Quality of wheat starch films with amylose-lipid nanomaterials

by

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Declaration

I, Prelen Moodley, declare that this dissertation, which I hereby submit for the degree MSc Food Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature:

Date:

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Abstract

Starch is used in the food industry for many applications, for example, as a thickener and stabiliser. Novel uses of starch include making biodegradable plastic materials. These are environmentally friendly alternatives as they have a lower carbon footprint than synthetic plastics. However, starch plastics have poor mechanical and water vapour barrier properties, and as such have limited applications. Several researchers have noted that modification of starches can result in plastics with increased performance. Some modifications include the additions of filler material, which is thought to improve starch plastic's performance. Filler materials can include materials such as inorganic clays (kaolin, montmorillonite and sepiolite). An alternative material can be naturally occurring amylose-lipid complexes. These complexes can be made by modifying starch, such as wheat starch, with lipid material, such as stearic acid. This project investigated the effect of making plastic materials from wheat starch modified with stearic acid after different pasting times to produce modified starch with amylose-lipid complexes. The plastic's mechanical, thermal and barrier properties are determined. The work also investigated the effect of adding amylose-lipid nanomaterial to wheat starch films and determined the plastic's mechanical, thermal and barrier properties.

Wheat starch was modified, with 0.5% and 1.5% stearic acid, and films were then made from the modified wheat starch after pasting with a RVA (Rapid Visco-Analyser). After 30 minutes pasting time, the wheat starch films modified with 0.5% stearic acid resulted in significantly increased tensile properties compared to the other films, including maximum stress and strain as well as stress at break. These films also showed lower oxygen and water vapour permeability. However, when starch films were made with modified wheat starch during pasting for 120 minutes, a lower tensile stress and higher water vapour and oxygen permeability was obtained. The differences can be attributed to amorphous amylose-lipid complexes formed in situ during short (30 minutes) pasting compared to semi-crystalline amylose-lipid complexes formed during extended (120 minutes). The semi-crystalline amylose-lipid complexes can act as weak point and pin holes for crack initiation and propagation. The presence of amorphous amylose-lipid complexes in the films was suggested to be the main factor that increased the films' tensile properties. In addition, when amylose takes part in complexation with stearic acid, it is not available to form junction zones for network development. Consequently, weaker films are formed.

Amylose-lipid material was isolated from wheat starch that had been modified with stearic acid. This was then characterised to ensure that the isolated material was in fact amylose-lipid material. The amylose-lipid material was found to exist at nano-scale. These nanomaterials were then added to wheat starch paste and films were made. Wheat starch films made with 5% amylose-lipid nanomaterial addition resulted in the highest increases in the films' tensile properties in terms of the tensile stress and modulus, increased barrier properties in terms of water vapour and oxygen, and most stable thermal properties in terms of glass transition and dynamic mechanical thermal analysis. It was suggested that there is an interaction between the amylose-lipids materials and wheat starch polymers which improved the mechanical properties. The improved mechanical property is thought to arise from the amylose-lipid nanomaterial having acted as a nano filler. By acting as a filler, the nanomaterial provided a tortuous pathway which increased the oxygen and water vapour barrier properties.

Amylose-lipid complexes formed in situ during wheat starch pasting with stearic acid or through exogenous addition of amylose-lipid complexes have the potential to improve biodegradable wheat starch films.

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1. Introduction

1.1 Statement of Problem

Packaging materials have played a significant role in protecting food material, minimizing food losses and maintaining the safety of food. Packaging material is also a key contributor in ensuring that the shelf life of food products is retained for as long as possible whilst keeping the quality intact (reviewed by Zhao, Torley and Halley, 2008). A study done by the *European Bioplastics Organisation* showed that in 2013, there was a demand for 1.62 million tons of bioplastics and that this figure would have increased fourfold to yield a demand of 6.72 million tons by 2018 (European Bioplastics, 2013) This further emphasizes the importance of plastic materials for food products.

Plastic, for example, polyethylene terephthalate (PET), polypropylene (PP) and poly (vinylidene chloride) (PVDC) are mainly used as packaging material for many food products. These materials are derived from non-renewable petroleum-based resources and are considered as non-biodegradable materials (Zhao *et al.*, 2008). This has a drastic effect on the environment as only a small proportion of these materials are recycled, while the rest is combusted in the environment or dumped as landfill (as reviewed by Hopewell, Dvorak, Kosior, 2009). Combustion releases large amounts of gas into the environment, specifically CO_2, which adds to the greenhouse effect (Scott, 2002). A study on the impact of dumping plastics as landfill showed that a high amount of leaching out of minerals such as zinc from plastic materials can contaminate water systems (Mwiganga and Kansiime, 2005). Other factors such as attracting vermin or flies and the production of odours also have negative environmental impacts.

Several studies have demonstrated the potential use of starch in producing biodegradable plastics, but their low performance compared to synthetic plastic has limited their application (Bastioli, 2001; Sozer and Kokini, 2008). Some of these limitations are poor mechanical properties and high water transmission (Psomiadou, Arvanitoyannis, Biliaderis, Ogawa and Kawasaki, 1997; Avérous and Fringant, 2001). The incorporation of nanomaterials in bioplastics have shown improved physical, chemical and biological properties (Chivrac, Angellier-Coussy, Guillard, Pollet and Avérous, 2010).

However, these nanomaterials, for example, montmorillonite (Sozer and Kokini, 2008) and sepiolite (Fukushima, Abbate, Tabuani, Gennari and Camino, 2009), are often inorganic in nature. Furthermore, these nanomaterials-cannot be added within food systems to confer structural integrity or act as a barrier, as they are not food grade material (Zhao *et al*., 2008).

Amylose-lipid complexes are food-grade materials (Gelders, Goesaert and Delcour, 2006). These complexes exist at nano-scale (0.8–2.93 nm) and are nanomaterials (reviewed by Putseys, Lamberts and Delcour, 2010). These nanomaterials can be naturally enhanced in various ways, such as during biphasic pasting (Wokodala, Ray and Emmambux, 2012). Biphasic (two-phase) pasting curves result when starch is pasted with controlled settings for time and temperature, using instruments such as RVA. This means that two peaks result as a consequence of two distinct phases. The first peak is seen after pasting for a short time (<15 minutes), and the second peak is seen after a longer pasting time (>30 minutes) (Nelles, Dewar, Bason, Taylor, 2000). During biphasic pasting, starch modified with lipids exhibited a more stable second peak after pasting using RVA. The stable second peak has been attributed to amylose-lipid complexes (Wokodala *et al*., 2012; Nelles *et al*., 2000).

This project aimed to produce biodegradable starch films with amylose-lipid complexes formed in situ during starch biphasic pasting, as well as through exogenous addition of the amylose-lipid complexes.

2. Literature review

In this chapter, existing literature on the chemistry of starch and amylose-lipid complexes is first discussed. Following this, the properties, limitations and ways of improving starch films is also discussed.

2.1 Starch

Starch is considered to be one of the most abundant organic compounds, as reviewed by Mohd, Amini, Masri, (2016). It can be sourced from numerous botanical sources, such as cereal grains, tubers and fruits (Alavi, 2003). Starch is made up of the essentially linear amylose polymers and the branched amylopectin polymers (Bornet, 1993), and has a characteristic white powdery appearance (BeMiller and Whistler, 2009). Wheat starch is one of the main commercially used starches after maize starch (Swinkels, 1985). Wheat starch granules have dimensions that range between 2–24 µm *(Figure 2.1)*. Wheat starch granules are made up of A and B type granular structures; consequently, they have a bimodal starch distribution of > 9.9 µm and < 9.9 µm, respectively. The functional properties of starch depend on several factors such as its polymer composition (amylose:amylopectin content), molecular structure, internal organization and minor constituents such as lipids (BeMiller and Whistler, 2009).

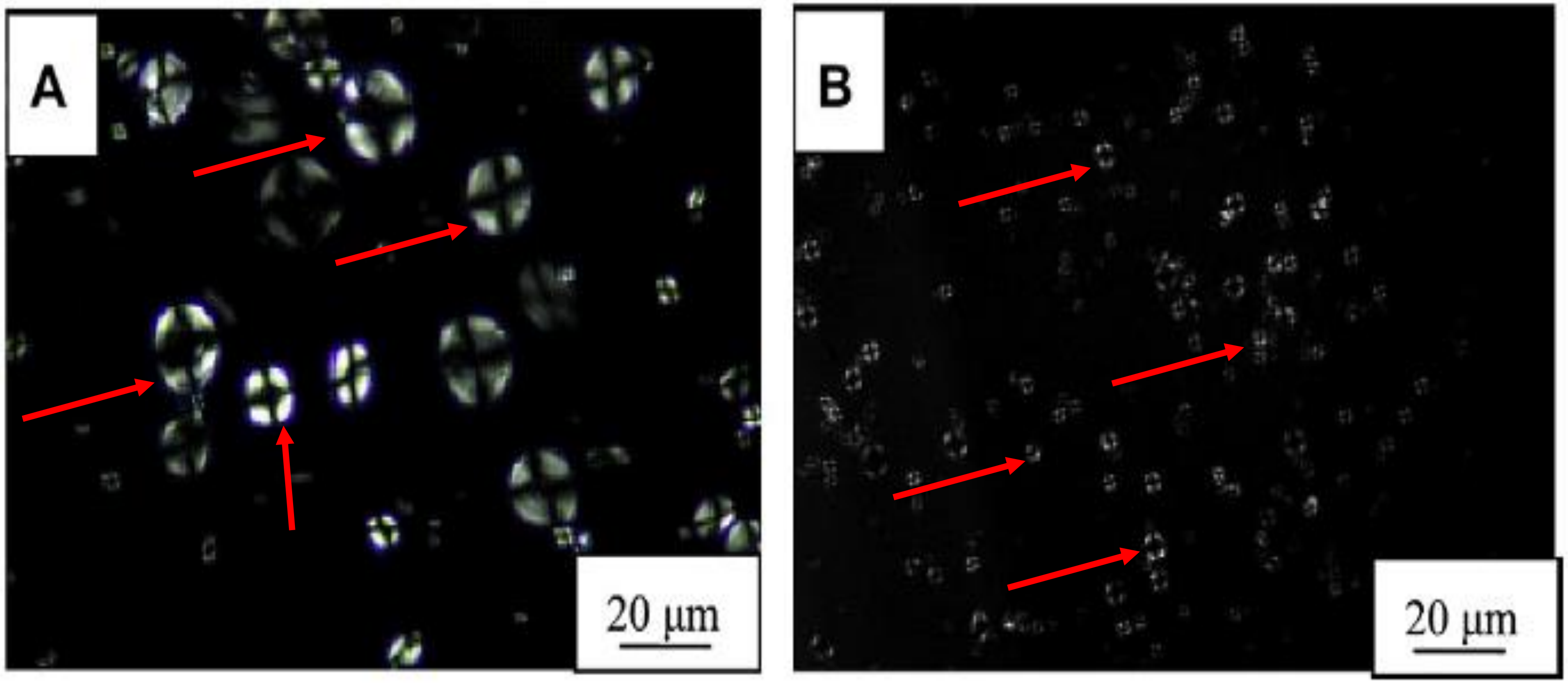

Figure 2.1: Polarized light microscopic images of A: A-type and B: B-type wheat starch granules, as indicated by arrows **(Zhang, Li, Liu, Xie, Chen, 2013).**

2.1.1 Amylose

The average content of amylose in wheat starch is about 28% (Swinkels, 1985). Amylose refers to the essentially linear portion of starch molecules (BeMiller and Whistler, 2009) that consist of α-D-glucopyranosyl units linked mainly via α (1-4) glycosidic linkages with occasional α (1-6) linkages (Oates, 1997). In wheat starch, the degree of polymerization (DP) of amylose varies from 200–1200 glucose units with an average of 800 glucose units (Swinkels, 1985). Using the average DP, the average molecular weight of amylose is 129 x 10^3 D (Swinkels, 1985 and BeMiller and Whistler, 2009).

Amylose molecules can form a single or a double helix (Winger, Christen and van Gunsteren, 2009). A single helix is characterised as having 6 glucose residues per turn and a hydrophobic core due to the presence of many hydrocarbon (CH) groups. The presence of glycosyl hydroxyl (OH) groups makes the exterior of the helix hydrophilic (Zobel, Young and Rocca, 1988). Amylose molecules can form helices depending on their conformation (BeMiller and Whistler, 2009). Amylose single helices can take part in complexation with iodine and fatty acids (Eliasson and Wahlgren, 2004). The amylose double helix is characterized as being left-handed with parallel strands (BeMiller and Whistler, 2009). The bonding of hydrogen with oxygen (O-2 and O-6) allows for a double helix conformation. Moreover, these double helixes are bound so tightly that there is no cavity in the helix to allow for complexation reactions.

2.1.2 Amylopectin

Amylopectin is a macromolecule of starch, and makes up the larger portion of wheat starch. According to Swinkels, (1985) the degree of polymerization value of amylopectin in wheat starch is around 2 x 10^6. It is a highly branched polymer, consisting of α (1-4) glycosidic linkage and up to 5% α (1-6) glycosidic linkages (Miles, Morris, Orford and Ring, 1985). Waxy starch, which has minimal (<1%) amylose but high amounts of amylopectin (> 99%), showed little to no ability to form films, and thus it was noted that amylose present in starch is the polymer that for the most part enables starches to make films (Romero-Bastida, Bello-Perez, Garcia, Martino, Solorza-Feria and Zaritzky, 2005).

2.1.3 Structural organisation of starch

Starch granules exhibit a crystalline-amorphous structure organised at various length scales (Cornuéjols and Pérez, 2010). The organisation is based on glucose building blocks, right up until the starch granule *(Figure 2.2).* Amylose-lipid complexes (discussed later in Chapter 4 and 5) may be found in the crystalline lamellae and can be responsible for a further level of organisation, in which starch polymers are arranged into semi-crystalline structures (BeMiller and Whistler, 2009).

Powder X-ray diffraction can be used to identify types of starch based on their crystallinity. Four distinctive patterns are given off from starch: A, B, C and V *(Figure 2.3).* The pattern depends on the degree of crystallinity of the starch. Wheat starches, along with other cereal grain starches, are reported to have an A type pattern, whilst tuber starches have a B type pattern. C type patterns are a combination of A and B patterns and are characteristic starches from legumes (Zobel *et al.*, 1988). The V-pattern is characteristic of starch-lipid complexes (Putsey *et al.,* 2010).

Figure 2.2 shows the organisation in starch, in which polymerization of glucose (a) molecules allows for a double helix confirmation (b) to be formed. The double helix then forms the lamella (c) region in layers where there are 2 outer crystalline layers surrounding an amorphous layer (c). This lamella region can be seen using transmission electron microscopy. The lamella region forms a structured superhelix (d), which is the building block for blocklets (e). Blocklets are layers of small spherical structures in which each blocklet contains alternating crystalline and amorphous lamellae. The alternating regions are made up of semi-crystalline (soft shells) and crystalline layers (hard shells). Blocklets can be seen using atomic force microscopy. The blocklets further organise in concentric growth rings (f) which increase due to the successive addition of alternating semi-crystalline and crystalline layers. These growth rings can be seen using transmission electron microscopy. The final organisation is the granular level (g), which is the characteristic form of starch.

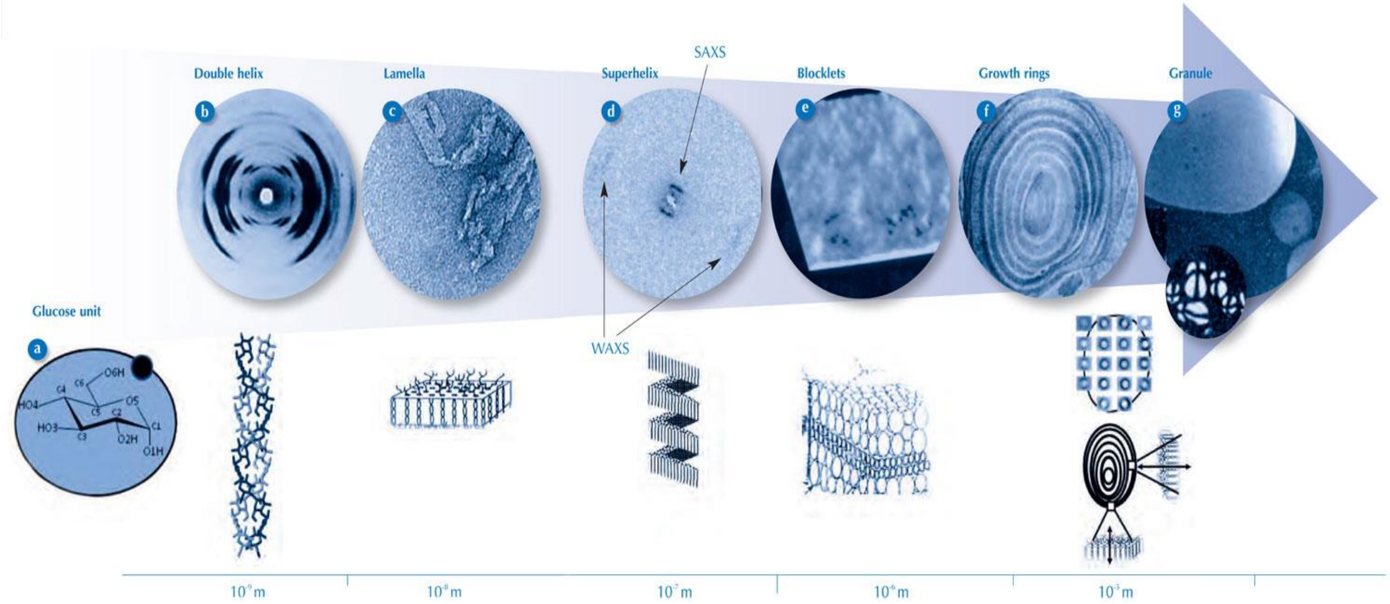

Figure 2.2: Levels of starch organisation, **(Cornuejols and Perez, 2010).**

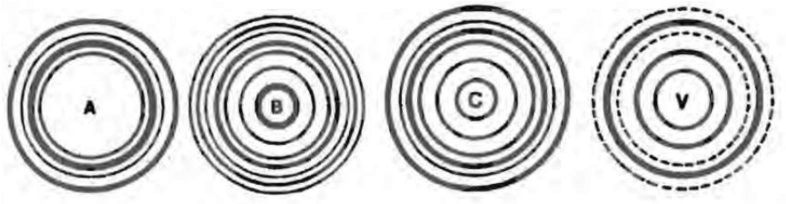

***Figure 2.3: X-ray diffraction patterns of A, B, C and V starch crystallinity* (Zobel *et al.*, 1988).**

2.2 Heat processing of starch

When starch is cooked in the presence of water, the following phenomena are experienced: gelatinisation, pasting and retrogradation.

Gelatinisation and pasting

Gelatinisation is the loss of molecular order within the starch granule when cooked in the presence of water (BeMiller and Whistler, 2009). It can be characterized by several irreversible changes which can include: granular swelling; native crystalline melting; loss of birefringence; and starch solubilisation (Blazek and Gilberta, 2011).

When starch is heated in water, 3 phases can occur:

The first phase is characterized as a reversible process in which water is gradually absorbed by granules and swelling is limited. This phase occurs at temperatures below 55 degrees Celsius ($\leq 55^{o}$C). The viscosity of this suspension does increase noticeably and the granules can return to their original state if dehydrated (Li, Zhang, Wei, Zhang, and Zhang, 2014). Upon further heating the onset of the second phase starts.

During the second phase the granules absorb large amounts of water resulting in an increase in their volume (BeMiller and Whistler, 2009). Larger amounts of water can only enter the starch granules when the temperature increases and hydrogen bonds in the starch granule are broken (Blazek and Gilberta, 2011). Subsequently the starch granules lose their molecular order, i.e. lose their birefringence. During this phase there is also an increase in viscosity and some starch becomes solubilized: thus this phase is not reversible (Li *et al*., 2014). Upon further heating the onset of the third phase starts.

During the third phase, more soluble portions of the starch tend to leach out the starch granules (Li *et al*., 2014); this is the onset of pasting. Pasting is the phenomenon following gelatinisation in the dissociation of starch. It involves granular swelling, exudation of molecular components from the granule; and eventually the total disruption of the granules (BeMiller and Whistler, 2009). The starch granules are said to be in a formless state (Li *et al*., 2014). A study done by Sandhu and Singh (2007) showed characteristics of starch that had been pasted. They reported an increase in viscosity as the most notable observation.

Retrogradation

Retrogradation is the reaction that takes place in gelatinised/pasted starch during cooling. The amylose and amylopectin chains start to align themselves, which causes the gelatinised starch to adopt a more ordered state. This is known as recrystallisation, and the starch moves from an amorphous state to a more ordered gel-like state (Tian, Xu, Xie, Zhao and Jin, 2011). The amylose and amylopectin chains align themselves in a parallel manner, and the formation of hydrogen bridges as junction zones allow for a more stable gel to be formed. The associations can be amylose-amylose, amylose-amylopectin or amylopectin-amylopectin. The associations will also affect the firmness of the gel being formed, in which amylose associations will tend to form more firm gels, whilst amylopectin associations will tend to form a softer but stable gel (Gudmundsson, 1994).

2.3 Starch biphasic pasting

Biphasic pasting is a phenomenon that has been characterized as the development of 2 peaks during controlled starch pasting, using RVA (Nelles *et al.*, 2000). The first peak is developed during cooking for a short time (<15 minutes), whilst a prolonged cooking period (>30 minutes) would give rise to a second peak or a continuous rise in viscosity during the holding period. The second peak is characteristically higher than the first peak due to an increase in viscosity, as seen in Figure 2.4 (D'Silva, Taylor and Emmambux, 2011). A study done by Nelles *et al.* (2000) hypothesized that the second peak was due to the formation of amylose-lipid complexes. The lipids form associations with the amylose portion of the starch, making amylose-lipid complexes. It was hypothesized that these complexes formed a stable network, in which water could be held in more tightly (Lin, Huang, Chang, Anderson and Hu, 2010). This could then explain the increased viscosity and the formation of a more stable second peak. D'Silva *et al.* (2011) noted that an increase in the amount of stearic acid added to starch that was pasted resulted in more pronounced second peak formation. It was also observed that the amount of free amylose was lowered during pasting for a prolonged period when lipids where added to it. It was hypothesized that the amylose took place in complexation with the lipids to form amylose-lipid complexes.

Wokodala *et al.* (2012) found that stearic acid addition to maize and teff starches allowed for interactions between the amylose portion of starch and the polar stearic acids, which enabled amylose-lipid complex formation. During 30 minutes of pasting, amorphous Type I amylose-lipid complexes are formed, while during long pasting of 120 minutes, semi-crystalline Type II amylose-lipid complexes are formed. These authors used DSC and XRD to show the evidence of these complexes. They also hypothesized that high viscosity is due to the fact that amylose-lipid complexes are at nanoscale and thus can have stronger interaction for higher molecular order.

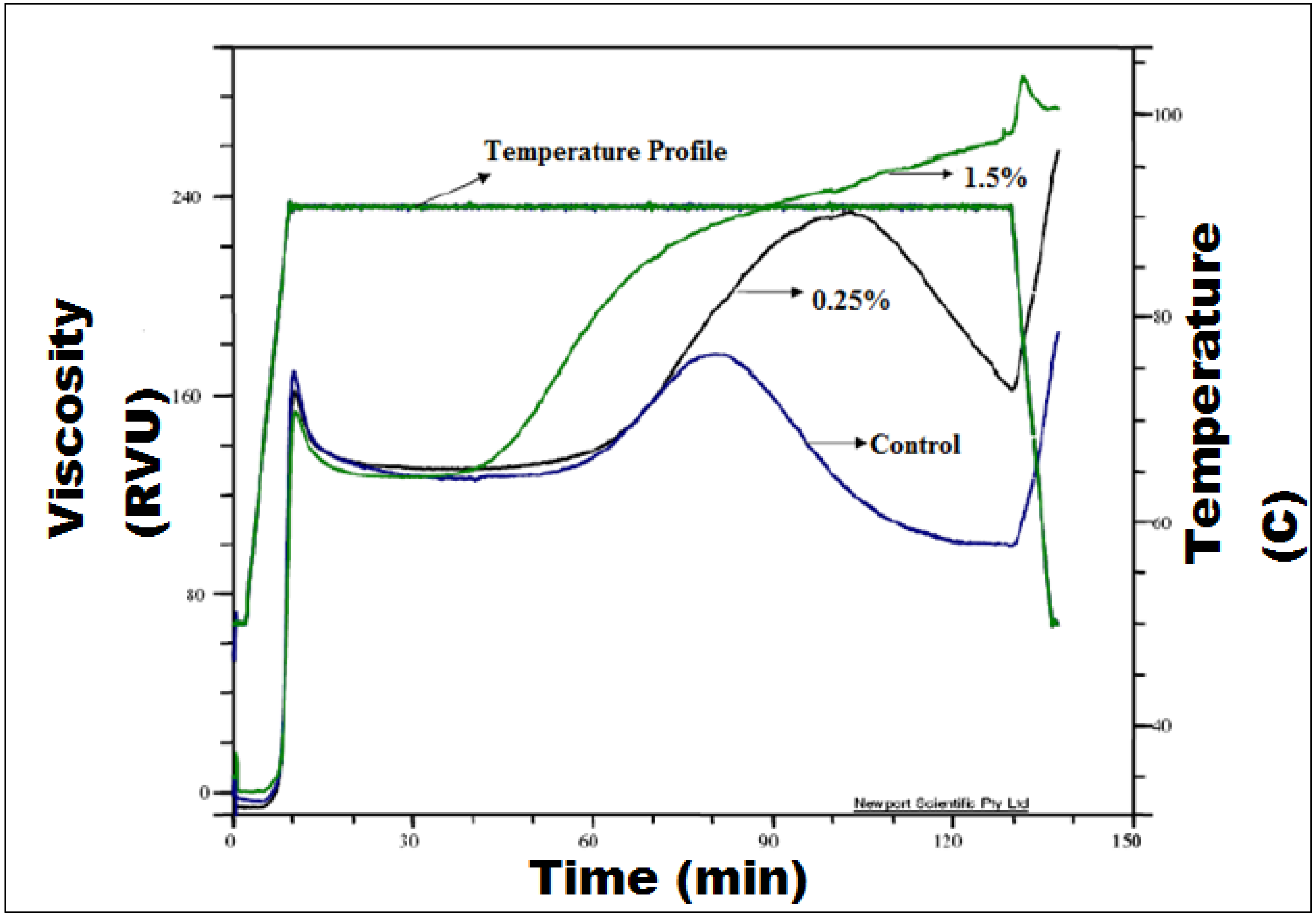

Figure 2.4: Biphasic pasting curves with or without added stearic acid found using a Rapid Visco-Analyser, (D'Silva *et al.*, 2011).

2.4 The chemistry, structure and isolation of amylose-lipid complexes

Amylose structures are characterised as single stranded, left-handed helices (Gelders *et al.*, 2006), with an internal cavity where the complexed ligand, such as fatty acids, can reside *(Figure 2.5)*. These structures are called V-amylose structures and are formed due to a collapse of the double helices forming the left-handed single helices arrangement with 6 glycosyl residues per turn (Putseys *et al.*, 2010). Several authors have reported that the lipid molecules are located with their aliphatic chain inside the helix cavity and that the carboxyl groups were outside the helix due to steric and electrostatic repulsions (Godet, Buleon, Tran and Colonna, 1993). Following the formation of the complex, the fatty acid is immobilized within the amylose helix (Karkalas and Raphaelides, 1986).

X-ray diffraction studies also indicate that amylose-lipid complexes have several conformations, based on glucose residues per amylose helix turn. There can be 6, 7 or 8 glycosyl residues per turn depending on the ligand being used as well as the degree of hydration (Fanta, Kenar, Byars, Felker and Shogren, 2010). These conformations determine the morphology of the amylose-lipid complexes. The helices can be stabilised by various associations, such as intra- and inter-helical Van der Waals interactions or hydrogen bonds. However, due to the conformation of the amylose helix, which is hydrophilic on the outside with a hydrophobic cavity, the formation of hydrophobic interactions with fatty acids inside the core is favoured (Putseys *et al.*, 2010). Amylose-lipid complexes have dimensions on the range of 0.80–2.93 nm, depending on factors such as the degree of hydration or the size and volume of the ligands used for complexing (Gelders *et al*., 2006). These amylose-lipid complexes can crystallize, and melting temperatures vary depending on the order of the complexes (Becker, Hill, and Mitchell, 2001). Furthermore, the complexes can form suprastructures in which the individual complexes form aggregates with each other. These aggregates also exist at nanoscale or micron scale (reviewed by Obiro, Ray, and Emmambux, 2011).

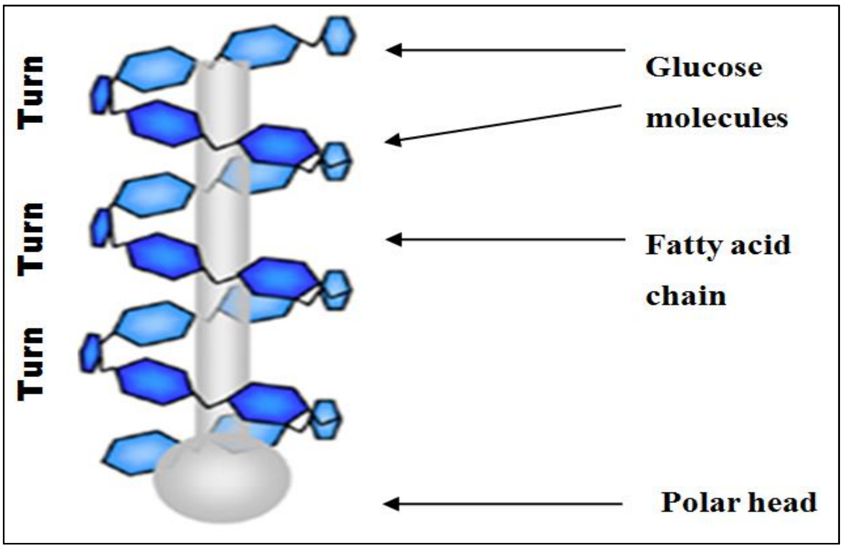

Figure 2.5: A complexed amylose structure with the ligand's aliphatic chain located on the inside of the helix whilst the polar head is located outside **(Putseys *et al.*, 2010).**

Complexing starches with unsaturated fatty acids is challenging, as the fatty acids have *cis*-double bonds, making them inflexible. Added to this, fatty acids have an internal cavity and can result in steric hindrance (Putseys *et al.*, 2010). This allows only partial inclusion of ligands in the internal cavity, making the formation of amylose-lipid complexes minimal. However, the unsaturated fatty acid may adopt a change in confirmation and allow for rotation of the C—C bonds adjacent to C=C bonds, causing the amylose chain to have an increased diameter. This can then enable complexation of unsaturated fatty acids to occur (Karkalas and Raphaelides, 1986).

Furthermore, V-amylose can exist in 2 forms, i.e. in dry (V_a) and hydrated (V_h) form (Putseys *et al.*, 2010). The main difference between these forms is that the V_h-amylose contains 2 water molecules that are hydrogen bonded to each other, inside the helix (reviewed by Obiro *et al.*, 2011). The dry form shows smaller unit dimensions than the hydrated form (Putseys *et al.*, 2010). Characterisation of V_a and V_h amylose using X-ray diffraction gives characteristic peaks, where peaks are observed at 12 Å, 6.8 Å and 4.4 Å for hydrated V_h complexes and at 11.3 Å, 6.5 Å and 4.3 Å for V_a type (Kugimiya, Donovan, Wong, and Berkeley, 1980).

The fatty acid orientation within the amylose helix may also differ. Godet *et al.* (1993) describe six possible positions in which each position is 60° apart and corresponds to three main planes. The hydrogen atoms on the fatty acids orientate towards a less crowded region near the glycosidic oxygen on the amylose molecule. This conformation allows for minimal movement, as the amylose helix and the fatty acid have complementary geometries (Gelders *et al*., 2006).

A review by Obiro *et al*. (2011) indicated that through characterization of amylose-lipid complexes, it was found that these complexes exist at a nanoscale. The complexes can exist between sizes of 0.80–2.93 nm. Furthermore, it was found that the complexes can form superstructures in which the individual complexes form aggregates with each other. These aggregates also exist at nanoscale. This indicates that amylose-lipid complexes are nanomaterials.

2.4.1 Factors affecting the properties of amylose-lipid complexes

Chain length

Fatty acid:

The fatty acid chain length directly influences the degree of complexation, in which a longer fatty acid chain promotes an increased degree of complexation. More specifically, a chain length of 10 carbon (10C) atoms of fatty acids is a minimum requirement for complexation; this is due to the nature of fatty acids being more soluble in a crystallisation medium (Putseys, Derde, Lamberts, Goesaert and Delcour, 2009). Moreover, it was observed that a fatty acid with a longer chain length will give a more stable complex (Gelders *et al*., 2006). Long chain fatty acids will allow for more hydrophobic interactions, resulting in higher temperature requirements to break these interactions (Putseys *et al*., 2010).

Amylose:

Research has shown that an increase in amylose chain length promotes greater complexation (Shogren, Fanta and Felker, 2006). It was observed that a longer amylose chain will also yield a more stable complex (Godet *et al*., 1993), owing to a longer chain that can bind to more ligands, such as fatty acids. This will allow for a more crystalline complex to be formed, which will have a higher melting point (Putseys *et al*., 2010).

A study done by Blazek and Gilberta (2011) on the interactions between wheat starch and lipids during heating showed that the viscosity of the pastes depended on the saturation of the fatty acids as well as the fatty acid chain lengths. This can also affect the number of starch-lipid complexation levels.

Fatty acid saturation level

Saturated fatty acids that have been complexed with amylose have shown improved tensile properties as opposed to those complexed with unsaturated fatty acids (Becker *et al*., 2001). Some of the properties include a lower pasting peak viscosity, greater resistance to breakdown, and reduced final viscosity. Studies done by Karkalas, Ma, Morrison and Pethrick (1995) showed that amylose-lipid complexes made with unsaturated fatty acids lead to lower dissociation temperatures. This was attributed to the fatty acids having a non-linear structure, making the insertion of the cis-double bonds on the unsaturated fatty acid into the linear helix cavity of the amylose more difficult. Complexation can occur due to the ability of the carbon atoms adjacent to the double bond to rotate.

Concentration of fatty acid

The level of fatty acid addition can have an impact on the rheological properties during the complexation process (Morrison, 1988). An increase in fatty acids can lead to reduced first peak viscosity, a longer pasting time and reduced viscosity during cooling. A higher degree of complexation between the amylose and fatty acids was also noted with an increased concentration of fatty acids (Karkalas and Raphaelides, 1986). Furthermore, a longer pasting time during starch pasting in which fatty acids are incorporated resulted in the formation of a more stable higher second peak viscosity (discussed earlier).

Effect of heat and pH

The temperatures used during complexation can affect the crystal structures that are formed (Biliaderis, Page, Slade and Sirett, 1985). Temperature can also affect the yield of complexes that can be formed. At lower temperatures (≈ 60ºC) complexes known as Type I complexes form random crystals due to rapid nucleation. Type I complexes result in randomly orientated complexes with individual helical segments (Putseys *et al.,* 2010). These complexes can be dissociated at temperatures of between 95ºC and 105ºC (Gelders *et al*., 2006). Amylose molecules have a tendency to form long helical chains. These helical chains can be longer than the ligand that it is being complexed with, resulting in some sections of amylose that are not complexed. These regions allow for random distribution in Type I complexes, explaining why lower temperatures are required to dissociate these complexes. The random orientation does not allow for ordered superstructures be formed, as nucleation happens rapidly (Biliaderis *et al*., 1985).

At higher temperatures (≈ 90ºC), annealing occurs, which confers a more crystalline order to the complexes (de Pilli, Derossi, Talja, Jouppila and Severini, 2011). These complexes are known as Type II complexes. Furthermore, the fatty acid chain length can affect the heating rate as well as the rate at which complexes care formed. After the initial molecular association between amylose and the fatty acid, there can be varying degrees of order (Putseys *et al.,* 2010; Gelders *et al*., 2006). The two types are the less ordered Type I and semi-crystalline Type II complexes (Le Bail, Bizot, Ollivon, Keller, Bourgaux and Buleon, 1999). Type II complexes can further be divided into Type IIa and Type IIb.

The majority of amylose-lipid complexes are of this type and are known to be amorphous but may be annealed to form the crystalline Type II. Type II complexes can be dissociated at temperatures of around 115ºC (Putseys *et al.,* 2010). The order thus governs the thermal stability of the amylose-lipid complex. Type II complexes are described as superstructures of several Type I complexes crystallized together (Gelders *et al*., 2006).

Type II complexes can also take on two forms, namely Type IIa and Type IIb. Type IIa is formed during gelatinisation and is semi-crystalline, while Type IIb, which is formed after gelatinisation with continuous heating, is fully crystalline and forms ordered superstructures. Rappenecker and Zugenmaier (1981) suggested that Type IIb is the most stable form of the amylose-lipid complex. Wokodala *et al.* (2012) reported that these complexes are possibly present in starch pastes pasted with extended time at high temperatures *(Figure 2.6)*. Type IIa has a melting temperature above 114ºC–121ºC, whilst Type IIb has higher melting temperatures (>121ºC) (Karkalas *et al.,* 1995). Type I and II complexes can be characterized by their respective dissociation temperatures. Using thermal analysis, such as Differential Scanning Calorimetry (DSC) *(Figure 2.6)* the dissociation temperatures can be identified using the endothermic peak (Biliaderis *et al*., 1985). The pH level can also have an effect on complex formation (Zhang, Huang, Luo and Fu, 2012). Complexes can be formed if a neutral fatty acid is used in a neutral complexing medium. It was also found that at a higher pH, complexes with short chain fatty acids are more readily formed, whilst at a lower pH, complexes with longer chain fatty acids are formed more readily.

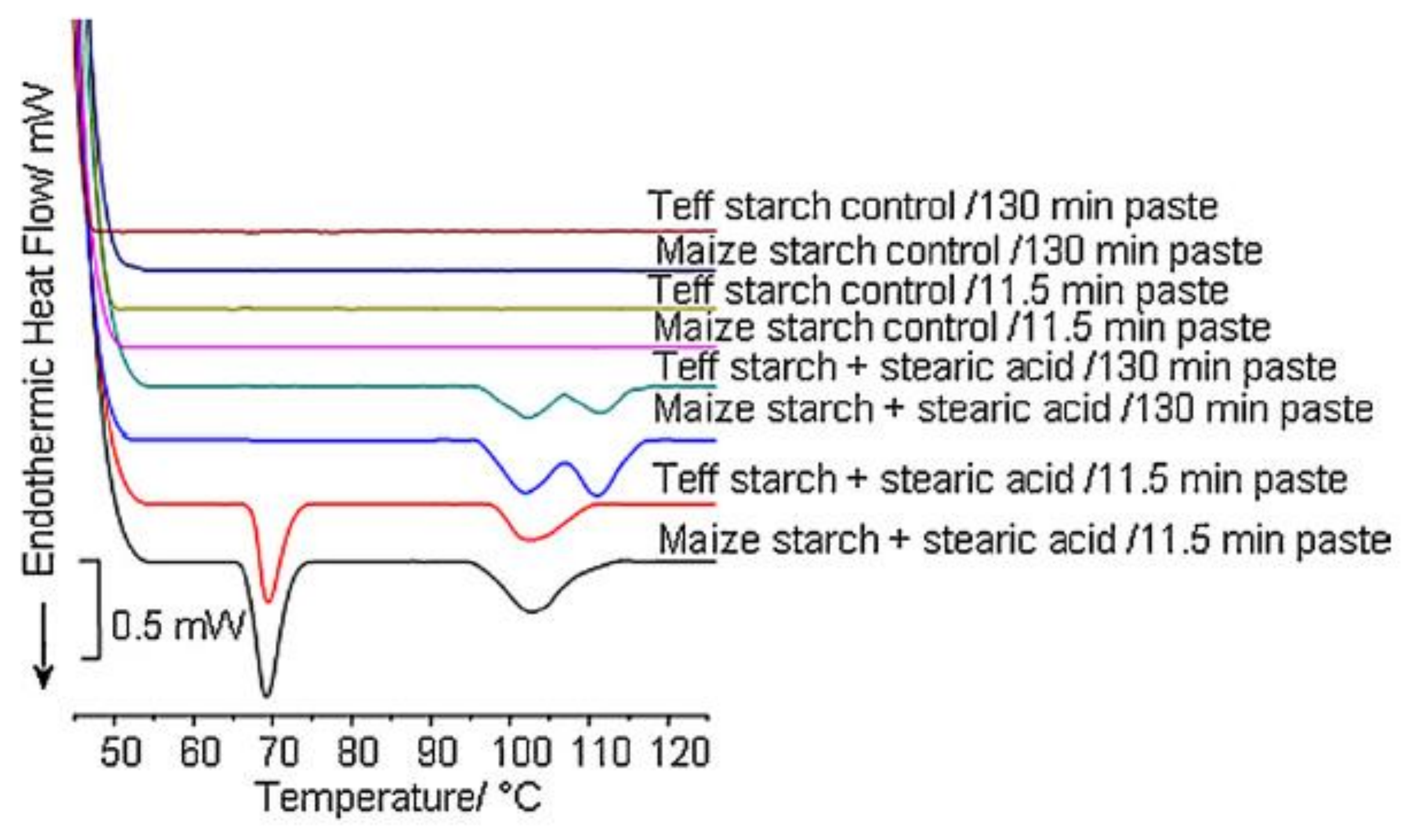

Figure 2.6: Differential Scanning Calorimetry of teff and maize starch complexed with stearic acid **(Wokodala *et al.*, 2012).**

2.5 Isolation of V-amylose complex

There are various methods that can be used to make amylose-lipid complexes (Putseys *et al.*, 2009). These preparation techniques include the use of heat in combination with shear treatments as well as a more standard method. Some of these methods include the use of extrusion, steam and pasting methods. Heating as a method to produce amylose-lipid complexes makes use of gelatinisation to promote the disruption of the starch granule (Nelles *et al.*, 2000). When gelatinisation of starch occurs, the amylose polymers of starch are freed from the ordered starch granule (Zhang *et al*., 2012), allowing them to interact with other constituents such as lipids. Studies showed that a second pasting peak is exhibited due to the formation of amylose-lipid complexes (as stated earlier), which can result in a more viscous paste (D'Silva *et al*., 2011; Wokadala, Ray, and Emmambux, 2012). The addition of external sources of lipids to starch that is being pasted produces a more stable second peak. This was attributed to more stable amylose-lipid complexes being formed. With an extended holding time after the second pasting peak, more organisation is experienced between the lipids and amylose, enabling more stable Type IIb amylose-lipid complexes to be formed (Putseys *et al.*, 2010). These amylose-lipid complexes can be isolated (a patented process has been developed: see Ray**,** Cuthbert and Emmambux, 2014).

Another method involves mixing amylose with lipid molecules. First, starch is dissolved in solvents, such as potassium hydroxide (KOH). Lipids are then added to the mixture and incubated at elevated temperatures (60^{o}C–90^{o}C) to allow association between amylose and lipids to occur (Seneviratne and Biliaderis, 1991). Crystallisation of the complexes is promoted by a cooling stage (retrogradation reaction). The pH can also be neutralized to promote crystallisation, but a cooling stage is usually used to allow for crystallisation of the complexes to occur (Kugimiya *et al.*, 1980). The last step of preparing amylose-lipid complexes is to isolate the complexes, which is usually done by means of centrifugation techniques. The complexes that are formed using this technique are usually not consistent, as variations in the complex types were found (Zabar, Lesmes, Katz, Shimoni, and Bianco-Peled. 2010). This method differs from heat treatments as no shear forces are applied in the procedures.

2.6 Starch films

Factors such as low cost, biodegradability and renewability have made starch a strong contender for packaging applications (Sorrentino, Gorrasi and Vittoria, 2007; Avérous and Fringant, 2001). However, starch first has to be modified (e.g. heat treatment), as starch in its native form has limited applications (Zhao *et al.*, 2008). Modification of starch can be done in the form of granule disruption or chemical modification. When heated, starch can form a molten thermoplastic material (Avérous and Fringant, 2001). However, films formed from pure starch are brittle in nature due to the strong cohesive nature of the starch polymers (Zhang *et al.*, 2013). The addition of plasticisers such as glycerol (10%–40% w/w of starch) to the starch before the heating step can improve the starch film. This improvement is in terms of improved physical and/or mechanical properties (Nejad, Ganster and Volkert, 2010). The starch films formed with plasticisers showed increases in molecular mobility, lowered glass transition temperature (T_g) and decreases in the degree of crystallinity. The plasticiser that is used should be compatible with the starch polymers. It was also noted that the plasticisers increased the flexibility of the starch film, probably because they hindered intermolecular interactions between adjacent polymeric chains (Pushpadass, Marx and Hanna, 2008).

Plasticised starch could be suitable for numerous packaging applications, for example injection moulding or thermoforming (Bastioli, 2001). However, there are various limitations in the utilisation of plasticised starch in packaging applications. These limitations include, but are not limited to, poor mechanical properties (low tensile strength), water sensitivity and poor barrier properties (indicated by large amounts of water allowed to permeate through film) (Avérous and Fringant, 2001; Psomiadou *et al.*, 1997). Studies done by Muscat, Raju, Stafford, Qipeng, and Benu (2013) showed that some of the limitations of using starch films are due to their hydrophilic nature and weak water barrier properties. This could also promote other limitations such as poor flexibility e.g. tensile properties, due to their hydrophilic nature.

2.6.1 Bio-composite material as a way to improve starch films

Many researchers have attempted to improve the functionality of starch films by using filler materials (Chivrac, Pollet, and Averous, 2009). These filler materials are usually biodegradable composite materials and at nanoscale (between 1–100 nm) (de Azerdo, 2009). Several authors have reported the benefits of bio-nanocomposite materials (Sorrentino *et al*., 2007; Sozer and Kokini, 2008; Chivrac *et al*., 2010; Fukushima *et al*., 2009; Bastioli, 2001; Zhao *et al*., 2008). This material is a combination of bio-based polymers and nano-sized fillers *(Figure 2.7)*. The nano filler material can be inorganic or organic in nature (Spiridon, Teaca and Bodirlau, 2010).

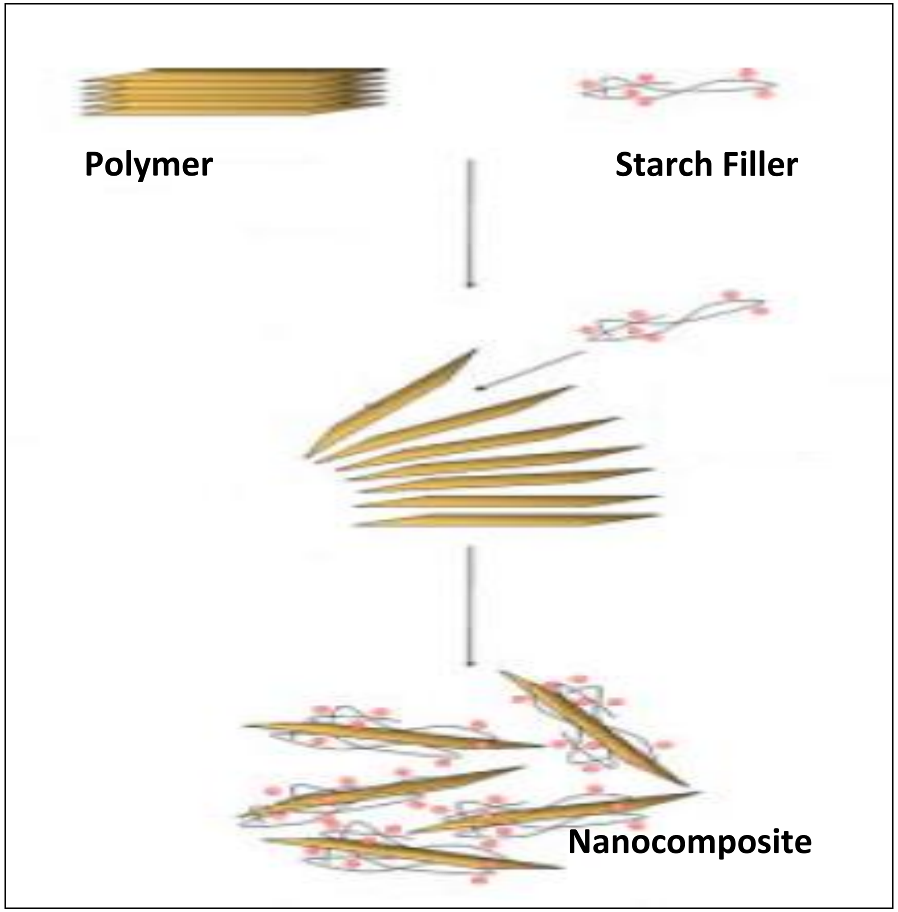

Figure 2.7: An example of a preparative technique to make a nanocomposite material **(Chivrac *et al.*, 2009).**

Clay materials such as montmorillonite and sepiolite can be used as inorganic filler materials at nanoscale (Fukushima *et al*., 2009). When these nano fillers were incorporated in the composite materials, it was observed that improvements to the mechanical, thermal and gas barrier properties could be attained (Nejad *et al.*, 2010). Chivrac *et al.* (2009) reported that an evenly distributed filler material will yield the best results. The main factor is that the large interface area leads to strong interactions between the polymer matrix and the nano fillers (Chivrac *et al*., 2010). These interactions allow for a stronger network to be formed through hydrogen linkages between the matrix and filler (Lin *et al*., 2010). It has been hypothesized that they improve the permeability properties. Owing to these interactions, several advantages have been observed. The molecular mobility is lowered due to the strong interaction between the matrix and nano filler. A lower mobility allows for improved barrier properties, as diffusion through the film needs more energy (activation energy) to diffuse through the material (Miller and Krochta, 1997). The relaxation state of the material is also lower (i.e. it relaxes faster), as the tight binding of the filler and the matrix helps the material to resume its original form more easily under stress conditions (Sorrentino *et al*., 2007). The formation of a tortuous pathway due to the binding of the filler and the matrix, *(Figure 2.8)* has also been used to show that diffusion will have to follow an indirect pathway through the material, thus improving the barrier properties. Lin *et al*. (2010) had also noted that an increase in the starch content used to make the filler material would allow for a decreased permeability to compounds like water and oxygen, based on the tortuosity of the material.

Nanocomposites can also have other mechanisms that act to reinforce its structure. A 3-phased structure, as seen in Figure 2.9, in which there is an interfacial region between the nano filler and polymer matrix, can reinforce the nanocomposite (Lin *et al.*, 2010). Reactions occur between the interfacial region, the polymer matrix and the nano filler. These reactions can include Van der Waals forces and hydrogen bonding, depending on the type of material used. These reactions are thought to stabilise the material by improving the adhesion between the nano filler and polymer matrix. This in turn will improve properties of the nanocomposite materials, such as enhanced mechanical properties.

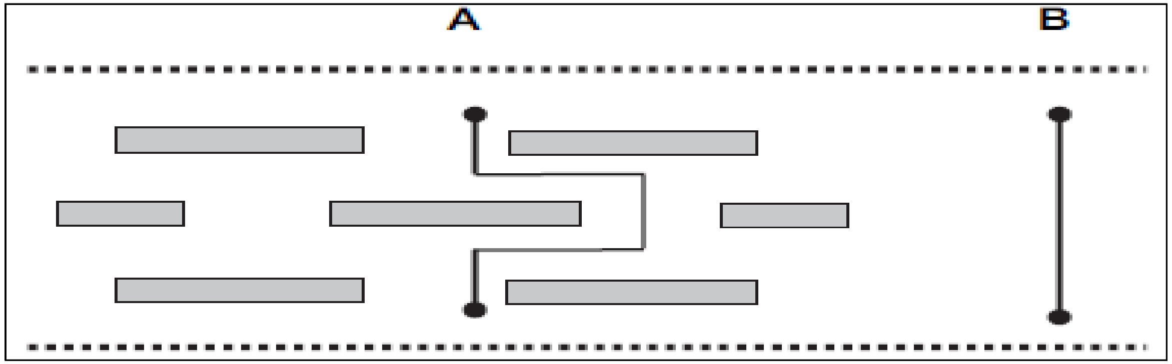

Figure 2.8: (A) exhibits the tortuous pathway due to incorporation of nano filler material in a polymer. (B) is the unfilled polymer **(Sorrentino *et al.*, 2007).**

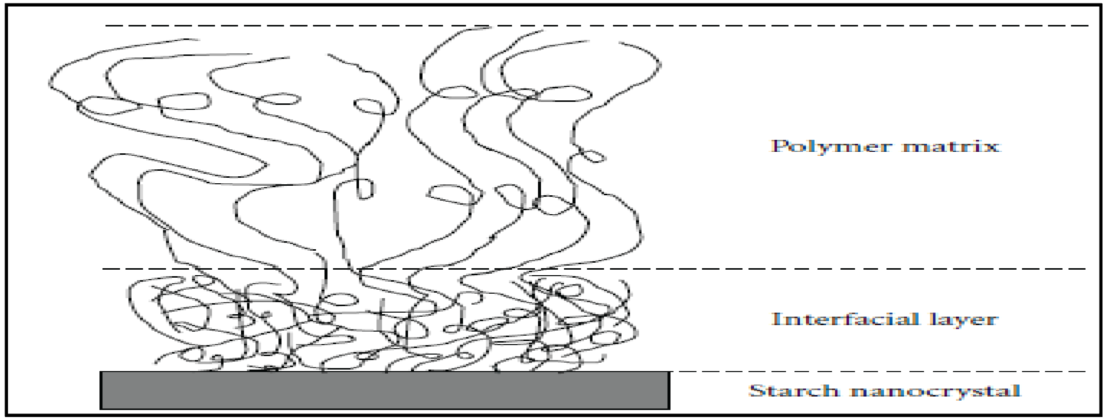

Figure 2.9: A schematic illustration showing a 3-phase structure model **(Lin *et al.*, 2010).**

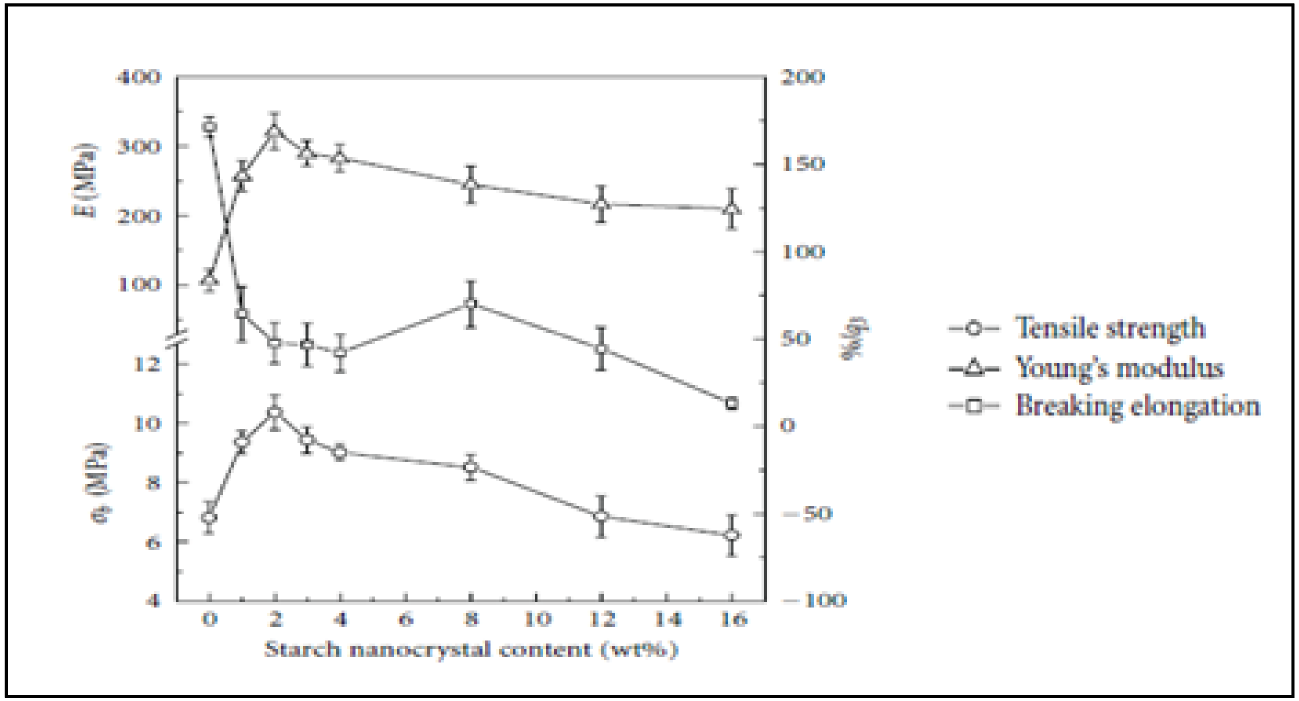

Figure 2.10: The effect of nanocrystal contents on the performance of a nanocomposite material (Lin *et al.*, 2010).

In a study done by Lin *et al.* (2010), it was noted that levels of 1%–3% of nano filler material (starch nanocrystals) increased tensile strength as well as the Young’s modulus *(Figure 2.10)*. The nano filler material was shown to improve properties of the film.

Tables 2.1 and 2.2 show the water vapour permeabilities and mechanical properties of starch nanocomposite films, respectively, as well as their controls, as reported by Muller, Laurindo and Yamashita (2011). It can be seen that the incorporation of a nanomaterial (sodium bentonite) into the film decreases the water vapour permeability and increases both the tensile strength and Young’s modulus. The elongation at break was reduced. These results further indicate the improvements expected as a result of incorporating nanoparticles to produce nanocomposite films.

Research has shown that incorporating filler materials such as nano fillers into the bio-based matrices, for example in Polylactic Acid (PLA), to produce nanocomposite material can improve the mechanical and barrier properties (Chivrac *et al*., 2010). Figure 2.11 shows that the incorporation of a nano filler material (nano clay) into the composite material results in decreased water vapour permeability with an increase in clay content, owing to the creation of a more tortuous pathway.

Table 2.1: Water vapour permeability (K^w), solubility coefficient (β), and diffusivity (D^w) of water in starch nanocomposite films **(Muller *et al.*, 2011).**

Film:	**K^w x 10^7 (g m/ m^2 h Pa)**	**RH**	**β x 10^5 (g water/ g solid Pa)**	**Flux (g water/ m^2 h)**	**D^w x 10^{12} (m^2/s)**
Control (no added nanocomposite)	7.8 ± 0.7	2%	8.78	4.58	2.13
		75%	26.90		
Nanocomposite	6.2 ± 0.6	2%	7.20	3.33	1.72
		75%	24.59		
Extruded nanocomposite	3.0 ± 0.2	2%	6.94	1.51	0.83
		75%	18.33		

- **Data are mean of ten replicate determinations ± standard deviations**
- **p-value ≤ 0.05**
- **Nanomaterial is sodium bentonite**

Table 2.2: Tensile strength (T), elongation at break (ε), and Young's modulus (Y) of starch nanocomposite films **(Muller *et al.*, 2011).**

Film:	**T (MPa)**	**ε (%)**	**Y (MPa)**
Control (no added nanocomposite)	0.96 ± 0.13	63.3 ± 11.5	16 ± 4
Nanocomposite	1.45 ± 0.14	72.93 ± 6.99	42 ± 5
Extruded nanocomposite	16.47 ± 1.17	2.60 ± 0.49	789 ± 42

- **Data are mean of ten replicate determinations ± standard deviations**
- **p-value ≤ 0.05**
- **Nanomaterial is sodium bentonit**

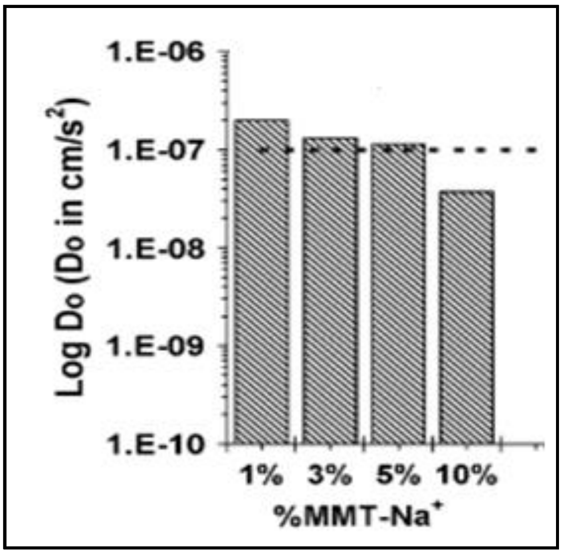

Figure 2.11: The diffusivity of water vapour in a composite material, using clay as filler material at various levels **(Sorrentino *et al.*, 2007).**

2.6.2 Preparation techniques for nanocomposites:

Various methods have been used to make layered silicate-containing polymer nanocomposite materials *(Figure 2.12).* In situ polymerisation refers to a method in which core materials (nanomaterials and monomers) are polymerized in a continuous phase and no reactants are included. This allows for the fabrication of a composite material in which all materials exist in a continuous phase. Template method refers to a method in which a solid template is used for composite materials with a monodispersed size distribution. This allows for the fabrication of composite materials with a predefined size distribution as well as homogenous particle distribution.

For composites that are organic-inorganic solid layer silicates, intercalation and exfoliation are the 2 major steps in nanocomposite formation (Sorrentino *et al.*, 2007). The difference between these methods is the way in which these composites are aggregated with the matrix. A review done by Sozer and Kokini (2008) stated that the use of exfoliation to produce nanocomposite material resulted in a material with significantly improved properties, such as increased tensile strength.

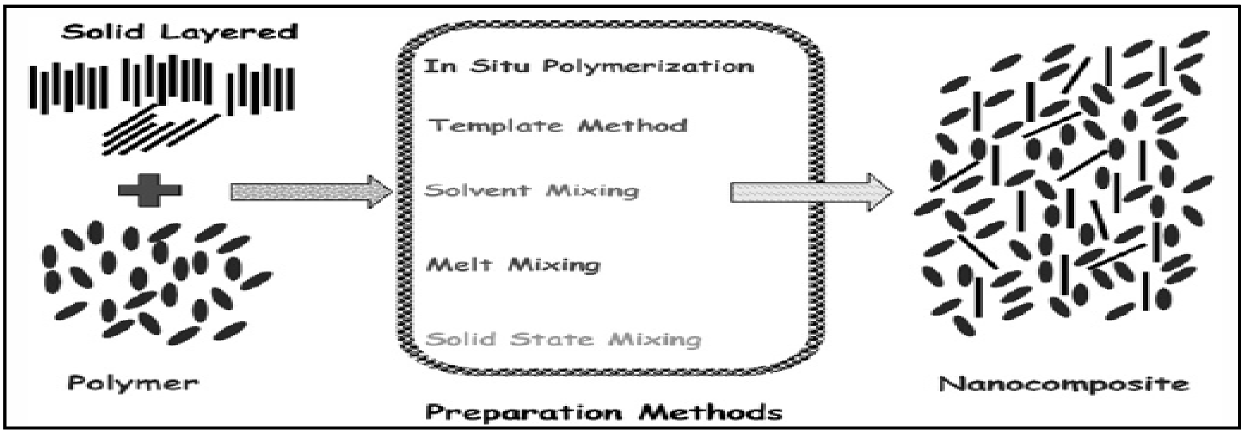

***Figure 2.12: Examples of preparation techniques for polymer composite materials* (Sorrentino *et al.*, 2007).**

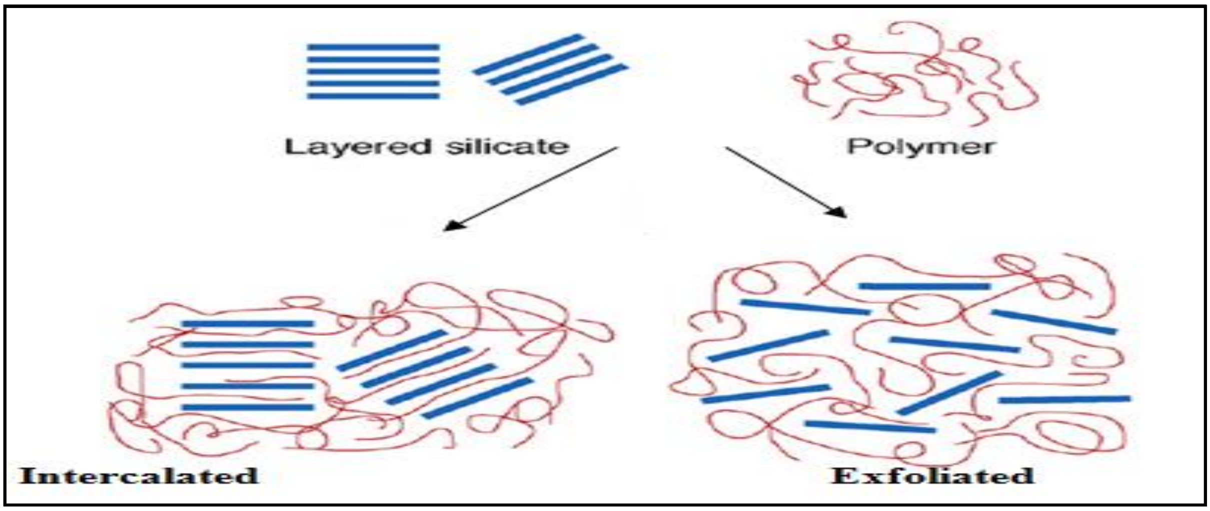

***Figure 2.13: Illustrations of intercalated and exfoliated nanocomposite materials* (Sozer and Kokini, 2008).**

Polymer chains can be intercalated by inserting a monomer between the layered sheets. Following this, the monomers have to be polymerised, which can be done by adding energy to the systems, with heat or radiation being the usual method (Ray and Okamoto, 2003). An alternate approach is directly inserting a polymer into the layered sheets provided that they are compatible. The polymer matrix can be in a molten state, or a solvent can be used to allow for insertion (Sorrentino *et al.*, 2007). The formed composite can either be an intercalated or an exfoliated layer silicate *(Figure 2.13).*

An alternate method used to make nanocomposites is the melt intercalation technique *(Figure 2.14)*. During the intercalation step, a large amount of molecules has to be released so that associations between the molecules and polymers can be achieved (Ray and Okamoto, 2003). Advantages of using this method include being able to make specific hybrid type composites, which are more environmentally and economically viable, as no solvent is needed to facilitate the exfoliation steps.

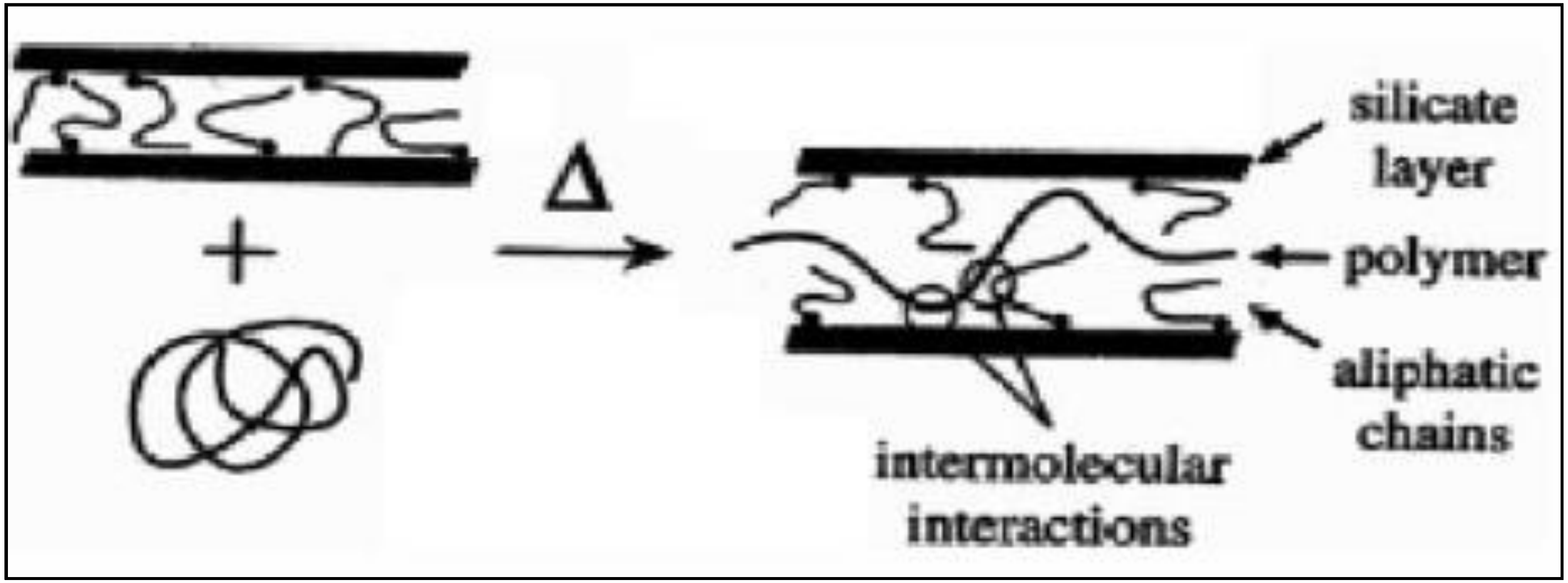

Figure 2.14: Illustration of a melt intercalation (Ray and Okamoto, 2003).

2.7 Concluding remarks

Starch can form a thermoplastic material that allows it to have an increased functionality in terms of a film. During prolonged starch pasting using RVA instrumentation, a biphasic peak can be seen, and the addition of lipids to the starch allows for more prominent and stable peaks to be formed. The lipids improve the starch's stability by allowing for associations with the amylose portion of starch. These complex amylose-lipid materials enable a stable network to be formed. The amylose-lipid complexes are seen to be nano size (1–100 nm) materials based on size. Various methods, such as RVA pasting using modified starches) or in situ polymerization (solvent, template or layering technique), can be used to produce these complexes. These methods can yield different types of complexes, i.e. Type I or Type IIa and Type IIb.

When the starch paste is cast, it can form a film. Although these films show poor barrier and mechanical properties, by incorporating nanomaterials such as clays into them, a composite material can be formed. These composites have been shown to increase the barrier properties and mechanical properties of the film due to several factors. For example, they create a stable 3-phased network, increase the surface area for binding, and create an indirect path for water and oxygen molecules to pass through the film. The materials used are mainly inorganic clays, but they are not edible. Organic based nanomaterials such as the amylose-lipid complexes can be used instead of inorganic nano fillers.

3. Hypotheses and objectives

3.1 Hypotheses

3.1.1 Wheat starch that is modified with stearic acid (using various percentages of stearic acid addition amounts) will produce films with varying mechanical (tensile stress and strain), thermal (increase in T_g) and barrier properties (lower moisture and oxygen barrier) when the modified starch is analysed after being pasted for either 30 minutes or 120 minutes.

Defatted starch produces a single pasting peak, but the incorporation of an external source of lipids to the starch results in the formation of a stable second peak. D'Silva *et al.* (2011) noted that an increased amount of stearic acid added to starch, that was pasted, resulted in a more pronounced second peak formation. It was also observed that the total amount of amylose in the system was lowered during pasting for a prolonged period when lipids were added to it. Wokadala *et al*. (2012) showed that amylose-lipid complexes are formed during the biphasic pasting and that these complexes are at nanoscale. The complexes can form a stable network, due to the high surface area and higher molecular order of the complexes when the films are produced. Thus the production of nanosize amylose-lipid complexes during pasting can act as a nano filler to improve wheat starch film properties.

3.1.2 Isolated amylose-lipid nanomaterial incorporated into wheat starch films can improve the mechanical (increase in the tensile stress and strain), thermal (increase in T_g) and barrier properties (lower moisture and oxygen barrier) of the material, as compared to materials that do not have amylose-lipid nanomaterial.

Nanoparticles incorporated into a packaging material system allow for improved barrier properties as molecular diffusion, of water or oxygen for example, follows a tortuous pathway (Sorrentino *et al.*, 2007). Several authors (Zhao *et al.*, 2008; Chivrac *et al.*, 2010; Psomiadou *et al.* 1997) report that there is a large surface area between the nanoparticles and the polymer matrix into which it is incorporated, allowing for stronger interactions between the nanoparticles and the matrix. This makes the system more tight, which lowers the molecular mobility and improves the relaxation behaviour of the film (Lin *et al.*, 2010).

This incorporation further imparts improved mechanical properties and an increase in thermal stability to the packaging material. The tight binding of the matrix with the nanoparticles also accounts for higher activation energy to facilitate diffusion of compounds, like oxygen and water, through the film (Miller and Krochta, 1996). The film will thus have lower permeability to diffusion and further improved barrier properties.

3.2 Objectives

3.2.1 To determine the effect of pasting time and stearic acid addition on the mechanical, thermal and barrier properties of wheat starch films.

3.2.2 To determine the effect of amylose-lipid nanomaterial on the mechanical, thermal and barrier properties of wheat starch films.

4. The effect of stearic acid addition and pasting times on the properties of wheat starch films

Abstract

Wheat starch was modified with stearic acid at 0.5% and 1.5% addition (i.e. modified wheat starch). The modified wheat starch was pasted using RVA instrumentation, for either 30 minutes or 120 minutes. Films were then made from this modified wheat starch paste. The films' properties were tested in terms of mechanical, thermal and barrier properties. Wheat starch films modified with 0.5% stearic acid resulted in increased tensile properties, including maximum stress and strain, and increased stress at break, as compared to the other films that were tested (i.e. the control film or modified wheat starch films with 1.5% stearic acid). Furthermore, there was a significant increase in the thermal stability indicated by increased glass transition (T_g). There was also a significant decrease in the water vapour permeation as compared to the other films tested (i.e. the control film or modified wheat starch films with 1.5% stearic acid). Wheat starch films made with wheat starch modified with 1.5% stearic acid after 30 minutes and 120 minutes pasting seemed to be weaker, as indicated by decreased stress, strain and T_g values. Films made after 120 minutes pasting time generally also had decreased stress, strain and T_g values. The results achieved indicated that pasting time as well as modifying wheat starch with stearic acid can affect films' properties. These include significant changes/improvement to the films’ tensile, barrier and thermal properties, as indicated above. The presence of amylose-lipid complexes in the films is suggested to be the main change factor. During short pasting, amorphous amylose-lipid material formed improved the film properties. The semi-crystalline amylose-lipid nanomaterial formed during extended pasting can also produce films, although with inferior properties.

4.1 Introduction

Packaging materials play a fundamental role in ensuring that food quality is maintained during transport and storage. A vast amount of petroleum based packaging materials are used in the food industry. The disposal of this synthetic packaging material has a negative effect on the environment due to several factors such as pollution and excessive landfill (Zhao *et al.*, 2008). As a result, great interest has arisen in producing environmentally friendly packaging materials suitable for food packaging.

Biodegradable packaging materials can be made using renewable resources such as starches (Sozer and Kokini, 2008). These materials, however, have poor mechanical and permeability performance as compared to materials made from non-renewable petroleum based resources (Sorrentino *et al.*, 2007). Starches can be modified to overcome these limitations and then be made into packaging materials (Avérous and Fringant, 2001). Chemical modification, using chemicals such as anhydrides or oxides, is widely used to modify starch. However, these chemicals are synthetic and may leave harmful residues in the packaging materials, causing a negative effect on food products. Food grade materials such as fatty acids can be used to modify the starch for packaging materials (Putseys *et al.*, 2010).

Stearic acid is a fatty acid that has been used to modify starches such as teff and maize during starch pasting (Wokadala *et al.*, 2012). Heat treatment, such as pasting, allows starch to form a thermoplastic material. Thermoplastics can be cast to form a film. Pasting of starch with the incorporation of stearic acid for longer periods shows that starch has a biphasic nature. Biphasic pasting is a phenomenon that has been characterized as the development of two pasting peaks during starch pasting, with RVA instrumentation, in the presence of lipids such as fatty acids. The first peak develops during cooking for a short time (< 15minutes), whilst a prolonged cooking period (>30 minutes) gives rise to a second peak. The second peak is characteristically higher than the first peak due to an increase in viscosity (D'Silva *et al.*, 2011).

Wokadala *et al.* (2012) noted that an increase in the amount of stearic acid added to starch resulted in more pronounced second peak formation during pasting for 120 minutes. This was attributed to the formation of semi-crystalline Type II amylose-lipid complexes as determined by XRD and DSC techniques. However, Type I amorphous amylose-lipid complexes were produced during pasting for 30 minutes. It was shown that the amylose participated in complexation with the lipids. The objective of this chapter was to determine the effect of stearic acid addition and different pasting times on the mechanical, barrier and thermal properties of wheat starch films.

4.2 Experimental

4.2.1 Materials

Commercial wheat starch (South African durum wheat supplied by Ruto Mills) was used, with 0.3% protein and 0.1% fat (proximate analysis done). Stearic acid (analytical grade, ≥ 98.5% assay) obtained from Sigma-Aldrich was used. All other chemicals, supplied by Sigma-Aldrich, were of analytical grade.

4.2.2 Methods

a. Stearic acid starch mixture preparation

Stearic acid [0%, 0.5%, 1.5% w/w (of total starch weight)] was mixed in excess ethanol until dissolved. Wheat starch, 100g, was thoroughly mixed into the ethanol-stearic acid solution in a shaking water bath at 45^{o}C for 45 minutes. The solution was then dried in a forced draught oven to evaporate all the ethanol at 40^{o}C–45^{o}C.

b. Pasting of wheat starch

Stearic acid and 100g wheat starch mixture (0%, 0.5%, 1.5%) and 35% glycerol (w/w of starch) was added to a RVA canister and made up to a volume of 28 ml with distilled water. The stearic acid and wheat starch mixtures were pasted for 30 minutes and 120 minutes.

Pasting for 30 minutes:

The RVA temperature setting used ensured that the start temperature was increased at a ramp rate of 5 ^{o}C/minute until the set point of 90^{o}C was reached. The mixture's initial stirring rate was 960 rpm for 30 seconds after the temperature of the mixture reached 50^{o}C. Once the temperature of 50°C was obtained, the stirring rate was decreased to 160 rpm for the remaining pasting period (30 minutes).

Pasting for 120 minutes:

The RVA temperature setting used ensured that the start temperature was increased at a ramp rate of 5 °C/minute until the set point of 90°C was reached. The mixtures initial stirring rate was 960 rpm for 30 seconds after the temperature of the mixture reached 50°C. Once the temperature of 50°C was obtained, the stirring rate was decreased to 160 rpm for the remaining pasting period (120 minutes).

c. Casting of film

The paste (holding at 30 minutes or 120 minutes at 90°C) was immediately diluted (1 part paste:2 parts boiling distilled water) and was homogenized for 1 minute at 1800 rpm. A magnetic stirrer was used to gently stir the paste for 15 minutes, whilst on a heated stirring plate with temperature set at 70°C. The gentle stirring of the paste allowed for the removal of air bubbles. A closed system using a volumetric flask, sealed with parafilm and foil, and an ice-pack on top was used to allow for condensation to occur, resulting in negligible water loss. The paste (16 ml) was then cast on sterilized petri dishes (8cm diameter) and dried in a vacuum oven for 8 hours at 50°C temperature and 400 mBar pressure. The resultant film was then conditioned in a RH chamber, using settings of 50% RH and 25°C.

4.2.3 Analyses

a. Mechanical properties

The films' tensile properties were tested according to ASTM method D882-10 (American Society for Testing and Materials, 1999). An EZ-LX Test texture analyser (Shimadzu, Kyoto, Japan) fitted with a tensile grip was used. Strips (60 mm long and 6 mm wide) were cut using a scalpel and the strips were conditioned in a RH chamber using settings of 50% RH and 25°C. A micrometer was used to measure the thickness at 5 different points before fitting them to tensile grips 40 mm apart. A crosshead speed of 0.4 mm/s was used. Several properties were tested for: maximum stress (σ); maximum strain (ε); strain at break (ε_b), stress at break (δ_b). The Young's modulus was calculated from the linear region of the stress and strain curves.

b. O_2 permeability

The films' oxygen (O_2) permeability was tested using a Mocon Oxtran 2/21 Modular System (Minneapolis, Minnesota, USA). Films were conditioned in a RH chamber using settings of 50% RH and 25ºC before testing. The films were masked with a foil mask to expose only 5 cm^2 area and 2.52 cm diameter for testing. The system was greased at the sealing rim with high vacuum grease before each test. Two films (same treatment) were loaded into the system at a time. A flow rate of 100 ml/minute for the carrier gas (moist N_2) and a temperature of 25ºC was used for each test. The pressure was set to give a 50% RH for the test. Films were conditioned at these temperature and RH settings for 24 hours in the module before the test started. Two cycle-testing was used in which the test stopped when two consecutive results were within 1% of each other. The results were reflected as permeation ($g\ m\ m^{-2}\ day^{-1}$) and then converted to a permeability result ($g\ mm\ m^{-2}\ h^{-1}\ kPa^{-1}$).

c. Water vapour permeability

The films' water vapour permeability was tested using a Mocon Permatran-W3/33 Modular System, (Minneapolis, Minnesota, USA). Films were conditioned in a RH chamber using settings of 50% RH and 25ºC before testing. The films were masked with a foil mask to expose only 5 cm^3 area and 2.52 cm diameter for testing. The system was greased at the sealing rim with high vacuum grease before each test to ensure proper sealing. Two films were loaded into the system at a time. Wet nitrogen gas was used as a carrier gas and a flow rate of 100 ml/minute was used. A temperature of 25ºC was used for each test. Films were conditioned at this temperature setting for 24 hours in the module before the test started. Two cycle testing was used in which the test stopped when 2 consecutive results were within 1% of each other. The permeation results were given and then converted to a permeability result.

d. Dynamic- Mechanical Analysis (DMA)

The films' mechanical properties were tested using a Perkin Elmer DMA 8000 (940 Waltham, Massachusetts, USA). The films were conditioned in a RH chamber with settings of 50% RH before testing. The films were cut into strips (with a scalpel) to fit in between the equipment sample holder (i.e. a metal envelope, approximately 1mm x 12mm) using a tweezer. The sample holder containing the film was then inserted into the DMA machine. The instrument was operated in dynamic mode, using single cantilever geometry. Temperature scans were performed at -30°C–130°C using a 2°C/minute ramp rate. A strain of 0.05% and a multi strain frequency of 1 Hz and 10 Hz was used. The glass transition (T_g) of the films was taken as the temperature in which a modulus change from a glassy to an amorphous state occurred (Roth and Dutcher, 2005). T_g was determined by a transition in tan δ and taken at the tan δ peak. This was verified by applying linear regression to fit a line at the glassy state and a line at the transition. The intersections between these two lines were taken as the T_g temperature, as explained by Andrews and Grulke (2005).

The effect of humidity on the films was investigated. A DMA with a humidity controller was used to test films using the same processing conditions as above (strain: 0.05% and frequency: 1Hz and 10 Hz). The humidity was increased step wise from 10% to 60% at a constant temperature of 25°C. Storage modulus (E′), loss modulus (E″) and tan δ at different RH (30%–60 %) were recorded.

4.2.4 Statistical analysis

Data was analysed using Analysis of Variance (ANOVA) using SAS v8 ® software (SAS Institute Inc., Cary, NC). This determined if there were significant ($p < 0.05$) differences between the different treatments in terms of pasting times (30 minutes and 120 minutes) and stearic acid addition (0%, 0.5%, 1.5%).

4.3 Results and discussion

4.3.1 Pasting properties of wheat starch and stearic acid mixture

Figure 4.1 shows the pasting properties of wheat starch and stearic acid mixture after short (30 minutes) and long (120 minutes) pasting times. From the results, it was observed that a single pasting peak was achieved for all wheat starch after a short pasting time. Wheat starch that had 0.5% stearic acid added to it resulted in the first peak at ca130 RVU. Wheat starch that had 1.5% stearic acid added to it resulted in the first peak at ca140 RVU. Wheat starch with no stearic acid addition had the first peak developing at ca120 RVU. After short pasting, wheat starch with 0.5% stearic acid addition as well as wheat starch pasted with 1.5% stearic acid addition seemed to have the highest peak develop after 30 minutes pasting time. The control seemed to develop the lowest peak after 25 minutes and then it plateaued until the short pasting was done.

During long pasting (120 minutes), two prominent pasting peaks were formed in wheat starch with 0.5% and 1.5% stearic acid addition. A single prominent peak was formed in the control. Wheat starch with 1.5% stearic addition resulted in higher pasting peaks as compared to the control and wheat starch with 0.5% stearic acid addition. The second pasting peaks seemed to be formed after 100 minutes at ca440 RVU for wheat starch with 1.5% stearic acid addition. The second pasting peaks seemed to be formed after 105 minutes at ca380 RVU for wheat starch with 0.5% stearic acid addition. The control seemed to have plateaued at ca110–120 RVU after the first peak at 35 minutes for the duration of the long pasting time.

D’Silva *et al.* (2011) suggested that the addition of stearic acid to maize and teff starches during pasting will allow for the amylose portion of starch to complex with the lipid portion of stearic acid. Wokodala *et al*. (2012) attributed the formation of more prominent and stable peaks to the formation of amylose-lipid complexes. After 30 minutes pasting time, a less ordered, amorphous, Type I complex will form, and after 120 minutes pasting time a more ordered and stable Type II, semi-crystalline complex can be formed. It was also noted that Type I complexes can be formed quickly, due to the random order of the structures (Wokodala *et al*., 2012). However, after 30 minutes pasting a small quantity (10%–15%) of amylose-lipid can be formed. Type II would take a longer time to form due to the ordered nature of the structure. After 120 minutes pasting a larger quantity (25%–30%) of amylose-lipid can be formed (Obiro *et al.*, 2011). This could explain why a more prominent peak was formed after a short pasting time when stearic acid was added to the wheat starch. The stearic acid was complexing with the amylose portion of the wheat starch to form Type I amylose-lipid complexes in situ. Using DSC analysis, Type I and Type II amylose-lipid complexes were characterized, in which Type I amylose-lipid complexes were observed to have an endotherm (Tp ≈ 100°C) and Type II were observed to have an endotherm of (Tp ≈ 110°C) (Wokodala *et al*., 2012). After an extended pasting time, the stearic acid was able to form more stable complexes with the amylose portion of the wheat starch, which could explain why two prominent peaks were seen when stearic acid was added to wheat starch and pasted for 120 minutes.

These complexation reactions between the stearic acid and the amylose portion of the wheat starch are the reason for the more prominent peaks during the 30 minute and 120 minute pasting times. It therefore indicates that the starch and stearic acid undergo some modification. From now on this starch and stearic acid complexed material will be referred to as modified wheat starch.

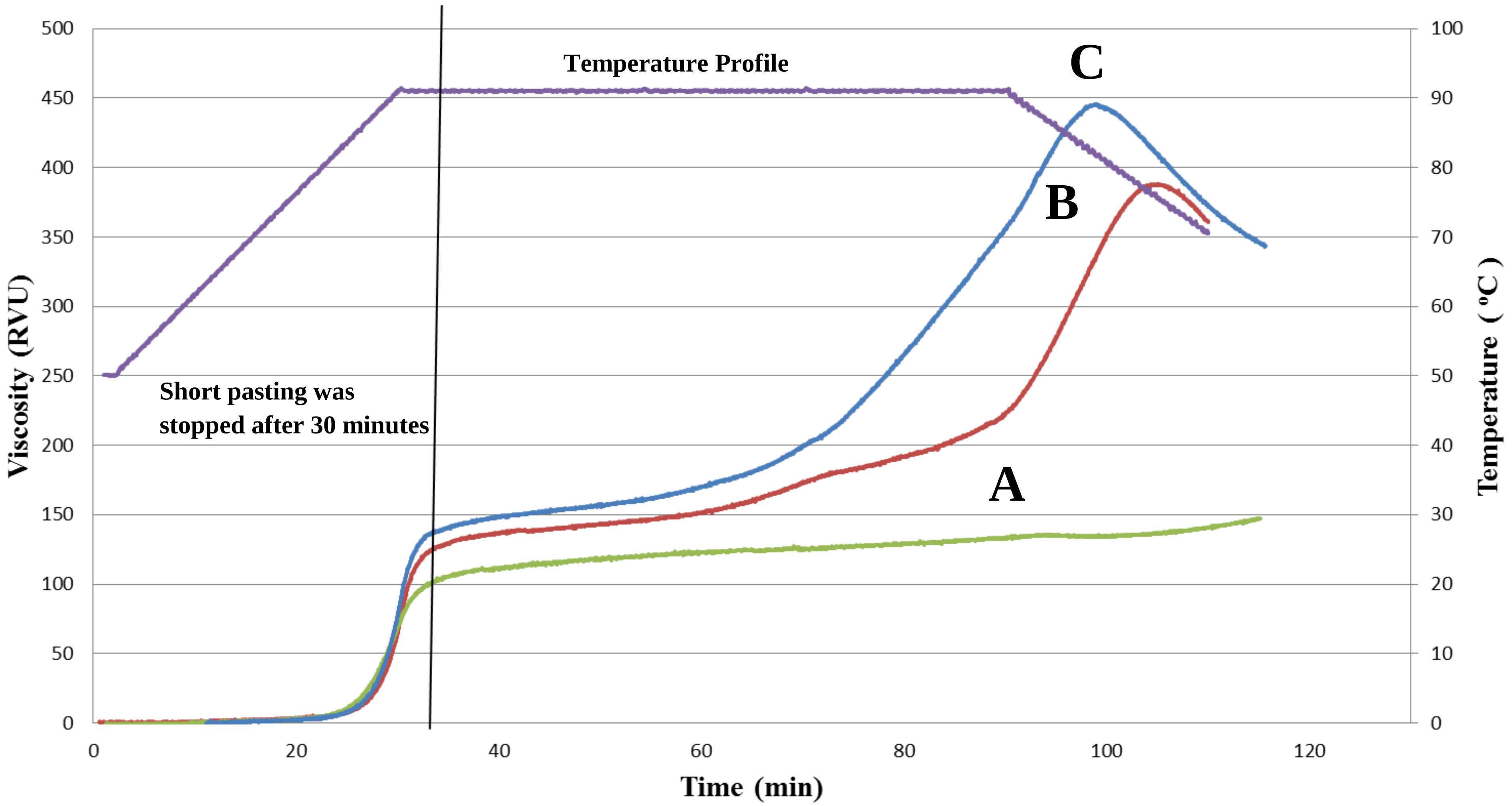

Figure 4.1: The effect of stearic acid addition on the pasting properties of wheat starch (A: 0% stearic acid, B: 0.5% stearic acid addition, C: 1.5% stearic acid addition).

4.3.2 Mechanical properties of modified wheat starch films after pasting for 30 minutes and 120 minutes

Figure 4.2 and Table 4.1 show the effect of short pasting (30 minutes) and the addition of stearic acid on the tensile properties of wheat starch films. From the results, it was observed that after short pasting the control (films made with wheat starch and no stearic acid modification) and films made with wheat starch modified with 0.5% stearic acid were not significantly different in terms of maximum stress and stress at break. In films made with wheat starch modified with 1.5% stearic acid, the maximum stress decreased by ca50% and stress at break decreased by ca48% as compared to the control films.

After short pasting films made with wheat starch modified with 0.5% stearic acid showed a ca42% increase in maximum strain as compared to the control films. It was also observed that in films made with wheat starch modified with 1.5% stearic acid, the maximum strain increased by ca34% as compared to control wheat starch films. There was a ca96% increase in Young’s modulus in the films made with wheat starch modified with 0.5% stearic acid as compared to the control films. Films made with wheat starch modified with 1.5% stearic acid showed a ca74% increase in Young’s modulus as compared to the control films.

Figure 4.3 and Table 4.1 show the effect of long pasting (120 minutes) and the addition of stearic acid on the tensile properties of wheat starch films. From the results it was observed that after a long pasting time, the control films showed the highest values for maximum stress and stress at break and were significantly different from films made with wheat starch modified with both 0.5% and 1.5% stearic acid. Films made with wheat starch modified with 0.5% stearic acid showed a ca32% decrease in maximum stress and a ca35% decrease in stress at break as compared to the control films. Films made with wheat starch modified with 1.5% stearic acid showed a ca65% decrease in maximum stress and a ca66% decrease in stress at break as compared to the control films.

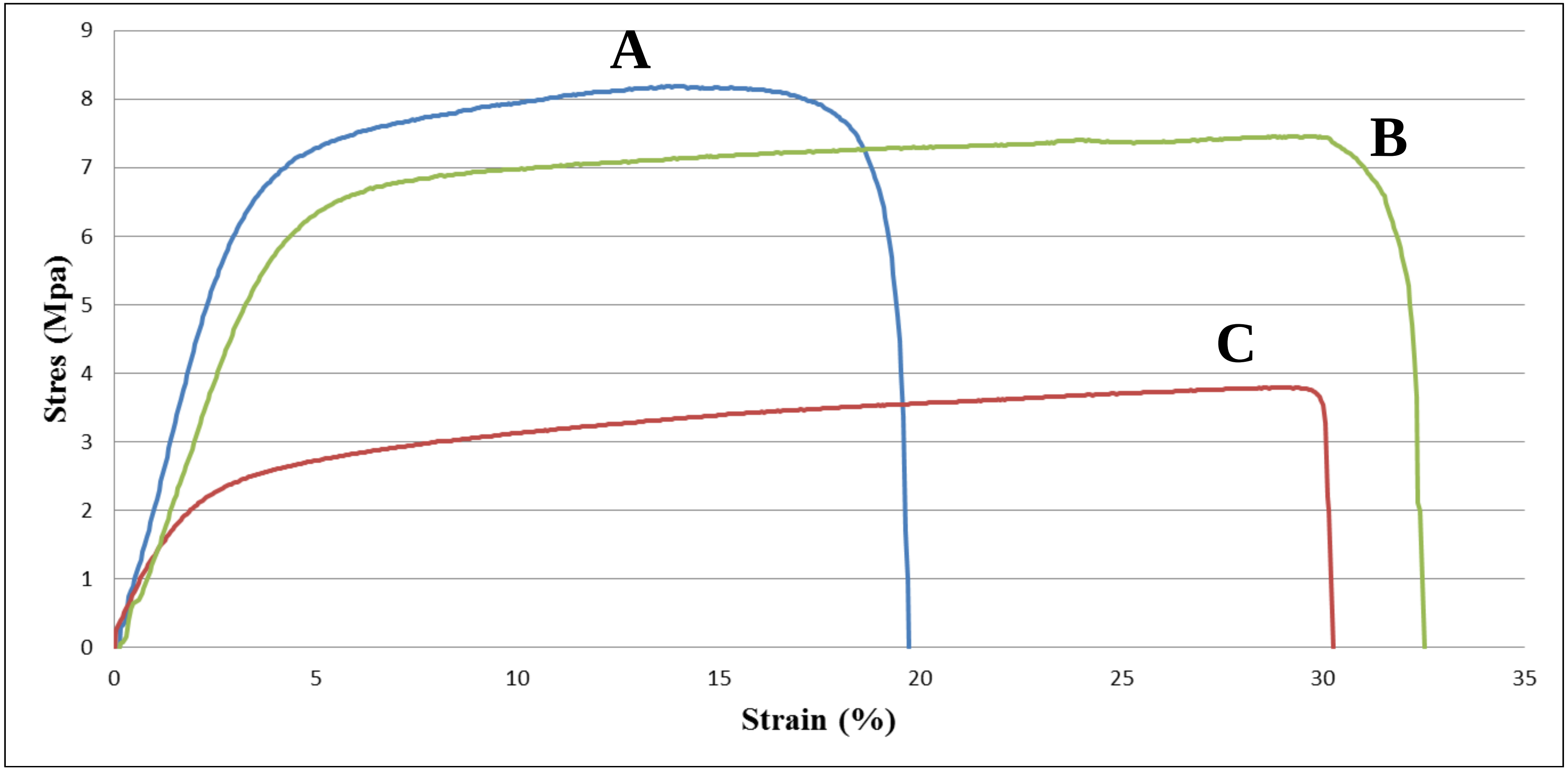

Figure 4.2: The tensile properties of films made with stearic acid modified starch during 30 minutes pasting; (A: 0% stearic acid addition, B: 0.5% stearic acid, C: 1.5% stearic acid).

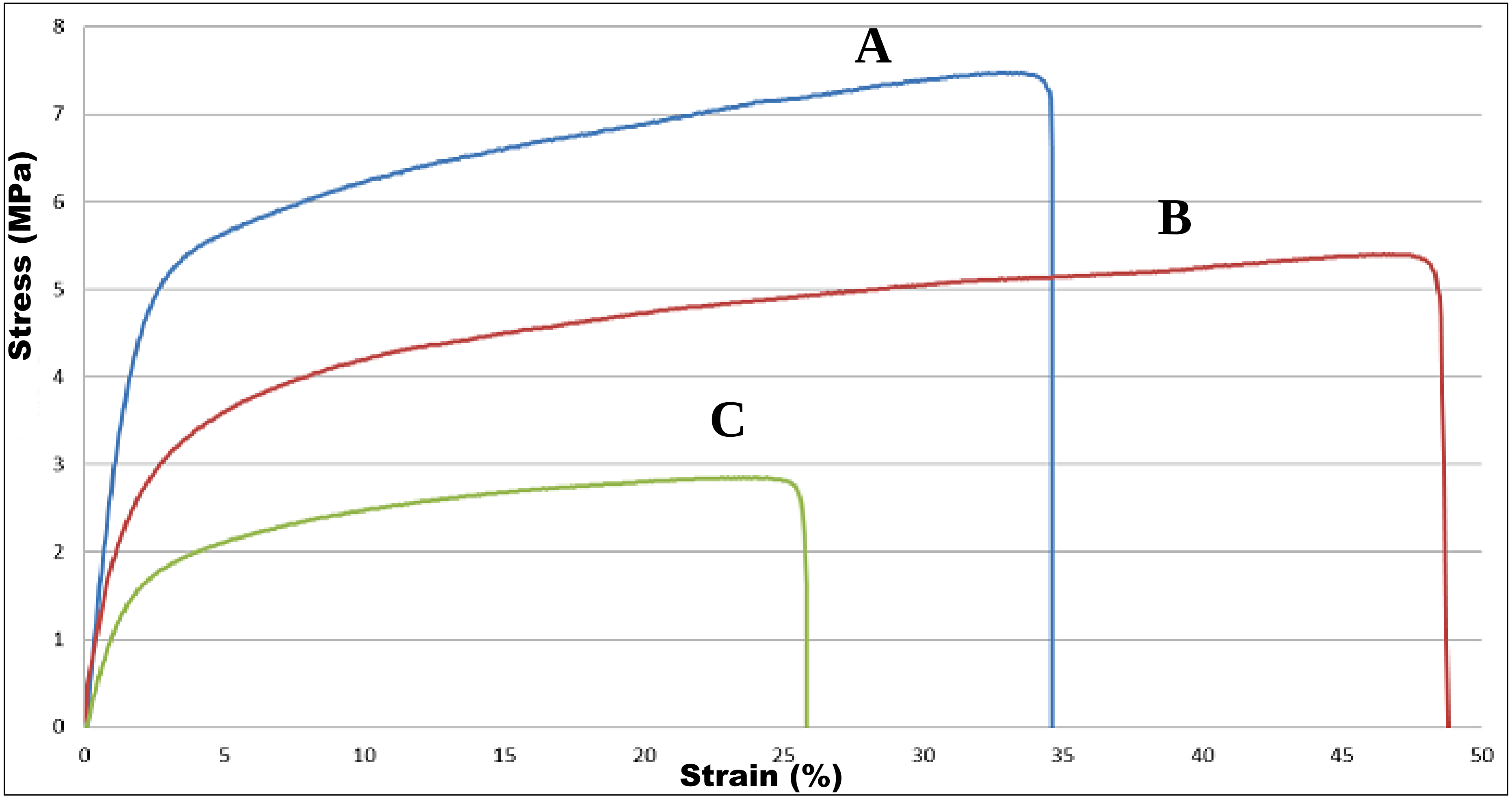

Figure 4.3: The tensile properties of films made with stearic acid modified starch during 120 minutes pasting; (A: 0% stearic acid addition, B: 0.5% stearic acid, C: 1.5% stearic acid).

Table 4.1: The maximum stress (σ), maximum strain (Ɛ), stress (σ) at break and Young's modulus (E´) of starch films with varying stearic acid modification (SA) and pasting times.

Pasting time:	Stearic-acid addition (%)	σ (MPa)*	Ɛ (%)*	σ at break (MPa)*	E´ (MPa)
30 minutes	0	8.5 ^{a} (0.41)	20.3 ^{a} (2.1)	7.6 ^{a} (0.58)	1.3 ^{a} (0.20)
	0.5	7.6 ^{a} (0.97)	35.2 ^{b} (2.85)	7.2 ^{a} (1.00)	34.6 ^{c} (6.55)
	1.5	4.0 ^{c} (0.38)	31.6 ^{b} (3.08)	3.8 ^{c} (0.49)	4.9 ^{a} (1.69)
120 minutes	0	8.0 ^{a} (0.39)	36.4 ^{b} (5.37)	7.7 ^{a} (0.44)	10.8 ^{b} (3.04)
	0.5	5.4 ^{b} (0.08)	50.9 ^{a} (3.76)	5.0 ^{b} (0.18)	30.4 ^{c} (0.86)
	1.5	2.8 ^{d} (0.04)	26.1 ^{c} (4.94)	2.6 ^{d} (0.08)	15.3 ^{b} (1.60)

- **Data are means of 3 replicates with standard deviation in brackets**
- *** a, b, c, d show different levels of variation at a p-value of 0.05**

After long pasting, films made with wheat starch modified with 0.5% stearic acid showed a ca28% increase in maximum strain as compared to the control and were significantly different to the other films. However, films made with wheat starch modified with 1.5% stearic acid showed a ca28% decrease in maximum strain as compared to the control films. Films made with wheat starch modified with 0.5% stearic acid had the highest Young's modulus value with an increase of ca65% as compared to the control and were significantly different to the other films. Films made with wheat starch modified with 1.5% stearic acid showed an increase of ca30% in Young's modulus as compared to the control.

From Table 4.1 it was observed that after short pasting, films made with wheat starch modified with 0.5% stearic acid showed higher maximum stress and stress at break as compared to the films made with wheat starch modified with 1.5% stearic acid. After short pasting, films made with wheat starch modified with 0.5% stearic acid, showed the highest maximum strain values as compared to the other films. It was also observed that after short pasting, the films made with wheat starch modified with 1.5% stearic acid showed higher values for maximum stress and stress at break as compared to the films made with wheat starch modified with 1.5% stearic acid, after long pasting.

From the results described above it was observed that films made from modified wheat starch and pasted for a short time showed higher tensile values compared to long pasting. It was also observed that wheat starch modified with 0.5% stearic acid seemed to have shown the highest increases in the films' tensile properties as compared to the control. These increases could be due to several factors such as the pasting time and stearic acid addition. It is suggested that starch and lipids can associate after pasting and form amylose-lipid complexes, as mentioned earlier. After short pasting times, more amorphous Type I complexes are more likely to form. Long pasting times will favour the formation of more semi-crystalline Type II complexes, as shown by Wokadala *et al.* (2012), this was further researched in Chapter 5 of this study. There were also fewer amylose-lipid complexes for short pasting compared to long pasting. Based on these findings, it can be inferred that after short pasting, wheat starch films modified with stearic acid addition will tend to have a more amorphous form, whilst films made after a long pasting time will tend to have a more crystalline nature of amylose-lipid complexes. Furthermore, after short pasting time, wheat starch films modified with stearic acid addition will have a lower amount of amylose-lipid complex formation, whilst more complexation reactions will occur after a long pasting time and result in more amylose-lipid complexes. It is suggested that films with a more amorphous form yield higher maximum stress and stress at break values as compared to films with a crystalline form, as seen in the results discussed earlier. It is also suggested that films with a lower amount of amylose-lipid complex will tend to have a less crystalline form as compared to films with a higher amount of amylose-lipid complexes. This could explain why wheat starch films modified with 0.5% stearic acid yielded higher maximum stress and stress at break values as compared to the wheat starch films modified with 1.5% stearic acid addition. Type II amylose-lipid complexes can further aggregate to form spherulite structures, as suggested by Bhosale and Ziegler, (*2010*). These spherulites can exist on a micro-scale. The spherulites or the more crystalline Type II amylose-lipid complexes can act as weak points. These weak points can initiate and propagate cracks during tensile testing for lower tensile stress and strain.

It is also noted that amylose is important for film formation (Le Corre, 2011). When amylose interacts with stearic acid to form amylose-lipid complexes, the amylose is not available to form the junction zones which allow for network formation. This is evident in non-gelling starches produced during pasting with stearic acid (D'Silva *et al.*, 2011). Thus, the higher the amount of amylose-lipid complexes in the film, for example during extended pasting, the less amylose is available for junction zone formation, and thus a weakened film is formed.

A study done by Schmidt, Porto, Laurindo and Menegalli (2013) showed that stearic acid addition to films made with cassava starch resulted in decreased tensile stress values. These decreases were attributed to several factors including stearic acid hydrophobicity and some micro-fractures (pinholes) developing in films with increased stearic acid addition. Furthermore, not all the stearic acid could have formed amylose-lipid complexes, which would lead to an excess amount of stearic acid in the films (Wokadala *et al.*, 2012). This could explain why films modified with 1.5% stearic acid addition seemed to have weakened films. The excess stearic acid could cause the development of weak points in the films and can cause micro-fractures more easily than when less stearic acid is present. These weak points could lead to crack initiation and crack propagation more easily and thus yield lower tensile values.

Films made after a short pasting time with wheat starch modified with 0.5% and 1.5% stearic acid addition both showed increases in the maximum strain values as compared to the control. This could be due to the formation of more amorphous Type I amylose-lipid complexes, as mentioned earlier. Films made after a long pasting time with 0.5% stearic acid yielded the highest maximum strain values as compared to all the other films. This may suggest that lower amounts of stearic acid addition may result in the formation of amylose-lipid complexes that are well dispersed in the films. These complexes form few or no aggregates and thus have fewer or no weak points (micro-fractures). These complexes can strengthen and improve the films' tensile properties. Amylose-lipid complexes have been found to form stable networks when incorporated into polymers (Putseys *et al.,* 2010). These stable networks can improve the structural properties of polymers and confer several improvements (Sorrentino *et al*., 2007). This could be the main factor behind the significant improvements to the films' mechanical strength.

Based on the mechanical properties tested above, it was decided that all other testing would be done on the films that showed the best results. Therefore, only films made with wheat starch modified with 0.5% stearic acid were tested further.

4.3.3 Barrier properties

Table 4.2 shows the barrier properties of films made with wheat starch and films made with wheat starch modified with 0.5% stearic acid after short pasting (30 minutes) and long pasting (120 minutes). It was observed that after short pasting, films made with wheat starch modified with 0.5% stearic acid showed decreased water vapour permeation of ca28% as compared to the control. The oxygen permeation through films made with wheat starch modified with 0.5% stearic acid, after short pasting, was decreased by ca14% as compared to the control. After long pasting, films made with wheat starch modified with 0.5% stearic acid showed a decrease in water vapour permeation of ca5% as compared to the control. The oxygen permeation through films made with wheat starch modified with 0.5% stearic acid, after long pasting, was increased by ca10% as compared to the control. It was also observed that the wheat starch films made with 0.5% stearic acid addition, after short pasting, generally showed the most significant decrease in terms of water vapour and oxygen permeation as compared to the other films.

A study done by Schmidt *et al.* (2013) showed that the incorporation of lipids to films made with wheat gluten resulted in films with improved permeability. In the study, it was noted that the improved barrier properties could be attributed to the complexation reactions between stearic acid and wheat starch forming amylose-lipid complexes. These complexes in turn can form networks with polymers (Putseys *et al.*, 2009).

Miller and Krochta (1997) suggested that diffusion through a film is controlled by factors such as size and solubility of permeate. Film properties in terms of polymer structure and compositions can affect the diffusion rate through the films. As mentioned earlier, after a short pasting time, the films are likely to have more amorphous Type I amylose-lipid complex formations than after a long pasting time, where films will have more crystalline Type II amylose-lipid complex formations. This suggests that films made after a short pasting time will be more amorphous in nature, and films made after long pasting time will be more crystalline. The formation of these complexes within the films could perhaps result in tighter network formation and thus will need higher T_g temperatures for a transition to occur (see below Chapter 4.3.4). This may reduce the diffusion of oxygen and water vapour permeability.

Table 4.2: Effect of stearic acid addition during short (30 minute) and long (120 minute) pasting times on Water Vapour Permeability and Oxygen Permeability of wheat starch films.

Pasting time	Stearic acid addition	Water Vapour permeability* ($g\ mm\ m^{-2}\ h^{-1} kPa^{-1}$)	Oxygen permeability* ($g\ mm\ m^{-2}\ h^{-1} kPa^{-1}$)
30 minutes	0% (Control)	2.2 (0.74) [a]	219.0 (8.52) [a]
	0.5%	1.6 (0.37) [c]	187.6 (11.26) [b]
120 minutes	0% (Control)	2.0 (0.65) [ab]	157.5 (10.61) [c]
	0.5%	1.9 (0.55) [b]	175.0 (9.85) [bc]

- **Data are means of 3 replicates (n=3) with standard deviation in parentheses**
- **a, b, c shows levels of variation at a p-value ≤ 0.05**

4.3.4 Thermal properties (Glass transition)

Figure 4.4 shows the thermal properties of films made with wheat starch and films made with wheat starch modified with 0.5% stearic acid after short pasting (30 minutes) and long pasting (120 minutes). The glass transition (T_g) temperature was taken as the peak of tan δ value, similarly to the methods described by Andrews and Grulke (2005). Wheat starch films modified with 0.5% stearic acid, after short pasting, showed higher T_g values as compared to the control films. There was an increase from 16.59°C (0% stearic acid addition, 30 minutes pasting) to 39.98°C (0.5% stearic acid addition, 30 minutes pasting). Films made with wheat starch modified with 0.5% stearic acid, after long pasting, showed no significant changes as compared to the control. It was also observed that after short pasting, wheat starch films modified with 0.5% stearic acid seemed to undergo another transition at ca115°C, indicated by the arrow. This transition could be due to the formation of amylose-lipid complexes which have peak transitional temperatures at 110 °C–118°C (Wokadala *et al.*, 2012).

Glass transition is described as a critical temperature that differentiates glassy and rubbery behaviour (Andrews and Grulke, 2005). It can also be an indicator of molecular motion of polymer chains or motion within a system (Roth and Dutcher, 2005). Several authors (Psomiadou *et al.*, 1997; Chivrac *et al.*, 2010; Zhao *et al.*, 2008) have reported that there can be large interfacial surface areas between polymers such as starch and materials such as amylose-lipid complexes. This may allow for stronger interactions between the complexes and the starch matrix. According to Putseys *et al.* (2010), amylose-lipids exist on a nanoscale of 0.8–2.93 nm. Thus, the amylose-lipid complexes seem to act as a nano filler material. This makes the films more stable due to the lowering of the molecular mobility as well as increasing the relaxation behaviour of the films (Lin *et al.*, 2010). Therefore, a higher T_g will be required due to the stability of the network, as mentioned earlier. This can further illustrate the stability of the network formed due to the higher energy inputs required to disrupt it, or the films can be said to have a lower molecular mobility. These factors impart improved mechanical properties and increased thermal stability to the wheat starch films with stearic acid addition. The tight binding of the starch with the amylose-lipid complexes also accounts for the higher activation energy required to facilitate diffusion of compounds, like oxygen and water vapour, through the film (Miller and Krochta, 1996). The film will thus have lower permeability to diffusion in terms of water vapour and oxygen permeation.

4.3.5 DMA at different RH conditions

Figure 4.5 summarizes the DMA results at different RH of films made with wheat starch as well as films made with wheat starch and modified with 0.5% stearic acid after short (30 minutes) and long (120 minutes) pasting times. At 30% RH, films made with wheat starch and modified with 0.5% stearic acid had a higher E′ as compared to the control for both short and long pasting times. This indicates that the modified wheat starch films can store more elastic energy. When the RH was increased to 40%, 50% and 60%, all the modified wheat starch films still showed higher E′ as compared to the unmodified wheat starch films. Films made after a short pasting time showed the highest E′ values after 30%, 40%, 50% and 60% RH conditions as compared to unmodified films as well as the films pasted for a long pasting time.

As the RH increased, there was a decrease in E′, indicating moisture absorption in all the films. There was a significantly higher decrease in E′ in films made with wheat starch, after short pasting, as compared films made with wheat starch modified with stearic acid addition. After a short pasting time, the control films showed a significant decrease in E′ as the RH was increased. The values dropped from 10.8 GPa at 30% RH to 8.6 GPa at 60% RH *(Figure 4.5)*. The decrease in E′ for films made with wheat starch modified with 0.5% stearic acid was not significantly different between 30%, 40% and 50%, in which values dropped from 12 GPa to 11.4 GPa. At 60% RH, there was a significant drop in E′ values. The same trend was seen for films made with wheat starch and modified with 0.5% stearic acid after long pasting. These results indicate that wheat starch films modified with stearic acid improves the films' mechanical stability at higher RH levels. Furthermore, all the films showed a decrease in E′ values as RH conditions increased; however, the modified wheat starch films showed the least overall decrease as compared to unmodified wheat starch films. In this study, the higher E′ values imply that the modified wheat starch films were more stable at higher RH conditions than the unmodified wheat starch films. These results could be due to several factors, such as a lowered molecular mobility or the stable structure within the film due to large interfacial area (as discussed earlier).

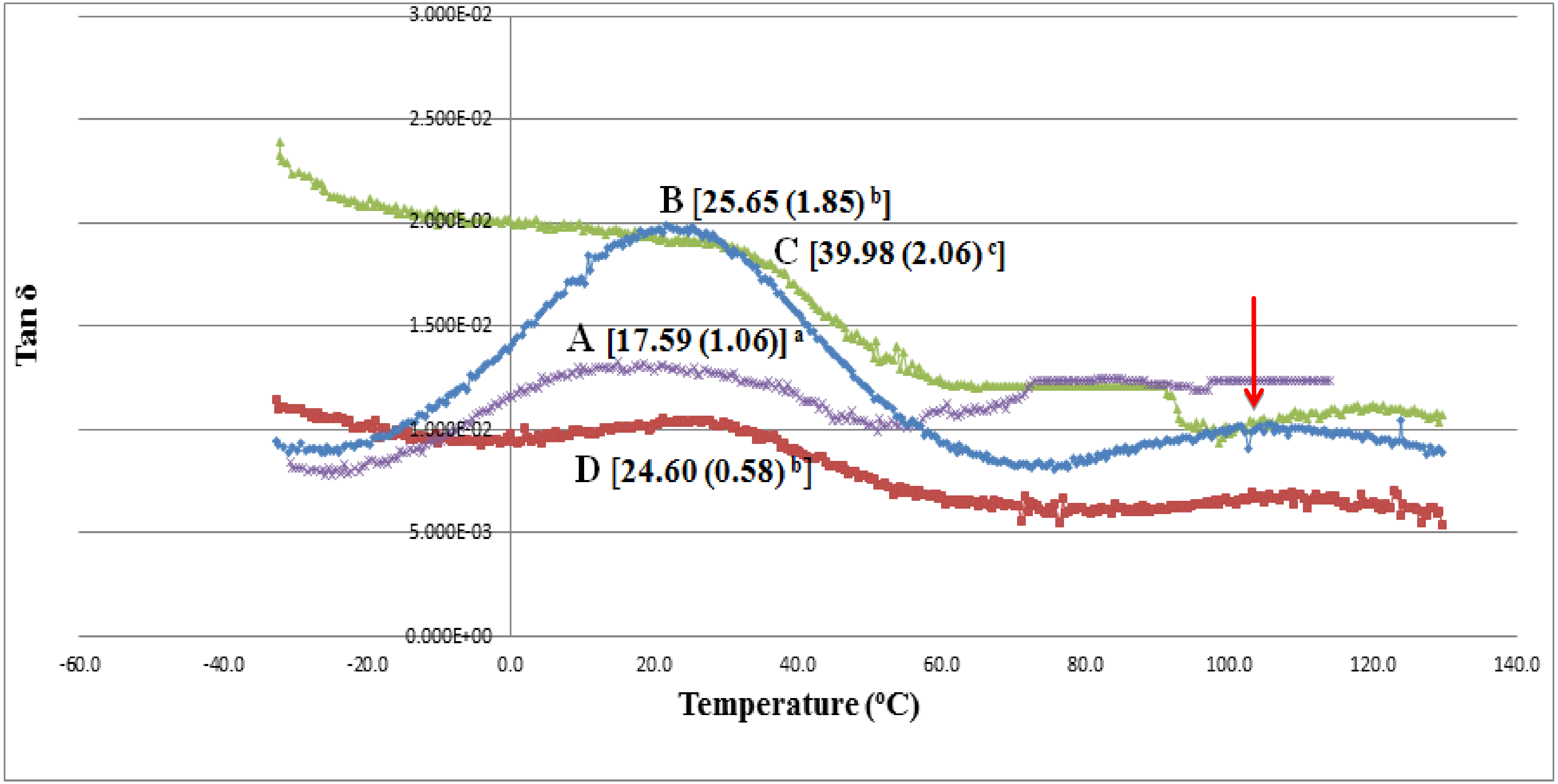

Figure 4.4: The effect of pasting time on the glass transition (Tg) of wheat starch films modified with 0.5% stearic acid tested at a strain of 0.05% and frequency of 10 Hz; (A: control film and 30 minutes pasting, B: control film and 120 minutes pasting, C: wheat starch modified with 0.5% stearic acid films and 30 minutes pasting, D: wheat starch modified with 0.5% stearic acid films and 120 minutes pasting, arrow indicates another transition in the control film and 120 minutes pasting).

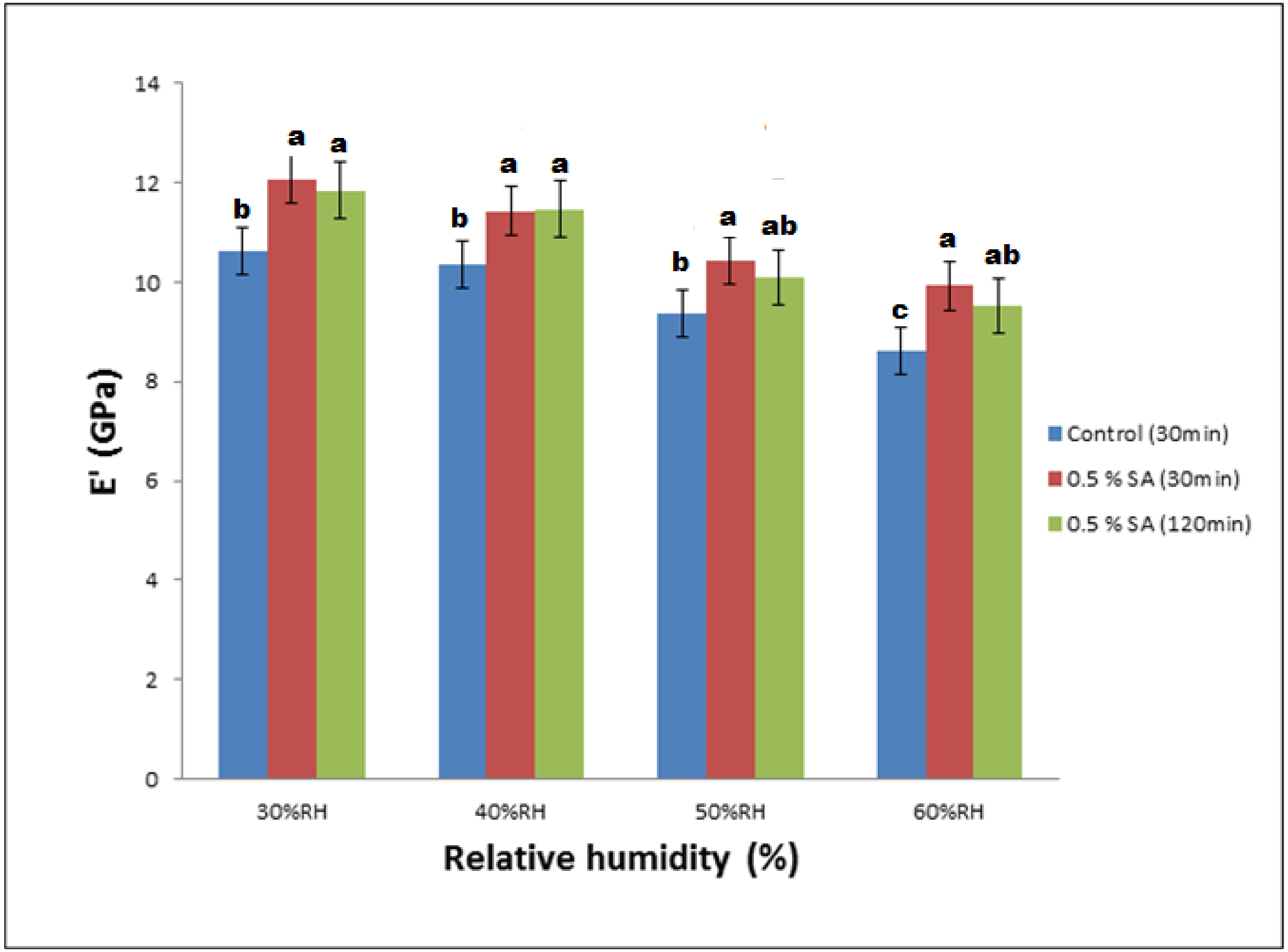

*Figure 4.5: The effect of pasting time on the Storage modulus (E′) of wheat starch films during DMA analysis under changing RH. Error bars shows standard deviations from corresponding means (*a, b, c shows levels of variation at a p-value ≤ 0.05).*

4.4 Conclusions

The results achieved in this study seem to indicate that pasting time as well as stearic acid modification of wheat starch can affect the films' properties. These affects include increased mechanical, barrier and thermal properties. However, the degree of improvement depends on the amount of stearic acid used to modify wheat starch films as well as length of pasting time. Wheat starch films modified with 0.5% stearic acid, pasted for 30 minutes, showed the highest increase in mechanical and increased barrier properties as well as lower molecular mobility as compared to the other treatments. This could be related to amorphous Type I amylose-lipid complexes produced in situ during pasting, this was further researched in Chapter 5 of this study. The molecular mobility within the stearic acid modified films can be responsible for the increase in tensile stress and Young's modulus, as well as improved barrier properties. This further implies that these film will be better suited as a packaging material alternative, as compared to the control film, due to the increased mechanical, barrier and thermal properties.

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5. The effect of amylose-lipid material on the quality of wheat starch films

Abstract

Bio-based packaging materials are a good alternative to synthetic packaging materials as they are environmentally friendly. Several studies demonstrate the potential use of starch in the manufacture of biodegradable plastics, but their low performance (poor mechanical and barrier properties) has limited the applications. However, the incorporation of nanomaterials in starch films can improve performance. The purpose of this study was to determine the effect of amylose-lipid nanomaterials on the mechanical, thermal and barrier properties of wheat starch films. The addition of nanomaterial to wheat starch films improved the films' mechanical properties in terms of increased tensile stress and Young's modulus and decreased oxygen and water vapour barrier properties. Films made using amylose-lipid nanomaterial showed a higher glass transition temperature (T_g) as compared to the control films. This suggests there is an interaction between the amylose-lipid nanomaterials and wheat starch polymers that improve the mechanical properties, with the amylose-lipid nanomaterial acting as a nano filler. Sorrentino *et al.* (2007) had shown that nanomaterial used as filler will provide a tortuous pathway to decrease the oxygen and water vapour barrier properties. Therefore, amylose-lipid complexes, occurring naturally at nanoscale, have the potential to improve inherent properties of biodegradable wheat starch films.

5.1 Introduction

Packaging material plays a significant role in protecting food material, minimizing food losses, and maintaining the safety of food in the food industry (Zhao *et al.,* 2008). Packaging material is also a key contributor in ensuring that the shelf life of food products is retained for as long as possible by controlling factors such as water and oxygen permeation (Smolander and Chaudhry, 2010). Most of these packaging materials are derived from non-renewable sources such as the plastics: polyethylene terephthalate (PET), polypropylene (PP), and polyvinylidene chloride (PVDC). These synthetic materials have a negative effect on the environment. Thus, there is growing concern about protecting the environment and many researchers have the aim of producing environmentally friendly packaging materials (de Azeredo, 2009; Zhao *et al*., 2008).

Packaging materials made from renewable materials, such as starches, can be compostable and leave a low carbon footprint (Sorrentino *et al.*, 2007). Starch can be made into a film as it is a thermoplastic material (BeMiller and Whistler, 2009). However, due to the poor mechanical and barrier properties of starch films as compared to synthetic plastics, they have limited applications (Psomiadou *et al.*, 1997; Avérous and Fringant, 2001).

Nanomaterials such as montmorillonite (Sozer and Kokini, 2008) and sepiolite (Fukushima *et al.*, 2009) can be incorporated into biodegradable films to improve the films' inherent properties (Chivrac *et al.*, 2010). Incorporation of nanomaterials into biodegradable films creates a large interface between nanomaterial and starch material (Lin *et al.*, 2010). Nanomaterials have a proportionally larger surface area which favours the formation of a more stable matrix (de Azeredo, 2009). Nanomaterials were also found to lower the relaxation state of the films (Sorrentino *et al.*, 2007) and create a stable three phase matrix (forming a tortuous pathway) in order to help lower permeation of water vapour and oxygen (Lin *et al.*, 2010).

However, these nanomaterials, for example montmorillonite (Sozer and Kokini, 2008) and sepiolite (Fukushima *et al*., 2009), are often inorganic in nature and are not edible. Therefore, these materials cannot be incorporated to make edible food coatings or added within food systems for specific functionality (Zhao *et al*., 2008). An alternate approach is to incorporate organic based nanomaterials into the films to make a composite material (Bastioli, 2001). These complexes are potentially food compatible materials, for example amylose-lipid complexes. These complexes are found to be in the range of 0.08–2.93 nm and are thus considered as nanomaterials (Putseys *et al*., 2010). This research will therefore investigate the properties of starch films with the incorporation of amylose-lipid nanomaterials.

5.2 Experimental

5.2.1 Materials

Commercial wheat starch (South African durum wheat supplied by Ruto Mills) was used, with 0.3% protein and 0.1% fat (proximate analysis done). Stearic acid (analytical grade, ≥ 98.5% assay) obtained from Sigma-Aldrich was used. All other chemicals, supplied by Sigma-Aldrich, were of analytical grade.

5.2.2 Methods

a. Modifying starch and preparation of amylose-lipid nanomaterial

Stearic acid was incorporated into wheat starch as stated in Chapter 4. Modified wheat starch was pasted for 2 hours and 10 minutes at 90^{o}C. The amylose-lipid complexes were then isolated according to a patented procedure developed in the Department of Food Science, University of Pretoria (Ray *et al.*, 2014).

b. Characterization of isolated starch material

Differential Scanning Calorimetry (DSC):
The thermal properties of the isolated amylose-lipid nanomaterial were confirmed using a high pressure DSC system (HPDSC-827^e, Mettler Toledo, Greifensee, Switzerland). Isolated material (10 mg) was mixed with 30 mg distilled water in a 100 µl DSC pan. The pan was allowed to equilibrate for at least 4 hours at room temperature. Scanning was done from 40^{o}C–125^{o}C at a rate of 2^{o}C/minute. Indium (T_p = 156.61^{o}C, 28.45 J g^{-1}) was used as a standard to calibrate DSC and an empty pan was used as a reference. The results were analysed using STARe software (Mettler Toledo).

Atomic Force Microscopy (AFM) imaging:
Isolated material was visualised using an AFM (Topometrix Corporation, Essex, London, UK). Isolated material was suspended in distilled water and then sonicated (25^{o}C and 20 x 10^3 Hz) for at least 30 minutes to have a uniform suspension. The sample was then drawn into a pipette and a few drops were added to a sample holder. The sample holder was spin

coated at high resolution to fix the complexes and remove excess water. The sample was dried overnight in a forced dried oven at 50^{o}C. Samples were then analysed.

c. Dynamic Mechanical Thermal Analysis (DMTA) of isolated material

Thermal properties of isolated material was confirmed using DMTA techniques. Using a spatula, a portion of the material was placed in a sample holder (i.e. a metal envelope approximately 1mm x 12mm). The sample holder containing the material was then inserted into the DMA machine. The instrument was operated in dynamic mode, using single cantilever geometry. Temperature scans were performed at 0^{o}C–180^{o}C using a 2^{o}C/minute ramp rate. A strain of 0.05% and a multi strain frequency of 1 Hz and 10 Hz were used. The glass transition (T_g) of the films was taken as the temperature at which a modulus change from a glassy to an amorphous state occurred (Roth and Dutcher, 2005). T_g was determined by a transition in tan δ and taken at the tan δ peak.

d. Pasting of wheat starch for film production

Wheat starch was pasted for 30 minutes using the same pasting methods as mentioned in Chapter 4.

e. Casting of films

The paste was then immediately diluted (1 part paste:2 parts boiling distilled water), and homogenized for 1 minute at 720 RCF rpm. The presumed isolated amylose-lipid material (0%, 2%, 5% and 10%) was added and mixed for 3 minutes at 390 RCF. A magnetic stirrer was used to gently stir the paste for 15 minutes, whilst on a heated stirring plate with temperature set at 70^{o}C. The gentle stirring of the paste allowed for the removal of air bubbles. The paste, 16ml, was then cast in sterilized petri dishes (8cm diameter) and dried in a vacuum oven for 8 hours at 50^{o}C temperature and 400 mBar pressure. The resultant film was then conditioned with a RH chamber, using settings of 50% RH and 25^{o}C.

5.2.3 Analyses

a. X-Ray Diffraction (XRD)

Starch film with isolated amylose-lipid material added to it was analysed using an X'Pert PAN analytical diffractometer (Eindhoven, Netherlands). The analysis was run using the following settings: 45 kV, 40 mA and CuK_1 (0.154 nm). Scattering angles were scanned from 5° to 30 ° (2θ) with an exposure time of 16 minutes and 14 seconds, step size of 0.026° and a time/step ratio of 229.5 s. The peaks of the total integrated area above the baseline were taken as the degree of crystallinity, which confirmed the complexation of nanomaterial within the starch film (Andrews and Grulke, 2005).

b. Mechanical properties

The films' mechanical properties were tested according to ASTM method D882-10, (American Society for Testing and Materials, 1999). An EZ-LX Test texture analyser (Shimadzu, Kyoto, Japan) fitted with a tensile grip was used. Strips (60 mm long and 6 mm wide) were cut using a scalpel and a micrometer was used to measure the thickness at 5 different points. The strips were conditioned in a RH chamber, using settings of 50% RH, overnight before fitting them in between two tensile grips which were 40 mm apart. A crosshead speed of 0.4 mm/s was used. Results were expressed as maximum stress (σ), maximum strain (ε), strain at break (ε_b) and stress at break (σ_b). The linear region of the stress and strain curves was used to calculate the Young's modulus.

c. O_2 permeability

O_2 permeability was analysed using the same methods as stated in Chapter 4.

d. Water vapour permeability

Water vapour permeability was analysed using the same methods as stated in Chapter 4.

e. Dynamic- Mechanical Analysis (DMA)

DMA analysis was done using the same methods as stated in Chapter 4. DMA tests were done at different RH (30%–60%) and temperatures (-30ºC–130ºC).

5.2.4 Statistical analysis

Data was analysed using analysis of variance (ANOVA) using SAS v8 software (SAS Institute Inc., Cary, NC). This determined if there were significant ($p < 0.05$) differences between the pasting times and the addition of presumed isolated amylose-lipid material addition (0%, 2%, 5%, 10%).

5.3 Results and discussion

5.3.1 Characterisation of isolated material

Thermal analysis

Figure 5.1 shows the DSC thermogram of starch complexed with stearic acid and hydrolysed with α-amylase enzyme. The thermogram shows two endothermic peaks, one at 110^{o}C and the other at 118^{o}C. These endothermic temperatures have been assigned to amylose-lipid complexes (Biliaderis *et al.,* 1985). Similarly, Wokadala *et al.* (2012) suggested that amylose-lipid complexes made from teff starch and maize starch after a long pasting cycle had peak temperature approximately at 110^{o}C. The endothermic peaks from the thermogram therefore suggest that the unhydrolysed residue or the isolated material contains amylose-lipid complexes.

A study done by de Pilli *et al.* (2011) illustrated that amylose-lipid complexes formed a less stable Type I at temperatures of 60^{o}C or less. Furthermore, it was reported that these complexes have a less ordered crystalline structure and melt at temperatures of 95^{o}C–105^{o}C. More stable complexes (Type IIa) are formed at temperatures of at least 90^{o}C and these complexes have a more structured crystalline order (Le Bail *et al*., 1999). The melting temperature of these complexes are higher (110^{o}C–115^{o}C). Further annealing can result in the formation of even more stable complexes (Type IIb) with melting temperatures of 115^{o}C or higher (Raphaelides and Karkalas, 1988). Upon thermal analysis these complexes give an endothermic reaction indicating the temperatures at which the complexes would dissociate. The DSC thermogram *(Figure 5)* indicated two endothermic peaks at 110^{o}C and 118^{o}C for the isolated material, it can consequently be inferred that it has a stable crystalline order of Type IIa and Type IIb amylose-lipid complexes.

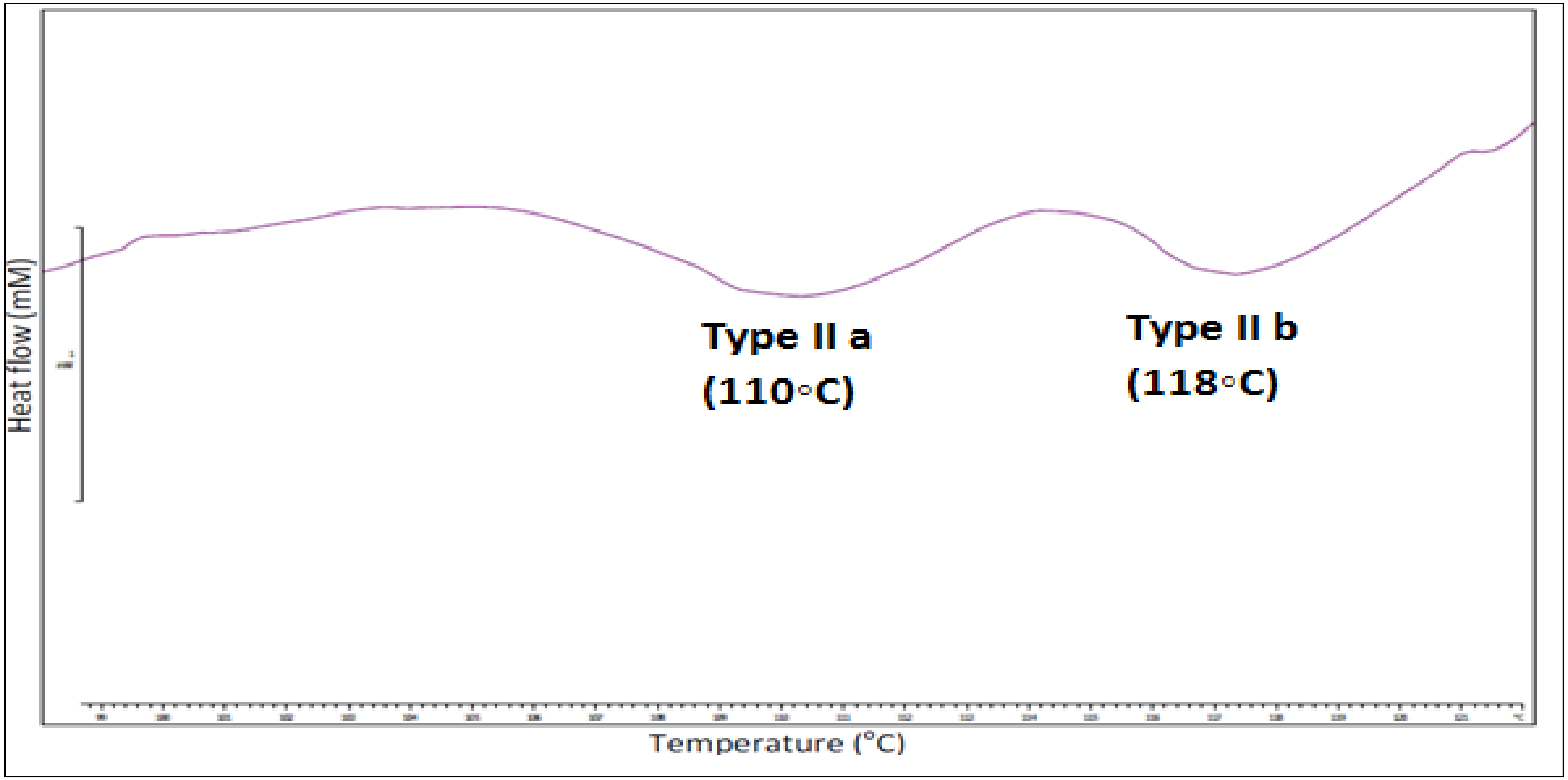

Figure 5.1: Differential Scanning Calorimetry (DSC) thermogram of isolated material (according to a patented process).

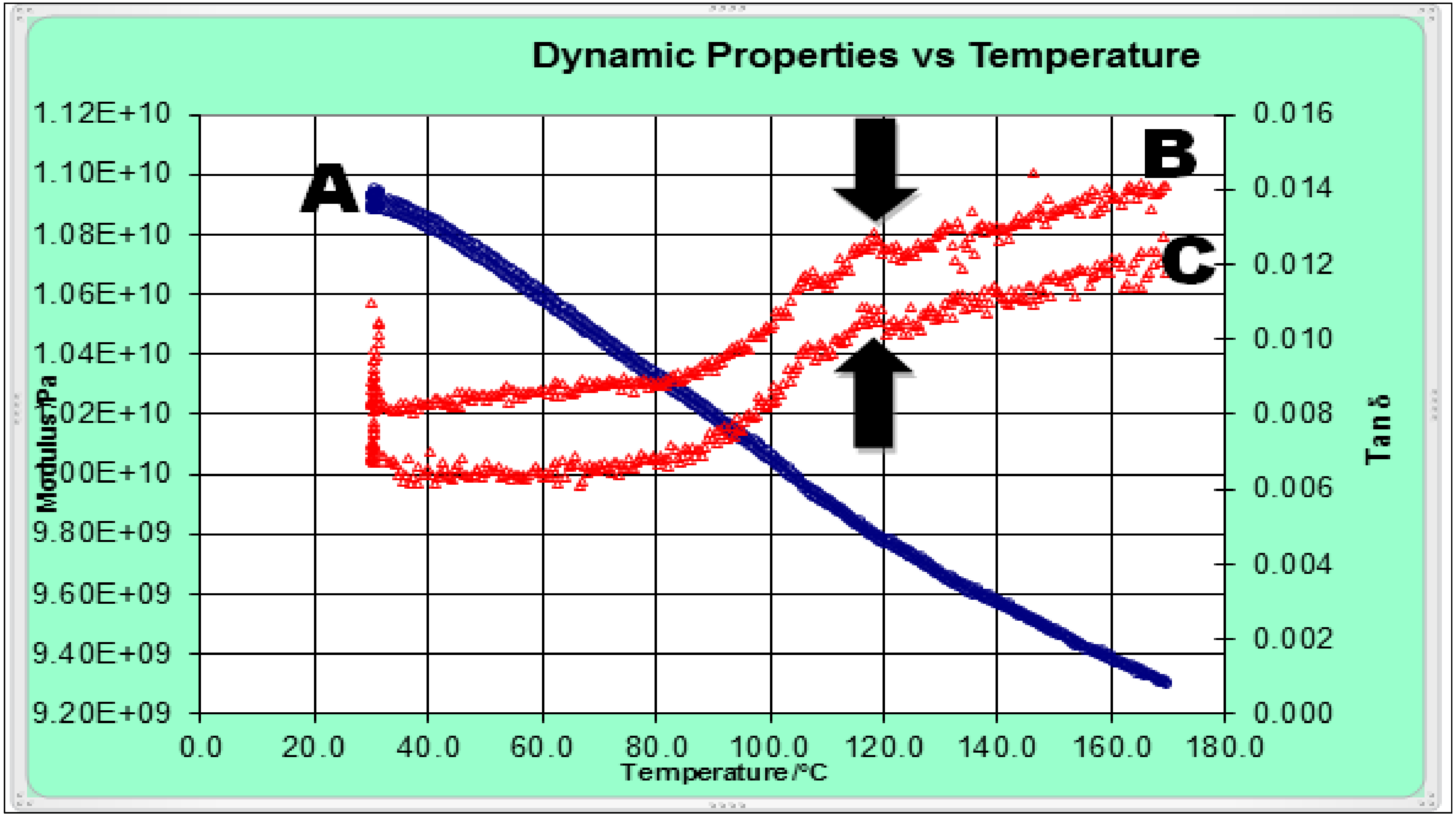

Figure 5.2: Dynamic Mechanical Thermal Analysis on modified isolated material (according to a patented process) (A: Storage Modulus (Pa), B: Tan δ (10 Hz), C: Tan δ (1 Hz), arrows indicate temperature at which a phase change occurs).

Figure 5.2 shows a Dynamic Mechanical Thermal Analysis (DMTA) done on modified wheat starch paste after enzymatic hydrolysis and freeze drying. The thermogram shows the storage modulus (A) and tan δ at different strain frequencies: 10 Hz (B) and 1 Hz (C). At 120°C there is a peak in tan δ for both frequencies (shown by the arrows). Andrews and Grulke (2005) stated that a maximum tan δ specifies a thermal transition. This suggests that the isolated materials experienced a thermal transition at about 120°C during heating at the two frequencies. The complexes are undergoing a transition in which they become more rubbery in nature (Andrews and Grulke, 2005). This possibly indicates melting or transition of some semi-crystalline materials at a temperature of ca120°C, suggesting the presence of Type II amylose-lipid complexes. The DMTA results of the isolated complexes seem to show similar results to the DSC thermoform.

Atomic Force Microscopy (AFM)

Isolated materials containing amylose-lipid complexes were analysed using AFM techniques. Using a 3D scanning configuration, the height of these materials could be seen visually *(Figure 5.3)*. The diameter ranges of the materials were between 16.7–60.7 nm. This suggests that the isolated materials were at nanoscale. Amylose-lipid complexes have been found to be about 0.80–2.93 nm (Gelders *et al*., 2006). Studies done by Zabar *et al.* (2010) reported that amylose-lipid material can form agglomerates. Thus, Figure 5.4 seems to shows the agglomerates of the amylose-lipid materials. These were mainly due to nucleation reactions, as mentioned earlier, causing more stable complexes to be formed, i.e. Type II. These will in turn cause the amylose-lipid nanomaterial to form aggregates and therefore agglomerate. The difference in size of the amylose-lipid materials could therefore be attributed to agglomeration. This can be visually seen in Figure 5.4 where the arrows show individual material as well as the agglomerated amylose-lipid materials.

Thus, based on the above three characterisation techniques (DSC, DMTA, and AFM), it can be confirmed that the isolated materials were mostly amylose-lipid materials at nanoscale. Hereinafter, these will be referred to as “amylose-lipid nanomaterial”.

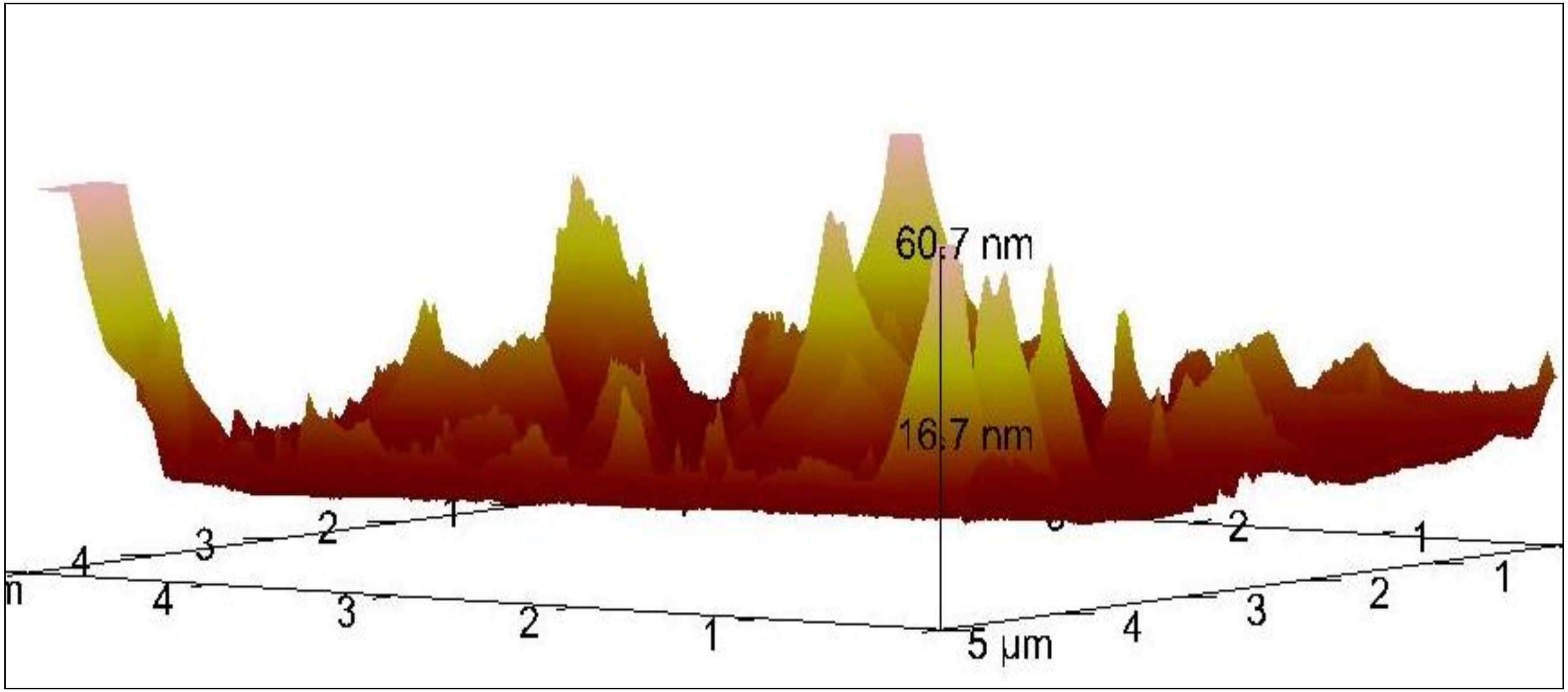

Figure 5.3: Atomic Force Microscopy (AFM) on modified isolated material (according to a patented process), using the height function indicating the range of the isolated material.

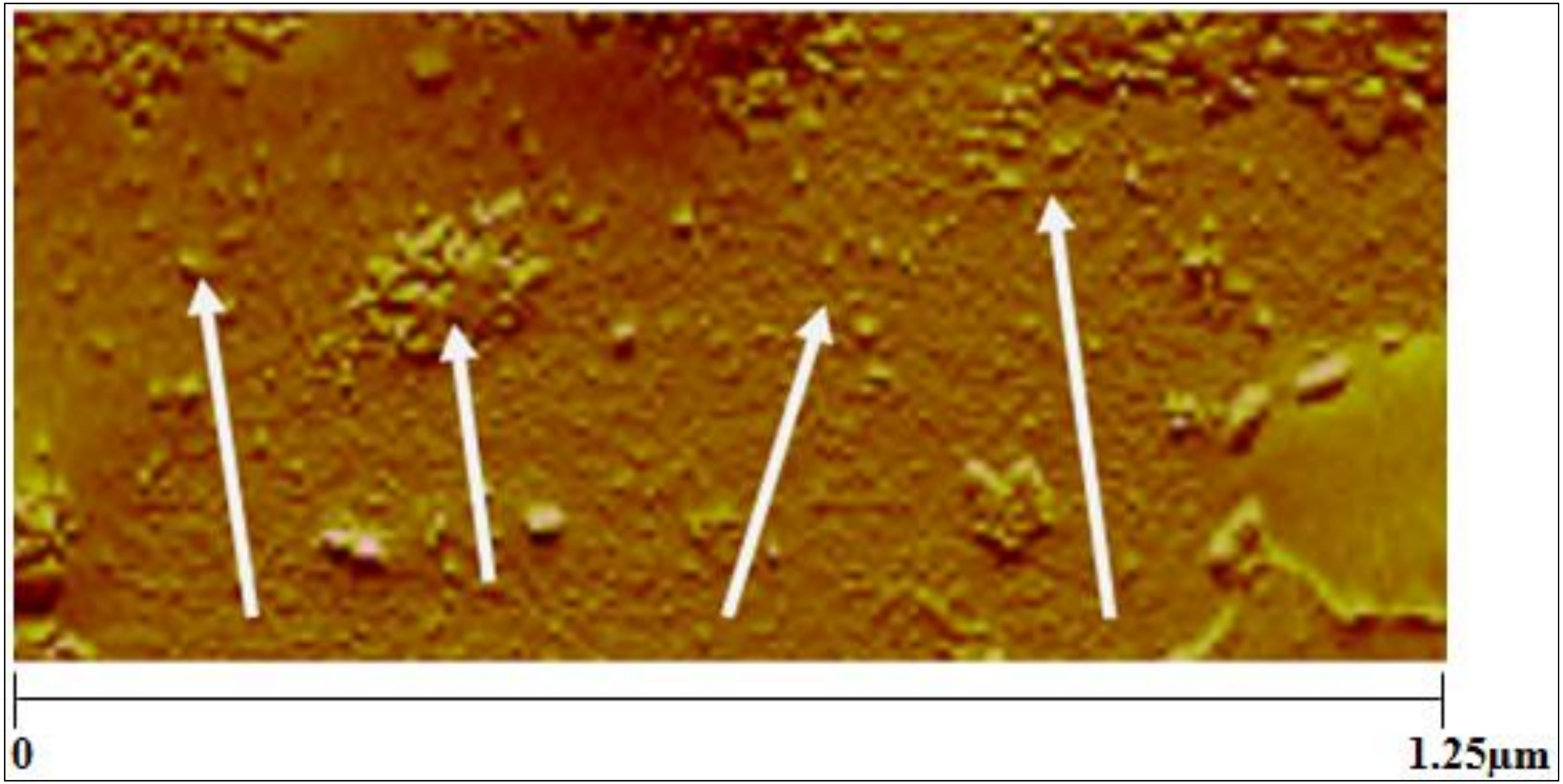

Figure 5.4: Atomic Force Microscopy (AFM) on modified isolated material (according to a patented process).

5.3.2 X-Ray Diffraction

Figure 5.5 shows the peaks of XRD analysis done on wheat starch films. The peaks on the films with 5% and 10% nanomaterial addition show more prominent peaks at $2\theta = 17^{o}$ and 19.9^{o}. According to Brisson, Chanzy and Winter (1991), peaks appearing at these angles are characteristic of V-type crystalline structures of amylose-lipid complexes at nano-scale. Thus, the XRD results show that there was no change to the amylose-lipid complexes when added to the films' matrix. This is important because an even and well distributed matrix is an essential step in improving the thermal, mechanical and barrier properties of wheat starch films.

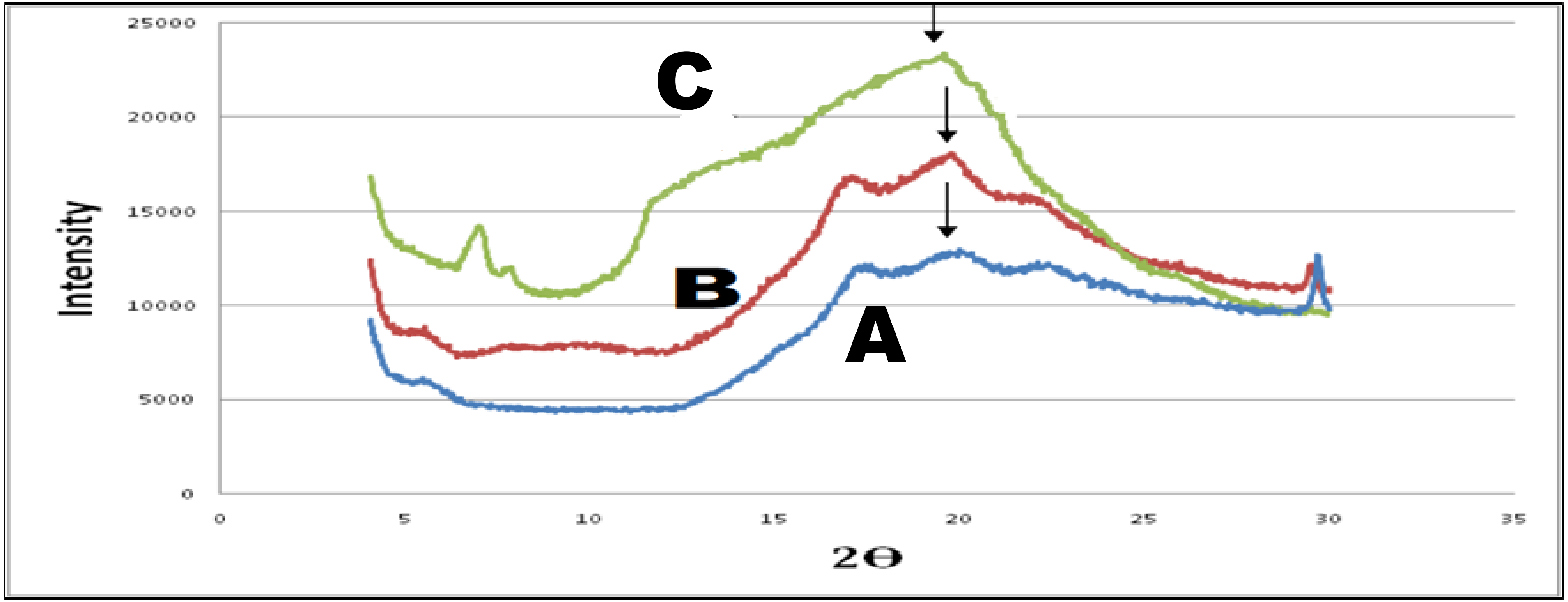

Figure 5.5: The effect of amylose-lipid nanomaterial addition on wheat starch films analysed using X-Ray Diffraction (A: 0% nano addition, B: 5% nano addition and C: 10% nano addition) (arrows indicates peak intensities).

5.3.3 Mechanical properties of starch films with amylose-lipid nanomaterial addition

The effect of amylose-lipid nanomaterial addition on the tensile properties of wheat starch films are illustrated in Figure 5.6 and Table 5.1. As the concentration of amylose-lipid nanomaterials addition was increased from 0% to 10% (by weight of starch), there appeared to be significant ($p \leq 0.05$) increases in the mechanical properties of the wheat starch film in terms of maximum tensile stress and Young's modulus. At 2% nanomaterial addition, the tensile stress was more than double as compared to the control film. A 5% addition of the amylose-lipid nanomaterials showed a ca59% increase in the maximum tensile stress as compared to the control. A 10% addition also showed an increase of ca60% in maximum tensile stress as compared to the control.

The stress at break values increased as the amount of amylose-lipid nanomaterial added was increased *(Figure 5.5 and Table 5.1)*. At 2% nanomaterial addition, the increase in the stress at break value was more than double as compared to the control. At 5% addition, there was a ca59% increase in the stress at break values as compared to the control. Addition of 10% nano filler showed a ca64% increase in the stress at break values as compared to the control.

de Carvalho, Curvelo, and Agnelli (2001) reported that the addition of clay nanomaterials (kaolin) to starch films showed increased tensile strength on the starch film in terms of increased maximum stress. Furthermore, it was reported that maximum stress can increase by up to ca50% along with an increase of 1.5 fold to the modulus with loading levels of 60 (parts per hundred) of kaolin to thermoplastic starch.

There were no significant differences ($p \geq 0.05$) in maximum strain when nanomaterial at 2% and 5% addition was added to wheat starch films as compared to the control *(Figure 5.5 and Table 5.1)*. When 10% nanomaterial was added to the wheat starch films, there was a 15% decrease in the maximum strain as compared to the control. This decrease in strain values was only experienced by the films with 10% amylose-lipid nanomaterial addition, whilst films made with 2% and 5% amylose-lipid nanomaterial additions did not experience decreases in strain.

Results reported by de Carvalho *et al.* (2001) showed that elongation and strain at break values had decreased by up to 50% upon an increase of nano clay addition. Studies done by Petersson and Oksman (2006) reported that although nanomaterial (bentonite) showed significant improvements to film strength, the elongation (strain) can be decreased with an increased nanomaterial loading level. It was reported that an addition of 5% of bentonite resulted in decreases of up to ca30% in elongation values.

Table 5.1: Effect of amylose-lipid nanomaterial addition on the maximum stress, maximum strain, stress at break, and Young's modulus of wheat starch films.

Nanomaterial addition (w/w starch)	Maximum Stress (MPa)*	Maximum Strain (%)*	Stress at break (MPa)	Young's modulus (MPa)*
0%	4.15^{c} (0.70)	33.33^{a} (2.94)	3.63^{b} (0.44)	3.63^{b} (0.43)
2%	9.58^{a} (0.46)	30.39^{ab} (2.26)	8.26^{a} (0.08)	13.96^{a} (1.74)
5%	10.14^{ab} (0.46)	31.63^{ab} (3.61)	8.78^{a} (0.15)	18.62^{a} (2.88)
10%	10.56^{b} (0.46)	27.58^{b} (0.88)	9.97^{c} (0.36)	58.68^{c} (6.24)

- **Data are means of 3 replicates (n=3) with standard deviation in parentheses**
- **a, b, c shows levels of variation at a p-value ≤ 0.05**

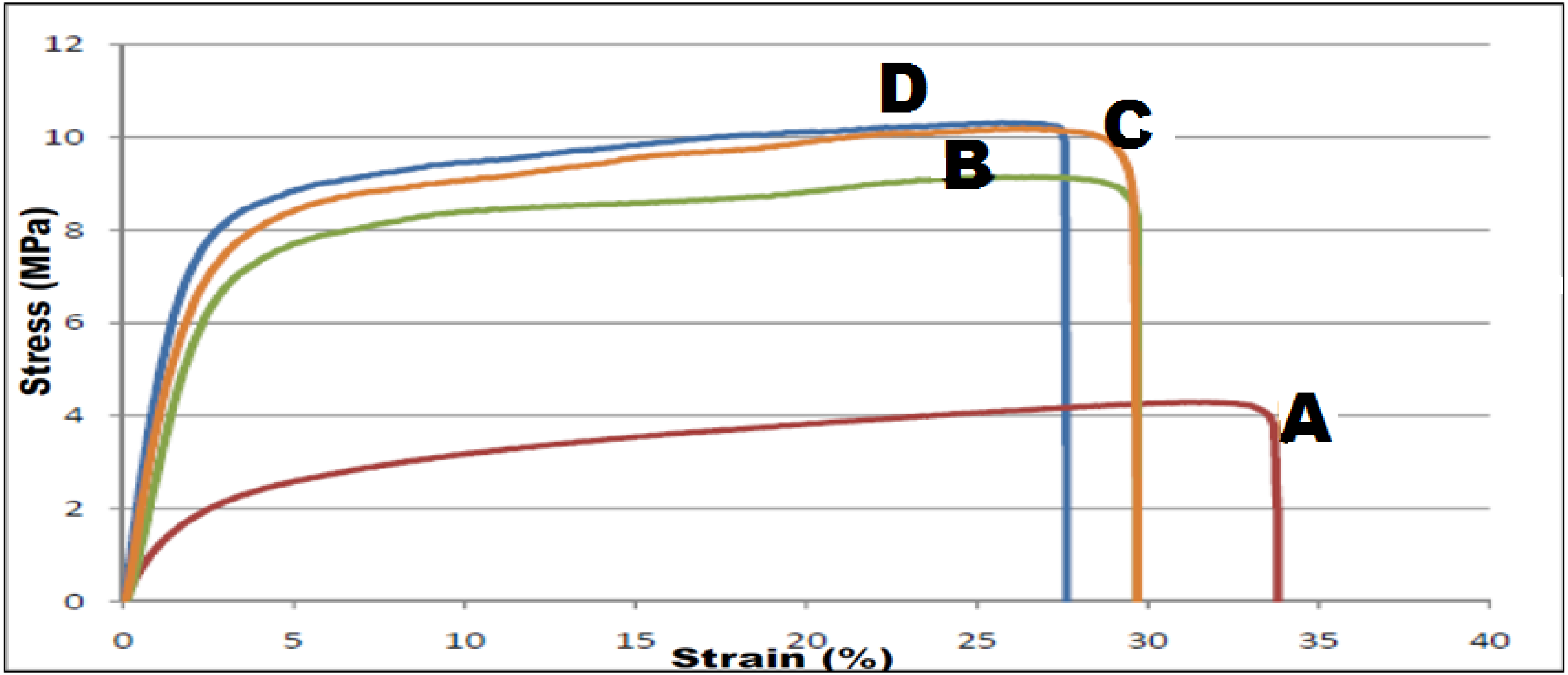

Figure 5.6: Effect of amylose-lipid nanomaterial addition on the tensile properties of wheat starch films (A: 0 % nano addition, B: 2% nano addition, C: 5% nano addition, D: 10% nano addition).

5.3.4 Barrier properties

The barrier properties of wheat starch films with amylose-lipid nanomaterials addition were tested in terms of water vapour permeability (WVP) and oxygen permeability (OP) *(Table 5.2)*. The addition of amylose-lipid nanomaterial significantly ($p \leq 0.05$) reduced the WVP and OP as compared to the control. Wheat starch films with 5% amylose-lipid nanomaterial addition resulted in a decrease of ca50% in the amount of water vapour that passed through the film compared to control. Films made with 10% amylose-lipid nanomaterial addition resulted in a decrease of ca40% in the amount of water vapour that passed through the film compared to control. Wheat starch films with 5% amylose-lipid nanomaterial addition resulted in a decrease of ca28% in the amount of oxygen that passed through the film compared to control. Films made with 10% amylose-lipid nanomaterial addition resulted in a decrease of ca14% in the amount of oxygen that passed through the film compared to control.

Studies done by Zhoa *et al.* (2008) reported that films made with nanomaterial (montmorillonite) addition showed significant improvements in water vapour and oxygen barrier properties. The incorporation of amylose-lipid nanomaterials to wheat starch films allows for stable and structured binding to occur, allowing for less permeation through the film. According to Lin *et al.* (2010), nanomaterial binds to the polymers of the starch film in a 3-phase structure, in which there is an interfacial layer between the nanomaterial and the polymers. This binding between nanomaterial and starch polymers creates an indirect pathway for permeation to occur, known as a tortuous pathway, as described by Sorrentino *et al.* (2007). This is one of the main factors that could explain why wheat starch films with nanomaterial addition have better barrier properties. There are other factors, such as molecular mobility, can also contribute to improved barrier properties. The amylose-lipid nanomaterial can lower the molecular mobility in wheat starch films due to a larger surface area allowing for more stable binding to occur (Zhao *et al.*, 2008). At lower molecular mobility in wheat starch films (discussed in detail later), the oxygen and water molecules have less opportunity to permeate through the film (Sorrentino *et al.*, 2007), which improves the barrier properties. The tight binding of the nanomaterial and starch film requires higher activation energy to facilitate diffusion of compounds (Miller and Krochta, 1997), thus making it harder for oxygen or water vapour to diffuse through the film.

Table 5.2: Effect of amylose-lipid nanomaterial addition on the water vapour permeability (WVP) and oxygen permeability (OP) of wheat starch films.

Nanocomplex addition (%)	*WVP (g mm $m^{-1}d^{-1}kPa^{-1}$)**	*OP (cm^3 µm m^{-2} d^{-1} kPa^{-1})**
0 (Control)	2.48 (0.15) [a]	219.03 (8.52) [a]
5	1.17 (0.07) [c]	157.54 (10.61) [c]
10	1.48 (0.21) [b]	187.58 (10.98) [b]

- Data are means of 3 replicates (n=3) with standard deviation in parentheses
- a, b, c shows levels of variation at a p-value ≤ 0.05

5.3.5 Thermal properties of wheat starch films

Figure 5.7 shows the effect of amylose-lipid nanomaterial addition on the thermal properties of wheat starch films during a DMTA analysis. The peak (tan δ) in Figure 5.8 is regarded as the temperature range in which a transition occurred, as mentioned earlier. This transition is a phase change, in which a material becomes more rubbery, thus moves from a glassy to a rubbery state (Angell, 1996), and this can be considered as T_g of the film. According to Andrews and Grulke (2005), the glass transition is a critical temperature that differentiates glassy and rubbery behavior. Furthermore, it is indicative of molecular motion of polymer chains or motion within a system (Roth and Dutcher, 2005). The wheat starch films with nanomaterial addition have a higher T_g than wheat starch films with no nanomaterial addition. The T_g increased from 13.83^{o}C (no nanomaterial addition) to 25.65^{o}C at 5% nanomaterial addition. Higher T_g values may indicate that it takes more energy to disrupt the molecular order and interactions created between the wheat starch films and nanomaterials. It further indicates that the wheat starch films with nanomaterial addition had a lower molecular mobility. Molecular mobility can be defined as the capacity of molecules present in a system to move by rotational or transitional movements (Fennema, 1996). Factors such as thermal energy and particle/molecule size can affect a system's molecular mobility. A 5% nanomaterial addition showed the highest T_g values. This further suggests that wheat starch films with 5% nanomaterial addition had the lowest molecular mobility. A low molecular mobility within the wheat starch films is a crucial factor to improve the mechanical, thermal and barrier properties of the films. Improvements in the stability of the wheat starch films upon nanomaterial addition can be seen due to the improved T_g results.

5.3.6 DMA of the films at different relative humidity (RH)

DMA results (at different RH levels) of wheat starch films with various amounts of amylose-lipid nanomaterial addition can be seen in Figure 5.8. It was observed that wheat starch films with 5% amylose-lipid nanomaterial addition had a higher E′ than the control. However, with a 10% amylose-lipid nanomaterial addition, the E′ was lower than the control film. When comparing the E′ values of wheat starch films without added nanomaterial, the E′ was significantly larger at 30% RH and 40% RH than it was at 50% RH and 60% RH. The same trend could be seen for wheat starch films with 5% and 10% nanomaterial addition.

Wheat starch films made with 5% nanomaterial addition resulted in the E′values of wheat starch films decreasing from ca11.8 GPa at 30% RH to ca10.3 GPa at 50% RH. However, at 60% RH, the E′ decreased significantly to ca9.7 GPa for the films with a 5% nanomaterial addition. Wheat starch films made with 10% nanomaterial addition resulted in the E′ values of wheat starch films decreasing from ca10.5 GPa at 30% RH to ca10.2 GPa at 60% RH. The control films also displayed decreased E′ values as well, where the E′ was ca11.0 GPa at 30% RH, it decreased significantly to ca9.4 GPa at 60% RH. These results indicate that nanomaterial addition to wheat starch films improves their mechanical stability at higher RH levels. This can be seen by the lower decreases in E′ for wheat starch films with nanomaterial addition as compared to the control films. It was noted that a change from a glassy to a more rubbery state of a film, as the RH increased, could potentially explain the drop in E′ values (Emmambux, Stading, and Taylor, 2004). Furthermore, significant decreases were seen at 50% RH and 60% RH. These decreases could be attributed to more moisture adsorption resulting in a decrease in elasticity, i.e. lower E′ values, as well as the anti-plasticization effect of water on the films.

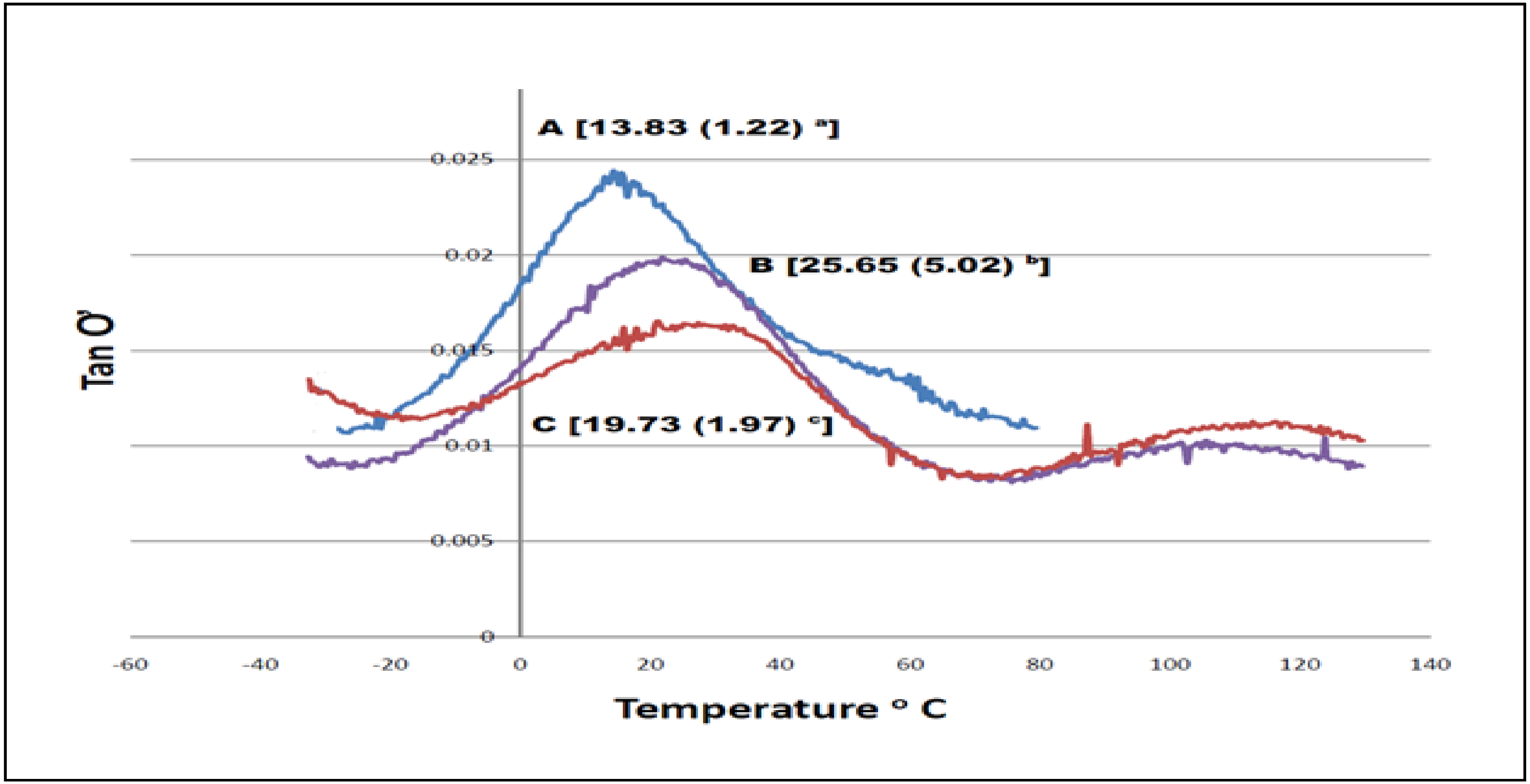

Figure 5.7: The effect of addition of amylose-lipid nanomaterials on the thermal properties of wheat starch films during Dynamic Mechanical Thermal Analysis (DMTA) (A: 0% nano addition, B: 5% nano addition, C: 10% nano addition).

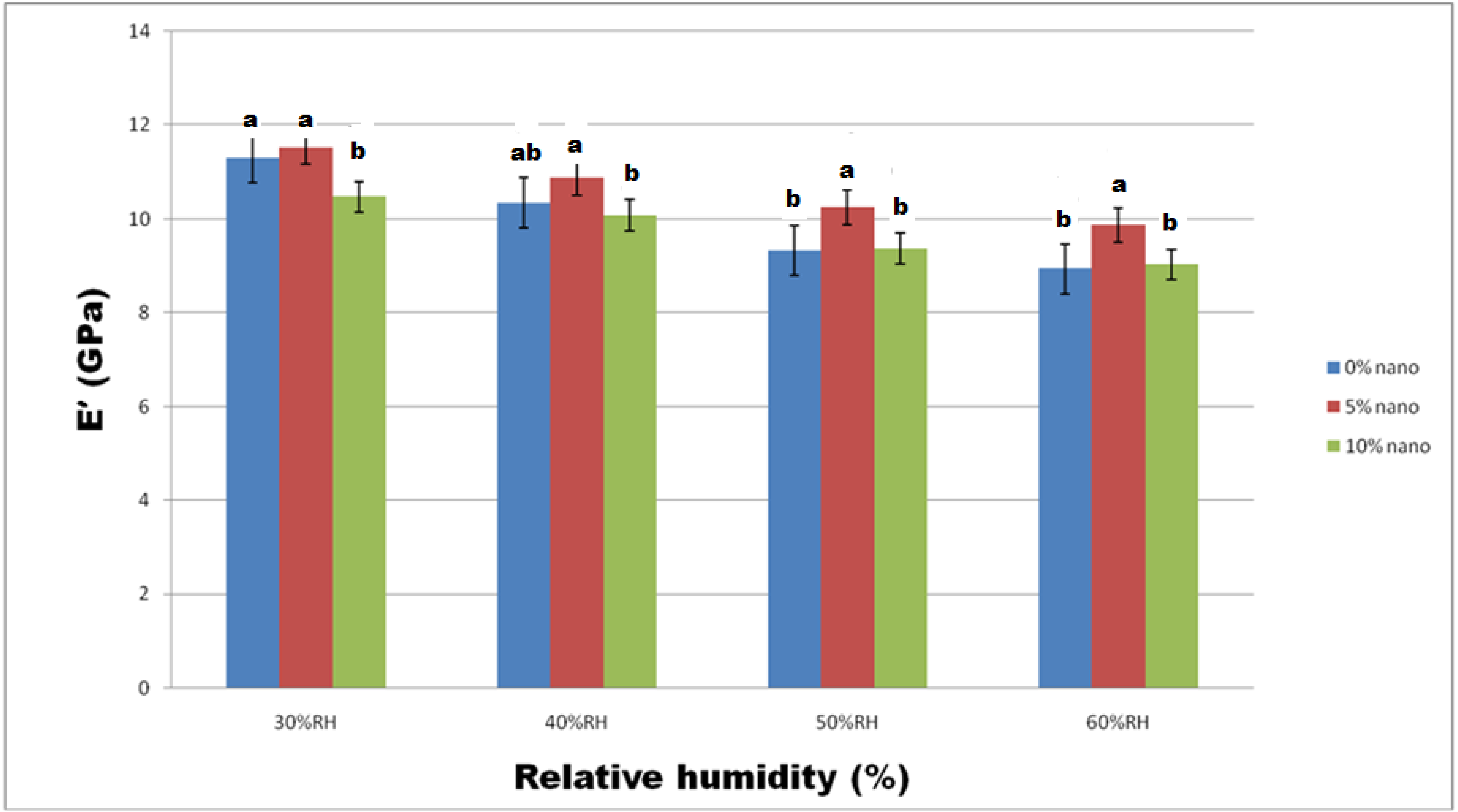

Figure 5.8: Effect of amylose-lipid nanomaterials on the Storage modulus (E′) of wheat starch films during DMA analysis under changing RH. Error bars shows standard deviations from corresponding means (a, b, c shows levels of variation at a p-value ≤ 0.05).

5.4 Conclusions

Amylose-lipid nanomaterial can act as a nano filler material in wheat starch films, yielding films with improved properties such as higher tensile properties, lower barrier properties and lower molecular mobility. These improvements suggest stronger interactions as well as the formation of molecular order. The presence of amylose-lipid nanomaterials dispersed at nanoscale within the wheat starch polymer matrix resulted in improvements to the relaxation behaviour of the films. These films are also environmentally friendly, as they are made from organic materials, allowing for environmentally friendly packaging material to be produced. Furthermore, the materials used to make the films are also all food grades in nature, making the films edible.

5.6 References

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6. General discussion

This chapter presents a critical review of the methods used during this research, explains the potential reasons why addition of amylose-lipid nanomaterial to wheat starch films leads to several improvements, and examines some potential applications of the wheat starch film in the food industry.

6.1 Methodology

Stearic acid was used to modify wheat starch. The stearic acid was first distributed into ethanol and then wheat starch was added to the solution. The solution was well dispersed in a shaking water bath at 45°C for 45 minutes. Several authors (Wokadala *et al.*, 2012; D'Silva *et al.*, 2011, Zhao *et al.*, 2008) have used this method to successfully incorporate stearic acid into starch and then form a modified starch during pasting. Thus, it is assumed that the stearic acid was well dispersed among the starch granules.

A RVA (Rapid Visco-Analyser) measures the viscosity changes within a starch suspension as a result of heating under constant stirring in the presence of water (Juhasz and Salgo, 2008). Different concentrations of modified starch (0%, 0.5%, 1.5%) and 35% glycerol (w/w) of starch were added to a RVA canister and made up to a volume of 28 ml with distilled water. Clumping and settling of starch could lead to variation in the pasting curves (Juhasz and Salgo, 2008). A paddle was used to agitate the suspension to prevent clumping and settling. The mixtures were stirred at an initial rate of 960 rpm to well disperse the solution and then a constant stirring rate of 160 rpm was used for the entire pasting period. A 30 minute pasting time was used for a short pasting period and a 120 minute pasting time was used for a long pasting period. It can be noted that there were some outliers on the pasting curve and only repeatable measurements were taken for reliable data.

After pasting, for 30 minutes and 120 minutes, the starch paste was immediately diluted two times with boiling distilled water before film casting (as mentioned in Chapter 5). This was done as the starch paste was highly viscous (100–500 RVU), and films that were cast from this viscous paste could not produce consistent films. Films made with a viscous paste were not of uniform thickness, had a large amount of air bubbles, and did not dry properly. This resulted in the formation of air bubbles within the film. These air bubbles were found to cause weak points in the films after casting. The bubbles had been removed by stirring the paste on a heated plate at 70°C for 15 minutes. The solution had been kept in a closed system by using parafilm and foil to seal the openings, which helped to prevent any moisture from escaping. Casting of the paste was done on sterilized petri dishes. It was also found that 6.5 ml of starch paste cast into a petri dish with a diameter of 8 cm resulted in the most uniform films upon drying. The films' thickness was measured with a micrometer to verify the uniformity of the films. Their thickness ranged from 0.048 mm to ±0.0036 mm.

Amylose-lipid material could be added to the films using a similar procedure as above. Wheat starch was first pasted and then diluted in a similar manner as above. However, the amylose-lipid material was added (0%, 2%, 5% and 10%) and was then dispersed into the solution. The air bubbles were removed and the films were cast in the same manner as above. In the current study, it was assumed that the amylose-lipid nanomaterials were well dispersed, but this was not confirmed. A better indication of the distribution of the amylose-lipid material within the films could be achieved using DSC or XRD techniques. The films could be cut into five sections as indicated in Figure 6.1. These five individual sections could then be tested using XRD or DSC techniques, to give an indication of the presence of amylose-lipid material in the films and this could then also indicate the distribution of the amylose-lipid within the films.

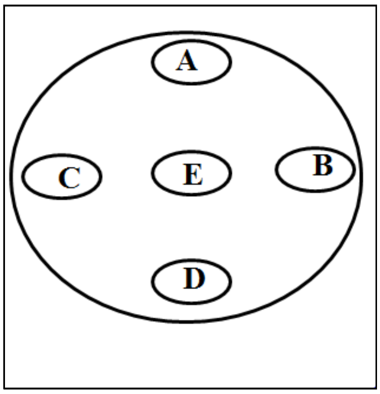

Figure 6.1: An example of random sampling to indicate the distribution of the amylose-lipid material within modified wheat starch films.

A texture analyzer fitted with a tensile grip was used to test the tensile properties of the wheat starch films. The film had to be cut into strips of (60 mm long and 6 mm wide) and then conditioned in a RH chamber with settings of 50% RH. The films were analysed at room temperature conditions of about 25°C and a RH between 20% and 30% as monitored for each film tested. It would be ideal to have a controlled testing environment with temperatures maintained at 25°C and 50% RH. Furthermore, the tensile grips caused some slippage of the films during testing and gave variable results in terms of stress and strain. Similar findings were observed by Anyango, Taylor and Taylor (2011). An adhesive strip was used to provide more grip (i.e. less slippage) between the films and the tensile grip. Only results where the films broke in the middle were used.

The films' barrier properties were tested using a Mocon Oxtran Modular system for oxygen transmission rate testing and a Mocon Permatran Modular system for water vapour transmission rate testing. The films were first conditioned in a RH chamber before testing, using settings of 50% RH. A layer of high vacuum grease was applied to the outer rim of the films and the sealing ring to ensure an airtight system. This technique has been used by several researchers as well as industries to test barrier properties of packaging materials (Nair, Zhu, Deng, Ragauskas, 2014). The results in the project show that amylose-lipid nanomaterials reduce the level of oxygen and water vapour permeability. Further microscopy work, such as TEM (Transmission Electron Microscopy), could be done to determine the microstructure and nanostructure of the films. This could perhaps explain the presence of the tortuous pathway for oxygen and water vapour diffusion through the films. This will allow for a better understanding of the mechanism used and can thus lead to the future development of films with better barrier properties.

6.2 Discussion of results

Table 6.1 summarizes all the results obtained from the two research chapters (i.e. Chapter 4 and 5). In Chapter 4, it was observed that wheat starch films modified with 0.5% stearic acid addition, after 30 minutes pasting time, resulted in films with increased mechanical, thermal and barrier properties compared to films made with more stearic acid addition or pasted for 120 minutes. Films made after 120 minutes pasting time with 0.5% stearic acid addition showed decreased mechanical properties, whilst the barrier and thermal properties were still higher than in the control films. Starch modified with stearic acid resulted in a paste with a higher viscosity and more prominent peaks especially after extended pasting times (mentioned earlier). These prominent peaks are suggested to be formed due to the in situ formation of amylose-lipid complexes (Wokadala *et al.*, 2012).

Table 6.1: A summary of all film treatments in terms of the mechanical, barrier and thermal properties in relation to the control films

		Tensile stress	**Tensile strain**	**Tensile modulus**	**Oxygen and water vapour barrier**	**Glass transition temp**
Pasting time	**Film treatment**					
30 minutes	Properties compared to control					
	0.5% Stearic acid	Stayed the same	Increase	Increase	Increase	Increase
	1.5% Stearic acid	Decrease	Increase	Increase	-	-
120 minutes	Properties compared to control					
	0.5% Stearic acid	Decrease	Increase	Increase	Increase	Increase
	1.5% Stearic acid	Decrease	Decrease	Stayed the same	-	-
30 minutes	Properties compared to control					
	2% Amylose-lipid material addition	Increase	Stayed the same	Increase	-	-
	5% Amylose-lipid material addition	Increase	Stayed the same	Increase	Increase	Increase
	10% Amylose-lipid material	Increase	Decrease	Increase	Decrease	Increase

Note: - indicates test were not done on treatment

Figure 6.2 is a schematic illustration of the different stages of pasting and complexation reactions between wheat starch and stearic acid. After a short pasting time, the complexation reactions result in a few amylose-lipid complexes which are somewhat amorphous in nature (Wokadala *et al*., 2012). After a long pasting time, there is more association between stearic acid and starch to form more amylose-lipid complexes. These amylose-lipid complexes are noted to be more crystalline in nature (Wokadala *et al.*, 2012). It can thus be assumed that films made after a short pasting time have more amorphous complexes, whilst films made after long pasting times have more crystalline complexes. Films made from the amorphous form of complexes showed improved properties whilst the films made from semi-crystalline complexes showed inferior properties. This could be due to the crystalline nature of the complexes that make the films more brittle and may cause some stress micro-fractures (pinholes) in the films (Schmidt *et al*., 2013). Furthermore, the presence of more amylose-lipid complexes, such as after long pasting, can aggregate to form spherulites (Fanta, Felker, Shogren and Salch, 2008). These could also cause micro-fractures (pinholes) in the films. Further research, such as DSC and optical microscopy, can be done to better explain the aggregation and formation of spherulites of amylose-lipid complexes. The amylose-lipid complexes will also prevent junction zone formation of amylose and can result in better film formation (Le Corre, 2011).

From Table 6.1 it was observed that the addition of 5% amylose-lipid material to the wheat starch films had resulted in changes which seemed to improve their mechanical, thermal and decrease in barrier properties. Amylose-lipid material addition, at 10%, resulted in changes that seemed to weaken the mechanical, thermal and decrease in barrier properties of the films. Isolated amylose-lipid complexes were added to wheat starch films and these complexes were semi-crystalline in nature. The incorporation of the amylose-lipid material is assumed to be done by phase separated and intercalated techniques (Sozer and Kokini, 2008) as indicated by Figure 6.2. Further research, such as additional microscopy analysis, could be done to further explain how the amylose-lipid material is dispersed between starch polymers in the films.

The addition of 10% amylose-lipid nanomaterial as compared to 5% means that these films had more semi-crystalline material within them. Higher amounts of crystalline materials can result in the formation of weak points in the film, as mentioned earlier. However, the addition of 5% amylose-lipid material resulted in improvements in the films' properties. The films' barrier properties were decreased with the addition of amylose-lipid material. The assumption made is that incorporation of amylose-lipid nanomaterials to wheat starch films allows for stable and structured binding to occur. This allows for the formation of a network with a 3-phase structure (as mentioned earlier). On this basis, it was inferred that the binding between nanomaterial and starch polymers creates an indirect pathway for permeation to occur, known as a tortuous pathway, as described by Sorrentino *et al.* (2007). This is one of the main factors that could explain why wheat starch films with nanomaterial addition have better barrier properties. It will also give a better indication of the films' lower molecular mobility and several other factors such as barrier, mechanical and thermal properties.

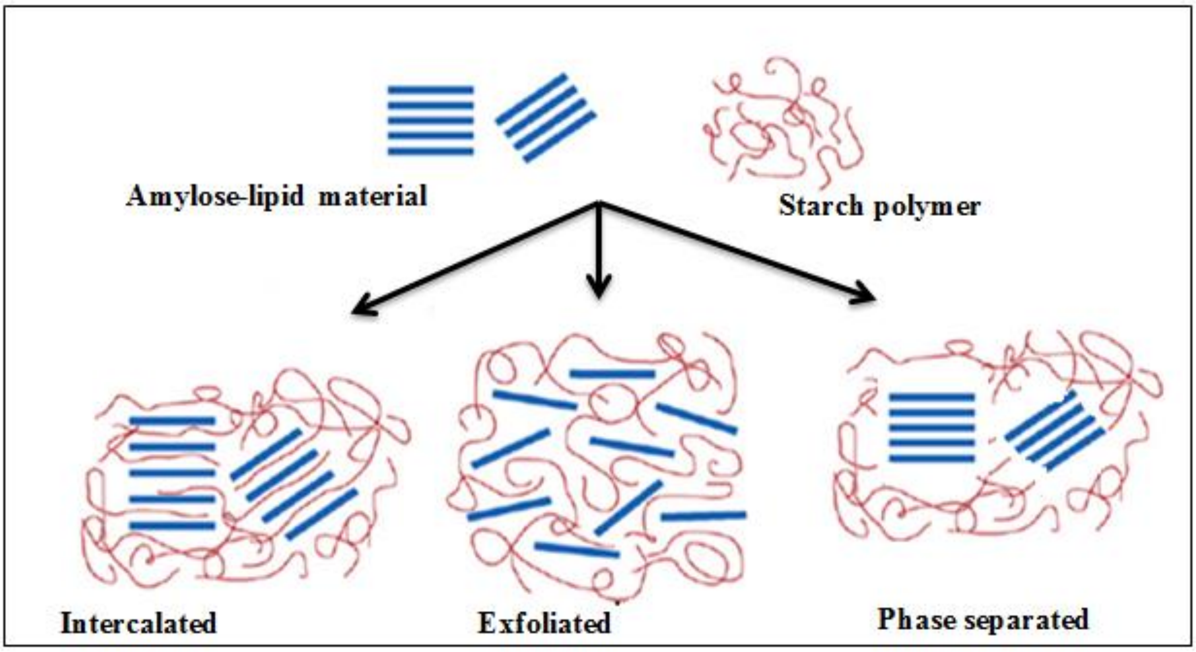

Figure 6.2: Schematic diagrams to indicate the techniques that amylose-lipid material can be incorporated into starch polymers (Sozer and Kokini, 2008).

The films produced from paste made during extended pasting with stearic acid showed different mechanical properties compared to short pasting. This has been explained in terms of the possibility that during long pasting, there are more semi-crystalline amylose-lipid complexes compared to the amorphous ones formed during short pasting (Wokadala *et al.*, 2012). However, when the same amylose-lipid nanomaterials formed during extended pasting are added in films at 2% and 5%, there was an increase in tensile properties, but at 10% there was a slight decrease. This suggests that the amount of amylose-lipid nanomaterial addition is an important factor in improving film properties. During extended pasting, about 25%–30%

amylose-lipid materials are formed (Wokadala *et al.*, 2012). Thus this amount may have a negative effect on film properties.

The thermal property test results showed that wheat starch films with nanomaterial addition had a higher T_g value. This indicates that more energy was used to disrupt the network within the films once amylose-lipid nanomaterial was added; thus the films are more stable. It further implies that there is a lowered amount of molecular motion of polymer chains or motion within the films. A low molecular mobility within wheat starch films can be the fundamental reason for several improvements seen in the films, such as improved mechanical, thermal and barrier properties. The T_g results could be further confirmed by using modulated DSC techniques in addition to DMTA.

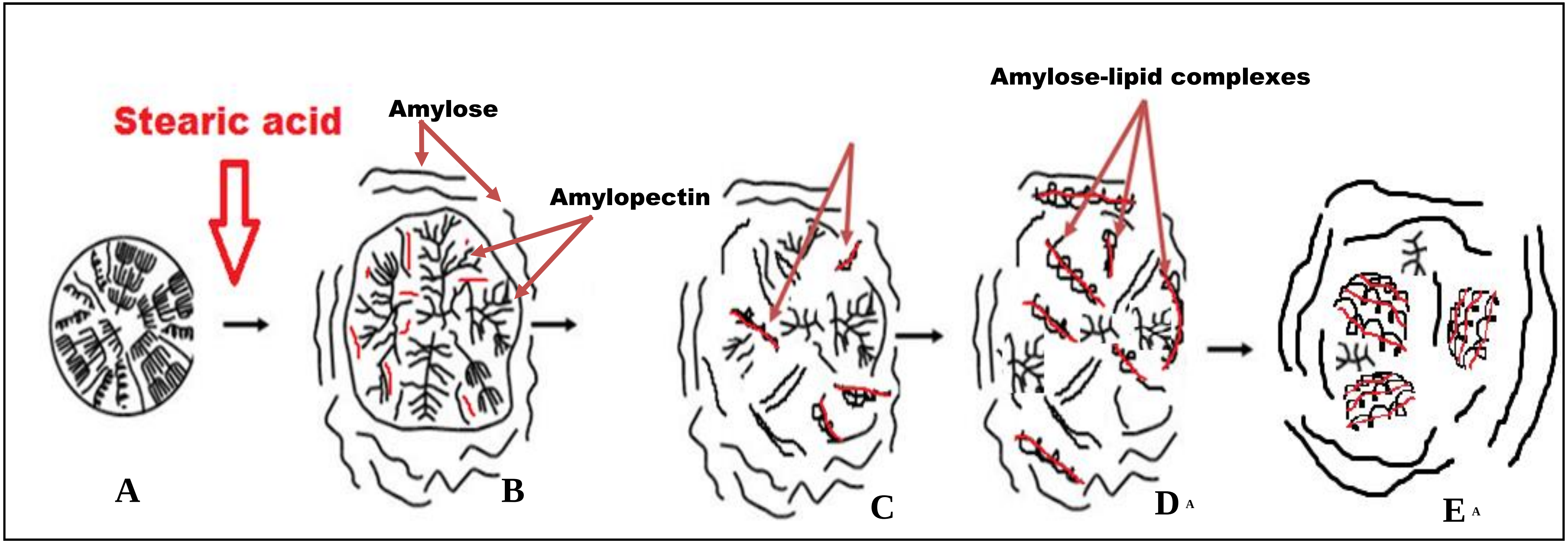

Figure 6.3: A schematic diagram showing pasting of wheat starch with stearic acid addition. (A= Native Starch, B= Gelatinized Starch, C= organisation and some complexation reactions after 1st peak pasting, D= organisation and a lot more complexation (arrows) reactions after 2nd peak pasting, E= spherulite formation).

Starch is known as a hygroscopic material (BeMiller and Whistler, 2009). The addition of nanomaterial to the wheat starch films indicated that the films were more stable at higher RH, as shown by significantly higher E′ values. The notion is that the films are more stable, as seen by the better barrier and thermal properties. It was inferred that the same mechanisms that improved the films' barrier and thermal properties improved the films' stability at varying RH conditions. A better understanding of the above mechanisms could further explain the improvements observed when amylose-lipid material is added to wheat starch films.

6.3 Production of wheat starch and amylose-lipid films in the food industry

The functional, organoleptic, nutritional and mechanical properties of an edible film can be modified by the addition of various chemicals in minor amounts. Sorrentino *et al.* (2007) describe edible films or coatings as continuous layers of edible material used as a coating or as a film placed between food components to provide a barrier of mass transfer. These materials are formed first, and then applied directly onto the food source (Miller and Krochta, 1997). They can be formed by casting or by thermoforming techniques, such as extrusion. The incorporation of organic filler materials, such as amylose-lipid nanomaterials, with the edible film or coating will yield a bio-nanocomposite material (Ghanbarzadeh, Almasi and Entezami, 2010). These exhibit changes to the properties of the material, such as improved barrier functioning. Amylose-lipid complex can alternatively be used in edible nanocomposite materials. However, the composite materials featured in the current study were produced through the casting method. It will be important to use thermoforming extrusion techniques to form films and determine their properties.

Garcia, Pereira and de Luca Sarantopoulos (2010) reported that edible films applied to strawberries did not affect its mechanical properties and furthermore the coatings were able to decrease the respiration rate and increase the water vapour resistance of the strawberries. Bertuzzi, Vidaurre, Armada and Gottifredi (2007) also commented that edible films are known to be an effective barrier to gas transport (O_2 and CO_2). Sozer *et al.* (2008) reported several applications for these edible materials (such as coatings in fresh fruits and vegetables, bakery products, and confectionery), where they might protect the food from moisture, lipids, gases, off-flavours and odours. This would imply that amylose-lipid complexes can be used to make a nanolaminate material. When used as a complex form, lipids are reported to have a good moisture barrier (Weiss, Takhistov and McClements, 2006). However, they are also reported as having poor mechanical strength. Due to these factors, research is being done to find an alternative, in which improved functional properties of edible films and coatings can be achieved (Mensitieri *et al.*, 2011). Some examples of materials with these capabilities include polyols, micelles, and fibers.

Based on their thin size, nanolaminates would be a suitable food coating. They would be applied to food by being dipped in a solution, which allows the laminate to adhere to a food surface. An alternate way would be to spray them directly onto the food product (Miller *et al.*, 1997). Several factors can also affect the properties of the laminates in terms of their thickness, properties and structure, such as the amount of dipping steps, the pH and temperatures of the solutions used or the order of dipping (Sorrentino *et al.*, 2007). Based on these factors, the future of nanolaminate as packaging holds several benefits. The addition of active ingredients with targeted and specific functions within the laminate would allow for tailor made functioning. For example, if ingredients such as antimicrobials or antioxidants are added, the benefits to the food product can be increased in terms of an increased shelf life as well as the quality of the food (Weiss *et al.*, 2006).

Amylose-lipid nanomaterial and wheat starch films are both edible materials. Thus, they could potentially be used to form edible films or coatings (with layers of edible material used as a coating) or as a film placed between food components to provide a barrier of mass transfer, as described by Sorrentino *et al.* (2007). These types of films could have several applications such as coatings in fresh fruits and vegetables, bakery products and confectionery, where they might protect the food from moisture, lipids, gases, off-flavours and odours. They are also biodegradable materials and as such will allow for environmentally friendly films to be made (Zhao *et al.*, 2008). The techniques used to make the films can also be done with fewer production steps, and as such can have potential monetary savings.

6.4 Conclusions and recommendations

The results achieved indicate that the choice of pasting time as well as modifying wheat starch with stearic acid can result in changes to film properties. Wheat starch films modified with 0.5% stearic acid, pasted for 30 minutes, showed some significant improvements to the films' properties, including increased mechanical properties, lower barrier properties and lower molecular mobility. These improvements could be attributed to the production of in situ nano size amylose-lipid complexes which can act as a nano filler material to increase wheat starch film properties.

When amylose-lipid complexes were added in situ to wheat starch films, it resulted in wheat starch films with properties such as “better” tensile properties, lower barrier properties and lower molecular mobility. These improvements suggest stronger interactions as well as molecular order being formed. Further research could be done to understand the mechanism by which amylose-lipid complexes act as nano filler material in wheat starch films. This could further help to understand the improvements seen in wheat starch films as well as additional ways to increase their mechanical, thermal and barrier properties.

Wheat starch films made with amylose-lipid complexes are also organic and edible in nature. Further research can be conducted to help unlock potential uses and find a commercial potential for these films. For example, the impact that amylose-lipid nanomaterial packaging material has on shelf life of food products. Alternatively, the effect of crystallisation and microfractures on the amylose-lipid nanomaterial for packaging material, could also be researched.

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