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# Effect of Acid Hydrolysis on Starch Structure and Functionality: A Review

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**Abstract:**

Acid hydrolysis is an important chemical modification that can significantly change the structural and functional properties of starch without disrupting its granular morphology. A deep understanding of the effect of acid hydrolysis on starch structure and functionality is of great importance for starch scientific research and its industrial applications. During acid hydrolysis, amorphous regions are hydrolysed preferentially, which enhances the crystallinity and double helical content of acid hydrolysed starch. This review discusses current understanding of the effect of acid hydrolysis on starch structure and functionality. The effects of acid hydrolysis on amylose content, chain length distribution of amylopectin molecules,

 

molecular and crystalline organization (including lamellar structure) and granular morphology are considered. Functional properties discussed include swelling power, gelatinization, retrogradation, pasting, gel texture and in vitro enzyme digestibility. The paper also highlights some promising applications of acid hydrolysed starch (starch nanocrystals) in the preparation of biodegradable nanocomposites, bio-hydrogen and slowly digestible starch-based healthy foods.

**Keywords** starch, acid hydrolysis (Lintnerization), amylose, amylopectin, *in vitro* digestibility, application

## 1. Introduction

Starch is the main storage reserve polysaccharide of higher plants and a biopolymer of considerable significance for humans. It is a renewable, cheap and biodegradable natural raw material used widely in the food industry, in many non-food applications and as a source of energy after conversion to bioethanol (Röper, 2002; Balat et al., 2008; Burrell, 2003). Plant starches are synthesized inside plastids by coordinated interactions of multiple biosynthetic enzymes and deposited in storage organs, such as roots, tubers, fruits, grains and seeds, as well as in leaves and stems (Tetlow, 2011). Native starch occurs naturally in the form of insoluble, semi-crystalline granules, which are made up of two glucose homopolymers: amylose and amylopectin. Despite its seemingly simple chemical composition, native starch is highly variable in structure and functionality between and within plant species, and even from the same plant cultivar grown under different conditions. This variability is evident in granule morphology (size, shape), amylose content, amylopectin architecture (chain length distribution and placement of branches), and the way these two macromolecular constituents are arranged into crystalline and amorphous regions within granules. The variability in starch functionality derives from variability of structure, which is due to diversity in the genes that encode the starch biosynthetic enzymes and environmental factors that act on the genes and enzymes concerned during plant growth. As has been described elsewhere, the functional properties of starch, such as swelling, gelatinization and retrogradation, pasting, and susceptibility to enzymatic digestion, are very important for food processing and human nutrition (Copeland et al., 2009; Wang et al., 2011).

The wide range of botanical sources provides native starches that meet the requirements for a multitude of different requirements. However, native starches are not always suitable for specific industrial applications due to functional limitations such as low resistance to shear stress, poor thermal properties, and a high tendency towards retrogradation (Hermannsson and Svegmark, 1996). These natural limitations can be improved greatly by starch modification, which generally involves physical (heat-moisture treatment, annealing, pre-gelatinization, high pressure treatment, radiation, sonication), chemical (cross-linking, substitution, acid hydrolysis, oxidation/bleaching) or enzymatic processes (BeMiller and Huber, 2011; Miyazaki et al., 2006; Jobling, 2004). Enzymatic modification is performed most commonly by hydrolyzing starch granules with amylolytic enzymes into smaller fragments called dextrins. The enzymatically modified starches are used widely for food and pharmaceutical industries.

Physical modification by moisture, heat, high pressure or radiation has been gaining wider acceptance because no by-products of chemical reagents are present in the modified starch, making it as a natural and highly safe ingredients in food applications (Zavareze and Dias, 2011). Chemical modification has been the subject of intensive research throughout the last century. Many kinds of chemically modified starches have been developed, and applied for food, paper, and textile industries (Miyazaki et al., 2006; Singh et al., 2007). The oldest but still commonly used chemical modification method is acid hydrolysis, which dates back to when Nägeli in 1874 and Lintner in 1886 used sulphuric and hydrochloric acids, respectively, to produce hot water “soluble starch”. These processes were adopted for the commercial production of acid-hydrolyzed starch in the late 19th century (Daniel et al., 2008). Acid-hydrolyzed starches are

normally prepared by treatment of starch granules with dilute aqueous or alcoholic solutions of mineral acid (hydrochloric or sulphuric acid) below the gelatinization temperature for various time periods. When the desired degree of hydrolysis is achieved, as usually judged by the viscosity of the cooked modified starch, the resultant modified starch is recovered by neutralizing acid solution with alkali followed by washing or only by washing until neutrality, and subsequent drying. Acid hydrolysis alters the structure of starch granules and in turn their functional properties.

To the best of our knowledge, there is only one review paper focusing on the effect of acid hydrolysis on starch structure and functionality (Hoover, 2000). It is worthwhile to re-examine the recent advancement regarding the effect of acid hydrolysis on starch structure and functionality. Considering the similar behaviour of starch hydrolysed in the aqueous and alcoholic solutions, we only review the acid hydrolysis of starch in the presence of aqueous solution. A brief summary of starch structure and functionality is given, but it is not intended in this article to review extensive literature on these topics. For more detailed information on starch structure and functionality, readers are referred to the recent comprehensive reviews and book chapters on starch structure (Pérez and Bertoft, 2010; Pérez et al., 2009; Jane, 2009) and functionality (BeMiller and Huber, 2011; Biliaderis, 2009).

**2. Overview of starch structure**

Amylose (AM) and amylopectin (AP) together form insoluble, semi-crystalline starch granules with an alternating grow ring structure (Fig. 1). The granule size, shape, crystallinity and internal molecular organisation vary with the botanical source. Normal native starches have 20-30% AM and 70-80% AP by weight. AM is an essentially linear macromolecules with mostly α-(1-4)-linked D-glucopyranosyl units and less than 0.5% of the glucoses in α-(1-6) linkages.



AM has a molecular weight in the range of $10^5$ to $10^6$, corresponding to between 500 and 6,000 glucose units. AM is not essential for the construction of starch granules, as seen from the starch granule morphology not varying greatly with widely differing AM contents. AM chains are thought to be predominantly in a single helical state, although a small proportion of them may participate in double helices with AP branches or are entangled within the intricate architecture of the starch granule. AM occurs as two different forms in native starch granules: free AM and lipid-complexed AM, which contribute differently to starch functionality and digestibility, especially for cereal starches (Copeland et al., 2009; Delcour et al., 2010).

AP is a highly branched macromolecule with an estimated molecular weight between $10^7$ and $10^9$. AP is composed of backbone chains linked by α-(1-4) glucosidic bonds containing 10-60 glucose units and side chains with 15-45 glucose units linked by an average of 5% of α-(1-6) bonds (branch points) (Buleon et al., 1998b). AP is classified into three different types of chains based on the chain length distribution, namely A-, B- and C-type chains (Hizukuri, 1986). A-type chains are outmost unbranched chains. B-chains are further subdivided into B1, B2, B3 and B4, of which B1 is singly branched and linked with unbranched A chains. Any AP molecule contains

a single C chain which possesses the sole reducing end. AP is considered to be responsible for the regular granule formation of native starches, as evidenced by the fact that different granular morphologies result from the disruption or interference in amylopectin biosynthesis (Fulton et al., 2002, Keeling and Myers, 2009). Within the starch granule, amylopectin molecules are oriented radially with the non-reducing ends of chains pointing towards the outer surface. Although the exact molecular architecture is still not clear, a cluster model for the structure of AP, proposed by Robin et al. (1974) and further refined by other researchers (Gallant et al., 1997; Myers et al., 2000), is now widely accepted. This model, which is based on our knowledge of the combination of chain length distribution, branching frequency and branching pattern, proposes a treelike structure in which clusters of chains occur at regular intervals along the axis of the molecule. In this model, A and B1 chains form ordered double helices arranged in discrete clusters, whereas longer B2, B3 and B4 chains span two, three or four clusters depending on their chain lengths, respectively.

The relative location and state of AM in native starch granules continues to be a matter of much debate and remains to be further elucidated. AM was initially suggested to be located in bundles among AP clusters in amorphous regions (Blanshard, 1986; Nikuni, 1969). However, there is little experimental evidence to support such a model, which merely considers the need for AM to be synthesized in bundles to avoid the action of the starch branching enzymes during biosynthesis. Considering there are minimal helical interactions between AM and AP, Gidley (1992) proposed that AM chains are oriented transversely to the lamellar stacks, penetrating the amorphous lamellae and introducing disorder and crystalline defects within AP clusters. Some

"tie-chain" AM molecules were proposed to occur in a straightened conformation in crystalline lamellae and in a disordered conformation in amorphous regions (Kozlov et al., 2007; Matveev et al., 1998). Chemical cross-linking studies using normal potato and corn starch showed that individual AM molecules are interspersed among the AP clusters in both the semi-crystalline and amorphous regions (Jane et al., 1992; Kasemsuwan and Jane, 1994). Using small-angle X-ray scattering to study the effect of varying AM content on the internal structure of starch granules, Jenkins and Donald (1995) proposed that AM is predominantly located in the amorphous regions (growth rings) of starch granules, with a small number of chains involved in co-crystallization with AP. A recent study of iodine complex formation with acid-hydrolysed corn and potato starches showed that AM is partially involved in the B-type crystallites of potato starch, but independent from A-type crystallites of corn starch (Saibene and Seetharaman, 2010).

Chemical surface-gelatinization of normal potato and maize starches indicated that AM is more concentrated at the periphery than at the core of the granules, and that AM near the surface has a smaller chain length than that at the centre (Jane and Shen, 1993, Pan and Jane, 2000). In contrast, on the basis of iodine staining, the AM component of transgenic potato starches was proposed to be synthesised within the nascent amylopectin matrix and largely confined to a central region of the granule (Tatge et al., 1999).

Semi-crystalline native starch granules display a hierarchical structure, which has been revealed by enzymatic or acidic hydrolysis. The concentric pattern of readily degradable and more resistant layers can be readily observed by scanning and transmission electron microscopy

(French 1984; Gallant et al., 1997; Li et al., 2003; Wang, et al., 2012; Wang et al., 2008a; Yamaguchi et al., 1979). The more resistant layers are generally referred to as semi-crystalline growth rings and have a thickness between 120-400 nm. The more rapidly degradable layers, termed amorphous growth rings, were proposed to be at least as thick as the semi-crystalline rings based on small angle X-ray scattering (SAXS) studies (Cameron and Donald, 1992; Jenkins and Donald, 1995; Tester et al., 2004).



Based on findings from the study of acid hydrolysis of pea starch, Wang et al. (2012) proposed that AM is predominantly present in the core of the granules, forming the bulk amorphous region, although a small number of AM chains with the low degree of branching may be interspersed randomly among the AP clusters oriented towards the outer surface of the granules. Wang et al. (2012) proposed that in acid-hydrolyzed pea starch the semi-crystalline growth rings decrease gradually in thickness towards the periphery, whereas the amorphous growth rings have a more uniform width throughout the granule. These structural features are illustrated in Figure 1, which is based on the model published by Wang et al. (2012). The model, which is not drawn to scale, depicts an amorphous core surrounding the hilum composed mainly of amylose molecules and amylopectin molecules not organised into crystalline arrays. The core is surrounded by concentric semicrystalline growth rings of decreasing width towards the periphery, alternating with amorphous growth rings of more uniform thickness. Some amylose chains, which are represented as radiating from the core in a spoke-like pattern, may reinforce the granule structure.

The semi-crystalline growth rings are characterized by alternating crystalline and amorphous lamellae presenting a repetition period of 9-10 nm (Blanshard et al., 1984; Cameron and Donald, 1992; Oostergetel and van Bruggen, 1989; Sterling, 1962). In contrast, the amorphous growth rings are not well characterized, except that this is where amylose is mainly located. The crystalline lamellae are thought to be formed by double helices of AP side chains packed into a crystalline lattice, whereas the amorphous lamellae contain AM and the AP branch points. When hydrated, the AP is considered to have characteristics of a liquid crystalline polymer (Donald, 2000; Waigh et al., 1998; Waigh et al., 2000). The alternating crystalline and amorphous lamellae are proposed to be organized into near-spherical structures termed blocklets, which can be observed under atomic force microscopy and scanning electron microscopy. The blocklet level of crystalline structure is of a scale between that of the large growth rings and AP lamellae, and varies in diameter between 20 and 500 nm (Baldwin et al., 1998; Gallant et al., 1997; Pérez and Bertoft, 2010; Pérez et al., 2009). Based on the estimated average diameter of an amylopectin side chain cluster, the structure revealed in the AFM images is postulated to be amylopectin side chain clusters grouped into blockets (Dang and Copeland, 2003; Pérez et al., 2009).

Although the so-called spherical blocklets are really observed and thought to be really present in starch, the relationship between the proposed structures and blocklets has not been fully understood (Pérez and Bertoft, 2010; Zeeman et al., 2010). In addition to the peripheral growth rings, a loose filamentous area is also observed by SEM and TEM at the centre of granules of native, acid- or enzyme-treated barley, maize, and pea starches (Li et al., 2003; Li et al., 2004a,

Li et al., 2004b; Pilling and Smith, 2003; Wang et al., 2008a; Wang et al., 2008c; Wang et al., 2012). This less ordered core region, which is composed mainly of AM and AP chains disordered at the reducing ends, has been proposed to be the main bulk amorphous area in starch granules (Fig. 1c) (Wang et al., 2012). The proportion of the granule occupied by this bulk amorphous region may be dependent on the AM content of starch; waxy maize starch granules had the smallest amorphous core area compared to normal and high amylose maize starches (Li et al., 2003).



Amylopectin chains that are unbranched (A chains) and singly branched (B1 chains) and that have more than 10 glucose units may form double helices, which are arranged into either A- or B- crystalline structures that can be differentiated by powder X-ray diffraction (XRD) or solid state $^{13}C$ nuclear magnetic resonance (NMR) spectra (Buléon, et al., 1998b; Gidley, 1987; Gidley and Bociek, 1985; Imberty et al., 1991; Veregin et al., 1986). The A-type crystal structures are densely packed with only four water molecules in the unit cell, whereas B-type structures are more open with 36 water molecules in a hydrated core. The A-type polymorphs are found in most cereal starches, whereas the B-type occur in some tuber and root starches, high AM cereal starches, and retrograded starches. Legume grains and some roots and fruits contain starches that are designated as C-type, which are characterised by XRD spectra that include both A- and B-type patterns (Wang et al., 2006a; Wang et al., 2006c; Wang et al., 2011). Whether the C-type starch is a mixture of the A- and B-type crystallites or a distinct form is not clear. The proportion of B-type polymorphs within C-type starches ranges from 4 to 49% in pea starches, depending on starch sources (Ratnayake et al., 2002, Wang et al., 2011). The A- and B-type crystalline

polymorphs co-exist within the same granule, but their relative location may vary in different C-type starches. In pea and yam starches, B-type polymorphs are considered to be located predominantly in the centre surrounded by peripheral A-type polymorphs (Bogracheva et al., 1998; Buléon et al., 1998c; Wang et al., 2007a; Wang et al., 2007b). However, the B-type polymorphs are proposed to be situated mainly at the periphery surrounding the central A-type polymorphs in C-type high-amylose starch from a rice mutant (Wei et al., 2010).



**3. Starch Functionality**

The functional properties and applications of starch in the food and non-food industries are determined mainly by changes that starch undergoes when it is heated in water. The changes include granule swelling, gelatinization, retrogradation and pasting. Starch functionality is determined by starch structure, and expanding our understanding of this relationship could lead to improvements in processing methods and the quality of both new and traditional food products (Hermansson and Svegmark, 1996; Delcour and Hoseney, 2010).

Upon heating in excess water, native starch granules undergo a series of events including water uptake, swelling, irreversible disruption of internal molecular order through dissociation of double helices and melting of crystallites, leaching of polysaccharides and disruption and eventual disintegration of swollen granules. These events, which are referred to collectively as gelatinisation, are accompanied by loss of birefringence and dramatic changes in granule morphology and leading to paste or gel formation. The conditions for these events, and the extent

to which they occur, depend on the starch source and its structural properties. Gelatinization behaviour is generally monitored by differential scanning calorimetry (DSC) in combination with other complementary techniques XRD, small angle X-ray scattering (SAXS), NMR), light and scanning electron microscopy. Starch gelatinization has been the subject of intensive research, resulting in a vast body of published literature on this topic. Similar DSC traces have been observed in almost all studies of starch-water systems at the same starch concentration. However, the exact nature of the thermal transitions that are represented by the DSC traces is still not well understood and there is as yet no universally accepted explanation of these phase transitions (Goldstein et al., 2010; Ratnayake and Jackson, 2009).



Upon cooling and centrifuging the gelatinised starch dispersion, a supernatant containing solubilised starch (mostly leached amylose) is separated from the sedimented swollen granules, which are enriched in amylopectin. The sedimented fraction retains water in a swollen three-dimensional network held together by hydrogen bonds. The water-holding capacity of the swollen starch granules is usually referred to as swelling power or swelling factor, which is a property of amylopectin and varies greatly with starch source (Konik-Rose et al., 2001; Leach et al., 1959; Tester and Morrison, 1990a, 1990b).

The dissociated AM and AP chains in the gelatinized starch paste can recrystallise gradually on storage into an ordered structure in a process referred to as retrogradation. Retrogradation is accompanied by increase in the degree of molecular order and gel firmness, exudation of water (syneresis), and the appearance of a B-type XRD pattern (Hoover et al., 2010). Short-term

storage leads to retrogradation of mainly AM, whereas recrystallization of AP side chains is much slower. As a consequence, amylose retrogradation determines the initial hardness of a starch gel, while the long-term development of gel structure and crystallinity of processed starch is determined by regrogradation of amylopectin (Delcour and Hoseney, 2010). Retrogradation of starch pastes and the mechanical properties of starch gels have been studied by many experimental methods, including rheological techniques, texture profile analysis, DSC, turbidimetry, syneresis, XRD, NMR, Raman and Fourier transform infra red spectroscopy (Devesa and Martinez-Anaya, 2003; Karim et al., 2000). The extent of gelatinization during heating and retrogradation during storage is the main determinant in the texture and digestibility of starch-rich food products.

Under controlled temperature profiles and shear forces, the changes that occur in starch-water systems throughout gelatinization and granule disruption are defined as pasting. The pasting properties of starch are commonly quantified by measuring changes in viscosity during the heating and cooling of starch dispersions. The Rapid Visco Analyser (RVA) is a heating and cooling viscometer that is commonly used for routine analyses (Crosbie and Ross, 2007). During a cycle of heating and cooling, the RVA trace provides information on pasting temperature, the peak viscosity, breakdown (stability of hot paste to shear forces), setback (initial retrogradation of the starch paste on cooling), and the final viscosity. The breakdown of the hot starch paste viscosity is caused by shear-induced disruption of the swollen granules and leached starch components orienting themselves in the direction of stirring. These processes contribute to shear

thinning, which is an important property of starch pastes of practical relevance for many food systems (Delcour and Hoseney, 2010).

In addition to the above physical aspects, starch functionality is also determined by its susceptibility to enzymatic digestion, which is of increasing interest for human nutrition and health. Starch digestibility is assessed by *in vitro* or *in vivo* methods, which are complementary rather than alternatives. *In vitro* methods measure the physico-chemical processes of starch breakdown, whereas *in vivo* methods, such as the glycemic index, reflect a physiological process that includes starch breakdown, glucose absorption into and clearance from the bloodstream. *In vitro* studies have shown that the rate of enzymic breakdown of starch is determined by its extent of gelatinisation, with gelatinised starch broken down more rapidly than native granules or starch that has undergone retrogradation. According to the *in vitro* digestibility, starch is classified into three different fractions, namely rapidly digested starch (RDS), slowly digested starch (SDS) and resistant starch (RS) (Englyst et al., 1992), which are considered useful in predicting the nutritional value of the starchy food products. For example, RDS is highly desirable for cost effectiveness of animal feed, whereas for the human diet, SDS and RS are considered to play an important role in reducing risk factors for diet-related diseases. Trying to produce starches with slow-digesting properties is an important objective for the food industry.



## 4. Effect of acid hydrolysis on starch structure

### *4.1. Hydrolysis kinetics of starch granules*

Hydrolysis kinetics of starch granules is most commonly monitored in two different ways: the content of soluble sugar in the solution and the recovery yield of insoluble residues. Almost all starches exhibit a two-stage hydrolysis pattern: a fast initial rate followed by a slower subsequent rate (Biliaderis, et al., 1981; Espinosa-Solis et al., 2011; Genkina et al., 2009; Gerard et al., 2002; Jane et al., 1997; Jayakody and Hoover, 2002; Jiang et al., 2010; Kim et al., 2012; Le Corre et al., 2010; Le Corre et al., 2011; Muhr et al., 1984; Robin et al., 1974; Srichuwong et al., 2005; Wang et al., 2008d; Wang et al., 2012). The relatively fast initial rate is thought to correspond to the hydrolysis of the amorphous parts within starch granules, whereas the slow process is attributed to the concomitant hydrolysis of the amorphous and crystalline regions (Wang et al., 2012). Amorphous regions within starch granules are thought to be more accessible to acid attack due to the loose packing of starch chains compared to the crystalline regions (Kainuma and French, 1971; Robin et al., 1974). The first stage of hydrolysis in the amorphous regions is influenced by the granule size, pores on the surface, amylose content, and the amount of lipid-complexed amylose chains. The second step of hydrolysis, when both amorphous and crystalline regions are attacked, is influenced by the amylopectin content, the distribution of α(1→6) branches between the amorphous and crystalline lamellae, and degree of packing of the double helices within the crystallites (Le Corre, et al., 2010). Some authors have proposed a three-stage, rapid, slow and very slow hydrolysis pattern corresponding to hydrolysis of amorphous, semi-crystalline and crystalline regions, respectively (Li et al., 2007). Two hypotheses have been proposed to account for the lower hydrolysis rate of the crystalline regions of starch granules. Firstly, the dense packing of starch chains within the crystallites does not readily allow rapid penetration of

hydrogen ions into these regions. Secondly, the transformation of glucopyranose rings from the chair to half-chair conformation (required for hydrolysis of the glucosidic bonds) occurs slowly due to immobilization of the sugars in the starch crystallites. All glucosidic oxygens are buried in the interior of the double helix and are, therefore, less accessible to acid attack (Kainuma and French, 1971).



High-amylose starches show low susceptibility to acid hydrolysis, whereas those with low amylose content are easily hydrolysed (Jayakody and Hoover, 2002; Nakazawa and Wang, 2003; Vasanthan and Bhatty, 1996). The lower susceptibility of high-amylose starches to acid hydrolysis is attributed to either the greater extent of starch inter-chain associations resulting in the amorphous regions being more compactly organized (Hoover and Manuel, 1996; Vasanthan and Bhatty, 1996), or slower penetration of hydrogen ions into the granules due to the restricted swelling of high-amylose starch (Nakazawa and Wang, 2003). The greater susceptibility of waxy starch to hydrolysis could be due to more loosely packed double helices within the crystallites (Jayakody and Hoover, 2002). However, there are inconsistent results on the susceptibility to acid hydrolysis of starches with different crystalline polymorphs. The differences in rates of acid hydrolysis between A- and B-type starches have been proposed to be influenced by the distribution of α(1→6) branch points in amorphous and crystalline regions of amylopectin (Jane et al., 1997) and the degree of packing of the double helices within the crystalline region (Jayakody and Hoover, 2002).

*4.2. Effect of acid hydrolysis on amylose content*

Two different methods are used frequently for the determination of amylose content in native starch granules: iodine binding and concanavalin A (Con A) precipitation. The former method is based on the binding capacity of amylose with iodine, whereas the latter involves determining AM enzymically after specific precipitation of amylopectin by Con A. Amylose content is usually overestimated by the colorimetric method due to the formation of complexes between long AP side chains and iodine. Similarly, Con A precipitation method may also result in the overestimation of AM content due to incomplete precipitation of AP with a low degree of branching or defective side branches. The difficulties of accurately determining AM content by these and other methods are discussed by Zhu et al. (2008) and Vilaplana et al. (2012).

Amylose content, as determined by iodine binding method, has been shown to decrease significantly during the early stage of acid hydrolysis (Abdorreza et al., 2012; Atichokudomchai et al., 2001; Biliaderis et al., 1981; Franco et al., 2002; Sandu et al., 2007; Singh et al., 2009a; Singh et al., 2009b; Wang et al., 2003; Wang et al., 2012). The decrease in AM content is attributed predominantly to the preferential hydrolysis of amorphous regions within starch granules, which is consistent with the hypothesis that AM is largely located in the amorphous regions of starch granules (Jenkins and Donald, 1995). The AM content becomes undetectable after extensive hydrolysis (Atichokudomchai et al., 2001; Biliaderis, et al., 1981; Sandu et al., 2007; Wang et al., 2012), indicating that the length of residual linear chains is too short to form a blue iodine complex (Wang et al., 2012). However, using the iodine binding method, the AM content was found to increase initially on acid hydrolysis (Amaya-Llano et al., 2011; Bectancur

and Chel, 1997), which was explained by either the rapid depolymerisation of AP and the liberation of more linear chains, or by the formation of intramolecular and intermolecular linkages between residues of AM increasing chain length and hence capacity of AM to form complexes with iodine (Amaya-Llano et al., 2011).

In contrast, the AM content as determined by the Con A method, increases rapidly during the early stages of acid hydrolysis (Li et al., 2007; Wang et al., 2012). Li et al. (2007) interpreted the increase in AM content of high-AM maize starch as indicating preferential hydrolysis of AP, which was assumed to be predominantly in the amorphous regions. This assumption is inconsistent with the general view that amylopectin is mainly responsible for the crystallinity of native starch granules. However, considering the relationship between degree of branching (Goldstein et al., 1965) and external chain length (Colonna et al., 1985) on the binding affinity between Con A and branched polysaccharides, the initial rapid increase in apparent AM content is more likely to be due to an overestimation because of a significant loss of binding affinity of Con A for partially degraded AP molecules (Wang et al., 2012). The observations that acid hydrolysis caused a rapid loss of both iodine and Con A binding ability of the starch provides evidence that the acid attacks amylose and amylopectin simultaneously in the initial stage of hydrolysis.

*4.3. Effect of acid hydrolysis on chain length distribution of amylopectin*

In native starch granules, amylopectin chains have a polymodal distribution with A (chain length (CL) 12-16), and B chains of various lengths, i.e., B1 (CL 20-24), B2 (CL 42-48), B3 (CL 69-75) and B4 (CL 104-140) (Belloperez et al., 1996; Hizukuri, 1986; Peat et al., 1952; Tester et al., 2004). After acid hydrolysis, two main populations of chains with peak maxima at DP 13-15 and 25-27, are distinguished in various lintnerised starches  Further, small peaks (DP < 12) and peak shoulders (DP >12), originating from branched products, are detected between the two main peaks (Angellier-Coussy et al., 2009; Bertoft, 2004; Biliaderis et al., 1981; Bogracheva et al., 1999; Franco et al., 2002; Gerard et al., 2002; Jacobs et al., 1998; Jane et al., 1997; Jiang et al., 2010; Lauro et al., 1997; Kim et al., 2012; Maningat and Juliano, 1975; McPherson and Jane, 1999; Miao et al., 2011; Morrison et al., 1993; Robin et al., 1974; Shi and Seib, 1992; Song et al., 2010; Vermeylen et al., 2004). Lintners of cereal starches, but not of pea and potato starches, show additional shoulders (DP 35) and peaks (DP 50 and ~65) (Jacobs et al., 1998; Jane et al., 1997; McPherson and Jane, 1999; Vermeylen et al., 2004). These shoulders and peaks were suggested to arise from the multiply branched chains (Jacobs et al., 1998; Morrisson et al., 1993). Some longer chain residues (DP 77-130) are also detected from lintnerised starches and attributed to the double helices from retrograded free AM or segments of lipid-complexed AM. During the early stage of hydrolysis, amorphous free AM is partially hydrolysed into materials (DP <120) that retrograde into double helices (with B-type crystallinity) and are resistant to acid hydrolysis (Jacobs et al., 1998; Morrison et al., 1993).

Following debranching treatment with isoamylase, the position of the first population (DP 13-15) remain essentially unchanged, with the second one (DP 25-27) largely disappearing. The two

main populations at DP 13-15 and DP 25-27 in the lintnerised residues were assumed to originate from the double helices formed by externally unbranched A- and singly branched B1-chains in amylopectin of native starch. Interactions both within and between double helices do not allow acid to readily attack the crystalline lamellae (Jayakody and Hoover, 2002; Vermeylen et al., 2004). The survival of the singly branched B1-chains (DP 25-27) after extensive hydrolysis were interpreted as indicating that α-(1→6) branch points of B1 chains are predominantly located within crystalline lamellae and protected from acid hydrolysis (Biliaderis et al., 1981; Robin et al., 1974; Srichuwong et al., 2005; Watanabe and French, 1980). The long B2- B3- and B4-chains, which traverse two, three, or four crystalline lamellae (clusters), respectively, do not survive extensive acid hydrolysis, and are cleaved in the vicinity of α-(1→6) branch points where the structure is mostly amorphous (Morrision et al., 1993).

*4.4 Effect of acid hydrolysis on molecular structure of starch granules*

$^{13}$C CP/MAS-NMR is mostly used to analyse the molecular structure of starch granules at the single chain (amorphous) and double helix (ordered) structural levels (Gidley and Bociek, 1985; Veregin et al., 1986). NMR spectroscopy is a short-distance range probe that is considered to detect order corresponding to double-helix content, in contrast to XRD, which detects double helices that are packed into crystalline arrays (Cooke and Gidley, 1992). The $^{13}$C NMR resonance for C-1 (90-110 ppm) is related to the crystalline structure of starch granules: A-type starch shows a triplet C-1 resonance, B-type starch displays a doublet C-1 resonance, whereas the C-1 resonances of C-type starch are largely dependent on the relative proportion of A- and B-

type polymorphs. For example, if the B-type polymorph predominates over the A-type, the C-type starch shows a doublet C-1 resonance (Bogracheva et al., 2001; Gidley and Bociek 1985; Veregin et al., 1986).

Several major changes in molecular structure of starch granules during the course of acid hydrolysis have been monitored by $^{13}$C CP/MAS-NMR spectra (Fig. 2) (Atichokudomchai et al., 2004; Morrison, et al., 1993; Wang et al., 2008b; Wang et al., 2009; Wei et al., 2010; Yu et al., 2009). In the C-1 region of the spectrum (90-110 ppm), intensity increases gradually in the range 99-102 ppm (characteristic of double helices) and decreases in the range 93-99 ppm and at 103 ppm, which are assigned to the C-1 resonances of amorphous regions. The signal at 82.5 ppm arising from amorphous domains for C-4 becomes less pronounced and then almost disappears after prolonged hydrolysis. Major changes in the $^{13}$C CP/MAS-NMR spectra also occur in the C-2, C-3, C-4, C-5 region (68-78 ppm), where the signals become increasingly sharp and split into four well-resolved peaks corresponding to one crest peak at 72.7 ppm and three shoulder peaks at 71.5, 74.6 and 76.2 ppm. These four peaks are typical of A-type starches at high moisture content (30%) (Brogracheva et al., 2001). One more notable change is the substantially increased intensity relative to native starch of the resonance at 62 ppm, which is attributed to the C-6. The resonances for C-1 of Chinese yam starch have been shown to transform progressively from doublet to triplet with acid hydrolysis, indicative of preferential hydrolysis of B-type polymorphs within the granules (Wang et al., 2008b; Wang et al., 2009). However, a C-type high-amylose rice starch with a triplet C-1 resonance displayed a characteristic doublet after 20 days of acid hydrolysis, which was ascribed to the preferential hydrolysis of A-type polymorphs (Wei et al.,

2010). Acid hydrolysis results in a substantial initial increase in double helix content, which is considered to be due to the preferential hydrolysis of amorphous regions and retrogradation of free amylose that is released (Atichokudomchai et al., 2004; Morrison et al., 1993). In contrast to normal starches, there was relatively little change in double helix content of waxy starch on acid hydrolysis, which was suggested to be due to an overall transformation from a double-helix/non-ordered composite to a predominantly double-helix/V-type-conformation composite (Morrison et al., 1993).



*4.5 Effect of acid hydrolysis on crystalline structure of starch granules*

The semi-crystalline nature of starch granules is commonly characterized by using XRD, SAXS or neutron scattering (SANS) techniques (Blazek and Gilbert, 2011; Lopez-Rubio and Gilbert, 2009). When starch granules are subjected to acid hydrolysis, the relative crystallinity increases with increasing hydrolysis time, as shown by increasing XRD peaks centred in the region 15-30° 2θ. Several hypotheses have been proposed for the increased crystallinity in the initial stages of acid hydrolysis. Firstly, the cleavage of some of the amylose chains running through the amorphous regions may allow reordering of the newly released chain ends into a more crystalline structure (Kainuma & French, 1971; Robin et al., 1974). Secondly, the reordering of crystalline structure during acid hydrolysis would result in the increased crystallinity by partial filling of water channels in the crystallite cavities with double helices (Wu and Sarko, 1978). Thirdly, increased crystallinity may also result from the retrogradation of hydrolysed free amylose into double helices (Morrison et al., 1993), which rearrange into crystalline regions that are resistant

to acid hydrolysis (Atichokudomchai et al., 2004; Jane and Robyt, 1984). As mentioned previously, hydrated amylopectin molecules within starch granules may behave as a liquid-crystalline polymer. Decoupling of individual double helices from the amylopectin backbone by acid hydrolysis could remove spatial constraints and allow double helices to alignment into more crystalline structures (Atichokudomchai et al., 2004; Vermeylen et al., 2004; Wang et al., 2012). The increased crystallinity of acid-hydrolysed starches has been generally considered to indicate preferential hydrolysis of amorphous regions during the early stage of hydrolysis, resulting in the increase in relative proportion of crystalline regions. Nevertheless, it has been proposed that crystalline regions are also hydrolysed simultaneously with amorphous regions, albeit more slowly (Le Corre et al., 2010). No methods are presently available to evaluate accurately the proportion of amorphous and crystalline materials that have actually been hydrolysed after a particular period of acid treatment. Although the crystallinity of Lintnerised starch increases with hydrolysis time, it does not approach 100% even after very extensive hydrolysis. This indicates the presence of acid-resistant amorphous regions in native starch granules, which presumably arise from the amorphous lamellae that are protected by crystalline lamellae.

Acid hydrolysis not only increases the crystallinity of starch granules but it may also trigger polymorphic transitions in the XRD spectra depending on the starch source and degree of acid hydrolysis. In most cases, acid hydrolysis does not change the crystalline polymorphs of A- and B-type starch, although the transition of XRD patterns from A to C or A to B have been noted for barley (Morrison et al., 1993), cassava (Garcia et al., 1996), wheat (Buléon et al., 1987; Planchot et al., 1997; Vermeylen et al., 2004), and tapioca starches (Vermeylen et al., 2004) after acid

hydrolysis. These lintnerised starches show a far greater amount of B-crystallites than their native starch counterparts, as demonstrated by the increased peak intensity at the 5.6° 2θ, the decreased peak intensity at 15° 2θ, transition of the 17-18° doublet to the 17° singlet, and broadening of the 23° 2θ peak. Two hypotheses have been proposed for this transition of XRD patterns (Garcia et al., 1996). Firstly, some A-type crystallites could be metastable and the removal of a part of A-type crystallites would cause the remaining chains to reorganize into the more stable crystalline B-type. The second hypothesis is based on the assumption that some B-type polymorphs could have been present in the native starch but in amounts too small to be detected by X-ray diffraction. The preferential hydrolysis of A-type polymorphs results in an increase in relative proportion of B to A polymorphs. Additionally, the retrogradation of hydrolysed free amylose could also be responsible for the occurrence of B-type polymorphs (Biliaderis, 1991; Gidley, 1989; Morrisson et al., 1993).

The gradual progression towards the B-type polymorphs from C-type high-amylose rice (Wei et al., 2010) and maize mutant starches (Gerard et al., 2002) was also reported during acid hydrolysis. This polymorphic transition is attributed to the preferential hydrolysis of A-type polymorphs, resulting in the increased amount of B-type polymorphs, which were assumed to be intrinsically more resistant to acid hydrolysis. This explanation is consistent with the experimental observations that A-type wheat starch has the greater susceptibility to acid hydrolysis than B-type potato starch (Muhr et al., 1984), and that A-type maize mutant starches are more susceptible to acid hydrolysis than B-type ones (Gerard et al., 2002). However, Jane et al. (1997) and Nakazawa and Wang (2003) observed that A-type normal maize and wheat

starches have lower susceptibility to acid hydrolysis than B-type potato starches. Based on the branch-structure differences in A- and B-type Naegeli dextrins, Jane et al. (1997) proposed that branch points of A-type starch are scattered in both amorphous and crystalline regions and those in crystalline regions might be protected from acid hydrolysis. In contrast, the B-type starch has most branch points scattered in the amorphous regions, making them more susceptible to the acid hydrolysis. This proposal is in general agreement with the structural stability of the densely packed A-type crystallites and loosely packed B-type crystallites (Imberty et al., 1988; Imberty and Pérez, 1988).

Transitions in the XRD patterns also occur from B to A or B to C in the course of acid hydrolysis of sweet potato (Mcpherson and Jane, 1999) and potato (Buléon et al., 1998a) starches, or from C to A for yam (Mcpherson and Jane 1999), pea (Wang, et al., 2008b, Wang et al., 2012) and Chinese yam starches (Wang et al., 2006b). These polymorphic transitions can be attributed to the rearrangement from B- to A-type polymorphs due to the reduction in the chain lengths of double helices by acid hydrolysis. This explanation is consistent with shorter double helices being preferentially organized into the A-type polymorphs (Hizukuri, 1985). Since potato starch is considered to contain pure B-type polymorphs, the preferential hydrolysis of B-type polymorphs inducing this transition can be ruled out. The gradual evolution towards the A-type XRD pattern from C-type yam, pea and Chinese yam starches was interpreted as indicating the preferential hydrolysis of B-type crystalline polymorphs (McPherson and Jane, 1999; Wang et al., 2008b; Wang et al., 2006). This explanation is consistent with that B-type polymorphic structure

is more susceptible to acid hydrolysis (Jane et al., 1997), and that the B-type polymorph has a loosely packed structure (Imberty et al., 1988; Imberty and Pérez, 1988).

According to Waigh et al. (1998), hydrated amylopectin molecules within starch granules could behave as a liquid crystalline polymer. Removal of amorphous amylopectin backbone and covalent linkages between double helices by acid hydrolysis could allow the rearrangement of double helices, not only by relief of the entropy driven effects exerted by the amylopectin backbone (Waigh et al., 1998) but also by cancelling the influence of internal amylopectin chain length on the lateral distance between double helices (O'Sullivan and Pérez, 1999). The rearrangement of double helices involves the lateral or axial translation of double helices, which would lead to the polymorphic conversion from A to B or B to A by filling the water channels in the crystallite cavities with more water molecules or double helices. As a consequence, the transition of XRD patterns from B to A(C) or A to B (C) during acid hydrolysis must involve the rearrangement of decoupled double helices (Fig. 3). However, the transition from C to A or C to B could be predominantly attributed to the preferential hydrolysis of one polymorphs followed by possible rearrangement of decoupled double helices

Small angle X-ray scattering has also been used effectively to study changes in the crystalline structure of starch granules following acid hydrolysis. The intensity of the scattering peak at $q \approx$ 0.06-0.07 $Å^{-1}$ increases initially, reaches a maximum and decreases gradually, and eventually disappears into the increased background (Jenkins and Donald, 1997; Muhr et al., 1984; Oostergetel and van Bruggen, 1989; Wang et al., 2012). Using a model fitting technique, Jenkins

and Donald (1997) attributed these changes mainly to the decrease in electron density of the amorphous growth rings, and to a lesser extent of the amorphous lamellae, as a result of preferential hydrolysis of these regions. The electron density of amorphous growth rings was proposed to decrease more rapidly than that of amorphous lamellae, which are less accessible to acid attack due to the protection from crystalline lamellae. In a subsequent study (Wang et al., 2012), the changes in the lamellar peak were interpreted as indicating preferential hydrolysis of the amorphous parts of the lamellae followed by the concomitant hydrolysis of amorphous and crystalline lamellae. The lamellar *d*-spacing remained essentially unchanged within the first six days of hydrolysis. The subtle changes in position of the lamellar peak within the first 6-12 days of hydrolysis followed by the peak centre shifting gradually to higher *q* (corresponding to a lamellar *d*-spacing decrease from 10.8 nm to ca. 8.4 nm after 35 days of hydrolysis) was interpreted as due to the adjacent crystalline layers coming closer together as a result of the hydrolysis of amorphous lamellae and partial disruption of crystalline lamellae. Additionally, the low-*q* scattering increased up to 6-12 days of hydrolysis, after which it decreased somewhat, although the intensity remained higher than that of native starch. This was attributed to the preferential hydrolysis of other amorphous regions (amorphous growth rings and central bulk amorphous regions) followed by the disruption of larger structures (blocklets or superhelices) than the semi-crystalline lamellae (Wang et al., 2012). Interestingly, the interhelix repeat signal at around 3.9 $nm^{-1}$ (corresponding to the distance between the 100 crystallographic planes in the hexagonal unit cell of B-crystalline starch) increased initially and then decreased as a function of hydrolysis time (Wang et al., 2012). This observation suggests an initial increase and a subsequent gradual decrease in the number of interhelical repeats, which supports the proposed

distribution of A- and B-polymorphs in pea starch (Fig.1a). Based on their combined XRD and SAXS results, Wang et al (2012) concluded that the initial increase in relative crystallinity is due to the faster degradation of amorphous regions (bulk amorphous core, amorphous growth rings), and that after the central bulk amorphous regions and amorphous growth rings are substantially degraded, there is little or no change in relative crystallinity due to the concomitant hydrolysis of crystalline and amorphous lamellae.



*4.6 Effect of acid hydrolysis on granule morphology*

Starches isolated from different botanical sources show characteristic granule morphology, including different shape (round, oval, polyhedral), varied particle size from submicrons to 100 μm in diameter and particle size distribution (unimodal, bimodal, trimodal) as well as features on the granule surface, such as pores evident on cereal starch surfaces (Buléon et al., 1998; Miao, et al., 2011; Pérez and Bertoft, 2010, Tester, et al., 2004; Vandeputte and Delcour, 2004). The effect of acid hydrolysis on granule morphology of starch has been shown to vary from starch sources and degree of acid hydrolysis. Low degree of hydrolysis does not significantly alter the granular morphology of starch (Ahmed and Auras, 2011; Atichokudomchai et al., 2000; Amaya-Llano et al., 2011; Campanha and Franco, 2011; Franco, et al., 2002; Gao et al., 2012; Jayakody and Hoover, 2002; Sandhu et al., 2007; Singh et al., 2009a; Singh et al., 2009b; Pang et al., 2007; Wang and Wang, 2001; Wang et al., 2007b; Wei et al., 2010). The starch granules still remain intact, although the outer surface becomes roughened and have an open, lace-like appearance at high magnification (Wang et al., 2012). The extensive pitting on the inner surface covered by

lace-like structure that represents the formation of pores or channels was also observed (Wang et al., 2012). Extensive hydrolysis results in the damage of the internal part of starch granules, generating the visible peripheral alternating growth rings and central filamentous areas (Fig. 1a) (Li et al., 2003; Wang et al., 2008a; Wang et al., 2012). More extensive hydrolysis leads to the destruction of starch granules into platelet nanocrystals (Campanha and Franco, 2011; Le Corre et al., 2010; Le Corre et al., 2011; Putaux et al., 2003). As the granular morphology of the acid-hydrolyzed starch changes, the particle size distribution as measured by laser-light scattering according to Wang et al. (2011) also changes accordingly (Fig. 3).



## 5. Effect of acid hydrolysis on functionality of starch granules

### *5.1 Effect of acid hydrolysis on swelling power*

Acid hydrolysis has been shown to greatly change the swelling power of starch granules, although the effect is not consistent (Abdorreza et al., 2012; Betancur and Chel, 1997; Gao et al., 2012; Jayakody and Hoover, 2002; John eta l., 2002; Komiya and Nara, 1986; Singh et al., 2009a; Singh et al., 2009b; Tester and Morrison, 1990b; Wang and Copeland, 2012b). Swelling power of waxy maize (Jayakody and Hoover, 2002), waxy rice (Tester and Morrison, 1990b), arrowroot (John et al., 2002), sorghum (Singhet al., 2009), sago (Abdorreza et al., 2012) and pea starch (Wang and Copeland, 2012b) granules is almost completely lost after 24 h of hydrolysis. The intact structure of amylopectin was proposed to play an important role in starch granule swelling and water-holding ability (Tester and Morrison, 1990b). Once the amylopectin structure is

disrupted, an intact network cannot form and the damaged chains tend to dissolve as they no longer can entrap water (Wang & Copeland, 2012b). Bectancur and Chel (1997) suggested that acid-hydrolyzed starch granules are fragile and fragment instead of swelling on heating in water. A few studies showed that swelling power of potato starch decreases initially and then subsequently increases slightly (Komiya and Nara, 1986), or that swelling power of normal cereal starches increases initially and decreases thereafter (Jayakody and Hoover, 2002). The initial increase in swelling power was attributed to the interaction of water molecules with hydrolysed amylose chains that remain within the granule; these amylose chains were considered to be originally associated with each other in the native granule and unable to interact with water (Jayakody and Hoover, 2002).

*5.2. Effect of acid hydrolysis on starch gelatinization*

The thermal properties and gelatinization characteristics of starches have been shown to be greatly influenced by the acid hydrolysis (Abdorreza et al., 2012; Ahmed and Auras, 2011; Amaya-Llano et al., 2011; Atichokudomchai et al., 2002; Biliaderis et al., 1980; Campanha and Franco, 2011; Donovan, 1979; Donovan and Mapes, 1980; Espinosa-Solis et al., 2011; Gao et al., 2012; Garcia et al., 1996; Genkina et al., 2007; Jacobs et al., 1998; Jayakody and Hoover, 2002; Jenkins and Donald, 1997; Jiang et al., 2010; John et al., 2002; Kim, et al., 2012; Komiya and Nara, 1986; Miao et al., 2011; Morrison et al., 1993; Muhr et al., 1984; Perera et al., 2001; Shi and Seib, 1992; Singh et al., 2009a; Singh et al., 2009b; Tester and Morrison, 1990b; Thirathumthavorn and Charoenrein, 2005; Wang and Copeland, 2012a; Wootton and

Bamunuarachchi, 1979). The main endothermic transition peak in DSC traces of acid hydrolysed starch is generally shifted to higher temperatures compared to native starch (Abdorreza et al., 2012; Amaya-Llano et al., 2011; Atichokudomchai et al., 2002; Campanha and Franco, 2011; Donovan, 1979; Donovan and Mapes, 1980; Espinosa-Solis et al., 2011; Garcia et al., 1996; Jacobs et al., 1998; Komiya and Nara, 1986; Miao et al., 2011; Muhr et al., 1984). This shift is considered to reflect an increase in gelatinization temperature as a result of the increased molecular order or crystallinity in acid hydrolysed starch. Other interpretations include that the preferential hydrolysis of amorphous regions attenuates the destabilizing effect of swelling in amorphous regions on the melting of the crystallites (Donovan, 1979; Donavan and Mapes, 1980), or that longer amylopectin double helices may form as a result of the removal of branch points (Morrison et al., 1993). In contrast, a decrease in the onset and peak temperature of the endothermic transition has been reported for waxy starches (Perera et al., 2001; Shi and Seib, 1992; Tester and Morrison, 1990b). A general feature of the DSC traces of acid hydrolysed starch is a broadening of the transition temperature range, considered to be due to the heterogeneity of the crystallites formed from different entities, such as crystalline amylopectin side chains, retrograded amylose and amylose-lipid complexes, after acid hydrolysis (Jacobs et al., 1997; Morrison et al., 1993).



The observations on the effect of acid hydrolysis on enthalpy of gelatinization are more conflicting. In most studies, gelatinization enthalpy was observed to decrease with increasing hydrolysis time (Amaya-Llano et al., 2011; Biliaderis et al., 1980; Blanshard, 1987; Campanha and Franco, 2011; Komiya and Nara, 1986; Muhr et al., 1984; Sandhu et al., 2007; Singh et al.,

2009; Tester and Morrison, 1990b; Thirathumthavorn and Charoenrein, 2005), which has been explained on the basis that enthalpy change is mainly contributed by amorphous regions that are preferentially hydrolysed by acid (Muhr et al., 1984). Another explanation is based on the hypothesis that enthalpy changes for the dissolution of short chains in acid-hydrolyzed starches are not measured by DSC and hence not included in the gelatinization enthalpy (Muhr et al., 1984). An increase in gelatinization enthalpy following acid hydrolysis has also been noted, and attributed to an increase in double-helix content or crystallinity (Jayakody and Hoover, 2002; Miao et al., 2011; Morrison et al., 1993). Furthermore, some researchers reported that gelatinization enthalpy is not affected greatly even after prolonged acid hydrolysis (Atichokudomchai et al., 2002; Donovan and Mapes, 1980; Jacobs et al., 1997; Jenkins and Donald, 1997; John et al., 2002), which was explained as being due to acid hydrolysis having little effect of on the crystalline structure (Donovan and Mapes, 1980).

Wang and Copeland (2012a) studied the effect of acid hydrolysis on gelatinization properties of pea starch granules by applying a novel concept of "semi-preparative" DSC, which involves subjecting starch to thermal DSC transitions and subsequent morphological characterization by scanning electron microscopy of samples quenched after heating. The endothermic transition broadened and the endothermic peak was shifted to higher temperature after 1 day of acid hydrolysis. The typical narrow endothermic transition disappeared after two days and the DSC traces showed a very broad endotherm. After observing the associated morphological changes, Wang and Copeland (2012a) proposed that endothermic enthalpy changes were due to a combination of swelling of relatively intact starch chains and dissolution of partially degraded

chains. The DSC behaviour of acid-hydrolysed starch was explained in terms of the transition through several phases: from swelling of amylose and amylopectin chains, to swelling of starch chains that are relatively intact and dissolution of partially degraded chains, and finally to the predominant dissolution of degraded chains. In general, the nature of the thermal transitions of acid-hydrolysed starch (i.e., the shift and extent of broadening of the endothermic transition, changes in endothermic enthalpy) are likely to depend on the sequence and extent of multiple thermal events, which are determined by the structure of starch chains as well as water availability to these chains.



*5.3. Effect of acid hydrolysis on starch retrogradation*

Several researchers have studied the effect of acid hydrolysis on the retrogradation of corn (Singh et al., 2007; Wang and Wang, 2001; Wang et al., 2003), potato (Wang and Wang, 2001), arrowroot (John et al., 2002), rice (Kang et al., 1997; Thirathumthavorn and Charoenrein, 2005; Wang and Wang, 2001), sago (Abdorreza et al., 2012) and fruit starches (Espinosa-Solis et al., 2011). Generally, acid hydrolysis increases the DSC onset and peak temperatures of the endothermic transition of retrograded starch gels. In contrast, changes in conclusion temperature and enthalpy of endothermic transition of retrograded starch gels do not show a consistent trend as acid hydrolysis proceeds. Two factors were proposed to influence the retrogradation process of acid-hydrolysed starch and its thermal transition behaviour. Removal of α-(1→6) branch points in amylopectin clusters (Kang et al., 1997) and hydrolysis of amylose (Wang and Wang, 2001, Wang et al., 2003) with acid could accelerate the retrogradation of starch gels. On the

other hand, small molecules left in the acid-hydrolyzed starch residues could have a disordering effect on the recrystallization of acid-hydrolyzed starch gels (Thirathumthavorn and Charoenrein, 2005).

*5.4. Effect of acid hydrolysis on starch pasting properties and starch gel structure*



Acid hydrolysis greatly alters the pasting profile of starch granules, resulting in decreased peak, trough, and final viscosities, and breakdown and setback (Amaya-Llano et al., 2011; Aparicio-Saguilan et al., 2005; Ozturk et al., 2011; Polesi and Sarmento, 2011; Sandhu et al., 2007; Singh et al., 2009a; Singh et al., 2009b; Thirathumthavorn and Charoenrein, 2005; Wang et al., 2003; Wang and Copeland, 2012b). The pasting temperature of acid-hydrolyzed starch was shown to increase for corn starch (Sandhu et al., 2007; Wang et al., 2003) and Hylon starches (Ozturk et al., 2011), and decrease for sorghum starch (Singh et al., 2009), maize and Jicama starches (Amaya-Llano et al., 2011) . The decrease in the peak viscosity was attributed to the hydrolysis of amorphous regions and production of low molecular weight dextrins (Polesi and Sarmento 2011; Singh et al., 2009). Low molecular weight dextrins tend to dissolve rather than swell during heating in water, resulting in the low viscosity profiles of acid-hydrolyzed starches (Wang and Copeland, 2012b). The low setback of acid-hydrolyzed starches may be due to the Newtonian behaviour of the corresponding gel and due to the insufficient time for the starch molecules to align themselves with the direction of the flow during the measurement

(Thirathumthavorn and Charoenrein, 2005). The effects of acid hydrolysis on pasting temperature may depend on the conditions and extent of hydrolysis. The increase in pasting temperature could be attributed to decreased swelling power of acid-hydrolyzed starch retarding the development of viscosity, whereas a decrease in pasting temperature observed for sorghum starch could be explained on the basis that water penetrates more readily into the disrupted starch granules.



The mechanical properties of starch gels depend on various factors, including the rheological characteristics of the amylose matrix, the volume fraction and rigidity of gelatinized starch granules, as well as the interactions between dispersed and continuous phases of the gel (Biliaderis, 1998). Amylose and amylopectin determine the short-term and long-term gel structure development, respectively (Miles et al., 1985). An initial great increase in gel hardness of acid hydrolysed starch was considered to suggest the removal of negative effects of the branching structure of intact amylopectin on gel formation (Singh et al., 2009b; Wang et al., 2003) since the amorphous regions hydrolysed preferentially is composed of mainly amylopectin branches. The subsequent decrease in gel hardness following extensive hydrolysis was attributed to a lower number of high molecular weight amylose molecules (Wang et al., 2003). Acid hydrolysis was also shown to decrease the elastic modulus of starch gels. The lower elastic modulus of acid-hydrolyzed starch gel was attributed to the hydrolysis of amylose, which reduces the rigidity of the starch granular structure (Ahmed and Auras, 2011).

*5.6. Effect of acid hydrolysis on in vitro digestibility of starch*

Several studies have shown that acid hydrolysis influences the *in vitro* digestibility of starch granules (Espinosa-Solis et al., 2011; Miao et al., 2011; Ozturk et al., 2011; Planchot et al., 1997; Song et al., 2010; Srichuwong et al., 2005; Zhang et al., 2006). Mild acid hydrolysis does not significantly change the *in vitro* digestibility of normal corn (Song et al., 2010) and Hylon starch (Ozturk et al., 2011). However, severe hydrolysis has been shown to greatly alter the *in vitro* digestibility of starch granules (Espinosa-Solis, et al., 2011; Miao et al., 2011; Srichuwong et al., 2005; Zhang et al., 2006). Acid hydrolysis greatly increases RDS content and decreases RS content as compared to the native counterpart, but the effect on SDS is largely dependent on starch sources and hydrolysis degree. The increased RDS in acid-hydrolysed starches was attributed to either the disruption of granular structure, resulting in an increase in effective surface area (small particle size lintners) for enzyme absorption and binding (Zhang et al., 2006); or the removal of α -(1-6) branching points and inter-cluster chains in the amorphous lamellae, increasing the accessibility of substrate to the enzyme and allowing α –amylase to penerate internal structures more readily (Srichuwong et al., 2005). The changes in SDS content during acid hydrolysis may demonstrate that the slower hydrolysis rate of starch is only affected by the inherent granular structure and interplay between crystalline and amorphous lamellae within a semi-crystalline ring (Miao et al., 2011). For the acid-hydrolyzed starches, the increase in RS content with increasing hydrolysis degree was attributed to the increasing tightly packed crystalline structure due to the preferential hydrolysis of amorphous regions (Miao et al., 2011). Acid-hydrolyzed starches with A-type crystallinity are more susceptible to amylolysis than those with B-type (Planchot et al., 1997; Zhang et al., 2006).

**6. Application of acid-hydrolysed starch**

Acid hydrolysis modifies the granular structure of starches, making them display a different behaviour upon heating in water and produce pastes with lower intrinsic viscosity values, reduced hot paste viscosity, increased gel strength, increased water solubility, and better film-forming capabilities. Acid-hydrolyzed starches have been used widely for many years in many industries. Food industry applications of acid-hydrolysed starch include: (i) as gelling agents in the manufacture of gum candies (e.g., jelly beans, gummy bears and orange slices) and processed cheese loaves (Wurzburg, 1986); (ii) as fat replacers/fat mimetic in low-fat butter spread/margarine, low-fat mayonnaise, low-fat milk type products and low-fat ice cream (Sajilata and Singhal, 2005); (iii) as resistant starch-rich powder in slow digestible cookies (Sagulian et al., 2007). Examples of non-food industry uses of acid-hydrolyzed starches are: (i) as a premodification step for the production of cationic and amphoteric starches (Wurzburg, 1986); (ii) as warp sizes to increase yarn strength and abrasion resistance in the weaving operation in textile manufacture (Wurzburg, 1986); (iii) as adhesives to bond plaster and paper together in the manufacture of gypsum board for dry wall construction (Wurzburg, 1986); (iv) as paper sizes in paper and paperboard manufacture (Wurzburg, 1986); (v) as a pretreatment step for production of boiling-stable granular resistant starch (Brumovsky and Thomson, 2001); (vi) as fillers in direct-compression tablet preparation (Atichokudomchai and Varavinit, 2003). (vii) as raw materials in the preparation of biodegradable film (Singh et al., 2009a); (vii) as a pretreatment step for fermentative bio-hydrogen production (Kapdan and Kargi, 2006; Kapdan et

al., 2009); (ix) as reinforcing agent in polymeric matrices to improve their mechanical and barrier properties (Le Corre et al., 2010).

## 7. Concluding comments and future directions

Acid hydrolysis has been proved to be an efficient approach in revealing the microstructure of starch granules and modifying their functional properties. Acid hydrolysis preferentially attacks the surface of granules followed by the loosely packed amorphous regions within starch granules, which are mainly composed of central amorphous areas and peripheral amorphous growth rings. The preferential hydrolysis of amorphous regions results in the increase in relative proportion of crystalline regions and double helices. Both amylose and amylopectin are located on the surface of the granules and are attacked simultaneously in the early stages of acid hydrolysis. As acid hydrolysis progresses, functional properties of native starch are altered significantly, which can be used for different industrial applications.

As an important modification method, acid hydrolysis of native starch is of high interest due to its wide industrial applications. Recently, the increasing interest in preparation of biodegradable nanocomposites with enhanced mechanical and barrier properties and bio-hydrogen production from acid-hydrolyzed starch highlights the importance of acid hydrolysis of starch in the production biomaterials and bioenergy (Kapdan and Kargi, 2006; Kapdan et al., 2009; Le Corre et al., 2010). In addition to single acid modification, there are also increasing interests in studying the effect of acid hydrolysis in combination with other types of modifications on starch

structure and functionality, especially for the formation of resistant starch. Acid hydrolysis with hydrothermal modifications (HMT and ANN) has a beneficial effect on the formation of resistant starch (Zavareze and Dias, 2011). Also, the combination of acid hydrolysis with autoclaving and subsequent β-amylolysis could greatly reduce the starch digestibility (Song et al., 2010). These combined modifications would further extend industrial applications of modified starches in food and non-food sectors.

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## Figure Legends

**Figure 1**. Model of pea starch granule organization. The panels show an SEM image of a pea starch granule after 2 days of acid hydrolysis (a) and a schematic description of the growth ring model (b) and the chain distribution model (c). The growth ring and chain distribution models are not to scale and are intended to depict a stylised cross-section through a pea starch granule. Panels (a) shows a split granule in cross-section; the core is seen to be extensively damaged, whereas the surrounding crystalline and amorphous rings are substantially intact. A channel from the surface to the core is clearly evident. Panels (b) and (c) depict an amorphous core surrounding the hilum composed mainly of amylose molecules and amylopectin molecules not organised into crystalline arrays. The core is surrounded by concentric semicrystalline growth rings of decreasing width towards the periphery, alternating with amorphous growth rings of more uniform thickness. The semi-crystalline growth rings are composed mainly of crystalline amylopectin interspersed with amylose molecules, whereas the amorphous growth rings are composed mainly of extended chains of amylopectin interconnecting the crystalline regions and interspersed amylose molecules. The amylose and amylopectin chains are likely to be packed more densely *in situ* than depicted. (modified from a figure in Wang et al., 2012 reproduced with permission from Elsevier).



 

**Figure 2**. $^{13}C$ CP/MAS NMR spectra of native and acid-hydrolyzed Chinese Yam starch at various hydrolysis times. (a) native starch, (b) 2days, (c) 4 days, (d) 8 days, (e) 16 days, (f) 32 days, (g) 40 days (reproduced from Wang et al., 2008b, with permission from Elsevier).

**Figure 3**. Possible mechanism of polymorphic transition during acid hydrolysis (a: from A- to B-polymorphs; b: from B- to A-polymorphs).

**Figure 4.** Effect of acid hydrolysis on particle size distribution of Kaspa pea starch (S. Wang, unpublished results).



 

**Figure 1**

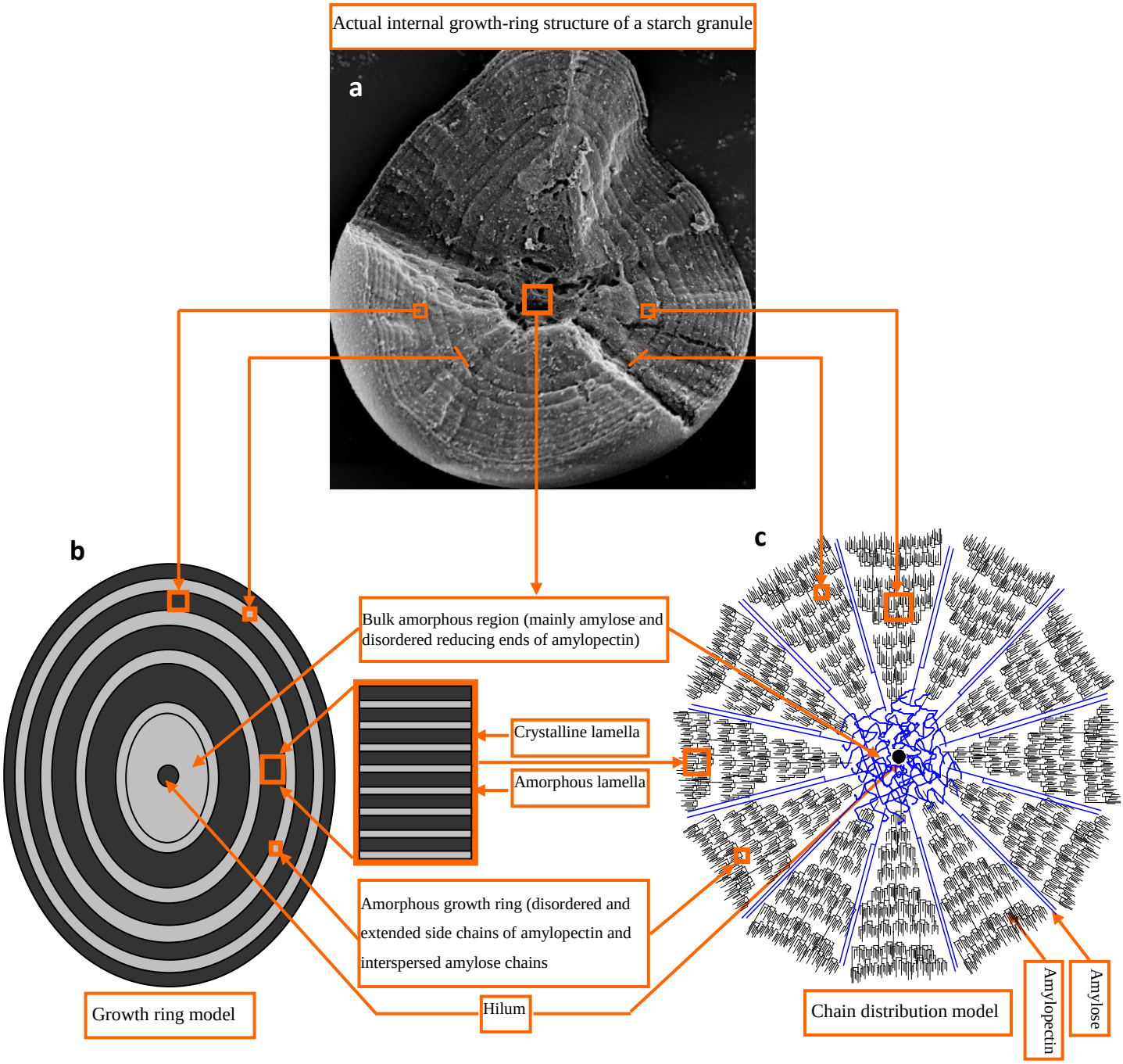




 

Figure 2.

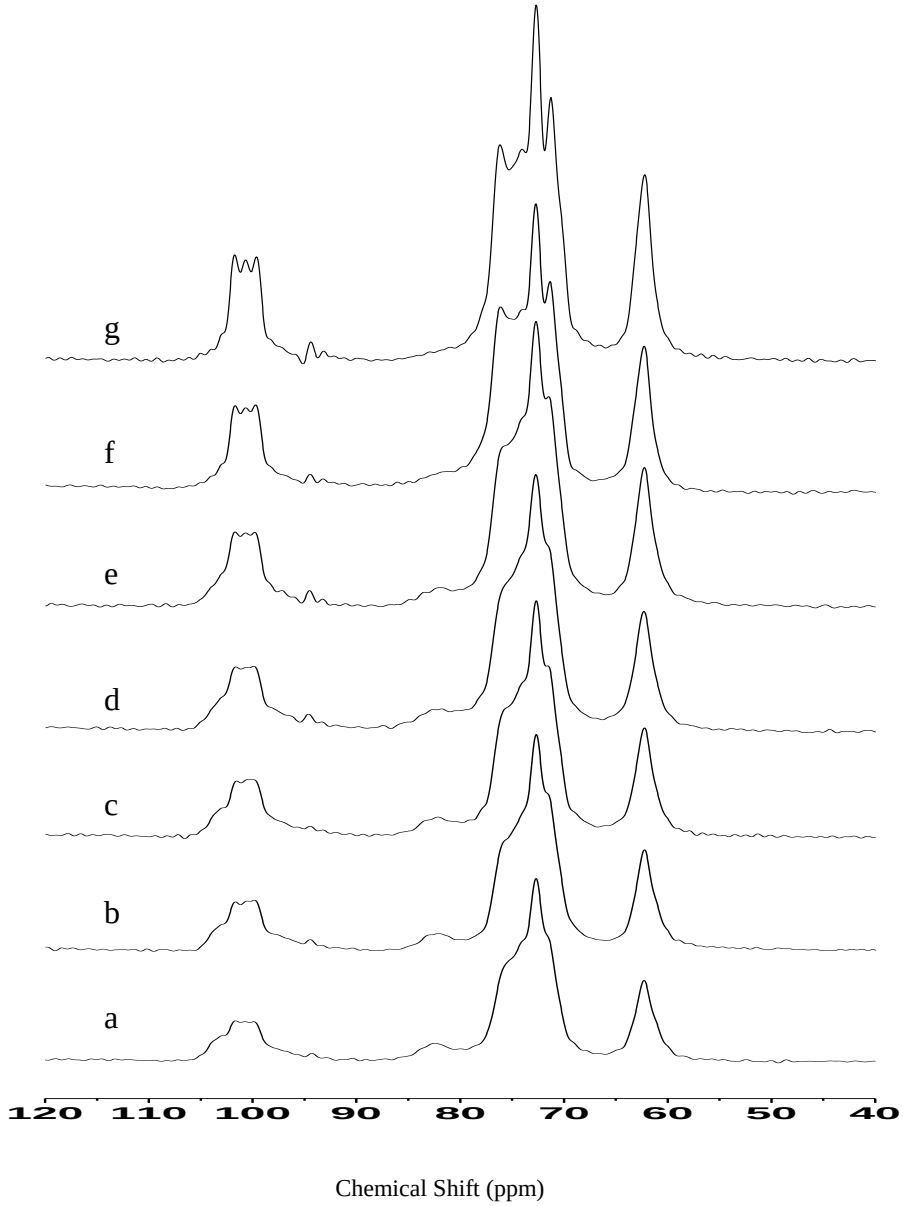

Figure 3

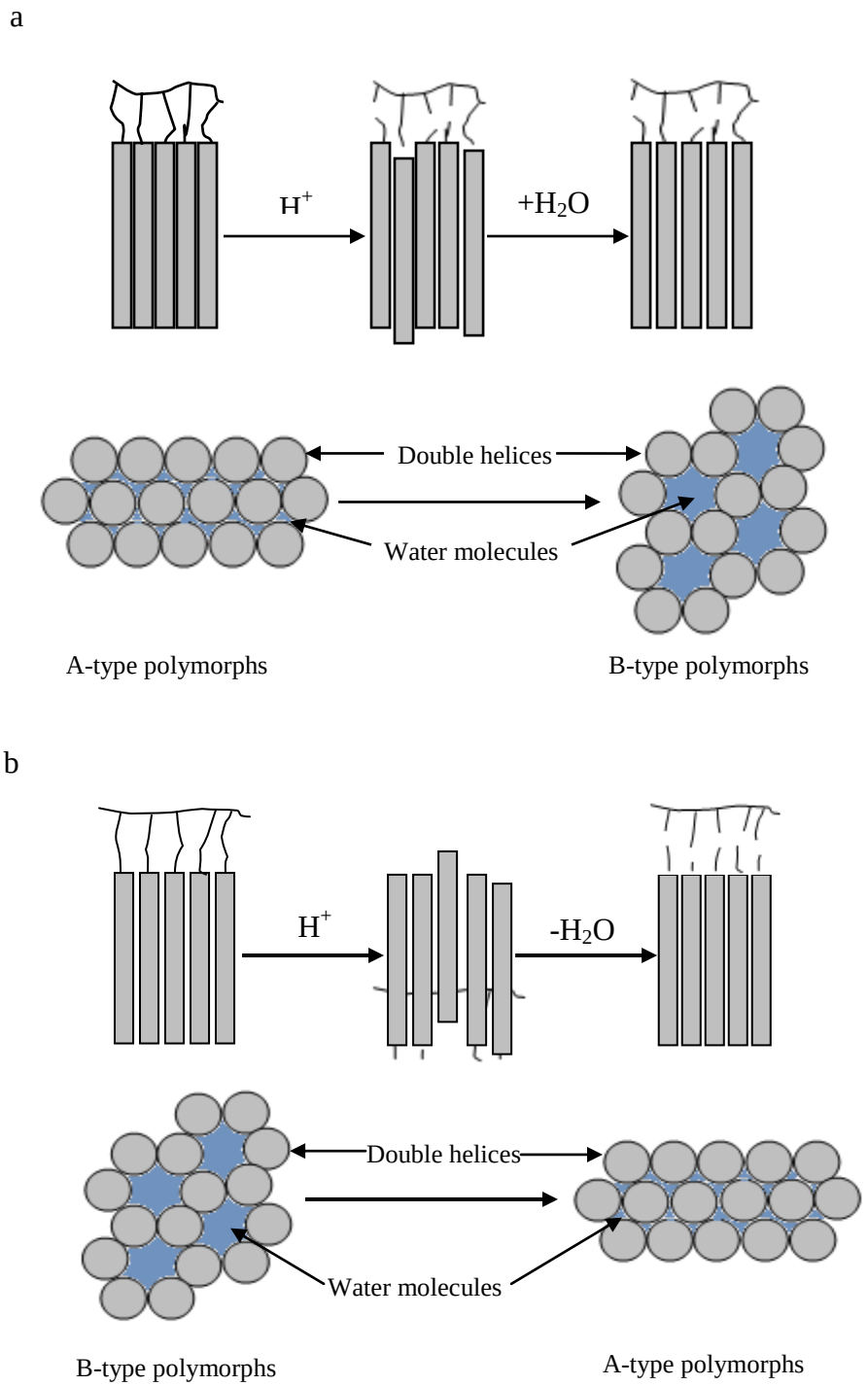

Figure 4