute to the semi-crystalline nature of starch granules. Small-angle X-ray scattering (SAXS) has shown that the semi-crystalline growth rings have a periodicity of about 9-10 nm with alternating crystalline and amorphous lamellae. While the wide-angle X-ray diffraction had illustrated that the arrangement of the amylopectin double helical structure give rise to two distinctive polymorphs namely A- and B-type. It is believed that amylose is concentrated towards the periphery of the granule and mostly concentrated in the amorphous lamellae. Other authors suggested that amylose was predominantly located at the core area of the starch granules and formed the bulk amorphous region with discorded amylopectin chains (Wang & Copeland, 2012), however, the exact position of amylose in granules remains to be established (Pérez & Bertoft, 2010).

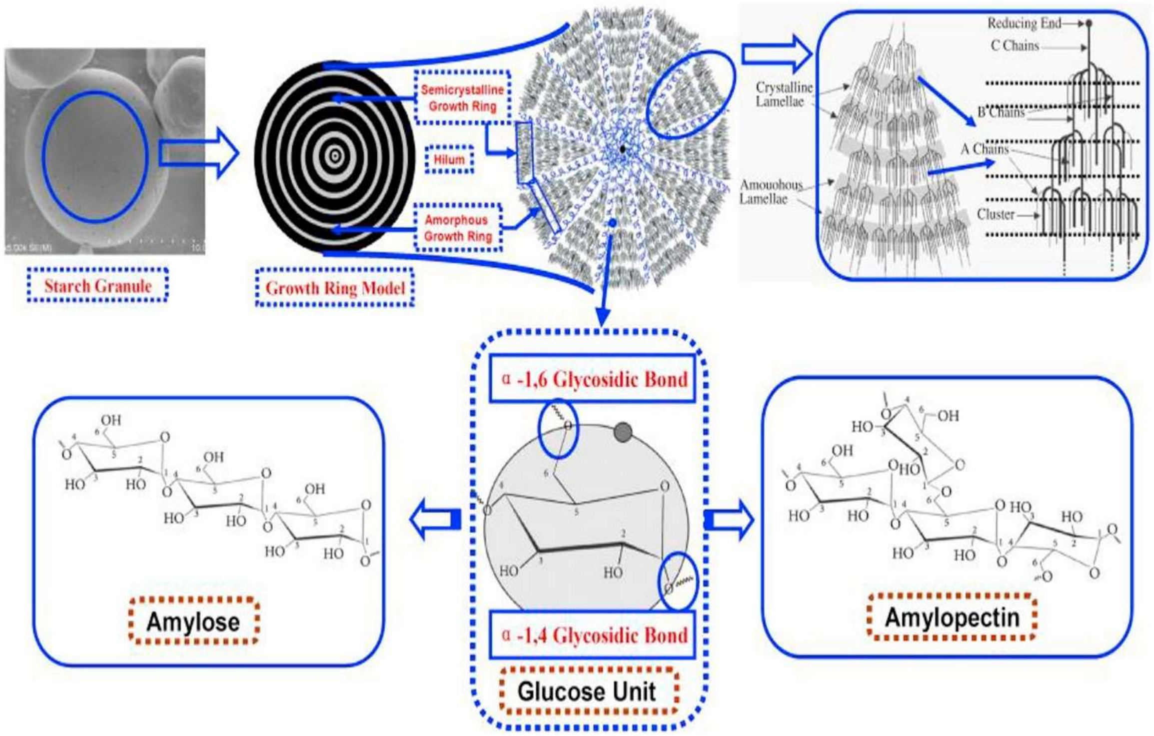


Figure 2. 1: Schematic diagram of structure starch (Liu, Gu, Hong, Cheng & Li, 2017).

2.2 Principles of sonication

Ultrasound is a sound wave with a frequency above the threshold of human ear hearing (>16 kHz). It may be divided into three frequency ranges; diagnostic ultrasound (1 – 10 MHz), high frequency ultrasound (100 kHz - 1 MHz) and power ultrasound (16 - 100 kHz) (Patist & Bates, 2008). The use of high frequency ultrasound and power frequency ultrasound to modify starch has increased in the past two decades.

Most applications of ultrasound in starch modifications are liquid-solid systems with water as solvent or a medium in the system. The ultrasound is generated by either piezoelectric or magnetostrictive transducers that initiate high-energy vibrations that are amplified and then transferred to probe, which is in direct contact with the liquid-solid system (Fig 2.1). Furthermore, the acoustic energy that results from ultrasound cannot be absorbed by molecules; it is therefore transformed into unstable form of phenomenon via cavitation. Sinusoidal ultrasound from the probe passes through the liquid-solid medium resulting in a continuous wave-type motion. Therefore, pressure resulting from the waves induces motion and mixing within the fluid medium (at low intensities, amplitude). As intensity increases, local pressure in the expansion phase of the cycle drops below the vapour pressure, initiates tiny bubbles from the existing gas already in the fluid as nuclei and keeps on expanding. This further increases negative transient pressures and bubbles expand further and produces new cavities by tensioning (Mason, 1998).

During the compression cycles, bubbles shrink, and some are dissolved back into liquid, while some remain the same due to their big surface area and continue to expand. Bubbles expand until their wall oscillation matches that of applied sound frequency causing bubbles to collapse and explode. The consequent collapse of the bubbles and compression and refraction process comprise a phenomenon "well-known" as cavitation, which induces shear forces and turbulence with great energy waves that gradually increase the temperature of the medium. Furthermore, cavitation induces micro-jets, where bubbles collapse in a jet of water shooting onto granules and modify the system chemically when free radicals such as hydroxide (-OH) and hydrogen (-H) radicals from the dissociation of water molecules (Czechowska-Biskup, Rokita, Lotfy, Ulanski & Rosiak, 2005; Riesz & Kondo, 1992).

Pioneer works of ultrasound have reported and shown that the effect of ultrasound on starch depends on temperature, power of the ultrasound, frequency, time of treatment, type of sonication, concentration of solid and type of starch (Table 2.1 and Table 2.2). These factors make it difficult to compare data between papers. Nevertheless, numerous research papers have shown that ultrasound has effect on structure of starch granules, physicochemical properties and molecular structure of starch and therefore yields modified starch (Table 2.1).

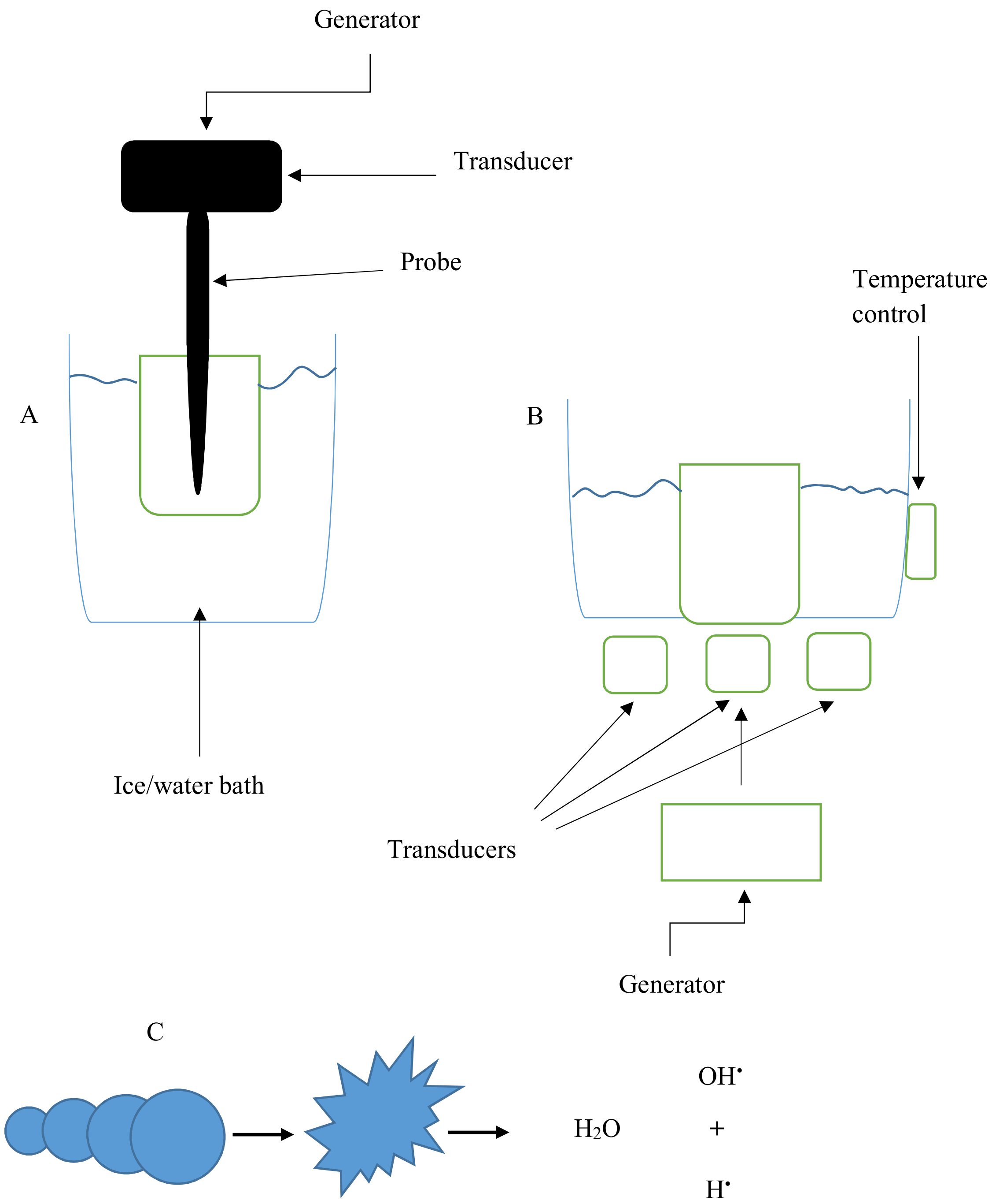


Figure 2. 2: Effect of ultrasound on physical structure of starch granules

2.3 Effects of sonication on physical structure starch

2.3.1 Morphology of starch granules

The effects of ultrasound on physical structure of starch granules have been examined by scanning electron microscopy, light microscopy, transmission electron microscopy and other techniques such as specific surface area (S_{BET}) (Table 2.2). In general, ultrasound leads to formation of cracks, pores and other damages to the granules regardless of botanical origin (Table 2.2). The types of sonication used play an important part on the effect of ultrasound on general morphology of starch granules. For instance, there was no significant change, apart from the decrease in size of the granules, when granules were treated in bath-type ultrasound but probe-type caused the formation of pores at the same experimental conditions (Jambrak, Herceg, Šubarić, Babić, Brnčić, Brnčić, Bosiljkov, Čvek, Tripalo & Gelo, 2010). Factors such as concentration of solids (starch), frequency, power, temperature and botanical origin also have particular effect on morphology of granules.

The collapse of bubbles and shear forces induced by ultrasound are responsible for rupturing and mechanical damage to starch granules (Jambrak *et al.*, 2010). Furthermore, temperature during sonication showed effect on physical damage of starch granules. Some reported papers had revealed that lower temperatures (<25 °C) cause more profound pitting to starch granules as shown in Table 2.2. On the contrary, others reported that sonication at low temperatures did not cause significant damage of starch granules (Zuo, Knoerzer, Mawson, Kentish & Ashokkumar, 2009; Linfeng & Ya-Jane, 2004). These differences are probably due to difference in experimental conditions such as power, frequency, botanical origin and concentration of solids. High power and prolonged time of exposure appeared to have significant effect of morphology of starch regardless of starch botanical origin (Sujka & Jamroz, 2013).

Sujka & Jamroz (2013) reported that some small starch granules from maize and rice were less damaged compared to large starch granules for example potato and wheat starch granules due to difference in surface area. This suggests that the extent of damage on starch granules can be affected by starch botanical origin since one of the differences are starch granule sizes (Torres, Troncoso, Vega & Wong, 2015; Wahlund, Leeman & Santacruz, 2011). Furthermore, this is consistent with the results reported by Sujka (2017), where rice, maize, wheat and potato starch granules were all observed to have been damaged consistently.

Table 2. 2: Effect of ultrasound on morphology of the granules examined by different techniques

Ultrasound conditions	Starch source	Technique (s)	Observations	References
5% (w/w) in distilled water 25 kHz, 20 kHz and duel 25 kHz + 20 kHz, 400 W, 40 min	Maize starch	SEM[a]	Starch granules that were treated with dual frequencies appeared to have depressions and starch granules reduced in size compared to those that were treated by single frequency	(Hu, Jiao, Zheng, Li, Fan, Chen & Zhang, 2015)
1% (w/w) slurry Milli Q water[c] 850 kHz at power 0.2, 2 and 3.7 W 0.05 MHz at power 2 W 0.85 MHz at power 2 W 2.5 to 120 min	Potato starch Waxy potato starch	SEM LM[b]	Ultrasound sound caused pitting and the maximum pits per granules on smaller granules were found to be higher compared to larger granules.	(Bai, Hébraud, Ashokkumar & Hemar, 2017)
30 % (w/v) in distilled water 40 kHz at power 420 W for 40 min, 50 C 40 kHz at power 480 W for 30 min, at 40 C	Maize starch	SEM PLM[d]	Increase in ultrasonic intensity resulted in increased starch damage. Starch granules had notched, grooved and visible erosion appearance on the surface. PLM revealed that ultrasound eliminated or reduced the polarised cross, indicating the damage to crystal structure.	(Li *et al.*, 2018)

40 kHz at power 540 W for 20 min, at 60 °C				
30 % (w/v) in distilled water or 99.5 % ethanol 20 kHz at power 170 W for 30 min at 20 °C	Potato starch Wheat starch Corn starch Rice starch	SEM TEM[e]	Some rice and maize starches had small cracks. All treated starches had small fissures and depressions, when sonicated in water than in ethanol. Large potato starch granules had numerous small depressions compared to small ones. TEM revealed that ultrasound led to formation of central cavities of irregular shapes, in respective of botanical origin	(Sujka & Jamroz, 2013)
10, 15, 20 % (w/w) in distilled water 24 kHz for 5, 10, 15 min, at amplitude 0, 50, 100 %	Maize starch	SEM LM	Lower sonication temperatures appeared to cause profound damages as compared to higher temperatures. LM revealed that lower temperatures caused higher amount of damage but there were no significant changes in granule size.	(Mohammad Amini, Razavi & Mortazavi, 2015)

5 % (w/w) in deionised water 211 kHz at power 2.5 and 4.1 W for 60 min at 25C to 70 °C	Waxy rice starch	SEM	Some granules only started to lose integrity and formed a polymeric matrix Further increasing temperature led to pasted starch granules and decrease in size	(Zuo *et al.*, 2009)

[a] Scanning electron microscopy

[b] Light microscopy (LM)

[c] Starch was first washed 6 times with Sodium dodecyl sulphate or ethanol or Milli Q water before being treated

[d] Polarised light microscopy

[e] Transmission electron microscopy

2.3.2 Crystallinity and polymorphism of starch granules

Native starch is semi-crystalline in nature and this plays an important role in functional properties of starch. Crystallinity and polymorphism of starch granules are evaluated by wide-angle X-ray diffraction analysis (WAXS) and small-angle X-ray diffraction analysis (SAXS). Sonication affects the crystallinity of starch to an extent, depending on experimental conditions, starch type and amount of amylose.

Sonication has little notable effects on crystallography pattern of native maize starch since the pattern of the peaks from treated starch are similar to the native starch, though the relative crystallinity slightly decrease with sonication time (Li *et al.*, 2018). The decrease in relative crystallinity suggests that ultrasound has depolymerised amylopectin, destroying the binding force between double helix in maize starch (Li *et al.*, 2018). The amount of amylose also appears to have important role in crystallinity of starch. For instance, ultrasound resulted in a complete loss of crystallinity in waxy (>99% amylopectin) starch compared with a significant decrease crystallinity without complete loss for standard starch (70% amylopectin) (Boufi, Bel Haaj, Magnin, Pignon, Impéror-Clerc & Mortha, 2018). These results are similar to the ones that were reported by Bel Haaj *et al.* (2013). The complete loss of crystallinity in waxy starch may be due to excessive decrease in the crystalline size, which arises from reduction of granule size (Bel Haaj *et al.*, 2013). Surprisingly, V-type diffraction peaks were not affected by the ultrasound in standard starch.

Ultrasound does not seem to change the type of crystalline polymorph for A-type maize starch crystalline structures and potato starch (B-type) (Zhu, Li, Chen & Li, 2012). As aforementioned, relative crystallinity may slightly decrease but the polymorph patterns are not affected by ultrasound. In a study conducted by Qiang *et al.* (2007), relative crystallinity increased after maize starch of 30 % (w/v) in distilled water was exposed to ultrasound 500 W output power for three minutes but a significant decreased in relative crystallinity was noted after a prolonged exposure of 15 minutes. The initial increase could be due to disruption of amorphous region and further decrease could be attributed to degradation of crystalline region in the granules (Qiang *et al.*, 2007).

2.3 Effect of ultrasound on physiochemical properties of starch

There are different ways used to evaluate physiochemical properties of starch after an ultrasound treatment. Table 2.3 shows some of them.

Table 2. 3: Effect of ultrasound on physiochemical properties of starch.

Starch property	**Starch type**	**Treatment**	**Major findings**	**Reference**
Pasting, thermal	Maize starch	30 % (w/v in water), 500 W[a], unknown frequency	Maximum viscosity decreased, breakdown reduced, and setback value increased. DSC analysis showed an initial increased in gelatinisation temperature and ΔH and later dropped with increasing degree of hydrolysis	(Qiang *et al.*, 2007)
Pasting, thermal, swelling, solubility and freeze-thaw stability	Normal maize, waxy maize, amylomaize V starches	70 % moisture content, 100 W, 30 min at 30 °C	Viscosity decreased; waxy maize < normal maize < amylomaize V. Swelling power and solubility increased in all starches; amylomaize V > normal maize > waxy maize. T_o increased; amylomaize V > normal maize > waxy maize, ΔH decreased (amylomaize V > waxy maize ≈ normal maize) by ultrasound.The degree of syneresis was in this order; waxy maize > normal maize > amylomaize V	(Zhigang *et al.*, 2008)
Pasting	Waxy rice starch	5 % (w/w) water[b], 2.5 W, 4,1 W, 211 kHz, 25 °C to	Peak and final viscosities of starch sonicated at temperatures near onset temperature of gelatinisation were lower that untreated samples	(Zuo *et al.*, 2009)

		70 °C, up to 60 min		
Swelling, solubility	Tapioca starch	3 % (w/w) water , 400 W, 24 kHz, 10, 15 and 30 min	Ultrasound led to an increase in swelling power and solubility, solubility increased significantly with sonication time but swelling power did not.	(Manchun, Nunthanid, Limmatvapirat & Sriamornsak, 2012)
Pasting	Maize starch	5 % (w/w) water, 20 kHz, 25 kHz, 25 kHz + 20 kHz, 400 W	Peak viscosity decreased up to 19.61 %, dual frequency decreased peak viscosity most.	(Hu *et al.*, 2015)
Pasting, thermal		30 % (w/v) water, 40 kHz, 420 W, 40 min, 50 °C 40 kHz, 480 W, 30 min, at 40 °C. 40 kHz, 540 W, 20 min, 60 °C	Pasting temperature was not affected by ultrasound while peak, trough, breakdown, final viscosities decreased significantly. DSC analysis revealed that gelatinisation temperature and ΔH decreased significantly.	(Li *et al.*, 2018)

[a] Output power
[b] Distilled water

2.3.1 Thermodynamic properties

Most studies use Differential Scanning Calorimetry (DSC) to study the effect of sonication on starch gelatinization (Li *et al.*, 2018; Yu, Zhang, Ge, Zhang, Sun, Jiao & Zheng, 2013). Enthalpy change (ΔH) and gelatinization temperatures such as onset (T_o), peak (T_p) and conclusion (T_c) have been commonly used. Principles of DSC on thermodynamic properties of starch are based on the assumption of a rapid scanning from room temperature to a certain above-boiling temperature accurately representing the entire phase transition process, where ΔH represents net sum of all endothermic processes that take place during heating process. Previous studies have shown that source of starch is the main determinant of thermal properties (Wang, Chao, Xiang, Zhang, Wang & Copeland, 2018).

Some studies have shown that ultrasound has effect on thermodynamic properties of starch as shown in Table 2.3 (Yu *et al.*, 2013; Zhigang *et al.*, 2008; Qiang *et al.*, 2007). On the contrary, Jambrak *et al.* (2010) reported that ultrasound treatment did not induce a significant effect on gelatinization temperatures and ΔH. Sonication resulted in an increase gelatinization temperatures of maize starch (Qiang *et al.*, 2007), decrease in gelatinization in rice starch (Yu *et al.*, 2013) and narrowed range for the gelatinisation endotherm for maize starches with varying amylose content (Table 2.3). Qiang *et al.* (2007) observed that sonication hydrolyzed maize starch and ΔH increased at low degree of hydrolysis and decreased at higher degree of hydrolysis An increase in ΔH values suggested that amorphous regions were degraded before the crystalline regions and a decrease in ΔH values suggested that some double helices in crystalline and non-crystalline regions may be disrupted during ultrasound treatment (Qiang *et al.*, 2007). A decrease in gelatinization temperatures could be attributed to the distorted crystalline region in starch granules prior to a reversible hydration of the amorphous phase which gradually led to destruction of granule structure (Jambrak *et al.*, 2010). These differences are probably due to experimental conditions and it should be noted that temperature was not strictly regulated, thus, starches were perhaps pre-heated during ultrasound treatment leading to lowering of gelatinization endotherm (Wang *et al.*, 2018).

2.3.2 Swelling and solubilisation

Starch molecules absorb water in the presence of excess water and pass through several stages before starch granules are disintegrated. During heating in excess of water, the semi-crystalline regions are disrupted; amylose and amylopectin expose hydroxyl groups form hydrogen bonding with water molecules. This process causes swelling and increases granule size and solubility (Singh, Singh, Kaur, Sodhi & Gill, 2003). The evaluation of swelling power and solubility is the evidence of the magnitude of interaction between starch chains in both amorphous and crystalline regions of the starch granules. The extent of the magnitude is influenced by the ratio of amylose to amylopectin, degree of branching, branch length, molecular weight, phosphorus content and distribution of amylose and amylopectin (Hoover, 2001).

Sonication generally increases swelling power and solubility of starch (Table 2.3). Swelling and solubility appear to be affected not only by experimental conditions of sonication but also by the source of starch amylose content. For instance when potato, maize, wheat and rice starch were exposed to ultrasound for 30 minutes at a frequency of 20 kHz and power of 170 W, treated potato starch had the highest value of swelling power and solubility (Sujka & Jamroz, 2013). This may be due to a high content of phosphate groups on amylopectin (Sujka & Jamroz, 2013). Zhigang *et al.* (2008) reported that the swelling power and solubility of ultrasound treated starches were affected by amylose content, in the following order amylomaize V > normal maize > waxy maize starch. This suggests that amylose is easily disrupted by ultrasound compared to amylopectin (Zhu, 2015). The increase in both swelling and solubility could be attributed to damage of the crystalline molecular structure, which leads to hydroxyl groups of amylose and amylopectin exposed to bind more water through hydrogen bonds (Sujka & Jamroz, 2013).

2.3.3 Rheological properties

2.3.3.1 Pasting

Sonication affects pasting properties of starch (Table 2.3). Rapid Visco-analyser (RVA) is commonly used to investigate pasting properties of starch, and some researchers use other techniques such as Brabender Visxograph/Amylograph/Viscoamylograph. All these techniques use the same principles to investigate pasting properties of starch. During pasting, viscosity development is noted when starch is heated and cooled at constant shear for a certain period. Many reports have shown that ultrasound treatment reduces viscosity during pasting, irrespective of the source of starch (Table 3). Previous studies have proposed many hypotheses for reduction of viscosity. Li *et al.* (2018) suggested that reduction in viscosity was probably due to breakage in starch chains. Majzoobi *et al.* (2015) suggested that granule remnants also play an important role. Ultrasound physically disrupts starch granules, facilitating the penetration of water for hydration and resulting in granules with less swelling capacity. In contrast, Chan *et al.* (2010) reported that ultrasound increased the peak viscosity of maize, mug bean and potato starches and little effect on sago starch. The increase in peak viscosity of maize starch could be attributed to the removal of protein layers in starch, helping to increase swelling capacity of starch (Chan, Bhat & Karim, 2010), while the increase in potato starch was probably due to phosphate group content.

2.3.3.2 Retrogradation

Retrogradation refers to the reorganisation and disorganisation of macromolecules of starch components (amylose and amylopectin) in the presence of excess of water. This happens when gelatinised starch begins to cool. Retrogradtion is an on-going process that initially involves the re-association of amylose and later the re-crystallisation of amylopectin (Shujun, Caili, Les, Qing & Shuo, 2015).

Zhigang *et al.* (2008) reported that ultrasound treatment led to a decrease (and increase) in syneresis, water exuded after freezing and thawing. Syneresis decreased in waxy maize starch and normal starch and increased in amylomaize after sonication. Amylose content could have influenced the above results and it is also possible that ultrasound disrupted starch chains in amorphous regions in both waxy maize and normal starch, this allowed an extensive reordering of the chain segments (Zhigang *et al.*, 2008).

The effect of ultrasound on retrogradation of starch appears to be dependent on the type of sonication used. For example, maize starch that was treated with probe-type ultrasound had higher adhesiveness, hardness and cohesiveness than the one that was treated with bath-type sonication (Herceg, Jambrak, Subaric, Brncic, Brncic, Badanjak, Tripalo, Jezek, Novotni & Herceg, 2010). This suggests that starch molecules become more disrupted by the probe-type than bath-type ultrasound.

2.4 Effect of ultrasound on the molecular structure of starch

Previous studies have shown that ultrasound may have effect on scission of starch molecules, and this appears to be affected mainly by experimental conditions (Zheng, Li, Hu, Yang, Lu, Zhang & Lin, 2013; Iida, Tuziuti, Yasui, Towata & Kozuka, 2008). Diverse techniques have been used to study the effect of ultrasound on starch molecules scission. Iodine-starch complexes (blue value) is one of the techniques commonly used to evaluate the lengths and branching pattern of amylose and amylopectin. On the other hand, chromatography and other reducing power methods are used to evaluate the effect of starch molecule scission in detail.

Sujka and Jamroz (2013) have observed that blue value, A_{680}/A_{545} ratio (A_{680} and A_{545} indicates the absorbance of the starch solution at the wave length of 680 nm and 545 nm, respectively) and λ_{max} of maize, potato, wheat and rice starches had decreased after being exposed to ultrasound for 30 minutes at frequency 20 kHz and power 170 W. This suggests that ultrasound caused degradation of starch molecules; while Zheng *et al.* (2013) observed that dual frequencies were more effective than single frequency of the ultrasound.

Chromatography has been used to study in details of starch chains scission caused by ultrasound. For instance, Iida *et al.* (2008) evaluated molecular weight of gelatinised waxy starch after being exposed to ultrasound and reported that molecular weight sharply decreased after being treated by ultrasound for 30 min, from 2.9×10^6 to 1.4×10^6 and slowly decreased thereafter (1.4×10^6 to 1.0×10^6 in 30 min). These results suggest that ultrasound at power 120 W (frequency unknown) caused depolymeration of starch chains as confirmed by other techniques such as static light scattering method and nuclear magnetic resonance spectroscopy (NMR spectroscopy) (Iida *et al.*, 2008). Depolymeration of starch chain by ultrasound could be a result of shear force, increasing temperature and high pressure that were created by cavitation (Zhu, 2015). In addition, Czechowska-Biskup *et al.* (2005) demonstrated that free radicals that are produced during ultrasound are responsible for starch chain scansion. This could be the reason why water as solvent reduced molecular weight of starch significantly than ethanol as a solvent (Sujka & Jamroz, 2013).

Some previous studies have reported that sonication had little or no effect on molecular weight of starch (Zuo *et al.*, 2009; Chung, Moon, Kim & Chun, 2002). These contradictions could be due to difference in experimental conditions. For instance, (Chung *et al.,* 2002) sonicated mung bean, potato and rice starches up to 5 min (frequency and power unknown). It is probable that the time of sonication was short to have effect on molecular weight, or power or perhaps frequency was not efficient compared to 20 kHz and 170 W that were used by Sujka and Jamroz (2013).

2.5 Principle of acid hydrolysis

Acid hydrolysis has been used as one of the chemical methods to modify starch to get certain functional properties. Hoover (2000) proposed the mechanism in which acid hydrolysis occurs in starch (Fig 2.3). The α-(1, 4) glycosidic bonds are firstly attacked by the hydroxonium ion (H_3O^+) (Fig 2.3A). This is followed by shift of one of the electrons in carbon-oxygen bonds onto the oxygen atom (Fig 2.3B) to generate unstable high-energy carbocation intermediate, Lewis acid (Fig 2.3C). Since this high-energy carbocation intermediate is Lewis acid, it will therefore react with water (Fig 2.3D), Lewis base, leading to regeneration of a hydroxyl group (Fig 2.3E).

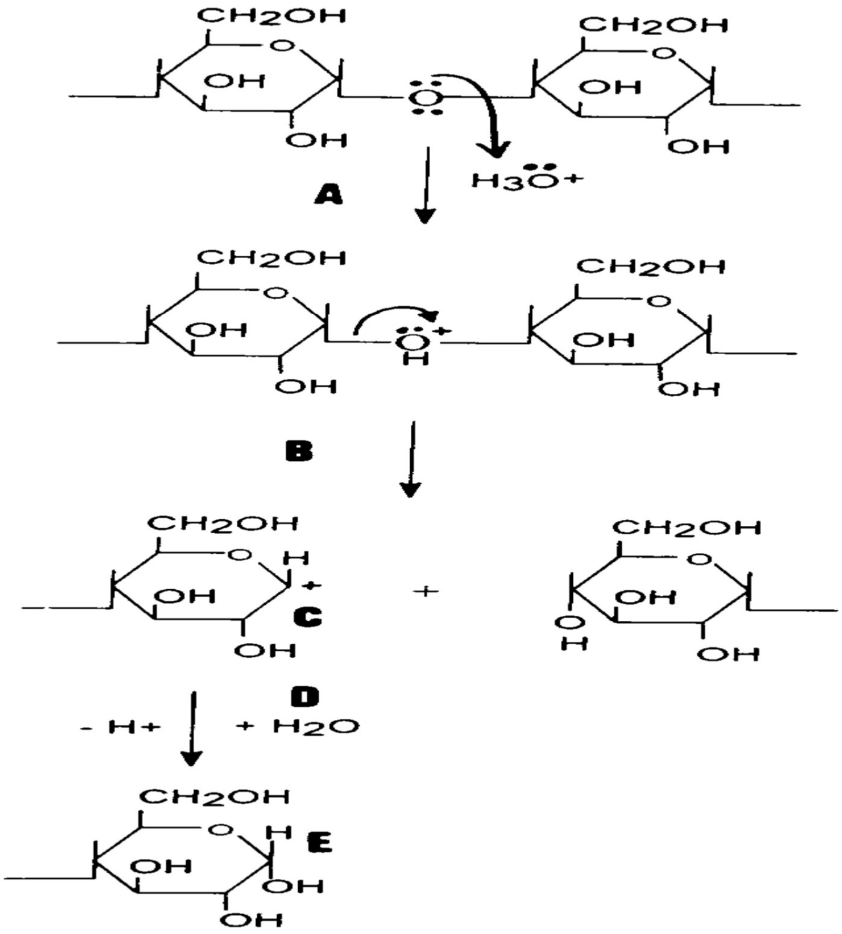


Figure 2. 3: Acid hydrolysis of starch (Hoover, 2000).

Pioneer studies have been using two different ways to monitor starch hydrolysis: content of soluble sugars in a solution and recovery of insoluble content of starch (Jiang, Hong, Gu, Cheng, Li & Li, 2018; Sanchez De La Concha *et al.*, 2018). The first stage is fast and involves the hydrolysis of the amorphous regions of the starch granules. The second stage, slow stage, involves the hydrolysis of the crystalline regions. It is believed that the amorphous region is more susceptible acid hydrolysis due to amylose that is loosely packed compared to the crystalline region. Therefore, the rate of the first hydrolysis is influenced by amylose content, granule size, pores on the surface and amount of amylose lipid complexes (Shujun *et al.*, 2015; Hoover, 2000). The latter is influenced by amount of amylopectin, distribution of α-(1,6) branches between the amorphous and crystalline lamellae, and the degree of packing of the double helices within the crystallites (Le Corre, Bras & Dufresne, 2010). Other authors reported an addition stage of acid hydrolysis, “three stage” hydrolysis (Wang, Blazek, Gilbert & Copeland, 2012; Li, Corke & Beta, 2007), which involved the hydrolysis of the amorphous (I), semicrystalline (II) and crystalline layers of structure of starch (III). Wang *et al.* (2012)

observed that acid hydrolyse both amorphous region and crystalline region at the later stage of hydrolysis.

2.6.1 Effects of acid on physical structure of starch granules

2.6.1.1 Morphology of starch granules

The time of acid hydrolysis appears to be the main factor that affects the morphology of starch (Wang & Copeland, 2012; Espinosa-Solis, Sanchez-Ambriz, Hamaker & Bello-Pérez, 2011)Like ultrasound treated starch, SEM and TEM and some confocal laser scanning microscopy (CLSM) commonly examine acid hydrolysed starch. Only in few papers, PLM and LM were to examine the morphology of starch granules after acid hydrolysis. Hydrochloriic acid (2 M) did not cause significant changes in waxy and high-amylose starches after two days of acid hydrolysis, however, prolonged exposure caused surface and endo-corrosion in both waxy starch but endo-corrosion was not observed in high-amylose starch (Chen, Xie, Zhao, Qiao & Liu, 2017). The starch molecules surrounding the hilum could be attributed to the endo-corrosion of waxy since it had been reported that central area is most vulnerable to acid hydrolysis due to being least packed (Wang *et al.*, 2012). CLSM showed that prolonged acid hydrolysis led to an increased number of reactive ends when the fluorescence was more visible (Chen *et al.*, 2017).

Sulphuric acid (H_2SO_4) appears to have more capability of damaging starch granules of starch as compared to HCl. Comparing the two, H_2SO_4 hydrolyse starch granules to nano-sized particles faster than HCl. For example, Angellier, Choisnard, Molina-Boisseau, Ozil and Dufresne (2004) reported that H_2SO_4 produced 15 %(w/w) of nano-sized particles from starch granules after five days, while HCl produced 0.5 % (w/w) of starch nano-sized particles. This suggests that strong acid damages starch granules effectively than weak acid. As such, weak organic acid such as acetic acid has been used to slightly hydrolyse starch to facilitate other modifications (Zhao *et al.*, 2018; Chi, Xu, Wu, Chen, Xue, Song, Zhang & Wang, 2008).

2.6.1.2 Crystallinity and polymorphism of starch granules

Acid hydrolysis affects the crystallinity of starch by increasing the relative crystallinity as shown by the XRD diffraction analysis (Fig. 4). Many previous studies have reported that acid hydrolysis increases relative crystallinity with increasing time, and this is usually observed in diffraction angle of 2θ 15-20°. Authors have proposed different hypotheses for this behavior. The first one states that cleaved bonds from amylose that are running through amorphous regions may allow the reordering of newly released end chain into the crystalline region and therefore making it more crystalline (Kainuma & French, 1971). The second one state that this reordering would increase the relative crystallinity by partially filling water channels in the crystallite cavities with double helices (Hsein-Chih & Sarko, 1978). Thirdly, free amylose that results from acid hydrolysis may be retrograde into double helices, which may also rearrange to be resistant to acid hydrolysis (Wang & Copeland, 2015).

Acid hydrolysis not only increases the relative crystallinity but may also affect the polymorphism of starch to a certain extent depending on the degree of hydrolysis and source of starch. In general, acid hydrolysis does not cause obvious changes to starch polymorphism (Jeong & Shin, 2018; Utrilla-Coello, Hernández-Jaimes, Carrillo-Navas, González, Rodríguez, Bello-Pérez, Vernon-Carter & Alvarez-Ramirez, 2014) On the contrary, some have reported transition of XRD patterns (Vermeylen, Goderis, Reynaers & Delcour, 2004). Wheat starch was initially A-type and became C-type after acid hydrolysis, while acid hydrolysis did not cause significant difference in XRD pattern of normal, waxy, cassava, broad bean and smooth pea starch (Vermeylen *et al.*, 2004). Transitions in the XRD patterns were also observed in pea, from B to A (Wang *et al.*, 2012). There are two hypotheses that were suggested for this XRD transition. The first one assumes the B-type polymorph was present in native starch in small amount that could not be detected by XRD and the second one is based on the A-type polymorph being susceptible to acid hydrolysis and some parts that are being removed from A-type crystalline and reorder into a more stable crystalline B-type (Shujun *et al.*, 2015).

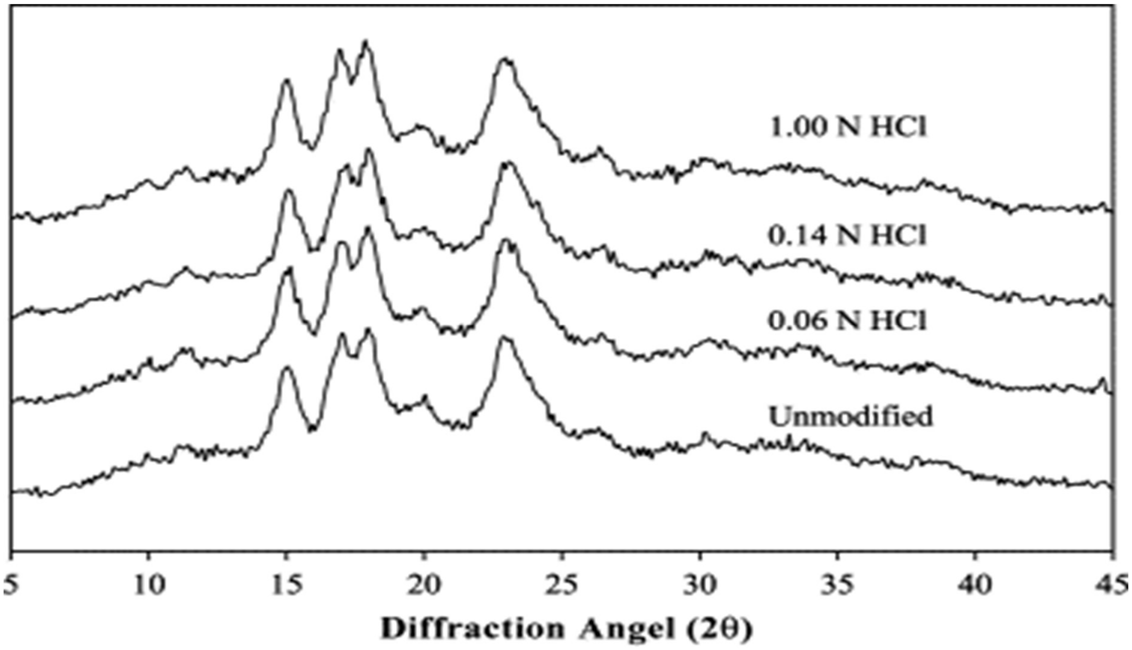


Figure 2. 4: XRD diffraction on the effect of acid hydrolysis on relative crystallinity of starch (Wang, Truong & Wang, 2003).

2.6.2 Effect of acetic acid on physiochemical properties of starch

2.6.2.1 Thermodynamic properties

Various studies showed that acid hydrolysis increased peak (T_p) and conclusion (T_c) temperatures of rice starch with increasing time of hydrolysis, however, onset (T_o) temperature decreased (Le Corre *et al.*, 2010), broadened thermogram peaked and decrease in gelatinisation temperatures and ΔH (Jeong & Shin, 2018; Wang & Copeland, 2015) had little influence on gelatinisation temperatures of deffated waxy and high-amylose starch (Wei, Hu, Zhang, Li, Xu, Jin & Tian, 2013). In contrast, other authors had noted the increase in ΔH with hydrolysis time in the following order; amylomaize > rice > oat > normal maize > waxy maize starch (Jayakody & Hoover, 2002). Increase in T_p may be attributed to the removal of the amorphous regions, due to the melting of the remaining crystalline regions without altering the content and order of the double helices of amylopectin (Utrilla-Coello *et al.*, 2014). The decrease in ΔH may be attributed to the disruption of crystalline regions by acid hydrolysis (Singh & Adedeji, 2017).

2.6.2.2 Swelling and solubilisation

Acid hydrolysis influences the swelling and solubilisation of starch. The effect of acid hydrolysis on swelling power appears to be inconsistence. For instance, swelling power of starch in general initially increases and later decreases with time of hydrolysis (Jayakody & Hoover, 2002; John, Raja, Rani, Moorthy & Eliasson, 2002), increased with hydrolysis time

(Xing, Liu, Li, Wang & Adhikari, 2017), decreased with hydrolysis time (Astuti, Widaningrum, Asiah, Setyowati & Fitriawati, 2018; Gao, Vasanthan, Hoover & Li, 2012).These inconsistencies may be attributed to the amylopectin content of starch. It is generally accepted that amylopectin plays an important role in water-binding of the starch (Tester & Morrison, 1990). As such, acid hydrolysed starch loses the ability to bind water once the amylopectin is disrupted, an intact network cannot form therefore starch cannot bind water. However, Gao *et al.* (2012) suggested that amorphous region, amylose, may have an effluence on swelling power since acid hydrolysis increases relative crystallinity while swelling power decreases. Initial increase of swelling power could be attributed to interactions of hydrolysed amylose that which remained in the granules, however, these did not interact with water in native starch because they were interacting with each other (Jayakody & Hoover, 2002).

Most previous studies have shown that swelling power acid hydrolysed starch decreased with increasing solubility of starch (Singh & Adedeji, 2017; Xing *et al.*, 2017; Wang & Copeland, 2012; Singh, Bawa, Riar & Saxena, 2009a; Singh, Sodhi & Singh, 2009b; Jayakody & Hoover, 2002; John *et al.*, 2002; Betancur & Chel, 1997). Figure 4 shows a typical example of solubilisation of starch from different sources. It appears that the acid hydrolysis can solubilise starch to a certain extent and it can be noted that solubilisation rates (hydrolysis rates) of starch due to acid hydrolysis were depended on the source of starch. This difference in hydrolysis rate may be attributed to the size of the granule (Vasanthan & Bhatty, 1996), formation of lipid-complexes (Morrison, Tester, Gidley & Karkalas, 1993), amylose content (Hoover & Manuel, 1996) and pores on the granule surface (Jayakody & Hoover, 2002).

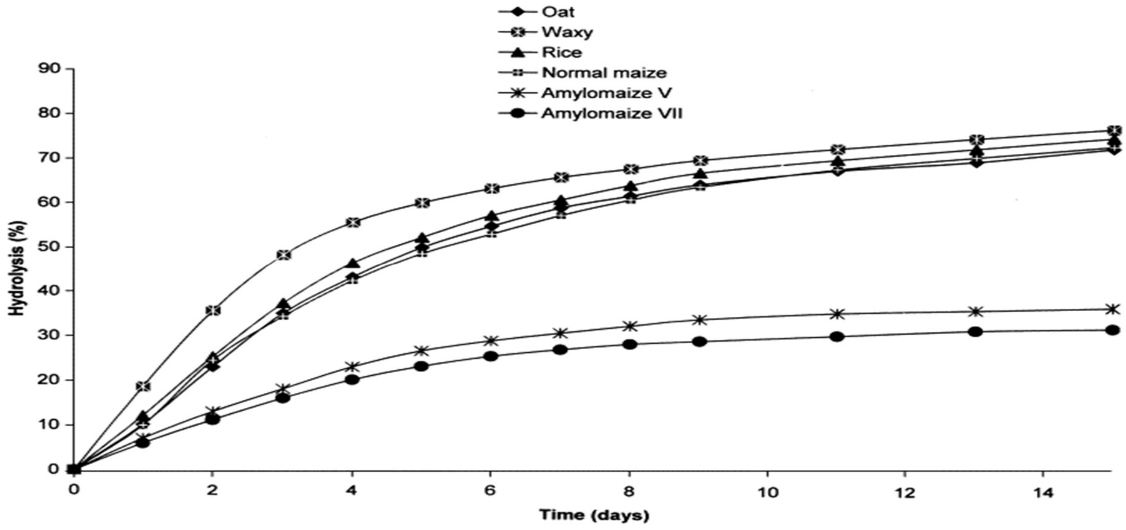


Figure 2. 5*:* Acid hydrolysis (2.2 N HC1, 35 ℃) profiles (solubilized carbohydrates as a function of time) of native cereal starches (Jayakody & Hoover, 2002).

2.6.3 Effect of acid hydrolysis on amylose content

Many studies have shown that acid hydrolysis deceases amylose content using either one of two methods; iodine colorimetric or concanavalin A (Con A) precipitation. There are some limitations about these and other techniques that are used to determine amylose content. Con A is based on the enzyme (Con A) that precipitates highly branched molecules (amylopectin). As such, amylose content may be overestimated due to incomplete precipitation of low branched amylopectin (Vilaplana, Hasjim & Gilbert, 2012). Similarly, iodine colorimetric method may overestimate due to the formation of complexes between long amylopectin side chains and iodine.

Acid hydrolysis increased the amylose content of Hylon VII and Hi-maize starches from 65. 10% to 99.15% and from 66.30% to 99.60%, respectively after one day, as relieved by Con A method (Li *et al.*, 2007). The effect of the type of acid on the amylose content and observed that HCl increased amylose content more as compared to HNO_3 and HSO_4 while H_3PO_4 decreased amylose content (Singh & Ali, 2000). A decrease in amylose content in maize starch (Utrilla-Coello *et al.*, 2014) and *Cynamchum auriculatum* starch (Wang, Wen, Zhang, Shen, Jiang & Liu, 2017) after three days and no significant decrease thereafter, as revealed by iodine colorimetric mothed. Acid type, amylose content and type of methods used to determine amylose content may be the reason why above authors reported different outcomes. Rapid hydrolysis of amylose in the first three days was more likely for occur in the amorphous region since amylose is readily available and free (Wang *et al.*, 2017). Whereas, amylopectin appears to be more resistance to acid hydrolysis since it is located in the crystalline region and cannot be easily hydrolysed by hydrogen ions (Utrilla-Coello *et al.*, 2014).

2.6.4 Rheological properties

2.6.4.1 Pasting properties

Pasting temperature, peak viscosity, final viscosity, setback viscosity and breakdown viscosity of starch are affected by acid hydrolysis. Acid hydrolysis reduces peak, final setback and breakdown viscosity of starch regardless of the starch source (Xing *et al.*, 2017; Wang *et al.*, 2003) and had little or no effect on pasting temperature on proso millet starch (Singh & Adedeji, 2017; Polesi & Sarmento, 2011), slightly increased in maize starch (Xing *et al.*, 2017; Wang *et al.*, 2003), decreased in sorghum (Singh *et al.*, 2009b), maize and Hylon starches (Amaya-Llano, Martínez-Bustos, Alegría & De Jesús Zazueta-Morales, 2011). The reduction of acid hydrolysed starch was due to disruption of the amorphous region of the starch granules (Khan, Ali & Hasnain, 2014) and reduction in setback viscosity may be attributed to formation of the short chain amylose by acid hydrolysis (Khan *et al.*, 2014). These could be the possible reason for decrease in pasting temperature and may be attributed to faster rate of water penetration into disrupted starch granules. Whereas an increase in pasting temperature may be attributed to the decrease in swelling power of acid-hydrolysed starch preventing the development of viscosity (Wang & Copeland, 2015).

2.6 Conclusion remarks

- Sonication can be used to modify starch physiochemical properties
- Effects of sonication on starch mainly depends on experimental conditions such as output power, type of sonication (bath vs probe), exposure time, botanical origin of starch and temperature. Sonication can also be used to produce starch nano-materials.
- Acids are used to chemically modify starch. The degree of hydrolysis by acids mainly depends on acid used, time of exposure and botanical origin of starch. H_2SO_4 and HCl have higher degree of hydrolysis but are not environmentally friendly and cannot be considered as “clean label”.
- Acetic acid is weak organic acid that is used to partially hydrolyse starch to facilitate in other processes as such esterification but has lower degree of hydrolysis as compared to H_2SO_4 and HCl. However, acetic acid can be declared as “clean label” since can be removed via distillation.
- By far, no known literature has reported the effect of sonication of maize starch when acetic acid is solvent instead of distilled water to modify starch physically and physiochemically.

3 HYPOTHESIS AND OBJECTVIES

3.1 Hypothesis

3.1.1 Maize starch sonicated in acetic acid will have increased peak viscosity when exposed for short period of time. Jambrak *et al.,* (2010) reported that sonication in distilled water resulted in pitting or erosion of maize starch granules when probe type of 24 kHz with different output power intensities of 34, 55, 73 W cm^{-2}. Li *et al.,* (2018) also reported severe physical damage with prolonged time of sonication, maize starch sonicated at 40 min at 420 W. Ultrasound waves will create microscopic pores or pitting as a cavitation that creates high pressure locally and shear force (Bai *et al.*, 2017; Hu, Lu, Zheng, Sun, Yang, Zhang, Zhang & Lin, 2013). These microscopic pores will facilitates water penetration into starch granules, resulting in increased water absorption and increased peak viscosity (Zuo *et al.*, 2009)**.**

Acetic acid will assist with the penetration of water by hydrolyzing glycosidic bonds α-(1,4) in the amorphous region. Previous studies reported that acetic acid decreases pH of the solution by dissociating into conjugate base and hydrogen ions that hydrolyse glycosidic bonds of amylose in the amorphous region (Majzoobi, Kaveh & Farahnaky, 2016; Majzoobi, Seifzadeh, Farahnaky & Mesbahi, 2015b).

3.1.2 Acetic acid will dissociate into conjugate ($C_2H_3O_2^-$) base and hydrogen ions and ultrasound will induce the formation of free radicals by decomposing water into hydroxide (-OH) and hydrogen (-H) radicals. These free radicals will make Carbon 6 in starch molecule to be active. Conjugate base from dissociated acetic acid will react with OH in Carbon 6 to incorporate acetyl group. Czechowska-Biskup *et al.* (2005) have observed formation of free radical from water molecules as a results of collapsing bubble from the cavitation. While Carbon 6 have been reported to be more active than Carbon 3 and Carbon 2 because Carbon 6 OH located on the exterior surface is readily available and reacts easily with dissociated conjugate base of acetic acid.

3.2 Objectives

3.2.1 To determine the effect of sonication alone in distilled water as solvent and in combination with acetic acid as solvent on physiochemical properties of maize starch at 3, 5 and 10 days.

3.2.2 To determine the effect of simultaneous expose of sonication and acetic acid on maize starch, with the aim of incorporation acetyl groups without the use of any chemical catalyst.

4 Experimental design

Figure 4.1 shows the experimental design of the research. Distilled water and acetic acid were used as solvents to make starch slurry. Both mixtures were sonicated for 3, 5 and 10 days from 60 min while kept in shaking water bath. Starch slurry were distilled water was used as a solvent was preserved with sodium azide.

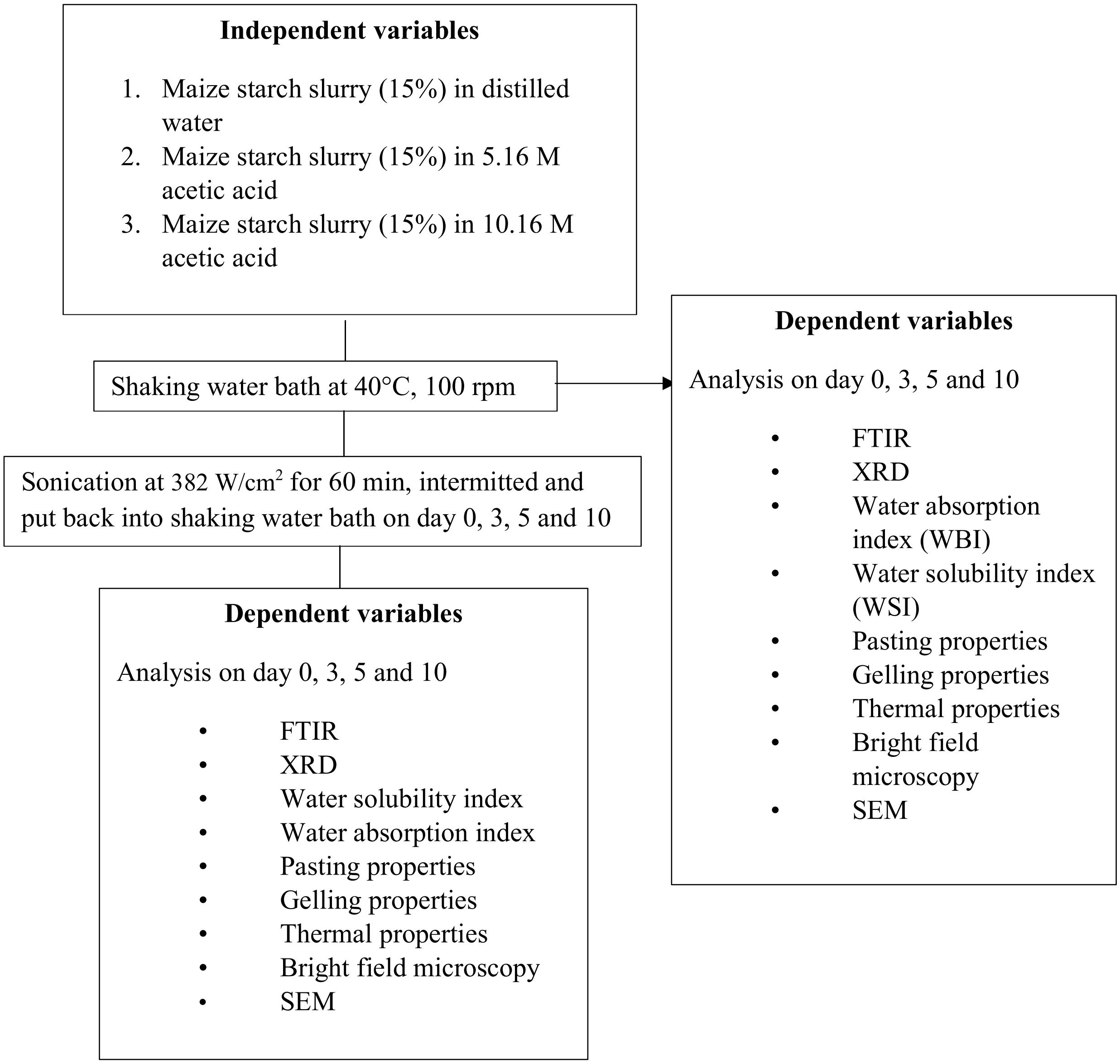


Figure 4. 1: Experimental design of research

4.1 Material

White maize starch was supplied by Tongaat Hulett (Edenvale, South Africa) and Glacial acetic acid (CAS 64-19-7) was obtained from (Merck, Pretoria, South Africa)

4.2 Methods

A 22.5 KHz Microson Ultrasound cell disruptor (Misonix Incorporated, New York, USA) coupled with a horn (tip diameter of 3 mm) was used to treat starch slurry. Maize starch (25.05g, db) was suspended in distilled water (preserved with 0.02 % sodium azide), 5.16 M and 10.16 M to make starch slurry of 15% (w/v). The temperature of the slurry was adjusted to 30°C prior to ultrasound treatment. The probe (3 mm) was immersed at the centre of the container with 2.5cm depth in the slurry and kept in ice-bath to prevent temperature from rising above 40 °C during treatment. Starch slurry was treated at intermitted times, that is, ultrasound equipment (output power 27 W) was allowed to sonicate starch slurry for 5 min and then a rest of 1 min. The total sonication time was 60 min. After each treatment, starch slurry was placed in shaking water bath at 40 °C and 100 rpm. Starch slurry in distilled water or in acetic acid (5.16 M and 10.16 M) were exposed to ultrasound treatment daily as explained above and samples were taken on 3, 5 and 10 days.

Treated starch slurry in distilled water and in acetic acid (5.16 M and 10.16 M) after 0, 3, 5 and 10 days with or without sonication were neutralized with sodium hydroxide and washed successively and freeze-dried.

4.2.1 Output power calculation

Output power record without sample was 3 W and output power when probe was immersed in starch slurry was 30 W. Therefore, about of power delivered to samples can be calculated as follows;

30 W – 3 W = 27 W was delivered to the sample.

Area of 3 mm probe: 3 mm/10 = 0.3 cm with a radius of 0.15cm.

Power density; Power/π r^2

$$27/0.0707 = 381.9 \text{ W/cm}^2 \text{ or } 382 \text{ W/cm}^2$$

4.3 Analysis

4.3.1 Pasting properties

Tretated and untreated starches were pasted with an Anton Paar Rheomter (Physica MCR 101 Ostildern, Germany). Starch (1.7 g, db) was suspended in distilled water and adjusted to make total suspension weight of 17 g. The suspension was initially heated 50 °C for 30 s while stirring at 940 rpm and the stirring was held at 160 rpm for the rest of the cycle. Thereafter, suspension was heated from 50 °C to 90 °C at a heating rate of 5.5 °C/min and then held at 90 C for 15 min. The suspension cooled down to 90 °C at a cooling rate of 5.5 °C/min. Pasting curve with parameters such as pasting temperature (PT), Peak viscosity (PV), breakdown viscosity (BV), setback viscosity (SV) and final viscosity were recorded.

4.3.2 Texture properties

Starch gel texture properties were determined using texture analyzer (EZ-Test) (Schninazu Corp, Kyoto, Japan) with cylinder probe of 20 mm diameter. Starch paste that was prepared as above was immediately poured into small, circular plastic containers (11.6 mm height × 38.7 mm diameter) and stored at for 24 h at 23 °C. The texture of the pasted starch was determined using a penetration test at a pre-speed of 50 mm/min, at a pre-force of 0.02 N and a test speed of 10 mm/min. The maximum force (N) during penetration was recorded as firmness and the negative force during the retraction of the probe as stickiness.

4.3.3 Water solubility index and water absorption index

Water solubility and swelling power of untreated starch and starch sonicated in acid, water was determined using a method described by Ocloo, Minnaar and Emmambux (2014). In brief, starch (0.125 g, dry basis) was heated in 10 ml distilled water at 50 and 90°C for 30 min in shaking water bath (rpm of 150) and then centrifuged at 25°C for 15 min at 3000 × g. The supernatant was dried at 105 °C for 16 h in an air-oven while the supernatant was decanted. Water solubility is the ratio of the weight of the supernatant to the initial weight of starch and expressed as percent (%). Swelling power is the weight of the residue obtained after centrifugation.

4.3.4 Thermal properties

Thermal properties of all starches were determined using methods described by Cuthbert, Ray and Emmambux (2017) with some changes. In short, starch (10 mg, db) was mixed with distilled (30 mg) and then equilibrated for at least 2 h at room temperature. Scanning was done from 30 °C to 120 °C with nitrogen flow rate of 100 ml/min and heating rate of 60°/min and pressure of 40 bar. The standard that was used was Indium (T_p= 156.61 °C, 28.45 J/g), while empty aluminum pan was used a reference.

4.3.5 X-ray diffraction

The crystalline nature of treated and untreated starch was evaluated by XRD with X'Pert PANanalytical diffractometer (Malven Panalytical, Eindhoven, Netherlands). The treated and untreated starches were equilibrated at 25°C in equilibration chamber (glass desiccator) to make 50% relative humidity. The instrument was operating with target voltage, current and wave length of 45kV, 40mA and CuKα1 (0.154 nm), respectively. Scanning was ranging 5-30° 2Θ with an exposure time of 16 min 14 s, step size of 0.026° and time a time/step ratio of 229.5. The relative crystallinity was estimated by the ratio of the integrated area of crystalline peaks to the total integrated area above straight baseline.

4.3.6 FTIR- ATR Spectroscopy

Treated and untreated starches were also analyzed by Fourier transform infrared (FTIR) using a procedure according to Chu, Wei and Zhu (2015) with some modifications. Absorbance spectra of starch were determined using Pekin Elmer Spectrum 100 with a universal attenuated total reflectance accessory (Perkin Elmer, Connecticut, USA). Samples were scanned over a wavelength range of 4000 cm^{-1} to 500 cm^{-1} and averaged from a total of 32 scans with a resolution of 8 cm^{-1}.

4.3.7 Light microscopy (LM)

Treated and untreated starch samples were observed under bright field and polarized light using Nikon Optiphot Transmitted Light Microscope (Tokyo, Japan). Starch samples (100 mg) were added in 5 ml of 30 % glycerol in water solution. Three drops of the suspension were taken and placed onto a slide and cover applied for polarized light observation and observed under × 20.

4.3.8 Scanning electron microscopy

The shape and surface morphology of the treated and untreated starches were observed using scanning electron microscopy (SEM). Starch samples were placed onto stubs using conductive double-sided carbon tape and coated with carbon on EMITECH carbon evaporator. Prepared samples were viewed in a SEM JSM-7500F (Tokyo, Japan) at an accelerating voltage of 3 KV. Observations were done at 2000 × magnifications.

4.3.9 Statistical analysis

The experiments were done in triplicate. Multivariate analysis of variance (MANOVA) using SPSS for Windows version 20 (IBM CA, USA) and Fisher's least significant difference (LSD) test was used as a statistical tool. The statistical analysis was done with a 95 % confidence interval ($p \leq 0.05$). Principal component analysis (PCA) of measured was used to evaluated differences and similarity of some measured parameters of all samples. Sonication parameters (output power and frequency), time of treatment and acetic acid were independent variables. While all measured properties of starch were dependent variables.

5 Results

5.1 Pasting properties

Figure 5.1, Table 5.1 and Table 5.2 show the pasting properties of maize starch sonicated with acetic acid and water for 0 (Native starch), 3, 5 and 10 days. The main effect in Table 5.2 shows that all three variables (solvent, sonication and days of treatment) had a significant ($p < 0.05$) effect on peak viscosity (PV), pasting temperature (PT), breakdown viscosity (BV) and setback viscosity (SV) of maize starch. The two-way and three-way interaction of solvent x sonication and solvent x sonication x days, respectively, had a significant ($p < 0.05$) effect on all four above measured pasting properties variables as shown in Table 5.2.

There was no significant increase in pasting temperatures of non-sonicated starches in water with day as shown by data for 0, 3, 5 and 10 days. On the contrary, starches sonicated in water had a decrease in pasting temperatures from 0 to 5 days but then significantly ($P < 0.05$) increased from 5 to 10 days. Pasting temperatures of starch sonicated in two acetic acid concentrations and starches treated with two acetic acid concentrations without sonication decreased with time (0-10 days).

Peak viscosity of non-sonicated starch did not increase significantly ($p > 0.05$) as compared to starch sonicated in water (Table 5.1). The peak viscosity of starch sonicated in water increased from day 0 to day 3 and then decreased from days 3 to 10, but the peak viscosity was still higher than that of native and untreated starches even after 10 days of treatment. Similarly, sonicated and non-sonicated starches in 5.16 M and 10.16 M acetic acid had a significant ($p < 0.05$) increase in peak viscosity from 0 to 3 days and a significant ($p < 0.05$) decrease from days 3 to 10. However, unlike starch sonicated in water, the peak viscosity was lower than native and untreated starches after 10 days. Peak viscosities of starches sonicated in 5.16 M and 10.16 M values were lower than those starches treated with the same acetic acid concentration.

In general, setback viscosity of some non-sonicated and sonicated starches increased from 0 to 3 days and from 3 to 5 days, with few exceptions in some starch samples. For instance, there was a significant ($p < 0.05$) increase in setback viscosity from 0 to 3 days but decreased from 3 to 10 days for starch sonicated in 10.16 M acetic acid. Values of setback viscosities of starch sonicated in water were higher than setback viscosities of untreated starches for day 3, 5 and 10.

Final viscosity of starch sonicated in water decreased significantly ($p < 0.05$) with days 0-10 days compared to untreated starch where a significant increase was observed between 0 and 3 days and then decrease between 5 and 10 days (Table 5.1). Starch sonicated in water had higher values of final viscosity as compared to untreated starch in all recorded days. There was a significant ($p < 0.05$) increase in setback viscosity from 0 to 3 but a significant decrease was also noted between 3 to 10 days in starch sonicated and non-sonicated starch in 5.16 M and 10.16 M acetic acid.

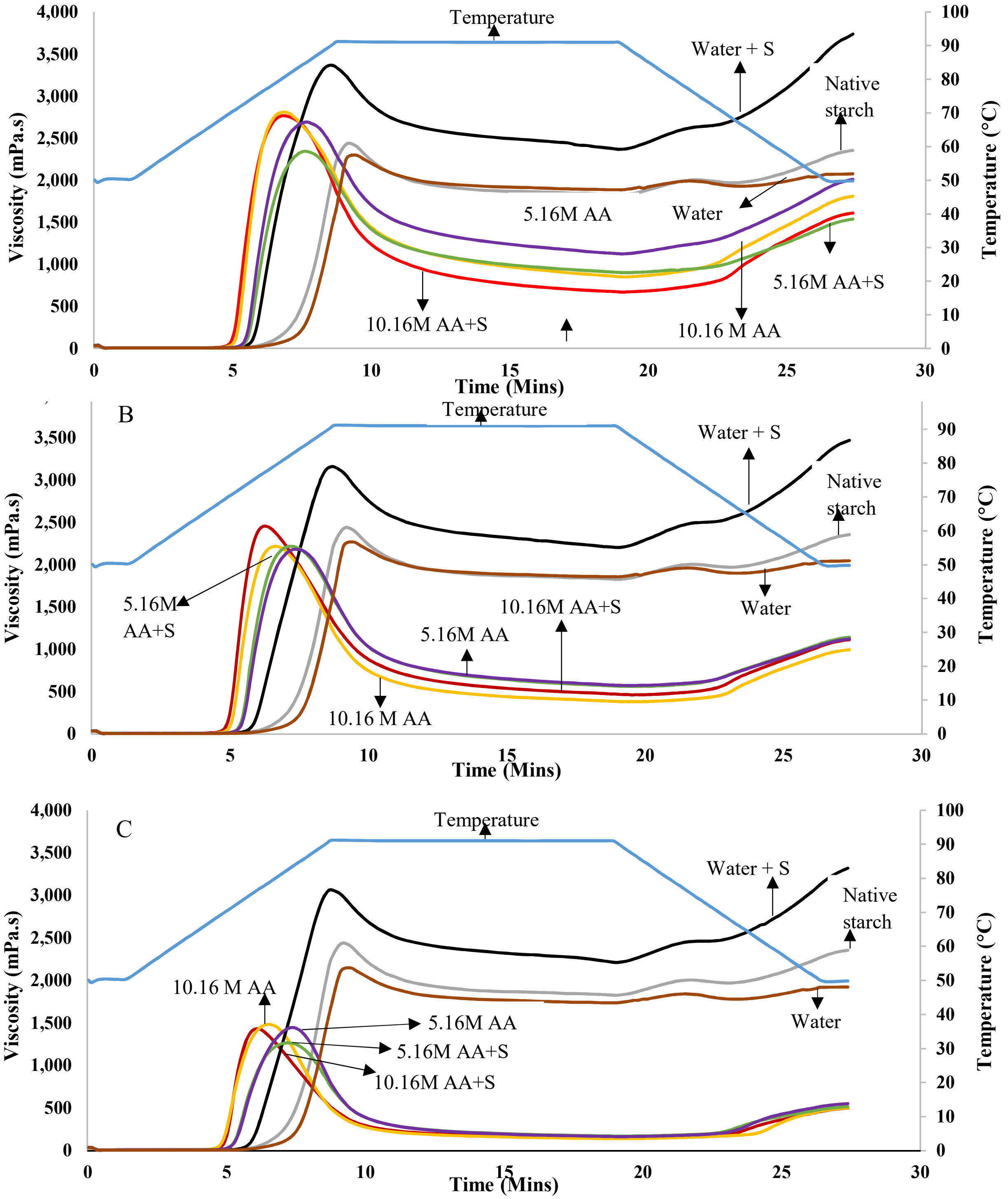


Figure 5. 1: Effects of acetic acid with and without sonication on pasting properties of maize starch for 3 (A), 5 (B) and 10 (C)
AA is acetic acid
10.16 M and 5.16 M is the molarity of acetic acid
S is probe type sonication at 382 W/cm^2 for one hour per day at 40 °C

Table 5. 1: Effects of acetic acid with and without sonication on the pasting properties of maize starch incubated for 3, 5 and 10 days at 40 °C

Solvent	Sonication	Time (Days)	Pasting temperature (°C)	Pasting viscosity (mPa.s)	Breakdown viscosity (mPa.s)	Setback viscosity (mPa.s)
Water	No sonication	0	76.6±0.5^{a}	2034.3±1.5^{a}	196.3±0.5^{c}	550.7±0.6^{a}
		3	79.1±0.3^{a}	2298.9±0.3^{a}	411.3±0.6^{a}	188.6±0.4^{b}
		5	78.7±0.3^{a}	2269.6±0.5^{a}	411.0±0.1^{a}	187.6±0.7^{b}
		10	78.4±0.3^{a}	2149.2±0.6^{a}	382.9±0.7^{b}	157.5±1.0^{c}
	Sonication	0	76.6±0.5^{a}	2034.3±1.5^{d}	196.3±0.5^{d}	550.7±0.6^{d}
		3	73.8±0.2^{b}	3452.0±2.7^{a}	1084.0±1.7^{a}	1493.7±3.8^{a}
		5	73.8±0.5^{b}	3154.3±1.5^{b}	1000.3±0.5^{b}	1316.2±2.9^{b}
		10	74.1±0.5^{c}	3064.3±4.5^{c}	793.7±0.5^{c}	1047.3±1.5^{c}
5.16M AA	No sonication	0	76.6±0.5^{a}	2034.3±1.5^{c}	196.3±0.5^{a}	550.7±0.6^{c}
		3	72.1±0.5^{b}	2663.7±1.5^{a}	1537.7±0.5^{c}	889.3±2.3^{a}
		5	72.1±0.5^{b}	2174.3±2.1^{b}	1621.7±0.7^{b}	553.0±2.1^{b}
		10	70.7±0.4^{c}	1446.0±1.7^{d}	1280.4±1.4^{d}	389.4±2.9^{d}
	Sonication	0	76.6±0.5^{a}	2034.3±1.5^{c}	196.3±0.5^{d}	550.7±0.6^{c}
		3	72.4±0.5^{b}	2342.3±3.3^{a}	1441.7±2.0^{b}	956.6±2.6^{a}
		5	71.5±0.5^{b}	2161.7±3.1^{b}	1613.0±0.1^{a}	608.8±2.6^{b}
		10	70.7±0.4^{c}	1243.3±3.5^{d}	1068.6±1.4^{c}	442.8±3.3^{d}
10.16M AA	No sonication	0	76.6±0.5^{a}	2034.3±1.5^{c}	196.3±0.5^{d}	550.7±0.6^{c}
		3	70.2±0.0^{b}	2796.0±2.7^{a}	1942.2±0.3^{a}	956.6±2.6^{a}
		5	70.5±0.5^{b}	2210.3±4.9^{b}	1826.9±0.8^{b}	608.8±2.6^{b}
		10	68.2±0.5^{b}	1863.3±5.8^{d}	1708.0±1.0^{c}	442.7±3.3^{d}
	Sonication	0	76.6±0.5^{a}	2034.3±1.5^{c}	196.3±0.5^{d}	550.7±0.6^{c}
		3	70.7±1.3^{b}	2665.7±5.8^{a}	1836.6±0.3^{b}	863.1±1.3^{a}
		5	69.1±0.5^{c}	2475.3±2.1^{b}	2012.9±1.0^{a}	656.6±0.9^{b}
		10	68.8±0.5^{c}	1467.0±2.7^{c}	1318.0±1.3^{c}	354.9±1.2^{d}

The values are means ± standard deviation of three replicates. The means with different letter in a column differs significantly ($p < 0.05$)
PT is Pasting Temperature
PV is Peak Viscosity
BV is breakdown Viscosity

SV is Setback Viscosity
AA is acetic acid
10.16 M and 5.16 M is the molarity of acetic acid
S is probe type sonication at 382 W/cm^2 for one hour per day at 40 °C

Table 5. 2: Three-way anova results for the effect of sonication, acid concentration and days on the effect of pasting properties of maize starch

Source	PT(°C)	PV(mPa.s)	BV(mPa.s)	SV (mPa.s)
Solvent	*	*	*	*
Sonication	*	*	*	*
Days	*	*	*	*
Solvent x Sonication	*	*	*	*
Solvent x Days	NS	*	*	*
Sonication x Days	NS	*	*	*
Solvent x Sonication x Days	*	*	*	*

*significant at $p < 0.05$
Sonication is probe type at 382 W/cm^2 for one hour per day at 40 °C
Time is in days

5.2 Gel strength

The effects of acetic acid with sonication on gel strength of maize starch for 0, 3, 5 and 10 days are presented in Table 5.3 and 5.4. Table 5.3 showed that the gel strength was measured in terms of firmness and stickiness while Table 5.4 showed the effects individual variables on each measured variable. The firmness of starch sonicated in water decreased with increasing number of days compared to untreated starch in water as shown in Table 5.3. It appeared that neither sonication in water and/or in acetic acid caused any significant changes in the stickiness of maize starch as shown in Table 5.3 and 5.4. The gel firmness of maize starch significantly ($p < 0.05$) decreased from 4.4 N to 3.8 N when sonicated in water from day 0 to day 10. The firmness of starch sonicated in both acetic acid concentrations and non-sonicated starches decreased significantly ($p < 0.05$) from 0 to 3 days and from 3 to 5 days. However, there was no significant from 5 to 10 days in starches sonicated and non-sonicated in 5.16 M acetic acid as compared to sonicated and non-sonicated starches sonicated in 10.16 M acetic acid where there was no significant ($p < 0.05$) decrease from 3 to 10 days. Table 5.3 showed that the solvent and time of treatment influenced firmness and the interaction between the two also had a significant effect ($p > 0.05$) on firmness of starch.

Table 5. 3: Effects of sonication with acetic acid on gel strength of maize starch for 3, 5 and 10 days

Solvent	Sonication	Time (days)	Firmness (N)	Stickiness (N)
Water	No Sonication	0	4.4±0.2^{a}	0.3±0.3^{a}
		3	5.0±0.2^{a}	0.1±0.1^{a}
		5	3.8±0.6^{a}	0.3±0.3^{a}
		10	4.8±0.8^{a}	0.2±0.1^{a}
	Sonication	0	4.4±0.2^{b}	0.3±0.3^{a}
		3	4.8±0.8^{ab}	0.2±0.1^{a}
		5	4.0±0.8^{ab}	0.2±0.2^{a}
		10	3.8±0.8^{a}	0.2±0.0^{a}
5.16M	No Sonication	0	4.4±0.2^{a}	0.3±0.3^{a}
		3	1.9±0.6^{b}	0.2±0.1^{a}
		5	0.4±0.0^{c}	0.1±0.0^{a}
		10	0.2±0.0^{c}	0.0±0.0^{a}
	Sonication	0	4.4±0.2^{a}	0.3±0.3^{a}
		3	1.2±0.1^{b}	0.1±0.0^{a}
		5	0.5±0.1^{c}	0.1±0.0^{a}
		10	0.3±0.0^{c}	0.1±0.0^{a}
10.16M	No Sonication	0	4.4±0.2^{a}	0.3±0.3^{a}
		3	0.8±0.1^{b}	0.1±0.0^{a}
		5	0.2±0.0^{b}	0.1±0.1^{a}
		10	0.2±0.2^{b}	0.0±0.1^{a}
	Sonication	0	4.4±0.2^{a}	0.3±0.3^{a}
		3	0.5±0.0^{b}	0.2±0.1^{a}
		5	0.3±0.1^{b}	0.1±0.0^{a}
		10	0.2±0.0^{b}	0.2±0.3^{a}

The values are means ± standard deviation of three replicates. The means with different letter in a column differs significantly ($p < 0.05$)
PT is Pasting Temperature
PV is Peak Viscosity
BV is breakdown Viscosity
SV is Setback Viscosity
AA is acetic acid
10.16 M and 5.16 M is the molarity of acetic acid

S is probe type sonication at 382 W/cm^2 for one hour per day at 40 °C

Table 5. 4: Three-way Anova results for the effects of type of treatment, acid acetic acid and days on the effect of gel strength of maize starch

Source	Firmness (N)	Stickiness (N)
Solvent	*	NS
Sonication	NS	NS
Time (days)	*	NS
Solvent x Sonication	NS	NS
Solvent x Time	*	NS
Sonication x Times	NS	NS
Solvent x Sonication x Time	NS	NS

*significant at $p < 0.05$
NS indicates no significant at $p > 0.05$
Time in days
Sonication at 382 W/cm^2 for one hour per day at 40 °C

5.3 Water solubility index (WSI) and Water absorption index (WAI)

The effects of sonication in acetic acid on water solubility index and water absorption index of maize starch for 0, 3, 5 and 10 days is presented in Table 5.5 and 5.6. Both water solubility index and water solubility index starch appeared to be strongly depended to temperature, that is, values of water solubility index at 90 °C were higher than those obtained at 50 °C and values of water absorption index were higher at 90 °C than at 50 °C (Table 5.5). Water solubility index of starch sonicated in water decreased from 0 to 10 days as compared to untreated starch at 50 °C. The opposite was observed at 90 °C since the water solubility was observed to be increasing from 0 to 10 days in the same starch samples, as shown in Table 5.5. Water absorption index of starch treated with sonication in water increased significantly ($p < 0.05$) from 0 to 3 days then decreased thereafter (from 3 to 10 days) compared to untreated starch in water.

In general, water solubility index of sonicated and non-sonicated starches in both acetic acid concentrations increased from 0 to 10 days in both temperatures as shown in Table 5.5. Non-sonicated starch in 5.16 M acetic acid had higher values than starch sonicated in 5.16 M acetic acid at the end of day 10 at 50 °C and 90 °C .On the contrary, starch sonicated in 10.16 M acetic acid had higher values than non-sonicated starch in 10.16 M acetic acid at 50 °C but the opposites was observed at 90 °C after 10 days. Table 5.6 showed that the solvent and time of treatment had significant ($p < 0.05$) effect on water solubility index at both temperatures, while sonication had no significant ($p > 0.05$) effect.

A significant ($p < 0.05$) increase in water absorption index in starch sonicated in water was only noted after 10 days of treatment while a significant ($p < 0.05$) increase in untreated starch was noted after 3 days at 50 °C. However, water absorption index values of starch sonicated in water were lower than those of untreated starch in 3, 5 and 10 days (Table 5.5). There was a significant ($p < 0.05$) increase in water absorption index in starch sonicated in water from 0 to 3 days and then a significant ($p < 0.05$) decrease thereafter (3 to 10 days) at 90 °C. Similar to starch sonicated in water, all sonicated and non-sonicated starches in both acetic acid concentrations at 50 °C increased from 0 to 3 days and then decreased from 3 to 10 days of treatment. However, at 90 °C, the water absorption index of sonicated and non-sonicated starches in 5.16 M and 10.16 M acetic acid decreased from 0 to 10 days. Table 5.6 showed that solvent and time of treatment had a significant ($p < 0.05$) effect water absorption index at 90 °C but not significant ($p > 0.05$) effect at 50 °C, and sonication had no significant ($p >0.05$) effect at both temperatures.

Table 5. 5: Effects of sonication with acetic acid on water solubility index (WSI) and water absorption index (WAI) on maize starch for 3, 5 and 10 days

			WSI (%)	**WAI (g/g)**	**WSI (%)**	**WAI (g/g)**
Solvent	**Sonication**	**Time (days)**	**50 °C**		**90 °C**	
Water	No sonication	0	0.6 ± 0.2^{a}	2.1 ± 0.1^{a}	20.0 ± 1.4^{a}	16.8 ± 1.2^{a}
		3	0.6 ± 0.4^{a}	2.9 ± 0.1^{b}	20.5 ± 2.1^{a}	16.8 ± 0.5^{a}
		5	0.6 ± 0.1^{a}	2.6 ± 0.2^{b}	21.4 ± 0.3^{a}	18.1 ± 0.8^{a}
		10	0.6 ± 0.3^{a}	2.8 ± 0.4^{b}	21.2 ± 0.3^{a}	17.4 ± 0.4^{a}
	Sonication	0	0.6 ± 0.2^{ab}	2.1 ± 0.1^{a}	20.0 ± 1.4^{b}	16.8 ± 1.2^{b}
		3	0.9 ± 0.2^{a}	2.5 ± 0.1^{a}	22.6 ± 1.2^{a}	19.8 ± 0.3^{a}
		5	0.8 ± 0.2^{a}	2.1 ± 0.1^{a}	19.5 ± 1.7^{b}	16.6 ± 1.2^{b}
		10	0.4 ± 0.1^{b}	1.6 ± 1.1^{b}	20.0 ± 1.2^{b}	15.7 ± 1.4^{b}
5.16M	No sonication	0	0.6 ± 0.2^{c}	2.1 ± 0.1^{b}	20.0 ± 1.4^{d}	16.8 ± 1.2^{a}
		3	2.5 ± 0.5^{b}	2.3 ± 0.1^{ab}	46.0 ± 0.7^{c}	14.8 ± 1.4^{a}
		5	2.9 ± 0.5^{ab}	2.5 ± 0.0^{a}	74.1 ± 0.8^{b}	9.9 ± 1.2^{b}
		10	4.0 ± 0.8^{a}	2.2 ± 0.2^{ab}	88.0 ± 0.9^{a}	4.7 ± 0.3^{c}
	Sonication	0	0.6 ± 0.2^{a}	2.1 ± 0.1^{a}	20.0 ± 1.4^{d}	16.8 ± 1.2^{a}
		3	2.3 ± 0.8^{b}	2.7 ± 0.8^{a}	52.8 ± 1.7^{c}	13.6 ± 1.5^{b}
		5	2.7 ± 0.9^{b}	2.3 ± 0.1^{a}	70.0 ± 1.7^{b}	10.0 ± 1.5^{c}
		10	3.0 ± 0.6^{b}	2.2 ± 0.0^{a}	87.1 ± 1.9^{a}	5.5 ± 0.5^{d}
10.16M	No sonication	0	0.6 ± 0.2^{c}	2.1 ± 0.1^{a}	20.0 ± 1.4^{d}	16.8 ± 1.2^{a}
		3	2.6 ± 0.2^{b}	2.5 ± 0.5^{a}	55.5 ± 1.6^{c}	13.5 ± 1.6^{b}
		5	3.1 ± 0.3^{b}	2.3 ± 0.2^{a}	75.4 ± 2.1^{b}	10.5 ± 0.5^{c}
		10	3.8 ± 0.2^{a}	2.1 ± 0.1^{a}	86.1 ± 1.8^{a}	5.3 ± 0.3^{d}
	Sonication	0	0.6 ± 0.2^{b}	2.1 ± 0.1^{c}	20.0 ± 1.4^{d}	16.8 ± 1.2^{a}
		3	2.6 ± 1.7^{a}	2.3 ± 0.1^{b}	54.9 ± 0.8^{c}	15.1 ± 0.4^{b}
		5	3.1 ± 0.3^{a}	3.0 ± 0.1^{a}	78.7 ± 0.5^{b}	10.4 ± 0.9^{c}
		10	3.2 ± 2.4^{a}	2.5 ± 0.1^{b}	87.2 ± 1.6^{a}	5.2 ± 0.9^{d}

The values are means ± standard deviation of three replicates. The means with different letter in a column differs significantly ($p < 0.05$)

WSI is water solubility index in percentage (%)

WAI is water absorption index (g/g)

10.16 M and 5.16 M is the molarity of acetic acid

Sonication is probe type at 382 W/cm^2 for one hour per day at 40 °C

Table 5. 6: Three-way Anova results for the effects of type of treatment, acid acetic acid and days on the effect of water solubility index and water absorption index of maize starch

	WSI (%)	**WAI (g/g)**	**WSI (%)**	**WAI(g/g)**
Source	**50 °C**		**90 °C**	
Solvent	*	NS	*	*
Sonication	NS	*	NS	NS
Time	*	NS	*	*
Solvent x Sonication	NS	*	NS	NS
Solvent x Time	NS	NS	*	*
Sonication x Times	NS	*	*	*
Solvent x Sonication x Time	NS	NS	*	*

*significant at $p < 0.05$

NS indicates no significant at $p > 0.05$

Time in days

Sonication at 382 W/cm^2 for one hour per day at 40 °C

5.4 Thermal properties

Thermal properties of maize starch that was sonicated in acetic acid and water for 0, 3, 5 and 10 days are presented in figure 5.2, Table 5.7 and Table 5.8. The endothermic peak (corresponding to gelatinisation) sonicated and non-sonicated starches in water were similar and also similar to endothermic peak of native starch as shown in Figure 5.2. Endothermic peaks of starch sonicated in either 5. 16 M or 10. 16 M were broader than endothermic peaks of non-sonicated starches in 5. 16 M and 10. 16 M acetic acid (Fig. 5.2). The T_o (onset temperature) T_p (peak temperature), T_c (endset temperature) and ΔH (enthalpy) at day 0 or native starch were 65.6, 70.2, 76.6 °C and 21.8 J/g, respectively. The T_o of sonicated and non-sonicated starch in water and of two concentration of acetic acid decreased significantly ($p < 0.05$) after 3 days of treatment as compared to untreated starch and native starch. Similar to non-sonicated starch in water, there was no significant ($p > 0.05$) difference in T_o of starch sonicated in water from 3 to 10 days (Table 5.7). There were also no significant ($p > 0.05$) changes in T_o of starch sonicated in 5. 16 M and 10.16 M from 3 to 10 days as compared to non-sonicated in 5. 16 M and 10. 16 M where a significant decrease was noted mainly after 5 days of treatment. Table 5.8 shows that the solvent and time of treatment had a significant ($p < 0.05$) effect on T_o.

Similarly, T_o, T_p and T_c generally decreased with increasing time of treatment in most starches as a general trend (Table 5.7). The T_p of both non-sonicated and sonicated starches in water significantly ($p < 0.05$) decreased from 0 to 3 days and no significant ($p > 0.05$) was noted after 3 days to 10 days. A significant decrease ($p < 0.05$) that was noted in T_p of non-sonicated starch in 5.16 M after 10 days was also noted after 3 days in starch sonicated in 5.16 M. The opposite was observed in starch sonicated in 10.16 M acetic acid when a significant ($p < 0.05$) decrease observed after 10 days in starch sonicated in 10.16 M compared to a significant decrease that was observed after 3 days. The T_c of starch sonicated in water and non-sonicated starch in water decreased significantly ($p < 0.05$) from 0 from 3 days and did not change significantly thereafter, however, values of starch sonicated in water were lower than values of T_p of non-sonicated starch in water for 3, 5 and 10 days (Table 5.7). There were no significant changes in T_p of sonicated and non-sonicated starch with 5.16 M acetic acid. There was a significant ($p < 0.05$) increase in T_p of non-sonicated starch with 10.16 M from 0 to 3 days and decreased significantly (3 to 10 days) as compared to sonicated starch in 10.16 M where a significant ($p < 0.05$) was noted from 3 to 10 days of treatment as shown in Table 5.8.

The ΔH of starch sonicated in water decreased from 0 to 3 days from 21.8 to 20.1 J/g and then increased from 3 to 10 days (from 20.1 to 24.3 J/g) as compared to non-sonicated starch in water for 3, 5 and 10 days (Table 5.7). ΔH of sonicated and non-sonicated in 5.16 M acetic acid decreased from 0 to 5 days and increased from 5 to 10 days (Table 5.7). The ΔH of non-sonicated starch in 10.16 M decreased from only 0 to 3 days and increased from 3 to 10 days but the ΔH of starch sonicated in 10.16 M decrease from 0 to 5 days and increased from 5 to 10 days (Table 5.7). Solvent, sonication and time of treatment had significant ($p < 0.05$) effect on T_p, T_c and ΔH as shown in Table 5.7.

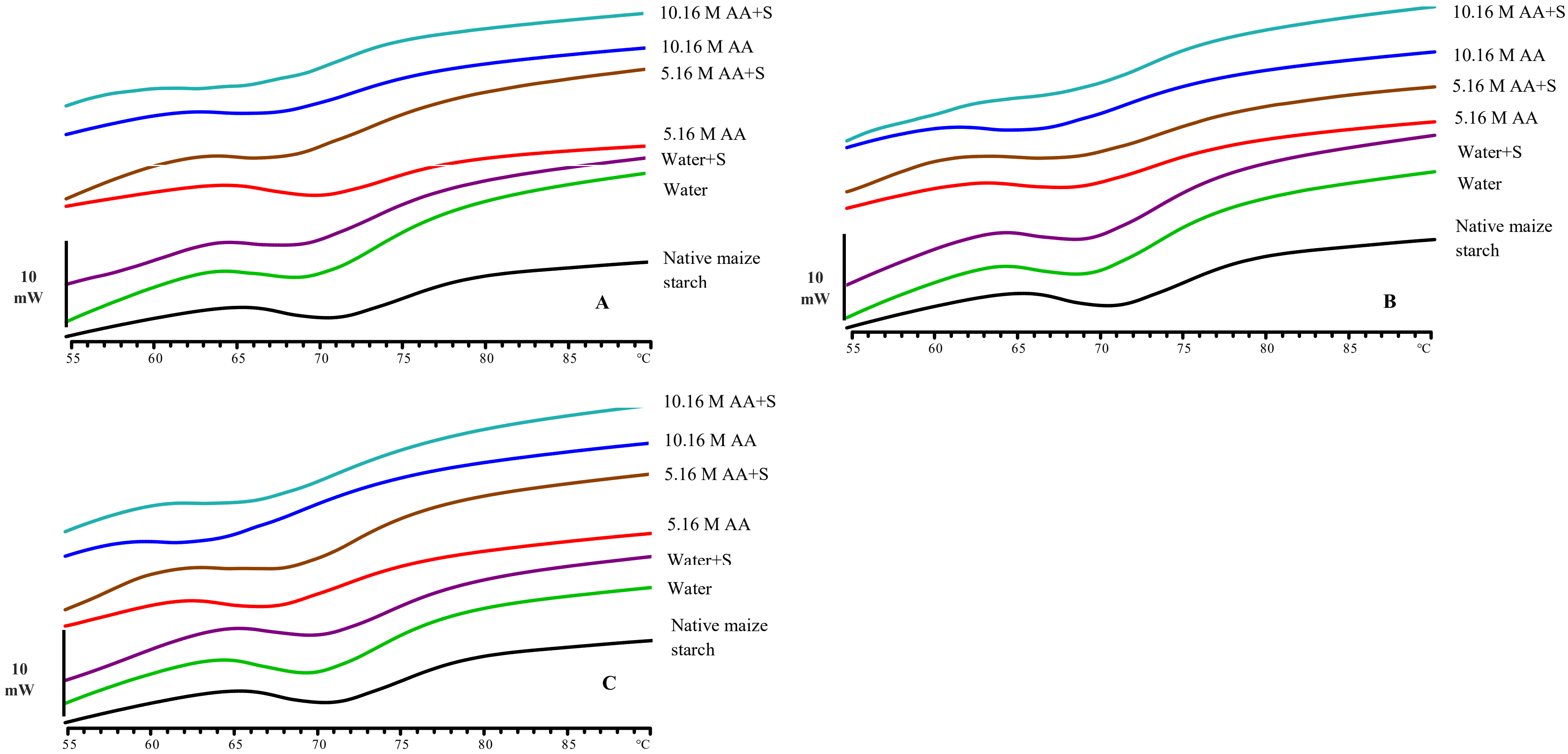


Figure 5. 2: Effects of acetic acid with and without sonication on pasting properties of maize starch for 3 (A), 5 (B) and 10 (C)
AA is acetic acid
10.16 M and 5.16 M is the molarity of acetic acid
S is probe type sonication at 382 W/cm^2 for one hour per day at 40 °C

Table 5. 7: Effects of acetic acid and sonication on thermal properties of maize starch for 0, 3, 5 and 10 days

Solvent	Sonication	Time (Days)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
Water	No sonication	0	65.6 ± 0.5^{a}	70.2 ± 0.6^{a}	76.6 ± 0.7^{a}	21.8 ± 0.1^{a}
		3	66.7 ± 0.8^{a}	71.5 ± 0.1^{b}	80.2 ± 0.7^{b}	21.3 ± 1.4^{a}
		5	66.2 ± 1.0^{a}	71.5 ± 0.3^{b}	79.9 ± 0.8^{b}	21.3 ± 2.6^{a}
		10	66.6 ± 0.5^{a}	71.3 ± 0.1^{b}	79.2 ± 0.5^{b}	21.4 ± 0.8^{a}
	Sonication	0	65.6 ± 0.5^{a}	70.2 ± 0.6^{a}	76.6 ± 0.7^{a}	21.8 ± 0.1^{ab}
		3	64.3 ± 0.1^{b}	68.7 ± 1.0^{b}	75.0 ± 0.3^{b}	20.1 ± 2.6^{a}
		5	64.3 ± 0.0^{b}	68.3 ± 0.2^{b}	75.2 ± 0.2^{b}	23.9 ± 1.5^{b}
		10	64.4 ± 0.5^{b}	69.5 ± 0.6^{ab}	75.7 ± 0.1^{b}	24.3 ± 1.7^{b}
5.16M	No sonication	0	65.6 ± 0.5^{c}	70.2 ± 0.6^{a}	76.6 ± 0.7^{a}	21.8 ± 0.1^{a}
		3	64.3 ± 1.1^{b}	69.8 ± 0.3^{a}	76.5 ± 0.4^{a}	19.9 ± 0.3^{b}
		5	63.1 ± 0.8^{ab}	68.9 ± 1.0^{ab}	75.3 ± 0.5^{a}	16.2 ± 1.0^{b}
		10	62.6 ± 0.1^{a}	67.7 ± 3.4^{b}	74.9 ± 1.9^{a}	18.6 ± 3.4^{b}
	Sonication	0	65.6 ± 0.5^{a}	70.2 ± 0.6^{a}	76.6 ± 0.7^{a}	21.8 ± 0.1^{a}
		3	63.5 ± 0.5^{b}	68.3 ± 1.6^{b}	75.3 ± 0.7^{a}	19.4 ± 0.4^{b}
		5	62.5 ± 0.5^{b}	67.9 ± 0.7^{b}	75.0 ± 0.8^{a}	16.5 ± 0.5^{c}
		10	63.0 ± 1.3^{b}	68.9 ± 0.2^{ab}	75.3 ± 1.7^{a}	23.2 ± 1.9^{a}
10.16M	No sonication	0	65.6 ± 0.5^{a}	70.2 ± 0.6^{a}	76.6 ± 0.7^{b}	21.8 ± 0.1^{a}
		3	62.5 ± 0.4^{b}	69.5 ± 0.1^{a}	77.4 ± 0.1^{a}	12.3 ± 0.7^{d}
		5	61.5 ± 0.5^{c}	66.3 ± 1.5^{b}	73.8 ± 0.3^{c}	16.6 ± 2.6^{c}
		10	58.7 ± 0.5^{d}	65.5 ± 0.0^{b}	72.8 ± 0.6^{d}	19.7 ± 0.1^{b}
	Sonication	0	65.6 ± 0.5^{a}	70.2 ± 0.6^{a}	76.6 ± 0.7^{a}	21.8 ± 0.1^{a}
		3	63.1 ± 2.2^{b}	69.2 ± 1.7^{ab}	74.4 ± 2.1^{b}	17.4 ± 0.9^{b}
		5	62.5 ± 0.7^{b}	68.4 ± 1.3^{ab}	73.3 ± 0.8^{b}	13.1 ± 2.4^{c}
		10	62.0 ± 0.6^{b}	67.8 ± 0.6^{b}	73.5 ± 0.9^{b}	17.2 ± 0.6^{b}

The values are means ± standard deviation of three replicates. The means with different letter in a column differs significantly ($p < 0.05$)

T_o is onset temperature
T_p is peak temperature
T_c is endset temperature
ΔH is enthalpy of the endothermic peak

AA is acetic acid
10.16 M and 5.16 M is the molarity of acetic acid
S is probe type sonication at 382 W/cm^2 for one hour per day at 40 °C

Table 5. 8: Three-way Anova results for the effect of type of treatment, acid concentration and days on the effect of thermal properties of maize starch

Source	T_o (ºC)	T_p (ºC)	T_c (ºC)	ΔH (J/g)
Solvent	*	*	*	*
Sonication	NS	*	*	*
Time (days)	*	*	*	*
Solvent x Sonication	*	*	*	*
Solvent x Time	*	*	*	*
Sonication x Time	*	*	*	NS
Solvent x Sonication x Time	NS	NS	NS	*

*significant at $p < 0.05$
NS: not significant ($p > 0.05$)
Sonication is probe type at 382 W/cm^2 for one hour per day at 40 °C
Time is in days

5.5 X-ray diffraction

The effects of acetic acid and sonication on X-ray diffraction pattern and relative crystallinity of maize starch for 0, 3, 5 and 10 days is presented in Figure 5.3, Table 5.9 and Table 5.10. Starch that was sonicated in water for 3, 5 and 10 days had X-ray diffraction patterns that were similar to native starch and non-sonicated starch for 3, 5 and 10 days as shown in Figure 5.3. Table 5.9 shows that the relative crystallinity of both sonicated and non-sonicated starches in water did not increase significantly ($p > 0.05$) from 0 to 10 days and Table 5.10 also shows that solvent and sonication had overall significant ($p < 0.05$) effect on relative crystallinity. As such, X-ray pattern of starch sonicated in 5.16 M for 3, 5 and 10 days were also similar to non-sonicated starches in 5.16 M acetic acid at the same time interval.

There was a change in X-ray diffraction pattern of starch sonicated in 10.16 M after 3, 5 and 10 days of treatment as compared to non-sonicated starch in 10.16 M acetic acid. As indicated with arrows in Figure 5.3, there was a peak between diffraction 2Θ ~17 ^{o} and ~18 ^{o} that appeared to be disappearing with time of treatment in starch sonicated in 10.16 M acetic acid. The relative crystallinity of sonicated and non-sonicated starches in both 5.16 M and 10.16 M acetic acid concentrations increased significantly ($p < 0.05$) after 3 days of treatment (Figure 5.3 and Table 5.9). The relative crystallinity then decreased from 3 to 5 days and increased from 5 to 10 days of treatment in sonicated and sonicated starches in 5.16 M acetic acid and in 10.16 M acetic acid. The same trend was observed in non-sonicated in 5.16 M. On the contrary, relative crystallinity of non-sonicated starch in 10.16 M increased from 39.9 to 40.1 % and decreased from 40.1 to 36.8 %, from 3 to 5 and 5 to 10 respectively.

Table 5. 9 : Effect of acetic acid with and without sonication on the relative crystallinity of maize starch incubated at 40 ºC for 3, 5 and 10 days

Solvent	Sonication	Time (Days)	Relative crystallinity (%)
Water	No sonication	0	37.7±0.4^{a}
		3	37.9±1.3^{a}
		5	37.4±1.7^{a}
		10	37.5±0.5^{a}
	Sonication	0	37.7±0.4^{a}
		3	38.1±1.7^{a}
		5	39.5±1.8^{a}
		10	38.8±2.1^{a}
5.16M	No sonication	0	37.7±0.4^{b}
		3	40.7±1.7^{a}
		5	39.2±1.0^{ab}
		10	41.3±1.1^{a}
	Sonication	0	37.7±0.4^{b}
		3	42.5±0.9^{a}
		5	40.5±0.9^{a}
		10	42.0±1.3^{a}
10.16M	No sonication	0	37.7±0.4^{b}
		3	39.9±1.3^{a}
		5	40.1±1.3^{a}
		10	36.8±0.2^{b}
	Sonication	0	37.7±0.4^{a}
		3	42.2±1.3^{b}
		5	40.5±1.5^{b}
		10	41.9±2.8^{b}

The values are means ± standard deviation of three replicates. The means with different letter in a column differs significantly ($p < 0.05$)

AA is acetic acid

10.16 M and 5.16 M is the molarity of acetic acid

S is probe type sonication at 382 W/cm^{2} for one hour per day at 40 °C

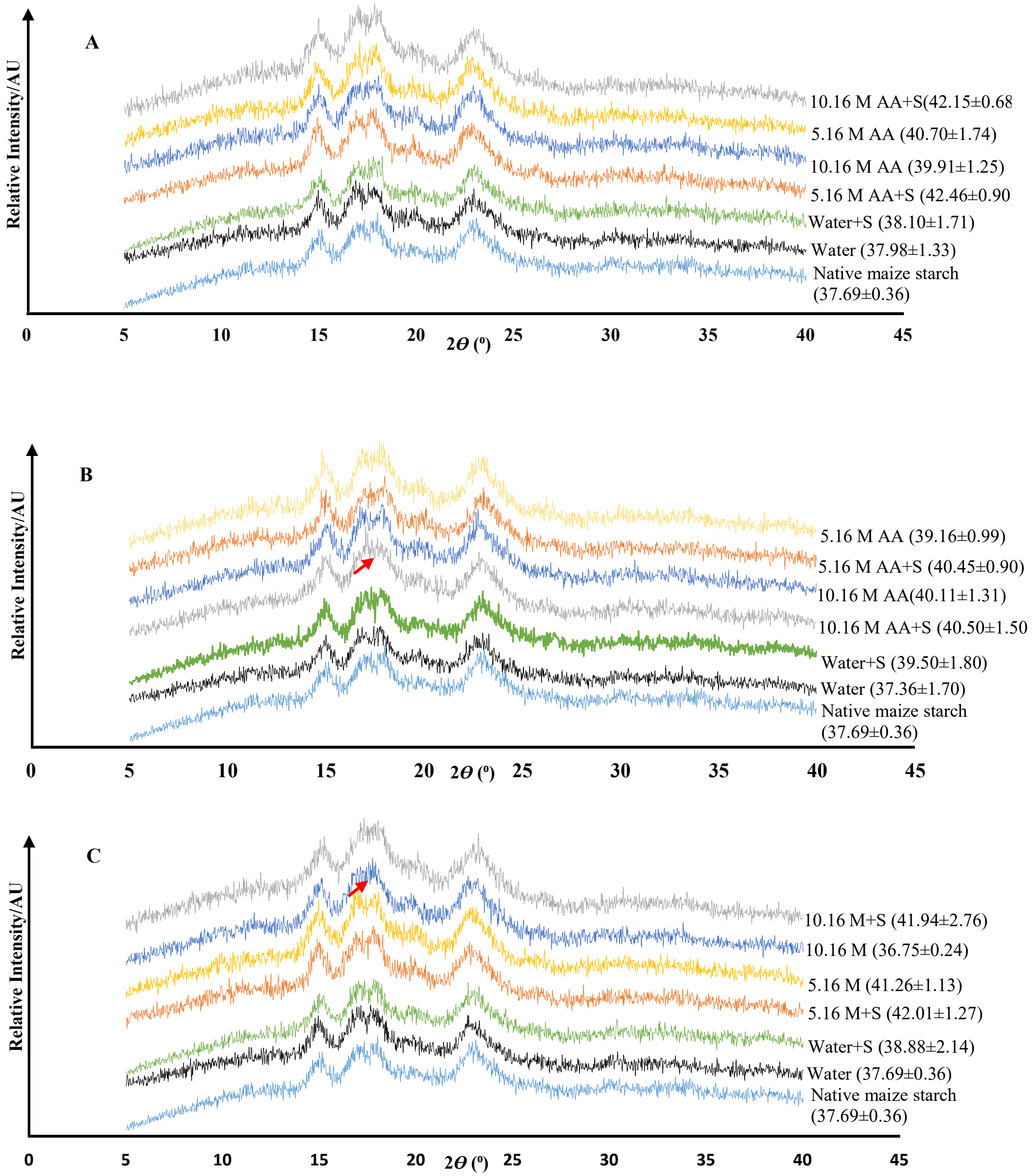


Figure 5. 3: Effect acetic acid with and without sonication on X-ray diffraction of maize starch for 3 (A), 5 (B) and 10 (C)

*Values in parenthesis represents mean relative crystallinity in percentage (%) ±SD

AA is acetic acid

10.16 M and 5.16 M is the molarity of acetic acid

S is probe type sonication at 382 W/cm^2 for one hour per day at 40 °C

Table 5. 10: Three-way Anova results for the effect of type of treatment, acid concentration and days on the effect of relative crystallinity of treated maize starch.

Source	Relative crystallinity (%)
Solvent	*
Sonication	*
Time	NS
Solvent x Sonication	*
Solvent x Time	*
Sonication x Time	NS
Solvent x Sonication x Time	NS

*significant at $p < 0.05$
NS: not significant
Time in days
10.16 M and 5.16 M is the molarity of acetic acid
S is probe type sonication at 382 W/cm^2 for one hour per day at 40 °C

5.6 FTIR spectra

Figure 5.4 showed FTIR spectra of acetic acid and native white maize starch. FTIR spectra of sonicated and non-sonicated starches in water and in acetic acid for 0, 3, 5 and 10 days is presented Figure 5.5. Native starch spectra were different from acetic acid spectra as shown in Figure 4. The main peak that differentiate the two was the peak 1722 cm^{-1} on acetic acid which was not in starch spectra. The spectra of all sonicated and non-sonicated starches appeared to be similar to native starch (Fig. 5.5) but there was slight diminution in their intensities and few newly formed peaks.

The intensities of both sonicated and non-sonicated starches in water decreased from 3 to 10 days with no obvious formation or disappearing of peaks as compared to native starch spectra (Fig. 5.5). Similarly, sonicated and non-sonicated starches in 5.16 M acetic acid had no formation nor disappearance of peaks. However, spectra of starch sonicated in 10.16 M acetic acid had a formation of two peaks, first one at 1720 cm^{-1} and the second one at 1566 cm^{-1} (as indicated in arrows). The first peak appeared after 3 days and increased in intensity from 3 to 10 days, while the second one only appeared from 5 to 10 days of treatment and also increased in intensity as shown in Figure 5.5. Furthermore, acetic acid spectra in Figure 5.5 had the peaks that were close to the wavelength of those two newly formed peaks in starch sonicated in 10.16 M acetic acid.

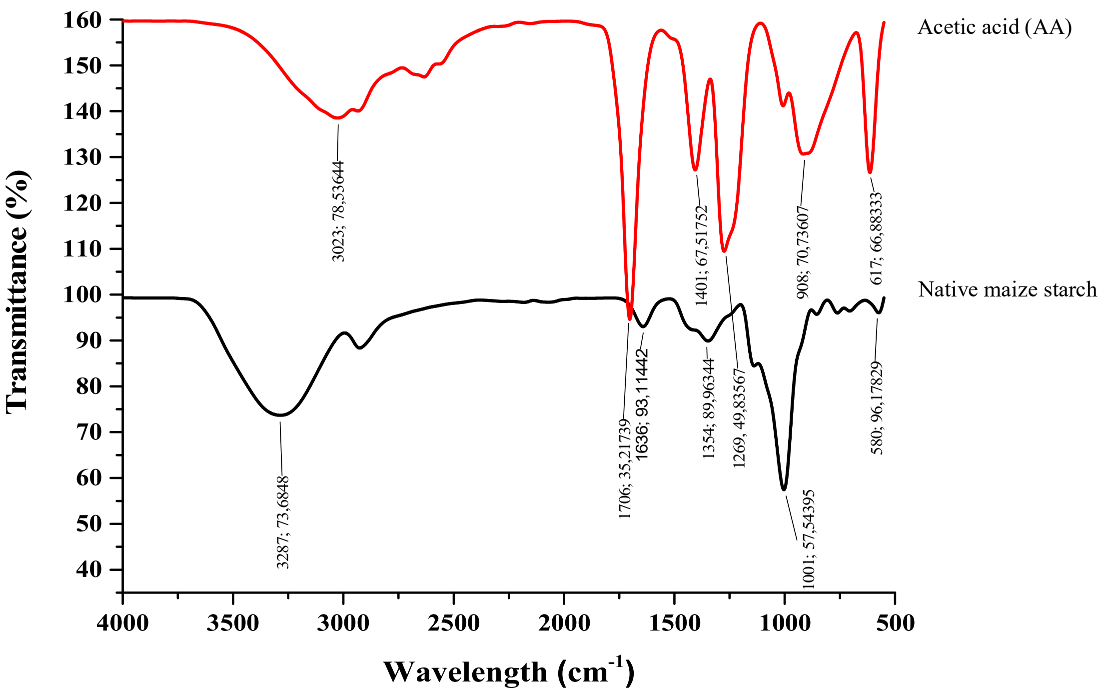


Figure 5. 4: FTIR spectra of acetic acid and native maize starch.

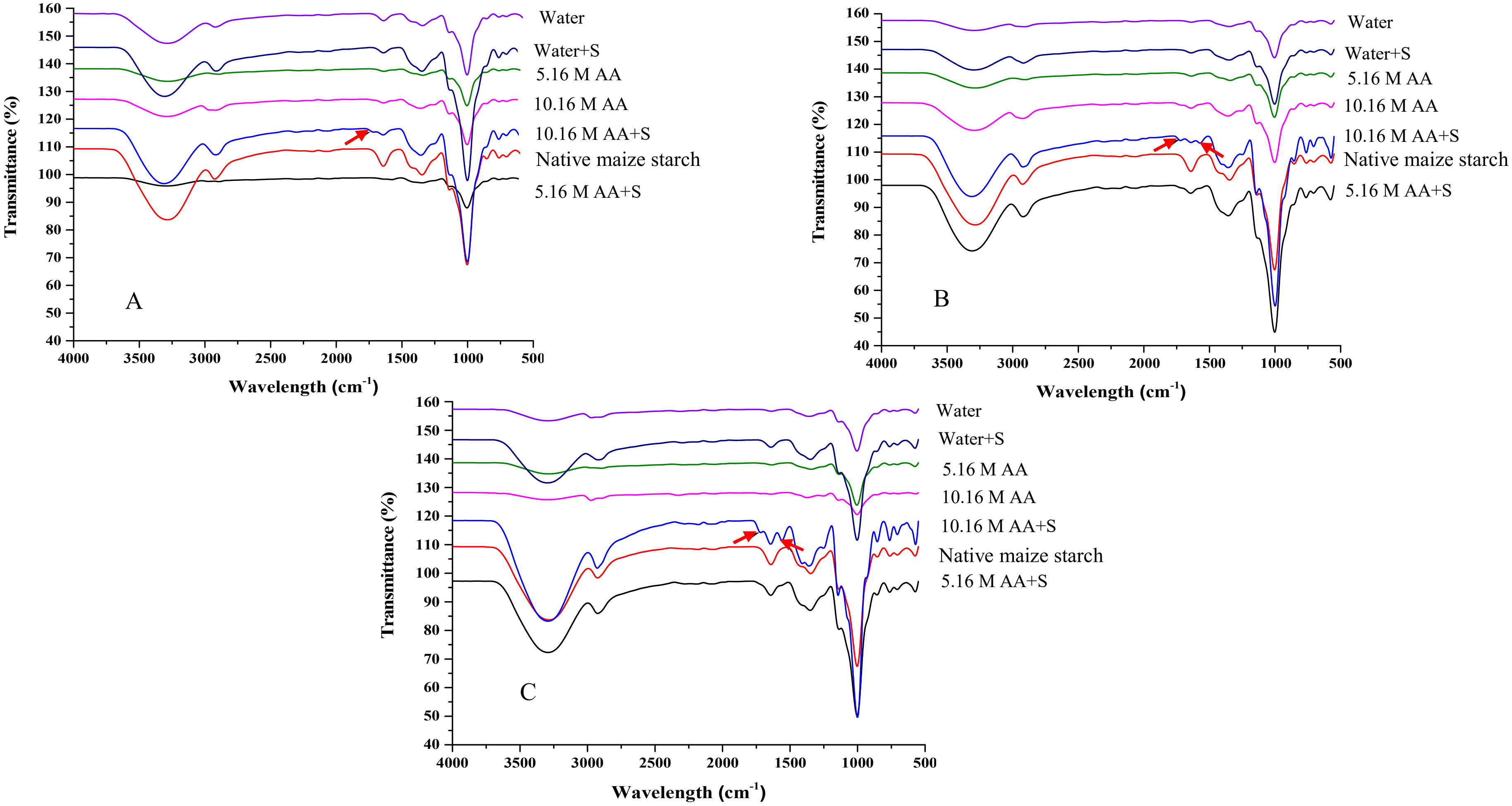
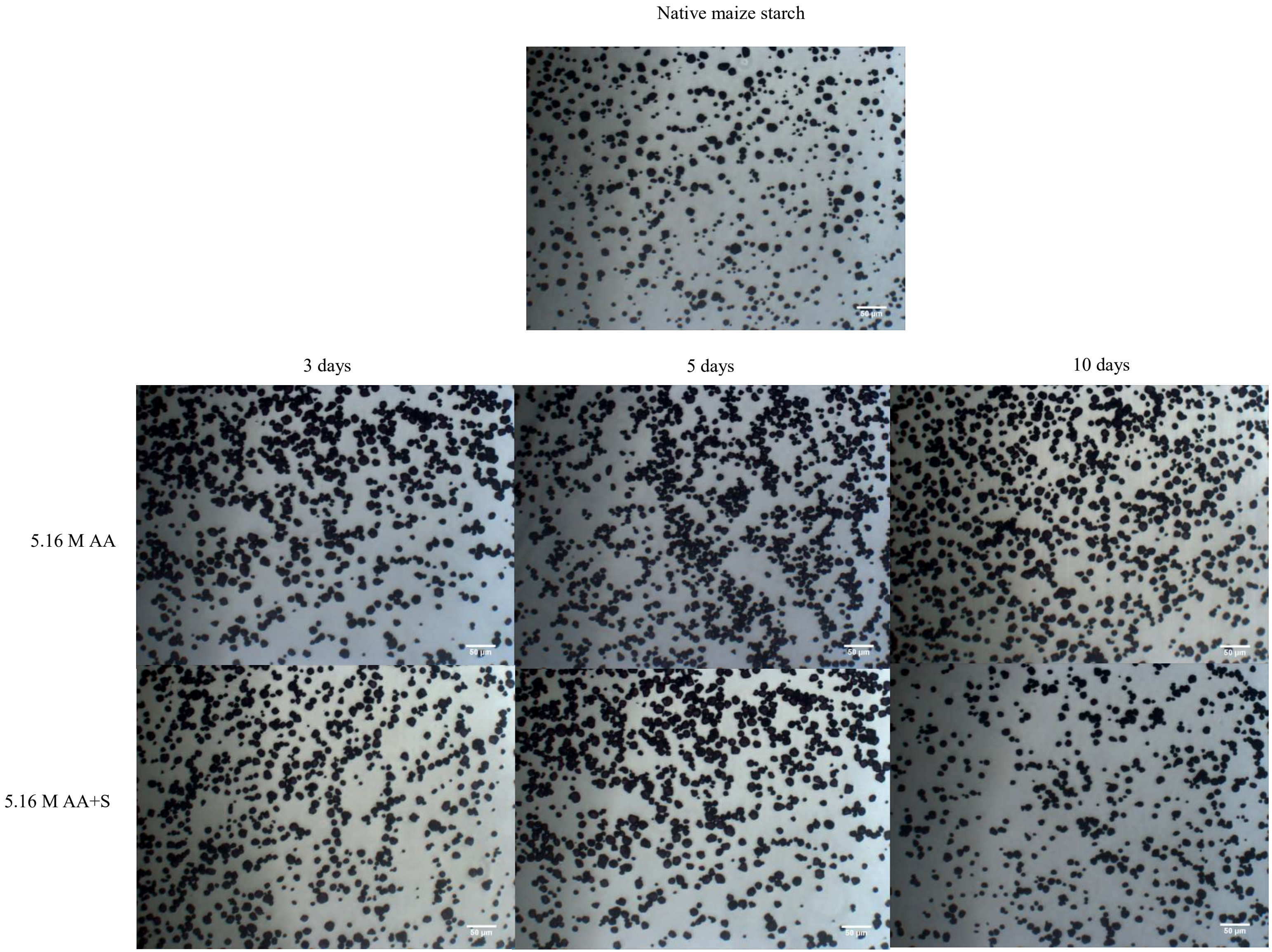


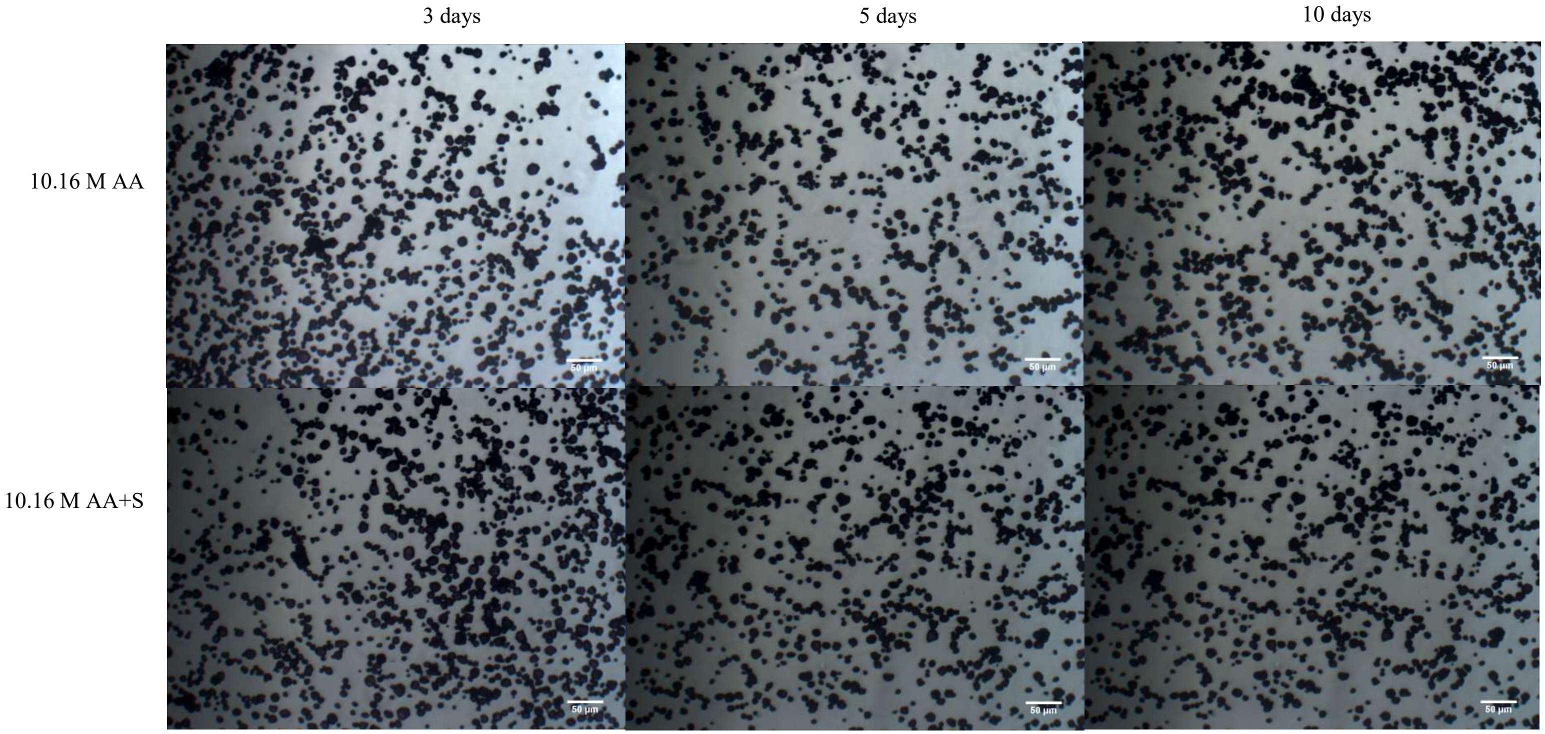
Figure 5. 5: Effects of acetic acid with and without sonication on molecular structure starch for 3 (A), 5 (B) and 10 (C)
AA is acetic acid
10.16 M and 5.16 M is the molarity of acetic acid
S is probe type sonication at 382 W/cm^2 for one hour per day at 40 °C

5.7 Microscopy

Figure 5.6 and 5.7 shows the microstructure of sonicated maize starch acetic acid and water and non-sonicated maize starch for 0, 3, 5 and 10 days under light microscopy and under polarised light microscopy, respectively. It can be seen (Fig. 5.6) that stained native starch granules are similar to sonicated and non-sonicated starch granules in 5.16 M and 10. 16 M acetic acid for 3, 5 and 10 days. Stained native starch granules are also similar to sonicated and non-sonicated in water for 3, 5 and 10 days (Fig. 5.6). Under polarised microscopy, Maltese cross of non-sonicated starch granules in both acetic acid concentrations was bigger as compared native starch granules (indicated arrows, Fig. 5.7). There was an increase in size of Maltese cross of non-sonicated as compared to sonicated starch granules for 3, 5 and 10 days, thus, Maltese cross of non-sonicated starch granules appeared to be larger than sonicated starch granules. On the contrary, the Maltese crosses of sonicated starch granules disappeared in some starch granules in 5. 16 M and 10. 16 M acetic acid with time of treatment, from 3 to 10 days (Fig. 5.7) as indicated by white arrows. There were no obvious differences in Maltese cross between 5. 16 M and 10. 16 M acetic acid concentrations (Fig. 5.7).

Figure 5.8 shows the microstructure of maize starch sonicated in acetic acid and non-sonicated maize starch 0, 3, 5 and 10 days under scanning electron microscopy. These figures show a detail microstructure of the effect of acetic acid with sonication on maize starch granules. As such, it can be seen that 5. 16 M and 10. 16 M with and without sonication at 382 W/cm^2 showed significant changes on the surface of starch granules compared to native starch (Fig. 5.8). The overall shape of the granules appeared not to have been affected by 5. 16 M and 10. 16 M acetic with and without sonication. As indicated by red arrows in Figure 5.8, non-sonicated starch granules exposed to 5. 16 M acetic acid appeared to have pitting after 3 days of treatment compared to 10. 16 M acetic acid at that particular time interval, 3 days. Prolonging the treatment from 3 to 10 days did not show visible pitting as compared to the treatment after 3 days in non-sonicated starch granules in water. However, non-sonicated starch granules exposed to 5. 16 M acetic acid showed more pitting than those exposed to 10.16 M after 3 days. Contrary to non-sonicated starch granules in Figure 5.8, sonicated starch granules appeared to have mostly exo-corrosion, corrosion that is on the surface of starch granule, rather than pitting as in non-sonicated granules as indicated by white arrows. Pitting in sonicated starch was observed to be in random granules. Corrosion was being intense with prolonging of time of treatment (3 to 10 days), since the greatest erosion was 10 days in both acetic acid but mostly in 10. 16 M than in 5. 16 M at the same time frame (Fig. 5.8).


Native maize starch
50 µm
3 days
5 days
10 days
5.16 M AA
5.16 M AA+S
50 µm


3 days
5 days
10 days
10.16 M AA
10.16 M AA+S
50 µm

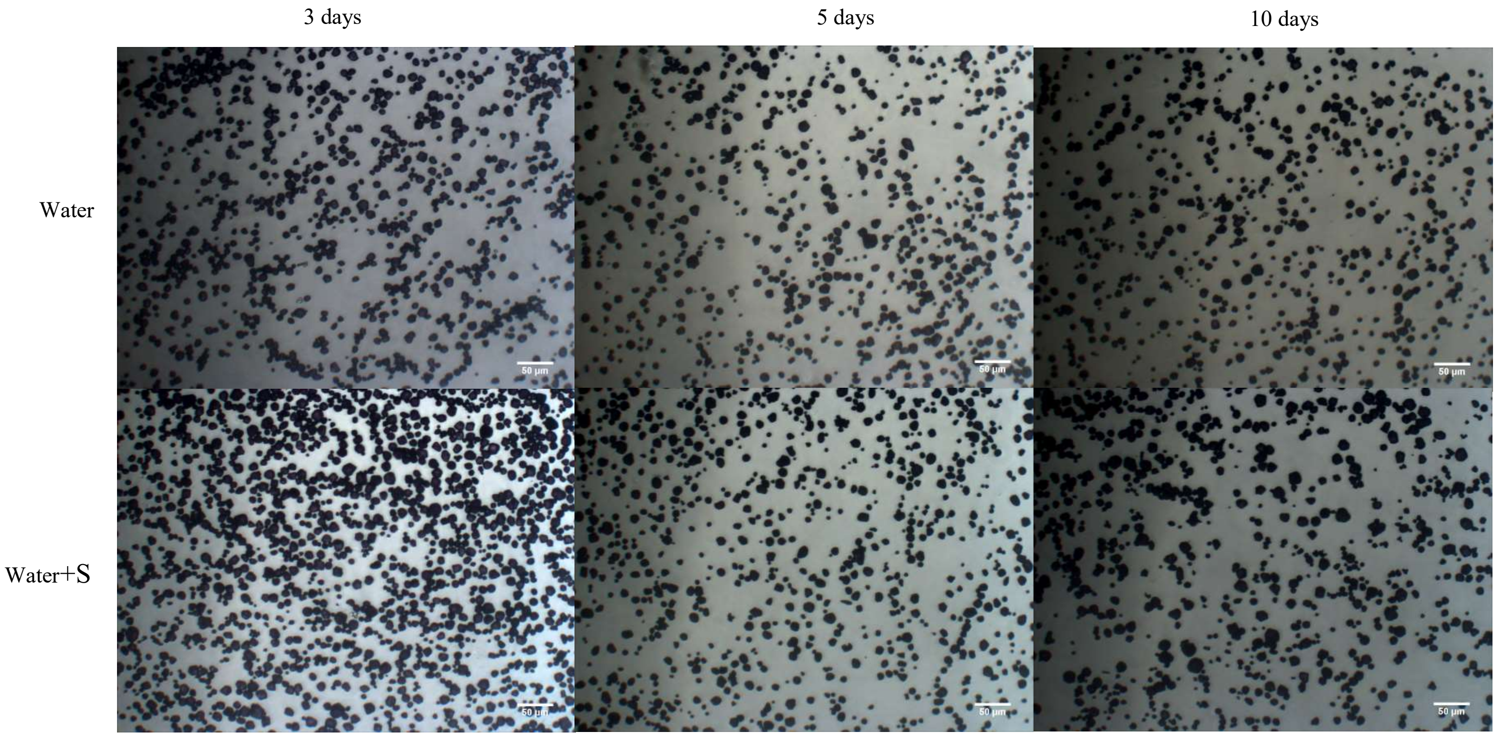
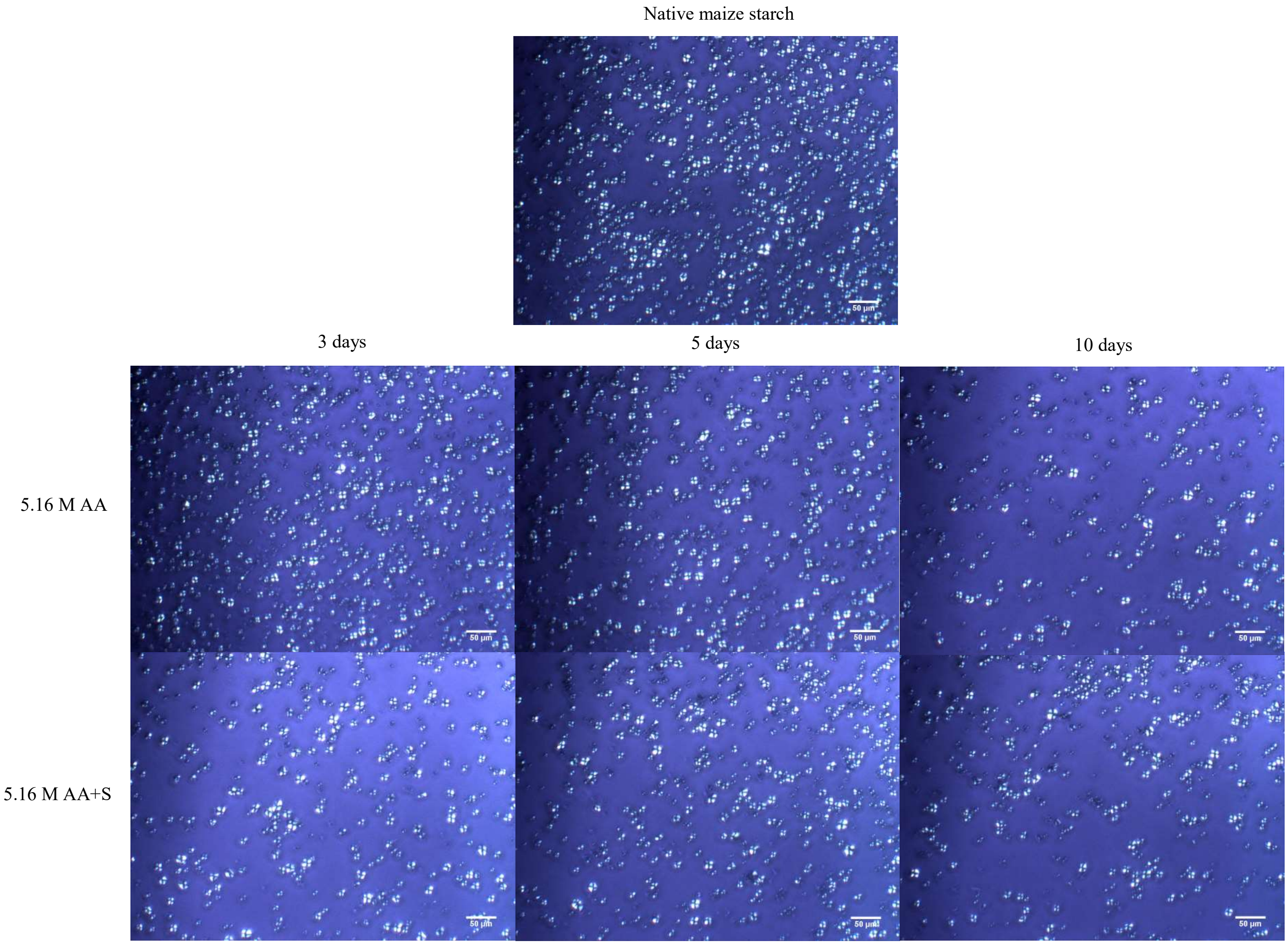
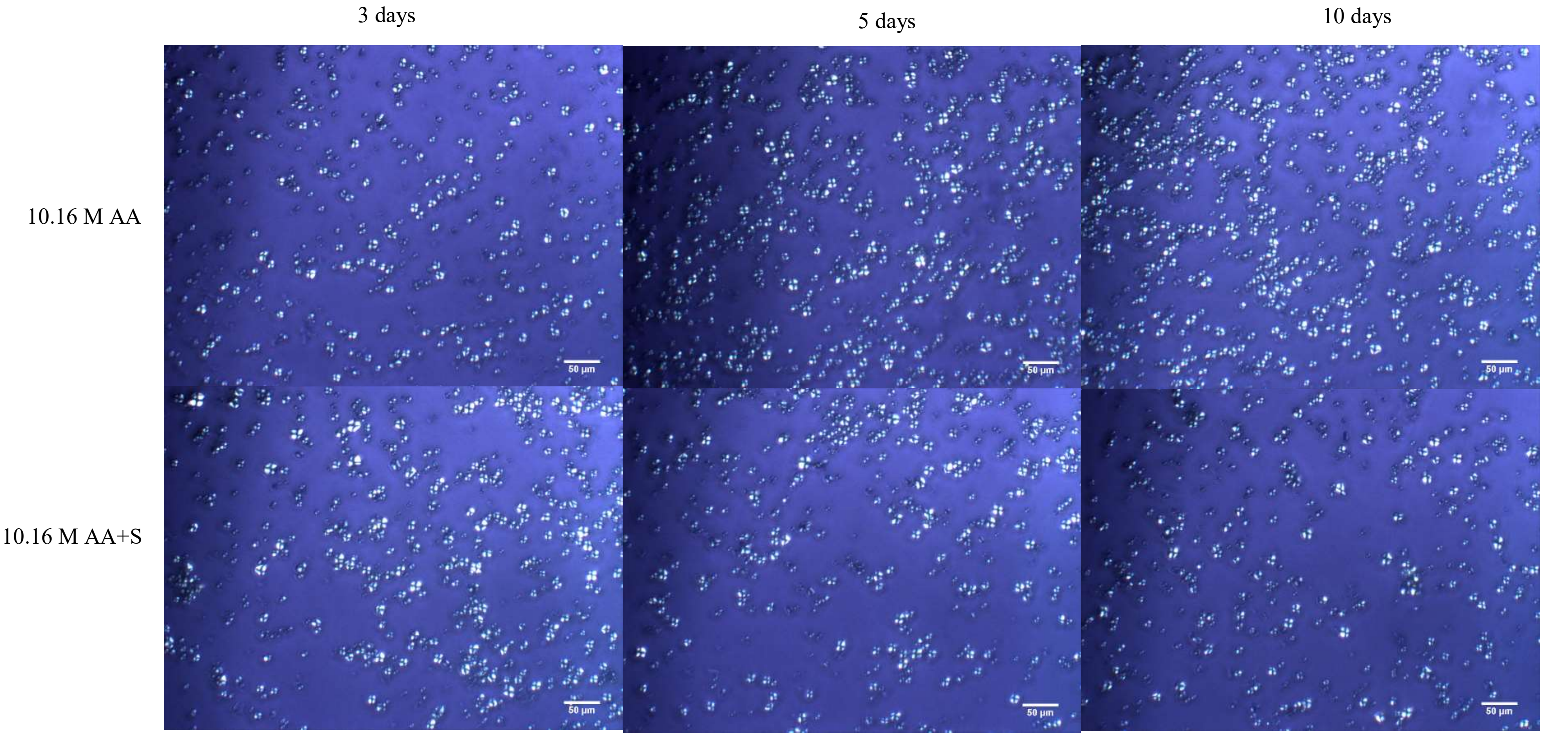


Figure 5. 6: Effects of acetic acid with and without sonication on microscopic structure starch with iodine stain for 3 and 10 days
AA is acetic acid
10.16 M and 5.16 M is the molarity of acetic acid
S is probe type sonication at 382 W/cm^2 for one hour per day at 40 °C


Native maize starch
3 days
5 days
10 days
5.16 M AA
5.16 M AA+S
50 µm


3 days
5 days
10 days
10.16 M AA
10.16 M AA+S
50 µm
50 µm
50 µm
50 µm
50 µm
50 µm

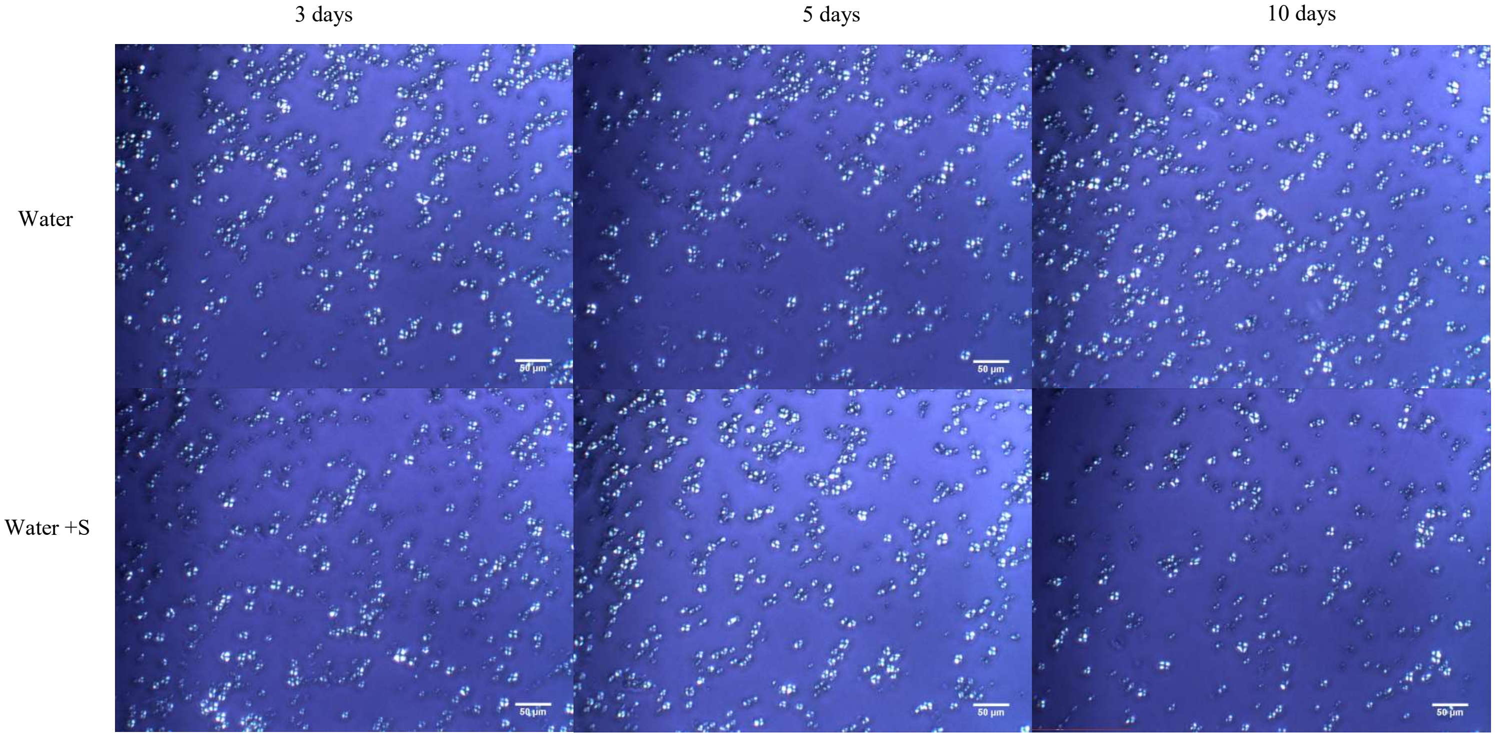
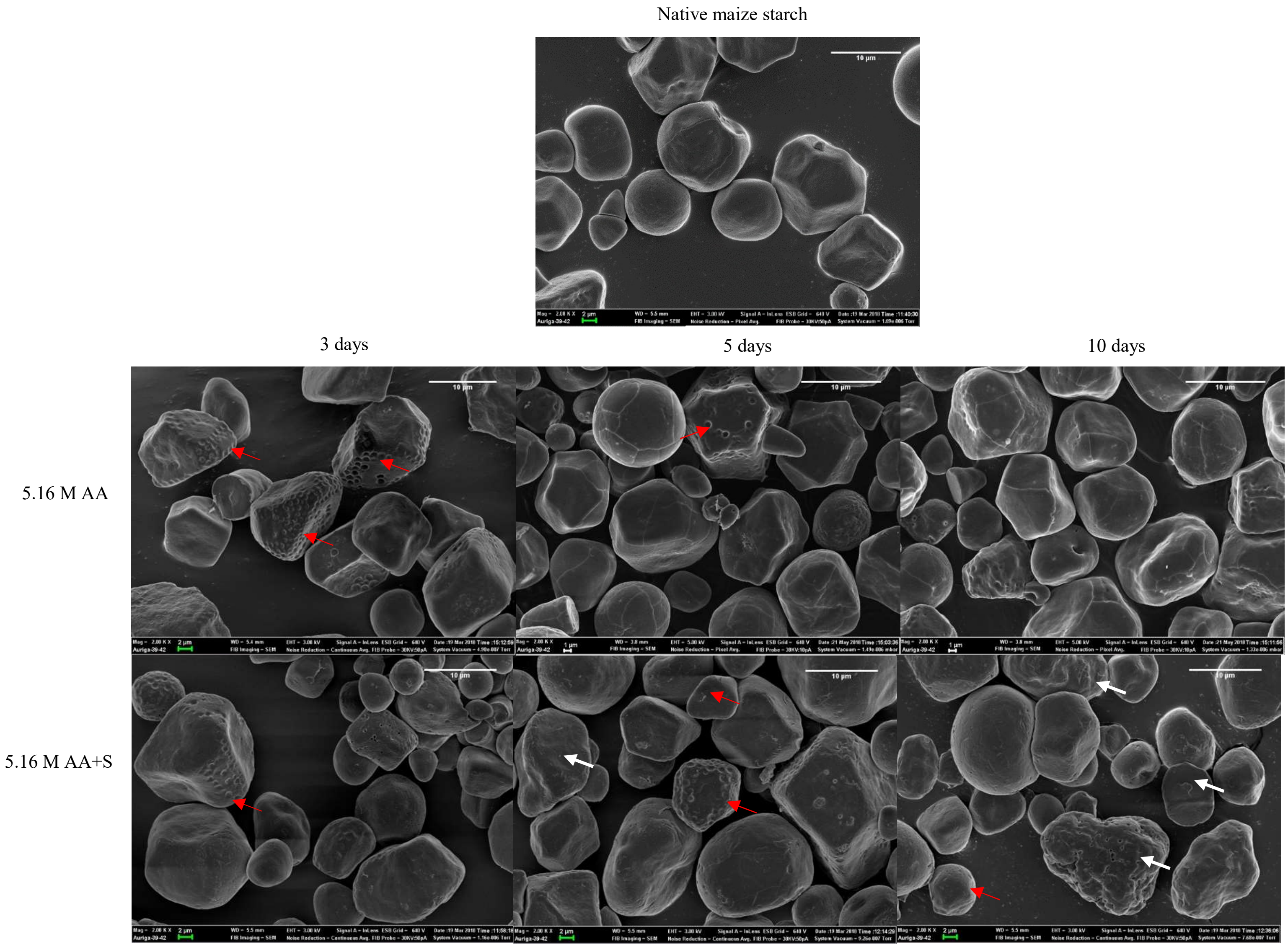


Figure 5. 7: Effects of acetic acid with and without sonication on microscopic structure starch under polarize light microscopy for 3 and 10 days
AA is acetic acid
10.16 M and 5.16 M is the molarity of acetic acid
S is probe type sonication at 382 W/cm^2 for one hour per day at 40 °C



Native maize starch

3 days

5 days

10 days

5.16 M AA

5.16 M AA+S

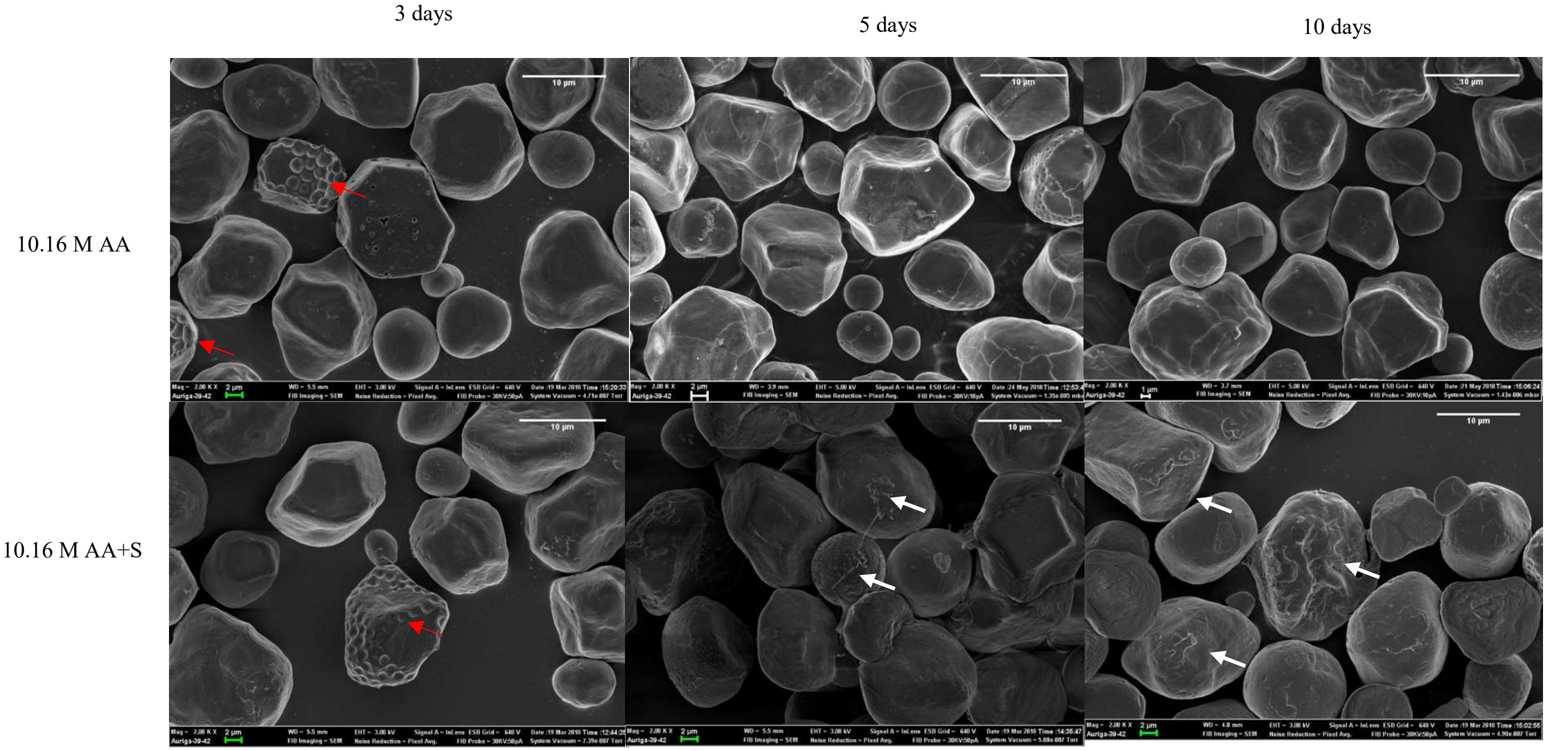


Figure 5. 8: Effects of acetic acid with and without sonication on microscopic structure starch under scanning electron microscopy for 3 and 10 days
AA is acetic acid
10.16 M and 5.16 M is the molarity of acetic acid
S is probe type sonication at 382 W/cm^2 for one hour per day at 40 °C

6 Discussion

The first section of this discussion critically evaluated and discusses methodologies used in this research in terms of principles, strengths and weakness.

6.2 Experimental methodology: Weakness and strengths of the experimental methodology

For this study, acetic acid was used as solvent and compared with water. Acetic acid is regarded as weak organic acid with pKa of 4.74 (Narendranath, Thomas & Ingledew, 2001) and can referred as in clean labelling regulations in most developed counties worldwide (Do Nascimento, Paes & Augusta, 2018). As such, the main objective of this research was to modify maize starch using a solvent that can be regarded as a clean label and safe for human consumption. However, this research showed that acetyl groups can be incorporated in starch without the use of any chemical catalysts such as acetic anhydride as previous study has also shown (Luo & Shi, 2018).

Sonication at an output power of 383 W/cm^2 of a probe type ultrasound apparatus was used in the research. Sonication type was selected based on the effectiveness of probe type as compared to bath type. The probe was inside starch slurry releasing ultrasound waves directly to starch slurry and therefore limiting irradiation distance to a certain area to the probe. Previous studies have reported that acetic acid has high heating rate compared to water (Zhao *et al.*, 2018). As such, an output power of 383 W/cm^2 was applied to standardize heating rate of acetic acid starch slurry and water starch slurry. Results from preliminary trails shows that sonication for 5 min in both acetic acid concentrations shows that the temperature did not exceed 40 ^{o}C, the literature also reported that sonication in ice-bath does not increase the temperature significantly (Monroy, Rivero & García, 2018). Again, preliminary trials also proved to the difficulty of using cold water to control final temperature after 5 min as the temperature would exceed 40 ^{0}C just after 3 min. Ice bath was used to optimize the process as little time as possible. It would have been desirable to measure and record temperatures during sonication process, but this was not possible because the dynamic probe thermometer would also be sonicated and gradually being damaged, hence temperature were only assessed in preliminary trails. Other devices as thermocouple would as also give inaccurate reading because the probe would have been placed inside the slurry after sonication. If any metal probe was to be placed inside during sonication, ultrasound waves would probably erode the probe (São José, Andrade, Ramos, Vanetti, Stringheta & Chaves, 2014) and this would interfere with the analysis such Fourier transform infrared and X-ray diffraction.

In this study, starch in either 5.16 M or 10.16 M acetic acid or water at intermitted times (5 min sonicated and 5 min cooling) for 60 min per day for 3, 5 and 10 days and left in shaking water bath at 40 ºC for 24 h. The temperature of shaking water bath was specifically chosen based in the literature (Muir & O'dea, 1992), with the objective to facilitate modification process. At the temperature of 40 ºC, starch molecules in amorphous region may become more rigid and less mobile that can be related to annealing process (Tester & Debon, 2000).

In this study, sodium hydroxide (NaOH) was used to neutralise acetic acid. Acetic acid is a weak organic acid, that is, does not completely dissociate in water. One mole of NaOH neutralise one mole acetic acid;

$$HC_2H_3O_2 + NaOH \rightarrow C_2H_3O_2NA + H_2O \text{...} (1)$$

This reaction produces sodium acetate that was washed away with 3 successive washing with water since sodium acetate soluble in water, hence NaOH was selected as an effective to remove acetic acid from sonicated and non-sonicated starch. Starch sonicated in water was preserved by 0.02% sodium azide to ensure that microbial growth does not affect the results.

Fourier transform infrared with attenuated total reflection (FTIR-ATR) technique was used in the study. FTIR involves the use of infrared (IR) light. It is basically based on the changes in vibrational and rotational status of the molecules as a result of absorption in the infrared region. As such, absorption intensity depends on the effectiveness of the proton energy transferred to the molecules that ultimately depends on the change in the dipole moment that occurs as a result of molecular vibration, whereas absorption frequency depends on the vibrational frequency of the molecules.

There are limitations that are associated with FITR. One limitation that stand out and significantly affects the results is weak diffuse reflectance (Grdadolnik, 2002). This is due to a decrease in scattering with an increasing wavelength while mid-IR absorptions (4000 to 400 cm^{1}) are orders of magnitude stronger than those in the near-IR. FTIR was used with attenuated total reflection (ATR) technique to improve the accuracy of the results. ATR is based up on internal reflectance as shown in Figure 6.1. A trapezoidal block of transparent material (ATR crystal in Figure 6.1) with relatively high index of refraction in contact with sample with lower index. This ATR crystal internally reflects the infrared beam so that all the infrared beam does not leave crystal and reflected off the internal surface of the crystal. As a results, ATR improved FTIR peaks and the accuracy of the reading on FTIR (Smith, 2011).

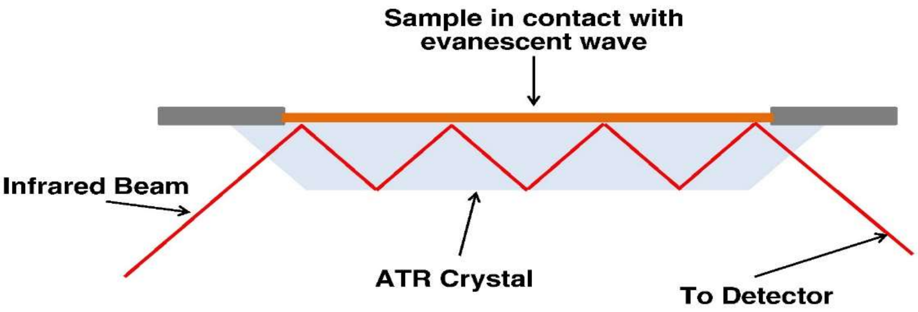


Figure 6. 1: Schematic diagram of an attenuated total reflectance accessory (Shai, 2013)

The infrared beam passes through the attenuated total reflection crystals. Once inside, an evanescent wave will be set up and this evanescent wave scans the material outside the crystal with a distance called depth of penetration (dp). In this way, infrared beam can scan the sample multiple times to get more amplified spectra. Radiation is not transmitted through the samples, making FTIR results more consistence since the thickness of the samples will not affect the outcome of the analyses. One of the limitations on FTIR is that it lacks the ability of quantifying different compound in a sample; this means that a mixture of starch and other polymers such as proteins would only give one FTIR spectra.

The crystallinity of treated and untreated starch were evaluated by X-ray diffraction. Electromagnetic waves strikes the lattice of the atoms in the samples to produce contrastive interference and scatter at specific angles from each set of lattice planes in a sample. The atomic position within the planes of the sample determines intensities of X-ray diffraction peaks (Doyle, 1992).

This method of analyses was selected particularly because starch is a semi-crystalline polymer with a single type of crystal, therefore, simplifying results interpretation. Since starch is semi-crystalline, XRD has also provided more details on the amorphous part of starch. However, XRD has limitations that can lead to faulty results. Various factors can affect the starch crystallinity analysis by XRD, especially water content of starch (Bogracheva, Wang, Wang & Hedley, 2002; Cheetham & Tao, 1998). High water content of starch may decrease the relative crystallinity as compare to high water content (Bogracheva *et al.*, 2002).Amount of disordered amylose and amylopectin which are available to form crystal nuclei increases due to increasing water content and the mobility speed of these two molecules also increase, resulting in more amylose and amylopectin chains rearranged and forming crystals (Ding, Zhang, Tan, Fu & Huang, 2019). In short, starch samples that are exposed to the same treatment with different water content may give varying relative crystallinity. To prevent this, all untreated (including) and treated samples were placed in controlled moisture chamber that was made by 50 % glycerol for 7 days at room temperature to make relative humidity approximately 50 % (Forney & Brandl, 1992) . This was to ensure that all samples were at the same water content after the equilibration process. There were some results that had high coefficients of variation (CV), which indicates great variation of results. This could be as results of the different size of starch granules not being exposed equally to ultrasound waves (No & Son, 2019).

The equilibration was also done samples for thermal properties of treated starch. Firstly, water content of all samples were determined in order to calculate water needed to make a ratio of 1:3 of water. These samples were allowed to equilibrate for 2 h at room to hydrate starch. It was evident that acetic acid as weak acid causes physical damage of starch granules, facilitating water penetration (Diop, Li, Xie & Shi, 2011; Ohishi, Kasai, Shimada & Hatae, 2007). As such, shorter period of equilibration, 2 h, was particular selected for this reason as done by Wokadala, Ray and Emmambux (2012) .

Differential scanning calorimetry (DSC) is the most accepted method used to measure change in enthalpy and gelatinisation temperatures. DSC measures the heat flow and temperature in relative to time under controlled atmosphere. Excessive heat applied to starch induces gradual disruption of semi-crystalline and crystalline region of starch and this process is referred as ‘gelatinisation’ (Bertoft, 2017). DSC was used to analyse this transition over a certain period of time, with the aim of understanding thermal properties of treated starch in contrast to native starch.

Samples in excess amount of water inside aluminium pan were heated in elevated temperatures in comparison to reference (empty aluminium pan). Anything change in transition in the sample was monitored by DSC. As a result, starch ordered structure gradually became disordered structure and absorb and release energy as a result of this transition. DSC mainly analyse semi-crystalline and crystalline region parts of the starch and provide less information about the amorphous region or glass transition of the amorphous regions (Xie, Liu, Liu, Wang, Halley & Yu, 2010). As such, DSC provide details about crystalline region, this means that any change to gelatinisation temperature could indicated acetic acid with ultrasound induced changes to the crystalline region. However, the discontinuity in heat capacity at glass transition is often too small to be measured by DSC heated in excess water (Xie *et al.*, 2010). Therefore, other analyses such XRD had to be done to compliment DSC. Modulated temperature DSC would have been alternative method to further study glass transition of the amorphous regions of gelatinised starch as studied by Xie *et al.* (2010).

6.3 Discussion of results

Pasting properties of maize starch treated with both acetic acid concentrations (5.16 and 10.16 M) and without sonication were similar to pasting properties to maize starch sonicated in both acetic acid concentrations. Pasting temperatures of starch treated with both sonication and acetic acid decreased with treatment time. This trend was also observed in water absorption and water solubility indexes. The reflected effect of the combination of acetic acid and sonication on starch was time dependent. The time based trend of decreasing peak viscosity (PV), breakdown viscosity (BV), setback viscosity (SV) and final viscosity (FV) observed here were similar to that reported by Shimada and Hatae (2007). These changes in pasting properties indicates that there was modification either in chemical, microstructure and physical properties.

Pasting temperatures of all treated starches decreased regardless of time of treatment as shown in Rapid viscosity analyses (RVA) in figure 8. The DSC shown that there was decrease in onset temperature on all treated starches. This difference could be as a result on the method used. Viscosity of starch increases as soon as starch starts to absorb water and swell, this was observed in RVA. While DSC analyzed this process at the molecular level when heat and water disrupt the crystalline starch of starch. Majzoobi, Seifzadeh, Farahnaky and Mesbahi (2015b) observed that pasting temperatures decreases with increasing concentration of acetic acid and lactic acid in both wheat and cross-linked wheat starches when analyzed by DSC. Pasting temperatures had decreased in rice starch sonicated in distilled water with 100, 500 and 1000 W output power and frequency of 24 kHz. A decrease in pasting temperatures indicates the disruption in amorphous region of starch. Lin, Lin, Zeng, Wu and Chang (2018) observed a decrease in molecular weight of starch molecules when acetic acid as added to starch solution. Molecular weight of potato, wheat, rice and maize starches were reported to have decreased sonication (20 kHz, 170 W for 30 min) when stained with iodine. XRD and polarized light microscope also provided insight in the decrease in pasting temperature.

There were increases in relative crystallinity analyzed by XRD in treated starches. An increase in relative crystallinity could be as results of disrupted amorphous region which made crystals more rigid. Maltese crosses observed under polarized light microscopy appeared to not have changed when compared to native starch. This also suggests that crystalline region was not significantly affected by acetic acid and sonication.

The peak viscosity of starches sonicated in 5.16 M and 10.16 M acetic acid and in water, increased for the first 3 days of treatment and decreased thereafter. The increase in peak viscosity could be due to an increase in water absorption of treated starches as observed in water absorption index. Sonicated waxy rice starch was reported to have a slight increase in peak viscosity and swelling power with 0.18 W/cm^2 under treatment temperatures of 25 and 63 °C (Jenny Yue Zuo, 2009), while sonicated maize starch was observed to have decreased peak viscosity and swelling power with 400 W power and frequencies of 40 kHz, 25 kHz and 20 kHz (Hu *et al.*, 2015). The variation in these observation could be due to power, temperature, starch source and frequency of the ultrasound (Zhu, 2015).

As such, an initial increase in peak viscosity and water absorption index could also be attributed by microscopic pores/depressions that facilitated water penetration inside starch granule, resulting in increase in water absorption. These microscopic pores were observed under scanning electron microscopy (Fig. 5.8). Figure 6.2 shows schematic diagram of increased peak viscosity as a result of microscopic pores that were observed on SEM in starch granules with sonication. While figure 6.3 shows microscopic pores facilitated water penetration inside the granules, resulting in increased swelling of the granules. Sonicated starch granules in 5. 16 and 10.16 M acetic acid had lower peak viscosity compared to starch sonicated in distilled water probably due to the increased pore size and severe physical damage caused by acetic acid and sonication. These two factors made starch granules to be fragile and easily disrupted during stirring.

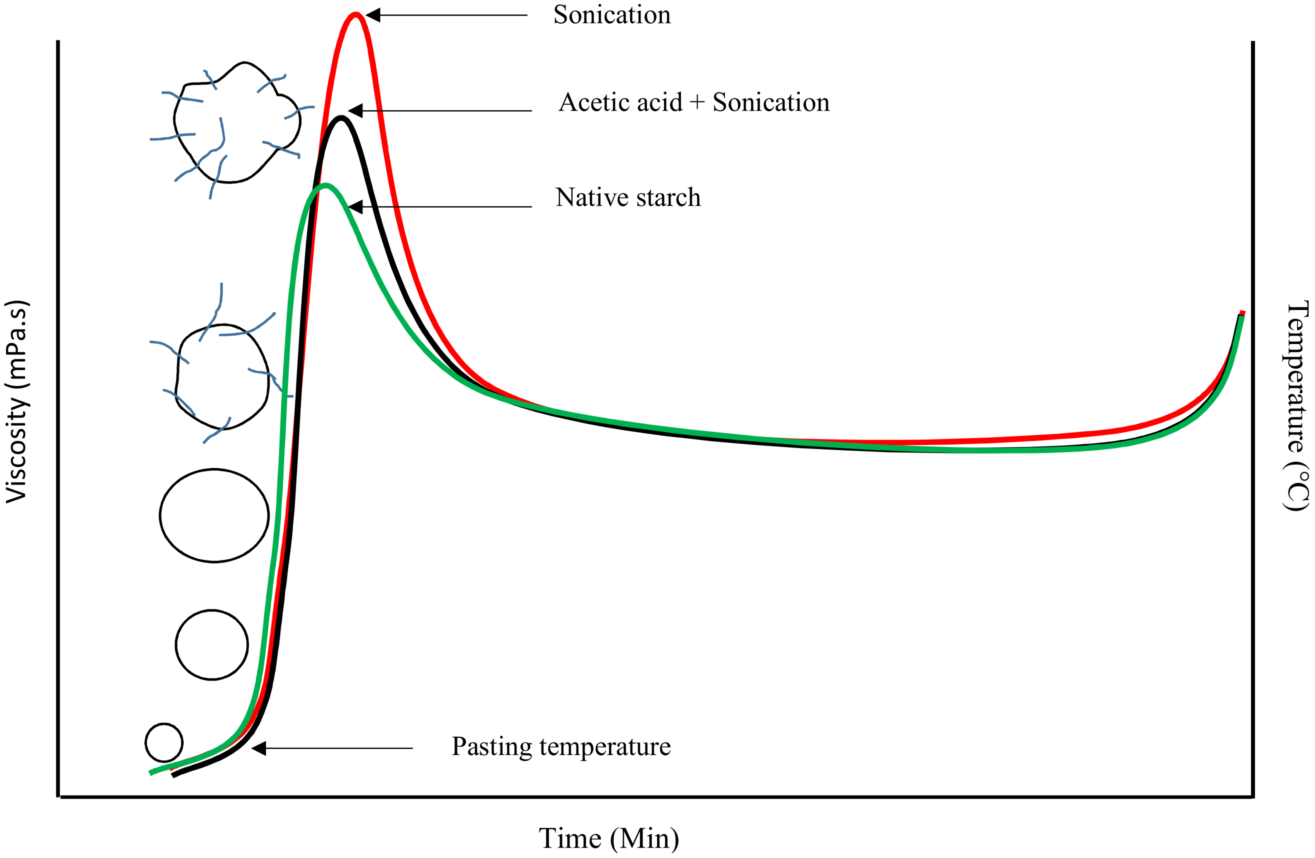


Figure 6. 2: Schematic diagram of starch granules with microscopic pores with increased peak viscosity in pasting profile.

Several authors reported that ultrasound caused physical damage to starch granules due to the cavitation that creates high pressure locally and shear force of the ultrasound waves (Bai *et al.*, 2017; Hu *et al.*, 2013). Sonication could have mainly attributed to the increase in peak viscosity and water absorption index that later decreased while acetic acid was partially hydrolyzing amylose and amylopectin. Hu *et al.* (2013) reported that ultrasound at 0.17 W/cm^2 intensity decreased viscous resistance of starch due to disruption of macromolecular starch chains and crystalline region. In addition, the microscopic pores were probably caused by powerful shock waves in acetic acid and starch solution, and also by micro jet produced by rapid collision of ultrasonic cavitation bubbles around starch granules.

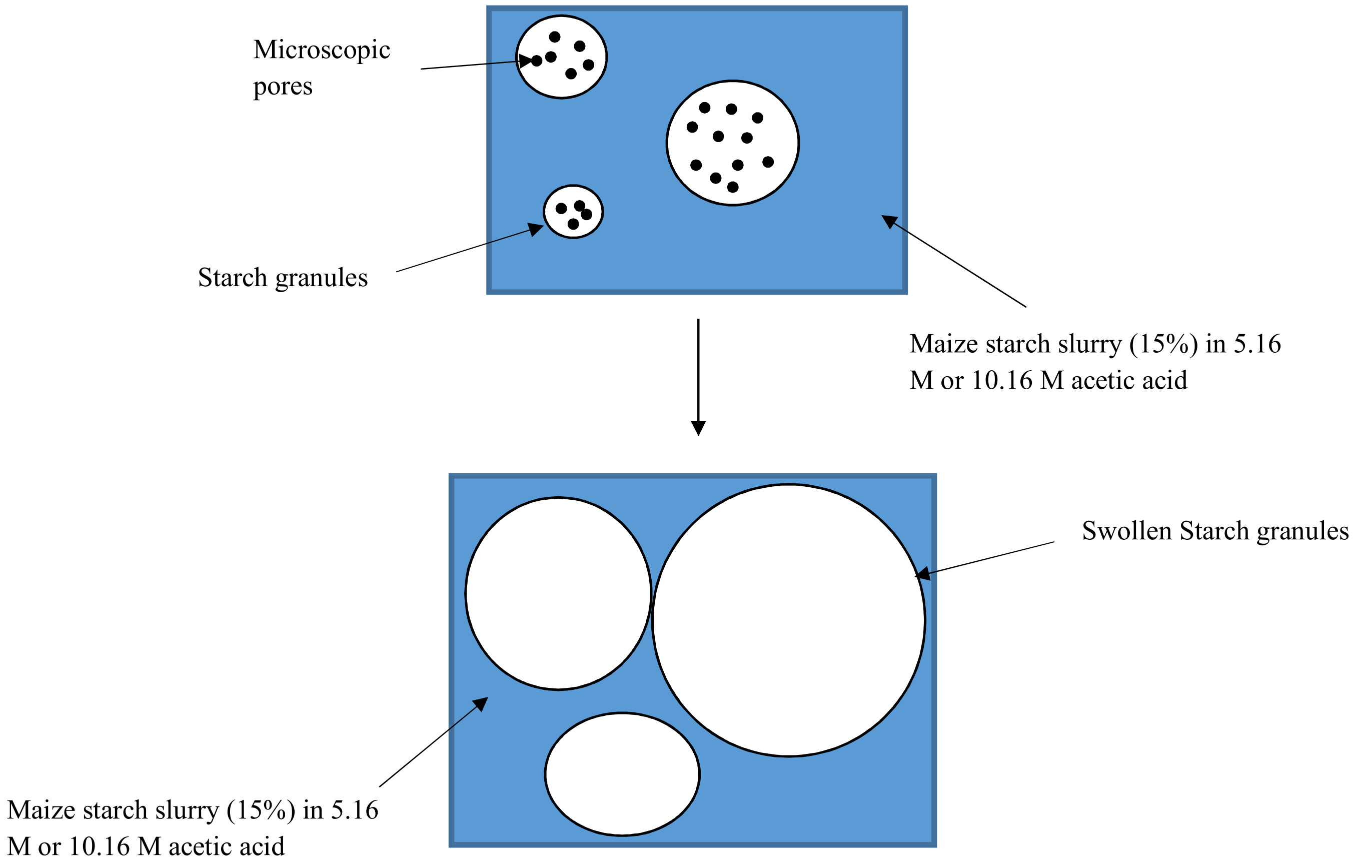


Figure 6. 3: Schematic diagram of starch granules with microscopic pores that assist with water penetration resulting in increased swelling.

Furthermore, initial increase (increased from 0 to 3 days then decreased) in peak viscosity could be as a result of microstructural change by sonication with and without acetic acid. Several research have hypothesed and shown that starch granules do not show complete swelling until amylose has been leached out of the granules (Karim, Nadiha, Chen, Phuah, Chui & Fazilah, 2008; Bowler, Williams & Angold, 1980). Thus, it seems that amylose in the study was able to leach out of the granules with the assistance of these microscopic pores and increasing the peak viscosity and water absorption index of starch granules.

Peak viscosity decreased after increasing from 3 days in starches sonicated in 5.16 M and 10.16 M acetic acid and in water. Water absorption index also decreased at this time. The decrease in peak viscosity suggests the degradation of amylopectin in the crystalline region. Crystalline analyses by XRD showed that the relative crystallinity generally increased, especially in starch sonicated in 10.16 M acetic acid. Decrease in peak viscosity could be as a result of physical damage to starch granule. SEM images showed that some starch granules had pitting or erosion on the surfaces. Physical damage caused ultrasound resulted in fragile starch granules which were easily disrupted during stirring. Mohammad Amini, Razavi and Mortazavi (2015) observed that sonication induced physical damage to maize starch granules and swelling of starch granules increased as well, this was depended on temperature and exposure. Acetic acid has also been reported to partially cause damage to maize starch granules (Majzoobi *et al.*, 2016; Majzoobi *et al.*, 2015b). Acetic acid can hydrolyse starch polymers causing a lower molecular weight polymer. This lower molecular weight could have caused a lower viscosity. The molecular weight of the starch polymers was not determined in the study, thus there is merit to determine the molecular weight in future studies.

Breakdown viscosity, setback viscosity and final viscosity of starch sonicated in 5.16 M and 10.16 M were lower than those of starch exposed to sonication alone. Similar results had been reported in maize starch in acetic acid (Ohishi *et al.*, 2007) in rice, and for maize starch in distilled water exposed to sonication with output nominal power of 150 W, temperature ranging from 25-65 °C and ultrasound amplitude of 50 and 100 % (Mohammad Amini *et al.*, 2015). Breakdown viscosity was also observed to decrease in all treated starches, suggesting that starch granules were fragile to stirring.

The solubility index of all treated starches generally increased more with higher temperatures (90 °C) than lower temperatures (50 °C), suggesting that treated starch was susceptible to heat. Water solubility index of starch treated with 5.16 M and 10.16 M acetic acid with and without sonication showed significant increase than starch that was sonicated in distilled water, suggesting that acetic acid attributed more this effect. There was also a gradual decrease in gelatinization temperatures of starch that was treated in both acetic acid concentrations with and without sonication. Zhigang, Xiong, Xiaowei, Faxing, Qunyu and Shujuan (2008) reported an increase in solubility of normal maize, waxy and amylomaize starches with increasing temperature (55-95 °C) after sonication with 100 W output powder. An increased in water solubility of starch indicates possible disruption of the amorphous region and could be attributed by hydrolysis of intermolecular bonds of starches and granular arrangements were less compact (Zhigang *et al.*, 2008).

Acetic acid dissociates in the presence of water and produce protons that can results in destabilization and hydrolysis of glycosidic bonds (Mahsa Majzoobi, 2016). Acetic acid as weak acid releases the hydrogen ions H^+ proton as a results of electronegativity differences of oxygen and hydrogen in OH group in acetic acid and dipole interaction with water as indicated:

$HC_2H_3O_2 \rightleftharpoons H^+ + C_2H_3O_2^-$……………………………………………………………..(2)

Where H^+ is hydrogen ion and $C_2H_3O_2^-$ is conjugated base (acetate ions) of acetic acid. In the presence of water, Acetic acid donates H^+ to water molecule to form Hydronium ions, since protons do not exist naked in solution:

$HC_2H_3O_2 + H_2O \rightleftharpoons H_3O^+ + C_2H_3O_2^-$…………………………………………......(3)

This consequently decrease the pH of the solution and further hydrolyze starch molecules. Hydrolyses could have taken place in the α-(1,4) glycosidic bonds mostly in amorphous region (Fig. 6.4).

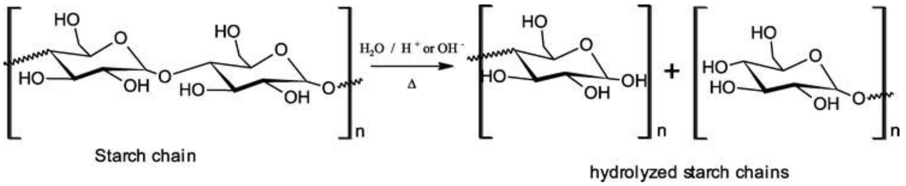


Figure 6. 4: Hydrolysis of α(1,4) glycosidic bond as a results of dissociation of acetic acid (Egharevba, 2019)

Furthermore, free radicals that were produced as a results of ultrasound may cause depolymerization of amylopectin (Iida *et al.*, 2008). Treated starches and native starch showed increased water solubility index at high temperature (90 °C) due to partial hydrolysis of hydrogen bond between amylose and amylopectin and therefore exposing hydroxyl groups. Denardin and Silva (2009) reported that hydrogen bond between amylose and amylopectin are broken during gelatinization process, exposing hydroxyl groups and increases solubility.

Final viscosity of starch sonicated in 5.16 and 10.16 M acetic acid and in distilled water decreased compared to native starch final viscosity, reflecting to a partial hydrolyses of amylose in the amorphous region. There was an increase in relative crystallinity of starch treated in 5.16 M and 10.16 M acetic acid with and without sonication but mostly in starch sonicated in acetic acid. Amylose are responsible for short term retrogadation or firmness of the starch gel (Xu, Fan, Ning, Wang, Jin, Lv, Xu & Xu, 2013)**,** while amylopectin is responsible for long term retrogadation (Wang, Li, Copeland, Niu & Wang, 2015). As such, final viscosity can be associated with re-association of amylose. Final viscosity of starches sonicated in 5.16 and 10.16 M acetic acid and in distilled water generally decreased with time of treatment**.** Majzoobi, Kaveh, Blanchard and Farahnaky (2015a) observed a decrease in firmness and cohesiveness of maize and wheat pregelatinized starches with increasing concentration of acetic acid. Zhigang, Xiong, Xiaowei, Faxing, Qunyu and Shujuan (2008) reported that sonicated native starch had a decreased syneresis, amount of water released during retrogadation. Soft gel behavior of starch sonicated in 5.16 M and 10.16 M acetic acid observed here are similar to gel behavior of acetylated starch gels (Sun, Zhang & Ma, 2016). The observed decrease in final viscosity and soften gel behavior could also be attributed by new formed peak on observed in FTIR in Figure 5.5. This new peak as discussed suggested some acetyl groups substituted starch to reduce gelling.

Starch sonicated in 5.16 M and 10.16 M acetic acid for 3, 5 and 10 days were observed on FTIR to have a new peak (1720 cm^{-1}) which could be associated with acetyl groups as reported in the literature (Xu, Miladinov & Hanna, 2004). More profound peaks were noted on starch sonicated in 10.16 M acetic acid. Most studies report acetylated starch with the assistance of acetic anhydride (Colussi, Pinto, El Halal, Vanier, Villanova, Marques E Silva, Da Rosa Zavareze & Dias, 2014). However, acetylation here did not happen with the assistance of any catalyst. Sonication may have assisted with acetylation. Ultrasound waves causes the bubbles that were initially created to collapse, resulting in decomposition of water molecules and formation of free radicals as such hydroxide (-OH) and hydrogen (-H) radicals. Hydroxyl groups can interact to be substituted with acetyl group, and hydroxyl groups have been reported to differ in their reactivity with Carbon 6 having the most reactive followed by Carbon 2 and 3 due to steric hindrance of glucose molecules (Wojeicchowski, Siqueira, Lacerda, Schnitzler & Demiate, 2018). Carbon 6 OH located on the exterior surface in starch polymer is readily available and reacts easily with dissociated conjugate base of acetic acid, while two other OH on Carbon 2 and Carbon 3 located in interior surface of starch formed hydrogen bonding with OH groups of neighboring glucose units. Radicals that were created during sonication may have assisted in altering the starch and facilitate the penetration of acetic acid into starch granules. Shabana, Prasansha, Kalinina, Potoroko, Bagale and Shirish (2019) reported that free radicals produced during sonication may depolymerize glycosidic polysaccharides bonds in amylose within the amorphous regions since this area is less ordered, and Luo and Shi (2018) reported that the process of acetylation mostly happens in the amorphous region with acetic anhydride assisting in penetration. As such, free radicals' formation and physical damage of starch due to sonication may have facilitated the acetylation process.

Acetylation of starch sonicated in 10.16 M may had occurred as follows; acetic acid dissociated into conjugated base and hydrogen ion which was donated to water molecule to form hydronium ions. Free radicals produced from water as a results of ultrasound waves partially hydrolyze starch molecules and activates OH on Carbon 6. Conjugated base of acetic acid reacts with OH in Carbon 6 to add acetyl group:

$$HC_2H_3O_2 + H_2O \rightleftharpoons H_3O^+ + C_2H_3O_2^-$$

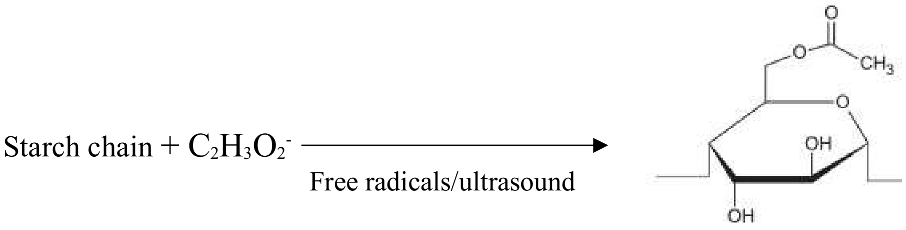


Figure 6. 5: Schematic diagram of proposed mechanism of acetylation of starch using the assistance of sonication. Acetylated starch adapted from Teodoro, Mali, Romero and De Carvalho (2015).

As stated above, thermal properties and relative crystallinity of starch treated in 5.16 M and 10.16 M acetic acid with and without sonication appeared to be affected. The increase in relative crystallinity of starch was consistent with results observed by (Qiang *et al.*, 2007) on sonicated maize starch but different from those reported by Zheng *et al.* (2013) on sonicated maize starch**.** Decrease in gelatinization temperatures were similar to gelatinization temperatures of sonicated maize starch at output power of 500 W reported by Qiang *et al.* (2007) but contrary to those obtained by Yu, Zhang, Ge, Zhang, Sun, Jiao and Zheng (2013) on sonicated rice starch with an output power of 100-1000W. Results were different probably due applied power of sonication.

T_p which indicates the quality of the crystallinity related to double helix length (Sang, Bean, Seib, Pedersen & Shi, 2008) generally decreased indicating that acetic acid with sonication partially disrupted double helix length in the crystalline region. Peak temperature (T_p) and endset temperature(T_c) may have been influenced by the structural architecture of crystalline region, which correspond to the distribution of the length of amylopectin and not the proportions of crystalline region (Miao, Zhang, Mu & Jiang, 2011), as measured by XRD and observed under polarized light microscopy**.** Low T_p and T_c of native starch reflect the presence of abundant short amylopectin chains (Miao, Jiang, Zhang, Jin & Mu, 2011). Acetic acid with sonication may have resulted in shorter molecules of amylopectin. The FTIR showed that there was an incorporation of acetyl group possibly in the amorphous region. Decreased enthalpy suggest less pronounced thermal energy to long amylopectin double helices during gelatinization. The change is enthalpy is mainly due to disruption of double helices rather than the loss of crystallinity, quality and/or amount to crystallites of amylopectin (Cooke & Gidley, 1992). A decrease on ΔH was attributed by the incorporation of acetyl groups in starch molecule to increase the free volume inside the chain, reducing the tension caused by intermolecular hydrogen bonds (Diop *et al.*, 2011)..

Lower firmness of starches treated with both sonication and acetic acid and treated with acetic acid alone reduced probably due to partial hydrolysis of starch molecules, especially amylose in the amorphous regions and this was probably caused by acetic acid, and high pressure created by cavitation and shear force of ultrasound probably had less effect on degradation of starch molecules (Hu *et al.*, 2013). Amylose in the amorphous region was probably partially hydrolyzed by acetic acid resulting in smaller molecules and then were incorporated in crystalline region creating more crystallinity as observed in XRD analyses. Lin, Lin, Zeng, Wu and Chang (2018) reported acetic acid produced small molecules that were more stable in water. The decrease in firmness and final viscosity could also be explained by the incorporation of acetyl groups in starch molecules as reported in acetylated starches (Luo & Shi, 2018; Colussi *et al.*, 2014). The incorporation was observed mostly in starches that were sonicated in both acetic acids, but mostly in 10.16 M acetic acid this study. The incorporation of acetyl groups may have prevented the re-association of both intra- and inter-molecular hydrogen bonding as shown Fig 18. Since amylose and amylopectin cannot re-associate due to acetyl groups, junction zones formation were prevented and water could easily escape figure 6.6. The degree of incorporation increased with time of treatment as shown by FTIR.

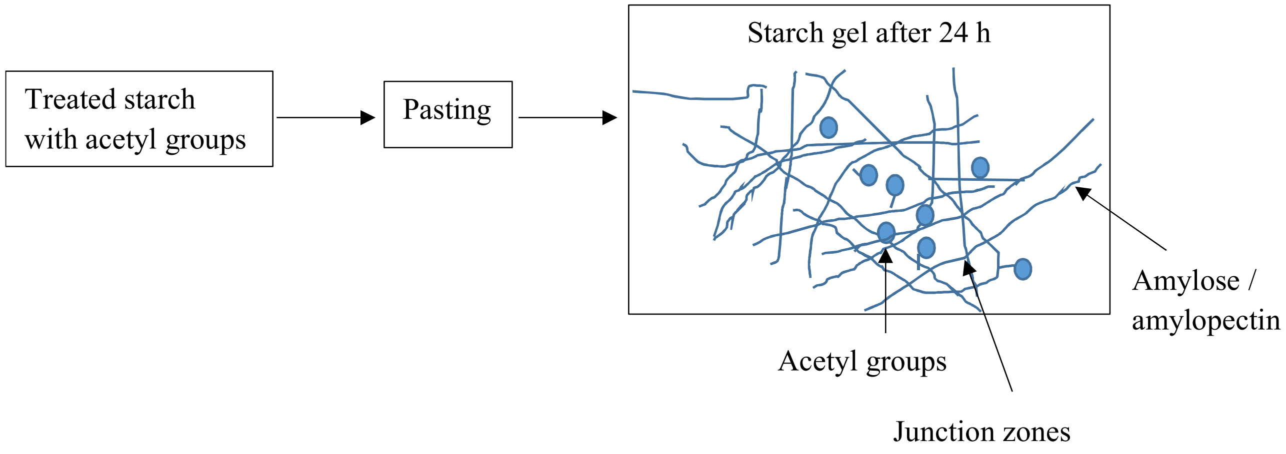


Figure 6. 6: Schematic diagram of treated starch with acetyl group incorporated in starch after pasting to form a gel after 24 h.

7 Conclusions

Ultrasound of maize starch in water causes some mircostructural changes as pitting and micropores as a result micro-jet and shear force resulted from collapse bubbles which were induced by ultrasound waves. These microstructural changes result on higher water absorption to cause a higher peak viscosity during pasting.

Acetic acid with sonication appears to lower pasting temperatures of maize starch. This is due the disruption of amorphous region as a result of dissociation of acetic acid into conjugated base in the form of acetate ions and hydrogen ions in the form of hydronium ions. This consequently drops the pH of the solution. At the same time, collapsing bubbles as results of ultrasound waves decompose water molecules into free radicals. Hydronium ions together with free radicals from decomposition of water depolymerize α-(1,4) glycosidic bond of amylose in amorphous region. This also decreases the gelatinization temperatures of maize starch.

Prolonging exposure of both acetic acid and sonication to maize starch increases relative crystallinity of maize due to reorganization of small molecules hydrolyzed from amorphous region. These small molecules are able to bind in the crystalline region via hydrogen bonding. Free radicals also assist in disruption of the amorphous region by hydrolyzing α-(1,4) glycosidic bonds in amylose to produce small molecules.

Acetyl group incorporation in starch is responsible for softness of the gel. Acetyl groups prevents the formation of junction zones that usually form as a results intra and inter re-associations of amylose and amylopectin via hydrogen bonding. As such, acetyl group interface with this association, resulting in soft gel.

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